

Mapping and Ordering of Fragments of BK Virus DNA Produced by Restriction Endonucleases

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Received for publication 27 July 1978

A total of 51 restriction sites were recognized within the BK virus genome by the combination of 10 different restriction endonucleases. These sites were mapped and oriented relative to one another as well as to the five fragments generated by the digestion of BK virus DNA with *Hind*III and *Eco*RI. The result was a comprehensive physical map suitable for in-depth characterization of the functions of BK virus at the molecular level.

BK virus (BKV), a human papovavirus, was first isolated by Gardner from the urine of a renal allograft patient (8). The ubiquitous nature of BKV has been demonstrated by the high frequency (80 to 90%) of persons throughout the populations of England (7) and the United States (28) exhibiting antibodies against BKV.

Like simian virus 40 (SV40), the most thoroughly studied of the small DNA viruses, BKV has been shown to transform BHK cells in culture (11, 20, 27) as well as induce tumors in hamsters (5, 23). The T antigens of BKV and SV40 have been shown to be nearly indistinguishable by their immunological cross-reactivities (28); however, the structural proteins of the two viruses appear to differ considerably. This has been shown both by immunological methods (10) and by tryptic digest mapping (29, 30).

The genomes of BKV and SV40 show between 5 and 20% homology by DNA-DNA reassociation (11; Fiori and di Mayorca, unpublished results). The DNA of SV40 has been mapped extensively, using many different restriction endonucleases (1, 25, 26). This extensive mapping has proven a valuable tool in in-depth studies of the biochemistry and genetics of SV40.

In this paper we describe the cleavage sites produced in the DNA of BKV with 10 different restriction endonucleases. The restriction endonucleases used in this study were: *Hae*III, from *Haemophilus aegyptius*; *Hin*fI from *H. influenzae*, serotype f; *Ava*II; from *Anabaena variabilis*; *Bam*HI, from *Bacillus amyloliquefaciens*; *Hpa*II, from *H. parainfluenzae*; *Hha*I, from *H. haemolyticus*; *Kpn*I, from *Klebsiella*

pneumoniae; *Hph*I from *H. parahaemolyticus*; *Pvu*II, from *Proteus vulgaris*; and *Bgl*II from *B. globigii*.

The genome of BKV had been previously mapped with *Hind*III from *H. influenzae* (10), which recognizes four sites on the DNA. These sites were positioned relative to a single *Eco*RI site (10, 19), which, as in SV40, is designated as position zero on the physical map. We positioned the 51 sites recognized by the 10 different restriction endonucleases with respect to these *Hind*III and *Eco*RI sites as well as to one another.

MATERIALS AND METHODS

Cells and virus. Prototype BKV, obtained from Sylvia Gardner, Central Public Health Laboratory, London, England, was propagated in human embryonic kidney (HEK) cells cultured before infection in Dulbecco modified Eagle medium (DME) supplemented with 10% fetal calf serum and maintained after infection in DME with 2% fetal calf serum (29, 30). These cells were infected with 0.01 PFU of plaque-purified BKV per cell to limit the accumulation of defective genomes. Plaque-purified SV40 was propagated in BSC-1 cells.

DNA extraction. Viral DNA was extracted by the method of Hirt (9). Covalently closed form I DNA was purified from the Hirt supernatant by equilibrium centrifugation in CsCl (1.56 g/ml)-ethidium bromide (150 µg/ml). After isopropanol extraction and dialysis, the DNA was further purified by density centrifugation through 5 to 20% neutral sucrose.

Preparation of highly ³²P-labeled DNA. Incorporation of [α -³²P]CTP and [α -³²P]TTP (ICN Chemical and Radioisotope Division, Irvine, Calif.) into the DNA was accomplished by the "nick translation" method developed by Rigby et al. (21) and modified by Maniatis et al. (17), using *Escherichia coli* DNA nucleotidyltransferase I (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). The high-specific-activity (10⁷ cpm/µg) DNA was shown to be mostly full-length open circular DNA by electrophoresis in 0.8% agarose gels.

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Preparations of fragments of BKV DNA by using restriction endonucleases. Restriction endonucleases were purchased from New England Biolaboratories, Beverly Mass., and Bethesda Research Laboratories, Rockville, Md. The initial sample of *AvaII* used in this study was a gift from Joan Macy of Bethesda Research Laboratories.

