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Characterization of the 5′ and 3′ Ends of the 16S Ribonucleic Acid from T_1-Ribonuclease-Treated 30S Ribosomes*

Melvin Santer and Ursula V. Santer

ABSTRACT: Three RNA fragments, obtained from T_1-RNase-treated ^{32}P-labeled 30S ribosomes, were completely digested with T_1-RNase and separated on a two-dimensional electropherogram. One fragment is about 120 nucleotides long and comes from the 5′ end of the 16S RNA; it contains the sequence pAAAUUGp. One fragment about 25 nucleotides long comes from the 3′ end of the 16S RNA and contains an oligonucleotide with the following partial sequence: (AUC,C)-(AC, UC)UUCA$_{OH}$. A third RNA fragment, 65 nucleotides long, has been characterized. Its position in the 16S RNA chain is not known.

T_1-Ribonuclease treatment of 30S ribosomes hydrolyzes rRNA at a limited number of sites producing RNA fragments of a wide size range (Fellner *et al.*, 1970b; Santer and Székely, 1971). These RNA fragments must come from portions of the RNA which are inaccessible to nuclease action, probably because they are complexed with protein molecules and/or are buried in the "interior" of the ribosome. T_1-RNase treatment of ribosomes offers an easy way of obtaining RNA molecules for sequence study.

In this paper, we report on the isolation of three RNA fragments from T_1-RNase-treated 30S ribosomes. Two of the fragments are the 5′ and 3′ ends of the 16S RNA. The third RNA fragment arises from some as yet unknown portion of the 16S RNA. None of these fragments is contained in the large pieces isolated by Fellner *et al.* (1970b) or Ehresmann *et al.* (1970), although they do contain some of the T_1-RNase-generated oligonucleotide fragments which are produced from complete T_1-RNase digests of free 16S RNA (Fellner *et al.*, 1970a).

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 mCi of [^{32}P]P_i (Sanger *et al.*, 1965). ^{32}P-Labeled cells were lysed by freezing and thawing in the presence of lysozyme followed by treatment with sodium deoxycholate, according to Ron *et al.* (1966). 70S ribosomes obtained from cell-free supernatant were dissociated into 50S and 30S units by suspending them in pH 7.8 buffer containing 0.01 M Tris–0.001 M magnesium acetate and 0.1 M NH_4Cl, and shaking in the cold for 24 hr (Atsmon *et al.*, 1969). 50S and 30S ribosomes were separated on 5–30% sucrose gradients, made up in the same buffer, centrifuged in a SW25 rotor at 21,000 rpm for 17 hr at 4°. Peaks were located by counting fractions. The 30S ribosome peak was collected, the Mg^{2+} concentration brought to 0.005 M (the sucrose concentration thus diluted to one-half), and the ribosomes were centrifuged for 20 hr at 100,000*g*.

Enzyme Treatment and Preparation of RNA. The 30S pellet was resuspended in pH 7.8 buffer containing 0.01 M Tris, 0.005 M magnesium acetate, and 0.01 M KCl. These ribosomes were treated with T_1-RNase in a ratio of 1–2 μg of enzyme/ODU for 1 hr at 23°, in a volume of about 0.3 ml. At the end of the incubation period, the RNA was obtained from ribosomes as previously described (Santer and Székely, 1971). The final RNA precipitate was lyophilized to remove the last traces of alcohol which was found to interfere with the layering of the RNA on top of the gel prior to electrophoresis. The [^{32}P]RNA bands were separated by electrophoresis in flat gels according to Adams *et al.* (1969). The gel contained 10% acrylamide and 0.5% bisacrylamide in 0.04 M Tris (pH 8.4) with 7 M urea.

Sequence Determination. The methods used in sequence determination were those devised by Sanger and associates. An RNA band obtained from polyacrylamide gels was completely digested with T_1-RNase and the products were separated by two-dimensional fractionation techniques (Brownlee and Sanger, 1967). Radioautography was used to locate these products, as well as the products of all subsequent treatments.

