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Recombinogenic Properties of Herpes Simplex Virus Type 1 DNA Sequences Resident in Simian Virus 40 Minichromosomes

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In a previous work, it was demonstrated that the bacterial transposon Tn*5* is capable of undergoing sequence inversion via recombination between its duplicated IS*50* elements when replicated by the herpes simplex virus type 1 (HSV-1) origin ori_s but not by the simian virus 40 (SV40) origin ori_{sv}. Further analysis of the latter phenomenon indicated that this lack of recombination was the result of topological constraints imposed by the SV40 minichromosome, such that recombination events could be readily detected in Tn*5* derivatives in which the IS*50* elements were arranged in a direct rather than inverted orientation. With this information, a second set of experiments were carried out to examine how the highly recombinogenic sequences which mediate the inversion of the long (L) and short (S) components of the HSV-1 genome behave in an SV40 minichromosome. Tandem copies of the L-S junction of the HSV-1 genome were observed to promote deletions in an SV40 shuttle plasmid at a frequency that was considerably greater than that of duplicated bacterial plasmid vector DNA. However, the presence of superinfecting HSV-1 did not enhance the frequency of these recombination events. These results support our previous findings that HSV-1 genome isomerization is mediated by a homologous recombination mechanism which is intimately associated with the act of viral DNA synthesis. Moreover, they demonstrate that the sequences which comprise the L-S junction appear to be inherently recombinogenic and, therefore, do not contain specific signals required for HSV-1 genome isomerization.

The herpes simplex virus type 1 (HSV-1) genome is a 152-kilobase (kb) linear double-stranded DNA molecule which comprises a 108-kb long (L) component and a 13-kb short (S) component. The L and S components are each flanked by the inverted repeat sequences *b* (9 kb) and *c* (6.5 kb), respectively, and both are flanked by the inverted repeat sequence *a* (250 to 500 base pairs). Thus, the HSV-1 genome is organized in the following arrangement:

$$a - b \text{ —— } L \text{ —— } b' - a' - c' \text{ —— } S \text{ —— } c - a$$

(9, 34, 40).

The L and S components of the HSV-1 genome are able to invert relative to each other at high frequency, resulting in the appearance of four equimolar isomeric populations of viral DNA (7, 9). It has been previously suggested that these inversions are due to a site-specific recombination event which occurs at the *a* sequence (4, 22–25). However, recent evidence now indicates that HSV-1 genome isomerization is the result of homologous recombination between the inverted repeats which flank the L and S components (41).

Although the inversion events which occur in the HSV-1 genome do not appear to be mediated by a site-specific process, studies with engineered duplications of viral restriction fragments have nevertheless demonstrated that some HSV-1 DNA sequences are more recombinogenic than others. For example, the L-S junction (23, 25, 35), the *a* sequence (4, 39), a subfragment of the *c* sequence (39), the *Bam*HI L fragment (29), the HSV type 2 glycoprotein C gene (29), the *Hin*dIII O fragment (27), and the ICP4 gene promoter (13, 14, 28) (but not the HSV-1 glycoprotein C gene [20], the glycoprotein D gene [8], the thymidine kinase gene [30], and three *Sst*I fragments derived from the *Bam*HI F and J fragments [23]) were able to promote detectable recombination events when duplicated in the HSV-1 genome. Most notably, small subfragments of sequences comprising the L-S junction have been observed to direct sequence inversions over great distances within the viral genome (4, 39). The mechanism of this enhanced recombinogenicity is unclear, but it may involve the families of tandem GC-rich reiterations which are scattered throughout the L-S junction (32, 39).

In a previous study, it was determined that the generalized recombination which results in HSV-1 genome isomerization was mediated by the complex of gene products which replicates the viral DNA rather than by a specific recombinase protein (41). A critical observation which led to this conclusion involved the bacterial transposon Tn*5*, which was found to undergo a high frequency of sequence inversion in the HSV-1 genome caused by recombination between its duplicated IS*50* elements. However, Tn*5* inversion was not observed in DNA which lacked the HSV-1 origin of replication ori_s, nor in DNA in which ori_s was replaced by the simian virus 40 (SV40) origin of replication ori_{sv}, even if HSV-1 gene products from superinfecting virus were present in *trans*. These results indicated that the putative viral recombinase had a strict requirement for DNA replication, specifically HSV-1 DNA replication. Subsequent experiments demonstrated that the viral DNA synthesis enzymes carried this recombinase function, since they could mediate recombination events in the absence of any other viral proteins (41).

