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Comparative Analysis of GS and BK Virus Genomes

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Analysis of heteroduplexing between the genomes of GS virus, a BK-group virus, and the prototype BK virus revealed one region of nonhomology. Further analysis by cleavage of viral DNA with the restriction endonucleases *Eco*RI, *Hin*dIII, and *Hae*III revealed that base changes in the GS virus genome spanned 0.6 to 0.7 map unit from the *Eco*RI site. Large T and small t antigens of GS virus appear to be similar in size to the BK virus antigens.

Serological studies have shown that 80 to 100% of the adult human population has antibodies against the papovavirus BK virus (11, 19, 26). The virus has been isolated only from the urine of renal transplant patients (7, 12) and from the urine or brains of patients with the Wiskott-Aldrich syndrome (13).

Two BK-related viruses (JM and MM) were isolated (13) from the urine of two patients with Wiskott-Aldrich syndrome, a disease characterized by both humoral and cellular immunodeficiencies. These isolates were shown to be identical serologically to the prototype BK virus. Although the genomes of these viruses were shown to be identical to that of BK virus by reassociation kinetics, differences were found in the number of restriction sites in one of the isolates, MM (13). Another BK-like virus, RF, has also been isolated from the urine of a kidney transplant patient and has been shown to be immunologically indistinguishable from BK virus (18). The genome of this virus was shown to share 88% homology with BK virus (18). Minor differences were found, however, in the peptide patterns of two major capsid proteins (31).

Another BK-type virus, GS, isolated from the urine of a kidney transplant patient by S. D. Gardner, has been shown to be serologically identical to BK virus (31). Also, the tryptic peptides of two major virion proteins were indistinguishable. In this study we compared the DNA structure of this isolate to that of BK virus and found a lack of homology in a small region of the genome.

MATERIALS AND METHODS

Cells and virus. BK and GS viruses were grown in human embryonic kidney cells cultured in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum. The cells were infected with 0.01 PFU of plaque-purified virus per cell to eliminate the accumulation of defective viruses (32). The viruses were banded in CsCl twice and resuspended in TNE buffer (10 mM Tris-10 mM NaCl-1 mM EDTA, pH 8).

Preparation of viral DNA. The viruses were lysed in 1% sodium dodecyl sulfate, extracted once with phenol and twice with chloroform-isoamyl alcohol (24:1, vol/vol), and precipitated with ethanol. Viral DNA was banded in CsCl (1.56 g/cm^3)-ethidium bromide (200 μg/ml) as described by Radloff et al. (22). Form I DNA was collected and further purified in a 5 to 20% neutral sucrose gradient. The DNA was ethanol precipitated and resuspended in TNE.

In vitro labeling of viral DNA. Viral DNAs were labeled in vitro with ^{32}P by the nick-translation procedure (15, 16, 24) without DNase. Specific activities were about 2×10^7 to 4×10^7 cpm/μg. Labeled DNA was separated from the free triphosphate on Sephadex G50.

Cleavage of BK and GS virus DNA with restriction endonucleases. Samples of labeled DNA (about 10^5 cpm per gel slot) were digested with various restriction endonucleases. Restriction endonuclease *Eco*RI was prepared by the method of R. N. Yashimori (Ph.D. thesis, University of California, San Francisco, 1972). *Hin*dIII was prepared by chromatography on substituted Sepharose. The reaction mixture for digestion with *Hin*dIII contained 7 mM Tris-hydrochloride, pH 7.5, 10 mM $MgCl_2$, 7 mM 2-mercaptoethanol, 60 mM NaCl, and 0.5% bovine serum albumin. The reaction mixture for combined *Hin*dIII-RI was the same except that it contained 0.1 M Tris-hydrochloride and no 2-mercaptoethanol. Incubations were for 1 h at 37°C in the presence of 2 to 3 U of enzyme. The reaction mixture for *Hae*III (New England Biolabs) was the same as that for *Hin*dIII except that the concentration of NaCl was lowered to 6 mM. Electrophoresis was in a buffer containing 50 mM Tris-acetate, pH 7.8, 20 mM sodium acetate, and 2 mM EDTA. *Hin*dIII and *Hin*dIII-RI fragments were separated by electrophoresis in 1% agarose gels for 3 h at 120 V. *Hae*III fragments were separated by electrophoresis in a composite gel consisting of 4% polyacrylamide in the top two-thirds and a 12% polyacrylamide trap in the bottom for 12 to 16 h at 150 V.

