Localization of a series of RNA-protein cross-link sites in the 23S and 5S ribosomal RNA from *Escherichia coli*, induced by treatment of 50S subunits with three different bifunctional reagents

Monika Oßwald, Barbara Greuer and Richard Brimacombe*
Max-Planck-Institut für Molekulare Genetik, Abteilung Wittmann, Berlin-Dahlem, FRG

Received October 17, 1990; Revised and Accepted November 5, 1990

ABSTRACT

50S ribosomal subunits were reacted with *bis*-(2-chloroethyl)methylamine, 2-iminothiolane or methyl p-azidophenyl acetimidate, and RNA-protein cross-link sites on the RNA were localised using our published procedures. The degree of precision with which these sites could be determined was variable, depending on the particular protein or RNA region concerned. The following positions in the 23S RNA were identified as encompassing the individual cross-link sites (numbered from the 5'-end, with asterisks denoting sites previously reported): L1, 1864–67, 1876–78,2119–33, 2163–72*; L2, 1819–20*; L3, 2832–34; L4, 320–25*; 613–17*; L5, 2307; L6, 2473–81*; L9, 1484–91; L11, 1060–62; L13, 547–50; L14, 1993–2002; L17, 1260–95; L18, 2307–20; L19, 1741–58; L21, 544–48*; 1198–1248; L23, 63–65, 137–41*; L24, 99–107*; L27, 2272–83, 2320–23*; 2332–37*; L28, 195–242, 368–424; L29, 101–02*; L30, 931–38; L32, 2878–90; L33, 2422–24. Cross-links to 5S RNA were observed with L5 (positions 34–41), and L18 (precise site not localised).

INTRODUCTION

RNA-protein cross-linking is a well-established method for studying ribosome structure. In the case of the *E. coli* 30S ribosomal subunit, the application of the technique has led to the identification of a series of cross-link sites on the 16S RNA involving many of the 30S ribosomal proteins (1, 2), and this data set played a prominent role in the recent development of models for the three-dimensional arrangement of the 16S ribosomal RNA *in situ* in the 30S subunit (3, 4); the RNA-protein cross-links provide a 'link' between the phylogenetically-established secondary structure of the ribosomal RNA (5, 6) and the topographical arrangement of the ribosomal proteins, as determined by neutron scattering (7).

For some time we have been accumulating a corresponding set of RNA-protein cross-link data for the *E. coli* 50S ribosomal subunit (8, 9), using the same reagents as those that were applied to the 30S subunit, namely *bis*-(2-chloroethyl)-methylamine (2, 'nitrogen mustard'), 2-iminothiolane (10), and methyl p-azidophenyl acetimidate (1, 'APAI'). The first of these is a symmetrical bifunctional reagent which leads to cross-linking in a single incubation step, whereas the other two reagents react first with the lysine groups of proteins and the cross-linking to RNA is then achieved by a brief exposure to ultraviolet irradiation. The more complex nature of the 50S subunit made it necessary to introduce some refinements into our methodology for the identification of the cross-link sites, and these have been described elsewhere (11, 12). The purpose of the present paper is simply to document the cross-link sites that we have identified, which now total 30 sites in the 23S RNA, and two in the 5S RNA.

MATERIALS AND METHODS

^{32}P-labelled 50S ribosomal subunits from *E. coli* strain MRE 600 were isolated and subjected to cross-linking with nitrogen mustard, 2-iminothiolane or APAI as described in ref. 11. Non-cross-linked protein was removed by centrifugation through sucrose gradients containing SDS, according to ref. 12, and the peak of 23S RNA plus 23S RNA-protein complexes was precipitated with ethanol. Partial digestion of the RNA was then carried out using ribonuclease H together with sets of oligodeoxynucleotides as described in ref. 12, with the exception that both sets of deoxynucleotides (12) were added simultaneously, and the digestion was carried out at 55° under the slightly modified conditions of ref. 13. RNA-protein complexes were separated from 'free' RNA fragments by glass fibre filtration (12, 14), and after elution from the filters the individual cross-linked complexes were separated by two-dimensional gel electrophoresis as in ref. 11. Radioactive spots of interest were excised from these gels and subjected to final purification (with concomitant identification of the protein contained in the cross-linked complex) by antibody affinity chromatography, again as in ref. 12, using antibodies to the individual 50S proteins. The ^{32}P-labelled RNA fragments were released from the cross-linked protein and thus eluted from the agarose by treatment with proteinase K (12), and each sample

* To whom correspondence should be addressed

was then subjected to oligonucleotide analysis by total digestion with ribonuclease A or T_1, followed by two-dimensional thin-layer chromatography on polyethyleneimine cellulose plates (15), according to our standard procedures (11, 12). Secondary digests of the separated oligonucleotides were made in the usual manner (11, 12), and finally the oligonucleotide sequence data were fitted to the 23S RNA sequence of Brosius *et al.* (16).

