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An Unusual Precursor of 50S Ribosomes in a Mutant of *Escherichia coli*

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Escherichia coli strain 15–28 is a mutant with a defect in ribosome synthesis that leads to the accumulation of large amounts of ribonucleoprotein ('47S') particles during exponential growth. These particles are precursors to 50S ribosomes, but are distinct from precursors detected by pulse-labelling of the parent strain and also from ribosome precursors that accumulate during inhibition of growth by $CoCl_2$. Either ribosome assembly in the mutant differs from that in the wild-type strain, or 47S particles represent a hitherto unstudied stage in the synthesis of 50S ribosomes.

Pulse-labelling techniques have been used extensively to study ribosome biosynthesis in exponentially growing cultures of *Escherichia coli*. These procedures detect two (Nomura, 1970) or three (Lindahl, 1975) stages in the formation of 50S ribosomes. However, the precursors are normally present in such small absolute amounts that their isolation for detailed study presents considerable technical difficulties. Inhibition of growth by selective means can transiently increase the concentration of ribosome precursors (Osawa *et al.*, 1969; Blundell & Wild, 1969*b*). Larger quantities of material are then available, but ribosome assembly is being studied under abnormal conditions.

Ribonucleoprotein particles distinct from ribosomes are also found in large amounts in some mutant strains with defects in ribosome formation (MacDonald *et al.*, 1967; Lewandowski & Brownstein, 1969; Lopez-Revilla & Bastarrachea, 1971; Nashimoto *et al.*, 1971; Buckel *et al.*, 1972). The mutations involved are either conditional, so that particles accumulate under restrictive conditions, or 'leaky', in which case particles are present during exponential growth. Although the relationship of such particles to the ribosome precursors in wild-type cells is often unclear, the use of mutants has the advantage that particles are more readily available for characterization. Moreover, in leaky mutants, no interference with metabolism (other than that originating from the genetic lesion) is required to produce the particles.

In the present paper we describe an unusual component present in a mutant of *E. coli* that has a defect in ribosome metabolism (MacDonald *et al.*, 1967). Extracts prepared from exponentially growing cells of this strain contain large amounts of a ribonucleoprotein particle that is a precursor to 50S ribosomes, but is distinct from precursors detected by pulse-labelling of the parent strain and also from at least one type of inhibitor-induced particle.

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Experimental

Growth and harvesting of bacteria

Bacterial strains were *E. coli* 15 *thy⁻pro⁻* (15TP) and the mutant 15–28 derived from it (MacDonald *et al.*, 1967). *E. coli* strain M.R.E. 600 was also used. Cultures were grown in a shaking incubator at 37°C in medium (Blundell & Wild, 1969*a*) containing 0.2mg of glucose/ml and sometimes supplemented with 0.1mg of vitamin-free casamino acids (Difco Laboratories, West Molesey, Surrey, U.K.)/ml. Thymine (10μg/ml) and proline (50μg/ml) were added as necessary. For most experiments, an overnight culture (2ml) was used to inoculate fresh medium to $E_{450} = 0.01$–0.03. For continuous labelling, radiochemicals (see the Figure legends) were then added, and growth was continued to $E_{450} = 0.3$–0.5.

In medium containing casamino acids, the generation time of strain 15TP was 35min; that of strain 15–28 varied between 75 and 95min. Faster growing variants are sometimes produced by strain 15–28; each culture was therefore started with a single colony from a nutrient-agar plate. Cultures with a generation time of less than 75min were discarded.

Growth was stopped by pouring cultures on crushed ice. Cells were collected by centrifuging at 0°C, resuspended in buffer (see below) containing 10μg of deoxyribonuclease/ml and broken in a chilled Aminco–French pressure cell operated at about 44MPa (450kg/cm^2). Unbroken bacteria and

debris were removed by centrifuging at 40000*g* for 10min. For some experiments, cells were lysed as described by Blundell & Wild (1969*b*); such lysates were not clarified.

Centrifuging and radioactivity measurements

Two buffers, both of pH7.4, were used in the preparation of cell-free extracts and sucrose gradients: (i) 10mM-Tris/HCl/10mM-magnesium acetate/100mM-KCl (THMK buffer); (ii) 10mM-Tris/HCl/0.1mM-magnesium acetate (TM buffer). The examination of sedimentation profiles and the preparation of isolated particles from sucrose gradients was as described by Blundell & Wild (1969*b*). Buffers containing isolated particles were changed, where necessary, by dialysis for 8–12h at 4°C.

For the determination of radioactivity in whole cells, portions (1ml) of cultures were precipitated with 5% (w/v, final concn.) trichloroacetic acid at 0°C and filtered through Whatman GF/C glass-fibre filter discs. The discs were washed with ice-cold 5% trichloroacetic acid and then with ethanol, dried and the radioactivity was measured.

