Identification of a rRNA/Chloramphenicol Interaction Site within the Peptidyltransferase Center of the 50 S Subunit of the Escherichia coli Ribosome*

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We have used oligodeoxyribonucleotide probes to investigate possible interactions between chloramphenicol and portions of the rRNA contained within the peptidyltransferase center of the Escherichia coli ribosome. Oligodeoxyribonucleotide probes complementary to bases 2448-2454, 2468-2482, and 2497-2505 of 23 S rRNA were hybridized to 50 S subunits in situ. Probe binding was qualitatively assessed by sucrose gradient centrifugation. Each probe was shown to bind specifically with its intended binding site through digestion of the rRNA within the RNA/DNA heteroduplexes with RNase H and analysis of the digestion fragments using gel electrophoresis. Competitive binding experiments were conducted between each probe and the antibiotics chloramphenicol and erythromycin. The binding of a probe complementary to bases 2497-2505 was attenuated by 70% upon the binding of chloramphenicol. A probe complementary to bases 2468-2482 showed an increase in binding of 14% while binding of a probe complementary to bases 2448-2454 was not affected by chloramphenicol binding. Erythromycin did not affect the binding of any of these probes to 50 S subunits. These results suggest that bases within the 2497-2505 region of 23 S rRNA in E. coli may be involved in a chloramphenicol/rRNA interaction.

Ribosome targeting antibiotics have served as powerful tools in the elucidation of the functions of specific ribosomal components. The study of antibiotic/ribosome interactions has also provided an increased understanding of the mechanisms by which these antibiotics exert their inhibitory activity. With the increasing incidence of bacterial resistance to many antibiotics, knowledge of precise antibiotic/ribosomal interaction sites will be fundamental in the design and synthesis of new ribosome targeting antibacterials.

Several antibiotics have been shown to bind directly to ribosomes and in most cases their binding sites have been localized to either the 50 S or 30 S subunit. In several cases, there is strong evidence that the binding sites are composed of ribosomal RNA (rRNA), at least in part (for a review see Cundliffe, 1981).

The binding site of chloramphenicol, a bacteriostatic antibiotic which inhibits peptide bond formation in bacteria (Gale and Folkers, 1953; Lamborg and Zamccnick, 1960), mitochondria (Wintersberger, 1965; Kroon, 1965), and chloroplasts (Ellis, 1969), has been localized on the large ribosomal subunit (Vazquez, 1964). The electrophilic affinity analogs of chloramphenicol, monooiodoamphenicol and monobromoamphenicol, specifically label the ribosomal proteins L2, L6, L16, L24, and L27 (Pongs et al., 1973; Sonnenberg et al., 1973). Antigenic determinants for each of these ribosomal proteins were mapped within the peptidyltransferase center of the 50 S subunit (Stoffler et al., 1980). Since chloramphenicol inhibits peptide bond formation (a reaction ascribed to the peptidyltransferase center of the ribosome) it is not surprising that the binding site of chloramphenicol would be localized in this region of the 50 S subunit.

In addition to ribosomal proteins, rRNA has been implicated as a component of the chloramphenicol binding site. A loss of sensitivity to chloramphenicol occurs in mitochondria following point mutations at specific base pairs in the large subunit rRNA (Dujon, 1980; Kearsley and Craig, 1981; Blanc et al., 1981). The analogous positions for these point mutations map at bases 2447, 2451, 2452, 2503, and 2504 in 23 S rRNA of the Escherichia coli 50 S subunit. The generation of a chloramphenicol-resistant phenotype, as a result of base substitutions, suggests that these residues may be part of the chloramphenicol binding site or that they are required for the inhibitory action of chloramphenicol.

The postulated secondary structure maps of the large subunit rRNAs organize the rRNA into six structural domains (Glotz et al., 1981; Branlant et al., 1981; Noller et al., 1981). The bases implicated in chloramphenicol sensitivity all lie within the central loop of domain V (Fig. 1). This central loop region of domain V comprises the major rRNA portion of the peptidyltransferase center. A comparison of both the sequence and secondary structure of the large subunit rRNAs in chloroplasts, mitochondrial, eukaryotic, and prokaryotic ribosomes reveals that this region of rRNA is highly conserved phylogenetically (Gutell and Fox, 1988).

