Klausner, R. D., & Harford, J. B. (1989) *Science 246*, 870-872.
Koeller, D. M., Casey, J. L., Hentze, M. W., Gerhardt, E. M., Chan, L. L., Klausner, R. D., & Harford, J. B. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 3574-3578.
Kuo, D. J., & Rose, I. A. (1987) *Biochemistry 26*, 7589-7596.
Luzzati, V. (1953) *Acta Crystallogr. 6*, 142.
Matthews, B. W. (1968) *J. Mol. Biol. 33*, 491-497.
Ogston, A. G. (1948) *Nature 162*, 963.
Plank, D. W., & Howard, J. B. (1988) *J. Biol. Chem. 263*, 8184-8189.
Phillips, M. A., Fletterick, R., & Rutter, W. J. (1990) *J. Biol. Chem. 265*, 20692-20698.
Robbins, A. H., & Stout, C. D. (1985) *J. Biol. Chem. 260*, 2328-2333.
Robbins, A. H., & Stout, C. D. (1989a) *Proteins 5*, 289-312.
Robbins, A. H., & Stout, C. D. (1989b) *Proc. Natl. Acad. Sci. U.S.A. 86*, 3639-3643.
Robbins, A. H., Stout, C. D., Piszkiewicz, D., Gawron, O., Yoo, C. S., Wang, B. C., & Sax, M. (1982) *J. Biol. Chem. 257*, 9061-9063.
Roncero, M. I. G., Jepsen, L. P., Streman, P., & Heeswijick, R. V. (1989) *Gene 84*, 335-343.
Rose, I. A., & O'Connell, E. L. (1966) *J. Biol. Chem. 242*, 1870-1879.
Rosenthal, E. R., & Calvo, J. M. (1990) *Nucleic Acids Res. 18*, 3072.
Rouault, T. A., Tang, C. K., Kaptain, S., Burgess, W. H., Haile, D. J., Samaniego, F., McBride, O. W., Harford, J. B., & Klausner, R. D. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 7958-7962.
Rouault, T. A., Stout, C. D., Kaptain, S., Harford, J. B., & Klausner, R. D. (1991) *Cell 64*, 881-883.
Schloss, J. V., Porter, D. J. T., Bright, H., & Cleland, W. W. (1980) *Biochemistry 19*, 2358-2362.
Schloss, J. V., Emptage, M. H., & Cleland, W. W. (1984) *Biochemistry 23*, 4572-4580.
Schrag, J. D., Li, Y., Wu, S., & Cygler, M. (1991) *Nature 351*, 761-764.
Sprang, S., Standing, T., Fletterick, R. J., Stroud, R. M., Finer-Moore, J., Xuong, N. H., Hamlin, R., Rutter, W. J., & Craik, C. S. (1987) *Science 237*, 905-909.
Switzer, R. L. (1989) *BioFactors 2*, 77-86.
Telser, J., Emptage, M. H., Merkle, H., Kennedy, M. C., Beinert, H., & Hoffman, B. M. (1986) *J. Biol. Chem. 261*, 4840-4846.
Walsh, C. (1979) in *Enzymatic Reaction Mechanisms*, pp 525-532, W. H. Freeman and Co., San Francisco, CA.
Werst, M. M., Kennedy, M. C., Beinert, H., & Hoffman, B. M. (1990a) *Biochemistry 29*, 10526-10532.
Werst, M. M., Kennedy, M. C., Houseman, A. L. P., Beinert, H., & Hoffman, B. M. (1990b) *Biochemistry 29*, 10533-10540.
Whitlow, M., Howard, A. J., Finzel, B. C., Poulos, T. L., Winborne, E., & Gilliland, G. L. (1991) *Proteins 9*, 153-173.
Yeates, T. O., & Rini, J. M. (1990) *Acta Crystallogr. A46*, 352-359.
Zheng, L., Andrews, P. C., Hermodson, M. A., Dixon, J. E., & Zalkin, H. (1990) *J. Biol. Chem. 265*, 2814-2821.
Zheng, L., Kennedy, M. C., Beinert, H., & Zalkin, H. (1992) *J. Biol. Chem.* (in press).

Probing Dynamic Changes in rRNA Conformation in the 30S Subunit of the *Escherichia coli* Ribosome†

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ABSTRACT: Ribosomal RNA molecules within each ribosomal subunit are folded in a specific three-dimensional form. The accessibility of specific sequences of rRNA of the small ribosomal subunit of *Escherichia coli* was analyzed using complementary oligodeoxyribonucleotides, 6-15 nucleotides long. The degree of hybridization of these oligomers to their RNA complements within the 30S subunit was assessed using nitrocellulose membrane filter binding assays. Specifically, the binding of short DNA oligomers (hexameric and longer) complementary to nucleotides 919-928, 1384-1417, 1490-1505, and 1530-1542 of 16S rRNA was monitored, and in particular how such binding was affected by the change in the activation state of the subunit. We found that nucleotides 1397-1404 comprise an unusually accessible sequence in both active and inactive subunits. Nucleotides 919-924 are partially available for hybridization in active subunits and somewhat more so in inactive subunits. Nucleotides 1534-1542 are freely accessible in active, but only partially accessible in inactive subunits, while nucleotides 1490-1505 and 1530-1533 are inaccessible in both, under the conditions tested. These results are in general agreement with results obtained using other methods and suggest a significant conformational change upon subunit activation.

Within the 2 subunits of the *Escherichia coli* ribosome, there are over 50 different proteins and 3 rRNA molecules. It is becoming clear that the rRNA performs an active role in the ribosomal function. For instance, rRNA has been shown to have a role in mRNA placement (Shine & Dalgarno, 1974; Calogero et al., 1988) and tRNA alignment (Prince et al.,

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1982; Ofengand et al., 1986; Moazed & Noller, 1986; Marconi & Hill, 1989) during protein biosynthesis. rRNA is also involved in the interactions between ribosomal subunits (Santer & Shane, 1977; Herr et al., 1979; Tapprich & Hill, 1986), and 5S rRNA may play a role in the peptidyl transferase reaction (Erdmann & Wolters, 1986).

An arsenal of techniques has been used to define the rRNA–rRNA, protein–protein, and rRNA–protein interactions within ribosomal subunits as well as the three-dimensional structure of the whole assembly. On the basis of the results emanating from these studies, several models have been proposed, describing the structural contribution of rRNA to the overall architecture of the subunit (Noller et al., 1986; Noller & Lake, 1984; Expert-Bezancon & Wollenzien, 1985; Brimacombe et al., 1988; Stern et al., 1988; Ericson & Wollenzein, 1989).

The resolution of this image is not high, and the placement of single-stranded regions of rRNA is still obscure. At the same time, information on these rRNA regions would be especially meaningful, since they clearly have the potential to interact with the single-stranded polynucleotides which participate in protein synthesis and so are likely candidates for centers of functional activity.

Nuclease accessibility and chemical modification data have been used to define regions of 16S rRNA that are exposed and single-stranded in the 30S subunit in various functional states (Brow & Noller, 1983; van Stolk & Noller, 1984; Moazed et al., 1986; Meier & Wagner, 1985; Baudin et al., 1989). Because it is likely that functionally important sites are highly constrained from variation in evolutionary terms, phylogenetic comparisons have been made using the 16S rRNA sequences of many organisms (Woese, 1980, 1987; Woese et al., 1983; Gutell et al., 1985; Gutell & Woese, 1990). It has been found that the regions determined by chemical means to be single-stranded are also among the most highly conserved, reinforcing the supposition that they are of functional importance.

The hybridization of complementary DNA oligomers to selected sites in rRNA has been used to assay the function of the 3′-end of 16S rRNA (Backendorf et al., 1980, 1981), to obtain topographical information on the position of rRNA sequences in the intact subunit (Lasater et al., 1988), and to establish tRNA interaction sites (Marconi & Hill, 1989), subunit-association interaction sites (Tapprich & Hill, 1986), or antibiotic binding sites (Marconi et al., 1990). Short oligodeoxyribonucleotides 6–8 nucleotides in length give cDNA/rRNA hybrid binding energies sufficiently large to be stable under the experimental conditions, yet small enough to allow competition with normal ligands of the amino acid polymerization reactions, such as mRNA or tRNA.

