The Atypical Ribosomal RNA Complement of *Rhodopseudomonas spheroides*

By T. G. LESSIE*

Microbiology Unit, Department of Biochemistry, University of Oxford

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SUMMARY

Ribosomes and phenol-purified RNA were prepared from *Rhodopseudomonas spheroides* and were characterized by sucrose density-gradient centrifugation and sedimentation-velocity centrifugation. The ribosomes extracted from *R. spheroides* correspond to the 30S, 50S and 70S ribosomes derived from other bacteria. Under appropriate conditions polyribosomes were extracted. The primary ribosomal RNA of *R. spheroides* corresponds to the 16S particle of other bacteria. *Rhodopseudomonas spheroides* was devoid of the 23S ribosomal RNA typically found in other bacteria. Preparations of phenol-purified *R. spheroides* RNA contain, in addition to 16S RNA, smaller amounts of an RNA component which sediments more slowly than the 16S unit, with a sedimentation coefficient of about 14–15S. Of several other Athiorhodaceae examined, only *Rhodopseudomonas capsulata* had an unusual RNA complement like that of *R. spheroides*.

INTRODUCTION

The ribosomal RNA isolated from bacteria consists typically of components with sedimentation coefficients of 16S and 23S. In *Escherichia coli* the 16S particle derives both from 30S and 50S ribosomes; the 23S particle derives only from 50S ribosomes (Kurland, 1960). The 30S and 50S ribosomes aggregate in appropriate magnesium concentrations to form 70S ribosomes which function in protein synthesis.

The studies of Aronson & McCarthy (1961) have suggested that 16S and 23S ribosomal RNAs are built from multiples of a single subunit, the 23S particle being viewed as a dimer of 16S RNA. This view is supported by the work of Spahr & Tissieres (1959), which showed that the 30S and 50S ribosomes of *Escherichia coli* had similar nucleotide compositions, and the work of Petermann & Pavlovec (1963), which indicated that there was interconversion between the 18S and 28S RNA particles of rat liver ribosomes. On the other hand, Aronson (1962, 1963) showed for several bacteria that the RNAs from 30S and 50S ribosomes differed in base sequence. Yankofsky & Spiegelman (1963) reported that 16S and 23S ribosomal RNAs hybridized with different sites on the bacterial genome. These observations imply that 16S and 23S RNA particles are distinct units. The results reported in the present work show that *Rhodopseudomonas spheroides* contains a usual complement

* Present address: Department of Biological Sciences, Purdue University, Lafayette, Indiana.

of ribosomes although it has only one of the usual ribosomal RNA particles, namely that corresponding to the 16S ribosomal RNA of *E. coli*. This suggests that ribosomes can be built up from the 16S unit.

METHODS

Organisms. The strains of *Rhodopseudomonas spheroides* studied were: strain L (NCIB 8253); strain L-81, which was derived from strain L and does not form photopigments; strain Ga, a spontaneous green mutant deriving from strain 2.4.1 of Professor C. B. van Niel's collection; strain M-25, which derived from strain 2.4.7 of Professor van Niel's collection, and requires uracil for growth. Strain M-25 does not grow when orotic acid is substituted for uracil; presumably its pyrimidine synthesis is blocked at some step after the formation of orotic acid.

Rhodopseudomonas spheroides strains Ga and M-25 were obtained from Professor W. R. Sistrom. All other bacteria were obtained from the collection of Dr June Lascelles. Other Athiorhodaceae used were: *Rhodospirillum rubrum* (NCIB 8255); *Rhodopseudomonas palustris*, 2.1.7; *Rhodopseudomonas gelatinosa*, 2.2.13; *Rhodopseudomonas capsulata*, 2.3.11. The last three organisms were originally from Professor van Niel's collection.

The *Escherichia coli* used was laboratory strain 88.

Growth of organisms. All the bacteria were grown to a concentration equivalent to about 100 μ g. protein/ml. in the glutamate medium of Lascelles (1959) with succinate substituted for malate (medium SG). For growth of *R. spheroides* strain M-25, the medium was supplemented with uracil (5 μ g./ml.).

