Intragenic Complementation by Gene 42 Amber Mutations of Bacteriophage T4

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Phage T4 amber mutants defective in gene 42 (dCMP hydroxymethylase) were shown by in vivo and in vitro experiments to participate in both positive and negative intragenic complementation. This argues that incomplete polypeptide chains can participate in subunit interaction.

Chiu and Greenberg (4) have observed intragenic complementation in ts × ts mixed infections by using ts mutants defective in gene 42 of phage T4, the gene that specifies dCMP hydroxymethylase (13). In the present study, am × ts and am × am* complementation tests were carried out with gene 42 mutants both in vivo and in vitro, and positive as well as negative complementation reactions were observed. These assays were done to test whether incomplete polypeptide chains can participate in subunit interaction with sufficient specificity either to restore partially or to reduce the function of the homologous complete polypeptide.

A recombinational map of gene 42 is shown in Fig. 1, giving the sites of 5 ts mutations and 13 am mutations. The map indicates that gene 42 is a small gene about two map units long. O'Farrell et al. (10) determined the molecular weight of dCMP hydroxymethylase to be 25,000. This determination was made under conditions in which multimers are dissociated, so that the molecular weight determined is for the monomer. Pizer and Cohen (11) determined the molecular weight of phage T6 dCMP hydroxymethylase to be 68,000 under conditions in which multimers are not dissociated. Their estimate in phage T6 could be consistent with the molecular weight of phage T4 dCMP hydroxymethylase if in these T-even phage this enzyme is a multimeric protein consisting of two to three subunits.

The am × ts mixed infections were carried out between five ts mutants and four am mutants (Table 1) to identify combinations that showed positive or negative complementation. The infections were carried out in Escherichia coli B/5 (an Su- host) at 35 C, a partially permissive temperature for the ts mutants. A high burst size of 110 was obtained on infection by tsA53 alone, and a burst size of 63 was obtained with tsl40 alone. In the four tsA53 × am and the four tsl40 × am mixed infections, burst sizes of 2.8- to 5.5-fold lower than in the respective infections by the ts mutants alone were obtained, indicating negative complementation. Mutants tsA64 and tsl66 (which alone gave burst sizes of 10 and 10) showed no apparent positive or negative complementation. In the case of tsA79 (which alone gave a burst size of 5), there were two- to threefold increases in burst size in the mixed infections with amNG352, amNG93, and amNG498, suggesting positive complementation.

Table 2 shows the levels of dCMP hydroxymethylase activity observed in extracts of phage-infected cells. Infections were carried out at 37 C, and enzyme activity was measured at 37 and 42 C. An extract of wild-type-infected cells (extract no. 1) shows comparable levels of activity at 37 and 42 C, indicating that the dCMP hydroxymethylase specified by wild-type phage is not thermolabile within this temperature range. An extract of cells infected by amNG93 (no. 2) had about 60% of the wild-type level of activity when assayed at 37 C but only 12% when assayed at 42 C. This suggests that the polypeptide fragment formed by amNG93 has thermolabile hydroxymethylase activity. Mutant amNG498 (no. 3) produced 30% of the wild-type level of activity when assayed at 37 C and 32% when assayed at 42 C. The amber mutants were tested for plating efficiency on E. coli S/6 prior to these in vitro assays, and no plaques were found among approximately 104 phage plated.

