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Alignment of the Genome of Monkey B-Lymphotropic Papovavirus to the Genomes of Simian Virus 40 and BK Virus

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We located the origin of DNA replication of African green monkey B-lymphotropic papovavirus DNA by analyzing pulse-labeled form I DNA. With the replication origin used as a reference point, the B-lymphotropic papovavirus genome was aligned with the genomes of simian virus 40 and BK virus from DNA homology between specific fragments hybridized under low-stringency conditions. From the results of these experiments, it was possible to deduce the correlation between the physical and functional maps of the B-lymphotropic papovavirus genome.

Lymphotropic papovavirus (LPV), isolated from a B-lymphoblastoid cell line of an African green monkey (12), shows an interesting host range in virtro that is distinct from those of other polyomaviruses. Its growth is restricted to certain continuous lines of monkey or human B-lymphoblasts (1, 2, 11). LPV has a number of characteristics common to polyomaviruses, but its transforming capacity is yet to be proved and its pathogenicity is unknown at present. Serological surveys (1, 11) have shown that antibody against monkey LPV is detectable in humans, apes, and monkeys, providing strong evidence that unidentified viruses antigenically related to LPV occur widely among primates.

Monkey LPV has been adapted to grow in a human B-lymphoblast cell line, BJA-B (2, 11). Since LPV DNA obtained from infected BJA-B cells was heterogeneous in size (2, 11), the DNA was molecularly cloned in *Escherichia coli*, and the various cloned DNAs were characterized. The longest LPV molecule (5.1 kilobases [kb] long) was found to be nondefective, and its restriction endonuclease cleavage maps were constructed (11). For further biological and biochemical studies, it was necessary to correlate the physical and functional maps of the LPV genome.

In the present study, we located the origin of LPV DNA replication by analyzing the newly synthesized form I DNA and also examined the homology between specific DNA fragments of LPV and simian virus 40 (SV40) and of LPV and human polyomavirus BK (BKV). From these data the LPV genome was aligned with those of SV40 and BKV, whose entire DNA sequences and functional maps have been correlated (5).

The replication origin of LPV DNA was located by the method described for SV40 by Danna and Nathans (3). BJA-B cells, cultivated as previously described (11), were infected with a stock of nondefective LPV, designated LPV-02 (11). After 5 days, when 40% of the cells were positive for LPV antigens as determined by the indirect immunofluorescence test, the infected cells (10^7 cells) were labeled with 170 μCi of [^{3}H]thymidine per ml for 5, 10, or 15 min or uniformly labeled with 9 μCi of [^{3}H]thymidine per ml for 22 h. DNA was extracted immediately after the labeling period as described by Hirt (4). The Hirt supernatant fractions were mixed with unlabeled, purified LPV DNA (2 μg), and form I DNA was purified by the ethidium bromide-CsCl buoyant density method (8). Purified, pulse-labeled (for 5, 10, or 15 min), and uniformly labeled (for 22 h) DNAs were digested with *Bam*HI, which cleaves LPV DNA once (11), and with *Hpa*II, which cleaves LPV DNA at four sites (11). The restriction endonucleases used for all experiments were purchased from Bethesda Research Laboratories, Rockville, Md., and used as recommended by the manufacturer. After *Bam*HI-*Hpa*II digests were subjected to electrophoresis in a 4% polyacrylamide slab gel in Tris-borate buffer (89 mM Tris, 89 mM boric acid, pH 8.2) at 120 V for 6 h. The DNA bands were visualized by staining with ethidium bromide, cut out from the gel, and assayed for incorporation of [^{3}H]thymidine. The radioactivity of each pulse-labeled fragment relative to that of the corresponding, uniformly labeled fragment was calculated, normalized, and plotted at the midpoint of each fragment (Fig. 1).

Combined digestion of 5.1-kb LPV DNA with *Bam*HI and *Hpa*II generated five fragments, arranged in the uncleaved circular DNA in the

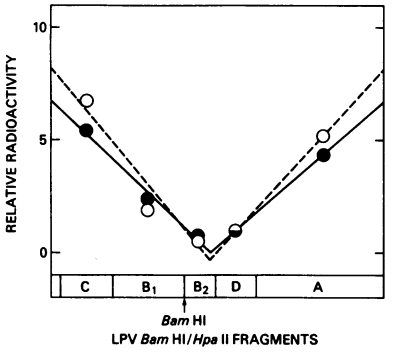

FIG. 1. Relative radioactivities of LPV DNA fragments generated by cleavage of pulse-labeled LPV form I DNA with restriction endonucleases *Bam*HI and *Hpa*II. The radioactivity of each isolated fragment derived from pulse-labeled DNA was divided by the radioactivity of the corresponding fragment from uniformly labeled DNA. The ratio thus obtained was normalized to the value for fragment D and plotted at the midpoint of each fragment. The total radioactivities of the DNA analyzed were 8,450 cpm for the 10-min pulse-labeled sample (○), 12,140 cpm for 15-min sample (●), and 1.16×10^6 cpm for the uniformly labeled sample. Data obtained for the 5-min sample (950 cpm) were not included here because incorporation was low.

