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Presence of precursor ribosome in the ribosomal preparation from chloramphenicol-treated *Escherichia coli* AB301/I05 (RNase III –).

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Abstract. On sucrose gradient centrifugation, the ribosomal preparation from chloramphenicol-treated ^{32}P labelled *Escherichia coli* AB301/105 (RNase III$^-$) showed the presence of a radioactive peak moving slower than the 70S ribosome; this peak disappeared on treatment with RNase III. The presence of precursor 30S RNA was shown in such preparations by affinity chromatography on a lysine-sepharose 4B column as well as polyacrylamide gel electrophoresis. Dialysis against low Mg^{2+} concentration followed by sucrose density gradient electrophoresis. Dialysis against dissociation of 70S ribosome into its subunits, did not lead to the dissociation of the precursor ribosome. However, the dissociation took place upon treatment with RNase III. A tentative model of coupled rRNA transcription and ribosome assembly has been presented.

Keyword. Ribosomes; precursor; ribosomal RNA; RNase III; ribosome assembly.

Introduction

It is well established that *Escherichia coli* ribosomal RNAs are transcribed as a single unit (30S RNA) which is subsequently processed by RNase III to precursors of 16S and 5S RNAs (Dunn and Studier, 1973; Nikolaev *et al.,* 1973, 1974; Ginsburg and Steitz, 1975). Duncan and Gorini (1975) showed the presence of a ribo-nucleoprotein particle (46S) in the lysate of *Escherichia coli* AB301/105 (RNaseIII$^-$) and presented some evidence to indicate that this is composed of precursor rRNA (30S) and almost all the ribosomal proteins. Similarly Nikolaev *et al.* (1975) working with lysates of AB301/105 showed that the newly formed 30S preribosomal RNA moved in a complex with proteins as a particle having a sedimentation constant of 53S. A similar particle was also formed from purified RNA and 30S and 50S ribosomal proteins and could be cleaved by RNase III to yield 30S and 48S particles. There are several reports in the literature indicating that the defect in some proteins of one of the two subunits may be reflected in the assembly of the other subunit (Lewandowski and Brownstein, 1969; Kreider and Brownstein, 1971; Geyl *et al.,* 1977). These reports raise the question of whether the assembly of 50S and 30S ribosomes is coupled under *in vivo* conditions. The experiments of Duncan and Gorini (1975)

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have been repeated in this laboratory and further evidences are produced to show that the 46S ribonucleo-protein particle is indeed the precursor of 50S and 30S ribosomes. On the basis of the above, a speculative model regarding the coupled transcription and precursor ribosome assembly is presented.

Materials and methods

Escherichia coli MRE600, a RNase I^- strain was obtained from Dr. U. Maitra of Albert Einstein College of Medicine, Bronx, New York, USA. *Escherichia coli* AB301/105, a RNase I^- and RNase III^- strain, was a generous gift from late Luigi Gorini, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Masschusettes, USA. RNase-free DNase was the product of Worthington Biochemical Corporation, New Jersey, USA. Carrier-free $H_3{}^{32}PO_4$ solution was procured from Bhabha Atomic Research Centre, Bombay. Lysine-sepharose 4B and chloramphenicol were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden and Sigma Chemical Company, St. Louis, Missouri, USA respectively.

Escherichia coli MRE600 (RNase I^-) and *Escherichia coli* AB301/105 were respectively grown in synthetic medium (Datta and Burma, 1972) and enriched medium (Nikolaev *et al.,* 1975). Ribosomes were prepared from MRE600 by ultracentrifugation as described by Datta and Burma (1972). For preparation of ribosomes from the chloramphenicol-treated AB301/105 the cells were grown at 30°C to an absorbancy of 0.4 O.D. at 610 nm in half-diluted Luria broth and the cell pellet was suspended in 0.4 volume of TG medium (0.12M Tris-HCl, pH 7.4, 0.2 mM $CaCl_2$,0.002 mM $FeCl_3$, 0.02 M KCl, 0.08 M NaCl, 0.02 M NH_4C1,0.002 M $MgSO_4$ and 1% glucose) containing chloramphenicol (0.4 mg/ml). For preparation of radioactive ribosomes, carrier-free $H_3{}^{32}PO_4$ solution (40 μCi/ml) was added at this stage. The cell suspension was kept shaking for 90 min at 30°C and then chilled with the simultaneous addition of an equal volume of 0.5M NaCN and 0.075M potassium phosphate buffer pH 7.0. The suspension was centrifuged and the cell pellet was collected and washed with 10 mM tris-HCl buffer pH 7.0. Ribosomes were prepared by grinding the cell pellet for 90 min at 4°C with twice its (wet) weight of alumina (A-305) and small quantities of TMK1 buffer 0.02 M Tris-HCl, pH 7.4, 0.01 M magnesium acetate , 0.03M KCl and 0.2mM mercaptoethanol). Alumina and cell debris were removed by centrifugation at 20,000x *g* for 20 min and the supernatant was treated for 7 min at 30°C with RNase-free DNase (100 μg per 10 gm of cells). The treated extract was re-centrifuged at 106,000 *g* for 3 h at 4°C. The sediment was resuspected in a minimum volume of TMK1 buffer.