However, with the same SV40 shuttle plasmid system developed in the previous study (41), it was discovered that Tn*5* failed to invert not only when the transposon was replicated by ori_{sv} but also when it was replicated by the ColE1 origin of the pUC18 plasmid vector (P. C. Weber, unpublished observations). In addition, other examples of

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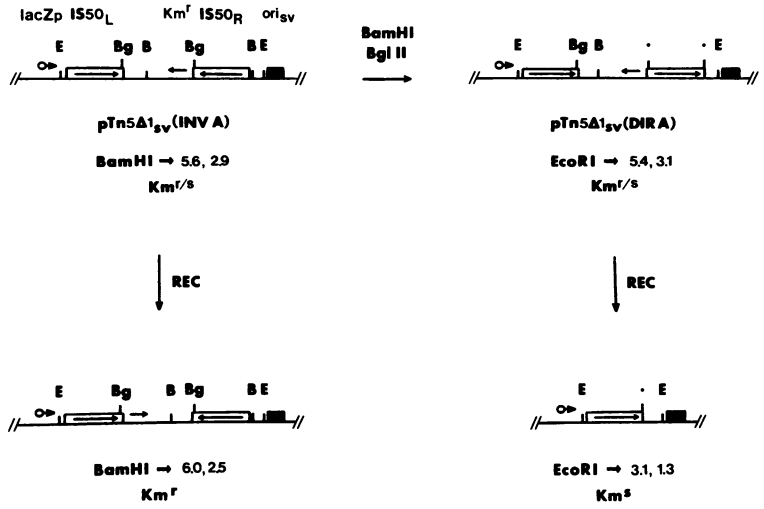

FIG. 1. Construction of SV40 shuttle plasmids containing Tn*5* derivatives with IS*50* elements arranged in direct or inverted orientations. Details of the constructions are described in Materials and Methods. The predicted products of recombination between the IS*50* elements of $pTn5\Delta1_{sv}$ (INV A) and $pTn5\Delta1_{sv}$ (DIR A) in COS7 cells are shown at the bottom. The sizes of the *Bam*HI or *Eco*RI fragments and the kanamycin resistance phenotypes conferred upon *E. coli* DHI (MICs of antibiotic were >150 μg/ml for Km^r, 25 μg/ml for Km^r/Km^s, and <10 μg/ml for Km^s [41]) for each plasmid are indicated. Abbreviations: lacZp, *lacZ* gene promoter of pUC18; $IS50_L$ and $IS50_R$, insertion sequences contained within Tn*5*; Km^r, kanamycin phosphotransferase gene of Tn*5*; ori_{sv}, SV40 origin of replication; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI.

inhibition of recombination between inverted repeat sequences in SV40 minichromosomes have been recently described (10). Thus, the observation that Tn*5* inversion is prohibited in supercoiled covalently closed circular DNA such as the SV40 minichromosome and an *Escherichia coli* plasmid but not in linear DNA such as the HSV-1 genome (41), the bacteriophage λ genome (43), and the *E. coli* chromosome (1) suggests that it may be mechanistically impossible for any recombinase, including that encoded by HSV-1, to promote inversion events in this form of DNA. The existence of such topological constraints within this SV40 shuttle vector indicates that it should not be employed in an analysis to determine whether the recombinase built into the HSV-1 DNA replication machinery can also function through other DNA replication systems.

The experiments in this work were designed initially to explore the role of DNA topology in the suppression of recombination in SV40 minichromosomes. This information was then applied towards the redevelopment of the SV40 shuttle vector system, so that it could once again be used in the analysis of the *cis*- and *trans*-acting functions required for HSV-1 genome isomerization. These studies confirmed and extended the results of our previous work (41) in demonstrating that the viral gene products which mediate recombination in HSV-1 will not act on a template that is replicated by a non-HSV-1 origin and that sequences which are highly recombinogenic in HSV-1 DNA behave similarly in a different viral genome.

