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Regulation of Herpes Simplex Virus Gene Transcription In Vitro

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We used partially purified RNA polymerase II from uninfected (Pol II) and from herpes simplex virus type 1 (HSV-1) infected HEp-2 cells (Pol II-H) to transcribe HSV-1 DNA in vitro. Gel electrophoretic analysis of the products produced from native HSV-1 DNA yielded weight average chain lengths of 4.0 and 3.5 kb for the Pol II and Pol II-H products, respectively. Blot hybridization analyses of the HSV DNA transcripts showed that both enzymes transcribed RNA from essentially all regions of the genome. However, Pol II preferentially transcribed regions coding for the immediate-early or alpha mRNAs, whereas Pol II-H preferentially copied regions coding for the early (β) and late (γ) gene products. Transcriptional analyses of the cloned HSV-1 Bam HI-Q fragment (containing the thymidine kinase (TK) gene) and its subfragments showed that (1) the major transcripts produced by Pol II-H were distinctly different from those produced by Pol II; (2) Pol II and Pol II-H utilized different promoters for the synthesis of major transcripts; (3) both enzymes produced three minor transcripts that were partially overlapping and in opposite direction to the TK gene; and (4) only Pol II-H initiated transcription from the TK promoter. In contrast, both Pol II and Pol II-H generated an identical set of transcripts from an adenovirus 2 early region DNA fragment. The sizes of the products suggest that RNA processing may be occurring in vitro. These results show that HSV-1 infection alters the in vitro transcriptional specificity of RNA polymerase II and demonstrate that this system should be useful for studying in vitro the regulation of gene transcription.

Key words: in vitro transcription, HSV-1, regulation, RNA polymerase II

Recently, two soluble RNA polymerase II systems have been described that give accurate transcription of eukaryotic genes in vitro [1,2]. The system of Weil et al [1] contains a crude cellular extract (S-100) and purified RNA polymerase II from KB cells, whereas the preparation of Manley et al [2] consists of a concentrated whole-cell extract from HeLa cells. Although both of these preparations have been shown to transcribe RNA accurately and selectively from a variety of eukaryotic and viral gene promoters [eg 1–6], generally they have failed to exhibit the transcriptional regulation

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that is observed in vivo [eg 4,7–9]. Thus these systems appear to contain general transcriptional control factors, but lack demonstrable tissue or viral specific transcriptional control activities in vitro.

Herpes simplex virus type-1 (HSV-1) infected cells provide a useful system for studying transcriptional regulation in vitro. Although viral transcription is mediated by the host cell RNA polymerase II throughout the infectious cycle [10,11], the synthesis of (HSV-1) RNA is coordinately and sequentially regulated so as to produce three major classes—the immediate-early (α), early (β), and late (γ) mRNAs [12–16].

Several lines of evidence indicate that a positive transcriptional control mechanism activates the switch from immediate-early to early gene expression. Experiments of Leung [17] suggested that an immediate-early protein synthesized by thymidine kinase minus virus could act in "trans" to activate transcription of the early class thymidine kinase gene of a superinfecting virus. Studies by Preston [18,19], using a temperature sensitive mutant of HSV-1 (tsK) that synthesizes mainly immediate-early gene products at the nonpermissive temperature, indicated that the immediate-early polypeptide affected by the tsK lesion is required for transcription of the thymidine kinase gene and other early and late genes. Subsequently Watson and Clements [20] showed by temperature shift experiments that the protein affected by the tsK lesion is required continuously for the synthesis of early and late mRNAs.

To study the transcriptional regulation of herpes simplex virus, we have developed a partially purified RNA polymerase II system from both uininfected (Pol II) and HSV-1 infected (Pol II-H) HEp-2 cells. The purification of this enzyme system and some of its general transcriptional properties have been described elsewhere [21].

In this communication we describe some of the transcriptional differences between these two RNA polymerase II preparations using both whole and cloned fragments of HSV-1 DNA. With whole viral DNA as template we have found that Pol II, in contrast to Pol II-H, shows a marked preference for transcribing immediate-early gene regions. Furthermore, using the cloned Bam HI-Q fragment of HSV-1 DNA (containing the viral thymidine kinase, TK, gene), we have shown that the two enzyme preparations produce different major transcripts, with only Pol II-H able to initiate synthesis from the TK gene promoter. These results show that several features of in vivo transcriptional regulation of (HSV-1) gene expression are preserved in this in vitro system.

MATERIALS AND METHODS

Purification of RNA Polymerase II

RNA polymerase II was prepared from uninfected HEp-2 cells or HEp-2 cells infected with HSV-1(F) at 10 pfu/cell and harvested 8 hr postinfection. RNA polymerases were solubilized by sonication in hypertonic medium, fractionated by chromatography on DEAE-cellulose as described previously [21], and stored in 50% glycerol (v/v) at $-70°C$.

Isolation of HSV DNA

Whole HSV DNA was prepared from purified virus as described previously [21,22]. HSV DNA for Southern blot analysis was prepared by digestion with restriction endonuclease Sal I, fractionation by electrophoresis through a horizontal 1% agarose gel, and transfer of fragments to nitrocellulose filters [23] as described elsewhere [21].

Preparation of DNA Restriction Fragments

The EcoRI C fragment of Ad2 DNA was a generous gift of Dr. Stephen Zimmer (University of Kentucky). Plasmid pTK5, containing the HSV-1 Ban HI Q fragment inserted into pBR322, was obtained from Dr. Saul Silverstein (Columbia U.) and was prepared and digested with restriction as described previously [21]. In some cases, DNA fragments were purified by electrophoresis through a preparative 1% agarose gel, electroelution of the fragments, and precipitation with ethanol. The fragments were then extracted twice with phenol-chloroform-isoamyl alcohol (50:48:2) and twice precipitated with ethanol. The precipitated DNA was rinsed with 80% ethanol, dried in vacuo, and suspended in 10 mM Tris·HCl (pH 8.0) and 1 mM EDTA at about 500 μg/ml.

