The Composition of an Unusual Precursor of 50S Ribosomes in a Mutant of *Escherichia coli*

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Escherichia coli strain 15–28 is a mutant which during exponential growth contains large amounts of a '47S' ribonucleoprotein precursor to 50S ribosomes. The '47S particles' are more sensitive to ribonuclease than are 50S ribosomes. The 23S RNA of 47S particles may be slightly undermethylated, but cannot be distinguished from the 23S RNA of 50S ribosomes by sedimentation or electrophoresis. Isolated particles have 10–15% less protein than do 50S ribosomes; proteins L16, L28 and L33 are absent. Comparison with precursor particles studied by other workers in wild-type strains of *E. coli* suggests that the assembly of 50S ribosomes in strain 15–28 is atypical.

Escherichia coli strain 15–28, first described by MacDonald *et al.* (1967), is a mutant that accumulates large amounts of unusual ribonucleoprotein ('47S') particles during exponential growth. We (Markey & Wild, 1976) previously showed that 47S particles are precursors to 50S ribosomes, but differ from the precursor particles detected in the parent strain during pulse-labelling and from precursors accumulated during inhibition of the growth of *E. coli* by CoCl_2 (Blundell & Wild, 1969). Here we describe a more detailed characterization of 47S particles, including an examination of their protein composition. There are major differences from other precursors (insofar as the composition of the latter is known). Our conclusion is that assembly of 50S ribosomes in strain 15–28 differs from that in wild-type strains.

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). Growth of cultures [in a glucose-minimal medium containing 10 µg of thymine/ml, 50 µg of proline/ml, 0.1 mg of vitamin-free casamino acids/ml (Difco Laboratories, West Molesey, Surrey, U.K.), and where required, radiochemicals noted in the Table and Figure legends], harvesting of bacteria and the preparation of extracts by using an Aminco-French pressure cell were as described previously (Markey & Wild, 1976).

Centrifuging

The buffers (pH 7.4) used to make extracts and sucrose gradients were (i) 10 mM-Tris/HCl/10 mM-magnesium acetate/100 mM-KCl (THMK buffer),

(ii) 10 mM-Tris/HCl/10 mM-magnesium acetate (THM buffer) and (iii) 10 mM-Tris/HCl/0.1 mM-magnesium acetate (TM buffer). The examination of sedimentation profiles after centrifuging in sucrose-density gradients and the preparation of isolated ribosomes and 47S particles were as previously described (Blundell & Wild, 1969; Markey & Wild, 1976). Buffers containing ribosomes or 47S particles were changed, when necessary, by dialysis for 8–12 h at 4°C. For the preparation of material for analysis of protein composition, portions (1.0 ml) of clarified extract (E_{260} about 200) were centrifuged through 38 ml linear 15–30% (w/w) sucrose gradients for 13 h at 95000g (Spinco SW27 rotor). These gradients were sometimes collected in about 40 discrete fractions by piercing the bottom of the tube: radioactivity was measured in portions of the fractions and appropriate fractions were then pooled. Alternatively, gradients were collected by using an ISCO model 185 Density Gradient Fractionator, by using the E_{280} to locate ribosomes and 47S particles.

'Chloramphenicol particles' (Kono & Osawa, 1964) were isolated from extracts made from *E. coli* strain 15TP that had been grown for 30 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.

Isopycnic centrifuging of 50S ribosomes and 47S particles in CsCl gradients after fixation with 7% formaldehyde was as described previously (Markey & Wild, 1976).

Extraction and electrophoresis of RNA

For extraction of RNA, ribonucleoprotein from sucrose gradients was precipitated with 2 vol. of ice-

