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The *Bam*HI J Fragment (0.706 to 0.737 Map Units) of Pseudorabies Virus Is Transcriptionally Active during Viral Replication

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The *Bam*HI J fragment of the pseudorabies virus (PRV) genome has not been associated with any viral transcripts during viral replication. In this report, data are presented to show that a portion of *Bam*HI-J is transcribed during a productive infection. Four oligo(dT)-cellulose-selected RNA species were detected by hybridization with probes derived from *Bam*HI-J. These RNAs were partially colinear, and they were transcribed in the opposite orientation with respect to the immediate-early gene (IE180) of PRV. At least one of the transcripts overlapped (antisense) the coding sequence of IE180 by 450 nucleotides. Expression of these RNAs was sensitive to phosphonoacetic acid, indicating that they are transcripts of PRV late genes. There were several similarities between these RNAs and the latency-associated transcripts detected in the trigeminal ganglia of swine latently infected with PRV (A. K. Cheung, J. Virol. 63:2908–2913, 1989).

The pseudorabies virus (PRV) genome is a linear, duplex DNA molecule with a molecular weight of approximately 90 $\times 10^6$ (2). It is estimated that the genetic material is capable of coding for 50 to 100 viral genes. The transcription pattern of PRV in infected cells is extremely complex; however, the genes are expressed in a coordinated and temporally regulated manner (8, 9, 12, 20). In general, herpesvirus genes are categorized into three classes: immediate-early (IE), early, and late genes. The IE genes are transcribed immediately upon infection and do not require de novo protein synthesis. Transcription of early genes depends on IE protein expression and occurs before viral DNA replication. The late genes are transcribed after the onset of viral protein and DNA synthesis.

More than 80 PRV-specific RNAs have been detected (there are some duplications) in PRV-infected cells, and their general locations have been ascribed to various regions of the viral genome (2). The entire viral genome, with the exception of the *Bam*HI J fragment, has been associated with RNAs of one or more classes. *Bam*HI-J is approximately 4.8 kilobases (kb) and is located at 0.706 to 0.737 map units (Fig. 1). Since *Bam*HI-J contains a portion (approximately 1.8 kb) of the internal repeat sequence, the same transcription inactivity reported is implied for the *Bam*HI O fragment, the duplicated terminal repeat sequence at 0.99 to 1.00 map units.

Recently, I demonstrated that at least two virus-specific latency-associated transcripts (LATs) were detected in the trigeminal ganglia of swine latently infected with PRV (5). These RNAs were transcribed in the opposite polarity with respect to the PRV IE gene (IE180). They contained *Bam*HI-J DNA sequences and overlapped the IE180 mRNA at the 3′ end. It is interesting that the PRV LATs differ from their herpes simplex virus type 1 LAT analog at least in two respects. While both viruses are members of the alphaherpesvirus group and exhibit many common structural and biological properties, the LATs of PRV are polyadenylated and the LATs of herpes simplex virus type 1 are reported to have a varying degree of polyadenylation (19, 23, 25). Herpes simplex virus type 1 LATs are antiparallel (i.e., opposite in polarity) and overlap ICP0 and not ICP4 (14, 22–27). Although both ICP0 and ICP4 are herpes simplex virus type 1 IE genes, ICP4 is the homolog equivalent of PRV IE180 (6).

One of the questions that arises is whether the LATs are expressed only during latency, representing a new class of RNAs, thus not following the expression cascade described for PRV, or whether the LATs are actually expressed during replication and belong to one of the gene classes, i.e., IE, early, or late, but have so far eluded the attention of researchers. The mechanisms that control viral gene expression during replication and latency are intimately associated. It is crucial that the transcription activity or inactivity around the *Bam*HI J region during replication be firmly established. In the present work, experiments were done to reexamine whether *Bam*HI-J, which is transcribed during latency in the antiparallel orientation with respect to the IE180 mRNA, is also transcriptionally active during a PRV infection. The data obtained indicated that the RNAs derived from *Bam*HI-J during viral replication share some common characteristics with the LATs previously described (5).

(Part of this research was presented at the 14th International Herpesvirus Workshop, Nyborg, Denmark, 20–26 August 1989.)

MATERIALS AND METHODS

Virus and cell culture. The Indiana-Funkhauser (In-Fh) strain of PRV was propagated on Madin-Darby bovine kidney (MDBK) cells. The MDBK cell cultures were maintained in Eagle minimum essential medium containing 10% fetal bovine serum (18).

Time course experiment. Parallel cultures of MDBK cells were set up and then infected with PRV (In-Fh) at a multiplicity of infection of 1.5. At various times postinfection (p.i.), the medium was removed and a guanidinium isothiocyanate-containing solution (7) was added to monolayer culture for total cellular RNA preparation. To study the effect of cycloheximide (100 μg/ml) or phosphonoacetic acid (PAA) (200 μg/ml) on RNA accumulation, I incubated the cells with drug-containing medium 1 h before and during the first hour of PRV infection.

