U_L27.5 Is a Novel γ2 Gene Antisense to the Herpes Simplex Virus 1 Gene Encoding Glycoprotein B

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An antibody made against the herpes simplex virus 1 U_S5 gene predicted to encode glycoprotein J was found to react strongly with two proteins, one with an apparent Mr of 23,000 and mapping in the S component and with a herpes simplex virus protein with an apparent Mr of 43,000. The antibody also reacted with herpes simplex virus type 2 proteins forming several bands with apparent Mr's ranging from 43,000 to 50,000. Mapping studies based on intertypic recombinants, analyses of deletion mutants, and ultimately, reaction of the antibody with a chimeric protein expressed by in-frame fusion of the glutathione S-transferase gene to an open reading frame antisense to the gene encoding glycoprotein B led to the definitive identification of the new open reading frame, designated U_L27.5. Sequence analyses indicate the conservation of a short amino acid sequence common to U_S5 and U_L27.5. The coding sequence of the herpes simplex virus U_L27.5 open reading frame is strongly homologous to the sequence encoding the carboxyl terminus of the herpes simplex virus 2 U_L27.5 sequence. However, both open reading frames could encode proteins predicted to be significantly larger than the mature U_L27.5 proteins accumulating in the infected cells, indicating that these are either processed posttranslationally or synthesized from alternate, nonmethionine-initiating codons. The U_L27.5 gene expression is blocked by phosphonoacetic acid, indicating that it is a γ2 gene. The product accumulated predominantly in the cytoplasm. U_L27.5 is the third open reading frame found to map totally antisense to another gene and suggests that additional genes mapping antisense to known genes may exist.

In this report, we describe the identification of a new open reading frame (ORF) in the genomes of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2). What makes this ORF particularly interesting is its location antisense to U_L27, the gene encoding glycoprotein B (gB) (5, 22). The initial objectives of this study were quite different from its outcome.

The sequence of the HSV-1 genome (22) corroborated the laboratory reported two sets of antisense genes. Thus, ORFs O and P map antisense to the γ3.45 gene, and the U_L43.5 gene maps antisense to the U_S43 gene (6, 20, 26). The striking feature of the antisense genes is that their expression is mutually exclusive. Thus, derepression of ORF P leads to its expression early in infection and grossly reduces the normal expression of the γ3.45 gene (27). U_S43 and U_L43.5 are also expressed at different times after infection (6). The gB/U_L27.5 genes are the third set of genes located antisense to each other. gB is expressed relatively early in infection, whereas the U_L27.5 gene belongs to the γ2 group in that its expression is totally dependent on viral DNA synthesis (14, 15).

MATERIALS AND METHODS

Cells and viruses. Rabbit skin cells and Vero cells were obtained from John McLaren and American Type Culture Collection, respectively, and were maintained in Dulbecco's modified Eagle medium supplemented with 5% newborn calf serum. BHK(TK-+) cells (American Type Culture Collection) and 143TK- cells (obtained from Carlo Croce) were maintained in the same medium supplemented with 5% fetal bovine serum. Infected cells were maintained in mixture 199 supplemented with 1% calf serum (199V) unless indicated otherwise. HSV-1(F) and HSV-2(G) are prototypes of HSV-1 and HSV-2 strains, respectively, used in this laboratory (10). Intertypic recombinants HSV-1(F) × HSV-2(G) were described previously (1, 8). In R7015 the HSV-1 S component was replaced by the homologous HSV-2(G) sequences. HSV-1(F)Δ305 was derived from HSV-1(F) and has a 501-bp deletion in U_L23 (thymidine kinase) and U_L24 genes (25). The HSV-1(KOS) mutant, lacking the U_S26 gene (11), was the generous gift of Steven Weinheimer (Bristol-Myers Squibb).

Antibodies. Mouse monoclonal antibody CH28 to the human cytomegalovirus (HCMV) gB epitope or G1102 to ICP15 was purchased from Goodwin Institute (Plantation, Fla.). ICP0 polyclonal antibody was described previously (18). Goat anti-mouse or anti-rabbit immunoglobulin G alkaline phosphatase-conjugated secondary antibodies were purchased from Bio-Rod (Hercules, Calif.).

