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Temporal regulation of herpes simplex virus type 2 transcription and characterization of virus immediate early mRNA's

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ABSTRACT

Nuclear and cytoplasmic virus RNAs, synthesized in cells infected with herpes simplex virus type 2 at early and late times post-infection, and in the continuous presence of the protein synthesis inhibitor cycloheximide (immediate early), have been analyzed by blot hybridization to virus DNA fragments generated by Bam HI and Eco RI restriction endonucleases. Polyadenylated immediate early mRNAs were separated on denaturing gels containing CH_3HgOH giving three virus-specific mRNA bands of estimated sizes 4.7, 3.4 and 1.75 kb, and these have been mapped to five discrete regions of the genome. The polypeptides produced by *in vitro* translation of the HSV-2 immediate early mRNA's have been identified. Orientations of immediate early mRNA's on the virus genome have been determined by mapping cDNAs complementary to the 3'termini of the mRNAs.

INTRODUCTION

Herpes simplex viruses comprise two distinct serotypes (HSV-1 and HSV-2), each with a linear duplex DNA genome of about 150 kbp(1, 2). The HSV-1 and HSV-2 genomes show approximately 50% cross homology (3, 41), and have a similar complex structure (4, 5). The two serotypes can be easily distinguished by the characteristically different DNA fragment patterns generated by cleavage with various restriction endonucleases (6, 7, 42).

At least some of the HSV-1 and HSV-2 gene products are functionally interchangeable, as intertypic complementation has been observed (8). Furthermore, exchange of genetic material has been shown by production of intertypic recombinants containing genomes, parts of which are derived from each parent (9, 10, 11, 12, 13, 23). Analysis of intertypic

recombinants allowed a correlation of the genetic and physical maps of the two serotypes, and demonstrated the colinearity of equivalent genes on the two DNAs (42).

Both HSV-1 and HSV-2 have been shown to be capable of inducing the biochemical and morphological transformation of cells cultured *in vitro* (24, 25, 26, 27, 28, 40, I. Cameron and J.C.M. Macnab, personal communication). HSV-2 also has been implicated in the aetiology of human cervical carcinoma (14, 29, 30).

Hence the evidence available indicates close similarities between HSV-1 and HSV-2 in terms of the molecular organization, regulation and expression of their genomes.

Previous studies on HSV transcription have been concerned primarily with HSV-1, and have shown that transcription is subject to temporal regulation (31, 33, 34). Inhibitors of virus protein synthesis, or infection of cells at the non-permissive temperature with certain HSV-1 temperature-sensitive mutants, resulted in accumulation of immediate early (IE) RNA which hybridized only to certain restricted regions of the genome (33, 35, 36). By contrast, at both early and late times (before and after the onset of viral DNA replication) the virus transcripts mapped throughout the genome (33). At least one virus gene product is involved in effecting the switch from the restricted IE stage of transcription (37).

On the basis of liquid hybridization studies, Frenkel *et al* (15) have proposed a radically different transcriptional programme for HSV-2 as compared to HSV-1. The present communication investigates the transcriptional programme of HSV-2 using the Southern blot procedure (16, 33), and also characterises the HSV-2 IE mRNAs.

MATERIALS AND METHODS

Cells and virus

Baby hamster kidney cells (BHK 21-C13) were grown to confluent monolayers in 2 litre roller bottles, or on 90 mm plastic tissue culture plates as described previously (17, 33), and infected with HSV-2 (strain HG52) at a multiplicity of 50 pfu/cell.

For purification of radioactively labelled RNA, confluent monolayers were incubated for 16 hr prior to infection in phosphate-free Eagles medium containing 2% calf serum. After 1 hr virus absorption, the cells were labelled with 0.25 - 0.75 mCi (^{32}P)-orthophosphate/ml of medium at 37°C. For preparation of IE RNA, monolayers were pre-treated with cycloheximide (200 µg/ml) for 30 min at 37°C prior to infection. Infection and subsequent maintenance of monolayers was carried out in the continous presence of 200 µg/ml cycloheximide. RNA was isolated 6 hr after the virus absorption period.

Cell fractionation and isolation of RNA

Nuclear and cytoplasmic cell fractions were prepared and RNA was isolated essentially as described by Kumar and Lindberg (18). In general, RNA preparations to be hybridized to blot strips were further purified by Cs_2SO_4 equilibrium density centrifugation (33). Nuclear IE RNA was also prepared without Cs_2SO_4 banding, by two cycles of DNAase treatment (20 µg/ml) for 1 hr at 37°C followed each time by Sephadex G-50 filtration. Polyadenylated RNA was selected as described previously (36).

In vitro labelling procedures

Cytoplasmic IE RNA was labelled with (γ-^{32}P)-ATP (3000 Ci/mmol; Radiochemical Centre, Amersham) using polynucleotide kinase (17). HSV-2 DNA was labelled to high specific activity with (α-^{32}P) deoxyribonucleoside triphosphates (300 Ci/mmol) by the method of "nick translation" (19). ^{32}P-labelled HSV-2 complementary DNA (cDNA) was prepared as previously described (17), using an oligo $(dT)_{10}$ primer.

Gel electrophoresis

RNA samples were fractionated on denaturing 1.5% agarose gels containing 5 mM CH_3HgOH as described previously (36). RNA bands were excised from preparative gels, and the gel slices were homogenised in 2 ml formamide. Gel homogenates were adjusted to 50% formamide, 3 x SSC, 1 x Denhardt's solution (0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin), 100 µg/ml yeast RNA, and were incubated overnight at 45°C with shaking prior to hybridization.