Isopycnic centrifuging

Samples for isopycnic centrifuging were prepared in buffers containing 5mM-triethanolamine in place of 10mM-Tris: buffer EHMK is thus analogous to buffer THMK. Samples were fixed overnight at room temperature (about 20°C) with 7% (v/v) formaldehyde (pH7) and then centrifuged in partially pre-formed CsCl gradients (Brunck & Leick, 1969). Each gradient was prepared from a lower step of 2.50ml of 65% (w/w) CsCl in EHMK buffer plus 0.38ml of 40% (v/v) formaldehyde (pH7), and an upper step of 1.10ml of 65% CsCl in EHMK buffer, 0.38ml of 40% formaldehyde (pH7), 0.2ml of 10% (w/v) Brij 58 and 0.30ml of sample. Gradients were overlayered with liquid paraffin and centrifuged at 22°C for 20–24h at 84000*g* in a Spinco SW50.1 rotor, and fractionated in the same way as sucrose gradients (Blundell & Wild, 1969*b*). Densities were measured by weighing portions (25μl) of the fractions.

Analytical ultracentrifuging

A Spinco model E centrifuge, with u.v.-absorption optics, was used at 20°C and 44770rev./min. Photographs were taken at 2min intervals after reaching speed, and boundary positions measured by scanning the negatives with a Joyce–Loebl double-beam microdensitometer. Concentrations of ribosomes and 47S particles were estimated by assuming that 1mg of either/ml has an E_{260} of 14 (Hill *et al.*, 1969).

Chemicals

Radioactive chemicals ([5-^{3}H]uracil, [2-^{14}C]uracil and L-[1-^{14}C]leucine) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Deoxyribonuclease I was from Worthington Biochemical Co., Freehold, N.J., U.S.A.; Brij 58 was from Honeywill and Stein Ltd., Carshalton, Surrey, U.K.; Whatman products were obtained from H. Reeve Angel and Co., London E.C.4, U.K.

Results

Characteristics of E. coli 15–28

E. coli 15–28 was isolated as a mutant that, although slow-growing, had an RNA/protein ratio (measured chemically) greater than that of the parent strain 15TP; extracts made from exponentially growing cultures of strain 15–28 contained a ribonucleoprotein component referred to as '43S particles' (MacDonald *et al.*, 1967). The present work confirmed that the generation time of strain 15–28 is 2–2.5 times that of strain 15TP in the same medium (see the Experimental section). To verify that strain 15–28 has a higher RNA/protein ratio than strain 15TP, cultures were grown in medium containing [^{14}C]leucine and [^{3}H]uracil (by using the same concentrations and specific radioactivities for each strain). Samples were taken at intervals for measurement of trichloroacetic acid-insoluble radioactivity. In Fig. 1, ^{3}H radioactivity is plotted against

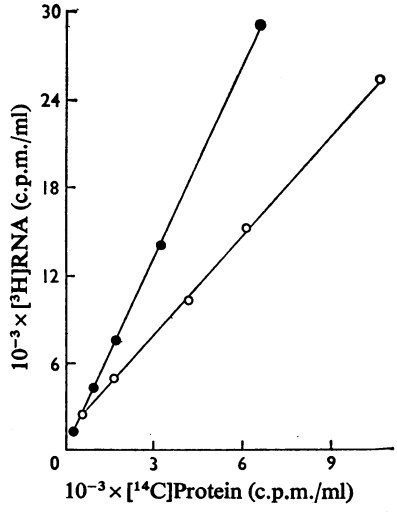

Fig. 1. *Synthesis of RNA and protein in strains* 15*TP* (●) *and* 15–28 (○)

Cultures (50ml) were grown in minimal-glucose medium. At an E_{450} of 0.05 for strain 15–28 and 0.1 for strain 15TP, [^{3}H]uracil (300nCi/11μg per ml) and [^{14}C]leucine (30nCi/15μg per ml) were added. Samples (1.0ml) were taken at intervals for determination of radioactivity insoluble at 0°C in 5% (w/v) trichloroacetic acid.

