J. Indian Inst. Sci., Jan.-Feb. 1989, **69**, 13-24.

Antibody probes of RNA structure in the ribosome

D. G. GLITZ, L. LASATER, L. MONTESANO, P. CANN AND H. M. OLSON
Department of Biological Chemistry, UCLA School of Medicine, University of California, Los Angeles, CA 90024, USA.

Received on February 22, 1988.

Abstract

The RNA of each ribosomal subunit is folded in a specific and precise conformation. We have induced antibodies that are directed against individual naturally methylated nucleosides, against reagents used in the chemical modification of unique sites in the RNA, and against antigenic determinants on synthetic oligodeoxynucleotides that complement specific ribosomal RNA sequences. All of these antibodies serve as probes of the RNA conformation in the ribosome. Immunoelectron microscopy—the visualization in electron micrographs of antibody-subunit complexes—has been used to localize several nucleotides or short RNA sequences at specific sites on the surface of the small ribosomal subunit. These results are used to generate an increasingly detailed map of the conformation of the RNA within the three-dimensional structure of the *Escherichia coli* ribosome.

Key words: Antibody, ribosome, ribosomal RNA, RNA conformation.

1. Introduction

The ribosome plays a central role in protein biosynthesis. It provides the physical surface upon which all proteins are assembled. More significantly, it is the organizational center at which specific interactions occur between and among messenger and transfer RNA molecules, the non-ribosomal initiation, elongation and termination protein factors, and other molecules that function in the translational process and its regulation. A knowledge of ribosome structure and the relationship of the structure to the many aspects of protein biosynthesis is a necessary component of any molecular-level comprehension of translation.

The ribosomes of different species are structurally similar; all consist of two subunits, and all include a few specific RNA molecules and a larger number of proteins in particles of defined size, shape, and topology. In *Escherichia coli* the smaller (30S) subunit is composed of one (16S) RNA molecule of 1542 nucleotides plus 21 different proteins, while the larger (50S) subunit includes one 23S RNA of 2904 nucleotides, one 5S RNA of 120 nucleotides, and more than 30 protein species. Each subunit shows characteristic projections in electron micrographs, and three-dimensional models that help to define subunit morphology and explain aspects of ribosome function are widely accepted[1].

Abbreviations used: m^7Guo, 7-methylguanosine; m^6Ado, N^6-methyladenosine; m^6_2Ado, N^6,N^6-dimethyladenosine; ε-Ado, 1,N^6-ethenoadenosine; HPLC, high-performance liquid chromatography.

Considerable effort has been expended in positioning individual ribosomal proteins at specific loci on the particle[2–4]. It is now possible to place most of the 30S proteins and several 50S proteins at unique sites on the surface of the subunits. It is often also possible to relate such placements to specific functional sites and activities. Much less is known about the three-dimensional distribution of RNA within each subunit or the functional roles the RNA must play. RNA makes up about two-thirds of the mass of each ribosomal subunit; it both determines the size and shape of each subunit[5,6] and provides a scaffolding upon which the proteins of the subunits are arranged. The RNA is also involved in an active functional sense; *e.g.* the Shine and Dalgarno[7] sequence near the 3′-end of 16S RNA complements and binds messenger RNA so as to correctly place an appropriate AUG codon for the initiation of protein biosynthesis.

We are concerned with describing the structure and function of the ribosomal RNA as it is folded within each ribosomal subunit. Our primary technique is immunoelectron microscopy–the visualization in electron micrographs of complexes that are formed between ribosomal subunits and antibodies directed against a specific component of the ribosome. We have used antibodies that specifically recognize unique methylated nucleotides of the ribosomal RNA to place these components within the structure of the subunit. We have also used specific chemical modification to create an antibody-recognizable structure in the RNA. Most recently, we have used chemically synthesized oligodeoxynucleotides that complement ribosomal RNA sequences to first probe the subunit for conformationally available RNA segments and then, after modification to permit interaction with an antibody, to place these sequences by electron microscopy. Here we summarize our progress in these experiments.

2. Experimental procedures

Ribosomes and ribosomal subunits were isolated from various strains of *Escherichia coli* essentially as described by Traub *et al*[8]. Preparation of ribosomes from pea chloroplasts[9] and wheat germ[10] has also been described. The synthesis of nucleoside-protein conjugates and methods of antibody induction and purification have been detailed by Eichler and Glitz[11]. Antibody characterization in membrane filter binding assays was modified[12] from Humayun and Jacob[13]. Properties of specific antibody preparations are detailed as follows: N^6,N^6-dimethyladenosine, Politz and Glitz[12]; N^6-methyladenosine, Montesano and Glitz[14]; 7-methylguanosine, Trempe *et al*[15]; 1,N^6-ethenoadenosine, Frink *et al*[16]; 2,4-dinitrophenol, Olson and Glitz[17]. Analysis of modified nucleosides in ribosomal RNA has been described[9,10,14,15].

