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The binding site for ribosomal protein L2 within 23S ribosomal RNA of *Escherichia coli*

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Ribosomal protein L2 from *Escherichia coli* binds to and protects from nuclease digestion a substantial portion of 'domain IV' of 23S rRNA. In particular, oligonucleotides derived from the sequence 1757-1935 were isolated and shown to rebind specifically to protein L2 *in vitro*. Other L2-protected oligonucleotides, also derived from domain IV (i.e. from residues 1955-2010) did not rebind to protein L2 *in vitro* nor did others derived from domain I. Given that protein L2 is widely believed to be located in the peptidyl transferase centre of the 50S ribosomal subunit, these data suggest that domain IV of 23S rRNA is also present in that active site of the ribosomal enzyme.
Key words: peptidyl transferase/ribosomal protein L2/ribosomal RNA activity/ribosomal RNA-protein interaction

Introduction

The ribosome is a complex ribonucleoprotein enzyme with an undetermined number of active sites, and even those that have been identified (e.g. the peptidyl transferase centre - the site of peptide bond formation) have been only poorly characterized. Various techniques have been employed in attempts to achieve this goal ranging from dissociation-reconstitution analysis, aimed at identifying proteins indispensable for peptidyl transferase activity (for review, see Nierhaus, 1980), to affinity labelling with derivatives of the substrates or putative inhibitors of peptidyl transferase (for reviews, see Cooperman, 1980; Ofengand, 1980). The latter studies were typically designed to identify components (RNA or protein) adjacent to the 3'-aminoacyl terminus of tRNA bound in the ribosomal A or P site, while antibiotic derivatives were employed in the belief that the native drug molecules normally bind into active sites within the ribosomal enzyme. The resultant literature is extensive and not without inconsistencies but, nevertheless, the end product is a reasonably cohesive pattern. A distinct group of proteins within the 50S ribosomal subunit (i.e. L2, L11, L15, L16, L18 and L27) have been most commonly 'hit' by probes aimed at the peptidyl transferase and also, depending upon their chemical characteristics, some of those probes attached to 23S RNA. To this group of proteins may be added L23, which was identified as the principal target when the antibiotic puromycin or derivatives thereof were photo-incorporated into the 50S particle (Jaynes *et al.*, 1978; Nicholson *et al.*, 1982). In quite dissimilar studies in which 50S ribosomal subunits lacking specific groups of proteins were assembled *in vitro* and then assayed for peptidyl transferase activity, proteins L2, L3, L4, L15, L16, L18 and 23S rRNA were specifically present in all preparations that yielded active particles, whereas other components did not appear indispensable (Hampl *et al.*, 1981). More recent studies have simplified this list by eliminating protein L15, which is absent from the ribosomes of certain mutant strains of *E.coli* (Lotti *et al.*, 1983). In summary, a coherent and interwoven body of data appears to link specific ribosomal proteins to the peptidyl transferase and, of these, protein L2 has probably been most frequently implicated. It may therefore be significant in this context that L2 appears to be the most highly conserved of all the ribosomal proteins; homologues from all three phylogenetic kingdoms scored positive in Western analysis using antibody raised against protein L2 from *E.coli* (Schmid *et al.*, 1984).

In addition to specific ribosomal proteins, 23S rRNA is also believed to be involved (perhaps even fundamentally so) in peptidyl transferase activity, largely as a result of studies involving antibiotics that inhibit protein synthesis. Thus, ribosomal resistance to various such drugs (including erythromycin and other macrolides, chloramphenicol or lincomycin) can be brought about by methylation or mutational alteration of specific nucleosides located within the so-called 'domain V' (for nomenclature see Noller, 1984) of eubacterial 23S RNA or 23S-like RNA from other organisms or organelles (for review, see Cundliffe, 1987). Moreover, when bound to 50S ribosomal subunits, those antibiotics variously protect specific bases within domain V from chemical attack (Moazed and Noller, 1987). The fact that such inhibitors interact with this defined portion of 23S RNA clearly places the latter within the peptidyl transferase centre of the ribosome and has fuelled speculation that rRNA might be intimately involved in the catalysis of peptide bond formation. Entirely consistent with such speculation is the labelling of domain V of 23S RNA by a photo-labile derivative of peptidyl-tRNA that took part in peptide bond formation following covalent attachment (Barta *et al.*, 1984). What is not yet clear, however, is whether other (and, if so, which) portions of 23S RNA might also be involved in ribosomal peptidyl transferase activity. In this context, the binding sites for particular ribosomal proteins within 23S RNA assume considerable significance - particularly that of L2, for the reasons given above.

