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Herpes Simplex Virus mRNA Species Mapping in *Eco*RI Fragment I

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We described the detailed characterization and high-resolution mapping of nine herpes simplex virus type 1 mRNAs encoded in *Eco*RI fragment I. Four of these mRNAs are partially colinear and encode the same sized polypeptide in vitro. Nucleotide sequence analysis of the DNA around the 5′ ends of these mRNAs suggested that the larger may encode a small (ca. 100-dalton) polypeptide not resolvable by in vitro translation.

The general properties of herpes simplex virus type 1 (HSV-1) mRNA have been described by this and other laboratories over the past decade, and the basic outlines of the temporal sequence of gene expression are clear. Such work is presented in several recent reviews (18; E. Wagner, *in* G. Klein, ed., *Advances in Viral Oncology*, vol. II, in press). It suffices to state here that three basic temporal classes of viral mRNA are expressed: immediate-early (α), prior to any virus-directed protein synthesis; early (β), requiring the action of α genes and abundant in the absence of viral DNA replication; and late (βγ and γ), which are abundant only after viral DNA replication. Rigorous inhibition of viral DNA synthesis leads to abundant levels of early (β) and low levels of some late (βγ) mRNAs, whereas other late (γ) mRNAs are not seen at all in the absence of viral DNA synthesis (10, 11). Proper use of DNA synthesis inhibition thus allows a ready assignment of the temporal class of any given viral mRNA species.

We have described methods for the isolation, translation, and high-resolution (±50 bases) mapping of HSV-1 mRNA species (1, 4, 8). In particular, we have described early and late mRNAs mapping in the 18-kilobase (kb) region contained in *Hin*dIII fragments K (0.527–0.592) and L (0.592–0.647). A striking feature of these mRNAs is that the 5′ ends of most early and late mRNAs map in such a way as to preclude any but the most minimal splices. Although one mRNA family has spliced members (8), further characterization has shown that the unspliced member of this family is an abundant one (R. J. Frink and E. K. Wagner, manuscript in preparation). Subsequently, we have confirmed the lack of splicing of several of the mRNAs mapping in this region by nucleotide sequence analysis (9; K. G. Draper and E. K. Wagner, J. Virol., in press; Frink and Wagner, in preparation).

These findings, along with sequence and transcription initiation analyses for a number of HSV-1 genes carried out by us and by others (9, 14), lead to a simple model for the "typical" HSV-1 gene. Such a gene should have its control elements or promoters very near the actual protein coding sequence encoded and should contain only very small introns, if any, only near the 5′ end.

In spite of this simplicity and general lack of splicing, we have found several properties of HSV-1 transcription which can lead to multiple mRNA overlapping species capable of encoding the same or a different polypeptide. In some instances, an mRNA species may have a second promoter close by, upstream or downstream of the major one, giving rise to a resolvable mRNA species larger or smaller than the major one. Such an mRNA would be different in the length of its leader sequence. One potential example of this lies in two minor colinear mRNAs seen to map in *Hin*dIII fragment L (0.592–0.647) (8). A second case was found in which a minor promoter appears to lie about 450 bases upstream of the major one for an abundant early (β) mRNA mapping in *Hin*dIII fragment K (0.527–0.592) (9).

Another mechanism for the generation of multiple mRNAs is found when an mRNA species is inefficiently terminated at a given polyadenylation site. Transcription then proceeds downstream to the next polyadenylation site. Here, two resolvable mRNA species were found which are colinear on their 5′ ends and encode the same polypeptide and translation termination signals, but the larger mRNA contains nontranslated sequences beyond the nominal polyadenylation sites. An example is seen with 1.9- and 7-kb late (γ) mRNA species mapping in *Hin*dIII fragment K (0.527–0.592) (1).

In this report, we describe the properties of nine viral mRNAs (some overlapping) mapping in the 11.5-kb region between the *Hin*dIII site at 0.647 and the *Eco*RI site at 0.721. Only one of these mRNAs is characterized by a possible

splice. Four other mRNAs overlap colinearly and encode the same sized polypeptide on the basis of in vitro translation. The overlap and size of polypeptides encoded suggested to us that these mRNAs might be "redundant"; however, nucleotide sequence analysis of the DNA around the 5′ ends of these mRNAs suggested that the larger species could encode a small polypeptide in their unique 5′ sequence. The larger species may, thus, encode a separate viral function.

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 enzymes were obtained from Bethesda Research Laboratories; digestion was carried out in buffers recommended by that supplier. Phage T4 polynucleotide kinase was used for 5′ phosphate exchange as described by Maxam and Gilbert (13).

Isolation, labeling, and size fractionation of polyribosomal RNA. Monolayer cultures of HeLa cells (2×10^7 cells per 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. Viral RNA synthesized in the absence of HSV-1 DNA synthesis (early RNA) was prepared from cells pretreated for 1 h and incubated for 6 h postinfection with 1.5×10^{-4} M adenosine arabinoside and 3.7×10^{-6} M pentostatin, as described previously (10). The drugs were a gift of C. Shipman of the University of Michigan, Ann Arbor.

Polyribosomes were isolated from the cytoplasm of HSV-1-infected cells by the magnesium precipitation method of Palmiter (1, 16). Polyadenylic acid-containing mRNA [poly(A) RNA] was isolated from total rRNA by oligodeoxythymidylic acid-cellulose (Collaborative Research, Inc.). Details of this procedure were presented elsewhere (1, 4, 8). This poly(A) mRNA is hereafter termed infected-cell mRNA.

RNA was size fractionated by electrophoresis on 1.2% agarose gels containing 10 mM methylmercury hydroxide (2) as previously described.

Recombinant DNA. All recombinant DNA clones described in this paper were derived from a clone of *Eco*RI fragment I (0.633–0.721) cloned in a λ WES·B vector (7), the kind gift of L. Enquist and G. Vande Woude. Procedures for cloning HSV-1 DNA fragments in the pBR322 vector were described previously. DNA fragments cloned were named as described previously and located by their map coordinates on the P arrangement of the HSV-1 genome.

In situ RNA blots. The method for in situ RNA blots was described to us by Inder Verma of the Salk Institute and H. Fan of the University of California, Irvine. Samples (7 μg) of infected-cell mRNA were fractionated on methylmercury gels and dried onto Whatman 3MM paper with vacuum. The agarose film was floated off the paper in water and hybridized with appropriate ^{32}P-labeled DNA probes in 50% formamide containing 0.4 M Na^+, 0.1 M HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 8.0), 0.005 M EDTA, and Denhardt solution (5) at 50°C for 36 h. Blots were rinsed in 0.1× SSC (1 × SSC = 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 50°C for 3 to 4 h and then autoradiographed.

In vitro ^{32}P-labeled DNA was made by nick-translating appropriate DNA clones with DNA polymerase I (Boehringer-Mannheim) and 50 μCi of [α-^{32}P]dCTP (3000 Ci/mmol; Amersham).

Isolation of restriction fragment-specific mRNA. Restriction 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 (1).

In vitro translation. Translation of size-fractionated 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 (12) were described in several previous papers (1, 4, 8). Gels were dried with vacuum, and radioactive bands were localized by autoradiography with Kodak NS-2T film.

Nuclease mapping of HSV-1 RNA. S1 nuclease and exonuclease VII analyses of RNA were carried out essentially as described by Berk and Sharp (3) and as described previously (1, 4, 8). Appropriate HSV-1 DNA clones (2 μg) were restricted at the desired site with the appropriate enzyme. The DNA then was 5′-labeled with ^{32}P, using polynucleotide kinase (13) to a specific activity of 4,000 to 10,000 cpm/μg of DNA. Samples containing DNA and 10 μg of infected-cell mRNA were dissolved in 50 μl of hybridization buffer containing 80% recrystallized formamide, 0.4 M Na^+, 0.1 M HEPES (pH 8), and 0.005 M EDTA. Details of denaturation, hybridization, and nuclease digestion were as described.

After digestion, samples were fractionated on 1.2% agarose gels in 30 mM NaOH–2 mM disodium EDTA. Electrophoresis was for 90 min at 150 V (500 mA). (These conditions gave superior resolution of fragments of <1,000 bases.) Size standards were derived from an *Hin*dIII, *Bam*HI, *Pvu*II digest of *Hin*dIII-*Bam*HI fragment L-O (0.592–0.602) cloned in pBR322. This 1,500-base HSV-1 DNA fragment has two *Pvu*II sites 1,250 bases apart, and digestion of the pBR322 gives fragments 2,325 and 1,690 bases long. A 1,450-base band was the result of partial digestions at the HSV-1 *Pvu*II site nearest the *Hin*dIII site. This digested material was 5′ end-labeled with ^{32}P by kinase treatment as described above.

Several experiments were performed with strand-separated HSV-1 DNA. This DNA (5′ end labeled) was denatured and strand separated on a nondenaturing 5% acrylamide gel. The separated strands (from 2 μg of cloned DNA) were hybridized with 10 μg of infected-cell mRNA in 0.1 M Na^+–0.1 M HEPES (pH 8.0)–0.01 M EDTA at 65°C for 2 h in a 10-μl volume and then digested with S1 nuclease in buffer containing 25 μg of denatured calf thymus DNA per ml. Material was fractionated on a denaturing 5% acrylamide gel with 5′ end-labeled *Hin*fI-digested pBR322 DNA frag-

ments as a size standard or nucleotide sequence gels as size standards. All procedures were based on those of Maxam and Gilbert (13).

