Journal of Virology

Herpes simplex virus type 1 DNA replication is specifically required for high-frequency homologous recombination between repeated sequences.

R E Dutch, V Bianchi and I R Lehman

Updated information and services can be found at:
http://jvi.asm.org/content/69/5/3084

These include:

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: **http://journals.asm.org/site/misc/reprints.xhtml**
To subscribe to to another ASM Journal go to: **http://journals.asm.org/site/subscriptions/**

Journals.ASM.org

Herpes Simplex Virus Type 1 DNA Replication Is Specifically Required for High-Frequency Homologous Recombination between Repeated Sequences

REBECCA ELLIS DUTCH, VERA BIANCHI,† AND I. R. LEHMAN*

*Department of Biochemistry, Beckman Center, Stanford University
School of Medicine, Stanford, California 94305*

Using an assay for recombination that measures deletion of a β-galactosidase gene positioned between two directly repeated 350-bp sequences in plasmids transiently maintained in COS cells, we have found that replication from a simian virus 40 origin produces a high frequency of nonhomologous recombination. In contrast, plasmids replicating from a herpesvirus origin (ori$_s$) in COS cells superinfected with herpes simplex virus type 1 (HSV-1) show high levels of homologous recombination between the repeats and an enhanced recombinogenicity of the HSV-1 *a* sequence that is not seen during simian virus 40 replication. When the same assay was used to study recombination between 120- to 150-bp repeats in uninfected Vero cells, the level of recombination was extremely low or undetectable (<0.03%), consistent with the fact that these repeats are smaller than the minimal efficient processing sequence for homologous recombination in mammalian cells. Recombination between these short repeats was easily measurable (0.5 to 0.8%) following HSV-1 infection, suggesting that there is an alteration of the recombination machinery. The frequency of recombination between repeats of the U$_c$-DR1 region, previously identified as the only segment of the HSV-1 *a* sequence indispensable for enhanced *a*-sequence recombination, was not significantly higher than that measured for other short sequences.

The linear 152-kb double-stranded DNA genome of herpes simplex virus type 1 (HSV-1) is composed of two unique sequences, U$_L$ and U$_S$, each flanked by inverted repeat regions such that the final structure can be represented as $a_n b$-U$_L$-$b'a_m'c'$-U$_S$-ca (37). During the course of viral infection, the two unique regions invert relative to each other, producing four equimolar isomers of the virus (11, 16, 43). The HSV-1 *a* sequences are very likely required for this inversion event (20, 30, 34, 46). However, the mechanism of inversion through the *a* sequences remains unclear, and both site-specific and homologous recombination models have been invoked (30, 53).

Although the fine structure of the HSV-1 *a* sequence varies from strain to strain, with lengths ranging from 200 to 500 bp, all are approximately 85% G+C and share the same general organization. They contain 20-bp direct repeats at each end (DR1) and two unique segments (U$_b$ and U$_c$) separated by arrays of repeats that always include a set of 12-bp DR2 repeats but can also include other repeats (8, 29, 31, 51, 52). The *a* sequences are especially recombinogenic; recombination between *a* sequences on a plasmid transiently maintained in HSV-1-infected cells occurs at approximately twice the frequency of that seen between any other sequences (12, 13). The novel DNA conformation of the DR2 repeats (57, 58) and their cleavage by a host-encoded endonuclease (56) are not the cause of this recombinogenicity, since addition of endonuclease cleavage sites (13) or deletion of the DR2 repeats (13, 40), which are the sites of endonuclease cleavage, had no effect on high-level *a*-sequence recombination. The *a* sequences also contain the sites for cleavage and packaging of HSV-1 DNA (9, 31), and it has been suggested that this process may lead to

enhanced recombination at the *a* sequences (40). However, by replacing segments of the *a* sequence with DNA fragments of similar length, it was shown that the U$_b$-DR1 region, containing the HSV-1 pac1 signal essential for packaging (10, 52), was not required for the enhanced frequency of recombination observed with the *a* sequences. Instead, only the 95-bp U$_c$-DR1 segment was indispensable for high-level *a*-sequence-mediated recombination (13).

In addition to the *a*-sequence-mediated inversions, HSV-1-infected cells display a high level of homologous recombination that gives rise to the frequent generation of recombinant virus during coinfections with different strains of HSV-1 or with HSV-1 and HSV-2 (3, 4, 17, 41, 55); high-frequency integration of DNA fragments containing HSV-1 sequences into the viral genome (24, 25, 33, 48); and inversion events mediated by repeated copies of the HSV-1 *Bam* L sequence (35), the Tn5 repeats (53), and the HSV-1 *b* sequence (28).

High-level recombination between repeated sequences in HSV-1-infected cells requires replication of the recombining DNA. Mocarski and Roizman (32) demonstrated that recombination between stably integrated inverted repeats of the *a* sequence occurred only in conjunction with HSV-1 infection and subsequent amplification of the region. High-level recombination between repeats contained on plasmids requires both an HSV-1 origin of replication on the plasmid and subsequent HSV-1 infection or transfection with the genes needed for HSV-1 DNA replication (12, 53).