RESULTS

The two most significant refinements in our methodology — which as mentioned above were developed for the identification of RNA-protein cross-link sites in the 50S subunit — are (a) the use of ribonuclease H in combination with a set of specific oligodeoxynucleotides to digest the cross-linked RNA into fragments of a suitable size for analysis (12, 13), and (b) the use of the immuno affinity chromatography method with antibodies to the individual ribosomal proteins (9, 12) as a final purification step in the isolation of the cross-linked complexes.

An example of a two-dimensional gel separation of ^{32}P-labelled cross-linked RNA-protein complexes after digestion by the ribonuclease H method (see Materials and Methods and ref. 12) is shown in Figure 1. In this case the 50S subunits had been crosslinked by treatment with 2-iminothiolane (8). The gel system (11) involves the use of high salt concentrations in both dimensions, to prevent aggregation of the RNA-protein complexes, and the first dimension is a high-percentage gel run in the presence of urea and SDS, whereas the second dimension is a low-percentage gel run in the presence of urea and the zwitterionic detergent 'CHAPS'. The step-down in gel concentration between the two dimensions makes it necessary to cut the first-dimension gel into slices, and to elute each slice before application to the second dimension. Each second dimension gel slot in Figure 1 thus represents the eluate from a 1.7 mm slice in the first dimension. The gel shows the usual features (cf. 9, 11, 12) of a 'diagonal' of free RNA fragments (which are never completely removed by the glass fibre filtration step — see Materials and Methods), with the cross-linked RNA-protein complexes appearing above this diagonal.

If the gel of Figure 1 is compared with the corresponding gels from older experiments (e.g. Fig. 1 of ref. 9, in which the RNA had been subjected to limited digestion with cobra venom nuclease (17)), it is clear that the ribonuclease H method leads to a much better defined and less heterogeneous spectrum of 23S RNA fragments. However, since the digestion of the RNA is essentially quantitative with ribonuclease H, there is much less material remaining in the upper part of the first-dimension gel (not shown in Fig. 1), and the regions of the gel containing complexes of a useful size for analysis are correspondingly more 'crowded'. (Running the second-dimension gels for longer times does not significantly improve the separation, as the bands merely tend to smear out.) As a result, the final analysis of the individual cross-linked complexes is crucially dependent on the second technical improvement mentioned above, namely the use of immuno affinity chromatography (9, 12). The gel of Figure 1 shows several examples of the successful application of this technique; in one case no less than three different cross-linked complexes, arising from entirely different regions of the 23S RNA and cross linked respectively to proteins L11, L14 and L24, could be identified from a single fraction extracted from the gel, and in two other cases pairs of complexes (containing L29 and L30, and L29 and L33, respectively) could similarly be analysed. In those gel fractions where only a single complex was present, the immuno affinity chromatography step serves to confirm the identity of the cross-linked protein and in general also leads to much 'cleaner' oligonucleotide analyses of the cross-linked RNA.

The fact that the same protein (with the same cross-link site on the RNA) sometimes appears in two different places on the

