

Determination and Analysis of the Complete Nucleotide Sequence of Human Herpesvirus 7†

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Human herpesvirus 7 (HHV-7) is a recently isolated betaherpesvirus that is prevalent in the human population, with primary infection usually occurring in early childhood. HHV-7 is related to human herpesvirus 6 (HHV-6) in terms of both biological and, from limited prior DNA sequence analysis, genetic criteria. However, extensive analysis of the HHV-7 genome has not been reported, and the precise phylogenetic relationship of HHV-7 to the other human betaherpesviruses HHV-6 and human cytomegalovirus has not been determined. Here I report on the determination and analysis of the complete DNA sequence of HHV-7 strain JI. The data establish that the close biological relationship of HHV-6 and HHV-7 is reflected at the genetic level, where there is a very high degree of conservation of genetic content and encoded amino acid sequences. The data also delineate loci of divergence between the HHV-6 and HHV-7 genomes, which occur at the genome termini in the region of the terminal direct-repeat elements and within limited regions of the unique component. Of potential significance with respect to biological and evolutionary divergence of HHV-6 and HHV-7 are notable structural differences in putative transcriptional regulatory genes specified by the direct-repeat and immediate-early region A loci of these viruses and the absence of an equivalent of the HHV-6 adeno-associated virus type 2 *rep* gene homolog in HHV-7.

Human herpesvirus 7 (HHV-7) was first isolated from the blood of a healthy donor and has since been detected in and isolated from the blood and saliva of other individuals (12, 38, 154). Like human herpesvirus 6 (HHV-6), HHV-7 displays a predominantly CD4⁺ T lymphotrophism for productive infection, both in vivo and in vitro (11, 38, 74). While HHV-6 infects the majority of individuals prior to 1 year of age, with concurrent immunogenic reaction against HHV-6 antigens and possible fever and rash (exanthem subitum), primary infection with HHV-7 seems to occur slightly later (but typically prior to 2 years of age) and may have similar disease associations (6, 21, 47, 59, 108, 132, 155, 156). In vitro, both HHV-6 and HHV-7 infection of primary T cells or T-cell lines causes a cytopathic effect characterized by marked enlargement of infected cells (cytomegaly) and formation of syncytia, features that are typical of betaherpesvirus (cytomegalovirus) infection (11, 12, 38, 49, 73). Sequence analyses of the genomes of HHV-6 and HHV-7 have confirmed that these viruses belong to the betaherpesvirus subgroup (11, 33, 43, 65, 87, 92, 95, 122).

The division of herpesviruses into three major subgroups based originally on a diverse collection of in vivo and in vitro biological properties is reflected unambiguously at the genetic level, at least for herpesviruses of higher vertebrates (birds and mammals) (43, 49, 79–81, 112, 113). Within all characterized herpesvirus genomes, there are conserved genes, representing each kinetic class, that make up 25 to 70% of the genes within a particular virus. Many of these genes are clustered and can be recognized as “gene blocks” within different herpesvirus genomes. These gene blocks, containing similarly arranged homologous genes, are orientated and ordered identically in appropriately aligned genomes of the same subgroup (2, 14, 15, 43, 62, 79, 82, 133–135). The conserved herpesvirus genes are generally better conserved among members of the same sub-

group than they are between subgroups. Furthermore, members of the same subgroup possess genes common and specific to the subgroup, and these genes, which form 15 to 25% of the total genome content, are positionally conserved. Noncoding, subgroup-specific features of the viral genomes may also be present, e.g., local CpG suppression of betaherpesvirus major immediate-early (MIE) gene loci (50, 92) or arrangements of specific repetitive elements. Previous partial sequencing of HHV-7 has provided evidence that HHV-7 is a member of the betaherpesvirus subgroup on the basis of such genetic criteria (11, 87, 122).

This report presents the main findings from the analysis of the complete nucleotide sequence of HHV-7 strain JI. The data show that there is very high conservation of genetic content and encoded protein products between HHV-7 and HHV-6, but they also identify localized regions of genetic divergence. These regions include the terminal direct-repeat (DR) sequences, which specify multiple US22 open reading frames (ORFs) of different coding capacities and structures between HHV-6 and HHV-7, and a locus toward the right end of the unique component, which in HHV-6 specifies the adeno-associated virus type 2 (AAV-2) *rep* gene homolog (139); there is no *rep* homolog in HHV-7. The product of HHV-6 *rep* mediates both DNA replicative and transcriptional regulatory activities (141), while specific members of the US22 gene family encode IE and/or transactivating proteins (43).

MATERIALS AND METHODS

Recombinant plasmids. Sequencing of HHV-7 was carried out with a series of overlapping and adjoining lambda phage and plasmid subgenomic clones as sources of DNA fragments for which sequences were derived and assembled to give the contiguous viral sequence. Isolation, characterization, and mapping of the HHV-7 subgenomic clones used in this study have been described elsewhere (115) and are illustrated in Fig. 1.

PCR. PCR techniques were used to obtain short DNA fragments spanning junctions between nonoverlapping subgenomic clones, using either relevant recombinant lambda phage or HHV-7-infected-cell DNA templates. Oligonucleotides corresponding to determined HHV-7 sequence at fragment termini were synthesized with noncomplementary 5' extensions containing restriction endonuclease cleavage sites, subsequently used for cloning of the PCR fragments into

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† This article is dedicated to the memory of Carol Newman.

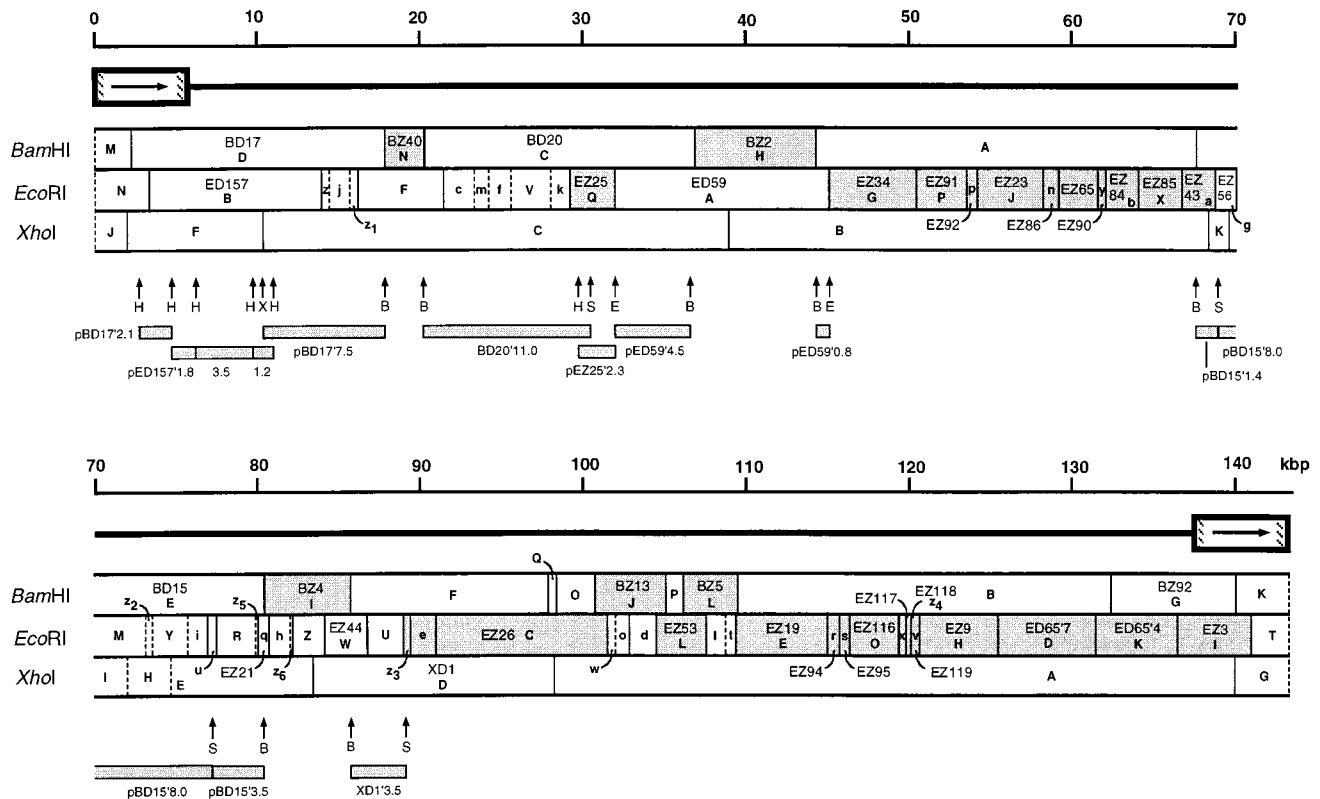


FIG. 1. Restriction fragments used for the sequencing of HHV-7 strain JI. The HHV-7 genome is represented diagrammatically, together with the positions of *Bam*HI, *Eco*RI, *Xho*I, *Sma*I, and *Sal*I sites mapped previously (115). Various λ phage-cloned restriction fragments are prefixed with ED, EZ, BD, BZ, or XD (indicating their terminal restriction sites and the vector, λ DASHIII or λ ZAPII, into which they were initially cloned [115]). Plasmid subclones of these fragments are shown below. Sequenced restriction fragments are indicated by shading.

appropriately cleaved M13 vectors. PCRs were carried out with approximately 10 ng of lambda phage or infected-cell DNA template by 30 cycles of denaturation (95°C for 1 min), annealing (50°C for 1 min), and extension (72°C for 1 to 3 min). PCR products were cloned into M13 vectors, and single-stranded DNA was prepared and sequenced by established techniques (see below) to confirm the joins between adjacent restriction endonuclease fragments (Fig. 1). Junctional sequences were also obtained directly from junction-spanning lambda phage templates by use of appropriate oligonucleotide primers and application of linear amplification sequencing (28).

