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Location of the Structural Genes for Glycoproteins gD and gE and for Other Polypeptides in the S Component of Herpes Simplex Virus Type 1 DNA

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To map the structural genes for the gD and gE polypeptides and for other viral products encoded in the S component of herpes simplex virus type 1 DNA, we selected mRNAs capable of hybridizing to cloned viral DNA fragments and translated the mRNAs in vitro to determine which polypeptides were encoded therein. The gD and gE polypeptides were identified by immunoprecipitation with appropriate monoclonal and monospecific antibodies, whereas the other polypeptides were characterized only by their electrophoretic mobilities in polyacrylamide gels. We found that gD mRNA hybridized to a single *Sac*I subfragment of *Bam*HI fragment J, whereas gE mRNA hybridized to an adjacent *Sac*I subfragment of *Bam*HI fragment J and also to BamHI fragment X. These and other results permit the conclusion that the structural gene for gD is located between map coordinates 0.911 and 0.924, and the gene for gE is between map coordinates 0.924 and 0.951. We also found that mRNAs for polypeptides of 55,000, 42,000, 33,000, and 22,000 molecular weight hybridized to DNA fragments spanning the regions from map coordinates 0.911 to 0.924, 0.897 to 0.911, 0.939 to 0.965, and 0.939 to 0.965, respectively. Finally, in accord with the results of others, we found that mRNA for a 68,000-molecular-weight polypeptide hybridized to the two noncontiguous *Bam*HI fragments N and Z, which share a reiterated DNA sequence.

Herpes simplex virus (HSV) specifies the production of several glycoproteins in infected cells. As constituents of the virion envelope and the infected cell surface, these glycoproteins play essential roles in virion infectivity, in virus-induced cell fusion, and probably also in other aspects of viral replication and cytopathology (36).

Among the glycoproteins specified by HSV type 1 (HSV-1), four major species designated gA/gB, gC, gD, and gE have been identified (3, 7, 35). Most of these species are antigenically and functionally related but structurally nonidentical to glycoproteins specified by HSV-2 (7, 8, 15, 23, 26, 27). Because the related glycoproteins specified by HSV-1 and HSV-2 exhibit characteristic differences in electrophoretic mobility, it has been possible to map the regions of the HSV genome that determine these differences by analysis of the genomes of, and the proteins specified by, HSV-1 × HSV-2 recombinant viruses. By using this approach, genes determining properties of gD and gE have previously been mapped to the small or S component of the HSV genome, and genes determining properties of gA/gB and gC have been mapped to the large or L component (15, 20, 26, 33).

To verify that the HSV DNA sequences determining the electrophoretic mobilities of the glycoproteins indeed contain the structural genes encoding the polypeptide precursors and to map these structural genes more precisely, we have undertaken to identify the cloned viral DNA fragments capable of hybridizing to mRNA for each of the glycoproteins. Identification of the mRNAs is based on their ability to direct the synthesis in vitro of polypeptides that are immunoprecipitable by monoclonal or monospecific antibodies directed against the appropriate glycoprotein.

In this study, we have focused on gD and gE. The physiological roles of these two glycoproteins are not known, although it has been shown that gE binds to the Fc portion of immunoglobulin G (IgG) (3) and is probably responsible for the Fc-binding receptors present on HSV-infected cells (40) and on virions (24). We have found, in accord with previously published results (15, 20, 26, 33), that the structural genes for HSV-1 gD and gE are located in the S component of the HSV-1 genome, specifically in adjacent but ap-

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parently nonoverlapping regions of the unique sequence. During the course of this work, we also showed that the structural genes for at least four other HSV-1 polypeptides are located in the unique sequence of the S component and confirmed the map location of a fifth gene.

MATERIALS AND METHODS

Cells and viruses. Vero (African green monkey kidney) cells and HEp-2 (human epidermoid carcinoma-2) cells were used in this study. Their source and maintenance have been described previously (24). Vero cells were used in most of the experiments because their yield of translatable viral mRNA was higher, although experiments where both cell lines were used provided identical results. HSV-1 strains F (9) and mP (12) were used. They specify the production of all the viral glycoproteins identified to date. Results obtained with either virus strain were comparable.

