Herpesvirus Papio DNA Is Similar in Organization to Epstein-Barr Virus DNA

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*Eco*RI, *Hind*III, *Sal*I, and *Xba*I restriction endonuclease maps of herpesvirus papio (HVPapio) DNA were derived by determining the fragment sizes and the linkage relationships between fragments generated by the different enzymes. The data indicate that HVPapio DNA has a single molecular arrangement which is similar to that of Epstein-Barr virus DNA. The size of the DNA was 110×10^6 to 114×10^6 daltons. Restriction fragments from both ends varied in the number of repeats of a 4×10^5 -dalton sequence, TR, and hybridized to each other. This suggests that there is an identical repeating unit, TR, at both ends of the DNA. There were usually six tandem repetitions (range, 1 to 11) of a 2×10^6 -dalton sequence, IR, within the DNA. IR separated the DNA into two domains of largely unique sequence complexity, a 9×10^6 -dalton segment, U_S, and an 88×10^6 -dalton segment, U_L. There was homology between DNA fragments which mapped at 25×10^6 to 29×10^6 and at 91×10^6 to 95×10^6 daltons in U_L.

Chimpanzees and baboons are frequently infected with herpesvirus pan (HVPan) and herpesvirus papio (HVPapio), which are similar but not identical to human Epstein-Barr virus (EBV) (5-9, 13, 22, 24, 28, 36). The host range of the viruses in vitro is restricted to primate B lymphocytes (5, 7, 8). Lymphocytes infected with HVPan or HVPapio are transformed into cells capable of growth as continuous cell lines. Most of the cells in the lines established after infection with the primate herpesviruses are not permissive of virus replication (7).

The viral capsid, membrane, and early antigens of HVPan and HVPapio are recognized by human sera which are reactive with the analogous antigenic determinants of EBV (5-9, 13, 24, 28, 36). Chimpanzee or baboon sera react with each of the EBV antigenic determinants (6-9, 13, 24). The intranuclear antigens induced by HVPan and HVPapio in latently infected, growth-transformed cells differ, however, from the nuclear antigen induced by EBV (6-9, 28). HVPan and HVPapio DNA have approximately 40% homology to EBV DNA as shown by DNA-DNA reassociation kinetics (5, 8). The biological and DNA sequence relatedness between EBV and the primate herpesviruses suggest that genetic interactions among these genomes may be feasible.

The EBV genome has been previously shown to consist of two components of unique sequence complexity, U_L and U_S, which are separated by varying numbers of a tandemly reiterated 3,300-base-pair sequence, IR (10, 11, 15, 18, 31, 33).

The DNA is bounded at both ends by a varying number of direct repeats of a 500-base-pair sequence, TR (10, 12, 14, 18, 19, 31). Nothing was previously known about the structural organization of the primate herpesviruses. The objective of this study was to derive a restriction endonuclease map of the DNA of a HVPapio isolate to determine whether this DNA was similar in organization to EBV DNA.

MATERIALS AND METHODS

Cell culture and virus purification. HVPapio was obtained from a virus-producing baboon lymphoid cell line, baboon 65. Baboon 65 is a continuous cell line established from circulating lymphocytes of a normal baboon, *Papio cynocephalus* (7). Cells were grown in media consisting of RPMI 1640 supplemented with 10% fetal calf serum (both obtained from GIBCO Laboratories, Grand Island, N.Y.). Some cell cultures were treated with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) at a concentration of 20 ng/ml for 7 to 9 days before harvest of the media for virus purification (40). The procedures used in purifying virus from the supernatant media of cell cultures have been described (4). Treatment with 12-*O*-tetradecanoylphorbol-13-acetate usually resulted in an increase in the yield of virus.

Preparation of viral DNA. The final virus pellet was resuspended in 0.1 M NaCl, 0.05 M Tris-hydrochloride (pH 7.6), and 0.01 M EDTA. Sodium dodecyl sulfate (SDS) was added to 1% (wt/vol). The DNA solution was extracted with phenol at 56°C and then extracted with chloroform containing 2% (vol/vol) isoamyl alcohol (26). The DNA was dialyzed against 0.3 M NaCl, 0.01 M EDTA, and 0.05 M Tris-hydrochloride (pH 7.6) and then stored in 1 mM Tris-hydrochloride (pH 7.6) and 0.1 mM EDTA at 4°C.

