STIMULATION OF ^{14}C-LEUCINE INCORPORATION INTO PROTEIN IN VITRO BY RIBOSOMAL RNA OF ESCHERICHIA COLI

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Among RNAs of E. coli, only RNA fractions to be identified with messenger RNA have so far been known to stimulate the protein synthesis in vitro (cf. Gros et al., 1963). They sediment as a broad 14S peak. Meanwhile, ribosomal RNA has been considered only as a structural component of ribosomal particles, because of its failure to show any 'messenger' activity in vitro after isolation from ribosomes (cf. Gros et al., 1963; Lipmann, 1963; Crick, 1963). However, before we can safely conclude that the ribosomal RNA lacks information for protein synthesis, possible factors should be considered which might inactivate ribosomal RNA, even if the RNA carried genetic codes. One of such factors would possibly be the fairly well-ordered structure of ribosomal RNA (cf. Spirin, 1963) which would prevent its proper attachment on the ribosome in a cell-free system for protein synthesis.

We have recently demonstrated that the ribosomal particles are formed via several intermediate steps starting from a set of free ribosomal RNAs with nearly the same sedimentation coefficients as the mature RNAs (Kono & Osawa, 1964). It has also been shown that the ribosomal RNA which has not yet been incorporated into mature ribosomal particles has a secondary structure distinct from, and probably less ordered than, that of mature ribosomal RNA (Mitsui et al., 1963; Suzuki & Hayashi, 1964). Such a 'premature' ribosomal RNA might

accomodate itself properly on the ribosome and can direct the protein synthesis. In this note, we will report some experimental results which support the above view.

Throughout this paper, RNA to be tested for stimulation of amino acid incorporation into protein was prepared from cells of E. coli B(H) subjected to our 'shift-up' culture conditions (Mitsui et al., 1963). Advantage of using this system lies in the selective supression of messenger RNA synthesis during the first 20 min of the shift-up, starting from the starved cells which have the minimum content of preformed messenger RNA. In order to accumulate the 'premature' ribosomal RNA by preventing the formation of mature ribosomal particles, chloramphenicol (CM) was added at the outset of shift-up culture. RNA was prepared from such cells by the Method II described previously (Mitsui et al., 1963).

Total RNA prepared from cells 10 min after the shift-up or fraction of 16S region (see below) thereof was active for the stimulation of ^{14}C-leucine incorporation into protein in a cell-free system of E. coli which was similar to that of Nierenberg and Matthaei (1961),

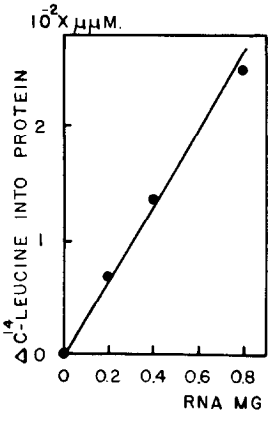

Fig.1. Stimulation of ^{14}C-leucine into protein by E. coli 16S ribosomal RNA fraction

The RNA preparation used was Fr.II described in legend of Fig.3. The reaction mixture contained, in a total volume of 0.25 ml, 0.04 ml of S-30 fraction (Nierenberg and Matthaei,1961), 0.25 mg of E. coli sRNA, 0.62 μmoles of phosphoenol pyruvate, 5 μg of pyruvate kinase, 0.25 μmoles of ATP, 0.062 μmoles of GTP, 0.1 μc of ^{14}C-L-leucine (2.5 x 10^5 cpm), each 0.05 μmoles of 19 amino acids, 86 mM of KCl, 25 mM of Tris buffer (pH 7.8), 12.5 mM of Mg acetate, and 6 mM of mercaptoethanol. Samples incubated at 37 C for 30 min, were deproteinized with hot 5% TCA-0.25% Na tungustate (pH 2). Protein precipitate was washed on a Millipore filter with TCA-tungustate. Radioactivity was measured in Packard Tri-Carb Scintillation Spectrometer.

and Gilbert (1963). Under the conditions employed, the amount of RNA added to the system was proportional to the amount of leucine incorporated (Fig. 1).

