*Proc. Natl. Acad. Sci. USA*
Vol. 92, pp. 10555–10559, November 1995
Genetics

# Genetic and comparative analyses reveal an alternative secondary structure in the region of nt 912 of *Escherichia coli* 16S rRNA

J. STEPHEN LODMELL*, ROBIN R. GUTELL†, AND ALBERT E. DAHLBERG*

*Department of Molecular and Cell Biology and Biochemistry, Division of Biology and Medicine, Brown University, Providence, RI 02912; and †Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309

*Communicated by Harry F. Noller, University of California, Santa Cruz, CA, August 2, 1995*

**ABSTRACT** **Mutations at position 912 of *Escherichia coli* 16S rRNA result in two notable phenotypes. The C → U transition confers resistance to streptomycin, a translational-error-inducing antibiotic, while a C → G transversion causes marked retardation of cell growth rate. Starting with the slow-growing G912 mutant, random mutagenesis was used to isolate a second site mutation that restored growth nearly to the wild-type rate. The second site mutation was identified as a G → C transversion at position 885 in 16S rRNA. Cells containing the G912 mutation had an increased doubling time, abnormal sucrose gradient ribosome/subunit profile, increased sensitivity to spectinomycin, dependence upon streptomycin for growth in the presence of spectinomycin, and slower translation rate, whereas cells with the G912/C885 double mutation were similar to wild type in these assays. Comparative analysis showed there was significant covariation between positions 912 and 885. Thus the second-site suppressor analysis, the functional assays, and the comparative data suggest that the interaction between nt 912 and nt 885 is conserved and necessary for normal ribosome function. Furthermore, the comparative data suggest that the interaction extends to include G885-G886-G887 pairing with C912-U911-C910. An alternative secondary structure element for the central domain of 16S rRNA is proposed.**

rRNA has been shown to be an active participant in many ribosomal functions, including initiation of translation, decoding of the mRNA, and modulation of translational fidelity (for review, see refs. 1 and 2). One approach to assigning particular functions of the ribosome to specific nucleotides or groups of nucleotides within the rRNA involves mutagenesis. We have reexamined an interesting mutation at position 912 in 16S rRNA of *Escherichia coli*, which was described as slow-growing and prone to reversion (3), although the underlying cause of the slow growth was not determined.

Position 912 is of particular interest as a potential functional site for several reasons. (*i*) Genetic (4–12), footprinting (13), and crosslinking data place the binding site for streptomycin, a translational error-inducing antibiotic (14), at or near position 912 (15) and near ribosomal protein S5 (16). (*ii*) Ribosomal proteins S5 and S12 (both implicated in the modulation of translational accuracy) have been mapped to the vicinity of position 912 by chemical footprinting (17) and by crosslinking (18), and mutations in 16S rRNA near position 912 have been shown to influence translational fidelity (8, 19, 20). (*iii*) Position 912 lies near the convergence of the three major domains found in all small-subunit rRNAs (see Fig. 1). Because this three-domain structure is conserved throughout nature, it has been proposed that the domains could serve as functional modules of the ribosomal machinery (21). Position 912 is located at a potential hinge site at the junction of these modules and is in close proximity to three pseudoknot structures that have been proven experimentally (22–24). (*iv*) Although the reactivity toward chemical modification of position 912 itself is not altered upon tRNA binding or subunit association, the reactivities of nearby residues A892, A908, and A909 are affected, implying that the region is directly or indirectly involved in these binding activities (25, 26). Still, the function of the nt-912 region remains obscure.

Several laboratories have made mutations at position 912 in *E. coli* since the discovery that a C912 → U transition confers streptomycin resistance in *Euglena* chloroplasts (3–5, 8, 22, 27). Indeed, U912 confers streptomycin resistance in *E. coli*, although the resistance appears to be recessive when both wild-type (C912) and mutant (U912) ribosomes are translationally active (22). In addition, one laboratory has made the reciprocal mutation (U912 → C) in yeast, which are naturally resistant to streptomycin, and this induced increased sensitivity to the antibiotic (28). Because of the apparent connection of the nt-912 region with streptomycin binding, most of the published experiments have investigated antibiotic resistance associated with U912.

Frattali *et al.* (3) constructed all possible mutations at position 912 (A, G, U, and deletion) and measured growth rates and streptomycin resistance. They found that whereas the C912 → U or A mutations did not affect growth rate significantly, the C912 → G mutation caused very slow growth. Here we have employed random mutagenesis to generate a second site mutation in the plasmid-borne rRNA operon that restores normal growth to the G912 mutant. This suppressor mutation, a G → C transversion at position 885, supports a recent prediction of base pairing between positions 912 and 885 (21). The genetic suppressor analysis and the comparative sequence analysis provide compelling evidence for an alternative structure in current 16S secondary structure maps (e.g., refs. 21 and 29) that would incorporate base pairing of G885-G886-G887 with C912-U911-C910 (Fig. 1).

