Takai, K., Hashimoto, S., and Muramatsu, M. (1975), *Biochemistry 14,* 536.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244,* 4406.
Westphal, H., and Crouch, R. J. (1975), *Proc. Natl. Acad. Sci, U.S.A. 72,* 3077.
Wu, M., and Davidson, N. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 4506.

Nucleotide Sequences of Accessible Regions of 23S RNA in 50S Ribosomal Subunits†

Winship Herr‡ and Harry F. Noller*

ABSTRACT: Nucleotide sequences around kethoxal-reactive guanine residues of 23S RNA in 50S ribosomal subunits have been determined. By use of the diagonal paper electrophoresis method (Noller, H. F. (1974), *Biochemistry 13,* 4694–4703), 41 ribonuclease T_1 oligonucleotides, originating from about 25 sites, were identified and sequenced. These sites are single stranded and accessible in free 50S subunits, and are thus potential sites for interaction with functional ligands during protein synthesis. Examination of these sequences for potential intermolecular base-pairing reveals the following: (1) There are 19 possible complementary combinations between exposed sequences in 16S and 23S RNA containing more than 4 base pairs: 15 containing 5 base pairs and 4 containing 6 base pairs. Nine of these complementary combinations contain 16S RNA sequences which we have previously shown to be protected from kethoxal by 50S subunits (Chapman, N. M., and Noller, H. F. (1977), *J. Mol. Biol. 109,* 131–149). (2) One of the exposed sites in 23S RNA has a sequence which is complementary to the invariant GTψCR sequence in tRNA.

Elucidation of the functional role of ribosomal RNA in protein synthesis should benefit from a knowledge of the exposed, unpaired nucleotide sequences in ribosomes and ribosomal subunits. Such information is also valuable in the critical assessment of secondary or tertiary structural models. We have used the guanine-specific reagent kethoxal (2-keto-3-ethoxybutyraldehyde) as a probe of the accessibility of sequences in 16S and 5S RNA in 30S, 50S, and 70S ribosomes (Noller, 1974; Noller and Herr, 1974; Chapman and Noller, 1977). Here we report the use of this method to identify accessible 23S RNA sequences in 50S ribosomal subunits. Some of these sequences contain regions of complementarity with accessible sequences in 16S RNA and with the GTψCR sequences of transfer RNA.

Materials and Methods

Buffers are as follows: (buffer A) 0.05 M NH_4Cl, 0.5 mM $MgCl_2$, 0.006 M β-mercaptoethanol, 0.05 M Tris-HCl, pH 7.6; (buffer B) 0.02 M $MgCl_2$, 0.1 M potassium cacodylate, pH 7.0; (buffer C) 0.15 M NaCl, 0.01 M EDTA, 0.015 M sodium citrate, 0.025 M sodium borate, pH 7.0.

Ribonucleases T_1 and U_2 (Sankyo) were obtained from Calbiochem, ribonuclease A and snake venom phosphodiesterase were from Worthington, and bacterial alkaline phosphatase was from Sigma. Silkworm nuclease was a gift from Dr. John Abelson, and ε-carboxymethyllysine-41-ribonuclease A was a gift from Dr. Gary Paddock.

Preparation of ^{32}P-Labeled 50S Subunits. Cells (*Escherichia coli,* strain MRE 600) were labeled with 20 mCi of [^{32}P]orthophosphate in a volume of 50 mL as previously described (Chapman and Noller, 1977). The cells were lysed and the 70S ribosomes pelleted as described by Noller (1974). The 70S ribosome pellet was resuspended in 1 mL of buffer A by gentle shaking for 1 h at 0 °C, layered on a 35-mL 5–20% sucrose gradient in buffer A, and centrifuged for 13.5 h at 20 000 rpm (Beckman L3-40 centrifuge, SW-27 rotor) at 6 °C. Ten-drop fractions were collected and ribosomal subunits peaks were located by measurement of the radioactivity of each fraction. Fractions containing 50S subunits were pooled, made 10 mM in $MgCl_2$, and precipitated by addition of 0.65 volume of ethanol. The mixture was immediately centrifuged at 15 000 rpm (Sorvall RC-2B, SS-34 rotor) at 0 °C for 45 min. The pellet of ^{32}P-labeled 50S subunits was resuspended in 0.5 mL of buffer B, quick-frozen in dry ice–acetone, and stored overnight at −70 °C. The yield was typically $1–2 \times 10^9$ cpm of 50S subunits (specific activity $4–5 \times 10^6$ cpm/μg).

Kethoxal Reaction and Isolation of Modified RNA. Labeled 50S subunits ($1–2 \times 10^9$ cpm in 0.5 mL of buffer B) were incubated for 10 min at 37 °C prior to addition of kethoxal. Fifty microliters of a saturated solution of kethoxal in 20% (v/v) ethanol was then added, and incubation was continued for 1 h at 37 °C. Unreacted kethoxal was removed by precipitation of the ribosomes with 0.65 vol of ethanol. The precipitate was resuspended in 0.5 mL of buffer C and extracted three times with an equal volume of buffer C saturated phenol. RNA was recovered from the final aqueous phase by precipitation six times with 2 vol of ethanol. The final precipitate was dissolved in 50 μL of water and lyophilized.

