A Conformational Switch in *Escherichia coli* 16S Ribosomal RNA During Decoding of Messenger RNA

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Direct evidence is presented for a conformational switch in 16S ribosomal RNA (rRNA) that affects tRNA binding to the ribosome and decoding of messenger RNA (mRNA). These data support the hypothesis that dynamic changes in rRNA structure occur during translation. The switch involves two alternating base-paired arrangements apparently facilitated by ribosomal proteins S5 and S12, and produces significant changes in the rRNA structure. Chemical probing shows reciprocal enhancements or protections at sites in 16S rRNA that are at or very near sites that were previously crosslinked to mRNA. These data indicate that the switch affects codon-anticodon arrangement and proper selection of tRNA at the ribosomal A site, and that the switch is a fundamental mechanism in all ribosomes.

The predecessors of ribosomes, unlike their modern-day ribonucleoprotein descendants, were probably composed entirely of RNA [the emergence of the ribosome from the pre-biotic "RNA world" has been discussed (*1*)]. The basic functions of the ribosome, namely tRNA selection, catalysis of peptidyl transfer, and translocation of tRNA and mRNA through the ribosome, probably have their origins in an all-RNA apparatus. In present day ribosomes, proteins are thought to fine-tune the catalytic functions of the rRNA. For example, 50S ribosomal subunits heavily depleted of their ribosomal proteins retain significant peptidyltransferase activity (*2*). Likewise, in prokaryotes, the 3′ end of 16S rRNA plays a direct role in binding of mRNA to initiating ribosomes via the so-called Shine-Dalgarno interaction (*3*). Furthermore, a small RNA analog of the decoding region of 16S rRNA interacts with antibiotics in a way that is biochemically virtually indistinguishable from their interactions with rRNA in the intact ribosome (*4*).

Although these processes provide insight into the catalytic functions of rRNA, they do not address the dynamic aspects of protein synthesis. It has been recognized that alternative conformations in rRNA must occur during the different stages of translation, and switch mechanisms have been proposed (*5*). However, although a catalytic RNA switch has been described in the *Tetrahymena* group I intron ribozyme (*6*), up to now no specific switch site that is associated with a particular ribosomal function appears to have been identified.

We have demonstrated that the potential to form the C912·G885 base pair in the central region of 16S rRNA was vital for effective translation and normal ribosome function (*7*). However, certain combinations of mutations behaved anomalously and led us to conclude that the existence of a stable helix between nucleotides 912-910 and 885-887 is not all that is required for normal ribosome function. We now present evidence that a second structure, involving base pairing between 912-910 and 888-890, also exists and a switch between the structures occurs during translation. The site of this switch, the 912 region of 16S rRNA, is centrally located at the junction of the three major domains of 16S rRNA, near three pseudoknot structures, and is involved in the binding of ribosomal proteins S5 and S12 as well as the antibiotic streptomycin, all of which affect translational fidelity.

In one of the two conformations, nucleotides C912, U911 and C910 are paired with G888, A889, and G890 (designated the "912-888 conformation") (Fig. 1). In the alternative conformation, nucleotides 912-910 are paired with G885, G886, and G887 ("912-885 conformation"). The former arrangement was shown in secondary structure maps derived by several groups (*8*), whereas the latter arrangement was initially suggested (*9*) on the basis of a comparative analysis of rRNA sequences, and was subsequently shown to be physiologically important by genetic methods (*7*). In our study, we used site-directed mutagenesis of a 16S rRNA gene in conjunction with chemical structure probing to determine that both the 912-888 and the 912-885 conformations are physiologically relevant structures in *Escherichia coli* ribosomes, and represent alternate conformations during protein synthesis.

Functional consequences of mutations in these structures included perturbations in translational fidelity, tRNA binding, and compatibility with mutations in ribosomal proteins S5 and S12. Changes in 16S rRNA structure were observed both locally and in more distant regions of the rRNA where mutations have been shown to affect decoding of mRNA (*10*). More specifically, the sites match many of the positions crosslinked to the coding region of mRNA. Together these data suggest that this switch mechanism may influence positioning of the mRNA for proper selection of tRNA at the ribosomal A site.

Evidence for two base pairing arrangements in rRNA mutants. The model of two alternating conformations in the 912 region of 16S rRNA (Fig. 1) was empirically derived from the observation that mutations