M13 shotgun and directed sequencing. Nucleotide sequences of cloned subgenomic fragments of HHV-7 were determined by the dideoxynucleotide chain termination method (119) to sequence M13-cloned sonicated subfragments to generate data which were assembled to provide contiguous sequence stretches, as described previously (9, 118). Sequences of specific regions of ambiguous "single-stranded" sequence and nonoverlapping junctions were confirmed by directed sequencing on double-stranded templates, using a modification of the M13 sequencing method (95) or using PCR techniques (see above). The use of junction-spanning cDNA clones to confirm joins between adjacent genomic restriction fragments has been described elsewhere (115).

Assembly and analysis of the sequence data. Sequence data generated from sequencing of the M13 shotgun clones were assembled by using the sequence assembly program (SAP) of Staden, based on the previous DB system (128). Analysis of the individually assembled sequence stretches (from each of the sequence subgenomic clones) and contiguous HHV-7 sequence was carried out with the nucleotide interpretation program (NIP) of Staden (128), and encoded protein sequences were analyzed with various options in the Genetics Computer Group program package (32). Database searches for homologous protein sequences were carried out with the FASTA (99), BLITZ (124), and BLAST (4) methods applied directly to the most current protein databases at the time of the search (searches with all HHV-7 ORF-encoded sequences were carried out during December 1995). Positional base preference analyses were undertaken on small ORFs to assess their likelihood of being protein coding, using the NIP package (126–128). Regions of the HHV-7 sequence encoding only very small ORFs (<300 nucleotide) within a significantly larger nucleotide stretch were analyzed by BLASTX searches (4) to determine whether they encoded translation products with homology to known sequences.

RESULTS

Assembly of the contiguous HHV-7 sequence. The HHV-7 genome was sequenced by the M13 shotgun sequencing method described by Bankier et al. (9) applied to cloned and mapped subgenomic restriction fragments of HHV-7. The fragments used for the project have been described previously (115) and are shown in Fig. 1. Junctions between adjacent, nonoverlapping restriction fragments were confirmed by sequencing across these regions with appropriate cloned PCR fragments, junction-spanning cDNA clones (115) or lambda phage clones as templates for directed sequencing (see Materials and Methods). The sequences of the terminal DR elements were derived by compiling partial sequences from DR_L and DR_R to generate the complete DR sequences. The positions of the left and right genome termini were based on previously published data (122). Data from this laboratory have identified three regions of the HHV-7 genome that appear to display sequence heterogeneity (115). Two of these occur at each end of the DR elements and contain telomeric repeat [(GGGTTA)_n] motifs, while the third occurs at a locus now known to contain repetitive elements homologous to the *Kpn*I repeats of HHV-6 (43, 77, 78). Sequences of each of these regions were derived from single, representative clones containing these loci (pED157*1.2, pEZ3, and pED65*7 [Fig. 1]).

Identification of HHV-7 protein-coding ORFs. The contiguous 144,861-bp HHV-7 nucleotide sequence was analyzed to identify potentially protein-coding ORFs by using the NIP

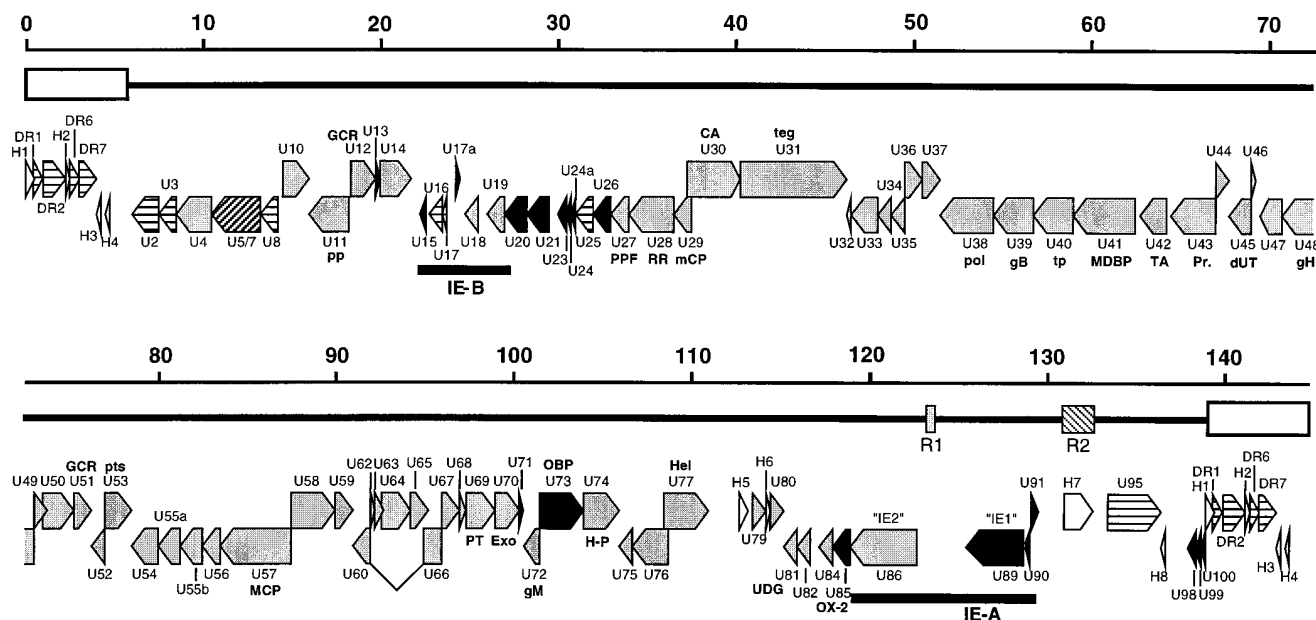


FIG. 2. ORFs identified in HHV-7. ORFs within the 144,861-bp sequence were identified with the Nucleotide Interpretation Program (NIP) of Staden, and homologs of HHV-6 genes (solid areas) and HHV-6/HCMV genes (shaded areas) were determined by FASTA, BLASTP, BLASTX, BLITZ, and GAP analyses (see Materials and Methods). Genes apparently unique to HHV-7 are unshaded. Lightly shaded ORFs (horizontal lines) correspond to members of the US22 gene family (DR1, DR2, DR6, DR7, and U95 are not conserved in HCMV). HHV-7 ORFs with homologs in HHV-6 are named after their HHV-6 counterparts; HHV-7-unique ORFs are prefixed with "H" and successively numbered (from left to right on the genome). ORF U5/7 encodes a translation product with N-terminal homology to HHV-6 U7 (and HCMV U28) and C-terminal homology to HHV-6 U5 (and HCMV U27). Abbreviations refer to gene identities and functions as follows: pp, pp100 structural phosphoprotein; GCR, G-protein-coupled receptor; PPF, DNA polymerase processivity factor; RR, ribonucleotide reductase; mCP, minor capsid protein; CA, capsid assembly protein; teg, large tegument protein; pol, DNA polymerase; gB, glycoprotein B; tp, transport protein; MDBP, major DNA-binding protein; TA, herpesvirus conserved transactivator; Pr., primase; dUT, dUTPase; gH, glycoprotein H; pts, protease/assembly protein; MCP, major capsid protein; PT, phosphotransferase; Exo, alkaline exonuclease; gM, glycoprotein M; OBP, origin binding protein; H-P, helicase-primase complex component; Hel, helicase; UDG, uracil-DNA glycosylase; OX-2, OX-2 membrane antigen.

program package of Staden (128). The criteria used for identification of these ORFs were essentially those that I have applied previously in similar analyses (see, e.g., reference 92). Briefly, ORFs of more than 300 nucleotides were considered to be significant; where there were extensively overlapping large ORFs, the larger/largest was considered to be protein coding; in regions of intergenic gaps, encoding only small ORFs (fewer than 300 nucleotides), ORFs were considered significant if they had high positional base preference scores (126, 127) or if they encoded products with homology to previously determined translation products. Searches for homologs of HHV-7 ORF-encoded proteins were carried out by a combination of FASTA, BLITZ, BLASTP, and BLASTX searches of the available protein databases. BLASTX searches of the "genpept" database were particularly useful for identifying very small ORFs coding for homologs of HHV-6 proteins or exon-encoded sequences (e.g., regions of gp82/105 spliced late gene [102]). In most cases, FASTA searches with larger translation products enabled unambiguous identification of homologous sequences in the database. BLASTP searches identified some additional homologous sequences (e.g., HHV-6 DR1 and DR6 homologies to HHV-7 DR1). The ORFs and homologs identified are illustrated in Fig. 2 and listed in Table 1.