Preparation of RNAs. Whole-cell total RNAs were prepared from PRV (In-Fh)-infected MDBK cells by the guanidinium isothiocyanate (7) and CsCl cushion centrifugation

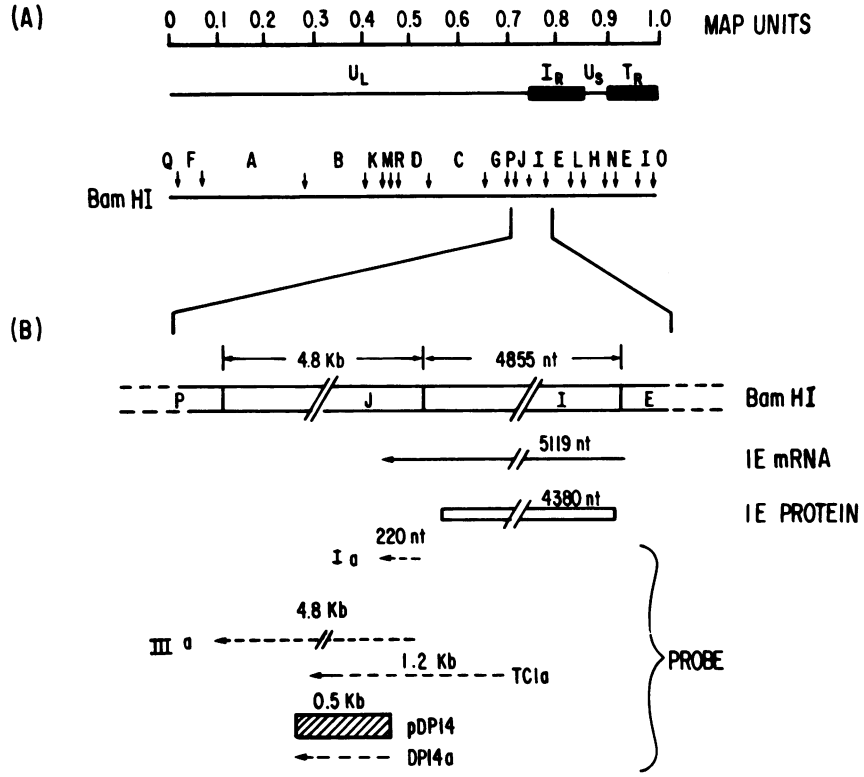

FIG. 1. (A) Schematic representation of the PRV genome and *Bam*HI restriction enzyme map. (B) Expanded diagram of *Bam*HI-I and *Bam*HI-J with the transcription and translation of the PRV IE gene. Size and polarity (arrows) of each single-stranded probe are also indicated. ▨, Nick-translated probe. U_L, Unique long region; I_R, internal repeat; U_S, unique short region; T_R, terminal repeat.

method (10). Poly(A)$^+$ RNAs were selected by oligo(dT)-cellulose chromatography (1).

Single-stranded RNA probes. [^{35}P]UTP-labeled runoff RNA transcripts of known specificity were generated from Bluescript plasmids (Stratagene, La Jolla, Calif.) containing PRV DNA as previously described (5). Since identical probes were used to study the PRV LATs, their designations are retained in this report (Fig. 1). Probe Ia was derived from a plasmid containing the rightmost 220 nucleotides (nt) of *Bam*HI-J, which, in fact, is the extreme 3′ end of the IE180 gene.

Probe IIIa was derived from the entire *Bam*HI J fragment. Probe TCla was derived from a plasmid containing approximately 1,250 nt of the *Bam*HI-I/*Bam*HI-J junction sequences, 550 nt from *Bam*HI-J and 700 nt from *Bam*HI-I. Probe DP14a was generated from plasmid pDP14 (see below). During the synthesis of single-stranded probes, 5′ and 3′ sequences of plasmid origin were also transcribed; thus, sizes of the probes are usually greater than the PRV-specific sequences present in the plasmid. The polarity of all four probes is identical to that of the PRV IE180 mRNA. These probes were used for the detection of RNAs transcribed in the opposite orientation with respect to the IE180 mRNA.

Nick-translated probe. Plasmid pDP14 contains approximately 500 nt of the *Bam*HI J fragment (Fig. 1) and was nick translated (21) in the presence of [α-^{32}P]dCTP for hybridization. The rightmost 60 nt of pDP14 overlaps probe Ia. Therefore, pDP14 is located about 150 nt from the *Bam*HI-I/*Bam*HI-J junction and extends leftward as indicated.

