Studies on Lambda Virulent Mutants
II. Anti-Repression and Vir-Repression Function of $\lambda_{virC}$ and $\lambda_{virCvirR}$

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Summary. The clearish plaque mutants $\lambda_{virC}$ which were isolated from $\lambda$ true-virulent, $\lambda_{virLvirCvirR}$ ($\lambda_{virLCR}$), do not complement CI mutants but CII, CIII and $\lambda$ mutant ($\lambda_{sus}$) for lysogenization. No complementation for lysogenization was observed between $\lambda_{virC}$ and any CI, CII, CIII or y mutants. No lysogen was obtained when $\lambda_{virC}$ or $\lambda_{virC}$ carrying $\lambda_{susN}$, $\lambda_{susO}$ or $\lambda_{susP}$ was infected to $\lambda$-sensitive sup - host. This was also true for $\lambda_{virC}$. Infection of $\lambda d^{-}$ lysogen with $\lambda_{virCRsusNO(P)}$ or $\lambda_{virC}^{susNO(P)}$ results in marked prophage induction. Effect of $\lambda_{virCRsusNO(P)}$ on prophage induction is stronger than that of $\lambda_{virC}^{susNO(P)}$. These results suggest the existence of gene(s) for anti-repressor. When $\lambda_{virC}^{susNO(P)}$ or $\lambda_{virCRsusNO(P)}$ was infected to W3350 sup - at high m.o.i., lysogen in anti-immune state and that in weak-immune state was obtained, respectively. Wild type $\lambda$ phage forms clear plaque on $\lambda_{virC}^{susNO(P)}$ lysogen with e.o.p. of one and no plaque on $\lambda_{virCRsusNO(P)}$ lysogen. T4rII can plate on both lysogens. This weak-immunity caused by $\lambda_{virCRsusNO(P)}$ prophage is different from CI immunity and not abolished by irradiation of ultraviolet light (hereafter this is referred to as the $\lambda$-immunity). Action of anti-immunity and $\lambda$-immunity are almost $\lambda$ specific. Possible functional sites for anti- and $\lambda$-immunity substances are suggested to be $\lambda_{virL}$ and $\lambda_{virR}$ regions. A hypothesis was presented that the $\lambda$-immunity may caused by the overproduced anti-immunity substance coded from $\lambda$ region.

Introduction

Weak-virulent mutants, $\lambda_{virLvirR}$ ($\lambda_{virLR}$), of coli-phage $\lambda$ producing turbid plaques on sensitive cells can grow in $\lambda$CIts lysogen synthesizing a temperature-sensitive repressor but not in $\lambda$CI + lysogen synthesizing a normal repressor. Virulent mutants, $\lambda_{virLvirCvirR}$ ($\lambda_{virLCR}$), producing clear plaques on wild type $\lambda$ lysogen develop in the presence of normal repressor (Horiuchi et al., 1969).

In the previous paper, it was suggested that the $\lambda$IR mutation which is located at the left end of the immunity region (Fig. 1) is an operator mutation of the left hand operon including the N gene, and the $\lambda$IR and $\lambda$Vir are those of the right hand operon including the $\lambda$ region (Eshima et al., 1972).

In the present paper, it is demonstrated that production of an anti-repressor promoted by the effect of $\lambda$IR mutation antagonizes CI-repressor and over-production of the anti-repressor causes a different repression system for super-infecting $\lambda$ phage ($\lambda$-repression).

Materials and Methods

Bacteria and Phage Strains. E. coli K12 strains used were: C600 (Appleyard, 1954) and W3350 (Campbell, 1961), permissive and nonpermissive host, respectively, for the $\lambda_{sus}$

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nonsense mutants, and B582 (originally from Dr. A. D. Kaiser) which carries a large deletion of the galE, attB, bio, ureB and chlA. For bacterial mating HfrH (Hays, 1953), W3350 carrying F- gal (Jacob and Wollman, 1961), and W3350 gal str were used.

The phage strains used were λ1, λ217, λ258, λ27, λ293, λ297 (Kaiser, 1957), λC17 (Thomas and Bertani, 1964; Pereira da Silva and Jacob, 1968), λind- (Jacob and Campbell, 1959), λsus8, λsus10, λsusP (Campbell, 1961), λimm84 (Kaiser and Jacob, 1957), λimm21 (Liedek-Kulke and Kaiser, 1967), φ80 (Matsushiro, 1963), hgsimm (hybrid: constructed by Dr. K. Matsubara) and T4rII12. All λ virulent derivatives, λvirL, λvirR, λvirC, λvirC4, λvirL, λvirL, λvirC, λvirL, λvirL, λvirC, λvirC were described in previous papers (Horiiuchi et al., 1969; Koga et al., 1970).

Media. Media for the growth of cells and for λ phage assays have been described previously (Horiiuchi et al., 1969). For phage assays of T4rII, tryptone plates were made of tryptone broth medium containing 1% Difco-Tryptone, 0.5% NaCl and 1.2% agar. Tryptone soft agar was the same as tryptone agar, but contained 0.5% agar. Penassay broth for bacterial crosses contained Difco Antibiotics Medium 3 Bacto-Penassay. Eosin-methylene blue (EMB) agar with 0.5% galactose contains 200 μg dihydrostreptomycin/ml.

Complementation Test. The procedure used was followed to Kaiser (1957). A loopful of λ phage (~10^9/ml) was cross-streaked on λ agar plate seeded with sensitive bacteria and incubated overnight at 37°C. The occurrence of complementation can be made with more growth of bacteria in the area of overlap than in the other streak-region.

Measurement of Frequency of Lysogenization. A culture of cells growing exponentially at 37°C at a cell density of about 5 × 10^6 cells/ml was washed with 0.01 M-MgCl2 and aerated for 30 minutes at 37°C. These starved bacteria were infected with phage at the desired multiplicity. After 15 minutes at 37°C, the infected cells were diluted into broth, incubated for 30 minutes at 37°C and plated for colony survivors. Colonies were isolated and tested for the immunity by spotting with toothpick on a plate seeded with λ1 (3 × 10^6 phages/plate). In this condition, sensitive bacteria can not form colonies while λ lysogens form normal colonies. Percentage of lysogens/infected cells was used as lysogenization frequency. The test whether or not the bacteria carries the phage genome of λvirC or λvirC was performed as follows. λ wild type phages were plated on bacteria to be tested. Plaques were not detected when the bacteria is lysogenic for λvirCNO(P), while small clear plaques were appeared when the bacteria is lysogenic for λvirCNO(P). When the bacteria is resistant for λ, hgsimm was used instead of λ. T4rII produces plaque on both lysogens but not on λ wild type lysogen.

Bacteria Crosses. Crosses were done with slow shaking at 37°C in Penassay broth at cell concentrations of 1 × 10^9 donors/ml and 3 × 10^8 recipients/ml in a total volume of 0.8 ml. Mating was interrupted after 60 minutes by treatment for 30 seconds on a vortex mixer.