In vitro translation

Total cytoplasmic RNA samples were translated in vitro

as described by Preston (20).

Cloning procedures

Restriction endonuclease fragments of HSV-2 DNA (7) were cloned under Category II containment within the Institute of Virology as specified by the U.K. Genetic Manipulation Advisory Group. The cloning vector was pAT 153, and the host bacterium, E.coli K12 HB101 (39).

Blot hybridization procedures

Nitrocellulose blot strips containing HSV-2 DNA fragments generated with various restriction endonucleases were prepared as previously described (33). The strips were pre-incubated overnight at 45°C in 50% formamide, 3 x SSC, 1 x Denhardt's solution containing 100 µg/ml yeast RNA. Hybridizations were performed under the same conditions at 45°C for 48 hr. The blot strips were washed and autoradiographed as described previously (33).

RNA was transferred onto DBM paper according to the method of Alwine et al., (44). The DBM strips were then hybridized and washed as described for the nitrocellulose strips with the addition of 1% glycine in the pre-incubation mixture.

RESULTS

The HSV DNA molecule consists of long unique (U_L) and short unique (U_S) regions, each flanked by inverted repeated sequences (TR_L/IR_L, TR_S/IR_S) joined at IR_L - IR_S (4, 5, 6, 7). The genome also contains a true terminal redundancy, designated the "a" sequence, which is present at the junction of the internal repetitions in one or more copies in different DNA molecules (43). Fragment Bam HI $\bar{g}$ (Fig. 1, tracks 1, 8 and 10) consists of the joint fragment, derived from those DNA molecules in the population which contain two internal "a" sequences. Four genome arrangements, resulting from all of the possible combinations of inversions of the two unique regions, are present in DNA preparations in approximately equal amounts (4, 6). A further consequence of this structure is that a number of fragments produced by digestion with a restriction endonuclease may contain sequences in common (7, 42).

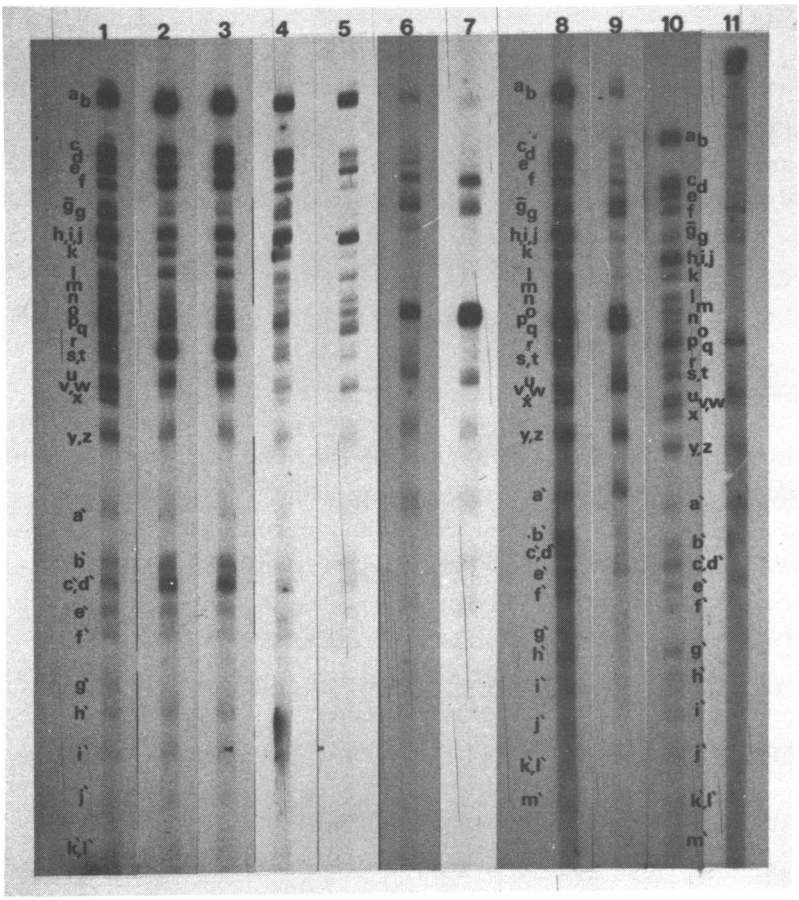


Figure 1: Fluorographs of IE, early and late RNA samples hybridized to the Bam HI fragments of HSV-2 DNA. 1. Nick-translated total HSV-2 DNA the control strip for tracks 2 to 7. 2. Early nuclear RNA. 3. Early cytoplasmic RNA. 4. Late nuclear RNA. 5. Late cytoplasmic RNA. 6. IE nuclear RNA. 7. IE cytoplasmic RNA. 8. Nick-translated total HSV-2 DNA the control stip for track 9. 9. In vitro labelled IE cytoplasmic RNA. 10. Nick-translated DNA the control strip for track 11. 11. Unbanded IE nuclear RNA.

Mapping of HSV-2 transcripts

Nuclear and cytoplasmic RNA samples were isolated from infected cells labelled post-absorption with (^{32}P)-orthophosphate from 0-2 hr (early RNA), 0-10 hr (late RNA), or 0-6 hr in the continuous presence of cycloheximide (IE RNA).

