Either Orientation of the L Segment of the Herpes Simplex Virus Genome May Participate in the Production of Viable Intertypic Recombinants

(Accepted 27 August 1982)

SUMMARY

We have analysed the genome structures of 90 recombinant viruses produced by coinfection of baby hamster kidney cells with the DNA of a herpes simplex virus type 1 temperature-sensitive mutant (tsD) and specific DNA fragments of wild-type herpes simplex virus type 2 at non-permissive temperature. Crossovers were located predominantly in regions of greatest intertypic homology, and we conclude that primary recombination occurred with the L segment of the genome in either orientation.

Recombination between herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2) has been described by Wildy (1955) and Timbury & Subak-Sharpe (1973). Recombination may occur throughout the genome, and several laboratories have made excellent use of intertypic recombinants to map physically HSV mutations and gene functions on the genome by virtue of the distinctive restriction endonuclease fragments and polypeptide profiles exhibited by each serotype. Recombinants have been isolated as a result of marker rescue by mixed virus infection or by DNA co-infection. Little is known of the recombination mechanism, although intratypic recombination in HSV-1 seems to be a stochastic process involving the formation of partial heterozygotes (Brown & Ritchie, 1975).

HSV-1 and HSV-2 DNAs are homologous in approximately 50% of their sequences (Kieff et al., 1972), and both a genetic approach (Esparza et al., 1976) and studies of DNA homology (Wilkie et al., 1979; A. J. Davison & N. M. Wilkie, unpublished results) indicate that the two genomes are colinear. The two viruses share a common genome structure which comprises two covalently linked segments [L, 120 kilobase pairs (kbp) and S, 25 kbp], each of which consists of a unique sequence (UL, 99 kbp; US, 13 kbp) bounded by inverted repeats (TRL and IRS, 10-5 kbp; TRS and IRS, 6-0 kbp). Double-stranded virion DNA contains equimolar amounts of the four genome arrangements which result from inversion of the L and S segments about their mutual joint. The arrangements are termed P (prototype), IL (L inverted), IS (S inverted) and ISL (L and S inverted). Whereas earlier indirect experiments suggested that only arrangements containing one particular orientation of L are able to participate in the generation of viable recombinants, we show directly that both are able to take part in marker rescue of a mutant genome by restriction fragments.

The lesion in the HSV-1 strain 17 temperature-sensitive mutant tsD maps in the Vmw IE 175 gene in TRS/IRS (Preston, 1981). Monolayers of BHK-21 C13 cells in fifteen 50 mm Petri dishes were co-infected with tsD DNA and an equimolar mixture of HSV-2 strain HG52 EcoRI b, c, d and e fragments isolated from agarose gels (Cortini & Wilkie, 1978). These four restriction fragments span the HSV-2 L-S joint, extending into UL and US, and each originates from one of the four genome arrangements. Coinfection was done by calcium phosphate precipitation and dimethylsulphoxide boost (Stow & Wilkie, 1976). The monolayers were incubated at the non-permissive temperature (38.5 °C) under the Glasgow modification of Eagle's medium (Busby et al., 1964) containing 5% (v/v) pooled human serum, and 6 well-separated plaques were picked from each dish. The 90 plaques were subjected to two further rounds of plaque purification at 38.5 °C. Virus stocks were prepared in BHK cell monolayers on 50 mm Petri dishes containing the Glasgow modification of Eagle's medium plus 5% (v/v) calf serum. The stocks were titrated, and in vivo-labelled DNA was prepared using [32P]orthophosphate (PBS 11; Amersham International) by the method of Preston et al. (1978). Labelled DNA from HSV-1, HSV-2 and recombinant viruses was digested with XbaI, HindIII, EcoRI, BglII, Hpal, KpnI or BamHI.
Table 1. Genome arrangement of HSV-1 tsD rescued by HSV-2 EcoRI fragments

<table>
<thead>
<tr>
<th>Arrangement of genome</th>
<th>Rescuing HSV-2 EcoRI fragment</th>
<th>Crossovers</th>
<th>Number of recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>c</td>
<td>U_L and U_S</td>
<td>1</td>
</tr>
<tr>
<td>L</td>
<td>e</td>
<td>U_L and U_S</td>
<td>0</td>
</tr>
<tr>
<td>I_L</td>
<td>b</td>
<td>U_L and U_S</td>
<td>13</td>
</tr>
<tr>
<td>I_SL</td>
<td>d</td>
<td>U_L and U_S</td>
<td>0</td>
</tr>
<tr>
<td>L in P arrangement, S unspecified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L inverted, S unspecified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S in P arrangement, L unspecified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S inverted, L unspecified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neither orientation determined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninterpreted structures</td>
<td>Undetermined</td>
<td>Undetermined</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>90</td>
</tr>
</tbody>
</table>

(Bethesda Research Laboratories) and the products were subjected to agarose gel electrophoresis. The gels were dried and autoradiographed, and genome structures were analysed by scoring the presence or absence of parental restriction sites, as described by Preston et al. (1978). All the crossovers mapped within the EcoRI fragments b, c, d and e of the rescuing HSV-2, and the genome structures of six isolates from any single Petri dish suggested that they were not related.

