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Area of 16S Ribonucleic Acid at or near the Interface Between 30S and 50S Ribosomes of *Escherichia coli*

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To determine the region of 16S ribonucleic acid (RNA) at the interface between 30 and 50S ribosomes of *Escherichia coli*, 30 and 70S ribosomes were treated with T1 ribonuclease (RNase). The accessibility of 16S RNA in the 5′ half of the molecule is the same in 30 and 70S ribosomes. The interaction with 50S ribosomes decreases the sensitivity to T1 RNase of an area in the middle of 16S RNA. A large area near the 3′ end of 16S RNA is completely protected in 70S ribosomes. The RNA near the 3′ end of the molecule and an area of RNA in the middle of the molecule appear to be at the interface between 30 and 50S ribosomes. One site in 16S RNA, 13 to 15 nucleotides from the 3′ end, normally inaccessible to T1 RNase in 30S ribosomes, becomes accessible to T1 RNase in 70S ribosomes. This indicates a conformational change at the 3′ end of 16S RNA when 30S ribosomes are associated with 50S ribosomes.

All cells contain two ribosomal subunits which are physically, chemically, and functionally differentiated (32, 33). In procaryotic cells, peptide bond formation (i.e., chain elongation) depends on the formation of 70S ribosomes from 50 and 30S subunits, although the initiation of protein synthesis takes place on 30S ribosomes (13). The association of the two subunits is, therefore, a crucial step in protein synthesis, and it is of some interest to know how the two subunits interact. There are, in theory, a number of ways in which they could be held together, and they involve either protein:protein, protein:ribonucleic acid (RNA), or RNA:RNA interactions or some combination of these three. Although we do not know what proteins participate in subunit interactions, there are a number of 30 and 50S proteins at or near the interface between the two subunits that are available, at the very least, to play such a role (5, 8, 9, 11, 12, 17, 29). There are no comparable data that indicate that areas of 16S RNA are at the interface between 30 and 50S ribosomes. There are, however, a number of lines of evidence which demonstrate that restricted parts of 16S RNA are on the "surface" of the 30S subunit. (i) Colicin E3 treatment of 70S ribosomes creates one hydrolytic split in 16S RNA, 48 nucleotides from the 3′ end of the chain. These ribosomes are inactive in protein synthesis (2, 25). (ii) The number of G residues in 30S ribosomes that react with kethoxal is limited; however, a significant number are located in the 3′ half of the chain (18). (iii) T1 ribonuclease (RNase) treatment of 30S ribosomes releases a variety of oligonucleotides from widely separated areas on the 16S RNA molecule; about 50% of these oligonucleotides come from an area near the 3′ end of the chain (24) contained in sections A and J (6; see Fig. 5). By this criterion, the largest unprotected area of RNA is near the 3′ end of the chain (24, 34). (iv) There is a polypyrimidine tract at the end of 16S RNA of 30S ribosomes of all procaryotes, which Shine and Dalgarno (26, 27) have suggested base-pairs with a polypurine cluster preceding the initiating codon of many messenger RNA molecules, suggesting a role for the 16S RNA in the initiation of protein synthesis.

In an elegant experiment, Steitz and Jakes (28) have demonstrated that the short polypurine sequence preceding the AUG codon in the messenger RNA for the R17 A protein base-pairs with the polypurine tract in the 16S RNA of 30S ribosomes, which is in accordance with the hypothesis of Shine and Dalgarno (26, 27).

Thus, a small portion of RNA on the surface of the 30S ribosome at the 3′ end of the chain appears to play a direct role in the initiation of protein synthesis.

While this report was being prepared, Van Duin et al. (31) suggested that 30S ribosomes interact with 50S ribosomes by way of complementary sequences near the 3′ ends of both 16 and 23S RNA. In this paper, we suggest that an area near the 3′ end of the 16S RNA molecule is

at the interface with 50S ribosomes. These data offer support for the hypothesis of Van Duin et al. (31).

MATERIALS AND METHODS

The growth of *Escherichia coli* MRE 600 on ^{32}P medium and the isolation of 70, 30, and 50S ribosomes and the RNA from them have been previously described (23). The various buffers used are as follows. Buffer 5 consisted of 5 mM Mg acetate, 10 mM Tris-hydrochloride, and 10 mM KCl (pH 7.8); buffer 10 consisted of 10 mM Mg acetate, 30 mM NH_4Cl, 10 mM Tris-hydrochloride, and 6 mM β-mercaptoethanol (pH 7.5); buffer N consisted of 40 mM Mg acetate, 50 mM Tris-hydrochloride, 1 mM dithiothreitol, and 1 M NH_4Cl (pH 7.4).

Uniformly labeled 70S ribosomes were obtained from S30 extracts of *E. coli* by centrifugation at 39,000 rpm for 75 min in a no. 40 Spinco rotor. Fifty units of ribosomes measured at an optical density of 260 nm were washed one time with 3 ml of buffer 10, spun at 39,000 rpm for 60 min, resuspended in 1.0 ml of buffer 10, and treated with 250 μg of T1 RNase for 5 min at 23°C. Salt-washed 70S ribosomes were prepared by suspending 50 units of ribosomes measured at an optical density of 260 nm in 1 M NH_4Cl buffer (buffer N) and shaking in the cold for 24 h. The ribosomes were centrifuged at 100,000 $\times$ *g* for 60 min, resuspended, and washed one time in 1.0 ml of buffer 10. The 70S ribosomes were finally resuspended in 1.0 ml of buffer 10 and treated with 250 μg of T1 RNase for 5 min at 23°C. Polyacrylamide gel electrophoresis of RNA fragments and retrieval of the fragments were carried out as previously described (1). All procedures for two- and one-dimensional electrophoretic separation of oligonucleotides have also been previously described (1, 22).

T1 RNase (EC 2.7.7.26) was purchased from Calbiochem. Pancreatic RNase (EC 2.7.7.16) was obtained from Worthington Biochemical Corp. Cellulose acetate strips (Cellogel) were obtained from Kalex Scientific Co.