Plasmids and the isolation of plasmid DNA. The plasmids used in this study are described in Fig. 1. All except pRB123-3 were kindly provided by B. Roizman, University of Chicago, Ill. The arrangement of reiterated and unique DNA sequences in the HSV-1 genome (indicated near the top of Fig. 1) is as described by Wadsworth et al. (38). The *Bam*HI map shown here is that published by Locker and Frenkel (18) as modified by Post et al. (29). We confirmed the order of the X and Z fragments established by Post et al. (29); in other reports (18, 31), these two fragments were either named differently or shown in the opposite order. Preston (31) recently reported the existence of and mapped several small *Bam*HI fragments (<0.3 × 10^6 molecular weight) from the S component of HSV-1 DNA; these fragments are not indicated in Fig. 1. The positions of the *Sac*I recognition sites and *Sac*I subfragments in the *Bam*HI J fragment were established by performing single and double digestions of pRB123, pRB123-3, pRB308, and pRB309 DNAs with the appropriate restriction endonucleases; we found the positions of the *Sac*I sites to be the mirror image of that published by Mocarski et al. (21).

To isolate plasmid DNA for mRNA selection, *Escherichia coli* C600SF8 containing each of the different plasmids were grown in L broth, and the plasmids were amplified by the addition of chloramphenical at 150 μg/ml during the late-logarithmic phase. Cleared lysates were prepared, and plasmid DNA was purified by two cycles of CsCl isopycnic centrifugation (5). The authenticity of each plasmid was verified by restriction enzyme digestion and by hybridization of the labeled plasmid to restriction enzyme digests (*Bam*HI, *Sal*I, *Hin*dIII, and *Eco*RI) of HSV-1 DNA blotted on nitrocellulose filters (34).

Preparation and selection of viral mRNA. Near-confluent cells were infected with HSV-1 F or mP at 20

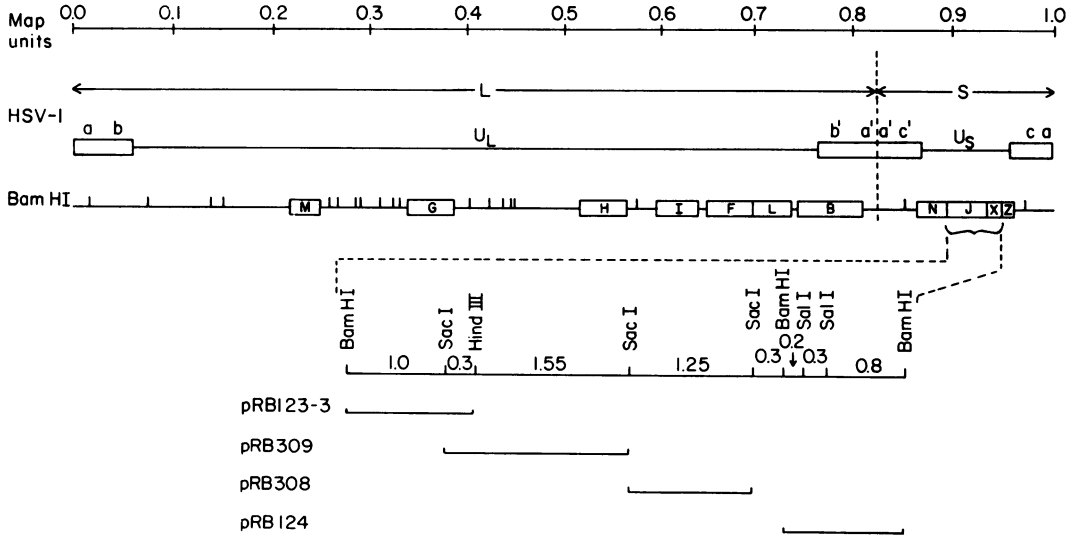

FIG. 1. Viral DNA fragments used to select mRNAs for in vitro translation. The *Bam*HI restriction fragments of HSV-1 F DNA which were tested for their ability to hybridize to gD and gE mRNAs are indicated by the boxes shown on the *Bam*HI restriction map. These fragments were cloned by Post et al. (29) in pBR322 to generate the following recombinant plasmids containing the *Bam*HI fragments indicated within parentheses: pRB107 (B), pRB128 (F), pRB102 (G), pRB127 (H plus L), pRB130 (I plus M), pRB123 (J), pRB126 (L), pRB125 (M), pRB114 (N), pRB124 (X), and pRB122 (Z). Subcloned DNA sequences from *Bam*HI fragment J are shown on the enlarged map of *Bam*HI fragments J and X. Mocarski et al. (21) constructed the plasmids pRB308 and pRB309 by inserting the indicated *Sac*I fragments into the unique *Sac*I site of pRB103 (*Bam*HI fragment Q of HSV-1 F DNA [map units, 0.294 to 0.317] cloned into pBR322). We constructed pRB123-3 for this study by deleting the HSV-1 and pBR322 DNA sequences between the two *Hin*dIII sites in pRB123. The numbers shown above the line in the enlarged map indicate the molecular weights (×10^6) of the DNA fragments.

