At Least Two Types of Control Regions Can Be Found among Naturally Occurring BK Virus Strains

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The various strains of BK virus (BKV) exhibit a remarkable degree of heterogeneity in the transcriptional control region, which may affect the biological characteristics of a BKV strain. We describe the detection and sequencing of BKV control regions directly from urine samples and after propagation in cell culture. A BKV strain [BKV (TU)] with a control region anatomy not described earlier, as well as a BKV (WW)-like strain [BKV (WWT)], was detected in urine samples by direct sequencing of polymerase chain reaction products. Urine inocula containing BKV (WWT) yielded BKV (TU) upon one passage in cell culture, while BKV (TU) did not change its control region during propagation in cell culture. Analysis of the nucleotide sequence of the transcriptional control regions revealed a partial deletion and duplication in BKV (TU) compared with BKV (WWT). In addition, the control region of BKV (TU) contains two point mutations relative to BKV (WWT). This indicates that both virus strains were probably present in the BKV (WWT)-dominated urine inocula, rather than that BKV (WWT) genomes were rearranged into BKV (TU) genomes during cell propagation. The heterogeneity of the control region of BKV strains is discussed in relation to both confirmed and putative transcription factor-binding sites.

The ubiquitous human polyomavirus BK (BKV) efficiently infects populations all over the world (5). Most primary infections occur in early childhood, and by young adult age, serum antibodies can be detected in 70 to 80% of the individuals (16, 23, 24, 56). Important parts of the natural history and epidemiology of BKV are unknown or uncertain. Indications of respiratory symptoms during primary childhood infections have been reported (27, 44), while reactivated infections have been linked to cases of hemorrhagic cystitis (1, 2, 33) and uretic stenosis (14). BKV can establish latent infections within the human organism. All of the target organs and cell populations have not been identified, but the kidneys seem to be included (11, 34). Latent virus can be reactivated, e.g., during immunosuppressive disease or treatment (13, 22, 26, 36, 44, 65), but the mechanisms for reactivation are virtually unknown.

BKV virus has a potential for transformation of cell cultures from various species, including man, and for induction of tumors in laboratory animals (30, 51, 65). BKV nucleic acids and polypeptides have also been detected in human tumors and in cell lines established from tumor tissues (6, 15, 20, 41, 65).

BKV was first isolated from the urine of a kidney transplant patient in 1971 (25). This strain, BKV (Gardner), has since been commonly used in experimental studies together with the closely related strain BKV (Dunlop) (55). A number of other strains have also been reported (for a review, see reference 65). These strains have generally been subjected to repeated cell culture passages before detailed characterization was initiated. Repeated passages in cell culture may lead to rearrangements and deletions of control region sequences (4, 37, 55, 61, 64). However, for one strain, BKV (WW), viral DNA was cloned directly from several liters of urine collected from the same patient (10, 47, 53). DNA sequencing of BKV strains has revealed a striking sequence conser-

vation in the polypeptide-encoding regions of the genome (55, 60, 64). However, in the approximately 400-base-pair (bp)-long noncoding control region, which includes the origin of replication and the regulatory region for early and late transcription, a high degree of heterogeneity has been demonstrated (65). BKV (WW) has been proposed as the archetypal strain of BKV (53). The transcriptional control region of BKV (WW) has arbitrarily been divided into three transcription factor-binding regions called P (68 bp), Q (39 bp), and R (63 bp), to aid visualization of the rearrangements (45). All other sequenced BKV strains contain control regions with partial duplications and/or deletions of these blocks, compared with BKV (WW) (45). At present, it is impossible to know whether these rearrangements existed in the original wild-type BKV strains or have accumulated during cell culture isolation and passage. Since rearrangements of the control region may occur during propagation in cell culture, we have no comprehension of the repertoire of BKV strains circulating under natural conditions. This is an important point, since the anatomy of the control region may strongly influence the biological characteristics of a BKV strain with regard to host cell permissivity and transforming potential (for a review see reference 65). Consequently, in order to perform studies relevant for human pathology, there is an obvious need for knowledge about the structure of BKV control regions present in the human body.

In this article we describe studies aimed at the detection, cloning, and sequencing of BKV from urine samples. The strategy allowed comparison of BKV control regions present in the urine with those found after cell culture propagation of the urine-derived virus. Differences in the control region between BKV strains are also discussed in relation to both proven and potential transcription factor-binding sites.