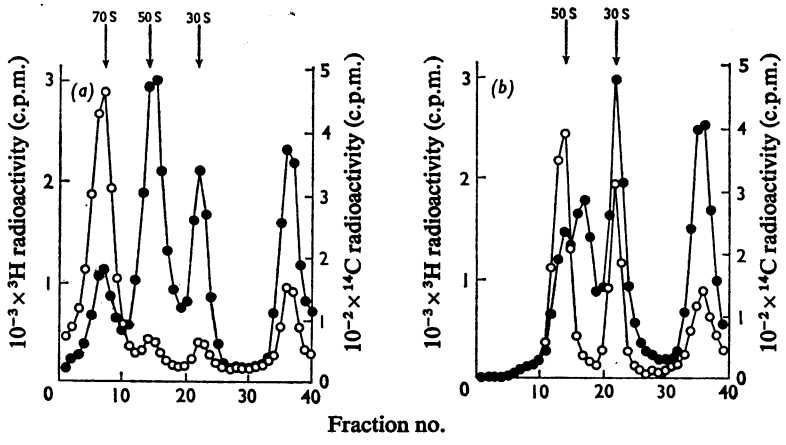

Fig. 2. *Sedimentation of extracts of strains* 15TP *and* 15–28

Cultures (50ml) were grown in minimal-glucose medium supplemented with casamino acids. At an E_{450} of 0.03, strain 15–28 was labelled with [^{14}C]uracil (20 nCi/5 μg per ml) and strain 15TP with [^{3}H]uracil (200 nCi/5 μg per ml). At E_{450} = 0.3, the cells were harvested, extracts made and centrifuged through sucrose gradients in (*a*)THMK buffer, (*b*) TM buffer. Sedimentation profiles are superposed: ●, ^{14}C radioactivity (strain 15–28); ○, ^{3}H radioactivity (strain 15TP).

^{14}C radioactivity during growth of each strain for 2.5 generations. Incorporation of uracil relative to leucine is 1.85 times higher in strain 15–28 than in strain 15TP.

Fig. 2 compares the sedimentation profiles of extracts made from cultures of strains 15TP and 15–28 that had been labelled for four generations with [^{3}H]- and [^{14}C]-uracil respectively. The profiles of the parent strain are normal. In THMK buffer (Fig. 2*a*) most of the RNA in strain 15TP sediments as 70S ribosomes and tRNA, with small peaks of free ('native') 50S and 30S subunits. In contrast, the extract from strain 15–28 contains relatively few 70S ribosomes: there is an excess of tRNA, together with '43S' particles and 30S subunits. In TM buffer (Fig. 2*b*), 70S ribosomes of both strains dissociate into 'derived' subunits, but there is an additional component in strain 15–28, sedimenting between the positions of the two normal subunits.

The extra component in strain 15–28 in TM buffer [called '43S particles' by MacDonald *et al.* (1967)] is the same material that, in THMK buffer, differs little in sedimentation properties from native 50S ribosomes (see Fig. 2*a* and below). To avoid confusion with other ribosome precursors to which the term '43S particles' has been applied (see Nomura, 1970), we have renamed the extra component in strain 15–28 '47S particles', in accordance with their sedimentation coefficient in THMK buffer (see below).

Comparison of 47*S particles with* 50*S ribosomes*

The 47S particles, isolated from extracts of strain 15–28 made in THMK buffer, were compared with other particles by sedimentation through sucrose gradients. In neither THMK nor TM buffer do 47S particles co-sediment with native or derived 50S ribosomes (Fig. 3). Differences are more marked at the lower Mg^{2+} concentration, although in TM buffer 47S particles are less well separated from native than from derived 50S subunits. This may reflect a difference in behaviour of native and derived subunits; alternatively native 50S ribosomes may inevitably be contaminated by slower-sedimenting precursors to 50S ribosomes, which are present in the steady state in strain 15TP. Nevertheless, the distinction between 47S particles and native 50S ribosomes is clear.

Sedimentation coefficients of 47S particles and derived 50S ribosomes measured in an analytical ultracentrifuge (Table 1) establish that changes in sedimentation properties of the former are responsible for the increased separation from 50S ribosomes noted in TM buffer (Fig. 3*a*). Derived 50S subunits have sedimentation coefficients of about 53S in both buffers: values for 47S particles are 47S in THMK buffer and 36S in TM buffer. The samples were dilute (30–70 μg/ml), so that no variation of sedimentation coefficient with concentration is expected.

Fig. 4 demonstrates the effect of mixing extracts of strain 15–28 in THMK buffer with isolated 50S or 30S subunits from strain 15TP. The 47S particles are completely unable to associate with added 30S ribosomes (Fig. 4*a*); however, about 65% of the free 30S subunits in strain 15–28 can form 70S particles when 50S ribosomes are added (Fig. 4*b*). Thus the un-

