Isolation and Properties of a Conditionally Lethal Bacteriophage $\lambda$ Mutated in the $x$ Region

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Summary. A thermosensitive $\lambda$ phage mutant was isolated which can grow at high temperature only in the presence of the $\lambda x$ gene product supplied in trans. This mutation $tn$ was mapped within the $x$ region, and $\lambda tn$ phage expressed the $pRoR-0P$ operon only poorly at high temperature.

Effects of the $tn$ mutation on expression of other operons were also examined and compared with those observed with $cro_{37}$ or some tof mutations.

Introduction

In the normal growth process of bacteriophage $\lambda$ the early expression of three operons of $\lambda$: $pLoL-N-cIII-exo$ operon, $pRoR-tof-0-P$ operon and $pre-cl-rex$ operon (Fig. 1) is observed. Their gene products are needed for stimulation of transcription, phage-specific DNA synthesis, establishment of repressor synthesis, etc. (for reviews, see Hershey, 1971).

Recent studies showed that transcription of the $pLoL-N-cIII-exo$ operon ("leftward transcription system") and the $pre-cl-rex$ operon are regulated by the product of a gene located in the $x$ region of $\lambda$ genome: a gene called $cro$ (Eisen et al., 1970) or $Ai$ (Calef et al., 1971) codes for a substance which negatively controls expression of the $pre-cl-rex$ operon. A $\lambda$-lysogen grown for several generation in the absence of repressor accumulates the $cro$ gene product, and this inhibits resumption of production of $cl-rex$ products upon return to a permissive condition. On the other hand, $cro$ mutant prophage readily resumes the synthesis of $cl-rex$ products. A gene called $tof$ (Pero, 1970) or $fed$ (Franklin, 1971) was discovered which controls leftward transcription, turning it off at about 10–15 min after initiation of phage growth. A $tof$ mutant phage cannot turn off expression of the $pLoL$-controlled gene products, e.g. exonuclease, even late after infection. The $cro$ and $tof$ genes are likely to be the same gene, as some $\lambda$ $cro$ prophage behave like $\lambda$ $tof$ prophage (Eisen et al., 1970), and vice versa (Franklin, 1971).

Takeda et al. (1975) have recently suggested that some of their newly isolated $tof$ mutations affect expression of the $pRoR-tof-0-P$ operon ("rightward transcription system"), whose promoter-operator ($pRoR$) is located within the $x$ region. It seems, therefore, that the $x$ region of $\lambda$ genome plays key roles in coordinating the expression of the phage operons.

In order to further elucidate these problems, we attempted to isolate conditionally lethal mutants of $\lambda$ whose mutation falls within the $x$ region. For this purpose, a new procedure was devised which does not rely upon the already known $cro$ or $tof$ phenotypes, but makes use of the fact that such mutants grow at high
temperature only in the presence of active tof products provided in trans. This paper describes the properties of a mutant obtained by this technique.

Materials and Methods

Bacterial and Phage Strains

All the bacterial strains used are derivatives of E. coli K12 and originated from the collection of A. D. Kaiser; W3102, W3350 are nonpermissive hosts for sus nonsense mutants of λ (Campbell, 1961). C600 carries a suI suppressor and is thus a permissive host. C600S penR is a derivative of C600 resistant to penicillin and was obtained from Tomizawa. Strain WGS6, a lysogen with a defective prophage on an F' trp episome, was obtained from Spiegelman. This prophage carries the cl587 mutation and is defective because of a deletion that leaves only the N-oLpL-rex-cI-pRoR-x segment (Spiegelman, 1971). The plasmid, referred to as PWGS6 in this work, was transferred to W3102. It can express only N, rex, cI and x functions (see Fig. 1).

The phage strains, λ susNNs, λ susO s, λ clsN and λ immN cI originated from A. D. Kaiser's collection. λ sIs57cro27 (Eisen et al., 1970) and λ susN58dsαTXz5 (Roberts, 1969) were obtained from A. B. Oppenheim, M. Gottesman and T. Horiuchi, respectively. Recombinants and lysogens were prepared from these mutants as needed.

Media

PB contains 10 g of polypepton (Daigo-Eiyo Chemicals, Tokyo) 1 g of Bonito extract (Katayama Chemical, Kyoto) and 2.5 g NaCl per l of water. For plaque assay, PB was solidified with 0.5% or 1.2% agar for the top layer or the bottom layer, respectively.

Mutagenesis

λ susN58 was diluted 1:5 in 2.5 M NH₂OH-HCl solution containing 2 M NaCl, 2 mM MgSO₄ and 0.15 M Na₂HPO₄ adjusted to pH 7.5 with NaOH, and incubated at 37°. At various times, aliquots were taken out and the reaction was stopped by the addition of 4 volumes of cold solution containing 2% acetone, 0.5% of peptone and 6% of NaCl. The mixture was then immediately diluted and plated with various indicators. After 6 hr incubation, the titer of plaque formers was reduced to about 10⁻³.

Assay of Exonuclease Synthesis

Exponentially growing E. coli C600 in PB containing 0.2% Maltose and 3 mM MgCl₂ at 37° was chilled in an ice bath and infected with λ phage. After adsorption at 0° for 20 min, the infected bacteria were transferred and incubated at 41° (time zero). At various times after infection, cells (1 ml) were pipetted out into a small chilled test tube at 0°, centrifuged, suspended in 0.1 ml of “lysis medium” which consists of 50 mM glycine-KOH buffer pH 9.5, 2 mM EDTA, 200 μg/ml of lysozyme, 0.5% Brij and 2 mM KCN, and then incubated at 37° for 5 min to prepare lysates. To each lysate, 0.2 ml of “assay mixture” which consists of 50 mM glycine-KOH buffer pH 9.5, 1.3 mM MgCl₂ and ³H labeled E. coli DNA (1 × 10⁶ cpm), was added and then incubated at 37° for 30 min. The reaction was stopped by chilling at 0° and adding an equal volume of 1.75% Percloric acid. Acid-soluble ³H was counted in toluene-based liquid scintillator.

Results

Isolation of the tn Mutant

A stock of mutagenized phage λ susN58 was plated with a 1:1 mixture of W3102/F' trp and W3102/F' WGS6 on PB agar and incubated at 41° overnight. Under the plating condition used, this phage shows clear plaques, whereas mutants whose growth depends upon the tof product would be expected to show turbid plaques,