JOURNAL OF VIROLOGY, Oct. 1986, p. 185–193
0022-538X/86/100185-09$02.00/0

Vol. 60, No. 1

Use of λgt11 To Isolate Genes for Two Pseudorabies Virus Glycoproteins with Homology to Herpes Simplex Virus and Varicella-Zoster Virus Glycoproteins

ERIK A. PETROVSKIS, JAMES G. TIMMINS, AND LEONARD E. POST*

The Upjohn Company, Kalamazoo, Michigan 49001

Received 7 April 1986/Accepted 15 June 1986

A library of pseudorabies virus (PRV) DNA fragments was constructed in the expression cloning vector λgt11. The library was screened with antisera which reacted with mixtures of PRV proteins to isolate recombinant bacteriophages expressing PRV proteins. By the nature of the λgt11 vector, the cloned proteins were expressed in *Escherichia coli* as β-galactosidase fusion proteins. The fusion proteins from 35 of these phages were purified and injected into mice to raise antisera. The antisera were screened by several different assays, including immunoprecipitation of [^{14}C]glucosamine-labeled PRV proteins. This method identified phages expressing three different PRV glycoproteins: the secreted glycoprotein, gX; gI; and a glycoprotein that had not been previously identified, which we designate gp63. The gp63 and gI genes map adjacent to each other in the small unique region of the PRV genome. The DNA sequence was determined for the region of the genome encoding gp63 and gI. It was found that gp63 has a region of homology with a herpes simplex virus type 1 (HSV-1) protein, encoded by US7, and also with varicella-zoster virus (VZV) gpIV. The gI protein sequence has a region of homology with HSV-1 gE and VZV gpI. It is concluded that PRV, HSV, and VZV all have a cluster of homologous glycoprotein genes in the small unique components of their genomes and that the organization of these genes is conserved.

The glycoproteins of herpesviruses are incorporated into the membranes of infected cells and the viral envelope and have a variety of functions. Herpesvirus glycoproteins are best characterized in the prototype alphaherpesvirus, herpes simplex virus (HSV) (for a review, see reference 44). The functions of the HSV glycoproteins include binding the virus to the surface of cells (16, 41) and fusing infected cells (34). The glycoproteins are important in interactions with the host immune system. They are not only the targets of the host immune response (for a review, see reference 43), but individual HSV glycoproteins have been shown to interact with specific components of the immune system. HSV gE is a receptor for the Fc portion of immunoglobulins (1), and gC is a receptor for the C3b component of complement (15).

The structural and functional characterization of the HSV glycoproteins has focused primarily on the major glycoproteins, gB, gD, gE, and gC of HSV type 1 (HSV-1) and gG of HSV-2. Recent work has begun the characterization of low-abundance glycoproteins such as gG (38) and gH (7) of HSV-1. These minor glycoproteins were not discovered until specific monoclonal antibodies were isolated. In the course of sequencing large regions of the HSV genome, McGeoch et al. (28) discovered open reading frames that could code for unknown HSV glycoproteins. A detailed analysis of the sequence data led McGeoch to conclude that there very likely are additional HSV glycoproteins remaining to be characterized (27).

The glycoproteins of the porcine herpesvirus pseudorabies virus (PRV) are much less well characterized than those of HSV. One PRV glycoprotein gene, that for gII, codes for three PRV glycoproteins derived by posttranslational processing (19) and has been shown to have homology with the HSV gB gene (A. K. Robbins, R. J. Watson, and L. W. Enquist, European patent application 0162738). The gene for another major glycoprotein, gIII, has been mapped (40) and shown to have homology with the HSV gC gene (39). The gene for a minor PRV glycoprotein, gp50, was mapped by marker rescue of a mutation conferring resistance to a monoclonal antibody (48). The sequence of the gp50 gene showed that gp50 has a region homologous to HSV gD (36). It is not known whether gp50 is identical to gIV or gV characterized by Hampl et al. by mobility on 2-dimensional electrophoresis gels (19). The gene for the PRV secreted glycoprotein, gX, has been sequenced (37), but no homology with HSV glycoproteins has been noted. An additional PRV glycoprotein gene, that for gI, has been mapped (30), but homology to HSV glycoprotein genes has not been reported.

In view of the homology between the HSV and PRV glycoproteins characterized to date and the number of known and predicted glycoproteins in HSV, it seemed likely that there would be additional PRV glycoproteins beyond those already characterized. To isolate PRV glycoprotein genes we have used the λgt11 vector system of Young and Davis (50, 51). This system expresses inserted genes as β-galactosidase fusion proteins. This allows the isolation of genes for which suitable antibodies are available. Since the clones are isolated by expression of a protein in *Escherichia coli*, once they are isolated it becomes possible to purify these fusion proteins for use in raising monospecific antisera against the proteins. Therefore, if PRV genes are isolated by cloning in the expression cloning vector λgt11, such antisera could be raised to characterize the proteins coded by the cloned genes by analysis of the reactive proteins in PRV-infected cells. In particular, antisera raised against fusion proteins containing PRV glycoprotein sequences could be expected to react with [^{14}C]glucosamine-labeled proteins from PRV-infected cells.

In this report we describe the use of λgt11 to isolate a collection of PRV genes. By raising antibodies to the fusion proteins, we identified three glycoprotein genes: the previ-

* Corresponding author.

ously characterized gene for gX (37); the gene for gI, which on sequencing showed homology with the HSV gE gene; and the gene for a previously unidentified glycoprotein with homology to an HSV open reading frame for which no protein product has been detected.

MATERIALS AND METHODS

Cells and viruses. PRV Rice was originally obtained from D. P. Gustafson, Purdue University, West Lafayette, Ind. It was propagated on Vero cells, as previously described (37).

Construction and screening of λgt11 library. PRV Rice DNA was prepared as previously described (37). The DNA was sheared to a size range of 0.5 to 3.0 kilobases by sonication. After treatment with T4 DNA polymerase and the addition of *Eco*RI linkers, fragments in this desired size range were isolated by agarose gel electrophoresis. The fragments were purified from the agarose slices by the method of Vogelstein and Gillespie (46). The library was constructed by ligation of approximately 500 ng of PRV DNA fragments with 750 ng of *Eco*RI-digested λgt11 DNA in a 10-μl reaction mixture containing 50 mM Tris hydrochloride (pH 7.4), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM spermidine, 1 mM ATP, and 400 U of T4 DNA ligase (New England BioLabs, Inc.). After the ligation reaction proceeded overnight at 15°C, the DNA was packaged in bacteriophage lambda particles by using Packagene extract (Promega Biotec). The result was a library of 300,000 phages, 20% of which gave white plaques on X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) plates.