Generation of U_S5 polyclonal antibody. The U_S5 maltose binding protein expression plasmid pRB5154, containing the entire coding region of U_S5, was

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transfected into *Escherichia coli*. The induction and purification procedure of maltose binding fusion protein was done as recommended by the manufacturer (New England BioLabs, Beverly, Mass.). A New Zealand White female rabbit was injected subcutaneously five times, each time with 250 to 400 μg of fusion protein and an adjuvant.

Detection of viral proteins in lysates electrophoretically separated in a denaturing polyacrylamide gel. Mock-infected or HSV-1-infected Vero or BHK(TK+) cells were harvested in disruption buffer (50 mM Tris-HCl [pH 7.0], 2% sodium dodecyl sulfate (SDS), 0.1 M β-mercaptoethanol, 2.5% sucrose), electrophoretically separated in a denaturing polyacrylamide gel cross-linked with N,N’-diacytardiamid, electrophoretically transferred to a nitrocellulose membrane (Schleicher & Schuell), and probed with appropriate antibodies. The immunoblotting procedures was as described elsewhere (7). The molecular size marker was obtained with the LMW electrophoresis calibration kit (Pharmacia Biotech, Uppsala, Sweden).

**Generation of plasmids and recombinant viruses.** All molecular cloning was done by standard techniques as described elsewhere (29). The maltose binding protein-US5 chimeric construct was cloned as pRB5154 and contained the entire U5 coding sequence. The U5 coding sequence with an insertion at the EcoO119 site of an oligomer encoding the HCMV β epitope (5′ AAAAGGG ACAGAAGGCCACCTGCTAGGACTGGCGACACCGCAAAAACGG GTACCACGACC 3′) was ligated to the U25.6 promoter sequence (~599 to +44, relative to the transcription start site of the U25.6 gene) to create pRB4152. The U25.6 promoter-U5 construct was cloned into the HSV-1(F) thymidine kinase (tk) gene. A BamHI/EcoRI fragment from pRB4454 containing the a27.4 cassette was cloned into the BamHI/EcoRI sites of pRB4428 to create pRB4463. pRB4463 contains the 3′ terminus of the U25.6 gene and the inserted a27.4 cDNA. Subsequently, a Nari fragment containing an a4 promoter (−604 to +25 relative to the transcription start site of the U25.6 gene) and the inserted a27.4 terminal sequence from pRB4080 was cloned into the Nari site of pRB4463 to create pRB4492. pRB4351 and pRB4492 were recombined into the HSV-1(F)A305 gene to create recombinant viruses R4351 and R4492, respectively.

The a27.4 fragment subcloned from pAV1 (a kind gift from Aviron, Mountain View, Calif.), containing HSV-2 genes U25 to U27 genes (Fig. 1C, lane 6), was cloned into pGEM3Zf(+) as pRB812. pRB812 was recombined into the R4492 viral genome to create a series of intertypic recombinant viruses (K-2 to K-7, K-9, and K-10).

All recombinant viruses made for these studies were generated by homologous recombination. Rabbit skin cells were transfected with viral DNA and cotransfected with appropriate plasmid DNA as previously described (7). The transfection was carried on 1536 cells and selected either for the tk+ progeny virus, e.g., R4351 and R4492 in HAT medium (15 μg of hypoxanthine, 0.2 μg of aminopterin, and 5 μg of thymidine per ml) or tk mutant progeny viruses (e.g., R5175) in medium containing 40 μg of bromodeoxyuridine per ml. Individual isolates were plaque-purified on Vero cells, and their genome sequences and gene expression were verified by Southern blot and immunoblot analyses, respectively.