We have investigated the postulated interaction of chloramphenicol with single-stranded regions of rRNA within the peptidyltransferase center of the E. coli 50 S ribosomal subunit. DNA probes complementary to bases 2448-2454, 2468-2482, and 2497-2505 of the 23 S rRNA were synthesized and hybridized to 50 S subunits. The binding interactions were quantified and demonstrated to be specific. If the rRNA contained within these regions is involved in the binding of chloramphenicol, competition for the same rRNA binding site will be reflected by either an attenuation of probe binding or a decrease in chloramphenicol binding upon the binding of the competing molecule.

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Upon the binding of chloramphenicol, the binding of a probe spanning bases 2497-2505 was attenuated by 70%, while the binding of a probe complementary to bases 2468-2482 was stimulated by 14%. The binding of a probe complementary to bases 2448-2454 was not affected. These results suggest that bases contained within the 2497-2505 region of 23S rRNA may be involved in the binding of chloramphenicol. In addition, the binding of chloramphenicol may induce conformational changes in the rRNA which result in an increased binding of the 2468 probe. Finally, the data presented here do not support previous suggestions of a direct chloramphenicol interaction with bases 2448 and 2452 (Kearsey and Craig, 1981; Blanc et al., 1981).

**EXPERIMENTAL PROCEDURES**

**Isolation of Ribosomes and Ribosomal Subunits**

Ribosomes and ribosomal subunits were isolated from mid-log phase E. coli (MRE-600) as described previously (Marconi and Hill, 1988). One A260 unit of 50 S subunits was taken to equal 39 pmol. The homogeneity of the ribosome and ribosomal subunit preparations were checked by analytical ultracentrifugation in a Spinco model E ultracentrifuge equipped with Schlieren optics. The integrity of the rRNA was analyzed by electrophoresis on a 5% (7M urea) polyacrylamide gel (35:1 acrylamide/bis, 89 mM Tris borate, 1 mM EDTA, pH 8.3) for 5 h at 12.5 mA, followed by staining with 0.025% methylene blue.

The integrity and activity of the ribosomal preparations was indirectly assayed by quantifying the activity of both the 50 S subunits and the 70 S ribosomes to bind tRNA. The tRNA binding experiments were conducted as described previously (Marconi and Hill, 1989).

The ribosomal preparations were also assayed for their ability to carry out poly(U) directed polypeptide synthesis essentially as described by Traub et al. (1981). The [3H]phenylalanine (513 mCi/mmol) was from Du Pont-New England Nuclear.

**Synthesis, Purification, 5' End Labeling, and Sequencing of the cDNA Probes**

All probes were synthesized on a Biosearch model 8000 automated DNA synthesizer and deblocked according to the manufacturer's protocol. All chemicals were from Biosearch with the exception of high performance liquid chromatography grade acetonitrile and methylene chloride (J. T. Baker Chemical Co.). Probes were purified both before and after removal of the 5'-dimethoxymethyl group by reverse phase high performance liquid chromatography (Marconi and Hill, 1988). The probes are designated by the numerical assignment given to the rRNA bases to which they are complementary. Probes 2448, 2468, and 2497 are complementary to bases 2448-2454, 2468-2482, and 2497-2608, respectively. Labeling of the 5'-terminus of the probes with [γ-32P]ATP (Du Pont-New England Nuclear) and polynucleotide kinase (United States Biochemical Corp.) was according to Chaconas and van de Sande (1980). After labeling, the homogeneity of the probe preparations were checked by electrophoresis on a 20% (7M urea) polyacrylamide gel at 40 mA for 50 min followed by autoradiography (gel composition was as described above). Probes were partially sequenced to within two bases of their 3' terminus by the enzymatic method of Black and Gilham (1985).

**Probe Hyridization Assays**

**Nitrocellulose Filtration Techniques—**Probe binding to 50 S subunits was measured by incubating increasing amounts of radiolabeled probe (up to a ratio of probe:50 S subunits of 40:1) with 29 pmol of 50 S subunits in 50 µl of binding buffer (10 mM Tris-HCl, pH 7.4, 10 mM MgCl2, and 150 mM KCl) at 0 ℃ for 4 h. Reactions were diluted to 1 ml with ice-cold binding buffer, layered onto nitrocellulose filters (HAWP, 0.45 µm, 25 mm, Millipore), washed twice with 1 ml of binding buffer, and the radioactivity retained measured by liquid scintillation counting in a Packard Tri-Carb 1500. To account for nonspecific retention of radiolabeled probe on the filters, equal amounts of probe were incubated without subunits and treated as described above. The radioactivity retained for each point was subtracted from the values obtained for each reaction.

