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The use of 2-iminothiolane as an RNA-protein cross-linking agent in Escherichia coli ribosomes, and the localisation on 23S RNA of sites cross-linked to proteins L4, L6, L21, L23, L27 and L29

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SUMMARY

When E. coli ribosomal subunits are reacted with 2-iminothiolane and then subjected to a mild ultraviolet irradiation, an RNA-protein cross-linking reaction occurs. About 5% of the total protein in each subunit becomes cross-linked to the RNA, and a specific sub-set of proteins is involved in the reaction. In the case of the 50S subunit, the sites of cross-linking to the 23S RNA have been determined for six of these proteins: protein L4 is cross-linked within an oligonucleotide comprising positions 613-617 in the 23S sequence, L6 within positions 2473-2481, L21 within positions 540-548, L23 within positions 137-141, L27 within positions 2332-2337 and L29 within positions 99-107.

INTRODUCTION

For several years we have been concerned in this laboratory with the development of RNA-protein cross-linking techniques, as a tool for investigating the structural organisation of the E. coli ribosome (1-7). Our objective has been to introduce such cross-links in situ into the ribosomal subunits, and then to determine the precise sites of cross-linking on both protein and RNA, in order to build up a detailed topographical map of RNA-protein contacts and neighbourhoods within the particles. Up to now this research has been pursued in two directions, firstly the search for suitable bifunctional cross-linking reagents (1,2,4,6) and secondly the development of techniques for the analysis of the cross-link sites (3,5,7).

A number of symmetrical bifunctional reagents were found which could be used as RNA-protein cross-linking agents (2,4, and cf. 8), but only recently has a genuine hetero-bifunctional reagent been developed for the ribosome, namely p-azido phenyl-

acetic imidoester (APAI, 6). This type of compound has distinct advantages which have already been discussed (6); the cross-linking proceeds in two stages, involving firstly a reaction of the imidoester function with the ε-amino group of a lysine residue, which is then followed by mild ultraviolet irradiation to activate the azide group, leading to reaction with the RNA. A similar reagent has since been described by Millon et al. (9).

Techniques for cross-link site analysis were worked out using direct ultraviolet irradiation of the subunits as a test system, under conditions where the RNA-protein cross-linking reaction involved predominantly a single protein in each subunit (1). This led to the finding that methionine-115 in protein S7 is cross-linked to uridine-1240 in the 16S RNA within the 30S subunit (3,5), and that tyrosine-35 in protein L4 is similarly linked to uridine-615 in the 23S RNA within the 50S subunit (7). More important, in the latter case, the technology was developed with a view to future analyses of multiple simultaneous cross-link sites, which is the situation normally pertaining after reaction with a bifunctional chemical cross-linker. In this paper we describe for the first time such a multiple analysis of protein cross-link sites on 23S RNA.

The reagent which we have used in this instance was not APAI (see above), but rather a compound which reacts in a very similar fashion. This compound is the well-known protein-protein cross-linker 2-iminothiolane (10,11, previously designated methyl 4-mercaptobutyrimidate), which we have found acts very effectively as an RNA-protein cross-linking agent, if the reaction with the imino function is followed by a mild ultraviolet irradiation. This compound has the advantage over APAI that it is commercially available, and its use was suggested by the observation made fifteen years ago (12) that ultraviolet irradiation causes cross-link formation between cysteine and uridine.

MATERIALS AND METHODS

30S and 50S ribosomal subunits from E. coli MRE 600 were prepared as previously described (13,14,6). The subunits were either unlabelled, or labelled with ^{32}P in the RNA moiety, or labelled with ^{3}H in the RNA and ^{14}C in the protein moiety.

For reaction with 2-iminothiolane (obtained from Pierce Chemical Co.), the subunits were dialysed into 50 mM KCl, 5 mM magnesium acetate, 6 mM 2-mercaptoethanol, 25 mM triethanolamine-HCl, pH 7.8, and the concentration was adjusted to 20 A_{260} units/ml. 2-iminothiolane was dissolved in 0.5 M triethanolamine to a concentration of 0.5 M (10), and this solution was added to the subunit solution to give a final reagent concentration of 20 mM. Incubation was for 20 min at 22°, after which the subunits were precipitated with ethanol to remove excess reagent, and were resuspended in 50 mM KCl, 5 mM magnesium acetate, 50 mM triethanolamine-HCl, pH 7.8, to a concentration of 5 A_{260} units/ml. The solution was subjected to ultraviolet irradiation for 3 min under the conditions previously described (1), and was then made 3% in 2-mercaptoethanol and incubated for 30 min at 37°. The cross-linked subunits were concentrated by precipitation with ethanol, and resuspended in dodecyl sulphate buffer (1). The type and amount of subunits subjected to cross-linking in the particular experiments was as follows:

(a) To determine the extent of reaction, aliquots of double-labelled 30S or 50S subunits (1-2 A_{260} units) were used. After cross-linking as described above, the reaction mixtures were applied to 3-8% polyacrylamide gels containing dodecyl sulphate (1,2,6). The percentage of RNA-protein cross-linking was determined from the amount of ^{14}C-protein co-migrating with the peak of ^{3}H-RNA in the gel.

