Expression of Herpes Simplex Virus Type 1 Latency-Associated Transcripts in the Trigeminal Ganglia of Mice during Acute Infection and Reactivation of Latent Infection

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Herpes simplex virus type 1 (HSV-1) establishes a latent infection in the trigeminal ganglia of mice infected via the eye. In these ganglia three viral transcripts, of 2.0, 1.5, and 1.45 kilobases (kb), which are at least partially colinear, have been identified by Northern (RNA) blot analysis. These RNAs partially overlap ICP0, but are transcribed in the opposite direction (J. G. Spivack and N. W. Fraser, *J. Virol.* 61:3841-3847, 1987). The accumulation of these latency-associated transcripts, as well as other viral RNAs, was studied during an acute infection and the reactivation of a latent HSV-1 infection in mice. The 2.0-kb latency-associated transcript was detected in trigeminal ganglia of mice as early as 4 days postinfection, and the 1.45- and 1.5-kb RNA doublet was detected at 14 days postinfection. The levels of these latency-associated transcripts increased steadily over a 60-day period. In contrast, other HSV-1 transcripts were detected at 2 to 3 days postinfection, reached a peak on day 4, and rapidly declined below detectable levels by day 7. The data indicate that the temporal expression of the latency-associated genes during acute infection in the trigeminal ganglia of mice is different from the temporal expression of genes involved in HSV-1 replication. During the reactivation of latent HSV-1 from explanted trigeminal ganglia, the latency-associated RNAs decreased about twofold, but were present at significant levels even after HSV-1 DNA increased and infectious virus was recovered. The decrease of the latency-associated transcripts occurred when reactivation was blocked by phosphonoacetic acid or novobiocin, which suggests that this decrease may be an early event in the entry of latent HSV-1 into the viral replication cycle.

Latent herpes simplex virus type 1 (HSV-1) resides within the neurons of sensory ganglia (6, 24). Reactivations of latent HSV-1 to produce recurrent disease (2, 13, 41) are common (25) and can be life threatening in immune-suppressed individuals. To understand the herpesvirus-host relationship, it is important to identify and characterize the viral genes expressed during the pathogenesis of acute, latent, and reactivated infections.

Virus-specific transcripts have been detected during HSV-1 latency by in situ hybridization in the central and peripheral nervous systems of mice (7, 8, 10, 33, 42, 43) and during HSV-2 latency in guinea pig (44) and human sensory ganglia (11, 12). The viral transcripts in the sensory ganglia of latently infected mice hybridized to the long repeat region of HSV-1 (7, 10, 33, 42), which encodes several genes, including the immediate-early gene ICP0. However, the viral RNA present during HSV-1 latency is transcribed from the DNA strand complementary to that encoding ICP0 (33, 37, 42). Three transcripts, of 2.0, 1.5, and 1.45 kilobases (kb), present during HSV-1 latency in the trigeminal ganglia of mice originate from a 3.0-kb region contained within the internal long repeat region that partially overlaps the 3' terminus of ICP0 (37). These latency-associated transcripts are distinct from any that have been previously identified during the lytic replication cycle of HSV-1 (33, 37, 42).

The systems available to detect virus that has reactivated from latently infected animals have limitations for studying the reactivation process. Reactivation can be induced in vivo, but occurs with a variable time course in only 20 to 50% of the animals (13, 41). HSV-1 reactivation is assayed in

these systems retrospectively by the shedding of virus or the appearance of lesions. Reactivation can also be induced in vitro by explant cocultivation of sensory ganglia from latently infected animals and cells permissive for HSV-1 replication (for a review, see references 13 and 41). An explant reactivation model that is efficient, quantitative, and time dependent has been developed to study the molecular events during reactivation that occur prior to the appearance of infectious HSV-1 (38).

In the present study the expression of the latency-associated transcripts in the trigeminal ganglia of mice was monitored during the acute infection and reactivation of the latent infection. These data demonstrate that (i) during an acute infection the transcripts of a number of HSV-1 genes involved in the lytic replication cycle rapidly increase and decrease over a 7-day period; (ii) the latency-associated transcripts are first expressed during the acute infection, and their levels continue to increase thereafter; (iii) during reactivation of the latent infection the latency-associated transcripts decrease, but remain at significant levels even after reactivated virus is detectable; and (iv) the decrease in the latency-associated transcripts occurs even when reactivation is blocked by phosphonoacetic acid or novobiocin, suggesting that the decrease may be a critical early event in the reactivation process, rather than a secondary consequence of reactivation.

