JOURNAL OF VIROLOGY, Dec. 1987, p. 3841–3847
0022-538X/87/123841-07$02.00/0

Vol. 61, No. 12

Detection of Herpes Simplex Virus Type 1 Transcripts during Latent Infection in Mice

JORDAN G. SPIVACK* AND NIGEL W. FRASER
The Wistar Institute, Philadelphia, Pennsylvania 19104

Received 25 June 1987/Accepted 10 September 1987

A latent infection can be established in the trigeminal ganglia of mice after corneal inoculation of herpes simplex virus type 1 (HSV-1). With a virion DNA probe, three transcripts (2.0, 1.5, and 1.45 kilobases [kb]) were detected by Northern blot (RNA blot) analysis of RNAs isolated from the ganglia of latently infected mice. All three transcripts hybridized to a nick-translated HSV-1 DNA probe from *Bam*HI restriction fragment B (strain F). These RNAs were mapped with subfragments of *Bam*HI-B and with strand-specific probes. They are at least partially colinear with each other, map to a 3.0-kb *Pst*I-*Mlu*I subfragment of *Bam*HI-B, and are transcribed from left to right. The latent HSV-1 RNAs partially overlap the 3′ end of ICP0 mRNA but are transcribed in the opposite direction. The latent RNAs were not as extensively poly(A)$^+$ as actin mRNA. The HSV-1 transcripts detected in latently infected trigeminal ganglia did not correspond with any that have been previously identified in permissively infected cells in tissue culture. However, the 2.0-kb HSV-1 RNA present during latency was detectable at reduced levels in the trigeminal ganglia of acutely infected mice and in infected tissue culture cells. The data indicate that the pattern of viral gene expression during HSV-1 latency in the trigeminal ganglia of mice does not represent restriction of the genes actively transcribed during the lytic replication cycle in tissue culture.

After a primary infection, herpes simplex virus type 1 (HSV-1) often persists in a latent state for the life of the individual (2, 9, 15, 40). The latent virus resides within neurons of sensory ganglia that innervate the primary site of infection (8, 24). Periodic reactivations of latent herpesvirus to produce recrudescent disease are frequent (25) and can be life threatening in individuals who are immune suppressed, such as organ transplant recipients and cancer chemotherapy and acquired immunodeficiency syndrome patients.

The transcription pattern of HSV-1 in infected cells is complex (45). Herpesvirus genes are expressed in a coordinated and temporally regulated fashion during the viral replication cycle (17, 33). HSV-1 may encode as many as 50 to 100 genes, which are transcribed from both DNA strands (33, 46). There are spliced and unspliced mRNAs and overlapping coterminal nested sets of transcripts (30). Virus-specific transcripts have been detected during HSV-1 latency in the central and peripheral nervous systems of mice by in situ hybridization (10, 12, 41, 42) and during HSV-2 latency in guinea pig (42) and human (13, 14) sensory ganglia. Some of these studies, using probes from defined regions of the genome, have determined that the transcripts present in the sensory ganglia of latently infected mice hybridize to the long repeat region (10, 12, 41), which encodes infected cell protein 0 (ICP0). ICP0 is one of five HSV-1 immediate-early genes (17) that encode proteins required to activate the rest of the viral replication cycle during infection of tissue culture cells (33). However, the viral RNA present during HSV-1 latency has been reported to be transcribed from the DNA strand opposite that encoding ICP0 (41).

Evidence is presented in this report that at least three HSV-1-specific transcripts are present in the trigeminal ganglia of latently infected mice and map within *Bam*HI restriction fragment B. The transcripts are distinct from the immediate-early HSV-1 mRNAs ICP0 and ICP27, which have been mapped to *Bam*HI-B (7, 22, 46). The most abundant of the latent HSV-1 RNA species (2.0 kilobases [kb]) was present in reduced amounts in the ganglia of acutely infected mice and infected CV-1 cells. During the course of this study, additional 1,100- and 900-base transcripts that also map to *Bam*HI-B were detected in infected CV-1 cells. The potential role of the viral genes expressed during latency in the pathogenesis of acute and latent HSV-1 infections is discussed.

MATERIALS AND METHODS

Cell culture, virus titration, and preparation of HSV-1 stocks. Subconfluent monolayers of CV-1 cells, a monkey kidney cell line, grown in Eagle minimum essential medium supplemented with 5% fetal bovine serum at 37°C with 5% CO_2 were infected with HSV-1 (strains F [from B. Roizman, University of Chicago], KOS [16], and HFEM [from G. Cohen, University of Pennsylvania]) at 0.01 PFU per cell to make virus stocks or at 1 PFU per cell to produce virus for the infection of mice, as previously described (10, 32). HSV-1 was titered on CV-1 cells by addition of fresh medium containing human immune serum globulin (0.5 mg/ml; Armour Pharmaceuticals) at the end of the absorption period, and the plaques were stained with 1% methylene blue and counted 2 days later as previously described (38, 39). To isolate HSV-1 (F) RNA, CV-1 cells were infected at 5 PFU per cell, the inoculum was removed after 1 h, and the cells were trypsinized at 4 to 5 h postinfection. After low-speed centrifugation, RNA was isolated from the cell pellet as described below.

Infection of mice. Following corneal scarification, 4- to 6-week-old female BALB/c mice were infected with approximately 10^6 to 10^7 PFU of HSV-1 strain F, KOS, or HFEM (10, 32) per eye. A minimum of 4 weeks after infection, latently infected mice were sacrificed by cervical dislocation. All mice tested were latently infected as determined by explant reactivation assay (21, 37).

RNA extraction and poly(A)$^+$ selection. Trigeminal ganglia were rinsed with phosphate-buffered saline and homoge-

* Corresponding author.

nized for 20 s with a Polytron homogenizer (Brinkmann Instruments, Inc.; setting 5) in 4 M guanidinium thiocyanate–0.5% sodium-*N*-lauroylsarcosine–100 mM β-mercaptoethanol–25 mM sodium citrate–0.1% Antifoam A (Sigma Chemical Co.) (pH 7.0) (3). 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. After centrifugation, the supernatant was carefully removed by aspiration, the clear glassy pellet was suspended in H_2O, and A_{260} was measured. The RNA (4 to 5 μg per ganglion) was stored as an ethanol precipitate at −20°C.

