A COMPARISON OF THE NATIVE AND DERIVED 30S AND 50S RIBOSOMES OF *Escherichia coli*

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ABSTRACT Four types of ribosomes occurring in *E. coli* have been separated by sucrose gradient centrifugation. These are the 30S and 50S particles occurring in *E. coli* extracts (native particles), and the 30S and 50S particles which are the subunits of 70S ribosomes (derived particles). Two criteria were used in comparing these particles: (1) The type of RNA contained in each, as determined by sedimentation velocity in the analytical ultracentrifuge. (2) The ability of mixtures of 30S and 50S ribosomes (derived 30S + derived 50S, native 30S + native 50S) to undergo the reaction:

$$30S + 50S \xrightarrow{10^{-3}\,M\,Mg^{++}} 70S$$

Native and derived 30S particles were found to contain 16S RNA. Derived 50S particles contained 23S RNA and a small amount of 15 to 20S RNA, whereas native 50S ribosomes contained only 16S RNA. Derived 30S and 50S particles combined to form 70S particles. However, under identical conditions, native 30S and 50S particles did not form 70S ribosomes.

The principal types of ribonucleoprotein particles (ribosomes) present in extracts of *Escherichia coli* have sedimentation coefficients of approximately 30S, 50S, 70S, and 85 to 100S (1). The 85 to 100S particle appears to be a dimer containing two 70S particles (2). S^{35}-labeling experiments *in vivo* (3) and C^{14} amino acid incorporation experiments *in vitro* (4) strongly suggest that the 70S ribosomes are the primary site of protein synthesis.

Of considerable relevance both to the structure of 70S ribosomes and to their possible mode of biosynthesis is the observation that, at Mg^{++} concentrations below 10^{-3} M, 70S ribosomes dissociate into subunits having sedimentation coefficients of approximately 50S and 30S (1, 5, 6). These "derived" 30S and 50S particles recombined to form 70S particles when the Mg^{++} concentration is increased to 10^{-2} M. Since the formation of 70S particles from a mixture of derived 30S and 50S ribosomes is nearly complete (1), (also *cf.* Fig. 4, E, F) the very existence of

"native" 30S and 50S ribosomes in an extract of *E. coli* at 10^{-2} M Mg^{++} suggested a possible difference between the native and derived particles.

We have undertaken a detailed comparison of the native and derived 30S and 50S ribosomes. The criteria used for comparison were of two types: (1) The sedimentation coefficient of RNA extracted from the various particles, (2) the ability of native and of derived particles to form 70S ribosomes *in vitro*.

MATERIALS AND METHODS

1. *Isolation of Ribosomes*

Ribosomes were obtained from *Escherichia coli* strain B grown in a glucose-salts medium (C medium) (7). The cells were harvested while in the log phase, at a density of 10^9 cells/ml, and washed with 0.01 M tris (hydroxymethyl) aminomethane buffer at pH 7.3 containing 0.05 M KCl (tris-KCl). The cells were disrupted by alumina grinding. The grindate was suspended in tris, 0.01M $MgCl_2$, and DNAase was added (10 μg/ml). Alumina, unbroken cells, and membranous material were removed by centrifugation for 15 minutes at 12,000 RPM in an SS-34 rotor. The supernatant was then centrifuged for 60 minutes at 38,000 RPM in the 40.3 rotor of a Spinco preparative ultracentrifuge, thereby pelleting the majority of the 70S and 100S ribosomes.

