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Nucleotide Sequence and Structural Features of a Novel U_S-*a* Junction Present in a Defective Herpes Simplex Virus Genome

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Defective genomes generated during serial propagation of herpes simplex virus type 1 (Justin) consist of tandem reiterations of sequences that are colinear with a portion of the S component of the standard viral genome. We determined the structure of the novel U_S-*a* junction, at which the U_S sequences of one repeat unit join the *a* sequences of the adjacent repeat unit. Comparison of the nucleotide sequence at this junction with the nucleotide sequence of the corresponding U_S region of the standard virus genome indicated that the defective genome repeat unit arose by a single recombinational event between an L-S junction *a* sequence and the U_S region. The recombinational process might have been mediated by limited sequence homology. The sequences retained within the U_S-*a* junction further define the signal for cleavage and packaging of viral DNA.

The herpes simplex virus (HSV) DNA genome is composed of two components, L and S, each consisting of unique sequences (U_L and U_S) bracketed by inverted repeats in the sequence arrangement ab-U_L-b'a'c'-U_S-ca. The L and S components invert relative to each other, generating four equimolar isomeric forms of standard virus DNA (reviewed in reference 23). The inversions appear to involve site-specific recombination between inverted copies of the *a* sequence (17–20, 24).

Previous analyses of serially passaged stocks of HSV type 1 (HSV-1) (Justin) revealed the presence of defective genomes composed of head-to-tail reiterations of simple subsets of the standard viral DNA sequences (5, 7, 8, 13). Three families of repeat units (see Fig. 1) differing in size were recognized in these defective viral DNA preparations (13). All three types of repeat units contained contiguous sets of sequences corresponding to the DNA sequences that constitute the right terminus of the viral genome (when displayed in the prototype orientation). These sequences included the entire *ca* inverted repeat as well as various amounts of the adjacent U_S sequences. The three families of repeat units had a common structure in which the *a* sequence was linked to a region in U_S through a novel U_S-*a* junction. Defective virus genomes with a similar general structure (class I defective genomes) have been characterized in stocks of other HSV-1 and HSV-2 strains after undiluted propagation in culture (reviewed in reference 5).

In the presence of helper virus DNA, monomeric repeat units of defective genomes as well as constructs derived by cloning of the corresponding standard virus DNA fragments can be propagated in virus stocks as concatemeric defective virus genomes (1, 25, 27, 32). Two *cis*-acting functions were found to be essential for this propagation, a DNA replication origin and a cleavage-packaging signal (5, 6, 25a, 28, 29, 32, 33). The HSV-1 (Justin) defective genomes were shown to contain the replication origin 1 (ori-1 or Ori_S) located in the *c* inverted repeat sequence (19, 27, 28, 32). The cleavage-packaging signal in the Justin defective genome repeats recently has been shown to reside within the U_S-*a* sequences constituting the junctions between adjacent repeat units (6; L. P. Deiss and N. Frenkel, manuscript in preparation).

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Earlier studies on the structure of the *a* sequence in standard HSV-1 (F) DNA established that the L-S junction (with the structure *bac*) had an *a* sequence in the arrangement DR1-U_b-$(DR2)_{19}$-$(DR4)_3$-U_c-DR1 (18, 20). The DR1 (20 base pairs [bp]), DR2 (12 bp), and DR4 (37 bp) elements corresponded to stretches of directly repeated sequences, whereas U_b (64 bp) and U_c (58 bp) corresponded to unique sequences located on the *b* and *c* sides within the *a* sequence, respectively (see Fig. 3).

From analysis (20) of the termini of HSV-1 (F) DNA, it appeared that the 20-bp DR1 element contained the target sequence for the cleavage of circular or concatemeric DNA. Thus, genomic termini could be generated by a single base-pair-staggered cleavage between nucleotides 18 and 19 of DR1 (see Fig. 4), leaving 1½ bp of the DR1 sequence (with a 3' single base extension) at the S terminus and the remaining 18½ bp of the DR1 sequence at the L terminus of the standard viral genome (20).

We present here the detailed structure of the *a* sequence present in a cloned HSV-1 (Justin) defective genome repeat unit. This work further defines the structure of a functional cleavage-packaging signal and the nature of the recombinational event leading to the formation of the defective virus genomes.

MATERIALS AND METHODS

Cells and virus. The derivation of the HSV-1 (Justin) series by serial undiluted virus propagation in HEp-2 cells has been described previously (7).

