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The *a* Sequence of the Cytomegalovirus Genome Functions as a Cleavage/Packaging Signal for Herpes Simplex Virus Defective Genomes

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Although herpes simplex virus (HSV) 1 and human cytomegalovirus (CMV) differ remarkably in their biological characteristics and do not share nucleotide sequence homology, they have in common a genome structure that undergoes sequence isomerization of the long (L) and short (S) components. We have demonstrated that the similarity in their genome structures extends to the existence of an *a* sequence in the CMV genome as previously defined for the HSV genome. As such, the *a* sequence is predicted to participate as a *cis*-replication signal in four viral functions: (i) inversion, (ii) circularization, (iii) amplification, and (iv) cleavage and packaging of progeny viral DNA. We have constructed a chimeric HSV-CMV amplicon (herpesvirus *cis* replication functions carried on an *Escherichia coli* plasmid vector) substituting CMV DNA sequences for the HSV cleavage/packaging signal in a test of the ability of this CMV L-S junction sequence to provide the *cis* signal for cleavage/packaging in HSV 1-infected cells. We demonstrate that the *a* sequence of CMV DNA functions as a cleavage/packaging signal for HSV defective genomes. We show the structure of this sequence and provide a functional demonstration of cross complementation in replication signals which have been preserved over evolutionary time in these two widely divergent human herpesviruses.

Human cytomegalovirus (CMV) and herpes simplex virus (HSV) are biologically and biochemically distinct herpesviruses. They exhibit so few common characteristics that they are prototypes of separate subfamilies within the *Herpesviridae* (28, 29). Although the CMV genome, which is 240 kilobase pairs (kbp) (4, 6, 12, 35), shares no detectable sequence homology (10) with the 150-kbp HSV-1 genome (11, 43), both viruses are composed of long (L) and short (S) components that invert during viral replication such that virion DNA consists of four sequence isomers differing in the orientation of the L and S components (7, 12, 13, 30, 42, 43; reviewed in reference 27). Each component is composed of unique sequences (U_L and U_S) bracketed by inverted repeats. In HSV-1, the repeats bracketing U_L have been designated *ba* sequences (approximately 10 kbp), and those bracketing U_S are called *ca* sequences (6 kbp; references 30, 42, 43). The structure of the HSV-1 genome may thus be schematically diagrammed as *ab*-U_L-*b'a'c'*-U_S-*ca*. In CMV (Towne) DNA, the repeated sequences bracketing U_L and U_S are 11 and 2 kbp (13), respectively, but these regions vary in size between strains (7, 34).

In HSV-1 the *a* sequence is present in direct orientation at both genomic termini and in an inverted orientation at the L-S junction (21, 42, 43) and thus is the only sequence shared by both the L and S components. The *a* sequence plays a key role in viral replication as a *cis* signal for inversion (18–21), cleavage/packaging of progeny viral DNA (21, 40, 41), and circularization of the viral genome (43). In addition, the *a* sequence undergoes an amplification such that multiple tandem copies may be present at the L-S junction and L terminus of the HSV-1 genome (15, 19, 21, 44).

Several lines of evidence suggest the existence of a sequence in CMV which has some properties similar to those of the HSV *a* sequence. (i) In exonuclease digestion experiments similar to those which originally defined the *a* sequence on the HSV-1 genome, a terminal direct repeat has been demonstrated on CMV DNA (7). (ii) The CMV genome has a sequence that is amplified at the L-S junction as well as at the genomic termini (13, 33, 34, 37; see below). (iii) We have recently shown that the CMV (Towne) L-S junction can replace an HSV *a* sequence as a *cis* signal for cleavage/packaging into virions with an HSV defective virus amplicon construct (33).

There are two *cis* signals necessary for replication and packaging of defective HSV DNA (32a, 36, 40, 41): the cleavage/packaging signal within the *a* sequence and an origin of DNA replication. When these two signals are included on a plasmid clone, the entire construct, termed an amplicon, can be propagated in *Escherichia coli* or in HSV-infected mammalian cells (32). By substituting CMV L-S junction sequences for the HSV *a* sequence in an amplicon, we showed that *trans*-acting HSV-1 functions can recognize and cleave within CMV L-S junction sequences (33).

In this communication, we report the structure of the CMV L-S junction-spanning fragment (which includes an *a* sequence), identify a short region of homology with HSV *a* sequences, and establish the structural similarities between the CMV (Towne) and HSV *a* sequences.

MATERIALS AND METHODS

Cells and viruses. Vero (African green monkey kidney) cells and HEp-2 (human epidermoid carcinoma) cells were purchased from the American Type Culture Collection. HSV-1(m̄P)*ts*HA1 (2) was obtained from B. Roizman. CMV (Towne) was obtained from M. Stinski.