MATERIALS AND METHODS

Urine samples. A total of 335 urine samples were collected from 163 patients, mostly children, visiting the University

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Hospital of Tromsø. Among these were 201 samples collected during hospitalization and follow-ups from 37 children undergoing chemotherapeutic treatment for leukemias or solid tumors. The samples were stored at -70°C until further analyzed.

Cell culture and virus propagation. Urine samples (2 ml) were diluted fivefold with cell culture medium and inoculated on subconfluent monolayers of Vero cells (ATCC CCL 81) and low-passage human embryonic kidney cells (HEK) (Whittaker M.A. Bioproducts, Inc., Walkersville, Md.; no. 70-151) in 75-cm² flasks (Nunc, Roskilde, Denmark). The inocula were allowed to adsorb for 2 h. Vero cells were maintained in Eagle minimum essential medium supplemented with 2% fetal calf serum, 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 100 μg of streptomycin per ml, and 200 U of penicillin G per ml. HEK cells were maintained in Eagle minimum essential medium supplemented with 0.5% Ultrosor G (GIBCO Laboratories, Grand Island, N.Y.), 20 mM HEPES, nonessential amino acids, 2 mM L-glutamine, and antibiotics as described above. Inoculated cell cultures were inspected weekly for development of cytopathogenic effect (CPE), with mock-infected Vero and HEK cells serving as controls. Cultures were maintained in a humidified 5% CO₂ atmosphere at 37°C . The medium was changed weekly.

Immunocytochemical detection of BKV in cell culture. An indirect immunoperoxidase staining method was applied for detection of BKV antigens in infected Vero and HEK cells (21). Subconfluent cell monolayers in 24-well polystyrene plates (Nunc) were inoculated with supernatants from cell cultures displaying CPE. A cross-reacting monoclonal antibody directed against simian virus 40 large T antigen (PAB 1614) (3) and rabbit antisera against purified BKV virions (12) were used as primary antibodies.

DNA manipulations. Plasmids containing full-length genomes of BKV strain Gardner (pBKV33-1) and JC virus (JCV) strain Mad-1 (pJCV1-4) were obtained from ATCC (ATCC 45024 and 45027). A 975-bp *SacI*-*HindIII* subfragment of BKV containing part of the large T antigen exon 2 region was subcloned into pGEM-3 blue (Promega Biotec, Madison, Wis.), yielding pGEMBKV-3. A 957-bp *PstI*-*BamHI* subfragment of JCV containing part of the large T antigen exon 2 region was also subcloned into pGEM-3 blue, yielding pGEMJCV-5. Before use as hybridization probes, viral DNA inserts were separated from vector DNA by restriction endonuclease digestion and agarose gel electrophoresis and purified from agarose gel bands by using GENECLAN (BIO 101, La Jolla, Calif.). Subcloning of specific restriction fragments, nucleotide sequencing by the dideoxynucleotide procedure, probe labeling by random priming, and Southern blotting were performed as described previously (38, 39).

Slot blot hybridization. Portions (400 μl) of urine samples were immersed in boiling water for 5 min and cooled on ice before addition of 20 μl 10 M NaOH to each sample and application into the wells of a slot blot apparatus (Minifold II; Schleicher & Schuell, Inc., Keene, N.H.). GeneScreen-Plus membranes (Dupont, NEN Research Products, Boston, Mass.) were used, and UV cross-linking of wet membranes was performed. Prehybridization was for at least 4 h at 65°C in $4\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.5% sodium dodecyl sulfate– $5\times$ Denhardt solution–100 μg of denatured, sonicated salmon sperm DNA per ml. Filters were hybridized for 20 h at 42°C in a solution containing $4\times$ SSC, 0.5% sodium dodecyl sulfate, 10% dextran sulfate, $5\times$ Denhardt solution, 50% deionized form-

amide, and 100 μg of denatured, sonicated salmon sperm DNA per ml (melting temperature, 58°C). Membranes were hybridized sequentially to three different DNA probes. Purified viral inserts from pGEMBKV-3 and pGEMJCV-5 and *EcoRI*-digested pGEM-3 blue were used as probes. Following hybridization, filters were washed at 65°C in $2\times$ SSC–0.5% sodium dodecyl sulfate for 30 min and in $0.1\times$ SSC–0.5% sodium dodecyl sulfate for 3 h. Wet filters were autoradiographed for 48 h by using Cronex-4 films (Du Pont Co., Wilmington, Del.) at -70°C with two intensifying screens. Between the three separate hybridizations, probes were removed from the filters as described by the manufacturer (E-94749; Dupont NEN Research Products) and the filters were checked by autoradiography for complete removal of probes.

