JOURNAL OF VIROLOGY, Dec. 1994, p. 7900–7908
0022-538X/94/$04.00+0

Vol. 68, No. 12

# Varicella-Zoster Virus (VZV) Transcription during Latency in Human Ganglia: Construction of a cDNA Library from Latently Infected Human Trigeminal Ganglia and Detection of a VZV Transcript

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Received 3 June 1994/Accepted 24 August 1994

**The entire varicella-zoster virus (VZV) genome appears to be present in latently infected human ganglia, but the extent of virus DNA transcription is unknown. Conventional methods to study virus gene transcripts by Northern (RNA) blotting are not feasible, since ganglia are small and VZV DNA is not abundant. To circumvent this problem, we prepared radiolabeled cDNA from ganglionic RNA, hybridized it to Southern blots containing VZV DNA, and demonstrated the presence of a transcript within the *Sal*I C fragment of the virus genome (R. Cohrs, R. Mahalingam, A. N. Dueland, W. Wolf, M. Wellish, and D. H. Gilden, J. Infect. Dis. 166:S24–S29, 1992). To further map VZV transcripts, in the work described here we constructed a cDNA library from poly(A)$^+$ RNA obtained from latently infected human ganglia. Phage DNA isolated from the library was used in PCR amplifications to detect VZV-specific inserts. The specificity of the PCRs was provided by selection of a primer specific for VZV gene 17, 18, 19, 20, or 21 and a second vector-specific primer. VZV gene 21-specific sequences were identified by PCR amplification. The PCR product contained the *Xho*I cloning site and poly(A)$^+$ sequences between vector and VZV gene 21 sequences. The sequence motif at the 3′ end of VZV gene 21, determined by cloning and sequencing of the PCR product, consisted of 49 to 51 nucleotide bases of 3′-untranslated DNA, the termination codon for the VZV gene 21 open reading frame, and DNA sequences reading into the VZV gene 21 open reading frame.**

Varicella-zoster virus (VZV), a member of the neurotropic α-herpesvirus family, is a ubiquitous human pathogen. Primary VZV infection causes varicella and is usually benign. After primary infection, VZV establishes latency in multiple ganglia at all levels of the human neuraxis (15). Decades later, VZV reactivates to produce zoster. Zoster and its various attendant neurologic complications affect more than 300,000 elderly Americans annually (10). An understanding of VZV latency is essential to control, if not prevent, VZV reactivation. Although the entire VZV genome appears to be present in latently infected ganglia (15), the extent of VZV DNA transcription is unknown.

Identification of VZV genes transcribed during latency is fraught with obstacles. VZV is latent only in human ganglia, which are small, and the virus burden is only ca. 6 to 31 copies per $10^5$ cells (14). Techniques to identify single transcripts, such as in situ hybridization, Northern (RNA) blot analysis, or RNase protection assays, are not easily applied to VZV latency since the virus contains over 68 unique open reading frames (ORFs), and ganglionic RNA is quickly exhausted. Therefore, we constructed a cDNA library from intact mRNA extracted from well-characterized human trigeminal ganglia latently infected with VZV. The library contained cDNA in amounts sufficient to search for multiple virus gene transcripts. Since our earlier work showed VZV-specific RNA transcribed from *Sal*I C DNA sequences in latently infected ganglia, our initial search focused on the five ORFs contained within this fragment of the virus genome (4). Phage DNA was extracted from the cDNA library and used in a PCR to identify VZV cDNA transcripts specific for the *Sal*I C fragment. The DNA sequence of the 3′ end of the VZV transcript was determined from the PCR-amplified product.

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## MATERIALS AND METHODS

**Cells, virus, and antibody.** The propagation of VZV in African green monkey kidney (BSC-1) cells and the extraction and purification of VZV DNA have been described previously (9, 11). VZV antibody in human sera was detected by enzyme immunoassay (7).

**Human tissue.** Trigeminal ganglia were aseptically removed within 24 h after death, washed twice in Dulbecco's modified Eagle's medium, cut into two unequal portions, and quick-frozen in liquid nitrogen.

**Nucleic acid extraction.** Total DNA was extracted from a small piece of each of the two trigeminal ganglia from all 10 subjects as described previously (15). From each of the 10 subjects, the two large pieces of frozen trigeminal ganglia were pooled, and total RNA was extracted with guanidine isothiocyanate–acid-phenol followed by treatment with RNase-free DNase, as described previously (4). Poly(A)$^+$ RNA was selected from the total human trigeminal ganglionic RNA by affinity chromatography on oligo(dT)-cellulose spin columns (Clontech, Palo Alto, Calif.).

**Oligonucleotide primers.** Synthetic oligonucleotide primers (Operon, Inc., Alameda, Calif.) for various regions of the VZV genome (6), human β-actin (17), and lambda-ZAP (Strat-

TABLE 1. Oligonucleotide primers

| Specificity | Name | Sequence (5′ to 3′) | Location | Orientation[a] |
|---|---|---|---|---|
| VZV gene: | | | | |
| 17 | 17-GSP-1 | TCATCCTCCCCATTGGATAA | −241[b] | Sense |
| | 17-GSP-2 | GCAAAATACGGCAGACCCATA | −130[b] | Sense |
| 18 | 18-GSP-1 | AATCGTTTATCACTGTGCCCGC | −286[b] | Sense |
| | 18-GSP-2 | GATTCGGACTTTCCACTTGCA | −5[b] | Antisense |
| 19 | 19-GSP-1 | TGGTACAGGTTAATTCGCCTCC | −213[b] | Sense |
| | 19-GSP-2 | CGTCTGGGCGTTCCTCAATAAA | −153[b] | Antisense |
| 20 | 20-GSP-1 | CCTCTAACCAAATAGTTGGCACGT | −246[b] | Sense |
| | 20-GSP-2 | ACTTCCCACACAATTATCCTG | −156[b] | Sense |
| 21 | 21-GSP-1 | ACAAGGCAGCAGTTTCATTCG | −266[b] | Sense |
| | 21-GSP-2 | CCGACGCTGATAATAGGACAA | −84[b] | Sense |
| 29 | 29-GSP-1 | AGAGACTTGGAGGAGTTACACG | −294[b] | Sense |
| | 29-GSP-2 | GCCATGACACCACATCTAACGT | −131[b] | Sense |
| 40 | 40-GSP-1 | TCACACACAATCGGATGTTGC | −430[b] | Sense |
| | 40-GSP-2 | ATACGGTGACAGGCTATACAACGGAA | −360[b] | Sense |
| | f1 | TCACACACAATCGGATGTTG | −448[b] | Sense |
| | r1 | ATCGCTTGAGCATAGTGGTG | −114[b] | Antisense |
| | r3 | ATACGGTGACAGGCTATACAACGGAA | −379[b] | Antisense |
| 62 | 62-GSP-1 | ACGACAGAGAACACGCTTTGG | −272[b] | Sense |
| | 62-GSP-2 | GACGAGGACGAGGACAACAGC | −64[b] | Antisense |
| β-Actin | Act-GSP-1 | GATGCATTGTTACAGGAAGT | −376[c] | Sense |
| | Act-GSP-2 | TCATACATCTCAAGTTGGGGG | −166[c] | Antisense |
| Lambda-ZAP | VSP-1 (UNVL)[d] | TAAAACGACGGCCAGTGAATTGT | +45[e] | Antisense |
| | VSP-2 (T7)[f] | AATACGACTCACTATAG | +28[e] | Antisense |
| | VSP-3 (REV)[g] | GGAAACAGCTATGACCATGATTACG | −96[e] | Sense |