Late RNA

Both nuclear and cytoplasmic late RNA hybridized to all the DNA fragments generated by Bam HI (Fig. 1, tracks 4 and 5). Comparison of the nuclear and cytoplasmic hybridization patterns from this and other experiments revealed consistent differences in the relative amounts of hybridization to several DNA fragments. Late nuclear RNA showed greater hybridization to Bam HI g, f and c, as compared with late cytoplasmic RNA. A similar situation, to a lesser extent, can be seen with Bam HI k, m and x.

Early RNA

As with late RNA, early nuclear and cytoplasmic RNAs hybridized to all the Bam HI fragments (Fig. 1, tracks 2 and 3). No major consistent differences were observed between the hybridization patterns of nuclear and cytoplasmic early RNAs. However, the hybridization patterns of early RNA differed quantitatively from the patterns obtained with late RNA. For example, early RNA showed greater relative hybridization to Bam HI e, f and s t as compared to late RNA, and reduced hybridization could be seen to Bam HI d and m.

Immediate early RNA

As compared to late and early RNA, the pattern of hybridization of nuclear and cytoplasmic IE RNA is severely restricted (Fig. 1, tracks 6, 7 and 9). Some small differences appeared to be present between the nuclear and cytoplasmic RNA samples; there was no apparent hybridization of cytoplasmic RNA to Bam HI e, h i j or k. However, these sequences must be present in cytoplasmic RNA preparations, as they can be detected in RNA selected on oligo-dT cellulose (Fig. 5, track 2), and as minor species in IE cDNA (Fig. 7, track 6).

In order to ensure that the restricted hybridization of IE RNA was not due to preferential labelling in vivo of certain RNA species or to selective fractionation of RNAs, IE cytoplasmic RNA was labelled in vitro with polynucleotide kinase and then hybridized (Fig. 1, track 9). In addition, in vivo ^{32}P-labelled IE nuclear RNA was hybridized without fractionation on Cs_2SO_4 (Fig. 1, track 11). Both hybridization profiles were qualitatively similar to those of IE RNA selected in the normal way

(Figs. 1, tracks 9 and 11).

Summary of RNA mapping data

The RNA mapping data are summarized in Fig. 2. Significant hybridization to a fragment is indicated by a single continuous line, while low levels of hybridization are indicated by a broken line.

Characterization of IE mRNAs

Translation products of HSV-2 IE mRNAs

The HSV-2 IE polypeptides translated *in vitro* have apparent molecular weights of 178 x 10^3, 140 x 10^3, 116 x 10^3 and 65 x 10^3 (Fig. 3). These values are similar, but not identical to those of the equivalent polypeptides found *in vivo*. The *in vivo* labelled HSV-2 polypeptides have molecular weights of 182 x 10^3, 142 x 10^3, 118 x 10^3, 67 x 10^3, 65 x 10^3 and 12.3 x 10^3 (13). The slightly lower molecular weights of some of the polypeptides when translated *in vitro* can be attributed to their absence of normal post-translational modification, as found previously for HSV-1 (20). No *in vitro* translation product equivalent to the 67 x 10^3 polypeptide found *in vivo* was detected, possibly

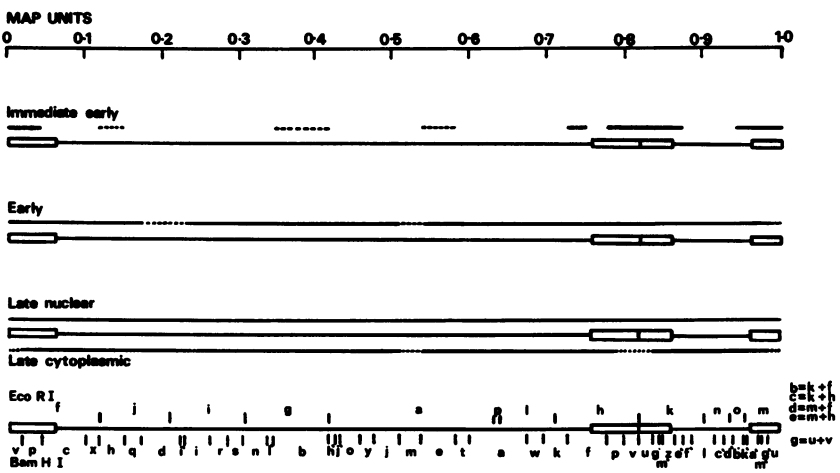


Figure 2: Summary of the RNA mapping data and physical maps for HSV-2 DNA fragments generated by restriction endonucleases Bam HI and Eco RI. The maps are shown for the standard orientation of U_L and U_S (7, 42).

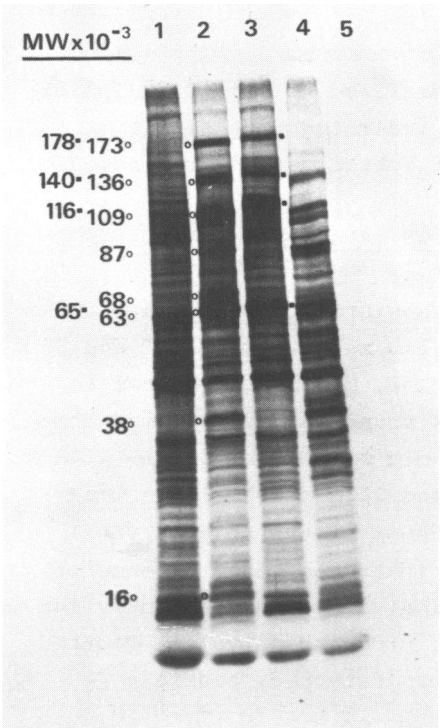


Figure 3: PAGE separation of the in vitro translation products of HSV-2 IE mRNAs. 1. Mock-infected RNA. 2. HSV-1 IE RNA. 3. HSV-2 IE mRNA. 4. HSV-1 late RNA. 5. E.coli rRNA. HSV-1 specific IE polypeptides (o). HSV-2 specific IE polypeptides (■).