In Vitro Transcription and Purification of RNA

Transcription was performed in a total volume of 50 μl containing 50 mM Tris·HCl (pH 8.0), 7 mM$MgCl_2$, 120 mM KCl, 0.6 mM each of ATP, GTP, and UTP, and 50 μM [α-^{32}P] CTP at 5–10 μCi/nmole (New England Nuclear), 50 μg/ml DNA and 20 μl of RNA polymerase II containing 56–140 units of enzyme [21]. Transcription mixtures were incubated at 37°C for 60 min and terminated by chilling on ice. RNA was purified by extraction with phenol-chloroform-isoamyl alcohol, DNase treatment for 5 min at 37°C and ethanol precipitation as described previously [21]. RNA was denatured with glyoxal as described by McMaster and Carmichael [24] and analyzed by electrophoresis through 1.6% agarose (Bethesda Research Laboratories, Rockville, Maryland) at 50 V for 12 hr unless otherwise specified. The gels were dried by heating in vacuo on Whatman 3 MM paper and were exposed to X-ray film (Kodak XRP-1) at −70°C with an intensifying screen (Quanta III, E.I. Dupont De Nemours and Co).

RESULTS

Size Analysis of RNA Synthesized From HSV DNA by Pol II and Pol II-H

To determine the size of RNA synthesized in vitro from HSV DNA, RNA was isolated as described in the Materials and Methods, denatured with glyoxal, and analyzed by electrophoresis through an agarose gel. A densitometer scan of the autoradiograph of the gel and the position of rRNA size standards are shown in Figure 1. These results demonstrate that both Pol II and Pol II-H synthesize high molecular weight RNA ranging in size from about 600 to greater than 5,000 nucleotides. When synthesis was carried out in the presence of alpha-amanitin (1 μg/ml), no RNA was produced. From these data we have calculated weight average chain lengths of 4,000 and 3,500 nucleotides for Pol II and Pol II-H products, respectively. These values are strikingly close to the average UV target sizes of the immediate early (4,070 base pairs) and early (3,480 base pairs) transcription units as calculated from the data of Millette and Klaiber [25].

Hybridization Analysis of RNA Synthesized From HSV DNA by Pol II and Pol II-H

To determine if Pol II and Pol II-H show differences in transcriptional selectivity on HSV DNA, RNA products generated in vitro by the two enzymes were hybridized to Sal I restriction fragments of HSV DNA immobilized on nitrocellulose filters. RNA synthesized in the presence of a-amanitin, ^{32}P-labeled nick-translated HSV

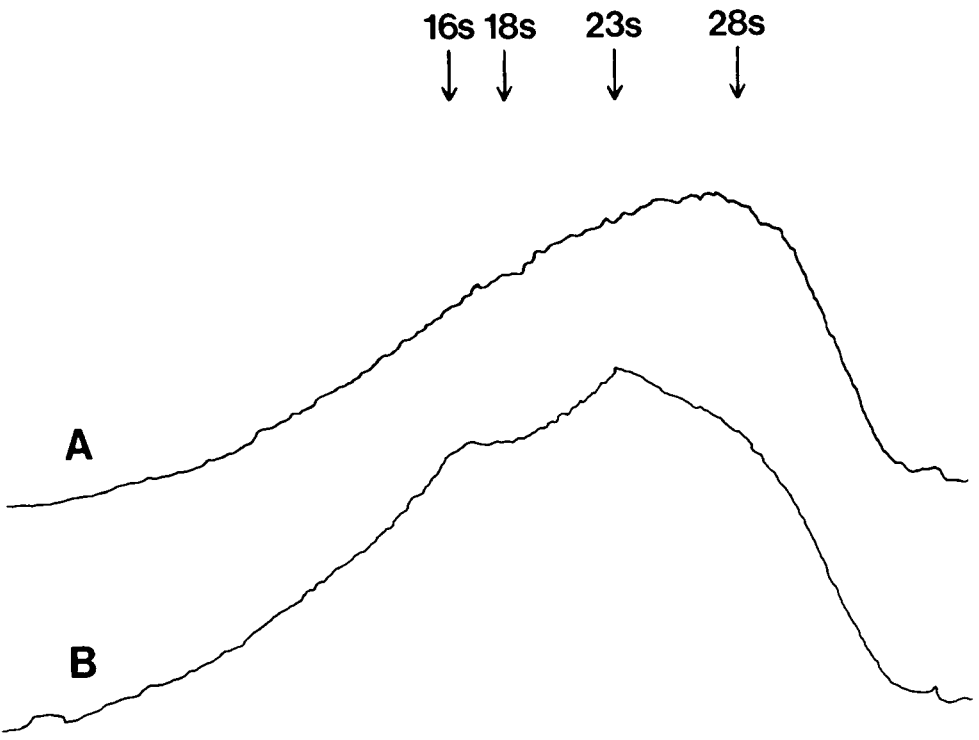

Fig. 1. Electrophoretic analysis of RNA transcribed in vitro from HSV-1 DNA by Pol II and Pol II-H. Transcription mixtures were prepared and incubated as described in Materials and Methods. RNA products were extracted, denatured, and electrophoresed through a 1.6% agarose gel. The figure shows densitometer tracings of an autoradiogram from the individual gel tracts containing RNA transcribed in vitro by Pol II (A) and Pol II-H (B). The position of 28S, 23S, 18S, and 16S ribosomal RNAs in the gel are indicated by arrows. Weight average chain lengths were calculated from the electrophoretic mobility relative to the ribosonal RNA markers.

DNA, and ^{32}P-labeled HEp-2 cell RNA served as controls. Shown in Figure 2 are the densitometer tracings of the blot hybridizations and the Sal I restriction map of HSV-1(F). Pol II-H (Fig. 2B) transcribed all regions of the HSV DNA and the hybridization pattern is similar to that produced by ^{32}P-labeled HSV DNA (Fig. 2A). However, there appears to be some preferential transcription of RNA homologous to Sal I fragments r, s, t, and u (Table I). This is in general agreement with the mapping data of Clements et al [14] and Jones and Roizman [13] that showed that RNA present at early and late times during HSV infection hybridized to all regions of the HSV genome but with more abundant transcripts mapping approximately at coordinates 0.1–0.42 and 0.60–0.83.

