ISOLATION AND CHARACTERIZATION OF TWO HERPES SIMPLEX VIRUS TYPE 1 VARIANTS CONTAINING DUPLICATION OF SEQUENCES WITHIN THE UNIQUE LONG COMPONENT OF THEIR GENOMES

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Summary. – This paper reports the first spontaneous isolation of two DNA duplication variants in the unique long (U_L) component of herpes simplex virus type 1 (HSV-1) strain 17^+ genome, one (1719) with a duplication of 7.5 kb DNA sequences centered around Ori_L and the other (1740In) with a 356 bp DNA duplication between the UL19 (MCP) and UL20 open reading frames (ORFs). The variant 1719 is stable with the rare isolation of a wild type (strain 17^+) genome presumably generated by the excision of the duplicated sequences during homologous recombination. Because of the 7.5 kb duplication, UL29 (DBP) is diploid and UL30 (DNA pol) is present as one complete and one partial copy. Although duplication in the variant 1740In involved sequences from the UL20 ORF, the virus produces an intact UL20 gene product. Both variants show normal growth characteristics when compared with the parental viruses. DNA duplications in these variants suggest a link between replication and recombination in HSV-1.

Key words: herpes simplex virus type 1; DNA duplication variants; DNA replication; recombination

Introduction

The genome of HSV-1 consists of two covalently linked components, long (L) and short (S), each comprising a unique sequence (U_L and U_S) bounded by inverted repeats (TR_L/IR_L; IR_S/TR_S). The genome contains three origins of replication; one (Ori_L) situated in the middle of U_L at nucleotide position (np) 62475/6 and one each (Ori_S) in IR_S and TR_S at np 131999 and 146234, respectively.

The isolation of either spontaneous or constructed HSV deletion and/or insertion variants has facilitated the identification of a number of genes non-essential for HSV DNA replication *in vitro* (Post and Roizman, 1981; Harland and Brown, 1985, 1989; Brown and Harland, 1987; MacLean and Brown, 1987b,c; Barker and Roizman, 1990; Baines and Roizman, 1991; MacLean, AR *et al.*, 1991; MacLean CA *et al.*, 1991; Fareed, 1992; Fareed and Spivack, 1994). The removal of an entire ORF or deletion/insertion disrupting an ORF is used to determine the essential/non-essential nature of a particular gene. Most of the previously reported spontaneous variants of HSV have involved rearrangement of the repeat elements along with part of the adjacent unique sequences. The hypothesis proposed for the frequency of these occurences, is that the repeat elements promote recombination and may contain hot spots for homologous recombination or a site-specific recombinase (Dutch *et al.*, 1992, 1994; Umene, 1993). Since two copies of each repeat is present within the viral genome, one copy is easily deleted or rearranged without having any effect on the viability of the virus (Harland and Brown, 1985, 1989; MacLean and Brown, 1987b,c). In this paper, we report the isolation

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Abbreviations: DNA pol = DNA polymerase; HSV = herpes simplex virus; IR = inverted repeat; *M* = molarity; mc = map coordinate; MDBP = major DNA binding protein; MOI = multiplicity of infection; np = nucleotide position; ORF = open reading frame; Ori = origin of replication; PAGE = polyacrylamide gel electrophoresis; PBS = phosphate buffered saline; p.i. = post infection; PMSF = phenylmethylsulfonyl fluoride; SDS = sodium dodecylsulfate; TR = terminal repeat; U_L,U_S = unique component long, short; WT = wild type; BSA = bovine serum albumin

of two HSV-1 strain 17$^+$ variants containing duplicated copies of specific unique sequences with no involvement of any of the repeat elements. The variant 1719 contains a tandemly duplicated 7.5 kb sequence around Ori_L whereas the variant 1740In duplicates a 356 bp sequence whose 5'-end point starts from 21 bp downstream of the 3'-end of UL20. The structure of these variants suggests a possible linkage between the processes of DNA replication and recombination in HSV.

Materials and Methods

Cells. Baby hamster kidney 21 clone 13 (BHK21/C13) cells (MacPherson and Stoker, 1962) were grown in Eagle's medium containing twice the normal concentration of vitamins and amino acids, 10% (v/v) tryptose phosphate broth and 10% (v/v) calf serum (ETC10).

Viruses. HSV-1 Glasgow strain 17 syn$^+$ (Brown *et al.*, 1973) was the parental wild type (WT) virus used in most experiments. The variants 1702 (MacLean and Brown, 1987a), devoid of the four normally occuring *Xba*I sites, 1708 (MacLean and Brown, 1987b) containing an additional *Xba*I site at 0.74 map coordinates (mc) and the variants desribed in the present work were all derived from the parental strain 17 syn$^+$.

The nucleotide numbers used throughout this paper represent the numbering system of HSV-1 strain 17 syn$^+$ DNA sequence, as described by McGeoch *et al.* (1988). The numbers given represent the first base of the respective restriction endonuclease cleavage site or the first base of the respective ORF/gene and/or specific sequence of HSV-1 DNA.

Preparation of virion DNA was done by the method of Wilkie (1973) as described by Fareed and Spivack (1994).

