JOURNAL OF VIROLOGY, Sept. 1996, p. 5975–5989 Vol. 70, No. 9
0022-538X/96/$04.00+0

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

JOHN NICHOLAS*

Johns Hopkins Oncology Center, Baltimore, Maryland 21231

Received 18 March 1996/Accepted 13 May 1996

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 subgroup 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

* Mailing address: Johns Hopkins Oncology Center, 418 N. Bond St., Baltimore, MD 21231.

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

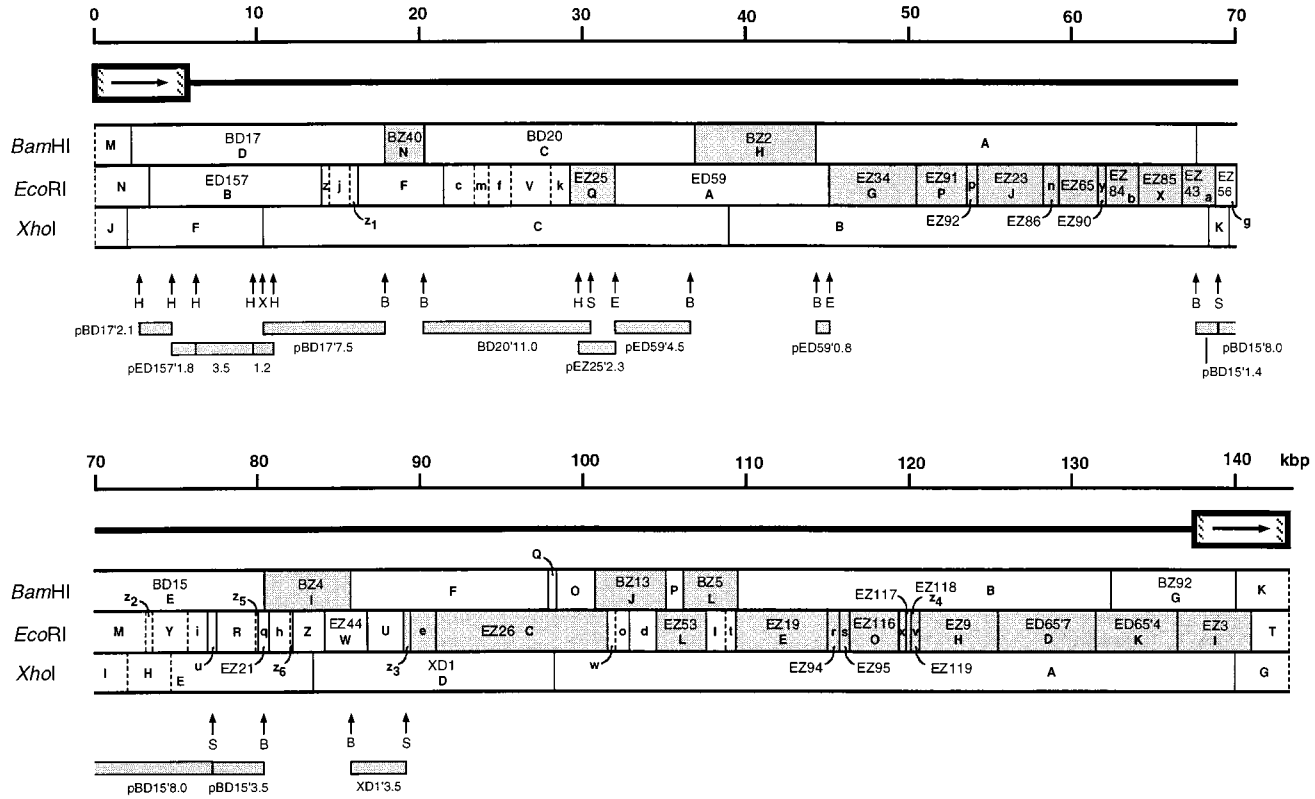


FIG. 1. Restriction fragments used for the sequencing of HHV-7 strain JI. The HHV-7 genome is represented diagrammatically, together with the positions of *Bam*HI, *Eco*RI, *Xho*I, *Sma*I, and *Sal*I sites mapped previously (115). Various λ phage-cloned restriction fragments are prefixed with ED, EZ, BD, BZ, or XD (indicating their terminal restriction sites and the vector, λDASHII or λZAPII, 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

