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Two distant regions of the Epstein-Barr virus genome with sequence homologies have the same orientation and involve small tandem repeats

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The two regions of the Epstein-Barr virus genome (DS_L and DS_R) carrying homologous sequences at distant parts of the long unique region are described. Cleavage of cloned DNA containing the DS_R region with restriction endonucleases revealed a so far unrecognized small tandem repeat of ∿120 base pairs present in ∿20 copies. Heteroduplexes of the DNA of two clones containing DS_L and DS_R respectively, visualized in the electron microscope by cytochrome c spreading, revealed that the region of homology is ∿2.5 kb long, involves small tandem repeats, and has the same orientation in the viral genome. Mica adsorption of the heteroduplex showed, that the homologous region consists of ∿1.5 kb with only partial homology including the small internal repeats and 0.9 kb with well-matched duplexes. When DNA containing the DS_L region reanneals, it can give rise to two single-stranded loops of the same size at different positions suggesting the presence of a row of tandem repeats also in this region.

Key words: Epstein-Barr virus/heteroduplex/tandem repeats/recombinant plasmids/hybridization

Introduction

Epstein-Barr virus (EBV) is carried latently by most human adults. Primary infection with the virus leads to inapparent infection or to infectious mononucleosis. Additionally, the virus is associated with two human malignancies, Burkitt's lymphoma and nasopharyngeal carcinoma (for review, see Epstein and Achong, 1979).

The linear double-stranded DNA genome purified from EBV particles has a molecular weight (mol. wt.) of 120 x 10^6. It consists of terminal tandem repeats of 400 – 500 base pairs (bp) (Given *et al.*, 1979, Kintner and Sugden, 1979), a short and a long unique region of 9 and 90 Md respectively, joined by a variable number of 5 – 12 internal tandem repeats of 2 Md (Rymo and Forsblom, 1978; Given and Kieff, 1979; Hayward *et al.*, 1980). A linkage map of EcoRI, HindIII, and SalI fragments has been provided by Given and Kieff (1978). Comparison of different isolates revealed a great similarity among the DNA of most isolates (Bornkamm *et al.*, 1980; Heller *et al.*, 1981; Fischer *et al.*, 1981) with two exceptions: the non-transforming P3HR-1 strain has a deletion of sequences in the long unique region adjacent to the internal 2 Md repeat, and B95-8 (EBV) (a virus previously regarded as an EBV prototype) has a deletion of 8 Md in the right part of the long unique region (Delius and Bornkamm, 1978; Raab-Traub *et al.*, 1978, 1980; Bornkamm *et al.*, 1980, Heller *et al.*, 1981). An interesting observation was made by Raab-Traub *et al.* (1980), that sequences located at the left-hand side of the long unique region in BamHI-H share homology with sequences ∿70 Md apart at the right-hand side of the long unique region, denoted left and right duplicated sequence (DS_L and DS_R). DS_R is located within the 8 Md, which are deleted in B95-8 virus (Raab-Traub *et al.*, 1980). Interestingly, the region carrying DS_R has been reported to be actively transcribed in Burkitt's lymphoma tumor cells (Dambaugh *et al.*, 1979). Here we describe the homologies between both regions in more detail. We used DNA of M-ABA (EBV), a virus originally derived from the tumor cells of a nasopharyngeal carcinoma (Crawford *et al.*, 1979) and shown to have the genomic organization of EBV wild-type or prototype (Bornkamm *et al.*, 1980).

Results

A novel small internal repeat in EBV DNA

The structural organization of M-ABA (EBV) DNA including HindIII, EcoRI, and SalI sites is shown in Figure 1. The deletion in B95-8 virus DNA is located within the EcoRI-C fragment (Raab-Traub *et al.*, 1978). Two recognition sites for each of BamHI, HindIII, and SalI, are known to be missing in B95-8 virus DNA (Raab-Traub *et al.*, 1980, Bornkamm *et al.*, 1980). The DS_R region, which lies within the deletion of B95-8 virus, is located within the HindIII-D_2 fragment.

In an attempt to identify smaller fragments which contain the complete DS_R region, we have mapped BamHI, BglII, and PstI sites in the M-ABA (EBV) HindIII-D_2 fragment, which had been cloned in pACYC 184 (Chang and Cohen, 1978). Compilation of the mol. wts. of the viral fragments after BamHI and BglII digestion revealed a mol. wt. of 8.9 x 10^6 for HindIII-D_2, which is slightly smaller than the value reported earlier (Bornkamm *et al.*, 1980). PstI cleavage revealed a remarkable feature: the sum of the mol. wts. of the PstI fragments is only 7.45 x 10^6, and a small PstI fragment of 0.08 Md is present at ∿20-fold molar excess. This indicates that the small fragment represents small tandem repeats. Partial digestion of HindIII-D_2 with PstI, in addition to complete cleavage with HindIII, showed a ladder-like pattern spanning from 0.08 Md up to ∿1.6 Md and revealed ∿20 repeats (Figure 2). The mol. wt. of the repeat was estimated from the partial PstI digests in Figure 2. Additionally, the partial digestion with PstI revealed the order of PstI fragments as shown in Figure 3. The 1.8 Md and 3.3 Md fragments are involved in ladder or smear formation above their respective mol. wts. and are thus neighbouring the small repeats. Partial digestion products of 2.3 and 4.1 Md can well be discriminated indicating that the 2.1 Md, 0.2 Md, and 1.8 Md fragments lie adjacent to each other.