RESULTS

General characterization of mRNA species mapping between 0.647 and 0.721. Figure 1 shows a restriction map for the region being studied (Fig. 1A), the gross distribution and enumeration of viral mRNA species mapping in this region (Fig. 1B), and a high-resolution map of specific mRNAs (Fig. 1C) based on studies described in the next section. Note that in Fig. 1A the *Kpn*I site at 0.703 and the *Sma*I site at 0.712 are not the only sites for these enzymes in this region, but are shown because the *Kpn*I site is the only one in *Xho*I fragment W (0.689–0.706) and the *Sma*I site is the only one in *Xho*I-*Eco*RI fragment B-I (0.712–0.721); thus, they are convenient for S1 nuclease mapping experiments as described below.

Cloned HSV-1 DNA fragments were nick-translated to make probes for RNA localization. *Hin*dIII-*Pvu*II fragment D-E′ (0.647–0.653) and *Hin*dIII-*Sal*I fragment D-Q (0.647–0.674) are homologous to two major mRNA species, one 5.0 kb and the other 2.7 kb in length (Fig. 1B, tracks i and ii). These sizes include the poly(A) tails of the mRNA known to be around 200 bases in length (19). For clarity, we refer to the mRNA size as isolated for our studies, but have indicated the actual coding size of the mRNAs in the high-resolution maps. Two minor species of ca. 2 and 1.5 kb were seen under heavy exposures (see track i), but not with lower exposures (track ii). These mRNA species were not further characterized. We previously found the 3′ ends of these major mRNAs to extend 200 bases to the

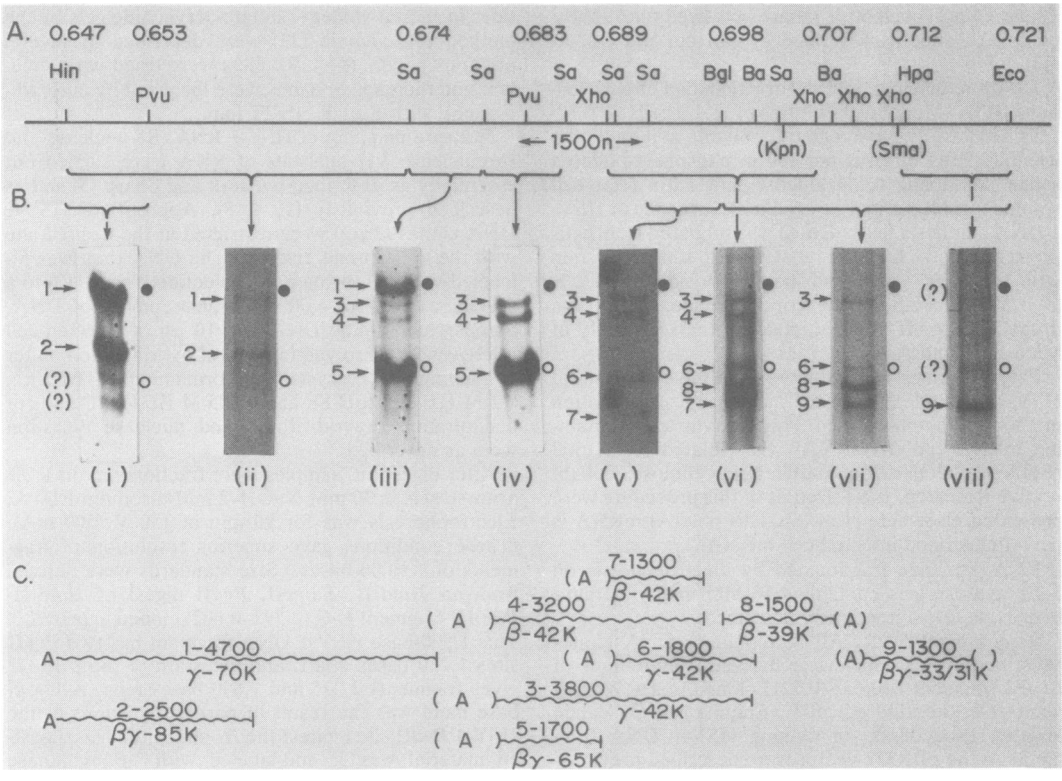

FIG. 1. Distribution of mRNA species mapping between 0.647 and 0.721 on the P arrangement of the HSV-1 genome. (A) Restriction endonuclease cleavage sites: Hin = *Hin*dIII, Pvu = *Pvu*II, Sa = *Sal*I, Xho = *Xho*I, Bgl = *Bgl*II, Ba = *Bam*HI, Kpn = *Kpn*I, Sma = *Sma*I, Hpa = *Hpa*I, Eco = *Eco*RI. Map coordinates are based on HSV-1 DNA being 150 kb in length and setting the *Hin*dIII site at 0.647. (B) mRNA localization by in situ RNA hybridization. Gels of infected-cell mRNA were hybridized in situ (see the text) with [^{32}P]cDNA made to HSV-1 DNA subclones as described in the text. The solid circles indicate the position of 28S rRNA (5.2 kb; 15), and the open circles indicate that of 18S rRNA (2.0 kb; 20), run in parallel as a size standard. (C) High-resolution transcription map. Specific HSV-1 mRNA species were localized by S1 nuclease and exonuclease VII mapping procedures outlined in the text. mRNA species were arbitrarily numbered (see text). The size of the encoded region [i.e., without the poly(A) tail] in nucleotides is shown above each transcript; time of appearance and polypeptide encoded are shown below (see Fig. 2 and Table 1).

left of the *Hin*dIII site at 0.647 (8; unpublished data), so these mRNAs are in part colinear. For convenience, we have designated these mRNAs as species 1 (5 kb) and species 2 (2.7 kb). The 5′ end of mRNA species 1 extends into *Sal*I fragment J′ (0.674–0.681; Fig. 1A, track iii).

The mRNA species 1 and 2 were isolated by preparative hybridization of infected-cell poly(A) polyribosomal RNA (infected-cell mRNA) with *Hin*dIII-*Sal*I fragment D-Q (0.647–0.674) bound to cellulose, followed by gel fractionation. In a reticulocyte lysate system, mRNA species 1 translated into a 70,000-dalton (d) polypeptide (Fig. 2A, track iii), and mRNA species 2 translated into an 86,000-d one (Fig. 2A, track iv). The 70,000-d translation product of mRNA species 1 also was seen when this mRNA was isolated with *Sal*I fragment J′ (0.674–0.681) bound to cellulose (Fig. 2B, track vi). This confirmed the extension of mRNA species 1 into this fragment.

Three other mRNA species were found to be homologous to DNA in *Sal*I fragment J′ (0.674–0.681; Fig. 1B, track iii). One is 4 kb long (species 3), one is 3.4 kb long (species 4), and one is 1.9 kb long (species 5). These three mRNA species also were homologous to DNA from *Sal*I fragment F′ (0.681–0.687; Fig. 1B, track iv). The mRNA species 3 and 4 were homologous to DNA in *Xho*I fragment W (0.689–0.707; Fig. 1B, track vi), and mRNA species 3 extended into *Bgl*II-*Eco*RI fragment F-I (0.698–0.721; Fig. 1B, track vii). We detected mRNA species 3 and 4 by using cDNA made to *Sal*I-*Bgl*II fragment D′-G (0.693–0.698; Fig. 1B, track v) and also saw two further mRNA species, one 2.0 kb in length (species 6) and one 1.5 kb in length (species 7). We confirmed that the 2-kb mRNA species 6 was not identical to the 1.9-kb species 5 by in vitro translation (see below).

We used *Sal*I fragment J′ (0.674–0.681) to preparatively hybridize mRNA species 3, 4, and 5 for in vitro translation. mRNA species 3 and 4 both encoded 42,000-d polypeptides (Fig. 2B, tracks vii and viii), whereas mRNA species 5 encoded a 63,000-d one (Fig. 2B, track ix). We isolated mRNA species 3 and 4 by using *Xho*I fragment W (0.689–0.707; Fig 1B, track vi) and confirmed that they translated into 42,000-d polypeptides (Fig. 2C, tracks xii and xiii). Furthermore, we isolated the three smaller mRNAs seen homologous to *Xho*I fragment W (0.689–0.707)—species 6 (2 kb), species 7 (1.5 kb), and species 8 (1.7 kb)—and translated them in vitro. Although the bands were not fully resolved, it was clear that mRNA species 6 and 7 both encoded 42,000-d polypeptides (Fig. 2C, tracks xiv and xvi), whereas mRNA species 8 encoded a 39,000-d one (Fig. 2C, track xv).

The mRNA species 3 and 6 were homologous to a portion of *Bgl*II-*Eco*RI fragment F-I (0.698–0.721; Fig. 1B, track vii), and we confirmed the fact that they can be translated into 42,000-d polypeptides in vitro (Fig. 2D, tracks xviii and xix) when isolated with this DNA fragment. In both cases, other polypeptides also were seen due to lack of full resolution of the mRNA species and to the fact that at least two other mRNA species around 4 and 2 kb in length map in this DNA fragment (see Fig. 1B, track viii).

