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Mutations in the New Gene stIII of Bacteriophage T4B Suppressing the Lysis Defect of Gene stII and Gene *e* Mutants

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Mutations of bacteriophage T4B were found which suppress the lysis defect of both gene stII mutants and gene *e* mutants. The suppressor mutations belong to a new gene, stIII, of phage T4B. Gene stIII is located on the genetic map of T4B between genes stI and *e*. stIII mutants sometimes form star plaques on *Escherichia coli* B. The latent period on *E. coli* 594, but not *E. coli* B, is shorter with stIII mutants than that with wild-type phage. The premature lysis of *E. coli* 594 infected with stIII phage does not depend on the expression of both stII$^+$ and *e*$^+$ function. stIII allele is dominant over the stIII$^+$ with respect to both the ability to suppress the stII defect and the early lysis of infected *E. coli* 594 cultures.

Lysis of *Escherichia coli* cells infected with bacteriophage T4 occurs in two steps. The gene stII of T4B (homologous to the t gene of T4B) (9) controls the breakdown of the cytoplasmic membrane (6, 9). stII mutants do not lyse *E. coli* K-12 and its derivatives, but lyse poorly *E. coli* B (7, 8). The *e* mutants do not lyse bacteria of either strain. The *e* gene lysis defect is suppressed by spackle gene mutations (3). The stII lysis defect is suppressed by rII gene mutations. The suppressor effect of rII mutations is displayed on *E. coli* B, but not on *E. coli* K-12 (7, 8). *E. coli* mutants were found permissive for *e* and t mutants (11).

In the present investigation we found phage mutations suppressing the lysis defect of stII mutants on *E. coli* K-12 and suppressing the lysis defect of *e* mutant on *E. coli* B and on *E. coli* 594. This paper presents the results of the genetic and physiological study of the suppressor mutations.

MATERIALS AND METHODS

Bacteriophages. T4B$^+$ wild-type phage and its mutants were used. The mutations in stII gene were temperature-sensitive missense mutant *st2* (8) and *am* mutant 14 (9). The amZ25 is an amber mutant in gene *e*. The amber mutants in other genes of T4B phage were obtained from V. Gordeev (Moscow University).

Bacterial strains. For isolating mutants of T4B phage carrying a suppressor mutation and for analyzing these mutations the following bacterial strains were used: *E. coli* B, the usual host of T4 phage; *E. coli* 594 (su$^-$), a restrictive host and a derivative of *E. coli* K-12; *E. coli* K-12S, a nonlysogenic derivative of *E. coli* K-12, carrying a weak amber suppressor. *E. coli* CR63 was used for obtaining stocks of phages, as a plating indicator and as a host in phage crosses.

All phage and bacterial strains were obtained from the collection of our Institute.

Media. The Hottinguer broth (pancreatic digest of meat) and M9 medium used in our experiments were described previously (8, 9). The solid media for the bottom layer was the broth with 1.2% agar and the semisolid media was the broth with 0.7% agar. To obtain pronounced differences in morphology of plaques of different phages, the bottom layer contained not less than 40 ml of the medium and the top layer contained 1.0 to 1.5 ml. The plates were incubated overnight at 37 C.

Phage crosses were carried out by the method of Edgar and Steinberg (2).

Physiological experiments. The physiological experiments were carried out at 37.0 C. The minimal duration of the latent period was determined in four to eight experiments for each mutant. Control experiments were carried out simultaneously with the wild-type phage or with single mutants. The single-step growth experiment was carried out by the method of Adams (1).

Permeability of infected bacteria. A culture of *E. coli* 594 was grown in M9 medium with a mixture of ^{14}C-labeled amino acids (4 μCi/ml). The bacteria were washed free of label by centrifuging and diluted into fresh M9 medium without label to 5×10^7/ml. The bacteria were infected with phage at a multiplicity of infection (MOI) of 5 and were incubated at 37 C. The samples were withdrawn at different times, chilled in a water-ice bath, and centrifuged at 5 C at $5{,}000 \times g$ for 15 min. Radioactivity of the pellet was measured.

