4234–4238 Nucleic Acids Research, 1995, Vol. 23, No. 21

Nonsense suppressor and antisuppressor mutations at the 1409–1491 base pair in the decoding region of *Escherichia coli* 16S rRNA

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Received September 12, 1995; Accepted September 21, 1995

ABSTRACT

Using a genetic selection for suppressors of a UGA nonsense mutation in *trpA*, we have isolated a G to A transition mutation at position 1491 in the decoding region of 16S rRNA. This suppressor displayed no codon specificity, suppressing UGA, UAG and UAA nonsense mutations and +1 and –1 frameshift mutations in *lacZ*. Subsequent examination of a series of mutations at G1491 and its base-pairing partner C1409 revealed various effects on nonsense suppression and frameshifting. Mutations that prevented Watson–Crick base pairing between these residues were observed to increase misreading and frameshifting. However, double mutations that retained pairing potential produced an antisuppressor or hyperaccurate phenotype. Previous studies of antibiotic resistance mutations and antibiotic and tRNA footprints have placed G1491 and C1409 near the site of codon-anticodon pairing. The results of this study demonstrate that the nature of the interaction of these two residues influences the fidelity of tRNA selection.

INTRODUCTION

Interactions between rRNAs and tRNAs play a critical role during the decoding process. Chemical protection studies (1,2) have identified nucleotides in rRNA participating in such interactions. While many of these protections probably represent direct contacts between rRNA and tRNAs, the functional significance of such contacts is largely unknown. One approach to addressing this issue is to identify mutations in rRNA at sites of putative rRNA–tRNA interactions, and to examine their effects on the accuracy of tRNA selection.

Using a genetic selection for nonsense suppressors we have isolated a G to A transition mutation at position 1491 of 16S rRNA (Fig. 1). This nucleotide and its base pairing partner C1409 have been localized to the A site, near the site of codon-anticodon pairing, by both biochemical and genetic studies. Nucleotides A1408, A1492, A1493 and G1494 are protected from chemical modification by A site-bound aminoacyl-tRNA or by an oligonucleotide corresponding to the anticodon arm of tRNA (1,2). Nucleotides A1408, G1491 and G1494 are protected by aminoglycoside antibiotics known to interfere with decoding in the A site (3). Resistance of various organisms to these antibiotics can be conferred by mutations at positions homologous to G1491 or U1495 (4), C1409 (5) or by methylation of nearby bases homologous to G1405 or A1408 (6). An A to G transition mutation at the 1492 homologue in human mitochondrial 12S rRNA, which forms an additional base pair in this helix, results in aminoglycoside hypersensitivity (7).

Mutations introduced into *Escherichia coli* 16S rRNA at C1409 and G1491 have been characterized with regard to their effects on sensitivity to aminoglycosides (8, 9). Our isolation of the A1491 mutation as a nonsense suppressor prompted us to examine the ability of some of these mutations to promote nonsense readthrough and frameshifting. While mutations which disrupted base pairing between positions 1409 and 1491 acted as nonsense and frameshift suppressors, two double mutations which retain Watson–Crick base pairing behaved as antisuppressors, decreasing readthrough of some nonsense mutations. These experiments indicate the importance of the interaction between residues 1409 and 1491 for optimal translational fidelity and suggest an important role for the conformation of this helical element in modulating codon–anticodon interaction.

MATERIALS AND METHODS

Bacterial strains and plasmids

Mutations in the *rrnB* operon of *E.coli* were isolated in FTP1860 [*his* Δ(*tonB trpAE15*)/F′ *trpA*(UGA243)], kindly provided by E.J. Murgola. Analysis of translational accuracy *in vivo* of 1409/1491 mutants was performed in EF41 [F⁻ Δ(*lac-pro*) *thi recA1*] (10). EF42 is a derivative of EF41 containing F′100, a wild type revertant of the F′ *proA*⁺*B*⁺*lacZ*(GGG461) episome from CSH102 (11).

