JOURNAL OF BACTERIOLOGY, June, 1966
Vol. 91, No. 6

Suppression of *amber* and *ochre* rII Mutants of Bacteriophage T4 by Streptomycin

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Received for publication 21 February 1966

ABSTRACT

ORIAS, E. (University of California, Santa Barbara), AND T. K. GARTNER. Suppression of *amber* and *ochre* rII mutants of bacteriophage T4 by streptomycin. J. Bacteriol. **91**:2210–2215. 1966.—Streptomycin-induced suppression of *amber* and *ochre* rII mutants of phage T4 was studied in a streptomycin-sensitive strain of *Escherichia coli* and four nearly isogenic streptomycin-resistant derivatives of this strain, in the presence and in the absence of an *ochre* suppressor. Most of the 12 rII mutants tested were suppressed by streptomycin in the streptomycin-sensitive su^- strain. This streptomycin-induced suppression in the su^- strain was eliminated by the independent action of at least two of the four nonidentical mutations to streptomycin resistance. In two of the su^+ *str-r* strains, streptomycin markedly augmented the suppression caused by the *ochre* suppressor. In those su^- *str-r* hosts in which significant streptomycin-induced suppression could be measured, the *amber* mutants were more suppressible than the *ochre* mutants.

Streptomycin can cause phenotypic suppression of a variety of mutations in either streptomycin-resistant or streptomycin-sensitive *Escherichia coli* (1, 15, 16, 19). Streptomycin also can cause ambiguity of translation in an in vitro protein-synthesizing system which utilizes synthetic polyribonucleotide as messenger ribonucleic acid with ribosomes from either streptomycin-resistant or streptomycin-sensitive cells (1, 11). Mutations to streptomycin resistance alter the structure of the 30*S* component of the 70*S* ribosome (9, 10) and concomitantly lower the level of streptomycin-induced ambiguity of translation in vitro (1, 11). Therefore, it seems likely that phenotypic suppression caused by streptomycin occurs at the ribosome during protein synthesis (1, 11).

Mutations to streptomycin resistance differ from one another in their effects on (i) the suppressibility of mutations in *E. coli* by streptomycin (15, 16), (ii) the level of ambiguity in an in vitro protein-synthesizing system (1), and (iii) the suppressibility by an *ochre* suppressor of an *ochre* mutant of *E. coli*, some *amber* mutants of phage T7, and some *amber* and *ochre* mutants of phage T4 (14). The reduced genetic suppression of the *amber* and *ochre* mutants in certain streptomycin-resistant strains has been ascribed to a decreased rate of translation of the *amber* and *ochre* mutant codons (14).

In this study, streptomycin-induced suppression of *amber* and *ochre* mutants was investigated because these classes of mutations and their suppression have been extensively studied. The *amber* and *ochre* mutant codons have been identified as uridine-adenine-guanine (UAG) and uridine-adenine-adenosine (UAA), respectively, at the level of messenger ribonucleic acid (7). Both of these mutations create nonsense codons (4, 5, 12). Some but not all *amber* and *ochre* mutations in multicistronic messenger ribonucleic acid cause polarity effects (17). The *amber* codon characteristically is suppressible by both *amber* suppressors and *ochre* suppressors. The *ochre* codon is suppressible only by *ochre* suppressors (5). The well-characterized *amber* suppressors suppress with high efficiency, whereas *ochre* suppressors appear less efficient (5). Suppression of the *amber* codon in *E. coli* strain S26R1E is caused by a new or altered serine transfer ribonucleic acid molecule (8). Suppression of the *ochre* codon in *E. coli* strain 2320 (λ)-15B either is caused by an altered ribosome or indirectly causes alterations of ribosome structure (18).

The purpose of this study was to characterize streptomycin-induced suppression of *amber* and *ochre* rII mutants of the phage T4 in streptomycin-sensitive and streptomycin-resistant strains of *E. coli* in the absence and presence of a known *ochre* suppressor.

