A NEW METHOD FOR THE ISOLATION OF A 5 S RNA COMPLEX WITH PROTEINS L5, L18 AND L25 FROM *ESCHERICHIA COLI* RIBOSOMES

Ursula CHEN-SCHMEISSER and Roger A. GARRETT
Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Berlin-Dahlem, Germany

Received 9 December 1976

1. Introduction

Many studies on *Escherichia coli* ribosomes have recently been concerned with the structures of the individual protein and RNA components, or discrete groups of components, with a long-term view to build up a complete model of the ribosome (reviewed in [1,2]). A 5 S RNA–protein complex is one such unit that has been prepared by reconstituting either single proteins [3–5] or total 50 S subunit proteins [6,7] with the 5 S RNA. The former studies demonstrated that proteins L18 and L25 each bind directly to 5 S RNA. The latter showed that these two proteins, together with small amounts of a few other proteins, including L5, assemble as a group with the RNA. There is also evidence that the protein L5 binds independently and weakly to 5 S RNA [8]. Moreover, it has been implicated, although at the time incorrectly identified as L6 [3,5] in the coupling of 5 S RNA with 23 S RNA. A reconstituted 5 S RNA–protein complex, containing predominantly proteins L18 and L25, exhibited ATPase and GTPase activities [9,10] and the latter activity was indirectly attributed to L18 [11]. However, whether these enzymic activities have any biological relevance is not known (e.g., [12–14]).

In the present work, for the first time, the large scale preparation of a complex of 5 S RNA with proteins L5, L18 and L25, directly from the 50 S subunit, is described. The method involves a very mild treatment of the 50 S subunits with carrier-bound ribonuclease, in the presence of magnesium, with the subsequent separation of the 5 S RNA–protein complex in a sucrose gradient containing EDTA. A possible structural significance of this extraction procedure is discussed.

2. Materials and methods

2.1. *Preparation of the 5 S RNA–protein complex*

50 S Ribosomal subunits (4 mg/ml) were treated with carrier-bound RNAase A (Boehringer) at an enzyme : 23 S RNA ratio of 1:3000 (w/w) in 0.01 M Tris–HCl, pH 7.5, 1 mM $MgCl_2$, 0.1 M KCl using the procedure of Allet and Spahr [15], modified as described earlier [16]. The digestion was for 30 min at 0°C and the enzyme was removed by centrifugation and by agarose gel filtration [16]. 50 mg of the treated 50 S subunits were centrifuged through a 5–20% (w/w) sucrose gradient in 30 mM Tris–HCl, pH 7.5, 1 mM EDTA in a zonal rotor [16]. Three peaks were resolved containing the 13 S ribonucleoprotein, the 18 S ribonucleoprotein and the 5 S RNA–protein complex. At least 800 mg subunits can be applied to the gradient if the 5 S RNA–protein complex, only, is required. High percentage yields of the 5 S RNA and proteins were obtained.

2.2. *Preparation of 5 S RNA and proteins*

5 S RNA was prepared either by a standard procedure [17] or from the 5 S RNA–protein complex by two phenol–dodecylsulphate extractions [16]. Proteins were extracted by acetic acid extraction of the 5 S RNA–protein complex [18] with a subsequent fractionation [19] on a Sephadex G-100 column in 15% acetic acid (3 × 200 cm). The fractionated proteins were dialysed against 2% acetic acid and

lyophilised. They were identified and checked for purity by two-dimensional gel electrophoresis [20]. Protein concentrations were determined using the Folin reagent [21].

2.3. *Binding of the isolated proteins to 5 S RNA*

10 μg 5 S RNA and 10 μg unfractionated tRNA from *E. coli* were dissolved in 40 μl TMK reconstitution buffer (30 mM Tris–HCl, pH 7.4, 20 mM $MgCl_2$ and 0.30 M KCl, 6 mM 2-mercaptoethanol). Proteins were added at a 3- to 5-fold molar excess and the solution was incubated at 34°C for 45 min, cooled on ice and 10 μl 50% sucrose (w/w) containing bromophenol blue and TMK buffer was added. Complex formation was assayed by modifying the electrophoretic procedure of Schaup et al. [22]. The complex was electrophoresed in tubes (5 mm diameter) containing 5% polyacrylamide gels in 30 mM Tris–HCl, pH 7.4, 10–20 mM $MgCl_2$ and 0.2 M KCl, for 5 h at 4°C and 10 mA/tube with circulating buffer. Duplicate samples were stained overnight for RNA with Pyronin G and for protein with Coomassie Brilliant Blue, as described earlier [23].

