Mutations at Two Invariant Nucleotides in the 3’-Minor Domain of Escherichia coli 16 S rRNA Affecting Translational Initiation and Initiation Factor 3 Function*

Matthew A. Firpoš, Mercedes B. Connelly1, Dixie J. Gossi, and Albert E. Dahlbergt

From the 1Section of Biochemistry, Brown University, Providence, Rhode Island 02912 and the 2Department of Chemistry, Hunter College, New York, New York 10021

We have investigated the highly conserved GAUCA sequence of small subunit ribosomal RNA. Within this region, the invariant nucleotides G1530 and A1531 of Escherichia coli 16 S RNA were mutagenized to A1530/G1531. These base changes caused a lethal phenotype when expressed from a high copy number plasmid. In low copy number plasmids, the mutant ribosomes had limited effects when expressed in vivo but caused significant deficiencies in translation in vitro, affecting enzymatic tRNA binding, non-enzymatic tRNA binding, subunit association, and initiation factor 3 (IF3) binding. Mutant 30 S ribosomal subunits showed a 10-fold decrease in affinity for IF3 as compared to wild-type subunits but showed an increased affinity for IF3 when in 70 S ribosomes. Additionally, IF3 did not promote dissociation of 70 S ribosomes, which had mutated subunits as monitored by light-scattering experiments. However, extension inhibition experiments (toeprinting) showed that IF3 retained its ability to discriminate between initiator and elongator tRNAs on mutated subunits. The results indicate that the two functions of IF3, tRNA discrimination and subunit dissociation, are separable and that the invariant nucleotides are important for correct subunit function during initiation.

Ribosomal RNA (rRNA) plays a significant role in the process of translation, and specific RNA regions have been implicated in several translational functions (1, 2). The best evidence for direct involvement of rRNA in the base-paired interaction between the purine Shine-Dalgarno (SD)1 sequence in mRNA and the polyuridylic anti-SD region at the extreme 3’-end of 16 S rRNA during translational initiation (3) and elongation (4, 5). The SD interaction does not occur in eucarya, and the anti-SD region is conserved only in archaeal and bacterial rRNA (6). However, the GAUCA sequence (nucleotides 1530–1534 in Escherichia coli 16 S rRNA, see Fig. 1) immediately upstream of the anti-SD region is highly conserved in all three domains (8) and includes invariant nucleotides at positions 1530 and 1531 (9).

* This work was supported by National Institutes of Health Grant GM19756 (to A. E. D.) and National Science Foundation Grant GER-3023681 (to D. J. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by National Institutes of Health Predoctoral Training Grant GM07601. Present address: Howard Hughes Medical Institute, University of Utah, Salt Lake City, UT 84112. To whom correspondence should be addressed. Tel.: 401-863-2223; Fax: 401-863-1182.

1 The abbreviations used are: SD, Shine-Dalgarno; IF3, initiation factor 3; FITC, fluorescein isothiocyanate.

The conserved nature of the GAUCA sequence implies a functional significance for these nucleotides. However, little is known about the role of this site. In experiments done with E. coli ribosomes, kethoxal modification of nucleotide G1530 moderately inhibited subunit association (10) while nucleotide A1531 displayed enhanced reactivity toward chemical modification upon 50 S subunit binding (11). Nucleotide A1531 also displayed enhanced modification upon subunit inactivation (12), a reversible conformation change associated with monovalent or divalent cation depletion. The site is protected from nuclease attack by initiation factor 3 (IF3) binding (13), and the adjacent stem structure has been cross-linked to both IF3 (14) and 23 S rRNA (15). Recently, nucleotide G1530 has been cross-linked to mRNA between the Shine-Dalgarno region and the AUG start codon (16). Based partially on the conserved nature of the region, Kössen et al. (17) proposed that an interaction occurs between the GAUCA sequence and the 5’-end of 16 S rRNA as a discrete functional state during elongation, whereas Thanaraj and Pandit (18) have proposed that the GAUCA sequence functions as a translational enhancer by base pairing with a complementary sequence in mRNA upstream of the start codon.

The conserved nature of the GAUCA sequence and its proximity to the anti-SD region, the decoding site, and the terminal helix place it in the center of a very important functional region of the 30 S subunit. Here, we describe experiments designed to investigate the function of the GAUCA sequence, especially the invariant G1530 and A1531 residues. We have switched the order of these two purines on a plasmid-borne copy of the rnb operon and assayed the effects of the mutations in vivo and in vitro. We found that expression of mutant 16 S rRNA affected several subunit functions in vitro, including initiation complex formation, subunit association, and IF3 binding.