Plasmid cloning and nucleotide sequencing. The 8.6-kilobase (kb) repeat units of the HSV-1 (Justin) defective genomes were purified from an agarose gel after electrophoresis of *Eco*RI-digested, passage-15, HSV-1 (Justin) DNA. The *Eco*RI-cleaved monomers (see Fig. 1) were cloned into the *Eco*RI site of pACYC184 (2), yielding the Justin defective (JD) cloned plasmid pJD101. The 8.5-kb *Hin*dIII-*Eco*RI fragment from standard HSV-1 (Justin) DNA spanning coordinates 0.910 to 0.965 was inserted between the *Hin*dIII and *Eco*RI sites of pBR322 (see Fig. 1), yielding the plasmid pJ1. A 370-bp *Sal*I fragment spanning the region of U_S involved in the generation of the defective genome was subcloned from pJ1 into the *Sal*I site of pUC9 (31). This clone was designated pON101. All cloning was performed by

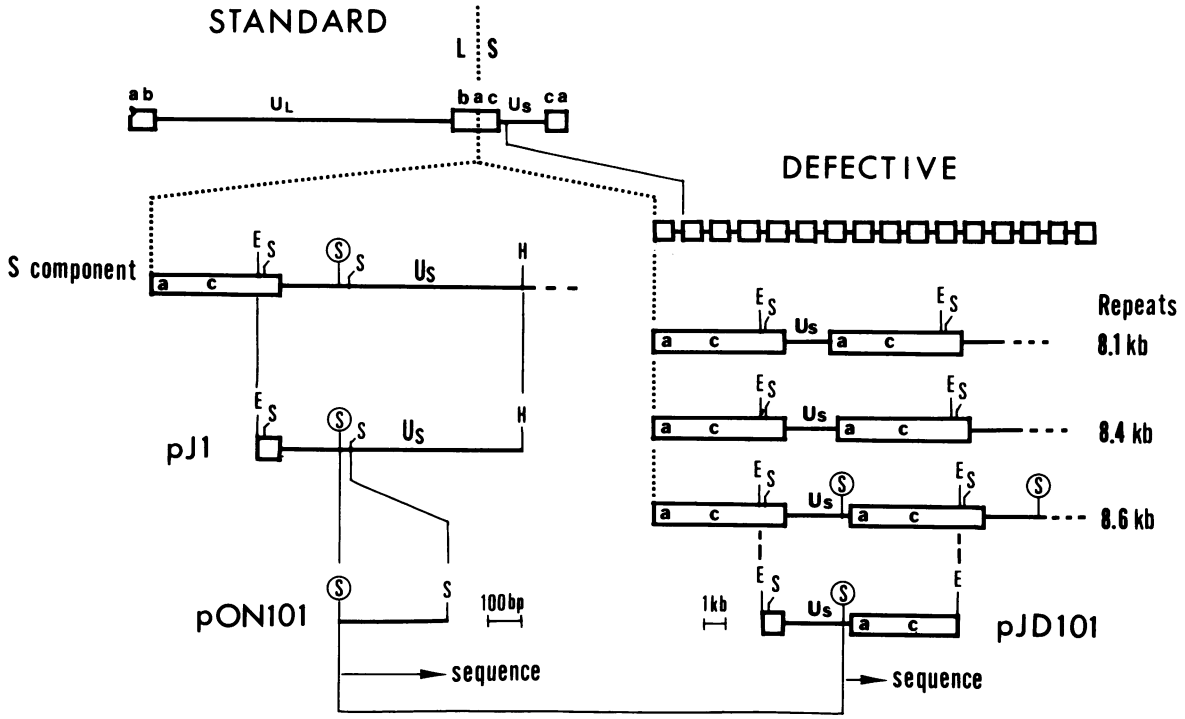

FIG. 1. Structure and origin of the defective HSV-1 (Justin) genomes and the derivation of the standard and defective virus clones. Left, the pJ1 and pON101 clones were derived by cloning segments of the S component as described in the text. Right, the concatemeric structure of the defective virus DNA molecules. Repeat unit sequences correspond to the standard virus DNA sequences from the right side of the S component when displayed in the P or I_L arrangements or the left side of S when displayed in the I_S or I_{SL} orientations (as shown in this figure). Three major size families of repeat units (8.1, 8.4, and 8.6 kb) differing in the amount of U_S sequences have been described previously (13). pJD101 was cloned as described in the text. S, *Sal*I; E, *Eco*RI; H, *Hin*dIII. The circled S denotes the *Sal*I site serving as the reference point for nucleotide sequencing. The arrows point the 5'-to-3' direction of the sequences shown in Fig. 2.

procedures described previously (21, 25). The nucleotide sequence of ^{32}P-end-labeled DNA fragments was determined by the procedures of Maxam and Gilbert (16). The sequence of relevant regions of the plasmid pJD101 was determined starting from the *Sal*I site in U_S and the *Hin*fI site in the *c* sequence (18, 20). The sequence of the plasmid pON101 was determined from the *Bam*HI and *Hin*dIII sites adjacent to the insert in the polylinker of pUC9 (31).

