A SUPPRESSOR* IN THE GENOME OF PHAGE T4 INHIBITING PHENOTYPIC EXPRESSION OF MUTATIONS IN GENES *46* AND *47*

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IN studies with conditional lethal mutants of phage T4, it has been shown that genes *46* and *47* control cytosine-DNA specific deoxyribonuclease activity (WIBERG 1966). Moreover during T-even phage infection of *Escherichia coli*, synthesis of other new nucleases is known to occur. The genes which control these nucleases, however, are not known and the enzymes coded for by genes *46* and *47* have not yet been identified biochemically (KUTTER and WIBERG 1968).

In the process of evolution new genes can arise as a result of duplication of preexisting genes. It is possible that some of the supposed nuclease genes of phage T4 originated from the same gene. If this assumption is correct, the possibility that a nuclease gene after "successful" mutation will function as another nuclease gene cannot be excluded. It could be manifested by modification of the enzyme specifity in biochemical tests. In genetic tests this phenomenon may be revealed by gene-specific suppression. On the basis of these general considerations, we have attempted to select phage mutations which specifically suppress amber mutations in genes *46* and *47*.

MATERIALS AND METHODS

Bacterial strains: Escherichia coli B and *E. coli* CR-63. The strain *E. coli* B was used as a restrictive plating indicator for selecting and assaying revertants and in order to estimate the recombinants in crosses. Permissive strain *E. coli* CR-63 was used as a plating indicator for assaying amber mutants and as host in the phage crosses.

Phages: Phage T4, a wild type, was kindly supplied by S. BENZER in 1961. Mutant *r1* in gene *r1* was isolated in this laboratory. The amber mutants of phage T4B in different genes were kindly supplied by V. GORDEEV (Moscow University); UGA mutant *336Fy* in gene *46* was kindly supplied by T. METREVELY.

Media: Nutrient broth after HOTTINGUER ("broth") from a typtic digest of meat containing 100 mg% of amine nitrogen and 5 g NaCl per liter was used in all of our experiments. Broth top-layer agar (0.7%) and broth bottom agar (1.2%) were used for plating.

Phage crosses were carried out according to STEINBERG and EDGAR (1962).

The studies of mixed infections of *E. coli* B by either *am*+ or *am su* and *am* with different multiplicities of *am* were performed according to SNUSTAD (1968).

The general techniques for assaying phage were described by ADAMS (1959).

* A previous designation of *Su A* (KRYLOV and PLOTNIKOVA 1970) has been replaced here by the symbol *sua* in order to avoid confusion with two different suppressor mutations already known by that name in *E. coli*.

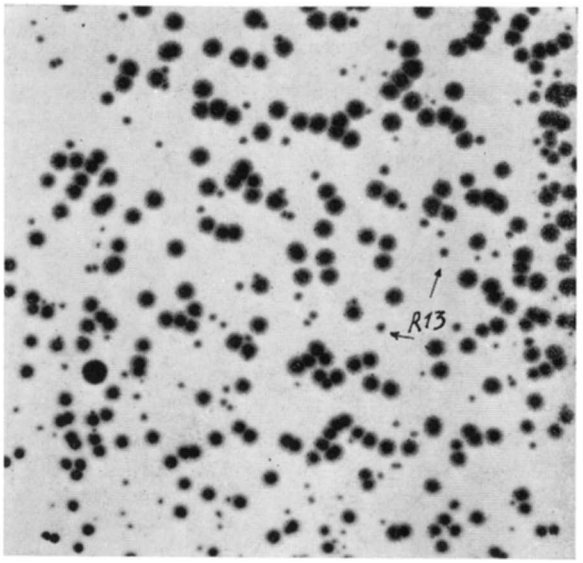

FIGURE 1.—Plaques of wild-type phage and phage R13 on *E. coli* B.

RESULTS

On *E. coli* B revertants of *am 765* (amber mutant of phage T4B in gene *46*) form several types of plaques. One of the revertants tentatively marked as R13 forms small plaques on *E. coli* B and wild-type plaques on *E. coli* CR-63 (Figure 1).

