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Identification of Proteins Encoded by a Fragment of Herpes Simplex Virus Type 2 DNA That Has Transforming Activity

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Cloned *Bgl*II fragment N (map units 0.58 to 0.625) of herpes simplex virus type 2 DNA has been shown to transform rodent cells to an oncogenic phenotype (Galloway and McDougall, J. Virol. **38**:749–760, 1981). RNA homologous to this fragment directs the synthesis of five polypeptides in a cell-free translation system. The approximate molecular weights of these proteins are 140,000, 61,000, 56,000, 35,000, and 23,500. The 35,000-dalton protein is the major species late in infection and is the only species detected before the onset of viral DNA replication. The arrangement of the sequences encoding these proteins along the herpes simplex virus type 2 genome was determined by hybridization of the RNA to cloned *Pst*I fragments of *Bgl*II-N and to single-stranded DNA segments cloned into M13mp7. Both the hybridization experiments and immunoprecipitation with monoclonal antibodies suggested that the 140,000- and 35,000-dalton proteins are at least partially colinear and share antigenic determinants.

The oncogenic potential of herpes simplex virus type 2 (HSV-2) has been well documented (31); nevertheless, the identification of a viral protein(s) that mediates transformation has remained elusive. A number of HSV-specific antigens have been reported in transformed cells, including membrane glycoproteins gA/gB (6; R. Courtney, personal communication) and CP-1, which is probably equivalent to gD (33), nonstructural proteins VP143 (10), thymidine kinase (26, 32), and ICP 10 (20), and a number of other polypeptides with presumed viral counterparts (16, 22, 39). In the absence of transformation-deficient mutants of HSV-2, it has not been possible to demonstrate that any of these proteins plays an essential role in transformation.

Cells transformed by UV-inactivated HSV retain variable regions of the genome (11, 13). Workers in two laboratories have reported that a fragment of HSV-1 DNA located between 0.31 and 0.42 map unit (1.0 map unit = 15 kilobases) is able to transform hamster embryo cells (6, 34). Two different regions of the HSV-2 genome have been reported to have transforming potential; neither of these regions is homologous to the HSV-1 sequences. Reyes et al. (34) used *Bgl*II fragment N of HSV-2 strain 333, which maps between positions 0.58 and 0.625, to alter the growth properties of hamster embryo cells and BALB 3T3 cells. Jariwalla et al. (20) selected transformed cells which grew out of continuous passage after exposure to fragments of HSV-2 strain 333 or S_1 DNA. These experiments identified a fragment of DNA with transforming potential located between positions 0.43 and 0.58 on the HSV-2 genome.

The use of cloned *Bgl*II fragment N of HSV-2 DNA in transformation experiments has demonstrated unambiguously that sequences of viral DNA located between map positions 0.58 and 0.625 on the HSV-2 genome can alter the phenotypes of primary rat cells and NIH 3T3 cells in a fashion which allows the cells to grow in medium containing low serum concentrations, to reach high saturation densities, to form colonies in semisolid media, and to produce tumors in nude mice and newborn animals (14). Therefore, located within 7.1 kilobases of the HSV-2 genome is all the viral information required to initiate and maintain the transformed state. One protein from this region, which had a molecular weight of 37,800 (37.8K), was identified by hybrid-arrested translation experiments (9). To identify other proteins specified by these sequences, RNA prepared from lytically infected cells was selected by hybridization to *Bgl*II-N and was translated in a rabbit reticulocyte cell-free system. By analogy with other DNA tumor viruses, it might be expected that a potential oncogenic protein would be synthesized in the absence of viral DNA replication. To determine whether proteins of the α or β class (19) are encoded by *Bgl*II-N sequences, RNA synthesized in the presence of anisomycin, cycloheximide, or cytosine arabinoside was analyzed. We found that a number of polypeptides with molecular weights ranging from 140,000 to 23,500 can be translated from RNA homologous to this

region of the genome and that only one major species, a 35K protein, is synthesized early in infection. A preliminary map locating the viral polypeptides was constructed by selection of RNA to small fragments of *Bgl*II-N that had been recloned into pBR322 or into single-stranded phage M13mp7. These studies identified several proteins which may be involved in transformation by HSV-2.

