Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 4927–4931, July 1989
Biochemistry

Mutation at position 791 in *Escherichia coli* 16S ribosomal RNA affects processes involved in the initiation of protein synthesis

(initiation factor 3/subunit association/site-directed mutagenesis)

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Communicated by Bernard Davis, April 13, 1989 (received for review February 15, 1989)

ABSTRACT **A single base was mutated from guanine to adenine at position 791 in 16S rRNA in the *Escherichia coli rrnB* operon on the multicopy plasmid pKK3535. The plasmid-coded rRNA was processed and assembled into 30S ribosomal subunits in *E. coli* and caused a retardation of cell growth. The mutation affected crucial functional roles of the 30S subunit in the initiation of protein synthesis. The affinity of the mutant 30S subunits for 50S subunits was reduced and the association equilibrium constant for initiation factor 3 was decreased by a factor of 10 compared to wild-type 30S subunits. The interrelationship among the region of residue 790 in 16S rRNA, subunit association, and initiation factor 3 binding during initiation complex formation, as revealed by this study, offers insights into the functional role of rRNA in protein synthesis.**

Several regions of rRNA have been shown to be involved in the process of translation, primarily regions that are single stranded and highly conserved phylogenetically (1, 2). The locations of these sequences in *Escherichia coli* 16S rRNA have now been placed in three-dimensional models of the 30S ribosomal subunit (3, 4). In general there is good agreement between structure and function since many single-stranded highly conserved rRNA regions, proposed to carry out related functions in translation, are clustered in the same area of the subunit (5).

One example of a region with a structural placement consistent with its proposed function is the 790 loop of 16S rRNA. In this 9-base loop in the central domain of 16S rRNA, 6 of the bases are universally conserved and 2 additional bases are strongly but not universally conserved (6). The loop has been localized to the platform of the subunit in the area that interfaces with the 50S subunit (3, 4, 7). Several studies have established that many of the bases in the 790 loop are exposed in 30S subunits and inaccessible in 70S ribosomes (8–13). Some of these same studies have implicated this region in the process of subunit association (9–11).

The chemical modification studies by Moazed and Noller (13, 14), in which rRNA accessibilities were probed in the presence of tRNA or antibiotics, showed that the 790 loop influences several aspects of protein synthesis aside from subunit association. To further define its functional role, the universally conserved guanine at position 791 was mutated to an adenine by site-directed mutagenesis. Here we report on the structural and functional characteristics of the resulting mutant 30S subunits.

MATERIALS AND METHODS

Mutagenesis and Expression. The single-base mutation at position 791 was constructed by oligonucleotide-directed mutagenesis in M13 as described by Zoller and Smith (15). The *Eco*RI–*Xba* I fragment from *rrnB* was cloned into M13mp19 and the template was isolated from the *ung*$^-$ *dut*$^-$ strain RZ1032 to increase the frequency of mutagenesis (16). After mutagenesis M13 isolates were screened by DNA sequencing (17) and the *Bgl* II–*Xba* I fragment containing the mutation was cloned into the expression vector pKK3535 (18) and transformed into *E. coli* strain HB101. The mutation in HB101 transformants was confirmed by backcloning into M13 and sequencing.

The level of expression of mutant 16S rRNA was determined by sequencing total cellular RNA by using reverse transcriptase (13) and scanning the autoradiograms. The same analysis was used to monitor the incorporation of mutant rRNA into 30S subunits, 70S ribosomes, and polyribosomes.

Maxicells. Transcripts from wild-type and mutant plasmids were labeled specifically with ^{32}P in maxicells as described (19). Ribosomal particles and rRNA were analyzed by agarose/acrylamide composite gel electrophoresis (20).

Ribosomes and Ribosomal Subunits. Plasmid-containing cells growing in LB medium with ampicillin (200 μg/ml) were harvested in the logarithmic growth phase (OD_{600} = 0.6) and the cells were broken by grinding with alumina in 20 mM Tris·HCl, pH 7.6/100 mM KCl/10 mM $MgCl_2$/1 mM dithiothreitol (DTT). Alumina and cell debris were cleared by consecutive centrifugations at 10,000 and 16,000 × *g* for 15 min and 90 min, respectively. Ribosomes were pelleted from the cleared supernatant by centrifugation at 50,000 rpm for 3 hr at 4°C in a Beckman 60 Ti rotor. Ribosomal pellets were resuspended in 20 mM Tris·HCl, pH 7.6/0.5 M NH_4Cl/10 mM $MgCl_2$/1 mM DTT, cleared of insoluble material by low-speed centrifugation, repelleted, redissolved, and then centrifuged for 16 hr at 35,000 rpm in a 60 Ti rotor through a 20-ml 10%/30% (wt/vol) sucrose cushion in the same buffer. The final ribosome pellets were dissolved in 20 mM Tris·HCl, pH 7.6/60 mM KCl/6 mM $MgCl_2$/1 mM DTT and stored frozen in small aliquots at −70°C. Tightly coupled 70S ribosomes and free subunits were isolated by fractionating a 5–30% sucrose gradient that was centrifuged for 14 hr at 20,000 rpm in a Beckman SW 28 rotor. The ribosomal particles were recovered from the pooled fractions by pelleting for 16 hr at 40,000 rpm in a Beckman 60 Ti rotor.

