Ribosomal Protein L7/L12 Cross-links to Proteins in Separate Regions of the 50 S Ribosomal Subunit of Escherichia coli

(Received for publication, June 21, 1983)

Robert R. Traut, John M. Lambert, and James W. Kenny

From the Department of Biological Chemistry, School of Medicine, University of California, Davis, California 95616

The 50 S ribosomal subunits from Escherichia coli were modified by reaction with 2-iminothiolane under conditions in which 65 sulfhydryl groups, about 2/3 of protein, were added per subunit. Earlier work showed that protein L7/L12 was modified more extensively than the average and that nearly all 50 S proteins contained sulfhydryl groups. Mild oxidation led to the formation of disulfide protein-protein cross-links. These were fractionated by urea gel electrophoresis and then analyzed by diagonal gel electrophoresis. Cross-linked complexes containing two, three, and possibly four copies of L7/L12 were evident. Cross-links between L7/L12 and other ribosomal proteins were also formed. These proteins were identified as L5, L6, L10, L11, and, in lower yield, L9, L14, and L17. The yields of cross-links to L5, L6, L10, and L11 were comparable to the most abundant cross-links formed. Similar experiments were performed with 70 S ribosomes. Protein L7/L12 in 70 S ribosomes was cross-linked to proteins L6, L10, and L11. The strong L7/L12-L5 cross-link found in 50 S subunits was absent in 70 S ribosomes. No cross-links between 30 S proteins and L7/L12 were observed.

Ribosomal protein L7/L12 of Escherichia coli large ribosomal subunits is of particular interest in studies of ribosome structure and function. Protein L7 is the NH2 terminally acetylated form of L12 (1), and there are four copies of the monomer/50 S ribosomal subunit. It is the only ribosomal protein present in multiple copies (2, 3). The monomers form stable, elongated dimers in solution (4), and all four copies can be isolated from ribosomes in a stable complex with a fifth component, protein L10 (5, 6). The sequence of the protein has been determined (1). The results of physical studies indicate that the protein has an NH2-terminal domain containing a high a-helix content and a COOH-terminal domain with a globular structure (7-9). X-ray crystallographic studies have provided the structure of the COOH-terminal globular domain at 2.6 A resolution (9).

These ribosomal proteins play a unique functional role in protein synthesis. They participate in the partial reactions in which GTP cleavage to GDP takes place: the binding of the initiation factor G-GTP complex, and the termination factors to the ribosome (10-13). In each case, the rate of GTP hydrolysis associated with the interaction is greatly reduced in protein-deficient ribosomes specifically lacking L7/L12. The L7/L12-deficient core particles are inactive in protein synthesis. The presence of one dimer of L7/L12 fully restores the rate of GTP hydrolysis (14, 15) but two dimers are needed to restore the maximum rate of polyphenylalanine synthesis (15). A nucleotide-binding site in the COOH-terminal portion of L7/L12 has been postulated from the crystal structure (9). A GTPase activity in the presence of the isolated protein and elongation factor Tu has been reported (16). Cross-linking experiments have shown the proximity of initiation factor 2 (17), elongation factor G (18, 19), and termination factor 2 (20) to L7/L12.

Proteins L7/L12 occupy a unique location within the 50 S structure. The image of the 50 S subunit observed by electron microscopy shows three characteristic protruberances from the rounded body of the particle, one central and one on each side (21-24). The narrower and longer of the side protuberances is directed away from the particle and is specifically absent from particles from which proteins L7/L12, and only those proteins, have been removed (25). Antibodies against protein L7/L12 have been found by immune electron microscopy to decorate the narrower side protuberance. These findings have led to the conclusion that the side protuberance contains all four copies of protein L7/L12. It has been called, therefore, the L7/L12 stalk (25). Similar structures have been observed in other proaryotic ribosomes and substantial conservation of primary structure of L7/L12 homologs has been observed (26, 27).

There is evidence to suggest that all four copies of L7/L12 do not always occupy the position attributed from electron microscopy, with the L7/L12 stalk extending away from the body of the particle (28-30), and that portions of L7/L12 comprise a uniquely flexible domain in the ribosomal subunit (31). We have recently completed a detailed analysis of the disulfide-linked 50 S protein cross-links formed by oxidation of 50 S subunits and 70 S ribosomes lightly modified with 2-iminothiolane. We have identified a number of cross-links in which one member is L7/L12. Locations for most of the proteins cross-linked to L7/L12 have been proposed by others from immune electron microscopy and affinity labeling studies. Comparison of cross-links to L7/L12 and the locations imputed to them suggests that the two L7/L12 dimers, or portions thereof, are not invariably in the stalk extending away from the body of the particle and that contacts with the central region are made.

