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Ribosomal Proteins L11 and L10.$(L12)_4$ and the Antibiotic Thiostrepton Interact with Overlapping Regions of the 23 S rRNA Backbone in the Ribosomal GTPase Centre

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The *Escherichia coli* ribosomal protein (r-protein) L11 and its binding site on 23 S ribosomal RNA (rRNA) are associated with ribosomal hydrolysis of guanosine 5′-triphosphate (GTP). We have used hydroxyl radical footprinting to map the contacts between L11 and the backbone riboses in 23 S rRNA, and to investigate how this interaction is influenced by other ribosomal components. Complexes were characterized in both naked 23 S rRNA and ribosomes from an *E. coli* L11-minus strain, before and after reconstitution with L11. The protein protects 17 riboses between positions 1058 and 1085 in the naked 23 S rRNA. Within the ribosome, L11 also interacts with this rRNA region, although the protection effects are subtly different and extend to nucleotide 1098. The pentameric r-protein complex L10.$(L12)_4$ binds to an adjacent site on the rRNA, protecting riboses at positions 1043, 1046 to 1049, 1053 to 1055 and increasing the accessibility of position 1068. The overlap in the positions affected by r-proteins L11 and L10.$(L12)_4$, and the increase in protection between positions 1078 and 1084 when they are bound at the same time, reflect the mutually co-operative nature of their interaction with the rRNA. The data support a model for the tertiary configuration of the rRNA region, in which two stem-loop structures fold so that the loops lie in close proximity, with the main ribose interactions of L11 within the minor groove of one of the stems. The conformation of the rRNA-L11 interaction is modulated by L10.$(L12)_4$ and other proteins within the ribosome. The antibiotics thiostrepton and micrococcin inhibit the catalytic functions of this region by slotting in between the accessible loops and interacting with nucleotides there.

Keywords: 23 S rRNA; r-protein L11; RNA-protein interaction; thiostrepton; ribosomal GTPase

1. Introduction

Ribosomal RNA has been shown to be involved in all the functional interactions that occur during protein synthesis (Noller, 1991). The most important processes carried out by the 50 S ribosomal subunit are those associated with guanosine 5′-triphosphate (GTP‡) hydrolysis and the formation of the peptide bond. These functions have been mapped to structures within 23 S rRNA domains II and V, respectively. The rRNA probably plays a direct, catalytic role in protein synthesis as peptide bond formation still occurs after most of the r-proteins have been removed (Bernabeu *et al.*, 1979; Noller *et al.*, 1992). The r-proteins can be envisaged as playing important auxiliary roles, such as tuning local rRNA structure, supporting interactions between rRNA domains, and facilitating conformational changes in the rRNA.

The *Escherichia coli* r-protein L11 binds between nucleotides 1052 and 1112 in the GTPase centre of 23 S rRNA (Schmidt *et al.*, 1981). This region is associated with A-site-dependent steps of protein synthesis including those involving GTP hydrolysis. The performance of these different functions probably requires changes in the conformation of the L11-rRNA complex (Cundliffe, 1986, 1990). Several approaches have shown that L11 interacts with bases (Egebjerg *et al.*, 1990; Karaoglu & Thurlow, 1991; Ryan *et al.*, 1991), and possibly with riboses (Ryan *et al.*, 1991) in this rRNA region. These nucleotide contacts are probably modulated by other r-proteins including the L10.$(L12)_4$ pentameric complex that binds co-operatively with L11 (Dijk *et al.*, 1979), and perturbed by binding of the

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‡ Abbreviations used: GTP, guanosine 5′-triphosphate; DMS, dimethyl sulphate.

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peptide antibiotics thiostrepton and micrococcin, that block the function of this region (Cundliffe & Thompson, 1981; Cundliffe, 1990).

In this study, we have used a footprinting approach with hydroxyl radicals to define the contacts L11 makes with the backbone riboses in 23 S rRNA. We have studied these interactions in naked rRNA as well as in ribosomes isolated from an L11-minus strain (Stöffler *et al.*, 1980), and have determined how the L11 interaction is influenced by binding of L10.$(L12)_4$ or antibiotics. The radicals are small and highly reactive, and provide a sharp definition of the region of rRNA-protein interaction. This has enabled us to orient the contact sites of L11 within the tertiary structure proposed for the GTPase centre. Slight changes in this interaction on addition of L10.$(L12)_4$ and within the ribosome indicate the structural basis for the co-operative interactions between L11 and other r-proteins. The site of antibiotic interaction has been accurately placed as only few ribose contacts are made by the drugs, and this provides insight into their mechanism of inhibition.