[a] Sense, same (5′-to-3′) direction as the ORF; antisense, opposite (5′-to-3′) direction as the ORF.
[b] Location of 3′ nucleotide with respect to the ORF termination codon.
[c] Location of 3′ nucleotide with respect to the actin 3′ terminus.
[d] Universal primer on the lambda-ZAP vector.
[e] Location of 3′ nucleotide with respect to the cDNA insertion site.
[f] T7 primer on the lambda-ZAP vector.
[g] Reverse primer on the lambda-ZAP vector.

agene, La Jolla, Calif.) were selected. Table 1 gives the DNA sequences and locations of oligonucleotide primers. VZV and human β-actin primers were chosen to produce similar-size PCR products.

**PCR.** Standard 100-μl PCR mixtures contained 1 μg of DNA in 50 mM KCl–10 mM Tris-HCl (pH 8.3)–200 μM (each) dATP, dCTP, dGTP, and dTTP–1 to 5 mM $MgCl_2$. Primers were used at a final concentration of 1 μM. Samples were overlaid with mineral oil, heated to 95°C for 10 min, and cooled to 80°C before the addition of 2.5 U of *Taq* DNA polymerase (Promega, Madison, Wis.). PCR was performed in an automated DNA thermal cycler (Perkin-Elmer Cetus, Emeryville, Calif.) programmed for 40 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 2 min, and polymerization at 72°C for 3 min. The final cycle included a polymerization step at 72°C for 7 min. Amplified DNA fragments were separated by electrophoresis in 2% agarose gels, transferred to Zeta-probe membranes (Bio-Rad Laboratories, Hercules, Calif.), and detected by Southern blot hybridization to $^{32}$P-end-labeled oligonucleotide probes located internal to the amplified DNA segment.

**cDNA cloning.** Total RNA from latently infected human trigeminal ganglia was treated with RQ-1 DNase (Promega) until no amplifiable VZV DNA could be detected. Poly(A)$^+$ RNA was extracted from total RNA. Double-stranded (ds) cDNA was synthesized from 4 μg of poly(A)$^+$ RNA with 20 U of Moloney murine leukemia virus reverse transcriptase. Single-stranded DNA containing oligo(dT) and *Xho*I restriction endonuclease recognition sequences (ZAP cDNA Synthesis Kit; Stratagene) was used as a primer. To quantitate the amount and the size distribution of cDNA, 2.0 μl of [α-$^{32}$P] dATP (~3,000 Ci/mmol; Amersham, Arlington Heights, Ill.) was included in the second-strand synthesis reaction. The hemimethylated, blunt-ended ds-cDNA was ligated to *Eco*RI linkers and digested with *Xho*I. Adapted cDNAs were separated from linker fragments by chromatography on a Bio-Gel A-50 column (32 cm long; internal diameter, 0.2 cm; flow rate, 1 drop per 3 min [12]). Column fractions 14 to 26 were collected, pooled, extracted with phenol-chloroform, and ethanol precipitated. From 4 μg of poly(A)$^+$ RNA, 1.6 μg of adapted ds-cDNA was synthesized. After the size distribution was determined by agarose gel electrophoresis and autoradiography, the adapted cDNA was ligated to *Eco*RI- and *Xho*I-digested lambda-ZAP arms at 8°C for 48 h. The ligated cDNA was packaged in vitro, and the resultant phage was quantitated by plaque titration in *Escherichia coli* XL1-Blue MRF′ (Strat-

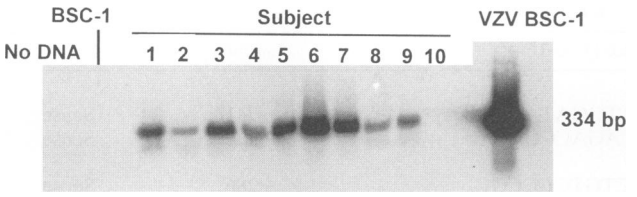


FIG. 1. Detection of VZV DNA in normal human trigeminal ganglia by PCR amplification. Ganglionic DNA (1 μg) from subjects 1 to 10 was amplified with VZV gene 40-specific primers. A 334-bp VZV gene 40-specific amplification product was detected in 9 of 10 subjects. PCR controls included no DNA and DNA extracted from uninfected (BSC-1) and VZV-infected (VZV BSC-1) BSC-1 cells.

agene) on NZY agar plates containing 5.0 mg of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) per ml and 3.0 μM IPTG (isopropyl-β-D-thiogalactopyranoside). The stock library was prepared by plate lysate propagation of the phage in *E. coli* XL1-Blue MRF′ on NZY agar plates (279 plates with diameters of 150 cm).

**Phage DNA isolation.** Recombinant phage particles were concentrated from 1.4 liters by polyethylene glycol precipitation in the presence of 1 M NaCl and banded twice on CsCl density gradients, and DNA was isolated by sodium dodecyl sulfate-pronase followed by phenol-chloroform extraction (20).