(b) To identify the cross-linked proteins, aliquots of up to 150 A_{260} units of unlabelled 30S or 50S subunits were mixed with ca. 3 A_{260} units of double-labelled subunit. After cross-linking, the reaction mixtures were applied to sucrose gradients in dodecyl sulphate (1,2,6). The peaks of RNA plus RNA-protein cross-linked complex isolated from the gradients were in each case subjected to digestion with ribonucleases A and T_1 as previously described (2). Oligonucleotides were removed by several precipitations with ethanol, and the cross-linked protein-oligonucleotide complexes were examined on the gel system of Mets and Bogorad (15) or that of Laemmli and Favre (16), exactly as described (2,6).

(c) To analyse the sites of cross-linking on the ribosomal

RNA, reaction mixtures were prepared containing 25 A_{260} units of ^{32}P-labelled 50S subunit (1-2 x 10^9 counts/min total). The RNA-protein cross-linked complexes were isolated on sucrose gradients as above, and 5 A_{260} unit aliquots were then subjected to a partial digestion with ribonuclease T_1 (1.3 - 2 units enzyme per A_{260} unit RNA) for 15 min at 37°, in 30 µl of 0.1% Triton X-100, 2.5 mM EDTA, 6 mM 2-mercaptoethanol, 10 mM Tris-HCl, pH 7.8. After incubation the samples were heated for 5 min at 80°, and applied to the Triton X-100/dodecyl sulphate two-dimensional gel electrophoresis system previously described (7). The ^{32}P-labelled RNA-protein complexes were located by autoradiography, and were extracted from the gel, yields at this stage being of the order of 10,000-50,000 counts/min per individual complex. Where necessary a further purification step was made, on 17% polyacrylamide gels containing urea as well as dodecyl sulphate, exactly as described (7). Each isolated RNA-protein complex (see text) was subjected to protein and oligonucleotide analysis.

The identity of the protein was established by two methods. Firstly, an aliquot of the RNA-protein complex was fully digested with ribonucleases A and T_1, and applied to the gel system of Mets and Bogorad (15) or of Laemmli and Favre (16), together with a sample of total 50S protein. The gels were stained and autoradiographed, exactly as described (7). This method was sometimes sufficient for an unequivocal identification of the protein in the cross-linked complex, but more often served only to identify a group of possible candidates. In such cases a second aliquot of the isolated RNA-protein complex (without further digestion) was precipitated twice with ethanol to remove dodecyl sulphate, and the complex was dissolved in 40 µl of 8 M urea, 10 mM Tris-HCl, pH 7.8. An aliquot of total 50S protein was added, and the sample subjected to Ouchterlony double-diffusion tests (17) against antibodies to the appropriate individual ribosomal proteins, supplied by Dr. G. Stöffler. The Ouchterlony plates were autoradiographed to locate the precipitation line corresponding to the ^{32}P-labelled cross-linked complex.

Oligonucleotide analysis of the RNA-protein complexes was carried out as described previously (7), using the two-dimen-

sional thin layer system of Volckaert and Fiers (18) to separate the oligonucleotides released after total digestion with ribonuclease T_1. No proteinase K treatment was made (cf. 7). Secondary digestions with ribonuclease A were made as before (7), and the oligonucleotides thus identified were fitted to the 23S RNA sequence of Brosius et al. (19). Further digestions of the oligonucleotide remaining attached to the protein after ribonuclease T_1 digestion were also made as previously described (5,7), using ribonuclease A or T_2.

RESULTS AND DISCUSSION

As already noted in the Introduction, the properties of 2-iminothiolane (10,11) as an RNA-protein cross-linker are very similar to those of p-azidophenylacetic imidoester (APAI, 6). In both cases the first step in the cross-linking process is the reaction of the imido function with lysine residues, which proceeds rapidly at room temperature and neutral pH; preliminary tests showed that this reaction was already complete within ten minutes, although a reaction time of 20 minutes was allowed (see Materials and Methods, and cf. ref. 10). The extent of RNA-protein cross-linking as a function of the reagent concentration is shown in Fig. 1, from which it can be seen that the reaction reaches a well-defined plateau at a 2-iminothiolane concentration of about 20 mM. Both 30S and 50S subunits showed almost identical saturation curves, the level of RNA-protein cross-linking being determined from experiments with ^{3}H- and ^{14}C-labelled subunits as described in Materials and Methods (cf. 1).