MATERIALS AND METHODS

Cell culture and media. Transfections of ori_{sv}-containing plasmids were done into T-antigen-expressing COS7 cells. HSV-1 (strain KOS) stocks used in superinfections were propagated in Vero cells. Both cell lines were grown in minimum essential medium supplemented with 10% calf serum.

Plasmids. All plasmid constructs employed the vector pCW522, which consists of an ori_{sv}-containing *Pst*I-*Hin*dIII fragment cloned into pUC18 (41). The construction and characterization of $pTn5\Delta1_{sv}$ (INV A), which contains the IS*50* elements of Tn*5* in their normal inverted orientation, have been described previously (41). $pTn5\Delta1_{sv}$ (DIR A), which contains the IS*50* elements of Tn*5* in direct orientation, was generated by inversion of the IS*50*-containing, 1.3-kb *Bam*HI-*Bgl*II fragment of $pTn5\Delta1_{sv}$ (INV A) (Fig. 1).

pCW101 contains the L-S junction of the HSV-1 genome inserted into pCW522 and was constructed as follows. pSG1-EK1, a cloned *Eco*RI-*Kpn*I fragment (31) which contains the ICP4 gene of the *c* sequence, two tandem *a* sequences (P. C. Weber, unpublished observations), and a portion of the *b* sequence, was mutagenized with Tn*5* by the λ467::Tn*5* carrier phage method of deBruijn and Lupski (6) to create new restriction sites which could be utilized in subsequent constructions. pSG1-EK1::Tn*5* (#58) contained a Tn*5* insertion in the ICP4 gene which was 1.3 kb from the closest *a* sequence; it therefore contained a *Hin*cII site (derived from an IS*50* element of the transposon) which was 1.5 kb from the nearest *a* sequence. This *Hin*cII site, along with an *Sst*I site in the *b* sequence 3.0 kb away, were used to clone the *b-a-a-c* sequences of the L-S junction into pCW522 to create plasmid pCW101 (Fig. 2).

A pCW101 dimer was generated by electrophoresing undigested pCW101 DNA which had been isolated from Rec^+ *E. coli* C600 on a low-melting-point agarose gel, purifying the dimeric form of the plasmid from the agarose by phenol extraction, and transforming this DNA into *recA E. coli* DH1. The pCW101 dimer was stable in this strain, since the *recA* mutation prevented resolution of the plasmid into monomeric DNA. *Sma*I digestion revealed that one of the four *a* sequences in the pCW101 dimer had been spontaneously deleted during construction, so that the plasmid actually consisted of an intact 6.0-kb pCW101 monomer with two *a* sequences plus a 5.8-kb deletion derivative of the pCW101 monomer, called pCW101(Δ), which contained only one *a* sequence (Fig. 2).

The pCW101 dimer was partially digested with *Eco*RI, and the 8.8-kb products were isolated and ligated to yield pCW102 and pCW103 (Fig. 2). This treatment resulted in the