**PCR amplification and cloning of VZV-specific cDNAs.** DNA extracted from the human ganglionic cDNA library was PCR amplified by using primers specific for vector and for VZV ORFs contained within *Sal*I C (genes 17 to 21), and any amplified products were cloned into plasmid vector by TA cloning (Invitrogen, San Diego, Calif.). The unmodified PCR fragment (50 ng) was ligated to 50 ng of the plasmid pCRII overnight at 16°C and transformed into *E. coli* INVαF′ (Invitrogen). A portion of white colonies on Luria-Bertani agar plates containing 50 μg of kanamycin and 40 μg of X-Gal per ml was lysed in 50 μl of 2% Triton X-100–10 mM Tris-HCl (pH 8.0)–1 mM EDTA for 5 min at 100°C. After centrifugation at 12,000 × *g* for 5 s, 20 μl of supernatant was PCR amplified by using internal primers. The orientation of the PCR insert was determined by restriction endonuclease digestion and agarose gel electrophoresis.

**DNA sequencing.** The DNA sequences of PCR-generated fragments were determined by dideoxy-chain termination with thermostable DNA polymerase (cycle sequencing; Gibco-BRL, Gaithersburg, Md.) and affinity-purified amplified DNA (Qiagen, Inc., Chatsworth, Calif.), using internally located $^{32}$P-end-labeled oligonucleotide primers. The DNA sequences of plasmid inserts were obtained by dideoxy-chain termination (Sequenase; United States Biochemical Corp., Cleveland, Ohio).

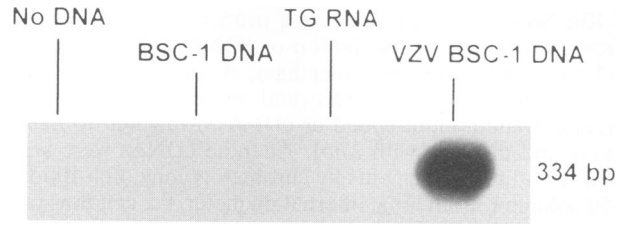


FIG. 2. Absence of VZV DNA in human trigeminal ganglionic RNA. Total RNA extracted from pooled trigeminal ganglia of subjects 1 to 10 was treated with DNase and amplified with VZV gene 40-specific primers. A 334-bp VZV gene 40-specific amplification product was detected in DNA extracted from VZV-infected BSC-1 cells (VZV BSC-1 DNA) but not in that extracted from DNase-treated pooled ganglionic RNA (TG RNA), from uninfected BSC-1 cells (BSC-1 DNA), or when DNA was not added to the reaction mixture (No DNA).

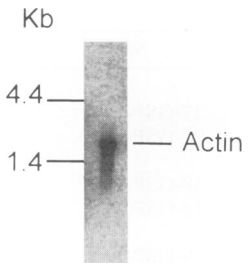


FIG. 3. Northern blot analysis of poly(A)$^+$ human trigeminal ganglionic RNA. Poly(A)$^+$ RNA was extracted from pooled ganglionic total RNA from subjects 1 to 10 and probed for β-actin transcripts. A discrete β-actin-specific 2.2-kb band was detected. Mobilities of size markers are indicated on the left.

## RESULTS

**Identification of human trigeminal ganglia latently infected with VZV and isolation of mRNA.** Table 2 gives the age, sex, VZV serological status, and underlying disease for the 10 subjects whose ganglia were used in these studies. None of the subjects was immunocompromised before death, and there were no skin lesions characteristic of recent varicella or zoster at autopsy. Total DNA was extracted from a small portion of each trigeminal ganglion from each individual, and 1 μg of DNA from each ganglion was analyzed by PCR for VZV DNA. A 334-bp DNA segment within VZV gene 40 was amplified in trigeminal ganglionic DNA from 9 of the 10 subjects (Fig. 1). In three separate amplifications, VZV gene 40 DNA was detected in trigeminal ganglia of subjects 1 to 9; the ganglia of subject 10 revealed VZV gene 40-specific DNA in two of three amplifications.

The larger portions of frozen trigeminal ganglia from each of the 10 subjects were pooled, and total RNA was extracted

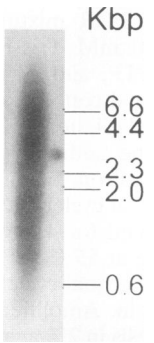


FIG. 4. Size distribution of in vitro-synthesized trigeminal human ganglionic cDNA. In vitro-synthesized cDNA was resolved on a neutral agarose gel and detected by autoradiography. After adaption of the cDNA termini and fractionation on a Bio-Gel A-50 column, the cDNAs ranged in size from approximately 500 bp to >7 kbp. Lambda DNA digested with *Hin*dIII served as size markers.

TABLE 2. Subject history[a]

| Subject no. | Age/sex[b] | Cause of death |
|---|---|---|
| 1 | 53/M | Amyloidosis |
| 2 | 54/M | Chronic renal disease |
| 3 | 83/F | Metastatic cancer |
| 4 | 70/M | Cirrhosis |
| 5 | 63/M | Emphysema |
| 6 | 67/F | Congestive heart failure |
| 7 | 66/F | Myocardial infarction |
| 8 | 61/M | Esophageal cancer |
| 9 | 79/F | Alzheimer's disease |
| 10 | 81/F | Cerebrovascular disease |

[a] Subjects 1 through 9 were VZV antibody positive; VZV antibody status was not available for subject 10.
[b] M, male; F, female.

and treated with DNase. No VZV DNA was amplifiable after DNase treatment of RNA extracted from the pooled human ganglia (Fig. 2). Poly(A)$^+$ RNA was extracted from the pooled ganglionic RNA, free of latent VZV DNA, and the integrity of the poly(A)$^+$ RNA was determined by Northern blot analysis. A discrete 2.2-kb band was visualized after hybridization of poly(A)$^+$ RNA to a human β-actin probe (Fig. 3).

**cDNA library construction.** Poly(A)$^+$ RNA from pooled latently infected human trigeminal ganglia was used to construct the cDNA library. Figure 4 shows the size distribution of in vitro-synthesized ds-cDNA after ligation to *Eco*RI linkers, *Xho*I digestion, and column chromatography. From the initial 4 μg of poly(A)$^+$ RNA, 1.6 μg of adapted ds-cDNA, ranging in size from 500 bp to >7 kbp, was synthesized. In vitro packaging of the adapted ds-cDNA ligated to lambda-ZAP resulted in a primary library consisting of $10.4 \times 10^6$ recombinant phage. Nearly all (>99.6%) phage contained inserts as determined by blue-white plaque screening on NZY agar plates containing X-Gal and IPTG. PCR amplification was performed on 20 randomly selected plaques from the primary library by using vector-specific oligonucleotide primers flanking the cDNA insertion site (VSP-1 and VSP-3; Table 1). Most cDNA inserts ranged in size from approximately 800 bp to >3 kb (Fig. 5).

