Effect of Mutation to Streptomycin Resistance on Amber Suppressor Genes

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Three classes of nonidentical streptomycin-resistant mutations were distinguished in *Escherichia coli* by their effect on the efficiency of suppression by an amber suppressor gene, *sup E*. The first class of mutation caused a strong restriction in efficiency of suppression of an amber codon in various cistrons of phage λ and in an alkaline phosphatase structural gene of *E. coli*. The second class caused weak restriction, and the third class caused no restriction. The restrictive effect of the streptomycin resistance mutation of the first class on the *sup E* gene was reduced by addition of streptomycin. This mutation had little effect on efficiencies of suppression by amber suppressor genes *sup D* and *sup F*. Analyses on the alkaline phosphatase formed in the suppressor strain indicated that mutation to restrictive streptomycin resistance causes a reduction in translation of the amber codon in the alkaline phosphatase structural gene.

Mutation to streptomycin resistance imposes a significant restriction on the efficiencies of suppressor genes which act in streptomycin-susceptible strains (3, 9-11). However, the mode of the restrictive effect of the streptomycin resistance mutation on the function of these suppressor genes is uncertain, because little is known about the mechanism of action of these suppressors. Recently, it was shown that each of the three amber suppressor genes, *supD*, *supE*, and *supF*, incorporates a particular amino acid into a site specified by the amber codon in the alkaline phosphatase gene of *Escherichia coli* (8, 23, 24) and by the amber codon in a ribonucleic acid (RNA) phage (15) and in phage T4 (21). The *supD* gene incorporates serine, the *supE* gene incorporates glutamine, and the *supF* gene incorporates tyrosine. There is also evidence (1, 2, 6) that the *supD* gene acts at the level of seryl soluble RNA (sRNA) and that the *supF* gene acts at the level of tyrosyl sRNA (19).

Therefore, the present study concerned the mode of action of the streptomycin resistance mutation on the suppression, by the amber suppressor genes, *supD*, *supE*, and *supF*, of nonsense mutations in various cistrons of phage λ and in the alkaline phosphatase gene of *E. coli*. This paper reports that one type of streptomycin resistance mutation causes restriction of function of the amber suppressor *supE* gene, but has

little, if any, effect on the efficiencies of suppression by the *supD* and *supF* genes.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study (Table 1) were all derived from *E. coli* K-12 and all carry an amber mutation in the alkaline phosphatase structural gene or in the *z* cistron of the *lac* operon.

Phage strains. All phages used were suppressor-sensitive (*sus*) mutants of phage λ . Phages A11, B10, E4, G9, J6, N7, N53, O8, P3, P72, R5, R54, R60 were kindly supplied by A. Campbell; O205, P207, and R216 were from R. Thomas. P1001 was isolated in our laboratory.

The efficiencies of plating of phages G9, N53, P3, and R216 are shown in Table 2; from the results, the amber suppressor genes can be classified as *supD*, *supE*, and *supF*.

Media. TG medium contained 0.1 M tris(hydroxymethyl)aminomethane (Tris; pH 7.2), 0.2% glucose, 8×10^{-2} M NaCl, 2×10^{-2} M KCl, 2×10^{-2} M NH_4Cl , 3×10^{-3} M Na_2SO_4 , 10^{-3} M MgCl_2 , 2×10^{-4} M CaCl_2 , and 2×10^{-6} M FeCl_3 . As a "high phosphate" medium, 6.4×10^{-4} M KH_2PO_4 was added; a "low phosphate" medium, 3.2×10^{-5} M KH_2PO_4 was added.

Tryptone broth contained tryptone (Difco), 10 g; yeast extract, 5 g; NaCl, 5 g; glucose, 1 g; 1 N NaOH, 1 ml; and water to 1 liter.

