The genome of herpesvirus papio 2 is closely related to the genomes of human herpes simplex viruses

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Infection of baboons (Papio species) with herpesvirus papio 2 (HVP-2) produces a disease that is clinically similar to herpes simplex virus (HSV-1 and HSV-2) infection of humans. The development of a primate model of simplexvirus infection based on HVP-2 would provide a powerful resource to study virus biology and test vaccine strategies. In order to characterize the molecular biology of HVP-2 and justify further development of this model system we have constructed a physical map of the HVP-2 genome. The results of these studies have identified the presence of 26 reading frames that closely resemble HSV homologues. Furthermore, the HVP-2 genome shares a collinear arrangement with the genome of HSV. These studies further validate the development of the HVP-2 model as a surrogate system to study the biology of HSV infections.

In 1985 an epizootic outbreak of a simplex-like virus occurred in a captive baboon colony (Eberle et al., 1995; Levin et al., 1988). The virus associated with this outbreak was serologically similar to simian herpesvirus SA8. However, DNA sequence analysis of genes predicted to encode glycoproteins B, D and J (gB, gD and gJ) revealed that this virus was genetically distinct from SA8. As a result, the baboon isolates were designated herpesvirus papio 2 (HVP-2) (Eberle et al., 1995). HVP-2 infection of baboons produces a disease that is clinically similar to herpes simplex virus (HSV) infection of humans. Adult animals develop genital lesions whereas oral lesions represent the predominant form of disease in younger animals. (Levin et al., 1988). Acquisition of genital infection is primarily associated with the onset of sexual activity in baboons. Spontaneous reactivation from latency has been demonstrated, a pattern consistent with HSV infection of humans (Martino et al., 1997; D. Martin, unpublished). Finally, studies have demonstrated that the baboon is a natural reservoir for HVP-2 as 90% of a group of wild-caught baboons were seropositive for the virus (Eberle et al., 1997, 1998).

The objective of this study was to characterize the genome of HVP-2. The results of this study are important for several reasons. First, further characterization of the HVP-2 genome is necessary in order to validate the HVP-2 system as a surrogate model for HSV studies. Second, characterization of the genome will identify and target specific genes for future study. Finally, characterization of the genome of a non-human primate simplexvirus will ultimately provide additional insight into the evolutionary biology of herpesviruses. Consistent with these objectives, we have developed a physical map of the HVP-2 genome.

Our working hypothesis for these studies was that the HVP-2 genome would be collinear with the HSV genome. In order to map the HVP-2 genome we chose to use a modification of the procedure of Thomson & Smith (1999). Concatenemic HVP-2 strain 860 DNA was digested with BamHI or KpnI. Fragments were identified alphabetically by descending size (Table 1). The results of BamHI restriction analyses demonstrate that the size of the HVP-2 genome is approximately 161 kb. By comparison, DNA sequence analysis has demonstrated that the HSV-1 genome is 152 kb while restriction analysis has demonstrated that the herpes B virus genome is estimated to be 165 kb (Harrington et al., 1992; McGeoch et al., 1988). Current restriction and DNA sequence data suggest that the difference in size between HSV-1 and HVP-2 may be due to larger repeat regions. Detailed DNA sequence analysis will be required to precisely define the size of the HVP-2 genome.

HVP-2 BamHI and KpnI fragments were shotgun-cloned into pUC19 resulting in the production of two genomic libraries. Twenty-four BamHI clones were initially identified that contained more than 75% of the viral genome (Table 1). The KpnI library contains more than 79% of the genome (Fig. 1), and includes clones that contain the majority of BamHI fragments A and C, which were refractory to cloning as intact fragments. The termini of the respective clones were subjected to DNA sequence analysis. The resulting sequence data were compared to known simplexvirus sequences using the "BLASTX" (translated BLAST search) comparison engine at the NCBI BLAST website. The description of each clone is given in Table 1 including the HSV protein with which the given construct has homology (presuming expression of the open reading...
Table 1. HVP-2 BamHI genomic library

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*Name of genomic fragment; fragments named based on decreasing size starting with A and arranged in order on the genome template by homology with HSV-1 protein.
†Name of clone containing genomic fragment.
‡HSV protein with which the terminus of the fragment has homology based on BLASTx. NH, no homology, or homologous to several proteins. These tend to be in areas of high GC content or intergenic regions.
§‘Expect’ value obtained from BLASTx analysis.
‖Method by which continuity with fragment to the right was proven. SAJ, sequencing across the junction (the BamHI site); Sn, Southern analysis; Special, see authors’ website for discussion.
††These termini are identical by sequence. The amount of sequence data available for K27B did not provide a significant match by BLAST analysis whereas more extensive sequence data for clone 83 revealed homology to ICP0.

frame). Consistent with the method of Thomson & Smith (1999), the fragments were aligned using the HSV-1 genome as a template. A summary of this alignment is shown in Fig. 1. Details of the analysis of specific fragments and proof of their arrangement on the genome can be found on-line at http://www.sfbr.org/sfbr/departments/virology/hvp2.html.