Nuclease Digestion and Diagonal Electrophoresis. The lyophilized RNA sample was dissolved in 25 μL of a solution containing 1 mg/mL RNase T_1 and 5 mg/mL alkaline phosphatase in 10 mM Tris-HCl, pH 8.0, 20 mM potassium borate, pH 8.0, 0.01 mM $ZnCl_2$, and digested for 1 h at 37 °C. The digest was spotted 10 cm from the end of a 12×110 cm strip

† From Thimann Laboratories, University of California, Santa Cruz, California 95064. *Received July 20, 1977.* Supported by U.S. Public Health Service Grant No. 17129 from the National Institute of General Medical Sciences.
‡ Present address: The Biological Laboratories, Harvard University, Cambridge, Mass. 02138.

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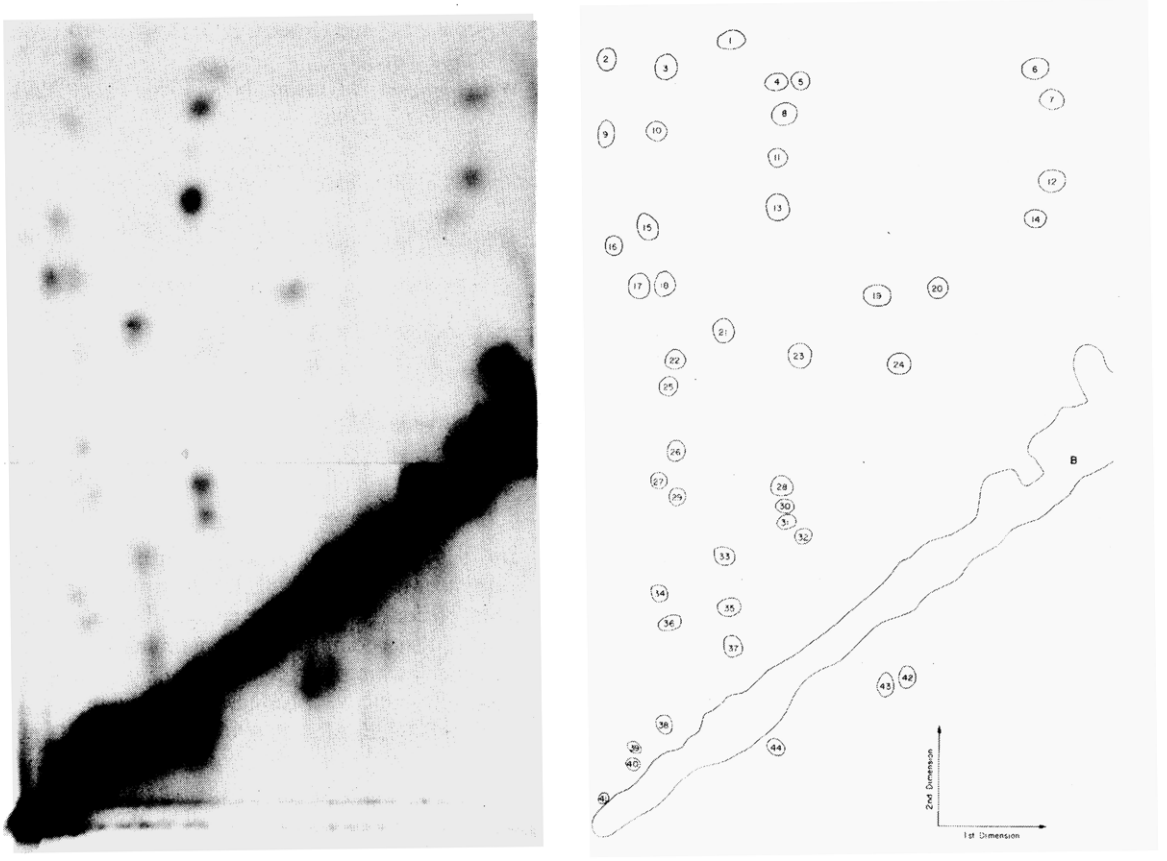

FIGURE 1: Autoradiograph of diagonal electropherogram showing oligonucleotides from sites of reaction of kethoxal with 23S and 5S RNA in 50S ribosomal subunits. ^{32}P-labeled 50S subunits were reacted with kethoxal; the RNA was extracted, digested, and subjected to diagonal electrophoresis as described in Materials and Methods, and by Noller (1974). Autoradiography was for 45 min with Kodak XR-5 film. A tracing of the autoradiograph is shown at the right with the number of each oligonucleotide spot indicated. The position of the blue dye marker (xylene cyanol FF) is indicated by the letter B.

of Whatman DE-81 DEAE paper, and electrophoresed in 7.7% formic acid for 23.5 h at 1400 V. Unblocking and redigestion were carried out as described by Noller (1974). A 4 × 46 cm strip containing the partially fractionated oligonucleotides was sewn onto a 46 × 85 cm sheet of DEAE paper and electrophoresed for 14 h at 1000 V in 7.7% formic acid. Oligonucleotides were located by autoradiography.

Repurification and Sequencing of Oligonucleotides. Oligonucleotide spots were cut out and the radioactivity was determined by Čerenkov counting in a Beckman LS-230 scintillation counter. They were then eluted with triethylamine carbonate (Barrell, 1971) and repurified. Oligonucleotides having a mobility greater than that of the blue tracking dye (xylene cyanol FF) were repurified by electrophoresis on DEAE paper in pyridine–acetate (pH 3.5). Those having mobilities slower than the blue dyes were repurified by homochromatography on 20 × 40 cm DEAE-cellulose thin-layer plates (Analtech, Inc., Newark, Del.) using homomix c (Barrell, 1971).