Polyribosomes were prepared by the procedure of Godson and Sinsheimer (21) and fractionated on a 10–35% sucrose gradient in 25 mM Tris·HCl, pH 7.6/60 mM NH_4Cl/10 mM $MgCl_2$/1 mM DTT by centrifugation for 8 hr at 20,000 rpm in a Beckman 28 SW rotor.

RNA Sequencing. The sequence of rRNA, isolated by phenol extraction of ribosomal particles or cell lysates, was determined by primer extension using reverse transcriptase (13, 22). The annealing mixture (10 μl), containing 5 pmol of rRNA and 2.5 pmol of a 17-base primer in 50 mM Tris·HCl, pH 8/100 mM KCl/20 mM $MgCl_2$, was heated at 90°C for 30

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Abbreviations: IF-3, initiation factor 3; DTT, dithiothreitol.

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sec and then 50°C for 20 min. The annealing mixture was divided into four aliquots and added to an extension mixture. The final concentrations in the 10-μl extension mixtures were 0.2 mM dCTP, 0.2 mM dGTP, 0.2 mM dTTP, 0.25 μCi ^{35}S-labeled dATP (500 Ci/mmol; 1 Ci = 37 GBq), 50 mM Tris·HCl (pH 8), 125 mM KCl, 25 mM $MgCl_2$, 10 mM DTT, and 1 unit of reverse transcriptase (Promega). Each of the four extension mixtures also contained one of the following dideoxy (dd) nucleoside triphosphates: 0.2 mM ddATP, 0.066 mM ddCTP, 0.033 mM ddGTP, or 0.2 mM ddTTP. The extension reactions were incubated for 20 min at 42°C and then for another 20 min after the addition of 2 μl of chase solution [50 mM Tris·HCl, pH 8/2 mM DTT/10% (vol/vol) glycerol/dNTPs at 1.2 mM each]. The extension reactions were stopped by the addition of 5 μl of 98% (vol/vol) formamide/10 mM EDTA/0.2% bromphenol blue/0.2% xylene cyanol. Samples were electrophoresed on an 8% sequencing gel. Autoradiograms of the sequencing gels were scanned using an LKB Ultroscan XL laser densitometer. The relative intensities of the bands from wild-type and mutant tracks were compared to determine the ratio of wild-type and mutant rRNA.

***In Vitro* Protein Synthesis.** Ribosomes were assayed in cell-free translation systems utilizing the synthetic mRNA poly(U) (23) and natural mRNA transcribed from plasmid DNA. The transcription/translation system was modified from Kaltschmidt *et al.* (24) using pBR322 as a DNA template. The S100 enzyme fraction and the initiation factor fraction for both the poly(U) and transcription/translation systems were prepared according to Traub *et al.* (23).

Initiation Factor 3 (IF-3) Binding. IF-3 was fluorescently labeled with fluorescein isothiocyanate (Molecular Probes). The protein was incubated with a 5-fold molar excess of dye for 30 min at 4°C in 50 mM Tris·HCl, pH 7.5/50 mM KCl/0.5 M NH_4Cl/5% glycerol. The total volume was 50 μl. The sample was applied to a 1-ml Sephadex G-10 column equilibrated with the same buffer to separate free dye. The molar ratio of fluorescein to protein was determined by using $E_{490} = 8.51 \times 10^4\ cm^{-1} \cdot M^{-1}$ for bound fluorescein and the protein concentration assay of Bradford (25). The dye/protein ratio was 0.87. Experiments were performed in buffer 1 (10 mM Tris·HCl, pH 7.8/50 mM KCl/6 mM 2-mercaptoethanol) containing $MgCl_2$ as indicated.

