Complementation Between BK Human Papovavirus and a Simian Virus 40 tsA Mutant

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Complementation tests between BK human papovavirus and SV40 temperature-sensitive mutants tsA58 and tsB11 were performed. Under the reported experimental conditions, BKV complemented the "early" mutant tsA58 but failed to complement the "late" mutant tsB11.

BK human papovavirus, originally isolated from the urine of a renal transplant patient (4) and subsequently from the urines of three patients with the Wiskott-Aldrich syndrome (14), has been the subject of much recent investigation. Of particular interest is its relatedness, now partially characterized, to the oncogenic simian papovavirus SV40. BKV and SV40 share identical or closely related tumor (T) antigens as monitored by immunofluorescence (15). Cross-reacting viral (V) antigens of BKV and SV40 have also been demonstrated by immunofluorescence, immunoelectron microscopy, and neutralization (4, 11, 15). The BKV and SV40 genomes have approximately 20% polynucleotide sequence homology (5). The major area of homology has been localized within the late region of the SV40 genome, with only a small amount detectable in the early region in the SV40 Hin-B fragment (6; Howley et al., manuscript in preparation). BKV, like SV40, is oncogenic for hamsters (13); it transforms hamster cells in vitro, and the transformed cells subsequently induce tumors in hamsters (9, 12; L. M. Näse, M. Kärkkäinen, and R. A. Mäntyjärvi, *Acta Pathol. Microbiol. Scand.*, in press). Purified BKV DNA also transforms hamster cells, which, again, are tumorigenic for hamsters (13a).

This study was undertaken to investigate further biological relatedness between BKV and SV40. In the studies reported herein, the ability of BKV to complement temperature-sensitive (ts) mutants of SV40 was tested. The progeny resulting from co-infection were also analyzed for possible recombinants. BKV(MM), an isolate from the urine of a Wiskott-Aldrich patient (14), was selected for these studies, since the DNA of this virus is considerably more homogeneous than the prototype BKV (5). This virus is indistinguishable from prototype BKV in nucleic acid hybridization experiments and has

been passaged solely in human cells (5). SV40 ts mutants tsA58 and tsB11 (generously supplied by P. Tegtmeier) were selected for complementation tests with BKV(MM). tsA58 is an "early" mutant, in a functional group of mutants ts for the ability to initiate (but not to complete) viral DNA replication and, to a lesser extent, the induction of host DNA synthesis in productive infection (1-3, 16). A mutants also are ts for the initiation, but not maintenance, of late viral RNA synthesis (3, 10). By marker rescue experiments, this mutant maps in the Hin-I region of the SV40 genome (7, 8). tsB11 is ts for production of V antigen and viral particles (16, 17, 18). This mutant is defective in the Hin-G region (8) and displays the BC phenotype in complementation experiments (8).

BKV(MM) was grown and assayed by plaque titration in human embryonic kidney cells, purchased from Flow Laboratories, Rockville, Md. Stocks of tsA58 and tsB11 were grown in CVC-WT clone 2 cells, derived from the TC-7 clone of the CV-1 line (supplied by P. Tegtmeier). Complementation tests and assay of the progeny of complementation were performed in CV-1 cells. All cells were grown and maintained in Eagle medium supplemented with 10% fetal bovine serum.

In preliminary experiments, the induction of T and V antigens by BKV(MM) at 33 and 41 C was assayed by indirect immunofluorescence. This was done to examine the growth characteristics of BKV(MM) under the experimental conditions. CV-1 cells grown on cover slips in petri dishes (60 by 15 mm) were infected with 0.5 ml of undiluted BKV(MM) (titer 2.5×10^6 PFU/ml). At 24, 48, and 72 h, cover slips were fixed in 50% acetone-50% methanol and stained for T and V antigens by using the standard indirect procedures.

As shown in Table 1, T antigen induced by BKV(MM) appeared earlier at 41 than at 33 C.

TABLE 1. Induction of T and V antigens in BKV(MM)-infected CV-1 cells at 33 and 41 C

Hours after infection	Incubation temp			
	33 C		41 C	
	T ^a	V	T	V
24	0	0	10%	0
48	25%	0	40-50%	Positive (<1%)
72	75%	0	75%	Positive (<1%)

^a Percent positive by fluorescent antibody tests.

However, at 72 h 75% of cells were positive at the two temperatures. At 33 C all cells were negative for V antigen. At 41 C rare cells (less than 1%) were positive for V antigen at 48 and 72 h. Seventy-two hours was therefore chosen as the duration of BKV(MM) infection before infection with the ts mutants. These experiments indicate that CV-1 cells are semipermissive hosts for BKV(MM), and most of the cells are abortively infected.

