A 30S Precursor of 30S Ribosomes in a Mutant of *Escherichia coli*

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Escherichia coli 15–28, a mutant with a defect in ribosome metabolism, accumulates a ribonucleoprotein particle that is indistinguishable from 30S subunits by sedimentation but contains the precursor form of 16S RNA. This particle is probably a precursor of 30S ribosomes.

Precursors of 30S ribosomes in *Escherichia coli* that sediment more slowly than completed subunits have been detected by pulse-labelling of growing cultures (Mangiarotti *et al.*, 1968; Osawa *et al.*, 1969). Similar if not identical species accumulate during inhibition of growth by antibiotics and other agents (Osawa *et al.*, 1969; Blundell & Wild, 1969b), or when appropriate conditional mutants are transferred to their restrictive temperatures (Nashimoto & Nomura, 1970; Rosset *et al.*, 1971). These '26S' precursors can be distinguished not only by their sedimentation properties but also because they contain '17S' RNA, which is up to 10% longer than the mature 16S RNA in completed subunits (Hayes & Hayes, 1971). The maturation of 17S RNA has therefore been thought to be associated with the conversion of 26S particles into 30S ribosomes. However, Lindahl (1973, 1975) suggested that in exponentially growing *E. coli* B (AS19) there are two species of precursor to 30S ribosomes. One intermediate is indistinguishable in sedimentation properties from completed subunits but contains 17S RNA. Mangiarotti *et al.* (1974) also detected, by pulse-labelling, 17S RNA in both native 30S ribosomes and polyribosomes of *E. coli* D10. Because only about 2% of the total 16S-type RNA is in the two precursors (Lindahl, 1975), their characterization, particularly under conditions uncomplicated by the kinetics of pulse-labelling of RNA or protein, presents considerable difficulties. In the present paper we show that during exponential growth about 10% of the total ribosome complement of *E. coli* strain 15–28 (MacDonald *et al.*, 1967) is 30S material containing 17S RNA. If, as seems likely, this component is a 30S ribosome precursor similar to that described by Lindahl (1973, 1975), strain 15–28 has considerable practical advantages for the further study of the assembly of 30S ribosomes.

Experimental

Bacterial strains used were *E. coli* 15 *thy⁻pro⁻* (15TP) and the mutant 15–28 derived from it. Growth and harvesting of bacteria, preparation of extracts and sedimentation methods have been described previously (Blundell & Wild, 1969a,b). The two buffers (pH 7.4) used to make extracts and for sucrose gradients were (i) 10 mM-Tris-HCl-10 mM-magnesium acetate-100 mM-KCl (THMK buffer) and (ii) 10 mM-Tris-HCl-0.1 mM-magnesium acetate (TM buffer). 'Chloramphenicol particles' were isolated from extracts made from *E. coli* strain 15TP that had been grown for 35 min in the presence of 100 µg of chloramphenicol/ml (added to the culture at E_{450} 0.4); the extracts were prepared in TM buffer and centrifuged through sucrose density gradients in this buffer. Under these conditions 'chloramphenicol particles' have sedimentation coefficients of about 18S and 25S. For examination of RNA, ribonucleoprotein particles isolated from sucrose density gradients were precipitated with 2 vol. of ice-cold ethanol and redissolved in 10 mM-Tris-HCl buffer, pH 7.4, containing 10 mM-magnesium acetate and 0.25% (w/v) sodium dodecyl sulphate. RNA was extracted with phenol (Kurland, 1960), precipitated with ethanol, and redissolved at 1 mg/ml (E_{260} 24) in 50 mM-NaCl containing 2 mM-EDTA (disodium salt). Electrophoresis was in polyacrylamide-gel slabs made with a 2–7% (w/v) gradient of acrylamide dissolved in Tris-borate electrophoresis buffer (Peacock & Dingman, 1967) and containing *NN'*-methylenebisacrylamide at 5% of the acrylamide concentration. The apparatus of Margolis & Kenrick (1968) was used. Gels were pre-run for 30 min at 10 V/cm and slots then loaded with 5 µg of RNA. Electrophoresis was at 5°C and 10 V/cm for 8 h. Gels were stained with 0.2% Methylene Blue (Peacock & Dingman, 1967) and de-stained electrophoretically. ^{32} P radioactivity in gel slices was measured by Čerenkov radiation in 10 ml of water.

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Fig. 1. Sedimentation profiles of cell-free extracts from *E. coli* strains 15TP and 15-28

Cultures (50 ml) were grown in minimal glucose medium supplemented with 0.1 mg of casamino acids (casein hydrolysate)/ml and 10 μ g of thymine/ml. At E_{450} 0.03, strain 15-28 was labelled with [^{14}C]uracil (20 nCi/5 μ g per ml) and strain 15TP with [^3H]uracil (200 nCi/5 μ g per ml). At E_{450} 0.3, the cells were harvested, and extracts were made by using an Aminco French pressure cell and centrifuged through sucrose density gradients in THMK buffer. Sedimentation profiles are superimposed. ●, ^{14}C (strain 15-28); ○, ^3H (strain 15TP).

