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Deletion of the Nontransforming Epstein-Barr Virus Strain P3HR-1 Causes Fusion of the Large Internal Repeat to the DS_L Region

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The nontransforming Epstein-Barr virus (EBV) strain P3HR-1 is known to have a deletion of sequences of the long unique region adjacent to the large internal repeats. The deleted region is believed to be required for initiation of transformation. To establish a more detailed map of the deletion in P3HR-1 virus, *Sal*I-A of the transforming strain M-ABA and of P3HR-1 virus was cloned into the cosmid vector pHC79 and multiplied in *Escherichia coli*. The cleavage sites for *Bam*HI, *Bgl*II, *Eco*RI, *Pst*I, *Sac*I, *Sac*II, and *Xho*I were determined in the recombinant plasmid clones. Analysis of the boundary between large internal repeats and the long unique region showed that in M-ABA (EBV) the transition is different from that in B95-8 virus. The map established for *Sal*I-A of P3HR-1 virus revealed that, in contrast to previous reports, the deletion has a size of 6.5 kilobase pairs. It involves the junction between large internal repeats and the long unique region and includes more than half of the rightmost large internal repeat. The site of the deletion in the long unique region is located between a *Sac*I and a *Sac*II site, about 200 base pairs apart from each other. The sequences neighboring the deletion in the long unique region showed homology to the nonrepeated sequences of the DS_R (duplicated sequence, right) region. Sequences of the large internal repeat are thus fused to sequences of the DS_L (duplicated sequence, left) region in P3HR-1 virus DNA under elimination of the DS_L repeats. Jijoye, the parental Burkitt lymphoma cell line from which the P3HR-1 line is derived by single-cell cloning, is known to produce a transforming virus. Analysis of the Jijoye (EBV) genome with cloned M-ABA (EBV) probes specific for the sequences missing in P3HR-1 virus revealed that the sequences of M-ABA (EBV) *Bam*HI-H2 are not represented in Jijoye (EBV). In Jijoye (EBV) the complete DS_L region including the DS_L repeats is, however, conserved. Further analysis of Jijoye (EBV) and of Jijoye virus-transformed cell lines will be helpful to narrow down the region required for transformation.

Epstein-Barr virus (EBV) is widespread within the human population and usually leads to inapparent infection or to the clinical symptoms of infectious mononucleosis. Additionally, the virus is associated with two human malignancies, Burkitt's lymphoma and nasopharyngeal carcinoma (for review see reference 11).

Epidemiological evidence, obtained for Burkitt's lymphoma, indicates that one important factor in the development of the disease is contributed by the virus (10). The cells of both tumors carry Epstein-Barr virus DNA (34, 49), mainly as covalently closed episomal DNA (25, 29), and express the virus-specific nuclear antigen EBNA (30, 40). Viral transcripts could also be demonstrated in Burkitt tumor biopsies (7). Transformation of human B lymphocytes in vitro by EBV (21, 31, 36) and experimental induction of malignant lymphoproliferation in marmosets by EBV (32, 44) have demonstrated the oncogenic potential of the virus.

EBV DNA purified from virus particles is a double-stranded linear DNA of 120 megadaltons (Md). It is composed of identical short tandem repeats of about 450 base pairs (bp) present at both ends (17, 27) and a short and a long unique region of 9 and 90 Md, respectively, joined by a variable number of large internal repeats (16, 19, 42). A linkage map of *Eco*RI, *Hin*dIII, and *Sal*I fragments has been provided for two strains by Given and Kieff (15) and has been extended to a number of other isolates (2, 12, 20). Two regions of the genome, DS_L and DS_R (duplicated sequence, left and right, respectively), located about 70 Md apart from each other within the long unique region, have been shown to have some sequence homology (37). These regions are present in the same orientation in the viral

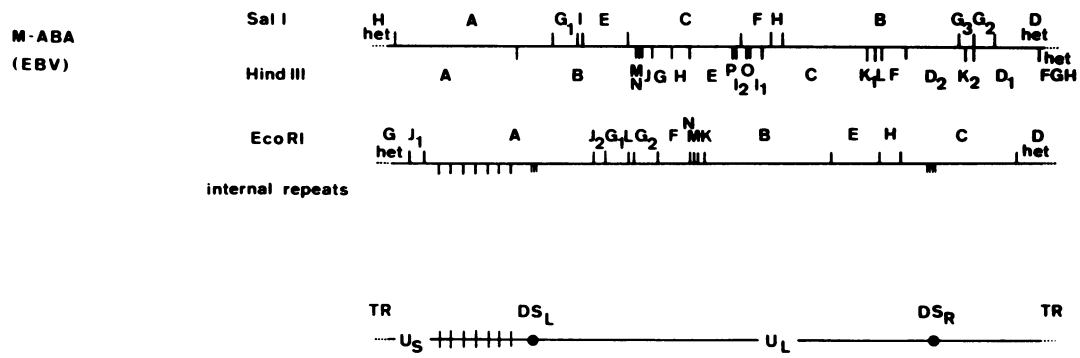


FIG. 1. Schematic diagram of the EBV genome, with restriction sites for *Eco*RI, *Hin*dIII, and *Sal*I. The DNA consists of terminal repeats (TR) and a short (U_S) and a long unique region (U_L) joined by large internal repeats (IR). Two regions of the long unique region show sequence homologies (DS_L and DS_R).

genome and consist of about 0.9 kilobase pairs (kbp) with good homology and 1.5 kbp with partial homology (24). The region of partial homology involves a 102-bp small internal repeat (DS_R repeat), which is partially conserved in DS_L (24). The partially homologous part of the DS_L region also consists of short tandem repeats (18a). A schematic drawing of the viral genome including restriction sites for *Eco*RI, *Hin*dIII, and *Sal*I is shown in Fig. 1.

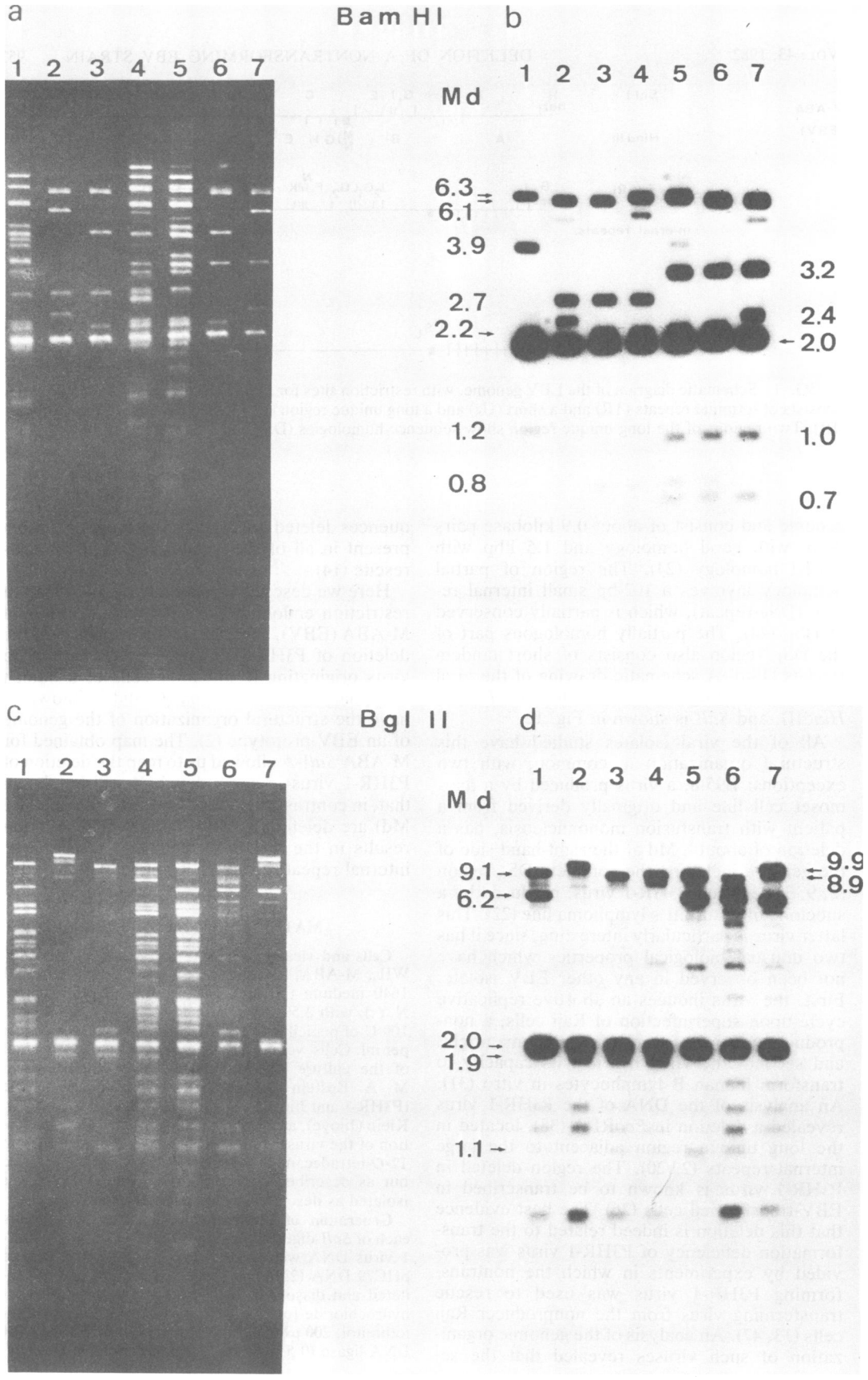
All of the viral isolates studied have this structural organization in common, with two exceptions: B95-8, a virus produced by a marmoset cell line and originally derived from a patient with transfusion mononucleosis, has a deletion of about 8 Md at the right-hand side of the genome including the complete DS_R region (2, 9, 37, 38) and P3HR-1 virus, produced by a subclone of a Burkitt's lymphoma line (22). This latter virus is particularly interesting, since it has two unusual biological properties which have not been observed in any other EBV isolate. First, the virus induces an abortive replicative cycle upon superinfection of Raji cells, a nonproducer cell line of Burkitt's lymphoma origin; and second, the virus has lost its capacity to transform human B lymphocytes in vitro (31). An analysis of the DNA of the P3HR-1 virus revealed a deletion in *Eco*RI-A (38), located in the long unique region adjacent to the large internal repeats (2, 20). The region deleted in P3HR-1 virus is known to be transcribed in EBV-transformed cells (26). The best evidence that this deletion is indeed related to the transformation deficiency of P3HR-1 virus was provided by experiments in which the nontransforming P3HR-1 virus was used to rescue transforming virus from the nonproducer Raji cells (13, 47). An analysis of the genomic organization of such viruses revealed that the sequences deleted in P3HR-1 virus were invariably present in all of the isolates obtained by virus rescue (14).