## MATERIALS AND METHODS

**Bacterial Strains.** Strains used were DH1 (30), MDA6646 (*pcnB*, described in ref. 31), and CSH116 (obtained from Cold Spring Harbor Laboratory). CSH116 contains the *mutD* allele of the ε subunit of DNA polymerase III and was used for random mutagenesis. This mutation interferes with an editing function necessary for accurate replication of DNA (32); therefore, errors are introduced randomly throughout genomic and plasmid DNA within the cells. MDA6646 contains the *pcnB* allele, which reduces the copy number of ColE1-type multicopy plasmids, effectively reducing the gene dosage of plasmid-encoded rRNA in transformed cells. This strain was used to maintain plasmids that were very deleterious at high copy number.

**Plasmids.** Plasmids were derivatives of pSTL102, which contains the *rrnB* rRNA operon and two additional antibiotic-resistance markers, U1192 in 16S rRNA (spectinomycin resis-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: IPTG, isopropyl β-D-thiogalactopyranoside.

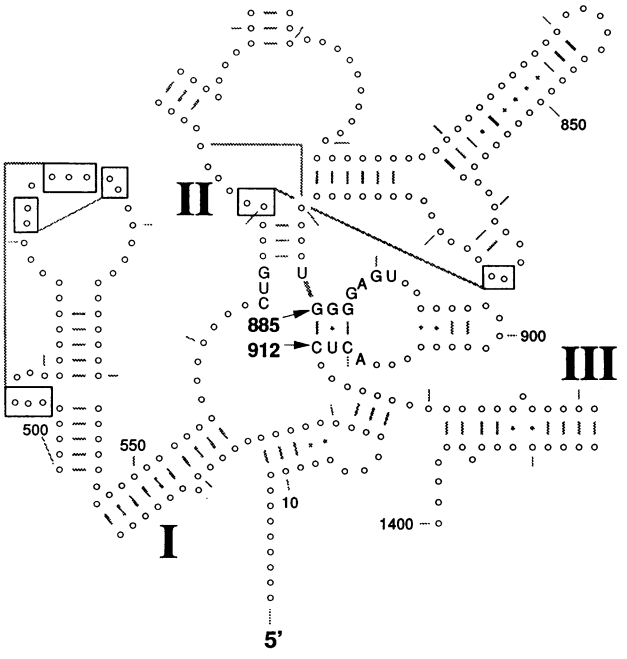


FIG. 1. Secondary structure maps of the central portion of *E. coli* 16S rRNA. Every 10th position has a tick mark, every 50th position is numbered. Roman numerals are domain numbers. Previous secondary structure maps showed pairing or juxtaposition between positions 888–891 and 909–912 and between positions 564–566 and 884–886 (see text for details).

tance) and G2058 in 23S rRNA (erythromycin resistance) in a pBR322 vector (33). Mutations U912 and G912, originally expressed (3) in plasmid pKK3535 (34), were subcloned into pSTL102 by using the *Bgl* II–*Xba* I fragment.

**Mutagenesis.** Selection of a second-site suppressor of G912 was accomplished by transforming CSH116 with a plasmid containing G912. Transformants were used to inoculate 2.5-ml liquid cultures [LB broth/ampicillin (200 μg/ml)], which were diluted and grown to saturation several times. Aliquots of the liquid culture were plated after each growth cycle onto LB/ampicillin (200 μg/ml)/spectinomycin (10 μg/ml)/erythromycin (50 μg/ml) plates. After 18–24 h at 37°C, colonies were scraped from the plates and used to prepare plasmid DNA, which was then used to transform DH1 cells on LB/ampicillin (200 μg/ml) plates. Fast-growing transformants were then screened by streaking onto LB/ampicillin (200 μg/ml)/spectinomycin (100 μg/ml) plates. Plasmid DNA was isolated from strains surviving the selection and screening, and mutations were mapped and identified by subcloning and sequencing. Site-directed mutagenesis (35) was used to make mutations at positions 912 and 885 in the *Eco*RI–*Xba* I fragment of pSTL102 via an M13mp18 intermediate.

**Expression of Mutant rRNA.** The relative expression of plasmid-encoded and host chromosomal rRNA was determined by using the primer-extension method of Sigmund *et al.* (36). A primer complementary to 16S rRNA immediately downstream of position 1192 was used to measure the relative proportions of U1192 (plasmid encoded) and C1192 (chromosomally encoded) rRNA in cell lysates or gradient fractions.