RESULTS

RNA fragments from 30 and 70S ribosomes. To demonstrate that ribosomal RNA (rRNA) from 30S ribosomes is protected from nuclease attack by 50S ribosomes, we have treated 70S as well as 30S ribosomes in buffer 10 and separated the RNA fragments by polyacrylamide gel electrophoresis. The various gel patterns are shown in Fig. 1. Column 1 is a "standard" RNA band pattern that was obtained from 30S ribosomes after T1 RNase treatment in buffer 5. These RNA fragments represent areas that are completely resistant to T1 RNase hydrolysis under these experimental conditions. The bands are designated according to our previous numbering system (23, 24) and include the following components. (i) Band 5′ is a fragment of an RNA molecule that is about 120 nucleotides long and includes the 5′ end of the 16S rRNA. It is equivalent in composition to section L of 16S RNA (6). To help in our analysis of the data, Fig. 2 contains the 16S RNA molecule organized in lettered sections (6). The meaning of the hatched and clear boxes above the lettered sections and the arrows below the sections has been deferred until the discussion. (ii) Band 7 is an RNA fragment that is 66 nucleotides long and includes the unique oligonucleotides found in section D′ (6). (iii) Band 3′ is a piece of RNA which is the final 25 nucleotides of the 3′ end of the 16S RNA chain and is about one-half the length of both section J (6) and the colicin E3 fragment (2). (iv) Low-molecular-weight fractions I and II includes a collection of unique oligonucleotides that come from various parts of the 16S rRNA chain; a large percentage of these unique oligonucleotides comes from sections A and J (6, 24) near the 3′ end of the chain. They range in size from 6 to 12 nucleotides. The RNA at the top of the gel is composed of very large RNA fragments, some of which have been previously characterized (24). In column 2 of Fig. 1 is the RNA obtained from 30S ribosomes treated in buffer 10, since 70S ribosomes are also treated with T1 RNase in buffer 10. The band pattern is identical to that in column 1. Column 3 contains the RNA obtained from 70S ribosomes. Column 4 is a diagram of the 16S RNA components present in column 3; the contribution of RNA from 50S ribosomes has been removed. RNA fragments were assumed to come from 23S RNA when their unique oligonucleotides, i.e., those with six or more nucleotides, could not be assigned to the 16S RNA catalogue of oligonucleotides (6, 34). The allocation of RNA bands to 30S ribosomes was judged by their oligonucleotide composition after complete T1 RNase digestion. It was also possible to eliminate the contribution of 23S rRNA because little of this RNA is converted to fragments in the 20 to 120 nucleotide size range (M. Santer, unpublished observation). The largest contribution is a 5S RNA molecule that co-migrates in the gel with band 5′. The 5S RNA molecule apparently emerges intact from T1 RNase-treated 50S ribosomes (7).

If we compare the gel patterns of RNA derived from 70S ribosomes and free 30S ribosomes (Fig. 1, columns 2 and 4), we see a number of differences and two basic similarities. The similarities are: (i) the presence in every preparation of a fragment with the same mobility as band 5′ and (ii) the presence of a low-molecular-weight fraction II. The differences between free 30 and 70S ribosomes are as fol-

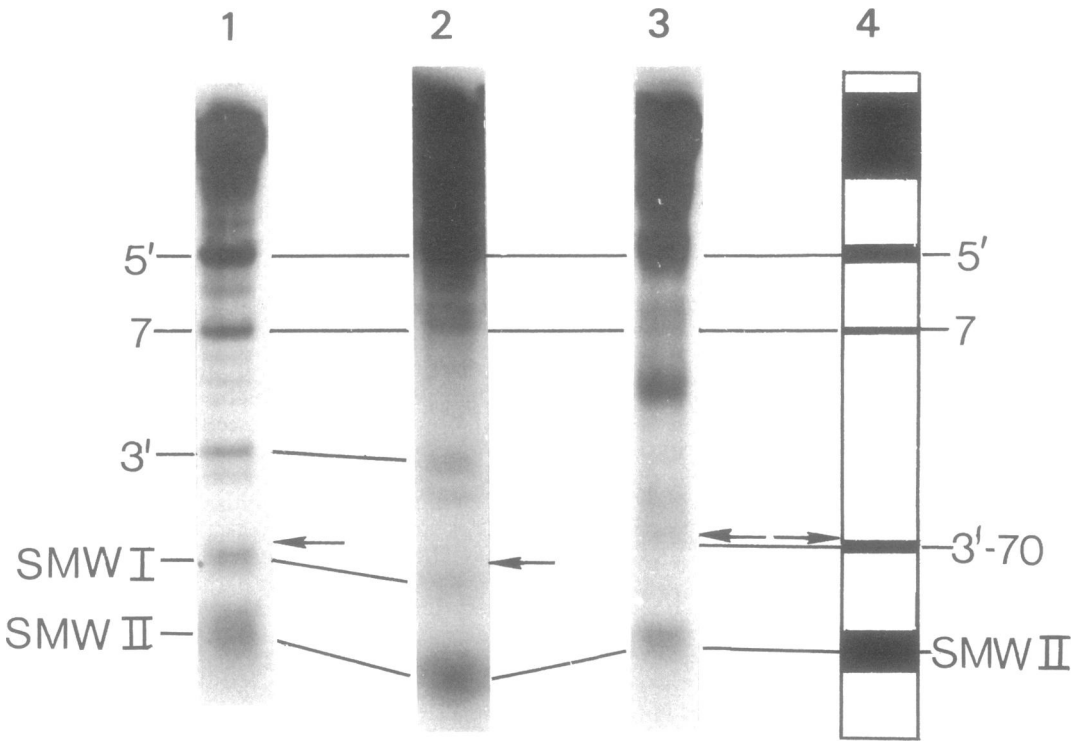

FIG. 1. *Radioautograph and diagram of polyacrylamide gel electrophoretic separation of RNA fragments generated by T1 RNase treatment of 30 and 70S ribosomes. Column 1 represents RNA from 30S ribosomes treated with buffer 5. The various fractions have been previously characterized (23, 24). Column 2 represents RNA from 30S ribosomes treated with buffer 10. Column 3 represents RNA from 70S ribosomes treated with buffer 10. Column 4 only diagramatically represents the RNA components from 30S ribosomes found in column 3. Their composition is described in Results. Arrows indicate the position of the bromophenol blue dye marker in each gel. SMW, Small (low) molecular weight.*

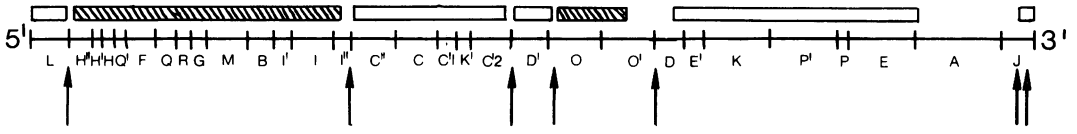

FIG. 2. *Lettered sections of 16S rRNA (6). Clear bars indicate RNA fragments produced by T1 RNase treatment of 30S ribosomes (24). Hatched bars indicate lengths of RNA which appear to remain intact after T1 RNase treatment, since none of their unique oligonucleotides appears in either smaller RNA fragments or in low-molecular-weight fractions. Clear spaces between bars are sections of 16S rRNA converted to low-molecular-weight RNA. Arrows below the lettered segments indicate T1 RNase sites of hydrolysis in 70S salt-washed ribosomes. The availability of sites between C′2-D′ and D′-0 are considerably reduced in 70S ribosomes.*

lows. In 70S ribosomes, the concentration of band 7 is greatly reduced (column 3), whereas band 3′ is missing and a low-molecular-weight fraction I is replaced by a fraction labeled 3′-70. Fraction 3′-70 migrates with the bromophenol blue dye marker in 10% polyacrylamide gels, indicating a fragment of RNA about 13 to 15 nucleotides long, and is differentiated from fraction I by its oligonucleotide composition.

Identity of band 5′. In every instance of T1 RNase treatment of either 30 or 70S ribosomes, a fragment of RNA with a mobility identical to that of band 5′ is generated. The identity of band 5′ was confirmed by complete digestion with T1 RNase and separation of the digest by two-dimensional electrophoresis (Fig. 3). All the oligonucleotides previously found in the standard band 5′ oligonucleotide "fingerprint" (23; Fig. 3A) are found in a band having the same electrophoretic mobility from 70S T1

RNase-treated ribosomes (Fig. 3B). In addition to the oligonucleotides of band 5', a unique set of oligonucleotides derived from 5S RNA is also in the band (4; Fig. 3B) because the 5S RNA of the 50S subunit co-migrates with 5', since they are both 120 nucleotides long.