RNA analysis. S1 nuclease protection analysis (15, 28) and Northern (RNA) blot analysis (17) of PRV-specific transcripts were done as previously described (5). In all cases, equivalent amounts of RNA (determined spectrophotometrically) were used at each time point of the same experiment. Size estimates of the probes and the protected fragments were derived from RNA molecular weight standards purchased from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.).

Size fractionation of RNAs. Total cellular RNAs (100 μg in 100 μl) were incubated at 50°C for 5 min in the presence of 80% formamide, 10 mM Tris hydrochloride (pH 7.5), 1 mM EDTA, and 0.1% sodium dodecyl sulfate. The samples were diluted threefold with water and then layered onto a 10 to 30% linear sucrose gradient containing 20 mM Tris hydrochloride (pH 7.4), 40 mM NaCl, 1.0 mM EDTA, and 0.1% sodium dodecyl sulfate. Sample fractions were collected at the bottom of the tube after centrifugation (110,000 × *g*, 15.5 h, 18°C) in a Beckman SW40 rotor. The RNA samples were precipitated in ethanol in the presence of 300 mM sodium acetate (pH 5.2).

RESULTS

Transcription from internal repeat sequence of *Bam*HI-J. RNA transcription from the *Bam*HI J fragment during a PRV infection was analyzed by S1 nuclease protection experiments. A single-stranded probe (probe Ia) containing the extreme 3′ end (220 nt) of the IE180 transcript was used to analyze total cellular RNAs isolated from PRV-infected cells at the indicated times p.i. (Fig. 2). The relative abundance of *Bam*HI-J-derived RNAs present throughout the infection cycle was estimated by using an equivalent amount (25 μg) of RNAs from each time point. No virus-specific fragments

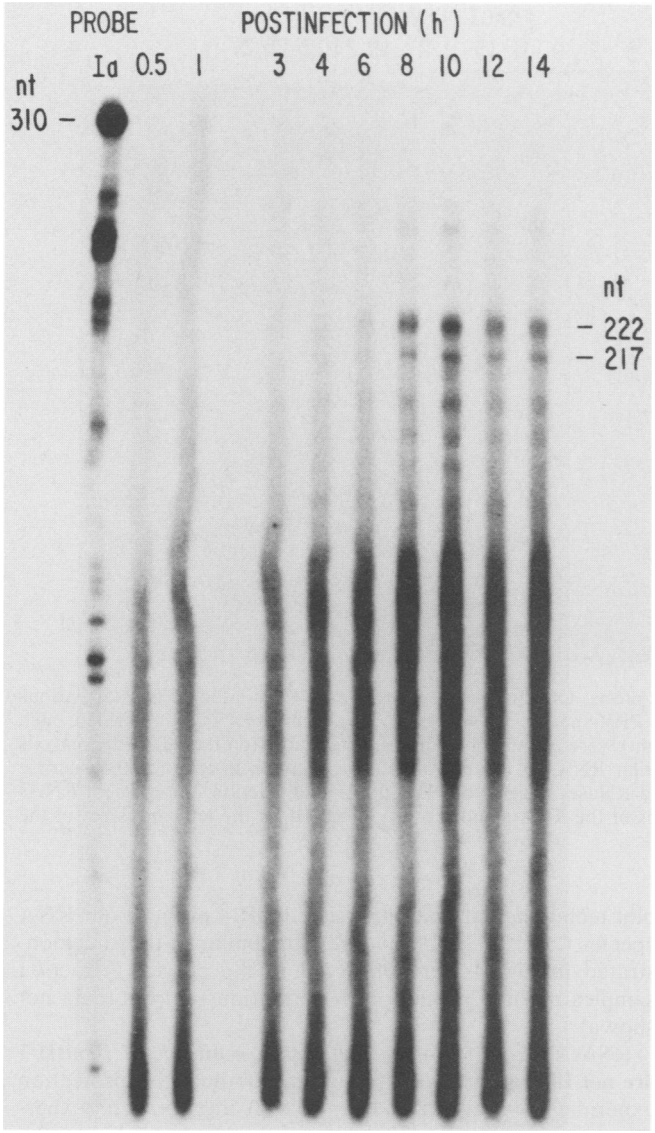

FIG. 2. Detection of RNA transcript with probe Ia. Total cellular RNAs isolated from PRV-infected cells at various times p.i. were analyzed by the S1 nuclease technique and electrophoresed on a 6% sequencing gel (16). Sizes of the input probe and protected bands derived from a known sequencing ladder are indicated on the left and right, respectively.