MATERIALS AND METHODS

Cell culture, HSV-1 growth, and titration. Subconfluent monolayers of CV-1 cells, a monkey kidney cell line, grown in Eagle minimum essential medium supplemented with 5% fetal calf serum at 37°C with 5% CO₂ were infected with HSV-1 (strain F; from B. Roizman, University of Chicago)

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at 1 PFU per cell, and the virus was concentrated from the medium, as previously described (7, 32, 38). Virus stock titers were determined on CV-1 cells, and the plaques were stained with 1% methylene blue 2 days later (39, 40).

Infection of mice and explant reactivation. Following corneal scarification, 4- to 6-week-old female BALB/c mice were each infected with approximately 10^6 to 10^7 PFU of HSV-1 F per eye (32). At a minimum of 4 weeks after infection, latently infected mice were sacrificed by cervical dislocation. The trigeminal ganglia were rapidly dissected and incubated for 4 days in Eagle minimal essential medium supplemented with 5% fetal calf serum at 37°C in 5% CO₂, Dounce homogenized, and titrated (38).

RNA extraction, agarose gel electrophoresis, Northern blot transfer, hybridization, and washing of Northern blots. At each time point after explant, 10 to 12 trigeminal ganglia were homogenized in 4 M guanidinium thiocyanate–0.5% sodium *N*-lauroyl sarcosine–100 mM β -mercaptoethanol–25 mM sodium citrate (pH 7.0)–0.1% Antifoam A (Sigma Chemical Co.) (4), and total RNA was extracted as previously described (37). The denaturation of RNA with glyoxal, agarose gel electrophoresis, and Northern (RNA) blot transfer to GeneScreen Plus (Du Pont Co.) were performed as described previously (37). RNA markers were purchased from Bethesda Research Laboratories Inc. Heat-denatured ³²P-labeled nick-translated probes were added to Northern blots and hybridized overnight (37). The filters were given two 30-min washes in each of decreasing concentrations of SSC to $0.1\times$ ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–1% sodium dodecyl sulfate at 65°C, covered with plastic wrap, and autoradiographed with XAR-5 film (Eastman Kodak Co.) at –70°C with an intensifying screen (Du Pont). The autoradiographs were scanned with a laser densitometer Ultrosan XL; LKB Instruments, Inc.). The probes were removed in $0.01\times$ SSC–0.01% sodium dodecyl sulfate, and the Northern blots were rehybridized as recommended by Du Pont.

Preparation of ³²P-labeled probes. Total HSV-1 DNA was isolated from virions and purified by CsCl gradient centrifugation (3). The *Bam*HI fragments of HSV-1 F cloned into pBR322 were obtained from B. Roizman (28). Subfragments of *Bam*HI-B were isolated from restriction digests by gel electrophoresis and electroelution into dialysis tubing (21). An actin DNA probe was provided by R. Weinmann (16). Restriction enzymes were purchased from Boehringer Mannheim Biochemicals or Bethesda Research Laboratories and used as recommended by the manufacturers. DNA probes were nick-translated by standard procedures (21) and were separated from unincorporated nucleotides by passage through mini-spin Sephadex G-50 columns (Boehringer Mannheim). The specific activities of the probes were 1×10^8 to 5×10^8 cpm/ μ g of DNA.

DNA extraction from tissues. Trigeminal ganglia were rinsed with phosphate-buffered saline and Dounce homogenized in ice-cold 160 mM NaCl–50 mM EDTA–5 mM Tris (pH 8.0) (32, 38). Sodium dodecyl sulfate and pronase were added to 0.5% and 1 mg/ml, respectively, and the mixtures were incubated at 37°C overnight. The digests were extracted with phenol, phenol-chloroform, and chloroform, ethanol precipitated, suspended in 10 mM Tris–1 mM EDTA (pH 7.4), incubated with RNase (100 μ g/ml) at 37°C for 2 h and proteinase K (100 μ g/ml) at 37°C for an additional 2 h, extracted with phenol, phenol-chloroform, and chloroform, and ethanol precipitated. DNA amounts were measured by A_{260} .

