Intemolecular Recomeration of the Herpes Simplex Virus Type 1 Genome Analysed Using Two Strains Differing in Restriction Enzyme Cleavage Sites

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SUMMARY

Intemolecular recombination of herpes simplex virus type 1 (HSV-1) was studied by analysing the segregation of strain-specific restriction enzyme cleavage sites among progeny viruses produced after co-infection by two HSV-1 strains differing in eight restriction enzyme cleavage sites. Out of 93 progeny viruses examined, 51 clones were recombinant, and crossover sites of the recombinants were mapped on the HSV-1 genome. These sites were distributed evenly in the unique sequence of the L component (UL) and the recombination frequency in UL was estimated to be 1-12 per genome length, or 0-007 per kilobase pair. No evidence was obtained to support the existence of enhanced intermolecular recombination events in the regions containing inverted repeats and the L-S junction in comparison with the recombination frequency in UL. The finding of recombinants in an arrangement that minimized the number of crossover events suggested the participation of both of two arrangements of the L component of parental DNA (P or Is, and IL or ISL) in the generation of the recombinants. The possibility of a preference for P or Is over IL or ISL arrangements remains to be determined.

INTRODUCTION

Homologous intermolecular recombination may occur between viral genomes. For example, recombination between two temperature-sensitive (ts) simian virus 40 mutants in the same complementation group was observed at a frequency of $2 \times 10^{-4}$ (Dubbs et al., 1974), and pronounced homotypic recombination between two human adenovirus type 5 ts mutants was observed at a frequency of $3.1 \times 10^{-7}$ at 54 h post-infection (Young & Silverstein, 1980). Linkage maps of herpes simplex virus type 1 (HSV-1) have been constructed on the basis of ts mutants (Brown et al., 1973; Schaffer et al., 1974). When these were compared with a physical map of the HSV-1 ts mutations determined by marker rescue experiments (Stow et al., 1978) the recombination frequencies of the linkage maps did not accurately reflect distances on the physical map. Honess et al. (1980), investigating recombination between structural and regulatory genes of HSV-1, used many different markers: (i) ts markers, (ii) plaque morphology (syn), phosphonoacetate resistance (P') and thymidine kinase (TK) phenotypes, and (iii) electrophoretically distinct variants of the polypeptides. Mean two-factor recombination frequencies ranged from 2% to 40% and were influenced by the relative contributions of parental viruses to the mixed infection. As a measure of linkage, they studied the segregation of unselected markers (identifiable polypeptides) among the recombinant progenies. The use of multiple unselected markers for such studies was recommended rather than measurements of frequencies of recombination between selected markers (Honess et al., 1980). Also, it has been suggested that restriction enzyme site deletion mutants might facilitate study of recombination (Brown et al., 1984).

In the present work, intermolecular genetic recombination of two HSV-1 strains, differing in restriction enzyme cleavage sites, was studied using restriction fragment polymorphism as the
unselectable marker. Intermolecular recombination was observed uniformly on the HSV-1 genome and no definable increase of the recombination frequency was detected, even in the region containing the L-S junction.

METHODS

Cells and viruses. Vero cells were grown in Eagle's MEM supplemented with 5% calf serum.

HSV-1 strains used were Patton (Enquist et al., 1979) and Isolate No. 3 (Umene et al., 1984a); these two strains differ in at least eight restriction enzyme cleavage sites (Fig. 1). In the present report, strain Patton and Isolate No. 3 will be abbreviated as strain A and strain B, respectively.

Recombination and single clonal isolation of progeny viruses. A Vero cell monolayer (1 x 10^6 cells in a Falcon no. 3013 plastic tissue culture flask) was infected with the two HSV-1 strains, each at input multiplicity of 20 p.f.u./cell (Honess et al., 1980). After adsorption for 2 h at 37 °C, the infected monolayer was washed three times with MEM containing 2% foetal bovine serum (FBS), and overlaid with 5 ml MEM containing 2% FBS. After incubation at 37 °C for 14 h, progeny viruses were harvested.