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The sequence or partial sequence of each oligonucleotide was determined using combinations of the following procedures. (1) Complete pancreatic RNase digest followed by electrophoresis was carried out according to Adams *et al.* (1969). (2) Complete reaction of *N*-cyclohexyl-*N'*-(β-morpholinyl-(4)-ethyl)carbodiimide–methyl *p*-toluenesulfonate with uracil and guanine of the respective nucleotides (carbodiimide reaction) was carried out according to Brownlee *et al.* (1968), except that the addition reaction was carried out at pH 7.5. The addition product was treated with pancreatic RNase and the products were separated by electrophoresis according to Adams *et al.* (1969). The products were individually eluted from paper by shaking with 30% triethylamine carbonate (pH 9.5). After the triethylamine carbonate was removed by freeze-drying, the fragment was treated with 0.2 N ammonia to remove the carbodiimide reagent, and the small oligonucleotide was again treated with pancreatic RNase and electrophoresed according to Adams *et al.* (1969). (3) Partial pancreatic RNase digestion. Buffer (10 μl; 0.01 M Tris–0.001 M EDTA, pH 7.4) containing an enzyme to substrate ratio of $1:2 \times 10^4$ by weight was incubated for 30 min at 37°. The partial digestion products were separated by electrophoresis on DEAE-cellulose paper in 7% formic acid for 4 hr at 1500 V. The products were hydrolyzed with alkali (procedure 5), and the ratios of the mononucleotides determined by liquid scintillation counting. (4) Partial snake venom phosphodiesterase digest after alkaline phosphatase treatment, followed by analysis of digestion products, was carried out according to Brownlee and Sanger (1967). (5) Alkaline hydrolysis was carried out by incubating the oligonucleotide fragment in 0.3 N KOH at 37° for 18 hr. The products were separated by electrophoresis in pH 3.5 acetate buffer on either Whatman No. 52 paper, or DEAE-cellulose paper. (6) Spleen phosphodiesterase treatment was carried out according to a modification of the method of Sanger *et al.* (1965). The enzyme concentration was 0.2 mg/ml in 0.1 M ammonium acetate buffer (pH 7.5) containing 0.002 M EDTA. Products were separated by electrophoresis on DEAE-cellulose paper in 7% formic acid.

Reagents. T_1-RNase (EC 2.7.7.26) was purchased from Calbiochem. Pancreatic RNase (EC 2.7.7.16) was obtained from Worthington Biochemical Corp. Alkaline phosphatase (3.1.3.1), snake venom phosphodiesterase (3.1.4.1), and spleen phosphodiesterase (3.1.4.1) also were purchased from Worthington. Cellulose strips (Cellogel) were obtained from Colab, Glenwood, Ill.

Results

Separation of RNA Fragments. ^{32}P-Labeled 30S ribosomes were purified after dissociation of 70S ribosomes in the presence of 0.1 M NH_4Cl. The 30S ribosomes are treated with T_1-RNase and the RNA fragments separated by electrophoresis in 10% polyacrylamide gels. A typical pattern, as revealed by autoradiography, is shown in Figure 1; this pattern has been reproducible in 12 separate experiments. Figure 1 shows three clearly distinguishable bands, which appear to be the only major ones resolved in a 10% gel. The band believed to contain the 5′ end of the 16S RNA molecule (band 5′) is larger than tRNA; band number 7 is smaller; the band believed to contain the 3′ end (band 3′) is considerably smaller.

