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# Molecular Genetics of the BK Virus

*Christopher L. Cubitt and Gerald L. Stoner*

Polyomavirus-associated nephropathy is a complication of kidney and bone marrow transplantations arising most commonly, if not exclusively, from reactivation of latent BK virus (BKV) infection in the kidney. The molecular genetics of BKV is consonant with that derived from studies of the highly homologous polyomaviruses, JC virus and SV40. Genomic sequencing of BKV provides information on naturally occurring sequence variations, on the rearrangement state of the viral regulatory region, as well as on the predicted serotype of BK viral strains. Mutations within coding regions of BKV and other polyomaviruses might be associated with altered disease incidence and/or progression. Viral regulatory region rearrangements in the kidney consisting of deletions and duplications are frequently associated with BKV-induced disease, while the transmissible form of BKV excreted in the urine contains the non-rearranged archetypal form of the regulatory region. In this review, we focus on DNA sequence determination of viral genetic elements that might influence BKV-associated disease.

BK virus (BKV) is a polyomavirus that is widespread in the human population and infects multiple tissues, including the kidneys, where it usually remains in an asymptomatic state for the life of the host. More than 80% of the world population is believed to be infected with the virus based on serological studies. BKV spread occurs human to human. In the context of immunosuppression, the virus has been found largely responsible for renal stenosis<sup>1</sup> and interstitial nephritis in kidney transplant patients (BK nephropathy, BKN) and hemorrhagic cystitis in bone marrow transplant patients.<sup>2</sup>

## Genomic Structure

Much of our knowledge pertaining to the molecular biology of BKV has been obtained from the two most studied polyomaviruses, SV40 and JC virus (JCV). BKV shares a 75% sequence identity with JCV and a 69% identity with SV40.<sup>3,4</sup> Like SV40 and JCV, the genome of BKV is a closed circular, double-stranded molecule of DNA approximately 5 kb in size. The relatedness of BKV with SV40 and JCV is clearly evident in their genomic organization (Fig. 1). The genome of BKV consists of the genetically conserved coding region and the

hypervariable 300 to 500 bp noncoding regulatory region (NCRR). The coding region is functionally divided between the early and late gene coding regions. The early genes that encode the large T (TAg) and small t antigens (tAg) are expressed soon after infection of the host cell. As for SV40 and other polyomaviruses, TAg is a multifunctional transactivating factor necessary for regulating the transcription and replication of the viral genome (reviewed by Moens and Rekvig<sup>5</sup>). TAg autoregulates its own transcription<sup>6</sup> and is largely responsible for the cell-transforming potential of BKV.<sup>7</sup> TAg and tAg are differentially translated by alternative splicing of the early mRNA transcript. Removal of the TAg intron splices the first exon with the next exon, allowing translation of TAg. Alternatively, retention of the intron allows translation to reach a termination codon within the intron, resulting in tAg. The late genes, consisting of the structural proteins VP1, VP2, and VP3 and the agnoprotein genes, are predominately expressed after genomic replication has been initiated. VP2 and VP3 share a coding sequence that is translated from the late transcript in the same reading frame, and the VP1 gene is translated in a separate reading frame (Fig. 1).

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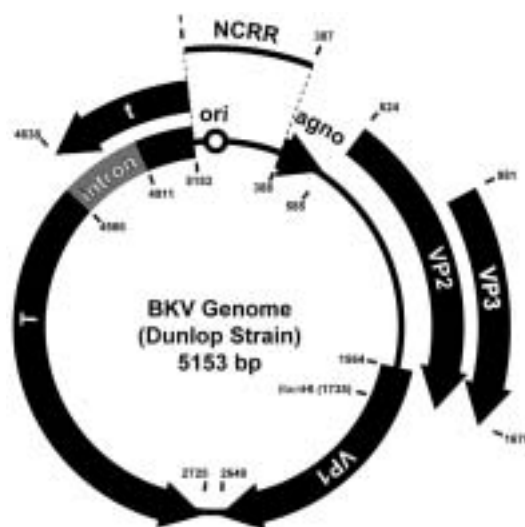


Figure 1. Genomic map of BK virus Dunlop strain. The BKV genome is a closed circular, double-stranded DNA molecule ~5 kb in size. The coding regions for the early genes, large and small T antigens (T and t), are transcribed in a counterclockwise direction, and the late genes, agnoprotein (agno) and VP1 through VP3, are transcribed in a clockwise direction. The non-coding regulatory region (NCCR) is ~387 bp and includes the origin of replication (ori). Genomes of BKV contain a unique BamH1 site located in VP1 that is useful for whole-genome cloning.