MATERIALS AND METHODS

Bacterial Strains and Plasmids—Strain SU1675 (F’ lac’ lacI q Tn::5 kan’ reABC Δ(lac-pro) thi ara), a derivative of CH32 (19) was used randomly as host in this study. Strain MDA6646 (F’ ilvB1202 ilvH2202 rbs221 ara thi Δ(lac-pro) Δgpt pcmB ΔrreA) (a gift from Dr. E. J. Murgola) was used during the initial cloning to reduce plasmid copy number. Strain XL-1 Blue (Stratagene) was used to propagate M13 phage, and the ung dut strain Cj 236 (Bio-Rad) was used to prepare uracil containing M13 DNA. The intact rnb operon was carried in pKK3535, a pBR322-derived high copy plasmid (20), and in pMO11, a derivative of pSC101, a low copy plasmid (21). A single C to U nucleotide change at position 1192 in the 16 S rRNA gene in both pKK3535 and pMO11 confers spectinomycin resistance yielding pKK1192 and pMM1192, respectively. The A1530/G1531 16 S rRNA mutations were carried on plasmids pMF161 (a derivative of pCK1192) and pMF161 (a derivative of pMM1192). The rnb operon in all plasmids was transcribed from the constitutive P2 promoters.

Mutagenesis and Ribosome Preparation—The A1530/G1531 mutation was constructed and ribosomes and ribosomal subunits were prepared basically as described (22). Polyribosomes were prepared as de-
scribed (23) and separated by sucrose density gradient centrifugation using polysome buffer (20 mM Tris-HCl, pH 7.6, 100 mM KCl, 15 mM MgCl₂, 1 mM dithiothreitol). Fractions were collected and precipitated with ethanol. The proportion of plasmid-encoded rRNA in total cellular RNA, 30 S subunits, 70 S ribosomes, and polysome preparations was determined by the method of Sigmund et al. (24). A 20% S̅-labeled primer complementary to 16 S rRNA nucleotides 1193-1236 was used as described in the legend of Fig. 2. Autoradiograms were scanned using an LKB Ultrascan XL laser densitometer.

In Vitro Assays—Aminoacylation and formylation of tRNAATAFT (Sigma) were carried out as described (25) except that a commercial synthetase mix was used (Sigma). Binding of hF1[Met]tRNA ATAFT to 30 S subunits was carried out as described (26) except that the rRNA used was a random copolymer of adenosine, guanosine, and uracil (poly(A,G,U), Sigma, 50 pmol), which contained a random distribution of AUG start codons as well as SD sequences. IF1, IF2, and IF3 were gifts from Dr. Claudio Gualerzi.

The ability of 30 S subunits containing mutant rRNA to associate with 30 S subunits to form 70 S ribosomes was analyzed as described (27) except that MgCl₂ concentrations indicated in the Fig. 2 were used. Toeprint analysis was carried out as described (28, 29) using free 30 S subunits (93% mutant rRNA) and bacteriophage T4 gene 32 rRNA.

IF3 Binding Experiments—IF3 was labeled with 5-fluorescein isothiocyanate (FITC) (Molecular Probes). IF3 (1 μg/ml), dissolved in 0.01 n NaHCO₃, was incubated with a 5-fold molar excess of FITC solution in freshly distilled Me₂S at 4°C. The total volume was 50 μl. The sample was applied to a 1-ml Sephadex G-25 column equilibrated with 50 mM Tris-HCl, pH 7.6, 250 mM KCl, 0.5 mM NaCl, 5% glycerol. The molar ratio was determined as described previously (30). Experiments were performed in 10 mM Tris-HCl, pH 7.8, 50 mM KCl, 6 mM MgCl₂, 1 μM dithiothreitol. Fractions were collected and precipitated with ethanol. The molar ratio was determined as described previously (30). Experiments were performed in 10 mM Tris-HCl, pH 7.8, 50 mM KCl, 6 mM MgCl₂, 1 μM dithiothreitol. Fractions were collected and precipitated with ethanol. The molar ratio was determined as described previously (30).