The progeny virus stock was titrated, diluted appropriately, added to Vero cell suspensions, and distributed in microtest plates of 96 wells (Falcon no. 3072). Each well received a volume of 0.2 ml, containing 5 x 10^6 cells and 0.3 p.f.u. of virus. The cloned progeny viruses were recovered after the cytopathic effect became apparent (Manservigi, 1974).

Small-scale rapid isolation of HSV-1 DNA. A Vero cell monolayer (5 x 10^6 cells/culture) infected with a cloned HSV-1 stock was scraped off the culture bottle after confluent cytopathic effect, and the infected cells were collected by low-speed centrifugation in a microfuge tube (1.5 ml). The pellet was resuspended in 0.4 ml 0.01 M-Tris-HCl pH 8.0, 0.01 M-EDTA and lysed by adding 10% SDS to a final concentration of 0.6%. Five M-NaCl was added to a final concentration of 1.0 M, and the lysate was maintained overnight at 4 °C (Walboomers & Schegget, 1976). After centrifugation at 4 °C for 30 min at 15000 r.p.m., the cellular pellet was removed from the microfuge tube using a toothpick. The supernatant was extracted once with phenol, twice with phenol/chloroform/isoamyl alcohol (25:25:1), and once with chloroform. Two vol. ethanol was added to the final aqueous phase and the mixture was stored at -70 °C to precipitate the DNA.

Phages and plasmids. Hybrid phages (Enquist et al., 1979) and hybrid plasmids (Goldin et al., 1981) carrying HSV-1 DNA fragments and used for the preparation of probe DNA for Southern hybridization analysis, were as described (Umene et al., 1984a).

Restriction enzyme digestion, agarose gel electrophoresis and Southern hybridization. Restriction endonucleases were purchased from the Takara Shuzo Company (Kyoto, Japan) or the Nippon Gene Company (Toyama, Japan), and digestion conditions were as recommended by the manufacturers.

Agarose gel electrophoresis procedures were according to Denniston et al. (1981).

Southern hybridization was carried out on a Biodyne A transfer membrane (Pall Ultrafine Filtration Corp.) as described (Umene et al., 1984c).

RESULTS

Restriction fragment polymorphism and its stability

Restriction fragment polymorphism among HSV-1 isolates was analysed, and 13 distinctly variable regions, VR-1 to VR-13, were identified where a strain had a different restriction fragment compared to the prototype (Umene et al., 1984a). Of the 15 HSV-1 isolates analysed, strain Patton (strain A) and Isolate No. 3 (strain B) had at least seven differing restriction enzyme cleavage sites after digestions with BamHI [VR-2b, VR-6 on line (2) of Fig. 1], KpnI [VR-3, VR-9, VR-10 on line (3) of Fig. 1], and SacI [VR-8, VR-11 on line (4) of Fig. 1]. VR-11 is on the inverted repeat of the L component, and hence occurs twice in a HSV-1 genome. In addition to the seven restriction enzyme cleavage sites, a SacI site present in the DNA of strain B and absent from the DNA of strain A was found in the unique region of the S component [vertical arrow under line (1) of Fig. 1]. This SacI site is designated as SacI* in this work.

The stability of the restriction fragment polymorphism was examined by analysing the DNAs of 24 progeny viruses recovered after adding strain A or strain B to Vero cells at 40 p.f.u./cell. All progeny viruses had a restriction enzyme cleavage pattern identical to that of the parent virus.

