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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 10^5 phage plated.

The high activity retained by these *am* mu-

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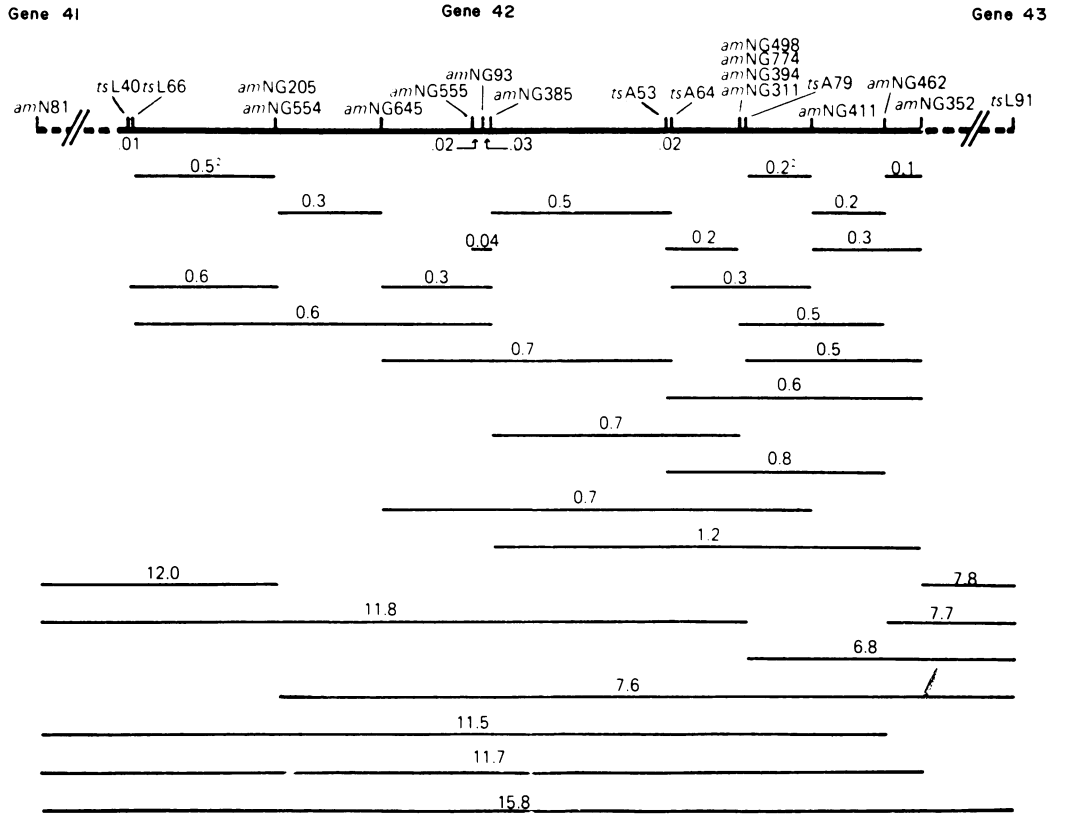

FIG. 1. *Recombinational map of gene 42. The map was constructed on the basis of two-factor crosses performed on E. coli CR63 at 25 C. In these assays, 0.5 ml of phage was added to 0.5 ml of bacteria at about 2 × 10⁸/ml to give a multiplicity of infection of 13. A 0.1-ml portion of the culture was diluted 4 × 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.*

TABLE 1. *Burst sizes in am × ts mixed infections and in infections by each ts mutant alone*[a]

Mutant	*ts* or *ts*$^+$ alone	Mixed infection			
		amNG352	*amNG93*	*amNG498*	*amNG205*
tsA53	110	20	21	37	40
tsL40	63	23	26	16	21
tsA64	10	8	6	12	8
tsL66	10	12	16	10	13
tsA79	5	10	13	15	5
ts$^+$	366				

[a] Burst sizes were obtained upon infection of *E. coli* B/5 at 35 C. The infection protocol and multiplicity of infection are described in the legend to Fig. 1. The burst sizes from infections with each of the *am* mutants alone were negligible (i.e., less than 0.1 phage per bacterium). To verify that the *am* mutants used here were true nonsense mutants, these phage were tested on three *Su*$^-$ strains (W3110 [1], K12-594 [2], and S/6 [5]) and on an "isogenic" set of three *E. coli* W3110 derivatives containing *SuI*$^+$, *SuII*$^+$, and *SuIII*$^+$, respectively, as well as on *E. coli* O11′ (containing *SuII*$^+$ [14]). The *am* mutants did not form plaques on the *Su*$^-$ strains and generally plated with high efficiency on the *Su*$^+$ strains.