PFU per cell and incubated at 34°C in medium 199 supplemented with 1% fetal calf serum. Preliminary studies showed that de novo synthesis of viral glycoproteins in the infected cells was detectable at either 8 or 18 h after infection as determined by immunoprecipitation of pulse-labeled glycoproteins with appropriate monoclonal antibodies. When the yield of viral glycoprotein mRNA isolated from cells harvested at 6, 8, and 12 h after infection was determined by in vitro translation and immunoprecipitation, it was found that 12 h-infected cells provided the highest yield. Therefore, cells were harvested relatively late (at 14 h after infection) to isolate cytoplasmic RNA for most of the experiments described below.

To isolate cytoplasmic mRNA, the infected cells were scraped off the growth surface, washed once in cold phosphate-buffered saline, and swollen in 5 cell volumes of hypotonic buffer (10 mM NaCl, 25 mM Tris-chloride [pH 8.3], 1.5 mM $MgCl_2$ with 0.1% [vol/vol] diethylpyrocarbonate added immediately before use) on ice for 5 min. Nonidet P-40 (NP-40) was added to a final concentration of 0.5% (vol/vol) to lyse the cells. Nuclei were pelleted at 1,500 × *g* for 10 min at 4°C. The cytoplasmic extract was adjusted to 0.5% (wt/vol) sodium dodecyl sulfate (SDS) and 150 mM NaCl, and RNA was purified by phenol extraction and ethanol precipitation. Polyadenylated RNA was isolated by oligodeoxythymidylate-cellulose (T-3, Collaborative Research) chromatography.

To select mRNA with sequence homology to specific viral DNA fragments, 25 μg of recombinant plasmid DNA was immobilized to each diazobenzyloxymethyl filter disk (1.1 cm in diameter, Schleicher and Schuell) as described by Goldberg et al. (10). Approximately 50 to 70% of the input DNA was retained by the filter as monitored by using radioactive marker DNA. Cytoplasmic polyadenylated RNA isolated from 10^8 infected cells was solubilized in 0.8 M NaCl, 20 mM 1,4-piperazinediethanesulfonic acid (PIPES), pH 6.5, 1 mM EDTA, 0.5% SDS, 1 mg of rat liver tRNA per ml, and 50% (vol/vol) formamide, applied to a DNA-containing filter, and allowed to hybridize at 56°C for 6 h. After extensive washing, the filter-bound RNA was eluted with 96.5% (vol/vol) formamide in 10 mM PIPES, pH 6.5, and 0.5% SDS at 75°C (19) and recovered by ethanol precipitation in the presence of 10 μg of rat liver tRNA. One-fifth of the yield was used in each in vitro translation reaction.

In vitro translation. In vitro translation by reticulocyte lysate (New England Nuclear Corp., Boston, Mass.) was carried out exactly as specified by the supplier. The radioactive tracer used for all in vitro reactions was [^{35}S]methionine (1,000 Ci/mmol [50 μCi/25-μl reaction]). A 1-μl amount of the total reaction mixture of 25 μl was removed for analysis of the total translation products by polyacrylamide gel electrophoresis. The rest of the reaction mixture was prepared for immunoprecipitation.

Antibodies and immunoprecipitation. Hybridoma cell lines producing antibodies directed against HSV glycoproteins were isolated by procedures to be described in detail elsewhere (M. F. Para, R. Sprague, and P. G. Spear, manuscript in preparation). Seven different hybridoma antibodies specific for gD and one specific for gE were tested for possible use in this study. All hybridoma antibodies identified as anti-gD were shown to precipitate the same polypeptides (the multiple electrophoretic forms of gD) from infected-cell lysates as did the anti-gD hybridoma antibody designated HD1 and described by Pereira et al. (27). The hybridoma antibody identified as anti-gE (II-481) was shown to precipitate the same polypeptides (multiple forms of gE) as did rabbit serum S24, a monospecific anti-gE serum previously described (25).

