JOURNAL OF VIROLOGY, Nov. 1979, p. 684-687
0022-538X/79/11-0684/04$02.00/0

Vol. 32, No. 2

Physical Maps of Bovine Papillomavirus Type 1 and Type 2 Genomes

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Received for publication 13 February 1979

Physical maps of bovine papillomavirus type 1 and type 2 (BPV-1 and BPV-2) DNA were constructed from analysis of the electrophoretic mobilities of restriction endonuclease cleavage fragments from dual digests. BPV-1 DNA was sensitive to *Hin*dII, *Hin*dIII, *Eco*RI, *Hpa*I, and *Bam*HI, with all but *Hin*dII yielding single scissions. BPV-2 DNA was resistant to *Eco*RI, and *Hin*dIII had one cleavage site whereas *Hpa*I, *Bam*HI, and *Hin*dII yielded multiple fragments. Of four BPV-1 isolates examined, DNA from one isolate was resistant to *Hin*dIII, and another DNA isolate was resistant to *Bam*HI. The three BPV-2 isolates examined were uniformly sensitive to the restriction endonucleases employed.

The *Papillomavirus* genus of the papovaviruses represents a group of viruses which produce benign self-limiting neoplasms (warts) in the host species. Recently, two classes of bovine papillomavirus (BPV) which are related but not identical have been described. The viruses share 45 to 58% of their DNA sequences, cross-react antigenically, and agglutinate mouse erythrocytes with different efficiencies (7). The lesions produced by these viruses appeared histologically identical, and no differences were noted in anatomic location. The oncogenic potential of the viruses appears to be similar since DNAs from both virus types have been identified in hamster tumors induced by untyped BPV and also in naturally occurring equine connective tissue tumors (8). To characterize these two virus types further, cleavage maps of the genomes were constructed by using the *Eco*RI, *Hin*dII, *Hin*dIII, *Hpa*I, and *Bam*HI restriction endonucleases.

The results of cleavage of BPV type 1 (BPV-1) and BPV type 2 (BPV-2) DNAs with the restriction endonucleases used in this study are shown in Fig. 1 and 2. The numbers of fragments produced by each enzyme and dual digests, the percent molecular weight of each fragment, and the total molecular weight are given in Tables 1 and 2. Molecular weights of multiple cleavage products were estimated by comparing mobilities with *Hin*dIII-, *Eco*RI-, and *Hin*dII-restricted human papillomavirus type 1 DNA run in parallel in the same gel. Molecular weights for human papillomavirus type 1 DNA restriction enzyme fragments were taken from Favre et al. (2). The molecular weight of unit length BPV DNA was determined from *Eco*RI- and *Hin*dIII-digested λ DNA as standards by using the molecular weight values reported by Parker et al. (11). The BPV-1 and BPV-2 cleavage maps are shown in Fig. 3, and the evidence for their construction is summarized below.

To order the cleavage sites of the restriction enzymes that yielded single scissions on BPV-1 DNA, the *Eco*RI product was digested with *Hpa*I, *Bam*HI, or *Hin*dIII (Fig. 1f to h), and the *Hin*dIII product was digested with *Hpa*I or *Bam*HI. From the estimated molecular weights of the digestion products (Table 1), the cleavage sites for these endonucleases were placed in the following order: *Hin*dIII-*Bam*HI-*Eco*RI-*Hpa*I. *Hin*dII cleaved BPV-1 DNA at three sites (Fig. 1a). To order these sites, *Hin*dII-fragmented DNA was further digested with *Eco*RI and compared with the *Hpa*I/*Eco*RI digest (Fig. 1e and f). Since *Hpa*I cleavage represents a subset of the *Hin*dII cleavage sites (4) and the *Eco*RI/*Hpa*I B fragment was equal in percent molecular weight to the sum of the *Eco*RI/*Hin*dII B and C fragments (Table 1), the *Eco*RI site would be in *Hin*dII-A, and the order of the *Hin*dII fragments would be A-B-C. To localize the *Hin*dIII site, a comparison was made of *Hin*dII, *Hin*dIII/*Hin*dII, and *Hin*dIII/*Hpa*I digests (Fig. 1a, b, and d). The *Hin*dIII/*Hpa*I digest indicated *Hin*dIII to be in either *Hin*dII-A or -B; however, if *Hin*dIII cleaved within *Hin*dII-B, then the *Hin*dIII/*Hin*dII digest would show an alteration in migration of the B fragment as compared with the *Hin*dII digest. Careful comparison of the *Hin*dII and *Hin*dIII/*Hin*dII digests, however, indicates a small but detectable difference in molecular weight of the A fragments (Fig. 1a and b and Table 1). Therefore, *Hin*dIII cleaves