The second step in the cross-linking process is the mild ultraviolet irradiation treatment. Here again the conditions are very similar for both 2-iminothiolane and APAI, although the reaction mechanisms must be different. With APAI, the irradiation causes photoactivation of the azide group, leading to radical formation and hence to the cross-linking reaction. In the case of 2-iminothiolane it is presumably a nucleic acid residue (most probably a uridine) which becomes activated and then subsequently reacts with the thiol group (cf. 12), although it must be emphasized that we have no direct evidence that this is indeed the reaction which occurs here. As with APAI, an irradia-

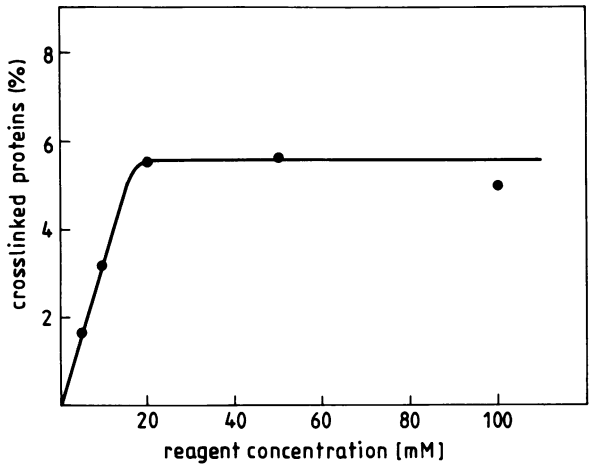


Figure 1: Percentage of RNA-protein cross-linking in 50S subunits, as a function of 2-iminothiolane concentration. Subunits subjected to irradiation (see Materials and Methods), but without 2-iminothiolane treatment, showed approximately 1.5% cross-linking of protein to RNA; this "background" has been subtracted in the Figure.

tion time of 3 minutes under our "standard" conditions (1) was sufficient for this step of the reaction; longer irradiation times did not increase the reagent-dependent component of the cross-linking, but only led to an increase in the direct ultraviolet-induced RNA-protein cross-linking reaction (cf. 1). In the absence of reagent, an irradiation treatment of 3 minutes leads to a cross-linking of approximately 1.5% of the total protein to RNA (cf. Fig. 1), and this should be compared with our previous experiments, where irradiation times of up to 30 minutes were used to generate the highly specific RNA-protein cross-links involving protein S7 in the 30S and L4 in the 50S subunit (1,3,5). In short, as was the case with APAI, 2-iminothiolane can be used to generate a reasonable level of RNA-protein cross-linking in ribosomal subunits under extremely mild conditions. The effect of both imidoester treatment and the irradiation process on the activity of the subunits in poly-Phe-synthesis has already been described (6).

The pattern of proteins cross-linked by both reagents was also very similar, a typical example from subunits cross-linked

with 2-iminothiolane on the gel system of Laemmli and Favre (16) being shown in Fig. 2 (cf. 6). For this type of analysis, non-cross-linked proteins were removed by centrifugation through sucrose gradients containing dodecyl sulphate, and the isolated RNA-protein cross-linked complexes were subjected to ribonuclease digestion followed by oligonucleotide removal (see ref. 2 and Materials and Methods) before being applied to the gel. It can be seen from Fig. 2 that in the control samples (irradiated but not treated with 2-iminothiolane) only protein S7 in the 30S subunit and L4 (together with some L2) in the 50S subunit are

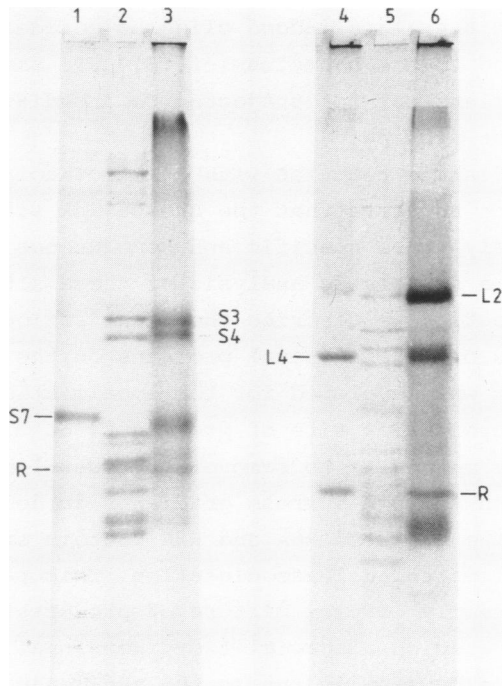


Figure 2: Analysis of cross-linked proteins on the gel system of Laemmli and Favre (16). Slot 1: Cross-linked proteins from 30S subunits, subjected to irradiation treatment only. Slot 2: Total 30S protein. Slot 3: Cross-linked proteins from 30S subunits, treated with 2-iminothiolane and irradiated. Slot 4: Cross-linked proteins from 50S subunits, irradiated only. Slot 5: Total 50S protein. Slot 6: Cross-linked proteins from 50S subunits, treated with 2-iminothiolane and irradiated. "R" denotes ribonuclease. Each sample of cross-linked protein was derived from 40 A_{260} units of ribosomal subunit.