MATERIALS AND METHODS

Cells and viruses. BHK-21 cells were cultured in a 10% CO_2 atmosphere by using Dulbecco modified Eagle medium (GIBCO Laboratories) containing 10% fetal calf serum (Microbiological Associates) in plastic tissue culture plates (Falcon Plastics). Plaque-purified HSV-2 strain 333 was used for all infections.

Infection of cells. Six different RNA preparations were made from BHK-21 cells infected with HSV-2 at a multiplicity of 20 PFU/cell. The cells were exposed to the virus for 60 min in Dulbecco modified Eagle medium without serum and subsequently maintained in medium containing 2% fetal calf serum. Untreated infected cell RNA was prepared from cells harvested 10 h postinfection. To obtain immediate early RNAs, 30 μg of cycloheximide per ml (Sigma Chemical Co.) or 100 μM anisomycin (a gift from Pfizer, Ltd.) was present in the medium continuously from the time of exposure to the virus and subsequent incubation until 4 h postinfection. To obtain β class early RNAs, 20 μg of cytosine arabinoside (Sigma) per ml was present in the medium throughout exposure of the cells to virus and subsequent incubation for 6 h postinfection. Alternatively, 30 μg of cycloheximide per ml or 10 μM anisomycin was added 2 h postinfection, and the cells were maintained in the presence of the drug for 4 h postinfection. To prepare radiolabeled extracts of viral proteins, BHK-21 cells were infected with HSV-2 strain 333 at a multiplicity of 10 PFU/cell. The cells were exposed to virus for 1 h in Dulbecco modified Eagle medium containing 2% fetal calf serum but no methionine. After absorption, the inoculum was replaced with Dulbecco modified Eagle medium containing 2% fetal calf serum, one-fifth the usual amount of methionine, and 25 μCi of [^{35}S]methionine (New England Nuclear Corp.) per ml. Cultures were harvested 24 h after infection.

Isolation of viral RNA and proteins. The infected cells were rinsed, scraped into ice-cold phosphate-buffered saline, collected by centrifugation, and washed twice with ice-cold phosphate-buffered saline. Total cytoplasmic RNA was prepared as described by Anderson et al. (1). Cell extracts were prepared for immunoprecipitation by suspending cell pellets in extraction buffer (phosphate-buffered saline containing 0.1% sodium dodecyl sulfate [SDS], 0.5% sodium deoxycholate, and 1% Nonidet P-40, pH 7.5) at a concentration of 10^7 cells per ml. The cell suspension was sonicated with a probe sonicator twice for 15 s on ice and centrifuged at 2,500 rpm for 10 min. The supernatant was collected and frozen at −70°C.

Enzymes, electrophoresis, nick translation, and blot hybridization. All restriction endonucleases and T4 ligase were purchased from Bethesda Research Laboratories and were used according to the conditions suggested by the manufacturer. Agarose gel electrophoresis was carried out in vertical slab gels by using 0.7% agarose to fractionate the DNA from the recombinant plasmids. The gels were stained with ethidium bromide and photographed. DNA was denatured in situ and transferred to nitrocellulose sheets (37) as described previously (5). DNA was made radioactive by the nick translation method (36), using DNA polymerase I (New England Nuclear Corp.). Acrylamide gel electrophoresis was carried out in vertical slab gels by using 9, 10, or 15% acrylamide with either bisacrylamide or *N,N'*-diallyltartardiamide (DATD) as a linker.

Recombinant DNA clones. The construction and characterization of plasmid pDG401, which contains *Bgl*II fragment N of HSV-2 DNA, have been described previously (14, 15). Subclones of pDG401 were obtained by digesting the plasmid and vector pBR322 with *Pst*I. The two DNAs were mixed in a ratio of 10:1, ligated, transfected into *Escherichia coli* HB101 by using the $CaCl_2$ method, and selected on agar plates containing 30 μg of tetracycline per ml, as described previously (15). The colonies were screened for sensitivity to ampicillin, and plasmid DNA was prepared by the rapid alkaline extraction procedure (3) for restriction analysis with *Pst*I. Large preparations of plasmid DNA were prepared by centrifugation in CsCl containing ethidium bromide.

