NOTES

Colinearity Between the DNAs of Epstein-Barr Virus and Herpesvirus Papio

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Epstein-Barr virus (EBV) and herpesvirus papio (HVPapio) DNAs share a common format and 40% homology. Labeled cloned fragments of EBV DNA were hybridized to blots of *Xba*I, *Eco*RI, *Hind*III, and *Sa*I fragments of HVPapio DNA. EBV fragments which mapped from 2×10^6 to 54×10^6 and from 59×10^6 to 106×10^6 daltons hybridized to fragments at identical map positions in HVPapio DNA. Regions of nonhomology were demonstrated at 0×10^6 to 2×10^6 , 54×10^6 to 59×10^6 , and 106×10^6 to 115×10^6 daltons.

Epstein-Barr virus (EBV) of humans shares many biological and chemical properties with a related virus of baboons, herpesvirus papio (HVPapio) (3, 4, 5, 23). Latent infection with these viruses is prevalent. Their host range in vitro is restricted to primate B lymphocytes (3, 5). Primate B lymphocytes infected with EBV or HVPapio can be grown as continuous lymphoblastoid cell lines which contain an intranuclear antigen, Epstein-Barr nuclear antigen (25) or HVPapio nuclear antigen (23). The viral capsid and membrane antigens of both viruses react with antibody from infected humans or baboons (3, 5).

EBV and HVPapio DNAs have a common format (see Fig. 2) which distinguishes this subgroup of herpesviruses (2, 13, 14, 19). The DNAs have tandem repeats of a 0.35×10^6 - to 0.40×10^6 -dalton sequence, TR, at both ends (2, 8, 10, 13, 16); tandem direct repeats of a 2×10^6 -dalton sequence, IR, which separates the genomes into unique sequence components of 9×10^6 daltons, U_S , and 88×10^6 daltons, U_L (2, 8, 9, 12, 23a, 26); homologous sequences which map in U_L at 26×10^6 to 28×10^6 and 93×10^6 to 95×10^6 daltons in EBV DNA and at 25×10^6 to 29×10^6 and 91×10^6 to 95×10^6 daltons in HVPapio DNA (13, 23a); and a size of 115×10^6 dalton (2, 13, 23a). HVPapio DNA hybridizes to 40% of labeled EBV DNA at 30°C below the T_m of EBV DNA (3).

The level of relatedness between EBV and HVPapio DNAs is similar to that between herpes simplex virus type 1 and type 2 DNAs (15). Analysis of recombinants between HSV-1 and HSV-2 and between adenoviruses has indicated that these DNAs are colinear and has

permitted mapping of many of the viral polypeptides and mutant phenotypes (17, 21, 30, 31). Similar approaches could be useful for mapping EBV functions, although the limited repertoire of known phenotypic characteristics and difficulties in propagating the virus are constraints.

The objective of this series of experiments is to evaluate the colinearity of EBV and HVPapio DNAs. The restriction endonuclease maps of HVPapio and EBV DNAs are shown in Fig. 2 (13, 19). The B95-8 isolate was the previous prototype EBV DNA but differed from other isolates in the deletion of 9×10^6 daltons of DNA contained in the *Bam*HI fragments B1, W1, and I1 of the EBV (AG876) strain (23a). All of the *Bam*HI fragments of B95-8 and the *Bam*HI B1, W1, and I1 fragments of AG876 have been cloned in pBR322 (2, 23a). The *Eco*RI fragment D-I, J het, containing the fused ends of circular intracellular B95-8 DNA, has been cloned in charon 4A (2). The strategy used in this study was to hybridize each of the labeled EBV DNA fragments under stringent conditions ($T_m - 30^\circ\text{C}$) to blots of *Xba*I, *Eco*RI, *Hind*III, or *Sa*I fragments of HVPapio DNA to map the location of homologous sequences in HVPapio DNA. The results are shown in Fig. 1 and summarized in Fig. 2.