Restriction endonuclease analysis of virus genomes was carried out by a modification of the method described by Lonsdale (1979). Cells were infected in the presence of [^{32}P] orthophosphate in phosphate-free Eagle's medium containing 1% (v/v) calf serum and incubated at 31 °C for at least 48 hrs. ^{32}P-labelled virus DNA was extracted with SDS and phenol and precipitated with ethanol. The DNA was subjected to digestion with appropriate restriction endonucleases using the manufacturer's recommended conditions. The digests were analysed by electrophoresis on agarose gels of the appropriate concentrations (0.5 to 1.2%) in TBE buffer pH 7.8 (89 mmol/l Tris-HCl, 89 mmol/l boric acid, 2 mmol/l EDTA). The gels were air-dried and exposed to Kodak XS1 film for 24 to 72 hrs.

DNA-DNA hybridization. DNA fragments from restriction endonuclease digests were transferred from agarose gels to Hybond nylon membranes (Amersham) and hybridized with nick-translated DNA by the method of Southern (1975). Nick-translated DNA was prepared from the plasmid containing the appropriate HSV-1 restriction endonuclease fragment (Sambrook *et al.*, 1989). Hybridization was carried out as described by MacLean AR *et al.* (1991).

Virus growth properties. Single-cycle growth experiments were carried out as described by Harland and Brown (1989). Multiple-cycle growth experiments were performed as described by MacLean and Brown (1987a).

DNA sequence determination. As described previously (Junejo *et al.*, 1991; MacLean, AR *et al.*, 1991), DNA sequence was determined by dideoxynucleotide sequencing using the chain termination reaction method of Sanger *et al.* (1987). The appropriate restriction endonuclease fragments were initially cloned into the multiple cloning regions of pGEM vectors, positive clones identified and analysed by restriction endonucleases and/or Southern blotting (Sambrook *et al.*, 1989). The cloned fragments were either used directly in sequence determination or where necessary, sequencing of their small subfragments cloned into M13mp18/19 vectors was carried out.

Immunoprecipitation was carried out as previously described (MacLean CA *et al.*, 1991). Briefly, BHK21/C13 cell monolayers were grown in 50 mm Petri dishes, infected at a multiplicity of infection (MOI) of 20 PFU/cell in 0.5 ml Eagle's medium containing 5% newborn calf serum and the virus adsorbed at 37 °C for 1 hr. The inoculum was removed and the monolayers washed twice with Eagle's medium containing one-fifth the normal concentration of methionine and 2% calf serum (Emet/5C2) before being maintained in 2 ml Emet/5C2 at 37 °C. At 4 hrs post infection (p.i.), 100 µCi of [^{35}S]methionine (Amersham) per plate was added and at 24 – 29 hrs p.i., cells were washed twice with PBS before being harvested in 0.5 ml of extraction buffer [100 mmol/l Tris-HCl pH 8.0, 10% (v/v) glycerol, 0.5% (v/v) NP40, 0.5% (w/v) sodium deoxycholate, 0.2 mmol/l PMSF]. The cell extracts were sonicated and centrifuged at 11,000 x g for 10 mins in a Beckman microfuge. The supernates were then stored at -70 °C until used. Extracts (100 µl each) were incubated with 100 µl of extraction buffer and 25 µl of anti-peptide serum in the presence of 10 µg of the relevant peptide or unrelated control peptide at 37 °C for 30 mins and then at 4 °C overnight. 60 µl of a 50% (v/v) suspension of protein A-Sepharose was added to each sample and the samples were incubated on ice for 45 mins with occasional mixing. The immunoprecipitates were then pelleted by centrifugation and the pellets washed 4 times with extraction buffer. The bound proteins were eluted by boiling for 5 – 10 mins in 60 µl of elution buffer (50 mmol/l Tris-HCl pH 6.7, 2% SDS, 70 mmol/l 2-mercaptoethanol, 10% glycerol and 1% bromphenol blue) and analysed by SDS-PAGE (Marsden *et al.*, 1978).

Preparation and analysis of HSV-infected cell polypeptides. Confluent monolayers of BHK21/C13 cells in 35 mm Petri dishes were infected at a MOI of 20 PFU/cell. The monolayers were treated as described under immunoprecipitation (see above). However, the samples were harvested in 0.75 ml of sample buffer [151 mmol/l Tris-HCl pH 6.7, 6.28% (w/v) SDS, 0.15% (v/v) 2-mercaptoethanol, 0.31% (v/v) glycerol, 0.1% (w/v) bromphenol blue] and heated at 80 °C for 5 mins. Immediately prior to use, the extracts were boiled for 10 mins and analysed by SDS-PAGE (Marsden *et al.*, 1978).

Results

Introduction of mutations within the HSV genome requires cotransfection of cells with intact viral DNA and linearised plasmid DNA carrying the desired mutation. The resultant progeny virus is titrated, individual plaques picked,

small scale virus stocks grown and their ^{32}P-labelled DNA analysed with restriction endonucleases (Lonsdale, 1979). During such analyses two variants showing aberrant restriction enzyme profiles were identified.