Genetic structure of R13 phage: The specific appearance of R13 plaques can be accounted for by plaque-type mutation in am^+ revertants, or alternatively, by a suppressor mutation in the *am 765* genome with partial suppressor effect. To reveal the genetic structure of R13 phage, crosses R13 × wild-type phage have been performed. Some phage stocks isolated from progeny plaques grown on *E. coli* CR-63 turned out to be *am* mutants. These *am* mutants neither complement nor recombine with phage *am 765*. Thus R13 is suppressed and must carry a suppressor mutation which we termed *su*α. Therefore the genetic structure of R13 phage can be represented by the following formula: *am 765 su*α.

Isolation of suα *mutation*: The progeny of the cross R13 × wild type forms on *E. coli* B plaques of three types: Two types are indistinguishable from those of R13 and wild type, respectively. The third type is slightly more turbid than those of wild type. This third phage was crossed with *am 765* and progeny were plated on *E. coli* B. Small plaques identical to those of R13 were found. Thus the results suggest that the third type phage from the cross R13 × wild type has the *su*α genotype. On *E. coli* CR-63 phage, *su*α forms wild-type plaques.

Localization of suα *on the genetic map of phage T4*: In order to localize *su*α, the following five crosses have been performed: $+ + \times$ *am 765 su*α, *r1 am 765* $+ \times + +$ *su*α, *am 765* $+ \times +$ *su*α, *r1 am 765* $+ \times +$ *am 765 su*α, $+$ *am 765*

TABLE 1

Results of crosses used to localize suα *on the genetic map of phage T4B*

Crosses						Number of crosses	Genotype of recombinants			Percent recombination*
+	+	×	..	*am 765*	*suα*	3	*am*	+	..	20.3
am 765	+	×	..	+	*suα*	12	*am*	*su*	..	24.1
+ *am 765*	*suα*	×	*r1*	+	+	2	*r*	*am*	+	18.7
r1 am 765	+	×	+	*am 765*	*suα*	7	*r*	*am*	*su*	19.4
r1 am 765	+	×	+	+	*suα*	2	*r*	*am*	*su*	1.9
							+	*am*	*su*	11.6
am 765	+	×	+	*am 50*	(gene *47*)	23	+	+	..	12.7
am 50	+	×	+	*am 1582*	(gene *55*)	5	+	+	..	5.0
am 765	+	×	+	*am 1582*		2	+	+	..	17.6
am 1582	+	×	+	*am 132*	(gene *49*)	2	+	+	..	10.3
am 50	+	×	+	*am 132*		10	+	+	..	13.7
am 132	+	×	+	*r1*	(gene *rI*)	5	+	+	..	12.1

* Percent observed recombinants, doubled. It is assumed that reciprocal recombinants occur with equal frequencies in a lysate of the population of cells.

suα × *r1* + + ; the results of these and some other crosses are summarized in Table 1.

According to the data in Table 1, map positions of all markers tested are given in Figure 2. The *suα* mutation is localized in a new gene in the "early" region of the genetic map in the relatively vacant space between genes *55* and *49*.

Specificity of suppressor effect of suα: In order to reveal *suα* suppressor specificity, a number of crosses of *suα* with amber mutants in genes *10, 18, 20, 23, 26, 30, 42, 43*, and *49* have been performed. In no case did progeny of these crosses form R13 type plaques on *E. coli* B. It can be concluded, therefore, that either *suα* is unable to suppress the amber mutations examined, or alternatively, this suppression is complete. The second assumption appears to be unlikely. It can

