JOURNAL OF VIROLOGY, May 1996, p. 2684–2690 Vol. 70, No. 5
0022-538X/96/$04.00+0

A Novel Herpes Simplex Virus 1 Gene, U_L43.5, Maps Antisense to the U_L43 Gene and Encodes a Protein Which Colocalizes in Nuclear Structures with Capsid Proteins

PATRICIA L. WARD, DAVID E. BARKER, AND BERNARD ROIZMAN*

The Marjorie B. Kovler Viral Oncology Laboratories, The University of Chicago, Chicago, Illinois 60637

Received 5 December 1995/Accepted 19 January 1996

An open reading frame mapping antisense to the U_L43 gene of herpes simplex virus 1 encodes a protein with an apparent M_r of 38,000. The protein was detected in wild-type-infected cells with rabbit monospecific polyclonal antibody directed against a fusion protein containing all of the sequences encoded by the open reading frame. The antibody did not react with mutants from which the open reading frame was deleted. Expression of this gene, designated U_L43.5, was grossly decreased or abolished in infected cells incubated in medium containing inhibitory concentrations of phosphonoacetic acid, suggesting that it is regulated as a γ gene. U_L43.5 is dispensable in cell culture. U_L43.5 protein colocalized with the major capsid protein (infected cell protein 5) and the capsid scaffolding proteins (infected cell protein 35) in nuclear structures situated at the periphery of the nucleus. The predicted amino acid sequence indicates that the U_L43.5 protein is a highly hydrophilic protein. The colocalization of U_L43.5 protein with capsid proteins in discrete nuclear structures suggests that the former may be involved in assembly of viral particles in an accessory role in cells in culture.

The 152+-kbp herpes simplex virus 1 (HSV-1) DNA genome consists of two covalently linked components, L (large) and S (small). The L component consists of a unique sequence, U_L, flanked by inverted repeats *ab* and *b′a′*, whereas the S component consists of a unique sequence, U_S, flanked by inverted repeats *a′c′* and *ca*. Available data indicate that the genome contains 84 open reading frames (ORFs) encoding proteins. Of these, 60 map in U_L, 14 map in U_S, 4 map in each of the repeats flanking U_L, and one maps in each of the repeats flanking U_S. In addition, a number of transcripts that are expressed either during the viral lytic cycle or during latency, but for which no proteins have as yet been identified, have been found. This report concerns a region of the genome mapping between map units 0.6 and 0.65. McGeoch et al. (23) assigned to this region an ORF designated U_L43, but no protein product that corresponds to the U_L43 ORF has yet been reported. This ORF is antisense to two transcripts reported earlier by Frink et al. (15) as arising from this region. The two transcripts were reported as migrating as a doublet in denaturing gels, and therefore they were thought to be colinear but to differ in their 5′ termini. The 5′ ends of these transcripts mapped to the right of map unit 0.621 and upon in vitro translation yielded a polypeptide with an apparent M_r of approximately 85,000. The properties of the U_L43 ORF will be described elsewhere (8b). This report concerns one ORF mapping antisense of U_L43. To differentiate this ORF from U_L43 as described by McGeoch et al. (23), we have designated it U_L43.5.

We report the existence of a HSV-1 protein recognized by a polyclonal antiserum made against an amino acid sequence encoded by an ORF antisense to the U_L43 gene. This protein, designated U_L43.5, is regulated as a γ gene. U_L43.5 protein colocalized in nuclei with infected cell protein 5 (ICP5), the major capsid protein, and ICP35, the capsid scaffolding protein, in structures distinct from those associated with viral DNA synthesis. U_L43.5 protein appears to be an accessory protein associated with structures involved in capsid assembly.

* Corresponding author. Mailing address: The Marjorie B. Kovler Viral Oncology Laboratories, The University of Chicago, 910 E. 58th St., Chicago, IL 60637. Phone: (312) 702-1898. Fax: (312) 702-1631.

MATERIALS AND METHODS

Cells and viruses. HSV-1 strain F [HSV-1(F)] is the prototype strain used in our laboratories (13). HSV-1(F)Δ305 is a recombinant virus from which 500 bp had been deleted in the thymidine kinase (*tk*) gene (27). Recombinant viruses described in this report were derived from HSV-1(F)Δ305. Viral stocks of HSV-1(F) and HSV-1(F)Δ305 were made in HEp-2 cells; stocks of recombinant viruses were made and their titers were determined in Vero cells. Transfections were done in rabbit skin cells originally obtained from J. McClaren. Selection of tk^- and tk^+ viruses were done in 143TK$^-$ cells originally obtained from Carlo Croce. The cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with either 5% newborn calf serum (rabbit skin, Vero, or HEp-2 cells) or 5% fetal calf serum (143TK$^-$ cells). R7032 lacks the entire U_S8 gene, which encodes glycoprotein E (gE) (24); it was used in immunofluorescence studies to avoid nonspecific immunofluorescence caused by binding of immunoglobulin G (IgG) to the Fc receptor expressed by gE.

Nucleotide numbers refer to the published sequence of strain HSV-1(17)*syn*$^-$ (23) and such subsequent corrections as they appear in GenBank.

Plasmids. Restriction endonucleases were obtained from New England Biolabs, Beverly, Mass. T4 DNA ligase was obtained from U.S. Biochemical, Cleveland, Ohio. pRB172 contains the 6.6-kb HSV-1 *Bam*HI I fragment cloned into the *Bam*HI sites of pGEM3Z vector (Promega). pRB172 was digested with *Pst*I, and the 1.8-kbp fragment containing the U_L43.5 ORF (Fig. 1, line 2) was ligated into the *Pst*I site of the expression vector pMal-c (Promega) such that the U_L43.5 coding sequence was in frame with the gene encoding the bacterial maltose-binding protein (MBP). The resulting plasmid was designated pRB4418. pRB4061 contains the entire U_L43 and U_L44 genes in the form of a 4.9-kbp *Hpa*I-to-*Bam*HI fragment subcloned from pRB172 and ligated into pGEM3Z from which the *Eco*RI site in the polylinker had been deleted (Fig. 1, line 2). pRB4061 was digested with *Eco*RI and *Eco*RV, and the 0.9-kbp fragment was replaced with a 2.15-kbp *Bam*HI fragment derived from plasmid pRB3964 (6) containing the coding domain of the HSV-1(F) *tk* gene driven by the α27 promoter to yield pRB4062 (Fig. 1, line 3). To delete both ORFs encoded by the strand antisense to the U_L43 and U_L44 genes, pRB4061 was digested with *Nsi*I and *Eco*RV, the ends were treated with T4 polymerase, and the plasmid was religated to yield plasmid pRB4246 (Fig. 1, line 4). To delete solely the U_L43.5 ORF, pRB4061 was digested with *Hin*dIII present in the polylinker and *Mlu*I, the ends were blunted with T4 polymerase, and the plasmid was religated to make the remaining *Pst*I site in the plasmid unique. This plasmid was designated pRB4285. pRB4285 was digested with *Nsi*I and *Pst*I, and the compatible ends were religated to yield plasmid pRB4286 (Fig. 1, line 5).