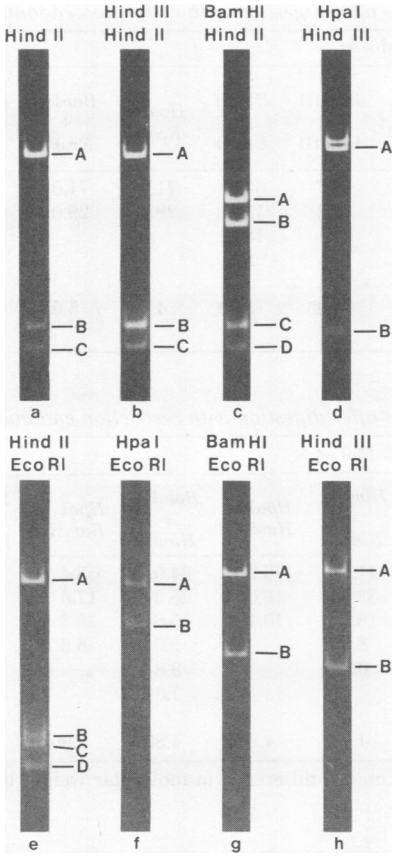

FIG. 1. *Electropherograms of restriction endonuclease cleavage products of BPV-1 DNA. DNA samples (0.15 to 0.25 μg) were incubated with 1 U of enzyme for 90 min at 37°C in a total volume of 25 μl. Reactions were stopped by the addition of 10 μl of a solution containing 7 M urea, 50% sucrose, 25 mM EDTA, and 0.025% bromophenol blue. Samples were electrophoresed at 5 V/cm in 1% agarose slab gels in the E buffer of Loening (10). Slots a to d were from the same gel and electrophoresed for 2 h; slots e to h were from another gel electrophoresed for 1.5 h. Gels were photographed after staining with ethidium bromide (0.5 μg/ml in E buffer). The fragment above A in slot d resulted from incomplete cleavage.*

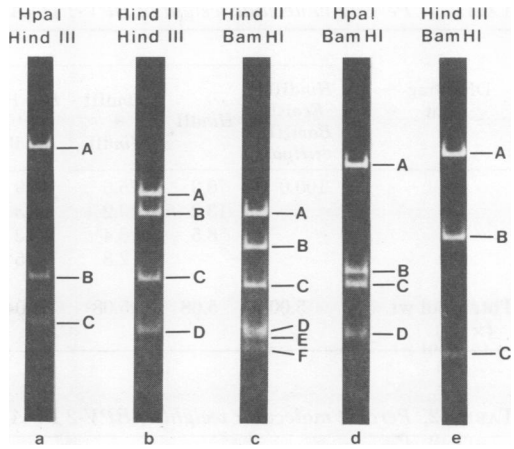

FIG. 2. *Electropherograms of restriction endonuclease cleavage products of BPV-2 DNA. DNA concentrations and conditions for cleavage were the same as described in the legend to Fig. 1. Slots a to e were from the same gel electrophoresed for 2 h.*

near one of the *Hin*dII sites bordering the A fragment. The *Hin*dIII/*Hin*dII D fragment was visualized by electrophoresis for a shorter period of time at higher DNA concentrations (Fig. 4); although subtle, the *Hin*dIII/*Hin*dII A fragment migrated slightly ahead of the *Hin*dII A fragment, thus indicating the *Hin*dIII site to be within *Hin*dII-A. A *Bam*HI/*Hin*dII dual digest indicated the *Bam*HI site to be within the *Hin*dII A fragment (Fig. 1c). Orienting the restriction endonuclease cleavage sites in relation to the unique *Hin*dIII site was arbitrary.

