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Primary Structure of the Alcelaphine Herpesvirus 1 Genome

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Alcelaphine herpesvirus 1 (AHV-1) causes wildebeest-associated malignant catarrhal fever, a lymphoproliferative syndrome in ungulate species other than the natural host. Based on biological properties and limited structural data, it has been classified as a member of the genus *Rhadinovirus* of the subfamily *Gammaherpesvirinae*. Here, we report on cloning and structural analysis of the complete genome of AHV-1 C500. The low GC content DNA (L-DNA) region of the genome consists of 130,608 bp with low (46.17%) GC content and marked suppression of CpG dinucleotide frequency. Like in herpesvirus saimiri, the prototype of the rhadinoviruses, the L-DNA is flanked by approximately 20 to 25 GC-rich (71.83%) high GC content DNA (H-DNA) repeats of 1,113 to 1,118 nucleotides. The analysis of the L-DNA sequence revealed 70 open reading frames (ORFs), 61 of which showed homology to other herpesviruses. The conserved ORFs are arranged in four blocks collinear to other *Rhadinovirus* genomes. These gene blocks are flanked by nonconserved regions containing ORFs without similarities to known herpesvirus genes. Notably, a spliced reading frame with a coding capacity for a 199-amino-acid protein is located in a position homologous to the transforming genes of herpesvirus saimiri at the left end of the L-DNA. A gene with homology to the semaphorin family is located adjacent to this. Despite common biological and epidemiological properties, AHV-1 differs significantly from herpesvirus saimiri with regard to cell homologous genes, probably using a different set of effector proteins to achieve a similar T-lymphocyte-transforming phenotype.

Malignant catarrhal fever (MCF) is a usually fatal disease in various ruminants. It occurs in two distinct epizootological forms: (i) wildebeest-associated MCF (WA-MCF), which is widespread in southern and eastern Africa; and (ii) sheep-associated MCF (SA-MCF), which has an almost worldwide distribution. The causative agent of WA-MCF has been isolated from asymptomatically infected blue wildebeest (*Connochaetes taurinus taurinus*) (61). It was classified as alcelaphine herpesvirus 1 (AHV-1). Preliminary characterization has shown that AHV-1 should be included in the genus *Rhadinovirus* of the subfamily *Gammaherpesvirinae* (15). Closely related herpesviruses were isolated from several other species of genuine antelopes (68). Attempts to isolate the agent of SA-MCF were unsuccessful, but partial genomic sequences of a virus with a close relationship to AHV-1 have been characterized from lymphoblastoid cells of diseased cattle, deer, and rabbits (16). This agent has been designated ovine herpesvirus 2. The agent is readily demonstrated by serology (42) and PCR (9, 78) in samples from SA-MCF. The symptoms of both forms include fever, ocular and nasal discharge, corneal opacities, and diarrhea. The pathology of MCF is characterized by a combination of lymphoproliferation and degenerative symptoms in the affected animals. A certain variation of the predominant morphological changes has been noted between different affected species, from lymphosarcoma-like changes in deer (12) to vasculitis and necrosis predominating in other species (64). The lymphoid and lymphoblastoid cells present in MCF have been shown to be T lymphocytes of a CD8 or CD4 phenotype (17, 22, 48, 49, 77). MCF and the diseases caused by infection of marmosets with herpesvirus saimiri show remarkable similarities. Both of the causative agents belong to the genus *Rhadinovirus*, both are apathogenic in their natural hosts, and both cause lymphoproliferative disease in other species. Both viruses can induce a similar pathology after infection of rabbits. The lymphocytes involved are of an analogous phenotype, and lymphocytic cell lines can be established from naturally and experimentally infected animals (26, 36, 45, 72).

Attracted by the striking similarities of MCF to herpesvirus saimiri-induced pathology and lymphoproliferative syndromes, we decided to identify AHV-1 genes associated with transformation and lymphoproliferation. Thus, we have cloned and sequenced the complete genome of AHV-1. The genome organization is essentially collinear with the other well-characterized rhadinoviruses, herpesvirus saimiri (3), equine herpesvirus 2 (EHV-2) (73), and human herpesvirus 8 (HHV-8 [Kaposi's sarcoma-associated herpesvirus]) (51, 65). Marked differences were found in the regions interspersed between the blocks of conserved herpesvirus genes. There were no homologs to the known cytokine-related or transformation-associated genes of HHV-8 and herpesvirus saimiri.

MATERIALS AND METHODS

Cell lines and viral culture. The bovine epithelial kidney cell line MDBK (ATCC CCL 22) was propagated in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 μg of gentamicin per ml, and 350 μg of L-glutamine per ml. AHV-1 strains C500 (62) and WC11 (63) were serially propagated by infection of fresh MDBK cells with aliquots of infected MDBK cells showing cytopathic changes in more than 80% of the cells. AHV-1 C500 was obtained from H. W. Reid (Moredun Research Institute, Edinburgh, United Kingdom), and the attenuated WC11 strain was obtained from D. W. Verwoerd (Veterinary Research Institute, Onderstepoort, South Africa). For the preparation of virions, tissue culture supernatant was precleared by centrifugation at 2,000 × *g*. Strain C500 virions (passage 5) were pelleted by centrifugation at 50,000 × *g* with an SW28 rotor. The viral DNA was extracted from the pellet with phenol-chloroform and precipitated with ethanol. WC11 DNA was further purified by density gradient centrifugation as described previously (37).