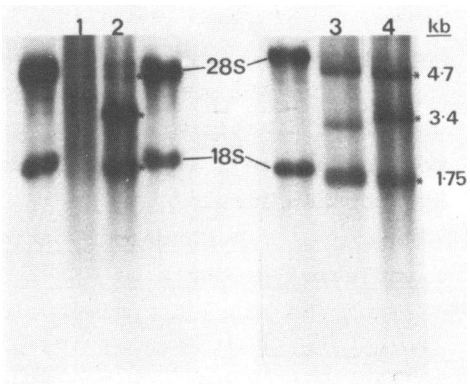


Figure 4: Fluorographs of HSV-1 and HSV-2 mRNAs separated by electrophoresis through CH_3HgOH agarose gels. 1. Mock-infected RNA. 2. HSV-2 IE RNA. 3. HSV-1 IE RNA. 4. HSV-2 IE RNA.

because the unmodified polypeptide comigrates with a host polypeptide band. It is not expected to observe the 12.3 x 10^3 polypeptide, as it would migrate with globin, an inherrent contaminant of the reticulocyte translation system (20).

Sizes of IE mRNAs

Fractionation of ^{32}P-labelled cytoplasmic polyadenylated IE mRNA on denaturing CH_3HgOH agarose gels revealed three bands which were not present in polyadenylated cytoplasmic RNA from mock-infected cells (Fig. 4). Different preparations of poly (A) +RNA contained variable small amounts of 28S ribosomal RNA, and

the relative proportions of the three virus RNA bands varied somewhat (Fig. 4, tracks 2 and 4). These bands had sizes of 4.7 kb, 3.4 kb, and 1.75 kb, consistent with the HSV-1 situation (36), though clear size differences can be seen between the HSV-1 and HSV-2 IE mRNAs (Fig. 4, tracks 3 and 4).

Genome map locations of HSV-2 IE mRNAs

The hybridization pattern of total polyadenylated cytoplasmic IE RNA (Fig. 5, track 2) was similar to that of unselected cytoplasmic IE RNA (Fig. 1, track 6). Individual polyadenylated RNA bands excised from a preparative CH_3HgOH gel were hybridized to blot strips containing the Bam HI fragments (Fig. 5, tracks 3, 4 and 5).

The 4.7 kb RNA band hybridized predominantly to Bam HI g (and $\bar{g}$), u, y z, a', and also to g', and m' (Fig. 5, track 3); some hybridization could also be seen to Bam HI b, e and p. The major hybridization indicates the 4.7 kb mRNA (designated IE mRNA IV) maps within TR_S/IR_S as indicated in Fig. 9.

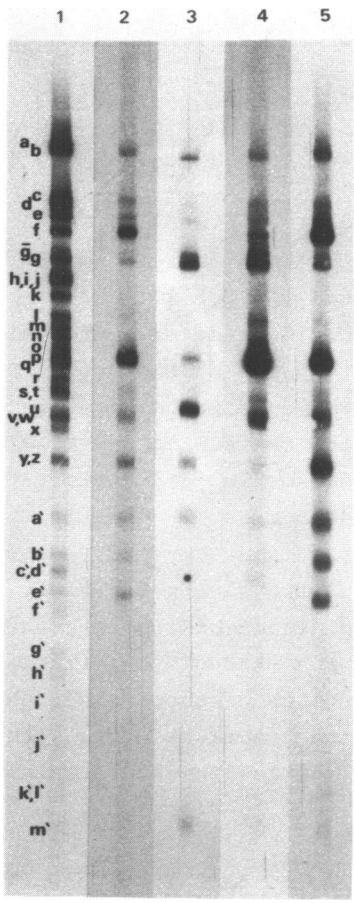
The 3.4 kb RNA band showed strong hybridization to Bam HI g, p, and v w (Fig. 5, track 4), with faint hybridization to Bam HI c and f as well as to certain fragments represented in the larger, 4.7 kb mRNA. Since the region containing Bam HI w is not represented in IE RNA (Fig. 2), and Bam HI p and v are contiguous, this indicates that the 3.4 kb mRNA (IE mRNA I) maps entirely within TR_L/IR_L (Fig. 9).

The 1.75 kb RNA band hybridized strongly to Bam HI f, y z, a', b', e' and k' l', and also to DNA fragments represented in the 3.4 kb mRNA (Fig. 5, track 5). The strong hybridization was to fragments from three distinct regions of the genome; at U_L near the junction with IR_L, and at the junctions of U_S with both IR_S and TR_S. This indicated that the 1.75 kb band contained at least three individual mRNAs (IE mRNAs III, V and VI respectively; Fig. 9).

IE mRNAs IV and V both hybridized to Bam HI z, while IV and VI hybridized to Bam HI a'. This does not necessarily imply that these RNAs overlap, but more likely that the ends of the two relevant mRNAs lie within the Bam HI fragment, and are not separated by a Bam HI cleavage site. The equivalent HSV-1 IE mRNAs which map in these locations do not overlap (36) and these

regions of uncertainty are represented by a discontinuous line in Fig. 9.

