IMMATURE 50 S SUBUNITS IN *ESCHERICHIA COLI* POLYRIBOSOMES

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1. Introduction

The assembly of bacterial ribosomes is followed by a maturation process whose significance and timing are still uncertain [1]. We have shown that newly-formed 30 S subunits can enter polyribosomes while still containing a precursor form of RNA [2]. Here we report that 50 S subunits entering polyribosomes for the first time can be distinguished from mature particles both for their sedimentation properties and their susceptibility to RNase attack. Thus maturation of both ribosomal particles may occur after they have joined messenger RNA.

2. Materials and methods

[^{14}C]Uracil (61 mCi/mmol), [^{3}H]uridine (29 Ci/mmol) and [^{3}H]oleic acid (2 Ci/mmol) were purchased from Amersham. Ribonuclease A was from Sigma.

Escherichia coli D10 cells were grown at 37°C in medium MS9 [3] supplemented with 0.5% casamino acids and 0.2% glucose (doubling time 40 min). They were harvested by adding 0.5 mg/ml chloramphenicol and pouring the culture immediately onto crushed ice. Cells collected by centrifugation were lysed by the method in [4], in the presence of 0.2 mg/ml chloramphenicol. Polyribosomes and ribosomal subunits were isolated as described in figure legends.

3. Results and discussion

Polyribosomes were isolated by zonal sedimentation from lysates of *E. coli* D10 cells as described in fig.1. For the purpose of our analysis it was important to exclude the fact that ribosomal subunits not belonging to polyribosomes moved into the polyribosomal region of the gradient because of some artifact, such as non-specific aggregation or binding to membrane fragments. To exclude these two possibilities we grew cells for two generations in the presence of [^{14}C]uracil to label polyribosomes, and in the presence of [^{3}H]oleic acid to label membranes [5]. As shown in fig.1, no ^{3}H-label, and therefore no membrane fragment, moved into the polyribosomal region of the gradient. On the other hand, polyribosomes were completely converted into 70 S monosomes by a mild treatment with RNase, suggesting that they were not contaminated by non-specific ribosomal aggregates.

In line with this evidence is the kinetics of entry of newly-formed ribosomes into polyribosomes. To label new ribosomes, cells pre-labelled with [^{14}C]uracil received a 1 min pulse of [^{3}H]uridine, followed by several minutes chase with cold uridine. Ribosomal subunits derived from polyribosomes were displayed on sucrose gradients as shown in fig.2. No ^{3}H-labelled 50 S particles were found in polyribosomes 2.5 min after the addition of [^{3}H]uridine. At this time the whole lysate already contained a large amount of ^{3}H-labelled 50 S precursor particles (data not shown).

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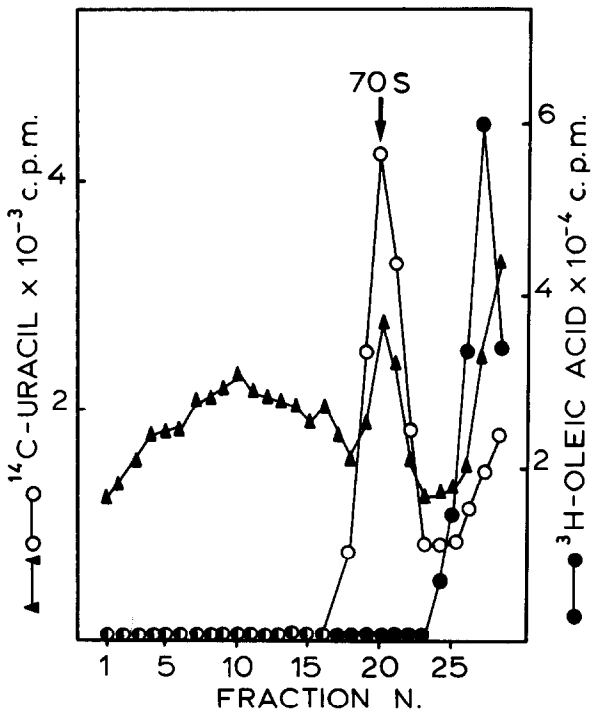

Fig.1. Isolation of polyribosomes. A 20 ml culture was labelled for 2 generations with 0.1 μCi [^{14}C]uracil and 25 μCi [^{3}H]oleic acid. Cells were harvested and lysed as in section 2. The lysate was centrifuged through a 10–30% sucrose gradient in 10^{-2} M $MgCl_2$, 10^{-2} M Tris–HCl, pH 7.6 and 3 X 10^{-2} M NaCl, at 40 000 rev./min for 50 min at 4°C in an International SB283 rotor. Fractions collected through a polystaltic pump were counted directly in Bray solution ((▲——▲) [^{14}C]uracil; (●——●) [^{3}H]oleic acid). An aliquot of the same lysate was incubated at 37°C for 10 min in the presence of 0.01 μg pancreatic RNase A before being centrifuged through a sucrose gradient under the same conditions as above. Only the [^{14}C]uracil pattern (○——○) is shown for this gradient. The symbol (◉) has been used where an open circle coincides with a closed circle. The direction of sedimentation is from the right to the left.