In this paper, we report on the relative ability of 16S rRNA regions 919–928, 1384–1417, 1490–1505, and 1530–1542 to hybridize with cDNA oligomers of lengths 6–15 nucleotides. The relative degree of accessibility of these regions in the active compared to the inactive conformations of 30S subunits is assessed.

Experimental Procedures

Ribosomes and Subunits. Ribosomes were prepared from *E. coli* strain MRE600 (Grain Processing Co., Muscatine, IA) by the methods of Hill et al. (1969). Ribosomal subunits were then purified using zonal centrifugation, as outlined by Tam and Hill (1981), except that the subunits were pelleted out of the sucrose using centrifugation rather than using ethanol precipitation. Tight-couple ribosomes were separated from crude ribosomes by the method of Noll et al. (1973) as modified by Hill and Tassanakajohn (1987). Ribosomes and ribosomal subunits were stored in small aliquots at −70 °C.

Subunits were checked for homogeneity by sedimentation velocity centrifugation using schlieren optics in a Spinco Model E analytical ultracentrifuge. The integrity of the rRNA was monitored by electrophoresis on a 5% polyacrylamide gel containing 8 M urea, 89 mM Tris–borate, pH 8.3, and 1 mM EDTA.

Activation and Inactivation. Under our isolation conditions, ribosomal subunits begin in an inactive state. Inactive subunits, suspended in the appropriate buffer, were used directly for all assays calling for inactive subunits. Activated subunits were prepared by incubating a sample of 30S subunits in activation buffer containing 15 mM $MgCl_2$, 150 mM KCl, 10 mM Tris-HCl, pH 7.4, and 2 mM DTT at 37 °C for 20 min (Zamir et al., 1971).

Activity of the subunits was assayed using the poly(U)-directed $tRNA^{Phe}$ binding activity assay as follows: deacylated $tRNA^{Phe}$ (Boehringer-Mannheim) was labeled with ^{32}P according to the method of Lill et al. (1986); 15 pmol of activated 30S subunits, 5 μg of poly(U), and 30 pmol of $tRNA^{Phe}$ were incubated in 50 μL of the activation buffer at 4 °C for 30 min, spotted onto nitrocellulose membrane filters, washed 5 times with 1-mL aliquots of activation buffer, then immersed in Scintiverse (Fisher Scientific) scintillation fluid, and counted in a Packard scintillation counter. Activated subunits gradually lose tRNA binding activity when they are stored at 4 °C. Under the conditions used in the experiments outlined below, a 4-h incubation at 4 °C generally reduced the tRNA binding activity to 80–85% of the original value.

Only homogeneous subunits which showed both intact rRNA and poly(U)-directed $tRNA^{Phe}$ binding of 0.45–0.80 (mol/mol) for activated subunits and poly(U)-directed $tRNA^{Phe}$ binding of less than 0.02 (mol/mol) for inactive subunits were used for further experiments. Reassociated subunits were also assayed for their ability to carry out in vitro protein synthesis (Traub et al., 1979).

Synthesis and Purification of Oligodeoxyribonucleotides. Oligodeoxyribonucleotides were synthesized on a Biosearch Model 8600 automated DNA synthesizer utilizing β-cyanoethyl diisopropylphosphoroamidite chemistry. All reagents were obtained from Biosearch with the exception of HPLC-grade acetonitrile (Baker) and methylene chloride (Baker). The cDNA oligomers were deblocked according to the manufacturer's protocol and purified both before and after removal of the 5′-dimethoxytrityl (DMT) blocking group by reverse-phase high-performance liquid chromatography (RP-HPLC) (Gilson).

Labeling of the cDNA Oligomers. Purified DNA oligomers were then labeled at the 5′ terminus by using [γ-^{32}P]ATP (New England Nuclear) and T4 polynucleotide kinase (United States Biochemical) according to the method of Chaconas and Van de Sande (1980). The labeled probes were separated from unincorporated nucleotides and salts using Nensorb-20 columns (DuPont) according to the supplier's instructions.

Binding of cDNA Oligomers to 30S Subunits. (A) Filter Binding Assays. The binding of cDNA probes to 30S subunits was assayed by incubating the subunits with 5′-^{32}P-labeled cDNA (with specific activities between 300 and 1000 cpm/pmol) for 4 h on ice in 50 μL of activation buffer. To allow comparison of cDNA binding to active versus inactive 30S subunits, the subunits were brought to a concentration of 1 pmol/μL in activation buffer, and half was kept at 4 °C, which prevents activation, while the other half was incubated at 37 °C for 20 min (Zamir et al., 1971) as outlined above. For each experimental point, 25 pmol of subunits was incubated

with increasing ratios of cDNA oligomer, to yield a saturation binding curve. At the end of the incubation period, the reaction mixtures were diluted with 1 mL of activation buffer and immediately filtered through HAWP 0.45-μM nitrocellulose filters (Millipore) followed by 2–4 1-mL washes of the filter with activation buffer. The amount of complex formation between radiolabeled cDNA oligomer and 30S subunit was measured by liquid scintillation counting of the filters. All reactions were performed in triplicate in order to correct for variations in the experimental procedure, and each experiment with a given cDNA oligomer was done at least 2 separate times.

In addition, all cDNA oligomers were incubated with 50S subunits as a control for nonspecific binding of probe to a protein–nucleic acid complex (computer sequence searches did not reveal any full sequence identities in accessible regions of 23S rRNA for any of the probes used).

(B) Gradient Binding Assays. Active and inactive 30S subunits (100 pmol) were incubated for 30 min with a 20:1 excess of the indicated cDNA having a specific activity of about 1000 cpm/pmol, in a volume of 100 μL of activation buffer. The mixtures were layered onto 5-mL 5–25% linear sucrose gradients (DEPC-treated sucrose, gradients made up in 1× activation buffer) and centrifuged in a Beckman SW50.1 rotor at 45 000 rpm (300000g) for 2.5 h. Gradients were dripped into 15 equal fractions, and both the A_{260} and cpm of each fraction were monitored.

Binding Specificity. To determine the site(s) of hybridization, 50 μg of purified 16S rRNA was incubated with 500 pmol of a given cDNA oligomer (approximately a 12:1 molar ratio) in 50 μL of RNase H buffer (20 mM Tris-HCl, pH 7.9, 15 mM $MgCl_2$, and 150 mM KCl) and 2–4 units of RNase H (Pharmacia) for 30–60 min at 37 °C, according to Donis-Keller (1979). After incubation, the reactions were extracted with phenol, and the nucleic acid fragments were analyzed by electrophoresis on 8 M urea/10% polyacrylamide gels in TBE buffer on 15 cm × 0.8 cm gels, run at 200 V for 7 h.

The smaller fragment produced by RNase H digestion of 16S rRNA and a given cDNA oligomer in the region 1530–1542 would be only one to seven nucleotides in length. As it is difficult to recover and resolve such a small fragment, a "secondary" cDNA oligomer giving a larger digestion product was co-incubated with the reaction, essentially a "double-digest" as performed on DNA with restriction endonucleases. These digestions were done using 500 pmol of cDNA 1396–1403 or 500 pmol of cDNA 1394–1408 as the "secondary cDNA", along with the "primary cDNA", i.e., the cDNA to the 1530–1542 target region. The smaller rRNA fragments produced by digestion of the hybrids formed with these secondary cDNA oligomers were about 130 nt in length, a size easily resolvable on a 10% polyacrylamide–7.5 M urea gel, and the size change in the reference fragment (produced by the secondary cDNA), due to digestion at the primary cDNA hybridization site, was more readily apparent.

Results

Oligodeoxyribonucleotide Synthesis. The secondary structure map proposed by Noller (1984) and modified by Woese et al. (1990) (see Figure 1) was used to design the sequence of DNA oligomers complementary to the 3′-terminal domain of 16S rRNA. The cDNA sequences are listed in Table I and shown in Figure 4a. In most cases, the target sites are proposed to be single-stranded. The cDNA oligomers have been identified according to the sequence to which they are complementary. For example, the cDNA designated 1534–1541 is complementary to 16S rRNA nucleotides 1534–1541.

