NOTES

Decreasing the Number of 68-Base-Pair Tandem Repeats in the BK Virus Transcriptional Control Region Reduces Plaque Size and Enhances Transforming Capacity

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Wild-type BK virus, which has three 68-base-pair (bp) elements tandemly repeated in its transcriptional control region, forms clear, large plaques in human embryonic kidney cell cultures but rarely transforms hamster or rat cells. From this BK virus DNA, deletion mutants with fewer than three 68-bp elements were constructed and characterized. The mutant DNA with two 68-bp elements formed small, turbid plaques in human cell cultures and transformed rat 3Y1 cells inefficiently. The mutant DNA with one 68-bp element formed minute, turbid plaques in human cell cultures and transformed rat cells efficiently.

The structure of the transcriptional control region of BK virus (BKV) affects the productive infection of human cells and the transformation of hamster or rat cells. The wild-type BKV (wt-501, a plaque isolate from the Gardner prototype strain), which has three 68-base-pair (bp) tandem repeats in its transcriptional control region (8, 13), grows well and forms clear, large plaques in human embryonic kidney (HEK) cells but rarely transforms hamster or rat cells in cultures (10). By contrast, a plaque morphology mutant (pm-522) rescued from a hamster pineocytoma (11) is highly tumorigenic (7, 11) and efficiently transforms hamster or rat cells (10), although it grows somewhat less efficiently and forms small, turbid plaques in HEK cell cultures. Our previous studies (8, 10) have shown that the DNA rearrangement (deletions and duplications) that occurred in the pm-522 control region are responsible for the distinct biological characteristics of the mutant and that the pm-522 control region contains only one complete 68-bp element and shorter 37-bp repeats. Thus, it seems that the inability of BKV wt-501 to transform hamster or rat cells is due to the characteristic structure of its control region containing triplication of the 68-bp elements.

In the present study, we compared the biological activities in human and rat cells of deletion mutants with one and two 68-bp elements constructed by removing these elements from BKV wt-501 DNA (Fig. 1). For construction of the mutant with two 68-bp elements, plasmid pBK501, consisting of pBR322 and BKV wt-501 DNA ligated at their BamHI sites, was partially digested with MstII (New England BioLabs, Inc., Beverly, Mass.), which cleaves wild-type BKV DNA at four sites, one between the BamHI and EcoRI sites and three each within a 68-bp repeat, but does not cleave pBR322 DNA. The partially digested plasmids were used to transfect Escherichia coli HB101 after ligation with T4 ligase (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). The clones thus obtained were screened for plasmid size, and an appropriate clone, pBK503 (Fig. 1), was selected. For construction of the mutant with one 68-bp element, pBK501 was completely digested with MstII, and the two longer fragments were isolated by preparative agarose gel electrophoresis. The isolated fragments were ligated and used for transfection of strain HB101. The clones thus obtained were screened for the orientation of the two fragments, and one clone, pBK504 (Fig. 1), was selected for

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FIG. 1. Construction of BKV deletion mutants by digestion with MstII. The structure at the top represents the noncoding region near the DNA replication origin of the wild type (wt-501, a plaque isolate of the Gardner strain). The nucleotide sequence of the wt-501 noncoding region (8) is identical with that of the Gardner strain, the sequence of which has been partially determined (13). Numbering was as described previously (8), and nucleotide number 0 is cytosine at the center of the symmetrical region of the DNA replication origin. l and II refer to T-antigen-binding sites. The larger rectangles represent 68-bp repeating elements. The middle one of the three has a deletion of 18 bp. Symbols: ▲, 5'CCTCC3' (putative promoter core); ▲, 5'TGGA(T)A(T)A(T)3' (simian virus 40 enhancer core [12]) in two orientations; ▲, 5'AGGAATTGAA3' (adenovirus E1A enhancer core [3]) in the anti-sense orientation. MstII cleavage sites (5'CC/TNAGG3') within 68-bp repeats are indicated by vertical arrows. BKV DNA has another MstII site (not shown) near the EcoRI site. Deletions generated by MstII digestion and ligation (see text) are indicated by broken lines.
further characterization. The recombinant DNAs, pBK501, pBK503, and pBK504, were digested with BamHI (Bethesda Research Laboratories) to release viral genomes (wt-501, dl-503, and dl-504) and with HpaII (Bethesda Research Laboratories) to digest pBR322 at 26 sites for biological assays. The recombinant DNA pBK522 (containing the pm-522 genome that can efficiently transform rat cells (10)) was included in the study for comparison. The methods used for DNA preparation and molecular cloning were as described previously (10). For transfection, the DEAE-dextran (5) and calcium phosphate (2) methods were used for HEK and rat 3Y1 cells, respectively, as described previously (10).

Table 1 summarizes the results of the biological characterization of the mutants constructed. In HEK cell cultures, all of the DNAs induced production of BKV-specific T antigen as determined by staining with anti-T hamster serum and examination by indirect immunofluorescence 3 days after infection. The specific plaque-forming activity of dl-504 DNA was the lowest among those of the mutants and was about 1/10 that of the others. The average size of the plaques formed by the DNA in HEK cell cultures decreased from large to small to minute as the number of 68-bp repeats was reduced from three to one. When one of the repeats was removed, the plaques became turbid as examined on days 17 and 18 (turbid plaques eventually became clear on later days). Thus, reduction of the number of 68-bp repeats seemed to slow virus growth and impair the functions required to kill infected human cells. On the other hand, BKV DNA could efficiently express its transforming functions when two of the three 68-bp elements were removed from the transcriptional control region.