PCR amplification and sequencing of BKV DNA from urine and cell culture. Urine samples (100 μl) were pelleted in a microcentrifuge, and the pellets were diluted in the same volume of distilled H₂O and boiled for 5 min. Supernatants (100 μl) from cell cultures showing CPE were collected and boiled for 5 min. A 1- to 5- μl portion of these samples was used for standard double-stranded polymerase chain reaction (PCR) amplification (54). The same amount of sample was used to generate single-stranded PCR products (31). Oligonucleotide primers (Operon Technology, Inc., San Pablo, Calif.) complementary to the T antigen (BKTT1; 5'-AAGGTCCATGAGCTCCATGGATTCTTCC-3') and VP2 (BKTT2; 5'-CTAGGTCCCCCAAAGTGCTAGAGCAGC-3') coding regions of BKV and JCV were used (see Fig. 2). Oligonucleotides complementary to the noncoding regions, BKTT4 (5'-ACAAGGCCAAGATTCTAGGCTCGC-3') and BKTT10 (5'-CCAGGTTTTACCACTTTCACTGAAGC-3'), as well as BKTT2, were used as primers for DNA sequencing. For single-stranded PCR, 50 pmol of BKTT-1 and 0.5 pmol of BKTT-2 were used in a 100- μl reaction containing 2.5 U of *Taq* polymerase and 40 cycles of 1 min at 94°C , 2 min at 55°C , and 3 min at 72°C were performed by using a thermocycler (Ericomp, San Diego, Calif.). For double-stranded PCR, 50 pmol of each primer was used. Nucleotide sequencing by the dideoxynucleotide method was carried out by using Sequenase (United States Biochemicals Corp., Cleveland, Ohio). To eliminate sources of contamination, all reagents were made and stored in new disposable containers. The general precautions cited by Kwok and Higuchi (42) were followed. All reagents used were first assayed for the presence of BKV and JCV DNA, and all experiments were conducted in parallel with positive and negative controls.

Computer analysis of nucleotide sequences. DNA sequences were analyzed by using the Geneus (32) and UWGCG (version 6.0) (17) software packages on a VAX/VMS computer.

RESULTS

Identification of BKV-related DNA sequences in urine samples. A slot blot hybridization assay was used to screen urine samples for the presence of BKV and JCV. The sensitivity of this assay allowed detection of 0.1 to 0.2 pg of BK or JC viral DNA. No cross-hybridization was detected between BKV and JCV DNAs with the probes used (Fig. 1). The specificity was also evaluated by analyzing urine samples from four patients with a lower-urinary-tract infection. Significant bacteriuria ($\geq 10^5$ bacteria per ml), with *Escherichia coli*, *Staphylococcus saprophyticus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, was detected in these four urine samples.

FIG. 1. Detection of BKV DNA by slot blot hybridization with a 32 P-labeled BKV DNA subfragment probe (see Materials and Methods). (A) Twofold dilutions of BK (1) and JC (2) viral DNA standards (12.5 to 0.08 pg). No cross-hybridization was detected. (B) An autoradiogram of some of the urine samples. Samples yielding a clearly detectable signal after 48 h of exposure with no signal from the negative controls (JCV DNA and samples with significant bacteriuria) were scored as BKV positive.

No hybridization signals were observed in these urine samples (data not shown).

The results from screening urine samples for the presence of BKV DNA are presented in Table 1. Positive BKV DNA signals were detected in 39 urine samples from 30 patients. An example of slot blot hybridization signals is shown in Fig. 1.

Virus propagation and antigenic identification. Twenty-nine of 39 BKV DNA-containing urine samples were inoculated on Vero and HEK cell cultures. Slowly progressive CPE was observed in one or both cell types for a total of 19 urine samples during an observation period of 150 days. Mock-infected cell cultures were negative with regard to CPE throughout the observation period. The mean propagation time from inoculation until CPE was 45 days in HEK (32 to 78 days) and 72 days (32 to 136 days) in Vero cells. The difference is not significant ($P = 0.08$; Student paired t test done two sided). The prolonged incubation time was in accordance with other observations (14, 25, 26, 59, 65). No correlation between intensities of hybridization signals and propagation times in cell culture was observed. During passage in Vero and HEK cell cultures, all the viral isolates were antigenically identified as BKV by using immunoperoxidase staining with antibodies directed against BKV structural proteins and also with a monoclonal anti-simian virus 40 T-antigen antibody (data not shown).

