VIROLOGY 118, 448-450 (1982)

SHORT COMMUNICATIONS

Bacteriophage Lambda Containing Two Temperature-Sensitive Mutations in the *cI* Gene Produces Clear Plaque at 30°

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Received June 29, 1981; accepted December 18, 1981

When λ*cIts2Psus3* was crossed with λ*Nsus7sus53*cI857, clear plaque formers at 30° were obtained among the λN^+P^+ recombinants. This clear recombinant of λ was shown to contain the two *cIts* mutations, 2 and 857, of the parents. It was also observed that crosses between any two λ*cIts* mutants, from a list of eight, produced recombinants forming clear plaques at 30°. These suggest that no pair of *ts* mutations of the eight tested are compatible with the functioning of the repressor even at 30° when they are present in *cis*.

The repressor of bacteriophage λ functions as a dimer originating from the same polypeptide chain coded by the *cI* gene (for a review, see Ref. *1*). This monomeric protein molecule contains functionally two distinct domains; the amino terminal domain has the specificity for binding to operator DNA while the carboxy terminal domain favors the subunit interaction (*2, 3*). There are several temperature-sensitive (*ts*) mutations which map in the two regions of the *cI* gene corresponding to the above two functional domains of the repressor molecule (*4, 5*). While the renaturability of the heat-inactivated *ts* repressors *in vivo* depends on which of the above two regions the mutational sites map in (*4*), their renaturability *in vitro* does not bear any such relationship (*6*). Certain λ*cIts* mutants with defects in the amino terminal half of the repressor can complement certain others having defects in the carboxy terminal half (*4, 7*) to produce less heat-labile repressor. This indicates that the hybrid dimers formed by two different *ts* monomers in question are stable at 43°. But the λ mutants having *ts* mutations, cI857 and U9, in the region corresponding to the DNA binding half of the repressor molecule are not complemented by any other *cIts* mutants (*7*). Though the complementation between two *cIts* mutants takes place in certain cases as mentioned above, nothing is known about the effect of the simultaneous presence of two *ts* mutations in the *cI* gene of λ on the functional behavior of the repressor.

In an attempt to make such a double *cIts* mutant of λ by genetic cross, we initially chose λ*cIts2* and λ*c*I857 mutants because the lysogens of these two phages possess certain distinguishing properties (*4, 8*) by which the two mutations could be identified, if needed. For easy screening, λ*cIts2Psus3* was crossed with λ*Nsus7-sus53c*I857, and the N^+P^+ recombinants (see Fig. 1) were selected on su^- bacteria at 30°. It was observed that a fraction of the plaques produced under the above conditions was of clear phenotype. All the clear plaques obtained so far had similar morphology and they appeared with a frequency of 0.2% which was much higher than that of spontaneous appearance of clear plaque mutants (less than 0.001%) in the individual populations of the two parent phages. This clear phenotype λ, when crossed separately with λ*Nsus7-sus53c*I^+ and λ*c*I^+*Psus3* phages, could produce two λ*cIts* recombinants which were identical, in several properties, with λ*cIts2* and λ*c*I857 (*4, 8*), respectively. This established the presence of two mutations, *cIts2* and *c*I857, of the parents in *cis* in the above clear phenotype N^+P^+ recombinant.

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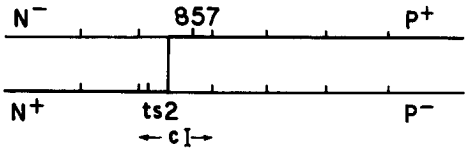

FIG. 1. Cross between λ*Nsus7sus53*cI857 and λc*Its2Psus3*. Cross was done in *E. coli* 594 (*su*⁻). The input m.o.i. was 5 for each parent. The λN^+P^+ recombinants were selected on the same bacteria, and the total phage in the lysate were assayed on C-600 (*su*⁺). The heavy line indicates the formation of N^+P^+ recombinants which may have two *ts* mutations in the *c*I gene. Cross between λc*Its2* and λcI857 also yielded clear recombinants with nearly the same frequency as that obtained with their P^- and N^- derivatives, respectively.

The above result led us to test whether λ with combinations of any two known c*Its* mutations produces clear plaques at 30°. The results of crosses between the pairs of different λc*Its* phages having mutations either in the same domain or in the two different domains of the *c*I protein are shown in Table 1. The data indicate that in all the cases tested so far, phages producing clear plaques at 30° appeared. From the observed direct relationship between the relative distances of two *ts* sites in question and the frequencies of occurrence of these phages in the cross lysates in most cases (Table 1), we conclude that these clear plaque phages are the recombinants carrying in *cis* the two corresponding c*Its* mutations of their parents. It may further be noted that all the clear recombinants isolated at 30° produced clear plaques even at 25°.

Each of the c*Its* mutants 1, U46, and U50 complements each of 2, 1-22, and U51 in *trans* (*7*). This study shows that when any of the former three mutations is present in *cis* with any of the latter three, the corresponding λ produces clear plaques at 30° (Table 1). There are several mutants of λ each having single missense mutations at different sites in the *c*I gene, and these mutants produce clear plaques at temperatures ranging from 25 to 43° (*9*). Since the formation of clear plaques is attributable to the loss of function of the repressor of λ, one can conclude that the simultaneous presence of two different *ts* defects in this protein molecule makes it nonfunctional at 30°. This loss of function of the *c*I protein by the *cis* combination of

TABLE 1

FORMATION OF CLEAR PLAQUE RECOMBINANTS BY CROSSES[a] BETWEEN PAIRS OF DIFFERENT λc*Its*[b] PHAGES

Parent phage No1	Frequency[c] of clear recombinants[d] formed with (parent 2)[e]							
	ts2	1-22	U51	*ts1*	857	U46	U9	U50
ts2	x	0.013	0.096	0.19	0.20	0.32	*0.18*	0.32
1-22		x	0.08	0.24	*0.06*	*0.12*	0.30	0.24
U51			x	0.026	*0.04*	0.09	0.138	0.14
ts1				x	0.036	0.042	0.046	0.11
857					x	0.018	0.013	0.048
U46						x	0.062	0.109
U9							x	0.05
U50								x

[a] Crosses were done in *E. coli* 594 using an input m.o.i. of 5 for each parent. Results, which are in italics, were obtained by crosses done in *E. coli* C-600. For the details of the cross between *ts2* and 857, see Fig. 1; for all other crosses, respective c*Its* phages with wild type *N* and *P* were used, and the clear recombinants were selected on 594 lawn.

[b] For the map positions of these mutations, see Ref. (*5*).

[c] Frequencies are shown as percentage of total phage in the lysate which produced clear plaque at 30°.

[d] All the 28 clear recombinants obtained from various crosses also produced clear plaques at 25°.

[e] Mutations, *ts2*, 1-22, and U51, map in the same domain of subunit interaction, while others in the domain of DNA binding (Refs. *4*, *5* and *7*).

two *ts* mutations seems to be independent of whether the two mutations in question map in the same domain or in the two different domains of the protein molecule. Whether this behavior of double-*ts* repressors of λ is due to their inability to form stable oligomers, or is due to their failure to show stable interaction with the operator DNA after oligomer formation, is not known.

ACKNOWLEDGMENTS

We thank Prof. M. Lieb, Department of Microbiology, University of Southern California School of Medicine, Los Angeles, for the λ*clts* mutants. Thanks are also due to Prof. B. B. Biswas, Head of the Department of Biochemistry, for showing interest, and to Prof. S. Ghosh for valuable comments during the progress of this work. This work was supported by a grant from the Department of Atomic Energy, Government of India, Bombay.

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