JOURNAL OF VIROLOGY, Aug. 1991, p. 4275–4283
0022-538X/91/084275-09$02.00/0

Vol. 65, No. 8

Oligomer Formation of the gB Glycoprotein of Herpes Simplex Virus Type 1

STEVEN L. HIGHLANDER,[1] WILLIAM F. GOINS,[2] STANLEY PERSON,[2] THOMAS C. HOLLAND,[3] MYRON LEVINE,[4] AND JOSEPH C. GLORIOSO[2]*

Unit for Laboratory Animal Medicine[1] and Department of Human Genetics,[4] University of Michigan Medical School, Ann Arbor, Michigan 48109; Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261[2]; and Department of Microbiology and Immunology, Wayne State University Medical School, Detroit, Michigan 48120[3]

Received 8 January 1991/Accepted 19 April 1991

Oligomer formation of the gB glycoprotein of herpes simplex virus type 1 was studied by sedimentation analysis of radioactively labeled infected cell and virion lysates. Fractions from sucrose gradients were precipitated with a pool of gB-specific monoclonal antibodies and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Pulse-labeled gB from infected cells was synthesized as monomers and converted to oligomers posttranslationally. The oligomers from infected cells and from virions sedimented as dimers, and there was no evidence of higher-molecular-weight forms. To identify amino acid sequences of gB that contribute to oligomer formation, pairs of mutant plasmids were transfected into Vero cells and superinfected with a gB-null mutant virus to stimulate plasmid-specified gene expression. Radioactively labeled lysates were precipitated with antibodies and examined by SDS-PAGE. Polypeptides from cotransfections were precipitated with an antibody that recognized amino acid sequences present in only one of the two polypeptides. A coprecipitated polypeptide lacking the antibody target epitope was presumed to contain the sequences necessary for oligomer formation. Using this technique, two noncontiguous sites for oligomer formation were detected. An upstream site was localized between residues 93 and 282, and a downstream site was localized between residues 596 and 711. Oligomer formation resulted from molecular interactions between two upstream sites, between two downstream sites, and between an upstream and a downstream site. A schematic diagram of a gB oligomer is presented that is consistent with these data.

Glycoproteins located in the envelopes of viruses and in the membranes of infected cells aid in the transport of nucleocapsids into cells, out of cells, and from cell to cell. They are also targets of the host immune system and function in modulating host immunity. For herpes simplex virus type 1 (HSV-1), seven glycoproteins, designated gB, gC, gD, gE (see, for example, reference 53), gG (45), gH (2), and gI (31, 32), have been identified. Open reading frame analysis of the genome sequence reveals several additional candidate glycoproteins (35, 36). Of these, gB, gD, and gH are essential for viral growth in tissue culture (16, 30, 50, 51). Deletion mutants that lack gB (8) or gD (30) form enveloped virions, as does a temperature-sensitive mutant of gH when grown at the nonpermissive temperature (16).

Virus entry is initiated by fusion between viral and plasma membranes and appears to be a complex process as regards HSV-1 glycoproteins. Glycoprotein gB of HSV-1 promotes virus entry (33); single-amino-acid differences within the gB polypeptide alter the rate of entry (4, 15, 25) and the extent of virus-induced cell fusion (4, 6). Glycoprotein gD seems to be essential for cell fusion (30), although no gD-coding sequences have been identified that induce cell fusion (20). Deletion mutants that lack gB (8) or gD (30) are defective in virus entry. Neutralizing monoclonal antibodies (MAbs) to gD (38, 40) and to gH (22) have been identified that block cell fusion. MAbs to gB do not block cell fusion but have been found to alter the rate of virus entry (24). Antibodies to gB, gD, or gH have been reported that reduce plaque size, which indicates that these glycoproteins affect the spread of virus (22, 24). Although experiments to date have focused on individual glycoproteins, glycoproteins may interact with each other and/or perhaps with other proteins in the performance of their biological functions. Recently, Gompels and Minson (23) have reported that the transport of gH to the cell surface requires additional viral factors.

HSV-1 gB consists of 904 amino acids including a 30-amino-acid cleaved signal sequence, an external N-terminal domain, a long hydrophobic transmembrane region (residues 727 through 795), and a 109-residue C-terminal cytoplasmic domain (3, 5, 7, 12). To date, the only gB functions identified are related to virus entry and cell fusion. Wild-type gB has been shown to be required for cell fusion. Mutations that increase the cell fusion function are located in the cytoplasmic domain; those that decrease cell fusion are located in the external domain. The fusion and essential functions of gB have not yet been separated (see, for example, reference 6).

The gB gene is conserved among all herpesviruses examined. These include the five common human herpesviruses (3, 4, 14, 28, 36, 42, 43, 55), human herpesvirus 6 (40a), several simian herpesviruses (18), equine herpesvirus 1 and equine herpesvirus 4 (46, 57), bovine herpesvirus 1 (39, 59), feline herpesvirus (52a), Marek's disease virus (48), and pseudorabies virus (47). Ten homologs have been sequenced. The high degree of conservation between them suggests a common role for gB, likely in virus entry.

Complementation inhibition data indicated that the functional state of gB was an oligomer (7). Oligomers of gB have been reported based on polyacrylamide gel electrophoresis (PAGE) analysis of infected cell lysates. In these studies, heat-denatured gB migrated as a dimer, although higher-molecular-weight forms were sometimes observed (10, 11,

* Corresponding author.

49). Dimers have also been observed, based on sedimentation experiments, as the primary oligomer form of the gB homolog of pseudorabies virus (58). The purpose of the present research was to determine the number of monomers in gB oligomers of HSV-1 and to map the oligomerization site.

MATERIALS AND METHODS

Cells, viruses, and plasmids. Vero cells were grown in Eagle's minimum essential medium supplemented with 10% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.). Virus stocks of KOS (HSV-1) and of K082, a gB-null mutant, were prepared as described previously (8). D6, a gB-transformed Vero cell line, was used as the host for K082 since gB is essential for viral replication (8).

Plasmid pKBXX (7, 8) contains a 4.0-kb fragment of KOS DNA, including the 2.7-kb gB-coding region and 790 bp of 5'- and 498 bp of 3'-flanking sequences, inserted into the pUC9 vector. All mutant plasmids are derivatives of pKBXX, and all the constructs, except pKΔSs, contain insertions of 6-bp *Hpa*I linkers into pKBXX (7). pKΔSs is an in-frame *Sst*II deletion within the gB-coding sequence of pKBXX. Deletion mutants pKΔ5C and pKΔ6C were constructed by cloning plasmids to remove sequences between *Hpa*I insertions. Sequences between the *Hpa*I insertions in pK082 (after codon 43) and in pK177 (after codon 595) were removed in the construction of pKΔ5C. Sequences between the *Hpa*I insertions in pK082 and in pK271 (after codon 569) were removed in the construction of pKΔ6C. To destroy the chain termination mutations, 12-bp *Cla*I linkers were inserted at the *Hpa*I junctions. The structures of most of the plasmids used in this study are shown in Fig. 7. Enzymes and linkers were from New England BioLabs, Inc. (Beverly, Mass.) and Bethesda Research Laboratories, Inc. (Gaithersburg, Md.).