Herpesvirus conserved genes identified in HHV-7. Homologs to almost all of the HHV-7 ORFs shown in Fig. 2 were identified among the collection of previously identified herpesvirus genes. These homologs are listed in Table 1. For the genes conserved among all the currently sequenced herpesviruses, the encoded HHV-7 translation products showed much closer similarity to betaherpesvirus (HHV-6, human cytomegalovirus [HCMV]) homologs than to those of the alphaherpes-

viruses (equine herpesvirus 1 [EHV-1], varicella-zoster virus, herpes simplex virus type 1 [HSV-1]) and gammaherpesviruses (herpesvirus saimiri Epstein-Barr virus equine herpesvirus 2 [EHV-2]). The degree of conservation between HHV-7 and HHV-6 proteins was particularly high, ranging from 41 to 75% amino acid sequence identity for the "core" herpesvirus gene products (Table 1). The relative arrangements of the HHV-7 herpesvirus-conserved genes were identical to those of homologous genes in HHV-6 and HCMV, as expected for a betaherpesvirus genome (Fig. 3) (43). Thus, conserved gene blocks I to VII contained homologous genes in the same orders and orientations and were positioned and orientated identically between HHV-7, HHV-6, and HCMV. Especially striking was the lack of extensive genetic divergence at intervening loci between HHV-7 and HHV-6, in contrast to the divergence seen between conserved gene blocks, e.g., II and III, in HCMV and HHV-7/HHV-6.

The deduced functions of the various HHV-7 genes for which homologs were identified are listed in Table 1. These functional assignments are based on previous experimental findings with other herpesviruses or on functional characterization of cellular gene homologs. Brief descriptions of the roles of various classes of HHV-7 homologous gene products are given below.

(i) **IE/regulatory genes.** HCMV is the best characterized of the betaherpesviruses, and several immediate-early (IE) regulatory genes have been identified. These occur either within one of two complex IE gene loci (MIE, UL36-38) or as single genes in the short unique (U_s) and short repeat (IR_s/TR_s) regions of the genome (17, 53, 61, 129, 136, 149). Partially conserved equivalents of the HCMV MIE locus have been

TABLE 1. HH7 ORFs and homologs in other sequenced herpesviruses

ORF ^a	Sense ^b	Position ^b			N-Term Met?	Poly(A)	Length (aa) ^c	Homology with HHV-6 (%Sim/%Id) ^d	Homolog(s) ^e										Comments	Reference(s)
		Start	Stop	Homology with HHV-6 (%Sim/%Id) ^d					HHV-6	HCMV	HVS	EBV	EHV-2	EHV-1	VZV	HSV-1				
																	Homology (s) ^f			
H1	+	33	542	N	853*	169	57.1/44.0	DR1										US22 gene family, DRI1/DR6 homology		
DR1	+	368	826	N	853*	152	57.1/44.0	DR1										US22 gene family		
DR2	+	898	2100	N	3917*	400	58.1/37.0	DR2										US22 gene family, DRI1/DR6 homology		
H2	+	2267	2506	Y	3917*	79	71.6/58.8	DR6										US22 gene family, transactivator	55, 137	
DR6	+	2562	3050	Y	3917*	161	71.6/58.8	DR6										US22 gene family, transactivator		
DR7	+	3122	3910	Y	3917*	262	77.8/60.5	DR7										US22 gene family		
H3	-	4224	3976	Y	3040	82												Structural phosphoprotein	54, 88, 101	
H4	-	4745	4449	Y	3040	98												GCR homolog, chemokine receptor		
U2	-	7417	6338	Y	6248*	359	69.6/51.1	U2	UL23									HCMV UL25/35 gene family		
U3	-	8732	7578	Y	7514	384	65.4/49.3	U3	UL24									IE-B, transactivator (spliced to U17)	61, 95	
U4	-	10382	8754	Y	7514	542	75.5/59.2	U4	UL27									IE-B, transactivator (spliced to U16)	61, 95	
U5/7	-	13004	10407	Y	10411 ⁺	865	71.2/49.7	U5	UL27									IE-B, homologous to HCMV IE glycoprotein	61	
U8	-	14262	13174	Y	11850	362	80.2/60.7	U7	UL28									IE-B	61, 136	
U10	+	14608	15963	Y	15885*	451	69.0/51.0	U8	UL29									Ig gene family?		
U11	-	18249	15982	Y	15971	755	71.6/51.5	U10	UL31									Glycoprotein		
U12	+	18396	19436	Y	19481	346	55.6/30.7	U11	UL32									Glycoprotein, EHV-1 gJ homology	135	
U13	+	19521	19817	Y	20424*	98	67.7/45.7	U12	UL33									Glycoprotein		
U14	+	19885	21831	Y	21925*	648	54.6/37.1	U13	UL35									US22 gene family, transactivator	95	
U15	-	22244	22564	Y	22075	106	67.9/53.5	U14	UL35									DNA polymerase processivity factor	44, 98	
U16	-	23547(A)	22772	N	22754	264	84.9/64.1	U15	UL36x2									Ribonucleotide reductase (large subunit)	96	
U17Ex	-	23836	23620(D)	Y		72	74.8/56.5	U16	UL36x1									Minor capsid protein (mCP)	90, 91, 138	
U17	-	23836	23570	Y	22754	88	55.5/41.7	U17Ex	UL36x1									Capsid assembly, myosin		
U17a	+	24318	24587	Y	24707	89	54.5/39.8	U17	UL38									Large tegument protein	10, 42	
U18	-	25600	24713	Y	24578	295	46.6/29.3	U17a	UL37x3									Virion protein	76, 83	
U19	-	26922	25945	Y	25504	325	71.4/43.9	U18	UL38									Virion protein?		
U20	-	28211	27036	Y	27027	391	59.9/40.1	U19	UL38									Probable virion protein	23, 83	
U21	-	29494	28202	Y	28127	430	53.2/23.4	U20	UL38											
U23	-	30418	29903	Y	29904 ⁺	171	55.8/32.2	U21	UL38											
U24	-	30772	30524	Y	30455*	82	55.6/29.4	U23	UL38											
U24a	-	31129	30776	Y	30671	117	63.2/36.8	U24	UL43											
U25	-	31898	30936	Y	30671	320	57.1/33.9	U24a	UL43											
U26	-	32869	31988	Y	31959	293	70.7/48.9	U25	UL43											
U27	-	33951	32857	Y	31959	364	60.4/30.4	U26	UL44	(59)	(18)	(16)	(UL42)							
U28	-	36484	34064	Y	34059	806	83.8/67.6	U27	UL44	(59)	(18)	(16)	(UL42)							
U29	-	37347	36487	Y	36457	286	70.2/47.1	U28	UL45	61	21	19	UL39							
U30	+	37562	40178	Y	37548	938	77.5/53.3	U29	UL46	62	22	20	UL38							
U31	+	40179	46358	Y	46994	2,059	66.0/45.9	U30	UL47	63	(23)	(21)	(UL37)							
U32	-	46627	46355	Y	46385*	90	65.3/46.2	U31	UL48	64	24	22	UL36							
U33	-	48041	46608	Y	46385*	477	80.7/65.9	U32	UL49	(66)	(25)	(23)	(UL35)							
U34	-	48768	47992	Y	47906	258	78.5/59.4	U33	UL49	67	(26)	(24)	(UL34)							
U35	-	49119	48805	Y	48775	104	75.0/59.1	U34	UL50	67	27	25	UL33							
U36	+	49118	50575	Y	51089*	485	78.6/58.3	U35	UL51	68	28	26	UL32							
U37	+	50577	51356	Y	51613*	259	77.3/58.2	U36	UL52	68	28	26	UL32							
							80.5/63.0	U37	UL53	69	29	27	UL31							