Equation 1 defines the binding equilibrium constant:

\[ K = \frac{[C][B]}{[AB]} \]  
(Eq. 1)
rRNA from each 30 and 70 S fraction is shown in Fig. 2. The data indicate that the 30 S peaks were enriched with mutant rRNA. Thus, subunits containing the A1530/G1531 mutations were deficient in the ability to form 70 S ribosomes. It is important to note, however, that these subunit re-association assays were carried out in vitro, performed in the absence of translation factors, mRNA, or tRNA. Sucrose density gradients of polysome samples (see Table I) showed mutant rRNA was present in ribosomes and polysomes. Apparently, during initiation of translation in vivo, the subunit association defect of the mutant subunits was suppressed.

In Vitro Initiation Complex Formation on A1530/G1531 Subunits—Given the proximity of the mutant nucleotides to the anti-Shine-Dalgarno region and the IF3 cross-link site (14), it was possible that the A1530/G1531 mutations would affect events of translational initiation. We therefore analyzed the ability of mutant subunits to form the initiation complex in vitro. Activated salt-washed 30 S subunits were used in poly-(A,G,U)-directed [3H]Met-tRNA\textsuperscript{Met} binding assays in the presence of the three initiation factors. The mutant subunit preparation used in this experiment contained 65% plasmid-encoded (mutant) rRNA. As can be seen in Fig. 3, tRNA binding to mutant subunits was severely reduced relative to wild-type subunits. Indeed, the overall reduction in tRNA binding was almost equal to the amount of mutant rRNA present in the subunit preparation, suggesting that the A1530/G1531 mutations resulted in a complete inability to bind [3H]Met-tRNA\textsuperscript{Met} in vitro.

Initiation Factor 3 Binding to A1530/G1531 30 S Subunits and 70 S Ribosomes—Because IF3 has a central role in translational initiation and has been cross-linked very near (nucleotides 1506–1529) to the mutagenized nucleotides (14), the binding of IF3 was examined in more detail. Steady-state experiments were carried out in which the change in fluorescence intensity as FITC-labeled IF3 bound to ribosomes and 30 S subunits was monitored to determine equilibrium binding constants. In these experiments, we took advantage of the propensity of mutant ribosomes to dissociate at lower magnesium ion concentration to obtain a subunit fraction enriched in mutant rRNA. Samples of dissociated mutant ribosomes, which con-

![Fig. 1. Location of the A1530/G1531 mutations. Secondary structure map of E. coli 16 S rRNA (7) with the G to A base change at position 1530 and the A to G base change at position 1531.](image)

**Table I**

| Plasmid | Sequence         | Plasmid-encoded rRNA | %       |
|---------|------------------|----------------------|---------|
|         |                  | Total RNA | 30 S | 70 S | Polysomes |
| pMM 1192| G1530/A1531 (wt) | 72        | 80   | 75   | 69        |
| pMF-M161| A1530/G1531 (mut)| 70        | 79   | 67   | 62        |
Effects of A1530/G1531 Mutations in E. coli 16 S rRNA

**Fig. 2.** Primer extension analysis of A1530/G1531 subunit re-association gradient fractions. Ribosomal RNA was isolated from the 30 and 70 S fractions of subunit re-association gradients containing 1.5, 5, 10, or 15 mM MgCl₂. The samples were analyzed by the primer extension method of Sigmund et al. (24) using the entire amount of rRNA isolated from each peak, an excess of labeled primer, and dCTP, dGTP, dTTP, and ddATP. A one-base extension corresponded to U1192 (plasmid-encoded rRNA), if a cytidine residue was encountered by the polymerase at position 1192 (chromosomally encoded rRNA). If a cytidine residue was encountered by the polymerase at position 1192 (chromosomally encoded rRNA), extension continued to the next uridine residue, at position 1189, resulting in a four-base extension.

**Fig. 3.** Enzymatic [³H]Met-tRNAᵐet binding to wild-type and A1530/G1531 mutant subunits. Increasing amounts of [³H]Met-tRNAᵐet were bound to a constant amount of activated 30 S subunits from cells containing wild-type or mutant rRNA and 32 mRNA, tRNA, and 30 S subunits halts the extension of a primer annealed downstream on the mRNA and results in a characteristic band (the toeprint) when the assay is examined by gel electrophoresis (28, 29). Under the conditions used in this experiment, the toeprint was strictly dependent on bound tRNA.In the presence of tRNA, tRNA discrimination function of IF3 was not disrupted by the A of the AUG start codon as +1 was seen corresponding to the tRNA Met bound in the P-site, and a second stop was at +19 corresponding to tRNA Met bound at the P-site. As increasing amounts of IF3 were added to mutant subunits in the presence of both tRNAs, the tRNA discrimination function of IF3 was not disrupted by mutant subunits.