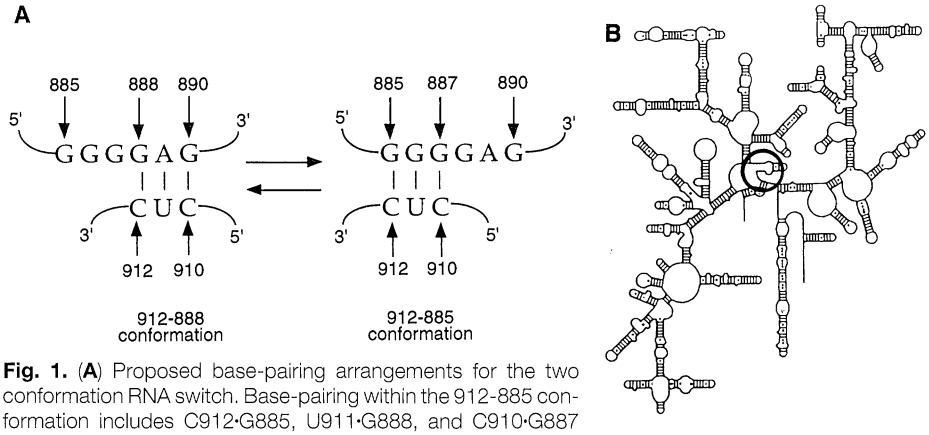

Fig. 1. (**A**) Proposed base-pairing arrangements for the two conformation RNA switch. Base-pairing within the 912-885 conformation includes C912·G885, U911·G888, and C910·G887 base pairs. Pairing in the 912-888 conformation includes C912·G888, U911·A889, and C910·G890. (**B**) Location of 912 region in secondary structure model of 16S rRNA (*43*).

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in the 912 region cause two distinct fidelity phenotypes. A rigorous test of the model by site-directed mutagenesis showed that these results could be explained in terms of the relative stabilities of the proposed conformers. In the wild-type sequence, C912, U911, and C910 can base pair with both G885, G886, and G887 and with G888, A889, and G890. The equilibrium between the two conformations was manipulated by site-directed mutations designed to interfere with base pairing in one or the other structure. The relative stabilities of the two conformations were predicted from the assumption that, in general, canonical (A·U or G·C) or wobble (G·U) base pairs create more stable helices than other "mismatched" pairs (G·G, C·C, and A·C, for example).

According to the model, rRNA mutants in the 912 region enhance the stability of one or the other of the proposed conformations (Fig. 2). The mutations that favored the 912-888 conformation increased fidelity and were designated "restrictive" mutants (called "restrictive" because they restrict suppression of stop codons) (Fig. 2A). In the examples shown, a G to C mutation at position 885 (G885C) interfered with the 912·885 base pair but not with the 912·888 pair. Thus, the equilibrium between the two conformers was shifted toward the 912-888 conformation, which had three canonical base pairs compared to one canonical, one wobble, and one mismatch pair in the 912-885 conformation. Likewise, the double mutant C912G-G888U favored the 912-888 conformation, but this time without alteration at position 885. Several of these mutants were cold-sensitive and grew only at 42°C (Table 1). Unlike the cold-sensitive mutants of Dammel and Noller (*11*), these mutants did not appear to have assembly defects.

All of the error-prone rRNA mutants were more stable in the 912-885 conformation (Fig. 2B). The C912G-G885U double mutant allowed base pairing at 912·885, but interfered with base pairing at 912·888. The same effect was observed with the U911C mutation. Here, the natural U911·A889 base pair was replaced with a C·A mismatch, whereas the U911·G886 wobble pair was replaced with a C·G canonical pair, thereby enhancing the stability of the 912-885 conformation.

The simultaneous introduction of three mutations, at positions 912, 885, and 888, effectively reestablished a balance between the two proposed conformers (Fig. 2C). The C912G-G888C double mutant favored the 912-888 conformation and was cold sensitive but viable at elevated temperature. The C912G-G885C double mutant favored the 912-885 conformation. Combining these three mutations apparently restored viability by restoring the equilibrium between the proposed conformers. Similar results were obtained by constructing the analogous mutations at positions 910, 887, and 890 (Table 1).

Translational fidelity and interaction with ribosomal protein mutants. The translational fidelity of rRNA mutants was assayed by measuring the rate of aberrant read-through of in-frame stop codons within the reporter gene *lacZ* (Table 1). In this assay decreased translational fidelity results in increased expression of *lacZ*. Overall, mutations that favored the 912-885 conformation (for example, C912G-G885U, C912G-G885C, U911C, and C910G-G887C) had higher rates of reading through an in-frame stop codon. Mutations that favored the 912-888 conformation (for example, C912G and C910G; the cold-sensitive restrictive mutants were too unstable to assay) had lower stop codon read-through rates. However, the mutations favoring 912-888 had an elevated rate of frameshifting (*12*). Empty A-site ribosome pausing has been implicated in promoting frameshifting events (*13*), which may indicate that these mutant ribosomes are deficient in A-site tRNA binding.