RESULTS

The present study was undertaken to determine the nucleotide sequence of a functional cleavage-packaging signal within defective HSV-1 DNA and to define the structural organization of the novel U_S-*a* junction found between adjacent repeat units of the defective virus genomes. We expected that the analysis of the structure of this novel junction would elucidate both the nature of recombinational events which led to the formation of the defective HSV genome and the structure of the cleavage-packaging signal. By restriction enzyme analyses, we have previously (13) distinguished three major size families of repeat units in the HSV-1 (Justin) defective genomes (8.1, 8.4, and 8.6 kb; Fig. 1). In that study we suggested that the three classes of repeat units represented the products of recombinational events linking the *a* sequence to different subsets of the U_S sequences. Subsequent replication (most likely by a rolling circle mechanism) of the three types of repeat units yielded the concatemeric defective genomes. As summarized in Fig. 1, the two smaller classes of repeat units present in the HSV-1 (Justin) defective genome did not contain the *Sal*I site located at position 0.944 of standard virus DNA, whereas the largest type of repeat units included this site.

To study the arrangement of sequences at the novel U_S-*a* junction, we digested the Justin defective genomes with *Eco*RI (this enzyme cleaves once within each repeat) and cloned the resultant monomeric repeat units into a plasmid vector. The clone pJD101 carried the largest type of repeat units (8.6 kb), as defined by the presence of the *Sal*I site adjacent to the U_S-*a* junction (Fig. 1). In addition, a 370-bp *Sal*I fragment containing the relevant U_S sequences of standard virus DNA was cloned into pUC9 plasmid to generate the clone pON101. The nucleotide sequence for the corresponding cloned defective and standard virus DNA was determined by using the common *Sal*I site (Fig. 1) as a reference point. All sequences were determined for both DNA strands by the procedures of Maxam and Gilbert (16).

Figure 2 displays the nucleotide sequences of pJD101 and pON101 aligned from the reference *Sal*I site, which occupies position 0.944 on the standard virus DNA. Figure 3 shows the *a* sequence portion of pJD101 displayed alongside the previously determined nucleotide sequence of the *a* region of standard HSV-1 (F) DNA (18, 20). The results of the nucleotide sequence analyses may be summarized as follows. (i) The U_S sequences in the pJD101 defective genome were homologous to the corresponding U_S sequences of standard virus for 174 bp from the reference *Sal*I site. (ii) The remaining sequences from nucleotide 175 to the end of the sequenced region corresponded to an *a* sequence resembling the *a* sequence of HSV-1 (F). (iii) The point of transition between the U_S and the *a* sequence is denoted by an asterisk in Fig. 2 and is displayed in greater detail in Fig.

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          SalI
pJD101    GTCGACGCCT TAATACCGAC TGTTGGCGGC CCATGCGTAC GAGGAAGTCG 50
pON101    GTCGACGCCT TAATACCGAC TGTTGGCGGC CCATGCGTAC GAGGAAGTCG

pJD101    TTGGCCGCCT CGTCTTCGCT TTCCGAGTAG TAGGCTTCGG CCGAAACTGG 100
pON101    TTGGCCGCCT CGTCTTCGCT TTCCGAGTAG TAGGCTTCGG CCGAAACTGG

pJD101    CGAGGCCGTG GGATAAAGCG GCACGGACAT GTCGGATCGC GCTGAGGAGT 150
pON101    CGAGGCCGTG GGATAAAGCG GCACGGACAT GTCGGATCGC GCTGAGGAGT

                                  *
pJD101    TGGGATCGGA GAGCCGGGAC GTCACTGCCG CCGCCGCTTT AAAGGGCCGC 200
pON101    TGGGATCGGA GAGCCGGGAC GTCATCGAGG CCGGAAGAAA GCTCCGGGTG

pJD101    GCGCGACCCC CGGGGGGTGT GTTTCGGGGG GGGCCCGTTT TTGGGGTCTG 250
pON101    GGAAGTTGCG GTCGCAGTGA CCTCACGATT TTTAATTTGC TGCGGCTAGG

pJD101    GCCGCTCCTC CCCCGCTCCT CCCCGTCTGT GGGTGGGGCT CCTCCCCGTC 300
pON101    CGGACCACCG GCCCTTTATG CGCCTCGGGC AATTGACGTC ACATACCACG

pJD101    TGTGGGTGGG GCTCCTCCCC GCTCCCGCGG CCCCGCCCCC CACGCCCGCC 350
pON101    CAATCCCACA CAGGCGGCCC CCAAGCCGGA AGCCCCCCGG AGCCACCGAG

pJD101    GCGCGCGCGC ACGCCGCCCG GACCGCCGCC CGCCTTTTTT GCGCGCCGCC 400
pON101    CGGCCGAGG....