DNA analysis. (i) Spot blot of DNA. DNA samples in 10

mM Tris–1 mM EDTA (pH 7.4) were denatured with 0.4 M NaOH for 10 min at room temperature. After they had cooled on ice, an equal volume of 2 M sodium acetate was added (15). The samples were filtered through nitrocellulose (pore size, 0.45 μ m; BA-85; Schleicher & Schuell, Inc.) in a Schleicher & Schuell 96-well filtration manifold.

(ii) **Agarose gel electrophoresis and Southern blot transfer of DNA.** Agarose gel electrophoresis and Southern blot transfer were performed by standard techniques (21), as previously described (32, 38). Spot blots were hybridized and washed essentially as described for Southern blots (32, 38). After being washed, the filters were air dried, covered with plastic wrap, and autoradiographed with XAR-5 film at –70°C with intensifying screens. The radioactivity of spot blots was quantitated by counting in a liquid scintillation counter with Econofluor (New England Nuclear Corp.).

RESULTS

Mice were infected with approximately 10^6 to 10^7 PFU of HSV-1 F per eye after corneal scarification. Infectious virus could be detected in trigeminal ganglia homogenates from 2 to 7 days postinfection, with a peak at 4 days (18; unpublished observations). The amount of HSV-1 DNA detected by spot blot analysis followed a similar time course (10). Mice were considered latently infected 4 or more weeks after inoculation, when infectious virus was no longer detectable, but could be reactivated from 100% of the trigeminal ganglia by explant cultivation (8, 32, 38). The lack of infectious virus at the time of explant and the ability to reactivate HSV-1 from the ganglia form an operational definition of latency (13, 41).

Expression of HSV-1 latency-associated transcripts during the acute phase of infection. Three transcripts (2.0, 1.5, and 1.45 kb) have been detected in the trigeminal ganglia of latently infected mice by Northern blot analysis (37). These transcripts map to a 3.0-kb *Pst*I–*Mlu*I fragment contained within *Bam*HI-B (37) (Fig. 1). The accumulation of the latency-associated transcripts was studied from 0 to 60 days postinfection by Northern blot analysis of RNA isolated from the trigeminal ganglia of mice (Fig. 2). The 2.0-kb latency-associated transcript was detectable as early as 4 days postinfection, which was the peak of the acute infection, and increased steadily over the 60-day time course. The 1.45- and 1.5-kb RNA doublet was barely detectable at 14 days (visible in overexposures; data not shown) and continuously increased thereafter (Fig. 2). This doublet is noticeable in the 21- and 30-day samples and was detected in previous work (37). It should be noted that a faint band at the position of ICP0 mRNA (2.6 to 2.7 kb) was detectable only at day 4 (Fig. 2).

Detection of HSV-1 transcripts during the acute phase of infection. To investigate the expression of HSV-1 genes during the acute stage of infection, we hybridized Northern blots with nick-translated probes of virion DNA or *Bam*HI fragments Y, N, or X (see Fig. 1 for locations). *Bam*HI-Y encodes a portion of the immediate-early gene ICP4 (5, 19, 46), *Bam*HI-N encodes a portion of the immediate-early gene ICP22 (5, 19, 46) and 35 nucleotides of ICP4 (5, 19, 46), and *Bam*HI-X encodes a portion of five RNAs (23, 31). In order of decreasing size, the RNAs that hybridize to *Bam*HI-X are glycoprotein E, the immediate-early gene ICP47 (5), a DNA-binding protein (molecular mass, 18 kilodaltons), a minor infected-cell protein (34 kilodaltons), and an uncharacterized protein (10 kilodaltons) (23, 31). The virion DNA probe hybridized to RNA from ganglia at 2 to 3 days

FIG. 1. Map of the HSV-1 genome and location of the latency-associated transcripts. (A) HSV-1 genome. Symbols: —, the unique long and short regions (U_L and U_S); □, the internal and terminal long and short repeats (IR_L , TR_L , IR_S , and TR_S). (B) *Bam*HI fragments of HSV-1 DNA (28). Symbol: ■, fragments positive by in situ hybridization to the trigeminal ganglia of latently infected mice (7). The fragments referred to in the present work are labeled with capital letters. (C) Map of *Bam*HI-B. The approximate positions of restriction sites (1, 19, 27, 35) and RNAs for ICP0 (5, 19, 27, 46), ICP27 (5, 19, 46), the 22- and 20-kilodalton proteins (20, 37), and the latency-associated transcripts (37) are indicated with arrows pointing in the direction of transcription. The 5' end of ICP0 mRNA is in *Bam*HI SP. The splice junctions of ICP0 mRNA are not shown (5, 27). Symbols: *, RNA termini that have not been precisely mapped; zzz, approximate boundary between the unique and repeat regions of the HSV-1 genome (18a). The three latency-associated transcripts (37) are represented by a single line 2.0 kb long.

