JAPAN. J. GENETICS Vol. 47, No. 3: 171-178 (1972)

LAMBDA PHAGE MUTANTS INSENSITIVE TO TEMPERATURE-SENSITIVE REPRESSOR* IV. EFFECTS OF *VIR*L MUTATIONS ON THE EXPRESSION OF GENES N AND *EXO*

SETSUKO FUJII, NATSUKO ESHIMA, TERUO MIYAUCHI AND TADAO HORIUCHI

Faculty of Pharmaceutical Sciences, Kyusyu University, Fukuoka 812

Received January 21, 1972

Previously we have reported (Horiuchi *et al.* 1969) the isolation and genetic analysis of weak-virulent mutants of coli-phage λ which can grow on the bacteria carrying a mutant prophage producing temperature-sensitive λ repressor (Horiuchi and Inokuchi 1967) but cannot grow on the lysogen producing normal repressor. We have found that the weak-virulent mutant has at least two mutations. One is located on the left side of cI gene (*vir*L) and the other is located on the right side of cI gene (*vir*R) (Horiuchi *et al.* 1969). λ recombinants carrying single mutation, *vir*L or *vir*R have been isolated.

In this paper, we will report the effect of the *vir*L mutation on the gene expression. Results indicated that the *vir*L may be the mutation of operator gene controlling the left-hand operon including gene N.

MATERIALS AND METHODS

Bacteria and phage strains: *E. coli* strains used and their relevant characteristics were: C600 su^+ and W3350 su^-, permissive and nonpermissive host, respectively, for the λ*sus* mutants (Campbell 1961). W3101 thy^- was used for the preparation of isotopically labelled DNA.

The phage strains used were λ*sus*N_7, λ*sus*0_8 (Campbell 1961), λ, λc_{47} (Kaiser 1957), λc_{17} (Thomas and Bertani 1964; Pereira da Silva and Jacob 1968), λ*ind*$^-$ (Jacob and Campbell 1959), λ*imm*^{434} (Kaiser and Jacob 1957), λ*vir* (Jacob and Wollman 1954), λcI*ts*$_{389}$, λ*vir*L, λ*vir*R (Horiuchi *et al.* 1969). Recombinants were prepared from these mutants as needed.

Media: λ-broth contains 10g of polypeptone (Daigo-Eiyo Chemicals, Tokyo) and 2.5g NaCl per 1 of water. For plaque assay λ-broth was solidified with 0.5% agar for the top layer and 1.2% agar for the bottom layer. M-9 (Kornberg *et al.* 1959)—maltose—casamino acid medium was used for the preparation of ^{3}H-thymidine labelled W3101

* This work was partly reported at the XIIth International Congress of Genetics, held in Tokyo, on August 23, 1968.

thy⁻ DNA.

Gene expression experiments: (a) Phage complementation: *E. coli* strain W3350 (λind^-) was grown in λ-broth to 1×10^9 cells/ml, diluted with λ-broth to 5×10^8 cells/ml. The cells were simultaneously infected with the λ phage to be tested at a multiplicity of about 4 phages/cell and with either $\lambda imm^{434} sus N_7 cI$ or $\lambda imm^{434} sus 0_8 cI$ at a multiplicity of about 0.4 phage/cell. After 15 minutes adsorption at 37°C, the infected cells were treated with λ antiserum and aerated at a concentration of 1×10^5 cells/ml for 90 minutes at 37°C in λ-broth. The cultures were chloroformed, and assayed for phage on the *E. coli* strains, C600 (λ) and C600 (λimm^{434}).

(b) Prophage complementation: Each lysogenic strain was grown in λ-broth to 1×10^9 cells/ml. The cells were infected with either $\lambda imm^{434} sus N_7 cI$ or $\lambda imm^{434} sus 0_8 cI$ at a multiplicity of 0.3 phage/cell. The next procedures were as in (a). The cultures were assayed for phage on C600 (λ) and C600 (λimm^{434}).

Replication inhibition experiments: W3350 (λind^-) and W3350 were grown, washed, resuspended, as in *Gene expression experiments* and were infected with phage $\lambda imm^{434} cI$ and the λ phage to be tested at a multiplicity of infection of about 3 of each type. After 90 minutes, the cultures were chloroformed and assayed for phage on C600 (λ) and C600 (λimm^{434}).