The hyperaccurate and error prone phenotypes of the rRNA mutants resemble the phenotypes associated with mutations in several ribosomal proteins. Mutations in protein S12 confer resistance to or dependence on the error-inducing antibiotic streptomycin, retard translation rate, and increase accuracy. Mutations in ribosomal proteins S4 and S5 confer the opposite phenotype; they increase the basal translational error rate (called *ram* for ribosomal ambiguity); they can also suppress the streptomycin dependence of restrictive S12 mutants (*14, 15*). The similarity of phenotypes suggested that the rRNA and ribosomal protein mutations might be interactive. Ribosomal proteins S4, S5, and S12 protect

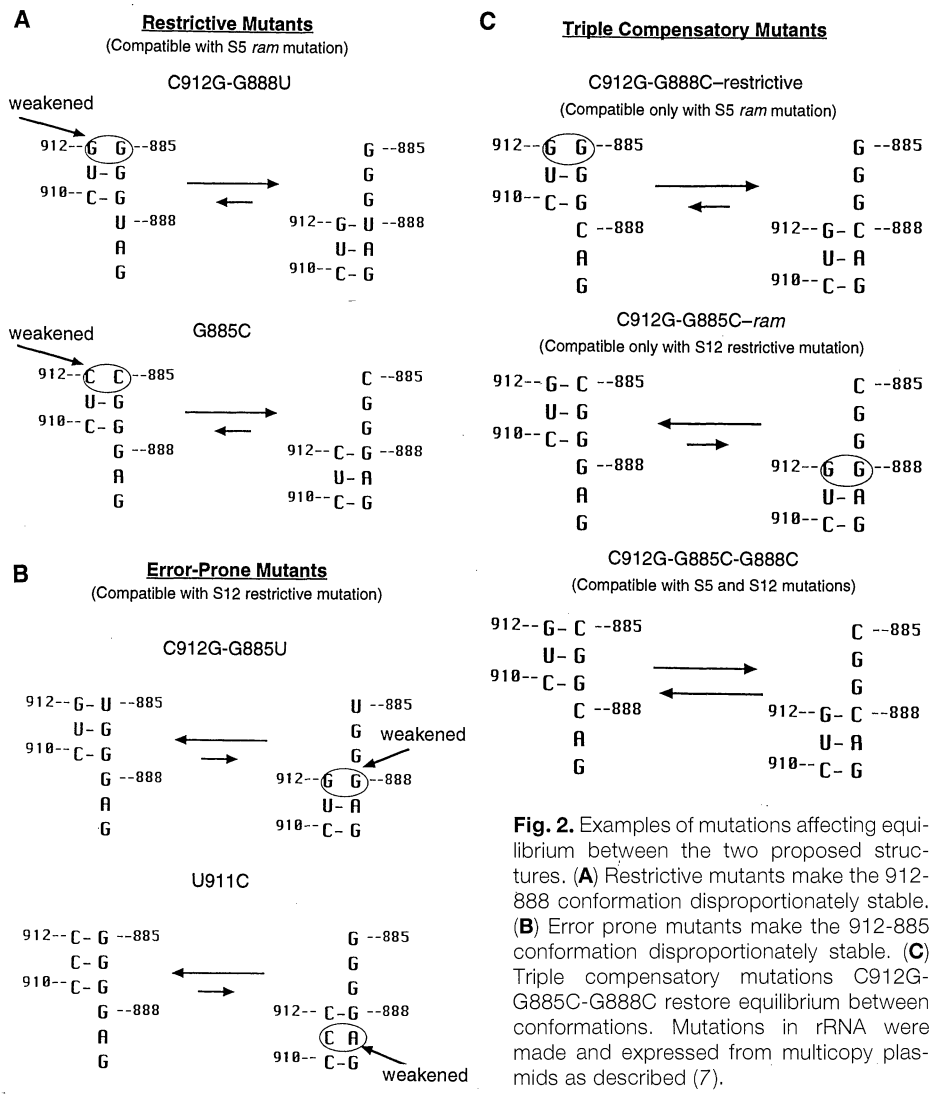

Fig. 2. Examples of mutations affecting equilibrium between the two proposed structures. (**A**) Restrictive mutants make the 912-888 conformation disproportionately stable. (**B**) Error prone mutants make the 912-885 conformation disproportionately stable. (**C**) Triple compensatory mutations C912G-G885C-G888C restore equilibrium between conformations. Mutations in rRNA were made and expressed from multicopy plasmids as described (*7*).

nucleotides in the 912 region from chemical attack, and mutations in S4 and S12 alter the structure of this region (*16*). In addition, streptomycin interacts with this region (*17*) although recent evidence suggests that it binds in the 1400 region (*18*). The compatibility of the rRNA mutations with mutations in ribosomal proteins S5 and S12, measured by transformability (Table 1), indicated that compatibility depended on the ability of the rRNA mutants to form base pairs in either or both of the proposed conformations (Fig. 1). The restrictive mutants, compromised in their ability to form the 912-885 conformation but able to form the 912·888 base pair, were compatible only with an S5 *ram* mutation [strain UD11F7, (*15*)], and not with hyperaccurate S12 mutations [strains UK317 and UK235 (*19*) (Fig. 2A)]. The *ram* mutations in ribosomal protein S4 and elongation factor Tu (EF-Tu) did not improve growth characteristics of these rRNA mutants, nor did other non-*ram* mutations in protein S5 (that is, spectinomycin resistance mutations). Thus, this is not a general suppression effect caused by restoring translational fidelity, but rather represents a specific protein-rRNA interaction consistent with results indicating that certain portions of protein S5 are in proximity to the 912 region (*20*).