Transient expression and analysis of gB polypeptides. Vero cells in 60-mm-diameter culture dishes were transfected with DEAE-dextran (500,000 molecular weight average) (Sigma Chemical Co., St. Louis, Mo.) and approximately 2 μg of plasmid DNA as described previously (7). At 24 to 48 h after transfection, cultures were infected with K082 at a multiplicity of infection of approximately 1 PFU per cell to stimulate gB polypeptide expression. At 6 h postinfection, viral proteins were labeled for 1 h with 50 μCi of [^{35}S]methionine per ml in methionine-free minimum essential medium. Cultures were washed with phosphate-buffered saline and treated with 1 ml of lysis buffer containing 1% Nonidet P-40 (Sigma), 20 mM Tris HCl (pH 8.0), 150 mM NaCl, 1 mM tosyl lysyl chloro-methyl ketone, phenylmethylsulfonyl fluoride (Sigma), and 5% powdered milk. After centrifugation in a microcentrifuge (15,000 × *g*, 15 min, 4°C), immunoprecipitation was started by the addition of a pool of gB-specific MAbs (equal volumes of B2, B3, B4, and B6) (34), B6 antibody alone, or polyclonal rabbit antiserum isolated against the cytoplasmic domain of gB (26a). Immune complexes were sedimented after binding to protein A-Sepharose CL-4B beads (Sigma), washed six times with lysis buffer without powdered milk, and subjected to sodium dodecyl sulfate (SDS)-PAGE as described previously (27). Gels were treated with 1 M sodium salicylate in 0.1 M Tris and dried as described previously (26).

Sedimentation analysis of gB monomers and oligomers. Vero cells in 60-mm-diameter culture dishes were infected with KOS at a multiplicity of infection of 10. At 6 h postinfection, viral proteins were labeled for 10 min with 200 μCi of [^{35}S]methionine in 200 μl of Tricine-buffered saline (TBS). Labeled cultures were harvested immediately or after a 1-h chase interval in growth medium to allow oligomer formation (21). Cultures were harvested by scraping the cell monolayer into TBS, and the cells were pelleted and lysed in 200 μl of a lysis solution similar to that used above (20 mM Tris HCl [pH 6.5], 150 mM NaCl, 1 mM tosyl lysyl chloromethyl ketone, 1% Triton X-100). After clarification by centrifugation (15,000 × *g*, 15 min, 4°C), supernatants were layered onto 10.8-ml 8 to 20% (wt/wt) sucrose gradients prepared in the same lysis solution. Proteins were sedimented through the gradients at 40,000 rpm for 16 h at 4°C in an SW41 rotor. Fractions were collected, immunoprecipitated with a pool of gB specific MAbs, and analyzed by SDS-PAGE.

To analyze gB from virions, Vero cells in a 150-cm^2 culture flask were infected with KOS at a multiplicity of infection of 10 and labeled from 5 to 24 h after infection with 100 μCi of [^{35}S]methionine per ml in methionine-free minimum essential medium (GIBCO) supplemented with 2% fetal calf serum. Intracellular and released virus were pelleted along with 10^{10} PFU of unlabeled KOS as a carrier and sedimented through a 10 to 30% (wt/wt), Dextran T-10 (Pharmacia) gradient in TBS containing 2% (vol/vol) fetal calf serum. Virions from the visible band were collected, diluted in TBS, and pelleted. Virions were then lysed, layered onto sucrose gradients, and analyzed as described above.

RESULTS

Sedimentation analysis of radioactively labeled gB from infected cells and virions. To determine the number of subunits present in gB oligomers, [^{35}S]methionine-labeled proteins from KOS-infected cells and from virions were sedimented through sucrose gradients. Gradient fractions were exposed to a pool of gB-specific MAbs, and the resulting immune complexes were analyzed by SDS-PAGE. Since multimers sediment more rapidly than monomers, they should be found further from the axis of sedimentation (higher fraction numbers in Fig. 1). For the pulse-labeled infected cells, most of the radioactivity was detected in fraction 5, with a small amount present in fraction 9 (Fig. 1A). Sedimentation of proteins of known molecular masses in parallel gradients indicated that proteins with molecular masses of 110 and 200 kDa are expected to be in these fractions. When a chase interval was added after pulse-labeling, most of the radioactivity appeared in fraction 9 (Fig. 1B). A similar pattern was observed for gB extracted from purified virions (Fig. 1C). Multiple bands for fractions from pulse-labeled, and from pulse-labeled and chased, infected cells are due to multiple states of glycosylation of gB. These bands persist during the 50-min chase interval since normally not all gB precursors are converted to fully glycosylated gB. The band of slowest mobility in lanes 7 to 10 of Fig. 1B corresponds to fully glycosylated gB. Precursor bands were not observed in virion fractions (Fig. 1C) because the labeling interval was from 6 to 24 h after infection and because precursor forms are not generally present in virions. On the basis of these experiments, it appears that the majority of gB oligomers sediment as dimers. For virions, including an analysis of released virus (data not shown), the amount of radioactivity present in fraction 5 varied from trace amounts up to that shown in Fig. 1C.

Coprecipitation of mutant and wild-type polypeptides. A coprecipitation assay, diagrammed at the bottom of Fig. 2,

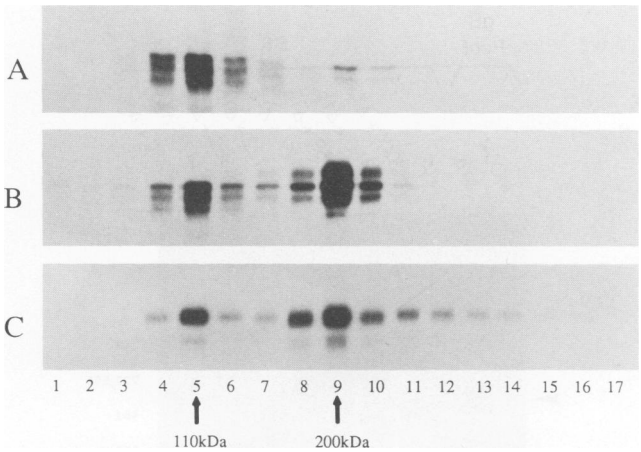

FIG. 1. Sedimentation analysis of [^{35}S]methionine-labeled gB from infected cells and virions. KOS-infected cells were pulse-labeled at 6 h postinfection and lysed in a nonionic detergent solution either immediately (A) or after a 1-h chase interval (B). Lysates were layered onto sucrose gradients, and fractions were collected after sedimentation. Fractions were treated with a pool of gB-specific MAbs, and immune complexes were analyzed by SDS-PAGE. Purified virions (C) were also lysed and subjected to the same procedures. The direction of sedimentation is from left to right. Multiple bands for fractions from labeled infected cells are probably due to multiple states of glycosylation, and the slowest-sedimenting bands in fractions 7 through 10 of the chased protocol correspond to fully glycosylated gB.