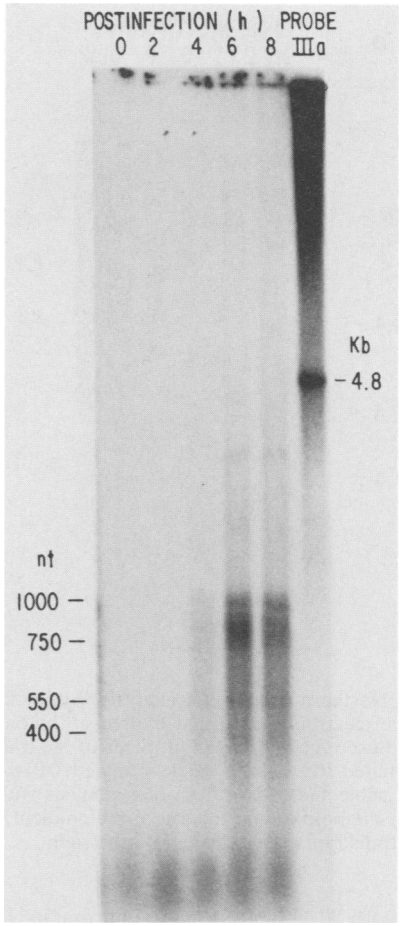

FIG. 3. Confirmation of *Bam*HI-J-derived RNA(s) during viral replication with probe IIIa. S1 nuclease protection analysis with probe IIIa was done with total RNAs isolated from PRV-infected cells at the indicated times p.i. The samples were analyzed on a 1% agarose gel after glyoxal-dimethyl sulfoxide treatment and heat denaturation (4). Size of the input probe IIIa is indicated on the right, and sizes of the protected fragments are indicated on the left.

were protected at 3 h p.i. At 4 and 6 h p.i., small amounts of several S1 nuclease-resistant fragments could be detected. As the infection proceeded, these protected bands became more evident. Among them, two protected fragments of 217 and 222 nt (in close agreement with the PRV-specific sequences of 220 nt present in probe Ia) were the most prominent. They were maintained at approximately the same level for the rest of the infection cycle.

S1 nuclease protection analysis with probe IIIa (approximately 4.8 kb). To confirm and extend the S1 nuclease analysis results obtained with probe Ia, I used the much larger probe IIIa of the same polarity in similar experiments. Selected RNA samples (15 μg) from the indicated time p.i. were analyzed (Fig. 3). No protected fragments were observed at 0 and 2 h p.i. At later time points (4, 6, and 8 h p.i.), four S1 nuclease-resistant fragments of 400, 550, 750 (a cluster), and 1,000 nt were observed. In other experiments, the same S1-protected fragments, but in a reduced amount, have been detected as early as 3 h p.i. (data not shown). These data suggest that multiple RNAs are transcribed from *Bam*HI-J in the same orientation and/or that the RNA(s) detected is spliced.

Northern (RNA) blot analysis. An RNA blot of oligo(dT)-selected RNAs (5 μg per time point) isolated from PRV-infected MDBK cells throughout the infectious cycle was analyzed with a nick-translated pDP14 probe (Fig. 4a). No specific hybridization was detected at early stages of the infection (0 to 4 h p.i.). From 6 to 12 h p.i., four RNA species of sizes greater than 9.5, 8.2, 4.4, and 2.0 kb hybridized with probe pDP14. Identical results were obtained when similar Northern blot analysis with probe pDP14 was performed with corresponding total cellular RNA samples before oligo(dT)-cellulose selection (date not shown).

An additional experiment was done to confirm and establish the polarity of the RNA species detected here. Total cellular RNAs at 8 h p.i. were size fractionated on a sucrose gradient. A portion of a sample from the indicated fraction collected was subjected to Northern blot analysis. After hybridization with the single-stranded DP14a probe, the blot

