The involvement of base 1054 in 16S rRNA for UGA stop codon dependent translational termination

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ABSTRACT

The deletion of the highly conserved cytidine nucleotide at position 1054 in *E. coli* 16S rRNA has been characterized to confer an UGA stop codon specific suppression activity which suggested a functional participation of small subunit rRNA in translational termination. Based on this structure-function correlation we constructed the three point mutations at site 1054, changing the wild-type C residue to an A, G or U base. The mutations were expressed from a complete plasmid encoded rRNA operon (*rrnB*) using a conditional expression system with the lambda P_L- promoter. All three altered 16S rRNA molecules were expressed and incorporated into 70S ribosomal particles. Structural analysis of the protein and 16S rRNA moieties of the mutant ribosomes showed no differences when compared to wild-type particles. The phenotypic analysis revealed that only the 1054G base change led to a significantly reduced generation time of transformed cells, which could be correlated with the inability of the mutant ribosomes to specifically stop at UGA stop codons *in vivo*. The response towards UAA and UAG termination codons was not altered. Furthermore, *in vitro* RF-2 termination factor binding experiments indicated that the association behaviour of mutant ribosomes was not changed, enforcing the view that the UGA stop codon suppression is a direct consequence of the rRNA mutation. Taken together, these results argue for a direct participation of that 16S rRNA motif in UGA dependent translational termination and furthermore, suggest that termination factor binding and stop codon recognition are two separate steps of the termination event.

INTRODUCTION

The participation of ribosomal RNA motifs in the intrinsic function of the translational particle is a well documented phenomenon (1). Ribosomal RNA domains are known to be involved in the initiation and elongation steps of protein biosynthesis and more recently, structural motifs in small subunit rRNA could be identified to participate in UGA and UAA dependent translational termination (2, 3, 4, 5). The rRNA termination domains are located in highly conserved sequence regions, such as the 530 domain (*E. coli* numbering) for UAA dependent termination and helix 34 (helix numbering according to Maly and Brimacombe (6)) for UGA dependent termination. The latter secondary structural element specifies a high degree of molecular flexibility (7) which is clearly in line with the paradigm of a translating particle that functions via structural changes within specific ribosomal domains in a highly cooperative manner.

A mechanistic attempt to explain the involvement of 16S rRNA sequences in UGA dependent termination was primarily based on the study of a deletion mutant at cytidine 1054 within helix 34 (2) and led to a hypothetical 'base pairing' model (3). This model describes the interaction of two tandem UCA triplets (bases 1199–1204) juxtaposed of the 1054 site to interact with the UGA stop codon on the mRNA in an antiparallel fashion, thus forming an intermolecular stem structure between mRNA and 16S rRNA. To examine the specific involvement of base 1054 in translational termination directly, we decided to complement the already existing deletion mutant with the three other possible base changes at that site and study the behaviour of altered ribosomes in translational termination.

Here we show that the three altered rRNAs are expressed in transformed cells and are incorporated into 70S ribosomes. The three different base changes cause no structural perturbation within the steady state conformation of helix 34 and the mutant ribosomes contain a full complement of ribosomal proteins bound to the rRNA in a manner indistinguishable from wild-type. Upon induction of mutant rRNA transcription, only cells containing the 1054G mutant plasmid show a significantly reduced cell generation time which is due to a specific suppression of UGA stop codons, as demonstrated *in vivo*. Neither the specificity for UAA and UAG stop signals is altered, nor is the *in vitro* stop codon dependent binding of RF-2 termination factor. Together, these results are entirely consistent with the suggestion that helix 34 in 16S rRNA is involved in UGA dependent translational termination and, that the availability and structural flexibility of the helix 34 stem structure is a key step in the onset of the termination event.

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MATERIALS AND METHODS

Bacterial strains and plasmids

All bacterial strains used in this study were *E. coli* K12 derivatives. Strain XL1-Blue (8) was used as the host for phage M13 and its derivatives. The bacteria used to assay readthrough levels of the nonsense codons were derivatives of strain KL16 *Hfr thi-1 relA1 spoT1* (9) and have the following pertinent genotypes: DEV1−KL16 *lacZ105* (UAG), DEV14−KL16 *lacZ659* (UAA) and DEV15−KL16 *lacZ* (UGA). Strain CJ236-*dut-1, ung-1, thi-1, relA-1* with plasmid pCJ105 (Cm^r) was used for the mutagenesis procedure (10). Strain pop2136 contains the chromosomal cI857 allele and was used as the host for plasmids with the lambda P_L promoter (11). SU1675 with pPY1025 and pKB4 (syn RF-2) is an RF-2 protein overproducing strain.