HVPapio DNA which was to be labeled in vitro by nick translation was further purified by density equilibrium sedimentation in CsCl. The DNA solution was adjusted to contain 25 mM Tris-hydrochloride (pH 7.6), 2.5 mM EDTA, 0.2% (wt/vol) Sarkosyl, and 1.715 g of CsCl per cm³ (Eastern Chemical, Hauppauge, N.Y.). The solution was centrifuged in a VTi 50 rotor (Beckman Instruments, Inc., Palo Alto, Calif.) at 40,000 rpm for 18 h at 20°C. Fractions with a density between 1.730 and 1.707 g/cm³ were pooled and dialyzed against 1 mM Tris-hydrochloride (pH 7.6) and 0.1 mM EDTA.

Restriction endonucleases. Restriction endonucleases *Xba*I, *Eco*RI, *Hind*III, and *Sa*II were purchased from Bethesda Research Laboratories, Rockville, Md. Enzyme digestion was carried out under conditions specified by the manufacturer.

Gel electrophoresis and isolation of DNA fragments. Electrophoresis of digested DNA samples was performed in 0.3 or 0.4% agarose [Seakem HGT(P), Marine Colloids, Rockland, Maine] or 0.8% agarose (Seakem ME) containing ethidium bromide in a vertical slab gel 27 by 22 by 0.6 cm in size. Sample slots (5 or 10 cm wide) were used to make preparative Southern blots of each enzyme digest. The vertical slab gel apparatus used permits use of very low percentage agarose gels, since the level of buffer in each buffer chamber is the same and therefore the gel experiences no net hydrostatic force. (The design principle was developed and suggested to us by Marvin Stodolsky, Loyola University, Chicago, Ill.) The buffer system contained 0.036 M Tris, 0.03 M NaH₂PO₄, and 1 mM EDTA (16).

Electrophoresis was carried out for 20 h at 50 to 60 V at 4°C. The ethidium bromide-stained DNA bands were visualized with UV light and were sliced from the gels. In cases where fragments were similar in molecular weight and migrated in the same region of the gel, the region was sliced intact from the gel, embedded in agarose in a horizontal slab gel, and electrophoresed again to improve the separation of these fragments.

DNA was eluted from individual agarose slices either by repeated cycles of freeze-thaw or by electrophoresis of the agarose in dialysis tubing filled with 4 ml of buffer. The eluted DNA was extracted with butanol to remove ethidium bromide and concentrated by vacuum dialysis against 1 mM Tris-hydrochloride (pH 7.6) and 0.1 mM EDTA.

Radioactive labeling of DNA and hybridization to nitrocellulose filters. Viral DNA fragments were immobilized on nitrocellulose filters (Millipore Corp., Bedford, Mass.) by the transfer technique of Southern (35). Identical filter strips of a restriction endonuclease digest were made by slicing preparative Southern blots into strips 2 mm wide.

³²P-labeled DNA fragment probes were made by nick translation of 25 to 50 ng of each fragment with *Escherichia coli* DNA polymerase I (Boehringer-Mannheim Corp., Indianapolis, Ind.) and [α -³²P]dCTP (350 to 400 Ci/mmol, Amersham Corp., Arlington Heights, Ill.) (25, 32). ³²P-labeled HVPapio DNA total probe was made by nick translation of 0.25 μ g of DNA purified on CsCl. Labeled DNA was separated from unincorporated nucleotide by chromatography on a column of Sephadex G-50 (Pharmacia Fine Chemicals,

Inc., Piscataway, N.J.) in 10 mM Tris-hydrochloride (pH 7.4), 1 mM EDTA, and 0.1% (wt/vol) Sarkosyl or by dialysis against this buffer. The labeled DNA had a specific activity of 1×10^8 to 2×10^8 cpm/ μ g.