pJD101    CGCCGCGGGG GGCCCGGGCT GC...c sequence
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FIG. 2. Alignment and nucleotide sequence of the standard and defective HSV-1 (Justin) DNA in the region of the U_S-*a* junction. The two sequences are aligned at the corresponding *Sal*I site at coordinate 0.944 on the standard viral genome. The point of transition from U_S to *a* sequences is marked by an asterisk. The 174 bp of U_S sequences are shown in bold type.

4. The defective genome *a* sequence retained only 4 bp of the DR1 element adjacent to the U_b sequences, and these 4 bp were joined to U_S sequences at a single point, generating the U_S-*a* junction. In contrast, the boundary between *a* and *c* sequences had a complete 20-bp DR1 element that was identical to that found in standard HSV-1 (F) DNA (20). Thus, the novel U_S-*a* junction could be described as the product of a single recombinational event linking the U_S

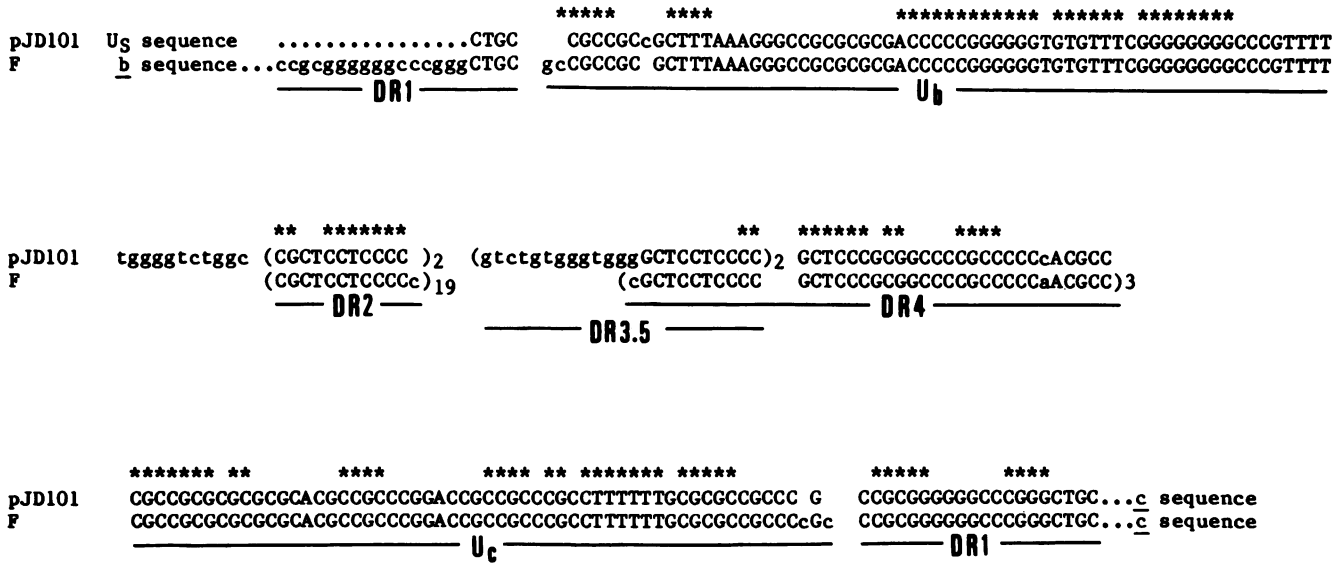

FIG. 3. Alignment of the HSV-1 (Justin) defective genome *a* sequence with that of standard HSV-1 (F) DNA. The nucleotide sequence for the HSV-1 (F) *a* sequence is taken from previous studies by Mocarski and Roizman (18, 20). Nonhomologous sequences are shown in small letters, and homologous sequences are shown in capital letters. The designations for direct repeats (DR) and unique (U) elements are those used previously (18, 20) with the addition of DR3.5, a repeat that is absent from the HSV-1 (F) *a* sequence. Asterisks denote sequences conserved in all HSV-1 and HSV-2 strains sequenced to date that are available, i.e., the HSV-2 strain HG52 (3), the HSV-1 strains F (18, 20), USA-8 and 17 (3), and Justin (this paper).

