RNA–PROTEIN INTERACTIONS IN THE RIBOSOME. BINDING OF PROTEINS L1, L3, L6, L13 AND L23 TO SPECIFIC FRAGMENTS OF THE 23S RNA*

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1. Introduction

Ten of the 33 proteins found in the 50 S subunit of the *Escherichia coli* ribosome associate independently and specifically to the 23 S ribosomal RNA [1]. The location of the binding sites for these proteins has been studied in two different ways.

Branlant et al. [2,3] have isolated ribonucleoprotein particles resulting from extensive nuclease digestion of complexes between proteins L1, L20, L23, L24 and the 23 S RNA. Sequence analysis of the RNA regions protected from digestion by the proteins enabled these authors to locate the binding sites on the 23 S RNA molecule.

On the other hand, Spierer et al. [4] have tested the association of each of the ten binding proteins with two segments of the 23 S RNA produced by limited ribonuclease digestion of the 50 S subunit. One segment sediments at 13 S, covers 40% of the 23 S RNA sequence, and derives from the 5′ end of the molecule. It contains the specific binding sites for proteins L4, L20 and L24. The other segment, which sediments at 18 S, encompasses about 60% of the 23 S RNA sequence and arises from the 3′ end of the molecule. It contains the specific binding sites for proteins L1, L2, L3, L6, L13, L16 and L23.

We report here the preparation of fragments of the 18 S RNA segment, and the distribution of binding sites for proteins L1, L3, L6, L13 and L23 on these fragments.

2. Materials and methods

^{3}H-Labelled ribosomal proteins were extracted from purified 50 S subunits with 67% acetic acid and separated by ion-exchange chromatography according to Zimmermann and Stöffler [5]. ^{14}C- or ^{32}P-labelled 18 S segments were prepared by limited digestion of 50 S subunits with immobilized pancreactic ribonuclease, following which the RNA was extracted by phenol and the 18 S segment was purified on sucrose gradients as previously described [4].

Frgaments of the 18 S RNA were produced by limited T1 ribonuclease digestion. From 50 to 500 μg of the 18 S RNA were preincubated for 20 min at 40°C in TMK buffer (50 mM Tris, pH 7.6; 20 mM $MgCl_2$; 350 mM KCl; 6 mM 2-mercaptoethanol), then chilled and incubated for 10 min at 0°C with T1 ribonuclease (Sankyo) added to a final concentration of 8 μg/ml. The reaction mixture was layered on a 12-ml 3 to 15% sucrose gradient in TMK buffer and

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sedimented 12 h at 37 000 rpm in an IEC SB283 rotor at 4°C. After fractionation of the gradient, the tubes corresponding to the various peaks were pooled and the RNA was precipitated with ethanol. The fragments were then repurified by a second round of sucrose gradient centrifugation. ^{32}P-labelled RNA fragments were fingerprinted by two-dimensional electrophoresis after complete digestion with T1 ribonuclease in the presence of alkaline phosphatase [6].

Protein–RNA complexes were prepared by incubating 50 to 100 μg of ^{14}C-labelled 18 S RNA (30–80 cpm/μg) with two molar equivalents of ^{3}H-labelled protein (800–2200 cpm/μg) for 20 min at 40°C in TMK buffer. Digestion and sucrose gradient resolution of the fragments was performed as above. Fractions of the gradient were analysed for TCA-precipitable radioactivity.

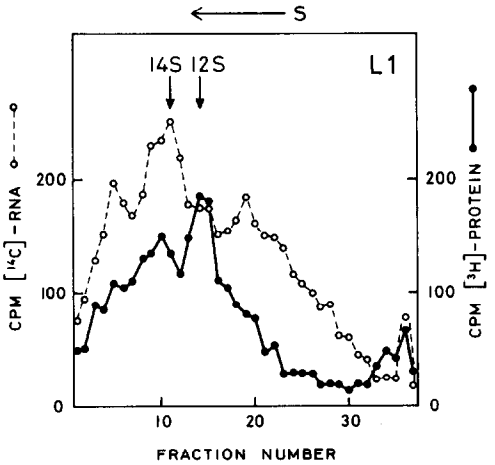
3. Results

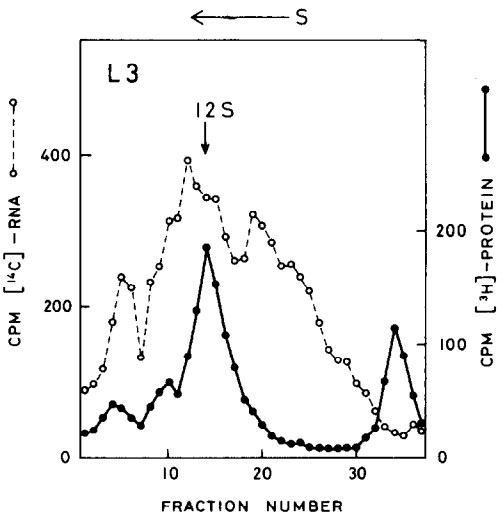
3.1. *Association of protein L1, L3, L6, L13 and L23 fragments of the 18 S segment of the 23 S RNA*

