Ribosomal Protein Differences in a Strain of *E. coli* Carrying a Suppressor of an Ochre Mutation

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In this paper we show that a strain of *Escherichia coli* harboring a suppressor of an "operator negative" mutation in the lactose (*lac*) operon has a different electrophoretic pattern of ribosomal proteins than that of the non-suppressor parent from which it was derived by single step mutation. It has previously been suggested that the basis of suppression may be the result of altered ribosome structure (1).

Materials and Methods

Escherichia coli K12 strain 2320 (λ) O_2° S^r Su^-, its suppressor derivative 15B O_2° S^r Su^+ and *E. coli* B/5 were used in this investigation. As previously described (3), strain 15B was derived from strain 2320 (λ) by a single mutational step which confers the ability to suppress the O_2° mutation of the *lac* operon and a variety of ochre mutations (2) of the rII region of phage T4. Strain B/5, a phage T1,T5 resistant derivative of strain B, was obtained from Dr. R. S. Edgar.

Cells were grown and harvested, ribosomes prepared and electrophoresis performed as described by Leboy, Cox and Flaks (4) with the following modifications:

1. Strain 15B was grown in small batches which were concentrated at harvest as described below. This was done to circumvent the problem caused by the high rate of reversion of strain 15B to cell types with an increased growth rate. Each batch of 15B was prepared by picking an isolated colony

from a tryptone B1 plate (3) into 1.0 ml of tryptone B1 broth (3) and distributing 0.1 ml of this suspension into each of six 250 ml Erlenmeyer flasks containing 75 ml of LC-broth minus $CaCl_2$ (5). The cultures were grown with shaking at 37°C and harvested by concentrating the contents of two such groups of six flasks to 1-2 X 10^9 cells/ml in 200 ml 0.85% NaCl. Only cultures that gave a 15B phage suppressor pattern (3) and had $\leq$10% fast growing revertants were used to prepare ribosome extracts.

2. Cells were resuspended in 9.0 ml of the standard buffer of Nirenberg and Matthaei (5) and extracts prepared by sonication (MSE ultrasonic disintegrator, Instrumentation Associates, 17 W. 60th Street, New York 23, N. Y.). The duration of sonication was five minutes at 1.6$\pm$ 0.1 amperes with 1 1/2 minute intervals of cooling between each minute of sonication. The temperature rise in one minute of sonication starting from 2.0$\pm$ 1.0°C was 10.0$\pm$ 1.0°C.

At selected steps in the isolation of ribosomal proteins, the concentration of protein was determined by the method of Lowry (7), the concentration of RNA by the orcinol method of Ceriotti (8), and the concentration of total phosphorus by the method described by Spitnik-Elson (9). The analysis of four ribosome extractions showed that about 5% of the phosphorus and about 3% of the RNA in the ribosome preparation remained in the extracted ribosomal protein. These values agree with those reported by Spitnik-Elson (6).

The following experiment was performed five times. Two independently grown and harvested cultures of strain 2320 (λ) and two independently grown and harvested cultures of strain 15B were processed in parallel. After the initial high speed centrifugation, the ribosome fraction and the supernatant obtained from each crude extract were saved. The ribosomes of one 15B crude extract were then sedimented through the supernatant of the 2320 (λ) crude extract and vice versa. As a control, the ribosomes of the other 2320 (λ) crude extract and those of the other 15B crude extract were sedimented through their own supernatants. Following this cycle of centrifugation, the ribosomes were

washed with the buffer of Leboy, Cox and Flaks (4) and further extraction was performed as previously described (4).

