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Homology of the HSV-2 "a-Sequence" to Cellular Sequences

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Abstract

Bgl-II fragments of the genome of Herpes simplex virus type 2 (HSV-2) HG-52 were cloned into the vector p-Neo and were used to screen the complete HSV-2 genome for regions cross-hybridizing with the genome of HEL cells. Most extensive cross-hybridizing activity was observed with a 530 bp *Sst*II subfragment of the viral *Bam*HI G DNA-fragment (contained in *Bgl* II F), which spans the joint and the viral a-sequence. From a λ-L47 library, a cellular 15 kb *Hin*dIII DNA fragment was subcloned in pBR 322 which contained a 1920 bp *Sst*II subfragment having strong cross-hybridizing activity with the 530 bp *Sst* II fragment of HSV-2 *Bam*HI G. Within this 1920 bp *Sst* II fragment the cross-hybridizing activity was confined to a 230 bp *Bgl* I/*Hpa* II subfragment. This 230 bp fragment (including the flanking sequences) was analyzed in comparison to the viral a-sequence. Sequence data revealed a (G + C) content of 66% in the cellular and 81% in the viral DNA fragment, which is mainly determined by an extremely (G + C) rich 16-fold direct repeat (DR2) at the 5'-end. The homology between both DNA-fragments varies between 56% and 79% within the L-S inversion region. Both sequences, furthermore, show homology to the human c-myc protooncogene.

Introduction

The genome of Herpes simplex virus type 2 (HSV-2) consists of a linear, double-stranded DNA of approximately 148 kilobase pairs (kbp) (1,2). It is composed of two large unique sequences, a long U_L and a short U_S region, each flanked by terminal repeats and linked by inverted repeats (3,4). The U_L and U_S region can invert relative to each other, producing four equimolar populations of the genome.

In several previous studies, cross-hybridization between segments of the HSV genome and mammalian DNA of various species was observed (5,6). Predominantly the inverted repeat regions of the HSV-2 genome and the *Bgl* II C fragment within the U_L region were identified as sequences with apparent homology to cellular DNA (7,8). It was estimated that cellular sequences with cross-hybridizing activity are reiterated in 10^3–10^5 copies per cell (5) and it has been suggested that such sequences reflect an evolutionary relation between the viral and the cellular sequence (6). In further studies, however, cross-hybridization signals were found to be suppressed under conditions of high stringency. This led to the conclusion that the observed cross-hybridization was due to nonspecific binding of (G + C) rich cellular and viral sequences rather than to a true sequence homology (9). Previous findings of our laboratory, however, indicated that cross-hybridization between cellular and HSV-2 DNA occurred even under conditions of high stringency, namely suppressing nonspecific hybridization of (G + C) rich sequences.

Since cross-hybridization events may reflect a functionally important homology between HSV-2 and the host cell genome concerning initiation of DNA replication, recombination events or transcriptional regulation of virus genes, we decided to analyze the DNA sequences involved in such interactions on a molecular level. For this purpose viral and cellular DNA segments exhibiting strong cross-hybridization activity were subcloned, their DNA sequence was determined, and comparatively analyzed by computer-aided alignment studies.

Materials and methods

Cells and viruses

Human embryonic lung (HEL)-cells strain WI-38 and RC-37 (African green monkey kidney) cells were grown in basal medium Eagle (BME) (Biochrom, Berlin) supplemented with 10% fetal calf serum (FCS) (Biochrom).

The HSV-2 strain HG-52 was serially passaged at a low multiplicity of infection (m.o.i.) of 0.01 pfu/cell to avoid the production of defective interfering particles (10). Preparation of purified virus was performed as previously described (11). Briefly, RC-37 cells were infected at an m.o.i. of 5 pfu/cell. Supernatants were collected after 40 hr and clarified by low speed centrifugation at 3000 ×g for 15 min. Supernatants were subjected to ultracentrifugation at 100,000 ×g for 1 hr at 4°C in an SW-27 rotor (Beckman Instruments, Heidelberg). Viral pellets were resuspended in TBS/1 mM phenylmethylsulfonyl fluoride (PMSF) and virions were purified on linear 20–70% sucrose gradients at 100,000 × g for 90 min in a SW41 rotor (Beckman Instruments, Heidelberg). Virions formed a visible band at 50% sucrose. Finally, virions were harvested from sucrose gradients, diluted in PBS and again centrifuged at 100,000 ×g for 45 min (SW41 Ti). The resulting viral pellet was resuspended in 1 ml TNE buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM Na_2-EDTA, pH 7.5).

Extraction and purification of HSV-2 DNA

Virions purified as described above were incubated overnight at 37°C in TNE buffer containing 1% sarcosyl and 200 µg/ml proteinase K (Merck, Darmstadt). Proteins were removed by extraction with phenol, phenol-chloroform and chloroform-isoamylalcohol. The DNA was precipitated by addition of sodium acetate, pH 5.0, to a final concentration of 0.3 M and 2.5 volumes ethanol and overnight incubation at -20°C.