*Bgl*II-*Eco*RI fragment F-I (0.698–0.721) was used to isolate mRNA species 8 and a further species 1.5 kb in length (species 9), as seen in Fig. 1B (track vi). Translation of species 8 in vitro yielded the 39,000-d polypeptide described above (Fig. 2D, track xx), and translation of species 9 yielded two major polypeptide bands, one 33,000 d and one 31,000 d in size (Fig. 2D, track xxi). *Xho*I-*Eco*RI fragment B-I (0.711–0.721) was used to isolate mRNA species 9 free of species 8 (Fig. 1B, track vii), and its translation also yielded the 33,000- and 31,000-d polypeptides (Fig. 2A, track v).

The general location, time of appearance, and translation products of the nine mRNA species described are summarized in Table 1. We classified these mRNA species as early (β) or late (βγ or γ) by using Northern blots of infected-cell mRNA from cells in which DNA synthesis was

TABLE 1. General properties of HSV-1 mRNA mapping between 0.647 and 0.721

mRNA		Approx. map coordinates	Time of appearance[a]	Size of polypeptide encoded (10^3 d)
Species[b]	Size[c] (kb)			
1	5.0	0.646–0.674	γ	70[d]
2	2.7	0.646–0.660	βγ	85[e]
3	4	0.675–0.702	γ	42[f]
4	3.4	0.675–0.697	β	42[g]
5	1.9	0.675–0.688	βγ	63[h]
6	2	0.688–0.702	γ	42[i]
7	1.5	0.688–0.697	β	42[j]
8	1.7	0.698–0.708	β	39[k]
9	1.5	0.709–0.719	βγ	33, 31[l]

[a] Assignment of temporal class is discussed in the text and previously (1, 8, 10).

[b] Arbitrarily numbered as shown in Fig. 1B.

[c] Size is based on comigration with 28S (5.2-kb) and 18S (2.0-kb) HeLa cell rRNA (15, 20) and includes the length of the poly(A) tail (ca. 200 bases; 19).

[d] Fig. 2A (track iii); Fig. 2B (track vi).

[e] Fig. 2A (track iv).

[f] Fig. 2B (track vii); Fig. 2C (track xii); Fig. 2D (track xviii).

[g] Fig. 2B (track viii), Fig. 2C (track xiii).

[h] Fig. 2B (track ix).

[i] Fig. 2C (track xiv); Fig. 2D (track xix).

[j] Fig. 2C (track xvi).

[k] Fig. 2C (track xv); Fig. 2D (track xx).

[l] Fig. 2D (track xxi); Fig. 2A (track v).

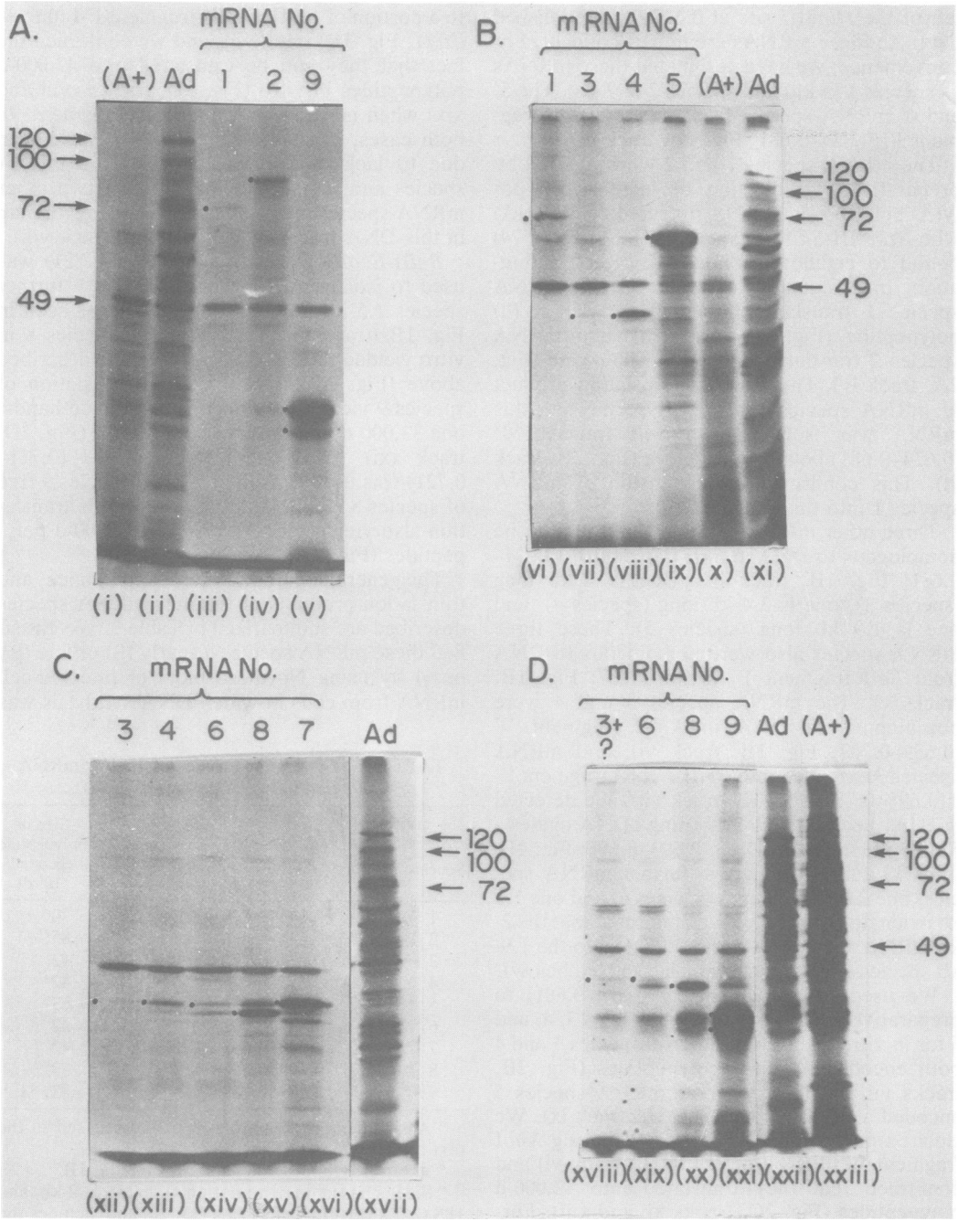

FIG. 2. In vitro translation of specific mRNA species. Viral mRNA species were isolated by preparative hybridization to appropriate HSV-1 DNA subclones bound to cellulose, and individual species were isolated by gel electrophoresis. Translation products discussed in the text are marked with dots (·). In vitro translation was carried out with [^{35}S]methionine and a commercial reticulocyte lysate system. Adenovirus mRNA was translated as a size marker as described previously (1, 4, 8), and sizes of particular polypeptide bands are indicated. In some cases, total infected-cell poly(A) mRNA also was translated [marked A(+)]. (A) Translation of mRNA species 1 and 2 isolated with *Hin*dIII-*Sal*I fragment D-Q (0.647–0.674) and mRNA species 9 isolated with *Xho*I-*Eco*RI fragment B-I (0.712–0.721). (B) Translation of mRNA species 1, 3, 4, and 5 isolated with *Sal*I fragment J′ (0.674–0.681). (C) Translation of mRNA species 3, 4, 6, 7, and 8 isolated with *Xho*I fragment W (0.689–0.707). (D) Translation of mRNA species 3, 6, 8, and 9 isolated with *Bgl*II-*Eco*RI fragment F-I (0.698–0.721).

inhibited (not shown). The mRNA species 6 (1.5 kb) and 8 (1.7 kb) were readily detectable before viral DNA synthesis; therefore, we classified them as early (β). It should be noted, however, that species 6 was never as abundant as species 8. The mRNA species 4 (3.4 kb) was readily detectable in the absence of viral DNA replication, and although it was never a highly abundant species, we also tentatively classified it as an early mRNA. The mRNA species 2 (2.7 kb), 5 (1.9 kb), and 9 (1.5 kb) were detectable without viral DNA replication, but were major species only after its inception; therefore, we classified them as late (βγ). The mRNA species 1 (5 kb), 4 (4.0 kb), and 7 (2 kb) were only seen after viral DNA replication; therefore, we classified these as "true" late (γ). Our assignments for mRNA species 4 and 7 should be considered tentative due to the relatively low abundance of these mRNAs at late times.

High-resolution mapping of the individual HSV-1 mRNA species. We used S1 nuclease and exonuclease VII mapping procedures adapted by us from the Berk-Sharp procedures (3) as described previously (1, 4, 8) to localize precisely the viral mRNA species described above. These data are summarized in Fig. 1C, and specific details are described below.