Purification of viral DNA and construction of recombinant plasmids. HSV and CMV DNAs were prepared by centrifugation to equilibrium on NaI gradients as previously described (18, 23, 45). The recombinant plasmid pON301 was constructed by inserting the 5.0-kilobase *Eco*RI/*Pvu*II CMV

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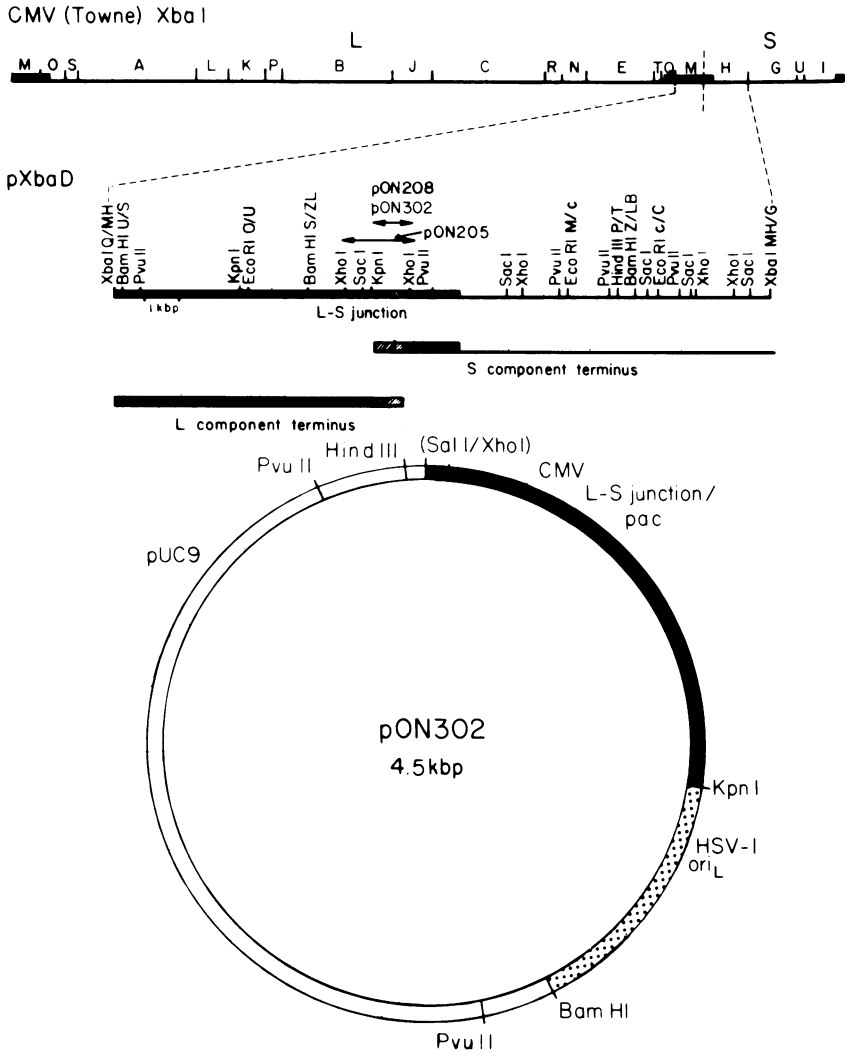


FIG. 1. Plasmid clones used in structural and functional studies of the L-S junction region of the CMV (Towne) genome. (Top) *Xba*I map of CMV (Towne) genome (R. L. LaFemina and G. S. Hayward, personal communication). (Middle) *Bam*HI, *Pvu*II, *Eco*RI, *Xho*I, *Sac*I, and partial *Kpn*I map of the *Xba*I MH fragment carried on the plasmid clone pXbaD (38), showing the L-S junction-spanning fragments cloned in pON302, pON208, and pON205 as described in the text. The terminal fragments, *Xba*I-M and -H, were aligned through common restriction sites with the *Xba*I MH junction fragment to approximate the position of the junction. The solid thick line indicates those sequences derived from the inverted repeats of the L and S components, and the thin line indicates sequences derived from the unique region of the S component of the viral genome. The hatched section illustrates the region of overlap when the termini are aligned, a region that gives rise to 750-bp ladders at the L-S junction and termini. (Bottom) HSV-CMV chimeric amplicon pON302 used to analyze the function of the CMV L-S junction as a cleavage/packaging site. The CMV L-S junction fragment is shown in solid black, the HSV-1 ori_L DNA replication origin is shown stippled, and the plasmid vector, pUC9, is open. Restriction sites destroyed in the process of cloning are in parentheses.

