**λcI Mutants: Intragenic Complementation and Complementation with a cI Promoter Mutant**

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**Summary.** Complementation for the maintenance of lysogeny was studied by superinfecting λcI<sup>ts</sup> lysogens at 34 ° C, and then heating to 43 ° C. With certain exceptions,<sup>1</sup> <sup>ts</sup> mutants with defects in the left half of the repressor complemented <sup>ts</sup> mutants with defects in the right half to produce a less heat-labile repressor (Fig. 3). All cI<sup>amber</sup> mutants failed to complement cI<sup>ts</sup> mutants. The cI mutant c50 complements all <sup>ts</sup> mutants. Mutations in Pre (cy) or genes cII and cIII do not significantly affect the expression of cI by a superinfecting λ genome in an immune lysogen.

Mutants with very heat-labile repressors failed to complement λcI<sup>cy42</sup> for the establishment of lysogeny at elevated temperatures, while those with less heat-sensitive repressors apparently did complement cy. According to a suggested model, the left side of the cI product is concerned primarily with subunit aggregation, while operator binding is the function of the right side of the oligomer.

**Introduction**

Intragenic complementation is often observed when the active gene product contains two or more identical subunits. The aggregation of monomers coded by different mutants of the same gene can result in the formation of hybrid oligomers. In many instances, the activity of a hybrid enzyme is closer to wild-type than the activity of the enzyme formed by either mutant alone (Fincham, 1966). The repressor coded by the cI gene of λ is an oligomer (Pirrota et al., 1970). Kaiser (1957) first observed complementation between two cI mutants, c47 and c50. Complementation between different cI<sup>ts</sup> mutants for the maintenance of lysogeny was reported by Lieb (1966a, 1969) and Green (1967), but Horiuchi and Inokuchi (1967) failed to detect complementation in a set of cI<sup>ts</sup> mutants isolated in their laboratory.

The establishment and maintenance of stable lysogens of bacteriophage λ requires the continuous presence of the repressor. Immediately after infection, transcripts of gene cI originate at the “early promoter”, Pre. After repression has been established, cI is transcribed, at a slower rate, from the “maintenance promoter”, Prm (Reichard and Kaiser, 1971). Since the concentration of cI product is much higher immediately after infection than in lysogens, it would appear that more repressor is required during the initial “decision” for lysogeny than to maintain λ in the prophage state. Reasoning that it would be easier to detect complementation for prophage maintenance than for lysogenization, I have concentrated my studies on the heat induction of λcI<sup>ts</sup> lysogens superinfectected with λcI<sup>ts</sup> mutants. Pairs of mutants that complemented each other were then tested for complementation during lysogenization. Complementation between cI<sup>ts</sup> and Pre mutants (Brachet and Thomas, 1959) was also re-examined.

The data presented here and in the accompanying reports (Lieb, 1976; Mandal and Lieb, 1976) suggest a model for the functions of different parts of the λ repressor molecule.

**Materials and Methods**

**Phage and Bacterial Strains.** Most strains are described in the accompanying paper (Lieb, 1976). E. coli strain K12S, which contains an amber suppressor, is derived from strain W1 294 (Buchman, 1972). Strain 1200 is su<sup>+1</sup>, endo<sup>-1</sup>, RNase I (Dürwald and Hoffmann-Berling, 1968).

**Media and Culture Methods.** Described in Cross and Lieb, 1967.

**Preparation of λcI<sup>ts</sup> Lysogens.** E. coli strains were grown in a broth medium (TBM) to a concentration of about 2 × 10<sup>8</sup> bacteria/ml centrifuged, and resuspended in K buffer. The culture was aerated for 60 min at 34 ° C and then mixed with λ to give a multiplicity of 3–10 phage particles per bacterium. After 20 min at 34 ° C, the bacteria were plated. The resulting colonies were
replicated to a lawn of sensitive bacteria (strain C600). Lysogenic colonies produce replicas surrounded by a halo of lysed lawn. Lysogens were cloned, restested for the presence of phage, and then heated to confirm the heat-inducible phenotype of the prophage(s).

Unless another strain is cited, all data presented here were obtained with lysogens of the sa strain 594. A large number of complementation tests between clts mutants were also done in M3S lysogens. There were slight differences in the results obtained with the two strains.

Complementation Test for Maintenance of Immunity. A culture containing a particular clts prophage was grown in TBM at 34° C to a concentration of 2.4 × 10⁸ bacteria/ml. A small volume of bacteria was mixed with superinfecting phage to give a multiplicity of 15-20 phage particles per bacterium. After absorption at 34° C for 10 min, the bacteria were diluted 1:100 into TB and incubated at 34° C for an additional 30 min to allow expression of the cl genes of the superinfecting λ genomes. The cultures were chilled, and held on ice for up to 30 min. The bacteria were then diluted 1:10 into TB previously brought to 43° C.

Samples of the superinfected bacteria were removed before heating and after incubation at 43° C. The bacteria were diluted, spread on TA plates, and incubated at 34° C.

Inactivation of all (or most) of the repressor molecules in a lysogen leads to induction of phage replication, resulting in death of the bacterium. Thus, the colony-forming bacteria are those lysogens which still contain sufficient active repressor to prevent derepression of any of the phage genomes that they contain. For complementation studies, loss of bacterial viability is a more sensitive and convenient measure of induction than is assay of the number of lysogens producing phage.

Complementation Test for Establishment of Lysogeny. The following method is based on that used by Belfort et al. (1975). About 10⁸ phage particles and 10⁶ bacteria were plated in 2.5 ml of soft agar on a TA plate. If clts mutants were to be tested, the plates were then warmed to about 42° C on a special heating block. Phages to be tested, diluted to about 10⁶ particles/ml, were spotted onto the agar surface, using a loop delivering about 0.005 ml. During this procedure, plates were maintained at about 42° C. After spotting, the plates were incubated at 42° C or 37° C. As a control, λcll67 was spotted on all plates.

Results

Distinguishing Complementation from Additive Effects

When a superinfecting λ phage genome enters an immune lysogen, its cl and rex genes are expressed. It is important to be able to distinguish an increase in the number of repressor molecules in a lysogen from the appearance of a more heat-stable repressor due to complementation between different cl products. The synthesis of additional clts product, even if it is identical to that produced by the prophage, increases the resistance of a λts lysogen to derepression at 43° C (in Fig. 1 compare curve A to curve B and curve C to curve D). The addition of phages identical to the prophage will be called “homologous superinfection”. The addition of clts mutant genomes that produce a repressor that is more heat-resistant than the repressor produced by the prophage will obviously increase the heat-resistance of the lysogens even more than homologous superinfection. For every heterologous superinfection, homologous superinfections with the two phages were used as controls. Two mutants are considered to be complementing only when the surviving fraction of the heterologously infected lysogen after 40 min at 43-44° C is at least twice that of either of the control superinfections. For example, the data shown in Figure 1 do not support the conclusion that mutants tsU51 and ts1-22 complement each other (curve E).

Complementation between clts Mutants for Maintenance of Immunity

An example of the type of result that indicates positive complementation is given in Figure 2, and Figure 3 summarizes the data obtained with clts mutants in bacterial strain 594. When complementation occurred between a given mutant prophage and a heterologous superinfecting mutant, it was usually also observed when the positions of the mutants were reversed. As indicated by dots in Figure 3, nonreciprocal results were obtained with ts mutants U37, U51 and 71. All three mutants appeared to complement certain lysogens as superinfecting phages but were not complemented by superinfection with the complemented mutants. One can only speculate as to the basis of nonreciprocal recombination. One possibility is that