JOURNAL OF VIROLOGY, May 1981, p. 483–496
0022-538X/81/050483-14$02.00/0

Vol. 38, No. 2

Characterization of a Major Late Herpes Simplex Virus Type 1 mRNA

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A major, late 6-kilobase (6-kb) mRNA mapping in the large unique region of herpes simplex virus type 1 (HSV-1) was characterized by using two recombinant DNA clones, one containing *Eco*RI fragment G (0.190 to 0.30 map units) in λ·WES·B (L. Enquist, M. Madden, P. Schiop-Stansly, and G. Vandl Woude, Science **203**:541–544, 1979) and one containing *Hin*dIII fragment J (0.181 to 0.259 map units) in pBR322. This 6-kb mRNA had its 3′ end to the left of 0.231 on the prototypical arrangement of the HSV-1 genome and was transcribed from right to left. It was bounded on both sides by regions containing a large number of distinct mRNA species, and its 3′ end was partially colinear with a 1.5-kb mRNA which encoded a 35,000-dalton polypeptide. The 6-kb mRNA encoded a 155,000-dalton polypeptide which was shown to be the only one of this size detectable by hybrid-arrested translation encoded by late polyadenylated polyribosomal RNA. The S1 nuclease mapping experiments indicated that there were no introns in the coding sequence for this mRNA and that its 3′ end mapped approximately 800 nucleotides to the left of the *Bgl*II site at 0.231, whereas its 5′ end extended very close to the *Bam*HI site at 0.266.

The herpes simplex virus type 1 (HSV-1) genome is large (150 kilobase pairs) and has a complex arrangement (reviewed in reference 27). Expression of HSV-1 mRNA is a highly regulated process requiring both virus-directed protein synthesis and viral DNA replication. Concomitant with viral DNA replication, a large number of specific mRNA's appear which are not readily detectable before this (16, 17). Some of these late mRNA's are considerably larger than 5 kilobases (kb) in length. We have used hybridization of size-fractionated polyribosomal polyadenylated [poly(A)] mRNA to Southern blots of endonuclease-restricted HSV-1 DNA to grossly localize many of these mRNA species (5, 16, 17; E. K. Wagner, K. P. Anderson, R. H. Costa, G. B. Devi, B. H. Gaylord, L. E. Holland, J. R. Stringer, and L. L. Tribble, *in* Y. Becker, ed., *Herpesvirus DNA: Recent Studies on the Internal Organization and Replication of the Viral Genome*, in press) and have used HSV-1 DNA restriction fragments bound to cellulose to isolate and partially characterize individual HSV-1 mRNA species (3–5, 16, 17).

From our studies on the characterization of individual HSV-1 mRNA species, it is evident that several regions of the viral genome are not expressed as abundant mRNA until late after infection (16, 17). One region of particular interest is that between approximately 0.18 and 0.3 map units on the HSV-1 genome. This region encodes a large (6 kb) mRNA which in long-term labeling regimens is the most abundant viral mRNA seen in the polysomes (5). This mRNA is of interest because it maps in the region where the major capsid polypeptide (155,000 daltons in size) of the HSV-1 virion has been localized by use of intertypic recombinants (20, 22).

Our mRNA isolation techniques and the availability of recombinant DNA technology in the study of HSV-1 have allowed us to precisely characterize the map position, the direction of transcription, and the polypeptide encoded by this 6-kb HSV-1 mRNA species. It also has demonstrated that, as has been seen with other mRNA's mapping in other areas of the viral genome, this mRNA is partially colinear with at least one smaller species. These studies suggest that the procedure reported here, in addition to those previously reported, will be of great value in obtaining an increasingly complete picture of the properties and expression of HSV-1 mRNA.

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MATERIALS AND METHODS

Cells and virus. Monolayer cultures of HeLa cells were grown at 37°C in Eagle minimal essential medium containing 10% calf serum and no antibiotics. Plaque-purified virus of the KOS strain of HSV-1 was used for all infections.

Enzymes. All restriction endonucleases were obtained from New England Biolabs, and digestion was carried out in buffers recommended by that supplier. Each microgram of DNA was digested with 0.25 to 0.5 U of the appropriate enzyme for 3 h at 37°C. Phage T4 ligase was obtained from Bethesda Research Laboratories. The ligation reaction was essentially as recommended by the supplier. A solution of 10 μg of restricted HSV-1 and 1 μg of pBR322 DNA in 25 μl of solution was incubated with each unit of ligase in 66 mM Tris (pH 7.6), 6.6 mM $MgCl_2$, 10 mM dithiothreitol, and 0.4 mM ATP for 6.5 h at 4°C. Then the solution was diluted to 250 μl, and incubation was continued for a further 20 h. Reverse transcriptase from avian myeloblastosis virus was supplied by Joseph Beard. The incubation was carried out as described previously (3, 4, 16) with a 1,000-fold excess of random oligodeoxyribonucleotide primer (34) or a 100-fold excess of oligodeoxythymidine [oligo(dT_{12-18}); Collaborative Research, Inc.]. Incubation mixtures were in 50 μl and contained: 50 mM Tris (pH 8.1), 50 mM KCl, 8 mM $MgCl_2$, 50 μCi of [^{32}P]TTP (350 Ci/mmol; New England Nuclear Corp.), 0.2 mM each dATP, dCTP, and dGTP, 4 mM dithiothreitol, 10 μg of actinomycin per ml, 0.1 to 1 μg of denatured DNA or RNA template primer, and 2 U of reverse transcriptase. Incubation was for 60 min at 37°C. For random primed reactions, an additional 1 U of enzyme and 0.1 μmol each of dGTP, dCTP, and dATP were added after 1 h, and incubation was carried out for a further 1 h at 37°C. The S1 nuclease was obtained from Boehringer Mannheim Corp. as a lyophilized powder. Samples of 5×10^5 U, as supplied, were dissolved in 0.5 ml of S1 buffer (0.25 M NaCl, 0.03 M NaOAc, pH 4.6, and 0.001 M $ZnCl_2$) containing 50% glycerol. For each digestion, 10^4 U was used, and samples containing 10 μg of poly(A) RNA, 10 μg of carrier tRNA, and 2 to 3 μg of [^{32}P]DNA (specific activity, 50,000 to 60,000 Cerenkov cpm/μg; 28) were digested in S1 buffer containing 5% glycerol for 30 min at 45°C.

Isolation, labeling, and size fractionation of polyribosomal RNA. Monolayer cultures of HeLa cells (2×10^7 cells/flask) were infected for 30 min at a multiplicity of 10 PFU of virus per cell in phosphate-buffered saline containing 0.1% glucose and 1.0% fetal calf serum. For ^{32}P-labeled RNA, the cells were overlaid with 200 μCi of $^{32}P_i$ per ml (2.5 to 3.0 mCi/culture; New England Nuclear Corp.) in Eagle minimal essential medium containing a 1/10 normal concentration of phosphate and 5% dialyzed calf serum. Time of infection was measured after the 30-min absorption period.

Polyribosomes were isolated from the cytoplasm of HSV-1-infected cells by the magnesium precipitation method of Palmiter (24). Polyribosome-associated RNA was purified by phenol-chloroform extraction after proteinase K digestion of the polyribosomes. Poly(A) mRNA was isolated from total rRNA by the use of oligo(dT)-cellulose (Collaborative Research, Inc.). Details of this procedure have been presented elsewhere (3, 4, 16, 30).

The RNA was size fractionated by electrophoresis on 1.2% agarose gels containing 10 mM methylmercury hydroxide (6) as previously described (3–5, 16, 17). Electrophoresis was through vertical slabs (12 by 15 by 0.15 cm) at 25 mA/gel for 4 to 6 h. After electrophoresis, the gels were soaked in 50 mM β-mercaptoethanol for 20 min before further workup. The RNA was eluted from appropriate gel slices by dissolving the agarose in 2 to 3 ml of phenol at 60°C and then extracting twice with equal volumes of 0.1 M NaCl, 0.01 M Tris, pH 7.4, and 0.001 M EDTA. The RNA in the aqueous phase was then ethanol precipitated and repurified by oligo(dT) chromatography. Overall yields of RNA were in the range of 50%.