Almost all of the labeled EBV DNA fragments hybridized to HVPapio DNA. In most instances the map location of the EBV probe fragments and the homologous HVPapio DNA fragment(s) corresponded. The IR of EBV DNA has a single *Bam*HI site (2, 9, 26), whereas that of HVPapio DNA has a single *Xba*I site (13). Digestion of EBV DNA with *Bam*HI and HVPapio DNA with *Xba*I therefore generates fragments

EBV cloned fragment probes hybridized to blots of Herpesvirus papio DNA (HVPap)

A. HVPap Xba I blots

B. HVPap Eco RI blots

Fig. 1. Cross-hybridization of labeled EBV DNA fragments to Southern blots of HVPapio DNA. EBV (B95-8 or AG876) DNA *Bam*HI fragments cloned in pBR322, the *Eco*RI-D-I, J *het* fragment (106×10^6 to 2×10^6 daltons of the circular, intracellular form of B95-8 DNA) cloned in charon 4A, B95-8 *Bgl*II A (0×10^6 to 11×10^6 daltons), and HVPapio DNA (TP) were labeled by nick translation with [α - 32 P]dCTP and *E. coli* DNA polymerase I and hybridized at 74°C to identical nitrocellulose filters containing the separated *Xba*I (A), *Eco*RI (B), *Hind*III (C), or *Sal*I (D) restriction endonuclease fragments of HVPapio DNA (2, 8, 13, 28). The blot strips were exposed to X-ray film for autoradiography to identify HVPapio DNA fragments homologous to the EBV DNA probes. The probe fragment is indicated at the top of each blot strip.

FIG. 2. Summary of homology between EBV and HVPapio DNA. Maps of the BamHI fragments of EBV (B95-8 or AG876) DNA (2, 23a) and the XbaI, EcoRI, HindIII, and SalI fragments of HVPapio DNA (13) are shown. The relative order of HVPapio XbaI-R and -K has not been determined; XbaI-R and -K are arbitrarily placed to the left and right of IR (13). The hybridization results in Fig. 1 are indicated by arrows from the EBV probe fragments to the homologous HVPapio DNA fragments. The symbol ⊕ indicates that a labeled EBV probe did not hybridize to HVPapio DNA.

BamHI-C and -X and XbaI-R and -K, which contain only part of the IR to the left or right of the cut site, and BamHI-V and XbaI-P, which are multiple copies of the complete reiteration (Fig. 2) (2, 9, 12, 13, 23a). As a consequence of homology between components of the IR, BamHI-C has homology to BamHI-X (9; Cheung et al., in preparation) and XbaI-K hybridizes to XbaI-R (13). This homology appears to be conserved between EBV and HVPapio DNAs, since labeled BamHI-C and BamHI-X hybridized to both HVPapio XbaI-K and -R (Fig. 1A and 2). Similarly, the sequences duplicated at 25×10^6 to 29×10^6 and 91×10^6 to 95×10^6 daltons in HVPapio DNA and at 26×10^6 to 28×10^6 and 93×10^6 to 95×10^6 daltons in EBV DNA are conserved, since labeled BamHI-H and BamHI-B1 hybridized with sequences at both locations in HVPapio DNA, XbaI-I and -A (Fig. 1A), EcoRI-H and -A (Fig. 1B), and HindIII-A and -E (Fig. 1C). The EBV probes

hybridized predominantly to the HVPapio fragments which are colinear, and to a lesser extent with HVPapio fragments which map at the other location.

Two regions of nonhomology were detected in these experiments. First, labeled BamHI-E (54×10^6 to 59×10^6 daltons) did not hybridize to any HVPapio XbaI, EcoRI, HindIII, or SalI DNA fragment (Fig. 1 and 2). This BamHI-E probe did hybridize to blots of EBV DNA fragments which were present in the same hybridization vessel. Also, the HVPapio blots which did not hybridize with BamHI-E were rehybridized with labeled HVPapio DNA to confirm that they had all of the HVPapio fragments, including those which mapped at 54×10^6 to 59×10^6 daltons (data not shown). Second, the TR and adjacent unique sequences of HVPapio and EBV DNAs did not have detectable homology. Labeled EBV BamHI-A (102×10^6 to 110×10^6 daltons) hybridized to HVPapio XbaI-G and -D

