JOURNAL OF VIROLOGY, July 1985, p. 96–100
0022-538X/85/070096-05$02.00/0

Vol. 55, No. 1

Monkey B-Lymphotropic Papovavirus Mutant Capable of Replicating in T-Lymphoblastoid Cells

TADAHITO KANDA†* AND KENNETH K. TAKEMOTO

Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205

Received 13 November 1984/Accepted 22 March 1985

Monkey B-lymphotropic papovavirus (LPV) DNA present as free copies in LPV-transformed hamster embryo cells was molecularly cloned in *Escherichia coli*. Twenty-two of 24 cloned DNAs were 4.9 kilobases long and shorter than the wild-type LPV DNA (5.1 kilobases). The shorter DNA was nondefective and generated infectious virus (designated LPV-76) upon transfection of human B-lymphoblastoid BJA-B cells. LPV-76 DNA had a small deletion in the early region and a deletion and an insertion in the control region for transcription. LPV-76 VP-1 was apparently larger than that of the wild-type LPV. LPV-76 could grow in human T-lymphoblastoid MOLT-4 cells, whereas the wild-type LPV replicated only in B-lymphoblastoid cells. Characterization of constructed recombinant viruses between wild-type LPV and LPV-76 showed that the mutation responsible for the extended host range of LPV-76 was within the *PstI* B fragment, which includes the VP-1 coding region. These data strongly suggest that the mutation of VP-1 altered the host range of LPV.

Monkey B-lymphotropic papovavirus (LPV) was isolated from a B-lymphoblastoid cell line derived from the lymph node of an African green monkey (19). Although LPV has characteristics common to polyomaviruses (14, 19), it is distinct from other polyomaviruses in two aspects: the wide distribution of antibody among primates and its unique host range in vitro. Serological surveys have shown that monkeys, apes, and humans have anti-LPV antibody, indicating that viruses closely related to LPV are widely prevalent among primates (1, 14). Replication of LPV is confined exclusively to certain B-lymphoblastoid cell lines (1, 2, 14).

The physical map of LPV (14) was correlated to its functional map from location of the DNA replication origin (7), hybridization of LPV and simian virus 40 DNAs (7), and determination of the DNA sequence around the replication origin (4). Initiation of replication of circular 5.1-kilobase (kb) LPV DNA is at a point within *Bam*HI/*Hpa*II-B2. The LPV DNA has partial homology over the entire genome of simian virus 40 or BK virus, as revealed by low-stringency hybridization. DNA sequencing has shown that, despite its unique structure in the control region, the structure of the DNA replication origin and the genomic organization of LPV resemble those of other polyomaviruses. Therefore, it was possible to align the LPV DNA to the simian virus 40 genome with the replication origin as the reference point.

LPV was recently demonstrated to be capable of transforming hamster embryo cells (15). The transformed cells contained about 10 free copies of LPV DNA per cell (15). In the present study we molecularly cloned the free LPV DNA in the transformed cells. The cloned DNA was infectious and, upon transfection of B-lymphoblastoid BJA-B cells, yielded infectious virus, designated LPV-76. This mutant was found to be capable of growing in both B- and T-lymphoblastoid cells. This paper describes characterization of the new host range mutant of LPV rescued from LPV-transformed hamster cells.

* Corresponding author.

† Present address: Department of Enteroviruses, National Institute of Health, Shinagawa-ku, Tokyo 141, Japan.

MATERIALS AND METHODS

Virus. The wild-type LPV stock virus (LPV-02) was a pool of nondefective virus obtained from molecularly cloned LPV DNA (14). The infectivity titer was approximately 10^6 50% infective doses per ml.

Cells. Human BJA-B cells, a B-lymphoblastoid line (8), were obtained from C. Yee, National Cancer Institute, Bethesda, Md. Human MOLT-4 cells, a T-lymphoblastoid line (13), were kindly provided by Y. Hinuma, Kyoto University, Kyoto, Japan. These lymphoblastoid cells were grown in RPMI 1640 medium supplemented with 5% fetal calf serum. LPV-transformed hamster embryo cells [HE-LPV(E)] (15) were grown in Eagle minimum essential medium supplemented with 5% calf serum.