**Table II**

| IF3 binding to 30 S subunits and tight couple ribosomes | Kₑₒₑ, M⁻¹ × 10⁶ |
|--------------------------------------------------------|------------------|
| Wild type                                              | 26.7 ± 7.0⁶     |
| Mutant*                                                | 6.8 ± 4.0        |
| Wild type                                              | 0.31 ± 0.06⁶    |
| Mutant*                                                | 10.0 ± 5.0       |

* Errors in Kₑₒₑ fit by ENZFITTER are due to the uncertainty in concentration (±5%) of FITC-IF3.

† Within agreement of previously reported Kₑₒₑ value of 18 × 10⁻⁶ (31).

‡ 93% mutant rRNA.

§ Previously reported (31).

visited both chromosomally encoded (wild-type) rRNA and plasmid encoded (mutant) rRNA, were re-associated at 10 mM MgCl₂. Under these conditions, chromosomally encoded 30 S subunits readily associated with 50 S subunits to form 70 S ribosomes leaving the 30 S fraction enriched in mutant subunits. The resulting mutant 30 S samples contained 95% plasmid-encoded (mutant) rRNA. However, tight couple 70 S ribosomes used in steady-state experiments contained only 50% mutant rRNA. The FITC-IF3 ribosome binding constants are summarized in Table II. FITC-IF3 bound to wild-type 30 S subunits with a Kₑₒₑ of 27 × 10⁶ M⁻¹ and to wild-type 70 S ribosomes with a Kₑₒₑ of 0.30 × 10⁶ M⁻¹. Both of these equilibrium constants agree with previously reported values (27, 31). FITC-IF3 bound to mutant 30 S subunits (95% mutant rRNA) with an equilibrium association constant of 7 × 10⁶ M⁻¹. As increasing amounts of IF3 were added to mutant subunits in the presence of both tRNAs, the tRNA discrimination function of IF3 was not disrupted by mutant subunits.

**Discussion**

Expression of the A1530/G1531 mutant rRNA produced severe effects on translation. It was lethal when expressed from a high copy number plasmid, and even at low copy it had significant, yet subtle, effects on growth rate. In vitro, the
Effects of A1530/G1531 Mutations in E. coli 16 S rRNA

mutant subunits displayed explicit functional defects, the most dramatic involving IF3 binding and function. IF3 is bifunctional. It promotes proper selection of the initiator tRNA by recognizing specific determinants on tRNA\textsuperscript{Met} and promotes subunit dissociation by preferential binding to free 30 S subunits (32–34). IF3 is an elongated protein consisting of two separate domains (35) and has been shown by footprinting (13, 36) and cross-linking (14) studies to interact with the 30 S subunit at both the central and the 3′-minor domains of 16 S rRNA (see Ref. 2 and references therein). The central domain binding site includes the 700 region, the 790 loop, and the 840 stem while the 3′-binding site includes the 1500 region and the 3′-terminal helix. These sites of IF3 interaction with 16 S rRNA correlate well with IF3 function as the 700 region, the 790 loop, and the terminal helix have all been implicated in subunit association and are clustered in the vicinity of the P-site (2). Additionally, a mutation at 791 not only resulted in a 10-fold decrease in IF3 binding, an effect remarkably similar to the effect seen in the A1530/G1531 mutant, but also resulted in decreased subunit association (27), further linking the two functions to the central domain. The 3′-end of 16 S rRNA has also been implicated in subunit association (2, 9, 14, 37, 38). In the present study, the toeprint data (Fig. 5) indicate that the A1530/G1531 mutation does not alter IF3’s ability to discriminate between initiator and elongator tRNAs. However, subunit dissociation is affected. The binding of IF3 does not cause dissociation of mutant 70 S ribosomes, a particularly interesting result since mutant ribosomes have a propensity for dissociation. Thus, it appears that the mutations at 1530 and 1531 uncouple the two functions of IF3 on the 30 S subunit.

