A ribonuclease-resistant region of 5S RNA and its relation to the RNA binding sites of proteins L18 and L25

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ABSTRACT

An RNA fragment, constituting three subfragments of nucleotide sequences 1-11, 69-87 and 89-120, is the most ribonuclease-resistant part of the native 5S RNA of Escherichia coli, at 0°C. A smaller fragment of nucleotide sequence 69-87 and 90-110 is ribonuclease-resistant at 25°C.

Degradation of the L25-5S RNA complex with ribonuclease A or T2 yielded RNA fragments similar to those of the free 5S RNA at 0°C and 25°C; moreover L25 remained strongly bound to both RNA fragments and also produced some opening of the RNA structure in at least two positions.

Protein L18 initially protected most of the 5S RNA against ribonuclease digestion, at 0°C, but was then gradually released prior to the formation of the larger RNA fragment. It cannot be concluded, therefore, as it was earlier (Gray et al., 1973), that this RNA fragment contains the primary binding site of L18.

INTRODUCTION

The complex of 5S RNA and its binding proteins, L5, L18 and L25 (1-7) is an integral part of the 50S ribosomal subunit. Its presence in the subunit is essential for ribosomal function (8,9), and it is situated near the interface region which is generally considered to be the main functional centre of the ribosome (10). Structurally, the complex has been considered as a microcosm of the ribosome and has provided an excellent system for studying protein-RNA interactions and protein-RNA assembly (1-7, 11, 12). Prime attention has been drawn to identifying the interacting sites of the proteins and RNA.

Gray et al. (1,2) first identified an RNA region which was "protected" by proteins L18 and L25 against ribonuclease A digestion. This region occurred within the sequences 1-11 and 69-120. More recent studies by Zimmermann and Erdmann (13), using
a less direct approach, indicated that the nucleotide sequence 58-116 was "protected" by the two proteins. The validity of the above results depends on the following assumption: if an RNA molecule, and its protein-RNA complex, are digested under identical conditions and a resistant RNA fragment is generated only by the complex, then the fragment constitutes the primary binding site of the protein. The recent demonstration that L18 and L25 produce major and minor conformational changes, respectively, in the 5S RNA (4,5) led us to question this assumption. Therefore, we compared the digestion of 5S RNA, and its complexes with proteins L18 and L25, using newly developed methods for detecting RNA-bound proteins (12,14). We demonstrate that whereas the above assumption is valid for the L25-5S RNA complex it is not valid for the L18-5S RNA complex.

MATERIALS AND METHODS

Preparation of ribosomal components. 32P-labelled and unlabelled 5S RNA were prepared as described earlier from E. coli MRE600 or K12 RNase I10 (1). The RNA was renatured by heating at 55°C for 10 min in HMK reconstitution buffer (30 mM Hepes-OH, 20 mM MgCl2, 300 mM KCl, pH 7.6) and slowly cooling (15). The presence of the native A-conformer was established electrophoretically (15) and by the specific binding of proteins L18 and L25 (2,16). More than 90% of the native conformer was invariably present.

Ribosomal proteins L18 and L25 were prepared either by the method of Hindennach et al. (17), or that of Chen-Schmeisser and Garrett (18) with a final fractionation of the proteins on a phosphocellulose column (6). L25 was also prepared by treating ribosomes at pH 3.8 as described by Brosius (19). Fraction 1 proteins, which correspond to a 2 M LiCl-split fraction from 50S subunits, were prepared and identified, as described earlier (2, 20).

Complexes were formed by incubating 7.5 μg 5S RNA or 4 μg RNA fragment with protein, in 30 μl HMK buffer, pH 7.6, for 40 min at 33°C with slow cooling. L18 (M.Wt. = 12,770) and L25 (M. Wt. = 10,700) were added at a 3:1 molar excess (cf. 6,7). A similar molar excess was estimated, approximately, for the fraction 1 proteins.
Ribonuclease treatment of 5S RNA and its protein complexes. 5S RNA, or its protein complexes, were adjusted to a concentration of 7.5 μg RNA in 30 μl HMK buffer, pH 7.6. RNase A (Worthington, 2 x crystallised) was added at increasing concentrations in the range 1:2,000 to 1:1 (w/w). Digestion was for 10 min at 0°C. In some experiments the digestion temperature was 25°C. Digestion with RNase T2 (Calbiochem) was performed on about 10 μg 32P-5S RNA or protein-5S RNA complex in 50 μl TMK buffer (in which HEPES was replaced by Tris) with 10 or 50 RNase T2 units for 15 min at 25°C. Samples were rapidly loaded onto a 12% polyacrylamide slab gel, at 4°C, generally containing 30 mM Tris-Cl and 10 mM MgCl₂, pH 7.6. Electrophoresis was for 16 hr at 30 mA and 4°C with circulating buffer. The RNA was detected autoradiographically.