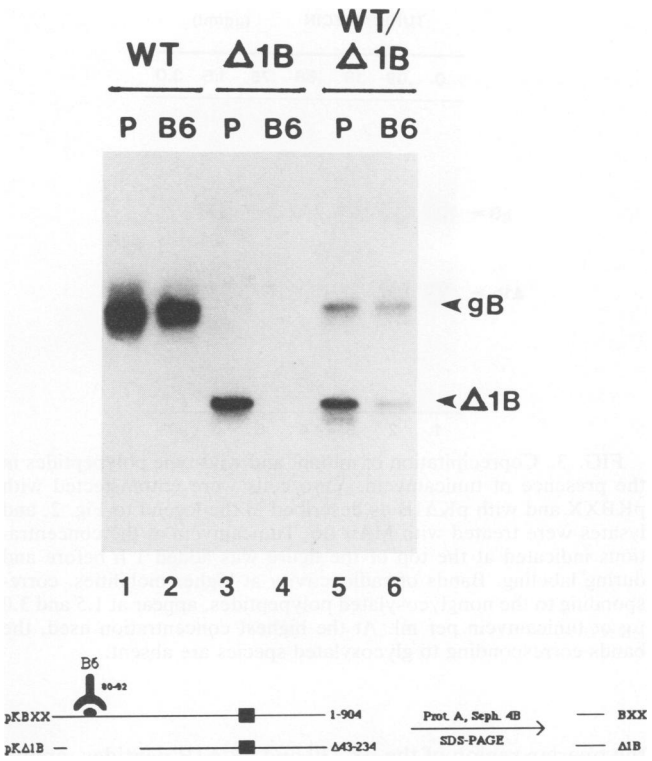

FIG. 2. Coprecipitation of mutant and wild-type (WT) polypeptides. Cells were transfected with pKBXX or with pKΔ1B individually (lanes 1 to 4) or in a cotransfection experiment (lanes 5 and 6) and infected with K082 to stimulate plasmid gene expression. Infected cells were labeled for 1 h with [^{35}S]methionine at 6 h postinfection. Lysates were treated with MAb B6 (lanes 2, 4, and 6) or with a pool of gB-specific MAbs (P, lanes 1, 3, and 5), and immune complexes were analyzed by SDS-PAGE. The mobilities of the wild-type (gB) and mutant polypeptides are indicated. A diagram depicting coprecipitation of the pKΔ1B polypeptide with that of pKBXX by MAb B6 is indicated at the bottom (left) of the figure. Potential interaction sites are indicated by cross-hatched rectangles. A break in the horizontal line for pKΔ1B indicates the region of the deleted sequences, and the corresponding amino acid residues deleted are indicated at the right side of the line, i.e., amino acids 43 through 234 are not present in the pKΔ1B polypeptide. The amino acid residues present in pKBXX are indicated at the right side of that plasmid. MAb B6 and its combining site (amino acid residues 80 through 92) are indicated above the pKBXX plasmid. Prot. A, protein A; Seph. 4B, Sepharose 4B.

was devised to identify domains of gB that contribute to oligomer formation. A mutant plasmid, pKΔ1B (containing a deletion corresponding to codons 43 through 234), and pKBXX (specifying wild-type gB) were transfected individually and together into Vero cells. The cells were superinfected with a gB-null virus (K082) to stimulate plasmid-specified gene expression. After labeling with [^{35}S]methionine, cells were lysed and treated with an MAb (B6) whose target epitope has been mapped to amino acid residues 80 to 92 (sequences that are missing on the deletion polypeptide) (24, 37). Immune complexes were dissociated and analyzed by SDS-PAGE. Multiple bands were often observed for each polypeptide owing to the presence of N-linked glycosylation sites. Results are shown in Fig. 2. After transfection with the individual plasmids, the wild-type polypeptide was precipitated by B6 and by the MAb pool (lanes 2 and 1, respectively), whereas the mutant polypeptide was precipitated by the MAb pool but not by B6 (lanes 3 and 4, respectively). However, in the cotransfection experiment, both polypeptides were detected after treatment with either the antibody pool or B6 (lanes 5 and 6, respectively). This suggests that an interaction occurs between the pKΔ1B and wild-type polypeptides, facilitating coprecipitation of the mutant polypeptide.

The same two plasmids were used in cotransfection experiments using the same labeling protocol in the presence of the glycosylation inhibitor tunicamycin (Fig. 3). Glycosylation was inhibited at concentrations of 1.5 and 3.0 μg of tunicamycin per ml as evidenced by the appearance of higher-mobility bands on the gels. However, tunicamycin did not inhibit coprecipitation of the pKΔ1B-specified polypeptide (Fig. 3, lanes 6 and 7).

Oligomer site mapping. Complementation inhibition studies of Cai et al. (7) identified gB amino acid residues between 463 and 791 as essential for oligomer formation. To delineate the C-terminal boundary of that oligomer interaction site, we used a set of polypeptide chain termination plasmids isolated by Cai et al. (7) that contained the linear B6 epitope (residues 80 to 92) in cotransfection experiments with pKΔ1B. As shown above, the pKΔ1B polypeptide was coprecipitated along with the wild-type polypeptide. It should also be coprecipitated by B6 along with any polypeptide that includes an intact oligomer site. Therefore, the longest truncation polypeptide that effects coprecipitation of the pKΔ1B polypeptide would define the C-terminal limit of the oligomer site. The results of these experiments are shown in Fig. 4. Surprisingly, the pKΔ1B peptide was coprecipitated with each truncation polypeptide tested, the shortest of which terminates after amino acid residue 282 (pK230). One interpretation of these data is that the oligomer site is located in

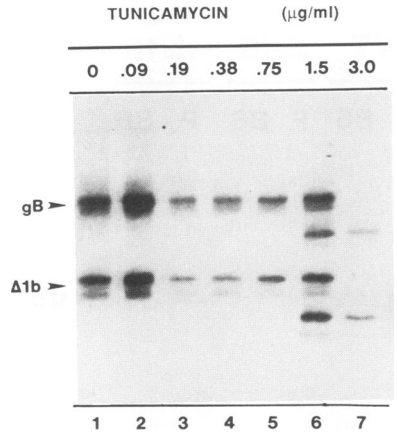

FIG. 3. Coprecipitation of mutant and wild-type polypeptides in the presence of tunicamycin. Vero cells were cotransfected with pKBXX and with pKΔ1B as described in the legend to Fig. 2, and lysates were treated with MAb B6. Tunicamycin at the concentrations indicated at the top of the figure was added 1 h before and during labeling. Bands of radioactivity at higher mobilities, corresponding to the nonglycosylated polypeptides, appear at 1.5 and 3.0 μg of tunicamycin per ml. At the highest concentration used, the bands corresponding to glycosylated species are absent.

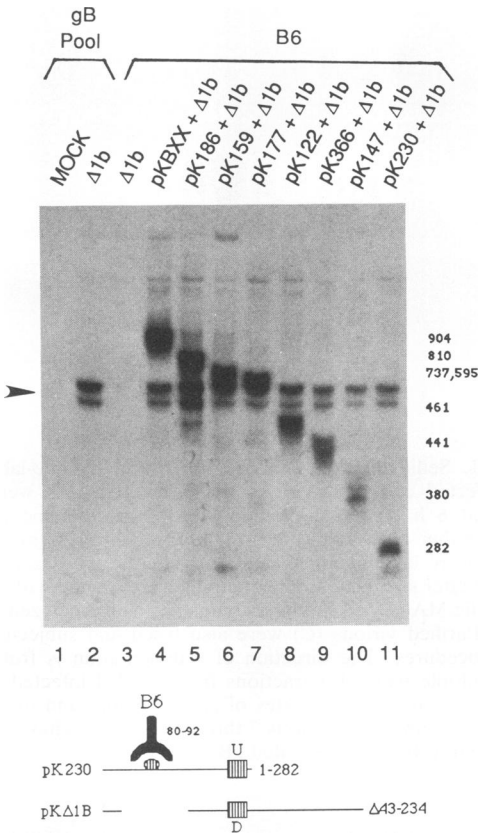

FIG. 4. Coprecipitation of pKΔ1B and chain termination polypeptides. Vero cells were transfected or cotransfected with the plasmids indicated at the top of the figure. Virus infection, labeling, and analysis were as described in the legend to Fig. 2. Lysates were treated with either a pool of gB-specific MAbs (lanes 1 and 2) or with B6 (lanes 3 to 11). The mobility of the pKΔ1B polypeptide is indicated by an arrowhead at the left side of the lanes. The length of the chain termination polypeptides (in amino acids) is indicated at the right side of the figure. The coprecipitation of the pKΔ1B polypeptide with that of pK230 by MAb B6 is indicated at the bottom of figure. pK230 specifies polypeptide chain termination after codon 282. The putative oligomer interaction is depicted as being between an upstream site (U) specified by pK230 and a downstream site (D) specified by pKΔ1B, rather than between overlapping residues 31 to 42 and/or 234 to 282 (see text).

the overlap region of the pK230 and pKΔ1B peptides, amino acids 235 to 282. However, given the data of Cai et al. (7), who located a site between amino acid residues 463 and 791, it is also possible that residues in two noncontiguous sites interact to form oligomers, an upstream site specified by the truncation mutants and a downstream site specified by pKΔ1B.