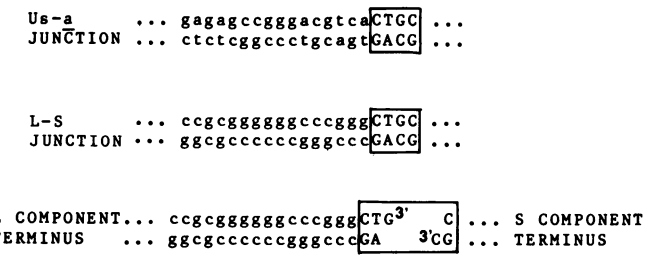

FIG. 4. Comparison of the U_S-*a* junction with the DR1 element at the L-S junction, and the sequences located at the L and S termini of standard HSV-1 (Justin) DNA. Purified virion DNA was end labeled with terminal deoxynucleotidyl transferase and ^{32}P-labeled cordycepin triphosphate. The *Bam*HI terminal fragments were purified after electrophoresis in an agarose gel, digested with *Ava*I, and subjected to electrophoresis on a DNA sequencing gel as previously described for HSV-1 (F) DNA (20). The boxed sequences denote the 4 bp of the DR1 element retained in the U_S-*a* pJD101 junction.

sequence to an *a* sequence, with the crossover point residing within the left (b-side) DR1 element. (iv) Although closely related, the *a* sequences of HSV-1 (Justin) and (F) DNAs differ in several respects. As shown in a previous report (14), the size of the HSV-1 (Justin) *a* sequence was approximately half (264 bp) that of the HSV-1 (F) sequence (501 bp). The presence of only two copies of DR2 in the Justin *a* sequence (compared with 19 copies in the *a* sequence of F) and only a single copy of a portion of DR4 (versus 3 copies in F) accounted for the size differences. In addition, the pJD101 *a* sequence had two copies of a repeat which we termed DR3.5 because it occupied the equivalent position between the DR3 and DR4 elements described for the *a* sequence of F DNA. Although 10 of the 23 bp constituting DR3.5 closely resembled the DR2 repeat, the remaining 13 bp had no counterpart in the HSV-1 (F) *a* sequence. Interestingly, the DR3.5 element was present in six copies within the *a* sequence of the HSV-1 strain USA-8 studied by Davison and Wilkie (3) but was absent from the *a* sequence of the HSV-1 strain 17 (3). The presence of these sequences in the Justin and USA-8 strains might reflect a close relationship between these two strains of HSV-1.

DISCUSSION

The results of the studies presented in this paper address the nature of events leading to the generation of defective HSV genomes and the structure of the *a* sequence in relation to its function in the cleavage-packaging process.

Three points can be made regarding the generation of defective virus genomes. First, it appears that the defective HSV genomes evolved as a consequence of a recombinational process involving an *a* sequence. This conclusion rests on the observation that the point of transition from U_S to *a* sequence in the defective genome repeat unit occurred within the *a* sequence itself.

Second, our studies have revealed that the *a* sequence which became incorporated into the Justin defective genome repeat unit was originally derived from an L-S junction (Fig. 1) rather than from an S end. This conclusion rests on results of studies which were designed to determine the structure of the L and S termini of standard HSV-1 (Justin) DNA. Specifically, these studies (E. S. Mocarski, unpublished data) were done with ^{32}P-labeled cordycepin triphosphate and terminal deoxynucleotidyl transferase to label the termini of standard HSV-1 (Justin) DNA. The labeled end fragments were then analyzed by using protocols similar to those previously used to analyze the termini of standard HSV-1 (F) DNA (20). The deduced structure of the L and S termini is displayed in Fig. 4. The Justin S end, like its F counterpart (20), contained an *a* sequence terminating with 1 bp of the DR1 element and an additional 3′ protruding base, whereas the L end terminated with the remaining 18½ bp of the DR1 sequence. The novel U_S-*a* junction which retained 4 bp of the DR1 sequence could not have been formed by recombination involving the S terminus because that terminus retained only 1½ bp of DR1. Therefore, the original recombinational event leading to the joining of the U_S and *a* sequences within the pJD101 repeat unit most likely involved an *a* sequence situated within an L-S junction.

