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Detailed structural analysis of two spliced HSV-1 immediate-early mRNAs

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ABSTRACT

The structures of two HSV-1 immediate-early mRNAs have been determined by nuclease-digestion procedures using 5' and 3' end-labelled DNA probes. These mRNAs, which map across the junctions between the short unique (U_S) and short repeat (IR_S and TR_S) genome regions, have common 5' portions located in IR_S and TR_S. The 3' portions, which extend into opposite ends of U_S, are unique. The DNA sequence encoding the common 5' portions largely comprises a 247 base pair (bp) leader region and a single intron of variable size. The variation in intron length is due to different copy numbers of a 22 bp tandem reiteration. A small proportion of the mRNA population is unspliced, but otherwise is identical to the more abundant spliced species.

INTRODUCTION

Transcription in herpes simplex virus (HSV) infected cells has been divided into three temporal classes, namely immediate-early (IE), early and late (1, 2, 3). The earliest (IE) class is defined as viral RNA made in the absence of viral polypeptide synthesis and is therefore the result of transcription by a pre-existing (α-amanitin sensitive) cellular RNA polymerase (4). Furthermore, at least one of the IE mRNAs encodes a polypeptide which mediates the switch-on and the maintenance of subsequent virus transcription (5, 6). Hence, study of IE transcription and the IE to early switch provides a good viral model for transcriptional regulation of virus genes and also may provide information on the expression of cellular genes.

The IE transcriptional phase has been analysed by infecting cells in the presence of protein synthesis inhibitors (1, 7) or with certain temperature-sensitive mutants which accumulate IE transcripts at the non-permissive temperature (8). From these studies, 5 major IE mRNAs have been characterised and their locations and orientations on the HSV genome have been determined (9, 10, 11). This mapping data has revealed an interesting relationship between two of these mRNAs (termed IE mRNAs-4 and -5 (10)),

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which is a function of the virus genome structure. The HSV genome consists of two unique regions (U_L and U_S) which are flanked and joined by different sets of inverted repeats (TR_L and IR_L; TR_S and IR_S respectively) (12, 13). Previous mapping experiments have shown that IE mRNA-4 spans the IR_S/U_S junction while IE mRNA-5 spans the U_S/TR_S junction. These two mRNAs share common 5' sequences which are specified by IR_S and TR_S whereas the 3' portions, which extend into opposite ends of U_S, are unique (10, 14, 15).

This situation therefore poses interesting questions regarding the control of expression of these two mRNAs and the nature of the polypeptides encoded. The present study describes the precise map locations and structures of IE mRNAs-4 and -5. This information is correlated with DNA sequence data for these genome regions (Murchie and McGeoch (16)).

MATERIALS AND METHODS

Cells and virus

Baby hamster kidney 21 (C13) cells were grown as monolayers in rotating 80 oz bottles.

For the production of IE mRNA, cell monolayers were infected with HSV-1 (Glasgow strain 17) at a multiplicity of infection of 50 p.f.u./cell. The cell monolayers were pretreated and maintained in medium containing cycloheximide as previously described (1).

Preparation of cytoplasmic RNA

Cytoplasmic RNA was prepared using the procedure of Kumar and Lindberg (17).

Enzymes

All enzymes were obtained from Bethesda Research Laboratories or New England Biolabs with the exception of T4 polynucleotide kinase (P-L Biochemicals) and nuclease-S1 (Boehringer Corporation Limited). The digestion procedures used were those outlined by the manufacturers.

Cloning Procedures

Fragments of HSV-1 DNA generated using restriction endonucleases were cloned under Category I containment (U.K. Genetic Manipulation Advisory Group). The host bacterium was E.coli K12 HB101 and the cloning vector was pAT 153. Isolation of cloned virus DNA was as described by Davison and Wilkie (18).

Purification and end-labelling of DNA fragments

Purification of DNA fragments from agarose or polyacrylamide gels and labelling of the 5' and 3' ends was carried out as described by McLauchlan and

Clements (19).

Structural analysis of mRNAs

Structural analysis was performed using the nuclease digestion procedures of Berk and Sharp (20) which were modified by using either 5' or 3' end-labelled DNA probes (21) instead of uniformly-labelled DNA.

Either 5'- or 3'-labelled DNA (less than 1 µg) was co-precipitated with known amounts of cytoplasmic RNA from infected or mock-infected cells. The DNA/RNA pellet was resuspended in 20 µl of 90% (v/v) formamide (deionised with Amberlite monobed resin MB-1), 0.4 M NaCl, 40 mM PIPES, pH 6.8, 1 mM EDTA. This mixture was heated to 90°C for 3 min then incubated at 57°C or 57.5°C (when single-stranded DNA probes were used, the hybridisation temperature was reduced to 37°C) for either 5 h or 16 h. Prior to nuclease treatment, the hybridisation mixtures were rapidly quenched on ice.

Nuclease-S1 digestion was performed at 37°C for 1h in 200µl of 0.25M NaCl, 30mM NaOAc, pH 4.5, 1 mM $ZnSO_4$ with 4000 units of nuclease-S1. The nuclease-S1-digested hybrids were extracted with phenol/chloroform then precipitated with ethanol. The digestion products were analysed by gel electrophoresis.