The library was screened by plating 20,000 plaques per 150-mm petri dish containing *E. coli* Y1090 by the procedure of Young and Davis (50, 51). The only antisera that successfully detected expression of PRV genes in the λgt11 library were the gel-slice antisera described previously (45). Positive plaques were picked, plaque purified, and rescreened until homogeneously positive. High-titer stocks of each positive phage were prepared by making plate lysates (25) on *E. coli* Y1090.

Antisera. To raise antisera, β-galactosidase fusion proteins were prepared from the λgt11-PRV recombinant phages. A fresh overnight culture of *E. coli* K95 (Sup^- λ^- Gal^- Str^r *nusA*; obtained from D. Friedman [14]) was diluted 1:50 in 250 ml of L broth and grown at 30°C to an A_{550} of 0.5. The culture was then infected at a multiplicity of infection of 5 and incubated in a shaking water bath at 42°C for 25 min followed by 37°C for 2 to 3 h. Before lysis began, the cells were harvested by centrifugation and suspended in 1 ml of 0.1 M Tris hydrochloride (pH 7.8)–0.3 M NaCl. An equal volume of 2× sodium dodecyl sulfate (SDS) electrophoresis sample buffer (33) was added, and the sample was boiled for 10 min followed by brief sonication to reduce viscosity. The extract was layered onto a 3-mm-thick 9% SDS-polyacrylamide electrophoresis gel (33). The band containing the β-galactosidase-PRV fusion protein was excised, and the protein was eluted as previously described (37).

Approximately 50 to 75 μg of fusion protein was suspended in complete Freund adjuvant and injected subcutaneously and intraperitoneally in female CD-1 mice. After 3 weeks and every 10 days thereafter the immunizations were repeated with incomplete Freund adjuvant until immunoprecipitating antiserum was obtained. An average of three to five immunizations with a fusion protein yielded a precipitating antiserum. Once it was determined that a mouse was producing a useful antiserum, an ascites tumor was induced to collect 20 to 50 ml of polyclonal antibodies per mouse (42).

Immunoprecipitations were performed with 10 to 20 μl of mouse antiserum (or ascites fluid) mixed with a [^{14}C]glucosamine-labeled extract of PRV-infected cells. These procedures were previously described (37), except for the following. The extracts were incubated with normal mouse serum and then with washed *Staphylococcus aureus* cells and centrifuged for 30 min in a Beckman SW50.1 rotor at 40,000 rpm. After the extracts were incubated with monoclonal or polyclonal antiserum plus *S. aureus* cells, the cells were washed three times in 10 mM Tris hydrochloride (pH 7.0)–1 mM EDTA–0.1 M NaCl–1% Nonidet P-40–0.5% deoxycholate. The immunoprecipitated proteins were analyzed on 11% SDS-polyacrylamide electrophoresis gels (33).

DNA analysis. The DNA cloning methods were standard techniques (25). The genomic DNA used for sequencing was plasmid pPR28, a clone of the *Bam*HI 7 fragment of PRV Rice (37), and subclones thereof. DNA sequencing was done totally by the method of Maxam and Gilbert (26), with all sequences determined at least twice on both strands. Because of the high G+C content of some regions of the sequence, it was necessary to run sequencing gels as warm as possible to obtain an unambiguous sequence.

RESULTS

Isolation of λgt11 recombinants expressing PRV glycoprotein genes. A library of PRV fragments cloned into λgt11 was constructed as described in Materials and Methods. The library was constructed to isolate genes for known glycoproteins and for minor glycoproteins that have not been characterized. To retain all possibilities, appropriate antisera for screening the library were polyclonal sera that reacted with many PRV proteins. Screening with sera from pigs which had recovered from PRV infection was unsuccessful because of the high background of antibody binding to all plaques, even after preadsorption with *E. coli* extracts. Sera from mice immunized with purified PRV virions or from rabbits immunized with PRV-infected cells detected no positive plaques and were negative even on a specially constructed λgt11 recombinant known to express a β-galactosidase-gX fusion protein. These negative results were presumed to be due to the inability of antibodies raised against native PRV proteins to recognize PRV proteins in the denatured form produced in *E. coli* (45). The antisera used to successfully detect λgt11 plaques producing β-galactosidase-PRV fusion proteins were the gel-slice antisera. These antisera were produced against molecular weight fractions of the total proteins from PRV-infected cells after the fractions were eluted from SDS-polyacrylamide electrophoresis gels (45). With the gel-slice sera, 43,000 λgt11-PRV recombinants were screened and 60 antibody-positive plaques were selected and plaque purified for further analysis.

Because the gel-slice sera reacted with many if not most PRV proteins, the positive plaques were identified only as synthesizing PRV-specific proteins, with no information as to which PRV proteins were being expressed. To identify the genes cloned and being expressed, *E. coli* cells infected with recombinant phages were used to produce fusion proteins. Examples of β-galactosidase-PRV fusion proteins are shown in Fig. 1; fusion proteins of various molecular weights were obtained depending on the size of the fused open reading frame. To obtain the level of fusion protein expression shown in Fig. 1, it was necessary to use an alternative *E. coli* host other than strain Y1089 (50). We found that, for unknown reasons, *E. coli* K95 (14) produced substantially higher levels of fusion proteins than strain Y1089 did. The

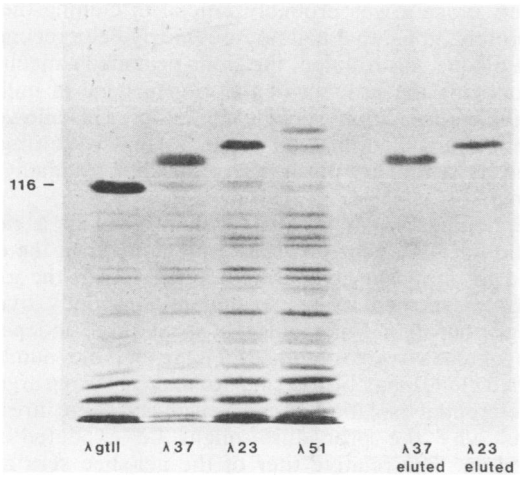

FIG. 1. SDS-polyacrylamide gel electrophoresis of *E. coli* extracts containing PRV-β-galactosidase fusion proteins. The first four lanes contained samples of extracts prepared from phage-infected *E. coli*, as described in Materials and Methods. The phages used to prepare the extracts are indicated. The two lanes on the right contained samples of fusion protein prepared for immunizations. The position of the 116-kDa marker, β-galactosidase, is indicated. A Coomassie blue-stained 9.25% polyacrylamide gel is shown.