Following precipitation, the viral DNA was washed twice in ice-cold 70% ethanol (v/v) and was redissolved in TE buffer (10 mM Tris-HCl, 1 mM Na_2EDTA, pH 7.5). CsCl was added to a final density of 1.718 g/cm^3 and the DNA was centrifuged for 72 hr at 100,000 ×g and 20°C in a 50 Ti rotor (Beckman Instruments). After centrifugation the DNA fractions at a specific density of 1.718 g/cm^3 were recovered, the DNA was diluted in 3 volumes TE buffer and precipitated with ice-cold ethanol. Finally, $E_{260/280}$ of the DNA sample was determined and the DNA concentration calculated.

Extraction of cellular DNA

For preparation of WI-38 DNA, cells were harvested from culture bottles by gentle agitation with glass beads, washed three times in PBS and were resuspended in TNE buffer. Subsequently, the DNA was extracted essentially as described above.

Preparation of plasmid DNA

E.coli (strain K514(12)) containing recombinant plasmids were grown in LB-medium supplemented with 50 µg/ml ampicillin at 37°C. When cultures reached an O.D. of 0.8, bacterials were pelleted at 4000 ×g (4°C) and were incubated with lysozyme (15 mg/ml; Merck) for 30 min on ice. Subsequently, a 2-fold volume of 0.2 M NaOH/1% $NaDodSO_4$ was added, the solution was gently shaken for 5 min, sodium acetate pH 5.0 was added to a final concentration of 1 M and the solution was incubated for 2 hr at 0°C. The solution was centrifuged at 15,000 ×g (4°C) for 30 min, the supernatant was extracted with phenol, phenol-chloroform, chloroform-isoamylalcohol and the DNA was precipitated with a 2.5-fold volume of cold ethanol. The DNA was redissolved in TE-buffer containing 1 mg/ml ethidium bromide, CsCl was added to a final density of 1.496 g/cm^3 and samples were subjected to ultracentrifugation for 20 hr at 150,000 ×g (20°C) in a VTi 65 rotor (Beckman Instruments). Subsequently, form I DNA (13) was collected, ethidium bromide was removed by butanol extraction, and the plasmid DNA was precipitated with ice-cold ethanol.

Vectors and cloning strategies

Plasmids pBR322, pNEO and pNO1523 were derived from Pharmacia (Freiburg, FRG). Phage λ-L47 DNA was purchased from Amersham (Braunschweig, FRG), *Hin*d III cut DNA of λ-L47 came from BRL (Neu-Isenburg, FRG).

Restriction enzymes, T4-DNA ligase, T4-DNA polymerase were purchased from BRL (Neu-Isenburg).

For the preparation of recombinant plasmids vector DNA was cut with the appropriate restriction enzyme and subsequently digested with calf intestinal phosphatase (CIP; Boehringer, Mannheim) to suppress self-ligation. Enzymes were removed by phenol-chloroform extraction followed by chromatography on Sephadex G-75 columns (Pharmacia, Freiburg). Finally, the linearized plasmid DNA was ethanol-precipitated.

For cloning of specific DNA fragments, the DNA was cut with the respective restriction enzymes, subjected to gel electrophoresis in low melting point (LMP) agarose (Gibco, BRL, GmbH, Eggenstein, FRG) and stained with ethidium bromide. The respective DNA fragments were excised from the gel and were recovered by phenol-chloroform extraction, concentrated with 2-butanol and precipitated with ethanol. Ligations were carried out with 10–100 ng of cellular or viral DNA, 10–100 ng 5′-dephosphorylated plasmid DNA and 1–3 U of T4 DNA ligase in the appropriate buffers for 15–20 h at 15°C.

E.coli strain K514 was transformed with recombinant plasmids according to the procedure described by Mandel and Higa [14]. Briefly, *E.coli* strain K514 was grown in overnight cultures, incubated for 10 min on ice, centrifuged at 2500 × g 4°C for 10 min and were resuspended in a small volume of 100 mM ice-cold $CaCl_2$ solution. After 1 hr incubation on ice, bacterials were spun down, 0.001 volumes of 100 mM $CaCl_2$ were added and bacterials were incubated for a further 24 hr on ice. Then 2–5 µl of the ligation mixture was added to 100 µl $CaCl_2$-treated *E.coli*. Bacterials were incubated for 30 min on ice, then for 5 min at 42°C and, after the addition of 900 µl LB-medium, for 1 hr at 37°C. One hundred–250 µl of the bacterial suspension was seeded on amp^+ agar plates and incubated overnight at 37°C.

Recombinant bacteriophages were prepared as follows. The λ-L47 DNA was digested with *Hin*dIII, subjected to electrophoresis in a 0.5% LMP-agarose gel and the right and left arm of the λ-L47 genome was recovered from the gel. One–3 × 10^{-9} mol of *Hin*dIII cut cellular DNA was ligated with the right and left arm of λ-L47 DNA. The relation of insert DNA to λ-L47 DNA varied from 1:1 to 1:5 (15). In vitro packaging of recombinant λ-L47 DNA was performed as described by Hohn and Murray (16). Briefly, cell extracts from bacterial strains capable of in vitro packaging were mixed with up to 1 µl of recombinant phage λ-L47 DNA and incubated for 2 hr at room temperature. Subsequently, 500 µl of bacteriophage buffer (10 mM Tris-HCl pH 7.5, 10 mM $MgSO_4$, 0.01% gelatine) and 10 µl chloroform were added and samples were stored at 4°C.