For hybridization, blot strips were preincubated for 1 h at 72°C in 6 $\times$ SSC (pH 7.4) (1 $\times$ SSC is 0.15 NaCl plus 0.015 M sodium citrate) containing 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 0.2% Ficoll, 1 mM EDTA, 50 μ g of salmon sperm DNA per ml, and 0.2% SDS (3, 21) in glass tubes heat sealed at one end and closed with a silicone rubber stopper at the other. The ³²P-labeled DNA probes were heat denatured at 110°C for 6 min and chilled on ice. The probe solution was made 6 $\times$ SSC containing 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 0.02% Ficoll, 1 mM EDTA, 50 μ g of salmon sperm DNA per ml, and 0.2% SDS. The pre-hybridization solution was poured off the blots, probe solution was added, and hybridization was carried out at 72 to 74°C with shaking for 8 to 20 h. After hybridization, the nitrocellulose strips were washed with sequential changes of 2 $\times$ SSC and 0.2% SDS, 0.5 $\times$ SSC and 0.2% SDS, and 0.2 $\times$ SSC and 0.2% SDS for 2 to 4 h at 72°C. The filters were dried and exposed to SB-5 X-ray film (Eastman Kodak Corp., Rochester, N.Y.) for autoradiography.

Molecular weight estimates of restriction endonuclease fragments of HVPapio DNA. Portions (0.25 μ g) of HVPapio DNA were separately digested with *Eco*RI, *Hind*III, *Sa*II and *Xba*I restriction endonucleases. Bacteriophage lambda DNA (New England Biolabs, Beverly, Mass.) was digested with *Eco*RI and *Hind*III restriction enzymes to generate DNA fragments of known molecular weight. Intact lambda DNA and adenovirus type 2 (Ad2) DNA (Ad2 DNA was the kind gift of Maurice Green, St. Louis University, St. Louis, Mo.) were added to the completed lambda digests to provide higher-molecular-weight DNA markers (29). The lambda DNA digests were heated for 1 min at 70°C to denature fused end fragments formed due to annealing of the cohesive ends of lambda DNA. The DNA fragments of each digest were separated by electrophoresis in parallel lanes in a 0.4% agarose vertical slab gel. The DNA fragments were transferred to a nitrocellulose sheet by Southern blotting. The filter was sealed in a plastic bag (Seal N'Save, Sears Roebuck and Co., Chicago, Ill.), and the fragments were stained by hybridization with a mixture of ³²P-labeled lambda DNA and HVPapio DNA probes prepared by nick translation.

RESULTS

Four site-specific restriction endonucleases, *Eco*RI, *Hind*III, *Sa*II, and *Xba*I, were used to cleave HVPapio DNA into specific fragments. The organization of these fragments in HVPapio DNA was determined from linkage relationships and from the size of the fragments. Linkage relationships were established by hybridizing a labeled restriction fragment with blots of fragments generated by other enzymes.

Size and molar abundance of *Eco*RI, *Sa*II, *Xba*I, and *Hind*III fragments of HVPapio

DNA. The sizes of the *EcoRI*, *SalI*, *XbaI*, and *HindIII* fragments of HVPapio DNA were determined from the electrophoretic mobility of the fragments in agarose gels relative to lambda or Ad2 DNA and to *EcoRI* or *HindIII* fragments of lambda DNA (Table 1). An autoradiogram of a Southern blot of *EcoRI*, *HindIII*, *SalI*, and *XbaI* fragments of HVPapio DNA and of lambda DNA which were subjected to electrophoresis in an agarose gel and hybridized to labeled HVPapio and lambda DNAs is shown in Fig. 1.

HindIII-B and -C, *SalI*-B and -C, and *XbaI*-K, -L, and -M were overrepresented to the same extent in photographs of ethidium bromide-stained gels and in autoradiograms of blots hybridized to labeled HVPapio DNA. The fragments are therefore assumed to consist of two or more different, unique sequence fragments.