significantly cross-linked (1). On the other hand, samples from subunits both treated with iminothiolane and irradiated show a number of bands corresponding to cross-linked protein. S3 and S4 are among the most prominent in the 30S subunit, whereas in the 50S subunit the main bands are L2, L4 and a group of proteins running as a broad band slightly ahead of ribonuclease. A more detailed analysis by chromatography on Sephadex G-75 followed by gel electrophoresis (cf. 7) indicated that most of the ribosomal proteins were cross-linked to some extent to the RNA (data not shown). In experiments similar to that of Fig. 2 using ^{32}P-labelled subunits, the pattern of ^{32}P-radioactivity observed corresponded precisely to the stained pattern in Fig. 2, demonstrating that there are indeed oligonucleotides remaining covalently attached to the proteins (cf. 2,4,6). Samples treated with 2-iminothiolane but not subjected to irradiation showed no RNA-protein cross-linking.

The cross-linking reaction with 2-iminothiolane is very reproducible, which suggests that the cross-link sites on protein and RNA are likely to be specific and homogeneous. We have therefore undertaken a systematic analysis of these sites, and in the following section we describe the localisation of six such cross-link sites of 50S ribosomal proteins on the 23S RNA, using the system which was developed for the localisation of the ultraviolet-induced cross-link site of protein L4 on 23S RNA (7). The principle of the method is to remove non-cross-linked proteins by centrifugation through sucrose gradients in dodecyl sulphate, and to subject the isolated RNA and RNA-protein cross-linked complexes to a limited nuclease digestion. This partial digestion leads to an extremely complex mixture of products, which can however be separated on a special two-dimensional gel system, in which the first dimension is run in the non-ionic detergent Triton X-100 and the second dimension in dodecyl sulphate. The system has already been described in detail (7), its central feature being that the cross-linked RNA-protein complexes appear as a set of "reverse diagonals", running roughly at right angles to the "normal" diagonal of free RNA fragments. A typical example of such a gel from ^{32}P-labelled 50S subunits cross-linked with 2-iminothiolane is illustrated in Fig. 3. Individual spots

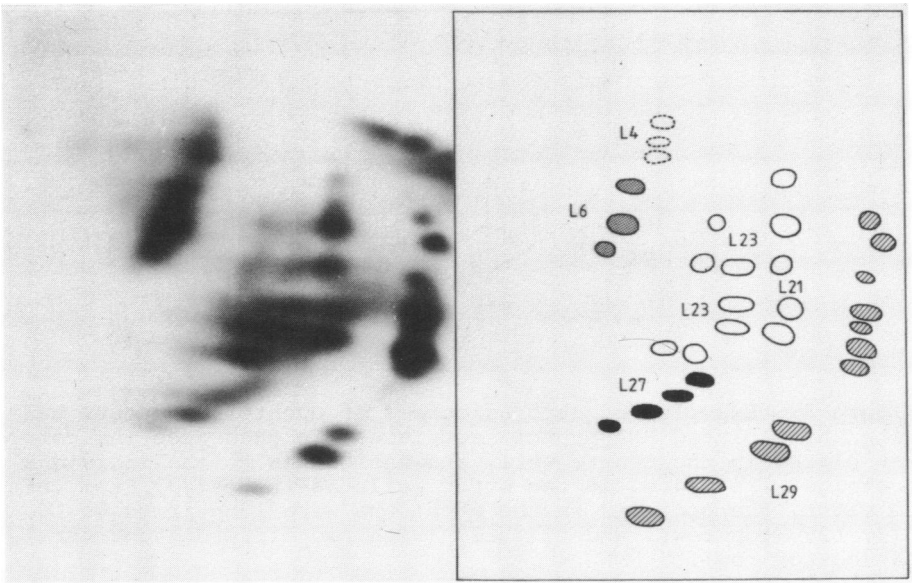


Figure 3: Triton X-100/dodecyl sulphate two-dimensional gel of partially digested ^{32}P-labelled RNA-protein complexes from 50S subunits cross-linked with 2-iminothiolane. The left-hand side of the diagram shows the relevant part of the autoradiogram of the gel (cf. 7); the key diagram on the right indicates which spots form a "reverse diagonal" corresponding to a particular protein. Spots containing proteins L21 and L23 were intermingled. Direction of electrophoresis was from left to right (1st dimension), and top to bottom (2nd dimension). The radioactivity in the upper right-hand corner of the autoradiogram corresponds to the end of the "diagonal" of free RNA fragments (cf. 7).

were extracted from this gel, and where necessary subjected to a further electrophoretic purification step (see Materials and Methods). Each isolated complex was then submitted to both oligonucleotide analysis and protein analysis, the key diagram on the right of Fig. 3 showing the identities of the proteins found in the various spots.