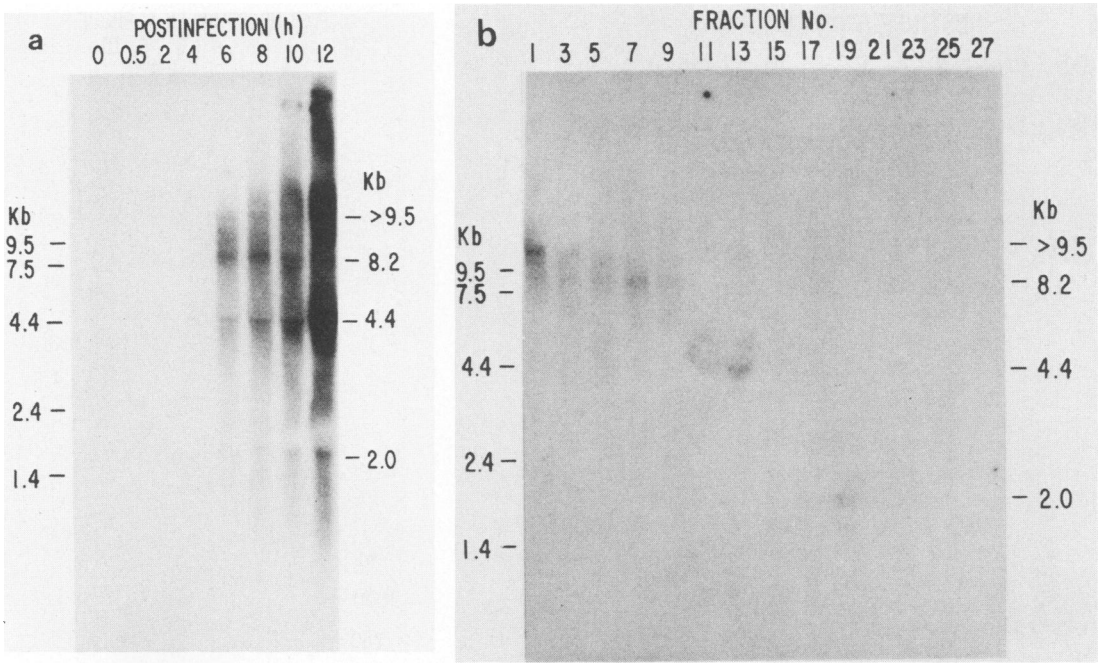

FIG. 4. (a) Northern (RNA) blot analysis with nick-translated pDP14 probe. Oligo(dT)-selected RNAs of PRV-infected cells at various times p.i. were electrophoresed and blotted on nylon membranes. The PRV-specific DNA present in plasmid pDP14 was purified, nick translated, and used for hybridization. Sizes of the transcripts (on the right) were estimated from RNA standards (on the left). (b) Analysis of size-fractionated RNAs with single-stranded DP14a probe. Total cellular RNAs at 8 h p.i. after centrifugation in a 10 to 30% sucrose gradient were subjected to Northern blot analysis with DP14a probe and RNase A digestion. Fraction 1 and fraction 27 represent RNAs collected from the bottom and the top of the gradient, respectively. Sizes of the RNA standards are indicated on the left, and sizes of the PRV-specific transcripts are indicated on the right.

was treated with 80 U of RNase A (Pharmacia, Inc., Piscataway, N.J.) per ml at room temperature for 20 min before autoradiography. The results (Fig. 4b) showed that all four RNA species (>9.5, 8.2, 4.4., and 2.0 kb) have the same polarity and are antiparallel to the IE180 mRNA. These data further indicated that the RNAs detected are partially colinear.

Confirmation of partially colinear transcripts and evidence for antisense sequence to coding region of PRV IE180 gene. Oligo(dT)-selected RNAs (5 μg) from various times p.i. were subjected to S1 nuclease protection analysis with probe TCla. Multiple protected bands were observed, with the 1.2- and 0.35-kb fragments being more prominent during the infection. A protected fragment of 1.2 kb was observed at 4 h p.i. (Fig. 5). As the infection proceeded, this 1.2-kb fragment, together with a 0.35-kb fragment, became more abundant, although minor bands were also observed. The 1.2-kb protected fragment demonstrated that one of the transcripts must contain nucleotide sequences complementary to the entire PRV-specific sequences present in the TCla probe. Furthermore, the 3′ end of this particular transcript overlapped IE180 mRNA for about 850 bases. Since the size of the protected bands together exceeded the size of viral sequences in probe TCla, multiple transcripts must have derived from the same region and they shared at least 350 identical nucleotides.

Poly(A)$^+$ versus poly(A)$^-$ RNAs. Total RNAs from two time points, 6 and 8 h p.i., were separated into poly(A)$^+$ and poly(A)$^-$ fractions after recycling each sample through an oligo(dT)-cellulose column three times and washing the column extensively before elution. Equal amounts (10 μg) of RNAs from each fraction were analyzed by the Northern blot technique with nick-translated DP14 probe. Four RNA species (>9.5, 8.2, 4.4, and 2.0 kb) similar to those demonstrated in Fig. 4 were observed in the poly(A)$^+$ fraction samples but not in the poly(A)$^-$ fraction samples (data not shown).