**Comparative Sequence Analysis.** A prokaryotic 16S rRNA alignment (Ribosomal Database Project, ref. 37; ≈1500 sequences) and eukaryotic (≈700 sequences) and mitochondrial (≈230 sequences) 16S-like rRNA alignments (S. Damberger and R.R.G., unpublished dataset) were analyzed by using correlation algorithms (refs. 38–40; S. Damberger and R.R.G., unpublished data) to identify positional covariance in 16S and 16S-like rRNAs.

**Growth Rates.** The optical density of cells growing in LB/ampicillin at 37°C with shaking was followed by using a Klett–Summerson colorimeter (filter 54; 500–570 nm).

**Translation Rate.** The translation rate assay measures the amount of time required to produce β-galactosidase *in vivo* after induction with isopropyl β-D-thiogalactopyranoside (IPTG). Assays were performed as described in refs. 41 and 42.

**Ribosome Gradients.** The relative proportions of 70S ribosomes and ribosomal subunits in lysates of cells transformed with mutant or wild-type plasmids were determined as described (43). From 15 to 30 $A_{260}$ units of cell lysates were loaded onto a 5–35% sucrose gradient and centrifuged for 18 h at 17,000 rpm in a Beckman SW28 rotor. The gradients were analyzed by using an ISCO density gradient fractionator/detector.

## RESULTS

**Genetic Selection of a Suppressor of the G912 Mutation.** Cells transformed with a multicopy plasmid containing the G912 mutation grew very slowly (Table 1) and, when combined with a C1192 → U mutation (which normally confers resistance to spectinomycin), the G912/U1192 mutants remained spectinomycin-sensitive. To examine the possibility that an essential secondary or higher-order structure interaction involving position 912 was disrupted in the G912 mutant, we sought to isolate second-site suppressor mutations. Random mutagenesis of the G912/U1192 plasmid and subsequent selection for faster growth and resistance to spectinomycin were used to isolate plasmid-encoded suppressor mutations. Two groups of suppressors were isolated. The first group was the expected G → C reversion to wild type at position 912. More interesting was a G → C mutation at position 885 in 16S rRNA. When the G912/C885 double mutant was constructed by site-directed mutagenesis, it gave the same phenotype as the

Table 1. Growth rates, antibiotic resistances, and translation rates of transformed DH1 cells

| Plasmid | Doubling time, min | Cell viability on spectinomycin | Streptomycin MIC, μg/ml | Translation rate of β-gal induction, sec |
|---|---|---|---|---|
| C912/G885 (wt) | 39 ± 2 | + | 8 | 123 ± 10 |
| G912/G885 | 78 ± 9 | − | 16* | 156 ± 11 |
| U912/G885 | 44 ± 3 | + | 32 | 129 ± 6 |
| G912/U885 | 59 ± 7 | + | 4 | 146 ± 6 |
| G912/C885 | 41 ± 3 | + | 8 | 132 ± 8 |

Growth rates were measured in LB medium supplemented with ampicillin (200 μg/ml). Viability on spectinomycin was determined by streaking cells onto LB plates containing ampicillin (200 μg/ml) and spectinomycin (100 μg/ml). Minimal inhibitory concentrations (MIC) of streptomycin were measured by streaking cells onto plates containing ampicillin (200 μg/ml), spectinomycin (60 μg/ml), and streptomycin (0, 2, 4, 8, 16, or 32 μg/ml). Translation rate was measured as the time required to translate β-galactosidase (β-gal) after induction of cell cultures with IPTG (see text).
*G912 mutants did not grow in the absence of streptomycin.

original G912/C885 isolate, indicating the C885 mutation alone was sufficient to produce the suppressor phenotype.

**Comparative Sequence Analysis of the Regions of Positions 912 and 885.** Comparative sequence analysis is based on the simple concept that different sequences performing similar structural functions will adopt a comparable three-dimensional structure. Base pairings are inferred when two positions in an alignment of homologous sequences vary in a coordinated manner. This positional covariation is the cornerstone of comparative sequence analysis and has been applied to several RNA molecules including 16S rRNA (21, 29, 44–47, 56). However, in highly conserved sequences, as are often found in functionally important regions, the level of variation may be too low to provide sufficient covariation data.