E.coli strains WL 66 and WL 95 were propagated in overnight cultures (LB

medium, 0.2% maltose, 37°C), centrifuged at 2500 × g and 4°C for 10 min and resuspended in 10 mM cold $MgSO_4$ solution. Infection of bacterials was performed by incubating 0.10-10 µl of the in vitro packaged recombinant bacteriophages with 100 µl of bacterials prepared as described above for 20 min at 37°C. Finally, samples were suspended in 5 ml top agar (0.7% agarose type Seakem ME in LB medium, 10 mM $MgSO_4$). Plates were incubated at 37°C and observed for the appearance of plaques.

Selection of recombinant phage clones was achieved by in situ plaque hybridization as described by Benton and Davis (17). For the propagation of recombinant bacteriophages 100 µl of *E.coli* strain WL-66 or WL-95 were infected with phages harvested from single plaques and layered in top agar onto solid agar plates. Plates were incubated at 37°C until almost complete lysis of bacterials. Then 5 ml of SM medium (5.8 g/l NaCl, 2.5g/l $MgSO_4$, 0.01% gelatine, 0.05 M Tris-HCl pH 7.5) was pipetted onto agar plates and plates were gently shaken for 2 hr at room temperature. The phage-containing medium was removed, and, after addition of 100 µl chloroform, was centrifugated at 4000 ×g for 10 min. The supernatant was stored after the addition of a few drops of chloroform at 4°C.

Recombinant phage DNA was obtained by precipitation of phages in 20% polyethyleneglycol, 2 M NaCl in SM medium and centrifugation at 15,000 × g and 4°C for 20 min. The resulting pellet was resuspended in SM medium containing 0.1% $NaDodSO_4$, 5mM Na_2EDTA and 100 µg/ml proteinase K and incubated for 1 hr at 68°C. Finally, the DNA was extracted with phenol-chloroform and precipitated with ethanol.

Southern blot and hybridization

DNA fragments separated in 1% agarose gels were transferred to nitrocellulose filters according to Southern (18) and Wahl et al. (19). Nitrocellulose filters were dried, baked 2 h at 80°C, rewetted in 6× SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate) for 15 min and prehybridized for 3 h at 68°C in 10× Denhardt's solution (20), 6× SSC, 0.1% SDS and 100 µg/ml denatured herring sperm DNA. In cross-hybridization experiments, hybridization was carried out for 48 hr at 68°C in 1 M NaCl, 50 mM sodium phosphate buffer pH 6.5, 5 mM Na_2EDTA, 10× Denhardt's solution, 0.5% $NaDodSO_4$ and 100 µg/ml of denatured herring sperm DNA. DNA probes used for hybridization were labelled by nick translation (21) with α^{32}P-dCTP to a specific activity of 10^8 cpm/µg DNA. Filters were washed once for 3 h at 68°C in 1 M NaCl, 50 mM Tris-HCl pH 8.5, 2 mM Na_2EDTA and 1% SDS and twice for 90 min at 68°C in 0.5 m NaCl, 50 mM sodium phosphate buffer pH 6.5, 2 mM Na_2EDTA and 0.5% $NaDodSO_4$. Subsequently, filters were rinsed with 6× SSC, dried, and subjected to autoradiography on Kodak XAR5 film using intensifying screens.

To suppress nonspecific hybridization between DNA regions of high (G + C) content, 50 µg/ml of polyG and polyU,G were added in prehybridization and hybridization experiments performed under high stringency conditions. In these ex-

periments filters were additionally washed for 2 hr at 68°C in 0.5x SSC, 0.1% SDS.

DNA sequencing

Nucleotide sequencing was performed as described by Maxam and Gilbert (22). The 3′ ^{32}P-labelled DNA reisolated from agarose gels was divided in two 5 µl and two 10 µl portions each and was incubated with the appropiate reaction mixtures (according to the Maxam and Gilbert method). Reactions were stopped, DNA samples were ethanol-precipitated, washed twice in 70% ethanol, redissolved in 50 µl 1 M piperidin and incubated for 45 min at 90°C. Finally, samples were lyophilized to remove remaining piperidin. Sequencing gels were 0.4 mm 6–20% polyacrylamide gels and were run at a constant temperature of 55°C and 40 V/cm. Following electrophoresis, gels were washed twice in 10% acetic acid, dried, and subjected to autoradiography.