To stabilize the in vitro-packaged recombinant phage, the primary library was propagated in *E. coli* (XL1-Blue MRF') to yield a stock cDNA library containing approximately $10 \times 10^{12}$ phage. To determine the quality of the cDNA library, the relative proportion of recombinant phage containing β-actin transcripts was determined by nucleic acid hybridization. The detection, in duplicate, of approximately 100 plaques in phage lifts containing 50,000 plaques after probing for β-actin transcripts indicated that the human ganglionic cDNA library contained about 0.2% β-actin cDNA transcripts.

**Identification of a VZV transcript within *Sal*I C in the cDNA library.** Total DNA extracted from $5.2 \times 10^{12}$ phage from the cDNA library was PCR amplified with primers specific for the predicted ORFs within the *Sal*I C fragment of VZV DNA (gene 17, 18, 19, 20, or 21) in combination with a vector-specific primer. Figure 6A shows the locations and orientations of the five ORFs contained within the 12.5-kbp *Sal*I C fragment of VZV DNA; Fig. 6B schematically shows the 3' ends of the putative cDNA inserts from these ORFs within the library. Amplification using primers specific for vector with and without various VZV gene-specific primers revealed the presence of VZV gene 21 but not VZV genes 17 to 20 (Fig. 7). In DNA extracted from the cDNA library, VZV gene 21-specific sequences could be amplified only by the use of a vector-specific primer and a VZV gene 21-specific primer; no VZV gene 21-specific sequences were amplified from DNA extracted

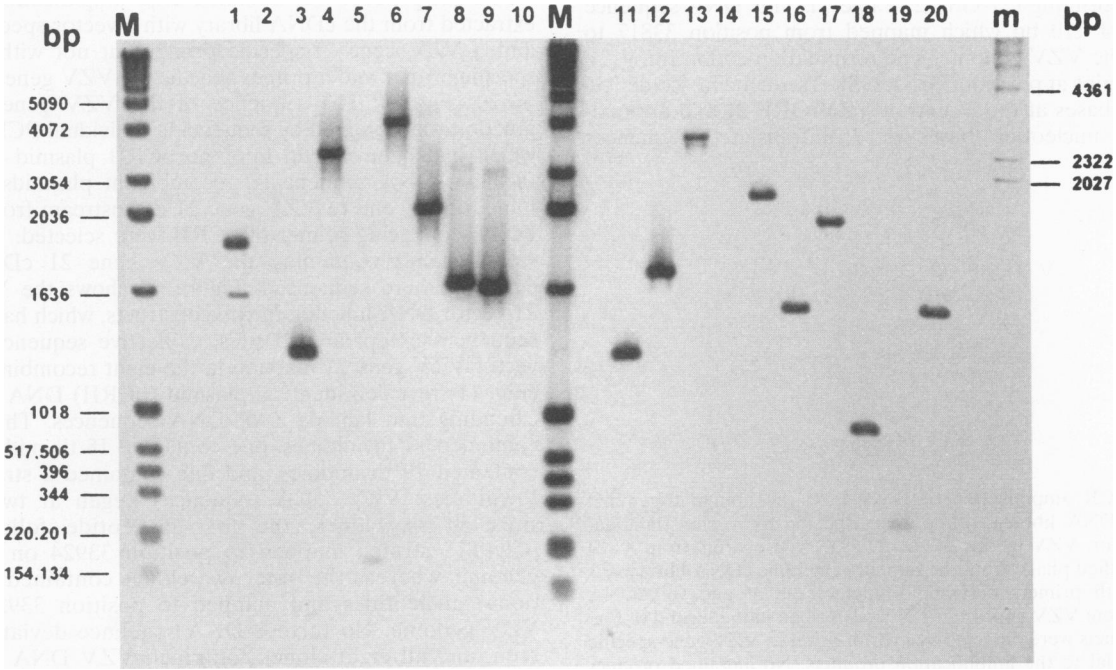


FIG. 5. Size distribution of human trigeminal ganglionic cDNA inserts. Phage DNA from 20 randomly selected clear plaques (lanes 1 to 20) on NZY agar plates containing X-Gal and IPTG was amplified by using primers flanking the cDNA insert. PCR products representing cDNA inserts from approximately 800 bp to >3 kb were resolved by agarose gel electrophoresis and photographed after ethidium bromide staining. Size markers included a 1-kb ladder (lanes M) and lambda DNA digested with *Hin*dIII (lane m).

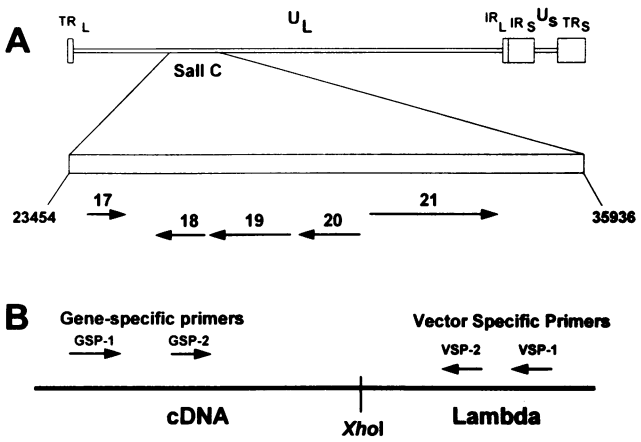


FIG. 6. Locations and directions of predicted ORFs within the *Sal*I C fragment of VZV DNA and PCR primers (virus and vector specific) used to detect virus cDNA inserts in the cDNA library. (A) The VZV genome consists of two covalently linked segments, a unique long ($U_L$) segment and a unique short ($U_S$) segment, bounded by inverted repeat sequences ($IR_L/TR_L$ and $IR_S/TR_S$). The expanded 12.5-kbp *Sal*I C fragment of VZV DNA and the locations and directions of five predicted ORFs are shown. (B) Junction of cDNA and lambda vector at the *Xho*I site and primers specific for VZV (GSP-1) and vector (VSP-1) used for PCR amplification of DNA extracted from the cDNA library. The DNA sequence of the PCR product was obtained by using internal primers specific for the VZV cDNA insert (GSP-2) and for vector (VSP-2).