Tapprich and co-workers (27) showed that the 10-fold decrease in IF3 binding seen for the mutation at 791 was entirely due to a 10-fold increase in off-rate ($k_\text{-off}$), with on-rate ($k_\text{on}$) remaining unchanged. This led to their proposal that IF3 can interact with either binding site but requires a specific set of interactions, including G791, to form a stable complex. This model is supported by studies in which IF3 was bound to 30 S subunits lacking the 3′-colicin E3 fragment (nucleotides 1493–1542) in which only weak IF3 interactions were seen (39). Our data are consistent with a model in which IF3 first interacts with the 3′-domain binding site and is then stabilized by cooperative interactions with the central domain binding site. The loss of the cooperative interactions in subunits containing the A1530/G1531 mutant rRNA could account for the 10-fold decrease in IF3 binding. This model also provides a mechanism for IF3 release upon 50 S binding to the 30 S initiation complex. After proper initiation occurs, 50 S subunits can bind the central domain, eliminating the cooperative interactions and reversing the process of IF3 binding.

The decrease in enzymatic poly(A,G,U) f\textsuperscript{3H}[\text{Met}\text{-tRNA}^{\text{Met}}] binding may be due, in part, to the inability of mutant subunits to reform the active conformation. Activation, defined by the ability of the subunit to bind N-acetyl-Phe-tRNA\textsuperscript{Phe} in the presence of poly(U) (40), is associated with a conformational change in the 3′-minor domain (12). In fact, one of the mutated sites, A1531, has been shown to have enhanced reactivity in inactive subunits (12).

Finally, the conserved nucleotides between 1529–1534 have been proposed to base pair with mRNA and function as a translational enhancer (the TP interaction, Ref. 18). Using compensatory base change analysis, we have compared the in vivo expression of a lacZ reporter gene containing a TP site upstream of the SD sequence, which was complementary to either the wild-type or A1530/G1531 mutant 16 S rRNA. No significant change in expression was observed (data not shown). Furthermore, if the region was functioning as a translational enhancer, we might expect differences in spot intensities on two-dimensional protein gels corresponding to increased or decreased complementarity to specific mRNAs, much as was seen in experiments involving Shine-Dalgarno mutants (41). The fact that we see no differences in relative intensities for proteins in two-dimensional gels (data not shown) together with the results of compensatory base change analysis indicates that expression of different proteins was not altered and does not support the translational enhancer model.

Acknowledgments—We thank Anna La Teana, Steve Ringquist, and Ruth Van Bogelen for procedural assistance and Claudio Gualerzi for providing initiation factors. We also thank Michael O’Connor, William...
Tapprich, and Kathy Lieberman for helpful suggestions and critical reading of the manuscript and Mary Sue Purzycki, Stephen Lodmell, Steven Gregory, Don Van Ryk, Carleen Brunelli, and George Q. Pen- nable for numerous discussions.

