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Effects of Mutations to Streptomycin Resistance on the Rate of Translation of Mutant Genetic Information

T. K. GARTNER AND E. ORIAS
Department of Biological Sciences, University of California, Santa Barbara, California

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ABSTRACT

GARTNER, T. K. (University of California, Santa Barbara), AND E. ORIAS. Effects of mutations to streptomycin resistance on the rate of translation of mutant genetic information. J. Bacteriol. 91:1021-1028. 1966.—The effects of mutations to streptomycin resistance of independent origin upon the translation of suppressible mutant information were studied in an isogenic series of strains of *Escherichia coli*. The group of suppressible mutants included 1 mutation in the *z* gene of the *lac* operon of *E. coli* (O^0_2 allele), 12 mutations distributed among the two *r*II cistrons of T4, and 13 mutations distributed among at least five cistrons of phage T7. It was concluded that the mutations to streptomycin resistance cause a significant decrease in the rate of translation of the suppressible codons, and that this effect is limited to a few types of codons.

Mutations which in a single step confer resistance to high levels of streptomycin are closely linked in *Escherichia coli* (19, 27). These mutations affect the 30*S* component of the ribosome (14, 15). Since ribosomes participate in the translation of genetic information, mutations to streptomycin resistance might affect this process. Evidence which could be interpreted in terms of modifications of the process of translation of genetic information resulting from mutations to streptomycin resistance has been previously reported by Gorini and Kataja (18) and by Lederberg, Cavalli-Sforza, and Lederberg (22).

In this paper, we report the effects of mutations to streptomycin resistance on the efficiency of the suppression of *amber* and *ochre* mutations in *E. coli* and in phages T4 and T7. All of the mutations studied are suppressible by certain extragenic suppressors of the $O_2°$ mutation of the *z* gene of the *lac* operon of *E. coli*. The suppressor systems used permitted (i) detection of restrictive effects of mutations to streptomycin resistance on the translation of the suppressible mutant codons, (ii) identification of at least four nonidentical mutations to streptomycin resistance on the basis of their restrictive effects, and (iii) the conclusion that the basis of the restrictive effects is a decreased rate of translation of a few types of codons.

MATERIALS AND METHODS

Symbols. The following symbols are used: *str-r* and *str-s*, streptomycin resistance and sensitivity, respectively; *lac*, *mal*, *mel*, and *xyl*, utilization of lactose maltose, melibiose, and xylose, respectively; *z* and *y* genes of the *lac* operon which determine the structure of β-galactosidase and lactose permease, respectively; i^+ and i^-, wild-type and constitutive alleles of the regulator gene of the *lac* operon, respectively; $O_2°$, allele of the *z* gene of the *lac* operon carrying an *ochre* (polar nonsense) mutation; *oss*, mutants of phage T7 which are suppressed by suppressors of the $O_2°$ mutation.

Strains. Strain 2320(λ) (28) is *str-r*, mal^+, xyl^-, i^-, $O_2°$. Strain SBO is *str-s*, mal^+, xyl^+, i^-, $O_2°$. It was constructed by conjugation of *str-s*, mal^+, xyl^+, donor strain JC182 (13) with strain 2320(λ) and selection for xyl^+ recombinants. Strain SBO is lac^- and mel^- as a result of carrying the $O_2°$ mutation of the *z* gene (20) originally present in strain 2320. A mal^- derivative of SBO was obtained by selecting mutants resistant to phage λ (21).

The T4 phage mutants used in this study belong to the *amber* and *ochre* classes of *r*II mutants of phage T4 (10); these classes of mutants are suppressible by suppressors of the $O_2°$ mutation (2, 28). The T4 mutations map in the A or B cistrons of the *r*II region. All of the *r*II mutants, with the exception of *r*GU63, were obtained from S. P. Champe. Mutant *r*GU63 is a mutant induced by 5-bromodeoxyuridine, and was isolated in our laboratory. The T7 mutants were induced by 2-aminopurine, and were isolated in our laboratory; the mutations are distributed throughout at least five cistrons of T7 (Orias and Gartner, *unpublished data*). Some cistrons of T7 are represented by mutations at more than one site.