Isolation of streptomycin-resistant mutants. A 0.2-ml sample of an overnight culture was diluted in tryptone broth to a total volume of 10 ml and aerated at 37 C. Log-phase cells were harvested by centrifugation, suspended in 3.5 ml of 0.9% NaCl, and exposed to ultraviolet (UV) light from a 15-w Toshiba germicidal lamp for 60 sec at a distance of 60 min; 0.1-ml

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TABLE 1. *Bacterial strains used^a*

Strain ^b	Genotype	Phenotype (Str)	Origin and source
CA85	<i>lac</i> ⁻	Str ^S	E. R. Signer
BE910	<i>pho</i> ⁻ <i>supD</i>	Str ^S	S26R1E (λ) ⁻ ; A. Garen
BE900	<i>pho</i> ⁻ <i>supE</i>	Str ^S	S26R1D (λ) ⁻ ; A. Garen
F960	<i>pho</i> ⁻ <i>supF</i>	Str ^S	H12R8a (λ) ⁻ ; A. Garen
CA5013	<i>lac</i> ⁻ <i>supD</i>	Str ^S	E. R. Signer
BE1007	<i>lac</i> ⁻ <i>supE</i>	Str ^S	P1 (BE900) + CA85
BE1008	<i>lac</i> ⁻ <i>supF</i>	Str ^S	P1 (F960) + CA85
BE667	<i>pho</i> ⁻ <i>supE</i>	Str ^{RR}	BE900 Str ^{RR} by UV
BE694	<i>pho</i> ⁻ <i>supE</i>	Str ^R	BE900 Str ^R by UV
BE1512	<i>pho</i> ⁻ <i>supD</i>	Str ^{RR}	P1 (BE667) + BE910
BE2002	<i>pho</i> ⁻ <i>supE</i>	Str ^{RR}	P1 (BE667) + BE900
BE1516	<i>pho</i> ⁻ <i>supF</i>	Str ^{RR}	P1 (BE667) + F960
BE1510	<i>lac</i> ⁻ <i>supD</i>	Str ^{RR}	P1 (BE667) + CA5013
BE1406	<i>lac</i> ⁻ <i>supE</i>	Str ^{RR}	P1 (BE667) + BE1007
BE1419	<i>lac</i> ⁻ <i>supF</i>	Str ^{RR}	P1 (BE667) + BE1008
BE2070	<i>supE</i> (λ sus R5)	Str ^S	BE900 (λ sus R5)
BE2073	<i>supE</i> (λ sus R5)	Str ^{RR}	BE2002 (λ sus R5)

^a The following abbreviations are used: *lac*, lactose; *pho*, alkaline phosphatase; *sup*, suppressor; Str, streptomycin; R, nonrestrictive resistant; RR, restrictive resistant; S, susceptible.

^b Hfr strains of *E. coli*. CA85, CA5013: Hfr Hayes. BE910, BE900, F960: Hfr Cavalli.

quantities of the culture were transferred to test tubes containing 5 ml of tryptone broth (30 tubes) and aerated overnight at 37 C to allow expression of mutants. Samples were then spread on tryptone-agar medium containing streptomycin (100 µg/ml) and incubated at 37 C for about 20 hr. To eliminate the possibility of picking up the same clone, only one streptomycin-resistant strain was isolated from a single plate.

Measurement of burst size. Overnight cultures were diluted 50-fold with tryptone broth and aerated at 37 C until the cell density was about 3×10^8 cells per ml. The cells were then centrifuged and resuspended in one-third of the original volume of 0.01 M Tris buffer (pH 7.2) containing 0.01 M MgSO₄. A fraction of the suspension was infected with λ phage to give a multiplicity of infection of 0.05. After 15 min of incubation at 37 C to allow adsorption of the phage, the infected bacteria were diluted and plated on a C600 indicator strain. Unadsorbed phage was always determined by plating a chloroform-treated sample from the cultures. Infected bacteria were diluted 10⁴-fold in tryptone broth, incubated for 150 min at 37 C, and plated with C600 indicator bacteria. The average burst size was obtained by dividing the total number of progeny phage by the total number of infective centers.