2. Materials and Methods

(a) *Strains*

E. coli strain DH1 (Sambrook *et al.*, 1989) and AM68 (Stöffler *et al.*, 1980) were grown in liquid LB-medium (Sambrook *et al.*, 1989) at 37°C. Strain DH1 contains wild-type ribosomes, whereas AM68 lacks the ribosomal protein L11.

(b) *Preparation of ribosomes and rRNA*

Cells were grown to an A_{450} of 0·4, and were harvested by centrifuging at 8000 revs/min for 15 min. The cells were washed by resuspending, in half the original culture volume of 50 mM Tris·Cl (pH 7·8), 10 mM $MgCl_2$, 100 mM NH_4Cl (TMN buffer), and recentrifuging. The cells were resuspended in 10 ml of TMN buffer and lysed by sonication; cell debris was removed by centrifugation at 15,000 revs/min for 15 min. The supernatants were transferred to Ti 50 tubes, and ribosomes were pelleted by centrifugation at 18,000 revs/min for 16 h. All the steps were carried out at 4°C. Ribosomes were stored in TMN buffer at −80°C. 23 S rRNA was isolated by extracting ribosomes three times with phenol/chloroform, extracting once with chloroform, and precipitating with ethanol.

(c) *Binding of L11 and antibiotics to ribosomes*

30 μg of L11-minus ribosomes were pre-incubated in 100 μl of binding buffer (50 mM Hepes KOH, (pH 7·6), 10 mM $MgCl_2$, 100 mM KCl, 5 mM DTT and 0·1 unit RNasin [Promega]) for 15 min at 30°C, prior to addition of a 1·5-fold molar excess of L11 (Egebjerg *et al.*, 1990) and incubation for 20 min at 30°C. In the antibiotic binding experiments, thiostrepton or micrococcin (dissolved in DMSO) was added in a 3-fold molar excess relative to ribosomes, and incubated for a further 20 min at 30°C. The final concentration of DMSO did not exceed 1%.

(d) *Binding of proteins to 23 S rRNA*

Naked 23 S rRNA (10 μg per sample in 25 μl binding buffer) was renatured by heating at 50°C for 5 min, followed by slow cooling to 37°C. Ribosomal proteins (Egebjerg *et al.*, 1990) were added in a 1·5-fold molar excess, and samples were incubated for 30 min at 37°C and then placed on ice.

(e) *Monitoring protein and antibiotic binding to rRNA and ribosomes*

Enzymatic digestions with RNases T_1, T_2 (Sigma), and V_1 (Pharmacia), and modification with dimethyl sulphate (DMS) were performed as previously described to check the binding of the r-proteins (Egebjerg *et al.*, 1990) and the antibiotics (Egebjerg *et al.*, 1989).

(f) *Hydroxyl radical probing*

Hydroxyl radical probing was carried out essentially as described by Hüttenhofer & Noller (1992). Ribosomes were placed on ice after binding of protein L11 and antibiotics as described above. The hydroxyl radical mixture, consisting of 5 μl 50 mM $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 5 μl 100 mM EDTA, 5 μl 250 mM ascorbate and 5 μl 2·5% H_2O_2 per reaction, was prepared immediately prior to its addition to 30 μg ribosomes in 25 μl binding buffer. The reactions were carried out for 4 min on ice, and were stopped by precipitation with 0.3 M NaOAc and 2·5 volumes of ethanol. Naked 23 S rRNA and protein-23 S rRNA complexes were probed with 4 μl of freshly prepared hydroxyl radical mixture per sample, and the reactions were stopped after 10 min by precipitation.

(g) *Primer extension*

Primer extension analyses of 23 S rRNA were carried out as described by Moazed *et al.* (1986) using 3 deoxyoligonucleotides: the 1109-primer, 5′-CGCGCAGGAAGACTCG, complementary to nucleotides 1109 to 1124; the 1170 primer, 5′-CCCAACAACGCATAAGCGT, complementary to nucleotides 1169 to 1187; the 2800-primer, 5′-CCTTCAGGACCCTTAAAG, complementary to nucleotides 2805 to 2812.