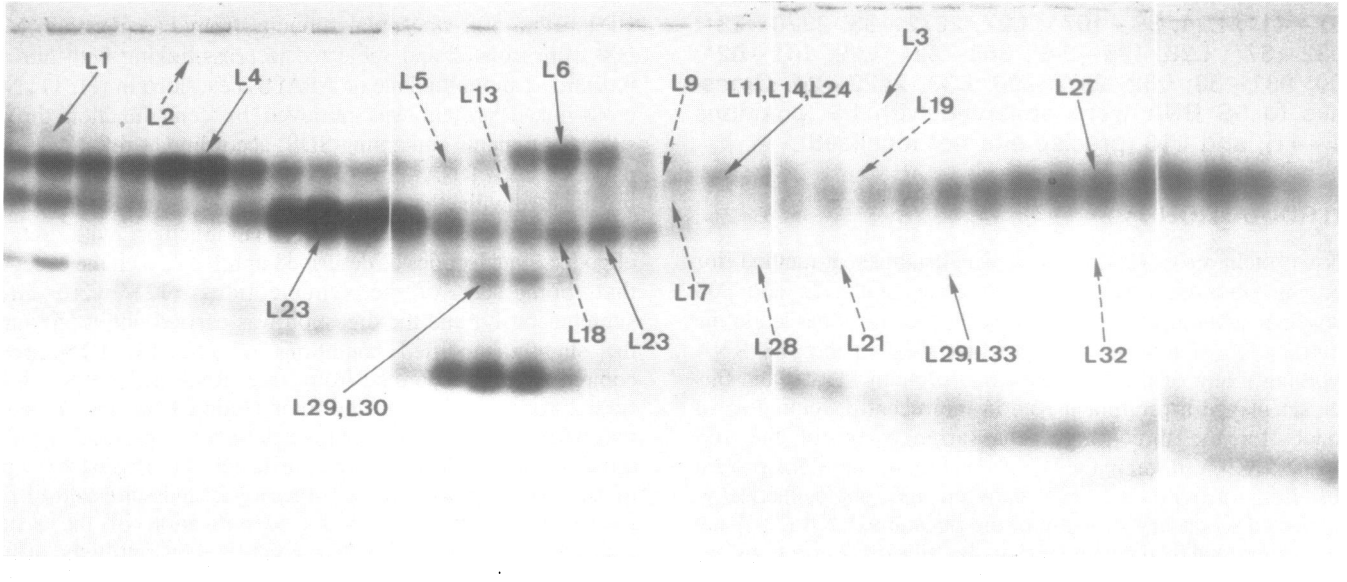

Figure 1. Two-dimensional gel separation of RNA-protein cross-linked complexes. Direction of the first dimension is from left to right, and that of the second dimension from top to bottom. Each second dimension gel has ten slots, containing the individual eluates from the first-dimension gel slices; four such gels are combined to give the complete pattern. The rows of RNA-protein complexes lie above the 'diagonal' of free RNA, and are marked with arrows giving the identity of the protein(s) found in each group of spots. Arrows with solid lines denote proteins found in this particular experiment (in which the 50S subunits were cross-linked with 2-iminothiolane). Arrows with dotted lines indicate the approximate positions on the gel of proteins in complexes observed in other experiments, or with the other two cross-linking reagents.

gel (e.g. L29 or L23 in Fig. 1) indicates that the ribonuclease H digestion is not entirely homogeneous and that RNA fragments of different lengths may be generated from the same sequence region. For this and other reasons (see below) some variability in the gel patterns was observed, and not every cross-linked protein was found in every experiment with a particular cross-linking reagent. Nonetheless the final analyses of the actual cross-link sites on the RNA were in general highly consistent and reproducible. The gel patterns of cross-linked products obtained with the other two reagents (nitrogen mustard (2) or APAI (1)) were qualitatively very similar to the iminothiolane pattern shown in Figure 1, although other groups of proteins were represented; the positions where these other cross-linked complexes appeared on the gels are indicated by the dotted lines in Figure 1.

The results of a large number of cross-link site analyses, derived from all three cross-linking reagents, are summarized in Table 1. This data set was collected over a relatively long period, during which the methodology for cross-link site analysis was still being developed. The procedure given in Materials and Methods represents the latest version, and most of the cross-link sites in Table 1 were determined using this procedure. A number of the sites, however, including those already published (8, 9), were repeatedly observed at different stages of the methodological development. As an exception, it should be noted that the two cross-link sites to 5S RNA (involving proteins L5 and L18, Table 1) were only observed using the older procedure of ref. 11. (In the new procedure, where non-cross-linked protein is removed by sucrose gradient centrifugation in SDS, cross-linked complexes with 5S RNA run together with the free proteins at the top of the gradient, and are therefore effectively lost.)

The yield and frequency of observation of the individual cross-linked complexes listed in Table 1 varied considerably. Apart from the obvious and expected heterogeneity in the cross-linking reactions themselves (cf. Fig. 1), the amount of recoverable complex is dependent on many factors, such as the efficiency of the ribonuclease H digestion in the 23S RNA region concerned (already mentioned above), the tendency of a particular protein or complex to aggregate in the separation systems, or the ability of the individual antibodies to recognize their corresponding protein in the cross-linked complexes. The degree of precision with which the positions of the various cross-link sites could be determined within the 23S RNA sequence was also very variable. In general, a cross-link site is indicated by the absence of a particular oligonucleotide from the ribonuclease T_1, or ribonuclease A fingerprint (cf. 1, 2, 9). This absence should in theory be accompanied by the concomitant appearance of a 'new' spot on the fingerprint corresponding to the cross-linked oligonucleotide-oligopeptide residue. In practice, however, this cross-linked moiety is often heterogeneous (as a result of incomplete digestion of the protein during the proteinase K treatment used to release the cross-linked RNA from the agarose-antibody matrix—see Materials and Methods), and is in consequence not always visible on the fingerprints. In other cases the cross-link site may be obscured simply by lying in an unfavourable position in the RNA sequence; these questions have been discussed in detail elsewhere (e.g. 1). The precision of the individual cross-link site analyses can be discerned from Table 1, the following being a brief summary of the data.