Assay of λ exonuclease: The lysogen W3350 ($\lambda cI ts_{389} sus 0_8$) was grown in λ-broth to 3.5×10^8 cells/ml at 28°C, harvested, and resuspended in 1/10 volume of 0.01M $MgSO_4\cdot\lambda$ phages to be tested were added at a multiplicity of 3–4 and allowed to adsorb. The infected cells were diluted 10 fold into λ-broth and shaken at 28°C. Crude extracts from these infected cultures for enzyme assay were prepared by the method of Sly and Rabideau (1969). The assay of λ exonuclease was similar to that of Little, Lehman and Kaiser (1967) with the following modification: DNA used as the substrate was prepared from the cells W3101 thy⁻ labelled with 2 μCi/ml of ^{3}H-thymidine (5.0 Ci/mμmole) for 12 hrs at 37°C by the method of phenol extraction (Saito and Miura 1963) with the procedure of charcoal treatment (Lehman 1960). The radioactivity of the DNA in a assay tube was about 4×10^4 c.p.m. (specific activity 47,500 c.p.m./μgm). One unit of activity of λ exonuclease was defined as 10^4 c.p.m. solubilized in 30 minutes per mg of protein at 37°C.

RESULTS

Effects of virL mutation on the expression of the N or 0 gene function

As shown in Fig. 1, two operons related to the early function of λ phage development are known: the left operon contains gene N and the right operon contains genes CII and 0. The left operon is transcribed from right to left and the right operon is transcribed from left to right (Eisen *et al.* 1966; Taylor *et al.* 1967; Doerfler and Hogness 1968).

The fact that the *vir*L mutational sites locate on the right side of, and very close to gene N suggests strongly that the *vir*L mutation is the operator mutation of the left-hand operon. We have tested λ*vir*L for the ability to synthesize the product of gene N or 0 in the presence of λ repressor by phage complementation test (Thomas

and Bertani 1964) and by prophage complementation test (Thomas 1966). Two among several *vir*Ls mutants, *vir*L_{18} and *vir*L_{112}, have been used in this paper. In the case of phage complementation test, a *λind*$^-$ lysogen was coinfected with *λvir*L and heteroimmune phage *λimm*^{434}cI bearing *sus* mutation in gene N or 0. Phage *λimm*^{434}sus progeny will develop if the infecting *λvir*L can supply the N or 0 gene product in the presence of λ repressor.

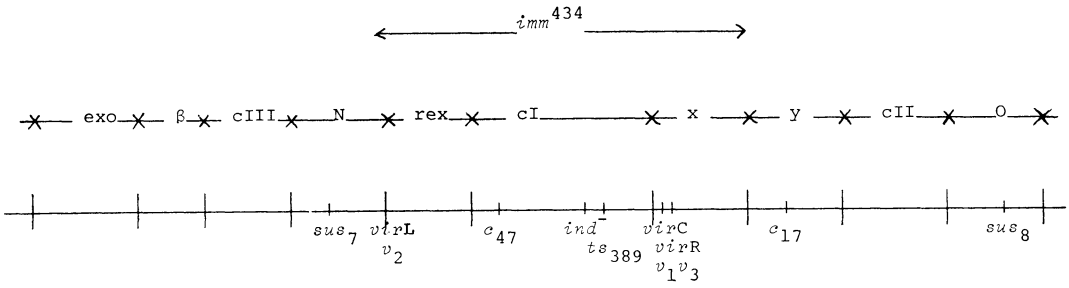

Fig. 1. Diagrammatic representation of a portion of the λ genome. *imm*^{434} indicates the region of non-homology between λ and *λimm*^{434}.

As shown in Table 1, *λvir*L supplied the N gene product for the coinfected *λimm*^{434} cI*sus*N but not the 0 gene product for the coinfected *λimm*^{434}cI*sus*0. *λvir*L itself did not develop significantly in this condition. On the other hand, *vir*R_{112} supplied both the N and 0 gene products, and c_{17} bearing a mutation in y region which creates a new promotor for 0 gene (Packman and Sly 1968; Roberts 1969) expressed the 0 gene function but did not express the N gene function under the same condition. We conclude

Table 1. The expression of N and 0 genes of λ virulent derivatives by the method of phage complementation