U38	-	54401	51363	Y	51353	1,012	81.6/66.7	U38	UL54	9	BALF5	9	30	28	UL30	DNA polymerase	64, 151
U39	-	56869	54401	Y	53919*	822	72.1/56.4	U39	UL55	8	BALF4	8	33	31	UL27	Glycoprotein B (gB)	20, 26, 125
U40	-	58997	56832	Y	56686	721	75.4/56.0	U40	UL56	7	BALF3	7	32	30	UL28	Transport protein (tp)	100
U41	-	62395	59000	Y	58773*	1,131	84.1/63.3	U41	UL57	6	BALF2	6	31	29	UL29	Major DNA-binding protein	16
U42	-	64352	62772	Y	62714*	526	75.7/57.0	U42	UL69	57	BMLF1	57	5	4	UL54	Transactivator	45, 84, 103, 123, 150
U43	-	67086	64501	Y	64470	861	76.9/61.2	U43	UL70	56	BRLF1	56	7	6	UL52	Primase	29, 58
U44	+	67143	67754	Y	67750	203	77.0/58.5	U44	UL71	55	BSRF1	55	(8)	(7)	(UL51)	dUTPase	67, 109
U45	-	68898	67759	Y	67763+	379	69.3/51.2	U45	UL72	54	BLLF2	54	9	8	UL50		
U46	+	68930	69190	Y	69186+	86	72.8/53.1	U46	UL73	53	BLRF1	53	(10)	(9A)	(UL49a)		
U47	+	70579	69638	Y	69235*	313	46.6/25.4	U47	UL74								
U48	-	72889	70817	Y	70714	690	63.7/39.0	U48	UL75	22	BXLF2	22	39	37	UL22	Glycoprotein H	27, 71
U49	+	73003	73722	Y	73765*	239	68.6/51.9	U49	UL76	20	BXRF1	20	37	35	UL24	Fusion protein	116
U50	+	73538	75202	Y	76287	554	73.4/54.9	U50	UL77	19	BVRF1	19	36	34	UL25	Virion protein	1
U51	+	75304	76188	Y	76287	294	64.6/36.1	U51	UL78	74						GCR, opioioid ^R homolog	2, 43, 94
U52	-	76949	76185	Y	75932	254	79.9/55.5	U52	UL79	18	BVRF1.5a/b	18				Protease/assembly protein	31, 39, 146, 147
U53	+	76957	78495	Y	79010	512	67.1/52.4	U53	UL80	17	BVRF2	17	35	33	UL26	Tegument protein transactivator	70, 114
U54	-	79870	78503	Y	78443	455	66.3/41.4	U54	UL82/83							Replication function?	53, 98
U55A	-	81201	79918	Y	79891	427	56.8/32.6	U55	UL84							Replication function?	53, 98
U55B	-	82577	81285	Y	79891	430	46.4/20.7	U55	UL84							Replication function?	53, 98
U56	-	83511	82630	Y	82431	293	82.6/65.2	U56	UL85	26	BDLF1	26	43	41	UL18	Capsid protein	90, 91
U57	-	87551	83514	Y	83098	1,345	82.6/68.4	U57	UL86	25	BGLF1	25	42	40	UL19	Major capsid protein (MCP)	68
U58	+	87563	89890	Y	90111*	775	75.7/61.3	U58	UL87	24	BcRF1	24					
U59	+	89838	90881	Y	90942*	347	58.9/38.7	U59	UL88								
U60	-	92005(A)	90878	N	90793	394	86.9/75.7	U60	UL89x2	29b	BDRF1	29b	44	42	UL15x2	Late spliced gene (U60/U66) DNA packaging	107
U62	+	92017	92244	Y	92564	75	81.7/50.7	U62	UL91	30	BDLF3.5	30					
U63	+	92216	92851	Y	93008	211	84.5/71.8	U63	UL92	31	BDLF4	31					
U64	+	92829	94148	Y	94363*	439	64.5/40.7	U64	UL93	32	BGLF1	32	45	43	UL17		
U65	+	94111	95103	Y	95318	330	77.1/60.1	U65	UL94	33	BGLF2	33	46	44	UL16		
U66	-	95985	95122(D)			309	76.2/62.3	U66	UL89x1	29a	BGRF1	29a	47	45	UL15x1	Late spliced (U60/U66) DNA packaging	107
U67	+	95984	97024	Y	97041	346	70.3/51.7	U67	UL95	34	BGLF3	34	(48)	(46)	(UL14)		
U68	+	97024	97868	Y	97971*	114	71.1/48.9	U68	UL96	35	BGLF3.5	35					
U69	+	97371	99011	Y	99096*	546	71.8/54.0	U69	UL97	36	BGLF4	36	49	47	UL13	Phosphotransferase	18, 69, 131
U70	+	99013	100455	Y	100554	480	72.5/52.0	U70	UL98	37	BGLF5	37	50	48	UL12	Alkaline exonuclease	
U71	-	100392	100613	Y	100609+	73	64.4/53.4	U71	UL100	39	BBRF3	39	52	50	UL10	Integral membrane protein (gM)	8, 104
U72	+	101676	100636	Y	100640+	346	81.1/59.0	U72	UL102	(41)	(BBLF3)	(41)	(54)	(52)	UL9	Origin-binding protein (OBP)	35, 52, 66, 145
U73	+	101693	104456	Y	104067	787	75.4/58.2	U73	UL103	42	BBRF2	42	55	53	UL7	Helicase/primase complex	29, 98
U74	+	104007	105986	Y	106055	659	64.4/40.6	U74	UL104	43	BBRF1	43	56	54	UL6	Virion protein?	83, 148
U75	-	106743	105973	Y	105977+	256	64.9/45.6	U75	UL105	44	BBLF4	44	57	55	UL5	Helicase	29, 48, 157
U76	-	108589	106667	Y	106633*	640	78.5/59.7	U76	UL104	43	BBRF1	43					
U77	+	108435	110897	Y	111009	820	85.7/74.9	U77	UL105	44	BBLF4	44					
H5	+	112811	113311	N	114614	166			UL112								
U79	+	113502	114203	Y	114614	233	70.6/44.7	U79	UL112								
H6	+	114257	114505	N	114614	82	64.1/48.7	U79(C)									
U80	+	114557	115189	N	115347*	210	57.0/40.4	U80	UL113								
U81	-	115948	115184	Y	115161	254	79.1/58.5	U81	UL114	46	BKRF3	46	61	59	UL2	Uracil-DNA glycosylase	67, 97, 152
U82	-	116778	116038	Y	115986	246	62.1/40.0	U82	UL115	(47)	(BKRF2)	(47)	(62)	(60)	(UL1)	Glycoprotein L	51, 56, 72
U84	-	118043	117111	Y	116463*	310	65.7/46.5	U84	UL117								
U85	-	118913	118071	Y	117885	280	60.8/37.7	U85	UL122								
U86	-	122708	119091	Y	119082	1205	57.0/41.4	U86	UL122								
U89	-	128668	125420	Y	125424+	1,082	60.7/36.4	U89									

Continued on following page

TABLE 1—Continued.

U90	U91	U95	H7	H8	U98	U99	U100	H1'	DR1'	DR2'	H2'	DR6'	DR7'	H3'	H4'	IE-A, exon in HHV-6	120
-	129051	128776	N	-	138451	137945	N	-	139415	139945	141147	+	142169	+	-	IE-A, exon in HHV-6	120
U90	129122	129625	Y	U90	136293	136204	Y	U95	139900*	142964*	141553	+	143271	-	-	IE-A, exon in HHV-6	120
H7	130829	132112	Y	U95	136257	136307	Y	U98	139900*	142964*	142097	+	143792	-	-	DraI repeats	60, 86
U95	133382	136204	Y	U98	136257	136307	Y	U99	139900*	142964*	142097	+	143792	-	-	MCMV IE2 homolog, US22 gene	60, 86
H8	136579	136307	Y	U99	136257	136307	Y	U100	139900*	142964*	142097	+	143792	-	-	Homology to HHV-6 gp82/105	102
U98	138451	137945	N	U100	139900*	138375	N	DR1	139900*	142964*	142097	+	143792	-	-	Homology to HHV-6 gp82/105	102
U99	138692	138375	N	DR1	139900*	138751	Y	DR2	139900*	142964*	142097	+	143792	-	-	Homology to HHV-6 gp82/105	102
U100	138999	138751	Y	DR2	139900*	139589	N	DR6	139900*	142964*	142097	+	143792	-	-	US22 gene family, DRI/DR6 homology	55, 137
H1'	139080	139589	N	DR6	139900*	139873	N	DR7	139900*	142964*	142097	+	143792	-	-	US22 gene family, DRI/DR6 homology	55, 137
DR1'	139415	139873	N	DR7	139900*	139873	N	DR7	139900*	142964*	142097	+	143792	-	-	US22 gene family, transactivator	55, 137
DR2'	139945	141147	N	DR7	139900*	139873	N	DR7	139900*	142964*	142097	+	143792	-	-	US22 gene family, transactivator	55, 137
H2'	141314	141553	Y	DR7	139900*	139873	N	DR7	139900*	142964*	142097	+	143792	-	-	US22 gene family, transactivator	55, 137
DR6'	141609	142097	Y	DR7	139900*	139873	N	DR7	139900*	142964*	142097	+	143792	-	-	US22 gene family, transactivator	55, 137
DR7'	142169	142957	Y	DR7	139900*	139873	N	DR7	139900*	142964*	142097	+	143792	-	-	US22 gene family, transactivator	55, 137
H3'	143271	143023	Y	DR7	139900*	139873	N	DR7	139900*	142964*	142097	+	143792	-	-	US22 gene family, transactivator	55, 137
H4'	143792	143496	Y	DR7	139900*	139873	N	DR7	139900*	142964*	142097	+	143792	-	-	US22 gene family, transactivator	55, 137

^a HHV-7 ORFs are named after their HHV-6 homologs (43); ORFs unique to HHV-7 are prefixed with "H" and numbered 1 to 8.

^b The positions and orientations of the ORFs are indicated, together with the positions of the first downstream polyadenylation signals (AATAAA, ATTAAA*). Polyadenylation signals overlapping ORF C-terminal sequences are indicated (+). Determined (U16/U17Ex) and predicted (U60/U66) splice donor (D) and acceptor (A) sites are indicated.

^c The sizes of ORF translation products (starting at the N-terminal methionines where these occur) in amino acids (aa) are shown.

^d The values for percent similarity (%Sim) and percent identity (%Id) between HHV-7 and HHV-6 homologs are based on BESTFIT alignments (32), with gap and length weights set at 3.0 and 0.1, respectively. ^e Homologous genes were identified by database searches and pairwise alignments (see Materials and Methods). Listings of homologous genes based on these analyses and on data from comparisons of other herpesvirus proteins (see, e.g., references 2, 17, 43, and 79). Genes that show only limited sequence similarities to HHV-7/HHV-6/HCMV genes but are colinear (and in some cases functionally analogous) are given in parentheses.

identified in other betaherpesviruses including simian CMV, murine CMV, and rat CMV. They all display the feature of CpG suppression, are structurally conserved, encode analogously spliced transcripts, and specify transregulatory functions (25, 46, 50, 57, 85, 106, 117, 130). The MIE locus of HHV-6 (IE-A) is notably divergent from those of other betaherpesviruses with respect to overall size (11 kbp; over twice as large as other MIEs) and coding capacity, although there are structural similarities and partial homology (43, 92, 120). At least one transactivating function has been mapped to the HHV-6 IE-A locus (77). In HHV-7, homologs of the major HHV-6 IE-A ORFs have been identified, with counterparts of HHV-6 U86/87 and U89 being present in HHV-7. (Note that previous HHV-6 ORFs U86 and U87 are now known to form a single ORF. The corrected HHV-6 nucleotide sequence contains an extra nucleotide, G, at position 128132 relative to the originally submitted sequence ([43, 92a]). The previously characterized proximal MIE ORFs (IE1) of betaherpesviruses show very little, if any, amino acid sequence similarity, despite the conservation of splicing patterns (92). However, HHV-7 and HHV-6 encode clearly homologous although notably divergent, proximal IE-A gene products, in addition to relatively highly conserved distal IE-A gene products that are significantly diverged from equivalent IE2 proteins specified by the other characterized betaherpesviruses (92). The IE-A loci of HHV-6 and HHV-7 each contain intergenic (dissimilar) repetitive motifs (HHV-6 R2, HHV-7 R1) between U86 and U89 and show CpG suppression within proximal IE-A sequences (92) (Fig. 3). Conserved repetitive elements of 105 bp are present upstream of the IE-A ORFs in HHV-6 (R3 or *KpnI* repeats [77, 78]) and HHV-7 (R2 or *DraI* repeats [Fig. 2 and 3]).