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cold ethanol and redissolved in 10mM-Tris/HCl buffer (pH 7.4) containing 10mM-magnesium acetate and 0.25% (w/v) sodium dodecyl sulphate. RNA was extracted with phenol (Kurland, 1960) and precipitated with ethanol at 0°C. RNA to be examined by sucrose-gradient sedimentation was redissolved in 10mM-sodium acetate buffer (pH 4.6) containing 100mM-NaCl and centrifuged for 5.5h at 234000g through 15–30% (w/w) sucrose gradients made in this buffer. RNA for electrophoretic analysis was redissolved at 1mg/ml ($E_{260}=24$) in 50mM-NaCl containing 2mM-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 total acrylamide concentration. Gradients were formed in the cold from solutions of 2 and 7% acrylamide containing 3.7 μ l of 10% (w/v) ammonium persulphate/ml plus 2.0 μ l and 7.0 μ l of *NNN'*-tetramethylethylenediamine respectively/ml of gel solution. Polymerization was then allowed to proceed at room temperature (about 20°C). The Gradipore apparatus (Universal Scientific Ltd., London E13 0QU, U.K.), which is that described by Margolis & Kenrick (1968), was used. Gels were pre-run for 30min at 10V/cm and then loaded with 3–6 μ g of RNA per slot. Electrophoresis, at 5°C, was for 8–10h at 10V/cm; gels were stained with 0.2% Methylene Blue in 0.4M-acetic acid containing 0.4M-sodium acetate (Peacock & Dingman, 1967) and destained electrophoretically.

Extraction and electrophoresis of proteins

The 50S ribosomes or 47S particles were either pelleted by centrifuging for 5–7h at 135000g or precipitated at 0°C with 2vol. of ethanol. They were then resuspended in THMK buffer at a concentration of 2–3mg/ml. Proteins were then extracted with acetic acid (Kaltschmidt & Wittmann, 1972), dialysed against water and freeze-dried. No differences in protein composition were noted as a result of these alternative procedures. Two-dimensional electrophoretic separation of proteins was carried out by the method of Kaltschmidt & Wittmann (1970a) (by using a gel containing 8% acrylamide, pH 8.6, in the first dimension) with the modifications suggested by Howard & Traut (1973), in which, for the first dimension, two separate half-length rod gels are run in opposite directions and urea is omitted from the electrode buffer. Proteins were dissolved (at 5mg/ml) in sample buffer (pH 8.6) which contained, per litre: urea, 480g; Tris, 5g; boric acid, 3.2g; EDTA (disodium salt), 0.85g. Samples (about 250 μ g of protein) were applied to the top of duplicate rod gels and the ends of the tubes then plugged with 0.5% (w/v) agarose dissolved in electrode buffer. One of each

pair of gels was inverted so that electrophoresis in the two tubes was under identical conditions but in opposite directions. After electrophoresis in the second dimension, gel slabs were stained for 4–8h in 0.1% (w/v) Coomassie Brilliant Blue in 50% (v/v) methanol containing 7.5% (v/v) acetic acid, and destained electrophoretically in 7.5% acetic acid.

Chemicals

Radiochemicals ([5-³H]uracil, [2-¹⁴C]uracil, L-[methyl-³H]methionine and L-[4,5-³H]lysine) were from The Radiochemical Centre, Amersham, Bucks., U.K. Pancreatic ribonuclease (EC 3.1.4.22), crystallized five times, was from Koch-Light Laboratories, Colnbrook, Bucks., U.K.

Results

The 23S RNA of 47S particles

Chromatography on kieselguhr coated with methylated albumin showed that 47S particles contain 23S RNA (MacDonald *et al.*, 1967). We have confirmed this by sedimentation analysis of RNA extracted from 47S particles and cannot resolve the 23S RNA of the particles from that of 50S ribosomes. However, neither of these techniques satisfactorily distinguishes precursor ('p23S') RNA from the 'mature' form. Chloramphenicol particles contain 16S and 23S RNA that are largely in precursor forms (Kono *et al.*, 1964) and can be distinguished from the mature species by electrophoresis in polyacrylamide gels (Hecht & Woese, 1968; Pace *et al.*, 1970). This procedure resolves 23S RNA from both 50S and chloramphenicol particles into three components. Fig. 1 compares RNA extracted from four sources: 70S ribosomes and 47S particles from strain 15–28, derived 50S subunits and chloramphenicol particles

Fig. 1. Analysis of RNA in polyacrylamide gradient gels

RNA was extracted, as described in the Experimental section, from (i) derived 50S ribosomes of strain 15TP, (ii) 'chloramphenicol particles' from strain 15TP, (iii) 47S particles from strain 15–28 and (iv) 70S ribosomes of strain 15–28, and analysed by electrophoresis in 2–7% polyacrylamide gels. The drawing of the stained gels is based on three separate experiments. In each case the distance from the origin to the 16S RNA band was about 3.5cm.

from strain 15TP. Analysis was by electrophoresis in polyacrylamide gradient gels, as described in the Experimental section. The p23S RNA in chloramphenicol particles was clearly separated from the mature 23S RNA of 70S and 50S ribosomes; the RNA in 47S particles from strain 15-28 was indistinguishable from that in either 70S ribosomes of the mutant or 50S subunits of the parent strain, and so by this criterion is 'mature'.

