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The EB virus genome in Daudi Burkitt's lymphoma cells has a deletion similar to that observed in a non-transforming strain (P3HR-1) of the virus

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Epstein-Barr virus (EBV) DNA isolated from the frequently studied and unusual Burkitt's lymphoma cell line, Daudi, contains a 7.4-kb deletion, similar to (but larger than) that found in a non-transforming isolate of the virus, P3HR-1. A comparison of EBV sequence in Daudi cells with that from a comparable region in a wild-type, transforming strain of the virus (B95-8) indicates that at least two of the previously identified RNAs, a highly repetitive sequence, and other interesting coding or structural features should be absent in Daudi EBV DNA as a consequence of the deletion. The information removed by the deletion, as well as that which might be generated by juxtaposition of two regions of the genome that are not adjacent in most strains of the virus are discussed.
Key words: B-lymphoma/Daudi/deletion variant/EBV/transformation

Introduction

The lymphotropic herpesvirus, Epstein-Barr virus (EBV), can be detected in latent form from early childhood onwards in most of the human population. Where infection has been delayed until adolescence, the virus can produce infectious mononucleosis (IM). Further, EBV is strongly associated with two malignancies, Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC). Many studies have been carried out to relate changes in the viral genome of EBV strains of different origins with the various diseases. To date, however, these attempts have proved largely disappointing. That is, regardless of the source, sufficient similarities have been observed among the different EBV strains to make it unlikely that there are disease-specific viral sub-types (Rymo *et al.*,1979; Bornkamm *et al.*,1980; Fischer *et al.*,1981; Heller *et al.*,1981). Nevertheless, considerable attention has been focussed on a sub-clone, P3HR-1 (Hinuma *et al.*,1967), of a BL line (Jijoye) that is the only known source of an EBV which lacks the ability to immortalise human immature B-lymphocytes, with the hope that such studies will lead to an understanding of the mechanism of cellular transformation by EBV (Miller *et al.*,1974; Bornkamm *et al.*,1980, 1982; Heller *et al.*,1981; Hayward *et al.*,1982; Heston *et al.*,1982; Hudewentz *et al.*,1982; King *et al.*,1982; Rabson *et al.*,1982; Jeang and Hayward, 1983; Stoerker *et al.*,1983). The conclusion reached is that a deletion in the EBV genome adjacent to the large internal repeat sequence of the virus is of importance for events that lead to initiation of cellular transformation. For example, Rabson *et al.* (1982) showed that the only modification detectable by restriction enzyme mapping which distinguished the non-transforming P3HR-1 strain of virus from its parental (Jijoye) transforming virus, was a deletion of $\sim 2.4 \times 10^6$ daltons of information from sequence residing within the *Bam*HI-H and -Y fragments in the latter viral genome (for a map, see Figure 4A). The P3HR-1 viral strain is heterogeneous (Fresen *et al.*, 1977; Heston *et al.*, 1982), and this fact complicates the interpretation of all data obtained so far except those mentioned above (Rabson *et al.*, 1982), in which cloned cell lines of P3HR-1 were used. In fact, studies by Gerber *et al.* (1982), using P3HR-1 cells from several laboratories, indicated the persistence of both transforming and non-transforming viruses. These authors suggested that, in general, virus from P3HR-1 cells which lacks transforming activity at the normal temperature (37°C) may regain it at reduced temperature (32°C), i.e., at least some of the EBV isolates from P3HR-1 may be temperature sensitive with respect to transformation. In spite of the reservations suggested by these data, studies on the P3HR-1 strain(s) of EBV provide a promising approach for analysing cellular transformation mediated by the action of EBV. The deletion present in one strain of this virus has recently been characterised by DNA sequence analysis (Jeang and Hayward, 1983).

We have been investigating the EBV of another BL line, Daudi, whose unusual properties have made it of broad general interest. Daudi continuous cell lines were established by Klein *et al.* (1968) from a young, male African with Burkitt's lymphoma. They were initially found to display a very strong surface accumulation of IgM molecules, without demonstrable cytoplasmic IgM. The cells carry the 8:14 chromosomal translocation typical of many BL lines (Manolov and Manolova, 1972; Zech *et al.*, 1976; Lenoir *et al.*, 1982) which results in some of the immunoglobulin V_H genes becoming associated with chromosome 8 (Erickson *et al.*, 1982). They apparently have also lost the capacity to synthesise β2-microglobulin, which makes them unique among lymphoid lines (Nilsson *et al.*, 1973). Moreover, they have been of considerable interest in interferon studies in that they are particularly susceptible to the growth inhibitory effects of this agent (Adams *et al.*, 1975; Gewert *et al.*, 1981; Silverman *et al.*, 1982). Tovey *et al.* (1982) report that although interferon can greatly enhance the expression of early viral antigens in Daudi cells, a similar effect was not observed in the same cells superinfected with P3HR-1.

Daudi cells contain many copies of EBV DNA (200–400 copies/cell; Adams, 1979) present mainly as episomal forms. They are naturally weak virus producer lines but can be induced to greater production with sodium butyrate (Luka *et al.*, 1979). These and other studies suggest that among the many valuable BL lines, the Daudi cell line may have particular advantages for investigating the molecular biology of EBV