Under defined conditions of mild nuclease digestion, 23S RNA within intact 50S ribosomal subunits can be cleaved into three fragments of ~1200, 800 and 1000 nucleotides derived, respectively, from the 5' terminal, central and 3' terminal portions of the native molecule (Allet and Spahr, 1971; Spierer *et al.*, 1975, 1976). By hindsight, these RNA fragments appear to encompass the folded domains I plus II, III plus IV and V plus VI respectively. It was also observed (Spierer *et al.*, 1979) that protein L2 bound selectively to the central RNA subfragment although the attachment site was not further characterized at that time. We therefore decided to undertake such studies in the belief that protein L2 might lead us to another portion of 23S

rRNA, other than domain V, that is involved in the peptidyl transferase centre. We were also aware that protein L23 (the target for photo-incorporation of puromycin) had also been shown to bind, independently of L2, to that same central fragment of 23S RNA (Spierer *et al.*, 1976). Accordingly, these two ribosomal proteins were used to protect specific portions of 23S RNA from nuclease digestion. The results are presented below.

Results

In preliminary studies, uniformly labelled 23S rRNA was digested with ribonuclease T1 both in the presence and absence of protein L2; in the latter case, L2 was added to the mixture after digestion. Then, RNA fragments were selectively fractionated by filtration of the mixture through nitrocellulose followed by elution with a combination of LiCl plus SDS. Using a similar protocol, discrete sets of protected oligonucleotides had previously been recovered when 23S RNA was digested in the presence of ribosomal proteins L11 and/or L8 (Beauclerk *et al.*, 1984), and again (see below) using L1 or L23, whereas none were retrieved in this way when any of these proteins were added to pre-digested RNA prior to filtration. However, with protein L2, the results were in total contrast to those observed earlier. Thus, a remarkable number of oligonucleotides was recovered in eluates from filters loaded with 'protected' nuclease digests of 23S rRNA and even in controls, when protein L2 was added to pre-digested RNA, a significant yield of oligonucleotides was obtained (data not given, but see below). Nevertheless, there were sufficient and reproducible differences between the two sets of RNA fragments to indicate that protein L2 was protecting specific sequences within 23S rRNA.

Protection of 23S RNA by protein L2

For the present studies, the experimental protocol was modified somewhat from that used previously (Beauclerk *et al.*, 1984), in that digestion fragments were produced from unlabelled 23S rRNA and then radioactively end-labelled following elution from the filter. These were then resolved by electrophoresis on a denaturing polyacrylamide gel prior to sequence analysis. The gels (Figure 1) revealed two populations of oligonucleotides. Digestion of 23S rRNA carried out in the presence of protein L2 generated quite a few fragments (Figure 1, track a) that had no migrational counterparts in the control digests (Figure 1, track b) and therefore appeared to have been specifically protected by protein L2. On the other hand, some oligonucleotides appeared to be present in both tracks. Accordingly, as many of the RNA fragments as possible were eluted from gel slices (taken from either track) and subjected to sequence analysis (data not given). The results (summarized in Table I) revealed that all the oligonucleotides thus obtained were derived from three defined regions of the 23S RNA primary sequence and these lay within two specific domains of the folded, secondary structure (Noller, 1984).

By far the majority of the RNA fragments that were specifically protected by protein L2 (i.e. were present in track a but not track b of Figure 1), together with others that were much more prominent in the 'protected' track compared with the control, were derived from domain IV of 23S RNA (see Figure 2). These oligonucleotides (numbers 6–26 plus 30 and 32) constituted a 'nest' of overlapping

