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Structural changes in the 530 loop of *Escherichia coli* 16S rRNA in mutants with impaired translational fidelity

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ABSTRACT

The higher order structure of the functionally important 530 loop in *Escherichia coli* 16S rRNA was studied in mutants with single base changes at position 517, which significantly impair translational fidelity. The 530 loop has been proposed to interact with the EF-Tu–GTP–aatRNA ternary complex during decoding. The reactivity at G530, U531 and A532 to the chemical probes kethoxal, CMCT and DMS respectively was increased in the mutant 16S rRNA compared with the wild-type, suggesting a more open 530 loop structure in the mutant ribosomes. This was supported by oligonucleotide binding experiments in which probes complementary to positions 520–526 and 527–533, but not control probes, showed increased binding to the 517C mutant 70S ribosomes compared with the non-mutant control. Furthermore, enzymatic digestion of 70S ribosomes with RNase T1, specific for single-stranded RNA, substantially cleaved both wild-type and mutant rRNAs between G524 and C525, two of the nucleotides involved in the 530 loop pseudoknot. This site was also cleaved in the 517C mutant, but not wild-type rRNA, by RNase V1. Such a result is still consistent with a more open 530 loop structure in the mutant ribosomes, since RNase V1 can cut at appropriately stacked single-stranded regions of RNA. Together these data indicate that the 517C mutant rRNA has a rather extensively unfolded 530 loop structure. Less extensive structural changes were found in mutants 517A and 517U, which caused less misreading. A correlation between the structural changes in the 530 loop and impaired translational accuracy is proposed.

INTRODUCTION

Translation of messenger RNA by the ribosome is a major step in gene expression and involves a series of complex reactions. While many of the details of ribosome structure and function remain unclear, it has become apparent that ribosomal RNA plays a central role in most of the step-wise functions of translation. Experimental evidence suggests rRNA is involved in subunit association (1–3), binding of mRNA (4,5) and tRNA (6–8), as well as elongation factors (9), and, more recently, in peptide bond formation (10). Of these activities one of the most important and complex is the decoding of messenger RNA.

Codon–anti-codon interactions occur in the decoding region, centered around nucleotide C1400 (*Escherichia coli* numbering) of 16S rRNA (6,11,12), while several lines of evidence have implicated the highly conserved 530 loop region in 16S rRNA in proof-reading aatRNAs entering the ribosomal A-site. Mutagenesis of G530 abolishes EF-Tu–tRNA–GTP ternary complex–ribosome interactions (13). In addition, base substitutions at position 517 allow read-through of all three stop codons, as well as increasing +1 and –1 frame shifting *in vivo* (14). Mutations in proteins S4 and S12, both of which interact with the rRNA at the 530 loop region (15,16), decrease and increase translational fidelity respectively (17). Finally, several mutations in the 530 loop result in resistance to streptomycin (18,19), an antibiotic that induces misreading.

Specific bases in the 16S rRNA have been shown to be protected from modification by chemical probes upon binding of tRNA to the ribosome (7,8). In the 530 loop nucleotides 530 and 531 are protected from attack by kethoxal and 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluene sulfonate (CMCT) respectively by A-site bound tRNA, while P-site tRNA protects A532 from dimethyl sulfate (DMS). More recent experiments have suggested that these protections may be due to other ligands, instead of a direct protection by the tRNA. For example, synthetic messenger RNAs containing thioU at position +11 (where +1 is A in the AUG initiation codon) have been cross-linked by UV irradiation to A532 (20–24). Furthermore, Noller and co-workers have shown that base substitution mutations at G530 severely inhibit enzymatic binding of tRNA to the A-site, although non-enzymatic binding is unaffected (25). They suggest that the 530 loop functions by interacting with the EF-Tu–GTP–aatRNA ternary complex, rather than with tRNA *per se*, and that the ribosome may actually play an active role in such processes as tRNA selection, possibly through conformational changes.

Mutations at position 517 in the 530 loop of *E.coli* 16S rRNA have been shown to affect tRNA selection by increasing the translational error rate (14). In the present study we have

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investigated the secondary and tertiary RNA structure of the 530 loop in these 517 mutants in an attempt to define the relationship between higher order structure and function in this region. We found that any base substitution at G517 (A, C or U) resulted in an increased level of chemical modification at positions 530, 531 and 532 in 70S ribosomes. No changes were noted anywhere else in the 16S rRNA. Base substitutions at U534, to which G517 had been previously proposed to base pair, did not affect translational fidelity (14) and caused no detectable structural effects. Experiments using oligonucleotide probes specific for the 530 loop showed enhanced binding to both sides of the loop structure in the 517C mutant 70S ribosomes compared with wild-type ribosomes. Finally, ribonuclease digestion of these 70S ribosomes demonstrated substantial changes in the 530 loop structure, particularly in 517C mutant ribosomes, suggesting that the pseudoknot may not be a stable feature of the 530 loop. Taken together these results show that mutations at position 517 introduced significant changes in the complex structure of the 530 loop, which may account for the reduced translational accuracy.