MATERIALS AND METHODS

2-Iminothiolane was purchased from Pierce and stored over Drierite.

1 R. R. Traut, J. M. Lambert, and J. W. Kenny, unpublished results.

This work was supported by United States Public Health Service Grant GM 17924. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Division of Tumor Immunology, Dana-Farber Cancer Institute, Boston, MA 02115.
§ Present address: Calgene Inc., Davis, CA 95616.
ite in a vacuum desiccator at 4°C. Triethanolamine was obtained from Matheson, Coleman, and Bell. Acrylamide (practical grade) and methylenebisacrylamide were from Eastman and used without further recrystallization. BisTris\(^2\) and iodoacetamide were from Sigma, glycine from Eastman, sodium dodecyl sulfate from Serva. Urea (ultra pure), sucrose (ultra pure) and Amido black 10B were purchased from Schwarz-Mann. 2-Mercaptoethanol was from BDH Chemicals. Co-\(omalum\) of G-250 was obtained from Pierce. \(\text{H}_2\text{O}_2\) and \(\text{LiCl}\) were Mallinkrodt (analytical grade). KI was purchased from Fisher and catalase from Boehringer/Mannheim. The iodogen was from Pierce and \(12^5\%\) (carrier-free, 100 mCi/ml) was from Amersham.

Preparation of Ribosomes and Ribosomal Subunits—50 S \(E.\) coli ribosomal subunits were prepared as described previously (32). They originated from midlog, quickly cooled cells and were washed in 500 mM \(\text{NH}_4\text{Cl}\) prior to recovery of subunits by sucrose gradient centrifugation. The 70 S ribosomes were tight couples prepared from slowly cooled cells as described previously (33).

Cross-linking of 70 S Ribosomes and 50 S Subunits and Extraction of the Cross-linked Proteins—Cross-linking procedures employed in this paper follow exactly those described previously in detail (32, 33). Particles, generally 3 to 5 mg at 3 mg/ml, were incubated with 25 mM 2-iminothiolane for 2.5 h at 0°C, oxidized, incubated with 40 mM iodoacetamide, and extracted with an equal volume of 8 M urea, 6 M \(\text{LiCl}\). This solution containing protein and RNA was adjusted to 66% (v/v) isopropanol to precipitate completely the RNA. The proteins were dialyzed against 6% acetic acid and lyophilized. The cross-linked 70 S ribosomes were purified first by sucrose gradient centrifugation to remove ribosome dimers and a small proportion of 50 S subunits present.

Fractionation of Cross-Linked Proteins prior to Analysis by Dodecyl Sulfate-diagonal Gel Electrophoresis—The lyophilized cross-linked protein from 70 or 50 S particles was fractionated by electrophoresis in gels containing 6 M urea at pH 5.5. The tube gels were sliced into segments of 0.5 cm which were used directly as the samples for diagonal gel electrophoresis. The methods have been described in detail previously (33). The separation achieved by electrophoresis of cross-linked proteins at acid pH depends in part on the relative isoelectric points of the various proteins and cross-linked complexes. Basic species migrate more rapidly and acidic species more slowly.

The acidic character of L7/L12 and of complexes containing them led to their presence in the gel segments nearest the origin. The entire series of 20 0.5-cm gel segments were used as samples for diagonal gel electrophoresis.

Two-dimensional Diagonal Polyacrylamide-Dodecyl Sulfate Gel Electrophoresis—The segments were dried, rehydrated with sample buffer for dodecyl sulfate gel electrophoresis and embedded at the origin of the tube gel for electrophoresis in the first dimension run under oxidizing conditions. These gels were removed, soaked in reducing agent, and embedded at the origin of gel slabs containing dodecyl sulfate for electrophoresis in the second dimension run under reducing conditions. The details of these procedures have been given previously (32, 33). The apperance of L7/L12 below the diagonal, indicative of cross-linked species containing L7/L12, was limited to the first 5 0.5-cm urea gel segments from the origin (see Ref. 33).

The total diagonal gel pattern for 50 S particles is shown in Fig. 1. The molecular weight scales were established from the amino acid sequences of individual ribosomal proteins (34). Past experience has shown that the mobility and apparent molecular weight of a cross-linked complex is that expected from the sum of the two components. Protein L7/L12 is the only exception to this general pattern. Protein L7/L12 migrates as a monomer with an apparent molecular weight of about 9,500 compared to the scale established from the other ribosomal proteins. Dimers containing L7/L12 have apparent molecular weights predicted from its sequence (12,200) and that of the second component. Since L7/L12 migrates at a unique position in the gel (Fig. 1, position \(P\)), its aberrant behavior poses no problem in interpreting the cross-links.