Infecting phage		Phage in lysate			*imm*^{434} test*
imm$^{\lambda}$	*imm*^{434}cI	*imm*$^{\lambda}$	prophage	*imm*^{434}	*imm*^{434} control
c_{47}	+*sus*N_7	6.3×10^3	$<10^3$	4.5×10^4	1.0
c_{47}	+*sus*0_8	2.1×10^5	1.2×10^4	1.9×10^4	1.0
c_{47}*vir*L_{18}	+*sur*N_7	6.7×10^4	$<10^3$	5.0×10^5	11.1
c_{47}*vir*L_{18}	+*sus*0_8	3.0×10^5	9.0×10^3	1.7×10^4	0.9
c_{47}*vir*L_{112}	+*sus*N_7	2.9×10^4	$<10^3$	3.5×10^5	7.7
c_{47}*vir*L_{112}	+*sus*0_8	1.5×10^5	2.0×10^3	1.9×10^4	1.0
c_{47}*vir*R_{112}	+*sus*N_7	2.8×10^5	$<10^3$	4.6×10^5	10.3
c_{47}*vir*R_{112}	+*sus*0_8	5.0×10^5	7.0×10^3	2.5×10^5	13.2
c_{17}	+*sus*N_7	3.5×10^4	5.0×10^2	3.9×10^4	0.8
c_{17}	+*sus*0_8	2.3×10^5	2.7×10^4	8.3×10^4	4.5

The procedure is described in *MATERIALS AND METHOD*. The concentration of the infected *λind*$^-$ lysogens was 1×10^5 cells/ml and multiplicity of infection of λ is about 4 and that of *λimm*^{434} was about 0.4. The cultures were assayed for phage on the *E. coli* strains C600 (λ) and C600 (*λimm*^{434}).

*This value was expressed as the ratio of the number of *λimm*^{434} produced in each case to that produced when the infecting λ phage was *λc*$_{47}$.

that the *vir*L region controls the expression of the left-hand operon.

As described in the previous paper, λ*vir*L and λ*vir*R can lysogenize *E. coli* (Horiuchi *et al.* 1969). We have tested the effect of *vir*L on the expression of the N or 0 gene function in the prophage state. The results were presented in Table 2.

λ*vir*L mutants in the prophage state express the N gene function constitutively, while λ*vir*R_{112} expresses the N and 0 gene functions constitutively. We confirmed the the conclusion obtained in the phage complementation test that the *vir*L region controls the expression of the left-hand operon.

Table 2. The expression of N and 0 genes of λ virulent derivatives by the method of prophage complementation

Prophage imm^{λ}	Superinfecting phage imm^{434}cI	imm^{434}	Phage in lysate imm^{434} test / imm^{434} control	Prophage
wild type	+ *sus*N_7	1.1×10^4	1.0	5.9×10^4
wild type	+ *sus*0_8	2.1×10^4	1.0	7.2×10^4
virL_{112}	+ *sus*N_7	3.8×10^4	3.5	5.2×10^4
virL_{112}	+ *sus*0_8	2.0×10^4	1.0	4.3×10^5
virL_{18}	+ *sus*N_7	3.8×10^5	34.6	1.8×10^5
virL_{18}	+ *sus*0_8	5.7×10^4	2.7	3.3×10^5
virR_{112}	+ *sus*N_7	3.2×10^5	29.1	1.4×10^5
virR_{112}	+ *sus*0_8	2.3×10^6	110	5.0×10^6

The procedure is described in *MATERIALS AND METHOD*. The results were expressed as the ratio of the number of λimm^{434} produced in each case to that produced when the prophage was wild type λ.

λimm^{434}*sus*$^+$ recombinants or revertants were less than 0.01 to that of calculated infective centers of λimm^{434} in all experiments.

We could not succeed to obtain λ*vir*R_{18} lysogen, so far.

Table 3. Test of replication inhibition of λ virulent derivatives

Phage imm^{434}cI plus λ	Bacterium W3350 (λ*ind*$^-$) λ/λimm^{434}	W3350 λ/λimm^{434}
c_{47}	0.05	0.70
c_{47}*vir*L_{18}	0.05	0.56
c_{47}*vir*L_{112}	0.08	0.63
c_{47}*uir*R_{112}	0.28	0.89
c_{17}	0.50	0.59
c_{17}*sus*0_8	0.64	0.70

The procedure is described in *MATERIALS AND METHODS*. The concentration of the infected cells was 1×10^5 cells/ml and multiplicity of infection was about 3 for each of λimm^{434}cI and λ. The results were expressed as the ratio of λ to λimm^{434} phage in each lysate.

Effects of λvirL on replication inhibition

Coinfection of a λ lysogen with λ and λimm^{434} results in the production of large excess of λimm^{434} phages among the progeny (Thomas and Bertani 1964). The low yield of λ phage is accounted for by the fact that the λ genomes appearing in the burst have undergone only limited replication (Ptashne 1965; Green *et al.* 1967).

We have carried out this experiment using *λvir*L. As shown in Table 3, *λvir*L did not develop in the *λind*$^-$ lysogen. On the other hand, $\lambda virR_{112}$ develops considerably and λc_{17}(Packman and Sly 1968) develops extensively in this condition.