The 16S rRNA secondary structure models have evolved as the number and diversity of sequences have increased, and our covariance algorithms became more sophisticated. In the center of the older 16S rRNA secondary structure models were two base-paired helices, nt 564–566 base-paired to nt 886–884, flanked on the 3′ side by nt 888–891 base-paired to nt 909–912, which were proposed (but not considered proven) based on nucleotide pairing compatibility in the rRNA sequences known at the time. With increases in the number of available sequences, a growing number of noncompensated changes were identified, thus eliminating these pairings from the secondary structure model. Visual analysis of the sequences in this region suggested three alternative base pairings, nt 885·912, nt 886·911, and nt 887·910 (21). Comparative support for these pairings was initially within the smaller mitochondrial data set and these pairings were considered marginal (21). With improvements in the covariance algorithms (refs. 38 and 39 and S. Damberger and R.R.G., unpublished data) and a significantly larger set of mitochondrial sequences (≈230 as of May 1995), we have found that positions 885 and 912 and positions 886 and 911 correlate best with one another by using algorithms measuring mutual information (38) and phylogenetic events (39). No single significant correlation was found for positions 887 or 910 although they did have a weak correlation with one another (data not shown). Due to a greater conservation of these nucleotides in the prokaryotic and eukaryotic alignments, neither correlation algorithm identified significant correlations at any of these positions, but within the Eucarya, there was a minor covariance between nt 887 and 910, lending credence for this putative base pair.

Table 2 reveals the percentages and phylogenetic events occurring at the nt 912·885,. nt 911·886, nt 910·887, and nt 912·886 pairings. Note for the prokaryotic/chloroplast and eukaryotic data sets, no compensatory base changes are known, except for the nt 887·910 pair in the Eucarya, where a few phylogenetically independent examples of mutual change underlie the G·C and C·G base pairs. In contrast, within the mitochondrial data set, many phylogenetic events underlie the canonical and G·U base pairs at these three pairings. The nt 886·912 pairing is suggestive in the prokaryotic/chloroplast data set due to the number of phylogenetic events associated with the canonical and G·U pairings. However, in the mitochondria this pairing is suspect, thus we believe that the apparent correlation is due to independent identity constraints on positions 912 and 886.

**Construction of Additional Mutants at Positions 885 and 912.** To test the hypothesis that base pairing between positions 912 and 885 is necessary for normal ribosome function, mutants were constructed in which the base-pairing potential varied considerably. These included base pairs C·G, G·C, U·G, G·U, G·G, and C·C (nt 912·885). In cells transformed with each plasmid, the plasmid-encoded rRNA accounted for >70% of the total 16S rRNA, and it was equally represented in the 30S, 70S, and polysome fractions for each sample as measured by primer-extension analysis. Only the C912/C885 mutant was not viable at high expression levels and was not used further.

Table 2. Comparative analysis of base pairing between positions 912–910 and 885–887

| Base pairs | | | |
|---|---|---|---|
| nt 885·912 | nt 886·911 | nt 887·910 | nt 886·912 |
| (eu)Bacteria, Archaea, and chloroplasts (≈1500 sequences) | | | |
| G·C (93) | G·U (96) | G·C (100) | G·C (91) [3] |
| G·U (7) | A·U (4) | | G·U (5) |
| | | | A·U (2) [3] |
| | | | A·C (2) [2] |
| Eucarya (≈700 sequences) | | | |
| G·U (100) | G·U (100) | G·C (93) [2] | G·U (100) |
| | | C·G (4) [1] | |
| | | A·C (2) | |
| | | N·N (1) | |
| Mitochondria (≈230 sequences) | | | |
| G·C (87) [7] | A·U (61) [9] | G·C (81) [5] | A·C (54) [1] |
| A·U (5) [3] | G·C (28) [8] | A·C (11) | G·C (33) [3] |
| C·G (3) [4] | G·U (9) | G·U (2) [1] | A·U (5) [3] |
| G·U (2) [1] | A·C (1) | U·G (2) [3] | A·G (3) [1] |
| U·A (1) [1] | U·A (1) [1] | A·U (1) [2] | G·U (3) [1] |
| N·N (1) | | C·G (1) [1] | N·N (2) |
| | | U·A (1) [1] | |
| | | G·A (1) | |

Numbers in parentheses are percent of total sequences examined. Numbers in brackets, if given, are the approximate numbers of phylogenetically independent examples of mutual change resulting in that base-pair type. N·N represents other nucleotide combinations.

**Growth Rates of Mutants at Positions 912 and 885.** The growth rates of DH1 cells transformed with wild-type and mutant plasmids were measured and are shown in Table 1. The U912/G885 and the G912/C885 mutants had doubling times of 44 and 41 min, respectively, comparable to the wild-type C912/G885 value of 39 min. However, cells containing the G912/U885 and G912/G885 mutations grew more slowly, with doubling times of 59 and 78 min, respectively. The growth rate data suggest that the ability to form base pairs between positions 912 and 885 is important for cell growth.

**Antibiotic Resistance of Mutants at Positions 912 and 885.** Spectinomycin resistance was assessed by streaking transformed cells onto LB/ampicillin (200 μg/ml)/spectinomycin (100 μg/ml) plates. All of the plasmids tested contained U1192 (which normally confers spectinomycin resistance when the majority of 16S rRNA within the cells contains U1192) in addition to mutations at positions 912 and/or 885. Cells transformed with each of the mutant plasmids except for G912/G885 formed normal colonies on spectinomycin plates. Therefore, base pairing capability between nt 912 and nt 885 appears to be essential for cell viability under these conditions.