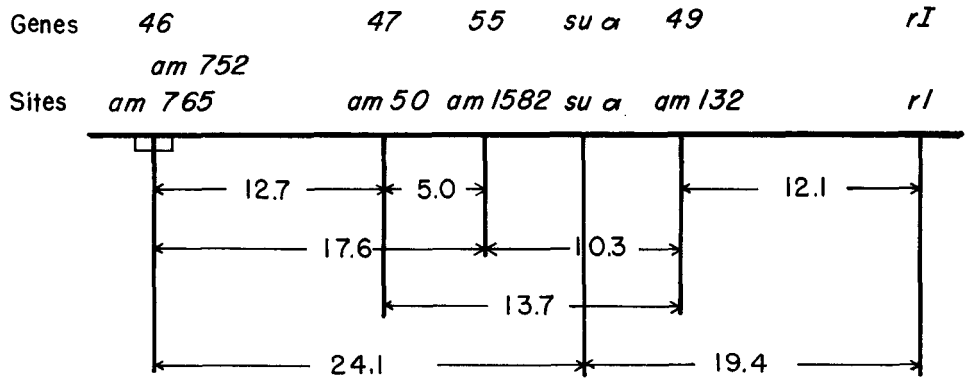

FIGURE 2.—Localization of the *suα* mutation on the genetic map of phage T4B (intervals expressed in map units).

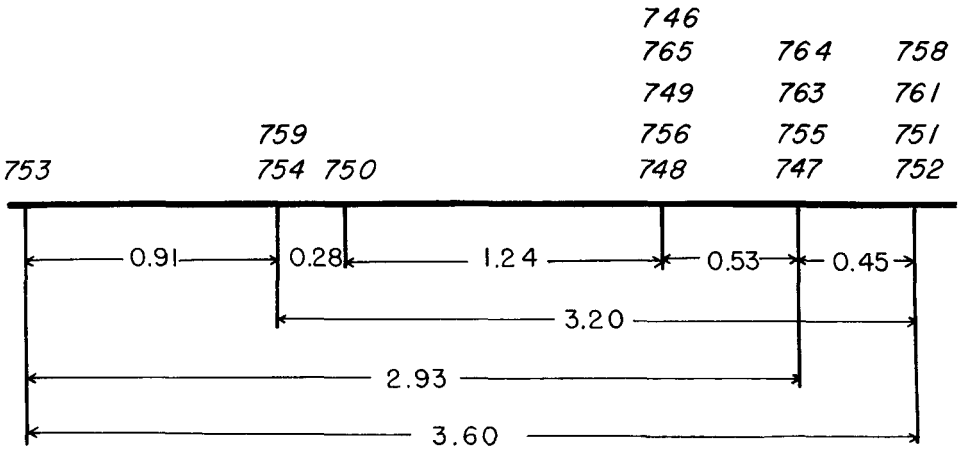

FIGURE 3.—Localization of *am* mutations in gene *46* (intervals expressed in map units).

hardly be imagined that all of the *am* mutations studied are completely suppressed by *suα*.

The suppression of amber mutations in gene 47 *of phage T4B*: Three mutants were located in different sites of the genetic map of gene *47*: *am 50*, *am C49*, and *am C133*. All tested mutants were suppressed by *suα* with the same efficiency. It had previously been reported (KRYLOV and PLOTNIKOVA 1970) that *suα* was unable to suppress the mutation *am 720* in gene *47*. Mutation *am 720* in gene *47* appears to be multiple, and it contains other *am* mutations in unidentified genes. It seems possible that this multiplicity was just the reason why we could not observe small plaques of the R13 type in progeny from the cross *am 720* × *suα*.

Suppression of different mutations in gene 46: The map of gene *46* has been constructed by crossing different *am* mutants in gene *46*. The resulting map is presented in Figure 3. The orientation of gene *46* on the genetic map of T4B was determined in the cross *am 765* + + × + *am 752 r1*, in which frequencies of + + + and + + *r1* recombinants were compared. *suα* mutants were crossed with *am* mutants from each site in gene *46*. It was found that small plaques with R13 phenotype were formed on *E. coli* B in the progenies of all the crosses. Therefore *suα* suppresses all *am* mutations in different sites of gene *46*. *suα* suppresses the UGA mutation in gene *46*(*336 Fγ*) also. This lack of allele specificity provides additional evidence for a different mechanism of suppression by *suα* than by bacterial or phage (YANOFSKY and ST. LAWRENCE 1960; MCCLAIN 1970) nonsense suppressors.