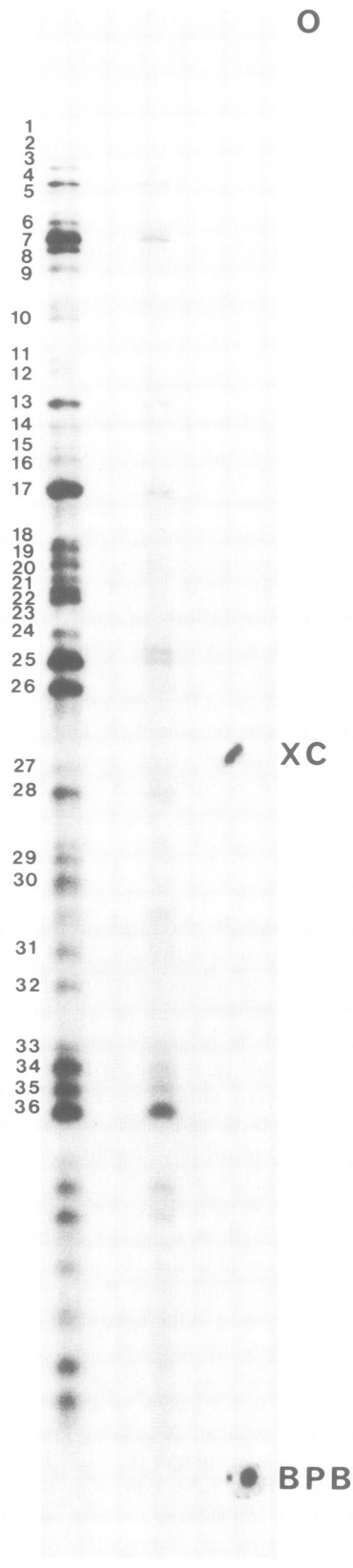

Fig. 1. Autoradiogram of a urea–polyacrylamide gel containing ribonuclease digests of *E.coli* 23S rRNA. Nuclease digestion was carried out in the presence (**a**) or absence (**b**) of protein L2. (In the latter case, protein L2 was added *after* digestion of the RNA.) Digests were passed through cellulose nitrate filters, which were then eluted with LiCl plus SDS before loading onto the gels (for details, see Materials and methods). Dye markers were xylene cyanol FF (XC) and bromphenol blue (BPB). 0 is the origin.

Table I. Fragments of 23S RNA protected by ribosomal protein L2

Oligonucleotide[a]	Sequence within 23S RNA
1	10– 247
2	10– 230
3	10– 220[b]
4	10– 214/215[b]
5	10– 194[b]
6	1757–1922
7	1777–1935[b]
8	1777–1930
9	1777–1922
10	1777–1904
11	1757–1869
12	1777–1884
13	1757–1857[b]
14	1777–1869
15	1771–1860
16	1771–1857
17	1777–1857[b]
18	1777–1850
19	1777–1849
20	1777–1846
21	1777–1842
22	1777–1840
23	1777–1839
24	1777–1835
25	1777–1831
26	1777–1828
27	?
28	?
29	1969–2010
30	1793–1828
31	?
32	1777–1807
33	?
34	1955–1980
35	?
36	507– 530[b]

[a]For numbering scheme, see Figure 1.
[b]Oligonucleotides recovered from both protected and unprotected digests (see text).

fragments derived from residues 1757–1935 of 23S RNA and included most of those that had been recovered in relatively high yield. Even more striking was the observation that almost all of these RNA fragments originated at residue 1777 (only four out of 23 did not; of the remainder, three began at residue 1757) and none of them terminated prior to residue 1828. Beyond residue 1828, fragments were observed terminating at (but not originating from) G residues as far as 1869, after which the longer fragments mainly extended beyond the 'modified' loop (residues 1911–1919; see Figure 2). These data strongly suggested that protein L2 binds to and most strongly protects a folded 'core' of RNA, embracing residues 1777–1828 of 23S RNA, with less strong (but none the less significant) protection extending outwards from the core in either direction. That conclusion was confirmed when the ability of selected oligonucleotides to rebind to protein L2 was assayed (see below).

In addition to the nested family of RNA fragments detailed above, others were also recovered from L2-protected digests of 23S RNA. For example, fragments 29 and 34 (the latter a fairly prominent band in Figure 1, track a) were also