Genome structure of recombinant viruses

Vero cells were co-infected with strains A and B, each at a multiplicity of infection of 20 p.f.u./cell. After single-clonal isolation from the progeny viruses generated by co-infection,
Fig. 1. Maps of HSV-1 DNA. (1) Structure of the HSV-1 genome. HSV-1 DNA consists of two covalently linked components designated L and S, comprising 82% and 18%, respectively, of the viral DNA. Each component consists of unique sequences (UL and US) bracketed by inverted repeat sequences (TRL, IRL, IRs and TRs). A short sequence, a, is repeated directly at the termini and is also present in the inverse orientation at the L-S junction (Roizman, 1979). A SacI site (SacI*) present in strain B and not present in strain A is shown by a vertical arrow [see panel (A) in Fig. 2] (Umene & Enquist, 1981). Strain A therefore had a 6.7 kb SacI fragment instead of two SacI fragments of 4.3 kb and 2.2 kb present in strain B. (2) BamHI cleavage sites differing between strains A and B (Umene et al., 1984a). The BamHI digests of DNA of strain A contain the 5.2 kb and 9.5 kb fragments instead of the 1.54 kb (A') and 11.2 kb (A) fragments of strain B (VR-2b). The site at which BamHI cleaves the 2.6 kb BamHI fragment (W + K') into two fragments of 2.1 kb (W) and 0.5 kb (K') is absent in DNA of strain A and present in strain B (VR-6). (3) KpnI restriction sites differing between strains A and B (Umene et al., 1984a). The KpnI site at which the 5.1 kb KpnI fragment (M) is cleaved into two fragments of 4.1 kb and 1.0 kb is present in DNA of strain A and absent in strain B (VR-3). The KpnI sites between the 10.0 kb (Aa) and 7.0 kb (Ab3) KpnI fragments, and the 7.0 kb (Ab3) and 1.5 kb (Y) KpnI fragments are present in DNA of strain B and absent from strain A (VR-9 and VR-10). (4) SalI restriction sites differing between strains A and B (Umene et al., 1984a). The SalI site which cleaves the 6.7 kb (K + C') SalI fragment into two fragments of 5.3 kb (K) and 1.4 kb (C') is present in DNA of strain A and absent in strain B (VR-8). The SalI sites between the 5.5 kb (I) and 9.3 kb (C) SalI fragments and the 5.5 kb (I) and 7.1 kb (F) SalI fragments on IRs are absent in DNA of strain A and present in strain B (VR-11). (5) Genome locations of EcoRI D, H, N and O fragments used as a probe for Southern hybridization analyses in Fig. 2 (Umene et al., 1984a).

DNAs of the cloned isolated viruses were analysed by digesting with SacI, BamHI, KpnI and SalI. Of 93 progeny clones examined, 22 clones had the same restriction enzyme cleavage pattern as strain A, and 20 clones had the same pattern as strain B. Fifty-one clones had patterns showing recombination between the two strains; these clones were numbered with the prefix N.

In Fig. 2 an example of an analysis of the genome structure of a recombinant clone, N.16, is presented. The SacI digests of N.16 contained 6.7 kb fragments as in strain A and did not contain the 4.3 kb and 2.2 kb fragments of strain B (panel A of Fig. 2). These findings indicated that the SacI* site of N.16 was derived from strain A. The BamHI cleavage pattern of N.16 was
Fig. 2. Restriction enzyme cleavage patterns characteristic of strains A and B, and a profile of restriction enzyme cleavage patterns of a recombinant clone with sequences derived from DNAs of strains A and B. (a) Southern hybridization profiles of SaeI (panel A), BamHI (panel B), KpnI (panel C) and SalI (panel D) digests of strain B (lanes 1), recombinant clone N. 16 (lanes 2) and strain A (lanes 3). The EcoRI H fragment of HSV-1 was used as a probe to the SacI digests (panel A). The EcoRI D and EcoRI O fragments of HSV-1 were used as a probe to the BamHI digests (panel B). The EcoRI N and KpnI (Ab3 + Y) fragments of HSV-1 were used as a probe to the KpnI digests (panel C). SalI C, SalI K and SalI C' fragments of HSV-1 were used as a probe to the SalI digests (panel D). (b) Schematic representation of the Southern hybridization profiles shown in (a). Sizes of the fragments are shown in kilobase pairs. Sizes of fragments that are characteristic of strains A and B are underlined.