The overlap region also includes residues 31 to 42. It is unlikely that these residues form part of an oligomer site. The pKΔ4B polypeptide, deleted in sequences corresponding to codons 43 through 711 but containing residues 31 to 42, was not coprecipitated with those of pKΔ1B, pKBXX, or pK230 (Table 1, line 10, and see Fig. 6, lanes 1 to 4). In addition, 7 of these 12 amino acids are not conserved between HSV-1 and HSV type 2 (HSV-2) gB, although the entire molecules are 90% identical (3). Therefore, these residues, 31 to 42, have not been considered to be part of the oligomer binding sequences.

To test for the existence of an upstream site, we constructed deletion mutants that should lack this site. These mutants, pKΔ5C (deleted in codons 43 to 595) and pKΔ6C (deleted in codons 43 to 569), were cotransfected in Vero cells along with pK366, which generates a polypeptide chain that is terminated after codon 441. Following cotransfection with either of the deletion plasmids and pK366, cell lysates were treated with MAb B6 to test for coprecipitation of the deletion polypeptides (Fig. 5). Polypeptides specified by pKΔ5C and pKΔ6C lack the B6 epitope and were not precipitated by B6 in the absence of the pK366 polypeptide (Fig. 5, lanes 5 and 6). However, they were coprecipitated by B6 in the presence of the pK366 polypeptide (Fig. 5, lanes 2 and 4). Therefore, an upstream oligomer site exists, and the coprecipitates in Fig. 5 are due to an interaction between the upstream site specified by pK366 and a downstream site specified by pKΔ5C and pKΔ6C. Based on these experiments, the N terminus of the downstream site begins at or following amino acid 596 (the first residue after the deletion by pKΔ5C). Cell lysis and immune complex formation in the absence of powdered milk and a low signal-to-noise ratio probably account for the numerous background bands that are present in this autoradiograph.

Results of further experiments also support the identification and characterization of an upstream site (Fig. 6). pKΔSs is a plasmid that is deleted in sequences specifying residues 93 through 576. The pKΔSs polypeptide was precipitated by an antibody specific for the cytoplasmic domain of gB (Fig. 6, lane 8). In a cotransfection with pKΔSs and pK230, the cytoplasmic domain antiserum also coprecipitated the pK230 truncation polypeptide (Fig. 6, lane 9). The mobility of the pK230 polypeptide is shown in lane 3. The coprecipitation result could be due to interactions of the upstream portion (1 to 92) or the downstream portion (577 to 904) of the pKΔSs polypeptide with that of pK230. To distinguish between these two possibilities, we cotransfected pKΔSs and pKΔ5C into Vero cells and treated expressed polypeptides with MAb B6 (whose target sequence is specified by

TABLE 1. Coprecipitation tests for polypeptides specified by the indicated plasmids[a]

Polypeptide	BXX (1–904)	Δ318 (Δ616–711)	Δ1B (Δ43–234)	ΔAv (Δ65–421)	ΔSs (Δ93–576)	230 (1–282)
1. BXX (1–904)						
2. Δ318 (Δ616–711)	+					
3. Δ1B (Δ43–234)	+[b]	+				
4. ΔAv (Δ65–421)	ND	ND	+			
5. ΔSs (Δ93–576)	+	ND	+	+		
6. 230 (1–282)	ND	ND	+[b]	+	+[b]	
7. 122 (1–461)	ND	ND	+[b]	+	ND	+ (B3)
8. 366 (1–441)	ND	ND	+[b]	+	ND	ND
9. Δ3 (Δ462–711)	+	ND	+	ND	ND	ND
10. Δ4B (Δ43–711)	−	ND	−	ND	ND	−[b]

[a] The gB codons deleted, or the last codon translated, is indicated for deletion and truncation polypeptides, respectively. The antibody used, if not B6, is indicated. +, coprecipitate formed; ND, not done; −, coprecipitate not formed.
[b] From data shown in Fig. 2 to 6.

pKΔSs) (Fig. 6, lane 10). If the pKΔSs-pK230 polypeptide interaction was due to amino acids N terminal to residue 93, then the pKΔ5C polypeptide cannot be coprecipitated with that of pKΔSs, since the pKΔ5C deletion begins at codon 43.

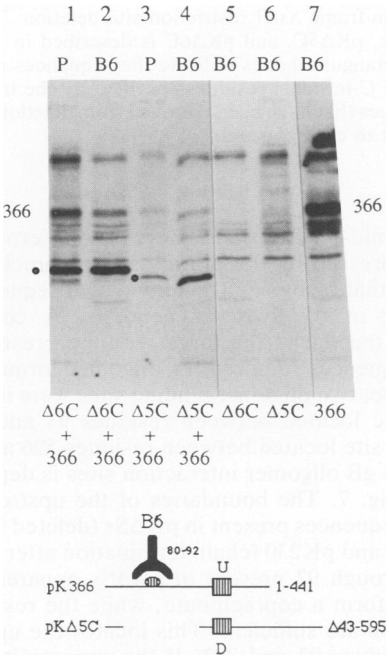

FIG. 5. Coprecipitation of pKΔ5C, pKΔ6C, and a chain termination polypeptide. Vero cells were transfected or cotransfected with plasmid pKΔ5C, pKΔ6C, and pK366. pKΔ5C is deleted from codons 43 to 595; pKΔ6C is deleted from codons 43 to 569; pK366 is a truncation plasmid that terminates after amino acid residue 441. Cells were infected, labeled, and assayed as described in the legend to Fig. 2. Lysates were treated either with a pool of gB-specific MAbs (P) or with B6 as indicated at the top of the figure. The mobility of the pK366 polypeptide is shown at the sides of the figure (366), and the mobilities of the pKΔ6C and pKΔ5C polypeptides are indicated by circles to the left of lanes 1 and 3, respectively. Data for Fig. 5 and 6 were obtained with a minigel apparatus which allowed less resolution than for data shown in Fig. 1 to 4. A diagram depicting the coprecipitation of the pKΔ5C polypeptide with that of pK366 by MAb B6 is shown at the bottom of the figure. The interaction is depicted as being between an upstream site (U) specified by pK366 and a downstream site (D) specified by pKΔ5C. Amino acid residues deleted in the pKΔ5C polypeptide are indicated at the right side of the line representing the pKΔ5C plasmid.