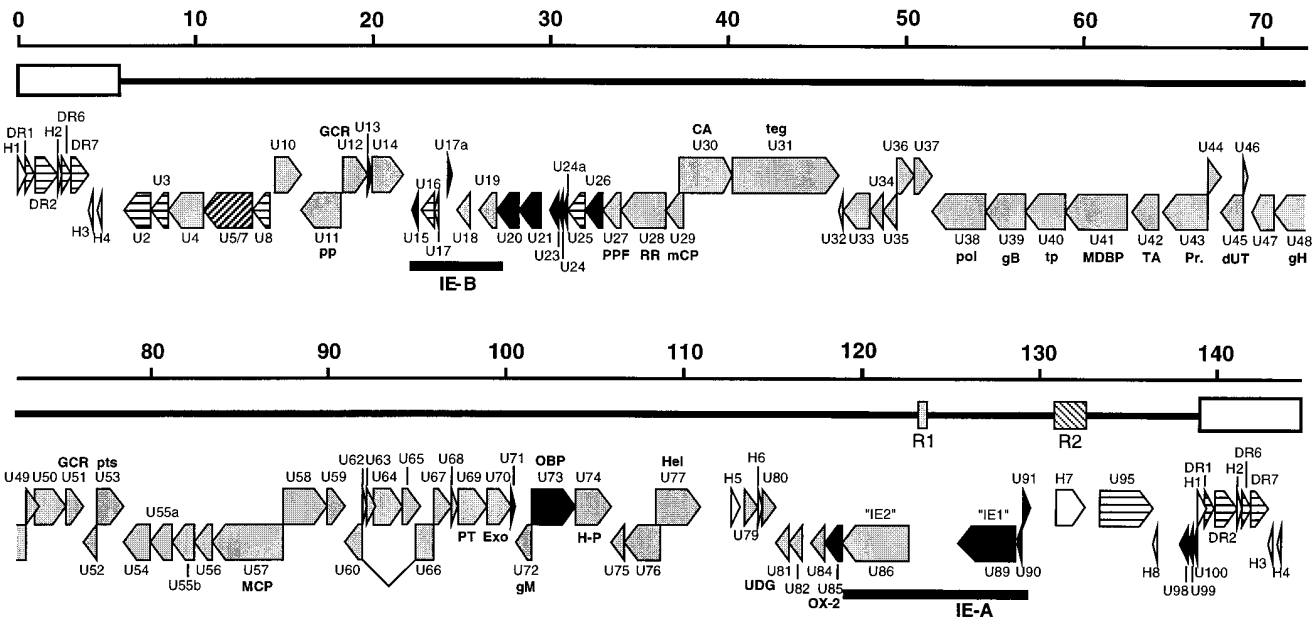


FIG. 2. ORFs identified in HHV-7. ORFs within the 144,861-bp sequence were identified with the Nucleotide Interpretation Program (NIP) of Staden, and homologs of HHV-6 genes (solid areas) and HHV-6/HCMV genes (shaded areas) were determined by FASTA, BLASTP, BLASTX, BLITZ, and GAP analyses (see Materials 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 alphaherpesviruses (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]				Length (aa)[c]	Homology with HHV-6 (%Sim/%Id)[d]	Homolog(s)[e]								Comments	Reference(s)
		Start	Stop	N-Term Met?	Poly(A)			HHV-6	HCMV	HVS	EBV	EHV-2	EHV-1	VZV	HSV-1		
H1	+	33	542	N	853*	169											
DR1	+	368	826	N	853*	152	57.1/44.0	DR1								US22 gene family, DR1/DR6 homology	
DR2	+	898	2100	N	3917*	400	58.1/37.0	DR2								US22 gene family	
H2	+	2267	2506	Y	3917*	79											
DR6	+	2562	3050	Y	3917*	161	71.6/58.8	DR6								US22 gene family, DR1/DR6 homology	
DR7	+	3122	3910	Y	3917*	262	77.8/60.5	DR7								US22 gene family, transactivator	55, 137
H3	−	4224	3976	Y	3040	82											
H4	−	4745	4449	Y	3040	98											
U2	−	7417	6338	Y	6248*	359	69.6/51.1	U2	UL23							US22 gene family	
U3	−	8732	7578	Y	7514	384	65.4/49.3	U3	UL24							US22 gene family	
U4	−	10382	8754	Y	7514	542	75.5/59.2	U4	UL27								
U5/7	−	13004	10407	Y	10411+	865	71.2/49.7	U5	UL27							US22 gene family (HHV-6 U7)	
							80.2/60.7	U7	UL28								
U8	−	14262	13174	Y	11850	362	69.0/51.0	U8	UL29							US22 gene family	
U10	+	14608	15963	Y	15885*	451	71.6/51.5	U10	UL31								
U11	−	18249	15982	Y	15971	755	55.6/30.7	U11	UL32							Structural phosphoprotein	54, 88, 101
U12	+	18396	19436	Y	19481	346	67.7/45.7	U12	UL33							GCR homolog, chemokine receptor	
U13	+	19521	19817	Y	20424*	98	54.6/37.1	U13									
U14	+	19885	21831	Y	21925*	648	67.9/53.5	U14	UL35							HCMV UL25/35 gene family	
U15	−	22244	22564	Y	22075	106	84.9/64.1	U15									
U16	−	23547(A)	22772	N	22754	264	74.8/56.5	U16	UL36x2							IE-B, transactivator (spliced to U17)	61, 95
U17Ex	−	23836	23620(D)	Y		72	55.5/41.7	U17Ex	UL36x1							IE-B, transactivator (spliced to U16)	61, 95
U17	−	23836	23570	Y	22754	88	54.5/39.8	U17									
U17a	+	24318	24587	Y	24707	89	46.6/29.3	U17a									
U18	−	25600	24713	Y	24578	295	71.4/43.9	U18	UL37x3							IE-B, homologous to HCMV IE glycoprotein	61
U19	−	26922	25945	Y	25504	325	59.9/40.1	U19	UL38							IE-B	61, 136
U20	−	28211	27036	Y	27027	391	53.2/23.4	U20								Ig gene family?	
U21	−	29494	28202	Y	28127	430	55.8/32.2	U21								Glycoprotein	
U23	−	30418	29903	Y	29904+	171	55.6/29.4	U23								Glycoprotein, EHV-1 gJ homology	135
U24	−	30772	30524	Y	30455*	82	63.2/36.8	U24								Glycoprotein	
U24a	−	31129	30776	Y	30671	117	57.1/33.9	U24a									
U25	−	31898	30936	Y	30671	320	70.7/48.9	U25	UL43							US22 gene family, transactivator	95
U26	−	32869	31988	Y	31959	293	60.4/30.4	U26									
U27	−	33951	32857	Y	31959	364	83.8/67.6	U27	UL44	(59)	(BMRF1)	(59)	(18)	(16)	(UL42)	DNA polymerase processivity factor	44, 98
U28	−	36484	34064	Y	34059	806	70.2/47.1	U28	UL45	61	BORF2	61	21	19	UL39	Ribonucleotide reductase (large subunit)	96
U29	−	37347	36487	Y	36457	286	77.5/53.3	U29	UL46	62	BORF1	62	22	20	UL38	Minor capsid protein (mCP)	90, 91, 138
U30	+	37362	40178	Y	37548	938	66.0/45.9	U30	UL47	63	BOLF1	63	(23)	(21)	(UL37)	Capsid assembly, myosin	
U31	+	40179	46358	Y	46994	2,059	65.3/46.2	U31	UL48	64	BPLF1	64	24	22	UL36	Large tegument protein	10, 42
U32	−	46627	46355	Y	46385*	90	80.7/65.9	U32		(66)	(BFRF2)	(66)	(25)	(23)	(UL35)		
U33	−	48041	46608	Y	46385*	477	78.5/59.4	U33	UL49							Virion protein	
U34	−	48768	47992	Y	47906	258	75.0/59.1	U34	UL50	67	BFRF1	67	(26)	(24)	(UL34)	Virion protein?	76, 83
U35	−	49119	48805	Y	48775	104	78.6/58.3	U35	UL51				27	25	UL33		
U36	+	49118	50575	Y	51089*	485	77.3/58.2	U36	UL52	68	BFLF1	68	28	26	UL32	Probable virion protein	23, 83
U37	+	50577	51356	Y	51613*	259	80.5/63.0	U37	UL53	69	BFLF2	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	BSLF1	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)		
U45	−	68898	67759	Y	67763^{+}	379	69.3/51.2	U45	UL72	54	BLLF2	54	9	8	UL50	dUTPase	67, 109
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, opioid^{R} homolog	2, 43, 94
U52	−	76949	76185	Y	75932	254	79.9/55.5	U52	UL79	18	BVRF1.5a/b	18					
U53	+	76957	78495	Y	79010	512	67.1/52.4	U53	UL80	17	BVRF2	17	35	33	UL26	Protease/assembly protein	31, 39, 146, 147
U54	−	79870	78503	Y	78443	455	66.3/41.4	U54	UL82/83							Tegument protein transactivator	70, 114
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	BcLF1	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						
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									
U72	−	101676	100636	Y	100640^{+}	346	81.1/59.0	U72	UL100	39	BBRF3	39	52	50	UL10	Integral membrane protein (gM)	8, 104
U73	+	101693	104456	Y	104067	787	75.4/58.2	U73						51	UL9	Origin-binding protein (OBP)	35, 52, 66, 145
U74	+	104007	105986	Y	106055	659	64.4/40.6	U74	UL102	(41)	(BBLF3)		(54)	(52)	(UL8)	Helicase/primase complex	29, 98
U75	−	106743	105973	Y	105977^{+}	256	64.9/45.6	U75	UL103	42	BBRF2	42	55	53	UL7		
U76	−	108589	106667	Y	106633*	640	78.5/59.7	U76	UL104	43	BBRF1	43	56	54	UL6	Virion protein?	83, 148
U77	+	108435	110897	Y	111009	820	85.7/74.9	U77	UL105	44	BBLF4	44	57	55	UL5	Helicase	29, 48, 157
H5	+	112811	113311	N	114614	166											
U79	+	113502	114203	Y	114614	233	70.6/44.7	U79	UL112							HCMV replication, spliced (UL112/UL113)	98, 153
H6	+	114257	114505	N	114614	82	64.1/48.7	U79(C)								HHV-6 U79 homology (C terminus)	43, 92
U80	+	114557	115189	N	115347*	210	57.0/40.4	U80	UL113							HCMV replication, spliced (UL112/113)	98, 153
U81	−	115948	115184	Y	115161	254	79.1/58.5	U81	UL114	46	BKRF3		61	59	UL2	Uracil-DNA glycosylase	67, 97, 152
U82	−	116778	116038	Y	115986	246	62.1/40.0	U82	UL115	(47)	(BKRF2)		(62)	(60)	(UL1)	Glycoprotein L	51, 56, 72
U84	−	118043	117111	Y	116463*	310	65.7/46.5	U84	UL117							Spliced in HCMV	
U85	−	118913	118071	Y	117885	280	60.8/37.7	U85								OX-2 homology, glycoprotein	22, 92
U86	−	122708	119091	Y	119082	1205	57.0/41.4	U86	UL122							IE-A, HCMV IE2 homology	17, 19, 85, 105, 130
U89	−	128668	125420	Y	125424^{+}	1,082	60.7/36.4	U89								IE-A, transactivator	77, 120

Continued on following page

TABLE 1—*Continued.*

U90	−	129051	128776	N		91	69.6/39.1	U90		IE-A, exon in HHV-6	120
U91	+	129122	129625	Y	130111	167	47.7/27.0	U91			
H7	+	130829	132112	Y	132157	427				DraI repeats	
U95	+	133382	136204	Y	136293	940	52.8/29.7	U95		MCMV IE2 homolog, US22 gene	60, 86
H8	−	136579	136307	Y	136257	90					
U98	−	138451	137945	N		168	52.2/31.2	U98		Homology to HHV-6 gp82/105	102
U99	−	138692	138375	N		105	61.3/37.6	U99		Homology to HHV-6 gp82/105	102
U100	−	138999	138751	Y		82	44.4/23.5	U100		Homology to HHV-6 gp82/105	102
H1′	+	139080	139589	N	139900*	169					
DR1′	+	139415	139873	N	139900*	152	57.1/44.0	DR1		US22 gene family, DR1/DR6 homology	
DR2′	+	139945	141147	N	142964*	400	58.1/37.0	DR2		US22 gene family	
H2′	+	141314	141553	Y	142964*	79					
DR6′	+	141609	142097	Y	142964*	161	71.6/58.8	DR6		US22 gene family, DR1/DR6 homology	
DR7′	+	142169	142957	Y	142964*	262	77.8/60.5	DR7		US22 gene family, transactivator	55, 137
H3′	−	143271	143023	Y	142087	82					
H4′	−	143792	143496	Y	142087	98					