The same as that of strain A (panel B of Fig. 2), thereby indicating that VR-2b and VR-6 of N.16 were derived from strain A. The KpnI cleavage pattern of N.16 was a mixture of those of strains A and B (panel C of Fig. 2). The KpnI digests of N.16 contained the 4-1 kb KpnI fragment characteristic of VR-3 of strain A and not the 5-1 kb KpnI fragment characteristic of VR-3 of strain B. In contrast, the KpnI digests of N.16 contained the 7-0 kb KpnI fragment characteristic of VR-9 of strain B and not the 18-6 kb KpnI fragment characteristic of VR-9 of strain A. The 1-5 kb KpnI fragment characteristic of VR-10 of strain B was also present in the KpnI digests of N.16. The SalI cleavage pattern of N.16 was also a mixture of those of strains A and B (panel D of Fig. 2). The SalI digests of N.16 contained the 6-7 kb SalI fragment characteristic of VR-8 of strain B and not the 5-3 kb and 1-4 kb SalI fragments characteristic of VR-8 of strain A. The ladder-like SalI fragments of 20-2 kb, 18-0 kb, 14-8 kb and 12-6 kb characteristic of VR-11 of strain A were present in the SalI digests of N.16 and the 9-3 kb and 7-1 kb SalI fragments characteristic of VR-11 of strain B were absent in the SalI digests of N.16. By way of summary, VR-2b, VR-3 and VR-6 were from strain A, VR-8, VR-9 and VR-10 were from strain B, and VR-11 and the SacI* site were from strain A. The recombinant clone N.16 was assumed to be generated after two crossover events between strains A and B, in regions between VR-6 and VR-8, and VR-10 and VR-11 (ninth line of Fig. 3).

The structures of the genomes of the recombinant clones were inferred from the patterns of segregation of the polymorphism markers as described above, and are summarized in Fig. 3. The number of crossover events was minimized in all recombinants. Some recombinant clones have similar structures; for example, the four recombinants N.33, N.89, N.109 and N.114 could not be differentiated. There were several pairs of recombinant clones which might be generated by a reciprocal crossover: N.3, N.86 to N.4, N.102, N.110; N.12, N.17 to N.84, N.99; N.33, N.89, N.104, N.114 to N.92, N.116; N.50, N.104 to N.52.
On the other hand, ambiguities remained for the genome structures of the recombinant clones with a heterozygous inverted repeat of the L component, as described below.

**Genome structure of a recombinant clone with an odd number of crossovers in UL and a heterozygous inverted repeat of the L component (pattern III)**

An example of this is the genome structure of recombinant clone N. 6, represented by symbols (two closed circles and parentheses) in the third line of Fig. 3. Four possible structures of this genome are shown in Fig. 4. N. 6 had two heterozygous VR-11. If the VR-11 from strain A was linked to VR-10 and the VR-11 from strain B was linked to VR-2b, the crossover event on the L component would occur only once between VR-3 and VR-6 [structures in lines (2) and (3) of Fig. 4]. On the other hand, if the VR-11 from strain A was linked to VR-2b and the VR-11 from strain B was linked to VR-10, two other crossover events would have to occur [structures in lines (4) and (5) of Fig. 4]. The structures in lines (2) and (3) were more probable than those in lines (4) and (5), though there was the possibility of an occurrence of two extra crossover events.

The SacI* site in the S component of N. 6 can be linked to either the VR-11 from strain A or the VR-11 from strain B. If the VR-11 from strain B was linked to the SacI* site from strain B, there would be no further crossover events [structures in lines (2) and (5) of Fig. 4]. If the VR-11 from strain A was linked to the SacI* site from strain B, there must have been a crossover event [structures in lines (3) and (4) of Fig. 4]. The parentheses indicate the existence of at least two possibilities, the presence or absence of the crossover event between the VR-11 and the SacI* site. The structure in line (2) of Fig. 4 had a minimum number of crossover events.

**Genome structure of a recombinant clone with zero or an even number of crossovers in UL and a heterozygous inverted repeat of the L component (pattern IV)**

An example of this is the genome structure of recombinant clone N. 8, represented by symbols (two open circles, a horizontal arrow and parentheses) in the fourth line of Fig. 3. N. 8 had a heterozygous VR-11 and there were two possible explanations. One was that the VR-11 from strain A was linked to VR-10 and the VR-11 from strain B (represented as a horizontal arrow) linked to VR-2b [structures in lines (2) and (3) of Fig. 5]. The other was that the VR-11 from strain A was linked to VR-2b and the VR-11 from strain B was linked to VR-10 [structures in lines (4) and (5) of Fig. 5]. The parenthesis between VR-11 and the SacI* site of N. 8 indicates two possibilities; one was the presence of a crossover between the VR-11 and the SacI* site [structures in lines (3) and (5) of Fig. 5] and the other was the absence of the crossover [structures in lines (2) and (4) of Fig. 5]. Structures which minimized the number of crossover events were those in lines (2) and (4) of Fig. 5.

