Intragenic Complementation Between Temperature-Sensitive Mutants of Gene 42 (dCMP Hydroxymethylase) of Bacteriophage T4

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Interallelic complementation between certain temperature-sensitive mutants of gene 42 of bacteriophage T4 was demonstrated by measuring the incorporation of labeled thymine into DNA.

As part of a study of the apparently complex nature of dCMP hydroxymethylase of T4 bacteriophage (2, 6), we have investigated some of the characteristics of temperature-sensitive (ts) mutants of gene 42. During the course of our studies, we observed intragenic complementation between some ts mutants of gene 42.

DNA synthesis was followed by measuring the incorporation of labeled thymine into the acid-insoluble fraction. In other experiments it was shown that prior alkali treatment to remove RNA gave the same incorporation values. The method of infection, the assay techniques, and the mutants used were described earlier (2). A Thy⁻ strain was used as the host (2). The map of gene 42 is shown in Fig. 1. *L66*, *L40*, and *L13* appear to map at a common site. However, from temperature profile studies of DNA synthesis, *L66* is clearly different from *L40* or *L13* (unpublished data).

Mutants of gene 42 are of the DO type, i.e., they do not form DNA. DNA synthesis in cultures mixedly infected with several combinations of equal numbers of two mutants at 42°C is shown in Fig. 2. Repeated complementation studies gave essentially the same results. Complementation between two mutants was considered to be positive when the mixedly infected culture showed a rate of DNA synthesis greater than the average of the rates observed on single infection by the two mutants.

Complementation was clearly demonstrable between *tsLB1* and *tsLB3* and between *tsLB1* and *tsL13*. The value obtained from complementation between *LB1* and *LB3* was about 5.5 times higher than the average obtained from *LB1* and *LB3* on independent infection and 30% of that obtained with the wild-type control (not

shown), measured at 30 min after infection. *LB1* and *L13* also complemented to a level six times higher than the average obtained with independent infections by *LB1* and *L13*. *LB3* did not complement with *L13*, the DNA synthesis of the doubly infected cells being exactly equal to the average of the values for the singly infected cultures.

Other studies were carried out (data not shown). In these investigations, *L40* behaved very much like *L13*, i.e., it complemented with *LB1* but not with *LB3* or *L13*. However, *L66*, reflecting its difference from *L13* and *L40*, did

FIG. 1. Genetic map by J. Flatgaard (personal communication) of gene 42 showing the mutants used in this study. The units are in percentage of recombination. The order of *L13*, *LB3*, and *LB1* was confirmed by D. Bowden by three-factor crosses (4). *AmE21* was mapped by J. Wiberg (personal communication).

not complement with *LB1*, *LB3*, or *L13*. As a control, *L13* and *L159* (a ts mutant in gene 45 which is also a DO gene) gave complete complementation, i.e., DNA synthesis reached the wild-type level.

It is clear from Fig. 2 that at 42°C *LB3* is leaky as measured by DNA synthesis, such that it gradually reaches a rate which is about 10% of that shown by the wild-type phage. This value is obviously far less than that of the complementing system, *LB3* + *LB1*. A slight rate of DNA synthesis was also detected transiently in

FIG. 2. Complementation in cultures mixedly infected with *ts* mutants of gene 42 at 42°C as measured by incorporation of labeled thymine into DNA. Thymine-2- ^{14}C with a specific activity of 7.4×10^6 counts per min per μmol at a concentration of 130 nmol/m of culture was added at the time of infection. In the singly infected cultures a multiplicity of 8 phage/cell was used, and in the mixedly infected cultures multiplicities of 4 of each of the two phage were used. The incorporation of ^{14}C -thymine is expressed as counts per minute in the acid-insoluble fraction from 1 ml of culture containing 5×10^8 cells.

cultures infected at 42°C by LB1, but no synthesis occurred in the case of L13.

A number of T4 genes show intragenic complementation (1). Because gene 42 mutants show intragenic complementation, dCMP hydroxymethylase must be considered to be mul-

timeric (3). The molecular weight of the T6 enzyme has been reported to be 68,000 (5). While our experiments followed DNA synthesis, E. Kutter and J. Wiberg independently have found intragenic complementation between gene 42 mutants measuring phage formation (J. Wiberg, personal communication).

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