Here we describe a relatively detailed map of restriction endonuclease sites within *Sal*I-A of M-ABA (EBV), a fragment which spans over the deletion of P3HR-1 virus. M-ABA (EBV) is a virus originating from tumor cells of a human nasopharyngeal carcinoma (6) and is known to have the structural organization of the genome of an EBV prototype (2). The map obtained for M-ABA *Sal*I-A allowed us to map the deletion of P3HR-1 virus in some detail. It will be shown that, in contrast to previous reports, 6.5 kbp (4.3 Md) are deleted in P3HR-1 virus. The deletion results in the fusion of sequences of the large internal repeat to sequences of the DS_L region.

MATERIALS AND METHODS

Cells and viruses. P3HR-1, B95-8, CC34-5, QIMR-WIL, M-ABA, and Jijoye cells were grown in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.), with 2.5% fetal calf serum and 8% calf serum, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. Cells were refed once weekly by replacing half of the culture. Initial cultures were obtained from M. A. Epstein (M-ABA), G. Henle and W. Henle (P3HR-1 and Jijoye), G. Miller (B95-8 and CC34-5), G. Klein (Jijoye), and J. Pope (QIMR-WIL). The purification of the viruses and viral DNA after induction with 12-*O*-tetradecanoyl-phorbol-13-acetate was carried out as described previously (2). Cellular DNA was isolated as described by zur Hausen et al. (49).

Generation of plasmid clones. Three micrograms each of *Sal*I-digested M-ABA (EBV) DNA and P3HR-1 virus DNA were mixed with 3 μg of *Sal*I-cleaved pHC79 DNA (23). The samples were ethanol precipitated and dissolved in ligation buffer (66 mM Tris-hydrochloride [pH 7.5], 6.6 mM $MgCl_2$, 10 mM dithiothreitol, 200 μM ATP) in a total volume of 10 μl. T4 DNA ligase (0.5 μl) (New England Biolabs, Beverly,


a
Bam HI
b
1 2 3 4 5 6 7
Md
6.3
6.1
3.9
2.7
2.2
1.2
0.8
3.2
2.4
2.0
1.0
0.7
c
Bgl II
d
9.1
6.2
2.0
1.9
1.1
9.9
8.9

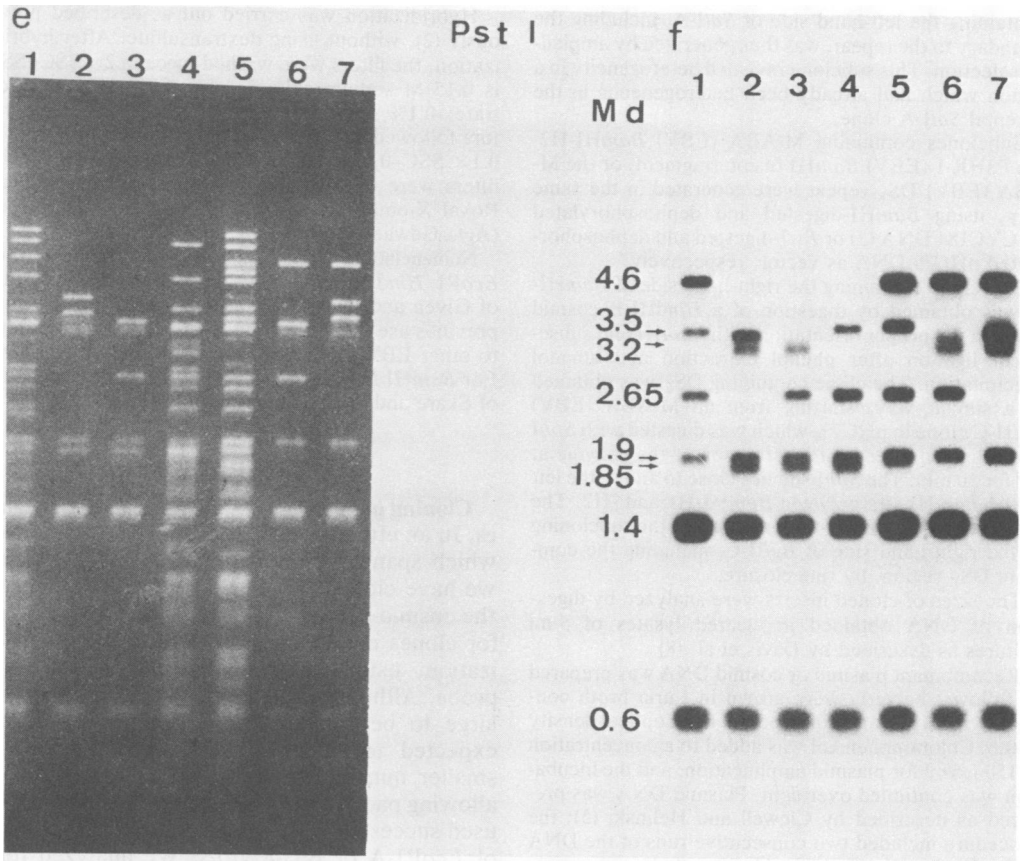


FIG. 2. Comparison of cloned P3HR-1 (lane 3) and M-ABA (lane 6) (EBV) DNA fragments with fragments of viral DNA of B95-8 (lane 1), P3HR-1 (lane 4), and M-ABA (EBV) (lane 5) after double digestion with *Bam*HI/*Sal*I (a), *Bgl*II/*Sal*I (c), and *Pst*I/*Sal*I (e). Lanes 2 and 7 contained cloned P3HR-1 and M-ABA fragments, cleaved only with *Bam*HI to identify the fragments joined to the vector. After being transferred to nitrocellulose, the blots were hydridized to cloned ^{32}P-labeled M-ABA (EBV) *Sal*I-A, from which the vector had been removed by electrophoresis (b, d, and f). The submolar fragments observed in (d) are due to overdigestion with *Bgl*II, resulting in lower site specificity of the enzyme as described for *Eco*RI (35).

Mass.) was added, and the samples were incubated overnight at 12°C and then again for 24 h at 4°C. The DNA became very viscous upon ligation. One microliter of ligated DNA was packaged into phage lambda head proteins as described by Hohn and Collins (23). *Escherichia coli* HB101 served as the recipient for the in vitro-packaged cosmid DNA. Clones containing recombinant cosmid DNA were selected on Luria broth agar plates with 100 μg of ampicillin per ml.

Clones containing *Sal*I-A were identified by colony hybridization (18) with ^{32}P-labeled insert of pSL9, a recombinant plasmid containing the *Bam*HI 3.1-kbp repeat in a pBR322 vector, which was kindly provided by S. D. Hayward and G. S. Hayward.

Subclones containing the boundaries of the large internal repeat to the short and long unique regions, respectively, were obtained by partial digestion of the M-ABA (EBV) *Sal*I-A clone with *Bgl*II and, after phenol extraction and ethanol precipitation, by subsequent ligation into the *Bgl*II site of pHC79 DNA which had been treated with calf intestine phosphatase (Boehringer Mannheim Corp., West Germany) for 2 h at 37°C. The dephosphorylated vector was phenol extracted three times and then precipitated with ethanol. The ligation mixture contained 250 ng of DNA from the M-ABA *Sal*I-A clone and 25 ng of phosphatase-treated pHC79 DNA in a total volume of 20 μl. After being incubated overnight at 12°C and then again for 24 h at 4°C, 10 μl of the ligation mixture was used to transform 100 μl of *E. coli* K-12 (strain 490A), which was made competent and then frozen as described by Morrison (33). After being placed in ice for 1 h and heat shocked for 90 s at 42°C, 1 ml of Luria broth was added, and the cultures were shaken in a water bath for 2 h at 37°C. Samples were plated on Luria broth agar plates containing 15 μg of tetracycline per ml or 100 μg of ampicillin per ml. Clones selected by tetracycline resistance contained *Bgl*II fragments of *Sal*I-A cloned into the *Bgl*II site of pHC79. Ampicillin selection, however, revealed clones carrying the terminal *Sal*I-*Bgl*II fragment, generated by ring closure without the participation of the added pHC79 DNA. A clone

containing the left-hand side of *Sal*I-A, including the boundary to the repeat, was thus generated by ampicillin selection. This subclone revealed heterogeneity in a region which had already been heterogeneous in the parental *Sal*I-A clone.