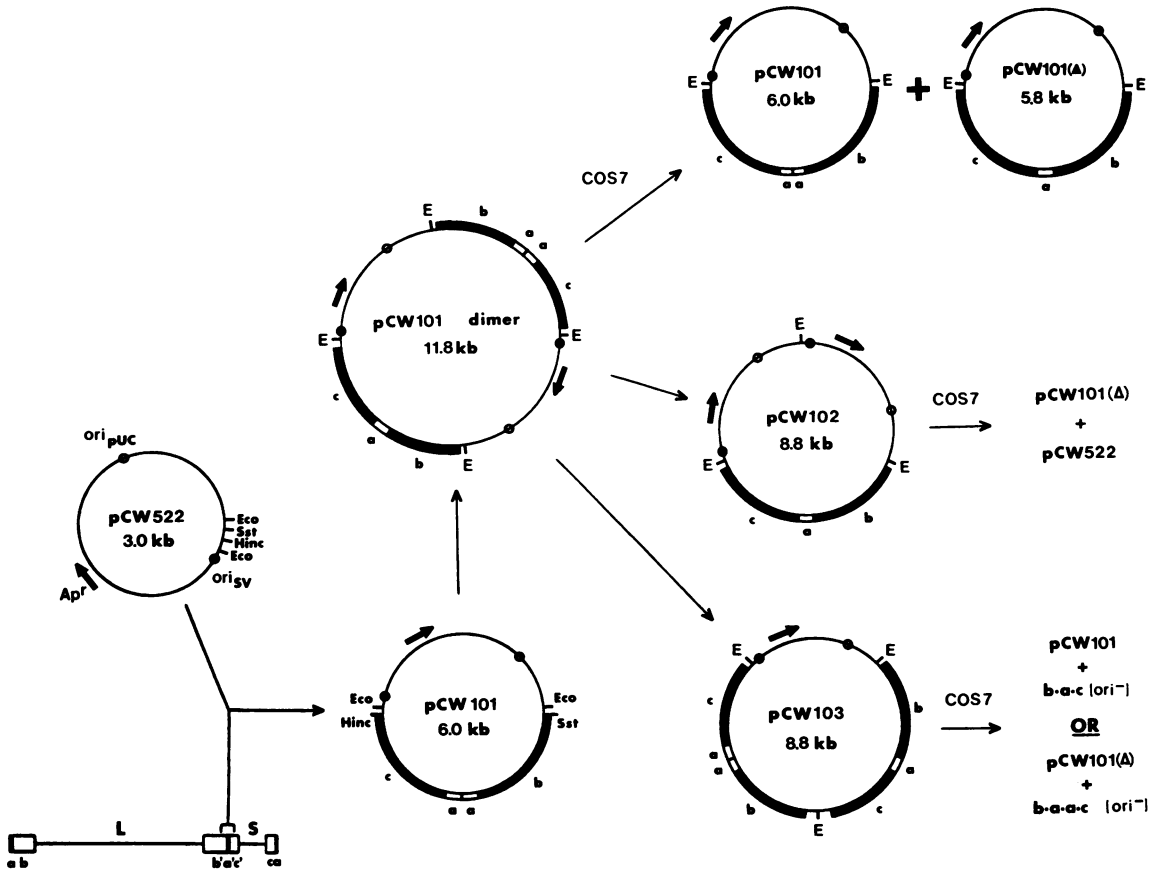

FIG. 2. Construction of SV40 shuttle plasmids containing copies of the L-S junction of the HSV-1 genome. Details of the constructions are described in Materials and Methods. A schematic representation of the structure of the HSV-1 genome, including the L and S components and the *a*, *b*, and *c* inverted repeat sequences, is shown at lower left. The predicted products of recombination for the pCW101 dimer, pCW102, and pCW103 in COS7 cells are indicated at right. The *Eco*RI (E) restriction sites, pUC18 origin of replication (○), pUC18 ampicillin resistance gene (indicated by thick arrows) and SV40 origin of replication (●) are included in each plasmid illustration.

loss of the *b-a-a-c* sequence in pCW102, leaving the *b-a-c* sequence and two tandem repeat pCW522 vectors, and the loss of a pCW522 vector in pCW103, leaving the other pCW522 vector and the *b-a-c* and *b-a-a-c* sequences as tandem repeats.

Rescue of transfected plasmid DNA by Hirt extraction. Five micrograms of plasmid DNA was transfected into COS7 cells by calcium phosphate precipitation and a 25% dimethyl sulfoxide shock as described previously (12). Transfected cells were mock or HSV superinfected (multiplicity of infection, 10) after 24 h. After 48 h, the transfected plasmid DNA was rescued by Hirt extraction (11), purified by RNase A, proteinase K, and phenol-chloroform treatment, digested with *Dpn*I to eliminate unreplicated DNA, and transformed into *E. coli* DHI as described previously (41). This treatment normally resulted in 10^2 to 10^3 transformants per microgram of Hirt extract DNA. Plasmids lacking ori_{sv} were used as controls to monitor the *Dpn*I digestion step and typically yielded less than five transformants per microgram of Hirt extract DNA. No significant differences in the number of transformants were observed between mock- and HSV-superinfected transfections, indicating that the presence of HSV-1 does not interfere with SV40 DNA replication. Plasmid DNA was isolated from pooled transformants by the procedure of Birnboim and Doly (2).