The first ^{3}H-labelled 50 S subunits appeared in polyribosomes after 3–3.5 min labelling. Their rate of sedimentation was slightly, but reproducibly lower than that of mature ^{14}C-labelled particles (fig.2a). The difference in the sedimentation profile was not due to the presence of ^{3}H-labelled mRNA sedimenting on the light side of 50 S particles, since no counts hybridizable as mRNA were found in this region of the gradient (data not shown).

The ^{3}H and ^{14}C sedimentation profiles were affected in a very different way by preincubating the same preparation of derived subunits with an appropriate amount of RNase. As shown in fig.2b,

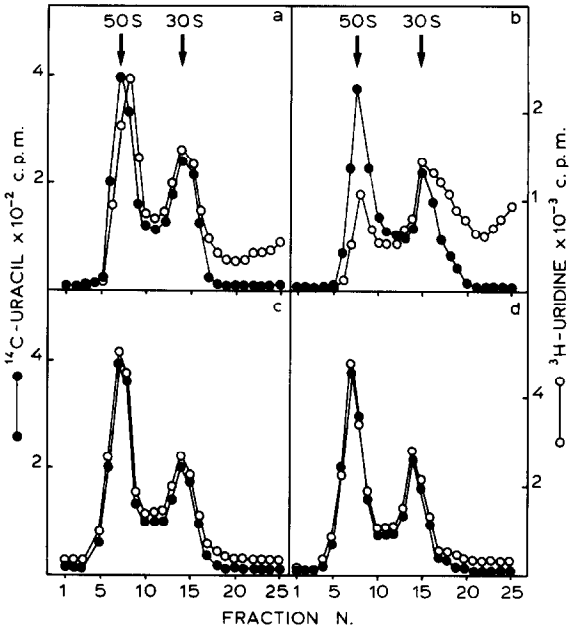

Fig.2. Properties of newly-formed subunits derived from polyribosomes. Polyribosomes were prepared as described in fig.1 from 2 cell cultures which had been labelled for 2 generations with 6 μCi [^{14}C]uracil. One received 0.4 mCi [^{3}H]uridine 3.5 min (panels a, b), the other 5 min (panels c, d) before harvesting. To both cultures 0.2 mg/ml cold uridine was added 1 min after the ^{3}H-label. Fractions 2–15 of the 2 preparative gradients were pooled. Polyribosomes were precipitated by adding polyethylene glycol (100 mg/ml) [6] and collected by centrifugation at 100 000 X *g* for 20 min. The pellets were resuspended and dialysed for 5 h against buffer A, containing 2 X 10^{-4} M $MgCl_2$ and 10^{-2} M Tris–HCl, pH 7.6 to dissociate ribosomal subunits. To remove most mRNA, the dialysed preparations were layered on top of 5–20% sucrose gradients made in buffer A and stratified over a 0.5 ml cushion of 1.6 M sucrose. The tubes were centrifuged long enough (7 h at 40 000 rev./min) to allow ribosomal particles (but not mRNA) to reach the sucrose cushion at the bottom of the tubes. The bottom 1 ml sucrose solution was collected from each tube and dialysed for 3 h against buffer A. The sedimentation profile of the particles thus derived from polyribosomes was analysed through a final sucrose gradient (5–20% in buffer A, 3 h run) both directly (panels a, c) or after exposure to 5 ng pancreatic ribonuclease for 10 min at 37°C (panels b, d).

^{14}C-labelled 50 S subunits remained intact, while the ^{3}H-labelled ones were largely destroyed.

Maturation of 50 S particles, judged on the basis of their sedimentation properties and RNase sensitivity, must follow shortly after the entry of the particles into polyribosomes. The ^{3}H and ^{14}C profiles of 50 S subunits derived from polyribosomes obtained from cells harvested 5 min after the addition of the label were perfectly superimposable, both before and after RNase treatment (fig.2c,d).

Results similar to those reported in fig.2a–d were obtained with ribosomal subunits derived not directly from polyribosomes, but from 70 S monosomes isolated from polyribosomes fragmented by mechanical shearing.

From this data we conclude that at least some steps in the maturation of ribosomal subunits occur after they have entered polyribosomes. This opens the possibility that these steps are not a prerequisite, but rather a consequence of the functioning of newly formed ribosomes in protein synthesis.

Acknowledgements

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