Plasmid pMO10 is a kanamycin resistance, pSC101-derived, low copy number construct carrying the *rrnB* operon transcribed from the P_1P_2 promoter (12). pKK3535 consists of pBR322 containing the *rrnB* operon transcribed from the P_1P_2 promoter (13). pNO2680 is a pBR322 derivative carrying the *rrnB* operon in which the P_1P_2 promoters have been replaced by the P_L promoter of bacteriophage λ (14). pLG857 (15) is a kanamycin resistance plasmid expressing the temperature sensitive *857* allele of the bacteriophage λ *c*I repressor. pSG25 and its derivatives are derived from pACYC184 and carry the *lacZ* gene transcribed from the P_{tac} promoter (15).

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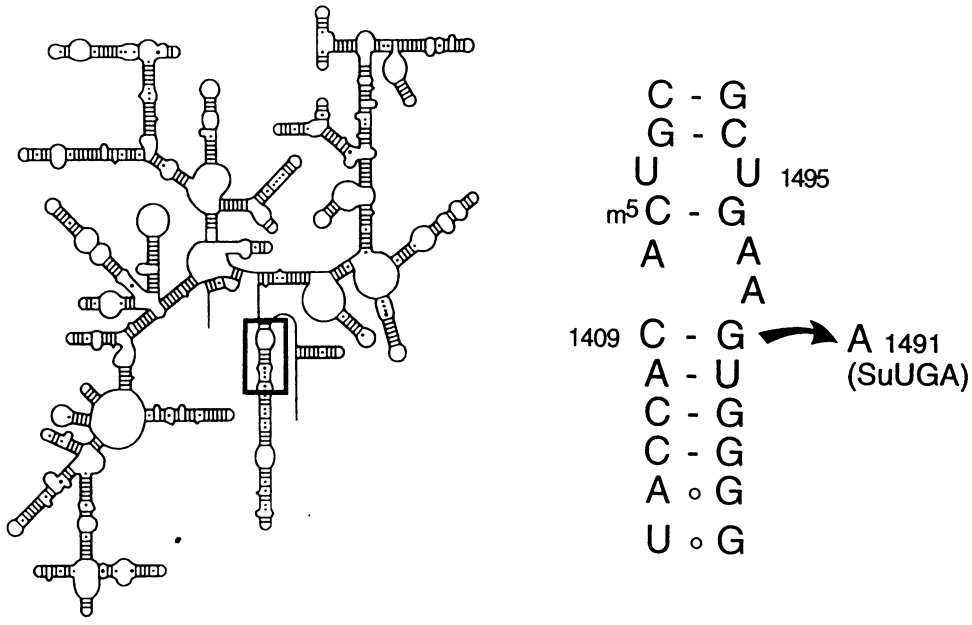

Figure 1. Secondary structure model of *E.coli* 16S rRNA (26) and the decoding region around the 1409–1491 base pair. Indicated is the site of the suppressor of *trpA*(UGA243) isolated in this study.

Mutagenesis and selection of mutants

FTP1860 containing plasmid pMO10 was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine essentially as described by Miller (11) with the following modification. After mutagenesis, 0.1 ml of washed culture was plated directly onto M9 glucose minimal plates (11) containing 0.1 % casamino acids and 50 μg/ml kanamycin.

Assay for β-galactosidase activity

β-galactosidase activity was assayed by the method of Miller, with modifications (16). Strain EF41 carrying plasmid pMO10 or pMO10(A1491) and mutant or wild-type pSG25 was grown in LB plus 50 μg/ml kanamycin and 12.5 μg/ml tetracycline at 37°C to mid log phase and assayed. EF41 carrying mutant or wild type plasmid pKK3535 and mutant or wild type pSG25 was grown in LB plus 100 μg/ml ampicillin and 12.5 μg/ml tetracycline at 37°C to mid log phase and assayed. EF41 containing plasmid pLG857, mutant or wild type plasmid pNO2680 and mutant or wild type pSG25 was grown in LB plus 50 μg/ml kanamycin, 100 μg/ml ampicillin and 12.5 μg/ml tetracycline at 30°C overnight, diluted into the same medium, grown to mid log phase at 42°C and assayed.