FA procedure. The indirect fluorescent-antibody (FA) procedure with anti-LPV serum made in hamsters and fluorescein-conjugated goat anti-hamster immunoglobulin was used. This anti-LPV serum contained both anti-T and anti-V antibodies (15). Infected cells were centrifuged at 2,500 rpm for 5 min. The pelleted cells were suspended in a small volume of phosphate-buffered saline (pH 7.2) and smeared onto cover slips. The air-dried cover slips were fixed in ethanol for 3 min and stained after drying.

Enzymes. Restriction endonucleases *Bam*HI, *Hin*dIII, *Hha*I, *Hpa*II, and *Pst*I and T4 ligase were purchased from Bethesda Research Laboratories Inc., Gaithersburg, Md., and reactions were carried out according to the instructions of the manufacturer.

Preparation of LPV DNA. Viral DNA was extracted from LPV-infected BJA-B cells or LPV-transformed hamster embryo cells (15) by the method of Hirt (6). Supercoiled form I DNA was purified by the ethidium bromide buoyant density method (11).

Molecular cloning. The LPV DNA cleaved with *Bam*HI was cloned in *Escherichia coli* plasmid pBR322 at a *Bam*HI site and then used to transform *E. coli* K-12 strain HB101, essentially as described previously (14). Recombinant DNA was extracted from chloramphenicol-amplified bacterial cul-

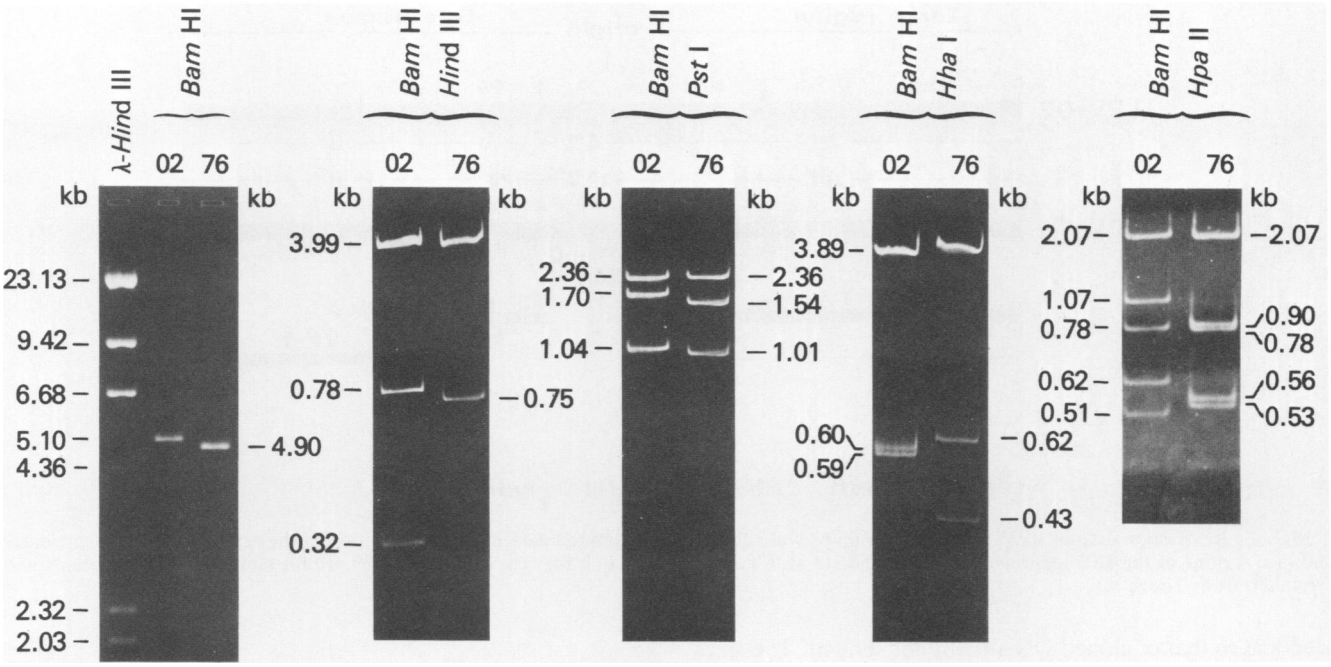

FIG. 1. Comparison of LPV-02 and LPV-76 DNA. Purified form I DNA from LPV-02- or LPV-76-infected BJA-B cells was digested with the restriction endonucleases indicated and electrophoresed. Agarose gels, 0.8%, were used for *Bam*HI-digested samples and polyacrylamide gels, 3.5%, were used for the others. DNA bands were stained with ethidium bromide.

tures (3) by a modification of the clear lysate procedure (5) and purified by the dye-buoyant density method (11).