Exonuclease VII digestion was performed at 37°C for 1h in 200 µl of 6.7 mM KPO_4, pH 7.9, 8.3 mM EDTA, 10 mM 2-mercaptoethanol with 0.5 units of exonuclease VII. The exonuclease VII-digested hybrids were extracted with phenol/chloroform, desalted on a Pharmacia PD-10 Sephadex G25M column and precipitated with ethanol. The digestion products were analysed by gel electrophoresis.

Analysis of small hybrids on denaturing gels often revealed a microheterogeneity of the bands. This stutter is due to the imprecise cleavage at hybrid ends by nuclease-S1 and exonuclease VII. Sizes quoted are those which align the RNA with the appropriate recognition site on the DNA sequence.

Due to the processive nature of the exonuclease VII activity, this enzyme will leave several undigested nucleotides at a hybrid end (22). This accounts for the slightly larger size of the exonuclease VII-resistant bands compared to the equivalent nuclease-S1-resistant bands.

Gel Electrophoresis

Samples were electrophoresed either on non-denaturing 1.5% (w/v) agarose gels in a buffer containing 90 mM Tris, 90 mM boric acid, pH 8.3, 1 mM EDTA or on alkaline 1.5% (w/v) agarose gels in 30 mM NaOH, 2 mM EDTA. Electrophoresis was carried out at room temperature for 16 h at 50 V.

Two-dimensional analysis of hybrids was performed on 1.5% (w/v) agarose gels as described by Favaloro et al. (23). All gels were then dried down and the bands visualised by autoradiography at -70°C using Kodak X-Omat-S film.

Denaturing polyacrylamide gels, essentially as described by Maxam and Gilbert (24), were run in 90 mM Tris, 90 mM boric acid, pH 8.3, 1 mM EDTA and the gels contained 9 M urea. Samples were dissolved in deionised formamide and denatured at 90°C for 2 min before loading. Electrophoresis was carried out at room temperature for 3-6 h at 40 watts. The radiolabelled bands were detected by autoradiography.

RESULTS AND DISCUSSION

We have used the nuclease digestion procedures of Berk and Sharp (20) to examine the structures of HSV-1 IE mRNAs-4 and -5, the map locations of which are given in Fig. 1. Previous work had shown that the two mRNAs have common 5' ends mapping within the IR_S and TR_S regions of HSV-1 DNA. It was further shown that the 3' ends of IE mRNAs-4 and -5 map within Hind III n and Bam HI z respectively (10, 14). To analyse the structures of these mRNAs, we have used the four sub-clones of Bam HI n, x and z as indicated in Fig. 1.

Structure of IE mRNA-5

The 3' end of IE mRNA-5 was located using pGX55 DNA which was 3'-labelled at the Bam HI site. This probe was hybridised to different amounts of total cytoplasmic IE RNA, the resultant hybrids were digested with nuclease-S1

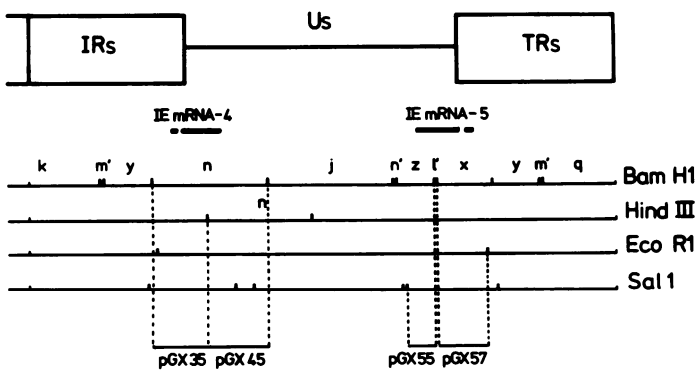

Fig. 1 Restriction endonuclease cleavage maps of the HSV-1 strain 17 short region. Approximate map locations of IE mRNAs-4 and -5 are given. The cloned restriction enzyme fragments used to analyse the 5' (pGX35 and pGX57) and 3' (pGX45 and pGX55) portions of each mRNA are shown.

and the digestion products analysed on neutral, alkaline and two-dimensional gels (Fig. 2). A single virus RNA-dependent product of 580 bases (b) was seen, the intensity of which was proportional to the amount of IE RNA present. The size of the protected fragment on alkaline gels consistently was larger than on neutral gels. This was due to differential migration with respect to the markers of the DNA/RNA hybrid and the single-stranded DNA. As there was no size reduction on alkaline gels, the 580 b 3'-terminal region appeared to be unspliced. This was confirmed by the two-dimensional analysis in which the spot representing the hybrid did not migrate to a position below the diagonal formed by the markers, as would be the case with a spliced mRNA (Fig. 2).

Examination of the 5' end made use of pGX57 DNA 5'-labelled at the Bam HI site. On an alkaline gel a single band was seen, while on neutral gels two bands of 1030 base pairs (bp) and 780 bp were present (Fig. 3). The 780 bp band was equivalent to the single band found on alkaline gels and resulted from nuclease-S1 cut-through of the RNA strand opposite a nick in the