MATERIALS AND METHODS

Bacterial strains and plasmids

Escherichia coli strain MC127 (*F*$^-$Δ(*lac-pro*)*thi*$^-$, *recA*$^-$, *srL*::Tn*10*) was used. Plasmid pSTL102 (28), a derivative of pKK3535 (29), contains the mutations 1192U (in 16S rRNA) and 2058G (in 23S rRNA), which confer resistance to spectinomycin and erythromycin, respectively. Plasmids with mutations at positions 517 and 534 of 16S rRNA in pSTL102 were a gift from M. O'Connor (14).

Preparation of tight-couple 70S ribosomes

Ribosomes were prepared as described by Moazed and Noller (30), using *E.coli* MC127 that had been transformed with pSTL102 carrying the indicated mutations in the 530 loop of 16S rDNA. After the final high salt wash ribosome pellets were resuspended in 50 mM Tris–HCl, pH 7.6, 10 mM $MgCl_2$, 100 mM NH_4Cl, 6 mM 2-mercaptoethanol, 0.5 mM EDTA, diluted to ~10 pmol/μl, quick frozen and stored at –70°C.

The ratio of host wild-type and plasmid-encoded mutant ribosomal RNA was determined by primer extension (31). Autoradiograms were scanned using a LKB Utroscan XL laser densitometer.

Chemical modification of 70S ribosomes

Binding of poly(U) and deacylated *E.coli* tRNA^{Phe} (Subriden RNA) to tight-couple 70S ribosomes was followed by chemical modification with DMS, kethoxal or CMCT and primer extension as described by Moazed and Noller (30). The extent of modification was quantified by densitometry of autoradiographic bands using a LKB ultroscan XL laser densitometer.

Binding of DNA oligonucleotides to 70S ribosomes

Synthetic DNA oligonucleotide probes complementary to 16S rRNA were purchased from Operon Technologies Inc. (Alameda, CA). The procedures for labeling and binding the probes to ribosomes are essentially those of Weller and Hill (32). Briefly, 70S ribosomes in hybridization buffer (10 mM Tris–HCl, pH 7.4, 15 mM $MgCl_2$, 150 mM KCl, 2 mM DTT) were activated by incubation at 42°C for 20 min, followed by 37°C for 20 min and then placed on ice. The oligonucleotide probes were 5′-end-labeled using T4 polynucleotide kinase, purified and diluted to 500–1000 c.p.m./pmol. The binding assays were carried out by incubating 100 pmol probe with 10 pmol ribosomes in 50 μl final volume at 0°C for 4 h. Where indicated, 4 μg poly(U) and 20 pmol *E.coli* tRNA^{Phe} were bound to 70S ribosomes as described (30), prior to addition of the probes. After incubation the reaction mixtures were filtered through HAWP 0.45 μm nitrocellulose filters (Millipore), washed twice with 1 ml ice-cold hybridization buffer and the radioactivity remaining on the filter determined using a Beckman liquid scintillation counter. The oligonucleotide probes used in this study were: oligo 1 (520–526), 5′-GGCTGCT; oligo 2 (527–533), 5′-TTACCGC; oligo 3 (1397–1403), 5′-GGCGGTG; oligo 4 (1534–1541), 5′-AAGGAGGT.

Nuclease digestion of 70S ribosomes

Digestions were carried out by adding 0.5 U RNase V1 or 10 U RNase T1 to 50 pmol activated ribosomes or subunits in 50 μl DMS modification buffer (80 mM potassium cacodylate, pH 7.2, 20 mM $MgCl_2$, 100 mM NH_4Cl, 1.5 mM DTT) or RNase V1 reaction buffer (20 mM Tris–HCl, pH 7.2, 10 mM $MgCl_2$, 200 mM NaCl) at 37°C. Where indicated, 20 mg poly(U), with or without 100 pmol *E.coli* tRNA^{Phe}, were bound to 70S ribosomes as described (30). At the indicated times aliquots were removed and added to an equal volume of SDS solution (0.3% SDS, 0.14 M NaCl, 0.05 M sodium acetate, pH 5.1), followed by extraction with phenol and chloroform (98:2 chloroform:isoamyl alcohol). The resulting RNA fragments were analyzed by primer extension as previously described (7,8,30).