Cloning and sequencing of the control region of BKV DNAs

TABLE 1. Detection of BKV DNA in 39 out of 335 urine samples compared to the detection of JCV and plasmid-related DNA by means of slot blot hybridization^a

No. of samples	JCV +		JCV -	
	Plasmid +	Plasmid -	Plasmid +	Plasmid -
BKV +, 39	4	5	0	30
BKV -, 296	1	2	9	284

^a+, Positive hybridization signal; -, negative hybridization signal.

FIG. 2. Physical and functional map of the BKV (Gardner) genome. (A) Thick lines represent the coding sequences for BKV proteins. The putative agnoprotein is omitted. The broken lines represent the spliced-out regions. The numbering system is that used by Seif et al. (55), with the first nucleotide in the start codon for large T and small t antigen as nucleotide number 5196. Restriction enzyme sites used for subcloning, *Bam*HI (B) and *Pst*I (P), are indicated. (B) The 748-bp fragment amplified by PCR with the primers BKTT1 and BKTT2 is shown. Restriction enzyme sites *Sac*I and *Hind*III used for subcloning are indicated (S and H, respectively). The location of the start codons for the T and t antigens and the putative agnogene are indicated by angled arrows. The locations and orientations of primers used for PCR are indicated by horizontal arrows. The location of the origin of replication (origin) and the sequence blocks of the transcriptional regulatory region, P and Q, are shown.

obtained from one passage in cell culture. Hirt supernatants (35) from cell cultures with CPE (isolates 1 through 9) were analyzed by agarose gel electrophoresis. Agarose gel bands corresponding in size to supercoiled BKV DNAs were excised, and the purified viral DNAs (isolates 1 through 9) were digested with *Hind*III, *Hind*III plus *Sac*I, and *Bam*HI plus *Pst*I and analyzed by Southern blotting. The numbers and sizes of fragments seen were exactly as expected from the sequence of the BK Dunlop genome (55) (data not shown). The 3.4-kilobase *Bam*HI-*Pst*I fragment of these isolates was then first cloned into pGEM-1 (Promega Biotech). The $\approx$ 500-bp *Sac*I-*Hind*III subfragment (Fig. 2) containing the control region was further subcloned into M13mp18 and M13mp19, and both strands of the inserts were sequenced. All nine isolates displayed the same new type of sequence organization of the control region. In order to link this viral isolate to its geographical origin, we named this strain BKV (TU) (Tromsø University). Eight of the nine isolates were identical in the sequence of the 494-bp *Sac*I-*Hind*III fragment, whereas isolate nine (named TUD) differed from the others by a 31-bp deletion located between the origin of replication and the start codon of the T and t antigens (Fig. 3).

PCR amplification and sequencing of BKV control region DNAs from urine samples and cell culture supernatants.

Table 2. The PCR products from all culture media consisted of BKV control regions with BKV (TU) sequences identical to the sequences of the cloned viral DNAs. However, three of the corresponding urine samples (isolates 4, 11, and 13) contained BKV with a WW control region anatomy. These three isolates differed from the WW strain reported by Rubinstein et al. (53) by five single nucleotide changes (Fig. 3). We named this type of strain BKV (WWT) (Tromsø). In addition to the partial duplication and deletion in the BKV (TU) control region sequence relative to BKV (WWT), there are two single-base differences between these two strains (Fig. 3). In one of the urine specimens, the PCR products also consisted of JCV control regions (isolate 13). Both BKV and JCV DNAs were detected by slot blot hybridization of this urine. Since the cell lines we used have not been successful for primary isolation of JCV, only BKV sequences were found in the cell culture medium.

TABLE 2. BKV strains with variant control region sequences identified directly from urine and after one passage in cell culture

Isolate	Propagation time (days) ^a		Control region type ^b		
	HEK	Vero	Urine PCR	Cell culture	
				PCR	Cloning
1	32	32	—	TU	TU
2	32	32	TU	TU	TU
3	38	92	—	TU	TU
4	42	104	WWT	TU	TU
5	55		—	TU	TU
6	58	92	—	TU	TU
7	32	33	TU	TU	TU
8	32	46	TU	TU	TU
9	33		—	TU	TUD ^c
11	78		WWT	TU	ND
13	47	65	WWT/JCV	TU	ND
14	35		TU	TU	ND

^a Time before development of CPE after inoculating cells with urine samples containing BKV DNA.

