Genome Analysis of MG Virus, a Human Papovavirus

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The genome of MG virus, a variant of the human papovavirus BK virus, consists of two molecules, M1 and M2. M1 and M2 have deletions which correspond to 0.33 to 0.55 map unit and 0.77 to 0.85 map unit, respectively, from the *EcoRI* site on the BK virus genome. Restriction enzyme analysis of the DNAs of these viruses revealed many alterations in both DNA species. Both M1 and M2 DNAs have three recognition sites for *EcoRI*. M1 has a single recognition site for *HindIII* and five sites for *PvuII*. M2 has a single recognition site for *PvuII* and three sites for *HindIII*. The sites of these and several other restriction enzymes on each DNA molecule were mapped after the cloning of M1 and M2 DNAs into pBR322 at their unique *HindIII* and *PvuII* sites, respectively.

The human papovavirus BK virus (BKV) was first isolated from the urine of a kidney transplant patient by Gardner et al. in 1971 (3). Since then several variants of this virus have been isolated (2, 7, 13, 16). Although the variants are antigenically similar to BKV, differences have been found at the DNA level. DNA from two variants, GS and MM, have base differences around the origin of replication, about 0.6 to 0.7 map unit from the *EcoRI* site (11, 15, 18). The DNA from another isolate (JL) has an additional *EcoRI* site (13). The DNA of RF virus has 88% homology to BKV DNA (9), and its genome consists of two complementary defective molecules (12). A rather different virus, MG virus (MG), has been isolated by Lecatsas et al. (6). Antisera against BKV react weakly with this virus. Also, comparison of the peptides from the capsid proteins of the two viruses has revealed several differences (17). In the present study we compared the DNA of the MG and BK viruses and found that the genome of the MG consists of two molecules. We detected extensive base changes in both DNA species.

Plaque-purified BK and MG viruses were prepared, and their DNAs were prepared as before (11). Viral DNAs were nick translated (8, 11, 14), and the sizes of the two DNAs were compared. As can be seen in Fig. 1, the linear form of MG DNA is slightly smaller than that of BKV DNA. We digested MG DNA with *EcoRI*, which cleaves BKV DNA at a single site (5). As can be seen (Fig. 1), *EcoRI* cleaved MG DNA into six fragments. The total molecular weight of the fragments added up to more than that expected for a linear molecule of the size of BKV DNA. Digestion with *SstI* produced seven fragments

(Fig. 1). The total molecular weight of these fragments was also larger than that expected for a BKV size genome.

We then digested MG DNA with the restriction enzymes *HindIII*, *PvuII*, and *XbaI*. As can be seen (Fig. 2), digestion of MG DNA with *HindIII* produced four fragments, one of which (M1) has the same size as the linear MG DNA. The combined sizes of the other fragments (2A, 2B, and 2C) were also the same size as the linear MG DNA. Digestion of MG DNA with *PvuII* produced six fragments (Fig. 2). One of the fragments (M2) was the same size as the linear MG DNA. The combined sizes of the other five fragments (1A, 1B, 1C, 1D, and 1E) were the same size as the linear MG DNA. Digestion of MG DNA with *XbaI* (Fig. 2) produced three fragments. The size of one fragment (M1) was the same as the linear MG DNA, and the combined sizes of the other two fragments (2A and 2B) were the same size as the linear MG DNA. These results suggest that MG DNA consists of two molecules (M1 and M2), which are of equal size, but have different numbers of sites for various restriction enzymes.