Study of complementation and dominance. *E. coli* 594 at 5×10^7/ml were infected with a mixture of mutants so that each of the tested phage was at an MOI of 5. In control tests, cells were infected with each of the phages at an MOI of 10. In experiments the length of the minimal latent period was determined.

RESULTS

Isolation of pseudorevertants of stII mutants. Upon plating stII mutants on an *E. coli* 594 lawn, some plaques were observed to arise with a low frequency (10^{-6}). It was found that the phages from the plaques harbor parental stII mutations. Thus, the phages are pseudorevertants, carrying suppressor mutations which promote the lysis of *E. coli* 594. We analyzed one pseudorevertant of mutant st2 and nine pseudorevertants of mutant l4. All the pseudorevertants had the same plaque morphology; they formed tiny plaques on *E. coli* 594, K-12, and K-12S and star plaques on *E. coli* B. The phages isolated from the halo of star plaques of pseudorevertants on *E. coli* B carried rII mutations in different sites of the rII region.

Phages carrying a suppressor mutation. Phages carrying only a suppressor mutation were found among recombinant progeny of crosses of st2 and l4 pseudorevertants with the wild-type phage. Suppressor mutations were isolated from each of 10 pseudorevertants. Suppressor phage plaques were smaller than wild-type plaques and had no blue opalescence typical for wild-type plaques. All of the 10 suppressor phages formed similar plaques on *E. coli* B, CR63, 594, K-12, and K-12S. Every suppressor mutation was able to suppress both the l4 and st2 mutations. Only parental type phages were found in the progeny of crosses of a phage carrying a suppressor mutation with the wild-type phage. The suppressors seemed to be due to mutation at a single site.

About 10% of the plaques of suppressor phages had a star morphology. We termed the gene carrying the suppressor mutations the stIII gene. The secondary phages isolated from the halo of star plaques of stIII mutants on *E. coli* B carried rII and rI mutations.

Dominance of stIII mutations. Ninety percent of *E. coli* 594 bacteria coinfected with 14stIII-1 and 14stIII$^+$ phages were able to form plaques on *E. coli* CR63. The efficiency of plating of *E. coli* 594 infected with the l4stIII mutant on *E. coli* CR63 was 15%. Bacteria *E. coli* 594 coinfected with l4stIII-1 and l4stIII$^+$ phages were able to be lysed, and the lysis started on the 18th to 20th min, as if they were infected with the l4stIII-1 mutant only (Fig. 1). A similar result was obtained for l4stIII-2 mutant. Thus allele stIII dominated allele stIII$^+$ in both features: the ability to suppress the lysis defect of stII mutants upon infection of *E. coli* 594 and the ability to prematurely lyse infected cells. Because of the dominance of stIII mutations, it was not possible to conclude whether these stIII mutations were situated in one or different cistrons.