Mutations in rRNA that favored pairing in the 912-885 conformation had the opposite compatibility pattern. They were incompatible or marginally compatible with the error-enhancing protein S5 mutation, but were fully compatible with the S12 restrictive mutation (Fig. 2B). Comparing the translational fidelity data of the rRNA mutants with the mutant protein compatibility data reveals, perhaps not surprisingly, that the error prone rRNA mutants are compatible only with restrictive S12 mutations, whereas the restrictive rRNA mutants are compatible only with the *ram* S5 alleles. This reciprocity between the 16*S* rRNA and ribosomal protein mutations supports our proposal that these are not simply two static structures of 16*S* rRNA, but, in fact, a switch between the structures does occur during translation, and it is facilitated by proteins S5 and S12. Evidence for a switch is further supported by the fact that extremely restrictive mutants in both 16*S* rRNA and protein S12 (the cold-sensitive mutants such as C912G-G888C and the streptomycin-dependent mutants in S12, respectively) are suppressed by the same mutation in ribosomal protein S5.

Physical evidence for the two conformations and differences in tRNA binding. In order to examine base pairing arrangements more directly, we probed mutant and wild-type ribosomes by chemical modification, using kethoxal and dimethyl sulfate (DMS), which specifically modify guanine and adenine not involved in Watson-Crick base pairing. The C912G mutation (Fig. 3A, lane 2) causes an increase in reactivity of nucleotides G885, G886, and G887 to kethoxal, an effect that is exacerbated in the C912G-G888C double mutant (Fig. 3A, lane 3). Similar effects on G885, G886, and G887 occur upon mutagenesis of C910 (Fig. 4A, lane 2). Since G885, G886, and G887 become more reactive with mutations at positions 910 and 912 but are less reactive in wild-type ribosomes, we concluded that C912, U911, and C910 are predominantly base paired to G885, G886, and G887 (the 912-885 conformation) in isolated wild-type ribosomes.

To detect base pairing in the 912-888 conformation, we examined the reactivity of G910 in combination with either G890 or G890C. If nucleotide 910 does form a base pair with nucleotide 890, it should be less reactive to kethoxal modification in the C910G-G890C double mutant (Fig. 4A, lane 3) than in the C910G single mutant (lane 2). However, if nucleotides 910 and 890 do not normally interact, the identity of nucleotide 890 should not affect the chemical reactivity of G910. In fact, the reactivity of G910 was diminished, thus providing evidence that at least some of the ribosomes were base-paired in the 912-888 configuration (compare intensity of 910 band in Fig. 4A, lanes 2 and 3). Similar results were obtained with the analogous mutations at positions 912, 885, and 888. Whereas these and earlier data (*7*) establish the importance of the 912-885 conformation with functional studies, our data also provide structural evidence for the existence of the alternative 912-888 conformation.

In vivo translational fidelity assays and mutant ribosomal protein complementation assays indicated that ribosomes biased toward one or the other of the proposed conformations had a higher or lower stringency of tRNA selection. By definition, ribosomes that accept noncognate tRNAs in the A site and use them in the peptidyl transfer reaction at a higher than normal rate are

Table 1. Compatibility of rRNA mutants with mutations in ribosomal proteins S5 and S12. Mutations were made in the pBR322-based vector pSTL102 (*39*) as described (*7*). Translational misreading was measured by co-transforming MC140 cells with wild-type or mutant rRNA plasmids and a *lacZ* reporter gene plasmid (pSG3/4UGA) containing an in-frame UGA stop codon within the *lacZ* gene. The frequency of aberrant read-through of the UGA stop codon (and subsequent production of β-galactosidase) is proportional to misreading by mutant and wild-type ribosomes (*40*). Translational misreading values are units of β-galactosidase activity normalized to the rate of wild-type read-through. The rRNA mutants classified as "restrictive" either by increased translational fidelity or by compatibility only with S5 mutation are shown in boldface. The rRNA mutations classified as "*ram*" are shown in plain type. Mutant plasmids were transformed into (i) wild-type MC140 cells (*41*), (ii) UD11F7 cells, which harbor a mutation in the gene for ribosomal protein S5 resulting in a translational error-prone ("*ram*") phenotype (*42*), or (iii) UK317 cells, which have a mutation in the gene for ribosomal protein S12, conferring a hyperaccurate (restrictive) phenotype (*19*). Resulting transformants were classified as viable (+), nonviable (−), or unstable (+/−) if the transformants were viable but extremely slow-growing and prone to reversion. Ribosomal RNA mutants C912G-G888C, C912G-G888U, and C910G-G890C were cold-sensitive (cs) and would not grow in MC140 cells at or below 37°C (C912G-G888C, C912G-G888U) or 30°C (C910G-G890C). rRNA mutant G885U was presumably restrictive but too unstable to be assayed for translational fidelity. It was viable only at low plasmid copy number or in the S5 *ram* mutant strain UD11F7. ND, not determined.