Total RNA from the ganglia of 50 latently infected mice was poly(A)$^+$ selected by one round of oligo(dT)-cellulose chromatography (23). Total cellular RNA in H_2O was heat denatured for 5 min at 65°C, adjusted to 20 mM sodium citrate (pH 7.6)–0.5 M NaCl–1 mM EDTA–0.1% sodium dodecyl sulfate, and loaded onto an oligo(dT)-cellulose column equilibrated with the same buffer. The flowthrough was heat denatured and reloaded onto the column. After washing with 5 to 10 volumes of loading buffer and 5 to 10 volumes of loading buffer containing 0.1 M NaCl without sodium dodecyl sulfate, the poly(A)$^+$ RNA was eluted with 5 volumes of 10 mM Tris–1 mM EDTA (pH 7.4). The second flowthrough solution and all of the washes were combined as the poly(A)$^-$ RNA fraction. Since there was not sufficient RNA to pass through two rounds of selection, the poly(A)$^+$ fraction was not completely free of rRNA. The yield was 2 to 3% poly(A)$^+$ RNA.

Agarose gel electrophoresis and Northern blot (RNA blot) transfer of RNA. RNA was denatured by incubation with 1 M deionized glyoxal–10 mM Na_2PO_4 (pH 7.0)–50% dimethyl sulfoxide at 50°C for 1 h (23). After cooling to room temperature, 0.25 volume of 50% glycerol–10 mM Na_2PO_4 (pH 7.0)–0.4% bromophenol blue was added. The RNA was electrophoresed through 1.2% agarose in 10 mM Na_2PO_4 (pH 7.0) with constant buffer recirculation (23) and capillary blotted to Gene Screen Plus (Du Pont Co.), as directed by the manufacturer. After blotting and air drying, the filter was placed in 20 mM Tris (pH 8.0) at 100°C and allowed to cool to room temperature to reverse the glyoxylation. The RNA markers used were purchased from Bethesda Research Laboratories.

Hybridization and washing of Northern blots. The filters were prehybridized for 4 h at 50°C in 50% formamide–10% dextran sulfate–1× Denhardt solution–1% sodium dodecyl sulfate–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–1 mM EDTA (pH 8.0). A ^{32}P-labeled nick-translated or single-strand-labeled probe was added and hybridized for ≥16 h. After hybridization, the filters were washed with gentle shaking in decreasing concentrations of SSC to 0.1× with 1% sodium dodecyl sulfate at 65°C for 30 min per wash. The filters were air dried, covered with plastic wrap, and autoradiographed with XAR-5 film (Eastman Kodak Co.) at −70°C with an intensifying screen (Du Pont).

Nuclease controls. After Northern transfer and reversal of glyoxylation, the Gene Screen Plus membranes were incubated in 6× SSC plus 10 mM $MgCl_2$–2 mM $CaCl_2$ for 1 h at 37°C with 10 μg of DNase 1 or RNase A (Worthington Diagnostics) per ml or without added enzymes. The membranes were washed in the same buffer without nucleases and air dried before hybridization.

Preparation of ^{32}P-labeled probes. Total HSV-1 DNA was isolated from virions and purified by CsCl gradient centrifugation (5). The *Bam*HI B and SP fragments of HSV-1 (F) cloned into pBR322 were a generous gift of Bernard Roizman (27). An actin DNA probe was kindly provided by Roberto Weinmann (19). Subfragments of *Bam*HI-B were obtained from restriction digests after gel electrophoresis and electroelution of the bands of interest into dialysis tubing (23) or onto DEAE-paper (NA-45; Schleicher & Schuell, Inc.; manufacturer protocol). Restriction enzymes were purchased from Boehringer Mannheim Biochemicals, Bethesda Research Laboratories, or New England BioLabs, Inc., and used as recommended by the manufacturers. DNA probes were nick translated by standard procedures (23). Single-strand-labeled probes were synthesized from cloned HSV-1 DNA in M13 mp18 and mp19 vectors with the Klenow fragment of DNA polymerase and a random primer as previously described (23). The probes were separated from unincorporated nucleotides by passage through a minispin Sephadex G-50 column (Boehringer). The specific activities of the nick-translated probes were 1×10^8 to 5×10^8 cpm/μg of DNA.

RESULTS

Characterization of latently infected mice. Mice were infected with 10^6 to 10^7 PFU of HSV-1 (F) per eye after corneal scarification. After the primary infection, which lasted for 7 to 10 days, infectious virus could no longer be recovered from homogenates of eyes, trigeminal ganglia, or central nervous system tissue (data not shown). HSV-1 DNA and RNA can be detected during latency by Southern blot analysis (11, 31, 32) and in situ hybridization (10, 12, 41, 42), respectively. HSV-1 can be reactivated from explanted intact trigeminal ganglia of latently infected mice by 4 days of incubation with Eagle minimum essential medium and 5% CO_2 at 37°C and can be detected by titration of HSV-1 in homogenates of these ganglia (21, 37).

Detection of HSV-1 RNA in latent trigeminal ganglia. Total cellular RNAs were isolated from the trigeminal ganglia of acutely (5 days postinfection) and latently infected mice, Northern blotted (5 μg per lane), and hybridized to a nick-translated DNA probe of purified HSV-1 DNA. No specific hybridization to RNAs extracted from uninfected mice (Fig. 1, lanes 2) was observed. Although many bands were observed with RNA (2 μg) from CV-1 cells infected in tissue culture (Fig. 1A, lane 1), only one major band of hybridization, of approximately 2.0 kb, and a minor band, of 1.5 kb, were detected in ganglion RNA samples from latently infected mice (Fig. 1A, lane 3). The 2.0-kb band was also present at reduced levels in RNAs isolated from the ganglia of acutely infected mice (Fig. 1A and B, lanes 4).

