Temperature-sensitive Mutants of Vesicular Stomatitis Virus; Homology and Nomenclature

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SUMMARY

Reciprocal complementation experiments in mouse L cells and BHK-21 cells show that the complementing temperature-sensitive (ts) mutants of vesicular stomatitis virus (VSV), Indiana serotype, isolated from the HR wild-type strain, belong to groups I, III and IV (Flamand & Pringle, 1971). Since the homologies of ts mutants isolated from three different wild-type strains growing in different host cells can be established by cross-complementation, proposals are made for a uniform nomenclature of ts mutants of VSV Indiana.

INTRODUCTION

Temperature-sensitive (ts) mutants of the Indiana serotype of vesicular stomatitis virus (VSV) have been isolated independently in different laboratories (Flamand, 1969, 1970; Holloway, Wong & Cormack, 1970; Pringle, 1970a,b; Wong, Holloway & Cormack, 1971; A. F. Howatson, personal communication) and classified into groups by complementation tests. The physiological properties of these mutants are being investigated in detail, either to provide a picture of the organisation of the genome of VSV or to provide experimental tools for investigation of virus multiplication (Lafay, 1969, 1971; Lafay & Berkaloff, 1969; Deutsch, 1970; Martinet & Printz-Ane, 1970; Cormack et al. 1971; Deutsch & Berkaloff, 1971; Pringle & Duncan, 1971; Printz & Wagner, 1971; Reichman, Pringle & Follett, 1971; Wunner & Pringle, 1972; Szilágyi & Pringle, 1972; Wong, Holloway & Cormack, 1972; Závada, 1972). Consequently, it is important to establish the precise genetic homologies of these mutants to facilitate comparison of results obtained in different laboratories.

Flamand & Pringle (1971) have shown that the four groups of induced mutants isolated in BHK-21 cells (Pringle, 1970a,b) correspond to four of the five groups of spontaneous mutants isolated in chick embryo cells (Flamand, 1970), despite differences in restrictive temperature and strain of origin.

We now report comparison of complementing ts mutants isolated from a third wild-type strain in mouse L cells (Holloway et al. 1970) with representatives of the five established complementation groups of VSV, Indiana serotype. The results indicate that these mutants also can be accommodated in the existing classification of complementation groups and that a uniform nomenclature of VSV ts mutants is possible. It is proposed therefore that the
complementation groups of VSV Indiana serotype should be designated by Roman numerals as groups I, II, III, IV and V, and that individual mutants should be numbered according to convenience but preceded by a letter indicating the laboratory of origin (e.g. G, Glasgow; O, Orsay; W, Winnipeg).*

METHODS

Virus. Mutants ts G11, ts G22, ts G31 and ts G41 from the collection of induced mutants isolated in BHK-21 cells were used to represent complementation groups I, II, III and IV, respectively. These mutants are derived from the Indiana-c strain of VSV. Mutant ts O45, isolated originally in chick embryo cells by Flamand (1970) and subsequently cloned and propagated in BHK-21 cells, was used to represent group V.

Mutants ts W10 and ts W16B (group A), ts W29 (group B) and ts W4, 11, 14 and 28 (group C) were used to represent the complementation groups described by Wong, Holloway & Cormack (1972). These mutants are derived from the HR strain of VSV Indiana.

Complementation experiments in L cells. Mouse L cells (L 60 line) were maintained in a medium of CMRL 1066 supplemented with 5% foetal calf serum. One-day-old monolayers (in 60 mm Petri dishes) of approximately 2.5 x 10⁶ cells were infected at an input multiplicity of 20 p.f.u./cell. The total input multiplicity was the same in both single and mixed infections. The infected monolayers were washed with 10 ml PBS after an adsorption period of 25 min at 39 °C. Medium (5 ml) was added and the monolayers were incubated at 39 °C for 5 h. The infectivity of the medium was assayed at 30 °C at this time, and the results expressed as a complementation index, which is the ratio

\[
\frac{\text{yield (m1 + m2) at 39 °C}}{\text{yield m1 at 39 °C} + \text{yield m2 at 39 °C}}
\]

where m1 and m2 are any pair of mutants and the yield (leak + revertant) of m1 at 39 °C exceeds that of m2 at 39 °C.