### The NCCR of BKV and Its Rearrangements

Within the NCCR is the origin of replication as well as the binding sites for numerous regulatory factors involved in transcription and replication. Replication begins at ori (Dunlop strain sequence <sup>97</sup>GAGGCA GAGGCG GCCTCG GCCTC<sup>119</sup>) in both directions and is completed when the replication forks meet on the opposite side of the genome (Fig. 1).

The first NCCR DNA sequences of the BKV genome were obtained from virus preparations derived from urine and brain samples and propagated in cell cultures.<sup>8</sup> Because these virus stocks were cultured in the laboratory prior to DNA sequence analysis, the NCCR contained various deletions and duplications that may have arisen during adaptation to growth in vitro. It was not until cloning techniques and direct PCR amplification became available for amplifying the NCCR from urine samples that the nonrearranged form of the NCCR was discovered.<sup>9-11</sup> It is this prerearranged form

### Key Points

- The BK virus (BKV) genome is a double-stranded, circular DNA molecule with genetic organization similar to other polyomaviruses and high homology to the JC virus (JCV) and SV40.
- The archetypal form of the BKV noncoding regulatory region (NCCR) is predominant in the urine. Rearranged forms of the NCCR are found in kidney and other tissues, often in association with disease.
- BKV strains can be assigned to genotype/serotype groups based on sequence variation in the VP1 gene. Sequencing of the complete genome will enhance phylogenetic studies.
- Genotypic differences and naturally occurring mutations in BKV may correlate with the incidence and/or severity of a disease.
- Success in human polyomavirus research will require that BKV and JCV each be considered in relation to the other.

(now termed the *archetype*) of the NCCR that predominates in the urine<sup>11-14</sup> and therefore is the transmissible or infectious form of the virus. A similar pattern has been found for JCV. When other tissues, such as the brain and kidneys, are screened for polyomavirus infection, JCV- and BKV-rearranged NCCR sequences predominate. It remains unclear how the archetype form of BKV is preferentially secreted in the urine, even though both rearranged and archetype sequences can be found in the BKN kidney. This differential secretion pattern could be explained if the archetype form actively replicates in the tubular epithelial cells and rearranged forms are anatomically restricted to other parts of the kidney.

The archetype NCCR has been arbitrarily divided into regions O (142 bp), P (68 bp), Q (39 bp), and R (63 bp), based on the origin and regulatory binding sites, and region S (63 bp), the late leader sequence.<sup>14,15</sup> This scheme helps in visualizing the