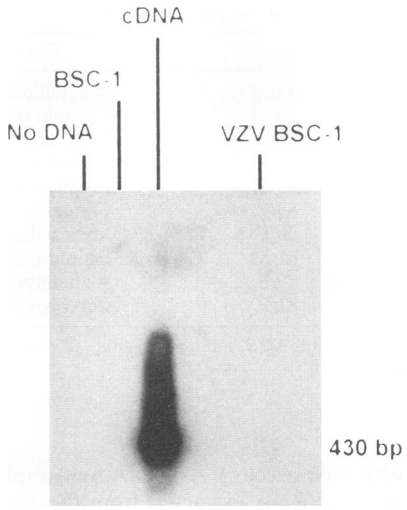


FIG. 8. Specificity of PCR amplification used to detect VZV gene 21 in the human trigeminal ganglionic cDNA library. DNAs extracted from BSC-1 cells, VZV-infected BSC-1 cells, and the human ganglionic cDNA library were amplified with primers specific for vector and VZV gene 21, and the PCR products were probed for VZV gene 21-specific DNA by hybridization. Amplification of a 430-bp DNA fragment was detected in DNA extracted from the lambda-ZAP cDNA library (cDNA) but not in DNA extracted from uninfected (BSC-1) or VZV-infected (VZV BSC-1) BSC-1 cells or when DNA was not added to the PCR (No DNA).

from VZV-infected or uninfected BSC-1 cells or when DNA was omitted from the PCR mixture (Fig. 8).

**DNA sequence of the 3′ end of VZV gene 21 from a cDNA library.** Figure 9A shows the DNA sequence obtained by dideoxy-chain termination of the PCR product after VZV gene 21-specific priming (21-GSP-2; Table 1). This DNA sequence consisted of 110 bp which mapped from position 33817 to 33927 on the VZV genome. The termination codon for VZV ORF 21 begins at position 33872; GSP-2 sequencing yielded 55 nucleotide bases at the 3′ end of VZV ORF 21 and approximately 50 nucleotide bases of 3′-untranslated sequences

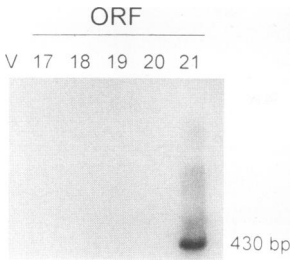


FIG. 7. PCR amplification of DNA from the human trigeminal ganglionic cDNA library with primers specific for vector (lambda-ZAP) and for VZV genes 17 to 21. DNA prepared from CsCl gradient-purified phage from the human ganglionic cDNA library was amplified with primers designed to detect the 3′ ends of cDNA transcripts from VZV genes 17 to 21 as described in the legend to Fig. 6. PCR products were detected by hybridization to VZV gene-specific probes internal to the amplification products. No amplified product was detected after PCR with a vector-specific primer pair (V) or primers specific for vector and VZV gene 17, 18, 19, or 20 (lanes 17 to 20, respectively). A 430-bp DNA fragment was detected after PCR with primers specific for vector and VZV gene 21 (lane 21).

(UTL). The poly(A)$^+$ stretch starts directly after the UTL. The DNA sequence obtained by dideoxy-chain termination of the PCR product after vector-specific priming (VSP-2; Table 1) consisted of the *Xho*I cDNA insertion site extending into a poly(T)$^+$ stretch (Fig. 9B).

A PCR product was obtained after amplification of DNA extracted from the cDNA library with a vector-specific primer and a VZV gene 21-specific primer but not with a vector-specific primer and a primer specific for VZV gene 17, 18, 19, or 20 (Fig. 7). The sequence of the VZV gene 21-vector junction was obtained by sequencing individual PCR products which had been cloned into the pCRII plasmid vector. To facilitate DNA sequencing, recombinant plasmids that contained the 3′ end of VZV gene 21 downstream from the SP6 DNA sequencing primer of pCRII were selected. Eight individual clones containing the VZV gene 21 cDNA-vector construct were sequenced. Figure 10 shows the VZV gene 21-vector DNA junction of two constructs, which had identical sequences except at two sites. Collective sequencing of the vector-VZV gene 21 junction in the eight recombinant clones (Fig. 11) revealed identical plasmid (pCRII) DNA sequences extending into lambda-ZAP DNA sequences. Three clones contained 17 thymidines, one contained 18 thymidines, three contained 19 thymidines, and one contained a stretch of 20 thymidines. VZV DNA sequences began at two distinct sites; in six clones, the first nucleotide following the poly(T)$^+$ stretch mapped to position 33924 on the VZV genome, whereas the other two clones contained two additional nucleotides and mapped to position 33926 on the VZV genome. No further DNA sequence deviations were seen, and all eight clones contained VZV DNA which did not diverge from genomic sequences to the extent of analysis (position 33671).