Oligodeoxynucleotides were synthesized using a Dupont/Vega Biotechnologies Coder 300 synthesizer and the phosphoramidite approach of Matteuci and Caruthers[18], except with β-cyanoethyl nucleotide derivatives[19]. The oligomers were deblocked in concentrated ammonia for 18-24 h at 55°C and purified by ion exchange HPLC using a BioRad Laboratories TSK DEAE 5PW column and a hyperbolic gradient of NaCl in 0.02 M Tris-HCl, pH 7.2. Purified oligodeoxynucleotides were desalted by dialysis against distilled water. Incorporation of a 5′-terminal phosphate (sometimes radiolabeled) was modified

from Donis-Keller *et al*[20]. Chemical modification of the 5'-terminus with dinitrophenol and addition of a 3'-terminal residue of 1,N^6-ethenoadenosine to synthetic oligodeoxynucleotides is described by Glitz *et al*[21]. Binding of ^{32}P-labeled oligomers to ribosomal subunits was measured in a nitrocellulose filter assay[22], and assay of poly(U)-stimulated binding of phenylalanyl-tRNA followed Zamir *et al*[23].

Samples for electron microscopy included *ca* 15 pmol of ribosomal subunits (and, if appropriate, *ca* 30 pmol of terminally modified oligodeoxynucleotide incubated up to 16 h on ice). Then 15-60 pmol of antibody-combining capacity (equivalent to 7.5-30 pmol of IgG) in up to 60 μl of 10 mM Tris-HCl, pH 7.2, 150 mM NH_4Cl, 1-10 mM $MgCl_2$ buffer were added. Samples were sometimes incubated at 37°C for 5-15 min; all samples were incubated for 0.5-18 h on ice. The reaction mixtures were then fractionated by size-exclusion HPLC using a Beckman TSK-3000 column in the cold (or in earlier experiments a small column of Sepharose 6B). The peak corresponding to ribosomal subunits and complexes was collected, and samples were adsorbed to thin carbon films and stained with 1% uranyl acetate using the double-carbon technique first described by Valentine *et al*[24]. Electron micrographs were obtained using JEOL 1200 EX or 100B electron microscopes at an accelerating voltage of 80,000 and a nominal magnification of 60-80,000 X. Micrographs were interpreted using the model described by Lake[25].

3. Results

3.1. *Specificity of anti-nucleoside antibodies*

In order to be useful as structural probes for immunoelectron microscopy, antibodies directed against modified nucleosides must be of sufficient specificity to recognize a single residue of a methylated nucleotide in a sequence that contains several hundred residues of the parent nucleotide. Moreover, the affinity of the antibody must be sufficient to permit survival of the immune complex through purification and sample dilution, adsorption to a carbon support, negative staining, and drying. Quantitation of radioligand binding using a nitrocellulose membrane filter assay (based on Humayun and Jacob[13]) allows evaluation of antibodies from individual rabbits and bleedings. Figure 1 shows a typical result with antibodies to 7-methylguanosine; the antibodies recognize both nucleoside and nucleotide well, and show very little affinity for the parent guanosine or GMP. The chemically modified (ring-opened) guanosine derivative shows intermediate affinity. Similar measurements with a variety of anti-nucleoside antibodies are summarized in Table I. We conclude that these preparations of antibodies to methylated purines have characteristics that should allow their use as immunoelectron microscopic markers. Not all antibody preparations are suitable; in particular, although we have induced antisera to modified pyrimidines, the antibodies have not been useful as probes because of insufficient selectivity relative to the unmodified nucleoside.