3. Results

(a) *Hydroxyl radical probing of naked 23 S rRNA and its interaction with L11 and L10.$(L12)_4$*

Upon binding to naked 23 S rRNA, protein L11 reduced the reactivities of nucleotides, between positions 1058 and 1085, towards hydroxyl radicals (Fig. 1 and Table 1). These protection effects are most pronounced in helix 3 (Fig. 2b). Binding of the L10.$(L12)_4$ protein complex to naked 23 S rRNA reduced the reactivities of riboses at positions 1043, 1046 to 1049, 1053 to 1055, and increased the reactivity of position 1068. All these positions were protected when L11 was bound together with the L10.$(L12)_4$ complex, and, additionally, the riboses between positions 1078 and 1084 became more strongly protected.

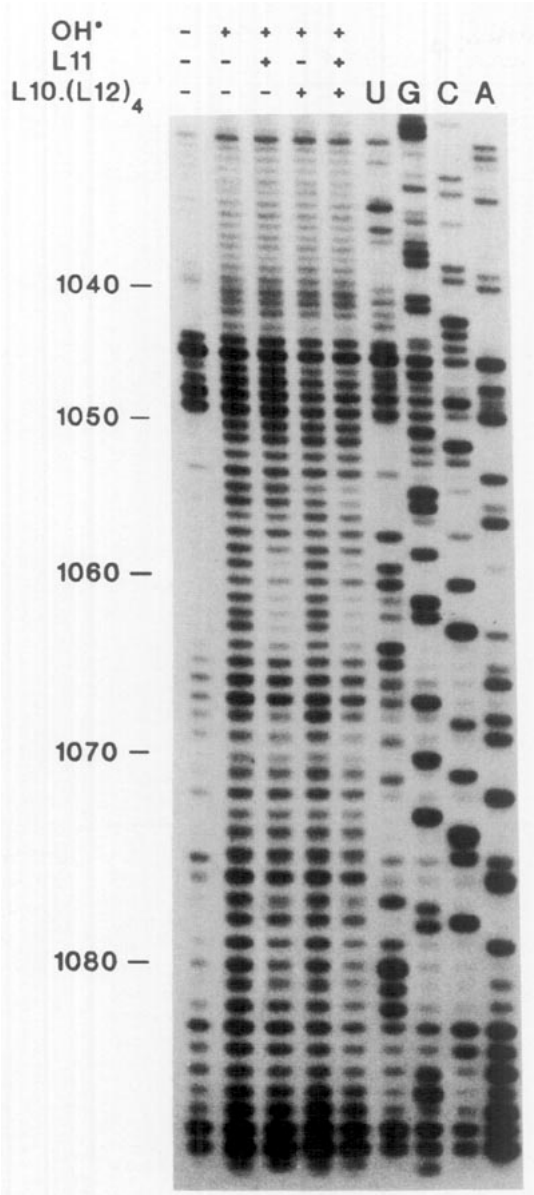

Figure 1. Autoradiograph of primer extensions reactions using the 1109 primer. Modification with hydroxyl radicals (OH*) was carried out on naked 23 S rRNA, or on naked rRNA complexed with the r-proteins as indicated. Unmodified rRNA was used as the template for the dideoxy sequencing reactions. (The modification and sequence bands are displaced by one nucleotide).

(b) *Determination of whether L11-minus ribosomes reconstitute r-protein L11*

Ribosomes from the *E. coli* strain AM68 (L11-minus ribosomes) were probed with a double-strand specific (V_1) and single-strand specific (T_1 and T_2) RNases, and a single-strand specific chemical probe (DMS). Bases within the GTPase region, that are inaccessible in wild-type ribosomes, are reactive to the probes in the L11-minus ribosomes. Modification of the L11-minus ribosomes reconstituted with L11 produced a footprint pattern identical to that of wild-type ribosomes. Figure 3 illustrates that 1088A and 1089A are highly reactive to DMS in L11-minus ribosomes, and that these bases become protected by bound protein L11. The RNase footprinting results similarly showed that the L11-minus ribosomes bind protein L11 at a stoichiometric ratio close to 1:1, and that the structure of the reconstituted ribosomes is indistinguishable from that of wild-type ribosomes (data not shown). These modification and digestion patterns match those previously reported by Egebjerg *et al.* (1990) for wild-type ribosomes.