Plasmid pNO2680 carries the entire *E. coli rrnB* transcriptional unit cloned behind the repressible lambda P_L promoter (12). Mutated derivatives of pNO2680 were designated as pNO292 (C1054 to A), pNO293 (C1054 to G) and pNO294 (C1054 to A). Plasmid DNA preparation and transformations as well as media and other genetic procedures were as described (13).

Site-directed mutagenesis

Site-directed mutagenesis was performed using the mismatched oligodesoxynucleotide technique (14) with the modifications developed by Kunkel et al. (10). The single stranded DNA template was isolated from a recombinant M13 mp11 derivative, containing a 1050 bp EcoRI/XbaI insert comprising the coding region of the 3′-domain of the 16S rRNA cistron. The screening for mutants was done by directly sequencing single stranded DNA isolates from transformants (15). Each mutation was confirmed after back cloning into the plasmid vector by plasmid sequencing, as well as by directly sequencing the cellular 16S rRNA moiety using primer-directed reverse transcriptase reactions (16).

β-Galactosidase activity determination

For assaying readthrough levels of *lacZ*-UGA, -UAA and -UAG, β-galactosidase measurements were performed (17). Whole-cell assays were carried out in a final volume of 1.0ml Z buffer at 28°C and were started by the addition of 0.2ml O-nitro-phenyl-β-D galactopyranoside (4mg/ml). The reaction was terminated by the addition of 0.5ml 1M Na_2CO_3. The assay tubes were centrifuged before reading the A_{420}. The whole cell unit definition is β-gal units = $[10^3 (A_{420})] / [OD_{600} \times \text{time (min)} \times \text{vol. (ml)}]$.

Preparation of ribosomes and RNA

70S tight couple ribosomes were isolated from transformed bacteria usually 2 or 5 hours after induction of episomal transcription (19). Ribosomal RNA was extracted from 70S ribosomes by 3 consecutive phenol extractions followed by ethanol precipitation. Total RNA isolates were prepared from lysozyme pretreated cells in a detergent mix by freeze-cracking (20).

Analysis of ribosomal proteins

Ribosomal proteins were extracted from 70S ribosomes by acetic acid extraction (21) and separated on two dimensional gels (22). The gels were stained with Coomassie Brilliant Blue and the intensities of the separated protein spots were quantified by 2D-laser densitometry.

RF-2 protein isolation

RF-2 termination factor was isolated from the overproducing strain SU1675 pPY1025 pKB4 (syn RF-2). Upon induction of

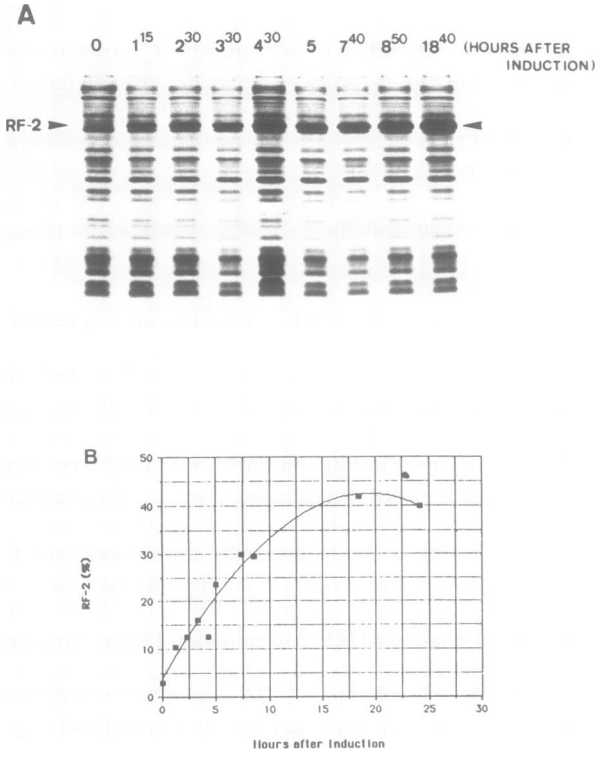

Figure 1. RF-2 termination factor overproduction in *E. coli* SU1675 pPY1025 pKB4 (syn RF-2), kindly provided by R. Weiss and L. Wang. (A) One dimensional gel separation of cell lysates at various time points after induction of episomal transcription. The protein band that corresponds to the release factor protein is marked with an arrow head. (B) Results from a densitometric analysis of the induction experiments showing the time course of RF-2 synthesis.