When the transcription of HSV DNA was carried out with Pol II, the RNA produced again hybridized to all regions of the HSV genome (Figure 2C). However, regions corresponding to Sal I fragments a, j_1, j_2, and k were transcribed preferentially. Quantitation of the level of hybridization within these regions relative to the

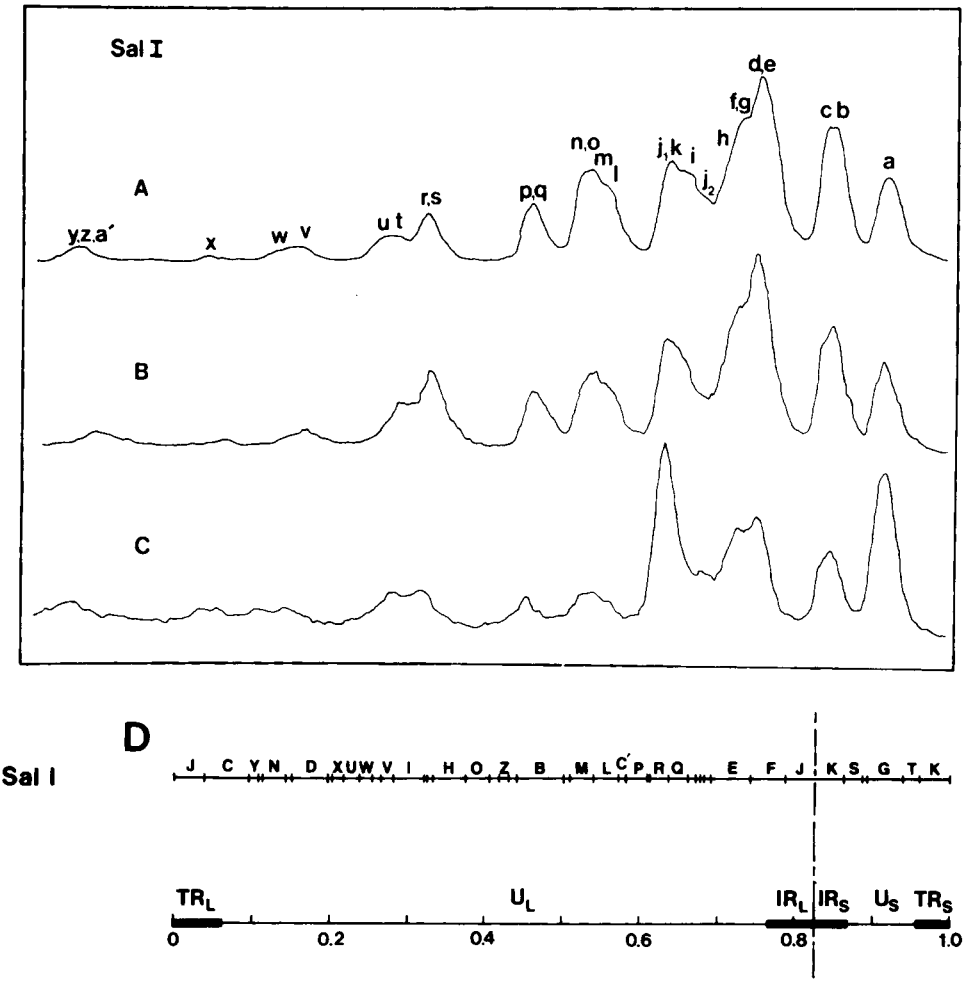

Fig. 2. Blot hybridization analysis of RNA transcribed in vitro from HSV DNA by Pol II and Pol II-H. RNA was transcribed in vitro as described in Materials and Methods. RNA products were extracted and hybridized to Sal I restriction fragments of HSV-1 DNA immobilized on nitrocellulose strips. The densitometer tracing of the resulting autoradiogram shows the hybridization patterns for (A) ^{32}P-labeled nick-translated HSV-1 DNA, (B) ^{32}P-RNA transcribed by Pol II-H and (C) ^{32}P-RNA transcribed by Pol II. The input radioactivities were 58,400, 38,000, and 33,200 cpm, respectively. The position of the Sal I restriction fragments are indicated by letters a–z. The Sal I restriction map for HSV-1 DNA, from Locker and Frenkel [36], is shown in Figure 2D. Fragment a, the IL_L–IR_S junction fragment, is equal in size to fragments j+k.

HSV DNA hybridization control revealed a two-fold greater transcription from these regions (Table I). We have also consistently observed a small amount of preferential transcription from the region of Sal I fragment t. The genomic regions showing enhanced transcription map primarily in the internal (IR_L, IR_S) and terminal (TR_L, TR_S) repeat units, and adjacent regions. Several investigators [26,27] have shown that these are the regions that contain the immediate-early genes.

TABLE I. Quantitation of Hybridization to Sal I Restriction Fragments of HSV-1 DNA

Sal I fragment	Total radioactivity hybridized			Ratio of radioactivity hybridized	
	Pol II-H products (%)	Pol II products (%)	^{32}P-HSV DNA	Pol II-H/DNA	Pol II/DNA
a	8.9 ± 1.2	18.7 ± 1.0	7.6 ± 0.6	1.17	2.46
b, c	13.2 ± 1.0	9.4 ± 0.6	12.7 ± 2.1	1.04	0.77
c, e	14.0 ± 2.4	9.7 ± 1.1	15.1 ± 1.4	0.93	0.64
f, g, h	15.3 ± 0.1	11.9 ± 2.0	16.1 ± 1.0	0.95	0.74
j_2, i, j_1, k	14.6 ± 2.1	28.4 ± 1.4	14.3 ± 0.3	1.02	1.99
l, m, n, o	12.0 ± 0.4	7.3 ± 0.5	14.5 ± 0.7	0.83	0.50
p, q	6.4 ± 0.3	3.0 ± 0.7	6.2 ± 1.5	1.03	0.48
r, s	7.7 ± 0.9	4.3 ± 0.3	5.3 ± 0.7	1.50	0.81
t, u	4.7 ± 0.9	4.1 ± 0.2	3.3 ± 0.2	1.50	1.24
v, w	1.4 ± 0.8	1.6 ± 0.2	2.5 ± 0.9	0.56	0.64
x	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.0	1.00	1.25
y, z, a	1.4 ± 0.7	1.1 ± 0.5	2.0 ± 0.7	0.70	0.55

RNA polymerase products from HSV DNA transcription and nick-translated ^{32}P-HSV DNA were hybridized to blots of Sal I restricted HSV-1 DNA as described in Experimental Procedures. Values shown are the percentage of total radioactivity hybridized and are given as the mean ± standard deviation for two autoradiograms of two separate hybridizations.

These findings are in general agreement with the results of hybridization studies of Clements et al [14] and Jones and Roizman [13] that showed that RNA, which accumulates in infected cells in the absence of protein synthesis, is derived mainly from the internal and terminal repeats regions. From these results we conclude that Pol II, in contrast to Pol II-H, transcribes with greater preference the immediate-early regions of the HSV genome.