**Analyses of viral DNA by hybridization.** Cytoplasmic DNAs were purified from infected cells as described elsewhere (16), digested with either BamHI or EcoRI, and separated by agarose gel electrophoresis, and transferred to a nylon membrane (Schleicher & Schuell), and probed with appropriate antibodies. The immunoblot analyses. Cytoplasmic DNAs from infected cells were digested with either the BamHI or the EcoRV enzyme, separated on an agarose gel, transferred to a nylon membrane, and hybridized with appropriate probes. As illustrated in Fig. 1C, lines 2 and 3, the replacement of the BglII/EcoNI fragment of the tk gene with a U26.5 promoter-U5-HCMV tag construct increased the size of HSV-1(F) BamHI-O from 3.5 to 4.2 kb, as detected when 32P-labeled pRB165 was used as the probe (Fig. 2B). To verify the predicted genomic structure of R4351 and R4492, electrophoretically separated EcoRV digests of the recombinant virus DNAs were probed with a 32P-labeled HSV-1(F) EcoR V D fragment. There are two closely positioned EcoRV sites in the a27.4 construct. The EcoRV-D probe showed, as predicted, that insertion of the a27.4 fragment and the a4 promoter into R4492 (Fig. 1C, lines 4 and 5) replaced the original EcoRV D fragment (14.8 kb) with two fragments of 2.4 and 14.6 kb in length (Fig. 2A, lanes 1 and 3). In the same fashion, the insertion of the a27.4 cassette into the R4351 viral genome (Fig. 1C, lane 6) resulted in the replacement of the EcoRV D fragment with two fragments of 4.6 and 11.8 kb in length (Fig. 2A, lane 2). We conclude that the three recombinant viruses made in these studies show the expected genotype.

U5 rabbit polyclonal antibody reacted with two viral proteins by immunoblot analyses. Although the rabbit polyclonal antibody was made against the U5 protein, in immunoblot assays the anti-U5 polyclonal antibody reacted with two bands containing different proteins. Mock-infected or HSV-1(F)-infected BHK(TK+) cells were harvested in disruption buffer at 18 h after infection, electrophoretically separated in an SDS-15% polyacrylamide gel, electrophoretically transferred to a nitrocellulose sheet, and probed with the U5 polyclonal antiserum. The results (Fig. 3A, lane 5) reproducibly showed that the serum reacted with two virus-specific HSV-1 proteins with apparent Mr of 43,000 and 23,000, respectively (designated band 2 and band 3, respectively). The antibody did not react with lyses of mock-infected cells (Fig. 3A, lane 1). In HSV-2(G)-infected cell lysate, only one virus-specific signal, with a mobility on a denaturing gel equivalent to that of HSV-1(F) band 2, was detected as a heterogeneous group of bands with apparent Mr ranging from 43,000 to 50,000. The HSV-2 equivalent of HSV-1(F) band 3 was not detected (Fig. 3A, lane 3). Overdeveloped immunoblots occasionally showed another faint band with an apparent Mr of greater than 100,000 (designated band 1).

The reactive band 3 was encoded by a gene residing in the U5 sequence of HSV-1(F) inasmuch as the signal was missing in the intertypic recombinant virus R7015 (Fig. 3, lane 4). As noted in Materials and Methods, in this recombinant, the U5 sequence of HSV-1(F) was replaced with the corresponding...
The hypothesis that the protein contained in band 3 is encoded by the US5 gene was supported by studies of recombinant R5175. In this recombinant, a second copy of US5 coding sequence tagged with an HCMV gB epitope tag (line 9). FIG. 1. (A) Schematic diagram of genome structures of HSV-1(F) and HSV-2(G) intertypic recombinants. Line 1, HSV-1(F) genome arrangement. Open rectangles represent inverted repeats, and the single lines in between represent unique long (UL) and unique short (US) regions of the genome. Lines 2 to 7, schematic diagram of genome arrangement of intertypic recombinants used in this study. Lines labeled 1 represent HSV-1(F) sequences, and lines labeled 2 represent HSV-2(G) sequences. Crossovers are indicated by bold-faced lines. R7015 (line 2) has the HSV-1(F) unique short region replaced with the HSV-2(G) unique short sequence. Lines 3 to 7 are a set of intertypic recombinants that are basically HSV-1(F), with various portions between map units 0.3 and 0.45 replaced with the HSV-2(G) sequence. (B) Schematic diagram of HSV-1(F) and various recombinants used in this study. Line 1, genome arrangement of HSV-1(F) and location of ORFs U23 to U28. Arrow indicates the polarity and position of each ORF. Line 2, position of the HSV-2(G) KpnI H fragment relative to the location of ORFs. Lines 3 to 7, positions of proposed new ORF U27.5 in the HSV-1(F) and HSV-2(G) genomes, respectively. Lines 5 to 9, genome organization of various recombinants. Short vertical lines represent deletions (lines 5, 7, and 9). Triangles represent insertions (lines 6, 8, and 9). Filled rectangle represents HCMV epitope tag (line 9). (C) Line 1, sequence arrangement of HSV-1(F). Line 2, ORFs in HSV-1(F) BamHI Q fragment. Line 3, in RS175, an 0.8-kb EcoNI/BglII fragment within the U23 to U24 region is replaced by a 1.5-kb U26.5 promoter-US5 construct. Line 4, ORF arrangement in HSV-1(F) EcoRV D fragment. Line 5, in R4492, a 2.2-kb α27tk-a4 promoter construct is inserted between the U25 and U26 genes. Line 6, in R4351, a 1.6-kb α27tk construct is inserted between the U26 and U27 genes. B, BamHI; Bu, BglII; E, EcoRV; En, EcoNI; N, NcoI.