The interaction of the antibiotics thiostrepton and micrococcin with ribosomes is dependent on the presence of r-protein L11 (Stöffler *et al.*, 1980), and this was used as an additional test for whether the L11-minus ribosomes reconstituted with L11. Addition of the drugs to L11-minus ribosomes did not change the accessibility of bases (Fig. 3). In ribosomes reconstituted with protein L11, however, the drugs affected the accessibility of multiple nucleotides in the 1067 to 1098 region. The protected positions are the same as those previously found for wild-type ribosomes (Egebjerg *et al.*, 1989), and the intensities of some of the protection effects are actually greater in the reconstituted ribosomes. This is shown for the DMS probe in Figure 3, where the drugs can be seen to protect several bases, most notably there is complete protection at 1070A and 1095A; additionally, the accessibility of 1067A is reduced by thiostrepton and enhanced by micrococcin, as has been reported previously (Egebjerg *et al.*, 1989).

(c) *Hydroxyl radical probing of L11 and antibiotic interactions with ribosomes*

Riboses within L11-minus ribosomes were accessible to hydroxyl radicals in the L11 binding region of 23 S rRNA. Binding of L11 to these ribosomes protected tracts of riboses between positions 1058 and 1098 (Figs. 2c, 3 and Table 2). Addition of thiostrepton or micrococcin to ribosomes prior to reconstitution with L11 had no effect on ribose accessibility (Fig. 3). The drugs bound to reconstituted ribosomes, however, and thiostrepton protected ribose positions 1067 to 1069 within loop B, and 1096, 1097 in loop D, and enhanced the accessibility of position 1103 (Table 2). Micrococcin affected the same bases, but to a lesser degree (Fig. 3).

(d) *Footprinting of the α-sarcin region of 23 S rRNA*

The region encompassing the α-sarcin loop, which has functional links with the GTPase centre, was probed by using a primer complementary to nucleotides 2805 to 2812 of *E. coli* 23 S rRNA. This region was generally less accessible than the GTPase centre to reaction with hydroxyl radicals. Binding of L11 and/or antibiotics to L11-minus ribosomes had no

Table 1
Reactivities of nucleotides in naked 23 S rRNA

Nucleotide position in 23 S rRNA	23 S rRNA	23 S rRNA + L11	23 S rRNA + L10.$(L12)_4$	23 S rRNA + L11 + L10.$(L12)_4$
C 1043	+ +		0·2	0·2
A 1046	+ + +		0·3	0·4
G 1047	+ + + +		0·5	0·6
A 1048	+ + + +		0·5	0·6
C 1049	+ + + +		0·5	0·6
C 1053	+ + +		0·6	
A 1054	+ + +		0·3	0·3
G 1055	+ + +		0·4	0·3
U 1058	+ + +	0·5		0·6
G 1059	+ + +	0·1		0·2
U 1060	+ + +	0·6		
U 1061	+ + +	0·2		0·2
G 1062	+ + +	0·2		0·2
G 1063	+ + +	0·1		0·1
C 1064	+ + +	0·4		0·4
G 1068	+ + +	0·5	1·8†	
A 1069	+ + +	0·5		0·5
A 1077	+ + +	0·4		0·4
U 1078	+ + +			0·6
C 1079	+ + +	0·4		0·3
A 1080	+ + +	0·4		0·2
U 1081	+ + +	0·3		0·3
U 1082	+ + +	0·5		0·3
U 1083	+ + +	0·6		0·4
A 1084	+ + +	0·6		0·4
A 1085	+ + +	0·5		0·5

Hydroxyl radical modification sites in naked 23 S rRNA that are affected by binding of L10.$(L12)_4$ and L11. For naked rRNA, the reactivities of the riboses are average visual estimates of autoradiograph bands from at least 3 experiments, and are graded using a + system (+ + + + is the most strongly reactive). Changes in reactivities on protein binding were measured by laser densitometer scanning of bands, and are standardized against positions 1038, 1040, 1088 and 1095 outside the binding region, and additionally at 1067 (†). Band intensities are shown as a fraction of the reactivity of the same position in the naked rRNA (0·5 represents 50% protection, and 0·0 complete protection) and are rounded off to the nearest 0·1. Only the positions where the r-proteins change reactivity to below 0·7 or above 1·3 relative to the naked rRNA are shown, a blank space indicates that there was no change exceeding these values.