Samples of nick-translated DNA were digested with various restriction endonucleases. The reaction mixtures for digestion by *HindIII*, *BamHI*, and *HhaI* contained 7 mM Tris-hydrochloride (pH 7.5), 10 mM MgCl₂, 7 mM 2-mercaptoethanol, 60 mM NaCl, and 0.5% bovine serum albumin. The reaction mixture for *HaeIII*, *HinI*, *AvaII*, *PvuII*, *HphI*, *HpaII*, and *KpnI* was the same as that above, except the NaCl concentration was lowered to 6 mM. The combined digestion of BKV DNA with *HindIII* and *EcoRI* was carried out in 0.1 M Tris-hydrochloride (pH 7.5)-10 mM MgCl₂-60 mM NaCl-0.5% bovine serum albumin. Approximately 2 U of enzyme (1 U digests 1 µg of DNA in 15 min at 37°C) was used per µg of DNA to insure complete digestion. Incubations were carried out at 37°C for 10 to 16 h. After incubation, sucrose and bromophenol blue marker dye were added to each sample, and the samples were loaded onto vertical polyacrylamide slab gels (3). For most separations, a composite gel consisting of 4% polyacrylamide in the top two-thirds and a 12% polyacrylamide trap in the bottom third of the gel was used (25). For the larger digestion products, such as those produced by *HindIII* or *AvaII* digestion, 3% polyacrylamide was used. Electrophoresis was carried out at 150 to 175 V for 12 to 16 h in a buffer containing 40 mM Tris-acetate (pH 7.8), 20 mM sodium acetate, and 2 mM EDTA (15). The labeled DNA fragments were located by autoradiography, excised from the gel, and homogenized in 0.1× SCC (1× SCC is 0.15 M NaCl-0.015 M sodium citrate) with 1 mM EDTA. After incubation at 50°C for 10 to 12 h the acrylamide was pelleted by successive centrifugations at 3,000 rpm for 10 min. The fragments were precipitated from the supernatant by the addition of 2 volumes of cold ethanol in polyallomer tubes and stored at -20°C overnight. The DNA was recovered by centrifugation at 40,000 rpm in Beckman type 50.1 rotor. After drying the DNA in vacuo, the fragments were redissolved in 10 mM Tris (pH 7.5)-1 mM EDTA.

Secondary digestions. Secondary digestions were carried out under the same reaction conditions as the primary digestions.

Partial digestions. Partial digestion products of BKV DNA with *HaeIII* were obtained by digesting the DNA with 1 U of undiluted enzyme per µg of DNA at 4°C for 30 min. Since we used much less labeled DNA than 1 µg, we added unlabeled BKV DNA to bring the total to 1 µg. After partial digestion products were obtained and purified, the DNA was digested to completion with an excess of enzyme and incubation at 37°C for 10 to 12 h.

RESULTS

The enzymes used in this study are listed in Table 1 along with their numbers of cleavages within BKV DNA. Enzymes which were tested

TABLE 1. Restriction endonucleases tested in this study

Restriction endonuclease	No. of cleavages on BKV DNA
<i>HaeIII</i>	21
<i>HinI</i>	13
<i>AvaII</i>	7
<i>HphI</i>	2
<i>PvuII</i>	2
<i>BglII</i>	2
<i>BamHI</i>	1
<i>HpaII</i>	1
<i>HhaI</i>	1
<i>KpnI</i>	1
<i>HindIII</i>	0
<i>HpaI</i>	0
<i>HaeII</i>	0
<i>BglI</i>	0
<i>TaqI</i> ^a	0

^a From *Thermus aquaticus*.

but which failed to cleave BKV DNA are also listed.

Size estimations of the *HaeIII* and *HinI* fragments of BKV DNA. Figure 1 shows the digestion patterns of BKV DNA with *HaeIII* and *HinI*, as compared with the *HaeIII* and *HinI* fragments of SV40 DNA. Some of the bands appeared to be much more intense than the others; this represented comigration of DNA fragments of similar size. The *HaeIII* fragments which comigrated were the J and K and the M and N fragments. In the *HinI* digest, the K and L fragments migrated together.

The digestion patterns of *AvaII* and the combined digest of *HindIII* with *EcoRI* are shown in Fig. 2.

Size estimations of the *HinI* and *HaeIII* fragments of BKV DNA were made by comparing their migrations relative to those of the SV 40 *HaeIII* and *HinI* fragments whose sizes are known (25). Figure 3 shows the numbers of base pairs versus the electrophoretic mobilities. The sizes of the *HinI* and *HaeIII* fragments of BKV DNA were derived from this plot and are listed in Table 2. The sizes of the *HindIII* and *AvaII* fragments were calculated similarly, and the values were rechecked and confirmed by summing up the sizes of the *HinI* or *HaeIII* fragments present internally within the *HindIII* and *AvaII* fragments (Table 2).

Positions of the *HinI*, *HaeIII*, and *AvaII* fragments relative to the *HindIII* and *EcoRI* sites. The five fragments produced by cleavage of prototype BKV DNA with *HindIII* and *EcoRI* have been previously ordered (10). The positions of the *HaeIII*, *HinI* and *AvaII* fragments were first determined relative to these five fragments. The method used was that of