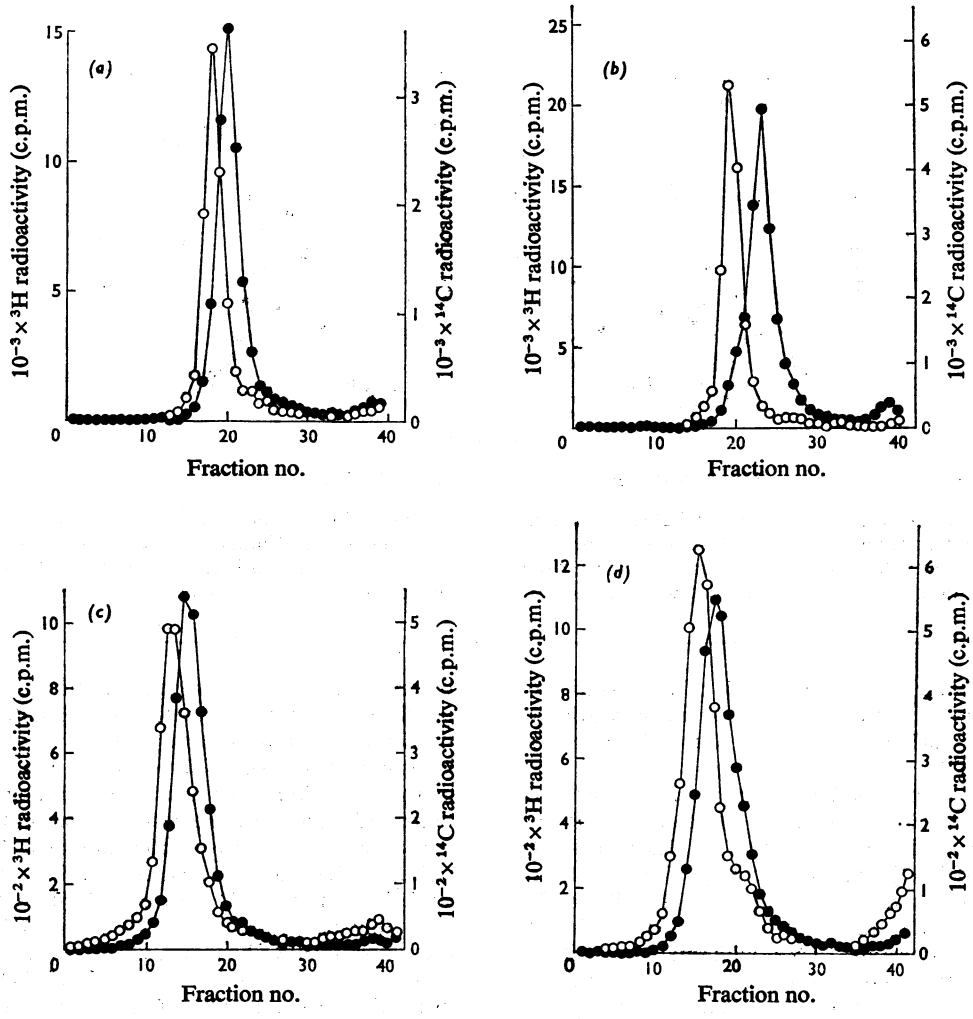

Fig. 3. *Comparison of 47S particles and 50S ribosomes*

An extract was made in THMK buffer from strain 15TP that had been grown for 3.5 generations with [^{3}H]uracil (5 μCi/5 μg per ml). Native 50S ribosomes were isolated from a portion of this extract after centrifuging through a sucrose gradient in THMK buffer. A second portion was diluted threefold with TM buffer and centrifuged through a sucrose gradient in TM buffer to prepare derived 50S ribosomes. An extract was also prepared in THMK buffer from strain 15–28 that had been labelled for four generations with [^{14}C]uracil (120 nCi/5 μg per ml). 47S particles were isolated after centrifuging through a sucrose gradient made in THMK buffer. Portions of each preparation were dialysed separately against THMK or TM buffer; mixtures (0.1 ml) were then centrifuged through sucrose gradients in the appropriate buffers. 47S particles (●) are compared with derived 50S ribosomes (○) (*a*) in THMK buffer, (*b*) in TM buffer, and with native 50S ribosomes (○) (*c*) in THMK buffer, (*d*) in TM buffer.

usual sedimentation profile of strain 15–28 ribosomes in THMK buffer derives primarily from the properties of 47S particles and not from a defect in the free 30S subunits.

Metabolic status of 47S *particles*

Gel electrophoresis shows that 47S particles contain 23S RNA, indistinguishable from that in 50S ribosomes (F. Markey & D. G. Wild, unpublished work). Thus 47S particles are related to 50S ribosomes: the following experiment establishes the nature of this relationship.

A culture of strain 15–28 was labelled for five generations with both [^{3}H]- and [^{14}C]-uracil; the

Table 1. *Analytical ultracentrifuging of 47S particles and 50S ribosomes*

Particles and ribosomes were isolated from sucrose gradients and centrifuged as described in the text.