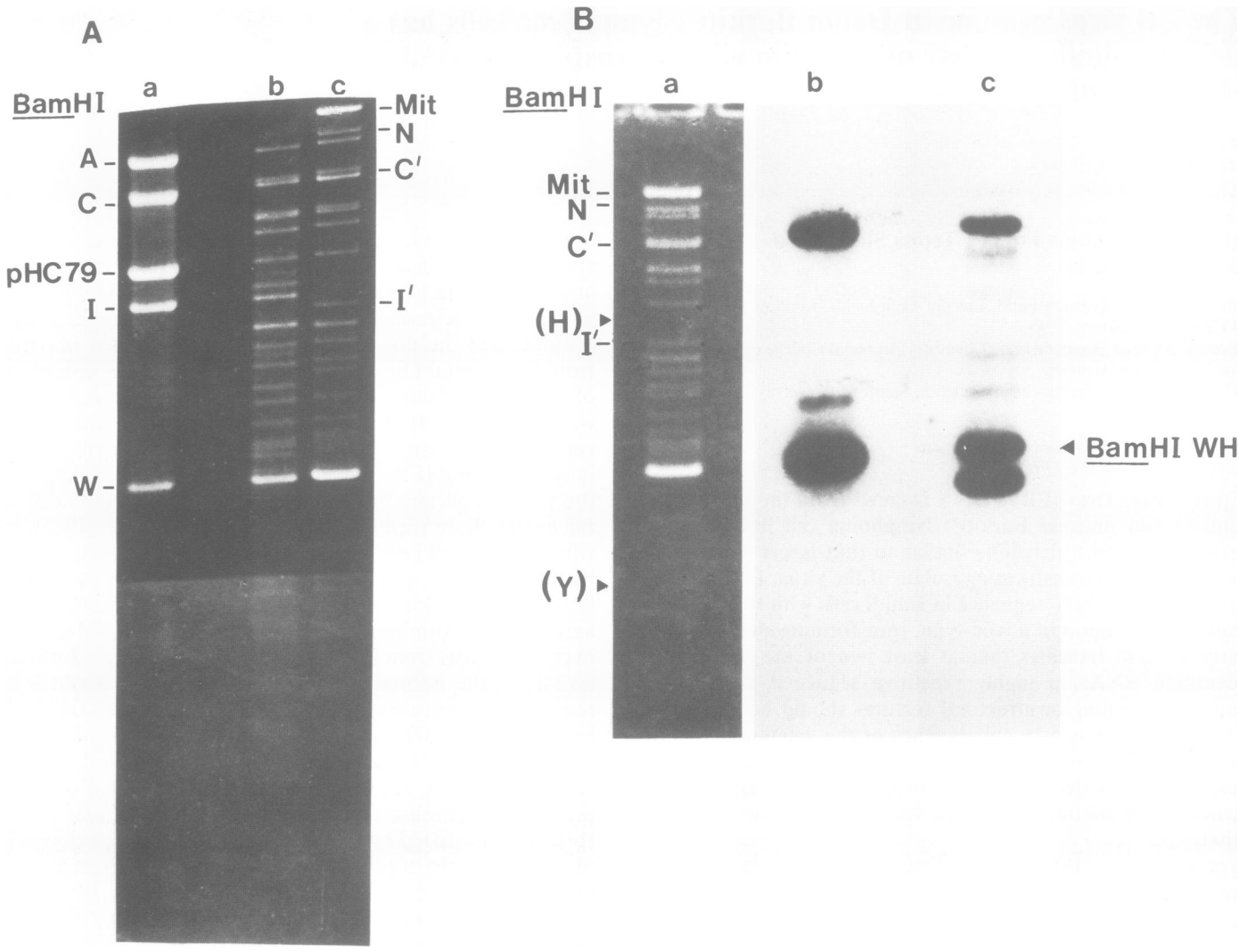


Fig. 1. (A) Photograph of *Bam*HI-cleaved linear DNA from B95-8 virions **(lane b)** and episomal EBV DNA from Daudi cells **(lane c)**, separated on a 0.8%/1.2% agarose gel by electrophoresis in the presence of ethidium bromide, and visualised under u.v. light. For size markers, the cosmid clone p65 **(lane a**; Griffin and Karran, *Nature*, in press), which contains *Bam*HI fragments A, C, I and W and the cosmid pHC79, was cleaved and included on the gel. Notably absent in the lane containing Daudi DNA is a band corresponding to *Bam*HI-H. Moreover, *Bam*HI-Y is also missing (see below). Novel bands among the Daudi fragments include (from top to bottom): mitochondrial DNA (Mit), a band derived from circularisation of 'ends' (N), a band that co-migrates with *Bam*HI-C (C′) and a *Bam*HI I-related fragment (I′). The latter two bands are derived from a region within the *Eco*RI-C fragment present in most EBV isolates (Raab-Traub *et al.*, 1980; Fischer *et al.*, 1981), but not in the B95-8 strain. **(B)** *Bam*HI-cleaved Daudi DNA **(lane a**, similar to **lane c**, in **A)**, hybridised against ^{32}P-labelled nick-translated DNA (Rigby *et al.*, 1977) from cloned B95-8 *Bam*HI fragment H **(lane b)** and, following removal of most of this probe, against ^{32}P-labelled *Bam*HI fragment W **(lane c)**. The arrowheads (at left) indicate sites at which normal (B95-8) fragments H and Y should appear and, (at right) the novel band (*Bam*HI-WH) formed by a deletion of 7.4-kb from Daudi DNA that fuses sequences from *Bam*HI-W to -H.

and its interaction with human B-lymphocytes.

Since the deletion found in the P3HR-1 strain of EBV has been assumed to be unique among viral isolates and to have resulted as an accidental consequence of propagation and cloning of the host cells in the laboratory (Miller *et al.*, 1974), we were surprised to find a similar, but larger, deletion in DNA derived from Daudi cells. This paper describes the deletion that exists in Daudi EBV DNA and the corresponding sequence found in the *Bam*HI-H fragment from 'wild-type' strain B95-8 virion DNA.

Results

Episomal (covalently closed circular, ccc) EBV DNA was isolated from Daudi cells, cleaved with *Bam*HI, and the fragments resolved by electrophoresis on agarose gels. When the results were compared with data from supercoiled EBV DNA of Raji cells (not shown) or linear DNA of B95-8 virions (Griffin *et al.*, 1981), a number of differences were observed (Figure 1A). Some of the variations between B95-8 virion DNA and Daudi ccc DNA can be ascribed to the known deletion in the *Eco*RI-C fragment in the former (Raab-Traub *et al.*, 1980), and a new band which corresponds to the end-joined fragments in the latter. In these respects, EBV DNAs from Raji and Daudi cells were similar. Other discrepancies, such as a possible small decrease in size in *Bam*HI-P of Daudi EBV DNA resemble changes described for the P3HR-1 and

Jijoye strains of virus (Rabson *et al.*, 1982). The most conspicuous and surprising difference observed was the apparent absence of the two fragments corresponding to *Bam*HI-H and -Y in the Daudi isolate. Although a deletion in this region is known to exist in virion DNA from the non-transforming strain P3HR-1, it has not been observed so far in EBV DNA from other sources. To confirm this finding, Daudi ccc DNA was cleaved with *Bam*HI and the resulting fragments separated by agarose gel electrophoresis, transferred to nitrocellulose, and probed with a [^{32}P]nick-translated recombinant clone of B95-8 EBV DNA, *Bam*HI-H (Arrand *et al.*, 1981). Two major bands were observed (Figure 1B, lane b), one corresponding in approximate size with *Bam*HI-B (or -C) and the other being marginally larger than *Bam*HI-W, a fragment present in multiple copies comprising the large internal repeat found in all strains of EBV virion DNA (Rymo and Forsblom, 1978) as well as in episomal forms of the virus (see Griffin *et al.*, 1981). To confirm that the smaller of the two fragments observed here was not simply a copy of the repetitive sequence, the nitrocellulose filter was washed to remove most of the hybridised material, then re-examined with a ^{32}P-labelled probe derived from a clone of B95-8 *Bam*HI-W (Jones and Griffin, 1983). The results are shown in Figure 1B (lane c). Of the three bands now observed, only the smallest had initially failed to hybridise to B95-8 *Bam*HI-H; it could be assigned as corresponding to the *Bam*HI-W fragment. Since hybridisation failed to reveal the presence of either normal B95-8 *Bam*HI-H or -Y, and since the fragment of ~3.5-kb hybridised to both *Bam*HI-H and W (see Figure 1B, band labelled *Bam*HI WH), it presumably represents a fusion of the normal fragments and a deletion over part of this region.