(Towne) DNA fragment derived from pXbaD (38) into the corresponding sites of plasmid pKC7 (25) as previously described (33). The recombinant pON205 was constructed by cloning the 1,800-base pair (bp) junction-spanning *Xho*I fragment (Fig. 1) derived from pON301 into the unique *Sal*I site of pUC9 DNA (39). pON302 was constructed by substituting a 750-bp *Bam*HI/*Kpn*I fragment containing the class II HSV-1 origin (ori_L) of replication (32, 32a) for the CMV DNA sequences between the *Bam*HI and *Kpn*I sites of pON205 (Fig. 1). pON208 was derived from pON205 by collapsing the region between the *Bam*HI and *Kpn*I sites (Fig. 1). All bacterial cloning was done in *E. coli* HB101, transformed by the calcium shock method (16). Plasmids from ampicillin-resistant colonies were prepared by the rapid boiling method (9) and analyzed with restriction enzymes purchased from New England BioLabs, Inc. Large-scale preparations of plasmid DNA were made by the cleared lysate procedure followed by centrifugation to equilibrium in cesium chloride-ethidium bromide gradients (1).

Transfection and isolation of infected cell DNA. Transfections of Vero cells were done as described previously (8) except that the monolayer was washed once with phosphate-buffered saline containing 200 μg of DEAE dextran per ml before application of the precipitated DNAs (18). Carrier DNA was not used. The cells were glycerol shocked 4 h posttransfection (22). Propagation and analyses of the transfection-derived series were as previously described (32) except that infected cell DNA was not radiolabeled before purification. Transfer to nitrocellulose filters after restriction enzyme digestion and electrophoresis in 0.5 to 1% agarose

gels were by standard techniques (31). Complete digestion was monitored by observing characteristic helper virus DNA restriction profiles in the samples.

Preparation of radioactively labeled probes and hybridization. Plasmid or viral DNA was radioactively labeled in vitro by nick translation (26) with [α^{32}P]dCTP (Amersham Corp.). Hybridizations to immobilized CMV DNA were performed at 65°C in a solution of 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.2% bovine serum albumin, 0.1% sodium dodecyl sulfate, 25 μg of salmon sperm DNA per ml with 30% formamide (18, 23). All other hybridizations were performed in an identical solution without formamide. Hybridizations were washed and autoradiographed as previously described (18, 23).

Nucleotide sequence determination and analysis. DNA fragments were end labeled with polynucleotide kinase (New England BioLabs) and [γ^{32}P]ATP (Amersham) or DNA PolI, Klenow fragment (Boehringer Mannheim Biochemicals), and [α^{32}P]dCTP or [α^{32}P]dATP and sequenced by procedures previously described (17, 19). Analysis of sequence was performed with Intelligenetics programs.

RESULTS

Authenticity of the junction-spanning clones. The criteria used to locate the L-S junction within the CMV (Towne) genome were: (i) revealing a region of overlap by aligning terminal and L-S junction-spanning fragments through common restriction sites and (ii) identifying restriction sites preserving the 750-bp ladder at the L-S junction. Radiolabeled pON208, carrying an L-S junction-spanning fragment, was used to probe CMV DNA digests electrophoresed in lanes adjacent to the plasmid-cloned derivatives of L-S junction sequences, pON205 and pON302. The results of this analysis, shown in Fig. 2, established that: (i) the cloned fragments represented authentic CMV DNA fragments as judged by their comigration with counterparts in the CMV genome; (ii) the heterogeneity which exists at both ends and at the L-S junction of the CMV (Towne) genome (13) was preserved when viral DNA was digested with *Kpn*I or *Xho*I, thus demonstrating that the restriction enzymes did not cut within amplified sequences; (iii) the sequences cloned from pXbaD into pON302 or pON205 represented the smallest member of the L-S junction-spanning family of fragments, and (iv) both L- and S-terminal fragments were shorter than the smallest L-S junction (compare, for example, the T_S fragment in the *Xho*I or *Kpn*I/*Xho*I digest of CMV DNA with the L-S junction fragment in the *Kpn*I/*Xho*I-digested CMV DNA). This last point indicated that cleavage of genome-length molecules occurred within sequences delimited by the *Kpn*I and *Xho*I restriction sites. As depicted in Fig. 1, the ends of the CMV (Towne) genome overlap by about 750 bp when aligned with the L-S junction by using common restriction sites. In addition and as noted by others (13), the ladder of fragments resulting from amplification of a sequence at the L-S junction and both ends of the genome was in 700- to 800-bp increments. These properties were reminiscent of *a* sequence amplification in the HSV-1 genome (15, 19, 44) and led to our studies below.

