λ CI vir CR変異株によって生産される新しいリプレッサー

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SHORT COMMUNICATION

A NEW REPRESSOR PRODUCED BY λ*CIvirCR* MUTANT

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The weak-virulent mutants (λ*virLvirR*) of coli-phage λ can grow on the λ*CIts* lysogen producing a temperature-sensitive repressor, but can not grow on the wild type λ lysogen. The virulent mutants able to grow on the wild type λ lysogen were obtained from the weak-virulent mutant by an additional clear plaque mutation, *virC*. *VirL*, *virR* and *virC* mutations have been mapped as shown in Fig. 1 (Horiuchi *et al.* 1969; Koga *et al.* 1970).

The *virCvirR* is a promotor and operator 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; Koga and Horiuchi in preparation).

Surprisingly, λ*CIvirCvirRsusNsusO* (λ*CIvirCRsusNO*) or λ*CIvirCsusNO* which cannot produce a repressor coded by *CI* gene is able to lysogenize a bacterium. The genome of λ*CIvirCRsusNO* or λ*CIvirCsusNO* transferred in accompany with gal^+ marker (which is located very close to att^{λ} marker) from donor lysogen (HfrH, gal^+ str^s carrying λ*CIvirCRsusNO* or λ*CIvirCsusNO*) to recipient cell (F^-, gal^- str^r). The phage λ*CIvirCRsusNO* or λ*CIvirCsusNO* cannot lysogenize the strain B582 which has deleted chromosome at att^{λ} site, and these phage genomes cannot be eliminated by the treatment of acridine orange (Hirota 1960).

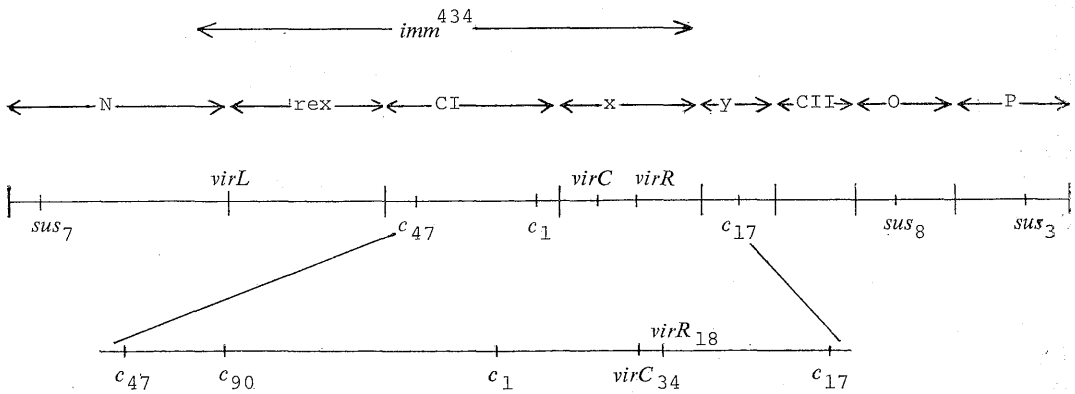

Fig. 1. Diagrammatic representation of a portion of the λ genome. Capital letters indicate clear plaque cistrons (*CI* and *CII*) and *sus* cistrons (*N*, *O* and *P*). imm^{434} indicates the region of nonhomology between λ and λimm^{434}.

Table 1. Effects of UV-irradiation on the transmission coefficients of λc_1 infected on cells lysogenic for λCI^+susNO or for $\lambda CIvirCRsusNO$ (A) and plating efficiencies of λ virulent derivatives, lambdoid and T4rII (B)

			Bacterial hosts	
			W3350 (λCI^+susNO)*	W3350 ($\lambda CIvirCRsusNO$)*
(A)	Transmission coefficients of λc_1	−UV	0.01	0.05
		+UV	0.83	0.14
(B)	Relative plating efficiencies	λc_1	$<10^{-10}$	1×10^{-5}
		$\lambda virL_{18}$	2×10^{-7}	6×10^{-3}
		$\lambda virR_{18}$	1×10^{-8}	2×10^{-4}
		$\lambda virC_{34}$	5×10^{-7}	2×10^{-5}
		$\lambda virL_{18}R_{18}$	2×10^{-6}	0.3
		$\lambda virL_{18}C_{34}$	1×10^{-5}	0.03
		$\lambda virC_{34}R_{18}$	1×10^{-5}	4×10^{-4}
		$\lambda virL_{18}C_{34}R_{18}$	1	1
		λimm^{434}	1	1
		λimm^{21}	1	1
		$T4rII_{1241}$	$<10^{-10}$	1 (1)**

(A) Exponential phase cultures (about 5×10^8 cells/ml) were irradiated with UV-light (200 ergs/mm^2), incubated for 30 min at 37°C, and infected with λc_1 at about multiplicity of 0.01 in 0.01M-$MgCl_2$ for 15 min at 37°C. After neutralization of uninfected phages by λ-antiserum, cultures were immediately plated on strain W3350. Transmission coefficients were expressed as the ratio, titers/input phage titers.

(B) 1.2% Tryptone agar plates were used for plaque assays of T4rII, and 1.2% λ agar plates for those of lambdoid phages.

* The lysogens each carry a λ prophage with $\lambda susN_7susO_8$, or $\lambda c_{90}virC_{34}virR_{18}susN_7susO_8$.