Transcription of the Cloned HSV Bam Fragment Containing the Viral Thymidine Kinase Gene

To obtain more definitive evidence for differences in transcriptional specificity of the polymerase preparations, we used the (HSV-1) Bam HI Q fragment (containing the viral thymidine kinase gene) cloned in pBR322 (pTK5) as a template for in vitro transcription. The DNA sequence of much of this fragment has been determined recently [28,29]. Plasmid pTK5 was cleaved with Bam HI and transcribed in vitro with Pol II and Pol II-H. The RNA products were analyzed after denaturation with glyoxal by electrophoresis in a 1.6% agarose gel (Fig. 3). From this template Pol II generated major transcripts of 2.12 and 1.53 kb (Fig. 7, lanes 2–4). In addition, minor species of 1.19, 1.03, and 0.63 kb were occasionally observed. (We will refer to the Pol II transcripts as RNAs 2.12, 1.53, 1.19, 1.03, and 0.63.) Low concentrations of alpha-amanitin (1 μg/ml) added to the reaction mixture appeared to not completely inhibit production of the major RNAs (lane 5).

Transcription of pTK5 by Pol II-H resulted in the production of a completely different set of major transcripts having sizes of 2.84, 1.62, and 1.47 kb (Fig. 7, lanes 6–8). These will be subsequently referred to as RNAs 2.84, 1.62, and 1.47. RNA 2.84 has the correct size for a run-off transcript from the TK gene promoter. The two predominant larger bands are the result of end-to-end transcription of the plasmid

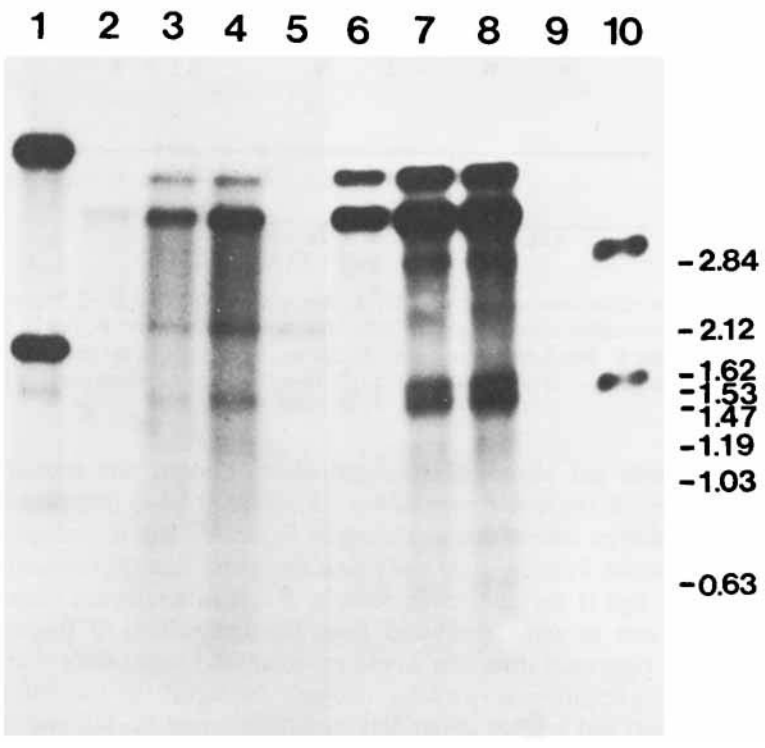

Fig. 3. Electrophoretic analysis of transcripts produced from the Bam HI-Q fragment of HSV-1 DNA. Transcription conditions and product analysis are described in Materials and Methods but with reaction mixtures containing 50 μg/ml pTK5 cleaved with Bam HI and various amounts of Pol II or Pol II-H. Lanes 2-5 show products of Pol II, lanes 6-9 products of Pol II-H using 20 units (lanes 2 and 6), 40 units (lanes 3 and 7), 60 units (lanes 4 and 8), and 60 units of enzyme plus 1 μg/ml alpha-amanitin (lanes 5 and 9). Lanes 1 and 10 contain marker HEp-2 cell and E coli ribosomal RNAs.

fragments. It should be noted that the minor species observed with Pol II are also present in RNA generated by Pol II-H (Fig. 3, lanes 6–8). When transcription was performed in the presence of alpha-amanitin, the production of all Pol II-H transcripts was fully inhibited (lane 9).

As shown in Figure 3, the pattern of RNAs produced by both enzyme preparations is independent of enzyme concentration in the range of 20 to 60 units per 50 μl reaction. The patterns remain unchanged even with 140 units of polymerase (data not shown). No RNA was produced in the absence of DNA, and varying the DNA concentration from 25 to 100 μl/ml produced no changes in the transcript patterns of relative band intensities (data not shown). These results demonstrate that the infected cell RNA polymerase II (Pol II-H) differs markedly from the uninfected cell enzyme (Pol II) in its ability to transcribe the Bam HI-Q fragment and in the products produced from this cloned fragment of HSV-1 DNA.

Truncated Template Mapping of Pol II Products

In order to map the in vitro transcripts produced by Pol II, we subcut the Bam Q fragment with Bgl II, EcoRI or Sma I, purified the appropriate subfragments by

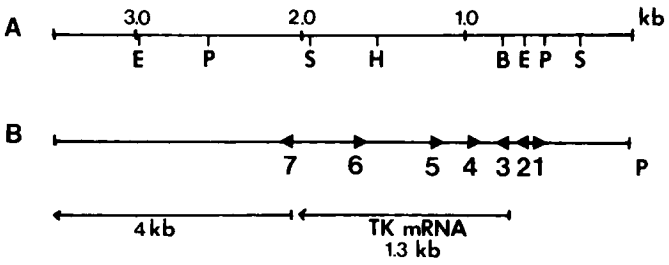

Fig. 4. Map of restriction sites and potential RNA start sites on the Bam HI-Q fragment of HSV-1 DNA. (A) Map of restriction enzyme cleavage sites. The symbols represent: B, Bgl II; E, EcoRI; P, Pvu II; H, Hinf I; and S, Sma I restriction sites. (B) Shows the location of the major in vivo mRNAs and possible RNA polymerase II initiation sites (P1–P7) based on DNA sequence analysis [30].