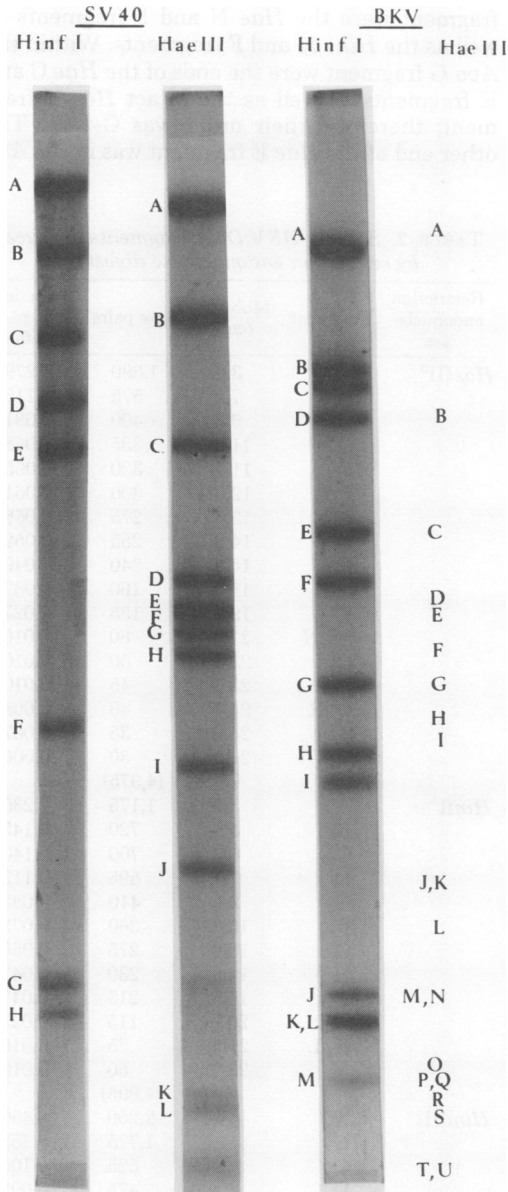


FIG. 1. Electrophoretic patterns of digestion products of BKV and SV40 DNAs with *HinfI* and *HaeIII* restriction endonucleases. Separation was accomplished in a 4/12% composite polyacrylamide gel.

reciprocal digestion (26). In the first step, the five fragments generated by the digestion of BKV DNA were separated by electrophoresis in 3% acrylamide (Fig. 2), eluted from the gel, and then redigested with *HaeIII*, *HinfI*, and *AvaII*. The digests were then separated by electrophoresis through a 4/12% composite polyacrylamide gel, and the resulting products were visualized

by autoradiography (Fig. 4). The *HaeIII*, *HinfI*, and *AvaII* fragments located within each of the five *HindIII/EcoRI* fragments are shown in Table 3.

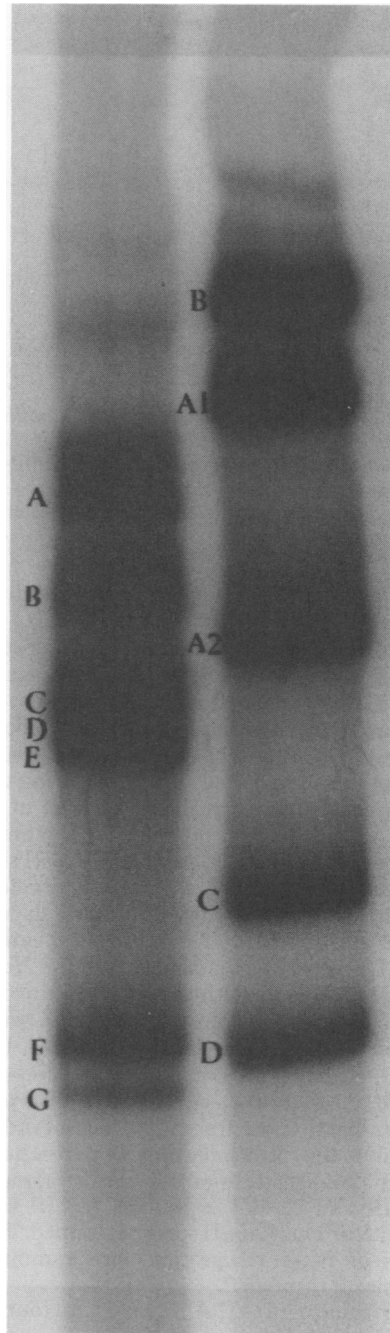


FIG. 2. Electrophoretic patterns of *AvaII* (left) and combined *HindIII/EcoRI* (right) digests of BKV DNA in a 3% polyacrylamide gel.

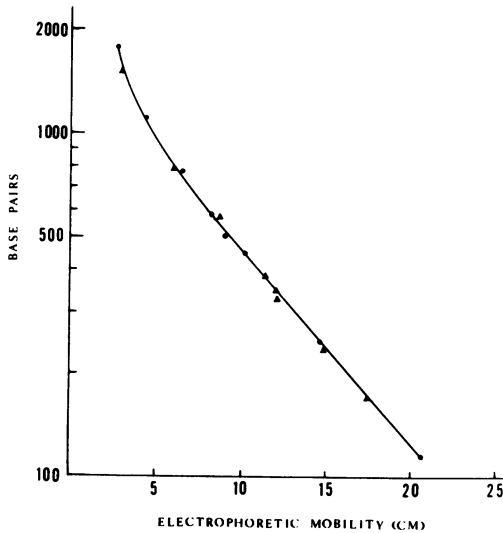


FIG. 3. Plot of the electrophoretic mobilities of the *HinfI* (●) and *HaeIII* (▲) fragments of SV40 DNA, as shown in Fig. 1, versus the numbers of base pairs. The sizes of the *BKV* digestion products were determined from this plot by their relative electrophoretic mobilities.