In this report, we show that HSV-1 DNA replication is specifically required for both high-level homologous recombination and the enhanced frequency of recombination conferred by the *a* sequence. Replication from a simian virus 40 (SV40) origin in COS cells leads primarily to nonhomologous recombination between 300-bp repeats, and no enhanced recombinogenicity of the *a* sequence. However, replication from a herpesvirus origin after HSV-1 infection of COS cells leads

to levels of homologous recombination similar to those previously described for HSV-1-infected Vero cells (12, 13). Recombination in Vero cells between small, 120-bp repeats was also examined. Whereas essentially no recombination between repeats of this size could be detected in uninfected cells, consistent with the fact that the repeats are smaller than the minimum efficient processing size for homologous recombination in mammalian cells (2, 26, 38), recombination between these repeats was easily detectable in HSV-1-infected cells. However, the frequency of recombination between repeats containing the U_c-DR1 region of the a sequence is not significantly higher than that seen between two other sets of repeats, indicating that the U_c-DR1 region by itself is not sufficient for high-level recombination between the HSV-1 a sequences.

MATERIALS AND METHODS

Cells and viruses. Vero cells (African green monkey kidney fibroblasts) were obtained from the American Type Culture Collection and were propagated in Dulbecco modified Eagle minimum essential medium supplemented with 10% fetal calf serum, 0.1 mM nonessential amino acids, and 2 mM glutamine. COS cells were grown in Dulbecco modified Eagle minimum essential medium supplemented with 5% fetal calf serum. The HSV-1 strain F(Δ305) (36) was used.

Plasmid construction. All restriction enzymes and linkers used in plasmid construction were obtained from New England Biolabs or the United States Biochemical Corp. Plasmids were prepared by the alkaline lysis procedure and then purified on Qiagen columns according to the manufacturer's instructions.

Plasmids for analysis of recombination in COS cells were constructed by addition of an SV40 origin of replication to the plasmids pRD105 and pRD110 (12). pRD105 and pRD110 contain two selectable markers, the ampicillin resistance gene and the complete β-galactosidase gene; the pUC19 origin of replication, which permits replication in *Escherichia coli*; the *Sma*I fragment of HSV-1 ori$_s$, which allows replication in HSV-1-infected cells; and two direct repeats on opposite sides of the *lacZ* gene. In pRD105, the repeats are copies of the a sequence from HSV-1 strain KOS, and in pRD110, they are copies of a portion of the *dsg* (d-signalling) gene from *Myxococcus xanthus* (6). The plasmids pRD105-6 and pRD110-11 were created by inserting the minimal origin of SV40 replication (ori$_{sv}$) obtained from plasmid S301 (14) as a *Sal*I-*Hin*dIII fragment of 120 bp into the unique *Bsm*I sites in pRD105 and pRD110. These plasmids are therefore able to replicate in COS cells which constitutively express the SV40 large T antigen (15).

Plasmid pRD107 was used as the parent vector for construction of plasmids containing 120- to 150-bp direct repeats. This plasmid is the same as pRD105 and pRD110 described above, except that it lacks the repeats flanking the β-galactosidase gene. Plasmids containing the small repeats were prepared by placing a copy of the desired insert into both the *Bam*HI and *Xba*I sites of pRD107, such that the inserts were in directly repeated orientation relative to each other. For all three constructs, the inserts were prepared by digestion with the appropriate restriction enzymes, addition of *Xba*I linkers, and insertion into the *Xba*I site, followed by addition of *Bam*HI linkers to the *Xba*I fragment and insertion in the *Bam*HI site. Purification of fragments was performed by electrophoresis through a 3.5% Metaphor agarose (FMC Biochemicals) gel and elution from the gel with an Elutrap electrophoresis chamber (Schleicher & Schuell). The insert in U_c-100-2 was a fragment from the a sequence of HSV-1 strain KOS obtained by cleavage of a 250-bp *Bam*HI-*Dra*I fragment from pRD105 (12) with the restriction enzyme *Mnl*I, to give an *Mnl*I-*Bam*HI fragment of 100 bp spanning the U_c-DR1 portion of the a sequence. U_b-100-2 contains two copies of a 95-bp insert formed by digestion of the 317-bp *Bam*HI fragment from pRD105 with *Eae*I, creating a *Bam*HI-*Eae*I fragment with the DR1-U_b portion of the a sequence. The inserts in dsg-100-2 were created by digestion of pRD110 (12) with *Xho*I and *Stu*I to give a 126-bp fragment containing bp 436 to 562 of the *dsg* gene of *M. xanthus* (6).

Transfection, infection, and DNA isolation. Actively growing COS cells at a concentration of 3×10^6 cells per sample were electroporated (7) at 150 V with 1 μg of the desired plasmid. Vero cells at a concentration of approximately 7×10^6 cells per ml were electroporated at 220 V with 20 μg of the desired plasmid. For studies of HSV-1 infection, transfected cells were allowed to recover for approximately 24 h and then infected with 10 PFU of HSV-1 strain F(Δ305) per cell in 3 ml of medium or mock infected with 3 ml of medium. Virus and medium were removed after 1 h, the cells were washed twice with phosphate-buffered saline (Gibco), and the medium was replaced.

DNA was extracted from both Vero and COS cells by the RAPP procedure described previously (12). DNA obtained from COS cells was digested with *Dpn*I for 3 h after isolation to remove the high background of unreplicated DNA.