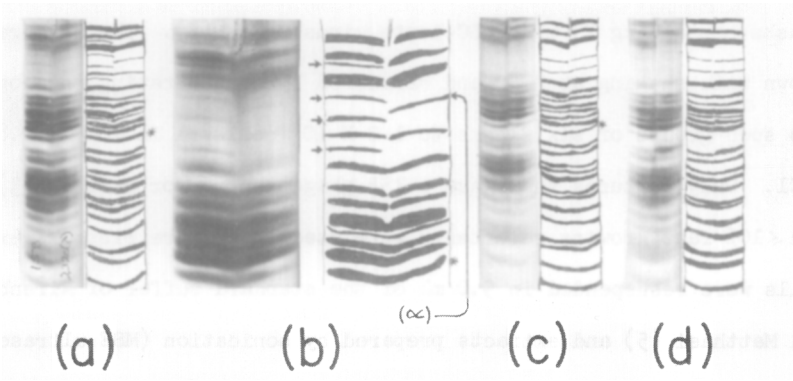

Figure I

Electrophoretic patterns of ribosomal proteins from various strains of E. coli. Two samples were run on the same gel column by the "split gel" technique (4). Each picture is accompanied by its diagrammatical representation; (a) left: 15B; right: 2320 (λ). (b) close up of the upper portion of the gel shown in (a); arrows indicate band differences. (c) left: 2320 (λ), ribosomes sedimented through 15B supernatant; right: 2320 (λ), ribosomes sedimented through their own supernatant (see text). (d) left: 2320 (λ); right: B/5; the "K" character (present in K strains and absent in B strains of E. coli) as previously demonstrated (4), is marked with an * in each gel.

Results

Figures 1a and 1b show the electrophoretic patterns produced by ribosomal proteins of strains 2320 (λ) and 15B as seen in five different experiments. Four relatively faint bands in the 15B pattern are not visible in the 2320 (λ) pattern. In addition, one band of the 2320 (λ) pattern is not visible in the 15B pattern. The electrophoretic pattern of the ribosomal protein of strain 2320 (λ) did not change as a consequence of exposing 2320 (λ) ribosomes to a 15B supernatant (figure 1c); analogously, the electrophoretic pattern of the ribosomal proteins of strain 15B did not change as a consequence of exposing 15B ribosomes to a 2320 (λ) supernatant. As a further control on our technique, the difference in electrophoretic pattern of the ribosomal protein of K12 and B strains (4) were verified by comparing strains 2320 (λ) and B/5 (figure 1d).

Conclusions and Discussion

On the basis of the following characteristics of strain 15B (3), it was concluded that the suppressor in that strain affects the translation of other genetic information in addition to that of the nonsense (0°) codon of the lac operon:

(1) The specific growth rate of 15B is one-half that of 2320 (λ) in tryptone Bl medium at 37°C.

(2) Strain 15B has a reduced resistance to streptomycin in tryptone Bl medium despite the presence of the unaltered allele for resistance to streptomycin.

(3) The pleiotropic effects of strain 15B are not due to a deletion.

(4) The suppressor mutation in strain 15B is not co-transducible with either the 0° mutation or the locus for streptomycin resistance and sensitivity.

The multiple differences in the ribosomal protein electrophoretic pattern of 15B and 2320 (λ) support the conclusion that the suppressor affects the translation of other genetic information in addition to the nonsense codon of the lac operon.

The suppressor in strain 15B could cause ribosomal protein differences by primarily altering either a) a non-ribosomal component of the protein synthetic system necessary for the translation of ribosomal protein M-RNA, or b) a ribosomal component, as a direct consequence of an alteration in a structural gene for ribosomal protein. Whatever cellular component is the suppressor molecule, multiple band differences might be expected as a consequence of errors in the translation of M-RNA for the ribosomal proteins. The following argument favors the idea that the suppressor molecule is an altered ribosomal component. In the cases studied, suppressors of the ochre mutation are known to operate at low efficiency (2). Assuming a low efficiency of suppression by the 15B suppressor, no protein bands should disappear as a consequence of infrequent translation errors caused by the suppressor; i.e., the 15B pattern should contain not only those bands

representing proteins altered by suppression, but also all the bands of the 2320 (λ) pattern. However, one band of the 2320 (λ) pattern is absent (or displaced) in the 15B pattern (band ∝). It follows, therefore, that the absence of a band in the pattern of strain 15B is probably the primary consequence of a mutation in a structural gene for ribosomes.

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Explanation of Symbols

(λ) = lysogenic for phage lambda.

O° = "operator negative" mutation.

S^{r} = resistance to high levels of streptomycin (500 /ml).

Su^{-} = restrictive allele of suppressor gene.

Su^{+} = permissive allele of suppressor gene.

RNA = ribonucleic acid.

M-RNA = messenger ribonucleic acid.