MOLECULAR HETEROGENEITY OF RNA IN RIBOSOMES OF *E. COLI* WITH RESPECT TO TURNOVER RATE IN THE MAXIMUM CONCENTRATION OF THE CELLS.*

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It has been recognized that the turnover of RNA and of protein is more marked in resting than in growing microorganisms (Mandelstam, 1960). It was also shown that the RNA in ribosomes represents a significant part in the turnover (Mandelstam and Halvorson, 1960; Goldstein and Brown, 1960). The present experiment was undertaken to study the turnover of ribonucleic acid in ribosomes with special reference to its heterogeneity in a culture of maximum concentration (M-conc.**) of *E. coli* B, strain F-2 (uracil or cytosine and arginine requiring).

The cells were grown at 37° C for 17 hours in a casamino acids-lactate medium supplemented with uracil (0.1 g per l). As shown in Fig. 1, when harvested at the stationary phase and resuspended in a fresh medium to make 16 times the original population, cells almost ceased to grow, showing a generation time of 10 hours as estimated by viable count. The total amount of RNA degraded by about 10 % during 10 hours' incuba-

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** M-conc., maximum concentration of bacteria defined by Bail (1929).

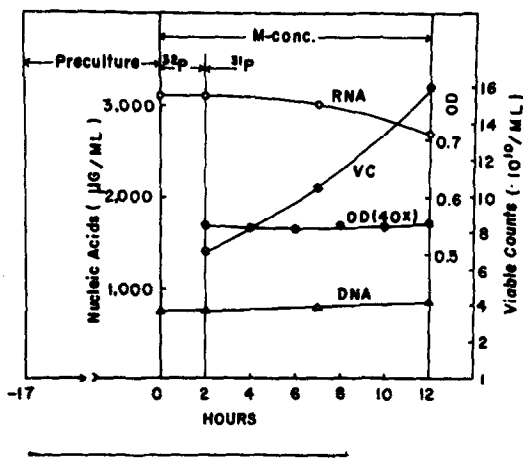

Fig. 1. Degradation of RNA in M-conc. Cells pre-incubated for 17 hours in a medium (Difco casamino acids 10 g, lactate 5 g, tris(hydroxymethyl)amino-methane 7.3 g, uracil 0.1 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, $CaCl_2$ 0.01 g and KH_2PO_4 1.30 g per 1) were made M-conc. in a fresh medium: the amounts of RNA(o—o) and DNA(Δ—Δ), viable counts(⊖—⊖) and cells observed by O.D. at 650 mµ(●—●).

tion, while DNA increased slightly. No net increase of protein was demonstrated during the period studied, whereas a slight synthesis of protein could be detected by experiments in which labeled amino acids were incorporated into the hot trichloro-acetic acid precipitable fraction and also β-galactosidase was induced by the addition of melibiose at any stage of the incubation.

When $^{32}PO_4$ was added at 0 time in the M-conc., it was incorporated almost linearly into the RNA fraction. After 2 hours' uptake of ^{32}P, cells were collected and disintegrated by passing French pressure cell and the RNA was prepared by phenol method from ribosome fraction of the cells (precipitate of centrifuge at 100,000 x g for 90 minutes). In order to examine whether the newly sythesized RNA forms separate molecules distinct from the preexisting ones or merely a terminal extension of them, the RNA extracted was digested with snake venom, resulting in a release of radioactivity and of 260 mµ absorbing material at nearly identical rate (Fig. 2). This shows the former possibility to be the case.

The labeled RNA was then subjected to a swinging bucket sedimentation analysis in a sucrose gradient as described by