Classification of the suppressible mutants used. Amber and *ochre* mutations have been studied extensively. The mutant codon corresponding to an

amber mutation has the nucleotide composition UAG and the mutant codon corresponding to an *ochre* mutation is UAA (10). Both of these mutations are nonsense codons (6, 7, 16). The *amber* codon is characteristically suppressible by both *amber* suppressors and *ochre* suppressors. The *ochre* codon is suppressible by *ochre* suppressors, but not by *amber* suppressors (7). The *amber* suppressors which are sufficiently characterized suppress with high efficiency, whereas *ochre* suppressors appear to suppress with low efficiency (7). The *amber* suppressor in *E. coli* strain S26R1E appears to be a serine soluble ribonucleic acid (sRNA) molecule (11). The *ochre* suppressor in *E. coli* strain 2320(λ)-15B either is an altered ribosome or indirectly causes alterations of ribosome structure (30).

The suppressor in strain SBO-22, a strain whose isolation is described below, appears to be an *ochre* suppressor because it can suppress both *ochre* and *amber* mutants. The $O_2°$ mutation of the *z* gene is an *ochre* mutation (7). The T4 mutants *r*N21, *r*360, *r*UV375, *r*N24, *r*N17, *r*N7, *r*SD160, *r*UV199, and *r*AP53 are *ochre* mutants (7). The T4 mutant *r*B94 belongs to the class of ambivalent mutants of type I (5); this class is now known to be identical to the *amber* class (7). The T4 mutant *r*GU63 is probably also an *amber* mutant, because it is suppressible by strain C600(λ) (Orias and Gartner, *unpublished data*); strain C600(λ) is a lysogenic derivative of strain C600, a strain which possesses an *amber* suppressor (9). The T4 mutant *r*609 is probably an *ochre* mutant because it is suppressible by strain SBO-22 and not by either strain C600(λ) or CR63(λ). It is possible, however, to incorrectly classify an *amber* mutant as an *ochre* mutant as a result of the lack of an appropriate *amber* suppressor. The T7 mutants probably are *amber* mutants because all of them are suppressible by both strains SBO-22 and C600(λ).

Media. The compositions of the media used were previously described (17).

Isolation of strain SBO-22, carrying a suppressor of the $O_2°$ mutation. *Lac*$^+$ revertants of SBO were isolated and screened for their ability to suppress T7 mutants. A 0.1-ml sample of an overnight culture of SBO started with a small inoculum and grown with aeration in tryptone B1 medium at 37 C was plated on lactose minimal medium. *Lac*$^+$ colonies were picked, suspended in 1 ml of tryptone B1 medium, and incubated overnight at 37 C without aeration. A 0.4-ml sample of each of these cultures was then plated on each of two tryptone B1 agar plates, and the bacterial lawn on each of these plates was spotted with the phage mutants as described below; one of these plates was incubated overnight at 25 C and the other at 42 C. SBO-22 is a *lac*$^+$ and *mel*$^+$ derivative of SBO isolated by this procedure. This strain is capable of suppressing some of the *amber* and *ochre* mutants of phages T4 and T7 referred to above.

Isolation of streptomycin-resistant derivatives of SBO-22. Streptomycin-resistant derivatives of SBO-22 were isolated by the following method. Samples of 0.1 ml of an overnight culture of SBO-22, grown at 37 C with aeration, were each diluted in trytpone B1 medium to a total volume of 5 ml and the mixture was filtered (Millipore filter, 0.45-μ average pore size). The filter containing the organisms was placed on a tryptone B1 agar plate, and the plate was incubated at 37 C. After 6 hr of incubation, the filter was transferred to a tryptone B1 agar plate supplemented with 50 μg/ml of dihydrostreptomycin sulfate (Nutritional Biochemicals Corp., Cleveland, Ohio), and incubation was continued at room temperature. Colonies were picked and screened by spot tests for resistance to or dependence on streptomycin by using tryptone B1 agar plates supplemented with 500 μg/ml of dihydrostreptomycin. The different streptomycin-resistant mutants of SBO-22 are designated by the prefix SBO-22 followed by a number which reflects the order of their isolation; subsequently, each strain will be referred to simply by this last number.

In addition to the streptomycin-resistant mutants obtained by the method described above, a streptomycin-resistant derivative of SBO-22 (designated SBO-22-*str-r*) was constructed by transduction of the *str-r* allele of strain 2320(λ).