(i) mRNA species 1 and 2. We hybridized infected-cell mRNA to *Hin*dIII-*Sal*I fragment D-Q (0.647–0.674) 5′-labeled with ^{32}P at the *Hin*dIII site at 0.647. S1 nuclease or exonuclease VII digestion always yielded two major protected DNA fragments, one 4,200 bases long and one 2,250 bases long. An example of an exonuclease VII digestion is shown in Fig. 3A (track ii). The 4,200 bases corresponds to the full length of the fragment and is due to hybridization of mRNA species 1, whereas the 2,250-base DNA band is due to mRNA species 2. We also hybridized infected-cell mRNA to this clone 5′-labeled at the *Pvu*II site at 0.653, 1,000 bases to the right of the *Hin*dIII site at 0.647. In this case, mRNA species 1 protected a 3,200-base fragment of DNA from S1 nuclease or exonuclease VII digestion, and mRNA species 2 protected a 1,250-base piece. An example of an S1 nuclease digestion is shown in Fig. 3B (track iv).

We precisely localized the 5′ end of mRNA species 1 by hybridizing strand-separated *Sal*I fragment J′ (0.674–0.681) DNA 5′-labeled at 0.674 to infected-cell mRNA. S1 nuclease digestion yielded a protected DNA band 260 bases in length (Fig. 3C, track v), positioning the 5′ end of mRNA species 1 to be this number of bases to the right of the *Sal*I site at 0.674.

We confirmed the localization of the 5′ ends of mRNA species 1 and 2 by hybridizing infected-cell mRNA with DNA from *Hin*dIII-*Bgl*II fragment D-G (0.647–0.698) 5′-labeled at the *Hin*dIII site at 0.647. Exonuclease VII and S1 nuclease digestion (not shown) gave a protected DNA band 4,500 bases in length, corresponding to the full length of the coding sequence of mRNA species 1. This confirms the lack of splices in this mRNA. Also, a band 2,250 bases long, corresponding to the length of the coding sequence of mRNA species 2, was seen with equivalent intensity. This suggested that any noncontiguous leader sequence for mRNA species 2 mapping near the 5′ end of mRNA species 1 was too short to hybridize under standard conditions. Such a conclusion was confirmed by the fact that probe made to *Sal*I fragment J′ (0.674–0.681) did not show homology to mRNA species 2 by blot hybridization (Fig. 1B, track iii).

(ii) mRNA species 3, 4, 5, 6, and 7. The contiguous 5′ regions of mRNA species 3, 4, and 5 could be readily visualized and mapped by hybridizing infected-cell mRNA with DNA from *Hin*dIII-*Bgl*II fragment D-G (0.647–0.698) 5′-labeled at both *Pvu*II sites (0.653 and 0.683). The S1 nuclease or exonuclease VII digestion yielded a number of protected DNA bands. An example of an S1 nuclease digestion is seen in Fig. 4A (track ii). Comparison of these bands with those shown in Fig. 3B allowed us to assign the 3,500-base band to the 5′ region of mRNA species 1 and the 1,250-base band to the 5′ region of mRNA species 2. Fragments migrating with a size of 2,200 to 2,600 bases comprise the DNA protected by mRNA species 3 and 4, and the 900-base piece represents the 5′ region of mRNA species 5. The diffuse radioactivity migrating more slowly than the 1,250-base band was not reproducibly seen. Such artifactual "bands" have been described previously (see references 1, 4, and 8).

We established the direction of transcription of mRNA species 3, 4, and 5 in two ways. First, hybridization of infected-cell mRNA with *Sal*I fragment J′ (0.674–0.681) DNA, 5′-labeled at 0.682 (the complementary strand to the one used in section i above), yielded no nuclease protection. This indicated that no mRNA transcribed from right to left maps through this point. It was then clear that all the protected DNA fragments seen using DNA 5′ end-labeled at the *Pvu*II site at 0.683 were due to mRNA transcribed from the right of this point. Second, we hybridized infected-cell mRNA to *Hin*dIII-*Xho*I fragment D-A (0.674–0.689) DNA, 5′ end-labeled at the *Pvu*II sites at 0.653 and 0.683. Here, S1 nuclease and exonuclease VII digestions gave protected fragments 3,500 bases long (from mRNA species 1), 1,250 bases long (from mRNA species 2), and 900 bases long (from mRNA species 5 and from the truncation of the DNA protected by mRNA species 3 and 4) (Fig. 4A, track iv).

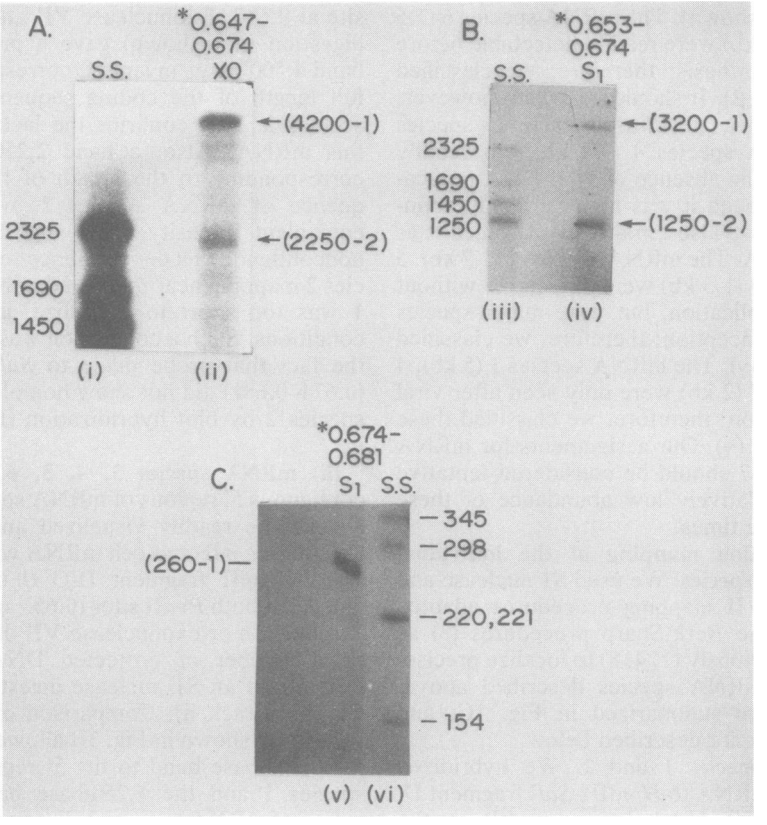

FIG. 3. S1 nuclease and exonuclease VII mapping of mRNA species 1 and 2. All details of hybridization, nuclease digestion, alkaline gel fractionation, and size standards (tracks i, iii, and vi) are described in the text. (A) DNA from *Hin*dIII-*Sal*I fragment D-Q (0.647–0.674), 5′ end-labeled at 0.647, was hybridized to infected-cell mRNA and digested with exonuclease VII (track ii). The 4,200-base band due to mRNA species 1 and the 2,250-base band due to mRNA species 2 are indicated. (B) As (A), except the DNA was 5′ end-labeled at the *Pvu*II site at 0.653 (track iv) and an S1 nuclease digestion is shown. The 3,200-base band from mRNA species 1 and the 1,250-base band from mRNA species 2 are shown. (C) Localization of the 5′ end of mRNA species 1. DNA from *Sal*I fragment J′ (0.674–0.681) was 5′ end-labeled at both ends and strand separated; the strand bearing the label at 0.674 was hybridized with infected-cell mRNA and digested with S1 nuclease. The nuclease-resistant DNA (260 bases long) was fractionated on a denaturing acrylamide gel (track v) along with *Hin*fI-digested pBR322 DNA, 5′ end-labeled, as a size marker (track vi).

The above experiment also confirmed that mRNA species 5 had its 5′ end very near the *Xho*I site at 0.689, and the lack of ability to isolate the mRNA by using DNA fragments mapping to the right of this site indicated that there was no detectable noncontiguous leader sequence to this mRNA.

We hybridized infected-cell mRNA to *Hin*dIII-*Bgl*II fragment D-G (0.647–0.698) DNA 5′ end-labeled at the *Xho*I site at 0.689. S1 nuclease and exonuclease VII digestion always yielded two bands, one 900 bases in length due to the protection by mRNA species 4 and 7, and one 1,350 bases long due to the protection of the region between the *Xho*I site at 0.689 and the *Bgl*II site at 0.698 by mRNA species 3 and 6. Furthermore, the relative intensity of the bands generally reflected the abundance of these mRNAs. An example of an exonuclease VII digestion is shown in Fig. 4B (track v).