Selection of recombinant viruses. Recombinant virus R7113 was derived by cotransfection of rabbit skin cells with HSV-1(F)Δ305 viral DNA along with

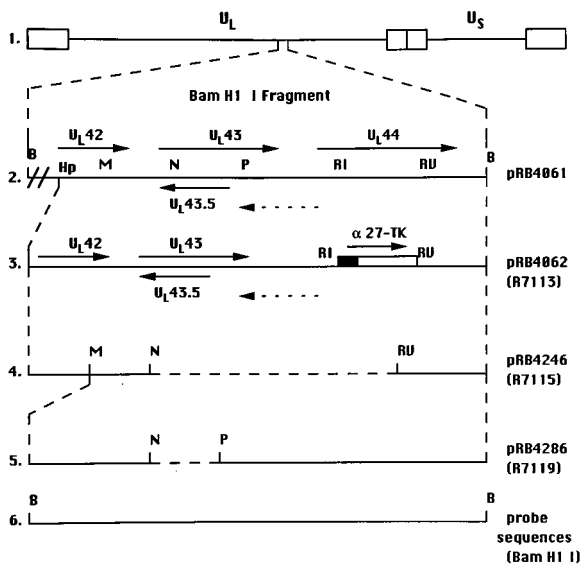


FIG. 1. Schematic representations of the sequence arrangements of HSV-1 DNA and of the various plasmids used to construct recombinant viruses in these studies. Lines 1 and 2, locations within the HSV-1(F) genome of the *Bam*HI I fragment, of the U_L42, U_L43, U_L43.5, U_L44 ORF, and of a second putative ORF (dashed line) which lies antisense to U_L43 and U_L44. Line 3, sequence arrangement after insertion of the α27-*tk* gene within the coding sequence of U_L44 as in pRB4062. This plasmid was used to construct the recombinant virus R7113. Line 4, sequence arrangement of plasmid pRB4246 used to construct the deletion mutant virus R7115, which lacks U_L43, U_L43.5, the second ORF (dashed line), and most of U_L44. Line 5, sequence arrangement of plasmid pRB4286 used to construct the deletion mutant virus R7119. This virus lacks U_L43 and U_L43.5. Line 6, diagram of probe pRB172, which contains the entire HSV-1(F) *Bam*HI I fragment. B, *Bam*HI; Hp, *Hpa*I; M, *Mlu*I; N, *Nsi*I; P, *Pst*I; RI, *Eco*RI; RV, *Eco*RV.

pRB4062 plasmid DNA and subsequent selection of tk^+ virus in Dulbecco's modified Eagle's medium containing hypoxanthine, thymidine, and aminopterin. This virus contains the α27-*tk* chimeric gene within the coding sequence of the U_L44 gene. Recombinant virus R7115 was derived by cotransfection of R7113 viral DNA with pRB4246. Recombinant virus R7119 was derived by cotransfection of R7113 viral DNA with pRB4286. tk^- progeny viruses were selected on 143TK$^-$ cells in the presence of bromodeoxyuridine as previously described (22). R7115 lacks the U_L43 and U_L44 coding sequences and the ORFs encoded by the strand antisense to these genes. R7119 lacks the U_L43 and U_L43.5 genes. After two rounds of selection in medium containing bromodeoxyuridine, the progeny were plated on Vero cells, and individual plaques were picked for further studies.

Purification of viral DNA. DNA intended for transfections was prepared from potassium acetate gradients as previously described (17). All other DNA samples were purified by phenol-chloroform extraction of cytoplasmic extracts of infected Vero cells. Nick translations were done with the aid of a kit in accordance with the recommendations of the manufacturer (DuPont, Wilmington, Del.). Separation of DNA fragments and transfer to nitrocellulose were done by the method of Southern (31) as modified by Longnecker and Roizman (20). Hybridization conditions were as previously described (18).

Production of the U_L43.5 rabbit antiserum. New Zealand White rabbits were inoculated with 250 μg of purified U_L43.5-MBP fusion protein in complete Freund's adjuvant and then given three immunizations with the fusion protein in incomplete Freund's adjuvant at 2-week intervals. The bacterial fusion protein was produced by standard methods as recommended by the manufacturer of the expression vector (Promega).

Antibodies. The polyclonal rabbit antiserum to U_L43.5 was used at dilutions of 1:1,500 for immunoblotting and 1:50 for immunofluorescence assays. The mouse monoclonal antibodies to ICP5 and ICP35 described previously (19) were purchased from Goodwin Biotechnology Inc. (Plantation, Fla.). The goat anti-rabbit Ig alkaline phosphatase-conjugated antibody was purchased from Bio-Rad Laboratories (Richmond, Calif.). The goat anti-rabbit Ig fluorescein isothiocyanate-conjugated antibody was purchased from Sigma Chemical Co. (St. Louis, Mo.). The goat anti-mouse and anti-rabbit Texas red-conjugated antibodies were purchased from Molecular Probes, Inc. (Eugene, Ore.).

Polyacrylamide gel electrophoresis and immunoblotting. Infected cell lysates were electrophoretically separated in denaturing gels consisting of 11 or 12.5% polyacrylamide cross-linked with *N,N′*-diallyltartardiamide and 0.1% sodium dodecyl sulfate (SDS), electrically transferred to nitrocellulose sheets, and reacted with antibody as previously described (32).

Immunofluorescence. Approximately 5×10^4 Vero cells were seeded onto glass slides (Cell-line Inc., Newfield, N.J.) and allowed to attach overnight. Cells were infected with 10 PFU of virus per cell and fixed in ice-cold methanol at 16 to 18 h after infection. Slides were blocked in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) for 1 h at room temperature, reacted for 2 h at room temperature with primary antibody diluted in PBS supplemented with 1% BSA, rinsed extensively with PBS, reacted for 1 h with a 1:400 dilution of anti-rabbit or anti-mouse Ig conjugated to Texas red, rinsed extensively, and mounted in 90% glycerol in PBS containing 1 mg of *p*-phenylenediamine per ml. The U_L43.5 polyclonal antiserum was used at a dilution of 1:50, and the ICP35 and ICP5 monoclonal antibodies were used at a dilution of 1:500. The slides were examined in a Zeiss confocal fluorescence microscope; digitized images of the fluorescent antibody-stained cells were acquired with software provided with the Zeiss confocal microscope and printed by a Codonics CP210 digital printer.

