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Arrangement of the Genome of the Human Papovavirus RF Virus

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DNA from plaque-purified RF virus, a variant of BK virus, was found to contain two species of molecules. Hybridization of each DNA species to the fragments of BK virus DNA revealed that one species had a deletion corresponding to at least 50% of the late region and the other had a deletion corresponding to at least 40% of the early region of BK virus DNA. Analysis by cleavage of each RF virus DNA species with restriction endonucleases *Eco*RI, *Hin*dIII, *Ava*II, and *Pvu*II, when compared with BK virus DNA, revealed that the size and number of fragments were different. These results suggest the loss of some restriction sites and the appearance of new sites, probably as a result of base changes in each RF virus DNA species. Furthermore, analysis of the restriction map of each DNA molecule revealed an insertion(s) in both DNA species.

Since the initial isolation of human BK virus (BKV) from the urine of a renal allograft recipient (5), several strains of this virus have been isolated (4, 7, 9, 14, 19, 22). Although immunological differences have been detected among a few variants (10), most of the differences detected have been changes in the DNA close to the origin of replication (13, 14, 17, 26).

RF virus (RFV), a variant of BKV isolated by Dougherty and diStefano (4), has been shown to be immunologically indistinguishable from the the prototype BKV (4, 12) but more oncogenic when injected into newborn hamsters (3). The peptide patterns of two major capsid proteins are slightly different from those of BKV (22). The genome of this virus has 80% homology to that of BKV as shown by liquid hybridization (4). In the present study we found that the DNA isolated from this virus consists of two species, one with a deletion corresponding to the early and the other with a deletion corresponding to the late region of BKV DNA. We also found base changes and insertions in both DNAs as compared with BKV DNA.

MATERIALS AND METHODS

Cells and virus. Prototype BKV (5) and RFV were grown in human embryonic kidney cells as described (13). The three preparations of RFV had been plaque purified separately in 1975, 1976, and 1978 [RF(A), RF(B), and RF(C), respectively, in Fig. 7].

Preparation of viral DNAs. Viruses banded in CsCl were extracted with phenol, and the DNAs were further purified by banding in CsCl-ethidium bromide gradients (13). Viral DNAs were labeled with ^{32}P by the nick-translation procedure (11, 15) as before (13).

Cleavage of viral DNAs with restriction endonucleases. Digestion of viral DNA with *Eco*RI and *Hin*dIII was as previously described (13). Digestion with *Ava*II and *Mbo*I was as suggested by New England Biolabs. Digestion with *Pvu*II was as suggested by Bethesda Research Laboratories. *Hin*dIII, *Hin*dIII-*Eco*RI, and *Pvu*II fragments were separated by electrophoresis through 1.2% agarose gels (13). *Ava*II and *Mbo*I fragments were separated by electrophoresis through a composite gel containing 4% polyacrylamide in the top two-thirds and a 12% polyacrylamide trap in the bottom (13). DNA fragments were isolated from agarose and acrylamide gels by electroelution (23).

Hybridization of ^{32}P-labeled viral DNAs with BKV DNA fragments blotted onto filter papers. Unlabeled BKV DNA was cleaved with *Hin*dIII-*Eco*RI and separated by electrophoresis through 1% agarose horizontal gels. Vertical acrylamide gels were used for separating *Mbo*I fragments of BKV DNA. The DNA was denatured, transferred onto nitrocellulose filters, and hybridized to ^{32}P-labeled viral DNA (13, 18, 20; M. M. Pater et al., 6th Annual Cold Spring Harbor Conference on Cell Proliferation, in press). The method of Whal et al. (21) was used for the transfer of *Mbo*I fragments of BKV DNA onto diazobenzyloxymethyl paper.