Static Experiments. The effect of labeled IF-3 on wild-type ribosomal subunit equilibrium was determined by light-scattering as described (26). Monitoring fluorescence intensity changes when labeled IF-3 bound to ribosomes was used to determine values of the equilibrium constant for the IF-3–ribosome interaction. IF-3 (0.05 μM) was titrated with increasing concentrations of ribosomes in buffer 1 containing 1 mM $MgCl_2$. Fluorescence exitation was at 490 nm and emission was monitored at 520 nm. Data were fit by a Fletcher–Powell (27) sum of squares minimization routine according to a simple single-equilibrium step. Equations for fitting such a fluorescence intensity change are given elsewhere (28). Errors in the parameters were obtained from the variance–covariance matrix.

Kinetic Experiments. Association and dissociation rate constants were determined by changes in fluorescence intensity. Experiments were performed using a SF-51 stopped-flow apparatus (Hi-Tech Scientific, Wiltshire, U.K.). The light source for the stopped-flow was a 200-W Hg–Xe lamp. Exitation was at 490 nm and emission was monitored using a cut-off filter that transmitted wavelengths above 510 nm. Data collection, which allowed averaging of replicate runs, was with a Zenith Z-100 computer. Averages of a minimum of four replicate runs were used to determine the rate constants reported.

For association experiments, the 30S subunits (0.12 μM) were mixed in the stopped-flow apparatus with an equal volume of IF-3 (0.09 μM). Both samples were in buffer 1 containing 1 mM $MgCl_2$. Data were fit to a simple second-order association reaction as described (26). Dissocation reactions were monitored by dilution experiments in the stopped-flow. Unequal driving syringes were used to give a 1:6 dilution. The IF-3–30S complex (0.32 μM) was mixed with buffer 1 containing 1 mM $MgCl_2$. The data were fit to a relaxation reaction, 30S–IF-3 $\rightleftarrows$ 30S + IF-3. Control experiments and data analysis were as described (29) for eukaryotic IF-3 binding to 40S subunits.

RESULTS

Construction and Expression of Mutant rRNA. The single-base mutation, guanine to adenine at position 791 in 16S rRNA, was produced by oligonucleotide-directed mutagenesis and cloned into the expression vector pKK3535 to produce the mutant plasmid pKK791A. The plasmid was transformed into *E. coli* strain HB101 and the transformed cells grew significantly slower (90-min doubling time) than cells transformed with wild-type plasmid pKK3535 (55-min doubling time). The cellular concentration of plasmid-coded, mutant rRNA was 58% (42% was wild-type from the host *rrn* operons), as determined by primer-extension sequencing of total 16S rRNA by using reverse transcriptase.

Processing of the mutant rRNA and its assembly into 30S subunits were analyzed by specific labeling with ^{32}P in maxicells (19) and electrophoresis in agarose/acrylamide composite gels (20). The mutant 16S rRNA gave the same results as wild-type rRNA, showing no alterations in size, stability, processing, or assembly into 30S-like particles (data not shown). The ribosomal protein compositions of mutant and wild-type 30S subunits were identical as determined by one-dimensional SDS/polyacrylamide gel electrophoresis. Thus there was no evidence of any deficiency in processing or assembly of the mutant rRNA.

Reduced Presence of Mutant 30S Subunits in 70S Ribosomes and Polyribosomes *in Vivo*. The distribution of free subunits and 70S ribosomes in cells containing the mutant and wild-type plasmids was determined by sucrose density gradient centrifugation. Ribosomes were sedimented in sucrose gradients containing 1.5, 6, or 10 mM $MgCl_2$. Virtually all of the ribosomes from cells with the wild-type plasmid were 70S ribosomes at 6 and 10 mM $MgCl_2$. Significant amounts of free subunits appeared only when the magnesium ion concentration was reduced below 6 mM. In contrast ribosomes from cells containing the mutant plasmid displayed free subunits even at 10 mM $MgCl_2$ and the proportion of free subunits remained greater than for wild type as the magnesium ion concentration was reduced. Fig. 1 shows the gradient profiles at 6 mM $MgCl_2$.

Reverse transcriptase sequence analysis of rRNA from the 30S and 70S regions of the gradients in Fig. 1 showed that at 6 mM $MgCl_2$ very little mutant rRNA was associated with 70S ribosomes (17 ± 4% of the 16S rRNA in the 70S pool, representing approximately 15% of the mutant rRNA in the cell). The free 30S subunits were predominantly mutant (87 ± 6%, representing approximately 85% of the mutant rRNA in the cell; Table 1). Analysis of a polyribosome fraction at a higher magnesium ion concentration (10 mM) showed 28 ± 4% of the 16S rRNA was mutant (representing approximately 30% of the mutant rRNA in the cell). It should be noted that reverse transcriptase sequence analysis can be somewhat variable, hence the relatively large error bars. The means and errors reported represent the data from at least three replicate sequence analyses on independent ribosomal samples.