MATERIALS AND METHODS

Strains. The origin and method of isolation of the bacterial strains used have been described (14). The genotypes of, and the relationships among, these strains are shown in Fig. 1. Table 1 lists the T4 phage mutants used, their classification as *amber* and *ochre* mutants, and the sources of these strains. Mutants *r*N24A and *r*AP53A were obtained in our laboratory from 2-aminopurine-treated lysates of the *ochre* mutants *r*N24 and *r*AP53, respectively. They were selected on the basis of their ability to form plaques on strain C600(λ) and not on strain SBO. Strain C600(λ) is a lysogenic derivative of strain C600, which carries an *amber* suppressor (6). Since *amber* suppressors suppress *amber* but not *ochre* mutants, *r*N24A and *r*AP53A probably are *amber* mutants, which arose by a transition from the UAA (*ochre*) codon to the UAG (*amber*) codon (5). Thus, the mutant codons in mutants *r*N24A and *r*AP53A probably occupy the same position in the messenger ribonucleic acid of the B cistron as the mutant codons of *r*N24 and *r*AP53, respectively.

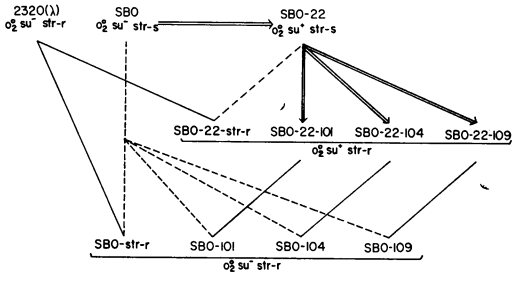

FIG. 1. *Genotypes of and relationships among bacterial strains. Double lines and arrowhead connecting two strains indicate a single mutational step. Strains whose designation appears below an intersection of two single lines were obtained by transduction. Broken lines indicate recipient strains; solid lines, donor strains. The O_2^c mutation is a polar nonsense mutation at the z gene of the lactose operon, otherwise designated lac_2 (5).*

Media. The burst size measurements and plate assays were made on tryptone B1 medium (13) in the absence or in the presence of streptomycin (dihydrostreptomycin sulfate; Nutritional Biochemicals Corp., Cleveland, Ohio).

Burst size measurements. The burst size measurements were done as previously described (14), with one modification. Immediately after dilution of the infected bacteria in the adsorption tube, a 2.5-ml sample of the contents of the growth tube was added to 2.5 ml of broth supplemented with enough dihydrostreptomycin to give the desired final concentration.

RESULTS

Suppression by streptomycin of a variety of *amber* and *ochre* rII mutants was studied in bacterial strains which differed either at the streptomycin-resistance locus or at a locus determining an *ochre* suppressor.

Suppression of amber and ochre mutations by streptomycin in a streptomycin-sensitive strain. For concentrations of streptomycin between 10 and 100 μg/ml, the burst size of *ochre* mutant *r*N24 in strain SBO increased relative to the burst size of the wild-type control as a function of the increasing amount of streptomycin in the growth medium (Fig. 2). The onset of suppression occurred abruptly at 5 to 10 μg/ml of streptomycin; this was also the concentration at which the burst size of wild-type T4 began to be decreased by streptomycin. By contrast, neither the very low burst size of *r*N24 (<0.01; not shown in

TABLE 1. *Characterization of the rII mutants of phage T4*

Mutant	Map segment	Type	Reference	Source
*r*638	B	Deletion	2	R. S. Edgar (from S. Benzer)
*r*HB118	A2	*amber*	3, 7	R. Hill (from S. Benzer)
*r*N11	A4	*amber*	3, 7	R. Hill (from S. Benzer)
*r*N24A	(B1)*	*amber**		Isolated in our laboratory
*r*B94	B4	*amber*	3	S. P. Champe
*r*GU63	B	*amber*	14	Isolated in our laboratory
*r*AP53A	(B9)*	*amber**		Isolated in our laboratory
*r*N21	A6	*ochre*	7	S. P. Champe
*r*360	B1	*ochre*	7	S. P. Champe
*r*UV375	B1	*ochre*	7	S. P. Champe
*r*N24	B1	*ochre*	7	S. P. Champe
*r*N17	B4	*ochre*	7	S. P. Champe
*r*N7	B6	*ochre*	7	S. P. Champe
*r*609	B7	*ochre*	14	S. P. Champe
*r*SD160	B7	*ochre*	5	S. P. Champe
*r*UV199	B7	*ochre*	5	S. P. Champe
*r*AP53	B9	*ochre*	7	S. P. Champe

* See Materials and Methods.