Cross-link L1a was identified within the sequence region encompassing positions 1850–1905 by the presence of sub-molar amounts of two oligonucleotides in the ribonuclease T_1 fingerprint (at positions 1864–67 and 1876–78); these positions lie opposite one another in helix 68 of the 23S RNA secondary structure (see Fig. 2, below). The exact location of cross-link L1b varied from one experiment to another within the region of positions 2119–33; this type of situation was not uncommon with the cross-links induced by nitrogen mustard. Cross-link L1c

Table 1: Locations of RNA-protein cross-link sites in 23S (and 5S) RNA. The position of each cross-link site is given generally in terms of the ribonuclease T_1-oligonucleotide (ending with G) or ribonuclease A-oligonucleotide (ending with C or U) which was 'missing' in the fingerprint analysis. Where it is necessary to show a longer sequence, a missing T_1-oligonucleotide is indicated by underlining and a missing A-oligonucleotide by overlining. Double underlining indicates the cross-linked bases which could be identified by secondary digestion of a cross-linked oligopeptide-oligonucleotide residue on the fingerprint (see text). The cross-linking reagent with which each cross-link site was found is denoted by 'X' in the appropriate column, '(X)' indicating that the same sequence region was found with another reagent, but not necessarily analysed in detail. 'NM' is nitrogen mustard, '2-IT' is 2-iminothiolane, and 'APAI' is methyl p-azidophenyl acetimidate. Sequence regions in the 23S RNA (16) are numbered from the 5'-end.

	Cross-link site		Reagent		
Protein	Oligonucleotide	Sequence region	NM	2-IT	APAI
L1a	UCAG[a,b]	1864-67			X
	AAG[b]	1876-78			
L1b	AGGUGGGAGGCUU$^{A}_{U}$G[c]	2119-33	X		
L1c	ACCUUG	2163-68		X	
	GAAAU[d]	2168-72	X		
L2	UAUACG[e]	1818-23		X	
L3	UUG	2832-34		X	
L4a	AUACAG[e]	320-25	(X)	X	(X)
L4b	AAUAG[f]	613-17		X	(X)
L5	UCGGAC	2305-10	X		
L5 (5S)	ACCCCAUG	34-41 (5S)			X
L6	UUCAUAUCG[f]	2473-81	(X)	X	(X)
L9	UUUUCCAG	1484-91			X
L11	UUG	1060-62		X	
L13	CUUAGGC	544-50	X		
L14	UCUCCACCCG	1993-2002		X	X
L17	- - -[g]	1260-95	X		
L18	GGACAUCAGGAGGU[c]	2307-20	X		
L18 (5S)	- - -[g]	1-120 (5S)	X		
L19	- - -[g]	1741-56			X
	GAAGAU[h]	1753-58	X		
L21a	CUUAG[f]	544-48		X	
L21b	- - -[g]	1198-1248	X		X
L23a	CUAAUCUG	61-68	X		
L23b	UUUCG[f]	137-41	(X)	X	(X)
L24	UUAUAACCG[e]	99-107		X	
L27a	UAACG	2272-76			X
	GGAGGAGC	2276-83	X		
L27b	UUAG[e]	2320-23		X	
L27c	CAUAAG[f]	2332-37		X	
L28a	- - -[g]	195-242	X		(X)
L28b	- - -[g]	368-424	X		
L29	UUAUAACCG[f]	99-107	(X)	X	
L30	UUAUCACG[j]	931-38		X	
L32	UACUAAUG	2878-85		X	
	AACCG	2886-90			X
L33	CUCAACG	2422-28	(X)	X	(X)

a. The sequence of Brosius *et al.* (16) has UUAG at this position.
b. The sequence region found covered nucleotides 1850–1905; both oligonucleotides indicated were only present in sub-molar amounts.
c. Several sites were observed in this oligonucleotide region in different experiments.
d. Reported by I.Wower, (Ph. D. thesis, Leeds, England, 1983).
e. Reported in ref. 9.
f. Reported in ref. 8.
g. The precise cross-linking site could not be localised.
h. This site was only observed in one experiment.
j. This site was only observed in one experiment, but in several different fragments on the gel (see text).

