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 λ *imm*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/mm²), incubated for 30 min at 37°C, and infected with λc_1 at about multiplicity of 0.01 in 0.01M-MgCl₂ 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 *λCIts₈₅₇susNO* 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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