RESULTS

Effects of DNA topology on recombination within an SV40 minichromosome. The shuttle plasmid developed in an earlier work (41) to study Tn*5* inversion in an SV40 minichromosome, $pTn5\Delta1_{sv}$ (INV A), is shown in Fig. 1. The structure of Tn*5* in this plasmid has been manipulated so that the kanamycin phosphotransferase gene of the transposon has lost its normal promoter but can be expressed by the *lacZ* gene promoter of the pUC18 vector if a sequence inversion event takes place (Fig. 1). Thus, the degree of Tn*5* inversion which occurs in $pTn5\Delta1_{sv}$ (INV A) when the plasmid is replicated as an SV40 minichromosome can be rapidly and accurately assessed by calculating the frequency of kanamycin-resistant transformants obtained after rescue of *Dpn*I-resistant Hirt extract DNA from transfected COS7 cells. The occurrence of an inversion event can also be readily verified by *Bam*HI digestion of recovered plasmid DNA (Fig. 1). However, as discussed above, no Tn*5* inversion could ever be detected in this plasmid by this system of analysis (41).

The possibility that this absence of recombination within the SV40 minichromosome was specific for the inverted arrangement of the IS*50* sequences was examined by using the plasmid $pTn5\Delta1_{sv}$ (DIR A). This derivative of $pTn5\Delta1_{sv}$

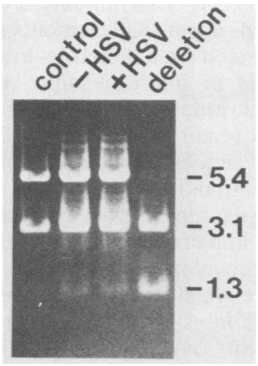

FIG. 3. Rescue of SV40 shuttle plasmid pTn*5*Δ1$_{sv}$ (DIR A) into *E. coli* after replication in COS7 cells. pTn*5*Δ1$_{sv}$ (DIR A) was transfected and mock (−HSV) or HSV superinfected (+HSV) as indicated. Plasmid DNA isolated from pooled transformants derived from the rescue of Hirt extract DNA was digested with *Eco*RI. Untransfected DNA was included as a control in the first lane, and a purified deletion product plasmid is shown in the final lane. Sizes of *Eco*RI fragments are indicated on the right.

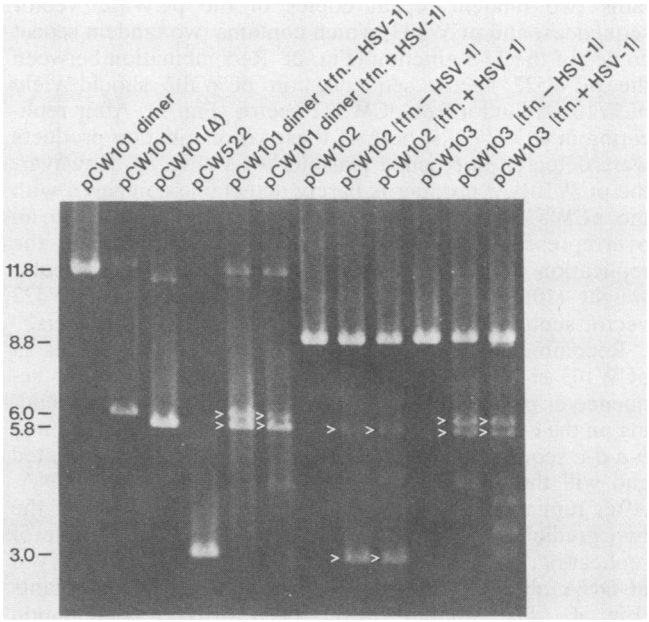

FIG. 4. Rescue of SV40 shuttle plasmids containing copies of the L-S junction of the HSV-1 genome into *E. coli* after replication in COS7 cells. pCW101 dimer, pCW102, and pCW103 were transfected and mock or HSV superinfected as indicated. Plasmid DNA, isolated from pooled transformants derived from rescue of Hirt extract DNA, was electrophoresed undigested. Untransfected pCW101 dimer, pCW101, pCW101(Δ), pCW522, pCW102, and pCW103 were included as reference plasmids, and their sizes are indicated on the left. Arrows identify products of intramolecular recombination.