Digestion and fractionation of oligonucleotides using pancreatic RNase A, RNase T_2, and snake venom phosphodiesterase were performed as described by Barrell (1971). Digestion with U_2 ribonuclease, mixed digestion with silkworm ribonuclease and alkaline phosphatase, and fractionation of the resulting products were done as previously described (Noller, 1974). Overdigestion with RNase T_1 was carried out in 10 μL of a solution containing 10 mg/mL of T_1 ribonuclease, 0.01 M Tris-HCl, 0.001 M EDTA (pH 7.8), for 16 h at 37 °C in a sealed capillary. Products were fractionated by electrophoresis on DEAE paper (46 × 110 cm) in 7.7% formic acid for 16 h at 1100 V. Digestion with ϵ-carboxymethyllysine-41-pancreatic RNase A was carried out at a ratio of enzyme: RNA of 1:25 (Contreras and Fiers, 1971), in a solution containing 1.26 mg/mL enzyme, 0.001 M EDTA, 0.01 M NaOAc, pH 4.0, for 30 min at 37 °C. The digest was fractionated by electrophoresis on DEAE paper (110 cm long) at pH 1.9 for 8 h at 2000 V. All oligonucleotide products from secondary digestions were subjected to complete digestion to mononucleotides with RNase T_2 and with snake venom phosphodiesterase, to determine nucleotide compositions and 5′- and 3′-terminal nucleotides.

Results

Reaction of kethoxal with ^{32}P-labeled 50S ribosomal subunits followed by diagonal electrophoresis of the modified RNA gives rise to the pattern shown in Figure 1. Spots lying off the diagonal arise from nucleotide sequences containing guanine residues modified by kethoxal (Noller, 1974). After repurification, 45 oligonucleotides were judged to be present in significantly high amounts (0.1 mol or greater, by comparison with spot 3) to warrant nucleotide sequence analysis. These oligonucleotides are numbered according to the scheme in the drawing in Figure 1.

Determination of the sequences of the oligonucleotides is summarized in Table I. In nearly all cases, the deduced sequences were unambiguous. Immediately obvious is the pres-

TABLE I: Sequence Determination of Ribonuclease T_1 Oligonucleotides.[a]

Oligonucleotide no.	T_2	Venom	Panc	Other	Sequence
1	U	pG	U		U-G_{OH}
2	G	G	G		Gp
3	G	G	G		Gp
4	G	G	G		Gp
5	U, C	pC, pG	$U_{1.0}$, $C_{0.8}$		U-C-G_{OH}
6	G	G	G		Gp
7	A, U	pA, pG	$U_{1.1}$, A-$G_{OH1.0}$		U-A-G_{OH}
8	A, U	pA, pG	$U_{1.2}$, A-$G_{OH1.0}$		U-A-G_{OH}
9	C, G		C, G		C-Gp
10	U, A, C	pA, pC, pG	$U_{1.1}$, A-$C_{1.0}$		U-A-C-G_{OH}
11	A, U	pU, pG	A-U		A-U-G_{OH}
12	$C_{2.6}$, $G_{1.0}$	pC, pGp	$C_{2.5}$, $G_{1.0}$		C-C-Gp
13	$C_{2.1}$, $A_{0.9}$, $U_{1.1}$	$pC_{1.7}$, $pU_{1.4}$, $pG_{0.8}$	$C_{2.1}$ A-$U_{1.0}$	U_2:A, (U,C)G_{OH}	A-U-C-C-G_{OH}
14	A, U	pA, pU, pG	A-$U_{1.0}$, A-$G_{OH0.9}$	U_2:A, U-A	A-U-A-G_{OH}
15	A, C	pA, pC, pG	$C_{0.9}$, A-A-$C_{1.0}$	U_2:A, C-C-G_{OH}	A-A-C-C-G_{OH}
16	A	pA, pG	A-A-A-G_{OH}		A-A-A-G_{OH}
17	A, U		A-$G_{OH0.8}$, A-A-$U_{1.0}$	U_2:$A_{2.0}$, U-$A_{0.7}$	A-A-U-A-G_{OH}
18-1	A, G	pA, pGp	A-A-Gp		A-A-Gp
18-2	A, C	pA, pG	$C_{1.0}$, A-$G_{OH0.8}$		C-A-G_{OH}
19	C	pC, pG	C		C-C-G_{OH}
20	C	pC, pG	C		C-C-G_{OH}
21	A, C, U	pA, pC, pU, pG	$C_{1.2}$, A-$G_{OH0.8}$,	U_2:A, U-C-A	A-A-U-C-A-G_{OH}
22	A, C, U		$C_{0.9}$, A-$C_{1.0}$, A-$G_{OH0.7}$, A-$U_{1.1}$	U_2:A, C-A, U-C-A,	A(C-A,U)C-A-G_{OH}
23	A	pG	A-G_{OH}		A-G_{OH}
24	C	pG	C		C-G_{OH}
25	A, G		A-A-A-Gp		A-A-A-Gp
26	C, U	pC, pU, pG	$U_{2.2}$, $C_{1.0}$	ODT_1:C-G_{OH} SWP: U-U-C	U-U-C-G_{OH}
27	C, U	pC, pU, pG	U, C	ODT_1:C-C-G_{OH}	U-U-C-C-G_{OH}
28	U, G	pGp	$U_{1.2}$, $G_{1.0}$		U-Gp
29	C, Um-G	pUm, pGp	$C_{0.8}$, Um-$G_{1.0}$		C-Um-Gp
30	A, U	pA, pU, pG	U, A-G_{OH}		U-U-A-G_{OH}
31	C, U	pC, pU, pGp	$U_{1.0}$, $C_{0.9}$	ODT_1:U-G_{OH}, C-U-U, C-C-U, C(C,U)U	C-C-U-U-G_{OH}
32	C*, U, G		C*-U, G		C*-U-Gp
33	C, U, G	pC, pU, pGp	$U_{1.0}$, $C_{2.1}$, $G_{1.3}$	ODT_1:C-U, C-C-U, C-U-Gp	C-C-U-Gp
34	A, C, U	pA, pC, pU, pG	$C_{3.3}$, $U_{1.7}$, A-A-$G_{OH1.0}$	U_2:A, (C_3,U_2)A	C(C_2,U_2)A-A-G_{OH}
35	A, U	pA, pU, pG	$U_{0.8}$, A-$G_{OH0.9}$, A-A-$U_{1.3}$	U_2:U-A, U-A-A, U-A-G_{OH}	U-A-A-U-A-G_{OH}
36	A, C, U	pA, pC, pU, pG	$U_{1.0}$, $C_{2.0}$, A-$U_{1.1}$, A-A-$G_{OH0.9}$	U_2:C-A, A-G_{OH}, U(C,U)A, U(C,U)A-A CMP:C, A-A-G_{OH}, A(U,C)U	C-A-U-C-U-A-A-G_{OH}
37	A, C, U, G	pA, pC, pU, pGp	$U_{1.0}$, $G_{0.9}$, A-$C_{2.2}$	U_2:$A_{1.0}$, C-A$_{1.0}$, C-U-$Gp_{1.2}$	A-C-A-C-U-Gp
38	A, C, U	pA, pC, pU, pG	$C_{2.0}$, $U_{1.9}$, A-$U_{1.1}$	U_2:U-A, U(C_2,U)G_{OH} ODT_1:C(U,C)G_{OH}, U(C_2,U)G_{OH}, A(C_2,U_2)G_{OH} SWP:U-A, U-A-U, N-U-G_{OH},[b] U(A,U)C, U(C_2,U)G_{OH}	U-A-U-C-C-U-G_{OH}
39	A, C, U, G	pA, pC, pU, pGp	C, U, G, A-A-C	U_2:A, C-C-Gp, U-U-A-A ODT_1:C-C-Gp, U(A,U)A SWP:U-U-A, A-C-C-Gp	U-U-A-A-C-C-Gp
40			C, U, G!, A-A-C		U-U-A-A-C-C-Gp!
41	C, U, G	pC, pUpGp	U, C, G	ODT_1:C-Gp, U-U-U	U-U-U-C-Gp
42	A, C, U, G	pA, pC, pU, pGp	$C_{1.2}$, $U_{1.0}$, $G_{0.9}$, A-A-A-C	U_2:A, C-A, A-A, C-A-A, C-U-Gp	C-A-A-A-C-U-Gp
43	A, C, U, G	pA, pC, pU, pGp	$C_{3.0}$, $G_{1.1}$, A-$C_{0.9}$, A-$U_{0.9}$	U_2:A, U-Gp, C-C-C-C-A	A-C-C-C-C-A-U-Gp
44	A, C, U, G	pA, pC, pGp	$C_{1.0}$, $U_{1.8}$, A-$Gp_{1.2}$	ODT_1:A-Gp, C-U-U, C-U-U-A	C-U-U-A-Gp