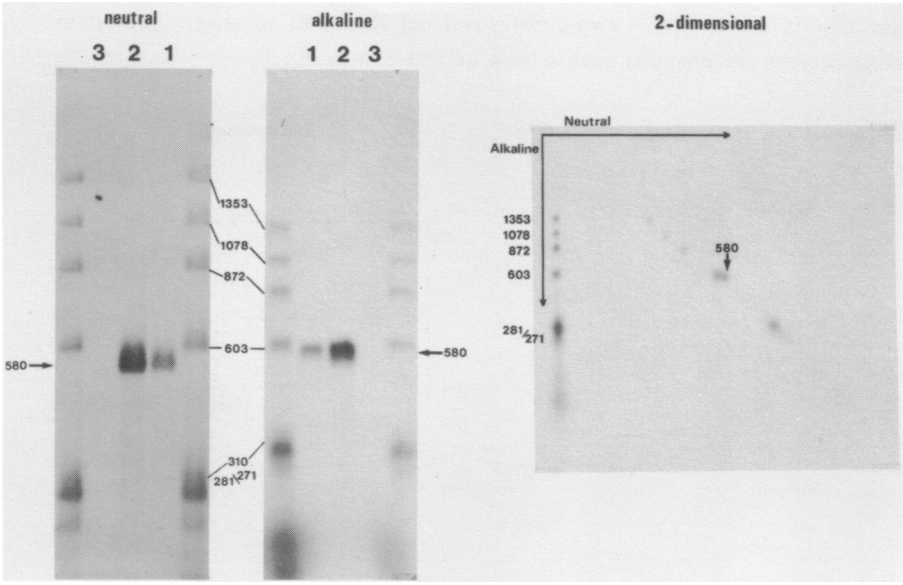

Fig. 2 Analysis of the 3' portion of IE mRNA-5. pGX55 DNA was 3'-labelled at the Bam HI site and was hybridised to the following RNA samples: 1. 5 µg of IE cytoplasmic RNA. 2. 15 µg of IE cytoplasmic RNA. 3. 20 µg of mock-infected cytoplasmic RNA. The nuclease-S1-resistant material was electrophoresed on a 1.5% (w/v) neutral agarose gel, and on a 1.5% (w/v) alkaline agarose gel. Sample 2 was analysed further on a 1.5% (w/v) two-dimensional agarose gel. End-labelled Hae III digested ØX 174 DNA was used as a size standard.

DNA strand corrresponding to the location of the unhybridised intron DNA (23). Therefore, the 780 bp band locates the splice acceptor site on the DNA. The 1030 bp hybrid was the result of the 780 b unspliced region being linked to a leader of about 250 b. This relationship was shown by the two-dimensional gel in which the 250 b leader sequence was dissociated from the 780 b fragment which resulted in the major spot migrating below the diagonal (Fig. 3).

Structure of IE mRNA-4

IE mRNA-4 was examined in a similar manner to IE mRNA-5. DNA from pGX45, 3'-labelled at the Hind III site, gave a single nuclease-S1-resistant band of 475 b on neutral and alkaline gels (Fig. 4). This unspliced 3'-terminal region therefore migrated as a single spot lying on the diagonal of a two-dimensional gel (Fig. 4).

The 5' end was analysed using pGX35 DNA which was 5'-labelled at the Hind III site. Two nuclease-S1-resistant products analogous to those found for IE mRNA-5, with sizes of 1200 bp and 950 bp were detected on neutral gels (Fig. 5) and a single band corresponding to the 950 bp hybrid was present on alkaline gels (Fig. 5). On a two-dimensional gel the 1200 bp spot migrated to a position below the diagonal with a size of 950 b (Fig. 5).

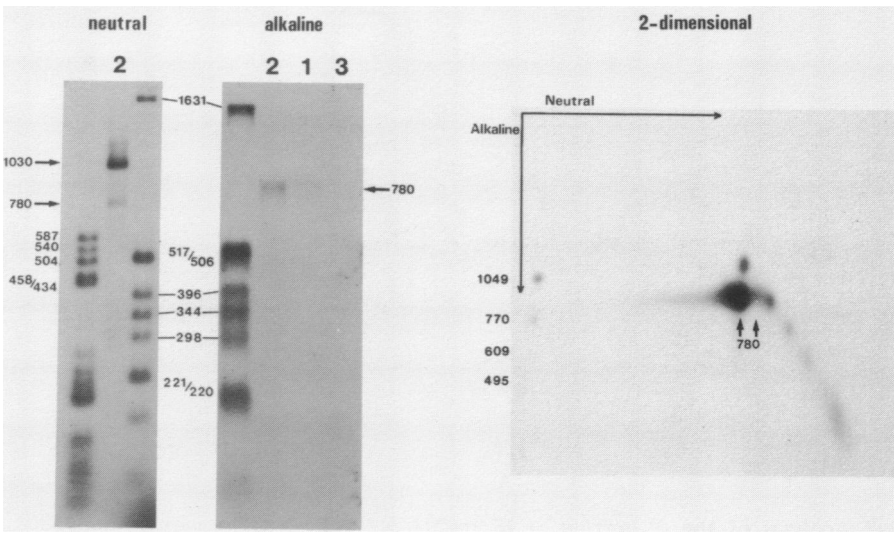

Fig. 3 Analysis of the 5' portion of IE mRNA-5. This analysis was carried out in the same manner as described for the 3' portion of IE mRNA-5 (Fig. 2). pGX57 DNA, which was 5'-labelled at the Bam HI site, was used as the DNA probe. The size standards used were Hae III digested pBR322 DNA (neutral gel), Hinf I digested pBR322 DNA (neutral and alkaline gels) and Hinc II digested ØX 174 DNA (two-dimensional gel).

Therefore the structures of IE mRNAs-4 and -5 are similar, each consisting of a short leader joined through a single splice to a longer 3' unspliced region of 1425 b for IE mRNA-4 and 1550 b for IE mRNA-5.