Recombinant DNA and isolation of restriction fragments. All HSV-1 restriction fragments are referred to by their accepted letter designations where appropriate [i.e., *Eco*RI, fragment G (0.19 to 0.30)] and their coordinates in map units on the prototype arrangement of the HSV-1 genome (3, 27). The relationship of the prototype arrangement of the HSV-1 genome to the other possible arrangements has been described previously (27). All recombinant DNA experiments were done according to current National Institutes of Health guidelines.

*Eco*RI fragment G (0.190 to 0.30) is a clone in λ·WES·B. This clone was a gift from L. Enquist and G. Vande Woude of the National Institutes of Health and was grown according to their procedures (14). The phage stock supplied was plaqued on *E. coli* DP50 SupF (a gift of D. Tiemier) grown in Luria broth containing 100 μg of diaminopimelic acid and 5 mg of thymidine per ml (SupF medium). An individual plaque was used to prepare a high-titer lysate (2.3×10^{10} PFU/ml) by infection of 0.05 ml of an overnight culture of bacteria with a single plaque and dilution to 5 ml in growth medium containing 10 mM $MgSO_4$. Samples of 10^9 PFU of this phage stock were used to infect 1 to 2 ml of an overnight culture which was grown to lysis in 200 ml of SupF medium containing 10 mM $MgSO_4$. Phage was prepared by cesium chloride centrifugation. We routinely obtained about 100 μg of phage DNA from a 200-ml lysate.

*Hin*dIII fragment J (0.181 to 0.259) was cloned in pBR322 (clone J-2) in our laboratory. The procedure is essentially that of Bedbrook et al. (7); pBR322 was restricted with *Hin*dIII, mixed with *Hin*dIII-restricted HSV-1 DNA, and ligated. The 10 μl of ligated mixture containing 4 μg of DNA was transfected into 5×10^8 cells of *E. coli* strain X-1776 by the $CaCl_2$ method (11). Transfected cells were spread on detergent-free nitrocellulose filters (Millipore Corp.) and grown on 1.5% agar plates containing X-1776 broth (X-1776 broth contains, per liter, 25 g of tryptone, 7.5 g of yeast extract, 20 mM $MgSO_4$, 50 mM Tris, pH 7.5, 100 mg of diaminopimelic acid, and 5 g of thymidine) supplemented with 40 μg of ampicillin per ml. After overnight growth, colonies were replica plated onto fresh filters, and these were subjected to colony hybridization (15). We carried out colony hybridization by using 2×10^6 to 3×10^6 Cerenkov cpm of [^{32}P]complementary DNA (cDNA) made to isolated *Hin*dIII fragment J (0.181

to 0.259) purified by electrophoresis through agarose gels. Hybridization conditions are described below. Positive colonies were located by autoradiography and then respread and replated for low-density colony hybridization. After the second round of colony hybridization, individual colonies were picked and streaked on 1.5% agarose containing X-1776 broth with ampicillin, and individual colonies were picked for growth in 50-ml liquid cultures. The plasmid was isolated from these cultures by the method of Clewell and Helinski (10), and about 5 μg of plasmid was isolated. This was characterized by restriction endonuclease digestion and electrophoresis against appropriate size standards. When intactness of the cloned HSV-1 DNA fragment was confirmed, the plasmid was used to transfect *E. coli* LE392 (a gift of B. Moss). The colony of transfected bacteria then was treated as above to confirm the presence of the proper fragment, and 100- to 200-ml cultures were grown in Luria broth, and the plasmid was isolated by the procedure of Tanaka and Weisblum (33) for chloramphenicol amplification. The LE392 grown in 40 μg of ampicillin yielded between 0.5 and 1 μg of plasmid DNA per ml of culture. Each preparation was rechecked by restriction digestion before further use.

Individual restriction fragments of the DNA clones were prepared by restriction enzyme digestion followed by fractionation by electrophoresis in 40 mM Tris (pH 7.8), 5 mM sodium acetate, and 2 mM EDTA on horizontal 0.7% agarose slabs (20 by 20 by 0.7 cm) at 30 mA/gel, as described previously (3–5, 16, 17). Agarose bands containing DNA of interest were dissolved in phenol at 65°C, and agarose was removed by repeated extraction of the phenol with a buffer containing 0.1 M NaCl and 0.002 M EDTA, pH 7.4. Recovery of DNA was between 50 and 60% of theoretical yield.

Subclones of the original cloned DNA fragments were made by isolating the specific DNA restriction fragment (0.5 to 2 μg) and ligating a 10-fold excess of this with appropriately digested pBR322 followed by transfection directly into LE392 and colony screening as described.

Isolation of fragment-specific mRNA. Fragment-specific mRNA was isolated from poly(A) polyribosomal RNA by preparative hybridization to the appropriate DNA covalently coupled to cellulose. Details of coupling of DNA to cellulose and preparative hybridization were as described previously (3–5). Hybridization was in 80% recrystallized formamide containing 0.4 M Na^+, 0.1 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 8.0, and 0.005 M EDTA at 57°C. Rinsing of cellulose and elution of hybridized RNA were as described elsewhere (5).

Synthesis of cDNA. The cDNA was synthesized from either RNA or DNA as described in previous reports (3, 4, 16, 17). Total cDNA was synthesized by using random oligodeoxyribonucleotides prepared from calf thymus DNA as a primer for reverse transcriptase (34). The 3'-cDNA was made by use of oligo(dT_{12-18}) (Collaborative Research, Inc.) as a primer. Partial alkaline digestion of the RNA and reselection on oligo(dT)-cellulose columns were as described previously (4, 17).

Preparation and hybridization of DNA (Southern) blots. Southern blots of DNA were prepared as described previously (3–5) according to the basic method of Southern (29). Hybridization of RNA to Southern blots was carried out in 65% formamide, 0.4 M Na^+, 0.1 M HEPES, pH 8.0, and 5 mM EDTA (hybridization buffer) at 45°C for 24 to 48 h. Hybridization of cDNA to blots was carried out at 37°C for 24 to 48 h in hybridization buffer containing Denhardt solution (0.02% Ficoll, 0.2% bovine serum albumin, and 0.02% polyvinylpyrrolidone; 13). All blots were rinsed twice for 1 h in hybridization buffer under hybridization conditions and subjected to autoradiography on Kodak X-Omat-R film.

Preparation and hybridization of RNA (Northern) blots. After fractionation by methylmercury-containing agarose gel electrophoresis, RNA was blotted onto diazotized paper according to the method of Alwine et al. (2). The cDNA was hybridized to such RNA blots exactly as described for hybridization to Southern blots.

S1 nuclease analysis of HSV-1 RNA. S1 nuclease analysis of RNA was carried out essentially as described by Berk and Sharp (8, 9). Cultures (100 ml) of *E. coli* LE392 containing the appropriate restriction fragment of HSV-1 DNA in pBR322 were grown in low-phosphate morpholinepropanesulfonic acid medium (23, 32) containing 2 to 3 μCi of $^{32}P_i$. The DNA had a specific activity of 50,000 to 60,000 Cerenkov cpm/μg. The DNA was linearized by digestion with appropriate restriction enzymes, and 2-μg portions were added to 10 μg of infected-cell polyribosomal poly(A) RNA and then precipitated with ethanol. The mixed pellets were dissolved in 100 μl of hybridization buffer containing 80% recrystallized formamide, denatured by incubation for 10 min at 78°C, and immediately transferred to a 60°C water bath for 3.5 h of hybridization. The hybridization was terminated by addition of 1.0 ml of ice-cold pH 4.6 buffer containing 250 mM NaCl, 30 mM sodium acetate, 1 mM $ZnCl_2$, and 5% glycerol. A 10-μl portion (~10^4 U) of S1 nuclease was added, and digestion was carried out for 30 min at 45°C. At this time, 20 μg of tRNA was added as carrier to each sample, and the material was ethanol precipitated.