Subclones containing M-ABA (EBV) *Bam*HI-H2, the P3HR-1 (EBV) *Bam*HI fusion fragment, or the M-ABA (EBV) DS_R repeat were generated in the same way, using *Bam*HI-digested and dephosphorylated pACYC184 DNA (3) or *Pst*I-digested and dephosphorylated pHC79 DNA as vector, respectively.

The clone containing the right-hand side of *Bam*HI-Y was obtained by digestion of a *Hin*dIII-B cosmid clone in the proper orientation with *Bam*HI and subsequent ligation after phenol extraction and ethanol precipitation. The clone containing DS_L was obtained in a similar way, starting from an M-ABA (EBV) *Bgl*II-C clone in pHC79, which was digested with *Sph*I and religated after heat inactivation of the enzyme at 70° for 10 min. The *Sph*I site lies close to and to the left of the *Bam*HI site between *Bam*HI-H1 and -H2. The orientation of the *Bgl*II-C clone allowed the subcloning of the right-hand side of *Bgl*II-C, including the complete DS_L region, by ring closure.

The sizes of cloned inserts were analyzed by digestion of DNA obtained in cleared lysates of 5-ml cultures as described by Davis et al. (8).

Recombinant plasmid or cosmid DNA was prepared as follows: bacteria were grown in Luria broth containing 100 μg of ampicillin per ml to an optical density of 0.6. Chloramphenicol was added to a concentration of 150 μg/ml for plasmid amplification, and the incubation was continued overnight. Plasmid DNA was prepared as described by Clewell and Helinski (5); the procedure included two consecutive runs of the DNA to equilibrium in cesium chloride-ethidium bromide gradients.

Digestion with restriction enzymes and gel electrophoresis. Restriction enzymes were purchased from Boehringer Mannheim, West Germany, Bethesda Research Laboratories, Rockville, Md., and New England Biolabs, Beverly, Mass. Digestion was carried out usually with 2 to 4 U of enzyme per μg of DNA for 1 to 3 h in the buffers indicated by the manufacturers. DNA fragments were separated in horizontal agarose gels run at 30 to 40 V for 12 to 18 h in buffer containing 40 mM Tris-acetate (pH 7.8)–1 mM EDTA. Adenovirus type 2 *Eco*RI and *Bam*HI fragments (48) and φX174 replicative form *Hae*III fragments were used as size markers. The gel was stained in 0.5 μg of ethidium bromide per ml, and bands were visualized on a 254-nm transilluminator (UV Products, San Gabriel, Calif.) and photographed with a Polaroid camera through a Kodak Wratten 23A filter.

Separated fragments were transferred to nitrocellulose filters as described by Southern (46), except washing of the filters after the transfer was omitted so that small fragments would not be lost.

Isolation of restriction enzyme fragments. Restriction enzyme fragments were isolated by the method of Koller et al. (28), using electrophoresis of fragments onto malachite green coupled to polyacrylamide (Boehringer Mannheim), with the modifications described previously (2).

In vitro labeling and blot hybridization. DNA was labeled with 25 to 50 μCi of [^{32}P]dCTP by nick translation (Amersham Corp., Arlington Heights, Ill.; 400 Ci/mmol) in a total volume of 20 to 50 μl (41).

Hybridization was carried out as described previously (2), without using dextransulfate. After hybridization, the filters were washed twice in 2× SSC (SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at room temperature followed by four consecutive washes for 30 min in 0.1× SSC–0.1% sodium dodecyl sulfate at 50°C. The filters were then air dried and exposed to a Kodak Royal X-omat AR film, using an intensifying screen (Agfa-Gewaert MR600).

Nomenclature of restriction enzyme fragments. For *Eco*RI, *Hin*dIII, and *Sal*I fragments, the nomenclature of Given and Kieff (15) was used. In contrast to our previous use (2), *Sal*I-A and -B are denoted by analogy to other EBV strains and not according to their size. For *Bam*HI fragments, we followed the nomenclature of Skare and Strominger (45) and Arrand et al. (1).

RESULTS

Cloning of *Sal*I-A of M-ABA and P3HR-1 viruses. In an attempt to clone EBV DNA sequences which span over the deletion of P3HR-1 virus, we have cloned B95-8 (EBV) *Sal*I fragments in the cosmid vector pHC79 (23) and have selected for clones containing *Sal*I-A by colony hybridization, using the large internal repeat as a probe. Although the size of B95-8 *Sal*I-A is too large to be packaged into lambda heads, we expected to select for molecules carrying a smaller number of large internal repeats, thus allowing packaging. A similar approach has been used successfully by Arrand et al. (1) for cloning of *Eco*RI-A of B95-8 virus. We analyzed four clones obtained in this way. All of these had deletions to the right of the large internal repeat. It could not be discriminated whether these clones represented a minority of deletion derivatives already present in the B95-8 virus DNA population or whether they had deleted the sequences during the cloning procedure.

It was assumed that *Sal*IA of M-ABA virus, which carries only five to six repeats, would fit more easily into the cosmid. Thus, *Sal*I fragments of M-ABA (EBV) were cloned into pHC79 DNA, and the clones containing *Sal*I-A were identified as described above. Three analyzed clones had identical inserts.

*Sal*I fragments of P3HR-1 virus DNA were cloned in an identical manner. Six of six clones analyzed had identical *Sal*I-A inserts. One from each of the M-ABA and P3HR-1 virus *Sal*I-A clones was studied in detail and is described below.

Sizes of internal and terminal *Bam*HI, *Bgl*II, and *Pst*I fragments of cloned M-ABA and P3HR-1 virus *Sal*I-A and comparison with viral DNA fragments. The DNA of M-ABA and P3HR-1 virus *Sal*I-A clones was digested by *Bam*HI, *Bgl*II, and *Pst*I, with and without *Sal*I, to identify fragments adjacent to vector DNA. Double digests of B95-8, M-ABA, and P3HR-1 virus DNA with *Bam*HI/*Sal*I, *Bgl*II/*Sal*I, and *Pst*I/

TABLE 1. Hybridization of ^{32}P-labeled P3HR-1 *Bam*HI/*Sal*I fragments of the P3HR-1 (EBV) *Sal*I-A clone to separated *Bam*HI/*Sal*I, *Bgl*II/*Sal*I, and *Pst*I/*Sal*I fragments of viral origin and of the M-ABA (EBV) and P3HR-1 (EBV) *Sal*I-A clones[a]

Fragment	P3HR-1 clone	P3HR-1 virus	M-ABA clone	M-ABA virus	B95-8 virus	CC34-5 virus	QIMR-WIL virus
2.2 Md *Bam*HI	2.2	2.2	2.2	2.2	2.2	2.2	2.2
*Bgl*II						11[b]	
	8.9	9.1	8.9	9.1	9.1	9.1	9.1
*Pst*I	2.65	2.65	2.65	2.65	2.65	2.65	2.65
6.1 Md *Bam*HI	6.1	6.3	6.1	6.3	6.3	6.3	6.3
						2.8[c]	
	2.0	2.0	2.0	2.0	2.0	2.0	2.0
*Bgl*II						11[b]	
	8.9	9.1	8.9	9.1	9.1	9.1	9.1
	2.0	2.0	2.0	2.0	2.0	2.0	2.0
*Pst*I	3.2	3.5	3.2	3.5	3.5	3.5	3.5
	2.65	2.65	2.65	2.65	2.65	2.65	2.65
	1.9	1.9	1.9	1.9	1.9	1.9	1.9
	1.4	1.4	1.4	1.4	1.4	1.4	1.4
	0.4	0.4	0.4	0.4	0.4	0.4	0.4
	0.3	0.3	0.3	0.3	0.3	0.3	0.3
2.7 Md *Bam*HI		(6.4)[d]		(6.4)		(6.6)	(6.6)
	2.7[e]	2.7[e]	3.2	3.2	3.9	4.5	3.9
	2.0	2.0	2.0	2.0	2.0	2.0	2.0
			1.0	1.0	1.2	1.2	1.2
*Bgl*II			6.2	6.2	6.2	6.6	6.1
		(3.4)		(3.4)		(3.6)	(3.6)
	2.0	2.0	2.0	2.0	2.0	2.0	2.0
	1.9[e]	1.9[e]					
	1.15	1.15	1.15	1.15	1.15	1.15	1.15
*Pst*I		(3.6)		(3.6)		(3.6)	(3.6)
	1.85[e]	1.85[e]	4.6	4.6	4.8	4.8	4.7
	1.9	1.9	1.9	1.9	1.9	1.9	1.9
	1.4	1.4	1.4	1.4	1.4	1.4	1.4
	0.6	0.6	0.6	0.6	0.6	0.6	0.6
	0.55	0.55	0.55	0.55	0.55	0.55	0.55
0.8 Md *Bam*HI	0.8	0.8	0.8	0.8	0.8	0.8	0.8
*Bgl*II		1.4		1.4	1.4	1.4	1.4
	1.15	1.15	1.15	1.15	1.15	1.15	1.15
*Pst*I		1.1		1.1	1.1	1.1	1.1
	0.55	0.55	0.55	0.55	0.55	0.55	0.55

[a] The molecular sizes of fragments are given in megadaltons.
[b] Fragments generated by partial digestion with *Sal*I.
[c] Fragments pointing to heterogeneity in CC34-5 virus DNA.
[d] Fragments containing the DS_R region, which hybridized to the P3HR-1 (EBV) fusion fragment, are in parentheses.
[e] Fusion fragments.