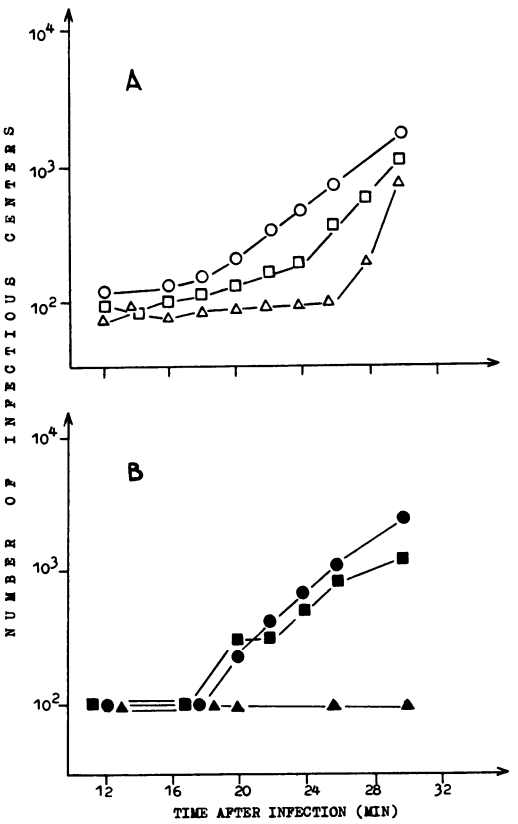

FIG. 1. *Dominance of the stIII-1 allele over the stIII$^+$ allele. The dominance of the stIII-1 allele is determined by the start of lysis of a mixedly infected bacterial culture at 37 C. E. coli 594 at 5 × 10^7/ml was infected with a mixture of mutants at an MOI of 5 of each phage type. At the same time single infections with each of the mutants at a MOI=10 were also carried out. At 7 min, anti-T4 serum was added to K = 3/min. At 12 min, the mixture was diluted at least 10^4-fold, and was plated at different times. Lysis curves of infected bacteria are shown: (A) infecting phages carried the stII$^+$ allele; (B) infecting phages carried the l4 mutation. Symbols: Δ, wild-type T4B$^+$ phages; O, stIII-1 mutant; □, the mixture of the wild-type phage and stIII-1 mutant; ▲, l4 mutant; ●, l4stIII-1 mutant; ■, the mixture of l4 and l4stIII-1 mutants.*

Mapping of suppressor mutations and gene stIII. In mapping stIII mutations we used the morphological differences between plaques of phage stIII and plaques of the wild-type phage. stIII mutational sites were located between genes *e* and stI (Table 1, Fig. 2). The distance between the nearest stI and stIII mutations was about 3 map units. Four independently isolated stIII mutants were distributed at three sites on

the genetic map. The orientation of the stIII locus with respect to adjacent genes was determined by three-factor crosses.

Physiological study of phages carrying stIII mutations. The double mutant z25stIII-1 was obtained to elucidate an interaction between gene stIII and *e* mutations. The stIII-1 mutation restored the ability of the mutant l4 to lyse *E. coli* 594 (Fig. 3) and the ability of mutant z25 to lyse *E. coli* 594 and *E. coli* B (Fig. 3 and 4). The time of the start of the lysis of *E. coli* B was the same for the stIII mutant and the wild-type phage. *E. coli* 594 were lysed with mutant stIII-1 6 to 8 min earlier than with the wild-type phage. The start of lysis of *E. coli* 594 infected with stIII mutant phage did not depend on the functioning of the *e* or stII gene. Spontaneous liberation of phage progeny was stopped at 40 min, when 50 to 60% of the total progeny was liberated for the z25 stIII-1 mutant and 70 to 80% was liberated for the l4stIII-1 mutant.

Mutant z25stIII-1 did not form visible plaques on *E. coli* B and *E. coli* 594 restrictive for amber mutants at any temperature (30, 37, 42 C). After incubation, the presence of the z25 stIII-1 phage in the lawn of *E. coli* B was determined, but no phage was found. Thus, the stIII-1 mutation suppressed the *e* lysis defect in the liquid but not on the solid medium. To explain this difference we studied the growth of the z25 stIII-1 mutant on overnight cultures (Fig. 4). The latent period of development was prolonged upon infection of the overnight culture of *E. coli* B with the z25stIII-1 mutant, and only 10% of phage were liberated spontaneously. Thus, it is possible that growth inability of the mutant on the solid media was due to the physiological age of the bacteria.

Permeability of E. coli 594 cells infected with stIII and the wild-type phage. The permeability of infected cells was determined to elucidate a possible effect of stIII mutations on the lytic process (Fig. 5). No label was found in the medium before lysis. The liberation of label and mature phage coincided in time. Thus, the effect of stIII mutations on the lysis did not appear to be a permeability alteration of infected cells.

TABLE 1. *Results of crosses of mutant stIII-1 with mutants in different genes to locate the stIII mutation on the genetic map of T4B phage*

Mutant	Gene	Frequency of recombination (%)[a]
am10 am765	42-46	8
am10 am753	42-56	9
am1311 am937 r1272	38-30-rII	13
am937 XF16	30-32	9
am937 am398	30-63	15
X7-4 X7-6	48-25	12
XF19 am 610	16-11	17
am765	46	20
am753	56	18
am689	52	19
amP74	34	30
XF16-1	33	24
XF18-1149	6	23
am730	1	21
am132	49	11
rI	rI	10.4
z25	*e*	9.3
st3-4	stI	5.6
4st-1	stI	3.4

[a] Frequency of recombination was measured as twice the percent of wild-type recombinants in progeny of cross on *E. coli* CR63.

