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Expression of Herpes Simplex Virus Type 1 (HSV-1) Latency-Associated Transcripts and Transcripts Affected by the Deletion in Avirulent Mutant HFEM: Evidence for a New Class of HSV-1 Genes

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During latent herpes simplex virus type 1 (HSV-1) infection in the trigeminal ganglia of mice, three virus-specific transcripts, 2.0, 1.5, and 1.45 kilobases (kb), are detectable by Northern (RNA) blot analysis, but only the 2.0-kb transcript can be detected in HSV-1-infected tissue culture cells (J. G. Spivack and N. W. Fraser, J. Virol. 61:3842–3847, 1987). Since these latency-associated genes map to a diploid region of the genome, transcription from the deletion mutant HFEM, which contains only one complete copy of these genes, was investigated to determine the effect of gene dosage. The 4.1-kb HFEM deletion is located between the α genes ICP0 and ICP27. ICP0 mRNA and the 2.0-kb latency-associated transcript were present at normal levels during HFEM infection, but ICP27 mRNA and 0.9- and 1.1-kb transcripts that map near the deletion were not readily detectable. The levels of expression of one or more of these genes might be an important determinant of HSV-1 virulence in animal hosts. ICP27 mRNA accumulated when protein synthesis was inhibited before HFEM infection, implying that the deletion may affect ICP27 regulatory rather than coding elements. Expression of the 2.0-kb latency-associated transcript was characterized in infected CV-1 cells with metabolic inhibitors and strand-specific probes. On the basis of metabolic inhibitor studies, the gene encoding the 2.0-kb latency-associated transcript is not an α gene. During HSV-1 replication in infected tissue culture cells, the β and γ genes require the prior expression of α gene products. However, the latency-associated RNAs are expressed in the absence of detectable levels of α transcripts in latently infected mice. Thus, this latency-associated gene family appear to be regulated quite differently than α, β, or γ genes. For these reasons, and because the latency-associated genes may perform latent rather than replicative functions, we propose that they should be considered members of a new HSV-1 gene class, the λ genes.

After a primary infection, herpes simplex virus type 1 (HSV-1) can remain in a latent state for the life of the individual (2, 16, 46). Virus-specific transcripts have been detected by in situ hybridization during HSV-1 latency in the central (10, 12, 48) and peripheral (9, 47) nervous systems of mice and rabbits (33), in human trigeminal ganglia (8, 45), and during HSV-2 latency in guinea pig (49) and human (13, 14) sensory ganglia. The HSV-1 transcripts present in the sensory ganglia of latently infected animals originate from the repeat regions (9, 10, 12, 32, 40, 41, 47), which are present in two copies per genome. Three viral transcripts, 2.0, 1.5, and 1.45 kilobases (kb), which are present during HSV-1 latency in mice map to a 3.0-kb region contained within *Bam*HI-B and -E and the long repeat region (Fig. 1) (41). These RNAs partially overlap the 3′ terminus of ICP0 (41) and are transcribed in the opposite direction (33, 41, 47). Only the 2.0-kb transcript has been detected, at low levels, in HSV-1-infected cells (41).

By definition, the α genes, ICP0, -4, -22, -27, and -47, are transcribed in HSV-1-infected cells in the absence of protein synthesis (17, 22, 34, 51); β and some γ_1 genes require protein synthesis but not viral DNA synthesis, and γ_2 gene transcription is strictly dependent upon both protein and viral DNA synthesis (17, 34). During HSV-1 replication in tissue culture, β and γ genes require the prior expression of α genes (17, 34). The latency-associated transcripts are expressed in the absence of detectable levels of α or β RNAs during latent infection in mice (8, 35, 41, 47) and in humans (45). In this study, the 2.0-kb latency-associated transcript was characterized in infected CV-1 cells with metabolic inhibitors and strand-specific probes. The data indicate that the latency-associated RNAs are not transcribed from α genes. Since they are expressed with characteristics different from those encoded by α, β, or γ genes, we propose that they be termed λ (lambda) genes.