*Sal*I were made for comparison (Fig. 2a, c, and e).

In an attempt to demonstrate that the *Sal*I-A clones of M-ABA and P3HR-1 viruses contain authentic *Bam*HI, *Bgl*II, and *Pst*I fragments, ^{32}P-labeled M-ABA (EBV) *Sal*I-A, free of vector DNA, was hybridized to nitrocellulose blots of the same gels. The autoradiograms are shown in Fig. 2b, d, and f.

It is obvious that all of the M-ABA and P3HR-1 fragments derived from the *Sal*I-A clones comigrated with fragments of the viral DNA, with one exception. The *Bam*HI-C fragments of M-ABA, P3HR-1, and B95-8 viruses have the same size; the corresponding fragments of both M-ABA and P3HR-1 *Sal*I-A clones, however, are about 0.2 to 0.3 Md smaller than the viral fragments and are, additionally, heterogeneous in size. The same observation was made for the 9.1- and 3.5-Md fragments of *Bgl*II and *Pst*I, respectively. The size difference between viral and cloned fragments is most prominent after *Pst*I digestion (Fig. 2e and f, lanes 3 through 6).

Linkage of M-ABA (EBV) *Bam*HI, *Bgl*II, and *Pst*I fragments of *Sal*I-A. By following the linkage map of *Bam*HI fragments of P3HR-1 virus

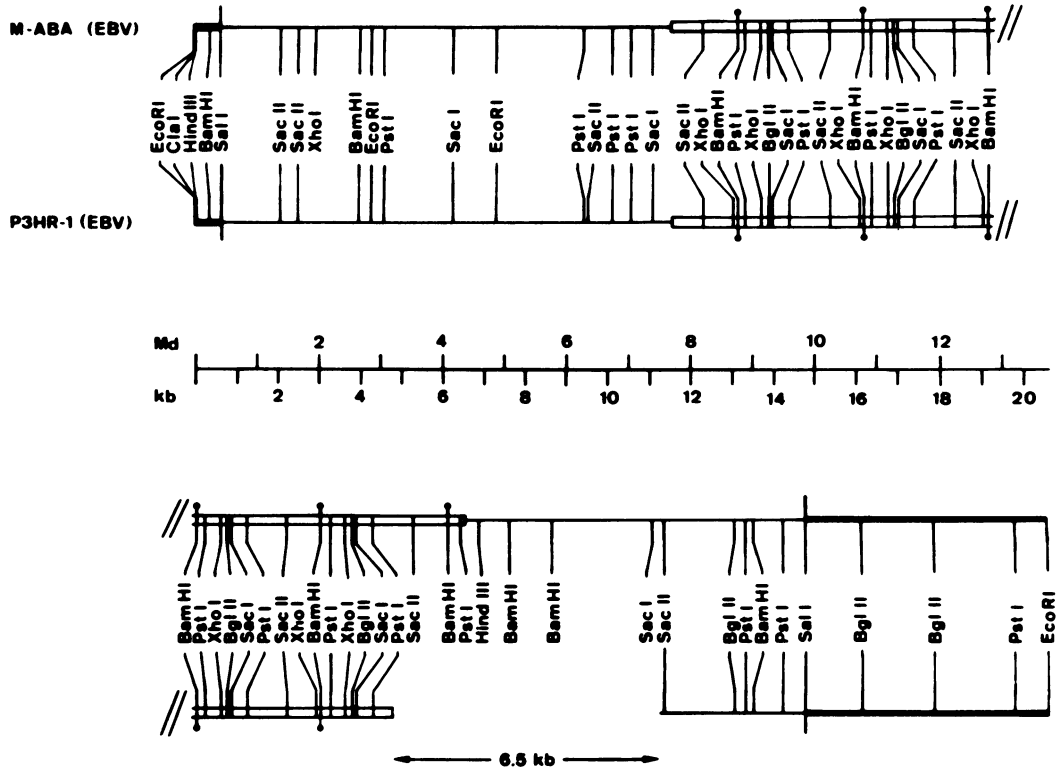


FIG. 3. Restriction enzyme maps of cloned M-ABA (EBV) and P3HR-1 (EBV) *Sal*I-A. The size heterogeneity generated during cloning is located between an *Eco*RI and a *Pst*I site 4.4 and 5.8 Md apart from the *Sal*I site, respectively, in the short unique region. The boundary between the large internal repeat and the short unique region is drawn arbitrarily. It is located within 0.6 Md to the left of the *Sac*II site of the leftmost large internal repeat; the *Pst*I site of the repeat to the left of the *Sac*II site is already missing. The *Xho*I sites in M-ABA (EBV) *Sal*I-A were deduced from the sizes of fragments generated by multiple enzyme digestion (see Fig. 4). The *Xho*I sites in P3HR-1 (EBV) *Sal*I-A were determined from Fig. 5, lanes g and h.

DNA provided by Heller et al. (20), the *Bam*HI fragments of the P3HR-1 *Sal*I-A clone can be ordered from left to right as follows: 2.2-Md terminal fragment, *Bam*HI-C, *Bam*HI-W (large internal repeat), 2.7-Md *Bam*HI-H fusion fragment, and 0.8-Md terminal fragment. Accordingly, the M-ABA (EBV) *Bam*HI fragments can be similarly arranged, with small variations compared with those of the B95-8 virus, which only concern the region to the right of the large internal repeat. *Bam*HI-Y, adjacent to the repeat, is only 1.0 Md compared with 1.2 Md in the B95-8 virus, and *Bam*HI-H has an additional *Bam*HI site, thus giving rise to two fragments of 3.2 Md (*Bam*HI-H1) and 0.7 Md (*Bam*HI-H2).

To establish the linkage map of *Bgl*II and *Pst*I fragments, *Bam*HI fragments of the P3HR-1 *Sal*I-A clone were isolated from the gel, labeled with ^{32}P by nick translation, and hybridized to blots containing separated *Bam*HI/*Sal*I, *Bgl*II/*Sal*I, and *Pst*I/*Sal*I double-cut fragments of *Sal*I-A clones and of viral origin.

Table 1 summarizes the results of the hybridization with labeled P3HR-1 (EBV) *Bam*HI fragments. The linkage of fragments resulting from the hybridization data of Table 1 is shown in the map of the M-ABA (EBV) *Sal*I-A clone (upper part of Fig. 3).

The only fragment of the *Sal*I-A clones which was not recognized by any of the labeled fragments was the M-ABA *Bam*HI 0.7-Md fragment. Since the P3HR-1 *Bam*HI 2.7-Md fusion fragment has the right-hand side in common with the M-ABA *Bam*HI-H1 fragment, the 0.7-Md *Bam*HI-H2 fragment must be located between *Bam*HI-Y and *Bam*HI-H1. In the inverse experiment this fragment did not hybridize to any P3HR-1 virus DNA fragment (see Fig. 10c).

A fragment which turned out to be particularly useful in further studies was the 6.2-Md M-ABA (EBV) *Bgl*II C fragment. This fragment, located to the right of the large internal repeat, carries more than four-fifths of the repeat and spans over the whole region deleted in P3HR-1 virus DNA.

Boundary between the large internal repeat and

the long unique region in M-ABA and B95-8 (EBV). The boundary between the large internal repeat and the long unique region of B95-8 (EBV) has been determined by restriction endonuclease mapping (18a) and by sequencing (4) to be located between the *Xho*I and the *Bgl*II sites of the large internal repeat. To analyze the junction M-ABA (EBV), we mapped the *Xho*I sites in M-ABA (EBV) *Sal*I-A by single, double, and triple digestions with *Xho*I alone, *Xho*I/*Sal*I, and *Xho*I/*Sal*I/*Bam*HI, *Xho*I/*Sal*I/*Bgl*II, and *Xho*I/*Sal*I/*Pst*I, respectively. Triple digests with *Xho*I were compared with double digests without *Xho*I (Fig. 4). Additional digestion with *Xho*I did not change the sizes of the *Bam*HI, *Bgl*II, and *Pst*I fragments containing the junction between the repeat and the long unique region. This indicated that the *Xho*I site of the large internal repeat, which lies nearest to the boundary to the long unique region, is located to the left of the *Bgl*II site. Thus, the M-ABA (EBV) 7.3-Md *Xho*I fragment contains almost an entire repeat unit.