The extent of the methylation of 23S RNA in precursors to 50S ribosomes is unclear. Osawa *et al.* (1969) reported that '40S' particles detected by pulse-labelling of *E. coli* were about 60% methylated by comparison with 23S RNA from completed 50S subunits. Forget & Varricchio (1970) noted that when strains of *E. coli* K12 were grown rapidly, extracts contained a '43S' precursor to 50S ribosomes which could be detected spectrophotometrically after density-gradient centrifuging. The methylation of the 23S RNA in this precursor was shown to be not significantly different from that of 'mature' 23S RNA from 50S ribosomes. Methylation of the 23S RNA in 47S particles of strain 15-28 was measured as follows. A culture of this mutant was grown for four generations to $E_{450} = 0.5$ in minimal medium containing L-[methyl- ^3H]methionine ($6.5\mu\text{Ci}/5\mu\text{g}$ per ml) and [^{14}C]uracil ($20\text{nCi}/10\mu\text{g}$ per ml). Portions of extracts made in THM buffer were centrifuged through sucrose-density gradients made with this buffer and fractions containing 70S ribosomes and 47S particles retained for isolation of RNA. Sedimentation of the RNA preparations through sucrose gradients enabled the relative extent of methylation to be determined from the ratio ^3H radioactivity/ ^{14}C radioactivity in peak fractions of 23S RNA. Five separate experiments (in each of which the ratio for 23S RNA from 70S ribosomes was set at 100%) gave values for the 23S RNA in 47S particles ranging between 86 and 96%, with a mean of 89%. Thus RNA from 47S particles apparently lacks about 10% of its full complement of methyl groups. This corresponds to a deficiency of about two methyl groups per RNA molecule (Fellner, 1969).

In general, ribosome precursors are more sensitive to degradation by ribonuclease than are completed ribosomal subunits (see, e.g. Hayes & Hayes, 1971). To test whether this applies to 47S particles, their rate of degradation was compared with that of derived 50S ribosomes from strain 15TP and the RNA isolated from the latter. The [^3H]uracil-labelled ribonucleoproteins and RNA were prepared in TM buffer and used at the same concentration of RNA. Incubation was at 30°C with 20ng of pancreatic ribonuclease/ml; degradation was monitored by measurement of residual radioactive material insoluble at 0°C in 5% (w/v) trichloroacetic acid. Fig. 2 shows that 47S particles are degraded more rapidly and extensively than are 50S ribosomes, but more

slowly than is free RNA. These results do not directly compare the accessibility of the RNA in ribosomes and 47S particles to ribonuclease, because the appearance of acid-soluble radioactivity requires both degradation of RNA and release of fragments from ribonucleoprotein complexes. However, they do indicate a further difference between 47S particles and 50S ribosomes and a general similarity of 47S particles to other ribosome precursors.

Composition of 47S particles

When strain 15-28 is grown for several generations in medium containing [^3H]lysine and [^{14}C]uracil, organisms contain ribosomes and 47S particles whose RNA and protein is uniformly labelled. The relative composition of isolated components can then be compared by their ratio of ^3H radioactivity to ^{14}C radioactivity. An extract of strain 15-28 in THMK buffer was centrifuged through a sucrose gradient in this buffer and 70S ribosomes and 47S particles were isolated. After dialysis against TM buffer, the 70S ribosomes (now dissociated into 50S and 30S subunits) were re-centrifuged through a sucrose gradient in TM buffer. Portions of the preparation of 47S particles were dialysed against TM and THMK

Fig. 2. Degradation by ribonuclease

47S particles from strain 15-28 and derived 50S ribosomes from strain 15TP were prepared in TM buffer; both were labelled with [^3H]uracil at about 25000 c.p.m./ μg of ribonucleoprotein. RNA was extracted from a portion of the same preparation of 50S ribosomes. Samples (1.1 ml) of the three preparations were brought to 30°C . A portion (0.2 ml) was withdrawn for measurement of radioactivity insoluble at 0°C in 5% (w/v) trichloroacetic acid, and 0.1 ml of pancreatic ribonuclease (final concentration 20 ng/ml) then added. Further samples (0.2 ml) were taken at intervals. ●, 50S ribosomes; ▲, 47S particles; ○, RNA.