The replicative form (RF) of M13mp7 was cleaved with *Pst*I and mixed with individual *Pst*I subclones of pDG401 which had been similarly cleaved. The DNAs were ligated, used to transfect *E. coli* JM101, and plated onto agar plates in the presence of isopropyl thio-β-galactoside and 5-bromo-4-chloro-3-indolyl-D galactoside. The recombinants, which were detected as white plaques, were picked and grown for 8 to 12 h with *E. coli* JM101, and the phage were purified from the culture supernatant by precipitation with 10% polyethylene glycol. The DNA was released from the phage by deproteinization with proteinase K (10 μg/ml) and purified by phenol extraction and ethanol precipitation. The identity of the recombinant phage was determined by restriction analysis and sizing of the RFs on agarose gels, hybridization of the phage DNAs to each other to establish pairs, and hybridization of radiolabeled RF DNA to *Pst*I digests of pDG401 DNA.

Selection hybridization and in vitro translation. Linearized, denatured plasmid DNA or single-stranded phage DNA was applied to nitrocellulose and used to select complementary mRNA essentially as described by Ricciardi et al. (35). In a typical experiment we used two 4-mm squares of nitrocellulose to which approximately 20 μg of DNA and 250 to 500 μg of cytoplasmic RNA in a total hybridization mixture of 100 μl had been applied. The eluted RNA was used to program cell-free translation (total volume, 12.5 μl) in a micrococcal nuclease-treated reticulocyte lysate, as described by Pelham and Jackson (29). The products of cell-free translation were labeled with [^{35}S]methionine and were analyzed by SDS-polyacrylamide gel electrophoresis as described by Laemmli (24), followed by fluorography.

Preparation of monoclonal antibodies and immunoprecipitation. Hybridoma cell lines producing monoclonal antibodies were isolated by the method of Kohler and Milstein (23). Spleen cells from C57BL/6 mice infected with HSV-2 strain 333 were fused with BALB/c NSI myeloma cells by using procedures de-

scribed elsewhere (23; Goldstein et al., manuscript in preparation). Culture supernatants were assayed for anti-HSV antibodies by an antibody-binding assay (28), using ^{125}I-labeled protein A against HSV-1- or HSV-2-infected cell antigens. Hybridomas 6-A6 and 6-H11 produced antibody specific for HSV-2 and were cloned by limiting dilution.

Immunoprecipitation reaction mixtures contained 100 μl of ascites fluid diluted 1:100 and 100 μl of [^{35}S]methionine-labeled cell lysate or in vitro translation products incubated for 1 h on ice. Each reaction was terminated by incubation for 1 h with 100 μl of *Staphylococcus aureus* (Formalin fixed; 10% [vol/vol] in extraction buffer containing 0.1% ovalbumin) (21). The precipitate was collected by centrifugation in a microfuge, and the pellet was washed five times in 0.1 M Tris-hydrochloride–0.5 M LiCl–1% β-mercaptoethanol (pH 8.0) (41). The precipitated proteins and antibodies were solubilized in 0.05 M Tris-hydrochloride (pH 7.0)–2% SDS–5% β-mercaptoethanol–0.005% bromophenol blue at 100°C for 2 min for analysis by SDS-polyacrylamide electrophoresis in 9% slab gels (17, 24, 38). Autoradiography was enhanced by fluorography, using the commercial reagent EnHance (New England Nuclear Corp.).

RESULTS

Cell-free translation of RNA preparations from HSV-2-infected cells directs the incorporation of [^{35}S]methionine into numerous proteins (data not shown). mRNA selected by hybridization to pDG401, a recombinant clone which contains *Bgl*II fragment N of HSV-2 DNA, encoded a much smaller set of proteins, which had approximate molecular weights of 140,000, 61,000, 56,000, 35,000, and 23,500 (Fig. 1, lanes a, d, and i). If plasmid pBR322 DNA was used for hybridization (Fig. 1, lane b) the products of cell-free translation were indistinguishable from those obtained when no RNA was added (Fig. 1, lane e). The predominant species were the 35K, 61K, and 56K proteins, indicating that the messages for these proteins were more abundant or were translated more efficiently. The 140K and 23.5K proteins were less prominent, which may indicate that the mRNAs of these proteins were less abundant or were encoded only partially by

