JOURNAL OF VIROLOGY, Sept. 1985, p. 867–869
0022-538X/85/090867-03$02.00/0

Vol. 55, No. 3

Enhancement of the Transforming Capacity of BK Virus by Partial Deletion of the 68-Base-Pair Tandem Repeats

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Received 27 February 1985/Accepted 15 May 1985

We constructed a set of deleted BK viral DNAs that lack part of the triplicated 68-base-pair (bp) sequences (the central unit has an 18-bp deletion) and assayed them for capacity to transform hamster kidney cells. Deletion of one of the two 68-bp units remarkably enhanced transforming capacity.

Since its isolation in 1971 by Gardner et al. (2), human papovavirus BK (BKV) has been shown to transform various rodent cells in culture (reviewed in reference 5). It has turned out, however, that prototype BKV (10, 13) and a wild-type virus (*wt*-501) cloned from it (13) are weak agents for in vitro transformation. Studies by Watanabe et al. (11, 13) with a viable deletion mutant of BKV (*pm*-522) which has a high transforming capacity have suggested that local DNA rearrangement near the origin of replication can affect the transforming capacity of BKV. Thus, the apparent discrepancy in the transforming capacity of BKV could be accounted for by the genomic difference probably existing among the virus stocks used for transformation.

BKV mutant *pm*-522 has undergone DNA rearrangement in a region including the tandem triplication of a 68-base-pair (bp) sequence (11). In consequence, *pm*-522 has one complete 68-bp unit and two shorter sequences containing part of that unit. Thus, partial loss of the tandem repeats appears to be correlated to the enhanced transforming capacity of *pm*-522. Alternatively, change of some other sequences may have stimulated the transforming capacity, because *pm*-522 also has deleted and amplified small streches of nucleotides near the repeated region (11). In the present study, we constructed various deleted BKV DNAs that lack part of the tandem repeats and compared their transforming capacity. We found that deletion of one of the two 68-bp sequences enhanced the transforming capacity of BKV DNA.

All procedures for molecular cloning were done by the standard techniques described by Maniatis et al. (4). For the assay of transforming capacity, secondary hamster kidney cells from 12- to 14-day-old hamsters were cultured in Dulbecco modified Eagle medium (DME) supplemented with 10% fetal bovine serum. Hamster kidney cells grown on a 60-mm dish were treated at 37°C for 5 h with a DNA-calcium phosphate suspension prepared by the procedure of Graham and van der Eb (3). Then 5 ml of DME supplemented with 10% fetal bovine serum was added to the cultures and incubation continued. Two days later, the cells were replated at a density of 2.5×10^5 cells per 90-mm dish and cultured in DME with 10% calf serum. Three days posttransfection, the medium was replaced with DME containing 3.5% calf serum. Twice a week thereafter the cultures were refed with fresh DME containing 3.5% calf serum. Three weeks posttransfection, the dishes were fixed and stained with Giemsa, and densely stained foci were counted.

For construction of a set of deletion mutants that lack some of the repeating units, we took advantage of the specificity of restriction enzyme *Mst*II which recognizes 5'CCT*N*AGG3' and cleaves between C and T within this sequence. The wild-type BKV DNA has four such sequences, one (5'CCT*C*AGG3') in each of the triplicated tandem repeats and one (5'CCT*T*AGG3') in the middle of the late region (8, 15). Vector pBR322 has no *Mst*II site (6, 9). Partial digestion with *Mst*II and subsequent self-ligation of a recombinant plasmid containing the entire wild-type genome (p8001) generated, in addition to the parental recombinant, three kinds of recombinants with a reduced number of the repeating units; those with one 68- and one 50-bp units (p8110, p8127), two 68-bp units (p8209, p8225), and only one 68-bp unit (p8303, p8326) (Fig. 1). Digestion of these recombinants with *Mst*II showed that each recombinant had lost specific segments, either or both the 68- and 50-bp-long units (data not shown).

We examined the transforming capacity of recombinant DNAs that carry wild-type DNA (p8001), *pm*-522 DNA (p3004), and the deletion mutant DNAs obtained above. Viral inserts excised from the vector were introduced into hamster kidney cells by the calcium phosphate method (3), and the numbers of foci generated were scored 3 weeks posttransfection (Table 1). Clones carrying only one 68-bp unit (p8326, p8303) or one 68-bp unit and a sequence(s) carrying part of the 68-bp unit (p8110, p8127, p3004) gave rise to significant numbers of foci. In contrast, clones with two 68-bp units (p8001, p8209, p8225) yielded no or very few foci. When foci were assayed at 4 weeks after transfection with these clones, no appreciable number of foci was observed (data not shown). Difference in transformation efficiency between experiments 1 and 2 was probably a result of the condition of the cells used for the assay. Several foci produced by p8110, p8127, p8326, and p8303 were tested for the presence of BKV T antigen by immunofluorescence; all foci examined showed typical nuclear T antigen (data not shown).