Escherichia coli was grown aerobically in Erlenmeyer flasks containing volumes of media equal to 1/10 their nominal capacities. The cultures were shaken at 30° on a reciprocal shaker. Aerobic cultures of *R. spheroides* were grown under the same conditions.

Anaerobic cultures of Athiorhodaceae were incubated at 34° in flat medicine bottles or Roux bottles filled to capacity, and were illuminated at the surface of a water bath with a light intensity of about 1200 ft.-c. The cultures were aerated with a gas mixture of 5% (v/v) CO₂ + 95% (v/v) N₂.

Harvesting of cultures. Organisms were centrifuged, washed in 5×10^{-3} M-tris buffer (pH 7.4) containing 10^{-2} M-MgCl₂, and then frozen.

Radioactive labelling of bacterial RNA. *Rhodopseudomonas spheroides* strain M-25 or *Escherichia coli* was grown in SG medium containing per ml. 5 μ g. uracil and 0.3 μ c. 2-¹⁴C uracil (obtained from the Radiochemical Centre, Amersham, Buckinghamshire). The bacteria were centrifuged, washed three times in 5×10^{-3} M-tris buffer (pH 7.4) containing 10^{-2} M-MgCl₂, and then frozen.

Estimation of protein and ribonucleic acid. Cell protein was determined by the Folin phenol method (Lowry, Rosebrough, Farn & Randall, 1951) after digestion of organisms in N-NaOH for 2 hr at 40°. RNA was estimated by the orcinol method of Schneider (1945) following extraction from samples with 5% (w/v) trichloroacetic acid for 30 min. at 90°.

Preparation of bacterial extracts and phenol-purified RNA. Frozen organisms were thawed and suspended to a concentration equivalent to between 6 and 15 mg. protein/ml. in 5 ml. of 5×10^{-3} M-tris buffer (pH 7.4) containing 10^{-2} M-MgCl₂ and

2 μ g. DNAase/ml. (DNAase from Worthington Biochemical Co., New Jersey, U.S.A.). The suspensions were passed through a cold French press under 6000 lb./in.² pressure. The pressed material was incubated for 10 min. at room temperature (about 23°) to allow DNA degradation to proceed. All further steps were carried out at 4°. A portion of the pressed material was centrifuged for 10 min. at 10,000g to remove unbroken organisms and debris. The supernatant fluid was pipetted off, dialysed overnight against 1000 times its volume of 5×10^{-3} M-tris buffer (pH 7.4) containing specified amounts of MgCl₂, and used as a source of ribosomes. Under these conditions at least 80 % of the bacterial RNA was recovered in the dialysed supernatant fluid.

The portion of the pressed material left uncentrifuged was shaken with 1/25 its volume of sodium laurylsulphate for 10 min. to dissociate protein from RNA, and the solution deproteinized by the phenol method of Schramm & Gierer (1956). The phenol-purified RNA thus obtained was dialysed overnight against 1000 times its volume of 5×10^{-3} M-tris buffer (pH 7.4) containing specified amounts of MgCl₂. Samples of phenol-purified RNA were occasionally frozen before use; this did not alter the sedimentation behaviour of the RNA.

Preparation of polyribosome extracts. Bacteria were suspended to a concentration equivalent to about 50 mg. protein/ml. in 5 ml. of 5×10^{-3} M-tris buffer (pH 7.4) containing 2×10^{-3} M-MgCl₂ and 2 μ g. DNAase/ml. The bacterial suspension was passed through a cold French press under a pressure of about 1000 lb./in.², and the pressed material was incubated for 10 min. at room temperature to allow DNA degradation to proceed. All further steps were carried out at 4°. The pressed material was centrifuged for 10 min. at 10,000g. The supernatant fluid was discarded, and the pellet was suspended in 3 ml. of 5×10^{-3} M-tris buffer (pH 7.4) containing 2×10^{-3} M-MgCl₂ and 0.3 % (w/v) sodium deoxycholate. After incubation for 5 min. to solubilize the RNA, the suspension was centrifuged for 10 min. at 10,000g. The pellet was discarded, and the supernatant fluid used as a source of polyribosomes. 5–10 % of the bacterial RNA was recovered in the supernatant fluid.