The high activity retained by these am mu-
Fig. 1. Recombinational map of gene 42. The map was constructed on the basis of two-factor crosses performed on E. coli CR63 at 25 C. In these assays, 0.5 ml of phage was added to 0.5 ml of bacteria at about 2 x 10^9/ml to give a multiplicity of infection of 13. A 0.1-ml portion of the culture was diluted 4 x 10^-4-fold, and growth was allowed to occur for 2 h at 25 C (Fig. 1) and 1.5 h at 35 C (Table 1). At these times a few drops of chloroform were used to lyse the cells. The designations of the ts and am mutations are given above the bold line. Because of the long distances to neighboring genes, the orientation of gene 42 mutations with respect to these genes is uncertain. Where superscripts are given, they indicate how many repeated determinations were used to calculate the indicated average recombination percentages. All phage strains used in this study and E. coli strains CR63, S/6, and B/5 were from the California Institute of Technology stock collection, under the care of W. B. Wood. E. coli strains K12-594, DM53 (SuI^+), DM54 (SuII^+), and DM55 (SuIII^+) were provided by D. Mount, the University of Arizona. E. coli W3110 was from the laboratory of R. L. Sinsheimer, California Institute of Technology, and was supplied by L. H. Smith, California Institute of Technology. E. coli 011' was a gift from C. Bernstein, the University of Arizona.
The level of activity of tsA53 (extract no. 4) declined from 111% of the wild-type extract at 37 C to 11% at 42 C. Similarly for tsA79 (no. 5), the level declined from 135 to 14%. Thus, both of the ts mutant extracts appear to contain high levels of a thermosensitive enzyme.

The combination tsA79 and amNG498, which gave positive complementation in terms of burst size (Table 1), was further tested by assaying for dCMP hydroxymethylase activity in an extract of mixedly infected cells. The activity obtained at 42 C (Table 2, no. 10) was 60 U or 56% of wild-type activity. This compares to an expected value of 23% if there were no complementation. The expected value is the average of the activities obtained in the individual infections with tsA79 and amNG498 (Table 2, no. 5 and 3, respectively). Complementation was assayed further by mixing in various proportions extracts of cells infected separately by the two mutants. Clear positive complementation was again observed (Fig. 2a). In another experiment (Table 2, no. 11), in which equal volumes of the extracts were mixed, a similar result was seen.

The combination tsA53 and amNG93 showed clear negative complementation in terms of burst size at 35 C (Table 1). However, the activity of an extract of a mixed infection when assayed at 42 C gave somewhat higher activity (Table 2, no. 6) than the average of the activities in the individual infections by tsA53 and amNG93 (Table 2, no. 4 and 2, respectively). When extracts of separately infected cells were combined in varying proportions (Fig. 2b) or in equal proportions (Table 2, no. 7), negative complementation was again observed.

It was reasoned that if am mutants can show negative complementation with ts mutants a similar effect might be observed between am mutants and the wild type. Clear negative complementation was observed when extracts of amNG498-infected cells and wild-type-infected cells were mixed in varying proportions (Fig. 3a). With amNG93, only a marginal effect was observed (Fig. 3b). The combination tsA53 and tsA79 also showed marginal negative complementation when activity was measured in an extract of a mixed infection (Table 2, no 8) or a mixture of extracts (Table 2, no. 9). Also, amNG93 and amNG498 showed no significant complementation in a mixture of extracts (Table 2, no. 12).

To determine whether the infections with mutant phage under restrictive conditions were successful, the levels of dihydrofolate reductase activity in extracts of infected cells were measured by the procedure of Mathews and Sutherland (9). These assays were performed by C. K. Mathews. In all cases, levels of activity were considerably greater than in extracts of wild-type-infected cells. The results reported here suggest that polypeptide fragments produced by am mutants can have sufficient specificity to interact with, and either partially restore or reduce, the function of the homologous complete polypeptide. These results, along with those of Chiu and Greenberg (4), also imply that in its natural state dCMP hydroxymethylase is a multimeric protein.
Table 2. Deoxycytidylate hydroxymethylase activity in extracts and in mixed extracts from phage-infected cells