(INV A) contained two IS*50* elements which were situated in a direct rather than inverted orientation but still maintained the proper spacing of the parental plasmid (Fig. 1). Recombination between the IS*50* elements in pTn*5*Δ1$_{sv}$ (DIR A) should result in a deletion event rather than sequence inversion; this process can be readily monitored by both the loss of the kanamycin resistance phenotype and the replacement of the 5.4-kb *Eco*RI fragment of the plasmid with a 1.3-kb fragment (Fig. 1).

When transfected pTn*5*Δ1$_{sv}$ (DIR A) DNA was rescued by Hirt extraction and analyzed in *E. coli*, the predicted 1.3-kb deletion product could be detected in plasmid DNA isolated from pooled transformants (Fig. 3). Thus, recombination between IS*50* elements could occur in an SV40 minichromosome if the sequences were arranged in a direct instead of an inverted orientation, demonstrating that the mammalian cell recombinase which mediates this process is sensitive to the topology of the template DNA. When plasmids that were 4.4 kb in size were purified from this pooled population by isolation of supercoiled DNA from a low-melting-point agarose gel, they were all found to contain the 3.1- and 1.3-kb *Eco*RI fragments of a plasmid which had undergone the predicted deletion event (Fig. 3). As expected, these derivatives conferred a kanamycin-sensitive phenotype upon their hosts.

The fact that recombination within an SV40 minichromosome could now be detected by using pTn*5*Δ1$_{sv}$ (DIR A) meant that studies to determine whether the HSV-1-encoded recombinase could function through the SV40 DNA replication machinery were finally possible. pTn*5*Δ1$_{sv}$ (DIR A)-transfected COS7 were therefore superinfected with HSV-1 to supply the viral proteins required for DNA replication and recombination; the plasmid DNA was then rescued and analyzed in *E. coli*. No enhancement of recombination caused by the presence of HSV-1 proteins was apparent in these experiments, since similar levels of the 1.3-kb deletion product could be detected in plasmid DNA derived from both mock- and HSV-superinfected transfections (Fig. 3). These results demonstrate that the viral proteins which mediate recombination in DNA which is replicated by an HSV-1 origin will not act on DNA which is replicated by a non-HSV-1 origin such as ori$_{sv}$. This observation supports the conclusion from a previous work (41) that recombination within the HSV-1 genome is inexorably linked to the act of viral DNA replication.

Behavior of highly recombinogenic HSV-1 sequences in an SV40 minichromosome. The successful redevelopment of the SV40 shuttle vector system for HSV-1 recombination studies enabled additional sequences to be examined for their recombinogenic abilities in an SV40 minichromosome. Of particular interest were the *b-a-c* sequences of the L-S junction of the HSV-1 genome, which have been shown to be highly recombinogenic when duplicated at different locations in the viral DNA (4, 23, 25, 35, 39). To analyze whether the recombinogenicity of these sequences was manifest in non-HSV-1 DNA as well, a 3.0-kb restriction fragment containing the L-S junction was cloned into the SV40 shuttle vector pCW522 to yield pCW101 (Fig. 2). A dimeric version of this plasmid, which consisted of a complete pCW101 molecule containing two *a* sequences and a pCW101 derivative molecule containing only one *a* sequence [pCW101(Δ); Fig. 2], was generated so that the amount of recombination across the two directly repeated pCW101 monomeric units could be analyzed. After replication in COS7 cells and rescue into *E. coli*, almost all of the pCW101 dimer had been resolved into its pCW101 and pCW101(Δ) monomer components (Fig. 4), indicating that a high degree of recombination can occur in an SV40 minichromosome which contains direct repeat copies of the L-S junction. However, this analysis does not demonstrate that the pCW101 dimer is being resolved by recombination occurring preferentially at the *b-a-c* sequences.

To map this resolution site(s), two deletion derivatives of the pCW101 dimer were constructed: pCW102, which con-

tains two tandem repeat copies of the pCW522 vector sequences, and pCW103, which contains two tandem repeat copies of the L-S junction (Fig. 2). Recombination between the pCW522 vector sequences in pCW102 should yield pCW101(Δ) and a free pCW522 vector (Fig. 2). After replication in COS7 cells, both of these recombination products were detected in rescued plasmid DNA (Fig. 4). However, the pCW101(Δ) product is barely detectable compared with the pCW522 product; this is almost certainly due to an overrepresentation of the latter plasmid by virtue of the replication advantage conferred by its smaller molecular weight (10). Thus, recombination between the pCW522 vector sequences in pCW102 occurred at very low levels.