The other complex betaherpesvirus IE locus corresponds to the UL36–38 region of HCMV, called IE-B in HHV-6 (17, 61, 95, 136). Like the MIE locus, the UL36–38 transcription unit gives rise to multiply spliced transcripts encoding distinct proteins, some of which effect transcriptional activation in experimental systems (25, 53). The HHV-6 UL36 homolog (U16/U17) also has been shown to encode a transactivating function, as have sequences corresponding to the proximal half of the HHV-6 UL36–38 homologous locus, IE-B (40, 95). The IE-B loci are highly conserved between HHV-6 and HHV-7, although somewhat diverged from their HCMV counterpart (95) (Fig. 3). Homologs of HCMV UL36 exons 1 and 2 are present in HHV-6 and HHV-7 and have positionally well conserved functional splice donor/acceptor sequences (Table 1) (16a, 17, 95, 144a).

The UL36 homologous proteins are members of the so-called US22 family. There are multiple (11, a partially overlapping set) US22 genes in HCMV, HHV-6, and HHV-7, and these appear to be specific to the betaherpesvirus subgroup (17, 43). The translation products of these genes differ greatly in length (143 to 788 amino acids), but they each contain at least one of four US22 amino acid sequence motifs (17, 33). This family includes the HCMV genes IRS1/TRS1 that have been shown to be transcribed with IE kinetics and to encode transactivating functions (129). The DR7 and U25 US22 genes of HHV-6 also have been reported to encode transactivators (55, 95, 137). Direct equivalents of these genes are present in HHV-7, as are other US22 genes that are conserved between HHV-6 and HCMV (HHV-7 U2, U3, U5/7 [N-terminal region], U8) or between HHV-6 and MCMV (HHV-7 U95). Other US22 coding sequences are present within the DR elements of HHV-6 and HHV-7. Values for amino acid sequence conservation between the different HHV-7 encoded US22

translation products are given in Table 2. US22 motifs identified in these proteins are shown in Fig. 4.

(ii) **Replication genes.** All sequenced herpesviruses contain a set of conserved replication genes that are essential for viral DNA replication during productive infections of host cells. Other genes necessary for DNA replication are specific to herpesvirus subgroups and/or particular viruses. The core replication genes are those encoding DNA polymerase, DNA polymerase processivity factor, single-stranded DNA-binding protein (or major DNA-binding protein), and components of the heterotrimeric helicase-primase complex (helicase, primase, helicase-primase associated factor) (16). In HHV-6 and HHV-7, these genes correspond to ORFs U38, U27, U41, U77, U43, and U74, respectively (43) (Fig. 2). The alphaherpesvirus-homologous origin-binding protein gene present in HHV-6 (U73) is conserved in HHV-7 and presumably is also functionally analogous (35, 52, 66, 145). The elegant work of Anders and colleagues (5, 53, 98) has identified genetic loci in addition to the core replication genes that are necessary for origin-dependent replication in HCMV. The UL84 and the UL112 and UL113 genes are likely to encode essential replication functions, while other loci important for the transient-replication assays used probably act to enhance expression of the replication genes (53). HHV-7 encodes two ORFs, U55A and U55B, specifying proteins with homology to UL84. The HCMV UL112 and UL113 spliced ORFs are weakly or partially conserved in HHV-6 and HHV-7 (43) (Fig. 3); therefore, the U79 and U80 (and possibly HHV-7 H6) ORFs may encode replication functions also. It is noteworthy that the AAV-2 *rep* gene homolog identified in HHV-6, which has been shown to specify transregulatory and AAV-2 DNA replication functions (139, 141), is absent in HHV-7. In light of the generally close genetic conservation between HHV-6 and HHV-7 (Fig. 3), this may indicate that HHV-6 *rep* does not represent an essential replication (or transregulatory) protein.

(iii) **Nucleotide metabolism and DNA repair.** HHV-7 homologs of herpesvirus gene products that are involved indirectly in DNA replication, by providing nucleotide substrates and DNA repair functions, include ribonucleotide reductase (U28), dUTPase (U45), phosphotransferase (U69), alkaline exonuclease (U70), and uracil-DNA glycosylase (U81). In common with the other sequenced betaherpesviruses (HHV-6, HCMV), the HHV-7 ribonucleotide reductase homolog is encoded by a single ORF specifying a single protein (17, 43, 95). Characterized members of the alpha- and gammaherpesvirus subgroups specify distinct catalytic and regulatory subunits that form $\alpha_2\beta_2$ structures, as occurs among their cellular counterparts (7, 24, 37, 96). The phosphotransferase gene present in HCMV (UL97) is capable of phosphorylating nucleosides, an activity that accounts for the efficacy of ganciclovir in suppressing HCMV infections (69, 131). Homologs of the thymidine kinase genes found in the alpha- and gammaherpesviruses are absent in HCMV, HHV-6, and HHV-7 (17, 43). The HHV-7 dUTPase and uracil-DNA glycosylase homologs presumably specify enzymatic activities involved in excision of uridine residues from DNA, by analogy to bacterial and eukaryotic counterparts (67, 97, 121), although there is notable sequence divergence between the HHV-7, cellular, and other herpesvirus dUTPase homologs.

(iv) **Glycoproteins.** Within the sequenced alpha-, beta-, and gammaherpesviruses is a core of herpesvirus genes that encode homologous glycoprotein species. These genes, which encode glycoprotein B (gB), glycoprotein H (gH), glycoprotein M (gM), and glycoprotein L (gL), are conserved in HHV-7 also (ORFs U39, U48, U72, and U82, respectively). The structures and roles of these proteins were determined initially for the HSV-1-specified species; characterization of several homo-

FIG. 3. Alignment of the genomes of HCMV (AD169), HHV-6, and HHV-7, showing homologous (shaded) and nonconserved (open) genes in these betaherpesviruses. Solid areas indicate genes conserved between HHV-6 and HHV-7 but not present in HCMV. The herpesvirus-conserved gene blocks (I to VII) are indicated. Major divergence loci between HCMV and HHV-6/HHV-7 correspond to terminal sequences, regions between HCMV UL38-UL43 and UL57-UL69, and sequences within and around the MIE locus (UL122/123). HHV-6/HHV-7 US22 genes U95, DR2, and DR7 have approximate positional counterparts in HCMV, but these corresponding genes are not necessarily the nearest homologs. The locus at which additional genes (UL133 to UL151) have recently been identified in HCMV strain Toledo (15b) is indicated.

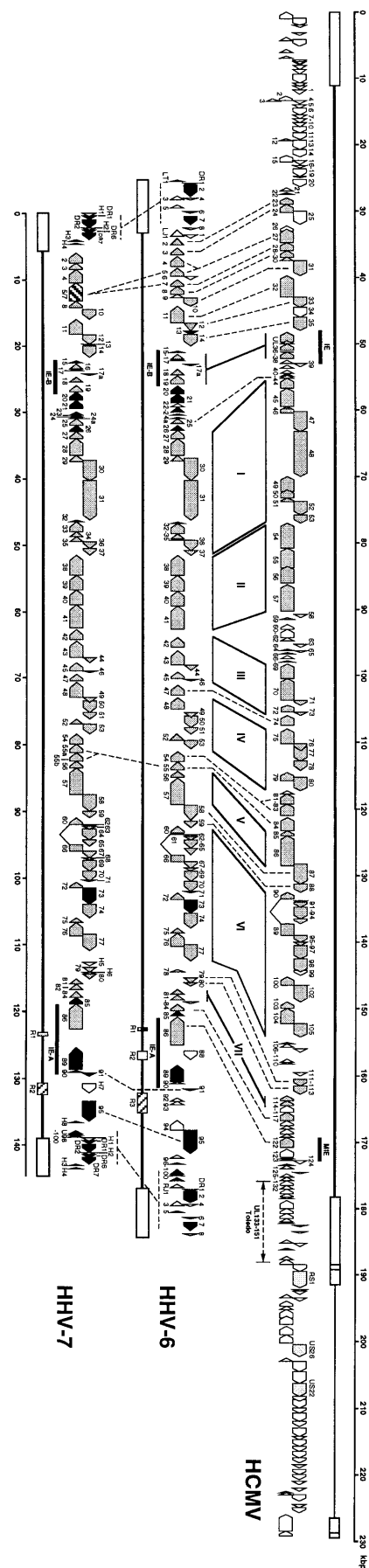


TABLE 2. Sequence similarities between HHV-7 US22 gene products

Protein	% Similarity/% identity with ^a :									
	DR2	DR6	DR7	U2	U3	U5/7	U8	U17/16	U25	U95
DR1	44.8/24.1	43.1/28.5	43.6/26.2	42.8/21.4	37.6/16.8	44.7/16.7	34.9/18.4	42.3/19.0	43.1/17.5	45.8/23.2
DR2		41.0/16.8	47.1/26.3	41.4/18.3	39.0/15.6	43.7/17.9	41.7/18.5	43.8/18.9	44.3/16.0	43.4/18.6
DR6			40.3/17.0	46.5/18.7	43.0/19.2	42.9/17.9	43.3/19.1	43.3/16.6	42.5/15.6	37.1/16.4
DR7				45.9/17.9	41.3/20.5	45.7/19.8	47.4/21.5	44.4/20.3	42.2/14.8	41.4/20.3
U2					50.6/23.7	45.1/17.6	48.9/17.8	49.3/21.9	47.4/21.3	47.6/21.1
U3						48.6/19.5	48.1/22.4	48.8/22.9	42.1/19.9	50.4/21.3
U5/7							48.3/22.3	45.4/18.7	45.3/19.9	42.6/15.7
U8								45.1/21.4	44.1/16.3	42.3/20.5
U17/16									41.8/21.8	45.1/19.6
U25										46.2/21.9