Table I: Synthetic Oligodeoxyribonucleotides Used To Probe 30S Ribosomal Subunits, and Some Predicted Physical Constants[a]

cDNA sequence	16S rRNA sequence	probe length	% GC	ΔG (kcal)	T_d (°C)
GTC AAT	919–924	6	33	−9.6	21
CCC CGT	923–928	6	83	−22.2	27
GGC CCG	1384–1389	6	100	−25.0	29
GTA CAA	1390–1395	6	33	−9.6	21
GTG TAC	1392–1397	6	50	−10.2	23
TGT GTA	1393–1398	6	33	−9.6	21
GTG TGT	1394–1399	6	50	−10.2	23
GGT GTG	1395–1400	6	67	−13.0	25
CGG TGT	1396–1401	6	67	−16.2	25
GCG GTG	1397–1402	6	83	−16.2	27
GGC GGT	1398–1403	6	83	−22.2	27
GGG CGG	1399–1404	6	100	−25.0	29
CGG GCG	1400–1405	6	100	−25.0	29
ACG GGC	1401–1406	6	83	−22.2	27
GAC GGG	1402–1407	6	83	−19.4	27
TGA-CGG	1403–1408	6	67	−16.2	25
GGC GGT G	1397–1403	7	86	−24.0	31
GCG GTG TGT	1394–1402	9	67	−25.2	35
GGC GGT GT	1396–1403	8	75	−26.6	33
ACG GGC GG	1399–1406	8	87	−44.4	35
TGACGGGCGTGTGT	1394–1408	15	67	−47.8	55
GTG ATC	1530–1535	6	50	−10.6	23
AGG TGA	1532–1537	6	50	−13.8	23
GAG GTG	1533–1538	6	67	−13.8	25
GGA GGT	1534–1539	6	67	−16.6	25
AGG AGG	1535–1540	6	67	−16.6	25
AGG TGA TC	1530–1537	8	50	−17.8	29
AAG GAG GT	1534–1541	8	50	−20.0	29
TAA GGA G	1536–1542	7	43	−14.6	25
TAAGGAGGTGATC	1530–1542	13	46	−30.2	43

[a] ΔG calculations from Tinoco et al. (1973). T_d from Wallace et al. (1979). ΔG is the free energy when all bases are Watson–Crick hydrogen-bonded. T_d is the temperature at which half of the duplex is dissociated for probes less than 14 nt in length. It was assumed that the stability of an RNA–DNA duplex and the effect of lower monovalent cation with magnesium approximately equaled each other.

Physicochemical Properties of Oligonucleotides. The degree of binding in a complex molecule is partially due to the physicochemical properties of the hybrid being formed. Table I provides a compilation of some of the predicted physical constants for the cDNA/rRNA hybrids we have studied. The cDNA binding to 30S subunits in our study ranged from 0.0 to 1.2 mol/mol, except in two cases, detailed below, in which unusually high binding was seen. The free energy value, ΔG, calculated from Tinoco et al. (1973), was not a particularly useful predictive index of the ability of a cDNA oligomer to hybridize to its target site. However, when binding was observed, the free energy did seem to relate to the molar excess of probe required to achieve binding saturation when binding was observed. Longer probes generally required lower excess to reach binding saturation than did shorter probes spanning the same region.

Binding of Oligodeoxyribonucleotides to 30S Subunits. The extent of interaction between radiolabeled cDNA oligomer and 30S ribosomal subunits was assayed by a nitrocellulose membrane filter binding assay. Oligodeoxyribonucleotide retention by filters in the absence of subunits varied depending upon the particular cDNA and the cDNA concentration, so the retained counts in the absence of subunits were determined for every experiment and these background values subtracted from the specific binding data. The numbers reported are averages of from 6 to 18 measurements with the range of values as noted in Table II. We found that for all of the cDNA oligomers tested, the binding to 50S subunits was almost negligible, amounting to less than 0.1 (mol/mol) in most

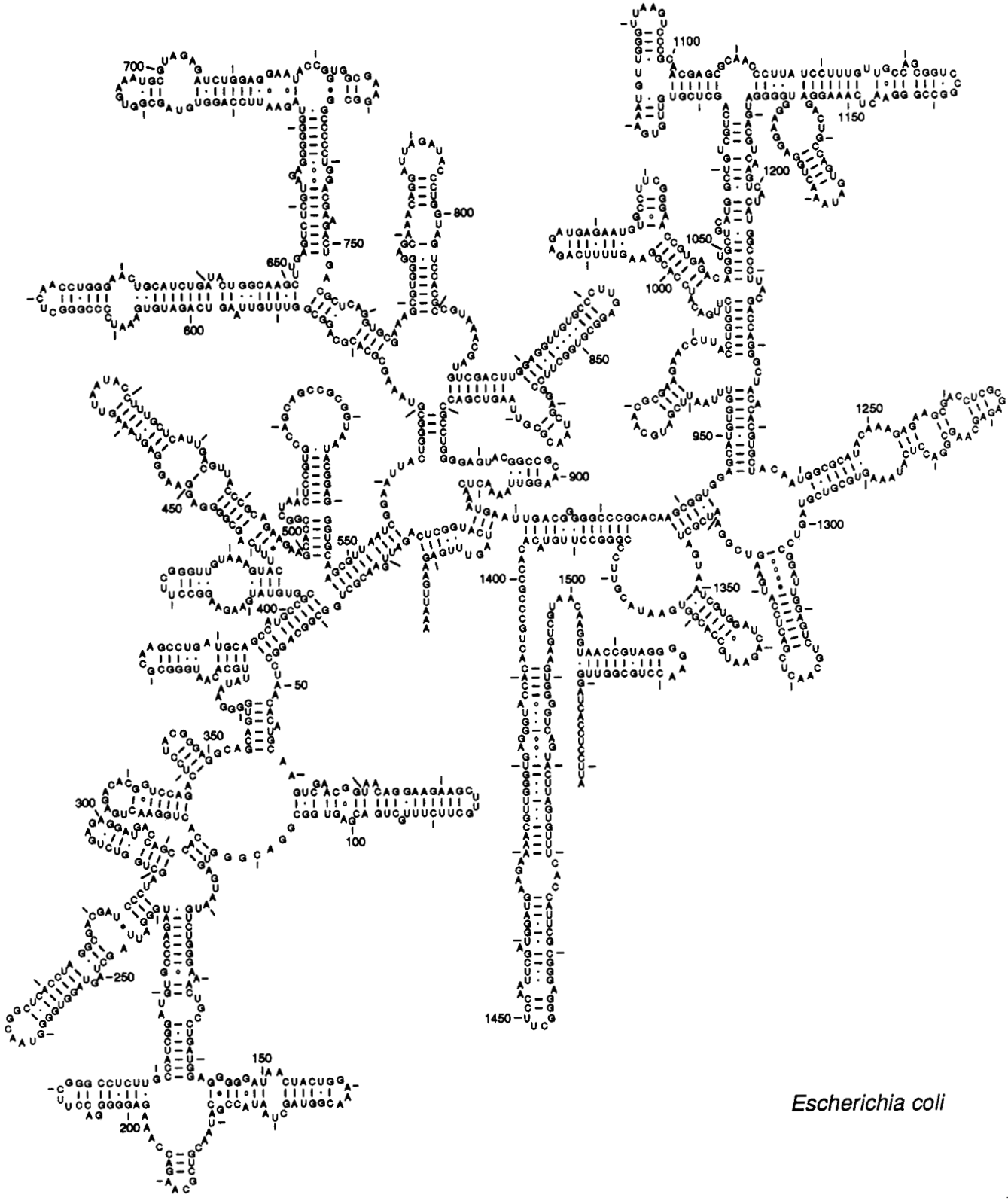

FIGURE 1: Secondary structure map of 16S rRNA. From Woese et al. (1990).

cases, even at the highest incubation ratios (data not shown).