Size and Yield of Band 5′. A more accurate estimation of the size of band 5′ has been obtained by comparing the migration of 5S RNA obtained from 50S ribosomes of *E. coli* and band 5′. In gels made with 5% acrylamide–0.25% bis-

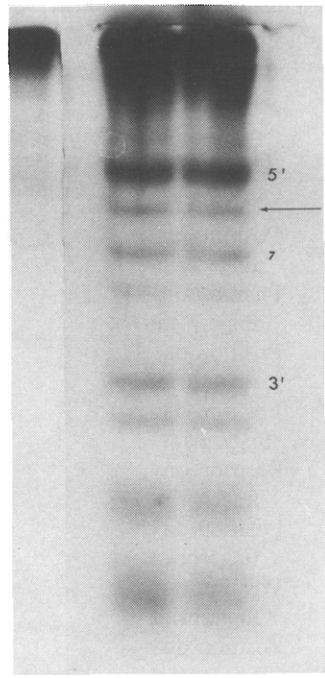

FIGURE 1: Radioautograph of polyacrylamide gel electrophoretic separation of RNA obtained from T_1-RNase-treated 30S ribosomes. The procedures for treatment of ribosomes and for the preparation of RNA are found in the Materials and Methods section. The arrow indicates the position of a ^{32}P *E. coli* tRNA marker. The bands labeled 5′, 3′, and 7 refer to the bands described in this paper. The extreme left-hand column contains the control RNA obtained from ^{32}P 30S ribosomes whose RNA was purified in the presence of T_1-RNase without prior incubation with the enzyme.

acrylamide, band 5′ has almost the same R_F as 5S RNA, which contains 120 nucleotides (Brownlee *et al.*, 1968).

The molar yield of band 5′ has been estimated by the following experiment. RNA from T_1-RNase-treated ^{32}P-labeled 30S ribosomes was separated by electrophoresis in disc gels containing 5% acrylamide–0.166% bisacrylamide. The disc gels were cut into 1-mm slices and each slice was counted by liquid scintillation counting. Duplicate gels were sliced longitudinally and autoradiographs were obtained. Band 5′ was located on the disc gel by comparing its R_F with that established on the flat gel (Adams *et al.*, 1969). The "flat gel" was used to show that all the RNA from T_1-RNase-treated 30S ribosomes enters the 5% acrylamide–0.166% bisacrylamide gel and that bands 5′, 7, and 3′ are clearly resolved. (Much larger fragments of RNA, which appear to be at or near the origin in 10% gels, are also resolved in this gel.) The amount of isotope in band 5′ was 6% of the total label in the disc gel (8400 cpm out of a total of 139,000 cpm). If band 5′ contains about 120 nucleotides and emerges in molar amounts from the 16S RNA, it should comprise about 7% of the approximately 1700 nucleotide-containing 16S RNA (Fellner, 1969). It appears that band 5′ is present in almost molar quantities.

Composition of Band 5′. Figure 2 shows the oligonucleotides of band 5′, after complete T_1-RNase digestion, separated on a two-dimensional electropherogram (fingerprint). Table I presents the various oligonucleotide sequences found in band 5′ and their molar ratios. The purity of each of the larger oligonucleotides is checked by counting all of the products after pancreatic RNase digestion or alkaline hydrolysis. The ratios of products are calculated on the basis of one G or G-containing residue per oligonucleotide. For example, spot a in Figure 2 was found to contain both AACG and CAAG

TABLE I: Oligonucleotides Obtained by T_1-RNase Treatment of Band 5′ Isolated from T_1-RNase-Treated 30S Ribosomes.

Oligonucleotide	Method for Sequence Determination[a]	Products	Molar Ratios	Oligonucleotide	Method for Sequence Determination[a]	Products	Molar Ratios
G	Position on fingerprint		8.7	pAAAUUG (23)	5	2A,2U,G,pAp[d]	1.0
					1	pA_3U,U,G[e]	
AG	Position on fingerprint		5.9		4	A	
					f	*f*	
AAG	Position on fingerprint		4.5	UCUG (53) (f)	2	UC,UG	1.1
				UAUG (46) (g)	1	AU,U,G	2.1
CG	Position on fingerprint		1.9		4	U	
				UAACAG (c)	1	AAC,U,AG	1.0
UG	Position on fingerprint		1.0		2	UAAC,AG	
				(C,UC)AG (77) (b)	1	AG,U,2C	1.0
AUG	Position on fingerprint		2.5		2	UC,C,AG	
CUG	Position on fingerprint		2.0	(UAAC,C,C)-ACAUG (d)	1	AAC,AU,AC,-U,2C,G	1
UCG	Position on fingerprint		1.6		2	UAAC,AUG,-AC,2C	
UUUG (20)[b]	1		2.0		3	ACAUG	
ACG	1	AC,G	2.1	AUCAUG (34) (e)	1	2AU,C,G	0.7
AACG (98) (a)	1	AAC,G	3.8[c]				
CAAG (99) (a)	1	C,AAG			2	AUC,AUG	