Reduced Affinity of Mutant 30S Subunits for 50S Subunits *in Vitro*. Studies were performed *in vitro* to examine the effect of the mutation on the interaction between 30S and 50S subunits. The association of mutant and wild-type 30S sub-

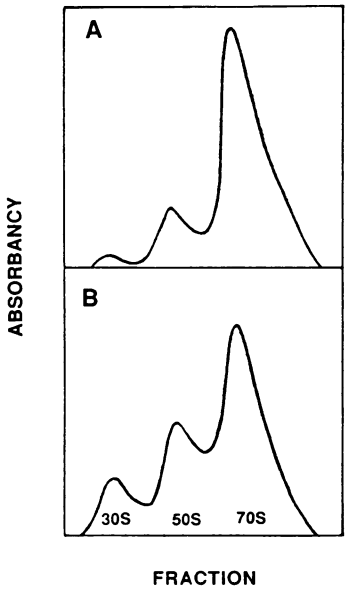

FIG. 1. Sucrose gradient profile of ribosomes from cells containing wild-type and mutant plasmids. Isolated ribosomes (5 mg) were layered onto a 5–30% sucrose gradient in 20 mM Tris·HCl, pH 7.6/60 mM KCl/6 mM $MgCl_2$ and centrifuged for 14 hr at 20,000 rpm in a Beckman SW 28 rotor. The profiles represent the absorbancy (260 nm) of the 70S ribosomes and 50S and 30S subunits of the wild-type (*A*) and the mutant (*B*) ribosomes.

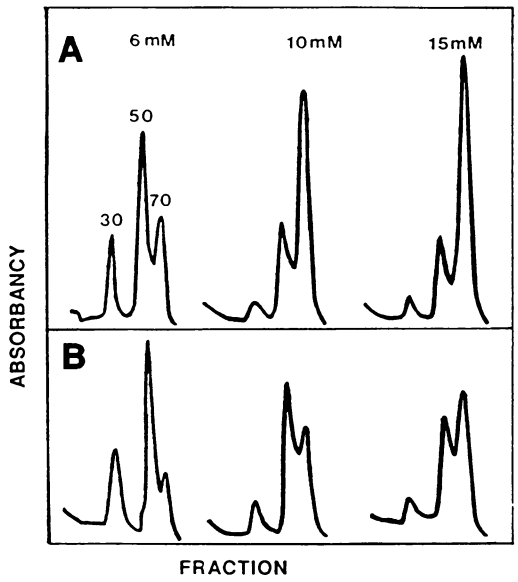

FIG. 2. Subunit association *in vitro*. Isolated wild-type and mutant 30S subunits were incubated with equimolar quantities of 50S subunits (75 pmol of each) at 37°C for 50 min in 20 mM Tris·HCl, pH 7.6/60 mM KCl and $MgCl_2$ as indicated. The mixtures were layered onto a 5–30% sucrose gradient in the same buffer and centrifuged for 4.5 hr at 35,000 rpm in a Beckman SW 41 rotor. The profiles represent the absorbancy (260 nm) for wild-type (*A*) and mutant (*B*) ribosomes.

units with 50S subunits was analyzed by sucrose density gradient centrifugation at various concentrations of magnesium ion. Mutant 30S subunits for these experiments were obtained from the 30S subunit region of a gradient as in Fig. 1 (87% mutant). Equimolar amounts of free 30S and 50S subunits were incubated in buffer containing 6, 10, or 15 mM $MgCl_2$ and then sedimented in sucrose gradients under the same ionic conditions. As shown in Fig. 2*B*, increasing the magnesium ion concentration did increase the incorporation of mutant subunits into 70S ribosomes, but the degree of association remained significantly below that for wild-type subunits (Fig. 2*A*) at all concentrations of $MgCl_2$ tested. These results demonstrate that the mutation at position 791 in 16S rRNA renders the 30S subunits deficient in subunit association, but increasing the concentration of magnesium ion does increase the number of mutant 30S subunits in 70S ribosomes.