2. *Separation of Different Types of Ribosomes*

(*a*) *Complete separation procedure.* The resulting supernatant from section 1 was centrifuged for 3 hours at 38,000 RPM to pellet the native 30S and 50S ribosomes. The pellet was dissolved in tris, 0.01 M $MgCl_2$, and separation of the remaining 70S ribosomes from the 30S and 50S particles was achieved by sucrose density gradient centrifugation. 1.5 ml of the ribosome solution + 1.5 ml of 2 per cent sucrose were layered onto 26 ml. of a 3 to 20 per cent sucrose gradient. All solutions were 0.01 M in both tris and $MgCl_2$. The ribosomes were sedimented through the gradient by centrifugation for 8 hours at 24,000 RPM in the SW-25 rotor. Collection of fractions from the tube and analysis of ultraviolet absorbancy were done as previously described (8). Those fractions containing 30S and 50S particles were pooled and concentrated by centrifugation in the 40.3 rotor and resuspension of the pellets in a smaller volume of tris, 0.01 M $MgCl_2$. Fractions containing 70S ribosomes were similarly pooled and concentrated. The 70S ribosomes were dissociated to form derived 30S and 50S particles by dialysis of the concentrated solution against tris, 10^{-4} M $MgCl_2$ for 7 hours. A second sucrose gradient centrifugation produced virtually complete resolution of the 50S and 30S ribosomes (Fig. 1).

(*b*) *Partial separation procedure for preparation of concentrated native 30S and 50S ribosomes.* This method and the one immediately following were used solely for studying the combining properties of native and derived 30S and 50S ribosomes.

The resulting supernatant from section 1 was further enriched for native 30S and 50S ribosomes by a sucrose density gradient centrifugation. The gradient was formed by placing 2.5 ml of 10 per cent sucrose in the mixing chamber and 2.5 ml of ribosomes in the other chamber. Both solutions were in tris, 0.01 M $MgCl_2$. The ribosomes were sedimented through this 0 to 10 per cent sucrose gradient for 12 hours at 38,000 RPM in the SW-39 rotor. Approximately 0.5 ml fractions were collected by placing a siphon just above the pellet. The fractions were then examined by ultracentrifugation employing

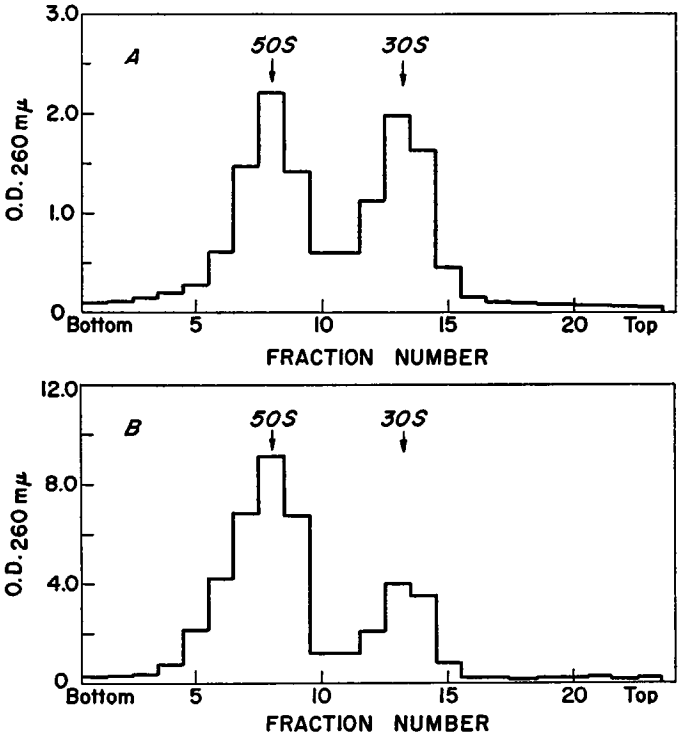

FIGURE 1 Separation of 30S and 50S ribosomes by sucrose gradient centrifugation.
(A) Native particles, Mg^{++} concentration 10^{-2} M,
(B) derived particles, Mg^{++} concentration 10^{-4} M.

the ultraviolet optical system of the model E ultracentrifuge, and those which contained primarily 30S and 50S particles were pooled.