For oligonucleotide analyses, the faster migrating fragment was prepared from 5S RNA, L25-5S RNA and L18-5S RNA by digesting the complexes at an RNase A to 5S RNA ratio of 1:10 w/w for 10 min at 0°C. The slow migrating intermediate RNA fragment was prepared from the L18-5S RNA complex at a ratio of 1:20.

Assay for 5S RNA-bound and rebound proteins. The 5S RNA-protein complex or its ribonuclease digest were electrophoresed in duplicate cylindrical 8% polyacrylamide gels for 8-10 hr at 6 mA/tube. The electrophoresis buffer was 20 mM Tris-Cl, 20 mM MgCl₂ and 50 mM KCl, pH 7.6. All experiments were repeated in a high salt buffer containing 250 mM KCl with the same results. All cylindrical gels shown in the Figures contain the low salt buffer. After electrophoresis, one gel was stained for RNA with pyronin G or toluidine blue and the other was stained exclusively for protein with Coomassie brilliant blue (21,22). In some experiments, the RNA-bound protein was identified by dodecylsulphate gel electrophoresis as described earlier (12,14).

In rebinding experiments 10 μg 5S RNA were digested with RNase A at a 1:10 enzyme:RNA ratio for 15 min at 0°C. The reaction was stopped by precipitating for 2 hr at -30°C with 2.5 volumes of ethanol. The precipitate was dissolved in 20 μl TMK buffer and incubated with a 2.5-fold molar excess of L18 or L25. The complex was then precipitated with 1.8 volumes of ethanol for 2 hr, dissolved in 20 μl electrophoresis buffer and electro-
phoresed in cylindrical gels as described above.

Oligonucleotide sequence analyses of the RNA fragments. The $^{32}$P-labelled fragments were located in slab gels autoradiographically and the gel pieces were excised. The RNA fragments were analysed by two methods. (a) The RNA was eluted onto Whatman DEAE 81 paper, electrophoretically (23). It was then eluted from the paper with triethylamine carbonate, pH 10, and dried. $T_1$ RNase digestion, and one dimensional analysis on DEAE paper, were performed according to Bellemare et al., (16). Two-dimensional fingerprinting, and secondary analyses with RNase A and $T_2$ were performed as described by Barell (24). (b) The gel piece was soaked for 3 hr in 8 M urea in water to dissociate base-paired regions, and then set on a composite 15 to 20% polyacrylamide gel containing 0.09 M Tris-borate, pH 8.3, 2.5 mM EDTA and 7 M urea; a short upper layer contained 0.1% dodecylsulphate (23). The RNA subfragments, resolved by the electrophoresis, were eluted onto DEAE 81 paper electrophoretically (23) and further analysed as above.

RESULTS

Digestion of 5S RNA and the protein complexes. The ribonuclease digestion characteristics of 5S RNA and the two protein complexes were compared at increasing RNase A concentrations (Fig. 1). The free 5S RNA produced an RNA fragment. A similar digestion pattern was also obtained with $T_2$ RNase (data not given) which showed that the production of a fragment was independent of the nuclease specificity.

The protein complexes also produced fragments, in high yields, migrating with similar Rf values (Fig. 1); the Rf value of the fragment from the L25-5S RNA complex was slightly less than the others. The L25-5S RNA was more readily digested than the L18-5S RNA complex. The latter yielded more complex digestion characteristics, in particular a slow migrating product was produced (indicated by an arrow in gel C) prior to formation of the RNA fragment.

Proteins bound to the RNA fragments and rebinding experiments. The presence of proteins on the RNA fragments was investigated by
Figure 1: RNase A digestion of 5S RNA, L25-5S RNA and L18-5S RNA. Samples were co-migrated in 12% polyacrylamide slab gels (see Methods). The ribonuclease:RNA ratios were: A - no enzyme, B - 1:500, C - 1:60, D - 1:10 and E - 1:2 w/w. Digestion was for 10 min at 0°C. The positions of the 32p-5S RNA complexes, and 32p-RNA fragments, are indicated. Small amounts of aggregates were present at the origin of the L18-5S RNA sample. The arrow on gel C of the latter indicates the slow migrating intermediate digestion product.

staining selectively for protein. The results are given in Fig. 2. For the L25-5S RNA, L25 was bound to both the 5S RNA and the RNA fragment (gels C and D). The L18-5S RNA complex was stained for protein (gel E) but when it was digested under conditions intermediate between those used in gels C and D of Fig. 1, no protein was present on the RNA fragment. However, a small amount of L18 remained bound to both undigested 5S RNA and to a degradation product migrating behind the 5S RNA (gel F; Fig. 2).

