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Action of Ribonuclease T1 on 30S Ribosomes of *Escherichia coli* and Its Role in Sequence Studies on 16S Ribonucleic Acid

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Two large ribonucleic acid (RNA) fragments have been obtained from T1-RNase-treated 30S ribosomes of *Escherichia coli*. One fragment, about 475 nucleotides long, contains all the unique oligonucleotides found by Fellner and associates in sections of 16S RNA designated P, E, E', and K, and one-half the large oligonucleotides of section A. The other large fragment is about 300 nucleotides long and contains the oligonucleotides found in sections C, C', C''. The isolation of these large fragments seems to confirm the arrangement of sections within 16S RNA. There are also recovered from nuclease-treated ribosomes three small fragments, one (120 nucleotides long) from the 5' end, one (26 nucleotides long) from the 3' OH end of the chain, and another section (66 nucleotides long) from the middle of the 16S RNA chain. Small molecular weight material is also generated by nuclease treatment, and about half this material is derived from a region close to the 3' OH end of the 16S RNA chain. This indicates that the most accessible part of the rRNA of *E. coli* 30S ribosomes is a region 100 to 150 nucleotides long near the 3' end of the chain. A general scheme is proposed to explain the generation of the various-sized RNA products from the rRNA of the 30S ribosome.

Ribonuclease T1 (T1 RNase) makes a number of breaks in the primary structure of the 16S ribonucleic acid (RNA) of the intact 30S ribosome of *Escherichia coli* (5, 23). Gel electrophoresis of RNA derived from enzyme-treated ribosomes revealed that specific areas of 16S survive RNase treatment. Only three of the RNA fragments that are enzyme resistant are relatively small, and contain between 26 and 120 nucleotides; they have been previously described (21), and are the 5' and 3' ends of the RNA and a fragment designated band 7 from the middle of the 16S RNA (4, 6).

In these earlier experiments, most of the RNA obtained from T1-RNase-treated 30S ribosomes remained near the origin in 10% polyacrylamide gels; that is, consisted of sections of RNA even larger than 120 nucleotides. These large protected areas of RNA are of interest because they may be involved in binding to ribosome proteins (10, 16, 17, 18). These large RNA fragments have been resolved by electrophoresis in 5% polyacrylamide gels, and two have been characterized.

The first band is about 475 nucleotides long and contains all the oligonucleotides found by Ehresmann et al. (4), in sections P, E', E, and K, and about one-half of the large oligonucleotides found in section A. These sections make up the major portion of the 3' OH side of the 16S RNA. The second RNA fragment is about 300 nucleotides long and contains oligonucleotides found in bands C, C', and C''. A third large RNA fragment, only incompletely analyzed, contains some of the oligonucleotides found in the 5' half of the 16S RNA.

In addition to these large "protected" areas, there is RNA that is hydrolyzed to sections smaller than 15 nucleotides. This material contains oligonucleotides found in widely varying sections of 16 S RNA; however, among these oligonucleotides are all the larger oligonucleotides found in fragment A and half the oligonucleotides found in section J, which is the 3' end section of 16S RNA.

Fellner and associates (4, 6) have reported the sequence of about 75% of the 16S RNA of *E. coli*. Their major approach has been to treat free 16S RNA with T1 RNase to generate various lengths of RNA for sequence studies. They have designated their various fragments with capital letters, L, R, Q, H, G, M. B, I, C, O, D, E, P-K,

A, and J, going from the 5′ end to the 3′ end of the molecule. We shall continue to refer to their nomenclature throughout this paper. Since we have used only T1 RNase treatment of 30*S* ribosomes to generate RNA bands, we shall be comparing RNA sections obtained in different ways from 16*S* RNA. These comparisons enable us to differentiate between more protected and less protected areas of RNA in the intact ribosome.

MATERIALS AND METHODS

Ribosomes. ^{32}P-labeled ribosomes were prepared from *Escherichia coli* MRE 600 cells grown in a low-phosphate medium in the presence of 10 to 30 mCi of [^{32}P] P_i (20). 30*S* ribosomes were obtained from cell extracts by procedures previously described (21).

Enzyme treatment and preparation of RNA. Treatment of 30*S* ribosomes with T1 RNase was carried out as before (21) and the RNA bands were separated by gel electrophoresis according to Adams et al. (1). The gel was 40 cm long and contained 5% acrylamide and 0.125% bisacrylamide in 0.04 M tris(hydroxymethyl)aminomethane (Tris) (pH 8.4) buffer with 7 M urea. The electrophoresis was carried out at 4 C at 300 V (19 mA) for 19 h.

Sequence determination. The methods used in sequence determination were those devised by Sanger and associates (20) and Adams et al. (1), and have been described in detail in a previous paper (21).