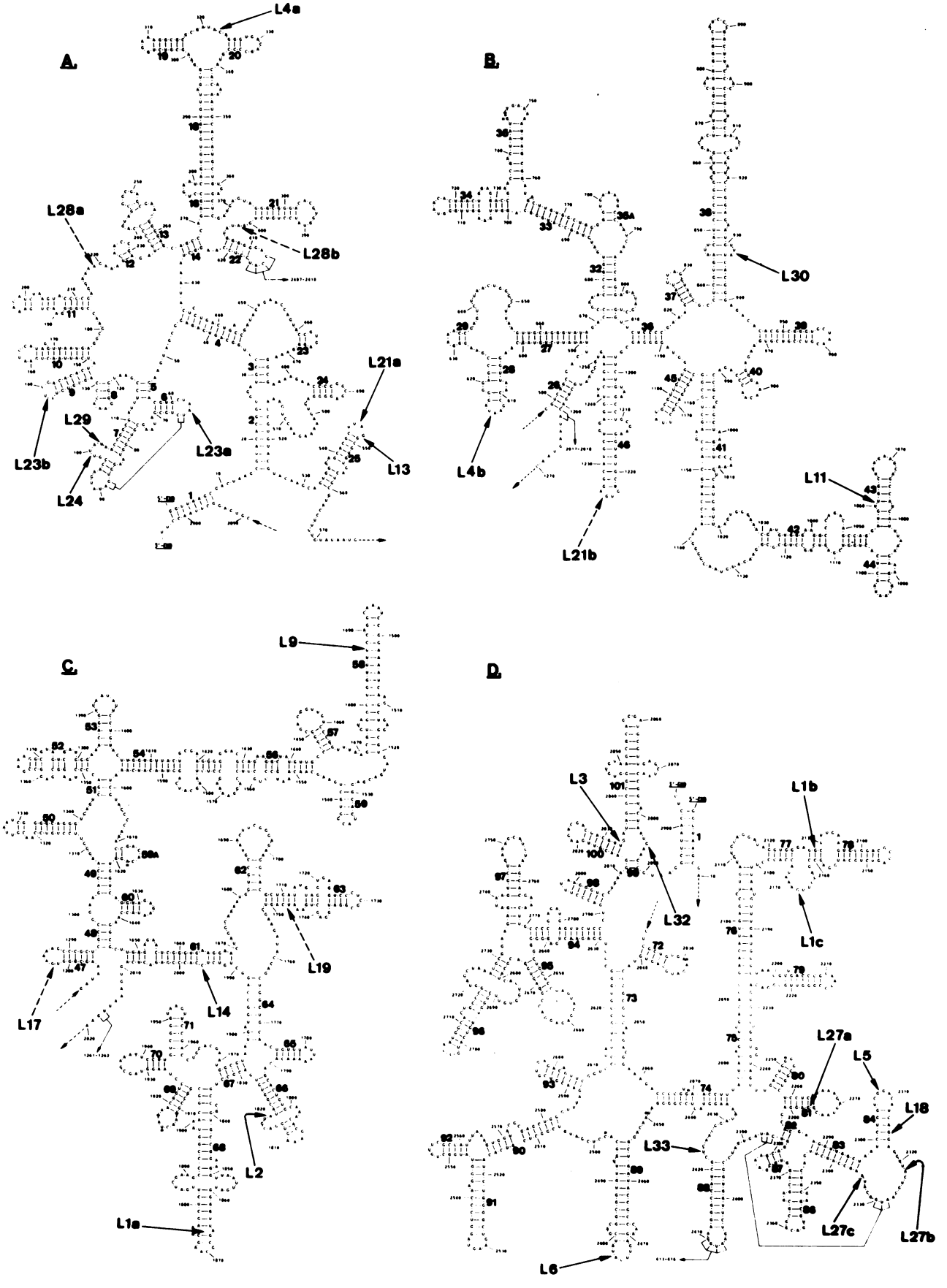

Figure 2. Secondary structure of 23S RNA (6,18), showing the locations of the RNA-protein cross-link sites listed in Table 1. Dotted lines for a particular cross-link indicate that the RNA region containing the cross-link has been identified, but not the site within that region (see Table 1 for details).