Figure 2 summarizes the structure of the tandem-repeated region and the transforming capacity of the various viral DNAs examined in this study. It is clear that, although one copy of the 68-bp sequence was probably essential for transformation, the presence of the second 68-bp sequence suppressed the transforming capacity of BKV DNA (Fig. 2A and C). The extra 68-bp sequence was poisonous for transformation, whether it was located adjacent to (C) or distant from (A) the essential 68-bp sequence. Shorter sequences which contain part of the 68-bp sequence appear to have had

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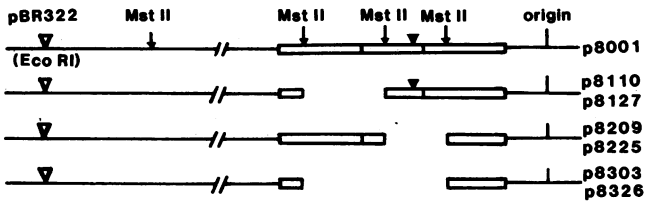

FIG. 1. Construction of deleted viral DNAs. Recombinant plasmid p8001 containing the entire wild-type BKV genome cloned at the *Eco*RI site was partially digested with *Mst*II and self-ligated. Transformation of *Escherichia coli* HB101 with the ligation mixture gave rise to recombinant plasmids that lack one or two *Mst*II fragments within the repeated region. BKV sequences are represented by solid lines, and the repeated sequences are represented by rectangles. Solid arrowheads indicate deletions (18 bp) in the central repeating unit.

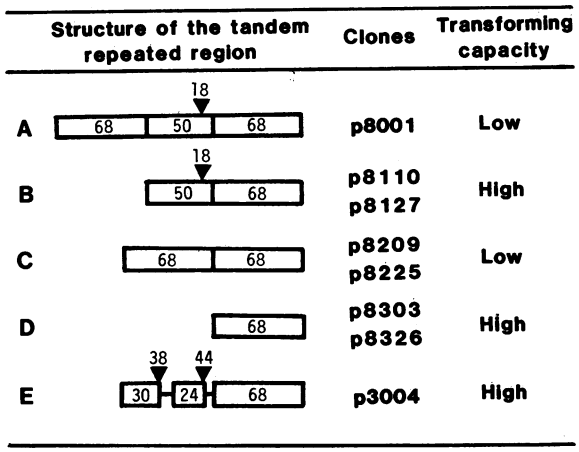

FIG. 2. Correlation between the organization of the repeated region and the transforming capacity of BKV DNA. The results presented in this study are summarized. Rectangles indicate the repeated sequences, and the numbers inside them are the sizes of the sequences (in base pairs). Arrowheads indicate sites of deletions, and the numbers above them are the extent of the deletions (in base pairs).

no or slight suppressive effect on transformation (B and E). It should be noted that *pm*-522, with a high transforming capacity, contains only one complete 68-bp sequence (E).

In the present study, we showed that duplication of the 68-bp sequence near the origin of replication on the BKV genome can lead to strong suppression of transforming capacity. We conclude that for this reason the wild-type BKV carrying two copies of the 68-bp sequence reveals a low transforming capacity. On the contrary, some, if not all, BKV stocks that have a high transforming capacity are expected to contain only one complete 68-bp sequence, although most of them, other than *pm*-522 and its derivatives (11), have not yet been examined for the structure of the repeated regions.

It is not now known how duplication of the 68-bp sequence inhibits transformation of hamster cells. One possible explanation is that in rodent cells the extra 68-bp sequence inhibits the essential 68-bp sequence from enhancing the transcription of the viral early gene. However, the triplicated tandem repeats of BKV when inserted into a transient expression vector can enhance equally the expression of the chloramphenicol acetyltransferase gene in human and mouse cells (7). Furthermore, the initial expression of T antigen in rat 3Y1 cells infected by a wild-type BKV which contains double 68-bp sequences was not significantly different from that in 3Y1 cells infected by *pm*-522, which contains a single 68-bp sequence (12). Therefore, in hamster cells the second 68-bp sequence does not appear to inhibit the enhancer activity of the essential 68-bp sequence. Recently, de Villiers et al. (1) reported that replication of polyomavirus DNA can be activated by the homologous and heterologous enhancer sequences. The BKV 68-bp sequence may also have the ability to stimulate the replication of viral DNA, and the second 68-bp sequence would interfere with this activity in hamster cells but not in human cells. It remains to be studied, however, whether viral DNA replication is one of the steps that determine the efficiency of transformation of hamster cells.

TABLE 1. Transforming capacity of deleted viral DNAs[a]

Expt no.	Viral DNA derived from:	No. of foci per microgram of DNA
1	p8001	0.5
	p8110	110
	p8209	2.5
	p8326	172
	p3004	110
2	p8001	0
	p8127	28.5
	p8225	0.5
	p8303	34.5
	p3004	17.5

[a] Viral DNAs (2 μg), excised from their plasmid vectors and isolated by agarose gel electrophoresis, were introduced into hamster kidney cells grown on a 60-mm dish by the calcium phosphate method (3). Densely stained foci were scored 3 weeks posttransfection. Recombinant p3004 contains the entire genome of deletion mutant *pm*-522 (14) cloned at the *Bam*HI site.

This investigation was supported in part by grants-in-aid for cancer research from the Ministry of Education, Science, and Culture of Japan.

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