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

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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^a allele), 12 mutations distributed among the two rII 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 30S 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^a 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^a, allele of the z gene of the lac operon carrying an ochre (polar nonsense) mutation; os, mutants of phage T7 which are suppressed by suppressors of the O^a mutation.

Strains. Strain 2320(λ) (28) is str-r, mal^+, xyl^−, i^−, O^a. Strain SBO is str-s, mal^+, xyl^+, i^+, O^a. 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^a 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 rII mutants of phage T4 (10); these classes of mutants are suppressible by suppressors of the O^a mutation (2, 28). The T4 mutations map in the A or B cistrons of the rII region. All of the rII mutants, with the exception of rGU63, were obtained from S. P. Champe. Mutant rGU63 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
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(X).

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 reversibility 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 × 107 particles per milliliter for the T4 mutants, and 5 × 106 particles per milliliter for the T7 mutants.

Burst size measurements. Burst size measurements of the RII mutants were done by the method previously described (28). Wild-type T4D and RII mutant r638, 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 Megason (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 mMole of α-nitrophenol in 1 min at pH 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 rII 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 rII 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 rII 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 rII mutants. For the rII 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 meliobiore 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 × 10−4). 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 $2 \times 10^9$ 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.
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
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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**