RESULTS

RFV DNA and its homology to BKV DNA. First, the sizes of RFV DNA and BKV DNA were compared. Both DNAs were nick translated and run by electrophoresis through 1.2% agarose gels (Fig. 1). The nick-translated BKV DNA was in forms II and III. RFV DNA contained forms II and III of two DNA species (R1 and R2). As can be seen in Fig. 1, this preparation contained more R1 than R2. However, other preparations had more R2 than R1 DNA or equal amounts of both DNAs. Linear (form III)

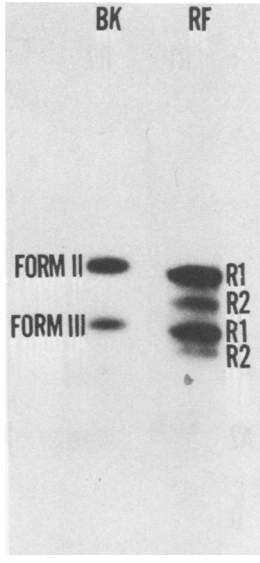

FIG. 1. *Separation of R1 and R2 DNAs on agarose gels. BKV and RFV DNAs were nick translated and run by electrophoresis through 1.2% agarose gels for 20 h at 50 V. The bands were detected by autoradiography of the gel.*

R1 was about 100 base pairs smaller than linear BKV DNA, whereas linear R2 DNA was 620 base pairs smaller than BKV DNA (Table 1).

Since both DNA species were smaller than BKV DNA, it was of interest to see whether specific regions of BK genomes were missing in each DNA species. Unlabeled BKV DNA was digested with combined restriction endonucleases *Eco*RI and *Hin*dIII which cleaved BKV DNA into fragments B, A_1, A_2, C, and D (Fig. 2). These fragments were separated through 1% agarose gels and were then blotted onto nitrocellulose filters and hybridized with nick-translated R1, R2, and BKV DNAs separately. As shown in Fig. 3A, R1 DNA hybridized very little with the *Hin*dIII-*Eco*RI fragment A_1 of BKV DNA. This fragment is located in the late region of prototype BKV DNA (6). The band containing *Hin*dIII fragment B or BKV DNA was less intense than the band containing fragment A_1 when nick-translated R2 DNA was hybridized (Fig. 3A). This suggests that R2 DNA lacks part of this fragment which is located in the early region of BKV DNA.

To examine whether the deleted regions could be located more precisely, we digested BKV DNA with the restriction endonuclease *Mbo*I which cleaved prototype BKV DNA into 12 fragments (one fragment, H in Fig. 3B, more than MM DNA as reported by Yang and Wu [24]). The fragments were then separated by electrophoresis and hybridized with nick-translated R1, R2, and BKV DNA separately. As can be seen in Fig. 3B, R1 DNA lacked J, K, and possibly E fragments of BKV DNA, which are

TABLE 1. *Sizes of R1, R2, and BKV DNA and their fragments produced by HindIII, AvaII and PvuII digestion*

Restriction endonuclease	RF fragment	Base pairs[a]	BK fragment	Base pairs[a]
None[b]	Linear R1	5,070	Linear	5,153
	Linear R2	4,540		
*Hin*dIII[b]	1A	1,680	A	2,301
	1B	1,530	B	1,925
	1C	1,110	C	554
	1D	710	D	416
		(5,030)		(5,153)
	2A	2,330		
	2B	870		
	2C	750		
	2D	570		
		(4,520)		
*Ava*II[b]	1A	1,510	A	1,152
	1B	1,150	B	931
	1C	1,040	C	783
	1D	930	D	769
	1E	440	E	694
		(5,070)	F	433
	2A	830	G	434
	2B	780		(5,153)
	2C	730		
	2D	690		
	2E	650		
	2F	440		
	2G	410		
		(4,530)		
*Pvu*II[b, c]	1A	3,540	A	3,245
	1B	1,480	B	1,951
		(5,020)		(5,153)
	2A	1,980		
	2B	1,820		
	2C	750		
		(4,550)		

[a] Totals in parentheses.

[b] Fragment sizes calculated by comparing the mobility of the fragments after electrophoresis in 1.2% agarose gels with BKV DNA markers on a semilog plot. Sizes of BKV DNA markers were determined from the base sequence of BKV DNA (17). To obtain the sizes of *Ava*II and *Hin*dIII fragments of R1 and R2 DNAs, labeled RFV DNA was subjected twice to electrophoresis in 1.2% agarose gels to separate form II DNAs of R1 and R2. These were cleaved, and the fragments were separated in 1.2% agarose and 4%/12% acrylamide gels with *Ava*II, *Hae*III, and *Hin*dIII fragments of BKV DNA used as markers.

[c] R1 and R2 DNAs were digested with *Pvu*II and BKV *Sst*I; *Pvu*II and *Pvu*II-*Kpn*I fragments were used as markers.