Because positions 912 and 885 have been implicated in streptomycin resistance, different combinations of nucleotides at positions 912 and 885 were tested for streptomycin resistance on plates containing ampicillin, spectinomycin, and increasing amounts of streptomycin (Table 1). Spectinomycin was included to remove the host encoded (wild type) ribosomes from the translating pool and avoid the dominant streptomycin sensitivity that would otherwise affect the assay (22). U912/G885 mutants were resistant to streptomycin up to 32 μg/ml. C912/G885 (wild type) and G912/C885 mutant cells were inhibited at streptomycin concentrations >4 μg/ml, and G912/U885 was inhibited at >2 μg/ml. The streptomycin effects were more complex for the G912/G885 mutant. With streptomycin at 0 and 2 μg/ml, cells could not be streaked to single colonies, but with streptomycin at 4–8 μg/ml, single colonies formed. This mutant therefore can be described as streptomycin dependent under these conditions. At concentrations >8 μg/ml, growth was again inhibited.

**Translation Rates of the Mutants at Positions 912 and 885.** The translation rate assay measured the amount of time

required for the first molecules of functional β-galactosidase to be produced after induction with IPTG (41). The results demonstrated a correlation between growth rate and translation rate inasmuch as mutations causing the slowest growth rates (e.g., G912 and G912/U885) also had the longest induction times (Table 1). Likewise, the faster growing mutants, U912 and G912/C885, had induction times close to that of the wild type.

**Sucrose Gradient Ribosome/Subunit Profiles.** Lysates from cells containing mutant or wild-type plasmids were fractionated by sucrose density gradient centrifugation, and the relative proportions of subunits and 70S ribosomes are shown in Fig. 2. Wild-type C912/G885 and U912/G885 and G912/C885 mutants gave similar profiles, with the 70S peak much larger than the subunit peaks. However, the G912/G885 profile showed an abundance of subunits relative to 70S ribosomes and the G912/U885 profile was intermediate between the wild-type profile and the G912/G885 profile. Thus, the potential for base pairing between positions 912 and 885 correlated well with the relative proportions of subunits and intact 70S ribosomes in the sucrose gradient profiles.

## DISCUSSION

In this work we present genetic and comparative evidence for the base-pairing interaction of positions 885 and 912 of 16S rRNA of *E. coli*. Further, we propose that this interaction involves three base pairs, nt 885·912, nt 886·911, and nt 887·910, not depicted in previous secondary structure maps of 16S rRNA (e.g., refs. 21 and 29). We show that disruption of potential base pairing between positions 912 and 885 perturbs normal ribosome function and is manifested in slow cell growth, altered antibiotic sensitivity, decreased translation rate, and abnormal 70S ribosome/ribosomal subunit ratios in sucrose gradient profiles. Restoring potential base pairing to these two positions largely corrects these defects. Because the interaction was identified with a genetic selection, we believe the interaction to be of physiological relevance.

An objective of the present work was to determine why a C → G substitution at position 912 causes slow cell growth whereas the C → A and C → U mutations do not (3). In an effort to identify a specific interaction between position 912 and another nucleotide (or ribosomal protein) that is necessary for normal ribosome function *in vivo*, we used genetic suppressor analysis. We found that a G → C transversion at position 885 eliminated the slow growth rate associated with the G912 mutation. However, the C885/C912 double mutant was not viable, suggesting that pairing potential between positions 912 and 885 is important, not strictly the nucleotide identity at either position. In light of earlier experiments, this result suggested that the reason that C, U, and A are tolerated

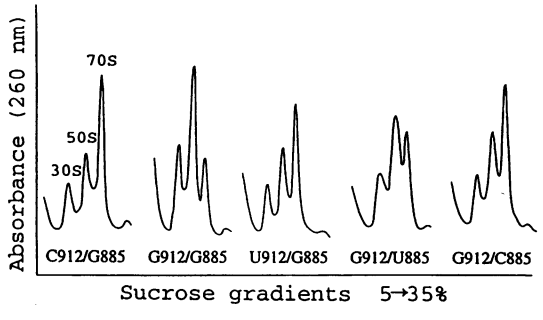


FIG. 2. Sucrose density gradient ribosome/subunit profiles of lysates from DH1 cells transformed with plasmids indicated. Cell lysates were subjected to centrifugation through 5–35% linear gradients. Primer-extension analysis indicated that the proportion of plasmid-encoded rRNA in each fraction was approximately equal (see *Results*).