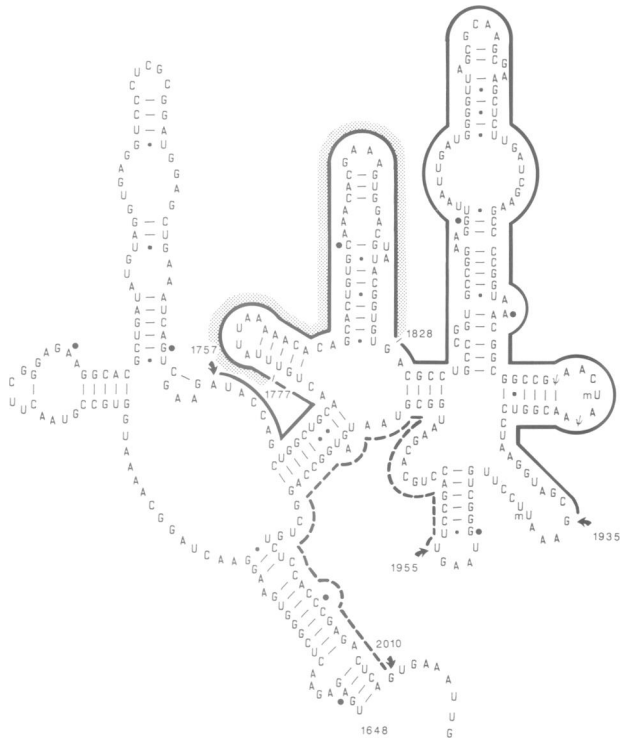

Fig. 2. Secondary structure of domain IV of *E.coli* 23S rRNA. Sequences 1757–1935 (——) and 1955–2010 (- - -) are protected by protein L2. The 'core' of the L2 binding site (residues 1777–1828) is indicated by shading. This secondary structure model for domain IV was kindly provided by Dr Niels Larsen, Kemisk Institut, University of Aarhus. Sequences of 25 species of 23S-like rRNA (six from eukaryotes, eight from archaebacteria, nine from eubacteria and two from chloroplasts) were aligned by the sequence editor ALIAS (S.Thirup and N.Larsen, manuscript in preparation), and edited and plotted by EDSTRUC and PLSTRUC (N.Larsen and S.Thirup, manuscript in preparation).

derived from domain IV (residues 1955–2010) where they are believed to form secondary interactions with other sequences also recovered here (see Figure 2) and with yet others that we have not retrieved (e.g. 1648–1663 and 1945–1950).

Not all of the RNA fragments retrieved in these experiments were derived from domain IV of 23S RNA; e.g. oligonucleotides numbered 1–5 plus 36 in Figure 1(a) were derived from domain I (see Table I). Interestingly, these were present also in unprotected digests of 23S RNA from which they had been recovered selectively following the addition of protein L2 (Figure 1, track b). Other bands visible in Figure 1, track (b) also had migrational counterparts in track (a) and were also excised and analysed. The majority of these RNA fragments, isolated from the control track, did not yield unambiguous sequences since they were not sufficiently pure, possibly reflecting the absence of protein L2 which might otherwise have conferred additional specificity during nuclease digestion. However, those that did so were closely similar to their counterparts in the protected track and are marked in Table I. Finally, there were also present in L2-protected digests a few oligonucleotides that did not yield unambiguous sequences as it was not possible to isolate them in a sufficiently pure state, despite several attempts to do so. It *was* possible, however, to reproduce the data given in Figure 1 and Table I with two independent preparations of protein L2.

Table II. Rebinding of isolated fragments of 23S RNA to ribosomal proteins

RNA fragment[a]	Location of fragment within 23S RNA	Rebinding of RNA fragment (% of input) to protein[d]			
		L2	L1	L11	L23
4[b]	10– 214/215	5	7	3	4
5[b]	10– 194	2	5	3	5
7	1777–1935	34	7	3	4
13	1757–1857	38	<1	<1	3
17	1777–1857	19	1	<1	<1
26	1777–1828	23	<1	<1	1
29[c]	1969–2010	<1	<1	<1	<1
36[b]	507– 530	<1	<1	1	<1

[a]See Figure 1 for numbering scheme.
[b]RNA fragment derived from 23S RNA domain I; otherwise domain IV.
[c]Rebinding not stimulated by presence of fragment 13 with which fragment 29 is believed to pair within 23S RNA (see Figure 2).
[d]10 pmol of each protein was used.

Table III. Summary of RNA protection data with three ribosomal proteins

Protein used to protect 23S RNA	Location of key protected fragments within the 23S RNA sequence	Total number of RNA fragments analysed
L2	1757–1922 1777–1935	22[a]
	1955–1980	1
	1969–2010	1
L1	2084–2234 or 2235	3[a]
L23	1304–1324 1325–1426 1574–1613	8[a]

[a]Other oligonucleotides analysed proved to be subfragments of 'key' sequences.