** Relative plating efficiency of T4rII when plated on W3350 lysogenic for $\lambda CI^+virCRsusNO$.

The $\lambda CIvirCRsusNO$ lysogen is immune for superinfecting λ phage, but not for other phages, for example, λimm^{434} and λimm^{21} (Table 1). This immunity for phage λ is different from that caused by *CI*-repressor (*CI*-immunity) as shown in Table 1, and therefore, will be referred as *vir*-immunity or *vir*-repressor for later description.

As can be seen in Table 1, irradiation of ultraviolet light on the lysogens inactivates *CI*-repressor almost completely, but hardly inactivates *vir*-repressor.

T4rII phage can develop in the λCI^+rex^- lysogen but not in the λCI^+rex^+ lysogen (Howard 1967). In our system, T4rII phage cannot develop in the λCI^+rex^+susNO lysogen but it can in the $\lambda CI^+rex^+virCRsusNO$ lysogen. This result indicates that the *CI* and *rex* genes do not function in the $\lambda virCRsusNO$ lysogen.

Unexpectedly, infection by $\lambda CIvirCRsusNO$ to the λind^- lysogen markedly induces development of prophage λind^- at 2-3 hrs after infection. The degree of development of superinfecting $\lambda CIvirCRsusNO$ was much less than that of prophage type in this condition (Koga and Horiuchi, in preparation). This result suggests that $\lambda CIvirCRsusNO$ produces anti-repressor which antagonizes the *CI*-repressor synthesized by λind^- prophage. Similar results were observed when λind^- lysogen was infected with $\lambda CIvirCsusNO$ but

the inducing effect of *λCIvirCsusNO* on prophage is weaker than that of *λCIvirCRsusNO*. It is to be noted that cells lysogenic for *λCIvirCsusNO* or for *λCIvirRsusNO* are anti-immune, in the sense that wild type *λ* phage produces clear plaques on these lysogens, while *λCIvirCRsusNO* lysogen is immune as described before. Further, partial diploid cell, in which the phages, *λCIvirCsusNO*, integrate into both the att^{λ} sites of F'1 and of host chromosome of *E. coli*, is immune like *λCIvirCRsusNO* lysogen. (Lysogen in which *λCIvirCsusNO* integrates only into the F'1 of att^{λ} is anti-immune). From these results, our current working hypothesis is that the anti-repressor becomes the *vir*-repressor when it is produced in a large amount.

Weak-virulent mutants, *λvirLvirR* are able to develop in the presence of *vir*-repressor (Table 1), but *λvirL*, *λvirR*, and weak-virulent, *λvirLvirC* are not. This result suggests that the functional sites of action of *vir*-repressor are *virL* and *virR* regions.

Since regions including *virCR* are transcribed from left to right (Taylor *et al.* 1967), and in the absence of active *N* gene product the rate of transcription of regions including *y-CII* is markedly decreased (Kumar *et al.* 1970), this *vir*-repressor would be coded from the *x* region. The *vir*-immunity may be similar to but much weaker than the super-immunity produced by *λdv* carrier cell (Matsubara and Kaiser 1968; Matsubara, personal communication). Recently, it was also found that an anti-repressor, which promotes lytic response of superinfecting *λ* phage, is produced by $\lambda CIts_{857}susNO$ lysogen (Oppenheim *et al.* 1970; Neubauer and Calef 1970; Eisen *et al.* 1970) and by defective lysogen carrying a prophage deleted for all the right sides of *y* region, and for all of the late genes (Heinemann and Spiegelman 1970; Spiegelman 1971).

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LITERATURE CITED

Eisen, H., P. Brachet., L. Pereira da Silva, and F. Jacob, 1970 Regulation of repressor expression in *λ*. Proc. Natl. Acad. Sci. **66**: 855-862.

Heinemann, S. F., and W. G. Spiegelman, 1970 Control of transcription of the repressor gene in bacteriophage lambda. Proc. Natl. Acad. Sci. **67**: 1122-1129.

Hirota, Y., 1960 The effect of acridine dyes on mating type factors in *Escherichia coli*. Proc. Natl. Acad. Sci. **46**: 57-64.

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. Molec. Gen. Genetics **104**: 51-58.

Howard, B. D., 1967 Phage lambda mutants deficient in rII exclusion. Science **158**: 1588-1589.

Koga, H., T. Miyauchi, and T. Horiuchi, 1970 Lambda phage mutants insensitive to temperature-sensitive repressor. II. Genetic character of *λvirC* mutant. Molec. Gen. Genetics **106**: 114-122.

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.

Matsubara, K., and A. D. Kaiser, 1968 *λ*dv: An autonomously replicating DNA fragment. Cold Spring Harbor Symp. Quant. Biol. **33**: 769-775.

Neubauer, Z., and E. Calef, 1970 Immunity phase-shift in defective lysogens: Non-mutational hereditary change 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.

Spiegelman, W. G., 1971 Two states of expression of genes *CI*, *rex*, and *N* in Lambda. Virology **43**: 16-33.

Taylor, K., Z. Hradecna, and W. Szybalski, 1967 Asymmetric distribution of the transcribing regions on the complementary strands of coli-phage λ DNA. Proc. Natl. Acad. Sci. **57**: 1618-1625.