For preparation of subunits, the ribosomal preparation was dialysed against low magnesium containing TMK2 buffer (20 mM Tris-HCl, pH 7.4, 0.3 mM magnesium acetate, 0.06M KCl and 1.4 mM mercaptoethanol) for 24 h. The density gradient centrifugation of this preparation on 15-40% sucrose containing TMK2 was carried out for 8 h at 130,000 *g* in the swing-out rotor of a Vac 601 ultra-centrifuge. When dissociation was not desired, the gradient contained TMK1 buffer. The fractions (0.25 ml) were collected and radioactivity was measured by Cerenkov radiation (Clausen, 1968).

Ribosomal RNA (rRNA) was prepared by repeated phenol extraction of the ribosome followed by ethanol precipitation as described earlier (Datta and Burma,

1972). For separation of rRNAs, the affinity chromatography was carried out on a lysine Sepharose 4B column as described by Jones *et al.,* (1976). A column (0.8×10 cm) of lysine-Sepharose was equilibrated with 20 mM Tris-HCl, pH 7.4 containing 10 mM $MgCl_2$. The sample of RNA (usually 20 A_{260} units) was loaded on the column which was eluted with the equilibrating buffer containing NaCl of increasing concentration (a linear gradient of 0.05M to 0.5M). Fractions (1 ml) were collected and their absorbancy (A_{260}) and radioactivity were measured.

RNase III was purified according to the method of Suryanarayana and Burma (1975) with some modification. The treatment of ribosome (25 A_{260} units and 4×10^4 counts/min) or RNA (15 A_{260} units and 3×10^4 counts/min) with 6 units of purified RNase III was done at 37°C for 30 min in a total volume of 0.25 ml containing 40 µmol glycine-KOH, pH 10, 100 µmol of KCl and 5 µmol of magnesium acetate. After incubation, the mixture was cooled to 0°C and then layered on the top of a 15-40% sucrose gradient. The subsequent procedure was the same as described before.

Results

It is clear from the results presented in the figure 1A and B that the absorbancy profiles of the ribosome from *Escherichia coli* AB301/105 treated with chloramphenicol and *Escherichia coli* MRE 600 are similar showing the presence of 70S ribosomes. However, the radioactivity profile shows an extra peak sedimenting at a slower rate in the ribosomal preparation from AB301/105 (figure 1B). This peak disappears when the preparation is treated with RNase III (figure 1C). This peak seems to have a sedimentation rate between 50S and 30S and is presumably due to 46S ribosomes as detected by Duncan and Gorini (1975) in the lysate of AB301 cells.