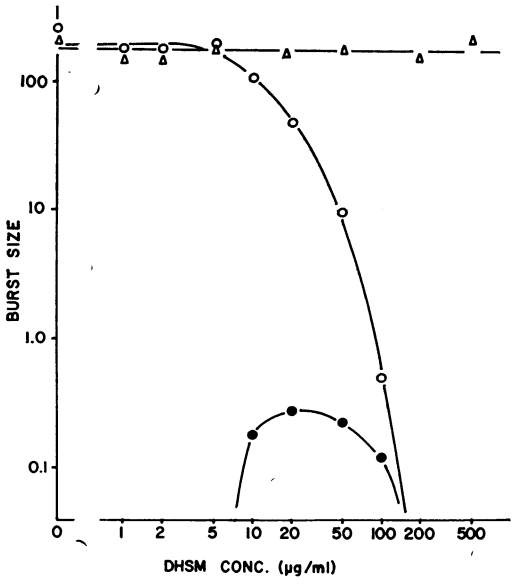

FIG. 2. *Burst sizes of the wild-type strain of T4 and of mutant rN24 grown on bacterial strains SBO and SBO-str-r at 25 C in the presence of various concentrations of streptomycin.* (○)*T4 r*$^+$ *in strain SBO;* (●)*T4 rN24 in strain SBO;* (△)*T4 r*$^+$ *in strain SBO-str-r.*

TABLE 2. *Comparison of the burst sizes of various amber and ochre mutants when grown at 25 C in strain SBO in the absence and in the presence of 50 μg/ml of streptomycin*

Mutant	Percentage burst size*	
	Without streptomycin	With streptomycin
T4B r^+	100	100
*r*638	0.001	<0.01
*r*B94	0.01	28
*r*N21	0.01	0.05
*r*360	0.002	0.03
*r*UV375	0.001	0.35
*r*N24	0.02	1.9
*r*N17	<0.001	0.02
*r*N7	0.001	0.01
*r*609	<0.002	0.10
*r*SD160	0.001	0.22
*r*UV199	<0.001	0.07
*r*AP53	0.03	7.4
Uncorrected T4B r^+	150	19

* In this and subsequent tables, the burst size of each T4 mutant is expressed as the percentage of the burst size of the wild-type control. The actual burst size of wild-type T4 is shown in the bottom row.

Fig. 2) nor the normal burst size of T4r^+ (around 200) was affected by streptomycin when the phage was grown in strain SBO-*str-r*, a streptomycin-resistant derivative of SBO.

Table 2 shows the effect of streptomycin (50 μg/ml) on the burst sizes of various *amber* and *ochre* mutants in strain SBO, at 25 C. All of the *r*II mutants, with the exception of *r*N21, gave at least a 10-fold increase in the percentage burst size.

Suppression of amber and ochre mutations by streptomycin in streptomycin-resistant strains. Table 3 shows the effect of streptomycin (500 μg/ml) on the burst sizes of various phage mutants in strain SBO and four streptomycin-resistant derivatives at 37 C. Suppression by streptomycin was completely abolished in two of the streptomycin-resistant mutants (SBO-*str-r* and SBO-104), even for those phage mutants which are most suppressible by streptomycin in the streptomycin-senstitive strain. This elimination of streptomycin-induced suppression is a consequence of the mutation to streptomycin resistance. A low but significant level of suppression by streptomycin occurred in the other two streptomycin-resistant hosts (SBO-109 and SBO-101). Suppression was significantly higher in strain SBO-109 than in strain SBO-101. In strain SBO-109, the *amber* mutants as a class were suppressed more than the *ochre* mutants. The burst size of wild-type T4 was not significantly altered either by the mutations to streptomycin resistance or by streptomycin in the streptomycin-resistant strains.