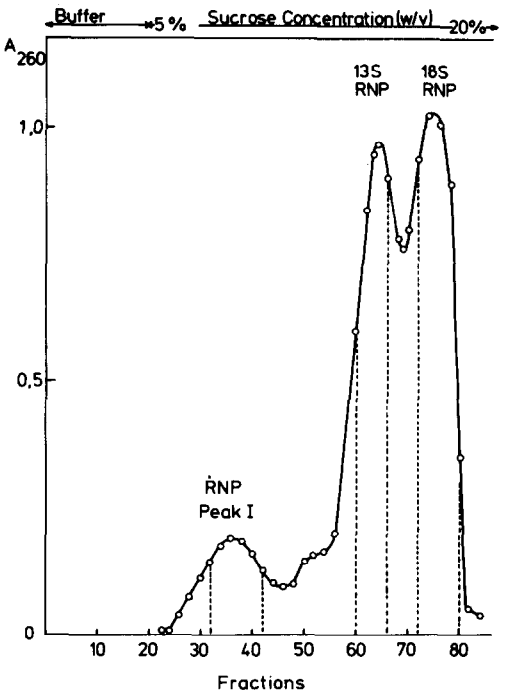


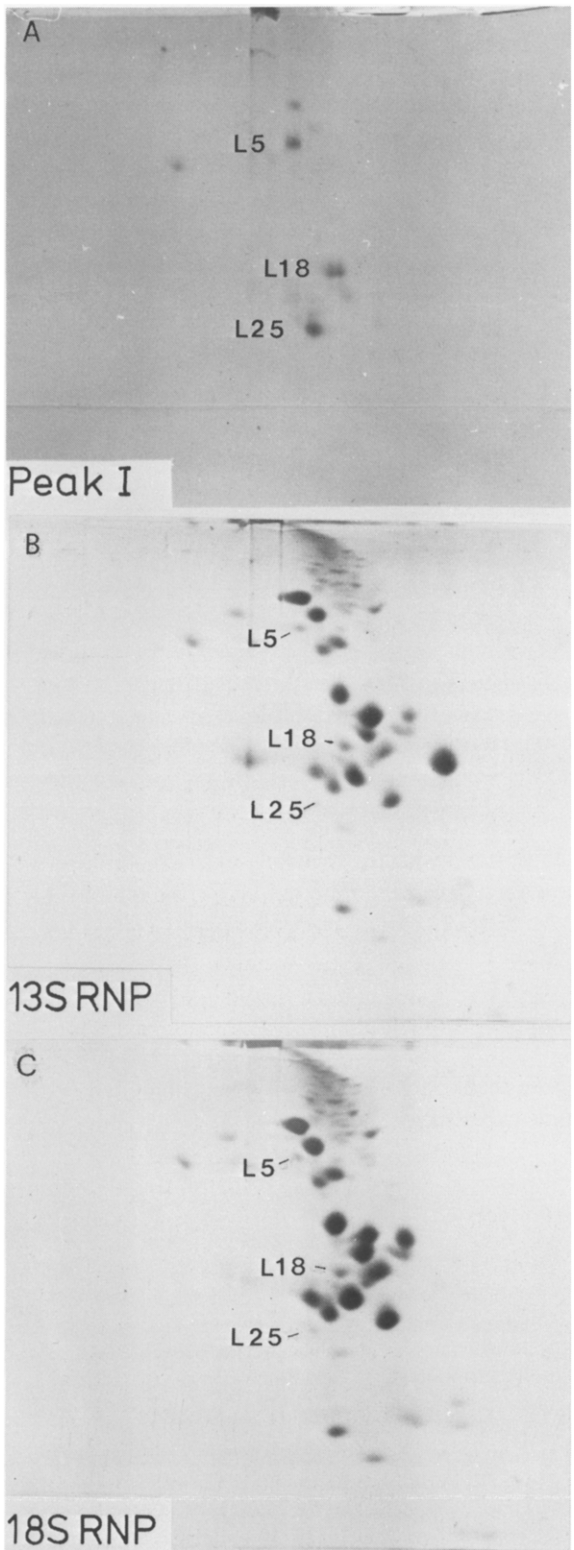
Fig.1. Separation of the 5 S RNA–protein complex (peak I) from the 13 S and 18 S ribonucleoproteins by centrifuging through a sucrose gradient in a zonal rotor.