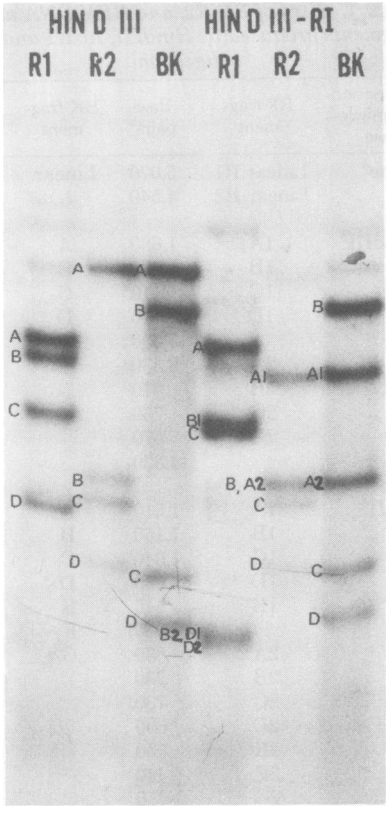

FIG. 2. *Cleavage of R1 and R2 DNAs with HindIII and combined HindIII-EcoRI. ^{32}P-labeled RFV DNA was run by electrophoresis as in Fig. 1 to separate R1 and R2 DNAs. The regions of the gel containing R1 and R2 bands were located by autoradiography and excised for electroelution. R1 and R2 DNAs thus separated and BKV DNA were cleaved with the restriction endonucleases HindIII and combined HindIII-EcoRI. These were run by electrophoresis through 1.2% agarose gels. The faint bands are due to partial digestion by the enzymes.*

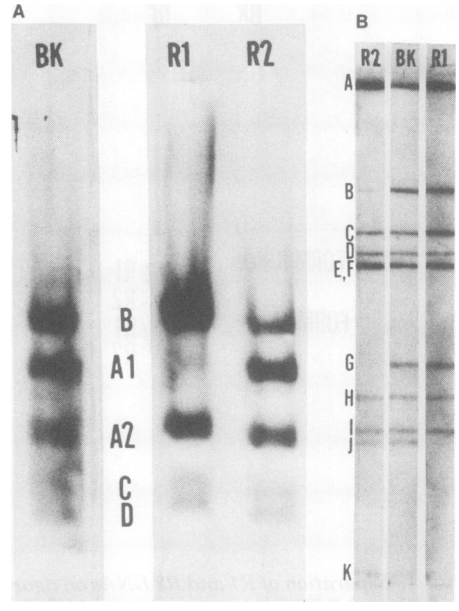

FIG. 3. *Hybridization of labeled R1 and R2 DNAs with unlabeled BKV DNA immobilized on filters. For Fig. 3A BKV DNA (100 ng per filter) was cleaved with the combined restriction endonucleases HindIII-EcoRI and separated on a 1% agarose gel. The DNA was then blotted onto nitrocellulose filters and hybridized separately to ^{32}P-labeled R1, R2, and BKV DNA, and the radioactive bands were detected by autoradiography. For Fig. 3B BKV DNA was cleaved with MboI, separated by electrophoresis through 4%/12% acrylamide gels, blotted onto diazobenzyloxymethyl paper, and hybridized with labeled R1, R2, and BKV DNAs. The slight hybridization of MboI B fragment of BKV DNA to ^{32}P-labeled R2 DNA and of HindIII-EcoRI fragment A1 to R1 DNA could be due to minor cross-contamination of R1 and R2 DNA.*

in the coding part of the late region (Fig. 4). R2 DNA lacked all of G and B and part of C fragments of BKV DNA, which are in the DNA coding for T antigen in the early region (Fig. 4).

Restriction endonuclease analysis of R1 and R2 DNAs. The results presented in the previous section revealed that R1 DNA lacked approximately 50% of the late region and R2 DNA lacked approximately 50% of the early region of BKV DNA. It was, therefore, of interest to see whether other changes due to alterations in base sequences could also be detected by analysis with restriction endonucleases. To examine this possibility, we cleaved ^{32}P-labeled R1 and R2 DNAs with several restriction enzymes and separated the resulting fragments by electrophoresis through agarose and acrylamide gels. The results of digestion with restriction endonucleases *Hin*dIII and combined *Hin*dIII-*Eco*RI are shown in Fig. 2. As can be seen, *Hin*dIII cleaved both R1 and R2 DNA into four fragments. The sizes of all four *Hin*dIII fragments of R1 DNA were different from those of R2 and BKV DNA (Fig. 2 and Table 1). *Hin*dIII fragment A of R2 DNA was the same size as *Hin*dIII fragment A of BKV DNA. However, the sizes of the other three *Hin*dIII fragments of R2 DNA were different from those of BKV DNA.