Identification of Components of Cross-linked Complexes Isolated from Diagonal Gels—The position of components beneath the diagonal, indicative of cross-linked complexes containing L7/L12, was limited to the lettered rows at the right. The molecular weight calibration for both dimensions was established using the values determined by their amino acid sequence (summarized by Wittmann (34)). Total 50 S proteins (50 \(\mu\)g in 10 \(\mu\)l of sample buffer) was applied to the top of the first dimension gel tube 20 min before the end of electrophoresis and this appears to the left of the diagonal gel. The arrow from zone \(P\) identifies the row of L7/L12 spots derived from cross-links. The positions of dimers, trimers, and tetramers of L7/L12 are indicated by brackets.

**RESULTS**

Fig. 1 shows the two-dimensional polyacrylamide-dodecyl sulfate gel pattern for total cross-linked 50 S proteins. Position \(P\) on the diagonal contains monomeric protein L7/L12 and the horizontal line across the diagonal at level \(P\) contains only L7/L12. There are concentrations of material at positions with apparent molecular weights of approximately 24,000 and 36,000. Some more faint material is found also with molecular weights above 45,000. Similar patterns were observed when cross-linked material from 70 S ribosomes was examined. The material indicated by *rounded brackets* (Fig. 1) is attributed to the formation of cross-linked homodimers and trimers. The *square bracket* indicates the position at which tetramers would appear. The dimers and trimers of L7/L12 are very distinct when oxidized cross-linked samples are stained and dried for radioautography.

2 The abbreviation used is: BisTris, 2-(2-hydroxyethyl)aminomethyl-2-(hydroxymethyl)-propane-1,3-diol.

---

**TABLE 1.** Two-dimensional polyacrylamide-sodium dodecyl sulfate diagonal gel electrophoresis of proteins extracted from 50 S ribosomal subunits cross-linked with 2-iminothiolane.

| Protein | \(M_r \times 10^{-3}\) |
|---------|-----------------|
| L7/L12  | \(50 \pm 1\) |
| L7      | \(25 \pm 1\) |
| L12     | \(25 \pm 1\) |
| L7L12   | \(40 \pm 1\) |
| L7L12L12| \(60 \pm 1\) |
| etc.    |                 |

Proteins were extracted from 3.0 mg of 50 S subunits with 3 M \(\text{LiCl}\), 4 M urea, and 40 mM iodoacetamide. Acetic acid was added to 66% to precipitate RNA. The protein solution was dialyzed against 6% acetic acid and lyophilized. The cross-linked, alkylated protein (800 \(\mu\)g) was applied to a discontinuous 17.5% dodecyl sulfate gel and incubated with 40 mM iodoacetamide for 6 h at 1 mM. Following electrophoresis in the first dimension, the proteins were incubated with reducing conditions by incubation of the gel in 3% 2-mercaptoethanol. The gel was then exposed to autoradiography in the absence of reducing reagents and electrophoresed for 6 h at 1 mA. The proteins were visualized by staining with Coomassie blue G-250 and dried for autoradiography.
Cross-links Containing Ribosomal Proteins L7/L12

1) and their molecular weights are shown for reference. Spots or regions of complex spots that were dissected into several sections are indicated by numbers and brackets. The material in these excised sections was recovered and identified by radiolabeling as described under “Materials and Methods.” Representative examples are shown in Fig. 5. Fig. 5a shows the stained pattern of the proteins separated in the two-dimensional acid-urea gel and Fig. 5b shows autoradiographs of proteins from spots on diagonal gels. Zone P (Figs. 1 and

FIG. 2. Diagonal gel electrophoresis of purified fractions of cross-linked ribosomal protein. Twelve mg of 50 S ribosomal subunits were cross-linked with 2-iminothiolane. Initial fractionation of the cross-links recovered from the cross-linked subunits was achieved by electrophoresis in a polyacrylamide-acid-urea gel at pH 5.5, after which the tube gel was sliced into 20 segments. The protein contained in each segment was then analyzed by two-dimensional polyacrylamide-sodium dodecyl sulfate electrophoresis.

analyzed on isoelectric focusing dodecyl sulfate gels.3

The masking of other cross-links by the predominant cross-linked dimers and trimers necessitated additional steps for fractionating the cross-linked material. Protein L7/L12 is the most acidic protein in the 50 S subunit. The mixture of cross-linked 50 S proteins was fractionated by gel electrophoresis at pH 5.5, and individual 0.5-cm slices were then examined by diagonal gel electrophoresis. Only slices near the origin containing more acidic proteins and cross-linked complexes showed the presence of L7/L12. Fig. 2 shows the diagonal gels of the second to fifth 0.5-cm slices from the origin of the acid-urea gel. The only material present below the diagonal in addition to that in line P (L7/L12) was in lines A, H, I, and J. Fig. 3 shows enlarged areas of the diagonal gels shown in Fig. 2. The components found in zones E, H, I, J, and P (Fig.