Infection with LPV DNA. Molecularly cloned LPV DNA was cleaved with *Bam*HI, released from bacterial plasmid pBR322, and tested for infectivity with BJA-B cells. BJA-B cells were centrifuged and suspended at a concentration of 10^6 cells per ml in serum-free RPMI 1640 medium containing 100 μg of DEAE-dextran per ml. A 2-μg portion of viral DNA was added to the cells, and the preparation was shaken and allowed to incubate at room temperature for 30 min. The cells were then centrifuged and suspended in RPMI 1640 medium containing 5% serum.

Gel electrophoresis. For determination of cleavage patterns, DNA samples digested with various restriction endonucleases were adjusted to 10% glycerol and subjected to electrophoresis in either agarose or polyacrylamide slab gels, depending on the size of DNA to be analyzed. The running buffer was Tris-borate (pH 8.2; 89 mM Tris, 89 mM boric acid, 2.5 mM EDTA). DNA bands were stained with ethidium bromide (0.5 μg/ml for 30 min) and photographed on a UV transilluminator.

Analysis of viral proteins by immunoprecipitation. BJA-B cells (10^7 cells) infected with LPV for 3 to 5 days were labeled with [^{35}S]methionine for 3 h, and cell extracts were immunoprecipitated with anti-LPV hamster serum. The immunoprecipitates were electrophoresed in a 12.5% sodium dodecyl sulfate-polyacrylamide gel and fluorographed according to previously described procedures (15).

Construction of recombinant DNA. The cloned LPV DNA containing pBR322 was digested with *Bam*HI and *Pst*I and electrophoresed in a 1.2% agarose slab gel. The separated fragments (A1, A2, and B from LPV-02 and A1′, A2′, and B′ from LPV-76) were extracted by the method of McDonell et al. (10). Four mixtures of fragments (A1 + A2, A1 + A2′, A1′ + A2, and A1′ + A2′) were incubated with T4 ligase at 12°C for 14 h and cleaved with *Pst*I to eliminate DNA molecules ligated at the *Pst*I site, and then the B or B′ fragment was ligated. All samples were digested with *Bam*HI and inserted into pBR322 at the *Bam*HI site. Orientation of each fragment in constructed recombinants was determined from the *Hpa*II digestion patterns, and recombinants containing the complete viral genomes were selected.

RESULTS

Rescue of virus from LPV-transformed hamster cell line. LPV was rescued by transfection of BJA-B cells with molecularly cloned LPV DNA from HE-LPV(E) cells containing about 10 copies of free LPV DNA per cell (15). Form I LPV DNA from HE-LPV(E) cells at passages 12 to 15 was extracted and purified. This DNA was digested with *Bam*HI and inserted into bacterial plasmid pBR322 at the *Bam*HI site. Viral DNAs from 2 of 24 clones were about 5.1 kb long and showed the same *Hpa*II cleavage pattern as that of LPV-02, which was initially used to transform hamster embryo cells (15) (data not shown). DNAs of the remaining 22 clones were about 4.9 kb long, and their *Hpa*II cleavage patterns were the same among them but were different from that of LPV-02 DNA (data not shown).

The shorter 4.9-kb DNA cloned from HE-LVP(E) cells was nondefective. One of the 22 clones (pL76) was transfected to BJA-B cells by the DEAE-dextran method after cleavage with *Bam*HI. Viral antigens were detected by the FA procedure 3 days after infection, and 14 days after infection more than 60% of the cells were positive. The culture fluid was collected and used as a virus stock (designated LPV-76) for further characterization. The infectivity titer was approximately 10^6 50% infective doses per ml.