The region of the genome expressed during latency has been localized to the internal and unique long terminal repeats by in situ hybridization (10, 12, 41). This positive region is contained in *Bam*HI fragments B and SP (10; see Fig. 2B). The adjacent *Bam*HI restriction fragments did not hybridize to latently infected trigeminal ganglia by in situ hybridization (10). A *Bam*HI-B probe hybridized to all of the latent RNAs detected with the virion DNA probe (compare Fig. 1A and B, lanes 3). Two additional RNAs of about 900 and 1,100 bases were also detected in infected cells with the *Bam*HI-B probe (Fig. 1B, lane 1). These results indicate that HSV-1 transcripts present during latency and detectable by Northern blot analysis mapped within *Bam*HI-B. The minor latent transcript was resolved into a 1.45-1.5-kb doublet (Fig. 1B). The hybridization signal was abolished by treatment of the filters with RNase H between Northern blot transfer and probe hybridization but was not affected by DNase digestion (data not shown). A *Bam*HI-SP probe was positive by in situ

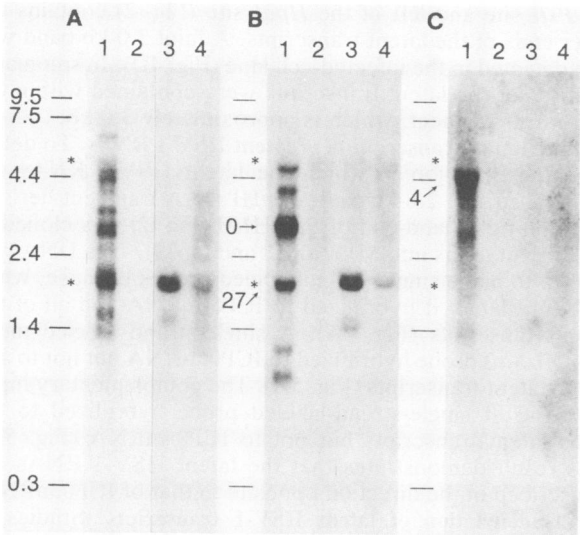

FIG. 1. Detection of HSV-1 RNA in the trigeminal ganglia of latently infected mice by Northern blot analysis. Lanes: 1, 2 μg of RNA from HSV-1-infected CV-1 cells (multiplicity of infection, 5) 4 to 5 h postinfection; 2, 5 μg of RNA from uninfected mouse brain; 3, 5 μg of RNAs from the trigeminal ganglia of mice latently infected with HSV-1; 4, 5 μg of RNAs from the trigeminal ganglia of mice 5 days postinfection with HSV-1. Panels: A, HSV-1 DNA probe; B, *Bam*HI-B probe; C, *Bam*HI-SP probe. The autoradiograph was made with XAR-5 film (Kodak). The positions of ICP0, ICP4, and ICP27 RNAs are marked by arrows. The positions of RNA markers are labeled in kilobases on the left, and the positions of 28S and 18S rRNAs are labeled by asterisks.

hybridization (10) but did not hybridize to Northern blots of RNAs isolated from ganglia of latently infected mice (Fig. 1C). This result suggests that Northern blots detect only the most abundant latent transcripts.

Localization of latency transcripts within *Bam*HI-B. The transcripts mapped to *Bam*HI-B are from two immediate-early genes, ICP0 and ICP27 (7, 22, 46; Fig. 2C). ICP0 and ICP27 mRNAs were easily resolved and detected in infected cells with *Bam*HI-B (Fig. 1B). The four *Hpa*I fragments of *Bam*HI-B (22, 27; Fig. 2C) were used as probes to further localize the latent RNAs. The 2.4-kb *Hpa*I fragment hybridized to ICP27 RNA and the 900-base transcript in infected cells but did not hybridize to any transcripts present in the trigeminal ganglia of latently infected mice (Fig. 3A). The 1.2-kb *Hpa*I fragment hybridized to 900- and 1,100-base transcripts in infected cells but did not hybridize to any latent RNAs (Fig. 3B). The 3.8-kb *Hpa*I fragment did not hybridize significantly to transcripts in infected cells but did hybridize to all of the latency transcripts (Fig. 3C). The 3.1-kb *Hpa*I fragment hybridized to ICP0 RNA in infected cells and to all of the transcripts present in the trigeminal ganglia of latently infected mice (Fig. 3D). A faint band was detectable in the infected-cell lane at the position of the 2.0-kb latent transcript (Fig. 3D). These results indicate that the transcripts present during latency are distinct from ICP0 and ICP27 RNAs. (i) They map to a different location than ICP27, (ii) they are smaller (2.0, 1.5, and 1.45 kb) than ICP0 RNA, and (iii) they hybridize across an *Hpa*I restriction site across which ICP0 does not hybridize, so they, at most, only partially overlap the transcribed region of ICP0. Since the latent HSV-1 RNAs all crossed the same *Hpa*I site, they at least partially overlap one another.

To map the transcripts present during latency more accurately, the 3.1-kb *Hpa*I fragment of *Bam*HI-B was subdivided to probe Northern blots. The 3.1-kb *Hpa*I-*Bam*HI fragment was cut with *Sal*I or *Mlu*I (Fig. 2C). The 2.6-kb *Sal*I-*Bam*HI (22, 27) fragment hybridized to all of the latency transcripts (Fig. 4A). The 1.9-kb *Mlu*I-*Bam*HI (1) fragment hybridized to ICP0 RNA (Fig. 4B, lane 1) but did not hybridize to any of the latent transcripts (lane 3). This result indicates that all of the latent HSV-1 transcripts have termini between the *Sal*I and *Mlu*I sites, a distance of approximately 700 bases (1).