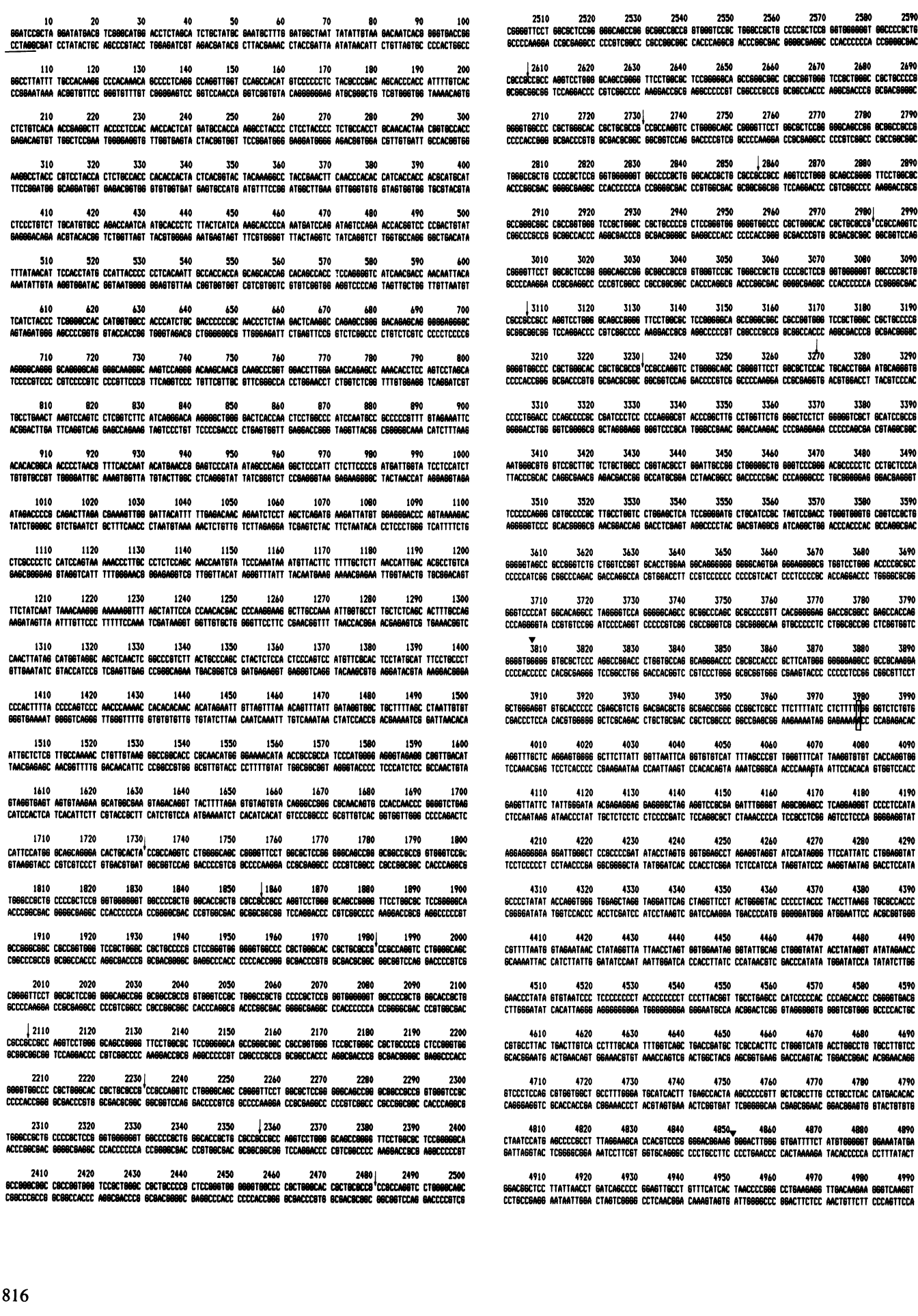
To confirm this, *Bam*HI fragments of Daudi-derived EBV ccc DNA were cloned in the vector, pAT153 (Twigg and Sherratt, 1980) by standard procedures. Recombinant clones which hybridised to both B95-8 fragments *Bam*HI-Y and H (note that *Bam*HI-Y will also hybridise to *Bam*HI-W since it contains part of the same repetitive sequence, Jones and Griffin, 1983) were selected, and one of them (designated 10G) was used to prepare DNA which was further cloned into single-stranded M13 vectors and sequenced by the dideoxy nucleotide method (Sanger *et al.*, 1980). To compare the sequence from clone 10G with corresponding sequences from B95-8, most of *Bam*HI-Y and all of *Bam*HI-H from the latter virus strain were also sequenced. The primary structure of *Bam*HI-W had been determined previously (Cheung and Kieff, 1982; Jones and Griffin, 1983). The sequence of *Bam*HI-H is given in Figure 2. When the recombinant clone containing *Bam*HI-H was partially digested with *Not*I, it was found to contain 12 complete copies of this repeat (data not shown) and these are included in the total DNA sequence of the fragment. The primary structure of Daudi clone 10G, designated Daudi *Bam*HI-WH, is given in Figure 3. The data show that Daudi ccc DNA contains a deletion of ~7.4-kb in a region encompassed by *Bam*HI fragments W, Y, and H in B95-8 virion DNA. The sequence data are analysed schematically in Figures 5 and 6. It is notable that all the sequence 'unique' to the *Bam*HI-Y fragment in B95-8 virion DNA – that is, not also found in *Bam*HI-W – is missing in Daudi ccc DNA, as well as a presumptive leftward promoter, the long repetitive sequence characterised by the *Not*I repeat (Hayward *et al.*, 1982; Jones and Griffin, 1983; Jeang and Hayward, 1983), and a portion of the DS_L region from *Bam*HI-H. Overall, the deletion is somewhat larger than that which has recently been defined for the P3HR-1 strain of the virus (Jeang and Hayward, 1983).