at position 912 in *E. coli* is that they could all form base pairs with G885 (A·G pairs, though less common than C·G and U·G pairs, do occur in rRNA), whereas G912 would force a G·G interaction, a relatively rare and probably thermodynamically less stable structure in rRNA (21, 48). Based on older secondary structure maps (e.g., refs. 47 and 49), one might have expected to isolate a suppressor at position G888, which was depicted as base-paired with C912. In fact, based on differences between ribosomes containing only U912 and those containing both U912 and A888 in *in vitro* translation assays, it has been suggested that there might be base pairing between these positions (50). Although our results do not exclude the possibility that nt 912 interacts with other nucleotides, our *in vivo* selection and phylogenetic analysis suggest the nt 912·885 interaction is the most important.

The sucrose gradient profiles shown in Fig. 2 demonstrate that perturbation of the nt 912·885 interaction results in an increase of subunits relative to 70S ribosomes in cell lysates. A possible interpretation is that ribosomes compromised in their ability to form nt 912·885 base pairs have a defect at initiation which results in the accumulation of 30S subunits relative to actively translating 70S ribosomes. However, if mutant (plasmid encoded) 30S subunits were inefficient at initiation, mutant 16S rRNA would be overrepresented in the 30S subunit fraction and underrepresented in the 70S and polysome fractions. In fact, we found that the proportion of plasmid-encoded vs. chromosomally encoded rRNA is roughly equal in all three fractions. Thus, these mutant ribosomes do not appear to have an initiation defect *per se*. Rather, initiation may be affected by elongating ribosomes moving away from the ribosomal start site abnormally slowly. Indeed, the translation rate results show that induction time for *lacZ* expression after addition of IPTG is longer in mutants compromised in nt 912·885 base-pairing potential (Table 1). Thus these data suggest that pairing between positions 912 and 885 is necessary for efficient elongation, and that mutations that perturb this interaction exert their deleterious effects by slowing the overall translation rate.

Over the last 10 years, much of the interest in the nt-912 region of 16S rRNA has centered around its apparent involvement in binding streptomycin, a translational error-inducing antibiotic. Point mutations at positions equivalent to position 912 in various organisms confer streptomycin resistance (4–7, 10, 22). Notably, a recent report (10) showed that a point mutation at position 885 in *Nicotiana plumbaginifolia* chloroplasts also afforded streptomycin resistance, consistent with a functional linkage between these two positions. In this study, we assayed the streptomycin resistance of cell strains harboring several combinations of mutations at positions 912 and 885. Both wild-type (C912/G885) and the reciprocal mutant G912/C885 were inhibited by streptomycin at >4 μg/ml. Consistent with previous observations, U912/G885 mutants were significantly resistant to streptomycin (3, 5, 22, 50–52), but we found that G912/U885, the reciprocal mutant, was streptomycin-sensitive. The nonequivalence of these two mutants is interesting and probably relates to differences in the local RNA structure. In particular, several studies have shown that the stability of G·U and U·G pairs is quite context-dependent, and often G·U pairs are not equivalent to U·G pairs (refs. 53–55; see Fig. 3). Interestingly, the G912/G885 mutant would not grow on ampicillin/spectinomycin plates without streptomycin, nor did it grow on these plates at high concentrations of the drug, but it did form colonies on these plates containing streptomycin between 4 and 8 μg/ml. Thus, the G912/G885 mutant is dependent upon streptomycin for growth in the presence of spectinomycin.

These streptomycin-resistance data provide clues into the nature of the interaction of streptomycin with 16S rRNA. We hypothesize, based on several lines of evidence, that streptomycin stabilizes a normally dynamic interaction of nt 885–887

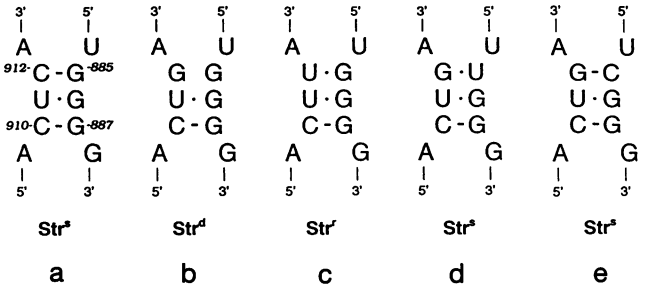


FIG. 3. Base-pairing combinations between positions 912–910 and 885–887 with plasmids C912/G885 (wild type) (*a*), G912/G885 (*b*), U912/G885 (*c*), G912/U885 (*d*), and G912/C885 (*e*). Streptomycin-sensitive (s), -dependent (d), and -resistant (r) phenotypes are indicated.

with nt 912–910. (*i*) Streptomycin causes nt 912 to be protected from chemical modification (13). This effect may be manifested via the stabilization of base pairing between positions 912 and 885 by streptomycin. (*ii*) Mutations at both positions 912 and 885 confer resistance to streptomycin (discussed above). These mutations could exert their effects directly, by disrupting the antibiotic binding site, or indirectly, by weakening normal base pairing between these two regions, thereby lessening the stabilizing effect of streptomycin. (*iii*) In the present work, we find that under certain conditions, the deleterious G912/G885 mutant grows better in the presence of a low concentration of streptomycin. In this case, it is possible that streptomycin stabilizes the nt 912·885 interaction, which was weakened by the G912 mutation.