DNA of the rescued virus (LPV-76). The LPV-76 DNA was analyzed by restriction endonuclease cleavage of the viral DNA purified from LPV-76-infected BJA-B cells (Fig. 1). The *Hpa*II/*Bam*HI cleavage pattern (Fig. 1, right lane) was

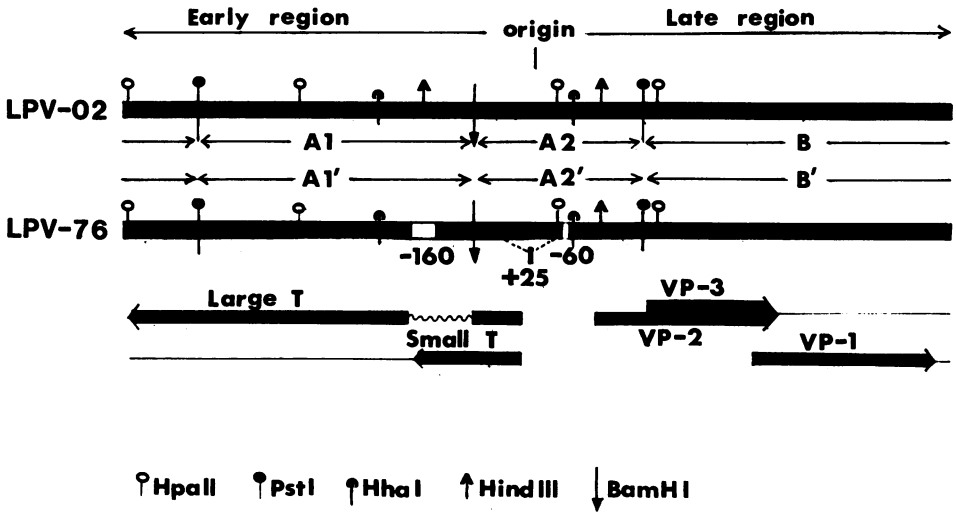

FIG. 2. Restriction map of LPV-76 DNA. The maps were constructed from the data in Fig. 1. Location of the replication origin, the early and late regions of the LPV genome, is from Kanda et al. (7) and Furuno et al. (4). The coding regions are for simian virus 40 proteins and are taken from Tooze (16).

identical to that of cloned DNA (data not shown). The sizes of LPV-76 DNA fragments were determined with reference to LPV-02 DNA and its fragments, the sizes of which were based on previously reported data (14), and to λDNA fragments obtained by *Hin*dIII digestion. From the results shown in Fig. 1, the LPV-76 genome size was estimated to be 4.9 kb in length and its physical map was constructed (Fig. 2). These data revealed three changes in LPV-76 DNA. These consisted of a 160- to 170-base-long deletion in the early region, an insertion of approximately 20 bases, and a 50- to 60-base-long deletion in the control region for transcription.

Viral proteins of the rescued virus (LPV-76). The sizes of LPV-76 viral proteins were compared with those of LPV-02 (Fig. 3). Cell extracts from [^{35}S]methionine-labeled BJA-B cells infected with LPV-02 or LPV-76 were immunoprecipitated with anti-LPV serum, and precipitates were electrophoresed in sodium dodecyl sulfate-polyacrylamide slab gels. Although the sizes of large T antigen (84,000 daltons [84K]), VP-2 (35K), and VP-3 (26K) of LPV-76 were identical to those of LPV-02 (12), the major capsid protein (VP-1) of LPV-76 was 42K and apparently larger than the 41K VP-1 of LPV-02 (12).

Host range of LPV-76. The growth of LPV-76 in T- and B-lymphoblastoid cell lines was tested to examine whether LPV-76 had an altered host range. BJA-B (10^6) and MOLT-4 (10^6) cells were infected with 1 ml of LPV-02 or LPV-76 stock virus, and virus replication was monitored by the FA test at 3, 6, and 18 days postinfection. The results (Table 1) showed that in BJA-B cells both LPV-02 and LPV-76 grew well. By 6 days, over 60% of the cells were FA positive and cytopathic effects were observed. LPV-02 did not replicate in MOLT-4 cells as previously reported (14); however, FA-positive cells in the LPV-76-infected culture increased gradually and reached 37% by day 18. When the LPV-76 virus harvested from MOLT-4 cells was grown in fresh BJA-B cells, the resultant virus induced a higher percentage of V-antigen-positive cells (45%) in MOLT-4 cells. These results indicate that LPV-76 is a new host range mutant capable of replicating in both B- and T-cell lines.