Analysis of the primary DNA sequence of *Bam*HI-WH suggests that the deletion in the Daudi EBV genome removes a number of potential coding regions found in B95-8 DNA and deletes or rearranges some putative transcriptional control signals (Figures 5 and 6). A comparison of the DS_L sequence in *Bam*HI-H (Figure 2) with the DS_R sequence from *Eco*RI-C of Raji DNA (B.G.Barrell, personal communication) shows that although there are some minor sequence differences between the two homologous regions, the (leftward) putative promoter control signals are unaltered. However, the part of DS_L that is deleted in the Daudi DNA, although leaving the 'CAT' boxes intact, removes the potential 'TATA' box. From the sequence data (see Figure 2), it would appear that over a large part of the DS_L the DNA is non-coding.

Discussion

The main question to answer in considering the EBV DNA deletion in Daudi cells is whether it can be correlated with specific viral functions lost in the cells relative to properties of isolates, such as EBV from the marmoset B95-8 line, which retain the region. Alternatively, have unusual viral functions been created by the deletion?

The complete DNA sequences of B95-8 *Bam*HI fragments W (Cheung and Kieff, 1982; Jones and Griffin, 1983) and H (see Figure 2), and the partial sequence of *Bam*HI-Y (our unpublished data, and B.G.Barrell, personal communication), and the sequence of Daudi EBV *Bam*HI WH fragment (see Figure 3), coupled with transcriptions and marker rescue experiments (cited below) allow us to address these questions. The data of van Santen *et al.* (1981) and Hummel and Kieff (1982) suggest that at least two mRNAs may be encoded in the region considered here. One, a major transcript 3.0–3.1 kb in size, appears to be derived from sequences present in *Bam*HI fragments W, Y and H. The other, 2.5-kb in size, is derived solely from *Bam*HI-H. Jeang and Hayward (1983) have defined the limits of the latter and shown that it includes the *Not*I repeat sequence within *Bam*HI-H. No product has been directly associated with the 2.5-kb mRNA. [However, recent data of Glaser *et al.* (1983) indicate that the early antigen, EA-R, is encoded entirely within *Bam*HI-H and this may be the 2.5-kb mRNA function.] When the primary DNA sequence is analysed, in a left to right direction, on a standard physical map (see Figure 4A), three areas with long open reading frames are observed. (i) The first encompasses information from *Bam*HI fragments W, Y and H. This could encode >370 amino acids (~42 K) from *Bam*HI-H alone, as shown in frame 2, Figure 5A. It may correspond to the 3.0–3.1 kb mRNA mentioned above. It should be noted that although this region in *Bam*HI-H is deleted in P3HR-1 cells, it also appears to be deleted in Jijoye cells, a line that produces transforming virus (King *et al.*, 1982). Although no protein has yet been assigned to this area, the open reading frame in *Bam*HI-H, if translated, would produce a polypeptide with a very high proline (~25%) content. Similarly the region in *Bam*HI-Y into which it extends would yield a very



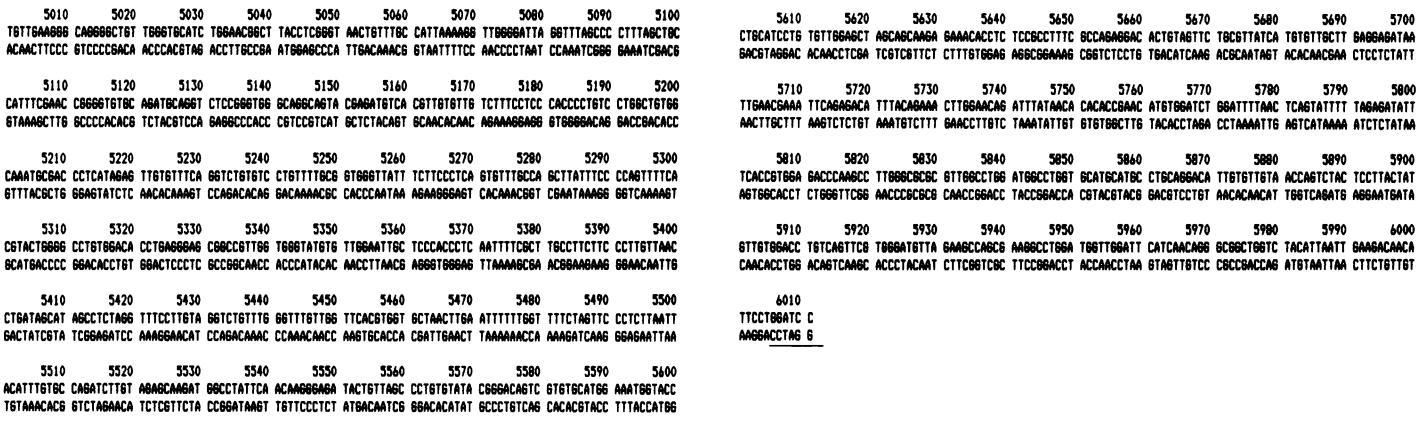


Fig. 2. The 6005-bp complete DNA sequence of *Bam*HI-H from B95-8 EBV DNA, shown in the 5′ – 3′ direction that corresponds to the conventional restriction enzyme map (Skare and Strominger, 1980). The *Bam*HI site (repeated) is underlined. Bornkamm *et al.* (1980) report a second *Bam*HI site in EBV DNA isolated from the M-ABA cell line in the region covered by the *Bam*HI-H fragment; this results in a cleavage of 'normal' *Bam*HI-H into two unequal-sized fragments. A change of the CG base pair to TA at position 1088 would account for the M-ABA data. The location of the small repetitive sequences (↓) and the DS_L sequence (▼) are indicated. In our recombinant clone, 12 complete repeats were observed by partial digestion with *Not*I; the DNA sequence further identifies one incomplete (30%) repeat. This is similar to the observation of a non-unit repeat number for the large (*Bam*HI-W) internal repeat (Hayward *et al.*, 1982). A sequence comparison of the DS_L with the homologous DS_R from *Eco*RI-C (Bornkamm *et al.*, 1980; B.G.Barrell, personal communication) reveals 99% homology between the two, with an overall difference of 1 bp (1044 relative to 1045 bp, respectively), and 11 mismatches. The site of the deletion in Daudi DNA (nucleotide 3978, see Figure 3) which removes a part of the DS_L is indicated (**box**). The corresponding deletion in EBV DNA from P3HR-1 cells (Jeang and Hayward, 1983) occurs at nucleotide position 3599. There are a number of changes between the DNA sequence as determined here and the part of *Bam*HI-H sequence recently reported (Jeang and Hayward, 1983). In seven cases, bases were not identified by Jeang and Hayward; their assignment is given here. In 10 cases, there are single base alterations between the two sequences. Since none of these changes results in the deletion or insertion of a termination codon they may be assumed to be of little significance. It should be noted, however, that there are some changes with respect to potential initiation codons, which could prove to be meaningful. Differences that produce frame-shifts are found, as noted: (i) position 4088 – 4089 bottom line, CA, is given as N by Jeang and Hayward (JH, position 10); (ii) position 3956 – 3958, CGA, is given as GT (JH, 140 – 141); (iii) position 2780, G, is AG (JH, 317 – 318); (iv) position 3637 – 3638, CC is C (JH, 460); (v) position 1550 – 1551, CC, is C (JH, 2420). One further and possibly significant difference is in the number of *Not*I repeats observed in *Bam*HI-H. We find 12.3 as against 11.3 previously reported. This difference would be significant if the region is translated.

