バクテリオファージλのclts変異株のマッピングおよびその一般性質

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MAPPING AND GENERAL CHARACTERIZATION OF cI*ts* MUTANTS IN BACTERIOPHAGE λ[1)]

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Infection of a cell with bacteriophage λ can elicit one of two responses; (1) lytic response which leads to progeny phage production and lysis of the host, (2) the lysogenic response in which the viral DNA becomes associated with the bacterial DNA and replicates with it, without self multiplication of λ DNA. Once lysogeny is established, repressor coded by the gene cI prevents the expression of the prophage genes as well as of homoimmune superinfecting phages.

Two types of mutation in gene cI resulting in temperature-sensitive regulation of prophage induction have been reported (Lieb 1966; Horiuchi and Inokuchi 1967): lysogens for λ cI*ts* type A (or type I) having mutational sites in the left part of gene cI are induced irreversibly even at low temperature after heat treatment, and lysogens for λ cI*ts* type B (or type II) mapped in the right part of gene cI are induced only when the culture is heated at a high temperature during growth. In the previous paper, Horiuchi *et al.* (1967) reported that significant complementation for lysogenization between some cI*ts*A and cI*ts*B mutants was not observed. However, weak complementation between some cI*ts*A and cI*ts*B mutants was observed by Lieb (1966) and Green (1966). This result may be due to intracistronic complementation, because molecular structure of cI-repressor is supposed to be possibly tetramer (Chadwick *et al.* 1970).

This communication confirms that several cI*ts*A and cI*ts*B mutants newly isolated were mapped in the left and right part of gene cI, respectively, and furthermore demonstrates that some cI*ts*B mutants which are located proximal to *x* region easily complement for lysogenization with some cI*ts*A mutants.

MATERIALS AND METHODS

Bacteria and phage strains: *E. coli* strains used and their relevant characteristics were; C600*su*$^-$ and W3350*su*$^-$, permissive and nonpermissive hosts, respectively, for the λ*sus* nonsense mutants (Campbell 1961). The phage strains used were λc_1, λc_{47}, λc_{97}, λc_{50}, λc_{68}, λc_{67} (Kaiser 1957), λt_{2-1} (Horiuchi and Inokuchi 1967), $\lambda sus\mathrm{N}_7$ and $\lambda sus\mathrm{O}_8$ (Cambell 1961). Recombinants desired were prepared from these mutants by the cross, as needed.

1) This work was briefly reported in Seikagaku **39**: 590 (1967).

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Media; λ-broth contains 10.0 g polypeptone (Daigo Eiyo Chemicals, Tokyo), 2.5 g NaCl, 1.0 *l* 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 buffer was used for heat induction of λcI*ts* lysogen. This buffer contains $MgSO_4$, 0.01 %; NH_4Cl, 0.1 %; Na_2HPO_4, 0.6 %; KH_2PO_4, 0.3 %; NaCl, 0.5 %, pH 7.0.

Phage crosses: Phage crosses were performed as follows. Exponentially growing C600 cultures in λ-broth were centrifuged, and the bacteria were suspended in 0.02 M-$MgCl_2$, and aerated for 60 minutes at 37°C. These starved bacteria (about 2×10^8 cells/ml) were then infected with 10 phages per cell of each of two mutant phages, which were previously irradiated with UV light allowing about 50 % survival. The infected cultures were kept for 15 minutes at 37°C, and then anti-λ serum was added and allowed to act for 20 minutes. The cultures were then diluted into λ-broth and incubated for 90 minutes at 37°C with vigorous aeration. Chloroform was added to the cultures to ensure complete lysis of infected cells and release of progeny particles. Lysates were diluted as required for plaque assays. When c*Its* mutants were used in the cross, the plates were incubated at 43°C overnight and then the numbers of plaques were counted. Determination of relative order in the fourfactor crosses were done by the detection of sus^+c^+ (wild type) recombinants on a lawn of W3350su^-.

