Abnormal 30S Ribosomal Subunits Determined by an Ochre Suppressor Mutation in Escherichia coli

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The results demonstrate that an ochre suppressor mutant of Escherichia coli K-12 produces abnormal 30S ribosomes. The production of these abnormal 30S ribosomes is probably a secondary consequence of the suppressor mutation.

Strain 2320(λ)-15B of Escherichia coli produces ribosomes with altered protein composition (7); it has a mutation which causes ochre suppressor activity, a decreased growth rate, and an increased sensitivity to streptomycin (2). Data presented reveal that: (i) strain 15B produces some ribonuclease-sensitive ribosomal subunits which are not present in detectable amounts in its su+ parental strain; (ii) these unusual ribosomal subunits of strain 15B are related to a normal 30S subunit; and (iii) a single mutation causes the ochre suppressor activity, the unusual physiology, and the altered 30S subunits which characterize strain 15B. These results are of interest in light of recent findings which indicate that the altered ribosomal subunits associated with the ochre suppressor are probably secondary effects of the suppressor mutation.

All the strains used in this study were derived from 2320(λ) and were previously described (2). Strain 15B is a phenotypically lac+ revertant of 2320(λ), a strain whose lac– phenotype is due to an ochre mutation in the z gene. The lac+ phenotype of strain 15B is due to an ochre suppressor mutation (su+). Strain 15B-L4 is a lac–(su+) revertant of 15B. Strain 15B-T9 was obtained by P1Kc-mediated transduction (6), with 15B as the donor and 2320(λ) as the recipient, selecting for lac+ (su+) transductants. The transduction to lac+ (su+) occurred with essentially the same frequency as the transduction of a second recipient to trp+ by using the same P1 lysate.

Ribosomes were obtained and treated as follows. Cells were grown, harvested, and sonically treated as previously described (7). Ribosomes were collected and washed as described by Cox, White, and Flaks (1). Ribosomal subunits were separated in linear 5 to 20% sucrose gradients (1). Ribosomal ribonucleic acid (RNA) was extracted with either 1% sodium lauryl sulfate (3) or phenol (8). Pancreatic ribonuclease (Worthington Biochemical Corp., Freehold, N.J.) treatment was done at a final concentration of 1 μg/ml; the mixture of ribonuclease and either ribosomes or ribosomal RNA was immediately analyzed in the analytical ultracentrifuge at 4°C. Ribosomal proteins were prepared (7) and polyacrylamide gel electrophoresis was performed in 8 M urea by use of the acetic acid–β-aminie buffer system at pH 4.5 (4).

Ribosome preparations in 10−4 M Mg++ from strains 2320(λ) and 15B appear different when analyzed in an analytical ultracentrifuge. The ratio of the area under the 30S Schlieren peak to the area under the 50S peak from strain 15B is approximately 70% higher than the corresponding ratio for strain 2320(λ) (Fig. 1a). The “30S” material from strain 15B, when purified in a sucrose gradient, reveals at least two components: 30S particles and slower-moving particles (approximately 28S; Fig. 2a). Treatment of a ribosome preparation from strain 15B with ribonuclease changes the ratio of 30S to 50S particles to a value very similar, if not identical, to that for 2320(λ) (Fig. 1d); a similar treatment does not appreciably affect the corresponding ratio for strain 2320(λ) (Fig. 1c) or the amount of 50S particles of strain 15B (Fig. 1d). Only the “28S” particles are sensitive to ribonuclease (Fig. 2b). These results indicate that ribonuclease-sensitive subunits are present in 15B su+ cells but not in parental su− cells.

The following two observations demonstrate that the “28S” particles are related to the normal 30S particles. The RNA extracted by either sodium lauryl sulfate or phenol from the mixture of “28S” and 30S ribosomes appears as a homogeneous 16S Schlieren peak (Fig. 2d and 2e). The protein extracted from the same mixture and analyzed by polyacrylamide gel electrophoresis gives a band pattern similar to that of protein from the 30S subunits of strain 2320(λ) (Fig.

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The Schlieren patterns of the ribosomal subunits of strains 2320 (A), 15B, and their derivatives. Samples were centrifuged at 4°C at 50,740 rev/min in 12-mm aluminum cells containing Epon double-sector centerpieces. The ribosome suspension, at a concentration of 2.6 to 3.0 mg of protein per ml, was placed in one sector; solvent was placed in the other sector. Two cells containing different samples or different treatments of the same sample were centrifuged simultaneously and photographed by using a 1° wedge window in one of the cells. Protein concentration was estimated by the method of Lowry et al. (5). The numbers associated with each peak in Fig. 1 and 2 represent approximate S_w values, corrected only for the difference in density and viscosity of water between the temperature of the centrifugation and 20°C. The value 0.64 was assumed for the partial specific volume of ribosomes (9). The S values are uncorrected for radial dilution, concentration, Johnston-Ogston effects, or the differences between the physical constants of water and those of the buffers used. The numbers to the right of each schlieren pattern represent the ratio of the area under the "30S" peak to that under the 50S peak. The ratio shown is the mean of the ratios obtained for each of two frames for each centrifugation. The areas were measured with a Compensating Polar Planimeter (model 39231, Gelman Instrument Co., Ann Arbor, Mich.). No account was taken of Johnston-Ogston effects in computing the ratios. (a) Top, 15B; bottom, 2320 (A). (b) Top, 15B; bottom, 15B-T9. (c) Top, 2320 (A); bottom, 15B-L4. (d) Top, 15B without ribonuclease; bottom, 15B with ribonuclease. (e) Top, 2320 (A) without ribonuclease; bottom, 2320 (A) with ribonuclease. (f) Top, 15B-T9 without ribonuclease; bottom, 15B-L4 with ribonuclease. (g) Top, 15B-L4 without ribonuclease; bottom, 15B-L4 with ribonuclease.

Fig. 2. Properties of the "30S" subunits of strains 15B and 15B-T9. (a) Schlieren pattern of the purified "30S" subunits of strain 15B. (b) Schlieren pattern of purified "30S" subunits of strain 15B. Top, without ribonuclease; bottom, with ribonuclease. (c) Schlieren pattern of the purified "30S" subunits of strain 15B-T9. Top, without ribonuclease; bottom, with ribonuclease. (d) Schlieren pattern of ribosomal RNA extracted with phenol from the same purified preparation of "30S" subunits of strain 15B whose pattern is shown in (a); bottom, RNA from a reconstituted mixture of purified 30S and purified 50S subunits of strain 2320 (A). (e) Schlieren pattern of ribosomal RNA extracted with phenol from the same purified preparation of "30S" subunits of strain 15B whose pattern is shown in upper half of (b). All of the RNA peaks in (d) and (e) were missing upon rerunning the samples in the presence of pancreatic ribonuclease. Conditions of centrifugation were the same as those described for Fig. 1, with the exception that the RNA preparations were centrifuged at 20°C. (f) Pattern of ribosomal proteins resolved by disc electrophoresis in polyacrylamide gels. Two samples were run in each of the two gels by using the "split gel" method previously described (4). (1) Left, proteins from purified 30S subunits of strain 2320 (A); right, protein from purified 30S subunits of strain 15B. (2) Left, proteins from purified 50S subunits of strain 2320 (A); right, same as 1, right.
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