postinfection, increased to a maximum at day 4, and rapidly declined by day 7 (Fig. 3A). At day 7 the virion DNA probe hybridized to a 2.0-kb RNA, which is probably the latency-associated transcript (compare Fig. 3A with Fig. 2). The 2.0-kb band was strongest at the peak of the viral infection, because a number of HSV-1 transcripts are 2.0 kb, including ICP27 and ICP22 (45). The probes *Bam*HI-Y and *Bam*HI-N hybridized to ICP4 and ICP22 mRNA, estimated at 4.7 and 2.0-kb, respectively (5, 46), with kinetics similar to that of the virion DNA probe (Fig. 3B and 3D). *Bam*HI-X hybridized to five transcripts on day 4 (Fig. 3C), which correspond to genes US8 to US12 predicted by sequence analysis of the unique short region (23). *Bam*HI-Y, *Bam*HI-N, and *Bam*HI-X did not hybridize to RNA from ganglia between 14 and 60 days postinfection (data not shown). These results indicate that the latency-associated transcripts are expressed with different kinetics in the trigeminal ganglia of mice than are a number of genes involved in the lytic replication cycle (compare Fig. 2 and 3). It should be noted that the data do not distinguish between the following two possibilities: (i) the latency-associated transcripts and RNAs involved in HSV-1 replication are expressed with different kinetics in the same cells; and (ii) one population of cells expresses the latency-associated RNAs, and a second population of cells expresses RNAs from the viral replication cycle.

Levels of HSV-1 DNA during latency and reactivation in trigeminal ganglia. To establish a time frame to study HSV-1 gene expression during the reactivation process, we determined the amount of HSV-1 DNA present during latency and reactivation by spot blot analysis of DNA extracted from latent trigeminal ganglia (Fig. 4). The cross-hybridization between HSV-1 and mouse DNA sequences (26, 29), minimized by the stringent hybridization and washing conditions, was subtracted as background. The hybridization was confirmed as HSV-1 specific by Southern blot analysis

(32, 38; data not shown). The HSV-1 DNA present during latency (Fig. 4A) was equal to a dilution of $(3.6 \pm 2.8) \times 10^{-6}$ (mass HSV-1 DNA:mass mouse DNA; three to five mice per experiment, nine independent experiments). Assuming a

FIG. 2. Detection of HSV-1 latency-associated transcripts in the trigeminal ganglia of mice by Northern blot analysis. Each lanes contains 5 μ g of RNA from the trigeminal ganglia of mice at the indicated times (in days) postinfection. The 3.1-kb *Hpa*I-*Bam*HI probe was used (Fig. 1). The autoradiograph was made with XAR-5 film (Kodak). The positions of RNA markers (in kilobases) are labeled on the left, and the positions of 28S and 18S rRNAs are labeled with asterisks.

FIG. 3. Detection of HSV-1 transcripts in the trigeminal ganglia of infected mice by Northern blot analysis. (A) Purified HSV-1 DNA probe (TV, total virion DNA); (B) *Bam*HI-Y probe; (C) *Bam*HI-X probe; (D) *Bam*HI-N probe. Each lane contains 5 μ g of RNA from the trigeminal ganglia of mice at the indicated times (in days) postinfection. The autoradiograph was made with XAR-5 film (Kodak). The positions of RNA markers (in kilobases) are labeled on the left, and the positions of 28S and 18S rRNAs are labeled with asterisks.

mass of approximately 10^8 daltons for HSV-1 DNA (17) and 3×10^{12} daltons for mouse DNA, this corresponds to 0.11 ± 0.08 genome equivalents of HSV-1 DNA per cell, which is comparable to published estimates (9, 30, 32).