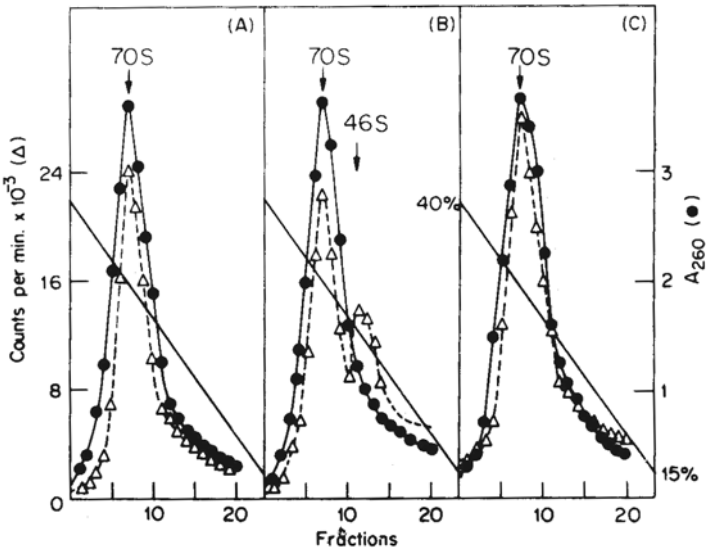

Figure 1. Conversion of precursor ribosome to 70S ribosome by the action of RNase III. ^{32}P labelled ribosomes from *Escherichia coli* MRE600(A) and chloramphenicol-treated *Escherichia coli* AB 301 (B) were isolated and layered on a 15-40%sucrose gradient. The centrifugation was done as described under Materials and Methods. The ribosomal preparation from *Escherichia coli* AB301 treated with RNase III (C) was similarly run. The details have been described under Materials and Methods.

In order to demonstrate the presence of precursor rRNA in such a ribosomal preparation, rRNA was isolated by phenol extraction as described under Materials and Methods. The presence of precursor RNA was checked by two independent methods (i) Polyacrylamide gel electrophoresis and (ii) affinity chromatography on a lysine-Sepharose 4B column. The results obtained with Polyacrylamide gel electrophoretic method are not being presented as the standard patterns reported by others (Dunn and Studier, 1973; Nikolaeve *et al.,* 1973) were obtained with the preparation from the chloramphenicol-treated AB301 cells. Other than 23S, 16S and 5S RNAs, an extra band moving slowest of all, was present. This was absent in the preparation from MRE600 and also disappeared on treatment of the preparation from AB301 with RNase III. Affinity chromatography on lysine-Sepharose 4B column has been introduced comparatively recently as an efficient method for the separation of rRNAs (Jones *et al.,* 1976). The presence of 30S RNA in the rRNA preparation could also be demonstrated by this technique (figure 2). As expected for such a column, 30S RNA is eluted last and clearly separated from 16S and 23S RNAs (figure 2A). Treatment with RNase III leads to a considerable reduction of this peak and peaks corresponding to 10S and 23S appear (figure 2B) indicating that this might be the precursor rRNA. This 30S RNA was isolated and treated with RNase III and it was found that this gives rise to 23S, 16S and 5S RNAs confirming to identity with precursur rRNA (results not presented). The presence of precursor 30S RNA in the ribosomal preparation confirms that it is a component of the ribosome.

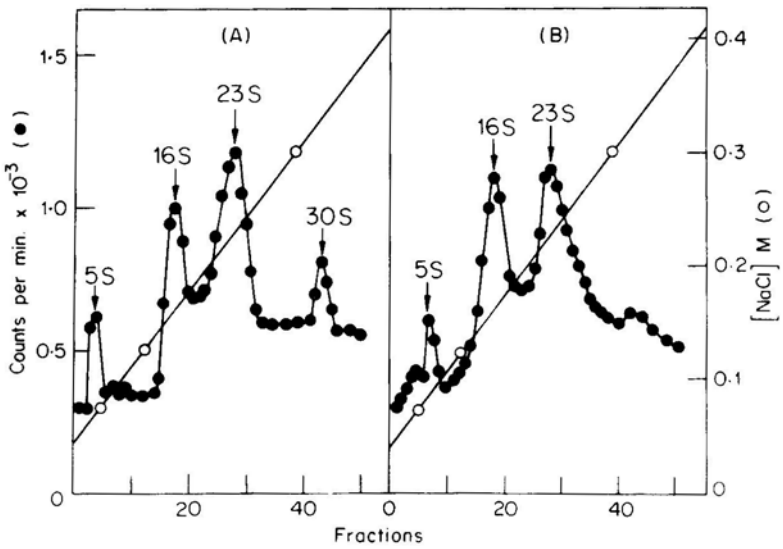

Figure 2. Identification of precursor rRNA present in the ribosomal preparation from the chloramphenicol -treated *Escherichia coli* AB301 cells.
Radioactive rRNAs were isolated from ^{32}P labelled *Escherichia coli* AB301 ribosome and loaded on a lysine-Sepharose 4B column (A) as described under Materials and Methods. The preparation treated with RNase III was similarly chromatographed (B).