Heteroduplexing. Viral DNA (2 μg) was digested with *Eco*RI for 1 h at 37°C. The DNA was precipitated with ethanol and resuspended in Tris-hydrochloride

(0.1 M, pH 7.3). The DNA was denatured by boiling for 4 min and cooling rapidly on ice-ethanol. Tris and NaCl were added to a final concentration of 0.1 M and 0.3 M, respectively. Reannealing was at 68°C for 10 min. Single-stranded DNA was digested with 300 U of S1 nuclease (21) for 30 min at 25°C. S1 nuclease, free from other nucleases, was prepared by a slight modification of the method of Vogt (30). The electrophoresis of the reannealed DNA was in 1% agarose as described above.

Analysis of viral tumor antigens. HEK cells were infected with each virus at 10 PFU/cell and then incubated for 96 h at 37°C. They were then labeled for 2 h at 37°C with 180 μCi of [^{35}S]methionine per ml in methionine-free Dulbecco modified Eagle medium. Antigens were extracted and gels were run as described by Rundell et al. (25). *Staphylococcus aureus* was used for immunoprecipitation as described by Cullen and Schwartz (5).

RESULTS

Sequence homology between the genomes of BK and GS viruses by heteroduplexing. Equal amounts of linear BK and GS virus DNAs (1 μg of each) were mixed, denatured, and renatured. The reannealed DNA was digested with single-strand-specific S1 nuclease and electrophoresed on agarose gels. The assumption was that, if all the regions of the two viral DNAs were homologous, then only one band would be detected since the size of the linear molecule is the same for both viruses. However, if certain regions of the two genomes were nonhomologous, these single-stranded regions would be cleaved and additional bands would be detected. From the number of bands obtained one could calculate the number of the nonhomologous regions.

Figure 1 shows that S1 nuclease digested the denatured DNA and had no nicking activity for supercoil DNA. Only one band, the linear molecule, was obtained when *Eco*RI-treated GS and BK virus DNA were denatured, reannealed, and then treated with S1. However, three bands were observed when equal amounts of BK and GS virus DNAs were mixed together and analyzed with S1. This means that the largest, linear-sized molecule is a homoduplex for each of BK and GS virus DNAs. The sizes of the two additional bands were calculated to total up to the size of BK virus genome, when linear BK virus DNA and *Hin*dIII fragments A and B were used as markers (Fig. 2).

Restriction endonuclease cleavage of BK and GS virus DNAs. The results of heteroduplexing of the *Eco*RI-digested mixed BK and GS virus DNAs indicated that the region of nonhomology between the genomes of these two viruses was restricted to one specific area. Heteroduplexing of the *Hpa*II-treated DNAs produced only one band (data not shown), indicating that the region of nonhomology had to be close to the *Hpa*II site (0.69 map unit). For *Hin*dIII, which cleaves BK virus DNA into four fragments, A, B, C, and D (13), the site of cleavage for *Hpa*II is in fragment C. To examine the areas containing base differences, both DNAs were cleaved with *Hin*dIII, and Fig. 3 shows that only three fragments, A, B, and C-D,

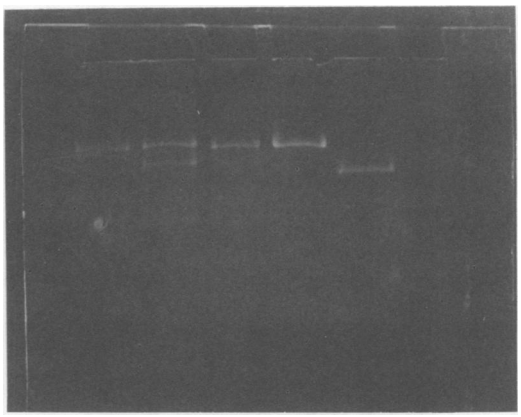

FIG. 1. *Heteroduplexing of EcoRI-treated (except where indicated) BK and GS virus DNAs. Samples were S1-treated and separated in 1% agarose gels. From left to right: reannealed BK DNA, mixed and reannealed BK-GS DNAs, reannealed GS DNA, native GS DNA, reannealed form I BK DNA, and denatured but not reannealed BK DNA.*