[a] The following abbreviations are used: T_2, total enzymatic hydrolysis with ribonuclease T_2, A, and T_1; venom, complete hydrolysis by snake venom phosphodiesterase; panc, digestion with pancreatic ribonuclease A; U_2, digestion by U_2 ribonuclease; SWP, combined digestion with silkworm nuclease and alkaline phosphatase; ODT_1, overdigestion by ribonuclease T_1; CMP, digestion by ϵ-carboxymethyllysine-41-pancreatic RNase. Conditions are as described in Materials and Methods. Oligonucleotide numbers are as shown in Figure 1. Upon repurification, spot 18 yielded two oligonucleotides, which were then called 18-1 and 18-2. Nucleotides are the 5′-OH, 3′-monophosphates, unless otherwise designated. The presence or absence of a 3′-phosphoryl group on 3′-terminal guanosines is specifically indicated. [b] The 5′-nucleotide residue of the 3′-terminal silkworm nuclease fragment of oligonucleotide 38 was not directly identified and is designated by N.

ence of the oligonucleotides surrounding the reactive sites G_{13} and G_{41} of 5S RNA (Noller and Herr, 1974), spots 7, 12, 19, and 43. In experiments where 23S was purified free of 5S RNA prior to diagonal electrophoresis, these spots were missing from the pattern (data not shown).

Some of the sequences require comment. Digestion of oli-

TABLE II: Oligonucleotide Sequences.