To assign the various fragments produced by cleavage with the restriction enzymes used in Fig. 1 and 2 to each DNA molecule, M1 and M2 DNAs were first cloned. M1 DNA was cleaved at its unique *HindIII* site and ligated to *HindIII*-cleaved pBR322 DNA. M2 DNA was cleaved at its unique *PvuII* site and blunt-end ligated to *PvuII*-cleaved pBR322 DNA and used to transform *Escherichia coli* strain HB101. The resulting ampicillin-resistant colonies were screened by the colony hybridization method of Grunstein and Hogness (4) with slight modifications. Pos-

FIG. 1. Cleavage of MG DNA with the restriction endonucleases RI (EcoRI) and SstI. Nick-translated (8, 11, 14) MG DNA was cleaved with EcoRI and SstI and run by electrophoresis through 1.2% agarose gels. BKV DNA was cleaved with the combined EcoRI-HindIII and run as marker. Undigested MG and BKV DNAs were also run for size comparison. The darker top band in undigested MG and BKV DNAs are form II, and the lighter lower bands are form III (linear) DNAs. The SstI fragments of M1 are 2,330, 1,120, 1,090, and 480 base pairs, and those of M2 are 2,640, 1,450, and 860 base pairs. The EcoRI fragments of M1 are 2,370, 1,590, and 1,060 base pairs, and for M2 they are 3,580, 1,040, and 330 base pairs.

itive colonies were detected by hybridization with ^{32}P -labeled BKV DNA (A. Pater, M. M. Pater, R. M. Dougherty, and G. di Mayorca, Virology, in press). Plasmids were isolated and

FIG. 2. Cleavage of MG DNA with HindIII, PvuII, and XbaI. Nick-translated MG DNA was digested with the restriction enzymes and run by electrophoresis through 1.2% agarose gels. BKV DNA was cleaved with SstI, HaeIII, and combined HindIII-EcoRI and run as marker for the estimation of molecular weight. The smallest PvuII fragment, 1E, ran off the gel. The HindIII fragments of M2 are 3,780, 840, and 330 base pairs. The PvuII fragments of M1 are 1,750, 1,230, 1,000, 840, and 200 base pairs. The XbaI fragments of M2 are 3,590 and 1,360 base pairs.

analyzed by cleavage with the appropriate restriction enzymes. Viral DNA was then separated from pBR322 DNA after cleavage with the appropriate restriction enzyme and separation by agarose gel electrophoresis. Each DNA molecule was redigested with a second restriction enzyme. The fragments were separated by electrophoresis, and from the size of the resulting fragments, the bands in Fig. 1 and 2 were designated M1 or M2.

To locate the sites of the various restriction enzymes on M1 and M2 DNAs, nick-translated viral DNA was separated from the pBR322 DNA after cleavage with the appropriate restriction enzyme. Each DNA was first digested with one restriction enzyme, and then the fragments were redigested with a second enzyme. From the size of the resulting fragments and the application of overlap analysis (1), the order of frag-

ments produced by each restriction enzyme was determined (Fig. 3).

Several observations can be made with regard to the restriction enzyme maps of M1 and M2 when compared with that of BKV. For M1 DNA the right side of the map (Fig. 3) shows homology to BKV DNA, whereas for M2 DNA the left side of the map shows homology to BKV DNA. When comparing homologous regions, we noted that M1 had an extra *EcoRI* site at 3,430 base pairs and an extra *PvuII* site at 3,780 base pairs. M2 had two extra *HindIII* sites at 1,630 and 2,470 base pairs, although lacking two BKV DNA *HindIII* restriction sites at 870 and 3,210 base pairs. Thus, of the 17 recognition sites in homologous regions between MG and BKV DNAs, 6 (35%) differed.

An overall comparison of the sites for the hexanucleotide-recognizing restriction enzymes

FIG. 3. Ordering of the fragments produced by the cleavage with restriction endonucleases *EcoRI*, *HindIII*, *PvuII*, *SstI*, and *XbaI*. The distances between the cleavage sites are given in base pairs to allow comparison. The left-to-right orientation corresponds to the clockwise direction of the circular map (12). The site of cleavage for *EcoRI* is designated as R, that for *HindIII* is designated as H, and so on. The *EcoRI* fragments are designated as RA, RB, and so on. Circled letters represent restriction sites different for BK and MG.

