Bovine herpesvirus 4 genome: cloning, mapping and strain variation analysis

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The restriction map of the bovine herpesvirus 4 (BHV-4) genome (V. Test strain) was established for the restriction enzymes EcoRI, BamHI and HindIII by analysis of clones from a lambda library (Sau3AI partial digestion) and from a plasmid library (EcoRI fragments). One genome unit was defined as the length of the unique central part, flanked at both ends by one of the terminal tandem repeats called polyrepetitive DNA (prDNA) and was estimated to be 113 ± 2 kbp. A restriction map of the prDNA of the V. Test strain showed internal 200 bp tandem repeats of different sequences. This region in the prDNA was highly polymorphic between BHV-4 strains, even in a viral DNA preparation from a plaque-purified strain. The right junction between the repeated and the unique sequence of the genome occurred at an almost constant site, but the left junction contained a modified prDNA and was variable between BHV-4 strains. The unique central part of the genome was very similar in the four strains under consideration, with a few variations due to the presence or absence of a restriction site and four length variations were observed, located at positions 0·006 to 0·034 (left end), 0·211 to 0·225, 0·864 to 0·881 and 0·962 to 0·984 (right end). The total length variation of 1 genome unit does not exceed 1 kbp.

Introduction

Bovine herpesvirus 4 (BHV-4) has been isolated from a variety of clinical cases and even from primary cell cultures of healthy cattle. Except for a few isolates, BHV-4 strains are mildly pathogenic or apathogenic for cattle (for review, see Thiry et al., 1989). The virus was first isolated in Europe from respiratory and ocular diseases by Bartha et al. (1966) and later in the U.S.A. by Mohanty et al. (1971). These isolates are antigenically closely related, show similar restriction patterns (Ludwig et al., 1983; Storz et al., 1984; Osorio et al., 1985; Dubuisson et al., 1988) and cross-hybridization (Henry et al., 1986).

The BHV-4 genome consists of a linear dsDNA of approximately 145 kbp which contains a unique central sequence of 110 kbp with a low G–C content, flanked at both ends by tandem repeats of a high G–C content, called polyrepetitive DNA (prDNA). The number of prDNAs varies at each end of the genome, but the overall number is relatively constant (about 15 prDNA units per genome for the 66-P-347 strain) (Ehlers et al., 1985). This genomic structure is characteristic of the class B herpesviruses (Roizman, 1982) and is frequently observed in the γ-2-Herpesvirinae (Honess, 1984), such as herpesvirus saimiri (Fleckenstein et al., 1975; Bornkamm et al., 1976), herpesvirus ateles (Fleckenstein et al., 1978), herpesvirus aotus type 2 (Fuchs et al., 1985), alcelaphine herpesvirus 1 (Bridgen et al., 1989) and herpesvirus sylvilagus (Medveczky et al., 1989).

The prDNA size varies between BHV-4 strains and this size variation is a consequence of the presence of a variable number of 200 bp fragments inside the prDNA (Ehlers et al., 1985). Thiry et al. (1989) defined two groups of BHV-4 strains on the basis of their restriction patterns: the Movar 33/63-like (MOV-like) strains and the DN 599-like (DN-like) strains. All the published restriction patterns of American strains belong to the DN-like group and of the European strains all but one (strain UT) belong to the MOV-like group.

In order to study the differences existing between the two groups of BHV-4 strains and to localize important genes in the BHV-4 genome, we have cloned and mapped the whole BHV-4 genome of the V. Test (VT)
strain and a comparison of several BHV-4 reference strains is also presented.

Methods

Cells and virus strains. Georgia bovine kidney cells were cultured in minimum essential medium and used for BHV-4 multiplication, as previously described by Dubuisson et al. (1987). The BHV-4 strains used were VT strain (Thiry et al., 1981), LVR 140 (LVR) strain (kindly provided by Dr G. Welleman, Brussels, Belgium) (Welleman et al., 1983), the European reference strain Movar 33/63 (MOV) (ATCC VR-842) (Bartha et al., 1966) and the American reference strain DN 599 (DN) (ATCC VR-631) (Mohanty et al., 1971) (the latter two were both obtained from the American Type Culture Collection). These strains were plaque-purified three times before use.