If the pKΔSs-pK230 interaction was due to sequences C terminal to residue 576 of the pKΔSs polypeptide, then the pKΔ5C polypeptide would be coprecipitated. Lane 10 of Fig. 6 shows that the pKΔ5C polypeptide was coprecipitated by B6 along with the pKΔSs polypeptide. Therefore, the pK230-pKΔSs coprecipitation did not involve sequences before residue 93 of pKΔSs. The coprecipitation the pK230 polypeptide by cytoplasmic domain antibodies in the presence of pKΔSs polypeptide is, therefore, an example of an

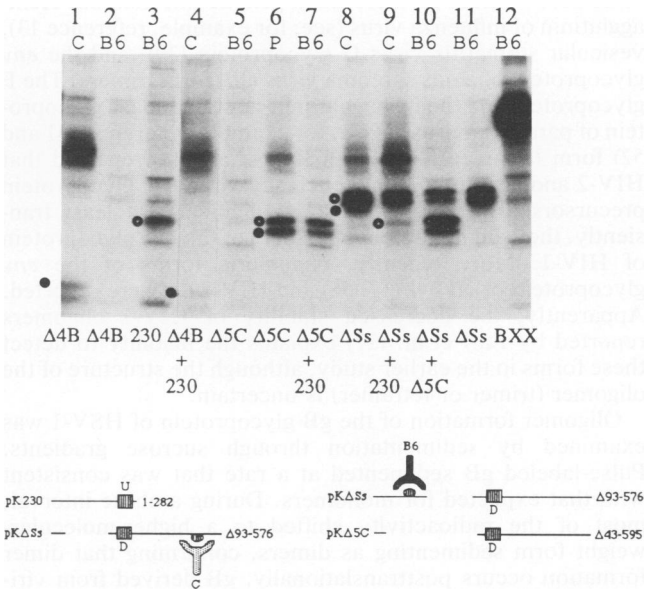

FIG. 6. Oligomer site mapping. Vero cells were transfected or cotransfected with the plasmids indicated at the bottom of the figure and were infected, labeled, and assayed as described in the legend to Fig. 2. Lysates were treated with cytoplasmic-domain-specific gB antiserum (C) or with B6 as indicated at the top of the figure. The structures of the plasmids are shown in Fig. 7. The region of radioactivity approximately one-third of the distance from the top to the bottom of the gel present in lanes 1, 4, 6, 8, and 9 is composed of glycoproteins gE and gI, whose structures include F_C immunoglobulin binding domains. These bands are evident when polyclonal antisera are used. The resolution on this gel allowed the separation of glycosylated (○) and nonglycosylated (●) species for pKΔ5C and pKΔSs polypeptides. The coprecipitation of the pK230 polypeptide with that of pKΔSs by gB cytoplasmic-domain-specific antibodies and of the pKΔ5C polypeptide with that of pKΔSs by MAb B6, is diagrammed at the bottom of the figure.

interaction between an upstream and a downstream site. The pKΔSs-pKΔ5C interaction is between two downstream sites.

Coprecipitation of the pKΔAv polypeptide (deleted in codons 65 through 421) by MAb B6 in the presence of the pK230, pK122, or pK366 polypeptide (Table 1, column 4, lines 6 to 8) is another example of upstream-downstream interactions. Coprecipitation of the pKΔ1B polypeptide by B6 in the presence of the same truncation mutant polypeptides could be due either to upstream-downstream or to upstream-upstream interactions. Similar interactions could occur for the coprecipitation of the pKΔ1B polypeptide in contransfections with that of pKΔ318 (deleted in codons 616 to 711) and with that of pKΔ3 (deleted in codons 462 to 711) (Table 1, column 2, line 3, and column 3, line 9). The complementation inhibition data of Cai et al. (7) are consistent with the interactions between two downstream sites, as is the coprecipitation of the pKΔAv polypeptide by B6 in the presence of pKΔSs (Table 1, column 4, line 5). The coprecipitation of the pK230 polypeptide by MAb B3 (localized to residues 381 to 441) in the presence of the pK122 polypeptide is the single example of an interaction between two upstream sites (Table 1, column 6, line 7).

DISCUSSION

Numerous viral glycoproteins exist as homo-oligomers. This serves to increase the variety of three-dimensional structures without an increase in genetic information. Hemagglutinin of influenza virus (see, for example, reference 13), vesicular stomatitis virus G glycoprotein (29), and the *env* glycoprotein of Rous sarcoma virus (19) form trimers. The F glycoprotein and the hemagglutinin-neuraminidase glycoprotein of paramyxoviruses (see, for example, references 41 and 52) form tetramers. Although Rey et al. (44) reported that HIV-2 and simian immunodeficiency virus *env* glycoprotein precursors formed SDS-resistant oligomers, at least transiently, they did not detect oligomers of the *env* glycoprotein of HIV-1. More recently, oligomeric forms of the *env* glycoprotein of HIV-1 (17, 56) and HIV-2 (9) were reported. Apparently, the decreased stability of HIV-1 oligomers reported by Earl et al. (17) explains the inability to detect these forms in the earlier study, although the structure of the oligomer (trimer or tetramer) is uncertain.

Oligomer formation of the gB glycoprotein of HSV-1 was examined by sedimentation through sucrose gradients. Pulse-labeled gB sedimented at a rate that was consistent with that expected for monomers. During a chase interval, most of the radioactivity shifted to a higher-molecular-weight form sedimenting as dimers, confirming that dimer formation occurs posttranslationally. gB derived from virions sedimented mainly as dimers. The amount of monomer in virions shown in Fig. 1C is the maximum amount observed. In some experiments, only trace amounts of virion gB were found as monomers. The presence of monomer may be due to dissociation of dimers during the assay procedure and/or contamination of virus preparations with plasma membranes. For infected cells and for virions, we never observed an increase in radioactivity at higher fraction numbers, for example, at fractions 13 and 17, which would have indicated the presence of trimers or tetramers. It remains possible that they are formed but are not stable in the assay conditions used. Our data are consistent with the sedimentation data reported recently for the gB homolog of pseudorabies virus (58).

The sites for oligomer formation were mapped by using gB mutant plasmids. After cotransfection of Vero cells, polypeptides expressed by the plasmids were precipitated with an antibody that recognized an amino acid sequence present in only one of the two polypeptides. A coprecipitated polypeptide that lacks the target epitope presumably contains the sequences required for oligomer formation. Using this technique, two oligomer binding sites were identified, an upstream site located between residues 93 and 282 and a downstream site located between residues 596 and 711. The extent of the gB oligomer interaction sites is depicted at the bottom of Fig. 7. The boundaries of the upstream site are defined by sequences present in pKΔSs (deleted in codons 93 through 576) and pK230 (chain termination after codon 282). Residues through 92 present in pKΔSs apparently are not sufficient to form a coprecipitate, while the residues specified by pK230 are sufficient. This locates the upstream site between residues 93 and 282. If the coprecipitation of the pKΔ1B polypeptide in the presence of pK230 is due to interactions between two upstream sites, then the upstream site is localized to amino acid residues 235 to 282 (the overlap region of the two polypeptides). pKΔ5C (deleted in codons 43 through 595), pKΔ6C (deleted in codons 43 through 569), and pKΔSs (deleted in codons 93 through 576) possess an intact downstream site, while pKΔ4B (deleted in codons 43 through 711) does not (Fig. 6, lane 4; Table 1, line 10). This locates the downstream site between residues 596 and 711.