Transformation, miniprep isolation of DNA, and restriction analysis. Transformation into frozen *E. coli* DH5α cells was performed as described previously (13). Miniprep isolation of plasmid DNA was performed by the modified boiling procedure (39), with 50 mM Tris-Cl (pH 8.0)–62.5 mM EDTA–0.4% Triton

X-100–2.5 M LiCl as the resuspension buffer. DNA either was digested with *Eco*RI or *Dra*I for 2 h and electrophoresed through 1% agarose gels in TAE or was digested with *Bam*HI and *Xba*I and electrophoresed through 3.5% Metaphor agarose gels in TAE.

Assay for recombination frequency. The assay employed to measure recombination frequency has been described previously (12). In brief, plasmids containing directly repeated sequences flanking a *lacZ* gene were introduced into either Vero or COS cells. After the appropriate times, and in some cases after HSV-1 infection, DNA was extracted by the RAPP procedure and then transformed into *E. coli* DH5α. The number of blue and white colonies was used to determine the frequency of *lacZ* deletion. To determine the percentage of white colonies that contained products consistent with deletion between the repeats, the DNA was isolated from a fraction of the white colonies by the miniprep procedure and examined by restriction analysis. Finally, the percentage of recombination between repeats was determined by multiplying the percent white colonies by the fraction that contained a correct deletion.

RESULTS

High-level homologous recombination and enhanced recombinogenicity of the HSV-1 a sequence require HSV-1 infection. To determine whether the high-level homologous recombination between directly repeated sequences specifically requires HSV-1 replication, plasmids were constructed in which the SV40 origin of replication (ori$_{sv}$) was introduced into the unique *Bsm*I site of pRD105, which contains two directly repeated copies of the HSV-1 a sequence, and pRD110, which contains two directly repeated copies of a portion of the *dsg* gene from *M. xanthus*, to create the plasmids pRD105-6 and pRD110-11, respectively. To ascertain that the presence of the 120-bp ori$_{sv}$ did not modify the behavior of the plasmids in the recombination assay (12), the recombination frequency in HSV-1-infected Vero cells was determined for these constructs. The resulting frequencies of 3.3 and 3.4% white colonies for duplicate samples of pRD110-11 and 5.5 and 5.5% white colonies for duplicates of pRD105-6 are in agreement with results of previous experiments using the parental plasmids pRD105 and pRD110 (12, 13). Thus, addition of the SV40 origin does not affect the high-level homologous recombination observed to occur in HSV-1-infected Vero cells, or the enhanced recombinogenicity of the a sequence.

Plasmids pRD105-6 and pRD110-11 were introduced into COS cells by electroporation, and samples were obtained daily for 5 days after transfection. DNA samples from COS cells were treated with the *Dpn*I restriction enzyme to ensure that only replicated DNA was examined. Samples from mock-electroporated cells that had been mixed with plasmid DNA in suspension and then plated in parallel with the electroporated samples were also obtained to measure the background of white-colony formation in *E. coli*. No white colonies were produced by plasmid DNA recovered from the mock-electroporated COS cells (data not shown), indicating that the events measured take place only when plasmids are transfected into the COS cells.

Table 1 shows the frequency of white colonies observed when plasmid replication was initiated from ori$_{sv}$ in COS cells, in two separate experiments. Although the values were somewhat lower in the second experiment, the two plasmids behaved similarly within each experiment. The frequency of white colonies was low on day 1 and then increased steadily over the next 4 days, possibly reflecting accumulation of deleted plasmids in COS cells. The percent white colonies observed by day 5 (approximately 5%) was in the same range as that observed when the parental plasmid, pRD105, replicated in HSV-1-infected cells. However, no difference between the pRD105-6 and pRD110-11 plasmids was apparent, indicating that there was no specificity for the a sequence.

When the DNA from white colonies obtained on each day of the time course experiment was extracted and examined by

TABLE 1. Recombination frequency in COS cells

Plasmid	Replication origin[a]	Day	% White colonies		Total colonies	Avg % white colonies	No. with correct deletion/total no.[b]	Recombination between repeats[c]
			Expt 1	Expt 2				
pRD110-11	SV40	1	0.3	0.2	7,507	0.2	7/21	0.1
		2	2.5	1.4	3,020	2.0	0/33	<0.1
		3	3.4	1.5	3,335	2.4	1/20	0.1
		4	3.3	2.3	2,958	2.8	2/16	0.4
		5	4.5	2.9	4,561	3.7	2/16	0.5
pRD105-6	SV40	1	0.2	0.1	9,514	0.2	3/21	0.1
		2	1.7	0.3	3,347	1.0	3/20	0.2
		3	2.1	1.5	1,680	1.8	0/21	<0.1
		4	3.1	2.3	3,635	2.7	1/20	0.1
		5	5.2	4.2	2,825	4.7	0/16	<0.3
pRD110	HSV-1	1	4.2	—[d]	1,222	4.2	13/18	3.0
pRD105	HSV-1	1	9.3	—	731	9.3	38/42	8.4

[a] All experiments were carried out with COS cells, which express the T antigen needed for replication from an SV40 origin of replication. The SV40 origin was contained on the plasmids pRD110-11 and pRD105-6, which were replicated by the SV40 mode of replication. The plasmids pRD110 and pRD105 do not contain an SV40 origin but do have an HSV-1 origin of replication. Cells were infected with HSV-1 as described in Materials and Methods.

[b] Number of white colonies containing a correct deletion, that is, a plasmid that is the product expected from deletion between the repeats, as judged by restriction digest analysis, divided by the total number of white colonies examined.