Plasmid	Compatibility with r-protein alleles			Translational misreading
	wt	S5 *ram*	S12 restr.	
Wild-type (C912,G885,G888)	+	+	+	1.00
C912G	+	+	−	0.86
C912G/G888C	− (cs)	+	−	ND
C912G/G888U	− (cs)	+	−	ND
G885C	−	+	−	ND
G888C	+	+/−	+	1.11
C912G/G885C	+	+/−	+	1.72
C912G/G885U	+	−	+	1.95
C912G/G885C/G888C	+	+	+	ND
C912G/G885U/G888U	+	+	+	ND
U911C	+	−	+	1.44
C910G	+	+	−	0.80
C910G/G890C	+ (cs)	+	−	ND
G890C	+	+/−	+	1.08
C910G/G887C	+	−	+	1.52
C910G/G890C/G887C	+	+	+	1.59

error prone. A possible mechanism for this phenomenon is that the A site in error prone ribosomes has a higher affinity for accepting noncognate tRNAs than the A sites in wild-type or restrictive ribosomes (*21*). Therefore, even if the codon-anticodon match is not perfect, the noncognate aminoacyl tRNA has a greater likelihood of remaining bound to the ribosome during selection.

Aminoacylated $tRNA^{Phe}$ ([^{14}C]Phe-$tRNA^{Phe}$) was bound to wild-type, *ram*, and restrictive ribosomes with and without polyuridylic acid [poly(U)], an mRNA analog. In the absence of mRNA [poly(U)], the error prone ribosomes bound [^{14}C]Phe-$tRNA^{Phe}$ to a much greater extent than either wild-type or restrictive ribosomes (Fig. 5A). The addition of poly(U) stimulated binding of [^{14}C]Phe-$tRNA^{Phe}$ to wild-type and mutant ribosomes but had less of a stimulatory effect on the error prone ribosomes (Fig. 5B). This suggests that the restrictive and wild-type ribosomes are more dependent on mRNA for tRNA binding, but it is evident that mRNA does not restore wild-type–like binding to the restrictive mutants. A third experiment measured [^{14}C]Phe-$tRNA^{Phe}$ binding to the A site after first filling the P site with (deacylated) $tRNA^{Phe}$ (*22*) (Fig. 5C). Essentially all of the bound tRNA was in the A site in these assays because only 5% of the tRNA was puromycin reactive. Here it was apparent that the binding by error prone ribosomes of [^{14}C]Phe-$tRNA^{Phe}$ to the A site was greater than that by the wild-type or restrictive ribosomes. (Presumably the actual difference is larger than measured because of the presence of 30 to 40 percent host encoded, wild-type ribosomes in the mutant preparations.)

Effects of mutations on mRNA and tRNA binding domains in 16S rRNA. As shown above, mutations in the 912 region affect tRNA binding and fidelity of translation, two aspects of mRNA decoding. Although two nucleotides in the immediate region, A892 and G926, have been implicated in tRNA binding (*23*), most of the sites involved in A- and P-site tRNA binding and codon-anticodon interactions lie outside the immediate 912 region (*24*). In structure-probing experiments, we found that mutations in the 912 region caused changes in other parts of 16S rRNA (Fig. 6), many of which were implicated previously in decoding of mRNA (*24*). The reactivity toward chemical attack of these remote nucleotides was modulated up or down, depending on whether the mutation or mutations in the 912 region caused a restrictive or error-prone phenotype. Where restrictive mutations caused an increase in reactivity of a nucleotide, the error-prone mutants caused little change or even a decrease in reactivity relative to the wild type and vice versa (for example, position 864, Fig. 3B). The effect of the conformational switch in the central domain of 16S rRNA on distal

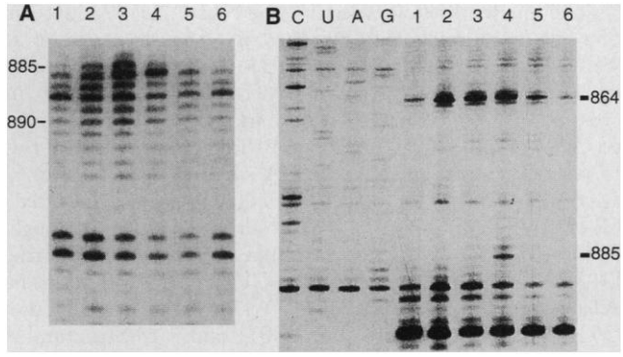

Fig. 3. Results of kethoxal (**A**) and DMS (**B**) probing of ribosomes containing either wild-type or mutant rRNA (*44*). (A) Disruption of the 912-885 conformation results in increased reactivity of nucleotides G885-G887 to modification by kethoxal. (B) Changes in chemical reactivity were also observed in nucleotides at positions remote from the 912 region such as modification of A864 by DMS. Ribosomes were isolated from MC140 cells transformed with the following plasmids: Lane 1, pSTL102 (wt); lane 2, pC912G (restrictive); lane 3, pC912G-G888C (very restrictive); lane 4, pC912G-G888C-G885C (like wild type); lane 5, pC912G-G885C (*ram*); lane 6, pC912G-G885U (*ram*). C, U, A, and G are sequencing lanes.