As outlined in Materials and Methods, preliminary identifications of the proteins were made by gel electrophoresis after total nuclease digestion of the complexes (cf. 7), and the final identifications were made by Ouchterlony double diffusion tests between the complexes and antibodies to the individual proteins. Fig. 4 shows some typical autoradiographs of the Ouchterlony

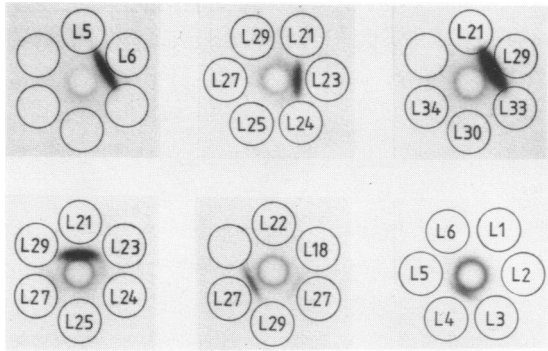


Figure 4: Examples of autoradiograms of Ouchterlony tests made with the isolated RNA-protein complexes. In each case the complex was placed in the centre well, and antibodies to the individual proteins were placed in the outer wells as indicated. From left to right, the proteins in the complexes were identified as L6, L23, L29 (top row), and L21, L27 (with two different antisera), L4 (bottom row).

plates, which unequivocally identify proteins L6, L23, L29, L21 and L27. The Ouchterlony test for protein L4 (Fig. 4, bottom right) was never very satisfactory, but this protein could be clearly identified by the gel analysis (cf. 7). Although the pattern of spots found on the Triton X-100/dodecyl sulphate two-dimensional gels (Fig. 3) was remarkably reproducible, it can be seen that the pattern of proteins found does not entirely correspond to that of the total cross-linked protein in Fig. 2. In particular, L2 was never observed on the two-dimensional gel (Fig. 3), although it is one of the strongest bands in Fig. 2. Similarly, protein L4 was only weakly observed, although it is also very strong in Fig. 2, and the subsequent oligonucleotide analysis (see below) suggested that the L4 cross-link site which we analysed from these gels corresponded to a background of the direct ultraviolet-induced cross-link previously found (7), and not to an iminothiolane-induced cross-link. This apparent contradiction between Figs. 2 and 3 could be due to a selective aggregation of some proteins in the Triton X-100/dodecyl sulphate gel system, but is more likely a result of the partial nuclease digestion conditions. If a protein is cross-linked at a particularly exposed site on the RNA, then even a mild partial digestion

could cut the attached RNA fragment down to a size such that the RNA-protein complex no longer carries a net negative charge at neutral pH; in such cases, the complexes would no longer enter the Triton X-100 gel. In this context it is noteworthy that it is the larger proteins (L2 and L4, which need a correspondingly larger attached RNA fragment in order to carry a net negative charge) which are lost or partially lost in this procedure, whereas the smaller ones (L21, L23, L27 and L29, which correspond to the proteins running ahead of ribonuclease in Fig. 2, slot 6) are found in high yields. Protein L6 was also found in good yield (Fig. 3), although this protein (which is the first distinct protein band below L4, Fig. 2, slot 5) seldom gave a strong band in gel analyses of the total cross-linked protein (cf. Fig. 2, slot 6). Clearly the partial digestion procedure and gel analysis (Fig. 3) leads to a non-random selection of cross-link sites in differing yields, and other conditions will need to be investigated if the complete set of 2-iminothiolane-generated cross-links is to be determined.

Oligonucleotide analysis of the individual RNA-protein complexes (Fig. 3) was made on polyethyleneimine thin-layer plates after ribonuclease T_1 digestion, and some typical examples of the fingerprints obtained are shown in Fig. 5. Due to the complexity of the gel patterns (Fig. 3), the fingerprints often showed a background of contaminating oligonucleotides, but (as can be seen in Fig. 5) the background spots were clearly distinguishable from the main digestion products. The principal spots on each fingerprint were extracted and subjected to a secondary digestion with ribonuclease A, and the oligonucleotide sequences deduced were then fitted to the 23S RNA sequence of Brosius et al. (19). Fig. 6 summarizes all the fingerprint data obtained for all six cross-link sites. As was previously found in the case of L4 (7), the RNA fragments are of various lengths, and in each case one oligonucleotide was clearly missing from near the centre of the fragment. The missing oligonucleotides must contain the cross-link sites (cf. 5,7), and in the examples in Fig. 5 the positions on the fingerprints are indicated where this missing oligonucleotide would have occurred. In some cases, where the missing oligonucleotide would be expected to co-mi-

