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A variant enhancer/regulatory region from a cloned human prototype BK virus genome

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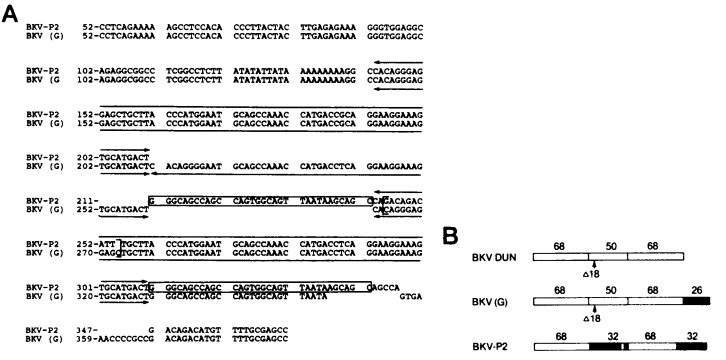
We have cloned the genome of a unique human BK virus strain, designated BKV-P2, from a preparation of prototype (Gardner) BK virus (ATCCVR837) grown in human embryonic kidney cells. Infectious virions can be generated from the cloned BKV-P2 DNA following transfection into primary human embryonic kidney cells, and the cloned DNA is capable of transforming BHK-21 cells (ATCC CCL10) to anchorage independence (growth in soft agar), with a transformation efficiency one half that of BKV Gardner. The sequence of the BKV-P2 regulatory region differs from that of the Gardner strain. Shown in Fig 1A are the DNA sequences of the Stu I to Avr II fragments of the BKV-P2 and Gardner (G) strains (1), encompassing the transcriptional regulatory regions and origin of replication [numbering convention of Seif et al. (1)]. The two sequences differ only in the repeat region which, in the Gardner and Dunlop strains, consists of an imperfect 68 bp triplication previously shown to be important for transcriptional enhancer activity (2). The repeat region in BKV-P2 is comprised of two nearly perfect 100 bp repeat sequences consisting of the 68 bp repeat (arrows) found in the Gardner and Dunlop strains, and an additional 32 bps (boxes). In the prototype Gardner strain, 26 bps out of the 32 are present in the unique 42 bps (1) to the late side of the last 68 bp repeat. The second 100 bp BKV-P2 repeat has a 10 bp region of nonidentity to the first (bracket). The organization of the 100 bp repeats relative to the Dunlop and Gardner strains is shown schematically in Fig 1B. The enhancer activity of the duplicated 100 bp sequence was assessed by inserting the Stu I to Avr II restriction fragment of BKV-P2 upstream of the SV40 early promoter, replacing the SV40 enhancer sequence in the expression vector pSV2 CAT (ATCC 37115). The level of chloramphenicol acetyltransferase (CAT) expression (3) from the resulting plasmid, pBS-CAT, ranged from 105 to 155% that of pSV2-CAT in the MK2 cell line (ATCC CCL7). In analogous constructions, the repeat sequences of the Dunlop and Gardner strains have stimulatory activity only 5.5 to 9.6% that of the SV40 enhancer (2,4). These data demonstrate that the unique 100 BKV-P2 repeat region is a strong transcriptional enhancer for the SV40 early promoter, equivalent to or better than the SV40 enhancer. We thank Stan Burgett, John Shepherd and Ivan Jenkins for sequence analysis of M13 subclones of the BKV-P2 genome.



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