The deletion mutant HFEM, which encodes only one complete copy of the latency-associated genes, was studied to examine the effects of gene dosage. HSV-1 strain HFEM, which was derived from HF (52), has a 4.1-kb deletion within *Bam*HI-B and the long internal repeat (of the prototype configuration of the genome) that is between the α genes ICP0 and ICP27 (Fig. 1) (36). The termini of the deletion have been precisely located by sequence analysis (19). HFEM replicates at 37°C in tissue culture (26) and is virulent in mice inoculated intracerebrally but avirulent in mice inoculated intraperitoneally, subcutaneously, or intravenously (1). HFEM can establish a latent infection in mice after peripheral inoculation (26, 41). Since replacement of the deleted region by *Bam*HI-B or a 3.8-kb *Hpa*I-*Hpa*I subfragment of B from strain F leads to virulent recombinants (1, 37) the gene(s) affected by the HFEM deletion is likely to be important in the pathogenesis of HSV-1 infections in experimental animal models.

We have shown that the viral transcripts present in mice latently infected with strain F are also present with strain HFEM (41). One copy of the latency-associated genes is

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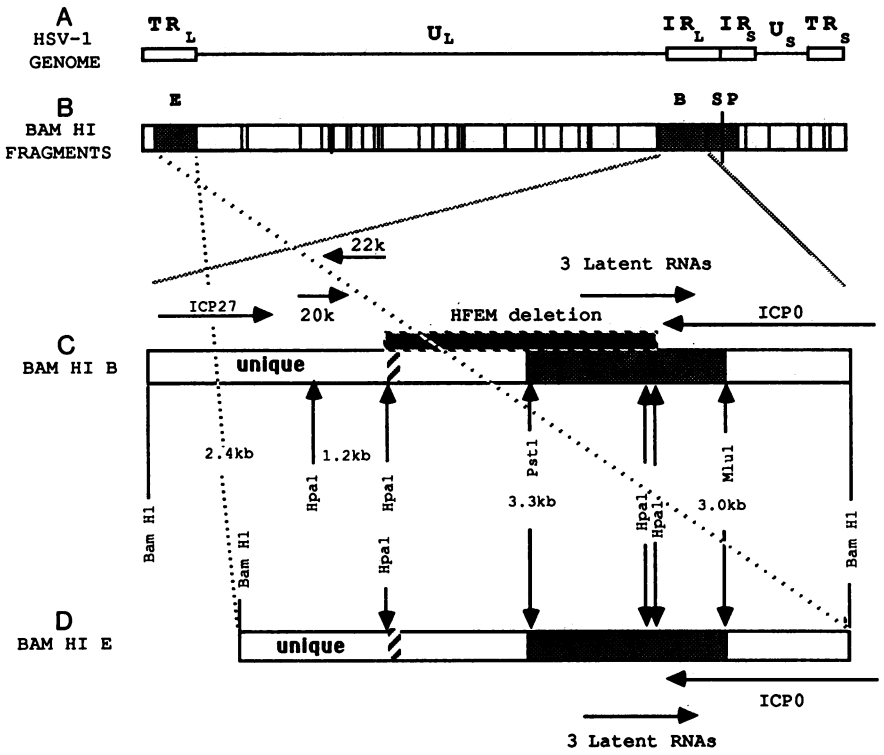

FIG. 1. Map of HSV-1 genome and transcripts within *Bam*HI-B. (A) The HSV-1 genome, illustrating the unique long and short (U_L and U_S) regions of the genome (——) bounded by the internal (IR) and terminal repeat (TR) regions (□). (B) The positions of the *Bam*HI restriction sites of HSV-1 strain F (29). The fragments that are positive by in situ hybridization during latency are labeled and shaded (9, 10). (C) Detailed map of *Bam*HI B. The sizes of the *Hpa*I fragments (McGeoch et al., unpublished data) and positions of other restriction sites used in this study are shown (1, 22, 28, 38). The approximate locations of ICP0, ICP27 (6, 22, 28), 0.9-kb (20k), and 1.1-kb (22k) (23, 41) mRNAs are marked by arrows that indicate the direction of transcription. The shaded area between the *Pst*I and *Mlu*I restriction sites hybridized to three overlapping HSV-1 latency-associated transcripts, which are represented by a single arrow, by Northern blot analysis (41). The hatched region is the boundary between the unique long region and long internal repeat (19). The 4.1-kb deletion in strain HFEM is marked by a shaded box above *Bam*HI-B (36). (D) Detailed map of *Bam*HI-E. *Bam*HI-E is shown in reverse orientation to emphasize that the region between the boundary of the long repeat and the *Bam*HI site on the right is also contained within *Bam*HI-B.