Oligonucleotide no.	Deduced sequence	Rel[a] molar yield	Probable location[b] Branlan et al. (1977) 13 S	Branlan et al. (1977) 18 S	Spierer et al. (1975) 13 S	Spierer et al. (1975) 18 S	Other
1	U-G_{OH}	0.3					
2	Gp	0.3					
3	Gp	1.0	F8				
4	Gp	0.4					
5	U-C-G_{OH}	0.2					
6	Gp	0.3		F-L1, G_{29}[c]			
7	U-A-G_{OH}	0.5					5S RNA, G_{13}[d]
8	U-A-G_{OH}	0.9		F-L1, G_{24}			
9	C-Gp	0.1					
10	U-A-C-G_{OH}	0.1					
11	A-U-G_{OH}	0.1					
12	C-C-Gp	0.4					5S RNA, G_{13}
13	A-U-C-C-G_{OH}	0.7					
14	A-U-A-G_{OH}	0.2		F-L1, G_{29}			
15	A-A-C-C-G_{OH}	0.2	F6(2X)	F3(2-3X)			
16	A-A-A-G-$_{OH}$	0.1					
17	A-A-U-A-G_{OH}	0.5	+				
18-1	A-A-Gp	0.4					
18-2	C-A-G_{OH}						
19	C-C-G_{OH}	0.6					5S RNA, G_{41}
20	C-C-G_{OH}	0.2					
21	A-A-U-C-A-G_{OH}	0.3	F7a				
22	A(C-A,U)C-A-G_{OH}	0.1					
23	A-G_{OH}	0.5					
24	C-G_{OH}	0.6					
25	A-A-A-Gp	0.1					
26	U-U-C-G_{OH}	0.2		55			
27	U-U-C-C-G_{OH}	0.1		52			
28	U-Gp	1.0		F-L1, G_{24}			
29[e]	C-Um-Gp	0.2					Methylated spot 2
30	U-U-A-G_{OH}	0.2	39 (F7a)	49			
31	C-C-U-U-G_{OH}	0.2		50 (F-L1)			
32	C*-U-Gp	0.1					
33	C-C-U-Gp	0.3	F7a				
34	C(C_2,U_2)A-A-G_{OH}	0.1		39	37		
35	U-A-A-U-A-G_{OH}	0.2	30		29		
36	C-A-U-C-U-A-A-G_{OH}	0.2		32 (F3)		32	
37	A-C-A-C-U-Gp	0.2					
38	U-A-U-C-C-U-G_{OH}	1.0	16 (F8)		17		
39	U-U-A-A-C-C-Gp	0.6	32b		31		
40	U-U-A-A-C-C-Gp!						
41	U-U-U-C-Gp	0.2	17 (F6)		21		
42	C-A-A-A-C-U-Gp	0.3					
43	A-C-C-C-C-A-U-Gp	0.3					5S RNA, G_{41}
44	C-U-U-A-Gp	0.7	37a	46	34		
45	A-C-A-C-A-C-U-A-U-C-A-U-U-A-A-C-U-G_{OH}		2 (F6)		2		

[a] Molar yields are calculated relative to oligonucleotide 3 = 1.0. [b] Locations of oligonucleotides in 23S RNA refer to the nomenclature scheme of Branlant et al. (1977) and Spierer et al. (1975). The 13S and 18S RNA fragments are as described by Allet and Spahr (1971). [c] Nucleotide positions in the ribosomal protein L1 binding site fragment refer to Branlant et al. (1976). The site of kethoxal reaction is indicated. [d] Identification of 5S RNA kethoxal sites is based on Noller and Herr (1974). [e] Identified as a methylated sequence by Fellner and Sanger (1968).

gonucleotide 29 with RNase A or with RNase T_2 gave rise to C and a resistant dinucleotide having a mobility (DEAE paper, pH 3.5) slightly slower than UGp (Woese et al., 1976). Hydrolysis with snake venom phosphodiesterase gave rise to pU, establishing a CUGp sequence in which the U-G phosphodiester linkage is unusually resistant to cleavage. Analysis of the products of venom phosphodiesterase digestion by two-dimensional thin-layer chromatography (Nishimura et al., 1967) revealed a spot migrating significantly faster than pU in both dimensions. The presence of a posttranscriptional modification is reflected also in the high mobility of spot 20 in the diagonal system (Figure 1). The properties of this oligonucleotide are consistent with the presence of a 2′-*O*-methyl(U) in the sequence. The final sequence, CU_mGp, is identical with a sequence previously identified in 23S RNA by Fellner and Sanger (1968).

Oligonucleotide 32 yielded U, G, and a mononucleotide with a mobility marginally slower than unmodified C (Whatman 3MM paper, pH 3.5), on digestion with RNase T_2. Pancreatic RNase digestion gave rise to G and a resistant C*-U. These results suggest the presence of a C that has been modified in the pyrimidine ring. Fellner and Sanger (1968) did not identify

TABLE III: Location of Kethoxal-Reactive Guanine Residues.[a]

Oligonucleotide no.	Sequence and location of reactive guanines	Location in 23S RNA	Rel molar yields
3/38	K | G-G-U-A-U-C-C-U-G	F8	1.0, 1.0
6/14	K | G-G-A-U-A-G	F-L1, G_{29}	0.3, 0.2
12/7	K | G-C-C-G-U-A-G	5S RNA, G_{13}	0.4, 0.5
13	K | G-A-U-C-C-G		0.7
15	K | G-A-A-C-C-G	13S, 18S	0.2
22	K | G-A(C-A,U)C-A-G		0.1
27	K | G-U-U-C-C-G	18S	0.1
28/8	K | G-U-G-U-A-G	F-L1, G_{24}	1.0, 0.9
31	K | G-C-C-U-U-G	F-L1	0.2
32/5	K | G-C*-U-G-U-C-G		0.1, 0.2
33/21	K | G-C-C-U-G-A-A-U-C-A-G	F7a	0.3, 0.3
34	K | G-C(C_2,U_2)A-A-G	18S	0.1
35	K | G-U-A-A-U-A-G	13S	0.2
36	K | G-C-A-U-C-U-A-A-G	F3	0.2
37/1	K | G-A-C-A-C-U-G-U-G		0.2, 0.3
39/17	K | G-U-U-A-A-C-C-G-A-A-U-A-G	13S	0.6, 0.5
41/45[b]	K | G-U-U-U-C-G-A-C-A-C-A-C-U-A-U-C-A-U-U-A-A-C-U-G	F6	0.2
42/24	K | G-C-A-A-A-C-U-G-C-G		0.3, 0.6
43/19	K | G-A-C-C-C-C-A-U-G-C-C-G	5S RNA, G_{41}	0.3, 0.6
44	K | G-C-U-U-A-G	13S, 18S	0.7

[a] Location in 23S RNA is from references given in Table II. Relative molar yields are from Table II. [b] Sequence 41/45 is derived from Table I and from Herr and Noller (1975).

a methylated oligonucleotide with the sequence C*UG, suggesting that the modification may be other than methylation.

