Effect of Streptomycin Deprivation on Enzyme Synthesis in Streptomycin-Dependent Escherichia coli

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During growth of streptomycin-dependent strains of Escherichia coli in the absence of streptomycin (deprived growth), both constitutive and inducible synthesis of β-galactosidase were preferentially inhibited. A similar preferential inhibition of constitutive and derepressed synthesis of alkaline phosphatase was observed. Catabolite repression accounted for part, but not all, of the inhibition of the inducible β-galactosidase synthesis. Serological experiments indicated that that part of the inhibition specifically associated with streptomycin deprivation was not a result of the induction of altered β-galactosidase. It is suggested that during deprived growth the ribosomes of streptomycin-dependent bacteria become impaired in their ability to translate certain messages.

Of the manifold effects of streptomycin and other aminoglycoside antibiotics on sensitive bacteria, inhibition of protein synthesis and interference with correct translation of the genetic code have, in recent years, been intensively investigated. Brock, in his excellent review (2), summarized the information regarding the effects of streptomycin on protein synthesis. Briefly, in vitro experiments have demonstrated that streptomycin inhibits polyuridylic acid-directed polyphenylalanine synthesis in cell-free systems in which ribosomes are derived from streptomycin-sensitive (SmS) cells (8, 9, 27). Polyphenylalanine synthesis is not inhibited when ribosomes from streptomycin-resistant (SmR) cells are used. Moreover, it is the 30S and not the 50S ribosomal subunit which is affected (3, 4). In addition to inhibiting specific amino acid incorporation, streptomycin promotes ambiguity in translation of the genetic code by allowing amino acids other than that dictated by a given codon to be incorporated into the growing polypeptide (5, 6, 10, 20). Apparently the step affected is the binding of aminoacyl soluble ribonucleic acid (RNA) to the ribosome (14, 24, 25).

Evidence that streptomycin can elicit ambiguous codon recognition in vivo comes from streptomycin-promoted suppression of some mutant genes (11, 12, 16, 21). Recently, Bissel (1) presented evidence that an SmS strain of Escherichia coli, induced with isopropyl-β-D-thiogalactopyranoside (IPTG) in the presence of neomycin or streptomycin, synthesized inactive protein which cross-reacted with antibody toward β-galactosidase.

The unitary hypothesis of streptomycin action proposed by Spotts and Stanier (29) assumes that the critical reaction (or activity) inhibited by streptomycin in SmS strains would require that antibiotic for normal activity in bacteria which have mutated to dependency. It has been shown (28) that there is a preferential inhibition of the synthesis of some enzymes, among them β-galactosidase, in a streptomycin-dependent (SmD) bacterium when it is deprived of the antibiotic, though growth and protein synthesis continue for a long period before finally ceasing. It will be shown in this report that alkaline phosphatase also undergoes preferential inhibition. If the primary effect of streptomycin in SmS strains is the promotion of miscoding, then it might be expected according to a strict interpretation of the unitary hypothesis that similar miscoding effects would be detected in dependent strains in those instances where removal of streptomycin causes a preferential inhibition of synthesis of certain enzymes. On the other hand, the miscoding found in SmS strains could be a secondary effect and the inhibition of enzyme synthesis in SmD strains might be due to any of a number of unrelated effects, including catabolite repression.

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inhibition of specific messenger synthesis, or even inability to translate certain codons.

We have investigated the effects of streptomycin deprivation on the synthesis of \( \beta \)-galactosidase and alkaline phosphatase by SmD strains of \( E. \) coli. Our results support the following conclusions: (i) the rates of synthesis of both \( \beta \)-galactosidase and alkaline phosphatase decline preferentially during deprived growth; (ii) catabolite repression accounts for part, but not all, of the inhibition of inducible \( \beta \)-galactosidase synthesis; (iii) the inhibition of \( \beta \)-galactosidase synthesis during deprived growth is not accompanied by the production of altered enzyme.