sequence of HSV-2(G). The hypothesis that the protein contained in band 3 is encoded by the U55 gene was supported by studies of recombinant RS175. In this recombinant, a second copy of U55 coding sequence tagged with an HCMV gB epitope tag (line 9).

FIG. 2. Autoradiography of electrophoretically separated DNA hybridized with 32P-labeled probe. DNA of HSV-1(F) or recombinant virus was digested with EcoRV (A) or BamHI (B), electrophoretically separated in agarose gel, transferred to a nylon membrane, and hybridized with labeled EcoRV D fragment (A) or BamHI Q fragment (B).
epitope was inserted into the tk gene. The polyclonal rabbit serum made against the US5 protein reacted with a fourth band in electrophoretically separated lysates of R5175 virus-infected cell lysates (Fig. 3A, lane 8, band 4). Band 4 comigrated with the signal detected in R5175 reacted only with the monoclonal antibody to the HCMV gB epitope (Fig. 3, compare lanes 8 and 9). The CMV epitope-tagged second copy US5 protein migrated with an apparent Mr of about 18,000, which is faster than the authentic US5 protein. One explanation for the discrepancy in the electrophoretic mobilities is that insertion of the epitope blocks the glycosylation of the protein. The studies with the gJ protein will be dealt with elsewhere.

The gene encoding protein in band 2 maps in UL. In this section, we describe three series of experiments designed to map the gene encoding band 2 protein. We took advantage of the difference in the electrophoretic mobilities of the HSV-1 and HSV-2 homologs of band 2 protein.

The first series of experiments was done with a series of HSV-1 x HSV-2 recombinants shown in Fig. 1A. These recombinants have been extensively studied for mapping studies, and the crossover sites are well known. Replicate Vero cell cultures were exposed to 10 PFU of a wild-type or recombinant virus, incubated at 37°C, harvested at 18 h after infection, solubilized in disruption buffer, electrophoretically separated in a denaturing polyacrylamide gel, transferred to a nitrocellulose sheet, and reacted with the anti-US5 antibody. The results shown in Fig. 4 indicate that the recombinant viruses RHIG7 and RHIG13 encoded the HSV-2(G) form of band 2 protein (Fig. 4, lanes 3, 4, and 6), whereas the recombinants RHIG8, RHIG44, and RHIG48 yielded the HSV-1(F) form of band 2 protein (Fig. 4, lanes 2, 5, 7, and 8). These results indicated that the sequence encoding the band 2 protein maps between map units 0.33 and 0.37, that is, within the genome domain encoding the U1 to U6 genes (Fig. 1A) (22).

