A Restriction Map of Bovine Papillomavirus Type-1 DNA

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SUMMARY

A highly detailed restriction map of bovine papillomavirus type-1 (BPV-1) DNA is presented. The cleavage sites of 15 restriction endonucleases were determined, providing the locations of 72 specific BPV-1 DNA fragments. This map will be useful for future physical and functional studies of the BPV-1 genome.

The papillomaviruses are members of the family Papovaviridae and have been shown to be the aetiological agents of warts (papillomas) in several species of animals including man (Orth et al., 1977; zur Hausen, 1978; zur Hausen & Gissmann, 1980). These double-stranded DNA-containing viruses are capable of inducing tumours which are generally benign, self-limiting neoplasms of the epidermis; however, under certain circumstances some apparently benign tumours may exhibit high rates of malignant conversion to cancerous lesions. The assessment of the oncogenic potential of these papillomaviruses has been hampered by the lack of suitable in vitro cell culture systems by which to propagate the viruses.

The bovine papillomaviruses (BPV) appear to be the most oncogenic of the papillomaviruses and are able to infect a number of species other than its host. For example, intracranial injection of BPV produces meningiomas in calves (Gordon & Olson, 1968), while subcutaneous injection of the virus induces fibromas in hamsters or C3H/eB mice (Boiron et al., 1964; Robl & Olson, 1968). BPV will also induce equine sarcomas (Lancaster et al., 1977). BPV is capable of in vitro transformation of cells of bovine (Black et al., 1963; Boiron et al., 1965; Meischke, 1979; Thomas et al., 1963), murine (Dvoretzky et al., 1980; Lowy et al., 1980; Thomas et al., 1964), and hamster origins (Gerald et al., 1969, 1970; Morgan & Meinke, 1980). Because of the obvious oncogenic potential of BPV a defined physical map of the BPV genome is required for more specific studies of BPV-induced transformation.

Four distinct types of bovine papillomaviruses have been defined by DNA sequence homology, their antigenic properties, and by the types of tumour induced (Campo et al., 1980; Pfister et al., 1979). EcoRI, HpaI, HindIII and BamHI all cleaved BPV-1 DNA at BPV-1, BPV-2 and BPV-3 genomes have been constructed (Lancaster, 1979; Jarrett et al., 1980; Pfister et al., 1979). EcoRI, HpaI, HindIII and BamHI all cleaved BPV-1 DNA at single sites while HindII produced three fragments (Lancaster, 1979). These five enzymes provided convenient reference points for the construction of a more detailed restriction map of the BPV-1 genome.

Presented in this study are the cleavage sites of 15 additional restriction endonucleases, which now provide the locations of 72 specific BPV-1 DNA fragments. The mol. wt. of the restriction fragments produced by cleavage with the various identified enzymes are presented in Table 1. The relative positions of these fragments are illustrated on the circular map (Fig. 1). The single EcoRI cleavage site was used as the zero reference point for all the restriction endonucleases examined.

BPV-1 was extracted from excised bovine papillomas; virus and supercoiled viral DNA were prepared by procedures described previously (Morgan & Meinke, 1980). Digestion of variable amounts (0.25 to 0.7 µg) of BPV-1 DNA with each restriction endonuclease was
### Table 1. Mol. wt. of the restriction fragments of BPV-1 DNA