We previously reported the nucleotide sequence around a

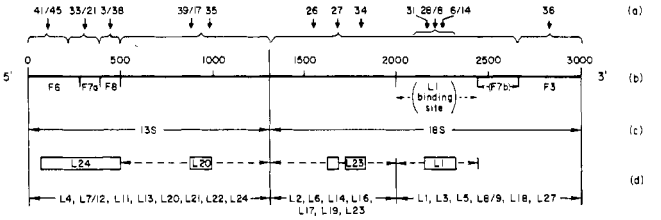

FIGURE 2: A preliminary map of the location of kethoxal-reactive sequences of 23S RNA in 50S subunits, based on the presently available partial sequence information (Branlant et al., 1975, 1976, 1977; Spierer et al., 1975). (a) Approximate position of the oligonucleotide sequences listed in Tables II and III. (b) The 23S partial digestion fragments as designated by Branlant et al. (1975). (c) RNA fragments produced by partial digestion as described by Allet and Spahr (1971). (d) Approximate location of 50S ribosomal protein binding and assembly sites (cf. Spierer and Zimmerman, 1976; Chen-Schmeisser and Garrett, 1976).

kethoxal-reactive G residue in 23S RNA (Herr and Noller, 1975). One of the oligonucleotides from this sequence appears as spot 41 in Figure 1, and the other, oligonucleotide 45 (Table II), has too slow a mobility in the diagonal electrophoresis system to be detected. We have discovered a discrepancy in the nucleotide sequence of spot 41 compared with our previously reported sequence, and have established it to be UUUCGp, rather than UUCUGp (Herr and Noller, 1975), and in agreement with the sequence reported by Branlant et al. (1975).

The sequence of oligonucleotide 38 was found to be UAUCCUG. Branlant and Ebel (1977) do not report this sequence in their catalog of 23S RNA T_1 oligonucleotides, but list the closely related sequence UAUCCCUG. Spierer et al. (1975) and D. Stahl and C. Woese (personal communication) have found the sequence UAUCCUG, but not UAUCCCUG, to be present in RNase T_1 digests of 23S RNA, in agreement with our findings.

The sequences of the oligonucleotides are listed in Table II, along with their relative molar yields and, where possible, their approximate location in 23S (or 5S) RNA, based on the presently available information on the distribution of oligonucleotide sequences within the 23S RNA chain (Branlant et al., 1975, 1976, 1977; Spierer et al., 1975).

Each kethoxal-modified guanine gives rise to a pair of off-diagonal spots, one of which has a phosphorylated 3′ terminus and the other a dephosphorylated terminus. Phosphorylated 3′-terminal guanosine residues are the sites of kethoxal substitution, and oligonucelotides containing dephosphorylated termini contain the sequences immediately to the 3′ side of kethoxal-reactive sites (Noller, 1974). Thus, among the sequences in Table II are both 3′-phosphoryl and 3′-hydroxyl termini. Exceptions to the pairing rule are (1) when adjacent oligonucleotides both contain kethoxal-substituted guanines, in which case additional phosphorylated oligonucleotides appear off the diagonal, (2) when the 3′ oligonucleotide is G, in which case the dephosphorylated product G_{OH} is not detected by autoradiography, and (3) when the alteration of mobility after redigestion with T_1 ribonuclease is such that one of the spots (usually the 3′-phosphorylated one) has a mobility that coincides with the diagonal. Identification of oligonucleotide pairs extends the sequence around each modified G residue, giving sequence overlaps that are often difficult to establish by conventional methods, and in some cases provides the additional sequence information needed to unambiguously locate a reactive site in the complete RNA sequence.

Oligonucleotide pair assignments are listed in Table III, along with sites of kethoxal substitution of sequences five or

TABLE IV: Potential Complementarity between Exposed Single-Stranded Sites in 16S and 23S RNA.[a]

Oligonucleotide no.		Sequence	No. of base pairs	16S sequence protected in 70S[b]
23S 10	(5′)	K GUACG	5	nd
16S 36-3/31a	(3′)	GCCACACAUGUUCCG K K		
23S 27	(5′)	K GUUCCG	5	+
16S 3/13	(3′)	GCUAAGG K		
23S 36	(5′)	K GCAUCUAAG	6	++
16S 37/27	(3′)	GUCCCAUAGAUUAG K		
23S 38	(5′)	K GUAUCCUG	5	+
16S 1a/32	(3′)	GUCAAAUAGG K		
23S 39	(5′)	K GUUAACCG	5	nd
16S 36a	(3′)	GAAUUG K		
23S 41	(5′)	K GUUUCG	5	+
16S 3/13	(3′)	GCUAAGG K		
23S 41	(5′)	K GUUUCG	6	+
16S (14a-2)(19/15)	(3′)	GUAAAGUG K		
23S 44	(5′)	K GCUUAG	6	++
16S 36a/6	(3′)	GCCCUGAAUUG K		
23S 45	(5′)	K GACACACUAUCAUUAACUG	5	nd
16S 23b/8a	(3′)	GCACCUGAUG K		
23S 45	(5′)	K GACACACUAUCAUUAACUG	5	++
16S 36a/6	(3′)	GCCCUGAAUUG K		