We had previously shown that band 5' emerges in molar quantities from T1 RNase-treated 30S ribosomes (23). When 70S ribosomes are used as substrate, it appears from preliminary estimates that band 5' also appears in molar quantities. It is, therefore, quite clear that even when 30S ribosomes are combined with 50S subunits, the T1 RNase-sensitive site, some 120 nucleotides from the 5' end of the 16S chain, remains completely accessible to the enzyme.

Quantitative reduction of band 7. Although the susceptibility of the band 5' area of 16S RNA to T1 RNase remains constant in 70S ribosomes, other parts of the 16S RNA become differently susceptible to nuclease action when 30S ribosomes are combined with 50S ribosomes. In the normal digest of 30S ribosomes, band 7 emerges in a molar ratio to band 5'; however, the amount of band 7 that is recovered from 70S ribosomes is estimated to be about 0.2 to 0.3 M. The oligonucleotide composition of the low-yield band 7 is nonetheless unchanged, except for some contaminating oligonucleotides (Fig. 4).

Alteration of band 3'. To clarify our analysis of events at the 3' end of the 16S rRNA molecule, we have provided the final 175 or so nu-

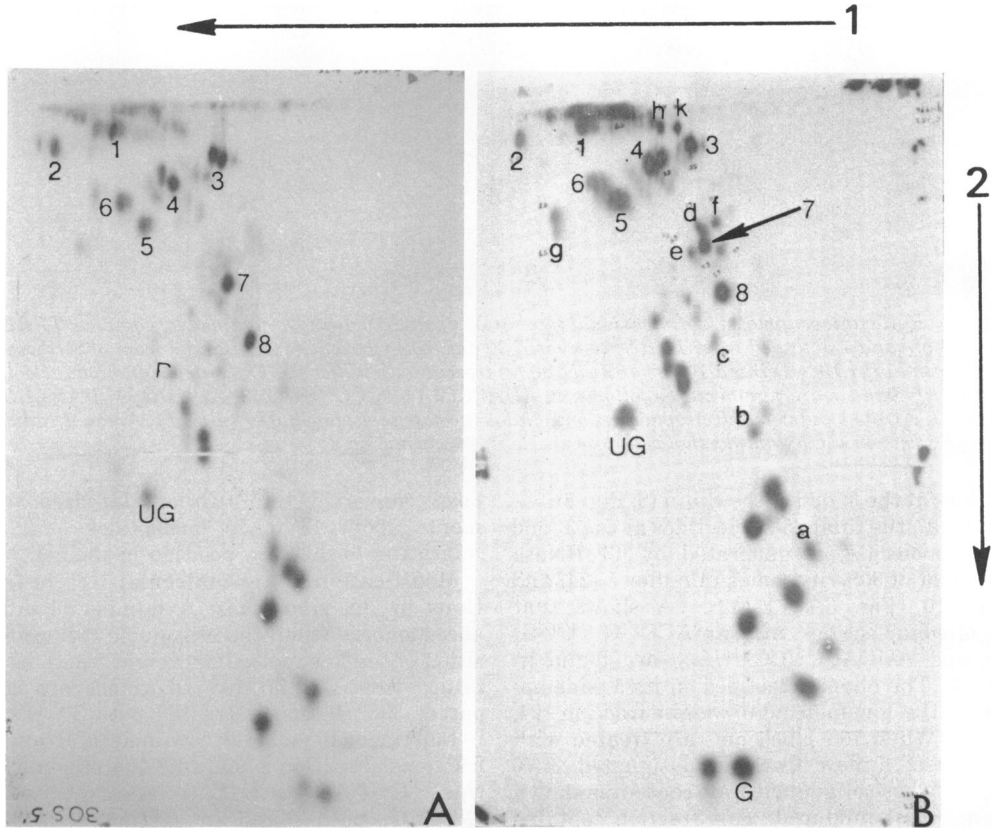

FIG. 3. *Radioautograph of two-dimensional electrophoretic separation of oligonucleotides obtained by T1 RNase digestion of band 5' obtained from T1 RNase-treated 30 and 70S ribosomes. (A) Band 5' from 30S ribosomes treated with buffer 10. (B) Band 5' from 70S ribosomes treated with buffer 10. Arrow 1 indicates electrophoresis on cellulose acetate in acetate buffer (pH 3.5). Arrow 2 indicates electrophoresis on diethylaminoethyl-cellulose sheets in 7% formic acid. The unique oligonucleotides which characterize band 5' are numbered, whereas the 5S oligonucleotides are lettered: 1, pAAAUUG; 2, UUUG; 3, CCUAACACAUG; 4, AUCAUG; 5, CUUG; 6, AUUG; 7, UAACAG; 8, CUCAG; a, CCAG; b, AAACG; c, CCUG; d, AACUCAG; e, AACUG; f, ACCCCAUG; g, pUG; h, UCUCCCCAUG; i, CCUUAG, included in spot 4; k, UCCCACCUG. The 5S oligonucleotide sequences are from Brownlee et al. (4).*