Sample	Buffer	Concentration (μg/ml)	s_{20} (S)
47S particles	THMK	30	47.2
		50	46.3
		63	46.4
47S particles	TM	48	36.3
		67	35.6
50S ribosomes	THMK	33	53.4
		53	53.7
		66	53.3
50S ribosomes	TM	32	52.5
		54	53.0
		72	53.4

cells were collected by centrifuging at room temperature (about 20°C) and resuspended at 37°C in medium containing only [^{3}H]uracil (at the same specific radioactivity as before resuspension). Samples taken from the culture for up to 3.5 generations showed that there was no loss of trichloroacetic acid-insoluble ^{14}C radioactivity from the cells. Extracts were prepared from portions of the culture at the time of resuspension and at intervals thereafter and centrifuged through gradients in THMK buffer: Fig. 5 shows the sedimentation profiles at zero time and two generations later. At the time of resuspension, the profiles of ^{3}H and ^{14}C radioactivity are identical: the ^{3}H profile remained unchanged during subsequent growth. The ^{14}C profile shows a marked loss of radioactivity from 47S particles and an increase in 70S ribosomes. Analysis of the distribution of ^{14}C radioactivity in all the samples taken (Fig. 6) shows that these changes are reciprocal in that loss of ^{14}C label from 47S particles is matched by a gain in 70S ribosomes. The simplest interpretation is that 47S particles, unable to combine with 30S ribosomes (Fig. 4*a*), are converted into 50S ribosomes which then appear as 70S material. There is no large change in the proportion of native 30S ribosomes, since these can exchange with 30S subunits in 70S ribosomes. The equivalence between the changes in content of 47S particles and 70S ribosomes suggests that the former are converted intact into 50S ribosomes.

Fig. 6 also reveals a slight decrease in the proportion of ^{14}C-labelled 30S ribosomes and a slight increase in 'soluble RNA' during continued growth. The latter is probably a consequence of the turnover of mRNA and the utilization of labelled uracil. The decrease in 30S ribosomes (complete within one generation) is significant. Native 30S ribosomes of strain 15–28 are heterogeneous in that they contain about 30% of a component that

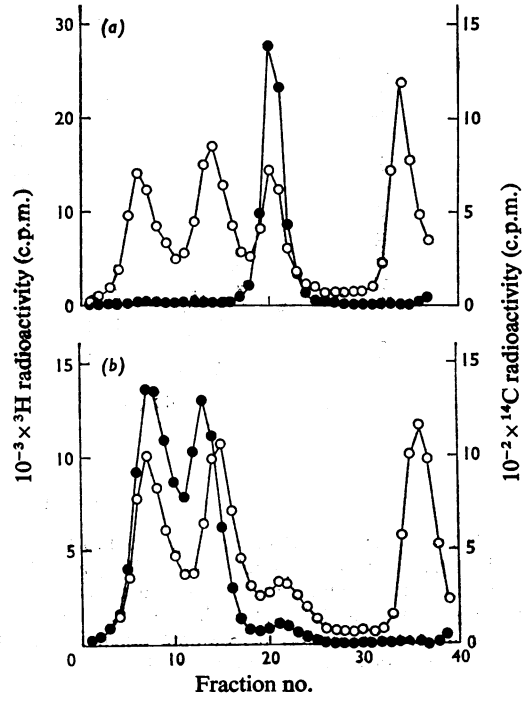

Fig. 4. *Sedimentation of extracts of strain 15–28 mixed with ribosomal subunits from strain 15TP*

An extract was made in TM buffer from strain 15TP that had been grown for four generations after the addition of [^{3}H]uracil (1.5μCi/ml with no additional carrier). Derived 50S and 30S subunits were obtained from this extract. Another extract was made in THMK buffer from strain 15–28 that had been labelled with [^{14}C]uracil (40nCi/5μg per ml) for four generations. Separate portions (20μl) of the extract of strain 15–28 (^{14}C radioactivity, ○) (containing about 5μg of 47S particles and 3.5μg of free 30S subunits) were then mixed with (*a*) 0.2ml (6μg) of 30S subunits from strain 15TP (^{3}H radioactivity, ●), and (*b*) 0.15ml (4μg) of 50S subunits from strain 15TP (^{3}H radioactivity, ●). The mixtures were centrifuged through sucrose gradients made in THMK buffer.

contains 17S RNA and is probably a precursor of 30S ribosomes, although not resolved from completed subunits by sedimentation analyses (Markey & Wild, 1975).