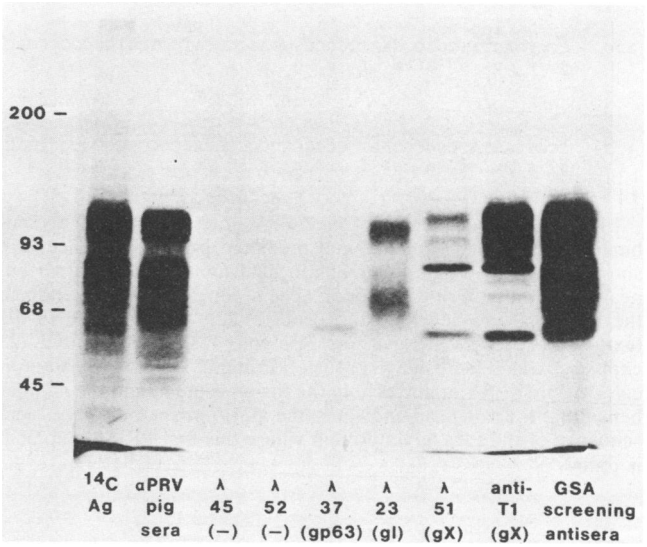

FIG. 2. Immunoprecipitation of PRV proteins with antisera raised against PRV-β-galactosidase fusion proteins. PRV-infected cells were labeled with [^{14}C]glucosamine, and an extract of these cells was used in the immunoprecipitation reactions. The extract is shown in the lane labeled ^{14}C Ag. The lane labeled αPRV pig sera shows the proteins immunoprecipitated with a mixture of sera from pigs which had recovered from PRV infection. The lanes labeled λ followed by a number indicate immunoprecipitation with an antiserum raised against the fusion protein produced by a particular λgt11-PRV recombinant. In parenthesis below the phage number is the name of the PRV protein recognized by the antiserum (if known). The lane labeled anti-T1 shows an immunoprecipitation with serum against a piece of the gX gene expressed in *E. coli* (37). The lane labeled GSA screening antisera shows an immunoprecipitation with the gel-slice antisera (45) used to screen the λgt11 library. An autoradiogram of an 11% polyacrylamide gel is shown. The positions of molecular size markers (in kilodaltons) are shown on the left.

expression level was sufficient to allow convenient preparation by preparative gel electrophoresis of milligram amounts of fusion proteins to immunize mice. Fusion proteins were prepared from 35 of the recombinant phages that produced substantial amounts of a fusion protein with a molecular weight significantly greater than that of β-galactosidase. These gel-isolated fusion proteins were then used for immunizations.

The antisera from the mice immunized with fusion proteins were screened by several methods. None of the antisera was capable of neutralizing PRV. The sera were used in Western blots of proteins from PRV-infected cells and in immunoprecipitation reactions with [^{35}S]methionine- and [^{14}C]glucosamine-labeled PRV proteins. By these methods, 19 of the sera were shown to recognize specific PRV proteins. Surprisingly, 15 of these 19 sera immunoprecipitated [^{14}C]glucosamine-labeled PRV proteins, indicating that most of the identified cloned genes coded for PRV glycoproteins.

Glycoproteins recognized by anti-fusion protein sera. The antisera that showed distinct patterns of immunoprecipitation of [^{14}C]glucosamine-labeled PRV proteins were selected for further analysis (Fig. 2). The glycoproteins immunoprecipitated by serum raised against the λ51 fusion protein (anti-λ51 serum) were similar to the various forms of gX found in infected cells (Fig. 2) (L. M. Bennett, J. G. Timmins, D. R. Thomsen, and L. E. Post, submitted for publication). Southern blots with ^{32}P-labeled λ51 DNA hybridized to PRV fragments showed that the DNA cloned into this phage mapped to the *Bam*HI and *Kpn*I fragments that include the gX gene (37). Because the gX gene was previously characterized, no further work was done with phages giving this immunoprecipitation pattern.

The second pattern of glycoprotein immunoprecipitation was that of anti-λ23 serum, which precipitated 110- and 75-kilodalton (kDa) glycoproteins. The DNA cloned in λ23 was mapped to *Bam*HI-7 by Southern blots. More detailed Southern blots showed that λ23 contained DNA spanning the *Sph*I cleavage site in *Bam*HI-7 (Fig. 3). Because of the pattern of immunoprecipitation and the map location of the cloned gene, it was concluded that λ23 represented the gI gene that was mapped by Mettenleiter et al. (30).

The third pattern of glycoprotein immunoprecipitation

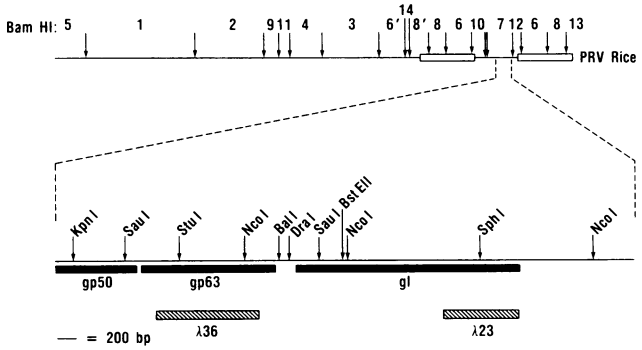

FIG. 3. Restriction enzyme cleavage sites in PRV genome region including the gp63 and gI genes. The upper line shows the map of the *Bam*HI cleavage sites of the PRV Rice genome (37), with the inverted repeats of the genome indicated by boxes. The lower line shows an expansion of the region of the genome which includes the gp63 and gI genes. The hatched bars under the expanded map indicate the regions of DNA cloned into the λgt11-PRV recombinants used to map and sequence the genes. The position of the gp50 gene is from reference 36. bp, Base pairs.

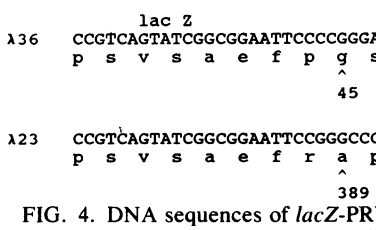

FIG. 4. DNA sequences of *lacZ*-PRV junctions in λgt11 recombinants. The DNA sequence and predicted protein sequences are shown, with the single-letter amino acid abbreviation shown beneath the first base of each codon. The reading frame is taken from the known sequence of β-galactosidase (22). As described in the text, λ36 expresses a β-galactosidase-gp63 fusion protein and λ23 expresses a β-galactosidase-gI fusion protein. The arrowhead indicates the first PRV amino acid in the fusion protein, and the number beneath the arrowhead indicates the position in the amino acid sequence of the PRV glycoprotein where this first PRV amino acid is found.

was that shown with anti-λ36 and anti-λ37 sera. These sera immunoprecipitated a glycoprotein of 63 kDa that is distinct from known PRV glycoproteins. We refer to this glycoprotein as gp63. The PRV DNA in λ36 and λ37 also mapped to the *Bam*HI 7 fragment of the PRV genome. Detailed Southern blotting with ^{32}P-labeled λ36 DNA showed that λ36 contained DNA from both sides of the *Stu*I site (Fig. 3) within *Bam*HI-7. This placed the gp63 gene between the gI and gX genes. Although the gp50 gene also is between the gX and gI genes, the gp50 gene does not extend to the *Stu*I cleavage site (36).