The stoichiometry of binding of cDNA oligomers to 30S subunit was measured using increasing levels of cDNA with a constant quantity of subunits, as shown in Figure 2. To test probe stability, we initially selected a pentamer (all G/C) in a region in which a longer probe was known to bind (Hill & Tassanakajohn, 1987), complementary to 1400–1404. This interaction was insufficiently stable to give saturation of binding even at a 40:1 molar ratio of probe to subunit. Similar experiments were done with pentamers targeted to the 1533–1537 and 1535–1539 sequences with identical results (data not shown). Hexameric probes in the regions containing nucleotides 1397–1404 and 1535–1541 were shown to saturate quite well and provide sufficient stability for these assays. Therefore, we chose to use hexameric probes in our binding studies since they were the shortest oligomers for which binding saturation was routinely obtainable, albeit at somewhat high molar ratios. As the size of the probe increased, the molar

Table II: Nitrocellulose Membrane Filter Binding Assays[a]

cDNA	max extent of cDNA binding (mol/mol of subunits) active	inactive	cDNA:30S ratio for max binding	cDNA binding to 30S at 4:1 probe ratio active	inactive
919–924	0.2	0.4	20:1	0.1	0.1
923–928	0	0	8:1	0	0
1384–1389	0.1	0.1	32:1	0	0
1390–1395	0.1	0.1	20:1	0	0
1392–1397	0	0	12:1	0	0
1393–1398	0	0	12:1	0	0
1394–1399	0	0.1	16:1	0	0
1394–1402	0	0.1	4:1	0	0.1
1394–1408	1.0 ± 0.1	0.6 ± 0.1	3:1	1.0	0.6
1395–1400	0.1	1.0 ± 0.1	15:1	0.1	0
1396–1401	0.1	0.3 ± 0.2	15:1	0	0.1
1396–1403	1.5	1.0	4:1	1.5	1.0
1397–1402	2.0 ± 0.2	2.0 ± 0.2	12:1	2.0	1.5
1397–1403	1.0	0.5	12:1	0.5	0.2
1398–1403	1.0	0.8	4:1	1.0	0.8
1399–1404	1.0	0.8	15:1	0.2	0.2
1399–1406	1.0 ± 0.1	1.0 ● 0.1	15:1	0.7	0.6
1400–1405	0.2	0.2	15:1	0.1	0.1
1401–1406	0	0.1	4:1	0	0.1
1402–1407	0.2	0.3 ± 0.1	8:1	0.1	0.2
1403–1408	0.1	0.1	12:1	0	0
1404–1409	0	0	12:1	0	0
1410–1417	0	0.1	9:1	0	0.1
1490–1497	0	0	20:1	0	0.1
1495–1503	0	0.1	20:1	0	0
1498–1505	0.1	0	20:1	0	0
1530–1535	0.1	0.1	13:1	0	0
1530–1537	0.1	0.1	17:1	0	0
1530–1542	1.0	0.2	2:1	1.0	0.2
1532–1537	0.1	0.1	16:1	0	0
1533–1538	0.1	0.1	16:1	0	0
1534–1539	1.0 ± 0.1	0.3	20:1	0.2	0
1534–1541	0.9 ± 0.1	0.3	2:1	0.9	0.2
1535–1540	1.0	0.6	20:1	0.2	0.1
1536–1542	0.5	0.2	12:1	0.4	0.2

[a] Values reported are numerical averages of from 6 to 18 measurements which had a range of less than ±20% unless otherwise noted.

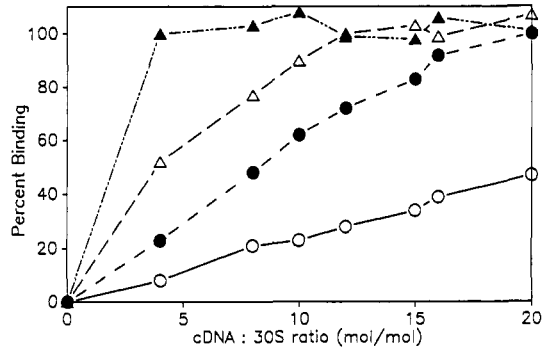

FIGURE 2: Nitrocellulose filter binding assays showing the binding of complementary oligodeoxyribonucleotide probes of varying lengths to 30S ribosomal subunits. Increasing concentrations of 5′-^{32}P-labeled cDNA (specific activities from 200 to 1000 cpm/pmol) were incubated with 25 pmol of activated 30S subunits in 50 μL of binding buffer (10 mM Tris-HCl, pH 7.4, 150 mM KCl, 15 mM $MgCl_2$, and 0.2 mM DTT) for 4 h on ice. Reactions were then diluted to 1 mL with binding buffer and immediately filtered through nitrocellulose membranes (0.45 μm HAWP, Millipore), washed twice with 1 mL of binding buffer, air-dried, and then counted using liquid scintillation. The symbols are as follows: cDNA 1400–1404, open circles; cDNA 1399–1404, closed circles; cDNA 1397–1403, open triangles; cDNA 1394–1408, closed triangles.

excess required to reach saturation of binding decreased.

These results are illustrated in Figure 2 for four cDNA oligomers complementary to portions of the 1400 region of 16S rRNA. Binding of the pentameric oligomer 1400–1404

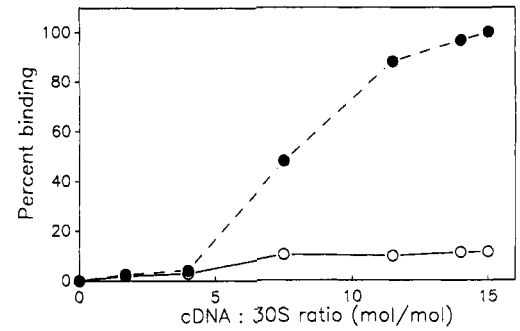

FIGURE 3: Nitrocellulose filter binding assay of binding of cDNA 1395–1400 to active 30S ribosomal subunits (open circles) and to inactive 20S subunits (closed circles). The subunits were activated by heating at 37 °C for 20 min prior to the addition of probe (see Experimental Procedures). Inactive subunits were left on ice. Buffer and filtration conditions are identical to those given in the legend to Figure 2.

did not saturate within the incubation concentrations used; the hexameric probe 1399–1404 reached a level of 1.0 mol/mol saturation, but required a 20:1 DNA:subunit ratio excess to achieve this, while the oligomer complementary to nucleotides 1394–1408 reached the same level of saturation with a 4:1 DNA:subunit ratio.

In the cases of longer cDNAs, we found that as long as such probes encompassed at least six bases complementary to an accessible or open region, the longer probes generally saturated at the same or higher binding maxima as the hexamers (except the anomalous binding of 1397–1402, discussed below). The length was roughly inversely proportional to the molar excess ratio of probe to subunit required to reach the binding maximum.

Oligodeoxyribonucleotide Binding Results. Because we were primarily interested in conformational changes which might be associated with the activation state of the 30S subunit, all cDNAs were assayed using both active and inactive 30S subunits. Since maximal tRNA binding to active 30S subunits has been reported to occur at 15 mM Mg^{2+} (Rheinberger & Nierhaus, 1987), 15 mM Mg^{2+} was used in all assays. However, we have observed no change in cDNA oligomer binding in the range from 5 to 15 mM Mg^{2+} (Marconi & Hill, 1989). The results of hybridizing cDNA oligomers to the 3′-end of 16S rRNA in active and inactive 30S subunits are summarized in Table II. In the region containing nucleotides 919–928, only the 919–924 region showed binding activity. Interestingly, the inactive conformation showed twice the binding activity for this cDNA than did the active form.

In the 1384–1408 region, the binding activity was almost wholly confined to the region containing nucleotides 1395–1406, and the subset containing nucleotides 1397–1404 was almost uniformly able to bind cDNAs at 1.0 mol/mol binding saturation in either subunit state. Nucleotides 1395 and 1396 seem to be considerably more exposed in the inactive conformation than in the active conformation (Figure 3). The contiguous nucleotides 1405–1408 were wholly unavailable in both conformations, as well as the regions spanning 1410–1417 and 1490–1505. Overall this seems to indicate that there is an eight-nucleotide "window" in this region of 16S rRNA that is accessible to an oligonucleotide in both the active and inactive conformations and this "window" may become somewhat larger in the inactive conformation.

It should be noted here that two probes in this region, 1397–1402 and 1396–1403, gave higher than stoichiometric binding. The saturation curves were not biphasic, the experiments were repeated many times, and RNase H digestion with native and melted 16S rRNA resulted in only single

digestion products with each of these probes. These are all indications that only a single site was being bound in each case. It is possible that the cDNAs may interact with themselves in some fashion such that more than one was bound at the same site for each subunit or there may be a specific interaction with a ribosomal protein. The results from these probes are unusual and need to be investigated more fully, but are not critical to the overall conclusions, nor do they contradict the results of neighboring probes. While curious, they are reported here for the sake of completeness.