preparative agarose gel electrophoresis and electroelution, and transcribed these fragments in vitro. A restriction map of the 3.5 kbp Bam HI-Q fragment of (HSV-1) DNA in the prototype orientation is shown in Figure 4. Bgl II cleaves at one site about 770 nucleotides from the right end of the fragment. Run-off transcripts initiated to the left of the Bgl II site and transcribed by Pol II in a leftward direction would have the same size as those produced from the intact Bam Q fragment. Those synthesized in a rightward direction would be about 770 nucleotides shorter. EcoRI cleaves the Bam Q fragment at two sites, one near the Bgl II site (ca. 640 nucleotides from the right end) and another about 500 nucleotides from the left end. Both Bgl II and EcoRI cleave within 80 nucleotides of the TK mRNA start site and therefore might inactivate this promoter. To test for initiation at the TK promoter, we used the isolated 1.63 kbp Sma I fragment extending from nucleotide 314 to nucleotide 1937. This fragment includes 409 nucleotides 5′ and 1214 nucleotides 3′ to the TK and mRNA start site.

From the Bgl II cleaved Bam Q fragment Pol II produced transcripts having sizes of 1.53, 1.35, 0.76, 0.61, 0.47, and 0.30 kb (Fig. 5A, lane 3). With the internal 2.4 kbp EcoRI fragment transcripts of approximately 1.35, 0.97, 0.57, and 0.35 kb were produced (Fig. 5A and B, lanes 4 and 2 respectively). With the isolated 1.6 kbp Sma I fragment we observed RNA products of about 0.90 and 0.70 kb but no RNAs large enough to have originated from the TK promoter (Fig. 5C, lane 1).

RNA 2.12 was not synthesized with the Bgl II cleaved template, suggesting that it is a rightward reading transcript initiated near 2.1 on the TK map. If this were true, a transcript of 1.36 kb should be produced from the Bgl II cleaved template. As shown in Figure 5A, a transcript of 1.35 kb is synthesized. Cleavage with EcoRI, which cuts near the Bgl II site, did not shift the mobility of this transcript significantly (Fig. 5A, lane 4). We, therefore, conclude that RNA 2.12 is initiated at 2.1 on the TK map and is transcribed in the rightward direction.

Another transcript produced from the Bgl II cleaved Bam HI-Q fragment comigrates with RNA 1.53. This suggests that this RNA may be transcribed in a leftward direction from position 2.0 on the TK map. Therefore, a transcript of about 1.0 kb should be synthesized from the EcoRI template. We observed, in fact, a transcript of approximately 0.97 kb (Fig. 5B, lane 2) with this template. Therefore, we conclude that RNA 1.53 is initiated near 2.0 (P7) on the TK map and is transcribed in a leftward direction.

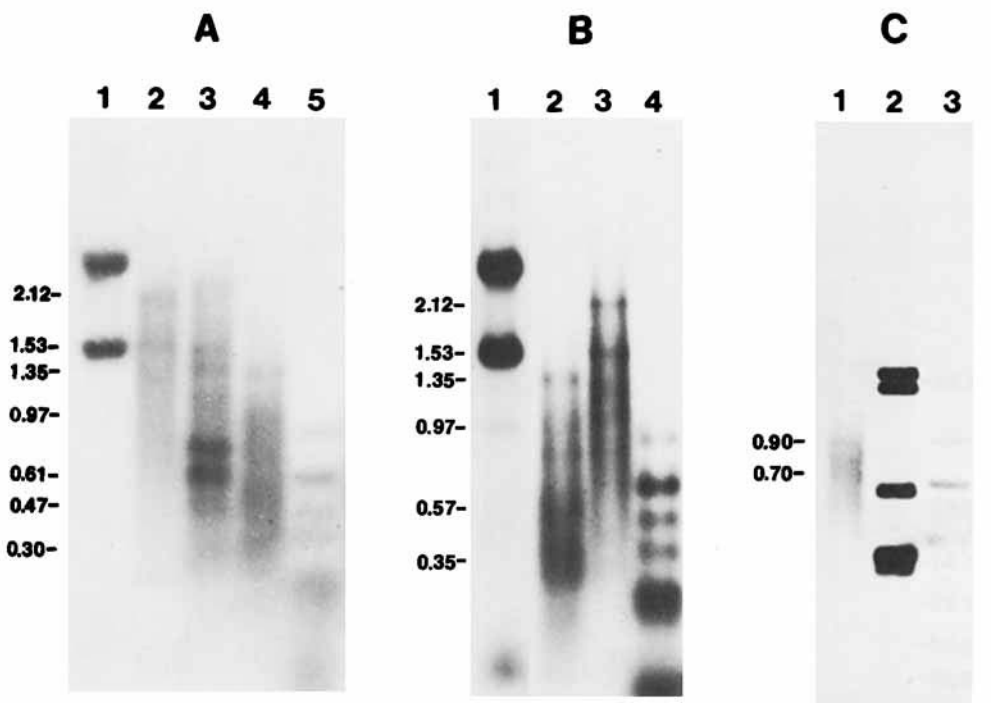

Fig. 5. Pol II transcription of truncated templates. RNA was synthesized in transcription mixtures containing 94 units Pol II and 50 μg/ml of the DNAs as follows: (A) Bam HI Q fragment (lane 2), Bam HI Q fragment cleaved with Bgl II (lane 3), 2.4 kbp EcoRI fragment, (lane 4). The markers were E coli ribosomal RNAs (lane 1) and pBR322 cleaved with Alu I (lane 5). Electrophoresis was performed for 4 hr at 100 V. (B) The DNA templates were 2.4 kbp EcoRI fragment (lane 2), Bam HI Q fragment (lane 3). The markers were E coli ribosomal RNAs (lane 1) and PBr322 cleaved with Alu I (lane 4). (C) The DNA template was the 1.6 kbp Sma I fragment (lane 1) and the markers were pBR322 cleaved with Taq I (lane 2) and Alu I (lane 3). Electrophoresis was performed for 3 hr at 100 V.

