The Genome of Caprine Herpesvirus 1: Genome Structure and Relatedness to Bovine Herpesvirus 1

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SUMMARY

Caprine herpesvirus 1 (CapHV-1) DNA was examined by electron microscopy, restriction site mapping and homology studies with bovine herpesvirus 1 (BHV-1) DNA. Although the restriction site maps differed significantly, we showed that the genome structures of CapHV-1 and BHV-1 were identical and that the DNAs shared a high degree of base sequence homology.

Caprine herpesvirus 1 (CapHV-1), also known as bovid herpesvirus 6 (BHV-6; Ludwig, 1983), is distributed worldwide (Kao et al., 1985) and causes a generalized disease in young kids affecting mainly the digestive tract (Saito et al., 1974; Mettler et al., 1979). Infection in adult goats remains inapparent, or may cause abortion (Berrios et al., 1975; Waldvogel et al., 1981), vulvovaginitis or balanoposthitis (Horner et al., 1982; Tisdall et al., 1984). Several viral properties have been examined, such as pathogenicity (Berrios et al., 1975; Waldvogel et al., 1981), behaviour in cell cultures (Berrios & McKercher, 1975; Engels et al., 1983), cross-neutralization between different strains (Kao et al., 1985) and physicochemical properties of the virion (Berrios & McKercher, 1975) and of the DNA (Engels et al., 1983). An antigenic relationship exists between CapHV-1 and bovine herpesvirus 1 (BHV-1) and is detected as a serological one-way cross-reactivity (Berrios et al., 1975; Mettler et al., 1979; Kao et al., 1985; Ackermann et al., 1986). Western blot analyses using monoclonal antibodies as well as polyclonal antisera revealed that the main capsid protein (VP4) and the viral glycoproteins VP7 and VP17 are responsible for the cross-reaction, whereas VP12 seems to be the most type-specific glycoprotein (Ackermann et al., 1986; Friedli & Metzler, 1987). These results as a whole point to a strong cross-reactivity of BHV-1 and CapHV-1, although a few significant differences in biological (Berrios et al., 1975) and genomic properties (Engels et al., 1983; Tisdall et al., 1984; Brake & Studdert, 1985) are apparent.

In this paper we present EcoRI, HindIII, BamHI and BstEII restriction site maps of CapHV-1 DNA using a Swiss isolate (strain E/CH, Engels et al., 1983) and a Californian isolate (strain McK/US, kindly supplied by Dr D. G. McKercher, Los Angeles, Ca., U.S.A.), both biochemically characterized earlier (Engels et al., 1983). Since restriction patterns of CapHV-1 DNA have been shown to be unique (Engels et al., 1983; Ludwig, 1983; Tisdall et al., 1984; Brake & Studdert, 1985) it was of interest to analyse the base sequence homology between CapHV-1 and BHV-1 DNA.

The virus strains were plaque-purified on Georgia bovine kidney cells and propagated on bovine foetal lung cells. Virus purification, DNA extraction and gel electrophoresis were carried out as described by Engels et al. (1983). Restriction site maps were constructed by conventional methods, i.e. by double digestion and crossed hybridization of single restriction fragments and by Bal 31 digestion for end fragment determination as described previously (Engels et al., 1986). The genome structure of CapHV-1 was shown by electron microscopy of self-annealed DNA single strands after protein-free spreading according to Vollenweider et al. (1975) and Sogo et al. (1984).
Cross-hybridization experiments between the DNAs of CapHV-1 and BHV-1 were performed by hybridizing $^{32}$P-labelled, cloned HindIII or EcoRI–HindIII fragments of BHV-1 strain K22 with nitrocellulose-bound BamHI and BstEII fragments of the CapHV-1 DNAs. Hybridization conditions have been described previously (Engels et al., 1986). Since no published information exists about the infectivity of BHV-1 and CapHV-1 DNAs, transfection experiments were conducted according to the method described by Van der Eb & Graham (1980).

