JOURNAL OF VIROLOGY, July 1989, p. 3195–3199
0022-538X/89/073195-05$02.00/0

Vol. 63, No. 7

Growth Efficiency of Naturally Occurring BK Virus Variants In Vivo and In Vitro

CHIE SUGIMOTO,[1] KAZUYA HARA,[1]* FUMIAKI TAGUCHI,[1] AND YOSHIAKI YOGO[2]

Department of Microbiology, School of Hygienic Sciences, Kitasato University, Sagamihara, Kanagawa 228,[1] and Department of Viral Infection, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 105,[2] Japan

Received 27 January 1989/Accepted 4 April 1989

We molecularly cloned a number of BK virus (BKV) DNAs from urine samples collected from a patient with systemic lupus erythematosus undergoing immunosuppressive therapy. On the basis of the structure of the noncoding regulatory region, cloned viral DNAs were classified into a major group and several minor groups. The major group contained a single 68-base-pair (bp) promoter-enhancer element and a 63-bp sequence identified in the genomes of many BKV strains. Most of the minor groups retained a variety of duplications within the transcriptional control region and the origin of DNA replication. We assayed various cloned viral DNAs for the capacity to induce viral growth in transfected human embryonic kidney cells. While major viral DNAs induced slow viral replication, a minor viral DNA retaining partial duplication of the 68-bp element induced rapid viral growth. We concluded that reiteration of the promoter-enhancer element, which is required for efficient growth of BKV in cell culture, is not advantageous for replication of BKV in natural hosts.

Human papovavirus BK (BKV) was initially identified by Gardner et al. (6) in the urine of an immunosuppressed renal transplant patient. Since then, several strains of this virus have been isolated, mainly from renal or bone marrow transplant recipients or patients with immunologic disorders (1, 3, 7, 10, 12, 14, 21). Complete DNA sequences determined for two BKV strains, Dun (19) and MM (29), have shown that the genome of BKV, like that of simian virus 40 (5, 15), consists of the early, late, and regulatory regions. Among the BKV strains analyzed, there exists significant diversity in the structures of their regulatory regions (13, 14, 17, 19, 23, 24, 29).

The BKV strain isolated by Gardner et al. contains three sets of the 68-base-pair (bp) element to the late side of the origin of DNA replication (19), and these reiterated elements function as enhancers in a transient expression assay (4, 16) and are required for efficient growth of BKV in human cells in culture (9, 25). Many other BKV strains contain reiterations of similar sequences (13, 14, 24, 29). Recent reports, however, have shown the occurrence of two BKV strains, Dik and WW, which retained a single 68-bp element and an extra 63-bp sequence (17, 23). The 63-bp sequence is not present in a few strains, including strain Gardner (14, 19, 24), but a complete or partial copy of this sequence has been identified in most BKV strains (13, 14, 17, 23, 29). It has been suggested that repeated enhancer-promoter elements in various BKV strains may have evolved from a putative prototype BKV similar to WW or Dik (17, 26).

In this study, we molecularly cloned a variety of BKV DNAs differing in the regulatory region from two urine samples which had been collected at an interval of 2 weeks from an immunosuppressed patient. After the structures of their regulatory regions were characterized, various cloned viral DNAs were assayed for the capacity to induce viral growth in transfected human embryonic kidney (HEK) cells.

A 17-year-old male with systemic lupus erythematosus had been treated with prednisolone and cyclophosphamide for 3 months (21). Urine samples collected on 9 August (sample 1) and 22 August (sample 2) 1977 were centrifuged at low speed to remove urinary sediment. The supernatant was centrifuged at 100,000 × *g* for 2 h, and the pellets obtained were suspended in phosphate-buffered saline and stored at −70°C for about 10 years. The suspension was digested with proteinase K, and the viral DNA was extracted with phenol and chloroform-isoamyl alcohol (24:1). The viral DNA was cleaved with *Bam*HI, ligated to *Bam*HI-digested and phosphatase-treated pAT153, and used to transform *Escherichia coli* HB101. Recombinant plasmids were extracted from the transformed bacteria and subjected to restriction analysis with *Hin*dIII.