Exonuclease VII analysis of the 5' portions of IE mRNAs-4 and -5

In initial experiments to determine the size of the introns, hybrids between IE mRNA and 5'-labelled pGX57 or pGX35 were digested with exonuclease VII. On an alkaline gel, single nuclease-resistant bands of 1330 b for pGX57 (IE mRNA-5) and 1450 b for pGX35 (IE mRNA-4) were present (Fig. 6). The 1330 b IE mRNA-5 band was 550 b larger than the 780 b nuclease-S1-resistant band and, allowing for the 250 b leader, this gives an intron size of 300 bp. Similarly, the 1450 b IE mRNA-4 band was 250 b larger than the 250 b leader and the 950 b nuclease-S1-resistant band combined. Sizes obtained for the different regions of these IE mRNAs were somewhat imprecise due to the limitations of the gel techniques and to the large size of the protected DNA fragments. Also, due to the nature of the DNA sequences forming the hybrids, an incorrect estimate of intron sizes was obtained with exonuclease VII

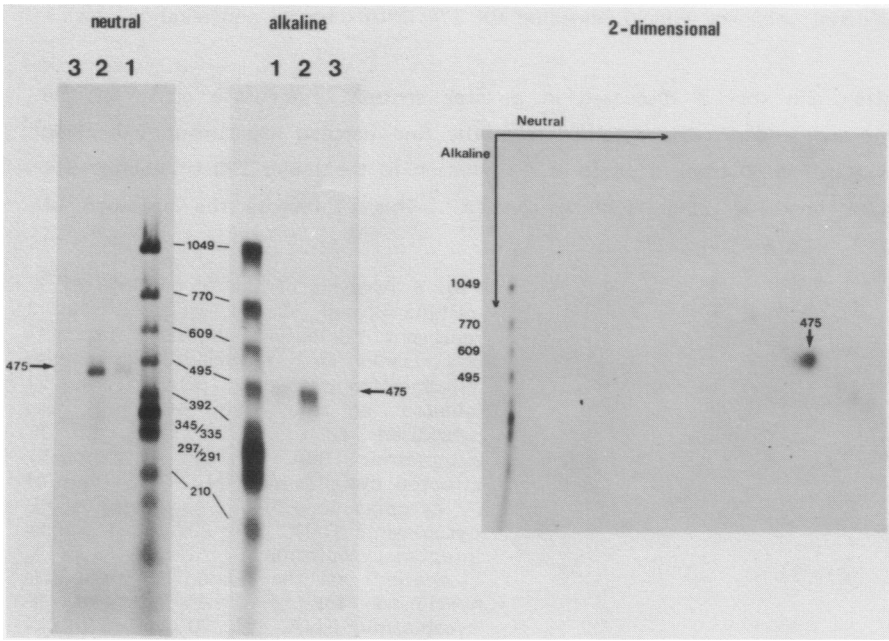

Fig. 4 Analysis of the 3' portion of IE mRNA-4. This analysis was carried out in the same manner as described for the 3' portion of IE mRNA-5 (Fig. 2). pGX45 DNA, which was 3'-labelled at the Hind III site, was used as the DNA probe. Hinc II digested ϕX 174 DNA was used as a size standard.

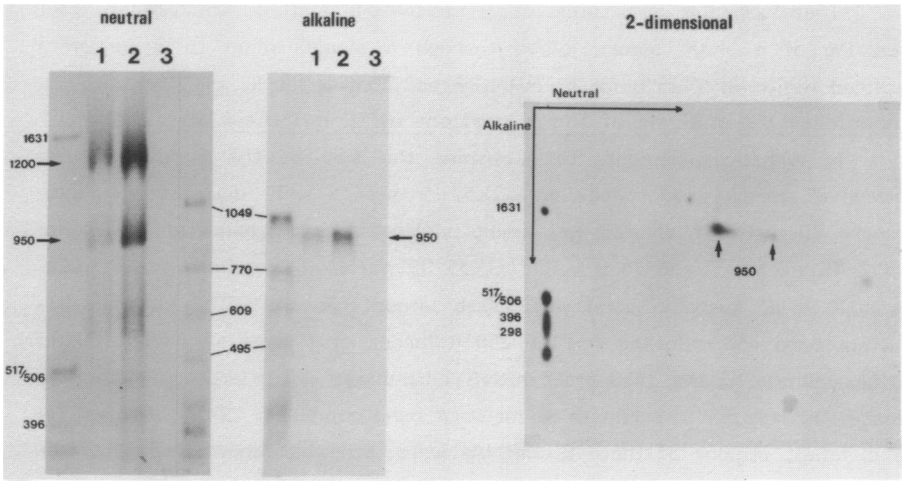

Fig 5 Analysis of the 5' portion of IE mRNA-4. This analysis was carried out in the same manner as described for the 3' portion of IE mRNA-5 (Fig. 2). pGX35 DNA, which was 5'-labelled at the Hind III site, was used as the DNA probe. The size standards used were Hinf I digested pBR322 DNA (neutral and two-dimensional gels) and Hinc II digested ØX 174 DNA (neutral and alkaline gels).

digestion and this is discussed in a later section. Accurate sizes for the introns and leaders were obtained from the fine-mapping experiments described below. In Fig. 6 track 1 there is, in addition to the major 780 b nuclease-S1-resistant band, a minor 1240 b species. This represents the presence of