Cell fractionation. To prepare nuclear and cytoplasmic extracts, infected 143TK$^-$ cells were rinsed in PBS, resuspended in 1 mM phosphate buffer (pH 7.4) containing 0.25 M sucrose, and placed on wet ice for 15 m. The plasma membrane was then lysed by the addition of 0.5% Nonidet P-40; the mixture was briefly vortexed, maintained on wet ice for an additional 15 min, and then centrifuged in a tabletop Beckman centrifuge to separate the nuclei from the cytoplasm. The cytoplasmic extract was poured into a separate tube, and the nuclear pellet was gently washed twice with 1 mM phosphate buffer. Disruption buffer containing 2% SDS and 0.5% β-mercaptoethanol was added to each fraction prior to sonication and electrophoresis on denaturing polyacrylamide gels.

RESULTS

An ORF antisense to U_L43 gene is expressed. An ORF oriented right to left with respect to the prototype genomic organization lies between nucleotides 95716 and 94783 (Fig. 1,

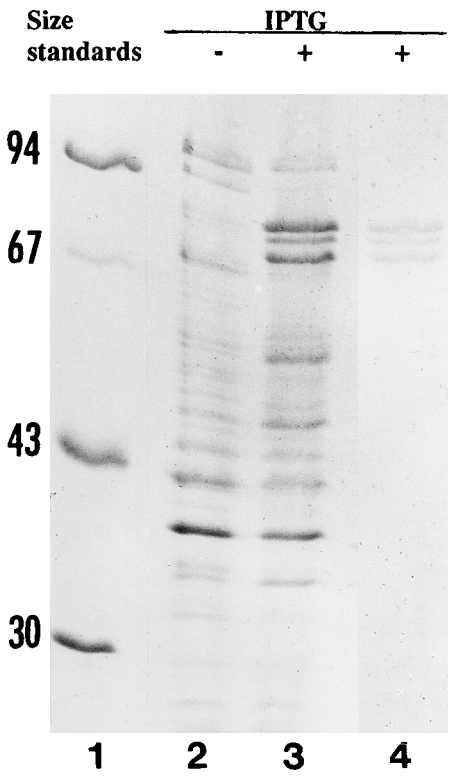


FIG. 2. Photograph of a Coomassie blue-stained polyacrylamide gel of electrophoretically separated bacterial proteins produced in *E. coli* BL21 cells transformed with the U_L43.5-MBP expression plasmid. Lane 1, molecular weight markers; lane 2, profile of bacterial proteins expressed after 2 h of growth at 37°C; lane 3, overexpression of U_L43.5-MBP following addition of IPTG; lane 4, U_L43.5-MBP purified from an amylose-coupled resin matrix. Sizes are indicated in kilodaltons.

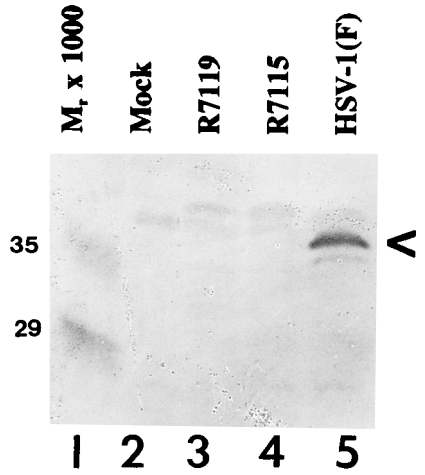


FIG. 3. Photograph of an immunoblot of electrophoretically separated lysates of 143TK$^-$ cells infected with the indicated viruses. Blots were probed with rabbit antiserum raised against U_L43.5-MBP. The arrowhead indicates the position of the U_L43.5-specific band.

line 2). We designated this ORF U_L43.5. We constructed a chimeric gene consisting of the entire U_L43.5 ORF fused to the gene encoding the bacterial MBP as described in Materials and Methods. *Escherichia coli* transformed with the U_L43-MBP plasmid and induced with isopropyl-β-D-thiogalactopyranoside (IPTG) overexpressed proteins which formed in denaturing gels three bands with apparent M_rs of 70,000, 75,000, and 80,000 (Fig. 2, lane 3). The molecular weights of the proteins in these bands roughly correspond to the expected molecular weight of a fusion protein consisting of M_r-43,000 MBP, the predicted 311-amino-acid U_L43.5 protein, and the 5′ noncoding region of the U_L43.5 gene, which would add approximately 58 additional amino acids. The overexpressed fusion protein was purified by addition of amylose-coupled resin and elution with maltose (Fig. 2, lane 4) and used as the immunogen for production of a rabbit polyclonal antiserum (see Materials and Methods). To assay for expression of U_L43.5 protein, 143TK$^-$ cells were mock infected or infected with HSV-1(F), R7119, or R7115. The lysates of cells harvested 20 h after infection or mock infection were electrophoretically separated in denaturing gels, transferred to a nitrocellulose sheet, and reacted with the U_L43.5 antiserum. As shown in Fig. 3, lane 4, the antibody reacted with a polypeptide with an apparent M_r of 38,000 produced in HSV-1(F)-infected cells. U_L43.5 protein was not detected in lysates of uninfected cells or in cells that had been infected with the deletion mutant virus R7119 or R7115 (Fig. 3). Attempts to detect the expression of the ORF located to the right of the U_L43.5 ORF in the prototype arrangement of HSV-1 DNA by epitope tagging were not successful (8a).

The recombinant virus R7119 was constructed so as to delete 75% of the U_L43.5 ORF (Fig. 4). R7115 was constructed to delete the U_L43.5 gene and almost the entire U_L44 gene. Viral DNAs prepared from cells infected with HSV-1(F) or with these deletion mutants were digested with *Bam*HI, electrophoretically separated in agarose gels, transferred to a Zetaprobe membrane (Bio-Rad), and probed with the *Bam*HI fragment of HSV-1(F). As could be predicted from the details of the construction of these viruses (Fig. 1), the nick-translated probe hybridized to the wild-type 6.6-kbp *Bam*HI I fragment in the HSV-1(F) and HSV-1(F)Δ305 digests (Fig. 4, lanes 1 and 2) and to the approximately 3.6- and 5.7-kbp fragments in R7119 and R7115 digests, respectively (lane 4, band 2; lane 5, band 3). Digestion of R7113 viral DNA with *Bam*HI results in an approximately 5.6-kbp fragment (lane 3, band 1) due to release of the α27-*tk* chimeric gene (see Materials and Methods).

Kinetics of expression of the U_L43.5 gene. To determine the kinetic class to which the U_L43.5 gene belongs, Vero cells were infected with HSV-1(F) or with the deletion mutant viruses in the absence or presence of phosphonoacetic acid (PAA; 300 μg/ml; Sigma), an inhibitor of viral DNA synthesis. U_L43.5 protein produced in HSV-1- or HSV-2-infected cells (not shown) was not detected in the presence of PAA and therefore appears to be a late protein (Fig. 5A).