proline-rich translation product (data not shown). (ii) The second has potential coding sequences in all three reading frames, encompassing the *Not*I repeats (Figure 5A, nucleotides 1700 to ~4000). Of these, the sequence in frame 1 covers a region that could linearly specify a polypeptide of >560 amino acids. However, transcriptional evidence suggests that the protein actually encoded by the *Not*I repeat region is derived from the other strand of the DNA (Jeang and Hayward, 1983). The deletion in the EBV genome in Daudi cells removes all of the open reading frames (Figure 5B) considered above. (iii) The third lies between 5523 and 6011 nucleotides (Figure 2). It has an ATG at position 5529 and allows the expression of a 161 amino acid polypeptide (see frame 3, Figure 5A). The ATG codon fulfils the criteria for an initiating codon (Kozak, 1981) since it is preceded by the sequence AAG and followed by G. This open reading frame continues into the adjacent *Bam*HI-F fragment and can be seen to be conserved in the *Bam*HI-WH fragment from the Daudi isolate. The weakness in suggesting this region as being actively transcribed is that the putative promoter sequences associated with it (TCAAT, 'CAT' box nucleotide position 5369 and GATA, 'TATA' box, position 5403) are not those commonly considered to be strong promoters. This might, on the other hand, explain the fact that no major mRNA has yet been mapped to this region. One interesting consequence of the deletion is that transcription of this open reading frame in Daudi cells could conceivably come under the control of the strong promoters in *Bam*HI-W (GCAAT and TATA, see Jones and Griffin, 1983), probably normally used to transcribe the 3.0 – 3.1 kb mRNA (van Santen *et al.*, 1981). Thus, the deletion may potentially result in increased amounts of a protein expressed in part from the end of *Bam*HI-H, which might provide an explanation for some of the unusual properties associated with Daudi cells. This possibility is being explored.

From a similar analysis of the sequence on the other strand of the DNA (Figure 6), it can be seen that the only large open reading frames are the three in the region that encompasses the *Not*I repeat. The longest uninterrupted reading frame would accommodate a polypeptide nearly 700 amino acids long. The data of Jeang and Hayward (1983) map the 2.5-kb mRNA to this region of the genome. Our sequence data can accommodate this mRNA if the potential 'CAT' (either CCAAT at position 4032, or CCACT at 4018, Figures 2 and 6) and 'TATA' (GATA, position 3970) boxes are utilised as promoters to specify a message with a cap site (CC) at position 3941, an initiation codon (ATG) at position 3867 and a polyadenylation signal at 1470. Such a message would be transcribed initially from frame 3, but would require a splice to remove the termination codon (TGA) at position 3762. There are potential splice sites (Mount, 1982) within the sequence though none have yet been directly identified. Although the region around the *Not*I repeat is deleted in both P3HR-1 and Daudi DNAs, the former retains the putative weak promoters whereas the latter loses the GATA (TATA) box (see Figure 5). Based on these data, possible functional differences between P3HR-1 and Daudi EBV DNAs exist.