*Hin*dIII cleaves the DNA of BKV Gardner at four sites to give rise to four fragments, A to D (11; Fig. 1A). However, all of the cloned BKV DNAs analyzed (104 and 80 clones from samples 1 and 2, respectively) had lost the *Hin*dIII cleavage site between C and D and, after *Hin*dIII digestion, generated fused fragments designated as C/D (data not shown). The sizes of C/D varied among recombinant clones, but most of the clones could be classified into four groups on the basis of the sizes of C/D (Table 1). The size of C/D in group I was about 55 bp shorter than the sum of the sizes of C and D in strain Gardner. The sizes of C/D in groups II, III, and IV were approximately 30, 140, and 230 bp longer, respectively, than that of group I. Group I encompassed most of the clones derived from each urine sample (63 and 61% from samples 1 and 2, respectively), while clones belonging to groups II to IV ranged from 3 to 19%, depending on the groups and samples. In addition, some clones contained a shortened A or B fragment (group V) and some lacked the *Hin*dIII cleavage site between A and C or B and D (group VI).

We sequenced fragment C/D for multiple clones of each group derived from sample 1 urine. Sequencing was performed by cloning with M13 phage and by the chain termination method (18). Fragment C/D contains part of the T-antigen-coding region, a 5′-terminal sequence of the agnogene, and the whole regulatory region (Fig. 1B). The partial T-antigen-coding region and the 5′-terminal sequence of the agnogene were identical not only between clones within the same group but also among different groups (data not shown). However, six base mismatches were detected in

* Corresponding author.

TABLE 1. Classification of BKV DNAs cloned from a patient with systemic lupus erythematosus

Group and *Hin*dIII cleavage pattern	No. of recombinant clones in:					
	Sample 1			Sample 2		
	Total (% of total)	*Bgl*I	*Bgl*I$^+$	Total (% of total)	*Bgl*I	*Bgl*I$^+$
I; fusion of C and D	66 (63)	36	30	49 (61)	35	14
II; fusion of C and D and size increase of C/D (30 bp)[a]	7 (7)	6	1	7 (9)	7	0
III; fusion of C and D and size increase of C/D (140 bp)[a]	10 (10)	8	2	2 (3)	2	0
IV; fusion of C and D and size increase of C/D (230 bp)[a]	15 (14)	7	8	15 (19)	11	4
V; fusion of C and D and size change of A or B	4 (4)	2	2	0 (0)	0	0
VI; fusion of C and D and fusion of A, C, and D or B, C, and D	2 (2)	2	0	7 (8)	6	1

[a] Approximate size increase of C/D compared with C/D of group I.

these regions between all of the clones examined and strain Dun, for which the complete DNA sequence was determined (19).

It turned out that group I was divided into two subgroups based on the presence or absence of a single base replacement located at the origin of DNA replication, nucleotide −6, although the two regulatory regions were otherwise identical (Fig. 2). This change converts BKV DNA to susceptibility to *Bgl*I, and the presence of this change could be readily detected with this enzyme. Clones with the *Bgl*I site were also present in other groups which contained significant numbers of clones (Table 1). We designated a group I virus that is resistant to *Bgl*I (*cl*6) as MT-1. The basal structure of the regulatory region of MT-1 was identical to that of strain Dik (23) or WW (17); that is, all of these viruses contained only one set of the 68-bp element but retained the 63-bp sequence (Fig. 2). There were, however, several mismatches between MT-1 and WW or Dik (Fig. 2). However, WW and Dik were different in only one base mismatch (17, 23).