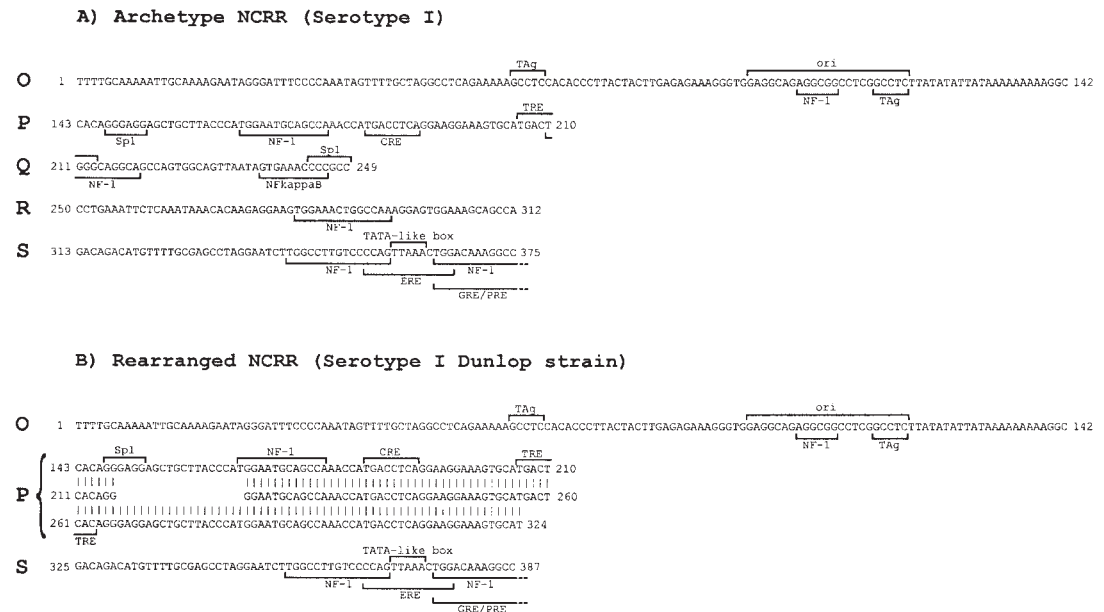


Figure 2. Comparison of archetype and rearranged noncoding regulatory region (NCRR) sequences. (A) Sequence of an archetypal serotype I strain (375 bp length). Putative and proven binding sites for TAg and cellular transactivating factors are identified with brackets over or underneath the sequence. The structure is divided into regions O, P, Q, R, and S, based on the boundaries of the duplications and deletions found in the rearranged form of BKV NCRR. (B) Sequence of a rearranged NCRR. The NCRR (387 bp length) of the Dunlop strain of BKV (serotype I) is shown as an example of a rearranged regulatory region. Regions Q and R are deleted in this strain. The P region is repeated twice with an 18-bp deletion in the first repeat and a 4-bp deletion at the 3' end of the second repeat. Vertical lines represent sequence homology due to sequence duplication.

archetype NCRR and its rearrangements. Figure 2 compares the NCRR from a rearranged strain originally isolated from peripheral blood from an immunocompetent individual (Dunlop strain, NCBI accession: PVBDUN) to a typical serotype I archetype sequence amplified from the urine of a renal transplant patient (Cubitt et al., manuscript in preparation). The archetype contains the full linear complement of regions O, P, Q, R, and S. The rearrangement of the NCRR found in the Dunlop strain is designated O<sub>142</sub>-P<sub>68</sub>-P<sub>50</sub>-P<sub>64</sub>-S<sub>63</sub>, as demonstrated in Figure 2. The P region is triplicated, and the entire Q and R regions are deleted. Rearrangement of most naturally occurring NCRRs involves duplication or triplication of the P region, including portions of the neighboring O and Q regions. Likewise, deletions are found to occur anywhere within the P, Q, R, or S regions, but frequently the deletion includes all or part of region R.

Both enhancement and suppression of transcription occur through binding of cellular transactivat-

ing factors. Jun/AP1 binding to the TRE element located at the P-Q junction has been shown to have a role in regulating both early and late transcription.<sup>15</sup> Moens et al.<sup>16</sup> have identified GRE/PRE binding sites in the NCRR S region, which may confer regulation of transcription and replication through the presence of the steroidal hormones such as progesterone or estrogen (Fig. 2).

The mechanism and functional significance of BKV regulatory rearrangements remain unknown. However, it is believed that recombination occurs between the two newly synthesized daughter strands at nonhomologous points during replication (reviewed by Yoshiike and Takemoto<sup>17</sup>). It is known that archetype forms of BKV replicate poorly in cell cultures, and NCRR rearrangements are necessary for efficient replication in vitro, suggesting that NCRR rearrangement are an adaptation of the virus for growth in diverse cell types. Rearrangement of the NCRR can delete or add a number of binding sites for transcription factors.