The second series of studies was designed to determine whether the gene encoding band 2 protein maps to U1 to U6 on the basis of the synthesis of band 2 protein in cells.
infected with mutants with changes in these genes. Thus, several recombinant viruses with mutations in this region, including deletions in the domains of U123 and U124 [HSV-1(F)Δ305 and R5175] and U126 [HSV-1(KOS)m100] (11) and disruption of the U125-to-U126 (R4492) and U126-to-U127 interfaces (R4351), were tested for alteration in the mobility of band 2 protein. In this series of experiments, Vero cells were infected and processed as described above. The results were that the electrophoretic mobility of band 2 protein specified by the recombinants tested in these studies was no different from that predicted (9, 17) or specified by HSV-1(F) (Fig. 3A, lanes 5 to 7, Fig. 3B, lanes 1 to 4, and Fig. 3C, lanes 1 to 3). Band 3 protein was detected in all of the recombinant viruses tested in this series of experiments.

We conclude from this series of experiments that the band 2 protein was not encoded by U123, U124, or U126 ORFs inasmuch as deletions in these genes had no effect on the mobility of band 2. Furthermore, the mobility of band 2 on denaturing polyacrylamide gel was different from what would be expected for U125 or U127 gene products (2, 3, 13, 22–24). Therefore, we conclude that band 2 protein is derived from a previously unidentified viral ORF between map units 0.33 and 0.37.

In the third series of experiments, we generated a series of recombinant viruses in which the HSV-1 ORFs U125 to U128 were replaced with the equivalent HSV-2(G) sequences. The strategy was to cotransfect the HSV-2 sequences in pRB812 with intact R4492 viral DNA and select for the TK- phenotype. In the process, the α27-αk chimeric gene inserted into the R4492 genome in the intergenic domain between U125 and U126 was replaced with the HSV-2(G) DNA sequences cloned in pRB812. Replicate Vero cell cultures, each exposed to 10 PFU of intertypic recombinant per cell, were then analyzed for the presence of the HSV-2(G) band 2 protein in the HSV-1(F) background. As shown in Fig. 5, the lysates of cells infected with isolates K-6, K-9, and K-10 exhibited band 2 proteins which comigrated with band 2 of HSV-2(G), that is, migrated more slowly than the HSV-1 band 2 protein present in the lysate from parent virus R4492 (Fig. 5, lane 1). The slower-migrating species, designated U127.5 in this figure for reasons detailed in the next section, appeared as a doublet similar to the band 2 in HSV-2(G)-infected lysate, although the upper band was not as prominent as in HSV-2(G)-infected lysate. Since the crossover could have occurred proximal to the location of the ORF encoding band 2 protein, not all of the recombinants in this series exhibited or were predicted to exhibit an HSV-2(G) band 2 phenotype. In no instance was the electrophoretic mobility of the band 3 (U125) protein affected. Based on the observation that an insertion between U126 and U127 (R4351) or between U125 and U126 (R4492) and a truncation in the amino terminus of the U126 gene (m100) had no effect on the mobility of band 2, we conclude that the new ORF could reside completely within the U125 ORF, within the carboxy-terminal portion of the U126 ORF, or within the region between U127 and U128 (Fig. 1B).

Sequence analysis predicts new ORF antisense to U127. We next searched for potential ORFs in the target regions stated above. We focused on new ORFs conserved in HSV-1 and HSV-2 with the capacity to encode at least 200 amino acids. As shown in Fig. 1B, lines 3 and 4, only one ORF, designated U127.5 and predicted to encode an HSV-1 protein of 575 amino acids, and an HSV-2 protein of 985 amino acids met these criteria. Sequence comparison showed that, except for the amino-terminal region of the predicted HSV-2 U127.5 ORF, the HSV-1 and HSV-2 U127.5 ORFs were homologous (Fig. 6). Since the U125 polyclonal antibody cross-reacted with the denatured band 2 protein, the cross-reacting epitope is predicted to be linear and potentially could be deduced by comparing primary amino acid sequences. Amino acid sequence comparison of HSV-1(F) and HSV-2(G) U127.5 and U125 revealed very limited sequence homology (Fig. 7). The sequence of the U125 gene predicts a hydrophobic protein. Therefore, the observation that the limited homology resided in a rather hydrophobic stretch of amino acids was not unexpected.