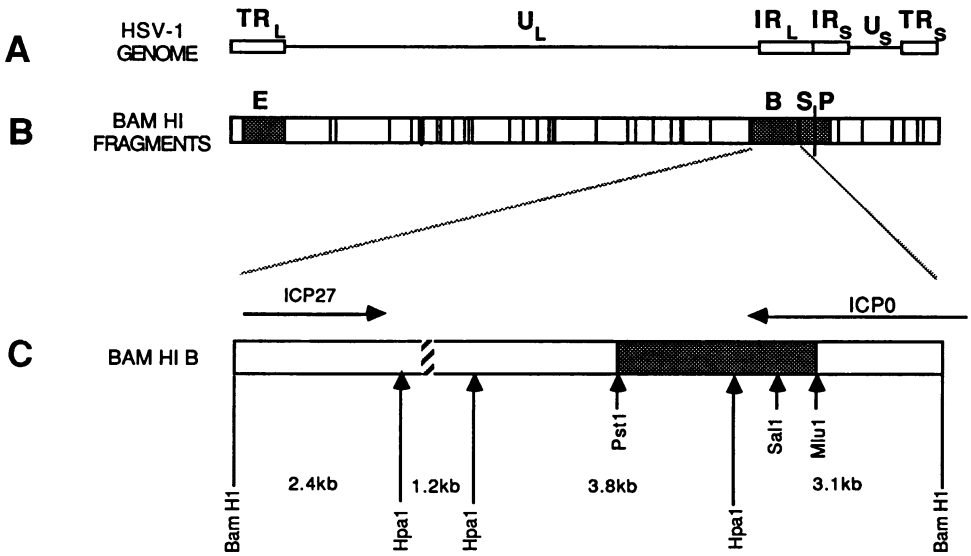

FIG. 2. Map of the HSV-1 genome and *Bam*HI-B. (A) Representation of the HSV-1 genome. The unique long and short regions (U_L and U_S lines) of the genome are bounded by internal repeat (IR) and terminal repeat (TR) regions (boxes). (B) Positions of the *Bam*HI restriction fragments of HSV-1 strain F (27). The fragments positive by in situ hybridization are labeled and shaded (10). (C) Detailed map of *Bam*HI-B. The positions and sizes of the *Hpa*I sites and other sites used in this study are shown (1, 22, 26, 39). The approximate locations of ICP0 and ICP27 mRNAs are marked by arrows (21, 32, 46). The shaded area is the region that hybridized to latent HSV-1 transcripts. The hatched region is the boundary between the unique region and the long internal repeat (22, 26).

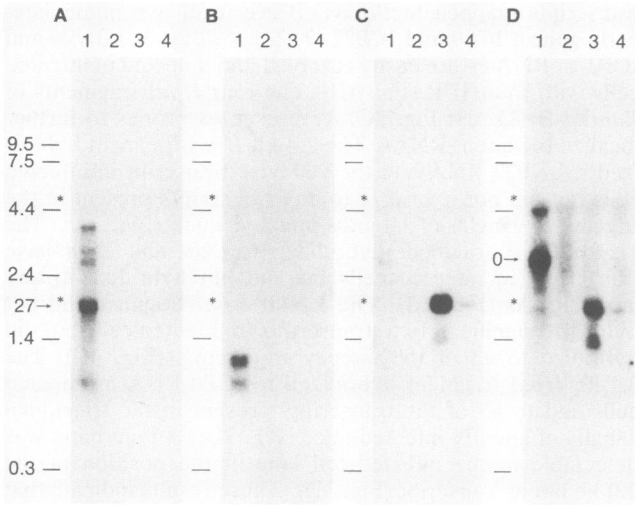

FIG. 3. Mapping of latent HSV-1 RNA to the *Hpa*I fragments of *Bam*HI-B. Lanes: 1, 2 μg of RNA from HSV-1-infected CV-1 cells (multiplicity of infection, 5) 4 to 5 h postinfection; 2, 5 μg of RNA from uninfected mouse brain; 3, 5 μg of RNAs from trigeminal ganglia of mice latently infected with HSV-1; 4, 5 μg of RNAs from trigeminal ganglia of mice 5 days postinfection with HSV-1. Panels: A, 2.4-kb *Bam*HI-*Hpa*I probe; B, 1.2-kb *Hpa*I-*Hpa*I probe; C, 3.8-kb *Hpa*I-*Hpa*I probe; D, 3.1-kb *Hpa*I-*Bam*HI probe (see Fig. 2C for probe locations). The RNA markers are as in Fig. 1. The autoradiograph was made with XAR-5 film.

To locate the left-hand boundary for the latent HSV-1 RNA, *Bam*HI-B DNA was digested with *Pst*I, which cuts *Bam*HI-B once. This site is in the *Hpa*I 3.8-kb fragment (36; Fig. 2C). The 4.9-kb *Pst*I-*Bam*HI fragment hybridized to all of the latent transcripts (Fig. 4D), but the 5.6-kb *Bam*HI-*Pst*I fragment did not (Fig. 4C). Thus, the region to the right of the *Pst*I site and left of the *Hpa*I site (Fig. 2) contains the other ends of the latent transcripts. A faint 2.0-kb band was also detected in the infected-cell lane (Fig. 4D). In summary, all three of the latent transcripts were contained within the *Pst*I-*Mlu*I fragment, which is approximately 3.0 kb.

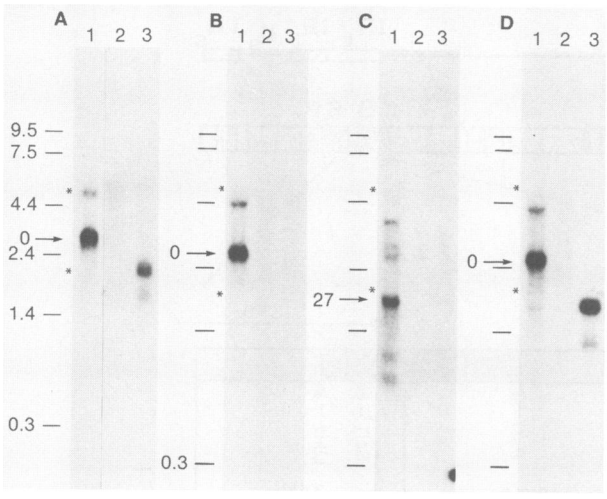

FIG. 4. Further mapping of latent HSV-1 RNA within *Bam*HI-B. Lanes: 1 to 3, as in Fig. 1 and 4; 1, HSV-1-infected-cell RNA; 2, uninfected mouse brain RNA; 3, latent mouse trigeminal ganglion RNA. Panels: A, 2.6-kb *Sal*I-*Bam*HI probe (within 3.1-kb *Hpa*I-*Bam*HI fragment [Fig. 2]); B, 1.9-kb *Mlu*I-*Bam*HI probe (within 3.1-kb *Hpa*I-*Bam*HI fragment); C, 5.6-kb *Bam*HI-*Pst*I probe (from left side of *Bam*HI-B); D, 4.9-kb *Pst*I-*Bam*HI probe (from right side of *Bam*HI-B). The RNA markers are as in Fig. 1. The autoradiograph was made with XAR-5 film.

