Transcription from the *Bam*HI J Fragment of Herpes Simplex Virus Type 1 (KOS)

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RNA transfer experiments (Northern analyses) were used to localize polyadenylated mRNA species made after herpes simplex virus type 1 infection to *Eco*RI and *Bam*HI fragments and subfragments from the short unique region of the herpes simplex virus type 1 (KOS) genome. Three predominant early mRNAs of 2.5, 1.3, and 0.9 kilobases map in the *Bam*HI J fragment. A detailed restriction map of the *Bam*HI J fragment was constructed.

Infection of permissive cells by herpes simplex virus type 1 (HSV-1) is followed by the sequential appearance of various classes of mRNAs (reviewed in reference 24). The five immediate-early (IE) mRNAs have been well characterized; two of these, IE4 and IE5, map partially in the short unique (*U_s*) region (Fig. 1) (1, 10, 28). At later times after infection, several different-sized mRNA species, of both the early and late classes, were localized to fragments from the *U_s* region (2, 7, 10). In these earlier studies, polyribosomal RNA was size fractionated by electrophoresis on methyl mercury-agarose gels and hybridized to Southern blots of HSV-1 (KOS) DNA restriction fragments (10).

We used blot hybridization (Northern analysis) of infected cell mRNA probed with ³²P-labeled HSV-1 DNA fragments to map more precisely the size classes and locations of the mRNAs transcribed from the *U_s* region. We concentrated on the *Bam*HI J fragment for several reasons. Mapping of intertypic recombinants between HSV-1 and HSV-2 indicated that the gene for glycoprotein D is located in the *U_s* region (13, 21). Second, Lee et al. (12) recently provided evidence that the genes for glycoprotein D (gD) and gE are contained within the *Bam*HI J fragment: a 2.7-kilobase (kb) *Sac*I subfragment of the *Bam*HI J fragment of HSV-1 strain F (Fig. 1) selected an infected cell mRNA that could be translated in vitro into a 50,000-molecular-weight non-glycosylated form of gD.

(A preliminary report of this research was presented at the American Society for Virology Meeting, Cornell University, Ithaca, N.Y., in August 1982.)

Most of our studies on HSV-1-infected cell mRNAs have utilized HSV-1 (KOS) (26), and we therefore obtained from M. Levine (9) a

clone carrying the *Eco*RI H fragment of HSV-1 KOS in plasmid pBR322. We subcloned the 6.2-kb *Bam*HI J fragment from the *Eco*RI H plasmid; to facilitate our analysis of the gD region of the *Bam*HI J fragment, we further subcloned a 3.4-kb *Sma*I fragment (which totally contains the 2.7-kb *Sac*I fragment used by Lee et al. [12]) into the unique *Sma*I site of plasmid pACYC177 (5). Strains carrying other *Bam*HI fragments from the *U_s* region of HSV-1 strain F cloned in pBR322 were obtained from the laboratory of B. Roizman (17).

The restriction map for the *Bam*HI J fragment (Fig. 2) was derived from two sets of experiments. First, complete digestion of the HSV-1 recombinant plasmids indicated the number of restriction sites within the cloned fragments as well as sizes of the fragments produced by each enzyme. Second, partial restriction digests of uniquely end-labeled fragments of *Bam*HI-J localized the restriction sites relative to the label; fragments labeled at the *Hind*III site as well as the *Bam*HI sites were used in these digestions. Our map for the *Bam*HI J fragment (Fig. 2) confirms the location of *Hind*III, *Hpa*I, and *Sac*I sites located earlier by other workers (2, 12). However, we found two *Pvu*II sites separated by roughly 500 base pairs, rather than the one site reported for HSV-1 (strain 17) (6).