^b BKV control region type identified by PCR and sequencing directly from urine, by PCR and sequencing in cell culture medium, or by sequencing of BKV DNA cloned from Hirt supernatants of infected cells. ND, Not determined; —, negative result.

^c TUD contains a 31-bp deletion relative to TU.

The 31-bp deletion in the earlier leader sequence of isolate 9 (TUD), cloned from intracellular BKV DNA, was not present in the PCR product from culture media. Thus, the cloning, where only one clone was sequenced, may have picked up a rare deletion occurring in cell culture.

All urine samples yielding single-stranded PCR products for direct sequencing were first shown to give double-stranded PCR products, and these products were analyzed by diagnostic restriction enzyme digestions to see whether more than one control region variant was amplified (Fig. 4). Heterogeneous products were not obtained by either of the PCR procedures.

Coding sequences flanking the control region. With both our sequencing strategies, parts of the early and late polypeptide-coding regions were sequenced together with the control region (Fig. 2). These sequences were identical for all BKV strains that we analyzed [BKV (Gardner), BKV (TU) isolates, and three BKV (WWT) isolates] and were also identical to those published for other BK viruses (55, 60, 64, 65), with the exception of BKV (AS), which has a deletion in the late leader (60).

Comparative analysis of BKV control region sequences. The organization of the control regions of BKV strains WW, Gardner, TU, and MM is shown in Fig. 5. Except for a few point mutations, the region between the early initiation codon and the start of the P block is identical for the virus strains we have sequenced, with the exception that the cloned isolate 9 (TUD) has a 31-bp deletion in the early leader. Identical sequences have also been published for other BKV strains (55, 64, 65). Also, the late leader is identical for our strains, with the exception that BKV (WWT) has a point mutation relative to BKV (TU) and BKV (WW) (53) as shown in Fig. 3. Differences do, however, become evident when the sequences between the AT-rich region and the late leader are compared. BKV (TU) retains the P block and the Q block. The sequences are identical to the corresponding blocks in BKV (WW), except for two point mutations indicated in Fig. 3. The Q block is followed by the proximal 12 bp from the R block. The distal 12 R

FIG. 4. Analysis of double-stranded PCR products from the control region of different BKV strains by diagnostic restriction enzyme digestion. The PCR products were analyzed on a 2% agarose gel by using a 1-kb ladder (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) as molecular weight markers (lane 10). Lane 1, BKV (WWT) cut with *Pst*MI (421- and 272-bp fragments); lane 2, BKV (WWT) cut with *Hind*III (451 and 242 bp); lane 3, uncut BKV (WWT) (693 bp); lane 4, BKV (TU) cut with *Pst*MI (365, 272, and 105 bp); lane 5, BKV (TU) cut with *Hind*III (500 and 242 bp); lane 6, uncut BKV (TU) (742 bp); lane 7, BKV (Gardner) cut with *Pst*MI (390 and 358 bp); lane 8, BKV (Gardner) cut with *Hind*III (506 and 242 bp); lane 9, uncut BKV (Gardner) (748 bp). Because *Pst*MI almost always gives partial digestion, additional bands can be seen in lanes 1, 4, and 7. BKV (Gardner) was amplified from plasmid DNA, while BKV (TU) and BKV (WWT) were amplified directly from urine samples.

block base pairs are also present, while the central 39 bp are deleted and replaced by an insertion of 88 bp. The insertion consists of the 53 distal base pairs of a P block, the proximal 34 bp of a Q block, and an additional T between the R and P block. BKV (MM) (59, 64), although markedly different, seems to be the strain most similar to BKV (TU) (see Fig. 5).

Computer analysis revealed that consensus and near-consensus sequence motifs for binding of transcription factors are found throughout the control region of BKV strains. These results are shown in Fig. 3 and 5.

DISCUSSION

We have demonstrated that BKV strains with two different types of noncoding control regions are found in the urines of patients from Norway. Some patients excreted BKV with a control region which differed by only five point mutations from the proposed archetypal strain BKV (WW). This strain was designated BKV (WWT). Others excreted virus with an earlier unreported control region anatomy. We have named this strain BKV (TU). During one passage in Vero or HEK cells, BKV (WWT)-dominated urine populations were converted into BKV (TU)-dominated populations. BKV (TU)-dominated urine populations were not changed during passage in Vero or HEK cells. If BKV (WW) is considered the archetype (45, 53), TU may have evolved through partial duplications and deletions within the