Marker rescue of HSV-1 tsD by HSV-2 DNA fragments which span the L-S junction resulted initially in regions of heterology between one or both sets of inverted repeats, but at the stage of genome analysis a primary crossover in one repeat was usually present in the complementary repeat because of secondary recombination events. Thus, if a primary crossover occurred in an inverted repeat of a segment it is not possible, in the great majority of cases, to state the orientation of that segment during primary recombination. Nevertheless, recombination events in U_L or U_S are unambiguously preserved regardless of further crossovers in the inverted repeats. Therefore, careful analysis of the recombinant genomes from marker rescue of tsD allowed either the orientation of both L and S at primary recombination to be deduced (crossovers in U_L and U_S), or the orientation of S only (crossovers in TR_L/IR_L and U_S), or the orientation of L only (crossovers in U_L and TR_L/IR_L and U_S), or neither orientation (crossovers in TR_L/IR_L and TR_S/IR_S, or TR_S/IR_S and TR_S/IR_S only).

Table 1 shows the numbers of recombinants representing each arrangement of the rescued genome. It is clear from these results that tsD was rescued with the genome in both the P and I_L arrangements, and that all the observed crossovers could have occurred between tsD and HSV-2 EcoRI b or c. It cannot be concluded, however, that recombination in the I_S or I_SL arrangements (with HSV-2 EcoRI d or e) did not occur, because the majority of recombinants possessed symmetrical crossovers in TR_S/IR_S and therefore might equally well have been the result of rescue by HSV-2 EcoRI d or e. The position of the lesion in tsD in TR_S/IR_S means that, upon rescue of this mutation, at least one crossover event in S is obligatory, whereas a concurrent event in L is optional. It may be concluded that at least the P and I_L arrangements of the genome participate in recombination.

A second feature of recombination is revealed by these results. About 10 times as many recombinants resulted from marker rescue of the I_L arrangement compared with the P arrangement, although HSV-2 EcoRI b and c differ in size by only about 5%. The distribution of primary crossover positions in U_L is shown in Fig. 1. Two recombinants possessed symmetrical crossovers in TR_L/IR_L, so it was assumed for the purposes of Fig. 1 that the primary event could have occurred with equal probability in either TR_L or IR_L. We have located the regions of greatest homology between the HSV-1 and HSV-2 genomes (A. J. Davison & N. M. Wilkie, unpublished results) and the crossovers seem to be located preferentially in a region adjacent to TR_L possessing greatest intertypic homology.
Previous analyses of the numbers of crossovers in recombinants have suggested that only one or both of the P and Iₜ arrangements participates in recombination (Morse et al., 1977; Preston et al., 1978; Roizman et al., 1979; Wilkie et al., 1979). The evidence is that genomes possessing an odd number of crossovers in L display the least number of crossovers in the P or Iₜ genome arrangements. The argument has three weaknesses, which were emphasized by its proposers. Firstly, it is based on a small number of recombinants with an odd number of crossovers in UL: 9/62 recombinants produced from genetic crosses in the studies of Morse et al. (1977), Preston et al. (1978), Marsden et al. (1978) and Halliburton et al. (1980). The assumption that an odd number (n) of recombination events in the genome is more likely to occur than an even number (n + 1) must be viewed with caution since the majority of recombinants (44/62) possess even numbers of crossovers. Secondly, undetected crossovers in recombinants with odd numbers of crossovers in L could reduce the number put forward as evidence. At least two have additional crossovers near the L terminus (A. J. Davison & N. M. Wilkie, unpublished results). Thirdly, the way in which recombinants were made may have biased the genome arrangements involved in recombination. Intertypic recombination in HSV appears to be homology-dependent, and therefore the structures of recombinant genomes are dictated not only by the particular mutants used in a genetic cross and by the sequences available for recombination in the case of marker rescue with restriction fragments, but also by the extent and distribution of homology within those sequences.

The second argument in favour of the hypothesis that only one orientation of L takes part in recombination stems from the work of Parris et al. (1980), who claimed to be able to correlate the genetically- and physically-determined mapping positions of several mutations. They concluded that only one or both of the Iₜ and Iₜₛ arrangements participates in genetic recombination. Clearly, this is the opposite conclusion from that attained by argument from recombinant genome structures.

We have shown that genome arrangements with L in either orientation are able to recombine. Although the mechanisms of recombination in mixed virus infection and DNA fragment marker rescue may differ, in the absence of any evidence to this effect we see at present no good
reason for supposing that not all four arrangements are able to recombine. Honess et al. (1980) presented a detailed genetic analysis of several HSV-1 markers, and concluded that recombination in HSV occurs via a circular or concatemeric mechanism. Two features of this model are that it better accommodates inclusion of all four arrangements in recombination and that all recombinants conceptually have an even number of crossovers.

We are grateful to Professor J. H. Subak-Sharpe for useful discussion. During the course of this work N.M.W. was a member of the staff of the MRC Virology Unit, Glasgow.

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REFERENCES


(Received 2 August 1982)