Before concentrating on the transcripts from the *Bam*HI J region, we used the large *Eco*RI H plasmid to examine transcripts from the *U_s* region. ³²P-labeled *Eco*RI H plasmid was hybridized to an RNA transfer (Northern) blot of polyadenylated [poly(A)] mRNAs taken at various times after infection of the transformed human cell line 293-31 with HSV-1 (KOS) (25, 26; R. M. Stenberg, Ph.D. thesis, University of Pennsylvania, Philadelphia, 1981). The results

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FIG. 1. HSV-1 U_s region and mRNA transcripts. The U_s region of the HSV-1 genome is shown in P orientation, with the internal and terminal repeats shaded. The *EcoRI* and *BamHI* fragments of HSV-1 (KOS) or HSV-1 (F) cloned in pBR322 are indicated; map locations are taken from the data of Anderson et al. (1) and Post et al. (17). The several small *BamHI* fragments (<0.5 kb) identified by Preston (18) are not shown. The immediate-early mRNAs are shown (1, 10, 27), as are the locations of the gD and gE genes (12). The *KpnI* and *HindIII* sites indicated in the *BamHI* N and J fragments, respectively, define subfragments used in this study.

Not cut by *Bgl II*, *Eco RI*, *Kpn I*, *Sal I*, *Xba I*, *Xho I*

FIG. 2. Restriction map of the *BamHI* J fragment. Analyses of restriction fragments on 1% agarose or 3.5 or 5% polyacrylamide gels were as described previously (22, 23); we used *SstI*, which is an isoschizomer of *SacI* used by Lee et al. (12). Labeling of DNA fragments by polynucleotide kinase and [γ - ^{32}P]ATP after alkaline phosphatase treatment was as described by Maxam and Gilbert (14). DNA fragments used as size markers were as follows: *EcoRI*-cleaved plasmids pBR322 (4) and pBR325 (3), *HinfI* and *HaeIII* digests of pBR322 (27), and *EcoRI* and *HindIII* digests of the *EcoRI* A fragment of HSV-1 (HF) in plasmid pBR325.

FIG. 3. Time-course Northern analyses of cytoplasmic poly(A) mRNA to fragments from the U_s region. Cytoplasmic poly(A) and nuclear poly(A) mRNA was extracted from HSV-1 (KOS)-infected 293-31 cells as previously described (26). Electrophoresis of glyoxal-treated mRNA on agarose gels, transfer to diazobenzylxymethyl paper, and hybridization conditions have been described previously (Stenberg, Ph.D. thesis). Size markers were glyoxal-treated DNA fragments of simian virus 40 plus hybridized transcripts of adenovirus from the IB region (Stenberg, Ph.D. thesis). The *Bam*HI fragments N (0.87 to 0.90 map units) and J (0.90 to 0.94 map units) in plasmid pBR322 were labeled by nick translation to 10^8 cpm/ μ g by using *Escherichia coli* DNA polymerase I and [α - 32 P]dCTP (20). The *Kpn*I-*Bam*HI (0.89 to 0.90 map units) segment of the *Bam*HI N fragment was gel purified before end labeling (14). Approximately 10^7 cpm was applied to each hybridization reaction.

showed that, as expected, mRNAs of roughly 1.8 kb appeared within 2 h postinfection and continued to increase through the 4-h period of infection (data not shown). Later, several mRNAs were seen ranging from 0.9 to 5.2 kb, with major bands appearing at 2.55 and 1.5 kb. The size classes of the nuclear poly(A) mRNA and cytoplasmic poly(A) mRNA were very similar, with no very large poly(A) messages being present in the nucleus (data not shown).

The mRNAs were further analyzed with *Bam*HI fragments that subdivide the unique short region into smaller segments. Figure 3 shows the hybridization of several of these *Bam*HI fragments to a cytoplasmic poly(A) mRNA transfer (Northern). The entire *Bam*HI N fragment (0.87 to 0.90 map units) hybridizes predominantly to an 1.8-kb mRNA that presumably codes for the immediate-early 68,000-molecular-weight protein (15, 19). Several less abundant mRNA species appear later after infection, whereas the 1.8-kb mRNA is still abundant from 3 to 4 h. Because of the reported location of the IE4 mRNA in *Bam*HI-N (1, 27, 28), the 1.6-kb *Kpn*I-*Bam*HI subfragment (0.89 to 0.90 map units) proximal to *Bam*HI-J should not contain sequences complementary to the IE4 mRNA. However, this subfragment did hybridize to a 1.8-kb mRNA and nearly as well

to several other mRNAs (Fig. 3). The 1.8-kb mRNA hybridizing to the *Kpn*I subfragment required a longer exposure for its detection and may be of much lower abundance than the IE4 mRNA. With the *Bam*HI J fragment as a probe in the Northern analyses, three predominant early mRNAs with sizes of 2.5, 1.3, and 0.9 kb were found from 3 h onward. Minor species of 1.0 and 5.2 kb also hybridized to the *Bam*HI J fragment.