was located by the absence of a ribonuclease T_1-oligonucleotide (at positions 2163–68, using iminothiolane) and an adjacent ribonuclease A-oligonucleotide (at positions 2168–72, using nitrogen mustard). The cross-link to L2 (9) was identified by the absence of a hexanucleotide from the T_1-fingerprint; a further localisation to the AU sequence at positions 1819–20 could be made from secondary analyses of the cross-linked oligopeptide-oligonucleotide moiety. The cross-link to L3 and the two cross-links to L4 (cf. 9) were all identified by the absence of the respective oligonucleotides (positions 2832–34, 320–25 and 613–17) from the T_1-fingerprints; cross-link L4a was found with all three reagents, and L4b with two out of the three. The cross-link to L5 in 23S RNA was pin-pointed by the absence of UCGp (2305–07) from the T_1-fingerprint and of GGACp (2307–10) from the A-fingerprint; the precise site is therefore at position G-2307, or possibly 2308. The corresponding cross-link to L5 in 5S RNA lies within the T_1-oligonucleotide at positions 34–41 of the 5S sequence. The cross-links to proteins L6, L9, L11 and L14 were all identified by the absence of the respective T_1-oligonucleotides (Table 1); the cross-link to L6 was found with all three reagents. In the case of L13, the T_1-oligonucleotide at positions 544–48 was only weakly present, whereas the ribonuclease A-oligonucleotide at positions 547–50 was entirely absent; the probable principal cross-linked nucleotide is therefore G-549.

The cross-link to protein L17 could only be approximately identified, and lies within the region 1260–95; this represents the sequence of the shortest fragment found containing the cross-link. The cross-link to L18 in 23S RNA was somewhat heterogeneous (between positions 2307–20), similar to the situation observed with cross-link L1b (above); the corresponding cross-link to L18 in 5S RNA could not be localised further. The cross-link to L19 — like that to L17 — could only be approximately localised, in this case to a region encompassing positions 1741–56 of the 23S RNA (using APAI); the corresponding nitrogen mustard cross-link (at positions 1753–58) was only seen in one experiment. For protein L21, in addition to the previously observed site at positions 544–48 (8), a new cross-linked RNA region (1198–1248) was also observed with both nitrogen mustard and APAI; a more precise localisation was not possible, however. L23 also gave a new cross-link (L23a) as well as the site previously seen at positions 137–41 (L23b, ref. 8); the new cross-link could be localised to the AAU sequence (63–65) within the missing ribonuclease T_1-oligonucleotide at positions 61–68, by secondary analysis of the cross-linked oligonucleotide-oligopeptide moiety.

The cross-link to L24 is that already published (9), within the T_1-oligonucleotide at positions 99–107; L29 was also previously shown (8) to cross-link to the same oligonucleotide, in this case to the AU sequence at positions 101–02. Protein L27 showed several cross-link sites; L27a was defined by a missing T_1-oligonucleotide at positions 2272–76 (using APAI) and a missing A-oligonucleotide at positions 2276–83 (using nitrogen mustard), cross-links L27b and L27c being those already published (9) at positions 2320–23 and 2332–37. Protein L28 showed cross-links to two distinct regions of the RNA; the shortest observed fragments containing these cross-links covered the sequence regions 195–242 and 368–424, respectively, but a more precise localisation was unfortunately not possible in either case. The cross-link to L30 was only seen in a single experiment; however, we feel confident in including this result, because the cross-linked complex appeared in several positions on the gel (cf. Fig. 1) with RNA fragments of different lengths, and in each case the T_1-oligonucleotide at positions 931–38 was clearly absent from the fingerprints. Protein L32 was cross-linked to two immediately adjacent regions, as indicated by the absence of the T_1-oligonucleotide at positions 2878–85 (using 2-iminothiolane) and that at positions 2886–90 (using APAI). Protein L33 was cross-linked to the T_1-oligonucleotide at positions 2422–28, and the site could be further localised to positions 2422–24 by analysis of the cross-linked oligonucleotide-oligopeptide residue. Finally, it should be mentioned that a number of further cross-links to various proteins were observed, which were either not sufficiently well-defined or reproducible to merit inclusion in Table 1.

DISCUSSION

The positions of all the RNA-protein cross-link sites (Table 1) in the secondary structure of the 23S RNA (6, 18) are shown in Figure 2. A number of points can be made concerning the distribution of these sites. First, there are several cases where two or more cross-links have been observed to individual proteins. In some instances, such as protein L23 (with cross-links in helices 6 and 9) or L27 (with cross-links in helix 81 and the region linking helices 83 and 84), the multiple cross-link sites lie close together in the secondary structure. In other cases, such as L4 (with site L4a in helix 20 and site L4b in helix 28) or L1 (with L1a in helix 68 and L1b, L1c in helices 77–78), the sites are widely separated and thus serve as strong constraints on the tertiary folding of the RNA; in the three-dimensional structure the various cross-link sites to any one protein must obviously be relatively closely neighboured. The multiple sites to protein L28 in the 5′-domain and to L21 (in helices 25 and 46) represent an intermediate situation, and proteins L5 and L18 — for which cross-links in both 23S RNA and 5S RNA were observed—should also be mentioned in this context.