following order: C (0.8 kb), B_1 (1.1 kb), B_2 (0.5 kb), D (0.6 kb), and A (2.1 kb), as reported previously (11). As shown for SV40 (3), fragment B_2, having the least relative radioactivity, must contain the origin of replication of LPV DNA. Replication of LPV DNA initiated from a site near the *Hpa*II-B/D junction within the B_2 fragment and, like the replication of SV40 DNA (3), proceeded bidirectionally and at approximately the same rate.

The nucleotide sequence homology between DNA fragments of LPV and SV40 and between those of LPV and BKV was examined by the method described by Howley et al. (6), with various concentrations of formamide used to change the stringency of hybridization conditions. Five *Bam*HI-*Hpa*II fragments of LPV DNA ligated to *Bam*HI linkers (obtained from Bethesda Research Laboratories) were inserted into bacterial plasmid pBR322 at the *Bam*HI site and then used to transform *E. coli* K-12 strain HB101, essentially as described previously (11). Recombinant plasmid DNAs containing each LPV fragment were purified (11), labeled with ^{32}P in vitro by the nick-translation method (9), and used as radioactive probes. SV40 virion DNA (a generous gift from Peter M. Howley, National Cancer Institute, Bethesda, Md.) was digested with *Bgl*I, *Eco*RI, and *Hpa*I and subjected to electrophoresis in 1.5% agarose slab gels in Tris-borate buffer at 100 V for 5 h. BKV (prototype) DNA, isolated from infected human embryonic kidney cells (4), was digested with *Bam*HI and *Hin*dIII and then subjected to electrophoresis. Separated DNA fragments were transferred to nitrocellulose filters by the method of Southern (10). Radioactive LPV DNA fragments denatured by heat were allowed to hybridize for 16 h to SV40 or BKV DNA fragments that had been denatured and immobilized on filter strips in the presence of 20, 40, or 60% formamide at 34°C (corresponding to T_m − 50°C, T_m − 36°C, and T_m − 21°C, respectively). The hybridization mixture contained 1 M NaCl, 0.01 M Tris (pH 7.4), 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 20% formamide. After hybridization, the strips were washed extensively in 6× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate) at 58.4°C. Hybridized DNA was detected with the autoradiograms exposed with intensifying screens at −80°C for 70 h.

Although homology between LPV and SV40 or BKV DNA was not detectable under higher-stringency (40 and 60% formamide) conditions (data not shown), it was detected under the lowest-stringency (20% formamide) conditions (Fig. 2). LPV fragment A hybridized to SV40 fragments B and D; LPV B_1 and B_2 hybridized only to SV40 A; LPV C hybridized to SV40 A and C; LPV D hybridized only to SV40 B (Fig. 2A). LPV fragment A hybridized to BKV fragments A_1 and A_2; LPV B_1 hybridized to BKV B; LPV B_2 hybridized to BKV C; LPV C hybridized to BKV B; and LPV D hybridized to BKV A_1 (Fig. 2B). Since the specific radioactivities of the probes were approximately the same, the degree of darkening of autoradiograms (within a certain exposure time) shows the amount of DNA hybridized, which depended on the size of the unlabeled DNA and the degree of homology between the probe and the unlabeled DNA.

From the results of the hybridization experiments, LPV DNA was aligned with SV40 and BKV DNAs with the replication origin used as a reference point. The nucleotide sequences of the genomes of these two viruses have been correlated with their functional maps (5). LPV *Bam*HI-*Hpa*II fragments C, B_1, and B_2 probably contain the early region and LPV fragments D and A the late region of the LPV genome, (Fig. 3).