Comparison of 47S particles with other ribosome precursors

Ribosome precursors accumulate when cultures of *E. coli* are inhibited with $CoCl_2$ (Blundell & Wild, 1969*b*). Fig. 7(*a*) compares the sedimentation profiles (in THMK buffer) of extracts made from strain 15–28 labelled with [^{14}C]uracil for 3.5 generations,

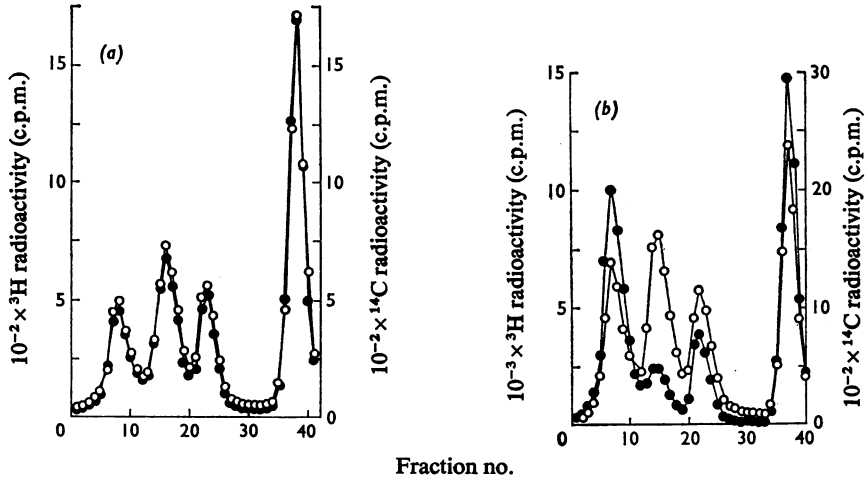

Fig. 5. *Metabolic fate of* 47S *particles*

Strain 15–28 was grown (from an E_{450} of 0.01 to 0.6) in 100ml of minimal-glucose medium supplemented with casamino acids and containing both [^{3}H]uracil (500nCi/10μg per ml) and [^{14}C]uracil (180nCi/10μg per ml). The culture was centrifuged at room temperature and the cells were resuspended in 200ml of the same medium but containing only [^{3}H]uracil. At intervals (see Fig. 6), samples (25ml) were harvested and portions of extracts in THMK buffer centrifuged through sucrose gradients made in THMK buffer. (*a*) Sample taken immediately after resuspension; (*b*) sample taken after 2.1 generations. ●, ^{14}C radioactivity; ○, ^{3}H radioactivity.

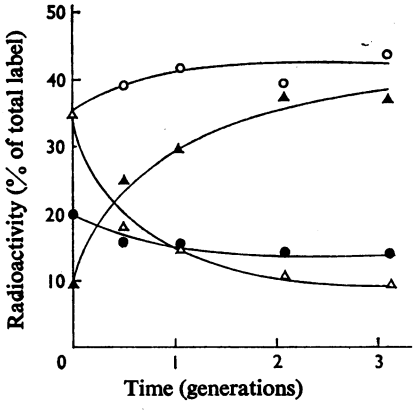

Fig. 6. *Distribution of radioactivity between components during growth of strain* 15–28

The ^{14}C radioactivity in Figs. 5(*a*) and 5(*b*) and in other samples taken in that experiment is apportioned between 70S ribosomes (▲), 47S particles (△), 30S ribosomes (●), and 'soluble RNA' (○). 'Soluble RNA' includes all material sedimenting more slowly than 30S ribosomes. 1 generation = 95min.

and *E. coli* M.R.E. 600 labelled with [^{3}H]uracil during inhibition for 30min with 0.3mM-$CoCl_2$. The latter profile shows the three 'cobalt particles' (Blundell & Wild, 1969*b*), of which the fastest sedimenting (the '44S particle') migrates less far than the 47S particles of strain 15-28. This distinction was confirmed by sedimentation of mixed isolated particles in TM buffer (Fig. 7*b*).

The 47S particles were also compared with '43S particles' present after pulse-labelling of strain 15TP for 5min (0.1 generation) in minimal-glucose medium without casamino acids. Difference in sedimentation properties were small, and a more sensitive distinction was obtained by centrifuging mixed particles (after fixation with formaldehyde) to equilibrium in CsCl gradients. The buoyant density of the pulse-labelled particles differs not only from that of 50S ribosomes (Fig. 8*a*), but also from that of 47S particles from strain 15–28 (Fig. 8*b*). Thus 47S particles are also distinct from the precursors identified by pulse-labelling of the parent strain, 15TP.