Cloning procedures. C500 DNA was restricted with *Bam*HI, *Bam*HI-*Sma*I, *Eco*RI, *Hin*dIII, *Pst*I, *Nsi*I, *Sst*I, and *Xba*I. The fragments were ligated into the respectively cut plasmids pBluescribe M13+ and pBluescript KSII+ (Stratagene,

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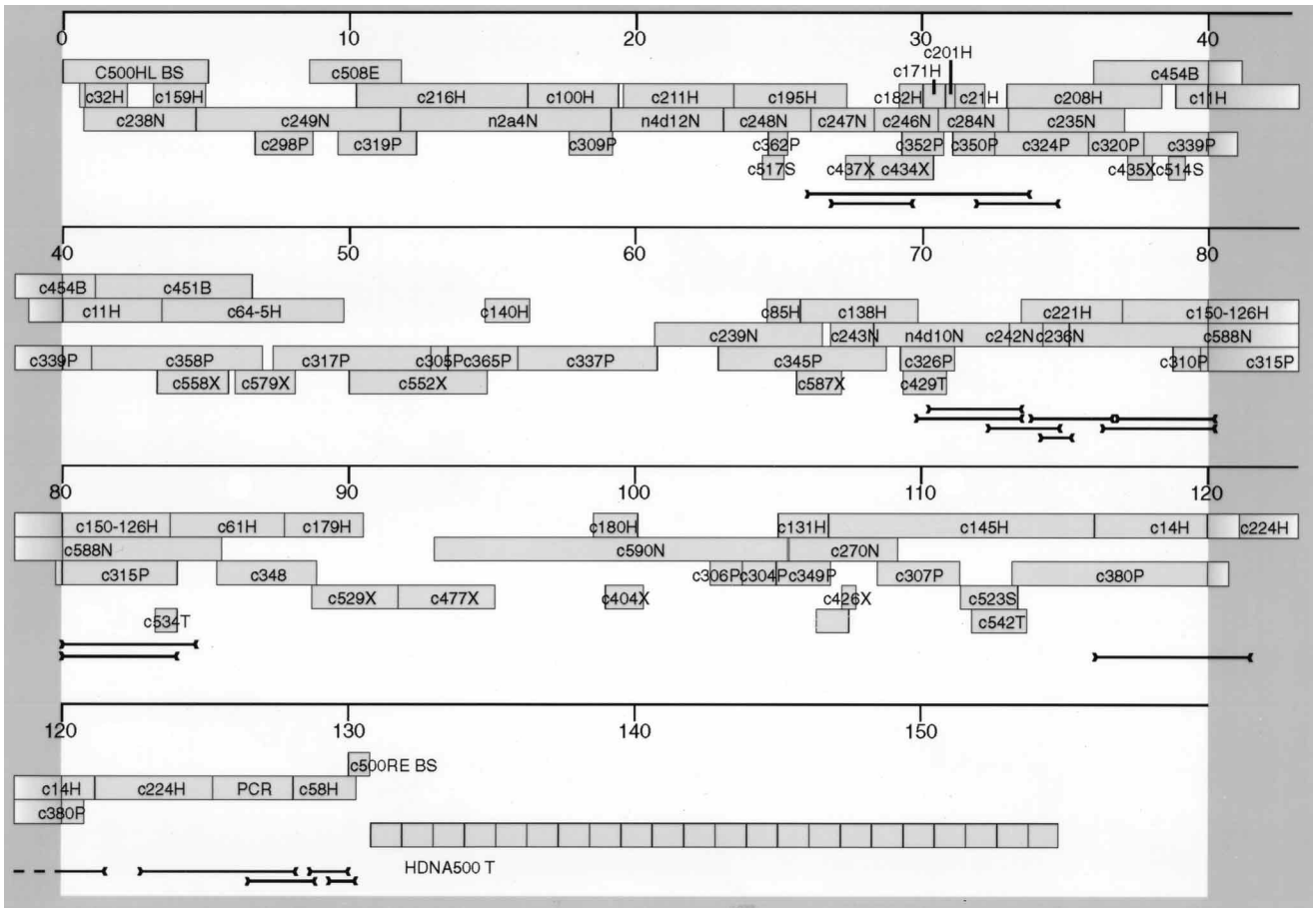

FIG. 1. Molecular cloning of the AHV-1 C500 genome. Clones were numbered consecutively in order of isolation, and a letter corresponding to the cloning sites used was added. B, *Bam*HI; E, *Eco*RI; BS, *Bam*HI-*Sma*I; H, *Hin*dIII; P, *Pst*I; N, *Nsi*I; S, *Sma*I; T, *Sst*I; X, *Xba*I. PCR-amplified fragments used to verify arrangements of clones and junctions between individual nonoverlapping clones are indicated below (>——<).

La Jolla, Calif.) and transformed into *Escherichia coli* K-12 DH5α (Life Technologies, Gaithersburg, Md.) by electroporation. Specific clones were identified by color selection with isopropyl-β-D-thiogalactopyranoside plus 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and a subsequent colony screening (6) with [α-^{32}P]dATP-labelled (24), gradient-purified DNA of AHV-1 WC-11. The cloned fragments were mapped to the viral genome by Southern blotting (70) and sequencing of their ends. Clones containing terminal low GC content DNA (L-DNA) fragments were identified by hybridizing *Bam*HI-*Sma*I and *Hin*dIII clones with a radiolabelled WC11 repeat probe prepared from supermolar small fragments of *Sac*II-digested viral DNA. A fragment of 4 kb missing in the initial cloning procedure was amplified by PCR with specific primers and cloned in pBluescript KSII+. Random subclones from suitable genomic clones were generated by sonication of inserts purified by preparative agarose gel electrophoresis (66). DNA from these shotgun clones was prepared either by modified alkaline lysis followed by precipitation with polyethylene glycol (Applied Biosystems, Foster City, Calif.) or by anion-exchange chromatography (Qiawell kit; Qiagen, Hilden, Germany).

PCR applications. PCR amplification of AHV-1 was done with 250 ng of DNA isolated from lytically infected MDBK cells. Fragments smaller than 2 kb were amplified with Ampli*Taq* (Perkin-Elmer, Weilderstadt, Germany); the missing 4-kb fragment (Fig. 1) was amplified with Vent-polymerase (New England Biolabs). Fragments larger than 2 kb and up to 8 kb were amplified with the Expand Long Template PCR System (Boehringer Mannheim). For the rapid amplification of cDNA ends (RACE), the Marathon kit (Clontech, Palo Alto, Calif.) was used. As starting material for RACE, poly(A)$^+$ RNA from AHV-1-infected MDBK cells was isolated with ferromagnetic poly(dT) beads (Dynal, Hamburg, Germany).