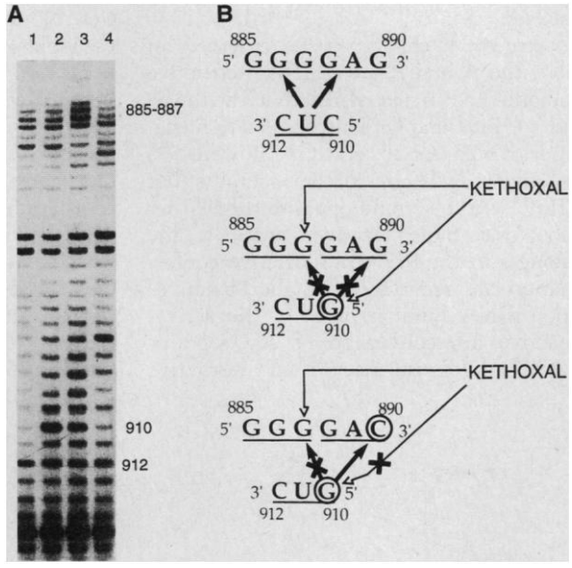

Fig. 4. G910 is protected from kethoxal modification by C890. (**A**) Results of kethoxal probing of ribosomes isolated from cells transformed with: lane 1, pSTL102 (wt); lane 2, pC910G; lane 3, pC910G/G890C; lane 4, pC910G-G890C-G887C. (**B**) Schematic for experimental design. If positions 910 and 890 are capable of base pairing in isolated ribosomes, then the identity of nucleotide 890 should affect the reactivity of C910G to kethoxal modification. Band intensity of 910 modification was reduced by 45 percent (by PhosphorImager analysis) between lanes 2 and 3, suggesting that nucleotides 910 and 890 do form a base pair.

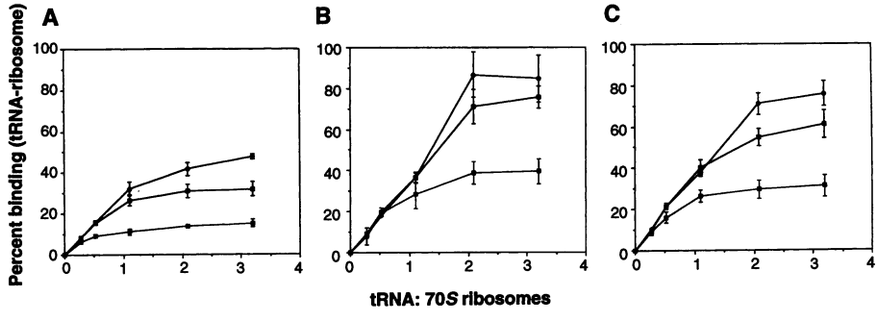

Fig. 5. [^{14}C]Phe-$tRNA^{Phe}$ binding to ribosomes (*22*). (**A**) [^{14}C]-Phe-$tRNA^{Phe}$ binding without poly(U). (**B**) ^{14}C-Phe-$tRNA^{Phe}$ binding with poly(U). (**C**) [^{14}C]-Phe-$tRNA^{Phe}$ binding after preincubation of ribosomes with 50 pmol of deacylated tRNA and poly(U), which blocks [^{14}C]-Phe-$tRNA^{Phe}$ binding to the P site. Ribosomes were from cells transformed with plasmids pSTL102 (wild type; open squares), pC912G/G885U (error prone; solid circles) and pC912G/G888C (restrictive; solid squares). Data are plotted as percent of ribosomes binding tRNA (ordinate) and molar ratio of tRNA to 70S ribosomes in incubation mix (abscissa).

portions of the secondary structure is consistent with a proposal that the 912 region links different functional regions in the ribosome, namely the 530 loop, helix 34, and the 3′ minor domain (*24, 25*).

The nucleotides affected by the 912-888-885 conformational switch (Fig. 6) were identical or adjacent to nucleotides in 16S rRNA shown previously to be crosslinked to mRNA (*10*). These included G925 and G926 [G926 was crosslinked to position +2 in mRNA, with A of the AUG start codon as position +1 (*10*)], G1401 (mRNA position +4 was crosslinked to C1402), G1053 (+6 crosslinked to U1052), G1392 (+7 crosslinked to C1395), A1196 (+8 or 9 crosslinked to A1196), G524 (+11 crosslinked to G530), and G1353 and G1355 (−3 crosslinked to U1360). Thus the locations of the structural changes observed in the restrictive and error-prone mutants are not randomly distributed throughout the 16S rRNA but occur in areas of the ribosome closely associated with mRNA.