The upstream site includes a single N-linked oligosaccharide addition signal, Asn-Tyr-Thr, at residues 141 to 143 and four cysteines at residues 116, 133, 207, and 271. The downstream site also contains a single N-linked glycosylation signal, Asn-Ile-Thr, at residues 674 to 676 and cysteines at residues 596 and 633. The four remaining oligosaccharide addition sites and cysteine residues are located outside the

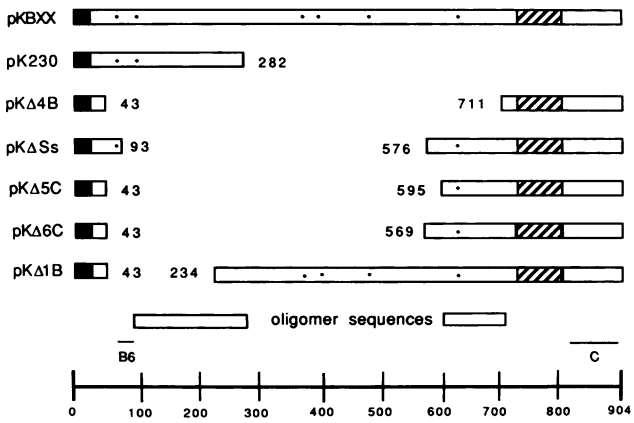

FIG. 7. Structure of representative mutant plasmids derived from pKBXX. pKBXX is a plasmid containing a 4.0-kb HSV-1 fragment that includes the 2.7-kb gB-coding sequence shown. The scale for the amino acid sequence is shown at the bottom of the figure. The locations of the signal sequence (solid box), N-linked glycosylation sites (●), and transmembrane domain (cross-hatched box) are shown for pKBXX and, if present, for the mutant plasmids. pK230, pKΔ1B, and pKΔ4B are from the collection of Cai et al. (7). pKΔSs is an in-frame *Sst*II restriction site deletion. The construction of pKΔSs, pKΔ5C, and pKΔ6C is described in Materials and Methods. Rectangular boxes indicate the sequences present in the plasmids. The C-terminal residues specified by the truncation mutant and residues that have been removed from deletion plasmids are listed adjacent to each plasmid.

putative oligomer formation sites. Since tunicamycin does not inhibit oligomer formation, it is unlikely that N-linked glycosylation is essential for oligomer formation. In addition, in pKΔ5C-pKΔSs cotransfection experiments (Fig. 6, lane 10), the nonglycosylated pKΔ5C polypeptide was coprecipitated along with the glycosylated form (pKΔ5C specifies sequences for a single glycosylation site at 674 to 676). Since almost all gB mutant polypeptides are retained in the endoplasmic reticulum (7), the two bands of different mobilities probably represent molecules containing zero or one high-mannose core oligosaccharide chains.

Cai et al. (7) did not use truncation mutants in their studies of complementation inhibition and did not observe the upstream site. Our data are consistent with theirs with the following exception: plasmids pK318, which is deleted in the DNA specifying codons 616 to 711, and pKΔ3, which is deleted in DNA specifying codons 462 to 711, did not inhibit complementation in mixed transfections with pKBXX even though they have intact upstream sites. In the present studies, the pKΔ1B polypeptide coprecipitated with either the pK318 or the pKΔ3 polypeptide, using MAb B6 (Table 1). The coprecipitation assay is more sensitive than the complementation inhibition assay since the former scores for the appearance of radioactivity (the appearance of a band in SDS-PAGE), while the latter measures the reduction in plaque number. Presumably, this is the source of the disagreement. Recently Qadrie et al. (43a) and Ali (1) have reported that plasmids that specify truncated polypeptides of 691 or 772 amino acids form homodimers when analyzed by sedimentation analyses following transfection. Dimers were not observed for plasmids that caused chain termination after residue 586 (1) or 610 (43a).

The coprecipitation assay employed a variety of mutant plasmids and utilized an indirect assay of oligomer formation. Therefore, we wondered whether the data regarding oligomer binding site mapping were due to a nonspecific factor that resulted in coprecipitation. It might be argued, for example, that the use of mutant polypeptides exposes hydrophobic or hides hydrophilic sequences, which causes the mutant polypeptide to precipitate in the presence of other relatively hydrophobic glycoproteins. In studies reported here, gC was precipitated with gC-specific antibodies and analyzed by SDS-PAGE. gB mutant polypeptides were never observed as contaminants in these precipitations (data not shown), which indicates that no such spurious interactions occurred. Alternatively, one might argue that an antibody such as B6 causes mutant polypeptides to be bound to endoplasmic reticulum proteins, causing nonspecific coprecipitation. This seems unlikely since consistent results were obtained with three different antibodies (MAbs B6 and B3 and polyclonal antiserum specific for the cytoplasmic domain of gB). Also note that pK122 and pK177, which cause polypeptide chain termination after codons 461 and 595, respectively, are processed normally (7). These polypeptides must appear native to the endoplasmic reticulum transport system, a finding that also argues against nonspecific coprecipitation of polypeptides.

Coprecipitates were demonstrated between two polypeptides containing upstream sites, between polypeptides that contain downstream sites, and between two polypeptides, one of which contains an upstream site and the other a downstream site. The compatibility of upstream and downstream sites suggests that molecular interactions at the two sites are further subdivided to provide complementary weak interactions between chemical groups within the two sites.

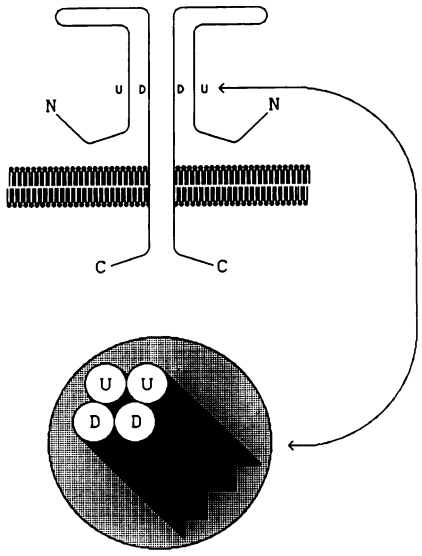

FIG. 8. Schematic diagram of a gB dimer. Monomers of gB are indicated as single continuous lines from the N terminus (N) to the C terminus (C). The upstream (U) and downstream (D) intermolecular interaction sites are shown in close proximity to each other. A cross-sectional view to demonstrate the possible spatial relationship of the sites is shown at the bottom of the figure. The loop structure of each monomer could give rise to T-shaped dimers consistent with structures, thought to be gB molecules, on the surface of virions (54).

There is no significant amino acid homology within the two sites.

A model of gB dimer formation characterized by upstream-downstream, upstream-upstream, and downstream-downstream interactions is shown in Fig. 8. It is possible that dimers result from only one class of interactions, for example, between upstream and downstream sites (as noted in the two-dimensional aspect of the model). However, the close proximity of all four chains shown in the cross-sectional view would allow all classes of interactions within a single dimer. It is impossible to specify which classes of interactions occur, on the basis of existing data. Dimers characteristic of both types of interactions may be formed, and one type may be energetically more favorable. It is tempting to speculate that the transition to the more stable form occurs at the time of virus entry and triggers fusion of the viral envelope with the cellular plasma membrane.

Mutant gB polypeptides are transdominant when expressed in the presence of wild-type polypeptides (7). Therefore, gB is a potential antiviral target. If the oligomer interaction region is sufficiently short, and if small gB polypeptides can prevent formation of wild-type oligomers, then these polypeptides could have antiviral activity.