Recombination between the L-S junction sequences in pCW103 should yield either pCW101 and a free *b-a-c* sequence or pCW101(Δ) and a free *b-a-a-c* sequence, depending on the crossover site (Fig. 2). Since the deleted *b-a-c* and *b-a-a-c* sequences lack ori_{sv}, they should not be propagated and will therefore be absent from rescued plasmid DNA. After replication in COS7 cells, equivalent amounts of the two predicted recombination products that are capable of replicating, pCW101 and pCW101(Δ), were readily observed in DNA obtained from pooled Hirt extract transformants (Fig. 4). The amount of the pCW101(Δ) recombination product generated by pCW103 was considerably greater than that of pCW102 and would have been even more pronounced if pCW103 did not also generate a second recombination product, pCW101, because of the different numbers of *a* sequences present in the two copies of the L-S junction. Since the pCW522 vector and L-S junction sequences present as tandem repeats in pCW102 and pCW103, respectively, are equivalent in size (3.0 kb), the differences in the levels of pCW101(Δ) recombination products clearly demonstrate that a much greater amount of recombination (estimated by scanning densitometry to be at least a sevenfold increase) is occurring at the L-S junction sequences of pCW103. This behavior in an SV40 minichromosome is identical to that in the HSV-1 genome (4, 23, 25, 35, 39), and together these observations indicate that the sequences which constitute the L-S junction are inherently recombinogenic.

When transfections of the pCW101 dimer, pCW102, and pCW103 were superinfected with HSV-1, no enhancement of recombination mediated by viral gene products was observed (Fig. 4), which was similar to the results obtained for $pTn5\Delta1_{sv}$ (DIR A) (Fig. 3). These observations offer a further demonstration of the inability of the HSV-1 recombination system to act on a template replicated by a non-HSV-1 origin, even when this DNA contains the normal target of the viral recombinase.

DISCUSSION

The SV40 minichromosome has proven to be a simple yet useful system for analyzing recombination in mammalian cells. Its high copy number enables it to be rescued from transfected cells by Hirt extraction and shuttled back into *E. coli*, which facilitates the characterization of individual recombination events within the plasmid. A shuttle vector developed in an earlier work (41), $pTn5\Delta1_{sv}$ (INV A), was used to explore the effects of DNA topology on recombination within an SV40 minichromosome. This analysis demonstrated that recombination events were permitted between direct repeat IS*50* sequences but were forbidden between IS*50* elements arranged in an inverted orientation. Similar results have been reported in studies with a chimeric ori_{sv}-oriP shuttle vector (10). This inhibition of recombination between the inverted repeat IS*50* elements in $pTn5\Delta1_{sv}$ (INV A) could not be reversed even if double-strand breaks, which promote high levels of intermolecular recombination between SV40 minichromosomes (19, 36) by an apparent double-strand break repair mechanism (38), were introduced into the duplicated sequences before transfection (Weber, unpublished observations).

Numerous other examples exist of recombinases which are mechanistically impaired by specific topological features within supercoiled covalently closed circular DNA. Invertases encoded by the *gin* system of phage Mu, the *cin* system of phage P1, and the *hin* system of *Salmonella typhimurium* prefer inverted repeats over direct repeats as substrates for recombination (15, 16, 26, 33). Transposon resolvases, however, such as the TnpR protein encoded by Tn*3* are specific for direct repeat sequences (17, 18). Finally, the activity of the *cer* locus in ColE1 plasmids mediates intramolecular but not intermolecular recombination (37). The topological restrictions in these systems appear to result from the requirement for a precise DNA-protein complex to form before recombination can take place. Supercoiling may assist the two target sites in attaining the necessary alignment within the plasmid with simultaneous recognition by the recombinase protein, so that two sites which are arranged in the incorrect orientation should be energetically unfavored for proper assembly into DNA-protein complexes (3, 5).