The variant 1719

The variant 1719 was isolated as a result of a marker transfer experiment involving intact HSV-1 strain 17 syn$^+$ DNA and a linearised plasmid containing HSV-1 *Bam*HI K fragment. The *Bam*HI K fragment was isolated from the HSV-1 strain 17 syn$^+$ variant 1714 and contained a 759 bp deletion spanning most of the RL1 (ICP 34.5 or gamma-34.5) ORF (MacLean AR *et al.*, 1991).

On *Bgl*II digestion (Fig. 1, lane 2), a *1M* band of 7.5 kbp was seen running above the M band, but no other alterations were apparent. On *Eco*RI digestion (Fig. 1, lane 4), a similar profile was seen; a novel 7.5 kbp, *1M* band but no further alteration. On a *Bam*HI digest (Fig. 1, lane 6), the normally *1M* 2.3 kbp V and 3.3 kbp R bands appeared to be *2M* and a novel 2 kbp, *1M* band was present above the 1.9 kbp Y band. On a *Kpn*I digest, (Fig. 1, lane 8), the molarity of the 1.95 kbp V and 1.15 kbp A' bands appeared to be *2M* and a novel *1M* band running above the 4.4 kbp P band was seen. The relevant restriction enzyme maps are illustrated in Fig. 2. Taken together these results suggested that 7.5 kbp of sequences, centred around 0.4 mc are reiterated. The insert appeared to start within the *Bam*HI G fragment (np 52588 - 60362) and terminated within *Bam*HI K' (np 66018 - 66238).

If the restriction enzyme used to digest the DNA cuts only once within the reiteration, the normal bands are unaltered with the only additional band being the same size as the reiterated sequences. This is exemplified by both *Bgl*II and *Eco*RI digestion. If the enzyme cuts multiple times within the reiteration, those fragments between sites which are entirely within the reiteration will be duplicated and thus become *2M*. The two fragments at the ends of the reiteration will be *1M* and a novel fragment consisting of covalently linked reiterated sequences from these two fragments will be seen. The additional fragments will add up to the size of the reiteration. This is exemplified on the *Bam*HI and *Kpn*I digests. When an enzyme does not cut within the reiteration, the fragment containing the reiterated sequence will

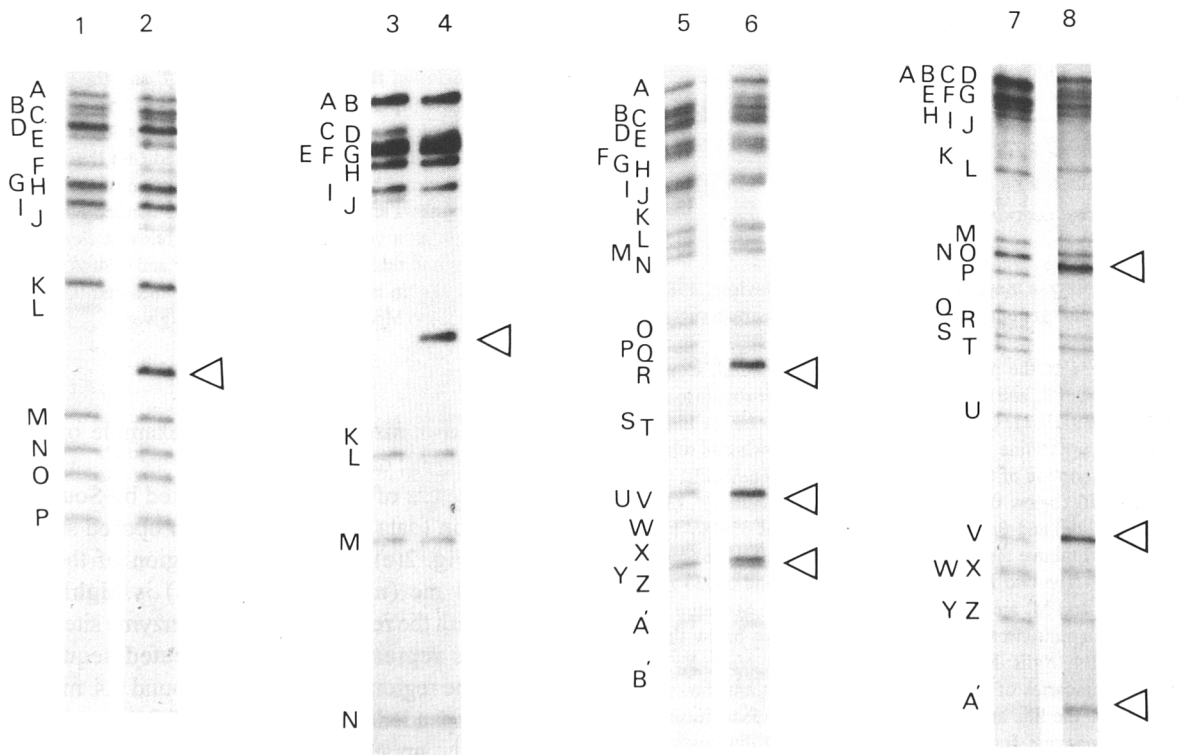

Fig. 1
Restriction endonuclease profiles of strain 17$^+$ (Davison, 1981; Wilkie, 1976) and the variant 1719 ^{32}P-DNAs labelled *in vivo*
WT strain 17$^+$ (lanes 1,3,5,7), variant 1719 (lanes 2,4,6,8). *Bgl*II (lanes 1,2), *Eco*RI (lanes 3,4), *Bam*HI (lanes 5,6), *Kpn*I (lanes 7,8). The WT bands are labelled on the left hand side. The novel bands in 1719 are indicated with open triangles.