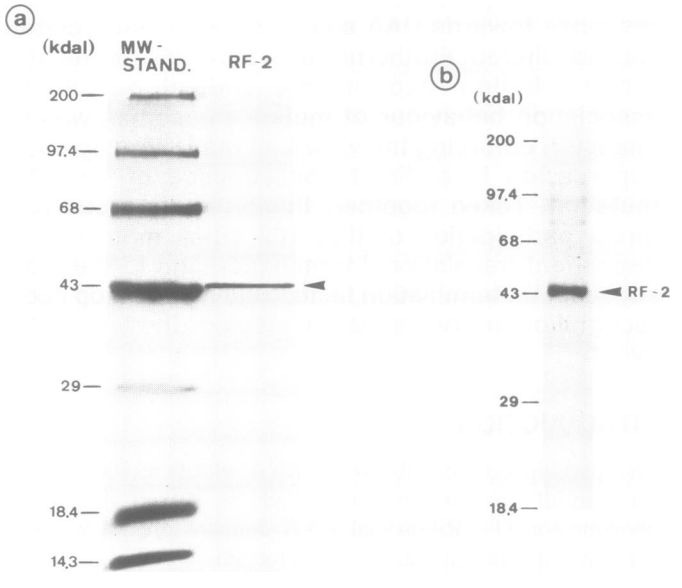

Figure 2. Purification of RF-2 termination factor. Panel (a) shows the one dimensional gel separation of a purified RF-2 protein isolate from the overproducing strain SU1675 pPY1025 pKB4 (syn RF-2). Panel (b) depicts an autoradiograph of an identical separation as above using *in vivo* [^{35}S]-methionine labelled RF-2, used for the termination factor binding studies (see Table 3).

transcription with IPTG the strain produces RF-2 protein up to a maximum of 40% of the soluble protein moiety (Figure 1). Following the isolation procedure of Caskey et al. (23) the termination factor was recovered to >98% purity as judged by electrophoresis on one and two dimensional polyacrylamide gels (see Figure 2a for a 1D separation). The activity of the protein factor was determined as release of Formyl-[^{14}C] methionine from preformed f[^{14}C]Met-tRNA-AUG-ribosome complexes (23). Protein isolates that were induced for more than 7 hours in rich medium or more than 12 hours in minimal medium did not result in an increase of the specific activity although the amount of RF-2 had not reached its maximum level. Apparently factor protein that is produced after that period is no longer functioning properly. Finally, RF-2 isolates were N-terminal sequenced on a gas-phase sequenator (Applied Biosystems, Model 477A) equipped with a Model 120 phenylthiohydantoin (PTH) amino acid analyzer (24).

Radioactively labelled RF-2 protein was isolated from the same strain as described above. Cells were grown in supplemented M9 minimal medium (17) lacking methionine and cysteine and containing 0.1mg/ml ampicillin and 0.05mg/ml spectinomycin for plasmid selection. After growth to 0.5A_{600}, episomal