Restriction endonuclease analysis. The production, extraction, digestion and electrophoresis of viral DNA were performed as described by Dubuisson et al. (1988). Cleavage products were calibrated with λ DNA HindIII fragments and φX174/RF HaeIII fragments. Viral DNA fragments shorter than 400 bp were rarely detected by restriction analysis or by hybridization and therefore were not investigated in this study.

Construction of a plasmid vector. The DNA of the VT strain was digested by EcoRI and ligated to the EcoRI-cleaved vector pSP18 (BRL). Competent Escherichia coli (HB101 strain) were transformed by the ligation products and the resulting clones identified by restriction analysis, as described by Maniatis et al. (1982).

Construction of a phage library. The DNA of the VT strain (10 μg) was partially digested with Sau3A1, loaded on a 5 to 25% sucrose density gradient and centrifuged in a Beckman SW56 rotor at 45,000 r.p.m. for 3 h at 20 °C. Twenty fractions were collected, dialysed and analysed by agarose gel electrophoresis. The DNA (approximately 400 ng) of the fraction containing fragments of 9 to 23 kbp was ligated to the vector arms (2 GEM-1 λ, Promega), packaged (Gigapack-gold, Stratagene) and the recombinants were titrated on the NM539 strain, as described by the suppliers. Phage DNA minipreparations were performed using NM539 according to Grossberger (1987).

Restriction mapping. Restriction maps of the viral EcoRI fragments were obtained by single and multiple digestions with EcoRI, BamHI and HindIII.

The prDNA maps were established by partial digestion of end-labelled prDNA. The plasmid carrying the prDNA at the EcoRI site was cleaved by HindIII (one site in the plasmid), digested by calf intestine phosphatase (Boehringer), end-labelled with the T4 polynucleotide kinase (Amersham) in the presence of [γ-32P]ATP (Amer sham), cleaved with SphI (one site in the plasmid) and loaded onto a 0.8% agarose gel. The fragment containing the insert was electro-eluted, partially cleaved by 14 enzymes (Fig. 3) and loaded onto a 1.5% agarose gel and an 8% polyacrylamide gel, as described by Maniatis et al. (1982). These maps were confirmed by analysing single or multiple digestion products of the cloned prDNA by the same enzymes on 1.5% agarose gel and 3-5% 5% or 10% polyacrylamide gels and by analysing the partial digestion products of a second end-labeling. The plasmid was digested with SstI (which cuts the insert at one site, but not the vector) and EcoRI, and then end-labelled at the EcoRI site with the Klenow fragment of the E. coli DNA polymerase I (Boehringer).

Results

Restriction maps of the EcoRI cloned fragments (VT strain)

Twelve of the 17 viral EcoRI fragments (fragments E to Q, except M, the right terminal fragment), were cloned as plasmids and mapped for the enzymes EcoRI, BamHI and HindIII; the fragment E was cloned from a recombinant phage DNA containing it. These restriction maps have been used to establish the map of the whole genome. One fragment was identified as prDNA due to its Mr, its presence in approximately half of the clones analysed, the fact that its restriction pattern was similar to that previously published by Ehlers et al. (1985) and by its hybridization with the hypermolar band of the EcoRI-cleaved VT DNA.

Hybridization of prDNA with the plasmid library allowed detection of the junction fragments between the unique and the repeated sequences, fragment G at the left and fragment I at the right. It also detected two fragments with restriction pattern similar to that of the prDNA, but with a size 200 bp greater or smaller (prDNA+ and prDNA-, respectively). The restriction maps of the fragments prDNA+, prDNA, prDNA and G for the enzymes BamHI, SstII and PstI are presented in Fig. 1.