^a Values for percent similarity and percent identity were derived from BESTFIT comparisons (32) of each protein pair. Gap and length weights were set at 3.0 and 0.1, respectively. U17 and U16 represent exons 1 and 2 of a single gene, homologous to HCMV UL36 (17).

gous proteins in other herpesviruses, including HHV-6, has subsequently been undertaken (34, 36, 71, 72, 75, 110). gB and gH are structurally highly conserved between herpesviruses, are membrane-bound glycoproteins, and appear to play roles in virus cell fusion and cellular spread of virus infection. Data relating to gL came from investigations of the HSV-1-, HCMV-, and HHV-6-encoded homologs and have determined that gL forms a physical association with gH precursor protein and may be required for gH transport and/or processing (51, 56, 71, 72). The so-called "integral membrane protein," gM, is fairly well conserved between herpesviruses and has been shown to be a component of the virus particle in HSV-1 and EHV-1 (8, 104). The herpesvirus gM proteins have multiple hydrophobic, putative membrane-spanning domains.

HHV-7 contains two genes (U12 and U51) that specify homologs of the G-protein-coupled receptor (GCR) family of proteins. HHV-7 U12 and U51 have positional and structural homologs in HHV-6 and HCMV (UL33 and UL78). A GCR homolog has also been reported in HVS, a gammaherpesvirus, and it is perhaps significant that HCMV UL78 and HHV-6/HHV-7 U51 show relatively high sequence similarity to the HVS GCR (43, 94). This could be an indication that they were derived from the same gene within a progenitor herpesvirus genome. The HHV-6 and HHV-7 U51 products show closest sequence similarity to cellular opioid receptors. HHV-7 U12 shows closest similarity to its positional homologs in HHV-6 (U12) and HCMV (UL33). All of the herpesvirus-specified GCRs are structurally related to chemokine receptors, and HCMV US28 has been shown to bind the CC-chemokine Hu MIP-1 α (2, 41, 43, 89).

In HHV-6, a highly spliced gene at the right end of the unique component of the genome has recently been characterized and shown to encode a glycoprotein species, gp82/105 (102). In strain GS, this gene contains 13 exons. Three HHV-7 ORFs (U98, U99, and U100) showing homology to regions of the gp82/105 amino acid sequence are listed in Table 1 and illustrated in Fig. 2 and 3. Other, very short ORFs with homology to gp82/105 have been identified by BLASTX searches of the database (data not shown). The potential splicing patterns for the HHV-7 gp82/105 homolog are too complex to infer with confidence from the sequence alone.

Homologs of HHV-6 ORFs U20, U21, U23, U24, and U85, predicted to encode glycoproteins (43, 92, 95), are present in HHV-7. HHV-6 U20 has been reported to show significant sequence similarity to the immunoglobulin E (IgE) C chain and therefore to be a member of the Ig superfamily (43). No very high-scoring matches to HHV-7 U20 (other than HHV-6 U20) were detected by FASTA, BLASTP, or BLITZ database

searches, although some low-scoring matches to Ig proteins were among potential homologs listed. The U85 genes of HHV-6 and HHV-7 encode homologs of OX-2 membrane antigen, which is a member of the Ig family (22). Like HHV-6 U24, HHV-7 U24 lacks an N-terminal signal sequence and may represent a glycoprotein exon (43). Finally, by using the BLASTP algorithm to screen the database for homologs of the U23 translation product, several fairly high-scoring matches were detected. One of these, EHV-1 glycoprotein J (gJ), showed 37.8% amino acid sequence identity with the U23 protein when these sequences were aligned by using the GAP algorithm (data not shown). This sequence similarity, together with the comparable lengths of the two proteins (172 and 116 amino acids for U23 and gJ, respectively) and the presence of potential signal sequence and glycosylation sites (NxT/S) in the U23 protein, suggests that HHV-7 U23 and EHV-1 gJ may indeed be homologous and functionally related.

(v) **Capsid, tegument, and virus assembly proteins.** Several of the ORFs identified in HHV-7 are homologous to herpesvirus genes encoding characterized or candidate structural proteins. These include homologues of the major capsid protein (MCP; U57), minor capsid protein (mCP; U29), large tegument protein (U31), and virion proteins specified by U33, U34, U36, U50, U56, and U76 (17, 30, 43, 68, 79, 90, 91, 135, 138) (Table 1). Betaherpesvirus-specific and conserved structural proteins are homologs of HCMV UL32 (antigenic phosphoprotein, pp150) and UL82/83 (tegument transactivator, pp65/72K), which in HHV-6 and HHV-7 correspond to U11 and U54, respectively. The HHV-6 U11 gene product is called pp100 and has been characterized as a major antigenic phosphoprotein and a component of the virion (88, 101). HHV-7 homologs of herpesvirus gene products involved in DNA packaging and capsid assembly have also been identified. These correspond to HHV-7 U29 (mCP), U30, U53, and U60/66. These genes appear to be conserved (at least positionally) in all of the sequenced herpesviruses (43, 134) (Table 1). The U60/66 ORFs correspond to the two exons of the late spliced gene characterized initially in HSV-1, which is likely to play a role in DNA packaging and capsid assembly (107). The splice junctions of the two exons of the genes are well conserved in the different herpesviruses, and this has allowed deduction of the functional splice donor and acceptor within U66 and U60 of HHV-7. U53 sequences code for the protease/assembly protein (assemblin) and the scaffolding protein; these proteins are derived through proteolytic cleavage (assemblin) and internal initiation (scaffolding protein). The sequences encoding the scaffolding protein are referred to as U53a (43) and correspond to HSV-1 UL26.5. From studies on HSV-1 UL26/26.5