*Eco*RI cleaved *Hin*dIII fragment A of BKV DNA once, producing the two smaller fragments, A_1 and A_2 (Fig. 2). *Eco*RI also cleaved *Hin*dIII fragment A of R2 DNA into two smaller fragments, A_1 and A_2, which were the same size as *Hin*dIII-*Eco*RI fragments A_1 and A_2 of BKV DNA (Fig. 2 and Table 1). *Eco*RI cleaved each

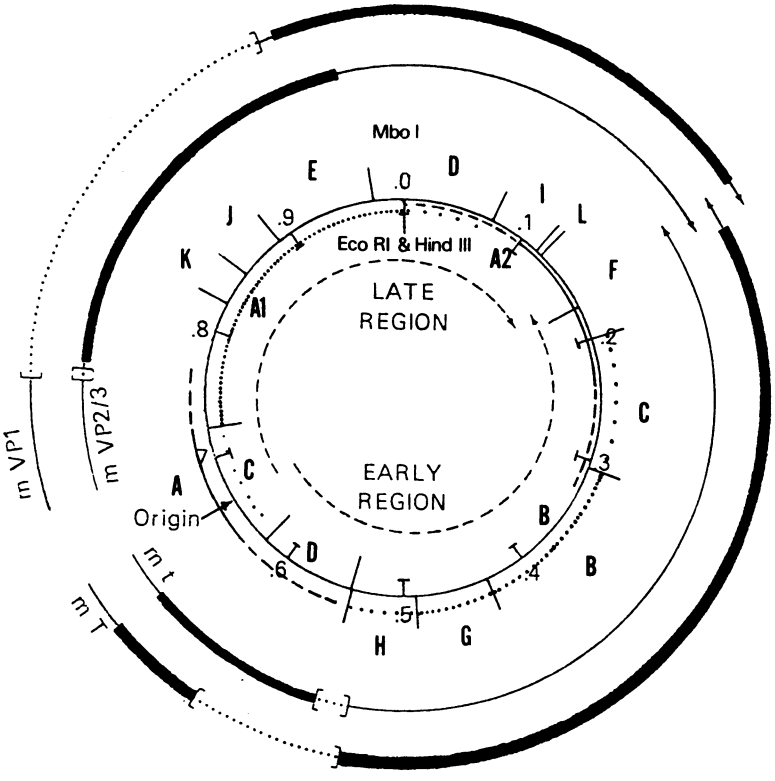

FIG. 4. *Physical map of BKV DNA depicting the deletions and insertions in R1 and R2 DNAs. Data showing the origin of replication, early and late regions, and mRNAs and their proteins are from Howley (6) and Seif et al. (17). MboI and EcoRI & HindIII maps were determined from the BKV DNA sequence (17). The regions of the genome known to be deleted in R1 and R2 DNAs are indicated by closely dotted lines, and the regions possibly deleted are indicated by dotted lines. The regions of the genome known to be repeated are indicated by solid lines, and the regions possibly repeated are indicated by dashed lines. The lines indicating insertions and deletions for R1 are inside the circular map; those for R2 are outside the circular map. The extent of the deletions was determined from the data in Fig. 3 and also from comparable hybridization data obtained with AvaII-digested BKV DNA (data not shown). The extent of the repetitions was determined from Fig. 6.*

of *Hin*dIII fragments B and D of R1 DNA into two smaller fragments.

We also cleaved nick-translated R1 and R2 DNAs with the restriction endonucleases *Ava*II and *Pvu*II and then separated the fragments by electrophoresis (Fig. 5). *Ava*II cleaved R1 DNA into five fragments and R2 DNA into seven fragments. The sizes of two of five *Ava*II fragments of R1 DNA were different from those of BKV DNA and the sizes of two of seven *Ava*II fragments of R2 were different from those of BKV DNA (Table 1). *Pvu*II cleaved BKV and R1 DNAs twice and R2 DNA three times. Only *Pvu*II fragment A of R2 DNA was the same size as the *Pvu*II fragment B of BKV DNA (Table 1).