The map positions of the major IE mRNA species were confirmed using RNA transferred to DBM paper. Total nick-translated HSV-2 DNA and cloned DNA probes were hybridized to the immobilized RNA. Bam HI g hybridized to both the 4.7 and 3.4 kb RNA bands (Fig. 6, track 2), while Bam HI p hybridized



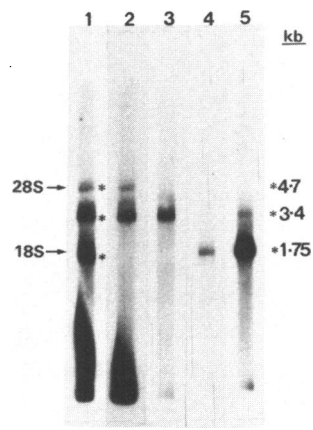


Figure 6: Fluorographs of nick-translated HSV-2 DNA fragments hybridized to IE poly (A)+RNA immobilized on DBM paper. 1. Total HSV-2 DNA. 2. Bam HI g. 3. Bam HI p. 4. Bam HI f. 5. Bam HI f.

Figure 5: Fluorographs of fractionated HSV-2 IE RNA bands hybridized to the Bam HI fragments of HSV-2 DNA. 1. Nick-translated total HSV-2 DNA. 2. Unfractionated cytoplasmic poly (A)+IE RNA. 3. 4.7 kb IE RNA band. 4. 3.4 kb IE RNA band. 5. 1.75 kb IE RNA band.

only to the 3.4 kb mRNA (Fig. 6, track 3). These results, in conjunction with those obtained by hybridizing isolated RNA bands to strips (Fig. 5) confirm the location of the 4.7 kb mRNA in TR_S/IR_S and the 3.4 kb mRNA in TR_L/IR_L. The hybridization observed using the Bam HI f probe confirms the location of a 1.75 kb mRNA at the junction of U_L and IR_L (Fig. 6, track 4). Overexposure of the DBM blot strip hybridized to Bam HI f, showed a small amount of hybridization to the 3.4 kb mRNA (Fig. 6, track 5). This indicates that this mRNA extends a small extent into Bam HI f, and explains the faint hybridization of the 3.4 kb mRNA band to Bam HI f (Fig. 5, track 4). The 3.4 kb mRNA also hybridizes to Bam HI c, as f and c share sequences from TR_L/IR_L (Fig. 2).

Orientation of IE mRNAs

cDNA synthesis initiated on polyadenylated HSV IE RNA using oligo $(dT)_{10}$ as primer results in a product of small size, which consists of sequences complementary to the 3'-termini of mRNAs. Therefore hybridization of this cDNA to blot strips allows these mRNAs to be oriented on the genome (17).

IE cDNA made from polyadenylated cytoplasmic RNA was hybridized to the Bam HI and EcoRI generated DNA fragments. The hybridization patterns of IE cDNA and of in vivo labelled total IE cytoplasmic RNA are shown in Fig. 7.

IE mRNA I There was abundant hybridization to Bam HI p, but much less to Bam HI v w (Fig. 7, track 6). This indicates that the direction of transcription of the 3.4 kb mRNA I in TR_L is rightwards as drawn in Fig. 9. Due to the structure of the HSV-2 genome, IE mRNA I synthesized from the IR_L template would be made on the complementary strand to that used in TR_L, and must therefore be transcribed in a leftwards direction (Fig. 9).

IE mRNA II In total nuclear (Fig. 1, track 6) and polyadenylated cytoplasmic IE RNA (Fig. 5, track 2), hybridization to Bam HI e and s t was observed, and this hybridization is also observed on overexposure with cytoplasmic IE RNA (data not shown) IE cDNA did not hybridize to Bam HI e, but hybridization was detected to Bam HI s t (Fig. 7, track 6). As Bam HI e and t are adjacent on the genome, this indicates that the minor II is transcribed rightwards (Fig. 9).

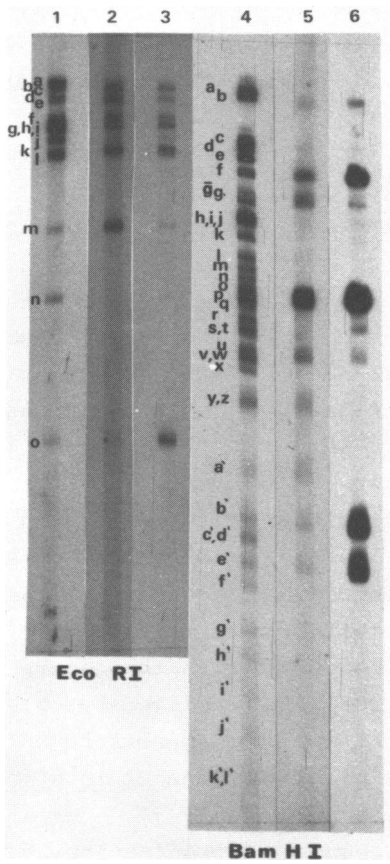


Figure 7: Fluorographs of HSV-2 IE cDNA hybridized to the Bam HI and Eco RI fragments of HSV-2 DNA. 1. Nick-translated total HSV-2 DNA. 2. IE cytoplasmic RNA. 3. IE cDNA. 4. Nick-translated total HSV-2 DNA. 5. IE cytoplasmic RNA. 6. IE cDNA.