***In Vitro* Translation by Mutant Ribosomes.** Ribosomes isolated from cells containing the mutant and wild-type plasmids were tested for protein synthetic activity using both poly(U) and natural mRNA in cell-free translation systems. Translation of natural mRNA by mutant ribosomes (actually 58% mutant and 42% wild type) was markedly reduced as compared to wild-type ribosomes (Fig. 3*A*), even at a rather high concentration of magnesium ion (10 mM). This is consistent with the *in vivo* result that shows about one-third of the mutant ribosomes were functioning (in polyribosomes). An even more striking difference was noted between mutant and wild-type ribosomes in the translation of poly(U) at low (6 mM) magnesium ion concentration (Fig. 3*B*), indicating that no mutant ribosomes were functioning. However, the difference in poly(U) translation disappeared when the magnesium ion concentration was raised to 10 or 15 mM (Fig. 3 *C* and *D*), presumably overcoming the effect of the mutation on subunit association. Although the mutant ribo-

Table 1. Percentage of mutant rRNA in ribosomes isolated from sucrose gradients

Gradient sample	Mg, mM	% mutant rRNA
30S	1.5	58 ± 8
30S	6	87 ± 6
70S	6	17 ± 4
Polyribosomes	10	28 ± 4

Ribosomes isolated from cells containing plasmid pKK791A were separated by sucrose density gradient centrifugation at the $MgCl_2$ concentrations indicated. The percentage of mutant 16S rRNA was determined by primer-extension sequence analysis.

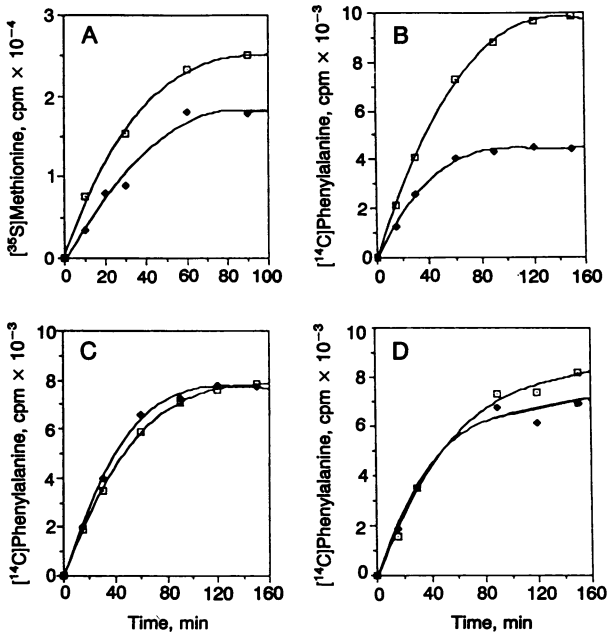

FIG. 3. *In vitro* protein synthesis using natural mRNA and poly(U). The incorporation of labeled amino acids into trichloroacetic acid-precipitable polypeptides by wild-type (□) and mutant (◆) ribosomes was measured in two *in vitro* systems. (*A*) Incorporation of [^{35}S]methionine in a transcription/translation system at 10 mM $MgCl_2$. (*B–D*) Effect of $MgCl_2$ on the incorporation of [^{14}C]phenylalanine in a poly(U)-dependent translation system at 6 mM $MgCl_2$ (*B*), 10 mM $MgCl_2$ (*C*), and 15 mM $MgCl_2$ (*D*).

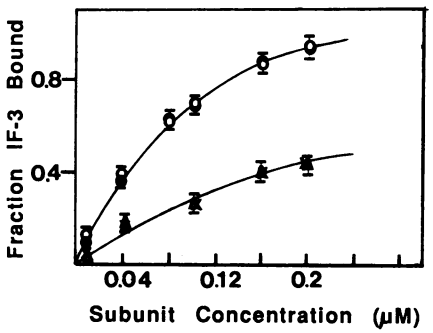

FIG. 4. Equilibrium binding of IF-3 to wild-type and mutant 30S subunits. The binding of IF-3 was monitored by changes in fluorescence intensity for fluorescein-labeled IF-3. The IF-3 concentration was 0.05 μM and the 30S concentration was as indicated. Buffer 1 containing 1 mM $MgCl_2$ was used. For each curve, two separate preparations of wild-type (●, ○) and mutant (x, △) subunits were used. For each data point shown, three samples were measured and averaged.

somes translated poly(U) well at 10 and 15 mM $MgCl_2$, they were deficient in translating natural mRNA under the same ionic conditions. This indicated that there was a second effect of the mutation at initiation in addition to subunit association. Given the relationship between IF-3 and subunit association, the effect of the mutation on IF-3 binding was investigated.