The amount of HSV-1 DNA in the trigeminal ganglia during the reactivation process was determined by spot blot analysis of DNA from ganglia in explant culture. The amount of HSV-1 DNA present began to increase at day 2 (Fig. 4B), which was the earliest time at which reactivated virus could be observed (38). By day 4, reactivated virus could be recovered from 100% of the ganglia (38), and the amount of HSV-1 DNA present was approximately 1,000-fold higher than during the latent period (Fig. 4B). Thus, HSV-1 DNA and infectious virus increased with similar kinetics during the reactivation process.

Detection of the latency-associated transcripts during the reactivation process. The time course of appearance of virus (38) and viral DNA (Fig. 4B) in trigeminal ganglia explanted from latently infected mice defined a 4-day interval for studying HSV-1 gene expression during the reactivation process. The three latency-associated transcripts were present at significant levels throughout the time course, but decreased in ganglia over a 96-h period (Fig. 5). The 3.1-kb *Hpa*I-*Bam*HI probe used to detect the latency transcripts (Fig. 1) also hybridized to ICP0 mRNA. This mRNA was apparent only in ganglia that had been explanted for 96 h or longer (Fig. 5). After probe removal, these Northern blots were rehybridized with an actin probe (16) as an internal standard to correct for variations in sample loading. The autoradiographs were quantitated by densitometer scanning, and the amounts of latency-associated transcripts were normalized relative to actin. The amounts of the 2.0-kb latency-associated transcript and the 1.45- to 1.5-kb doublet decreased to $70\% \pm 22\%$ (three determinations) and $49\% \pm 12\%$ (three determinations) of the latent value by 24 to 36 h postexplant, respectively, and $44\% \pm 13\%$ (three determinations) and $28\% \pm 10\%$ (three determinations) of the latent value by 96 to 120 h postexplant, respectively. The smaller band is most noticeable as a doublet in the 24-h sample, and was also found in previous work (37). These results demonstrate that reactivation can occur in tissues in the presence of the latency-associated transcripts, although the events in

FIG. 4. HSV-1 DNA in the trigeminal ganglia of mice during latency and reactivation. (A) DNA was extracted from the ganglia of latently infected mice. The samples and HSV-1 DNA standards were spotted onto nitrocellulose, hybridized to a total virion DNA probe, washed, and autoradiographed as described in Materials and Methods. Top row: each DNA sample (5 μ g) was extracted from the ganglia of three or more mice, from four independent infections. Bottom row: con. MB, 5 μ g of DNA from an uninfected mouse; the indicated HSV-1 DNA dilutions (5 μ g) were made in control mouse brain DNA. (B) DNA was extracted from 10 to 12 trigeminal ganglia from latently infected mice at the indicated times (hours) postexplant and spot blotted as in panel A. The HSV-1 DNA was quantitated by scintillation counting. Symbols: ◆, individual datum points; □, averages of four independent experiments.

FIG. 5. Detection of the HSV-1 latency-associated transcripts during the reactivation process by Northern blot analysis. Each lane contains 5 μ g of RNA from the trigeminal ganglia of latently infected mice at the indicated time postexplant. Lane C, RNA from an uninfected mouse brain; lane 0, RNA from the ganglia of a latently infected mouse; lanes 12, 24, 36, 48, 60, 72, and 96, RNA from the ganglia of latently infected mice at the respective times (hours) postexplant; lane PAA, explant reactivation of trigeminal ganglia for 96 h in the presence of 200 μ g of phosphonoacetic acid. The 3.1-kb *HpaI*-*Bam*HI probe was used (Fig. 1). The autoradiograph was made with XAR-5 film (Kodak). The positions of RNA markers (in kilobases) are labeled on the left, and the positions of 28S and 18S rRNAs are labeled with asterisks. ICP0 mRNA is marked with an arrow labeled O.

individual cells might vary. A comparable average decrease in the levels of the latent transcripts also occurred when 200 μ g of phosphonoacetic acid, an inhibitor of HSV-1 DNA synthesis (22) and reactivation in explanted ganglia (38), was added per ml at explant (Fig. 5). Similar results were obtained with 160 μ M novobiocin (data not shown), which also inhibits reactivation and viral replication in explanted trigeminal ganglia (38).

To examine the synthesis of other HSV-1 transcripts during reactivation, RNA samples from explanted trigeminal ganglia of latently infected mice were hybridized to nick-translated probes of *Bam*HI-X, *Bam*HI-Y, *Bam*HI-N, and purified viral DNA. These probes hybridized weakly to the 96-h sample, but hybridization to RNA from the earlier time points was below the level of detection (data not shown), even though infectious virus could be detected at 72 h in most of the ganglia (38). Thus, during the reactivation process HSV-1 transcripts, except for those expressed during latency, could not be detected by Northern blot analysis before the appearance of infectious virus.