RESULTS

Enhanced chemical reactivity of G530, U531 and A532 in 16S rRNA of ribosomes with mutations at G517

Structural analysis using chemical probes was carried out on ribosomes with mutations at positions 517 and 534 in 16S rRNA (Fig. 1). Although these experiments were done on a mixed population of ribosomes, containing both mutant and wild-type rRNAs, the high proportion of plasmid-encoded mutant ribosomes (85–90% as estimated by primer extension at U1192; see 31), expressed from the high copy number plasmid pSTL102 in *E.coli* strain MC127, made the use of an allele-specific primer unnecessary (33). The effects of the mutations at positions 517 and 534 on the 530 loop tertiary structure were analyzed by the chemical modification/primer extension method of Noller and co-workers (7,8,30). Salt washed, tight-couple 70S ribosomes with or without pre-bound poly(U) and *E.coli* tRNA^{Phe} were treated with kethoxal, DMS or CMCT, followed by primer extension of purified rRNA. An autoradiogram of CMCT-modified U531 is shown in Figure 2A as an example of the changes in accessibility to chemical reagents of the 530 loop as a result of mutations at position 517. The degree of chemical modification was quantified and standardized by laser densitometry of the autoradiographic bands using the non-specific reverse transcriptase stop at position 541 as an internal control. The results, summarized in Figure 2B, show clearly that G530, U531 and A532 are significantly more reactive to kethoxal, CMCT and DMS respectively in the 517 mutants, including the 517U/534G

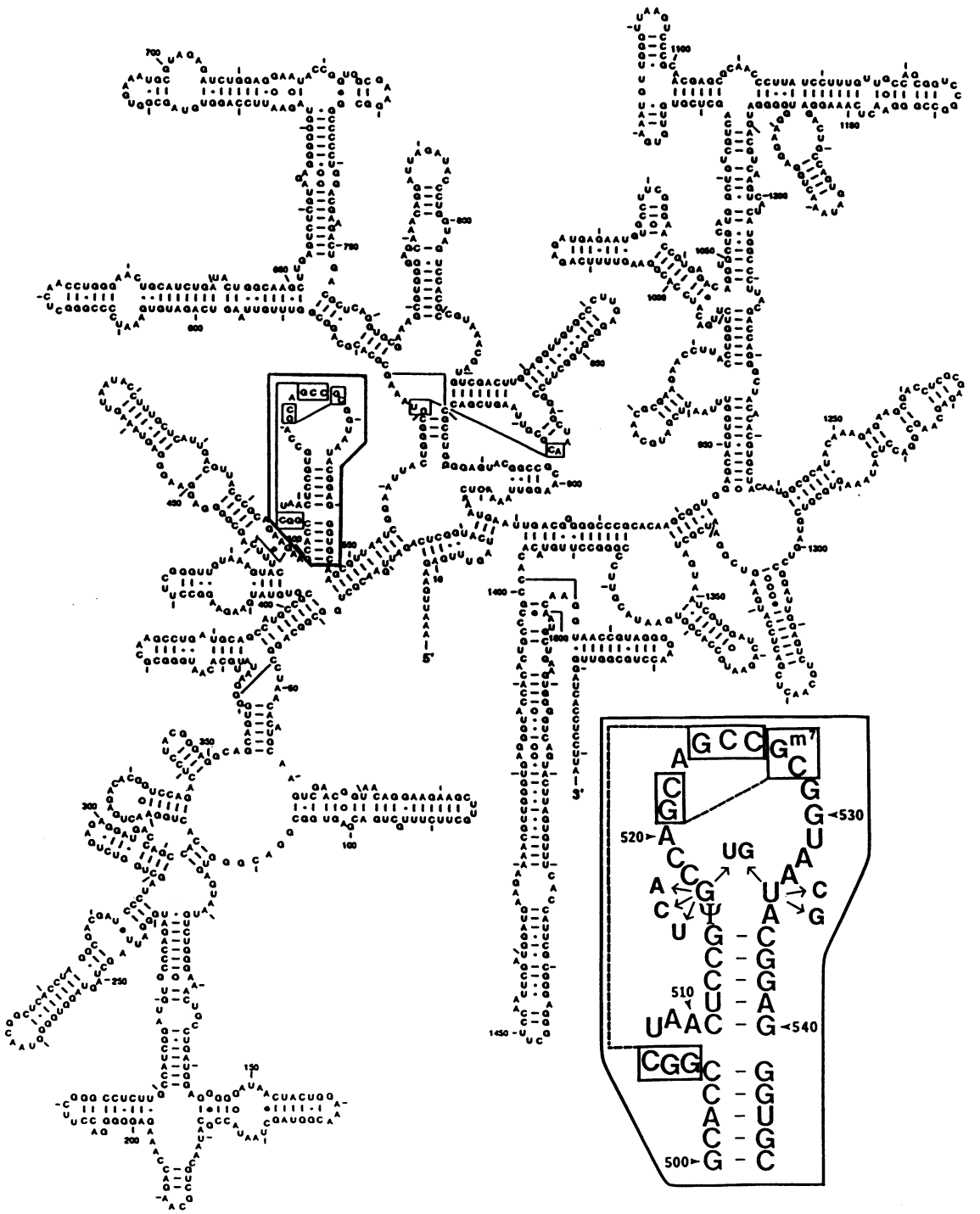
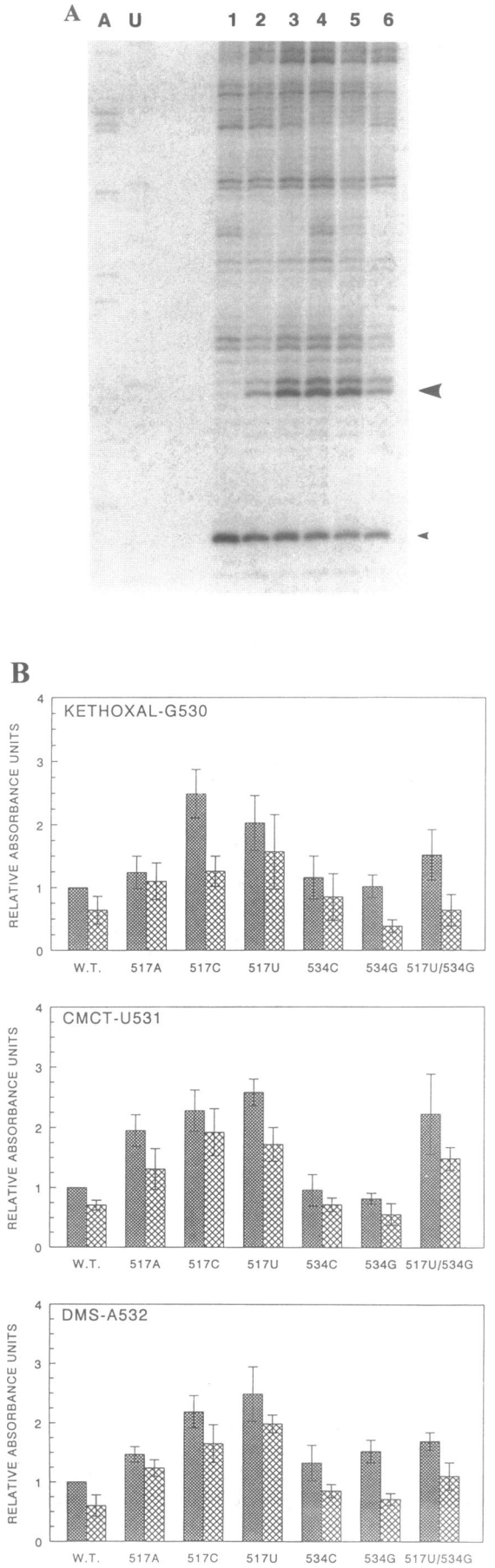