**Sucrose Gradient Centrifugation—**Radiolabeled probes (500,000 cpm) were incubated with 55 pmol of 50 S subunits in 50 µl of binding buffer as described above. After the 4-h incubation period, reaction mixtures were layered onto 5-20% sucrose gradients (in binding buffer) and centrifuged in a Beckman SW 60 rotor at 54,000 rpm for 1.75 h (4 ℃). All sucrose solutions were treated with diethyl pyrocarbonate by standard methods. The centrifuge tubes were punctured, 200-µl fractions collected, diluted to 500 µl with binding buffer, and the A260 and radioactivity of each fraction were measured.

**Hybridization Specificity**

**Computer Search Analysis of 23 S rRNA—**A computer search of the 23 S rRNA sequence was conducted to determine the location and number of additional sites of complementarity for each probe. Ribonuclease II Digestion of Probe-Subunit Complexes—Ribonuclease H (2.0 units, Pharmacia LKB Biotechnology Inc.) was incubated with 50 pmol of 50 S subunits and 150 pmol of probe as described previously (Marconi and Hill, 1988). After the incubation period the reactions were phenol-extracted, back-extracted, precipitated with 96% ethanol (ice-cold), washed with 70% ethanol, dried, resuspended in tracking dye (0.020% bromphenol blue, 0.020% xylene cyanol, in 7 M urea), and electrophoresed on a 5% (7 M urea) polyacrylamide gel at 12.5 mA for 5 h. RNA size markers (4.0 µg, Bethesda Research Laboratories) were mixed with an appropriate volume of tracking dye, heated at 70 ℃ for 5 min, cooled on ice, and loaded onto the gel.

**Chloramphenicol Binding to 50 S Subunits**

Increasing amounts of d-threo-[dichloroacetyl-1-14C]chloramphenicol (63 mCi/mmol, Amersham Corp.) were incubated with 25 pmol of 50 S subunits in 50 µl of binding buffer for 4 h at 0 ℃. Prior to

![Fig. 1. A portion of the secondary structure map of 23 S rRNA from E. coli. The diagram depicts the central loop region of domain V (i.e. the peptidyldtransferase center). Some of the bases implicated in antibiotic/rRNA interactions are indicated: chloramphenicol, chloramphenicol; ERM, erythromycin. The cDNA probe target sites are enclosed in brackets.](image-url)
use, a final specific activity of 100 cpmpmol was obtained by dilution with unlabeled chloramphenicol (United States Biochemical Corp.). The reactions were diluted with binding buffer, layered onto nitrocellulose filters, and treated as described above.

**Competitive Binding Experiments**

**32P-Labeled Probe Versus Unlabeled Chloramphenicol**—Increasing amounts of chloramphenicol were coincubated with 700 pmol of radiolabeled probe and 25 pmol of 50 S subunits in 50 ml of binding buffer for 4 h at 0°C. Reactions were diluted, filtered, and the radioactivity retained measured as described above.

**3'CHCloramphenicol Versus Unlabeled Probe**—Increasing amounts of probe were coincubated with 900 pmol of chloramphenicol and 25 pmol of 50 S subunits in 50 ml of binding buffer. Reactions were treated as described above.

**32P-Labeled Probe Versus Erythromycin**—Erythromycin (United States Biochemical Corp.) binding competition experiments were conducted as described for the chloramphenicol competition assays.

### RESULTS

#### Ribosomal Integrity and Activity

The ability of both the 50 S subunits and TC70S ribosomes used in this study to bind deacylated tRNA (either with or without poly(U)) has been previously described (Marconi and Hill, 1989). In summary, deacylated tRNA bound to 50 S subunits at levels of 45 and 30% with and without poly(U), respectively. These results are consistent with those previously reported by Gnirke and Nierhaus (1986) and demonstrate that the ribosome preparations employed were functionally intact, at least with respect to their ability to bind tRNA. The ribosomal preparations were found to be active in poly(U)-directed polyphenylalanine synthesis (data not shown).