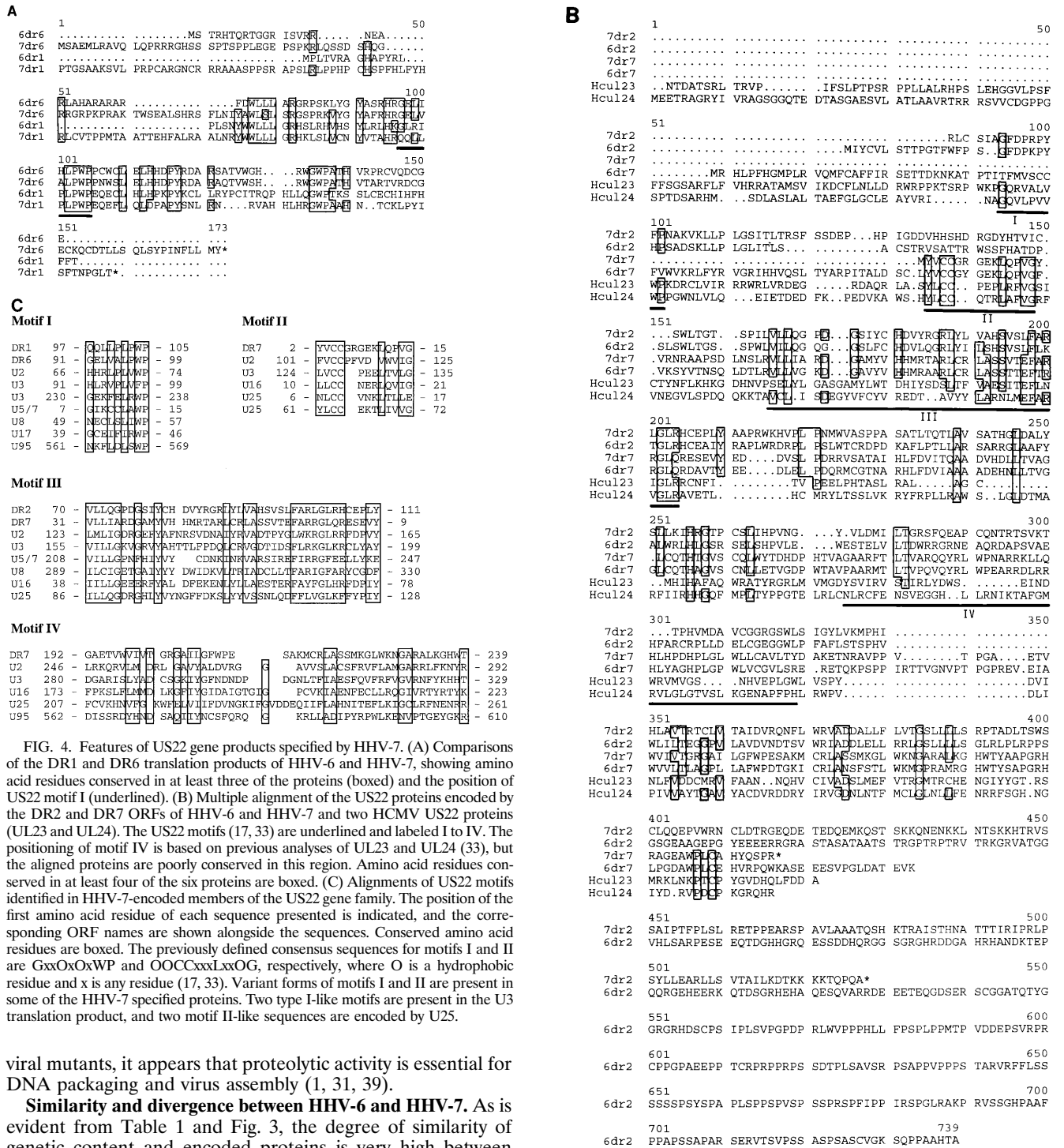


FIG. 4. Features of US22 gene products specified by HHV-7. (A) Comparisons of the DR1 and DR6 translation products of HHV-6 and HHV-7, showing amino acid residues conserved in at least three of the proteins (boxed) and the position of US22 motif I (underlined). (B) Multiple alignment of the US22 proteins encoded by the DR2 and DR7 ORFs of HHV-6 and HHV-7 and two HCMV US22 proteins (UL23 and UL24). The US22 motifs (17, 33) are underlined and labeled I to IV. The positioning of motif IV is based on previous analyses of UL23 and UL24 (33), but the aligned proteins are poorly conserved in this region. Amino acid residues conserved in at least four of the six proteins are boxed. (C) Alignments of US22 motifs identified in HHV-7-encoded members of the US22 gene family. The position of the first amino acid residue of each sequence presented is indicated, and the corresponding ORF names are shown alongside the sequences. Conserved amino acid residues are boxed. The previously defined consensus sequences for motifs I and II are GxxOxOxWP and OOCxxxLxxOg, respectively, where O is a hydrophobic residue and x is any residue (17, 33). Variant forms of motifs I and II are present in some of the HHV-7 specified proteins. Two type I-like motifs are present in the U3 translation product, and two motif II-like sequences are encoded by U25.

viral mutants, it appears that proteolytic activity is essential for DNA packaging and virus assembly (1, 31, 39).

Similarity and divergence between HHV-6 and HHV-7. As is evident from Table 1 and Fig. 3, the degree of similarity of genetic content and encoded proteins is very high between HHV-6 and HHV-7. With respect to coding content, there are only minor differences between the two betaherpesvirus genomes. Within the DR sequences, HHV-6 ORFs DR3, DR4, and DR5 appear to be unique, and HHV-7 ORFs H3 and H4 have no obvious counterparts in HHV-6. HHV-7 DR2 is C-terminally truncated relative to its HHV-6 homolog, and N-terminal splicing to supply an initiator ATG for expression of DR2-encoded sequences is predicted. HHV-6 DR2, by contrast, contains an N-terminal ATG codon (43). Similarly, while HHV-6 DR1 is assumed to represent an unspliced gene (43), HHV-7 DR1 lacks an N-terminal ATG codon and could con-

stitute an exon whose expression is dependent on splicing to upstream sequences to provide an initiator ATG codon. The homologous, albeit diverged, DR6 ORF of HHV-7 possesses an N-terminal ATG codon and is probably not spliced. It is notable that the DR7 ORF of HHV-7, although showing high amino acid sequence similarity to HHV-6 DR7 (Table 1), is N-terminally truncated relative to its HHV-6 counterpart (Fig. 4). DR1, DR2, DR6, and DR7 are all members of the US22

R1:

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1           ATGTACATATTACATAATATATGCTAGTAAATGATTACATGCACTAGTAAATATTTGCACATGCTAA (123407)
2 TGTGTTTCATGTGGGTATATGTACATATTACATAATATATGCTAGTAAATGATTACATGCACTAGCATATATTTGCACATACTAA (123492)
3 TGTGTTTCATGTGGGTATATGTACATATTACATAATATATGCTAGTAAATGATTACATGCACTAGCATATATTTGCACATACTAA (123575)
4           ATGTACATATTACATAATATATGCTAGTAAATGATTACATGCACTAGTAAATATTTGCACATGCTAA (123658)
          :
A (123507) ATATGTACATATTACATAA - n56 - TTATGTAATATGTACATAT (123599)
B (123570) TACTAGCATATATTTATGTA - n14 - TACATAATATATGCTAGTA (123620)

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R2:

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1 TCTTTGAAA CAACAGGAATTGCGGTTGTCTTCTCAGCAAGTTCTACACATTCCACTCGCTTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
2 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTACGGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
3 TATGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTACGGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
4 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
5 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
6 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
7 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
8 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
9 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
10 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
11 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
12 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
13 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
14 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
15 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA
16 CCCGTGAAAAACAACAGGAACTGCGGTTGCCTTCTCAGCAGGTTTTATGAGTTCTCACCCG TTTAGGCAGGAAAGACCCCAACCACATAACCCACTGACATTTAAA

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FIG. 5. Repetitive elements within the unique component of HHV-7. The two regions of reiterated sequences identified lie within (R1) and upstream (R2) of the IE-A locus. R1 comprises four sequence stretches, two of 67 bp flanking two of 84 bp, that are closely related. Repeats 1 and 4 are identical, and relative to comparable regions of repeats 2 and 3, there are two and three mismatches, respectively (indicated in boldface type). Within the R1 sequences, there are also two inverted repeats (A and B). The positions of the terminal nucleotides of each of the R1 repeat elements within the HHV-7 sequence are given. The R2 repeat unit comprises 16 closely related 105-bp elements flanked by partial repeats (not shown). Sequence variations between the 16 well-conserved elements are highlighted in boldface type. These sequence elements are related to but significantly diverged from the R3 (*Kpn*I) repeats of HHV-6 (43, 77, 78).

gene family, since they each contain at least one of the four US22 amino acid sequence motifs (17, 33, 43) (Fig. 4). The structural differences between the US22 genes encoded by the DRs of HHV-6 and HHV-7 might indicate functional divergence of these putative transcriptional regulators.

Differences within the unique components of HHV-7 include the "fusion" in HHV-7 of HHV-6 U5 and U7 sequences (to give HHV-7 U5/7, lacking HHV-6 U6 homologous sequences), the lack of an HHV-7 equivalent of HHV-6 U22, the duplication in HHV-7 of U55 (HCMV UL84 replication gene homolog [53, 98]), differences in ORFs identified within repetitive sequence elements within or close to IE-A, the absence of an AAV-2 *rep* gene homologue in HHV-7, and the presence of small unique ORFs within the right-terminal unique sequences of each virus (Fig. 3). Differences in the natures of the repetitive elements associated with the IE-A transcription units of each virus are described in more detail below. It is important to note that the U7-related sequences in HHV-6 and HHV-7 are homologous to the US22 gene family and that a direct counterpart of HHV-6 U7 is present in HCMV (UL28). The joining of U5 and U7 homologous sequences in HHV-7 may indicate that a unique regulatory function is encoded by this gene. The presence in HHV-7 of two homologs of HCMV UL84 (HHV-6 U55 homolog) is also of interest. UL84 has recently been shown to be an essential component of HCMV origin-dependent replication (5, 53, 98). Its function is unknown, but it appears as though UL84 equivalents are restricted to the betaherpesviruses (17, 43). HHV-7 U55A and U55B are considerably diverged from each other and from their HHV-6 counterparts, but FASTA searches of the database clearly revealed their relationships to HCMV UL84, and alignments of these gene products confirmed this finding (data not shown). The significance of duplicated but divergent UL84 homologs in HHV-7 is uncertain, but presumably at least one of the encoded gene products specifies an essential replication function. Also likely to be involved in replication are the U79/U80 ORF

products, which are homologous to HCMV replication gene UL112/113. HHV-7 H6 lies between U79 and U80 and shares amino acid sequence similarity with the C-terminal region of HHV-6 U79. By analogy with HCMV UL112/113, the HHV-7 U79, H6, and U80 coding sequences may represent exons of a spliced gene. However, the HHV-6 and HHV-7 U79 ORF products show only low amino acid sequence similarity to HCMV UL112, and the U80 proteins are N-terminally truncated relative to HCMV UL113. Thus, there appears to be considerable divergence among all three betaherpesviruses at this locus.

Major repetitive elements within the unique component of HHV-7. In common with HHV-6, HHV-7 has repetitive sequence motifs within and upstream of the IE-A locus, which encodes the partially conserved equivalent of the MIE transcription units identified in other betaherpesviruses (92). The HHV-7 R1 repeat unit occurs between U86 and U89 (adjacent ORFs), and it is notable that these repetitive sequences are distinct from the analogously positioned repetitive motifs (G-T repeats) in HHV-6 (43, 92, 140) (Fig. 5). The HHV-7 repeats at this locus are complex (two repeats of 84 bp and two "partial" repeats of 67 bp) and well conserved at the nucleotide sequence level. They occur between positions 123340 and 123658. Within and surrounding these repetitive motifs are multiple copies of the sequence TAAAT. The second set of repeats, R2, occurs upstream of the IE-A coding region (Fig. 2). With the exception of two of the HHV-7 R2 motifs, each of these elements contains a *Dra*I restriction endonuclease site. The length of the HHV-7 R2 (or *Dra*I) repeats is 105 bp, and there is a total of 16 well-conserved repeat elements (Fig. 5), with two partially conserved motifs within R2-flanking sequences. Because of the highly conserved nature of the *Dra*I repeats, the precise accuracy of assembly of the contiguous sequence across the R2 locus cannot be guaranteed; however, the number and types (sequence variants) of repeats within the sequenced clone, pED65'7, are believed to be correct. As re-

ported previously, some size heterogeneity within this region is apparent and may correspond to differences in numbers of *Dra*I repeats present within individually isolated clones (115). This may, in turn, reflect R2 heterogeneity among individual HHV-7 genomes. The HHV-7 R2 repeats are homologous to the 105-bp R3 (or *Kpn*I) repeats in HHV-6 (43, 77, 78).