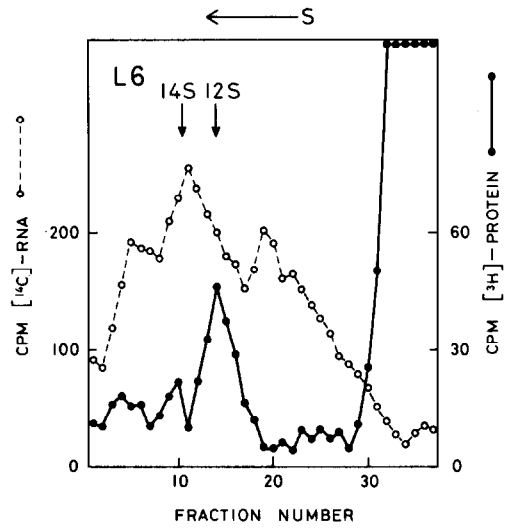
Limited digestion of the 50 S subunit with immobilized pancreatic ribonuclease cleaves the 23 S RNA into two segments which sediment at 13 S and 18 S. The 18 S segment contains the specific binding sites for L1, L2, L3, L6, L13, L16 and L23 [4]. Complexes between each of these proteins and the 18 S segment was reconstituted and subjected to limited digestion with T1 ribonuclease. Figure 1 shows the sucrose-gradient profiles of the reaction mixtures. The results for proteins L2 and L16 are not presented since significant binding of these proteins to the RNA fragments was not observed in these cases. Profiles for L1, L3 and L6 exhibit a sharp protein peak associated with RNA at about 12 S. Some binding is also noticeable at 18 S (the intact segment) and at about 14 S. On the other hand, the sedimentation profiles for L13 and L23 show protein peaks at about 8 S. Since the distribution of RNA fragments is similar in each of the five experiments, we conclude that the association of proteins L1, L3, L6, L13 or L23 does not affect the digestion of the 18 S RNA. The association of the protein peaks with different fragments indicates that the digestion products arise from specific regions of the 18 S RNA. Moreover, the fact that none of the proteins was found to bind to both the 12 S and the 8 S fragments strengthens the inference that these fragments represent different regions of the 18 S RNA. However, sequence analysis was necessary to establish this point and to locate the fragments in the 18 S RNA sequence.

3.2. *Characterization of the fragments of the 18 S RNA segment*

^{32}P-labelled 18 S RNA segment was subjected to limited T1 ribonuclease digestion under the same conditions used for the protein–RNA complexes. Figure 2 shows the sedimentation profile of the reaction mixture. Although the pattern looks complex, the







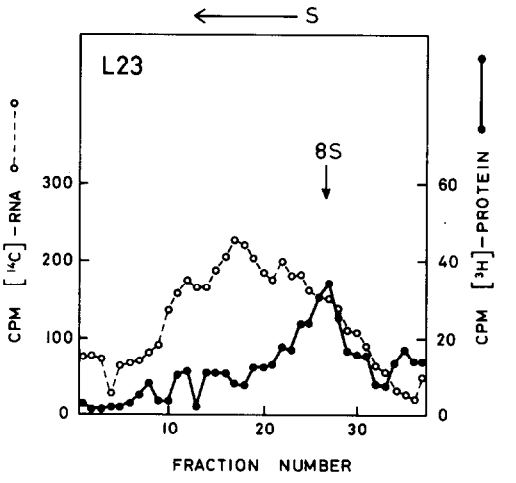
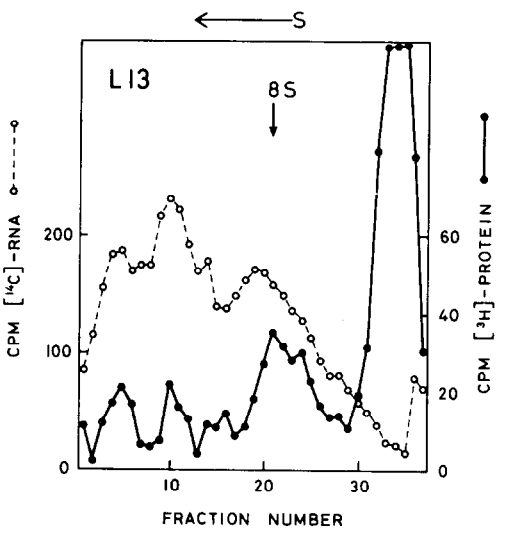