Transduction. All transductions reported in this paper were done with phage P1kc by the method of Luria, Adams, and Ting (26). For transduction to streptomycin resistance, phenotypic expression (23) of the infected cells occurred on a Millipore filter (average pore size, 0.45 μ) kept for 6 hr at room temperature on an agar plate of the appropriate nutrient composition but lacking streptomycin. Selection occurred at 37 C after transfer of the filter to agar plates containing the appropriate medium and 500 μg/ml of streptomycin. In all transduction experiments, tests for sterility of the phage suspension and for the reversion of the recipient strain were included.

Spot tests for phage suppressor pattern. Spot tests for suppression of individual phage mutants were done by depositing approximately 0.01 ml of a phage suspension, by use of a capillary tube, on the lawns of the bacterial strains to be tested. The suspensions of the phage mutants contained approximately 5×10^7 particles per milliliter for the T4 mutants, and 5×10^6 particles per milliliter for the T7 mutants.

Burst size measurements. Burst size measurements of the *r*II mutants were done by the method previously described (28). Wild-type T4D and *r*II mutant *r*638, which sustains a deletion of the entire B cistron (3), were included in all experiments.

Assays for β-galactosidase. Assays for β-galactosidase were performed by the method described by Revel, Luria, and Rotman (31), as modified by Loomis and Magasanik (24). Absorbance at 420 mμ was measured with a Beckman DU spectrophotometer. A unit of β-galactosidase is defined as that amount responsible for the release of 1 mμmole of *o*-nitrophenol in 1 min at *p*H 7.0 at 28 C. The samples were taken during exponential growth of the cultures at 37 C in glycerol minimal medium in the absence of inducer. Assays for total protein were performed by the method of Lowry et al. (25). The growth rate of each culture used for the β-galactosidase assays was determined by measuring the optical density at 550 mμ.

RESULTS

Characterization of the streptomycin-resistant mutants. Four classes of streptomycin-resistant

mutants (which are represented by the mutant strains 101, 104, 109 and SBO-22-*str-r*) were clearly distinguished on the basis of suppression of T7 mutants (Fig. 1). These types of streptomycin-resistant mutants can be arranged in the following order on the basis of their decreasing permissiveness of suppression of the T7 mutants: 101, 109, SBO22-*str-r*, and 104. Mutant 101, at the one extreme, appears almost as permissive as SBO-22, the streptomycin-sensitive parental strain. Mutant 104, at the other extreme, suppresses significantly only one of the phage mutants. Figure 1 also displays a striking property of the streptomycin-resistant mutants studied: if a streptomycin-resistant host significantly suppresses a given phage mutant, that phage mutant is also suppressed by every streptomycin-resistant mutant of more permissive rank. The size of the isolated plaques of wild-type T7 (second spot, top row of each plate) indicates that none of these mutations to streptomycin-resistance limits the size of the wild-type plaque.

Similar results were obtained by studying *r*II mutants of phage T4, which are suppressible by strain SBO-22. The ability of each type of streptomycin-resistant derivative of SBO-22 to suppress the T4 mutants was determined by spot tests. The results allowed ordering of the streptomycin-resistant derivatives of SBO-22 on the basis of decreasing permissiveness of suppression of the *r*II mutants. The resulting hierarchy of streptomycin-resistant mutants is *identical* with that obtained by the criterion of permissiveness of suppression of the T7 mutants. The results of the spot tests of the *r*II mutants were confirmed by burst size measurements in SBO-22 and its streptomycin-resistant derivatives (Table 1). In Table 1, the streptomycin-resistant derivatives of SBO-22 are listed from left to right in the order of their decreasing permissiveness of suppression. The table confirms the results obtained by the use of spot tests; the burst size of *every mutant* decreases across the table from left to right. Strain 104 does not suppress any of the *r*II mutants. For the *r*II mutants showing the highest levels of suppression by SBO-22 (e.g., N24), the burst size in strain 104 was reduced by at least 5 orders of magnitude. Under the same conditions of measurement, the burst size of wild-type T4 does not vary by more than a factor of about 2.

The growth rates and the specific activities of β-galactosidase for SBO-22 and each of its streptomycin-resistant derivatives are shown in Fig. 2. No striking differences in the growth rates were caused by any of the mutations to streptomycin-resistance. The rank of each streptomycin-resistant derivative determined by the magnitude of its specific activity of β-galactosidase is identical to the rank previously determined by the criterion of permissiveness of suppression of the phage mutants.

