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Genetics

# The translational function of nucleotide C1054 in the small subunit rRNA is conserved throughout evolution: Genetic evidence in yeast

**(suppression/translational termination/release factor/*Saccharomyces cerevisiae*)**

YURY O. CHERNOFF*, GARY P. NEWNAM*, AND SUSAN W. LIEBMAN†

Department of Biological Sciences, University of Illinois Molecular Biology Research Facility, Laboratory for Molecular Biology, 900 South Ashland Avenue, Chicago, IL 60607

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**ABSTRACT** **Mutations at position C1054 of 16S rRNA have previously been shown to cause translational suppression in *Escherichia coli*. To examine the effects of similar mutations in a eukaryote, all three possible base substitutions and a base deletion were generated at the position of *Saccharomyces cerevisiae* 18S rRNA corresponding to *E. coli* C1054. In yeast, as in *E. coli*, both C1054A (*rdn-1A*) and C1054G (*rdn-1G*) caused dominant nonsense suppression. Yeast C1054U (*rdn-1T*) was a recessive antisuppressor, while yeast C1054Δ (*rdn-1Δ*) led to recessive lethality. Both C1054U and two previously described yeast 18S rRNA antisuppressor mutations, G517A (*rdn-2*) and U912C (*rdn-4*), inhibited codon-nonspecific suppression caused by mutations in eukaryotic release factors, *sup45* and *sup35*. However, among these only C1054U inhibited UAA-specific suppression caused by a UAA-decoding mutant $tRNA^{Gln}$ (*SLT3*). Our data implicate eukaryotic C1054 in translational termination, thus suggesting that its function is conserved throughout evolution despite the divergence of nearby nucleotide sequences.**

Recent evidence indicates that rRNA serves as a primary target for translational antibiotics (1) and retains peptidyltransferase catalytic activity *in vitro* in the absence of most ribosomal proteins (2, 3). In addition, several mutations in prokaryotic rRNA have been isolated that cause read-through of termination codons (4–9). These results suggest a direct involvement of rRNA in the determination of translational accuracy levels and/or in the efficiency of termination. Among these rRNA suppressors, mutations at "helix 34" [according to the numbering system of Maly and Brimacombe (10)], which includes nt 1046–1067 and 1198–1211 of *Escherichia coli* 16S rRNA, were found to be particularly intriguing. A mutation at C1054 was first reported to suppress only UGA and not UAA or UAG termination codons (4). The additional observation that the sequence exactly opposite C1054 in helix 34 consists of tandem UCA repeats (nt 1199–1204), which are complementary to UGA codons (see Fig. 1), led to the proposal that mRNA (5′-UGA-3′)·rRNA (5′-UCA-3′) base pairing is required for efficient termination at UGA codons (4). Mutations at position 1054 were proposed to alter the secondary structure of the helix, thereby preventing UCA·UGA base pairing. This elegant model has stimulated interest in the possible roles of rRNA·mRNA base pairing in protein synthesis (11, 12).

While the UCAUCA sequence at nt 1199–1204 is present in most prokaryotic small subunit rRNAs, it is not found in eukaryotic (18S) counterparts (13, 14). In contrast, the C1054 residue is conserved in most eukaryotes (13, 14). Thus, according to the proposed base-pairing model, one might not expect mutations at position 1054 to cause UGA suppression in eukaryotes.

Until recently, it has been extremely difficult to study *in vivo* effects of eukaryotic rRNA mutations, because of the high copy number of eukaryotic rRNA genes. Now, we have applied a plasmid system, developed by E. Morgan (15), to the study of yeast rRNA mutations affecting translational accuracy (15). This system allows us to construct yeast strains in which most or all wild-type rRNA genes are substituted with artificially engineered mutant rRNA genes. By using this approach, we have previously generated and characterized two antisuppressor mutations in yeast 18S rRNA, which are located at positions corresponding to nt 517 and 912 of *E. coli* 16S rRNA (15) (Fig. 1).

Here, we report our finding that mutations at yeast position 1054 also affect translational fidelity. These results show that the functional importance of helix 34 is conserved throughout evolution, despite the absence of nearby UGA-complementary sequence. While this work was in progress, evidence has accumulated in *E. coli* that also suggests a more general involvement of helix 34 in translation (16, 17).

## MATERIALS AND METHODS

**Ribosomal DNA (rDNA) Sequences and Nomenclature.** rRNA sequences and alignment are from refs. 14, 15, and 18. The *E. coli* numbering system is used throughout the paper for simplicity.