HindIII-I het and -R het, *SalI*-H het and -L het, *XbaI*-D het, and *EcoRI*-H het were families of six to eight fragments differing in size by increments of 4×10^5 daltons. Components of the het fragments were markedly underrepresented in photographs of ethidium bromide-stained gels but were more abundant in representation in autoradiograms, indicating that these fragments contained sequences of lower complexity than the bulk of HVPapio DNA (1, 37). The finding that these fragments contained DNA of lower complexity, i.e., repeated DNA, and varied in size by 4×10^5 -dalton increments

suggested that these fragments contained variable numbers of copies of a 4×10^5 -dalton sequence.

XbaI-P and *EcoRI*-C were also more apparent in autoradiograms of blots hybridized to labeled HVPapio DNA than in ethidium bromide-stained gels, suggesting that these fragments also contained DNA of lower complexity than the bulk of HVPapio DNA. *EcoRI*-C was a family of fragments which varied in size by increments of 2×10^6 daltons. *XbaI*-P had a size of 2×10^6 daltons and was present in severalfold molar excess of other fragments in ethidium bromide-stained gels. These data suggested that *EcoRI*-C contained a variable number of tandem repeats of 2×10^6 -dalton sequence which had a single *XbaI* cleavage site.

Construction of a linkage map of restriction enzyme fragments of HVPapio DNA. Hybridizations between a labeled restriction endonuclease fragment and blots of fragments generated by digestion with other enzymes indicate which fragments contain common nucleotide sequences. To distinguish whether these fragments are colinear in HVPapio DNA or could share a common nucleotide sequence which is present at more than one map location in the DNA, the labeled fragment was also hybridized to blots containing separated fragments of the same enzyme digest. The results of hybridization of labeled *EcoRI*, *HindIII*, and *XbaI* fragments

TABLE 1. HVPapio DNA restriction enzyme fragments, summarized from data in Fig. 1

<i>XbaI</i>		<i>EcoRI</i>		<i>HindIII</i>		<i>SalI</i>	
Fragment	Mol wt $\times 10^{-6}$	Fragment	Mol wt $\times 10^{-6}$	Fragment	Mol wt $\times 10^{-6}$	Fragment	Mol wt $\times 10^{-6}$
A	12.3	A (TR)	22-24	A (IR)	24-26	A (IR)	32
B	12.1	B	16.0	B	12.6	B	20
C	9.8	C (IR)	14.7	C	12.6	C	20
D	6.8	D	12.0	D	10.0	D	12
E	5.9	E	7.6	E	8.5	E	9.0
F	5.0	F	7.2	F	5.2	F	5.0
G	4.85	G	6.6	G	5.2	G	4.2
H	4.6	H	5.4	H	4.35	H	3.15
I	4.0	I	5.2	I	4.0	I	2.0
J	3.55	J	4.3	J	3.5	J	1.8
K	2.6	K	2.6	K	3.15	K	1.4
L	2.6	L	1.6	L	2.4	H het (TR)	4.2-1.0
M	2.6	M	1.6	M	2.3	L het (TR)	
N	2.2	H het (TR)	5.2	N	1.75		
O	2.15			O	1.15		
P (IR)	1.95			P	1.10		
Q	1.75			Q	1.05		
R	1.4			R	1.0		
S	0.6			S	0.85		
T	0.4			T	0.75		
D hets (TR)	6.8			U	0.55		
	6.4			I het (TR)	5.2-0.9		
				R het (TR)

FIG. 1. Restriction endonuclease fragment profiles of HVPapio DNA. Portions (0.25 μ g) of HVPapio DNA or bacteriophage lambda DNA were cleaved with the indicated restriction endonuclease. The DNA fragments were separated by electrophoresis in parallel lanes on an agarose slab gel and transferred to a nitrocellulose filter. 32 P-labeled λ DNA probe (10^7 cpm) and HVPapio DNA probe (10^7 cpm) prepared by nick translation were hybridized to the filter, and the DNA fragments were visualized by autoradiography. Molecular weights of the DNA fragments are indicated in megadaltons. Sizes of the EcoRI and HindIII fragments of λ DNA used as molecular weight standards were obtained from the Bethesda Research Laboratories, Inc., 1979 catalog, p. 27.