Direction of transcription of latent HSV-1 RNAs. To determine the direction in which the latent HSV-1 RNAs are transcribed, the 2.6-kb *Sal*I-*Bam*HI DNA fragment derived from the right-hand end of *Bam*HI-B (Fig. 2) was cloned in both orientations into M13 (mp18 and mp19). This DNA was chosen to make single-strand-labeled probes because, when nick translated, it hybridized to ICP0 mRNA and all of the latent transcripts (Fig. 4A). A single-strand labeled mp19 *Bam*HI-*Sal*I probe hybridized to ICP0 mRNA but not to any of the latent transcripts (Fig. 5A). The complementary mp18 *Bam*HI-*Sal*I single-strand-labeled probe hybridized to the three latent transcripts but not to ICP0 mRNA (Fig. 5B). This result demonstrates that the latent HSV-1 RNAs are transcribed in the direction opposite to that of ICP0 mRNA.

Polyadenylation of latent HSV-1 transcripts. Studies of HSV-1 gene expression during latency by in situ hybridization have indicated that the highest density of grains is over the nuclei of latently infected neurons (41, 42). This nuclear localization of the transcripts may be due to (i) the kinetics of latent HSV-1 RNA synthesis or (ii) inhibited RNA processing. Thus, it was important to determine the extent of polyadenylation of the latent transcripts. Total RNAs from the ganglia of latently infected mice were poly(A)$^+$ selected by oligo(dT)-cellulose chromatography (23). The total, poly(A)$^+$ and poly(A)$^-$ RNA fractions (2 μg per sample) were electrophoresed in duplicate and hybridized with HSV-1 *Bam*B or an actin probe (19) as a control for a normally processed transcript (Fig. 6). The actin transcript

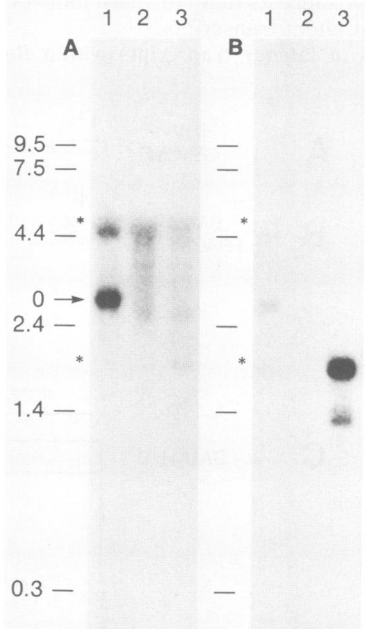

FIG. 5. Determination of the direction of transcription of latent HSV-1 RNA. Lanes: 1 to 3, as in Fig. 1, 4, and 5; 1, HSV-1-infected-cell RNA (2 μg); 2, uninfected mouse brain RNA (5 μg); 3, latent mouse trigeminal ganglion RNA (5 μg). Panels: A, single-strand-labeled 2.6-kb *Sal*I-*Bam*HI probe from M13 mp19; B, same probe from M13 mp18. The RNA markers are as in Fig. 1. The autoradiograph was made with XAR-5 film.

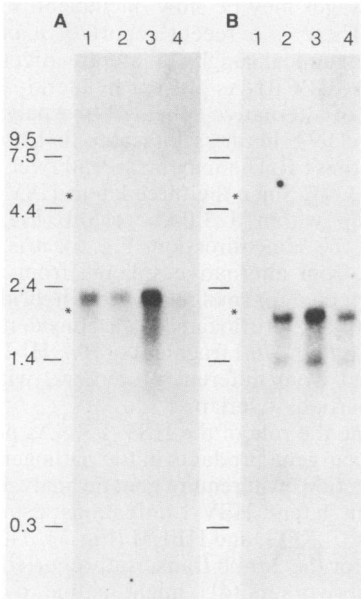

FIG. 6. Oligo(dT)-cellulose chromatography of RNAs from the trigeminal ganglia of mice latently infected with HSV-1. Lanes: 1, 2 μg of total RNA from an uninfected mouse brain; 2, 2 μg of total RNAs from trigeminal ganglia of mice latently infected with HSV-1; 3, 2 μg of poly(A)$^+$ RNAs from trigeminal ganglia of mice latently infected with HSV-1; 4, 2 μg of poly(A)$^-$ RNAs from trigeminal ganglia of mice latently infected with HSV-1. Panels: A, actin probe (23); B, *Bam*HI-B probe. The RNA markers are as in Fig. 1. The autoradiograph was made with XAR-5 film.

was much more abundant in the poly(A)$^+$ fraction (Fig. 6A, lane 3) than in the total-RNA sample (lane 2) and was barely detectable in the poly(A)$^-$ RNA sample (lane 4). This result is consistent with an actin mRNA that is efficiently processed to a poly(A)$^+$ message. The result for the latent HSV-1 transcripts was different than for actin. The signals for the 2.0-kb transcript and the 1.45-1.5-kb doublet were stronger in the poly(A)$^+$ fraction (Fig. 6B, lane 3) than in the total-RNA sample (lane 2), but the poly(A)$^-$ fraction (lane 4) contained almost as much HSV-1 RNA as did the total-RNA sample. This suggests that the latent transcripts are less extensively polyadenylated than actin mRNA. Since the same amounts of RNA and probe and autoradiograph exposure times were used in panels A and B, the levels of actin mRNA and the 2.0-kb HSV-1 transcript are within the same order of magnitude. It should be noted that the hybridization signals in the total-RNA lanes are not the sum of the poly(A)$^+$ and poly(A)$^-$ signals because 2 μg of poly(A)$^+$ RNA was derived from approximately 67 to 100 μg, not 2 μg, of total RNA. Thus, in these experiments, if a small percentage of an RNA was poly(A)$^+$, the poly(A)$^+$ signal would be enriched, whereas the total and poly(A)$^-$ signals would be comparable.