This is obviously in contrast to B95-8 virus, in which only 117 bp of the repeat are represented in the *Xho*I fragment containing the junction (4). This difference presumably accounts for the size differences of *Bam*HI-Y fragments of M-ABA (1.0 Md) and B95-8, CC34-5, and QIMR-WIL (EBV) (1.2 Md) (Table 1), since the remaining parts of *Bam*HI-Y between the *Hin*dIII and *Bam*HI sites appear to be identical in size.

Size of the deletion of P3HR-1 virus. Data obtained from hybridization of cloned labeled *Bam*HI fragments of B95-8 virus to blots containing separated *Bam*HI fragments (20) have been interpreted to indicate that part of the sequences of *Bam*HI-Y and -H are deleted in P3HR-1 virus DNA, giving rise to a *Bam*HI-YH fusion fragment of 2.7 Md. Heller et al. (20) assumed that the size of the deletion would be defined by the size difference of B95-8 (EBV) *Bam*HI-Y plus *Bam*HI-H to the P3HR-1 (EBV) fusion fragment and would thus be 2.5 Md.

From this conclusion, the size of the P3HR-1 (EBV) *Bgl*II fusion fragment could be predicted to be 3.7 Md. A fragment of this size, however, was not observed (Fig. 2 and 4). Hybridization with the ^{32}P-labeled P3HR-1 (EBV) *Bam*HI fusion fragment identified a 1.9-Md *Bgl*II fragment as the fusion fragment (Fig. 5, lane d).

An identical fragment was also observed in DNA derived from virus particles (Fig. 2 and Table 1). All other *Bgl*II fragments of the *Sal*I-A clones of M-ABA and P3HR-1 (EBV) were identical.

The only way to resolve the discrepancy between the predicted and the observed sizes of the *Bgl*II fusion fragment was to assume that the size of the deletion is indeed correctly defined by the difference in size between the M-ABA (EBV) and P3HR-1 (EBV) *Bgl*II fragments, and not by the size difference of *Bam*HI fragments, since a cleavage site for *Bam*HI lies within the

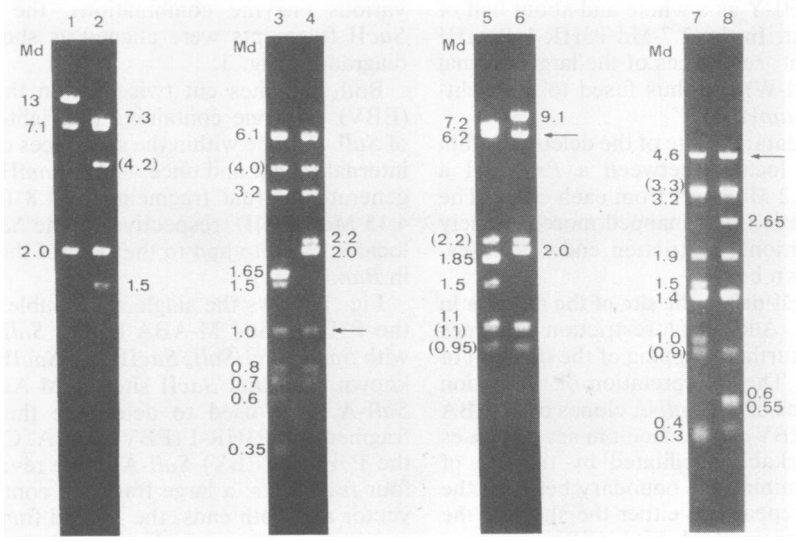


FIG. 4. Cleavage pattern of cloned M-ABA (EBV) *Sal*I-A after digestion with *Xho*I (lane 1), *Sal*I/*Xho*I (lane 2), *Bam*HI/*Sal*I/*Xho*I (lane 3), *Bam*HI/*Sal*I (lane 4), *Bgl*II/*Sal*I/*Xho*I (lane 5), *Bgl*II/*Sal*I (lane 6), *Pst*I/*Sal*I/*Xho*I (lane 7), and *Pst*I/*Sal*I (lane 8). Fragments were separated in 0.8% agarose. Parentheses indicate fragments containing vector DNA. The arrows point to the fragments containing the boundary between the large internal repeat and the long unique region. Note that *Xho*I digestion did not change the size of the *Bam*HI, *Bgl*II, and *Pst*I fragments containing the junction (lanes 3 through 8).

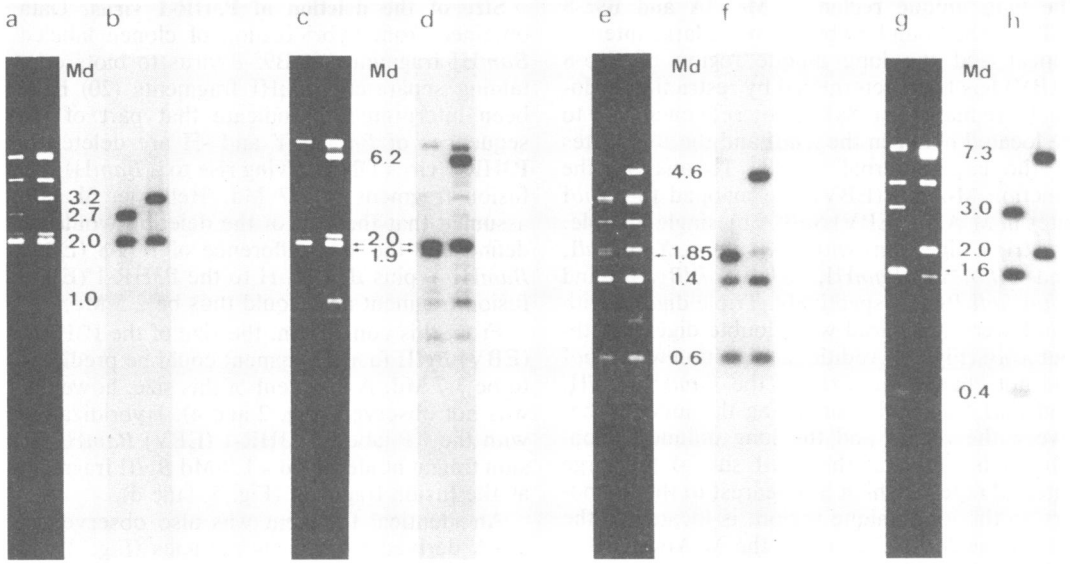


FIG. 5. Fragment pattern of cloned P3HR-1 (EBV) *Sal*I-A (left lanes) and cloned M-ABA (EBV) *Sal*I-A (right lanes) after double digestion with *Bam*HI/*Sal*I (a), *Bgl*II/*Sal*I (c), *Pst*I/*Sal*I (e), and *Xho*I/*Sal*I (g) and separation of fragments in 0.8% agarose. Fragments were transferred to nitrocellulose and hybridized to the ^{32}P-labeled P3HR-1 (EBV) *Bam*HI fusion fragment (lanes b, d, f, h). Note that P3HR-1 (EBV) has an additional *Xho*I site in the large internal repeat (g and h), which is also present in Jijoye (EBV) (data not shown).

deleted region. The deletion is thus 4.3 Md compared with M-ABA virus and possibly 4.5 Md compared with B95-8 virus instead of 2.5 Md reported by Heller et al. (20). The deletion involves *Bam*HI-Y as a whole and about half of one repeat unit. In the 2.7-Md P3HR-1 *Bam*HI fusion fragment, sequences of the large internal repeat (*Bam*HI-W) are thus fused to the right-hand side of *Bam*HI-H1.

From these data, the site of the deletion within the repeat is located between a *Pst*I and a *Bam*HI site, 1.2 Md apart from each other. The site of the deletion was mapped more precisely by use of additional restriction endonucleases, as will be shown below.

***Sac*I and *Sac*II define the site of the deletion in P3HR-1 virus.** Additional restriction enzymes were used for further mapping of the deletion of P3HR-1 virus. The interpretation of restriction enzyme patterns of the *Sal*I-A clones of M-ABA and P3HR-1 (EBV) was difficult in several cases but was remarkably facilitated by the use of subclones containing the boundary between the large internal repeat and either the short or the long unique region of M-ABA (EBV), respectively. Two enzymes, *Sac*I and *Sac*II, were particularly useful in defining the site of the deletion in P3HR-1 virus. To map *Sac*I and *Sac*II sites in M-ABA (EBV) *Sal*I-A, the subclones containing the boundaries were cleaved with different combinations of *Sac*I, *Sac*II, *Bgl*II, and *Sal*I. Fragments were separated in 0.8% agarose (Fig. 6). By identification of the fragments joined to the vector and by size determination of the fragments generated by the various enzyme combinations, the *Sac*I and *Sac*II fragments were aligned as shown in the diagram of Fig. 3.

Both enzymes cut twice within the M-ABA (EBV) subclone containing the right-hand side of *Sal*I-A, once within the sequences of the large internal repeat and once within *Bam*HI-H1, and generate internal fragments of 4.8 (*Sac*I) and 4.15 Md (*Sac*II), respectively. The *Sac*II site is located close to and to the right of the *Sac*I site in *Bam*HI-H1.