Rebinding of RNA fragments to ribosomal proteins

Clearly, the oligonucleotides recovered and sequenced thus far represented a non-random fraction of the total RNA digest, which argues powerfully for the specificity of their proposed interaction with protein L2. This point was reinforced when the ability of selected oligonucleotides to rebind to L2 and other ribosomal proteins was investigated (Table II). The 23S RNA fragments chosen for use in these experiments included the major protected bands (e.g. 7, 13, 17, 26, 35) derived from domain IV, other fragments arising from domain I (e.g. 4, 5, 35) plus 29, which was also derived from domain IV albeit not in such high yield. The results were highly suggestive. Only oligonucleotides that were derived from residues 1757–1935 of domain IV rebound to protein L2, and they did so specifically. In contrast, oligonucleotides from domain I of 23S RNA did not rebind to any of the proteins tested, nor did fragment 29 from domain IV (residues 1969–2010) even in the presence of other RNA sequences (e.g. fragment 13) with which fragment 29 is supposed to interact in models for the secondary structure (see Figure 2).

Protection of 23S RNA by ribosomal proteins L1 and L23

Protected fragments of 23S RNA were also generated in the presence of ribosomal proteins L1 or L23, with only minor variations on the present protocol (see Materials and methods for details) and the data are summarized in Table III. Essentially, they confirm to a remarkable extent results previously reported from other laboratories, particularly since those authors had used methods completely different from ours in the isolation of protected oligonucleotides. Thus, in our hands, protein L1 specifically protected three fragments of 23S RNA which were recovered in high yields, whereas none were recovered from unprotected digests to which L1 was subsequently added. Sequence analysis revealed that the largest oligonucleotide embraced the other two and its sequence was almost identical to that reported much earlier (Branlant *et al.*, 1980). We also recovered and sequenced eight specific oligonucleotides from L23-protected digests of 23S RNA but were forestalled by Vester and Garrett (1984) who published closely similar data. Interestingly, however, the L23-protected sequences covered by our 'key' fragments included residues 1325–1331 and 1417–1426, neither of which were detected by Vester and Garrett (1984). Accordingly, we would extend slightly their proposed binding site for protein L23 to encompass the helix 1420–1424/1574–1578 (see Noller, 1984 for the secondary structure).

Discussion

There are strong phylogenetic reasons for suspecting that domain IV of 23S RNA might play a particularly important role in ribosomal function. Thus, in a comprehensive review (Noller, 1984), 15 stretches of sequence within 23S-like rRNAs (ranging from 6 to 17 residues in length) were stated to be 'universal, or nearly so'. Six of those sequences lie within domain IV, five are represented among the L2-protected oligonucleotides described here and three are encompassed by RNA fragment 7, which rebinds specifically to protein L2 *in vitro*. Domain IV also contains a cluster of unpaired modified nucleosides (within the loop 1911–1919 plus residue 1939), again a suggestive landmark for functional significance. And even at the level of the gene, the sequence encoding domain IV stands out, being interrupted by intervening sequences in *Drosophila* and *Tetrahymena*. For these various reasons, plus the concentration of conserved helices within domain IV, others (Noller *et al.*, 1981) suggested some time ago that domain IV is 'likely to contain functionally indispensable regions'. We are now in a position to suggest what that function might be: the evidence that places protein L2 in the peptidyl transferase centre of the ribosome likewise places domain IV of 23S rRNA in that same active site.

Prior to the present work, the central loop or 'hinge' region of 23S RNA domain V had been implicated in ribosomal peptidyl transferase activity as a result of various studies, summarized above (see Introduction). Significantly, in the present context, that portion of domain V resembles domain IV in harbouring invariant sequences, a cluster of modified nucleosides and, at the genomic level in yeast mitochondria and *Physarum*, intervening sequences (see Noller *et al.*, 1981 for review). Such observations together with our present data lead us to conclude that both domains IV and V of 23S rRNA are involved in peptidyl transferase activity and ought, therefore, to interact functionally. That suggestion is consistent with the observation of a tertiary cross-link, established within intact 50S particles, between domains