[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 were 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 *Kpn*I repeats [77, 78]) and HHV-7 (R2 or *Dra*I 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 homolo-

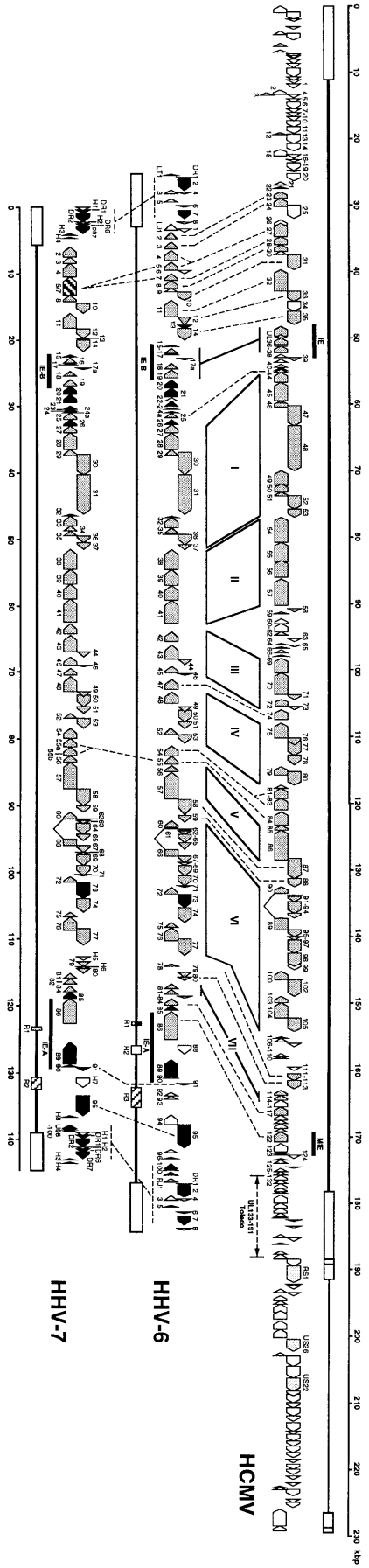


FIG. 3. Alignment of the genomes of HCMV (AD169), HHV-6, and HHV-7, showing homologous (shaded) and nonconserved (open) genes in these betaherpesviruses. Solid area indicates genes conserved between HHV-6 and HHV-7 but not present in HCMV. The herpesvirus-conserved gene blocks (I to VII) are indicated. 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 (15a) 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).

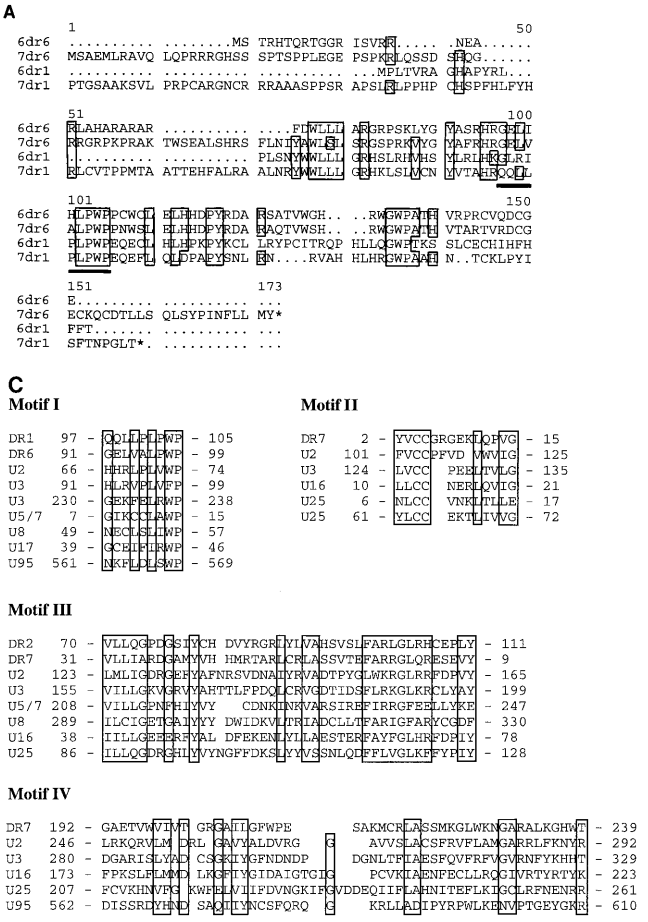
gous proteins in other herpesviruses, including HHV-6, has subsequently been undertaken (34, 36, 71, 72, 75, 110). gB and gH are structurally highly conserved between herpesviruses, are membrane-bound glycoproteins, and appear to play roles in virus cell fusion and cellular spread of virus infection. 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-1α (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



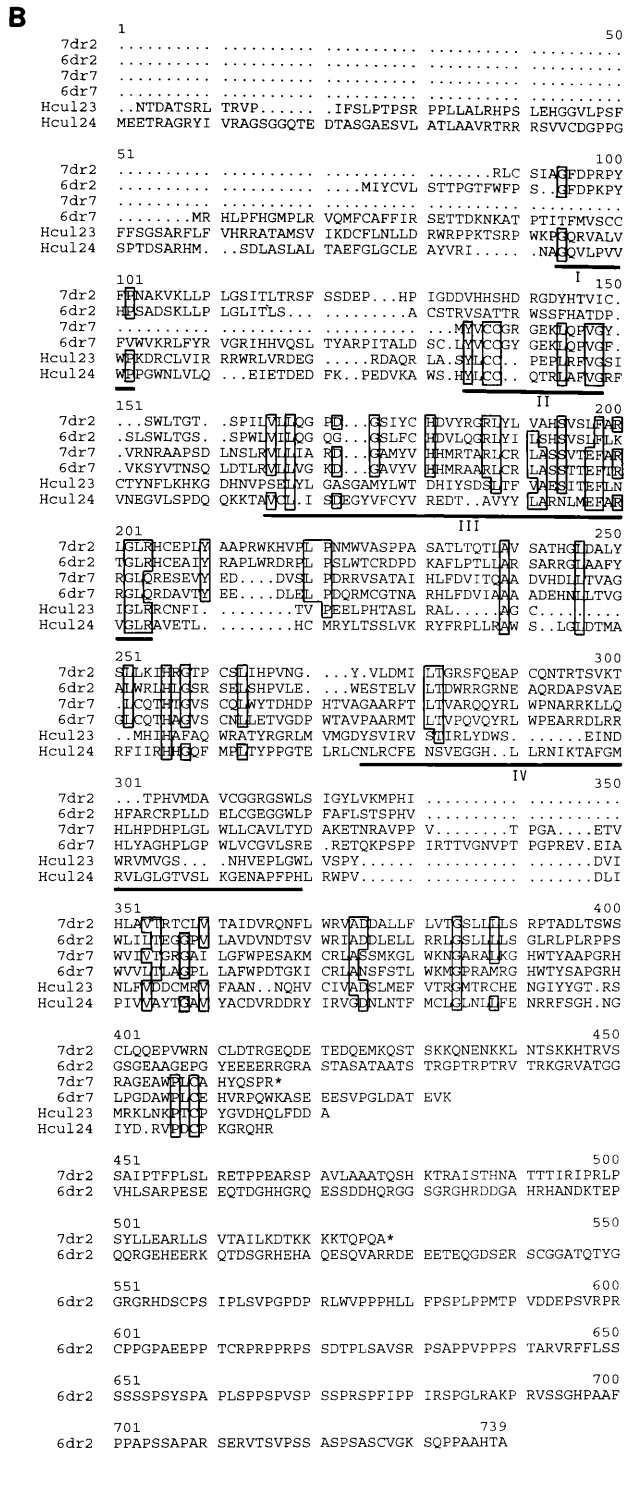


FIG. 4. Features of US22 gene products specified by HHV-7. (A) Comparisons of the DR1 and DR6 translation products of HHV-6 and HHV-7, showing amino acid residues conserved in at least three of the proteins (boxed) and the position of US22 motif I (underlined). (B) Multiple alignment of the US22 proteins 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 OOCCxxxLxxOG, 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 constitute 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

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 orientated. 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-chloramphenical 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 equivalent 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.