The data obtained in this study indicate that the BKV promoter-enhancer functions differently in human and rat cells. Although a single 68-bp element contains sequences resembling those in the simian virus 40 promoter (5'CCTCCC' of BKV and 5'CGGCC' of simian virus 40 [1, 8]), the simian virus 40 enhancer core (5'TGA[T][T]A[T][T][T]3' [12]), and the adenovirus type 5 E1A enhancer core (5'AGGAAGTAA3') [3], multiple 68-bp repeats seem to be required for BKV to be fully viable in human cells. The best structure for human cells, which probably evolved from a polyomavirus ancestor during adaptation to humans (6), was not necessarily efficient in rat cells. Interestingly, the structure least efficient in human cells (dl-504) was the most effective for transformation of rat cells.

BKV dl-504, which grew inefficiently in human cells, was unstable in human cells. HEK cell cultures transfected with BamHI-cleaved pBK501, pBK503, pBK504, and pBK522 (at 1 µg of DNA per 25-cm² culture) showed complete cytopathic effects ( detachment from plastic surface) on days 14, 16, 25, and 16, respectively, after infection. The virus stocks thus obtained were used to infect HEK cultures from which viral DNA was selectively extracted (4). Purified viral DNAs and parental plasmid DNAs were digested with HindIII (Bethesda Research Laboratories) and subjected to agarose gel electrophoresis (Fig. 2). Whereas HindIII C fragments from wild-type BKV, dl-503, and pm-522 were unchanged during transfection and passage (lanes 1 to 4, 7, and 8), HindIII-C of dl-504 became heterogeneous, as indicated by a less sharp band, and longer than that of the parental plasmid (lanes 5 and 6). The data indicate that DNA rearrangement occurred in HindIII-C of dl-504, probably in the transcriptional control region (8). Similar instability of HindIII-C has been observed with a naturally occurring viable deletion mutant (pm-526) from which various mutants had evolved (manuscript in preparation).

Expression of viral early functions in rat cells has been compared between nontransforming wild-type BKV (wt-501) and transforming pm-522 (9). The study has shown that production of T antigen continues in BKV pm-522 infection but declines in wild-type infection. This functional difference between the two viruses during the abortive infection seems to account for the difference in transforming capacity. Whether dl-504 behaves like pm-522 in rat cells and how T-antigen production declines in wild-type BKV-infected rat cells remains to be investigated. Comparative studies of these mutants and wild-type BKV may elucidate the role of the BKV transcriptional control region in the transformation of rodent cells by BKV.

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**TABLE 1. Biological activities of constructed BKV deletion mutant DNAs**

<table>
<thead>
<tr>
<th>Viral DNA</th>
<th>No. of 68-bp repeats</th>
<th>HEK cells</th>
<th>Transformation of rat 3Y1 cells (foci/µg of DNA)</th>
<th>Transformation of rat 3Y1 cells (foci/µg of DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plaque/ µg of DNA</td>
<td>Plaque size (mm) and morphology</td>
<td>1</td>
</tr>
<tr>
<td>wt-501</td>
<td>3</td>
<td>2 × 10³</td>
<td>2-7; clear</td>
<td>0</td>
</tr>
<tr>
<td>dl-503</td>
<td>2</td>
<td>9 × 10³</td>
<td>1-5; turbid</td>
<td>0</td>
</tr>
<tr>
<td>dl-504</td>
<td>1</td>
<td>7 × 10³</td>
<td>1-2; turbid</td>
<td>161</td>
</tr>
<tr>
<td>pm-522</td>
<td>1</td>
<td>10³</td>
<td>1-5; turbid</td>
<td>22</td>
</tr>
</tbody>
</table>

* Recombinant plasmids (pBR322-BKV) were digested with BamHI and HpaII to release viral genomes before biological assay.

* HEK cell cultures were infected with DNA by the DEAE-dextran method (5), and plaques were counted on days 16 to 18.

* Plaque size (millimeters in diameter) was recorded 17 and 18 days after inoculation.

* The transformation assay was as described previously for virions (10). Rat cells were transfected with DNA by the calcium phosphate method (2). Foci of transformed cells were stained and counted 25 days after infection. The number of foci per microgram of DNA was the average of three to six dishes. In experiment 3, DNA was purified by electrophoresis. A few colonies from the deletion mutants were isolated and examined by indirect immunofluorescence. All of the cell clones were positive for T antigen.

* The central element of the triplicated 68-bp repeats has a deletion of 18 bp (8).

* The structure of the pm-522 transcriptional control region has been determined (8).

FIG. 2. Cleavage of BKV deletion mutant DNAs with HindIII. Recombinant plasmids (pBR322-BKV) and virion DNAs obtained by transfection with viral DNAs released from recombinants were digested with HindIII and electrophoresed in a 1.6% agarose gel in E buffer (pH 7.8) at 100 V for 2 h. C and D refer to the positions for HindIII fragments C and D of wild-type BKV. Lanes: 1, pBK501 DNA; 2, wt-501 virion DNA; 3, pBK503 DNA; 4, dl-503 virion DNA; 5, pBK504 DNA; 6, dl-504 virion DNA; 7, pBK522 DNA; 8, pm-522 virion DNA.
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LITERATURE CITED