Table 1 | REPRESENTATIVE TYPING SITES FOR BKV

SEROLOGICAL GROUPS (STRAINS)	1760	1766	1767	1768	1769	1770	1775	1780	1784	1787	1792	1793	1794	1807	1809	1811	1824
I (DUN)	T	T	A	A	A	G	G	G	A	A	A	G	C	G	G	G	C
I (Yale)	T	T	A	A	A	G	G	G	A	A	A	G	C	G	A	G	C
I (PT)	T	T	A	A	A	G	G	G	A	A	A	G	C	G	G	G	C
I (MM)	T	T	A	A	A	G	G	G	A	A	A	G	C	G	G	G	C
I (GS)	T	T	A	A	A	G	G	G	A	A	A	G	C	G	G	G	C
II (SB)	A	T	A	A	A	G	C	G	A	C	G	A	C	G	C	A	T
III (AS)	A	A	G	C	A	C	G	G	A	C	G	A	G	G	C	G	T
IV (IV)	A	T	A	A	G	A	C	G	C	C	G	A	G	G	C	G	C
IV (MG)	A	T	A	A	G	A	C	G	C	C	G	A	G	G	C	G	—

For example, the triplication of the P region in Dunlop adds two more copies of the NF1 and CRE binding sites (Fig. 1). Kraus et al.<sup>18</sup> have shown that binding of NF1 proteins to NF1 binding sites in the NCRR results in repression of the BKV major late promoter when the template copy number is low and is relieved when the template copy number is high. It is possible that, through triplication of the NF1 binding sites, free NF1 proteins are bound, reducing local free NF1 concentrations and effectively mitigating NF1 repression of transcription.

Several clinical observations have demonstrated NCRR rearrangements that occur in association with disease. Boldorini et al.<sup>19</sup> have identified both archetype and rearranged forms of BKV in renal biopsies taken from patients suspected of kidney allograft rejection. We have identified BKV DNA in a leukemia patient with tubulointerstitial nephritis whose BKV disease led to meningoencephalitis.<sup>20</sup> The viral regulatory region in the polymerase chain reaction (PCR)-amplified urine sample was of the archetype form and was identical to the WWT strain. In the kidney, several NCRR rearrangements were found that involved duplication followed by sequence deletion. However, in the brain and cerebrospinal fluid (CSF), a single rearranged sequence dominated that contained a 94-bp deletion within each copy of a 71-bp tandem duplication, indicating the deletion preceded the duplication. Only one clone of eight sequenced from the brain and CSF showed a rearrangement that differed from that of the direct PCR product. No archetype sequences were identified within the brain or CSF. A review of the BKV literature gives the impression

that the NCRR of both BKV and JCV is progressively more rearranged with the progression of disease. Exactly which tissue(s) BKV rearrangement occurs in remains unknown, but recent evidence by Chatterjee et al.<sup>21</sup> suggests that rearrangement may occur outside the kidney. In that study, rearranged forms as well as the archetype form of BKV were found in peripheral blood leukocytes (PBLs) from healthy individuals.

### Genotyping BKV

Currently, there are four known serotypes of BKV in the human population. Among the four serotypes, there is nearly a 5% difference in sequence identity. Sequence variation within the VP1 gene accounts for the antigenic differences of the BKV serotypes.<sup>22</sup> Jin et al.<sup>22,23</sup> have developed a method for determining the serotype of a BKV isolate based on the sequence and type-specific restriction of endonuclease sites of a 327-bp PCR-amplified region of VP1. VP1 sites that can be used to distinguish BKV genotypes are shown in Table 1. Additional reliability in genotyping data could be achieved by using sequence data from the hypervariable NCRR and the noncoding region between the 3' ends of the VP1 and T-antigen genes (V-T intergenic region) to confirm the genotype (and therefore the serotype) of clinical BKV isolates. Sequencing these two regions has proven to be invaluable for genotyping JCV and is likely to be helpful for typing and subtyping BKV. It is interesting to note that there is only one serotype for JCV. This absence of multiple serotypes may be explained by the more highly conserved DNA sequence in JCV (approximately 1%–2% difference between the major geno-

types of JCV<sup>24</sup>). As progress is made in surveying BKV strains in the population, full genome sequence comparisons will be required for making accurate genotype assignments and for understanding the evolutionary relationships between them. Currently, there are only three BKV strains with full genome sequences available: AS<sup>25</sup> (NCBI accession: M23122), Dunlop<sup>8</sup> (NCBI accession: PVBDUN), and MM<sup>3</sup> (NCBI accession: M23122).