<table>
<thead>
<tr>
<th>Restriction endonuclease</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
<th>N</th>
<th>O</th>
<th>Total mol. wt. (x 10^-6)</th>
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<tr>
<td>PvuII</td>
<td>4.90</td>
<td>0.22</td>
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<td>5.12</td>
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<tr>
<td>ThaI</td>
<td>4.20</td>
<td>0.95</td>
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<td>5.15</td>
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<tr>
<td>BglII</td>
<td>2.96</td>
<td>1.64</td>
<td>0.41</td>
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<td>ClaI</td>
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<td>AccI</td>
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<td>AvaI</td>
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<td>HgiAI</td>
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<td>HpalI</td>
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<td>1.40</td>
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<td>0.28</td>
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<td>0.23</td>
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<td>0.18</td>
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<td>0.08</td>
<td>0.07</td>
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<tr>
<td>AvaII</td>
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<td>0.83</td>
<td>0.79</td>
<td>0.73</td>
<td>0.24</td>
<td>0.22</td>
<td>0.21</td>
<td>0.13</td>
<td>0.12</td>
<td>0.10</td>
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<td>0.73</td>
<td>0.58</td>
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<td>0.47</td>
<td>0.44</td>
<td>0.39</td>
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<td>0.21</td>
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<td>0.10</td>
<td>0.06</td>
<td>0.04</td>
<td>5.25</td>
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</table>
Fig. 1. Restriction map of BPV-1 DNA. Each circle represents the genome with the cleavage sites of one restriction endonuclease. The circular genome has been divided into tenths and the enzyme sites have been mapped according to the fractional lengths of the fragments produced by a given enzyme. The zero map unit was assigned to the single EcoRI site.

carried out according to the conditions recommended by the manufacturer. Single digests with all test enzymes were first analysed by electrophoresis in 1.2% agarose slab gels to determine the number and size of the fragments produced. Double-digestions by each test enzyme in the presence of previously mapped enzymes were analysed after electrophoresis both in 1.2% or 1.4% agarose and in 5% or 10% polyacrylamide-10% glycerol slab gels (Denniston-Thompson et al., 1977). Following electrophoresis, DNA bands were visualized by staining gels with ethidium bromide and by photography under u.v. light. Polyacrylamide gels were necessary to resolve low mol. wt. DNA fragments generated by enzymes which gave multiple cuts and to screen for the presence of minute DNA fragments undetectable in agarose gels. Fragments as small as about 70 base pairs could be well resolved in the acrylamide gels.

The mol. wt. of the BPV-1 DNA fragments were established with two sets of DNA markers. High mol. wt. markers consisted of either λ DNA digested with HindIII or φ29 DNA digested with EcoRI. Low mol. wt. markers comprised selected pBR322 DNA fragments produced by cleavage with various enzymes to provide a distribution ranging from 4362 to 127 base pairs.

In addition to the enzymes listed in Table 1, four did not cleave BPV-1 DNA: XhoI, SalI, SacI and BalI. These enzymes produced patterns indistinguishable from undigested BPV-1 DNA when examined in gels. Three other enzymes examined produced single cuts which
converted supercoiled and open-circular DNA to the linear form: Smal, KpnI and BstEII. These three enzymes became useful in mapping the cleavage sites of other restriction endonucleases.

HpaII, AvaII and HinII cleaved BPV-1 DNA into 11, 12 and 15 fragments respectively. Three HpaII fragments (I, J, K), four AvaII fragments (E, I, K, L) and three HinII fragments (J, K, O) could not be accurately positioned because double-digests failed to cleave these minor fragments. Nevertheless, these fragments were placed at unlabelled areas on the map based on their mol. wt. Verification of the positions of these remaining fragments is currently being determined by the method of Smith & Birnstiel (1976).

The total mol. wt. of the BPV-1 fragments generated by each enzyme utilized in this study averaged $5.2 \times 10^6$. This mol. wt. is slightly higher than the previously reported mol. wt. of $5 \times 10^6$ (Lancaster, 1979). The discrepancy probably arises from the different electrophoretic gel systems employed in the two studies. The acrylamide–glycerol gel system employed here proved to be more satisfactory in determining mol. wt. below $1 \times 10^6$ and in cases where numerous cuts were generated during double enzyme digestions.

The construction of the detailed restriction map reported here represents a more complete physical characterization of the BPV-1 genome. Experiments are currently in progress to assign functional roles to specific regions of the BPV-1 genome. BPV-1 restriction fragments are being used as probes to determine whether specific regions of the BPV-1 genome are producing messenger RNA in BPV-1-transformed cells. These regions of the BPV-1 DNA will then be sequenced. Restriction fragments of these genetically active regions will be used to infect cells growing in culture to precisely define the specific subgenomic fragments within the previously reported 69% of BPV-1 DNA capable of inducing transformation (Lowy et al., 1980). Finally, these studies will provide much information about the molecular events of bovine papilloma virus-induced transformation.

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REFERENCES


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