We confirmed the location of the 5′ ends of mRNA species 4 and 7 and positioned the 5′ ends of mRNA species 3 and 6 by hybridizing infected-cell mRNA to *Sal*I-*Bam*HI fragment D′-F (0.693–0.702) DNA 5′ end-labeled at the *Sal*I site at 0.693. In this case, S1 nuclease and exonuclease VII digestion yielded two bands, one approximately 750 bases long (from mRNA species 4 and 7) and one 1,250 bases long (from mRNA species 3 and 6) (Fig. 4C, tracks viii and ix). These results placed the 5′ ends of mRNA species 3 and 6 approximately 500 bases to the

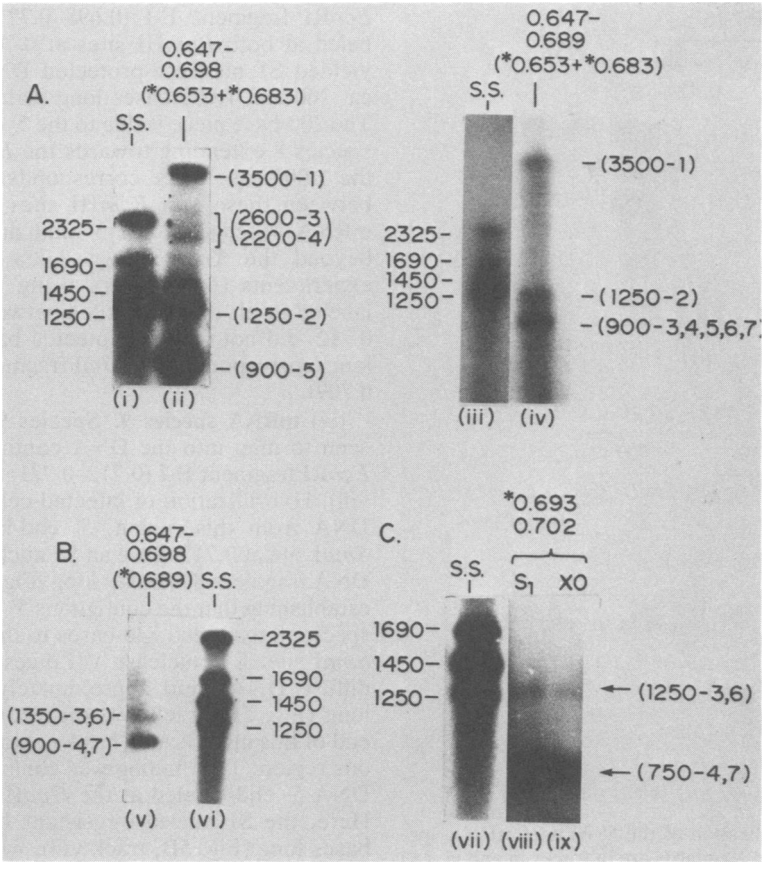

FIG. 4. S1 nuclease and exonuclease VII mapping of mRNA species 3, 4, 5, 6 and 7. Size standards (SS) are in tracks i, ii, v, and vii. (A) Track ii:DNA from *Hin*dIII-*Bgl*II fragment D-G (0.647–0.698) was 5′ end-labeled at *Pvu*II sites at 0.653 and 0.683, hybridized to infected-cell mRNA, and digested with S1 nuclease. The 3,500-base band is due to mRNA species 1; the multiple band 2,200 to 2,600 bases long is from mRNA species 3 and 4; the 1,250-base band is from mRNA species 2; and the 900-base band is due to mRNA species 5. In track iv, the same experiment was done with DNA from *Hin*dIII-*Xho*I fragment D-A (0.647–0.689). Here, the 900-base band is due to overlapping mRNA species 3, 4, 5, 6, and 7. (B) Hybridization was with the same DNA as in (A), track ii, 5′ end-labeled at the *Xho*I site at 0.689 and digested with exonuclease VII (track v). The 1,350-base band from mRNA species 3 and 6 and the 900-base band from mRNA species 4 and 7 are shown. (C) Hybridization was with *Sal*I-*Bam*HI fragment D′-F (0.693–0.702), 5′ end-labeled at 0.693 and digested either with S1 nuclease (track viii) or exonuclease VII (track ix). The 1,250-base band from mRNA species 3 and 6 and the 750-base band from mRNA species 4 and 7 are indicated.

right of the *Bgl*II site at 0.698. We exactly located the 5′ ends of these two sets of colinear mRNAs by doing a high-resolution S1 analysis run with sequence gels as size standards. We found the 5′ ends of mRNAs 4 and 7 to map exactly 590 bases to the right of the *Sal*I site at 0.693, while the 5′ ends of mRNA species 3 and 6 were located exactly 110 bases to the right of the *Bam*HI site at 0.700. This places this 5′ end 380 bases to the right of the *Bgl*II site at 0.698. These data are shown in the following section.

(iii) mRNA species 8. Early (β) mRNA species 8 mapped in DNA contained in *Xho*I fragment W (0.689–0.707) and lay to the right of the *Bgl*II site at 0.698. Hybridization of infected-cell mRNA with such DNA 5′ end-labeled at the *Kpn*I site at 0.703 gave an S1 nuclease-resistant DNA fragment 750 bases in length (Fig. 5A, track ii). This same result was found if DNA from *Bgl*II-*Eco*RI fragment F-I (0.698–0.721) was 5′-labeled at this *Kpn*I site (Fig. 5A, track i), so we concluded that mRNA species 8 had its 5′ end very near but not appreciably to the left of the *Bgl*II site at 0.698. We located this 5′ end within 200 bases of the *Bam*HI site at 0.700 by using DNA from *Sal*I-*Bam*HI fragment E′-F (0.693–0.700). Hybridiza-

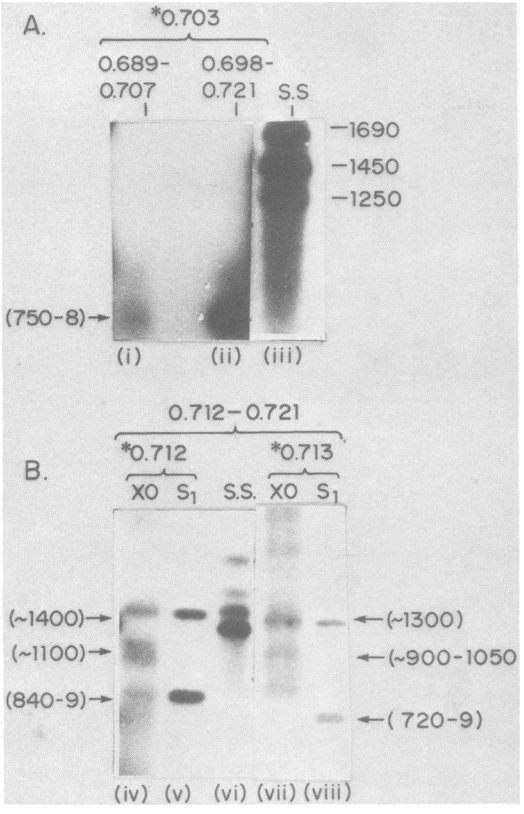

FIG. 5. Localization of the 5′ ends of mRNA species 8 and 9. Size standards are in tracks iii and vi. (A) Infected-cell mRNA was hybridized with DNA 5′ end-labeled at the *Kpn*I site at 0.703 and digested with exonuclease VII. Track i shows the 750-base band due to mRNA species 8 formed when DNA from *Xho*I fragment W (0.689–0.707) was used; track ii shows the same size band seen when DNA from *Bgl*I-*Eco*RI fragment F-I (0.698–0.721) was used. (B) Infected-cell mRNA was hybridized with DNA from *Xho*I-*Eco*RI fragment B-I (0.712–0.721) 5′ end-labeled at the *Sma*I site at 0.712 (tracks iv and v) or the *Hpa*I site at 0.713 (tracks vii and viii). S1 nuclease digestion shows the sharp full-length DNA fragment, as well as the band due to the mRNA species 9 (tracks v and viii), whereas exonuclease VII digestion yielded diffuse larger bands (tracks iv and vii).

tion of DNA 5′ end-labeled at the *Bam*HI site at 0.700 with infected-cell mRNA gave an S1-resistant fragment 200 bases long (not shown). We used high-resolution S1 analysis (next section) to precisely locate the 5′ end of mRNA species 8 to be 68 bases to the right of the *Bgl*II site at 0.698, since it mapped 12 bases to the left of a *Sma*I site 80 bases to the right of this *Bgl*II site.

The 3′ end of mRNA species 8 mapped to the right of the *Bam*HI site at 0.708, since hybridizing infected-cell mRNA with DNA from *Bgl*II-*Eco*RI fragment F-I (0.698–0.721), 5′ end-labeled at both *Bam*HI sites at 0.701 and 0.707, yielded S1 nuclease-protected DNA fragments ca. 200 and 1,000 bases long (data not shown). The 200-base piece is due to the 5′ end of mRNA species 8 extending towards the *Bgl*II site, and the 1,000-base piece corresponds to the region between these two *Bam*HI sites protected by mRNA species 8. The 3′ end did not extend beyond the *Xho*I site at 0.708 since similar experiments (not shown), using DNA 5′ end-labeled at all the *Xho*I sites between 0.706 and 0.712, did not yield a protected band 550 bases long, corresponding to *Xho*I fragment O′ (0.707–0.709).