Samples were fractionated by electrophoresis through horizontal neutral 0.9% agarose gels (20 by 20 by 0.5 cm) run in a pH 8.3 buffer containing 50 mM Tris, 40 mM sodium acetate, and 2 mM EDTA or on the same-size alkaline gels in 30 mM NaOH and 2 mM EDTA. The latter buffer was found to completely degrade ^{32}P-labeled RNA run as a test. Electrophoresis for 16 h at 40 mA was carried out by using restriction endonuclease-digested ^{32}P-cloned DNA in parallel tracks as size markers. The gels then were neutralized if needed and dried in vacuo, and bands were visualized by autoradiography on Kodak X-Omat-R film.

In addition to specific bands, which were reproducibly seen, there was often a variable amount of background radioactivity migrating heterogeneously throughout the gels. This background was specific for added infected-cell RNA since control experiments using as much as 100 μg of carrier HeLa cell RNA showed no residual radioactivity after mock hybridization and S1 digestion. Shorter digestion times with the enzyme increased this background, but longer

digestion times resulted in reduction of specific bands, especially larger ones. The background was usually reduced in alkaline gels and increased when hybridization was with DNA that could be expected to be nicked due to prolonged storage or manipulation. These observations led us to conclude that a major portion of the background was due to some nicking of DNA-RNA duplexes by the S1 nuclease. The digestion times chosen were the best compromise between loss of bands and too much background.

In vitro translation. Translation of viral mRNA was carried out in vitro by using a micrococcal nuclease-treated rabbit reticulocyte system (New England Nuclear Corp.) with [^{35}S]methionine (675 Ci/mmol) as the radioactive amino acid. Details of the procedure and fractionation of polypeptides in sodium dodecyl sulfate-acrylamide gels by the method of Laemmli (18) have been described in several previous papers (3, 4, 16). Gels were dried with vacuum, and radioactive bands were localized with autoradiography on Kodak NS-2T film. Hybrid arrest of in vitro translation was done by a modification of the procedure of Paterson et al. (25). Portions of 2 μg of total late polyribosomal poly(A) RNA were mixed with 0.5 μg of *Hin*dIII-digested clone J-2 DNA in hybridization buffer containing 80% recrystallized formamide. The mixture was incubated at 78°C for 5 min to denature the DNA and then annealed for 4 h at 57°C. Half of the sample was redenatured, volumes were adjusted to 20% formamide–0.1 M NaAc, and both halves were precipitated with 2 volumes of ethanol. RNA was collected by centrifugation and translated as described, and the resultant ^{35}S-labeled polypeptides were fractionated and subjected to autoradiography as described.

RESULTS

Restriction endonuclease map of the region 0.181 to 0.300. Clone Dec 28 contains the *Eco*RI restriction fragment G (0.19 to 0.300; 14), and we have cloned *Hin*dIII fragment J (0.181 to 0.259) in pBR322. Figure 1 shows the relative location of this region on the prototype arrangement of the genome. In the region between 0.18 and 0.30, the location of the *Eco*RI, *Hin*dIII, *Bgl*II, and *Bam*HI cleavage sites are shown. The *Bgl*II and *Bam*HI sites were localized by digestion of purified clone Dec 28 and J-2 DNA with various combinations of the four restriction endonucleases. Sizes of fragments were determined by comigration with wild-type phage λ (*c*I857*S*7) DNA, which had been digested with either *Eco*RI or *Hin*dIII. The sizes of individual wild-type λ DNA fragments were taken from the data of Thomas and Davis (35). Table 1 shows the sizes of the HSV-1 DNA fragments generated by endonuclease digestion of the cloned DNA, and Fig. 1 shows examples of the mixed digestions of the cloned fragments with *Eco*RI, *Hin*dIII, and *Bgl*II. Several restriction fragments generated by digestion of the cloned DNA were subcloned in pBR322 (Table 1). The location of restriction sites and their sizes were fully consistent with sizes determined by this and by other laboratories using HSV-1 DNA (19, 27, 36).

Gross localization of HSV-1 mRNA species mapping between 0.199 and 0.30. We used RNA transfer blots to localize the abundant mRNA species mapping between 0.199 and 0.30 to specific restriction fragments as follows. Portions of 10 μg of polyribosomal poly(A) RNA isolated from cells 6 h postinfection (hpi) were fractionated by electrophoresis through vertical methylmercury-agarose gel slabs. The slabs then were blotted onto diazotized paper as described in Materials and Methods and were hybridized under standard conditions with 2 × 10^6 to 4 ×

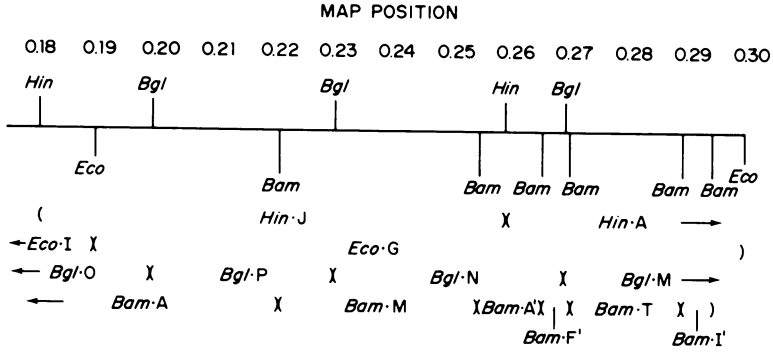


FIG. 1. *Restriction endonuclease cleavage sites in the region 0.18 to 0.3 on the prototype arrangement of HSV-1 (KOS). EcoRI fragment G (0.190 to 0.30) was cloned in λ·WES·B (14), and HindIII fragment J (0.18 to 0.259) was cloned in pBR322. These cloned fragments were digested with HindIII (Hin), EcoRI (Eco), BamHI (Bam), or BglII (Bgl) restriction endonucleases in various concentrations to determine the position of the sites shown. Fragments were sized by electrophoresis on 0.7 and 1.2% neutral agarose gels versus size standards derived by either HindIII or EcoRI digestion of phage λ (I85757) DNA (a gift of D. Wulff). The left-hand EcoRI site was set at 0.190. Names of restriction fragments are based on the following references: HindIII, EcoR1, and BglII (27, 31); BamHI (36).*

TABLE 1. *Size and map location of restriction fragments in the region 0.181 to 0.300*

Map location[a]	Fragment name[b]	Size[c] (base pairs)	Source[d]
0.190–0.300	*E*·G	16,500	1
0.181–0.259	*H*·J	11,700	2
0.181–0.190	*H-E*·J-D[e]	1,300	3
0.190–0.199	*E-Bgl*·G-O	1,300	3
0.199–0.222	*Bgl-Bam*·P-A	3,400	3
0.199–0.231	*Bgl*·P	4,800	4
0.222–0.255	*Bam*·M	5,100	4
0.231–0.259	*Bgl-H*·N-J	4,200	4
0.255–0.259	*Bam-H*·A′-J	500	3
0.255–0.266	*Bam*·A′	1,480	5
0.266–0.270	*Bam*·F′	760	3
0.231–0.269	*Bgl*·N	5,800	5
0.269–0.300	*Bgl-E*·M-G	4,500	6
0.270–0.289	*Bam*·T	2,900	6
0.289–0.294	*Bam*·I′	670	5

[a] Map location on the P arrangement of the HSV-1 genome was based on setting the *Eco*RI site at the left to 0.190 and a total size of the KOS strain of HSV-1 of 150,000 base pairs (31). Each 1,000 base pairs is then 0.00667 map units.

[b] Fragment names are based on the following references: *Hin*dIII (*H*) (27, 31); *Bam*HI (*Bam*) (19, 36); *Bgl*II (*Bgl*) (5); *Eco*RI (*E*) (3, 27).

[c] Size of restriction fragments based on comigration with *Eco*RI and *Hin*dIII digests of phage λ (*c*I857*S*7)DNA (35).

[d] (1) Obtained as a clone in λ·WES·B (14); (2) cloned in pBR322 (Materials and Methods); (3) derived by fractionation of a double restriction enzyme digest of *Hin*dIII fragment J (0.181 to 0.259); (4) restriction fragment from a digest of *Hin*dIII fragment J (0.181 to 0.259) subcloned in pBR322 (*Bgl*II sites were annealed into the *Bam*HI site); (5) restriction fragment from a digest of *Eco*RI fragment G (0.190 to 0.30) subcloned in pBR322 (*Bgl*II sites were annealed into the *Bam*HI site); (6) derived by fractionation of a double restriction enzyme digest of *Eco*RI fragment G (0.190 to 0.30).