FIG. 3. Enlargement of regions of the diagonal gels that contain cross-links involving L7/L12. The enlargements are of the two-dimensional gels of proteins contained in acid-urea gel slices 2 through 5 from the origin A–D, respectively, of 50 S proteins. Indicated in each panel by half-circles are the areas of the gel that were cut out. The protein was eluted from the slice and identified (see Fig. 5). Vertical lines with arrows indicate members of cross-linked complexes. Each component is numbered and the apparent molecular weights are given below the vertical lines. The lettered rows correspond to the lettering code of the diagonal gel shown in Fig. 1. Constituents of each lettered row are given, followed by their apparent molecular weight. The sections lettered a and b in gel B, and c and d in gel C, denote the positions of numerous cross-links involving dimers and trimers of L7/L12 which overlap due to their similar molecular weights. The three single unnumbered vertical arrows in gel B show the expected positions of the dimers, trimer, and tetramer of L7/L12.

FIG. 4. The corresponding series of gels of cross-linked 70 S proteins.

3 P. D. Butler and R. R. Traut, unpublished results.
3) contains only L7/L12 and it cannot readily be iodinated for identification since it lacks tyrosine and histidine. The identification of material in zone P as L7/L12 was done using L7/L12 from ribosomes labeled in vivo with $^{35}$S)sulfate. The material on line P was extracted and iodinated in order to detect any additional proteins (L25 or L28). No radiolabeled proteins were found.

Fig. 3A shows the enlargement of the entire lower part of the diagonal gel of Fig. 2A. Region 1 contains only L7/L12 and had an apparent $M_r$ consistent with its origin from 2 copies of L7/L12. There is no other material above it. B is an enlargement of the next diagonal in the series of Fig. 2. There is new material on lines H and I. Spot 2 contains L10 and L11. There is no material directly beneath it and its apparent $M_r$, 28,100 corresponds to a L10-L11 dimer. The overlapping but distinct spot 3 also contains both L10 and L11. Spot 3 is directed above the left side of the large spot I and the complexes yielding this part of the pattern were L10-L7/L12 and L11-L7/L12. The three arrows along line P show the positions of cross-linked dimers, trimers, and tetramers of L7/L12. The material in spots a and b on lines H and I is L10 and L11. The apparent $M_r$ of a is 39,200 and it is best explained by the trimers L10-2(L7/L12) and L11-2(L7/L12). The trimer L10-L11-L7/L12 has the apparent $M_r$, 41,800 and may also be formed. Spot b and material to its left have apparent $M_r$ values greater than 50,000 and are best explained by the complexes L10-3(L7/L12) and L11-3(L7/L12), $M_r$, 51,200-51,600, and by the complex L10-L11-2(L7/L12), $M_r$, 53,000. Since the various spots overlap, the assignments of specific higher complexes of L10, L11, and L7/L12 is difficult.

Fig. 3C is the enlarged area containing cross-links of the third diagonal gel in Fig. 2. Regions 2 and 3 are the same as in B. There is new material both on line E and also on lines H and I overlapping the lower part of region 3. Regions 4 and 5 are distinct but overlapping spots. Proteins L5 and L6 were found in both spots because of overlap. They originate from the complexes L5-L7/L12 and L6-L7/L12. As compared to B, there is clearly new material in zone P directly beneath L5 and L6 indicated by the arrow and line intersecting 33.3. This apparent $M_r$, is consistent with the composition of these complexes. Spot c contains both L5 and L6. The apparent $M_r$, ~40,000 could in principle represent the L5-L6 dimer. This is ruled out because the L5-L6 dimer is more basic and is separated in a different gel segment of the acid-urea gel (results not shown). Therefore, we conclude that spot c originates from L5-2(L7/L12) and L6-2(L7/L12). Spot d also contains both L5 and L6. The apparent $M_r$, although imprecise in this part of the gel, of the cross-linked complex is well above 50,000. There is L7/L12 beneath spot c and the results are most consistent with the formation of two tetramers L5-3(L7/L12) and L6-3(L7/L12) ($M_r$, 55,900). Region 6 represents material not present on the preceding gel slice (B) below spot a on line J and was found to contain protein L9 in addition to L11 from the overlapping spot a. The origin of L9 at a position with apparent $M_r$, 38,400 is best accounted for by the complex L9-2(L7/L12) ($M_r$, 37,800). Regions 7 and 8 on lines I and J on the underside of region 3 were not present in B. They are more distinct on D where there is less of the region 3 material (L10-L7/L12 and L10-L7/L12) and of the region 2 material (L10-L11). Region 8 is clearly to the right of region 7. This means that they cannot originate from a single cross-link with each other. Each did originate from a cross-link to L7/L12. Region 7 contained both L11 (from overlapping spot 3) and L14. Spot 8 contains L9. The identification gel in Fig. 5b, F, shows spot 7 from C where the overlapping spots 3 and 8 are also present so that L11, L14, and L9 are all observed. The evidence indicates that spot 7 originates from a L14-L7/L12 complex and spot 8 from a L9-L7/L12 complex.