There is also an anomalously low value for cDNA 1396–1401 in the inactive subunits. It is possible that there may be some interference with nucleotide 1396 which is not allowing the single nucleotide to be base-paired. When the cDNA oligomer goes to nucleotide 1395, this interference is mitigated.

At the 3′-end of 16S rRNA, certain regions appeared to be inaccessible in both the active and the inactive conformations, as demonstrated by the low binding of cDNA hexamers 1530–1535, 1532–1537, and 1533–1538 and the cDNA octamer 1530–1537. An abrupt change was observed with the adjacent hexameric cDNAs 1534–1539 and 1535–1540, which apparently had binding sites which were completely accessible in the active 30S conformation but which were considerably less accessible in the inactive 30S conformation. The octameric cDNA 1534–1541 showed a similar hybridization pattern, as did the oligomer 1530–1542, which spanned the entire region.

Binding of cDNA Containing a Mismatch. We modified two long probes in the 1400 region, one by creating a mismatch at position 1400 (in the middle of the binding window) and the other by creating a mismatch at position 1406. The first probe mismatched at position 1400 [probe 1394–1408 (m1400)] should have six nucleotides (1394–1399) at one end, and eight nucleotides (1401–1408) at the other which remain complementary to the target region and so are able to promote stable binding to 16S rRNA. However, this probe does not have six contiguous complementary bases in the region 1397–1404, previously demonstrated to be the most accessible to hexamers. This cDNA, when hybridized to active 30S subunits, decreased in binding from 100% to 17%, which suggests that the mismatch significantly destabilized the binding of the probe. It also reinforces the results obtained from the hexameric binding data, that nucleotides 1397–1404 are of great importance to the stability of binding of cDNA oligomers.

Probe 1394–1408 (m1406), which was mismatched at position 1406, was fully complementary to the accessible region, yet showed a moderate decrease in binding (from 100% to 67%). This suggests that some binding stabilization occurs as a result of the interactions of the distal ends of the probes. Another possibility is that complementarity in the distal regions causes a conformational change, such as straightening a kink in the rRNA, which promotes probe binding stability. This issue is discussed more fully below.

Specificity of Oligodeoxyribonucleotide–Subunit Interactions. Binding data are interpretable in terms of structure and function only if the binding site is known. Several lines of evidence suggested that the binding in the above instances was specific. The stoichiometry of cDNA binding to 30S subunits was consistent with single specific binding sites; saturation of subunit binding was observed, and cDNA–rRNA binding significantly exceeded 1.0 mol/mol in only two cases: cDNA 1397–1402 and cDNA 1396–1403. Also, in no case did we observe a biphasic curve, which would be an indication that a secondary site was beginning to fill.

Computerized sequence homology searches of both 16S rRNA and 23S rRNA identified rRNA sequences that were wholly or partially complementary to each cDNA oligomer. The probability of cDNA hybridization can be evaluated from a combination of the free energy calculations and the availability of the partially homologous sequence in the secondary structure rRNA maps of Noller et al. (1986). In most instances, the alternative targets are at least partially located in stable stem structures, suggesting that they are unlikely candidates to bind the cDNA oligomers. While such theoretical considerations are useful as a guideline, experimental evidence is preferable.

To obtain such evidence, 16S rRNA was isolated, melted, and hybridized to cDNA probes under the identical conditions used for subunit–cDNA hybridization, at a 12:1 probe:16S rRNA ratio, and RNase H digestion was performed as described under Experimental Procedures. Double digestions were performed when necessary, as described under Experimental Procedures, and the digestion products were analyzed by electrophoresis. In every case, digestion yielded a product of the expected size, and in the double digests, it was the expected reference fragment which was decreased in size upon the appearance of the fragment resulting from digestion with the primary cDNA. RNase H can hydrolyze RNA bonds at multiple sites within the hybrid, so the presence of more than one band clustered at the expected size was not unexpected. Oligomer 1530–1542 apparently did not provide a substrate for RNase H as no digestion product was observed in this case, perhaps because it formed a flush 5′-end with the 3′-end of 16S rRNA. However, incubation of this oligomer with either 16S rRNA or 30S subunits and RNase H did not yield any other fragments, suggesting that it did not bind stably enough to secondary sites to provide a substrate for RNase H. Incubation of intact subunits with cDNA and RNase H sometimes yielded no rRNA fragments at all, presumably because the cDNA–rRNA substrate was sequestered where the enzyme could not gain access. We did not have a filter binding assay for cDNA–16S rRNA binding saturation, so the 12:1 incubation ratio was arbitrarily chosen.

In all cases, only the expected band was observed for target site binding, even when the actual molar excess required for binding saturation with subunits was only 2:1. Under these conditions, one might have expected some minor binding sites to fill and be cleaved by the RNase H, particularly in the absence of shielding proteins, but such were not observed. Even in the cases where we obtained greater than 100% binding (cDNA 1396–1403 and 1397–1402), we observed no additional cleavages, which was surprising. It is possible that the conformation of 16S rRNA is sufficiently different in the ribosomal subunit that another sequence becomes available which is not present in the free rRNA.

Gradient Binding Assays. Another technique which demonstrates ligand binding specificity is the sucrose gradient comigration assay, or gradient binding assay (Backendorf et al., 1981). Because of the dilution effects as the 30S subunits migrated into the gradient, a high molar ratio of cDNA oligomer to subunit was used during the incubation. The difference in probe binding between active and inactive subunits is readily seen in the gradient binding assay, although the overall stoichiometry was different than that obtained in the filter binding assays (data not shown).

Discussion

We have looked at four different regions of 16S rRNA (nucleotides 919–928, 1384–1417, 1490–1505, and 1530–1542; see Figure 4a) using short, complementary DNA probes.

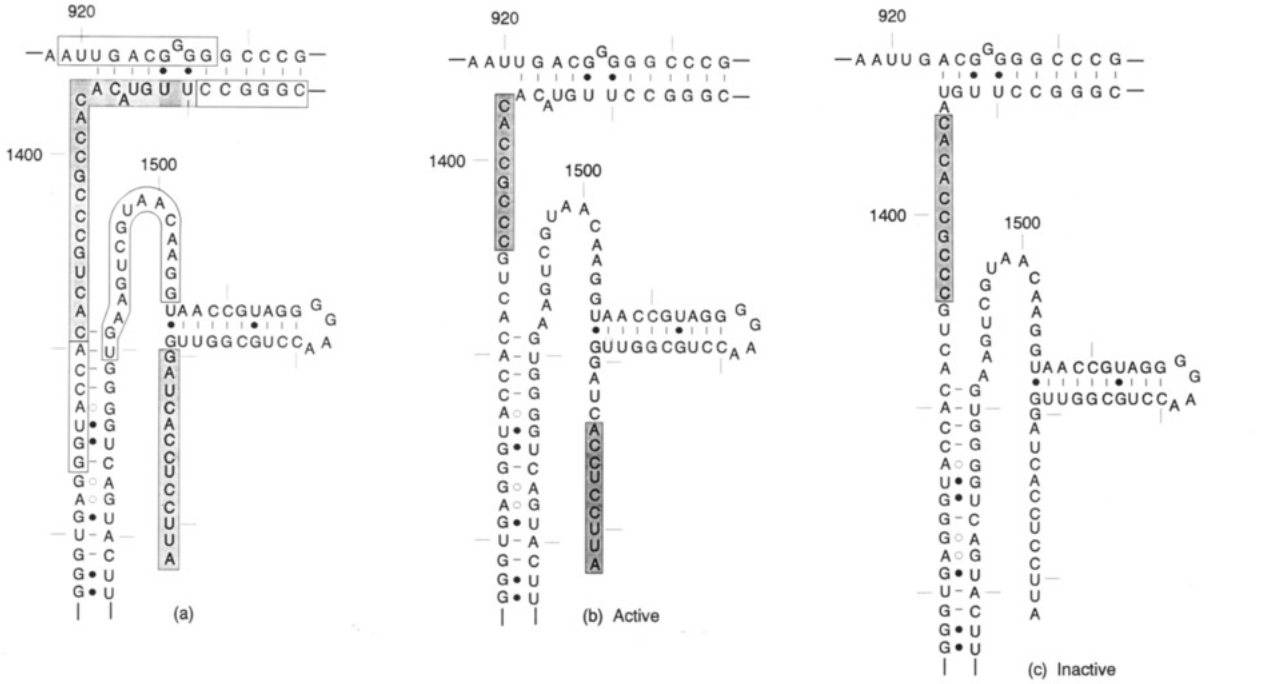

FIGURE 4: Detailed representation of the regions investigated in this study. Figure 4a shows the regions probed using complementary DNA oligomers. The shaded areas are those regions in which overlapping hexamers were used. Figure 4b is a representation of the structure postulated for the active conformation. Shaded areas are those regions in which the cDNA oligomers bound almost stoichiometrically. Figure 4c is a representation of a possible structure for the inactive conformation. The shaded area is the only region in which cDNA oligomers bound almost stoichiometrically. Regions not shaded in (b) or (c) showed either no binding or binding that was significantly less than stoichiometric (see Table II for the values).