The mutation to streptomycin resistance in strain 104 also results in a *mel*$^-$ phenotype. Since the metabolism of melibiose requires the activity of the lactose permease but not of β-galactosidase (1, 29), the *mel*$^-$ phenotype of strain 104 indicates that the specific activity of the lactose permease is also significantly decreased. The *lac*$^-$ and *mel*$^-$ phenotypes of strain 104 provided selective conditions for the isolation of the *lac*$^+$ and *mel*$^+$ transductants described in the next section.

Test of the mutations to streptomycin resistance for linkage. The four different streptomycin-resistant mutations were tested for linkage as follows. Lysates of phage P1kc were prepared on strains SBO, SBO-22, and on three of the streptomycin-resistant mutants: 101, 109 and SBO-22-*str-r*. Each of these strains was used as a donor in transduction experiments with strain 104 as the recipient. Either *lac*$^+$ or *mel*$^+$ transductants were selected. Transductants were tested both for sensitivity to streptomycin and for their phage suppressor pattern. In addition, *mel*$^+$ transductants were tested for their *lac* phenotype. The results are shown in Table 2. All of the transductants tested were streptomycin-sensitive when either SBO or SBO-22 was the donor and were streptomycin-resistant when strain 101, 109, or SBO-22-*str-r* was the donor. In all cases, the transductants also had the suppressor pattern expected from the interaction between the suppressor in SBO-22 and the allele for streptomycin sensitivity or resistance introduced from the donor. Thus, the *lac*$^+$ and *mel*$^+$ phenotypes resulted exclusively from the replacement of the restrictive allele for streptomycin resistance of strain 104 either by the allele for streptomycin sensitivity or by the permissive allele for streptomycin resistance of the donor. Streptomycin-sensitive *lac*$^+$ transductants resulting from recombination between two different mutations to streptomycin resistance were not observed. Thus, these mutations to streptomycin resistance either overlap or are very closely linked and, in all probability, constitute an allelic series.

Mapping of the suppressor in strain SBO-22. Transduction tests revealed that the suppressor in strain SBO-22 is loosely linked to the mutations to streptomycin resistance (frequency of cotransduction: 2.5×10^{-8}). The outcomes of the pertinent crosses are described in Table 3. The results indicate the order *su–str-r–mal.*

DISCUSSION

The results can be summarized as follows. Four nonidentical mutations to streptomycin resistance