**Materials and Methods**

**Bacterial strains.** The following strains were employed: \( E. \) coli K-12; CS-4, which is SmD, requires thiamine and uracil, and synthesizes \( \beta \)-galactosidase constitutively \((\text{lacZ}^+\), \text{lacI}^+) ; F, which is an alkaline phosphatase derepressed \((R_F)\) recombinant selected from two independent crosses \((C_{disp}^{0.32} \times \text{lacI}^+) \) and \((\text{lacI}^+)\) and SmD, requires uracil, and is inducible for \( \beta \)-galactosidase \((\text{lacZ}^+\), \text{lacI}^+) ; and \( E. \) coli K-12 HfrG (obtained from Neal Groman and originally from the collection of E. P. Goldschmidt), which is SmR, requires histidine, and is inducible for \( \beta \)-galactosidase \((\text{lacZ}^+\), \text{lacI}^+) . In some experiments, spontaneous T1 phase-resistant mutants of K-12 and F were employed. These are designated K-12/1 and F/1, respectively. The SmD strains are derivatives of strain CS-1 described previously \((28)\).

**Media and growth conditions.** Coli complete (CC) medium consisted of: yeast extract, 1 g; peptone, 5 g; MgSO\(_4\)-7H\(_2\)O, 0.2 g; KH\(_2\)PO\(_4\), 1 g; K\(_2\)HPO\(_4\), 2.1 g; and tap water 1 liter. Coli minimal (CM) medium, the mineral base used for all growth experiments, consisted of: NH\(_4\)Cl, 1.0 g; MgSO\(_4\)-7H\(_2\)O, 0.2 g; KH\(_2\)PO\(_4\), 2.7 g; K\(_2\)HPO\(_4\), 5.2 g; and distilled water, 1 liter. The medium was adjusted to pH 7 and autoclaved. Disodium succinate or glucose was sterilized separately and used at a final concentration of 0.6 or 0.4%, respectively. For growth of exponential cultures CM medium was supplemented with \( 5 \times 10^{-4} \) g of FeSO\(_4\)-7H\(_2\)O per liter. Growth factors were sterilized separately and used where appropriate in the following concentrations: uracil, 50 \( \mu g \)/ml; thiamine HCl, 1 \( \mu g \)/ml; histidine, 20 \( \mu g \)/ml; and streptomycin, 300 \( \mu g \)/ml, unless otherwise specified. A streptomycin concentration of 250 \( \mu g \)/ml was found to be the lowest amount of the antibiotic which allowed optimal growth of the dependent strains employed. In some cases, histidine was replaced by 0.2% Casamino Acids. For the derepressed synthesis of alkaline phosphatase by strain CS-4, low-phosphate CM (LPCM) medium was employed. This medium was similar to CM except that it contained only \( 10^{-1} \) M phosphate and was buffered with 0.1 M tris(hydroxymethyl)aminomethane (Tris)chloride at pH 7.4.

Growth was carried out in Erlenmeyer flasks at 37 C on a reciprocating water-bath shaker. The medium comprised no more than 20% of the flask volume. Culture turbidity was monitored with a Klett-Sum- merson photoelectric colorimeter with a green filter (no. 54). Appropriate corrections were made for nonlinearity of Klett response above a reading of 100. A Klett reading of 100 represented 3.6 \( \times \) 10\(^{6}\) cells/ml during exponential growth.

For the growth of cells in the absence of streptomycin deprivation, exponential-phase cultures were filtered (0.45 \( \mu \)m pore size; Millipore Corp., Bedford, Mass.) and were then washed with prewarmed unsupplemented CM medium; the filters were placed in fresh prewarmed medium without streptomycin. In some cases when the inoculum size was too great to allow efficient filtration, removal of streptomycin for deprivation was achieved by centrifugation and washing.

**Measurement of \(^{14}\)C-phenylalanine uptake.** In some experiments, cellular protein synthesis was monitored by the measurement of uptake of \(^{14}\)C-phenylalanine into trichloroacetic acid-precipitable material. In these instances, the growth medium contained 2 \( \mu g \) of unlabelled phenylalanine per ml plus a negligible quantity of medium-derived \(^{14}\)C-phenylalanine to give a specific activity of 0.0125 or 0.025 \( \mu c/\mu g \). Incorporated radioactivity was determined by removal of 0.5-ml samples into 2 ml of 10% trichloroacetic acid. The samples were then filtered (type B-6 membrane filters; Schleicher & Schuell Co., Keene, N.H.), washed with 5% trichloroacetic acid, and dried (their radioactivity) as determined in a Nuclear-Chicago liquid scintillation system. Under the conditions employed, 0.025 \( \mu c \) of \(^{14}\)C yielded 32,500 counts/min.