**Plasmids.** Each rDNA plasmid bears a yeast 2-μm DNA replicator and the whole yeast 9-kb rDNA repeat including the Pol I promoter, terminator, and 18S, 5.8S, 26S, and 5S rRNA sequences. Plasmids pRDN-wt, pRDN-2, pRDN-4 (all *TRP1 LEU2-d*), and pRDN-wt-U (*URA3*) were as described (15). The 18S rRNA gene in pRDN-2 and pRDN-4 contains mutations *rdn-2* (G517A) and *rdn-4* (U912C), respectively. These mutations each cause antisuppression and resistance to paromomycin and G418 (15). Plasmids pRDN-2-U and pRDN-4-U are identical to pRDN-wt-U, except that they contain *rdn-2* and *rdn-4* mutations, respectively. Construction of plasmids bearing mutations *rdn-1A, rdn-1G, rdn-1T*, and *rdn-1Δ*, which were named pRDN-1A, pRDN-1G, pRDN-1T, and pRDN-1Δ, respectively, is described below. *URA3* 2-μm plasmid pPXS, bearing a UAA-suppressor derivative of the $tRNA^{Gln}$ gene, was as described (19). Plasmid $pSlt^+$ (19) is identical to pPXS, except that $pSlt^+$ bears a wild-type $tRNA^{Gln}$ gene. The UAA-suppressor mutation (designated *SLT3*) was shown to change the UUG anticodon of $tRNA^{Gln}$ into the UAA-complementary UUA anticodon (19).

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Abbreviations: rDNA, ribosomal DNA (ribosomal RNA genes); 5-FOA, 5-fluoroorotic acid.

*Present address: School of Biology, Georgia Institute of Technology, Atlanta, GA 30332-0230.
†To whom reprint requests should be addressed.

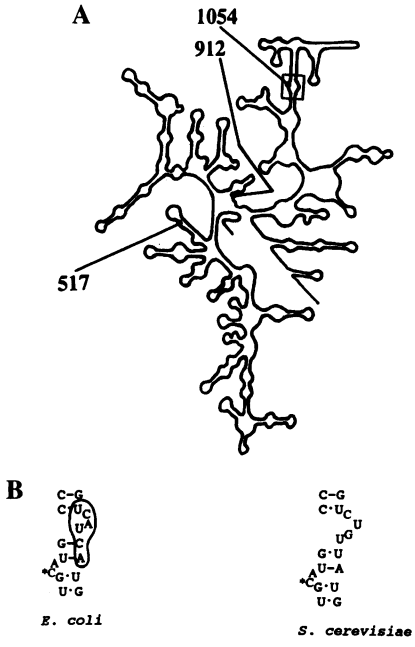


FIG. 1. Positions of mutations in the small subunit rRNA. (*A*) Positions of mutation sites are shown on the secondary structure of *E. coli* 16S rRNA. The boxed region is shown in more detail in *B*. (*B*) C1054 and the surrounding region in *E. coli* and *S. cerevisiae*. UGA-complementary triplets (in *E. coli*) are circled. *E. coli* C1054 and the corresponding position of the yeast rRNA are marked by asterisks.

**Yeast Strains and Cultivation Conditions.** Construction of L-1489 (*MAT***a** *ade1-14*$_{UGA}$ *his7-1*$_{UAA}$ *leu2-3,112 lys2-864*$_{UAG}$ *trp1-Δ1 ura3-52*) and L-1494 was as described (15). These strains are isogenic, except that L-1494 contains a large stable deletion of essentially the complete chromosomal rDNA cluster and is kept viable by the plasmid pRDN-wt. The previously reported (15) strains L-1495 and L-1496 and the newly constructed strains L-1521, L-1522, L-1523, L-1583, and L-1597 are isogenic to L-1494, except that they contain pRDN-2, pRDN-4, pRDN-wt-U, pRDN-2-U, pRDN-4-U, pRDN-1A, and pRDN-1T plasmids, respectively, instead of pRDN-wt. Recessive omnipotent suppressor mutants (including L-1524 and L-1526, which bear *sup45-R2* and *sup35-R8* alleles, respectively) were isolated as spontaneous derivatives of L-1521, in which both *ade1-14*$_{UGA}$ and *his7-1*$_{UAA}$ were simultaneously suppressed. This selection procedure is known (20) to preferentially uncover mutations at the *sup35* and *sup45* loci. The strain L-1592 (*sup35-R15*) was an L-1523 derivative, constructed in the same way. Complementation tests with *sup35* and *sup45* derivatives of strain 8A-P3532 (*MATα ade1-14*$_{UGA}$ *his7-1*$_{UAA}$ *met13-A1*, ref. 20) verified allelism of our omnipotent suppressor mutations.

Standard yeast media and cultivation procedures were used (21). Growth on appropriate medium was compared by inoculating an approximately equal number of cells in measured suspensions or by velveteen replica plating (21). Antibiotics, if not specifically mentioned, were added to organic complete (YPD) medium. Paromomycin was purchased from Sigma, and G418 (Geneticin) was purchased from Life Technologies (Grand Island, NY).