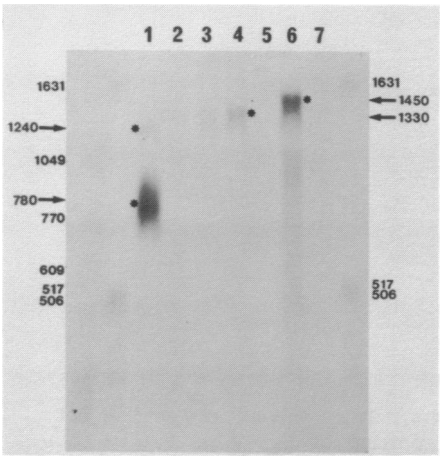

Fig. 6 Analysis of the 5' portions of IE mRNAs-4 and -5. Tracks 1 and 2 represent nuclease-S1-digestion products and tracks 3-7 represent exonuclease VII-digestion products. pGX57 DNA, 5'-labelled at the Bam HI site was hybridised to: 1. 15 µg of IE cytoplasmic RNA. 2. 20 µg of mock-infected cytoplasmic RNA. 3. 5 µg of IE cytoplasmic RNA. 4. 15 µg of IE cytoplasmic RNA. 5. 20 µg of mock-infected cytoplasmic RNA. pGX35 DNA, 5'-labelled at the Hind III site was hybridised to: 6. 15 µg of IE cytoplasmic RNA. 7. 20 µg of mock-infected cytoplasmic RNA. The nuclease-resistant material was electrophoresed on a 1.5% (w/v) alkaline agarose gel. Hinc II digested ØX 174 DNA and Hinf I digested pBR 322 DNA were used as size standards.

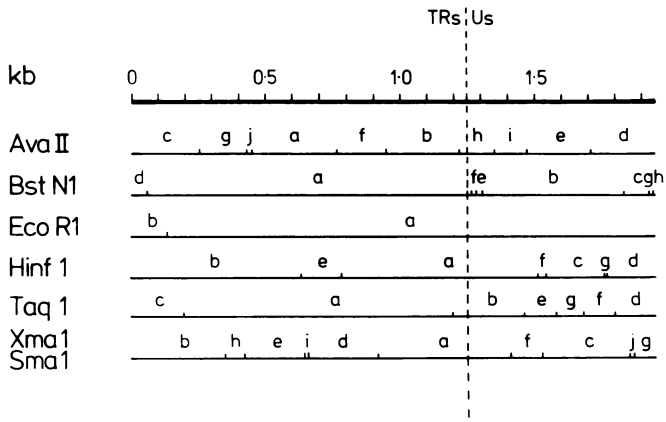

Fig. 7 Restriction endonuclease cleavage maps of HSV-1 strain 17, Bam HI x. This map is reproduced by permission of M.-J. Murchie.

unspliced mRNA and is described in more detail below.

Location of the 5' end

The 5' portions of IE mRNAs-4 and -5, including both the leader and intron sequences, extend 500 bp into the IR_S and TR_S regions respectively. More detailed examination of this region was performed using sub-fragments of Bam HI x (Fig. 7).

Using 5'-labelled Xma I d, a 202 b nuclease-S1-resistant band was detected (Fig. 8) while 5'-labelled Hinf I e gave rise to a 62 b band (data not shown). These two hybrids place the 5' end at the same position. The Hinf I band was electrophoresed alongside the DNA sequence for this fragment (data

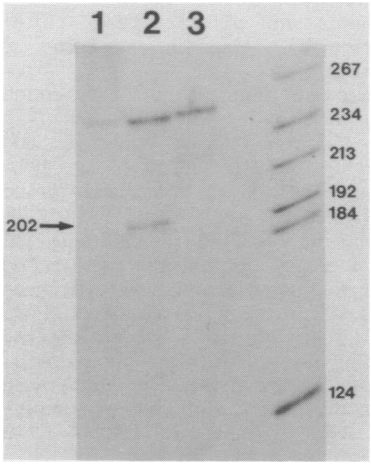

Fig. 8 Precise location of the 5' end. 5'-labelled Xma I d (Fig. 7) was used as the DNA probe. This probe was hybridised to: 1. unhybridised probe. 2. 15 µg of IE cytoplasmic RNA. 3. 20 µg of mock-infected cytoplasmic RNA. The nuclease-S1-resistant material was electrophoresed on a 6% denaturing polyacrylamide gel. Hae III digested pBR322 DNA was used as a size standard.

not shown) and the location of the 5' end at position 2563 on the DNA sequence is shown in Fig. 12.

Location of splice donor site

The splice donor site was determined with respect to two cleavage sites using 3'-labelled Hinf I a and 3'-labelled Xma I a. Xma I a gave nuclease-S1-resistant bands of 55, 47 (major species) and 35 b (Fig. 9A) while Hinf I a yielded bands of 196, 188 and 176 b (data not shown). The Xma I bands were electrophoresed alongside the DNA sequence for the fragment and their locations on the DNA sequence are shown in Fig. 12. The DNA sequence for this region contains 3 potential splice donor sites located at 62, 47 and 30 b from the Xma I site. Therefore, only the major 47 b nuclease-S1-resistant band exactly coincides with a splice donor site located at position 2810. The two remaining nuclease-S1 products, which are present in lower amounts, are slightly displaced relative to the potential donor sites and their locations coincide with relatively A+T rich regions. As Xma I a and Hinf I a map adjacent to the Xma I d and Hinf I e fragments used to locate the 5' end, summing the sizes of the hybrids obtained with these fragments gives a leader sequence length of 247 b.