Reagents. T1-RNase (EC 2.7.7.26) was purchased from Calbiochem. Pancreatic RNase (EC 2.7.7.16) and spleen phosphodiesterase (3.1.4.1) were obtained from Worthington Biochemical Corp. Cellulose acetate strips (Cellogel) were obtained from Colab, Glenwood, Ill.

RESULTS

Separation of RNA fragments. ^{32}P-labeled 30*S* ribosomes were treated with T1-RNase and the RNA fragments were separated by electrophoresis in 5% polyacrylamide gels. A typical pattern, as revealed by autoradiography, is shown in Fig. 1. Three rapidly migrating bands have been previously separated on 10% polyacrylamide gels (21), which are the 5′ and 3′ ends of the 16*S* RNA, 120 and 26 nucleotides long, respectively, and one band 66 nucleotides long. Two major bands are found in the upper part of the gel. They have been labeled 475 and 300, according to their approximate size, as judged by their mobility in gels (14). There is also RNA material that migrates more rapidly than band 3′ and consists of low molecular weight RNA. Band 47 is 47 nucleotides from the 3′ OH end of the 16*S* RNA. It appears that band 3′, 26 nucleotides long, is a major product of T1 RNase treatment of 30*S* ribosomes, whereas the yield of band 47 is about 10% of the yield of band 3′. Colicin E3 generates a fragment 50

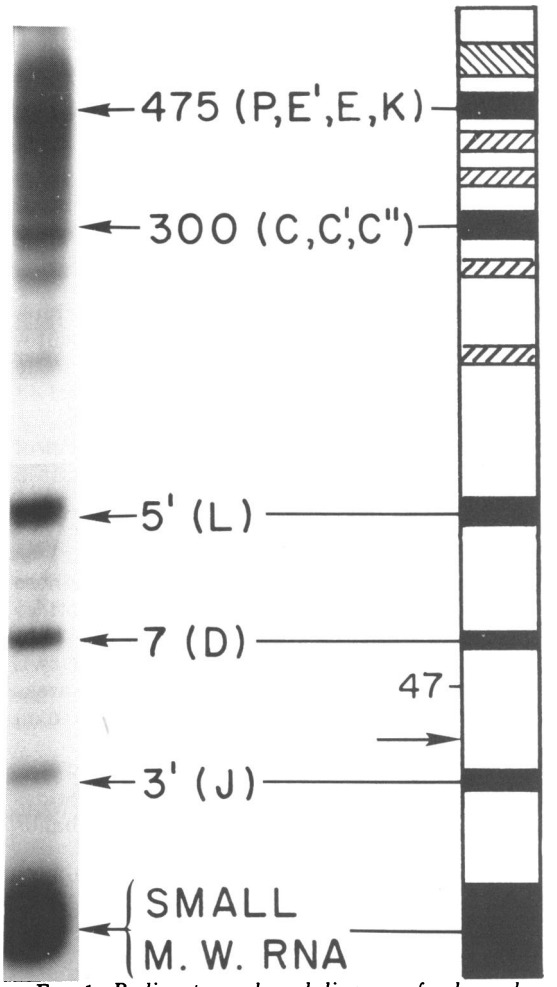

FIG. 1. *Radioautograph and diagram of polyacrylamide gel electrophoretic separation of RNA obtained from T1-RNase-treated ribosomes. The RNA was obtained from ribosomes by methods previously described. All alcohol precipitable RNA was subjected to electrophoresis on the gel. The arrow indicates the position of the brom phenol blue dye marker. The bands labeled 5′, 3′, and 7 (about 120, 26, and 66 nucleotides, respectively) have been previously described (21, 22). Next to these numbers are the letter designations of Ehresmann et al. (4). Band 3, is part of band J. The larger numbers refer to the approximate nucleotide number of that band and next to each number is the Ehresmann et al. (4) designation. The RNA band indicated by diagonal lines above band 475 is material not extensively characterized. The RNA bands indicated by diagonal lines, between bands 475 and 300 and between bands 300 and 5′, are derived mainly from band 475. The number 47 refers to an RNA fragment produced in very low yields which is the final 47 nucleotides of the 3′ OH end of the 16S RNA and is equivalent to band J (4). The picture of the radioautograph has been developed so that the larger bands are not as dark as they are on the original radioautograph, in order to make the larger bands more clearly defined.*

nucleotides long from the 3′ end of the chain (2), whereas the method of Fellner and associates yields band J, also 50 nucleotides long, from the 3′ end of 16S RNA. Analysis of all these fragments has provided the complete sequence of the final 50 nucleotides of the 3′ OH end of the 16S RNA.

A number of other RNA bands appear in the gel; analysis of their oligonucleotide composition, however, indicates that they are derivatives of the bands 475 and 300.