Complementation tests for lysogenization: A loopful of λ phage (about 10^9/ml) was cross-streaked each other on a plate seeded with sensitive bacteria and incubated overnight at 43°C. Judgements of complementation can be made with more growth of bacteria in the area of overlap than in the streak-region. As circumstances require, we used the methods of judgements of complementation by the more growth of bacteria in the overlap region of plaques of two phages when two phages (about 500/plate, respectively) were plated on sensitive bacteria and incubated overnight at 43°C.

Induction by heating: Bacteria lysogenic for λcI*ts* in the exponential phase of growth were divided into three portions. One portion was continued to incubate at 28°C, the other was incubated at 43°C. Another was centrifuged, washed with M9 buffer and suspended in one-tenth of the original volume of buffer. The suspension was heated at 47°C for 10 minutes and nine-tenth of the original volume of broth was added. Then the culture was incubated at 28°C. Bacterial density of each portion was measured as optical density at 660 mμ using Hitachi spectrophotometer model 101.

RESULTS AND DISCUSSION

1) *Isolation of c*Its *mutants*

Heat inducible mutants of λ were isolated from λ wild type irradiated with ultraviolet light to give a survival of 5×10^{-3} and then plated on ultraviolet-treated C600 bacteria to 60 % survival. The plates were incubated at 43°C and phages that gave clear plaques at 43°C and turbid plaques at 28°C were selected for further study. Thirteen mutants were isolated.

Lysogens for λcI*ts* obtained at 28°C lyses at 43°C, while wild type λ lysogen does not. When lysogenic cells for λcI*ts* in exponential phase at 28°C were heated in buffer at 47°C for 10 minutes, then incubated at 28°C after suspending cultures with growth

medium, there exist two types in their growing behavior. One is induced irreversibly even at 28°C after heat treatment, the other is not. We call the former as type A, the latter as type B, according to Lieb (1966). Of thirteen mutants tested, phage strains belonging to type A were 320, 327, 342, 417 and 495, and those to type B were 343, 389, 486, 503, 508, 511, 630 and 642.

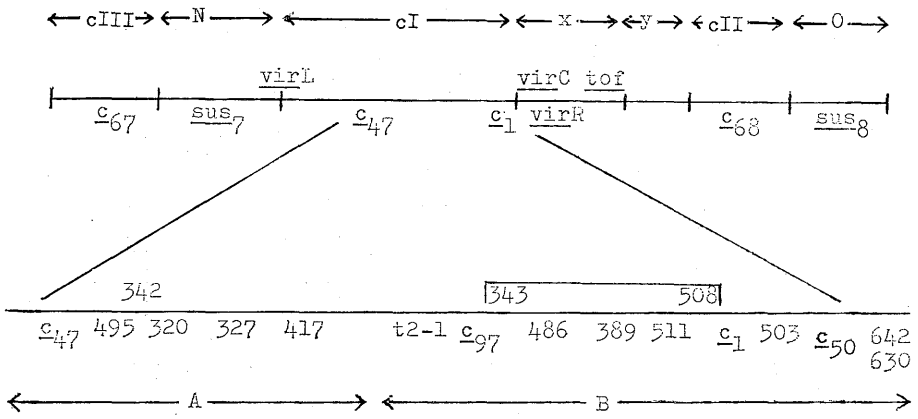

Fig. 1. Diagrammatic representation of a portion of the λ genome. Capital letters indicate clear plaque cistrons (cI, cII and cIII) and *sus* cistrons (N and O).

2) *Mapping of* cIts *mutants*

These cI*ts* mutants were crossed with each other and with λc_{47}, λc_{97} and λc_1 of the stocks of Kaiser (1957). Data are given in Table 1. It is evident that cI*ts* mutants belonging to type A or type B are all mapped in the left part or the right part of gene cI, respectively. These results confirm those of Lieb (1966). Here, it is interesting to recollect that the mutational sites of λind^- were all mapped in cI-A region (Eshima *et al.* 1972). The mapp orders of 343, 486, 389, 511 and 508 cannot be deduced from the data presented here. It may be concluded from these results that region of cI*ts* together extends from the vicinity of c_{47} to the right of c_1.