To determine the positions of the fragments which overlapped the *HindIII/EcoRI* cleavage sites, the fragments produced by digestion with *HinfI*, *HaeIII*, and *AvaII* were redigested with *HindIII* and *EcoRI*. The products were subjected to electrophoresis and autoradiographed (Fig. 5). The results of these redigestions are summarized in Table 4. By comparing the products in Table 4 with the end pieces in Table 3, the positions of the overlapping fragments with respect to the four *HindIII* and *EcoRI* sites were determined. From these data the complete order of the *AvaII* fragments was deduced as C-E-B-A-F-D-G-C, where C overlaps the *EcoRI* site. The partial orders of the *HaeIII* and *HinfI* fragments with the fragments overlapping the *EcoRI* site and moving clockwise, were B-(J, Q, S)-D-(F, N, A)-G-I-(M, O, H, T, U)-C-(L, E, P, K, R)-B for *HaeIII* and A-K-G-(B, H, J, L, M, F)-C-E-I-D-A for *HinfI*. The parentheses indicate those fragments not yet ordered. To resolve the positions of the parenthetic fragments, redigestions of the *AvaII* fragments with *AvaII* and *HaeIII* and of the *HaeIII* fragments with *HinfI* and *AvaII* were performed. The products of these redigestions are summarized in Tables 5 through 7.

Within the *AvaII* A fragment we found an end of *HaeA* and an end of *HinfH*. The other ends of these fragments were located in the *Ava* B fragment; therefore, these fragments overlapped the *Ava* A-B junction. Also, within the *Ava* B

fragment were the *Hae* N and F fragments as well as the *Hinf* M and F fragments. Within the *Ava* G fragment were the ends of the *Hae* C and E fragments as well as the intact *Hae* L fragment; therefore, their order was C-L-E. The other end of the *Hae* E fragment was in the *Ava*

TABLE 2. Sizes of *BKV* DNA fragments produced by restriction endonuclease digestion

Restriction endonuclease	Fragment	Mobility (cm)	Base pairs ^a	Fraction of genome
<i>HaeIII</i> ^b	A	3.0	1,390	0.279
	B	7.2	575	0.115
	C	9.8	400	0.081
	D	11.5	335	0.068
	E	11.7	320	0.065
	F	12.6	300	0.061
	G	13.5	275	0.056
	H	14.2	255	0.052
	I	14.8	240	0.049
	J, K	18.0	160	0.032
	L	19.2	135	0.027
	M, N	21.2	80	0.016
	O	23.1	50	0.010
	P	23.5	45	0.010
	Q, R	24.0	40	0.008
	S	24.6	35	0.007
T, U	26.0	30	0.006	
			(4,975)	
<i>HinfI</i> ^b	A	3.3	1,175	0.235
	B	5.9	720	0.145
	C	6.3	700	0.140
	D	7.1	595	0.113
	E	9.6	410	0.083
	F	10.8	360	0.073
	G	13.2	275	0.056
	H	14.8	230	0.047
	I	15.5	215	0.043
	J	20.8	115	0.023
K, L	21.5	75	0.015	
M	23.2	50	0.010	
			(4,995)	
<i>HindIII</i> ^c	A		2,250	0.450
	B		1,775	0.355
	C		525	0.105
	D		475	0.095
			(5,025)	
<i>AvaII</i> ^c	A		1,115	0.220
	B		820	0.160
	C		775	0.150
	D		710	0.142
	E		690	0.139
	F		450	0.085
	G		400	0.080
			(4,960)	

^a Totals in parentheses.

^b Fragment sizes derived from Fig. 3 relative to the electrophoretic mobilities of the SV40 DNA fragments.

^c Fragment sizes calculated as averages of the sums of the internal *HinfI* and *HaeIII* fragments.

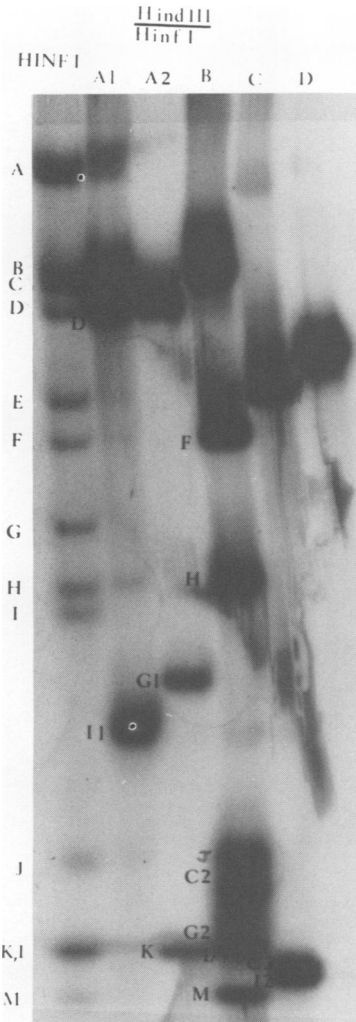


FIG. 4. Electrophoretic patterns of the redigestion products of the *HindIII/EcoRI* fragments with *HinfI* restriction endonuclease. Separation was on a 4/12% composite polyacrylamide gel.