tants is presumably due to residual function of the polypeptide chains synthesized by them. The presence of this activity, despite the conditional lethality of the mutants, is similar to the findings by Mathews and Kessin (8) and by Chiu and Greenberg (3) that the gene 42 mutant *tsL13*, on infection at 42 C, does not grow or form DNA yet does yield dCMP hydroxymethylase activity in extracts. Further work on this mutant (12) indicated that at 42 C the enzyme is not active in vivo, possibly because of inability to interact with another component to form an active complex.

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. The

TABLE 2. *Deoxycytidylate hydroxymethylase activity in extracts and in mixed extracts from phage-infected cells*[a]

Extract or extract mixture no.	Strain	nmol of HMdCMP/20 min		Expected activity[b]
		37 C	42 C	
1	Wild-type (WT)	119	108	
2	*amNG93*	71 (60)[c]	13 (12)	
3	*amNG498*	36 (30)	35 (32)	
4	*tsA53*	132 (111)	12 (11)	
5	*tsA79*	160 (135)	15 (14)	
6	*tsA 53 × amNG93*[d]		17 (16)	
7	*tsA53 & amNG93*[d]		9 (8)	13 (12)
8	*tsA53 × tsA79*		20 (19)	
9	*tsA53 & tsA79*		10 (9)	14 (13)
10	*tsA79 × amNG498*		60 (56)	
11	*tsA79 & amNG498*		89 (82)	25 (23)
12	*amNG93 & amNG498*		19 (18)	24 (22)

[a] *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 3×10^8/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 × *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 × *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 [H^{14}]CHO from New England Nuclear Corp., Boston, Mass. It was diluted with unlabeled HCHO to a specific activity of 120 counts/min per nmol.

[b] Expected activities were calculated by summing the activities of the two component extracts in the mixture.

[c] The values in parentheses are percentages.

[d] 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.

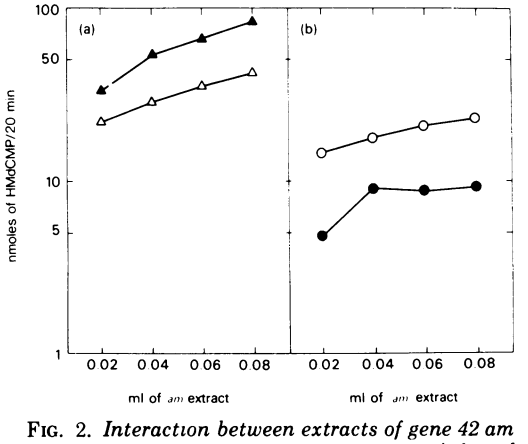

FIG. 2. *Interaction between extracts of gene 42 am mutant-infected cells and gene 42 ts mutant-infected cells. The infection protocol and multiplicity of infection are described in the footnotes to Table 2. Infections with either am or ts phage were carried out at 37 C; the extracts of the infected 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.*

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 interacts with a homologous, complete wild-type polypeptide it may reduce its function, whereas when it reacts with a complete, but severely

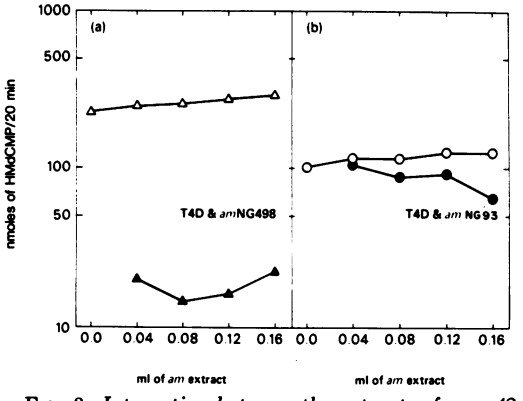

FIG. 3. *Interaction between the extracts of gene 42 am mutant-infected cells and wild-type phage-infected cells (a) amNG498 and wild type: observed (▲), calculated (△); (b) amNG93 and wild type: observed (●), calculated (○). The conditions and procedures in these experiments were the same as those described in the legend to Fig. 2. The infection protocol and multiplicity of infection are described in the footnotes to Table 2.*

defective homologous polypeptide, it may ameliorate the defect.

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