of HVPapio DNA to blots of fragments of HVPapio DNA are shown in Fig. 2, 3 and 4, and 5, respectively. The results are as follows. (i) Each labeled EcoRI, HindIII, or XbaI fragment specifically identified itself in blots of all of the EcoRI (Fig. 2A), HindIII (Fig. 3A), or XbaI (Fig. 5A) fragments. Minor hybridization to many other fragments was apparent with some of the probes. (ii) A provisional linkage map (Fig. 6A) was constructed from the hybridization of labeled EcoRI fragments to XbaI, HindIII, and SalI fragments (Fig. 2). Instances in which the order of fragments within a domain could not be determined are indicated by parentheses around the fragment (Fig. 6A). (iii) The linkage arrangements shown in Fig. 6A were confirmed and extended by hybridizations of labeled HindIII (Fig. 3 and 4) and XbaI (Fig. 5) fragments. The data are summarized in Fig. 6B and 6C. The maps of EcoRI, HindIII, XbaI, and SalI fragments are summarized in Fig. 6D.

Three ambiguities remain in the map (Fig. 6D). First, the relative order of SalI fragments H and K was not determined. Labeled XbaI-F

hybridized to SalI-A and -G (Fig. 5D) and labeled XbaI-C hybridized to SalI-G, -H, and -K (Fig. 5D). These results position SalI-H and -K to the right of fragment G (Fig. 6) but do not determine their order relative to each other. Second, the hybridization results which determined the relative order of HindIII-L and -Q were not completely consistent. Labeled HindIII-L hybridized to XbaI-Q and -H (Fig. 3B), EcoRI-D and -G (Fig. 4A), and SalI-C and -F (Fig. 4B), suggesting that it contains a cleavage site for each of these other enzymes. Labeled HindIII-Q hybridized to SalI-C (Fig. 4B). Placement of HindIII-Q to the left of fragment L, which was colinear with sequences in XbaI-Q and -H and EcoRI-D and -G, leads to the prediction that labeled HindIII-Q should hybridize to XbaI-Q and EcoRI-D. This is not supported by the data (Fig. 3B and 4A). The linkage map for HindIII-L and -Q shown in Fig. 6D is therefore tentative. Another explanation for the hybridization of HindIII-L to SalI-C and -F and the other data is that another HindIII fragment similar to size to HindIII-L maps to the right of

FIG. 2. Mapping of *Xba*I, *Hind*III, and *Sal*I restriction enzyme fragments relative to labeled *Eco*RI fragments of HVPapio DNA. Autoradiograms of Southern blot strips of *Eco*RI (A), *Xba*I (B), *Hind*III (C), and *Sal*I (D) restriction endonuclease fragments of HVPapio DNA which were hybridized with 32 P-labeled HVPapio DNA (TP) or 32 P-labeled *Eco*RI restriction endonuclease fragments of HVPapio DNA (designated by fragment letters at top of each blot).

*Hind*III-O. Third, the order of *Xba*I fragments R and K, which mapped near opposite ends of *Eco*RI-C could not be determined. The right *Eco*RI cleavage site in *Eco*RI-C mapped within *Xba*I-I (Fig. 2B, 5B, and 6). To determine whether the left *Eco*RI site was within *Xba*I-R or -K, which would order these fragments in the linkage map, or, alternatively, if the *Eco*RI site occurred within *Xba*I-O, HVPapio DNA was double digested with *Xba*I and *Eco*RI restriction enzymes or with *Xba*I alone. The fragments were separated in adjacent lanes in an agarose gel, transferred to a nitrocellulose filter, and hybridized with labeled *Xba*I-P. *Xba*I-P shared DNA sequences with *Xba*I-R and -K (discussed below) and therefore permitted the specific detection of the fragments on the filter. The results show that *Eco*RI digestion did not alter the size of *Xba*I-R or -K (Fig. 7A). Hybridization of the filter with labeled HVPapio DNA confirmed that the *Eco*RI-C-cut sites mapped in *Xba*I-O and -I (Fig. 7B).