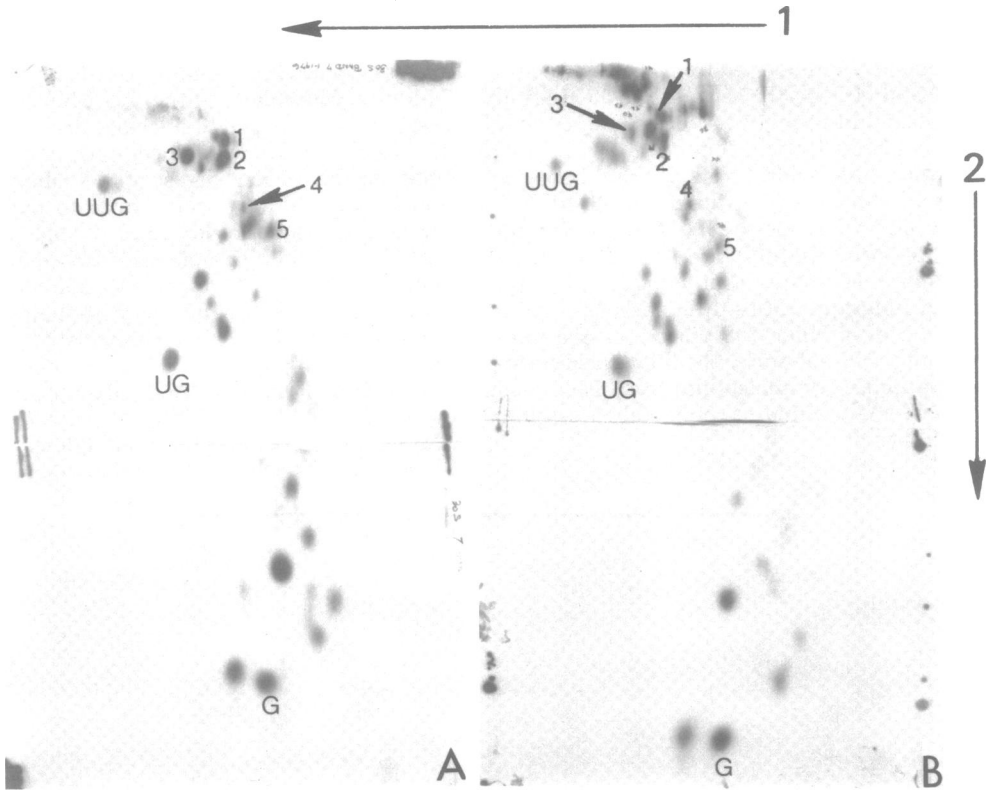

FIG. 4. *Radioautograph of two-dimensional separation of oligonucleotides obtained by complete T1 RNase digestion of band 7 obtained from T1 RNase-treated 30 and 70S ribosomes. (A) Band 7 from 30S ribosomes treated with buffer 10. (B) Band 7 from 70S ribosomes treated with buffer 10. The important oligonucleotides that identify band 7 are numbered as follows: 1, AUACCCUG; 2, CCCUUG; 3, ACUUG; 4, UAAACG; 5, UCCACG. Arrow 1 indicates electrophoresis on cellulose acetate in acetate buffer (pH 3.5). Arrow 2 indicates electrophoresis on diethylaminoethyl-cellulose sheets in 7% formic acid.*

cleotides at the 3′ end of the chain (6; Fig. 5).

Band 3′, the final 25 nucleotides at the 3′ end of the molecule, is generated by T1 RNase treatment of 30S ribosomes in buffer 5 (24) and buffer 10 (Fig. 6A). Figure 6A shows that the oligonucleotides $m_2^6Am_2^6ACCUG$, UUG, CG, and $AUCACCUCCUUA_{OH}$ are found in band 3′. The oligonucleotides on its 5′ side appear in the low-molecular-weight fraction (24; Fig. 5). When 70S ribosomes are treated with T1 RNase, a new fraction, designated 3′-70 (Fig. 1), whose oligonucleotide content is shown in Fig. 6B is produced. This fraction contains the following components: $m_2^6Am_2^6ACCUG$, CG, UUG, and $AUCACCUCCUUA_{OH}$. These oligonucleotides, if they were covalently linked to each other, as they normally are in 16S RNA and in band 3′ (Fig. 5), would span a region of RNA 25 nucleotides in length. Since a piece of RNA of this length would normally be found in the region occupied by band 3′, it appears that the final 25 to 30 nucleotides of the 3′ end have been converted into two similar-sized fragments about 13 to 15 nucleotides long, comigrating to this new position in the gel.

Modification of low-molecular-weight fractions in 70S ribosomes. A number of differences emerge when the oligonucleotide composition of the low-molecular-weight fractions obtained from 30 and 70S ribosomes are compared. 30S ribosomes treated with T1 RNase in buffer 5 or 10 yield two low-molecular-weight fractions. Fraction I contains the oligonucleotides CUUACCACUUUG and CUUAACCUUCG, both found in section A (Fig. 2 and 5), plus a number of small, nonunique (less than six nucleotides) oligonucleotides. The large oligonucleotides have been previously characterized (6, 24, 34) and were recognized by their positions on two-dimensional electrophoretic separation of oligonucleotides done according to the method of Sanger and associates (22). Fraction II contains the remainder of the oligonucleotides shown in Fig. 7A.

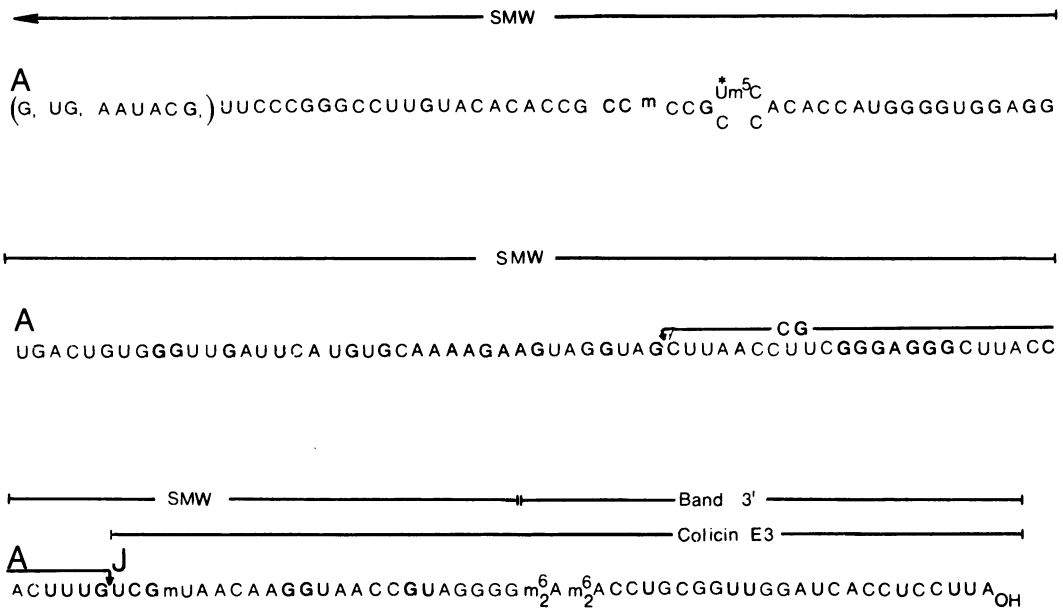

FIG. 5. *Primary structure of 16S RNA at the 3' end of the molecule (6). The sequence of the 3' OH oligonucleotide has been redrawn according to the data of Shine and Dalgarno (26, 27), Noller and Herr (19), Steitz and Jakes (28), and Woese et al. (34). The sequences of the oligonucleotides AUUCAUG and CC^{m}CCG have been revised (34). The diagram indicates that T1 RNase treatment of free 30S ribosomes in buffer 5 or 10 generates an intact band 3', whereas the remaining RNA designated SMW appears in low-molecular-weight fractions. The area of the colicin E3 fragment is also shown.*

This radioautograph actually contains all the oligonucleotides found in fractions I and II because this low-molecular-weight RNA fraction from 30S subunits was obtained from 5% polyacrylamide gels which do not resolve fractions I and II.

There are about 14 unique oligonucleotides, i.e., those with six or more nucleotides, plus smaller oligonucleotides containing methyl groups found in this fraction. Nine of these oligonucleotides, including the sequence mUAACAAG, are derived from sections A and J. One of the methylated oligonucleotides, spot 18, with five bases is also found in section A. Two oligonucleotides are found in section O'. Another oligonucleotide with the composition (3U,1C)G, may also come from section O' if its sequence is UCUUG. One oligonucleotide comes from section D, which is adjacent to O'. Two oligonucleotides are derived from section C''; one of them appears to be a methylated tetramer (spot 19). When 70S ribosomes are treated with T1 RNase, however, band 3'-70 replaces fraction I, whereas fraction II (Fig. 7B) is a simple mixture of small oligonucleotides three and four nucleotides in length. It contains none of the unique sequences found in fraction II obtained from 30S ribosomes.