Structural features of the CMV L-S junction: the *a* sequence. The strategy used and the deduced nucleotide sequence of the CMV L-S junction fragment is shown in Fig. 3. The fragment from the *Kpn*I site to the *Xho*I site was 966 bp and had a base composition of 71% G+C and 29% A+T. Although open reading frames exist throughout the sequence, the canonical features of a gene are not apparent. In common with the HSV *a* sequence, as previously described by Mocarski and Roizman (19) and Davison and Wilkie (3), this sequence is composed of many tandemly repeated elements (Fig. 3 and 4). Unlike the tandem repeats in HSV-1 (3, 19), those in CMV are not identical tandem copies but rather form a nested set of degenerate repeats (Fig. 4).

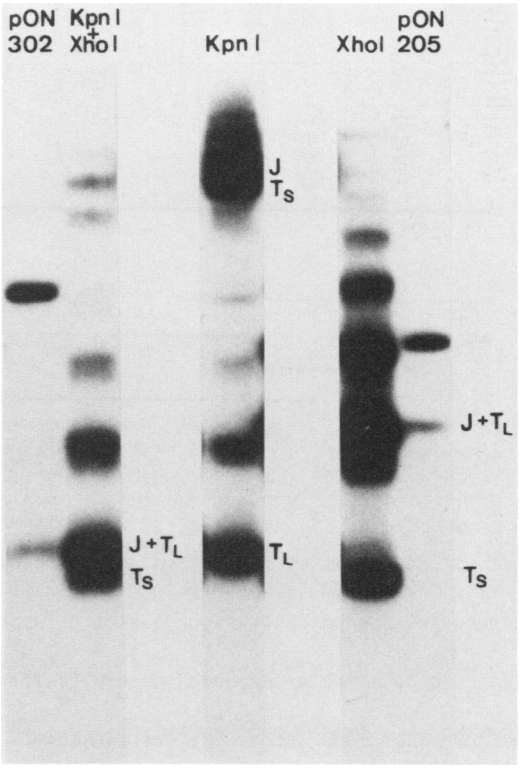
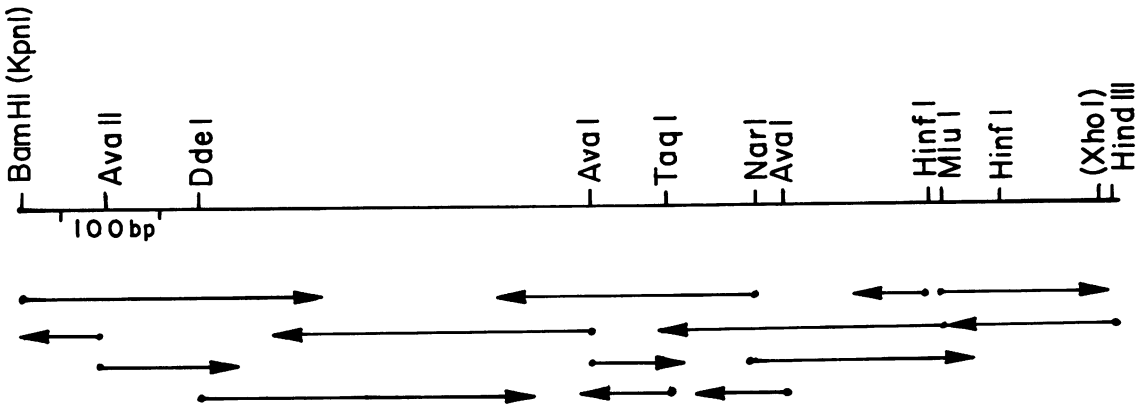


FIG. 2. Hybridization analysis of the CMV (Towne) L-S junction and termini demonstrating the authenticity of the plasmid-cloned L-S junction fragments and approximate boundaries of the CMV *a* sequence. CMV DNA was digested with *Kpn*I/*Xho*I (left lanes), *Kpn*I (middle lane), or *Xho*I (right lanes), subjected to electrophoresis in parallel with the *Kpn*I/*Hin*dIII CMV DNA fragment from pON302 and the *Bam*HI/*Hin*dIII fragment from pON205 in a 0.8% agarose gel, and transferred to nitrocellulose as described in the text. After transfer, the immobilized DNA was hybridized to a ^{32}P-labeled pON208 probe. The pON208 probe contained an L-S junction-spanning fragment because it hybridized to both L- and S-terminal fragments in addition to L-S junction fragments in *Bam*HI digests of CMV DNA (not shown). We used hybridization to identify L-S junction and terminal fragments in *Kpn*I/*Xho*I, *Kpn*I, and *Xho*I digests of CMV DNA. This analysis showed that sequences cloned into pON302 and pON205 were authentic copies of the smallest L-S junction-spanning *Kpn*I/*Xho*I and *Xho*I fragments. In addition, both *Kpn*I and *Xho*I cut outside the region, giving rise to 750-bp ladders, because this heterogeneity was preserved in all digests. The sizes of the smallest S terminal (T_S), L terminal (T_L), and junction (J) fragments were consistent with maps shown in Fig. 1.