Nucleotide sequence determination. DNA sequencing was performed with an ABI 373A automated sequencer and *Taq*-dye deoxy terminator chemistry (Applied Biosystems) in a combination of shotgun and primer-walking approaches (66). Random clones were sequenced with standard primers flanking the plasmid cloning sites. Regions of low redundancy and residual single-stranded regions were sequenced with virus-specific primers. All oligonucleotides were purchased from Eurogentec (Seraing, Belgium). The assembly of sequence readings was done with the program XBAP (21) on a Sparcstation 10 (SUN Microsystems, Mountain View, Calif.). Oligonucleotides were selected with the program OSP (33) implemented in XBAP.

Nucleotide and protein sequence analysis. The GCG package (28) with the FASTA (60) and the BLAST programs (4, 29) was used for analysis of nucleotide and amino acid sequences and for comparison with GenBank and SwissProt databases (versions 97.0 and 34.0, respectively). Potential coding regions were identified with the program GENEMARK (13) using matrices for human and gammaherpesvirus DNA (kindly compiled by William Hayes, Georgia Institute of Technology, Atlanta). Putative signal sequences were analyzed with the SignalP server with a neuronal network trained on eukaryotic sequences (58).

Nucleotide sequence accession number. The complete L-DNA sequence is available from GenBank as AF005370. The different repeat sequences are available under accession no. AF005363 to AF005368. The right-terminal H-L junction sequence with rearranged high GC content DNA (H-DNA) is available as AF005508. The RACE-amplified cDNA sequences of open reading frames (ORFs) A2 and 57 are available as AF005369 and AF005362, respectively. The left-terminal region is also separately available in the database under accession no. U18243.

RESULTS

Molecular cloning and sequencing of AHV-1 genome. The positions of cloned fragments used for sequencing of the genome are shown in Fig. 1. Sequencing of numerous short stretches indicated that the AHV-1 genome is largely collinear to herpesvirus saimiri and that the previously published mapping of AHV-1 strain WC11 (14) used the opposite orientation of the viral genome with respect to herpesvirus saimiri. For

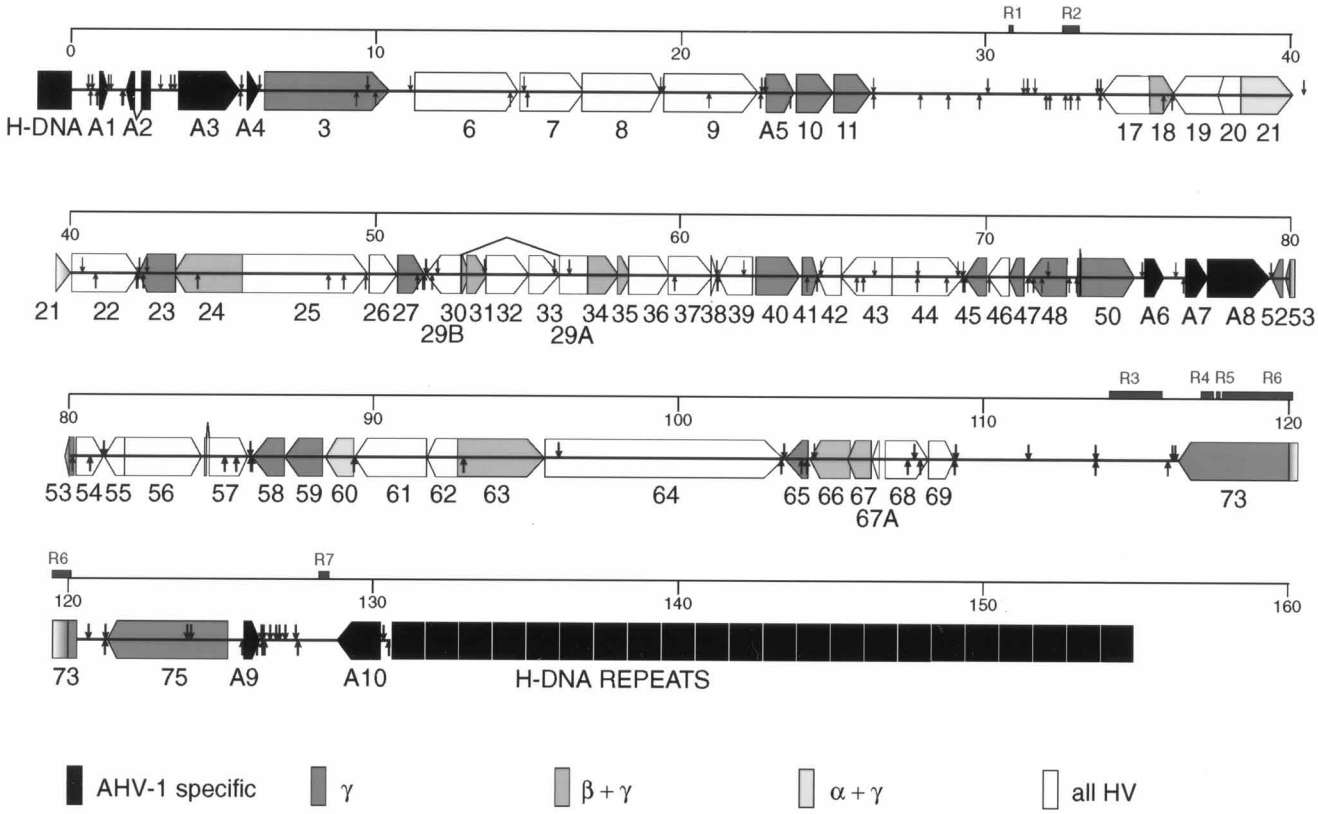

FIG. 2. Organization of the AHV-1 C500 genome. ORFs are as described in Table 1. Potential polyadenylation signals (AATAAA or AATTAAA) are given for the respective upper (↓) and lower (↑) DNA strands. Major repetitive regions (R1 to R7) are marked by shaded boxes. Shading of arrows representing the ORFs reflects the conservation among the three subfamilies (α, β, and γ) of herpesviruses (HV).