(iv) mRNA species 9. Species 9 mRNA was seen to map into the DNA contained in *Xho*I-*Eco*RI fragment B-I (0.712–0.721; Fig. 1B, track viii). Hybridization of infected-cell mRNA with DNA from this region, 5′ end-labeled at the *Sma*I site at 0.712, gave an S1 nuclease-resistant DNA fragment 840 bases long (Fig. 5B, track v), establishing that the contiguous 5′ end of mRNA species 8 extended 840 bases to the right of this *Sma*I site. Exonuclease VII digestion yielded a diffuse DNA band approximately 1,100 bases long (Fig. 5B, track iv), suggesting that the 5′ end of this mRNA may have a short noncontiguous region. This finding was confirmed by using DNA 5′ end-labeled at the *Hpa*II site at 0.713. Here, the S1 nuclease-resistant DNA was 720 bases long (Fig. 5B, track viii), whereas exonuclease VII digestion yielded diffuse bands ranging from 900 to 1,050 bases in length (Fig. 5B, track vii). Similar results were found when *Sal*I-*Eco*RI fragment E-I (0.703–0.721) DNA 5′-labeled at the *Xho*I sites at 0.707, 0.709, and 0.712 was hybridized to infected-cell mRNA (data not shown). In this latter case, S1 nuclease- and exonuclease VII-resistant DNA corresponding to *Xho*I fragment P′ (0.709–0.712) also was seen. This showed that the 3′ end of mRNA species 9 lay to the left of 0.709; the lack of protection of DNA the size of *Xho*I fragment O′ (0.707–0.709) indicated that this mRNA did not extend to the left of 0.707.

Any other mRNA mapping to the left of the *Eco*RI site at 0.721 must have its 5′ end to the right of this site, since all experiments hybridizing *Sal*-*Eco*RI fragment E-I (0.703–0.721) DNA that was 5′-labeled at the *Eco*RI site at 0.721 with infected-cell mRNA yielded no S1 nuclease- or exonuclease VII-resistant DNA fragments (data not shown). It should be noted, however, that hybridization of infected-cell mRNA with DNA 5′ end-labeled at the *Sma*I site or the *Hpa*I site around 0.712 always gave some protected DNA corresponding to the length of the DNA from the site in question to the *Eco*RI site at 0.721. This could be due to the

presence of one or more mRNA species colinear with the 1,300-base species 9. It should be remembered that several other RNA bands were seen homologous to this region (Fig. 1B).

Precise localization of the 5′ ends of mRNA species 3, 4, 6, 7, and 8. The close juxtaposition of the 5′ ends of mRNA species 3 and 6 and 4 and 7, and the apparent complementary overlap with species 8, made it of interest to precisely locate the 5′ ends of these mRNAs and to examine the nucleotide sequence of the DNA encoding them. A very high-resolution map of the region around where these 5′ ends map is shown in Fig. 6. We determined the nucleotide sequence of the DNA from about 200 bases to the right of the *Bam*HI site at 0.700 down to near the *Sma*I site at 0.696. We used 5′-labeled DNA fragments, and the strategy and overlapping sequencing are shown in Fig. 6 also. The precise sequence of both strands of the DNA is shown in Fig. 7, which is arbitrarily numbered from the right-hand end. This numbering also is indicated in Fig. 6.

We located the 5′ ends of mRNA species 3 and 6 by hybridizing infected-cell mRNA to strand-separated DNA 5′ end-labeled at the *Bam*HI site at 0.700. This DNA was isolated by *Bam*HI digestion of *Xho*I fragment W (0.689–0.707) and, after 5′ end-labeling, was further digested with *Hin*fI. The 5′ end-labeled piece was then isolated on a strand-separating gel and used for hybridization and sequence analysis. S1 nuclease digestion gave a resistant fragment 110 bases in length, indicating that the 5′ end of mRNA species 3 and 6 was located this far to the right of the *Bam*HI site at 0.700. Fractionation of the S1-digested hybrid beside the sequence gel of DNA 5′-labeled at the *Bam*HI site showed some stutter, a common finding in such analyses (9), but located this 5′ end as an AC dinucleotide at position 109–110 (Fig. 8A). This is 25 bases downstream of the sequence ATAAAA, a putative TATA box (Fig. 7). Note that in the gels shown the sequence seen is complementary to that of the mRNA sense strand.

The DNA of *Xho*I fragment W (0.689–0.707) was digested with *Sma*I and 5′ end-labeled, and the 325-base fragment spanning from 0.696 to approximately 0.699 was isolated to locate the 5′ ends of mRNA species 4, 7, and 8. Hybridization of strand-separated DNA 5′ end-labeled at 0.699 to infected-cell mRNA yielded an S1-resistant hybrid 12 bases long. Controls carried out without RNA indicated that this resistant fragment was specific and not due to the structure of the single-stranded DNA. We fractionated this S1 nuclease-resistant fragment next to a sequence gel of DNA 5′ end-labeled at this *Sma*I

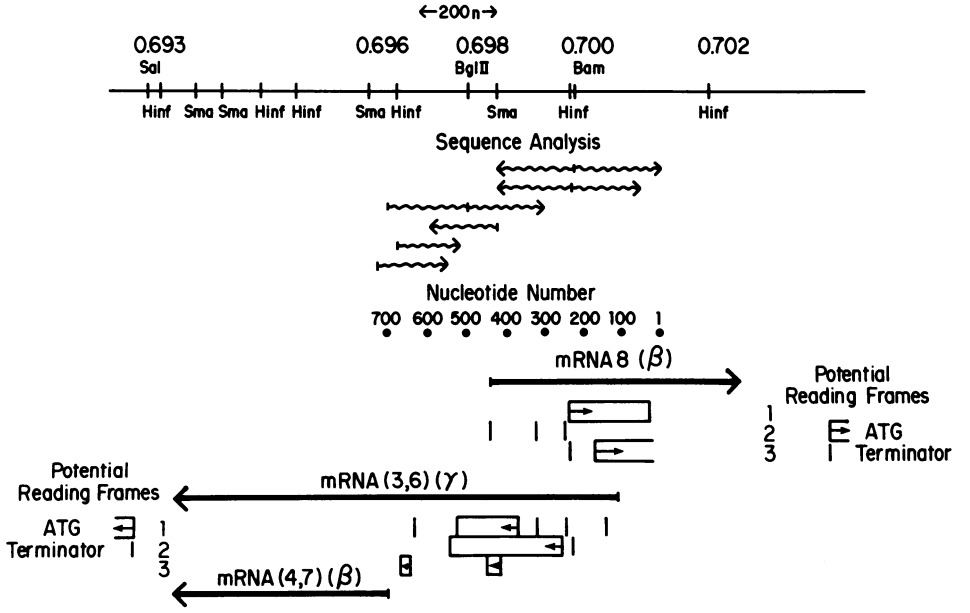

FIG. 6. High-resolution restriction map of HSV-1 DNA around the *Bgl*II site at 0.698. Sites shown are *Sma*I (Sma), *Hin*fI (Hinf), and *Bam*HI (Bam). The actual number of bases sequenced from particular 5′-labeled restriction sites are indicated (wavy arrows), and the numbering of the sequence data of Fig. 7 also is indicated. The location of the 5′ ends of mRNA species 3 and 6, 4 and 7, and 8 are shown. Potential translation reading frames, based on the sequence data of Fig. 7, are shown.

```
      1                                   30                                  60                                  90
(5')  GCGCAAAAGTCAGCCGGCATAGCCATTCGC AGGTCCAGAGAGACGCGCCCGACGGCCCAT CCGGAGTCCCCGCTGACCTTCGGCATAAAA
(3')  CGCGTTTTCAGTCGGCCGTATCGGTAAGCG TCCAGGTCTCTCTGCGCGGGCTGCCGGGTA GGCCTCAGGGGCGACTGGAAGCCGTATTTT

      91          (3,6) ⟶ 120                                 150                                 180
(5')  GCCACCGCGCGCCTGTTGACCAAGTTCAAG TTGCACGACTCCGCCCCCGCGAGTAGCGAC GGCCGTGTGCCAGTCGCCATCGTACCCCCG
(3')  CGGTGGCGCGCGGACAACTGGTTCAAGTTC AACGTGCTGAGGCGGGGGCGCTCATCGCTG CCGGCACACGGTCAGCGGTAGCATGGGGGC

      181                                 210                                 240                                 270
(5')  ACCCAAGCTGTCCGGCTGGACAAAGGAATA CGCTCCGGATCCCCACTGACTCATCTTCCT GTTAGGGACGATGGGCCCCCCCAGAAGGGT
(3')  TGGGTTCGACAGGCCGACCTGTTTCCTTAT GCGAGGCCTAGGGGTGACTGAGTAGAAGGA CAATCCCTGCTACCCGGGGGGGTCTTCCCA

      271                                 300                                 330                                 360
(5')  CTGTCGGGCGGGCCTGTTGTTTGTCTTGCT CGTCGCCTTAGCGGCGGGAGACGCGGGCCC GCACGGGGAGCCACCCGGCGAGGAGGGCGG
(3')  GACAGCCCGCCCGGACAACAAACAGAACGA GCAGCGGAATCGCCGCCCTCTGCGCCCGGG CGTGCCCCTCGGTGGGCCGCTCCTCCCGCC

      361                                 390                                 420                                 450
(5')  GCGCGATGGGATCGGGGACGCGCGGTGCGG GACCCAAAACGCTGGCCAATGTCGCCCCGG GGGCCCTGGTGCCCTTTTATGTAGGCATGG
(3')  CGCGCTACCCTAGCCCCTGCGCGCCACGCC CTGGGTTTTGCGACCGGTTACAGCGGGGCC CCCGGGACCACGGGAAAATACATCCGTACC
                                                                                      ⟵(8)
      451                                 480                                 510                                 540
(5')  CCTCGATGGGCGTGTGTATTATCGCACACG TCTGTCAGATCTGCCAGAGGCTACTGGCTG CCGGGCAGGCCTGAACCCGCCCTGTGTGGG
(3')  GGAGCTACCCGCACACATAATAGCGTGTGC AGACAGTCTAGACGGTCTCCGATGACCGAC GGCCCGTCCGGACTTGGGCGGGACACACCC

      541                                 570                                 600                                 630
(5')  GTGAGGGGTGGGGGTGGAGGGTGTCCCAGG ACTTCCCCTTCTTCGCGGAAACCGAGACCG TTTGGGGCGTGTCTGTTTCTTGGCCCCTGG
(3')  CACTCCCCACCCCCACCTCCCACAGGGTCC TGAAGGGGAAGAAGCGCCTTTGGCTCTGGC AAACCCCGCACAGACAAAGAACCGGGGACC

      631                                 660                                 690 (4,7) ⟶                         720
(5')  GGATTGGTTAGACCCATGGGTTGTCCATAT ATGCACTTCCTATAAGACTCTCCCCCACCG CCCACAGAGGGCCACTCACGCATCCCCAGT
(3')  CCTAACCAATCTGGGTACCCAACAGGTATA TACGTGAAGGATATTCTGAGAGGGGGTGGC GGGTGTCTCCCGGTGAGTGCGTAGGGGTCA
```