Fig.1. Sedimentation profiles of T1 ribonuclease digests of 18 S RNA–protein complexes.[14]C-Labelled 18 S RNA was incubated with two molar equivalents of [3]H-labelled protein L1, L3, L6, L13 and L23 (as indicated on each panel), digested with T1 ribonuclease, and sedimented on sucrose gradients as in Materials and methods.

distribution of peaks is very reproducible and is similar to those obtained with protein–18 S RNA complexes (fig.1). Fractions corresponding to the various peaks (designated by their approximate sedimentation coefficients) were pooled as indicated on the profile in fig.2. Each fragment was further purified by a second round of sucrose gradient centrifugation



and fingerprinted. Autoradiographs of the fingerprints of the 12 S and 8 S fragments are illustrated in fig.3. All the large and characteristic RNase T_1 digestion products from the 18 S fragment have been sequenced by Branlant and Ebel [7]; the nomenclature adopted on fig.3 is that of their catalogue. Upon limited digestion of the 50 S subunit with T_1 ribonuclease, Branlant et al. isolated a fragment denoted fragment II, which

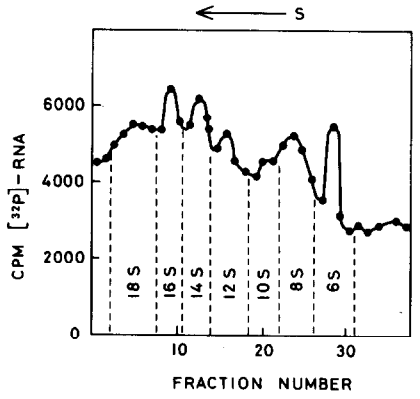


Fig.2. Digestion of [32]P-labelled 18 S RNA segment. [32]P-Labelled 18 S RNA segment was digested with T1 ribonuclease and sedimented on a sucrose gradient as in Materials and methods. Aliquots of each fraction were analysed for radioactivity and the fractions corresponding to the various peaks were pooled as indicated on the profile.