3.2. *Analysis of target nucleosides in ribosomal RNA*

The number of residues of a specific target nucleoside within the RNA of a ribosomal

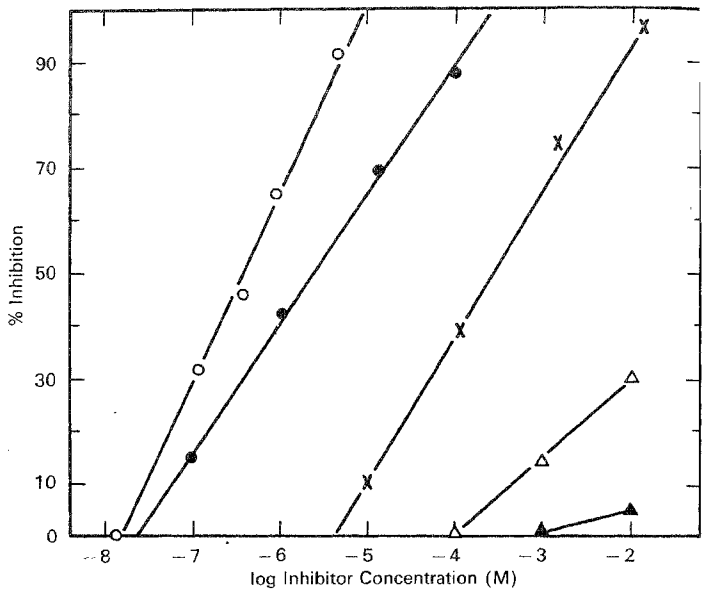

FIG. 1. Specificity of antibodies to 7-methylguanosine. Reaction mixtures (250 μl) included *ca* 20 pmol (5×10^4 cpm) of [^{3}H] 7-methylguanosine tri-alcohol, non-radioactive competitor as indicated, and anti-7-methylguanosine IgG in 0.01 M Tris-HCl, pH 7.2, 0.14 M NaCl buffer. Samples were incubated for 30 min at 37°C and then for 60 min on ice; they were then rapidly passed through Millipore type HA (0.45 μm) filters. Sample tubes and filters were each washed with four 2 ml portions of cold buffer, and nucleoside radioactivity retained on the filters was measured by liquid scintillation counting. The inhibitors used were: ○, 7-methylguanosine; ●, 7-methyl GMP; X, the ring-opened form of 7-methylguanosine, formed by treatment with ammonia; △, guanosine; ▲, GMP.

Table I
Affinity of anti-nucleoside antibodies

Antibody directed against	− Log ligand concentration at midpoint of inhibition curve					
	Ado	m^6Ado	m^6_2Ado	εAdo	Guo	m^7Guo
N^6,N^6-dimethyladenosine	2.5	5.2	7	n.d.	> 1	n.d.
N^6-monomethyladenosine	4.7	7	3.7	n.d.	> 1	n.d.
1,N^6-ethenoadenosine	> 1	1	> 1	7.4	> 1	n.d.
7-methylguanosine	> 1	n.d.	> 1	> 1	1	6.5
adenosine	4.2	n.d.	> 2	> 2	3	n.d.
guanosine	3	n.d.	> 2	n.d.	4.8	> 2

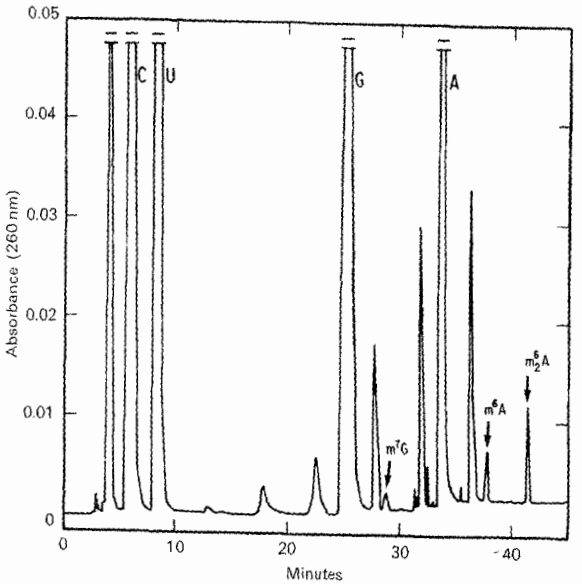

FIG. 2. Analysis of modified nucleosides in ribosomal RNA. RNA was isolated from purified wheat germ 40S ribosomal subunits by phenol extraction. About 0.1 mg was hydrolyzed to nucleosides with a mixture of bovine pancreatic ribonuclease, ribonuclease T1, snake venom phosphodiesterase, and *E. coli* alkaline phosphomonoesterase. Nucleosides were separated on a Supelco C-18 reversed phase column by high-performance liquid chromatography using the following program. 10 min, 100% buffer A (0.25 M ammonium acetate, pH 6); 35 min, to 75% buffer B (40/60 (v/v) acetonitrile/water) *via* a hyperbolic gradient (Waters Associates, curve 8); 5 min, to 100% buffer B *via* a linear gradient; 5 min, to 100% buffer A *via* a linear gradient. Absorbance was monitored at 260 nm and peak areas were integrated with a Shimadzu C-R3A Chromatopac.