In conclusion, *λvir*L is not subject to the loss of replication inhibition.

The λ specific exonuclease synthesis by λvirL in the presence of λ repressor

The cistron *exo*, which is located on the left side of N gene, is the structural gene of λ specific exonuclease (Radding *et al.* 1967) and the production of this enzyme is known to be induced by the N gene product (Radding and Echols 1968).

As shown in Fig. 2, the synthesis of the exonuclease in $\lambda cIts_{389}sus0_8$ lysogen superinfected with various λ virulent derivatives carrying $sus0_8$ has been studied.

The λ exonuclease level increased markedly when the superinfecting phage was

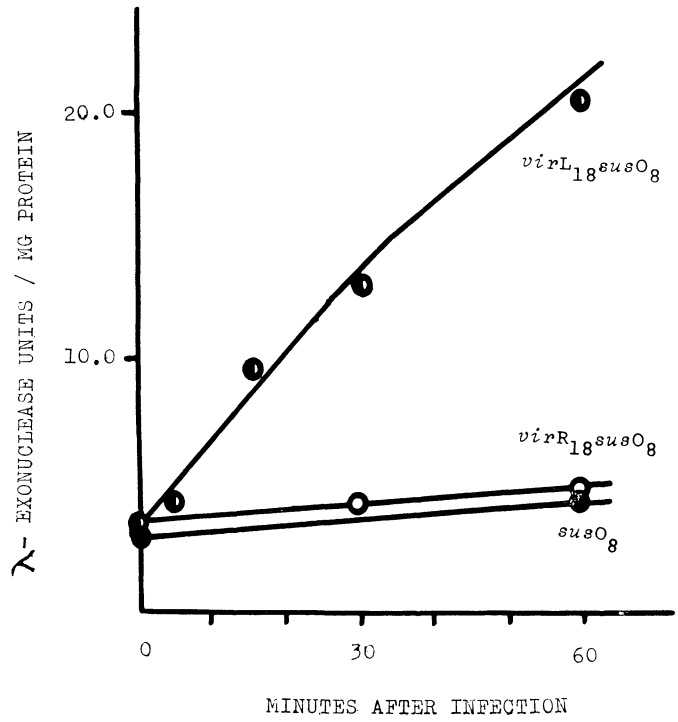

Fig. 2. λ exonuclease levels following infection of the lysogen W3350 ($\lambda cIts_{389}sus0_8$) by various λ virulent derivatives carrying $sus0_8$. Multiplicity of infection was 3-4 phage per bacterium for each of the mutants indicated. Preparation of extracts, and the λ exonuclease assay conditions are described in *MATERIALS AND METHODS*. Zero time refers to the time when the phage infected cells were diluted into 28°C growth medium.

$\lambda vir L_{18} sus 0_8$. However the λ exonuclease formation was not so significant when the superinfecting phage was $\lambda vir R_{18} sus 0_8$. The λDNA synthesis will be negligible during this stage because both prophage and infecting phage carry *sus*0 mutation. Similar tendency was observed when prophage or superinfecting phage carried the intact gene 0^+.

These results indicate that the *vir*L mutation is necessary to produce the N gene product the amount of which is enough to induce λ exonuclease synthesis in the presence of λc*Its* repressor.

DISCUSSION

As shown in section I, λvirL phage or prophage can supply the N gene product for the development of infected $\lambda imm^{434} sus$N in the presence of λind^- repressor. λvirL phage can also express the function of λ exonuclease gene, which is known to be induced by the N gene product, in the presence of temperature-sensitive λ repressor. The expression of the left-hand operon of λvirL in λ lysogen was also supported by the facts that λvirL synthesized preferentially mRNA of the left-hand operon in the λc*Its* lysogen and did not replicate in similar conditions (Sakakibara and Tomizawa 1971). These results indicate that the *vir*L region is an operator of the left-hand operon including gene N.

The effect of *vir*L is similar to that of v_2 mutation of the classical λvir ($\lambda v_1 v_2 v_3$) isolated by Jacob and Wollman (1954).

Ptashne and Hopkins (1968) and Pereira da Silva and Jacob (1968) have shown that λv_2 synthesizes the product of gene N in the presence of λ repressor and, moreover, isolated λ repressor has lower binding affinity for λv_2 DNA than for wild type λ DNA (Ptashne and Hopkins 1968).

It is to be mentioned that the recombinant, $\lambda vir L v_1 v_3$, shows virulency for λ lysogen (Koga *et al.* 1970).

Characters of *vir*R mutation will be described elsewhere.