DNA sequencing of the gp63 and gI genes. The *Sac*I-*Kpn*I fragments of λ23 and λ36 containing the inserted PRV DNA were subcloned into pUC18 (49) for convenient manipulation. By labeling the *Nde*I cleavage site in the β-galactosidase gene (22) with ^{32}P, we determined the sequence across the *lacZ*-PRV DNA junction by the method of Maxam and Gilbert (26). Since the reading frame of the *lacZ* gene is known, the reading frames of the gp63 and gI genes could be determined as the continuation of the *lacZ* frame. The DNA sequences of the *lacZ*-PRV DNA junctions in λ36 and λ23 are shown in Fig. 4.

The DNA sequences of the gp63 and gI genes were determined by sequencing the PRV genomic DNA surrounding the fragments that were cloned into λ36 and λ23 (Fig. 5). The gp63 open reading frame codes for 350 amino acids downstream from the first of a series of three possible initiation codons. The open reading frame for gI codes for 577 amino acids downstream from an initiation codon.

The gp63 amino acid sequence includes a region homologous to the protein encoded by the US7 open reading frame of HSV-1 (28) and to the gpIV glycoprotein of VZV (9, 10) (Fig. 6). The gI amino acid sequence has a region homologous to HSV-1 gE (28) and to VZV gpI (9, 10) (Fig. 7). Features of these homologies are discussed below.

DISCUSSION

The experiments presented in this report illustrate some of the most important strengths and limitations of the λgt11 cloning system. The most striking limitation is the previously reported importance of the quality of the screening antisera (45), which must recognize the target protein in the denatured form produced in *E. coli*. One of the strengths was the ability to screen the library with a polyclonal antiserum that reacted with many different PRV proteins. The use of such complex antisera was probably crucial in cloning the gene for a protein, gp63, that had not previously been recognized. Once a plaque was isolated, the clone provided a mechanism for producing the product of the cloned gene in milligram amounts to raise a monospecific antiserum. This allowed the straightforward identification of clones that were originally isolated as reactive with an antiserum against many PRV proteins.

The cloning procedure used did not isolate a random collection of PRV genes. A surprising number of the clones seemed to represent glycoprotein genes. Also, the gX and gp63 genes seemed to be isolated preferentially over the other glycoprotein genes since at least three independent clones of each were obtained. However, the number of identified clones may be too small to make a strong argument for preferential isolation of some genes. There are many reasons why the procedure might be expected to be nonrandom. The relative titer of the gel-slice sera against various proteins is not known. The study that led to the use of gel-slice sera showed that all antibodies that recognize a protein do not necessarily recognize antigen produced by a λgt11 clone (45). Conversely, antisera raised against the λgt11 fusion protein may not efficiently recognize the native protein; this could have prevented identification of some of the clones. Although there are many reasons why a given gene might not be cloned by the approach described in this paper, the method did allow cloning of a gene whose protein product was previously unknown.

Another useful feature of the λgt11 cloning system for this work was the identification of the reading frame in the DNA sequence that codes for the gene product. This is not a trivial problem in DNA such as that of PRV which is very G+C rich (3), since the result is that out-of-frame termination codons are rare (e.g., see references 36, 37, and 39 and Robbins et al., European patent application 0162738). It has been reported that a gene cloned and expressed in the λgt11 system oriented such that the β-galactosidase open reading frame was not fused to the gene of interest (8). However, the identification of the genes by raising antibodies to purified fusion proteins ensures that the gene of interest is fused in phase to the β-galactosidase reading frame.

Once the coding reading frames were identified, it was possible to determine the sequence of the gp63 and gI genes by sequencing PRV genomic DNA. In the gp63 gene, the reading frame is free of termination codons until far upstream of the λ36 inserted fragment. The ATG farthest upstream in that reading frame, however, is that indicated in Fig. 5. Interestingly, there are three consecutive ATG codons at that point. There is no evidence indicating which of these codons is used for initiation of gp63 translation. Similarly, the initiation codon farthest upstream in the open reading frame of the gI gene is indicated in Fig. 5. The assignment of these reading frames as coding for the gp63 and gI proteins rests on the assumption that the coding sequences for gp63 and gI are not produced by RNA splicing. Although unspliced mammalian transcripts are, in general, rare, most HSV mRNAs are unspliced (47). The unspliced HSV mRNAs include those coding for proteins with homology to gp63 and gI (discussed below), so the assignment of these reading frames as the coding sequences is most likely correct.

The predicted protein sequences for both gp63 and gI have features in common with most membrane proteins. Both have basic amino acids shortly after the initiator methiodine, which are followed by hydrophobic amino acids, like typical signal sequences of membrane proteins. Near the C-terminal