Map location of *Hin*dIII, *Eco*RI, *Ava*II, and *Pvu*II fragments of R1 and R2 DNAs. The locations of *Hin*dIII fragments of R1 and R2 DNAs were determined by partial digestion of separated R1 and R2 DNAs (7). Table 2 shows some of the partial digestion products and the redigestion products of these partial digestion products which were used for mapping. The products of *Hin*dIII partial digestion containing *Eco*RI sites were redigested with *Eco*RI, and from the molecular weights of the resulting fragments (Table 2) the position of *Eco*RI sites on each DNA species was determined. The order of *Hin*dIII-*Eco*RI fragments for R1 DNA was B1-D1-D2-C-A-B2 and for R2 DNA was A2-D-B-C-A1.

To map the position of *Ava*II and *Pvu*II fragments, we digested nick-translated R1 and R2 DNAs with each of the *Hin*dIII, *Ava*II, and *Pvu*II enzymes. The gel-separated fragments were then electroeluted and redigested with the other two enzymes. From the size of the resulting

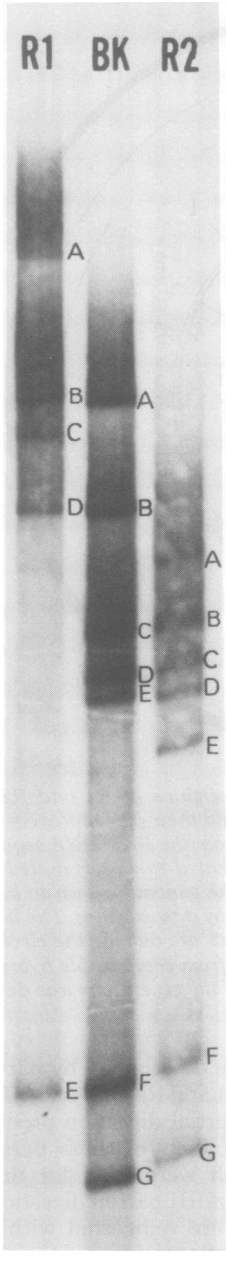

FIG. 5. *Cleavage of R1 and R2 DNAs with the restriction endonuclease AvaII. Labeled R1 and R2 DNAs were separated as in Fig. 1 and cleaved with AvaII; the fragments were separated by electrophoresis through 4% acrylamide gels at 180 V for 16 to 20 h. R1 DNA fragments are designated as 1A, 1B, 1C, 1D, and 1E. R2 DNA fragments are 2A, 2B, 2C, 2D, 2E, 2F, and 2G.*

fragments (Table 3) and the application of the overlap analysis (2), the order of fragments produced by *Ava*II and *Pvu*II was determined. The exception was the ordering of *Ava*II fragments B and G of R2 DNA, in which the positions were mapped by the additional digestion of these fragments by *Eco*RI. The map of all the four enzymes for R1 and R2 DNA is shown in Fig. 6.

TABLE 2. *Redigestion of partial HindIII digestion products of R1 and R2 with HindIII and EcoRI*[a]

Partial HindIII product[b]	Endonuclease of redigestion	Products obtained
1_1	*Hin*dIII	1A, 1B, 1C
1_2	*Hin*dIII	1A, 1B, 1D
1_5	*Hin*dIII	1A, 1B
1_5	*Eco*RI	2,100 and 1,120 base-pair fragments
1_6	*Hin*dIII	1A, 1C
1_7	*Hin*dIII	1B, 1D
1_7	*Eco*RI	1,460, 370, and 350 base-pair fragments
1_8	*Hin*dIII	1C, 1D
1_8	*Eco*RI	1,450 and 370 base-pair fragments
2_1	*Hin*dIII	2A, 2B, 2C
2_5	*Hin*dIII	2A, 2C
2_5	*Eco*RI	2,220 and 870 base-pair fragments
2_6	*Hin*dIII	2A, 2D
2_6	*Eco*RI	1,450 and 1,430 base-pair fragments
2_7	*Hin*dIII	2B, 2C
2_8	*Hin*dIII	2B, 2D

[a] Redigestions were subjected to electrophoresis in 1.2% agarose gels with RFV *Hin*dIII markers.