convenience, we have reversed the orientation of the genome and adapted the orientation of the L-DNA to the published genomes of herpesvirus saimiri (3), EHV-2 (73), and HHV-8 (51, 65). Altogether, 1,022 kb of raw data from 2,130 single readings were assembled into 130,608 bp of L-DNA sequence with an average redundancy of 7.83. The average content of GC nucleotides is 46.17%, with some significant local variations, particularly in the repetitive GC-rich regions. Like in herpesvirus saimiri, there is a marked suppression in the frequency of CpG dinucleotides. Junctions between adjacent, nonoverlapping restriction fragments were verified by direct sequencing of appropriate junction-spanning PCR fragments. The arrangement of regions represented by several smaller clones was also verified by PCR as indicated in Fig. 1.

Repetitive regions in the AHV-1 genome. A region of sequence heterogeneity was represented by clones c145H and c380P. A Southern blot showing multiple bands of genomic DNA suggested a repeat sequence of approximately 2 kb varying by ±500 bp. Random sequencing yielded 60 gel readings consisting exclusively of 29-bp repetitions, and there were only two types of junction sequences to the regular L-DNA. This indicated a uniform 29-bp GC-rich repeat region without other sequences interspersed. Because the exact number of repeats could not be determined, we have deliberately inserted a total of 59 units of this 29-bp element into the genome (positions 114100 to 115820 [R3] (Fig. 2). Each 29-bp unit contains an *amber* codon. Consequently, only a multiply spliced transcript from that region could possess coding potential. Several distinct repeats are localized within ORF73, as in the homologous genes of herpesvirus saimiri and HHV-8 (R4-6). All major repeats are shown in Fig. 2 (R1 to R7) and specified in the GenBank entry.

Eight independent H-DNA 1.1-kb *Sst*I repeat units were cloned and sequenced. The C500 repeat sequence varies from 1,113 to 1,118 bp between the individual clones. Differences occurred at two positions in the H-DNA, either with a variable number of TG dinucleotides (position 125) or with eight or nine thymidines (position 1075). The GC content of H-DNA is 71.83%, and there is no obvious CpG suppression.

The coding capacity of the virion DNA. Potentially protein-coding ORFs in the L-DNA sequence were defined by criteria similar to those previously applied to other herpesvirus genomes (3, 53): (i) ORF size larger than 60 amino acids (aa), (ii) presence of potential transcriptional start sites and polyadenylation sites, (iii) high GENEMARK score (>0.5), and (iv) homology to previously described herpesvirus or other genes. The longest of several overlapping ORFs was considered significant. The identified potential ORFs are shown in Fig. 2 and Table 1. The nomenclature of ORFs was also adapted to other rhadinovirus sequences (3, 65, 73). Thus, ORFs with homologs in herpesvirus saimiri were assigned the number of the related herpesvirus saimiri gene. ORFs with no homolog in herpesvirus saimiri were consecutively numbered, beginning from the left end of the L-DNA (with the prefix A for alcelaphine).

Genes with homology to other herpesvirus genes and nonconserved genes. Conserved herpesvirus genes are arranged in four blocks collinear to herpesvirus saimiri (Fig. 2 and Table 1). Interspersed are ORFs without homology to known herpesvirus genes located in the nonconserved regions. The left-terminal region is comprised of four ORFs. The short ORF A1