Oligonucleotide composition of various bands: band 475. Figure 2 is the radioautograph of the two-dimensional electrophoretic separation of a complete T1-RNase digest of band 475. Each spot on the "fingerprint" is labeled according to the numbering system of Ehresman et al. (4). Tables 1 and 2 contain a list of the oligonucleotides found in this band and the data used for deriving their sequences. Band 475 contains all the large oligonucleotides of sections E, E′, P, and K, but not all those found in band A. All the large oligonucleotides placed in section A are also found in the small molecular weight RNA fraction (see below). According to Fellner and associates, these areas are adjacent to each other, and are clustered on the 3′ OH half of the 16S RNA. Band 475 is a major product of T1 RNase digestion of 30S ribosomes, but many of the oligonucleotides found in 475 are recovered in RNA bands smaller than 475 (Fig. 1). Each of these bands has been partially characterized (Santer and Santer, unpublished data), and it is quite clear that both bands between 475 and 300 are derivatives of 475. The band that runs slightly ahead of 300, and which is about half the size of band 475, contains almost all the oligonucleotides found in 475 and we conclude that it represents two approximately equal-sized bands, i.e., the products of band 475 split almost in the middle. The production of band 475 and its derivatives appears to vary in different experiments.

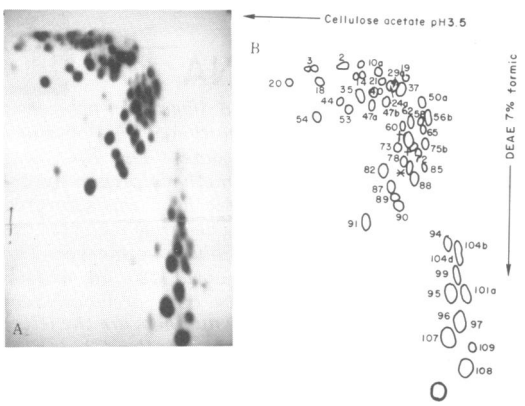

FIG. 2. *Radioautograph (A) of two-dimensional electrophoretic separation of oligonucleotides produced after complete T1 RNase digestion of band 475. Numbers next to each spot are according to Ehresmann et al. (4). See Tables 1 and 2 for characterization of each oligonucleotide. The asterisk in B indicates oligonucleotides 79, 81, 84; + indicates oligonucleotides 70a, 70b; † indicates oligonucleotides 66, 74. Product 55b runs slightly behind 56b. Spot 50b runs slightly behind 55b and is not recovered in pure form. The G residue has not been included in the photograph.*

Analysis of band 300. From its position in the gel, we calculate that this band has about 300 nucleotides. Figure 3 shows the diagram of a radioautograph of this band after T1 RNase digestion and two-dimensional electrophoretic separation of the products. Tables 3 and 4 contain the composition or sequence of each of these products. It is clear that almost all the oligonucleotides found in C, C′, C″ (4) are present in band 300.

Small molecular weight RNA. A rather diffuse band of small molecular weight RNA migrates ahead of band 3′. This material has an average nucleotide length of about 12 (Santer and Santer, unpublished data) and is precipitated by 66% ethanol. It is not known, however, whether during T1 RNase treatment it is completely hydrolyzed away from the 30S ribosomes or remains attached in some way. This material was completely digested with T1 RNase, and the oligonucleotides separated by the ordinary two-dimensional electrophoretic procedure. The list of oligonucleotides found in this fraction is given in Table 5. What is strikingly apparent is that half the unique oligonucleotides in this fraction come from sections A and J (4). All the large oligonucleotides in A are found in this fraction. The only portion of J that is missing is band 3′ (21, and Fig. 1), which is the protected portion of band J in the intact ribosome.

DISCUSSION

One of the aims in working on the sequence of the RNA of ribosomes is to gain a better understanding of the function of ribosomal RNA. Fellner and associates have accomplished the monumental task of sequencing 75% of the 16S RNA of *E. coli* (4, 6). Using their sequences and the data presented here, we can specify which sections of the RNA are protected from T1 ribonuclease and, conversely, which sections are vulnerable to digestion and thus presumably on the "surface" of the ribosome.

Figure 4 illustrates the action of T1 RNase on

TABLE 1. *Oligonucleotides obtained by T1 RNase treatment of band 475 isolated from T1-RNase-treated 30S ribosomes*