SUMMARY

The effect of *vir*L mutation on the gene expression was studied. λvirL mutant expresses the N gene function but not the 0 gene function as analysed by the method of hetero-immune phage complementation in the presence of λ repressor. The λ specific exonuclease which is induced by the N gene product was produced by λvirL in λc*Its* lysogen. *Vir*L region may be an operator region of the left-hand operon.

ACKNOWLEDGMENTS

We wish to thank Dr. Y. Sakakibara for his criticism in the preparation of the manuscript.

This work was aided in part by a research grant from Jane Coffine Childs Memorial Fund for Medical Research and in part by a research grant from the Ministry of Education of Japan.

LITERATURE CITED

Campbell, A., 1961 Sensitive mutants of bacteriophage λ. Virology **14**: 22-32.

Doerfler, W., and D. S. Hogness, 1968 Gene orientation in bacteriophage lambda as determined from the genetic activities of heteroduplex DNA formed *in vitro*. J. Mol. Biol. **33**: 661-678.

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.

Green, M. H., B. Gotchel, J. Hendershott, and S. Kennel, 1967 Regulation of bacteriophage lambda DNA replication. Proc. Natl. Acad. Sci. **58**: 2343-2350.

Horiuchi, T., and H. Inokuchi, 1967 Temperature-sensitive regulation system of prophage lambda induction. J. Mol. Biol. **23**: 217-224.

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 mutant. Mol. Gen. Genetics **104**: 51-58.

Jacob, F., and E. L. Wollman, 1954 Étude génétique d'un bactériophage tempéré d'*Escherichia coli* I. Le systèm génétique du bactériophage λ. Ann. Inst. Pasteur **87**: 653-673.

Jacob, F., and A. Campbell, 1959 Sur le systèm de repression assurant l'immunité chez la bacteriés lysogènes. C. R. Acad. Sci. Paris **248**: 3219-3221.

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., T. Miyauchi, and T. Horiuchi, 1970 Lambda phage mutants insensitive to temperature-sensitive repressor. II. Genetic character of λ*vir*C mutant. Mol. Gen. Genetics **106**: 114-122.

Kornberg, A., S. G. Zimmerman, S. R. Kornberg and J. Josse, 1959 Enzymatic synthesis of deoxyribonucleic acid. VI. Influence of bacteriophage T_2 on the synthetic pathway in host cells. Proc. Natl. Acad. Sci. **45**: 772-785.

Lehman, I. R., 1960 The deoxyribonucleases of *Escherichia coli*. I. Purification and properties of a phosphodiesterase. J. Biol. Chem. **235**: 1479-1487.

Little, J. W., I. R. Lehman, and A. D. Kaiser, 1967 An exonuclease induced by bacteriophage λ. I. Preparation of the crystalline enzyme. J. Biol. Chem. **242**: 672-678.

Packman, S., and W. S. Sly, 1968 Constitutive λ DNA replication by λc_{17}, a regulatory mutant related to virulence. Virology **34**: 778-789.

Pereira da Silva, L., and F. Jacob, 1968 Étude génétique d'une mutation modifiant lasen sibilité a l'immunité chez le bactériophage lambda. Ann. Inst. Pasteur **115**: 145-158.

Ptashne, M., 1965 Replication and host modification of DNA transferred during bacterial mating. J. Mol. Biol. **11**: 829-838.

Ptashne, M., and N. Hopkins, 1968 The operators controlled by the λ phage repressor. Proc. Natl. Acad. Sci. **60**: 1282-1287.

Radding, C. M., J. Szpirer, and R. Thomas, 1967 The structural gene for λ exonuclease Proc. Natl. Acad. Sci. **57**: 277-283.

Radding, C. M., and H. Echols, 1968 The role of the N gene of phage λ in the synthesis of two phage-specified proteins. Proc. Natl. Acad. Sci. **60**: 707-712.

Roberts, J. W., 1969 Promotor mutation *in vitro*. Nature **223**: 480-482.

Saito, H., and K. Miura, 1963 Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochem. Biophys. Acta **72**: 619-629.

Sakakibara, Y., and J. Tomizawa, 1971 Regulation of transcription of lambda bacteriophage operator mutants. Virology **44**: 463-472.

Sly, W. S., and K. Rabideau, 1969 The mechanism of λc_{17}cI virulence. J. Mol. Biol. **42**: 385-400.

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

1618-1625.

Thomas, R., 1966 Control of development in temperate bacteriophages. I. Induction of prophage genes following heteroimmune superinfection. J. Mol. Biol. **22**: 79-95.

Thomas, R., and L. E. Bertani, 1964 On the control of the replication of temperate bacteriophages superinfecting immune hosts. Virology **24**: 241-253.