ubunit is important both in the quantitation and the evaluation of the specificity of complexes that are seen in electron micrographs. Reversed-phase high-performance liquid chromatography allows accurate quantitation of the nucleosides in an RNA hydrolysate. Figure 2 shows an analysis of the 18S RNA of wheat germ; we detect 1.1 mol of m^7Guo^a, 1.0 mol of m^6Ado and 1.65 mol of m_2^6Ado per mol RNA (*ca* 1800 nucleotides). Similar analyses show N^2-methylguanosine to be absent from pea chloroplast 16S RNA but present in *E. coli* small subunit RNA (M. R. Trempe, unpublished) and m^6Ado to be absent from both chloroplast and *E. coli* 16S RNA.

3.3. *Binding of complementary oligodeoxynucleotide probes to ribosomal subunits*

Because the number of naturally occurring markers (such as methylated nucleosides) in ribosomal RNA is limited, we have explored the use of oligodeoxynucleotide probes that complement specific sequences within the RNA[22]. The interaction between an oligoxynucleotide and ribosomal subunits was quantitated using a nitrocellulose membrane filter binding assay. Parameters including subunit and oligodeoxynucleotide concentrations, magnesium ion levels, incubation time and temperature, and the activation state of the ribosomal subunits were independently varied. Figure 3 illustrates a typical experiment in which the effect of magnesium ion concentration is examined using three overlapping oligonucleotides that bind the 5′-terminal segment of the 16S RNA. Many experiments of this nature lead to the following generalizations:

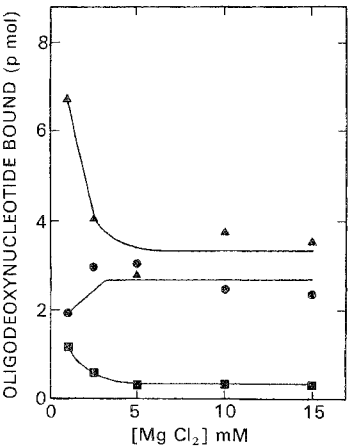

FIG. 3. Effect of magnesium concentration on oligodeoxynucleotide binding to ribosomal subunits. The reaction mixtures included 15 pmol of *E. coli* 30S ribosomal subunits and 30 pmol of ^{32}P-labeled oligodeoxynucleotide in 50 μl of buffer containing 0.01 M Tris-HCl, pH 7.5, 0.15 M NH_4Cl and varying amounts of $MgCl_2$. Samples were incubated overnight on ice, diluted to 1 ml with the appropriate buffer, and rapidly passed through pre-wet Millipore type HA (45 μm) filters. Sample tubes and filters were washed with three 2 ml portions of buffer and radioactivity retained by the filters was measured by liquid scintillation counting. The oligodeoxynucleotides used were: ●, the complement to 16S RNA nucleotides 1-11; ▲, the complement to RNA nucleotides 1-15; ■, the complement to RNA nucleotides 6-16.

FIG. 4. Electron micrograph of subunit-oligodeoxynucleotide-antibody complexes. The reaction mixture included (in 50 μl of 10 mM Tris-HCl, pH 7.2, 150 mM NH_4Cl, 1 mM $MgCl_2$) 20 pmol of activated *E. coli* 30S ribosomal subunits and 40 pmol of 5′-dinitrophenyl-oligodeoxynucleotide complementary to 16S RNA nucleotides 520-531. After 10 min at 37°C followed by overnight incubation on ice, 55 pmol (binding capacity) of an anti-dinitrophenol IgG preparation was added. Following 1 h on ice, the sample was subjected to size-exclusion high-performance liquid chromatography using a Beckman TSK-3000 column equilibrated with the same buffer at 0°C. The peak corresponding to subunits and complexes was used to prepare grids for electron microscopy. Arrows indicate immune complexes. Bar length: 100 nm.

1. Individual oligodeoxynucleotides differ significantly with respect to optimal binding conditions. Oligodeoxynucleotide chain length (over the range of 8-15 nucleotides) and the ΔG of the base-pairing interactions are partially responsible for the differences observed, but other factors clearly predominate.

2. In each instance in which significant levels of binding occur, a plateau in the saturation curve is seen at an oligodeoxynucleotide/subunit ratio of 1 to 1.5. No significant increases in binding are seen, even with large excesses of oligodeoxynucleotide. Non-specific binding, quantitated using 50S ribosomal subunits in the same assay, is not significant in any instance we have thus far examined.