ACKNOWLEDGMENTS

These studies would not have been possible without the extensive collection of linker-insertion mutants isolated by Weizhong Cai. We acknowledge discussions of data with colleagues Prashant Desai, Karen Dolter, Philip Gage, and Alexandra Krikos. We thank Dan Jaffurs and Brendon Wahlberg for help with preparation of the figures.

This research was supported by Public Health Service grants AI26937, AI21162, and KO4-AI0075 from the National Institutes of Health.

REFERENCES

1. **Ali, M. A.** 1990. Oligomerization of herpes simplex virus glycoprotein B occurs in the endoplasmic reticulum and a 102 amino acid cytosolic domain is dispensable for dimer assembly. Virology **178**:588–592.
2. **Buckmaster, E. A., U. Gompels, and A. C. Minson.** 1984. Characterization and physical mapping of an HSV-1 glycoprotein of approximately 115 × 10^3 molecular weight. Virology **139**:408–413.
3. **Bzik, D. J., C. DebRoy, B. A. Fox, N. E. Pederson, and S. Person.** 1986. The nucleotide sequence of the gB glycoprotein gene of HSV-2 and comparison with the corresponding gene of HSV-1. Virology **155**:322–333.
4. **Bzik, D. J., B. A. Fox, N. A. DeLuca, and S. Person.** 1984. Nucleotide sequence of a region of the herpes simplex virus type 1 gB glycoprotein gene: mutations affecting rate of virus entry and cell fusion. Virology **137**:185–190.
5. **Bzik, D. J., B. A. Fox, N. A. DeLuca, and S. Person.** 1984. Nucleotide sequence specifying the glycoprotein gene, gB, of herpes simplex virus type 1. Virology **133**:301–314.
6. **Cai, W., B. Gu, and S. Person.** 1988. Role of glycoprotein B of herpes simplex virus type 1 in viral entry and cell fusion. J. Virol. **62**:2596–2604.
7. **Cai, W., S. Person, C. DebRoy, and B. Gu.** 1988. Functional regions and structural features of the gB glycoprotein of herpes simplex virus type 1: an analysis of linker insertion mutants. J. Mol. Biol. **201**:575–588.
8. **Cai, W., S. Person, S. C. Warner, J. Zhou, and N. A. DeLuca.** 1987. Linker-insertion nonsense and restriction-site deletion mutations of the gB glycoprotein gene of herpes simplex virus type 1. J. Virol. **61**:714–721.
9. **Chakrabarti, S., T. Mizukami, G. Franchini, and B. Moss.** 1990. Synthesis, oligomerization, and biological activity of the human immunodeficiency virus type 2 envelope glycoprotein expressed by a recombinant virus. Virology **178**:134–142.
10. **Chapsal, J. M., and L. Pereira.** 1988. Characterization of epitopes on native and denatured forms of herpes simplex virus glycoprotein B. Virology **164**:427–434.
11. **Claesson-Welsh, L., and P. G. Spear.** 1986. Oligomerization of herpes simplex virus glycoprotein B. J. Virol. **60**:803–806.
12. **Claesson-Welsh, L., and P. G. Spear.** 1987. Amino-terminal sequence, synthesis, and membrane insertion of glycoprotein B of herpes simplex virus type 1. J. Virol. **61**:1–7.
13. **Copeland, C. S., K. P. Zimmer, K. R. Wagner, G. A. Healey, I. Mellman, and A. Helenius.** 1988. Folding, trimerization and transport are sequential events in the biogenesis of influenza virus hemagglutinin. Cell **53**:197–209.
14. **Cranage, M. P., T. Kouzarides, A. T. Bankier, S. Satchwell, K. Weston, P. Tomlinson, B. Barrell, H. Hart, S. E. Bell, A. C. Minson, and G. L. Smith.** 1986. Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. EMBO J. **5**:3057–3063.
15. **DeLuca, N. A., D. J. Bzik, V. C. Bond, S. Person, and W. Snipes.** 1982. Nucleotide sequences of herpes simplex virus type 1 (HSV-1) affecting virus entry, cell fusion, and production of glycoprotein gB (VP7). Virology **122**:411–423.
16. **Desai, P. J., P. A. Schaffer, and A. C. Minson.** 1988. Excretion of noninfectious virus particles lacking glycoprotein H by a temperature sensitive mutant of herpes simplex virus type 1: evidence that gH is essential for virion infectivity. J. Gen. Virol. **69**:1147–1156.
17. **Earl, P. L., R. W. Doms, and B. Moss.** 1990. Oligomeric structure of the human immunodeficiency virus type 1 envelope glycoprotein. Proc. Natl. Acad. Sci. USA **87**:648–652.
18. **Eberle, R., D. Black, and J. K. Hilliard.** 1989. Relatedness of glycoproteins expressed on the surface of simian herpes-virus virions and infected cells to specific HSV glycoproteins. Arch. Virol. **109**:233–252.
19. **Einfeld, D., and E. Hunter.** 1988. Oligomeric structure of a prototype retrovirus glycoprotein. Proc. Natl. Acad. Sci. USA **85**:8688–8692.
20. **Feenstra, V., H. Mojgan, and D. Johnson.** 1990. Deletions in herpes simplex virus glycoprotein D define nonessential and essential domains. J. Virol. **64**:2096–2112.
21. **Gething, M. J., K. McCammon, and J. Sambrook.** 1986. Expression of wild-type and mutant forms of influenza hemagglutinin: the role of folding in intracellular transport. Cell **46**:939–950.
22. **Gompels, U. A., and A. C. Minson.** 1986. The properties and sequence of glycoprotein H of herpes simplex virus type 1. Virology **153**:230–247.
23. **Gompels, U. A., and A. C. Minson.** 1989. Antigenic properties and cellular localization of herpes simplex virus glycoprotein H synthesized in a mammalian cell expression system. J. Virol. **63**:4744–4755.
24. **Highlander, S. L., W. Cai, S. Person, M. Levine, and J. C. Glorioso.** 1988. Monoclonal antibodies define a domain on herpes simplex virus glycoprotein B involved in virus penetration. J. Virol. **62**:1881–1888.
25. **Highlander, S. L., D. J. Dorney, P. J. Gage, T. C. Holland, W. Cai, S. Person, M. Levine, and J. C. Glorioso.** 1989. Identification of *mar* mutations in herpes simplex virus type 1 glycoprotein B which alter antigenic structure and function in virus penetration. J. Virol. **63**:730–738.
26. **Highlander, S. L., W. L. Sutherland, P. J. Gage, D. C. Johnson, M. Levine, and J. C. Glorioso.** 1987. Neutralizing monoclonal antibodies for herpes simplex virus glycoprotein D inhibit virus penetration. J. Virol. **61**:3356–3364.

26a. **Holland, T. C., et al.** Unpublished data.