Notably, the CMV sequence contains a short stretch of homology to the HSV-1 and HSV-2 *a* sequences (Fig. 5). The significance of this homology stems from the observation that this is also a region of high homology between HSV-1 and HSV-2, whose *a* sequences otherwise show little sequence homology (3, 19) but which nonetheless can be functionally interchanged (32a) as cleavage/packaging signals. We did not observe any direct repeats analogous or

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 KpnI                                                50                                                  100
GGTACCGGCG CGCCCAAAAA GCGCCGTGGA GACACCCGTA CAGAGATCCC TCAGCGCGAT GACGCCCCGC AAACCTCACG CAGTCCGGTC CCGCGAACAG

                                                    150                    DdeI                         200
ATACCGTGGG ACGACACGCA CCGGTAGTGC GCACAAAGGC AGCCGCGCTT ACGGGCCTCA AAGTTCCCTC AGCCCCGTCC CGCGCCGCGC TCGGGTTGGG

                                          DR2  250          DR3          DR3          DR3   300
TGTGCCGGGC GCGCGGCTGG GTGGGTGCGT GCGTGCCGGG TGTGTCGCGG GCGTCTGTCG GGTGTGTCGG TCGGGTGTGT CGGTCGGGTG TGTCGGTCGC

 DR3          DR4          DR3          DR2  350                                  DR3          400
GTGTGTCGGT CGGGTGTGCC GCGGGTGTGT CGGTCGGGTG TGTCGCGGGC GTGTGTCGGG CGCGTGGCGG GTGTGTCGCG GGTGTGTCGG TCGGGTGTGC

 DR4                                                450                    AvaI                         500
CGCGGGTGTG TCAGGGGTGT GTCAGCGGTG TGCGCGGCTT CGGGGTGCGT GTCGGCGGCC GGAGGGAACA ACAAGTGTGC CCCGGGGCCC GCGAGCCCCC

                                                    550                                                 600
CCCCCCTCCC CTCGGCCGGA CGCCTCTTCT GCGTGTGTCC TCGACGCGGG TCGCGCCGTA CTTTGCGGCC GTTGCTTCCC CCGCGGTCCC CAGGGTCGCG

 NarI          DR5                          AvaI  650    DR5                                            700
CGGCGCCGCG CGCTTCCTCT TTTCCGCGCG CGGCGTCCCC CCGGGGACTT CCTCTTTTCC GCGTCGTTTC CGCGTCGCTG GCCCCTGCGA GGCGTTCTTC

                                                    750            HinfI                MluI         800
GTGTGTCCCC GGGGACCCGC GCTGCCGTCG TCCCCTAGGG ACTTCCTCCG TTCCCCGGGG AATCAAACAG ACACAGACAC GCGTCTTCTT TTCGCCGTGC

                                                    850   HinfI    HinfI          HSV HOMOLOGY        900
GCGCCGCACG CCGCTTTTAT GCGCCGCCGC CGTCCCAACC GCACCGCAAC GCGACTCCAA GACTCCAAAT TTCACCCCCC CGCTAAAAAC ACCCCCCCGC

                                                    950            XhoI
CCCACGGGGA CCCAACACAC GGCCCGGAAT GGATGTCAGG CGTCCACCTA GATGACAGCG CTCGAG
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FIG. 3. Nucleotide sequence analysis of the *KpnI/XhoI* junction-spanning fragment. (Top) Restriction map of the L-S junction region indicating restriction sites used for nucleotide sequence determination. Arrows below the map indicate the radiolabeled sites and direction of sequencing. (Bottom) Nucleotide sequence of the 966-bp *KpnI-XhoI* L-S junction-spanning fragment. This segment has a base composition of 71% G+C and 29% A+T. Direct repeats (DR) and the region of sequence homology with HSV-1 and HSV-2 *a* sequences are identified.

homologous to DR1 of HSV (19), a 20-bp repeat that flanks copies of the HSV *a* sequence. Therefore, it appears that this structural characteristic of HSV *a* sequences is not preserved in the L-S junction of the CMV (Towne) genome.

To establish whether the CMV genome contains an *a* sequence structurally analogous to that of HSV, we determined whether sequences within the L-S junction were repeated at both genomic termini. As described above (Fig. 1 and 2), there is a 750-bp region of overlap where restriction fragments from the genomic termini are aligned with those from the smallest L-S junction. We selected a restriction fragment within the region of overlap between the direct repeats and the short stretch of sequence homology to HSV *a* sequences. By analogy with HSV, this would be entirely within an *a* sequence. This *MluI-HinfI* fragment (nucleotides 778 to 853 in Fig. 3) was radiolabeled and used to probe *XhoI*, *KpnI*, and *XhoI/KpnI* digests of CMV DNA (data not shown). A pattern identical to that shown in Fig. 2 was obtained, establishing that the region of overlap contained a sequence present at the L and S termini, as well as at the L-S junction: an *a* sequence.