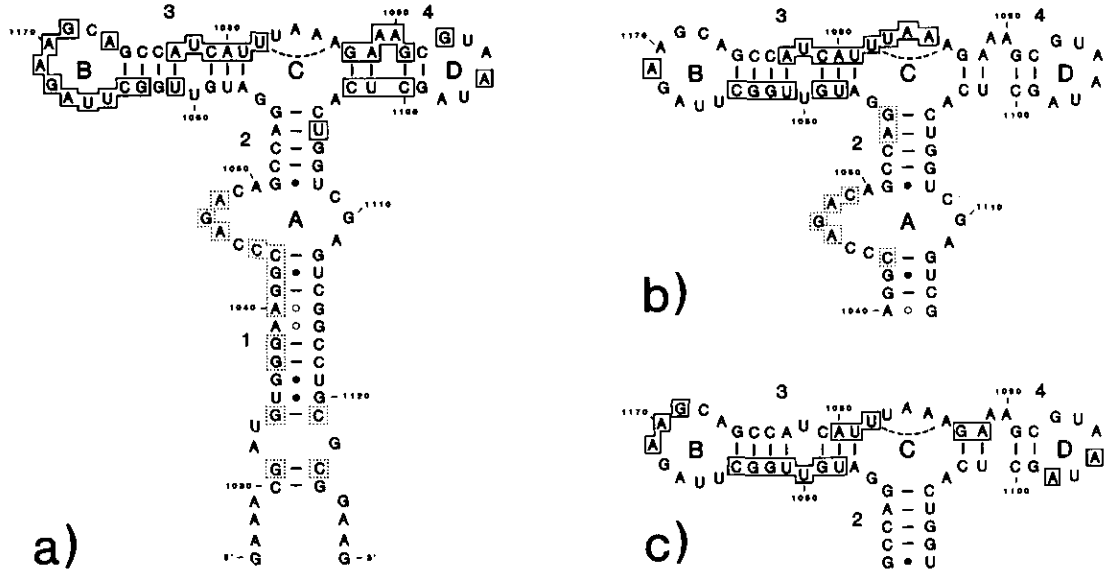

Figure 2. Proposed secondary structural model of 23 S rRNA in the region where the r-proteins L10.$(L12)_4$ and L11 interact. (a) The regions in naked 23 S rRNA protected from nucleases and chemical reagents by L10.$(L12)_4$ (dotted boxes) and L11 (continuous lines) (Egebjerg *et al.*, 1990). (b) Nucleotide positions protected from hydroxyl radicals when L10.$(L12)_4$ (dotted) and L11 (continuous) are bound together to naked 23 S rRNA. On binding the proteins individually, it was additionally observed that L10.$(L12)_4$ enhances the accessibility of 1068, whereas L11 protects this position. (c) Nucleotide positions (boxed) protected by L11 from hydroxyl radical modification within ribosomes.

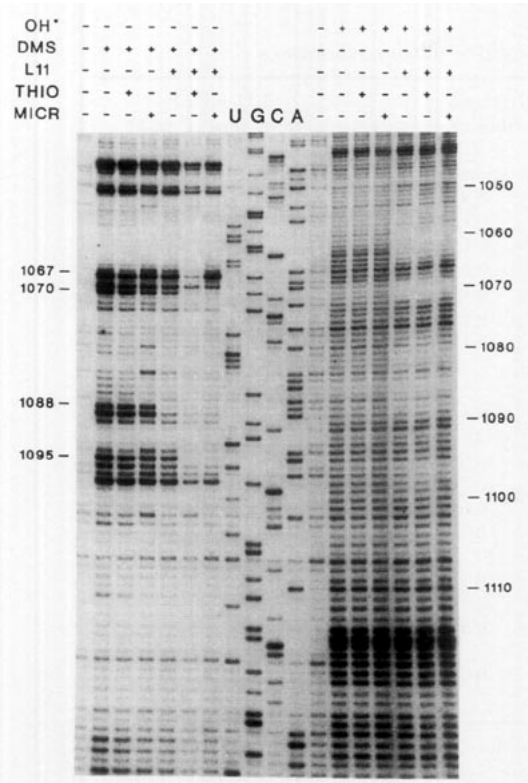

Figure 3. Autoradiograph of extension reactions from the 1170 primer showing altered reactivities to DMS or hydroxyl radicals (OH•) on binding of r-protein L11 or thiostrepton (thio) or micrococcin (micr) to L11-minus ribosomes. On binding of the drugs to reconstituted ribosomes in a 1 : 1 stoichiometry, 1095A is protected >95%, 1067A is protected 60% by thiostrepton and enhanced in reactivity 2·3-fold by micrococcin (DMS bands standardized against positions 1069 and 1098). The dideoxy sequencing reactions were performed on unmodified rRNA extracted from L11-minus ribosomes. The rRNA sequence was identical to that reported by Brosius *et al.* (1981).

effects on the accessibility of nucleotides within this region towards hydroxyl radicals or DMS (data not shown).

