A Host Range Mutant of Human Adenovirus Type 5
Defective for Growth in Hamster Cells

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SUMMARY

A host-range mutant of adenovirus type 5, which grows in human cells but not in hamster cells, has been isolated. This mutant is complemented in mixed infection in hamster cells at 38.5 °C by temperature-sensitive mutants of type 5 belonging to seventeen complementation groups, and may constitute a new group. In mixed infection of human cells at 32 °C with the host-range and temperature-sensitive mutants, recombination takes place and by a series of two factor crosses the host-range mutation has been approximately located on the adenovirus genetic map.

Temperature-sensitive (ts) mutants of human adenovirus type 5 (Ad5) have been isolated in this laboratory and characterized genetically and physiologically (Williams et al. 1971; Williams & Ustacelebi, 1971; Wilkie et al. 1973; Russell et al. 1974). By two-factor recombination analysis a genetic map of the Ad5 ts mutations has been obtained (Williams et al. 1974). In addition, several of these mutations have been mapped on the adenovirus genome by both restriction endonuclease analysis of intertypic recombinant DNAs (Grodzicker et al. 1974; Sambrook et al. 1975; Williams et al. 1975) and marker rescue using restriction fragments (E. Frost and J. Williams, unpublished results) and there is good agreement of the physical maps with the genetic map.

The genetic map is reasonably additive, but for closely aligned mutations (and there are many of these in the right half of the Ad5 map) the order is uncertain. This difficulty might be alleviated if an unselected third marker could be incorporated in the genetic crosses between ts mutants. For this purpose a heat stable (hs) mutation of Ad5 was crossed into the genetic background of a number of the ts mutants (Young & Williams, 1975) but because it is located near the left hand end of the genetic map it is not useful as a marker in three-factor crosses involving the many mutants in the right hand end. Ad5 replicates in hamster embryo cells (Takahashi, 1972; Williams, 1973) and host-range mutants which fail to replicate in hamster cells, but do transform them, have been described (Takahashi, 1972; Takahashi et al. 1974). In view of the fact that we had developed a plaque assay for Ad5 on hamster cells (J. F. Williams and T. J. Harrison, unpublished results), we believe that this type of host range marker would be potentially useful as an unselected marker in three-factor crosses, and could be easy to score in the progeny of such crosses. In this communication we report the isolation and preliminary characterization of such a hamster cell restricted mutant.

We find that when MgCl₂ is added to overlay medium (Williams, 1970), Ad5 plaques on BHK 21 C13 monolayers (Stoker & MacPherson, 1964) with an efficiency roughly 100-fold less than it does on HeLa cells. However, experience has shown us that the plaque assay on BHK 21 cells is variable and somewhat unreliable and in an attempt to improve this situation we developed a new line of hamster embryo fibroblasts (HECo) and isolated 10 clones from it (J. F. Williams, unpublished results). Ad5 grows and plaques in all of these clones and the parental line with more or less the same efficiency. We chose to use clone 9 cells for
most of this work, and in these cells Ad5 plaques with an efficiency roughly 30 to 100-fold less than on HeLa.

Virus was mutagenized by treatment of infected cells with N-methyl-N'-nitro-N-nitroso-guanidine (MNNG) and for this purpose HeLa cells infected with 100 p.f.u./cell were treated from 20 to 30 h post infection (32 °C) with 10 μg/ml MNNG. This was washed out, fresh medium added, and at 60 h post infection the virus was harvested and assayed on HeLa cells at 37 °C. The treatment resulted in a 4 log reduction in virus yield. From some 600 plaque isolates tested, one host-range mutant – hr 64 – was isolated. When grown in clone 9 cells this mutant gives a yield reduced 300 to 500-fold compared with the wild-type yield. In addition to growing poorly on clone 9 cells, hr 64 gives yields reduced up to 10-fold in HeLa cells compared with Ad5, suggesting that the mutation may be expressed to some extent in these latter cells. Mutant stocks have slightly higher particle/p.f.u. ratios than comparable wild-type stocks, so it is possible that a larger proportion of defective particles are generated in hr 64 stocks grown in HeLa cells. The growth rates of Ad5 in clone 9 cells, and of hr 64 in HeLa cells are reduced slightly compared to that of Ad5 in HeLa cells. Wild-type Ad5 plaques on clone 9 cells with an efficiency 30 to 100 times less than on HeLa cells, whilst the plaquing of hr 64 is reduced by a factor of around 105 on clone 9 cells compared to HeLa cells. A few small plaques, probably due to leakage, are seen occasionally when stocks of hr 64 are plated on clone 9 cells at low dilution. The progeny of twenty of these plaques were tested and found to be host-range; at present we do not know the reversion frequency of hr 64.