TABLE IV (*Continued*)

Oligonucleotide no.		Sequence	No. of base pairs	16S sequence protected in 70S[b]
23S 3/38	(5′)	K GGUAUCCUG	6	++
16S 37/27	(3′)	GUCCCAUAGAUUAG K		
23S 28/8	(5′)	K GUGUAG	5	nd
16S 31a	(3′)	GCCACACAUG K K		
23S 32/5	(5′)	K GC*UGUCG	5	nd
16S 23b/8a	(3′)	GCACCUG AUG K		
23S 32/5	(5′)	K GC*UGUCG	5	—
16S 23c/6e	(3′)	GUACG AUG K		
23S 32/5	(5′)	K GC*UGUCG	5	++
16S 33/6c	(3′)	GUAGCAAAUG K		
23S 37/1	(5′)	K GACACUGUG	5	—
16S 21a/6a	(3′)	GAUGCUG K		
23S 37/1	(5′)	K GACACUGUG	5	nd
16S 23b/8a	(3′)	GCACCUGAUG K		
23S 42/24	(5′)	K GCAAACUGCG	5	—
16S 21a/6a	(3′)	GAUGCUG K		
23S 42/24	(5′)	K GCAAACUGCG	5	nd
16S 23b/8a	(3′)	GCACCUGAUG K		

[a] Nucleotide sequences and nomenclature for 23S RNA are from Tables II and III, and those for 16S RNA are from Noller (1974) and Chapman and Noller (1977). [b] Protection of 16S sites from reaction with kethoxal by binding of 50S subunits, as reported by Chapman and Noller (1977). ++, strongly protected; +, moderately protected; –, not protected; nd, not determined.

TABLE V: Potential Base-Pairing between 23S RNA Site 33/21 and the GTψCR Sequence in tRNA

23S RNA	33/21	(5′)	GCCUGAAUCAG
			· | | | ·
tRNA		(3′)	GCψTG
23S RNA	33/21	(5′)	GCCUGAAUCAG
			| | | | ·
tRNA		(3′)	ACψTG

more long. Pair assignments must satisfy several criteria: (1) They must be aligned vertically with respect to the diagonal. (2) With the exceptions of the cases mentioned above, one oligonucleotide must contain a 3′-terminal phosphate, and the other a 3′-hydroxyl. (3) They must be present in similar molar amounts. (4) The distance of migration of the spots off the diagonal must be consistent with the alteration in the nucleotide composition after redigestion with T_1. The *M* values used by Sanger and his co-workers are useful in this regard (Barrell, 1971). (5) It is very helpful to have information that independently assigns the two oligonucleotides to the same region of the RNA chain, especially when more than one pairing arrangement satisfies the other criteria.

Discussion

A preliminary map of the position of kethoxal-reactive guanine residues in 23S RNA is given in Figure 2. Many of the sequences in Table II have not yet been identified in fragments of 23S RNA, and some of the sequences that have been identified may exist elsewhere in the 23S RNA chain. Thus, a complete assignment of the positions of kethoxal sites depends on a complete 23S RNA sequence. The sites which have been located appear to be distributed throughout the 23S chain, in contrast to 16S RNA, where most of the modified sites are found in the 3′ half (Noller, 1974). The total number of modified sites is about the same as found for 16S RNA, although there are twice as many nucleotide residues in 23S RNA. Since the extent of base pairing is about the same for the two RNA molecules, this result may indicate that more extensive RNA–RNA or RNA–protein interactions involving single-stranded regions occur in 23S RNA than in 16S RNA.

Since kethoxal reacts only with guanines that are single stranded, these data provide a test of RNA secondary structure within the ribosome. The only secondary structure model proposed for 23S RNA thus far is for the region which contains the binding site for ribosomal protein L1 (Branlant et al., 1976). Although most of the nucleotides in the model are base-paired, the two kethoxal sites that can be located in this fragment (oligonucleotides 6/14 and 28/8) are proposed to be single stranded, in accord with the kethoxal susceptibility of these sites. Two of the sites of T_1 ribonuclease attack on the L1 binding site fragment (Branlant et al., 1976) appear to be identical with the sites of kethoxal substitution, suggesting that this nucleoprotein fragment has a conformation similar to that of 23S RNA in the 50S ribosomal fragment.

A primary reason for determining the accessible nucleotide sequences in the 50S ribosomal subunit is that such sequences may be functionally important, and that their elucidation might provide information relevant to the understanding of the functional role of ribosomal RNA. During the course of the translation process, the 50S subunit interacts with a variety of ligands, including tRNA and 30S ribosomal subunits. It has often been suggested that the binding of such ligands to the 50S subunit involves RNA–RNA interaction based on complementary base pairing (see, for example, Forget and Weissman, 1967; Van Duin et al., 1976). Accordingly, we have searched the sequences in Tables II and III for possible complementarity with accessible regions in 16S RNA and with the "constant" regions of tRNA.