[a] Numbers refer to descriptions in Materials and Methods. [b] Number in parentheses next to larger oligonucleotide fragments refers to the oligonucleotide number in the paper by Fellner *et al.* (1970a). The letter refers to the spots shown in Figure 2. Molar ratios were obtained by determining the ^{32}P content of all the spots on the fingerprint and assuming that the 5′-end oligonucleotide, pAAAUUGp, is present once in the band. Spot d is presumed to exist once in the band from visual inspection of an autoradiograph. [c] Spot a is a mixture of 2 moles of AACG and 1 mole of CAAG. The resolution of this spot is described in the text. [d] On the DEAE paper the pAp spot is more tightly bound and does not migrate as rapidly as Up, Gp, or Ap. On Whatman No. 52 paper pAp is contaminated by Up, which travels more rapidly than both Ap and Gp. [e] Liquid scintillation counting of the pancreatic RNase digestion products revealed that there is one free Gp, one Up, and 5 equiv of ^{32}P in the spot which remains at the origin on DEAE-cellulose paper in the solvent system pH 3.5, acetate buffer. Alkaline hydrolysis (5) of the material at the origin yields pAp, 2Ap, and Up after electrophoresis on both Whatman No. 52 paper in pH 3.5 acetate buffer and DEAE-cellulose paper. Alkaline hydrolysis of the original material also yields pAp, 2Ap, Up, and Gp. [f] Oligonucleotides pApApApUpUpGp and ApUpCpApUpGp were both digested with 10 μl of alkaline phosphatase (0.1 mg/ml) for 1 hr at 37°, then electrophoresed on DEAE-cellulose paper in 7% formic acid. $^{32}P_i$ and each ^{32}P-labeled dephosphorylated oligonucleotide were located by autoradiography and their ^{32}P content was determined by liquid scintillation counting. The ratio $^{32}P_i$:^{32}P (dephosphorylated oligonucleotide) for pApApApUpUpGp is 1290:3288 cpm or 0.39. The theroetical value for a hexanucleotide with phosphate at both the 3′ and 5′ ends is 0.4. For the oligonucleotide ApUpCpApUpGp the ratio is 4229:19,802 cpm or 0.21. The theroetical value for a hexanucleotide with phosphate only at the 3′ end is 0.2.

when it yielded AAC, AAG, C, and G after pancreatic RNase treatment. Liquid scintillation counting of these products yielded the following results (counts per minute in thousands): AAC, 10.9; G, 3.0; AAG, 5.3; C, 1.5. This indicates that there are 2 moles of AACG and 1 mole of CAAG in this one spot. This is the only spot which showed this kind of mixture.

Two facts emerge from these analyses. (1) Band 5′ contains the sequence pAAAUUGp, which is the 5′ end of the 16S RNA molecule (Fellner *et al.*, 1970a). (2) The size of band 5′, calculated by adding the number of nucleotides in each component, is 124–133. (The ambiguity is due to variance in the yield of AAG, AUG, and UCG.) This corroborates the above-mentioned size estimate of 120 nucleotides. Moreover, band 5′ contains 19 different oligonucleotides after complete T_1-RNase digestion; the same number of components are obtained from a complete T_1-RNase digest of pure *E. coli* 5S RNA (Brownlee and Sanger, 1967). While there is no reason that two RNA fragments of the same size should have exactly the same number of components on a two-dimensional electropherogram, a mixture of two or more bands in this size range would be expected to have a much larger number of components.