The order of BamHI and BglII fragments of the HindIII-D_2 clone, which is shown in Figure 3, was deduced from double digestions with BamHI/PstI, BglII/PstI, and BamHI/BglII (data not shown).

Cleavage of the region containing the small internal repeats with a number of other restriction endonucleases revealed recognition sites in the repeat for AvaI and SacI, which includes an AluI site.

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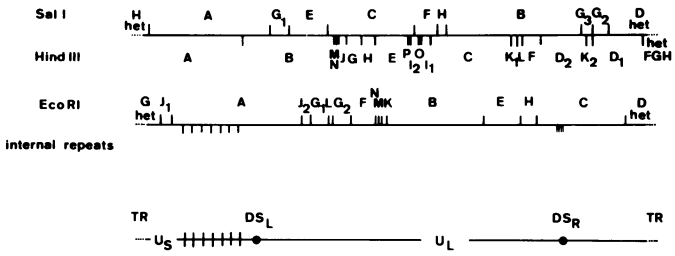

Fig. 1. Organization of EBV DNA and linkage of EcoRI, HindIII and SalI fragments of the M-ABA (EBV) strain. The viral DNA consists of terminal repeats (TR), a short and a long unique region (U$_S$-9 Md and U$_L$-90 Md) joined by a variable number of internal repeats (IR-2 Md). Two regions in the long unique region have sequence homologies (DS$_L$ and DS$_R$).

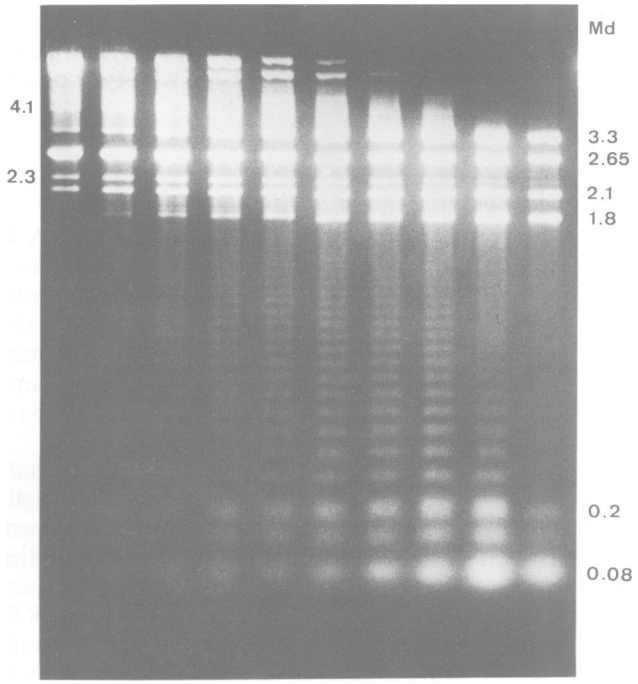

Fig. 2. Partial digestion of HindIII-D$_2$ with PstI. 5 μg of recombinant plasmid DNA containing HindIII-D$_2$ were digested to completion with HindIII in a volume of 50 μl before the addition of 1.5 units PstI. The mixture was incubated for 5, 10, 15, 20, 30, 40, 50, 60, 90, 120 min at 37°C (from left to right). In the aliquots the reaction was stopped by the addition of loading buffer at 0°C. Fragments were separated on a 1% agarose gel for 15 h at 30 V. The mol. wts. at the right indicate the size of HindIII-D$_2$. PstI/HindIII double cut fragments. The mol. wts. at the left side indicate the size of partial PstI digestion products.

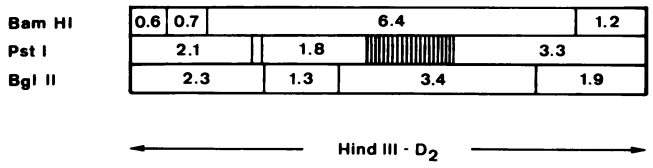

Fig. 3. Order and size of BamHI, BglII, and PstI framents in HindIII-D$_2$. The small PstI fragments are 0.2 and 0.08 Md.