**Recombination frequency**

The recombination frequencies on the HSV-1 genome were calculated as follows.

**Recombination frequency in the unique sequence of the L component**

There were six markers in UL and the distances between two neighbouring markers were considered as a percentage of the total genome length of HSV-1 (Table 1). The number of crossover events in UL of the recombinant clones in Fig. 3 was counted for each region separated by two neighbouring markers (Table 1). The distance from VR-2b to VR-10 was 51.8% of genome length and the total number of crossover events observed between VR-2b and VR-10 was 54 among the 93 isolates examined. The recombination frequency in UL was calculated to be 0.0112 (= 54/(93 x 51.8)) per 1% of genome length (1.12 per genome length) or 0.007 per kilobase pair. The expected numbers of crossover events between the two neighbouring markers were also calculated, on the hypothesis that recombination in UL occurs evenly (Table 1). To test the hypothesis, the Chi-square method was used (Stansfield, 1969). The value of $\chi^2$ was 4.85, and the number of degrees of freedom was 4. The $\chi^2$ value corresponds to the probability 0.30, greater than 0.05, and the data were regarded as being generally in agreement with the expectation. Therefore, a feasible hypothesis is that recombination in UL occurs evenly.
Fig. 3. Structures of recombinant clones between strains A and B. The number of crossover events is minimized. The recombinant numbers are given in the left column. The markers of restriction enzyme cleavage sites differing between the two strains are shown on the top line in the P arrangement (Roizman, 1979). The two markers of VR-11 on the inverted repeats of the L component are shown on the right side only. The sequence of a recombinant clone is shown by a horizontal line, and the higher and lower lines of each recombinant represent the sequences derived from strain A and strain B, respectively. Slanting dotted lines represent crossover regions. Parentheses represent a region between VR-11 and the SacI* site, where two lines facing each other at the parenthesis should be joined to
Intermolecular recombination of HSV-1

(1) Intermolecular recombination of HSV-I VR-2b VR-3 VR-6 VR-8 VR-9 VR-10 VR-11 S

(2) A B

(3) S VR-11 VR-2b VR-3 VR-6 VR-8 VR-9 VR-10 VR-11

(4) S VR-11 VR-2b VR-3 VR-6 VR-8 VR-9 VR-10 VR-11

(5) A B

Fig. 4. Possible genome structures of the recombinant clone having an odd number of crossover events in UL and a heterozygous IRL. (pattern III). (1) Representation of the genome of recombinant clone N. 6, using symbols as in the third line of Fig. 3. Four possible structures for N. 6, as a representative of this class of recombinants, are shown on lines (2) to (5). (2) The structure of N. 6 with the minimum number of crossover events (one crossover). The structure is in the I_{L} or I_{SL} arrangement. (3) The structure of N. 6 with an additional crossover event in the region between VR-11 and the SacI* site. The structure is in the P or I_{PL} arrangement. (4) The structure of N. 6 with additional crossover events in the regions between VR-11 and VR-2b, and VR-11 and VR-10, as well as between VR-11 and the SacI* site. The structure is in the I_{L} or I_{SL} arrangement. (5) The structure with crossover events in the regions between VR-11 and VR-2b, and VR-11 and VR-10, as well as the crossover in the region between VR-3 and VR-6. The structure is in the P or I_{PL} arrangement.

Recombination frequency in the region containing the inverted repeat of L component