The 200 bp difference between the three prDNA fragments was due to the repetition of the PstI fragments 8 and 9 (Fig. 1), which were present in prDNA-, prDNA and prDNA+ three, four and five times respectively. prDNA+ and prDNA− fragments were often observed in electrophoresis patterns of BHV-4 DNA EcoRI or BamHI digests (see Fig. 5 for the MOV and DN strains) and appeared as fragments either
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Fig. 1. Restriction maps for the enzymes BamHI (B), SstII (S) and PstI (P) of VT EcoRI fragments hybridizing with the prDNA used as a probe. These fragments were named prDNA+ (pr+), prDNA (pr), prDNA− (pr−) and G (left junction). Arrows indicate the suspected site of junction between two prDNAs, or between one prDNA and the unique central part at the left side of the VT genome.

Fig. 2. Restriction maps of the prDNA of the VT strain for 14 enzymes: (1) EcoRI, (2) StuI, (3) HinfI, (4) BamHI, (5) Sau3AI, (6) BglI, (7) SstII, (8) Apal, (9) PstI, (10) Bael, (11) Alul, (12) PvuII, (13) Asel and (14) Smal. The beginning of the prDNA was fixed 200 bp before the unique EcoRI site. Numbers are given for the fragments generated by enzymes 13 and 14 in the repeat part of the prDNA because the exact localization of the other fragments remains uncertain, due to the high number of sites found for these enzymes. PstI hypermolar fragment D was subdivided into D1 and D2 because of their different restriction maps for enzymes 10, 11, 12, 13 and 14. Sequences of the restriction sites have been noted and some of them are recognized by two enzymes (4 and 5, 11 and 12, 13 and 14). Arrows indicate the three regions which were sequenced to evaluate the G-C content of the prDNA.

slightly hypo- or hypermolar, depending on the DNA preparation. Therefore, in plaque-purified viral preparations, the size of the prDNAs was not homogeneous, despite the presence of a large amount of prDNA of the same size.

The prDNA sequence present in the left junction fragment (G) was slightly different from that of the other prDNAs; like the prDNA fragment it possesses four PstI fragments 8 and 9, but it does not contain the PstI fragment 7 and contained a new PstI fragment 5 (Fig. 1). Furthermore, the distance from the right SstII site to the site located after the last PstI fragment 8 of fragment G, or the site located after the PstI fragment 7 of prDNA fragments were identical. The exact junction point between the repeated and unique sequences at the left side of the VT genome was not established, but was estimated to be at the same place as found between two prDNA units, i.e. at approximately 200 bp before the EcoRI site, 120 bp after the last SstII fragment and therefore near this new PstI site (see arrow in Fig. 1).

Restriction maps of the prDNA

Restriction maps of the prDNA were constructed for 14 enzymes: EcoRI, StuI, HinfI, BamHI, Sau3AI, BglI, SstII, Apal, PstI, Bael, Alul, PvuII, Asel and Smal (Fig. 2). The beginning of the prDNA was fixed at 200 bp
before the EcoRI site by measuring the lengths of the terminal fragments obtained after EcoRI and BamHI cleavage (Fig. 6). The last six enzymes cut the repeated sequences of the prDNA, but the four repeated PstI fragments (E to D) were not homogeneous; indeed, the first D fragment (D1) had no site for AluI and PvuII, one site for BanI, one for AvaI and Smal, and these sites seemed to be present at the same position in the PstI fragment B. The three other D fragments (D2) had no site for BanI, one site for AluI and PvuII, and two sites for AvaI and Smal, and these sites were also present in the PstI fragment C. The region located between 1-35 kbp and 2-5 kbp (nearly all of the right side) from the beginning of the prDNA was therefore rich in similar, but not identical, repeated sequences.