deleted in HFEM, while the second copy is present intact in *Bam*HI-E (Fig. 1). In this report, evidence is presented that ICP27 mRNA and 0.9- and 1.1-kb RNAs that map to *Bam*HI-B (41) are not present at high levels in CV-1 cells infected with HFEM. The 1.1- and 0.9-kb transcripts partially overlap each other and are transcribed in opposite directions. The 0.9- and 1.1-kb transcripts are synthesized in HSV-1(F) infected cells when viral DNA synthesis is inhibited but not when protein synthesis is blocked, implying that they are β or γ_1 genes. The implications of these results for HSV-1 replication in tissue culture cells and the pathogenesis of infections in experimental animal models are discussed.

MATERIALS AND METHODS

Cell culture and HSV-1 growth and titration. Subconfluent monolayers of CV-1 cells grown in Eagle minimum essential medium with 5% fetal calf serum at 37°C with 5% CO_2 were infected with HSV-1 (strain F from B. Roizman, University of Chicago; HFEM from G. Cohen, University of Pennsylvania) at 1 PFU per cell, as previously described (9, 42).HSV-1 titers were determined on CV-1 cells, and the plaques were stained with 1% methylene blue and counted 2 days later (43, 44).

RNA extraction. To isolate HSV-1 RNA, CV-1 cells were infected at 5 PFU per cell. The cells were trypsinized at 5 to 6 h postinfection, and after low-speed centrifugation, the cell pellets were homogenized (Polytron, setting 5, 20 s) in a solution containing 4 M guanidinium thiocyanate, 0.5% sodium-*N*-lauroylsarcosine, 100 mM β-mercaptoethanol, 25 mM sodium citrate (pH 7.0), and 0.1% antifoam A (Sigma Chemical Co.) (4). The RNA was pelleted through a cushion of 5.7 M CsCl–0.1 M EDTA (pH 7.0) by centrifugation at 150,000 × *g* in a Beckman SW40.1 rotor for 20 to 24 h at 18°C (4, 40, 41). After centrifugation the RNA was suspended in H_2O and stored as an ethanol precipitate at −20°C. A_{260} was measured.

Agarose gel electrophoresis, Northern (RNA) blot transfer of RNA, and hybridization and washing of Northern blots. RNA was denatured with glyoxal, electrophoresed through 1.2% agarose, and capillary blotted to Gene Screen Plus (Du Pont Co.), as previously described (40, 41). RNA markers were purchased from Bethesda Research Laboratories, Inc. After blotting and air drying, the glyoxylation was reversed, as previously described (40, 41). The filters were prehybridized, and a ^{32}P-labeled nick-translated or single-strand-labeled probe was added to hybridize overnight (40, 41). After hybridization, the filters were washed in decreasing concentrations of SSC to 0.1× (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) with 1% sodium dodecyl sulfate at 65°C for two 30-min washes. The filters were covered with plastic wrap and autoradiographed with XAR-5 film

(Eastman Kodak Co.) and an intensifying screen (Lightning Plus; Du Pont) at −70°C.

Preparation of ^{32}P-labeled probes. The cloned *Bam*HI-B fragment of HSV-1(F) was obtained from B. Roizman (29). Subfragments of *Bam*HI-B were prepared by gel electrophoresis of restriction digests and electroelution into dialysis tubing (24) or onto DEAE paper (NA-45; Schleicher & Schuell, Inc.), as recommended by the manufacturer. DNA restriction enzymes were purchased from Boehringer Mannheim or Bethesda Research Laboratories and used as recommended. DNA probes were nick translated by standard procedures (24). Single-strand-labeled probes were synthesized from HSV-1 DNA cloned into M13 mp18 and mp19 vectors, by the method of Hu and Messing (18). The probes were separated from unincorporated nucleotides by passage through Sephadex G-50 mini-spin columns (Boehringer) and ethanol precipitation. Specific activities of the nick-translated probes were 1×10^8 to 5×10^8 cpm/μg of DNA.

Inhibitors. Stocks of phosphonoacetic acid (PAA) and cycloheximide (Sigma), 10 and 1 mg/ml, respectively, were made in phosphate-buffered saline, adjusted to pH 7.0, filter sterilized, and stored in 1-ml portions at −20°C. PAA (400 μg/ml) and cycloheximide (50 μg/ml) were added to cell cultures 30 to 60 min before infection and were present continuously until RNA extraction.