Fig. 4 shows a panel of gel slices from a similar separation of cross-linked 70 S proteins. The similarity with Fig. 3 is striking. These 70 S gels were analyzed exactly as described above with the same results with a single exception. Material on line E contained only L6 but not L5. There is no evidence for L5-L7/L12 formed in 70 S ribosomes.
Both 70 S ribosomes and 50 S subunits have been cross-linked using 2-iminothiolane. We have focused attention here on the cross-links involving the acidic protein of the 50 S subunit, L7/L12, which is present in four copies per particle. We do not distinguish between the acetylated and nonacetylated forms and use the notation L7/L12 for one copy of the protein.

The dimer of L7/L12 is considered to be the structural and functional unit in the ribosome. We have identified both cross-linked dimers and trimers of L7/L12 in yields similar to other relatively abundant cross-links. Tetramers were either formed in low yield or were absent. If a single L7/L12 were more likely to form a cross-link to the other member of the same dimer than to a member of the second dimer and if this were true for both dimers, then the favored formation of trimers over tetramers is difficult to explain. The relative abundance of dimers indicates not only that some part of the two dimers are located near each other, but that a single L7/L12 has a similar probability of forming a cross-link to its partner (from the same dimer) or to one member of the second dimer. If but one of the four monomers should utilize the same site for cross-link formation to either of the second monomers, then dimers and trimers but never tetramers would result. The formations of cross-linked multimeric complexes of L7/L12 is consistent with the existence of stable pentameric complexes consisting of four copies of L7/L12 and L10 (5, 6). However, the trimers of L7/L12 can be formed alone without being bridged by L10.

Four 50 S ribosomal proteins form cross-linked complexes in relatively high yield with L7/L12 in 50 S ribosomes. They are L5, L6, L10, and L11. The yield of these cross-links, estimated from intensity of staining, is similar to that of the most abundant cross-links. In addition, proteins L9, L14, and L17 form cross-linked dimers with L7/L12 in much lower yield. Earlier studies had shown a cross-link between L2 and L7/L12 (36). This cross-link was observed again in this study, but in quite low yield (results not shown). Cross-links between L7/L12 and L10 and L11 have been reported previously by Expert-Bazancon (27).

Proteins from cross-linked 70 S ribosomes were also analyzed to find cross-links to L7/L12. The major cross-links, L6, L10, and L11 to L7/L12 were found. The L5-L7/L12 cross-link was absent and the weak cross-links to L2, L9, L14, and L7 could not be identified. No cross-links between L7/L12 and 30 S proteins were found (33).

The cross-linking data concerning L7/L12 have importance in the evaluation of other conflicting and confusing data on the location of L7/L12 in the 50 S subunit. A body of evidence from electron microscopy comparing intact subunits with those lacking all four copies of L7/L12 shows the L7/L12 is responsible for the appearance of the asymmetric feature called the L7/L12 stalk and implies that all four copies of L7/L12 are located in the stalk (21). Immune electron microscopy shows that antibodies to L7/L12 bind exclusively to sites on the stalk (25). By contrast, other workers have interpreted images in electron micrographs as having antibody binding sites on both side protuberances (23) and on the central protuberance of the 50 S subunit (28).

Two reports from the laboratory of Möller have clearly defined alternative models for the location of L7/L12 distinct from that placing both dimers in the L7/L12 stalk. One model is based upon the measurement by fluorescence energy transfer of distances between Cys-70 of L10 and Lys-51 and the NH2 terminus of L7/L12. Protein L7/L12 can occupy two sites defined by these distance measurements. One site is the L7/L12 stalk and the other is proposed to be on the central protuberance, on the side proximal to the stalk. Both dimers would be anchored by attachment to L10 (29). A second model is based on the results of titrating the amount of L7/L12 added back to cores that lack L7/L12 and noting the restoration of the appearance of stalks, stimulation of GTP hydrolysis activity in coupled assays with factors, and stimulation of polyphenylalanine synthetic activity (15). One dimer/particle restored the appearance of stalks to the maximum extent as well as GTPase activity. Both dimers were required for maximum protein synthesis activity. The model proposed places the NH2 termini of both dimers in the stalk but allows the central and COOH-terminal regions of one dimer to turn inward to contact the central protuberance.