Although considerable amounts of sequence data from the regulatory region can be found in the BKV literature, relatively little VP1 sequence data allowing genotype assignments have been published. A study by Jin et al.<sup>26</sup> found that out of 41 BKV isolates, BKV type I (63.4%) was the most frequently found genotype, followed by types II (21.9%), IV (9.8%), and III (4.9%). Interestingly, in this study, 6 out of 11 bone marrow transplant patients had type II BKV, followed by 3 of type I and 2 of type IV BKV. Baksh et al.<sup>27</sup> identified BKV genotypes in the kidney tissues of 17 viral interstitial nephritis patients at the University of Pittsburgh Medical Center. Of these patients, 11 were genotype I, 1 was type II, and 5 were type IV based on the VP1 sequence.

### Association of Mutations and Genotypes with Disease

The sequencing and genotyping of BKV in the urine of patients with a polyoma-associated disease or healthy populations has the potential to provide information on type-specific disease incidence and prognosis. Several lines of evidence suggest that naturally occurring mutations in any of the BKV structural or regulatory proteins may enhance the ability of the virus to cause disease. Sequence variations may change the replication characteristics of the virus, allowing the virus to grow unchecked in other organs and tissues, or may help the virus to evade the host immune response. Bauer et al.<sup>28</sup> have found that a single amino acid substitution in VP1 can affect binding of the LID strain of the polyomavirus to its receptor and shorten the time to lethality by more than threefold in infected newborn C3H/Bi mice.

Koralnik et al.<sup>29</sup> have identified a JCV VP1 epitope (amino acid sequence ILMWEAVTL) recognized by cytotoxic T lymphocytes of progressive multifocal leukoencephalopathy (PML) patients.

This epitope is semiconserved in BKV (sequence LLMWEAVTV). Although this VP1 sequence has not been identified as an important epitope in BKV-associated kidney diseases, it is conceivable that mutations in this region could alter the ability of the immune response to contain the BKV infection.

Genotyping of JCV DNA in the brain of PML patients and urine of a non-PML control group in the United States has suggested a correlation between genotype and risk of disease.<sup>30</sup> It was found that 19 out of 53 PML patients had JCV type 2B, compared to a matched control group in which only 7 out of 119 people carried the type 2B DNA sequence. This translates into an approximately threefold higher risk of developing PML for those with a type 2B strain of JCV. One possible explanation for the observed differences in PML incidence is an amino acid change in TAg of Leu<sub>301</sub> to Gln two residues away from the first coordinating cysteine in a DNA-binding zinc finger.

A potentially more virulent strain of BKV (BKV(Cin)) has been identified in a AIDS patient in Cincinnati with severe tubulointerstitial nephritis.<sup>31</sup> BKV DNA was PCR amplified from the kidney of this patient and cloned. The sequenced NCRR of all clones were rearranged, the majority of which contained a 48-bp deletion and a 41-bp duplication. Sequencing of the TAg gene revealed a previously unidentified dinucleotide mutation (TG to AA) that would result in a predicted amino acid change of a hydrophilic Gln<sub>169</sub> to the hydrophobic Leu (the corresponding position is Ala in SV40). Based on SV40, this residue lies within the alpha-helical DNA-binding domain of TAg.<sup>32</sup> Because Leu, Glu, Met, and Ala are known to be strong helix formers, this amino acid mutation could change the dynamics of protein folding or the DNA-binding affinity of TAg. It is possible that a combination of both coding region mutations and regulatory region rearrangements, as found in BKV(Cin), are important virological factors contributing to the pathogenicity of this and other BKV strains in the immunocompromised host. It is unknown whether this fatally virulent strain of BKV was generated anew in this patient or is circulating in the population. However, the same TAg mutation has recently been found in the urine of a renal transplant patient in conjunction with an archetypal regulatory region.<sup>27</sup>