C fragment along with the *Hae* fragments P, K, R, and an end of B.

With the *Hinf* F fragment were ends of the *Hae* A and F fragments as well as the intact *Hae* N fragment. Therefore, the order of these *Hae* fragments was A-N-F. Within the *Hae* F fragment were the ends of the *Hinf* fragments F and C and the intact M fragment. Therefore, the order of these *Hinf* fragments was F-M-C. The *HaeIII* M fragment was cleaved by *HinfI* and found to contain the ends of the *Hinf* C and E fragments; therefore, the *Hae* M fragment spanned the *Hinf* C-E junction. From these reciprocal cleavage data the orders of the *HinfI* and *HaeIII* fragments could be further deduced as A-K-G-M-F-H-(B, J, L)-C-E-I-D-A for *HinfI* and B-(J, Q, S)-D-F-N-A-G-I-M-(O, H, T, U)-C-L-E-(P, K, R)-B for *HaeIII*.

Positions of single- and double-cleavage enzymes on the BKV physical map. Some restriction endonucleases were found to recognize only one (*Bam*HI, *Hpa*II, *Hha*I, *Kpn*I) or two (*Pvu*II, *Hph*I, *Bgl*II) sites on BKV DNA. These sites were positioned relative to the *Hind*III, *Eco*RI, *Hae*III, and *Hinf*I sites by combined digestion with these enzymes (Fig. 6). The results of these combined digestions are summarized in Table 8. Some of the cleavage sites were found to occur within the bracketed areas. *Kpn*I digested within *Hae* fragment P, *Hpa*II digested within *Hae* fragment H, and *Pvu*II digested within *Hae* fragments Q and C. Simultaneous digestion with these enzymes yielded four fragments which, upon redigestion with *HaeIII*, resolved the order of some of the bracketed fragments (Table 9). From these results we found the order of (J, Q, S) to be J-Q-S, that of (O, H, T, U) to be O-H-T-U, and that of (K, P, R) to be P-(K, R). This left only *Hae* fragments (K, R) and *Hinf* fragments (J, L, B) unordered.

Partial digestion of BKV DNA fragments. To resolve the order of *Hae* fragments (K, R),

TABLE 3. Redigestion of *HindIII/EcoRI* fragments with *HaeIII*, *HinfI*, and *AvaII*

<i>HindIII/EcoRI</i> fragment	Resulting fragments from redigestion with		
	<i>HaeIII</i>	<i>HinfI</i>	<i>AvaII</i>
A1	E, L, K, P, R, and 2 end pieces of 370 and 275 base pairs	D and 2 end pieces of 610 and 165 base pairs	G and 2 end pieces of 685 and 230 base pairs
A2	J, Q, S, and 2 end pieces of 300 base pairs each	K and 2 end pieces of 560 and 190 base pairs	E and 2 end pieces of 45 and 115 base pairs
B	A, F, N, and 2 end pieces of 45 and 30 base pairs	B, F, H, J, L, M, and 2 end pieces of 100 and 75 base pairs	2 end pieces of 1,070 and 735 base pairs
C	H, M, O, T, U, and 2 end pieces of 40 and 30 base pairs	E and 2 end pieces of 55 and 50 base pairs	2 end pieces of 470 and 50 base pairs
D	2 end pieces of 230 and 200 base pairs	Undigested; 430 base pairs	2 end pieces of 385 and 50 base pairs