Sucrose gradient centrifugation. Linear sucrose gradients (5 ml.) were prepared in plastic centrifuge tubes appropriate to the SW-39 swinging bucket Spinco rotor by using an apparatus like that described by Britten & Roberts (1960). Portions (0.2 ml.) of crude bacterial extract or phenol-purified RNA followed by 0.1 ml. of sucrose solution (4 or 5 % sucrose for gradients increasing, respectively, from 5 or 15 % sucrose) were drawn into a pipette with a capillary tip. The contents of the pipette were layered on to a sucrose gradient with the same buffer and Mg composition as the RNA sample, and the tubes were centrifuged for specified times at 38,000 rev./min. and 4° in a Spinco model L ultracentrifuge. After centrifugation the tubes were fitted with a stopper attached by tubing to a drawn 10 ml. syringe. The bottoms of the plastic tubes were coated externally with silicone grease and punctured with a small-bore syringe needle. Twenty to thirty 5-drop samples were collected from the gradients by gradually closing the syringe. Portions (0.05 ml.) of each sample were assayed for radioactivity (an Isotopes Development Laboratories liquid scintillation counter was used to measure radioactivity). The remaining fractions were diluted with water, and their absorbancy at 260 m μ was determined.

Analytical ultracentrifugation. Samples of phenol-purified RNA prepared in 5×10^{-3} M-tris buffer (pH 7.4) containing specified amounts of MgCl_2 were centrifuged at 20° in a Spinco model E ultracentrifuge. An optical cell with a light path of 12 mm. was used. For schlieren optics the RNA concentration was between 1 and 3 mg./ml., and the bar angle was set between 30 and 60° , depending upon the sensitivity required. For ultraviolet optics the RNA concentration was about $50 \mu\text{g./ml.}$ The photographic plates obtained from experiments with u.v. optics were analysed by means of a Spinco Analytrol photodensitometer equipped with a microanalyser attachment.

Rhodopseudomonas spheroides RNA, like *Escherichia coli* RNA, sediments more rapidly the higher the magnesium concentration and the smaller the RNA concentration. Since the experiments did not depend upon precise knowledge of sedimentation coefficients, apparent values have been given without correcting to infinite dilution or taking into account the effect of magnesium concentration.

RESULTS

Sucrose-gradient centrifugation

Phenol-purified RNA was prepared from *Escherichia coli* mixed with aerobically or anaerobically grown *Rhodopseudomonas spheroides*, and was characterized by sucrose-gradient centrifugation. The *R. spheroides* RNA included RNA corresponding to the 16S RNA particle of *E. coli* and smaller amounts of RNA which sedimented less rapidly than the 16S unit, but no RNA corresponding to the 23S *E. coli* unit. This is shown in Fig. 1*a* for a mixture of about 95% *E. coli* RNA and 5% ^{14}C -uracil-labelled RNA from *R. spheroides* strain M-25, in 10^{-4} M- MgCl_2 . The results were the same with RNA obtained from ^{14}C -uracil-labelled *E. coli* combined with excess *R. spheroides*. This observation rules out the possibility that the absence of *R. spheroides* 23S RNA was due to degradation by ribonuclease. In 10^{-2} M- MgCl_2 , *R. spheroides* RNA tended to aggregate to form RNA broadly sedimenting throughout the 23S region without a well-defined peak (see Fig. 1*b*).

Similar characterization of a mixture of ribosomes from mixtures of *Escherichia coli* and ^{14}C -uracil-labelled *Rhodopseudomonas spheroides* in 10^{-4} M- MgCl_2 indicated that *R. spheroides* had a complement of ribosomes corresponding to the 30S and 50S ribosomes of *E. coli* (see Fig. 2*a*). It should be noted that the 50S ribosomes of *R. spheroides* sedimented slightly behind the corresponding *E. coli* ribosomes. In 10^{-2} M- MgCl_2 , *R. spheroides* ribosomes aggregated to form a ribosomal RNA particle corresponding to the 70S unit of *E. coli* (see Fig. 2*b*). Like the 50S ribosomes, the *R. spheroides* 70S ribosomes sedimented behind the corresponding *E. coli* particles. Furthermore, the *R. spheroides* 70S ribosomes did not dimerize with the more abundant *E. coli* 70S ribosomes to form 100S particles.