[c] Recombination between repeats is the frequency at which homologous recombination occurs between the repeated regions, calculated by multiplying the average percentage of white colonies by the fraction of correct deletions.

[d] —, not done.

restriction analysis, only a small proportion derived from correct recombination between the direct repeats. No consistent product was detected among the other recombinant plasmids, indicating that there was no preferential site of recombination outside the direct repeats (Table 1; Fig. 1, lanes 9 to 16). When the percentage of recombination between repeats was calculated by multiplying the average percent white colonies by the fraction that contain a correct deletion, the frequency of homologous recombination between these repeats was low, ranging from 0.1 to 0.5%.

Transfection of plasmids pRD105-6 and pRD110-11 into COS cells was also performed by the calcium phosphate precipitation procedure (1), which may cause less extensive cell damage than electroporation and reduce the intracellular release of nucleases. The plasmids were extracted 48 h after dimethyl sulfoxide shock and tested for recombination. The frequency of white colonies was similar to that seen in the electroporation experiments: plasmid pRD105-6 gave 4.0% white colonies, and plasmid pRD110-11 gave 4.7%. However, restriction analysis of the DNA from these colonies again demonstrated that the majority resulted from illegitimate recombination (data not shown). Thus, the method of transfection is not responsible for the high level of nonhomologous recombination.

Another possibility was that the transformed phenotype of COS cells could be responsible for the high level of illegitimate recombination. We therefore examined the behavior of pRD105 and pRD110, the parental plasmids lacking ori_{sv}, in COS cells after transfection and superinfection with HSV-1. As shown in Table 1, a high percentage of white colonies was observed with both plasmids. pRD105, which contains the *a*-sequence repeats, gave a twofold higher frequency than did pRD110, containing the *dsg* sequence. Restriction analysis of the DNA showed that in this case the majority of the white colonies resulted from recombination between the repeated sequences (Table 1; Fig. 1, lanes 1 to 8). Since the frequency of recombination between the direct repeats in HSV-1-infected COS cells is approximately the same as that determined previously to occur in HSV-1-infected Vero cells (12), the transformed phenotype of COS cells is not the cause of the high level of illegitimate recombination seen with plasmids replicating from ori_{sv}. We therefore conclude that HSV-1 infection and replication from an HSV-1 origin are specifically required for a high level of homologous recombination between directly
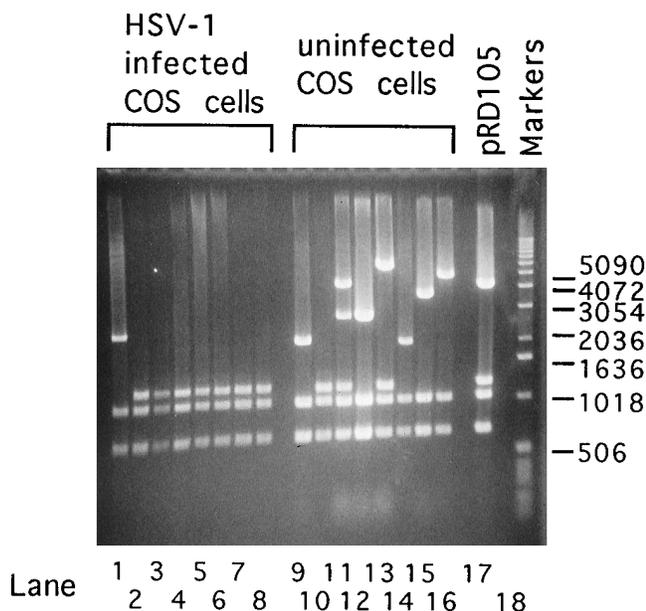

FIG. 1. *Dra*I restriction analysis of recombinants from uninfected and HSV-1-infected COS cells. Correct recombination through repeats should generate a plasmid lacking only the largest band of the parent plasmid pRD105. Lanes 1 to 8, plasmids obtained from white colonies after transformation with DNA extracted from HSV-1-infected COS cells transfected with pRD105; lanes 9 to 16, plasmids obtained from white colonies after transformation with DNA extracted from uninfected COS cells transfected with pRD105-6; lane 17, the parent plasmid pRD105; lane 18, 1-kb ladder. Numbers on the right indicate molecular size in kilobases.

TABLE 2. Recombination between small repeats

Plasmid	HSV-1 infection[a]	% White colonies in expt:						Total no. of colonies	Avg % white colonies	No. with correct deletion/total no.[b]	% Recombination between repeats[c]
		1	2	3	4	5	6				
U$_c$-100-2	−	—[d]	0.3 0.4	0.3 0.1	—	—	—	5,546	0.3	0/15	<0.02
	+	2.4 2.3	3.0 2.1	2.1 0.9	0.7 1.5 1.3	1.8 1.8	1.4	20,401	1.8	38/91	0.8
U$_b$-100-2	−	—	0.4 0.5	0.2 0.1	—	—	—	5,293	0.3	0/16	<0.02
	+	—	1.2 1.7	1.7 1.7	1.6 1.4 1.2	1.0 1.6	1.0	18,631	1.4	25/73	0.5
dsg-100-2	−	—	0.4 0.2	0.1 0.1	—	—	—	4,194	0.2	1/7	0.03
	+	0.4 1.0	1.0 1.4	2.4 1.2	0.8 0.5 0.8	1.0 1.2	0.5	20,102	1.0	35/68	0.5
pRD110	+	—	—	2.6	2.6	3.1	2.1	6,619	2.6		2.1[e]
pRD105	+	—	—	6.7	5.7	5.3	3.8	7,896	5.4		4.3[e]

[a] Transfected cells were either infected with HSV-1 (+) or mock infected (−).