FIG. 7. Nucleotide sequence of DNA encoding the 5′ ends of mRNA species 3 and 6, 4 and 7, and 8. Both strands are shown. The top sequence has its 5′ end at the upper left and is complementary to mRNA species 3 and 6, and 4 and 7; the lower sequence is complementary to mRNA species 8. Sequence analysis was carried out with 5′ end-labeled DNA. Sites labeled are indicated in Fig. 6. The sequence was arbitrarily numbered from 1 to 720 as shown in Fig. 6. The 5′ end of mRNA species 4 and 7 is at the AC at position 109–110; that of mRNA species 8 is at 430–431; and that of mRNA species 3 and 6 is at 694–695. The *Bam*HI site is at 219 and the *Bgl*II site is at 488. *Hin*fI sites are at 228 and 677. *Sma*I sites are at 418 and 747 (not shown). Potential translation initiation (ATG) and terminator (TAA, TGA, TAG) triplets are indicated in Fig. 6.

site (Fig. 8B). Although the bands are distorted in this region, due to their running with the dye front, the band of S1 nuclease-resistant material can be seen at nucleotides 430–431. The presence of a beginning of a string of four Ts at position 435 was confirmed by sequencing the other strand from the *Bgl*II site at 0.698. We have frequently noticed a very weak T band following a string of Cs (unpublished data).

We cannot rigorously exclude a multiple 5′ end of mRNA species 8 since an end even closer to the *Sma*I site at 0.699 cannot be resolved and resolution from the *Bam*HI site at 0.700 is insufficient. However, the end of mRNA species 8 that we have located maps 43 bases downstream of the sequence ATAATA and 10 bases below the sequence ATAAAA. Both, or either, are possible TATA boxes. Our data also indicate that the 5′ end of mRNA species 8 has 318 bases of complementary overlap with the 5′ end of mRNA species 3 and 6.

The 5′ end of mRNA species 4 and 7 was located at the AC dinucleotide number 694–695, which is 38 bases below the sequence ATATAT and 24 bases downstream of the sequence TATAA. Again, either or both are possible TATA boxes. This localization was done by hybridizing infected-cell mRNA with strand-separated DNA, 5′ end-labeled at the *Sma*I site at 0.696, and running the S1-resistant DNA next to a sequence gel from that site (Fig. 8C). The data are suggestive of a possible second 5′ end at position 704; the significance of this with regard to the two potential TATA boxes is unclear at this time. The 5′ end of mRNA species 4 and 7 is, then, about 585 bases downstream of the 5′ end of mRNA species 3 and 6.

We investigated the sequence data of Fig. 7 to locate potential translation reading frames starting with the ATG triplet. Our findings are summarized in Fig. 6. For mRNA species 3 and 6, there are two potential translation frames in the region upstream of mRNA species 4 and 7; one, starting at base 250, is open to base 542 and could encode a polypeptide of approximately 100 amino acids. There is a shorter open frame one base out of phase with this, running from base 366 to base 522, which could encode an approximately 50-amino acid polypeptide. The third potential reading frame is never open for more than 40 bases or so. From this, it is clear that mRNA species 3 and 6 cannot encode any polypeptide that begins upstream of the 5′ end of mRNA species 4 and 7 and continues into the DNA encoded by this mRNA family.

Although mRNA species 8 has a 318-base complementary overlap with mRNA species 3 and 6, its first potential open reading frame is

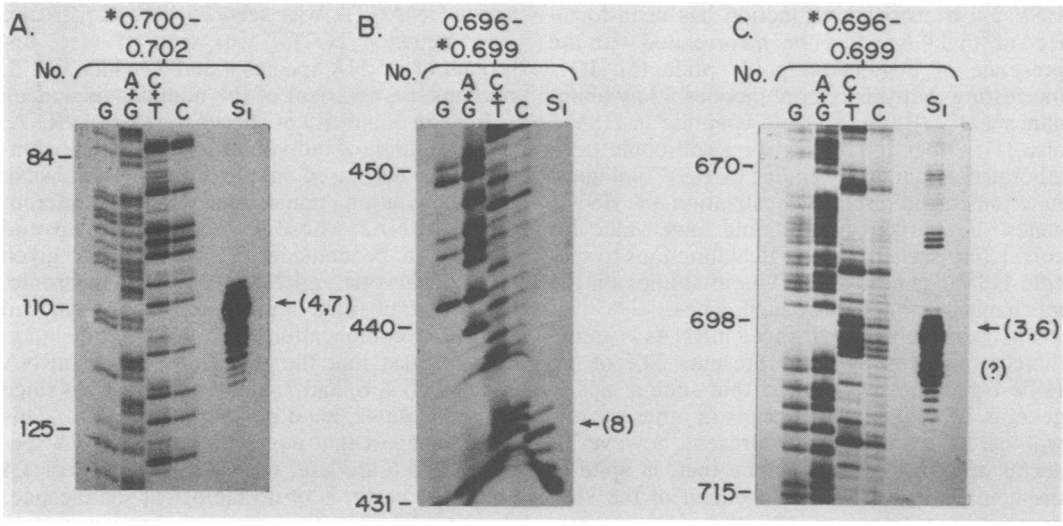

FIG. 8. Precise localization of the 5′ ends of mRNA species 3 and 6, 4, and 7, and 8. (A) Infected-cell mRNA was hybridized to strand-separated DNA labeled at 0.700. The S1-resistant DNA fragment was fractionated on a Maxam-Gilbert (13) 8% sequencing gel as described previously (9). The best location of the 5′ end is indicated. Nucleotide number in the sequence of Fig. 7 is indicated under the column marked "No." (B) As in (A), except DNA labeled at 0.699 was used. (C) As in (A), except DNA labeled at 0.696 was used. Note: The mRNA species indicated in (A) are incorrectly indicated as species (4,7); they are actually (3,6). The mRNA species indicated in (C) are incorrectly indicated as species (3,6); they are actually (4,7).

seen at position 170, which is 261 bases downstream from its 5′ end. Thus, there are two mRNAs that do not appear to encode any complementary peptide sequence information.

DISCUSSION

A high-resolution map of the 19 or so mRNA species mapping between 0.527 and 0.721 on the HSV-1 genome is shown in Fig. 9. These data summarize those reported earlier (1, 8) and the data of the present paper. The number of biological functions mapping in the HSV-1 genome is small but growing (reviewed in reference 18 and Wagner, in press). In the region between 0.527 and 0.721, host shutoff functions and glycoprotein C have been mapped. Furthermore, in

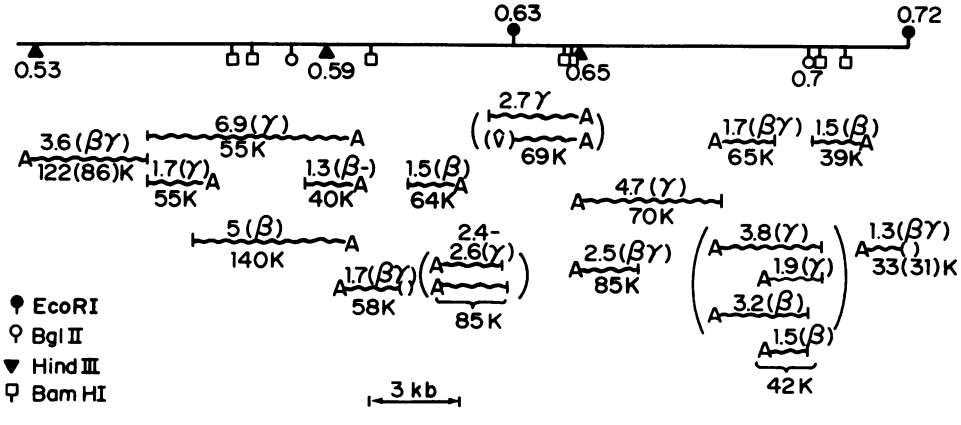

FIG. 9. Precise location of HSV-1 mRNA species mapping between 0.527 and 0.721 on the P arrangement of the genome. This map is based on data reported here, as well as previously (1, 8). The 3′ ends are indicated by A, and the 5′ ends are indicated by vertical bars. Two mRNAs have 5′ ends whose location appears to be noncontiguous with the body of the mRNA. These are indicated by the parentheses at the 5′ end. One mRNA family appears to contain spliced members as indicated. The size of the coding region in kb for each mRNA and its time of appearance is indicated above each one, and the size of the polypeptide(s) encoded in vitro are indicated below. *Hin*dIII (▼), *Bam*HI (⊓), *Bgl*II (♀), and *Eco*RI (●) cleavage sites are indicated.