No.[a]	Oligonucleotide	Molar ratio	Band[a]	No.[a]	Oligonucleotide	Molar ratio	Band[a]
2	(A-A-C,A-U,U_5,C_3)G	1	K	72	U-C-A-A-G	1	P
3	U-A-A-U-C-U-U-U-G	0.7	8S	73	A-A-U-G		[b]
10a	(A-A-C,U-U-A-C,U,C_2)G	0.5	E	74	U-A-A-C-G	1	N,Q
14	(A-U_2,C_2,U)G	1	P	75b	[A-C,(U-C)C_2]G	1	P
18	U-C-U-U-G	0.8	E'	78	A-C-U-G		K,A,C'
19	(A-C,A-U,U,C_3)A_3-G	0.5	E	79	C-A-U-G		E',A
21	A-U(U-A-A-A-C)G	0.7	P	81	C-U-A-G		E,Q
24a	(C,U-A-C)A-A-U-G	1	P	82	A-U-G	4	
29a	(A-C,U-C)C-A-U-G	1	E	84	U-C-A-G		E',C
35	A-A-U-U-G	1	A	85	U-C_3-G	1	K,E'
37	C-C-C(C_2,U-U-C)A-C-G	1	P	87	U-A-G	3	
40	U-A-A-U-C-G	1	E	88	(C,U-C)G		E
44	A-U-U-G	1	E	89	C-U-G	3	
47a	C-C-U-U-G	1	A	90	U-C-G	3	
47b	U-U-C-C-C-G	1	A	91	U-G	16	
50a	(A-A-C,U,C)A-A-A-G	1	K	94	A-A-A-G	1	
50b	C-A-U-A-C-A-A-A-G	—	P	95	A-A-G	8	
53	U-C-U-G	1	E (others)	96	A-C-G	3	
54	U-U-G	3		97	C-A-G	3	
55b	C(A-C_3,U)G		P	99	C-A-A-G	2	K (others)
56b	U(A-C_3,C_2)G	1	A	101a	C-C-A-G	1	K_2,C''
58	A-A-U(A-C,C)G	1	K	104b	(A-C,C)A-G	1	P,A
60	A-A-A-U-G	1	E',K	104d	(C,A-A-C)G	1	E'
62	A-A-U-A-C-G	1	A	107	A-G	21	
65	(A-A-C,C,U-C)G	1	E	108	C-G	18	
66	U-A-A-A-G	1	[c]	109	C-C-G	2	
70a	A-A-U-C-G	1	E		G	74	
70b	A-U-C-C-A-G	1	E				

[a] Fellner et al. (1970); Ehresmann et al. (1972), number and fragment allocation in the 16*S* RNA molecule. Where no letter appears in band column, it indicates that this oligonucleotide appears in many different sections of the 16*S* RNA. Where no number appears for an oligonucleotide, it indicates that it does not appear in the Ehresmann et al. (1972) catalogue of oligonucleotides in 16*S* RNA.

[b] Indicates that oligonucleotides 66 and 73 (Ehresmann et al., 1972) are not present in sections P,E,K, or A. However, Ehresmann et al. account for only 1 mol of 73 of 4 found in the 16*S*, and 2 mol of 66 out of 2.6 found in the entire 16*S*. Spots 56b and 55b have not been resolved in this separation procedure. In addition, spot 50b runs slightly behind 55b-56b, and contaminates 55b. Consequently, its content of A-C was never accurately determined. Spot 42, A-U-C-U-G, was sometimes present as a contaminant of spot 35, but this has not been a reproducible situation.

certain portions of the 16*S* RNA contained in the 30*S* ribosome. We shall discuss the data included in Fig. 4 section by section; that is, protected and unprotected areas (starting at the 3' OH end).

(i) Band 3' constitutes the last 26 nucleotides of the 3' OH end of the 16*S* RNA, which is inaccessible to enzyme action.

(ii) There is an area susceptible to T1 RNase, that extends from the 5' end of band 3' to the 5' end of section A (150 ± 25 nucleotides from the 3' OH end). All the oligonucleotides found in this area appear in the small molecular weight material. All these oligonucleotides are found in sections A and J (4). Not all the oligonucleotides of section A are invariably sensitive to enzyme treatment. Five oligonucleotides, which are clustered on the 5' side of section A, each of which occurs only once in the 16*S* RNA, sometimes appear as part of a very large RNA fragment, 475 nucleotides long. These oligonucleotides are those numbered 35, 47a, 47b, 56b, and 62 (see Tables 1 and 5). Thus, although it is possible to arrange that part of section A containing these oligonucleotides as a double helical structure (4), within the ribosome the RNA in section A appears to be susceptible to nuclease action.

(iii) From a region in section A until some point on the 5' side of section E (about 475 nucleotides away) there is limited enzyme hydrolysis. When there is no hydrolysis, band 475 is released. Smaller derivatives of band 475 are produced by enzyme hydrolysis of the 3' end of

TABLE 2. *Methods for sequence determination of large oligonucleotides in Table 1*