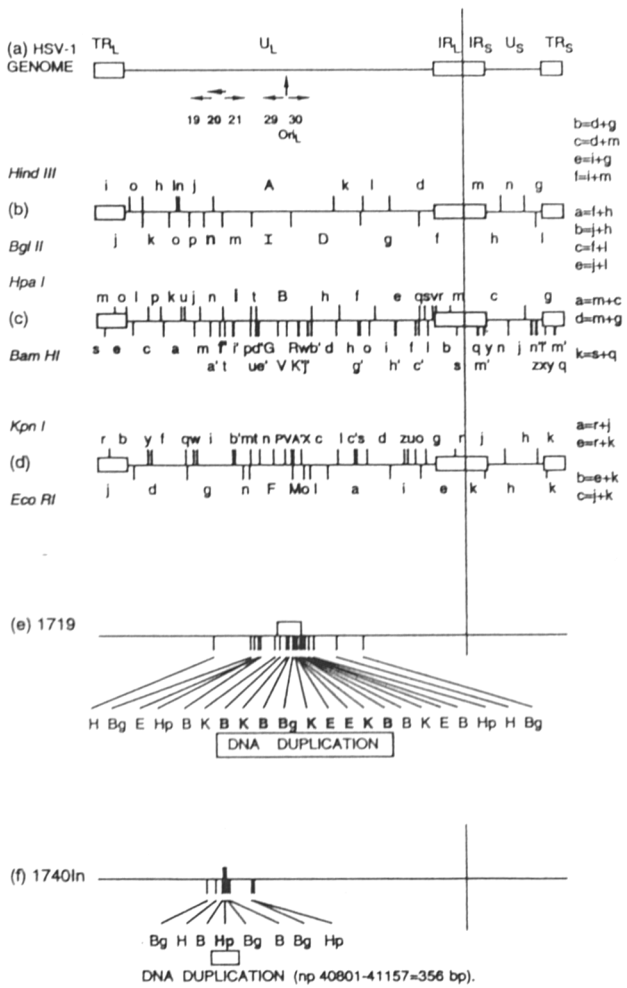

Fig. 2

Restriction enzyme maps of HSV-1 strain 17⁺ (Davison, 1981; Wilkie, 1976) and genome structures of the duplication variants 1719 and 1740In

(a) HSV-1 genome in the prototype orientation. U_L and U_S sequences bracketed with TR_L, TR_S and IR_L, IR_S are as shown. Approximate locations of the UL19, UL20, UL21, UL29 and UL30 genes and Ori_L are indicated with arrows below the line representing U_L. The arrowheads represent direction of transcription of the respective genes. (b) The *Hind*III (above the line) and *Bgl*II (below the line) maps of HSV-1 strain 17⁺. (c) The *Hpa*I (above the line) and *Bam*HI (below the line) maps of HSV-1 strain 17⁺. (d) The *Kpn*I (above the line) and *Eco*RI (below the line) maps of HSV-1 strain 17⁺. The two *Eco*RI sites at np 64121 and 64133 (12 bp apart; between F and M) are shown as a single site. (e) Structure of the variant 1719. The restriction endonuclease sites shown below the line are: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; Hp, *Hpa*I; K, *Kpn*I. Approximate boundaries of 7.5 kbp DNA duplication are shown with open boxes above the line and below the restriction endonuclease sites. The restriction enzyme fragments affected by 1719 duplication are highlighted with capital letters (upper case). (f) Location of the 356 bp DNA duplication in the variant 1740In. The *Bgl*II *n*, *Bam*HI *f* and *Hpa*I *i* fragments containing the 356 bp duplication are indicated with bold letters. The nucleotide positions (np) are those of HSV-1 strain 17⁺ DNA (McGeoch *et al.*, 1988).