The U_L43.5 protein localizes to the nucleus of infected cells. Two sets of experiments were done to determine the localization of the protein recognized by the anti U_L43.5 serum. In the first, 143TK$^-$ cells were mock infected or infected with HSV-1(F) or with the deletion mutant R7119. The nuclei and cytoplasm were separated as described in Materials and Methods, and each fraction was electrophoretically separated in dena-

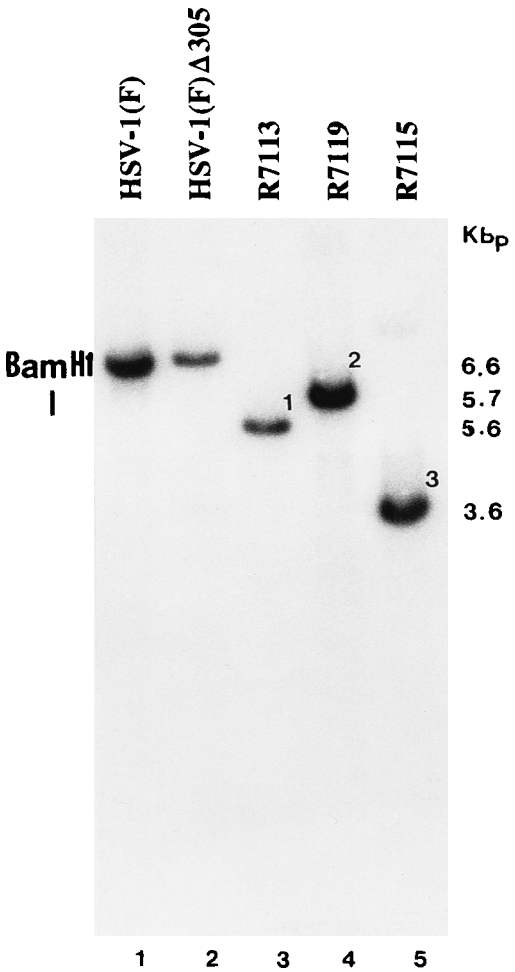


FIG. 4. Autoradiographic images of the electrophoretically separated restriction digests of the DNAs of HSV-1(F) (lane 1) and of the deletion mutant viruses R7119 (lane 2) and R7115 (lane 3). The DNAs were digested with *Bam*HI, separated on a 1.0% agarose gel, transferred to a Zetaprobe membrane, and probed with ^{32}P-labeled *Bam*HI I fragment of HSV-1(F). Band 1 represents the intact 6.6-kbp *Bam*HI I fragment; band 2 represents the 5.7-kbp fragment generated by deletion of 300 bp of sequence within the U_L43.5 gene; band 3 represents the 3.6-kbp fragment generated by deletion of 3.0 kbp of the *Bam*HI I fragment, which results in deletion of the U_L43, U_L43.5, and U_L44 genes.

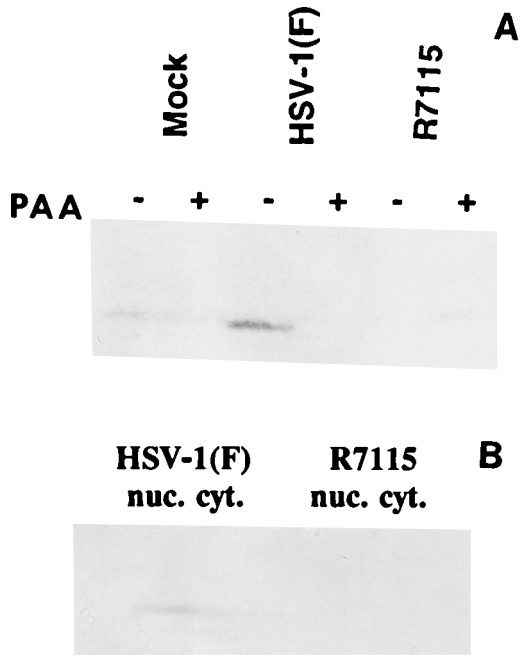


FIG. 5. Photograph of an immunoblot of electrophoretically separated lysates of infected 143TK$^-$ cells. Blots were probed with rabbit antiserum raised against U_L43.5-MBP. (A) Cells were treated with 300 μg of PAA, an inhibitor of viral DNA synthesis (+), or left untreated (−). (B) Infected cells were separated into nuclear and cytoplasmic fractions as described in Materials and Methods.

turing gels and reacted with the anti-U_L43.5 antiserum. The results show that U_L43.5 protein partitioned with the nuclear fraction (Fig. 5B). In the second set of experiments, slide cultures of Vero cells were infected with R7032 (gE$^-$) virus, fixed, and reacted with the U_L43.5 antiserum. U_L43.5 protein was distributed in bright fluorescent granules distributed within and near the periphery of the nucleus. These structures were absent from uninfected cells. Digitized 0.5-μm sections acquired from the basolateral surface (top left of Fig. 6) to the apical surface (bottom right of Fig. 6) showed that the structures were intranuclear and did not appear to be associated with the nuclear membrane.

U_L43.5 colocalizes with capsid proteins. The immunofluorescent pattern of U_L43.5 protein in the nucleus was reminiscent of what we have observed in assays using antibodies to the capsid proteins ICP5 and ICP35 (unpublished studies). To determine whether the U_L43.5 protein colocalizes with nucleocapsids, Vero cell slide cultures were reacted with rabbit polyclonal antibody to U_L43.5 protein and mouse monoclonal antibody to either ICP5 or ICP35. The distribution of U_L43.5 protein completely overlapped that of the major capsid protein ICP5 (Fig. 7b and c) and of the capsid scaffolding proteins ICP35 (Fig. 7a and d to f).

DISCUSSION

The studies described in this report were initiated to define the genes encoded in a relatively understudied region of HSV-1 DNA. As noted in the introduction, McGeoch et al. (23), on the basis of sequence analyses, assigned the designation U_L43 to an ORF for which no gene product was known. Earlier (15) studies identified two transcripts antisense to the U_L43 ORF, but the protein translated in vitro from these