Oligonucleotide no.	1[a]	2[b]	3[c]	4[d]
2	A-A-C,A-U; U_5,C_5,G	(U-U-U,A-U)C		
3	A-A-U,U_4,C,G	(A-A-U,U)C;U-G		
10a	A-A-C,A-C,U_3,C_2,G	U-U-A-C		
14	A-U_2,C_2,U,G	A-U-C		
18	U_3,C,G	U-U-G		
19	A-C,A-U,U,C_3,A-A-A-G			
21	A-A-A-C,U,G	U-A-A-A-C		
24a	A-A-U,A-C,U,C,G	U-A-C,A-A-U-G	A-A-U-G	
29a	A-C,A-U,U,C_2,G	U-C,A-U-G	C-A-U-G	
35	A-A-U,U,G			
37	C_6,U_2,A-C,G	U-U-C	(C_2,U-U-C)A-C-G	
40	A-A-U,U,C,G			
44	A-U,U,G	U-G		
47a	U_2,C_2,G			U_2,C_2,G
47b	U_2,C_3,G			U_2,C_3,G
50a	A-A-C,U,C,A-A-A-G			
50b	A-U,A-C,C,A-A-A-G			
53	U_2,C,G	U-C,U-G		
55b	A-C_3,U,C,G			
56b	U(A-C_3,C_2)G	U-A-C	(A-C,C_2)G	
58	A-A-U,A-C,C,G		(A-C,C)G	
60	A-A-A-U,G			
62	A-A-U,A-C,G	A-A-U-A-C		
70a	A-A-U,C,G	A-A-U-C		
72	U,C,A-A-G	U-C		
73	A-A-U,G			
75b	A-C,C_3,U,G	U-C		
78	U,G,A-C	U-G		
79	C,A-U,G	A-U-G		
81	U,C,A-G	U-A-G		
84	U,C,A-G	U		
85	U,C_3,G			
88	U,C_2,G	U-C		
96	A-C,G			
97	C,A-G			
99	C,A-A-G			
101a	C_2,A-G			
104d	C,A-A-C,G			

[a] Pancreatic RNase digestion products.

[b] Product or products obtained by pancreatic RNase digestion of oligonucleotide previously reacted with carbodiimide reagent.

[c] Product obtained by spleen phosphodiesterase treatment which contained the Gp residue of the remaining oligonucleotide fragment.

[d] Base composition of original oligonucleotide obtained by complete alkali hydrolysis.

this chain; when this occurs the cleaved material shows up in the small molecular weight fraction. Moreover, another single enzyme break occurs frequently in the middle of band 475, generating two pieces of approximately equal size (Fig. 1).

(iv) There is another T1-RNase-sensitive site that occurs 66 nucleotides away (21); cleavage at this site produced band 7(D).

(v) After the break at the 5′ side of band 7, enzyme hydrolysis is limited for about 300 nucleotides, when another break is made; this generates band 300, which encompasses sections C, C′, and C″ (4).

(vi) At the 5′ end of the 16*S* RNA (not shown in Fig. 4), there is a length of RNA (about 120 nucleotides long) which emerges intact in molar amounts from the T1-RNase-treated ribosomes (21). Between the break generating band 5′ and the break on the 5′ end of band 300, there may be a large RNA molecule (500 nucleotides long), which appears to be near the origin of the gel shown in Fig. 1. This material has not been analyzed completely. Almost none of it, how-