IF-3 Binding Studies. Since mutant ribosomes were shown to have reduced protein synthetic activity even when the altered subunit interaction was corrected by increasing the magnesium ion concentration, the binding of IF-3 was examined. Fluorescein-labeled IF-3 was tested with wild-type ribosomes and showed effects on the subunit association rate and equilibrium consistent with those reported earlier (26). In addition, unlabeled IF-3 was an effective competitor for ribosome binding, indicating the labeled factor was functional and bound to the same ribosomal binding site as native IF-3.

Fig. 4 shows the static titration curves for IF-3 binding to mutant and wild-type 30S subunits. For these experiments the mutant 30S subunits were greatly enriched for the position 791 mutation (87%) as described above. The affinity of IF-3 for mutant 30S subunits was decreased by a factor of 10 compared to wild-type subunits (Fig. 4 and Table 2). This difference in affinity resided mainly in the dissociation rate constant. The IF-3 association rate constants for mutant and wild-type subunits were the same within experimental error.

DISCUSSION

The compilation of rRNA sequences from a wide range of organisms has shown that nucleotides at certain positions are universally conserved (6). For the most part these nucleotides occur in single-stranded regions that are exposed on the surface of free subunits. Not surprisingly these same nucleotides repeatedly emerge as bases that are crucial to the function of the ribosome. The guanine at position 791 of *E. coli* 16S rRNA belongs to this group of bases. A number of functional activities have been indicated for G-791 (10, 11, 14, 30), and thus it was not unexpected that the single-base mutation at position 791 conferred a slow-growth phenotype on cells containing the mutant RNA.

Table 2. IF-3 binding to mutant and wild-type 30S subunits

30S subunit	k_1, $M^{-1}s^{-1}$ ($\times 10^{-6}$)	k_{-1}, s^{-1}	K_{eq}, M^{-1} ($\times 10^{-6}$)
Wild type	7.9 ± 0.5	0.44 ± 0.09	18 ± 4
Mutant	8.1 ± 0.5	4.0 ± 0.2	2.0 ± 0.3

Values of K_{eq} are calculated from the data in Fig. 4.

The guanine to adenine mutation at position 791 did not hamper the processing or assembly of rRNA into subunits, nor was elongation affected in an *in vitro* poly(U) translation system at 10 or 15 mM $MgCl_2$. Instead the primary effect of the mutation was on the initiation of protein synthesis. The maxicell analysis indicated that the mutant 30S subunits were deficient in subunit association and this was confirmed both by measuring the distribution of mutant subunits in 30S, 70S, and polyribosome fractions and by subunit association studies over a range of magnesium ion concentrations. These results are consistent with the findings using chemical (10) and oligonucleotide (11) probes that bases in the region of position 790 are directly involved in subunit association. The guanine to adenine mutation at position 791 creates three consecutive adenine residues (positions 790–792) that may stack differently than the wild-type AGA, distorting the loop structure sufficiently to alter its recognition by and association with 50S subunits. A sequence of six bases in 23S rRNA (positions 2753–2758) is complementary to the region of position 790 in 16S rRNA (positions 788–793) and has been proposed as a potential site for a 16S–23S rRNA base-pair interaction during subunit association (10). A compensatory mutation at position 2755 in 23S rRNA to restore this potential base pairing (and presumably subunit association) could be used to test this proposal.

The fact that mutant ribosomes efficiently translated poly(U) but not natural mRNA at high magnesium ion concentrations *in vitro* indicated that the mutation affected an initiation function in addition to subunit association. Indeed when mutant subunits were tested for IF-3 binding, it was shown that the affinity of IF-3 for mutant 30S subunits was reduced by a factor of 10 compared to wild-type subunits. The initial interaction between IF-3 and mutant subunits was not altered by the mutation at position 791 since the on-rate of IF-3 was identical for both mutant and wild-type subunits. Rather the 10-fold decrease in the association constant for mutant subunits was due to a 10-fold increase in the off-rate of IF-3. This indicates that the factor recognizes a specific structure on 30S subunits that includes G-791 to stabilize its interaction, but it does not necessarily imply that IF-3 binds directly to residue 791. Although direct IF-3–residue 791 interaction is the most straightforward interpretation, long-range conformational perturbations induced by the mutation could lead to the same results. Previous findings support multiple recognition sites for IF-3 on the 30S subunit since the factor interacts with the 3′-terminal colicin fragment of 16S rRNA (31) and with 30S subunits depleted of the colicin fragment (32). Two discrete crosslinks between IF-3 and 16S rRNA *in situ* are in agreement with these findings (33–35). Apparently IF-3 can interact with either binding site initially but requires a specific set of recognition signals, including G-791, to form a stable complex.