4. Discussion

(a) *Interaction between L11 and riboses in the naked 23 S rRNA*

The C1′ and C4′ positions of riboses, the presumed sites of hydroxyl radical attack (Wu *et al.*, 1983), are accessible within both single stranded and base-paired nucleotides (Hüttenhofer & Noller, 1992; Latham & Cech, 1989). All the riboses within the naked 23 S rRNA GTPase centre and flanking sequences (Fig. 1) are reactive towards hydroxyl radicals, indicating that none of these positions are occluded by tertiary conformation within the region. Protein L11 protects 17 riboses in naked 23 S rRNA between nucleotides 1058 and 1085, suggesting that extensive ribose contacts are involved in this interaction. A previous study (Egebjerg *et al.*, 1990) showed that L11 binding protected nucleotides between positions 1060 and 1105 from a range of chemical and enzymatic probes; nucleotides 1050 to 1059 were not reactive towards any of these probes (Fig. 2a).

Both sets of data are consistent in defining the L11 binding site. However, the small size and highly reactive nature of the hydroxyl radicals offer two major advantages over the other bulkier probes: first, the radicals are not as sterically hindered by L11 and the protection effects are thus more likely to reflect sites of protein-rRNA contact; second, the radicals gain access to the 1050 to 1059 region, showing additional protein interactions here. Based on this, we interpret our data in the most straightforward manner, i.e. that ribose protections reflect sites of direct interaction between protein (or drug) and the rRNA (although we cannot rule out that we are in some cases detecting conformational changes induced by ligand binding). The hydroxyl radicals give a sharp definition of the L11 contact site lying within helix 3 and loops B and C of the secondary structure in Figure 2.

A tertiary structure has been proposed for this region on the basis of the chemical and enzymatic probing together with phylogenetic data (Egebjerg *et al.*, 1990). In this model (Fig. 4), the orientation of helices 3 and 4 is constrained by a Watson-Crick base-pairing interaction between positions 1082 and 1086, and a base triplet interaction with 1056G, that bring loops B and D into close proximity. Subsequent confirmation of the 1082-1086 interaction has been obtained by site-directed mutagenesis (Ryan & Draper, 1991). The hydroxyl radical data place protein L11 on top of the model (relative to the plane of the page) in the orientation presented in Figure 4, with the main ribose interactions occurring through the minor groove of helix 3.

Other studies suggest that L11 makes additionally interactions within the 23 S rRNA GTPase region. Mutagenesis of an rRNA fragment showed that base changes in helix 4 and loop C reduce L11 binding to a greater extent than base changes in helix 3 (Ryan *et al.*, 1991), and these data are largely supported by a mutagenesis study with intact 23 S rRNA (our unpublished data). Present indications are that L11 interacts with both helices making mainly backbone contacts with helix 3 and base contacts with helix 4.

(b) *Binding of L10.(L12)$_4$*

The L10.(L12)$_4$ r-protein complex binds to naked 23 S rRNA, probably through its L10 moiety (Pettersson, 1979), protecting riboses at positions 1043 and 1046 to 1049 at the 5′ side of loop A, and 1053 to 1055 in helix 2. The ribose at position 1068 is rendered more reactive by L10.(L12)$_4$. Chemical and enzymatic footprinting of this interaction (Fig. 2a) showed numerous protections encompassing both sides of loop A and extending into helix 1, as well as within the L11 binding site,

Table 2
Reactivities of 23 S rRNA nucleotides within ribosomes

Nucleotide position in 23 S rRNA	L11-minus ribosomes	L11-minus ribosomes + L11	L11-minus ribosomes + L11 + thiostrepton
U 1058	+ +	0·3	0·4
G 1059	+ +	0·1	0·1
U 1060	+ +	0·1	0·0
U 1061	+ +	0·2	0·1
G 1062	+ +	0·1	0·1
G 1063	+ + +	0·2	0·2
C 1064	+ + +	0·4	0·5
A 1067	+ + +		[0·5]
G 1068	+ + +		[0·4]
A 1069	+ +	0·4	[0·1]
A 1070	+	0·2	0·2
G 1071	+ +	0·6	0·4
A 1080	+ +	0·2	0·3
U 1081	+ +	0·1	0·1
U 1082	+ +	0·6	0·5
A 1086	+	(0·7)	0·5
G 1087	+ +	0·4	0·4
A 1088	+ +	0·3	0·4
A 1096	+ +	0·5	[0·1]
U 1097	+ +		[0·2]
A 1098	+ +	0·6	0·4
A 1103	+		[1·9] †