Fig. 1 shows a sketch of the restriction patterns of the DNAs under study as revealed by digestion with the endonucleases EcoRI, HindIII, BamHI and BstEII. The total mol.wt. of the DNAs, as calculated by summation of the mol.wt. of single fragments, deriving from digestions with various endonucleases averaged 90.6 $\times$ 10$^6$ (137.3 kilobase pairs (kbp)) for CapHV-1 strain E/CH, 90.3 $\times$ 10$^6$ (136.8 kbp) for CapHV-1 strain McK/US and 92.3 $\times$ 10$^6$ (139.9 kbp) for BHV-1 strain K22. Minor differences in the restriction patterns between the two CapHV-1 DNAs were found. Four half-molar fragments were observed in the BamHI restriction pattern of strain E/CH indicating the existence of inverted repeat sequences. Electron microscopy of annealed single strands demonstrated the presence of a single set of inverted repeats, 11.0 $\pm$ 0.3 kbp long, flanking a short unique sequence (Us), 10.5 $\pm$ 0.4 kbp long. The results are consistent with those found for BHV-1 DNA. The length of the repeat sequences of the CapHV-1 DNAs is smaller than that of BHV-1 strain K22 DNA (12.15 $\pm$ 0.55 kbp), but not significantly different from average lengths measured for BHV-1 DNAs (Engels et al., 1986). The value measured for the length of Us of CapHV-1 DNA is slightly smaller than that of the BHV-1 DNAs and also smaller than expected theoretically. This may be due to the higher flexibility of DNA single strands, which therefore render measurements more difficult and to the fact that theoretical calculations are based on rather few restriction sites. The overall contour length of BHV-1 DNAs exceeds that of CapHV-1 DNA by about 2.5 kbp (Engels et al., 1983). The restriction site maps of the two CapHV-1 DNAs are depicted in Fig. 2. There is only one EcoRI and one HindIII site on these DNAs. The BstEII and BamHI maps reveal that the strain differences found in the restriction patterns are due to the absence of a restriction site for each of these enzymes in strain McK/US. The missing BamHI site explains why half-molar fragments are not recovered from this strain.

In addition, restriction site mapping revealed the existence of four half-molar BstEII fragments for the DNA of both strains. Although no common restriction sites could be detected in CapHV-1 and BHV-1 DNA, complete cross-hybridization occurred when the two genomic
Fig. 2. Restriction site maps of CapHV-1 strains E/CH and McK/US. The first line denotes the subdivision of the genome in map units. The second line demonstrates the genome structure (UL, unique long; US, unique short; IRs/TRs, internal and terminal repeat). The size of each fragment is indicated in kbp. The inversion of US caused by the genome structure is presented as a second line at the right hand end of the BstEII maps of each strain and the BamHI map of strain E/CH. Additional restriction sites in strain E/CH DNA are indicated by ▲.

DNAs were annealed even under stringent hybridization conditions (Fig. 3). This could indicate that the genome differences are due to point mutations or short sequence alterations. Thus the results concerning the degree of base sequence homology have to be interpreted cautiously, since more efficient experiments with more modification of the conditions will be necessary. Finally, as expected, the infectivity of CapHV-1 and BHV-1 DNAs could be confirmed.

The results presented allow an insight into the genomic relationship between the caprine and bovine herpesviruses which are closely related and which may have originated from a common ancestor in the course of evolution.

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Fig. 3. Cross-hybridization experiment using 32P-labelled cloned HindIII or EcoRI–HindIII (lower case letters) fragments of BHV-1 strain K22, shown in the restriction site map (a) and nitrocellulose-bound Southern blot (b) of BstEII fragments of CapHV-1 strain McK/US. Hybridizations were carried out in 5 × SSC either at 70 °C without formamide or at 52 °C with 50% formamide (Tm – 20 °C). Complete hybridization was observed in all experiments under both conditions. Hybridization between CapHV-1 and BHV-1 DNA fragments occurred in a colinear way.
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