Fig. 7 shows the single and double digests of the P3HR-1 and M-ABA (EBV) *Sal*I-A clones with *Sac*I, *Sac*I/*Sal*I, *Sac*II, and *Sac*II/*Sal*I. The known *Sac*I and *Sac*II sites in M-ABA (EBV) *Sal*I-A were used to determine the order of fragments in P3HR-1 (EBV) *Sal*I-A. Cleavage of the P3HR-1 (EBV) *Sal*I-A clone revealed only four fragments: a large fragment containing the vector and both ends, the 3.2-Md fragment heterogeneous in size, the large internal repeat, and the 1.95-Md fragment containing the junction between the large internal repeat and the short unique region (Fig. 7, lane 1). This indicates that the *Sac*I site in *Bam*HI-H1 is deleted in P3HR-1 virus. Additional cleavage with *Sal*I generated a terminal fusion fragment of 2.95 Md spanning

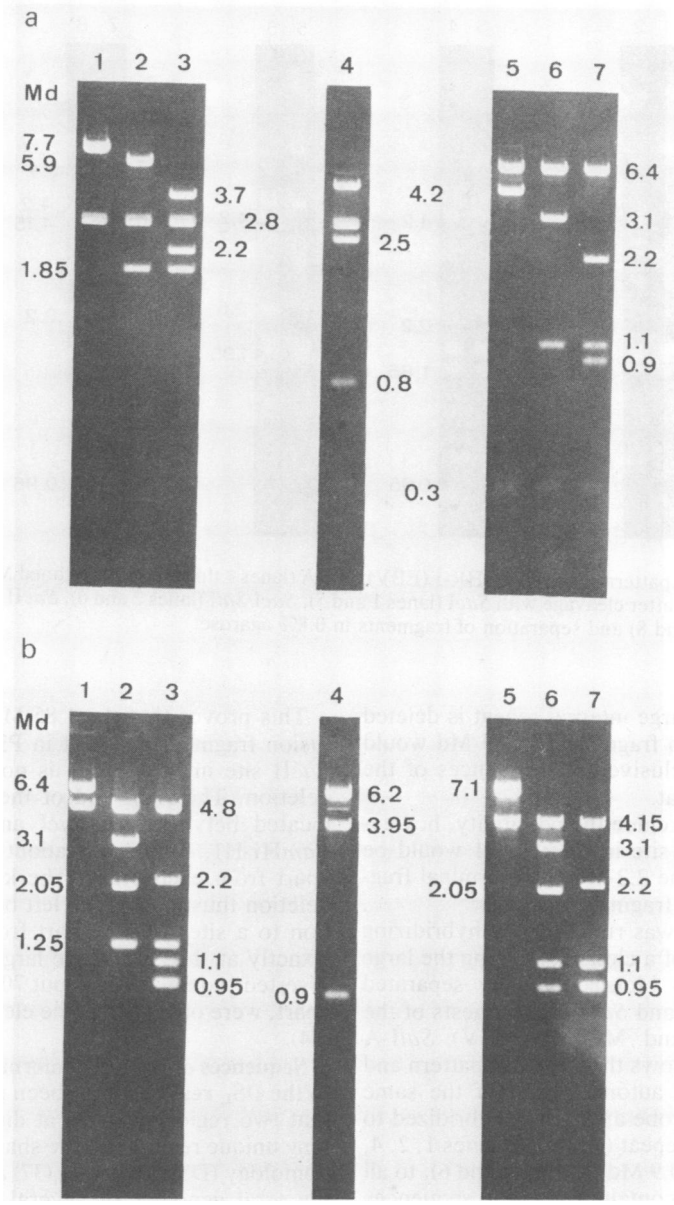


FIG. 6. Cleavage pattern of DNA of the M-ABA (EBV) subclones of *Sal*I-A containing the junction between large internal repeats and short (a) and long unique regions (b). Both clones extend from the *Sal*I sites to the *Bgl*II sites in the large internal repeat. DNA was digested with *Sac*I (lanes 1), *Sac*I/*Bgl*II (lanes 2), *Sac*I/*Bgl*II/*Sal*I (lanes 3), *Sac*I/*Sac*II (lanes 4), *Sac*II (lanes 5), *Sac*II/*Bgl*II (lanes 6), and *Sac*II/*Bgl*II/*Sal*I (lanes 7), and the fragments were separated in 0.8% agarose.

from the *Sac*I site in the large internal repeat to the *Sal*I site in the long unique region.

Cleavage of the P3HR-1 (EBV) *Sal*I-A clone with *Sac*II revealed five or six fragments (Fig. 6, lane 3): a large fragment containing the vector and both terminal fragments of 2.2 and 0.95 Md, a fragment heterogeneous in size of 4.5 Md, the large internal repeat, a presumably double molar fragment of 1.85 Md, and a small fragment of 0.3 Md, not seen in Fig. 7. Since the fragment heterogeneous in size is 6.4 Md in M-ABA (EBV) *Sal*I-A, an additional cleavage site was assumed to be present in P3HR-1 (EBV) *Sal*I-A, generating two fragments of about 4.5 and 1.85 Md. The right-terminal fragment of 2.2 Md appeared to be present in P3HR-1 (EBV), suggesting that the *Sac*II site in *Bam*HI-H1 would be conserved. This would imply that the rightmost

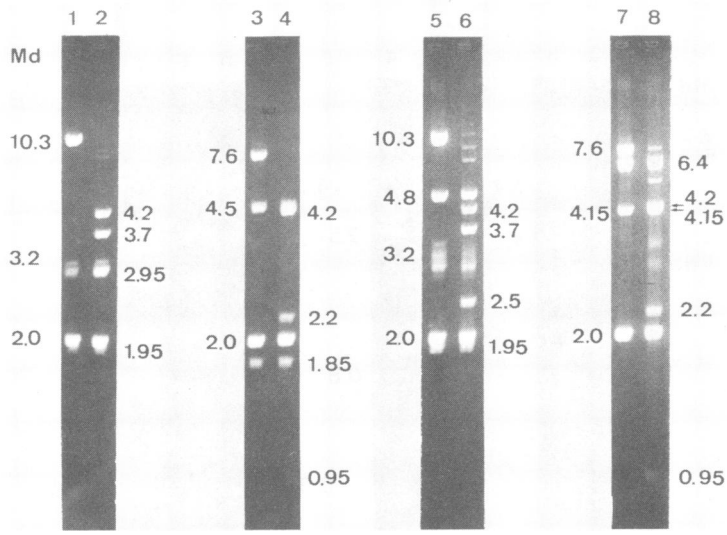


FIG. 7. Fragment pattern of cloned P3HR-1 (EBV) *Sal*I-A (lanes 1 through 4) and cloned M-ABA (EBV) *Sal*I-A (lanes 5 through 8) after cleavage with *Sac*I (lanes 1 and 5), *Sac*I/*Sal*I (lanes 2 and 6), *Sac*II (lanes 3 and 7), and *Sac*II/*Sal*I (lanes 4 and 8) and separation of fragments in 0.8% agarose.

*Sac*II site of the large internal repeat is deleted and that the fusion fragment of 1.85 Md would consist almost exclusively of sequences of the large internal repeat.

We wanted to exclude the possibility, however, that the *Sac*II site in *Bam*HI-H1 would be deleted and that the 2.2-Md right-terminal fragment is the fusion fragment.

This possibility was ruled out by hybridizing ^{32}P-labeled DNA of a clone containing the large internal repeat to blots containing separated *Sac*II/*Sal*I, *Sac*II, and *Sac*II/*Sac*I digests of the P3HR-1 (EBV) and M-ABA (EBV) *Sal*I-A clones. Figure 8 shows the fragment pattern and the corresponding autoradiogram of the same gel. The labeled probe apparently hybridized to the large internal repeat (2.0 Md in lanes 1, 2, 4, and 5 and 1.1 and 0.9 Md in lanes 3 and 6), to all of the fragments containing vector sequences (4.15 Md in lanes 1 and 4), and to the large fragments containing the vector and both termini (lanes 2, 3, 5, and 6). Additionally, the probe hybridized to the 4.15-Md fragment (lane 5) and the 3.95-Md fragment (lane 6) of M-ABA (EBV) containing the junction between large internal repeat and long unique region and weakly to the 6.4-Md fragment of M-ABA (EBV) containing the junction between repeat and the short unique region. In P3HR-1 (EBV), the probe hybridized to the 1.85-Md fragments containing the junction of the large internal repeat to the short and that to the long unique region; it did not hybridize, however, to the 2.2-Md fragment of P3HR-1 (EBV) (Fig. 8a and c, lanes 1).

This proves that the 1.85-Md fragment is the fusion fragment and that in P3HR-1 (EBV) the *Sac*II site in *Bam*HI-H1 is not involved in the deletion. The right end of the deletion is thus located between the *Sac*I and *Sac*II sites in *Bam*HI-H1, which lie about 200 nucleotides apart from each other. The known size of the deletion thus locates the left border of the deletion to a site 4.3 Md apart from the right end. Exactly at this site in the large internal repeat, inverted sequences of about 70 bp, about 290 bp apart, were observed in the electron microscope (24).