Figure 1. Secondary structure of *E.coli* 16S rRNA (42) with the 530 loop enlarged. The pseudoknot and the mutations at position 517 and 534 in the 530 loop are indicated.

double mutant, than in wild-type ribosomes. This difference, particularly at positions 531 and 532, is most apparent in ribosomes carrying the 517C and 517U mutations. On the other hand, rRNAs with single base substitutions (U to C or G) at 534 were unaffected, showing the same levels of chemical modification as the non-mutant ribosomes. The binding of poly(U) and $tRNA^{Phe}$ reduced the accessibility to chemical probes, but they all remained more accessible than wild-type sequences.

A similar pattern was observed when these experiments were repeated using ribosomes isolated from *E.coli* with mutations in ribosomal proteins S4 (*ram* strain D16) and S12 (restrictive strains *rpsL*-221, *rpsL*-224 and *rpsL*-222). In each case the 517



mutants caused an increase in chemical modification at positions 530–532 which was similar to that observed in *E.coli* with wild-type ribosomal proteins (results not shown).

Other nucleotides in the 16S rRNA are also protected from chemical modification upon binding of tRNA to the ribosomal A- and P-sites (7,8,30). These positions were examined by primer extension, with particular attention paid to G926, which is strongly protected by P-site bound tRNAs, and A1408, which is protected by binding tRNA to the A-site. No differences were found in the level of chemical modification at these other positions that could be attributed to rRNA mutations in the 530 loop (results not shown). This suggests that binding of tRNA to the ribosome was unaffected by the mutation, consistent with the results of Powers and Noller (25) using ribosomes mutagenized at G530.

Oligonucleotide binding to 70S ribosomes

To assess further structural changes in the 530 loop oligonucleotide binding experiments were performed on the wild-type and 517C mutant 70S ribosomes using short oligomers complementary to sequences within the 530 loop (oligos 1 and 2; see Materials and Methods). Both halves of the loop were probed separately. Oligo 1 (520–526) was designed to determine the availability of the bases involved in the pseudoknot (524–526), while oligo 2 (527–533) was used to probe the bases showing increased chemical reactivity in the 517C mutants (G530, U531 and A532). The level of binding for each probe was consistent with that reported by Hill and co-workers (for example see 34 for a review) for these regions of the 16S rRNA. As shown in Figure 3, the 517C mutant ribosomes bound more oligo 2 than the wild-type control. This was consistent with the enhanced chemical reactivity of the mutant rRNAs at positions 530–532 (see Fig. 2). Surprisingly, the 517C mutant 70S ribosomes also bound approximately twice as much oligo 1 as did the wild-type ribosomes. Pre-binding of poly(U) and $tRNA^{Phe}$ to the ribosomes reduced binding of both oligonucleotides to both wild-type and 517C mutant ribosomes, consistent with the reduction in chemical reactivity of bases 530, 531 and 532 under similar conditions.