The CMV L-S junction provides a *cis* recognition signal for HSV-1 cleavage/packaging functions. Because of the short stretch of sequence homology with HSV *a* sequences described above and the previous experiments demonstrating cross complementation between HSV-1 and HSV-2 *a* sequences (32a), we tested the ability of the CMV L-S junction fragment to function as a *cis* recognition signal for cleavage and packaging. We employed the HSV amplicon shuttle vector system described by Spaete and Frenkel (32). Briefly, plasmid clones containing both an HSV origin of replication and a cleavage/packaging function can be propagated as an HSV defective genome in the presence of helper virus. The ability of such a construct to be propagated in virus stocks is dependent on the presence of both functions. Removal of either the origin of replication or the cleavage/packaging site

198 GGGTGTGCC GGGGGCGCGGCTGGGT
GGGTGCGTG CGTGCC
GGGTGTGTC GC
GGGCGTGTG TC
GGGTGTGTC GGTC
GGGTGTGTC GGTC
GGGTGTGTC GGTC
GGGTGTGTC GGTC
GGGTGTGCC GC
GGGTGTGTC GGTC
GGGTGTGTC GC
GGGCGTGTG TC
GGGCGCGTG GC
GGGTGTGTC GC
GGGTGTGTC GGTC
GGGTGTGCC GC
GGGTGTGTC AG
GGGTGTGTC AGCGGTGTGCGCGGCTTCG
GGGTGCGTG TCGGCGGCCGGA 463

FIG. 4. Nucleotide sequence from within the *a* sequence illustrating the nested set of degenerate repeats starting at bp 198 and extending to bp 463. This segment is 75% G+C, 55% guanine, 20% cytosine, 24% thymine, and 1% adenine.

destroys the replication and propagation abilities of the defective genome (32a).

Our approach was to replace the HSV cleavage/packaging function with the CMV L-S junction fragment to test whether this sequence could provide the requisite cleavage/packaging function. The construct used, depicted in Fig. 1, was designated pON302. This chimeric plasmid carried an HSV-1 origin of replication, ori_L (32a), along with the CMV L-S junction. Vero cells were cotransfected with pON302 and intact HSV-1 (*ts*HA1) DNA to provide *trans*-acting helper functions. The resulting virus stocks were serially passaged three times on HEp-2 cells to test for propagation of defective genomes. Infected cell DNA was prepared from passages 2 and 3, digested with *Hin*dIII, electrophoresed in parallel with plasmid pON302 marker DNA, transferred to nitrocellulose, and probed with ^{32}P-labeled pUC9 DNA. This probe lacks homology with either HSV or CMV sequences. Figure 6 shows the hybridization results. The hybridization demonstrated that pON302 becomes incorporated in defective virus genomes capable of propagation within virus stocks. These stocks were referred to as HP2-302 in conformity with the previously established convention (32). The predominant fragment detected when HP2-302 DNA was digested with *Hin*dIII, *Eco*RI, or *Bam*HI, each of which cleave once in pON302, comigrated with the input linearized plasmid marker.

We have previously shown (33) that genome-length concatemers with homology to pUC9 were present in HP2-302

CMV(Towne) 897CGGGGGGGTGTTTTTAGCGGGGGGGT872

CMV(AD169) 23 26CGGGGGGGTGTTTTTAGCGGGGGGGT23 01

HSV-1(F) 23 7CCGGGGGGGTGTGTTTCGGGGGGGGCC263

HSV-2(HG52) 99CGGGGGGGGTGTTTTTTGGGGGGGGGC127

FIG. 5. Sequence homology between human herpesvirus *a* sequences. (Line 1) The region labeled HSV homology in the sequence shown in Fig. 3; (line 2) the comparable region from the CMV (AD169) L-S junction (37); (line 3) the homologous sequence from within the HSV-1 *a* sequence (19); and (line 4) the homologous sequence from within the HSV-2 *a* sequence (3). The superscript numbers refer to the position of the homology within each sequence.