These regions were selected because they had previously been suggested to be involved in some aspect of ribosome function. The results of this study suggest that only portions of these putatively single-stranded regions are actually accessible to short DNA oligomers and that the availability of portions of these sites varies, depending upon the activation state of the subunit.

We used complementary hexameric probes to "walk" through these regions and to determine which bases might be available for hydrogen bonding. We found that we could discriminate the availability of bases to single-base precision. For instance, in the active conformation, hexameric cDNA 1534–1539 was able to form a stable hybrid, suggesting that all six complementary nucleotides were available. Yet the adjacent hexameric cDNA, complementary to nucleotides 1533–1538, showed no binding, suggesting that nucleotide 1533 was not available for base-pairing.

There are several reasons that a nucleotide or a region may or may not be available for annealing. First, it is possible that the target region may be wholly or partially sterically hindered from accepting a complementary DNA oligomer, either due to base-pairing to other regions of rRNA or due to quaternary interactions, such as the presence of ribosomal proteins. It is also possible that the target region may have a constrained conformation, such as a bend or a kink, which makes the obligatory A-form double helix upon annealing impossible to achieve. Even more subtle hindrances could occur due to non-base-pair bonds (such as backbone phosphate hydrogen bonds to other bases) or similar interactions. The complementary DNA oligomers themselves may perturb the rRNA structure. Any or a mixture of the above constraints could render a site unavailable for hybridization to a hexameric probe. By comparison of the same site under varying conditions, our results can only indicate the gain or loss of such accessibility, from whatever cause. By "walking" hexameric probes through a region, we can determine, to some degree of accuracy, the specific nucleotides which are not available for base-pairing [see Hill and Tassanakajohn (1987)].

A more general question, dealing with the absolute stoichiometry of annealing, must also be addressed. It has been our experience in this study as well as previous studies that saturation of binding, as measured using filter binding assays, is significantly less than stoichiometric, at times only amounting to 20–30% subunits bound. At other times, binding approaches 100%. Saturation can occur at a 1:1 probe:subunit ratio or at considerably higher ratios. The pattern is generally the longer the probe, the lower the ratio. We have chosen to use hexameric probes since they are the minimal size which can uniformly provide binding to a target region (see discussion below). In so doing, a high ratio of probe to subunit is generally needed to provide saturation. Only in the "window" region (discussed below) have we found an exception.

The lack of stoichiometry can be due to thermodynamics or kinetics, or may indicate a heterogeneity of available sites. Since we use such short oligomers, unquestionably we are losing some binding as the filters are rinsed and the thermodynamic equilibrium changes. Additionally, the kinetic effects will vary for the various probes, since target sites will have varying accessibility. There are also numerous partially identical target sites which will compete with probe binding. Analysis of ligand binding to multiple, nonidentical sites is a foreboding task. The additional complexity of possible ribosomal substates presents another significant variable. Since the ribosome is dynamic, as this study clearly indicates, conformation fluctuations of various rRNA regions would be expected. Since the translational state of the ribosomes used cannot be controlled, such differences could modify the quaternary structure as well.

All of the above present themselves as potential reasons for obtaining less than stoichiometric binding of a short cDNA oligomer to a target region on rRNA within a subunit. By carefully obtaining data from side-by-side experiments,

changing a single variable at a time, differences attributable to that variable can be obtained. A starting point is to look at the minimal length a cDNA probe can be.

Under the hybridization conditions used, pentameric cDNAs did not show saturation of binding to 30S subunits, even at very high cDNA:subunit ratios. We interpret this fact as evidence that, under the conditions used, at least six available base pairs were generally necessary for stability. The hexamer or larger cDNA oligomer may compete successfully with interactions of lower stability, but it would necessitate a higher probe:subunit ratio for saturation than would be necessary otherwise. This is supported by our evidence showing drastic lowering of saturated binding when a single mismatch occurs in a much longer probe (see Results and discussion below).

It should be emphasized that all binding interactions were checked for specificity of binding using RNase H, an enzyme which digests the RNA in a DNA/RNA hybrid. Although it generally digests the most 3′-end of the RNA in the hybridized region first, it does show some nucleotide preference, and may also hydrolyze at more than one site on the RNA, giving rise to a cluster of products (Donis-Keller, 1979). RNase H digestion is a technique often used in the analysis of mRNAs (D'Alessio, 1982; Goldstein, 1989) and has been successfully used in this laboratory for the identification of rRNA fragments (Tapprich & Hill, 1986; Hill & Tassanakajohn, 1987; Marconi & Hill, 1988; Hill et al., 1988). In all of the cDNA probes tested in this study, only a single cleavage (cluster) occurred. Since there have been cases in which the RNase H could not penetrate the region of probe binding, we generally tested RNase H specificity using the protein-free rRNA rather than the intact subunits. This could possibly lead to spurious results, since the unconstrained rRNA may take a substantially different conformation than that found in the ribosomal subunits. Our results would indicate that such is not the case in the present study.

A variety of models have been proposed for the secondary structure of 16S rRNA at the 3′-end, some involving the formation of transient helices involving two or three base pairs between bases 919–928 and either the 1392–1396 and 1500–1505 or the 1392–1396 and 1530–1535 regions (Ericson & Wollenzein, 1989; Moazed et al., 1986; Brimacombe et al., 1986). One of the reasons for testing the region 919–924 was to compare the observed binding of this region with that which might be predicted from these structures. We tested the availability of the region containing nucleotides 919–928 using two slightly overlapping cDNA probes, one complementary to nucleotides 919–924 and the other complementary to nucleotides 923–928. The probe to the 923–928 region did not bind significantly in our experiments. However, the cDNA hexamer complementary to nucleotides 919–924 gave 20% binding in the active subunits and 40% binding in the inactive subunits, suggesting that structural changes upon inactivation allowed this region to become considerably more accessible. Moazed et al. (1986) reported that nucleotides 923 and 924 became slightly more available to chemical modification upon inactivation of the subunit. Our results also corroborate the secondary structure map used by Woese et al. (1990) which showed nucleotides 921–922 base-paired with nucleotides 1395–1396. However, our results would predict this base-pairing to be that generally found in the active conformation.

We also tested region 1497–1505 and found no binding to either active or inactive subunits (see Table II). The region encompassing nucleotides 1530–1534 did not appear to be available in either active or inactive conformation, but nucleotides 1395 and 1396 were much more available in the inactive conformation (see discussion below). The saturation binding level of cDNAs 919–924 and 923–928 was not altered in the presence of the 1534–1541 probe, nor was the binding to 1534–1541 affected by the presence of cDNA 919–924 or 923–928 (data not shown).

One of the most highly conserved regions in 16S rRNA extends from bases 1390–1417. This region is believed, on the basis of chemical modification and nuclease digestion studies, to have considerable single-stranded character (Noller, 1984; see Figure 1). It has also been shown to contain a site which can cross-link to tRNA in the P-site [C-1400 (Prince et al., 1982)] and which is essential to 16S processing and assembly (Denman et al., 1989a), and to 30S function (Denman et al., 1989b).