The final point regarding the evolution of the defective HSV genomes relates to the mechanism of the $U_S \times a$ sequence recombination. The structure of the U_S-*a* junction in the pJD101 defective genome repeat could be most simply explained by a single recombinational event linking the U_S and *a* sequences. It is intriguing, however, that the crossover site occurred close to the cleavage target within the *a* sequence, suggesting that the original repeat unit evolved as an aberrant product of an *a* sequence-mediated activity, most likely cleavage-packaging or inversion. Indeed, as depicted in Table 1 and Fig. 5, the corresponding U_S region in a standard virus DNA contained three types of homologies to the *a* sequence that might have facilitated the $U_S \times a$ recombination. First, several stretches of sequences closely resembling elements of the *a* region could be identified within the U_S region at a relatively distant location (50 to 200 bp) from the U_S-*a* junction point. These partly homologous sequences are listed in Table 1. One or more of these sequences might have played a role in the aberrant recognition of the corresponding U_S region by the cleavage-packaging or the inversion machinery, thereby facilitating subsequent interaction with *a*.

A second type of sequence similarity existed in close proximity to the U_S-*a* junction and included the partly homologous sequences depicted in Fig. 5B. These sequences might have played a role in stabilizing recombinational intermediates, although little homology could be found when the U_S and *a* sequences were aligned precisely at the crossover site (Fig. 5A). Finally, a stretch of U_S sequences, occurring in an inverted polarity 25 to 53 bp away from the U_S-*a* junction site exhibited complementarity to both the U_S and *a* sequences immediately flanking the junction (Fig. 5C). This sequence might have thus served to juxtapose the U_S and *a* sequences before an excision-ligation event. The juxtaposed sequence might have been stabilized by the base-paired stem structures depicted in Fig. 5C. Similar models for recombinations promoted by short sequence homology or by alignment through a third sequence have been previously proposed (see, for example, references 9 and 22).

We cannot predict whether the data and hypotheses presented above will be consistent with the structure of the smaller families of the Justin repeat units or with the structure of defective genomes generated from other HSV strains (4, 5, 10, 11, 15, 25). However, it is noteworthy that from restriction mapping data, many but not all, of these defective genomes appear to have evolved from an *a* sequence "invasion" of internal genomic sequences (11, 15), an event that most likely occurred in the pJD101 Justin repeat. The combined pressure to acquire the DNA replication origin as well as the cleavage-packaging signal and at the same time remain within the size constraints imposed on defective genome repeat units (12) undoubtedly plays a

TABLE 1. U_S sequences distal to the crossover point showing partial homology to sequences in *a*

Region[a]	Location[b]	Strand[c]	Sequence[d]	Homology[e] Exact (%)	Homology[e] Py/Pu (%)
U_S	−46/−61	2	^{129}TGTCCGTGCCGCTTTA^{114}	12/16	14/16
a (U_b)	+2/+17	1	^{176}TGCCGCCGCCGCTTTA^{191}	(75)	(87)
U_S	+96/+82	2	^{268}TAAAGGGCCG - GTG^{256}	12/14	13/14
a (U_b)	+16/+29	1	^{190}TAAAGGGCCGCGCG^{203}	(86)	(93)
U_S	+131/+147	1	^{305}CCCACACAGGCGGCCCC^{321}	13/17	14/17
a (DR4)	+144/+160	1	^{318}CCCGCTCCCGCGGCCCC^{334}	(76)	(82)
U_S	+168/+150	2	^{342}CTCCGGGGGGCTTCCGGCT^{324}	13/19	16/19
a (DR1)	−16/+2	1	CCGCGGGGGGCCCG-GGCT^{226}	(68)	(84)
U_S	+181/+131	2	^{355}GGCCGCTCGGTGGCTCCGGGGGGCTTCCGGCTTGGGGGCCGCCTGTGTGGG^{305}	32/51	36/51
a (U_b)	+21/+71	1	^{195}GGCCGCGCGCGACCCCCGGGGGGTGTGTTTCGGGGGGGGCCCGTTTTTGGG^{245}	(63)	(71)
U_S	+172/+145	2	^{346}GTGGCTCCGGGGGGCTTCCGGCTT-GGGG^{319}	20/29	23/29
a (U_b)	+45/+72	1	^{219}GTGTTTCGGGGGGGG-CCCGTTTTTGGGG^{246}	(69)	(79)

[a] The subregion within *a* containing the sequence shown is given in parentheses.
[b] The numbers refer to the position of the sequence relative to the U_S-*a* crossover point.
[c] Strand 1 has been designated as the strand shown in Fig. 2.
[d] The numbers refer to the position in the sequence shown in Fig. 2.
[e] Exact homology is scored for identical bases; Py/Pu homology is scored for pairs of pyrimidines and purines.

controlling role in the evolution of HSV defective genomes. The predictions of the model above, namely that various types of repeat units of defective virus genomes might contain one or more sequences homologous to *a* sequences, are currently under investigation.