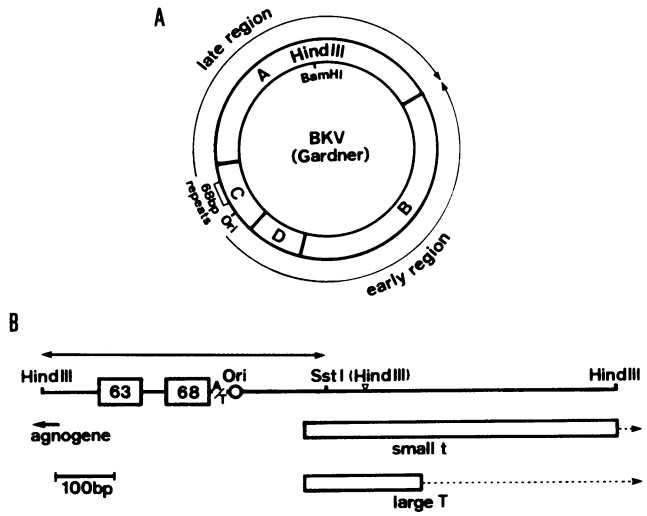

FIG. 1. Restriction enzyme cleavage map of BKV DNA. (A) *Hin*dIII fragments (A to D) are located on the genome of strain Gardner (10). Transcriptional regions, the replication origin, and 68-bp tandem repeats are shown outside the genome. (B) Restriction sites used in this study are shown above fragment C/D derived from MT-1, a BKV DNA clone obtained from the patient (see the text). Some other clones retain one more *Aat*I (*Stu*I) site because of duplication. Parentheses indicate loss of a restriction site. The coding region of small and large T antigens (exon 1; open rectangles) and the 5′ portion of the agnogene are shown below fragment C/D. Some other structural features of the C/D fragment revealed in this study, the replication origin (Ori), an AT-rich sequence (A/T), a 68-bp element, and a 63-bp sequence, are also indicated. The top line shows a subfragment of C/D which was used as a probe in blot hybridization.

We found that the size increase of fragment C/D in groups II to IV was due to duplication of segments within the 68-bp element (group II), segments extending from the replication origin to the 68-bp element (group III), or segments extending from the replication origin to the 63-bp sequence (group IV) (Fig. 3). Groups II to IV contained two or three subgroups which could be distinguished by location of duplication endpoints or by the presence or absence of the *Bgl*I site described above. In clones belonging to groups V and VI, we detected deletion of segments by analysis with restriction enzymes (data not shown). Furthermore, one clone (*cl*108) that was included in group I lacked a 7-bp sequence within the 68-bp element (Fig. 3).

To ensure that the variation detected in the regulatory region was not introduced during molecular cloning of viral DNA, we directly analyzed the viral DNA extracted from the urine by blot hybridization. The viral DNAs derived from the two urine samples and clones of groups I to IV were digested with *Hin*dIII and electrophoresed in a 1.4% agarose gel. DNA fragments separated in the gel were blotted to a nitrocellulose filter by the procedure of Southern (20) and hybridized with a ^{32}P-labeled subfragment of C/D indicated in Fig. 1B. In each urine sample, we detected three bands that corresponded to C/D of groups I and II, III, and IV (the C/D fragments of groups I and II were not resolved under the conditions used) (Fig. 4). Thus, it can be concluded that the size variation of the *Hin*dIII C/D fragment occurred within the patient. Similarly, variants carrying the *Bgl*I cleavage site, as well as those not carrying it, were detected in the urine by blot hybridization (data not shown).