If the cross-linking data are compared with the published binding sites or footprint sites for the various proteins, then a further set of correlations becomes apparent. Some cross-link sites, such as that to L11 or the two cross-links L1b and L1c, lie within the observed binding sites for those proteins (refs. 19, 20 and 21, respectively). Similarly, proteins L5 and L18 are well-known 5S RNA-binding proteins (e.g. 22), as well as showing *in situ* cross-links to 5S RNA, as just noted. Furthermore, these latter two proteins were found in a ribonucleoprotein fragment isolated from the 50S subunit (23), which contained the same 23S RNA region as that where the cross-link sites to L5 and L18 occur (Fig. 2). In contrast, the cross-link sites to protein L23 (in helices 6 and 9) are in a completely different area of the 23S RNA to the binding site for this protein, which encompasses helices 51 to 54 (24); this implies that these two RNA regions must be closely neighboured in the tertiary structure. A similar case is that of protein L9, which was found in a ribonucleoprotein fragment together with L1 and helices 75 to 79 of the 23S RNA (23), but which is cross-linked to helix 58. Again, 'intermediate' situations can be seen for proteins L3 and L24; L3 has a cross-link site in helix 100, but a principal footprint site in helix 94 (25), whereas L24 has a cross-link site in helix 7, but footprint sites on the opposite side of the 5′-domain in the region of helices 18, 22, 23 and 24 (26).

A final set of correlations can be made by comparing the RNA-protein cross-linking data with the known topographical arrangement of the 50S ribosomal proteins. In contrast to the 30S subunit (7), a complete neutron scattering map for the proteins of the 50S subunit is not yet available (see however ref.

27), but there is nevertheless a considerable amount of immuno electron microscopic and protein-protein cross-linking data, which have been incorporated by Walleczek *et al.* (28) into a map of the 50S protein arrangement. Although this map is preliminary, experience with the corresponding data for the 30S subunit has shown that the level of agreement between the neutron and electron microscopic data is in general extremely high (18).

A number of protein neighbourhoods in the Walleczek map (28) are also reflected in the RNA-protein cross-linking data (Fig. 2). Thus, L23 and L29 are close neighbours in the protein map, and have cross-link sites close together in the 23S RNA (in helices 6, 7 and 9). Similar pairs of neighboured proteins are L13 and L21 (with cross-links in helix 25), L14 and L19 (with sites in helices 61 and 63, respectively), or L3 and L32 (with sites in helices 100–101), and a conspicuous group is formed by proteins L5, L18, L27 and L33 (with cross-link sites in helices 81 to 88). As above, however, other pairs of neighboured proteins in the Walleczek map (28) have cross-link sites that are far apart in the 23S secondary structure, and — again — this type of data provides constraints on the tertiary folding of the RNA. Clear examples are the cases of L1 and L9 already mentioned above (with cross-link sites in helices 58, 68 and 78), or L6 and L11 (with sites in helices 89 and 43, respectively).

As was the case with the 30S subunit (3), the RNA-protein cross-linking data have played an important role in the construction of a first model for the three-dimensional folding of the 23S RNA (see refs. 13, 29 for a preliminary description). However, it must be borne in mind that the lack of any detailed knowledge concerning the shapes of the individual ribosomal proteins is a factor which severely limits the application of this type of data in model-building studies. RNA-protein cross-link sites (or footprint sites, cf. ref. 4) can thus only be used to give a rather crude orientation of a particular RNA region in the three-dimensional structure, and the more detailed RNA topography will have to be determined by other methods, such as intra-RNA cross-linking (see ref. 18 for further discussion). For this reason, we have decided to conclude our intra-subunit RNA-protein cross-linking studies at this point.

ACKNOWLEDGEMENTS

We are most grateful to Drs H.Bäumert and H.Fasold for synthesizing the APAI cross-linking reagent for us, and to Dr G.Stöffler for providing us with antibodies to the ribosomal proteins.