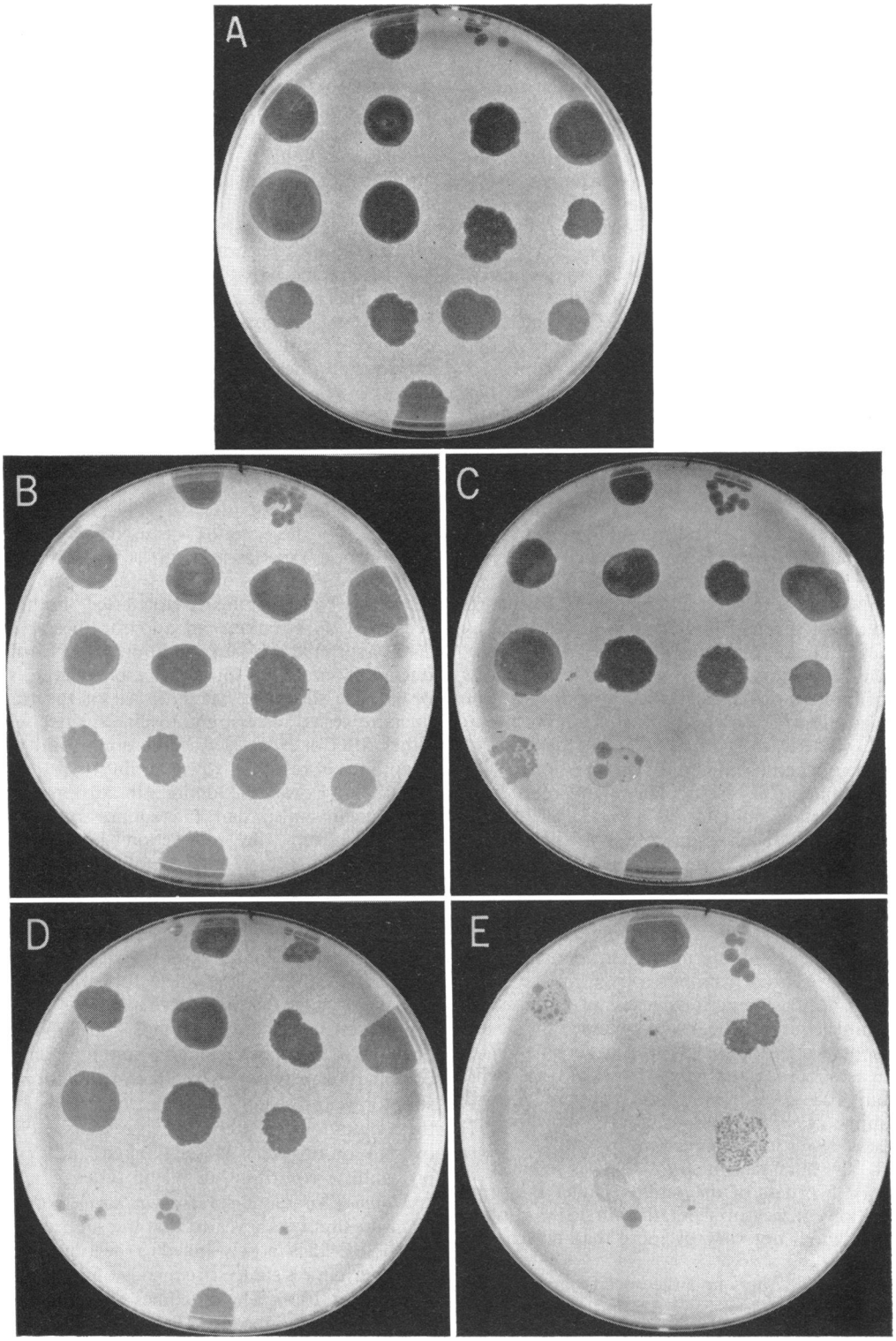

FIG. 1. *Spot tests of suppressible mutants of T7 on Escherichia coli strain SBO-22 and its streptomycin-resistant derivatives. (A) SBO-22, (B) 101, (C) 109, (D) SBO-22-str-r, (E) 104. Each bacterial lawn was obtained by plating 0.2 ml of an exponentially growing culture concentrated to about 2 × 10^9 cells per milliliter. The phage mutants were spotted at the concentration described in the text, and the plates were incubated at 25 C. The order of the spots is as follows, from left to right. Top row: wild-type T7 at the same concentration as that of the other mutants, wild-type T7 at low enough concentration to give isolated plaques; second row: oss 1, oss 12, oss 30, and oss 7; third row: oss 27, oss 20, oss 22, and oss 4; fourth row: oss 11, oss 13, oss 28, and oss 14; fifth row: oss 8.*

TABLE 1. *Burst sizes of amber and ochre rII mutants of T4 in strain SBO-22 and each of its streptomycin-resistant derivatives**

rII mutant	Map location	Avg percentage burst size				
		SBO-22	101	109	SBO-22-*str-r*	104
T4+		100.0	100.0	100.0	100.0	100.0
638	B deletion	0.00	0.03	0.01	0.00	<0.001
N21	A 6d	1.7	0.67	0.08	0.00	<0.001
360	B 1a	0.27	0.06	0.00	0.00	<0.001
UV375	B 1a	0.07	0.00	0.00	0.00	<0.001
N24	B 1b	54.0	39.0	9.7	0.07	<0.001
B94	B 4	60.0	30.0	5.6	0.06	<0.001
GU63	B	40.0	6.0	0.52	0.01	<0.001
N17	B 4	5.1	0.18	0.02	0.00	<0.001
N7	B 6	0.50	0.00	0.01	0.00	<0.001
609	B 7	14.0	0.29	0.03	0.00	<0.001
SD160	B 7	27.0	3.0	0.50	0.00	<0.001
UV199	B 7	33.0	3.2	0.36	0.00	<0.001
AP53	B 9b	73.0	60.0	53.0	10.0	<0.001
T4+, uncorrected		410.0	210.0	370.0	300.0	163.0

* The burst size of each T4 mutant is expressed as the percentage of the burst size of the wild-type T4 control, which is shown in the last row. The locations of the rII mutations were determined by Benzer (4). GU63 is an rII mutant isolated in our laboratory.