3. The maximum level of oligodeoxynucleotide binding observed ranges from zero to *ca* 0.4 mol/mol 30S under our assay conditions. In a very similar procedure[23], the poly(U)-stimulated binding of radiolabeled phenylalanyl-tRNA to active 30S subunits is *ca* 0.2 mol/mol.

4. Agents that affect ribosomal subunit conformation strongly affect the level of oligodeoxynucleotide binding. In each instance the effects we observe are most directly related to the activation state of the 30S subunit as defined by its activity in the poly(U)-stimulated phenylalanyl tRNA-binding assay[23]. Four classes of oligodeoxynucleotides have been defined.

A. Oligodeoxynucleotides that bind well (but not exclusively) to subunits that have been activated by incubation at 37°C in buffers containing 10 mM Mg^{2+};

B. Oligodeoxynucleotides that bind to activated subunits at a significant level but bind more effectively to subunits that have been reversibly inactivated by dialysis in the cold against buffers containing 1 mM Mg^{2+};

C. Oligodeoxynucleotides that bind reversibly inactivated 30S subunits exclusively or with very great preference over activated subunits;

D. Oligodeoxynucleotides that bind neither activated nor inactivated subunits. (In all instances but one we have observed binding of these oligodeoxynucleotides to either native or heat-denatured 16S RNA isolated from 30S subunits. Hence, their lack of binding to intact subunits shows the complementary RNA sequences to be unavailable.)

3.4. *Immunoelectron microscopy*

Antibody-subunit or antibody-oligodeoxynucleotide-subunit complexes are generated and excess (unbound or non-specific) IgG molecules are removed by size-exclusion HPLC. The complexes are then adsorbed to a thin carbon support, negatively contrasted with uranyl acetate, and examined by electron microscopy. Antibodies we have used in such experiments include those directed against N^6,N^6-dimethyladenosine[12], N^6-monomethyladenosine[14], and N^7-methylguanosine[15] residues found in the ribosomal RNA, against 1,N^6-ethenoadenosine[16] used to modify (and mark) the 3'-end of synthetic oligodeoxynucleotides[21], and against 2,4-dinitrophenol used to modify the 3'-end of ribosomal RNA[17] or the 5'-end of oligodeoxynucleotide probes[21]. An example of an electron micrograph is shown in fig. 4; in this instance an antibody to 2,4-dinitrophenol has been used to locate the DNP-modified

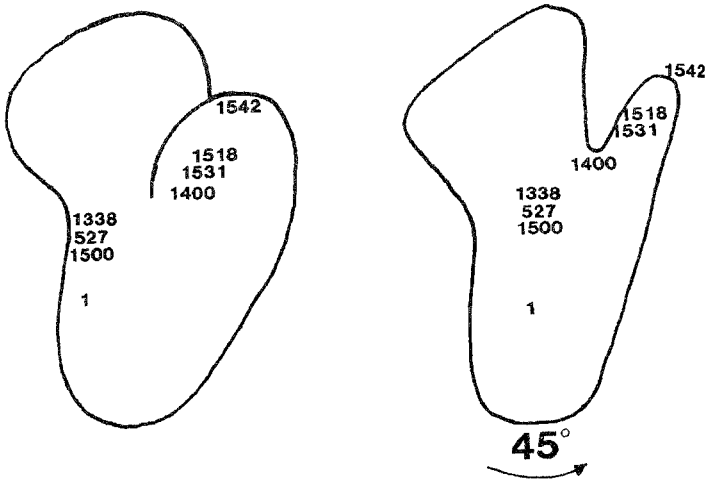

FIG. 5. RNA localizations within the 30S ribosomal subunit. The 5′-terminus was placed by Mochalova *et al*[26] and the sequence at position 1400 by Oakes *et al*[5]; all other localizations are from this laboratory. All numbers refer to positions in the *E. coli* 16S ribosomal RNA sequence[28]. Residues 1338 and 1550 are placed only by analogy of wheat germ and *E. coli* sequences.

5′-terminus of an oligodeoxynucleotide that is complementary to 16S RNA residues 520-531. Subunits are seen primarily in the three major projections described by Lake[25]. Subunit antibody complexes in fig. 4 are indicated by arrows.

Contact points between subunits and antibody in each subunit projection are quantitated, using at least 100 micrographs for each placement and at least two evaluators working independently. The specific contact point (and hence the location of the site being probed) is then placed using the three-dimensional model of the 30S subunit proposed by Lake[25]. Figure 5 summarizes several placements made by our laboratory and others.