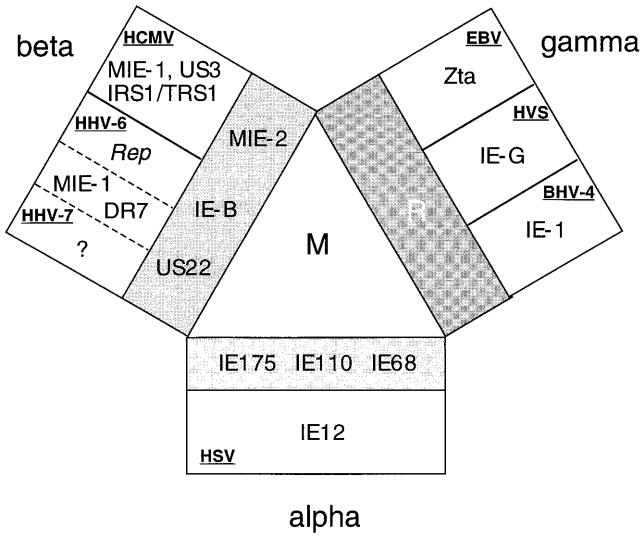


FIG. 6. Common and unique IE/regulatory genes between herpesvirus subgroups and individual herpesviruses. The diagram provides a simplified overview of the complements of IE genes or IE gene homologs present within the genomes of sequenced herpesviruses. Each herpesvirus contains a homolog of the EBV 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.

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.

ACKNOWLEDGMENTS

The work presented here was supported partly by a pilot project grant (JRG-11-33) and Junior Faculty Research Award (JFRA542) from the American Cancer Society, by Public Health Service grant R29 AID35483, and by the Johns Hopkins Oncology Center.

REFERENCES

1. **Addison, C., F. J. Rixon, J. W. Palfreyman, M. Ottara, and V. G. Preston.** 1984. Characterisation of a herpes simplex virus type 1 mutant which has a temperature sensitive defect in penetration in cells and assembly of capsids. Virology **138:**248–259.
2. **Albrecht, J. C., J. Nicholas, D. Biller, K. R. Cameron, B. Biesinger, C. Newman, S. Wittman, M. A. Craxton, H. Coleman, B. Fleckenstein, and R. W. Honess.** 1992. Primary structure of the herpesvirus saimiri genome. J. Virol. **66:**5047–5058.
3. **Albrecht, J. C., J. Nicholas, K. R. Cameron, C. Newman, B. Fleckenstein, and R. W. Honess.** 1992. Herpesvirus saimiri has a gene specifying a homolog of the cellular membrane glycoprotein CD59. Virology **190:**527–530.
4. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. F. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
5. **Anders, D. G., M. A. Kacica, G. S. Pari, and S. M. Punturieri.** 1992. Boundaries and structure of human cytomegalovirus *ori*Lyt, a complex origin for lytic phase DNA replication. J. Virol. **66:**3373–3384.
6. **Asano, Y., S. Suga, T. Yoshikawa, T. Yazaki, and T. Uchikawa.** 1995. Clinical features and viral excretion is an infant with primary human herpesvirus 7 infection. Pediatrics **95:**187–190.
7. **Bacchetti, S., M. J. Evelegh, and B. Muirhead.** 1986. Identification and separation of the two subunits of the herpes simplex virus ribonucleotide reductase. J. Virol. **57:**1177–1181.
8. **Baines, J. D., and B. Roizman.** 1993. The UL10 gene of herpes simplex virus 1 encodes a novel viral glycoprotein, gM, which is present in the virion and in the plasma membrane of infected cells. J. Virol. **67:**1441–1452.
9. **Bankier, A. T., K. M. Weston, and B. G. Barrell.** 1987. Random cloning and sequencing by the M13/dideoxynucleotide chain termination method. Methods Enzymol. **155:**51–93.
10. **Batterson, W., D. Furlong, and B. Roizman.** 1983. Molecular genetics of herpes simplex virus. VIII. Further characterization of a temperature-sensitive mutant defective in release of viral DNA and in other stages of the viral reproductive cycle. J. Virol. **45:**397–407.
11. **Berneman, Z. N., D. V. Ablashi, G. Li, M. Eger-Fletcher, M. S. Reitz, C. L. Hung, I. Brus, A. L. Komaroff, and R. C. Gallo.** 1992. Human herpesvirus 7 is a T-lymphotrophic virus and is related to, but significantly different from, human herpesvirus 6 and human cytomegalovirus. Proc. Natl. Acad. Sci. USA **89:**10552–10556.
12. **Berneman, Z. N., R. C. Gallo, D. V. Ablashi, N. Frenkel, G. Katsafanas, B. Kramarsky, and I. Brus.** 1992. Human herpesvirus 7 (HHV-7) strain JI: independent confirmation of HHV-7. J. Infect. Dis. **166:**690–691.
13. **Birney, E., S. Kumar, and R. Krainer.** 1993. Analysis of the RNA-recognition motif and RS and RGG domains: Conservation in metazoan pre-mRNA splicing factors. Nucleic Acids Res. **21:**5803–5816.
14. **Brunovskis, P., and L. F. Velicer.** 1995. The Marek's disease virus (MDV) unique short region: alpha-herpesvirus-homologous, fowlpox virus-homologous, and MDV-specific genes. Virology **206:**324–338.
15. **Bublot, M., P. Lamonte, A.-S. Lequarre, J.-C. Albrecht, J. Nicholas, B. Fleckenstein, P.-P. Pastoret, and E. Thiry.** 1992. Genetic relationships between bovine herpesvirus 4 and gammaherpesviruses Epstein-Barr virus and herpesvirus saimiri. Virology **190:**654–665.

15a. **Cha, T.-A., E. Tom, G. W. Kemble, G. M. Duke, E. S. Mocarski, and R. R. Speate.** 1996. Human cytomegalovirus clinical isolates carry at least 19 genes not found in laboratory strains. J. Virol. **70:**78–83.

16. **Challberg, M. D., and T. J. Kelly.** 1989. Animal virus DNA replication. Annu. Rev. Biochem. **58:**671–717.

16a. **Chandran, B.** Personal communication.