Complementation experiments in BHK-21 cells. These experiments were carried out according to the procedure described by Pringle (1970b). Monolayers of 3 x 10⁶ BHK-21 clone 13 cells in 30 ml screw-capped bottles were infected in duplicate at an input multiplicity between 5 and 20 p.f.u./cell. The inoculum was washed off by two changes of cold medium (Eagle's medium supplemented with 2% calf serum), after an adsorption period of 30 min at 4 °C. The cultures were incubated totally immersed in a water bath at 39 °C for 8 h. The cultures were frozen rapidly and later assayed at the permissive temperature. The results are expressed as a complementation index, which is the ratio

\[
\frac{\text{yield (m1 + m2) at 39 °C}}{\text{yield m1 at 39 °C} + \text{yield m2 at 39 °C}}
\]

where m1 and m2 are any pair of mutants. It should be noted that this formula differs from that quoted above. This reflects the different practices of the two laboratories; the difference has little effect on the results.

Values of greater than 1 indicate complementation, and can be tested for significance by comparison with the variation between the duplicates.

* These proposals were discussed informally at the International Rhabdovirus Colloquium held in Roscoff, France, during June 1972, and were generally acceptable.
Homology and nomenclature of VSV ts mutants

Table I. Complementation in L cells; 5 h at 39 °C

<table>
<thead>
<tr>
<th>Group</th>
<th>Mutant</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(1 x 10⁶)</td>
<td>(2 x 10⁷)</td>
<td>(2 x 10⁷)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6*</td>
<td>5*</td>
<td>6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8*</td>
<td>11*</td>
<td>1.6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0*</td>
<td>6*</td>
<td>600*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6*</td>
<td>2.0*</td>
<td>10*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

The values in the table are expressed as a complementation index (the ratio of the mixed infection yield to the yield of the more leaky parent), and are the means from duplicate determinations in 1 to 3 experiments.

* Values significantly greater than 1 (P < 0.05). The values in parentheses are the single infection yields in p.f.u./ml.

RESULTS

Growth of the Glasgow mutants in mouse L cells

Mutants ts G11, ts G22, ts G31 grew well in L cells at the permissive temperature (30 °C) and in 24 h gave titres comparable to those of the Winnipeg mutants and to their wild-type parent (the HR strain). These mutants produce wild-type plaques in 48 h at 30 °C. Mutant ts G41 grew more slowly than the others and gave distinctly smaller plaques. All four mutants showed a good differential between 30 and 39 °C growth in L cells, as indicated either by plaque formation or single-cycle yield.

Complementation between the Glasgow mutants in L cells

As indicated in Table I, clear evidence of complementation was obtained in all combinations of the mutants ts G11, ts G22, ts G31 and ts G41.

Comparison of the Glasgow and Winnipeg mutants in L cells

Table I indicates that the Winnipeg mutants ts W10 and ts W16B (group A) complement with ts G11, ts G22 and ts G31, but not with ts G41. The complementation index between ts W16B and ts G22, although small, is significantly greater than 1 (P < 0.05).

Mutants ts W29 (group B) complements with ts G11, ts G22 and ts G41, but not with ts G31.

Mutants ts W4 (group C) complements poorly ts G22 and ts G31, and fails to complement ts G11. An equivocal result was obtained with mutant ts G41. The other group C mutants, ts W11, ts W14 and ts W28, show clear evidence of complementation with ts G22, ts G31 and ts G41, but none with ts G11.

These results suggest that group A (ts W10) and group IV (ts G41), and group B (ts W29) and group III (ts G31), are homologous. Mutant ts W4 (group C) can be equated either with group I (ts G11) or group II (ts G22) but the other three mutants of group C clearly belong to group I.
Table 2. Plaque count of Winnipeg mutants after cloning in BHK-21 cells

<table>
<thead>
<tr>
<th>Mutant</th>
<th>p.f.u./ml at 31 °C</th>
<th>p.f.u./ml at 39 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>ts W4</td>
<td>$1.1 \times 10^9$</td>
<td>$2.0 \times 10^4$</td>
</tr>
<tr>
<td>ts W29</td>
<td>$2.0 \times 10^9$</td>
<td>$2.5 \times 10^8$</td>
</tr>
<tr>
<td>ts W16B</td>
<td>$1.5 \times 10^9$</td>
<td>$1.5 \times 10^8$</td>
</tr>
</tbody>
</table>