For representative clones of each group (groups I to IV), linear viral DNAs excised from the vector (0.1 μg each per 35-mm-diameter dish) were introduced into human embryonic kidney (HEK) cells by the calcium phosphate coprecipitation method (8). (It is known that endonuclease-cleaved linear viral DNA can be recircularized after transfection [2].) Viral DNAs of BKV Gardner (wt-501 [27]) derived from a recombinant DNA and a control were similarly transfected. Cells were collected when the maximum cytopathic effect due to wt-501 and *cl*51 occurred or on day 30 for clones which did not produce a detectable cytopathic effect and were subjected to titration of intracellular hemagglutinin (HA) activity (22), since we detected no extracellular HA activity in cultures transfected with most of clones (data not shown). A typical result is shown in Table 2. We detected a high HA titer in *cl*51-transfected cells, as expected from the rapid appearance of a cytopathic effect. *cl*51 retained a duplication of a 35-bp sequence (nucleotides 49 to 83) within the 68-bp element (Fig. 3). A low but significant HA titer was detected in cultures transfected with *cl*6 (group I, *Bgl*I$^-$) or *cl*8 (group I, *Bgl*I$^+$). However, a viral DNA containing a

```
Agnogene
      270                                                                                    63 bp sequence
MT-1  CATGGCCTTT GTCCAGTTTA ACTGGGGACA AGGCCAACAT TCCCAGGCTC GCAAAACATG TCTGTCTGGC TACTTTCCAC TCCTTTGGCC AGTTTCCACT
WW    CATGGCCTTT GTCCAGTTTA ACTGGGGACA AGGCCAAGAT TCCTAGGCTC GCAAAACATG TCTGTCTGGC TGCTTTCCAC TCCTTTGGCC AGTTTCCACT
Dik   CATGGCCTTT GTCCAGTTTA ACTGGGGACA AGGCCAAGAT TCCTAGGCTC GCAAAACATG TCTGTCTGGC TGCTTTCCAC TCCTTTGGCC AGTTTCCACT
      170
MT-1  TCCTCTTGTG TTTATTTGAG AATTTCATGG GCGGGGTTTC ACTATTAACT GCCACTGGCT GGCTGCCCAG TCATGCACTT TCCTTCCTGA GGTCATGGTT
WW    TCCTCTTGTG TTTATTTGAG AATTTTAGGG GCGGGGTTTC ACTATTAACT GCCACTGGCT GGCTGCCCAG TCATGCACTT TCCTTCCTGA GGTCATGGTT
Dik   TCCTCTTGTG TTTATTTGAG AATTTTAGGG GCGGGGTTTC ACTATTAACT GCCACTGGCT GGCTGCCCAG TCATGCACTT TCCTTCCTGA GGTCATGGTT
      70    68 bp element                                                  Origin    G
MT-1  TGGCTGCATT CCATGGGTAA GCAGCTCCTC CCTGTGGCCT TTTTTTTTAT AATATATAAG AGGCCGAGGC CGCCTCTGCC TCCACCCTTT CTCTCAAGTA
WW    TGGCTACATT CCATGGGTAA GCAGCTCCTC CCTGTGGCCT TTTTTTTTAT AATATATAAG AGGCCGAGGC CGCCTCTGCC TCCACCCTTT CTCTCAAGTA
Dik   TGGCTACAAT CCATGGGTAA GCAGCTCCTC CCTGTGGCCT TTTTTTTTAT AATATATAAG AGGCCGAGGC ..........  ..........  ..........
      -30
MT-1  GTAAGGGTGT GGAGGCTTTT TCTGAGGCCT AGCAAAACTA TTTGGGGAAA TCCCTATTCT TTTGCAAATT TTTGCAAAAA TG
WW    GTAAGGGTGT GGAGGCTTTT TCTGAGGCCT AGCAAAACTA TTTGGGGAAA TCCCTATTCT TTTGCAA-TT TTTGCAAAAA TG
Dik   ..........  ..........  ..........  ..........  ..........  ..........  ..........  .......... ..
                                                                                          T antigens
```

FIG. 2. Comparison of regulatory regions among BKV strains MT-1, WW, and Dik. The nucleotide sequences shown are from the T-antigen start sites to the agnogene start sites. The sequences of WW and Dik were from references 17 and 23, respectively. The 63-bp sequence, the 68-bp element, and the replication origin are underlined. The base replacement found in the BKV DNA clones analyzed in this study is indicated by an arrowhead. The nucleotide numbering system is that of Watanabe et al. (24). Symbols: *, base mismatch; —, gap introduced for alignment; · · ·, sequence not completed.