Construction of recombinant viruses between LPV-02 and LPV-76. We constructed recombinant viruses between

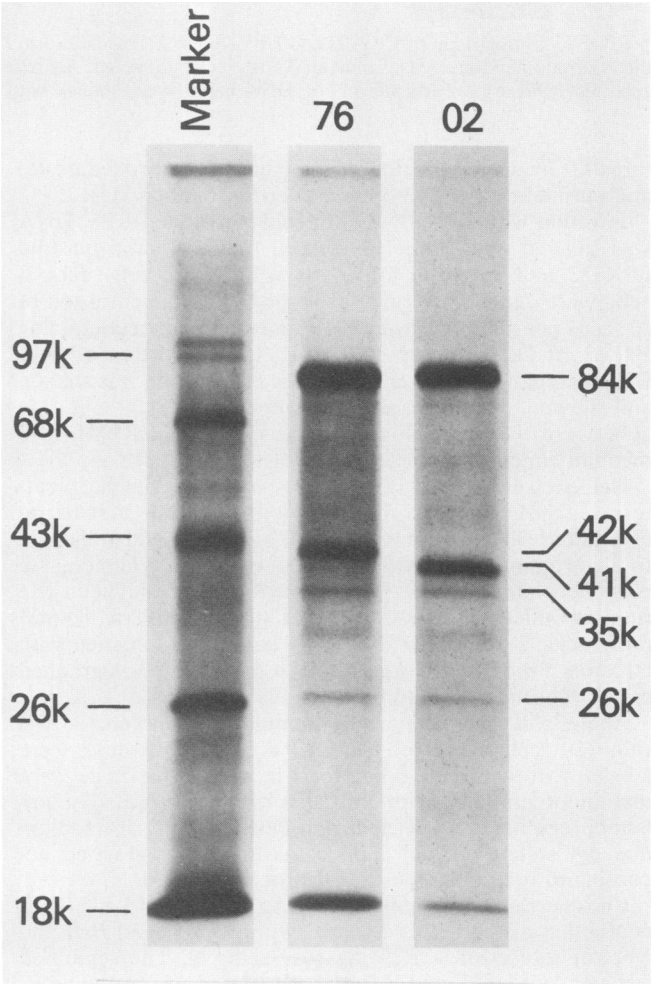

FIG. 3. Immunoprecipitation of LPV proteins in LPV-02- or LPV-76-infected BJA-B cells. The infected cells were labeled with [^{35}S]methionine, and cellular extracts were immunoprecipitated with anti-LPV serum.

TABLE 1. Growth of LPV-02 and LPV-76 in BJA-B and MOLT-4 cells

Virus	Days after infection	FA-positive cells (%)	
		BJA-B	MOLT-4
LPV-02	3	1	0
	6	62	1
	18	ND[a]	0
LPV-76	3	2	2
	6	68	12
	18	ND	37

[a] ND, Not done.

LPV-02 and LPV-76 to examine which part of the LPV-76 genome was responsible for its broadened host range. Digestion of LPV DNA with *Bam*HI and *Pst*I gave rise to three fragments: A1, A2, and B from LPV-02 DNA; A1′, A2′, and B′ from LPV-76 DNA (Fig. 1 and 2). Each segment of one virus was replaced with the corresponding fragment of the other, and the recombinants were molecularly cloned into pBR322 at the *Bam*HI site. Figure 4 shows the cleavage patterns of recombinants with *Bam*HI and *Pst*I. Orientation of the inserted fragments was examined by *Hpa*II digestion (data not shown), and recombinants containing complete viral genomes were used for transfection of BJA-B cells after *Bam*HI cleavage. The recombinant DNAs of all possible combinations were infectious in BJA-B cells, and culturefluids were collected for virus stocks when approximately 60% of the cells were FA positive.