[b] R1 and R2 were first purified twice by electrophoresis in 1.2% agarose gels. They were then partially digested by *Hin*dIII, and fragments were separated by electrophoresis in 1.2% agarose gels. Partial digestion products were designated according to size, 1_1 being the largest product of R1, 2_5 being the fifth largest product of R2, etc.

DISCUSSION

Data presented in this report show that plaque-purified RFV DNA (at least the virus grown in this laboratory) consists of two molecular species. Although the experiments reported in this paper used DNA from one RFV stock [Fig. 7, RF(C)], it should be noted that the DNAs isolated from three different virus preparations which had been grown independently in three different years were identical, as shown by their DNA band pattern when cleaved with restriction endonuclease *Hin*dIII (Fig. 7). Also, the relative amounts of R1 and R2 DNA varied from one preparation to another.

In Fig. 6 the map of R1 DNA shows similarities to both BKV and R2 DNAs. From the *Pvu*II fragment at 820 base pairs to the *Ava*II fragment at 3,540 base pairs, R1 is the same as BKV with

TABLE 3. *Redigestion of AvaII, HindIII, and PvuII fragments of R1 and R2 with AvaII, HindIII, and EcoRI*

First restriction endonuclease	Fragment[a]	Redigestion restriction endonuclease	Products of redigestion[b]
*Ava*II	1A	*Hind*III	1,440 and 130 base-pair end pieces
	1B		1,070 and 60 base-pair end pieces
	1C		Undigested
	1D		1D and 130 and 60 base-pair end pieces
	1E		Undigested
	2A		420 and 380 base-pair end pieces
	2B		Undigested
	2B	*Eco*RI	760 and 20 base-pair end pieces
	2C	*Hind*III	2D, 120, and 20 base-pair end pieces
	2D		Undigested
	2E		435 and 245 base-pair end pieces
	2F		Undigested
	2G		Undigested
*Hind*III	1A	*Ava*II	1C, 1E, and 130 and 70 base-pair end pieces
	1B		1,460 and 130 base-pair end pieces
	1C		1,000 and 80 base-pair end pieces
	1D		Undigested
	2A		2B, 2D, 2G, and 240 and 135 base-pair end pieces
	2B		2F and a 405 base-pair end piece
	2C		365 and 405 base-pair end pieces
	2D		Undigested
*Pvu*II	1A	*Ava*II	1B, 1D, 1E, and 830 and 220 base-pair end pieces
	1B		1,300 and 230 base-pair end pieces
	2A		2B, 2G, and 470 and 250 base-pair end pieces
	2B		2C, 2F, and 380 and 230 base-pair end pieces
	2C		400 and 390 base-pair end pieces
	1A	*Hind*III	1C, 1D, and 1,400 and 340 base-pair end pieces
	1B		1,170 and 330 base-pair end pieces
	2A		2,000 and 15 base-pair end pieces
	2B		2B, 2D, and 380 base-pair end pieces
	2C		750 and 15 base-pair end pieces

[a] *Ava*II and *Hind*III fragments were prepared by two methods. For the first method each of R1 and R2 DNAs was first purified twice by electrophoresis in 1.2% agarose gels and was then digested; fragments were isolated from a third 1.2% agarose gel. For the second method RFV DNA was digested with either *Ava*II or *Hind*III, and fragments were purified on 4%/12% acrylamide gels. For the latter method *Ava*II fragments 1E and 2F appeared as one band, and redigestion gave results consistent with those for separate fragments isolated by the first method. *Pvu*II fragments were isolated from 1.2% agarose gels.

[b] *Ava*II and *Hind*III redigestion products were analyzed on the type of gel used to isolate the fragments. *Pvu*II redigestion products were analyzed on 4%/12% acrylamide gels. Agarose gels contained *Ava*II and *Hind*III fragments of R1 and R2 as markers; acrylamide gels contained R1 and R2 products of the first restriction enzyme as well as BKV DNA digested with *Hae*III and *Hind*III-*Eco*RI.

the exception of three restriction enzyme site changes (an additional *Eco*RI site at 1,530 base pairs, an additional *Hind*III site at 1,780 base pairs, and a missing *Hind*III site at about 3,480 base pairs). This homology includes part or all of the BKV *Hind*III-*Eco*RI fragments B, A_2, C, and D, but not A_1, in agreement with the hybridization results of Fig. 3. The last part of the R1 map (4,350 to 5,060 base pairs) is a repeat of the pattern in the region of 820 to 1,530 base pairs. This part of the map includes single *Pvu*II, *Ava*II, *Hind*III, and *Eco*RI sites, which suggests that this region of R1 DNA is repeated. Such a hypothesis is consistent with the observations that a large part of R1 DNA did not hybridize to BKV DNA (Fig. 3A and B) and that R1 DNA was only slightly smaller than BKV DNA (Table 1).