**Restriction maps of the VT strain whole genome**

The total amount of recombinant phage obtained was approximately $3.5 \times 10^6$ p.f.u. and the analysis of about 100 clones allowed us to construct the restriction map of the VT strain DNA. This map and the location of some of the inserts in the recombinant phages are presented in Fig. 3. No cross-hybridization was detected between the phage vector DNA used as probes and the BHV-4 DNA,

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Fig. 3. Restriction maps of the whole BHV-4 genome of the VT, LVR 140, Movar 33/63 and DN 599 strains. Only three prDNAs were arbitrarily represented at each side of the unique sequence and this form does not represent the BHV-4 genome as it appears in the virion (see text). The numbers noted above the VT fragments correspond to their length in kbp, but the numbers have been noted above the fragments of the other strains only if their sizes were different from those of the corresponding VT strain. The localization of some recombinant phage inserts is shown in the upper part of the figure, those underlined were used as probes to confirm the VT map and to deduce the other maps. Variations observed between strains are noted in the lower part of the figure: a dot means variation in a restriction site; a filled bar, variation in the length of the unique part; an open bar, variation in the length of the repeated part of the genome.
also the hybridization of these vector DNAs with the lambda DNA used as the \( M_r \) marker provided a better calibration of the autoradiograms.

As the exact junction site between repeated and unique sequences at the left side of the genome remained unknown, position 0-0 was fixed at the beginning of the first prDNA copy, at the left junction and position 1-0 at the end of the first prDNA, at the right junction. This part of the VT genome has a size of 113 \( \pm \) 2 kbp. Southern blot hybridization of the HindIII-cleaved VT DNA with prDNA as a probe revealed two ladders of hypomolar fragments, corresponding to a variable number of prDNA units located at each end of the genome (Ehlers et al., 1985). Position 0-0 and position 1-0 correspond to the end of the smallest HindIII fragments at the left and the right part, respectively, of genomes present in the DNA preparation.

**Estimate of the G-C content of the unique and the repeated sequence**

The G-C content of the prDNA was first estimated by analysis of the frequency and the sequence of the restriction sites in the prDNA (Fig. 2). For a total of 348 bp, the G-C content was estimated at 82\% and it was then estimated as 62\% by sequencing three regions in the prDNA (region 1, 154 bp, 49-4\% G-C; region 2, 166 bp, 65-7\% G-C; region 3, 203 bp, 69-5\% G-C) (Fig. 3). The sequenced regions were located in the unique part of the prDNA (regions 1 and 2) and in the end of the repeated region (region 3), which was rich in short (5 to 6 bp) tandem repeats, sometimes separated by several base pairs and had a G-C content higher than that of the unique sequence of the prDNA. Owing to these local variations and the non-random distribution of restriction sites, the measurement of the G-C content of the prDNA is not accurate, but the estimate is about 70\%.

The G-C content of the unique part of the VT strain DNA was estimated to be 37\% by sequencing four different regions [position 0-367 to 0-369 (222 bp), position 0-554 to 0-556 (248 bp), position 0-570 to 0-572 (188 bp) and position 0-938 to 0-940 (202 bp)]. The G-C content was constant between these four different regions.

**Comparison of restriction patterns and maps of the four BHV-4 strains**

The restriction patterns of the VT, LVR, MOV and DN strains are presented in Fig. 4 and 5. These patterns were very similar and there were two kinds of strain differences observed on these patterns: the variations in relation to the prDNA and the variations independent of the prDNA.

The first type of variation was detected by the hybridization of prDNA on a Southern blot of the DNA of the four strains, after cleavage with EcoRI (Fig. 6a) and BamHI (Fig. 6b). The prDNA probe hybridized with the hypomolar band corresponding to cleaved prDNA, prDNA- and prDNA+ fragments, the end fragments and the junction fragments.

The major prDNA had a size of 2-65, 2-25, 1-585 and 2-25 kbp for the strains VT, LVR, MOV and DN, respectively. The prDNA of the four strains had one EcoRI site and one BamHI site, except that of the DN strain, which contained two sites for BamHI; this is characteristic of the DN-like strains (Thiry et al., 1989).