To prepare antigens from infected cells for immunoprecipitation, cell pellets from cultures labeled with [^{35}S]methionine were lysed in immunoprecipitation buffer (140 mM NaCl, 20 mM Tris-hydrochloride, pH 7.6, 1% [vol/vol] NP-40, 0.5% [vol/vol] sodium deoxycholate and ovalbumin [1 mg/ml]) and the lysates were cleared of particulate matter by centrifugation at 100,000 × *g* for 1 h. For the in vitro translation reaction mixtures, concentrated stocks of the above-mentioned components were added to yield the concentrations indicated for immunoprecipitation buffer. The amount of antibody used for the antigens made in vitro was that sufficient to precipitate over 90% of the respective glycoprotein extractable from 2.5 × 10^4 infected HEp-2 cells at 24 h after infection. Tripling this quantity of antibody did not enhance the detection of in vitro translation products of glycoprotein mRNA. The reaction of antigens with the antibodies was carried out at 0°C for 30 min. Sufficient amounts of Formalin-fixed *Staphylococcus aureus* (16) were then added to permit complete adsorption of IgG in the reaction. After an additional 10 min at 0°C, the immunoprecipitates were first washed in 140 mM NaCl, 20 mM Tris-chloride, pH 7.6, 1% (vol/vol) NP-40, 0.5% (wt/vol) sodium deoxycholate, 0.1% (wt/vol) SDS, followed by 140 mM NaCl, 20 mM Tris-chloride, pH 7.6, 0.1% (vol/vol) NP-40 and then 10 mM Tris-chloride, pH 7.6, and 0.1% NP-40. They were then eluted from the bacteria by boiling in SDS sample buffer for gel electrophoresis.

Polyacrylamide gel electrophoresis and peptide analysis by partial proteolysis. Electrophoresis on linear 10% polyacrylamide gel cross-linked with *N,N'*-diallyltartardiamide (11) was carried out to analyze polypeptides made in vitro. A linear 15% polyacrylamide gel was used to obtain profiles of peptides generated by partial proteolysis. Fluorography was carried out as described by Laskey and Mills (17).

Partial proteolysis was performed to verify the identity of polypeptides as described by Cleveland et al. (4). After gel electrophoresis, bands containing the polypeptide in question were sliced from the unfixed gels, equilibrated with 125 mM Tris-chloride, pH 6.8, 0.2% SDS, 1 mM EDTA, and reinserted into sample wells on the second gel with orientation rotated 90° so that the protein bands became vertical to the gel surface. *S. aureus* V8 protease was used at the concentrations indicated.

RESULTS

Use of antibodies to identify the in vitro products translated from gD and gE mRNAs. Seven hybridoma antibodies capable of precipitating gD from infected-cell lysates were tested for their ability to immunoprecipitate the in vitro translation product of gD mRNA present in unfractionated cytoplasmic polyadenylated RNA extracted from infected cells. Antibody II-

436 was chosen for use in this study because it was found to precipitate a polypeptide of 51,000 molecular weight, whereas the other monoclonal antibodies including HD1 described by Pereira et al. (27) reacted poorly, if at all, with any polypeptide made in vitro. The various forms of gD immunoprecipitable from infected cell lysates range in apparent molecular weight from 52,000 to 65,000 (6, 27, 35).

Previous work from this laboratory has showed that rabbit antiserum S24 immunoprecipitated gE made in cells infected with HSV-1 (25). The apparent molecular weights of the various forms of gE range from 66,000 to 80,000 (3, 25). From the total in vitro translation products, S24 immunoprecipitated a polypeptide of 66,000 molecular weight (Fig. 2A). Hybridoma antibody II-481 immunoprecipitated gE from infected-cell lysates (Para and Spear, manuscript in preparation) and also a 66,000-molecular-weight polypeptide made in vitro. The identity of the polypeptides recognized by S24 and II-481 was established by partial proteolysis as described by Cleveland et al. (4); Fig. 2B shows that the electrophoretic profiles of the digestion products were indistinguishable. Thus, we conclude that both S24 and II-481 react with the gE polypeptide made in vitro and, in addition, that it was the only polypeptide recognized by S24. Because S24 precipitated the 66,000-molecular-weight polypeptide more efficiently than did II-481, S24 was used as the primary tool for identifying the product of gE mRNA.