17. **Chee, M. S., A. T. Bankier, S. Beck, R. Bohni, C. M. Brown, R. Cerny, T. Horsnell, C. A. Hutchinson, T. Kouzarides, J. A. Martignetti, E. Preddie, S. C. Satchwell, P. Tomlinson, K. M. Weston, and B. G. Barrell.** 1990. Analysis of the protein coding content of the sequence of human cytomegalovirus strain AD169. Curr. Top. Microbiol. Immunol. **154:**125–169.
18. **Chee, M. S., G. L. Lawrence, and B. G. Barrell.** 1989. Alpha-, beta- and gammaherpesviruses encode a putative phosphotransferase. J. Gen. Virol. **70:**1151–1160.
19. **Chiou, C.-J., J. Zong, I. Waheed, and G. S. Hayward.** 1993. Identification and mapping of dimerization and DNA-binding domains in the C terminus of the IE2 regulatory protein of human cytomegalovirus. J. Virol. **67:**6201–6214.
20. **Chou, S., and G. I. Marousek.** 1992. Homology of the envelope glycoprotein B of human herpesvirus-6 and cytomegalovirus. Virology **191:**523–528.
21. **Clark, D. A., J. M. L. Freeland, P. L. K. Mackie, R. F. Jarrett, and D. E. Onions.** 1993. Prevalence of antibody to human herpesvirus 7 by age. J. Infect. Dis. **168:**251–252.
22. **Clark, M. J., J. Gagnon, A. F. Williams, and A. N. Barklay.** 1985. MRC OX-2 antigen: a lymphoid/neuronal membrane glycoprotein with a structure like a single immunoglobulin light chain. EMBO J. **4:**113–118.
23. **Coen, D. M., D. P. Aschman, P. T. Gelep, M. J. Retondo, S. K. Weller, and P. A. Schaffer.** 1984. Fine mapping and molecular cloning of mutations in the herpes simplex virus DNA polymerase locus. J. Virol. **49:**236–247.
24. **Cohen, E. A., J. Charron, J. Perret, and Y. Langelier.** 1985. Herpes simplex virus ribonucleotide reductase induced in infected BHK-21/C13 cells: biochemical evidence for the existence of two non-identical subunits, H1 and H2. J. Gen. Virol. **66:**733–745.
25. **Colberg-Poley, A. M., L. D. Santomenna, P. P. Harlow, P. A. Benfield, and D. J. Tenney.** 1992. Human cytomegalovirus US3 and UL36-38 immediate-early proteins regulate gene expression. J. Virol. **66:**95–105.
26. **Cranage, M. P., T. Kouzarides, A. T. Bankier, S. Satchwell, K. Weston, P. Tomlinson, B. Barrell, H. Hart, S. E. Bell, A. C. Minson, and G. L. Smith.** 1986. Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. EMBO J. **5:**3057–3063.
27. **Cranage, M. P., G. L. Smith, S. E. Bell, H. Hart, C. Brown, A. T. Bankier, P. Tomlinson, B. Barrell, and T. C. Minson.** 1988. Identification and expression of a human cytomegalovirus glycoprotein with homology to the Epstien-Barr virus BXLF2 product, varicalla-zoster virus gpIII, and herpes simplex virus type 1 glycoprotein H. J. Virol. **62:**1416–1422.
28. **Craxton, M.** 1991. Linear amplification sequencing, a powerful method for sequencing DNA. Methods **3:**20–26.
29. **Crute, J. J., T. Tsurumi, L. Zhu, S. K. Weller, P. D. Olivo, M. D. Challberg, and E. S. Mocarski.** 1989. Herpes simplex virus 1 helicase-primase: a complex of three herpes-encoded gene products. Proc. Natl. Acad. Sci. USA **86:**2186–2189.
30. **Davison, M. D., F. J. Rixon, and A. J. Davison.** 1992. Identification of genes encoding two capsid proteins (VP24 and VP26) of herpes simplex virus type 1. J. Gen. Virol. **73:**2709–2713.
31. **Desai, P., S. C. Watkins, and S. Person.** 1994. The size and symmetry of B capsids of herpes simplex virus type 1 are determined by the gene products of the UL26 open reading frame. J. Virol. **68:**5365–5374.
32. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
33. **Efstathiou, S., G. L. Lawrence, C. M. Brown, and B. G. Barrell.** 1992. Identification of homologues to the human cytomegalovirus US22 gene