If the species of ribosomes constituted of 30S RNA exists, then these are expected not to dissociate into subunits at very low Mg^{2+} concentration, unlike 70S ribosomes. In order to verify this, ribosomes were prepared in the usual way from the chloramphenicol-treated ^{32}P labelled *Escherichia coli* AB301/105 cells as described under Materials and Methods. This preparation was dialysed for 24 h against low Mg^{2+} containing buffer (dissociating buffer) in order to dissociate the subunits. As expected, the 70S ribosomal preparation from MRE600 gives rise to two peaks corresponding to 50S and 30S ribosomes (figure 3A). It is interesting to note that an extra peak (other than 50S and 30S ribosomes) is detectable under conditions causing subunit dissociation in the preparation from *Escherichia coli* AB301/105. The position of this peak is between 50S and 30S ribosomes and coincides with the precursor ribosomal peak as detected earlier (figure 1B). Thus even under dissociating conditions the precursor ribosome does not dissociate into 50S and 30S ribosome; this is to be expected if they are assembled on a single chain of RNA.

In order to verify whether the extra ribosomal peak is really the precursor of 50S and 30S ribosomes, the ribosomal preparation from chloramphenicol-treated *Escherichia coli* AB301 cells was treated with RNase III and then subjected to extensive dialysis under dissociating conditions followed by sucrose gradient centrifugation. The middle peak as obtained earlier (figure 3B) disappears and only two peaks corresponding to 50S and 30S ribosomes are obtained (figure 3C). It is quite clear that the precursor ribosome dissociated into 50S and 30S ribosomes following treatment with RNase III. This experiment strongly advocates the existence of a precursor ribosome and its conversion to 50S and 30S ribosomes.

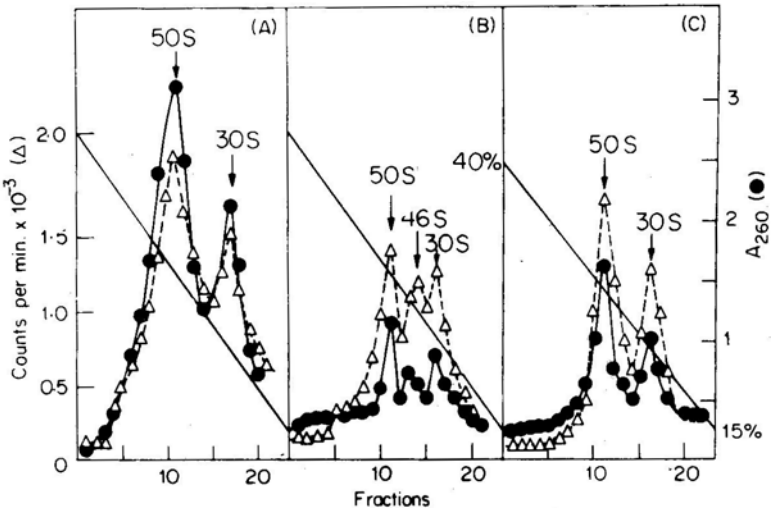

Figure 3. Demonstration of the integrity or the precursor ribosome under subunit dissociating conditions.

P^{32}-labeled ribosomes from *Escherichia coli* MRE600 (A) as well as *Escherichia coli* AB301 (B) were separately dialysed against 0.3mM Mg^{2+} containing TMK2 buffer and loaded on 15-40% sucrose gradients. *Escherichia coli* AB301 ribosomal preparation was also treated with RNase III and then dialysed (C) as described under Materials and Methods. The remaining procedure was the same as described earlier.

Discussion

As already mentioned, Duncan and Gorini (1975) produced evidence to indicate the presence of a ribosomal population which has a sedimentation constant of 46S and is constituted of precursor 30S RNA and almost all the ribosomal proteins, in chloramphenicol-treated *Escherichia coli* AB301/105 Nikolaev *et al.* (1975), on the other hand, showed the movement of precursor 30S RNA into a ribonucleoprotein particle with a sedimentation constant of 53S. They were also able to reconstitute the 53S particles from 30S RNA and ribosomal proteins (Nikolaev and Schlessinger, 1974). The present investigations produce further evidence to indicate the existence of precursor ribomes in the population of ribosomes isolated from the cloramphenicol-treated *Escherichia coli* AB301/105 cells. Not only does the precursor ribosome disappear on treatment with RNase III as observed by others (Duncan and Gorini, 1975; Nikolaev *et al.,* 1975) but also such a ribosomal population does not dissociate into subunits until and unless it is treated with RNase III. This is a direct demonstration of the existence of the two subunits in a linked form. Incidentally, affinity chromatography on a lysine-Sepharose 4B column has been shown here to be a very useful method like sucrose density gradient centrifugation and Polyacrylamide gel electrophoresis (Ginsburg and Steitz, 1975; Schlessinger *et al.,* 1974) in the detection of precursor RNA.