DISCUSSION

A picture of HSV gene expression during the pathogenesis of latent infections in mice is beginning to emerge. In situ hybridization studies have demonstrated that HSV-1 transcription in the trigeminal ganglia of latently infected mice appears to be restricted to a small portion of the genome (7, 10, 33, 42). Recently, three at least partially colinear HSV-1

transcripts, of 2.0, 1.5, and 1.45 kb, have been mapped by Northern blot analysis to a 3.0-kb *Pst*I-*Mlu*I fragment within the region positive by in situ hybridization (37) (Fig. 1). These transcripts partially overlap the 3' end of ICP0 mRNA which is transcribed in the opposite direction (37). The latent RNAs are distinct from any other RNAs previously identified as actively transcribed during the lytic replication cycle (33, 37, 42). Thus, there is an alternative pathway of HSV-1 gene expression during latency, and HSV-1 gene expression is partially restricted during lytic replication in tissue culture cells.

In the present paper, HSV-1 gene expression has been studied by Northern blot analysis during the acute, latent, and reactivation phases of infection. In trigeminal ganglia, infectious virus (18; unpublished observations) and viral DNA (10) quickly increased and declined in the first 7 days of infection. The expression of a number of viral RNAs also followed a similar pattern (Fig. 3). In contrast, the 2.0-kb latency-associated transcript was first detected at 4 days postinfection, the 1.45- 1.5-kb doublet was first detected at 14 days, and all steadily increased in abundance over a 60-day period (Fig. 2). The results demonstrate that in the trigeminal ganglia of mice the latency-associated transcripts are expressed with kinetics different than those of other HSV-1 RNAs during the acute stage of infection, when latency is established (36). The data do not address the issue of whether the acutely infected cells also express the latency-associated transcripts or whether there may be two populations of cells, one which succumbs to acute infection and the other which supports latent HSV-1 infection. The detection of a low level of the 2.0-kb latency-associated transcript in HSV-1-infected CV-1 cells (37) is evidence that at least one of the latency-associated RNAs may be transcribed in lytically infected cells.

Since the latency-associated transcripts continued to increase long after any infectious virus could be detected, it is clear that the latency-associated RNAs were not residual from the acute stage of infection. The accumulation of these transcripts was somewhat unexpected, since during a latent infection in mice, HSV-1 DNA does not increase between 1 and 5 months postinfection in the peripheral or central nervous systems of mice (9, 32; unpublished observations). It is possible that the accumulation of the latent transcripts from 0 to 60 days postinfection represents (i) an increase in transcription in each latently infected cell, (ii) a decrease in RNA turnover rates, (iii) an increase in the proportion of latently infected cells expressing the transcripts, or (iv) a combination of these possibilities. In any case, the data indicate that the host cell-latent HSV-1 relationship is an active one.

There are few estimates in the literature of the proportion of ganglion cells that harbor latent HSV-1 DNA, and it is not known what fraction of latently infected cells reactivate HSV-1 in explanted ganglia. Although there is evidence that reactivation occurs from neurons (6, 24), the possibility that latent HSV-1 DNA is present in extraneural sites cannot be rigorously excluded (for a review, see reference 13). The estimates for latent HSV-1 DNA are approximately 0.1 genome equivalent per cell (Fig. 4) (9, 30, 32). Although no conclusions about the number of HSV-1 DNA copies present in individual cells can be drawn, these estimates suggest the possibility that (i) approximately 10% of ganglion cells contain one copy of HSV-1 DNA or (ii) a smaller percentage of cells contain multiple HSV-1 DNA copies.

As a framework for the study of HSV-1 gene expression during the reactivation of a latent infection in mice, the time

course of accumulation of HSV-1 DNA in explanted ganglia was determined. An increase in the amount of HSV-1 DNA was first detected at 2 days postexplant and the DNA continued to increase with log linear kinetics until day 4 (Fig. 4). Since this was similar to the time course observed for the appearance of infectious virus (38), most of the lag period between the reactivation stimulus (i.e., explant) and the detection of reactivated virus also preceded the synthesis of viral DNA. In cell culture, viral DNA is synthesized near the middle of the HSV-1 replication cycle (14, 34, 45). Since HSV-1 DNA synthesis is initiated a few hours after infection (14, 34), it is possible that some of the events that occur between explant of trigeminal ganglia from latently infected mice and an increase in the amount of viral DNA are host cell changes rather than early events in the viral replication cycle.