The proteins were bound to the isolated RNA fragment (prepared from 5S RNA, see below) in order to confirm the specificity of the L25-RNA interaction. The RNA fragment was incubated, separately, with proteins L18 and L25, and fractionated from unbound protein by electrophoresis. The results in Fig. 3A demonstrate that L25 rebound to the RNA fragment. No binding of L18 was found (data not shown).

A further experiment was performed in order to establish whether L18, or any other protein, could bind to the RNA fragment in the presence of L25. 5S RNA was incubated with the fraction 1 proteins which contain L5, L18 and L25 and other proteins (see Methods). The complex was digested and electrophoresed. The
Figure 2: Assaying for proteins bound to 5S RNA and the RNA fragments. Samples were electrophoresed in cylindrical polyacrylamide gels (see Methods). A, B: 5S RNA before, and after, digestion, respectively, (RNase A:RNA - 1:10 w/w) and stained for RNA with pyronin G. C, D: L25-5S RNA before and after digestion, (RNase A:RNA - 1:10 w/w). E, F: L18-5S RNA before and after digestion (RNase A:RNA - 1:20 w/w). Gels C to F were stained for protein.

Figure 3: Rebinding of L25 to the RNA fragment and binding of the fraction 1 proteins. A. (a): RNA fragment stained for RNA, and (b): RNA fragment incubated with L25 and stained for protein. The Rf values of the RNA fragment, the L25-RNA fragment complex and the L25-5S RNA (gel not shown) were 0.31, 0.25 and 0.20, respectively. B. (c): 5S RNA-fraction 1 protein complex, and (d): after digestion at 1:10 w/w with RNase A. Both (c) and (d) were stained for protein. Samples were run in cylindrical gels as described in Methods. C. The band from gel (c) and bands 1 and 2 from gel (d) were excised and run in pockets (e), (f) and (g), respectively, of a dodecylsulphate slab gel (see Methods). O denotes the gel origins.
two bands formed (Fig. 3B) were analysed for their protein content. Band 1 which qualitatively resembled the product indicated by an arrow in gel C of Fig. 1 contained proteins L18 and L25; band 2 contained the RNA fragment and L25 (Fig. 3C). Therefore, neither L18, nor other proteins remained bound to the L25-RNA fragment complex.

Sequence of the RNA fragments. The contents of the RNA fragments, prepared from 5S RNA and the protein-RNA complexes (Fig. 1), were compared by co-migrating them in urea-polyacrylamide gels. The subfragment patterns were identical (Fig. 4). Sequences of the subfragments were determined by fingerprinting. Subfragments with identical Rf values (Fig. 4) produced essentially the same results (Table 1) and, therefore, analyses are shown only for the 5S RNA sample (Fig. 5A). The sequence of the 5S RNA is given in Fig. 6 with the positions of the T1 RNase oligonucleotides. The subfragments contained the following nucleotide sequences: a = 89 (90) to 120, b = 69-87 and c = 1-11. A minor difference was found for subfragment a from the L25-5S RNA complex which is considered further below.

2-D fingerprint analyses of the RNA fragments, extracted from the magnesium-containing gel (Fig. 1), were also made, in order to establish whether any oligonucleotides were not detected in the urea gel (Fig. 4). A 2-D fingerprint is shown for the

Figure 4: Subfragment compositions of the RNA fragments. The 32P-RNA fragments from the 5S RNA and complexes (Fig. 1) were excised from the gels, equilibrated with 8 M urea, and electrophoresed into a 15 to 20% compound polyacrylamide slab gel containing dodecylsulphate and 7 M urea (see Methods). RNA fragments from 5S RNA (gel A), L25-5S RNA (gel B) and L18-5S RNA (gel C) each yielded three main subfragments.
RNA fragment from 5S RNA in Fig. 5B. The results are summarised in Table 2 and correlate closely with those from one-dimensional analyses. A fingerprint of the fragment prepared with T2 RNase is also shown, in Fig. 5C, and the analyses (Table 2) reveal the same sequence region (nucleotides 1-10, 70-87 and 89-120).