Both *Hpa*I and *Bam*HI cleaved BPV-2 DNA at two sites (Table 2). Cleavage of these products with *Hin*dIII located the *Hin*dIII site within the *Hpa*I B fragment and the *Bam*HI A fragment (Fig. 2a and e). A dual digest of BPV-2 DNA with *Bam*HI and *Hpa*I (Fig. 2d) indicated one *Bam*HI site to be within *Hpa*I-A and the other in *Hpa*I-B since the sum of the percent molecular weights of the *Bam*HI/*Hpa*I A + C and B + D fragments was similar to the percent molecular weights of the *Hpa*I A and B fragments, respectively (Table 2). On the basis of this finding, the cleavage sites were placed in the following order: *Hpa*I-*Bam*HI-*Hpa*I-*Hin*dIII-*Bam*HI. To locate the *Hin*dII sites, *Hin*dIII-cleaved DNA was digested with *Hin*dII and compared with a *Hin*dIII/*Hpa*I digest (Fig. 2a and b and Table 2). The results indicated that *Hin*dII cleaved within the *Hin*dIII/*Hpa*I A and C fragments. A comparison of *Hin*dII and *Hin*dIII/*Hin*dII digests is shown in Fig. 4. As with BPV-1 DNA, *Hin*dIII cleaved near one of the *Hin*dII sites. *Hin*dII-C is slightly larger in molecular weight than *Hin*dIII/*Hin*dII-C, thus indicating that *Hin*dIII cleaves within the *Hin*dII C fragment. The *Hin*dIII/*Hin*dII E fragment was visualized as a smear in Fig. 4. The molecular weight of this fragment could not be determined from its electrophoretic mobility (Table 2). *Bam*HI digestion of the *Hin*dII products (Fig. 2c) indicated cleavage within the *Hin*dII A and C fragments. From the relationship of the *Bam*HI sites and the *Hpa*I and *Hin*dII sites to the *Hin*dIII site, a physical map of the BPV-2 genome was constructed. The BPV-2 map was oriented as shown due to the alignment of the

TABLE 1. *Percent molecular weight of BPV-1 DNA fragments after digestion with restriction endonucleases*

DNA fragment	% Mol wt									
	*Hin*dIII, *Eco*RI *Bam*HI, or *Hpa*I	*Hin*dII	*Hin*dIII + *Hin*dII	*Bam*HI + *Hin*dII	*Hpa*I + *Hin*dIII	*Bam*HI + *Hin*dIII	*Hin*dII + *Eco*RI	*Hpa*I + *Eco*RI	*Bam*HI + *Eco*RI	*Hin*dIII + *Eco*RI
A	100.0	78.3	75.6	43.9	88.5	68.7	63.5	71.9	71.0	60.0
B		13.2	13.2	34.3	11.5	31.3	15.0	28.1	29.0	40.0
C		8.5	8.4	13.3			13.1			
D			2.8	8.5			8.4			
Total mol wt ($\times 10^6$)	5.00	5.08	5.08	5.04	5.03	4.95	5.09	4.98	5.05	5.15

TABLE 2. *Percent molecular weight of BPV-2 DNA fragments after digestion with restriction endonucleases*