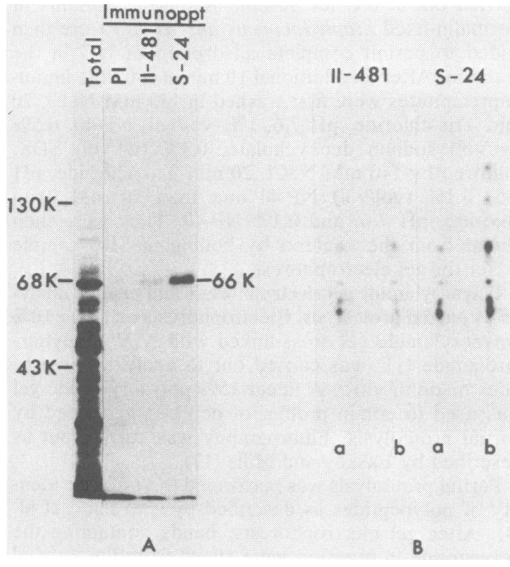

FIG. 2. Comparison by partial proteolysis of the in vitro translation products immunoprecipitated by antibody II-481 and immune serum S24. Cytoplasmic polyadenylated RNA isolated from cells infected with HSV-1 F was translated in vitro. Monoclonal antibody II-481 and monospecific serum S24 each immunoprecipitated a polypeptide with an apparent molecular weight of 66,000, whereas preimmune rabbit serum (PI) failed to react with this polypeptide (A). Polypeptides immunoprecipitated by each antibody were digested with 0.2 μg (a) and 0.08 μg (b) of *S. aureus* V8 protease as described in the text. The electrophoretic profile of the partially digested polypeptides in 15% polyacrylamide gel is shown in (B); all samples shown in (B) were analyzed on the same acrylamide gel slab, but exposure of the X-ray film was 8 weeks for the left lanes (II-481) and 3 weeks for the right lanes (S24).

Identification of the *Bam*HI fragments of HSV-1 DNA that hybridize to gD and gE mRNAs. Plasmid DNAs containing individual HSV-1 DNA fragments (each of the *Bam*HI fragments indicated in Fig. 1) were immobilized on DBM-filter disks and allowed to hybridize with cytoplasmic polyadenylated RNA isolated from cells infected with either HSV-1 F or mP. The mRNA retained by the filters was subsequently eluted and translated in vitro. The in vitro products were analyzed for the presence of the polypeptides translated from gD or gE mRNAs by immunoprecipitation with the monoclonal and monospecific antibodies described above. It was found that the mRNA capable of directing the synthesis in vitro of immunoprecipitable gD hybridized selectively to *Bam*HI fragment J (pRB123) (Fig. 3), whereas the mRNA directing the synthesis of gE in vitro hybridized to both *Bam*HI fragments J and X (pRB123 and pRB124) (Fig. 4). Neither gD nor gE could be immunoprecipitated from the in vitro products of mRNAs selected by the other *Bam*HI fragments tested (Figs. 1, 3, 4) (some of the data not shown).

HSV-1 DNA sequence homologous to gD mRNA. To identify the coding region for gD in *Bam*HI fragment J, plasmid DNAs containing subfragments of *Bam*HI fragment J (Fig. 1) were used to select gD mRNA by hybridization. Results shown in Fig. 3 identified the 1.85×10^6-dalton *Sac*I fragment (in pRB309) as the only subfragment of *Bam*HI fragment J capable of efficiently hybridizing to gD mRNA. Because pRB123-3 failed to hybridize to gD mRNA, we conclude that the coding sequence for the gD polypeptide is located between 0.911 and 0.924 map units on the HSV-1 genome (Fig. 5).

HSV-1 DNA sequence homologous to gE mRNA. A strategy similar to that used in mapping gD mRNA was used to map the coding region for gE in *Bam*HI fragments J and X. Results shown in Fig. 4 identified the 1.25×10^6-dalton *Sac*I fragment (in pRB308) as containing a sequence homologous to gE mRNA. The ability of the 0.3×10^6-dalton sequence located between this *Sac*I fragment and *Bam*HI fragment X to hybridize with gE mRNA was tested by comparing the hybridization efficiency of a