RESULTS

Determination of the kinetic class of the 2.0-kb latency-associated transcript. Of the three latency-associated transcripts present in latently infected mice (40, 41), only the 2.0-kb RNA is detectable during the acute stage of infection in mice (40, 41) and in infected tissue culture cells (41). This transcript was characterized in infected CV-1 cells in the presence and absence of PAA, which blocks viral DNA synthesis (25), and cycloheximide, which inhibits protein synthesis (Fig. 2). The 2.0-kb latency-associated transcript was detected in HSV-1-infected cells with a single-strand-labeled *Hpa*I-*Mlu*I probe (Fig. 2A, lane 2). In the presence of PAA (Fig. 2A, lane 3) or cycloheximide (lane 4), this RNA could not be detected. Because of the long exposure time, there was some cross-hybridization with the 2.7-kb ICP0 mRNA (6, 28, 51), especially in the cycloheximide-treated sample (lane 4). For comparison, the *Hpa*I-*Mlu*I single-strand probe labeled in the opposite orientation hybridized to ICP0 mRNA in HSV-1-infected cells in the absence (Fig. 2B, lane 2) or presence of PAA (lane 3) or cycloheximide (lane 4). These results demonstrate that the 2.0-kb latency-associated transcript is not synthesized in infected cells with characteristics of an α or β gene, in contradistinction to the α gene ICP0 (17, 34), which partially overlaps its 3′ end and is transcribed in the opposite direction (41).

Detection of HSV-1 transcripts that map to *Bam*HI-B in strains F and HFEM. One goal of this study was to analyze viral transcription in the region surrounding the HFEM deletion, which is located completely within *Bam*HI-B (Fig. 1). To localize the HSV-1 transcripts, *Bam*HI-B was cut with *Hpa*I to yield four major subfragments (Fig. 1). The 2.4-kb *Bam*HI-*Hpa*I probe hybridized to ICP27 mRNA (6, 22, 51) and a 0.9-kb transcript in CV-1 cells infected with strain F but did not hybridize to either transcript in cells infected with strain HFEM (Fig. 3A), except in very long exposures (data not shown). The 1.2-kb *Hpa*I-*Hpa*I probe hybridized to the 0.9- and 1.1-kb transcripts in cells infected with F but not in cells infected with HFEM (Fig. 3B). In very long exposures, the 0.9-kb RNA, but not the 1.1-kb RNA, was just

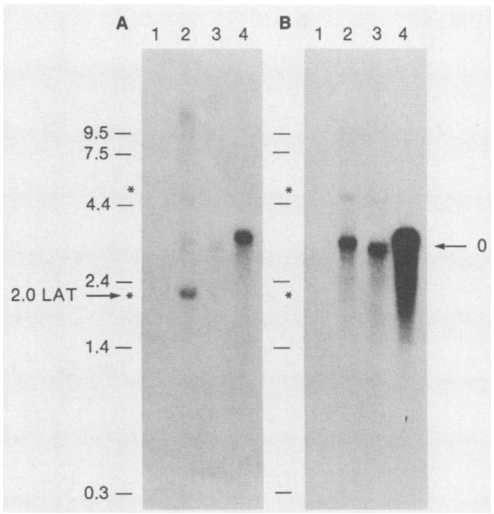

FIG. 2. Synthesis of the 2.0-kb latency-associated transcript in the presence and absence of PAA and cycloheximide. Lanes: 1, 5 μg of RNA from uninfected CV-1 cells; 2, 5 μg of RNA from HSV-1-infected CV-1 cells (multiplicity of infection, 5), 5 to 6 h postinfection; 3, 5 μg of RNA from HSV-1-infected CV-1 cells with 400 μg of PAA per ml; 4, 5 μg of RNA from HSV-1-infected CV-1 cells with 50 μg of cycloheximide per ml. The drugs were present continuously from 1 h before infection until the RNA was isolated at 5 to 6 h postinfection. (A) 1.2-kb *Hpa*I-*Mlu*I single-strand-labeled probe (mp18), 6-day exposure; (B) single-strand-labeled probe (mp19), 16-h exposure. The positions of the 2.0-kb latency-associated transcript (2.0 LAT) and ICP0 (0) mRNA are indicated by arrows. The positions of RNA markers (kilobases) are labeled on the left, and the positions of 28S and 18S rRNAs are indicated by asterisks.