family in human herpesvirus-6. J. Gen. Virol. **73:**1661–1671.
34. **Ellinger, K., F. Neipel, L. Foa-Tomasi, G. Campadelli-Fiume, and B. Fleckenstein.** 1993. The glycoprotein B homologue of human herpesvirus 6. J. Gen. Virol. **74:**495–500.
35. **Fierer, D. S., and M. D. Challberg.** 1992. Purification and characterization of UL9, the herpes simplex virus type 1 origin-binding protein. J. Virol. **66:**3986–3995.
36. **Foa-Tomasi, L., A. Boscaro, G. S. Di, and G. Campadelli-Fiume.** 1991. Monoclonal antibodies to gp100 inhibit penetration of human herpesvirus 6 and polykaryocyte formation in susceptible cells. J. Virol. **65:**4124–4129.
37. **Frame, M. C., H. S. Marsden, and B. M. Dutia.** 1985. The ribonucleotide reductase induced by herpes simplex virus type 1 involves minimally a complex of two polypeptides (136K and 38K). J. Gen. Virol. **66:**1581–1587.
38. **Frenkel, N., E. C. Schirmer, L. S. Wyatt, G. Katsafanas, E. Roffman, R. M. Danovich, and C. H. June.** 1990. Isolation of a new herpesvirus from human CD4+ T cells. Proc. Natl. Acad. Sci. USA **87:**748–752.
39. **Gao, M., L. Matusick-Kumar, W. Hurlburt, S. F. DiTusa, W. W. Newcomb, J. C. Brown, P. McCann, I. Deckman, and R. J. Colonno.** 1994. The protease of herpes simplex virus type 1 is essential for functional capsid formation and viral growth. J. Virol. **68:**3702–3712.
40. **Geng, Y., B. Chandran, S. F. Josephs, and C. Wood.** 1992. Identification and characterization of a human herpesvirus 6 gene segment that *trans* activates the human immunodeficiency virus type 1 promoter. J. Virol. **66:**1564–1570.
41. **Gerard, C., and N. P. Gerard.** 1994. C5A anaphylatoxin and its seven transmembrane-segment receptor. Annu. Rev. Immunol. **12:**775–808.
42. **Gibson, W.** 1983. Protein counterparts of human and simian cytomegaloviruses. Virology **128:**391–406.
43. **Gompels, U. A., J. Nicholas, G. Lawrence, M. Jones, B. J. Thomson, M. E. D. Martin, S. Efstathiou, M. Craxton, and H. A. Macaulay.** 1995. The DNA sequence of human herpesvirus-6: structure, coding content, and genome evolution. Virology **209:**29–51.
44. **Gottlieb, J., A. I. Marcy, D. M. Coen, and M. D. Challberg.** 1990. The herpes simplex virus type 1 UL42 gene product: a subunit of DNA polymerase that functions to increase processivity. J. Virol. **64:**5976–5987.
45. **Hardwicke, M. A., P. J. Vaughan, R. E. Sekulovich, R. O'Connor, and R. M. Sandri-Goldin.** 1989. The regions important for the activator and repressor functions of herpes simplex virus type 1 α protein ICP27 map to the C-terminal half of the molecule. J. Virol. **63:**4590–4602.
46. **Hermiston, T. W., C. L. Malone, P. R. White, and M. F. Stinski.** 1987. Identification and characterization of the human cytomegalovirus immediate-early region 2 gene that stimulates gene expression from an inducible promoter. J. Virol. **61:**3214–3221.
47. **Hidaka, Y., K. Okada, K. Kusuhara, C. Miyazaki, K. Tokugawa, and K. Ueda.** 1994. Exanthem subitum and human herpesvirus-7 infection. Pediatr. Infect. Dis. J. **13:**1010–1011.
48. **Hodgman, T. C.** 1988. A new superfamily of replicative proteins. Nature (London) **333:**22–23.
49. **Honess, R. W.** 1984. Herpes simplex and "the herpes complex": diverse observations and a unifying hypothesis. J. Gen. Virol. **65:**2077–2107.
50. **Honess, R. W., U. A. Gompels, B. G. Barrell, M. Craxton, K. R. Cameron, R. Staden, Y.-N. Chang, and G. S. Hayward.** 1989. Deviations from expected frequencies of CpG dinucleotides in herpesvirus DNAs may be diagnostic of differences in the states of their latent genomes. J. Gen. Virol. **70:**837–855.
51. **Hutchinson, L., H. Brown, V. Wargent, P. N. Davis, S. Pimorac, K. Goldsmith, A. C. Minson, and D. C. Johnson.** 1992. A novel herpes simplex virus glycoprotein, gL, forms a stable complex with glycoprotein H (gH) and affects normal folding and surface expression of gH. J. Virol. **66:**2240–2250.
52. **Inoue, N., T. R. Dambaugh, J. C. Rapp, and P. E. Pellett.** 1994. Alphaherpesvirus origin-binding protein homolog encoded by human herpesvirus 6B, a betaherpesvirus, binds to nucleotide sequences that are similar to ori regions of alphaherpesviruses. J. Virol. **68:**4126–4136.
53. **Iskenderian, A. C., L. Huang, A. Reilly, R. M. Stenberg, and D. G. Anders.** 1996. Four of eleven loci required for transient complementation of human cytomegalovirus DNA replication cooperate to activate expression of replication genes. J. Virol. **70:**383–392.
54. **Jahn, G., T. Kouzarides, M. Mach, B.-C. Scholl, B. Plachter, B. Traupe, E. Preddie, S. C. Satchwell, B. Fleckenstein, and B. G. Barrell.** 1987. Map position and nucleotide sequence of the gene for the large structural phosphoprotein of human cytomegalovirus. J. Virol. **61:**1358–1367.
55. **Kashanchi, F., J. Thompson, M. R. Sadaie, J. Doniger, J. Duvall, J. N. Brady, and L. J. Rosenthal.** 1994. Transcriptional activation of minimal HIV-1 promoter by ORF-1 protein expressed from the SalI-L fragment of human herpesvirus 6. Virology **201:**95–106.
56. **Kaye, J. F., U. A. Gompels, and A. C. Minson.** 1992. Glycoprotein H of human cytomegalovirus (HCMV) forms a stable complex with the HCMV UL115 gene product. J. Gen. Virol. **73:**2693–2698.
57. **Keil, G. M., A. Ebeling-Keil, and U. H. Koszinowski.** 1987. Sequence and structural organization of murine cytomegalovirus immediate-early gene 1. J. Virol. **61:**1901–1908.
58. **Klinedinst, D. K., and M. D. Challberg.** 1994. Helicase-primase complex of herpes simplex virus type 1: a mutation in the UL52 subunit abolishes primase activity. J. Virol. **68:**3693–3701.
59. **Knowles, W. A., and S. D. Gardener.** 1988. High prevalence of antibody to human herpesvirus-6 and seroconversion associated with rash in two infants. Lancet **ii:**912–913.
60. **Koszinowski, U. H., G. M. Keil, H. Volkmer, M. R. Fibi, A. Ebling-Keil, and K. Munch.** 1986. The 89,000-Mr murine cytomegalovirus immediate-early protein activates gene transcription. J. Virol. **58:**599–566.
61. **Kouzarides, T., A. T. Bankier, S. C. Satchwell, E. Preddy, and B. G. Barrell.** 1988. An immediate-early gene of human cytomegalovirus encodes a potential membrane glycoprotein. Virology **165:**151–164.
62. **Kouzarides, T., A. T. Bankier, S. C. Satchwell, K. Weston, P. Tomlinson, and B. G. Barrell.** 1987. Large-scale rearrangement of homologous regions in the genomes of HCMV and EBV. Virology **157:**397–413.
63. **Labow, M. A., P. L. Hermonat, and K. I. Berns.** 1986. Positive and negative autoregulation of the adeno-associated type 2 genome. J. Virol. **60:**251–258.
64. **Larder, B. J., S. D. Kemp, and G. Darby.** 1987. Related functional domains in virus DNA polymerases. EMBO J. **6:**169–175.
65. **Lawrence, G. L., M. Chee, M. A. Craxton, U. A. Gompels, R. W. Honess, and B. G. Barrell.** 1990. Human herpesvirus 6 is closely related to human cytomegalovirus. J. Virol. **64:**287–299.
66. **Lawrence, G. L., J. Nicholas, and B. G. Barrell.** 1995. Human herpesvirus 6 (strain U1102) encodes homologues of the conserved herpesvirus glycoprotein gM and the alphaherpesvirus origin-binding protein. J. Gen. Virol. **76:**147–152.
67. **Lindahl, T.** 1979. DNA glycosylases, endonucleases for apurinic/apyrimidinic sites, and base excision-repair. Prog. Nucleic Acid Res. **22:**135–192.
68. **Littler, E., G. Lawrence, M.-Y. Liu, B. G. Barrell, and J. R. Arrand.** 1990. Identification, cloning, and expression of the major capsid protein gene of human herpesvirus 6. J. Virol. **64:**714–722.
69. **Littler, E., A. D. Stuart, and M. S. Chee.** 1992. Human cytomegalovirus UL97 open reading frame encodes a protein that phosphorylates the antiviral nucleoside analogue ganciclovir. Nature (London) **358:**160–162.
70. **Liu, B., and M. F. Stinski.** 1992. Human cytomegalovirus contains a tegument protein that enhances transcription from promoters with upstream ATF and AP-1 *cis*-acting elements. J. Virol. **66:**4434–4444.
71. **Liu, D. X., U. A. Gompels, L. Foa-Tomasi, and G. Campadelli-Fiume.** 1993. Human herpesvirus-6 glycoprotein H and L homologs are components of the gp100 complex and the gH external domain is the target for neutralizing monoclonal antibodies. Virology **197:**12–22.
72. **Liu, D. X., U. A. Gompels, J. Nicholas, and G. Lelliott.** 1993. Identification and expression of the human herpesvirus-6 glycoprotein H and interaction with an accessory 30 kilodalton glycoprotein. J. Gen. Virol. **74:**1847–1857.
73. **Lusso, P., P. D. Markham, E. Tschachler, F. D. M. Veronese, S. Z. Salahuddin, D. V. Ablashi, S. Pahwa, K. Krohn, and R. C. Gallo.** 1988. In vitro cellular tropism of human B-lymphotropic virus (human herpesvirus-6). J. Exp. Med. **167:**1659–1670.
74. **Lusso, P., P. Secchiero, R. W. Crowley, A. Garzino-Demo, Z. N. Berneman, and R. C. Gallo.** 1994. CD4 is a critical component of the receptor for human herpesvirus 7: interference with human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **91:**3872–3876.
75. **Marsden, H. S., M. E. M. Campbell, L. Haarr, M. C. Frame, D. S. Parris, M. Murphy, R. G. Hope, M. T. Muller, and C. M. Preston.** 1987. The 65,000-M_r DNA-binding and virion *trans*-inducing proteins of herpes simplex virus type 1. J. Virol. **61:**2428–2437.
76. **Marsden, H. S., N. D. Stow, V. G. Preston, M. C. Timbury, and N. M. Wilkie.** 1978. Physical mapping of herpes simplex virus-induced polypeptides. J. Virol. **28:**624–642.
77. **Martin, M. E. D., J. Nicholas, B. J. Thomson, C. Newman, and R. W. Honess.** 1991. Identification of a transactivating function mapping to the putative immediate-early locus of human herpesvirus 6. J. Virol. **65:**5381–5390.
78. **Martin, M. E. D., B. J. Thompson, R. W. Honess, M. A. Craxton, U. A. Gompels, M.-Y. Liu, E. Littler, J. R. Arrand, I. Teo, and M. D. Jones.** 1991. The genome of human herpesvirus-6: maps of unit-length and concatemeric genomes for nine restriction endonucleases. J. Gen. Virol. **72:**157–168.
79. **McGeoch, D. J.** 1989. The genomes of the human herpesviruses: contents, relationships, and evolution. Annu. Rev. Microbiol. **43:**235–265.
80. **McGeoch, D. J.** 1990. Evolutionary relationships of virion glycoprotein genes in the S regions of alphaherpesvirus genomes. J. Gen. Virol. **71:**2361–2367.
81. **McGeoch, D. J., and S. Cook.** 1994. Molecular phylogeny of the alphaherpesvirinae subfamily and a proposed evolutionary timescale. J. Mol. Biol. **238:**9–22.
82. **McGeoch, D. J., C. Cunningham, G. McIntyre, and A. Dolan.** 1991. Comparative sequence analysis of the long repeat regions and adjoining parts of the long unique regions in the genomes of herpes simplex viruses types 1 and 2. J. Gen. Virol. **72:**3057–3075.
83. **McGeoch, D. J., M. A. Dalrymple, A. J. Davidson, A. Dolan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol. **69:**1531–1574.