In a recent set of studies (6; Diess and Frenkel, in preparation) we have fused the Justin U_S-*a* junction (derived by subcloning from the pJD101 plasmid) to a functional HSV DNA replication origin. Analyses of the ability of the resultant clone to become propagated into packaged defective genomes in the presence of helper virus DNA clearly showed that the U_S-*a* junction fragment contained a functional cleavage-packaging signal. Several points can therefore be made from the results of the present study regarding the cleavage-packaging process. First, our data extend earlier reports regarding variations in the *a* sequences of different HSV-1 strains. Specifically, the finding of a relatively small number of the DR2 and DR4 elements in the Justin *a* sequence indicated that a high degree of reiteration of these sequences was not essential for any of the known *a* sequence functions, a conclusion consistent with the previous results of Davison and Wilkie (3). In addition, the Justin *a* sequence contained two reiterations of the 23-bp DR3.5 sequence and in that respect resembled the *a* sequence of the HSV-1 strain USA-8 which contained six copies of this sequence (3). These results clearly indicate significant flexibility in the structural organization of the *a* sequence.

An additional point which can be made from our studies relates to the finding that the HSV-1 (Justin) defective genome repeat retained only 4 bp of the DR1 sequence at the U_S-*a* junction. Therefore, the presence of two complete copies of the DR1 sequence, one at each boundary of *a*, was not required for cleavage-packaging. Furthermore, as seen in Fig. 4 and 5, the U_S sequences at the novel junction differed considerably from the DR1 sequences which they replaced. There are currently two possible explanations for these findings. First, the DR1 portion which was retained in the defective genome repeat (4 bp) could contain the essential sequences recognized during the cleavage-packaging process. Alternatively, it is possible that the cleavage-packaging signal does not include any of the DR1 sequences. Rather, the cleavage-packaging process could involve the recognition of sequence elements present at internal location(s) within the *a* sequence, followed by cleavage within sequences located at a constant distance from the recognition signal itself. Although further work is required to test these hypotheses, it is noteworthy that there is a considerable lack of sequence homology between the DR1 element of HSV-2 (strain HG52 [3]) and the DR1 elements of HSV-1 strains (3, 18, 20; this paper). At the same time, results from our additional studies (5, 26) have revealed that HSV-1 *a* sequences (from strains Patton, F, and the Justin defective genomes) could be efficiently recognized for cleavage and packaging by *trans*-acting products specified by an heterologous HSV-2 helper virus. These observations strongly suggest that the DR1 sequence itself does not form the cleavage-packaging signal. Our finding that two complete copies of DR1 were not essential for the cleavage-packaging of the Justin defective genomes lends further support for this hypothesis.

In contrast to the divergence of the DR1 elements, certain internal subsets of the *a* sequence have been conserved in all the HSV-1 and HSV-2 strains sequenced to date. These conserved sequences have been marked with asterisks in Fig. 3. Of these various shared sequences, the ones within the U_b and U_c regions occupy in all HSV strains (including pJD101) a similar position relative to the L and S cleaved ends. Homology to the U_b conserved nucleotide sequences can be found in other herpesviruses as well, including human cytomeglovirus where it resides within the putative *a* sequence (30; R. R. Spaete and E. S. Mocarski, submitted for publication). It is noteworthy that the region of homology between these different herpesvirus *a* sequences is contained within the large hairpin loop shown in Fig. 5C. Therefore, we suggest that these sequences could serve as recognition signals for cleavage-packaging and that the cleavage process itself could involve a measuring function to defined distances away from these signals. The recent finding (26) that the