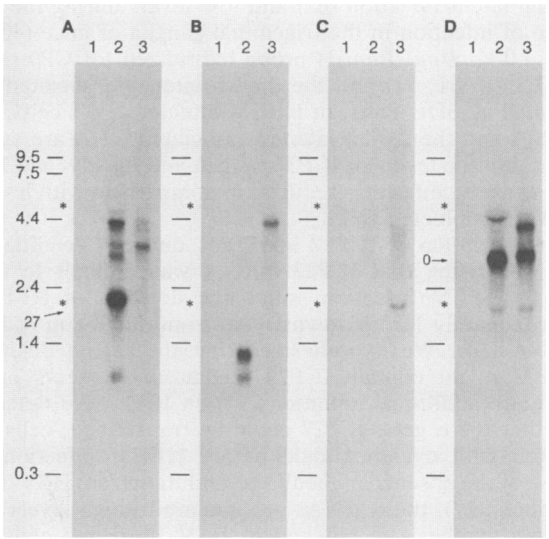

FIG. 3. Detection of HSV-1 RNAs that map to the *Hpa*I fragments of *Bam*HI-B in strains F and HFEM by Northern blot analysis. Lanes: 1, 2.5 μg of RNA from uninfected CV-1 cells; 2, 2.5 μg of RNA from HSV-1(F)-infected CV-1 cells; 3, 2.5 μg of RNA from HSV-1(HFEM)-infected CV-1 cells. (A) 2.4-kb *Bam*HI-*Hpa*I probe; (B) 1.2-kb *Hpa*I-*Hpa*I probe; (C) 3.3-kb *Hpa*I-*Hpa*I probe; (D) 3.0-kb *Hpa*I-*Bam*HI probe (see Fig. 1 for locations). The positions of ICP0 and ICP27 mRNAs are indicated by arrows. The positions of RNA markers (kilobases) are labeled on the left, and the positions of 28S and 18S rRNAs are indicated by asterisks.

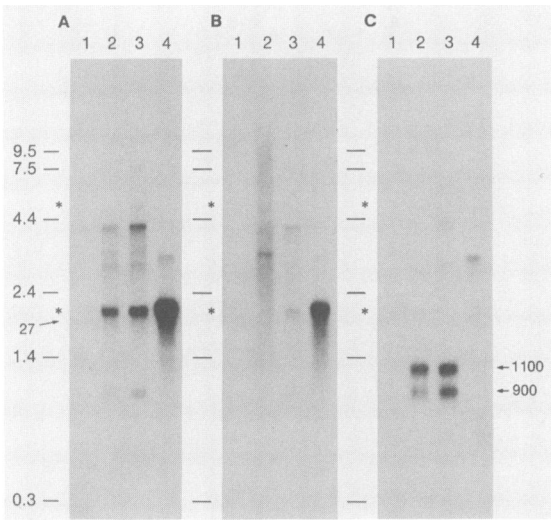

FIG. 4. Synthesis of ICP27 and the 0.9- and 1.1-kb transcripts in the presence and absence of PAA and cycloheximide. Lanes: 1, 2.5 μg of RNA from uninfected CV-1 cells; 2, 2.5 μg of RNA from HSV-1(F)-infected CV-1 cells; 3, 2.5 μg of RNA from HSV-1-infected CV-1 cells with 400 μg of PAA per ml; 4, 2.5 μg of RNA from HSV-1-infected CV-1 cells with 50 μg of cycloheximide per ml. (A) strain F, 2.4-kb *Bam*HI-*Hpa*I probe; (B) strain HFEM, 2.4-kb *Bam*HI-*Hpa*I probe; (C) strain F, 1.2-kb *Hpa*I-*Hpa*I probe. The positions of ICP27 and the 0.9-kb (900) and 1.1-kb (1100) RNAs are indicated by arrows. The positions of RNA markers (kilobases) are labeled on the left, and the positions of 28S and 18S rRNAs are indicated by asterisks.