In order to demonstrate that the differences between the wild-type and mutant 517C ribosomes were restricted to the 530

Figure 2. Chemical modification of 530 loop nucleotides in wild-type and mutant *E.coli* ribosomes. (**A**) Sample autoradiogram of CMCT-modified 16S rRNA extracted from tight-couple 70S ribosomes treated as described in Materials and Methods. The position of U531 is indicated by the large arrow, while the small arrow marks the position of the non-specific reverse transcriptase stop used to quantify and normalize the results. Unreacted control (lane 1) or CMCT-treated wild-type (lane 2), 517A (lane 3), 517C (lane 4), 517U (lane 5) and 534C (lane 6) rRNAs. The letters A and U represent sequence marker lanes. (**B**) Salt washed, tight-couple *E.coli* 70S ribosomes and ribosomes with poly(U) and $tRNA^{Phe}$ were treated with kethoxal, CMCT or DMS as described in Materials and Methods. Modified nucleotides were identified by primer extension using an oligonucleotide complementary to positions 559–575 of the 16S rRNA. Autoradiogram band intensities were measured by laser densitometry and normalized to a non-specific reverse transcriptase stop at position 541. The band intensity of wild-type 70S ribosomes at positions 530, 531 and 532 [in the absence of pre-bound poly(U) or $tRNA^{Phe}$] was given the arbitrary value of 1. Each value is the average of at least three separate experiments with each of two independently prepared ribosome samples. Error bars represent one standard deviation. Ribosome were isolated from *E.coli* MC127 transformed with pSTL102 carrying either wild-type (WT), 517A, 517C, 517U, 534C, 534G or 517U–534G mutant 16S rDNA. Small cross-hatch bars, 70S ribosomes; large cross-hatch bars, 70S ribosomes with poly(U) and $tRNA^{Phe}$.

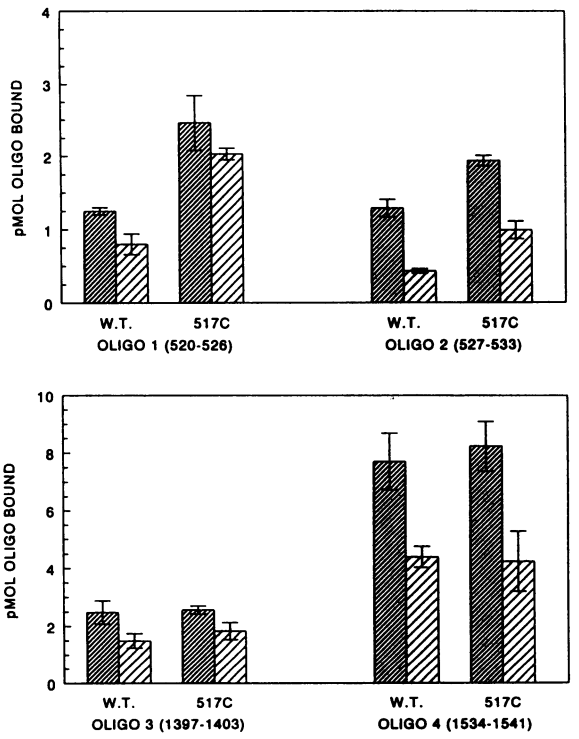


Figure 3. Oligonucleotide binding to the 530 loop of *E.coli* 16S rRNA. Salt washed, tight-couple 70S ribosomes or 70S ribosomes to which poly(U) and $tRNA^{Phe}$ were pre-bound were incubated with ^{32}P-end-labeled oligonucleotides complementary to positions 520–526 (oligo 1) and 527–533 (oligo 2) (top panel) or control oligonucleotides 1397–1403 (oligo 3) and 1534–1541 (oligo 4) (bottom panel) of 16S rRNA as described in Materials and Methods. The bars represent the total number of picomoles of oligomer that remain bound to nitrocellulose filters following hybridization to the ribosomes (100% binding = 10 pmol oligonucleotide). Each experiment was performed in triplicate and the results shown are from a representative experiment. Heavy shaded bars, 70S ribosomes; light shaded bars, 70S ribosomes plus poly(U) and $tRNA^{Phe}$.