Discussion

The 47S particles, present during exponential growth of *E. coli* 15–28, are different from other precursors of 50S ribosomes, but are themselves precursors. The position of these particles in the assembly of 50S ribosomes, and the relationship between assembly in the mutant and the wild-type, cannot yet be fully assessed. Nomura (1970) thought that the assembly of 50S ribosomes in exponentially

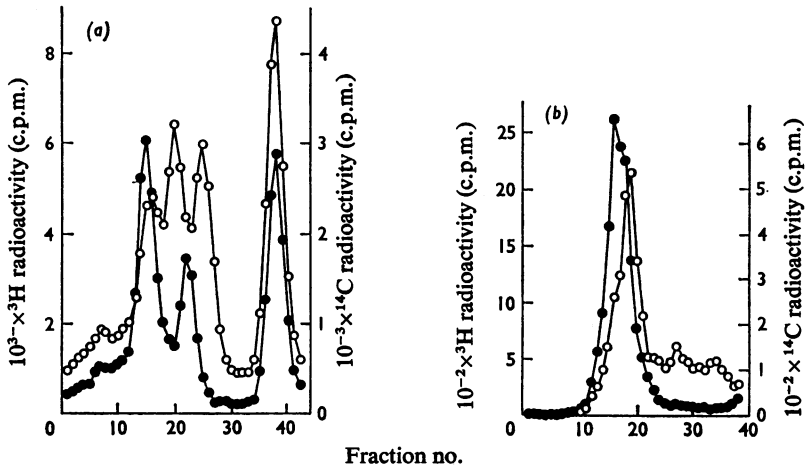

Fig. 7. *Comparison of 47S particles with 'cobalt particles'*

For (*a*), a culture (100ml) of *E. coli* M.R.E. 600 was grown in minimal-glucose medium containing 5μg of uracil/ml to $E_{450}=0.23$. It was then incubated for 30min with 0.3mM-$CoCl_2$ and 6μCi of [^{3}H]uracil/ml. The cells were then harvested and lysed. Cells from a culture (80ml) of strain 15–28 that had been labelled for 3.5 generations with [^{14}C]uracil (80nCi/5μg per ml) were lysed similarly. Portions of the two lysates were mixed and centrifuged through a sucrose gradient made in THMK buffer. For (*b*), lysates made as in (*a*) were centrifuged separately through sucrose gradients made in THMK buffer. The 47S particles (strain 15-28) and 44S 'cobalt particles' (*E. coli* M.R.E. 600) were isolated, dialysed against TM buffer and a mixture of them was centrifuged through a sucrose gradient made in TM buffer. ●, ^{14}C radioactivity (strain 15–28); ○, ^{3}H radioactivity (*E. coli* M.R.E. 600).

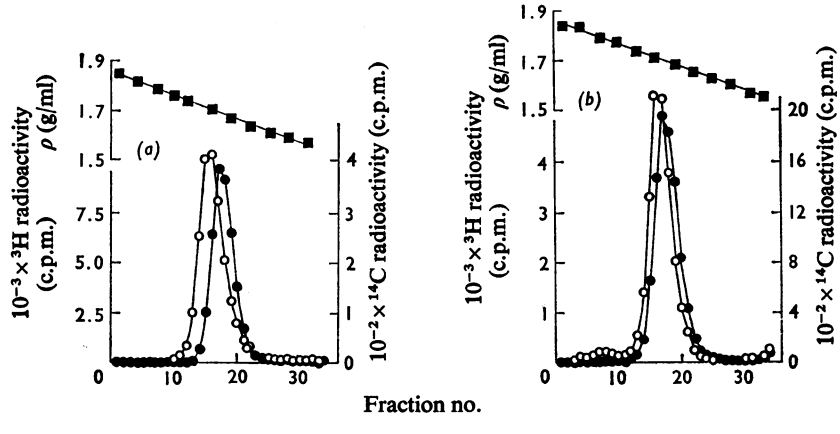

Fig. 8. *Comparison of pulse-labelled particles, 47S particles and 50S ribosomes by isopycnic centrifuging*

The 43S particles were prepared in EHMK buffer from a culture of strain 15TP labelled for 5min (0.1 generation) with [^{3}H]uracil (15μCi/1μg per ml). The 47S particles in EHMK buffer were prepared from an extract of strain 15-28 labelled with [^{14}C]uracil (120nCi/5μg per ml) for 3.5 generations. Derived 50S ribosomes were prepared from an extract made in EM buffer from strain 15TP grown in the presence of [^{14}C]uracil (40nCi/ml with no additional carrier). Samples were fixed with formaldehyde, portions mixed and centrifuged to equilibrium in gradients of CsCl [■, density (g/ml)]. ^{3}H-labelled (○) 43S particles are compared (*a*) with ^{14}C-labelled (●) 50S ribosomes, and (*b*) with ^{14}C-labelled (●) 47S particles.