**Construction of the rDNA Plasmids Bearing Mutations at Position 1054.** Mutations were created in the 1033-bp *Bss*HII fragment of the 18S rRNA gene cloned in M13mp18 phage as described (15). The U-DNA procedure (22) was used to generate oligonucleotide-directed mutations. The mutagenic oligonucleotide primers 5′-GGTGGTGGTGNATGGCCGTTC-3′ (where N is A, G, or T) and 5′-GGGTGGTGGTGΔATGGCCGTTTC-3′ (where Δ is C1054 deletion) were purchased from Integrated Life Technologies and Operon Technologies (Alameda, CA), respectively. Mutant *Bss*HII fragments were sequenced to verify the presence of the desired mutation and the absence of other changes and were then reinserted in the correct orientation into pGN1, which is a *Bss*HII-deletion derivative of pRDN-wt.

**Plasmid Shuffle.** Complete synthetic medium with 5-fluoroorotic acid (5-FOA; 0.7–1.0 mg/ml) (21) was used to select against *URA3*-containing rDNA plasmids as described (15). Primer extension analysis of rRNA (see below) and Southern blot analysis of DNA (in the case of *rdn-1A, rdn1-Δ, rdn-1G,* and *rdn-2*, which generate new sites for *Hph* I, *Hph* I, *Fok* I, and *Kpn* I, respectively) were used to check whether plasmid shuffle products contain only mutant 18S rRNA.

**Primer Extension Analysis of rRNA and rDNA.** Oligonucleotide primer 5′-ACTCCACCAACTAAGAACGG-3′ was purchased from AMITOF. Experimental procedures were as described (15). If wild-type rRNA or rDNA serves as the template in the presence of dideoxy-GTP, DNA synthesis terminates at C1054. If C1054 is removed or altered, DNA synthesis proceeds to the next cytidine, which is 21 bases farther away, enabling a distinction between the wild-type and mutant rRNA and rDNA. A similar analysis for *rdn-2* and *rdn-4* mutations was as described (15).

## RESULTS

**Effects of Mutations at Yeast Position 1054 in the Presence of Wild-Type rDNA.** *Saccharomyces cerevisiae* L-1489 and its rDNA deletion derivative L-1521, bearing plasmid pRDN-wt-U [*URA3* rDNA], were transformed individually with either wild-type rDNA plasmid pRDN-wt or *rdn-1* mutant plasmids pRDN-1A, pRDN-1G, pRDN-1T, and pRDN-1Δ. Each plasmid contained *TRP1* and *LEU2-d* yeast markers. Transformants selected on medium lacking Trp (-Trp medium) were transferred to medium lacking Leu (-Leu medium) to amplify the plasmids. Suppression of a particular nonsense mutation was detected by growth on medium, lacking the corresponding nutrient. Transformants, bearing the control plasmid pRDN-wt and the mutant plasmids pRDN-1T and pRDN-1Δ, showed no suppression, whereas plasmids pRDN-1G and pRDN-1A caused growth on medium lacking His (-His medium) (Fig. 2). In L-1489, growth was heterogeneous, indicating that only a subpopulation of cells (presumably, the cells with a higher copy number of the plasmid) expressed the suppressor phenotype. In L-1521, pRDN-1G also caused growth on medium lacking Ade (-Ade medium), whereas pRDN-1A caused papillation on -Ade medium (Fig. 2). Since *ade1* mutations cause the accumulation of a red pigment, suppression of *ade1-14*$_{UGA}$ was also confirmed by the light-pink color of the pRDN-1A and pRDN-1G transformants on YPD medium, in contrast to the red color of the pRDN-wt transformants. The third nonsense mutation, *lys2-L864*$_{UAG}$, was not suppressed.

Previously, we (15) and others (23, 24) have shown that plasmid-encoded rRNA is not expressed efficiently in the presence of chromosomal rDNA repeats. This explains the higher efficiency of suppression in strain L-1521, which lacks chromosomal rDNA. Primer extension results confirmed that the mutant 18S rRNA levels are very low in L-1489 transformants (data not shown), while significant levels of *rdn-1A, rdn-1G,* and *rdn-1T* mutant 18S rRNAs are accumulated in L-1521 transformants (Fig. 3). However, only an extremely low level of *rdn-1Δ* mutant 18S rRNA was detected in L-1521.