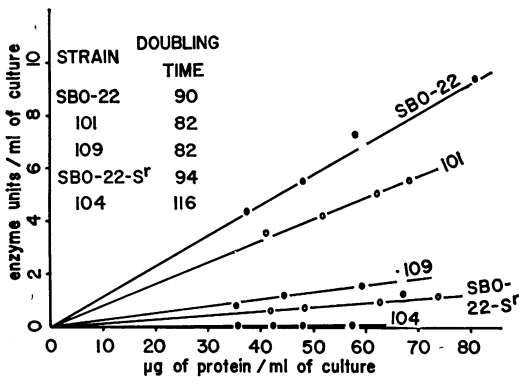

FIG. 2. *Specific activity of β-galactosidase and the growth rates of SBO-22 and each of its streptomycin-resistant derivatives, obtained from cultures growing exponentially at 37 C in glycerol minimal medium. The doubling times are expressed as minutes per generation.*

cause restrictions of suppression of *amber* and *ochre* mutations of at least five cistrons of phage T7, of both rII cistrons of phage T4, and of the *z* gene of the *lac* operon of *E. coli*. Different degrees of restriction of suppression are caused by these mutations to streptomycin resistance. The streptomycin-resistant mutants were unambiguously ranked in the same order by the criterion of decreasing permissiveness of suppression, regardless of the system in which suppression was measured. Furthermore, the mutations to streptomycin resistance do not cause serious alterations of the normal physiology of the streptomycin-resistant mutants; this was demonstrated by their nearly normal growth rates in glycerol minimal medium. These observations permit the conclusions given below.

The restrictive effects of the mutations to streptomycin resistance are exerted at the level of translation of the suppressible mutant codon. Since the mutant codons which are sensitive to restriction are located in a variety of phage and bacterial cistrons whose functions are not obviously related, it is unlikely that the mutations to streptomycin resistance affect the specific activity of the product of each of the suppressed genes subsequent to their synthesis. Therefore, the restriction must be exerted at a step not later than the translation of the mutant genetic information. It is likely that the restriction is caused at the level of translation, since it is not obvious how mutations which presumably alter ribosome structure (14, 15) can directly affect the transcription of messenger RNA molecules containing the mutant codons.

The restrictions caused by the mutations to SM resistance do not result primarily from amino acid substitutions during the process of translation of the mutant codons. Assume that the mutations to streptomycin resistance cause the substitution of an amino acid different from the amino acid coded by the mutant codon in the presence of the suppressor, without significantly changing the rate of translation of the mutant messenger RNA molecules. The observed

TABLE 2. *Test of linkage of the mutations to streptomycin resistance**

Donor	Recipient	Transductants			
		Selected phenotype	No. tested	Characteristics	
				Streptomycin-sensitive	Suppressor pattern†
Streptomycin-sensitive					
SBO	104	*mel*$^+$	100‡	100	SBO-22
SBO-22	104	*lac*$^+$	100	100	SBO-22
Streptomycin-resistant					
101	104	*lac*$^+$	100	0	101
109	104	*lac*$^+$	100	0	109
SBO-22-*str-r*	104	*lac*$^+$	100	0	SBO-22-*str-r*

* Lysates of phage P1kc were prepared on the donors indicated. *Lac*$^+$ and *mel*$^+$ transductants were selected and tested for streptomycin resistance or sensitivity and for their suppressor pattern.

† Characteristic suppressor pattern exhibited by all of the transductants tested (at least eight from each cross).

‡ All of the *mel*$^+$ transductants were also *lac*$^+$.

TABLE 3. *Mapping of the suppressor in SBO-22**

Donor	Recipient	Selected phenotype(s)	Number tested	*su*$^+$†	*mal*$^+$	Transduction frequency
101	SBO *mal*$^-$	*lac*$^+$ *str-r*	25	25	0	2.5×10^{-8}
101	SBO *mal*$^-$	*mal*$^+$ *str-r*	150	0	150	1.5×10^{-7}
101	SBO *mal*$^-$	*str-r*	100	0	0	3.4×10^{-5}

* The transduction frequency is expressed as the number of transductants for the selected marker(s) per plaque-forming unit. The *su*$^+$ or *su*$^-$ phenotype of every transductant was determined on the basis of the ability to suppress *r*II mutants N24 and AP53 in spot tests. The results indicate the order *su*$^-$–*str-r*–*mal*.