RNAs derived from internal repeat sequence of *Bam*HI-J are not IE transcripts. Although the S1 nuclease protection experiments (with probes Ia and TCla) and RNA blot experiments (with probe pDP14) consistently showed that the *Bam*HI-J derived transcripts were not synthesized at the initial stages of the infectious cycle, it was possible that they were made at an extremely low level and therefore not detected. Previous work (4, 13, 20) showed that cycloheximide inhibits protein synthesis and facilitates the accumulation of the PRV IE180 mRNA. Parallel cultures were set up and incubated in medium containing cycloheximide 1 h before PRV (In-Fh) infection. Cells were exposed to virus for 1 h, still in the presence of cycloheximide, and then the culture medium was replaced with fresh medium with or without cycloheximide. Total RNAs from cycloheximide-treated MDBK cells that were mock infected (Fig. 6, lane 1) or PRV infected (Fig. 6, lane 2) did not yield any protected fragment when examined by S1 nuclease analysis with probe IIIa. When cycloheximide was removed from the PRV-infected cultures and replaced with regular medium, the RNAs isolated 1 h after cycloheximide removal (Fig. 6, lane 3) remained negative for probe IIIa sequences. But by 4 h after cycloheximide removal (Fig. 6, lane 4), protected fragments of 400, 550, 750, and 1,000 nt were detected. These protected fragments were similar to those obtained in an earlier experiment (Fig. 3) with PRV-infected cultures

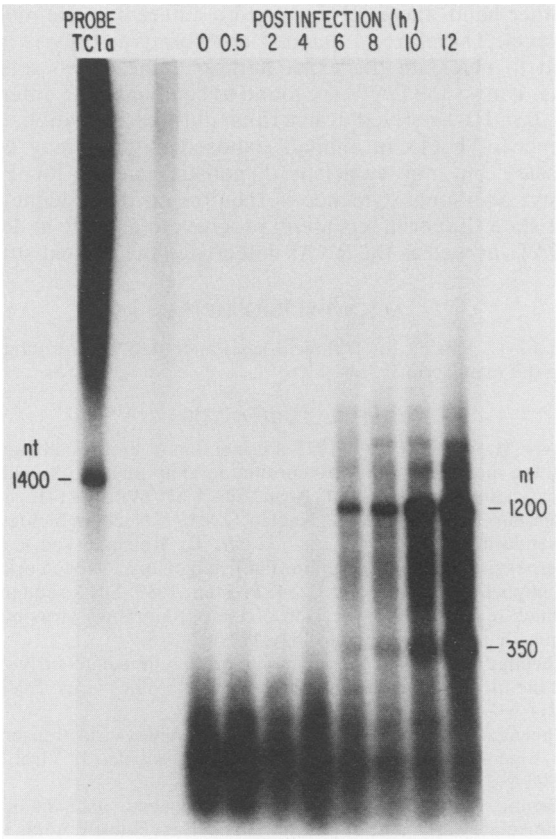

FIG. 5. S1 nuclease analysis with probe TCla. Poly(A)$^+$ RNAs at various times p.i. were analyzed with a probe (TCla) that contains sequences from both BamHI-I and BamHI-J. The samples were electrophoresed on a 1% agarose gel after chemical and heat denaturation. Molecular size of the input probe is indicated on the left, and sizes of the protected bands are indicated on the right.

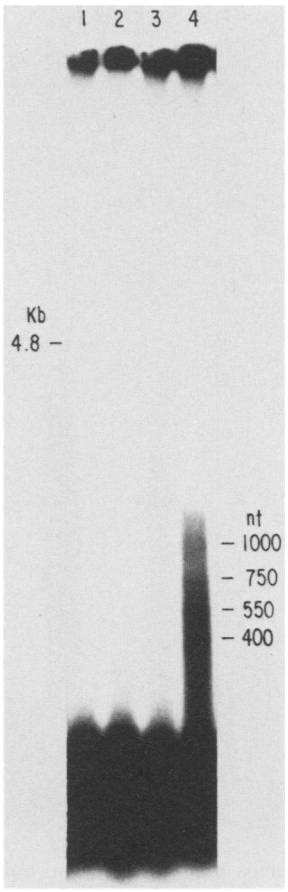

FIG. 6. Cycloheximide reversal experiment. Parallel cultures were treated with cycloheximide (100 μg/ml) 1 h before PRV infection. S1 nuclease analysis was done with total cellular RNAs isolated from mock-infected cells (lane 1) and cells at 4 h p.i. (lane 2). At 1 h p.i., cycloheximide was removed from the medium, and the cultures were washed three times and then maintained in regular medium for an additional 1 h (lane 3) and 4 h (lane 4), respectively, before harvest. Size of input probe IIIa is indicated on the left, and sizes of the protected fragments are indicated on the right.

that had not been exposed to cycloheximide at 4, 6, and 8 h p.i.