Attempts to obtain partial T_1-RNase digests of band 5′, in the presence of 0.01 M Mg, have not been successful thus far, which indicates that there is little or no secondary structure in this end of the chain.

Composition of Band 3′. Band 3′, after complete T_1-RNase digestion and separation on a two-dimensional electropherogram, yields only five major components. Band C of a pre-

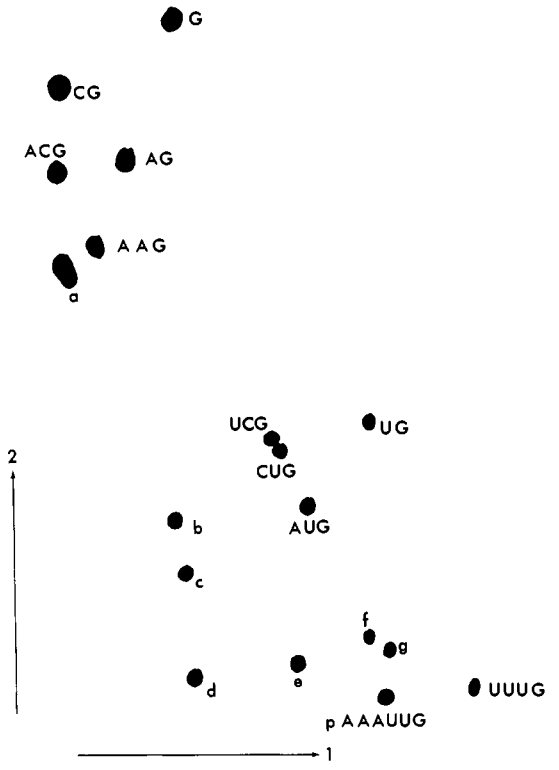

FIGURE 2: Two-dimensional separation of complete T_1-RNase digest of the 5′ end fragment of 16S RNA. The RNA fragment was obtained from polyacrylamide gels according to the methods of Adams *et al.* (1969) and completely digested with T_1-RNase; the oligonucleotides were separated by electrophoresis according to the methods of Brownlee and Sanger (1967). No. 1 indicates the cellulose acetate–pH 3.5 acetate–7 M urea buffer direction; no. 2 is the DEAE-cellulose–7% formic acid direction. Spot a is a mixture of AACG and CAAG; b is (C,UC)AG; c is UAACAG; d is (UAAC,C,C)ACAUG; e is AUCAUG; f is UCUG; g is UAUG.