FIG. 5. Comparison of the noncoding control regions of BKV (WW), BKV (Gardner), BKV (TU), and BKV (MM) with regard to P, Q, and R anatomy (45) and location of proven and potential transcription factor-binding sites. Single lines represent early-region DNA to the left and late-region DNA to the right. Putative binding sites for host cell proteins are indicated by numbers under single lines and in the different blocks (key: 1, ATTA [50]; 2, CACCC [49]; 3, CRE [40]; 4, ERE [63]; 5, GC box [18, 19]; 6, GRE-like [57]; 7, L1 [45]; 8, NF1 [45]; 9, Sp1 [40]; 10, TRE/TRE-like [40]). Numbers above the single lines and the blocks show the nucleotide numbers of the respective parts of the control regions. Short lines between blocks indicate additional nucleotides (1 to 3 nucleotides). The origin of replication is given (Or). All four BKV strains contain 119 nucleotides between the first nucleotide in the start codon for the T and t antigens and the A/T block. Furthermore, all four strains show a complete A/T block (20 bp) and a complete P block (68 bp). The MM strain has a deletion of the proximal 12 bp of the late leader sequence. Representative references for the different proven or putative factor-binding sites are indicated.

P-Q-R blocks, analogous to what has been proposed for other strains (45, 65). The exact timing of such events during the viral multiplication cycle is not known. It has been suggested, however, that rearrangements may arise as the result of unequal crossing over between daughter molecules after replication (65). The present studies demonstrate that rearrangements do not only occur during passage in cell culture. Viruses with rearranged control regions relative to BKV (WW) are also present within the human host. A particularly interesting point in this connection is presented by the three cases where urine inocula dominated by BKV (WWT) were turned into BKV (TU)-dominated populations during one cell culture passage. The question then arises whether both virus strains were present in the urine inocula or whether BKV (WWT) genomes were rearranged into BKV (TU) genomes during propagation in cell culture. We favor the former explanation, because the control region of the BKV (WWT) strains contained two point mutations relative to BKV (TU). If both strains were present in the urine inocula, it is possible that BKV (WWT) was present in large excess relative to BKV (TU) so that this strain was selectively amplified by PCR. Southern blot of PCR products cut with diagnostic restriction enzymes revealed only the BKV (WWT) variant, strongly suggesting that this control region was selectively amplified (data not shown). In cell culture, however, BKV (TU) is exclusively detected because it outgrows BKV (WWT) completely. In fact, hitherto there has been no report on successful propagation of BKV (WW) in cell culture. The presence of different control region variants in the urine from the same patient was also recently reported for another human polyomavirus, JCV (48). Cloning of one JCV variant from the brain and another variant from the kidneys of a patient with progressive multifocal leukoencephalopathy has previously been described (43). It is therefore not surprising that different BKV variants may also be present in the same patients as our results suggest.

In more general terms, our observations may indicate that naturally shed and transmitted BKV contains subpopulations with different control regions, permitting the viral species as such to adjust efficiently to the menu of transcription and replication factors offered by the host cells. The rearrangements and partial duplications and/or deletions of P-Q-R sequences seen upon comparison of various BKV strains have important implications for the number of putative factor-binding sites, as well as for the spacing between the sites (Fig. 5). In addition to different spacing between potentially important binding sites, the distances between them and transcription initiation sites are also affected.

A number of recent papers have addressed transcription control of BKV at the molecular level (7, 9, 28, 29, 46, 52, 62). However, studies of biological significance have mostly been confined to BK Gardner, and the cell types and culture conditions evaluated have had limited relevance to natural habitats for BKV. The transcription factors that have been focused upon are AP1, NF1, and Sp1. Indications have been given for all three as positive regulators of BKV transcription (7, 9, 19). For NF1, negative regulation is also suspected (7, 29). By deletion and linker scan mutant expression studies in reporter gene systems, other transcription factors have been implicated as important for transcription of BKV (7, 8, 18, 19). One of these is CREB (cAMP responsive element binding) factor (7). Recently, although the authors do not discuss it, direct proof for the *in vivo* importance of BKV CREs has been reported (58). A series of WW-like BKV variants, cloned directly from urine, were transfected into HEK cells. Upon analyzing the data of Sugimoto et al. (58), we find that the only variant for which HEK cells were reasonably permissive (cl51) differed from other similar variants that did not grow (cl97 and cl108) by the presence of a CRE. Indications of a positive effect of cAMP on both early and late transcription of BKV are accumulating also through other experimental approaches (U. Moens, A.

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Experiments designed to compare directly the biological characteristics of BKV (WW), BKV (TU), and BKV (Gardner) are in progress.

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