Complementation between SV40 ts mutants and BKV(MM) was measured as follows: CV-1 cells were infected with 0.5 ml of undiluted BKV(MM), a multiplicity of infection of approximately 1 PFU per cell; after 1 h of adsorption, cultures were washed three times and fresh medium was added; the cultures were held at 37 C; 72 h later the cultures were infected with a multiplicity of 1 PFU per cell of the ts mutants. One group of cultures was incubated at 33 C and another at 41 C. Uninoculated cells, cells inoculated with BKV(MM) or the ts mutants alone served as controls at each temperature. After 6 to 8 days of co-infection all cultures were harvested by freeze-thawing three times, clarified by centrifugation at 1,500 rpm for 10 min, and the titer at 33 and 41 C was determined.

Table 2 presents the results of complementation tests. BKV(MM) did not replicate under the experimental conditions. tsA58 and tsA58/BKV(MM) grew to a titer of 1.0×10^6 and 1.25×10^6 PFU/ml, respectively, at 33 C. At the nonpermissive temperature the titer of tsA58 was 3.5×10^2 PFU/ml. By contrast, in cells co-infected with BKV(MM), the titer of tsA58 was 1.2×10^6 PFU/ml, giving approximately a 4 log₁₀ increase in virus yield at the nonpermissive temperature. Similar levels of enhancement were obtained in repeated experiments.

On the other hand, BKV(MM) failed to complement tsB11 in several experiments. This failure of BKV(MM) to complement tsB11 is best explained by the growth behavior of BKV(MM) in CV-1 cells. As previously noted, CV-1 cells are semipermissive for BKV(MM)

TABLE 2. Complementation between BKV(MM) and tsA58

Virus ^a	Yield (PFU/ml) from cultures held at ^b	
	33 C	41 C
BKV(MM) ^c	0	0
tsA58	1.0×10^6	3.5×10^2
BKV(MM)/tsA58	1.25×10^6	1.2×10^6
tsB11	7.0×10^5	8.5×10^5
BKV(MM)/tsB11	7.5×10^5	3.25×10^6

^a Cultures were held at 37 C for 72 h after infection with BKV(MM) before infection with ts mutants.

^b Yield were titrated on CV-1 cells at 33 C.

^c BKV does not plaque on CV-1 cells.

growth and most cells were abortively infected by BKV(MM); therefore, factors required for assembly of tsB11 virions were not provided by BKV(MM).

In complementation experiments between tsA58 and BKV(MM), the possibility existed that SV40-BKV recombinant viruses could result from the cells infected with the two viruses. The types of possible recombinants that were considered were the following: (i) a hemagglutinating virus which could replicate in CV-1 cells, i.e., a virus which had the SV40 host range with BKV structural (hemagglutinating) proteins (this could be a true recombinant or a phenotypically mixed virus); (ii) a nonhemagglutinating virus that could grow in human embryonic kidney cells, i.e., a virus with BKV host range and SV40 (nonhemagglutinating) structural proteins; and (iii) a virus displaying the BKV or SV40 wild-type growth characteristics in CV-1 cells at 41 C.

For analysis of possible recombinants, virus obtained from mixed infection of BKV(MM) and tsA58 at 41 C was first tested for hemagglutinin by using human O erythrocytes at 4 C. The virus suspension failed to yield positive hemagglutination. When human embryonic kidney cells were infected with the progeny from mixed infection and held for 3 to 4 weeks, no cytopathic effects were noted. Similarly, the virus failed to grow in CV-1 cells at 41 C; the virus (first passage) titer was 1.2×10^5 PFU/ml at 33 C and less than 10^2 at 41 C. It was therefore concluded that mixed infection of BKV(MM) and tsA58 at 41 C did not result in recombinant viruses within the limits of the tests used for detection. This does not exclude the possibility that under proper conditions, perhaps with wild-type SV40 viruses, recombination between BKV(MM) and SV40 can occur. On the contrary, because of the related-

ness between the genomes of the two viruses, recombination would be expected to be a likely event in mixed infection.

The studies reported here reveal a further early functional relationship between SV40 and BKV. In other reports, these viruses were shown to induce an immunologically similar T antigen (12, 13, 15). U antigen, another nonstructural early antigen induced by SV40, has recently been shown to be synthesized in BKV-infected cells (13a). Thus, two of the early SV40 functions are shared with BKV.

Tegtmeier has reported that the SV40 tsA gene product acts as an initiator molecule in viral DNA replication and that "late" mutants are capable of supplying a factor capable of initiating the replication of the DNA of the A mutants (16). The ability of BKV(MM) to complement tsA58 therefore suggests that although the "early" regions of the genomes of the two viruses contain only limited detectable homology (6; Howley et al., manuscript in preparation), BKV(MM) is capable of supplying the SV40 A function required for growth of tsA mutants. The studies reported here thus provide further biological relatedness between the human and simian papovaviruses.

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