DNA fragment	% Mol wt								
	*Hin*dIII	*Hpa*I	*Bam*HI	*Hin*dII	*Hin*dIII + *Hin*dII	*Hpa*I + *Hin*dIII	*Bam*HI + *Hin*dII	*Hpa*I + *Bam*HI	*Bam*HI + *Hin*dIII
A	100.0	73.5	75.0	41.3	41.3	73.9	34.0	58.4	68.5
B		26.5	25.0	33.0	33.0	16.0	25.3	17.5	24.8
C				16.8	16.0	10.1	15.6	15.3	6.7
D				8.9	8.9		9.0	8.8	
E					0.8[a]		8.8		
F							7.3		
Total mol wt ($\times 10^6$)	5.00	5.05	5.00	4.94	4.94	4.84	4.80	4.90	4.96

[a] Percent molecular weight of *Hin*dIII/*Hin*dII-E was based on the difference in molecular weight between *Hin*dII-C (0.83×10^6) and *Hin*dIII/*Hin*dII-C (0.79×10^6).

*Bam*HI site on BPV-1 DNA with the *Bam*HI site at 0.31 map unit on BPV-2 DNA.

From the antigenic similarities and high degree of DNA sequence homology between BPV-1 and BPV-2, one would expect the physical maps of these virus DNAs to be closely related. Highly conserved sites are located at 0/1.0 (*Hin*dIII) and 0.31 (*Bam*HI) map unit, and the *Hin*dII sites near 0.90 and 0.97 map unit may also be conserved regions; however, the remaining sites show little similarity between the two genomes. These viruses are more closely related than depicted by the physical maps, since radiolabeled BPV-1 DNA was shown to hybridize to the four separated and immobilized BPV-2 *Hin*dII fragments under stringent conditions (melting temperature − 23°C) (9). Similarly, each radiolabeled *Hin*dII BPV-2 DNA fragment hybridized 50 to 60% to BPV-1 DNA in a liquid-phase system under standard stringent conditions (data not shown). These results would indicate that the DNA sequences shared by BPV-1 and BPV-2 are dispersed throughout the genomes.

Differences have been shown to exist among individual papillomavirus isolates with respect to restriction enzyme sensitivity. Gissmann et al. (5) reported the existence of an additional *Hin*dIII and *Bam*HI site in one of three human papillomavirus type 1 isolates that they examined. Similar results were obtained in this study, with DNA from one BPV-1 isolate being resistant to *Bam*HI and another isolate being resistant to *Hin*dIII; however, the three BPV-2 isolates studied gave identical cleavage patterns. Polynucleotide sequence heterogeneity has been observed for one of the four *Hpa*II cleavage sites in 40 to 50% of human papillomavirus type 1 DNA molecules isolated from a single plantar wart (3). Such heterogeneity was not observed in DNA from any of the BPV isolates digested with the restriction enzymes used. It will be necessary to examine additional isolates by using a larger battery of restriction enzymes to determine whether such heterogeneous DNA molecules exist for BPV.

The physical maps of BPV-1 and BPV-2 DNA can serve as primary reference for further, more detailed mapping with other restriction enzymes and positioning of RNA transcripts. From the high degree of relatedness between the virus genomes, these viruses may offer an interesting

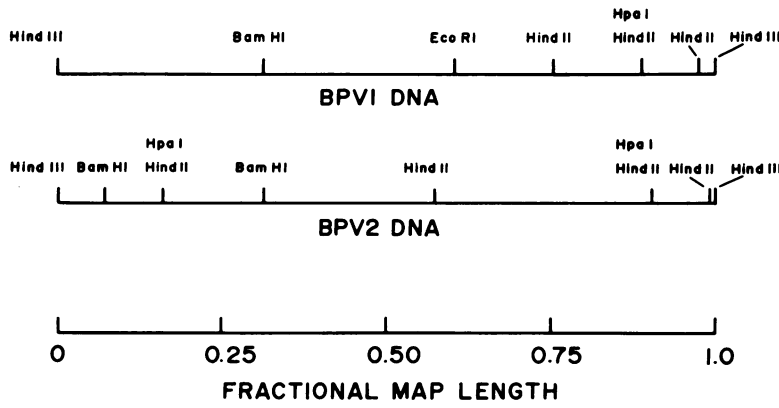

FIG. 3. *Restriction endonuclease cleavage maps of BPV-1 and BPV-2 DNA. The genomes are shown as linear structures opened at the HindIII (0/1.0 map unit) cleavage site.*