As shown in Table IV there are 4 possible pairings between the accessible sequences in 16S and 23S RNA that contain 6 base pairs each, and 15 that contain 5 base pairs. There are, of course, many more containing fewer than 5 base pairs (not shown). Sequences involved in 30S–50S subunit association would be expected to be less reactive toward kethoxal in 70S ribosomes. Nine of the examples listed in Table IV contain 16S RNA sequences that are reactive toward kethoxal in 30S subunits and become unreactive in 70S ribosomes (Chapman and Noller, 1977). Although this suggests that some of these complementary sequences might be involved in ribosomal subunit association, it is not yet known whether the corresponding 23S RNA sequences are also protected from modification by the 30S subunit. In addition, functional evidence is required to demonstrate, for example, that modification of these sites interferes with subunit association.

In the model recently proposed for the mutual base pairing of the 3′ termini of 16S and 23S RNA by Van Duin et al. (1976), interaction of the oligonucleotide AACCG in the 3′-terminal sequence of 23S RNA with CGGUU in 16S RNA helps to promote subunit association. We have not detected the oligonucleotide AACCGp as a major exposed sequence using the diagonal method, suggesting that its 3′-terminal G is not available for base pairing in the 50S subunit. The nucleotide sequence $AACCG_{OH}$ (oligonucleotide 15) is present, however, showing that the G preceding this sequence is unpaired in 50S subunits. However, since Branlant et al. (1975) report 4–5 copies of this sequence in 23S RNA, we cannot say whether the one we identify originates from the 3′-terminal sequence. It should also be pointed out that the results of kethoxal modification of 30S subunits show that part of the sequence of 16S RNA involved in this model is inaccessible in the 30S subunit (Chapman and Noller, 1977). Interaction of the 3′ termini of 16S and 23S RNA in this manner would thus require some structural alteration in both ribosomal subunits.

The constancy of the sequence GTψCR in transfer RNA molecules suggests that it may play a role in the interaction of tRNA with ribosomes, since all tRNA molecules must be accommodated by the same ribosome binding sites. When the complementary sequence CGAAC was discovered in 5S RNA (Brownlee et al., 1967; Forget and Weissman, 1967), it was suggested that tRNA might base pair with 5S RNA (Forget and Weissman, 1967). Subsequently, it was shown that the CGAAC sequence is not accessible in 50S or 70S ribosomes (Noller and Herr, 1974; Delihas et al., 1975) so that its involvement in base-pairing would be dependent on some prior structural rearrangement of the 50S subunit. We have examined the accessible sequences in 23S RNA (Tables II and III) for potential complementarity with the invariant tRNA sequence, and note that such a sequence exists (Table V). At the time the 5S RNA model was proposed, only the sequence GTψCG had been detected in tRNA. Since then, a number of tRNA species from *E. coli*, including the initiator tRNA, have been shown to contain the sequence GTψCA (Barrell and Clark, 1974). The exposed 23S RNA sequence UGAAU is complementary to both GTψCG and GTψCA. It should be pointed out that, whereas the complementary sequence in 5S RNA is inaccessible, the above 23S RNA sequence is likely to be free to base pair in the ribosome. Although we have no evidence to support possible base pairing between this site and

tRNA, the fact of its existence should be borne in mind in future consideration of tRNA binding site models.

References

Allet, B., and Spahr, P. F. (1971), *Eur. J. Biochem. 19,* 250–255.

Barrell, B. G. (1971), *Proced. Nucleic Acid Res. 2,* 751–779.

Barrell, B. G., and Clark, B. F. C. (1974), Handbook of Nucleic Acid Sequences, Oxford, Joynsson-Bruvvers, Ltd.

Branlant, C., and Ebel, J.-P. (1977) *J. Mol. Biol. 111,* 215–256.

Branlant, C., Sri Widada, J., Krol, A., Fellner, P., and Ebel, J. P. (1975), *Biochimie 57,* 175–225.

Branlant, C., Korobko, V., and Ebel, J. P. (1976), *Eur. J. Biochem. 70,* 471–482.

Brownlee, G. G., Sanger, F., and Barrell, B. G. (1967), *Nature (London) 215,* 735–736.

Chapman, N. M., and Noller, H. F. (1977), *J. Mol. Biol. 109,* 131–149.

Chen-Schmeisser, U., and Garrett, R. (1976), *Eur. J. Biochem. 69,* 401–410.

Contreras, R., and Fiers, W. (1971), *FEBS Lett. 16,* 281–283.

Delihas, N., Dunn, J. J., and Erdmann, V. A. (1975), *FEBS Lett. 58,* 76–80.

Fellner, P., and Sanger, F. (1968), *Nature (London) 219,* 236–238.

Forget, B. G., and Weissman, S. M. (1967), *Science 158,* 1695–1699.

Herr, W., and Noller, H. F. (1975), *FEBS Lett. 53,* 248–252.

Nishimura, S., Harada, F., Narushima, U., and Seno, T. (1967), *Biochim. Biophys, Acta 142,* 133–148.

Noller, H. F. (1974), *Biochemistry 13,* 4694–4703.

Noller, H. F., and Herr, W. (1974), *J. Mol. Biol. 90,* 181–184.

Spierer, P., and Zimmermann, R. A. (1976), *J. Mol. Biol. 103,* 647–653.

Spierer, P., Zimmermann, R. A., and Mackie, G. A. (1975), *Eur. J. Biochem. 52,* 459–468.

Van Duin, J., Kurland, C. G., Dondon, J., Grunberg-Manago, M., Branlant, C., and Ebel, J. P. (1976), *FEBS Lett. 62,* 111–114.

Woese, C., Sogin, M., Stahl, D., Lewis, B. J., and Bonen, L. (1976), *J. Mol. Evol. 7,* 197–213.