† *su*$^+$ indicates the mutant (suppressing) allele.

restrictions of suppression must then be the result of a decreased specific activity, resulting from the amino acid substitution in the product of each of the suppressed genes. Given this situation, it is not clear why an amino acid substitution would cause the observed congruence in the decrease of specific activity of each of the affected proteins, particularly since in the various suppressible mutants these hypothetical amino acid substitutions occur in different locations of one polypeptide as well as in different polypeptides. One solution to this problem is the assumption that all the mutations to streptomycin resistance cause the substitution of the *same amino acid* in the product of each of the suppressed genes; furthermore, the difference in the degree of restriction caused by the various mutations to streptomycin resistance requires the assumption that these substitutions occur with frequencies which are different but characteristic of each streptomycin-resistant mutant. Thus, in strain 104 this substitution must occur with high frequency to account for the extreme restriction of suppression characteristic of this strain.

The following arguments eliminate the possibility that restriction is simply the result of amino acid substitutions. The alteration of *r*II mutant N24 maps in a region of the B cistron (4) which can be deleted (e.g., by the alteration in mutant *r*1589) without decreasing the activity of the B cistron product to the point where it limits the burst size (12). Consequently, the burst size of N24 should be quite insensitive to most amino acid substitutions which possibly could result from the action of mutations to streptomycin resistance on the suppression of the mutant codon. However, the burst size of N24 in strain 104 is decreased by at least 5 orders of magnitude. Furthermore, the activity of lactose permease (determined by the *y* gene) should also be insensitive to amino acid substitutions, which possibly could occur during the translation of the mutant codon of the O_2° allele of the *z* gene. Strain 104, however, is *mel*$^-$ (permease-negative) as a consequence of the action of the restrictive mutation to streptomycin resistance.

Since the occurrence of amino acid substitutions does not explain either the *mel*$^-$ phenotype of strain 104 or the 10^5-fold decrease of the burst size of *r*II mutant N24 in this strain, these

restrictions must be caused by a significant decrease in the rate of translation of the respective mutant codons. The consequence of the decreased rate of translation of the mutant codon is a decrease in the number of complete gene products produced per unit time. This explanation can be extended to provide a basis for the congruence of the restrictions imposed by the four different mutations to streptomycin resistance: it is only necessary to assume that each mutation to SM resistance causes a different but characteristic rate of translation of the *amber* and *ochre* codons.

The restrictive effects of the mutations to SM resistance are limited to a few types of codons. The behavior of mutant 104 provides the best support for this conclusion. The growth rate of strain 104 in glycerol minimal medium and the burst size of wild-type T4 in that strain are not decreased to a physiologically significant extent. Thus, the mutation to streptomycin resistance in strain 104 does not severely restrict the rate of translation of any codon which specifies an amino acid in any of the indispensable proteins of phage T4 and of strain SBO-22 of *E. coli*. It follows that if most of the codons of *E. coli* specify amino acids, then only a few types of codons can be sensitive to the restriction caused by the mutations to streptomycin resistance. Possibly, only the *amber* and *ochre* codons (10) are sensitive to these restrictions.

In view of the work of Davies (15) and Cox, White, and Flaks (14), it is assumed that the mutations to streptomycin resistance described in this paper affect the structure of ribosomes. If this assumption is granted, the extreme specificity of the restrictions associated with these ribosomal alterations contrasts with the lack of specificity of ribosomes in mediating the translation of wild-type genetic information (8). This codon specificity may result either from an interaction between the suppressible codon and the altered ribosome, or from an interaction between the altered ribosome and the suppressor molecule required for the translation of the mutant codon.