#### Labeling and Sequencing of cDNA Probes

Each probe was partially sequenced and in all cases the observed sequence was correct (data not shown). The purified probes were 5'-end-labeled to specific activities between 2 and 4 x 10⁶ cpmpmol and then diluted to specific activities of approximately 500 cpmpmol prior to use in the hybridization and competition assays by the addition of unlabeled probe.

#### Hybridization of Probes to 50 S Subunits

At a ratio of 30:1 (probe:50 S subunits) probes 2448 (5'CTGTTAT), 2468 (5'TCGATATGAACTCTT) and 2497 (5'CATCGAGGT) bound to 50 S subunits in situ at levels of 22, 25, and 32%, respectively (Fig. 2).

A second approach which can be used to demonstrate the binding of probes to ribosomes or ribosomal subunits is sucrose gradient centrifugation. Probes 2448, 2497, and 2448 were all found to co-migrate with 50 S subunits upon centrifugation (data not shown). This technique also allows for a qualitative assessment of probe-subunit complex formation. It demonstrates that the formation of the probe-subunit complex does not result in any gross conformational changes.

**Chloramphenicol Binding to 50 S Subunits**

As assayed by the nitrocellulose filtration techniques, chloramphenicol bound to 50 S subunits at a maximum level of 23%. Binding saturation occurs at a ratio of [14C]chloramphenicol/50 S subunits of 10:1 (Fig. 3). These results are consistent with those reported by Vogel et al. (1971).

#### Hybridization Specificity

<table>
<thead>
<tr>
<th>Probe sequence</th>
<th>Location of additional 23 S single-stranded sites showing partial complementarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 2448-2454 5'-CTGTTAT</td>
<td>714-718</td>
</tr>
<tr>
<td>2. 2468-2482 5'-TCGATATGAACTCTT</td>
<td>None</td>
</tr>
<tr>
<td>3. 2497-2505 5'-CATCGAGGT</td>
<td>47-51, 1375-1380, 1727-1731</td>
</tr>
</tbody>
</table>
Fig. 4. RNase H digestion of the probe-subunit complexes. Products from the RNase H digestion assays were treated as described under "Experimental Procedures" and electrophoresed on a 5% polyacrylamide gel for 5 h at 12.5 mA. The gel was stained with 0.025% methylene blue. Lanes 1-4 are the digestion products generated in the presence of 2448 probe (lane 1), 2468 probe (lane 2), 2497 probe (lane 3), and no probe (lane 4). Lane 5 is RNA size markers (units in bases).

Table II

Summary of binding data

The percentage of 50 S ribosomal subunits bound at saturating probe concentrations are listed for each probe. The ratio of probe to subunit at saturating concentration is also listed for each probe.

<table>
<thead>
<tr>
<th>Probe</th>
<th>Probe sequence</th>
<th>Binding</th>
<th>Saturation ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>2448-2454</td>
<td>5'-CTGTAT</td>
<td>21</td>
<td>30:1</td>
</tr>
<tr>
<td>2497-2505</td>
<td>5'-CAGCGG</td>
<td>32</td>
<td>30:1</td>
</tr>
<tr>
<td>2489-2496</td>
<td>5'-GCTAAACA</td>
<td>4</td>
<td>16:1</td>
</tr>
<tr>
<td>2489-2499</td>
<td>5'-GCTCAGC</td>
<td>2</td>
<td>4:1</td>
</tr>
<tr>
<td>2506-2513</td>
<td>5'-TGAGCCAACA</td>
<td>7</td>
<td>8:1</td>
</tr>
</tbody>
</table>

*Indicates probes with double-stranded target sites.

Non-specific interactions between the probes and the 50 S subunits were not observed. The binding results of all probes employed in this study are summarized in Table II.

Competitive Binding Experiments

32P-Labeled Probe Versus Unlabeled Chloramphenicol—The effects of binding chloramphenicol on the binding of probes 2448, 2468, and 2497 were tested. Chloramphenicol caused a 70% attenuation in the level of 2497 probe binding to 50 S subunits. The binding of probe 2468 was stimulated by 14%, whereas the binding of the 2448 probe was not affected (Fig.

