SHORT COMMUNICATION

PROPHAGE INDUCTION BY THE PRODUCT OF X REGION OF SUPERINFECTED λ OPERATOR-MUTANTS

TOMOAKI MUROTSU AND TADAO HORIUCHI

Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka 812

Received August 6, 1971

Virulent mutants of coli-phage λ able to grow on the wild type λ lysogen has three mutations, *virL*, *virR* and *virC*, and these mutational sites have been mapped as shown Fig. 1 (Horiuchi *et al.* 1969; Koga *et al.* 1970). The *virCvirR* (*virCR*) region is an

Fig. 1. Diagrammatic representation of a portion of the λ genome. Capital letters indicate clear plaque cistrons (CI and CII (Kaiser 1957)) and *sus* cistrons (N and O (Campbell 1961)). *imm*⁴³⁴ indicates the region of nonhomology between λ and λ *imm*⁴³⁴ (Kaiser and Jacob 1957), and *x* region is described by Eisen *et al.* (1966).

operator (and promotor) of the right-hand operon including gene O, and the *virL* region is an operator of the left-hand operon including gene N (Eshima, Ishikawa, Miyauchi and Horiuchi, in preparation; Sakakibara, Koga and Horiuchi, in press). Recent result has shown that infection of W3350su⁻ (Campbell 1961) lysogenic for λ ind⁻ (Jacob and Campbell 1959) with λ *virC*₃₄*virR*₁₈*susN*₇*susO*₈ (*virCRsusNO*) or with λ *virC*₃₄*susN*₇*susO*₈ (*virCsusNO*) caused predominant production of the prophage (Koga and Horiuchi, in press). This result would suggest that these superinfecting phages produce an *anti*-repressor which antagonizes a repressor coded from CI gene (*CI*-repressor) and leads to the induction of the prophage. Since *virCR* region is an operator region (or a promotor-operator region) of x-O operon and *virC* or *virR* mutant is insensitive for *CI*-repressor on the expression of x-O operon, a gene coded for *anti*-repressor may be controlled by the *virCR* operator.

Fig. 2. Participation of the *tx* mutation on the production of prophage following infection of W3350 (λind^-) with λ virulent derivatives. Exponential phase cultures of W3350 (λind^-) were infected with various λ mutants for 15 min at 37°C in 0.01 M $MgCl_2$. After neutralization of uninfected phage by the addition of λ -antiserum, the cultures were diluted into λ -broth (Horiuchi *et al.* 1969) and incubated with aeration at 37°C. At various times indicated after infection, the cultures were chloroformed and assayed for λind^- (producing turbid plaque) on C600 (su^+) (Appleyard 1954).

- ; Infection with $\lambda C_{I}virC_{34}R_{18}tx+susN_7O_8$, multiplicity of infection (m. o. i.)=2.2
 ●—●; Infection with $\lambda C_{I}virC_{34}tx+susN_7O_8$, m. o. i.=4.1
 ×—×; Infection with $\lambda C_{I}virC_{34}tx_{58}susN_7O_8$, m. o. i.=5.9

Numbers of prophage produced/bacterial numbers at zero time is plotted on a scale *versus* time after infection of the phage.

Recently, Ishikawa and Horiuchi (in press) have isolated turbid plaque revertants ($\lambda virC_{tx}$) from $\lambda virC$. The recombinants, λtx , which produce very turbid plaque were constructed and the mutational sites of tx mutations have been mapped at the right side of $virC_{34}$ and within the imm^{434} region (Kaiser and Jacob 1957). This λtx develops very poorly on λ sensitive bacteria, but can grow normally when a CI mutation is added. The λtx lysogen over-produces λ specific exonuclease (Radding 1964) by several times higher than that of wild type λ lysogen upon induction by ultraviolet light. These results indicate that the tx mutation occurred in the gene *tof* (Pero 1970) or *cro* (Eisen *et al.* 1970) in x region (Eisen *et al.* 1966).

We have assumed that a product coded from the *tof* gene would be the *anti*-repressor mentioned above. To test this assumption, we have constructed $\lambda CI virC_{34} tx_{53} susN_7 O_8$ ($\lambda virC_{tx} susNO$) and the λind^- lysogen was superinfected with this mutant to see whether λind^- prophage can be induced or not. As shown in Fig. 2, infection of λind^- lysogen with $\lambda virCRsusNO$ or $\lambda virCsusNO$ result in production of prophage type at about 2 hrs after infection, and the magnitude of development of the prophage by $\lambda virCsusNO$ is lower than that by $\lambda virCRsusNO$. It seemed that the prophage induction is not due to overtitration of CI-repressor by the multiplication of infecting λ phage in the lysogen, because the ratio of phage yield (infecting phage/ λind^-) is less than 0.1 through the period of incubation. It should be mentioned that this inducing effect for the prophage by the infecting phages is λ specific, because λimm^{434} cannot be induced so much from λimm^{434} lysogen in the same condition (Koga and Horiuchi, in press). On the other hand, in the case of infection with $\lambda virC_{tx} susNO$, no induction of the prophage is observed. These results indicate that the gene harboring tx mutation cannot produce apparently the *anti*-repressor, and therefore, the gene coded for the *anti*-repressor is *tof* or *cro* gene. During this incubation (5 hrs), the cell number increased by a factor of ten, and some fractions of cells harboring the infecting phage can produce the prophage. The time of lag period (2 hrs) may be required for accumulation of the *anti*-repressor.