PAA inhibits synthesis of *Bam*HI-J-derived RNAs. PAA has been shown to interfere with DNA polymerase activity, inhibit DNA synthesis, and reduce the transcription of early and late genes of herpesviruses (11). Oligo(dT)-selected RNAs from parallel cultures of PRV-infected, PAA-treated, and PAA-untreated MDBK cells at various times p.i. were compared. Northern blot analysis with nick-translated pDP14 probe showed that all the previously detected *Bam*HI-J-specific RNAs (>9.5, 8.2, 4.4, and 2.0 kb) were no longer detected in cells treated with PAA (data not shown). The results obtained with the more sensitive S1 nuclease protection analysis with probe TCla are presented in Fig. 7. Except for an extremely low level of protected fragment (1.2 kb) in the 12-h p.i. PAA-treated sample, no protected bands were detected in the other PAA-treated samples from earlier time points. These data clearly demonstrated that the synthesis of *Bam*HI-J-specific RNAs was severely inhibited in the presence of PAA.

DISCUSSION

The experiments in this report employed S1 nuclease protection analysis, a very sensitive and specific method, to detect low levels of RNAs in PRV-infected cells. When RNAs synthesized during the entire PRV replicative cycle were analyzed, a portion of *Bam*HI-J was shown to encode virus-specific transcripts, and the transcribed RNAs could be detected at 3 to 4 h p.i. These transcripts were not detected at earlier stages of the infection cycle and did not accumulate in cycloheximide-treated cells, indicating that they are not IE gene transcripts. The synthesis of these RNAs was severely inhibited by PAA treatment, which suggests that the RNAs are probably late PRV transcripts. Four poly(A)$^+$ RNAs of >9.5, 8.2, 4.4, and 2.0 kb hybridized to probes (pDP14 and pDP14a) derived from *Bam*HI-J. The relationship between these RNA species is not known at the present time. Whether the higher-molecular-weight RNAs are precursors for the lower-molecular-weight RNAs has not been examined. S1 nuclease protection analysis with probe TCla confirmed that at least two RNAs shared at least 350 identical nucleotides and that these RNAs are transcribed in the opposite orientation with respect to the IE180 mRNA.

The exact location of the PRV IE gene has been mapped and the DNA nucleotide sequence has been determined (3, 4, 6). At least one of the RNAs detected here overlaps the IE180 mRNA by 850 nt in the antiparallel orientation.

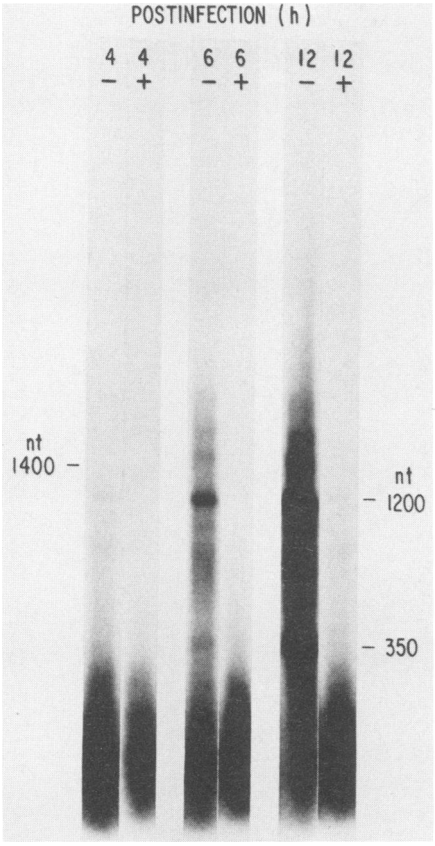

FIG. 7. PAA inhibits transcription from *Bam*HI-J. S1 nuclease analysis was done with oligo(dT)-selected RNAs at the indicated time p.i. with probe TCla. + and − denote the presence or absence of PAA, respectively, during the PRV infection. Sizes of the input probe TCla and the protected fragments are indicated on the left and right, respectively.

Therefore, this RNA extends into the coding region of IE180 by approximately 450 nt. Examination of the complementary DNA nucleotide sequence of the IE180 gene revealed that a consensus polyadenylation signal (AATAAA) occurs in *Bam*HI-J, 171 nt from the *Bam*HI-I/*Bam*HI-J juncture. No other polyadenylation signal is present in the entire 4.8 kb of *Bam*HI-I.