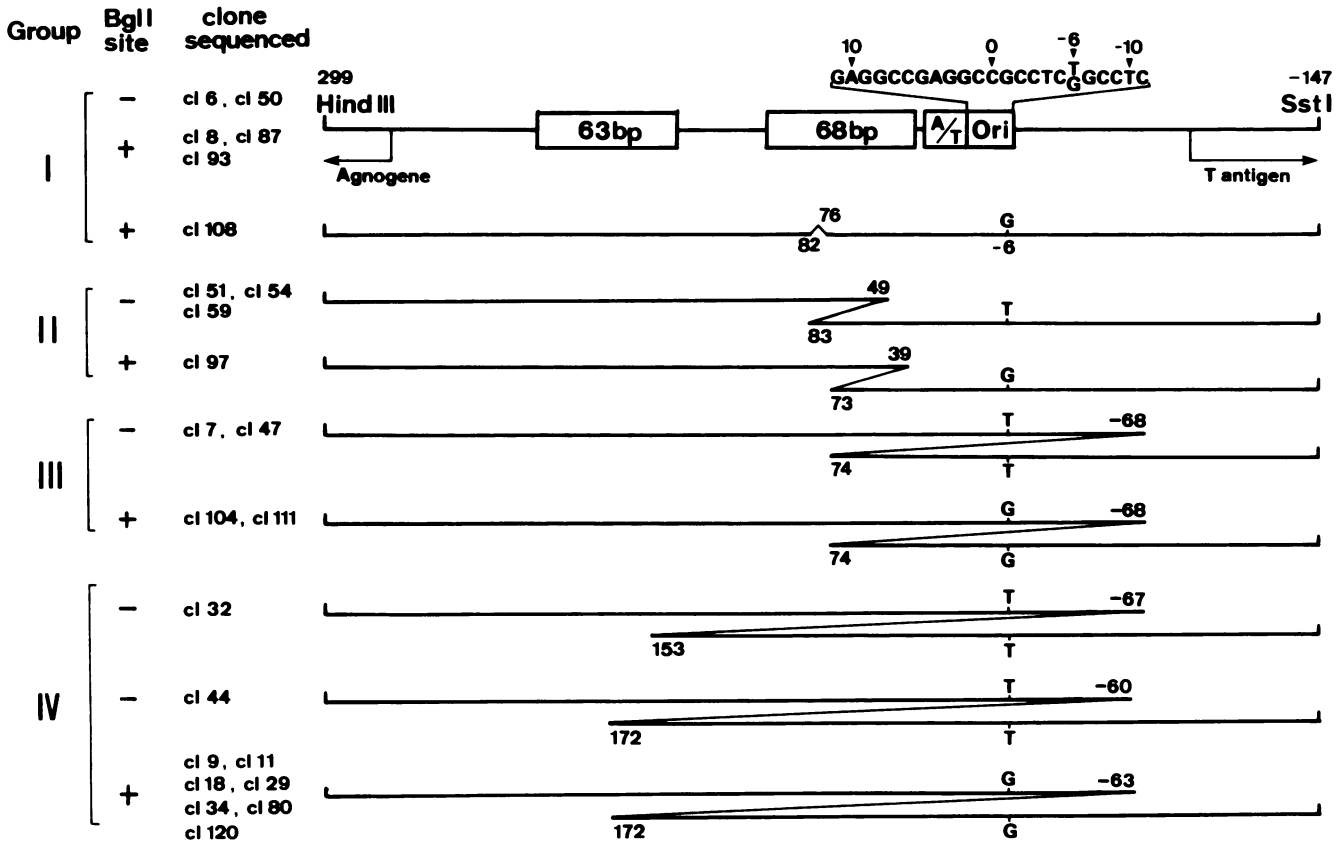

FIG. 3. Diagrammatic representation of the regulatory regions of various BKV DNA clones. The line at the top shows the regulatory regions of typical group I clones. Below are the regulatory regions of an exceptional clone of group I (*cl*108) and several typical clones of groups II to IV. Duplicated sequences are indicated by parallel lines connected with diagonal lines. Deletion of a sequence is represented by a caret. Endpoints of duplicated or deleted segments are shown by nucleotide numbers (the numbering system is that of Watanabe et al. [24]). T or G at the replication origin (Ori; nucleotide −6) on each line indicates the variable nucleotide (see the text); clones with T were not cleaved with *Bgl*I, while those with G were cleaved with *Bgl*I.