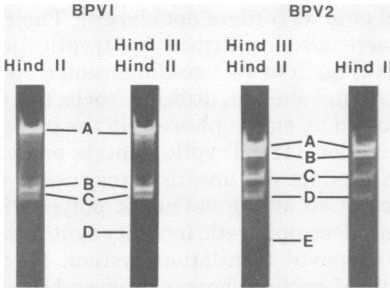

FIG. 4. *Electropherograms of HindII- and HindIII/HindII-digested BPV-1 and BPV-2 DNAs. DNA samples (0.5 μg) were electrophoresed in 1% agarose for 45 min at 5 V/cm.*

system for the study of evolution on the basis of polynucleotide sequence. Furthermore, the physical maps may be useful in determining relatedness between these BPVs and other papillomaviruses, such as the atypical BPV which produces a papilloma limited to epithelial involvement (1) and the recently described BPV which appears to be associated with alimentary tract papillomas and carcinoma (6).

This investigation was supported by Public Health Service grant CA-24505 from the National Cancer Institute.

LITERATURE CITED

1. **Barthold, S. W., L. D. Koller, C. Olson, E. Studer, and A. Holtan.** 1974. Atypical warts in cattle. J. Am. Vet. Med. Assoc. **165**:276–280.
2. **Favre, M., G. Orth, O. Croissant, and M. Yaniv.** 1975. Human papillomavirus DNA: physical map. Proc. Natl. Acad. Sci. U.S.A. **72**:4810–4814.
3. **Favre, M., G. Orth, O. Croissant, and M. Yaniv.** 1977. Human papillomavirus DNA: physical mapping of the cleavage sites of *Bacillus amyloliquefaciens* (*BamI*) and *Haemophilus parainfluenzae* (*HpaII*) endonucleases and evidence for partial heterogeneity. J. Virol. **21**: 1210–1214.
4. **Garfin, D. E., and H. M. Goodman.** 1974. Nucleotide sequences at the cleavage sites of two restriction endonucleases from *Hemophilus parainfluenzae*. Biochem. Biophys. Res. Commun. **59**:108–116.
5. **Gissmann, L., H. Pfister, and H. zur Hausen.** 1977. Human papilloma viruses (HPV): characterization of four different isolates. Virology **76**:569–580.
6. **Jarrett, W. F. H., P. E. McNeil, W. T. R. Grimshaw, I. E. Selman, and W. I. H. McIntyre.** 1978. A high incidence area of cattle cancer with a possible interaction between an environmental carcinogen and a papilloma virus. Nature (London) **274**:215–217.
7. **Lancaster, W. D., and C. Olson.** 1978. Demonstration of two distinct classes of bovine papilloma virus. Virology **89**:372–379.
8. **Lancaster, W. D., G. H. Theilen, and C. Olson.** 1979. Hybridization of bovine papilloma virus type 1 and type 2 DNA to DNA from virus-induced hamster tumors and naturally occurring equine tumors. Intervirology **11**:227–233.
9. **Law, M.-F., W. D. Lancaster, and P. M. Howley.** 1979. Conserved polynucleotide sequences among the genomes of papillomaviruses. J. Virol. **32**:199–207.
10. **Loening, U. E.** 1969. The determination of the molecular weight of ribonucleic acid by polyacrylamide-gel electrophoresis. Biochem. J. **113**:131–138.
11. **Parker, R. C., R. M. Watson, and J. Vinograd.** 1977. Mapping of closed circular DNAs by cleavage with restriction endonucleases and calibration by agarose gel electrophoresis. Proc. Natl. Acad. Sci. U.S.A. **74**:851–855.