(*c*) *Preparation of concentrated derived 30S and 50S particles.* The pellet containing 70S and 100S ribosomes (*cf.* section 1) was dissolved in tris, 10^{-4} M $MgCl_2$ and dialyzed against this solvent for 7 hours. This was sufficient time to produce complete dissociation of 70S particles to 30S and 50S particles (Fig. 4E).

3. *Preparation and Characterization of Ribonucleic Acid.* RNA was prepared from each type of purified ribosome (*cf.* section 2*a*) by treating the ribosome suspension with duponol and then phenol (9). Sedimentation velocity analysis of each RNA preparation was done at a concentration of 35 to 40 μg/ml with the ultraviolet absorption optical system of the model E ultracentrifuge.

RESULTS

1. *RNA Content of the Various Classes of Ribosomes.*

The results of the sedimentation velocity runs on the RNA isolated from the various ribosomes are depicted in Figs. 2 and 3. Whereas the derived 50S ribosomes

contained 23S RNA, as well as a small amount of heterogeneous material (approximately 15 to 20S), native 50S ribosomes contained only 16S RNA. The native and derived 30S ribosomes both contained only 16S RNA.

With regard to the type of RNA present in 30S particles and in derived 50S particles, these results are in agreement with the work of Kurland (10). From the molecular weight studies performed by him, and from the ribosome molecular weights reported by Tissieres, *et al.* (1), it is clear that both types of 30S particles contain one molecule of 16S RNA of molecular weight 5×10^5, and that a derived 50S ribosome has one molecule of 23S RNA of molecular weight 1.1×10^6.

If the 16S RNA present in native 50S ribosomes has a molecular weight proportional to its sedimentation coefficient, it follows that a native 50S ribosome must contain two RNA molecules of molecular weight 5×10^5. That the isolated 16S RNA does in fact represent the size of RNA molecules present in the intact particle rather than a product of the breakdown of 23S RNA appears certain for the following reasons:

1. The 23S RNA extracted from derived 50S particles was prepared concurrently and under the same conditions as the 16S RNA obtained from native 50S ribosomes.

2. The ultracentrifugal homogeneity of the 16S RNA indicates that it is not a product of random breakdown.

3. The observation of Kurland (10), which we have confirmed, that in the spontaneous breakdown of 23S RNA, discrete 16S RNA subunits do not result. Instead, fragments are produced which vary continuously in their sedimentation coefficients.

2. *Combining Ability of the Various Classes of Ribosomes.*

The native and derived ribosomes also differed in their ability to form 70S particles. Solutions enriched for native 30S and 50S ribosomes and at a concentration of 8 mg/ml were prepared as described in sections 1 and 2 *b* of Materials and Methods. As shown in Fig. 4, A-D; no increase in the amount of 70S particles resulted after 12 to 26 hours of incubation in 0.01 M Mg^{++}. This result was obtained for different preparations containing varying amounts of 70S ribosomes, thereby ruling out the possibility that combination did not take place due to an equilibrium having been established prior to the "zero time" ultracentrifuge run (Fig. 4, A, C).

In contrast, derived 30S and 50S ribosomes (prepared according to section 2 *c* of Materials and Methods) combined almost completely to form 70S and 85S particles under identical incubation conditions (Fig. 4, E, F). The most probable explanation for the persistence of a small 50S peak is that the amounts of derived 30S and 50S particles used in the experiment were not exactly stoichiometric, 50S particles being slightly in excess. The derived particles retained their ability to