TABLE 1. AHV-1 ORFs and homologs to other herpesviruses

Gene block[a]	AHV-1 ORF	Strand	Position of[b]:		Length (aa)	Position of[c]:		HVS		EBV	
			ATG start codon	Stop codon		TATA	Poly(A)	%id[d]	ORF[e]	%id	ORF
Left end	A1	+	918	1211	97	898	1248, 1308				
	A2	−	2230	1796	144	SA = 2230	1670				
	A2	−	2477	2313	55	2505 (?)	SD = 2313				
	A3	+	3492	5453	653		5590				
	A4	+	5732	6097	121	5665	6123				
	3	+	6333	10442	1,369	6286	11134	26.7	3	24.7	(BNRF1)
I	6	+	11260	14643	1,127	11224	15048	49.7	6	43.4	BALF2
	7	+	14725	16767	680			43.6	7	38.2	BALF3
	8	+	16751	19315	854	16699	19314	46.2	8	46.5	BALF4
	9	+	19428	22508	1,026	19405	22512	57.0	9	53.2	BALF5
	A5	+	22784	23692	302					16.3	BILF1
	10	+	23774	24988	404	23761		23.3	10	20.8	RAJILF1
	11	+	25002	26222	406	24985	27005	31.1	11	27.8	RAJILF2
II	17	−	35377	33803	524	35391	33780	38.6	17	36.5	BVRF2
	18	+	35337	36164	275	35305	36180	46.9	18	30.9	BVRF 1.5a and -b
	19	−	37808	36138	556	37855	36120	41.5	19	35.8	BVRF1
	20	−	38346	37594	250	38356	37410	39.2	20	33.7	BXRF1
	21	+	38345	40030	561	38304	39960	26.5	21	29.2	BXLF1
	22	+	40059	42260	733	40042	44450	27.8	22	28.0	BXLF2
	23	−	43458	42253	401		42219	33.7	23	32.6	BTRF1
	24	−	45653	43416	745	45666		40.0	24	39.9	BCRF1
	25	+	45655	49767	1,370	45642	49784	56.7	25	54.4	BCLF1
	26	+	49818	50738	306	49803	50765	46.9	26	48.8	BDLF1
	27	+	50745	51623	292	50735	51670, 51674	23.4	27	23.3	BDLF2
III	29	−			686			55.9	29	51.0	BDRF1/BGRF1
	29b	−	52824	51682	380	SA = 52824	51644		29b		DRF1
	30	+	52840	53097	85	52771	53601	24.0	30	26.2	BDLF3,5
	31	+	53016	53693	225	53010	54010	40.6	31	33.2	BDLF4
	32	+	53642	55066	474		55893	26.4	32	23.1	BGLF1
	33	+	55056	56063	335	55042	56379	33.3	33	34.5	BGLF2
	29a	−	56970	56053	306	57088, 56996	SD = 56053		29a		BGRF1
	34	+	56969	58000	343	56961		39.2	34	36.0	BGLF3
	35	+	57957	58415	152	57900		27.2	35	29.3	BGLF3,5
	36	+	58318	59682	454	58289	60870	32.5	36	32.5	BGLF4
	37	+	59615	61072	485	59590	61204	43.2	37	41.5	BGLF5
	38	+	61027	61206	59	61010	61204	31.0	38	33.9	BBLF1
	39	−	62374	61250	374	62350	61240	46.1	39	47.9	BBRF3
	40	+	62481	63917	478	62468	63954	18.9	40	18.9	BBLF2
	41	+	63996	64523	175	63961	64623	16.2	(41)	15.8	(BBLF3)
	42	−	65293	64520	257	65324	64502	35.3	42	40.1	BBRF2
	43	−	66950	65277	557	66973		54.2	43	47.4	BBRF1
	44	+	66940	69291	783	66906	69306	56.9	44	50.0	BBLF4
	45	−	70055	69348	235	70139	69284	20.9	45	24.9	BKRF4
	46	−	70846	70088	252	70858		56.3	46	50.4	BKRF3
	47	−	71285	70779	168	71295	70686	26.6	47	19.7	BKRF2
	48	−	72661	71402	419	72706	71412	22.5	48	20.7	BRRF2
	50				619						
	50×1	+	72825	72901	26		SD = 72901				
	50×2	+	73121	74903	593	SA = 73121	75159	17.2	50	20.2	BRLF1
	A6	+	75226	75858	210	75203	75865	21.4	A6/51	19.5	A6/BZLF1
	A7	+	76569	77300	243	75643		13.6	A7/51	17.1	A7/BZLF2
	A8	+	77293	79344	683		79388	26.9	A8/51	16.5	A8/BZLF3
IV	52	−	79775	79398	125	79800	79390	23.9	52	24.0	BLRF2
	53	−	80165	79854	103	80217	79850	32.2	53	29.7	BLRF1
	54	+	80228	81124	298	80211	81158	36.7	54	27.2	BLLF3
	55	−	81824	81162	220	81838	81134	42.0	55	46.9	BSRF1
	56	+	81823	84336	837		84370	42.0	56	36.7	BSLF1
	57a		84462	84513	16, 33	84408	SD = 84513				
	57b	+	84605	85863	418, 67	SA= 84605	85957	23.4	57b	20.7	BMLF1
	57				435						
	58	−	87077	86022	351	87115	85982	24.7	58	24.0	BMRF2
	59	−	88319	87084	411	88380, 88355		28.9	59	26.9	BMRF1
	60	−	89347	88430	305	89438	87830, 88407	59.0	60	58.6	BARF1
	61	−	91720	89378	780	71730	89349	51.2	61	42.4	BORF2
	62	−	92740	91736	334	92772		32.1	62	28.7	BORF1
	63	+	92739	95597	952	92701	95600, 96075	27.5	63	24.9	BOLF1
	64	+	95612	103432	2,606	95595	103457	25.2	64	24.0	BPLF1
	65	−	104231	103473	252	104257	103346	18.8	(65)	35.6	(BFRF3)
	66	−	105617	104304	437	105650	104022, 104197	29.5	66	26.5	BFRF2
	67	−	106312	105521	263	106345	105130	43.0	67	40.8	BFRF1
	67A	−	106573	106319	84	106582		49.4	67A[h]	33.3	67A/BFRF0.5
	68	+	106760	108166	468	106745	108241	42.9	68	33.6	BFLF1
	69	+	108189	109031	280	108158	109087	40.9	69	37.5	BFLF2
Right end	73	−	120278	116376	1,300	120290	116028	36.4	73		
	75	−	125243	121296	1,315	125290	121215	28.5	75	28.3	BNRF1
	A9	+	125771	126277	168	125720	126357	16.1	(16)	16.7	(BHRF1)
	A10	−	130229	128811	472	130295	127930, 127538				

[a] Blocks of conserved herpesvirus genes are separated by spaces and indicated by Roman numerals.
[b] The position of the respective ORF on the viral L-DNA is given from the first nucleotide of the first methionine codon to the last nucleotide of the stop codon.
[c] The first nucleotides of putative TATA boxes and polyadenylation signals (AATAAA or AATTAAA) are given. SD, splice donor; SA, splice acceptor.
[d] %id, percent identity of herpesvirus ORFs to the respective AHV-1 ORF (calculated with the GAP program [GCG, version 9.0] according to the parameters gapweight = 4 and lengthweight = 12 [matrix BLOSUM62]).
[e] ORFs in parentheses designate weak positional homologs or functional homologs at different positions on the viral genomes.
[f] The homologous ORFs of selected nonmembers of the subfamily *Gammaherpesvirinae* are provided for orientation. HCMV, human cytomegalovirus.
[g] Possible functions for the AHV-1 proteins are deduced from the homologous ORFs described in other herpesviruses (3, 7, 18, 44, 51, 53, 65, 73).
[h] After correction of a frameshift in the HVS sequence (G inserted after position 99117).