Ribosomes sedimenting more rapidly than the 70S units were obtained from *Rhodopseudomonas spheroides* by disrupting the organisms at low pressures in a French press and treating the particulate fraction of the product with sodium deoxycholate to solubilize the RNA particles (see Fig. 3). This class of ribosomes is analogous to the polyribosomal RNA of other investigators (see Schlessinger, 1963). When *R. spheroides* was disrupted at the usual pressures (about 6000 lb./in.²) in 2×10^{-3} M- MgCl_2 no ribosomes sedimenting ahead of the 70S units were detected

in either the sodium deoxycholate-treated fraction or the soluble fraction of the pressed material.

Sedimentation-velocity centrifugation

Ultraviolet optics. Resolution of *Rhodopseudomonas spheroides* ribosomal RNA by analytical ultracentrifugation and the use of ultraviolet (u.v.) optics confirmed that *R. spheroides* was devoid of the usual 23S ribosomal RNA. For example, preparations of phenol-purified RNA from *R. spheroides* strain Ga in 10^{-4} M-MgCl₂ appeared to contain a single species of ribosomal RNA (see Fig. 4a). This RNA corresponded

Fig. 1. $2\text{-}^{14}\text{C}$ -uracil-labelled organisms from 30 ml. of an anaerobic *Rhodopseudomonas spheroides* culture and unlabelled organisms from 300 ml. *Escherichia coli* culture were treated together to obtain a crude ribosome extract (see Methods). A portion of the ribosome extract was further treated to obtain phenol-purified RNA. One sample of the mixture of *E. coli* and *R. spheroides* phenol-purified RNAs was dialysed overnight against tris buffer containing 10^{-4} M-MgCl₂; another sample was dialysed against buffer containing 10^{-2} M-MgCl₂. Samples (0.2 ml.) of the RNA dialysed against 10^{-4} M-MgCl₂ (about 600 $\mu\text{g.}$ RNA) were centrifuged for 5 hr through linear 5–15% (w/v) sucrose gradient mixtures containing 10^{-4} M-MgCl₂ (Fig. 1a). Samples (0.2 ml.) of the RNA dialysed against 10^{-2} M-MgCl₂ were centrifuged for 4 hr through 5–20% (w/v) sucrose gradient solutions containing 10^{-2} M-MgCl₂ (Fig. 1b). Fractions were collected from the gradients. *R. spheroides* RNA (only about 5% of the RNA applied to the gradients) was assayed by determining radioactivities of the fractions. *E. coli* RNA was determined by measuring absorbance of the same fractions at 260 mμ.

to the 16S unit of *Escherichia coli* (see Fig. 4b). At the same magnesium concentration, 23S RNA comprised a major part of the *E. coli* ribosomal RNA. When *R. spheroides* RNA was prepared in 10^{-2} M-MgCl₂ the presence of trace amounts of RNA corresponding to *E. coli* 23S ribosomal RNA was noted (see Fig. 4c, d). Close examination of the sedimentation patterns of *R. spheroides* RNA revealed a slight

deflexion in the profile of the 16S boundary, indicating a heterogeneity of the '16S' RNA.

Schlieren optics. Characterization of *Rhodopseudomonas spheroides* RNA by analytical ultracentrifugation and schlieren optics confirmed that the occurrence of *R. spheroides* '23S' ribosomal RNA was strictly dependent upon high magnesium concentration (see Figs. 5, 6). In addition, sedimentation analysis with schlieren optics clearly showed the presence of a ribosomal RNA which sedimented less rapidly than did 16S RNA. In contrast, identical analyses of *Escherichia coli* RNA showed that 23S RNA occurred in solutions of low magnesium concentration (see Fig. 5). Furthermore, no particle corresponding to the slowly sedimenting *R. spheroides* ribosomal RNA was observed. We interpret these data to indicate that the basic unit of ribosomal RNA in *R. spheroides* is the 16S particle.