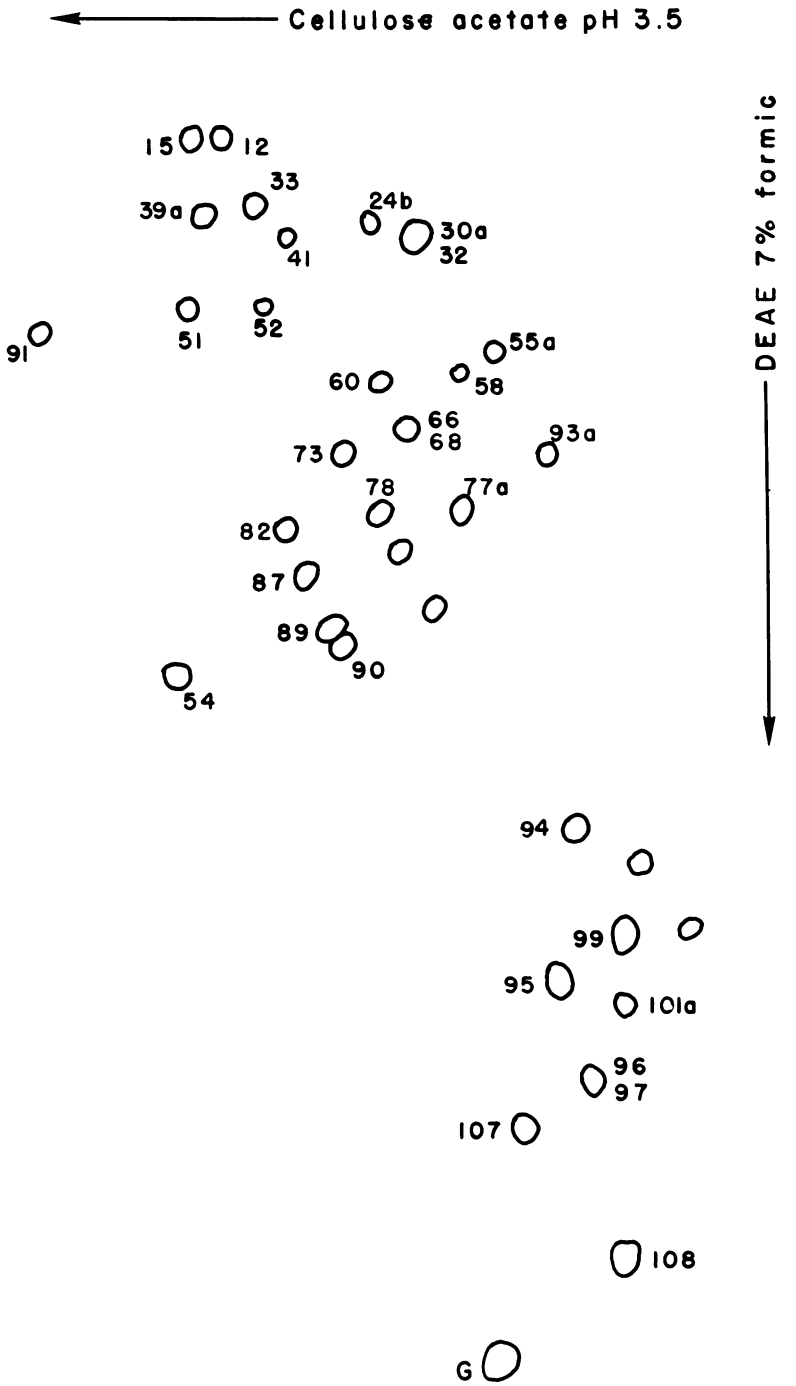

FIG. 3. *Radioautograph tracing of two-dimensional electrophoretic separation of oligonucleotides obtained by complete T1 RNase digestion of band 300. Numbers next to each spot are according to Ehresmann et al. (4).*

TABLE 3. *Oligonucleotides obtained by T1 RNase treatment of band 300 isolated from T1-RNase-treated 30S ribosomes*

No.[a]	Oligonucleotide	Molar ratio	Band[a]
12	A-A-U-U-A-C-U-G	1	C″
15	U-U-A-A-U-C-G	0.8	C″
20	U-U-U-G	0.5	C(L)
24b	[(A-A-U,U)C,C]A-G	1	C′
30a	C-C-U-A-A-C-C-C-U-G	1	C
32	C-U-A-A-C-U-C-C-G	1	C″
39a	U-U-A-A-G	2	C
54	U-U-G	2	
55a	A-A-A-U-C-C-C-C-G	0.7	C
58	(A-A-U-A-C,C)G	1	K
60	A-A-A-U-G	1	E′,K
66	U-A-A-A-G	1	C″ & I
68	(A-A-C,U)G	1	C
73	A-A-U-G	1	[b]
77a or 77b	(U,C_2)A-G	1	C′(L or M)
78	A-C-U-G	1	C′
84 or 81	(U,C)A-G	2	
86 or 88	(U,C_2,C)G	1	
82	A-U-G	2.5 ± 0.5	
87	U-A-G	2.5 ± 0.5	
89	C-U-G	1.5 ± 0.5	
90	U-C-G	1.5 ± 0.5	
91	U-G	10 + 1	
93a	C-C-C-C-C-C-U-G	0.4	C′
93c	(C,A-A-A-C)A-G	0.6	
94	A-A-A-G	1	C′(F)
95	A-A-G	4.0 ± 1	
96	A-C-G	3	
97	C-A-G	1	
99	C-A-A-G	2	
101a	C-C-A-G	1	C″(K)
107	A-G	12 ± 0	
109	C-C-G	1	
108	C-G	11 ± 1	
	G	55 ± 5	
	C-C-C-C-A-G	0.5	

[a] Where no letter appears in band column, it indicates that this oligonucleotide appears in many sections of the 16S RNA. The exception is product 93c, where there is no section allocation. Where no number appears, for an oligonucleotide, it indicates that it does not appear in the Ehresmann et al. (1972) catalogue.

[b] Indicates that oligonucleotide 73 (Ehresmann et al. 1972) is not present in section C. However, only 1 mol of 73, of the 4 found in the 16S, has been allocated by Ehresmann et al.

ever, shows up in the small molecular weight material. If we do not include this material in our total, we have accounted for 1,100 nucleotides in bands 475, 300, 5′, 7, 3′, and the small molecular weight material, or about 70% of the total nucleotides of the 16S RNA.

The above summary shows that the single most exposed length of RNA in the 30S ribosome of *E. coli* is approximately 150 nucleotides long and located near the 3′ OH end of the 16S RNA. The 3′ OH end has several other unique structural and functional features. Within this area are 9 of the 11 methyl groups found in the 16S RNA, including the 4 methyl groups on two adjacent bases found in oligonucleotide 71, seemingly responsible for kasugamycin sensitivity (4, 9). Fifty nucleotides from the 3′ OH end of the chain Colicin E3 makes one hydrolytic break in the RNA which results in the loss of ability of ribosomes to synthesize protein (2).