IE mRNA III The HSV-2 physical maps available were insufficiently detailed in this region to allow unambiguous orientation of this mRNA. Hence, the Hind III o fragment (0.737 -0.749 map units, Fig. 8) which contains this mRNA was inserted into pAT 153, and the recombinant plasmid was propagated in E.coli HB101. Fine maps of Hind III o were prepared using Pvu II and Sma I restriction endonucleases, and these are shown in Fig. 8. IE cDNA when hybridized to a blot strip containing the DNA fragments generated by a Pvu II/Sma I double-digest showed abundant

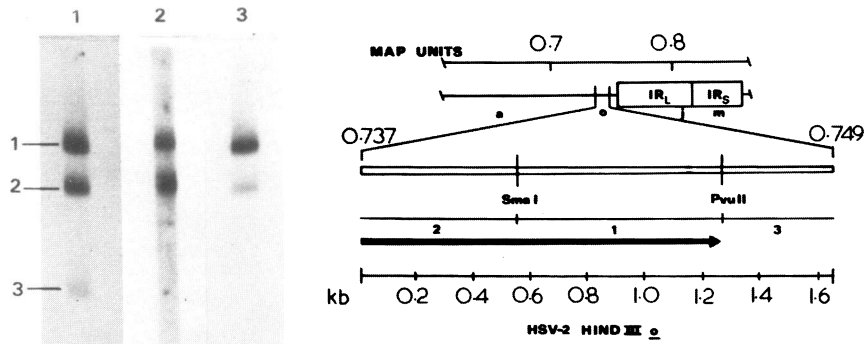


Figure 8: Fluorographs of IE cDNA hybridized to the Pvu II/Sma I fragments of the HSV-2 Hind III o DNA fragment, together with the physical map. 1. Nick-translated total HSV-2 DNA. 2. IE cytoplasmic RNA. 3. IE cDNA.

hybridization to fragment 1, with a small amount to fragment 2. This indicates that IE mRNA III is transcribed rightwards (Figs. 8 and 9).

IE mRNA IV IE cDNA hybridized to Bam HI u, but not to Bam HI g' or z (Fig. 7, track 6). This indicates that the direction of transcription of the 4.7 kb IE mRNA in IR_S is leftwards as shown in Fig. 9. Due to the structure of the HSV-2 genome, IE mRNA IV transcribed from the TR_S template is made from the complementary DNA strand, and therefore in a righwards direction (Fig. 9).

IE mRNA V IE cDNA hybridized to Bam HI e', but not to Bam HI z, (Fig. 7, track 6). Hence, IE mRNA V is transcribed rightwards from IR_S which serves as a template for IE mRNA IV (Fig. 9).

IE mRNA VI IE cDNA hybridized to Bam HI b', but not to Bam HI k' or a', and there was also abundant hybridization to Eco RI o, with little to Eco RI m (Fig. 7). The direction of transcription is therefore leftwards from TR_S to U_S from the strand complementary to that in TR_S which serves as a template for IE mRNA IV (Fig. 9).

3' termini of minor IE mRNAs Faint hybridization of IE cDNA was observed to Bam HI k, and also to Eco RI l (Figs. 2 and 7). As no in vivo labelled IE RNA was mapped to this region, this

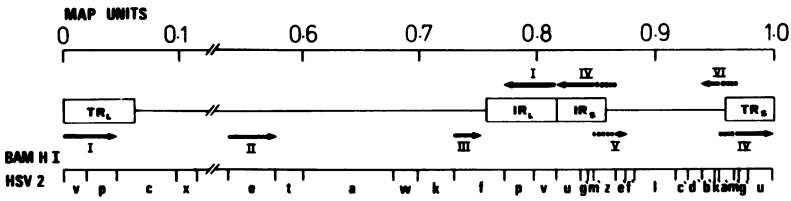


Figure 9: Genome locations and directions of transcription of HSV-2 IE mRNAs.

represents the 3' end of at least one previously undetected minor IE RNA.

As indicated earlier, the nuclear IE RNA hybridizing to Bam HI h i j was not detected in cytoplasmic IE RNAs (Fig. 1, track 7). However, cDNA prepared using a template of unlabelled cytoplasmic IE RNA did hybridize to Bam HI h i j and to Eco RI j, which overlaps with Bam HI h. This indicates that IE RNA from this region is present within the cytoplasm in low amounts, and also unambiguously locates it between 0.12 and 0.15 map units (Fig. 2).

DISCUSSION

The general pattern of HSV-2 transcription as determined by the Southern blot procedure appears directly analagous to that previously determined for HSV-1 using the same technique (33, 35, 37).

At early times, virus RNA hybridized to DNA fragments from all regions of the genome, as indicated by other studies (21, 22), and no major differences between the hybridization patterns of nuclear and cytoplasmic RNA were obtained. There were quantitative differences in the amount of early RNA that hybridized to the individual fragments, for example: the most abundant hybridization of early RNA was to Bam HI e and s t (0.54 to 0.60 map units), but little hybridization to the adjacent fragment Bam HI m, and also to Bam HI d was observed. This abundant hybridization to Bam HI e and s t is of interest, as this region is associated with morphological transformation of mouse cells and primary rat embryo cells cultured in vitro

TABLE 1. Sizes and genome locations of HSV-2 IE mRNAs.