In the next experiment, RNA was prepared from the cells 0, 5, 12, 30 and 50 min after the outset of the shift-up in the presence of CM. Each RNA preparation was then fractionated by sucrose density gradient centrifugation. Following measurement of u.v. absorbancy at 260 mμ, four fractions, Fr. I (20S), Fr. II (15-20S), Fr. III (7-15S) and Fr. IV (7S) were separately pooled as indicated in the right side of Fig. 2, and the RNA was precipitated with ethanol. Appropriate amount of RNA for testing amino acid incorporation was usually obtained by 4 to 6 runs of density gradient centrifugation. Stimulation of ^{14}C-leucine into protein was then tested for each fraction, and the results are illustrated in Fig. 2. All RNA fractions from zero time cells had no activity. In the preparations of 5 min and 12 min, about 45% and 35% of the activity were present in Fr. I and Fr. II, respectively. Therefore, 80% of the activity resided in the ribosomal RNA region. The rest of the activity was recovered from Fr. III (messenger RNA region), while no activity was found in Fr. IV. It should be recalled here that at these stages of the shift-up culture, most of the rapidly labelled RNA had a base ratio of ribosomal-RNA type and sedimented at nearly the same velocity as the mature ribosomal RNA (Mitsui et al., 1963). At 30 min, this distribution of activity was changed. The activity of Fr. I and Fr. II decreased, while that of Fr. III increased with appearance of some activity in Fr. IV. At 50 min, the messenger region (Fr. III plus IV) revaled more than half of the activity in the total RNA preparation. At this stage, the labelled RNA showed a DNA-type base ratio, and sedimented predominantly between 4S and 15S (Fig. 3; see also Mitsui et al., 1963).

From the above experiments, it is evident that the ribosomal RNA formed prior to the shift-up culture was inactive, and all the active

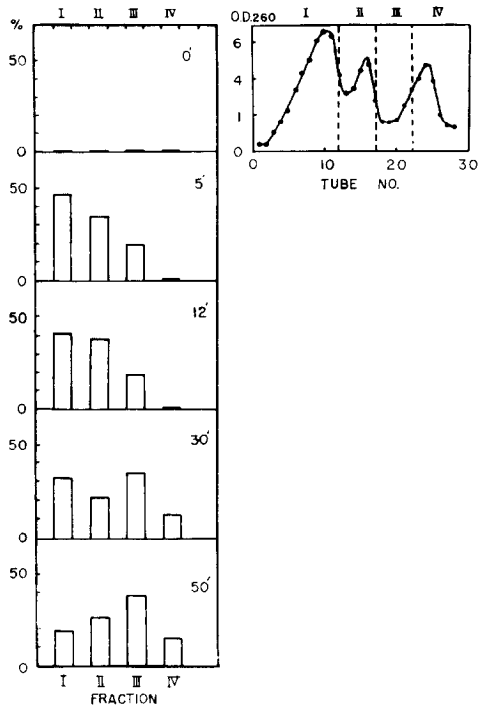

Fig. 2. Distribution of activity to stimulate ^{14}C-leucine incorporation into protein among subfractions of E. coli RNA prepared from cells of shift-up culture containing chloramphenicol

Each three liter portion of shift-up culture containing 200 μg/ml of CM were shaken for the time period indicated. The RNA prepared from the above cells were centrifuged at 25,000 rpm. for 15 hr in a sucrose density gradient (2.5 to 15%) containing 0.1 M NaCl-0.05 M sodium acetate (pH 4.8), and fractions I, II, III and IV were obtained. RNA was precipitated from each fraction with 2 volumes of ethanol, dissolved in 10 mM Tris buffer (pH 7.8), dialysed and tested for its ability to stimulate ^{14}C-leucine incorporation into protein. For the incubation conditions, preparation of radioactive protein and radioactivity measurement, see legend of Fig. 1.

