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Sequence of the ateline alphaherpesvirus 1 (HVA1) genome

R. Eberle¹ and Darla H. Black¹

¹Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA

Abstract

Here, we report the genome sequence of a spider monkey alphaherpesvirus (ateline alphaherpesvirus 1, HVA1) and compare it with that of other primate alphaherpesviruses. The HVA1 genome is 147,346 bp long and contains 67 predicted ORFs. The genetic layout of the HVA1 genome is similar to that of the squirrel monkey alphaherpesvirus (saimirine alphaherpesvirus 1, HVS1) in that it lacks inverted repeat regions flanking the unique long region and homologues of the UL43, UL49.5, US8.5 and US10-12 genes. Unlike HVS1, HVA1 also lacks a homologue of the RL1 (c34.5) gene and a replication origin near the end of the genome. Consistent with previous phylogenetic analyses, all predicted proteins of HVA1 are most closely related to those of HVS1.

The herpes simplex viruses of humans (HSV1, HSV2) are members of the order *Herpesvirales*, family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Simplexvirus*. Related viruses have been isolated from chimpanzees (chimpanzee herpesvirus, ChHV), baboons (papiine alphaherpesvirus 2, HVP2), vervets (cercopithecine alphaherpesvirus 2, SA8), macaques (macacine alphaherpesvirus 1, BV), squirrel monkeys (saimirine alphaherpesvirus 1, HVS1) and spider monkeys (ateline alphaherpesvirus 1, HVA1) [5, 7, 8]. Phylogenetic analyses separate these viruses into three distinct clades (human/ape, Old World monkey and New World monkey viruses), suggesting that these viruses have co-evolved with their hosts [2, 3]. Complete genome sequences have been determined for all of the primate alphaherpesviruses except HVA1 [9–14]. With the exception of HVS1, the primate alphaherpesviruses all have type E genomes and have the same overall genetic arrangement: unique long (U_L) and unique short (U_S) regions flanked by inverted repeat regions (R_L and R_S , respectively). In contrast, HVS1 has a type D genome that lacks inverted regions flanking the U_L region (so only two genome isomers occur instead of four), the US1 gene is located within the R_S region, and homologues of the UL43, 49.5, US8.5 and US10-12 genes are lacking. Here, we report the sequence of the HVA1 genome and compare it the genomes of other primate viruses.

Correspondence to: R. Eberle.

Compliance with ethical standards

Conflict of interest The authors declare that neither has any conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

HVA1 strain Lennette was originally isolated from a young spider monkey in a zoo that died of a generalized infection [6]. HVA1 DNA was purified from infected Vero cells on NaI gradients as described [1, 12]. Two separate DNA preparations were sent for Illumina sequencing. The HVS1 genome [12] was used as a scaffold for initial assembly of HVA1 sequences although only coding sequences were assembled. Subsequent assembly was done using a combination of *de novo* assembly and manual extension of sequence assemblies. All areas where gaps occurred in the assembled sequence were filled by Sanger sequencing of PCR products. Similarly, areas of repeated sequences and areas of the draft genome where the genetic structure deviated from that of the HVS1 genome were confirmed by PCR/sequencing. Termini of the genome were not experimentally determined; termini were inferred from a combination of similarity to the HVS1 genome (U_L terminus) and an abundance of sequence reads terminating at a single point (U_L and R_S termini). The HVA1 genome sequence has been deposited in the GenBank database under accession number KY385637.

The HVA1 genome is 147,346 bp long, has a G+C content of 75.5%, and is predicted to encode 67 ORFs (Fig. 1). Like HVS1, the HVA1 U_L region is not bounded by inverted repeats. At the U_L terminus, there is an inverted-duplicated 166-bp sequence that flanks 1053 bp of highly repeated sequences that have a very high C+T content (86%) on the positive strand. The region between the 166-bp terminal repeat and the UL56 ORF of HVA1 is only 365 bp, half of which consists of repeats (as compared to 646 bp in HVS1). Also, there was no DNA replication origin located in this area of the HVA1 genome, as occurs in the HVS1 genome. The arrangement of ORFs in the U_L region is the same as in HVS1 and other primate alphaherpesviruses in both gene order and orientation. Like HVS1, HVA1 lacks homologues of the UL43 and UL49.5 genes, and the RL2 (ICP0) gene, which has two exons, is located between the U_L region and the R_S repeats. However, HVA1 lacks a detectable homologue of the RL1 (c34.5) gene, which HVS1 does have. There is also a DNA replication origin palindrome located between the UL29 and UL30 genes as in all other primate alphaherpesviruses.

The short region of the HVA1 genome (both the U_S region and R_S inverted repeats flanking it) is organized in a manner similar to that of HVS1. Homologues of both the US1 (ICP22) and RS1 (ICP4) genes are located within R_S . The U_S region consists of the US2-9 genes, and as in HVS1, the US4 (glycoprotein gG) gene is much smaller than the US4 gene of HSV2, ChHV, and other monkey viruses. As in HVS1 one of the U_S/R_S junctions is located at the US2 termination codon, but in HVA1 the other R_S/U_S junction is located near the middle of the US9 ORF rather than at its terminus. Within the R_S region, there is a DNA replication origin palindrome and six areas of repeat sequences. These areas of repeats vary in the number of iterations (5-20) and size of the repeat unit (6-17 bp), although all had a high G+C content (73-85% G+C; average, 81%).

As observed previously for the UL27 (glycoprotein B) sequence [4], the level of amino acid sequence similarity between homologues of predicted HVS1 and HVA1 proteins was much lower than that seen among the Old World primate viruses (BV, SA8 and HVP2). Amino acid sequence identity between HVS1 and HVA1 varied from a low of 28.2% to a high of 84.0%, with an average of only 58.8%. The five most highly conserved proteins included

UL15 (DNA packaging terminase), UL18 and UL19 (capsid proteins), UL29 (single-strand DNA binding protein) and UL30 (DNA polymerase). The least conserved included UL56 (membrane protein), RL2 (ICP0; immediate early ubiquitin E3 ligase), US1 (ICP22; immediate early regulatory protein), US4 (glycoprotein G) and US5 (glycoprotein J).

In summary, unlike most other primate simplexviruses, only the U_S region of the HVA1 genome is bounded by repeats, resulting in a type D genome arrangement with two genomic isomers as found in varicelloviruses rather than the four isomer-type E genomes that the other primate simplexviruses have. The HVA1 US1 gene is present within the R_S repeats, but the US10 homologue present in the VZV and SVV R_S region is lacking in HVS1 and HVA1. Like HVS1, HVA1 also lacks homologues of several genes that are present in all other primate simplexviruses (US8.5, US10/11/12, UL43 and UL49.5). HVA1 proteins are clearly more closely related to analogous proteins of HVS1 than to those of varicelloviruses, such that phylogenetic analyses firmly place HVA1 within the primate simplexvirus clade, albeit on a separate branch together with HVS1.

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Fig. 1.
Genetic organization of the HVA1 genome. Open reading frames are identified by light grey arrows, and exons are darker grey. The R_S regions are identified by dark grey arrows