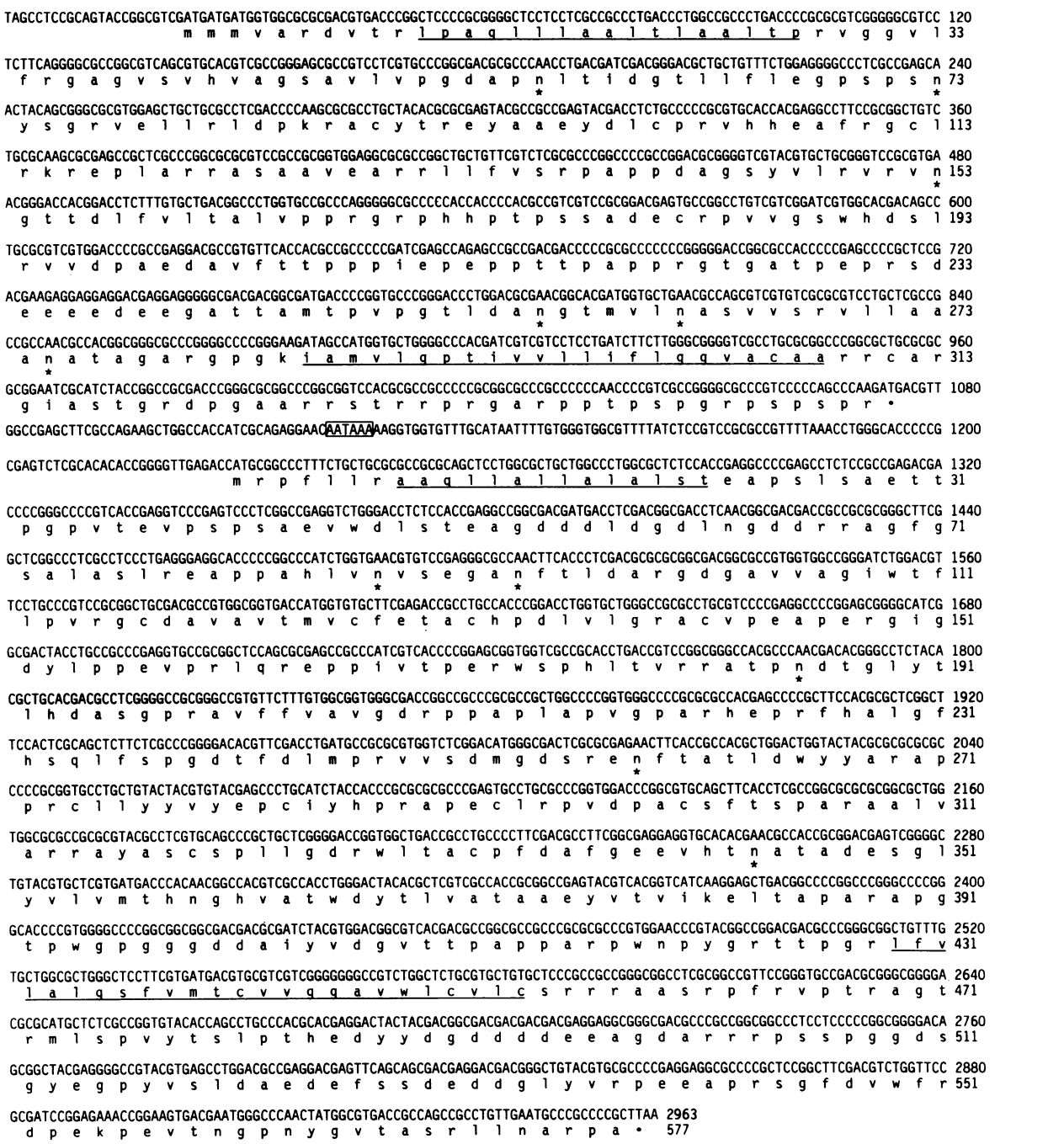

FIG. 5. DNA sequence of the region of the PRV genome encoding gp63 and gI. The DNA sequence is shown with the predicted amino acid sequence beneath it (the single-letter amino acid abbreviation is shown beneath the first base of each codon). The sequence begins with the termination codon of the upstream gp50 gene (36) and ends with the termination codon of the gI gene. Positions in the total DNA sequence and within each amino acid sequence are shown at the right of each line. The hydrophobic amino acid sequences which may constitute signal sequences or transmembrane domains of each protein are underlined. The asparagines which may be sites of N-linked glycosylation are indicated by asterisks.

ends of both gp63 and gI are very hydrophobic sequences, probably representing membrane-spanning segments, followed by hydrophilic sequences which probably are cytoplasmic domains. The cytoplasmic domain of gp63 is very basic, with 12 of 42 amino acids being arginine and with no acidic amino acids. The cytoplasmic domain of gI is long, consisting of 114 amino acids. In gI this domain is quite acidic, with 26 of 114 amino acids being aspartic or glutamic acid, although this large number of acidic residues is partially balanced by 15 basic amino acids.

As is true for the entire PRV genome (3), the gp63 and gI genes are extremely rich in guanine and cytosine. The gp63 and gI genes have G+C contents of 77 and 75%, respectively. The codon usage in these genes is overwhelmingly ... favor of codons with G or C in the third position. In the gp63 and gI genes 96 and 98% of the codons, respectively, have G

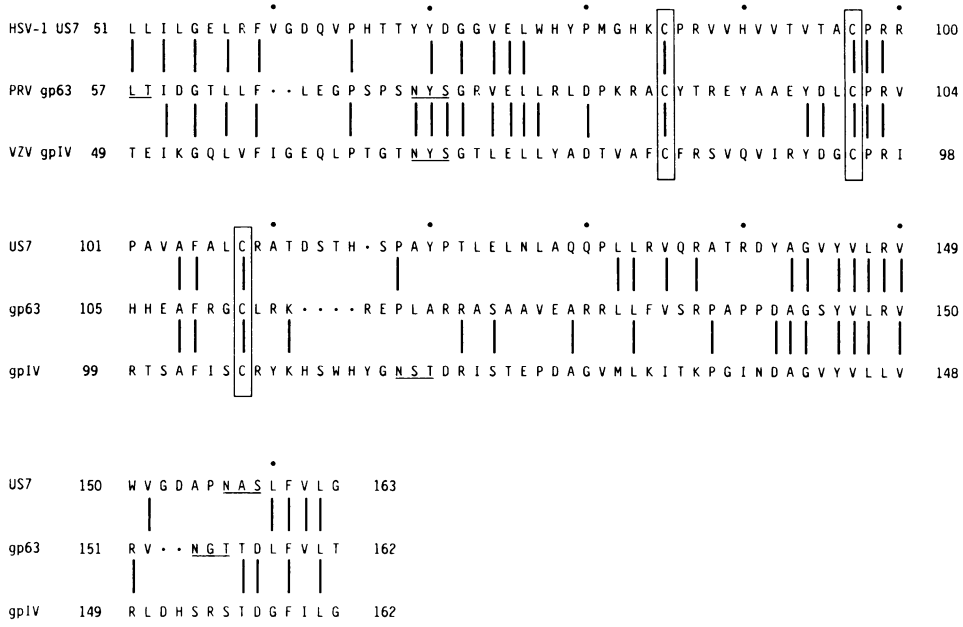

FIG. 6. Homology of gp63 with HSV-1 US7 and VZV gpIV. The sequences are aligned to illustrate the maximum homology, with dots indicating small gaps introduced in the sequence for the purpose of alignment. The US7 sequence is from reference 28, and the gpIV sequence is the 39-kDa sequence in reference 9. The numbers at the beginning and end of each line indicate the positions in the amino acid sequences. A line between an amino acid in gp63 and one in one of the other sequences indicates homology. Potential N-linked glycosylation sites are underlined, and cysteine residues are boxed.

or C in the third position. As observed for the gX (37) and gp50 (36) sequences, the G+C content is high enough to be reflected in more than the third position of the codon. Amino acids with G+C-rich codons are very abundant in these proteins. The predicted gp63 sequence has 13% alanine, 12% proline, and 11% arginine. The predicted gI sequence has 12% alanine, 11% proline, 8% glycine, and 8% arginine.