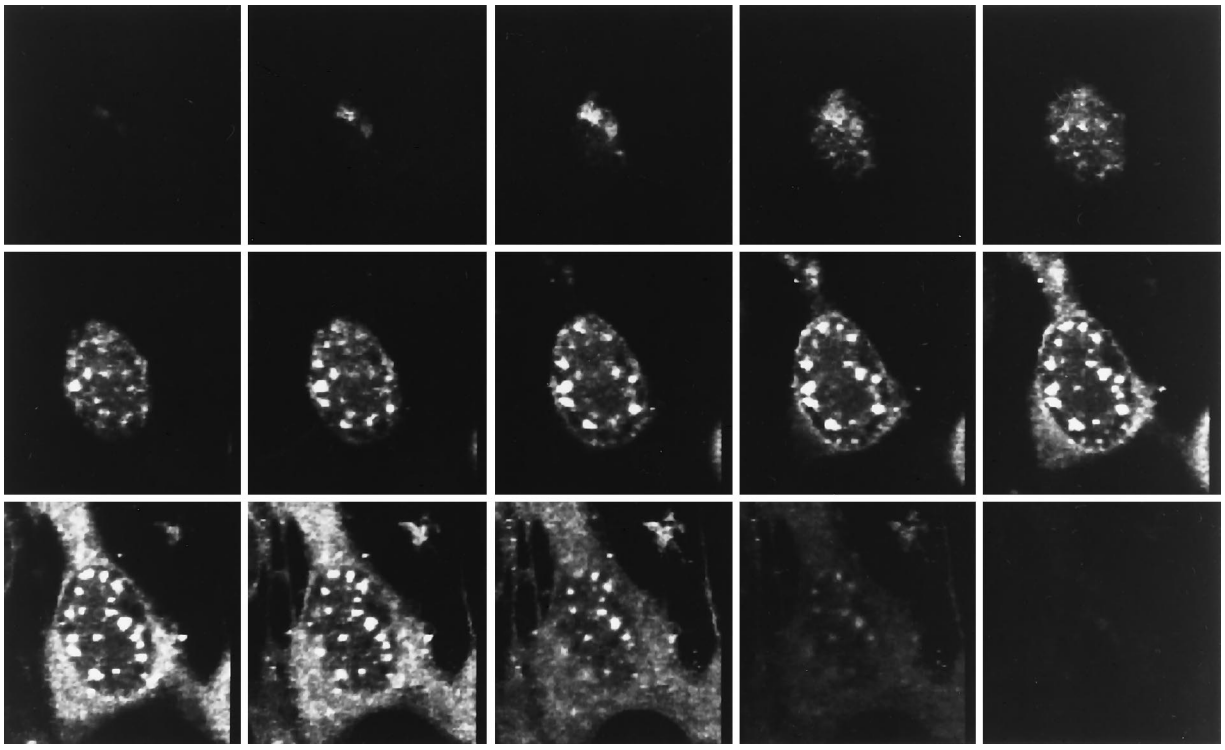


FIG. 6. Digital, unprocessed images of an R7032-infected Vero cell reacted with antibody to U_L20 and anti-rabbit Texas red-conjugated secondary antibody. Cells were infected, fixed, and stained as described in Materials and Methods. Fifteen 0.5-μm sections through the *z* axis were captured by a Codonics CP210 printer. The section in the top left panel is from the basolateral side of the cell.

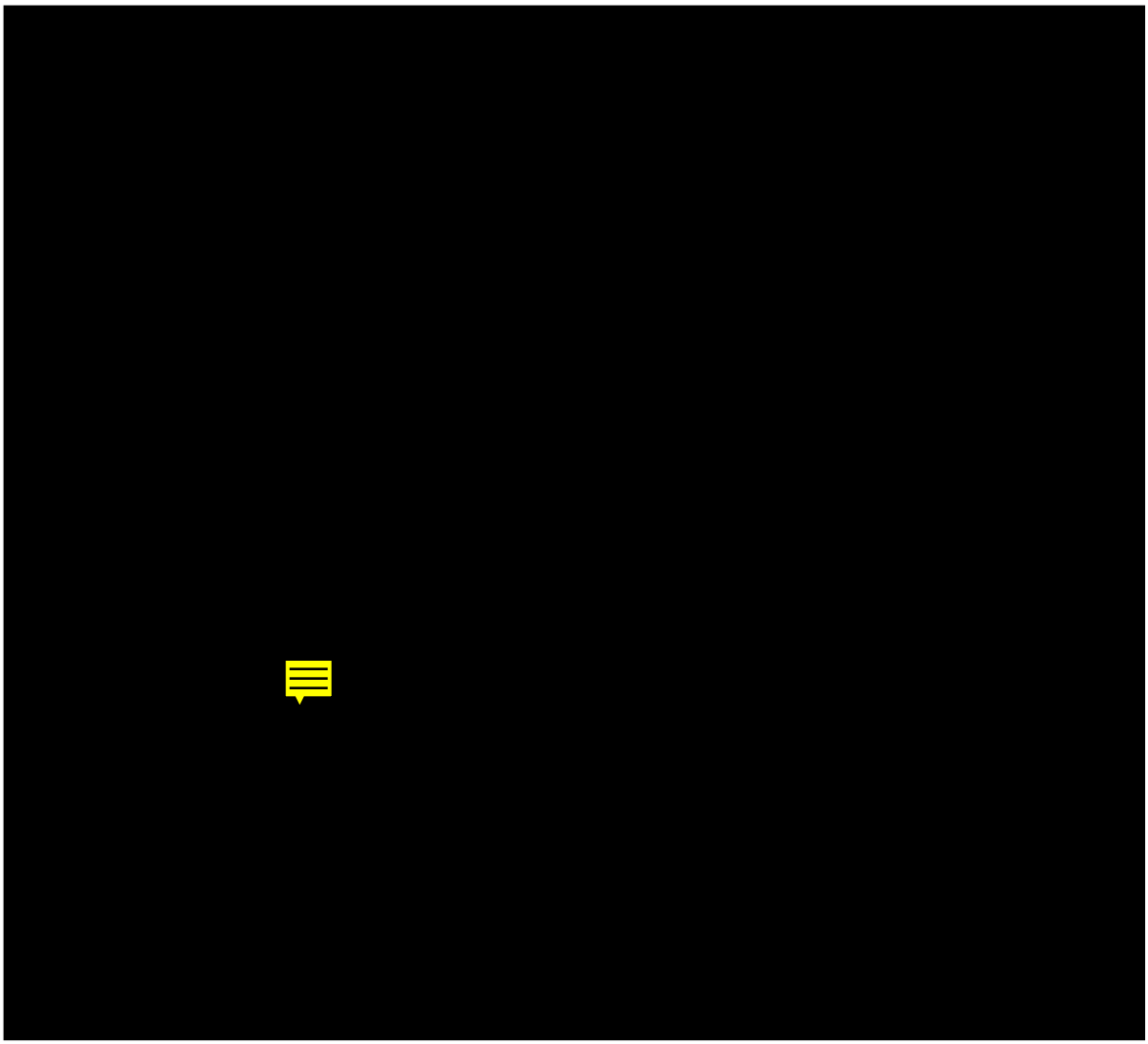


FIG. 7. Confocal, digital images of infected Vero cells double stained with rabbit antibody to $U_L43.5$ protein and with monoclonal antibodies to capsid protein ICP35 or ICP5. (a) ICP35 (top) and $U_L43.5$ (bottom); (b and c) $U_L43.5$ (top) and ICP5 (bottom); (d) ICP35 (red fluorescence); (e) $U_L43.5$ (green fluorescence); (f) overlay of images in panels d and e. The images were captured with software provided by Zeiss with the instrument and printed by a Codonics CP210 printer.

mRNAs was significantly larger than could be encoded by the any one of the ORFs in the region of the DNA strand from which they were reported to be derived. In fact, both U_L43 and an ORF antisense to it, designated as $U_L43.5$, are expressed. The properties of the U_L43 gene will be reported elsewhere (8b). In this report, we show the following.