Different numbers of small internal repeats account for the diversity in size among HindIII-D$_2$ fragments of different strains

It has been shown previously that the HindIII-D$_2$ fragment of M-ABA virus is 0.3 Md smaller than that of CC 34-5 and QIMR-WIL (EBV) (Bornkamm *et al.*, 1980). EcoRI-C and BamHI-B$_1$ fragments have also been reported to have slightly different sizes in different virus isolates (Rymo *et al.*, 1979;

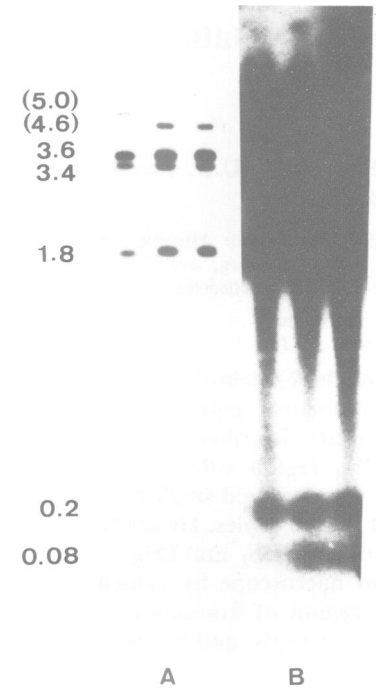

Fig. 4. Autoradiograms of blots containing separated PstI fragments of CC 34-5 (EBV), QIMR-WIL (EBV), and M-ABA (EBV) DNAs after hybridization with ^{32}P-labelled DNA of the recombinant plasmid containing HindIII-D$_2$. **a** is a short, and **b** a long exposure of the same autoradiogram. The mol. wts. put in parenthesis are those of the fragments containing the DS$_L$ region (5.0 Md of CC 34-5 virus, 4.6 Md of QIMR-WIL and M-ABA virus).

Raab-Traub *et al.*, 1980). BamHI-B$_1$ is located within HindIII-D$_2$ and the latter within EcoRI-C. All these fragments contain the small internal repeats. It was therefore assumed that the size variability in this region reflects the presence of different numbers of repeats in different virus strains. To test this hypothesis, viral DNA of CC 34-5, QIMR-WIL, and M-ABA (EBV) was digested with PstI and the fragments were separated in 0.8% agarose. The DNA was transferred to nitrocellulose and hybridized to ^{32}P-labelled DNA of the HindIII-D$_2$ clone. The result is shown in Figure 4. The interpretation is complicated by the fact that the labelled probe not only recognizes fragments carrying HindIII-D$_2$ sequences, but also the fragment containing the DS$_L$ region (4.6 Md for QIMR-WIL and M-ABA, and 5.0 Md for CC 34-5 virus). All PstI fragments of CC 34-5, QIMR-WIL and M-ABA (EBV) have the same size except for the fragment containing the DS$_L$ region, which is not derived from HindIII-D$_2$. Since all PstI fragments carrying HindIII-D$_2$ sequences have the same size among these strains, even though the size of the HindIII-D$_2$ fragments varies, we conclude that different numbers of small internal repeats account for the size variation observed in this part of the genome.

The cross hybridizing regions DS$_L$ and DS$_R$ have the same orientation and involve the small internal repeats

Raab-Traub *et al.* (1980) located the cross hybridizing region in two internal HinfI fragments of 1.06 and 2.1 Md within BamHI-H (DS$_L$) and in two internal HinfI fragments of 0.5 and 2.4 Md within BamHI-B$_1$ (DS$_R$) of the W91 (EBV) strain.

In an attempt to determine the site of the DS$_R$ region relative to the small internal repeats, we could narrow down the DS$_R$ region to the 3.4 Md BglII fragment shown in Figure 3. The DS$_L$ region was localized to 2.9 Md, overlapping bet-

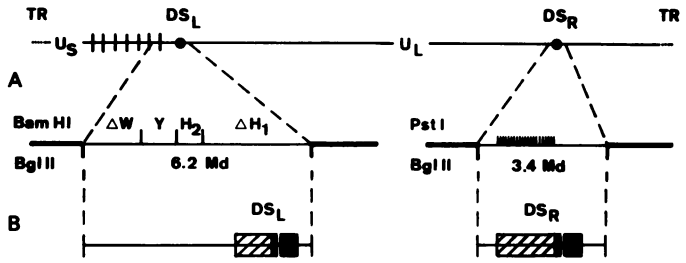

Fig. 5. Schematic representation of the recombinant plasmids containing DS_L and DS_R, linearized at the EcoRI site of the vector. The viral inserts are BglII fragments cloned into the BglII site of pHC 79. The result of the heteroduplex analysis is shown in **B**. The region of perfect homology is marked black, that of partial homology is dashed. Between both regions there may be a region of non-homology.

ween BamHI-H and a 6.2 Md BglII fragment.