Preparation of cell extracts. Exponentially growing cells were harvested by centrifugation, washed once with 0.05 M Tris buffer (pH 7.3) containing 0.02 M MgSO₄, and resuspended in the same buffer. The cells were ruptured by sonic disintegration (Kaijo Denki ultrasonic disintegrator, model TA-4201, 20 kc, 3 min) in an ice water bath and centrifuged at 15,000 × *g* for 20 min to remove debris. The resulting supernatant fraction was used for assay of alkaline phosphatase, for starch gel electrophoresis, and for

assay of immunological cross-reacting material (CRM).

Preparation of ¹⁴C-labeled alkaline phosphatase. Exponentially growing bacteria in high phosphate medium were harvested by centrifugation, washed twice with saline, and suspended in TG medium without added phosphate. After addition of 20 µC of ¹⁴C-proline (120 mc/mmole), the suspension was aerated at 37 C for 2 hr, centrifuged, and washed three times with 0.01 M Tris buffer (pH 8.0). Alkaline phosphatase was extracted by the procedure of Neu and Heppel (14) and dialyzed against 0.01 M Tris buffer (pH 7.3).

Assay of alkaline phosphatase activity. Enzymatic activity was measured by the rate of hydrolysis of *p*-nitrophenyl phosphate (2 mg/ml) in 1 M Tris buffer (pH 8.0). A sample (0.1 ml) was incubated with 2.6 ml of assay solution at 37 C for an appropriate time. The reaction was stopped by addition of 0.3 ml of K₂HPO₄ (12%), and the absorbancy at 410 mµ was measured. One unit of enzyme activity is defined as the amount causing a change of optical density at 410 mµ in 1 min per unit of cell turbidity (at 550 mµ) or per milligram (protein) of cell extract.

Assay of CRM. A standard concentration of serum was assayed by the agar gel method of Ouchterlony (18), by using serial dilutions of cell extract which had previously been diluted so as to give the same enzymic activity. The basic diffusion medium contained 1.5% washed agar, adjusted to pH 7.8 with HCl, and 0.01% Merthiolate. Melted agar solution (12 ml) was poured into a glass petri dish (diameter, 9 cm). Plates were incubated at 37 C for 20 hr to allow development of the precipitation reaction.

The relative amount of CRM in an extract was estimated by comparing the dilution titer of the extract from the streptomycin-resistant mutant, which

formed a minimal precipitation band in the agar, with that of the streptomycin-susceptible (*Str*^s) strain. The CRM value is given as the percentage of enzyme produced by the streptomycin-susceptible strain.

Protein determination. The amount of protein in extracts was determined by the method of Lowry et al. (13) with bovine serum albumin as a standard.

Electrophoresis. Vertical starch gel electrophoresis was carried out by the method of Smithies (20). The technique for detecting alkaline phosphatase was identical to that described by Levinthal et al. (12).

Chemicals. Streptomycin and kanamycin were purchased from Meiji Seika Kaisha, Ltd.; mitomycin C was supplied by Kyowa Hakko Kogyo Co., Ltd.

RESULTS

Growth of *sus* mutants of λ on streptomycin-resistant mutants from isogenic strains carrying the amber suppressor genes *supD*, *supF* and *supE*. To study the effect of mutation to streptomycin resistance on suppression of a variety of *sus* mutants of phage λ by amber suppressor genes, spontaneous streptomycin-resistant mutants and those induced by UV irradiation were isolated from strains BE910 (*supD*), BE900 (*supE*), and F960 (*supF*). These parent strains were originally isolated by A. Garen and are supposed to be isogenic except for their suppressor genes. The ability of the streptomycin-resistant mutants to suppress known *sus* mutants of λ phage was tested by determining the efficiency of plating of phages G9, N53, P3, and R216. Fifty-six independently isolated streptomycin-resistant mutants of BE900 were classified into three groups with respect to their restrictive effects on the efficiency of suppression of these λ *sus* phages. The mutants belonging to the first group supported growth of N53 and R216, but did not support G9 and P3. The second group of mutants supported growth of G9 and R216, but partially restricted growth of N53 and P3. The third group of mutants supported growth of all four *sus* mutants of λ . With respect to supporting growth of λ *sus* phage, the first group of streptomycin-resistant mutants was termed *Str*^{RR}, the second group was termed *Str*^{PR}, and the third group was termed *Str*^R. However, among the 44 mutants isolated independently from strains BE910 and F960, there were no mutants in which the efficiency of plating of these phages was reduced.