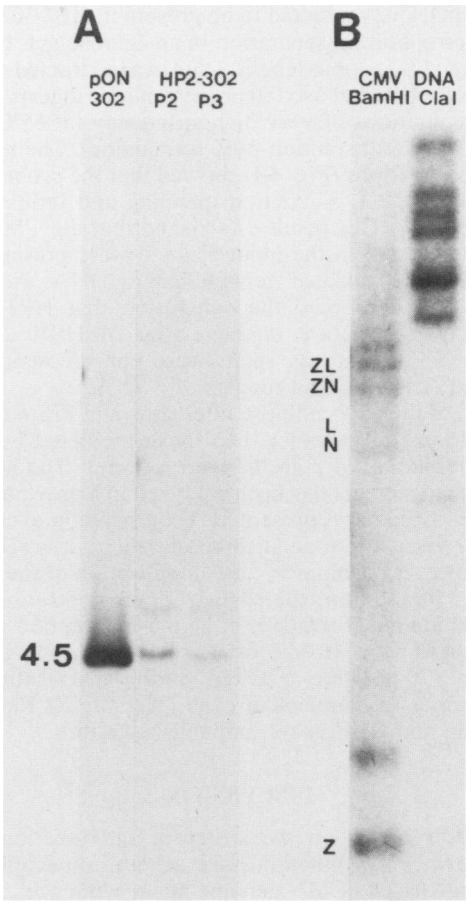

FIG. 6. DNA hybridization analysis of serially passaged HP2-302-derived virus stocks. DNA was prepared from cells infected with three serial passages of virus stocks generated by cotransfection of standard HSV-1 DNA along with pON302. (A) Propagation of pON302 in HSV-1 virus stocks. pON302 was cotransfected into Vero cells with standard HSV-1 (*ts*HA1) DNA, and the resultant virus stocks were serially passaged three times in HEp-2 cells. Vero cells were infected with the progeny of passage 2 (P2) and passage 3 (P3), total infected-cell DNA was prepared, and a portion was digested with *Hin*dIII, electrophoresed in parallel with *Hin*dIII-digested pON302 DNA, transferred to nitrocellulose, and hybridized with ^{32}P-labeled pUC9 DNA. Repair of sequences at the HSV ori_L in HP2-302 DNA accounts for the slightly slower migration relative to pON302. (B) The CMV *a* sequence is present in genome-length defective molecules. Infected cell DNA at passage 1 was digested with *Bgl*II, *Sac*I, *Sal*I, and *Xho*I (enzymes which do not cut in pON302), and uncut viral-legnth DNA was extracted from an agarose gel after electrophoresis, radiolabeled, and used to probe CMV DNA digests. The *Bam*HI and *Cla*I fragments showing homology to the probe are the junction and terminal fragments. A *Bam*HI restriction map of CMV (Towne) DNA, provided by R. L. LaFemina and G. S. Hayward (personal communcation), allowed the assignment of junction and terminal fragments. *Cla*I cuts outside of the inverted repeats of the L and S components (R. R. Spaete and E. S. Mocarski, unpublished data), giving rise to four junction and four terminal fragments.

stocks. The presence of the CMV L-S junction fragment within these repeat units was shown by the following experiment. Infected cell DNA (passage 1) was digested with *Bgl*II, *Sac*I, *Sal*I, and *Xho*I. The combined action of the four restriction enzymes reduced helper virus DNA to at least 100 fragments but did not cut within genome-length concat-

emers of pON302 predicted to be present in HP2-302 stocks. After electrophoretic separation in an agarose gel, high-molecular-weight (genome-length) DNA was extracted from the gel, radiolabeled, and used to probe genomic digests of CMV DNA at conditions of very high stringency (at 65°C, in 6× SSC–1× Denhardt solution–30% formamide). The results of these hybridizations (Fig. 6B) showed that the probe hybridized with all the L-S junction-spanning and terminal fragments of CMV. This result established that the CMV DNA sequences present in the input plasmid were present in the replicated and packaged genome-length DNA. Additional analyses (33) reinforced the conclusion that HP2-302 genomes had a structure characteristic of HSV defective genomes (5, 14) and, as such, were concatemeric DNAs comprised of head-to-tail repeats of pON302.

Data from the nitrocellulose filter shown in Fig. 6 revealed that species of DNA other than those predicted by simple concatemerization of pON302 were present. The nature of these rearrangements has been analyzed in a previous report (33) and most likely represented recombination events with the helper virus DNA, acquisition of helper virus sequences by defective viral genomes, and amplification of the CMV *a* sequence. In addition, the slightly slower migration of the amplicon monomers relative to the plasmid marker was due to deletions in the HSV-1 ori_L occurring during bacterial propagation that were repaired during propagation with HSV-1 helper in mammalian cells (32, 32a; R. R. Spaete, B. J. Lum, and N. Frenkel, unpublished data).

DISCUSSION

Our observations on the structure and function of the CMV (Towne) L-S junction have several implications and predictions. (i) The CMV genome has *a* sequences. (ii) The *cis* element for cleavage/packaging has been preserved over evolutionary time between widely divergent herpesviruses. (iii) The cleavage/packaging of progeny DNA probably follows a common mechanism in CMV and HSV. (iv) Other *cis*- and *trans*-acting functions with the capability of cross complementation are likely to be present in the HSV and CMV genomes.