HSV-1 gene expression was studied during the reactivation of latent infections by Northern blot analysis of RNA extracted from trigeminal ganglia in explant culture (Fig. 5). Even though infectious virus could be detected in some trigeminal ganglia from latently infected mice at 48 h postexplant (38) and an increase in HSV-1 DNA could also be detected at that time (Fig. 4), transcripts corresponding to ICP0 (Fig. 5) and other viral genes (data not shown) were not detectable by Northern blot analysis until 96 h. These results suggest that the assays for infectious virus and HSV-1 DNA are more sensitive than Northern blot analysis of RNA for studying the early events in the reactivation of latent HSV-1. The detection of viral transcripts in reactivated ganglia at 96 h postexplant probably represented viral RNA from cells secondarily infected by the spread of reactivated virus.

Even though other HSV-1 RNAs could not be detected during reactivation until 96 h postexplant, the levels of the latency-associated transcripts in explanted ganglia were monitored during the reactivation process. The latency-associated transcripts decreased, but were present at 96 h postexplant (Fig. 5): a time when all explanted ganglia were virus positive (38). The 55 to 70% decrease in the levels of the latency-associated RNAs during reactivation did not require viral DNA synthesis, since it occurred in the presence of phosphonoacetic acid or novobiocin. These results imply either that (i) reactivation and viral replication can occur in the presence of reduced levels of the latency-associated transcripts, or (ii) reactivation occurs in only a portion of the latently infected cells and expression of the latency transcripts is inhibited in these cells.

If reactivation occurs in some, but not all, of the latently infected cells, then viral transcription in cells in which latent HSV-1 reactivates would differ from transcription in cells in which latency is maintained. Theoretically, reactivation of a latent infection could be the result of events in a single latently infected cell. During reactivation in intact sensory ganglia explanted from latently infected mice, reactivation was detected in only a very small number of neurons (6, 24). McLennan and Darby (24) observed a total of nine reactivation foci in ganglia from three latently infected mice. These data suggest, but do not prove, that reactivation in explanted ganglia may occur in only a portion of the latently infected cells. The decrease in the amount of the latency-associated RNAs in trigeminal ganglia explanted from latently infected mice (Fig. 5) might be due to a switch from the latent to lytic viral transcription pattern in some of the latently infected cells.

Since the latent HSV-1 RNAs accumulated during the transition from the acute to the latent stage of infection and decreased during reactivation, it would be attractive to

propose that they have an important role in the maintenance of latent infection or the suppression of viral replication. However, determination of the functional significance of the latency-associated HSV-1 RNAs will require further analysis. Whether or not the latency-associated genes, their transcripts, or potential gene products play an essential role in the pathogenesis of HSV-1 infections or the control of viral replication, and determination of what the role(s) might be, remain to be established.

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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. 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.
4. 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.
5. 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.
6. Cook, M. L., V. B. Bastone, and J. G. Stevens. 1974. Evidence that neurons harbor latent herpes simplex virus. *Infect. Immun.* **9**:946-951.
7. 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.
8. Deatly, A. M., J. G. Spivack, E. Lavi, D. R. O'Boyle II, and N. W. Fraser. 1988. Latent herpes simplex virus type 1 transcripts in peripheral and central nervous system tissues of mice map to similar regions of the viral genome. *J. Virol.* **62**:749-756.
9. Efsthathiou, 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.
10. 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.
11. Galloway, D. A., C. 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.
12. 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.
13. Hill, T. J. 1985. Herpes simplex virus latency, p. 175-240. In B. Roizman (ed.), *The herpesviruses*, vol. 4. Plenum Publishing Corp., New York.
14. 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.
15. Kafatos, F. C., C. W. Jones, and A. Efstratiadis. 1979. Determination of nucleic acid sequence homologies and relative concentrations by a dot blot hybridization procedure. *Nucleic*