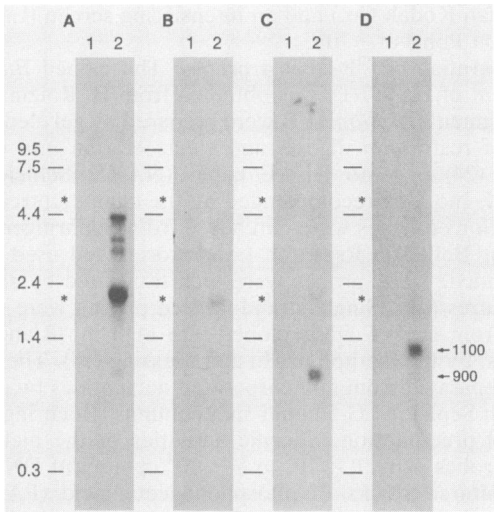

FIG. 5. Direction of transcription of the 0.9- and 1.1-kb HSV-1 RNAs. Lanes: 1, 2.5 μg of RNA from uninfected CV-1 cells; 2, 2.5 μg of RNA from HSV-1(F)-infected CV-1 cells (multiplicity of infection, 5), 5 to 6 h postinfection. (A) 2.4-kb *Bam*HI-*Hpa*I probe, mp19 single-strand labeled (15); (B) 2.4-kb *Bam*HI-*Hpa*I probe (mp18), single-strand labeled (15); (C) 1.2-kb *Hpa*I-*Hpa*I probe (mp19), single-strand labeled (15); (D) 1.2-kb *Hpa*I-*Hpa*I probe (mp18), single-strand labeled (15). The positions of the 0.9-kb (900) and 1.1-kb (1100) RNAs are indicated by arrows. The positions of RNA markers (kilobases) are labeled on the left, and the 28S and 18S rRNAs are indicated by asterisks.

barely detectable (data not shown). The 3.3-kb *Hpa*I-*Hpa*I probe hybridized faintly to a 2.0-kb transcript in cells infected with either F or HFEM (Fig. 3C). This is one of three latency-associated transcripts that are present at high levels during latent infection (41) and low levels during the acute stage of infection in the trigeminal ganglia of mice (40, 41). The 3.0-kb *Hpa*I-*Bam*HI probe hybridized to ICP0 mRNA (6, 22, 28, 31, 51) and the 2.0-kb latency-associated transcript (Fig. 3D). Thus, in HFEM-infected CV-1 cells, ICP0 mRNA and the 2.0-kb latency-associated RNA are synthesized, but the levels of ICP27 mRNA and the 0.9- and 1.1-kb RNAs are significantly reduced in comparison with levels in HSV-1(F)-infected cells.

Transcription of ICP27 in HFEM under α conditions. It was surprising that ICP27 mRNA was difficult to detect during HFEM infection, since the deletion in HFEM is approximately 1.5 kb downstream from the 5′ end of ICP27 mRNA (54). We hypothesized that the region deleted in HFEM might contain ICP27 regulatory elements or that there are additional mutations within ICP27. To determine whether the α gene ICP27 could be transcribed, cells were treated with cycloheximide before HFEM infection. Because of the absence of feedback inhibition from HSV-1 gene products (17), the α RNAs accumulate to high levels when protein synthesis is blocked in HSV-1-infected cells (34). ICP27 was transcribed in cells infected with strain F in the absence (Fig. 4A, lane 2) and presence (lane 3) of PAA. ICP27 mRNA accumulated when protein synthesis was blocked by cycloheximide (Fig. 4A, lane 4). In contrast, in cells infected with strain HFEM, ICP27 mRNA was virtually undetectable (Fig. 4B, lane 2). ICP27 mRNA was present at low levels when viral DNA synthesis was inhibited (Fig. 4B, lane 3) and at higher levels when protein synthesis was inhibited (lane 4). These results indicate that (i) ICP27 mRNA can be transcribed in HFEM infected cells, (ii) low levels of ICP27 mRNA may be sufficient for HFEM replication (38), and (iii) the mutation(s) in HFEM may affect the regulation of ICP27 expression.

Determination of the kinetic class of the 0.9- and 1.1-kb transcripts. The 0.9- and 1.1-kb transcripts that map within *Bam*HI-B in strain F (41) were synthesized at reduced levels, if at all, in cells infected with HFEM (Fig. 3). PAA and cycloheximide were used to determine whether their expression might be a prerequisite for the synthesis of ICP27 RNA. The 0.9- and 1.1-kb transcripts were synthesized when HSV-1(F) DNA synthesis was blocked with PAA but were not produced when protein synthesis was inhibited with cycloheximide (Fig. 4C). These results imply that the 0.9- and 1.1-kb RNAs are encoded by β or γ_1 genes. Since ICP27 is synthesized in cells infected with HFEM treated with cycloheximide and the 0.9- and 1.1-kb transcripts are not, they are not required for ICP27 expression. However, it is possible that they play a role in maintaining ICP27 mRNA levels.