One part of transfected cells with each recombinant DNA

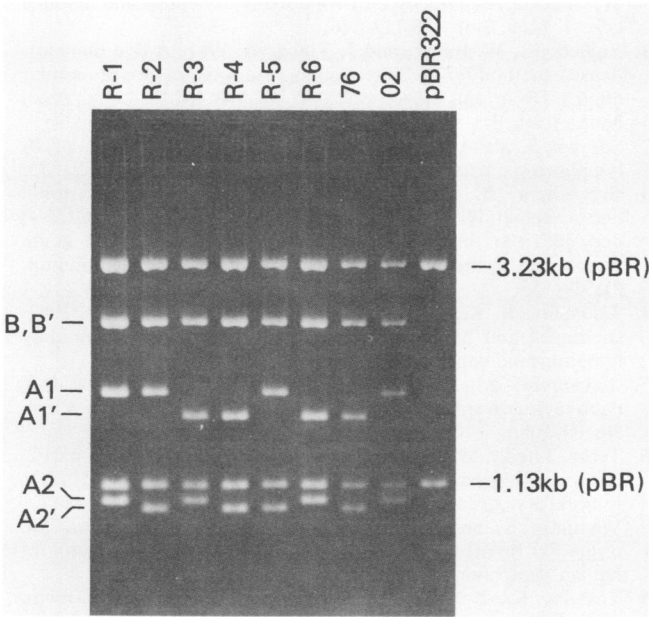

FIG. 4. Cleavage of recombinant DNAs between LPV-02 and LPV-76 with *Bam*HI and *Pst*I. Fragments B, A1, and A2 were derived from LPV-02 DNA, and fragments B′, A1′, and A2′ were derived from LPV-76 DNA. Recombinants R-1, R-2, and R-3 contained the B′ fragment, and R-4, R-5, and R-6 had the B fragment. All samples including pBR322 DNA were cleaved with *Bam*HI and *Pst*I and electrophoresed in a 1.2% agarose gel. DNA bands were stained with ethidium bromide.

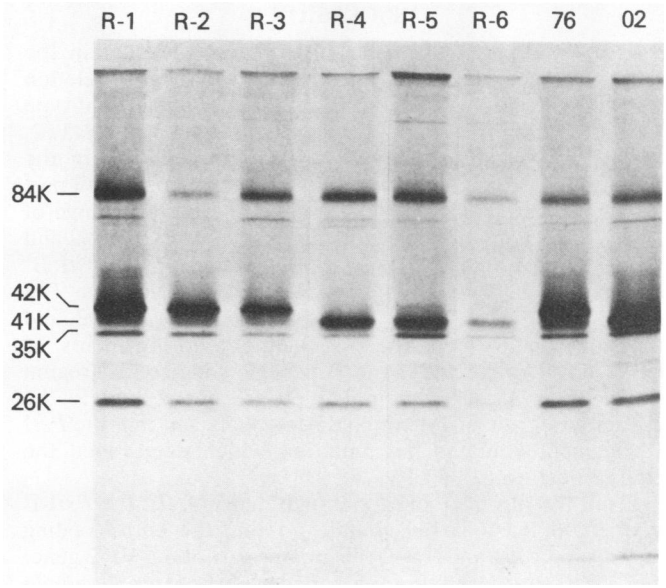

FIG. 5. Immunoprecipitation of LPV proteins in LPV-02, LPV-76, and the recombinant DNAs between LPV-02- and LPV-76-transfected BJA-B cells.

was labeled with [^{35}S]methionine, and the size of VP-1 was analyzed by immunoprecipitation (Fig. 5). Three recombinants (R-4, R-5, and R-6) that contained the *Pst*I B fragment derived from LPV-02 synthesized 41K VP-1, and three recombinants (R-1, R-2, and R-3) that had the *Pst*I B′ fragment derived from LPV-76 synthesized 42K VP-1. These results indicate that the *Pst*I B′ fragment carries the mutation responsible for the altered VP-1 of LPV-76.

Host range of recombinant viruses. Growth of recombinant viruses in MOLT-4 cells was tested (Table 2). Each virus stock (1 ml) was mixed with 10^6 MOLT-4 or BJA-B cells and the presence of FA-positive cells was examined. In BJA-B cells all viruses grew well, and FA-positive cells reached about 50 to 60% at 5 days after infection. In MOLT-4 cells, although the infection of LPV-02 R-4, R-5, and R-6 was abortive, FA-positive cells infected with LPV-76 R-1, R-2, and R-3 increased gradually to 30 to 40% by 18 days after infection. Thus, only recombinants that contained the *Pst*I B′ fragment derived from LPV-76 replicated in MOLT-4 cells.