A GST-U127.5 fusion protein expressing the U125 homologous region of U127.5 reacted with the U125 polyclonal antibody by immunoblot analysis. A GST-U127.5 fusion protein expressing the homologous region of the HSV-1 U127.5 coding sequence (Fig. 6, HSV-1 amino acids 299 to 412) was constructed, purified on affinity columns, and subjected to electrophoresis in denaturing polyacrylamide gels. The eluted GST-U127.5 chimeric protein, the chimeric protein remaining bound to the affinity resin and eluted by solubilization in disruption buffer, and the eluted GST protein were each separated on a 15% denaturing gel and stained with Coomassie blue (Fig. 8A, lanes 1, 2, and 3, respectively). Portions of the same preparation were electrophoretically separated in the same gel, electrophoretically transferred to a nitrocellulose membrane, and probed with U125 polyclonal antibody (Fig. 8B). The results were that the GST-U127.5 fusion protein migrated at an expected Mr of 42,000 on denaturing gel (panel A, lanes 1 and 2) (2). Both eluted GST-U127.5 and resin-associated GST-U127.5 reacted with the U125 polyclonal antibody on an immunoblot, whereas GST alone did not (Fig. 8B, lanes 1, 2, and 3). These results are consistent with the results of the mapping studies and indicate that U127.5 encodes a protein containing an epitope common to the U125 protein.

In vitro-translated product of the U127.5 coding sequence migrated as a protein with a molecular weight of 65,000. The
The primary sequence of the UL27.5 ORF has the capacity to encode a protein of 575 amino acids. The apparent molecular weight of band 2 on denaturing polyacrylamide gel was 43,000.

To test if the discrepancy between the predicted and observed size is due to the nature of the protein or to posttranslational modification, we translated the UL27.5 open reading frame with a coupled in vitro transcription translation system and analyzed the protein in a denaturing gel. As shown in Fig. 9, lanes 3 and 4, the only [14C]leucine-labeled species, supposedly the full-length product, migrated with an apparent Mr of 65,000. A minor protein band with an apparent Mr of 65,000 was also observed in immunoblots reacted with the US5 antibody (Fig. 4).

As a general rule, HSV proteins migrate with an apparent Mr larger than that predicted on the basis of their amino acid sequences. The apparent Mr obtained for the in vitro transcription-translation product is consistent with an HSV protein of 575 amino acids and suggests that either the domain translated is smaller than the ORF or that the protein is processed by cleavage in the environment of the infected cell.

UL27.5 is a g2 gene.

In this series of experiments, replicate Vero cultures were either mock infected or infected with HSV-1(F) and either left untreated or incubated in medium containing phosphonoacetic acid (PAA) (300 mg/ml of medium; a gift of Abbott Laboratories) throughout the course of infection. The cells were harvested at 18 h after infection, solubilized in disruption buffer, subjected to electrophoresis, electrically transferred onto a membrane, and probed with appropriate antibodies to viral proteins. As shown in Fig. 10B, lanes 2 and 4, the treatment of PAA was effective, as evidenced by the reduced accumulation of ICP35 (a g1 gene). In contrast to ICP0 and the ICP35 protein, U L27.5 was not detected in infected cells treated with PAA (Fig. 10C). We conclude therefore that UL27.5 is a g2 gene totally dependent on viral DNA synthesis for its expression.

UL27.5 protein accumulates in the cytoplasm. BHK(TK+) cells were either mock infected or exposed to 10 PFU of HSV-1(F) or of HSV-2(G) per cell and incubated at 37°C. The was also observed in immunoblots reacted with the U5 antibody (Fig. 4).

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cells were harvested at 18 h after infection. The cytoplasmic and nuclear fractions were collected as described in Materials and Methods, solubilized, electrophoretically separated onto a denaturing polyacrylamide gel, electrically transferred to a membrane, and reacted with the anti-US5 antibody. As shown in Fig. 11, lanes 1 and 2, the U L27.5 protein accumulated exclusively in the cytoplasmic fraction. In HSV-2(G)-infected cell lysates, the majority of the signal was detected in the cytoplasmic fraction. A slightly more rapidly migrating protein band was detected in both the nuclear and cytoplasmic fractions whereas a less abundant and more rapidly migrating protein band was detected only in the nuclear fraction (Fig. 11, lanes 3 and 4).