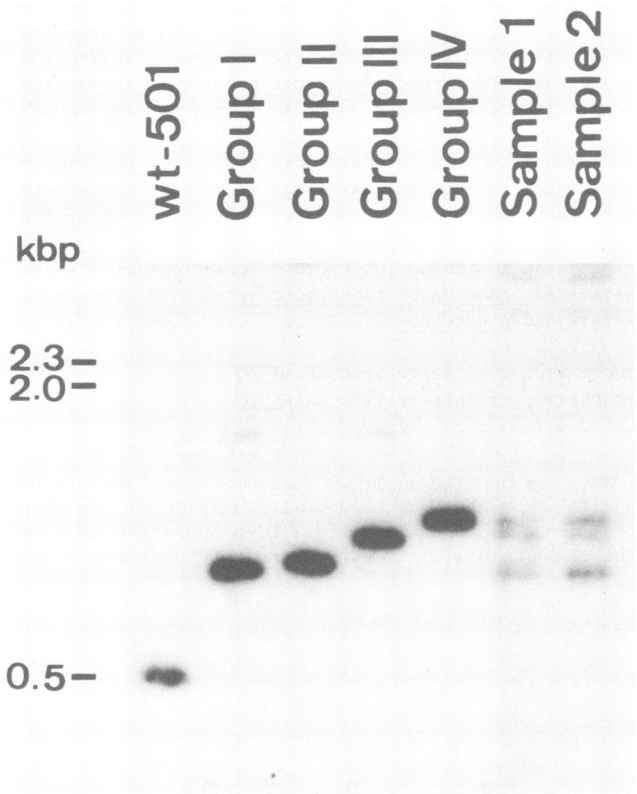

FIG. 4. Southern blot analysis of viral DNAs extracted from urine. Two nanograms each of a prototype BKV DNA clone (wt-501), a group I clone (*cl*6), a group II clone (*cl*51), a group III clone (*cl*7), and a group-IV clone (*cl*44) and 1 ng of viral DNAs extracted from urine samples 1 and 2 were digested with *Hin*dIII and electrophoresed in a 1.4% agarose gel. The DNA fragments separated were transferred to a nitrocellulose filter and hybridized with a ^{32}P-labeled subfragment of C/D derived from MT-1 (indicated in Fig. 1B) (28). The sizes of some *Hin*dIII fragments of lambda DNA are given in kilobase pairs (kbp) to the left.

duplication of a segment (nucleotides 39 to 73) within the 68-bp element (*cl*97), viral DNAs carrying duplications of the replication origin and the transcriptional control region (*cl*17, *cl*111, *cl*44, and *cl*120), and a viral DNA with a deletion within the 68-bp element (*cl*108) were incompetent for induction of viral growth under the conditions in which group I clones could induce detectable growth of BKV. We obtained an essentially identical result when a higher amount of each viral DNA (1 μg per dish) was introduced into HEK cells (data not shown).

TABLE 2. Appearance of BKV-specific HA activity in HEK cells after transfection with various cloned viral DNAs

Clone	Group (phenotype)	HA titer[a] at:		
		12 days	16 days	30 days
*cl*6	I (*Bgl*I$^-$)	—	—	16
*cl*8	I (*Bgl*I$^+$)	—	—	16
*cl*108	I (*Bgl*I$^+$ Del[b])	—	—	<2
*cl*51	II (*Bgl*I$^-$)	—	512	—
*cl*97	II (*Bgl*I$^+$)	—	—	<2
*cl*7	III (*Bgl*I$^-$)	—	—	<2
*cl*111	III (*Bgl*I$^+$)	—	—	<2
*cl*44	IV (*Bgl*I$^-$)	—	—	<2
*cl*120	IV (*Bgl*I$^+$)	—	—	<2
wt-501		2,048	—	—

[a] Linear viral DNA excised from the vector (100 ng/35-mm-diameter dish) with *Bam*HI was introduced into HEK cells by calcium phosphate coprecipitation (8). Intracellular HA activity was determined on the days indicated after transfection. —, Not done.

[b] Del, Deletion of a 7-bp sequence in the 68-bp element.