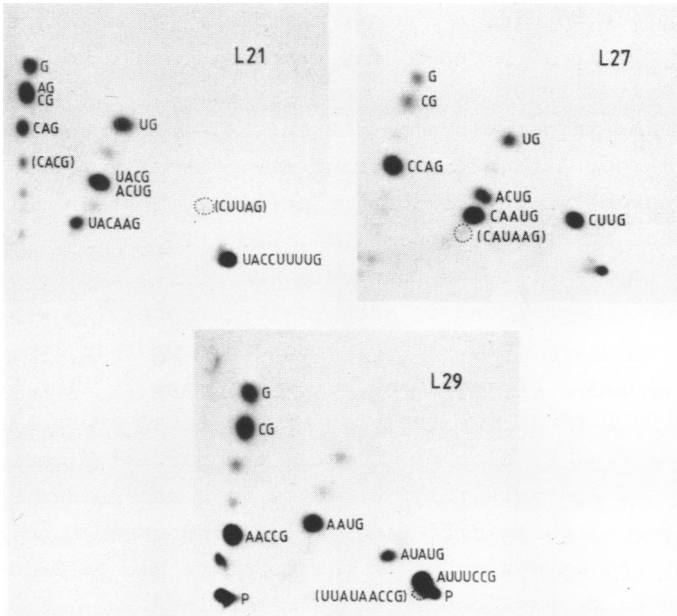


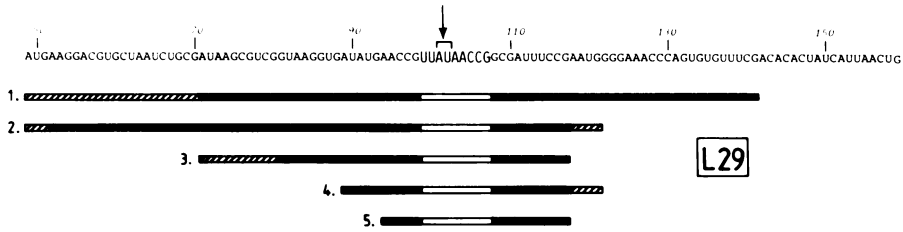
Figure 5: Examples of fingerprints of RNA-protein complexes on polyethyleneimine thin-layer plates (18). The first dimension ran from right to left, the second from bottom to top. Identities of the principal oligonucleotides are shown, those in brackets indicating the positions of the "missing oligonucleotides" (see text). "P" denotes a spot containing protein. The fingerprints should be compared with the sequences in Fig. 6.

grate with or near other oligonucleotides which were present, its absence was inferred from the ribonuclease A digestion data. For example, in the case of L29, Fig. 5, no A-A-C was found in the digest of the A-U-U-U-C-C-G spot; (A-A-C would have been expected if U-U-A-U-A-A-C-G had been present). On some fingerprints, spots with properties corresponding to the residual protein-oligonucleotide complex (cf. 5,7) could be observed (denoted by "P" in Fig. 5), running either at the origin of the thin-layer plate, or at the lower left-hand corner, or both. In other cases, this residual protein-oligonucleotide complex was insoluble and did not appear on the fingerprint.

In each case, attempts were made to localise the site of the cross-link further, within the missing oligonucleotide. As

before (5,7), this involved subjecting the RNA-protein complexes (Fig. 3) to a total digestion with ribonuclease T_1, isolating the resulting protein-oligonucleotide complex on a dodecyl sulphate gel, and then carrying out further digestions with ribonuclease A or T_2. The results of these analyses are also included in Fig. 6. In the case of protein L29, ribonuclease A digestion of the protein-oligonucleotide complex released 2U, C, G and A-A-C but no A-U, indicating that the cross-link lies within the A-U dinucleotide at positions 101-102. Further digestion with ribonuclease T_2 did not release a mononucleotide. With L23, such a further localisation of the cross-link site could not be made, since the RNA-protein complexes containing this cross-link (Fig. 3) needed the extra electrophoretic purification step (see Materials and Methods), and in consequence were only obtained in low yield. However, the U-U-U-C-G sequence at positions 137-141 (Fig. 6) was clearly absent from the RNA-protein complexes (Fig. 3). A second set of RNA fragments attached to L23 was also observed (complexes number 4 to 6, Fig. 6) where no missing oligonucleotide could be discerned; these fragments may represent a second cross-link site between L23 and an "uncharacteristic" T_1-oligonucleotide (such as the C-G at positions 109-110).