In the EcoRI pattern, the prDNA- fragment was located 200 bp below the prDNA fragment and therefore comigrated with the right end fragment. The intensity of the LVR prDNA- fragment was stronger than that found for the other strains and another faint band appeared on the autoradiogram 200 bp below the prDNA- fragment for the EcoRI pattern. This could be due to the fact that some LVR DNAs have a prDNA- fragment at the right end side. Indeed, this right terminal fragment was also visible on the BamHI pattern 200 bp below the right terminal fragment O. The prDNA+ fragments were often concealed by the large spot of the prDNA hypermolar fragment (see Fig. 6). A fragment of lower intensity was often visible 200 bp above the prDNA+ fragment and, when exposure times were longer, a ladder of bands appeared above and below the prDNA spot (data not shown). The size of prDNA in a viral DNA preparation was hence not homogeneous, despite the presence of a large amount of prDNAs of one size.

Since the EcoRI and the BamHI sites are located before the variable region of the prDNAs, the left end and the right junction fragments should be the same size for all the strains (Ehlers et al., 1985). The EcoRI left end fragment was never detected, probably due to its small size (around 200 bp) and the BamHI left end was the same size for the VT, LVR and MOV strains (fragment P, 0-7 kbp), but was smaller (fragment R, 0-4 kbp) for the DN strain because of the second BamHI site in the prDNA of this strain. The right junction fragments of the VT, LVR and MOV strains were identical in size for EcoRI and BamHI, whereas that of the DN strain was slightly (50 bp) longer.

The right end and the left junction fragments of the four strains should have a size proportional to the length of their respective prDNAs. This was the case for the right end fragments with EcoRI [fragments M (2-45 kbp), O (2-05), O (1-65) and M (2-05) for the VT, LVR, MOV and DN strains, respectively] and with BamHI [fragments O (1-95 kbp), O (1-55), O (1-15) and O (1-55) for the VT, LVR, MOV and DN strains, respectively]. Conver-
sely, the sizes of the left junction fragments were not proportional to that of their respective prDNAs with EcoRI [fragments G (4.9 kbp), H (5.05), I (4.35) and G (4.9) for the VT, LCR, MOV and DN strains, respectively] and with BamHI [fragments L (2.95 kbp), L (3.1), N (2.4) and L (2.95) for VT, LVR, MOV and DN, respectively]. This size variation in the left junction fragments was located either in the prDNA sequence present in these fragments, or at the beginning of the unique central sequence of the genome.

The variations independent of the prDNA observed in the restriction patterns of these four BHV-4 strains were of two types, i.e. the presence or absence of a restriction site and the length variation of some fragments. The first type of variation allows differentiation between MOV-like and DN-like strains; indeed, the MOV-like strains contained one additional EcoRI site (fragments F and L, E and M, and F and L of VT, LVR and MOV instead of fragment E of DN) and one additional HindIII site (fragments B and E of the VT, LVR and MOV strains replacing fragment A of DN) (Fig. 4 and 5). In the MOV-like group, the LVR strain had one additional EcoRI restriction site (new fragments F and G and no fragment B). The size variation of fragments was especially visible on the BamHI pattern [fragments I (5.5 kbp), I (5.6), I (5.4) and I (6.0) for the VT, LVR, MOV and DN strains, respectively] and the HindIII pattern [fragments U (1.45 kbp), V (1.24), V (1.33) and U (1.24) and for the VT, LVR, MOV and DN strains, respectively] (see Fig. 4 and 5).

The restriction maps of the four BHV-4 strains are presented in Fig. 3 and the location of the recombinant phage inserts that allowed the deduction of the other three maps are noted in the upper part of the figure. The map positions 0-0 and 1.0 were fixed following the same criteria as described for the VT strain (see above). This part of the genome had a length of 113, 112 and 113 (+2) kbp for the strains LVR, MOV and DN, respectively. The strain variations observed in the restriction patterns of these strains are summarized in Fig. 3. The additional