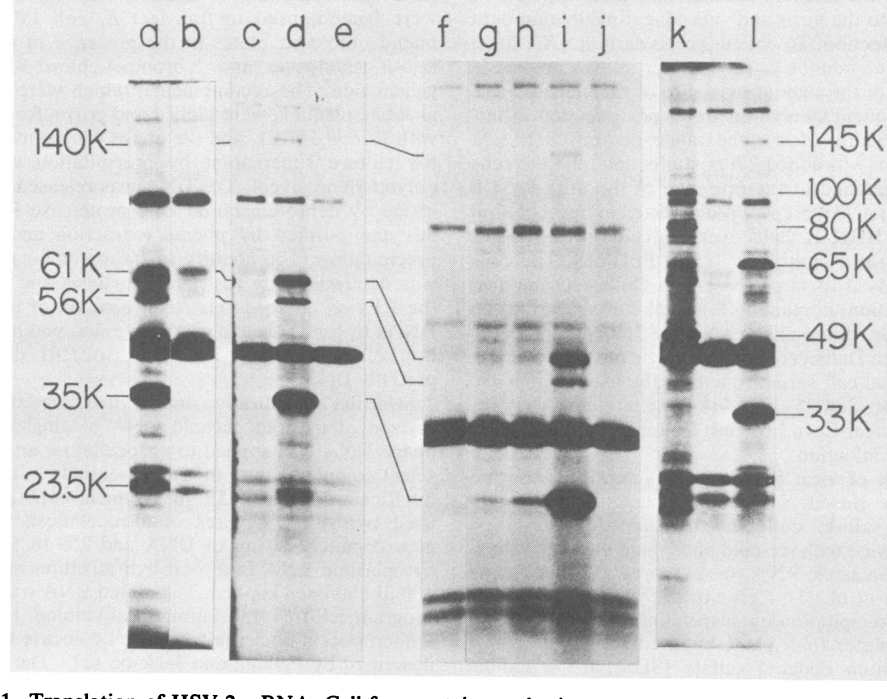

FIG. 1. Translation of HSV-2 mRNA. Cell-free protein synthesis was programmed by using RNA complementary to either pDG401 (lanes a, c, d, f, g, h, and i), pBR322 (lane b), pDG304 (lane k), or pDG305 (lane l). RNA from late in infection was used for some experiments (lanes a, b, d, i, k, and l), whereas in other cases RNA was prepared from cells treated before infection with 25 μg of cycloheximide per ml (lane c) or 100 μM anisomycin (lane f) or from cells treated 2 h postinfection with 10 μM anisomycin (lane g) or 20 μg of cytosine arabinoside per ml (lane h). No RNA was added to the control translations (lanes e and j). The products of cell-free translation were analyzed by SDS-polyacrylamide gel electrophoresis, using either 15% (lanes a through e and k through l) or 10% (lanes f through j) acrylamide gels. Fluorographed gels were exposed for 9.5 days (lanes a and b), 3 days (lanes c through e, k, and l), or 11 days (lanes f through j).

*Bgl*II-N and therefore were selected less efficiently. It is also possible that the 23.5K protein, as well as several other small polypeptides present in very minor amounts, represented premature termination of translation or the product of proteolytic degradation.

To indicate by comparison with other regions whether the abundance of late HSV-2 mRNAs complementary to pDG401 is typical of the HSV-2 genome, late HSV-2 RNA complementary to two other plasmids, pDG305 (*Bgl*II-G; 0.20 to 0.32 map unit) and pDG304 (*Bgl*II-K; 0.32 to 0.40 map unit), was prepared and translated. Selected by pDG304 (Fig. 1, lane k) was a major mRNA for a 49K protein, as well as mRNAs for 100K and 80K proteins, whereas pDG305 (Fig. 1l) encoded a major 33K protein, as well as 145K and 65K proteins. Several additional polypeptides present in minor amounts were observed among the translation products of mRNAs complementary to both plasmids.