In the case of L21, the C-U-U-A-G sequence (Fig. 6) was always absent from the RNA-protein complexes, and sometimes the neighbouring C-A-C-G was only present in reduced yield (cf. Fig. 5). Ribonuclease A digestion of the residual protein-oligonucleotide complex gave equimolar amounts of C, U and A-G, suggesting that the cross-link site is one of the two uridine residues at positions 545-546 (Fig. 6). In the case of L27, as with L23, no further localisation of the cross-link site was possible, although its location within the C-A-U-A-A-G sequence (positions 2332-2337, Fig. 6) was unequivocal (cf. Fig. 5). With L6, the missing oligonucleotide was very clearly U-U-C-A-U-A-U-C-G, and ribonuclease A digestion of the residual protein-oligonucleotide complex released U, C and G, but no A-U, suggesting that the cross-link lies within the A-U-A-U sequence (positions 2476-2479, Fig. 6). However, subsequent digestion with ribonuclease T_2 surprisingly did not release any mononucleo-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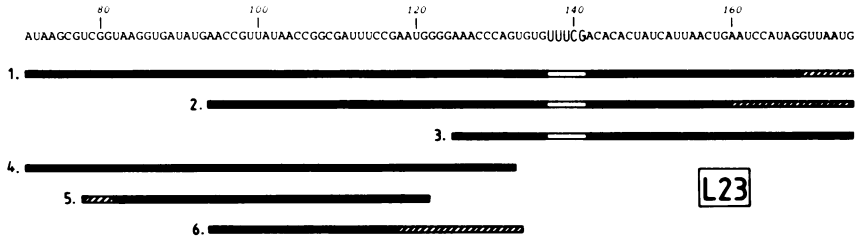
1.
2.
3.
4.
5.
L29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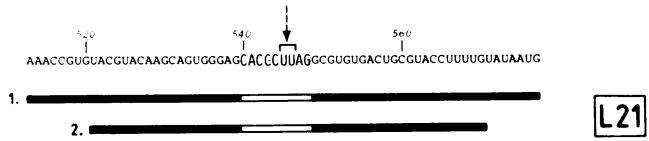
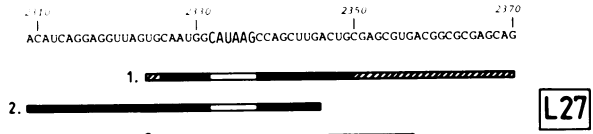
2.
3.
4.
5.
6.
L23


AAACCGUGUACGUACAAGCAGUGGGAGCACCCUUAGGCGUGUGACUGCGUACCUUUUGUAUAAUG
1.
2.
L21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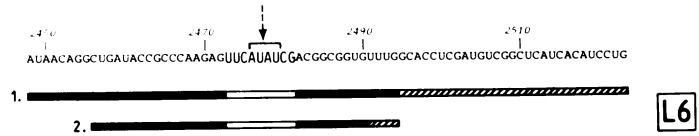
1.
2.
3.
L27


AUAACAGGCUGAUACCGCCCAAGAGUUCAUAUCGACGGCGGUGUUUGGCACCUCGAUGUCGGCUCAUCACAUCCUG
1.
2.
L6

tides, so this result must be regarded as tentative. Finally, with L4 the cross-link site analysis indicated precisely the same position as that previously observed (7), namely the A-A-U-A-G sequence at positions 613-617.

The positions of all six cross-linked oligonucleotides in the recently-determined secondary structure of 23S RNA (20) are illustrated in Fig. 7. It can be seen that all the sites occur in single-stranded loops of the structure, with the possible exception of the site for L27, although it should be noted that in the latter case there is no direct evidence (20) for the hydrogen-bonding between bases 2322-2334, and this segment may also exist as a single-stranded loop. Thus, the identification of the T_1-oligonucleotide encompassing the cross-link site in each case (Fig. 6) is sufficient to locate the cross-links within defined features of the secondary structure. The attempts to pinpoint the cross-link sites within these oligonucleotides, although tentative, are all consistent with the suggestion (see above) that the cross-linking reaction is most likely to involve uridine residues (cf. 12), although adenine is also a possible candidate in some cases.

Previous studies on the binding sites of 50S proteins on 23S RNA have located L4 and L21 within the 5'-1200 nucleotides (21,22), L23 between nucleotides 1500-1800 (23), and L6 and L27 within the 3'-1600 nucleotides (21,22). No data are available for protein L29. The only apparent contradiction with our data is the case of L23, which we find cross-linked within positions 137-141 (Fig. 6). We have however frequently pointed out (e.g.