TABLE 2. Growth of recombinant viruses in BJA-B and MOLT-4 cells

Virus	Composition of fragments[a]	FA-positive cells (%)		Size of VP-1 ($\times 10^3$)
		BJA-B[b]	MOLT-4[c]	
LPV-02	B + A1 + A2	55	0	41
LPV-76	B′ + A1′ + A2′	60	37	42
R-1	B′ + A1 + A2	53	34	42
R-2	B′ + A1 + A2′	48	31	42
R-3	B′ + A1′ + A2	65	39	42
R-4	B + A1′ + A2′	55	0	41
R-5	B + A1 + A2′	52	0	41
R-6	B + A1′ + A2	50	0	41

[a] Fragments A, A2, and B were derived from LPV-02 DNA, and fragments A1′, A2′, and B′ were derived from LPV-76.
[b] 5 days after infection.
[c] 18 days after infection.

DISCUSSION

LPV-76, derived from the LPV genome present in the HE-LPV(E) cell line (15), was found to be a viable deletion mutant with an extended host range. Whereas wild-type LPV is a strictly B-lymphotropic virus for replication (1, 2, 14), LPV-76 has acquired the ability to replicate in the T-lymphoblastoid cell line MOLT-4. To examine which part of the genome was reponsible for the altered host range of LPV-76, we constructed recombinants between LPV-02 and LPV-76 and found that only those containing the *Pst*I B′ fragment of LPV-76 could replicate in MOLT-4 cells. The data indicate that, from the correlation of physical and functional maps of LPV (4, 7), the rearrangements of LPV-76 DNA detected in the early region and control region (in the *Pst*I A′ segment in Fig. 2) are not related to the capacity to grow in T-lymphoblastoid cells and that the *Pst*I B′ segment contains the mutation which determined the altered host range of LPV-76.

From the physical and functional maps (4, 7), the *Pst*I B segment of LPV is believed to contain the entire coding regions for VP-1 and VP-3, the greater part of the VP-2 gene, and a part (the carboxyl-terminal end) of the large T-antigen gene. Thus, each of these genes may contain a mutation. Since the data on viral proteins (Fig. 3) clearly show that the mobility of VP-1 in electrophoresis has changed in LPV-76, the mutation in the VP-1 gene is probably responsible for the broadened host range of LPV-76. The precise location and type of mutation will be determined in future studies by sequencing *Pst*I B and *Pst*I B′ segments.

Apparently, LPV-76 originated from DNA constituting the major portion of the free viral DNA population in transformed hamster cells. The initial characterization of viral DNA in HE-LPV(E) showed that the free LPV DNA was indistinguishable in size and cleavage pattern from the wild-type LPV DNA from the input transforming virus (15). However, the major population of the free DNA isolated in this study was different from the DNA of the input virus. The DNA we analyzed previously was extracted from early-passage HE-LPV(E) (passage 3), but the DNA used in the present study was extracted from the cells at passages 12 to 15. LPV-76 DNA, therefore, seems to have become dominant in hamster cells during repeated cell passages, although it is not known whether LPV-76 emerged from the wild-type LPV before or after the establishment of cell transformation by LPV. Possibly, LPV-76 DNA may have some selective advantage over the wild-type LPV DNA in hamster cells.

The significance of rearrangements in LPV-76 DNA (Fig. 2) is not clear at present. A deletion of 160 to 170 bases occurs in the intervening sequence of large T mRNA or the carboxyl end of the probable small T-antigen coding region (4). If LPV has a small T antigen, the small T antigen of LPV-76 must be affected by this deletion. The DNA changes in the control region of LPV-76 may affect transcription and have different biological significance, since the DNA rearrangement in this region affects various biological properties of polyomaviruses, including the host range of mouse polyomavirus (9) and JC virus (18) and the transforming ability of BK virus (17). During the course of serial passage of LPV-76 in MOLT-4 cells, a variant emerged which replicated in MOLT-4 cells much more efficiently. This variant has an additional change in the control region (unpublished data). Comparative studies of the control regions of LPV-76 and its new variant are under way.

ACKNOWLEDGMENT

We thank Kunito Yoshiike, National Institute of Health, Tokyo, Japan, for critical review of the manuscript.

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