REFERENCES

1. Dahlberg, A. E. (1989) Cell 57, 525–529
2. Noller, H. F. (1991) Annu. Rev. Biochem. 60, 191–227
3. Shine, J., and Dalgarno, L. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 1342–1346
4. Weiss, R. B., Dunn, D. M., Dahlberg, A. E., Atkins, J. F., and Gesteland, R. F. (1988) EMBO J. 7, 1503–1507
5. Larson, B., Wills, N. M., Gesteland, R. F., and Atkins, J. F. (1994) J. Bacteriol. 176, 6842–6851
6. Neefs, J.-M., Van de Peer, Y., Hendriks, L., and De Wachter, R. (1990) Nucleic Acids Res. 18, 2237–2317
7. Woeser, C. R., and Pace, N. R. (1993) in The RNA World (Gesteland, R. F., and Atkins, J. F., eds) pp. 137–156, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
8. Raúé, H. A., Klootwijk, J., and Musters, W. (1988) Prog. Biophys. Mol. Biol. 51, 77–129
9. Noller, H. F. (1993) in The RNA World (Gesteland, R. F., and Atkins, J. F., eds) pp. 137–156, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
10. Herr, W., Chapman, N. M., and Noller, H. F. (1979) J. Mol. Biol. 130, 433–449
11. Baudin, F., Mougel, M., Romby, P., Eyermann, F., Ebel, J.-P., Ehresmann, B., and Ehresmann, C. (1989) Biochemistry 28, 5847–5855
12. Moazed, D., Van Stolk, B. J., Southwaite, S., and Noller, H. F. (1986) J. Mol. Biol. 191, 483–493
13. Widstrom, E. (1983) Nucleic Acids Res. 11, 2035–2052
14. Ehresmann, C., Moine, H., Mougel, M., Dondon, J., Grunberg-Manago, M., Ebel, J.-P., and Ehresmann, B. (1986) Nucleic Acids Res. 14, 4803–4821
15. Mitchell, P., Osswald, M., and Brimacombe, R. (1992) Biochemistry 31, 3004–3011
16. Rinke-Appel, J., Jünke, N., Brimacombe, R., Lavrik, I., Dokudovskaya, S., Donskova, O., and Bogdanov, A. (1994) Nucleic Acids Res. 22, 3018–3025
17. Kösel, H., Hoch, B., and Zaltz, P. (1990) Nucleic Acids Res. 18, 4803–4821
18. Thanneraj, T. A., and Pandit, M. W. (1989) Nucleic Acids Res. 17, 2979–2985
19. Miller, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
20. Brosius, J., Dull, T., Steeter, D., and Noller, H. F. (1981) J. Mol. Biol. 148, 107–127
21. O’Connor, M., and Dahlberg, A. E. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9218–9223
22. Tapprich, W. E., Göringer, H. U., De Stasio, E. A., and Dahlberg, A. E. (1990) in Ribosomes and Protein Synthesis (Spedding, G., ed) pp. 253–271, IRL Press, Oxford
23. Godson, G. N., and Sinheimer, R. L. (1967) Biochim. Biophys. Acta 149, 489–495
24. Sigmund, C. D., Edykebi, M., Bordon, A., and Morgan, E. A. (1988) Methods Enzymol. 164, 673–690
25. Tate, W. P., and Caskey, C. T. (1990) in Ribosomes and Protein Synthesis (Spedding, G., ed) pp. 81–100, IRL Press, Oxford
26. Canonicc, M. A., Pon, C. L., Pawlik, R. T., Calogero, R., and Gualerzi, C. O. (1987) Biochimie (Paris) 69, 957–963
27. Tapprich, W. E., Goss, D. J., and Dahlberg, A. E. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 4927–4931
28. Hartz, D., McPheeters, D. S., Traut, R., and Gold, L. (1988) Methods Enzymol. 164, 419–425
29. Hartz, D., McPheeters, D. S., and Gold, L. (1991) J. Mol. Biol. 218, 83–97
30. Tanford, C. (1967) Physical Chemistry of Macromolecules, p. 282, J ohn Wiley & Sons, Inc., New York
31. Goss, D. J., Parkhurst, L. J., and Wahba, A. J. (1982) J. Biol. Chem. 257, 10119–10127
32. Gualerzi, C. O., and Pon, C. L. (1990) Biochemistry 29, 5881–5889
33. Hartz, D., McPheeters, D. S., and Gold, L. (1990) in The Ribosome Structure, Function, and Evolution (Hill, W. E., Dahlberg, A. E., Garrett, R. A., Moore, P. B., Schlessinger, D., and Warner, J. R., eds) pp. 275–280, American Society of Microbiology, Washington, D. C.
34. Gualerzi, C. O., La Tena, A., Spario, R., Canonicc, M. A., Severini, M., and Pon, C. L. (1990) in The Ribosome: Structure, Function, and Evolution (Hill, W. E., Dahlberg, A. E., Garrett, R. A., Moore, P. B., Schlessinger, D., and Warner, J. R., eds) pp. 281–291 American Society of Microbiology, Washington, D. C.
35. Kycia, J. H., Biou, V., Shu, F., Gerchman, S. E., Graziano, V., and Ramakrishnan, V. (1995) Biochemistry 34, 6183–6187
36. Moazed, D., Serrahia, R. R., Gualerzi, C., and Noller, H. F. (1995) J. Mol. Biol. 248, 207–210
37. Politz, S. M., and Glitz, D. G. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 1468–1472
38. Brow, D. A., and Noller, H. F. (1983) J. Mol. Biol. 163, 27–46
39. Laughran, M., Dondon, J., and Grunberg-Manago, M. (1978) FEBS Lett. 91, 265–268
40. Zamir, A., Miskin, R., and Elson, D. (1971) J. Mol. Biol. 60, 347–364
41. Jacob, W. F., Sankel, M., and Dahlberg, A. E. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4767–4763
Mutations at Two Invariant Nucleotides in the 3'-Minor Domain of Escherichia coli 16 S rRNA Affecting Translational Initiation and Initiation Factor 3 Function
Matthew A. Firpo, Mercedes B. Connelly, Dixie J. Goss and Albert E. Dahlberg

J. Biol. Chem. 1996, 271:4693-4698.
doi: 10.1074/jbc.271.9.4693

Access the most updated version of this article at http://www.jbc.org/content/271/9/4693

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 33 references, 7 of which can be accessed free at http://www.jbc.org/content/271/9/4693.full.html#ref-list-1