[b] Number of white colonies containing a correct deletion, that is, a plasmid with the product expected from deletion between the repeats, as judged by restriction digest analysis, divided by the total number of white colonies examined.

[c] Recombination between repeats is the frequency at which recombination occurs between the repeated regions, calculated by multiplying the average percentage of white colonies by the fraction of correct deletions.

[d] —, not done.

[e] The ratio of recombination between repeats for pRD110 and pRD105 was calculated by using a correct-deletion frequency of 0.8, a number determined previously (12).

repeated sequences and the enhanced recombinogenicity of the *a* sequence.

HSV-1-infected cells can efficiently promote recombination between short repeated sequences; however, the U$_c$-DR1 segment of the *a* sequence does not display enhanced recombinogenicity when examined alone. We determined through replacement analysis that only the U$_c$-DR1 region is indispensable for the recombinogenic nature of the *a* sequence (13). We wished to ascertain whether this region also showed enhanced recombinogenicity when tested in the absence of flanking sequences. We therefore constructed plasmids containing direct repeats of either the U$_c$-DR1 region (124 bp), the DR1-U$_b$ region of the *a* sequence (120 bp), or a section of the *dsg* gene of *M. xanthus* (150 bp). The size of these repeats falls below the minimum efficient processing size (MEPS) for homologous recombination in mammalian cells of 200 to 300 bp (2, 26, 38), so that homologous recombination between them would be expected to be inefficient.

In mock-infected cells, the frequency of white-colony formation was extremely low or undetectable for all three constructs (Table 2). When the DNA from the white colonies was tested by restriction analysis, only one white colony from the dsg-100-2 samples was found to contain a plasmid with the correct restriction pattern for a deletion having occurred through the repeats, giving a frequency of 0.03% for homologous recombination between the 150-bp *dsg* repeats. No correct products were found for either U$_c$-100-2 or U$_b$-100-2. This recombination frequency is significantly lower than the average of 0.15% for recombination between 300-bp repeats in uninfected cells observed in 30 experiments (data not shown), consistent with the fact that the 120- to 150-bp repeats are smaller than the MEPS for mammalian cells.

After HSV-1 infection, the percentage of white colonies increased for all three plasmids, with U$_c$-100-2 giving the highest value. However, the difference between the U$_c$-100-2 (1.8%) and the U$_b$-100-2 or dsg-100-2 (1.4 and 1.0%, respec-

tively) plasmids is considerably less than that observed between the intact *a* sequence (pRD105) and the intact *dsg* sequence (pRD110), suggesting that other factors must play a role in the recombinogenic nature of the *a* sequence.

These findings do demonstrate that significant homologous recombination occurs between all three sets of small repeats. Analysis of the DNA from the white colonies showed that 35 to 50% contained the correct product for recombination between the repeats. These values correspond to frequencies of recombination between repeats of 0.8% for U$_c$-100-2 and 0.5% for the other plasmids. Thus, HSV-1-infected cells are capable of promoting recombination between repeats that are too small to undergo recombination efficiently in uninfected cells.

DISCUSSION

HSV-1-infected cells have long been known to display high levels of homologous recombination (3, 41, 55). Our results demonstrate that homologous recombination in HSV-1-infected cells has two special characteristics. First, HSV-1 infection and DNA replication are specifically required both for high-level homologous recombination between 300-bp repeats and for the enhanced recombinogenicity of the *a* sequence. Replication from an SV40 origin leads primarily to nonhomologous recombination. Second, HSV-1-infected cells can promote recombination between 120-bp repeats at a significant frequency (0.5 to 0.8%), in contrast to the extremely low frequency (<0.03%) observed for uninfected cells.

Nonhomologous recombination between repeated sequences on plasmids replicating from an SV40 origin has been shown to occur at a very high level (49, 50). The frequency of homologous recombination between repeated sequences is directly dependent on the amount of homology (23, 38, 50). Subramani and Berg detected a ninefold increase in the rate of homologous recombination between repeats as they increased in size from 237 to 943 bp (50). Kawasaki and coworkers (23),

screening for loss of the *galK* gene between two repeats, reported a threefold increase in loss of the screenable gene when the repeat size was increased from 350 to 960 bp, with approximately 50% of the loss attributable to homologous recombination when the repeats were 960 bp. Our finding that only about 10% of the recombinants generated during replication promoted by an SV40 origin result from homologous recombination between the 300-bp repeats is consistent with these findings, since the frequency of nonhomologous recombination is unlikely to be affected by the repeat size.

Weber and colleagues reported that the *b-a-c* junction of HSV-1 was more recombinogenic than a plasmid sequence of similar size when replication was in an SV40 minichromosome (54). This finding contrasts with our observation that there is no enhanced recombination between *a* sequences during SV40 replication. It is possible that the *b-a-c* junction is recombinogenic in a context in which the *a* sequence by itself is not. However, the large size of the repeats used by Weber and colleagues (3,000 bp) makes direct comparison with our results difficult.