Reactivities to hydroxyl radicals of 23 S rRNA nucleotides within ribosomes that are affected by the interaction of L11 and thiostrepton. The intensities of the autoradiograph bands were measured and are presented as described for Table 1. Bands were standardized against positions 1049, 1110 and additionally at 1102 (†). Only the positions that were significantly affected by L11 or thiostrepton binding are shown, a blank space indicates that there was no change greater than 30% relative to this position in L11-minus ribosomes. The additional effects caused by thiostrepton are boxed, the effects caused by micrococcin were similar but less intense (Fig. 3).

particularly around position 1068 (Egebjerg *et al.*, 1990). The hydroxyl radical data indicate that L10.$(L12)_4$ makes only few rRNA backbone interactions and binding to the rRNA probably involves more extensive protein-base or protein-phosphate contacts.

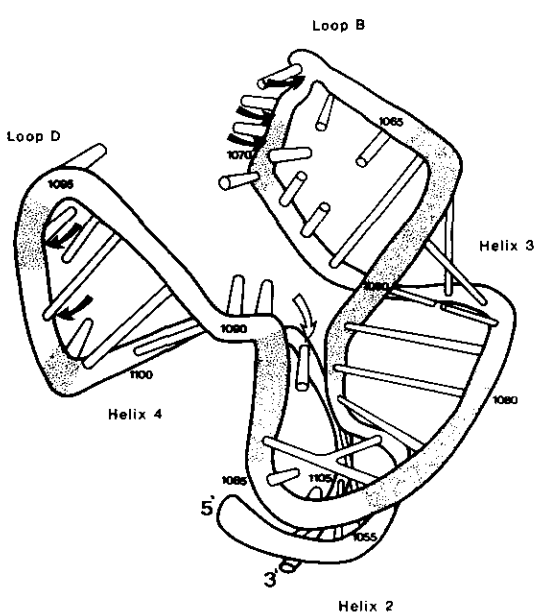

Figure 4. Proposed tertiary structural model of the L11 binding region (Egebjerg *et al.*, 1990). The shaded regions represent nucleotides that were shielded against hydroxyl radicals by protein L11 in ribosomes. Thiostrepton protected the riboses indicated by the filled arrows in loops B and D, and increased the accessibility of position 1103 shown by the open arrow.

The binding of L10.$(L12)_4$ and L11 has previously been shown to be mutually co-operative (Dijk *et al.*, 1979). The mechanism by which co-operativity occurs is indicated at ribose position 1068 where L10.$(L12)_4$ increases the accessibility of an L11 contact point. On binding of L11 and the pentameric complex to the rRNA, enhanced protein interaction occurs adjacent to the 1082-1086 pair which stabilizes the orientation of helices 3 and 4.

(c) *The structure of reconstituted ribosomes*

Enzymatic and chemical probing of L11-minus ribosomes reconstituted with protein L11 produced footprinting patterns identical to those of wild-type ribosomes. The antibiotics thiostrepton and micrococcin completely protected nucleotides in the reconstituted ribosomes at the same positions as in wild-type ribosomes (Egebjerg *et al.*, 1989). The more intense drug protections in reconstituted ribosomes could reflect that L11 is partially lost from wild-type ribosomes during isolation (Egebjerg *et al.*, 1990). It is concluded that the GTPase region of L11-minus ribosomes is accessible for interaction with L11, and the protein associates with this region in a stoichiometry of approximately 1 : 1, producing a structure that is indistinguishable from the wild-type ribosome containing L11.

(d) *L11 interactions with riboses within ribosomes*

The L11-ribose interactions in ribosomes are largely the same as those in naked rRNA, with the major protection sites located in helix 3 and loop B. However, there are several distinct differences. In the ribosome, there is a displacement in ribose positions that are protected in loop B, and there are fewer protections along the 3' side of helix 3 into loop C. The protection effects that remain (e.g. at positions 1060, 1080 and 1081) are generally stronger than in the naked rRNA, and additional weak protection effects are observed in helix 4 and loop D within ribosomes. These differences could be caused by either partial exclusion of hydroxyl radicals or Fe^{2+}-EDTA from the region, or by changes in the contacts between L11 and the rRNA backbone. As this region is freely accessible both in the naked rRNA and in L11-minus ribosomes, the latter explanation is the more likely and indicates that the L11-rRNA interaction is subtly influenced by other r-proteins in addition to the $L10.(L12)_4$ complex.