Ammonium-chloride-washed 70S ribosomes. It is possible that, in freshly prepared 70S ribosomes, the RNA of the 30S subunit is not only protected from T1 RNase hydrolysis by 50S ribosomes but also by extraribosomal components which, fortuitously or otherwise, bind to 16S RNA (20, 21). To test this possibility, 70S ribosomes were first washed with buffer containing 1 M NH_4Cl and then treated with T1 RNase. Isolated 50S ribosomes were also treated with T1 RNase to determine which oligonucleotides obtained from salt-washed 70S ribosomes might come from the 50S subunit.

The gel pattern of RNA derived from salt-washed 70S ribosomes is virtually identical to the pattern obtained from unwashed 70S ribosomes, which is shown in Fig. 1 (column 3). For example, band 3'-70 is produced and contains all the oligonucleotides found in band 3'. In addition, this fraction contains the following oligonucleotides: AACCUUACCUG, which is found in section O', and the oligonucleotide CUAACUCCG, which is derived from the 5' end of section C'' (6). No fraction I is produced; fraction II oligonucleotides from salt-washed 70 and 50S ribosomes are shown in Fig. 8A and 8B, respectively. It is clear that additional oligonucleotides are also found in fraction II from

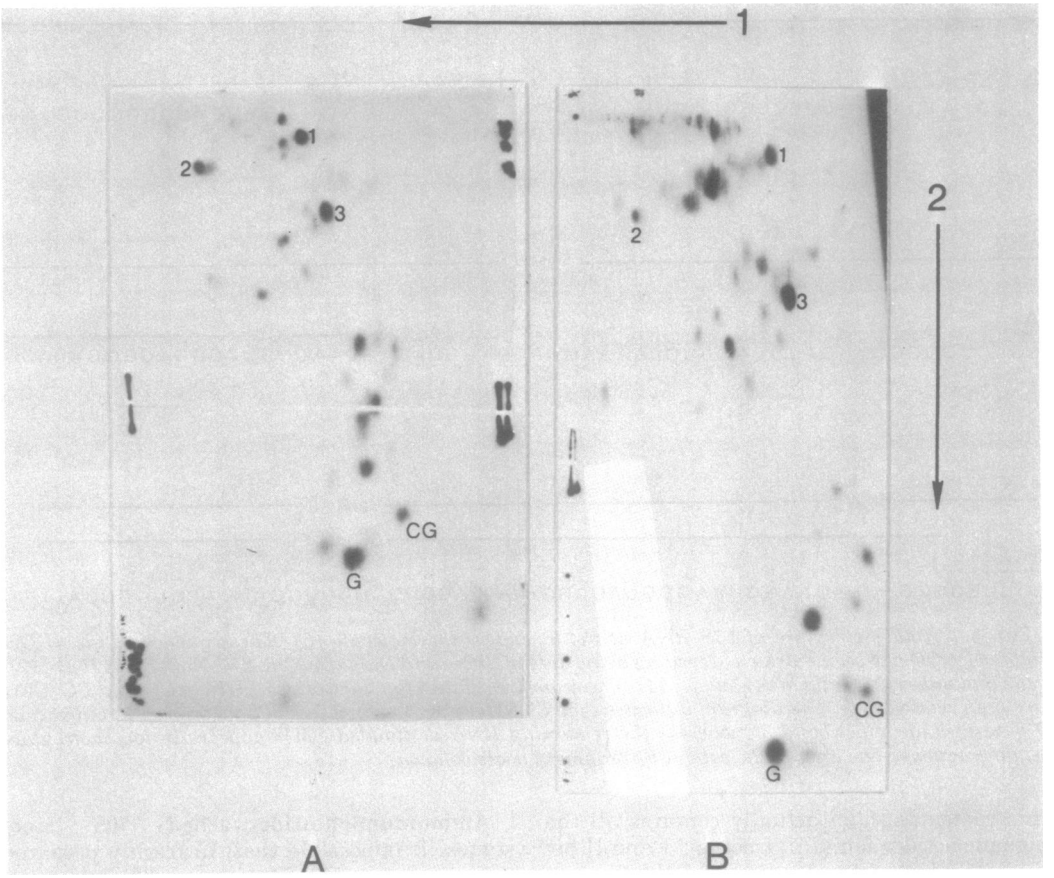

FIG. 6. *Radioautograph of two-dimensional electrophoretic separation of band 3' from T1 RNase-treated 30S ribosomes (A), and band 3'-70 from T1 RNase-treated 70S ribosomes (B). The key oligonucleotides have been numbered as follows: 1, AUCACCUCCUUA$_{OH}$; 2, UUG; 3, $m_2^6Am_2^6ACCUG$. Arrow 1 indicates electrophoresis on cellulose acetate in acetate buffer (pH 3.5). Arrow 2 indicates electrophoresis on diethylaminoethyl-cellulose sheets in 7% formic acid.*

salt-washed 70S ribosomes when compared to fraction II from unwashed 70S ribosomes (compare Fig. 8A to 7B).

Many of these oligonucleotides are derived from the rRNA of 50S ribosomes. A small but significant number are derived from the rRNA of 30S ribosomes and are among the oligonucleotides in fraction II obtained from T1 RNase-treated 30S ribosomes (Fig. 7A). For example, we find the sequence UUUUCAG (section D); also present in lesser amounts is the sequence UUUAAUUCG (section O'). Two other oligonucleotides are present with the following compositions: (2U,3C)G and (U,3C)G. Oligonucleotides with these compositions appear more than one time in 16S RNA, and until their sequences are determined, it is not possible to assign them to a particular region of the molecule. Another interesting oligonucleotide, number 7, is present in both "fingerprints," suggesting that it is derived from the rRNA of 50S ribosomes. Its composition is (3U,C)G. Both 16 and 23S RNA have an oligonucleotide with this nucleotide composition (3, 6; Fig. 7A). If all or part of this spot is derived from 16S RNA, its sequence might be UCUUG. This oligonucleotide is found in section O'.

Therefore, it is clear that some areas of 16S rRNA are revealed by salt-washing, but none of them are located in sections A or J.

DISCUSSION

Two important changes occur in 30S ribosomes when they are part of 70S ribosomes. A portion of the 16S rRNA near the 3' end is buried at the interface, whereas the final 25 or so nucleotides of the 3' end of the 16S RNA