TABLE 1—*Continued*

HHV-8		EHV-2		U or UL ORF[f]			Description[g]
%id	ORF	%id	ORF	HCMV	HHV-6 and 7	HSV-1	
							Exon 2 (144 aa)
							Exon 1 (55 aa)
							Semaphorin homolog, signal peptide position 60
							Signal peptide
23.8	(75)	25.9	3				Virion protein, FGARAT
50.2	6	49.3	6	UL57	U41	UL29	Major single-stranded DNA-binding protein
41.6	7	42.7	7	UL56	U40	UL28	Processing and transport protein
45.5	8	48.5	8	UL55	U39	UL27	Glycoprotein B
57.6	9	56.8	9	UL54	U38	UL30	DNA polymerase
		24.9	E6				Probable GPCR membrane protein, 7 transmembrane domains
19.0	10	19.9	10				Raji LF1 after reconstruction of deletion site in EBV Raji/B95-8
22.9	11	28.3	11				
34.8	17	35.9	17	UL80	U53	UL26	Protease and capsid protein (N terminus), minor capsid scaffold protein (C terminus)
49.2	18	46.3	18	UL79	U52		
40.6	19	41.6	19	UL77	U50	UL25	Virion tegument protein
36.8	20	36.6	20	UL76	U49	UL24	Fusion protein
24.2	21	26.5	21			UL23	Thymidine kinase
24.6	22	29.0	22	UL75	U48	UL22	Glycoprotein H, signal peptide
30.1	23	33.2	23				
39.6	24	36.7	24	UL87	U58		
56.7	25	56.3	25	UL86	U57	UL19	Major capsid protein
44.7	26	41.3	26	UL85	U56	UL18	Capsid protein vp23
26.0	27	25.0	27p				
52.4	29	53.0	29	UL89×1+UL89×2	U60+U66	UL15×1+UL15×2	Late spliced gene, DNA packaging protein, possible terminase
	29b		29b	UL89×2	U60	UL15×2	Late spliced gene, DNA packaging protein, possible terminase, exon 2
27.8	30	29.3	30	UL91	U62		
34.1	31	32.4	31	UL92	U63		
28.1	32	30.7	32	UL93	U64	UL17	
27.5	33	32.2	33	UL94	U65	UL16	
	29a		29a	UL89×1	U66	UL15×1	Late spliced gene, DNA packaging protein, possible terminase, exon 1
37.6	34	35.1	34	UL95	U67	UL14	
28.3	35	24.5	35	UL96	U68		
26.4	36	30.8	36	UL97	U69	UL13	Phosphotransferase—possible tyrosine kinase
41.0	37	45.5	37	UL98	U70	UL12	Alkaline exonuclease
22.4	38	33.9	38	(UL99)	(U71)	UL11	Myristylated in HSV
45.5	39	49.7	39	UL100	U72	UL10	Integral membrane protein, glycoprotein M (CMV)
20.5	40	20.8	40	(UL101)	(U73)	(UL9)	Helicase-primase complex
17.8	(41)	21.3	(41)	(UL102)	(U74)	(UL8)	Helicase-primase complex
32.9	42	32.0	42	UL103	U75	UL7	
48.6	43	50.6	43	UL104	U76	UL6	Minor capsid protein, virion protein
55.3	44	57.6	44	UL105	U77	UL5	Helicase
16.1	45	32.8	45				
52.4	46	59.1	46	UL114	U81	UL2	Uracil-DNA glycosylase
23.3	47	23.6	47	(UL115)	(U82)	(UL1)	Glycoprotein L (CMV)
17.6	48	23.9	48				
							Transcriptional control RTA homolog putative exons 1+2
							RTA homolog, (putative) exon 1
19.2	50	19.2	50				RTA homolog, exon 2
12.4	A6/K8	20.5	A6/51				
10.6	A7/K8	16.0	A7/51				Signal peptide
29.7	A8/K8	24.6	A8/51				Putative glycoprotein; structural similarity to EBV receptor
23.5	52	26.9	(52)				
34.0	53	38.2	53	UL73	U46	(UL49a)	Signal peptide
28.8	54	38.0	54	UL72	U45	UL50	dUTPase
44.2	55	50.5	55	UL71	U44	UL51	
41.4	56	43.2	56	UL70	U43	UL52	Helicase-primase complex, DNA replication protein primase
							Transcriptional control exon 1 IE52 homolog
23.8	57b	19.4	57	UL69	U42	UL54	Transcriptional control exon 2 IE52 homolog
							Transcriptional control IE52 homolog
24.0	58	27.4	58			(UL43)	
26.1	59	28.6	59	UL44	U27	(UL42)	Processivity factor/subunit of DNA polymerase
55.1	60	61.3	60			UL40	Ribonucleotide reductase, (small subunit)
50.0	61	49.9	61	UL45	U28	UL39	Ribonucleotide reductase, (large subunit)
33.8	62	33.7	62	UL46	U29	UL38	Probable capsid assembly and DNA maturation protein
26.9	63	26.5	63	UL47	U30	UL37	Tegument protein
24.8	64	21.9	64	UL48	U31	UL36	Large tegument protein
21.3	(65)	20.1	(65)			(UL35)	Capsid protein
31.7	66	30.3	66	UL49	U32		
39.5	67	45.2	67	UL50	U34	(UL34)	Virion tegument protein
34.6	67A	47.0	67A	UL51	U35	UL33	
36.1	68	39.4	68	UL52	U36	UL32	Probable major envelope glycoprotein
46.5	69	42.4	69	UL53	U37	UL31	
20.9	73						Glycine-rich, repetitive structure
28.9	75	28.6	75				Virion protein, FGARAT
20.5	(16)						Bcl2 homolog
							Possible glycoprotein

has a TATA box-like sequence upstream and a polyadenylation site downstream. ORF A2 may encode a 199-aa protein which has local similarity to the transcription factor ATF3 (19). ORF A3 is homologous to the semaphorin gene family (23). ORF A4 is located between the semaphorin and ORF 3 homologs, and it codes for a protein of 121 aa with a putative signal peptide cleavage site at position 19. The first conserved gene block consists of ORFs 6 to 11. ORF A5 is a homolog of