In a complementary experiment, the various classes of HSV-1 mRNA were examined by using cultures blocked by specific drugs at different stages in the replication cycle. Poly(A) cytoplasmic mRNA was extracted from HSV-1-infected cells treated with either cyclohexamide (to limit HSV-1 transcription to the immediate-early mRNA class) or phosphonoacetic acid (to block DNA replication and appearance of late mRNAs). The mRNAs extracted from these cells were denatured with glyoxal, electrophoresed on agarose, and transferred to diazobenzylxymethyl paper; various DNA probes from the U_s region were hybridized to the transferred RNAs (Fig. 4). The *Bam*HI N fragment hybridized to an immediate-early mRNA of 1.8 kb (CH tract) and to early mRNAs of 0.5 to 1.2 kb. The 1.9-kb portion of the *Bam*HI J fragment (0.90 to 0.91 map units) adjacent to *Bam*HI-N failed to

FIG. 4. Northern analysis of RNAs from specifically blocked cultures of infected cells. Conditions of cycloheximide (CH) and phosphonoacetic acid (PAA) treatment of cultures for isolation of poly(A) mRNA were as described by Stenberg and Pizer (26). The *Bam*HI N fragment (0.87 to 0.90 map units) in pBR322 was labeled by nick translation; the *Bam*HI J fragment was restricted with *Hind*III, and the separate fragments (0.90 to 0.91 and 0.91 to 0.94 map units) were gel purified and labeled by nick translation. Again, roughly 10^7 cpm of probe was used for each hybridization. Size markers were glyoxal-treated fragments of simian virus 40 DNA.

hybridize to immediate-early mRNAs, but did hybridize to a predominant mRNA species of 0.9 kb and to minor species ranging from 0.6 to 2.3 kb. The large (4.3-kb) *Hind*III-*Bam*HI J subfragment (0.91 to 0.94 map units) hybridized predominantly to a 1.35-kb mRNA, and weakly to 2.45-, 1.1-, and 0.97-kb mRNAs plus several minor species. In a separate experiment, the 2.7-kb *Sac*I fragment from the center of *Bam*HI-J hybridized primarily to 2.5- and 1.3-kb mRNAs.

The sizes of all of the mRNA species detected by these Northern analyses are summarized in Table 1 (the major species are in boldface type). There is good agreement between the major size classes of poly(A) cytoplasmic mRNAs hybridizing to a given fragment, as determined by the two different experiments, i.e., time course and specifically blocked cultures. The fact that the 1.8-kb immediate-early mRNA was the major RNA species from the *Bam*HI N and X fragments served as a control on our procedures; three early species of 2.5, 1.3, and 0.9 kb were the most abundant transcripts from the *Bam*HI J fragment. In the case of two adjacent fragments

hybridizing to mRNAs of equivalent size, it is tempting to assign that mRNA a position overlapping both fragments; this is true for the 1.1- and 1.3-kb mRNAs from the *Bam*HI J and X fragments. Several transcripts can be positioned over each DNA fragment, and we calculate that there are too many messages to be uniquely encoded by each fragment. We conclude that, as has been found for other regions of the HSV genome, the short unique segment encodes multiple transcripts. How these RNAs are related to each other is unknown.

We have confirmed and extended the observations of Holland et al. (10) on the location of early mRNA transcripts to the U_s region of HSV-1 (KOS) and have refined the location of mRNAs to the *Bam*HI N and J fragments through use of subfragments of these DNA segments. The 2.5- and 1.3-kb mRNAs that are the predominant species hybridizing to the *Bam*HI J fragment probably correspond to the 2.8- and 1.5-kb mRNAs previously mapped in the *Hind*III G fragment (10). We also detected several additional mRNAs of 0.6 to 5.2 kb hybridizing to the *Bam*HI J fragment. We, like Holland et al. (10), found a few (major) early mRNA species at 2 h postinfection, but we found at least five mRNAs hybridizing to each fragment by 3 to 4 h postinfection or in phosphonoacetic acid-treated cells. These differences are minor and could be due to the host cell used for infection, since HSV infection in 293-31 cells is rapid, with DNA synthesis detected between 2 and 5 h, structural proteins detected between 3 and 5 h, and progeny virions detected by 6 h (25, 26).