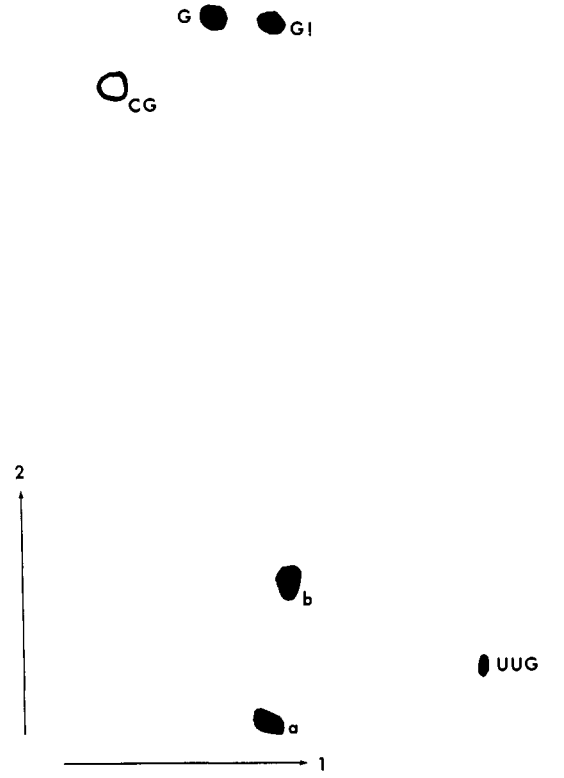

FIGURE 3: Two-dimensional separation of complete T_1-RNase digest of partial T_1-RNase digest of band 3′. Band 3′, obtained from the flat gel-like band 5′, was incubated with T_1-RNase (made in 0.01 M Tris buffer (pH 7.5) with 0.01 M $MgCl_2$), in an enzyme to substrate ratio of $1:5 \times 10^4$, for 10 min at 0° and electrophoresed on cellulose acetate in pH 3.5 acetate buffer with 7 M urea at 5 kV for 1 hr. The cellulose acetate strip was then blotted onto a thin layer of DEAE-cellulose:cellulose (1:7.5 ratio) and chromatographed in homomix A (Brownlee and Sanger, 1969) for about 5 hr at 60°. One major fragment was seen on an autoradiograph, eluted, and completely digested with T_1-RNase, and the oligonucleotides were separated according to Brownlee and Sanger (1967). No. 1 and 2 are as described in Figure 2. Spot b is $m_2^6Am_2^6ACCUG$; spot a is (AUC,C)(AC,UC)-$UUCA_{OH}$. Liquid scintillation counting of the ^{32}P-containing spots gives the following molar ratios on the assumption that spot a is present once and contains 11 ^{32}P phosphates: spot b 0.96; UUG 0.86; total G, 1.1; CG 0.56.

vious paper (Santer and Székely, 1971) contains these five components as well as several others; we believe band C was a mixture of band 3′ and other oligonucleotides, and the current method of preparing 30S ribosomes has enabled us to recover band 3′ in purer form.

To increase the purity of the 3′ end, band 3′ was subjected to a very mild T_1-RNase digestion and then fractionated by electrophoresis on cellulose acetate strips in one direction and by "homochromatography" (Brownlee and Sanger, 1969) in the second direction. Only one major radioactive spot was recovered; this was completely digested with T_1-RNase and separated on a two-dimensional electropherogram. Figure 3 shows that the product of mild T_1-RNase digestion still has five components: G, CG, UUG, and two spots named a and b. C, CG, and UUG are recognizable by their position on a fingerprint; the sequence UUG was verified by liquid scintillation counting of a complete pancreatic digest of the oligonucleotide, which gave a ratio of 2U:1G. The sequence of spot b, $m_2^6Am_2^6ACCUG$, was determined as follows. Complete pancreatic RNase digest yielded C, U, and G plus a "fast AAC" which did not electrophorese in the same position as standard AAC in the pH 3.5 acetate buffer using DEAE-cellulose paper (fast AAC, Bowman *et al.*, 1971). Carbodiimide addition and pancreatic RNase treatment revealed the presence of UG, fast AAC, and C, while partial snake venom phosphodiesterase and spleen phosphodiesterase treatment independently showed adenylic acid residues at the 5′ end. These data are consistent with the sequence of oligonucleotide 71 of Fellner *et al.* (1970a). Spot a, after pancreatic RNase treatment, gave the following products: AU, AC, 3U, 4C, and no G. These data are consistent with the analyses of Bowman *et al.* (1971), and Fellner *et al.* (1970a), which showed that this is the 3′ end of the 16S RNA molecule. We have assumed from their data that A_{OH} is at the 3′ end of the chain, although we have not tested this. Spot a, after carbodiimide addition and pancreatic RNase digestion, yielded the following products: C, AUC, UUC, UC, and AC. Partial venom phosphodiesterase treatment of spot a produced two partial digestion products. Alkaline hydrolysis of these products showed that the larger product lacked only the UUC portion of the original spot a, while the considerably smaller product contained C and AUC. These results indicate that the 5′ end of this oligonucleotide is either AUCC or CAUC, while the 3′ end of this oligonucleotide contains $UUCA_{OH}$. The partial sequence therefore is AUC,C(AC,UC)$UUCA_{OH}$. The mole ratio of each spot in Figure 3 was established by liquid scintillation counting and assuming that the non-G oligonucleotide is present once in the larger fragment. All the spots except CG are present once.