REFERENCES

1. Oßwald,M., Greuer,B., Brimacombe,R., Stöffler,G., Bäumert,H. and Fasold,H. (1987) *Nucl. Acids Res.* **15**, 3221–3240.
2. Greuer,B., Oßwald,M., Brimacombe,R. and Stöffler,G. (1987) *Nucl. Acids Res.* **15**, 3241–3255.
3. Brimacombe,R., Atmadja,J., Stiege,W. and Schüler,D. (1988) *J. Mol. Biol.* **199**, 115–136.
4. Stern,S., Weiser,B. and Noller,H.F. (1988) *J. Mol. Biol.* **204**, 447–481.
5. Noller,H.F. (1984) *Annu. Rev. Biochem.* **53**, 119–162.
6. Brimacombe,R. and Stiege,W. (1985) *Biochem. J.* **229**, 1–17.
7. Capel,M.S., Kjeldgaard,M., Engelman,D.M. and Moore,P.B. (1988) *J. Mol. Biol.* **200**, 65–87.
8. Wower,I., Wower,J., Meinke,M. and Brimacombe,R. (1981) *Nucl. Acids Res.* **9**, 4285–4302.
9. Gulle,H., Hoppe,E., Oßwald,M., Greuer,B., Brimacombe,R. and Stöffler,G. (1988) *Nucl. Acids Res.* **16**, 815–832.
10. Wower,I. and Brimacombe,R. (1983) *Nucl. Acids Res.* **11**, 1419–1437.
11. Brimacombe,R., Stiege,W., Kyriatsoulis,A. and Maly,P. (1988) *Methods Enzymol.* **164**, 287–309.
12. Brimacombe,R., Greuer,B., Gulle,H., Kosack,M., Mitchell,P., Oßwald,M., Stade,K. and Stiege,W. (1990) In Spedding,G. (ed.), *Ribosomes and Protein Synthesis – A Practical Approach*, IRL Press, Oxford, pp. 131–159.
13. Mitchell,P., Oßwald,M., Schüler,D. and Brimacombe,R. (1990) *Nucl. Acids Res.* **18**, 4325–4333.
14. Thomas,C.A., Saigo,K., McCleod,E. and Ito,J. (1979) *Analyt. Biochem.* **93**, 158–166.
15. Volckaert,J. and Fiers,W. (1977) *Analyt. Biochem.* **83**, 228–239.
16. Brosius,J., Dull,T.J. and Noller,H.F. (1980) *Proc. Nat. Acad. Sci. USA* **77**, 201–204.
17. Vassilenko,S.K. and Ryte,V.C. (1975) *Biokhimiya* **40**, 578–583.
18. Brimacombe,R., Greuer,B., Mitchell,P., Oßwald,M., Rinke-Appel,J., Schüler,D. and Stade,K. (1990) In Hill,W.E., Dahlberg,A., Garrett,R.A., Moore,P.B., Schlessinger,D. and Warner,J.R. (eds), *The Ribosome; Structure, Function and Evolution*, ASM Press, Washington DC, pp. 93–106.
19. Beauclerk,A.A.D., Cundliffe,E. and Dijk,J. (1984) *J. Biol. Chem.* **259**, 6559–6563.
20. Egebjerg,J., Douthwaite,S.R., Liljas,A. and Garrett,R.A. (1990) *J. Mol. Biol.* **213**, 275–288.
21. Branlant,C., Korobko,V. and Ebel,J.P. (1976) *Eur. J. Biochem.* **70**, 471–482.
22. Garrett,R.A. and Noller,H.F. (1979) *J. Mol. Biol.* **132**, 637–648.
23. Branlant,C., Krol,A., Sriwidada,J. and Brimacombe,R. (1976) *Eur. J. Biochem.* **70**, 483–492.
24. Vester,B. and Garrett,R.A. (1984) *J. Mol. Biol.* **179**, 431–452.
25. Leffers,H., Egebjerg,J., Andersen,A., Christensen,T. and Garrett,R.A. (1988) *J. Mol. Biol.* **204**, 507–522.
26. Egebjerg,J., Leffers,H., Christensen,A., Andersen,H. and Garrett,R.A. (1987) *J. Mol. Biol.* **196**, 125–136.
27. Nowotny,V., May,R.P. and Nierhaus,K.H. (1986) In Hardesty,B. and Kramer,G. (eds), *Structure, Function and Genetics of Ribosomes*, Springer-Verlag, New York, pp. 101–111.
28. Walleczek,J., Schüler,D., Stöffler-Meilicke,M., Brimacombe,R. and Stöffler,G. (1988) *EMBO J.* **7**, 3571–3576.
29. Brimacombe,R., Gornicki,P., Greuer,B., Mitchell,P., Oßwald,M., Rinke-Appel,J., Schüler,D. and Stade,K. (1990) *Biochim. Biophys. Acta* **1050**, 8–13.