To analyze heteroduplexes between fragments containing DS_L and DS_R in the electron microscope, BglII fragments containing DS_L and DS_R respectively, were cloned into the BglII site of the vector pHC 79 (Hohn and Collins, 1980). Maps of both clones are shown in Figure 5a. For heteroduplex formation DNA of both clones was linearized by a single EcoRI cut into the vector DNA, thus leaving 2.7 kb and 2.05 kb vector sequences at the ends.

Figure 6a shows a cytochrome spreading of a heteroduplex formed between DNAs from two clones containing the DS_L and DS_R region inserted into the vectors in the same sense with respect to their orientation in the viral genome. In this case the vector strands form a duplex at both ends, and homologies between the insert strands lead to duplex formation in the center of the structure. When heteroduplexes are formed between clones carrying the regions of sequence homology in the opposite sense, either the vector termini can reanneal while the insert regions show no interstrand homology (Figure 6b), or the homologous sequences in the center of the inserts can reanneal with each other (Figure 6c). The single strands of both DS_L and DS_R clones show some repetitive hairpin formation in the regions of the insert DNA as marked by the open arrows in Figure 6b and 6d. As shown in the schematic diagram (Figure 6e) the region of sequence homology is $\sim$2.5 kb in length. Small knobs and discontinuities in the double strand point to some mismatching in parts of the heteroduplex. Since the 3.4 Md BglII clone contains only $\sim$600 bp of EBV DNA to the left and 2100 bp to the right of the small internal repeat, the small repetitions of the DS_R region must participate in the heteroduplex formation and, therefore, must have a counterpart in the DS_L region. The very high standard deviations in all measurements involving the junction between the non-homologous parts to the left and the duplex involving the small repeats can probably be explained by reannealing of the small repeats out of frame. As a consequence looped-out regions will appear within the heteroduplex and varying numbers of the small repeats will participate in the duplex formation.

Another interesting feature emerging from the heteroduplex analysis is the presence of an inverted repeat of $\sim$400 bases in the 6.2 Md BglII clone. This inverted repeat is $\sim$1.4 kb away from the left BglII site and lies within the 2.5 kb derived from the large internal repeat of the EBV genome.

The cross hybridizing region consists of 0.9 kb with good and 1.5 kb with partial homology

To obtain more information about the degree of sequence homology between DS_L and DS_R, the heteroduplexes were visualized at higher resolution using mica adsorption (Portmann and Koller, 1976). Single-stranded DNA may be discriminated from double-stranded DNA with phage T_4 gene 32 protein, which binds to single strands and thus enlarges the diameter of single as compared to double strands (Priess *et al.*, 1980). Figure 7a shows heteroduplexes as visualized by the mica adsorption technique. The double strands at both ends consist of vector DNA, whereas the viral inserts start at the transition from double to single strands. As in the cytochrome c spreading, there is one large and one small loop of non-homologous sequences flanking the regions of homology. It becomes apparent, however, that the use of gene 32 protein leads to a more stringent discrimination between perfect and partial homology than obtained in cytochrome c spreadings. Adjacent to the small loop at the right-hand side there is a region of $\sim$900 bp which exhibit perfect duplex formation as judged by electron microscopy except for 115 $\pm$ 30 bases (diagram in Figure 7b). The degree of homology is markedly lower in the left part exhibiting a chain of small loops (95 $\pm$ 20 bases) interrupted by short double strands (37 $\pm$ 8 bp). On both sides of this chain single-stranded loops larger than 95 $\pm$ 20 bases, and variable in size are observed.

The small inverted repeat described above is also clearly discernable in the heteroduplex prepared by mica adsorption. It consists of a double-stranded stem of $\sim$70 bp and a single-stranded loop of $\sim$290 bases. The region of partial homology carrying the small loops undoubtedly represents the small internal repeats and their counterparts from DS_L. The loop larger than 95 $\pm$ 20 bases observed at the junction may either represent non-homologous sequences or may be formed by looping out some of the repeats. This may happen if the hybridization reaction started within the repeats, but out of the original frame.

Duplexes involving a frame-shift, are not only observed in heteroduplexes, but also occasionally, when the DNAs containing the DS_L or DS_R region are self-annealed. Self-annealing may lead to the appearance of two single-stranded loops of identical size on both sides of the matched region. This is shown for the clone containing the DS_L region in Figure 7c. Since these phenomena suggest the presence of tandem repeats, it is assumed that the DS_L region also contains small repetitive units which share about one third of their sequences with the small internal DS_R repeat. This presumed DS_L repeat was not detected by cleavage with restriction endonucleases. Out of 20 enzymes which were tested (AluI, AvaI, AvaII, BclI, BglI, BstEII, HinfI, HincII, HpaI, MboII, PvuII, PstI, SacI, SacII, SmaI, Sau3A, TaqI, XbaI, XhoI, and XorII) no restriction endonuclease was identified which generates a hypermolar fragment similar in size to the DS_R repeat.