Figure 6: Summary of the sequence data for the cross-linked complexes. The complexes are given in the order in which they appear in the 23S RNA sequence (19), namely L29, L23, L21, L27 and L6. The complexes with L4 (cf. 7) are not shown. The black bars denote the sequences found, with shaded regions indicating oligonucleotides which were sometimes present in the complex, sometimes not. Other minor variations were also found with slightly longer or shorter sequences at either end; these have been omitted for clarity. The white boxes indicate the missing oligonucleotides, which are shown in large letters in the sequence (cf. Fig. 5). The bracketed nucleotides and arrows indicate the location (solid arrow) or tentative location (dotted arrow) of the cross-linked site within the missing oligonucleotide (see text). The fingerprints shown in Fig. 5 are those of complex No. 4(L29), No. 2 (L21), and a complex similar to No. 3 (L27).

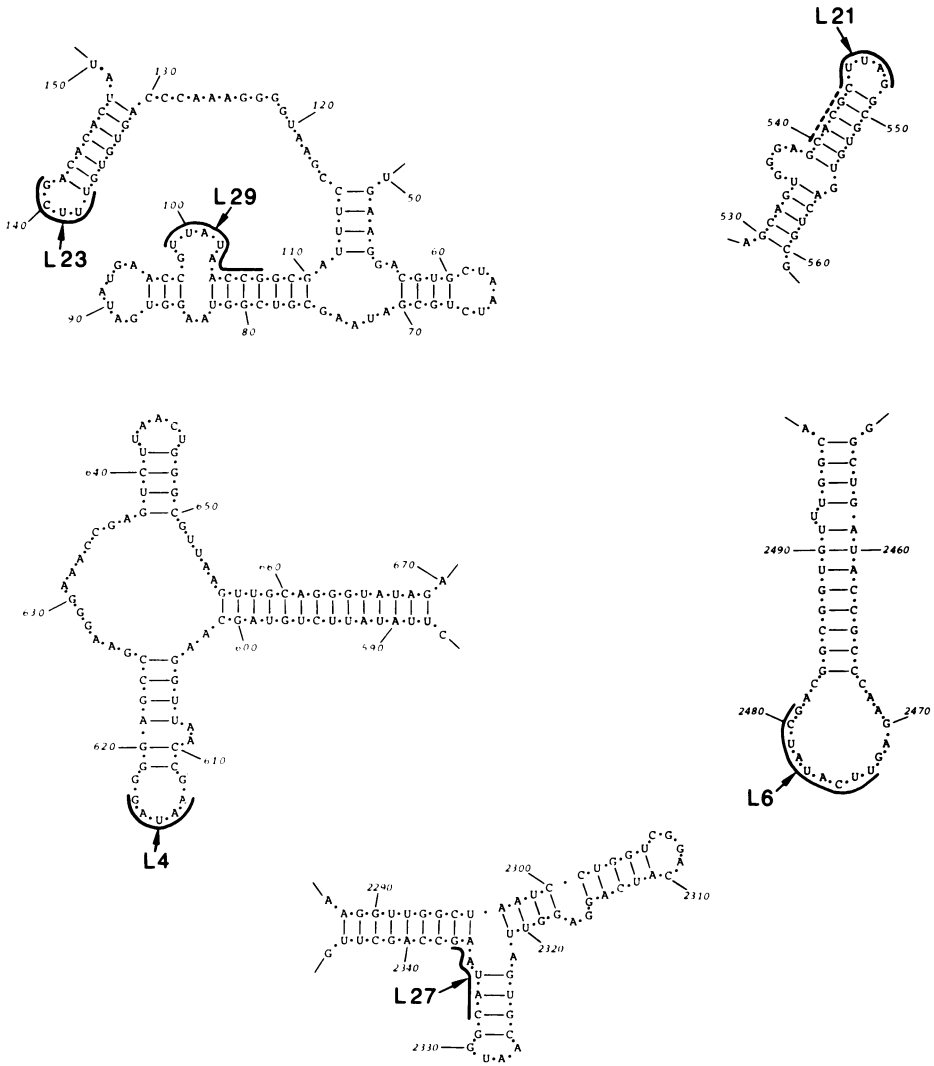


Figure 7: Location of the RNA-protein cross-link sites in the secondary structure of 23S RNA (20). The solid lines indicate the oligonucleotides encompassing each cross-link (see Fig. 6). The structures are drawn in the same orientation as that of ref. 20.

2) that cross-linking is a topographical probe, defining a neighbourhood between protein and RNA, which may or may not correspond to the principal binding site of the protein concerned.

The results described in this paper represent the first detailed multiple set of such topographical contacts between proteins and RNA within the 50S subunit, and demonstrate that it is indeed possible to extract such information from a complex mixture of cross-linked products. The analysis of the corresponding sites of cross-linking on the ribosomal proteins is currently in progress.

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