The 5S RNA base-pairing scheme of Fox and Woese (26) is shown in Fig. 7, which was derived from studying conserved base-pairing regions of different procaryotic 5S RNA’s, and is strongly supported by both ribonuclease digestion (27) and chemical modification studies (11). The structure is arranged as it was drawn earlier (11). The position of the RNA fragment is indicated.

Quantitation of digestion. The degree of RNA degradation, and the amount of RNA fragment formed, were determined, as a function of ribonuclease A concentration, at 0°C, for the 5S RNA and complexes (Fig. 8). The L25-5S RNA complex was degraded more readily than free 5S RNA (Fig. 8A) and the L25-RNA fragment was more readily formed; the latter was also marginally more resistant, at high ribonuclease concentrations, than the free RNA fragment (Fig. 8B).

Table 1: T2 RNase oligonucleotide compositions of the RNA subfragments a, b and c.

<table>
<thead>
<tr>
<th>Subfragment</th>
<th>free</th>
<th>L25-SS</th>
<th>L18-SS</th>
<th>theoretical</th>
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<tr>
<td>a (59-120 n)</td>
<td>1</td>
<td>G</td>
<td>2.4</td>
<td>3.3</td>
</tr>
<tr>
<td>b (69-87 n)</td>
<td>1</td>
<td>CC</td>
<td>1.0</td>
<td>1.0</td>
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<td>c (1-11 n)</td>
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<td>AG</td>
<td>1.9</td>
<td>2.0</td>
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</tbody>
</table>

*approximate molarity estimated visually.
Figure 5: Sequence analysis of the RNA subfragments. A: One dimensional T1 RNase analyses of the 32P-RNA subfragments a, b and c shown in Fig. 3A. Sample d is a total T1 RNase digest of 5S RNA. B, C: 2-D T1 RNase fingerprint of the RNA fragment prepared with RNase A and T2, respectively. The oligonucleotide nomenclature of Brownlee et al. (25) is used (see Fig. 6). Spot 31 contains a minor sequence heterogeneity in which C is replaced by U at position 3.

Figure 6: Sequence of 5S RNA from E. coli MRE600 (25) showing the positions of the T1 RNase oligonucleotides.
Table 2: Tj RNase oligonucleotide compositions of the RNA fragments determined from 2D fingerprints.

<table>
<thead>
<tr>
<th>Oligonucleotides</th>
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<th>RNase Tj digestion</th>
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<tr>
<td></td>
<td>0°C</td>
<td>25°C</td>
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<tr>
<td></td>
<td>found</td>
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<td>(nucleotides 1-11, 69-87, 89-120)</td>
<td>(nucleotides 69-87, 90-110)</td>
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<tr>
<td>1</td>
<td>10.3</td>
<td>10.2</td>
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<tr>
<td>2</td>
<td>1.5</td>
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<td>4</td>
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<td>8</td>
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<tr>
<td>9</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
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<td>1</td>
</tr>
<tr>
<td>16'</td>
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<td>0.2</td>
</tr>
<tr>
<td>15''</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>19</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>19</td>
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<td>1</td>
</tr>
<tr>
<td>14</td>
<td>0.8</td>
<td>0.1</td>
</tr>
<tr>
<td>AAC</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

The sequences of the Tj oligonucleotides are given in Table 1. Spot 31 in Figs. 5 and 12 contains a sequence heterogeneity at position 3 in the 5S RNA sequence (Fig. 6). It occurs in sub-molar amounts and was not quantitated. *Indicates that the molarity of C was estimated visually. †The presence of AAC in submolar amounts shows that in some of the fragment population the sequence extended to position 110.

indicating that the L25 protects the RNA fragment (see also below).

The L18-5S RNA complex was more resistant to degradation than the 5S RNA and L25-5S RNA, indicating that most of the 5S RNA molecule was initially protected by protein (Fig. 8A). At higher enzyme concentrations, however, the RNA fragment was produced in high yield (Fig. 8B).

When RNA fragments were prepared under identical digestion conditions, one consistent difference was observed between the fragment from the L25-5S RNA complex and those from 5S RNA and the L18-5S RNA complex. The analyses (Fig. 9; Table 1) reveal differences in the relative intensities of spots 16' and 12. Spot 16' (UCCCCAUG) is a derivative of oligonucleotide 16. Spot 12 contained 12 and 16'' (CCCCAUG) a smaller derivative of 16. The
yield of 16"" was invariably higher in the L25-RNA fragment complex, indicating that nucleotide 89 was preferentially excised. Therefore, L25 must produce some opening of this loop region (see Fig. 7).