This study demonstrated that a patient with systemic lupus erythematosus undergoing immunosuppressive therapy harbored multiple BKV variants which differed in the regulatory region of the viral genome. These variants, however, were closely related to each other, since they shared a number of base replacements in a portion of the T-antigen-coding region and in the regulatory region. There seems to be no doubt that viruses which had various duplicated segments in the control region were derived from group I viruses, because group I retained the simplest regulatory region and encompassed most of the cloned viral DNAs. It is not known, nevertheless, which of the group I viruses was the parental BKV which had primarily infected the patient. Because at least one BKV strain (GS [14]) has the *Bgl*I cleavage site at the origin of DNA replication, the possibility cannot be ruled out that a strain containing an origin similar to that of GS may be the parental virus, although most BKV strains lack the *Bgl*I site (13, 14, 17, 19, 23, 24, 29).

The major BKV variants identified in the patient (group I) grew poorly in HEK cells. Inefficient growth in cell culture appears to be characteristic of naturally occurring BKV variants without reiteration of the promoter-enhancer element, since Mew et al. (12) reported the failure of strain WW to propagate in cell culture. Most of the minor variants which gave no indication of productive growth in HEK cells had a variety of duplications encompassing the origin of DNA replication and the transcriptional control region. The reason why we could not detect the growth of these variants in cell culture is not clear, although some speculation is possible. For example, it is conceivable that the existence of two replication origins in close proximity interferes with early or late transcription, although it may not inhibit viral DNA replication as revealed by detection of significant amounts of these viral DNAs from the patient (Fig. 4). We are currently testing this possibility.

A BKV variant with a duplication within the enhancer-promoter element (*cl*51) could grow in HEK cells almost as efficiently as strain Gardner. The high growth capacity of *cl*51 in HEK cells is probably due to the duplication within the 68-bp element, because we previously demonstrated that duplication of a 33-bp segment which almost completely overlapped the 35-bp duplicated sequence of *cl*51 enhanced the growth capacity of BKV in HEK cells (9). Clone *cl*51 and its sister clones were included in group II, and the proportion of this group continued to be small throughout 2 weeks (7 and 9% in samples 1 and 2, respectively; Table 1). Therefore, a BKV variant containing *cl*51 DNA does not appear to have grown in the patient more efficiently than other viruses did. We conclude that reiteration of the promoter-enhancer element is not advantageous for growth of BKV in human hosts, although variants carrying a reiterated promoter-enhancer element, like *cl*51, may occasionally occur within patients.

This work was supported in part by grants for cancer research from the Ministry of Education, Science, and Culture of Japan.

LITERATURE CITED

1. **Beckmann, A. M., K. V. Shah, and P. Mounts.** 1985. Genetic heterogeneity of the human papovaviruses BK and JC. J. Med. Virol. **15**:239–250.