Two of the minor transcripts, RNAs 1.19 and 1.03, were not synthesized by Pol II from the 2.7 kbp Bgl II template (Fig. 5A, lane 3). This suggests that these RNAs might be initiated from P5 and P4, respectively, and transcribed in a rightward direction. Pol II initiation at P5 should result in transcripts of 0.88, 0.55, and 0.42 produced from the Sma I, EcoRI, and Bgl II templates, respectively. As shown in Figure 5, Pol II yields an RNA of 0.9 kb from the Sma I template, (Fig. 5C, lane 1) one of 0.57 kb from the EcoRI fragment (Fig. 5B, lane 2), and one of 0.47 from the Bgl II template (Fig. 5A, lane 3). This is consistent with initiation at P5. Similarly, initiation at P4 should yield RNAs of 0.65 and 0.33 kb from the Sma I and EcoRI templates, respectively. We observed RNAs of 0.7 and 0.35 in the products of these templates, (Fig. 5C, lane 1; Fig. 5B, lane 2) indicating that Pol II initiates at P4.

Initiation at P1 would explain the origin of the 0.63 minor transcript. This is supported by the synthesis of a 0.61 kb RNA from the Bgl II cleaved Bam HI-Q fragment (Fig. 5A, lane 3), and a band of 0.3 kb produced by Pol II from the Sma I fragment (latter data not shown). A summary of the mapping of the Pol II products is presented in Figure 7.

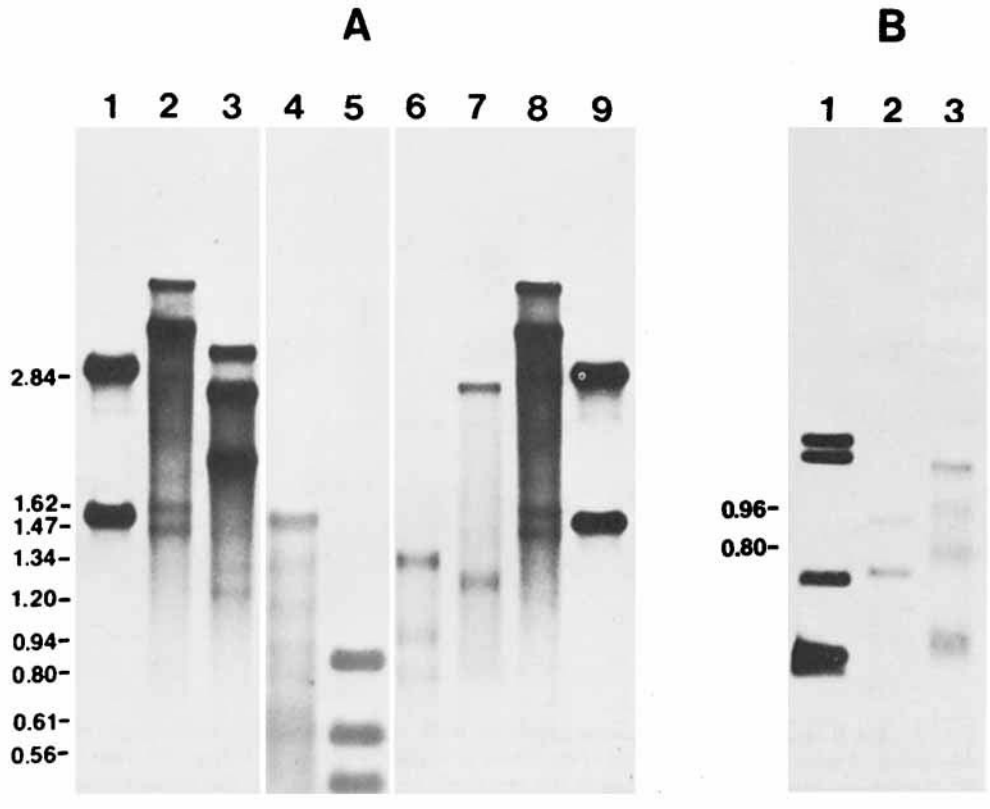

Fig. 6. Pol II-H transcription of truncated templates. Transcription mixtures containing 60 units Pol II-H and 50 μg/ml of DNA were prepared and incubated as described in Materials and Methods. (A) The DNA templates were pTK5 cleaved with Bam HI (lane 2), pTK5 cleaved with Pvu II (lane 3), 1.6 kbp Sma I fragment (lane 4), 2.4 kbp EcoRI fragment (lane 6), 2.7 kbp Bgl II fragment (lane 7), pTK5 cleaved with Bam HI (lane 8). The markers were E coli ribosomal RNAs (lanes 1 and 9), pBR322 cleaved with Alu I (lane 5). (B) The template was the 1.6 kbp Sma I fragment cleaved with Hinf I (lane 3). The markers and electrophoresis conditions were the same as described in the legend to Figure 5C.

Truncated Template Mapping of Pol II-H Products

In order to map the transcripts produced by Pol II-H from the Bam HI Q fragment, we followed the same approach used in the mapping of Pol II transcripts. Electrophoretic analysis of the products produced from various truncated templates (Fig. 6) may be summarized as follows: Pol II-H produced transcripts of 1.22 kb and 0.86 kb from the isolated 2.7 kbp Bgl II fragment (Fig. 6A, lane 7), transcripts of 1.34, 0.94, 0.80, and 0.56 kb from the 2.4 kbp EcoRI fragment (lane 6); transcripts of 1.34, 1.18, 0.96, and 0.71 kb from the 1.6 kbp Sma I fragment (lane 4); and transcripts of about 1.81, 1.47, 1.30, and 1.23 kb from the Pvu II cleaved pTK5 (lane 3).

First, it should be noted that, with the exception of an end to end transcript of circa 2.7 kb, none of the transcripts synthesized by Pol II-H from the Bgl II cleaved template comigrated with those produced from the Bam HI-Q fragment (Fig. 6A,

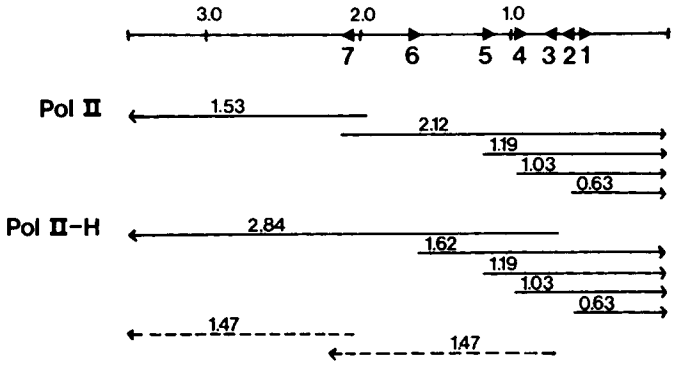

Fig. 7. Mapping of Pol II and Pol II-H in vitro transcripts on the Bam HI-Q fragment. RNA start sites are designated by black triangles, P1 through P7, on the HSV-1 Bam HI-Q fragment map. P3 coincides with the TK mRNA promoter. In vitro transcripts are designated by solid arrows; the dashed arrow shows tentative mapping for RNA 1.47.