Structural changes were observed in both the A and P site regions. Restrictive mutants had increased reactivity at the A site's G1491 and G1494. The P site nucleotides G925, G926, and G1401 were less reactive in the restrictive mutants but G1392, on the strand opposite the 925 region, was more reactive. Some of the changes in the 912-888 restrictive conformation are reminiscent of the "inactive" (non-tRNA binding) form of the active-inactive interconversion of 30S subunits (*26*). In both the inactive and restrictive forms, the capacity to bind tRNA is diminished (Fig. 5, compare G912·C888 to wild-type or G912·U885 binding), and both have diminished kethoxal reactivity at position G926 and increased reactivity at G1392. Recent three-dimensional reconstructions of active and inactive 30S subunits (*27*) revealed conformational changes during the inactive-to-active transition, which occurred primarily in the platform and neck of the 30S subunit. The anticodon binding sites for P and A site tRNAs have been localized to a channel running obliquely through the neck, which may be the conduit for the mRNA (*28*). Our data suggest that this may be the same region that is affected by the 912 switch.

Structural changes also occurred at or near all three pseudoknots in 16S rRNA. Restrictive rRNA mutations caused an apparent opening of the central pseudoknot (17-19 and 916-918), with increased reactivity at G917 and A19, whereas *ram* rRNA mutations decreased their reactivities. Normal base pairing in the central pseudoknot is vital for ribosome function (*29*), but there has been no direct evidence that it is a dynamic structure. Similarly, base pairing within the 570·866 pseudoknot is essential (*30*), but it too has not been shown to open and close during translation. Position A864, immediately adjacent to this pseudoknot, was hyperreactive toward DMS in restrictive mutants and less reactive in error-prone rRNA mutants (Fig. 3B), but no changes were noted in the 570 region to indicate an opening of this structure. Notably, A864 is also protected by protein S5 (*31*), and the increase in reactivity could reflect an altered S5 interaction. Finally, restrictive rRNA mutations also caused an apparent opening of the 505·524 pseudoknot in the 530 loop with increased reactivity at G524, G505, and G506. This pseudoknot has been implicated in streptomycin binding and modulation of translational fidelity (*32*).

The proposed switch: Its universality and function. It seems likely that the conformational switch that we propose here appeared very early in the evolution of rRNA. Examination of known rRNA sequences for patterns of covariation between nucleotides 912-910 and 885-890 revealed that positions 910-912 correlated best with 885-887 (*8*). However, the potential for base pairing between the highly conserved 910-912 and 888-890 sequences still existed (*33*). The rRNA sequences from several other organisms were superimposed on the switch model (Fig. 7A), and it appears that the switch could function in all cases. There are a few examples of noncompensated base changes in both of the proposed conformations, especially within the more divergent mitochondrial data set (*8, 33*). Because the proposed switch involves at least three base pairs in each conformation, a noncompensated base change at one of the base pairs could probably be tolerated. In support of the idea that this switch is conserved in other organisms, Liebman and colleagues have characterized rRNA mutants in yeast that behave in a manner consistent with our model (Fig. 7B). A site-directed mutation at the position equivalent to *Escherichia coli* 912 in yeast caused an antisuppressor (restrictive) phenotype (*34*), consistent with a shift in the equilibrium of structures toward the 912-888 conformation. Likewise, another site-directed yeast mutant, equivalent to *E. coli* G888A, appears to give an antisuppressor phenotype (*35*) which, according to our model, would serve to increase base pairing in the 912-888 conformation.

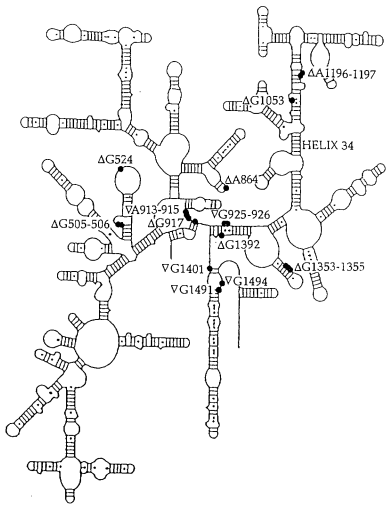

Fig. 6. Summary of sites whose reactivities toward dimethyl sulfate or kethoxal were altered by mutations in the 912 region. Up arrowheads indicate reactivities that were increased in the restrictive rRNA mutants. Down arrowheads indicate nucleotides whose reactivities were increased in the *ram* mutants.