het (Fig. 1A), *EcoRI*-A (Fig. 1B), and *HindIII*-D (Fig. 1C); *BamHI*-C (5×10^6 to 11×10^6 daltons) hybridized to *XbaI*-D het (Fig. 1A); whereas labeled EBV *EcoRI*-D-I, J het (106×10^6 to 2×10^6 daltons) did not hybridize to HVPapio *XbaI*-D het (Fig. 1A) or *HindIII*-J or -I het (Fig. 1C). Therefore, EBV DNA sequences to the right of 106×10^6 daltons and to the left of 2×10^6 daltons do not have homology to the corresponding regions of HVPapio DNA.

These hybridization data also resolve one previous ambiguity in the restriction endonuclease maps of HVPapio DNA and suggest a solution for another ambiguity. The relative order of *SalI*-H and -K was not previously known (13, 19). EBV *BamHI*-Q and -U hybridized only to *XbaI*-F and -C, respectively (Fig. 1A), whereas *BamHI*-Q hybridized to *SalI*-G and *BamHI*-U hybridized to *SalI*-G and -H (Fig. 1D), suggesting that *SalI*-G and -H are adjacent to each other (Fig. 2). *BamHI*-O hybridized to *HindIII*-B and -F (Fig. 1C) and to *SalI*-K and -C (Fig. 1D), confirming that *SalI*-K is adjacent to *SalI*-C and is to the right of *SalI*-H (Fig. 2). The second ambiguity was in the order of *HindIII*-L and -Q (Fig. 2) (13, 19). Previous mapping data suggested that *HindIII*-L mapped to the left and to the right of *HindIII*-Q (13). *BamHI*-Y and -K hybridized only to *SalI*-C and -F, respectively (Fig. 1D), whereas *BamHI*-Y linked *HindIII*-L and -Q (Fig. 1C), *BamHI*-R linked *HindIII*-Q and -O (Fig. 1C), and *BamHI*-K linked *HindIII*-O and -L (Fig. 1C). These results confirm the mapping of *HindIII*-L to the left of *HindIII*-Q in HVPapio DNA and suggest that another *HindIII* fragment identical in size to *HindIII*-L maps to the right of *HindIII*-O (Fig. 2).

The level of resolution of regions of homology and nonhomology between EBV and HVPapio DNAs is defined by the size of the EBV probe fragments and the density of *XbaI*, *SalI*, *HindIII*, and *EcoRI* sites in HVPapio DNA. Twenty-eight of the *BamHI* fragments of EBV DNA range in size from 0.8×10^6 to 6.4×10^6 daltons (2). The complexity of the 29th fragment was reduced from 8×10^6 to 4.6×10^6 daltons, since it shares 4.6×10^6 daltons with the *EcoRI*-D-I, J het fragment (2). The longest distance between restriction endonuclease sites in HVPapio DNA is 5×10^6 daltons (13). Regions of nonhomology or noncolinearity larger than 6×10^6 daltons would therefore be detected and, in most regions, the level of resolution is 1×10^6 to 2×10^6 daltons. The only long regions of nonhomology map at 0×10^6 to 2×10^6 , 54×10^6 to 59×10^6 , and 106×10^6 to 115×10^6 daltons in HVPapio and EBV DNAs. Since previous data indicate that overall only 40% of the DNAs

are homologous (3), the remainder of the non-homologous and homologous sequences must be interspersed. Homologous recombination could occur throughout these related regions of EBV and HVPapio DNAs (1, 27, 32).

The striking similarity in organization of HVPapio and EBV DNAs and the extent of colinearity between the genomes indicate that HVPapio and EBV evolved from a common ancestor. We have recently observed a similar extent of colinearity between EBV DNA and the DNA of herpesvirus pan, a chimpanzee herpesvirus isolate which has 40% homology to EBV DNA (6; M. Heller and E. Kieff, manuscript in preparation). These viruses and possibly also the antigenically related lymphotropic herpesviruses of gorillas, orangutans, and rhesus monkeys (3, 7, 11, 18, 22, 24, 29) are a distinct subgroup of herpesviruses.

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