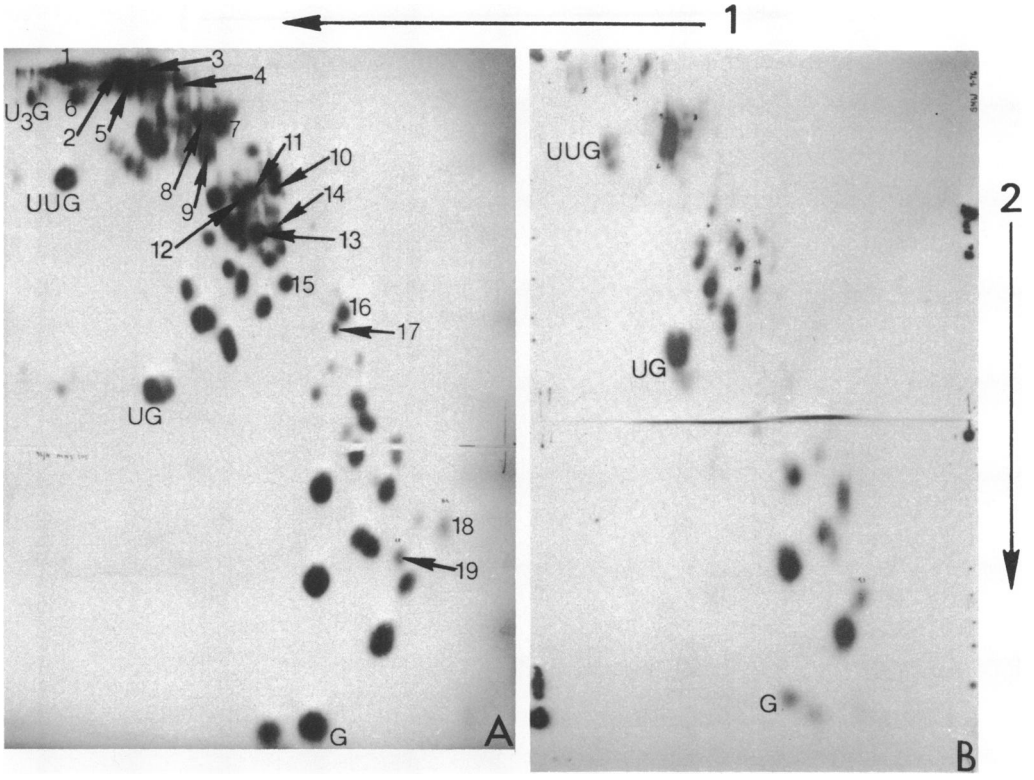

FIG. 7. *Radioautograph of two-dimensional electrophoretic separation of complete T1 RNase digests of low-molecular-weight fraction II obtained from T1 RNase-treated 30 and 70S ribosomes. (A) This radioautograph actually contains low-molecular-weight fractions I and II because the RNA was obtained from 5% polyacrylamide gels where fractions I and II are not resolved. (B) Low-molecular-weight fraction II from 70S ribosomes. The unique oligonucleotides found in Fig. 7A are numbered as follows: 1, mixture of UUUUCAG (D) and UUUAAUUCG (O'); 2, UUACCACUUUG (A); 3, CUUAACCUUCG (A); 4, AACCUUACCUG (O'); 5, AUUCAUG (A); 6, (3U,C)G[a]; 7, mixture of UC[m]ACACCUAUG (A) and CUAACUCCG[b] (C"); 8, mixture, not completely characterized; 9, (2U,3C)G; 10, UACACACCG (A); 11, AAUACG (A); 12, mUAACAAG (J); 13, UAACCG (J); 14, (U,2C,AC)G; 15, (U,3C)G; 16, CA$_3$CAG[c]; 17, CAAAAG (A). Oligonucleotides numbered 19 and 18 are assumed to have the sequences G[m]CCG and CC[m]CCG (34), respectively, because they correspond in electrophoretic properties to oligonucleotides previously characterized (30). Oligonucleotides 19 and 18 have been placed in section C" and A, respectively (6). Letter in brackets next to each oligonucleotide indicates its section location in 16S RNA (6). The sequence of the oligonucleotide represented as spot a is not certain. It may come from section O'. The oligonucleotide represented by spot b was characterized by its pancreatic RNase digestion products. Although there are two oligonucleotides in 16S RNA with this composition, we favor the sequence listed here because this is the oligonucleotide immediately adjacent to G[m]CCG (oligonucleotide 19) which also appears in this fraction, whereas the other sequence is in the middle of the large RNA fragment which is produced by T1 RNase treatment of 30S ribosomes and covers sections C" to C'2 (band 300; 24). There is no section allocation for the oligonucleotide represented by spot c (34). Arrow 1 indicates electrophoresis on cellulose acetate in acetate buffer (pH 3.5). Arrow 2 indicates electrophoresis on a diethylaminoethyl-cellulose-7% formic acid solvent.*

molecule, normally resistant to T1 RNase, appear to undergo a conformational change that alters its sensitivity to T1 RNase. We shall consider each of these phenomena in turn.

When free 30S ribosomes are exhaustively treated with T1 RNase, a range of RNA fragments is produced (24). Those which have been characterized are indicated by open bars that span the appropriate lettered regions (Fig. 2). Band 3', 25 nucleotides long, covers half of section J. A band about 475 nucleotides long includes sections E', K, P, P', and E. Part of J and most of A between these two fragments are converted to low-molecular-weight material. On the 5' side of section E' is an area including parts of sections O' and D, which are converted

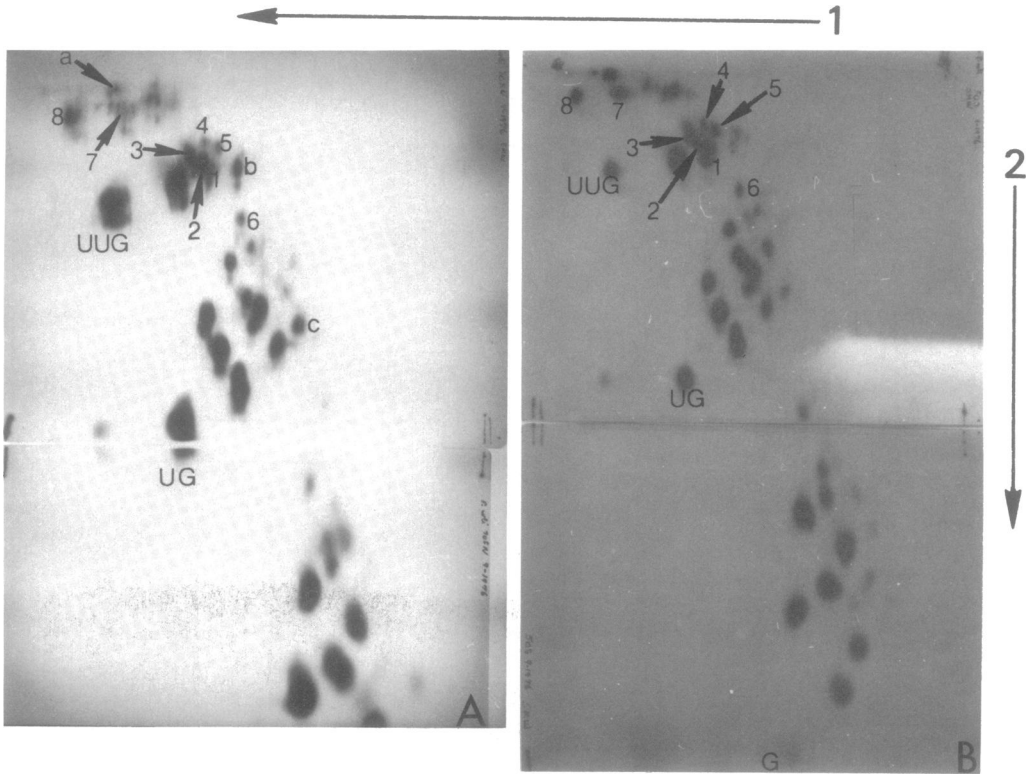

FIG. 8. *Radioautograph of two-dimensional electrophoretic separation of oligonucleotides derived from the low-molecular-weight fraction II obtained from T1 RNase-treated, salt-washed 70S ribosomes (A) and free 50S ribosomes (B). Oligonucleotides with identical numbers in both radioautographs have identical sequences or are composed of the same nucleotides; they are derived from 50S ribosomes. Spot 7 may come from 30S ribosomes. Other oligonucleotides are only found in 70S ribosomes (A). Spot a, UUUUCAG, is found in 16S RNA in section D; also present here, in small amounts, is UUUAAUUCG, found in section O'; spot b, $(U_2,C_3)G$; and spot c, $(U,C_3)G$. These four oligonucleotides are derived from the 30S component of salt-washed 70S ribosomes. Arrow 1 indicates electrophoresis on cellulose acetate in pH 3.5 acetate buffer. Arrow 2 indicates electrophoresis on diethylaminoethyl-cellulose paper in 7% formic acid.*