IE mRNA SPECIES	SIZE (kb)	MAP LOCATIONS
I	3.4	0.00 - 0.04 (TR_L) 0.77 - 0.82 (IR_L)
II	-	0.54 - 0.60 (U_L)
III	1.75	0.73 - 0.74 (U_L)
IV	4.7	0.82 - 0.86 (IR_S) 0.96 - 1.00 (TR_S)
V	1.75	0.85 - 0.88 (IR_S/U_S)
VI	1.75	0.94 - 0.97 (TR_S/U_S)

(40; I. Cameron and J.C.M. Macnab, personal communication). We have determined the orientation of an IE mRNA (IE mRNA II) which maps in this region (Fig. 9).

At late times, virus transcripts also hybridized to DNA fragments located throughout the genome consistent with HSV-1 and HSV-2 liquid hybridization experiments (15, 31, 34) and with HSV-1 blot hybridization experiments (33). Unlike the early situation, obvious differences in the hybridization patterns of late nuclear and cytoplasmic RNAs were observed. There was little late cytoplasmic RNA which hybridized to both sets of repetitions as compared with late nuclear RNA. This effect could be due either to sequestration of transcripts complementary to these repetitive sequences within the nucleus, or to their specific degradation in the cytoplsm. This has also been observed for HSV-1 (33, 34).

The early and late patterns are distinguished by quantitative differences in the relative abundance of RNA sequences hybridizing to the individual DNA fragments.

In contrast with the early and late RNAs, HSV-2 IE RNA hybridized to only certain DNA fragments, located in both repetitive regions, and in both unique regions. No major differences in the hybridization patterns of IE nuclear and cytoplasmic RNAs were observed, and this is consistent with the

observations reported for HSV-1 (33, 35). Similar restricted immediate early transcription has been observed for pseudorabies virus, a related herpesvirus (38).

The IE RNA results are not consistent with liquid hybridization data previously reported for HSV-2, which indicated that 45% of the genome was represented in total IE RNA (15). Liquid hybridization data described for HSV-1 indicated that 50% (32) to 30% (34) of the genome was represented in IE nuclear RNA, whereas only 13% was represented in the cytoplasm (34). Our current knowledge of the genome map locations, size and polypeptides specified by both HSV-1 (13, 33, 36) and HSV-2 IE mRNAs (Fig. 3 and 9), together with the molecular weights of those IE polypeptides shown to be unrelated due to breakdown (D. MacDonald and H.S. Marsden, personal communication), indicates that at least 20% of the HSV genome must be represented in the cytoplasm at the immediate early stage.

No additional IE nuclear sequences were detected with a DNAase treated RNA sample which was not fractionated on a Cs_2SO_4 gradient, or with cytoplasmic RNA labelled *in vitro*. These controls therefore demonstrate that the restricted hybridization patterns of nuclear and cytoplasmic IE RNA are not due to artefacts introduced by the methods of RNA isolation or purification.

HSV-2 IE mRNAs separated on denaturing gels showed three bands of similar, but not identical, size to those found with HSV-1 (36). The HSV-2 IE mRNAs mapped to genome regions equivalent to those of the respective HSV-1 IE mRNAs of a similar size.

The difference in size of the 3.4 kb HSV-2 IE mRNA I and its 3.0 kb HSV-1 equivalent may be related to the differences in molecular weight of the two equivalent polypeptides shown to map to this location (13). These are 118 x 10^3 for HSV-2 and 110 x 10^3 for HSV-1 (the polypeptides translated *in vitro*, and therefore unmodified, have molecular weights of 116 x 10^3 and 109 x 10^3 respectively (20)). The 3.4 kb HSV-2 IE mRNA I is sufficient to encode a polypeptide of 116 x 10^3 while the 3.0 kb HSV-1 mRNA is sufficient to encode a polypeptide of 110 x 10^3. The 4.7 kb IE mRNAs of HSV-2 and HSV-1 are both capable of encoding polypeptides of at least 182 x 10^3.

The orientations of the HSV-2 IE mRNAs are identical to those of the equivalent HSV-1 mRNAs (17). In addition, using a cloned DNA fragment, it was possible to orientate HSV-2 IE mRNA III, which was not orientated for HSV-1 (17). IE mRNA III is transcribed from the opposite DNA strand to that of IE mRNA I in IR_L, therefore this mRNA would appear to have a separate promoter. Hence it is unlikely that all the major HSV-2 IE mRNAs are transcribed using a single promoter located in TR_S/IR_S as suggested for HSV-1 (17).

In conclusion, the HSV-2 transcriptional programme closely parallels that of HSV-1. This is particularly evident in the immediate early phase, where individual mRNAs have been orientated on the genome. Late in infection there is sequestration of specific RNA sequences within the nucleus. Control of transcription appears to be exerted by an off/on mechanism, as in the switch from the immediate early to the early phase, and by abundance controls, as evidenced by differences in the relative abundance of RNAs which hybridize to various parts of the genome, both within, and between, the three replication phases studied. The data indicates that in HSV-2 there must be at least two distinct virus promoters which are recognised by an unmodified host cell RNA polymerase and we suggest that the same is likely to be true for HSV-1.

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REFERENCES

1. Becker, Y., Dym, H. and Sarov, I. (1968), Virology, 36, 184-192.
2. Kieff, E.D., Bachenheimer, S.L. and Roizman, B. (1971), J. Virol., 8, 125-132.
3. Kieff, E.D., Hoyer, B., Bachenheimer, S.L. and Roizman, B. (1972), J. Virol., 738-745.