DISCUSSION

The lysis of bacteria infected with T-even phage seems to be a complex process, the time and character of its realization being under control of many genes of the bacteriophage: 5 r genes (V. N. Krylov, Abstr. 1st Int. Symp. Genet. Indust. Microorg., 1971, p. 79), stI gene, ac gene (10), stII gene (6–9; Krylov, Abstr. 1st Int. Symp. Genet. Indust. Microorg. 1971, p. 79) *e*, and spackle genes (3, 4). In the present paper

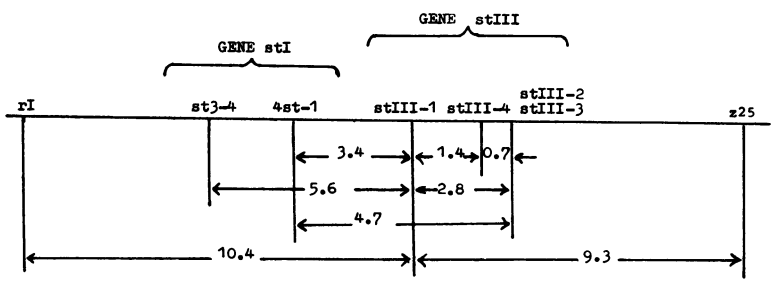

FIG. 2. *Localization of stIII mutations on the genetic map of phage T4B.*

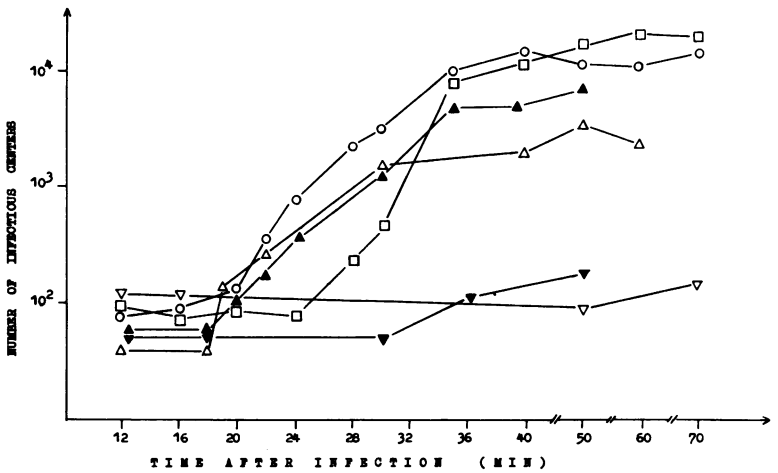

FIG. 3. *Single-step growth experiments with stII, stIII, e mutants and the wild-type T4B phage on E. coli 594. Bacteria at 10^8/ml were infected at 37 C with phage at MOI = 0.1 per cell (zero time). At 7 min, anti-T4 serum was added to K = 3/min. At 12 min, the mixture was diluted at least 10^4-fold and was plated at different times. Lysis curves of infected bacteria are shown. Symbols: □, the wild-type T4B phage; ▽, l4 mutant; ○, stIII-1 mutant; ▲, l4stIII-1 mutant; ▼, z25 mutant; △, z25stIII-1 mutant.*