**Enzyme assays.** \( \beta \)-Galactosidase was assayed in cell-free extracts in a 2-ml reaction mixture containing 3 \( \times \) 10\(^{-4}\) M \( \alpha \)-nitrophenyl-\( \beta \)-D-galactopyranoside (ONPG), 0.1 M sodium phosphate (pH 7.0), 0.1 M 2-mercaptoethanol (ME), and 10\(^{-4}\) M MgCl\(_2\). After incubation at 28 C, the reaction was stopped with 1 ml of 1 M Na\(_2\)CO\(_3\), and the optical density (OD) was determined at 420 \( \mu m\). One unit of enzyme activity is defined as that amount of \( \beta \)-galactosidase which hydrolyzes 1 \( \mu m\)ole of ONPG in 1 min. Under our assay conditions, 1 \( \mu g/mL \) of \( \alpha \)-nitrophenol has an OD of 0.0311. To assay cellular \( \beta \)-galactosidase, 0.5 ml of a culture deprived of a 0.5 ml or less of a culture was treated with toluene, and the assay was carried out as described above. After the reaction was stopped, the samples were centrifuged before the OD was read.

Alkaline phosphatase was assayed in a 2-ml reaction mixture containing 0.5 ml of toluene-treated cell, 1 ml of 1 M Tris chloride (pH 8.8), and 4 mg of \( \alpha \)-nitrophenyl phosphate disodium tetrahydrate (PNPP). After incubation at 37 C, the reaction was stopped by the addition of 1 ml of 1 M K\(_2\)HPO\(_4\). The mixture was centrifuged and the OD was determined at 420 \( \mu m\). Samples taken from phosphate-containing medium were washed by centrifugation with Tris chloride buffer (0.1 M, pH 7.8) prior to toluene treatment, and the cells were resuspended in the same buffer. One unit of enzyme activity is defined as that amount of alkaline phosphatase which hydrolyzes 1 \( \mu m\)ole of PNPP in 1 min. Under our assay con-
displace the creasing injection into enzyme a contained saline buffer: analysis, the in M

3.2 of The portion (13). to days 7, 14, and 28. Each rabbit received 25,000 of 0.1 M lactose, NaCl, 2 × 10⁻⁴ M MnCl₂, and 0.05 M ME. The cells were broken by passage through a French pressure cell, and the cell debris was removed by centrifugation at 25,000 × g for 40 min. Extracts were kept unfrozen at 0 C.

Anti-β-galactosidase serum (ABG). E. coli K-12 was contained 25 liters of C medium containing 0.5% lactose, and the cells were harvested by centrifugation. The cells (80 g wet weight) were suspended with a Waring Blender in 160 ml of 0.05 M potassium phosphate buffer (pH 7.2) and broken by passage through a French pressure cell. The enzyme was purified according to the method (with minor modifications) of Karlsson et al. (15) and Zabin et al. (31) but was not crystallized. The purified β-galactosidase used for injection into rabbits had a specific activity of about 6 × 10⁶ units/mg of protein. Three male San Juan rabbits (30) received primary subcutaneous injections of 3.2 mg of the antigen protein in Freund’s adjuvant. Each rabbit received subcutaneous booster injections of 0.75 mg of protein, also in Freund’s adjuvant, on days 7, 14, and 35. The rabbits were exsanguinated on day 39, and the sera were pooled and frozen at −15 C.

Serological test for altered β-galactosidase. Increasing amounts of the extract to be tested were added to a series of tubes, each containing a constant amount of ABG (final dilution of ABG was 1:600). A control series of tubes was prepared, each of which contained a 1:600 dilution of normal rabbit serum (NRS) instead of ABG. All dilutions were made in saline buffer: 0.1 M sodium phosphate (pH 7.0), 10⁻⁴ M MgCl₂, 5 × 10⁻⁴ M MnCl₂, 0.02 M ME, and 0.9% NaCl, and the volume in all tubes was brought up to 1.2 ml with this buffer. The tubes were incubated for 1 hr at 37 C, during which time they were shaken twice. Incubation for 4 days at 4 C followed; each tube was shaken twice a day. Finally, the tubes were centrifuged at 2,100 × g for 20 min, and samples of the supernatant fractions were assayed for β-galactosidase activity. The units of enzyme recovered in the supernatant fluid of the ABG tubes were plotted against those recovered in equivalent NRS tubes. The linear portion of the curve resulting from this plot was extrapolated to the abscissa, and this intercept was defined as the equivalence point. By this method of analysis, the presence of immunologically altered enzyme or of cross-reacting material (CRM) should displace the equivalence point toward the ordinate, resulting in an equivalence point of a lower value (13).