As mentioned earlier, at least two poly(A)$^+$ RNAs (2.0 and 0.95 kb) were detected in the trigeminal ganglia of latently infected swine that were experimentally exposed to the Becker strain of PRV (5). The LATs are transcribed in the opposite orientation with respect to the IE180 mRNA and contain sequences derived from the internal repeat portion of *Bam*HI-J, which is similar to the results obtained here with the In-Fh strain-infected cell RNAs. Indeed, a 2.0-kb RNA similar in size to one of the LATs was noted in this study. Thus, the data presented here and the results of LATs in latently infected swine (5) unequivocally demonstrated that the *Bam*HI J fragment is transcriptionally active during viral replication as well as during latency. However, it is not clear whether any identical transcripts are synthesized under these two apparently mutually exclusive conditions. On one hand, the similarities between these two sets of RNAs suggest that the PRV LATs are actually late PRV transcripts synthesized during viral replication and that latency is induced when the normal infection cycle is interrupted by specific factors after the synthesis of LATs. On the other hand, these RNAs may be different at the molecular level. Therefore, a detailed comparative study is warranted to elucidate the exact nature of these two sets of RNAs. Unless the LATs are found to be unique and different from *Bam*HI-J-derived transcripts during replication, the presence of LATs in animals exposed to PRV may be a necessary but not sufficient diagnostic marker for PRV latency; additional evidence is required to truly define the latent state. Experiments are in progress to analyze in detail the LATs as well as the RNAs detected in the present study.

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LITERATURE CITED

1. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA **69**:1408–1412.
2. **Ben-Porat, T., and A. S. Kaplan.** 1985. Molecular biology of pseudorabies virus, p. 105–173. *In* B. Roizman (ed.), The herpesviruses, vol. 3. Plenum Publishing Corp., New York.
3. **Campbell, M. E. M., and C. M. Preston.** 1987. DNA sequences which regulate the expression of the pseudorabies virus major immediate early gene. Virology **157**:307–316.
4. **Cheung, A. K.** 1988. Fine mapping of the immediate-early gene of the Indiana-Funkhauser strain of pseudorabies virus. J. Virol. **62**:4763–4766.
5. **Cheung, A. K.** 1989. Detection of pseudorabies virus transcripts in trigeminal ganglia of latently infected swine. J. Virol. **63**: 2908–2913.
6. **Cheung, A. K.** 1989. DNA nucleotide sequence analysis of the immediate-early gene of pseudorabies virus. Nucleic Acids Res. **17**:4637–4646.
7. **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.
8. **Feldman, L. T., J. M. DeMarchi, T. Ben-Porat, and A. S. Kaplan.** 1982. Control of abundance of immediate-early mRNA in herpesvirus (pseudorabies)-infected cells. Virology **116**:250–263.
9. **Feldman, L. T., F. J. Rixon, J. H. Jean, T. Ben-Porat, and A. S. Kaplan.** 1979. Transcription of the genome of pseudorabies (a herpesvirus) is strictly controlled. Virology **97**:316–327.
10. **Glisin, V., R. Crykvenjakov, and C. Byus.** 1974. Ribonucleic acid isolated by cesium chloride centrifugation. Biochemistry **13**:2633–2637.
11. **Honess, R. W., and D. H. Watson.** 1977. Herpes simplex virus resistance and sensitivity to phosphonoacetic acid. J. Virol. **21**:584–600.
12. **Ihara, S., L. Feldman, S. Watanabe, and T. Ben-Porat.** 1983. Characterization of the immediate-early functions of pseudorabies virus. Virology **131**:437–454.
13. **Jean, J. H., T. Ben-Porat, and A. S. Kaplan.** 1974. Early functions of the genome of herpesvirus. III. Inhibition of transcription of the viral genome in cells treated with cycloheximide early during the infective process. Virology **59**:516–523.
14. **Krause, P. R., K. D. Croen, S. E. Straus, and J. M. Ostrove.** 1988. Detection and preliminary characterization of herpes simplex virus type 1 transcript in latently infected human trigeminal ganglia. J. Virol. **62**:4819–4823.
15. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **68**:499–560.
17. **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.
18. **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.
19. **Puga, A., and A. L. Notkins.** 1987. Continued expression of a poly(A)$^+$ transcript of herpes simplex virus type 1 in trigeminal ganglia of latently infected mice. J. Virol. **61**:1700–1703.
20. **Rakusanova, T., T. Ben-Porat, M. Himeno, and A. S. Kaplan.** 1971. Early functions of the genome of herpesvirus. I. Characterization of the RNA synthesized in cycloheximide treated infected cells. Virology **46**:877–889.
21. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase. J. Mol. Biol. **113**: 237–251.
22. **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.
23. **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.
24. **Stevens, J. G., E. K. Wagner, G. B. Devi-Rao, M. L. Cook, and L. Feldman.** 1987. RNA complementary to a herpesvirus alpha gene mRNA is predominant in latently infected neurons. Science **235**:1056–1059.
25. **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.
26. **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.
27. **Wechsler, S. L., A. B. Nesburn, J. Zwaagstra, and H. Ghiasi.** 1989. Sequence of the latency-related gene of herpes simplex virus type 1. Virology **168**:168–172.
28. **Zinn, K., D. DiMaio, and T. Maniatis.** 1983. Identification of two distinct regulatory regions adjacent to the human β-interferon gene. Cell **34**:865–879.