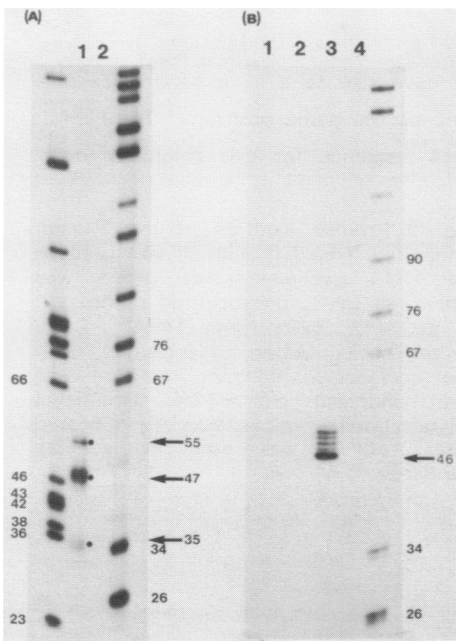

Fig. 9 (A) Precise location of the splice donor site. 3'-labelled Xma I a (Fig. 7) was hybridised to: 1. 15 µg of IE cytoplasmic RNA. 2. 20 µg of mock-infected cytoplasmic RNA. The nuclease-S1-resistant material was electrophoresed on a 8% denaturing polyacrylamide gel. Hpa II digested pBR322 DNA and 3'-labelled Hinf I fragments of pBR322 DNA, after digestion with Hae III were used as size standards.
(B) Precise location of the splice acceptor site. 5'-labelled Ava II b (Fig. 7) was strand-separated and each strand was used as a DNA probe. The slower migrating strand was hybridised to: 1. 15 µg of IE cytoplasmic RNA. 2. 20 µg of mock-infected cytoplasmic RNA. The faster migrating strand was hybridised to: 3. 15 µg of IE cytoplasmic RNA. 4. 20 µg of mock-infected cytoplasmic RNA. The nuclease-S1-resistant material was electrophoresed on a 8% denaturing polyacrylamide gel. Hpa II digested pBR322 DNA was used as a size standard.

Location of the splice acceptor site

The splice acceptor site was located using 5'-labelled Ava II b which, since it contains no restriction site suitable for recleavage, was strand separated on a 6% denaturing polyacrylamide gel. Only the faster migrating strand (subsequently sequenced to confirm the orientation of the hybrid) generated a hybrid (Fig. 9B) which placed the splice acceptor site at 46 bp from the Ava II b/h junction. To confirm this location, a 360 bp fragment resulting from Sma I digestion of 5'-labelled Bst NI a, was used. This fragment located the splice acceptor site at 86 bp from the Bst NI a/f junction (data not shown) in the same position as determined by the Ava II DNA probe. The Bst NI hybrid was electrophoresed alongside the DNA sequence for this fragment and the location of the splice acceptor site at position 3022 is shown in Fig. 12.

Evidence for unspliced mRNAs

In experiments where 5'-labelled Taq I a was used to precisely locate the splice acceptor site, a 19 b nuclease-S1-resistant product was generated. These experiments revealed an additional, minor 475 b viral RNA-dependent band on a 6% denaturing polyacrylamide gel (Fig. 10A). A size of 475 b is consistent with an unspliced mRNA with a 5' end at position 2563 where the 5' end of the spliced mRNAs is located.

To confirm the presence of unspliced mRNA hybridisations were performed with the larger fragment generated by EcoRI digestion of 5'-labelled Bst NI a. The major nuclease-S1-resistant product of 86 b corresponds to the location of the splice acceptor site (Fig. 10B, track 1) and the additional minor (<5% of protected label) 542 b band represents the unspliced mRNA with a 5' end at position 2563.

Exonuclease VII analysis; an unusual effect of the DNA sequence

Exonuclease VII treatment of hybrids generated with 5'-labelled Taq I a gave a minor 475 b band and a major product of 565 b (Fig. 10A, track 5). Similarly, exonuclease VII treatment of hybrids generated with the larger fragment produced by EcoRI cleavage of 5'-labelled Bst NI a yielded a minor 542 b band and a major 630 b band (Fig. 10B, track 3). The 475 b and 542 b bands place the 5' end at position 2563, however the larger sizes suggest that the 5' end lies about 90 bp upstream of this position. To examine this effect further, we used 5'-labelled Ava II a, which contains both the nuclease-S1-determined and the exonuclease VII-determined 5' ends. A single 48 b nuclease-S1-resistant product located the 5' end at position 2563, while exonuclease VII digestion gave a minor component of about 48 b together with

a major 136 b product (Fig. 11A). As this Ava II a DNA probe was labelled at both ends, the 136 b exonuclease VII-resistant band and the 48 b nuclease-S1-resistant band were excised and sequenced to confirm that they were labelled at the same position.

As the exonuclease VII data suggested the possibility of another splice extending beyond position 2563, the nuclease-S1 product generated with Ava II a was electrophoresed on a 5% non-denaturing polyacrylamide gel (Fig. 11B). The 55 bp hybrid observed was very similar in size to the 48 b band found on denaturing gels thus strongly suggesting that additional leader sequences were not present in the hybrid.