2. **Carbon, J., T. E. Shenk, and P. Berg.** 1975. Biochemical procedure for production of small deletions in simian virus 40 DNA. Proc. Natl. Acad. Sci. USA **72**:1392–1396.
3. **Chauhan, S., G. Lecatsas, and E. H. Harley.** 1984. Genome analysis of BK (WW) viral DNA cloned directly from human urine. Intervirology **22**:170–176.
4. **Deyerle, K. L., J. A. Cassill, and S. Subramani.** 1987. Analysis of the early regulatory region of the human papovavirus BK. Virology **158**:181–193.
5. **Fiers, W., R. Contreras, G. Haegeman, R. Rogiers, A. van de Voorde, H. van Heuverswyn, J. van Herreweghe, G. Volckaert, and M. Ysebaert.** 1978. Complete nucleotide sequence of SV40 DNA. Nature (London) **273**:113–120.
6. **Gardner, S. D., A. M. Field, D. V. Coleman, and B. Hulme.** 1971. New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet **i**:1253–1257.
7. **Goudsmit, J., M. L. Baak, K. W. Slaterus, and J. van der Noordaa.** 1981. Human papovavirus isolated from urine of a child with acute tonsillitis. Br. Med. J. **293**:1363–1364.
8. **Graham, F. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
9. **Hara, K., Y. Oya, H. Kinoshita, F. Taguchi, and Y. Yogo.** 1986. Sequence reiteration required for the efficient growth of BK virus. J. Gen. Virol. **67**:2555–2559.
10. **Howley, P. M.** 1980. Molecular biology of SV40 and the human polyomaviruses BK and JC. p. 489–550. *In* J. Klein (ed.), Viral oncology. Raven Press, New York.
11. **Howley, P. M., G. Khoury, J. C. Byrne, K. K. Takemoto, and M. A. Martin.** Physical map of the BK virus genome. J. Virol. **16**:959–973.
12. **Mew, R. T., G. Lecatsas, O. W. Prozesky, and E. H. Harley.** 1981. Characteristics of BK papovavirus DNA prepared directly from human urine. Intervirology **16**:14–19.
13. **Pagnani, M., M. Negrini, P. Reschiglian, A. Corallini, P. G. Balboni, S. Scherneck, G. Macino, G. Milanesi, and G. Barbanti-Brodano.** 1986. Molecular and biological properties of BK virus-IR, a BK virus variant isolated from a human tumor. J. Virol. **59**:500–505.
14. **Pater, A., M. M. Pater, L.-S. Chang, K. Slawin, and G. di Mayorka.** 1983. Multiple origins of the complementary defective genomes of RF and origin proximal sequence of GS, two human papovavirus isolates. Virology **131**:426–436.
15. **Reddy, V. B., B. Thimmappaya, R. Dhar, K. N. Subramanian, B. S. Zain, J. Pan, P. K. Ghosh, M. L. Celma, and S. M. Weissman.** 1978. The genome of simian virus 40. Science **200**:494–502.
16. **Rosenthal, N., M. Kress, P. Gruss, and G. Khoury.** 1983. BK viral enhancer element and a human cellular homolog. Science **222**:749–755.
17. **Rubinstein, R., N. Pare, and E. H. Harley.** 1987. Structure and function of the transcriptional control region of nonpassaged BK virus. J. Virol. **61**:1747–1750.
18. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
19. **Seif, I., G. Khoury, and R. Dhar.** 1979. The genome of human papovavirus BKV. Cell **18**:963–977.
20. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
21. **Taguchi, F., K. Hara, J. Kajioka, and D. Nagaki.** 1979. Isolation of BK virus from a patient with systemic lupus erythematosus (SLE). Microbiol. Immunol. **23**:1131–1132.
22. **Taguchi, F., K. Hara, J. Kajioka, and D. Nagaki.** 1980. BK virus haemagglutinin in urine of a patient with BK virus infection. Acta Virol. **24**:154.
23. **ter Schegget, J., C. J. A. Sol, E. W. Baan, J. van der Noordaa, and H. van Ormondt.** 1985. Naturally occurring BK virus variants (JL and Dik) with deletions in the putative early enhancer-promoter sequences. J. Virol. **53**:302–305.
24. **Watanabe, S., E. Soeda, S. Uchida, and K. Yoshiike.** 1984. DNA rearrangement affecting expression of the BK virus transforming gene. J. Virol. **51**:1–6.
25. **Watanabe, S., and K. Yoshiike.** 1985. Decreasing the number of 68-base-pair tandem repeats in the BK virus transcriptional control region reduces plaque size and enhances transforming capacity. J. Virol. **55**:823–825.
26. **Watanabe, S., and K. Yoshiike.** 1986. Evolutionary changes of transcriptional control region in a minute-plaque viable deletion mutant of BK virus. J. Virol. **59**:260–266.
27. **Watanabe, S., K. Yoshiike, A. Nozawa, Y. Yuasa, and S. Uchida.** 1979. Viable deletion mutant of human papovavirus BK that induces insulinomas in hamsters. J. Virol. **32**:934–942.
28. **Yogo, Y., A. Furuno, A. Nozawa, and S. Uchida.** 1981. Organization of viral genome in a T antigen-negative hamster tumor induced by human papovavirus BK. J. Virol. **38**:556–563.
29. **Young, R. C. A., and R. Wu.** 1979. BK virus DNA: complete nucleotide sequence of a human tumor virus. Science **206**: 456–462.