Sequencing of the cellular and viral DNA was performed as follows. The 1400 bp *Hpa* II subfragment of the 1.9 kbp genomic DNA insert of clone HEL 1920 was isolated and labelled with the Klenow fragment of *E.coli* DNA polymerase I at its 3′ ends. After *Eco*RI cleavage, the 3′*Eco*RI/*Hpa* II 430 bp subfragment was recovered from agarose gels and its nucleotide sequence was determined. The complementary strand was sequenced by *Eco*RI cleavage of the 1.9 kbp *Sst*II fragment, labelling of the resulting two subfragments at the 3′ end of their *Eco*RI site with Klenow enzyme and recovery of the 430 bp subfragment.

The 530 bp cross-hybridizing *Sst*II subfragment of the viral *Bam*HI G fragment was cleaved with *Hpa*II. The resulting 410 bp subfragment was labelled at the 3′ end of its *Hpa*II site by Klenow enzyme and the nucleotide sequence was determined. Thirty bp upstream the *Hpa*II site, an *Hin*f I recognition site was detected which divided the DNA fragment in a 5′ 370 bp and a 3′ 150 bp subfragment. Both subfragments were labelled at their *Hin*fI site and sequenced to their *Sst*II end.

Results

To investigate the cross-hybridization between the genome of HSV-2 and DNA of WI-38 cells, *Bgl* II-fragments of the DNA of HSV-2 strain HG52 were cloned into the plasmid pNEO. Recombinant plasmids comprising the complete genome of HSV-2 were selected and the inserts were used as probes on genomic blots of *Hin*d III cleaved cellular DNA under standard hybridization conditions. In these experiments no cross-hybridization was observed when viral DNA fragments of the U_L and U_S region were used. When the hybridization stringency was lowered, e.g., by hybridization at 55°C, extensive signals were obtained throughout the whole genomic DNA slot, which indicates the formation of nonspecific hybrids, probably between (G + C) rich sequences (data not shown).

Hybridization with viral fragments comprising the inverted repeat regions (*Bgl* II-A, B, E, F) also led to hybrid formation throughout the whole cellular DNA slot (Fig. 1). In addition, however, dominant bands were observed in these experiments at a position of approximately 15 kbp. In order to test the specificity of this hybridization, experiments were repeated under conditions which should suppress nonspecific hybrid formation by (G + C) rich tracks (9). This was achieved by addition of 50 µg/ml of polyG, polyU,G during hybridization and changing of washing conditions to high stringency conditions. Hybridization under these conditions led to a significant reduction of hybridization signals throughout the cellular genome, whereas the dominant band at 15 kbp was consistently observed (Fig. 1). Similar results were observed with all *Bgl* II fragments harboring the inverted repeat region, indicating possible sequence homologies between the respective viral and cellular DNA fragments.

To define more precisely such homologies on a molecular level, the 15 kbp region of *Hin*dIII digested HEL-cell DNA was recovered from LMP-agarose gels and cloned in bacteriophage λ L47 DNA. After in vitro packaging recombinant

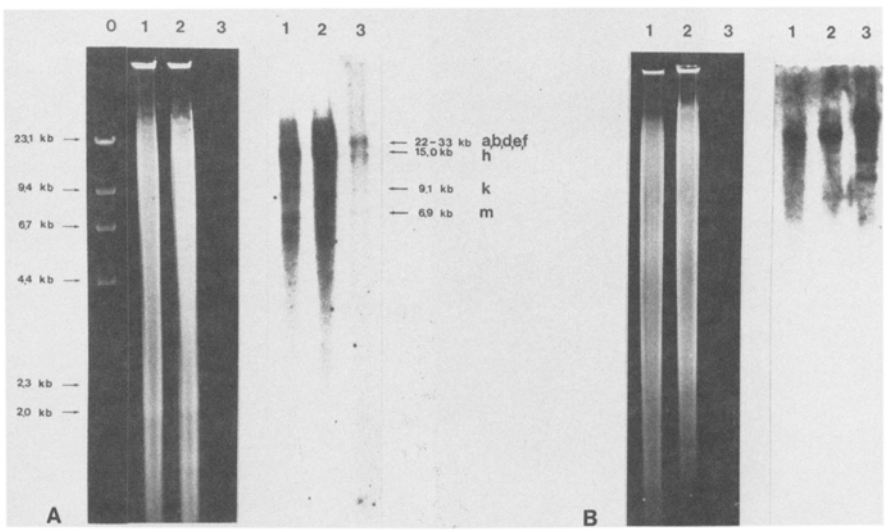

Fig. 1. Hybridization between genomic DNA and the *Bgl*II-B fragment of HSV-2. A: 50 µg *Hind*III digested HeLa DNA (lane 1), 50 µg *Hind*III digested WI-38 DNA (lane 2), and 10 ng *Bgl*II digested HSV-2 DNA (lane 3) were subjected to electrophoresis through a 1% agarose gel and visualized with ethidium bromide. Southern blots were hybridized with the nick-translated HSV-2 *Bgl*II-B fragment for two days at 68°C under normal conditions resulting in the autoradiograph shown. B: The same experiment was carried out under stringent conditions (including the addition of 50 µg/ml polyG and 50 µg/ml polyU,G during hybridization and a final washing step of 2 h at 68°C in 0.2× SSC/0.1% NaDodSO$_4$). Lane 0 represents 1 µg bacteriophage λ-DNA (*Hind* III-cut) as a molecular weight marker. (Autoradiographs of figures 1A and B were exposed for one and five days, respectively.)