4. Discussion

Electron microscopy is a particularly appropriate means through which to describe ribosomal subunit ultrastructure; resolution is high enough to permit examination of structural details, the experiments are reasonably direct, and electron microscopes are sufficiently accessible to allow several groups to independently attack the problems. Significant difficulties do arise in attempts to identify individual ribosomal components in electron micrographs. It is not possible to differentiate or even to distinguish specific

ribosomal macromolecules. Only the overall shape of the particles is seen, and then only as a variety of two-dimensional projections of the native three-dimensional structure.

Identification of individual ribosomal components in micrographs requires the use of highly specific probes or markers that are easily seen and identified. Antibodies are unusually attractive in this regard. Their unique specificity allows a selective interaction with individual structural components or elements of the ribosome, and their characteristic size and shape permit easy recognition of the antibody molecules in micrographs of immune complexes. The precise location of a ribosomal component is thus defined through the position of contact between the subunit and an antibody to the component.

Antibodies directed against ribosomal proteins have allowed the localization of many of the individual polypeptides of both the large and small subunits[1,3,4]. Initial results obtained by this technique were often controversial or conflicting, largely because of the impurity of the initial protein immunogens; contaminating ribosomal proteins induced antibodies of unknown or unexpected specificity resulting in localizations that were incorrect[1]. Such problems are now less serious because of better characterization of antibodies, or through the use of monoclonal antibodies[4] or antibodies directed against a hapten used to modify a specific (isolated) ribosomal protein[26] that is incorporated into reconstituted subunits.

Immunoglobulin probes of ribosomal RNA components are inherently more limited. Antibodies directed against the common nucleotides have too little specificity to have any value, and it is not likely to be possible to induce an antibody with specificity for the 7-10 nucleotide long sequence that would be necessary to ensure a unique recognition site. One successful approach has been through the use of antibodies directed against the naturally occurring modified nucleosides that occur at specific positions in ribosomal RNA. Antibodies directed against methylated purine nucleosides show the characteristics necessary for their use as probes. In several instances their specificity is sufficient to result in essentially exclusive interaction with the modified nucleotide in the RNA, with almost undetectable cross-reactivity with other RNA components (*e.g.* fig. 1). Equally important to these experiments, the absolute affinity of these antibodies is great enough to allow purification of complexes and their visualization by electron microscopy (Table I). Using such antibody preparations we have shown an essentially identical placement of N^6,N^6-dimethyladenosine on the platform of small subunits of ribosomes from *E. coli*[12], pea chloroplasts[9], and wheat germ[10]. Antibodies to 7-methylguanosine have been used to place this nucleoside at the junction of the segments of the small subunit in the same three cases[14,15]. This result is particularly interesting in that the position of the 7-methylguanosine in the linear sequence of *E. coli* 16S RNA (residue 527) is not equivalent to its position in the sequence of eukaryotic 18S RNA (*ca* 200 nucleotides from the 3'-terminus, equivalent to *E. coli* residue 1338), yet the three-dimensional placement is identical. An antibody to N^6-monomethyladenosine also permits its localization (only in wheat germ 40S) near the 7-methylguanosine site; the equivalent residue in the *E. coli* sequence occurs at position 1500 and is not methylated[14].

Other modified nucleotides occur in ribosomal RNA, but we have not yet been able to induce antibodies with the properties needed for their successful use. It has been possible

to exploit the unique chemical characteristics of each end of the RNA to place both the 3′ and 5′ termini[17,27]. Further investigation of specific RNA sites has required development of a different class of probes. We have used complementary oligodeoxynucleotides for this purpose[22]; the oligomers are readily synthesized using commercial automated equipment and easily purified by ion-exchange HPLC. Optimization of conditions for binding an oligodeoxynucleotide to ribosomal subunits (*e.g.* fig. 3) is necessary in each instance. In contrast, the methods for attaching an antibody-recognizable dinitrophenyl derivative at the 5′-end or a molecule of 1,N^6-ethenoadenosine at the 3′-end of an oligomer are generally applicable[21].

Immune electron microscopy with complementary oligodeoxynucleotide probes is probably more difficult than placement of methylated nucleotides. Formation of a ternary complex involving subunit, oligomer and antibody is less efficient than formation of a binary complex, and there may be a much higher probability that the ternary complexes will dissociate during purification or grid preparation. As a result we usually see only a few complexes per micrograph in these experiments, as is obvious in fig. 4. The major difficulty may thus be the necessity to expose and interpret up to several hundred micrographs in order to localize a single oligodeoxynucleotide with accuracy and confidence.