The extent of the host range of hr 64 was examined by infecting a number of hamster cell lines which are permissive for wild-type Ad5. All cell lines tested, including BHK 21, Ad5 transformed HT5 14a cells (Williams, 1973) and herpes simplex virus type 2 transformed HE/HSV2-333 cells (MacNab, 1974) proved to be non-permissive for hr 64. Furthermore, the mutant is unable to grow in clone 9 cells at 32 °C, so it is not temperature-sensitive in its host range. Finally, mutant 64 is totally neutralized by a 1/2000 dilution of rabbit antiserum to Ad5 (titre 1/6000) so it is Ad5 serotype, and has not arisen as a result of laboratory contamination with another virus (for example, type 12 which grows in human but not hamster cells). However, the physiological properties of this mutant have yet to be determined.

The Ad5 ts mutants fall into 17 groups on the basis of complementation tests in HeLa cells at 38 °C (Williams & Ustacelebi, 1971; Williams et al. 1974). To determine whether hr 64 fits into one of these groups, complementation tests were carried out in clone 9 cells at 38 °C – conditions non-permissive for both hr and ts mutants. The results (Table 1) indicate that hr 64 complements representative ts mutants from all 17 groups, and may constitute a new complementation group. The ts+ hr+ recombinant virus generated was measured by assay of the yield on clone 9 cells at 38 °C and found in all cases to be less than 1% of the total yield, and was therefore not corrected for in the calculation. In some cases complementation indices are low (less than 10-fold; see Williams & Ustacelebi, 1971), so that intracistronic complementation cannot be ruled out. However, lower complementation indices are not unexpected, since even with ts mutants which complement efficiently in HeLa cells, the complementation indices are greatly reduced in clone 9 cells (for example ts 1 × ts 2). We cannot explain the experimental variation in the yield of hr 64 in clone 9 cells; the yield of wt Ad5 in these cells also varies somewhat, but is always 300- to 500-fold greater than that of the mutant. Recently Minekawa et al. (1976) have reported the classification of 7 hamster cell hr mutants into 6 complementation groups and it would be of interest to determine which group, if any, hr 64 falls into. However, it should perhaps be pointed out here that,
Table 1. Complementation between hr 64 and Ad5 ts mutants in clone 9 cells at 38.5 °C*

<table>
<thead>
<tr>
<th>ts mutant</th>
<th>Single infection yields</th>
<th>Double infection yields</th>
<th>Complementation index</th>
</tr>
</thead>
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<tr>
<td>hr 64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
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</tr>
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</tr>
</tbody>
</table>

* For complementation tests, clone 9 cells were infected either with pairs of mutants each at an input multiplicity of 5 p.f.u./cell, or with single mutants at an input multiplicity of 10 p.f.u./cell. The infected cultures were incubated for 48 h at 38.5 °C, and the resultant yields were assayed on HeLa cells at 32 °C. In the table the yields are expressed as p.f.u./ml. The complementation index is the ratio of the yield of the double infection/the yield of the higher of the two single infections. A standard cross of ts1 x ts2 gave complementation indices of 59 and 2.3 x 104 when carried out in clone 9 and HeLa cells respectively.

because these authors were unable to plaque on hamster cells, they did not measure the ts+ hr+ component of their complementation yields, and recombination could largely explain their results.