When the streptomycin resistance marker from a *Str*^{RR} strain was transduced by phage P1kc to *Hfr* Cavalli and Hayes, which carry an amber suppressor gene, the restrictive effect on suppression by amber suppressor genes was observed only in the *supE* strains; no reduction in the efficiency of plating of the λ *sus* phages was seen in strains carrying the *supD* or *supF* gene (Table 2).

TABLE 2. Efficiency of plating of λ *sus* phages G9, N53, P3, and R216 on mutants of *E. coli* K-12 carrying amber suppressor genes

Strain ^a	G9	N53	P3	R216
BE910	1	1	1	<10 ⁻⁴
BE900	1	1	1	1
F960	1	1	<10 ⁻⁴	<10 ⁻⁴
CA5013	1	1	1	<10 ⁻⁴
BE1007	1	1	1	1
BE1008	1	1	<10 ⁻⁴	<10 ⁻⁴
BE1512	1	1	1	<10 ⁻⁴
BE2002	10 ⁻²	1	10 ⁻²	1
BE1516	1	1	<10 ⁻⁴	<10 ⁻⁴
BE1510	1	1	1	<10 ⁻⁴
BE1406	10 ⁻²	1	10 ⁻²	1
BE1419	1	1	<10 ⁻⁴	<10 ⁻⁴

^a Genotypic and phenotypic description of these strains is provided in Table 1.

The effect of the *Str*^{RR} mutation on amber suppressor genes was further confirmed by isolating *sup*⁺ strains from *lac*⁻ (CA85, X64, U131) and *lac*⁻ *Str*^{RR} strains which carry an amber mutation in the *z* gene of the *lac* operon. Lactose-positive revertants which appeared spontaneously from these strains were purified and tested for the presence of suppressor gene by infection of the revertants with *sus* mutants of phage λ . Among 224 revertants from the *Str*^R strain, 57 were able to support growth of G9, N53, P3, and R216, suggesting that they contained a suppressor gene similar to the amber suppressor *supE* gene. Among the 114 *lac*⁺ revertants from the streptomycin-resistant strain, however, no *sup*⁺ strains which supported growth of all four *sus* mutants of λ were isolated. Of these, 57 supported growth of N53 and R216, but did not support G9 and P3, indicating that the pattern of support of growth of λ *sus* phages by these *sup*⁺ revertants was the same as that of the *supE* *Str*^{RR}. Since the restrictive effect of the *Str*^{RR} mutation on the function of the amber suppressor genes was seen in the *supE* strain, the mechanism of the effect of the *Str*^{RR} mutation on the efficiency of suppression by the *supE* gene was studied further by use of mutants with the amber codon in some cistrons of phage λ and in the alkaline phosphatase structural gene of *E. coli*.

Growth of *sus* mutants of λ in a streptomycin-resistant *supE* strain. Streptomycin-susceptible and streptomycin-resistant mutants of *sup*⁺ strains were infected with various *sus* mutants of phage λ at a multiplicity of 0.05, and the average burst size was measured. When the *supE* *Str*^{RR} mutant was infected, the yields of some of the *sus* mutants (A11, B10, E4, G9, J6, L63, P3, P72,

P1001, R5, and R60) were greatly decreased. Average burst size of these phages was only slightly reduced in the *supD* Str^{RR} and *supF* Str^{RR} strains (Table 3).