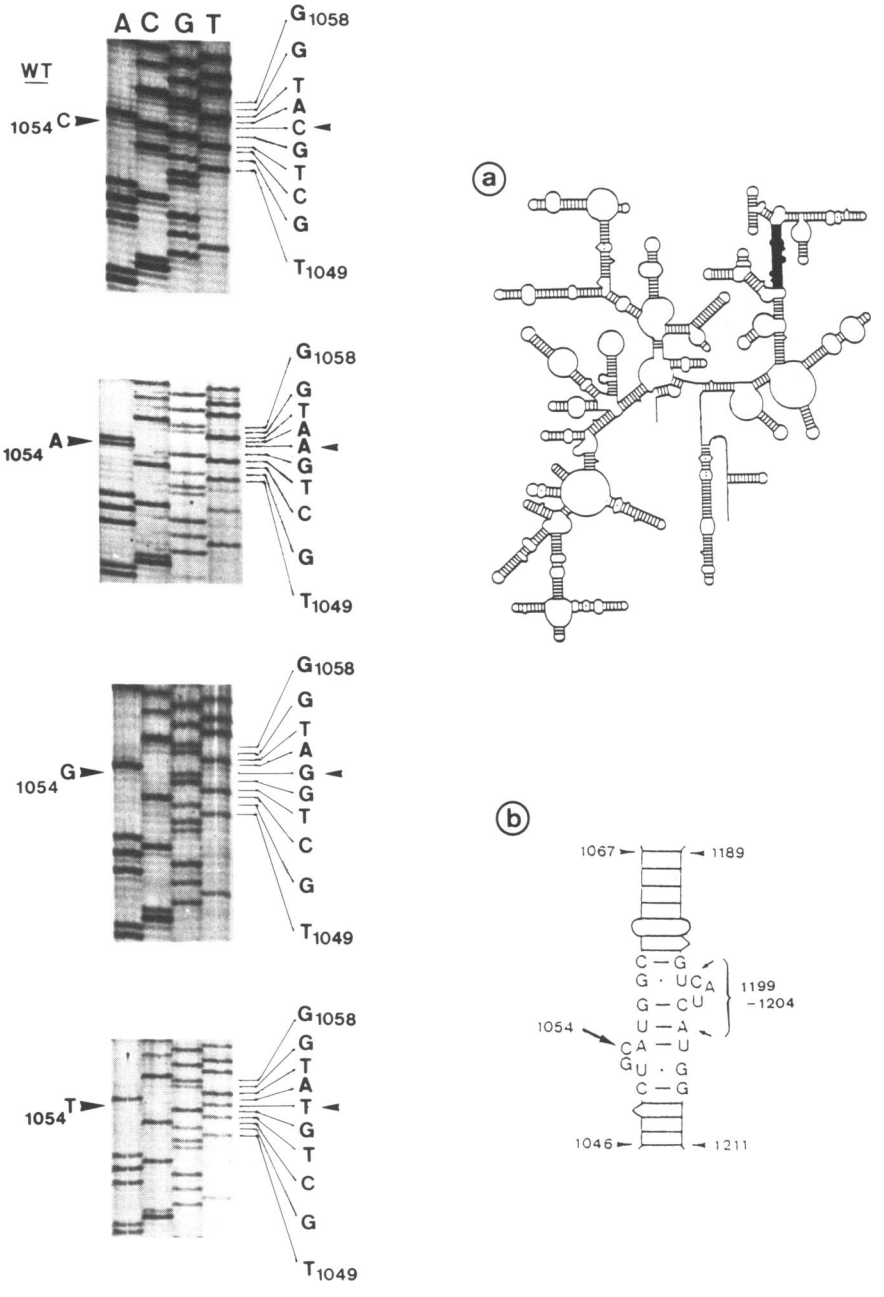

Figure 3. DNA sequence verification of all three base changes at position 1054 in helix 34. Gel autoradiographs from plasmid sequencing experiments (lanes A, C, G, T) are shown for wild-type plasmid pNO2680 (WT) as well as 1054A, 1054G and 1054T mutant plasmids. Altered positions are indicated by arrow heads and relevant nucleotide positions of *E. coli* 16S rRNA are denoted on the right. Panel (a) specifies the current secondary structure model of the entire *E. coli* 16S rRNA molecule (33) with helix 34 shaded in black (Note: The helix numbering system is according to Maly and Brimacombe (6)). Panel (b) details the secondary structure of helix 34 as derived from phylogenetic sequence comparison studies (33). The positions of the 'bulged out' cytidine 1054 and the tandem UCA sequence (bases 1199–1204) are marked by arrows.

transcription was started by adding IPTG to a final concentration of 1.5mM. Following 2 hours of induction [^{35}S]-methionine was added (0.5 mCi/100 ml cell culture) and cells were incubated at 37°C for an additional 10–12 hours. The termination factor was purified from freeze-cracked cell lysates (20) as described (23). The purity of the preparation was routinely checked by one- and two-dimensional polyacrylamide gel electrophoresis (for a 1D separation see Figure 2b). Estimates of the specific activity of the [^{35}S]-RF-2 protein preparations varied from 0.05–1.0μCi/nmole.

Binding of RF-2 to 70S ribosomes

The interaction between 70S ribosomes and RF-2 termination factor was standardly determined in 0.2ml TMK-buffer (20mM Tris-HCl pH 7.5, 20mM $MgCl_2$, 100mM KCl) containing 20–50pmoles heat activated (40°C, 30min) 70S ribosomes, 2.5nmoles termination codon (UGA or UAA) and varying amounts of [^{35}S]-RF-2 protein (2.5–500pmoles) in the presence of 6% (v/v) ethanol (25). Equilibrated termination complexes (0–4°C, 30min) were analyzed by isokinetic centrifugation on linear sucrose gradients (5–30% (w/v), Beckman SW 40 rotor) in TMK-buffer containing 6% (v/v) ethanol at 36000 rpm, 4°C for 4.5 hours. After sedimentation the gradients were fractionated (0.4ml fractions) and both the A_{260} and radioactivity profiles were determined. The recovery of [^{35}S] counts was in the range of 90–95%. Nonspecific binding of labelled RF-2 was determined using either no termination codon or the noncognate UAG triplet. The binding data were finally analyzed by applying a double reciprocal plot analysis.