**Relative abundance of VZV gene 21 cDNA in the human ganglionic cDNA library.** Figure 12 shows that compared with

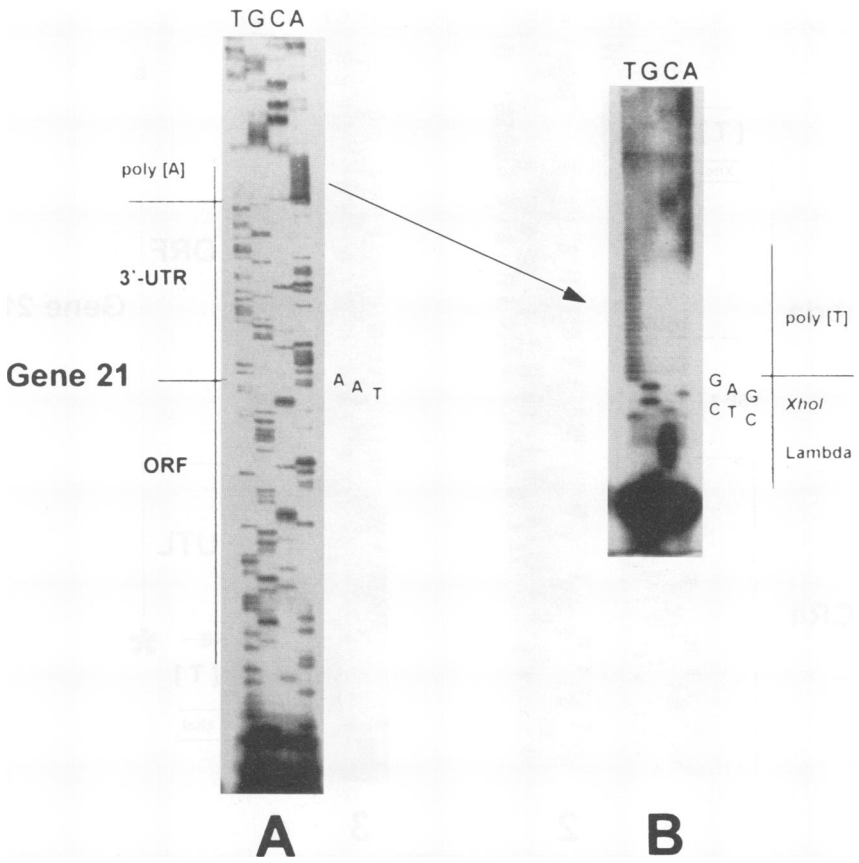


FIG. 9. Identification of the 3′ end of VZV gene 21-specific sequences in the human trigeminal ganglionic cDNA library. The VZV gene 21-specific PCR product shown in Fig. 8 was sequenced with two primers. (A) VZV gene 21-specific sequences with a primer internal to VZV gene 21 (GSP-2). (B) Vector-specific sequences with a primer internal to vector (VSP-2). DNA sequencing mapped the PCR product to the 3′ end of VZV gene 21, extending approximately 55 nucleotide bases into the 3′-UTL. Also shown is a poly(A)$^+$ stretch of approximately 20 nucleotide bases and the *Xho*I cDNA cloning site located on lambda-ZAP.

those of VZV gene 21, actin transcripts are approximately 10,000 times more abundant in the human ganglionic cDNA library, as determined by PCR on serial 10-fold dilutions of DNA extracted from the cDNA library.

**PCR amplification of DNA from the human ganglionic cDNA library for VZV gene 29 and 62 gene transcripts.** Transcripts expressed from VZV genes 29 and 62 have been identified by Northern blot analysis of RNA from latently infected human ganglia (16). To determine whether our cDNA library contained transcripts from these genes, DNA extracted from the human ganglionic cDNA library was amplified with either a VZV gene 29- or 62-specific primer and a vector-specific primer. At a sensitivity sufficient to detect VZV gene 21 cDNA transcripts, no cDNA transcripts from VZV gene 29 or VZV gene 62 were detected (Fig. 13). To demonstrate the ability of the PCR primers to amplify transcripts specific for VZV ORFs 17, 18, 19, 20, 21, 29, 40, and 62 along with cellular β-actin transcripts, a lambda-based cDNA library was constructed from poly(A)$^+$ RNA extracted from VZV-infected BSC-1 cells. DNA extracted from this cDNA library was used in PCR to amplify the 3′ end of each gene by using primer sets which combined a gene-specific primer with a lambda-specific primer. All PCR primer sets yielded amplification products specific for the ORFs amplified.

## DISCUSSION

This report describes the construction of a cDNA library prepared from human trigeminal ganglia latently infected with VZV and the detection of a VZV transcript within the library. Latently infected human ganglia were identified by PCR amplification with VZV gene 40-specific primers. The detection of amplifiable VZV DNA in each of 10 subjects confirms our earlier finding of VZV DNA in trigeminal ganglia from 13 of 15 individuals (15).

Since the small size of human ganglia precludes abundant RNA yields and since the amount of VZV DNA in latently infected ganglia is small (14), we prepared a cDNA library by using RNA extracted from these ganglia. Before cDNA synthesis, RNA extracted from ganglia was treated with DNase until no VZV DNA could be detected by PCR amplification. The cDNA library constructed from pooled human trigeminal ganglionic poly(A)$^+$ RNA contained β-actin transcripts at a frequency of 0.2%, corresponding favorably to the abundance of actin transcripts in human brain cDNA libraries (0.6%) (1) and in adult human thymus (0.11%) (18).

Because latent VZV DNA is present in only ~0.01% of ganglionic cells (15), we used PCR amplification to detect low-abundance VZV transcripts in the cDNA library. VZV