Table 1. Comparison of protein/RNA ratios in 47S particles and 50S ribosomes

An extract (0.75 ml) in THMK buffer was prepared from a culture (60 ml) of strain 15-28 grown to $E_{450} = 0.5$ in minimal-glucose medium without casamino acids but containing [^3H]lysine ($5 \mu\text{Ci}/6 \mu\text{g}$ per ml) and [^{14}C]uracil ($0.1 \mu\text{Ci}/5 \mu\text{g}$ per ml). Portions were centrifuged through sucrose gradients in THMK buffer to prepare 70S ribosomes and 47S particles. The 70S ribosomes were dialysed against TM buffer and portions of the preparation of 47S particles against both TM and THMK buffers. Samples were then centrifuged through further sucrose gradients in the buffer listed in the Table. The ratio ^3H radioactivity (in protein)/ ^{14}C radioactivity (in RNA) is the mean of the value for the three fractions at the peaks of radioactive material in the gradients.

Particle	Buffer	Protein/RNA ratio
		^3H radioactivity (c.p.m.)/ ^{14}C radioactivity (c.p.m.)
Derived 50S	TM	3.57
47S	TM	3.02
47S	THMK	2.94

buffers and then centrifuged through gradients in the buffer used for dialysis. Table 1 shows that the protein content (per unit of RNA) of 47S particles is essentially the same in both buffers and about 85% of that of the derived 50S ribosomes.

Isopycnic centrifuging is another method for determining the relative composition of ribonucleoprotein particles. Fig. 3 compares 47S particles from strain 15-28 with derived 50S ribosomes from strain 15TP. Both particles form sharp bands, with overlapping symmetrical peaks; 47S particles are denser than 50S ribosomes. The difference in buoyant density of 0.01 g/ml corresponds to a protein deficiency of about 12% in 47S particles. Banding in CsCl gradients also distinguishes 47S particles from pulse-labelled 43S precursors in strain 15TP (Markey & Wild, 1976). In this latter separation, 43S particles are the denser of the two. Thus 47S particles from strain 15-28 are intermediate in composition between pulse-labelled 43S precursors and completed 50S ribosomes, and lack 12-15% of the protein in mature subunits.

Proteins of 47S particles

Two-dimensional gel electrophoresis (Kaltschmidt & Wittmann, 1970a) was used to establish the pattern of proteins from derived 50S subunits of strain 15-28. For this, 70S ribosomes were first obtained by centrifuging portions of extract through sucrose gradients in THMK buffer. The ribosomes were precipitated with ethanol, dissolved in TM buffer and centrifuged through sucrose gradients in this buffer to obtain 50S subunits. The ribosomes were not washed with high concentrations of NH_4Cl because of the un-

Fig. 3. Isopycnic centrifuging of 47S particles

An extract was made, in TM buffer, of strain 15TP that had been grown in medium containing [^{14}C]uracil (40 nCi/ml with no additional carrier). From this, derived 50S ribosomes were isolated by gradient centrifuging in TM buffer. An extract was also made, in THMK buffer, of strain 15-28 that had been grown in medium containing [^3H]uracil ($2.5 \mu\text{Ci}/5 \mu\text{g}$ per ml); from this, 47S particles were isolated by gradient centrifuging in THMK buffer. The two preparations were mixed, dialysed against 5 mM-triethanolamine/HCl buffer (pH 7.4) containing 0.1 mM-magnesium acetate, then fixed with formaldehyde and centrifuged to equilibrium in a gradient of CsCl [Δ , density (g/ml)], as described by Markey & Wild (1976). $\circ$, ^3H radioactivity (47S particles); $\bullet$, ^{14}C radioactivity (50S ribosomes).