Table 1. Doubling times of transformants harbouring 1054 mutations

Temp.	WT	1054A	1054G	1054U
30°C	54	51	54	51
42°C	37	40	53	46

Growth behaviour of strain pop2136 transformed with plasmids containing either wild-type or 1054 mutant 16S rRNA genes. Cell doubling times are calculated in minutes from a minimum of 3 independent measurements (18). Relative errors are +/– 10%.

Table 2: Expression of mutant 16S rRNA

Mutant	total RNA (%)	70S ribosomes (%)
1054A	48	63
1054G	40	42
1054U	67	60

The percentage of mutant 16S rRNA within a mixture of wild-type and mutant molecules was determined by primer directed sequencing using AMV reverse transcriptase (16). The relative band intensities for both the wild-type and mutated nucleotide position was assessed by densitometry at 525nm from non-saturated autoradiographs. The values were normalized by comparison to neighboring bands. Relative errors were estimated from a minimum of three different experiments and were in the range of +/–15%. The values were measured 2 hours after induction of transcription.

Chemical modification

Chemical modification of 70S ribosomes was performed according to the general procedure described by Moazed et al. (26). Modified bases were identified by primer extension with reverse transcriptase following gel electrophoresis of the cDNA fragments. Both strands of helix 34 were tested using two different primer molecules (ATTGTAGCACGTGTGTAG; complementary to bases 1223–1240 and CGGGACTTAACCC-AACAT; complementary to bases 1082–1099 in 16S rRNA). Modification was done at 37°C (27) for 30 and 60min with thermo-activated 70S ribosomes (30min, 42°C). Control samples were treated identically throughout, except for the omission of modification reagent. Autoradiographs were finally analyzed by densitometry at 525nm.

RESULTS

Expression of the 1054 mutations

Site directed mutagenesis was used to introduce A, G, and T mutations at position C1054 in helix 34 of the 16S rRNA gene (Figure 3). The mutations were cloned into the *rrnB* operon unit in plasmid pNO2680 (12) where transcription is directed by the repressible lambda P_L-promoter. Mutant plasmids were transformed into strain pop2136 which harbours the cI857 temperature sensitive allele of the cI lambda repressor (11). Cell growth at permissive and nonpermissive temperature was measured in rich medium and the cell doubling times are listed in Table 1. At 30°C all three cell cultures carrying mutant plasmids grew identically to cultures transformed with wild-type plasmid pNO2680. Upon induction of episomal transcription (temperature shift to 42°C) the 1054G and 1054U mutant showed

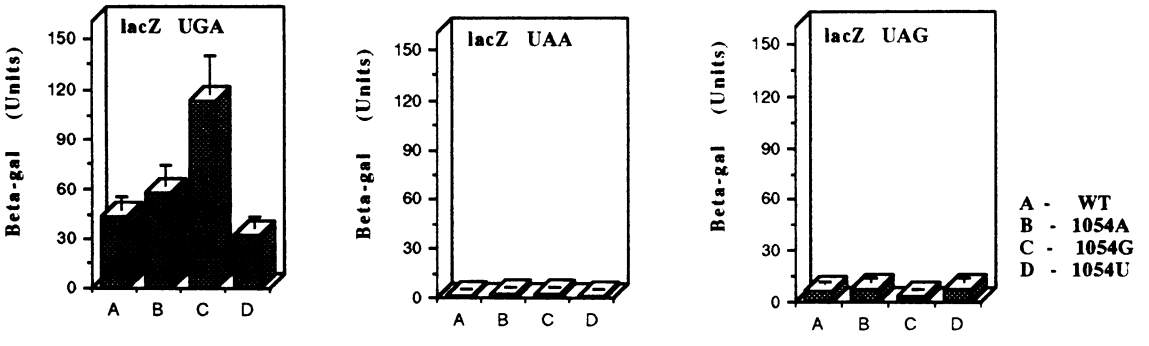

Figure 4. Termination codon suppression of 16S rRNA mutants *in vivo*. The bar graphs represent the synthesis of functional β-galactosidase in the DEV-*lac* Z strains (DEV15; *lac* Z-UGA / DEV14; *lac* Z-UAA / DEV1; *lac* Z-UAG) transformed with either the wild-type plasmid pNO2680 (A) or the mutant derivatives containing 1054A (B), 1054G (C) or 1054U (D) base changes. The enzyme level is dependent on suppression of the specified nonsense codons and is directly correlated to the suppression activity. Readthrough values were normalized according to the actual amount of mutated 16S rRNA present in 70S ribosomes (Table 2) and are the result of 5 independent experiments. β-gal expressions in the absence of IPTG were subtracted. Relative errors were determined as +/– 20% and are indicated as error bars.

a 46% and 24% increase in generation time respectively, indicative of an effect on translation. Revertants or aberrant cell morphologies of the mutant bacteria were not observed (28, 29).