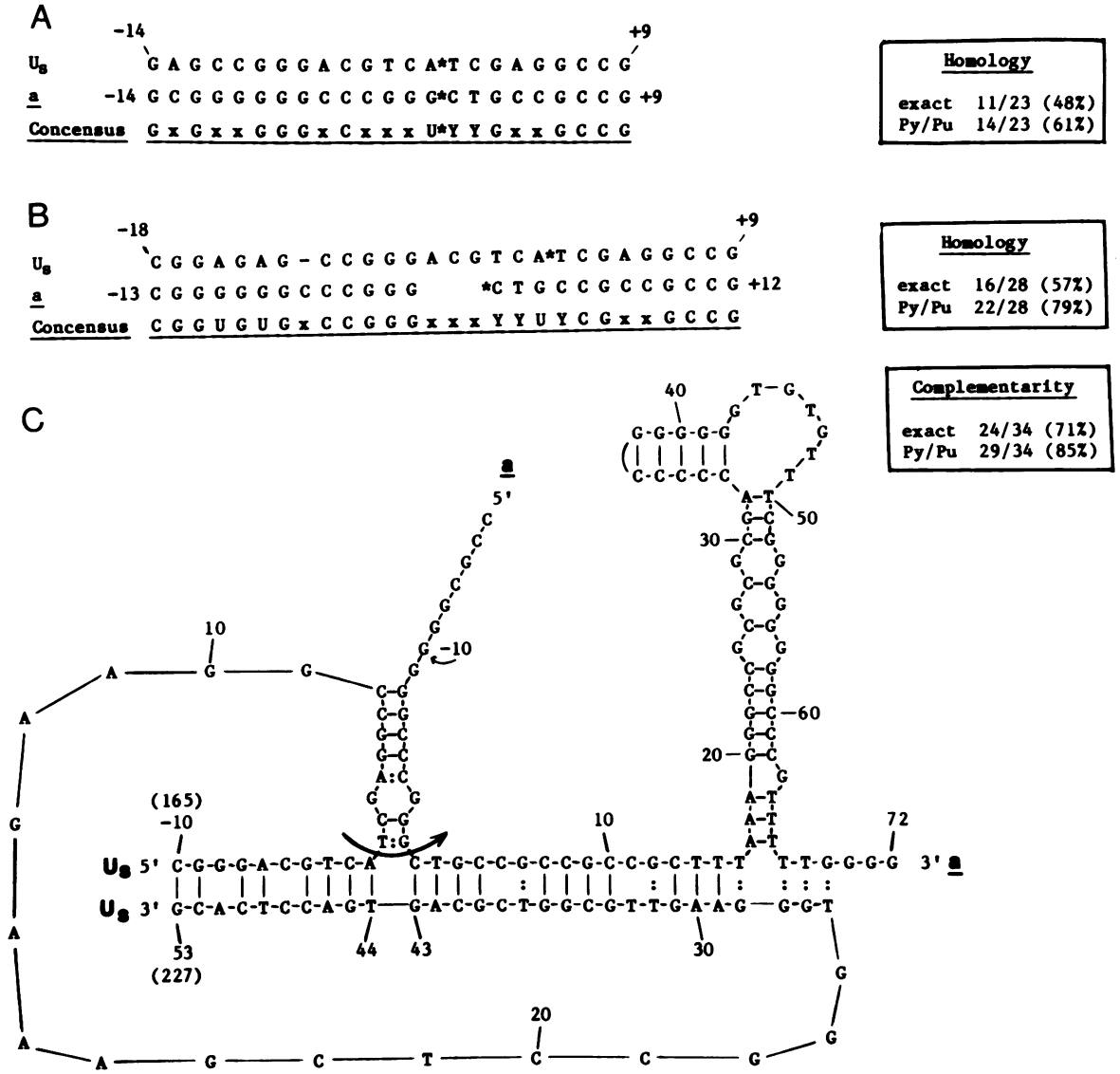

FIG. 5. The alignment of the U_S and *a* sequences surrounding the U_S-*a* crossover point. (A) When aligned precisely at the junction (denoted by asterisk), the U_S and *a* sequences show relatively poor homology. The numbers next to the sequences denote the sequence position relative to the crossover point. The sequence to the left of the asterisk in the U_S sequence and to the right of the asterisk in the *a* sequence are fused in the U_S-*a* junction. Exact homology refers to identical base pairs, whereas Py/Pu denote pyrimidines or purines present at an equivalent position. (B) More extensive homology is observed with the U_S and *a* sequences proximal to the U_S-*a* junction point are slightly misaligned relative to the U_S-*a* junction site to maximize homology. (C) A significantly more stable structure can be formed by juxtaposing the U_S and *a* junction points through their complementarity to a U_S sequence located 26 to 53 bp away from the crossover point. The arrow denotes the proposed recombinational event involving the excision-repair process. The level of complementarity (precise base pairs and Py-Pu pair) was calculated for the horizontal "backbone" sequence (the base-paired region positions 28 to 53 of U_S juxtaposing the U_S and *a* sequences) and the left stem formed by annealing of DR1 with the U_S sequence.

human cytomegalovirus *a* sequence can substitute for an HSV-1 *a* sequence as a cleavage-packaging signal in a constructed HSV-1 defective genome amplicon (25) reinforces this notion. Studies with constructed HSV defective genomes are currently in progress to further investigate the involvement of different portions of the HSV-1 *a* sequence in the cleavage-packaging process.

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

After the acceptance of this paper we learned that S. L. Varmuza and J. R. Smiley (Cell, in press) have independ-

ently shown that cleavage occurs at a constant distance from internal *a* sequences and that DR1 is not required for this process.

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