Measurement of protein. Protein concentration was determined by a modification of the method of Lowry et al. (17). Crystalline bovine plasma albumin (Armour Pharmaceutical Co., Kankakee, Ill.) was used as a standard.

RESULTS

Enzyme synthesis in deprived cultures. Synthesis of β-galactosidase in inducible and constitutive strains of SmD E. coli grown on succinate in the presence of streptomycin followed kinetics identical to those found in SmS strains grown in the absence of the antibiotic. Similarly, constitutive alkaline phosphatase synthesis was normal in strain F grown with streptomycin.

When streptomycin is removed from growing cultures of SmD strains of E. coli, the bacteria continue to grow exponentially for a short period and then lapse into a long period (16 to 20 hr) of arithmetic growth (deprived growth) during which time the organisms form filaments (28). During deprived growth of strain F/1, inducible β-galactosidase synthesis generally ceased several hours before linear growth stopped (Fig. 1). Moreover, when IPTG was added at various times to cultures of this strain, it was found that the initial rate of induced β-galactosidase synthesis decreased in relation to the elapsed time of deprived growth (Fig. 1). Evidence that streptomycin deprivation leads to inhibition of induction of β-galactosidase has been reported previously (7, 23, 26, 28). The differential rate of alkaline

![Fig. 1. Induction of β-galactosidase during deprived growth of streptomycin-dependent Escherichia coli strain F/1. Symbols: ●, turbidity; ○, β-galactosidase. At 0, 4, and 7 hr (arrows), 2 × 10⁻⁴ M IPTG was added to parallel cultures.](http://jb.asm.org/Downloaded_from http://jb.asm.org)
phosphatase synthesis in the derepressed strain F/I, and of β-galactosidase synthesis in the constitutive strain CS-4, also decreased during deprived growth (Fig. 2).

Differential rate of β-galactosidase synthesis during deprived growth. β-Galactosidase synthesis is notoriously sensitive to catabolite repression under conditions in which synthesis of macro-molecules is inhibited (18, 22). Such conditions exist during deprived growth of SmD bacteria, and Engelberg and Artman (7) have suggested that catabolite repression may be the cause of the decrease in rate of synthesis of β-galactosidase during arithmetical growth.

To examine the significance of catabolite repression relative to the decline in β-galactosidase inducibility during deprived growth, we employed the technique of Nakada and Magasanik (19), in which the differential rate of β-galactosidase synthesis in the absence of a carbon source is compared with the rate in its presence. At various times during deprived growth of F/I in succinate-CM medium, the cultures were filtered free from the carbon source and divided into parallel cultures with and without succinate. Both of these subcultures received 2 x 10^{-7} M IPTG (determined to be saturating under the conditions employed) and 14C-phenylalanine (0.025 μc/μg). Samples were removed at intervals and assayed for β-galactosidase and 14C-phenylalanine uptake as described in Materials and Methods. The differential rates of β-galactosidase synthesis determined from such experiments are plotted in Fig. 3. Inducibility, as measured by these differential rates, decayed exponentially with a half-life of about 2 hr in the presence of succinate and about 6 hr in its absence. We assume that in the absence of a carbon source catabolite repression is negligible; therefore, we attribute the decay observed in the absence of succinate to a second kind of inhibition, specifically associated with streptomycin deprivation. It is evident that, although catabolite repression accounted for a significant part of the inhibition of inducible β-galactosidase, it was not sufficient to explain the total inhibition observed.