The gp63 amino acid sequence has significant homology with the protein product of an open reading frame, US7, in the small unique region of the HSV-1 genome (28) (Fig. 6). The US7 open reading frame does not code for any known HSV proteins, but McGeoch predicted that it codes for a glycoprotein which he designated 41K (27). gp63 is also homologous to the protein encoded by an open reading frame in the small unique region of the VZV genome (Fig. 6). This open reading frame was designated 39 kDa by Davison (9) and shown to encode a glycoprotein (11) that was named gpIV in a new nomenclature system (10). Three cysteine residues align in the region of homology (Fig. 6). In addition, a fourth cysteine (residues 182 of gp63 and 41K) aligns in a comparison of the PRV and HSV proteins; a fourth cysteine (residue 200) is somewhat out of alignment in the case of VZV gpIV. gp63 and 41K have two and three cysteines, respectively, near the junction of the transmembrane and cytoplasmic domains, although these are not present in gpIV. Thus, the alignment of cysteines is not as complete as that observed between PRV gp50 and HSV gD (36). The positions of the N-linked glycosylation sites among the three proteins are not closely conserved, although the asparagines at positions 73 of gp63 and 67 of gIV fall in very close alignment with each other, as do the sites at positions 153 of gp63 and 156 of US7. It is important that the only region of significant homology between gp63 and the other glycoproteins is that shown in Fig. 6. The conservation of this region in three different viruses indicates that these sequences may be important in some common function of the three glycoproteins.

There is a region of significant homology between PRV gI and HSV-1 gE (Fig. 7) (28). The same region of gI is homologous to a region of the VZV protein designated 70K in the original sequencing report (9). In the new nomenclature system this protein is designated gpI (10) and is identified as one of the VZV glycoproteins that has been characterized by monoclonal antibodies (32) and expression in *E. coli* (13). By insertion of minor gaps in the alignment, six cysteine residues can be aligned, indicating the conservation of what are likely some important disulfide bonds for the structures of these glycoproteins. Again, the conservation of cysteines is not complete throughout the proteins since the four cysteines between amino acids 117 and 141 in PRV gI have no counterparts in HSV gE; there is a similar pattern of four cysteines between amino acids 246 and 274 of VZV gpI, however, that may serve a similar function. All three glycoproteins have cysteine residues in or near the transmembrane sequences. The positions of the sites for N-linked glycosylation are not conserved among these three glycoproteins. As in the case of the gp63 alignments, the region shown in Fig. 7 is the only significant region of homology of gI with the other two glycoproteins, perhaps indicating a special conservation of an important structure for the function of these glycoproteins. For VZV gpI, the region of homology appears in a different position in the protein sequence than that in the PRV or HSV proteins. HSV gE has been shown to function as a receptor for the Fc component of immunoglobulin molecules (1). There are conflicting reports on whether VZV produces an Fc receptor (17, 21, 35), and we are aware of no information on whether PRV encodes an Fc receptor. Clearly, PRV gI and VZV gpI would be candidates for Fc receptor molecules in these viruses.

We previously reported a region of homology between

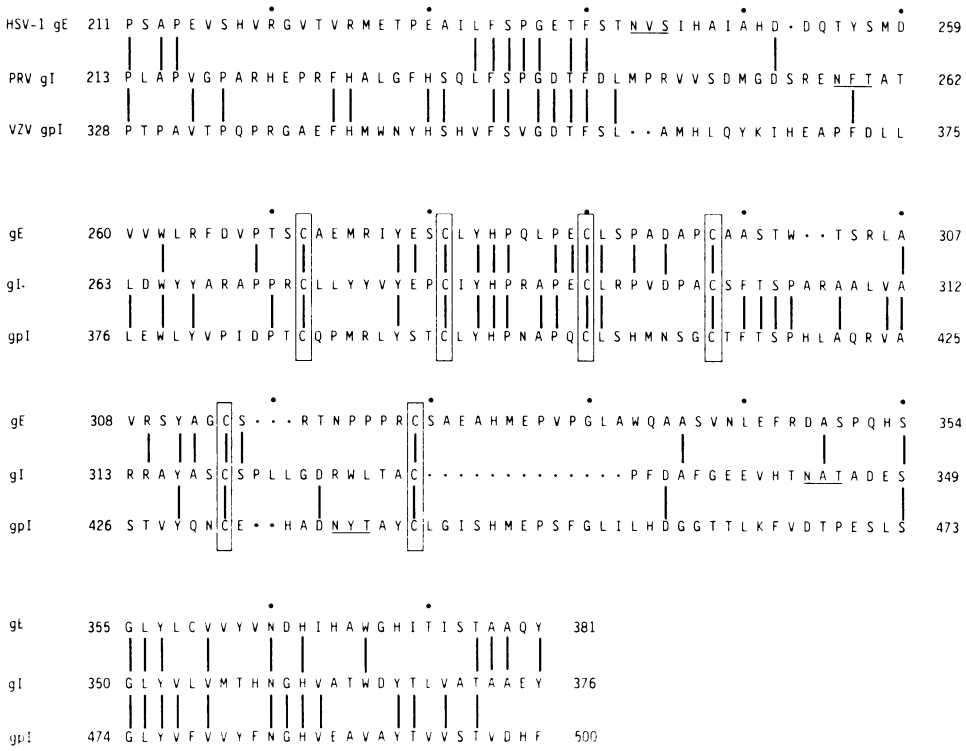

FIG. 7. Homology of gI with HSV-1 gE and VZV gpI. The sequences are aligned to illustrate the maximum homology, with dots indicating small gaps introduced in the sequence for the purpose of alignment. The gE sequence is from reference 28, and the gpIV sequence is the 70-kDa sequence in reference 9. The numbers at the beginning and end of each line indicate the positions in the amino acid sequences. A line between an amino acid in gI and one in one of the other sequences indicates homology. Potential N-linked glycosylation sites are underlined, and cysteine residues are boxed.

PRV gp50 and HSV gD (36). With the sequence homologies reported here, it is apparent that three glycoproteins encoded in the small unique components of the HSV and PRV genomes have homology. The genes for these homologous glycoproteins are arranged similarly on the genomes (Fig. 8). From what can be deduced from the DNA sequences, the transcriptional organization of these genes is probably also similar. In HSV, the gD gene has no polyadenylation signal immediately after it but instead is coterminal with the transcript for the 41K gene (28). Similarly, there is no AATAAA sequence, found in nearly all polyadenylation signals (for a review, see reference 6), between the gp50 and gp63 genes (Fig. 5). There is, however, an AATAAA sequence 46 bases after the termination codon of the gp63 gene (Fig. 5), and following that AATAAA there are three sequences, TGGTGTTT, TGTGGGTG, and GGCGTTTT, which are similar to the YGTGTTYY consensus sequence of polyadenylation signals (29). Therefore, it seems likely that there is a polyadenylation signal between the gp63 and gI genes, which is the polyadenylation signal for the coterminal gp50 and gp63 transcripts. Given the similar organization of gp50, gp63, and gI and their HSV counterparts, it is tempting to speculate that gX is a homolog of HSV gG or the US7 glycoprotein or both (28). However, we have been unable to find significant homology between gX and any sequenced HSV protein. The VZV gpIV and gpI genes have a similar arrangement on the genome to their homologs in PRV and HSV, although there is no homolog of the gp50 or gD gene in this region of the VZV genome. The conservation of the organization of these three homologous genes confirms early impressions that PRV and HSV may have similar organization of their genomes (4, 12). Given that VZV shares a similar organization of its two homologous genes, it seems likely that many alphaherpesviruses have a similar organization of some genes.