27. **Holland, T. C., S. D. Marlin, M. Levine, and J. C. Glorioso.** 1983. Antigenic variants of herpes simplex virus selected with glycoprotein-specific monoclonal antibodies. J. Virol. **45**:672–682.
28. **Keller, P. M., A. J. Davison, R. S. Lowe, M. W. Riemen, and R. W. Ellis.** 1987. Identification and sequence of the gene encoding gpIII, a major glycoprotein of varicella-zoster virus. Virology **157**:526–533.
29. **Kreis, T. E., and H. F. Lodish.** 1986. Oligomerization is essential for transport of vesicular stomatitis virus glycoprotein to the cell surface. Cell **46**:929–937.
30. **Ligas, M. W., and D. C. Johnson.** 1988. A herpes simplex virus mutant in which glycoprotein D sequences are replaced by β-galactosidase sequences binds to but is unable to penetrate into cells. J. Virol. **62**:1486–1494.
31. **Longnecker, R., S. Chatterjee, R. J. Whitley, and B. Roizman.** 1987. Identification of a herpes simplex virus 1 glycoprotein gene within a gene cluster dispensable for growth in cell culture. Proc. Natl. Acad. Sci. USA **84**:4303–4307.
32. **Longnecker, R., and B. Roizman.** 1987. Clustering of genes dispensable for growth in culture in the small component of the HSV-1 genome. Science **236**:573–576.
33. **Manservigi, R., P. G. Spear, and A. Buchan.** 1977. Cell fusion induced by herpes simplex virus is promoted and suppressed by different viral glycoproteins. Proc. Natl. Acad. Sci. USA **74**: 3913–3917.
34. **Marlin, S. C., T. C. Holland, M. Levine, and J. C. Glorioso.** 1985. Epitopes of herpes simplex virus 1 glycoprotein C are clustered in two distinct antigenic sites. J. Virol. **53**:128–136.
35. **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. **185**:1–13.
36. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Dolan, M. C. Frame, D. M. McNab, L. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the long unique region of the genome of herpes simplex virus type 1. J. Gen. Virol. **69**:1531–1574.
37. **Mester, J. C., S. L. Highlander, A. P. Osmand, J. C. Glorioso, and B. T. Rouse.** 1990. Herpes simplex virus type 1-specific immunity induced by peptides corresponding to an antigenic site of glycoprotein B. J. Virol. **64**:5277–5283.
38. **Minson, A. C., T. C. Hodgman, P. Digard, D. C. Hancock, S. E. Bell, and E. A. Buckmaster.** 1986. An analysis of the biological properties of monoclonal antibodies against glycoprotein D of herpes simplex virus and identification of amino acid substitutions that confer resistance to neutralization. J. Gen. Virol.

67:1001–1013.
39. **Misra, V., R. Nelson, and M. Smith.** 1988. Sequence of a bovine herpesvirus type-1 glycoprotein gene that is homologous to the herpes simplex gene for the glycoprotein gB. Virology **166**:542–549.
40. **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**:218–244.
40a. **O'Brian, S. S., C. A. Greenamoyer, E. D. Anton, and T. R. Dambaugh.** 1990. Abstr. 13th Int. Herpes Virus Workshop, Washington, D.C., p. 230.
41. **Parks, G. D., and R. A. Lamb.** 1990. Folding and oligomerization properties of a soluble and secreted form of the paramyxovirus hemagglutinin-neuraminidase glycoprotein. Virology **178**:498–508.
42. **Pellet, P. E., M. D. Biggin, B. Barrell, and B. Roizman.** 1985. Epstein-Barr virus may encode a protein showing significant amino acid predicted secondary structure homology with glycoprotein B of herpes simplex virus type 1. J. Virol. **56**:807–813.
43. **Pellet, P. E., K. G. Kousoulas, L. Pereira, and B. Roizman.** 1985. Anatomy of the herpes simplex virus 1 strain F glycoprotein B gene: primary sequence and predicted protein structure of the wild type and of monoclonal antibody-resistant mutants. J. Virol. **53**:243–253.
43a. **Quadri, I., D. Navarro, and L. Pereira.** 1990. Abstr. 13th Int. Herpes Virus Workshop, Washington, D.C., p. 216.
44. **Rey, M. A., B. Krust, A. G. Laurent, L. Montagnier, and A. G. Hovanessian.** 1989. Characterization of human immunodeficiency virus type 2 envelope glycoproteins: dimerization of the glycoprotein precursor during processing. J. Virol. **63**:647–658.
45. **Richman, D. D., A. Buckmaster, S. Bell, C. Hodgeman, 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.
46. **Riggio, M. P., A. A. Cullinane, and D. E. Onions.** 1988. Identification and nucleotide sequence of the glycoprotein gB gene of equine herpesvirus 4. J. Virol. **63**:1123–1133.
47. **Robbins, A. K., D. J. Dorney, M. W. Wathen, M. E. Whealy, C. Gold, R. J. Watson, L. E. Holland, S. D. Weed, M. Levine, J. C. Glorioso, and L. W. Enquist.** 1987. The pseudorabies virus gII gene is closely related to the gB glycoprotein gene of herpes simplex virus. J. Virol. **61**:2691–2701.
48. **Ross, L. J. N., M. Sanderson, S. D. Scott, M. M. Binns, T. Doel, and B. Milne.** 1989. Nucleotide sequence and characterization of the Marek's disease virus homologue of glycoprotein B of HSV-1. J. Gen. Virol. **70**:1789–1804.
49. **Sarmiento, M., and P. G. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. IV. Conformation of the virion glycoprotein designated VP7(B2). J. Virol. **29**:1159–1167.
50. **Schaffer, P. A., G. M. Aron, N. Biswal, and M. Benyesh-Melnick.** 1973. Temperature-sensitive mutants of herpes simplex virus type 1: isolation, complementation and partial characterization. Virology **52**:57–71.
51. **Schaffer, P. A., V. C. Carter, and M. C. Timbury.** 1978. Collaborative complementation study of temperature-sensitive mutants of herpes simplex virus types 1 and 2. J. Virol. **27**:490–504.
52. **Sechoy, O., J. R. Philippot, and A. Bienvenue.** 1987. F protein-F protein interaction within the Sendai virus identified by native bonding or chemical cross-linking. J. Biol. Chem. **262**:11519–11523.
52a. **Spatz, S. J., R. K. Maes, and C. E. Beisel.** 1990. Abstr. 13th Int. Herpes Virus Workshop, Washington, D.C., p. 233.
53. **Spear, P. G.** 1984. Glycoproteins specified by herpes simplex virus, p. 315–356. *In* B. Roizman (ed.), The herpesviruses, vol. 3. Plenum Publishing Corp., New York.
54. **Stannard, L. M., A. O. Fuller, and P. G. Spear.** 1987. Herpes simplex virus glycoproteins associated with different morphological entities projecting from the virion envelope. J. Gen. Virol. **68**:715–725.
55. **Stuve, L. L., S. Brown-Shimer, C. Pachl, R. Najarian, D. Dina, and R. L. Burke.** 1987. Structure and expression of the herpes simplex virus type 2 glycoprotein gB gene. J. Virol. **61**:326–335.
56. **Weiss, C. P., J. A. Levy, and J. M. White.** 1990. Oligomeric organization of gp120 on infectious immunodeficiency virus type 1 particles. J. Virol. **64**:5674–5677.
57. **Whalley, J. M., G. R. Robertson, N. A. Scott, G. C. Hudson, C. W. Bell, and L. M. Woodworth.** 1989. Identification and complete nucleotide sequence of a gene in equine herpesvirus 1 analogous to the herpes simplex virus gene encoding the major envelope glycoprotein gB. J. Gen. Virol. **70**:383–394.
58. **Whealy, M. E., A. K. Robbins, and L. W. Enquist.** 1990. The export pathway of the pseudorabies virus gB homolog gII involves oligomer formation in the endoplasmic reticulum and protease processing in the Golgi apparatus. J. Virol. **64**:1946–1955.
59. **Whitbeck, J. C., L. J. Bello, and W. C. Lawrence.** 1988. Comparison of the bovine herpesvirus 1 gI gene and the herpes simplex virus type 1 gB gene. J. Virol. **62**:3319–3327.