Suppression of nonsense mutation by streptomycin. As shown above, infection of the streptomycin-resistant *supE* strain with some of the sus mutants of λ resulted in only slight production of phage. However, if the recipient strain was infected with the sus mutant in the presence of streptomycin, the liberation of sus phage was greatly increased (Fig. 1). At concentrations of streptomycin between 100 and 1,000 μg per ml, the average burst size of the G9 mutant in BE1406 (*supE* Str^{RR}) increased as a function of the amount of streptomycin in the growth medium. In the presence of 1,000 μg of streptomycin per ml, G9 gave at least an 80-fold increase in the burst size. The burst size of G9 was not affected by kanamycin at concentrations between 0.3 and 30 $\mu\text{g}/\text{ml}$.

When strain BE2073, which is lysogenic for λ sus R5, was exposed to UV or mitomycin C and then aerated at 37 C, no lysis was observed in the absence of streptomycin. In the presence of streptomycin (1,000 $\mu\text{g}/\text{ml}$), however, lysis of the

bacteria occurred slightly later than that of a lysogenic streptomycin-susceptible strain, BE2070 (Fig. 2).

Effect of the Str^{RR} mutation on the suppression

FIG. 1. Burst sizes of sus mutants G9 and N53 grown on BE1406 in the presence of various concentrations of streptomycin. (●) G9; (○) N53.

TABLE 3. Burst size of sus mutants of λ in streptomycin-resistant mutants of *sup*⁺ strains^a

Phage	BE1512	BE1406	BE1419
A11	NA ^b	2.9	100
B10	38.5	3.8	44.8
E4	39.4	1.7	77.5
G9	11.5	1.5	86.5
J6	24.8	2.1	66.0
L63	23	1.2	74
N7	110	44	134
N53	95	95	92
O8	46	85	35
O205	NA	63	133
P3	23.8	4.9	— ^c
P72	NA	2.0	NA
P207	NA	105	280
P1001	NA	0.6	60
Q21	NA	22.7	275
Q73	NA	33	NA
R5	24	7.2	92
R54	NA	120	NA
R60	NA	6.1	NA
R216	—	102	—

^a The burst size of each sus mutant in the streptomycin-resistant mutants is expressed as the percentage of the burst size in the corresponding streptomycin-susceptible strain.

^b Not assayed.

^c No growth in the streptomycin-susceptible *sup*⁺ strain (Table 2).

FIG. 2. Lysis of BE2070 and BE2073 after induction with mitomycin C. Exponentially growing lysogenic strains in TG medium containing Casamino Acids (2.5 mg/ml) were exposed to mitomycin C at a concentration of 1 $\mu\text{g}/\text{ml}$ (17) and aerated at 37 C. The optical density at 550 $\text{m}\mu$ was read at intervals. (○) BE2070; (●) BE2073; (△) BE2073 plus streptomycin.

of alkaline phosphatase in *E. coli*. The efficiency of suppression of the amber codon by the *supE* gene was reduced by the *Str^{RR}* mutation to about one-fourth of that in the *Str^s supE* strain, whereas the *Str^R* mutation had no effect on the suppression by the *supE* gene (Table 4). The presence of streptomycin at a concentration of 1,000 $\mu\text{g/ml}$ abolishes the restrictive effect of the *Str^{RR}* mutation on the suppression by the *supE* gene and increases the level of alkaline phosphatase activity two- to threefold.

In Fig. 3, synthesis of alkaline phosphatase after removal of inorganic phosphate from the incubation medium is plotted as a function of time and of optical density of cells. Both in terms

of time and of cell density, enzyme synthesis in the *supE Str^R* strain was greatly decreased. The differential rates of enzyme synthesis by BE900, BE2002, and BE2002 (with streptomycin) were 1.76, 0.45, and 1.50 optical density units, respectively. No such decrease in enzyme synthesis was observed in BE694 (*supE Str^R*).