phages were selected from infected lysogenic strains of *E.coli* and were subjected to in situ hybridization with the HSV-2 *Bgl*II B fragment in order to detect recombinant phages with cellular inserts cross-hybridizing to viral DNA. Inserts of hybridization positive clones were subcloned into the *Hin*dIII site of plasmid pBR322 and their cross-hybridizing activity was again determined by Southern blotting with HSV-2 *Bgl*II fragments comprising the inverted repeat regions (e.g., *Bgl*II-B). Finally, a 15 kbp *Hin*dIII fragment of the genomic DNA (clone HEL63) with strong cross-hybridizing activity under stringent conditions was selected and further analyzed (figure 2).

Both the viral *Bgl*II-B fragment and the cellular insert of clone HEL63 were cut with the restriction enzymes *Eco*RI, *Bam*HI, *Bgl*II, *Sst*I, *Sst*II, and *Hpa* II to precisely determine the position of the cross-hybridizing region. A 4.5 kbp *Eco*RI subfragment, a 4.0 kbp *Bgl*I subfragment and a 4.0 kbp *Bgl*II subfragment of clone HEL 63 showed cross-hybridization with the HSV-2 *Bgl*II-B fragment. In further

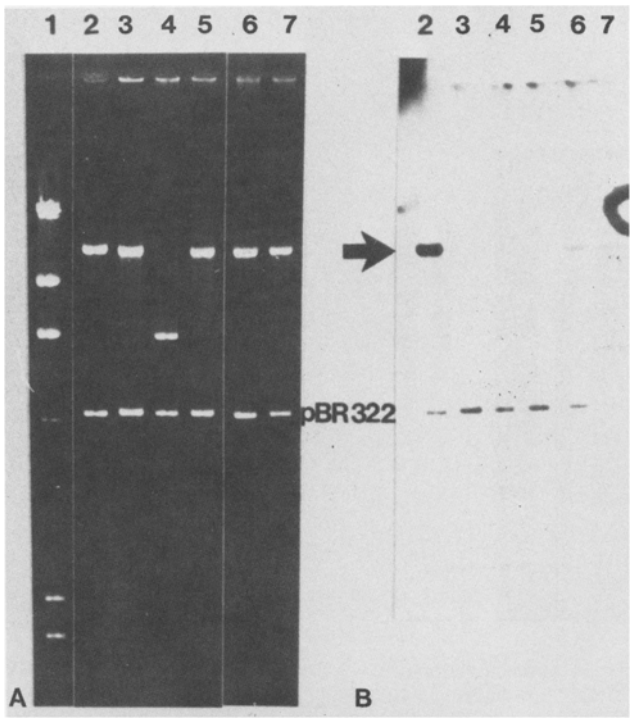

Fig. 2. Hybridization of different cellular DNA fragments cloned into pBR 322 with the *Bgl* II-B fragment of HSV-2. A: ethidium-bromide stain; B: autoradiograph. Recombinant plasmids were analyzed on 0.8% agarose gels, blotted on nitrocellulose filter and hybridized with a nick-translated HSV-2 *Bgl* II-B fragment under stringent conditions. Lane 1: 1 µg bacteriophage λ-DNA (*Hind* III-cut) as molecular weight marker; lane 2: 0.5 µg DNA of clone HEL63 (*Hind* III-cut); lanes 3 to 7: other cellular DNA fragments (*Hind* III-cut) without apparent cross-hybridization.

experiments, the cross-hybridizing region could be confined to a 1.9 kbp *Sst*II-subfragment of HEL63 which was cloned into the plasmid pNO1523 (clone HEL1920; data not shown). Vice versa, this cellular *Sst*II-subfragment under standard conditions was able to hybridize strongly with the HSV-2 *Bam*HI subfragments C, G and P, all harboring parts of the viral inverted repeat regions. Under stringent conditions, however, only the *Bam*HI-G subfragment showed positive hybridization signals (Fig. 3).

To further confine the region of homology between the two DNA fragments, clone HEL1920 was mapped with several restriction enzymes by hybridization