Other suppressors of genes 46 *and* 47: Analysis of *am 752* pseudorevertants has resulted in isolation of another suppressor mutation, *suβ*. Double mutant *am 765 suβ* has been constructed and crossed with mutant *am 765 suα*. No *am* phages have been found among 800 tested progeny plaques. Thus *suα* and *suβ* are closely linked: At a confidence level of 99%, *suα* must be within 0.4 map units of *suβ*. A third suppressor, *suγ*, was isolated in the course of analysis of revertants of *am C49*, a mutation in gene *47*. *suγ* does not differ from *suα*. Among

TABLE 2

Single infections or mixed infections of E. coli *B with two different phages at equal multiplicities (about 7.5)*

Infecting phages	Yield per infected cell
Wild type only	180.0
am 765 only	7.0
*su*α only	215.0
*am 765 su*α only	43.7
*su*α plus wild type	205.0
am 765 plus *su*α	226.0
am 765 plus wild type	155.0
*am 765 su*α plus *am 765*	24.5

the tested 600 plaques of the progeny of the cross *su*α *am 765* × *su*γ *am 765*, there were found no *am* phages.

Suppression of the double mutant: *su*α inhibits the defect of the double mutant, *am 753 am 50* (mutations in genes *46* and *47*, respectively). The degree of suppression as measured by the size of plaques and the yield of phage on *E. coli* B is the same for phage *su*α *am 753 am 50* as for both *su*α *am 753* and *su*α *am 50* phages.

Preliminary physiological studies of suα: There are no differences in latent period, yield, or adsorption rate between phage *am 765 su*α and either *am 765* or wild-type phages on *E. coli* CR-63. But on *E. coli* B, the yield of phage *am 765 su*α is about 25–40% that of the wild-type phage. Thus *su*α is a rather effective suppressor.

SNUSTAD (1968) has shown that the function of gene products may be characterized as catalytic or stoichiometric by mixed infection of *E. coli* B with am^+ and *am* phage mutants. The results obtained in mixed infections with the different phages of this study are presented in Table 2 and in Figure 4.

In the first experiment (Table 2), multiplicity of infection with both phages was the same. The multiplicity of the *am* mutant in the second experiment (Figure 4) was varied while holding the multiplicity of either wild type or *am 765 su*α constant (at 0.5). One can see that the *su*α mutation does not change the yield of phage in itself. Amber mutant *am 765* (like *am* mutants at other sites in gene *46*, results not shown) is leaky and makes 6–7 phages per cell of *E. coli* B. The decrease in yield of phage from singly infected cells is insignificant compared to the change in yield due to changing the multiplicity of infection of *am 765* in mixed infections of *E. coli* B with wild-type and *am 765* phage. This result is compatible with the conclusion of SNUSTAD (1968) that the function of gene *46* is a catalytic one. But the results are quite different under similar conditions of mixed infection of *E. coli* B with *am 765 su*α and *am 765*. In this case, the result is compatible with that expected for mixed infections with wild-type phage and a mutant in a gene with stoichiometric function (SNUSTAD 1968). The yield of phage obtained from mixed infections did not differ from that of *am 765* alone when the multiplicity of *am 765* was equal to 15.0 (Figure 4).