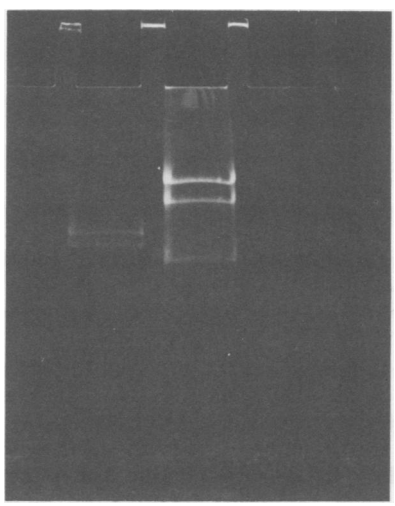

FIG. 2. *Size determination of BK-GS virus heteroduplex fragments obtained after digestion with S1 nuclease. Electrophoresis was in a 1% agarose gel. On the left, HindIII fragments A and B of BK DNA; on the right, reannealed BK-GS DNA.*

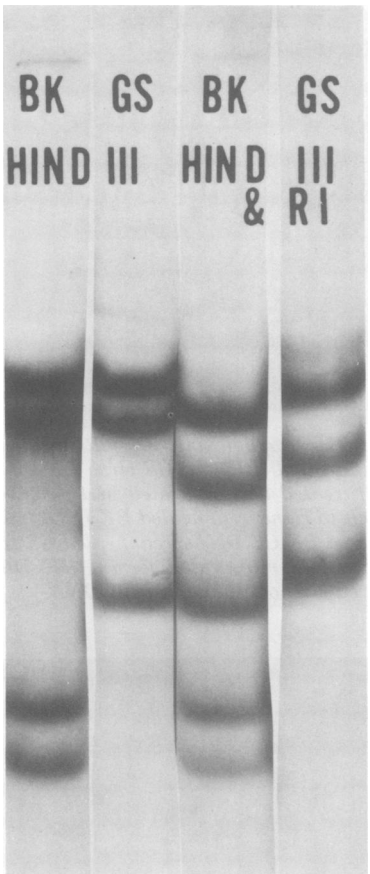

FIG. 3. *Electrophoretic patterns of BK and GS virus DNA after digestion with HindIII and HindIII-RI restriction endonucleases. Separation was in a 1% agarose gel.*

were obtained for GS virus DNA. The size of the C-D fragment was equal to fragment A_2, found after the digestion of BK virus DNA with combined *Hin*dIII-*Eco*RI; this confirms that the C-D fragment is approximately the size of the sum of the *Hin*dIII fragments C and D of BK virus DNA. Thus, base changes occurred both at one of the *Hin*dIII sites (0.62 map unit) and also near the *Hpa*II site (0.69 map unit).

*Hae*III cleaves BK virus DNA into 21 fragments. The position of each fragment has been determined on the physical map of BK virus DNA (10). To determine more exactly the region containing base differences between BK and GS virus genomes, we digested the DNA from these viruses with this enzyme. As can be seen in Fig. 4, BK virus DNA fragments H, M, and O were missing from the GS virus DNA and two new fragments appeared instead. The total molecular weight of these two new fragments was calculated to be the same as the sum of the molecular

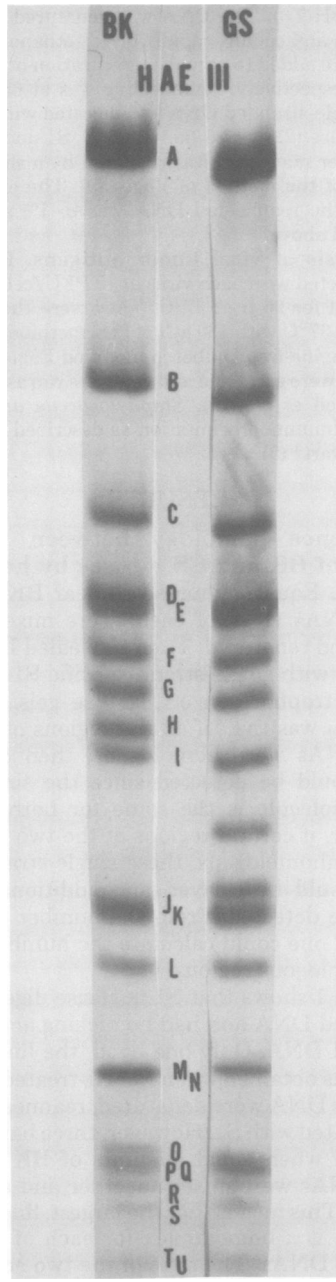

FIG. 4. *Electrophoretic patterns of the digestion products of BK and GS virus DNAs with restriction endonuclease HaeIII. Separation was in a 4/12% composite polyacrylamide gel.*

weights of the H, M, and O fragments. This suggests that the region containing base changes is within *Hin*dIII fragment C and extends slightly into *Hin*dIII fragment D or from about 0.62 to 0.69 map unit from the *Eco*RI site (Fig. 5).