Internal reiteration in HVPapio DNA. Labeled *Eco*RI-C (Fig. 2A), *Hind*III-A (Fig. 4A), and *Xba*I-P (Fig. 5B) defined a family of *Eco*RI-C fragments which varied in size by increments of 2×10^6 daltons. There were multiple copies of *Xba*I-P in HVPapio DNA, and its size was 2×10^6 daltons. These data and the lower kinetic complexity of *Eco*RI-C and *Xba*I-P, referred to previously, indicate that *Xba*I-P is a tandemly reiterated sequence, IR, which is present in varying numbers of copies in different molecules of HVPapio DNA.

Labeled *Xba*I-P hybridized to *Xba*I-K and -R (Fig. 5A), and *Xba*I-K (Fig. 5A) and -R (data not shown) hybridized to *Xba*I-P. *Xba*I-K and -R probably contained the components of the repeated sequence to the left or right of the *Xba*I cleavage site in IR. The smallest form of *Eco*RI-C was less than 5×10^6 daltons. *Xba*I-K and -R had a combined size of 4×10^6 daltons and together probably contained only a single complete copy of the reiterated sequence and ap-

proximately 2×10^6 daltons of unique DNA. Therefore, the 5×10^6 -dalton form of *EcoRI*-C probably contained a single complete copy of the reiterated sequence. The most abundant form of *EcoRI*-C was 10×10^6 daltons larger and therefore contained an additional five copies of the repeated sequence. Labeled *XbaI*-K (Fig. 5A) and -R (data not shown) hybridized to each other, suggesting that part of the sequences to the left and right of the *XbaI* site in IR had homology.

Terminal reiteration in HVPapio DNA. Labeled *EcoRI*-A hybridized to *EcoRI*-H het (Fig. 2A) and to *HindIII*-I het and -R het, *XbaI*-

D het, and *SaII*-H het and -L het (Fig. 2B-D). Heterogeneity in the size of *EcoRI*-A would not be detected in these experiments because of inadequate resolution of small differences between sizes of such large fragments in 0.4% agarose gels. A labeled 10^6 -dalton component of *HindIII*-R het hybridized to *HindIII*-I het and -R het, to all of the other het fragments, and to *EcoRI*-A (Fig. 3 and 4). These data indicate all of the het fragments shared a common sequence. Since both hets varied in size by increments of 4×10^5 daltons, the same 4×10^5 -dalton sequence is likely to be a component of both hets. The restriction fragment profiles of the terminal

FIG. 3. Mapping of *XbaI* restriction enzyme fragments relative to labeled *HindIII* fragments of HVPapio DNA. Autoradiograms of Southern blot strips of *HindIII* (A) and *XbaI* (B) restriction endonuclease fragments of HVPapio DNA which were hybridized with 32 P-labeled HVPapio DNA (TP) or with 32 P-labeled *HindIII* restriction endonuclease fragments of HVPapio DNA (designated by fragment letters at top of each blot). The symbols > and < refer to labeled fragments enriched for the indicated fragment of comigrating DNA fragments but still significantly contaminated with the other fragment.

FIG. 4. Mapping of *Eco*RI and *Sal*I restriction enzyme fragments relative to labeled *Hind*III fragments of HVPapio DNA. Autoradiograms of Southern blot strips of *Eco*RI (A) and *Sal*I (B) restriction endonuclease fragments of HVPapio DNA. Designations at the top of each blot are the same as those in Fig. 3.

FIG. 5. Mapping of *EcoRI*, *HindIII*, and *SalI* restriction enzyme fragments relative to labeled *XbaI* fragments of HVPapio DNA. Autoradiograms of Southern blot strips of *XbaI* (A), *EcoRI* (B), *HindIII* (C), and *SalI* (D) restriction endonuclease fragments of HVPapio DNA which were hybridized with ^{32}P -labeled HVPapio DNA (TP) or ^{32}P -labeled *XbaI* restriction endonuclease fragments of HVPapio DNA (designated by fragment letters at top of each blot).

heterogeneous fragments indicated that some of the components expected for a series of fragments which differ by increments of a 4×10^5 -dalton sequence were missing or underrepresented, particularly in the left-terminal *XbaI*-D het (*EcoRI*-J hybridization; Fig. 2A), *HindIII*-R het (*HindIII*-R het hybridization; Fig. 3A), and

EcoRI-H het (*HindIII*-R het hybridization; Fig. 4A).