RESULTS

Selection of UGA suppressor mutations in rRNA

Mutations in a plasmid-encoded copy of the *rrnB* operon were selected as suppressors of a UGA nonsense mutation at codon 243 of *trpA*. We had previously noted the inherent leakiness of this mutation and found it to be suppressible by a mutation in 23S rRNA having only moderate effects on translational accuracy (10). This suggested to us that relatively weak suppressors as well as highly efficient ones could be isolated using this strain.

To isolate mutants, cultures of the *trpA* mutant FTP1860 containing the *rrnB* plasmid pMO10 were mutagenized with nitrosoguanidine for 15 min and plated on M9 glucose minimal medium containing kanamycin and casamino acids. Thirteen independent mutageneses and a no-mutagen control were performed. Colonies arising from each of the mutagenized cultures were pooled and plasmid DNA was extracted. FTP1860 was transformed with plasmid DNA from each of the mutagenized cultures and plated onto selective medium. Trp^- microcolonies appeared on plates of FTP1860 transformed with unmutagenized DNA and as a background on plates containing large Trp^+ transformants. Three Trp^+ transformants from each mutagenesis were purified by restreaking and plasmid DNA was extracted by a standard miniprep procedure. The suppressor phenotype of the mutants was confirmed by a second round of transformations of FTP1860.

Mapping and identification of UGA suppressor mutations in 16S and 23S rRNA

Mutations were initially mapped to either the 16S or 23S rRNA genes by a series of restriction fragment exchanges between unmutagenized and suppressor-containing plasmids. Restriction fragments from mutant plasmids were then subcloned into M13mp vectors and sequenced. Plasmids from the same mutagenesis bearing different mutations were considered independent mutants whereas plasmids from the same mutagenesis bearing identical mutations were considered siblings. Three independent mutants carried a C to U transition at position 1054 of 16S rRNA, 10 carried a C to U transition at position 1200 of 16S rRNA, 11 mutants carried a G to A transition at position 1093 of 23S rRNA and one mutant carried a G to A transition at position 1491 of 16S rRNA. Mutations at C1054 (17,18) and C1200 of 16S rRNA (18) and at position G1093 of 23S rRNA (19) have been described previously by others and were not analyzed further in this study.

We proceeded to characterize the A1491 mutation for its effect on nonsense and frameshift suppression *in vivo*.

Effects of the A1491 mutation on translational accuracy *in vivo*

The A1491 mutation was initially characterized in the context of plasmid pMO10. This plasmid was introduced into the *recA* strain EF41 containing derivatives of plasmid pSG25. These plasmids carry a *lacZ* reporter gene bearing nonsense or frameshift mutations near the 5′ end. Increased readthrough or suppression of these mutations is an indication of decreased translational accuracy which can be conveniently quantitated by measurement of β-galactosidase specific activity. The results of this analysis are presented in Table 1.

Table 1. Effects of the A1491 mutation isolated in plasmid pMO10 on nonsense readthrough and frameshifting

lacZ mutation	β-galactosidase activity	
	G1491 (WT)	A1491 (SuUGA)
pSG34-11 (UGA)	30.5 ± 1.2	68.3 ± 2.0
pSG3/4 (UGA)	101 ± 5.7	275 ± 8.5
pSG12-6 (UAG)	19.1 ± 0.8	29.2 ± 0.4
pSG163 (UAG)	42.9 ± 0.7	68.0 ± 1.0
pSG853 (UAA)	4.7 ± 0.1	9.0 ± 0.5
pSG12DP (–1)	78.4 ± 2.8	109 ± 2.7
pSGlac7 (+1)	64.4 ± 2.6	86.0 ± 2.4
pSG25 (WT)	9590 ± 423	9320 ± 626

Values for β-galactosidase activity are given as Miller units (11). Each value represents mean ± SD of at least three experiments. Cultures of EF41 carrying pMO10 or pMO10(A1491) were incubated at 37°C to mid-log phase and assayed for β-galactosidase activity. SuUGA, suppressor of a UGA nonsense mutation.

The A1491 mutation was found to increase readthrough or suppression of all the UGA, UAG and UAA nonsense mutations and +1 and –1 frameshift mutations examined. No codon specificity was observed, indicating a general defect in translational accuracy.