3. Results

The separation of the 5 S RNA–protein complex from the 13 S and 18 S ribonucleoproteins in a sucrose gradient is demonstrated in fig.1. The protein content of the 5 S RNA–protein complex is shown in fig.2A. Clearly three main proteins were present, namely L5, L18 and L25. Very small amounts of other proteins, in particular L1 migrating behind the L5 (fig.2A), were also present. The almost complete removal of the three proteins from the 13 S and 18 S RNPs is shown in figs 2B and C.

In order to determine the stoichiometry of the proteins in this complex, the proteins were run in polyacrylamide disc-gels and the stained bands were densitometered using standard calibration curves for each protein. The molar ratios of the three proteins were estimated at 1:1:1 within experimental error limits of ± 15%.

The 5 S RNA complex prepared under these conditions was contaminated with low molar yields of fragments of 23 S RNA in the size range 200–400 nucleotides that contained only small amounts of bound protein. They were believed to arise from the central region of the 23 S RNA [16] and could be reduced by decreasing the extent of ribonuclease digestion without reducing the yield of the 5 S RNA–protein complex. The latter was concentrated by ultrafiltration, using an Amicon PM 10 membrane, and then purified by running through a Sephadex G-100 column (2 × 150 cm). The large fragments were excluded and the 5 S RNA–protein complex was included (see fig.3A). The 5 S RNA was deproteinised and electrophoresed in a 12% polyacrylamide gel (see fig.3B). It contained > 90% native 5 S RNA and < 10% denatured 5 S RNA. The latter could be renatured as described earlier [24]. In the presence of 8 M urea hidden breaks were not generally observed. This is consistent with the observation that 5 S RNA is highly resistant to ribonucleases in the 50 S subunit [25,26]. The protein content of the complex is shown in fig.3C. Some losses of proteins L5 and L18 occurred during the column run due to some unspecific protein migration in the presence of EDTA [16,27] to the



larger RNA fragments. However, no migration of L25 was detected. This indicates that the structure of the RNA binding site of L25 is exceptional in that it is not appreciably destabilized by removal of magnesium [16,27,28]. The molar ratio of L5:L18:L25 in the purified complex was 0.4:0.6:1.0. This was determined electrophoretically, as above and was reflected in the behaviour of the purified complex electrophoretically in that three complexes were resolved, namely (a) 5 S RNA–L5, L18, L25, (b) 5 S RNA–L18, L25 and (c) 5 S RNA–L25 (data not shown).

The proteins were fractionated by Sephadex G-100 gel filtration (see Materials and methods). In order to show that the native structures of the proteins were preserved their capacities to bind specifically to 5 S RNA were tested. Two criteria were invoked for the specificity of the protein–5 S RNA interaction.

(1) When the protein was incubated with a mixture of 5 S RNA and unfractionated tRNA, protein was detected exclusively on 5 S RNA.

(2) Ribosomal proteins known to complex exclusively with 16 S or 23 S RNA including S4, S8, L1, L2, L3 and L6, did not bind to either 5 S RNA or tRNA.

The results demonstrated that, by these criteria, proteins L18, L25 and L5 bound specifically to 5 S RNA.

4. Discussion

The present work demonstrates, for the first time, that a complex consisting of 5 S RNA and proteins L5, L18 and L25 can be isolated, in large quantities, directly from the *Escherichia coli* ribosome. Clearly, the present method can also be used for the large scale preparation of both 5 S RNA and the individual proteins L5, L18 and L25.

In an earlier study, it was shown that extensive EDTA treatment of 50 S subunits released only 5 S RNA and protein L25 [28]. The present results, in conjunction with earlier ones [16], show that the release of the 5 S RNA and proteins L5, L18 and L25

Fig.2. A, Protein contents of the ribonucleoprotein in peak I. B and C, illustrate the almost complete removal of these proteins from the 13 S and 18 S ribonucleoproteins.