[e] Names for fragments derived from double digests are as described elsewhere (27, 31).

10^6 cpm of [^{32}P]cDNA made to specific restriction fragments with avian myeloblastosis virus reverse transcriptase (Fig. 2 and Table 2).

When *Bgl*II fragment P (0.199 to 0.231) was used as a hybridization probe for the RNA transfer blots, mRNA bands ranging in size from 6.0 to 1.5 kb were seen. The 6-kb mRNA was by far the most abundant of these species. The abundant 6-kb mRNA was seen with probes extending to 0.266, but not beyond that. This 6-kb mRNA was not evident with probes made to the left of 0.199 (data not shown), so these experiments placed it between 0.199 and 0.266.

In addition to the 6-kb mRNA mapping in *Bgl*II fragment P (0.199 to 0.231), other species ranging in size from 1.5 to 6 kb were seen in various amounts. Of these, only the 1.5-kb species was seen as a clearly defined band extending to the right of 0.231, although some diffusely migrating material in the size range of 2.5 to 5 kb was seen in RNA transfer blots hybridized with the [^{32}P]cDNA probes homologous to the region roughly delineated by *Bgl*II fragment N (0.231 to 0.269). The 1.5-kb mRNA did not map to the right of 0.255 because cDNA probe made to *Bam*HI fragment A′ (0.255 to 0.266) did not light this band (Fig. 2). The overlap of this mRNA into *Bgl*II fragment P (0.199 to 0.231) suggested that it extended less than 1 kb to the right of the *Bgl*II site at 0.231.

A number of mRNA species mapped to the right of 0.266. Bands of mRNA 3.3 and >7 kb were seen when RNA transfer blots were hybridized to ^{32}P-labeled cDNA made to *Bam*HI fragment F′ (0.266 to 0.270; Fig. 2). Both extended to the right of 0.266 since they were seen when probes made to *Bam*HI fragment T (0.270 to 0.289) or *Bgl*II-*Eco*RI fragment M-G (0.269 to 0.300) were used (Fig. 2 and Table 2). Other mRNA species ranging from 1 to 4.5 kb were also seen with these probes. The mRNA species 1.0, 1.5, and 2 kb in length must map to the right of 0.289 since they were not seen with probes made to *Bam*HI fragment T (0.270 to 0.289). The faint mRNA band migrating with a size greater than 7 kb must overlap all of these smaller, more abundant species.

Isolation and in vitro translation of mRNA encoded between 0.199 and 0.259. An 80-μg portion of ^{32}P-labeled late poly(A) polyribosomal mRNA was preparatively hybridized to 100-μg equivalents of *Bgl*II fragment P (0.199 to 0.231) bound to cellulose to isolate the mRNA species mapping there. This specific mRNA then was size fractionated on a methylmercury-containing agarose gel by electrophoresis. The gel was sliced, and radioactivity in each slice was determined (Fig. 3A). The size distribution of the isolated mRNA had essentially the same complex pattern as seen when RNA transfer blots were used (Fig. 2A). A major band at 6.0 kb was seen, along with other bands ranging from 1.5 to 5 kb. The mRNA's corresponding to sizes of 6, 4, 2.8, 2, 1.7, and 1.5 kb were pooled as shown, eluted, reselected on oligo(dT)-cellulose, and then translated in vitro in a reticulocyte lysate system using [^{35}S]methionine (Materials and Methods). The 6-kb mRNA translated to a major polypeptide 155,000 daltons in size, as well as smaller amounts of species of many other sizes (Fig. 4A). The 4-kb mRNA was translated into a major species 80,000 daltons in size; the 2.8-kb mRNA pool translated into significant amounts of polypeptides of 85,000 and 77,000 daltons, as well as a product of about 33,000 daltons; the 2-kb

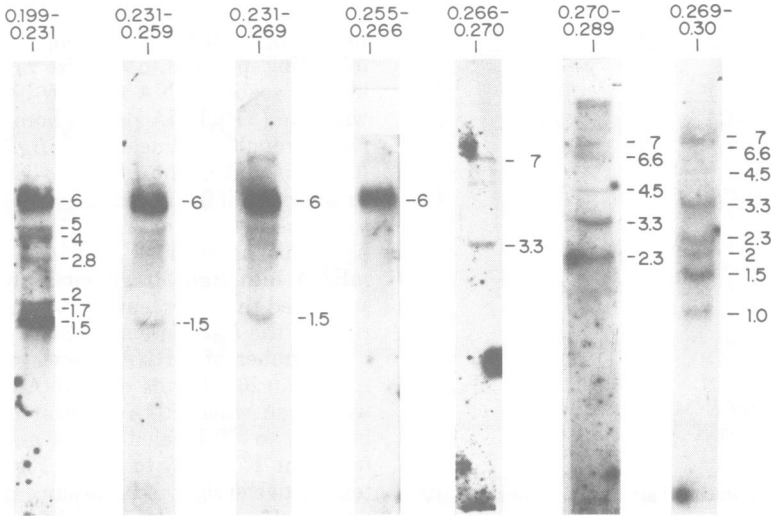


FIG. 2. *RNA transfer (Northern) blots of HSV-1 poly(A) mRNA homologous to regions of the viral genome between 0.199 and 0.30. As described in Materials and Methods, samples of 10 μg of late poly(A) polyribosomal RNA were fractionated by methylmercury-agarose gel electrophoresis and blotted onto diazotized paper. The blots were hybridized to 2 × 10^6 to 4 × 10^6 cpm of [^{32}P]cDNA made to (i) BglII fragment P (0.199 to 0.231); (ii) BglII-HindIII digest fragment N-J (0.231 to 0.259); (iii) BglII fragment N (0.231 to 0.269); (iv) BamHI fragment A' (0.255 to 0.266); (v) BamHI fragment F' (0.266 to 0.270); (vi) BamHI fragment T (0.270 to 0.289); and (vii) BglII-EcoII fragment M-G (0.269 to 0.30). Typical band separation and the specific restriction sites are shown in Fig. 1. Source of these fragments is shown in Table 1. The size of individual bands is shown on the right of each track and was based on the migration of 28S and 18S rRNA markers (5.2 and 2.0 kb; 21, 37) run in parallel tracks.*

mRNA yielded a complex polypeptide pattern migrating at approximately 65,000 to 68,000 daltons in size; the 1.7-kb band primarily translated into a polypeptide 40,000 daltons in size; and the 1.5-kb mRNA translated primarily into polypeptide products migrating diffusely at 35,000 daltons.

A similar experiment was carried out with the mRNA isolated by using *Bgl*II-*Hin*dIII fragment N-J (0.231 to 0. 259) bound to cellulose. Here, a major 6-kb and a minor 1.5-kb mRNA species were seen (Fig. 3B). The 6-kb mRNA translated into a polypeptide 155,000 daltons in size (Fig. 4B), the same as the major product of the 6-kb mRNA isolated by using *Bgl*II fragment P (0.199 to 0.231) as a reagent. Under longer exposures, minor amounts of smaller material migrating heterogeneously, like the minor ones seen with 6-kb mRNA from *Bgl*II fragment P (0.199 to 0.231), could be seen, but these were present in lower amounts. In different experiments, it was found that the relative proportions of these heterogeneously sized translation products varied greatly from translation to translation. We do not know what the origin of this polydispersed material is, although we suggest that it may be, in part, due to an artifact of translating such a large mRNA with such a high guanine plus cytosine content in our system.

The 1.5-kb mRNA isolated by using the double-digestion fragment N-J (0.231 to 0.259) translated to the same diffusely migrating polypeptide products with an average size of 35,000 daltons, as did the 1.5-kb mRNA isolated with *Bgl*II fragment P (0.199 to 0.231). These data indicate that both the 6-kb and the 1.5-kb mRNA overlap the *Bgl*II site at 0.231, as expected from the data of Fig. 2 and Table 2. This requires the 1.5-kb mRNA to be colinear with a portion of the 6-kb mRNA.