Comparison of latent transcripts of HSV-1 strains F, KOS, and HFEM. Recently, Stevens and co-workers (41) detected a transcript, estimated at 2.6 kb, in spinal ganglia of latently infected mice that had been inoculated via the footpad with HSV-1 (KOS). This transcript hybridized to a *Sal*I-*Bam*HI fragment derived from the right-hand part of *Bam*HI-B (Fig. 2). To determine whether HSV-1 strains F and KOS encoded different-size latent transcripts, RNA was isolated from the trigeminal ganglia of mice latently infected with KOS. In addition, mice were infected with HSV-1 strain HFEM, which is virulent when inoculated intracerebrally but avirulent when given intraperitoneally, subcutaneously, or intravenously (1). HFEM has a 4.1-kb deletion that corresponds approximately to the 3.8-kb *Hpa*I fragment derived from *Bam*HI-B (34; Fig. 2). The deletion in HFEM is within the long internal repeat. The second copy of this region is contained within *Bam*HI-E and is not deleted in HFEM (34). Since HFEM can establish latent infection in mice (24) and its deletion spans a portion of the area in which the latent transcripts map, it was of interest to determine which transcripts were present during latency in the trigeminal ganglia of mice latently infected with HFEM. The results of this comparison experiment are presented in Fig. 7. A transcript present in the trigeminal ganglia of mice latently infected with strain KOS (lane 4) was similar in size to the 2.0-kb transcript present with strain F (lane 3). HSV-1 transcripts were present in the trigeminal ganglia of mice latently infected with strain HFEM (lane 5) but were slightly smaller than those in mice infected with strain F. Upon longer exposure, a faint band at 1.5 kb was observed in the KOS sample, and a 1.4-kb band was seen in the HFEM sample (data not shown).

DISCUSSION

The nature of the interaction of latent herpesvirus with host cells is not well understood. The structure of latent HSV-1 DNA is different from the linear unit length genome found in virions (11, 31, 32). In situ hybridization studies have demonstrated that HSV-1 RNA is present in the sensory ganglia of latently infected mice (42) and is only

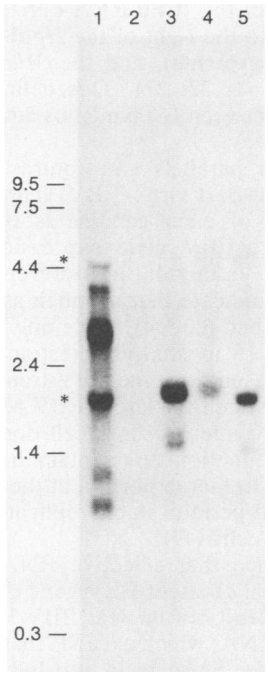

FIG. 7. Comparison of latent HSV-1 RNAs in mice infected with strains F, KOS, and HFEM. Lanes: 1, 2 μg of RNA from HSV-1-infected CV-1 cells (multiplicity of infection, 5) 4 to 5 h postinfection; 2, 5 μg of RNA from uninfected mouse brain; 3, 5 μg of RNAs from trigeminal ganglia of mice latently infected with HSV-1 (F); 4, 5 μg of RNAs from trigeminal ganglia of mice latently infected with HSV-1 (KOS); 5, 5 μg of RNAs from trigeminal ganglia of mice latently infected with HSV-1 (HFEM). The *Bam*HI-B probe was used. The RNA markers are as in Fig. 1. The autoradiograph was made with XAR-5 film.

transcribed from the region of the genome containing *Bam*HI-B, -E, and -SP (10, 12, 41; Fig. 2).

In the present study, three transcripts (2.0 kb and a 1.45-1.5-kb doublet) were identified by Northern blot analysis of RNAs that accumulate during HSV-1 latency in mice. The transcripts were barely detectable in infected CV-1 cells in tissue culture. Since these latent HSV-1 RNAs are present at much higher levels than during the acute stage of infection in mice, it is likely that they are actively synthesized during latency and are not residual transcripts from the primary infection. These transcripts are at least partially colinear with each other, since they all cross the same *Hpa*I and *Sal*I sites (Fig. 3 and 4) and are all transcribed from left to right in *Bam*HI-B (Fig. 5). The 3′ ends of all three latent transcripts map within a 700-base region between the *Sal*I and *Mlu*I sites (Fig. 4A and B). The latent HSV-1 RNAs map to a 3.0-kb region within *Bam*HI-B (Fig. 2C) which is contained within the region positive by in situ hybridization (10). It is possible that they are a subset of the latent viral transcripts detected by in situ hybridization, which may be a more sensitive technique for detecting low-abundance RNA. These transcripts overlap with the 3′ end of ICP0 mRNA but are clearly distinct from ICP0, ICP27, or any others that are present at high levels in infected CV-1 cells in culture. ICP27 mRNA is approximately 1.8 to 2.0 kb (5, 43, 44), is transcribed to the right (22, 46), and is completely contained with *Bam*HI-B (Fig. 2C). ICP0 mRNA is approximately 2.6 to 3.0 kb (7, 26, 45, 46) and is transcribed from a 5′ end in *Bam*HI-SP leftward into *Bam*HI-B (7, 22; Fig. 2C). The ICP0 gene has been sequenced in HSV-1 (strain 17), and its predicted size is 2,684 nucleotides (26). The distance between the 3′ end of the ICP0 RNA (29), which is approximately 240 bases to the right of the *Hpa*I site (between the 3.1- and 3.8-kb fragments), and the *Mlu*I site is approximately 950 bases (1, 22, 27). This defines the maximum possible overlap between ICP0 mRNA and any of the latent HSV-1 RNAs.

There are some parallels and contrasts between latent HSV-1 and Epstein-Barr virus (EBV) gene expression. Only a limited portion of each genome is transcribed during latency. However, EBV gene expression may be more extensive, since estimates in different EBV growth-transformed cells indicate that as much as 30 to 35% of the genome is transcribed (20, 44), while only 8% of the HSV-1 genome is positive by in situ hybridization (10). There are at least six RNAs present in some EBV-transformed cell lines (44). Since the host cell for latent EBV replicates, whereas the HSV-1 latently infected host cell does not, EBV may require more viral functions to maintain the latent viral DNA than does HSV-1. In fact, a portion of the EBV genome has been identified that permits stable replication of plasmids in EBV-transformed cells (47).

There is evidence that selective RNA processing is a factor in the control of latent EBV gene expression. During latency, the complexity of nuclear EBV RNA exceeds that of poly(A)$^+$ viral RNA, which exceeds that of polyribosomal EBV RNA (20, 35). Since in in situ hybridization studies most of the grains are localized over the nuclei of neurons in sensory ganglia of mice latently infected with HSV-1 (41, 42), there has been speculation that the transcripts expressed during latency might not be poly(A)$^+$ or might not be exported to the cytoplasm and that the protein products might not be produced. The HSV-1 transcripts present during latency were not as extensively polyadenylated as actin mRNA (Fig. 6). This may be because fewer of the HSV-1 transcripts are poly(A)$^+$ or because they have shorter poly(A)$^+$ tails. Although processing of latent transcripts to poly(A)$^+$ messages may be slow, inefficient, or inhibited, it does occur. There is a recent report of a poly(A)$^+$ viral transcript in trigeminal ganglia of latently infected mice (28).