HSV-2 a transforming function has been localized around 0.6 and has been correlated with the presence of a 40,000-d polypeptide (6). It is interesting that this region encodes a low-abundancy early (β) 40,000-d polypeptide in HSV-1 also (1). Other interesting data will come from laboratories pursuing studies of HSV biological functions. The precise localization of HSV-1 genes summarized here should have value for correlating particular biological functions to specific HSV-1 genes, an well as in studies on the mechanisms of action of such genes.

The virtual absence of sliced mRNAs is immediately apparent; as this represents 20% of the HSV-1 genome, we suggest that such a lack is general. Mapping of transcripts in other regions will test this conclusion. At present, however, it seems appropriate to conclude that, in spite of the complexity of the arrangement of the viral genome (see reference 17), the virus is aptly named as regards its mRNA processing.

The precise localization of the 5′ ends of mRNA species 3 and 6, 4 and 7, and 8 indicates that they all lie just downstream of TATA boxes; thus, we can reasonably expect these mRNAs to have individual promoters. We currently are carrying out a comparative analysis of the RNA sequence around the 5′ ends of early and late viral mRNAs (see reference 9 and Wagner, in press). We have found that three early (β) mRNAs have AC-rich regions ca. 100 to 110 bases upstream of their 5′ ends, and the two mRNA promoter regions characterized here (species 4 and 7, and 8) seem to follow this rule, although the extent of this region for mRNA species 4 and 7 is not particularly striking. This β mRNA is not as abundant as mRNA species 8, which may relate to this.

All of the mRNAs have sequences interpretable as TATA boxes nearby upstream. Generally, we have found these sequences 25 to 30 bases upstream of our indicated 5′ ends; however, the candidate TATA boxes for mRNA species 8 deviate from this rule somewhat. The significance (if any) of such a deviation is unclear, especially since there could be an undetected mRNA start 10 bases or so downstream.

We generally have located sequences recognizable as CAAT boxes 85 to 90 bases upstream of HSV-1 mRNA 5′ ends. This is the case for mRNA species 3 and 6, which have the sequences CATA 92 bases upstream and CATT 86 bases upstream. The situation with mRNA species 4 and 7 and 8 is less clear; no readily recognizable CAAT-like sequence was seen. For mRNA species 8, this could reflect an altered pattern of the nominal CAAT sequence, since the sequence TTCA was seen 92 bases upstream. It also could reflect our uncertainty or whether there is only one 5′ end, since the sequence AGTA was seen 75 bases upstream. The sequence TGTCT was seen 84 bases upstream of mRNA species 4 and 7, which could, perhaps, be a variant of the nominal sequence.

In spite of a rarity of splices in HSV-1 mRNA, the resolution of individual species can be complex. As discussed in the introduction, weak polyadenylation signals can lead to different sized mRNAs whose 5′ regions overlap and which can be considered "redundant," given that eucaryotic mRNAs are monocistronic. Close juxtaposition of promoter regions can lead to further complication. Our in vitro translation data suggest that the four overlapping mRNA species, 3, 4, 6, and 7, could be redundant since all encode a 42,000-d polypeptide in vitro. This would suggest that our finding that mRNA species 3 and 6 are late, whereas mRNA species 4 and 7 are early, is of no biological significance. Arguing against this are our sequence data showing translation frames open in the 600 or so bases upstream of the 5′ end of mRNA species 4 and 7 (Fig. 6). This suggests that this DNA may encode a specific late polypeptide of approximately 100 amino acids or even 50 amino acids, depending on the reading frame used. However, there is no evidence at present that a given open reading frame must be used. Such results do, however, point up the need to interpret in vitro translation data conservatively; they also demonstrate that nucleotide sequence data themselves do not necessarily lead to unambiguous data. It is clear that no polypeptide initiated upstream of the 5′ end of mRNA species 4 and 7 can be encoded by DNA sequences below this point since there are chain terminators in all three reading frames.

The situation with the multiple 5′ ends found with the four mRNAs described here is not confined to this one case. We have seen similar patterns in other regions of the genome (9; Wagner, in press; R. Costa and E. K. Wagner, unpublished data). This could reflect some limit on how small an HSV mRNA species effectively can be since few smaller than 1 kb have been seen (Wagner, in press). It also may reflect requirements for multiple promoters when mRNAs have 5′ sequences which share complementary overlap with others; this also is reasonably common with HSV-1. The present case, however, is potentially very interesting since the upstream promoters appear to be late acting, whereas those downstream are early. Thus, this region of the viral DNA may be of value for our in vitro and in vivo studies on HSV-1 promoter structure and function.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Anderson, K., R. Frink, G. Devi, B. Gaylord, R. Costa, and E. Wagner.** 1981. Detailed characterization of the mRNA mapping in the *Hin*dIII fragment K region of the HSV-1 genome. J. Virol. **37**:1011–1027.
2. **Bailey, J. M., and N. Davidson.** 1976. Methylmercury as a reversible denaturing agent for agarose gel electrophoresis. Anal. Biochem. **70**:75–85.
3. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell **12**:721–732.
4. **Costa, R., G. Devi, K. Anderson, B. Gaylord, and E. Wagner.** 1981. Characterization of a major late HSV-1 mRNA. J. Virol. **38**:483–496.
5. **Denhardt, D. T.** 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
6. **Docherty, J. J., J. H. Subak-Sharpe, and C. M. Preston.** 1981. Identification of a virus specific polypeptide associated with a transforming fragment (*Bgl*II-N) of herpes simplex virus type 2 DNA. J. Virol. **40**:126–132.
7. **Enquist, L., M. Madden, P. Schiop-Stansly, and G. Vande Woude.** 1979. Cloning of herpes simplex type 1 DNA fragments in a bacteriophage lambda vector. Science **203**:541–544.
8. **Frink, R. J., K. P. Anderson, and E. K. Wagner.** 1981. Herpes simplex virus type 1 *Hin*dIII fragment L encodes spliced and complementary mRNA species. J. Virol. **39**:559–572.
9. **Frink, R. J., K. G. Draper, and E. K. Wagner.** 1981. Uninfected cell polymerase efficiently transcribes early but not late herpes simplex virus type 1 mRNA. Proc. Natl. Acad. Sci. U.S.A. **78**:6139–6143.
10. **Holland, L. E., K. P. Anderson, C. Shipman, Jr., and E. K. Wagner.** 1980. Viral DNA synthesis is required for the efficient expression of specific herpes simplex virus type 1 mRNA species. Virology **101**:10–24.
11. **Jones, P. C., and B. Roizman.** 1979. Regulation of herpesvirus macromolecular synthesis. VIII. The transcription program consists of three phases during which both extent of transcription and accumulation of RNA in the cytoplasm are regulated. J. Virol. **31**:299–314.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
13. **Maxam, A, and W. Gilbert.** 1980. Sequencing end labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–559.
14. **McKnight, S. L., E. R. Gavis, and R. Kingsbury.** 1981. Analysis of transcriptional regulatory signals of the HSV thymidine kinase gene: identification of an upstream control region. Cell **25**:385–398.
15. **McMaster, G. K., and G. C. Carmichael.** 1977. Analysis of single and double stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc. Natl. Acad. Sci. U.S.A. **74**:4835–4838.
16. **Palmiter, R. D.** 1974. Mg^{++} precipitation of ribonucleoprotein complexes. Expedient techniques for the isolation of undegraded polysomes and messenger ribonucleic acid. Biochemistry **13**:3606–3614.
17. **Roizman, B.** 1979. The structure and isomerization of herpes simplex virus genomes. Cell **16**:481–494.
18. **Spear, P., and B. Roizman.** 1980. Herpes simplex virus, p. 615–746. *In* J. Tooze (ed.), Molecular biology of tumor viruses, 2nd ed., Part 2: DNA tumor viruses. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
19. **Stringer, J., L. Holland, R. Swanstrom, K. Pivo, and E. Wagner.** 1977. Quantitation of herpes simplex virus type 1 RNA in infected HeLa cells. J. Virol. **21**:889–901.
20. **Wellauer, P. K., and I. B. Dawid.** 1973. Secondary structure maps of RNA: processing of HeLa ribosomal RNA. Proc. Natl. Acad. Sci. U.S.A. **70**:2827–2831.