The proximity of IF-3 to certain ribosomal proteins (36) and rRNA (33–35) as well as electron microscopic visualization of the factor on the 30S subunit (37) allow for fairly precise localization of the binding site for IF-3 on the platform side of the cleft of the 30S subunit. The tertiary structure model for the arrangement of rRNA in the 30S subunit (3) is consistent with this localization, since it places the 3′-end crosslink region (33–35), the crosslink region of position 840 (35), and the region of position 791 of this study in the same general area of the 30S subunit. The clustering of these rRNA regions on the platform at the interface between 30S and 50S subunits is consistent with the functional relationships between IF-3 and 30S subunits during initiation complex formation. One function of IF-3 is to stimulate the interaction of mRNA and fMet-tRNA with the 30S subunit (38). The relationship between mRNA and the 3′ end of 16S mRNA is well known (39–41). It is also interesting to note that G-791 is the only nucleotide in 16S rRNA that is protected from

chemical probes when the synthetic mRNA poly(U) is bound to the ribosome (13). A second function of IF-3 is that of a ribosome anti-association factor with the capability of inhibiting the association of 30S subunits with 50S subunits until the 30S initiation complex is assembled (42–44). Nucleotides in the region of residue 790 in 16S rRNA are involved in subunit association (10, 11). It is possible that IF-3 controls subunit association by altering the accessibility of the 790 loop in free 30S subunits. One might expect that a mutation that alters IF-3 binding would result in an increase rather than the observed decrease of mutant subunits in 70S ribosomes. However, the fact that the mutation also reduces subunit association probably accounts for the absence of a large pool of mutant 70S ribosomes.

The 790 loop region of 16S rRNA is involved in a number of complex functions during protein synthesis. In this study we have shown that a single-base change at position 791 resulted in a deficiency in subunit association and IF-3 binding. The accessibility of G-791 to chemical modification is affected when poly(U) (13) or 50S subunits (10) bind to the 30S subunit. Neighboring bases such as A-790 are also protected from chemical probes by 50S subunits or by tRNA binding in free subunits, and A-794 and A-795 are protected by mRNA-dependent tRNA binding in both 30S subunits and 70S ribosomes (13). Interestingly A-792, unlike most of its neighbors, is not affected by 50S subunits or tRNA. Furthermore, a transition mutation (adenine to guanine) at this site produced no detectable effect on cell growth or subunit association. However, a transversion mutation (adenine to cytosine) did retard cell growth 20% and affected protein synthesis but it also had no effect on subunit association (M. Santer, personal communication). Thus although the 790 loop is a very active functional site in the 30S subunit, we are still somewhat removed from understanding its fine structure and function in detail.

We gratefully acknowledge John Hershey for generously providing IF-3. We thank Melvin Santer for his guidance and inspiration and for providing unpublished data. Helpful contributions were provided by H. U. Göringer, Elizabeth De Stasio, David Jemiolo, Jill Thompson, Catherine Prescott, Matthew Firpo, and George Q. Pennabble. This work was supported by grants from the National Institutes of Health (GM19756 to A.E.D.), the National Science Foundation (86007070 to D.J.G.), and an American Heart Association–New York City Investigatorship (D.J.G.).