Some of the EBV RNAs present in latently infected cells are the result of alternative splicing (4). Analysis of recombinants from cDNA libraries indicates that the exons contained within these RNAs may be spread over a distance of 80 to 100 kb (3, 4). Since the three latent HSV-1 transcripts appear to map within a 3.0-kb region (Fig. 4) and are transcribed in the same direction (Fig. 5), it is possible that (i) they arise from alternative splicing from a single promoter, (ii) they contain small exons that hybridize poorly in Northern blots and are located at a considerable distance from the 3.0-kb *Pst*I-*Mlu*I fragment of *Bam*HI-B, or (iii) they are transcribed from different promoters, with sequences that at least partially overlap.

To determine the role of the HSV-1 RNAs present during latency and their gene products in the pathogenesis of acute and latent infections will require genetic analysis. The RNAs are present in latent HSV-1 infections established with HSV-1 strains F, KOS, and HFEM (Fig. 7). The variation in size estimate for the 2.0-kb transcript, reported as 2.6-kb by Stevens and co-workers (41), might be due to differences in the transcripts expressed during latency in spinal ganglia and in trigeminal ganglia in mice or to technical differences. The slight size variation of the 2.0-kb transcript in mice latently infected with HFEM and the stringent hybridization and washing conditions used argue against the possibility that the latent RNAs are of cellular origin. However, the data do not exclude this alternative. These latent HSV-1 RNAs are the only transcripts known to map to the region deleted in HFEM. Since they map within the internal and long terminal repeat regions, the data suggest that two copies of the genes that encode the latent transcripts are present in HSV-1 (F) and only a single complete copy is present in strain HFEM. Whether or not they are regulatory RNAs or encode viral functions involved in (i) maintaining the latent state, (ii) suppressing the HSV-1 replication cycle, or (iii) a response to the presence or absence of neuronal transcription factors remains to be determined. Regardless of their function, it is clear that the HSV-1 genes expressed during latent trigeminal ganglion infection in mice are not merely a subset of the genes transcribed at high levels during the lytic replication cycle. Complete characterization of the viral RNAs present during HSV-1 latency should contribute to an understanding of their involvement in the pathogenesis of acute and latent HSV-1 infections.

ACKNOWLEDGMENTS

We thank Charles Sabino, Donald O'Boyle II, and Robert Carroll for expert technical assistance.

This work was supported by Public Health Service program project grant AI 23968 from the National Institutes of Health.

LITERATURE CITED

1. **Becker, Y., J. Hadar, E. Tabor, T. Ben-Hur, I. Raibstein, A. Rosen, and G. Darai.** 1986. A Sequence in *Hpa* 1-P fragment of herpes simplex virus-1 DNA determines intraperitoneal virulence in mice. Virology **149**:255–259.
2. **Blyth, W. A., and T. J. Hill.** 1984. Establishment, maintenance, and control of herpes simplex virus latency, p. 9–32. *In* B. T. Rouse, and C. Lopez (ed.), Immunobiology of herpes simplex virus infection. CRC Press, Inc. Boca Raton, Fla.
3. **Bodescot, M., O. Brison, and M. Perricaudet.** 1986. An Epstein-Barr virus transcription unit is at least 84 kilobases long. Nucleic Acids Res. **14**:2611–2620.
4. **Bodescot, M., and M. Perricaudet.** 1986. Epstein-Barr virus mRNAs produced by alternative splicing. Nucleic Acids Res.