The proteins associated with the 3′ OH end of the 16S RNA are proteins S13, 9, 19, 10, and 7, which are "late" in the ribosome assembly process (12, 11, 15, and Szekely, Brimacombe, and Morgan, personal communication), except for protein S7, which binds directly to 16S RNA.

It has been further demonstrated (R. Traut, personal communication) that initiation factor 2 (IF2), when present on 30S ribosomes, is physically close to proteins S13, 9, 19, 10, 1, and 14 because IF2 can be chemically cross-linked to all of these proteins.

What is suggested by these facts is that this portion of the ribosome "surface" near the 3′ OH end of the 16S RNA plays an important role in protein synthesis. The presence of a large area of rRNA on the "surface" of the ribosome in this same region may not be fortuitous; it may be involved in binding extraribosomal components. If we are treating ribosomes existing in different "states," with or without the "protective agent," this could explain the fact that the same area of section A is sometimes hydrolyzed to small molecular weight material and sometimes is part of band 475.

Additional evidence for a role for rRNA in protein synthesis is provided by the experiments of Noller and Chaires (13), who have produced totally inactive 30S ribosomal subunits by reacting them with kethoxal, which apparently has added specifically to 6 or 7 guanine residues. Also, Gualerzi and Pon (8) indicate that IF3 may bind directly to rRNA.

There are large areas of ribosomal RNA in 30S ribosomes that are relatively or completely resistant to nuclease treatment. Band 5′, for example, is produced in approximately molar amounts from 30S ribosomes treated with high levels of T1 RNase. Isolated band 5′, on the other hand, is invariably converted to its constituent oligonucleotides at very high dilution of enzyme, in high Mg^{2+} containing buffer, at 0 C. We have consequently found it very difficult to prepare partial digests of band 5′. Ehresmann et al. (4) have experienced similar difficulties. Yet the band in the ribosome is completely protected.

In the case of ribosome proteins, those which enter the ribosome "early" in the assembly process (10, 12) appear to be more protected

TABLE 4. *Methods for sequence determination of large oligonucleotides in Table 3*

Oligonucleotide no.	1[a]	2[b]	3[c]	4[d]
12	A-A-U,U_2,A-C,G	U-G		
15	A-A-U,U_2,C,G	(A-A-U,U_2)C	A-U-C-G	
24b	A-A-U,U,C_2,A-G	(A-A-U,U)C		A_3,U_2,C_2,G
30a[e]	A-A-C,U_2,C_4,G			
32[e]	A-A-C,U_2,C_3,G			
33	A-U,A-C,U,G	A-U-A-C; U-G		
39a	U_2,A-A-G			
41	A-U,C_2,U,G			
52	U_2,C_2,G	U-C		
55a	A-A-A-U,C_4,G		A-U-C-C-C-C-G	
58	A-A-U,A-C,C,G	A-A-U-A-C		
60	A-A-A-U,G			
66	A-A-A-G,U			
68	A-A-C,U,G	U-G		
77a	U,C_2,A-G	U-C		
93a	C_6,U,G	U-G		

[a] Pancreatic RNase digestion products.

[b] Product or products obtained by pancreatic RNase digestion of oligonucleotide previously reacted with carbodimide reagent.

[c] Product obtained by spleen phosphodiesterase treatment which contained the Gp residue of the remaining oligonucleotide fragment.

[d] Base composition of original oligonucleotide obtained by complete alkali hydrolysis.

[e] Oligonucleotides 30a and 32 were never completely resolved in the two-dimensional electrophoretic separation. The molar ratio of this combined oligonucleotide fraction always indicated that there were between 18 and 20 nucleotides present; that is, 2 mol of product per entire fragment.

TABLE 5. *Oligonucleotide composition of T1 RNase digest of small molecular weight RNA obtained from T1-RNase-treated 30S ribosomes*

No.[a]	Oligonucleotide[b]	Letter[a]
3	U-A-A-U-C-U-U-U-G	8S
4	C-U-U-A-C-C-A-C-U-U-U-G	A
6	U-U-U-U-C-A-G	D
8	C-U-U-A-A-C-C-U-U-G	A
13	A-U-A-U-U-C-G	A
20	U-U-U-G	L,C
26	U-m5C-A-C-A-C-C-A-U-G	A
35 or 39b	(A-A-U,U)G	A or H
39a	U-U-A-A-G	C,O
44 or 46	(A-U,U)G	I' and L, or E
47a	(U_2,C_2)G	A
47b	(U_2,C_3)G	A
53 or 51	(U_2,C)G	R,H',G,E,L,C
56b	U(A-C_3,C_2)G	A
57b	mU-A-A-C-A-A-G	J
62	A-A-U-A-C-G	A
66	U-A-A-A-G	I,C'' (475)
68 or 74	(U,A-A-C)G	M,C,O,N
69b	U-C-A-A-C-G	J
72	(U,C)A-A-G	P
76b	(U,C_2,A-C)G	D
77a	(U,C_3)A-G	L,C'
85	(U,C_3)G	K,E'
93b	C-A-A-A-A-G	A
	(U,A-U)A-G	
	(A-A-A-U,U)G	

[a] Fellner et al. (1970), and Ehresmann et al. (1972). Oligonucleotide and section allocation.