Complementation of hr 64 with ts 18 and ts 19 is low, and in view of the fact that these mutants are defective for interferon induction in chick embryo cells (Ustacelebi & Williams, 1972) and are more heat-labile than Ad5 wild-type (Ustacelebi, 1973), we tested hr 64 for these properties. In short, the mutant is neither defective in interferon induction nor more heat-labile than Ad5, and in these respects hr 64 is phenotypically unlike ts 18 or ts 19. Mutants ts 18 and ts 19 have not been mapped by analysis of intertypic recombinants but, on the basis of two-factor genetic crosses, ts 18 appears to map close to, although it is not clear on which side of, ts 2 (C. S. H. Young and J. F. Williams, unpublished results), whilst ts 19 appears to be between ts 1 and ts 2 (Williams et al. 1974).

Furthermore, complementation with ts 125 is also low, and 125 transforms rat embryo cells with a higher frequency than wild-type (Ginsberg et al. 1974). The transforming efficiency of hr 64 for rat embryo cells was therefore examined, but was found to be approximately equal to that of Ad5. In addition, despite its inability to grow on hamster cells, hr 64 transforms these cells with a frequency roughly equal to that of some transformation-positive Ad5 ts mutants (Williams, 1973).

In order to determine the approximate location of the hr 64 mutation on the genetic map, recombination experiments were carried out between hr 64 and Ad5 ts mutants. The tests were carried out in HeLa cells at 32 °C (Williams & Ustacelebi, 1971) and yields assayed on HeLa cells at 32 °C and 38.5 °C and clone 9 cells at 38.5 °C. Because wild-type plaques with a reduced efficiency on clone 9 cells compared to HeLa cells, it was necessary to correct the yields on clone 9 cells at 38.5 °C in order to calculate recombination frequencies. This
short communications

Fig. 1. The approximate location of the hr64 mutation on the Ad5 genetic map. The recombination frequencies in the upper half of the figure were obtained as follows: HeLa cells were infected as for complementation at total input multiplicities of 10 p.f.u./cell and incubated at 32 °C for 5 days. The resultant virus yields were assayed on HeLa cells at 32 °C, and on clone 9 cells at 38.5 °C. The recombination frequency is expressed as:

\[
\frac{\text{clone 9 yield at 38.5 °C} \times \text{correction factor}}{\text{HeLa cell yield at 32 °C}} \times 2 \times 100.\]

The correction factor is necessary because Ad5 plaques less efficiently on clone 9 cells than on HeLa cells; in this experiment the correction factor was 38. In all cases, cells infected singly gave very low infectivity yields at 38.5 °C compared with doubly infected cells, so that recombination frequencies did not require correction for reversion or leakiness. The factor of 2 corrects for production of double ts hr recombinants expected to occur with the same frequency as ts+hr+ recombinants. Yields were also assayed on HeLa cells at 38.5 °C to determine the output of the hr64 parent (plus a small amount of ts+hr+ recombinant). Comparison of these values with total yields measured on HeLa cells at 32 °C showed that the outputs of the two parental types (equal input) from the crosses were roughly equal. The values for recombination frequencies shown in the lower half of the figure are taken from Williams et al. (1974).

correction factor was the ratio of the plaquing efficiency of Ad5 (wild-type) on HeLa and clone 9 monolayers at 38.5 °C, and it was always determined on the same set of monolayers used to assay the recombination test yield. Recombination frequencies were calculated according to the formula:

\[
\frac{\text{clone 9 yield at 38.5 °C} \times \text{correction factor}}{\text{HeLa yield at 32 °C}} \times 2 \times 100\%.
\]

The recombination frequencies from a typical experiment are shown in the upper part of Fig. 1 (the map and values in the lower part of the figure are taken from Williams et al. 1974). It may be seen that the hr64 mutation appears to lie closest to those of ts 1 and ts 14, further away from ts 22, 36, and 49, and probably between the ts 1 and ts 14 mutations. Further analysis will be necessary to verify and define more clearly the location of hr64. Comparison with the physical map of the ts mutations indicates that hr64 probably lies within the coordinates 0.59 to 0.80, and it will be of great interest to map this mutation physically. Initial attempts to do this by marker rescue have, however, so far been unsuccessful (E. Frost, personal communication).
Because this host range marker is relatively easy to score, and lies in the region of the genome richest in ts mutations (group A, which includes ts1, contains some 20 mutants), it is potentially useful as a marker in three-factor crosses. If we can transfer this marker into the genetic background of the ts mutants it may be possible to carry out such crosses and order the many mutations in the right hand region of the map.

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REFERENCES


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