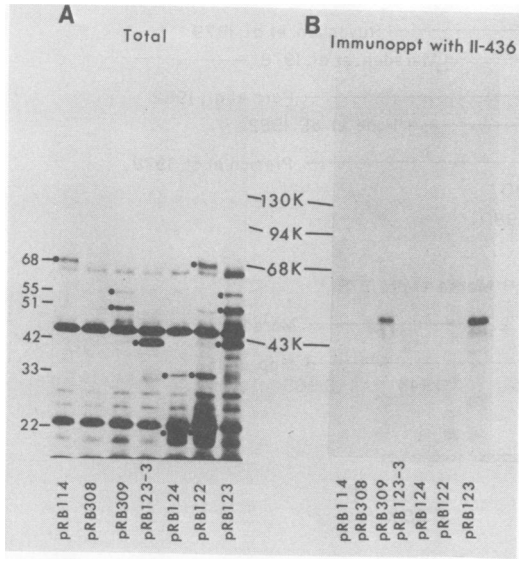

FIG. 3. Identification of viral DNA fragments capable of hybridizing to gD mRNA. Plasmid DNAs containing viral DNA fragments as depicted in Fig. 1 were immobilized on diazobenzyloxymethyl filter disks and allowed to hybridize with cytoplasmic polyadenylated RNA isolated from HSV-1 mP-infected cells. The mRNA species selected by each viral DNA fragment were translated in vitro and the products analyzed by polyacrylamide gel electrophoresis. The X-ray film was exposed for 2 days (A). The dots identify the polypeptides encoded by exogenous mRNA in each reaction. The apparent molecular weights of these polypeptides are indicated on the left border of the autoradiogram. The gD polypeptide made in vitro was identified by immunoprecipitation with monoclonal antibody II-436 followed by electrophoresis of the precipitates. This X-ray film was exposed for 6 weeks (B). β-galactosidase (130,000), phosphorylase b (94,000), bovine serum albumin (68,000), and ovalbumin (43,000) were used as molecular weight standards.

pRB123 derivative with only the 1.85×10^6- and 1.25×10^6-dalton *Sac*I fragments deleted to that of pRB123-3 and was found to be slightly above background (data not shown). The plasmid containing *Bam*HI fragment X (pRB124) was cleaved at its *Sal*I sites and recloned into pBR322. All the derived plasmids containing HSV-1 DNA also contained sequences capable of hybridizing with gE mRNA, with the plasmid containing the 0.8×10^6-dalton HSV-1 fragment showing highest hybridization efficiency (data not shown). We conclude that the coding sequence for the gE polypeptide is located between 0.924 and 0.951 map units on the HSV-1 genome (Fig. 5).

Other polypeptides encoded in HSV-1 DNA sequences from the S component. Electrophoretic analysis of the in vitro translation reaction mixtures (Fig. 3 and 4) revealed that at least five polypeptides in addition to gD and gE were synthesized from mRNAs homologous to particular HSV-1 DNA fragments from the S component of the genome. The DNA fragments that hybridized to the mRNAs for each of these polypeptides (68,000, 55,000, 42,000, 33,000, and 22,000 apparent molecular weights) are indicated in Fig. 5. The 45,000-molecular-weight polypeptide present in products of mRNA selected by pRB308 and pRB309 (Fig. 4) was excluded from consideration here because it was not found in products of mRNAs selected by pRB123 (Fig. 4) and is probably encoded in the *Bam*HI fragment Q sequences also contained in pRB308 and pRB309 (21). For some reason, this 45,000-molecular-weight polypeptide that was specifically selected by pRB308 and pRB309 DNAs was not detected when viral mRNAs were isolated from cells infected with HSV-1 mP (Fig. 3). Conversely, an mRNA encoding the

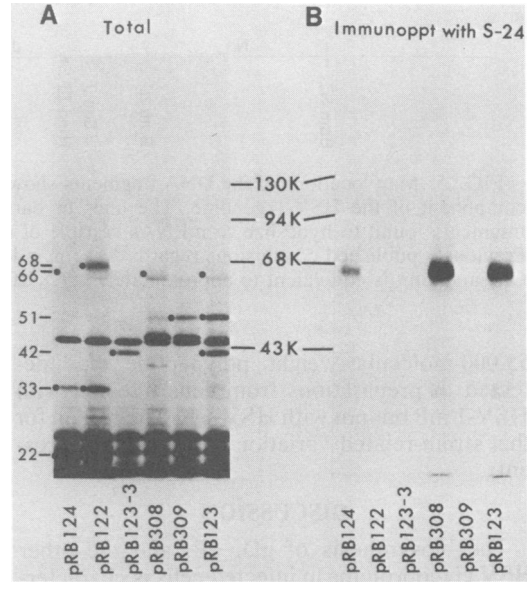

FIG. 4. Identification of viral DNA fragments capable of hybridizing to gE mRNA. Plasmid DNAs containing viral DNA fragments as depicted in Fig. 1 were immobilized on diazobenzyloxymethyl filter disks and allowed to hybridize with cytoplasmic polyadenylated RNA isolated from HSV-1 F-infected cells. The mRNA species selected by each viral DNA fragment were translated in vitro, and the products were analyzed by polyacrylamide gel electrophoresis. The X-ray film was exposed for 2 days (A). The gE polypeptide was identified by immunoprecipitation with antiserum S24 followed by electrophoresis of the precipitates. The X-ray film was exposed for 4 weeks (B). The molecular weight standards used are listed in the legend to Fig. 3.