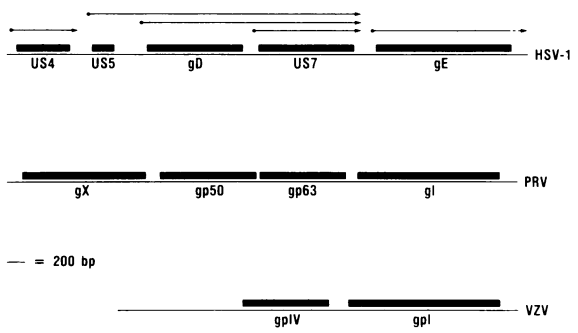

FIG. 8. Organization of glycoprotein genes in the small unique components of the genomes of three alphaherpesviruses. The organization of the HSV-1 genes, along with arrows indicating the transcripts, is taken from reference 28. The organization of the PRV genes is from this report and references 36 and 37. The organization of the VZV genes is from reference 9.

The gI gene is nonessential for PRV replication in culture, since it is deleted from the vaccine strains Norden and Bartha (23, 31). There is evidence that this gene is involved in the virulence of PRV (5, 24). Since the Bartha strain contains a deletion of nearly 4 kilobases within *Bam*HI-7 and this deletion has been reported to lie entirely in the *Bam*HI-*Kpn*I fragment containing the gI and gp63 genes (18, 20, 23,

24), it appears that at least part of the gp63 gene has been deleted in the Bartha strain. It is possible that gp63 is the function deleted in the Bartha strain, but not in the Norden strain, that is important for virus exit from some cell types (2). Given the homology of PRV gp63 and gI with glycoproteins of other herpesviruses, these biological data on PRV glycoproteins suggest some directions for experimentation with the other herpesviruses.

ACKNOWLEDGMENTS

We thank D. Court for helpful discussions that led to the use of *E. coli* K95 for fusion protein production and D. Friedman for providing strain K95; G. W. Bolton for providing sarcoma 180/TG cells for ascites production; R. Poorman for valuable advice on alignment of protein sequences; D. Thomsen, C. Marchioli, and M. Wathen for helpful comments on the manuscript; and K. Hiestand for typing the manuscript and figures.

LITERATURE CITED

1. **Baucke, R. B., and P. G. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. V. Identification of an Fc-binding glycoprotein. J. Virol. **32**:779–789.
2. **Ben-Porat, T., J. DeMarchi, J. Pendrys, R. A. Veach, and A. S. Kaplan.** 1986. Proteins specified by the short unique region of the genome of pseudorabies virus play a role in the release of virions from certain cells. J. Virol. **57**:191–196.
3. **Ben-Porat, T., and A. S. Kaplan.** 1962. The chemical composition of herpes simplex and pseudorabies virus. Virology **16**:261–266.
4. **Ben-Porat, T., R. A. Veach, and S. Ihara.** 1983. Localization of the regions of homology between the genomes of herpes simplex virus, type 1, and pseudorabies virus. Virology **127**:194–204.
5. **Berns, A., A. van den Ouweland, W. Quint, J. van Oirschot, and A. Gielkens.** 1985. Presence of markers for virulence in the unique short region or repeat region or both of pseudorabies hybrid viruses. J. Virol. **53**:89–93.
6. **Birnstiel, M. L., M. Busslinger, and K. Strub.** 1985. Transcription termination and 3′ processing: the end is in site! Cell **41**:349–359.
7. **Buckmaster, E. A., U. Gompels, and A. Minson.** 1984. Characterization and physical mapping of an HSV-1 glycoprotein of approximately 115 × 10^3 molecular weight. Virology **139**: 408–413.
8. **Dame, J. B., J. L. Williams, T. F. McCutchan, J. L. Weber, R. A. Wirtz, W. T. Hockmeyer, W. L. Maloy, J. D. Haynes, I. Schneider, D. Roberts, G. S. Sanders, E. Premkumar-Reddy, C. L. Diggs, and L. H. Miller.** 1984. Structure of the gene encoding the immunodominant surface antigen on the sporozoite of the human malaria parasite *Plasmodium falciparum*. Science **225**:593–599.
9. **Davison, A. J.** 1983. DNA sequence of the U_L component of the varicella-zoster virus genome. EMBO J. **2**:2203–2209.
10. **Davison, A. J., C. M. Edson, R. W. Ellis, B. Forghani, D. Gilden, C. Grose, P. M. Keller, A. Vafai, Z. Wroblewska, and K. Yamanishi.** 1986. New common nomenclature for glycoprotein genes of varicella-zoster virus and their glycosylated products. J. Virol. **57**:1195–1197.
11. **Davison, A. J., D. J. Waters, and C. M. Edson.** 1985. Identification of the products of a varicella-zoster virus glycoprotein gene. J. Gen. Virol. **66**:2237–2242.
12. **Davison, A. J., and N. M. Wilkie.** 1983. Location and orientation of homologous sequences in the genomes of five herpesviruses. J. Gen. Virol. **64**:1927–1942.
13. **Ellis, R. W., P. M. Keller, R. S. Lowe, and R. A. Zivin.** 1985. Use of a bacterial expression vector to map the varicella-zoster virus major glycoprotein gene, gC. J. Virol. **53**:81–88.
14. **Friedman, D. I., and L. S. Baron.** 1974. Genetic characerization of a bacterial locus involved in the activity of the N function of bacteriophage λ. Virology **58**:141–148.
15. **Friedman, H. M., G. H. Cohen, R. J. Eisenberg, C. A. Seidel, and D. B. Cines.** 1984. Glycoprotein C of herpes simplex virus I acts as a receptor for the C3b complement component on infected cells. Nature (London) **309**:633–635.
16. **Fuller, A. O., and P. G. Spear.** 1985. Specificities of monoclonal and polyclonal antibodies that inhibit adsorption of herpes simplex virus to cells and lack of inhibition by potent neutralizing antibodies. J. Virol. **55**:475–482.
17. **Gelb, L. D., W. J. Wellinghoff, J. H. Martin, and J. J. Huang.** 1981. Varicella-zoster virus fails to induce immunoglobulin G Fc receptors in infected human cells. Proc. Soc. Exp. Biol. Med. **168**:228–232.
18. **Gielkens, A. L. J., J. T. VanOirschot, and A. J. M. Berns.** 1985. Genome differences among field isolates and vaccine strains of pseudorabies virus. J. Gen. Virol. **66**:69–82.
19. **Hampl, H., T. Ben-Porat, L. Ehrlicher, K.-O. Habermehl, and A. S. Kaplan.** 1984. Characterization of the envelope proteins of pseudorabies virus. J. Virol. **52**:583–590.
20. **Herrmann, S.-C., B. Heppner, and H. Ludwig.** 1984. Psuedorabies viruses from clinical outbreaks and latent infections grouped into four major genome types, p. 387–401. *In* G. Wittmann, R. M. Gaskell, and H.-J. Rziha (ed.), Latent herpes virus infections in veterinary medicine. Martinus Nijhoff, Publishers, Dordrecht, The Netherlands.
21. **Ishak, R., W. A. Andiman, and G. Tucker.** 1984. Absence of IgG Fc receptors on varicella-zoster virus-infected cells. J. Med. Virol. **13**:261–267.
22. **Kalnins, A., K. Otto, U. Ruther, and B. Muller-Hill.** 1983. Sequence of the *lacZ* gene of *Escherichia coli*. EMBO J. **2**:593–597.
23. **Lomniczi, B., M. L. Blankenship, and T. Ben-Porat.** 1984. Deletions in the genomes of pseudorabies virus vaccine strains and existence of four isomers of the genomes. J. Virol. **49**:970–979.
24. **Lomniczi, B., S. Watanabe, T. Ben-Porat, and A. S. Kaplan.** 1984. Genetic basis of the neurovirulence of pseudorabies virus. J. Virol. **52**:198–205.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
27. **McGeoch, D. J.** 1985. On the predictive recognition of signal peptide sequences. Virus Res. **3**:271–286.
28. **McGeoch, D. J., A. Dolan, S. Donald, and F. J. Rixon.** 1985. Sequence determination and genetic content of the short unique region in the genome of herpes simplex virus type 1. J. Mol. Biol. **181**:1–13.
29. **McLaughlin, J., D. Gaffrey, J. L. Whitton, and J. B. Clements.** 1985. The consensus sequence YGTGTTYY located downstream from the AATAAA signal is required for efficient formation of mRNA 3′ termini. Nucleic Acids Res. **13**:1347–1368.
30. **Mettenleiter, T. C., N. Lukacs, and H.-J. Rziha.** 1985. Mapping of the structural gene of pseudorabies virus glycoprotein A and identification of two non-glycosylated precursor polypeptides. J. Virol. **53**:52–57.
31. **Mettenleiter, T. C., N. Lukàcs, and H.-J. Rziha.** 1985. Pseudorabies virus avirulent strains fail to express a major glycoprotein. J. Virol. **56**:307–311.
32. **Montalvo, E. A., R. T. Parmley, and C. Grose.** 1985. Structural analysis of the varicella-zoster virus gp98-gp62 complex: post-translational addition of *N*-linked and *O*-linked oligosaccharide moieties. J. Virol. **53**:761–770.
33. **Morse, L. S., L. Pereira, B. Roizman, and P. A. Schaffer.** 1978. Anatomy of herpes simplex virus (HSV) DNA. X. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 × HSV-2 recombinants. J. Virol. **26**:389–410.
34. **Noble, A. G., G. T.-Y. Lee, R. Sprague, M. L. Parish, and P. G. Spear.** 1983. Anti-gD monoclonal antibodies inhibit cell fusion induced by herpes simplex virus type 1. Virology **129**:218–224.
35. **Ogata, M., and S. Shigeta.** 1979. Appearance of immunoglobulin G Fc receptor in cultured human cells infected with varicella-zoster virus. Infect. Immun. **26**:770–774.