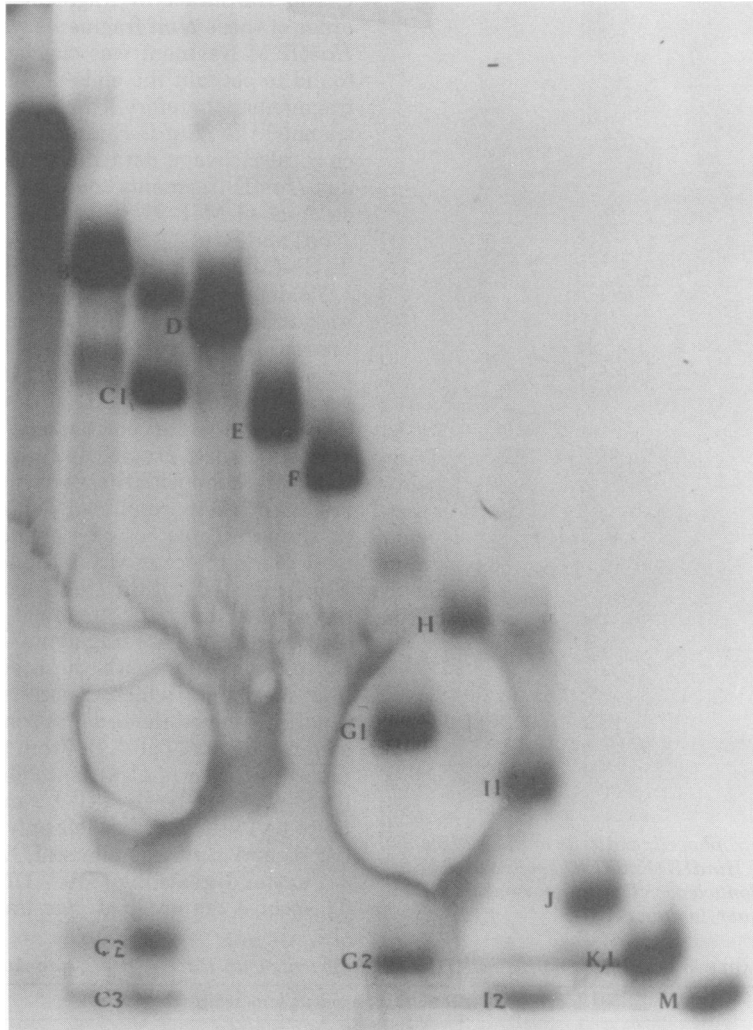


FIG. 5. Electrophoretic patterns of the redigestion products of the *HinI* fragments with *HindIII* in 4/12% polyacrylamide gel.

TABLE 4. Digestion of *HaeIII*, *HinfI* and *AvaII* fragments with *HindIII* and *EcoRI*

Fragment	<i>HindIII/EcoRI</i> digestion	
	Product	No. of base pairs ^a
<i>HaeIII</i>		
B	B1	300
	B2	275
C	C1	370
	C2	30
D	D1	300
	D2	30
G	G1	230
	G2	45
I	I1	200
	I2	40
<i>HinfI</i>		
A	A1	610
	A2	560
C	C1	430
	C2	100
	C3	55
G	G1	190
	G2	75
I	I1	150
	I2	50
<i>AvaII</i>		
A	A1	1,145
	A2	45
B	B1	735
	B2	50
C	C1	685
	C2	25
D	D1	510
	D2	280
E	Undigested	
F	F1	385
	F2	100
G	Undigested	

^a Size estimations based on electrophoretic mobilities relative to markers of known size.

the *HindIII/EcoRI* A1 fragment was partially digested with *HaeIII*, and the subsequent partial digestion products were redigested with *HaeIII* (Table 10). From the results we found that fragment R was contiguous with the end of fragment

B. Therefore, the order of the *HaeIII* fragments within the *HindIII/EcoRI* A1 fragment was B-R-K-P-E-L-C.

The positions of the *HaeIII*, *HinfI*, and *AvaII* sites as well as those of the single- and double-cleavage enzymes are shown in Fig. 7. The *HindIII* and *EcoRI* cleavage sites are also included for comparison.

TABLE 5. Redigestion of *AvaII* fragments with *HinfI* and *HaeIII*

<i>AvaII</i> fragment	Products of redigestion with:	
	<i>HaeIII</i>	<i>HinfI</i>
A	2 end pieces of 1,050 and 70 base pairs	B, J, L, and 2 end pieces of 135 and 65 base pairs
B	F, N, and 2 end pieces of 335 and 135 base pairs	F, M, and 2 end pieces of 160 and 215 base pairs
C	K, P, R, and 2 end pieces of 300 and 200 base pairs	2 end pieces of 115 and 700 base pairs
D	H, O, T, U, and 2 end pieces of 265 and 45 base pairs	E, I, and 2 end pieces of 75 and 60 base pairs
E	J, Q, S, and 2 end pieces of 275 and 45 base pairs	K and 2 end pieces of 590 and 50 base pairs
F	I and 2 end pieces of 180 and 90 base pairs	Undigested
G	L and 2 end pieces of 135 and 100 base pairs	Undigested

TABLE 6. Redigestion of *HinfI* fragments with *HaeIII* and *AvaII*

<i>HinfI</i> fragment	Products of redigestion with:	
	<i>HaeIII</i>	<i>AvaII</i>
A	B, J, K, Q, P, R, S, and 2 end pieces of 70 and 40 base pairs	2 end pieces of 700 and 595 base pairs
B	Undigested	Undigested
C	G, I, and 2 end pieces of 135 and 70 base pairs	F and 2 end pieces of 215 and 60 base pairs
D	L and 2 end pieces of 240 and 215 base pairs	G and 2 end pieces of 115 and 75 base pairs
E	H, Q, T, and 2 end pieces of 45 and 20 base pairs	Undigested
F	N and 2 end pieces of 185 and 50 base pairs	Undigested
G	2 end pieces of 230 and 50 base pairs	2 end pieces of 160 and 125 base pairs
H	Undigested	2 end pieces of 165 and 60 base pairs
I	2 end pieces of 180 and 30 base pairs	Undigested
J, K, L, M	Undigested	Undigested