14:7103–7114.
5. **Chen, M. S., D. C. Ward, and W. H. Prusoff.** 1976. Specific herpes simplex virus-induced incorporation of 5-iodo-5′-amino-2′,5′-dideoxyuridine into deoxyribonucleic acid. J. Biol. Chem. **251**:4833–4838.
6. **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.
7. **Clements, J. B., J. McLauchlan, and D. J. McGeoch.** 1979. Orientation of herpes simplex virus type 1 immediate early mRNAs. Nucleic Acids Res. **7**:77–91.
8. **Cook, M. L., V. B. Bastone, and J. G. Stevens.** 1974. Evidence that neurons harbor latent herpes simplex virus. Infect. Immun. **9**:946–951.
9. **Darby, G., and H. J. Field.** 1984. Latency and acquired resistance—problems in chemotherapy of herpes infections. Pharmacol. Ther. **23**:217–251.
10. **Deatly, A. M., J. G. Spivack, E. Lavi, and N. W. Fraser.** 1987. RNA from an immediate early region of the HSV-1 genome is present in the trigeminal ganglia of latently infected mice. Proc. Natl. Acad. Sci. USA **84**:3204–3208.
11. **Efstathiou, S., A. C. Minson, H. J. Field, J. R. Anderson, and P. Wildy.** 1986. Detection of herpes simplex virus-specific DNA sequences in latently infected mice and in humans. J. Virol. **57**:446–455.
12. **Fraser, N. W., A. M. Deatly, D. M. Mellerick, M. I. Muggeridge, and J. G. Spivack.** 1986. Molecular biology of latent HSV-1, p. 39–54. *In* C. Lopez and B. Roizman (ed.), Human herpesvirus infections: pathogenesis, diagnosis, and treatment. Raven Press, New York.
13. **Galloway, D. A., C. M. Fenoglio, and J. K. McDougall.** 1982. Limited transcription of the herpes simplex virus genome when latent in human sensory ganglia. J. Virol. **41**:686–691.
14. **Galloway, D. A., C. Fenoglio, M. Shevchuk, and J. K. McDougall.** 1978. Detection of herpes simplex RNA in human sensory ganglia. Virology **95**:265–268.
15. **Hill, T. J.** 1985. Herpes simplex virus latency, p. 175–240. *In* B. Roizman (ed.), The herpesviruses, vol. 4. Plenum Publishing Corp., New York.
16. **Holland, T. C., S. D. Marlin, M. Levine, and J. Glorioso.** 1983. Antigenic variants of herpes simplex virus selected with glycoprotein-specific monoclonal antibodies. J. Virol. **45**:672–682.
17. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpesvirus macromolecule synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **14**:8–19.
18. **Kafatos, F. C., C. W. Jones, and A. Efstratiadis.** 1979. Isolation and partial purification of the major abundant class rat seminal vesicle poly(A^+)-messenger RNA. Nucleic Acids Res. **7**:1541–1552.
19. **Khalili, K., C. Salas, and R. Weinmann.** 1983. Isolation and characterization of human actin genes cloned in phage lambda vectors. Gene **21**:9–17.
20. **King, W., V. Van Santen, and E. Kieff.** 1981. Epstein-Barr virus RNA. VI. Viral RNA in restringently and abortively infected Raji cells. J. Virol. **38**:649–660.
21. **Klein, R. J.** 1980. Effect of immune serum on the establishment of herpes simplex virus infection in trigeminal ganglia of hairless mice. J. Gen. Virol. **49**:401–405.
22. **Mackem, S., and B. Roizman.** 1980. Regulation of herpesvirus macromolecule synthesis: transcription-initiation sites and domains of alpha genes. Proc. Natl. Acad. Sci. USA **77**:7122–7126.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **McLennan, J. L., and G. Darby.** 1980. Herpes simplex virus latency: the cellular location of virus in dorsal root ganglia and the fate of the infected cell following virus activation. J. Gen. Virol. **51**:233–243.
25. **Overall, J. C.** 1979. Dermatologic diseases, p. 305–384. *In* G. J. Galasso, T. C. Merigan, and R. A. Buchanan (ed.), Antiviral agents and viral diseases of man. Raven Press, New York.
26. **Perry, L. J., F. J. Rixon, R. D. Everett, M. G. Frame, and D. J. McGeoch.** 1986. Characterization of the IE110 gene of herpes simplex virus type 1. J. Gen. Virol. **67**:2365–2380.
27. **Post, L. E., A. J. Conley, E. S. Mocarski, and B. Roizman.** 1980. Cloning of reiterated and nonreiterated herpes simplex virus 1 sequences as BamHI fragments. Proc. Natl. Acad. Sci. USA **77**:4201–4205.
28. **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.
29. **Rixon, F. J., M. E. Campbell, and J. B. Clements.** 1984. A tandemly reiterated DNA sequence in the long terminal repeat region of herpes simplex virus type 1 found in close proximity to immediate-early mRNA 1. J. Virol. **52**:715–718.
30. **Rixon, F. J., and D. J. McGeoch.** 1985. Detailed analysis of the mRNAs mapping in the short unique region of herpes simplex virus type 1. Nucleic Acids Res. **13**:953–973.
31. **Rock, D. L., and N. W. Fraser.** 1985. Latent herpes simplex virus type 1 DNA contains two copies of the virion DNA joint region. J. Virol. **55**:849–852.
32. **Rock, D. L., and N. W. Fraser.** 1983. Detection of HSV-1 genome in the central nervous system of latently infected mice. Nature (London) **302**:523–525.
33. **Roizman, B., and W. Batterson.** 1985. Herpesviruses and their replication, p. 497–526. *In* B. N. Fields (ed.), Virology. Raven Press, New York.
34. **Rosen, A., and G. Darai.** 1985. Mapping of the deletion in the genome of HSV-1 HFEM responsible for its avirulent phenotype. Med. Microbiol. Immunol. **173**:329–343.
35. **Rymo, L.** 1979. Identification of transcribed regions of Epstein-Barr virus DNA in Burkitt lymphoma-derived cells. J. Virol. **32**:8–18.
36. **Sacks, W. R., C. C. Greene, D. P. Aschman, and P. A. Schaffer.** 1985. Herpes simplex virus type 1 ICP27 is an essential regulatory protein. J. Virol. **55**:796–805.
37. **Spivack, J. G., D. R. O'Boyle II, and N. W. Fraser.** 1987. Novobiocin and coumermycin A_1 inhibit viral replication and the reactivation of herpes simplex virus type 1 from the trigeminal ganglia of latently infected mice. J. Virol. **61**:3288–3291.
38. **Spivack, J. G., W. H. Prusoff, and T. R. Tritton.** 1982. Inhibition of herpes simplex virus replication by methyl daunosamine. Antimicrob. Agents Chemother. **22**:176–179.
39. **Spivack, J. G., W. H. Prusoff, and T. R. Tritton.** 1982. Dissociation of the inhibitory effects of 2-deoxy-D-glucose on Vero cell growth and the replication of herpes simplex virus. Antimicrob. Agents Chemother. **22**:284–288.
40. **Stevens, J. G.** 1980. Herpetic latency and reactivation, p. 1–12. *In* F. Rapp (ed.), Oncogenic herpesviruses, vol. 2. CRC Press, Inc., Boca Raton, Fla.
41. **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.
42. **Stroop, W. G., D. L. Rock, and N. W. Fraser.** 1984. Localization of herpes simplex virus in the trigeminal and olfactory systems of the mouse central nervous system during acute and latent infections by *in situ* hybridization. Lab. Invest. **51**:27–38.
43. **Tenser, R. B., M. Dawson, S. J. Ressel, and M. E. Dunstan.** 1982. Detection of herpes simplex virus mRNA in latently infected trigeminal ganglion neurons by in situ hybridization. Ann. Neurol. **11**:285–291.
44. **Van Santen, V., A. Cheung, and E. Kieff.** 1981. Epstein-Barr virus RNA VII: size and direction of transcription of virus-specified cytoplasmic RNAs in a transformed cell line. Proc. Natl. Acad. Sci. USA **78**:1930–1934.
45. **Wagner, E. K.** 1985. Individual HSV transcripts: characterization of specific genes, p. 45–104. *In* B. Roizman (ed.), The herpesviruses, vol. 3. Plenum Publishing Corp., New York.
46. **Watson, R. J., C. M. Preston, and J. B. Clements.** 1979. Separation and characterization of herpes simplex virus type 1 immediate-early mRNA's. J. Virol. **31**:42–52.
47. **Yates, J., N. Warren, D. Reisman, and B. Sugden.** 1984. A *cis*-acting element from the Epstein-Barr viral genome permits stable replication of recombinant plasmids in latently infected cells. Proc. Natl. Acad. Sci. USA **81**:3806–3810.