HindIII-A and -J were the leftmost and rightmost molar fragments of HVPapio DNA which were used as probes in the determination of the fragment linkage map (Fig. 6). Labeled *HindIII*-A hybridized to *XbaI*-D het (Fig. 3B) and

FIG. 6. Summary of cross-hybridization data and restriction endonuclease fragment linkage for HVPapio DNA. The results of hybridization of ³²P-labeled EcoRI (A), HindIII (B), and XbaI (C) restriction enzyme fragments to Southern blots of XbaI, EcoRI, HindIII, and SalI restriction endonuclease fragments of HVPapio DNA were obtained from the data shown in Fig. 2 through 5. DNA fragments in A through C are not drawn in exact proportion to size. DNA fragments are drawn in proportion to size in D, which presents the linkage map of HVPapio DNA XbaI, EcoRI, HindIII, and SalI restriction enzyme fragments.

EcoRI-H het (Fig. 4A), but did not hybridize to the SalI-L het fragments (Fig. 4B). This established that a component of these leftward het fragments which was common to all the het fragments, as detected by the hybridization results with labeled HindIII-R het, maps to the left of all known molar DNA fragments. Labeled HindIII-J hybridized to XbaI-D het (Fig. 3B) and EcoRI-A (Fig. 4A) but not to the SalI-H het fragments (Fig. 4B). This establishes that a component of the rightward het fragments which was common to all the hets maps to the right of all known molar DNA fragments. These results suggested that HVPapio DNA contained the same variably reiterated 4×10^5 -dalton component at both termini.

Duplication of sequences in the long unique component of HVPapio DNA. Labeled fragments which included the map coor-

dinants 25×10^6 to 29×10^6 daltons and 91×10^6 to 95×10^6 daltons hybridized to each other (Fig. 6). Thus, labeled XbaI-A hybridized to XbaI-I and vice versa (Fig. 5A), labeled HindIII-A hybridized to HindIII-E and vice versa (Fig. 3A), and EcoRI-A hybridized to EcoRI-H and vice versa (Fig. 3A). These data were confirmed by the data obtained as each of these labeled fragments was hybridized to blots of the heterologous restriction enzyme fragments (Fig. 2B, 3B, 4, and 5B-D) and are summarized in Fig. 6.

DISCUSSION

The similarity between HVPapio and EBV DNAs revealed by these studies is quite striking. First, the size of HVPapio DNA, calculated from the sum of the size of its restriction endonuclease fragments, is 110×10^6 to 114×10^6 daltons, whereas the size of EBV DNA, calculated from

FIG. 7. Mapping of the *EcoRI*-C-cut sites relative to the corresponding *XbaI* DNA fragments. HVPapio DNA (0.5 µg) was digested with *XbaI* restriction endonuclease. Half of the digest was further digested with *EcoRI*. The DNA fragments were separated in adjacent lanes on a 0.8% agarose slab gel and transferred to a nitrocellulose filter. 32 P-labeled *XbaI*-P probe was hybridized to the blot, and the homologous DNA fragments were visualized by autoradiography (A). The blot was then hybridized with 32 P-labeled HVPapio DNA (B) to detect all of the viral fragments.

the sum of the size of its restriction endonuclease fragments, is 115×10^6 daltons (2, 14, 31).