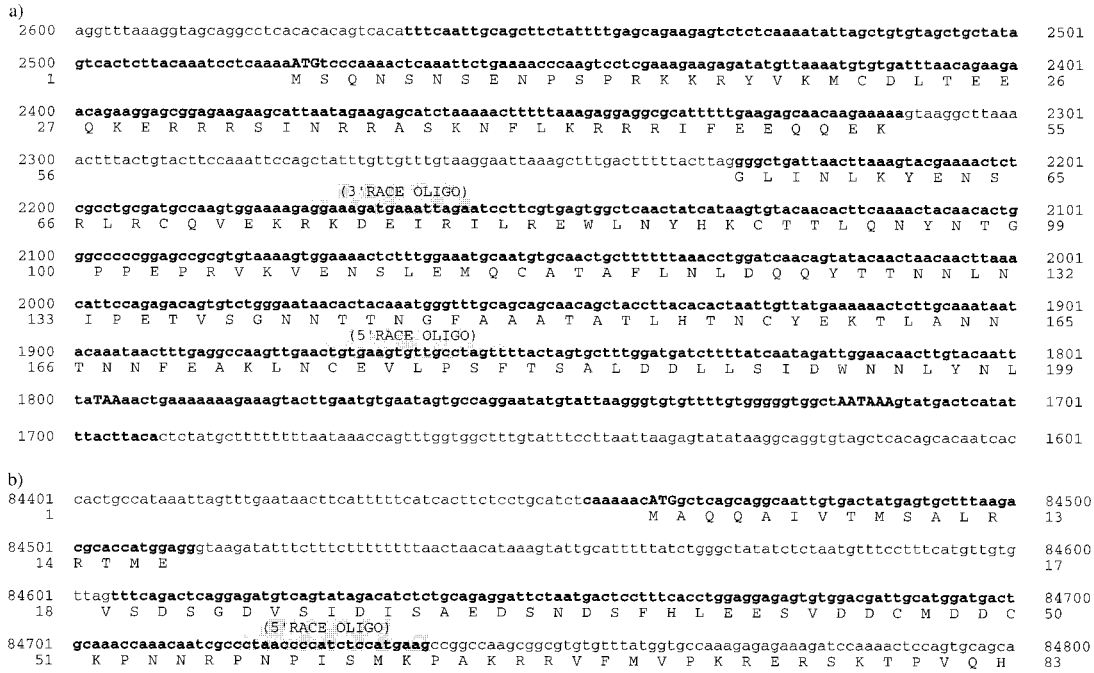

FIG. 3. RACE-amplified cDNAs from AHV-1 C500. The amplified cDNA sequences are shown in boldface letters on the genomic sequence. The numbering corresponds to the nucleotide positions on the genomic sequence and the amino acid residue numbers of putative translation products, respectively. Polyadenylation sites, translational start codons, and stop codons are indicated by capital letters. Positions of synthetic oligonucleotides used for RACE are shaded. (a) ORF A2. (b) ORF 57.

Epstein-Barr virus (EBV) BILF1 and EHV-2 E6. It is followed by the weakly conserved ORFs 10 and 11. Analysis of the 7.6-kb region between ORFs 11 and 17 failed to detect any similarities to cellular genes or herpesvirus immediate-early genes described in other gammaherpesviruses (3, 52, 56, 65, 73, 75). The next conserved gene block includes ORFs 17 to 27. AHV-1 is the only gammaherpesvirus devoid of at least a positional equivalent to ORF 28. The third large block is comprised of ORFs 29b to 50. ORF 37 is a putative phosphotransferase containing a perfect tyrosine kinase motif, LxHxDICxx-$N(L,I,V)_3$. ORF 48 lacks the repetitive region present in herpesvirus saimiri, and an ORF 49 homolog is absent. The putative R transactivator (RTA) homolog ORF 50 (BRLF1 in EBV) is only weakly conserved. ORF A6 codes for a protein with a size of 210 aa, and ORF A7 shows weak similarity to ORF 51 of herpesvirus saimiri and EHV-2, including a potential signal peptide cleavage site between the arginine and leucine residues at positions 19 and 20. ORF A8 is predicted to be a highly glycosylated protein of 683 aa. The following fourth gene block contains ORFs 52 to ORF 69. Between ORFs 67 and 68 is the short but significant ORF 67A, which was also described in EHV-2, human cytomegalovirus (UL51), HHV-6 and -7 (U35), and herpes simplex virus (HSV-1) 1 (UL33). The respective regions of EBV and HHV-8 also contain this ORF, but it was not described in the original publications (7, 65). In herpesvirus saimiri, resequencing of this region confirmed that this ORF was conserved among the herpesviruses (Table 1).

A 12.2-kb region containing solely ORF 73 lies between ORFs 69 and 75. This ORF has a coding potential for 1,300 aa and is similar in size to HHV-8 ORF 73, but it is much larger than the herpesvirus saimiri gene. It is characterized by a highly repetitive region. ORF 75 is homologous to formylglycineamide ribotide amidotransferase (FGARAT, EC 6.3.5.3) (8). This reading frame and its homolog close to the left terminus of L-DNA, ORF 3, are at conserved positions. At the right terminus of L-DNA, a region of 5.4 kb contains two ORFs, A9 and A10. ORF A9 is homologous to the Bcl2 family of apoptosis-regulating proteins. It contains only the well-conserved Bcl2 homology (BH) region BH1, whereas BH2 is hardly recognizable. The predicted protein also shares a hydrophobic carboxy terminus with the other Bcl2 homologs. ORF A10 has the potential to encode a 472-aa glycoprotein.

Prediction of splicing in AHV-1 ORFs. Two potential exons of ORF A2 were identified with GENEMARK and confirmed by 5′ and 3′ RACE techniques, which amplified a spliced cDNA with a coding capacity for a 199-aa protein (Table 1 and Fig. 3a). The transcripts for viral terminase ORF 29 and regulatory protein ORF 50 (31, 54, 76) homologs are spliced in other herpesviruses, and the locations of potential splice donor and acceptor sites were deduced by comparative sequence analysis (Fig. 2, Table 1). ORF 57 is also known to be encoded by a spliced message in EBV (55). A 193-bp 5′ RACE amplificate obtained with an exon 2-specific primer was directly sequenced and shown to encode the 5′ end of the spliced ORF 57 message (Fig. 3b and Table 1).