Effects of single and double mutations at positions 1409 and 1491 of 16S rRNA on translational accuracy *in vivo*

Mutations at positions 1409 and 1491 of *E.coli* 16S rRNA were previously constructed in this laboratory by oligonucleotide-directed mutagenesis to determine their effects on sensitivity to neomycin/kanamycin related aminoglycoside antibiotics (8,9). We examined the effects of several of these mutations on translational fidelity *in vivo*.

Mutant rRNA was expressed from high copy-number plasmids in order to maximize our ability to detect infrequent translational errors. As noted previously (9), some of these mutations are deleterious or lethal after prolonged high levels of expression and were therefore transcribed conditionally from the λ P_L promoter of plasmid pNO2680. Other mutations were expressed constitutively from plasmid pKK3535. The data from these experiments are presented in Table 2 and Table 3.

All three mutations at position 1491 (Table 2, C1409/C1491 and C1409/A1491; Table 3, C1409/U1491) caused increased misreading and frameshifting. Surprisingly, the C to G mutation at position 1409 (Table 2, G1409/G1491) also increased misreading and frameshifting contrary to the frameshift restrictive phenotype of the G1409 paromomycin resistance mutation in yeast mitochondria reported by Weiss-Brummer and Hüttenhoffer (20). We cannot account for this difference in our observations.

Double mutations which retain canonical Watson–Crick base pairing potential produced a hyperaccurate phenotype. Thus, while G/G, C/C, C/A, A/A and C/U juxtapositions increased misreading, G-C and U-A juxtapositions (Table 3, G1409/C1491 and U1409/A1491) had the opposite effect. This decrease in misreading was observed at UGA and UAG nonsense codons but not at the single UAA codon examined. No substantial effect on frameshifting was observed.

DISCUSSION

The isolation of the A1491 mutation as a nonsense suppressor is of interest because of the proximity of the C1409–G1491 base pair to the site of codon-anticodon interaction. Both mRNA (21) and tRNA (22) have been cross-linked to this region of 16S rRNA. Results from chemical modification experiments indicate that these nucleotides comprise part of the binding site for both the codon-anticodon complex (1,2) and aminoglycoside antibiotics (3). The discovery that mutations at C1409 and G1491 affect codon recognition as well as aminoglycoside sensitivity complements the structural data defining the A site and demonstrates the functional relevance of tRNA–16S rRNA interactions these studies have detected.

Aminoglycoside antibiotics interfere with accurate translation, presumably by binding to sites involved in decoding. It follows that aminoglycoside resistance mutations will arise at such sites, and in some cases, affect translational fidelity (23). Paromomycin resistance mutations in *Aspergillus nidulans* (24) and yeast mitochondrial rRNA (20) exhibited reduced levels of misreading, while paromomycin resistance mutations in *Podospora anserina* acted as weak suppressors (25). Several of the mutations analyzed in this study were originally characterized for resistance to aminoglycosides, including paromomycin (8,9). Mutations which disrupted base pairing potential between positions 1409 and 1491 conferred aminoglycoside resistance, while the G-C and U-A pairings showed little or no resistance. One interpretation of the correlation between aminoglycoside resistance and the nonsense and frameshift suppression observed in this study is that structural perturbations sufficient to prevent effective drug binding will also affect alignment of the A site substrate.

The two double mutations (G-C and U-A) were found to produce a hyperaccurate or antisuppressor phenotype. The decrease in misreading caused by the G-C and U-A mutations, though not observed with all *lac* mutants, was in some cases quite significant (Table 3). For instance, both double mutations restricted UGA readthrough with plasmid pSG34-11 from 28.6 to 17.7 U. This restriction was sufficient to be detected above the superimposed background of misreading by wild type ribosomes.