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**Fig. 4.** Restriction patterns of the VT (V), LVR 140 (L), Movar 33/63 (M) and DN 599 (D) strains for the enzymes EcoRI, BamHI and HindIII. Dotted lines correspond to the hypermolar band (prDNA) visible on electrophoresis, but fragments prDNA+ and prDNA− are not represented in this figure. Arrows indicate fragments generated by the gain or loss of a restriction site in the central part of the genome.
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EcoRI site of the MOV-like strains was located at position 0.342 to 0.344 and the HindIII site at position 0.864 to 0.870, depending on the strain. The additional BamHI site in the DN strain prDNA was located at positions 0.004 (left end) and 0.984 (right end) and the additional EcoRI site of LVR at position 0.647. The fragment size variations observed between strains were located at the left junction (positions 0.006 to 0.034, BamHI fragments L, L, N and L for the VT, LVR, MOV and DN strains, respectively) and at positions 0.211 to 0.225 (HindIII fragments U, V, V and U of the VT, LVR, MOV and DN strains, respectively) and 0.864 to 0.881. The beginning of this last region was fixed at the MOV-like strains' additional HindIII site (because a size variation was observed at the HindIII E fragments and not at the HindIII B fragments of these strains) to the end of the variable BamHI I fragments. The left border of this region for the DN strain is therefore uncertain and also this region of the DN strain was 400 bp to 600 bp longer than the same region in the MOV-like strains.

Discussion

BHV-4 has been isolated from a variety of samples and cells from diseased and healthy cattle and also from sheep (Van Opdenbosch et al., 1986) and cats (Kit et al., 1986). However, despite this broad range of origins, these viruses show a high antigenic relationship (Dubuisson et al., 1989) and a great similarity in their restriction patterns (Ludwig, 1983; Storz et al., 1984; Osorio et al., 1985).

Many of the differences observed between BHV-4 strains are located in the repetitive regions at both sides of the unique sequences. Up to eight different sizes of prDNA, varying from 1450 to 2850 bp, have been observed and this size variability seems to be due to the presence of a variable number of 200 bp fragments within the prDNA (Thiry et al., 1989). Despite the presence of many prDNA units of one or even two sizes in several strains (Thiry et al., 1989), a viral DNA preparation also contains prDNAs of different sizes to those of the major prDNA(s), by a multiple of 200 bp. This was also observed with the UT BHV-4 strain, which contains a minor prDNA 400 bp longer than that of the major prDNA (Ehlers et al., 1985). Two explanations could be given for this observation: either these different prDNAs are present in the same genome, or each genome contains prDNA of one size and the DNA preparation consists of a mixture of different populations of DNA molecules. No investigation was done to answer
Fig. 6. Representatives autoradiograms of Southern blot hybridizations of DNA of the VT (V), LVR 140 (L), Movar 33/63 (M) and DN 599 (D) strains cleaved with EcoRI (a) and BamHI (b), with the cloned prDNA as the probe. The left and the right junction fragments and also the left (not detected in the EcoRI patterns) and the right terminal fragments are visible (see Fig. 3 for their designations). The prDNA− (pr−), prDNA+ (pr+) fragments are shown in (a). The prDNA− fragments comigrate with the right terminal fragments and the prDNA+ fragments are represented by a dot in (b). The prDNA− fragments were contained in the large spot of the prDNA fragments. The arrows in (a) and (b) indicate the probable right terminal fragment of some LVR 140 genomes, which should have a prDNA− fragment at their right terminus and the high intensity of the prDNA− fragment of this strain can be seen.

this question for BHV-4, but similar observations have been made with herpesvirus saimiri DNA (a repetition of a 450 bp sequence at the right junction between L- and H-DNA and in some H-DNA units; Stamminger et al., 1987) and with herpesvirus aotus type 2 DNA, which has two H-DNA units, differing in size by 200 bp, that are present at relatively equal concentrations (Fuchs et al., 1985). In this last case, by analysing the dimer products of partial digestion with an enzyme that cleaves once in the H-DNA units, the authors proved that a genome could carry H-DNA units of different sizes. These observations, and the fact that these different prDNAs are also present in a DNA preparation of plaque-purified virus, favour the first hypothesis. These
repetitions within a prDNA of the VT strain do not have exactly the same sequence. Furthermore, these repeated sequences of ±200 bp are themselves rich in short tandem and inverted repeats (M. Goltz and H.-J. Buhk, personal communication).