DISCUSSION

Preliminary characterization and partial cloning of the viral genome of the tissue culture-adapted AHV-1 strain WC11 have been reported (14, 15), suggesting that AHV-1 should be included in the genus *Rhadinovirus* of the subfamily *Gammaherpesvirinae*. We have cloned the complete viral genome of low-passage strain C500. The DNA sequence was unambiguously determined in all but one highly repetitive region, which does not affect the coding potential of the DNA. The H-DNA repeat sequence of strain C500 is represented by a 1.1-kb supermolar *Sst*I fragment, in contrast to strain WC11, in which two distinct repeat subunits have been described (15). Minor variations of the C500 repeat occur at two positions: (i) in the

number of TG repeats and (ii) in the length of a stretch of thymidines. Sequences surrounding this second variable motif are reminiscent of the processing site for the viral terminase in herpesvirus saimiri H-DNA (71). Thus, the minor differences observed may reflect processing errors at viral genomic termini.

Homologs to 60 conserved herpesvirus ORFs are arranged collinear to herpesvirus saimiri (Table 1). The highly conserved ORFs 6 to 9 are followed by ORF A5, which has homologs in EBV BILF1 and EHV-2 E6 at the respective positions, but which is missing in herpesvirus saimiri and HHV-8; this hints at the presence of this ORF in an ancestral gammaherpesvirus and subsequent loss in the primate rhadinoviruses. It may code for a protein with characteristics of G protein-coupled receptors (GPCRs) with seven putative transmembrane domains. GPCR-like ORFs are generally present in the nonconserved regions of gamma-2 herpesviruses; they may serve as modulators of the host immune response (2) or even candidate oncogenes by delivering constitutive activation signals to transformed cells (5).

The putative transcriptional regulators encoded by ORF 50 (BRLF1 in EBV) and ORF 57 (55, 57) are only weakly conserved, presumably reflecting the changes necessary in the process of adaption of regulatory genes to different host environments. This may also apply to ORF 73, which shares structural similarity to herpesvirus saimiri and HHV-8 ORF 73. A partial cDNA encompassing the 3′ end of ORF 73 terminates in the 29-bp repetitive region, and a fusion protein derived from this cDNA is recognized by antisera from AHV-1-infected animals (40). The potential membrane protein with homology to herpesvirus saimiri ORF 75 and EBV BNRF1 has highly significant similarities to FGARAT (EC 6.3.5.3) (8), an enzyme catalyzing the fourth step in the de novo synthesis of purine bases. The human native protein is 150 kDa (74), in the same range as the respective EBV p140 and herpesvirus saimiri p160 proteins, thus supporting an analogous function for this protein. It is tempting to speculate about the therapeutic potential of prodrugs specifically metabolized by the viral proteins.

The nonconserved regions of most rhadinoviruses contain several homologs to cellular genes (51). These are thought to be responsible for modulating the host immune response to viral infection and for the establishment of lymphoid transformation. The ORFs detected in the left-terminal and other positions of AHV-1 show no sequence similarity to these reading frames. The positionally equivalent ORFs A1, A2, and A4 especially show no similarity to the herpesvirus saimiri transformation-associated proteins (10, 27, 47) and their presumed functions (11, 34, 35, 38, 46). The ORF A2 gene product is encoded by a spliced transcript and has a motif similar to nuclear localization signals and the basic domain of the stress-induced transcription factor ATF3 (19). ORF A3 is a gene with homology to the semaphorin family and a related gene of poxviruses (23). Semaphorins are a growing gene family of chemoattractant and/or repulsive factors with important roles in neuronal and lymphocyte development. The human T-cell semaphorin CD100 was recently shown to augment CD40/CD40-ligand interactions (30). The region including ORFs A6, A7, and A8 is similar in its arrangement of genes to EBV. Consequently, ORF A6 may be a regulatory protein like the BZLF-1 gene of EBV (59), whereas the putative glycoprotein A8 may be a candidate for interaction with a host cell receptor for AHV-1. Its positional equivalent EBV gp220 mediates attachment to the B-cell EBV receptor (CD21/CR2, C3d receptor) (25).

ORF A9 likely encodes a protein of 168 aa with homology to the Bcl2 family of regulators of programmed cell death or apoptosis (69). Bcl2 homologs have been described for iridoviruses (1) and the gammaherpesviruses EBV (BHLF-1) (32, 41), herpesvirus saimiri (ORF 16) (50, 69), HHV-8 (ORF 16) (20), and bovine herpesvirus 4 (ORF B2) (43). Although located at a different genomic position, ORF A9 is similar in size and contains a well-conserved BH1 region as well as a conserved hydrophobic carboxy terminus.

We found no ORFs or indications for putative exons in a region of 7.6 kb between the ORF 11 and 17 homologs and in the 7.3-kb region between ORFs 69 and 73. These regions contain homologs to cellular genes and regulatory genes in herpesvirus saimiri, HHV-8, and bovine herpesvirus 4. Large nonrepetitive regions of the viral genome that have no coding potential are unusual among the herpesviruses. Such regions occur only in EHV-2, in which large regions between ORFs 9 and E5, ORFs 13 and 17, and the region between the interleukin-10 homolog (E7) and the right terminus of the L-DNA display a relative paucity of genes. It appears that all rhadinovirus genes to the left of the conserved gene block ORFs 6 to 11 and those to the right of ORF 69 are to some extent derived from cellular homologs. Even the ORF 73 coding sequence has similarity to pyrimidine-rich regions found in various cellular genes, making cellular origin a possibility. The terminal regions of gammaherpesviruses are a hot spot for the integration of cellular genes, probably facilitated by the process of viral replication that originates in these areas of the genome (39, 67).

Thus, despite sharing many biological and epidemiological properties, AHV-1 differs from herpesvirus saimiri (and all other gammaherpesviruses) in the content of virus cell homologs, suggesting that different viral effector molecules achieve a similar phenotype. Taken together, the biological properties, genome structure, and organization of rhadinovirus ORFs confirm the classification of AHV-1 as a rhadinovirus.

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