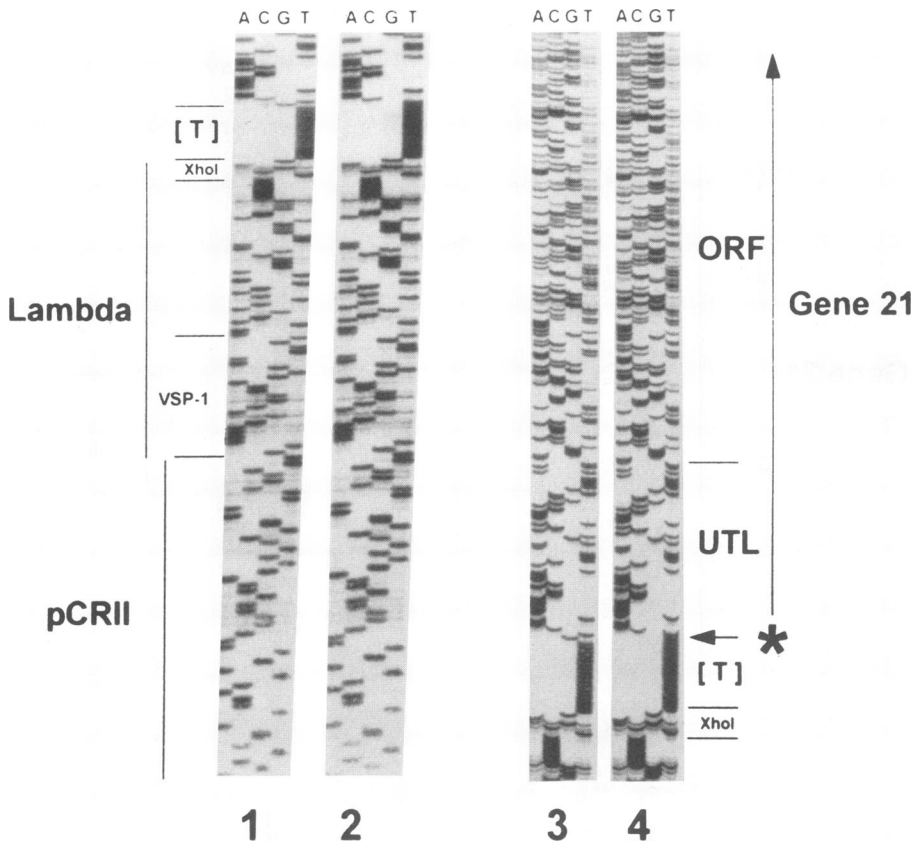


FIG. 10. DNA sequence analysis of the 3′ end of latently expressed VZV gene 21. The PCR product shown in Fig. 8 was inserted into the plasmid vector pCRII. (1 and 2) DNA sequences of two recombinants; (3 and 4) extensions of panels 1 and 2, respectively. The two recombinants show identical plasmid (pCRII) and lambda sequences. Panel 3 shows 17 thymidines, and panel 4 shows 20 thymidines (T). An asterisk indicates an additional 2 nucleotides at the 3′ end of VZV gene 21 that are seen in panel 3 and not in panel 4. The remaining VZV gene 21 DNA sequences for both clones are identical and include the VZV gene 21 UTL and ORF.

genes 29 and 62 and some or all of the *Sal*I C fragment of VZV DNA have been shown to be transcribed in latently infected ganglia (4, 16); therefore, we designed PCR primers to amplify cDNA constructs from these VZV genes. No amplification was detected with primers specific for vector and primers specific for VZV gene 17, 18, 19, 20, 29, or 62. However, the combination of a vector-specific primer and a VZV gene 21-specific primer yielded an amplification product which displayed the 3′ end of VZV gene 21, as determined by sequencing individual constructs containing the cloned PCR-

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----------------------pCRII----------------------/-------
gagctcgatccactagtaacggccgccagtgtgctggaattcggcttgtaaaacg

----------------------lambda----------------------------
acggccagtgaattgtaatacgactcactatagggcgaattgggtaccgggcccc

--XhoI--/<-----------VZV gene 21----3′-UTL-------------
ccctcgag[TTT...TTT]n(GT)CAATAAAAACCACAAATACTTTAATGTACAT

-------------------------------------ORF----------------
AAACACGCCTGTTTTATATTAAGGGTCACTCCCACTTGTATTCCCGGGTACGCCA

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AGACGCAAATCTACATTAGATAAAATCTCATCTGGACGAAGCCATCTTTGTCCTA

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TTATCAGCGTCG
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FIG. 11. Composite DNA sequence at the junction of VZV gene 21 and vector from eight clones. All clones showed identical pCRII (lowercase) and lambda DNA (lowercase italic) sequences. The number of thymidines varied from 17 to 20. Two clones contained an additional 2 bases (parentheses) in the 3′-UTL of VZV gene 21. The termination codon for ORF 21 is underlined, and the direction of translation is marked by an arrow.

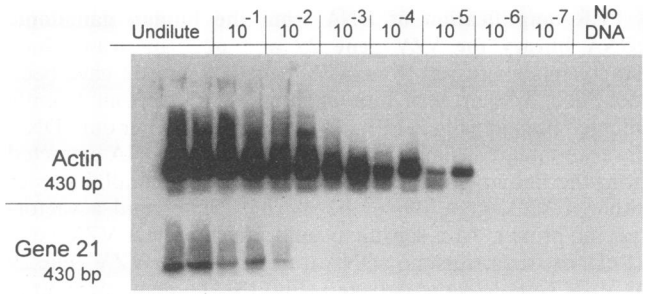


FIG. 12. Relative abundance of VZV gene 21 and actin cDNAs in the human ganglionic cDNA library. DNA extracted from the human ganglionic cDNA library was serially diluted 10-fold, and equal amounts were amplified with primers specific for vector (VSP-1; Table 1) and either human β-actin (Act-GSP-1; Table 1) or VZV gene 21 (21-GSP-1; Table 1). Gene-specific amplification was detected by probing with internally located primers specific for β-actin or VZV gene 21. Undiluted cDNA samples contained 1.4 μg of DNA. Actin-specific amplification was detected at a dilution of $10^{-5}$, and VZV gene 21-specific amplification was detected at a dilution of $10^{-1}$.

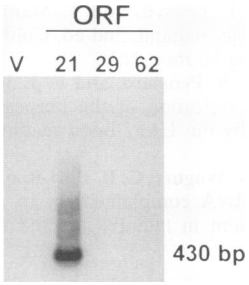


FIG. 13. PCR amplification of DNA from the human ganglionic cDNA library to detect VZV gene 29 and 62 transcripts. DNA from the human ganglionic cDNA library was amplified with primers specific for vector (VSP-1; Table 1) and either VZV gene 21, VZV gene 29, or VZV gene 62 (lanes 21, 29, and 62, respectively). Amplified DNA was detected by nucleic acid hybridization. No amplification was detected with a vector-specific primer pair (V) or a vector-specific primer in combination with either a VZV gene 29- or a VZV gene 62-specific primer; VZV-specific amplification was detected only when primers specific for vector and VZV gene 21 were used.

amplified DNA. All clones contained the predicted plasmid and lambda DNA sequences leading into a poly(A)$^+$ stretch of 17 to 20 residues. The major site of poly(A)$^+$ addition was at +49 with respect to the end of the VZV gene 21 ORF, and a minor site of poly(A)$^+$ addition was located at +51. No divergence from the VZV genomic DNA sequence (6) was seen in the 153-bp sequence of the VZV gene 21 cDNA obtained from the cDNA library.

The identification of a VZV transcript in the cDNA library from latently infected human ganglia not only confirms our previous detection of a VZV transcript within the *Sal*I C region of VZV DNA (4) but also shows that the region of *Sal*I C which is transcribed contains VZV gene 21 sequences. This same human ganglionic cDNA library revealed no transcripts for VZV genes 17 to 20, 29, or 62. Variations in the relative abundance or efficiency of cDNA synthesis of different latent VZV transcripts could account for the detection of VZV gene 21 but not gene 29 or 62 clones in the cDNA library. Also, the various primers may differ in PCR efficiency. In-depth analysis of cDNA synthesis and PCR efficiencies would involve reconstruction reactions in which known amounts of in vitro-synthesized VZV gene transcripts are added to a constant amount of control mRNA, cDNA is synthesized, and the limit of sensitivity is detected by PCR. Such determinations may explain the lack of detection of VZV genes 29 and 62 in the cDNA library. Furthermore, we cannot compare our detection of VZV gene 21 transcripts in the cDNA library with results of a previous study that identified multiple regions of the VZV genome transcribed during latency after in situ hybridization to sections of human ganglia (5); since that study used pooled VZV-specific probes, it was not possible to identify a specific region of VZV transcribed in latently infected ganglia.