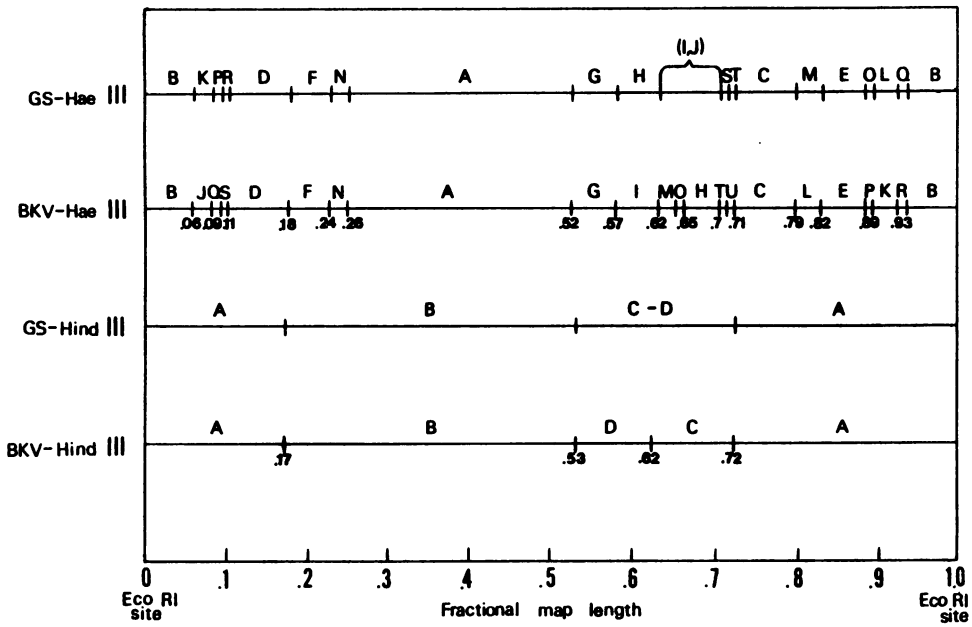

FIG. 5. *Comparison of the physical maps of BK and GS virus DNAs generated by digestion with restriction endonucleases HindIII and HaeIII.*

Tumor antigens in BK and GS virus-infected cells. The early region of simian virus 40 (SV40) DNA is known to code for at least two antigens, large T and small t. These antigens share N-terminal regions between 0.65 and 0.59 map unit from the *Eco*RI site (1, 4, 9, 14, 23). The early region of BK virus also codes for both large T and small t (6, 27). Since the region of nonhomology between BK and GS virus DNAs is in the early region (0.62 to 0.69 map unit), it was of interest to compare the amount and the size of the two antigens in infected HEK cells. As can be seen from Fig. 6, both large T and small t were expressed in about the same amount after infection and were the same size for both viruses. Thus, the region of nonhomology does not appear to alter the expression of either large T or small t.

DISCUSSION

Differences in genome organization have been found between the prototype BK virus and the variants isolated later (13). The question then is whether the changes in the genome found in the variants are random or restricted to certain regions. To answer this question, we undertook comparative studies of the genome of another BK virus variant, GS, which was also isolated from the urine of a renal transplant patient by Gardner and was shown to be serologically identical to BK virus (31). The tryptic peptides of two major capsid proteins of the two viruses have also been shown to be identical (31).

The results of the heteroduplexing and S1 digestion of the GS and BK virus DNA revealed one area of nonhomology between the two genomes. Analysis with restriction endonuclease *Hin*dIII revealed that the changes expanded into the junction between the *Hin*dIII fragments C and D of the prototype BK virus. Digestion with *Hae*III revealed that the area containing base differences is within 0.62 to 0.69 map unit from the *Eco*RI site.