Discussion

We have studied the two regions of homology in the long unique region of EBV DNA. The right part (DS_R) contains a small, so far unrecognized repeat of $\sim$120 bp, which is present in $\sim$20 copies within the cloned M-ABA HindIII-D_2 fragment. This small internal repeat has restriction cleavage sites for PstI, AvaI, and SacI. The latter includes an AluI site within its recognition sequence. Differences in the size of HindIII-D_2 fragments of different virus strains could be accounted for by different numbers of this repeat within this fragment. Heteroduplex analysis of cloned DNA fragments

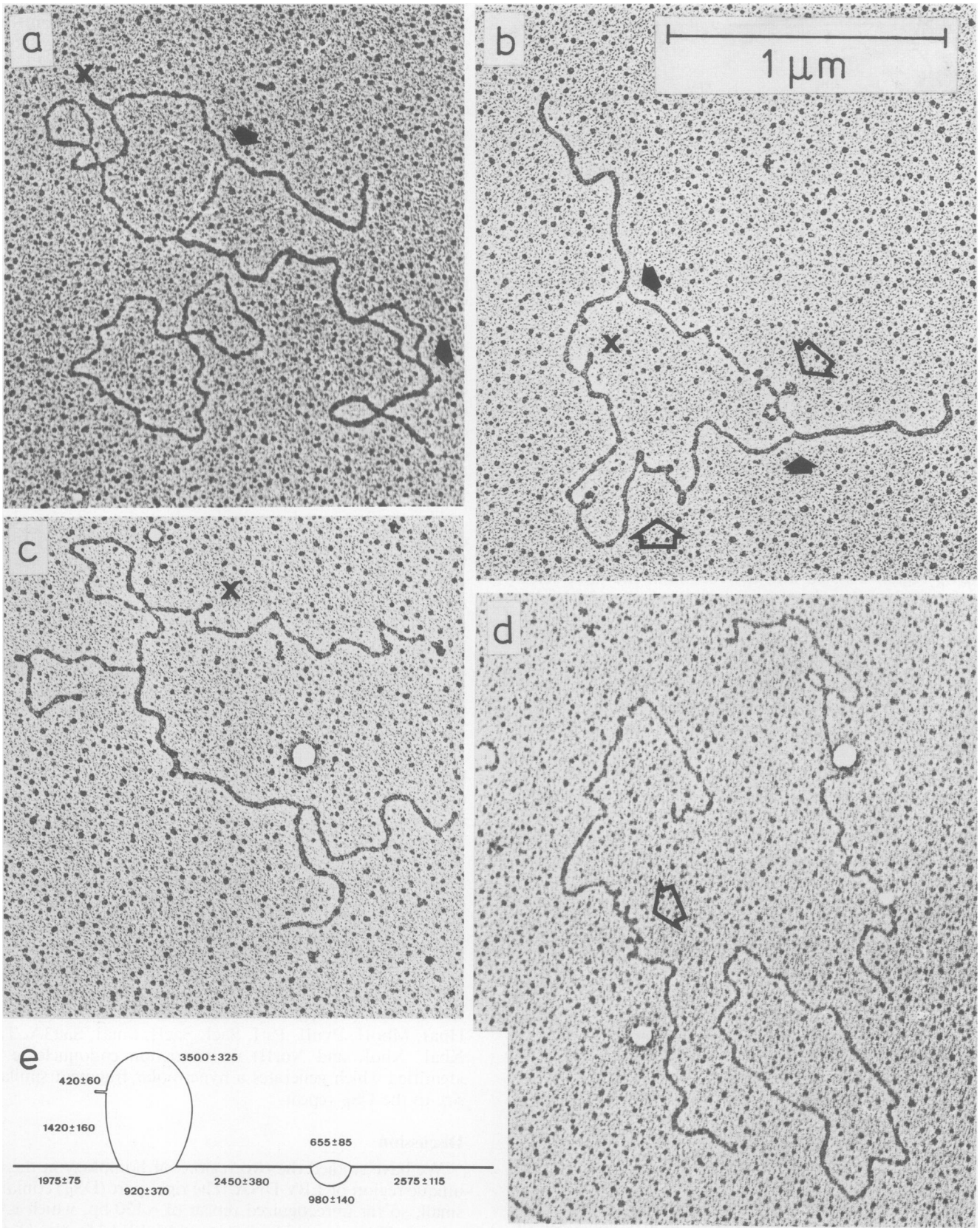

Fig. 6. Electron micrographs of heteroduplexes, visualized by cytochrome c spreading, formed between recombinant plasmid DNAs containing DS_L and DS_R. The two clones forming the heteroduplex in **a** contain DS_L and DS_R in the same sense with respect to their orientation in the viral genome. Heteroduplexes between clones carrying DS_L and DS_R in the opposite direction are shown in **b** and **c**, revealing either duplex formation of the vector **(b)** or of the insert **(c)**. The dark arrows point to the transitions between vector and insert sequences. **d**, single strands of the plasmid containing DS_R together with M13 DNA used as length standard. The open arrows point to hairpins and fold-back structures observed in the single strands of both clones **(b, d)**. The cross marks the position of an inverted repeat within the large internal repeat **(a, c)**. **e**, a schematic representation of length measurements obtained from heteroduplexes as shown in **a**. Sizes are given in nucleotides.