**Effects of Mutations at Yeast Position 1054 in the Absence of Wild-Type rDNA.** Loss of pRDN-wt-U [*URA3* rDNA] in L-1521 transformants, also bearing either pRDN-wt or each of the *rdn-1* mutant plasmids, was selected by growth on 5-FOA medium. In the case of pRDN-wt and pRDN-1T, the entire patch was covered with Ura$^-$ colonies after 3–4 days of

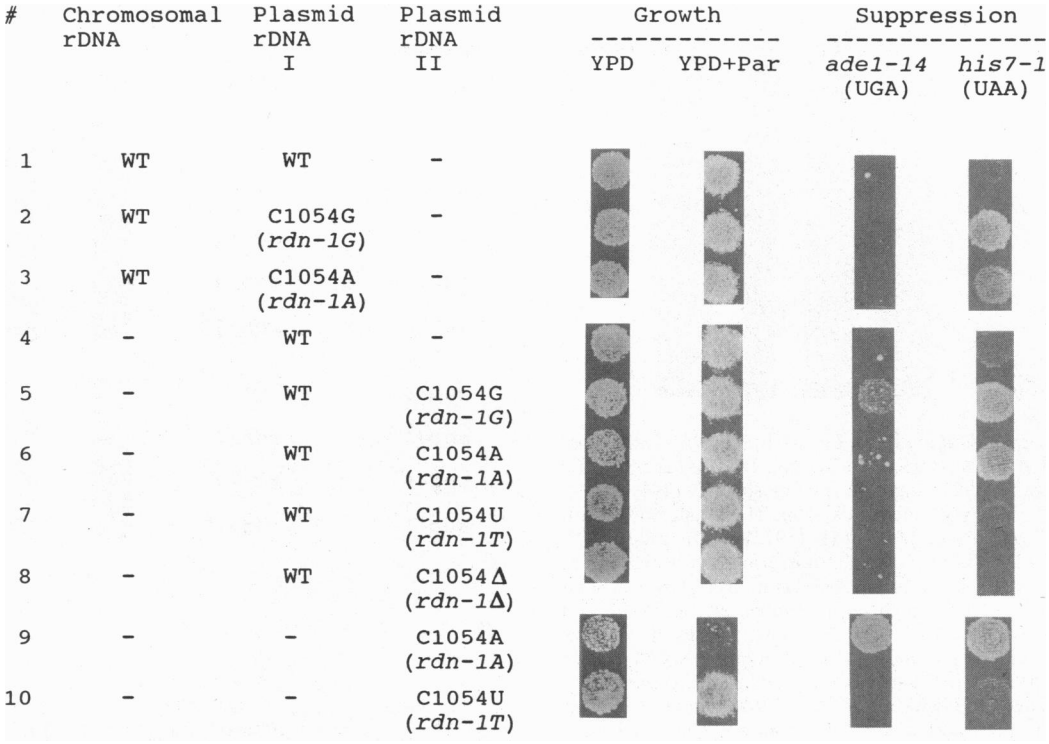


FIG. 2. Phenotypic effects of the mutations at yeast position 1054 (*rdn-1*). Construction of the yeast strains is described in the text. The strains are isogenic, except for their rDNA and rDNA-containing plasmids. Chromosomal rDNA is wild type in rows 1–3 and deleted in rows 4–10. Plasmid I is pRDN-wt (row 1), pRDN-1G (row 2), pRDN-1A (row 3), and pRDN-wt-U (rows 4–8). Plasmid II is pRDN-1G (row 5), pRDN-1A (rows 6 and 9), pRDN-1T (rows 7 and 10), and pRDN-1Δ (row 8). YPD is a complete organic medium, YPD+Par is the same medium containing paromomycin (0.1 mg/ml). Suppression of *ade1-14*$_{UGA}$ and *his7-1*$_{UAA}$ is scored by growth on synthetic medium lacking adenine (-Ade) and histidine (-His), respectively. YPD and YPD+Par plates were photographed after 3 days, and -Ade and -His plates were photographed after 1 week of incubation at 30°C. The spots on YPD look darker than on YPD+Par due to the accumulation of a red pigment caused by the *ade1-14* mutation, which is suppressed by paromomycin. The rDNA-deletion strains containing pRDN-1G (row 5) and pRDN-1A (rows 6 and 9) are lighter than the others due to suppression of *ade1-14* by the mutations *rdn-1G* and *rdn-1A*, respectively.