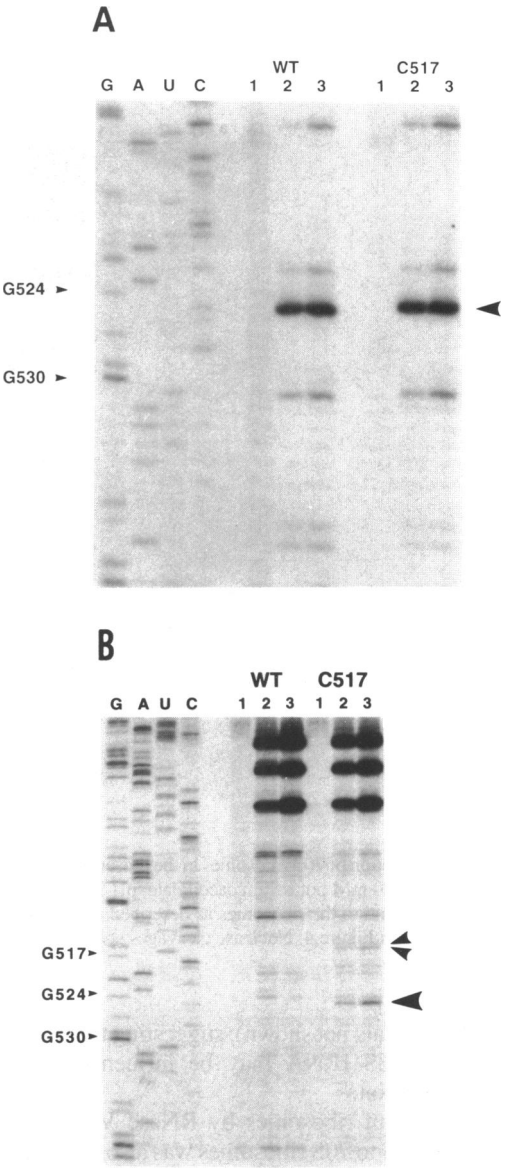


Figure 4. RNase T1 and V1 digestion of 70S ribosomes. Wild-type or mutant 517C 70S ribosomes (50 pmol) were incubated in 50 µl 20 mM Tris–HCl, pH 7.2, 10 mM $MgCl_2$, 200 mM NaCl at 37°C with 10 U RNase T1 (A) or 0.5 U RNase V1 (B). At 0 (lane 1), 15 (lane 2) and 30 min (lane 3) 15 µl aliquots were removed, the RNA extracted (see Materials and Methods) and nuclease cleavage sites identified by primer extension using AMV reverse transcriptase and a ^{32}P-labeled oligonucleotide complementary to positions 559–575 of the 16S rRNA. (**A**) RNase T1-treated 16S rRNA. The cleavage between G524 and C525 is indicated by the arrow. (**B**) RNase V1-digested 16S rRNA. Cleavages specific to mutant 517C are indicated by the large arrow for the cut between G524 and C525 and the small arrows for cuts between C514, G515 and U516.

loop, two additional oligonucleotide probes were tested which complemented structurally remote regions of the 30S subunit. Both oligo 3 (1397–1403) and oligo 4 (1534–1541) showed no significant differences in probe binding between wild-type and mutant 517C 70S ribosomes with or without poly(U) and $tRNA^{Phe}$.

Altered enzymatic digestion in the 530 loop of mutant 70S ribosomes

To address further the question of whether the mutations at base 517 resulted in a more open 530 loop structure wild-type and mutant 70S ribosomes were treated with RNase T1, which cleaves RNA at unpaired G residues, and RNase V1, which digests RNA at double-stranded or appropriately stacked regions. As shown in Figure 4A, RNase T1 readily cleaves the phosphodiester bond between G524 and C525 in both wild-type and 517C mutant ribosome preparations. The other mutant ribosomes were also cut to the same extent at this same position by RNase T1 (not shown). This was unexpected, since these two nucleotides, along with C526, have been implicated in the formation of a pseudoknot structure with residues G505, G506 and C507 (19,35). In contrast, while RNase V1 did not cleave wild-type RNA between G524 and C525, some digestion was noted at this position in mutants 517C (Figure 4B) and, to a lesser extent, 517U (not shown). An overall change in 530 loop structure in the 517C mutant ribosomes is also indicated by RNase V1 cleavages after C514 and G515. The absences of additional digestion in the stem could be due to bound ribosomal proteins or other steric hindrance effects. None of these cleavages was detected in RNase V1-treated, sucrose gradient-purified 30S subunits from wild-type or any of the