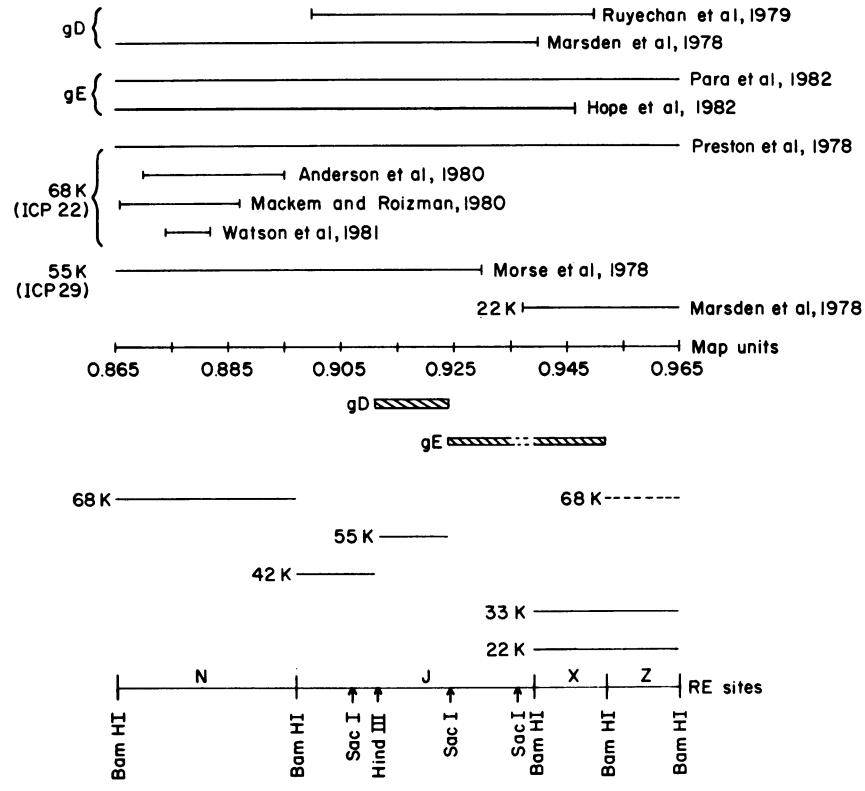

FIG. 5. Map locations of the DNA fragments shown to be homologous to specific mRNAs encoded in the S component of the HSV-1 genome. The lines or bars indicate the locations and sizes of the smallest DNA fragments found to hybridize to mRNAs capable of directing synthesis in vitro of the gene products named. Previously published conclusions regarding the map locations of the indicated gene products are summarized. One map unit is equivalent to approximately 1.7×10^3 base pairs of DNA.

55,000-molecular-weight polypeptide was detected in preparations from cells infected with HSV-1 mP but not with HSV-1 F. The reason for this strain-related variation is not clear at present.

DISCUSSION

The biosynthesis of gD, gE, and the other HSV glycoproteins in infected cells is characterized by multiple stages of posttranslational modification, some of which result in shifts in electrophoretic mobilities of the glycopolypeptides (3, 6, 7, 25, 26, 28, 35). For gD at least, the addition of carbohydrate probably begins before the polypeptide is released from the polysome, and the carbohydrate added includes preformed oligosaccharide transferred from a dolichol phosphate donor (37); this conclusion emerged from studies of Pizer et al. (28), who showed that newly synthesized gD made in the presence of tunicamycin had an apparent molecular weight of 50,000 and was not labeled by [^{3}H]mannose, whereas newly synthesized gD made in the absence of tunicamycin had an apparent molecular weight of 52,000 and had incorporated [^{3}H]mannose.

Based on the above considerations, one could predict that the glycoprotein polypeptides made in vitro might differ in apparent molecular weight and conformation (due to absence of carbohydrate moieties) from the newly synthesized glycopolypeptides made in infected cells. In fact, we found that the apparent molecular weight of the gD polypeptide made in vitro was only slightly less than that of the glycopolypeptide produced in vivo (51,000 versus 52,000 to 53,000). The conformation of the in vitro product was probably considerably different from that of the in vivo product, however, because several anti-gD monoclonal antibodies capable of immunoprecipitating gD from infected-cell lysates failed to react with the in vitro product.