Table 2. Effects of mutations at positions 1409 and 1491 of 16S rRNA expressed from pNO2680 on nonsense readthrough and frameshifting

lacZ mutant	β-galactosidase activity				
	C1409/G1491	G1409/G1491	C1409/C1491	C1409/A1491	A1409/A1491
pSG34-11 (UGA)	18.5 ± 0.8	21.0 ± 0.4	35.7 ± 0.3	28.6 ± 0.1	21.2 ± 0.6
pSG3/4 (UGA)	58.2 ± 1.7	76.7 ± 1.2	137 ± 2.8	110 ± 5.3	81.2 ± 5.7
pSG12-6 (UAG)	2.9 ± 0.1	3.3 ± 0.1	4.5 ± 0.3	6.2 ± 0.2	4.2 ± 0.2
pSG163 (UAG)	9.5 ± 0.7	13.9 ± 0.2	17.2 ± 0.9	25.0 ± 2.0	18.8 ± 0.8
pSG853 (UAA)	6.3 ± 0.1	11.3 ± 0.2	12.0 ± 0.5	18.9 ± 0.5	13.7 ± 1.2
pSG12DP (–1)	23.4 ± 0.9	33.1 ± 0.9	44.4 ± 1.1	64.2 ± 0.5	48.4 ± 0.7
pSGlac7 (+1)	19.5 ± 0.7	32.1 ± 0.1	30.9 ± 0.8	56.5 ± 1.4	42.3 ± 0.5
pSG25 (WT)	4840 ± 315	5200 ± 572	5350 ± 339	6670 ± 169	5300 ± 155
F'100 (WT)	854 ± 21.1	761 ± 83.5	1120 ± 32.5	958 ± 43.9	878 ± 18.0

Values for β-galactosidase activity are given as Miller units (11). Each value represents mean ± SD of at least three separate experiments. Cultures of EF41 carrying pNO2680 derivatives were incubated at 42°C for 2.5 h to induce mutant rRNA expression after which assays were performed. F'100 contains the wild type *lac* operon in EF42.

Table 3. Effects of mutations at positions 1409 and 1491 of 16S rRNA expressed from pKK3535 on nonsense readthrough and frameshifting

lacZ mutant	β-galactosidase activity			
	C1409/G1491	G1409/C1491	U1409/A1491	C1409/U1491
pSG34-11 (UGA)	28.6 ± 4.6	17.7 ± 1.8	17.7 ± 3.4	98.5 ± 61.8
pSG3/4 (UGA)	69.8 ± 6.2	52.1 ± 3.4	46.9 ± 6.3	165 ± 15.9
pSG163 (UAG)	51.2 ± 2.4	39.8 ± 5.9	41.5 ± 11.1	72.5 ± 9.6
pSG853 (UAA)	4.3 ± 0.6	5.4 ± 0.7	3.9 ± 0.3	7.7 ± 0.4
PSG12DP (–1)	74.8 ± 4.3	69.6 ± 6.1	62.9 ± 8.7	215 ± 69.3
PSGlac7 (+1)	86.0 ± 5.9	77.0 ± 5.1	81.5 ± 5.3	122 ± 6.5
PSG25 (WT)	7100 ± 921	6560 ± 658	6550 ± 324	ND
F'100 (WT)	513 ± 48.8	617 ± 53.4	540 ± 57.4	440 ± 63.9

Values for β-galactosidase activity are given as Miller units (11). Each value represents the mean ± SD of at least three experiments. Cultures of EF41 bearing pKK3535 derivatives were incubated at 37°C to mid-log phase and assayed for β-galactosidase activity. The F'100 episome carries the wild type *lac* operon. nd, not determined.

One curious observation was the increase in β-galactosidase activity from wild type pSG25 in the presence of the A1491 mutation on plasmid pNO2680 (Table 2). This increase was not observed when this mutation was carried on the low copy number plasmid pMO10 (Table 1) and is likely a function of the higher level of expression from pNO2680. The A1491 mutation has been found to be deleterious under these conditions (8,9). Such increases in β-galactosidase activity have been observed with other deleterious mutations due to defects in regulation of ribosome synthesis and elevated cellular ribosome content (18). The C-U juxtaposition was also deleterious and was unstable in the presence of pSG25 (Table 3), probably due to the combined stresses of the rRNA mutation and β-galactosidase overproduction.

The findings of this study demonstrate the importance of the 1409–1491 base pair in translational fidelity. It is of particular interest that the nature of the interaction between these nucleotides can affect fidelity in either a positive or negative manner. An explanation for this phenomenon awaits high resolution structure determination of the interaction of the codon–anticodon complex with the decoding region of the 30S subunit.