The placements summarized in fig. 5 identify the direct localizations of 16S RNA residues or segments that have been made in our laboratory and by others. The most obvious conclusions to be drawn relate to the relationships between the RNA conformation near its 3′-end and its interactions with other RNA molecules during protein synthesis. Our placement of the mRNA-complementary Shine and Dalgarno[7] sequence of 16S RNA (residues 1531-1542) suggests that the message runs (5′→3′) from the platform end down its inner surface. Deep in the cleft codon-anticodon interactions occur near 16S RNA residue 1400. The stem-loop structure that includes the dimethyladenosine residues therefore must extend from near the bottom of the cleft to midway on the platform, while residues on each side of the decoding site (residue 1400) are found at the cleft level but on the subunit body further from the base of the platform.

Although the absolute number of additional sites is small, it is clear that the methodology now exists that will allow many more placements in the future. It should equally be possible to extend these methods to the study of the *E. coli* 50S subunit and to ribosomes of other species.

Acknowledgements

This work has been supported by the National Science Foundation Grant DMB 86–12905 and the United States Public Health Service, National Institutes of Health.

References

1. WITTMAN, H. G. Architecture of prokaryotic ribosomes, *Annu. Rev. Biochem.*, 1983, **52**, 35–65

2. CAPEL, M. S., ENGELMAN, D. M., FREEBORN, B. R., KJELDGAARD, M., LANGER, J. A., RAMAKRISHNAN, V., SCHINDLER, D. G., SCHNEIDER, D.K., SCHOENBORN, B. P., SILLERS, I. Y., YABUKI, S. AND MOORE, P. B. — A complete mapping of the proteins in the small ribosomal subunit of *Escherichia coli*, *Science*, 1987, **238**, 1403–1406.

3. OAKES, M., HENDERSON, E., SCHEINMAN, A., CLARK, M. AND LAKE, J. A. — Ribosome structure, function, and evolution: mapping ribosomal RNA, proteins, and functional sites in three dimensions, in *Structure function and genetics of ribosomes*, 1986 (Hardesty, B., and Kramer, G., eds), pp. 47–67, Springer-Verlag.

4. TRAUT, R. R, TEWARI, D. S., SOMMER, A., GAVINO, G. R., OLSON, H. M. AND GLITZ, D. G. — Protein topography of ribosomal functional domains: effects of monoclonal antibodies to different epitopes in *Escherichia coli* protein L7/L12 on ribosome function and structure, in *Structure, function and genetics of ribosomes*, 1986 (Hardesty, B., and Kramer, G., eds), pp. 286–308. Springer-Verlag.

5. VASILIEV, V. D., SELIVANOVA, D. M. AND KOTELIANSKY, V. E. — Specific self packing of the ribosomal 16S RNA, *FEBS Lett.*, 1978, **95**, 273–276.

6. VASILIEV, V. D. AND ZALITE, O. M. — Specific compact self packing of the ribosomal 23S RNA, *FEBS Lett.*, 1980, **121**, 101–104.

7. SHINE, J. AND DALGARNO, L. — The 3'-terminal sequence of *E. coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites, *Proc. Natn. Acad. Sci. U.S.A.*, 1974, **71**, 1342–1346.

8. TRAUB, P., MIZUSHIMA, S., LOWRY, C. V. AND NOMURA, M. — Reconstitution of ribosomes from subribosomal components, in *Methods in enzymology*, 1971 (Moldave, K. and Grossman, L. eds). Vol. 20, pp. 391–407, Academic Press.

9. TREMPE, M. R. AND GLITZ, D. G. — Chloroplast ribosome structure: electron microscopy of ribosomal subunits and localization of N^6,N^6-dimethyladenosine by immunoelectron microscopy, *J. Biol. Chem.*, 1981, **256**, 11873–11879.

10. MONTESANO, L. AND GLITZ, D. G. — Wheat germ cytoplasmic ribosomes: structure of ribosomal subunits and localization of N^6,N^6-dimethyladenosine by immunoelectron microscopy, *J. Biol. Chem.*, 1988, **263**, 4932–4938.

11. EICHLER. D. C. AND GLITZ. D. G. — Nucleotide-specific antibodies as potential blocking agents in the structural analysis of nucleic acids, *Biochem. Biophys. Acta*, 1974, **335**, 303–317.