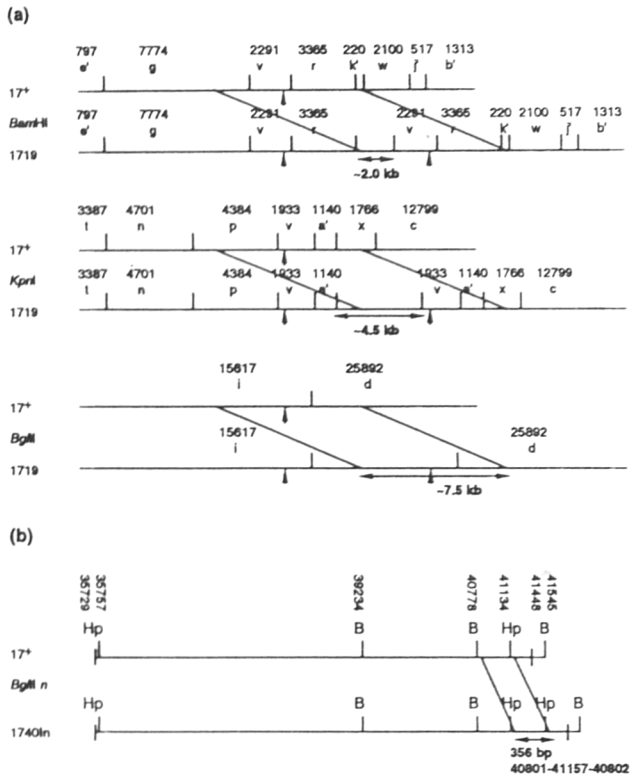

Fig. 3

Expanded region of the genomes of strain 17⁺ and the variant 1719 around 0.4 mc (np 52,000 - 70,000) (a), and of strain 17⁺ *Bgl*II n restriction fragment (np 35,729 - 41,448) (b)

(a) The diagonal lines before 17⁺ and 1719 indicate the extent of the reiterated sequences. The novel band at the junction of the reiteration is indicated with arrows and its approximate size is marked. The position of Ori_L is shown with a vertical arrow. (b) The relevant *Bam*HI (B) and *Hpa*I(Hp) sites and their np are shown for 17⁺ and 1740In. The 356 bp duplication in 1740In is shown with diagonal lines and the relevant np (McGeoch *et al.*, 1988) are given.

be increased in size by 7.5 kbp, an example of which is *Hpa*I (see Fig. 2; data not shown).

The structure of 1719 was confirmed by Southern blot hybridization (data not shown). The proposed structure is shown in Fig. 2(e) and 3(a). The region of the genome around 0.4 mc (np 39849 - 89364) is highlighted in Fig. 2(e) with the relevant restriction enzyme sites and with open boxes representing the duplicated sequences. In Fig. 3(a), the region of the genome around 0.4 mc (np 52 - 70 kb) is expanded and the position of Ori_L is marked with an arrow. The upper line for each restriction enzyme map represents the WT strain 17⁺ structure with the fragments and sizes marked; the lower lines show the proposed 1719 structure, with the positions of restriction sites and fragments; the reiterated sequence is marked by diagonal lines.

The boundaries of the reiterated sequence are delimited by the observed restriction enzyme profiles (see Fig. 1); the right hand boundary at one side by the reiteration of the *Bam*HI R/K' site giving a *2M* R band. The exact end points of the novel junction of G to K' are within the novel 2 kbp *Bam*HI fragment, presumably near the left hand end as this band contains at most 220 bp or probably considerably less of K' sequences as indicated by the weak intensity of hybridization to it (data not shown). The left hand boundary is indicated by the absence of the *Kpn*I X/C site within the reiterated ~4.5 kbp band, giving a *1M* X band. The left hand boundary is also indicated by the size of the reiteration (7.5 kbp) and the novel *Bam*HI 2 kbp band, which consists of between 1.8 and 2 kbp of *Bam*HI G. The reiteration in 1719 is therefore almost exactly centered around Ori_L, which is thus present twice. The whole of UL29 (MDBP) is now diploid and UL30 (pol) is present as one complete copy and one partial copy.

The growth characteristics of 1719 are similar to that of WT virus (data not shown). Virus stocks with titers greater than 10^9 PFU/ml have been obtained. In this respect it differs from defective amplicons generated by high multiplicity passage, which by definition must contain an Ori and are also dependent on helper virus for replication (Frenkel *et al.*, 1980; Spaete and Frenkel, 1982). Homologous recombination might be expected to occur between the directly repeated sequences in 1719 and it has been shown, on plaque purification that WT virus is generated at a frequency of 0.5 – 1%.

The variant 1740In

This variant was isolated during the analyses of recombinants from a cross between the variant 1708 containing an additional *Xba*I site at 0.74 mc (MacLean and