Suppression of amber and ochre mutations by streptomycin in a strain carrying an ochre suppressor and in its streptomycin-resistant derivatives. Strain SBO-22 carries an *ochre* suppressor. Evidence has been presented (4) that the rate of translation of the *amber* and *ochre* codons in the presence of this suppressor is lowered to a different extent by each of the mutations to streptomycin resistance utilized in the current study. Table 4 shows the effects of streptomycin (500 μg/ml) on the burst sizes of various *amber* and *ochre* *r*II mutants in strain SBO-22 and its streptomycin-resistant derivatives at 37 C.

The observations can be summarized as follows. (i) Streptomycin significantly increased the burst size of some of the phage mutants in all of the streptomycin-resistant derivatives of SBO-22. (ii) In strains SBO-22-109 and SBO-22-101, streptomycin-induced suppression markedly augmented the suppression caused by the suppressor alone in strain SBO-22, their streptomycin-sensitive parental strain. (iii) The streptomycin-resistant derivatives of SBO-22 can be arranged in the order SBO22-109, SBO-22-101, SBO-22-

TABLE 3. *Comparison of the burst sizes of various amber and ochre mutants when grown at 37 C in strain SBO and four streptomycin-resistant derivatives of strain SBO in the absence of streptomycin and in the presence of 500 μg/ml of streptomycin*

rII mutant	Without streptomycin					With streptomycin			
	SBO	SBO-109	SBO-101	SBO-*str-r*	SBO-104	SBO-109	SBO-101	SBO-*str-r*	SBO-104
T4B r+	100	100	100	100	100	100	100	100	100
*r*638	<0.001	<0.001	<0.002	<0.001	<0.001	<0.001	<0.007	<0.001	<0.001
*r*N24A	0.014	<0.001	0.002	<0.001	<0.001	0.95	0.05	<0.001	<0.001
*r*B94	0.025	<0.001	0.001	<0.001	<0.001	9.6	0.13	<0.001	<0.001
*r*GU63	0.004	<0.001	<0.001	<0.001	<0.001	8.4	0.017	<0.001	<0.001
*r*AP53A	0.12	<0.001	0.001	<0.003	<0.001	16	0.21	<0.005	0.001
*r*N21	0.007	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
*r*360	<0.001	<0.001	<0.001	<0.001	<0.001	0.024	<0.001	<0.001	<0.001
*r*UV375	0.005	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.008
*r*N24	<0.001	<0.001	0.002	<0.001	<0.001	0.005	<0.002	<0.001	<0.001
*r*N17	0.008	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.022
*r*N7	0.013	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001
*r*609	<0.001	0.001	0.001	0.001	0.001	<0.001	<0.001	<0.001	<0.001
*r*SD160	0.011	<0.001	0.003	0.001	<0.001	<0.002	<0.001	<0.001	0.029
*r*UV199	0.007	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.013	<0.001
*r*AP53	0.007	<0.001	0.001	<0.001	<0.001	0.14	0.013	<0.001	0.065
Uncorrected T4B *r*+	290	523	309	245	405	340	230	265	408

TABLE 4. *Comparison of the burst sizes of various amber and ochre rII mutants when grown at 37 C in strain SBO-22 and in four streptomycin-resistant derivatives in the absence and in the presence of 500 μg/ml of streptomycin*

rII mutant	Without streptomycin					With streptomycin			
	SBO-22	SBO-22-101	SBO-22-109	SBO-22-*str-r*	SBO-22-104	SBO-22-101	SBO-22-109	SBO-22-*str-r*	SBO-22-104
T4B r+	100	100	100	100	100	100	100	100	100
*r*638	0.002	0.026	0.008	0.022	<0.001	0.011	0.018	0.039	<0.001
*r*B94	60	34	5.5	0.055	<0.001	90	75	38	<0.001
*r*GU63	41	6.2	0.53	0.046	<0.001	81	88	16	0.16
*r*N21	1.7	0.79	0.09	0.018	<0.001	13	32	0.27	<0.001
*r*360	0.28	0.062	0.003	0.003	<0.001	2.5	13	0.19	<0.001
*r*UV375	0.065	<0.003	<0.002	0.033	<0.001	0.20	11	0.036	<0.001
*r*N24	78	44	10	0.32	<0.001	81	87	28	<0.001
*r*N17	4.8	0.19	0.018	0.008	<0.001	11	63	0.46	<0.001
*r*N7	0.50	<0.003	0.057	0.007	<0.001	0.28	1.5	0.14	<0.001
*r*609	0.13	0.41	0.031	0.019	<0.001	13.5	48	1.3	<0.001
*r*SD160	28	3.2	0.51	0.052	<0.001	30	70	4.1	<0.001
*r*UV199	13	3.2	0.35	0.035	<0.001	35	77	2.0	<0.001
*r*AP53	73	68	54	50	<0.001	100	96	120	0.43
Uncorrected T4B *r*+	400	198	334	130	143	142	228	120	189