(i) Polyclonal rabbit antibody made against a fusion protein containing the sequence encoded by the entire $U_L43.5$ ORF reacted with a protein species with an M_r of approximately 38,000, consistent with that predicted by the coding capacity of the $U_L43.5$ ORF. The protein was not detected in lysates of cells infected with mutants lacking all or most of the coding sequences of $U_L43.5$.

(ii) $U_L43.5$ protein was not detected in lysates of cells infected in the presence of PAA, and therefore it appears to be a γ gene. Since the amounts of $U_L43.5$ protein were low, a moderate decrease in the expression of the gene in the presence of PAA reflective of the γ_1 kinetic class would render the protein undetectable by current methods, and therefore we

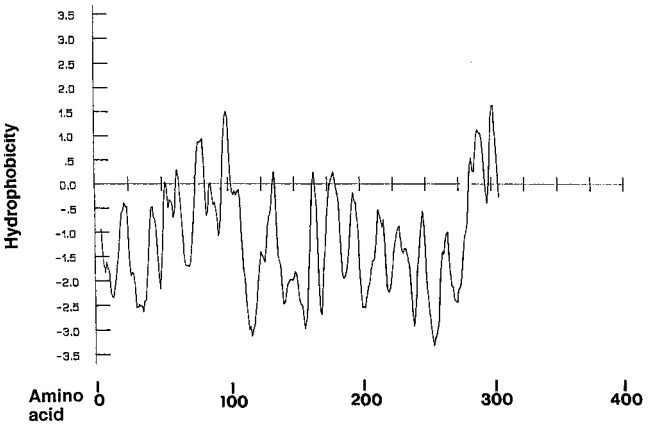


FIG. 8. Kyte-Doolittle hydrophobicity plot generated from the translated DNA sequence of the $U_L43.5$ gene by using a moving window of nine amino acids.

A. Predicted amino acid sequence of U_L43.5

```
  1  MASEKHLGPQ RQARARRSPG MFSHRLDGKR ARPQKGDHGH PDVFGPRDPG
 51  RCDLIGRRPV GSLGGRGGRG PGNPGSLWVL RGAAGKRLAC GDDTDAAVVG
101  ASPGKAVPGG GRPRARQHRR GQGGRGGPTD PPVARAASDR QPADDHPQGA
151  DEQGPRGEEK ERIDVIGEHQ ERRIGGAQCQ ALGGRARGRG PRLRAGLPET
201  PQNKGRSTGR QFEPGVYPRG GPREGRGCAH KAQHGAPDGE RPRRGIRVRP
251  ARHNDRSRKT QRGGLKPDMH EPPRQGPHAR EVWPSVVDPS LAVLRGHGPV
301  AVVAEHPAPW P
```

B. Analysis of amino acid sequence of U_L43.5

```
Consensus, amino-      F
acyl tRNA synthetase   Y  E( 4 or 12 X)R  H  E
Class II               HRXDXXXXXXXXXXXXHXXXFXXXD
UL43.5 sequence        HRLDgkrarpqkgdhgHpdvFgprD
```

FIG. 9. (A) Predicted amino acid sequence of the U_L43.5 protein. (B) Comparison of the consensus sequence of aminoacyl-tRNA synthetase class II conserved motif 2 (11, 14) with that of the motif found in U_L43.5 protein. The consensus sequence in U_L43.5 protein begins at amino acid 24.

cannot assign to it a more precise classification at the present time.

(iii) U_L43.5 protein was distributed in discrete structures located around the periphery of the nucleus of infected cells. The distribution of the U_L43.5 protein almost completely overlapped that of the nucleocapsid proteins ICP5 and ICP35.

Relevant to this report are the following observations.

(i) The U_L43 protein is made in relatively small amounts. One possible explanation for the paucity of the gene product may be related to the position of the gene. The existence of U_L43 is firmly established in the literature (21, 23). We anticipate the report of the studies on U_L43 (8b) to note that the discovery of U_L43.5 is the second instance of HSV-1 genes arranged almost completely antisense to each other. In the first instance, $\gamma_1$34.5 gene is regulated as a γ_1 gene, whereas ORF P is repressed during productive infection by ICP4, the major viral regulatory protein. Mutagenesis of the ICP4 binding site at the transcription initiation site of ORF P resulted in the overexpression of that gene but also in a profound decrease in accumulation of the $\gamma_1$34.5 protein (18, 18a).

(ii) U_L43.5 protein is a basic protein (pI of 12) and consists almost entirely of hydrophilic amino acids, as illustrated in a Kyte-Doolittle plot of hydrophobicity (Fig. 8). Examination of the predicted amino acid sequence of the U_L43.5 protein (Fig. 9A) revealed that U_L43.5 contains one of three motifs characteristic of the catalytic domains of class II aminoacyl-RNA synthetases (14), a group of enzymes which bind ATP and transfer activated amino acids to the appropriate tRNA molecule as a first step in biosynthesis of proteins (Fig. 9B). All three motifs appear to be required for enzymatic activity (11, 14), and while U_L43.5 protein contains only one of the three motifs, it is conceivable that the presence of this motif reflects a capacity of U_L43.5 protein to bind ATP, a potentially important step in capsid assembly. Comparison of the predicted amino acid sequence of the U_L43.5 protein with sequences in the Swiss protein database revealed that the U_L43.5 protein has approximately 25% homology with alpha collagen proteins from a number of species, which probably reflects the high glycine and proline content of the U_L43.5 protein. No other significant homologies have as yet been found. Bona fide homologs of the U_L43.5 gene in other herpesviruses have not been identified.

(iii) Studies carried out independently to define the locations of various viral proteins in infected cells led to the observation that several capsid proteins (e.g., ICP35 and ICP5) and proteins involved in assembly of capsids (e.g., U_L15 [1]) localize in dense nuclear structures located in the periphery of the nucleus. These dense nuclear structures do not colocalize with structures associated with viral proteins involved in DNA synthesis (e.g., ICP8, single-stranded-DNA-binding protein, and U_L42, the DNA polymerase accessory factor [33a]). These observations lead us to speculate that assembly of capsids and packaging of viral DNA may occur preferentially in defined nuclear structures close but not necessarily anchored to the nuclear membrane. The colocalization of U_L43.5 with ICP5 and ICP35 suggests that the former preferentially localizes to and is a component of this structure. U_L43.5 has not been detected in purified virions, but we cannot exclude the possibility that an amount of U_L43.5 too small to be detected is packaged in virions (data not shown).