DISCUSSION

A polyclonal rabbit antibody made against gJ reacted with a protein expressed by both HSV-1 and HSV-2. The gene encoding this protein has been mapped to an ORF antisense to the gene encoding gB. In this article, we report on the mapping of the gene and preliminary characterization of the product of the ORF. The salient features of this report are as follows.

(i) The protein is encoded by the U L27.5 gene on the basis of three kinds of data, i.e., mapping of two independently derived intertypic recombinants, analyses of insertion and deletion mutants, and direct reaction of the antibody with a chimeric protein containing the relevant domain of the ORF expressed in E. coli.

(ii) The U L27.5 ORFs predict an HSV-1 protein of 575 amino acids and an HSV-2 protein of 985 amino acids. The HSV-1 sequence predicts a single methionine codon, whereas the HSV-2 sequence predicts two methionines at positions 1 and 669. Translation in vitro of the entire HSV-1 ORF yielded a protein with an apparent M_r of 65,000. However, this observation does not prove that translation initiation occurs at the initiator methionine of the ORF, and there is no substantive evidence that the accumulating HSV-2 proteins are derived from a high-molecular-weight precursor. Initiation at the second methionine codon of the HSV-2 UL27.5 open reading frame would predict a protein much smaller than the HSV-2 protein accumulating in the infected cell. We cannot at this time exclude the possibility that translation initiation begins internally from an alternate translation initiation codon.

(iii) Most of the HSV-1 and HSV-2 U L27.5 accumulated in the cytoplasm. A small amount of HSV-2 U L27.5 protein migrating faster than the cytoplasmic protein partitioned in the nucleus. We should note that the amount of HSV-2 protein accumulating in infected cells is higher or reacts better with the antibody than the corresponding HSV-1 protein. It is conceivable that a small amount of HSV-1 U L27.5 also accumulates in the nucleus.

Because U L27.5 maps antisense to a gene essential for viral replication, it has not been possible at this time to determine whether the U L27.5 gene product is also essential.
ments now in progress should allow us to assess the role of this gene in cell culture and in an experimental animal system.

(iv) The discovery of a gene antisense to gB (UL27.5) was totally unexpected. Most analytical tools used to analyze nucleotide sequences are based on the assumption that a coding domain is present in the form of a linear array of nucleotides on one strand only. The gB-UL27.5 pair of antisense genes is the third set discovered within the HSV-1 genome (6, 20, 26). The ease with which they have been discovered in the past few years suggests that there may be more such pairs. Given the fact that the size of the capsids is conserved and virtually identical for all herpesviruses and that the capsids could package >240 kbp of DNA (e.g., the HCMV genome), the question arises as to why HSV encodes genes antisense to each other rather than stringing these ORFs in linear arrays. Among the many possible explanations, three are worth of further discussion.

The first, less-interesting hypothesis is that even the large herpesviruses contain genes arranged antisense to each other and that the actual number of genes in HCMV is grossly underestimated. It is conceivable that the antisense arrangements antedate the divergence of the primordial herpesvirus into the various subfamilies now in existence.

The second, more-attractive hypothesis is that the antisense arrangement is a form of regulation of gene expression that determines both the timing of synthesis and the abundance of the gene product. In the two preceding cases, that is, γ134.5/ ORF P and ORF O and UL43/UL43.5, we have found that the expression of the genes situated antisense to each other was sequential or even mutually exclusive (6, 20, 26). In this instance, gB is expressed very early in infection in abundant amounts, whereas the UL27.5 gene expression appears to be a late event, dependent on viral DNA synthesis. One test of the hypothesis would be to reverse the timing of the expression of the two genes to determine whether early expression of the UL27.5 is deleterious.

Lastly, the possibility that the sequences of two ORFs fit such that both proteins contain only the amino acid sequences essential for their function is probably remote. It is more likely that key amino acid domains of one protein correspond to neutral or linker domains in the product of the antisense ORF. Once the precise sequence encoding the UL27.5 protein is elucidated, it may be possible to probe more accurately the corresponding domains of the gB gene.

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