Fig. 5. Chloramphenicol/23 S rRNA Interactions

DISCUSSION

The results presented here provide evidence for a specific chloramphenicol/rRNA interaction within the peptidyltransferase center of the 50 S subunit. The attenuation of 2497 probe binding by 70% in the presence of chloramphenicol suggests that the rRNA region spanning bases 2497-2505 may contain residues involved in chloramphenicol binding. These results support previous studies in which residues 2503 and 2504 were implicated in chloramphenicol binding (Dujon, 1980; Kearsey and Craig, 1981). These residues are contained within the region spanning bases 2497-2505 which is the target site for the 2497 probe. In contrast to previous studies, we find no evidence of a chloramphenicol/rRNA interaction occurring at bases 2451 and 2452 (Kearsey and Craig, 1981; Blanc et al., 1981) which are contained within a site probed in this study spanning bases 2448-2454. In addition, the binding of chloramphenicol resulted in a 14% increase in the binding of the 2468 probe suggesting that the binding of chloramphenicol may induce conformational changes in the 23 S rRNA of the peptidyltransferase center.

The observed attenuation of binding of 2497 probe as a result of chloramphenicol binding may be interpreted in several ways. When the base substitution studies and the data presented here are considered together perhaps the most likely...
possibility is that direct competition is occurring for the same rRNA binding site. However, the attenuation of 2497 probe binding as a result of conformational changes in the rRNA, induced by the binding of chloramphenicol at local or distant site, cannot be excluded based solely on the data presented here. In fact the slight increase (14%) in 2468 probe binding suggests that the binding of chloramphenicol may indeed cause conformational changes in some regions of rRNA contained within the peptidyltransferase center. Such a conformational change may allow for the increased hybridization of the 2468 probe which was observed. It is possible that such conformational alterations in rRNA structure may be the basis for the inhibitory effects that chloramphenicol exerts on protein biosynthesis. Another possible, but seemingly unlikely, explanation for the increased binding of the 2468 probe is that chloramphenicol may alter the physical or ionic environment around these residues in such a way as to favor or stimulate probe binding.

With the cDNA probing technique utilized in this study as well as with other techniques, it is particularly difficult to assess the contribution that secondary effects may have on the observed results. Even the previous observations from base substitution studies, that residues 2503 and 2504 are involved in chloramphenicol sensitivity (Dujon, 1980, Kearsey and Craig, 1981) cannot exclude the possibility that the transition to chloramphenicol insensitivity resulted from conformational changes induced by the base substitutions. Single-base substitutions have been shown to have significant effects on the conformation and stability of an RNA hairpin loop structure (Tuerk et al., 1988).

However, when taken together, the base substitution data and the cDNA probing data presented in this study provide strong evidence that chloramphenicol is interacting directly with the 2497–2505 region of 23 S rRNA.

Two additional bases, 2451 and 2452, have been implicated through base substitution studies as components of the chloramphenicol binding site (Kearsey and Craig, 1981; Blanc et al., 1981). However, binding of a probe spanning bases 2448–2454 was not attenuated upon binding of chloramphenicol, and binding of [3H]chloramphenicol was not affected by probe binding, suggesting these bases are not part of the chloramphenicol binding site. The discrepancy between our results and those mentioned above may be attributable to differences between mitochondrial rRNA and that from E. coli, or it is possible that these residues may be necessary for chloramphenicol to exert its inhibitory effects but they may not be an integral component of its ribosomal binding site. As noted above, it is possible that base substitution of residues 2451 or 2452 may cause either short or long range conformational changes in the rRNA which prevents chloramphenicol binding. The loss of sensitivity due to these base substitutions may not then be suggestive of a chloramphenicol/rRNA interaction in this “general region” of the ribosome.

Competition experiments between the unlabeled probes and [3H]chloramphenicol revealed that the probes, when added up to a ratio of 1:1 (probe:chloramphenicol), were unable to displace or exclude the binding of chloramphenicol. This suggests that the binding constant of chloramphenicol is greater than that of the 2497 probe.

The antibiotic erythromycin as well as several additional macrolide antibiotics have been found to inhibit the binding of chloramphenicol to E. coli ribosomes (Vazquez, 1986). The dimethylation of an adenine residue in the 23 S rRNA of Bacillus stearothermophilus, which maps at position A-2058 in E. coli, results in erythromycin insensitivity (Skinner et al., 1983). Residue A-2058 of E. coli maps within the central loop region of domain V (i.e. the peptidyltransferase center). Taken together these results suggest that the binding sites for chloramphenicol and erythromycin may overlap.