Since the partial homology between LPV DNA and both SV40 and BKV DNAs was observed over almost the entire genomes (Fig. 2 and 3), we conclude that LPV is related to these two polyoma viruses and has probably diverged through evolution from a common polyomavirus ancestor (6). A different degree of darkening of the autoradiogram (Fig. 2) showed that the extent of conserved homology between LPV

and SV40 or BKV was different from region to region. Under the present hybridization conditions we could not detect homology between LPV DNA fragments and SV40 fragment E or BKV fragment D (Fig. 2), both of which contain the noncoding regions of the genomes (Fig. 3). Apparently the noncoding regions are less conserved than the coding regions. LPV fragment C, which overlapped only SV40 fragment C (Fig. 3), showed some homology to SV40 fragment A (Fig. 2A). The significance of this observation is unclear at present. It is possible that the gene region contained in LPV fragment C overlaps part of SV40 fragment A because LPV DNA is slightly shorter than SV40 DNA (11). A detailed comparison of the LPV genome with the SV40 and BKV genomes will be possible when the entire nucleotide sequence of LPV DNA has been determined.

Law et al. (7) colinearly aligned the genomes of primate polyomaviruses SV40, BKV, and JC virus (JCV) based on their homology, with the single conserved *Eco*RI site used as a reference point. Extensive homology was detected in all the gene regions of the three polyomaviruses when hybridization was performed in 40% formamide or at T_m − 36°C, whereas in our studies homology between LPV DNA and both SV40 and BKV DNAs was not detected under the same conditions. The region of strongest homology among the SV40, BKV, and JCV DNAs lay within a narrow segment (0.76 to 0.85 map units from the *Eco*RI site) that contains the codons for the N-terminal half of the minor capsid protein

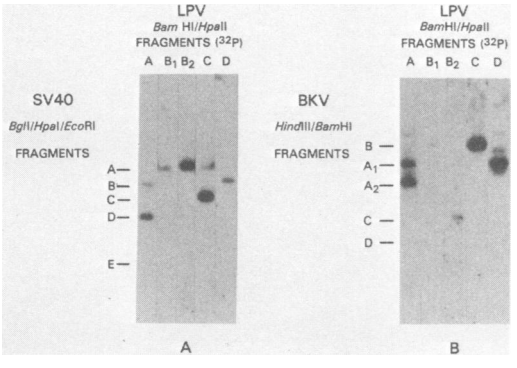

FIG. 2. Hybridization of ^{32}P-labeled LPV DNA fragments to DNA fragments of SV40 and BKV. (A) Filter strips (5 mm wide) containing unlabeled *BglI-EcoRI-HpaI* cleavage products of SV40 DNA (0.05 μg per strip) were hybridized with 2×10^5 cpm (specific activity, 10^8 cpm/μg) of in vitro-labeled, denatured fragments of LPV DNA in hybridization mixture (see text). (B) Filter strips containing *Bam*HI-*Hin*dIII-cleaved BKV DNA (0.05 μg per strip) were hybridized with ^{32}P-labeled LPV DNA and processed as described for SV40 DNA.

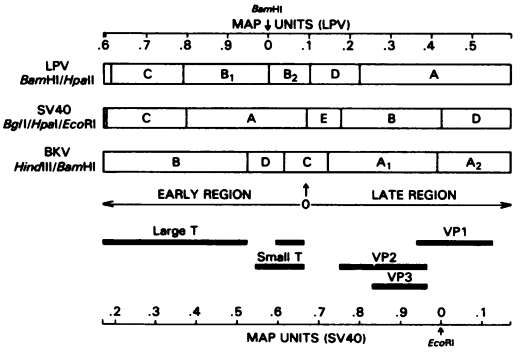

FIG. 3. Colinear alignment of the physical maps of the LPV, SV40, and BKV genomes. Five *Bam*HI-*Hpa*II DNA fragments of LPV, five *Bgl*I-*Eco*RI-*Hpa*I DNA fragments of SV40, and five *Bam*HI-*Hin*dIII DNA fragments of BKV are indicated on their respective genomes. The LPV genome was oriented to those of SV40 and BKV from the homology between the fragments (Fig. 2), with reference to the origin of DNA replication (Fig. 1), which is indicated by the arrow and the letter O. The cleavage maps of SV40 and BKV DNAs and the coding regions for SV40 proteins are taken from the published data (5).

VP2 (7). The LPV genome did not have such a region of strong homology with SV40 and BKV DNAs, although LPV protein(s) cross-reacts with antisera prepared with disrupted SV40 virions (11). The lack of DNA homology under stringent hybridization conditions shown in this study and the lack of T-antigen cross-reactivity (12) indicate that LPV is less closely related to SV40, BKV, and JCV. If all the polyomaviruses have diverged through evolution from a common ancestor (6), the monkey LPV must have diverged earlier than SV40, BKV, and JCV.

In summary, we located the replication origin of monkey LPV DNA, and from the nucleotide sequence homology we aligned the LPV genome with those of SV40 and BKV. The deduced correlation between the physical and functional maps of the LPV genome will provide the basis for further biological and biochemical studies of this interesting virus.

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