4. Sheldrick, P. and Berthelot, N. (1974), Cold Spring Harbor Symp. Quant. Biol., 39, 667-678.
5. Delius, H. and Clements, J.B. (1976), J. gen. Virol., 33, 125-133.
6. Clements, J.B., Cortini, R. and Wilkie, N.M. (1976), J. gen. Virol., 30, 243-256.
7. Cortini, R. and Wilkie, N.M. (1978), J. gen. Virol., 39, 259-280.
8. Stow, N.D. and Wilkie, N.M. (1978), Virology, 90, 1-11.
9. Timbury, M.C. and Subak-Sharpe, J.H. (1973), J. gen. Virol., 18, 347-357.
10. Esparza, J., Benyesh-Melnick, M. and Schaffer, P.A. (1976), Virology, 70, 372-384.
11. Morse, L.S., Buchman, T.G., Roizman, B. and Schaffer, P.A. (1977), J. Virol., 24, 231-248.
12. Morse, L.S., Pereira, L., Roizman, B. and Schaffer, P.A. (1978), J. Virol., 26, 389-410.
13. Preston, V.G., Davison, A.J., Marsden, H.S., Timbury, M.C., Subak-Sharpe, J.H. and Wilkie, N.M. (1978), J. Virol., 28, 499-517.
14. Jones, K.W., Fenoglio, C.M., Shevchuk-Chaban, M., Maitland, N.J. and McDougall, J.K. (1978) in Oncogenesis and Herpesviruses III, de The, G., Henle, W. and Rapp, F. Eds, IARC Scientific Publications No 24, part 2, pp 917-925.
15. Frenkel. N., Silverstein, S., Cassai, E. and Roizman, B. (1973), J. Virol., 11, 886-892.
16. Southern, E.M. (1975), J. Mol. Biol., 98, 503-533.
17. Clements, J.B., McLauchlan, J. and McGeoch, D.J. (1979), Nucleic Acids Res., 7, 77-91.
18. Kumar, A. and Lindberg, U. (1972), Proc. Natl. Acad. Sci. USA., 69, 681-685.
19. Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977), J. Mol. Biol., 113, 237-251.
20. Preston, C.M. (1977), Virology, 78, 349-353.
21. Oaks, J.E., Hyman, R.W. and Rapp, F. (1976), Virology, 75, 145-154.
22. Bodemer, W.W. and Bodemer, M. (1979), Virology, 92, 507-517.
23. Marsden, H.S., Stow, N.D., Preston, V.G., Timbury, M.C. and Wilkie, N.M. (1978), J. Virol., 28, 624-642.
24. Duff, R. and Rapp, F. (1971), J. Virol., 8, 469-477.
25. Duff, R. and Rapp, F. (1973), J. Virol., 12, 209-214.
26. Wilkie, N.M., Clements, J.B., Macnab, J.C.M. and Subak-Sharpe, J.H. (1974), Cold Spring Harbor Symp. Quant. Biol. 39, 657-666.
27. Subak-Sharpe, J.H., Brown, S.M., Ritchie, D.A., Timbury, M.C., Macnab, J.C.M., Marsden, H.S. and Hay, J. (1974), Cold Spring Harbor Symp. Quant, Biol., 39, 717-730.
28. Jamieson, A.T., Macnab, J.C.M., Perbal, B.and Clements, J.B. (1976), J. gen. Virol., 32, 493-508.
29. Nahmias, A.J., Naib, Z.M. and Josey, W. (1974), Cancer Res., 34, 1111-1117.
30. Ibrahim, A.N., Ray, M. Megaw, J., Brown, R. and Nahmias, A.J. (1976), Proc. Soc. Exptl. Biol. Med., 152, 343-347.
31. Frenkel, N. and Roizman, B. (1972), Proc. Natl. Acad. Sci. USA., 69, 2654-2658.

32. Kozak, M. and Roizman, B. (1974), Proc, Natl. Acad. Sci. USA., 71, 4322-4326.
33. Clements, J.B., Watson, R.J. and Wilkie, N.M. (1977), Cell, 12, 275-285.
34. Jones, P.C. and Roizman, B. (1979), J. Virol., 31, 299-314.
35. Watson, R.J. and Clements, J.B. (1978), Virology, 91, 364-379.
36. Watson, R.J., Preston, C.M. and Clements, J.B. (1979), J. Virol., 31, 42-52.
37. Watson, R.J. and Clements, J.B. (1980), Nature, 285, 329-330.
38. Feldman, L., Rixon, F.J., Jean, J.H., Ben-Porat, T. and Kaplan, A.S. (1979), Virology, 97, 316-327.
39. Boyer, H. and Roulland-Dessoix, D. (1969), J. Mol. Biol., 41, 459-472.
40. Reyes, G.R., La Femina, R. Hayward, S.D. and Hayward, G.S. (1979), Cold Spring Harbor Symp. Quant. Biol., 44, in the press.
41. Ludwig, H.O., Biswal, N. and Benyesh-Melnick, M. (1972), Virology, 49, 95-101.
42. Wilkie, N.M., Davison, A.J., Chartrand, P., Stow, N.D., Preston, V.G. and Timbury, M.C. (1979), Cold Spring Harbor Symp. Quant. Biol., 43, 827-840.
43. Wagner, M.J. and Summers, W.C. (1978), J. Virol., 27, 374-387.
44. Alwine, J.D., Kemp, D.J., and Stark, G.R. (1977), Proc. Natl. Acad. Sci. USA. 74, 5350-5354.