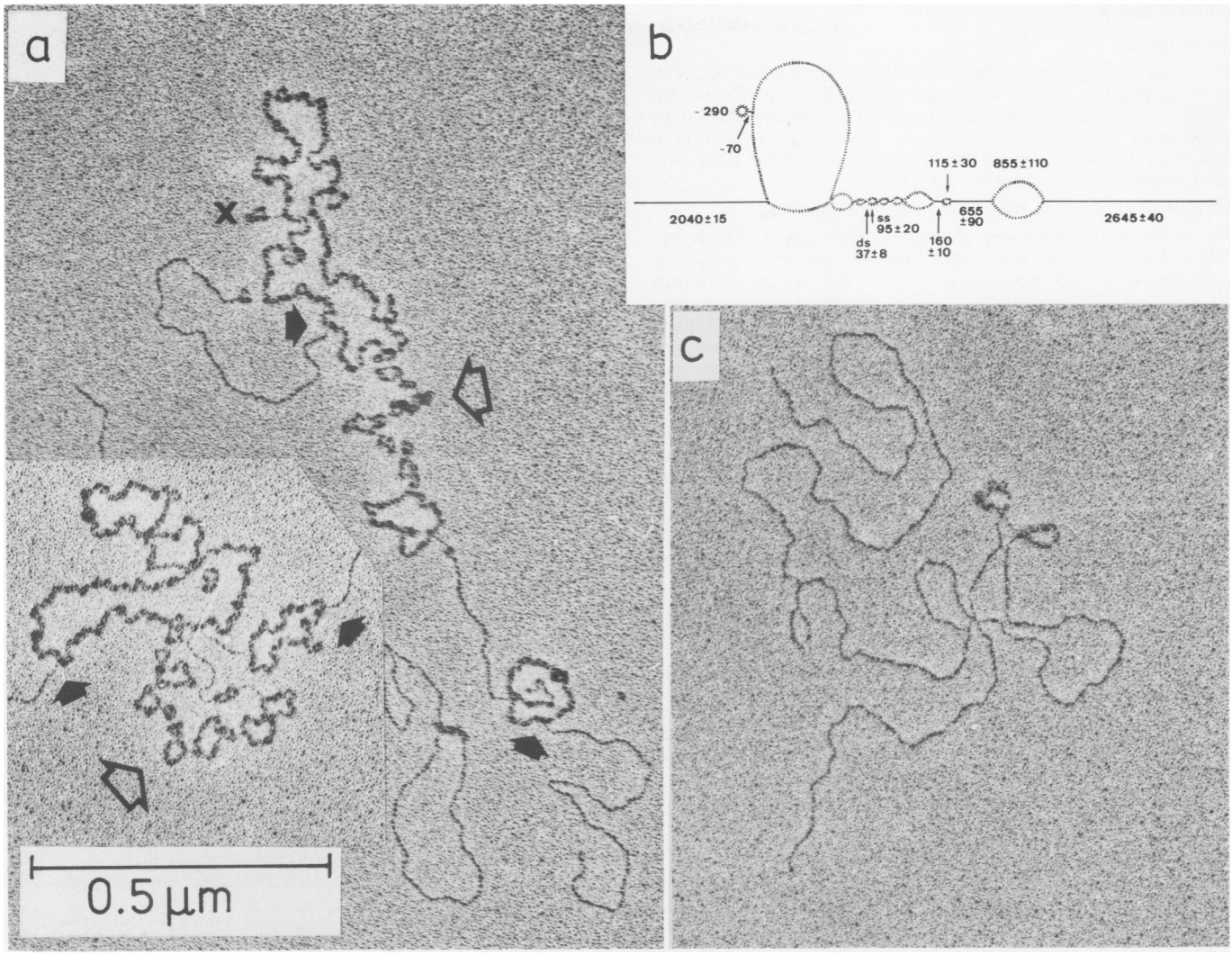

Fig. 7. Heteroduplexes visualized by mica adsorption. **a** Heteroduplexes formed between recombinant plasmids containing DS_L and DS_R in the same sense with respect to their orientation in the viral genome. The dark arrows point to the transitions between vector and insert sequences, the open arrows to the regions carrying the small internal repeats. The cross points to the inverted repeat as shown in Figures 6a and 6c. **b** Schematic representation of length measurements obtained from the heteroduplexes prepared by mica adsorption. **c** Self-annealed DNA of the recombinant plasmid containing DS_L. Two single-stranded loops suggest the presence of a row of tandem repeats.