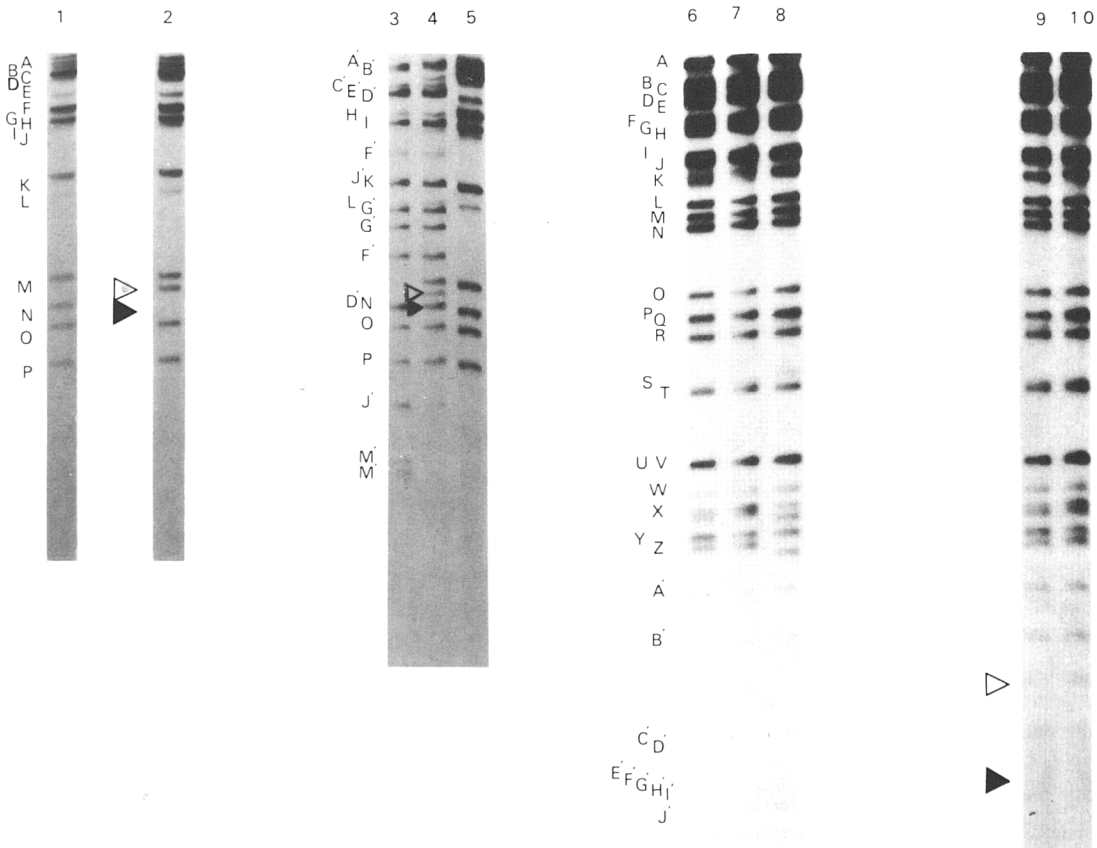

Fig. 4

Restriction endonuclease profiles of strain 17⁺ and the variants 1721 (Fareed, 1992; Fareed and Brown, unpublished data), 1708 (MacLean and Brown, 1987b) and 1740In ^{32}P-DNAs labelled *in vivo*

WT strain 17⁺ (lanes 1,6), variants 1721 (lanes 5,7), 1708 (lanes 3,7) and 1740In (lanes 2,4,9,10). *Bgl*II (lanes 1,2), *Bgl*II-*Xba*I (lanes 3-5), *Bam*HI (lanes 6-10). The WT bands are labelled on the left hand side of lanes 1,3 and 6. The missing bands are indicated with a filled triangle [the missing *Bam*HI F' band (lanes 9,10) can not be seen because of comigration with the E',G',H' and I'bands] and the novel bands with an open triangle.

Brown, 1987b) and the variant 1739 containing an additional *Hind*III site at 0.374 mc (Fareed and Brown, unpublished data). The variant 1739 was derived from the variant 1702 (Maclean and Brown, 1987a) lacking the four normally occuring *Xba*I sites and, therefore, contains no *Xba*I restriction endonuclease sites. On *Hind*III digestion, the DNA profile of 1740In was identical to that of 1739 whereas the *Xba*I profile indicated deletion of both 0.07 and 0.29 mc *Xba*I sites (Fig. 2; data not shown). On *Bgl*II-*Hind*III digestion, the *Bgl*II N' band was missing and a novel band was running slightly above the *Bgl*II J' band (data not shown). On *Bgl*II-*Xba*I digestion a novel band was seen migrating between M and the comigrating D' and N bands (Fig. 4, lane 4). On *Bgl*II digestion alone the N band was missing and a novel band was running below M (Fig. 4, lane 2). Taken together these profiles indicated that the genome of 1740In contained an insert of estimated M_r of about 0.32 x 10^6 (between 350 and 400 bp). The insert was further delimited to the *Bam*HI F' fragment within *Bgl*II N (Fig. 2; Fig. 4, lanes 9, 10). The *Bam*HI F' fragment from 1740In was cloned into pGEM and subsequently a *Hpa*I subfragment (see below) of *Bam*HI F' into M13mp19 and dideoxy sequencing carried out as described previously (Junejo *et al.*, 1991). Alignment of the sequence with that from the equivalent WT sequence revealed that the DNA insert in the variant 1740In was the result of a duplication of a 356 bp region between np 40801 and 41157 [(Fig. 2 (f)]. The duplication contained *Hpa*I N/I site ([see Fig. 2 (c); np 41134], thus generating a novel 356 bp *Hpa*I fragment [Fig. 3 (b)]. The 356 bp region was inserted between np 40801 and 40802, 21 bp downstream of the 3' end of the UL20 ORF. To determine whether 1740In produced normal levels of the UL20 protein immunoprecipitations were carried out (MacLean CA *et al.*, 1991). The 22 K protein was specifically precipitated from extracts of cells infected with HSV-1 strain 17^+ and the variants 1708, 1739 and 1740In. It can be seen that the insert in 1740In did not affect the production of the UL20 gene product