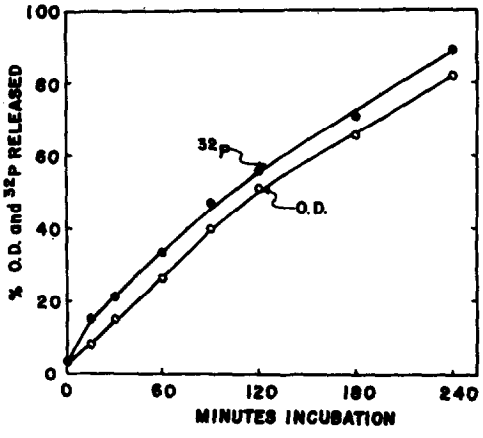

Fig. 2. The enzymatic hydrolysis of ^{32}P-labeled ribosomal RNA with snake venom. Incubation mixture contained labeled RNA (51 O.D. units at 260 mμ and 20,600 c.p.m.), 100 μmoles of glycine buffer of pH 9.4 and 100 μg of crude venom of Trimeresurus flavoviridis, a Crotalidae, in a final volume of 1.5 ml. The reaction mixture was incubated at 37° C. At the time intervals shown, 0.10 ml aliquots were removed and the reaction was stopped by the addition of 0.6 ml of carrier albumin (2.0 mg per ml) to insure complete precipitation and kept in the cold for more than 30 min. The precipitate was removed by centrifugation and appropriate aliquots were taken for radioactivity and UV absorbance determination.

Nomura et al (1961). It is seen from the results given in Fig. 3A that the bulk of RNA forms a major peak with sedimentation coefficient of 16 s with which half of the ^{32}P-RNA associated. the other half associated with the minor peak with sedimentation coefficient of 8 s which is known to exist loosely bound to ribosomes of growing E. coli cells (Nomura, et al., 1960).

Prelabeled cells for 2 hours in M-conc. were transfered to the medium with $^{31}PO_4$ (0.01 M) and incubated for further 10 hours. Ribosomal RNA was then prepared and subjected to the swinging bucket sedimentation analysis in the same way as above. The diagram shown in Fig. 3B clearly indicates that ^{32}P-RNA with sedimentation coefficient of 8 s turned over and disappeared in ^{31}P medium.

From these observations it may be concluded that the newly synthesized RNAs of ribosome in M-conc. form separate molecules distinct from preexisting ones and that the RNA which showed a rapid turnover has a sedimentation coefficient of about 8 s and is considerably smaller than the preexisting RNA.

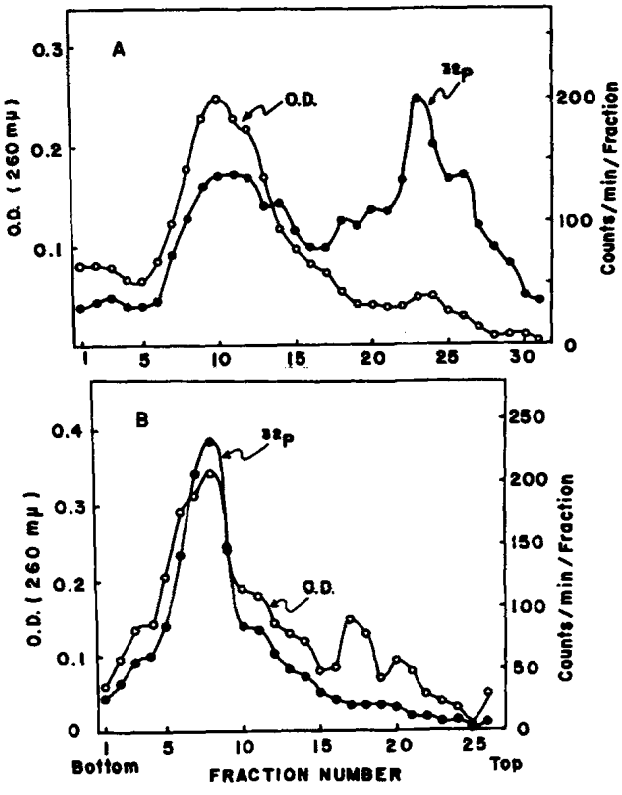

Fig. 3. Sedimentation diagram of ^{32}P-ribosomal RNA.
(A) from the cells, exposed to ^{32}P for 2 hours in M-conc.
(B) from the cells of 12th hour's culture in M-conc.

0.3 ml of RNA solution was layered carefully on 4.0 ml of sucrose solution (concentration gradient from 3 to 20 %), then centrifuged for 6.0 hours at 37,000 rev/min. Fractions were collected by dripping from the lower end of the tube. all solutions contained 0.03 M tris buffer at pH 7.3.

Full accounts of this work will be published elsewhere.

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