to low-molecular-weight RNA (Fig. 7). Three other fragments, indicated by open bars, have been characterized: band 7, 66 nucleotides long from section D'; a large fragment about 300 nucleotides in length covering sections C", C, C'1, K', and C'2; and band 5', 120 nucleotides long, a fragment that covers section L (24). Two other areas have been indicated by hatched bars; their composition has not been determined, but we assume they remain intact, since none of their unique oligonucleotides are found in low-molecular-weight RNA. These data show that there are two large areas of 16S RNA in the intact 30S ribosome which are hydrolyzed by T1 RNase and thus assumed to be single-stranded, surface components. They include sections A-J and O'-D. Smaller, equally sensitive sites are on either side of section D' (to generate band 7), on the 5' side of C", and between sections L and H" (Fig. 2). The area within band 7, section D', is also apparently single stranded although not cleaved by T1 RNase, since three closely positioned oligonucleotides are labeled with kethoxal (18).

When salt-washed 70S ribosomes are treated with T1 RNase, no oligonucleotides of section A or J are converted into low-molecular-weight fractions. Band 7 is produced, albeit in reduced amounts, indicating that the sites on either side of band 7 are less accessible in 70S ribosomes than in 30S ribosomes. All other sites sensitive to T1 RNase in 30S ribosomes, i.e., the section between L and H", the area on the 5' side of C" and the region O'-D, are sensitive in 70S ribosomes.

It is important to stress that band 5', 120 nucleotides long, from section L at the 5' end of the 16S RNA, is produced by T1 RNase treat-

ment of 70S ribosomes. Thus, whatever site was available to T1 RNase on 30S ribosomes, which led to the production of band 5', is still available when 30S ribosomes are combined with 50S ribosomes. We conclude that the 5' end of the 16S RNA is not at the interface between the two subunits, nor is its T1 RNase sensitivity influenced by the presence of 50S subunits.

It is clear that, of the six areas of rRNA of the 30S ribosome which can be shown to be surface components (Fig. 2), only section A and part of section J remain inaccessible to T1 RNase hydrolysis when 30 and 50S ribosomes are associated into 70S ribosomes. We conclude that sections A and J are at the interface between the two subunits.

Band 7 (section D') does not appear to be directly at the interface of the two subunits. Nevertheless, it may be near enough to it so that its sensitivity to nuclease is significantly influenced by the presence of 50S ribosomes. If section D' is at the interface, it suggests that distant portions of the same RNA molecule may be in close proximity in the intact ribosome. Such a model for ribosome organization is supported by two kinds of experiments. (i) S18 and S21 proteins can be cross-linked in intact 30S ribosomes (14). This indicates that these two proteins are physically close to each other in the 30S particle even when their respective binding sites on 16S RNA, section D'-O for S18 and the 3' end of the molecule for S21, are some 700 to 800 nucleotides apart. (ii) Individual ribosome proteins can bind in vitro to noncontiguous regions of 16S RNA (15), which suggests that distance portions of RNA molecules can be brought physically close by ribosomal proteins.

Section D', which has the interesting structural feature that three closely positioned oligonucleotides are reactive to kethoxal (18), is a region where the sequence is apparently highly conserved in procaryotic cells (34). It is likely that all these properties of section D', single-stranded character, position near or at the 30S-50S ribosome interface, and the highly conserved primary structure, are related to the role of 30S ribosomes in protein synthesis.

One of the oligonucleotides present in the low-molecular-weight fraction II obtained from 30S ribosomes is the sequence mUAACAAG (Fig. 7A). It is not released from 70S ribosomes by nuclease treatment. It appears in section J (Fig. 2 and 5), and we have concluded that it is at the interface between the two ribosomal subunits.

Van Duin et al. (31) have suggested that 30 and 50S ribosome association may be mediated by complementary sequences in 16 and 23S RNA. A part of this complementary sequence in 16S RNA involves the oligonucleotide mUAACAAG (31; Fig. 4). If that is the case, it is reasonable that this oligonucleotide appears at the 30S ribosome interface with 50S ribosomes. It is interesting to note this oligonucleotide is present in the 16S RNA of 27 procaryotic cells (34).

Structural changes at the 3' end of 16S RNA. The final 30 nucleotides at the 3' end of the chain appear to undergo some interesting structural modifications induced by interaction with 50S ribosomes. Although no enzymatic breaks are made within the final 25 nucleotides of the 3' end of the 16S RNA of free 30S ribosomes (Fig. 5), when 70S ribosomes are digested, one enzymatic break is made about 13 to 15 nucleotides from the 3' end and another cut is made, perhaps 26 to 30 bases from the 3' end. This digestion pattern indicates that these two sites are neither base-paired nor protected by the 50S-30S ribosome interaction. Furthermore, this fragment of RNA is quite unique in 70S ribosomes, since it contains the only sequences of RNA accessible to enzyme in this entire area, whereas in the free 30S ribosome it was the only sequence inaccessible to T1 RNase hydrolysis. These data explain why band 3' is not generated from 70S ribosomes.

These apparent structural changes at the 3' end of the 16S RNA of 30S ribosomes, induced by association with 50S ribosomes, raises the intriguing possibility that these conformational alterations reflect different functional states of the RNA and thus of the 30S ribosome.

After this paper was submitted for publication, we received a personal communication from Nora M. Chapman and Harry F. Noller (J. Mol. Biol., in press) in which they reported on the available kethoxal sites in 16S RNA in 30 and 70S ribosomes. They found that: (i) the reactivity of the sites in the 5' terminal 600 nucleotides is the same in 30 and 70S ribosomes; and (ii) two regions of 16S RNA, one near the middle of the molecule (section C-D') and another at the 3' terminus (sections A and J), are strongly protected in 70S ribosomes. Chapman and Noller suggest that regions C–D' and A–J lie at the interface between 30 and 50S ribosomes. The results reported in this paper support these conclusions. Chapman and Noller also suggest that conformational alterations in 16S RNA occurs when 30S ribosomes are associated with 50S ribosomes because some guanine sites become more accessible to kethoxal addition. This observation may be analogous to our finding that a new T1 RNase-

sensitive site, 13 to 15 bases from the 3′ terminus of 16S RNA, becomes available in 70S ribosomes.