Kaiser (1957) reported that among his stocks of λcI mutants, c_{47} and c_1 loci are located left-end and right-end in gene cI, respectively, and that c_{50} located on the right side of c_1 but not in *y* or cII gene (see Fig. 1) cannot lysogenize and shows unusual character on a point that it shows complementation for lysogenization with some of the cI mutants. Our mutants, cIts_{642} and cIts_{630}, are also expected to locate on the right side of λc_1, we performed four-factor crosses involving counterselectable marker, *sus*N and *sus*O, in order to determine relative order between mutants cIts_{642}, cIts_{630} and c_{50}. The lysates of the crosses were plated on the W3350su^- strain (Campbell 1961) to select for sus$^+$ recombinants, and turbid plaques among these recombinants were scored. The results given in Table 2 clearly show that the marker order is as follows: c_{50}-(cIts_{642}, cIts_{630}). The sites cIts_{642} and cIts_{630} are very closely linked or locate at the same site. These mapping results of relative gene order indicate that λc_{50} is the mutation in gene cI. Similar conclusion was also observed by Brachet (personal communication). Fur-

Table 1. Recombination frequencies between cI and cI*ts* mutants

		A					B										
	c_{47}	495	342	320	327	417	2-1	c_{97}	343	486	389	511	508	c_1	503	642	630
c_{47}		12	15	13	57	71	132	162	186	191	167	128	232	247	266	191	220
495								142						136			
342								122						214			
320								94						210			
327								93						200		206	
417							47	69	128	110	100	145		80		144	192
2-1								48						78			
c_{97}									30		26		38	39	52	37	94
343														63			
486														20			
389														17			
511														15			
508														15		19	34
c_1															25	26	18
503																	13
642																	<1
630																	

The numbers of wild type recombinants $\times 10^4$ are the average values from 2 to 5 times of crosses. Cultures of C600 were infected with a multiplicity of 10 of each parental phage which had been irradiated previously to increase recombination. After a 90-min growth period, the cultures were chloroformed and plated on C600. The plates were incubated at 43°C.

Table 2. Four-factor crosses to map relative order among c_{50}, cIts_{630}, and cIts_{642} mutants

	Crosses	$\frac{sus^+c^+ \text{ recombinants}}{sus^+ \text{ recombinants}}(\times 10^3)$	(a)/(b)
(a)	$susN_7c_{50}$ $\times$ cI$ts_{630}susO_8$	13.4	2.5
(b)	$c_{50}susO_8$ $\times susN_7$cIts_{630}	5.3	
(a)	$susN_7c_{50}$ $\times$ cI$ts_{642}susO_8$	8.2	3.7
(b)	$c_{50}susO_8$ $\times susN_7$cIts_{642}	2.2	
(a)	$susN_7$Its_{630} $\times$ cI$ts_{642}susO_8$	<0.01	
(b)	cI$ts_{630}susO_8 \times susN_7cIts_{642}$	<0.01	

Bacteria (C600su^+) were infected with a multiplicity of 10 of each parental phage which had been irradiated previously to increase recombination, and incubated for 90 minutes. The cultures were chloroformed and plated on C600su^+ and W3350su^-. The plates were incubated at 43°C and the number of turbid plaques and total plaques on strain W3350 were counted.

thermore, immune cells were obtained with a frequency of about 0.01 % (immune cells/input cells) when sensitive cells were infected with λc_{50} at a high multiplicity (m.o.i.= 13). This immune cell maintains its prophage stable and allows growth of weak-virulent mutants, λvirLvirR, able to grow on λcIts lysogen (Horiuchi *et al.* 1969; Koga *et al.*

1970). These results suggest that λc_{50} can lysogenize *E. coli* and produces a weak repressor like a temperature-sensitive one.