We "walked" hexameric probes from nucleotide 1390 to 1409, in each case overlapping all but one nucleotide at a time, in an effort to determine the availability of this region in both the active and inactive conformation. Only in the 1397–1404 region (which we call the "window" region) was it possible to consistently bind hexameric probes to give 100% binding to the active 30S ribosomal subunits, no matter what their state of activation (see Figure 4b,c). It was also possible to hybridize longer cDNAs to the region, as long as they included complementary nucleotides to at least six of the nucleotides in the "window" region. Most other regions of rRNA we have tested to date appear to be available only transiently during the translation process (Hill et al., 1990).

In the inactive conformation, the binding of probes to the "window" region was somewhat attenuated, but nonetheless quite high (see Table II). However, hexameric probes extending to nucleotide 1396 showed greatly increased binding over that seen in the active conformation, suggesting that nucleotides 1395 and 1396 became more available in the inactive 30S subunit conformation. The cDNA 1396–1401 did not bind particularly well, suggesting that some interference may be present at this nucleotide which could be successfully removed by cDNA 1395–1400. The chemical modification results of Moazed et al. (1986) show a similar anomaly for these two nucleotides.

These binding results are especially interesting in light of the most recent secondary structure map given by Woese et al. (1990) in which base pairs are shown between 921–922 and 1396–1395 (see Figures 1 and 4a). The previous map (Noller et al., 1986) had shown nucleotides 921–922 and 1393–1408 to be single-stranded (similar to Figure 4c). We emphasize that the base pairs between 921–922 and 1396–1395 would most likely be found in the active conformation according to our results (compare Figure 4b and Figure 4c).

Our results also corroborate the earlier results reported by Moazed et al. (1986) in which they showed that nucleotide 1395 became much more reactive to chemical modification in the inactive than in the active conformation and nucleotide 1396 slightly more reactive under the same conditions, suggesting a greater exposure of these nucleotides to the solvent environment. A secondary structure for this region suggested by them shows that nucleotides 923–924 might be base-paired with nucleotides 1393–1392 and nucleotides 1394–1395 base-paired to 1506–1505, respectively, in the active conformation. These interactions are not present in the inactive conformation. Although this model is somewhat different than the structure shown by Woese et al. (1990), our results, showing that cDNA can bind to the 919–924 region much more readily in the inactive conformation than in the active state, and cDNA 1395–1400 binding in the inactive conformation, but not in the active conformation would corroborate

either model. While we cannot conclude from our results that such base-pairing is actually occurring, structures such as those suggested by Moazed et al. or Woese et al. would explain our results in this region completely.

How is it possible for nucleotides 1397–1404 to remain continuously exposed in a cellular milieu containing ribonucleases? If we calculate the sizes of several cellular nucleases, RNase A would have a diameter of 31.6 Å, RNase III a diameter of 38 Å, and RNase H a diameter of 36.6 Å, assuming they are anhydrous spheres. If the RNA in the accessible region is helical, in the A-form typical for RNA, the translational distance per nucleotide is about 2.5 Å. Then the exposed region of 16S rRNA is approximately 20 Å in length. This "window" may be too small to accommodate the entrance of the nucleases, thus preventing the degradation of the rRNA. Yet this opening is large enough to provide a site for mRNA and/or tRNA interaction.

As noted above, it is possible to hybridize probes to this region which are longer than the "window". Most of the longer cDNAs require a lower cDNA:subunit ratio to reach the binding maximum, indicating that they form more stable hybrids with the 16S rRNA. For instance, cDNA 1394–1408, a pentadecamer, bound much more stably than the hexamers but required about the same cDNA excess to saturate binding as did the octamer, 1396–1403. Is this effect due to the longer probes displacing interactions in regions adjacent to the "window", or do the ends of such probes "dangle?"

To try to resolve this question, we hybridized cDNA, targeted at the 1394–1408 region, containing a mismatch both in the binding region and in the region that might "dangle", and we assayed the binding that resulted. Using cDNA 1394–1408, we put in a mismatch at base 1400 (m1400). This probe showed an 83% decrease in binding stability compared with the fully complementary cDNA 1394–1408. When the mismatch was put at base 1406, a 33% decrease was observed. These results suggest that the end(s) may compete to some degree with proteins or RNA interacting with the rRNA outside the "window" region, thereby stabilizing the hybrid.

The other accessible region of 16S rRNA that we have investigated in detail in this study is the region at the 3′-terminus of *E. coli* 16S rRNA. This region functions in positioning mRNA during the initiation of prokaryotic protein synthesis (Shine & Dalgarno, 1974). It has previously been found to be available for oligodeoxyribonucleotide binding (Backendorf et al., 1980; 1981; Canonaco et al., 1989) and has been located on the 30S ribosomal subunit by electron microscopy using an antibody to a 2,4-dinitrophenyl group attached to the 5′-end of a 12-nucleotide DNA oligomer complementary to nucleotides 1531–1542 of 16S rRNA (Olson et al., 1988).

Filter binding assay results for various cDNA oligomers complementary to this region containing nucleotides 1530–1542 confirmed the availability of portions of this region, and allowed us to define which 16S rRNA nucleotides are accessible. We found that hybridization did not occur with cDNAs 1530–1535, 1530–1537, 1532–1537, or 1533–1538 but that an abrupt change occurred in this pattern beginning with the cDNA oligomer 1534–1539. In active subunits, binding became nearly stoichiometric using oligomers complementary to bases 1534–1539, 1535–1540, 1534–1541, and 1530–1542 (see Table II and Figure 4b).

Our results coincide with those of Backendorf et al. (1981), in which cDNA oligomer 1534–1541 was shown to bind stoichiometrically to activated 30S subunits, but yielded a much lower stoichiometry of binding to inactive 30S subunits. These results are also in accordance with those reported by Moazed et al. (1986) in which they reported that nucleotides 1532–1538 became less reactive to chemical modification when the ribosomes were inactivated. However, our results are at variance with those reported by Lasater et al. (1988), in which cDNA oligomer 1530–1542 bound equally well to either active or inactive 30S subunits.

Our results in the 1530–1542 region suggest that although nucleotides 1534–1542 are a quite available for base-pairing, nucleotides in the 1530–1534 region are not. These results corroborate, in part, the structure proposed by Brimacombe et al. (1986), in which only nucleotides 1538–1542 at the 16S rRNA 3′-terminus are depicted as single-stranded. Our data would suggest that the single-stranded region should include nucleotides 1534–1537 as well, at least in the active conformation.

The inactive subunits show considerable decrease in probe binding in this region, suggesting that a substantial conformational change between active and inactive states occurs, which shields this 3′-terminus in the inactive state (see Figure 4c). This mobile end could be protected by creating temporary base pairs with another portion of the rRNA which would make the anti-Shine–Dalgarno region inaccessible in the inactive conformation. Although our results would corroborate such a model, this would not be the only possibility, since similar effects could be caused by quaternary interactions or other structural changes.

Thanaraj and Pandit (1989) have recently suggested that the 1530–1535 region is a secondary interaction site for mRNAs having a Shine–Dalgarno sequence. When we found the region to be unavailable in either the active or the inactive form, we hybridized a probe complementary to 16S rRNA nucleotides 1536–1542 to the 30S subunit or 70S ribosomes to see if the 1530–1535 region would then become available to probe binding. It did not. The addition of poly(U) or MS2 RNA were likewise ineffective in allowing the binding of cDNA 1530–1535 to 30S subunits or 70S ribosomes, suggesting that this region is not available for hybridization under any of the conditions we tested. Therefore, we cannot substantiate this interesting suggestion with our results.

In summary, we have used short oligodeoxyribonucleotides to probe portions of rRNA in situ. This technique has enabled us to precisely delineate the changes in the availability of 16S rRNA to complementary oligonucleotides, especially in the regions containing nucleotides 1390–1417 and 1530–1542, in the active and inactive conformational states of the 30S subunit. These results clearly indicate that there is a region of rRNA containing nucleotides 1397–1404 which is open and available to hybridization in both the active and the inactive state of the 30S ribosomal subunit. The results also show that the 3′-terminus of 16S rRNA, containing nucleotides 1535–1542, is quite available in the active conformation of the 30S subunits but is not as available in the inactive conformation. These results provide additional evidence for a dynamic ribosome.

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REFERENCES

Backendorf, C., Overbeek, G. P., Van Boom, J. H., Van Der Marel, G., Veeneman, G., & Van Duin, J. (1980) *Eur. J. Biochem. 110*, 599–604.