The eight recombinant clones represented as a structure with two X symbols in Fig. 3 had a definite crossover between VR-11 and VR-2b (pattern I). The five recombinant clones whose genome structure includes an oblique dotted line between VR-11 and VR-10 in Fig. 3 had a definite crossover between VR-11 and VR-10 (pattern II). The 15 recombinant genomes represented as a structure with two closed circles could have arisen in two ways: no crossover or a crossover both between VR-11 and VR-2b, and VR-11 and VR-10 (see Fig. 4) (pattern III). The 16 recombinant clones represented as a structure having two open circles had a crossover either between VR-11 and VR-2b, or VR-11 and VR-10 (see Fig. 5) (pattern IV). In the cases of patterns III and IV, it is difficult to differentiate the two crossover events between VR-11 and
Fig. 5. Possible genome structures of the recombinant clone having zero or an even number of crossover events in UL and a heterozygous IR \(_L\) (pattern IV). (1) Representation of the genome of recombinant clone N. 8, using symbols as in the fourth line of Fig. 3. Four possible structures for N. 8, as a representative of this class of recombinants, are shown on lines (2) to (5). (2) The structure of N. 8 with a crossover in the region between VR-2b and VR-11. The number of crossover events is the minimum. (3) The structure of N. 8 with an additional crossover in the region between VR-11 and the \(\text{SacI}^*\) site as well as the crossover in the region between VR-2b and VR-11. (4) The structure of N. 8 with a crossover in the region between VR-10 and VR-11. The number of crossover events is the minimum, as for (2). (5) The structure of N. 8 with an additional crossover in the region between VR-11 and the \(\text{SacI}^*\) site as well as the crossover in the region between VR-10 and VR-11.

Table 1. Crossover events in the unique sequence of the \(L\) component of HSV-1

<table>
<thead>
<tr>
<th>Markers on UL</th>
<th>VR-2b</th>
<th>VR-3</th>
<th>VR-6</th>
<th>VR-8</th>
<th>VR-9</th>
<th>VR-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance between two markers (% of genome length)</td>
<td>15.3</td>
<td>13.5</td>
<td>13.2</td>
<td>5.4</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Observed number of crossovers in UL</td>
<td>17</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Expected number of crossovers in UL</td>
<td>15.9</td>
<td>14.0</td>
<td>13.7</td>
<td>5.6</td>
<td>4.6</td>
<td></td>
</tr>
</tbody>
</table>

VR-2b, and VR-11 and VR-10. Therefore, the crossover events between VR-11 and VR-2b, and VR-11 and VR-10 were managed together in this study (Table 2). The recombination frequency between VR-11 and VR-2b, and VR-11 and VR-10 was designated \(x\) to calculate the crossover events of pattern III. The number of recombinant clones of pattern III having a crossover both between VR-11 and VR-2b, and VR-11 and VR-10 was estimated to be \(15x^2\) and the number of crossover events was \(30x^2\) as each recombinant had two crossover sites. The total number of recombinant clones of patterns I, II, and IV was 29. Thus, \(x = (30x^2 + 29)/93; x = 0.35, 2.75.\)
Table 2. Crossover events in the regions containing the inverted repeat of the L component and the L–S junction of HSV-1

<table>
<thead>
<tr>
<th>Distance between two markers</th>
<th>Observed number of crossovers</th>
<th>Expected number of crossovers</th>
</tr>
</thead>
<tbody>
<tr>
<td>VR-11 and VR-2b, VR-11 and VR-10</td>
<td>24.4 (29.5)†</td>
<td>12.4 (20.1)</td>
</tr>
<tr>
<td>VR-11 and SacI*</td>
<td>32.6</td>
<td>12</td>
</tr>
<tr>
<td>VR-11 and VR-10</td>
<td>25.4 (30.8)</td>
<td>12.9 (21.0)</td>
</tr>
</tbody>
</table>

† Figures in parentheses are the modified values calculated giving consideration to the diploidy of the inverted repeats of the L and S components, as explained in the text.

The number of recombinant clones of pattern III having a crossover event both between VR-11 and VR-2b, and VR-11 and VR-10 was calculated to be 18 (=15 × 0.35²), and the number of crossover events was 3.6. The observed number of crossover events between VR-11 and VR-2b, and VR-11 and VR-10 was estimated to be 32.6 (=3.6 + 29) (Table 2). Under the hypothesis that crossing over occurs evenly on the HSV-1 genome at the same frequency as that observed in U₅, the expected number of crossover events between VR-11 and VR-2b, and VR-11 and VR-10 was calculated to be 25.4 [= (54 × 24.4)/51.8] (Table 2). The existence of a pair of inverted repeats at both ends of the unique sequence would make it possible for the inverted repeats to face each other homologously and to recombine, even if two HSV-1 molecules had been arranged in opposing directions. Taking this into consideration, the distance between VR-11 and VR-2b, and VR-11 and VR-10 would be changed to 29.5% of genome length (from 24.4%), and the expected number of crossover events would be 30.8 [= (54 × 29.5)/51.8] (numbers in parentheses in Table 2). The observed number of crossover events in the regions between VR-11 and VR-2b, and VR-11 and VR-10 did not diverge from the expected number. These results suggest that the recombination frequency in the regions between VR-11 and VR-2b, and VR-11 and VR-10 was comparable to that observed in U₅.