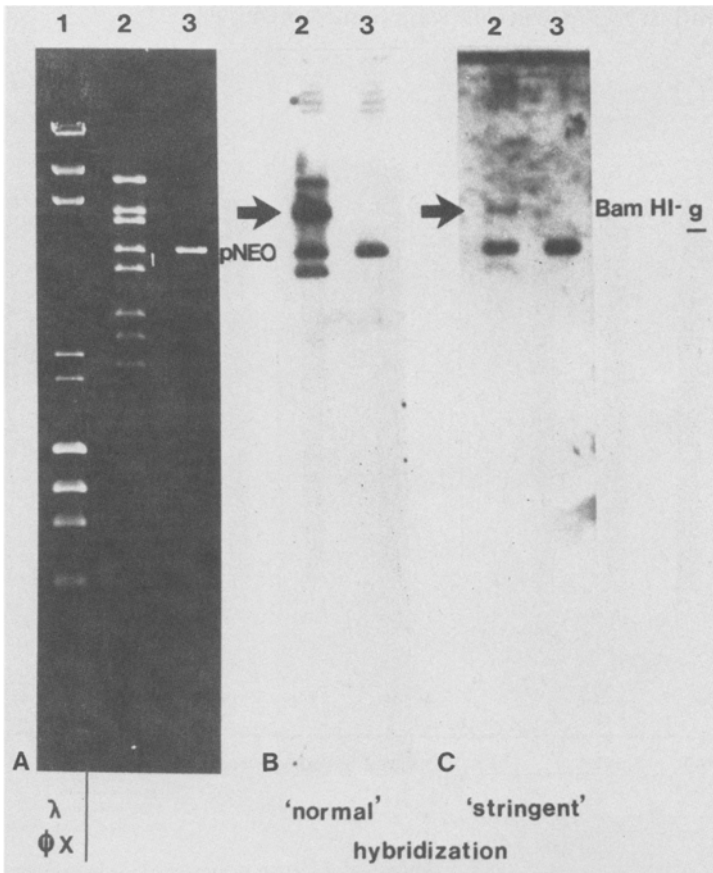

Fig. 3. Hybridization of the HSV-2 joint DNA-fragments (*Bgl*II-B cut with *Bam*HI) with cellular clone HEL1920. A: 1% agarose gel of phage λ/phage ϕ × 174 markers (lane 1), *Bam* HI fragments of HSV-2 *Bgl*II-B cloned in pNEO (lane 2), and *Bam*HI fragments of plasmid pNEO (lane 3). B: Southern blot of the left-hand gel hybridized with the nick-translated cellular clone HEL1920 (which is the cross-hybridizing 1.9 kbp *Sst*II subfragment of HEL63 cloned in plasmic pNO1523) under normal conditions. C: the same experiment under stringent hybridization conditions.

and partial digest analysis. Of the resulting subfragments, a 480 bp *Bgl*I-, a 680 bp *Eco*RI-, a 880 bp *Sst*I-, a 1100 bp *Bgl*II-, and a 1400 bp *Hpa*II-subfragment showed hybridization signals with the HSV-2 *Bam*HI-G fragment, indicating that the locus of homology would be the only region common to all these subfragments, which is a 230 bp DNA fragment between the unique *Bgl*I- and the 3'-neighboring *Hpa*II-restriction site (Fig. 4). The viral *Bam*HI-G fragment was cloned into pBR322 and cut with several restriction enzymes. The resulting subfragments were in turn hybridized with the cellular insert of clone HEL1920 under stringent conditions that led to the detection of a 530 bp *Sst*II subfragment with cross-hybridizing activity. It was interesting to note that this subfragment of *Bam*HI-G was located exactly at the joint between the long and the short region of the HSV-2 genome within the inverted repeats (data not shown).

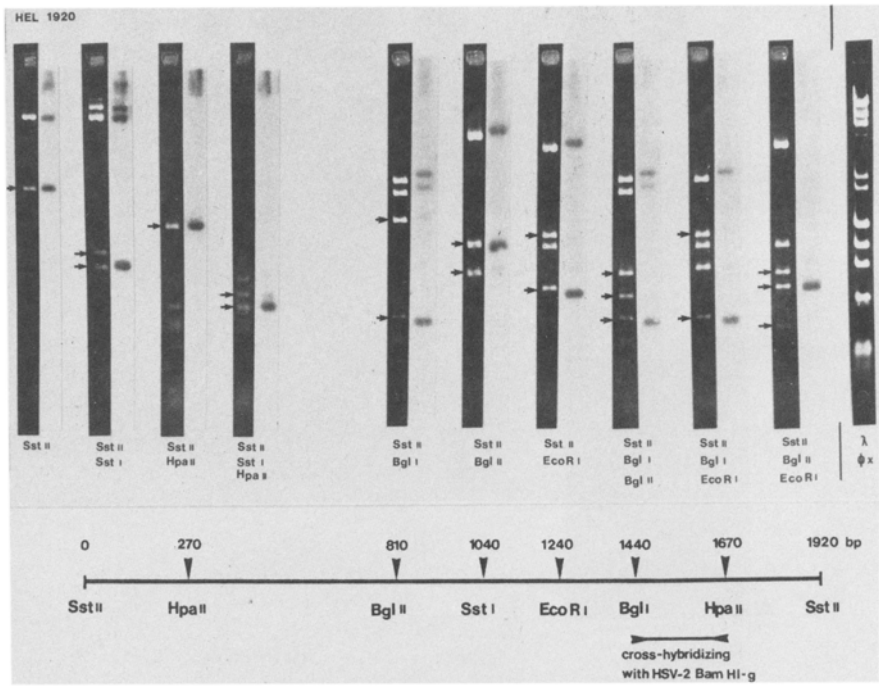

Fig. 4. Restriction enzyme analysis of cellular clone HEL1920 and cross-hybridization with HSV-2 *Bam*HI-G. The 1.9 kbp cellular insert of HEL1920 was separated from the vector and further digested with the restriction enzymes indicated. Arrows indicate subfragments of the 1.9 kbp *Sst*II-fragment. The agarose gel lanes were transferred to nitrocellulose filters and hybridized with the nick-translated, cloned HSV-2 *Bam*HI-G fragment. The information of this and other experiments led to the restriction map of the 1.9 kbp *Sst*II fragment shown. The region common to all cross-hybridizing subfragments is underlined.