containing DS_L and DS_R revealed, that the region of sequence homology has the same orientation, is 2.5 kb long and involves the small internal repeats. Visualization of the heteroduplexes in the electron microscope by mica adsorption without the use of cytochrome c revealed, that the cross-hybridizing region consists of 1.5 kb with only partial homology including the small internal repeats and 0.9 kb with much better matched duplexes (Figure 5b).

The involvement of the small internal repeat in the cross-hybridizing area raised the question, whether a similar repeat could be detected within DS_L. The formation of two single-stranded loops of identical size at different positions during homoduplex formation (Figure 7c) suggests that repetitive sequences, partially homologous to the DS_R repeat, are also present in DS_L. No enzyme was so far identified which generates a hypermolar fragment similar in size to the DS_R repeat. Analysis of the nucleotide sequence of both regions will answer the question of how the DS_R and the presumed DS_L repeat are related to each other. We are presently attempting to subclone both regions for sequencing.

The two regions of sequence homology, which we have studied here, are interesting in many respects. Firstly, the presumed DS_L repeat is involved in the deletion of P3HR-1 virus, which has eliminated the genes, presumably required for initiation of transformation (Fresen *et al.*, 1980). The right-hand side of this deletion maps within sequences partially homologous to the DS_R repeat (J. Hudewentz, U.K. Freese, U. Zimber, and G.W. Bornkamm, submitted). Secondly, it has been observed that the region deleted in B95-8 (EBV) containing DS_R is actively transcribed in Burkitt's lymphoma tumor cells (Dambaugh *et al.*, 1979). Abundant transcription from DS_R and DS_L has also been observed in virus producer cells and in Raji cells after induction with 12-O-tetradecanoyl-13-phorbolacetate (U.K. Freese, J. Hudewentz, U. Zimber, E. Schwarz, G. Laux, and G.W. Bornkamm, manuscript in preparation).

It will be of interest to determine whether the RNA is transcribed from the small internal repeats or from the adjacent sequences, and more importantly, whether the RNA is translated into a structural protein. By comparing the transcription of B95-8 (EBV) with that of other strains, it should be possible to decide whether DS_L and DS_R are functionally equivalent and what role they play, if any, in the transcription of neighbouring sequences.

Materials and methods

Cells and viruses

All cell lines were grown in RPMI 1640 (GIBCO, Grand Island, NY) with 2.5% fetal calf serum and 8% calf serum, 100 U/ml of penicillin and 100 μg/ml of streptomycin. Cells were re-fed once weekly by replacing half of the medium. Initial cultures were obtained from G. Miller (CC 34-5), J. Pope (QIMR-WIL) and M.A. Epstein (M-ABA). Virus and viral DNA were purified as described by Bornkamm *et al.* (1980).

Preparation of recombinant plasmids containing DS_L and DS_R

The recombinant plasmids were obtained by subcloning of cosmids containing M-ABA (EBV) SalI and HindIII fragments. The set of overlapping cosmid clones of M-ABA (EBV) and of subclones derived from the cosmids will be described in detail elsewhere. To obtain the desired subclone containing DS_L and DS_R respectively, 2 μg DNA of a cosmid containing SalI-A and of a cosmid containing HindIII-K_1, -F, -D_2, -K_2, and -D_1 were digested with BglII and HindIII, respectively. The digests were extracted twice with chloroform/isoamylalcohol (24:1) and precipitated with ethanol. DNA was redissolved in 10 mM Tris-HCl pH 7.4, 1 mM EDTA at a concentration of 50 μg/ml. pHC 79 DNA (Hohn and Collins, 1980) was used as a vector for cloning BglII fragments, pACYC 184 (Chang and Cohen, 1978) for cloning of HindIII fragments. Vector DNA was cleaved with the respective endonuclease and then treated with calf intestine phosphatase (Boehringer, Mannheim, FRG) for 2 h at 37°C. Enzymes were removed by four phenol extractions, followed by one chloroform/isoamylalcohol extraction and precipitation of the DNA with ethanol. Ligation was carried out in ligation buffer (66 mM Tris-HCl pH 7.5, 6.6 mM $MgCl_2$, 10 mM dithiothreitol, 200 μM ATP) in a volume of 20 μl with 200 ng viral, 20 ng phosphatase-treated vector DNA, and 0.5 units of T_4 DNA ligase (BRL). The mixture was incubated over night at 12–14°C and for 24 h at 4°C. 10 μl of the ligation mixture was used to transform 100 μl *Escherichia coli* K12 (strain 490 A), which were made competent and frozen as described by Morrison (1979). After 1 h in ice and a heat shock of 90 s at 42°C, 1 ml Luria broth was added and the cultures shaken in a water bath for 2 h at 37°C. Aliquots were plated on Luria-broth-agar plates containing 15 μg/ml tetracycline for selection of pHC 79 recombinants and 150 μg/ml chloramphenicol for selection of pACYC 184 clones. The size of cloned inserts was analyzed by digestion of DNA obtained in cleared lysates of 5 ml cultures as described by Davis *et al.* (1980). Recombinant plasmid DNA was prepared by the cleared lysate method (Clewell and Helinski, 1969) involving two consecutive runs of the DNA in CsCl-ethidium bromide.