incubation, indicating that each of these plasmids alone is sufficient to support yeast viability in the absence of both chromosomal rDNA repeats and pRDN-wt-U plasmid. Primer extension analysis (Fig. 3) confirmed that a Ura$^-$ derivative of a pRDN-1T transformant of L-1521 (named L-1597) contains only mutant 18S rRNA. However, rare Ura$^-$ colonies, recovered from pRDN-1A, pRDN-1G, and pRDN-1Δ transformants, still contained the wild-type 18S rRNA and, therefore, probably arose from gene conversion or plasmid rearrangement events. Apparently, *rdn-1* mutant plasmids pRDN-1A, pRDN-1G, and pRDN-1Δ are deleterious for growth in the absence of wild-type rDNA, making selection for loss of the wild-type rDNA plasmid difficult. To overcome this, plasmids pRDN-1A, pRDN-1G, and pRDN-1Δ were transformed individually into strain L-1522, which is isogenic to L-1521 except that it contains pRDN-2-U [*URA3 rdn-2*], instead of pRDN-wt-U. The *rdn-2* mutation causes a severe growth defect in the absence of wild-type rDNA (15) that decreases its competitive abilities compared to *rdn-1* mutations. Indeed, L-1522 transformants bearing pRDN-1A papillated efficiently on 5-FOA medium. Southern blot analysis (not shown) confirmed that the rDNAs of these 5-FOA-resistant Ura$^-$ cells contain the *rdn-1A* and not the *rdn-2* mutation. One such strain was named L-1583. In the cases of the *rdn-1G* and *rdn-1Δ* bearing plasmids, 5-FOA-mediated plasmid shuffle did not work even in L-1522, suggesting that these mutations cause recessive lethality.

The yeast strains bearing only *rdn-1A* or only *rdn-1T* mutant 18S rRNA were tested for nonsense suppression and antibiotic sensitivity. In the absence of wild-type rDNA, the *rdn-1A* mutation caused slow growth, extreme sensitivity to paromomycin, and efficient suppression of both *ade1-14*$_{UGA}$ and *his7-1*$_{UAA}$ but not of *lys2-L864*$_{UAG}$ (Fig. 2). In contrast, the *rdn-1T* mutation did not inhibit growth and did not cause any suppression. Moreover, phenotypic suppression of *ade1-14*$_{UAG}$ by paromomycin (0.2–0.5 mg/ml), which is normally observed in the isogenic wild-type strain (15), was not detected in the strain containing only *rdn-1T* mutant 18S rRNA. Therefore, *rdn-1T* appeared to be an antisuppressor (also see below) rather than a suppressor, which is similar to the previously described yeast 18S rRNA mutations *rdn-2* (G517A) and *rdn-4* (U912C) (15).

**Yeast rDNA Mutations *rdn-1T*, *rdn-2*, and *rdn-4* Inhibit Omnipotent Suppressors.** Yeast genes *SUP45* and *SUP35* encode eukaryotic release factors eRF-1 and eRF-3, respectively (25–27). Mutations in these genes cause growth defects, paromomycin sensitivity, and suppression of various nonsense mutations (for review, see ref. 28), including *ade1-14*$_{UGA}$ and *his7-1*$_{UAA}$ (20). To check whether antisuppressor rRNA mutations interfere with mutant release factors, either pRDN-wt or one of the antisuppressor rDNA plasmids (pRDN-1T, pRDN-2, and pRDN-4) were individually shuffled into two *sup35* and two *sup45* mutants derived from rDNA-deletion strain L-1521. 5-FOA-mediated plasmid shuffle was very efficient for all plasmids including pRDN-2, which causes a growth defect that reduces efficiency of plasmid shuffle in wild-type strains (15). Therefore, pRDN-2 can successfully compete with the wild-type rDNA plasmid only in the presence of a *sup35* or *sup45* mutation but not in a nonsuppressor background. This result indicates that defects caused by the antisuppressor mutation *rdn-2* and suppressor mutations *sup35* and *sup45*, partially complement each other. The suppressor efficiencies of both *sup35* and *sup45* mutations were significantly reduced in the mutant rRNA backgrounds, compared to

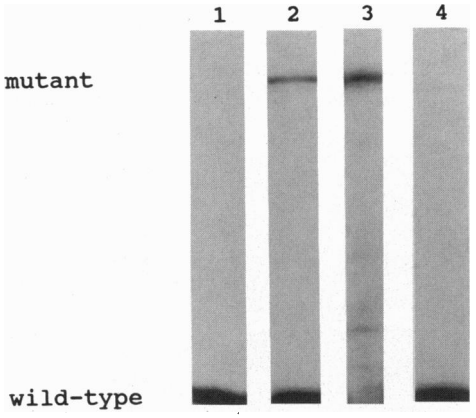


FIG. 3. Primer extension analysis of yeast 18S rRNA. Total yeast RNAs were isolated and analyzed (also see ref. 15) from the isogenic rDNA-deletion strains containing pRDN-wt plasmid (L-1494, lane 1), pRDN-wt-U and pRDN-1T plasmids (L-1521 transformed with pRDN-1T, lane 2), pRDN-1T plasmid (L-1597, lane 3), and pRDN-wt-U and pRDN-1Δ plasmids (L-1521 transformed with pRDN-1Δ, lane 4). RNAs for lanes 2 and 4 were isolated from cells grown on -Leu medium, which selects for a high copy number of pRDN-1T and pRDN-1Δ plasmids, respectively. DNA primer extension analysis (data not shown) indicates that the ratio of wild-type rDNA vs. mutant rDNA was about the same in the strains analyzed in lanes 2 and 4 (2.2:1 and 2.5:1, respectively). Positions of the RNA primer extension products on the 20% polyacrylamide gel are shown. The upper band corresponds to the mutant 18S rRNA primer extension product; the lower band corresponds to the wild-type 18S rRNA primer extension product.