On first consideration, it would seem bizarre that a major

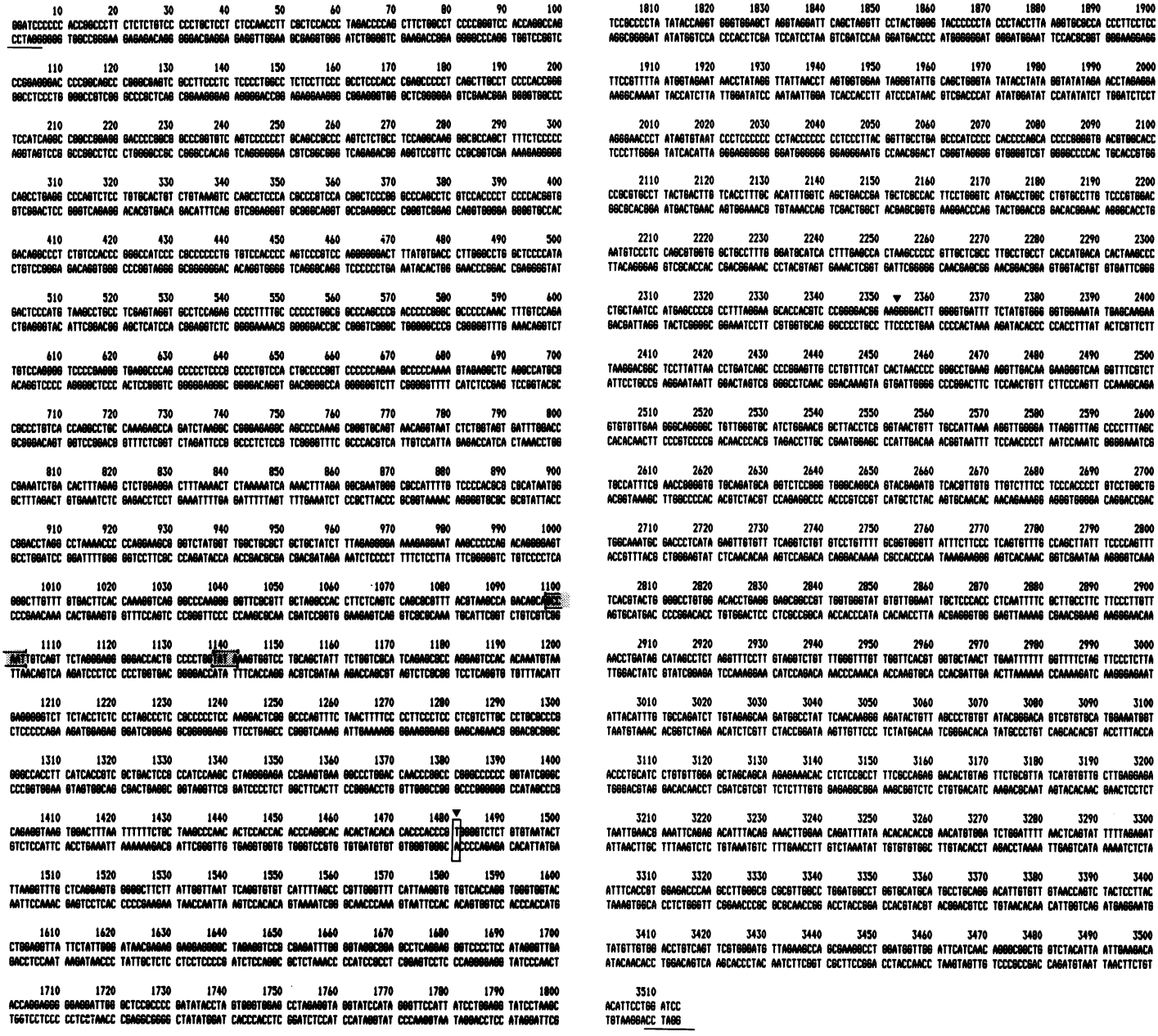


Fig. 3. The 3508-bp DNA sequence from a novel *Bam*HI fragment found in the Daudi EBV strain (designated Daudi *Bam*HI-WH) that corresponds to a deletion of sequence, found at the junction of *Bam*HI fragments Y and H in B95-8 DNA, which results in a juxtaposition of DNA from the large internal repeat, *Bam*HI-W, to sequence in *Bam*HI H. The 'join' was identified by determining the primary structure of *Pst*I-*Pvu*II and *Pst*I-*Kpn*I restriction fragments, as illustrated (Figure 4). The total sequence was derived from data in Figure 2, as well as from the sequence of *Bam*HI W described previously (Jones and Griffin, 1983). The potential promoter signals (from *Bam*HI W) and the DS_L sequence are indicated (hatching), and the fusion point of *Bam*HI-W with H noted (arrow). The deletion found in the Daudi EBV genome removes a number of open reading frames, all the *Not*I repeat sequences and part of the region (DS_L) that has homology with sequence (DS_R) in *Eco*RI-C. The resulting fusion of *Bam*HI W and H fragments does not create a new open reading frame. Further details are discussed in Figure 5.

viral protein should be encoded within a repetitive sequence, particularly one with such a high GC-content (nearly 85%) as the 125-bp *Not*I repeat (Jones and Griffin, 1983; Jeang and Hayward, 1983). Moreover, the number of nucleotides in the repeat also ensures that all triplets within the repeat are translated and re-iterated in the protein. However, it is interesting to note the recent report of a protein (*Plasmodium knowlesi* circumsporocoite) derived from DNA that has 12 copies of a 36-bp repeat unit which not only is translated but accounts for at least a third of the amino acid sequence of the total protein (Godson *et al.*, 1983).

This discussion has revolved around the potential coding sequences in an EBV transforming genome such as that found in the B95-8 strain of virus, and the consequences that may result from the deletion found in Daudi cells. Some of the properties of the latter might also, however, be a conse-

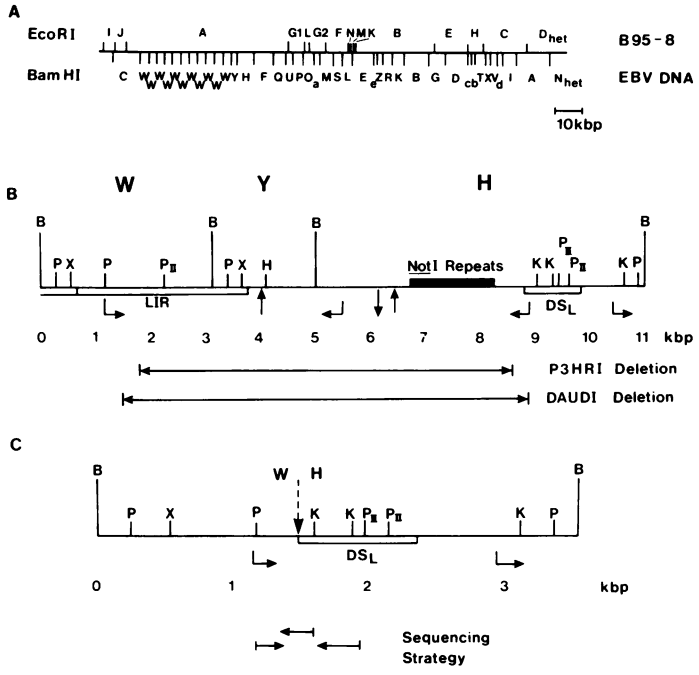


Fig. 4. (A) The *Eco*RI and *Bam*HI restriction maps of linear virion B95-8 EBV DNA. Data for the *Eco*RI map are derived from Given and Kieff (1978) and those for *Bam*HI from Skare and Strominger (1980). Daudi ccc DNA has an insertion of sequence in *Eco*RI-C similar to that observed with Raji EBV DNA (Raab-Traub *et al.*, 1980; Rymo *et al.*, 1981) and several viral strains derived from mononucleosis material (Fischer *et al.*, 1981), as well as from recently established Burkitt's lymphoma lines (A. Harris *et al.*, in preparation). **(B)** An expansion of the *Bam*HI W, Y, and H fragments that illustrates some of the restriction enzyme sites and some salient features in B95-8 DNA. Data from *Bam*HI-W are from Jones and Griffin (1983), those from *Bam*HI-Y are derived from our unpublished data and those of B.G.Barrell and colleagues (personal communication), and those from *Bam*HI-H from Figure 2. The scale in kbp is indicated, as are the large internal repeats (LIR), the *Not*I repeats, and the leftward duplicated sequence (DS_L). The region shown as deleted in Daudi EBV DNA is taken from data in Figure 3 and that in the P3HR-1 strain from the published sequence of Jeang and Hayward (1983). Symbols used are: B = *Bam*HI, P = *Pst*I, PII = *Pvu*II, H = *Hind*III, X = *Xho*I, K = *Kpn*I. Potential transcriptional control sequences: ⌞ (promoter, left to right), and ⌟ (right to left) and ↓ AATAAA (left to right) and ↑ (right to left). **(C)** A further expansion (note scale) of the portions of *Bam*HI-W and -H extant in Daudi DNA, showing some of the features prominent in **B** that are lost in the deletion and others that are retained. Particularly notable is the relocation of the potential promoter (⌞) from the large internal repeat (*Bam*HI-W). The arrows (at bottom) indicate the area whose DNA sequence was determined in order to localise the precise sites of the deletion. Abbreviations are as in **B**.

quence of possible structural alterations. Jeang and Hayward (1983) have drawn attention to hairpin-like structures present in B95-8 DNA in the region under consideration (which would be absent in EBV DNA from Daudi cells) and Henry *et al.* (1983) have mapped a putative origin of replication (*ars* sequence) onto this general region. Further, an interesting structural feature may be created in Daudi EBV DNA by the juxtaposition of two otherwise non-contiguous regions of the genome. That is, a 46-bp long AC-rich site from one of the large internal repeats (*Bam*HI-W) (CCCAACACTCCACC-ACACCCAGGCACACACTACACACACCCACCCG, nucleotides 1435–1480, Figure 3), with the potential for forming Z-DNA, becomes transposed from its normal positions in the genome to a site adjacent to the part of the EBV DS_L that remains in Daudi cells.