Backendorf, C., Ravensbergen, C. J. C., Vanderplas, J., Van Boom, J. H., & Van Duin, J. (1981) *Nucleic Acids Res. 9*, 1425–1444.

Baudin, F., Mougel, M., Romby, P., Eyermann, F., Ebel, J.-P., Ehresmann, B., & Ehresmann, C. (1989) *Biochemistry 28*, 5847–5855.

Brimacombe, R., Atmadja, J., Kyriatsoulis, A., & Stiege, W. (1986) in *Structure, Function, and Genetics of Ribosomes* (Hardesty, B., & Kramer, G., Eds.) pp 184–202, Springer-Verlag, New York.

Brimacombe, R., Atmadja, J., Stiege, W., & Schuler, D. (1988) *J. Mol. Biol. 199*, 115–136.

Brow, D. A., & Noller, H. F. (1983) *J. Mol. Biol. 163*, 27–46.

Calogero, R. A., Pon, C. L., Canonaco, M. A., & Gualerzi, C. O. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 6427–6431.

Canonaco, M. A., Gualerzi, C. O., & Pon, C. L. (1989) *Eur. J. Biochem. 182*, 501–506.

Chaconas, G., & van de Sande, J. H. (1980) *Methods Enzymol. 65*, 75–88.

D'Alessio, J. M. (1982) in *Gel electrophoresis of nucleic acids: A practical approach* (Rickwood, D., & Hames, B. D., Eds.) pp 173–197, IRL Press, Oxford and Washington, D.C.

Denman, R., Negre, D., Cunningham, P. R., Nurse, K., Colgan, J., Weitzmann, C., & Ofengand, J. (1989a) *Biochemistry 28*, 1012–1019.

Denman, R., Weitzmann, C., Cunningham, P. R., Negre, D., Nurse, K., Colgan, J., Pan, Y.-C., Miedel, M., & Ofengand, J. (1989b) *Biochemistry 28*, 1002–1011.

Donis-Keller, H. (1979) *Nucleic Acids Res. 7*, 179–192.

Erdmann, V. A., & Wolters, J. (1986) *Nucleic Acids Res. 14*, R1–R59.

Ericson, G., & Wollenzein, P. (1989) *J. Biol. Chem. 264*, 549–545.

Expert-Bezancon, A., & Wollenzien, P. (1985) *J. Mol. Biol. 184*, 53–66.

Goldstein, B. J. (1989) *Biochem. Biophys. Res. Commun. 159*, 664–669.

Gutell, R. R., & Woese, C. R. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 663–667.

Gutell, R. R., Weiser, B., Woese, C. R., & Noller, H. F. (1985) *Prog. Nucleic Acid Res. Mol. Biol. 32*, pp 155–216.

Herr, W., Champman, M. N., & Noller, H. F. (1979) *J. Mol. Biol. 130*, 433–449.

Hill, W. E., & Tassanakajohn, A. (1987) *Biochimie 69*, 1071–1080.

Hill, W. E., Rossetti, G. P., & Van Holde, K. E. (1969) *J. Mol. Biol. 44*, 263–277.

Hill, W. E., Camp, D. G., Tapprich, W. E., & Tassanakajohn, A. (1988) *Methods Enzymol. 164*, 401–418.

Hill, W. E., Weller, J. W., Gluick, T., Merryman, C., Marconi, R., & Tapprich, W. E. (1990) in *The Ribosome: Structure, Function and Evolution* (Hill, W. E., Dahlberg, A. E., Garrett, R. A., Moore, P. B., Schlessinger, D., & Warner, J. R., Eds.) pp 253–261, American Society for Microbiology, Washington, D.C.

Lasater, L. S., Olson, H., Cann, P. A., & Glitz, D. G. (1988) *Biochemistry 27*, 4687–4695.

Lill, R., Robertson, J., & Wintermeyer, W. (1986) *Biochemistry 25*, 3245–3255.

Marconi, R. T., & Hill, W. E. (1988) *Nucleic Acids Res. 16*, 1603–1615.

Marconi, R. T., & Hill, W. E. (1989) *Biochemistry 28*, 893–899.

Marconi, R. T., Lodmell, J. S., & Hill, W. E. (1990) *J. Biol. Chem. 265*, 7894–7899.

Meier, N., & Wagner, R. (1985) *Eur. J. Biochem. 146*, 83–87.

Moazed, D., & Noller, H. F. (1986) *Cell 47*, 985–994.

Moazed, D., Van Stolk, B., Douthwaite, S., & Noller, H. F. (1986) *J. Mol. Biol. 191*, 483–493.

Noll, M., Hapke, B., Schrier, M., & Noll, H. (1973) *J. Mol. Biol. 75*, 281–294.

Noller, H. F. (1984) *Annu. Rev. Biochem. 53*, 119–162.

Noller, H. F., & Lake, J. (1984) in *Membrane Structure and Function* (Bittar, E., Ed.) pp 218–289, John Wiley and Sons, New York.

Noller, H. F., Asire, M., Barta, A., Douthwaite, S., Goldstein, T., Gutell, R. R., Moazed, D., Normanly, J., Prince, J. B., Stern, S., Triman, K., Turner, S., Van Stolk, B., Wheaton, V., Weiser, B., & Woese, C. R. (1986) in *Structure, Function, and Genetics of Ribosomes* (Hardesty, B., & Kramer, G., Eds.) pp 143–163, Springer-Verlag, New York.

Ofengand, J., Ciesiolka, J., Denman, R., & Nurse, K. (1986) in *Structure, Function, and Genetics of Ribosomes* (Hardesty, B., & Kramer, G., Eds.) pp 473–494, Springer-Verlag, New York.

Olson, H. M., Lasater, L. S., Cann, P. A., & Glitz, D. G. (1988) *J. Biol. Chem. 263*, 15196–15204.

Pon, C. L., & Gualerzi, C. O. (1988) in *Gene Expression and Regulation: The Legacy of Luigie Gorini* (Bissell, M., Deho, G., Sironi, G., & Torriani, A., Eds.) pp 137–150, Elsevier, Amsterdam.

Prince, J. B., Taylor, B. H., Thurlow, D. L., Ofengand, J., & Zimmermann, R. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 5450–5454.

Rheinberger, H., & Nierhaus, K. H. (1987) *J. Biomol. Struct. Dyn. 5*, 435–446.

Santer, M., & Shane, S. (1977) *J. Bacteriol. 130*, 900–910.

Shine, J., & Dalgarno, L. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 1342–1346.

Stern, S., Weiser, B., & Noller, H. F. (1988) *J. Mol. Biol. 204*, 447–481.

Tam, M. F., & Hill, W. E. (1981) *Biochem. Int. 3*, 655–662.

Tapprich, W., & Hill, W. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 556–560.

Thanaraj, T. A., & Pandit, M. W. (1989) *Nucleic Acids Res. 17*, 2973–2985.

Tinoco, I., Borer, P. N., Dengler, B., Levine, M. D., Uhlenbeck, O. C., Crothers, D. M., & Gralla, J. (1973) *Nature (London), New Biol. 246*, 40–41.

Traub, P., Mizushima, S., Lowry, C. V., & Nomura, M. (1979) *Methods Enzymol. 20*, 521–538.

Van Stolk, B. J., & Noller, H. F. (1984) *J. Mol. Biol. 180*, 151–177.

Wallace, R. B., Shaffer, J., Murphy, R. F., Bonner, J., Hirose, T., & Itakura, K. (1979) *Nucleic Acids Res. 6*, 3543–3557.

Woese, C. R. (1980) in *Ribosomes: Structure, Function & Genetics* (Chambliss, G., Craven, G. R., Davies, J., & Davis, K., Eds.) pp 357–373, University Park Press, Baltimore.

Woese, C. R. (1987) *Microbiol. Rev. 51*, 221–271.

Woese, C. R., Gutell, R. R., Gupta, R., & Noller, H. F. (1983) *Microbiol. Rev. 47*, 621–669.

Woese, C. R., Winker, S., & Gutell, R. R. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 8467–8471.

Zamir, A., Miskin, R., & Elson, D. (1971) *J. Mol. Biol. 60*, 347–364.