The intermediate L18-RNA fragment. The intermediate digestion product of the L18-5S RNA complex (Fig. 1, gel C) was analysed. The appropriate gel piece was excised, soaked in 8 M urea and applied to a urea-polyacrylamide gel (see Methods). Four main subfragments were resolved (Fig. 10). They were eluted and ana-

![Figure 7: Secondary structure model of 5S RNA according to Fox and Woese (26) arranged in a Y-shape (28) as drawn by Noller and Garrett (11). The sequence of the RNA fragment is indicated by a broken line. The positions of the enzyme cuts are denoted by arrows.](image)

![Figure 8: A: Degradation characteristics of 5S RNA and the complexes and B: Yields of the RNA fragments produced at increasing RNase A concentrations. Digestions were performed as described for Fig. 1. •--• 5S RNA, o--o L25-5S RNA, Δ--Δ L18-5S RNA.](image)
Figure 9: A sequence difference in the L25-RNA fragment. Analyses are presented for subfragment c (Fig. 4) prepared from a: 5S RNA, b: L25-5S RNA and c: L18-5S RNA at an RNase A:RNA ratio of 1:10 w/w. The spots of differing intensity are at positions 16' and 12.

Analysed by one-dimensional paper electrophoresis (data not shown). The subfragments cover the sequence regions 2 = 1-35 (36), 3 = 42 (43)-87 and 4 = 89-120. 1 contains 42 (43)-120. Spot 34 (CCAUG - ref. 16) was present in band 3 in submolar amounts suggesting that the first excision in the central loop (Fig. 7) occurred after C-35 or C-36.

Smaller RNA fragment. In an earlier study (2), in which stronger digestion conditions were employed (30 min at 25°C instead of 10 min at 0°C), the predominant digestion product of the complexes lacked the stem region of the 5S RNA (nucleotides 1-11 and 111-120). The experiment was repeated for the L25-5S RNA complex in order to confirm the result and to establish whether protein was bound to this smaller fragment. The results which are shown in Fig. 11 demonstrated that L25 bound to, and strongly protected,
Figure 10: Analysis of the subfragments of the L18-5S RNA intermediate fragment (indicated by an arrow in Fig. 1, gel C) in a 20% polyacrylamide gel containing 7 M urea (see Methods).

Figure 11: Electrophoresis of the L25-32P-5S RNA before and after digestion at 25°C with RNase A. A: autoradiogram of L25-5S RNA and A': the complex stained for protein. B: autoradiogram of L25-5S RNA after digestion at an RNase A:RNA ratio of 1:2 w/w for 15 min at 25°C and B': the digested complex stained for protein.

Figure 12: T1 RNase fingerprints of RNA fragments prepared at 25°C with A: RNase T2 (enzyme:RNA ratio of 5 units enzyme per μg RNA), and B: RNase A (enzyme:RNA ratio of 1:2 w/w).
the smaller RNA fragment. Under stronger digestion conditions (>30 min at 25°C) the free 5S RNA was completely digested but the L25-RNA fragment complex was stable (thereby confirming the result in Fig. 8) although the band appeared more diffuse in the gel with increasing digestion times. This suggested that the structure became more heterogeneous possibly due to small sequence excisions (cf. 2). T1 RNase fingerprints of the smaller RNA fragment prepared with both RNase A and RNase T2 are shown in Fig. 12. The analyses are included in Table 2. The stem region was absent from both fragments.

DISCUSSION

A ribonuclease-resistant region of 5S RNA. The RNA fragment (nucleotides 1-11, 69-87 and 89 (90)-120) which is very resistant to RNase A and T2 digestion must be highly structured. The structural model of Fox and Woese (26) partially accounts for this in that it contains two double-helical regions, including the stem. However, there are also two sequences drawn single-stranded in Fig. 7 which must also be compactly folded into a tertiary structure (cf. tRNA (29)).

The RNA binding site of protein L25. The digestion experiments, at 0°C, demonstrated that the L25-5S RNA complex yielded the RNA fragment consisting of nucleotides 1-11, 69-87 and 90-120. It was shown that L25 was strongly and specifically bound to the RNA fragment by (1) staining the bound protein, (2) protein-RNA fragment re-binding experiments, and (3) demonstrating that only L25, of all the fraction 1 proteins (20), remained bound to the RNA fragment after digestion. At 25°C, the stem region (nucleotides 1-11 and 111-120) was rapidly excised but the L25 remained bound to the smaller RNA fragment (nucleotides 69-87 and 90-110). Therefore, the primary attachment site of L25 must lie within this 40 nucleotide fragment.