Recently, it was also found that an *anti*-repressor, which promotes lytic response of infecting wild type λ phage is produced by $\lambda CI ts_{857} susNO$ lysogen (Oppenheim *et al.* 1970; Neubauer and Calef 1970; Eisen *et al.* 1970), by defective lysogen harboring several early genes (Spiegelman 1971) and by $\lambda v_1 v_3$ of Jacob and Wollman (1954) (Sly and Rabideau 1969; Data were not shown in their paper). It is also suggested that the gene coded for the *anti*-repressor may be the *tof* or *cro* gene (Eisen *et al.* 1970; Kumar *et al.* 1970; Spiegelman *et al.* 1970).

The functional site for the *anti*-repressor was suggested to be both the *virL* and *virR* regions (Koga and Horiuchi, in press) and the relation between the *anti*-repressor and the *vir*-repressor produced by $\lambda CI virCRsusNO$ lysogen (Koga and Horiuchi 1971) will be discussed elsewhere.

The evidence presented here indicates that the *anti*-repressor which antagonizes the action of CI-repressor is coded from the x region, possibly from *tof* or *cro* gene.

We wish to thank Dr. H. Koga, Mrs. S. Fujii and Miss M. Sakamoto for helpful discussions. This work was partly supported by a Scientific Research Grant from the Ministry of Education of Japan.

LITERATURE CITED

- Appleyard, R. K., 1954 Segregation of lambda lysogenecity during bacterial recombination in *Escherichia coli* K12. *Genetics* **39**: 424-439.
- Campbell, A., 1961 Sensitive mutants of bacteriophage λ . *Virology* **14**: 22-32.
- Eisen, H., C. R. Fuerst, L. Siminovitch, R. Thomas, L. Lambert, L. Pereira da Silva, and F. Jacob, 1966 Genetics and physiology of defective lysogeny in K12(λ): Studies of early mutants. *Virology* **30**: 224-241.
- Eisen, H., P. Brachet, L. Pereira da Silva, and F. Jacob, 1970 Regulation of repressor expression in λ . *Proc. Nat. Acad. Sci. Wash.* **66**: 855-862.
- Heinemann, S. F., and W. G. Spiegelman, 1970 Control of transcription of the repressor gene in bacteriophage lambda. *Proc. Nat. Acad. Sci. Wash.* **67**: 1122-1129.
- Horiuchi, T., H. Koga, H. Inokuchi, and J. Tomizawa, 1969 Lambda phage mutants insensitive to temperature-sensitive repressor. I. Isolation and genetic analysis of weak-virulent mutants. *Mol. Gen. Genetics* **104**: 51-58.
- Jacob, F., and A. Campbèll, 1959 Sur le système de repression assurant l'immunité chez les bactériés lysogènes. *C. R. Acad. Sci. Paris* **248**: 3219-3221.
- Jacob, F., and E. L. Wollman, 1954 Étude génétique d'un bactériophage tempéré d'*Escherichia coli*. I. Le système génétique du bactériophage λ . *Ann. Inst. Pasteur* **87**: 653-673.
- Kaiser, A. D., 1957 Mutations in a temperate bacteriophage affecting its ability to lysogenize *Escherichia coli*. *Virology* **3**: 43-61.
- Kaiser, A. D., and F. Jacob, 1957 Recombination between related temperate bacteriophages and the genetic control of immunity and prophage localization. *Virology* **4**: 509-521.
- Koga, H., and T. Horiuchi, 1970 Lambda phage mutants insensitive to temperature-sensitive repressor. II. Genetic character of λ virC mutant. *Mol. Gen. Genetics* **106**: 114-122.
- Koga, H., and T. Horiuchi, 1971 A new repressor produced by λ CIvirCR mutant. *Japan. J. Genetics* **46**: 285-288.
- Kumar, S., E. Calef, and W. Szybalski, 1970 Regulation of the transcription of *Escherichia coli* phage λ by its early genes N and *tof*. *Cold Spring Harbor Symp. Quant. Biol.* **35**: 331-339.
- Neubauer, Z., and E. Calef, 1970 Immunity phase-shift in defective lysogens: Non-mutational hereditary changes of early regulation of λ prophage. *J. Mol. Biol.* **51**: 1-13.
- Oppenheim, A. B., Z. Neubauer, and E. Calef, 1970 The antirepressor: a new element in the regulation of protein synthesis. *Nature* **226**: 31-32.
- Pero, J., 1970 Location of the phage λ gene possible for turning off λ -exonuclease synthesis. *Virology* **40**: 65-71.
- Radding, C. M., 1964 Nuclease activity in defective lysogens of phage λ . *Biochem. Biophys. Res. Commun.* **15**: 8-12.
- Spiegelman, W. G., 1971 Two states of expression of genes cI, rex, and N in lambda. *Virology* **43**: 16-33.
- Spiegelman, W. G., S. F. Heinemann, P. Brachet, L. Pereira da Silva, and H. Eisen, 1970 Regulation of the synthesis of phage lambda repressor. *Cold Spring Harbor Symp. Quant. Biol.* **35**: 325-330.
- Sly, W. S., and K. Rabideau, 1969 Mechanism of c_{17} cI virulence. *J. Mol. Biol.* **42**: 385-400.