For the *Bam*HI N fragment, two different mRNAs of roughly 1.8 kb were found: the abundant immediate-early mRNA (IE4) and a nonoverlapping, much less abundant species mapping nearer the *Bam*HI J fragment. Because multiple mRNAs of nearly the same size can be transcribed from different segments of one restriction fragment, the use of small subfragments is of great value in the RNA mapping. As was done for each segment by Frink et al. (8) for the *Hind*III L and K fragments of the U_L region, analyses of the 5' and 3' ends of the messages are needed to precisely define the mRNA localizations.

As to which of the messages from the *Bam*HI J fragment we detected is the gD message, the 2.5-kb mRNA is the most likely. Viral proteins produced in the presence of tunicamycin, which blocks glycosylation (16), or by in vitro translation of mRNA (11, 12) indicate a size of approximately 50,000 molecular weight for the non-glycosylated derivative of gD. This protein would have about 400 amino acids and hence require at least a 1,200-nucleotide message, not including

TABLE 1. Summary of in vivo transcripts from U_s region

Fragment	Map units	Sizes of mRNAs (kb) at time after infection:				Sizes of mRNAs (kb) in cells treated with CH, PAA, or no drug ^a
		1 to 4 h	2 to 4 h	3 to 4 h	3 h only	
<i>Eco</i> RI-H	0.88–0.97	1.8^b	2.5	1.5, 1.3 , 1.0, 0.9	5.2	
<i>Bam</i> HI-N	0.87–0.90	1.8	2.5, 2.3	1.3, 1.0		1.8 , 1.1, 1.0, 0.8, 0.6
<i>Bam</i> HI-J	0.90–0.945		2.5	1.3 , 1.0, 0.9	5.2	
<i>Bam</i> HI-J– <i>Hind</i> III	0.90–0.91					2.4, 1.8, 1.5, 0.9 , 0.8, 0.6
<i>Bam</i> HI-J– <i>Hind</i> III	0.91–0.94					2.5 , 2.1, 1.8, 1.3 , 1.1, 1.0, 0.8, 0.6
<i>Bam</i> HI-X	0.94–0.96					2.5, 1.8 , ^c 1.3, 1.1, 1.0, 0.8, 0.6

^a CH, cycloheximide; PAA, phosphonoacetic acid.

^b Boldface type indicates predominant species.

^c Species made in the presence of cyclohexamide.

the roughly 200-nucleotide poly(A) tract, effectively eliminating the 1.3-kb message as a candidate. Nuclease S1 and *Exo*VII mapping data (Ikura and Pizer, manuscript in preparation) indicate that the start of transcription of the 2.5-kb mRNA is close to the *Hind*III site in the *Bam*HI J fragment (Fig. 2). Furthermore, this mRNA is unspliced and truncates near the downstream *Sac*I site (Ikura and Pizer, in preparation) for a calculated size of at least 2.0 kb for the transcribed region of the gD gene.

After these studies were completed, we learned of the sequencing of a 1.6-kb region of the *Bam*HI J fragment of HSV-1 (Patton) containing the gD gene by Watson and colleagues (30). The restriction sites on *Bam*HI-J (KOS) that we found agree very well with those predicted from the published sequence of the gD region of the *Bam*HI J fragment. Watson et al. (30) cite unpublished results that a 3.0-kb mRNA is the gD message. We saw no 3.0-kb mRNA hybridizing to *Bam*HI-J (KOS), but rather a 2.5-kb mRNA, a difference in molecular weight that may be strain dependent. We are currently sequencing the DNA on either side of the *Hind*III site in the *Bam*HI J fragment of HSV-1 (KOS) to compare the promoter sequence and translation signals of this strain with those reported for the Patton strain (30).

This research was supported by Public Health Service grants AI14592 and AI17873 from the National Institutes of Health. J.L.B. was the recipient of Public Health Service postdoctoral fellowship AI07209, and T.M.H. was the recipient of a Public Health Service predoctoral trainee grant, both from the National Institutes of Health.

We thank Rich Stenberg for preparing the time course mRNAs from HSV-1-infected cells and carrying out the electrophoresis separations. Also, we thank Dave Hare for helpful discussions.

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