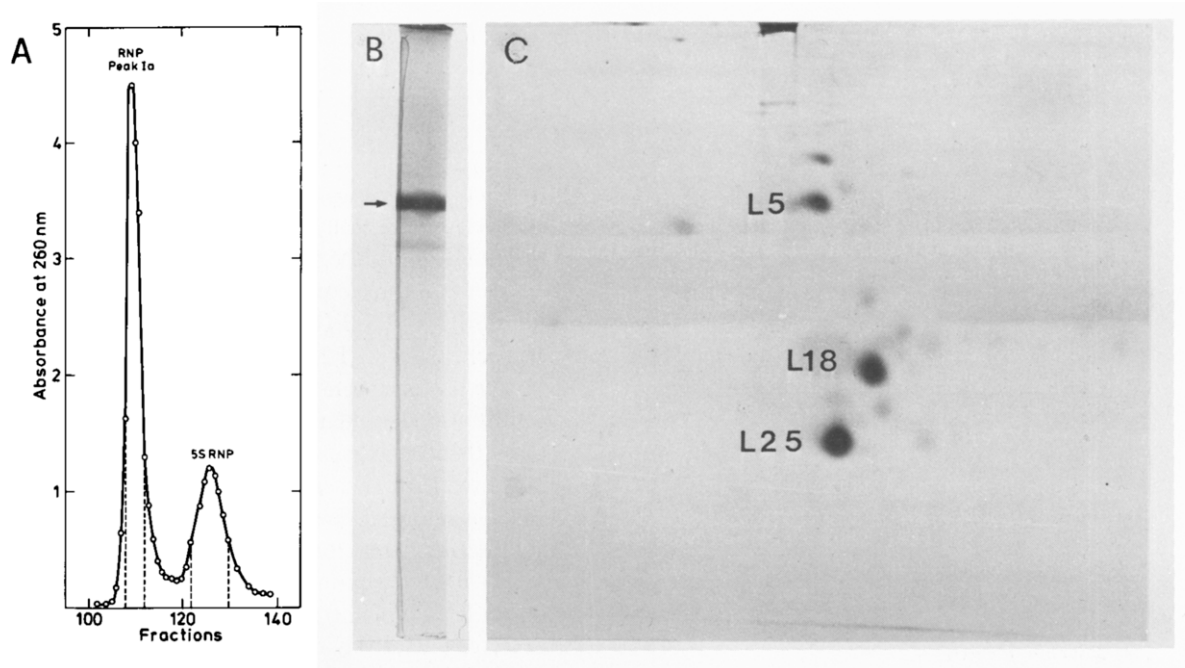


Fig.3. A, Purification of the 5 S RNA–protein complex on a Sephadex G-100 column in 40 mM Tris–HCl, pH 7.4, 1 mM EDTA at 4°C. B, 5 S RNA extracted from the 5 S RNA complex and electrophoresed in a 12% polyacrylamide gel containing 40 mM Tris–HCl, pH 7.5. The arrow indicates the 5 S RNA. The weaker, faster migrating band, contains 5 S RNA in the denatured conformation. C, The protein content of the purified 5 S RNA–protein complex.

requires, in addition, that a primary cut is made in the 23 S RNA some 1000 nucleotides from the 5′-end [16]. This suggests that the 5 S RNA is fairly intimately involved in the 50 S subunit structure: a conclusion that could explain two other results concerning the 5 S RNA–23 S RNA interaction. First, the 5 S RNA can be coupled, with the aid of proteins L5, L18 and L25, to the 3′-one-third of the 23 S RNA [3,16,29]. Second, a twelve consecutive base-complementarity exists between 5 S RNA and a sequence near the 5′-end of the 23 S RNA [30,31].

As the authors indicated [30], there is no reason why the complementarity should be significant if a better alternative pairing exists within the RNAs. Nevertheless, the proposed pairing is more impressive, energetically, than those so far proposed for intermolecular RNA–RNA interactions (e.g., [32]). If it is significant, then the 5 S RNA–protein complex could bridge the two halves of the 23 S RNA and facilitate its folding. Such a hypothesis would be compatible with the extended shape of the 5 S RNA [33], with its inaccessibility to ribonucleases in the ribosome [25,26] and with the relatively extreme conditions described here, that are required for the removal of the 5 S RNA–protein complex from the ribosome.

Acknowledgement

We thank Jack Hunter for his excellent help with some experiments.