To determine whether the pDG401-specific proteins are α or β gene products (19), we purified RNA from infected cells which had been treated with various metabolic inhibitors. RNA from cells which had been exposed at the time of infection to the protein synthesis inhibitor cycloheximide (25 μg/ml) (Fig. 1, lane c) or anisomycin (100 μM) (25) did not direct the synthesis of proteins encoded by *Bgl*II fragment N, indicating that no immediate early RNAs from this region could be detected by this method. To determine whether any of these RNAs were synthesized before the onset of viral DNA replication (β class), cells were infected in the presence of cytosine arabinoside (Fig. 1, lane h), or alternatively, anisomycin (10 μM) or cycloheximide (25 μg/ml) was added 2 h postinfection and the cells were harvested 4 to 6 h postinfection (Fig. 1, lane g). The 35K protein mRNA was present in the cells treated by all three protocols, but in amounts much smaller than at late times. In the absence of data measuring DNA synthesis in the presence of cytosine arabinoside, our conclusion that the 35K protein is encoded by a β-class transcript relies on the concurrence of the three inhibitors which were used. If other less abundant RNAs were synthesized in the early or immediate early class of transcripts, our methods would not have detected them. However, it is clear that at least one protein encoded

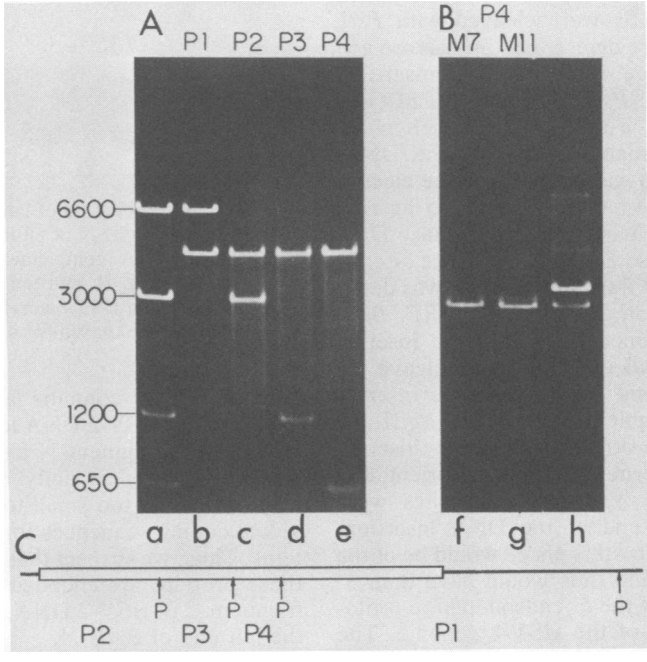

FIG. 2. Cloning of four *Pst*I fragments from a plasmid containing *Bgl*II fragment N of HSV-2 DNA. Plasmid pDG401, which contains *Bgl*II fragment N inserted into pBR322, was cleaved four times with *Pst*I (lane a). The four fragments were cloned by insertion into the *Pst*I site of pBR322, and the insertions were identified by cleavage of plasmids P1 through P4 with *Pst*I (A, lanes b to e) and electrophoresis through 0.7% agarose. Recombinants in M13mp7 which contained either strand of P4 were electrophoresed on a 1.4% agarose gel (B). When the two DNAs were hybridized at 68°C in 1 M NaCl for 30 min and chilled, the predominant band had a higher molecular weight, indicating a hybrid between the two complementary strands (lane h). (C) Sites of cleavage.

within the transforming region was synthesized, although in small amounts, in the absence of DNA replication.

Plasmid pDG401 was cleaved by *Pst*I at four sites to produce fragments of 6,600, 3,000, 1,200, and 650 base pairs (Fig. 2A, lane a). These fragments were recloned into the *Pst*I site of vector pBR322 (Fig. 2A, lanes b through e). Figure 2C shows the order of the *Pst*I fragments. The individual *Pst*I clones were used to select RNA for in vitro translation from cells infected with HSV-2. Plasmid P1 selected the mRNA for the 56K protein (Fig. 3, lane b), P2 selected the mRNA for the 140K, 61K, and 35K proteins (Fig. 3, lane C), P3 selected the mRNA for the 61K protein (Fig. 3, lane d), and P4 selected the mRNA for the 56K protein (Fig. 3, lane e). We were unable to select mRNAs for the 23.5K protein. The gene order deduced in part from this experiment is shown in Fig. 4 with respect to a map showing the *Pst*I fragments of HSV-2 *Bgl*II-N DNA used.