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LITERATURE CITED

1. BECKWITH, J. R. 1963. Restoration of operon activity by suppressors. Biochim. Biophys. Acta **76**:162–164.
2. BECKWITH, J. R. 1964. Restoration of operon activity by suppressors, p. 119–124. *In* H. Stubbe [ed.], Struktur und Funktion des genetischen materials. Akademie-Verlag, Berlin.
3. BENZER, S. 1959. On the topology of the genetic fine structure. Proc. Natl. Acad. Sci. U.S. **45**: 1607–1620.
4. BENZER, S. 1961. On the topography of the genetic fine structure. Proc. Natl. Acad. Sci. U.S. **47**: 403–415.
5. BENZER, S., AND S. P. CHAMPE. 1961. Ambivalent rII mutants of phage T4. Proc. Natl. Acad. Sci. U.S. **47**:1025–1038.
6. BENZER, S., AND S. P. CHAMPE. 1962. A change of nonsense to sense in the genetic code. Proc. Natl. Acad. Sci. U.S. **48**:1114–1121.
7. BRENNER, S., AND J. R. BECKWITH. 1965. *Ochre* mutants, a new class of suppressible nonsense mutants. J. Mol. Biol. **13**:629–637.
8. BRENNER, S., F. JACOB, AND M. MESELSON. 1961. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. Nature **190**:576–585.
9. BRENNER, S., AND A. O. W. STRETTON. 1964. The amber mutation. J. Cellular Comp. Physiol. **64** (Suppl. 1):43–50.
10. BRENNER, S., A. O. W. STRETTON, AND S. KAPLAN. 1965. Genetic code: the "nonsense" triplets for chain termination and their suppression. Nature **206**:994–998.
11. CAPECCHI, M. R., AND G. N. GUSSIN. 1965. Suppression *in vitro*: identification of a serine sRNA as a "nonsense" suppressor. Science **149**:417–422.
12. CHAMPE, S. P., AND S. BENZER. 1962. An active cistron fragment. J. Mol. Biol. **4**:288–292.
13. CLARK, A. J. 1963. Genetic analysis of a "double male" strain of *Escherichia coli* K-12. Genetics **48**:105–120.
14. 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.
15. 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.
16. GAREN, A., AND O. SIDDIQI. 1962. Suppression of mutations in the alkaline phosphatase structural cistron of *E. coli*. Proc. Natl. Acad. Sci. U.S. **48**:1121–1127.
17. GARTNER, T. K., AND E. ORIAS. 1965. Pleiotropic effects of suppressors of a *Lac*-"operator negative" mutation in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **53**:62–68.
18. GORINI, L., AND E. KATAJA. 1964. Phenotypic repair by streptomycin of defective genotypes in *E. coli*. Proc. Natl. Acad. Sci. U.S. **51**:487–493.
19. HASHIMOTO, K. 1959. Streptomycin resistance in *Escherichia coli* analyzed by transduction. Genetics **45**:49–62.
20. JACOB, F., AND J. MONOD. 1961. On the regulation of gene activity. Cold Spring Harbor Symp. Quant. Biol. **26**:193–209.
21. LEDERBERG, E. M. 1955. Pleiotropy for maltose fermentation and phage resistance in *Escherichia coli* K-12. Genetics **40**:580–581.

22. LEDERBERG, E. M., L. CAVALLI-SFORZA, AND J. LEDERBERG. 1964. Interaction of streptomycin and a suppressor for galactose fermentation in *E. coli* K-12. Proc. Natl. Acad. Sci. U.S. **51**: 678–682.
23. LEDERBERG, J. 1951. Streptomycin resistance: a genetically recessive mutation. J. Bacteriol. **61**: 549–550.
24. LOOMIS, W. F., AND B. MAGASANIK. 1964. The relation of catabolite repression to the induction system for β-galactosidase in *Escherichia coli*. J. Mol. Biol. **8**:417–426.
25. 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.
26. LURIA, S. E., J. N. ADAMS, AND P. C. TING. 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. Virology **12**:348–390.
27. NEWCOMBE, H. B., AND M. H. NYHOLM. 1950. The inheritance of streptomycin resistance and dependence in crosses of *Escherichia coli*. Genetics **35**:603–611.
28. ORIAS, E., AND T. K. GARTNER. 1964. Suppression of a class of *r*II mutants of T4 by a suppressor of a *Lac*-"operator" negative mutation. Proc. Natl. Acad. Sci. U.S. **52**:859–864.
29. PARDEE, A. B. 1957. An inducible mechanism for accumulation of melibiose in *Escherichia coli*. J. Bacteriol. **73**:376–385.
30. REID, P. J., E. ORIAS, AND T. K. GARTNER. 1965. Ribosomal protein differences in a strain of *E. coli* carrying a suppressor of an ochre mutation. Biochem. Biophys. Res. Commun. **21**:66–71.
31. REVEL, H. R., S. E. LURIA, AND B. ROTMAN. 1961. Biosynthesis of β-D-galactosidase by phage carried genes. I. Induced β-galactosidase biosynthesis after transduction of gene z^+ by phage. Proc. Natl. Acad. Sci. U.S. **47**:1956–1967.