We used hybrid-arrested translation to demonstrate that the 155,000-dalton polypeptide encoded by the 6-kb mRNA mapping in *Hin*dIII fragment J (0.181 to 0.259) is the only species of that size translated by total late polyribosomal poly(A) mRNA. Polyribosomal poly(A) RNA was isolated from cells at 6 hpi. Samples were hybridized to *Hin*dIII fragment J (0.181 to 0.259); one half of the sample then was denatured, and the other half was used as hybridized. Samples were translated in parallel with total 6-hpi polyribosomal poly(A) RNA (Fig. 4C). As has been consistently seen, the largest polypeptide observed in appreciable quantities in translation of total poly(A) RNA was 155,000 daltons in size, and a number of other bands between 140,000 and approximately 12,000 daltons also were seen. Translation of hybridized RNA

TABLE 2. *Size of mRNA species detected by Northern blot analysis with [^{32}P]cDNA made to different regions between 0.199 and 0.30 on the HSV-1 genome*

Location of DNA probe[a]	Size of mRNA seen[b] (kb)
0.199–0.231	6
	5[c]
	4
	2.8[c]
	2.0
	1.7
	1.5
0.231–0.259	6
	1.5[c]
0.231–0.269	>7[c]
	6
	1.5[c]
0.255–0.266	6
0.266–0.270	>7[c]
	3.3
0.270–0.289	>7[d]
	6.6[c,d]
	4.5[d]
	3.3
	2.3
0.269–0.30	>7[c,d]
	6.6[c,d]
	4.5[d]
	3.3
	2.3
	2
	1.5
	1[c]

[a] See Figure 1 and Table 1.

[b] Based on comigration with 28S (5.2 kb; 21) and 18S (2.0 kb; 37) HeLa cell rRNA (Materials and Methods). These RNA sizes include the poly(A) tails 200 to 300 bases in length (30).

[c] Faint band.

[d] Diffuse band.

showed that there was a general reduction in the amount of radioactive polypeptides migrating with a size larger than about 80,000 daltons in both the arrested and denatured samples. This may have been due to breakdown of the RNA during the hybridization. In spite of this complication, it was clear that no 155,000-dalton polypeptide was seen at all in the arrested sample, whereas it was translated in the denatured one. The amounts of all other bands migrating were essentially the same at all sizes between the arrested and denatured samples with two exceptions: a band migrating at 128,000 daltons was lost after hybridization, and a new band migrating at 107,000 daltons was seen. These could have been due to the interruption of translation of other region-specific mRNA species.

Determination of the direction of transcription of the 6-kb mRNA species mapping around 0.231. Since the 6-kb mRNA was detected in Northern blots when we used cDNA made to the regions on either side of 0.231 and 0.255, we performed several experiments to determine how far this major species extended. In one series, we determined whether it mapped to the right of 0.269. Cells were labeled with $^{32}P_i$ from 0 to 6 hpi, and polyribosomal poly(A) RNA was isolated. This RNA was hybridized to *Bgl*II fragment N (0.231 to 0.269; 200,000 cpm/μg) bound to cellulose, and the fragment-specific RNA was fractionated on a methylmercury-agarose gel. The 6-kb band was cut from a similar gel, and 50,000 cpm was hybridized to a Southern blot of *Bgl*II-digested HSV-1 DNA under the same conditions as with the RNA blot hybridization (0.4 M Na^+, 65% formamide, 45°C). No hybridization was observed in the *Bgl*II band spanning 0.269 to 0.311 (*Bgl*II fragment M) (Fig. 5A); therefore, we concluded that any portion of this mRNA mapping beyond 0.269 must be so short as to preclude hybridization under our criteria.

Since the 6-kb mRNA spans the *Bgl*II site at 0.231, we mapped its 3′ end to determine its direction of transcription. We made 3′-cDNA to mRNA isolated by hybridization to *Bgl*II fragment N (0.231 to 0.269) bound to cellulose and fractionated by electrophoresis on a methylmercury-containing agarose gel. The details of this procedure are outlined in Materials and Methods. The cDNA then was hybridized to Southern blots of *Bgl*II-digested HSV-1 DNA (Fig. 5B). It is clear that the 3′-cDNA of the 6-kb RNA species mapped in the restriction fragment spanning 0.199 to 0.231 (*Bgl*II fragment P). Since this mRNA must extend to the right of 0.231, it is clear that the 6-kb mRNA mapping in this region is transcribed from right to left on the prototype arrangement of the HSV-1 genome. Similar experiments using the 1.5-kb mRNA as a template for 3′-cDNA also suggested that this mRNA has its 3′ end to the left of 0.231; but in this case, the intensity of the hybridization was not great, and this conclusion must be regarded as tentative.

Lack of internal introns in the coding sequence for the major 6-kb mRNA. The RNA transfer blot data of Fig. 2 indicated that there were a large number of mRNA's mapping between 0.18 and 0.30, which is the region encompassed by our two major recombinant DNA fragments, *Eco*RI fragment G (0.19 to 0.30) and *Hind*III fragment J (0.183 to 0.259). Generally, an S1 analysis of such a complex mRNA pattern would be impossible to interpret unambiguously; however, the high abundance, large size, and gross localization of the 6-kb mRNA available through RNA transfer blots suggested to us that the portion of the cloned DNA protected by this

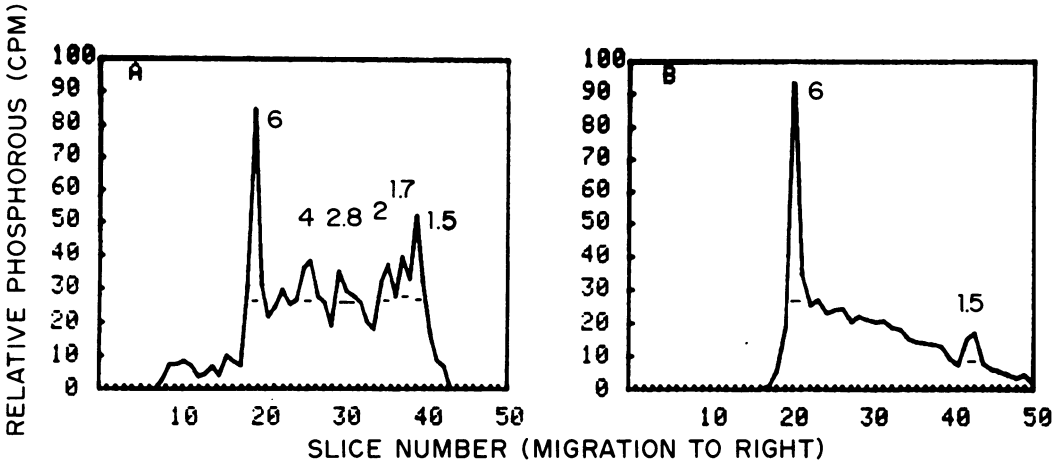
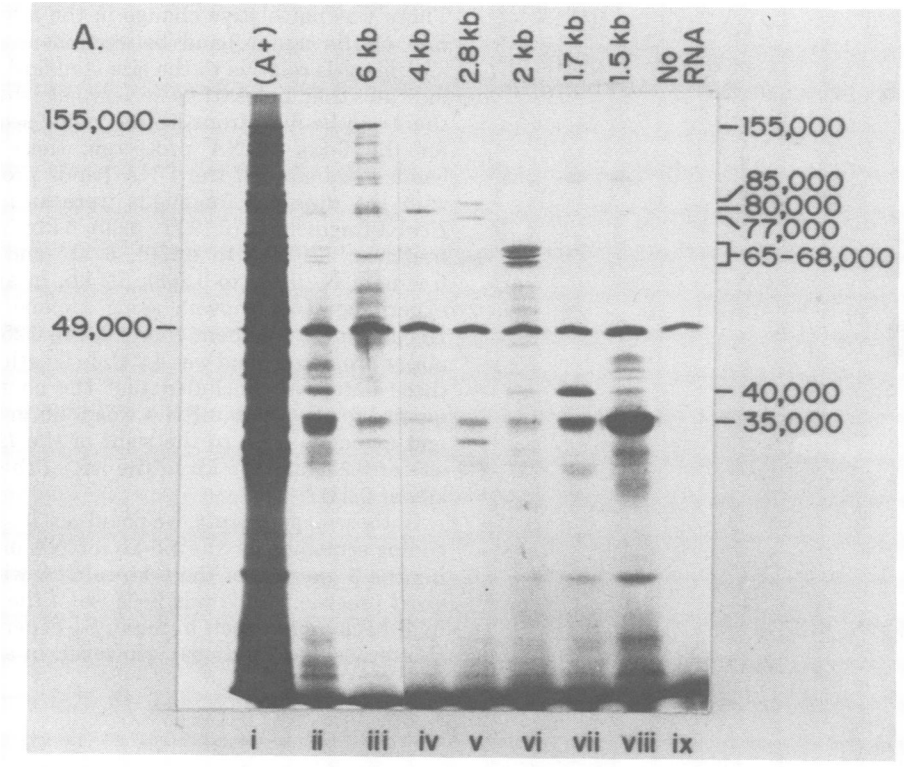