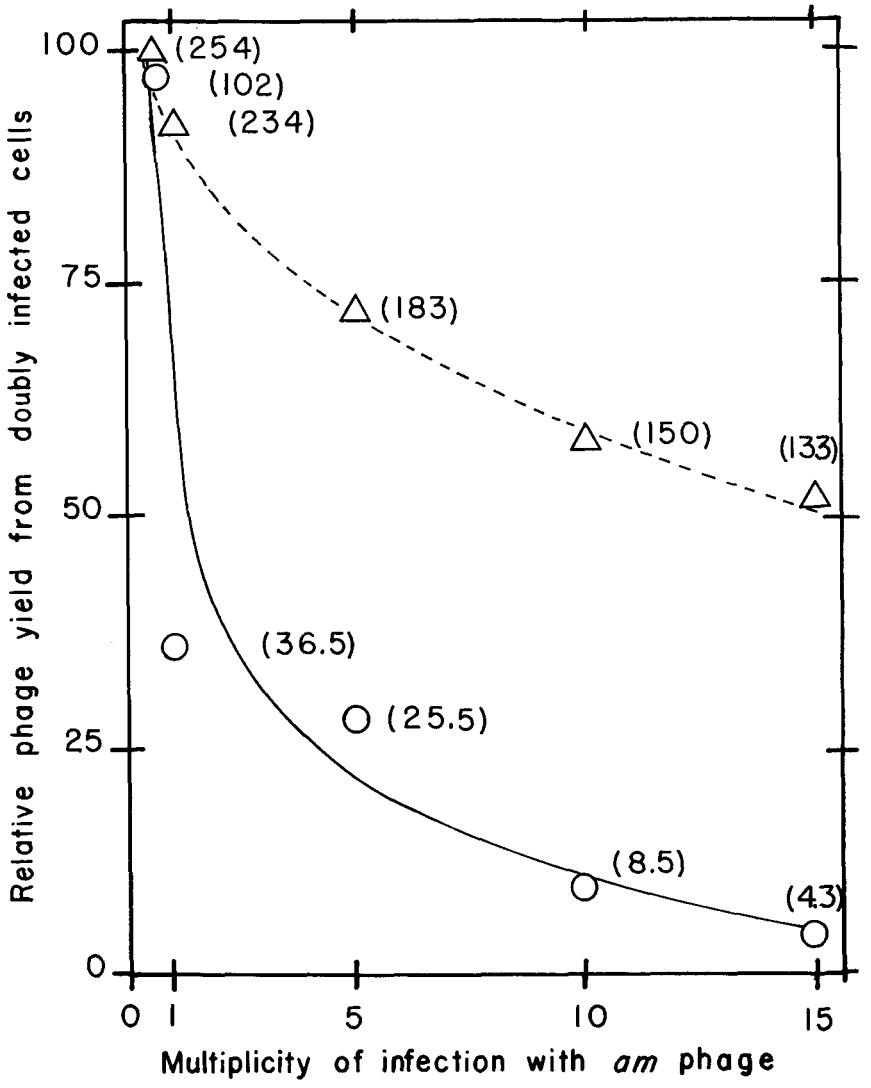

FIGURE 4.—Effect of varying the (*am*/wild type) and (*am*/*am sua*) input ratios on yields. The experimental procedure was to infect cells of *E. coli* B with a constant (approximately 0.5) multiplicity of T4B wild type or *am 765 sua* and to vary the multiplicities (0, 1, 5, 10, or 15) of *am 765*. Temperature was 30°C. T4 antiserum was added to the adsorption tubes 5 min after infection (at this time adsorption is about 90% complete). After 5 min incubation with antiserum, a 10^4 dilution from the adsorption tube into broth was performed (growth tubes). An aliquot from each growth tube was plated to titer total infective centers on *E. coli* B. After an additional 80 min incubation, the contents of the growth tubes were treated with chloroform and the total progeny phage were titered on *E. coli* CR-63 indicator. "Yield" is total progeny phage determined on *E. coli* CR-63 per titer total infective centers on *E. coli* B. "Relative yield" is percent yield relative to *am*+ or *am 765 sua* alone. The yield of particles of progeny phage per infected bacterium is given in parentheses.

△ *am*+ plus *am 765*
○ *am 765 sua* plus *am 765*

DISCUSSION

This report presents a new case of gene-specific, allele-nonspecific suppression in phage T4. *suα* suppresses all *am* mutations and a UGA mutation in gene *46*; it also suppresses amber mutations in gene *47*, but it does not suppress any *am* mutations investigated in other genes. One can explain the absence of small plaques of R13 type on *E. coli* B in the progeny of crosses of *suα* with *am* mutants in other genes by assuming perfect (i.e., complete) suppression in these cases; fully suppressed *am suα* phages do not differ from wild type. However, this assumption appears to be unlikely, since all the mutations in genes *46* and *47* were suppressed with equal efficiency.

Studies of the suppression of missense mutants in genes *46* and *47* could finally confirm the gene-specific character of the suppression. Unfortunately, we have not obtained missense mutations in genes *46* and *47*; their isolation appears to be rather difficult.