Single-stranded segments of *Bgl*II fragment N were obtained by cloning the P4 fragment of pDG401 into phage M13mp7. Four different phage were obtained; these represented the two strands of the pBR322 moiety of the molecule and the two strands of the pDG401 moiety. The double-stranded RFs were cleaved with *Pst*I, and their sizes were determined on agarose gels to identify the phage which contained insertions identical in size to *Pst*I fragment 4 of pDG401 (data not shown). To determine which phage contained complementary strands, the DNAs were hybridized to each other, and the electrophoretic mobility of duplex DNA on agarose gels was distinguished from that of phage DNA which did not reassociate (Fig. 2B). The orientation of the inserted fragment of DNA was determined by restriction analysis of the RF. *Bgl*II cleaves M13mp7 once outside of the inserted sequences, and *Sal*I and *Sac*I each cleave the recombinant plasmid once within the inserted sequences. By double digestion with *Bgl*II and *Sac*I or *Sal*I the orientation of the inserted fragment was determined, and this orientation indicated that the *Sal*I and *Sac*I sites were located at the 5′ end of the DNA insertion. Messages selected by this phage would be of the opposite polarity and thus would have their 3′ ends to the right of the 5′ ends along the prototype configuration of the HSV-2 genome. The single-stranded phage DNAs were used to select RNA for cell-free translation. Phage P4M11 selected mRNA for the 56K protein (Fig. 3, lane g), and P4M7 did not select any mRNA for the 56K protein (Fig. 3, lane f). This indicates that the message specifying the 56K protein is transcribed from left to right on the HSV-2 genome.

The plasmid containing the P2 fragment of

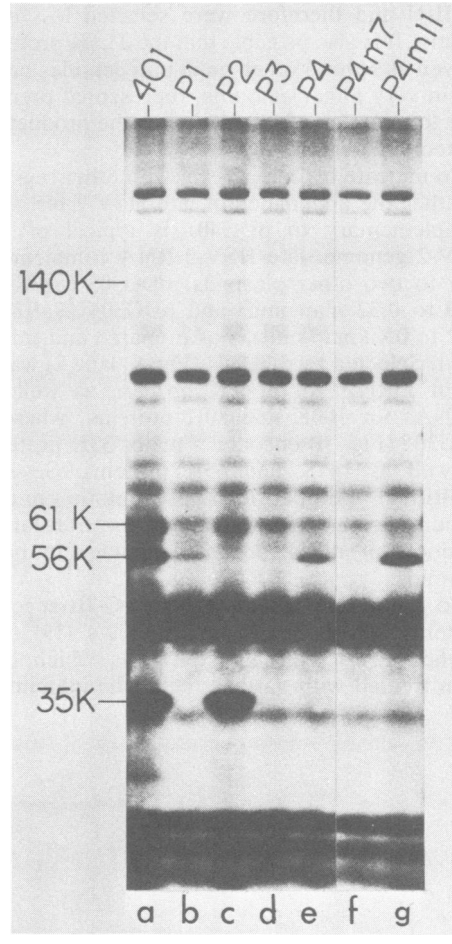

FIG. 3. Fractionation of late HSV-2 mRNAs complementary to *Bgl*II-N. Cell-free translation was directed with RNA complementary to the plasmids indicated (see Fig. 2), and the products of translation were analyzed on a 15% acrylamide gel. The fluorographed gel was exposed for 4 days.

pDG401 DNA contains approximately 2,100 base pairs of HSV-2 DNA located at the left end of the *Bgl*II fragment N and thus has a single-stranded coding capacity of about 700 amino acids, which is too small to accommodate individual coding sequences for 140K and 35K proteins. Thus, we suspect that either one or both of these proteins are encoded in part within *Bgl*II fragment C of HSV-2 DNA, which is adjacent to the left end of *Bgl*II-N.

Several monoclonal antibodies directed against type-specific HSV-2 proteins were screened for the ability to immunoprecipitate proteins encoded by *Bgl*II-N sequences. Two antibodies had nearly identical patterns of proteins precipitated from [^{35}S]methionine-labeled extracts of HSV-2-infected cells. Both antibodies precipitated a 140K protein and a 35K pro-