To determine whether the low enzymatic activity in BE2002 was a result of synthesis of inactive enzyme protein, BE900 and BE2002 were grown separately in low phosphate medium, and their extracts were assayed for enzymatic activity per unit of immunological CRM. The specific activities per unit of immunological CRM of the enzymes produced by BE900 and BE2002 were the

TABLE 4. Alkaline phosphatase activity produced in BE900 and in its streptomycin-resistant mutants^a

Strain	Genotype	Phenotype	Streptomycin	Enzymatic activity			
				As optical density ^b		As protein (mg) ^c	
				Expt 1	Expt 2	Expt 1	Expt 2
BE900	<i>pho⁻ supE</i>	<i>Str^R</i>	0	1.5	0.72	7.47	1.41
BE2002	<i>pho⁻ supE</i>	<i>Str^{RR}</i>	0	0.29	0.26	1.28	0.33
			1,000	0.66	0.51	4.55	0.74
BE694	<i>pho⁻ supE</i>	<i>Str^R</i>	0	1.69	0.89	6.1	
			1,000	1.70	0.92	3.8	

^a In experiment 1, cells were grown in low phosphate medium for 18 hr to achieve derepression of alkaline phosphatase. In experiment 2, cells were grown to the log phase in high phosphate medium, harvested by centrifugation, and suspended in Tris medium without added phosphate. After 2 hr of aeration with or without streptomycin, cells were collected by centrifugation.

^b The cells were treated with toluene 0.1M Tris (pH 8.9) containing 0.05% deoxycholate for 10 min and used for measuring enzymatic activity.

^c The cells were ruptured by sonic treatment and centrifuged at 15,000 $\times g$ for 20 min; the supernatant fraction was used for measuring enzymatic activity and protein determination.

FIG. 3. Synthesis of alkaline phosphatase in BE900 and BE2002. Cells grown to the log phase were harvested by centrifugation, washed twice with Tris medium without phosphate, and suspended in TG medium without added phosphate. Cells were aerated at 37 C and samples of the culture were removed at intervals for measurement of optical density (550 $m\mu$) and alkaline phosphatase. (●) BE900; (○) BE2002; (×) BE2002 plus streptomycin.

same (Table 5). In a second experiment, alkaline phosphatase was labeled with ^{14}C -proline in depressed conditions and extracted by the method of Neu and Heppel (14). The extracts were adsorbed on a column of diethylaminoethyl (DEAE)

TABLE 5. Specific activity in BE2002 per unit of immunological CRM of alkaline phosphatase produced in BE900

Strain	Streptomycin $\mu\text{g/ml}$	Enzymatic activity ^a	
		Expt 1	Expt 2
BE900	0	100	100
BE2002	0	130	100
	1,000	130	150

^a Induced as described in Table 4.

cellulose and eluted with a linear gradient of NaCl (0 to 0.25 M) buffered at pH 7.3. The chromatographic profile is shown in Fig. 4a. The fraction containing alkaline phosphatase was rechromatographed in the same way with an NaCl gradient from 0 to 0.15 M (Fig. 4b). Alkaline phosphatase activity, in relation to total ^{14}C radioactivity in the peak, is also shown in Fig. 4b. Assuming that inactive enzyme protein is also recovered in the same fraction by these procedures, we can conclude that there is no difference in specific activities of the enzyme protein fractions of BE900 and BE2002. These two experiments indicate that no inactive enzyme protein was synthesized in the *supE* Str^{RR} strain, suggesting that translation of the nonsense codon in the phosphatase gene was hindered in some way, resulting in synthesis of a smaller amount of active enzyme protein.