36. **Petrovskis, E. A., J. G. Timmins, M. A. Armentrout, C. C. Marchioli, R. J. Yancey, Jr., and L. E. Post.** 1986. DNA sequence of the gene for pseudorabies virus gp50, a glycoprotein gene without N-linked glycosylation. J. Virol. **59**:216–223.
37. **Rea, T. J., J. G. Timmins, G. W. Long, and L. E. Post.** 1985. Mapping and sequence of the gene for the pseudorabies virus glycoprotein which accumulates in the medium of infected cells. J. Virol. **54**:21–29.
38. **Richman, D. D., A. Buckmaster, S. Bell, C. Hodgman, and A. C. Minson.** 1986. Identification of a new glycoprotein of herpes simplex virus type 1 and genetic mapping of the gene that codes for it. J. Virol. **57**:647–655.
39. **Robbins, A. K., R. J. Watson, M. E. Whealy, W. W. Hays, and L. W. Enquist.** 1986. Characterization of a pseudorabies virus glycoprotein gene with homology to herpes simplex virus type 1 and type 2 glycoprotein C. J. Virol. **58**:339–347.
40. **Robbins, A. K., J. H. Weis, L. W. Enquist, and R. J. Watson.** 1984. Construction of *E. coli* expression plasmid libraries: localization of a pseudorabies virus glycoprotein gene. J. Mol. Appl. Genet. **2**:485–496.
41. **Sarmiento, M., H. Haffey, and P. G. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. III. Role of glycoprotein VP7 (B_2) in virion infectivity. J. Virol. **29**: 1149–1158.
42. **Sartorelli, A. C., D. S. Fischer, and W. G. Downs.** 1966. Use of sarcoma 180/TG to prepare hyperimmune ascitic fluid in the mouse. J. Immunol. **96**:676–682.
43. **Spear, P. G.** 1985. Antigenic structure of herpes simplex viruses, p. 425–443. *In* M. H. V. Van Regenmortel and A. R. Neurath (ed.), The basis for serodiagnosis and vaccines, vol. 23. Immunochemistry of viruses. Elsevier Science Publishers, Amsterdam.
44. **Spear, P. G.** 1985. Glycoproteins specified by herpes simplex viruses, p. 315–356. *In* B. Roizman (ed.), The herpesviruses, vol. 3. Plenum Publishing Corp., New York.
45. **Timmins, J. G., E. A. Petrovskis, C. C. Marchioli, and L. E. Post.** 1985. A method for efficient gene isolation from phage λgt11 libraries: use of antisera to denatured acetone-precipitated proteins. Gene **39**:89–93.
46. **Vogelstein, B., and D. Gillespie.** 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA **76**:615–619.
47. **Wagner, E. K.** 1985. Individual HSV transcripts. Characterization of specific genes, p. 45–104. *In* B. Roizman (ed.), The herpesviruses, vol. 3. Plenum Publishing Corp., New York.
48. **Wathen, M. W., and L. M. K. Wathen.** 1984. Isolation, characterization, and physical mapping of a pseudorabies virus mutant containing antigenically altered gp50. J. Virol. **51**:57–62.
49. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
50. **Young, R. A., and R. W. Davis.** 1983. Efficient isolation of genes by using antibody probes. Proc. Natl. Acad. Sci. USA **80**:1194–1198.
51. **Young, R. A., and R. W. Davis.** 1983. Yeast RNA polymerase II genes: isolation with antibody probes. Science **222**:778–782.