The HSV genome is divided into UL and US regions by three repeat regions denoted b, a, and c, with b and c being inverted with respect to their repeats (Fig. 1A) (Roizman, 1979; Umene, 1998; Wagner & Summers, 1978). As HSV replicates, virion-sized genomes are cleaved from concatemeric genomes at the a sequence and are subsequently packaged in the nucleocapsid. By extrapolation to the HSV genome, we would predict that the HVP-2 genome contains large segments of repeat that are cleaved during virus replication also. Indeed, the results of several studies have shown this to be the case. For example, cleavage of the HVP-2 genome at
an a sequence during genomic packaging would be expected to cut the BamHI A and C fragments into smaller pieces (17 and 4.5 kb fragments for A and 9.5 and 4.5 kb fragments for C, based on alignment shown in Fig. 1). Digestion of HVP-2 DNA with BamHI reveals bands of these sizes that are present at submolar ratios, indicating that these fragments may arise as the result of cleavage of the viral genome by the viral cleavage and packaging machinery (B', E' and K') (Fig. 2A). To demonstrate that these bands represent terminal fragments that result from genomic cleavage, HVP-2 DNA was treated with Exonuclease III followed by BamHI cleavage. Exonuclease III degrades DNA from free 3' ends. As a result, free termini that result from viral processing of genomic DNA would be degraded at a much greater rate than contiguous, concatemeric DNA. Analysis of an ethidium bromide-stained agarose gel clearly demonstrates that fragments B', E' and K' are readily degraded by Exonuclease III treatment relative to other genomic fragments. These results demonstrate that the B', E' and K' fragments represent the free genomic termini generated during genome packaging (Fig. 2A).

Sequence data obtained during the mapping of the genome showed that repeat DNA existed on either side of the

Fig. 1. Physical map of the HVP-2 genome. (A) General structure of the HSV genome. Note the unique long (UL) and unique short (US) coding regions and the repeats designated a, b and c. (B) The HVP-2 BamHI and KpnI (partial) restriction maps were generated as described. Alignment of the open reading frames generates a physical map that is collinear with the HSV genome. The size and locations of the subfragments B', E' and K' resulting from genome cleavage during packaging are shown above the HVP-2 map.

Fig. 2. Demonstration of genomic termini and repeat DNA. (A) HVP-2 genomic DNA was treated with Exonuclease III (ExoIII) to degrade free genomic termini. The DNA was subsequently digested with BamHI and resolved on a 0.8% agarose gel and visualized by ethidium bromide staining. Note the loss of the K' band after incubation with 10 U of ExoIII and the loss of the B' and E' bands after incubation with 100 U ExoIII. (B) Schematic of the HVP-2 genome demonstrating positions of KpnI probes and respective BamHI fragments compared with the US and repeat regions. (C) Southern analysis of HVP-2 repeat DNA. HVP-2 strain 860 DNA was BamHI digested, resolved on a 0.8% agarose gel, and subjected to Southern analysis using the constructs KB8 and K4 as probes. Note that the unique KpnI probes both hybridize to the same BamHI fragments demonstrating the repeat structure of the HVP-2 genome.
genomic packaging cleavage site (probable a repeat). Clone K27B has a terminus with sequence identical to a terminus of clone 83 (Fig. 1 and Table 1) and KpnI clone K4 (Fig. 1) has a terminus with sequence identical to a terminus of clone and K10+ (not shown). To demonstrate the presence of repeat DNA, Southern analysis of HVP-2 BamHI digested DNA was conducted using the K4 or KB8 (subclone of K10+), see Fig. 1) constructs probes (Fig. 2B). As K10+ (and therefore KB8) has one terminus that contains identical sequence to one terminus of K4, it is not surprising to see identical hybridization patterns when comparing the two Southern analyses. The patterns show that K4 and KB8 not only hybridize to the BamHI fragments from which they were cloned (A and C, respectively), but also to the fragment from which the other clone was generated and to the genomic termini subfragments B and E (Fig. 2C). Together, these data demonstrate that HVP-2 contains inverted repeat DNA on the US and UL sides of the genomic packaging cleavage site. This suggests that the HVP-2 genome is arranged similarly to the HSV-1 and HSV-2 genomes with repeat regions on either side of the genomic packaging cleavage site. While the previously mentioned data suggest that the HVP-2 genome may undergo isomerization, proof of genomic isomerization will require the identification of restriction sites that do not cut within the repeat regions in order to perform the appropriate analyses.

The results of this study demonstrate that the genome of HVP-2 is collinear with HSV and contains repeats on either side of a genomic cleavage site. These findings suggest that HVP-2 genome may contain b, a and c repeats, as does HSV. In addition, this study reports over 50 sequence analyses, including 26 open reading frames which have high degrees of homology between HVP-2 and HSV. Furthermore, these results provide extensive supporting evidence that the genomes of HVP-2 and HSV are closely related. The results of these studies combined with previous genetic, pathogenic, and serologic analyses further demonstrate the close relationship between HVP-2 and HSV (Bigger & Martin, 2002; Eberle et al., 1995, 1997, 1998; Levin et al., 1988).

Herpesviruses have co-evolved with their hosts throughout evolution (McGeoch et al., 1995). As such, the molecular biology of each virus has similarly co-evolved in the context of the molecular biology of its host. Therefore, certain aspects of viral pathogenesis may only be fully understood by studying a virus in the context of the natural host. HVP-2 infection of baboons provides us with a model to study the pathogenesis of a simplexvirus in the context of a natural, primate host. The characterization of the HVP-2 genome described in this study provides the foundation for future studies that will exploit this virus as a surrogate model system to study simplexvirus biology in a primate system.

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REFERENCES