the isogenic suppressor strains containing wild-type rRNA (Fig. 4*A*). The *rdn-2* and *rdn-4* mutations (but not the *rdn-1T* mutation) also compensated for the paromomycin sensitivity caused by *sup35* and *sup45* mutations (Fig. 4*A*). Compensation of paromomycin sensitivity by antisuppressor *rdn* mutations was detected even in the presence of wild-type rDNA (not shown). Since the *rdn-1T* mutation inhibits suppressor activity of *sup35* and *sup45* but does not compensate for the paromomycin sensitivity, it appears that paromomycin sensitivity of the *sup35* and *sup45* mutants is not a direct consequence of the general increase in translational suppression. It is worth noting that, in contrast to *rdn-2* and *rdn-4* (15), *rdn-1T* did not increase paromomycin resistance in the absence of suppressor mutations.

Judging from our observation that growth defects caused by *sup35* and *sup45* mutations are compensated by antisuppressor rRNA, one could predict the existence of very efficient *sup35* and *sup45* alleles that are viable only in the presence of antisuppressor rRNA. To check this hypothesis, we isolated *sup35* and *sup45* mutants from rDNA-deletion strains L-1522 and L-1523, which contain only *rdn-2* or *rdn-4* mutant rDNA, respectively. The frequency of spontaneous omnipotent suppressor mutations was reduced about 30-fold in L-1522 and about 3.5-fold in L-1523, compared to the isogenic strain L-1521 containing wild-type rRNA. This reduction is apparently due to a decrease of suppressor efficiency of the *sup35* and *sup45* mutations in the presence of antisuppressor rRNA mutations, as shown above. Six *sup35* and *sup45* mutants derived from L-1523 (*rdn-4*) were individually transformed with pRDN-wt or pRDN-4 plasmids. In five mutants, 5-FOA-mediated plasmid shuffle was efficient for both plasmids. However, one *sup35* mutant, L-1592 (*sup35-R15*), was able to exchange the resident pRDN-4-U plasmid for pRDN-4 but not for pRDN-wt. Therefore, the *rdn-4* mutation was essential for the viability of the *sup35-R15* allele.

**Interactions Between Yeast rDNA Mutations and a tRNA Suppressor.** *SLT3* is a mutant allele of the yeast $tRNA^{Gln}$ gene, with an anticodon that is complementary to the UAA stop