In ribosomes, the reactivity of riboses is generally lower than in naked rRNA. However, two sequences in ribosomes, at 1040 to 1042 and 1114 to 1116, are extremely reactive towards hydroxyl radicals (Fig. 3). In the naked rRNA, this high reactivity is absent, and nucleotide reactivity is unaffected by the binding of L11 and $L10.(L12)_4$, even though these sequences lie on opposite sides of helix 1, adjacent to the binding site of $L10.(L12)_4$. Nucleotide 1041G interacts with A-site bound tRNA (Moazed & Noller, 1989), suggesting that this region of helix 1 is exposed on the surface of the subunit.

(e) *Antibiotic interaction with ribosomes*

Thiostrepton and micrococcin bound to the reconstituted ribosomes (Fig. 3), and protected nucleotides between positions 1067 and 1098 from the DMS and enzymatic probes. The extensive protection effects are presumed to result from only a few direct drug contacts that tighten the structure of this rRNA region or strengthen the L11-rRNA interaction (Egebjerg *et al.*, 1989). This idea is supported by the hydroxyl radical data. The riboses protected by thiostrepton are limited to positions 1067 to 1069 in loop B, 1096 and 1097 in loop D, while position 1103 in loop C becomes slightly more exposed (Fig. 4). The effect of micrococcin binding is similar but weaker.

Other studies have also implicated loops B and D as the sites of drug interaction. The thiostrepton-producing organism *Streptomyces azureus* produces a methyl transferase that modifies the ribose 2'-OH at nucleotide 1067, lowering the binding constant of the drug by at least six orders of magnitude (Cundliffe, 1990). Transversion mutations at 1067A confer thiostrepton resistance in *E. coli* ribosomes (Thompson *et al.*, 1988). Furthermore, thiostrepton and micrococcin binding (as estimated by footprinting) is reduced in ribosomes containing 23 S rRNA mutagenised at 1067A or 1095A (our unpublished data).

(f) *Functional implications*

The r-proteins $L10.(L12)_4$ and L11 and their rRNA binding region are closely associated with ribosomal A-site-dependent processes including interaction with tRNA (Moazed & Noller, 1989) and with soluble protein factors (reviewed by Cundliffe, 1986, 1990). It has been suggested that during these different steps of protein synthesis, the rRNA in the ribosomal GTPase centre undergoes transition between discrete conformational states (Cundliffe, 1986). It is shown here that the protection pattern of L11 on the rRNA is modulated by the $L10.(L12)_4$ complex and other (as yet undetermined) r-proteins. These effects could be induced by conformational change in the rRNA, in L11 or in both components. Whether these changes in the L11-rRNA interaction are indicative of transitions between different functional conformational states during protein synthesis is not yet clear.

Close functional ties have been demonstrated between the GTPase centre within 23 S rRNA domain II and the region where the cytotoxin α-sarcin cleaves in domain VI (reviewed by Wool *et al.*, 1992). Elongation factor EF-G interacts with both of these domains (Sköld, 1983; Leffers *et al.*, 1988; Moazed *et al.*, 1988), and α-sarcin cleavage is partially blocked by thiostrepton binding to domain II (Miller & Bodley, 1991). These studies suggest that the two domains are probably folded so as to lie in close proximity within the 50 S subunit. Our data show no indication that L11 or thiostrepton have any direct effects upon the structure of the α-sarcin site. The inference from this is that other ribosomal components mediate the tertiary folding of domain II relative to domain VI.

The antibiotics thiostrepton and micrococcin bind to the GTPase centre and inhibit the steps of protein synthesis associated with this region (Cundliffe, 1990; Cundliffe & Thompson, 1981). The drugs protect only a few riboses here, making it possible to pinpoint the interaction sites on the rRNA. Our interpretation is that the drugs slot into the rRNA between loops B and D, making direct contact with nucleotides here. This increases the accessibility of loop C (Fig. 4), possibly by altering the orientation of stems 3 and 4. Drug binding inhibits the function of the region by either locking it in one conformation, as suggested by Cundliffe (1986), or by sterically blocking the accessibility of the region.

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