84. **McLauchlan, J., A. Phelan, C. Loney, R. M. Sandri-Goldin, and J. B. Clements.** 1992. Herpes simplex virus IE63 acts at the posttranscriptional level to stimulate viral mRNA 3′ processing. J. Virol. **66:**6939–6945.
85. **Messerle, M., B. Buhler, G. M. Keil, and U. H. Koszinowski.** 1992. Structural organization, expression, and functional characterization of the murine cytomegalovirus immediate-early gene 3. J. Virol. **66:**27–36.
86. **Messerle, M., G. M. Keil, and U. H. Koszinowski.** 1991. Structure and expresison of murine cytomegalovirus immediate-early gene 2. J. Virol. **65:**1638–1643.
87. **Mukai, T., Y. Isegawa, and K. Yamanishi.** 1995. Identification of the major capsid protein gene of human herpesvirus 7. Virus Res. **37:**55–62.
88. **Neipel, F., K. Ellinger, and B. Fleckenstein.** 1992. Gene for the major antigenic structural protein (p100) of human herpesvirus 6. J. Virol. **66:** 3918–3924.
89. **Neote, K., D. DiGregorio, J. Y. Mak, R. Horuk, and T. Schall.** 1993. Molecular cloning functional expression and signalling characteristics of a C-C chemokine receptor. Cell **72:**415–425.
90. **Newcomb, W. W., F. L. Homa, D. R. Thomsen, Z. Ye, and J. C. Brown.** 1994. Cell-free assembly of the herpes simplex virus capsid. J. Virol. **68:**6059–6063.
91. **Newcomb, W. W., B. L. Trus, F. P. Booy, A. C. Steven, J. S. Wall, and J. C. Brown.** 1993. Structure of the herpes simplex virus capsid: molecular composition of the pentons and triplexes. J. Mol. Biol. **232:**499–511.
92. **Nicholas, J.** 1994. Nucleotide sequence analysis of a 21-kbp region of the genome of human herpesvirus-6 containing homologues of human cytomegalovirus major immediate-early and replication genes. Virology **204:** 738–750.

92a. **Nicholas, J.** Unpublished data.

93. **Nicholas, J., K. R. Cameron, H. Coleman, C. Newman, and R. W. Honess.** 1992. Analysis of nucleotide sequence of the rightmost 43kbp of herpesvirus saimiri (HVS) L-DNA: general conservation of genetic organization between HVS and Epstein-Barr virus. Virology **188:**296–310.
94. **Nicholas, J., K. R. Cameron, and R. W. Honess.** 1992. Herpesvirus saimiri encodes homologues of G protein-coupled receptors and cyclins. Nature (London) **355:**362–365.
95. **Nicholas, J., and M. E. D. Martin.** 1994. Nucleotide sequence analysis of a 38.5-kilobase-pair region of the genome of human herpesvirus 6 encoding human cytomegalovirus immediate-early gene homologs and transactivating functions. J. Virol. **68:**597–610.
96. **Nikas, I., J. McLaughlan, A. J. Davison, W. R. Taylor, and J. B. Clements.** 1986. Structural features of ribonucleotide reductase. Proteins **1:**376–384.
97. **Olsen, L. C., R. Aasland, C. U. Wittwer, H. E. Krokan, and D. E. Helland.** 1989. Molecular cloning of human uracil-DNA glycosylase a highly conserved DNA repair enzyme. EMBO J. **8:**3121–3125.
98. **Pari, G. S., and D. G. Anders.** 1993. Eleven loci encoding *trans*-acting factors are required for transient complementation of human cytomegalovirus oriLyt-dependent DNA replication. J. Virol. **67:**6979–6988.
99. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
100. **Pellett, P. E., F. J. Jenkins, M. Ackermann, M. Sarmiento, and B. Roizman.** 1986. Transcription initiation sites and nucleotide sequence of a herpes simplex virus 1 gene conserved in the Epstein-Barr virus genome and reported to affect the transport of viral glycoproteins. J. Virol. **60:**1134–1140.
101. **Pellett, P. E., D. Sanchez-Martinez, G. Dominguez, J. B. Black, E. Anton, C. Greenamoyer, and T. R. Dambaugh.** 1993. A strongly immunoreactive virion protein of human herpesvirus 6 variant B strain Z29: identification and characterization of the gene and mapping of a variant-specific monoclonal antibody reactive epitope. Virology **195:**521–531.
102. **Pfeiffer, B., B. Thomson, and B. Chandran.** 1995. Identification and characterization of a cDNA derived from multiple splicing that encodes the envelope glycoprotein gp105 of human herpesvirus 6. J. Virol. **69:**3490–3500.
103. **Phelan, A., M. Carmo-Fonseca, J. McLauchlan, A. I. Lamond, and J. B. Clements.** 1993. A herpes simplex virus type 1 immediate-early gene product, IE63, regulates small nuclear ribonucleoprotein distribution. Proc. Natl. Acad. Sci. USA **90:**9056–9060.
104. **Pilling, A., A. J. Davison, E. Telford, and D. M. Meredith.** 1994. The equine herpesvirus type 1 glycoprotein M is a major constituent of the virus particle. J. Gen. Virol. **75:**439–442.
105. **Pizzorno, M. C., M.-A. Mullen, Y.-N. Chang, and G. S. Hayward.** 1991. The functionally active IE2 immediate-early regulatory protein of human cytomegalovirus is an 80-kilodalton polypeptide that contains two distinct activator domains and a duplicated nuclear localization signal. J. Virol. **65:** 3839–3852.
106. **Pizzorno, M. C., P. O'Hare, L. Sha, R. L. LaFemina, and G. S. Hayward.** 1988. *trans*-activation and autoregulation of gene expression by the immediate-early region 2 gene products of human cytomegalovirus. J. Virol. **62:**1167–1179.
107. **Poon, A., and B. Roizman.** 1993. Characterization of a temperature-sensitive mutant of the UL15 open reading frame of herpes simplex virus 1. J. Virol. **67:**4497–4503.
108. **Portolani, M., C. Cermelli, P. Mirandola, and D. DiLuca.** 1995. Isolation of human herpesvirus 7 from an infant with febrile syndrome. J. Med. Virol. **45:**282–283.
109. **Preston, V. G., and F. B. Fisher.** 1984. Identification of the herpes simplex virus type 1 gene encoding the dUTPase. Virology **138:**58–68.
110. **Qian, G., C. Wood, and B. Chandran.** 1993. Identification and characterization of glycoprotein gH of human herpesvirus-6. Virology **194:**380–386.
111. **Rittner, K., R. Heilbronn, J. A. Kleinschmidt, and G. Sczakiel.** 1992. Adeno-associated virus type 2-mediated inhibition of human immunodeficiency virus type 1 (HIV-1) replication: involvement of $p78^{rep}/p68^{rep}$ and the HIV-1 long terminal repeat. J. Gen. Virol. **73:**2977–2981.
112. **Roizman, B.** 1982. The family Herpesviridae: general description, taxonomy and classification, p. 1–23. *In* B. Roizman (ed.), The herpesviruses. Plenum Press, New York.
113. **Roizman, B., R. C. Desrosiers, B. Fleckenstein, C. Lopez, A. C. Minson, and M. J. Sduddert.** 1992. The family herpesviridae: an update. Arch. Virol. **123:**425–449.
114. **Ruger, B., S. Klages, B. Walla, J. Albrecht, B. Fleckenstein, P. Tomlinson, and B. Barrell.** 1987. Primary structure and transcription of the genes coding for the two virion phosphoproteins pp65 and pp71 of human cytomegalovirus. J. Virol. **61:**446–453.
115. **Ruvolo, V. R., Z. Berneman, P. Secchiero, and J. Nicholas.** Cloning, restriction endonuclease mapping and partial sequence analysis of the genome of human herpesvirus-7 strain JI. J. Gen. Virol., in press.

115a. **Ruvolo, V. R., P. Secchiero, and J. Nicholas.** Unpublished data.