- Acids Res. 7:1541-1552.
16. Khalili, K., C. Salas, and R. Weinmann. 1983. Isolation and characterization of human actin genes cloned in phage lambda vectors. *Gene* 21:9-17.
 17. Kieff, E. D., S. L. Bachenheimer, and B. Roizman. 1971. Size, composition and structure of deoxyribonucleic acid of herpes simplex virus subtypes 1 and 2. *J. Virol.* 8:125-132.
 18. Knotts, F. B., M. L. Cook, and J. G. Stevens. 1974. Pathogenesis of herpetic encephalitis in mice after ophthalmic inoculation. *J. Infect. Dis.* 130:16-27.
 - 18a. Kock, H.-G., A. Rosen, F. Ernst, Y. Becker, and G. Darai. 1987. Determination of the nucleotide sequence flanking the deletion (0.762 to 0.789 map units) in the genome of an intraperitoneally avirulent HSV-1 strain HFEM. *Virus Res.* 7:105-115.
 19. Mackem, S., and B. Roizman. 1980. Regulation of herpesvirus macromolecule synthesis: transcription-initiation sites and domains of α genes. *Proc. Natl. Acad. Sci. USA* 77:7122-7126.
 20. Maclean, A. R., and S. M. Brown. 1987. A herpes simplex virus type 1 variant which fails to synthesize immediate early peptide V_{mw} IE63. *J. Gen. Virol.* 68:1339-1350.
 21. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 22. Mao, J. C.-H., E. E. Robishaw, and L. R. Overby. 1975. Inhibition of DNA polymerase from herpes simplex virus infected Wi-38 cells by phosphonoacetic acid. *J. Virol.* 15:1281-1283.
 23. McGeoch, D. J., A. Dolan, S. Donald, and F. J. Rixon. 1985. Sequence determination and genetic content of the short unique region in the genome of herpes simplex virus type 1. *J. Mol. Biol.* 181:1-13.
 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. Peden, K., P. Mounts, and G. S. Hayward. 1982. Homology between mammalian cell DNA sequences and human herpesvirus genomes detected by a hybridization procedure with high complexity probe. *Cell* 31:71-80.
 27. Perry, L. J., F. J. Rixon, R. D. Everett, M. C. Frame, and D. J. McGeoch. 1986. Characterization of the IE110 gene of herpes simplex virus type 1. *J. Gen. Virol.* 67:2365-2380.
 28. Post, L. E., A. J. Conley, E. S. Mocarski, and B. Roizman. 1980. Cloning of reiterated and nonreiterated herpes simplex virus 1 sequences as *Bam*H1 fragments. *Proc. Natl. Acad. Sci. USA* 77:4201-4205.
 29. Puga, A., E. M. Cantin, and A. L. Notkins. 1982. Homology between murine and human cellular DNA sequences and the terminal repetition of the S component of herpes simplex type virus type 1 DNA. *Cell* 31:81-87.
 30. Puga, A., J. D. Rosenthal, H. Openshaw, and A. L. Notkins. 1978. Herpes simplex virus DNA and mRNA sequences in acutely and chronically infected trigeminal ganglia of mice. *Virology* 89:102-111.
 31. 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.
 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. 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.
 34. Roizman, B., and W. Batterson. 1985. Herpesviruses and their replication, p. 497-526. *In* B. N. Fields (ed.), *Virology*. Raven Press, New York.
 35. 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.
 36. Sekizawa, T., H. Openshaw, C. Wohlenberg, and A. L. Notkins. 1980. Latency of herpes simplex virus in absence of neutralizing antibody: model for reactivation. *Science* 210:1026-1028.
 37. Spivack, J. G., and N. W. Fraser. 1987. Detection of herpes simplex virus type 1 transcripts during a latent infection in mice. *J. Virol.* 61:3841-3847.
 38. 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 type 1 from the trigeminal ganglia of latently infected mice. *J. Virol.* 61:3288-3291.
 39. Spivack, J. G., W. H. Prusoff, and T. R. Tritton. 1982. Inhibition of herpes simplex virus by methyl daunomycin. *Antimicrob. Agents Chemother.* 22:176-179.
 40. 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.
 41. 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.
 42. Stevens, J. G., E. K. Wagner, G. B. Devi-Rao, M. L. Cook, and L. T. Feldman. 1987. RNA complementary to a herpesvirus α gene mRNA is prominent in latently infected neurons. *Science* 235:1056-1059.
 43. 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.
 44. 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.
 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.