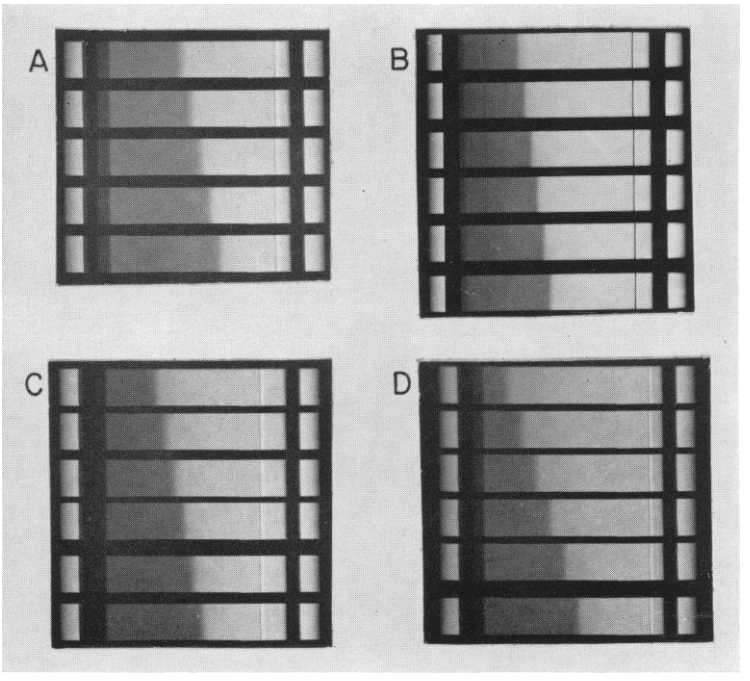

FIGURE 2 Sedimentation of RNA extracted from various purified ribosomes, ultraviolet absorption photographs.

(A) RNA from native 30S ribosomes.
Rotor speed, 59,780 RPM,
temperature of run, 5.3°C,
interval between pictures, 4 minutes,
$S_{20} = 16.8$ S.

(B) RNA from derived 30S ribosomes.
Rotor speed, 59,780 RPM
temperature of run, 12.1°C,
interval between pictures, 2 minutes,
$S_{20} = 16.1$ S.

(C) RNA from native 50S ribosomes.
Rotor speed, 59,780 RPM
interval between pictures, 4 minutes,
$S_{20} = 16.9$ S.

(D) RNA from derived 50S ribosomes.
Rotor speed, 59,780 RPM
temperature of run, 6.0°C,
$S_{20} = 23.7$ S[1]. Sedimentation proceeded from right to left in all pictures Time sequence of exposures from bottom to top.

[1] Only the sedimentation coefficient of the main component could be accurately measured.