In order to estimate the relative proportions of native and mutated ribosomes in the three mutant strains, we performed primer extension analysis of rRNA isolates from 70S particles. Clearly, upon 2 hours of induction of plasmid transcription, about 40–60% of the 70S ribosomes in the three mutant strains contained altered 16S rRNA molecules (Table 2), which is in good agreement with previous reports studying rRNA mutations and using similar expression systems (30, 31).

Stop Codon Suppression analysis

The behaviour of mutant ribosomes to recognize translational stop signals was analyzed *in vivo* using a system where the particles must translate through a stop codon in order to synthesize functional active β-galactosidase (9). To assay all three termination triplets, bacterial strains transformed with either wild-type plasmid pNO2680 or one of the three mutant derivatives were used. The β-gal activity can be directly correlated with the inability of the ribosomal particles in the cells to participate in a proper translational termination event and therefore is a direct

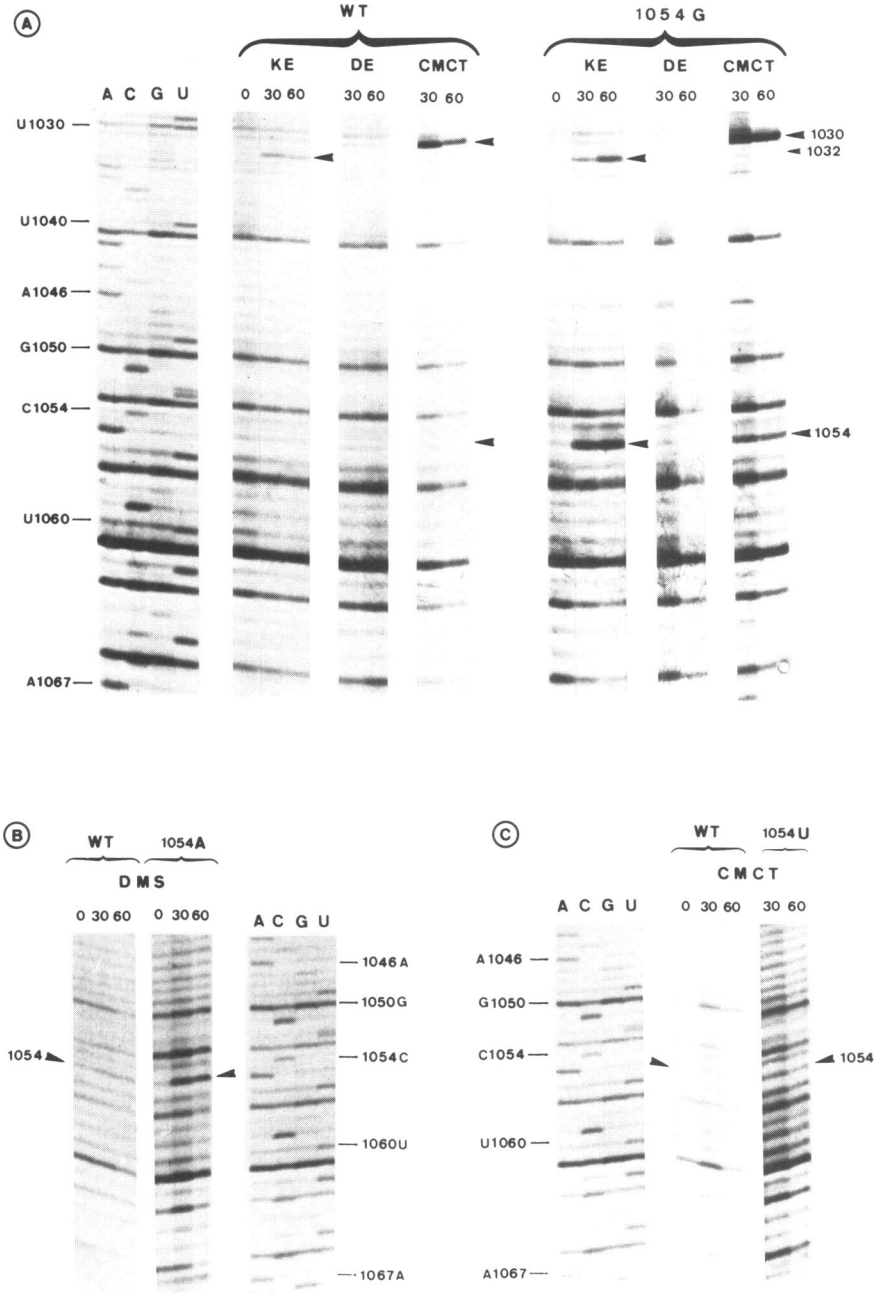

Figure 5. Structure probing of helix 34. 70S ribosomes prepared from pop2136 cells carrying either the plasmid pNO2680 (WT) or the 1054G (panel A), 1054A (panel B) and 1054U (panel C) derivatives were treated with DMS, Kethoxal (KE), DEPC (DE) and CMCT at 37°C for 30 or 60min, as indicated. The samples were deproteinized and subjected to reverse transcription using primer molecules complementary to 16S rRNA nucleotides 1223–1240 or 1082–1099. Sequence positions within the didesoxynucleotide sequencing lanes (A, C, G, U) using unmodified wild-type samples as a template are marked accordingly. Arrow heads and numbers denote reverse transcriptase stops indicating base modifications at sites one nucleotide behind the stop.