Alteration of the RNA structure by protein L25. A circular dichroism study (5) showed that L25 reduces the molar ellipticity of the 5S RNA on complex formation. It was concluded that a small decrease in secondary structure occurred. An effect of L25 on the
5S RNA structure was also suggested by a fluorescence study (4) in which it was demonstrated that the binding of L25 stabilised the interaction of L18 and 5S RNA. The present results support such a hypothesis in the following two respects.

1. The initial rate of degradation of the L25-5S RNA complex is faster than that of free 5S RNA (Fig. 8) which indicates that the internucleotide bonds 11-12 and/or 68-69 are rendered more accessible to ribonuclease by the protein. Chemical modification studies (6,11) support this conclusion, since the rate of modification of G69 with kethoxal is also higher in the presence of protein L25.

2. The nucleotide U-89 is more readily digested and, therefore, more accessible when L25 is bound. These results, shown in Fig. 9, thereby reinforce the qualitative observation of Gray et al. (2) that "a limited conformational change" occurs in this region on protein binding; the present data also establish, unequivocally, that the protein responsible is L25.

RNA binding site of protein L18. At high RNase A concentrations L18 apparently protects the RNA fragment. The mechanism of protection is different, however, from that of L25 (Fig. 8). L18 delays the degradation of 5S RNA but it is displaced before the RNA fragment is formed.

Analysis of an intermediate digestion product (Fig. 1C) showed that only the sequences in the loop regions h-35-42 and 87-88) are excised prior to release of L18. Therefore, L18 may have a fairly extended binding site on the 5S RNA, in contrast to L25. This hypothesis is compatible with the following properties of L18. (a) It can displace over half of the 5S RNA-bound ethidium bromide (4). (b) It induces a large increase in the molar ellipticity of 5S RNA, implying that it produces a change in the RNA conformation (5,30). (c) It prevents the kethoxal modification of several guanines when complexed with 5S RNA (6, 11). The effect of L25, in each case, is very small.

Comparison with earlier studies. The main difficulty in the earlier studies was in distinguishing between ribonuclease-resistant RNA and RNA protected by the protein. Two studies were
reported on complexes of *E. coli* 5S RNA with L18 and L25, using a ribonuclease probe, by Gray *et al.* (2) and Zimmermann and Erdmann (13); the latter also reported a heterologous study of *B. stearothermophilus* proteins with *E. coli* RNA (31). There are similarities between the reported protected sequences but also important differences. However, both studies need to be reexamined in the light of the present results.

The RNA region that was found by Gray *et al.* (2) to be protected by L25 has been essentially reproduced. In the earlier study all digestions were performed at 25°C such that a mixture of the RNA fragment and its sub-product were obtained. Moreover, some small sequence excisions were observed within the protected region which we have not observed in the present experiments. However, these excisions may occur as a result of prolonged digestion at 25°C and a systematic study is in progress to establish whether their occurrence impairs protein binding. Although evidence was also provided for an apparent protection of the RNA fragment (nucleotides 1-11, 69-87 and 90-120) by L18 (2), the present study demonstrates, unequivocally, that the RNA fragment cannot be considered as the main RNA binding site of L18.

Zimmermann and Erdmann (13) did not examine the L25-5S RNA complex, they digested complexes of L18-5S RNA and (L18 + L25)-5S RNA. For the former, the sequence 58-100 was resistant and for the latter, the sequence 58-116 was resistant; it was concluded that the primary "binding sites" of L18 and L25 were within the sequences 58-100 and 101 to 116, respectively. However, no direct evidence was presented to establish that the proteins remained bound to the resistant region, and our results indicate that L18 probably was not bound.

In conclusion, the ribonuclease-resistant region of 5S RNA corresponds to the binding site of protein L25. In this respect, it is comparable with the RNA binding site of protein S4 which constitutes a resistant region at the 5'-end of 16S RNA (32). In contrast, L18 which is released from the RNA during digestion may have a complex and more extensive interaction with the 5S RNA tertiary structure. Its release may be facilitated by a tendency of the RNA structure, on incurring a few enzyme cuts, to revert to the A-conformation of the free 5S RNA (4,5,30).
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