IV and V, involving (tentatively, according to Stiege *et al.*, 1983) a highly conserved sequence in domain V and what we now recognize as the core of the L2 binding site in domain IV. Our data also account for the cross-link established between protein L2 and the residues 1819–1820 of 23S RNA (Gulle *et al.*, 1988) and reveal its significance. It is less easy, however, to assess the significance of the apparent association of protein L2 with sequences located in domain I of 23S RNA since, in the present studies, L2 did not obviously protect residues 10–247 and 507–530 against nuclease digestion. Thus, oligonucleotides (numbers 1–5 and 36) representing those sequences were also recovered from unprotected digests to which protein L2 was subsequently added. On the one hand, there was specificity in the recovery process; the equivalent RNA fragments were not retrieved in earlier studies involving ribosomal proteins L8 or L11 under digestion conditions similar to those employed here (Beauclerk *et al.*, 1984). On the other hand, these fragments did not rebind to protein L2 following their recovery from polyacrylamide gels (Table II). Accordingly, we hold no fixed views concerning the significance, if any, of possible interactions between domain I of 23S RNA and ribosomal protein L2.

There have also been suggestions that 23S RNA domain III might be involved in the peptidyl transferase centre of the ribosome, following the demonstration that puromycin could be photo-incorporated into L23 within the 50S particle (Jaynes *et al.*, 1978) and that L23 binds to domain III (Vester and Garrett, 1984). Here, we have confirmed the latter data and have no doubt that a specific portion of domain III was correctly identified by those authors as containing the L23-binding site although, unlike them, we did not find any evidence for a fragment of domain V among the L23-protected oligonucleotides. More recently, however, puromycin has also been shown to photo-incorporate into domain V of 23S rRNA (Hall *et al.*, 1988) within the region already implicated in the peptidyl transferase centre. Does this in turn imply that domains III and V of 23S RNA form tertiary interactions? And what is the significance of the cross-link, established in intact 50S particles, between protein L23 and domain I of 23S RNA (Wower *et al.*, 1981; Gulle *et al.*, 1988)?

Without reaching any dogmatic conclusion, the balance of the evidence from affinity labelling studies using derivatives of peptidyl-tRNA suggests that protein L2 is located at or near the site where peptidyl transferase recognizes its donor substrate. Thus, our bias suggests to us that we have identified, in domain IV of 23S rRNA, one of the substrate recognition domains of the ribosomal peptidyl transferase—most probably the P site. In contrast, or by way of complementarity, the '2450' region of domain V (see Moazed and Noller, 1987) with its cluster of 'chloramphenicol sites' would ideally be involved in the recognition of A site substrates by the transferase—given that chloramphenicol appears to block that very recognition process (for review, see Gale *et al.*, 1981). Obviously, this model is (as yet) plausible rather than secure and we are actively seeking other evidence that might help to assess its validity.

Materials and methods

Materials

These were obtained as follows: cellulose nitrate filter (0.45 μm, 13 mm diameter) from Sartorius; ribonuclease T_1 from Sankyo; ribonucleases U_2 and *Phy*M and *Bacillus cereus* ribonuclease from Bethesda Research Laboratories; calf intestinal alkaline phosphatase from Boehringer; T4 RNA ligase and polynucleotide kinase (FPLC purified) from Pharmacia; radiolabelled materials from Amersham International.

Preparation of ribosomal proteins

Proteins L1, L2 and L23 were prepared as previously described (Dijk and Littlechild, 1979) and were stored at −70°C in HMK buffer plus 0.1 mM benzamidine and 0.1 mM phenylmethylsulphonyl fluoride. This buffer contained 10 mM Hepes–KOH, pH 7.45 at 20°C; 20 mM $MgCl_2$; 100 mM KCl; 3 mM 2-mercaptoethanol [Hepes; 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid].

Preparation of 23S rRNA

Total rRNA was extracted from 70S ribosomes using acetic acid plus urea (Hochkeppel *et al.*, 1976) and fractionated by density gradient centrifugation using a linear gradient of 15–30% (w/v) sucrose in 10 mM Tris–HCl, pH 7.5 at 20°C; 100 mM LiCl; 10 mM Na_2 EDTA; 0.2% (w/v) SDS. Centrifugation was at 25 000 r.p.m. for 20 h at 15°C in a Beckman SW 27 rotor. Fractions containing 23S rRNA were pooled and the RNA was precipitated with ethanol and redissolved in water. The 23S rRNA was then extracted with phenol and reprecipitated three times from 0.6 M potassium acetate, dried *in vacuo* and dissolved in water.