growing cells proceeds through two stages, comprising '32S' and '43S' particles. Lindahl (1975) provided evidence for an additional, possibly major, intermediate that co-sediments with 50S ribosomes but can be detected because the mobility of its 23S RNA during gel electrophoresis differs from that of the RNA of mature subunits. In Lindahl's (1975) experiments about 2% of the total 23S RNA of the bacteria was in the three species of precursor particle although the percentage of precursors depends both on growth rate and the strain used (Forget & Varricchio, 1970). Much therefore remains to be established about the assembly of 50S ribosomes in wild-type strains. However, there are essentially two interpretations of the results in the present paper. First, assembly of 50S ribosomes in strain 15–28 could proceed along a normal pathway and 47S particles represent a hitherto undetected stage in the process. This hypothesis predicts that 'normal' 32S and 43S particles should be stages in ribosome formation in the mutant: the absence of 23S RNA from preparations of 30S material from strain 15-28 (Markey & Wild, 1975) indicates that the steady-state content of 32S particles is low. Secondly, 50S ribosome assembly may be fundamentally different in the mutant strain. In this case, strain 15–28 is a valuable experimental tool in that functional 50S ribosomes, whose structure must be fairly rigorously defined, are assembled by an unusual pathway. With either possibility, one intermediate in the pathway is available for further study.

The nature of the lesion responsible for the accumulation of 47S particles in strain 15–28 is not established, but genetic manipulations have not separated the production of 47S particles from the slow growth rate and high RNA/protein ratio of the mutant (Turnock, 1969). Several other mutants have been described (Buckel *et al.*, 1972; Lewandowski & Brownstein, 1969; López-Revilla & Bastarrachea, 1971) whose phenotypes are superficially similar to that of strain 15–28. One (Buckel *et al.*, 1972) was selected by screening for increased amounts of $tRNA^{Ala}$ and also found to accumulate ribosome precursors during exponential growth: it has since been shown (Wittmann *et al.*, 1974) to contain an altered ribosomal protein S5. Others were isolated as ribosome mutants and then found to contain excess of tRNA. These characteristics suggest that alterations in ribosome assembly may affect the control of tRNA synthesis. Guanosine phosphates (ppGpp and pppGpp) produced on the ribosome (Haseltine *et al.*, 1972) are implicated in the control of stable RNA formation (Fiil *et al.*, 1972) so that interdependence of ribosome metabolism and RNA synthesis is already recognized. Mutants such as 15–28 widen the area in which this interdependence can be investigated.

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References

Blundell, M. R. & Wild, D. G. (1969*a*) *Biochem. J.* **115**, 207–212
Blundell, M. R. & Wild, D. G. (1969*b*) *Biochem. J.* **115**, 213–223
Brunck, C. F. & Leick, V. (1969) *Biochim. Biophys. Acta* **179**, 136–144
Buckel, P., Ruffler, D., Piepersberg, W. & Böck, A. (1972) *Mol. Gen. Genet.* **119**, 323–335
Fiil, N. P., von Meyenberg, K. & Friesen, J. D. (1972) *J. Mol. Biol.* **71**, 769–783
Forget, B. G. & Varricchio, F. (1970) *J. Mol. Biol.* **48**, 409–419
Haseltine, W. A., Block, R., Gilbert, W. & Weber, K. (1972) *Nature (London)* **238**, 381–384
Hill, W. E., Rossetti, G. P. & van Holde, K. E. (1969) *J. Mol. Biol.* **44**, 263–277
Lewandowski, L. J. & Brownstein, B. L. (1969) *J. Mol. Biol.* **41**, 277–290
Lindahl, L. (1975) *J. Mol. Biol.* **92**, 15–37
López-Revilla, R. & Bastarrachea, F. (1971) *Mol. Gen. Genet.* **113**, 99–113
MacDonald, R. E., Turnock, G. & Forchhammer, J. (1967) *Proc. Natl. Acad. Sci. U.S.A.* **57**, 141–147
Markey, F. & Wild, D. G. (1975) *Biochem. J.* **151**, 463–465
Nashimoto, H., Held, W., Kaltschmidt, E. & Nomura, M. (1971) *J. Mol. Biol.* **62**, 121–138
Nomura, M. (1970) *Bacteriol. Rev.* **34**, 228–277
Osawa, S., Otaka, E., Itoh, T. & Fukui, T. (1969) *J. Mol. Biol.* **40**, 321–351
Turnock, G. (1969) *Mol. Gen. Genet.* **104**, 295–312
Wittmann, H. G., Stöffler, G., Piepersberg, W., Buckel, P., Ruffler, D. & Böck, A. (1974) *Mol. Gen. Genet.* **134**, 225–236