12. POLITZ, S. M. AND GLITZ, D. G. — Ribosome structure: localization of N^6,N^6-dimethyladenosine by electron microscopy of a ribosome-antibody complex, *Proc. Natn. Acad. Sci. U.S.A.*, 1977, **74**, 1468–1472.

13. HUMAYUN, M. Z. AND JACOB, T. M. — Immunologic studies on nucleic acids and their components I. An analysis of the specificity of anti-deoxyadenylate antibodies by a membrane-binding technique, *Biochem. Biophys. Acta*, 1973, **331**, 41–53.

14. MONTESANO, L. AND GLITZ, D. G. — Wheat germ cytoplasmic ribosomes: localization of 7-methylguanosine and 6-methyladenosine by electron microscopy of immune complexes, *J. Biol. Chem.*, 1988, **263**, 4939–4944.

15. TREMPE, M. R., OHGI, K. AND GLITZ, D. G. — Ribosome structure: localization of 7-methylguanosine in the small subunits of *Escherichia coli* and chloroplast ribosomes by immunoelectron microscopy, *J. Biol. Chem.*, 1982, **257**, 9822–9829.

16 FRINK, R. J., EISENBERG, D. AND GLITZ, D. G — Localization of the site of adenylylation of glutamine synthetase by electron microscopy of an enzyme-antibody complex, *Proc. Natn. Acad. Sci. U.S.A.*, 1978, **75**, 5778–5782.

17. OLSON, H. M. AND GLITZ, D. G. — Ribosome structure: localization of 3′-end of RNA in small subunit by immunoelectron microscopy, *Proc. Natn. Acad. Sci. U.S.A.*, 1979, **76**, 3769–3773.

18. MATTEUCCI, M. D. AND CARUTHERS, M. H. — Synthesis of deoxyoligonucleotides on a polymer support, *J. Am. Chem. Soc*, 1981, **103**, 3185–3191.

19. SINHA, N. D., BIERNAT, J., MCMANUS, J. AND KÓSTER, H. — Polymer support oligonucleotide synthesis XVIII: use of β-cyanoethyl-N,N-dialkylamino-/N-morpholino phosphoramidite of deoxynucleosides for the synthesis of DNA fragments simplifying deprotection and isolation of the final product, *Nucleic Acids Res.*, 1984, **12**, 4539–4557.

20. DONIS-KELLER, H., MAXAM, A. M., AND GILBERT, W. — Mapping adenines, guanines and pyrimidines in RNA, *Nucleic Acid Res.*, 1977, **4**, 2527–2538.

21. GLITZ, D. G., CANN, P. A., LASATER, L. S. AND OLSON, H. M. — Antibody probes of ribosomal RNA, in *Methods in enzymology*, 1988 (Moldave, K., and Noller, H., eds) Vol. 164, pp. 493–503, Academic Press.

22. GLITZ, D. G., OLSON, H. M. AND LASATER, L. S. — Synthetic oligodeoxynucleotides as probes of RNA conformation within the ribosome, *Biophys. J.*, 1986, **49**, 11–13.

23. ZAMIR, A., MISKIN, R., VOGEL, Z. AND ELSON, D. — The inactivation and reactivation of *Escherichia coli* ribosomes, in *Methods in enzymology*, 1974 (Moldave, K., and Grossman, L., eds), Vol. 30, pp. 406–426, Academic Press.

24. VALENTINE, R. C., SHAPIRO, B. M. AND STADTMAN, E. R. — Regulation of glutamine synthetase. XII. Electron microscopy of the enzyme from *Escherichia coli*, *Biochemistry*, 1968, **7**, 2143–2152.

25. LAKE, J. A. — Ribosome structure determined by electron microscopy of *Escherichia coli* small subunits, large subunits and monomeric ribosomes, *J. Mol. Biol.*, 1976, **105**, 131–159.

26. OLSON, H. M., OLAH, T. V., COOPERMAN, B. S. AND GLITZ, D. G. — Immune electron microscopic localization of dinitrophenyl-modified ribosomal protein S19 in reconstituted *Escherichia coli* 30S subunits using antibodies to dinitrophenol, *J. Biol. Chem.*, 1988, **263**, 4801–4806.

27. MOCHALOVA, L. V., SHATSKY, I. N., BOGDANOV, A. A. AND VASILIEV, V. D. — Topography of RNA in the ribosome: localization of the 16S RNA 5′-end by immune electron microscopy, *J. Mol. Biol.*, 1982, **159**, 637–650.

28. NOLLER, H. F. — Structure of ribosomal RNA, *Annu. Rev. Biochem.*, 1984, **53**, 119–162.