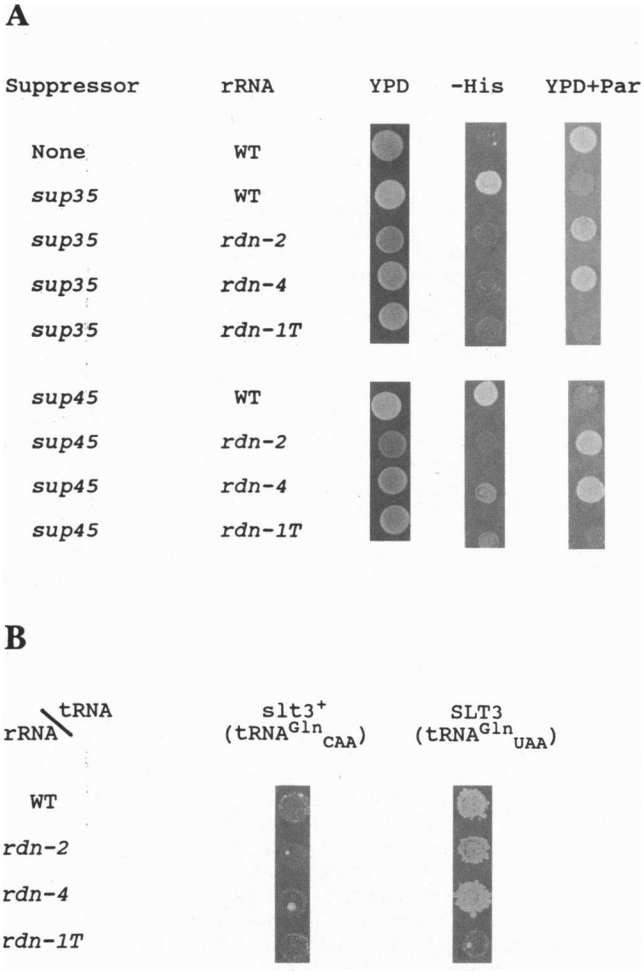


FIG. 4. Interactions between rRNA mutations and nonsense suppressors. (*A*) Interactions between omnipotent suppressor mutations *sup35-R8* and *sup45-R2* and antisuppressor *rdn* mutations. Construction of the rDNA-deletion strains, which are isogenic, except for the *SUP35/SUP45* loci and plasmid-localized rDNA, is described in the text. All rDNA-containing plasmids bear *TRP1* and *LEU2-d* markers. Media are the same as in Fig. 2, except that YPD+Par contains paromomycin (1.0 mg/ml). Plates were photographed after 3 days of incubation at 30°C. Suppression of $his7\text{-}1_{UAA}$ leads to growth on -His medium, while suppression of $ade1\text{-}14_{UGA}$ leads to growth on -Ade medium (data not shown) and white color on YPD (seen as a light shade). Strains in which suppression of $ade1\text{-}14_{UGA}$ does not occur are red on YPD (seen as a dark shade). (*B*) Interactions between rRNA mutations and $tRNA^{Gln}$ UAA-suppressor *SLT3*. Isogenic rDNA-deletion strains L-1494 (designated as wt), L-1495 (*rdn-2*), L-1496 (*rdn-4*), and L-1597 (*rdn-1T*) were transformed with either $pSlt^+$ (containing a wild-type allele of the $tRNA^{Gln}$ gene) or pPXS (containing a mutant UAA-suppressor allele of the $tRNA^{Gln}$ gene). Suppression of the $his7\text{-}1_{UAA}$ can be seen as growth on -His medium. Plates were photographed after 4 days of incubation at 30°C. There were no differences in growth on YPD and -Ura -Trp media (data not shown), except that *rdn-2* transformants grew slower than other strains, as reported (15).

codon (19). We have transformed yeast strain L-1494 and the isogenic strains L-1495, L-1496, and L-1597, bearing only mutant rDNA (*rdn-2, rdn-4*, and *rdn-1T*, respectively), with [*URA3*] plasmids pPXS (which contains a mutant *SLT3* allele of the $tRNA^{Gln}$ gene) and $pSlt^+$ (which contains a wild-type $tRNA^{Gln}$ allele). The mutation $his7\text{-}1_{UAA}$ was suppressed by *SLT3* in both the wild-type strain L-1494 and the *rdn-2* and *rdn-4* mutant strains, but not in the *rdn-1T* mutant strain (Fig. 4*B*). Thus, the *rdn-1T* (C1054U) mutation, but not the *rdn-2* (G517A) and *rdn-4* (U912C) mutations, inhibits read-through caused by UAA-suppressor $tRNA^{Gln}$.

## DISCUSSION

The effects of prokaryotic and eukaryotic small subunit rRNA mutations at position 1054 are compared in Table 1. In bacteria, strains containing only mutant rRNA have not been constructed. In the presence of the wild-type rRNA, C1054A and C1054G mutations cause UGA suppression in both *E. coli* and *S. cerevisiae*. This result confirms the importance of the C1054 position for translational accuracy and/or termination. Since UCAUCA triplets at nt 1199–1204 are not present in the corresponding region of eukaryotic 18S rRNA, the mRNA (UGA)·rRNA (UCA) base pairing model of Murgola *et al.* (4) cannot explain the suppressor effect of the mutations at position 1054 in yeast.

We have observed that yeast C1054A and C1054G mutations efficiently suppress UAA, in addition to UGA (Fig. 2 and Table 1). Although no effect of mutations at yeast position 1054 on UAG was shown, it cannot be excluded since *lys2-L864* was the only UAG mutation tested, and it is poorly suppressed by a variety of suppressors. Our data are in agreement with recent work in *E. coli*, reporting codon-nonspecific read-through caused by C1054G (17) and by C1054A during growth in enriched medium (16, 17). It has also been shown that C1054A and C1054G can alter the efficiency of UAA- and UAG-suppressor tRNAs in *E. coli* (8, 29) and that *E. coli* C1054A can suppress two frameshift mutations (16). However, since both frameshifts generate an in-frame nonsense codon, C1054A-mediated suppression of these frameshifts could result from a termination defect, as described for the *E. coli* release factor RF-2 gene (30). Thus, the evidence suggests the involvement of the 1054 base in the control of the general accuracy of translational termination and/or elongation, rather than in a specific codon·rRNA interaction, in both prokaryotes and eukaryotes.