It has not yet been possible to demonstrate the presence of such ribosomal populations in normal cells and therefore it may be argued that such ribosomal assembly takes place under unphysiological conditions (chloramphenicol treatment of RNase III$^-$cells) when precursor RNA (30S) accumulates. However, the observation that the assembly of 50S ribosome is interfered with, due to the mutation of a protein in the 30S subunit is a highly corroborating fact. For example, it is known that a 30S r-protein is altered in the spectinomycin-resistant mutant spec-49, an assembly defective mutant, but in this mutant the assembly of the 50S particle is also defective (Nashimoto and Nomura, 1970). The S2 and S3 supressor mutations supressed the defect in 50S assembly in spec-49 (Nashimoto and Uchida, 1975). The assembly mutants with altered S4 (Lewandowski and Brownstein, 1969; Kreider and Brownstein, 1971), S8(Geyl *et al.,* 1977) and S20 (Wittmann *et al.,* 1974; *Bock et al.,* 1974) also seemed to have defective 50S assembly. Furthermore, the cold sensitivity of the S8 mutant apparently could be supressed by mutations in a gene for a 50S r-protein, L30 (Geyl *et al.,* 1977). These observations suggest that 50S assembly may be coupled to 30S assembly. However, there are two assembly mutants that appeared to have a defect in 30S assembly only (Rosset *et al.,* 1971; Nomura, *et al.,* 1977). Thus it is still not clear whether there is any obligate coupling of 50S and 30S assembly *in vivo* (Nomura *et al.,* 1977). If it is assumed that the ribosomal assembly starts along with the transcription of 30S RNA it is most likely that the assembly of the small subunit will precede that of 50S, 16S rRNA being located in the 5′-end and a defect in 30S subunit may be reflected in the assembly of 50S subunit which is expected to assemble later (figure 4). It is, however, difficult to explain by such assumption, the results obtained with *erythro* 8 mutant which modifies the large subunit but blocks the assembly of both subunits (Pardo and Rosset, 1977). However, *in vitro.* transcriptionally-coupled assembly of *Escherichia coli* ribosomal subunits

has been recently demosntrated (De Narvaez and Schaup, 1979). The coupling of the transcription of rRNA and the assembly of ribosomal proteins will naturally be to the advantage of the cell.

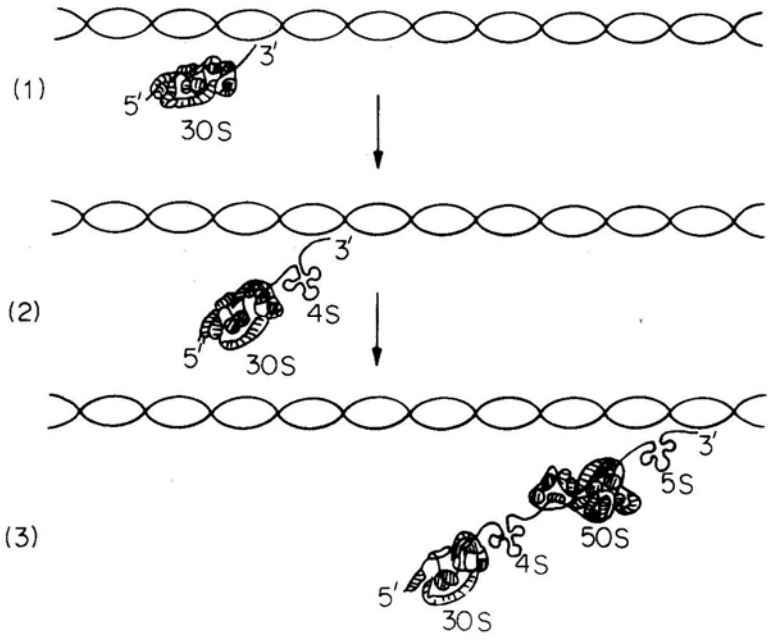

Figure 4. Model of coupled transcription and ribosome assembly. The details have been discussed in the text.

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