Some studies suggest a dynamic state of L7/L12. A fluorescent probe attached to the COOH-terminal residue of L7/L12 indicates flexibility of this region (30). Proton NMR spectroscopy of 50 S subunits and cores lacking L7/L12 show that the proteins comprise a unique mobile domain in the ribosomal subunit which remains mobile in the 70 S ribosome (30).

The cross-linking results can distinguish among the alternative models to the extent that there are firm locations assigned to the proteins to which L7/L12 becomes cross-linked. Fig. 6a shows a model of the 50 S subunit (21) with positions assigned the major proteins cross-linked to L7/L12. The evidence for the location of L10 at the base of the L7/L12 stalk is the formation of the pentameric complex composed of two L7/L12 dimers and L10. The NH2-terminal fragment of L7/L12 is the site of its interaction with L10 (38, 39). Protein L11 has been found to form in high yield a cross-linked dimer with L10 (Ref. 37 and this study). This places L11 near L10 and, therefore, near the NH2-terminal domain of L7/L12. Protein L6 has been cross-linked to 23 S RNA nucleotides 2473-2481 (40) near the 3' end of 23 S RNA. Since the 3' end of 23 S RNA has been localized near the base of the L7/L12 stalk by immune electron microscopy (24, 41), this places L6 in the general region of the anchorage for the stalk.

Protein L5 is one of three proteins (L5, L18, and L25) shown in many laboratories to bind to the 5 S RNA. We have found that a fourth protein, L31', is also a member of this complex (42). There is firm evidence from immune electron microscopy that the 5 S RNA is located in the central protuberance (41, 43). This necessitates the location of the four members of the complex in the central protuberance. If the location proposed for L5 is correct, as is highly likely from the 5 S RNA data, then L5 is too far from the L7/L12 stalk depicted in Fig. 6 to undergo cross-linking. The maximum length of a cross-link including the extended lysine side chains is approximately 24 A. This distance is represented by the bar drawn to the same scale as the ribosome model in Fig. 6a. No region of the stalk, extended as drawn to represent electron micrographs, is close enough to the central protuberance to account for the cross-link.

Protein L14 forms a weak cross-link to L7/L12. Protein L14 is the major site of cross-linking of elongation factor G to the large subunit (19). Elongation factor G also cross-links to L7/L12 (Ref. 19 and 44 and this laboratory) and has been shown by immune electron microscopy to be located near the base of the L7/L12 stalk. The antibiotic thiostrepton inhibits the binding of elongation factors by interacting with protein L11 also near the base of the stalk (45). All these observations support a location for L14 not far from the L7/L12 stalk.

Protein L2 has been shown by many affinity labeling studies (reviewed in Ref. 46), by reconstitution (47) and chemical modification (48) to be located in the peptidyltransferase domain. The results from immune electron microscopy, using...
antibodies against puromycin and ribosomal proteins considered to be constituents of the peptidyltransferase center, place this domain in a region encompassing the left-hand part of the central protuberance and the valley between the central and left protuberances (49, 50). Therefore, a location is proposed for L2 near the peptidyltransferase center distant from the L7/L12 stalk and its site of attachment.

The proteins cross-linked to L7/L12 are themselves interrelated by crosslinks formed between various pairs (51). The results, shown in Fig. 6b, are consistent with, and reinforce the locations proposed for, these proteins. Proteins L9 and L17, members of weak cross-links to L7/L12, also form weak cross-links to L2 (51) and accordingly are placed near L2 in Fig. 6. This location for L17, derived from its cross-linking both to L2 and L7/L12, differs significantly from that proposed by Lake and Stryczarski (52) based upon immune electron microscopy where it is located on the external surface of the subunit opposite the central protuberance, a site inconsistent with the cross-linking results.