lane 7). This suggested that most of the RNAs produced from Bam HI-Q were transcribed in a rightward direction. However, if RNA 2.84 were transcribed in a rightward direction, a transcript of 2.04 kb should be synthesized from the Bgl II template. This was not observed (lane 7). Therefore, RNA 2.84 must have been initiated from a site near 0.76 (ie, the TK promoter, P3) and be transcribed in a leftward direction. If this were correct a transcript of 1.2 kb should be produced from the Sma I template. As shown in Figure 6 (lane 4), a transcript of 1.18 kb is produced from this fragment. We further cleaved the Sma I fragment with Hinf I that cleaves about 810 nucleotides from the TK mRNA site. With this template a transcript of approximately 0.80 kb is produced (Fig. 6B, lane 3). We, therefore, conclude that RNA 2.84 is initiated at or near the TK promoter (P2).

If RNA 1.62 were a rightward reading transcript from P6, cleavage with Sma I, Pvu II, EcoRI C, and Bgl II should generate transcripts of 1.38, 1.18, 1.01, and 0.89 kg, respectively. With these templates transcripts of 1.42, 1.20, 0.94, and 0.86 kb are observed (Fig. 6). Therefore, we conclude that RNA 1.62 initiates at, or near, P6 and is transcribed in a rightward direction.

If RNA 1.47 were a leftward reading transcript initiated near P7, a transcript of 1.47 kb should be seen in the products from the Bgl II fragment. This, however, was not observed (Fig. 6, lane 7). If it were a rightward reading transcript, RNAs of 0.70 and 0.95 kb should be observed with the Bgl II and Pvu II cleaved fragments, respectively. However, these, too, were not observed. Therefore, the origin of this RNA is not clear. Pvu II cleaves pTK5 200 nucleotides upstream from the TK mRNA start site and about 540 nucleotides downstream from the Poly (A) site. It is therefore of interest that a transcript of about 1.47 is produced from this template. This would suggest that RNA 1.47 might arise from initiation at P2, transcription leftward past the poly (A) site and termination near 2.2 on the TK map. This possibility is being investigated.

The mapping of the minor transcript (RNAs 1.19, 1.03, and 0.63) produced by Pol II-H may be summarized as follows: No run-off transcripts having the sizes of these RNAs were observed with the isolated 2.7 kbp Bgl II fragment (Fig. 6A, lane

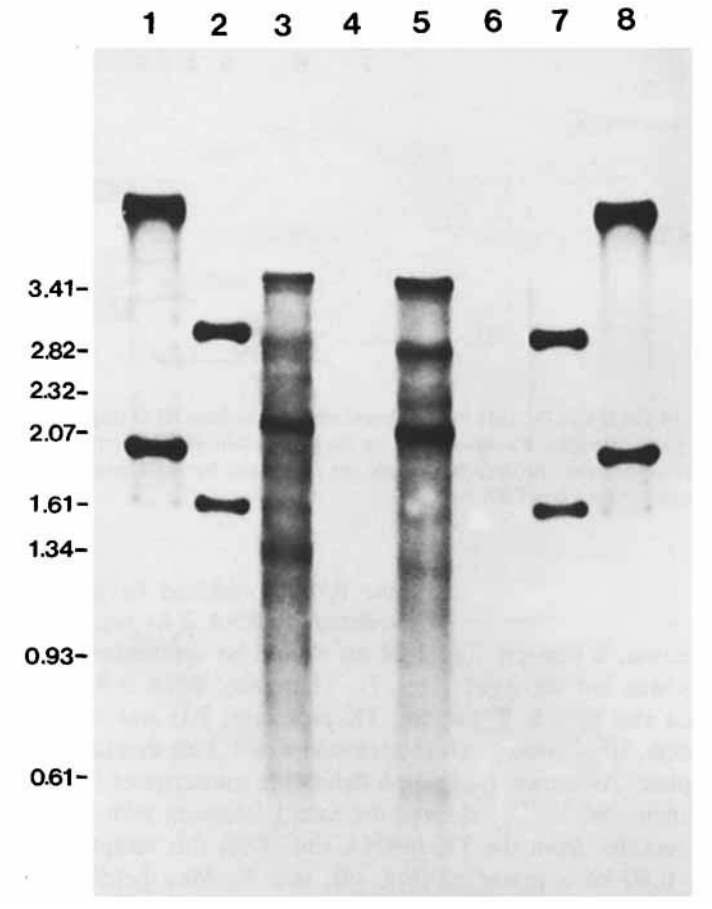

Fig. 8. Comparison of Pol II and Pol II-H transcripts from the Ad2 early region IV. Transcription mixtures containing 84 units Pol II or 34 units Pol II-H and 50 μg/ml of the Ad2 EcoRI C fragment in a total volume of 25 ml were prepared as described in Materials and Methods. The gel tracts contained RNA transcribed by Pol II (lane 3), Pol II in the presence of 1 μg/ml alpha-amanitin (lane 4), Pol II-H (lane 5), and Pol II-H in the presence of 1 μg/ml alpha-amanitin (lane 6). The markers were HEp-2 cell ribosomal RNAs (lanes 1 and 8), E coli ribosomal RNAs (lanes 2 and 7).

7). However, consistent with initiation at P5 and P4, transcripts of 0.94 and 0.65 were produced from the isolated 1.6 kbp Sma I fragment (lane 4) and a transcript of 0.55 kb was transcribed from the 2.4 kbp EcoRI fragment. Since Pol II and Pol II-H both produced the same sized minor transcripts from the intact Bam HI-Q and from the truncated templates, we conclude that both preparations initiate transcription at or near the same minor promoters within the Bam HI-Q fragment (ie, P5, P4, and P1). The mapping of the Pol II-H transcripts is summarized in Figure 7.