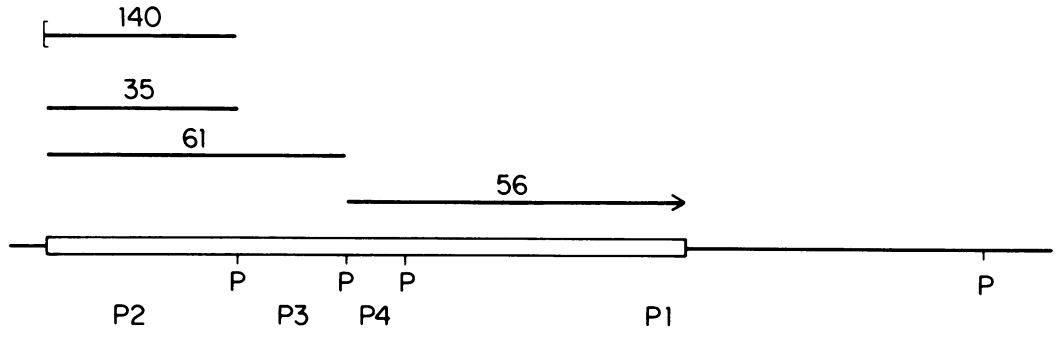

FIG. 4. Location of the polypeptides encoded by *Bgl*II fragment N. Plasmid pDG401, which contains *Bgl*II fragment N is shown on the bottom with the sites at which *Pst*I cleaves the plasmid. The box represents the sequences of HSV-2, and the line represents the pBR322 moiety of the molecule. The arrow below the 56K protein indicates that transcription proceeds from left to right. The bracket at the left of the 140K protein indicates that only a portion of the message is encoded within the *Bgl*II-N sequences.

tein as well as a series of minor species (Fig. 5). With one antibody (6-A6) the reaction to the 140K protein was stronger than to the 35K protein, whereas the opposite preference was observed with the other antibody. The antibodies precipitated the appropriate proteins from the products of cell-free translation directed by mRNAs homologous to *Bgl*II fragment N (Fig. 5). It is unlikely that the proteins coprecipitated because of aggregation. In other experiments (Goldstein et al., manuscript in preparation), when HSV-2-infected cell proteins were transferred from a gel to nitrocellulose paper and reacted with antibody and radiolabeled *S. aureus* protein, both 140K and 35K proteins reacted with the antibodies.

Taken together with the mapping data, these observations suggest but do not prove that the coding sequences for the 140K and 35K proteins are partially colinear and that the monoclonal antibodies recognize antigenic determinants common to both proteins.

DISCUSSION

We used cell-free translation of mRNA selected with specific fragments of HSV-2 DNA to map proteins encoded by *Bgl*II fragment N of the HSV-2 genome. The gene order shown in Fig. 4 assumes that herpesviral RNA is not transcribed from noncontiguous sequences. If that were the case, the RNA which hybridized to *Bgl*II fragment N could be remote from the main coding regions for these mRNAs. With the exception of two immediate early mRNAs (40), none of the HSV RNAs that have been studied in detail have readily detectable introns or noncontiguous leader sequences (2, 8, 30). Therefore, it seems reasonable to conclude that the genes for at least four polypeptides are encoded at least partially by the DNA sequences of *Bgl*II fragment N.

Of these species, the mRNA that is present in the greatest amount or is translated most efficiently is the mRNA for the 35K protein. This mRNA is present in small amounts before the onset of viral DNA replication and thus belongs to that set of β mRNAs that are enhanced at late times (18). The remaining species are detected only at late times and thus appear to be γ-class mRNAs (19), although if they were present at early times in reduced amounts, they might not have been detected in our experiments.

Our preliminary analysis of late mRNAs encoded by two other regions of the HSV-2 genome, represented by the viral insertions in pDG304 and pDG305 corresponding to *Bgl*II fragments J (0.32 to 0.40 map unit) and G (0.20 to 0.32 map unit), revealed a similar pattern of one prominent species and several less abundant species, encompassing a wide range of protein molecular weights. In all three regions of the genome, there are in addition to the species that we have noted in Fig. 1 several low-abundance, low-molecular-weight proteins. These could represent artifacts of translation or additional gene products. Further experiments, including DNA and protein sequence analyses and more extensive fractionation of RNA species, will be required to define fully the coding capacity of these genome segments.

The density of the coding sequences for the mRNAs in this small segment of the HSV-2 genome (5%) and the fact that different temporal classes of mRNA are in close proximity are not surprising considering the arrangement of mRNAs in other regions of the HSV genome that have been studied in detail (2, 8).