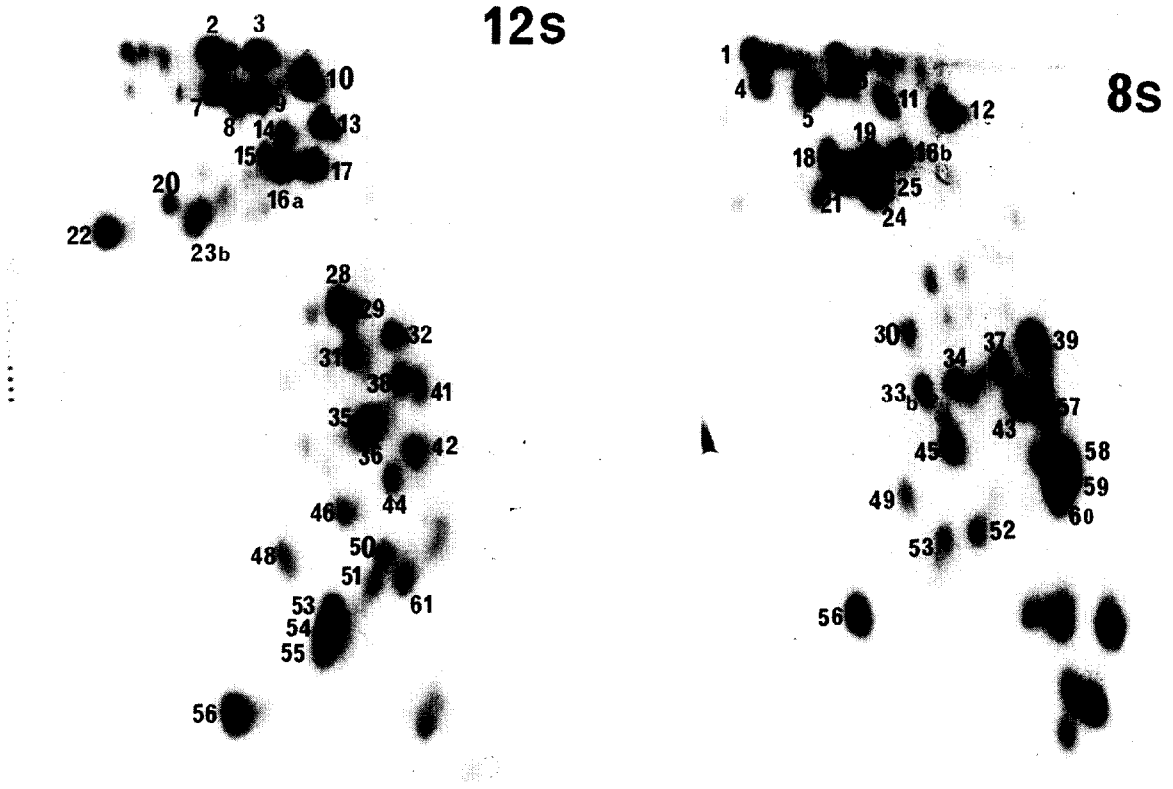


Fig.3. Fingerprints of 12 S and 8 S fragments. ^{32}P-Labelled 12 S and 8 S RNA fragments were prepared as described in Materials and methods and digested with T1 ribonuclease in the presence of alkaline phosphatase. The products were resolved by two-dimensional electrophoresis [6].

encompasses the 1000 nucleotides lying at the 3′ end of the 23 S RNA molecule (Branlant et al. in preparation). All the large RNase T_I digestion products recovered from the 12 S fragment are released by fragment II, and only few oligonucleotides from this latter are missing in the 12 S fragment. On the other hand the RNase T_I digestion products from the 8 S fragment are not released by fragment II. Since 8 S and 12 S fragments are fractionated by centrifugation in the presence of TMK buffer they could contain hidden breaks and even sequence excisions. But we can assume that the RNA sequences they contain belong: for the 12 S fragment to the 1000 nucleotides which lie at the 3′ end of the 18 S RNA segment and for the 8 S fragment of the 800 nucleotides at the 5′ end of this latter segment. Furthermore in each case the sequences represent about 90% of the corresponding section.

Since proteins L1, L3 and L6 bind to the 12 S fragment (fig.1), their binding sites must be located within the 1000 nucleotides which lies at the 3′ end of the 18 S segment. On the other hand the binding sites of proteins L13 and L23 which bind to the 8 S fragment must be located in the 800 nucleotides which lies at the 5′ end of this segment. Since according to fingerprint analysis there is no significant cross-contamination between the 12 S and 8 S fragments and since the molar ratio of protein to RNA was estimated in each case to be higher than 0.5 : 1, the assignment of a protein to a specific fragment on the sedimentation profile could not have been due to the association of the protein to a minor contaminant.

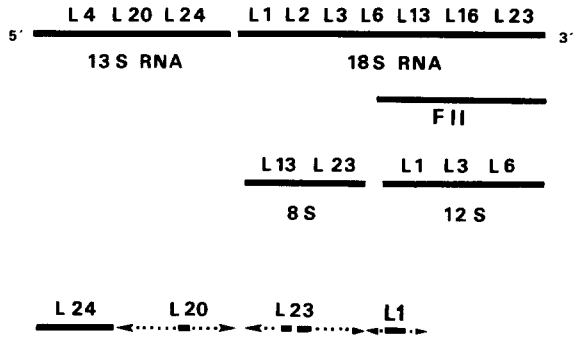


Fig.4. Relative placement of the 12 S and 8 S RNA fragment and of the binding sites for ten 50 S subunit proteins within the 23 S RNA. The upper part of the figure schematizes the distribution of protein binding sites on the 13 S and 18 S segments of the 23 S RNA molecule [4]. The results reported here enable us to position the binding sites for proteins L1, L3, L6, L13 and L23 on two sections of the 18 S segment as illustrated in the middle portion of the diagram. The location of the binding sites for proteins L1, L20, L23 and L24 as determined by Branlant et al. [2,3] is shown in the lower part of the figure.

4. Discussion

In summary, we have shown that the binding sites for proteins L1, L3 and L6 are located within the 3'-terminal 1000 nucleotides of the 18 S segment of the 23 S RNA, whereas the binding sites for proteins L13 and L23 are located within 800 nucleotides from the central portion of the 23 S RNA. These results are schematized in fig.4, together with the previous findings of Branlant et al. [2,3] and Spierer et al. [4]. There is a good agreement between this work and the report of Branlant et al. [3], even though these authors used different techniques for the localization of protein binding sites on the 23 S RNA. Thus, the scheme illustrated in fig.4 summarizes our present knowledge of the location of protein binding sites on the 23 S RNA.

Acknowledgements

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