Second, EBV and HVPapio DNA have variable numbers of repeats of a sequence, TR, at both termini. The size of the TR of EBV DNA is 3.5×10^5 daltons (2, 10, 12, 14, 18, 19, 31), whereas estimates of the size of the TR of HVPapio DNA suggest that it may be slightly larger, 4×10^5 daltons. The tandem repeats at both ends of EBV DNA are in a direct orientation relative to each other, based on the observation that the 3' ends of the DNA generated by treatment of EBV DNA with lambda exonuclease can anneal with each other to form small circles at the juncture between the ends (12). Current knowledge of the similarity between the ends of HVPapio DNA is limited to the observations that the TRs at both ends of the DNA are the

same size and hybridize to each other. The identification of a restriction endonuclease site within TR would permit more precise analysis of the size and organization of the termini.

EBV and HVPapio DNAs have tandem repetitions of a 2×10^6 -dalton sequence, IR, which divides the genomes into long and short components of largely unique sequence DNA, U_L and U_S (Fig. 6) (2, 10, 11, 15, 18, 31, 33). HVPapio DNA usually has six tandem repeats of IR, and a small portion of the molecules have from 1 to 11 copies of IR. The DNA of the B95-8 isolate of EBV usually has 10 or 11 tandem repeats of IR (10, 11, 15), whereas the AG876 isolate of EBV usually has 5 or 6 (31; M. Heller, T. Dambaugh, and E. Kieff, submitted for publication). The size of the U_S component of EBV and HVPapio DNAs is 8×10^6 to 9×10^6 daltons (2, 10, 11, 31). The size of the U_L component of HVPapio DNA and of DNA of the W91 and AG876 isolates of EBV is 88×10^6 daltons (31; Heller et al., submitted for publication), whereas the size of the U_L DNA of the B95-8 isolate of EBV is 80×10^6 daltons (2, 10, 11, 31). Recent data indicate that the DNA missing from the B95-8 isolate is present in most other EBV DNAs, including those of isolates from outside the Burkitt endemic region (17, 30, 31; Heller et al., manuscript submitted for publication). There is homology between sequences which map between 25×10^6 to 29×10^6 and 91×10^6 to 95×10^6 daltons in the U_L of HVPapio DNA and sequences which map between 26×10^6 to 28×10^6 and 93×10^6 to 95×10^6 daltons in the U_L region of EBV AG876 and W91 DNAs (31; Heller et al., submitted for publication). The conservation of this sequence homology within U_L and the larger size of U_L in HVPapio DNA and in EBV DNAs other than the B95-8 isolate indicate that these are important features of this family of DNAs.

In addition to these similarities in organization, HVPapio DNA has 40% homology to EBV DNA (5, 8). We have recently determined that the genomes are colinear by demonstrating that each cloned *Bam*HI fragment of EBV DNA hybridizes to the homologous map location in HVPapio DNA (17). This physical homology suggests that EBV and HVPapio DNAs may participate in genetic exchanges through homologous recombination (34).

Analysis of intertypic recombinants between adenoviruses (39) or between herpesviruses (20, 27, 38) has facilitated phenotypic mapping. Our data indicate that the application of a similar approach to functional mapping of EBV and HVPapio DNA may be feasible. Difficulties in propagating EBV and HVPapio in vitro will, however, be a major barrier.

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The death of our colleague Paul Gerber is sadly noted.

ADDENDUM

After this manuscript was prepared, a study of the DNA of another isolate of HVPapio was communicated to us by Lee et al. (23). Most of the *EcoRI* and *SalI* restriction enzyme fragments of the two isolates are similar in size and map position. Identical letter designations were assigned to *EcoRI* fragments in the studies, with the exceptions that *EcoRI*-J, -K, -L, and -H het (this study) correspond to *EcoRI*-K, -L, -N, and -J het in the paper by Lee et al. *SalI*-B and -C were both designated *SalI*-B in the study by Lee et al.; consequently, *SalI* fragments D, E, F, etc. (this study) were named C, D, E, etc., in that study. Also, many small *HindIII* fragments were not detected or not present in the analysis of HVPapio DNA by Lee et al.

The map position of *SalI*-H and -K relative to *SalI*-G and -C (Fig. 6, this study) has been determined to be *SalI*-G-H-K-C in a study of the colinearity of HVPapio and EBV DNAs (17). This result is consistent with the *SalI* linkage map for this region presented in the study by Lee et al.

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