Production of rRNA fragments protected by specific proteins

Ribosomal proteins (as below) were incubated with 23S rRNA for 20 min at 44°C and then for 5 min at 20°C in 100 μl buffer containing 20 mM Hepes–KOH, pH 7.5 (adjusted at 20°C); 4 mM $MgCl_2$; 380 mM NH_4Cl; 20 mM KCl; 3 mM 2-mercaptoethanol; 0.1 mM phenylmethylsulphonyl fluoride; 0.1 mM benzamidine; prior to nuclease digestion. Complexes formed between 23S RNA (50 pmol) and protein L2 (122 pmol) were incubated with T_1 ribonuclease (0.5 U) for 5 min at 37°C; those formed between 23S RNA (50 pmol) and protein L1 (50 pmol) were incubated at 37°C with T_1 ribonuclease (2.5 U) for 10 min; those involving 23S RNA (50 pmol) and protein L23 (200 pmol) were treated with T_1 ribonuclease (2 U) for 5 min at 37°C. In controls, the ribosomal protein (L1, L2 or L23) was added after digestion of 23S RNA with T_1 ribonuclease under the various conditions given above. Reactive mixtures were then filtered through cellulose nitrate discs (0.45 μm, 13 mm diameter), washed with 3 × 2 ml of incubation buffer (above) and eluted with 0.5 ml of 1 M LiCl/0.1% (w/v) SDS as described previously (Schmidt *et al.*, 1981). The eluates were immediately extracted with phenol and RNA fragments were precipitated from the aqueous phase with 2.5 vols of ethanol at −20°C and then reprecipitated from 0.6 M sodium acetate.

Preparation of terminally labelled [^{32}P]RNA fragments

Fragments, produced from unlabelled 23S rRNA as described above, were reprecipitated twice from 0.6 M sodium acetate and dissolved in water. They were then labelled either at the 5′ terminus using [γ-^{32}P]ATP and T4 polynucleotide kinase (Donis-Keller *et al.*, 1977), or, after treatment with calf intestinal alkaline phosphatase (Chaconas and van de Sande, 1980), at the 3′ terminus with 3′-[^{32}P]5′-cytidine bisphosphate and T4 RNA ligase (England *et al.*, 1980; D'Allessio, 1982). End-labelled RNA fragments were then resolved by electrophoresis at 300 V (constant voltage) for 20 h at 20°C in gels (35 cm × 1.5 mm; 6 mm wells) containing 12% (w/v) acrylamide and 7 M urea. Running buffer contained 90 mM Tris base; 90 mM boric acid; 2.8 mM Na_2 EDTA. RNA fragments in gels were visualized by autoradiography at −70°C using Fuji RX film and subsequently eluted from gel sections by maceration in 500 mM ammonium acetate; 10 mM magnesium acetate; 1 mM Na_2 EDTA; 0.1% (w/v) SDS followed by incubation at 20°C for 16 h. Pieces of gel were removed by filtration and the RNA fragments were precipitated three times from 0.6 M sodium acetate in the presence of 10 μg of tRNA as carrier before being finally dissolved in water.

Rebinding of RNA fragments to ribosomal proteins

Terminally labelled [^{32}P]RNA fragments eluted from gel slices were incubated at 20°C for 20 min with 10 pmol of ribosomal protein (either L1, L2, L11 or L23) in buffer (30 μl) containing 20 mM Hepes–KOH, pH 7.5 at 20°C; 4 mM $MgCl_2$; 380 mM NH_4Cl; 20 mM KCl; 3 mM 2-mercaptoethanol; 0.1 mM phenylmethylsulphonyl fluoride; 0.1 mM benzamidine. Reaction mixtures were filtered through cellulose nitrate discs (0.45 μm, 13 mm diameter) which were washed with 2 ml of incubation buffer and then subjected to liquid-scintillation counting.

Nucleotide sequence analysis

Terminally labelled [^{32}P]RNA fragments were partially degraded with RNase T_1 or U_2 (Donis-Keller *et al.*, 1977) with *Phy*M RNase (Donis-

Keller, 1980) or with *B.cereus* ribonuclease (Lockhard *et al.*, 1978) and the products were analysed on sequence gels (D'Allesio, 1982).

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Note added in proof

It has come to our attention that others have observed binding of L2 to a T7 transcript containing domain IV of 23S rRNA (Leffers,H., Egebjerg,J., Andersen,A., Christensen,T. and Garrett,R.A. (1988) *J. Mol. Biol.*, in press).