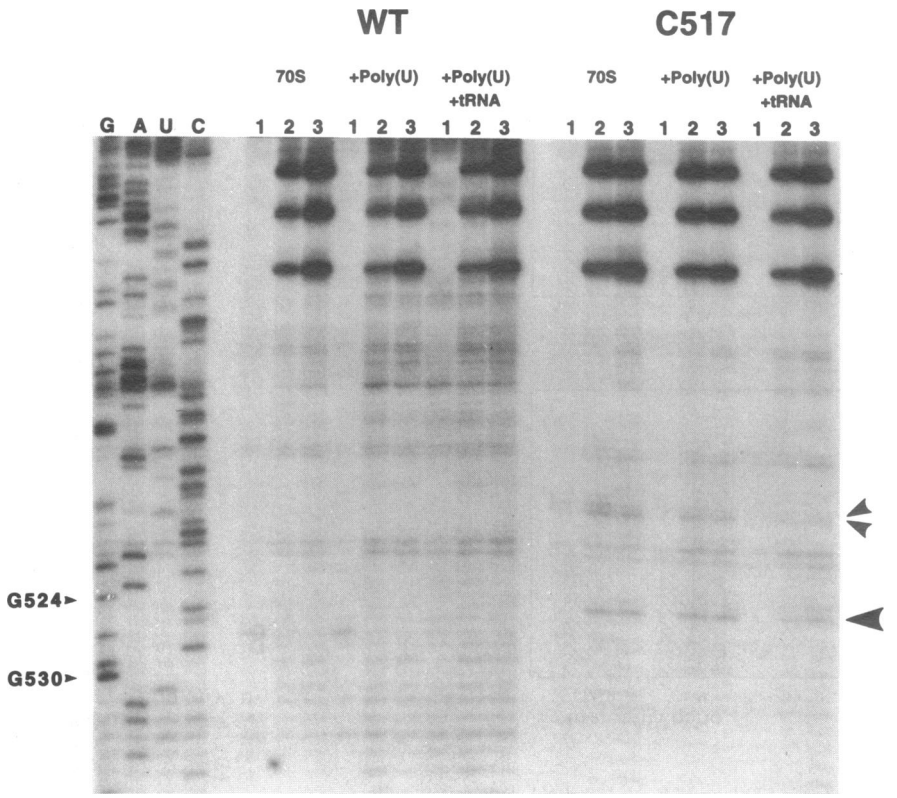


Figure 5. RNase V1 digestion of 70S ribosomes in the presence and absence of pre-bound poly(U) and $tRNA^{Phe}$. Wild-type and mutant 517C 70S ribosomes (50 pmol) were incubated in 50 µl 80 mM potassium cacodylate, pH 7.2, 20 mM $MgCl_2$, 100 mM NH_4Cl, 1.5 mM DTT at 37°C. Poly(U) (20 mg), with or without 100 pmol *E.coli* $tRNA^{Phe}$, was bound to the ribosomes as described (30) prior to the addition of 0.5 U RNase V1. Digestion, extraction of rRNA and primer extension were performed as described in Figure 4. Nuclease cleavages specific to mutant 517C are indicated by arrows, as described in Figure 4B.

16S rRNA mutants (data not shown), suggesting that the structure of this region of 16S rRNA may be influenced by subunit association/dissociation.

Digestion of mutant ribosomes by RNase V1 after binding poly(U) and $tRNA^{Phe}$ to 70S ribosomes was also investigated. As shown in Figure 5 (arrows), cleavage between G524 and C525 and between C514, G515 and U516 in the 517C mutant ribosomes was reduced by the addition of poly(U) and reduced further by poly(U) and $tRNA^{Phe}$. This was consistent with the effect of poly(U) and tRNA on chemical modification at positions 530, 531 and 532 shown in Figure 2.

DISCUSSION

Here we describe specific structural changes induced by a series of mutations in an important functional region of *E.coli* 16S rRNA, the 530 loop. Single base changes at position G517 had been reported to cause significant and varied effects on translational fidelity (14), but the structural basis for these effects had not been determined. As shown above, the extent of structural change correlated with the level of misreading in the different mutants and was greatest in mutant 517C. Residues G530, U531 and A532 within the 530 loop are functionally important and are normally protected in 70S ribosomes by A- or P-site bound tRNAs from attack by chemical probes (see 7,8,30). These residues became hyper-reactive when base 517 was mutagenized (see Fig. 2). By comparison, base substitution mutations at position 534, which had no effect on translational accuracy, did not affect the pattern of chemical modification in the 530 loop. These data clearly showed that significant changes in the higher order structure of the 530 loop occurred at functionally important nucleotides as a result of specific base changes at position 517.

The results of oligonucleotide binding experiments (Fig. 3) also indicate a more open structure of the 530 loop in 517C mutant ribosomes. Oligonucleotides complementary to both halves of the 530 loop showed increased binding in the error-prone 517C mutant ribosomes compared with the wild-type. These data are complemented by the reduced levels of oligonucleotide binding to both wild-type and mutant ribosomes to which tRNA had been pre-bound. This was consistent with an open structure which became more compact as a result of tRNA–ribosome interactions, although it might also reflect steric hindrance. These results would appear to conflict with those presented by Brakier-Gingras and co-workers (36). They showed that mutations at positions 13 and 914, which have a restrictive phenotype, also resulted in a more 'open' 530 loop structure as measured by oligonucleotide binding assays. Potential explanations for this inconsistency include the facts that they used isolated 30S subunits, while we used tight-couple 70S ribosomes, and their mutations were outside the 530 loop itself. In addition, they used a single probe specific for 520–531, while we used two separate probes for this region. Changes of only a few nucleotides can have significant effects on probe binding (34).