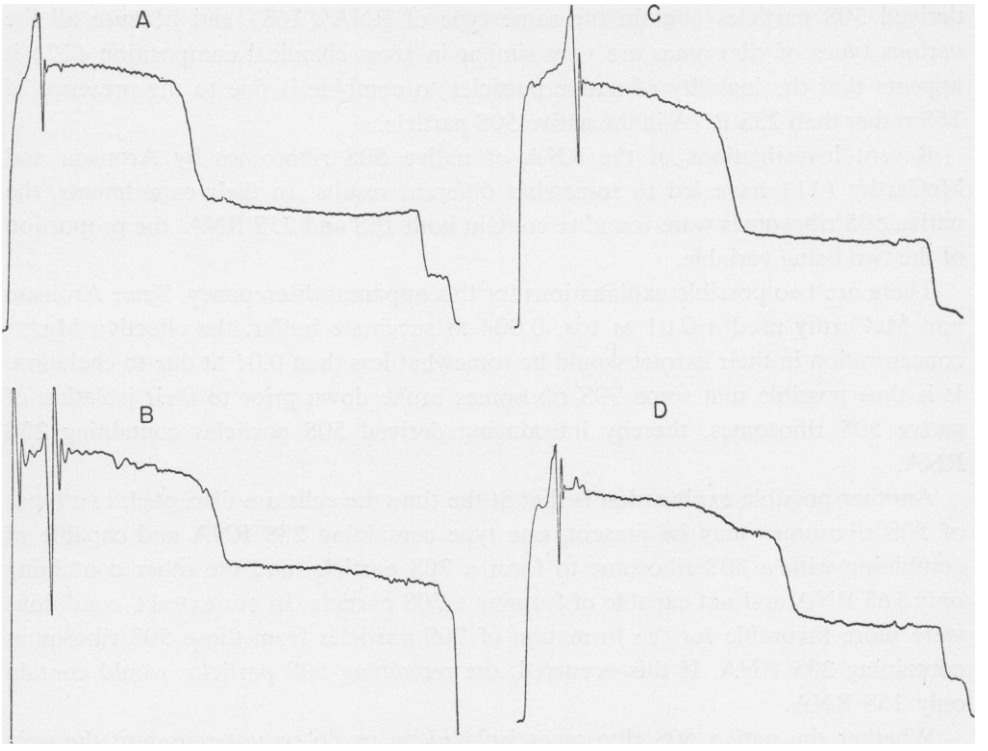

FIGURE 3 Sedimentation of RNA extracted from various purified ribosomes, densitometer tracings. (A) RNA from native 30S ribosomes. (B) RNA from derived 30S ribosomes, (C) RNA from native 50S ribosomes, (D) RNA from derived 50S ribosomes. Sedimentation boundaries are shown approximately 30 minutes after acceleration of the rotor was completed.

combine for at least 5 days, a period well in excess of that required for the separation of native 30S and 50S ribosomes from a bacterial extract. This effectively rules out the possibility that the observed difference in combining ability is due to the inactivation of a labile "combining factor."

DISCUSSION

The native and derived 50S ribosomes clearly differ with respect to the size of RNA contained in them—the native 50S particles containing exclusively 16S RNA and the derived 50S particles primarily 23S RNA. In addition we have noted that no 70S ribosomes are formed upon incubation of native 50S and 30S ribosomes in 10^{-2} M Mg^{++}, whereas formation of 70S ribosomes from derived 50S and 30S ribosomes is virtually complete under these conditions. Because the native and

derived 30S particles contain the same type of RNA (16S) and because all the various types of ribosomes are very similar in gross chemical composition (12) it appears that the inability of native particles to combine is due to the presence of 16S rather than 23S RNA in the native 50S particle.

Recent investigations of the RNA of native 50S ribosomes by Aronson and McCarthy (11) have led to somewhat different results. In their experiments, the native 50S ribosomes were found to contain both 16S and 23S RNA, the proportion of the two being variable.

There are two possible explanations for this apparent discrepancy. Since Aronson and McCarthy used a 0.01 M tris, 0.004 M succinate buffer, the effective Mg^{++} concentration in their extract would be somewhat less than 0.01 M due to chelation. It is thus possible that some 70S ribosomes broke down prior to their isolation of native 50S ribosomes, thereby introducing derived 50S particles containing 23S RNA.

Another possible explanation is that at the time the cells are disrupted, two types of 50S ribosomes may be present, one type containing 23S RNA and capable of combining with a 30S ribosome to form a 70S particle, and the other containing only 16S RNA and not capable of forming a 70S particle. In our extract, conditions were more favorable for the formation of 70S particles from those 50S ribosomes containing 23S RNA. If this occurred, the remaining 50S particles would contain only 16S RNA.

Whether the native 50S ribosomes isolated by us do or not represent the only type of free 50S ribosomes in *E. coli,* the nature of their RNA is of considerable interest. One of the major questions regarding ribosome biosynthesis is whether the native 30S and 50S ribosomes are precursors of the 70S ribosomes. Because the 70S ribosomes contain 23S RNA, and the isolated native 50S and 30S particles contain only 16S RNA, the following definite statement can be made:

Either the entry of native 50S particles into 70S ribosomes is accompanied by an increase in the degree of polymerization of their RNA ($16S + 16S \rightarrow 23S$) or native 50S ribosomes are not the precursors of 70S ribosomes.