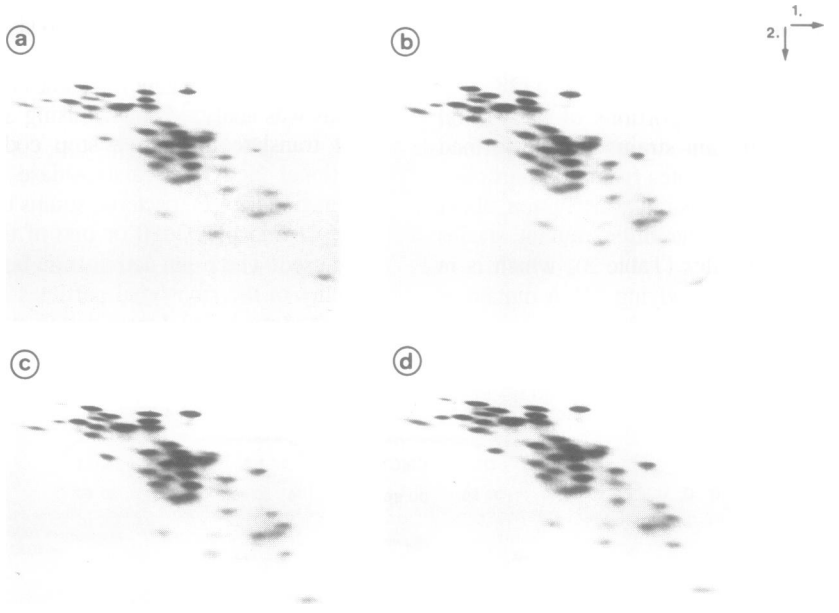

Figure 6. Two dimensional gel electrophoretic separation of ribosomal proteins. The proteins were isolated from strain pop2136 containing either wild-type plasmid pNO2680 (a) or the mutated derivatives 1054A (b), 1054G (c) and 1054U (d), five hours after induction of plasmid transcription. About 45 of the 52 individual ribosomal proteins have been resolved. For a numbering scheme of the various spots see (22).

measurement of the stop codon suppression activity. By sequence analysis, we were able to determine the actual fractions of wild-type and mutant 70S particles in transformed cells (see above), which enabled us to estimate suppression activities for mutant and wild-type ribosomes (Figure 4). Clearly, in the presence of either of the three mutant plasmids, UAA and UAG stop signals were recognized in a manner virtually indistinguishable from cells containing wild-type plasmid. For UGA stop codons however, mutant 70S ribosomes with 1054G 16S rRNA molecules suppress the termination signal by a factor of 2.6 when compared to wild-type ribosomes which were set to a value of 1.0. The 1054A and 1054U transformants gave relative values of 1.3 and 0.7, respectively. Considering the limits of resolution of the assay only the C→G base change can be considered to have a significant effect in this system, compatible with the aforementioned characteristic of that mutation on growth rate. Furthermore, it should be noted that the data additionally demonstrate that the ribosomes are not hampered with regards to translation initiation and elongation.