ACKNOWLEDGEMENTS

This study was supported by a grant (GM19756) from the National Institutes of Health to A.E.D. We are grateful to Michael O'Connor for providing F'100 and to J. Stephen Lodmell, Michael O'Connor and George Q. Pennable for critical reading of this manuscript. This work is dedicated to the memory of Paul Francis Fredrick.

REFERENCES

1 Moazed,D. and Noller,H.F. (1986) *Cell*, **47**, 985–994.
2 Moazed,D. and Noller,H.F. (1990) *J. Mol. Biol.*, **211**, 135–145.
3 Woodcock,J., Moazed,D., Cannon,M., Davies,J. and Noller,H.F. (1991) *EMBO J.*, **10**, 3099–3103.
4 Spangler,E.A. and Blackburn,E.H. (1985) *J. Biol. Chem.*, **260**, 6334–6340.
5 Li,M., Tzagoloff,A., Underbrink-Lyon,K. and Martin,N.C. (1982) *J. Biol. Chem.*, **257**, 5921–5928.
6 Beauclerk,A.A.D. and Cundliffe,E. (1987) *J. Mol. Biol.*, **193**, 661–671.
7 Hutchin,T., Haworth,I., Higashi,K., Fischel-Ghodsian,N., Stoneking,M., Saha,N., Arnos,C. and Cortopassi,G. (1993) *Nucleic Acids Res.*, **21**, 4174–4179.

8 DeStasio,E.A., Moazed,D., Noller,H.F. and Dahlberg,A.E. (1989) *EMBO J.*, **8**, 1213–1216.
9 DeStasio,E.A. and Dahlberg,A.E. (1990) *J. Mol. Biol.*, **212**, 127–133.
10 Gregory,S.T., Lieberman,K.R. and Dahlberg,A.E. (1994) *Nucleic Acids Res.*, **22**, 279–284.
11 Miller,J.H. (1992) *A Short Course in Bacterial Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
12 O'Connor,M. and Dahlberg,A.E. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 9214–9218.
13 Brosius,J., Ulrich,A., Raker,M.A., Gray,A., Dull,T.J., Gutell,R. and Noller,H.F. (1981) *Plasmid*, **6**, 112–118.
14 Gourse,R.L., Takebe,Y., Sharrock,R. and Nomura,M. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 1069–1073.
15 O'Connor,M., Gsringer,H.U. and Dahlberg,A.E. (1992) *Nucleic Acids Res.*, **20**, 4221–4227.
16 Weiss,R.B., Dunn,D.M., Atkins,J.F. and Gesteland,R.F. (1987) *Cold Spring Harb. Symp. Quant. Biol.*, **52**, 687–693.
17 Murgola,E.J., Hijazi,K.A., Gsringer,H.U. and Dahlberg,A.E. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 4162–4165.
18 Moine,H. and Dahlberg,A.E. (1994) *J. Mol. Biol.*, **243**, 402–412.
19 Jemiolo,D. and Murgola,E.J. (1995) *Proc. Natl. Acad. Sci. USA*, submitted.
20 Weiss-Brummer,B. and Hüttenhoffer,A. (1989) *Mol. Gen. Genet.*, **217**, 362–369.
21 Rinke-Appel,J., Jünke,N., Brimacombe,R., Dokudovskaya,S., Donstova,O.and Bogdanov,A. (1993) *Nucleic Acids Res.*, **21**, 2853–2859.
22 Ciesiolka,J., Gornicki,P. and Ofengand,J. (1985) *Biochemistry*, **24**, 4931–4938.
23 Gale,E.F., Cundliffe,E., Reynolds,P.E., Richmond,M.H. and Waring,M.J. (1981) *The Molecular Basis of Antibiotic Action*. John Wiley and Sons, London.
24 Martinelli,S.D. and Sheik (nee Zamir),A. (1991) *Curr. Genet.*, **20**, 211–218.
25 Dequard,M., Couderc,J.L., Legrain,P., Belcour,L. and Picard-Bennoun,M. (1980) *Biochem. Genet.*, **18**, 263–280.
26 Gutell,R.R., Larsen,N. and Woese,C.R. (1994) *Microbiol. Rev.*, **58**, 10–26.