TABLE 7. Redigestion of *HaeIII* fragments with *HinfI* and *AvaII*

<i>HaeIII</i> fragment	Products of redigestion with:		<i>HaeIII</i> fragment	Products of redigestion with:	
	<i>HinfI</i>	<i>AvaII</i>		<i>HinfI</i>	<i>AvaII</i>
A	B, H, J, L, and 2 end pieces of 185 and 50 base pairs	2 end pieces of 1,050 and 350 base pairs	F	M and 2 end pieces of 125 and 120 base pairs	Undigested
B	Undigested	2 end pieces of 75 and 30 base pairs	G	Undigested	2 end pieces of 150 and 130 base pairs
C	2 end pieces of 215 and 180 base pairs	2 end pieces of 265 and 135 base pairs	H, I, J, K, L	Undigested	Undigested
D	K and 2 end pieces of 200 and 40 base pairs	2 end pieces of 230 and 90 base pairs	M	2 end pieces of 80 and 30 base pairs	2 end pieces of 55 base pairs each
E	2 end pieces of 240 and 70 base pairs	2 end pieces of 230 and 80 base pairs	N, O, P, Q, R, S	Undigested	Undigested
			T	2 end pieces of 15 base pairs each	Undigested
			U	Undigested	Undigested

TABLE 8. Positions of double- and single-cleavage enzymes as compared with the *HindIII/EcoRI*, *HaeIII*, and *HinfI* fragments

Single- or double-cleavage enzyme	Combined digestion with:						Derived map position
	<i>HindIII/EcoRI</i>		<i>HaeIII</i>		<i>HinfI</i>		
	Product	No. of base pairs ^a	Product	No. of base pairs ^a	Product	No. of base pairs ^a	
<i>BamHI</i>	A1-1	1,250	B-1	700	A-1	740	0.98
	A1-2	700	B-2	175	A-2	460	
<i>HpaII</i>	C-1	430	H-1	210	E-1	380	0.695
	C-2	70	H-2	40	E-2	30	
<i>HhaI</i>	C-1	489	C-1	310	I-1	165	0.72
	C-2	20	C-2	30	I-2	45	
<i>KpnI</i>	A1-1	950	P-1	20	A-1	1,055	0.89
	A1-2	500	P-2	20	A-2	145	
<i>HphI</i>	A1-1	400	C-1	300	I-1	125	0.74
	A1-2	260	C-2	100	I-2	90	
	D-1	390	I-1	125	C-1	560	0.60
	D-2	50	I-2	115	C-2	135	
<i>PvuII</i>	A1-1	1,420	C-1	350	I-1	110	0.74
	A1-2	40	C-2	50	I-2	105	
<i>BglII</i>	A2-1	450	Q-1	20	A-1	460	0.095
	A2-2	360	Q-2	20	A-2	100	
	A2-1	600	G-1	250	D-1	300	0.125
	A2-2	200	G-2	25	D-2	35	
	D-1	455	C-1	580	G-1	230	0.526
	D-2	25	C-2	120	G-2	50	

^a Size estimations were based on electrophoretic mobilities relative to markers of known size.

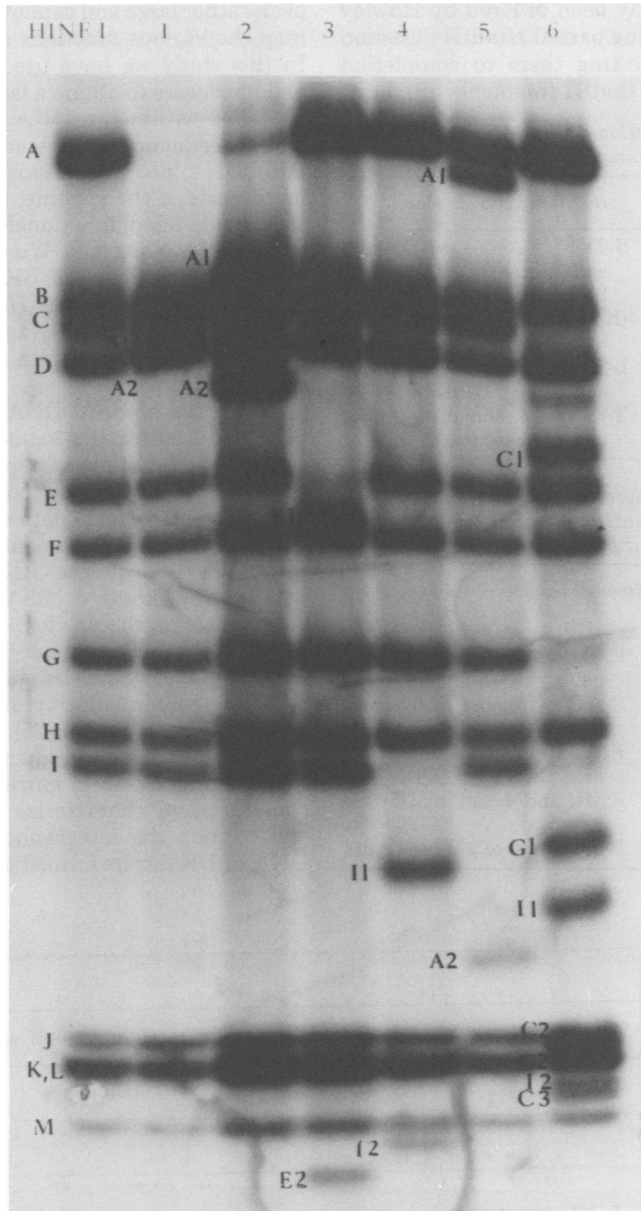


FIG. 6. Electrophoretic patterns of combined digestions of BKV DNA with *HinfI* and *EcoRI* (1), *BamHI* (2), *HpaII* (3), *HhaI* (4), *KpnI* (5), and *HindIII* (6) in a 4/12% composite polyacrylamide gel.