known effects of this procedure on the proteins of 47S particles. Electrophoresis showed that all 34 proteins recognized in the 50S ribosomes of *E. coli* were present and that protein L31 was more prominent in strain 15-28 (and strain 15TP) than in the strains studied by Kaltschmidt & Wittmann (1970b). One separation is shown in Plate 1(a). The intensity of weakly staining proteins (e.g. L7 and L12) could be increased by loading more protein on to gels, but this lowered the resolution and increased the apparent contamination of preparations with the more strongly staining proteins from 30S subunits. Not all the 34 proteins are visible in Plate 1. At pH 8.6, proteins L4 and L10 remain at or near the origin in the first dimension and tend to be lost during subsequent

EXPLANATION OF PLATE I

Electrophoresis of proteins from 50S ribosomes of strain 15-28

The experimental procedures followed are given in the text. For (a), electrophoresis was under standard conditions. For (b), the times of electrophoresis in the first and second dimensions were decreased and the load doubled in order to demonstrate more clearly the presence of protein L28; only those proteins moving towards the cathode in the first dimension are shown.

EXPLANATION OF PLATE 2

Electrophoresis of proteins from 47 S particles of strain 15-28

The experimental procedures followed are given in the text. For (a) and (b) electrophoresis was under the same conditions and carried out in the apparatus at the same time as the results in Plate 1(a) and 1(b).

dialysis: they were detected by the procedure of Knopf *et al.* (1975), in which electrophoresis at pH 3.7 precedes 'normal' electrophoresis in the second dimension. Protein L34 was detected by decreasing the time of electrophoresis in the first dimension.

The pattern of proteins from 47S particles of strain 15-28 is shown in Plate 2(a). The presence of some of the strongly staining proteins from 30S subunits is more noticeable than in the 50S ribosomes; purification of 47S particles by a further cycle of gradient centrifuging generally led to loss of material without markedly decreasing such contamination. In Plate 2(a), the multiple pattern of stained proteins in the area of the single L8/9 component of 50S ribosomes (Plate 1a) is not a significant difference between 47S particles and 50S ribosomes. Different preparations of proteins from 47S particles or 50S ribosomes gave either single (as in Plate 1a) or multiple (Plate 2a) spots in this region. Hardy (1975) has suggested that protein L8 may be an artifact caused by aggregation of proteins L7/12 and L10. Watson *et al.* (1975) have shown that aggregates involving proteins L7 and L12 may be contained in this area of gels.

However, by comparison with the 50S ribosomes of strain 15-28, 47S particles lack three proteins, L16, L28 and L33. The other L proteins present are not altered markedly in position or intensity of staining. Separate experiments, as described above, established the presence of proteins L4, L10 and L34 in 47S particles. Protein L28 was consistently absent from different preparations of 47S particles, but stains weakly under normal conditions. Plates 1(b) and 2(b) show separations for which the times of electrophoresis in the first and second dimensions were decreased and the load was doubled. Protein L28 can be seen among the proteins from 50S ribosomes but not from 47S particles.

The absence of proteins L16, L28 and L33 from 47S particles but not from 50S ribosomes was consistently observed. Electrophoresis was carried out on 13 separate preparations of the proteins of each component isolated from different batches of strain 15-28. There were slight differences between the preparations in the intensity of the weakly staining spots or in the degree of contamination with proteins from 30S ribosomes.

Ribosomal proteins were also isolated from derived 50S ribosomes of strain 15TP. These subunits were isolated from extracts that were made and centrifuged through sucrose gradients in TM buffer. After electrophoresis, protein patterns were not significantly different from those of 50S ribosomes of strain 15-28. Alterations in L proteins that might account either for the accumulation of 47S particles in strain 15-28 or for functional differences between completed 50S ribosomes from strains 15TP and 15-28 are therefore, if present, not detected by two-dimensional electrophoresis.