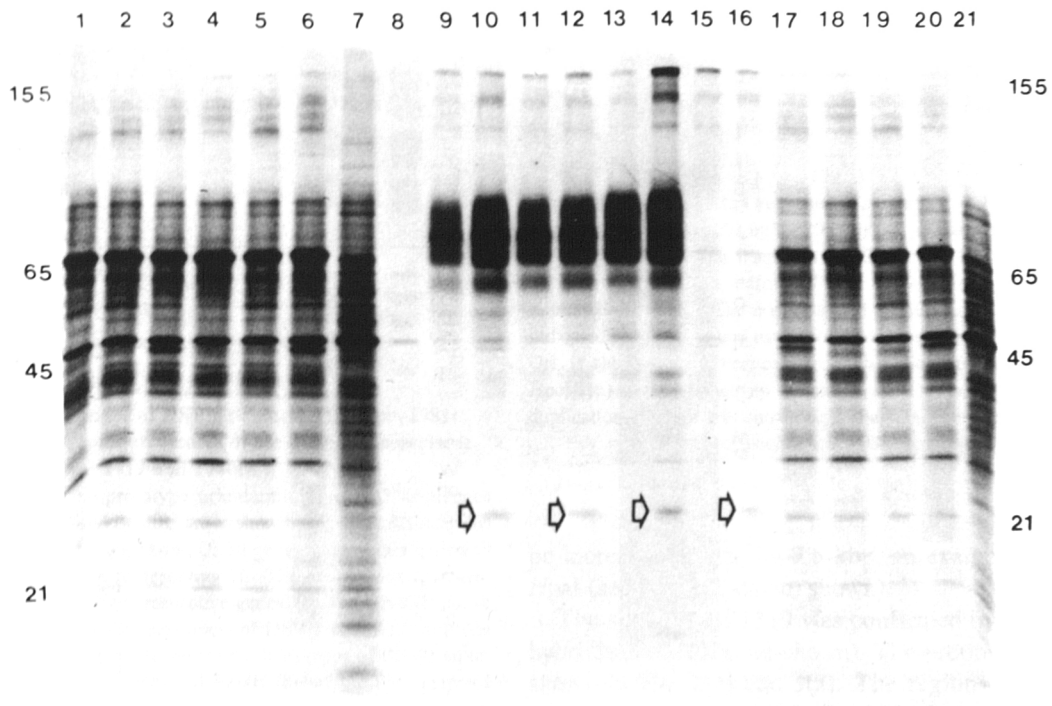

Fig. 5
Immunoprecipitation of the UL20 gene product

Antiserum was raised against a synthetic oligopeptide, NH_2-Gln-Met-Leu-Pro-Pro-Thr-Asp-Pro-Leu-Arg-Thr-Arg-Tyr-COOH, representing amino acids 168 to 179 of the UL20 ORF with a carboxy-terminal tyrosine residue added to facilitate coupling to BSA (MacLean CA *et al.*, 1991). Proteins from HSV-1 strain 17^+-infected (lanes 9,10), 1740In-infected (lanes 11,12), 1721-infected (lanes 13,14; Fareed, 1992), 1708-infected (lanes 15,16) or mock-infected (lane 8) cell extracts labelled with [^{35}S]methionine were precipitated using immune serum in the presence of 10 μg of the peptide against which the serum was raised (lanes 9,11,13,15) or an unrelated control peptide (lanes 8,10,12,14,16), and analysed on 5 – 12.5% SDS-PAGE. A band which appeared to be specifically precipitated is shown with an open arrowhead. Values of M_r (K) are shown on both sides of the figure. HSV-1 strain 17^+-infected (lanes 6,20), 1740In-infected (lanes 1,2,5,19), 1721-infected (lanes 4,18), 1708-infected (lanes 3,17) and mock-infected (lanes 7,21) cell extracts labelled with [^{35}S] methionine are also shown.

(Fig. 5). Single cycle (Fig. 6) and multiple cycle (data not shown) growth experiments showed that the insert had no effect on the viability of the variant.

Discussion

The process of recombination in HSV is complex being modulated by a number of factors. There are seven genes required for HSV-1 DNA replication (Wu *et al.*, 1988) which have been shown to direct recombination events independent of specific DNA sequence requirements (Weber *et al.*, 1988). One of these genes, UL29, has recently been shown to promote the renaturation of complementary single DNA strands (Dutch and Lehman, 1993) and to catalyse strand exchange (Bortner *et al.*, 1993), indicating that the major ssDNA binding protein (Ruyechan, 1983) may also play a role in HSV recombination. In addition, during viral replication, HSV also undergoes genome isomerisation resulting in four equimolar isomeric populations of DNA (Delius and Clements, 1976; Jenkins and Roizman, 1986). Families of tandemly reiterated short sequences in R_L (repeat component, long; Rixon *et al.*, 1984), the "a" sequence (Dutch *et al.*, 1992, 1994; Umene, 1993), manipulations such as transfection and restriction endonuclease treatment of DNA (Brown *et al.*, 1984; MacLean and Brown, 1987a; Fareed, 1992), use of high MOI in recombination experiments (Umene, 1986) and use of different cell types (Brown *et al.*, 1992) have also been implicated in enhancing the process of HSV recombination either directly or indirectly.