The presence of a VZV-specific polyadenylated transcript in human ganglia is novel among neurotropic α-herpesviruses. Herpes simplex virus type 1 (HSV-1), HSV-2, bovine herpesvirus type 1, and pseudorabies virus encode non-polyadenylated latency-associated transcripts. These transcripts accumulate in neurons of latently infected ganglia and have been mapped to the DNA strand opposite to the one encoding an immediate-early protein (3, 8, 13, 19, 22). Although a VZV gene 21-specific product has not been identified, VZV gene 21 is homologous (47%) to HSV-1 UL37 at the amino acid level (21). The putative HSV-1 homolog of VZV gene 21 encodes a phosphorylated late protein which complexes with the major HSV-1 DNA-binding protein (ICP8) (2). The detection of a polyadenylated, VZV gene 21-specific transcript in latently infected human ganglia provides a foundation to further analyze VZV gene expression during latency.

## ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grants AG 06127, NS 32623, and NS 07321 from the National Institutes of Health.

We are grateful to the Departments of Pathology at University Hospital, the Veterans Administration Hospital, and the Presbyterian-St. Luke's Medical Center in Denver for allowing us access to autopsy material. We thank B. Forghani for performing the enzyme immunoassays, Marina Hoffman for editorial review, and Cathy Allen for preparation of the manuscript.

## REFERENCES

1. **Adams, M. D., M. Dubnick, A. R. Kerlavage, R. Moreno, J. M. Kelley, T. R. Utterback, J. W. Nagle, C. Fields, and J. C. Venter.** 1992. Sequence identification of 2,375 human brain genes. Nature (London) **355**:632–634.
2. **Albright, A. G., and F. J. Jenkins.** 1993. The herpes simplex virus UL37 protein is phosphorylated in infected cells. J. Virol. **67**:4842–4847.
3. **Bratanich, A. C., N. D. Hanson, and C. J. Jones.** 1992. The latency-related gene of bovine herpesvirus 1 inhibits the activity of immediate-early transcription unit 1. Virology **191**:988–991.
4. **Cohrs, R., R. Mahalingam, A. N. Dueland, W. Wolf, M. Wellish, and D. H. Gilden.** 1992. Restricted transcription of varicella-zoster virus in latently infected human trigeminal and thoracic ganglia. J. Infect. Dis. **166**:S24–S29.
5. **Croen, K. D., J. M. Ostrove, L. J. Dragovic, and S. E. Straus.** 1988. Patterns of gene expression and sites of latency in human nerve ganglia are different for varicella-zoster and herpes simplex viruses. Proc. Natl. Acad. Sci. USA **85**:9773–9777.
6. **Davison, A. J., and J. E. Scott.** 1986. The complete DNA sequence of varicella-zoster virus. J. Gen. Virol. **67**:1759–1786.
7. **Forghani, B., K. W. Dupuis, and N. J. Schmidt.** 1984. Varicella-zoster viral glycoproteins analyzed with monoclonal antibodies. J. Virol. **52**:55–62.
8. **Fraser, N. W., T. M. Block, and J. G. Spivack.** 1992. The latency-associated transcripts of herpes simplex virus: RNA in search of function. Virology **191**:1–8.
9. **Gilden, D. H., A. R. Hayward, J. Krupp, M. Hunter-Laszlo, J. C. Huff, and A. Vafai.** 1987. Varicella zoster virus infection of human mononuclear cells. Virus Res. **7**:117–129.
10. **Gilden, D. H., R. Mahalingam, A. N. Dueland, and R. Cohrs.** 1992. Herpes zoster: pathogenesis and latency. Prog. Med. Virol. **39**:10–75.
11. **Gilden, D. H., Y. Shtram, and A. Friedmann.** 1982. Extraction of cell-associated varicella zoster virus DNA with Triton X-100-NaCl. J. Virol. Methods **4**:263–275.
12. **Huynh, T. V., R. A. Young, and R. W. David.** 1985. Constructing and screening cDNA libraries in λgt10 and λgt11, p. 49–77. *In* D. M. Glover (ed.), DNA cloning, a practical approach, vol. 1. IRL Press, Washington, D.C.
13. **Kutish, G., T. Mainprize, and D. Rock.** 1990. Characterization of the latency-related transcriptionally active region of bovine herpesvirus 1 genome. J. Virol. **64**:5730–5737.
14. **Mahalingam, R., M. Wellish, D. Lederer, B. Forghani, R. Cohrs, and D. Gilden.** 1993. Quantitation of latent varicella-zoster virus DNA in human trigeminal ganglia by polymerase chain reaction. J. Virol. **67**:2381–2384.
15. **Mahalingam, R., M. Wellish, W. Wolf, A. N. Dueland, R. Cohrs, A. Vafai, and D. H. Gilden.** 1990. Latent varicella-zoster viral DNA in human trigeminal and thoracic ganglia. N. Engl. J. Med. **323**:627–631.
16. **Meier, J. L., R. P. Holman, K. D. Croen, J. E. Smialek, and S. E. Straus.** 1993. Varicella-zoster virus transcription in human trigeminal ganglia. Virology **193**:193–200.

17. **Nakajima-Iijima, S., H. Hamada, P. Reddy, and T. Kakunaga.** 1985. Molecular structure of the human cytoplasmic beta-actin gene: interspecies homology of sequences in the introns. Proc. Natl. Acad. Sci. USA **82:**6133–6137.
18. **Patanjali, S. R., S. Parimoo, and S. M. Weissman.** 1991. Construction of a uniform-abundance (normalized) cDNA library. Proc. Natl. Acad. Sci. USA **88:**1943–1947.
19. **Priola, S. A., D. P. Gustafson, E. K. Wagner, and J. G. Stevens.** 1990. A major portion of the latent pseudorabies virus genome is transcribed in trigeminal ganglia of pigs. J. Virol. **64:**4755–4760.
20. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Shelton, L. S., M. N. Pensiero, and F. J. Jenkins.** 1990. Identification and characterization of the herpes simplex virus type 1 protein encoded by the UL37 open reading frame. J. Virol. **64:** 6101–6109.
22. **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.