Sequence Reiteration Required for the Efficient Growth of BK Virus

By KAZUYA HARA,1 YOSHIKAI OYA,1 HISASHI KINOSHITA,1 FUMIAKI TAGUCHI1 AND YOSHIKAI YOGO2.*

1Department of Microbiology, School of Hygienic Sciences, Kitasato University, Sagamihara, Kanagawa 228 and 2 Department of Viral Infection, The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 108, Japan

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SUMMARY

Compared with wild-type BK virus DNA having tandem triplication of a 68 base pair (bp) element in its transcriptional control region, a mutant viral DNA with a single copy of the 68 bp element induced remarkably delayed virus production in human embryonic kidney (HEK) cells. We molecularly cloned the DNA of progeny viruses using plasmid vector pAT153. Nucleotide sequence analysis of representative clones revealed that all of the altered viral DNAs examined duplicated various segments extending over origin-distal portions of the 68 bp element and their flanking regions. After transfection of HEK cells, most of these rearranged viral DNAs induced viral growth slightly slower than, or at the same rate as, the wild-type viral DNA. Comparison of the structures of these rearranged viral DNAs suggests that reiteration of a 13 bp sequence, which is located in an origin-distal portion of the 68 bp element, is required for the efficient replication of BK virus.

Triplicated tandem repeats of a 68 base pair (bp) sequence (the central copy is lacking an 18 bp sequence) (Seif et al., 1979; Yang & Wu, 1979) which has enhancer activity for transcription (Rosenthal et al., 1983) are located to the late side of the origin of replication on the BK virus (BKV) genome. Recently, Watanabe & Yoshiike (1985) have shown that multiplication of the 68 bp element is required for productive infection of BKV in human embryonic kidney (HEK) cells. BKV DNA with one copy of the 68 bp element, although able to induce expression of T-antigen, generates minute, turbid plaques less efficiently than does wild-type BKV DNA (Watanabe & Yoshiike, 1985). The viruses produced from this viral DNA appeared to be unstable and readily gave rise to altered viruses with DNA rearrangement in a segment containing the transcriptional control region (Watanabe & Yoshiike, 1985).

In the present study, we molecularly cloned the DNAs of viruses produced from the viral DNA with a single copy of the 68 bp element, before and after the second viral passage in HEK cells. Sequence analysis of the cloned BKV DNAs and their ability to induce a rapid infection has suggested that reiteration of a 13 bp sequence located in an origin-distal portion of the 68 bp element is essential for the efficient growth of BKV in HEK cells.

The method for preparation of cells from HEK was described previously (Uchida et al., 1976). The cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% foetal bovine serum. The wild-type BKV DNA (BK8001) and a deletion mutant DNA containing only one copy of the 68 bp element (BK8326) (Hara et al., 1985) were introduced into HEK cells by the calcium phosphate co-precipitation procedure (Graham & van der Eb, 1973). The BK8001-transfected culture rapidly developed cytopathic changes and the monolayer was severely affected by 13 days after transfection. By contrast, in the BK8326-transfected culture cytopathic changes were not obvious before day 13 but gradually developed throughout the culture and almost reached confluence by day 24. BKV haemagglutinin (HA) of titres 1:128 and 1:32 was

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detected in the supernatant fluids from the BK8001- and BK8326-transfected cultures, respectively, that showed maximum spread of c.p.e. In three independent experiments, delayed cytopathic changes and virus production were repeatedly observed in the BK8326-transfected cultures.

To examine whether new viruses capable of rapid growth had emerged after transfection with BK8326, we diluted the supernatant fluids obtained from the BK8001- and BK8326-transfected cultures showing maximum c.p.e. and used them to infect HEK cells at the same low input m.o.i. The two infected cultures rapidly developed a c.p.e.; the monolayers were completely destroyed on days 11 and 12 after infection with the supernatants from the BK8001- and BK8326-transfected cultures, respectively. BKV HA titres were equally high (1:256) in both cultures. These results imply that new viruses with efficient growth potential were generated after transfection of HEK cells with the viral DNA carrying a single copy of the 68 bp element.

The supernatant fluid of the BK8326-transfected culture that showed a significant HA activity was digested with proteinase K, treated with phenol and chloroform–isoamyl alcohol (24:1), and subjected to the ethidium bromide–CsCl equilibrium centrifugation. The form I DNA was collected, cleaved with EcoRI, and was used for cloning with plasmid vector pAT153. We designated recombinant plasmids obtained as pN, where N was a four-figure number, and the viral DNA which was excised with EcoRI from the plasmid as BKN. Forty-one recombinant plasmids containing BKV DNA were analysed with HindIII which cleaves BKV DNA into four fragments (Howley et al., 1975). From the size of the fragments, nearly half of the cloned viral DNAs were judged to be identical to the parental viral DNA (data not shown). The rest underwent size increases in the HindIII-C fragments containing the transcriptional control region but no alteration in the other HindIII fragments (data not shown). Predominant among the recombinants with sequence rearrangement were two species containing approximately 75 and 120 additional bp. Cloning and analysis of the viral DNAs obtained from a different culture transfected with BK8326 essentially confirmed the above results, but the major species of the rearranged viral DNAs had a HindIII-C fragment with an extra sequence of about 70 bp. DNA extracted from the viruses that had been generated from BK8326 and passaged once more through HEK cells were also cloned and analysed for their HindIII-C fragments. Among 30 recombinant clones examined, only one carried the parental HindIII-C fragment and most clones had rearranged HindIII-C fragments. A few new DNA species appeared which had not been obtained before the second passage of the viruses.