To determine exactly the extent of homology, the two cross-hybridizing regions were sequenced in both directions and, as far as possible, in overlapping fragments following the procedure of Maxam and Gilbert (22) as given in Materials and Methods. Fig. 5a gives the 422 bp nucleotide sequence between the *Eco*RI- and *Hpa*II- restriction site of the cellular 1920 bp *Sst*II fragment. Of the 530 bp cross-hybridizing *Sst*II subfragment of the viral *Bam*HI-G fragment, 498 bp were sequenced using the strategy described in Materials and Methods. This 498 bp nucleotide sequence is given in figure 5b and shows the viral "a-sequence" with an, at least, 16-fold direct repeat of 15 bp in 5′ direction which corresponds to DR 2 of HSV-1.

Both the cellular and the viral sequence are characterized by a high (G + C) content of 66% and 81%, respectively, and by multiple inverted repeats allowing the formation of various stem-loop structures.

The extent of homology between the two nucleotide sequences obtained (as determined by computer-aided alignment studies) was 56% for the viral "a-sequence" as compared to the cross-hybridizing cellular DNA sequence between the *Bgl*I- and the *Hpa*II-site. Within this alignment, a central, highly homologous region of 43 bp with 34 corresponding nucleotides was found that accounts for a homology of 79%. This region, however, is characterized by an extremely high (G + C) content of 86% and 93%, respectively, for the cellular and viral subsequence.

Discussion

In the present study, the occurrence of cross-hybridization could be demonstrated between DNA fragments of the HSV-2 genome comprising the viral inverted repeat region and cellular DNA. In contrast to previously published data (9), cross-hybridization between viral and cellular DNA of approximately 15 kb length after digestion with *Hin*d III was observed even under conditions suppressing nonspecific binding of (G + C) rich sequences. From the relative intensity of the hybridization signal, it was calculated that the cellular sequences responsible for hybridization are approximately 1000-fold repeated that is in accordance with earlier studies (5).

Most extensive cross-hybridization could be confined to a 530 bp *Sst*II subfragment of the *Bam*HI G fragment of HSV-2 and a 230 bp *Bgl*I/*Hpa*II subfragment within a cellular 15 kbp *Hin*dIII fragment.

DNA sequence analysis revealed a high (G + C) content of 81% and 66% of the viral and cellular DNA fragment, respectively. The 116 bp region toward the 3′ terminus of the 530 bp viral *Sst*II DNA fragment was found identical with the known nucleotide sequence of the HSV-2 HG52 "a-sequence" (23). It was interesting to note that, in the viral progeny, an at least 16-fold direct repeat of 15 bp was found at the 5′ end, that is likely to correspond to the DR2 of HSV-1 (24,25). Within this 15 bp direct repeat, a hexanucleotide sequence $\frac{5'GGGCGG3'}{3'CCCGCC5'}$ is located that