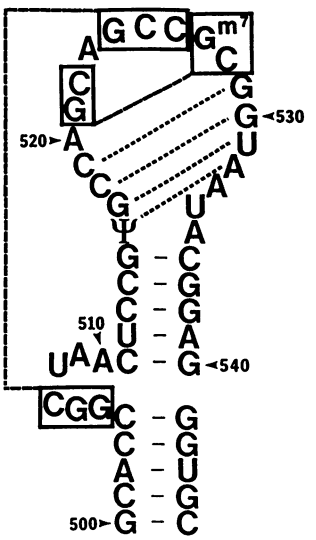


Figure 6. Potential transient base pairing interactions across the *E.coli* 530 loop of 16S rRNA between U516–C519 and G529–A532 are shown by the dashed lines.

RNase T1 cuts RNA at unpaired G residues, so the substantial cleavage between G524 and C525 in all ribosome preparations suggests that the pseudoknot, formed between G524-C525-C526 and G505-G506-C507 may not be a stable feature of the 530 loop. Data from other laboratories also suggest that the 530 loop can undergo structural transitions. For example, the reactivity of C525 to DMS is greatly enhanced when neomycin is bound to 30S subunits (37). In addition, mRNA has recently been cross-linked to G524 (22), showing that the bases involved in pseudoknot formation can interact with other ligands. Taken together these results suggest that the pseudoknot is not a permanent feature of the 530 loop structure and may alternate normally between folded and unfolded states during translation.

Cleavage of the phosphodiester bond between G524 and C525 by RNase V1 in 517C mutant ribosomes (see Fig. 4B) appears inconsistent with the possibility that this region may exist in a single-stranded form at least part of the time. However, it has been reported that this enzyme can recognize and cleave single-stranded RNA if the bases are appropriately stacked (26,27). A structural shift to a less compact form would also explain the RNase V1 cuts between residues C514, C515 and U516 of 517C mutant 16S rRNA, as shown in Figure 4B. The reduced cleavage by RNase V1 at all of these sites when tRNA was bound to the ribosomes (see Fig. 5) could then be explained as a structural transition that moved the 530 loop to a position that was less accessible to the enzyme.

The enhanced chemical modification of G530, U531 and A532 in the 517 mutant ribosomes suggests that these bases are at least partially protected in wild-type 70S ribosomes even in the absence of tRNA. It is possible that this protection could be the result of transient hydrogen bonding across the 530 loop between U516-C519 and G529-A532 (see Fig. 6). Position 534 is not involved in this alternate structure and mutations at this base neither affect translational fidelity nor alter the accessibility of the 530 loop to chemical probes. Disruption of base pairing potential caused by mutations of base 517, however, correlates well with the degree of chemical reactivity at positions 530–532. For example, mutants 517C and 517U, which would disrupt base pairing, give the highest level of chemical modification at position 530. On the surface it may appear that the G517→A mutation should reduce the levels of chemical modification at positions 530–532 as a G517–U531 wobble base pair would be replaced by 517A –U531. This might be expected in a protein-free environment, but the structure of the 530 loop is profoundly affected by interactions with ribosomal proteins, particularly S12. Recent results (38) have demonstrated that the ribose sugar at position 517 is strongly protected from iron–EDTA-induced cleavage by protein S12, suggesting direct contact. Such a close interaction would undoubtedly constrain the ability of 517A to rotate to a position that can effectively hydrogen bond to U531. The phylogenetic evidence for such an interaction is scant, owing to the high degree of conservation of the 530 loop. Only a few changes at these positions have been reported, some of which strengthen (e.g. U531→C in the mitochondrial ribosomes of *Rana catesbeiana*), while others weaken (e.g. C518→U in *Pyrodictium occultum*) these proposed interactions (39). However, since the 530 loop appears to undergo a shift between two distinct conformations which are affected/modulated by associated proteins, strict adherance to phylogeny in such cases may not be warranted. This model is not unreasonable in the light of the recent demonstration of transient base pairing in the α-sarcin loop, the 530 loop 'counterpart' in 23S rRNA (40).

The results presented here clearly demonstrate a change in the higher order structure of the 530 loop resulting from mutagenesis of G517. Whether these structural changes actually define a switch mechanism involved in 530 loop function is uncertain. It is apparent, however, that our data are not entirely consistent with a model for proof-reading involving the 530 loop recently proposed by Powers and Noller (41) They suggested that proof-reading involves, in part, a switch between 'open' and 'closed' states of the 530 loop and predicted that a more open 530 loop conformation would reduce the error frequency by binding more tightly to EF-Tu–GDP. Alternately, a shift to the closed form would result in a higher rate of factor release, which would be more permissive. We find, however, that mutations at position 517 which reduce translational fidelity result in a more open structure in the 530 loop. Despite this conflict, results from several laboratories now suggest that the 530 loop exists in at least two distinct structural forms, termed 'open' and 'closed'. It is tempting to speculate that a structural shift between these forms is involved in proof-reading and the rate of the shift, possibly dictated by the antagonistic activities of ribosomal proteins S4, S5 and S12, may be as important as the shift itself. Mutations in any of these components could alter the dynamics of this finely tuned process, which would affect translational fidelity.

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