DISCUSSION

Restriction endonuclease cleavage maps have proven useful in the biochemical and genetic analyses of SV40. Among these were the derivation of transcriptional (12) and translational maps, mapping of the origin of replication (2, 6), and the production and characterization of deletion mutations (13, 14, 24).

The methods of DNA sequencing developed recently by Maxam and Gilbert (18) and by Sanger and Coulson (22) rely on the existence of small DNA fragments produced by restriction endonuclease digestion. Using these methods, DNAs from several sources have been sequenced, and this has increased our understanding of events at the molecular level.

The four *HindIII* fragments of prototype BKV

DNA have previously been ordered by Howley et al. (10) by obtaining partial *Hind*III digestion products and redigesting them to completion with *Hind*III. The *Hind*III fragments are, how-

TABLE 9. Redigestion of *Hpa*II/*Pvu*II/*Kpn*I fragments with *Hae*III

<i>Hpa</i> / <i>Pvu</i> / <i>Kpn</i> fragment	<i>Hae</i> III digestion products
A	S-D-F-N-A-G-I-M-O and 2 end pieces of 210 and 20 base pairs
B	(K, R)-B-J and 2 end pieces of 20 base pairs each
C	L-E and 2 end pieces of 350 and 20 base pairs
D	T-U and 2 end pieces of 40 and 150 base pairs

TABLE 10. Partial digestion products of the *Hind* A1 fragment with *Hae*III and subsequent complete digestion

Partial <i>Hae</i> III product of <i>Hind</i> A1 fragment	Products upon digestion to completion with <i>Hae</i> III
A1 a	E, L, P, and 1 end of 370 base pairs
A1 b	E, L, and 1 end of 370 base pairs
A1 c	K, L, P, R, and 1 end of 275 base pairs
A1 d	K, R, and 1 end of 275 base pairs
A1 e	R and 1 end of 275 base pairs

ever, rather large and cannot be used to precisely map the various functions of the viral genome. In this study we have used several restriction endonucleases to obtain a large number of cleavage sites within the DNA of prototype BKV. The consequence of this study has been to cleave BKV DNA into several small fragments located in all parts of the genome, making it amenable to more detailed, functional studies.

Recently, Yang and Wu have published detailed physical maps of BKV (MM) DNA (31, 32). The positions of the *Hae*III, *Hpa*II, *Hha*I, *Kpn*I, and *Bam*HI cleavage sites which they have shown correlate with those which we describe in this paper.

Sequences of BKV DNA have also recently been deduced for the segments of the genome containing the origin of replication (4) and for part of the late region corresponding to the leader sequence of the late viral RNA (31).

One of the enzymes used in this study, *Hae*III, recognizes the sequence GGCC, which is present within the genome of BKV 21 times. A large percentage of these sites are within the late region of the BKV genome. This is similar to the SV40 genome, which also shows a predominance of these guanine-plus-cytosine-rich sequences within the same region (25).

The cleavage map produced by the 10 restriction endonucleases is currently being used to construct and characterize deletion mutations and to map the integration sites on the viral DNA of BKV-transformed cells.

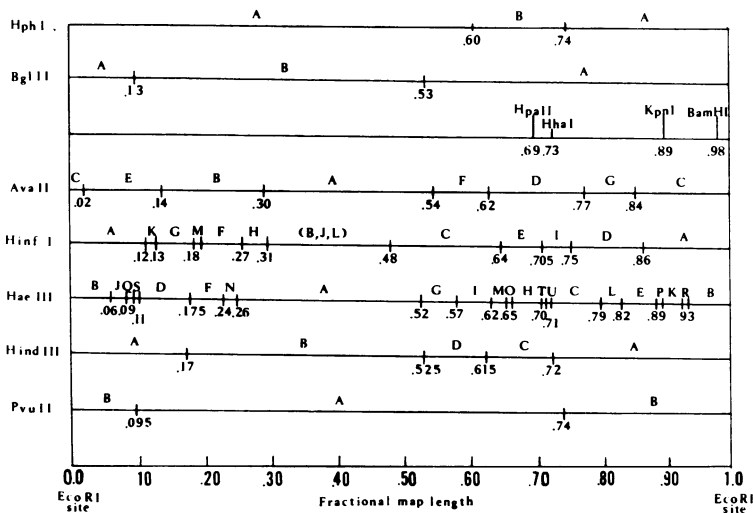


FIG. 7. Physical maps of BKV DNA derived in this study. BKV DNA, which is actually circular, is presented in a linear form for convenience in comparing the cleavage maps. Horizontal lines refer to the DNA and vertical lines indicate cleavage sites. The *Hind*III and *Eco*RI sites, derived by Howley et al. (10) are shown for comparison.

ACKNOWLEDGMENTS

This work was supported by Public Health Service contract NIH NO1 CP 43318 from the Virus Cancer Program of the National Institutes of Health to G. di M. and by National Cancer Institute Contract NCI NO1 CP 71061 to K.N.S.

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