Examination of the DNA sequence upstream of position 2563 reveals

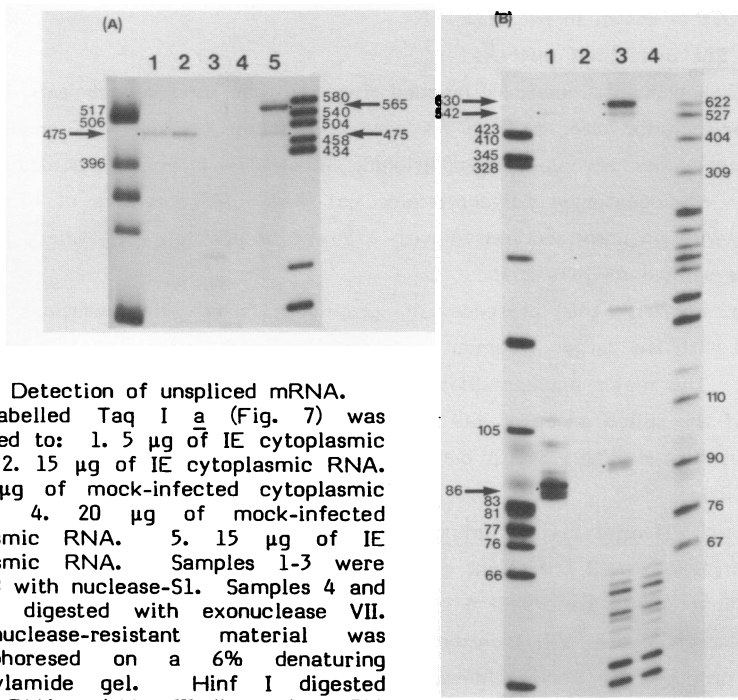

Fig. 10 Detection of unspliced mRNA.
(A) 5'-labelled Taq I a (Fig. 7) was hybridised to: 1. 5 µg of IE cytoplasmic RNA. 2. 15 µg of IE cytoplasmic RNA. 3. 20 µg of mock-infected cytoplasmic RNA. 4. 20 µg of mock-infected cytoplasmic RNA. 5. 15 µg of IE cytoplasmic RNA. Samples 1-3 were digested with nuclease-S1. Samples 4 and 5 were digested with exonuclease VII. The nuclease-resistant material was electrophoresed on a 6% denaturing polyacrylamide gel. Hinf I digested pBR322 DNA and Hae III digested pBR322 DNA were used as size standards.
(B) 5'-labelled Bst NI a (Fig. 7) was digested with Eco RI and the larger cleavage product was hybridised to: 1. 15 µg of IE cytoplasmic RNA. 2. 20 µg of mock-infected cytoplasmic RNA. 3. 15 µg of IE cytoplasmic RNA. 4. 20 µg of mock-infected cytoplasmic RNA. Samples 1 and 2 were digested with nuclease-S1. Samples 3 and 4 were digested with exonuclease VII. The nuclease-resistant material was electrophoresed on a 8% denaturing polyacrylamide gel. Hpa II digested pBR322 DNA and 3'-labelled Hinf I fragments of pBR322 DNA, after digestion with Hae III were used as size standards.

that the potential 5' end, as located by exonuclease VII at position 2476, immediately is preceded by a 42 bp G+C sequence. Localised DNA-DNA reassociation (either inter- or intra-strand) is likely to occur at this region which would prevent digestion with exonuclease VII but not with nuclease-S1. Furthermore, the exonuclease VII resistance of such a band would be IE mRNA-dependent as the absence of hybrid formation would allow the DNA strand to be digested at the labelled end.

A consequence of this effect was the overestimation of intron sizes determined from the exonuclease VII-resistant bands shown in Fig. 6. These sizes should be reduced by 90 bp, giving 212 bp for IE mRNA-5 and 168 bp for IE mRNA-4. The corrected intron sizes agree with the sizes determined from the DNA sequence.

CONCLUSIONS

IE mRNAs-4 and -5 possess a common 247 b leader, and both mRNAs have a single splice located in IR_S and TR_S respectively. Examination of the DNA sequence in TR_S which specifies the 5' portion of IE mRNA-5 (Fig. 12) reveals signals involved in initiation and processing of this mRNA. A 'TATA' box (25) is located 23 to 30 bp upstream of the 5' end, and appropriate splice donor and splice acceptor signals define the intron (26).