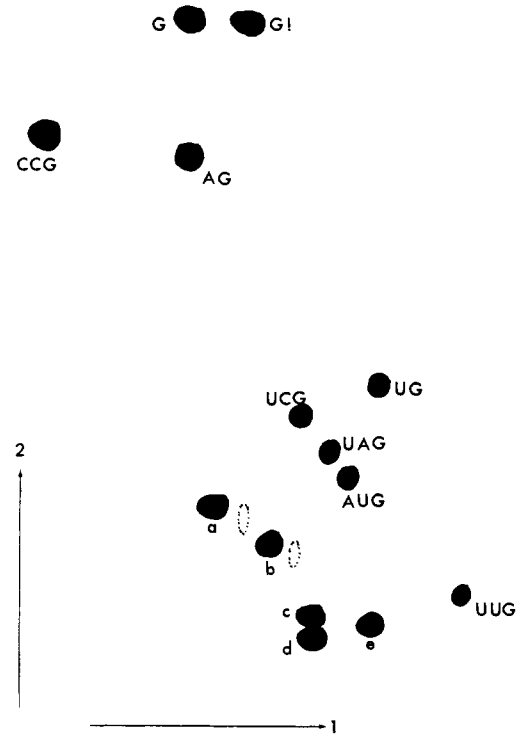

FIGURE 4: Radioautograph of two-dimensional separation of complete T_1-RNase digest of band 7. Band 7 obtained and treated in the same way as band 5′. Spot a is (UC,C)ACG; b is UAAACG; c is (UC,C)UG; d is AUAC(UC,C)G; e is UACUG. Dotted circles indicate cyclic products of spots a and b.

In the complete T_1-RNase digest of band 3′, there are 3 molecules of G and 1 mole of each of the other components. Because our results on the sequence of band 3′ are different from those of other workers (see Discussion), we have isolated a band corresponding to band 3′ from another culture of MRE600 received from Dr. Robert Traut. The complete T_1-RNase digest of this band gives a pattern identical with that seen in Figure 3. Liquid scintillation counting of the spots a, b, UUG, CG, and G gives a nucleotide ratio of 11:6:3:2:3 with a variance of 14%.

One additional RNA band, labeled 7 (Figure 1), appears as a single component in 10% polyacrylamide gels. Figure 4 shows the fingerprint pattern of this band, while Table II presents the evidence for the oligonucleotide sequences and the molar ratios of the various components. There are 65 nucleotides in this band.

Discussion

Under the conditions described in this paper, T_1-RNase cleaves the 16S RNA contained in the 30S ribosome at points approximately 125 nucleotides removed from the 5′ end and about 25 nucleotides from the 3′ end of the molecule. Other bands give rise to band 7 (65 nucleotides) and other fragments considerably larger than band 5′. The RNA fragments formed are protected from further enzyme hydrolysis by the ribosomal structure. When band 5′, for instance, is isolated and digested with very low concentrations of T_1-RNase (enzyme:substrate ratio, 1:20,000), it is completely digested to oligonucleotide fragments, whereas it is untouched in the ribosome at a 1:20 T_1-RNase:substrate ratio.

The conditions of ribosome isolation and treatment seem to affect the susceptibility of the 30S ribosome to T_1-RNase. A difference in the composition of the buffers used in the isolation of the 30S ribosomes from that used previously (Santer and Székely, 1970) results in differences in the RNA

TABLE II: Oligonucleotides Obtained by T_1-RNase Treatment of Band 7 Isolated from T_1-RNase-Treated 30S Ribosomes.