Thus, supercoiling within the SV40 minichromosome may also be responsible for the topological restrictions imposed upon the mammalian cell recombinase, since this protein(s) recognizes the IS*50* elements of Tn*5* when they are arranged in a direct but not inverted orientation. Interestingly, when the same two shuttle vectors employed in this study were analyzed for their ability to undergo recombination in *E. coli*, a situation identical to that in COS7 cells was observed: Tn*5* inversion could not be detected in $pTn5\Delta1_{sv}$ (INV A), but deletion events did occur at a low frequency in $pTn5\Delta1_{sv}$ (DIR A) (Weber, unpublished observations). These results suggest that the proteins which mediate generalized recombination in procaryotic and eucaryotic cells are restricted by the same topological features in supercoiled covalently closed circular DNA.

The ability of Tn*5* to undergo sequence inversion in the HSV-1 genome but not in the SV40 shuttle vector $pTn5\Delta1_{sv}$ (INV A) was interpreted as evidence that the viral recombinase had a strict requirement for HSV-1 DNA replication. This inference was substantiated by the observation that the HSV-1 recombinase activity was mediated by the seven viral gene products which constitute the minimum complement of DNA synthesis enzymes (41). However, the possibility remained that the recombinase component of the DNA replication apparatus could still act on Tn*5* sequences resident in an SV40 minichromosome but was suppressed by the same topological effects which inhibited the mammalian cell recombinase. Thus, the experiment designed in the previous work was repeated with $pTn5\Delta1_{sv}$ (DIR A), which contained a Tn*5* derivative capable of undergoing recombination. Since the HSV-1 DNA replication enzymes present in superinfected COS7 cells did not enhance the frequency of recombination in this plasmid, it was concluded that the viral recombinase cannot work through another replication system. Thus, recombination in the HSV-1 genome is mediated by a function that is built into the multiprotein complex which replicates the viral DNA.

A systematic dissection of the IS*50* elements in an earlier study demonstrated that no specific sequences were required

for Tn*5* inversion in the HSV-1 genome (41). This observation and previous reports of inversion events mediated by a variety of duplicated viral restriction fragments (4, 13, 14, 23, 25, 27–29, 35, 39) are inconsistent with the proposed role of the *a* sequence as the *cis*-acting signal through which HSV-1 genome isomerization is mediated (4, 22–25). Moreover, a recombinant virus capable of undergoing *a* sequence-independent isomerization has recently been described (21), and attempts at defining the critical recombination signal within the *a* sequence have yielded inconclusive, if not conflicting, results (4, 39). Together, these observations indicate that HSV-1 genome isomerization is mediated by generalized recombination between the inverted repeats which flank the L and S components of the viral DNA; that is, recombination can occur at the *b* and *c* sequences as well as the *a* sequence.

However, this conclusion is complicated by the fact that the sequences which constitute the L-S junction have been demonstrated to be highly recombinogenic when duplicated in the HSV-1 genome. To illustrate, an IS*50* derivative 600 base pairs in length could not mediate detectable recombination over a distance of 3 kb (41), while a subfragment of the *a* sequence 250 base pairs in length could do so over great distances within the viral genome (39). The *a* sequence and portions of the *b* and *c* sequences are composed of families of tandem reiterations (summarized in reference 32) which are predominantly G rich on one DNA strand and C rich on the other. Wohlrab et al. (42) have demonstrated that these reiterations are capable of forming a highly stressed secondary structure called anisomorphic DNA which may play a role in the recombination events mediated by these sequences. These results suggest that the high recombinogenicity associated with the L-S junction may be an inherent property of the DNA rather than an effect mediated by the HSV-1 recombinase.

To address this issue, the recombinogenic properties of an L-S junction replicated by a non-HSV-1 origin were examined by using the SV40 shuttle vectors pCW101, pCW102, and pCW103. The L-S junction was found to mediate deletion events between tandem repeats in an SV40 minichromosome at a much higher frequency than did an equivalent portion of pCW522 vector DNA. Moreover, this activity was manifest in the absence of, and could not be enhanced in the presence of, the HSV-1 recombinase function. These observations indicate that the L-S junction of the viral genome does indeed contain inherently recombinogenic sequences and that this DNA can mediate recombination events independently of any HSV-1-encoded proteins. Furthermore, these results provide additional evidence that the L-S junction does not contain specific signals required for HSV-1 genome isomerization.

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