<table>
<thead>
<tr>
<th>Extract or extract mixture no.</th>
<th>Strain</th>
<th>nmol of HMdCMP/20 min</th>
<th>Expected activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wild-type (WT)</td>
<td>119</td>
<td>108</td>
</tr>
<tr>
<td>2</td>
<td>amNG93</td>
<td>71 (60)*</td>
<td>13 (12)</td>
</tr>
<tr>
<td>3</td>
<td>amNG498</td>
<td>36 (30)</td>
<td>35 (32)</td>
</tr>
<tr>
<td>4</td>
<td>tsA53</td>
<td>132 (111)</td>
<td>12 (11)</td>
</tr>
<tr>
<td>5</td>
<td>tsA79</td>
<td>160 (135)</td>
<td>15 (14)</td>
</tr>
<tr>
<td>6</td>
<td>tsA53 \times amNG93 &amp;</td>
<td>17 (16)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>tsA53 &amp; amNG93 &amp;</td>
<td>9 (8)</td>
<td>13 (12)</td>
</tr>
<tr>
<td>8</td>
<td>tsA53 \times tsA79</td>
<td>20 (19)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>tsA53 &amp; tsA79</td>
<td>10 (9)</td>
<td>14 (13)</td>
</tr>
<tr>
<td>10</td>
<td>tsA79 \times amNG498</td>
<td>60 (56)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>tsA79 &amp; amNG498</td>
<td>89 (82)</td>
<td>25 (23)</td>
</tr>
<tr>
<td>12</td>
<td>amNG93 &amp; amNG498</td>
<td>19 (18)</td>
<td>24 (22)</td>
</tr>
</tbody>
</table>

* E. coli B was grown in 200 ml of the medium described by Fraser and Jerrell (6), containing glycerol and Casamino Acids. When the concentration of cells had reached \(3 \times 10^9\)/ml, the culture was infected with approximately 4 phage per bacterium. After 20 min of infection, the cultures were poured into chilled centrifuge bottles (250 ml) in ice and centrifuged for 15 min at 13,000 \(\times g\) in a Sorvall RC-2 refrigerated centrifuge. The pellet was kept on ice and suspended in 3 ml of 0.1 M potassium phosphate, pH 7.0. The suspended pellets were disrupted for 1.5 min (three 30-s intervals with intermittent chilling) by use of a Branson Sonifier (Branson Instruments, Inc., Stamford, Conn., model S110) on a setting of 4. The sonic extract was centrifuged for 20 min at 27,000 \(\times g\) in a Sorvall RC-2 centrifuge. The protein concentration in the supernatant was determined by the biuret method (7). The crude extract was used to assay for dCMP hydroxymethylase activity as described by Pizer and Cohen (11). The activities in 0.1 ml of extracts were measured. In mixtures of extracts, activity was measured after combining 0.05 ml of each extract. The labeled formaldehyde used in the assay was \([\text{H}^3\text{CHOH}\] from New England Nuclear Corp., Boston, Mass. It was diluted with unlabeled HCHO to a specific activity of 120 counts/min per nmol.

* Expected activities were calculated by summing the activities of the two component extracts in the mixture.

* The values in parentheses are percentages.

*Mixed infection between the two phage types shown. & Cells were infected separately with the individual phage types, and extracts of the infected cells were prepared and then mixed in the combination shown.

mutant amNG498, which shows negative complementation with the wild type (Fig. 3a), shows positive complementation with tsA79 (Fig. 2a). This can be explained by assuming that when an am polypeptide fragment reacts with a homologous, complete wild-type polypeptide it may reduce its function, whereas when it reacts with a complete, but severelyFig. 2. Interaction between extracts of gene 42 am mutant-infected cells and gene 42 ts mutant-infected cells. The infection protocol and multiplicity of infection are described in the footnotes to Table 2. Infections with either am or ts phage were carried out at 37°C; the extracts of the infected cells were then mixed in two combinations, (a) tsA79 and amNG498 (△) and (b) tsA53 and amNG93 (○), at 42°C. Increasing portions (0.02 to 0.1 ml) of the am mutant extract were added to 0.1 ml of the ts mutant extract, and dCMP hydroxymethylase activity was measured by the procedure described in Table 2. The levels of activity in the individual mutant extracts were also measured, and expected values were calculated, assuming no interaction, by summing individual activities in the extracts mixed together in each test. Symbols: △, calculated values for tsA79 and amNG498; ○, calculated values for tsA53 and amNG93. The levels of dCMP hydroxymethylase obtained in each of the single infections are listed in Table 2.
defective homologous polypeptide, it may ameliorate the defect.

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LITERATURE CITED