Finally, it should be noted that HHV-7 does not contain equivalents of the SSRA repeat motifs identified within the U86 translation product of HHV-6 (92). These result from a series of dodecameric repetitive nucleotide motifs. The HHV-7 U86 product does, however, contain a central basic, serine-rich region that corresponds to the location of the HHV-6 SSRA repeats. It has previously been proposed that such sequences may be indicative of an RNA interaction domain within the U86 encoded protein, because similar motifs have been identified within known RNA-binding proteins (13, 43).

DISCUSSION

The data presented in this report show clearly that HHV-7 is genetically very closely related to HHV-6, with only a few substantial differences in protein-coding content (Table 1). These differences are limited to specific regions of the DR components and sequences corresponding to HHV-6 U5-U7, U22, U78, U96, and U97 and HHV-7 U5/7, U55A/U55B, and H7. HHV-6 ORFs U88 and U92/U93 and HHV-7 ORF H9 occur within unique (HHV-6 R2) or partially conserved (HHV-6 R3, HHV-7 R2) repetitive sequences; their actual protein-coding potential is uncertain. HHV-6 LT1 and LJ1 and HHV-7 H1 (possibly representing an exon) contain telomeric repeat sequences at the DR termini but are oppositely oriented. These data are summarized in Fig. 3.

Analysis of the HHV-7 DR sequences has led to the identification of several ORFs encoding US22 translation products, as identified by FASTA searches of the database. The HHV-6 equivalents of two of these ORFs, DR2 and DR7, have previously been reported to be members of the US22 gene family (43). HHV-7 also encodes homologs of HHV-6 DR1 and DR6, which are homologous to each other (Fig. 4A) and which are related to the US22 gene family. It is important to note, however, that the respective translation products of HHV-7 DR1 and DR7 contain divergent US22 motifs I and II, respectively, and that while DR1 and DR6 contain only motif I, DR2 and DR7 lack this motif (Fig. 4). DR1, DR2, and DR6 lack N-terminal methionine codons, presumably indicating that these coding sequences are expressed through spliced mRNAs. Furthermore, DR7 and DR2 contain US22 motif II within their N-terminal sequences, and it is tempting to speculate that these ORFs are spliced to DR1 and DR6 upstream sequences, respectively, thereby joining motif I- and motif II-encoding ORFs. By analogy to characterized US22 proteins in HCMV and HHV-6, it seems likely that the DR-derived US22 proteins may play a role in gene regulation during productive replication. Indeed, HHV-6 DR7 has been demonstrated to function as a transcriptional activator in cotransfection assays involving long terminal repeat-chloramphenicol acetyltransferase plasmid and human immunodeficiency virus type 1 proviral targets (55, 137). Isolation of cDNA clones corresponding to DR2 and DR7 sequences from an HHV-7-infected cell cDNA library indicates that these genes are expressed and function during productive infections (115a).

One of the notable regions of divergence within the unique components of HHV-7 and HHV-6 corresponds to the position of HHV-7 U5/7. This ORF essentially forms a fusion between HHV-6 U5 and U7 homologous sequences; an equiv-

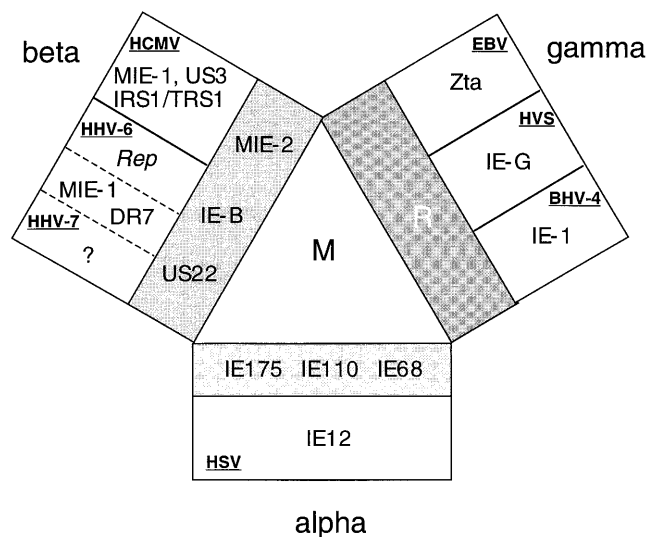


FIG. 6. Common and unique IE/regulatory genes between herpesvirus subgroups and individual herpesviruses. The diagram provides a simplified overview of the complements of IE genes or IE gene homologs present within the genomes of sequenced herpesviruses. Each herpesvirus contains a homolog of the EBV BMLF1-encoded transactivator, Mta (M); all members of each subgroup possess at least one IE/regulatory gene that is specific to the subgroup (e.g., gammaherpesvirus homologs of EBV BRLF1-encoded transactivator [R]), and each herpesvirus, appropriately analyzed, encodes at least one "unique" IE gene. Within the betaherpesvirus subgroup, there are both virus-common (HCMV UL23, UL24, UL28, UL29, UL36, UL43, and equivalents in HHV-6 and HHV-7) and nonconserved (e.g., HCMV IRS1/TRS1, and HHV-6/HHV-7 DR1, DR2, DR6, and DR7) US22 genes. The AAV-2 *rep* gene homolog is present only in HHV-6.

alent of HHV-6 U6 is not present in HHV-7 (Fig. 3). In HCMV, colinear homologs of HHV-6 U5 and U7 are present (UL27 and UL28). Interestingly, the HHV-6 and HHV-7 U4 ORFs represent additional UL27 homologs, essentially duplications of UL27-homologous U5 sequences. Therefore, deviations from colinearity between HHV-6, HHV-7, and HCMV occur at this locus. HHV-6 U7 and the N-terminal region of HHV-7 U5/7 (homologous to HHV-6 U7 and HCMV UL28) encode US22-related sequences (17, 33, 43). As for the DR US22 genes, the differences in the structures of the HHV-6 and HHV-7 US22 genes at the U7/U5 loci might indicate functional differences of the encoded, potentially transregulatory proteins. It can be speculated that the opportunity for such divergence within HHV-7 U5/7 sequences relates to the duplication of U5- and U7-type sequences within the genome. Thus, divergence of U5 and U7 may have been possible, without deleterious effects on replication efficiency, owing to complementing functions supplied by the homologous US22 and U4 genes. Therefore, while the HHV-7 U5/7 gene product may indeed encode unique regulatory functions, it is also conceivable that it is not expressed or is not biologically relevant.

Related to the issue of potentially novel transregulatory functions encoded by diverged US22 genes in HHV-6 and HHV-7 is the finding that HHV-7 lacks an equivalent of the HHV-6 AAV-2 *rep* gene homolog, U94 (139) (Fig. 3). The AAV-2 *rep* gene product is known to function as a transcriptional regulatory protein and is an essential component of the AAV-2 DNA replication machinery, activities that appear to be conserved in the HHV-6 *rep* homolog (63, 111, 141-143). It has been postulated, on the basis of the close amino acid sequence similarities of the AAV-2 and HHV-6 *rep* gene homologs, that HHV-6 U94 represents a gene acquired during coinfection of a single cell by AAV-2 and an HHV-6 progen-

itor genome (139). It is conceivable that such an event, leading to acquisition of a novel regulatory function, could be at least partially responsible for the biological and evolutionary divergence of HHV-6 and HHV-7. Other genetic differences giving rise to novel gene products and biological properties are also candidate effectors of "evolutionary drive" (biological divergence leading to herpesvirus speciation), as would be any intragenic or intergenic sequence variations leading to substantial alteration of gene function or expression. However, it is worth emphasizing that there is a correlation of herpesvirus IE/regulatory gene complements with herpesvirus subgroups and that each characterized herpesvirus contains at least one unique transregulatory gene (Fig. 6). Furthermore, such unique genes are located within or adjacent to unique gene clusters that may encode well-conserved, virus-specific cellular gene homologs (2, 3, 15, 92, 93, 95, 144). This is suggestive of recent acquisition of the unique IE genes at loci susceptible to and/or tolerant of genetic variation. Thus, it seems possible that acquisition of novel functions affecting the control and tropism of herpesvirus gene expression is an important determinant of biological properties effecting segregation and evolutionary divergence.

In summary, the data presented in this report demonstrate a close genetic relationship between HHV-6 and HHV-7 and have delineated specific, localized regions of divergence between these two genomes. These divergent regions include sequences specifying potential transregulatory and replication functions, in addition to colinear repetitive elements associated with the IE-A loci. The availability of the complete nucleotide sequences of HHV-6 and HHV-7 should allow rapid progress to be made on studies of gene function and on determinations of the common and unique properties of these human T-lymphotropic herpesviruses.

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