str-r, and SBO-22-104 by the level of streptomycin-induced suppression. This order is essentially congruent with the order SBO-109, SBO-101, and (SBO-*str-r* and SBO-104), which is based on the levels of suppression caused by streptomycin in the strains lacking the genetic suppressor (Table 3). The order is not congruent with the order SBO-22-101, SBO-22-109, SBO-22-*str-r*, and SBO-22-104 based on the levels of suppression in streptomycin-resistant derivatives of strain SBO-22, in the absence of streptomycin. The lack of congruence implies a streptomycin-induced differential increase of the efficiency of suppression caused by the *ochre* suppressor when it is functioning in strain SBO-22-109. This differential effect of streptomycin on physiological suppression probably is a result of the structure(s) of the ribosomes determined by the mutation to

streptomycin resistance in that strain. Thus, the special structure(s) of the ribosomes in strain SBO-22-109 seems to facilitate a streptomycin-*ochre* suppressor interaction which greatly enhances the efficiency of suppression.

DISCUSSION

These results clearly demonstrate that the *amber* and *ochre* codons are sensitive to suppression by streptomycin in most of the strains tested. In particular, streptomycin increased the burst size of some *amber* and *ochre* mutants in: a streptomycin-sensitive, su^- strain (SBO); two different streptomycin-resistant su^- strains and four streptomycin-resistant su^+ strains, each carrying a different mutation for streptomycin resistance. These results are consistent with the findings of other workers (1, 15); however, the nucleotide composition of the suppressible mutant codon(s) studied by these investigators was not known.

The possibility has been considered (15) that mutations of the wild-type allele for streptomycin sensitivity to streptomycin resistance may concomitantly create suppressor activity. The results presented above, and the in vitro results of Anderson, Gorini, and Breckenridge (1), indicate that the opposite is true. Thus, although suppressor mutations may alter a structural gene for a ribosomal component (18), such mutations probably do not concomitantly cause streptomycin resistance.

The mutations to streptomycin resistance used in this study were originally distinguished (14) on the basis of causing different degrees of restriction of the suppression of *amber* and *ochre* mutants by the suppressor in strain SBO-22. The present results show that the mutations to streptomycin resistance in strains SBO-109 and SBO-101 can also be distinguished by the levels of streptomycin-caused suppression which they allow.

In strain SBO-109 (su^-), phage mutants carrying an *amber* codon (UAG) appear to be more susceptible to streptomycin-induced suppression than mutants carrying an *ochre* (UAA) codon. This observation is of particular significance within those pairs (*r*N24 and *r*N24A; *r*AP53 and *r*AP53A) for which the two mutant codons presumably occupy identical positions in the messenger ribonucleic acid. This observation may be related to the observation that, as a class, *amber* mutations can be suppressed with higher efficiency by *amber* suppressors than *ochre* mutations by *ochre* suppressors (5).

There is abundant evidence that streptomycin can cause suppression by creating ambiguity in the translation of messenger ribonucleic acid as a consequence of interacting with the 30*S* ribosomal subunit (9–11). The overall susceptibility of the in vitro protein-synthesizing system to streptomycin-induced ambiguity is decreased in the presence of ribosomes from streptomycin-resistant cells (1, 11). In view of this observation, the efficient suppression caused by streptomycin in streptomycin-resistant bacteria carrying a known suppressor supports the possibility of the existence of other bases for streptomycin-induced suppression. For example, streptomycin may increase the efficiency of genetic suppression by interacting with a nonribosomal product of a suppressor gene.

ACKNOWLEDGMENTS

We are indebted to Linda J. Green for technical assistance.

This investigation was supported by grants GB 1389 and GB 2330 from the National Science Foundation.

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