Recombination frequency in the region containing the L–S junction

The S component may be connected to the L component in two different directions, and the average distance from a point in the unique sequence of the S component to the L–S junction is constant, that is 9% of genome length. Therefore, the distance between VR-11 and the SacI* site was 12.4% (= 3.4% + 9%) of genome length (Table 2). Eight recombinant clones had a definite crossover event in the region between VR-11 and the SacI* site, and 31 recombinant clones represented with parentheses (patterns III and IV) might have a crossover point in the region between VR-11 and the SacI* site (Fig. 4 and 5). The recombination frequency in the region between VR-11 and the SacI* site was designated y to calculate the crossover events on the recombinant clones represented with parentheses (patterns III and IV). Thus, y = (31y + 8)/93; y = 0.13.

The number of recombinant clones with a crossover event in the region between VR-11 and the SacI* site was calculated to be 12 [= (31 × 0.13) + 8] (Table 2). The expected number of the crossover events was 12.9 [= (54 × 12.4)/51.8], on the supposition that recombination in the region between VR-11 and the SacI* site occurs at the same frequency as that observed in U₅ (Table 2). The above calculation was made considering only two molecules arranged in the same direction. A homologous pair might also be formed between the inverted repeats of the L and S components, even if the two molecules should be arranged in opposite directions. The total distance between the VR-11 and the SacI* site of the two molecules arranged in either orientation was 20.1% of genome length, and the expected number of the crossover events in these directions was 21.0 [= (54 × 20.1)/51.8] (numbers in parentheses in Table 2). These results indicate that the recombination frequency in the region involving the L–S junction was similar to or less than that of U₅ of the HSV-1 genome, and that the presence of the L–S junction had no evident enhancing effect on intermolecular recombination.
DISCUSSION

The intermolecular recombination of HSV-1 was studied by analysing the segregation of eight restriction enzyme cleavage sites which differed between two strains, as unselectable markers, among progeny viruses generated after co-infection by the two strains. The linkage of the unselected markers in UL, measured by counting the crossover events, was in accord with the physical positions of the markers on the HSV-1 genome, and the results suggested a uniform occurrence of crossover events in UL (Table 1). Crossover events between two HSV-1 strains, differing in a restriction enzyme cleavage site and a deletion at a distance of 42% of genome length, were observed in 13 out of 40 progeny viruses examined, and the recombination frequency can be estimated to be 0.005 per kilobase pair (Roizman, 1979). In my work, the recombination frequency of HSV-1 was calculated to be 0.007 per kilobase pair, by using six markers on UL. The lower value for the recombination frequency in Roizman's work might be due to a loss of the recombinants having two crossover events between the two markers separated by 42% of the genome length. The recombination frequency between two temperature-sensitive mutants of human type 5 adenoviruses (H5ts1 and H5ts2) was reported to be 3.1% at 54 h post-infection, and the recombination frequency can be calculated as 0.005 per kilobase pair, if the distance between the two markers was 6.3 kb (Young & Silverstein, 1980). The recombination frequencies for these viruses are in good agreement, and a recombination frequency of 0.005 to 0.007 per kilobase pair might be general in homologous intermolecular recombination of viral DNAs in mammalian cells. The recombination frequency within the TK gene of HSV-1 was estimated to be 0.032 per kilobase pair as a minimal value, by counting the number of TK+ progeny after crosses between pairs of TK− mutants (Smiley et al., 1980). The discrepancy of the recombination frequencies may be due to differences in distances between markers, or to regional specificity. Owing to the loss of double crossover events, the recombination frequency obtained in this work may be underestimated, and there could be cryptic short stretches showing enhanced recombination, detectable only by analysing recombination frequency between closely situated markers.