Results and discussion

Fig. 1 shows the sedimentation profiles of material from *E. coli* strains 15TP and 15-28 in THMK buffer. The extract of strain 15-28 is characterized by an excess of tRNA and the presence of '47S particles'; these latter are precursors to 50S ribosomes (F. Markey & D. G. Wild, unpublished work). The large (by comparison with strain 15TP) symmetrical peak of native 30S ribosomes of strain 15-28 represents about 30% of the total ribosome complement of this strain. The apparent homogeneity of the 30S particles in strain 15-28 was also observed during sedimentation in buffers containing 2 mM- or 6 mM- Mg^{2+} . The 30S ribosomes of the two strains were not distinguishable by their sedimentation properties.

Electrophoretic analysis of RNA from the native 30S component of strain 15-28 is illustrated in Fig. 2(a), together with that of RNA from the 70S ribosomes of strain 15-28 and 'chloramphenicol particles' (from strain 15TP). The latter contain predominantly precursor forms of rRNA (Hecht & Woese, 1969); in our experiments, the particles were

not completely separated from 30S ribosomes, so that some 16S RNA is also present. Native 30S ribosomes from strain 15-28 contain both 16S RNA and a component indistinguishable from the 17S RNA of 'chloramphenicol particles': the intensity of staining of the bands suggested that the latter is about 25% of the total RNA. The 70S ribosomes of strain 15-28 contain no 17S RNA.

We have found (F. Markey & D. G. Wild, unpublished work) that the native 30S particles of strain 15-28 are not completely stable *in vivo*. After a culture of strain 15-28 had been labelled with [^{14}C]uracil for six generations, the radioactive uracil

Fig. 2. Analysis of RNA in polyacrylamide-gradient gels

(a) RNA was extracted from (i) 'chloramphenicol particles', (ii) native 30S particles of strain 15-28 and (iii) 70S ribosomes of strain 15-28 and analysed by electrophoresis in 2-7% polyacrylamide gels. The drawing of the stained gel is based on three separate experiments: in each case, the distance from the origin to the 16S RNA band was about 3.5 cm. For (b) and (c), a culture of strain 15-28 was grown for five generations in medium containing [^{32}P]phosphate (4 μCi /1.3 mg per ml). The cells were then resuspended in non-radioactive medium; extracts, prepared at intervals, were centrifuged through sucrose gradients in THMK buffer to obtain native 30S ribosomes. RNA extracted from these latter was electrophoresed; the gels were sliced and the radioactivity of slices was measured. The regions of the gels containing 16S and 17S RNA (located by staining) are shown. (b) Sample taken immediately after resuspension. (c) Sample taken after 1.5 generations.

was removed from the medium and growth allowed to continue. Analysis of the redistribution of ^{14}C radioactivity in extracts made in THMK buffer showed the precursor status of 47S particles, but also revealed that radioactivity in native 30S ribosomes decreased by about 25% during growth for one generation. This would be expected if the mutant, contained appreciable amounts of the '32S' precursor to 50S ribosomes, but this explanation is precluded by the absence of 23S RNA from the 30S material (Fig. 2a). In another experiment (Figs. 2b and 2c) strain 15-28 was grown with [^{32}P]phosphate for five generations and then transferred to non-radioactive medium. RNA isolated from the native 30S particles 0, 0.5 and 1.5 generations after the transfer was analysed by electrophoresis and ^{32}P radioactivity in gel slices was measured. At zero time 30% of the radioactivity was in 17S RNA; at 0.5 generations the value was 10%. No radioactivity was detected in the 17S-RNA band after 1.5 generations. In each sample the ratio of 17S to 16S RNA (about 0.5, estimated from the intensity of staining) was unaltered. The losses of radioactivity from the 30S fraction and 17S RNA during continued growth strongly suggest that the native 30S ribosomes of strain 15-28 are a mixture of 'genuine' ribosomes together with about 30% of a precursor that contains 17S RNA but is not resolved from the mature ribosomes by sedimentation analysis.

The protein content of 30S precursor particles is not known; it is possible that trimming of 17S RNA may not occur until a 30S ribosome is otherwise completed, or even until newly formed ribosomes participate in protein synthesis. The high

content of native 30S ribosomes and a precursor to them in strain 15-28 may be a direct consequence of the genetic lesion or may be associated with the delayed maturation of 50S ribosomes in this mutant.

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