Sequences of the large internal repeat are fused to the DS_L region. It has been shown previously that two regions located at distant parts of the long unique region of EBV share some sequence homology (DS_L and DS_R) (37) and are present in the viral genomes of several different isolates (20). This partially duplicated region has been shown to consist of about 0.9 kbp, with good sequence homology, and of 1.5 kbp, with partial homology, including small internal repeats of 102 bp (24). The small internal repeat originating from the right-hand side (DS_R repeat) appeared to be partially conserved in the left-hand cross-hybridizing region (DS_L).

To map the site of the deletion in the long unique region relative to the DS_L region, DNA of a clone containing the P3HR-1 (EBV) *Bam*HI fusion fragment was digested with *Bam*HI/*Eco*RI, and the fragments were separated in agarose. The fusion fragment was isolated from the gel, labeled by nick translation, and hybrid-

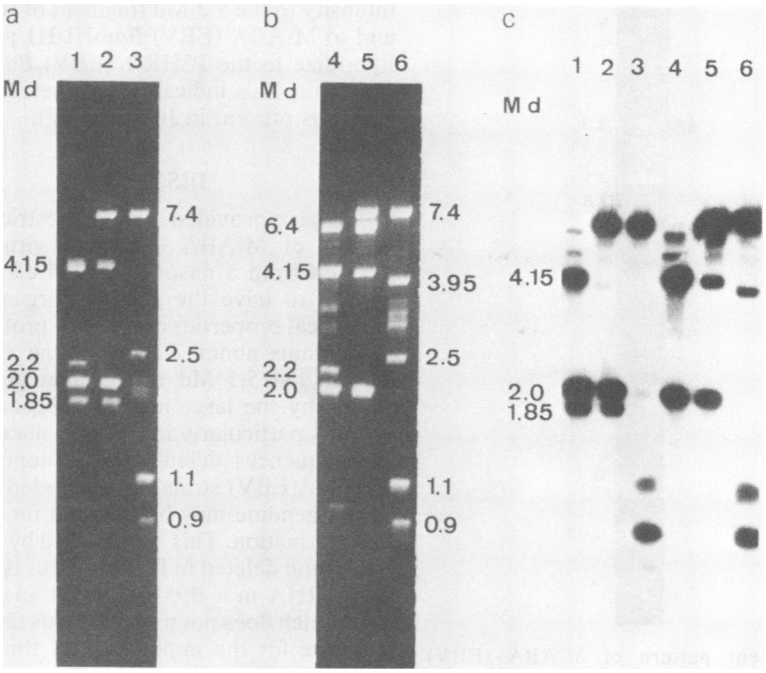


FIG. 8. Fragment pattern of cloned P3HR-1 (EBV) *Sal*I-A (a) and cloned M-ABA (EBV) *Sal*I-A (b) after cleavage with *Sac*II/*Sal*I (lanes 1 and 4), *Sac*II (lanes 2 and 5), and *Sac*I/*Sac*II (lanes 3 and 6) and separation of fragments in 0.8% agarose gels. The fragments shown in (a) and (b) were transferred to nitrocellulose and hybridized to ^{32}P-labeled DNA of a clone containing the large internal repeat (c).

ized to a nitrocellulose filter with separated *Pst*I fragments of a clone containing the DS_L region. The labeled fusion fragment hybridized efficiently to the 3.3-Md fragment to the right of the DS_R repeat, which is known to contain the region of good homology; it failed to hybridize, however, to the DS_R repeat (Fig. 9). In P3HR-1 virus, sequences of the large internal repeat are thus fused to the DS_L region.

Jijoye (EBV) contains the complete DS_L region, but misses sequences present in M-ABA (EBV). The P3HR-1 cell line was derived from the human Burkitt's lymphoma call line Jijoye by single-cell cloning (22). The Jijoye line has been reported to produce a virus which does not differ biologically from any prototype EBV. It has transforming capacity and is unable to induce the early antigen complex upon superinfection of Raji cells (39, 43).

To test whether the deletion in P3HR-1 virus does indeed correlate with the loss of the transforming capacity, we compared Jijoye (EBV) DNA to P3HR-1 virus DNA. This was done by hybridizing ^{32}P-labeled nick-translated M-ABA virus DNA and cloned M-ABA (EBV) probes representing the region deleted in P3HR-1 virus (see Fig. 11) to blots containing separated *Hin*dIII fragments of Jijoye and P3HR-1 DNA. The *Hin*dIII fragment pattern shown in Fig. 10a suggested that the *Hin*dIII site within *Bam*HI-Y, which joins *Hin*dIII-A and -B and which is deleted in P3HR-1 virus, is present in Jijoye (EBV). This was verified by hybridization with ^{32}P-labeled DNA of a clone containing the right half of M-ABA (EBV) *Bam*HI-Y (Fig. 10b). Hybridization with ^{32}P-labeled cloned M-ABA (EBV) *Bam*HI-H2, however, was totally negative. Therefore, M-ABA *Hin*dIII fragments were included as a positive control. Only M-ABA (EBV) *Hin*dIII-B hybridized strongly to cloned *Bam*HI-H2 DNA; none of the fragments of Jijoye and P3HR-1 DNA did (Fig. 10c).

For further analysis of the Jijoye (EBV) genome, blots containing separated *Bam*HI fragments of Jijoye, P3HR-1, and M-ABA were hybridized to labeled DNA of a clone, schematically described in Fig. 11, which contains the complete DS_L region. The DNA of this clone hybridized to M-ABA (EBV) *Bam*HI-H1, -H2, and the 6.4-Md fragment containing the DS_R region (Fig. 10d). In P3HR-1 DNA the probe hybridized with about the same intensity to the 2.7-Md *Bam*HI fusion fragment and to the fragment containing the DS_R region. In Jijoye, in addition to the DS_R-carrying fragment, the probe hybridized strongly to a 5.2-Md fragment and faintly to a 2.7-Md fragment. This latter fragment comigrates with the P3HR-1 (EBV)

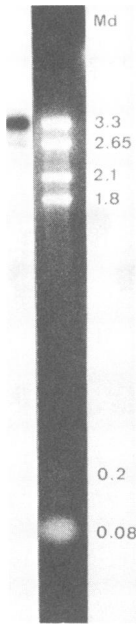


FIG. 9. Fragment pattern of M-ABA (EBV) *Hin*dIII-D2 cloned into the vector pACYC184 after double digestion with *Pst*I/*Hin*dIII and separation of fragments in 0.8% agarose (right lane). After being transferred to nitrocellulose, fragments were hybridized to the ^{32}P-labeled P3HR-1 (EBV) *Bam*HI fusion fragment, which had been isolated from the gel (left lane). The 3.3-Md fragment, which hybridized to the fusion fragment, is located to the right of the DS_R repeats and contains the region of good homology (24). A map of the *Hin*dIII-D2 clone has been described (24). The 2.65-Md vector fragment hybridized weakly due to contamination of the labeled probe with vector DNA sequences.

*Bam*HI fusion fragment and is presumably derived from the minority of cells in the Jijoye population which correspond to the P3HR-1 clone.

The probe used in Fig. 10b representing the right-hand side of M-ABA (EBV) *Bam*HI-Y hybridized to a *Bam*HI fragment of the same size (data not shown), indicating that *Bam*HI-Y and -H1 sequences are fused in the Jijoye (EBV) genome. The intensity of hybridization suggested that most of the *Bam*HI-H1 sequences would be present in the Jijoye (EBV) *Bam*HI-YH fusion fragment. To prove that the DS_L repeats are present in the Jijoye (EBV) 5.2-Md *Bam*HI fragment, the ^{32}P-labeled cloned DS_R repeat was hybridized to separated Jijoye, P3HR-1, and M-ABA *Bam*HI fragments. The DS_R repeat hybridized equally well to the fragments of Jijoye, P3HR-1, and M-ABA containing the DS_R region (Fig. 10e). The DS_R repeat hybridized with less intensity to the 5.2-Md fragment of Jijoye (EBV) and to M-ABA (EBV) *Bam*HI-H1 and failed to hybridize to the P3HR-1 (EBV) *Bam*HI fusion fragment. This indicates that the complete DS_L region is present in Jijoye (EBV).

DISCUSSION

We have provided a map of restriction sites in *Sal*I-A of M-ABA (EBV), a virus originally derived from a nasopharyngeal carcinoma and known to have the genomic organization and biological properties of an EBV prototype. *Sal*I-A contains about 7.2 Md of the short unique region and 5.5 Md of the long unique region joined by the large internal repeats. This fragment is particularly interesting since it contains the sequences deleted in the nontransforming P3HR-1 (EBV) strain. Genes coded by this part of the genome may be required for initiation of transformation. This is suggested by the fact that the region deleted in P3HR-1 virus is transcribed into mRNA in a B95-8 (EBV)-transformed cell line, which does not produce virus (26). The best evidence for the importance of this region for initiation of transformation was provided by the work of Fresen et al. (14), who isolated a number of recombinant viruses with transforming properties by infection of Raji cells with P3HR-1 virus. Analysis of the genome revealed that all of the rescued isolates had invariably picked up the region from the endogenous Raji (EBV) genome, which is deleted in P3HR-1 virus (14).