The specific requirement for HSV-1 replication for the high level of homologous recombination between 300-bp repeats and the enhanced recombination frequency between the *a* sequences could be due to a difference in the mode of replication; HSV-1 is thought to replicate predominantly by a rolling-circle mechanism (19), whereas SV40 undergoes theta-type replication (47). Alternatively, the absence of histones on DNA replicated by the HSV-1 replication proteins (45) may make it more accessible to the homologous recombination machinery. It is also possible that the HSV-1 replication proteins themselves may play an active role in recombination.

The MEPS is approximately 250 bp in mammalian cells (27, 38). The frequency of homologous recombination is linearly dependent on length for repeats larger than the MEPS and decreases exponentially for repeats smaller than the MEPS. Thus, little recombination should be observed between repeats of 120 to 150 bp in our Vero cell system. Indeed, the frequency of recombination between these repeats in uninfected cells is undetectable for the constructs U_c-100-2 and U_b-100-2 and extremely low for the construct dsg-100-2.

The situation in HSV-1-infected cells is quite different. For U_b-100-2 and dsg-100-2, 1.4 and 1.0% white colonies, respectively, are generated after incubation in HSV-1-infected cells. Analysis of the products demonstrated that a significant percentage of these white colonies contain products consistent with homologous recombination having occurred between the repeats. We did note that the level of nonhomologous recombination between the U_b-DR1 repeats was higher in all experiments (Table 2), and we have no explanation for this finding. However, both constructs give a homologous recombination frequency of 0.5%. Thus, HSV-1-infected cells can efficiently carry out homologous recombination between several sets of repeats that are smaller than the MEPS for mammalian cells. Though further experiments with repeats of a variety of sizes are needed to establish more exactly a MEPS for HSV-1-infected cells, our data indicate that it is lower than that previously determined for uninfected cells. It has been suggested that the MEPS represents a minimal size at which the recombination machinery can bind and act (44). A lower value for the MEPS in HSV-1-infected cells would therefore indicate that the recombination machinery following HSV-1 infection differs from that normally used in mammalian cells.

The frequency of both white-colony formation and recombination between repeats is only marginally higher for the plasmid U_c-100-2, which contains directly repeated copies of the U_c-DR1 region of the HSV-1 *a* sequence. We had previously reported that the 95-bp U_c-DR1 sequence is the only region of the *a* sequence indispensable for its high level of recombinogenicity (13), suggesting that it might be the site of action of a site-specific recombinase. However, the slightly enhanced recombination frequency seen with plasmid U_c-100-2 is considerably less than the difference between those of the intact *a* sequence and other sequences of similar length, indicating that it may not be the target for a site-specific recombinase. It is possible that flanking regions contain recombinational enhancers for a site-specific event, as seen in other systems such as V(D)J recombination (5, 42) and Hin/Gin inversion (18, 21, 22). Another possibility is that the U_c-DR1 region is a hot spot for homologous recombination, with significant enhancement detected only when the overall amount of homology is greater than the 120 bp tested.

ACKNOWLEDGMENTS

REFERENCES

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. S. Seidman, J. A. Smith, and K. Struhl.** 1994. Current protocols in molecular biology. Wiley and Green Associates Publishers, New York.
2. **Ayares, D., J. Spencer, F. Schwartz, B. Morse, and R. Kucherlapati.** 1985. Homologous recombination between autonomously replicating plasmids in mammalian cells. Genetics **111:**375–388.
3. **Brown, S. M., D. A. Ritchie, and J. H. Subak-Sharpe.** 1973. Genetic studies with herpes simplex virus type 1. The isolation of temperature-sensitive mutants, their arrangement into complementation groups and recombination analysis leading to a linkage map. J. Gen. Virol. **18:**329–346.
4. **Brown, S. M., J. H. Subak-Sharpe, J. Harland, and A. MacLean.** 1992. Analysis of intrastrain recombination in herpes simplex virus type 1 strain 17 and herpes simplex virus type 2 strain HG52 using restriction endonuclease sites as unselected markers and temperature-sensitive lesions as selected markers. J. Gen. Virol. **73:**293–301.
5. **Chen, J., F. Young, A. Bottaro, V. Stewart, R. K. Smith, and F. W. Alt.** 1993. Mutation of the intronic IgH enhancer and its flanking sequences differentially affect accessibility of the JH locus. EMBO J. **12:**4635–4645.
6. **Cheng, Y. L., L. B. Kalman, and D. Kaiser.** 1994. The *dsg* gene of *Myxococcus xanthus* encodes a protein similar to translation initiation factor IF3. J. Bacteriol. **176:**1427–1433.
7. **Chu, G., H. Hayakawa, and P. Berg.** 1987. Electroporation for the efficient transfection of mammalian cells with DNA. Nucleic Acids Res. **15:**1311–1326.
8. **Davison, A., and N. Wilkie.** 1981. Nucleotide sequence of the joint between the L and S segments of herpes simplex virus types 1 and 2. J. Gen. Virol. **55:**315–331.
9. **Deiss, L. P., J. Chou, and N. Frenkel.** 1986. Functional domains within the *a* sequence involved in the cleavage-packaging of herpes simplex virus DNA. J. Virol. **59:**605–618.
10. **Deiss, L. P., and N. Frenkel.** 1986. Herpes simplex virus amplicon: cleavage of concatemeric DNA is linked to packaging and involves amplification of the terminally reiterated *a* sequence. J. Virol. **57:**933–941.
11. **Delius, H., and J. B. Clements.** 1976. A partial denaturation map of herpes simplex virus type 1 DNA: evidence for inversion of the unique DNA regions. J. Gen. Virol. **33:**125–133.
12. **Dutch, R. E., R. C. Bruckner, E. S. Mocarski, and I. R. Lehman.** 1992. Herpes simplex virus type 1 recombination: role of DNA replication and viral *a* sequences. J. Virol. **66:**277–285.
13. **Dutch, R. E., B. V. Zemelman, and I. R. Lehman.** 1994. Herpes simplex virus type 1 recombination: the U_c-DR1 region is required for high-level *a*-sequence-mediated recombination. J. Virol. **68:**3733–3741.
14. **Fromm, M., and P. Berg.** 1982. Deletion mapping of DNA regions required for SV40 early region promoter function in vivo. J. Mol. Appl. Genet. **1:**457–481.
15. **Gluzman, Y.** 1981. SV40-transformed simian cells support the replication of early SV40 mutants. Cell **23:**175–182.
16. **Hayward, G. S., R. J. Jacob, S. C. Wadsworth, and B. Roizman.** 1975. Anatomy of herpes simplex virus DNA: evidence for four populations of