The isomerization caused by the inversion of the L and S components in the HSV-1 genome is a striking phenomenon (Mocarski et al., 1980; Mocarski & Roizman, 1981, 1982a, b; Davison & Wilkie, 1981, 1983a). Whether or not the L−S junction is a recombinational 'hot spot' is a subject of interest. The region containing the L−S junction was found to be similar to other regions of the HSV-1 genome, with regard to intermolecular recombination (Table 2), and this observation lends support to the idea that isomerization is caused by an intramolecular event rather than an intermolecular one (Roizman, 1979). Umene et al. (1984b) reported the presence of a variable number of the 15 bp tandem repeated DNA sequences in the BamHI Z fragment (map coordinates 0.936 to 0.949) of HSV-1 (Patton), and a novel derivative was isolated with tandem duplications generated by a non-homologous recombination between a site within the 15 bp tandem repeat and a site within the inverted repeat of the S component (Umene & Enquist, 1985). The observations suggested that the 15 bp tandem repeat was recombinogenic in the HSV-1 genome. Evidence supporting a high frequency of occurrence of intermolecular recombination in the S component was not obtained in this work, and it is assumed that the recombinations involving the 15 bp sequence occurred as intramolecular events rather than as intermolecular events, such as is the case with L−S inversion.

Here, studies were done to determine whether the recombinants represented in Fig. 3 were generated from one or from two arrangements of the L component. If the recombinants were generated from only the P or Is arrangements, then the recombination frequencies between the markers on UL and the SacI* site of the S component would not be uniform, but highest between VR-2b and the SacI* site and lowest between VR-10 and the SacI* site. The numbers of recombinant clones showing segregation of a marker on UL from the SacI* marker were 28 (VR-2b), 27 (VR-3), 23 (VR-6), 19 (VR-8), 21 (VR-9) and 22 (VR-10) (Fig. 3). The segregation frequencies were rather even on UL, leaving open the possibility that the segregation of VR-2b and VR-3 from the SacI* site might have been higher than that of others. Morse et al. (1977) and Preston et al. (1978) predicted that if recombinational events occur among all forms of HSV-1 DNA, there should be no single arrangement of recombinant DNAs with an odd number of
crossover events. In the case of intertypic (HSV-1 × HSV-2) crosses, recombinants arising from only the P or I₅ arrangements of parental DNA were isolated and the hypothesis that only one or two arrangements of the parental DNA participated in the generation of recombinants was proposed (Morse et al., 1977; Preston et al., 1978). In the present study, 15 recombinants having an odd number of crossover events in U₅ and heterozygous inverted repeats of the L component (represented by two closed circles and parentheses in Fig. 3) (pattern III, see Fig. 4) were isolated. Of the 15, five recombinants (N. 6, N. 21, N. 49, N. 61 and N. 51) were assumed to be generated from the I₅ or IₛL arrangements. For example, the recombinant N. 6 could have been derived from four possible structures, and the structure with a minimum number of crossover events could be generated from I₅ or IₛL arrangements, as shown on line (2) of Fig. 4. Similarly, ten other recombinants (N.10, N. 26, N. 50, N. 104, N. 52, N. 62, N. 70, N.103, N.106 and N. 94) were assumed to be from the P or I₅ arrangements. These results indicate that intratypic recombinants of HSV-1 can be generated from all four forms of DNA, although there is the possibility of a preference for P or I₅ arrangements over I₅ or IₛL conformations. This is in agreement with a report by Davison & Wilkie on intertypic recombination (1983b).

The mechanisms mediating intermolecular recombination of HSV-1 are unknown. It has been suggested that the genome might contain a gene encoding a general or site-specific recombination function, and a trans-acting viral gene product which might cause the L-S inversion has been proposed (Mocarski & Roizman, 1982a). Two HSV-1-induced proteins (21K and 22K) which interacted specifically with the a sequences of HSV-1 DNA (although their functions are unknown), have been identified by Dalziel & Marsden (1984). In addition to virus-encoded functions, genetic recombination pathways in host cells might affect recombination in HSV-1 (Dasgupta & Summers, 1980).

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REFERENCES


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