Direction of transcription of the 0.9- and 1.1-kb RNAs. To establish the direction of transcription of the 0.9- and 1.1-kb RNAs, the 2.4-kb *Bam*HI-*Hpa*I and 1.2-kb *Hpa*I-*Hpa*I subfragments of *Bam*HI-B (Fig. 1) were cloned into M13 in both orientations to synthesize single-strand-labeled probes (18). The 2.4-kb *Bam*HI-*Hpa*I mp19 probe hybridized to ICP27 RNA and the 0.9-kb transcript in HSV-1(F)-infected cells, while in the opposite orientation (mp18) it did not hybridize strongly to either RNA (Fig. 5A and B). Since ICP27 is read rightward (6, 22, 51), the 0.9-kb RNA is also transcribed from left to right. The 1.2-kb *Hpa*I-*Hpa*I probe hybridized to the 0.9-kb RNA in one direction (mp19) (Fig. 5C) and to the 1.1-kb transcript in the opposite direction (mp18) (Fig. 5D). Thus, these RNAs are transcribed from different DNA strands. The directions of transcription of the 0.9- and 1.1-kb

RNAs are indicated in Fig. 1, consistent with the prediction of DNA sequence analysis of this region (McGeoch et al., unpublished data).

DISCUSSION

The genes encoding the HSV-1 latency-associated transcripts, 2.0, 1.5, and 1.45 kb, are present in two copies per genome in F and one complete copy in HFEM. The 2.0-kb latency-associated RNA is the only one that has been detected in HSV-1-infected tissue culture cells (41). While it is possible that the virulence of HFEM in experimental animal models is influenced by a gene dosage effect, the current work demonstrates that the 2.0-kb latency-associated transcript is expressed at similar levels in F- and HFEM-infected cells (Fig. 3D). In addition, during latent infection in mice with HFEM (26, 41), strain F, or the virulent KOS strain (39, 50), the levels of latency-associated transcripts are comparable (41). Thus, the data indicate that although HFEM contains only one complete copy of the latency-associated genes, the levels of their expression in infected CV-1 cells and during latency in mice are not significantly diminished compared with expression levels of more virulent HSV-1 strains.

The 2.0-kb latency-associated transcript was synthesized in infected tissue culture cells but was not present when either viral DNA or protein synthesis was inhibited (Fig. 2). However, before assignment of this latency-associated transcript or the 1.45- and 1.5-kb latency-associated transcripts to a kinetic class, the data concerning their expression during the pathogenesis of infection in mice should be considered. In previous work, the following observations have been made. (i) During latent infection in mice, these RNAs appear to be expressed in the absence of detectable levels of the α genes (41, 47). (ii) The 2.0-kb latency-associated transcript is first detectable at the peak of the acute phase of infection (40) and is present at much higher levels during HSV-1 latency in the trigeminal ganglia of mice than during the acute phase of infection (40, 41). (iii) The 1.45- and 1.5-kb latency-associated transcripts are not detectable in HSV-1-infected cells (41) and are not detectable until after the acute phase of infection in mice (40). (iv) All three RNAs accumulate steadily from the time they first appear until at least 60 days postinfection (40).

Although the 2.0-kb latency-associated transcript does not appear to be an α gene, on the basis of inhibitor studies in infected cells, the data obtained with infected mice indicate not only that the latency-associated transcripts can be expressed in the absence of detectable levels of other HSV-1 messages (40, 41) but also that they are present at much higher levels during latency than during the acute stage of infection (40, 41). Thus, the data suggest that the latency-associated genes may be regulated in a different manner than genes that are involved in the lytic replication cycle. It is possible that the latency-associated genes are under cellular rather than viral transcriptional control and that they encode viral functions involved in the establishment or maintenance of latent infections rather than viral replication. Therefore, it may be inappropriate to categorize them with respect to the replicative HSV-1 gene classes. Hence, we propose that the latency-associated genes be considered members of a new class of HSV-1 genes represented by the Greek letter λ (lambda).