The discovery of a large (7.4-kb) deletion in the *Bam*HI-Y and -H fragments (see Figures 1 and 4B) of EBV episomal DNA in Daudi lymphoma cells clearly shows that the EVB deletion observed in P3HR-1 cells is not unique, as previously thought (see Introduction). Moreover, it exists in cells directly established from a tumour. The same deletion has been observed in the linear DNA of virions produced by Daudi cells (L.Karran and B.E.Griffin, unpublished results). It follows from studies on P3HR-1 that Daudi virions might be expected to be non-transforming. Preliminary experiments with EB virions from Daudi cells also indicate this to be the case (D.Crawford and B.E.Griffin, unpublished data), but these negative results require further confirmation.

Materials and methods

Cells

The EBV-transformed Daudi lymphoid cells, obtained from Professor G.Klein, Karolinska Institute, Stockholm, were the same as those studied by Rymo *et al.* (1979). They were propagated as suspension cultures in RPMI 1640 medium supplemented with 10% foetal calf serum, 100 U of penicillin and 100 μg of streptomycin/ml. Cells were subdivided to 2 x 10^5 cells/ml twice a week. B95-8 cells were the same as those used previously (Griffin *et al.*, 1981). Recombinant clones of EBV restriction enzyme fragments were taken from our EBV DNA library (Arrand *et al.*, 1981) and propagated as described.

Isolation and analysis of DNA

B95-8 virion DNA from EBV particles was isolated essentially as described by Adams (1975) from the spent medium of B95-8 cells that had been treated with 12-O-tetradecanoyl-phorbol-13-acetate (TPA), according to the procedure of zur Hausen *et al.* (1979). ccc DNA was prepared from Daudi cells as described by Griffin *et al.* (1981) except that for larger quantities of cells (~10^{10}), the chromosomal DNA, suspended in 1 litre of buffer (pH 12.4), was fragmented by a brief (5–10 s) mix in a Waring blender. This considerably reduced the work-up time with no marked deleterious effect on the yield of ccc DNA. For further purification prior to cloning, Daudi ccc DNA was separated from mitochondrial DNA on a neutral sucrose gradient in the presence of ethidium bromide. Analysis of the fastest migrating species by gel electrophoresis subsequent to cleavage with *Bam*HI showed that it consisted mainly of EBV DNA.

For comparison of genomes, linear EBV DNA isolated from B95-8 virions and ccc DNA isolated from Daudi cells were cleaved with a 5-fold excess of the *Bam*HI restriction endonuclease (Boehringer Mannheim Ltd) and digested according to the manufacturer's directions. Fragments were separated by electrophoresis on 0.8% agarose gels in the presence of ethidium bromide, as previously described (Griffin *et al.*, 1981). For hybridisation studies, viral DNA in gels was denatured, transferred to nitrocellulose, and hybridised against suitable ^{32}P-labelled probes, according to the procedure of Southern (1975).

Molecular cloning and sequencing of DNA from Daudi cells

Episomal EBV DNA from Daudi cells was digested to completion with the restriction enzyme *Bam*HI, and the resulting fragments cloned into a *Bam*HI-cleaved, and phosphatase-treated, pAT153 vector, essentially as described by Arrand *et al.* (1981). The library of Daudi EBV clones obtained (~300) was probed with [^{32}P]nick-translated B95-8 *Bam*HI fragment H. Six positive clones were obtained. Four clones contained an insert of ~3500 bp, which was further shown to contain sequences from EBV *Bam*HI fragment W (the large internal repeat unit). One of these (designated 10G) was extensively mapped with a variety of restriction enzymes using standard procedures. The remaining two clones were shown by restriction enzyme analysis to contain a larger insert that corresponded in size to a subfragment of *Eco*RI C; hybridisation against the analogous region of Raji DNA showed these to contain DS_R sequences.

The primary structure of the B95-8 *Bam*HI fragment H (clone number B7.12F) was determined by the random sonication, M13 cloning (Messing and Vieira, 1982) and dideoxy sequencing procedure (Sanger *et al.*, 1980), as pre-