The extent to which the gE polypeptide made in vitro differs from the newly synthesized polypeptide made in vivo is difficult to assess from our results because the apparent molecular

weight was the same (66,000) as we reported earlier for the in vivo product (3, 25) and both antibody preparations used reacted with both the in vitro and in vivo products.

The results presented here demonstrate that gD and gE mRNAs hybridize selectively to HSV-1 DNA sequences located between 0.911 and 0.924 map units and 0.924 and 0.951 map units on the viral genome, respectively, entirely within the unique sequence of the S component (Fig. 5). These findings are in accord with previously published results which were obtained by analyzing HSV-1 × HSV-2 recombinant viruses and which showed that genetic loci determining the electrophoretic difference between HSV-1 and HSV-2 gD and electrophoretic and antigenic differences between HSV-1 and HSV-2 gE mapped to the regions overlapping these sequences as indicated in Fig. 5 (15, 20, 26, 33).

The results presented here indicate that the structural genes for the gD and gE polypeptides determine most of the intertypic differences in these proteins previously mapped by use of HSV-1 × HSV-2 recombinant viruses as predicted earlier (20, 22, 33). Our results also permit more precise localization of the gD and gE genes. The genes are adjacent to each other or at least are very closely linked, but the DNA sequences homologous to the mRNAs apparently do not overlap; there was no reason to expect overlap of the coding sequences given the absence of any evidence for the sharing of structural determinants by gD and gE. Whether the transcriptional units for the gD and gE genes are overlapping remains to be determined. Physical characteristics of the gD and gE mRNAs are not known. Although several mRNAs homologous to appropriate regions of the S component of HSV-1 DNA have been described (2, 13), we have no basis for suggesting that any specific ones are the putative gD or gE mRNAs. It should be noted that greater quantities of translatable gD and gE mRNAs were isolated from infected cells at 12 h after infection than at 8 or 6 h, whereas most of the previously published work on mapping of HSV mRNAs was done with preparations of RNA extracted from cells before 8 h after infection.

Of the other polypeptides we detected by translation in vitro of selected mRNAs, considerable information has already been published by others about the 68,000-molecular-weight polypeptide and its mRNA. This α or immediate-early polypeptide was originally mapped to the S component of HSV DNA by analyses of HSV-1 × HSV-2 recombinant viruses (32). It has been shown that the 5′ end of the 1.7-kilobase mRNA encoding this polypeptide is located within the reiterated sequence of the S component designated c′ on Fig. 1 and that the 3′ end is located to the right in the U_S sequence (1, 19, 39). It has also been shown that this mRNA is spliced, with the intron being approximately 150 bases and located near the 5′ end (39). Although the role of the 68,000-molecular-weight polypeptide, designated ICP 22, is not known, it is of interest that deletions can be introduced into the gene, resulting in the production of truncated gene products without interfering with the ability of the mutated viruses to replicate in cultured primate or human cells (30).

Our finding that the mRNA for the 68,000-molecular-weight polypeptide hybridized to both the *Bam*HI fragments N and Z is explained by the fact that both fragments contain the reiterated sequence homologous to the 5′ end of the mRNA (Fig. 1). It is of interest that mRNA for an α polypeptide is still present in translatable form at 14 h after infection, given the evidence that α polypeptide synthesis is shut off or severely reduced coincident with the synthesis of β and γ polypeptides (14). Anderson et al. (1) also showed that translatable mRNA for α polypeptides could be isolated at late times after infection and, in addition, demonstrated continued synthesis of specific α mRNAs, including the one for the 68,000-molecular-weight polypeptide.

At the present time, it is not possible to state with certainty that the other polypeptides we mapped are identical to any previously characterized HSV-1 polypeptides. Possibly, the 55,000-molecular-weight polypeptide encoded between 0.911 and 0.924 map units is the 55,000-molecular-weight polypeptide (a β polypeptide designated ICP 29) that was mapped by Morse et al. (22), using HSV-1 × HSV-2 recombinant viruses, to a region of the genome between 0.78 and 0.93 map units. Similarly, the 22,000-molecular-weight polypeptide we showed to be encoded between 0.939 and 0.965 map units could be the 22,000-molecular-weight polypeptide localized by Marsden et al. (20) to the region from 0.94 to 0.99 map units. To our knowledge, mapping of the genes for the 42,000- and 33,000-molecular-weight polypeptides that we detected has not previously been described; our results permit localization of the structural genes for these polypeptides to regions of the HSV-1 genome between 0.897 and 0.911 map units and 0.939 and 0.965 map units, respectively.

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