With few exceptions, the gene orders of the HSV-1 and HSV-2 serotypes are generally thought to be identical (31). One puzzling difference is that at least two distinct sites in the long unique component of the HSV-1 and HSV-2

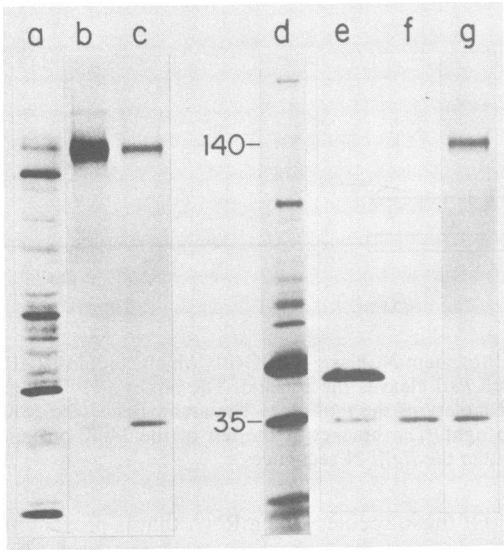

FIG. 5. Identification of HSV-2-specific proteins by using monoclonal antibodies. A large number of infected cell-specific proteins were identified by labeling BHK-21 cells infected with HSV-2 strain 333 with [^{35}S]methionine (lane a). When extracts from the infected cells were immunoprecipitated with monoclonal antibodies 6-A6 (lane b) and 6-H11 (lane c), two polypeptides with molecular weights of 140,000 and 35,000 were seen. The relative abundance of the proteins varied with the two antibodies, and minor species could be detected. The in vitro translation products specified by RNA selected on pDG401 showed five virus-specific polypeptides, including 140K and 35K proteins (lane d). The translation products were diluted 1:20 to reduce the SDS concentration (lane e) and were precipitated with 6-H11 (lane f) or 6-A6 (data not shown). The 35K protein was clearly precipitated, and after a very long exposure after immunoprecipitation with 6-A6, the 140K protein could be detected (data not shown). The locations of the two immunoprecipitated proteins are shown for comparison (lane g). The proteins were displayed on 9% polyacrylamide gels with a DATD linker, except for lane d, which was in 10% polyacrylamide with a bisacrylamide linker.

genomes are responsible for morphological transformation. Anderson et al. (2) and Frink et al. (12) have characterized the mRNAs which map to a region of the HSV-1 genome that partially overlaps the morphological transforming region defined by *Bgl*II fragment N. Of particular interest are a 5.2-kilobase mRNA and a 1.5-kilobase mRNA which may have colinear 3′ ends that map around position 0.60 on the HSV-1 genome. When translated in vitro, these RNAs specified 140K and 40K proteins, respectively. It is tempting to speculate that these proteins are the homologs of the 140K and 35K proteins encoded at the left end of HSV-2 *Bgl*II fragment N and that differences in the biogenesis of these RNAs between the two serotypes may be associated with the differences in transforming activity. Clements and McLaughlan (7) have determined the sequence of the DNA in this region of the HSV-1 genome and have concluded that the translation stop for the 140K protein precedes the translation initiation codon for the 40K protein. These results suggest a real difference between HSV-1 and HSV-2, in that precipitation with monoclonal antibodies indicates that the two HSV-2 polypeptides share coding sequences. More precise RNA mapping and greater analysis of the polypeptides are necessary to answer these questions.

In this study we identified the proteins that are encoded by a region of the HSV-2 genome which has the potential to transform rodent cells to a tumorigenic phenotype. If this process is mediated by a viral protein, we have described the possible candidates. Experiments are in progress to determine whether the 140K protein is identical to VP143, which has been found in transformed cells (10), or to the ICP 10 found in cells transformed by *Bgl*II fragment C (20). However, since this polypeptide is only partially encoded by *Bgl*II-N, a complicated mechanism would be required for it to have a role in transformation. Preliminary experiments to detect the 35K protein by immunofluorescence in a few HSV-2-transformed cell lines have been negative, as have experiments to detect virus-specific mRNA in these cell lines. At the moment, we cannot rule out the involvement of these proteins in the maintenance of the transformed phenotype. It is possible that they are present in transformed cells, but in amounts too low to be detected by our preliminary experiments. Alternatively, *Bgl*II-N gene products may be required for initiation but not for maintenance of transformation. Clearly, additional experiments, possibly including the isolation of transformation-deficient mutants, will be needed to define the gene product(s) involved in virally induced transformation.

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