The function of the CMV *a* sequence as a cleavage/packaging signal for HSV suggests that the signal has not undergone substantial evolutionary drift. Although these two viruses have diverged significantly in both sequence homology and biological characteristics, a comparison of nucleotide sequence revealed a short stretch of homology between the CMV (Towne) L-S junction and HSV *a* sequences (Fig. 5). Tamashiro et al. (37) have identified a similar region of homology with HSV *a* sequences in the CMV (AD169) L-S junction region. We speculate that this sequence, since it is the only region of homology, is responsible for the functional complementation we have observed. It is the only sequence which can be implicated on the basis of sequence comparison and is immediately adjacent to the site of cleavage of concatemers within HSV *a* sequences (21). In the otherwise divergent HSV-1 and HSV-2 *a* sequences (3, 19), this short homology is retained, consistent with the observations that an HSV-1 *a* sequence is recognized in *trans* by HSV-2 functions in both cleavage/packaging (32a) and inversion (20). In HSV-1 the actual cleavage event takes place within DR1 between two tandem copies of an *a* sequence (21), 33 bp from the region of homology. There is no sequence homology between DR1 of HSV-1 and HSV-2 (3, 19) and no sequence homology between these DR1 sequences and any region of the CMV L-S junction. Although speculation based on sequence comparisons directs attention to the sequences shown in Fig. 5, precise assignment of this or any other sequence as the recognition sequence for cleavage/packaging will require mutagenesis studies.

The CMV (Towne) L-S junction lacks a direct repeat structurally analogous to DR1 which brackets an *a* sequence in HSV. The significance of this feature with respect to the function of the *a* sequence is not apparent. Even in HSV, *a* sequences that do not contain two complete copies of DR1 are completely functional in inversion and cleavage/packaging (21). In addition, naturally occurring defective genomes studied in detail have *a* sequences which lack two complete copies of DR1, yet they maintain the cleavage/packaging function (E. S. Mocarski, L. P. Deiss, and N. Frenkel, J. Virol., in press). Therefore, it is unclear what role the flanking copies of DR1 play in *a* sequence function. The lack of analogous structures in the CMV (Towne) L-S junction further reduces the likelihood of a functional requirement for two copies of DR1. Our recent studies, (R. R. Spaete, A. C. Liu, and E. S. Mocarski, unpublished) on the genomic termini of CMV confirm the authenticity of the L-S junction fragment and support our observations reported here that the domain of the CMV (Towne) *a* sequence is within the 966-bp *KpnI*/*XhoI* L-S junction fragment.

Our data show that the CMV (Towne) genome contains an *a* sequence, that is, a sequence shared by the L-S junction and both genomic termini. The *cis* signals carried by the CMV *a* sequence may be similar to those of HSV-1 and include circularization of the viral genome after infection, inversion of the L and S components, amplification to yield tandem copies, and cleavage and packaging of progeny DNA into virions. We suggest that only one activity is different in CMV compared with HSV. Amplification of *a* sequences occurs at the L-S junction and L terminus in HSV (15, 19, 21, 44), whereas in CMV it occurs at both termini as well as the L-S junction (7, 13, 34). The mechanistic differences that confer this distribution of CMV *a* sequences remain to be determined.

Finally, in HSV-1, the maturation of unit-length DNA molecules packaged into capsids requires the *a* sequence as a *cis* element as well as other, less well-defined *trans*-acting elements (24). The process may involve initiation of packaging at an *a* sequence, measurement of a unit-length molecule, and cleavage at an *a* sequence once a specific amount of DNA has been packaged (5, 21). In the standard HSV-1 genome (21), as well as in defective genomes (L. P. Deiss and N. Frenkel, personal communication), at least one *a* sequence is present at both ends of the unit-length molecule. The presence of at least one *a* sequence at each terminus of mature viral genomes while nascent concatemers have predominantly single *a* junctions (15, 19, 21, 44) suggests that additional copies of *a* are generated during the cleavage/packaging process (L. P. Deiss and N. Frenkel, personal communication). Thus, in our present work, *a* sequence amplification would have occurred in the CMV *a* sequence carried in HP2-302 virus stocks. Some of the larger species seen on nitrocellulose blots (Fig. 6; reference 33) may have resulted from this amplification.

Our demonstration that *trans*-acting functions of HSV recognized a *cis* signal from the CMV genome suggests that the search for others might be fruitful. With the difficulty in studying CMV genome functions because of its slow growth, narrow host range, and lack of useful mutants, cross complementation may provide functional information that would otherwise be very difficult to obtain.

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