References

[1] Zimmermann, R. A. (1974) in: Ribosomes (Nomura, M., Tissières, A. and Lengyel, P. eds) pp. 225–269, Cold Spring Harbor Press, New York.
[2] Brimacombe, R., Nierhaus, K. H., Garrett, R. A. and Wittmann, H. G. (1976) Progress in Nucleic Acid Research and Molecular Biology (Cohn, W. ed) Vol. 18, 1–44.
[3] Gray, P. N., Garrett, R. A., Stöffler, G. and Monier, R. (1972) Europ. J. Biochem. 28, 412–421.
[4] Gray, P. N., Bellemare, G., Monier, R., Garrett, R. A. and Stöffler, G. (1973) J. Mol. Biol. 77, 133–152.

[5] Feunteun, J., Monier, R., Garrett, R., Le Bret, M. and Le Pecq, J. B. (1975) J. Mol. Biol. 93, 535–541.
[6] Horne, J. and Erdmann, V. A. (1972) Mol. Gen. Genet. 119, 337–344.
[7] Burrell, H. R. and Horowitz, J. (1974) FEBS Lett. 49, 306–309.
[8] Yu, R. S. T. and Wittmann, H. G. (1973) Biochim. Biophys. Acta 324, 375–385.
[9] Gaunt-Klöpfer, M. and Erdmann, V. A. (1975) Biochim. Biophys. Acta 390, 226–230.
[10] Horne, J. and Erdmann, V. A. (1973) Proc. Natl. Acad. Sci. USA 70, 2870–2873.
[11] Roth, H. E. and Nierhaus, K. H. (1975) J. Mol. Biol. 94, 111–121.
[12] Sander, G., Marsh, R. G., Voigt, J. and Parmeggiani, A. (1975) Biochemistry 14, 1805–1814.
[13] Schrier, P. I. and Möller, W. (1975) FEBS Lett. 54, 130–134.
[14] Nierhaus, K. H. and Dohme, F. (1976) Proc. Natl. Acad. Sci. USA 73, 2221–2225.
[15] Allet, B. and Spahr, P. F. (1971) Europ. J. Biochem. 19, 250–255.
[16] Chen-Schmeisser, U. and Garrett, R. A. (1976) Europ. J. Biochem. 69, 401–410.
[17] Monier, R. and Feunteun, J. (1971) in: Methods in Enzymology, Vol. 20, Part C, pp. 494–503, Academic Press, New York and London.
[18] Hardy, S. J. S., Kurland, C. G., Voynow, P. and Mora, G. (1969) Biochemistry 8, 2897–2905.
[19] Hindennach, I., Kaltschmidt, E. and Wittmann, H. G. (1971) Europ. J. Biochem. 23, 12–16.
[20] Kaltschmidt, E. and Wittmann, H. G. (1970) Analyt. Biochem. 36, 401–412.
[21] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[22] Schaup, H. W., Green, M. and Kurland, C. G. (1970) Mol. Gen. Genet. 109, 193–205.
[23] Schulte, C., Morrison, C. A. and Garrett, R. A. (1974) Biochemistry 13, 1032–1037.
[24] Aubert, M., Scott, J. F., Reynier, M. and Monier, R. (1968) Proc. Natl. Acad. Sci. USA 61, 292–299.
[25] Reynier, M. and Forget, B. G. (1970) Biochem. Biophys. Res. Commun. 39, 114–122.
[26] Feunteun, J. and Monier, R. (1971) Biochemie 53, 657–660.
[27] Newton, I., Rinke, J. and Brimacombe, R. (1975) FEBS Lett. 51, 215–218.
[28] Garrett, R. A., Schulte, C., Stöffler, G., Gray, P. and Monier, R. (1974) FEBS Lett. 49, 1–4.
[29] Branlant, C., Krol, A., Sriwidada, J. and Brimacombe, R. (1976) Europ. J. Biochem. 70, 483–492.
[30] Herr, W. and Noller, H. F. (1975) FEBS Lett. 53, 248–252.
[31] Branlant, C., Sriwidada, J., Krol, A. and Ebel, J.-P. (1977) Europ. J. Biochem., in the press.
[32] Branlant, C., Sriwidada, J., Krol, A. and Ebel, J.-P. (1976) Nucleic Acid Res. 3, 1671–1687.
[33] Österberg, R., Sjöberg, B. and Garrett, R. A. (1976) Europ. J. Biochem. 68, 481–487.