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LITERATURE CITED

1. **Adams, S. M., P. G. H. Jeppeson, F. Sanger, and B. G. Barrell.** 1969. Nucleotide sequence from the coat protein cistron of R17 bacteriophage RNA. Nature (London) **223**:1009–1014.
2. **Bowman, C. M., J. E. Dahlberg, T. Ikemura, J. Konisky, and M. Nomura.** 1971. Specific inactivation of 16S ribosomal RNA induced by colicin E3 *in vivo*. Proc. Natl. Acad. Sci. U.S.A. **68**:964–968.
3. **Branlant, C., J. S. Widada, A. Krol, P. Fellner, and J. P. Ebel.** 1975. Nucleotide sequences of the T1 and pancreatic ribonuclease digestion products from some large fragments of the 23S RNA of *Escherichia coli*. Biochemie **57**:175–225.
4. **Brownlee, G. G., F. Sanger, and B. G. Barrell.** 1968. The sequence of 5S ribosomal ribonucleic acid. J. Mol. Biol. **34**:379–412.
5. **Chang, F. N.** 1973. Conformational changes in ribosomal subunits following dissociation of the *Escherichia coli* 70S ribosome. J. Mol. Biol. **78**:563–568.
6. **Ehresmann, C., P. Stiegler, G. A. Mackie, R. A. Zimmerman, J. P. Ebel, and P. Fellner.** 1975. Primary sequence of 16S ribosomal RNA of *Escherichia coli*. Nucleic Acids Res. **2**:265–278.
7. **Forget, B. G., and M. Reynier.** 1970. Resistance of *E. coli* ribosomal 5S RNA to degradation by ribonuclease in reconstituted ribosomal particles. Biochem. Biophys. Res. Commun. **39**:114–122.
8. **Hawley, D. A., and L. I. Slobin.** 1973. The immunological reactivity of 30S ribosomal proteins in 70S ribosomes from *Escherichia coli*. Biochem. Biophys. Res. Commun. **55**:162–168.
9. **Highland, J. H., E. Ochsner, J. Gordon, J. W. Bodley, R. Hasenbank, and G. Stöffler.** 1974. Coordinate inhibition of elongation factor G function and ribosomal subunit association by antibodies to several ribosomal proteins. Proc. Natl. Acad. Sci. U.S.A. **71**:627–630.
10. **Huang, K. H., and C. R. Cantor.** 1972. Surface topography of the 30S *Escherichia coli* ribosomal subunit: reactivity towards fluorescein isothiocyanate. J. Mol. Biol. **67**:265–275.
11. **Huang, K. H., and C. R. Cantor.** 1975. Studies of 30S *Escherichia coli* ribosome reassembly using individual proteins labeled with an environmentally sensitive fluorescent probe. J. Mol. Biol. **97**:423–441.
12. **Lake, J. A.** 1976. Ribosome structure determined by electron microscopy of *Escherichia coli* small subunits and monomeric ribosomes. J. Mol. Biol. **105**:131–159.
13. **Lengyel, P.** 1974. The process of translation: a bird's-eye view, p. 13–52. *In* A. Tissières, M. Nomura, and P. Lengyel (ed.), Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Lutter, L. C., H. Zeichhardt, C. G. Kurland, and G. Stöffler.** 1972. Ribosomal protein neighborhoods. I. S18 and S21 as well as S5 and S8 are neighbors. Mol. Gen. Genet. **119**:357–366.
15. **Mackie, G. A., and R. A. Zimmerman.** 1975. Characterization of fragments of 16S ribonucleic acid protected against pancreatic ribonuclease digestion by ribosomal protein S4. J. Biol. Chem. **250**:4100–4112.
16. **Miller, R. V., and P. S. Sypherd.** 1973. Topography of the *Escherichia coli* 30S ribosome revealed by the modification of ribosomal proteins. J. Mol. Biol. **78**:539–550.
17. **Morrison, C. A., R. A. Garrett, H. Zeichhardt, and G. Stöffler.** 1973. Proteins occurring at, or near, the subunit interface of *E. coli* ribosomes. Mol. Gen. Genet. **127**:359–368.
18. **Noller, H. F.** 1974. Topography of 16S RNA in 30S ribosomal subunits. Nucleotide sequences and location of sites of reaction with kethoxal. Biochemistry **13**:4694–4703.
19. **Noller, H. F., and W. Herr.** 1974. Nucleotide sequence of the 3′ terminus of *E. coli* 16S ribosomal RNA. Mol. Biol. Rep. **1**:437–439.
20. **Ochoa, S., and R. Mazumder.** 1974. Polypeptide chain initiation, p. 1–51. *In* P. Boyer (ed.), The enzymes, 3rd ed., vol. 10. Academic Press Inc., New York.
21. **Pon, C. L., and C. Gualerzi.** 1976. The role of 16S RNA in ribosomal binding of IF-3. Biochemistry **15**:804–811.
22. **Sanger, F., G. G. Brownlee, and B. G. Barrell.** 1965. A two-dimensional fractionation procedure for radioactive nucleotides. J. Mol. Biol. **13**:373–398.
23. **Santer, M., and U. Santer.** 1972. Characterization of the 5′ and 3′ ends of the 16S ribonucleic acid from T1-ribonuclease-treated 30S ribosomes. Biochemistry **11**:786–791.
24. **Santer, M., and U. Santer.** 1973. Action of ribonuclease T1 on 30S ribosomes of *Escherichia coli* and its role in sequence studies on 16S ribonucleic acid. J. Bacteriol. **116**:1304–1313.
25. **Senior, B. W., and I. B. Holland.** 1971. Effect of colicin E3 upon the 30S ribosomal subunit of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **68**:959–963.
26. **Shine, J., and L. Dalgarno.** 1974. The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. U.S.A. **71**:1342–1346.
27. **Shine, J., and L. Dalgarno.** 1975. Determinant of cistron specificity in bacterial ribosomes. Nature (London) **254**:34–38.
28. **Steitz, J., and K. Jakes.** 1975. How ribosomes select initiator regions in mRNA: base pair formation between the 3′ terminus of 16S rRNA and the mRNA during initiation of protein synthesis in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72**:4734–4738.
29. **Sun, T. T., R. R. Traut, and L. Kahan.** 1974. Protein-protein proximity in the association of ribosomal subunits of *Escherichia coli*: crosslinking of 30S protein S16 to 50S proteins by glutaraldehyde or formaldehyde. J. Mol. Biol. **87**:509–522.
30. **Uchida, T., L. Bonen, H. W. Schaup, B. J. Lewis, L. Zablen, and C. Woese.** 1974. The use of ribonuclease U_2 in RNA sequence determination. J. Mol. Evol. **3**:63–77.
31. **Van Duin, J., C. G. Kurland, J. Dondon, M. Grunberg-Mangago, C. Branlant, and J. P. Ebel.** 1976. New aspects of the IF3-ribosome interaction. FEBS Lett. **62**:111–114.
32. **van Holde, K. E., and W. E. Hill.** 1974. General physical properties of ribosomes, p. 53–91. *In* A. Tissières, M. Nomura, and P. Lengyel (ed.), Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Wittman, H. G.** 1974. Purification and identification of *Escherichia coli* ribosomal proteins, p. 93–114. *In* A. Tissières, M. Nomura, and P. Lengyel (ed.), Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Woese, C. R., G. E. Fox, L. Zablen, T. Uchida, L. Bonen, K. Pechman, B. J. Lewis, and D. Stahl.** 1975. Conservation of primary structure in 16S ribosomal RNA. Nature (London) **254**:83–85.