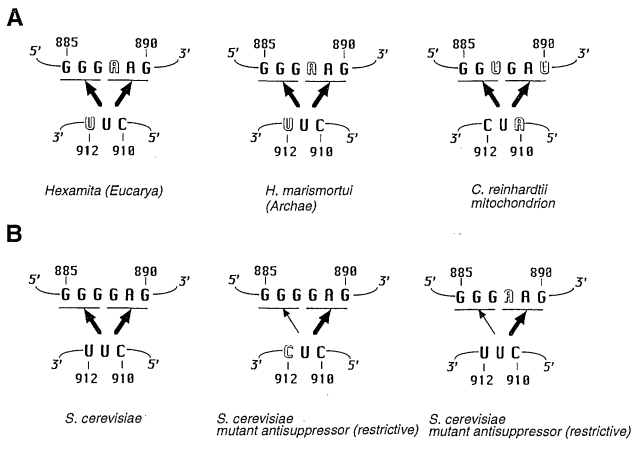

Fig. 7. Proposed conformations of small subunit rRNAs in *Hexamita*, *Halobacterium*, *Chlamydomonas*, and *Saccharomyces*. (**A**) Primary sequences of small subunit rRNAs are shown superimposed on the switch model. (**B**) Sequence of wild-type yeast 18S rRNA superimposed on the model (left); a site directed mutant in yeast shown to have a restrictive phenotype (*34*) (center); a site directed mutant in yeast that appears to have antisuppressor activity (*35*) (right). Open letters represent nucleotides that differ from the *E. coli* sequence (A), or are mutations in the yeast sequence (B). Bold arrows in (B) indicate conformations predicted by the phenotype.

Since altering the balance between the two conformations results in aberrations in translational fidelity, the most straightforward interpretation is that this region is involved in tRNA selection during decoding of the mRNA. However translocation cannot be ruled out, especially since mutants that favor the 912-888 conformation are hypersensitive to spectinomycin, an inhibitor of translocation (*12*, *36*). In addition, there is the curious parallel of a three-base shift both during translocation and the conformational switch.

How might the proposed switch affect or participate in decoding? The functional data (tRNA binding and fidelity) suggest that the 912-888 conformer may have a role in increasing the stringency of A site tRNA selection, perhaps by decreasing the affinity for tRNA in the A site. For example, during A-site tRNA selection the ribosome might shift transiently from the tRNA-receptive *ram* state (912-885) to the restrictive 912-888 conformation. The process could be triggered by codon recognition by the ternary complex (EF-Tu–GTP-aminoacyl tRNA) leading to GTP hydrolysis (*37*). The resulting switch in rRNA structure, particularly at those sites in close proximity to mRNA, could affect the mRNA structural orientation with respect to the tRNAs, disrupting nonspecific contacts between the ribosome and the tRNA and thereby enhancing the importance of the codon-anticodon interaction relative to the overall binding stability. In this restrictive state a noncognate tRNA would have a greater likelihood of diffusing away from the ribosome than would a cognate tRNA, providing an accuracy enhancing (proofreading) step prior to peptidyl transfer (*21*, *38*). Alternatively, the switch might be involved in an initial recognition event between the ribosome and the ternary complex of tRNA, EF-Tu, and GTP; then the conformational change would occur during initial discrimination between cognate and noncognate ternary complexes.

In conclusion, the 912 region of 16*S* rRNA, situated near the convergence of the conserved domains found in all small subunit rRNAs, appears to be an ideal location for a switch. Movement in a peripheral region of the 30*S* subunit could trigger a conformational change in this putative hinge region or, alternatively, manipulation of the 912 region could be used to synchronize movements in the peripheral domains. At present, we do not know the precise order of the reactions involved in the conformational change. However, phenotypes previously ascribed only to mutations in ribosomal proteins S4, S5, and S12 can now be mimicked by modulating the stability of each of the two switch conformations. It seems likely, then, that these proteins exert their effects by modulating the structure of the rRNA switch and thus may have assumed a role that at one time was entirely controlled by RNA-RNA interactions.

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22. Ribosomes (50 pmol, 1 A_{260} = 23 pmol of ribosomes) were incubated with or without 80 μg of poly(U) and unlabeled deacylated $tRNA^{Phe}$ in buffer 1 (80 mM potassium cacodylate, pH 7.2, 20 mM $MgCl_2$, 100 mM NH_4Cl) for 10 minutes at 37°C. Increasing amounts of [^{14}C]Phe-$tRNA^{Phe}$ {prepared as described by J. S. Lodmell, W. E. Tapprich, and W. E. Hill [*Biochemistry* **32**, 4067 (1993)], except that charged tRNA was not separated from uncharged; charging efficiency was about 55 percent} were added to a total volume of 100 μl. Reactions were incubated at 37°C for a further 10 min, then on ice for 1 hour. Portions (50 μl) were removed to tubes containing 2 μl of 25 mM puromycin and were held at room temperature for 1 hour. The remaining volumes were spotted onto nitrocellulose filters and washed three times with ice-cold buffer 1. Filters were dried, and radioactivity was counted in a scintillation counter. Puromycin reactions were extracted with 500 μl of ethyl acetate, and 350 μl of the ethyl acetate were removed for scintillation counting to determine the percentage of the bound [^{14}C]Phe-$tRNA^{Phe}$ that was in the P site. Prior incubation of ribosomes with stoichiometric amounts of deacylated tRNA blocks binding of acylated tRNA to the P site [S. Watanabe, *J. Mol. Biol.* **67**, 443 (1972)]. The proportion of Phe-$tRNA^{Phe}$ in the P site was measured by the puromycin reaction [H-J. Rheinberger, S. Schilling, K. H. Nierhaus, *Eur. J. Biochem.* **134**, 421 (1983)].
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