A Search for Recombination between Temperature-Sensitive Mutants of Vesicular Stomatitis Virus

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Studies of genetic recombination have been of considerable value in elucidating the structure and function of bacterial viruses and have also been of value for certain animal viruses (e.g. see Fenner, 1970). Pringle (1970) has reported recombination between ts mutants of vesicular stomatitis virus (VSV) when mutants from different complementation groups were used in mixed infections. We have looked for recombination in our collection of VSV mutants (Holloway, Wong & Cormack, 1970), also, and have obtained the results presented in this paper.

L-cell and virus stocks were maintained and assayed using the methods previously described (Holloway et al. 1970). For the present experiments, cell monolayers were prepared by seeding each of a number of 2 oz. Brockway bottles with $2.5 \times 10^6$ cells. For mixed infection $2.5 \times 10^7$ p.f.u. of each mutant were added in a total volume of 0.5 ml, giving a total m.o.i. of 20. For the single infections m.o.i. of 20 was used. The virus was allowed to adsorb for 25 min. at 38°C, the monolayer was then washed, 5 ml of medium was added and the infected bottles were incubated for 8-5 hr at 30°C, the permissive temperature for virus replication. The medium was then centrifuged at 500g for 5 min. to remove floating cells which might be mixedly infected and able to form a plaque at 38°C by complementation.

For convenience in the following discussion the plaques observed in the assay plate incubated at 30°C will be referred to as 30° plaques and those observed at 38°C as 38° plaques. Fig. 1 shows results obtained for single and mixed infections using mutants ts 14 and ts 16B and are expressed as the number of 38° plaques observed per dish plotted against the number of 30° plaques per dish. Mutants ts 14 and ts 16B have been shown to complement in mixed infections at 38°C (P. K. Y. Wong, A. F. Holloway & D. V. Cormack, unpublished results). The points in Fig. 1 (A) are for the medium harvested from cells mixedly infected with ts 14 and ts 16B and indicate that 0.8% of the progeny are able to form plaques at 38°C. Results (B) and (C) were obtained from single infections with ts 14 and ts 16B respectively. Re-plating of suspensions of these 38° plaques from the single infections showed that the viruses they contained were ts+, i.e. able to form plaques equally well at 38°C and 30°C and are presumably revertants of the ts parent. The number of 38° plaques is directly proportional to the number of 30° plaques and accounts for 0.02% and 0.001% of the total progeny for ts 14 and ts 16B respectively. In addition to those due to revertants, 38° plaques might be expected in the mixed infection yield by random co-infection of some cells on the assay plate by two complementing ts mutants. To investigate this effect with mutants ts 14 and ts 16B, stocks with equal titres were mixed and various dilutions were plated at 30°C and 38°C. The results are shown in Fig. 1 D. Whereas the data in A, B and C indicate a direct proportionality between 38°C plaques and 30° plaques the data in D are closely fitted by a 'two-hit' curve, i.e. one in which the number of 38°C plaques is proportional to the square of the number of 30° plaques. Such a relation is expected since at least two virus particles are required per cell in order to have complementation. The straight line in D was calculated assuming that there are $3 \times 10^6$ cells per assay plate and that every mixedly infected cell gives rise to a plaque. The actual number of cells per dish is about $2 \times 10^6$ cells and the dif-
ference between this and the value of $3 \times 10^6$ used in fitting the data in D probably reflects the fact that not all mixedly infected cells give rise to observable plaques. A comparison of curves D and A indicate that only a small percentage of the $38^\circ$ plaques observed in the products of mixed infection are due to complementation in cells on the assay plates.

Fig. 1. Number of plaques formed in monolayers incubated at $38^\circ$ as a function of the number of plaques observed or expected if the monolayer were incubated at $30^\circ$. $\bullet\bullet\bullet$, Mixed infection $ts14 \times ts16B$; $\triangledown\triangledown\triangledown$, infection with $ts14$; $\triangle\triangle\triangle$, infection with $ts16B$; $\times\times\times$, in vitro mixture of $ts14$ and $ts16B$ made before plating.

Table 1. Complementation levels and $38^\circ/30^\circ$ plaque ratios in mixed infections