R2 DNA contained a region in which all restriction enzyme sites were the same distance apart as they are in BKV DNA. This region extended rightward from 3,100 base pairs, through the *Eco*RI site, and continued to 890 base pairs on the R2 map. This region contained DNA homologous to *Hind*III-*Eco*RI fragments A1, A2, and C of BKV DNA, in agreement with the hybridization data of Fig. 3. The presence of *Hind*III-*Eco*RI fragment D in Fig. 3 was also expected because *Ava*II fragment F of BKV DNA (which contains *Hind*III fragment D) and *Ava*II fragment F of R2 DNA appear to be homologous. But in the remainder of the R2 map the *Hind*III site at 890 base pairs was much closer to the *Ava*II fragment F than the corresponding *Hind*III site is to the *Ava*II fragment in the BKV map. This suggests a big deletion in R2 DNA within *Hind*III-*Eco*RI fragment B, in agreement with the hybridization results of Fig. 3. A repeat pattern of *Ava*II, *Pvu*II, and *Hind*III sites was seen between 1,920 to 2,340 base pairs and 2,720 to 3,120 base pairs in R2 DNA, a region in which BKV DNA (1,920 to 2,340) contains the origin of replication. Such an insertion of approximately 400 base pairs was expected because the deletion in R2 DNA (Fig. 4) was at least 20% of the BKV genome, whereas R2 DNA was only 15% smaller than BKV DNA (Table 1).

Another relevant observation can be made by comparing the R2 map with the R1 and BKV maps. One of the three restriction enzyme site changes (at 1,530, 1,780, and 3,480 base pairs on the R1 map, as discussed earlier) observed in homologous regions of R1 and BKV DNA is not within the deleted region of R2 DNA. One *Hind*III site observed in BKV DNA is missing from its expected position at 3,480 base pairs on the R1 map. The map of R2 DNA also lacks this *Hind*III site (expected at 1,860 base pairs, within