molecules that differ in the relative orientations of their long and short components. Proc. Natl. Acad. Sci. USA **72:**4243–4247.

17. **Honess, R. W., A. Buchan, I. W. Halliburton, and D. H. Watson.** 1980. Recombination and linkage between structural and regulatory genes of herpes simplex virus type 1: study of the functional organization of the genome. J. Virol. **34:**716–742.

18. **Huber, H. E., S. Iida, W. Arber, and T. A. Bickle.** 1985. Site-specific DNA inversion is enhanced by a DNA sequence element in cis. Proc. Natl. Acad. Sci. USA **82:**3776–3780.

19. **Jacob, R. J., L. S. Morse, and B. Roizman.** 1979. Anatomy of herpes simplex virus DNA. XIII. Accumulation of head-to-tail concatemers in nuclei of infected cells and their role in the generation of the four isomeric arrangements of viral DNA. J. Virol. **29:**448–457.

20. **Jenkins, F. J., and B. Roizman.** 1986. Herpes simplex virus 1 recombinants with noninverting genomes frozen in different isomeric arrangements are capable of independent replication. Virology **59:**494–499.

21. **Johnson, R. C., and M. I. Simon.** 1985. Hin-mediated site-specific recombination requires two 26 bp recombination sites and a 60 bp recombinational enhancer. Cell **41:**781–791.

22. **Kahmann, R., F. Rudt, C. Koch, and G. Mertens.** 1985. G inversion in bacteriophage Mu DNA is stimulated by a site within the invertase gene and a host factor. Cell **41:**771–781.

23. **Kawasaki, I., Y. S. Bae, T. Eki, Y. Kim, and H. Ikeda.** 1994. Homologous recombination of monkey α-satellite repeats in an in vitro simian virus 40 replication system: possible association of recombination with DNA replication. Mol. Cell. Biol. **14:**4173–4182.

24. **Knipe, D. M., W. T. Ruyechan, and B. Roizman.** 1979. Molecular genetics of herpes simplex virus. III. Fine mapping of a genetic locus determining resistance to phosphonoacetate by two methods of marker transfer. J. Virol. **29:**698–704.

25. **Knipe, D. M., W. T. Ruyechan, B. Roizman, and I. W. Halliburton.** 1978. Molecular genetics of herpes simplex virus. Demonstration of regions of obligatory and non-obligatory identity in diploid regions of the genome by sequence replacement and insertion. Proc. Natl. Acad. Sci. USA **75:**3896–3900.

26. **Lin, F.-L. M., K. Sperle, and N. Sternberg.** 1984. Model for homologous recombination during transfer of DNA into mouse L cells: role for DNA ends in the recombination process. Mol. Cell. Biol. **4:**1020–1034.

27. **Liskay, R. M., A. Letsou, and J. L. Stachelek.** 1987. Homology requirement for efficient gene conversion between duplicated chromosomal sequences in mammalian cells. Genetics **115:**161–167.

28. **Longnecker, R., and B. Roizman.** 1986. Generation of an inverting herpes simplex virus 1 mutant lacking the L-S junction *a* sequences, an origin of DNA synthesis, and several genes including those specifying glycoprotein E and the α47 gene. J. Virol. **58:**583–591.

29. **Mocarski, E. S., L. P. Deiss, and N. Frenkel.** 1985. Nucleotide sequence and structural features of a novel U$_S$-*a* junction present in a defective herpes simplex virus genome. J. Virol. **55:**140–146.

30. **Mocarski, E. S., L. E. Post, and B. Roizman.** 1980. Molecular engineering of the herpes simplex virus genome: insertion of a second L-S junction into the genome causes additional inversions. Cell **22:**243–255.

31. **Mocarski, E. S., and B. Roizman.** 1981. Site-specific inversion sequence of the herpes simplex virus genome: domain and structural features. Proc. Natl. Acad. Sci. USA **78:**7047–7051.

32. **Mocarski, E. S., and B. Roizman.** 1982. Herpesvirus-dependent amplification and inversion of cell-associated viral thymidine kinase gene flanked by viral *a* sequences and linked to an origin of viral DNA replication. Proc. Natl. Acad. Sci. USA **79:**5626–5630.