FIG. 3. *Isolation of mRNA for characterization. (A) The mRNA species encoded by BglII fragment P (0.199 to 0.231). BglII fragment P bound to cellulose was used to hybridize ^{32}P-labeled poly(A) mRNA (Materials and Methods), and this specific RNA was fractionated by methylmercury-gel electrophoresis. The gel was sliced, and RNA bands were visualized by determining Cerenkov radiation. In addition to the major 6-kb band, bands at 5, 4, 2.8, 2, 1.7, and 1.5 kb can also be seen. The mRNA in bands indicated with the horizontal bars was isolated for in vitro translation. (B) The mRNA encoded by BglII-HindIII fragment N-J (0.231 to 0.259). Details are as in panel A.*

mRNA species should be readily observable. Accordingly, we carried out hybridizations between late polyribosomal poly(A) mRNA and ^{32}P-labeled clones of DNA mapping in the region of interest, digested the hybrids with S1 nuclease, and fractionated the protected DNA fragments through neutral and alkaline agarose gels (Materials and Methods). Figure 6 shows an example of such an experiment using *Hin*dIII fragment J (0.181 to 0.259) in which there was good resolution of many of the protected DNA bands. Major bands migrating with a size of 5 and 1.3 kb were seen, as well as numerous other bands migrating with sizes between these limits. We concluded that the major 5-kb band is the region of the DNA protected by the 6-kb mRNA extending leftward of 0.259; we tentatively assigned the 1.3-kb band to the 1.5-kb mRNA which was relatively abundant in RNA transfer blots probed with cDNA made to *Bgl*II fragment P (0.199 to 0.231). This mRNA was seen to extend to the right of 0.231, and a length of 1.3 kb would be expected for an mRNA bearing a poly(A) tail 200 to 300 bases long (30). This latter assignment must be regarded as tentative because more than one mRNA could be represented in the S1 band.

Exact sizes of the DNA segments protected by the 6-kb mRNA are best determined by using alkaline gels of S1 digests. Furthermore, comparison of such gels with neutral ones will indicate any interruptions of the DNA coding sequences for this mRNA due to introns. Figure 7 shows neutral and alkaline gels of S1 digests of

FIG. 4. *In vitro translation of specific mRNA species. (A) Specific mRNA samples (0.02 to 0.03 μg) were isolated from the gel of Fig. 3A by phenol extraction and reselection on oligo(dT)-cellulose. These samples were translated with [^{35}S]methionine in a commercial reticulocyte lysate system, and translation products were fractionated on 9% acrylamide gels. (i) Translation of total late poly(A) mRNA isolated from polyribosomes; (ii) translation products of 0.01 μg of unfractionated BglII fragment P (0.199 to 0.231)-specific mRNA; (iii–viii) translation products of the 6-, 4-, 2.8-, 2-, 1.7-, and 1.5-kb mRNA, respectively; (ix) no-RNA control. Sizes of polypeptides were based on migration of adenovirus-specific bands as described previously (not shown in panel A). The heavy band at 49,000 daltons is an endogenous translation product. (B) In vitro translation of the 6- and 1.5-kb mRNA species isolated by size fractionation of BglII-HindIII fragment N-J (0.231 to 0.259)-specific mRNA (Fig. 3B). Tracks i and ii contain translation products of the 6- and 1.5-kb mRNA species, respectively. Track iv contains the translation products of adenovirus-infected cell mRNA. Details are as in panel A. (C) Hybrid-arrested translation of total late poly(A) polyribosomal RNA from HSV-1-infected cells. Hybrid arrest was as described in the text. HindIII fragment J (0.181 to 0.259) was used for hybridization. Translation and electrophoresis of polypeptides are as in panel A. The arrow indicates the missing 155,000-dalton polypeptide in the arrested sample (compare tracks i and ii).*


A.
(A+)
6 kb
4 kb
2.8 kb
2 kb
1.7 kb
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No RNA
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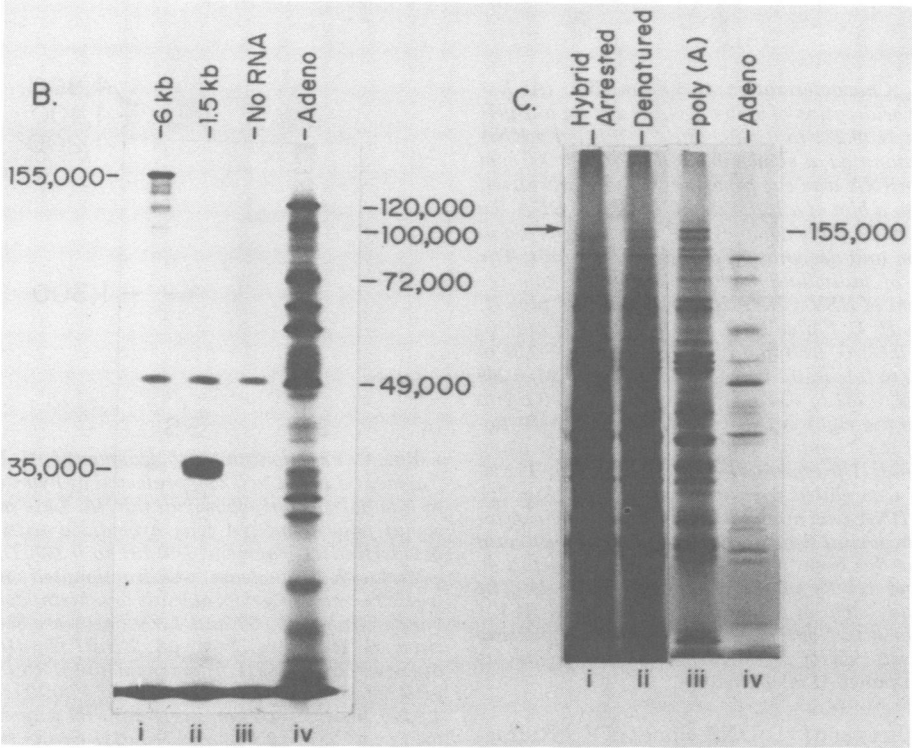
i ii iii iv v vi vii viii ix


B.
-6 kb
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-No RNA
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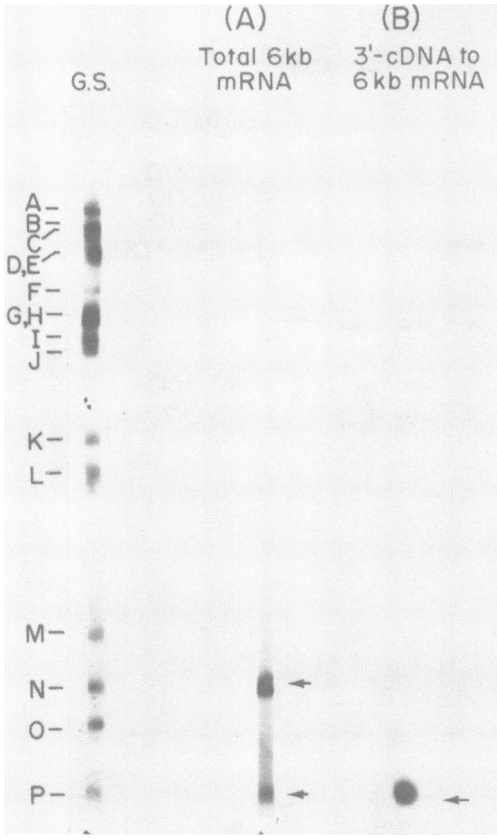