It has recently been shown (17) that the mutation at position 1054 originally found (4) to cause UGA suppression in *E. coli* is C1054A (not C1054Δ as previously believed). In contrast, *E. coli* C1054Δ does not show any suppression (17). In yeast, C1054Δ also does not show any phenotypic effect in the presence of wild-type rRNA (Fig. 2). In the absence of wild-type rRNA, this mutation is lethal. The extremely low level of the yeast C1054Δ mutant rRNA, compared to C1054A, C1054G, and C1054U mutant rRNAs (e.g., Fig. 3), suggests that deletion of C1054 probably affects 18S rRNA stability and/or its packaging into ribosomes. This explains both the recessive lethality and the absence of any dominant phenotypic effect of C1054Δ. The recessive lethality caused by another yeast mutation, C1054G, is probably due to the high level of suppression it induces. This hypothesis is supported by the observation that the less-efficient yeast suppressor mutation C1054A is not lethal but causes very poor growth in the absence of wild-type rRNA.

The C1054U mutation did not cause any suppression in either *E. coli* or *S. cerevisiae*. However, we were able to test the yeast C1054U mutation in the absence of the wild-type rRNA and found that the C1054U inhibits suppression caused by paromomycin or by release factor mutations, *sup35* and *sup45* (Fig. 4*A*). This effect is consistent with results obtained in prokaryotes, since involvement in translational termination has been proposed to be a primary function of helix 34 in *E. coli* (4, 11, 12). Yeast C1054A and C1054G mutations, which suppress nonsense codons, may be interpreted as being defective in termination. On the other hand, C1054U could increase the efficiency of termination, compensating for the termination defect caused by the mutant release factor(s). It is worth noting that different substitutions at the same position cause opposite effects, i.e., suppression (C1054A and C1054G) or antisuppression (C1054U).

Two other yeast rRNA antisuppressors, *rdn-2* (G517A) and *rdn-4* (U912C), also inhibited UAA and UGA read-through caused by release factor mutations (Fig. 4*A*). Recent cross-linking data (31, 32) suggest that the prokaryotic 530 region and helix 34 are located close to each other in the three-dimensional structure of the ribosome. Both the 530 region and the release factor binding site have been mapped to the "neck" of the 30S subunit (33). Finally, both the regions at positions 530 and 915 (including position 912) are involved in streptomycin binding, suggesting a possible structural interaction between these pieces of the 16S rRNA (34). It is worth noting that prokaryotic and mitochondrial rRNA G517A mutations cause suppression rather than antisuppression (5, 9). Moreover, UAA suppression mediated by the yeast mitochondrial G517A (*MSU1*) mutation is inhibited by overproduction of the mitochondrial release factor mRF-1 (35), indicating that *MSU1* may be defective in termination. However, the prokaryotic 530 region, which includes position 517, is also implicated in codon–anticodon recognition (36) and interaction with EF-Tu elongation factor (37).

A general model for the read-through of stop codons is that ribosomal or release factor mutations allow noncognate tRNAs to read stop codons. In yeast, the UAA-suppressor mutation *SLT3*, which is a G → A substitution at the UUG anticodon of the CAA-decoding $tRNA^{Gln}$, makes the $tRNA^{Gln}$ anticodon complementary to UAA codons (19). The C1054U antisuppressor mutation inhibited UAA suppression caused by the mutant $tRNA^{Gln}$ (Fig. 4*B*). However, two other rRNA antisuppressor mutations, G517A and U912C, had no effect on the $tRNA^{Gln}$ (*SLT3*)-mediated suppression (Fig. 4*B*). One explanation could be that C1054U generally increases the efficiency of termination, while G517A and U912C affect other steps in translation. For example, a mutation that reduced the error frequency during codon–anticodon recognition would be expected to act as an antisuppressor to mutations in release factors but not to suppressor tRNAs with anticodons that are complementary to stop codons.

Table 1. Comparative analysis of C1054 mutations in *E. coli* and *S. cerevisiae*

| | *E. coli* | | | *S. cerevisiae* | | | |
|---|---|---|---|---|---|---|---|
| | + rDNA-wt | | | + rDNA-wt | | − rDNA-wt | |
| Mutation | UGA | UAA | Ref(s). | UGA | UAA | UGA | UAA |
| C1054U | 0 | 0 | 6, 8 | 0 | 0 | − | − |
| C1054A | + | + | 4, 16, 17, 29 | + | + | + | + |
| C1054G | + | + | 6, 8, 17 | + | + | Lethal | |
| C1054Δ | 0 | 0 | 17 | 0 | 0 | Lethal | |

+, Suppression; −, antisuppression; 0, indistinguishable from wild type; rDNA-wt, wild-type rDNA.

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