Further evidence for the transient nature of this interaction lies in the comparative data that show that a wobble (G·U) or other noncanonical pair is usually present in the nt 912–910/885–887 helix, suggesting that both the paired and unpaired conformations are necessary during elongation. If the region were designed by nature to be static, one would expect more thermodynamically stable base pairing in this short helix. The existence of only very short helices and three pseudoknot structures surrounding the region of position 912 (21–24) may permit increased flexibility to accommodate alternative coaxial stacking and/or opening and closing of helical segments.

We are grateful to Steven Gregory, Michael O'Connor, and Angela Grant for critical reading of the manuscript and to George Q. Pennabble for experimental protocols. R.R.G. thanks the W.M. Keck Foundation for their generous support of RNA science on the Boulder campus. This research was supported by National Institutes of Health Grants GM48207 to R.R.G. and GM19756 to A.E.D. and National Institutes of Health Training Grant GM07601 to J.S.L.

1. Dahlberg, A. E. (1989) *Cell* **57,** 525–529.
2. Noller, H. F. (1991) *Annu. Rev. Biochem.* **60,** 191–227.
3. Frattali, A. L., Flynn, M. K., De Stasio, E. A. & Dahlberg, A. E. (1990) *Biochim. Biophys. Acta* **1051,** 27–33.
4. Montandon, P. E., Nicolas, P., Schurmann, P. & Stutz, E. (1985) *Nucleic Acids Res.* **13,** 4299–4310.
5. Montandon, P. E., Wagner, R. & Stutz, E. (1986) *EMBO J.* **5,** 3705–3708.
6. Etzold, T., Fritz, C. C., Schell, H. & Schreier, P. (1987) *FEBS Lett.* **219,** 343–346.
7. Harris, E. H., Burkhart, B. D., Gillham, N. W. & Boynton, J. E. (1989) *Genetics* **123,** 281–292.
8. Leclerc, D., Melancon, P. & Brakier-Gingras, L. (1991) *Nucleic Acids Res.* **19,** 3973–3977.
9. Honore, N. & Cole, S. T. (1994) *Antimicrob. Agents Chemother.* **38,** 238–242.
10. Yeh, K. C., To, K. Y., Sun, S. W., Wu, M. C., Lin, T. Y. & Chen, C. C. (1994) *Curr. Genet.* **26,** 132–135.
11. Fromm, H., Galun, E. & Edelman, M. (1989) *Plant Mol. Biol.* **12,** 499–505.
12. Meier, A., Kirschner, P., Bange, F.-C., Vogel, U. & Bottger, E. C. (1994) *Antimicrob. Agents Chemother.* **38,** 228–233.
13. Moazed, D. & Noller, H. F. (1987) *Nature (London)* **327,** 389–394.
14. Gorini, L. (1974) in *Ribosomes*, eds. Nomura, M., Tissiéres, A. & Lengyel, P. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 791–803.
15. Gravel, M., Melancon, P. & Brakier-Gringas, L. (1987) *Biochemistry* **26,** 6227–6232.
16. Abad, J. P. & Amils, R. (1994) *J. Mol. Biol.* **235,** 1251–1260.
17. Stern, S., Powers, T., Changchien, L.-M. & Noller, H. F. (1989) *Science* **244,** 783–790.
18. Osswald, M., Greuer, B., Brimacombe, R., Stoffler, G., Baumert, H. & Fasold, H. (1987) *Nucleic Acids Res.* **15,** 3221–3240.
19. Pinard, R., Cote, M., Payant, C. & Brakier-Gingras, L. (1994) *Nucleic Acids Res.* **22,** 619–624.
20. Pinard, R., Payant, C., Melancon, P. & Brakier-Gingras, L. (1993) *FASEB J.* **7,** 173–176.
21. Gutell, R. R., Larsen, N. & Woese, C. R. (1994) *Microbiol. Rev.* **58,** 10–26.
22. Powers, T. & Noller, H. F. (1991) *EMBO J.* **10,** 2203–2214.
23. Brink, M. F., Verbeet, M. P. & deBoer, H. A. (1993) *EMBO J.* **12,** 3987–3996.