Three models for the location of L7/L12 can be considered in relation to the cross-linking results. 1) The L7/L12 stalk contains both dimers and always extends away from the body of the 30 S subunit. 2) The stalk contains both dimers, and one or both dimers is sufficiently flexible to move close enough to the central protuberance to permit cross-linking to L5 and also to L2 and L9. 3) The stalk contains only one of the two dimers and the second is located on the central protuberance. In all cases, both dimers are anchored by interactions with L10. The cross-linking data, particularly the strong cross-links to L5, are inconsistent with the first model. The mobility of one or both dimers inward from the extended position observed by electron microscopy would adequately account for the formation of the L5 cross-link. Movement in the opposite direction, downward in the model depicted in Fig. 6, might account for a cross-link to L17 in the location imputed to it by Lake. Different positions for each of the two dimers with one of them on the central protuberance would also account for the cross-link to L5. We have clear evidence for cross-linked dimers and trimers of L7/L12 and suggestive evidence for the formation of tetrameric complexes containing one copy each of L5, L6, L10, and L11 and three copies of L7/L12. It is difficult to explain the L7/L12 trimers, formed without the intermediacy of another protein, if the two dimers are not both in the stalk. Our evidence is highly suggestive of tetrameric cross-linked complexes containing L5 and three copies of L7/L12. Should this be confirmed by isolation of such intact complexes, it becomes difficult to envision a model in which only one dimer moves inward to allow contact with the central region. The evidence from cross-linking leads to the conclusion that the two dimers are both in the stalk and that its mobility permits its approach to the central protuberance in the 50 S subunit and the formation of a cross-link to L5 and, to a lesser extent, to L2 and L17. The restriction of cross-linking to only these proteins suggests specificity in the movement and interaction.

The L7/L12 stalk retains its mobility in 70 S ribosomes, as judged from the NMR data, but does not form 30 S cross-links. The absence of the cross-link to L5 in the 70 S ribosome indicates that the 30 S subunit blocks its access to the central protuberance or that sites of cross-link formation of L5 are now engaged in cross-links to 30 S proteins (33). Alternatively, L7/L12 may undergo a conformational change upon joining of the 30 S subunit.

REFERENCES
1. Terhorst, C., Moller, W., Laursen, R., and Wittmann-Liebold, B. (1973) Eur. J. Biochem. 25, 13–19
2. Hardy, S. J. S. (1975) Mol. Gen. Genet. 140, 253–274
3. Subramanian, A. R. (1975) J. Mol. Biol. 96, 1–8
4. Moller, W., Groene, A., Terhorst, C., and Amors, R. (1972) Eur. J. Biochem. 25, 1–12
5. Pettersson, I., Hardy, S. J. S., and Liljas, A. (1976) FEBS Lett. 64, 135–138
6. Gudkov, A. T., Tamanova, L. G., Vovyninov, S. Yu., and Khechinashvilli, N. N. (1978) FEBS Lett. 93, 215–218
7. Moller, W., Castleman, H., and Terhorst, C. P. (1970) FEBS Lett. 8, 192–196
8. Luer, C. A., and Wong, C. P. (1980) Biochemistry 19, 178–183
9. Leijonmarck, M., Eriksson, S., and Liljas, A. (1980) Nature (Lond.) 286, 824–826
10. Hamel, E., Koka, M., and Nakamoto, T. (1972) J. Biol. Chem. 247, 805–814
11. Sander, G., Marsh, R. C., and Parmeggiani, A. (1972) Biochem. Biophys. Res. Commun. 47, 566–573
12. Koteliansky, V. E., Domogatsky, S. P., Gudkov, A. T., and Spirin, E. (1977) FEBS Lett. 78, 6–11
13. Brot, N., Tate, W. P., Caskey, C. T., and Weisshach, H. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 89–92
14. Lee, C. C., Cantor, C. R., and Wittmann-Liebold, B. (1981) J. Biol. Chem. 256, 41–48
15. Moller, W., Schier, P. I., Maassen, J. A., Zantema, A., Schop, E., and Reinhardt, H. (1983) J. Mol. Biol. 163, 553–573
16. Donner, D., Villens, R., Liljas, A., and Kurland, C. G. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3192–3195
17. Heimark, R. L., Hershey, J. W. B., and Traut, R. R. (1976) J. Biol. Chem. 251, 7779–7784
18. San José, C., Kurland, C. G., and Stőffer, G. (1976) FEBS Lett. 71, 133–137
19. Skold, S. E. (1982) Eur. J. Biochem. 127, 225–229
20. Stőffer, G., Tate, W. P., and Caskey, C. T. (1982) J. Biol. Chem. 257, 4203–4206
14598

Cross-links Containing Ribosomal Proteins L7/L12

21. Lake, J. A. (1980) in Ribosomes—Structure, Function and Genetics (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., and Nomura, M. eds) pp. 207–236, University Park Press, Baltimore

22. Kastner, B., Stoffler-Meilicke, M., and Stoffler, G. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6652–6656

23. Boubli, M., Hellmann, W., and Roth, H. E. (1976) J. Mol. Biol. 107, 479–490

24. Shatsky, I. N., Evstafieva, A. G., Bystrova, T. F., Bogdanov, A. A., and Vasiliev, V. D. (1980) FEBS Lett. 122, 251–255