To test whether there was any alteration in the

FIG. 4. DEAE cellulose column chromatography of alkaline phosphatase protein synthesized in BE900 and BE2002 in the presence and absence of streptomycin. Extracts were prepared and adsorbed on a column of DEAE. Elution was achieved with a linear gradient of NaCl (0 to 0.25 M) in 0.01 M Tris (pH 7.3). Fractions of eluate were assayed (Fig. 4a) for (^{14}C) proline (broken line) and for alkaline phosphatase activity (solid line). The alkaline phosphatase protein obtained from the enzyme peak was refractionated on a DEAE cellulose column (Fig. 4b). Elution was achieved with a linear gradient of NaCl (0 to 0.15 M in 0.01 M Tris, pH 7.3). Specific enzymatic activity (SA) is expressed as enzyme activity per 1,000 counts/min of ^{14}C -proline. (I) BE900; (II) BE2002; (III) BE2002 plus streptomycin (1,000 $\mu\text{g/ml}$).

FIG. 5. Starch gel electrophoresis of alkaline phosphatase produced by mutants of a *supE* strain. (A) BE900; (B) BE2002; (C) BE2002 plus streptomycin. Top, negative; bottom, positive.

alkaline phosphatase molecule produced by the *supE* Str^{RR} strain, extracts prepared from strains BE900, BE2002, and BE2002 grown in the presence of streptomycin were subjected to starch gel electrophoresis; there was no electrophoretic change in the enzyme formed in the *supE* Str^{RR} strain (Fig. 5). Since substitution of a single amino acid with a different electrophoretic charge causes a change in the electrophoretic mobility of the enzyme by this technique (7, 22), this result indicates that glutamine or a neutral amino acid was inserted into a peptide corresponding to the amber codon in the phosphatase gene.

DISCUSSION

The present work indicates that there are at least three classes of streptomycin resistance mutations with respect to effects on suppression by the amber suppressor *supE* gene. This was demonstrated by measuring suppression of an amber codon in some cistrons of phage λ and in the alkaline phosphatase structural gene of *E. coli*. The mutation to streptomycin resistance which exhibited the strongest restriction on suppression by the amber suppressor *supE* gene had little effect on the function of the amber suppressors *supD* and *supF*. Kuwano, Ishizawa, and Endo (10a) obtained similar results from an analysis of the growth of T4 amber mutants; their streptomycin-resistant mutants were classified into four groups.

If the streptomycin resistance gene causes amino acid substitutions for glutamine, without changing the rate of translation of messenger RNA with the amber codon, the low level of alkaline phosphatase and the small burst size of the phage should be a result of a decreased specific activity of the enzyme or phage protein. We can eliminate this possibility, however, since the specific activity of alkaline phosphatase pro-

duced in the streptomycin-resistant *supE* strain was the same as that in the streptomycin-susceptible strain. From the experiments on the differential rate of synthesis of alkaline phosphatase and on the specific activity of the enzyme per immunological CRM and per purified enzyme protein, we conclude that the restriction of the efficiency of suppression by the *supE* gene is due to a decrease in the rate of translation of the amber codon in bacteriophage and in *E. coli*, presumably resulting from an alteration of structure of the ribosome by mutation to streptomycin resistance (4, 5).