The structures of these HSV-1 strain 17 mRNAs closely resemble those

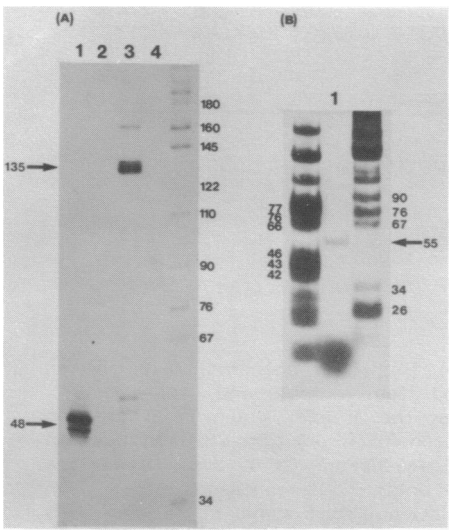

Fig. 11 Analysis of DNA regions encoding the 5' end.
(A) 5'-labelled Ava II a (Fig. 7) was hybridised to: 1. 15 µg of IE cytoplasmic RNA. 2. 20 µg of mock-infected cytoplasmic RNA. 3. 15 µg of IE cytoplasmic RNA. 4. 20 µg of mock-infected cytoplasmic RNA. Samples 1 and 2 were digested with nuclease-S1. Samples 3 and 4 were digested with exonuclease VII. The nuclease-resistant material was electrophoresed on a 8% denaturing polyacrylamide gel. Hpa II digested pBR322 DNA was used as a size standard.
(B) A portion of the nuclease-S1-digested sample shown in Fig. 11A, track 1 was electrophoresed on a 5% non-denaturing polyacrylamide gel. Hpa II digested pBR322 DNA and 3'-labelled Hinf I fragments of pBR322 DNA, after digestion with Hae III were used as size standards.

described by Watson et al. (15) for HSV-1 strain Patton. However, a major difference exists with respect to the introns. The intron size of IE mRNA-4 was determined as 168 bp using Bam HI n, and this was confirmed by DNA sequencing (16). By contrast, the intron size of IE mRNA-5 as determined from Bam HI x was 212 bp and the DNA sequence (Fig. 12) confirmed this. Watson et al. (27) determined the intron sizes of both mRNAs as 149 bp and stated that no size variability was detectable.

The HSV-1 strain 17 introns contain a 22 bp tandem reiteration which lacks a single bp but is otherwise identical to a 23 bp reiteration present in the strain Patton introns. For strain Patton the introns of IE mRNAs-4 and -5 each contained 4 copies of the 23 bp reiteration (27). For the strain 17 DNA clones sequenced, the intron size difference is due to the presence of 5 copies of the reiteration in Bam HI n and 7 copies in Bam HI x (16). Significantly, different clones of HSV-1 strain 17 and various HSV-1 field isolates show size variability of Bam HI n and Bam HI x (18, 28). This is likely to represent DNA molecules which contain different numbers of the intron reiteration.

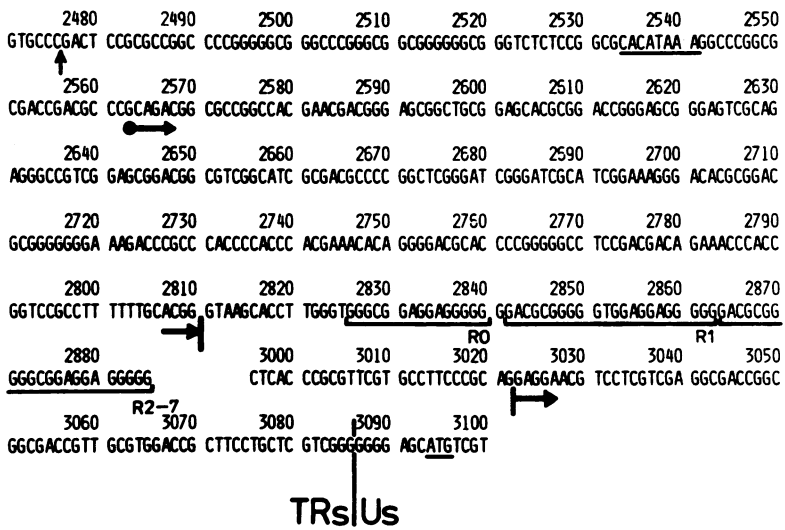

Fig. 12 Nucleotide sequence of the region of Bam HI x encoding the 5' portion of IE mRNA-5. This sequence is reproduced from Murchie and McGeoch (16). The 'TATA' signal 23 to 30 bp upstream of the 5' end, which is at position 2563, is underlined. The limits of the intron from position 2810 to position 3022 are indicated and the reiterated sequences within this intron are underlined. R_1 differs from R_2-R_7 by a single C-T transition. R_0 is an incomplete form of R_2-R_7. The ATG translational start signal at position 3095 within U_S is underlined. The arrow at position 2476 indicates the location of the potential 5' end as suggested by exonuclease VII digestion.

The IR_S and TR_S together with the IR_L and TR_L repeat regions are known to contain other areas of size variability which are related to the presence of different tandem reiterations. These different tandem reiterations are located outwith the genes present in the repeats. No function has yet been ascribed to these reiterations.

IE mRNAs-4 and -5 have a single intron, however the other IE mRNAs are unspliced (29, 30) as are the early and late mRNAs so far analysed (18, 31-33) (with one exception (34)). Adenoviruses also specify spliced and unspliced mRNAs but, in contrast to HSV-1, most adenovirus mRNAs are spliced (35).

Splicing in adenoviruses and papovaviruses serves to increase the coding capacity by generating different mRNAs from the same transcription unit. Splicing of IE mRNAs-4 and -5 serves only to remove introns from a 5' untranslated region and confers no additional coding capacity to the HSV-1 genome. As both 5' portions, including the introns, are located within IR_S and TR_S, splicing appears not to be involved in any differential expression of these mRNAs.

Recent experiments with deletion mutants of polyoma virus, SV40 and adenovirus 5 have shown that for certain mRNAs which normally are spliced, splicing is not essential to produce stable cytoplasmic mRNAs (36-38). Failure to splice IE mRNAs-4 and -5 would not alter their coding potential as no translational initation codons are present in the transcribed IR_S and TR_S regions. The unspliced forms which were detected in the cytoplasm therefore may serve as functional messages.

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