To determine the sites of the deletion of P3HR-1 virus, we cloned *Sal*I-A of M-ABA and P3HR-1 viruses. *Sal*I-A of M-ABA (EBV) spans completely over the deletion observed in P3HR-1 (EBV) *Sal*I-A. The restriction enzyme maps obtained for both cloned fragments (Fig. 3) allowed us to map the sites of the deletion in P3HR-1 virus. It has been reported previously by us and by others that sequences of the long unique region adjacent to the large internal repeat are deleted in P3HR-1 (EBV) (2, 20). That the P3HR-1 (EBV) *Bam*HI fusion fragment hybridized to *Bam*HI-Y and *Bam*HI-H was interpreted by Heller et al. (20) to mean that 2.5 Md from the right-hand side of *Bam*HI-Y and from the left-hand side of *Bam*HI-H would be deleted. Here we have provided evidence that the deletion is 4.3 Md compared with M-ABA (EBV) and involves the transition between the large internal repeats and the long unique region. The *Bam*HI fusion fragment of P3HR-1 virus thus consists of the left-hand side of *Bam*HI-W (the large internal repeat) and the right-hand side of *Bam*HI-H or *Bam*HI-H1 in M-ABA (EBV), respectively. The size of the deletion was determined by the size difference of M-ABA and P3HR-1 (EBV) *Bgl*II fragments, which span

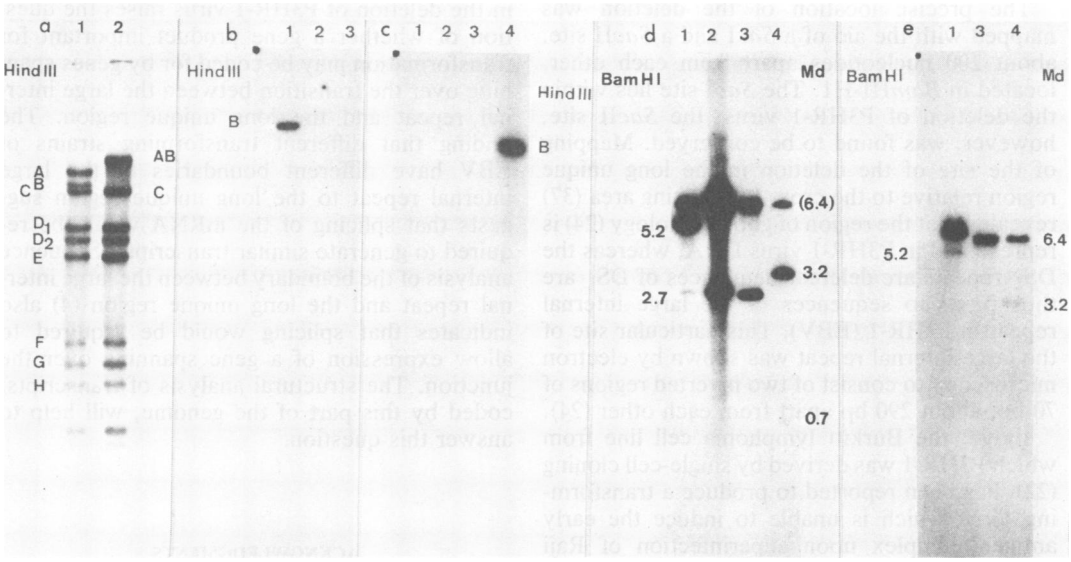


FIG. 10. Blots containing separated Jijoye (lanes 1), P3HR-1 cellular (lanes 2), P3HR-1 viral (lanes 3), and M-ABA cellular (lanes 4) *Hin*dIII fragments (a, b, and c) and *Bam*HI fragments (d and e) hybridized to ^{32}P-labeled M-ABA virus DNA (a), the cloned right-hand part of M-ABA (EBV) *Bam*HI-Y (b), the cloned M-ABA (EBV) *Bam*HI-H2 (c), the cloned right-hand part of M-ABA (EBV) *Bgl*II-C (d), and the cloned M-ABA (EBV) DS_R repeat (e). Cellular (8 μg) or viral (20 ng) DNA fragments were separated in 0.4% agarose before being transferred to nitrocellulose. The cloned viral sequences used as probes in b, c, and d are schematically described in Fig. 11.

completely over the deletion. The discrepancy between these results and the interpretation of the data by Heller et al. (20) is explained by the finding that an additional cleavage site, the rightmost *Bam*HI site of the large internal repeat, is also involved in the deletion. The hybridization of *Bam*HI-Y to the P3HR-1 (EBV) fusion fragment is explained by the fact that both *Bam*HI-Y and the fusion fragment contain sequences homologous to the large internal repeat.

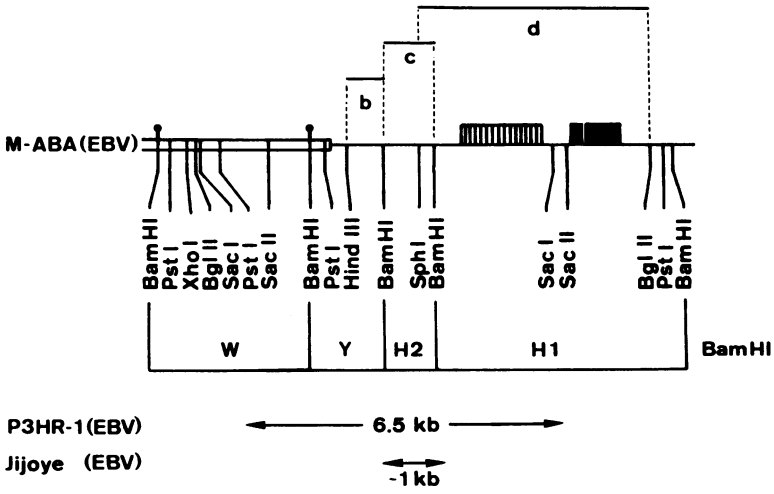


FIG. 11. Schematic description of the boundary between the large internal repeat and the long unique region of M-ABA (EBV). The DS_L region consists of sequences with good homology to DS_R (black) and the DS_L repeats, which have partial homology to the DS_R repeats. The cloned fragments used as labeled probes in Fig. 10b, c, and d are designated here as b, c, and d. The small arrow designates the minimum of sequences absent in Jijoye (EBV); the large arrow describes the deletion of P3HR-1 virus.

The precise location of the deletion was mapped with the aid of a *Sac*I and a *Sac*II site, about 200 nucleotides apart from each other, located in *Bam*HI-H1. The *Sac*I site lies within the deletion of P3HR-1 virus; the *Sac*II site, however, was found to be conserved. Mapping of the site of the deletion in the long unique region relative to the cross-hybridizing area (37) revealed that the region of good homology (24) is represented in P3HR-1 virus DNA, whereas the DS_L repeats are deleted. Sequences of DS_L are thus fused to sequences of the large internal repeat in P3HR-1 (EBV). This particular site of the large internal repeat was shown by electron microscopy to consist of two inverted regions of 70 bp, about 290 bp apart from each other (24).

Jijoye, the Burkitt lymphoma cell line from which P3HR-1 was derived by single-cell cloning (22), has been reported to produce a transforming virus which is unable to induce the early antigen complex upon superinfection of Raji cells (39, 43).

If the deletion in P3HR-1 virus has indeed eliminated the genes required for initiation of transformation, one would expect these genes to be present in Jijoye (EBV). Therefore, Jijoye DNA was analyzed with a number of cloned M-ABA viral probes specific for the region deleted in P3HR-1 virus. These experiments have demonstrated that most of *Bam*HI-Y, including the *Hin*dIII site between *Hin*dIII-A and -B and the complete DS_L region, is present in Jijoye (EBV). They also revealed, however, the unexpected finding that the sequences of M-ABA (EBV) *Bam*HI-H2 are absent from Jijoye virus. In Jijoye (EBV), sequences of *Bam*HI-Y are fused to sequences of *Bam*HI-H1, giving rise to a fusion fragment of 5.2 Md. Fusion of *Bam*HI-Y (1.2 Md) and *Bam*HI-H1 (3.2 Md) should result in a new fragment which is smaller than the sum of both. Why the Jijoye (EBV) *Bam*HI-YH fragment is larger is not understood. One obvious explanation could be that it contains a large number of DS_L repeats. Alternatively, the *Bam*HI sites limiting this fragment could be different from those in M-ABA (EBV). Finally, this Jijoye (EBV) fragment could contain sequences which are not present in M-ABA virus DNA.

The fact that sequences of about 1 kbp corresponding to M-ABA (EBV) *Bam*HI-H2 are not present in the transforming Jijoye virus stresses the importance of either the DS_L region or the boundary between the large internal repeat and the long unique region or even of both regions for the initiation of transformation. Further analysis of the Jijoye virus genome and its transcription in Jijoye (EBV)-transformed cells will be useful to narrow down the region required for transformation.

Partial involvement of the large internal repeat in the deletion of P3HR-1 virus raises the question of whether a gene product important for transformation may be coded for by genes spanning over the transition between the large internal repeat and the long unique region. The finding that different transforming strains of EBV have different boundaries of the large internal repeat to the long unique region suggests that splicing of the mRNA would be required to generate similar transcripts. Sequence analysis of the boundary between the large internal repeat and the long unique region (4) also indicates that splicing would be required to allow expression of a gene spanning over the junction. The structural analysis of transcripts, coded by this part of the genome, will help to answer this question.

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LITERATURE CITED

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