<table>
<thead>
<tr>
<th>Group</th>
<th>$ts$ mutants</th>
<th>$ts10$</th>
<th>$ts16B$</th>
<th>$ts12$</th>
<th>$ts4$</th>
<th>$ts8$</th>
<th>$ts11$</th>
<th>$ts14$</th>
<th>$ts18$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$ts10$</td>
<td>0.001</td>
<td>1.00</td>
<td>40.00</td>
<td>1000</td>
<td>26.00</td>
<td>20.00</td>
<td>30.00</td>
<td>9.00</td>
</tr>
<tr>
<td>I</td>
<td>$ts16B$</td>
<td>0.001</td>
<td>0.001</td>
<td>11.00</td>
<td>100.00</td>
<td>30.00</td>
<td>40.00</td>
<td>100.00</td>
<td>20.00</td>
</tr>
<tr>
<td>II</td>
<td>$ts12$</td>
<td>0.9</td>
<td>0.9</td>
<td>0.001</td>
<td>5.00</td>
<td>1.2</td>
<td>3.00</td>
<td>8.00</td>
<td>3.00</td>
</tr>
<tr>
<td>III</td>
<td>$ts4$</td>
<td>0.7</td>
<td>1.0</td>
<td>0.002</td>
<td>0.001</td>
<td>1.3</td>
<td>0.6</td>
<td>0.5</td>
<td>5.0</td>
</tr>
<tr>
<td>III</td>
<td>$ts8$</td>
<td>0.6</td>
<td>1.2</td>
<td>0.01</td>
<td>0.002</td>
<td>0.04</td>
<td>0.8</td>
<td>0.6</td>
<td>1.7</td>
</tr>
<tr>
<td>III</td>
<td>$ts11$</td>
<td>0.9</td>
<td>1.3</td>
<td>0.001</td>
<td>0.002</td>
<td>0.02</td>
<td>0.01</td>
<td>0.3</td>
<td>1.2</td>
</tr>
<tr>
<td>III</td>
<td>$ts14$</td>
<td>0.5</td>
<td>1.0</td>
<td>0.001</td>
<td>0.001</td>
<td>0.01</td>
<td>0.01</td>
<td>0.06</td>
<td>1.0</td>
</tr>
<tr>
<td>III</td>
<td>$ts18$</td>
<td>0.7</td>
<td>0.8</td>
<td>0.01</td>
<td>0.001</td>
<td>0.02</td>
<td>0.001</td>
<td>0.01</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The numbers in bold type are ratios of yields at $38^\circ$ in mixed infection and single infection. The other numbers give the ratios of number of $38^\circ$ plaques to $30^\circ$ plaques in the yield of a mixed infection at $30^\circ$.

The temperature-sensitivity of the viruses in the $38^\circ$ plaques from the mixed infection was then examined. Plaques arising from recombination would be expected to consist primarily of $ts^+$ particles. To our surprise, however, more than 90% of the viruses in the plaques tested (9/9) were unable to produce plaques when re-plated and incubated at $38^\circ$. Further analysis showed that most of these plaques contained virus of both mutant types, $ts14$ and $ts16B$. These results show that with these mutants few, if any, of the $38^\circ$ plaques are the result of genetic recombination during the mixed infection. Recombination at a frequency of $0.1\%$ or less might, however, have been undetected. The linear relation between the $38^\circ$ and $30^\circ$ assays indicate, however, that they can be produced by single infectious
units. These units may be mixed aggregates of virions of the two parental types or 'heteroploidy's', i.e. particles in which genomes of both parents are enclosed within one viral envelope (Fenner, 1970). These aggregates or 'heteroploidy's' may be related to the elongated particles and chains of particles observed during budding by Galasso (1967) and by Howatson (1970). In an attempt to dissociate possible aggregates the medium from the mixed infection was subjected to sonication or incubation at 37°C for 1 hr. These treatments failed, however, to produce appreciable changes in either the slope or position of curve A. It has therefore not been possible to distinguish between aggregates and 'heteroploidy's'.

Similar experiments were performed with various other pairs of ts mutants and the results are shown in Table 1. The values below the diagonal give the ratio of 38°C plaques to 30°C plaques after correcting for revertants. Complementation data (P. K. Y. Wong, A. F. Holloway & D. V. Cormack, unpublished results) for these same pairs of mutants are given above the diagonal, expressed as complementation levels (ratio of the yield at 38°C in mixed infection to that in single infection). Mutant ts10 and ts16B form a distinct complementation group, since they complement all other mutants and do not complement each other. The remaining mutants show some complementation among themselves but generally at a much lower level. The table shows a very similar pattern in the ratios of 38°C and 30°C plaques in the yields of mixed infection at 30°C. Here, also, ts10 or ts16B, when combined with any mutant outside their group give yields with net plaque ratios of about 1% whereas all other pairs give plaque ratios of 0-02% or less. As in the ts14 and ts16B mixed infection, the 38°C plaques from mixed infections with either ts10 or ts16B were found on re-plating to be made up largely of temperature-sensitive virus. There appears, therefore, to be no evidence of genetic recombination within this group of eight mutants representing the three complementation groups to which we have tentatively assigned our 21 ts mutants of VSV. The high proportion of 38°C plaques for mixed infections involving either ts10 or ts16B is probably another manifestation of the complementation behaviour of these mutants brought about by aggregation of virus particles or the presence of heteropolyploidy.

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REFERENCES


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