[b] Only oligonucleotides appearing once or twice in 16S RNA have been included in this table. Not all the oligonucleotides in this table were completely suquenced. In some cases, a simple pancreatic RNase digest of an oligonucleotide sufficed to establish its identity among the catalogue of oligonucleotides presented by Ehresmann et al. (1972). Examples of this are oligonucleotides 3, 4, 6, 8, 13, 85, and 56b. The sequence of the following oligonucleotides are simply established by their pancreatic RNase digestion products: 20, 39a, 66, and 93b. The sequences of 69b and 57b were previously established (22). Where no oligonucleotide number appears, it indicates that it did not appear in the Ehresmann et al., catalogue of oligonucleotides in 16S RNA. Preliminary evidence indicates that oligonucleotides 101c, m^4C_m-C-m_2C-C-G (section A) and 111, C-C-m^7G-C-G (Section C''), appear in this small molecular weight fraction.

from trypsin digestion (3), for example, than those proteins which are "late" in the assembly process. T1 RNase may act in a similar pattern on 16S RNA. It appears that the 5' side of the molecule initially interacts with proteins S4, 8, 15, and 20, which bind directly to RNA (7, 10, 17-19). These proteins and their related RNA may become inaccessible in the "interior" of the ribosome. For example, very few of the oligonucleotides found in the 5' side of the 16S RNA appear in small molecular weight RNA after T1 RNase treatment of 30S ribosomes (Table 5). It has been demonstrated (19, 24) that primary binding proteins S8 and S15 bind to parts of section C, which can be recovered from T1-RNase-treated ribosomes as part of band 300. Similarly, band 475, which embraces sections E, P, K, and A, may be the area involved in binding protein S7 and its assembly-related proteins (Szekeley, Morgan, and Brimacombe, personal communication; 15). The conclusion that emerges is that areas of RNA directly interacting with ribosomal proteins are the most

TABLE 6. *T1 RNase insensitive regions of 30S ribosomes and known protein binding areas on 16S RNA*

Band no.	Section allocation	Protein bound
475	K, E, P, A	S7 (S9, S13, S19)[a]
300	C, C', C''	S8, S15
"500"[b]	L through B	S4, S20 (S16, S17)

[a] 30S proteins in brackets are those proteins related in the "assembly sequence" to the protein (not bracketed) that binds directly and independently to 16S RNA.

[b] Band "500" has not been characterized. However, few of the oligonucleotides present in the 5' side of the 16S RNA (L through B) appear in small molecular weight material, indicating that most of it is insensitive to T1 RNase hydrolysis.

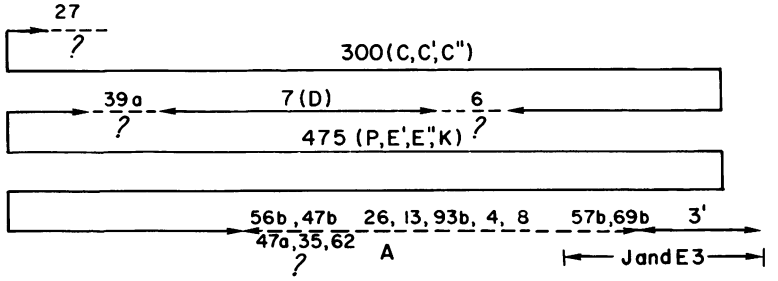

FIG. 4. *T1 RNase sensitive and insensitive sites on 16S RNA in the intact 30S ribosome. Solid lines indicate areas relatively resistant to digestion with T1 RNase and found primarily in large molecular weight material. Dashed lines indicate sensitive areas. Question marks below dashed line indicate that the extent of hydrolysis in these areas is unknown. Numbers next to letters indicate our band designation and those of Ehresmann et al. (4). Numbers above and below dashed lines refer to the oligonucleotides which Ehresmann et al. (4), located in the positions indicated, and which are found in small molecular weight RNA. E3 refers to the RNA fragment produced from 16S RNA by Colicin E3 (2). 3' refers to the protected 3' OH end piece of 16S RNA previously characterized (21, 22). Oligonucleotide number 27 has been inserted as a reference mark only.*

protected areas of the ribosome. Table 6 summarizes this conclusion.

On the other hand, "late" RNA, or the 3′ half of the molecule, may interact directly with only one protein, which influences the binding of several other proteins, leaving a considerable region of RNA free to participate in some way in protein synthesis.

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