Oligonucleotide	Method for Sequence Determination[a]	Products	Molar Ratios
G	Position on fingerprint		5.5
CCG	Position on fingerprint		1.1
AG	Position on fingerprint		2.2
UG	Position on fingerprint		2.1
UCG	Position on fingerprint		1.0
AUG	Position on fingerprint		0.9
UUG	1		1.8
UAG	1	U,AG	1.8
(UC,C)ACG (76b)[b] (a)	1	AC,2C,U,G	1
	2	UC,AC,C,G	
	4	UC,C at 5′ end	
UAAACG (63) (b)	1	AAAC,U,G	1
	2	UAAAC,G	
(UC,C)UG (c)	1	2U,2C,G	1
	2	UC,C,UG	
UACUG (e)	1	AC,2U,G	1
	2	UAC,UG	
AUAC(UC,C)G (28) (d)	1	AU,AC,U,2C,G	1
	2	AUAC,UC,C	
	6	Lost one A from 5′ end	
	4	AUAG at 5′ end[c]	

[a] Numbers refer to descriptions in Materials and Methods. [b] Numbers next to oligonucleotides refer to those of Fellner *et al.* (1970a); letters refer to the spots shown in Figure 4. The molar ratios are determined as in Table I, assuming that UCG is present once in band 7. Molar ratios of spots a through e were estimated by visual inspection of the autoradiograph. Subsequent work with partial T_1-RNase digests of band 7 have confirmed the quantitative estimate of the large oligonucleotides. [c] After partial venom phosphodiesterase treatment, the digest was separated by electrophoresis on DEAE paper in 7% formic acid. The fastest moving ^{32}P-containing spot was eluted and hydrolyzed with alkali, and the mononucleotides were separated by electrophoresis. Two Ap's and one Up were detected. Since the only known sequence in this molecule which contains two Ap residues is the sequence AUAC, it was concluded that this sequence is at the 5′ end of the molecule.

fragments produced after T_1-RNase treatment. T_1-RNase treatment of 30S ribosomes under another set of conditions (Fellner *et al.*, 1970b) again gives a different pattern of RNA cleavage. The susceptible areas of the 16S RNA therefore seem to differ according to the precise configuration of the 30S ribosome. This might be compared to the effect of different isolation procedures on the activity of 30S ribosomes (Zamir *et al.*, 1969).

The unique oligonucleotide used to identify band 3′ is identical with that previously reported by Bowman *et al.* (1971) and Hayes *et al.* (1971); we have derived a partial sequence, AUC,C(AC,UC)UUCA$_{OH}$. Bowman *et al.* (1971) have characterized the T_1-RNase-generated oligonucleotides from an RNA fragment which was removed *in vivo* from 30S ribosomes by colicin E3. This RNA fragment is 50 nucleotides long and from the 3′ end of the 16S RNA. All of the oligonucleotides of band 3′ are among those found by Bowman *et al.* (1971).

Hayes *et al.* (1971) give the complete sequence of an RNA fragment, 39 nucleotides long, from the 3′ end of the 16S RNA chain, although they have not provided the experimental details for this structure. Their sequence is as follows: GmUAACAAG G UAG GGG $m_2^6Am_2^6$ACCUG CUAACG
(71) (69)
G AU(AC,3U,4C)A$_{OH}$. (The numbers represent the oligo-
(10b)
nucleotide numbering system of Fellner *et al.* (1970); the m represents a methyl group.) This sequence differs in two respects from the data reported here. (1) The sequence UUG is missing from the Hayes *et al.* (1971) sequence although it has been found consistently in our band 3′ and also in several partial digests thereof. (2) Oligonucleotide 69 is not present in band 3′. We cannot explain this result on the basis of the size of our fragment, since band 3′ includes oligonucleotide 71. Although oligonucleotides 71 and 69 are isomers (except for the methylation of the adenylic acid residues), their sequences are readily distinguishable both by the carbodiimide addition reaction followed by pancreatic RNase and by snake venom phosphodiesterase treatment.

Several of the oligonucleotides reported here are not in the oligonucleotide list of Fellner *et al.* (1970a), *i.e.*, the sequence UAACAG in band 5′ and UACUG in band 7. Two oligonucleotides reported here (spot d of band 5′ and spot c of band 7) are isomeric but not identical with oligonucleotides 22 and 47a of Fellner *et al.* (1970a), respectively.

These differences may be due to variations among strains of bacteria; we have, however, used three independently received cultures of *E. coli* MRE600 with identical results.

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