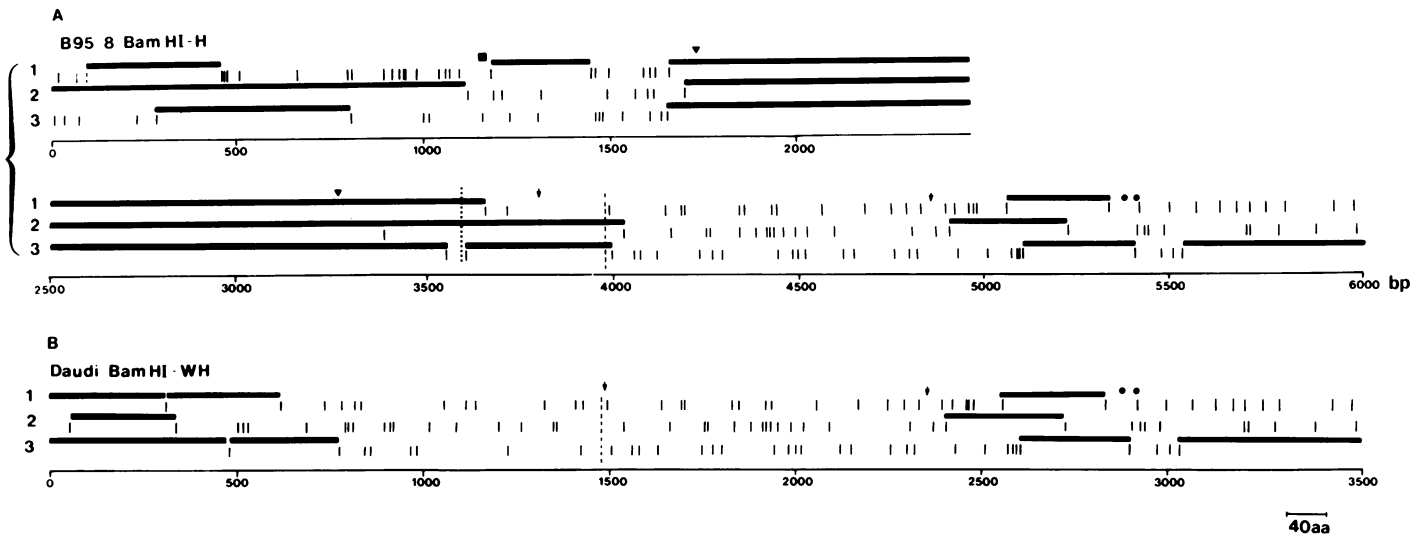


Fig. 5. (A) Some of the potential coding areas in B95-8 *Bam*HI fragment H, reading from left to right (5′→3′) on the conventional *Bam*HI restriction enzyme map (Skare and Strominger, 1980), as derived from the DNA sequence (Figure 2). The corresponding sequence from Daudi *Bam*HI-WH (from Figure 3) is shown for comparison in **B**. Some relevant transcriptional and structural features are noted. The number of base pairs (bp), taken from the sequences are indicated; reading frame 1 begins with the first nucleotide (G) of the *Bam*HI recognition (*G*GATCC) signal, frame 2 with the second, etc. Vertical bars = termination codons; black horizontal lines = open reading frames; ● = 'CAT' or 'TATA' boxes; ■ = AATAAA; ▼ = limits of *Not*I repeats; ↓ = limits of DS_L. The dashed vertical line shows the site of the deletion in *Bam*HI-H found in Daudi cells and the dotted line that in P3HR-1. Salient features found within *Bam*HI-H are: (i) The long open reading frame present at the beginning of the sequence in frame 2 of B95-8 DNA could code for 372 amino acids (42 kd) and terminate at nucleotide 1118 (Figure 2) with TAA. This polypeptide has a high (~25%) proline content. The initiation codon for this potential reading frame must originate elsewhere ('to the left') on the genome. A polyadenylation site (AATAAA) lies at nucleotide 1156. (ii) The entire region covered by the *Not*I 125-bp repeat contains no termination codons. The region adjacent to the repeats in all three reading frames also has coding capacity. (iii) The long open reading frame found at the end of the sequence in frame 3 (nucleotide 5529) has as its first codon an AUG initiation signal and within *Bam*HI-H could code for 161 amino acids. The putative promoter signals that precede this (TCAAT at nucleotide 5369 and GATA at nucleotide 5403) are not generally recognised as strong promoters. This potential coding sequence continues into *Bam*HI-F. **(B)** The possible coding areas found in the Daudi *Bam*HI-WH fusion fragment (in the left to right direction as above). The symbols used are the same as described in **A**. The sequence to the left of the vertical dashed line originates from *Bam*HI-W. It is noteworthy that the only long open reading frame retained from *Bam*HI-H is that described by (iii) above. The deletion removes ~190 bp from the DS_L.

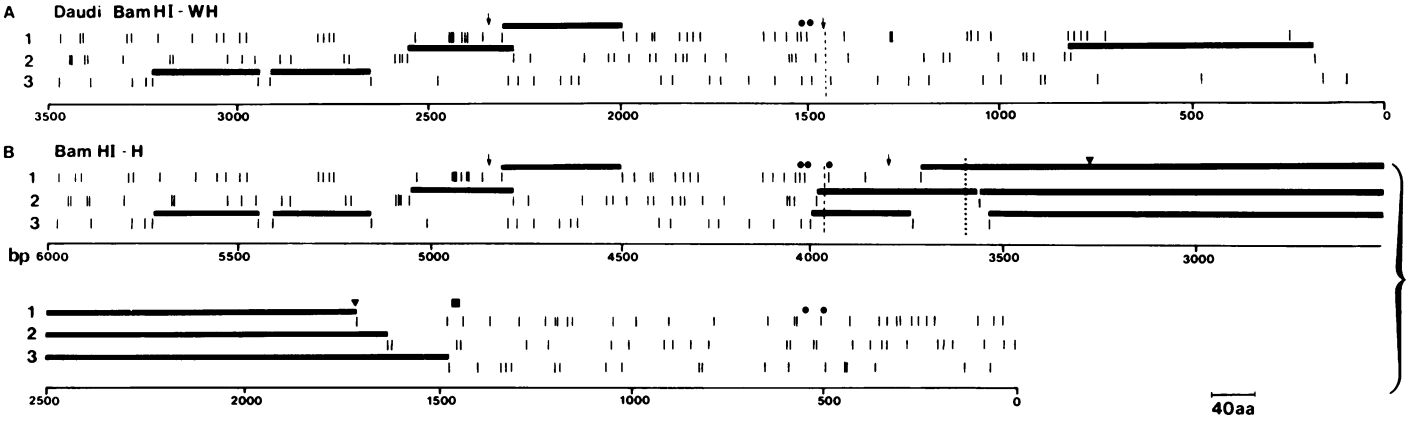


Fig. 6. Comparison of the potential coding areas for Daudi *Bam*HI-WH **(A)** and B95-8 *Bam*HI fragment H **(B)**, as derived from the DNA sequence (bottom to top, second strand, Figure 2), reading from right to left (5′→3′) on the conventional *Bam*HI restriction enzyme map (Figure 4A). As in Figure 5, for the *Bam*HI-H sequence frame 1 consists of amino acids that are initiated off the codon beginning with the first nucleotide (G) of the *Bam*HI recognition sequence, etc. The symbols used are given in the legend to Figure 5. The only obvious, long open reading frames in **B** are those that surround the *Not*I repeats. These are removed by the deletion that creates the Daudi *Bam*HI-WH fragment.

viously used for DNA sequence analysis of *Bam*HI-W (Jones and Griffin, 1983). Dideoxy sequencing was carried out using both [α-^{32}P]dATP and [α-^{35}S]thio-dATP labelling and 6% urea-polyacrylamide gels or 6% gradient polyacrylamide gels (Biggin *et al.*, 1983). To obtain the complete sequence, data were compiled from two libraries made from sonically fragmented DNA, and an average of 250–300 bases were read from individual gels. Over 180 clones were analysed to give the sequence of 39 000 bases, from which the total *Bam*HI-H primary structures (Figure 2) was deduced. Sequence was derived from both strands for 96.6% of the fragment, and more than one clone was analysed for deducing 97.8% of the sequence.

Specific fragments from Daudi clone 10G were separated by agarose gel electrophoresis, isolated by electro-elution, and subcloned into suitable M13mp vectors. The *Pst*I-*Pvu*II and *Pst*I-*Kpn*I restriction fragments that spanned the *Bam*HI-W and -H junctions were sequenced, as above.

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