Transcription of the Ad2 DNA EcoRI C Fragment Containing Early Region IV

To determine if Pol II and Pol II-H show transcriptional differences on an unrelated template, we used the EcoRI C fragment of adenovirus 2 (Ad2) DNA containing early region IV as a template. From mapping studies on the in vivo RNAs,

this region has been shown to contain one promoter and one poly(A) site and give rise to a family of about six mRNAs by differential splicing of the primary transcript [31,32]. From this template both Pol II and Pol II-H generate a similar pattern of transcripts. This consists of major RNAs of 3.41, 2.82, and 2.07 kb and several minor ones of 2.32, 1.61, 1.34, and 0.93 kb (Fig. 8, lanes 3 and 5). One of these, the 3.41 kb RNA, is of the size predicted for a run-off transcript from the EIV promoter [33], and the sizes of many of the products agree with those of spliced RNAs originating from this region in vivo [31–33]. Furthermore, all of the transcripts produced by Pol II and Pol II-H are completely inhibited by alpha-amanitin (1 μg/ml). These results indicate that the EcoRI fragment of Ad2 DNA, as opposed to the Bam HI-Q fragment of HSV-1 DNA, functions equally well as a template for both Pol II and Pol II-H in vitro.

DISCUSSION

In this report, we have utilized an in vitro transcription system, consisting of partially purified RNA polymerase II from HSV-1 infected (Pol II-H) and uninfected (Pol II) HEp-2 cells, to study the transcription of both whole and cloned fragments of HSV-1 DNA. The results presented show that this system preserves several of the features of HSV transcriptional regulation that are observed in vivo.

Evidence for Transcriptional Selectivity on Whole HSV-1 DNA

Analysis of transcripts produced from whole HSV-1 DNA demonstrated that both enzyme preparations generate high molecular weight RNA products. Moreover, the weight average size of the Pol II and Pol II-H products (4.00 and 3.50 kb, respectively) was found to be very close to the average size of the immediate-early (α) and early (β) viral transcription units, respectively, as calculated from our previous UV mapping data [25].

Southern blot hybridization analysis of the in vitro products transcribed from HSV DNA showed that the transcripts produced by the uninfected cell enzyme (Pol II) hybridized preferentially to fragments derived from the repeat regions of the genome, regions coding chiefly for the immediate-early gene products [26,27]. The products of the infected cell enzyme (Pol II-H), on the other hand, hybridized to all regions of the genome with some slight preference for certain portions in the unique long (U_L) regions. This pattern is consistent with observations on in vivo early (β) and late (γ) RNA transcription.

Although preferential transcription of the immediate-early sequences by Pol II was not as great as would be predicted from the in vivo transcription data of Clements [14], the blot hybridization pattern of our in vitro Pol II products agrees more closely with the hybridization data of Jones and Roizman [13]. Their results showed that immediate early (α) nuclear RNA hybridized to all fragments of HSV DNA analyzed, but with some preferential hybridization to sequences including the repeat regions.

Evidence for Transcriptional Regulation In Vitro Using the Cloned Thymidine Kinase Gene Fragment

In studies on the transcription of the cloned HSV-1 DNA fragment containing the early TK gene of HSV-1, we showed that Pol II-H generates major transcripts that are completely different from those produced by Pol II. By transcription of

truncated templates, the major transcripts produced by these two enzymes were shown to arise by initiation at different sites within the Bam HI Q fragment. Most importantly, only Pol II-H, the enzyme from infected cells, was shown to initiate with appreciable frequency at the TK gene promoter thus generating the expected sizes of run-off transcripts from truncated templates. Pol II, on the other hand, failed to produce detectable levels of RNA from the TK promoter. These results are compatible with what is known about TK gene regulation in productively infected cells. Thus TK transcription requires the prior synthesis of at least one immediate early gene product, $V_{mw}175$ or ICP4 [17–20]. Our findings are consistent with the interpretation that viral infection alters the specificity of RNA polymerase II allowing it to initiate transcription from the early (β) TK promoter.

The major Pol II transcripts (RNAs 2.12 and 1.53) were not completely inhibited by alpha-amanitin (1 μg/ml). This suggests that the RNAs synthesized by the Pol II preparation might be in part products of the small amount of alpha-amanitin resistent polymerase activity present in the preparation. Since these transcripts are fully inhibited with α-amanitin at 100 μg/ml, they could be RNA polymerase III products. Several in vitro studies have shown that RNA polymerase III sometimes generates transcripts from DNA fragments thought to contain only class II genes [2,34]. More interestingly, these results suggest that RNA polymerase II from uninfected cells (Pol II) per se may not efficiently utilize the promoter sites within the Bam HI-Q fragment.

Transcription of Adenovirus Early Region IV

Both Pol II and Pol II-H yield nearly identical transcript patterns from the EcoRI C fragment of Ad2 DNA containing early region IV. One of these (3.4 kb) has the expected size for a run-off transcript from the in vivo EIV promoter [31,33]. It is of interest that one major transcript (2.82 kb) has the correct size for a transcript initiated at the EIV promoter and terminated or processed at the Poly(A) addition site at 91.3 map units. The sizes of several of the other RNAs agree closely in size with the family of spliced, polyadenylated in vivo RNAs as determined by Chow et al [31] and Berk and Sharp [32].

Transcriptional Control of HSV-1 Gene Expression

At present, the mechanism of transcriptional control in HSV-1 infected cells is not clear. However, it is certain that the product of at least one viral gene, that coding for viral polypeptide $V_{mw}175$ (ICP4) is involved in the activation of transcription of the early (β) genes of HSV-1. Although the results presented here do not disclose the mechanism of this transcriptional regulation, they show that viral infection alters the in vitro specificity of RNA polymerase II allowing it to initiate transcription from the early TK gene promoter. The availability and transcriptional features of the partially purified RNA polymerase II system described here should now make it possible to identify and characterize the transcriptional control factors involved in the switch from immediate-early to early and late gene expression of HSV-1.

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NOTE ADDED IN PROOF

Read and Summers [30] recently published results on the in vitro transcription of the Bam HI-Q fragment of HSV-1 DNA using a Manley-type [2] system from uninfected cells. Our results with Pol II agree with theirs in the mapping of the three minor rightward reading transcripts and the leftward reading 1.5 kb RNA. The results differ in that 1) we find no evidence for tk gene transcription by the uninfected cell enzyme; and 2) we find a 2.12 kb rightward reading transcript initiated at approximately 2.1 kb on the Bam HI-Q map.