3) *Complementation for lysogenization among cI*ts *mutants*

In the usual complementation tests, lysogenization occurs when sensitive cells were simultaneously infected with two clear plaque mutants which belong to different genes, such as cII and cIII mutants. Lieb (1966), Green (1966) and Brachet and Thomas (1969) have observed complementation between some cI*ts*A and cI*ts*B mutants. We also performed complementation tests between cI*ts*A and cI*ts*B mutants by the detection of the production of turbidity as evidence of growth of lysogenic survivor cells at 43°C. Among λcI*ts*A or among λcI*ts*B mutants, no complementation for lysogenization occured, but weak complementation was observed between some cI*ts*B mutants, especially, which are mapped on the right-end regions of gene cI, and some cI*ts*A mutants (Table 3). As Chadwick *et al.* (1970) report that repressor acts in a oligomeric state, our results may be due to intracistronic complementation. Also, λc_{50} complements some cI*ts*A mutants, as shown in Table 3. Simple interpretation would be made that cI*ts*A

Table 3. Complementation tests for lysogenization among λcI*ts*, cI, cII and cIII mutants

	cI-A						cI-B											cII	cIII
	c_{47}	495	342	320	327	417	c_{97}	343	486	389	511	508	c_1	503	c_{50}	642	630	c_{68}	c_{67}
c_{47}	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	++	++
495		−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	++	++
342			−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	++	++
320				−	−	−	−	−	−	−	−	−	−	+	+	+	+	++	++
327					−	−	−	−	−	−	−	−	−	−	−	++	−	++	++
417						−	−	−	−	−	±	−	−	+	++	−	++	++	++
c_{97}							−	−	−	−	−	−	−	−	−	−	−	++	++
343								−	−	−	−	−	−	−	+	−	−	++	++
486									−	−	−	−	−	−	−	−	−	++	++
389										−	−	−	−	−	+	−	−	++	++
511											−	−	−	−	+	−	−	++	++
508												−	−	−	−	−	−	++	++
c_1													−	−	−	−	−	++	++
503														−	+	−	−	++	++
c_{50}															−	±	±	++	++
642																−	−	++	++
630																	−	++	++
c_{68}																		−	++
c_{67}																			−

A loopful of phage was cross-streaked each other on a lawn of C600 and incubated overnight at 43°C.

++: strong complementation
+: mediate complementation
±: weak complementation
−: no complementation

region is the site for the internal inducer by the observation that mutational sites of *ind*$^-$ are mapped in the left side of gene cI.

SUMMARY

(1) Mutants of phage λ occurred in gene cI which show clear plaque at high temperature but normal turbid plaque at low temperature, were isolated. (2) Lysogens for cI*ts*A are induced irreversibly even at low temperature after heat treatment, and lysogens for λcI*ts*B are induced only when the cultures are heated at a high temperature during growth. These results confirmed those of Lieb (1966) and Horiuchi and Inokuchi (1967). (3) λcI*ts*A and λcI*ts*B mutants were all mapped in the left and right part of gene cI, respectively, as reported by Lieb (1966). (4) Some λcI*ts*B which are located on the right-end region of gene cI complement λcI*ts*A for lysogenization.

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LITERATURE CITED

Brachet, P., and R. Thomas, 1969 Mapping and functional analysis of *y* and cII mutants. Mutation Res. **7**: 257–260.

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

Chadwick, P., V. Pirrota, R. Steinberg, N. Hopkins, and M. Ptashne, 1970 The λ and 434 phage repressors. Cold Spring Harbor Symp. Quant. Biol. **35**: 283–294.

Eshima, N., S. Fujii, and T. Horiuchi, 1972 Isolation of λ*ind*$^-$ mutants. Japan. J. Genetics **47**: 125–128.

Green, M. H., 1966 Inactivation of the prophage λ repressor without induction. J. Mol. Biol. **16**: 134–148.

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 mutants. Molec. Gen. Genetics **104**: 51–58.

Kaiser, A. D., 1957 Mutations in a temperate bacteriophage affecting its ability to lysogenize *Escherichia coli*. Virology **3**: 43–61.

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

Lieb, M., 1966 Studies of heat-inducible λ phage. I. Order of genetic sites and properties of mutant prophages. J. Mol. Biol. **16**: 149–163.