116. **Sanders, P. G., N. M. Wilkie, and A. J. Davison.** 1982. Thymidine kinase deletion mutants of herpes simplex virus type 1. J. Gen. Virol. **63:**277–295.
117. **Sandford, G. R., K. Ho, and W. H. Burns.** 1993. Characterization of the major locus of immediate-early genes of rat cytomegalovirus. J. Virol. **67:**4093–4103.
118. **Sanger, F., A. R. Coulson, B. G. Barrell, A. J. H. Smith, and B. A. Roe.** 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143:**161–178.
119. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
120. **Schiewe, U., F. Neipel, D. Schreiner, and B. Fleckenstein.** 1994. Structure and transcription of an immediate-early region in the human herpesvirus 6 genome. J. Virol. **68:**2978–2985.
121. **Seal, G., and M. A. Sirover.** 1986. Physical association of the human base-excision repair enzyme uracil-DNA glycosylase with the 70,000-dalton catalytic subunit of DNA polymerase α. Proc. Natl. Acad. Sci. USA **83:**7608–7612.
122. **Secchiero, P., J. Nicholas, H. Deng, T. Xiaopeng, N. van Loon, V. R. Ruvolo, Z. N. Berneman, M. S. Reitz, and S. Dewhurst.** 1995. Identification of human telomeric repeat motifs at the genome termini of human herpesvirus 7: structural analysis and heterogeneity. J. Virol. **69:**8041–8045.
123. **Smith, I. L., M. A. Hardwicke, and R. M. Sandri-Goldin.** 1992. Evidence that the herpes simplex virus immediate early protein ICP27 acts post-transcriptionally during infection to regulate gene expression. Virology **186:**74–86.
124. **Smith, T. F., and M. S. Waterman.** 1981. Identification of common molecular subsequences. J. Mol. Biol. **147:**195–197.
125. **Speate, R. R., A. Saxena, P. I. Scott, G. J. Song, W. S. Probert, W. J. Britt, W. Gibson, L. Rasmussen, and C. Pachl.** 1990. Sequence requirements for proteolytic processing of glycoprotein B of human cytomegalovirus strain Towne. J. Virol. **64:**2922–2931.
126. **Staden, R.** 1984. Graphic methods to determine the function of nucleic acid sequences. Nucleic Acids Res. **12:**521–538.
127. **Staden, R.** 1984. Measurements of the effects that coding for a protein has on a DNA sequence and their use for finding genes. Nucleic Acids Res. **12:**551–567.
128. **Staden, R.** 1987. Computer handling of DNA sequencing projects, p. 173–217. *In* M. J. Bishop and C. J. Rawlings (ed.), Nucleic acid and protein sequence analysis: a practical approach. IRL Press, Oxford.
129. **Stasiak, P. C., and E. S. Mocarski.** 1992. Transactivation of the cytomegalovirus ICP36 gene promoter requires the alpha gene product TRS1 in addition to IE1 and IE2. J. Virol. **66:**1050–1058.
130. **Stenberg, R. M., P. R. Witte, and M. F. Stinski.** 1985. Multiple spliced and unspliced transcripts from human cytomegalovirus immediate-early region 2 and evidence for a common initiation site within immediate-early region 1. J. Virol. **56:**665–675.
131. **Sullivan, V., C. L. Talarico, S. C. Stanat, M. Davis, D. M. Coen, and K. K. Biron.** 1992. A protein kinase homologue controls phosphorylation of ganciclovir in human cytomegalovirus-infected cells. Nature (London) **358:** 162–164.
132. **Tanaka, K., T. Kondo, S. Torigoe, S. Okada, T. Mukai, and K. Yamanishi.** 1994. Human herpesvirus 7: another causal agent for roseola (exanthem subitum). J. Pediatr. **125:**1–5.
133. **Telford, E., M. J. Studdert, C. T. Agius, M. S. Watson, H. C. Aird, and A. J. Davison.** 1993. Equine herpesvirus 2 and 5 are gammaherpesviruses. Virology **195:**492–499.
134. **Telford, E. A. R., M. S. Watson, H. C. Aird, J. Perry, and A. J. Davison.**

1995. The DNA sequence of equine herpesvirus 2. J. Mol. Biol. **249:**520–528.
135. **Telford, E. A. R., M. S. Watson, K. McBride, and A. J. Davison.** 1992. The DNA sequence of equine herpesvirus-1. Virology **189:**304–316.
136. **Tenney, D. J., and A. M. Colberg-Poley.** 1991. Expression of the human cytomegalovirus UL36-38 immediate-early region during premissive infection. Virology **182:**199–210.
137. **Thompson, J., S. Choudhury, F. Kashanchi, J. Doniger, Z. Berneman, N. Frenkel, and L. J. Rosenthal.** 1994. A transforming fragment within the direct repeat region of human herpesvirus type 6 that transactivates HIV-1. Oncogene **9:**1167–1175.
138. **Thomsen, D. R., L. L. Roof, and F. L. Homa.** 1994. Assembly of herpes simplex virus (HSV) intermediate capsids in insect cells infected with recombinant baculoviruses expressing HSV capsid proteins. J. Virol. **68:**2442–2457.
139. **Thomson, B. J., S. Efstathiou, and R. W. Honess.** 1991. Acquisition of the human adeno-associated virus type-2 rep gene by human herpesvirus type-6. Nature (London) **351:**78–80.
140. **Thomson, B. J., M. E. D. Martin, and J. Nicholas.** 1991. The molecular and cellular biology of human herpesvirus-6. Rev. Med. Virol. **1:**89–99.
141. **Thomson, B. J., F. W. Weindler, D. Gray, V. Schwaab, and R. Heilbronn.** 1994. Human herpesvirus 6 (HHV-6) is a helper virus for adeno-associated virus type 2 (AAV-2) and the AAV-2 rep gene homologue in HHV-6 can mediate AAV-2 DNA replication and regulate gene expression. Virology **204:**304–311.
142. **Tratschin, J. D., J. Tal, and B. J. Carter.** 1986. Negative and positive regulation in *trans* of gene expression from adeno-associated virus vectors in mammalian cells by a viral rep gene product. Mol. Cell. Biol. **6:**2884–2894.
143. **Trempe, J. P., and B. J. Carter.** 1988. Regulation of adeno-associated virus gene expression in 293 cells: control of mRNA abundance and translation. J. Virol. **62:**68–74.
144. **van Santen, V. L.** 1991. Characterization of the bovine herpesvirus 4 major immediate-early transcript. J. Virol. **65:**5211–5224.
144a. **Wan, X., and J. Nicholas.** Unpublished data.
145. **Weir, H. M., and N. D. Stow.** 1990. Two binding sites for the herpes simplex virus type 1 UL9 protein are required for efficient activity of the oriS replication origin. J. Gen. Virol. **71:**1379–1385.
146. **Welch, A. R., L. M. McNally, and W. Gibson.** 1991. Cytomegalovirus assembly protein nested gene family: four 3′ coterminal transcripts encode four in-frame, overlapping proteins. J. Virol. **65:**4091–4100.
147. **Welch, A. R., A. S. Woods, L. M. McNally, R. J. Cotter, and W. Gibson.** 1991. A herpesvirus maturational proteinase, assemblin: identification of its gene, putative active site domain, and cleavage site. Proc. Natl. Acad. Sci. USA **88:**10792–10796.
148. **Weller, S. K., D. P. Aschman, W. R. Sacks, D. M. Coen, and P. Schaffer.** 1983. Genetic analysis of temperature-sensitive mutants of HSV-1: the combined use of complementation and physical mapping for cistron assignment. Virology **130:**290–305.
149. **Weston, K.** 1988. An enhancer element in the short unique region of human cytomegalovirus regulates the production of a group of abundant immediate early transcripts. Virology **162:**406–416.
150. **Winkler, M., S. A. Rice, and T. Stamminger.** 1994. UL69 of human cytomegalovirus, an open reading frame with homology to ICP27 of herpes simplex virus, encodes a transactivator of gene expression. J. Virol. **68:** 3943–3954.
151. **Wong, S. W., A. F. Wahl, P.-M. Yuan, N. Arai, B. E. Pearson, K. Arai, D. Korn, M. W. Hunkapiller, and T. S.-F. Wang.** 1988. Human DNA polymerase α gene expression is cell proliferation dependent and its primary structure is similar to both prokaryotic and eukaryotic replicative DNA polymerases. EMBO J. **7:**37–47.
152. **Worrad, D. M., and S. Caradonna.** 1988. Identification of the coding sequence for herpes simplex virus uracil-DNA glycosylase. J. Virol. **62:**4774–4777.
153. **Wright, D. A., S. I. Staprans, and D. H. Spector.** 1988. Four phosphoproteins with common amino termini are encoded by human cytomegalovirus AD169. J. Virol. **62:**331–340.
154. **Wyatt, L. S., and N. Frenkel.** 1992. Human herpesvirus 7 is a constitutive inhabitant of adult human saliva. J. Virol. **66:**3206–3209.
155. **Wyatt, L. S., W. J. Rodriguez, N. Balachandran, and N. Frenkel.** 1991. Human herpesvirus 7: antigenic properties and prevalence in children and adults. J. Virol. **65:**6260–6265.
156. **Yamanishi, K., T. Okuno, K. Shiraki, M. Takahashi, T. Kondo, Y. Asano, and T. Kurata.** 1988. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet **i:**1065–1067.
157. **Zhu, L., and S. K. Weller.** 1992. The six conserved helicase motifs of the UL5 gene product, a component of the herpes simplex virus type 1 helicase-primase, are essential for function. J. Virol. **66:**469–479.