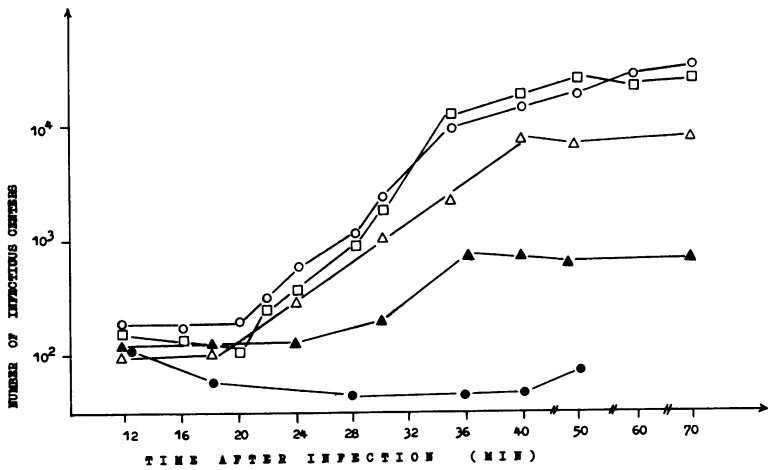

FIG. 4. *Single-step growth experiments of stIII, e mutants, and wild-type T4B phage on E. coli B. See the legend to Fig. 2. Lysis curves of infected bacteria are shown. Symbols: □, the wild-type phage; ○, stIII-1 mutant; △, z25stIII-1 mutant, logarithmic culture; ▲, z25stIII-1 mutant, overnight culture; ●, z25 mutant.*

we have shown that there is one more "lytic" gene, stIII.

We could not determine by complementation testing whether the stIII mutations were located in a single cistron because of the dominance of stIII alleles. Nevertheless, we think that all the stIII mutations belong to a single gene, as they have identical characteristics and are closely linked on the genetic map. We suppose that the dominance of stIII alleles was caused by a regulatory character of gene stIII function. It can be assumed that the stIII gene as well as some other genes (5, 13) participated in the control of membrane structure, leading normally to its strengthening.

StIII mutations suppress both stII-amber and stII-missense mutants. We suppose the stIII mutations were gene-specific suppressors, and that the suppression of the stII lysis defect was realized at the level of the expression of function. It is possible that the suppression of the stII and *e* mutants lysis defect by stIII mutations was accomplished by a lysis mechanism different from the normal one. It is possible that the stIII mutations could modify the effects of phage or bacterial genes controlling structure of

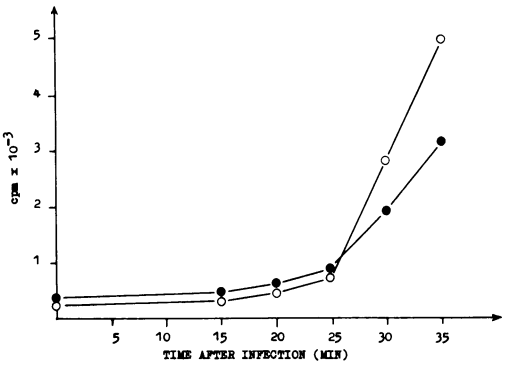

FIG. 5. *Permeability of stIII-1 and wild-type phage-infected E. coli 594. The culture of E. coli 594 grown on M9 medium with a mixture of ^{14}C-labeled amino acids (4 μCi/ml). The bacteria were washed from the label by centrifugation and resuspended in fresh M9 medium without label to 5 × 10⁷/ml. The bacteria were infected with phage at MOI = 5 and incubated at 37 C. The samples were withdrawn at different times, chilled in a water-ice bath and centrifuged at 5 C, 5,000 × g, for 15 min. The radioactivity of 0.5 ml of the pellet was measured. In a simultaneous growth experiment the start of the cultural lysis was determined. The activity of the pellet is shown. Symbols: O, the wild-type T4B phage; ●, stIII-1 mutant.*

the membrane and/or cell wall. *E. coli* mutants have been described which can be lysed upon infection with gene *e* or *t* mutants (11). It is possible that the stIII gene of phage T4B accomplished a function similar to that of *E. coli*. Emrich (3) found that the spackle mutation suppresses the *e* lysis defect. Thus, mutations of different genes are able to suppress the defect of *e* gene function. The fact that suppression of *e* defect with stIII mutation was manifested only in the liquid medium may be explained by a physiological "aging" of bacteria (the late stationary phase) on the lawn. It is interesting to note that the genes which were involved in the control of membrane structure and lysis (rI, rII, rV, ac, stI, stII, stIII) grouped in the two regions of the genetic map of T4. This arrangement may reflect the occurrence of at least two operons controlling these characters.

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