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LITERATURE CITED

1. Andoh, T., and A. Garen. 1967. Fractionation of a serine transfer RNA containing suppressor activity. *J. Mol. Biol.* 24:129-132.
2. Cappecchi, N. R., and G. N. Gussin. 1965. Suppression *in vitro*: identification of a serine SRNA as a "nonsense" suppressor. *Science* 149:417-422.
3. Couturier, M., L. Desmet, and R. Thomas. 1964. High pleiotropy of streptomycin mutations in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* 16:244-248.
4. Cox, E. C., J. R. White, and J. G. Flaks. 1964. Streptomycin action and the ribosome. *Proc. Natl. Acad. Sci. U.S.* 51:703-709.
5. Davies, J. 1964. Studies on the ribosomes of streptomycin-sensitive and resistant strains of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.* 51:659-664.
6. Engelhardt, D. L., R. E. Webster, R. C. Wilhelm, and N. D. Zinder. 1965. *In vitro* studies on the mechanism of suppression of a nonsense mutation. *Proc. Natl. Acad. Sci. U.S.* 54:1791-1797.
7. Gallucci, E., and A. Garen. 1966. Suppressor gene for nonsense mutations. II. The *su-4* and *su-5* suppressor genes of *Escherichia coli*. *J. Mol. Biol.* 15:193-200.
8. Garen, A., S. Garen, and R. C. Wilhelm. 1965. Suppressor genes for nonsense mutations. I. The *Su-1*, *Su-2* and *Su-3* genes of *Escherichia coli*. *J. Mol. Biol.* 14:167-178.
9. Gartner, T. K., and E. Orias. 1966. Effects of mutations to streptomycin resistance on the rate of translation of mutant genetic information. *J. Bacteriol.* 91:1021-1028.
10. Gorini, L., G. A. Jacoby, and L. Breckenridge. 1966. Ribosomal ambiguity. Cold Spring Harbor Symp. Quant. Biol. 31:657-664.

- 10a. Kuwano, M. M. Ishizawa, and H. Endo. 1968. Su-II specific restriction of *amber* suppression by mutation to streptomycin resistance. *J. Mol. Biol.* **33**:513-516.
11. Lederberg, E. M., L. Cavalli-Sforza, and J. Lederberg. 1964. Interaction of streptomycin and suppressor for galactose fermentation in *E. coli* K12. *Proc. Natl. Acad. U.S.* **51**:678-682.
12. Levinthal, C., E. R. Signer, and K. Fetherholf. 1962. Reactivation and hybridization of reduced alkaline phosphatase. *Proc. Natl. Acad. Sci. U.S.* **48**:1230-1237.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
14. Neu, H. C., and L. A. Heppel. 1965. The release of enzymes from *Escherichia coli* by osmotic shock during the formation of spheroplasts. *J. Biol. Chem.* **240**:3685-3692.
15. Notani, G. W., D. L. Engelhardt, W. Konigsberg, and N. Zinder. 1965. Suppression of a coat protein mutant of the bacteriophage f2. *J. Mol. Biol.* **12**:439-447.
16. Orias, E., and T. K. Gartner. 1966. Suppression of *amber* and *ochre* rII mutants of bacteriophage T4 by streptomycin. *J. Bacteriol.* **91**:2210-2215.
17. Otsuji, N., M. Sekiguchi, T. Iijima, and Y. Takagi. 1959. Induction of phage formation in the lysogenic *Escherichia coli* K-12 by mitomycin C. *Nature* **184**:1079-1080.
18. Ouchterlony, O. 1948. Antigen-antibody reaction in gels. *Arkiv Kemi Mineral. Geol.* **26B**:1-9.
19. Smith, J. D., J. N. Abelson, B. F. Clark, H. M. Goodman, and S. Brenner. 1966. Studies on amber suppressor tRNA. *Cold Spring Harbor Symp. Quant. Biol.* **31**:479-485.
20. Smithies, O. 1955. Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. *Biochem. J.* **61**:629-641.
21. Stretton, A. O. W., and S. Brenner. 1965. Molecular consequences of the amber mutation and its suppression. *J. Mol. Biol.* **12**:456-465.
22. Weigert, M. G., and A. Garen. 1965. Base composition of nonsense codons in *E. coli*. Evidence from amino-acid substitutions at a tryptophan site in alkaline phosphatase. *Nature* **206**:992-994.
23. Weigert, M. G., and A. Garen. 1965. Amino acid substitutions resulting from suppression of nonsense mutations. I. Serine insertion by the *Su-1* suppressor gene. *J. Mol. Biol.* **12**:448-455.
24. Weigert, M. G., E. Lanka, and A. Garen. 1965. Amino acid substitutions resulting from suppression of nonsense mutations. II. Glutamine insertion by the *Su-2* gene; tyrosine insertion by the *Su-3* gene. *J. Mol. Biol.* **14**:522-527.