Differential rate of alkaline phosphatase synthesis during deprived growth. Because the interpretation of the decay of β-galactosidase synthesis during deprived growth was complicated by catabolite repression, we investigated the preferential inhibition of alkaline phosphatase synthesis in strain CS-4 undergoing deprived growth. Synthesis of the latter enzyme is believed to be insensitive to catabolite repression (Jonathan Gallant, personal communication). At various times during deprived growth of CS-4 in glucose-LPCM medium, the cultures were filtered free from phosphate and resuspended in LPCM without phosphate and containing 14C-phenylalanine (0.0125 μc/μg). Samples were removed at inter-

![Fig. 2. Constitutive enzyme synthesis during growth of streptomycin-dependent Escherichia coli. Concentration of streptomycin prior to its removal was 200 μg/ml for CS-4 and 300 μg/ml for F/I. Dashed line represents theoretical differential rate for either enzyme during balanced growth.](image)
vals and assayed for alkaline phosphatase and 14C-phenylalanine uptake as described in Material
and Methods. The differential rates of alkaline phosphatase synthesis determined from such
experiments are plotted in Fig. 4. It is evident that the differential rate of derepressible synthesis
of alkaline phosphatase, like the inducible synthesis of β-galactosidase, declined during deprived
growth with a half life, in this case, of about 2 hr.

Serological analysis of β-galactosidase. The decay in β-galactosidase synthesis due specifically
to streptomycin deprivation could have been a result of the formation of altered or inactive
enzyme (CRM) caused by a paucity of streptomycin on the dependent ribosomes (G. Darland

Our data from serological titrations of β-galacto-
sidase synthesized under a variety of growth con-
tions indicate that this was not the case. β-Galactosidase from strain F/1 induced after 8 hr of deprived growth had essentially the same
equivalence point as the E. coli K-12 enzyme
(Fig. 5). Data from a number of experiments are summarized in Tables 1 and 2. In no case did the
equivalence points of the β-galactosidases syn-
thesized during deprived growth of either constitutive or inducible SmD strains differ signifi-
cantly from that of the normal K-12 enzyme
(the immunizing antigen).

Equivalence points of β-galactosidase synthe-
sized by either inducible or constitutive SmD strains growing in the presence of streptomycin
also were indistinguishable from that of the K-12
enzyme (Fig. 5; Table 1). A preliminary experi-
ment with partially purified β-galactosidase from
a nondeprived culture of strain F had suggested
that CRM was produced in the presence of streptomycin (experiment 1, Table 1). However,
subsequent experiments with crude extracts failed
to confirm this. We believe that the preliminary
finding was an artifact, possibly due to alteration of the enzyme during purification. The equiva-

cence points of β-galactosidase from nondeprived
SmD strains were independent of the streptomycin
concentration in the growth medium up to
3,000 μg/ml of the antibiotic (Table 1).

To ensure that our serological technique was
capable of detecting significant amounts of CRM,
we have confirmed the result of Bissell (1), who
found that wild-type E. coli B synthesized CRM
or altered β-galactosidase when induced in the
presence of streptomycin or neomycin. We
induced two SmS strains of E. coli K-12 in the
presence of streptomycin and found that the
equivalence points of their β-galactosidases were
significantly lower than those of the same strains
induced in the absence of streptomycin (Fig. 5;
Table 2).
TABLE 1. Serological equivalence points of β-galactosidase formed by streptomycin-dependent strains of Escherichia coli during normal and deprived growth

<table>
<thead>
<tr>
<th>Expt</th>
<th>Time of deprived growth before harvest (hr)</th>
<th>Strains</th>
<th>F or F′/1 (inducible)</th>
<th>CS-4 (constitutive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>800</td>
<td>1,210</td>
<td>1,400</td>
</tr>
<tr>
<td></td>
<td></td>
<td>920</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>830</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>950</td>
<td>1,220</td>
<td>1,030</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1,210</td>
<td>1,140</td>
<td>1,100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,330</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1,260</td>
<td>1,260</td>
<td>1,550</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,625</td>
<td></td>
<td>1,400</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,380</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>1,260</td>
<td>1,110</td>
<td>1,270</td>
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<td>6</td>
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<td>1,500</td>
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<td></td>
<td></td>
<td>1,380</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>1,200</td>
<td></td>
<td>1,040</td>
</tr>
</tbody>
</table>

* Equivalence point is defined in Materials and Methods.

† Inducer (where necessary) was added 6 hr prior to harvest.

‡ Concentration of streptomycin in growth medium was 500 μg/ml for strain F and 400 μg/ml for strain CS-4. The Fβ-galactosidase was partially purified.