Discussion

The 47S particles of *E. coli* strain 15-28 are unusual. They are precursors to 50S ribosomes (Markey & Wild, 1976) which are sensitive to degradation by ribonuclease but contain 23S RNA that is 'mature' by electrophoretic criteria. Lindahl (1973, 1975) described three sequential precursors to 50S ribosomes in exponentially growing cultures of *E. coli* B (AS19). Two of these ('p50S-1' and 'p50S-2' particles) probably correspond to the two stages in assembly that comprise the '32S' and '43S' particles detected by other workers (Nomura, 1970). The third precursor ('p50S-3') is probably the major intermediate and is not resolved from completed 50S subunits by sedimentation; however, it can be detected because the mobility of its 23S RNA during gel electrophoresis is less than that of the RNA from mature ribosomes, as is also the mobility of 23S RNA from intermediates p50S-1 and p50S-2. The implication is therefore that 47S particles are a 'late' stage in the assembly of 50S ribosomes: this is to some extent supported by the almost complete methylation of their RNA.

The 47S particles lack proteins L16, L28 and L33, the sum of whose mol.wts. is 41 000-46 000. The total mol.wt. of protein in a 50S ribosome has been estimated to be 510 000-640 000, calculated from the overall composition of the subunits, or about 555 000, calculated from the sum of the molecular weights of the individual proteins and assuming one copy of each per ribosome (Dzionara *et al.*, 1970). On this basis 47S particles should contain about 8% less protein than do 50S ribosomes. This value is less than those (12-15%) given by centrifuging in sucrose gradients and in CsCl, but better agreement cannot be expected. Hardy (1975) has suggested that *in vivo* and except for proteins L7 and L12, no ribosomal protein occurs in an amount significantly different from 1 molecule per ribosome. However, proteins are lost during most extraction procedures, so that isolated 50S ribosomes contain many proteins whose stoichiometry is not unity (Weber, 1972). Measurements of the overall protein deficiency of 47S particles, although showing that this exists, are at present only approximate.

Detailed comparison of the protein composition of 47S particles with that of other precursors is hindered by a lack of precise information on the latter. Osawa *et al.* (1969) compared the proteins of pulse-labelled '40S particles' with those of 50S ribosomes by chromatography on CM-cellulose. This method resolves 50S ribosomal proteins into nineteen components, twelve of which were present in 40S particles. This implies that a minimum of seven proteins is absent from 40S precursors; the missing proteins cannot all be allocated numbers according to the L protein nomenclature derived from electrophoresis,

although the correlation available (Wittmann, 1974) suggests that the missing proteins are L6, L7, L11, L12, L16, L25, L29 plus at least two others.

One group of workers, using *E. coli* B (AS 19), has twice reported the protein composition of '43S particles', which were operationally defined as material sedimenting between 50S and 30S subunits at low (1mM) Mg^{2+} concentrations (Homann & Nierhaus, 1971; Nierhaus *et al.*, 1973). The particles were isolated by zonal centrifugation of extracts made from exponentially growing cells and so may be equivalent to the 43S particles detected by pulse-labelling. The 43S particles were found to lack proteins L2, L6, L12, L16, L28, L31 and L32. Thus 47S particles contain more protein than do these 43S precursors to 50S ribosomes. Proteins L16 and L28 are apparently missing from both precursor species, although 47S, but not 43S, particles lack protein L33.

The protein composition, together with the size (and possibly methylation), of the 23S RNA of 47S particles suggests that the assembly of 50S ribosomes in strain 15-28 is atypical. Although 47S particles do not differ greatly from 50S ribosomes in their protein content, the missing proteins include L16, which binds specifically to 23S RNA (Stöffler *et al.*, 1971) and has been implicated in peptidyl transferase activity (Moore *et al.*, 1975), the binding of chloramphenicol to 50S ribosomes (Nierhaus & Nierhaus, 1973; Pongs *et al.*, 1973) and in binding of aminoacyl-tRNA (Pongs *et al.*, 1974). The 47S particles can therefore be predicted to lack several of the partial reactions of protein synthesis. Completed (70S) ribosomes from strain 15-28 are inefficient in protein synthesis (MacDonald *et al.*, 1967). Although there are no detectable differences between the protein components of 50S ribosomes from strain 15-28 and its parent, strain 15TP, the (undetected) alteration(s) produced by the genetic lesion in strain 15-28 may, besides giving rise to an unusual pathway of ribosome assembly, lead to the production of completed 50S subunits whose topography is slightly altered and so have decreased biological activity.

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