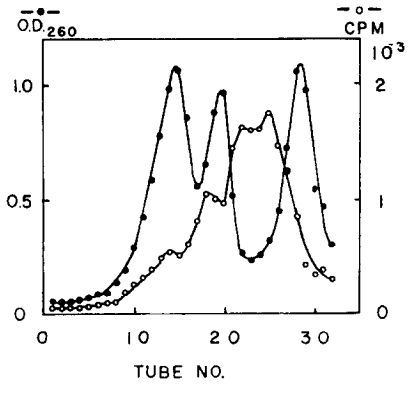

Fig. 3. Sedimentation analysis of RNA labelled for 20 sec with ^{3}H-adenosine 50 min after transfer to the shift-up medium containing chloramphenicol

200 μc of ^{3}H-adenosine were added to 140 ml of shift-up culture containing 200 μg/ml of CM. After 20 sec, cells were chilled and the RNA was prepared. Conditions of centrifugation were the same as in Fig. 2. After measurement of optical density at 260 mμ, an equal vol. of 20% TCA was added and the RNA was collected on a Millipore filter for radioactivity measurement.

RNA was synthesized after the shift-up. Some newly formed RNA in the ribosomal RNA region was active. Since no ribosomal particles were formed in the presence of CM, the activity can not be due to the mature ribosomal RNA derived from ribosome, but rather to the 'premature' ribosomal RNA. The above view was further supported by the following experiment.

From the cells taken 10 min after the shift-up culture in the presence of CM, Fr. I and Fr. II RNA were isolated by 6 runs of sucrose density gradient centrifugation. Fr. I and Fr. II were respectively centrifuged again. 2.3 ml fractions were collected into 12 tubes. Following optical density measurement, the RNA in each tube was precipitated with ethanol, dialyzed and tested for amino acid incorporation into protein. From tube no. 1 to 7 in the case of Fr. I RNA, and no. 1 to 8 in Fr. II RNA, the RNA could be collected quantitatively. No sufficient amount of RNA was collected from no. 8 to 12, and no. 9 to 12 of Fr. I and Fr. II RNA, respectively, and therefore their activity was not tested.

The results illustrated in Fig. 4a and b indicated that the activity curve for amino acid incorporation into protein coincided well with the optical density profile of 16S and 23S ribosomal RNA, suggesting that the active RNA was 'premature' ribosomal RNA rather than messenger RNA. The higher specific activity observed in the right side of the curve may be due to some contamination of messenger RNA. It was estimated that at least 30% of the optically detectable ribosomal RNA in Fig. 4 should be 'premature' ribosomal RNA from the CM-particles (Nomura & Watson, 1959; Kono & Osawa, 1964) plus 'free' ribosomal RNA (Kono & Osawa, 1964). It is at present not as yet decided whether the active RNA was represented by RNA from CM-particles, free RNA, or both.

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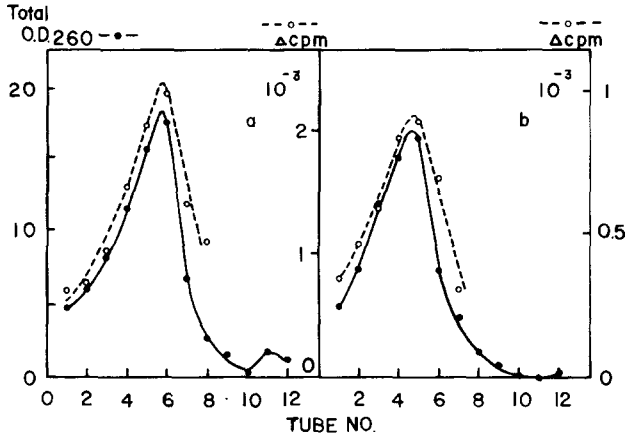

Fig. 4. Distribution of activity to stimulate ^{14}C-leucine incorporation into protein in the 16S and 23S ribosomal RNA region

Six liters of shift-up culture containing 200 μg/ml of CM were shaken for 10 min. Fractions I (23S region) and II (16S region) were obtained by the procedure described in the legend of Fig. 2. Fr. I and II were recentrifuged at 25,000 rpm. for 15 and 20 hr, respectively. 2.3 ml fraction was collected into 12 tubes. After measurement of optical density, RNA in each tube was precipitated with 2 vol. of ethanol, dissolved in 0.5 ml of Tris buffer (pH 7.8) and dialysed. Each RNA preparation was tested for its ability to stimulate amino acid incorporation into protein (see legend of Fig. 1). Total activity to stimulate ^{14}C-leucine incorporation by RNA in each tube was plotted in comparison with the distribution of OD_{260} of 16S (a) and 23S (b) ribosomal RNA.

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