During the genomic analyses of over 6000 individual plaques in the present study, 0.62% showing genomic rearrangements were identified (Fareed, 1992). This was in contrast to the spontaneous isolation frequency (0.02%) of deletion/insertion variants of HSV-1 strain 17^+ reported previously. (MacLean and Brown, 1987b,c). These figures are however low compared to our findings with HSV-2 strain HG52 which demonstrated a frequency of 24% variants with genomic deletions within a WT stock (Harland and Brown, 1985). Up to the present, the spontaneously isolated deletion/insertion variants have all had genomic rearrangements involving R_L, R_S (repeat component, short) and/or adjacent unique sequences and have possibly arisen as the result of illegitimate recombination events between tandemly reiterated sequences within these regions.

The origin of the variants 1719 and 1740In is open to speculation. 1719 was isolated during the screening of plaques from a marker transfer experiment [the fragment *Bam*HI K spanning the RL1 gene (joint region of HSV-1 genome) was used in marker transfer experiment (MacLean AR *et al.*, 1991) and, therefore, unlikely to take any part in the 7.5 kb DNA duplication which occured within the unique sequences approximately 60 kbp upstream of the RL1 ORF]

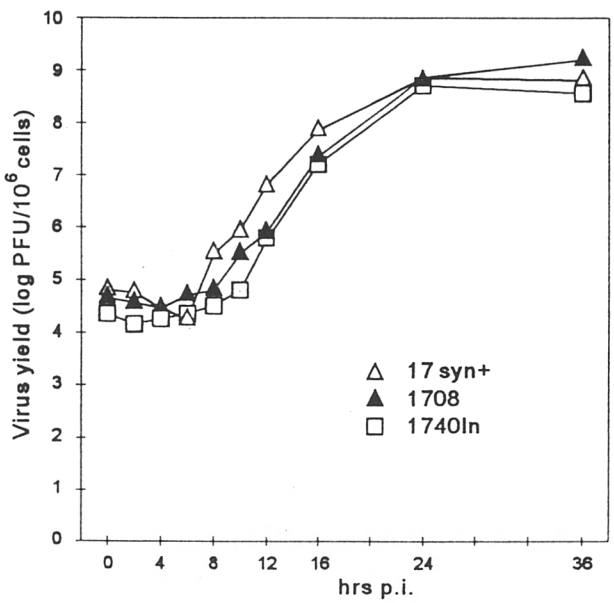

Fig. 6
Single-cycle growth curves of the strain 17 syn$^+$ and the variants 1708 and 1740In
BHK21/C13 cells were infected at a MOI of 5 PFU/cell and the viruses adsorbed at 37 °C for 45 mins. The cell monolayers were washed twice with PBS, overlaid with EMEM containing 10% calf serum and incubated at 37 °C. Cells were harvested at 0,2,4,6,8,10,12,16,24 and 36 hrs p.i. and viruses titrated on BHK21/C13 cell monolayers at 37 °C.

whereas 1740In was isolated during a recombination experiment. Both variants carry duplication of DNA within the U_L component of their genomes, indicating crossovers between partially replicated genomes during DNA replication. Recombination in HSV has been shown to occur after the onset of replication suggesting that the two processes are linked (Ritchie *et al.*, 1977). Moreover, Batra and Brown (1989) have demonstrated the occurence of preferential recombination between intact genomes and restriction endonuclease fragments containing an Ori. They have further demonstrated that large fragments which preferentially recombined always included the Ori region of the genome. DNA replication-mediated recombination in HSV-1 has also been demonstrated between Tn5 repeats within plasmids which occur only in the presence of the seven enzymes which constitute HSV-1 DNA replication machinery (Weber *et al.*, 1988). HSV replication machinery includes enzymes, such as DNA polymerase and MDBP which may carry out functions (Bortner *et al.*, 1993; Dutch and Lehman, 1993) similar to those of *E. coli* SSB (Christian and Baldwin, 1977), T4 gene 32 protein (Alberts and Frey, 1970) and *E. coli* RecA (McEntee, 1985) and RecT proteins (Hall *et al.*, 1993). The duplication of regions containing the entire UL29 gene (MDBP) and Ori_L along with partial duplication of UL30 (DNA pol) sequences in the variant 1719 implicate the pos-

sible involvement of DNA pol in repair synthesis and MDBP in renaturation of ssDNA as well as strand exchange to promote recombination between partially replicated genomes, each containing an intact copy of Ori_L. Although DNA duplication in 1740In has occurred between UL19 and UL20 at a distance of approximately 21.5 kbp from Ori_L, it could have arisen by a similar process. The isolation of 1719 and 1740In support a link between replication and recombination in HSV and suggest that the two processes involve common enzymes.

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