## Conclusion

The first description of an archetypal regulatory region sequence in a polyomavirus can be credited to Rubinstein et al.<sup>13</sup> They described the regulatory region of BKV obtained from urine and designated the structure the WW strain. The existence of an archetypal regulatory region in urinary JCV was first described by Yogo et al.,<sup>33</sup> and the variant forms found were designated MY and CY. These genotypes were subsequently redefined on the basis of their coding regions. Numerous studies of the rearranged regulatory region in the PML brain have confirmed that these structures are always constructed in a way that suggests derivation from the archetypal structure.<sup>34-39</sup> It now appears likely that the pattern in diseased tissue in BKV infection is much the same as for JCV. Individuals infected by strains with archetypal regulatory region sequences rearrange these in unknown tissues, apparently allowing selection of a more pathogenic viral variant. The progressive and pathogenic tissue infection occurring in PML or BKN is characterized by one or more extensive but localized regulatory rearrangements showing deletion and duplication of DNA sequence. These rearranged forms represent a dead-end infection. In neither virus do these rearrangements follow an easily identified pattern, in that no DNA sequence elements are regularly deleted or always duplicated.<sup>34</sup> However, it appears that the deletion step frequently precedes duplication, as the same deletion is often noted in both parts of a duplicated segment.

What can investigators of BKV pathogenesis in the kidney and other organs learn from the findings in the JCV field? Despite many studies, key questions remain unanswered. Nevertheless, five general principles regarding JCV rearrangements have emerged and are presented here as formulated in a review by Yogo and Sugimoto:<sup>40</sup>

1. JCV with the archetype regulatory sequence is circulating in the human population.
2. The archetype regulatory sequence is highly conserved, in marked contrast to the hyper-variable regulatory sequences of PML-derived isolates.
3. Each of the PML-type regulatory sequences is produced from the archetype by deletion and duplication or by deletion alone.
4. The shift of the regulatory region from archetype to PML type occurs during persistence in the host.
5. PML-type JCVs never return to the human population.

We suggest that analogous conclusions can be reached for BKV, for which rearrangement was originally thought to be merely an artifact of *in vitro* virus culture.

In the case of JCV, the original PML brain isolate, known as Mad-1,<sup>41</sup> was found to have 98-bp repeats,<sup>42</sup> which were assumed to play a major role in the biology of the virus. However, these were absent from the archetypal regulatory region described by Yogo et al.<sup>33</sup> and have never been found in urine. Nor has the identical Mad-1 structure been obtained from another PML brain. This has raised questions about the distribution and significance of this prototype strain. On the other hand, the availability of both Mad-1 and the closely related Mad-4 from the American Type Culture Collection (ATCC) means that most labs studying JCV are contaminated with one or both of these strains, and tissue searches for JCV using supersensitive PCR methods can easily be confounded by laboratory artifact. Reports of JCV Mad-1 or Mad-4 in human tumor tissues may be no more reliable than sightings of Bigfoot in the Pacific Northwest! To obviate this problem, we have suggested the use of a type 8 clone (pJCPNG-Ag, ATCC no. VRMC-24) obtained in Papua New Guinea.<sup>43</sup> DNA sequencing of the PCR product from reactions including this strain as a positive control can easily differentiate this laboratory strain from European, African, or other Asian genotypes likely to be present in infected or tumor tissues. Type 8 is unlikely to be found outside of Papua New Guinea and Melanesia in the Southwest Pacific. If similarly exotic strains of BKV can be identified for laboratory use, they would also be useful for ensuring the significance of BKV sequences amplified from infected or tumorous tissues and transformed cells.

The discoveries of BKV and JCV were originally reported in back-to-back articles in *Lancet*.<sup>44</sup> These closely related persistent human viruses have much to contribute to each other for a fuller understanding of their potential for disease. Whether

JCV contributes directly to polyomavirus nephropathy after transplantation is a different question that remains to be fully explored.<sup>45</sup>

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