25. Strycharz, W. A., Nomura, M., and Lake, J. A. (1978) J. Mol. Biol. 126, 123–140

26. Marquis, D. M., Fahnstock, S. R., Henderson, E., Woo, D., Schwinge, S., Clark, M. W., and Lake, J. A. (1981) J. Mol. Biol. 150, 121–132

27. Matheson, A. T., Möller, W., Amons, R., and Yaguchi, M. (1980) in Ribosomes—Structure, Function and Genetics (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., and Nomura, M. eds) pp. 297–332, University Park Press, Baltimore

28. Tischendorf, G. W., Zeichard, H., and Stoffler, G. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 4820–4824

29. Zantema, A., Maassen, J. A., Kriek, J., and Möller, W. (1982) Biochemistry 21, 3069–3082

30. Lee, C. C., Wells, B. D., Fairclough, R. H., and Cantor, C. R. (1981) J. Biol. Chem. 256, 49–53

31. Gudkov, A. T., Gongadze, G. M., Bushuev, V. N., and Okon, M. S. (1982) FEBS Lett. 138, 229–232

32. Kenny, J. W., Lambert, J. M., and Traut, R. R. (1979) Methods Enzymol. 59, 534–550

33. Lambert, J. M., and Traut, R. R. (1981) J. Mol. Biol. 149, 451–476

34. Wittmann, H. G. (1982) Annu. Rev. Biochem. 51, 155–183

35. Tolan, D. R., Lambert, J. M., Bouleau, G., Kenny, J. W., Vassos, A., and Traut, R. R. (1980) Anal. Biochem. 108, 101–109

36. Kenny, J. W., Sommer, A., and Traut, R. R. (1975) J. Biol. Chem. 250, 9434–9436

37. Expert-Bezançon, A., Barriault, D., Milet, M., and Hayes, D. H. (1976) J. Mol. Biol. 108, 781–787

38. Van Aethoven, A. J., Maassen, J. A., Schrier, P. I., and Möller, W. (1975) Biochem. Biophys. Res. Commun. 64, 1184–1191

39. Schop, E. N., and Maassen, J. A. (1982) Eur. J. Biochem. 128, 371–375

40. Wower, I., Wower, J., Meinke, M., and Brimascombe, R. (1981) Nucleic Acids Res. 9, 4285–4302

41. Stoffler-Meilicke, M., Stoffler, G., Odom, O. W., Zim, A., Kramer, G., and Hardesty, B. (1981) Biochemistry 78, 5538–5542

42. Fanning, T. G., and Traut, R. R. (1981) Nucleic Acids Res. 9, 993–1004

43. Shatsky, I. N., Evstafieva, A. G., Bystrova, T. F., Bogdanov, A. A., and Vasiliev, V. D. (1980) FEBS Lett. 121, 97–100

44. Acharya, A. S., Moore, F. B., and Richards, F. M. (1973) Biochemistry 12, 3108

45. Cundliffe, E. (1980) in Ribosomes—Structure, Function and Genetics (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., and Nomura, M. eds) pp. 555–581, University Park Press, Baltimore

46. Cooperman, B. S. (1980) in Ribosomes—Structure, Function and Genetics (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., and Nomura, M. eds) pp. 531–554, University Park Press, Baltimore

47. Nierhaus, K. H. (1980) in Ribosomes—Structure, Function and Genetics (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., and Nomura, M. eds) pp. 267–294, University Park Press, Baltimore

48. Dohme, F., and Fahnestock, S. R. (1979) J. Mol. Biol. 129, 63–81

49. Luhrmann, R., Bald, R., Stoffler-Meiliche, M., and Stoffler, G. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 7276–7280

50. Olson, H. M., Grant, P. G., Cooperman, B. S., and Glitz, D. G. (1982) J. Biol. Chem. 257, 2649–2656

51. Traut, R. R., Lambert, J. M., Bouleau, G., and Kenny, J. W. (1980) in Ribosomes—Structure, Function and Genetics (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., and Nomura, M. eds) pp. 89–110, University Park Press, Baltimore

52. Lake, J. A., and Strycharz, W. A. (1981) J. Mol. Biol. 153, 979–992
Ribosomal protein L7/L12 cross-links to proteins in separate regions of the 50 S ribosomal subunit of Escherichia coli.
R R Traut, J M Lambert and J W Kenny

J. Biol. Chem. 1983, 258:14592-14598.

Access the most updated version of this article at http://www.jbc.org/content/258/23/14592

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/23/14592.full.html#ref-list-1