1. Noller, H. F. (1984) *Annu. Rev. Biochem.* **53,** 119–162.
2. Brimacombe, R. & Steige, W. (1985) *Biochem. J.* **229,** 1–17.
3. Brimacombe, R., Atmadja, J., Steige, W. & Schüler, D. (1988) *J. Mol. Biol.* **199,** 115–136.
4. Noller, H. F., Stern, S., Moazed, D., Powers, T., Svensson, P. & Changchien, L.-M. (1987) *Cold Spring Harbor Symp. Quant. Biol.* **52,** 695–708.
5. Steige, W., Stade, K., Schüler, D. & Brimacombe, R. (1988) *Nucleic Acids Res.* **16,** 2369–2388.
6. Gutell, R. R., Weiser, B., Woese, C. R. & Noller, H. F. (1985) *Prog. Nucleic Acid Res. Mol. Biol.* **32,** 155–216.
7. Oakes, M., Scheinman, A., Rivera, M., Soufer, D., Shankweiler, G. & Lake, J. (1987) *Cold Spring Harbor Symp. Quant. Biol.* **52,** 675–686.
8. Santer, M. & Shane, S. (1977) *J. Bacteriol.* **130,** 900–910.
9. Chapman, N. M. & Noller, H. F. (1977) *J. Mol. Biol.* **109,** 131–149.
10. Herr, W., Chapman, N. M. & Noller, H. F. (1979) *J. Mol. Biol.* **130,** 433–449.
11. Tapprich, W. E. & Hill, W. E. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 556–560.
12. Moazed, D., Stern, S. & Noller, H. F. (1986) *J. Mol. Biol.* **187,** 399–416.
13. Moazed, D. & Noller, H. F. (1986) *Cell* **47,** 985–994.
14. Moazed, D. & Noller, H. F. (1987) *Nature (London)* **327,** 389–394.
15. Zoller, M. J. & Smith, M. (1982) *Nucleic Acids Res.* **10,** 6487–6500.
16. Kunkel, T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 488–492.
17. Sanger, F., Nicklen, S. & Coulsen, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
18. Brosius, J., Ullrich, A., Raker, M. A., Gray, A., Dull, T. J., Gutell, R. R. & Noller, H. F. (1981) *Plasmid* **6,** 112–118.
19. Stark, M. J. R., Gourse, R. L. & Dahlberg, A. E. (1982) *J. Mol. Biol.* **159,** 417–439.
20. Dahlberg, A. E., Dingman, C. W. & Peacock, A. E. (1969) *J. Mol. Biol.* **41,** 139–147.
21. Godson, G. N. & Sinsheimer, R. L. (1967) *Biochim. Biophys. Acta* **149,** 489–495.
22. Vester, B. & Garrett, R. A. (1988) *EMBO J.* **7,** 3577–3587.
23. Traub, P., Mizushima, S., Lowry, V. & Nomura, M. (1971) *Methods Enzymol.* **20,** 391–407.
24. Kaltschmidt, E., Kahan, L. & Nomura, M. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 446–450.
25. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
26. Goss, D. J., Parkhurst, L. J. & Wahba, A. J. (1982) *J. Biol. Chem.* **257,** 10119–10127.
27. Fletcher, R. & Powell, M. J. D. (1963) *Comput. J.* **6,** 163.
28. Goss, D. J., Parkhurst, L. J., Mehta, A. M. & Wahba, A. J. (1984) *Biochemistry* **23,** 6522–6529.
29. Goss, D. J. & Rounds, D. J. (1988) *Biochemistry* **27,** 3610–3612.
30. Moazed, D., Van Stolk, B. J., Douthwaite, S. & Noller, H. F. (1986) *J. Mol. Biol.* **191,** 483–493.
31. Wickstrom, E. (1983) *Nucleic Acids Res.* **11,** 2035–2052.
32. Laughrea, M., Dondon, J. & Grunberg-Manago, M. (1978) *FEBS Lett.* **91,** 265–268.
33. Van Duin, J., Kurland, C. G., Dondon, J. & Grunberg-Manago, M. (1975) *FEBS Lett.* **59,** 287–290.
34. Pon, C. L., Brimacombe, R. & Gualerzi, C. (1977) *Biochemistry* **16,** 5681–5686.
35. Ehresmann, C., Moine, H., Mougel, M., Dondon, J., Grunberg-Manago, M., Ebel, J. P. & Ehressmann, B. (1986) *Nucleic Acids Res.* **14,** 4803–4821.
36. MacKeen, L. A., Kahan, L., Wahba, A. J. & Schwartz, I. (1980) *J. Biol. Chem.* **255,** 10526–10531.
37. Stöffler, G., Bald, R., Kastner, B., Lührmann, R., Stöffler-Meilicke, M. & Tishendorf, G. (1980) in *Ribosomes: Structure, Function and Genetics*, eds. Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L. & Nomura, M. (University Park Press, Baltimore), pp. 171–205.
38. Gualerzi, C. O., Pon, C. L., Pawlik, R. T., Canonaco, M. A., Paci, M. & Wintermeyer, W. (1986) in *Structure, Function, and Genetics of Ribosomes*, eds. Hardesty, B. & Kramer, G. (Springer, New York), pp. 621–641.
39. Shine, J. & Dalgarno, L. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1342–1346.
40. Jacob, W. F., Santer, M. & Dahlberg, A. E. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4757–4761.
41. Hui, A. & deBoer, H. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4762–4766.
42. Subramanian, A. R. & Davis, B. D. (1970) *Nature (London)* **228,** 1273.
43. Godefrey-Colburn, T., Wolfe, A. D., Dondon, J., Grunberg-Manago, M., Dessen, P. & Pantaloni, D. (1975) *J. Mol. Biol.* **94,** 461–487.
44. Grunberg-Manago, M. & Gros, F. (1977) *Prog. Nucleic Acid Res. Mol. Biol.* **20,** 209–284.