Fig. 2. A portion of the mixture of *Escherichia coli* and *Rhodopseudomonas spheroides* ribosomes described in Fig. 1 was divided into two portions. One was dialysed against tris buffer containing 10^{-4} M-MgCl₂ and the other against buffer containing 10^{-2} M-MgCl₂. Samples (0.2 ml.) of the ribosome mixture dialysed against 10^{-4} M-MgCl₂ were centrifuged for 2.5 hr through 5–20 % (w/v) linear sucrose gradient solutions containing 10^{-4} M-MgCl₂ (Fig. 2a). Samples of the ribosome mixture dialysed against 10^{-2} M-MgCl₂ were centrifuged for 2.5 hr through 15–30 % sucrose gradient solutions containing 10^{-2} M-MgCl₂ (Fig. 2b). Fractions were collected, and their radioactivities and 260 mμ extinction were measured.

Ribosomal RNA of other Athiorhodaceae

Phenol-purified RNA specimens from *Rhodospirillum rubrum* and the *Rhodopseudomonas* species *R. palustris*, *R. gelatinosa*, and *R. capsulata*, as well as those of *R. spheroides* strains L and L-81, were characterized in 10^{-4} M-MgCl₂ by analytical ultracentrifugation and the use of u.v. optics. Only *R. capsulata* and the *R. spheroides* strains L and L-81 had the unusual RNA complement characteristic of *R. spheroides* strains Ga and m-25. The other Athiorhodaceae had a ribosomal RNA complement like that of *Escherichia coli*. Phenol-purified RNA from *R. capsulata* strain 2.3.11

was further characterized by sucrose density-gradient centrifugation and analytical ultracentrifugation with schlieren optics. Both these methods confirmed that *R. capsulata* had a ribosomal RNA complement like that of *R. spheroides*.

Fig. 3

Fig. 3. An anaerobic culture (900 ml.) of *R. spheroides* strain Ga (see Methods) was poured on to 300 g. crushed ice with 2.4 ml. M-MgCl_2 and 6 ml. $2 \times 10^{-1} \text{ M-NaN}_3$. The organisms were centrifuged, and washed in $5 \times 10^{-3} \text{ M-tris}$ buffer (pH 7.4) containing $2 \times 10^{-3} \text{ M-MgCl}_2$ and 10^{-3} M-NaN_3 . The washed bacteria were treated to obtain a polyribosome extract (see Methods). Portions (0.2 ml.) of the extract were applied to 15–30 % (w/v) linear sucrose gradient solutions containing $2 \times 10^{-3} \text{ M-MgCl}_2$. The gradient samples were centrifuged for 90 min. at 38,000 rev./min. Fractions were collected and their 260 $\text{m}\mu$ extinctions determined.

Fig. 4

Fig. 4. Organisms from 600 ml. of an anaerobic culture of *Rhodopseudomonas spheroides* were treated to obtain phenol-purified RNA. Phenol-purified RNA was also prepared from 600 ml. of a culture of *Escherichia coli*. One portion of each RNA sample was dialysed against tris buffer containing 10^{-4} M-MgCl_2 . Another portion of each sample was dialysed against buffer containing 10^{-2} M-MgCl_2 . The dialysed samples were diluted in buffers containing the same concentrations of MgCl_2 , to give final RNA concentrations of about 50 $\mu\text{g./ml.}$ The diluted samples were centrifuged at 50,740 rev./min. in an analytical ultracentrifuge fitted for u.v. optics. The sedimentation patterns shown were obtained 24 min. after reaching full speed. (a) *R. spheroides* RNA, 10^{-4} M-MgCl_2 ; (b) *E. coli* RNA, 10^{-4} M-MgCl_2 ; (c) *R. spheroides* RNA, 10^{-2} M-MgCl_2 ; (d) *E. coli* RNA, 10^{-2} M-MgCl_2 . The apparent sedimentation coefficients of the RNA components were as follows: (a), 5.5, 17.5; (b), 6.6, 16.3, 21.3; (c), 4.7, 21.8, 29.6; (d) 6.8, 20.6, 31.4. The boundary between the solutions and the air space in the centrifuge cells is indicated by m.