(iv) Of the 80 different genes currently known, at least 42 are dispensable for viral replication in cells in culture. As noted repeatedly elsewhere (29, 33), these genes are not truly dispensable, since deletion mutants have not been isolated from humans and most deletion mutants are at least partially disabled in experimental animal systems. At least some of these supplemental essential or accessory genes have been shown to play a significant role in enabling the virus to infect, replicate, or spread from cell to cell more efficiently or to block a host response to infection (2–4, 12, 21, 22, 30, 35). Although the U_L43.5 gene is dispensable for viral replication in cells in culture, the localization of its product suggests that it may play a role in virion assembly.

(v) In recent years, a number of new genes that were not predicted by analysis of the genomic sequence have been identified in the HSV-1 genome (5, 7, 9, 10, 16, 18, 19, 23). In addition, there is accumulating evidence that genes may be either required in some cells but not others (e.g., U_L20) or expressed (e.g., ORF P) under unusual regulatory conditions and therefore whose products may not be readily detected (8, 18, 34). The precise function of U_L43.5 may emerge from studies on experimental infected cell environments in which its role is essential and affects viral replication in a quantifiable fashion.

ACKNOWLEDGMENTS

These studies were aided by grants from the National Cancer Institute (CA47451) and the National Institute for Allergy and Infectious Diseases (AI124009) and by an unrestricted grant from the Bristol-Myers Squibb Program in Infectious Diseases.

REFERENCES

1. **Baines, J. D., A. P. W. Poon, J. Rovnak, and B. Roizman.** 1994. The herpes simplex virus 1 U_L15 gene encodes two proteins and is required for cleavage of genomic viral DNA. J. Virol. **68:**8118–8124.
2. **Baines, J. D., and B. Roizman.** 1991. The open reading frames U_L3, U_L4, U_L10, and U_L16 are dispensable for the replication of herpes simplex virus 1 in cell culture. J. Virol. **65:**938–944.
3. **Baines, J. D., and B. Roizman.** 1992. The U_L11 gene of herpes simplex virus 1 encodes a function that facilitates nucleocapsid envelopement and egress from cells. J. Virol. **66:**5168–5174.
4. **Baines, J. D., P. L. Ward, G. Campadelli-Fiume, and B. Roizman.** 1991. The U_L20 gene of herpes simplex virus 1 encodes a function necessary for viral egress. J. Virol. **65:**6414–6424.
5. **Baradaran, K., C. E. Dabrowski, and P. A. Schaffer.** 1994. Transcriptional analysis of the region of the herpes simplex virus type 1 genome containing the U_L8.5, U_L9, and U_L10 genes and identification of a novel delayed-early gene product, OBPC. J. Virol. **68:**4251–4261.
6. **Barker, D. E., and B. Roizman.** 1990. Identification of three genes nones-

sential for growth in cell culture near the right terminus of the unique sequences of long component of herpes simplex virus 1. Virology **177:**684–691.

7. **Barker, D. E., and B. Roizman.** 1992. The unique sequence of the herpes simplex virus 1 L component contains an additional translated open reading frame designated U_L49.5. J. Virol. **66:**562–566.
8. **Bohenzky, R. A., M. Lagunoff, B. Roizman, E. K. Wagner, and S. Silverstein.** 1995. Two overlapping transcription units which extend across the L-S junction of herpes simplex virus type 1. J. Virol. **69:**2889–2897.

8a. **Carter, K., and B. Roizman.** Unpublished data.

8b. **Carter, K., P. L. Ward, and B. Roizman.** Unpublished data.

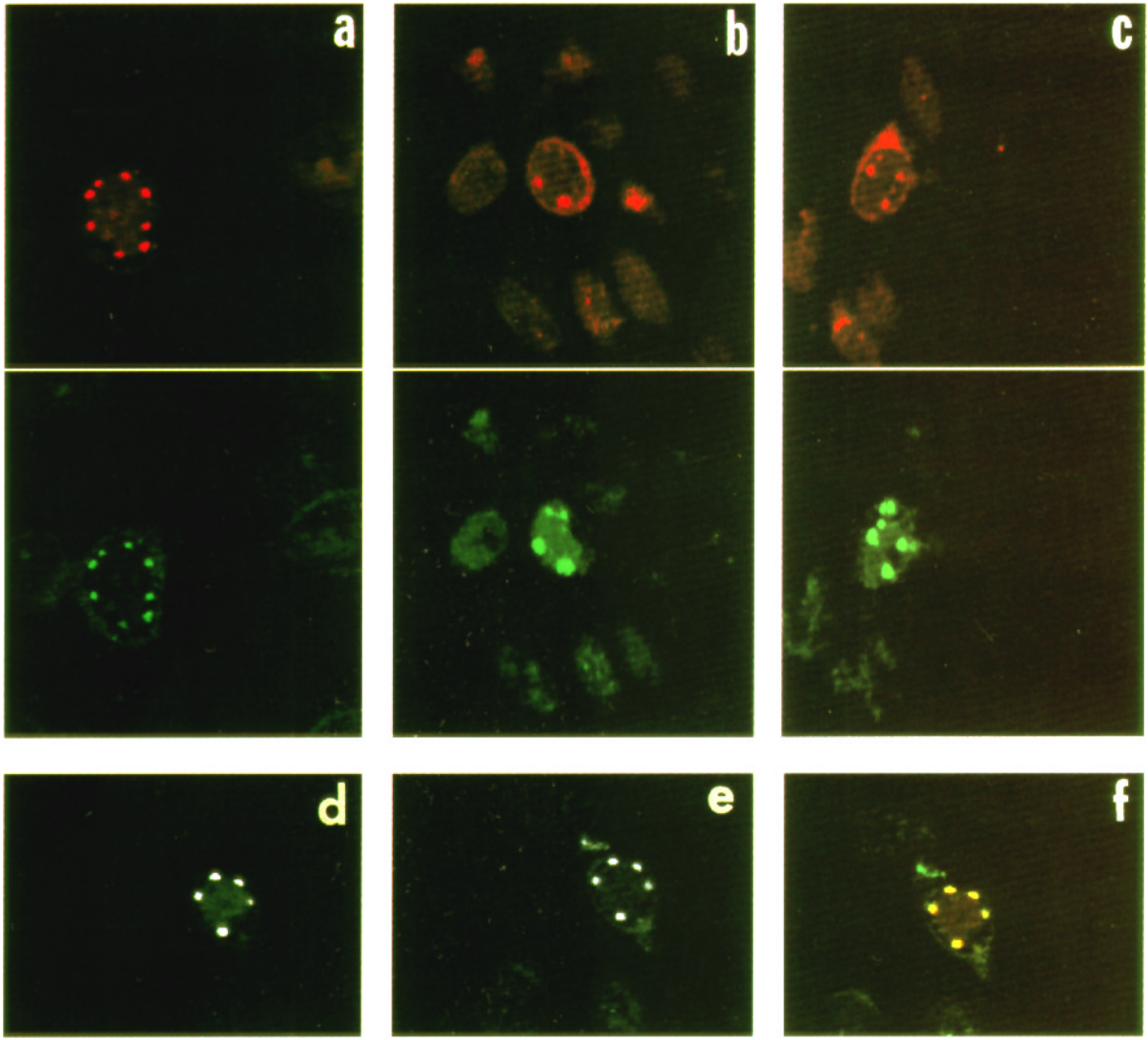
9. **Carter, K. L., and B. Roizman.** 1996. The promoter and transcriptional unit of a novel herpes simplex virus 1 α gene are contained in, and encode a protein in frame with, the open reading frame of the α22 gene. J. Virol. **70:** 172–178.
10. **Chou, J., and B. Roizman.** 1990. The herpes simplex virus 1 gene for ICP34.5, which maps in inverted repeats, is conserved in several limited-passage isolates but not in strain 17 syn+. J. Virol. **64:**1147–1156.
11. **Delarue, M., and D. Moras.** 1993. The aminoacyl-tRNA synthetase family: modules at work. Bioessays **15:**675–687.
12. **Dingwell, D. S., C. R. Brunetti, R. L. Hendricks, Q. Tang, M. Tang, A. J. Rainbow, and D. C. Johnson.** 1994. Herpes simplex virus glycoproteins E and I facilitate cell-to-cell spread in vivo and across junctions of cultured cells. J. Virol. **68:**834–845.
13. **Ejercito, P. M., E. D. Kieff, and B. Roizman.** 1968. Characterization of herpes simplex virus strains differing in their effects on social behavior of infected cells. J. Gen. Virol. **2:**357–364.
14. **Eriani, G., M. Delarue, O. Poch, J. Gangloff, and D. Moras.** 1990. Partition of tRNA synthetases into two classes based on mutally exclusive sets of sequence motifs. Nature (London) **347:**203–205.
15. **Frink, R. J., K. P. Anderson, and E. K. Wagner.** 1981. Herpes simplex virus type 1 *Hin*dIII fragment L encodes spliced and complementary mRNA species. J. Virol. **39:**559–572.
16. **Georgopoulou, U., A. Michaelidou, B. Roizman, and P. Mavromara-Nazos.** 1993. Identification of a new transcriptional unit that yields a gene product within the unique sequences of the short component of the herpes simplex virus 1 genome. J. Virol. **67:**3961–3968.
17. **Igarashi, K., R. Roller, and B. Roizman.** 1993. Construction and properties of a recombinant herpes simplex virus 1 lacking both S-component origins of DNA synthesis. J. Virol. **67:**2123–2132.
18. **Lagunoff, M., and B. Roizman.** 1994. Expression of a herpes simplex virus 1 open reading frame antisense to the $\gamma_1$34.5 gene and transcribed by an RNA 3′ coterminal with the unspliced latency-associated transcript. J. Virol. **68:** 6021–6028.