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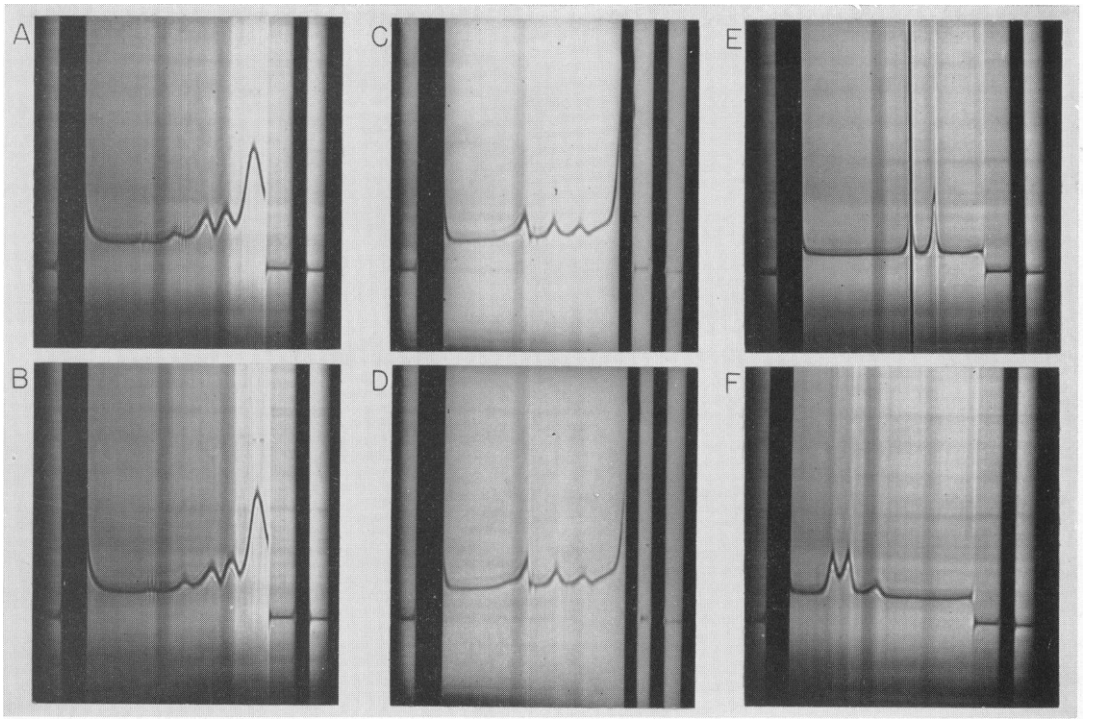

FIGURE 4 Sedimentation patterns of 30S and 50S ribosomes before and after incubation in 0.01M $MgCl_2$.

(A) Native 30S and 50S particles before incubation. Picture taken 18 minutes after acceleration was completed.

(B) Native 30S and 50S particles after 12 hours in 10^{-2}M Mg^{++}. Picture taken 18 minutes after acceleration was completed.

(C) Native 30S and 50S particles before incubation. Picture taken 16 minutes after acceleration was completed.

(D) Native 30S and 50S particles after 26 hours in 10^{-2}M Mg^{++}. Picture taken 16 minutes after acceleration was completed.

(E) Derived 30S and 50S particles before incubation. Picture taken 14 minutes after acceleration was completed. Measured sedimentation coefficients: 28S and 40S.[2]

(F) 70S and 85S particles resulting from 11 hour incubation of derived 30S and 50S ribosomes in 10^{-2}M Mg^{++}. Picture taken 14 minutes after acceleration was completed. Measured sedimentation coefficients: 52S, 67S, and 75S.[2] Rotor speed in all cases was 50,740 RPM. Temperature of runs, 3–6°C, ribosome concentration, 8 mg/ml. Sedimentation proceeded from right to left.

[2] The deviation of these values from the known s_w values of 30, 50, 70, and 85 can be attributed to concentration dependence of s.

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