Linear molecules of full genome size were obtained when heteroduplexes formed between *Hpa*II-treated BK and GS virus DNAs were digested with S1. Also, the combined sizes of the two heteroduplex fragments (70 and 30% of the genome) of *Eco*RI-treated, S1-digested BK-GS DNAs accounted for the total genome. This means that a very small region of the two genomes, close to the *Hpa*II site, is extensively nonhomologous and is removed after digestion with S1 nuclease. Additional base changes, which are not recognized by S1 nuclease under our conditions, span 7% of the genomes as shown by the comparison of the DNA fragments of GS and BK virus after cleavage with *Hae*III. The base changes in this region result in the loss of two *Hae*III restriction sites and the appearance of a new one. The number and the position of base changes can only be determined by DNA sequencing.

The area containing base changes between BK virus and another variant, MM, is also in the same region. The *Hae*III maps of both GS

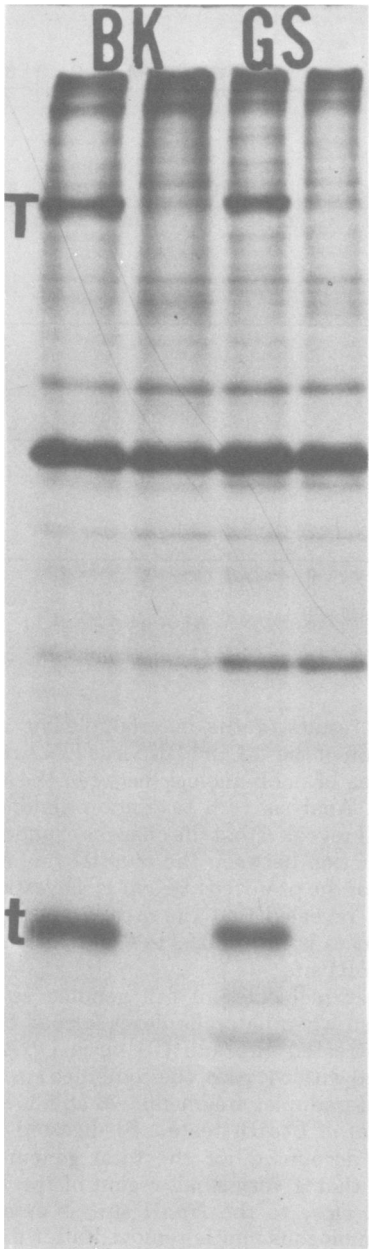

FIG. 6. *Comparison of the electrophoretic mobilities of large T and small t from BK and GS virus-infected cells. [^{35}S]methionine-labeled cell extracts were incubated in the presence of normal and anti-SV40 T serum followed by precipitation by Staphylococcus aureus. Precipitated proteins were subjected to electrophoresis through a 20% polyacrylamide slab gel, and the labeled protein bands were detected by autoradiography of the dried gel. The gel slots without antigen bands are from extracts precipitated with normal serum.*

(Fig. 5) and MM (33) differ from BK virus within and only within the map units of 0.57 to 0.71. This is within the *Hin*dIII fragments C and D of BK virus DNA. In this region both BK and MM viruses have four restriction sites for *Hae*III, but at least two and maybe four of these sites are at different locations on the map. GS shares two of four *Hae*III sites with BK virus and has one new site in this same region. All 15 *Hae*III sites outside the 0.57 to 0.71 region are identical for MM, GS, and BK viruses. It would be of interest to analyze other isolates to see whether this region of the BK virus genome is more susceptible to change in base sequences and whether these changes are of any significance in the evolution of the virus.

The small region of nonhomology (due to deletion or mismatching) detected by heteroduplexing is close to 0.69 map unit. This lies outside the early gene coding region. In both SV40 and BK virus genomes there is a noncoding region consisting of a set of tandem repeat sequences at approximately 0.69 map unit (6, 23). Deletions of portions of these tandem repeat sequences do not affect the viability of SV40 (23).

The origin of replication for BK virus is located around 0.67 map unit from the *Eco*RI site (P. M. Howley, M. A. Martin, C. J. Lai, and G. Khoury, personal communication), which is the same as in SV40 (see review by Fareed and Davoli [8]). The area of nonhomology between the BK and GS virus genomes contains this important region. Since GS replicates to the same extent in HEK cells as does BK virus, it is obvious that the functionality of the origin of replication is not affected by the possible base changes in this region. This 0.62 to 0.69 map region of the SV40 and BK virus genome also includes part of the coding region for large T and small t, which are involved in transformation (2, 3, 6, 17, 20, 27, 29, 30). The size and the amount of large T and small t in the GS virus-infected cells are the same as those in the BK virus-infected cells.

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