Suppression could be carried out in two ways, by inactivation or by modification of the normal product of the *suα* gene. If the inactivation possibility is correct, almost any mutation in the *su* gene would have some suppressor effect. In this case, different *su* mutations should be scattered over the map of the *su* gene. However, it has turned out that the three independently isolated suppressor mutations, *suα*, *suβ*, and *suγ* are located quite close together, or at the same site. We tentatively conclude that suppression arises from a modification of the normal product of *suα* gene. In this case, it is possible that the modified product of *suα* can act in place of the products of genes *46* and *47*. In particular, it is known that a certain enzyme carries out a preliminary degradation of host cytosine-containing DNA into large fragments during phage infection (KUTTER and WIBERG 1968). If this enzyme changed its specificity, it could perform the function of products of genes *46* and *47* (the degradation of host DNA into acid-soluble fragments). If this mechanism really exists, it is obvious that the function of gene *suα* must be a catalytic one. However, our experiments attempting to distinguish catalytic from stoichiometric functions for the *suα* gene gave an ambiguous result.

On the other hand, since all amber mutants in genes *46* and *47* investigated so far are leaky, one could make another postulate about the mechanism of suppression. It is possible that some other phage or bacterial nucleases are partially able to perform the function of genes *46* and *47*. It is possible that the *suα* product has some structural function; for example, the function of controlling the site on the cytoplasmic membrane where the nucleases are acting. In that case, suppression by *suα* may be the consequence of the increase of affinity of some other phage or bacterial nucleases with the post-infection cytoplasmic membrane.

The fact that the double amber mutants in genes *46* and *47* are suppressed by the *suα* suppressor confirms an earlier assumption (WIBERG 1966) about definite similarity of the functions of genes *46* and *47*. It also permits exclusion of one possible mechanism of suppression, namely, that the modified product of gene *suα* combines with the normal product of gene *47* and forms a partially active protein.

Although the results obtained cannot yet explain the mechanism of suppression,

nevertheless one may suppose that investigations of phage suppressors will promote the exposure of new genes. Mutation *suα* is located in a new gene between genes *55* and *49* in a "vacant region" of the genetic map.

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SUMMARY

A mutation of phage T4B, *suα*, suppresses nonsense mutations in genes *46* and *47*. *suα* is located in a new gene in the vacant region of the genetic map between genes *55* and *49*. The efficiency of suppression is from 25 to 40 percent. Suppression is possibly caused by modification of the normal product of gene *suα*. Mechanisms of suppression are discussed.

LITERATURE CITED

ADAMS, M., 1959 *Bacteriophages*. Interscience Publ., New York and London.

KRYLOV, V. N. and T. G. PLOTNIKOVA, 1970 *Su A* : A gene-specific suppressor inhibiting phenotypic expression of mutants in gene *46* of phage T4 (in Russian). Genetika **6**(7): 104–110.

KUTTER, E. M. and J. S. WIBERG, 1968 Degradation of cytosine-containing bacterial or bacteriophage DNA after infection of *E. coli* B with bacteriophage T4D wild type and with mutants defective in genes *46*, *47*, and *56*. J. Mol. Biol. **38**: 395–414.

MCCLAIN, W. H., 1970 UAG suppressor coded by bacteriophage T4. Federation European Biol. Soc. Letters **6**: 99–101.

SNUSTAD, P. D., 1968 Dominance interaction in *Escherichia coli* cells mixedly infected with bacteriophage T4D wild type and amber mutants and its possible implication as to type of gene-product function: catalytic *vs.* stoichiometric. Virology **35**: 550–563.

STEINBERG, C. M. and R. H. EDGAR, 1962 Critical test of a current theory of genetic recombination in bacteriophage. Genetics **47**: 187–208.

WIBERG, J. S., 1966 Mutants of bacteriophage T4 unable to cause breakdown of host DNA. Proc. Natl. Acad. Sci. U.S. **55**: 614–621.

YANOFSKY, C. and P. ST. LAWRENCE, 1960 Cited by S. BENZER and S. P. CHAMPE, 1961 In: Ambivalent *rII* mutants of phage T4. Proc. Natl. Acad. Sci. U.S. **47**: 1025–1038.