Fig. 5

Fig. 6

Fig. 5. The samples of *Rhodopseudomonas spheroides* and *Escherichia coli* used were those described in Fig. 4. Samples which had been dialysed against 10^{-4} M-MgCl₂ were diluted in buffer containing the same concentration of MgCl₂ to an RNA concentration of about 1.5 mg./ml. A mixture of *E. coli* and *R. spheroides* RNA was obtained by mixing equal volumes of the diluted samples. The mixture and the individual samples were each centrifuged at 50,740 rev./min. in an analytical ultracentrifuge with schlieren optics. The sedimentation patterns shown were obtained 32 min. after attaining full speed. (a) *R. spheroides* RNA; (b) *E. coli* RNA; (c) *R. spheroides* + *E. coli* RNAs. The apparent sedimentation coefficients of the RNA components were: (a) 3.7, 10.8, 12.8; (b) 5.6, 12.7, 17.2; (c) 3.6, 11.8, 14.2, 18.4. The *R. spheroides* RNA which sedimented between soluble RNA and 16S RNA is indicated by r.

Fig. 6. The *Rhodopseudomonas spheroides* and *Escherichia coli* RNA samples used were those described in Fig. 4. Samples which had been dialysed against tris buffer containing 10^{-2} M-MgCl₂ were diluted in buffer containing the same concentration of MgCl₂ to an RNA concentration of about 1.5 mg./ml. The mixture of *R. spheroides* and *E. coli* RNAs was prepared by mixing equal volumes of each of the diluted samples. The mixture of RNAs and the individual samples were centrifuged at 50,740 rev./min. The sedimentation patterns shown were obtained 24 min. after attaining full speed. (a) *R. spheroides* RNA; (b) *E. coli* RNA; (c) *R. spheroides* + *E. coli* RNAs. The apparent sedimentation coefficients of the RNA components were: (a) 3.2, 18.5, 19.6, 29.6; (b) 6.5, 19.5, 29.0; (c) 4.0, 17.0, 20.5, 29.0. The *R. spheroides* RNA which sedimented between soluble RNA and 16S RNA is indicated by r.

DISCUSSION

The results presented here strongly suggest that *Rhodopseudomonas spheroides* is able to form its ribosomes from a 16S ribosomal RNA unit. Studies of other bacteria which have suggested that two distinct ribosomal RNAs are necessary to form the usual complement of their ribosomes should be re-examined in the light of the present findings. The work of Petermann & Pavlovic (1963) indicated that there is conversion between the 18S and 28S ribosomal RNA of rat liver. This suggests that the difference between *R. spheroides* ribosomal RNA and that of other micro-organisms may be a difference in the ability of the respective 16S units to dimerize to 23S units. On the other hand, the RNA-DNA hybridization experiments of

Yankofsky & Spiegelman (1963) imply that 23S RNA is not a dimer of 16S RNA, but a distinct unit formed at a different site on the bacterial genome. This view is supported by data which show differences in the base sequences of the RNA from 30S and 50S ribosomes (Aronson, 1962, 1963).

The present observations that *Rhodopseudomonas spheroides* 70S ribosomes did not dimerize with an excess of *Escherichia coli* 70S particles to form 100S units, and that the 50S and 70S ribosomes of *R. spheroides* sedimented behind corresponding *E. coli* particles suggest that the *R. spheroides* 50S ribosome lacks some structural element which is present in the *E. coli* 50S particle. For several reasons it is attractive to speculate that such an element exists, and that it is a low molecular weight RNA. It is consistent with hybridization data, base sequence data, and data which suggest interconversion of 16S and 23S RNA to postulate that there are two classes of 50S ribosomes. One, represented by the 50S ribosome of *R. spheroides*, would be built from two 16S ribosomal RNA units. The other, represented by the *E. coli* 50S ribosome, might be derived from a 23S ribosomal RNA which consisted of two 16S units associated with an additional piece of RNA not present in the *R. spheroides* ribosome. This hypothesis would allow for the interconversion of 16S and 23S RNAs and for the exclusion of 16S RNA from hybridization sites available to 23S RNA.

I cannot offer any explanation for the presence in preparations of *Rhodopseudomonas spheroides* RNA of significant amounts of ribosomal RNA which sedimented more slowly than does 16S RNA. However, Yankofsky & Spiegelman (1963) observed a similar heterogeneity upon salt-gradient elution of *Bacillus megaterium* 16S RNA from methylated serum albumin columns.

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