§ Culture was grown in 500 μg of streptomycin per ml.

¶ Cultures were grown in 3,000 μg of streptomycin per ml.

‖ Culture was grown in 400 μg of streptomycin per ml.

TABLE 2. Serological equivalence points of β-galactosidase formed by streptomycin-sensitive strains of Escherichia coli induced in the presence and absence of the antibiotic

<table>
<thead>
<tr>
<th>Expt</th>
<th>Conc of streptomycin during induction (μg/ml)</th>
<th>Strains</th>
<th>K-12 or K-12/1</th>
<th>K-12 Hfr G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1,500</td>
<td>1,200</td>
<td>1,300†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,470</td>
<td>1,550</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1,015</td>
<td>1,120</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,440</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>670</td>
<td>600‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>580</td>
<td>1,000</td>
<td></td>
</tr>
</tbody>
</table>

* Inducer added 15 min after streptomycin.

† Graphs of these experiments are depicted in Fig. 5.

Discussion

We have shown that, during growth of SmD E. coli in the absence of Sm, induced and constitutive synthesis of β-galactosidase, as well as derepressed and constitutive synthesis of alkaline phosphatase, are preferentially inhibited relative to growth and protein synthesis. This inhibition was manifested in two ways: (i) the differential rate of enzyme synthesis decreased with time of deprived growth; (ii) enzyme synthesis ceased several hours before growth. Engleberg and Artman (7) also reported a decay in the rate of induced β-galactosidase synthesis during deprived growth of a SmD mutant of E. coli B/r, but could not demonstrate preferential inhibition of synthesis in this strain. The reason for this discrepancy is not clear but may reflect differences between the two strains.

Increasing catabolite repression accounts for much of the decline of inducibility of β-galactosidase. Yet the differential rate of synthesis also declined in deprived cultures induced in the absence of a carbon source. Moreover, synthesis of alkaline phosphatase, an enzyme presumably insensitive to catabolite repression, was also preferentially inhibited during deprived growth. Apparently the preferential decay of synthesis of these two enzymes is a specific consequence of streptomycin deprivation.

What is the cause of the deprivation-specific inhibition of synthesis of some proteins? We can consider two general hypotheses to account for this observation: (i) absence of streptomycin in dependent cells causes inhibition of synthesis of certain messengers; (ii) growth in the absence of streptomycin leads to a defect in the structure of ribosomes, resulting in ambiguity in translation of the genetic code.

The first of these hypotheses is unlikely since the SmD mutation probably occurs in a ribosomal cistron. Moreover, it would be expected according to this hypothesis that recovery after addition of streptomycin to a deprived culture would be
correlated with an increased production of new messenger RNA (mRNA) molecules. On the contrary, after readdition of streptomycin to deprived cells, at a time when the rate of protein synthesis had begun to increase noticeably, no detectable differences could be found, either qualitatively or quantitatively, between mRNA from deprived and recovering cells (A. P. Nygaard and C. R. Spotts, Intern. Congr. Biochem., 6th, 1964).

The second hypothesis leads to the prediction that CRM will be produced in those instances where preferential inhibition of synthesis of active enzymes is observed. This prediction has been tested in the specific case of β-galactosidase. Within the limits of our assay, we have not been able to detect any altered enzyme during either normal or deprived growth. Thus, unlike SmS E. coli in which gross misreading of mRNA occurs in the presence of streptomycin, the SmD mutant examined here appears in vivo to show no infidelity in translation of the code when grown either in the presence or absence of streptomycin.

In any case, gross miscoding would probably lead to a generalized inhibition of protein synthesis rather than the preferential inhibition actually observed.

It is possible, however, that deprived growth causes an alteration in the structure of ribosomes, which results not in gross miscoding but in their inability to recognize one or very few codons. When the critical codon occurs toward the beginning of the message, CRM might not be detected because of premature termination of the growing polypeptide. Preferential inhibition can also be explained by this modified hypothesis. If it is supposed that the critical codon is a relatively infrequent one, and occurs toward the end, or perhaps not at all, in some messages, then the proteins coded for by these messages will escape the inhibition.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Mary Bicknell and Jim Thomsen. We are indebted also to Jonathan Gallant for illuminating discussion and suggestions.

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