Cloning experiments were performed under $L3/B_1$ conditions in accordance with the German guide lines for recombinant DNA research.

Analysis of DNA fragments generated by restriction enzymes

Restriction enzymes were purchased from Bethesda Research Laboratories (Frankfurt, FRG) and Boehringer (Mannheim, FRG). Usually, 1 μg of DNA was incubated with 2–4 units of enzyme for 1–3 h at 37°C in the buffer indicated by the manufacturer. Gel electrophoresis was carried out in horizontal agarose gels at 30–40 V for 12–18 h in buffer containing 40 mM Tris-acetate (pH 7.8), 1 mM EDTA and 0.5 μg of ethidium bromide per ml. After long runs gels were stained additionally in the same buffer. Bands were visualized on a 254 nm transilluminator (UV products, San Gabriel, CA) and photographed with a Polaroid camera through a Kodak wratten 23 A filter. Adenovirus type 2 EcoRI and BamHI fragments (Yang and Flint, 1979) and ΦX174 RF HaeIII fragments were used as size markers. Fragments were transferred to nitrocellulose with a pore size of 0.1 μm (pH 79, Schleicher and Schüll, Dassel, FRG) according to Southern (1975) with the modifications described by Favaloro *et al.* (1980). DNA was fixed to the nitrocellulose by baking the filters for 4 h at 80°C. DNA was labelled by nick-translation with [^{32}P]dCTP (Amersham Corp.; 400 Ci/mmol) in a total volume of 20–50 μl (Rigby *et al.*, 1977; Maniatis *et al.*, 1975).

Hybridization was carried out as described by Bornkamm *et al.* (1980), however, without dextran sulfate. After hybridization, blots were washed twice in 2 x SSC (SSC is 0.15 M sodium chloride, 0.015 M sodium citrate), 0.1% sodium dodecylsulfate (SDS) at room temperature followed by four washes of 30 min each in 0.1 x SSC, 0.1% SDS at 50°C. Autoradiographs were made with a Kodak Royal X-omat AR film using an intensifying screen (Agfa-Gevaert MR 600).

Heteroduplex preparation and electron microscopy

The linearized DNAs were diluted to a concentration of 4 μg/ml into 60% formamide, 10 mM sodium phosphate, 1 mM EDTA, pH 7. They were denatured by immersing the tube for 60 s into boiling water. After the addition of CsCl to a final concentration of 0.2 M, the DNA was reannealed for 45 min at 37°C. Cytochrome spreading was done using a spreading mixture containing 30% formamide essentially as described by Davis *et al.* (1971). For the mica adsorption a buffer exchange was made on a Sephadex G-100 column equilibrated with 10 mM sodium phosphate, 1 mM EDTA, pH 7 before the addition of T4 gene 32 protein (100 μg/ml). The complexes were fixed with glutaraldehyde (0.1%) passed through a Sepharose CL-2B column into 4 mM magnesium acetate, 0.02% glutaraldehyde, and then adsorbed to mica as described earlier in more detail (Priess *et al.*, 1980).

Pictures were taken in a Zeiss EM 10A electron microscope at magnifications of 5000-fold (for cytochrome preparations) or 12 500-fold (for mica adsorptions). Measurements were done on the projected negatives using an X-Y coordinate digitizer (Brühl, Nürnberg) connected to a Cromemco CS-3 microcomputer system.

Nomenclature of restriction enzyme fragments

The nomenclature of EcoRI, HindIII, and SalI fragments of Given and Kieff (1978) was used. In contrast to our previous use, the large M-ABA (EBV) SalI fragments are denoted SalIA and SalIB in analogy to other EBV strains and not according to their size. For BamHI fragments we followed the nomenclature of Skare and Strominger (1980) and Arrand *et al.* (1981).

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Note added in proof

S.D.Hayward, S.G.Lazarowitz, and G.S.Hayward recently detected a NotI repeat of 135 bp in B95-8 (EBV) BamHI-H, which carries the DS_L region (personal communication).