It is also possible that the λ genes are under viral transcriptional control. In tissue culture, the host cell is lytically destroyed during HSV-1 infection, so that the λ genes do not have the opportunity to be expressed in infected cells in the absence of viral replication. While it is known that HSV-1 replicates in the nervous systems of mice during the acute phase of infection (11, 16, 32, 35, 39, 50), it has not been resolved whether or not viral replication actually has occurred in neurons that harbor the latent infection (7, 26). Estimates of the number of HSV-1 genome equivalents per cell in the trigeminal ganglia of latently infected mice suggest that there may be multiple DNA copies in each latently infected cell (30, 32, 35, 40). This may be the result of HSV-1 DNA replication or of a high-input multiplicity of infection. It is theoretically possible that some neurons survive the initial infection, that the 2.0-kb latency-associated transcript is first expressed late in infection, and that after the acute infection subsides the levels of all three latency-associated transcripts increase. Since the latency-associated transcripts are at least partially overlapping and colinear (Fig. 1) (37), and only the 2.0-kb latency-associated transcript is detectable in infected cells (Fig. 2 and 4) (37), an attractive hypothesis is that the replicative and latent transcription and RNA-processing patterns of the λ genes may be distinct.

The severity of acute disease produced by HSV-1 variants in animal models varies considerably (1, 3, 11, 27, 37, 39, 50). In some studies, genetic determinants of HSV-1 virulence have been localized to a region in the vicinity of the long internal repeat (1, 3, 37, 50). In the present study, HSV-1 transcription within the *Bam*HI-B restriction fragments of strains F and HFEM, which is less virulent than F in mice and tree shrews (1, 36), was compared. Until recently no HSV-1 transcripts had been mapped to the region of the HFEM deletion (41). This deletion is located between ICP0 and ICP27 (Fig. 1) (36). The 0.9- and 1.1-kb RNAs, which map near the left end of the HFEM deletion, and ICP27 mRNA (Fig. 1) are not synthesized at high levels in HFEM-infected cells (Fig. 3). Thus, the decreased expression of one or more of these genes might be factors that affect the virulence of HFEM in mice and tree shrews.

Although it was not part of the initial rationale for the study, it was observed that there are low levels of ICP27 transcription during HFEM infection (Fig. 3A), even though the deletion is 1.5 kb from the 3′ end of the message (54). A similar effect on ICP27 expression has been observed by MacLean and Brown with HSV-1 deletion mutants that have termini 500 bases downstream from the 3′ end of ICP27 (23). However, the ICP27 gene in HFEM can be expressed under α conditions (Fig. 4B), which is evidence that ICP27 is transcriptionally functional in HFEM. Although the herpesvirus α gene enhancer sequences that have been identified are 5′ elements (21, 53), it is also possible that there are 3′ enhancers located at a considerable distance from the poly(A) signal, as in β-globin (5, 15, 20). Genetic analyses of several temperature-sensitive ICP27 mutants indicate that ICP27 may be an essential α regulatory gene (38). Thus, we favor the hypothesis that although ICP27 may be essential for viral replication, low levels of ICP27 expression may be sufficient.

Genetic analysis has not been particularly successful in identifying HSV-1 functions involved in latent infections, in part because of the complexity of the virus-host interactions (for a review, see reference 35). However, because HFEM contains only one complete copy of the λ genes that encode the latency-associated transcripts (41), it may be a suitable starting point for engineering deletion mutants to determine whether λ gene expression is essential in the pathogenesis of latent infections. If an HSV-1 variant is obtained that is negative in explant reactivation assays (2, 16, 42, 46), the

following intermediate steps in the pathogenesis of latent infections should be studied independently. (i) Infectious virus can be measured during the acute stage of infection and during reactivation by plaque assay (2, 32, 42). (ii) HSV-1 DNA can be detected by dot blot and Southern blot analyses (32, 42). (iii) HSV-1 RNA can be detected by in situ hybridization (9, 10, 12, 33, 47, 48) and Northern blot analysis (33, 40, 41, 47). The abilities of HSV-1 mutants to multiply, maintain the stability of their genomes, and express viral transcripts during the acute, latent, and reactivation phases of infection can be monitored with a combination of these techniques and should provide valuable insights into the mechanism of HSV-1 latency.

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LITERATURE CITED

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