FIG. 5. *Characterization of 6-kb mRNA. (A) Extent of hybridization of 6-kb mRNA mapping in BglII fragment N (0.231 to 0.269). The ^{32}P-labeled mRNA was fractionated as shown in Fig. 3B, and 50,000 cpm of 6-kb mRNA was cut from the gel and hybridized at 45°C to a blot of a BglII digest of HSV-1 DNA. To the left is a guide strip (G.S.) showing the band resolution and designation of individual bands. The location of individual bands on the prototype arrangement of HSV-1 DNA is as follows: A, F plus H; B, J plus H; C, F plus L; D, 0.41 to 0.58; E, J plus L; F, 0.7 to 0.83; G, 0.58 to 0.7; H, 0.83 to 0.94; I, 0.31 to 0.41; J, 0 to 0.1; K, 0.1 to 0.17; L, 0.94 to 1.0; M, 0.269 to 0.31; N, 0.231 to 0.269; O, 0.17 to 0.199; P, 0.199 to 0.231. To the right is the experimental blot. (B) Location of the 3' end of 6-kb mRNA isolated by using BglII-HindIII fragment N-J (0.231 to 0.259). The 6-kb band was eluted from a gel such as in Fig. 3B, and 3'-cDNA was made by using reverse transcriptase after partial degradation of the RNA with mild alkali, as has been described elsewhere in detail (3, 4, 17) and briefly in Materials and Methods. The same guide strip of a blot of BglII-digested HSV-1 DNA hybridized with cDNA to HSV-1 DNA showing band resolution of BglII restriction fragments (as shown in panel A) is applicable.*

hybrids between [^{32}P]DNA clones of *Eco*RI fragment G (0.190 to 0.30), *Hin*dIII fragment J (0.181 to 0.259), and *Bgl*II fragment N (0.231 to 0.269). There was no relative change in the migration rate of the major band between neutral and alkaline gels relative to the size standards. This indicates that, unless they lie very near the ends, there can be no introns in the coding sequence for the 6-kb mRNA protecting these DNA bands. The sizes of the DNA bands protected with the different fragments were as follows: *Eco*RI fragment G (0.190 to 0.30), 6 kb; *Hin*dIII fragment J (0.181 to 0.259), 5 kb; and *Bgl*II fragment N (0.231 to 0.269), 5.2 kb. In another experiment (not shown), using a clone of the *Bgl*II-*Hin*dIII fragment N-J (0.231 to 0.259), the major protected band was 4.2 kb in length. From these data, we concluded that the coding sequence for the 6-kb mRNA was 6 kb in length and extended 1 kb to the right of the *Hin*dIII site at 0.259 and 0.8 kb to the left of the *Bgl*II site at 0.231.

In these experiments, we could not locate the coding sequence for the 1.5-kb mRNA underlying the 3' portion of the 6-kb mRNA with any great precision. This was because of the rather high background seen in many S1 experiments (Materials and Methods). However, in alkaline

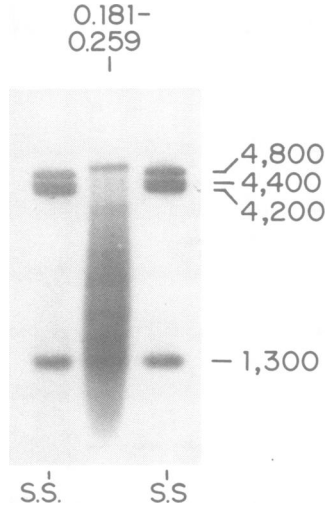

FIG. 6. *Fractionation of fragments of HindIII fragment J (0.181 to 0.259) protected by hybridization to late poly(A) polyribosomal mRNA. Late polyribosomal poly(A) mRNA was hybridized with ^{32}P-labeled HindIII fragment J (0.181 to 0.259) DNA, digested with S1 nuclease, and fractionated on a neutral 0.9% agarose gel (Materials and Methods). Major bands of about 5,000 and 1,300 bases are seen. Size markers (SS) are from an EcoRI-HindIII-BglII digestion of HindIII fragment J (0.181 to 0.259) in pBR322. Bands are BglII fragment P (0.199 to 0.231; 4.8 kb), pBR322 (4.4 kb), BglII-HindIII fragment N-J (0.231 to 0.259; 4.2 kb), and HindIII-EcoRI fragment J-D (0.181 to 0.190; 1.3 kb) combined with EcoRI-BglII fragment G-O (0.190 to 0.199; 1.3 kb).*

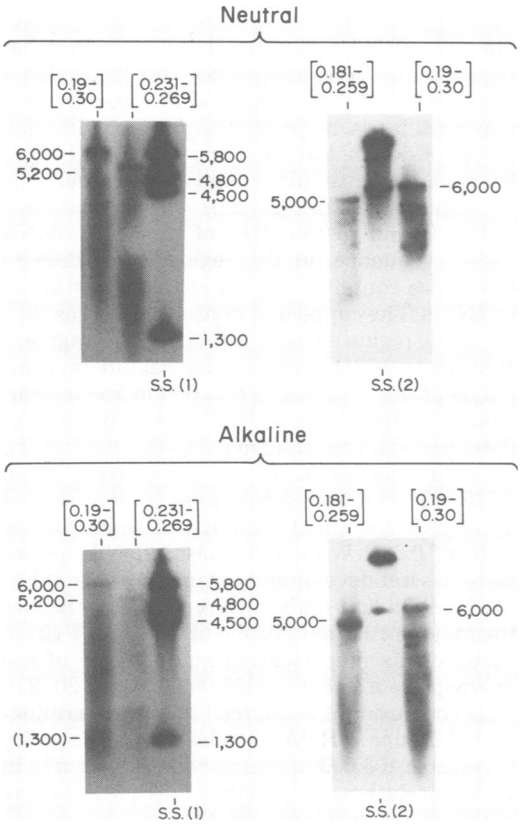

FIG. 7. *S1 nuclease analysis of the major 6-kb mRNA. As described in Materials and Methods, 10-μg portions of poly(A) polyribosomal RNA from cells 6 hpi were hybridized with ^{32}P-labeled EcoRI fragment G (0.190 to 0.30), HindIII fragment J (0.181 to 0.259), or BglII fragment N (0.231 to 0.269) DNA. After hybridization and S1 digestion, the material was fractionated on neutral or alkaline agarose gels with appropriate size markers. Neutral gels are shown at the top; alkaline ones are shown at the bottom. The panels to the left contain S1-digested hybrids made by using EcoRI fragment G (0.19 to 0.30) DNA and BglII fragment N (0.231 to 0.269) DNA. Size standards (S.S.) are from a BglII digest of ^{32}P-labeled EcoRI fragment G (0.19 to 0.30) which had been purified from an EcoRI digest of phage λ·WES·B. Bands are BglII fragment N (0.231 to 0.269; 5.8 kb), BglII fragment P (0.199 to 0.231; 4.8 kb), BglII-EcoRI fragment M-G (0.269 to 0.30; 4.5 kb), and EcoRI-BglII fragment G-O (0.19 to 0.199; 1.3 kb). Panels to the left contain S1-digested hybrids made by using HindIII fragment J (0.181 to 0.259) and EcoRI fragment G (0.19 to 0.30). Size standards (S.S.) are from an EcoRI digest of HindIII fragment J (0.181 to 0.259) in pBR322. This generates two chimeric fragments: one spans from the EcoRI site in pBR322 to the site at 0.19 in HindIII fragment J and is 10.3 kb in length; and the other spans from this site at 0.19, includes the HindIII site in pBR322 and all of that plasmid to the EcoRI site, and is 5.8 kb in length.*

gels of S1-digested hybrids of *Eco*RI fragment G, a band migrating at approximately 1.3 kb was rather consistently seen and may have corresponded to the 1.3-kb band seen in Fig. 6.