We analysed several representative viral DNA clones for the nucleotide sequences of the HindIII/SstI (SacI) fragments, 370 to 500 bp in size, including their transcriptional control regions. Fig. 1 shows the structures of the transcriptional control regions of seven rearranged viral DNA species, three major ones derived from two different BK8326-transfected cultures (BK1307, BK1322, BK1501) and four minor ones obtained before (BK1325) and after the second passage of the viruses through HEK cells (BK1405, BK1406, BK1412). The structural change of these rearranged viral DNAs was duplication of various segments either within the 68 bp element (BK1405, BK1406) or spanning the 68 bp element and its origin-distal flanking regions (BK1307, BK1322, BK1501, BK1325, BK1412).

The rearranged viral DNAs whose structural feature had been characterized above and BKV DNAs with one (BK8326) and three copies of the 68 bp element (BK8001) were introduced into HEK cells by calcium phosphate co-precipitation. Cultures transfected with one of the viral DNAs containing duplication (BK1412) developed cytopathic changes and produced BKV HA activity as rapidly as did BK8001 (Table 1, expt. 1). Cultures transfected with most of the other viral DNAs carrying duplication (BK1307, BK1322, BK1501, BK1406) induced somewhat slower cytopathic changes and cell-free BKV HA was detected 3 days later than in the BK8001-transfected culture (Table 1, expt. 1). In contrast, BK1405 with duplication of a short sequence (6 bp) and BK8326 lacking a duplication induced very slow cytopathic changes and HA was detected on day 25 in these cultures (Table 1, expt. 1).

To establish a correlation between the structural changes and efficient viral growth in HEK cells, the following replacement experiment was carried out. About 500 bp SstI-C fragments (Yogo et al., 1980) spanning the transcriptional control region were prepared from three
Fig. 1. Sequence duplication in cloned viral DNAs derived from BK8326. The DNAs of viruses produced from BK8326 in HEK cells were cloned using plasmid vector pAT153. The HindIII/SstI fragments of several representative clones were subcloned with M13mpl0 and sequenced by the dideoxy chain termination method (Sanger et al., 1977). The control region of three randomly selected clones that exhibited a HindIII restriction pattern identical to that of BK8326 was the same as that of BK8326, shown at the top. Cloned viral DNAs that had HindIII-C fragments longer than that of BK8326 were found to have tandem duplication of single segments in the control region, and therefore the duplicated segment in each clone (straight line below the top diagram) is shown relative to the control region of BK8326. The endpoints of duplications are indicated by the numbers below straight lines (nucleotide numbering of Seif et al., 1979). Nucleotide sequence above the 68 bp element of BK8326 represents the sequence which was duplicated in all rearranged viral DNAs that induced rapid viral growth in HEK cells with the exception of BK1405 (Table 1). A horizontal arrow indicates the direction of the late transcription.

Table 1. Appearance of BKV-specific HA activity in culture fluids after transfection of HEK cells with various viral DNAs*

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Viral DNA transfected</th>
<th>Days after transfection</th>
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<td>9</td>
</tr>
<tr>
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* HA activity was determined using human type O red blood cells.
† ND, Not determined.
representative rearranged viral DNAs, BK1501, BK1406 and BK1412, and were analysed for their nucleotide sequences. No change was observed other than that detected in each corresponding HindIII/SstI fragment (Fig. 1). The SstI-C fragments were introduced into a recombinant plasmid that contained the wild-type BKV DNA deprived of its SstI-C fragment. Viral DNAs (BK1501w, BK1406w, BK1412w) were excised from these plasmids, and assayed for virus production in HEK cells as described above. The reconstituted viral DNAs induced viral growth as rapidly as did the respective parental viral DNAs from which the SstI-C fragment was derived (Table 1, expt. 2). These results indicate that the changes detected in the control regions of the three rearranged viral DNAs are responsible for the efficient growth of BKV.

From the structure of the control regions of various BKV DNAs (Fig. 1) and their capacity to induce viral growth (Table 2), it can be concluded that the essential sequence whose duplication is required for the efficient, but not optimal, growth of BKV is located within the origin-distal portion of the 68 bp element. Duplication of the origin-proximal end of the 68 bp element is not required for efficient growth of BKV, because all of the rearranged viral DNAs capable of inducing efficient viral replication contained only one copy of this end (Fig. 1). Furthermore, duplication of origin-distal regions adjacent to the 68 bp element is apparently not required for the productive replication of BKV, because the viral DNA without repetition of these regions, BK1406, induced efficient replication of BKV.

By comparison of the repeated structures between BK1501 and BK1406, both having the potential for rapid viral replication, we may further narrow the region which is required in two copies on the BKV genome. Although BK1501 and BK1406 had unique duplications, both of their duplicated regions contained a 13 bp sequence, CATGACCTCAGGA, within the 68 bp element (Fig. 1). Other rearranged viral DNAs capable of inducing efficient viral growth contained two copies of the 13 bp segment. Sequences similar to the core sequences of simian virus 40 (SV40) enhancer (Weiher et al., 1983) and adenovirus E1A enhancers (Hearing & Shenk, 1983) are located outside this segment.

In SV40, revertant viruses have been isolated from non-viable SV40 DNAs lacking the enhancer region (Swimmer & Shenk, 1984; Weber et al., 1984; Herr & Gluzman, 1985). These revertants carry duplication of either the core sequence of enhancer (Herr & Gluzman, 1985) or sequences located outside the enhancer (Swimmer & Shenk, 1984; Weber et al., 1984). Despite the apparent absence of the enhancer core in the latter cases, the revertant DNAs had a high enhancer activity. It remains to be determined whether the duplication of the 13 bp sequence in the rearranged BKV DNA increased the enhancer activity.

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REFERENCES


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