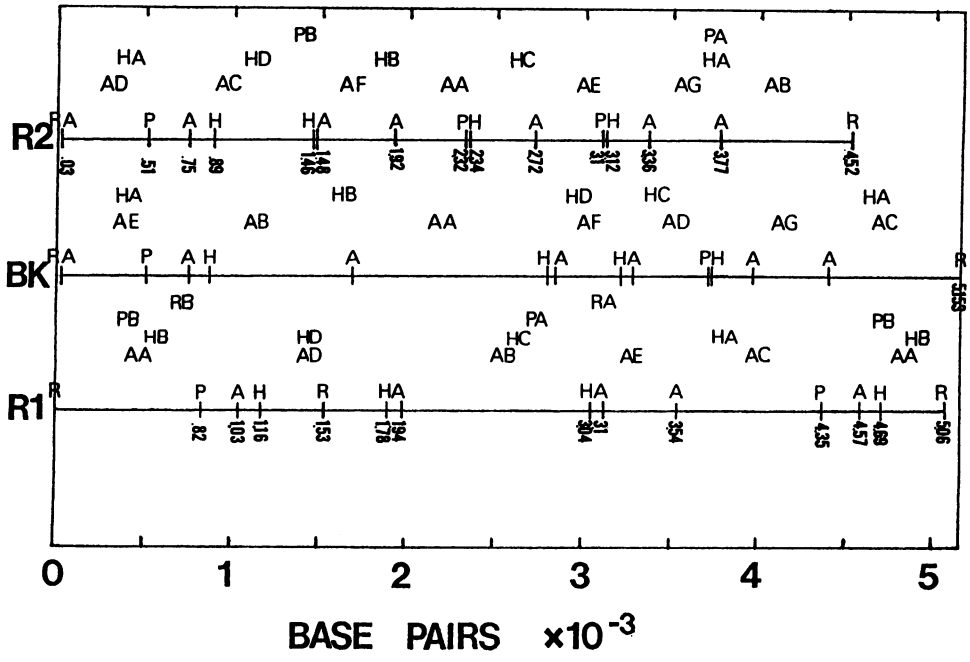

FIG. 6. *Ordering of the fragments produced by the cleavage with restriction endonucleases EcoRI, HindIII, AvaII, and PvuII. Since R1, R2, and BKV DNAs are of different sizes, the distances between the cleavage sites are given in base pairs rather than map units to allow direct comparison. (The left to right orientation in this map corresponds to the clockwise direction of the circular map in Fig. 4.) The site of cleavage for HindIII on the DNA is designated as H, for AvaII as A, for EcoRI as R, and for PvuII as P. The HindIII fragments are designated as HA, HB, HC, and HD. AvaII fragments are designated as AA, AB, AC, AD, etc. PvuII fragments are designated PA, PB, and PC. The map of restriction sites for BKV DNA was determined from the DNA sequence (17).*

*Ava*II fragment F). This suggests that both R1 and R2 DNAs present in RFV DNA could be derived from a single parental variant of BKV which differs from all BKV variants known to date. Based on Fig. 6, this putative parental variant would be expected to contain two *Eco*RI sites and to give four *Hin*dIII fragments, of which only the largest corresponded to a *Hin*dIII fragment of BKV DNA.

Defective animal viruses have been shown to arise during the cultivation of many viruses (8). The defective forms of simian virus 40 (SV40) have been used to elucidate the organization and expression of the SV40 genome (1, 16). Defective SV40s with deletions in the late region have been shown to be able to replicate their DNA in the absence of helper, to stimulate DNA synthesis of the host cell DNA, to produce tumor antigen, and to transform permissive and semipermissive cells with the same efficiency as the wild type. Defective SV40 with deletions in the early region cannot perform any of these functions. Thus, the early region of SV40 is necessary for these functions. The location of the early region of BKV DNA has been deduced from DNA sequence analysis (6, 17, 25). The biological functions of the early and late regions can now be best assigned by using each RFV DNA species for infection. These studies are presently under way.

The deletion in the R1 DNA is within 0.63 to 0.07 map unit on the BKV genome, which is in the coding region for the late genes (Fig. 4). The deletion in the R2 DNA is within 0.2 to 0.54 map unit on the BKV genome, the region coding for large T antigen but not small t antigen (Fig. 4). The small t antigen of BKV is not necessary for viral propagation or cell transformation (6). The biological functions of both large T and small t antigens will be further investigated by using R2 DNA alone and in combination with other BKV-type DNAs.

Since R1 DNA lacks part of the coding region for viral capsid proteins and R2 DNA lacks part of the coding region for the large T antigen, it is apparent that R1 and R2 DNAs are defective molecules and that they complement each other during infection. This phenomenon is being in-

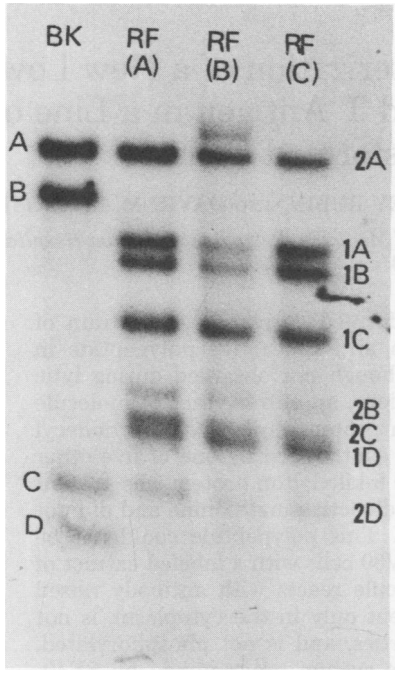

FIG. 7. *HindIII restriction pattern of three RFV DNA preparations after cleavage with HindIII and separation by electrophoresis in 1.2% agarose gels. RF(A) DNA was isolated from virus prepared in 1975, RF(B) DNA was from virus grown in 1976, and RF(C) DNA was from virus grown in 1978. The extra band in RF(B) DNA is either a partial digestion product or a fragment of a third DNA species.*

vestigated by infecting permissive cells with each of the DNA species separately and in combination.

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