in the BKV-type viruses revealed an interesting pattern. When the hypervariable region (0.62 to 0.69 map unit, 11) of the genomes was excluded from consideration, all of the 10 restriction sites for *EcoRI*, *SstI*, *PvuII*, *XbaI*, and *HindIII* in BKV (prototype; 18), BKV (MM; 19), BKV (Dun; 15), and GS (11 and our unpublished data) were identical, except that GS had one extra *SstI* site. However, when this group was compared with RF and MG DNAs, many differences were observed. Figure 4 shows a diagram of the differences and similarities of the maps for these five restriction enzymes. It is apparent that RF and MG have undergone more evolutionary divergence than the four BK-type viruses. One theory that the Fig. 4 data tend to support is that both MG and RF originated from BKV. The common *HindIII* site at 1,630 base pairs was generated and, afterwards, the two viruses diverged, and five differences appeared in their restriction enzyme maps.

Since the size (5,020 and 4,950 base pairs) of both M1 and M2 DNAs was smaller than that of BKV DNA (Fig. 1), it was possible that these molecules, like RF (12), had deletions. To examine this possibility, unlabeled BKV DNA was cleaved with the restriction endonuclease *AvaII*, which cleaves it seven times (Fig. 5). The DNA fragments were blotted onto nitrocellulose filter paper after their separation by electrophoresis through acrylamide gels. MG DNA was nick translated. Part of the sample was digested with *HindIII*, and the rest was digested with *PvuII*. Linear molecules (M1 and M2) were separated and used for hybridization with unlabeled *AvaII* fragments of BKV DNA. Figure 5A shows that

M1 DNA lacks sequences which correspond to the *AvaII* fragment A of BKV DNA. This corresponds to 0.33 to 0.55 map unit from the *EcoRI* site on BKV DNA and is more than 40% of the coding region for BKV large T-antigen. M2 DNA lacks the sequences which correspond to the *AvaII* fragment G of BKV DNA (Fig. 5A). This corresponds to 0.77 to 0.85 map unit from the *EcoRI* site on BKV DNA and is more than 30% of the coding region for VP2 and VP3. It is thus possible that, like RF (12), the genome of MG might consist of two complementary defective molecules. Experiments using M1 and M2 DNAs separately and in combination for transfection will be undertaken to examine the complementarity of these molecules. It should be noted that, although the deleted regions in both MG and RF were in the early and late regions, identical sequences were not deleted in both viruses. This is shown in Fig. 5, in which R1 is similar to M2 in that it did not hybridize with *AvaII*-G, but is different in that the deletion extended to most of *AvaII*-C. R2 had less DNA hybridizing to *AvaII*-B and more hybridizing to *AvaII*-A than did M1. Also, the restriction maps of MG DNAs (Fig. 3) were not identical to those of RF (12). Therefore, MG and RF are two different variants of the prototype BKV.

MG has been regarded as a different virus (10) due to its weak antigenicity when reacted with antisera prepared against BKV (7) and also due to differences in the peptides of capsid proteins of this virus and that of BKV (17). The large number of altered restriction sites that we observed between the DNAs of the two viruses are an obvious explanation for the observed differ-

FIG. 4. Diagram illustrating the number of similar and different *EcoRI*, *SstI*, *PvuII*, *XbaI*, and *HindIII* restriction sites among GS, MG, RF, and three other BKV DNAs. +RF DNA lacks the BKV *SstI* site at 1,830 base pairs (unpublished data). *GS DNA has a unique *SstI* site in *HindIII* fragment B (unpublished data).

FIG. 5. Hybridization of labeled M1 and M2 (A) and R1 and R2 (B) DNAs with unlabeled BKV DNA. BKV DNA was cleaved with *Ava*II and separated by electrophoresis through 4/12% acrylamide gels blotted onto nitrocellulose paper and hybridized with labeled M1, M2, R1, R2, and BKV DNAs (12). The *Ava*II fragments of BKV DNA are 1,152, 932, 783, 740, 726, 427, and 394 base pairs.

ences. It is, however, clear, as shown by our hybridization data (Fig. 5) and also by our hybridization data obtained under stringent conditions (50% formamide at 42°C, data not shown), that the genome of MG is closely related to that of BKV.

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