Table 3. Complementation in BHK cells: 8 h at 39 °C

<table>
<thead>
<tr>
<th>Mutant</th>
<th>A (ts W16B)</th>
<th>B (ts W29)</th>
<th>C (ts W4)</th>
<th>I (ts G11)</th>
<th>II (ts G22)</th>
<th>III (ts G31)</th>
<th>IV (ts G41)</th>
<th>V (ts O45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ts W16B</td>
<td>(2.1 \times 10^6)</td>
<td>1260*</td>
<td>1210*</td>
<td>48*</td>
<td>48*</td>
<td>114*</td>
<td>114*</td>
<td>1160*</td>
</tr>
<tr>
<td>ts W29</td>
<td>(\textless 5 \times 10^6)</td>
<td>19*</td>
<td>2-2*</td>
<td>14*</td>
<td>0-8</td>
<td>8-4*</td>
<td>1950*</td>
<td></td>
</tr>
<tr>
<td>ts W4</td>
<td>(\textless 5 \times 10^6)</td>
<td>0-6</td>
<td>4-4*</td>
<td>2410*</td>
<td>90*</td>
<td>940*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values in the table are expressed as a complementation index (the ratio of the mixed infection yield to the sum of the single infection yields). The values are the means of duplicate samples from a single experiment; *, values of 2:2 and over are significant. These results were verified by two additional experiments. The values in parentheses are the single infection yields as p.f.u./ml.

Growth of Winnipeg mutants in BHK-21 cells

The Winnipeg mutants ts W4, ts W16B and ts W29 were inoculated into BHK cells: ts W16B grew rapidly immediately, but the others poorly. Clones were established by isolating single plaques from this first passage in BHK-21 cells and stocks prepared by inoculating monolayers of 5 \times 10^6 cells in 30 ml bottles at an approximate input multiplicity of 0-1 p.f.u./cell. These stocks were used in complementation experiments and the plaque count at 31 °C (the permissive temperature) and 39 °C (the restrictive temperature) are given in Table 2.

Complementation between Winnipeg mutants in BHK-21 cells

Complementation was observed in each combination of the three Winnipeg mutants (Table 3). The complementation indices were comparable to those obtained with combinations of Glasgow mutants under similar conditions.

Comparison of Glasgow and Winnipeg mutants in BHK-21 cells

In general, cross-complementation between Winnipeg and Glasgow mutants was less efficient than complementation between combinations of mutants derived from the same wild-type parent, but nevertheless an unequivocal result was obtained.

Mutant ts W16B complemented ts G11 (group I), ts G22 (group II), ts G31 (group III) and ts O45 (group V), but not ts G41 (group IV).

Mutant ts W29 complemented ts G11 (group I), ts G22 (group II), ts G41 (group IV), and ts O45 (group V), but not ts G31 (group III).

Mutant ts W4 complemented ts G22 (group II), ts G31 (group III), ts G41 (group IV) and ts O45 (group V), but not ts G11 (group I).

These results indicate that the Winnipeg complementation group A (ts W16B), B (ts W29) and C (ts W4) are homologous with groups IV, III and I, respectively.
DISCUSSION

The reciprocal experiments in L cells and BHK-21 cells indicate that the complementation groups designated A, B and C by Wong et al. (1972) correspond to groups IV, III and I, respectively, described by Flamand & Pringle (1971).

Higher complementation indices were obtained in the BHK system probably because of the lower frequency of wild-type revertants in the cloned BHK stocks. The poorer cross-complementation observed between the Winnipeg and Glasgow mutants suggests that a number of minor genetic differences have built up in the two wild-type strains since they were derived from a common ancestral strain.

It appears that no mutants belonging to group II have been obtained from the HR strain by the Canadian group. No mutants belonging to group V are present in either the Winnipeg or Glasgow collection of mutants, all of which were obtained by chemical mutagenesis, whereas 4.2% of the spontaneous mutants isolated by Flamand belonged to group V. This difference may result from technical variations, but the three wild-type strains have distinct origins (the Indiana-c strain at least is derived from a different field isolate). Therefore the difference in mutability may be genetic.

The reliability of classification of \( ts \) mutants of VSV by complementation test is reinforced by the similarity of the functional properties of Winnipeg and Glasgow mutants belonging to homologous groups (Wong et al. 1972; Pringle & Duncan, 1970). For instance, mutants in group I and group C, and in group IV and group A, are unable to induce actinomycin-resistant RNA synthesis at the restrictive temperature. Mutants belonging to group III and group B, on the other hand, are able to synthesize near-normal amounts of virus-specific RNA.

Although a minority of non-complementing mutants occur in both collections of induced mutants, it seems that the majority of \( ts \) mutants of the Indiana serotype of VSV, whatever their origin, can be fitted into the existing classification. We propose therefore that the complementation groups should be numbered as at present by Roman numerals, and that individual mutants be numbered according to convenience, prefixed by a letter indicating the laboratory of origin. An additional letter may be required in the prefix where more than one wild-type strain is used in a laboratory.

This nomenclature cannot be extended to include the \( ts \) mutants of the New Jersey serotype of VSV isolated by Pringle, Duncan & Stevenson (1971), however, since it was not possible to obtain complementation between mutants belonging to different serotypes.

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REFERENCES


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