Lack of introns in the 5′ and 3′ coding sequences of the major 6-kb mRNA. The S1 data of the previous section indicated that the 5′ end of the major 6-kb mRNA extended approximately 1 kb to the right of the *Hin*dIII site at 0.259, whereas the RNA transfer (Northern) blot data of Fig. 2 and Table 2 indicated that it did not extend beyond the *Bam*HI site at 0.266. Therefore, we used ^{32}P-labeled *Bam*HI fragment A′ (0.255 to 0.266) cloned in pBR322 to hybridize to late poly(A) RNA to examine the region of the DNA protected by the 5′ end of the 6-kb mRNA. An alkaline gel of such an experiment (Fig. 8) showed that S1 nuclease digestion of hybrids yielded an ~1,480-nucleotide length of DNA resistant to digestion, nearly the whole length of *Bam*HI fragment A′ (0.255 to 0.266; 1,480 bases long). Although some rather diffusely migrating DNA was seen in the size range of about 1 to 1.4 kb long, the major protected species was the band migrating at 1,480 bases. This result indicated that there were no noncontiguous coding regions at the 5′ end for the bulk of the 6-kb mRNA and that the location of the 5′ end of the 6-kb mRNA was very near the *Bam*HI site at 0.266. This confirmed our conclusion that the coding sequence for the 6-kb mRNA extended 1 kb to the right of the *Hin*dIII site at 0.259.

The S1 nuclease data of Fig. 7 also suggested that the 3′ end of the 6-kb mRNA extended 800 bases to the left of the *Bgl*II site at 0.231. We performed similar experiments with *Bgl*II frag-

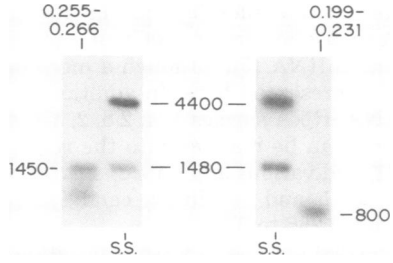

FIG. 8. *S1 analysis of hybrids made to the 5′ and 3′ ends of the 6-kb mRNA. Portions (5 μg) of late poly(A) polyribosomal RNA were hybridized with 1 μg of ^{32}P-labeled BamHI fragment A′ (0.255 to 0.266) or BglII fragment P (0.199 to 0.231). After hybridization and S1 digestion, the material was fractionated on an alkaline 0.9% agarose gel, using EcoRI- and BamHI-digested BamHI fragment A′ (0.255 to 0.266) cloned in pBR322 as a size marker (S.S.). Bands are pBR322 (3,700 bases) and BamHI fragment A′ (1,480 bases). The small 600-base fragment of pBR322 generated by the digestion is not visible.*

ment P (0.199 to 0.231) cloned into the *Bam*HI site of pBR322 to precisely determine the extent of overlap of the 3′ end of the 6-kb mRNA into this fragment. Figure 8 also shows an alkaline gel of a typical S1 digestion. A major band migrating with a rate corresponding to a size of 800 bases was readily apparent. Under much longer exposures, additional bands corresponding to coding sequences of other mRNA's mapping in the fragment could be seen. Since the 6-kb mRNA was by far the major mRNA mapping in this fragment, we assigned the 800-base length of DNA to the region protected by the 3′ end of this mRNA. The close agreement between the size of the major DNA fragment protected and that inferred from the S1 data of Fig. 8 confirmed this assignment and indicated that there were no measurable introns in the 3′ coding sequence for the 6-kb mRNA.

DISCUSSION

The region examined in this report, that between 0.199 and 0.30, encodes a number of late mRNA species. Yet, within this region the 6-kb length encoding the major 6-kb mRNA described here is not overlapped by any large amount of coding sequences for other mRNA's with the exception of its 3′ region, which is colinear with at least a portion of a less abundant 1.5-kb mRNA. This type of overlap of the 3′ region of a major mRNA with a less abundant, smaller mRNA has been found in another region (0.527 to 0.60) of the long unique portion of the HSV-1 genome (3a).

The coding region for the major 6-kb mRNA is flanked on both sides by regions which encode a large number of mRNA species. The RNA transfer blot data presented here are generally consistent with earlier data obtained with HSV-1 DNA restriction fragments bound to cellulose to isolate mRNA (5), although a more detailed picture is presented here. In addition to the 6-kb mRNA, RNA species 5, 4, 2.8, 2, 1.7, and 1.5 kb in size can be mapped into the region 0.199 to 0.231, and various amounts of species 6.6, 4.5, 3.3, 2.3, 2, 1.5, and 1 kb in size can be mapped in the region 0.266 to 0.30.

We carried out the in vitro translation of the size-fractionated *Bgl*II fragment P (0.199 to 0.231)-specific mRNA to demonstrate that the 6- and 1.5-kb mRNA's mapping there were identical to those mapping to the right of 0.231. Although the translation patterns of the other mRNA's were often complex and suggested multiple mRNA species, the data definitely indicate that these other mRNA's are functional in vitro, and their sequence distribution in the region from 0.199 to 0.231 (4,200 bases) must allow the overlap of at least some of them. Such overlapping of mRNA's has also been seen in other regions of the long unique segment (3a; Wagner et al., in press). The biological function of these polypeptides is at present unknown, although polypeptides in the size range seen here have been mapped into this general area by intertypic recombinant studies (20, 22).

The region to the right of the 6-kb mRNA coding sequence, in the region from 0.266 to 0.289, also contains a rather complex mixture of mRNA's. They appear as densely packed as they are in the region encompassed by *Bgl*II fragment P (0.199 to 0.231). Again, biological functions for polypeptides mapping in this region are unclear. The best-characterized HSV-1 gene product to date, thymidine kinase, maps to the right of the region in question, between coordinates 0.31 and 0.315 (12), and therefore cannot be represented by any of the small mRNA's mapping to the right of 0.289. What role these mRNA species have in viral development is unknown.

The abundant 6-kb mRNA probably encodes the major structural protein of the HSV-1 virion based on its map location and the size of the polypeptide it encodes (155,000 daltons; 20, 22). This conclusion is reinforced by our determination that this mRNA encodes the only readily detectable 155,000-dalton polypeptide seen in in vitro translation of total HSV-1 mRNA. The size of this polypeptide suggests that as much as 20% of the length of the 6-kb mRNA contains nontranslated sequences. This region would appear to be on the 3′ end of the 6-kb mRNA from the data reported here and is utilized to encode the 35,000-dalton polypeptide translated from the 1.5-kb mRNA mapping here. The function of this small polypeptide is unknown.

The alkaline S1 mapping data of Fig. 7 and 8 convincingly demonstrate that, unless introns lie extremely near the ends of the coding sequence for the 6-kb mRNA, there are none in this mRNA. In experiments described elsewhere (3a), we have found this to also be the case for a number of late mRNA's mapping between 0.527 and 0.602. This phenomenon would then appear to be common at least for a number of HSV-1 mRNA's mapping in the long unique segment. Only full nucleotide sequence data can convincingly demonstrate the absolute lack of introns in the coding sequences of any mRNA species, and it is significant that the HSV-1 thymidine kinase mRNA has been shown to lack splices (S. McKnight, unpublished data; M. Wagner, J. Sharp, and W. Summers, unpublished data).

Whatever the function of mRNA splicing, or its lack, it should be emphasized that at least

one immediate-early HSV-1 mRNA species mapping in the short region has a 150-base intron 260 bases inward from its 5′ end (36a). This suggests that splicing has a role in the maturation of at least some HSV-1 mRNA's. In light of the finding of both spliced and unspliced HSV-1 mRNA's, it is important to note that at least one functional adenovirus gene (gene IX) encodes an mRNA without introns (1). Therefore, even with this virus characterized by a large number of spliced mRNA's (26), splicing is not obligatory in mRNA maturation per se.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants CA11861 (from the National Cancer Institute) and AA03506 (from the Institute of Alcoholism and Alcohol Abuse). K.P.A. was a predoctoral trainee under Public Health Service training grant GM07311 from the National Institutes of Health.

We thank L. Tribble for technical assistance and J. Stringer and R. Frink for helpful discussions.

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