24. Vila, A., Viril-Farley, J. & Tapprich, W. E. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11148–11152.
25. Moazed, D. & Noller, H. F. (1986) *Cell* **47,** 985–994.
26. Allen, P. N. & Noller, H. F. (1989) *J. Mol. Biol.* **208,** 457–468.
27. Morgan, E. A., Gregory, S. T., Sigmund, C. D. & Borden, A. (1988) *ASI Ser. H.* **14,** 43–53.
28. Chernoff, Y. O., Vincent, A. & Liebman, S. W. (1994) *EMBO J.* **13,** 906–913.
29. Brimacombe, R. (1991) *Biochimie* **73,** 927–936.
30. Hanahan, D. (1983) *J. Mol. Biol.* **166,** 557–580.
31. O'Connor, M., Goringer, H. U. & Dahlberg, A. E. (1992) *Nucleic Acids Res.* **20,** 4221–4227.
32. Scheuermann, R., Tam, S., Burgers, P. M. J., Lu, C. & Echols, H. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7085–7089.
33. Triman, K., Becker, E., Dammel, C., Katz, J., Mori, H., Douthwaite, S., Yapijakis, C., Yoast, S. & Noller, H. F. (1989) *J. Mol. Biol.* **209,** 645–653.
34. Brosius, J., Ullrich, A., Raker, M. A., Gray, A., Dull, T. J., Gutell, R. R. & Noller, H. F. (1981) *Plasmid* **6,** 116–118.
35. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 488–492.
36. Sigmund, C. D., Ettayebi, M., Borden, A. & Morgan, E. A. (1988) *Methods Enzymol.* **164,** 673–690.
37. Maidak, B. J., Larsen, N., McCaughey, M. M., Overbeek, R., Olsen, G. J., Fogel, K., Blandy, J. & Woese, C. R. (1994) *Nucleic Acids Res.* **22,** 3485–3487.
38. Gutell, R. R., Power, A., Hertz, G. Z., Putz, E. J. & Stormo, G. D. (1992) *Nucleic Acids Res.* **20,** 5785–5795.
39. Gautheret, D., Damberger, S. H. & Gutell, R. R. (1995) *J. Mol. Biol.* **248,** 27–43.
40. Gutell, R. R. (1994) *Nucleic Acids Res.* **22,** 3502–3507.
41. Andersson, D. I., Bohman, K., Isaksson, L. A. & Kurland, C. G. (1982) *Mol. Gen. Genet.* **187,** 467–472.
42. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 352–359.
43. Tapprich, W. E. & Dahlberg, A. E. (1990) *EMBO J.* **9,** 2649–2655.
44. Woese, C. R., Fox, G. E., Bablen, K., Uchida, T., Bonen, K., Pechman, K., Lewis, B. J. & Stahl, D. (1975) *Nature (London)* **254,** 83–86.
45. Woese, C. R., Magrum, L. J., Gupta, R., Siegel, R. B., Stahl, D. A., Kop, J., Crawford, N., Brosius, J., Gutell, R., Hogan, J. J. & Noller, H. F. (1980) *Nucleic Acids Res.* **8,** 2275–2293.
46. Gutell, R. R. (1993) *Curr. Opin. Struct. Biol.* **3,** 313–322.
47. Woese, C. R., Gutell, R. R., Gupta, R. & Noller, H. F. (1983) *Microbiol. Rev.* **47,** 621–669.
48. Gautheret, D., Konings, D. & Gutell, R. R. (1994) *J. Mol. Biol.* **242,** 1–8.
49. Maly, P. & Brimacombe, R. (1983) *Nucleic Acids Res.* **11,** 7263–7286.
50. Bonny, C., Montandon, P.-E., Marc-Martin, S. & Stutz, E. (1991) *Biochim. Biophys. Acta* **1089,** 213–219.
51. Leclerc, D., Melancon, P. & Brakier-Gingras, L. (1991) *Biochimie* **73,** 1431–1438.
52. Leclerc, D., Melancon, P. & Brakier-Gingras, L. (1991) *FEBS Lett.* **279,** 171–174.
53. Serra, M. J., Axenson, T. J. & Turner, D. H. (1994) *Biochemistry* **33,** 14289–14296.
54. He, L., Kierzek, R., SantaLucia, J., Jr., Walter, A. E. & Turner, D. H. (1991) *Biochemistry* **30,** 11124–11132.
55. Freier, S. M., Kierzed, R., Caruthers, M. H., Neilson, T. & Turner, D. H. (1986) *Biochemistry* **25,** 3209–3213.
56. Woese, C. R. & Pace, N. R. (1993) in *The RNA World*, eds. Gesteland, R. F. & Atkins, J. F. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 91–117.