Competition experiments were conducted to determine if the binding sites for erythromycin and chloramphenicol might overlap in the rRNA region spanning bases 2497–2505. The addition of erythromycin to the 2497 binding reactions had no effect on 2497 probe binding suggesting that the competition between erythromycin and chloramphenicol is not attributed to an interaction at the 2497–2505 site.

In a previous study, a probe complementary to bases 2058–2062 was found to hybridize to 50 S subunits at a level of 11% (Marconi and Hill, 1988). This region of rRNA contains the A-2058 residue which has also been implicated in the binding of erythromycin. However, we were unable to demonstrate the specificity of the probe hybridization and therefore could not directly address the question of an erythromycin/rRNA interaction at this site.

The binding values reported here for the oligonucleotide/ribosomal subunit interactions are consistent with those observed at other rRNA sites (Lasater and Glitz, 1988; Tapprich and Hill, 1986; White et al., 1988). These binding values are also comparable with those previously reported for the binding of RNA to 50 S subunits (Marconi and Hill, 1989, Giurke and Nierhaus, 1986). [3H]RNA (poly(U)-directed) to activated 30 S subunits (Lasater and Glitz, 1988; Politz and Glitz, 1980; Zamir et al., 1971), and [3H]chloramphenicol to peptidyltransferase active 50 S subunits (Vogel et al., 1971).

We have utilized several approaches to determine if the interactions of the DNA probes with the ribosome are specific. It is important to demonstrate that the probes are not interacting with ribosomal proteins or with other regions of rRNA in addition to the intended target site.

Previously, we have shown that the binding of the 2497 probe is readily reversible (Marconi and Hill, 1988). In that study, competition experiments were conducted in which increasing amounts of unlabeled 2497 probe were added to a complex containing saturating amounts of the radiolabeled 2497 probe and 50 S subunits. The measurable binding of the labeled 2497 probe was found to decrease linearly with the addition of increasing amounts of unlabeled probe. In addition, we found no attenuation in probe binding when using unlabeled probes specific for regions containing dissimilar base sequences (results not shown). These results demonstrated that there was a site or sites which were sequence-specific for probe binding.

To further demonstrate specificity, several probes complementary to confirmed double-stranded regions of 23 S rRNA were tested for their ability to bind to 50 S ribosomal subunits measured by nitrocellulose filtration. None of these probes were found to bind to any significant extent to 50 S ribosomal subunits. The lack of binding clearly demonstrates that probes which do not have an accessible rRNA target site do not bind in a stable fashion to other regions of rRNA or to ribosomal proteins.

The specificity of the cDNA/rRNA hybridization can be demonstrated by ribonuclease H (RNase H) digestion experiments. RNase H cleaves RNA contained within an RNA/DNA heteroduplex (Donnis-Keller, 1979). The applicability of this approach in analyzing DNA/rRNA interactions has been previously demonstrated (Marconi and Hill, 1988, 1989; Tapprich and Hill, 1986; Hill and Tassanakajoohn, 1987). In this study only a single major digestion site was observed in the presence of each probe suggesting that each probe was hybridizing with a single site.

The identity of each cleavage site was determined by com-
paring the size of the smaller RNA fragment produced by cleavage with RNase H with known RNA size markers. The accuracy of this approach, under our conditions, is to within ±10 bases (Marconi and Hill, 1988). We have previously demonstrated the specificity of the 2497 probe/ribosome interaction by sequencing the 400-base rRNA fragment generated by digestion with RNase H (Marconi and Hill, 1989).

The smaller fragments generated upon RNase H digestion of the 50 S subunits in the presence of the 2448 and 2468 probes migrated closely with the fragment obtained in the presence of the 2497 probe. Since the 2448 and 2468 probes do not have any additional target sites which could result in fragments of the observed size upon RNase H digestion, and since the identity of the fragment generated in the presence of the 2497 probe has been established, we conclude that the 2448 and 2468 probe/ribosome interactions were site-specific as well.

The approach utilized in this study provides a sensitive and rapid means by which the specific regions of rRNA involved in the binding of various ribosome inhibiting antibiotics may be determined. The data presented here may also aid in defining the functional role of particular regions of rRNA in protein biosynthesis. A precise understanding of the interaction of these antibiotics with rRNA, and of the functional role of the sites to which they bind, may prove valuable in the conceptualization and development of new and novel antibiotics which could be employed to target the ribosome.

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