This repetitive region in the prDNA represents a ‘hot spot’ of recombination, which is not surprising because G–C-rich tandem repeats are known to be the site of frequent recombinations in the eukaryotic genome (Smith, 1976; Jeffreys & Harris, 1982). The unique part of the prDNA is well conserved between BHV-4 strains (Ehlers et al., 1985; Thiry et al., 1989) and it is suggested that this region contains the signal sequence necessary for the cleavage of mature genomes from intermediate concatemers during the packaging of the BHV-4 genome (Ehlers et al., 1985). Each prDNA unit should contain such a signal and the cleavage would then occur following a headful packaging mechanism, as described for herpesvirus saimiri (Stammeringer et al., 1987). The prDNA does not seem to play another role, as the sequence of the H-DNA unit of herpesvirus saimiri did not contain any sizeable open reading frame (Bankier et al., 1987) and no RNA derived from these repetitive sequences has been detected (Tracy & Desrosiers, 1980; Bankier et al., 1985). Sequencing of the junctions between two prDNAs and between the prDNA and the unique sequence at each side will permit comparison to be made with such conserved sequences of other herpesviruses (Hammerschmidt et al., 1988). Furthermore, it is still unknown whether the genomes of strains possessing short prDNAs contain an overall number of repeats greater than the number in the genomes of strains possessing large prDNAs.

The right junction between the unique and the repeated sequences of the BHV-4 genome is relatively constant between BHV-4 strains, but the left junction is not so well conserved. A similar observation was made for herpesvirus saimiri by Stammeringer et al. (1985), who showed that the transition from L- to H-DNA occurs abruptly at the same cleavage site, at the right end of the L-DNA component, but some rearranged restriction enzyme cleavage sites typical of H-DNA are found within the first 800 bp of the L-DNA sequence of the left H–L DNA junction. We have shown that the first prDNA at the left junction (VT strain) is different from the other prDNA units and these differences are located near the equivalent junction between two prDNAs.

The central part of the BHV-4 genome is well conserved between strains and the three major differences between MOV-like and DN-like strains (Thiry et al., 1989) are one in the unique part of the prDNA and two at positions 0-343 and 0-864 in the unique central part of the genome. Further epidemiological studies are needed to ascertain the classification of BHV-4 strains in these two groups. In addition to these variations due to the presence or absence of restriction sites, fragment length polymorphisms were detected in some regions and sequencing of these regions for several strains will give an explanation of this size heterogeneity. This may be the result of a variation in the number of repeated sequences in these regions, as is the case for many herpesviruses, such as herpes simplex virus (Davison & Wilkie, 1981), Epstein–Barr virus (Heller et al., 1982), varicella-zoster virus (Casey et al., 1985) and bovine herpesvirus 1 (Hammerschmidt et al., 1986).

The restriction map of the BHV-4 genome will be an important tool for studying the gene organization in this virus DNA. This virus may be suitable for use as a viral vector for recombinant bovine vaccines, as suggested by Kit et al. (1986), as its genome structure will allow the insertion of large pieces of foreign DNA, compensated by the loss of terminal repeated sequences. This follows a similar method described for herpesvirus saimiri (Grassmann & Fleckenstein, 1989).

BHV-4 has been considered to be a bovine cytomegalovirus (Storz et al., 1984), as it shares the same pathobiology with the other cytomegaloviruses (beta-herpesviruses). Nevertheless, it has a shorter genome, a lower species specificity and a higher homology between strains than do beta-herpesviruses and also its genome structure and thymidine kinase activity (Kit et al., 1986) are characteristic of the gammaherpesviruses (Honess, 1984). Preliminary sequencing data have shown a relative deficiency in CpG dinucleotides and a relative excess of TpG and CpA dinucleotides, which is also characteristic of this subfamily (Honess et al., 1989). To confirm these results further sequence data are needed.

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