33. **Parris, D. S., R. A. F. Dixon, and P. A. Schaffer.** 1980. Physical mapping of herpes simplex virus type 1 *ts* mutants by marker rescue: correlation of the physical and genetic maps. Virology **100:**275–287.

34. **Poffenberger, K. L., E. Tabares, and B. Roizman.** 1983. Characterization of a viable, noninverting herpes simplex virus 1 genome derived by insertion and deletion of sequences at the junction of components L and S. Proc. Natl. Acad. Sci. USA **80:**2690–2694.

35. **Pogue-geile, K. L., G. T.-Y. Lee, and P. G. Spear.** 1985. Novel rearrangements of herpes simplex virus DNA sequences resulting from duplication of

a sequence within the unique region of the L component. J. Virol. **53:**456–461.

36. **Post, L. E., S. Mackem, and B. Roizman.** 1981. Regulation of α genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with α gene promoters. Cell **24:**556–565.

37. **Roizman, B., and A. Sears.** 1990. Herpes simplex viruses and their replication, p. 1795–1843. *In* B. Fields and C. M. Knipe (ed.), Virology. Raven Press, New York.

38. **Rubnitz, J., and S. Subramani.** 1984. The minimum amount of homology required for homologous recombination in mammalian cells. Mol. Cell. Biol. **4:**2253–2258.

39. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a practical approach, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

40. **Sarisky, R. T., and P. C. Weber.** 1994. Requirement for double-strand breaks but not for specific DNA sequences in herpes simplex virus type 1 genome isomerization events. J. Virol. **68:**34–47.

41. **Schaffer, P. A., M. J. Tevethia, and M. Benyesh-Melnick.** 1974. Recombination between temperature-sensitive mutants of herpes simplex virus type 1. Virology **58:**219–228.

42. **Serwe, M., and F. Sablitsky.** 1993. V(D)J recombination in B cells is impaired but not blocked by targeted deletion of the immunoglobulin heavy chain intron enhancer. EMBO J. **12:**2321–2327.

43. **Sheldrick, P., and N. Berthelot.** 1975. Inverted repetitions in the chromosome of herpes simplex virus. Cold Spring Harbor Symp. Quant. Biol. **39:**667–678.

44. **Shen, P., and H. V. Huang.** 1986. Homologous recombination in *Escherichia coli*: dependence on substrate length and homology. Genetics **112:**441–457.

45. **Sinden, R. R., D. E. Pettijohn, and B. Francke.** 1982. Organization of herpes simplex virus type 1 deoxyribonucleic acid during replication probed in living cells with 4,5′,8-trimethylpsoralen. Biochemistry **21:**4484–4490.

46. **Smiley, J. R., B. S. Fong, and W.-C. Leung.** 1981. Construction of a double-jointed herpes simplex viral DNA molecule: inverted repeats are required for segment inversion, and direct repeats promote deletions. Virology **113:**345–362.

47. **Stillman, B.** 1989. Initiation of eukaryotic DNA replication in vitro. Annu. Rev. Cell Biol. **5:**197–245.

48. **Stow, N. D., J. H. Subak-Sharpe, and N. M. Wilkie.** 1978. Physical mapping of herpes simplex virus type 1 mutations by marker rescue. J. Virol. **28:**182–192.

49. **Subramani, S.** 1989. Analysis of recombination in mammalian cells using SV40 and SV40-derived vectors. Mutat. Res. **220:**221–234.

50. **Subramani, S., and P. Berg.** 1983. Homologous and nonhomologous recombination in monkey cells. Mol. Cell. Biol. **3:**1040–1052.

51. **Umene, K.** 1991. Recombination of the internal direct repeat element DR2 responsible for the fluidity of the *a* sequence of herpes simplex virus type 1. J. Virol. **65:**5410–5415.

52. **Varmuza, S. L., and J. R. Smiley.** 1985. Signals for site-specific cleavage of HSV DNA: maturation involves two separate cleavage events at sites distal to the recognition sequences. Cell **41:**793–802.

53. **Weber, P. C., M. D. Challberg, N. J. Nelson, M. Levine, and J. C. Glorioso.** 1988. Inversion events in the HSV-1 genome are directly mediated by the viral replication machinery and lack sequence specificity. Cell **54:**369–381.

54. **Weber, P. C., M. Levine, and J. C. Glorioso.** 1990. Recombinogenic properties of herpes simplex virus type 1 DNA sequences resident in simian virus 40 minichromosomes. J. Virol. **64:**300–306.

55. **Wildy, P.** 1955. Recombination with herpes simplex virus. J. Gen. Microbiol. **13:**346–360.

56. **Wohlrab, F., S. Chatterjee, and R. D. Wells.** 1991. The herpes simplex virus 1 segment inversion site is specifically cleaved by a virus-induced nuclear endonuclease. Proc. Natl. Acad. Sci. USA **88:**6432–6436.

57. **Wohlrab, F., M. J. McLean, and R. D. Wells.** 1987. The segment inversion site of herpes simplex virus type 1 adopts a novel DNA structure. J. Biol. Chem. **262:**6407–6416.

58. **Wohlrab, F., and R. D. Wells.** 1989. Slight changes in conditions influence the family of non-B-DNA conformations of the herpes simplex virus type 1 DR2 repeats. J. Biol. Chem. **264:**8207–8213.