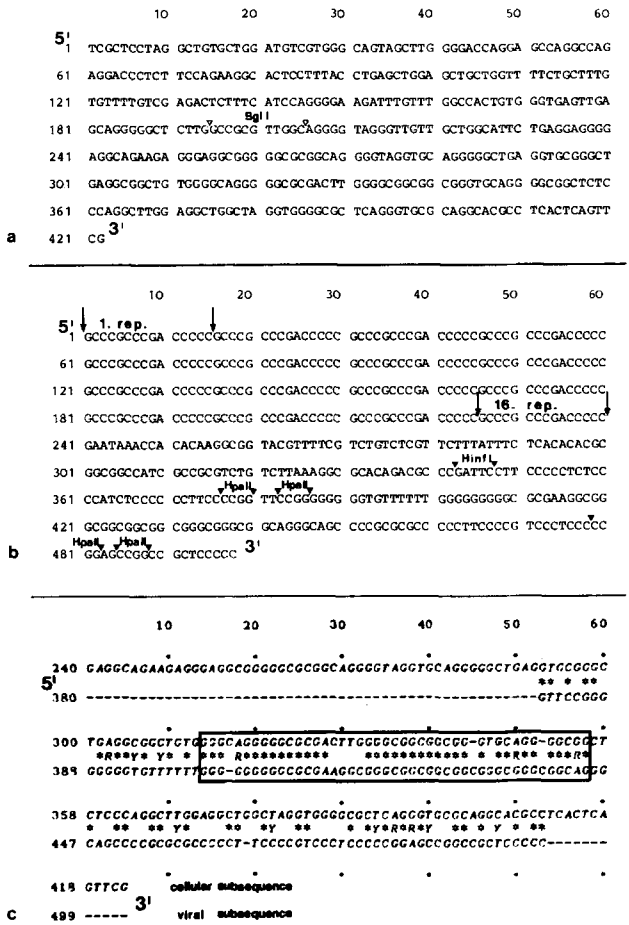

Fig. 5. A: Nucleotide sequence of the cross-hybridizing region of clone HEL1920. The 422 bp sequence is given from the *Eco*RI- to the *Hpa*II-site in (5′ → 3′) direction. The *Bgl*I-site indicates the beginning of the cross-hybridizing region toward the 3′ terminus. B: Nucleotide sequence of the cross-hybridizing region of HSV-2 *Bam*HI-G. The 498 bp sequence is given in (5′ → 3′) direction, with the at least 16-fold direct repeat of a sequence of 15 bases at the 5′ terminus, and the 116 bp part of the viral "a-sequence" at the 3′ terminus. The *Hin*fI- and the *Hpa*II-restriction sites are indicated. C: Alignment between the cross-hybridizing cellular and viral subsequences. The two aligned nucleotide sequences are shown in the same orientation as in figures 5a, b; the alignment was carried out between the cellular region flanked by the *Bgl*I- and the *Hpa*II site and the viral 116 bp region at the 3′ terminus which belongs to the "a-sequence" of HSV-2. The total homology of the two subsequences amounts to 56%, the indicated region of high homology represents 79% base matching within a 43 bp sector. *R* denotes purine analogues, *Y* denotes pyrimidine analogues.

is known to represent the consensus sequence for binding of the nuclear factor Sp 1. Recent studies have demonstrated that Sp 1 is involved in the transcriptional regulation of viral genes in mammalian cells (26) and Sp 1 binding sequences and in vitro binding of Sp 1 within the promoter region of viral genes was observed for HSV immediate-early (27) and early genes (28), for the SV-40 early promoter (29), and recently for HIV-1, HIV-2 and STLV-III/HTLV-IV (30). In addition, it has been shown that competent Sp 1 binding requires at least 10 bp and that effective binding of Sp 1 is widely dependent on the flanking sequences (31).

In this context it shall be noted that the terminal "a-sequence" of HSV-1 (F) contains the promoter-regulatory domain and the transcription initiation site of a diploid gene located in the viral "b-sequence" and probably regulated as γ_1-gene (32). According to its predicted size of 358 amino acids it was designated ICP34, 5. In the coding region of this gene a number of direct repeats was observed of which a 10-fold 9 bp direct repeat, designated R3, is homologous to the 16-fold direct repeat reported here. R3, however, does not contain a Sp-1 binding sequence. Furthermore, the inverted repeats flanking the L component could be demonstrated to contain the gene coding for ICPO (32,33). Thus, it may be speculated that the 16-fold 15 bp direct repeat is involved in the transcriptional regulation of adjacent genes.

The presented cellular sequence is not identical with any of the known eukaryotic nucleotide sequences and therefore represents a newly sequenced part of the human genome. It is interesting to note that the cellular DNA sequence between the *Bgl*I- and *Hpa*II- site as well as the viral "a-sequence" show a homology of 60% to several published sequences (34,35,36) of the cellular and viral myc oncogene. Similar data have been presented concerning the homology between the myc oncogene sequences and fragments of human cytomegalovirus (HCMV) DNA (37,38). According to McLachlan and Boswell (39), however, great care must be taken in the interpretation of data concerning homologies of not obviously related sequences. Although the sequence homology reported here appears to be significant in a binominal approximation, it may not be supported by the full analysis as proposed by these authors.

In computer-aided alignment studies we have found an overall homology of 56% between the HSV-2 "a-sequence" and the respective cellular DNA sequence. Both sequences, however, contain a 43 bp stretch with 34 corresponding nucleotides that accounts for a homology of 79%. This region is characterized by an extremely high (G + C) content of 86% and 93% for the viral and the cellular DNA, respectively. As the hybridization and washing conditions used in this study were chosen to permit renaturation of perfectly matched hybrids as well as of hybrids with up to 25% base mismatch, it should have been possible to detect regions of higher homology between the HSV-2 inverted repeats and the cellular genome if such were present. The cross-hybridization between (G + C) rich sequences observed in this study is in accordance with the hypothesis that HSV and the cellular genome share only limited sequence homologies in regions with high (G + C) content. The observation, however, that just the viral "a-seqeunce" is involved in

cross-hybridization to cellular DNA may be of some biological interest with respect to phenomena of latency and/or transformation. Thus, Puga et al. (40) have described the occurence of different oversized terminal DNA fragments during the latency of HSV that hints to an integration of the HSV genome into cellular DNA during the latent state. Thus, the relatively high homology between the central part of the a-sequence and a several hundred-fold repeated cellular subsequence may facilitate the integration of the virus genome at such sites.

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