18a. **Lagunoff, M., and B. Roizman.** Unpublished data.

19. **Liu, F., and B. Roizman.** 1991. The promoter, transcriptional unit, and coding sequences of herpes simplex virus 1 family 35 proteins are contained within and in frame with the U_L26 open reading frame. J. Virol. **65:**206–212.
20. **Longnecker, R., and B. Roizman.** 1986. Generation of an inverting herpes simplex virus 1 mutant lacking the L-S junction *a* sequences, an origin of DNA synthesis, and several genes including those specifying glycoprotein E and the α47 gene. J. Virol. **58:**583–591.
21. **MacLean, C. A., S. Efstathious, M. L. Elliott, F. E. Jamieson, and D. J. McGeoch.** 1991. Investigation of herpes simplex virus type 1 genes encoding multiply inserted membrane proteins. J. Gen. Virol. **72:**897–906.
22. **MacLean, C. A., L. M. Robertson, and R. E. Jamieson.** 1993. Characterization of the U_L10 gene product of herpes simplex virus type 1 and investigation of its role in vivo. J. Gen. Virol. **74:**975–983.
23. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Dolan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol. **69:**1531–1574.
24. **Meignier, B., R. Longnecker, P. Mavromara-Nazos, A. Sears, and B. Roizman.** 1988. Virulence of and establishment of latency by engineered deletion mutants of herpes simplex virus 1. Virology **162:**251–254.
25. **Mocarski, E. S., L. E. Post, and B. Roizman.** 1980. Molecular engineering of the herpes simplex virus genome; insertion of a second L-S junction into the genome causes additional genome inversions. Cell **22:**243–255.
26. **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.
27. **Post, L. E., S. Mackem, and B. Roizman.** 1981. Regulation of α genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with α gene promoters. Cell **24:**555–565.
28. **Post, L. E., and B. Roizman.** 1981. A generalized technique for deletion of specific genes in large genomes: α gene 22 of herpes simplex virus 1 is not essential for growth. Cell **25:**227–232.
29. **Roizman, B., and A. E. Sears.** 1995. The replication of herpes simplex viruses, p. 2231–2295. *In* B. N. Fields, D. M. Knipe, P. Howley, R. M. Chanock, M. S. Hirsch, J. L. Melnick, T. P. Monath, and B. Roizman (ed.), Virology, 3rd ed. Raven Press, New York.
30. **Sears, A. E., B. S. McGwire, and B. Roizman.** 1991. Infection of polarized MDCK cells with herpes simplex virus 1: two asymemetrically distributed cell receptors interact with different viral proteins. Proc. Natl. Acad. Sci. USA **88:** 5087–5091.
31. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
32. **Ward, P. L., G. Campadelli-Fiume, E. Avitabile, and B. Roizman.** 1994. Localization and putative function of the U_L20 membrane protein in cells infected with herpes simplex virus 1. J. Virol. **68:**7406–7417.
33. **Ward, P. L., and B. Roizman.** 1994. Herpes simplex genes: the blueprint of a successful human pathogen. Trends Genet. **10:**267–274.

33a. **Ward, P. L., and B. Roizman.** Unpublished data.

34. **Yeh, L., and P. A. Schaffer.** 1993. A novel class of transcripts expressed with late kinetics in the absence of ICP4 spans the junction between the long and short segments of the herpes simplex virus type 1 genome. J. Virol. **67:**7373–7382.
35. **York, I. A., C. Roop, D. W. Andrews, S. T. Riddell, F. L. Graham, and D. C. Johnson.** 1994. A cytosolic herpes simplex virus protein inhibits antigen presentation to CD8+ T lymphocytes. Cell **77:**525–535.


a
b
c
d
e
f