Structure probing of mutant 70S ribosomes

The higher order structure of helix 34 within the 70S ribosome was probed by chemical modification (26) to determine whether the introduction of the base changes at position 1054 were accompanied by detectable structural alterations within the steady state conformation of helix 34. The four bases were monitored at defined atoms with the following chemicals: Kethoxal, G (N-1, N-2); CMCT, U (N-3) > G (N-2); DEPC, A (N-7) and DMS, G (N-7) > A (N-1) > C (N-3). The pattern of reactivity was visualized following oligonucleotide directed reverse transcription by gel electrophoresis of the cDNA products (Figure 5). As mentioned before, we were using a mixed population of 70S ribosomes containing approximately 50% wild-type and 50% mutant 16S rRNA (see Table 2). The reactivity of base C1054 and the corresponding phosphate group was previously found to be very high in 30S subunits and is maintained upon 70S assembly

Table 3: RF-2 binding to mutant and wild-type 70S ribosomes

70S ribosomes	rel. $K_{app.}$
WT	1.0
1054A	1.1
1054G	1.0
1054U	1.0

Relative apparent association constants (rel. $K_{app.}$) were calculated from a double reciprocal plot analysis of RF-2 binding experiments with 70S ribosomes. The data were analyzed by a linear least square fit of the data points with the slope of the regression line representing $1/nK_{app.}$. The values are identical for UGA and UAA stop codons and are the average of 3 independent determinations. The value for wild-type ribosomes was set to 1.0. Relative errors were estimated as +/−25%. WT stands for wild-type.

(27). Similarly, A and G bases at that position in 70S ribosomes showed an accessibility and very high reactivity towards the different chemical probes: 1054G being reactive at its N1 and N2 position (Kethoxal and CMCT, Figure 5A) and 1054A at the N1 atom (DMS, Figure 5B). No N7 modification of 1054A with DEPC was found. 1054U was shown to be only marginal reactive (if at all) at its N3 site towards CMCT (Figure 5C). This might either reflect that it is involved in a new base pairing interaction or that the uracil participates in a strong stacking interaction with the neighboring purine bases thereby altering the accessibility for the carbodiimide. Since we were not able to determine a base pairing partner for 1054U (which would require major structural rearrangements) and furthermore no other structural differences were apparent in and around helix 34, we are in favour of the latter explanation. No additional new reactivities, qualitative or quantitative deviations from the wild-type modification pattern were found for any of the three mutants and we therefore concluded that the steady state structures of the mutated helices 34 are isomorphic to the conformation in wild-type ribosomes.

Additionally, since we used assembled 70S ribosomes for the probing study, the data show that the pattern of proteins associated with the mutated 16S rRNA molecules was indistinguishable from the situation in wild-type ribosomes. The latter fact was further substantiated by a quantitative protein analysis. Using a two dimensional gel system we were able to separate about 45 of the 52 individual ribosomal proteins. A comparison of the gel patterns from wild-type and mutant cells clearly demonstrated, that all three mutated ribosomes contain an identical (full) complement of ribosomal proteins (Figure 6).

In vitro RF-2 binding of mutant ribosomes

To examine further the *in vivo* termination suppression event, we analyzed the ability of the mutant 70S ribosomes to form termination complexes with RF-2 protein *in vitro*. The analysis was performed in the presence of [^{35}S] labelled termination factor, 70S ribosomes and termination triplet in a partial reaction of the termination event which requires the presence of 6% (v/v) ethanol (25). Relative apparent association constants were determined from a sedimentation assay and are summarized in Table 3. Within the experimental variation of the method all three mutant ribosomes showed an association behaviour identical to wild-type ribosomes and no difference was found using either UAA or UGA as a stop codon triplet. Obviously, the binding properties of the ribosomes with RF-2 factor was not altered in any of the mutants.

DISCUSSION

The deletion of cytidine 1054 in *E. coli* 16S rRNA has previously been shown to confer a UGA stop codon specific suppressor phenotype, suggesting the participation by small subunit rRNA in translational termination (2). Using *in vitro* mutagenesis techniques, we selectively changed cytidine 1054 to the three other possible nucleotides, in order to study more directly the involvement of base 1054 in translational termination. Using an *in vivo* conditional expression system, we were able to demonstrate that following induction of episomal transcription, all three mutant 16S rRNA molecules were expressed and assembled into 70S ribosomes (Table 2). Upon 2 hours following induction, about 40–60% of the cellular ribosome moiety contained mutated 16S rRNA molecules. Ribosomes isolated from each of the three mutants, apparently contained a full complement of ribosomal proteins (Figure 6) associated with the altered rRNA in a manner indistinguishable from wild-type particles. Employing the same approach as Powers and Noller (34), we were also able to demonstrate by chemical probing of mixed populations of 70S ribosomes, the mutations within helix 34 did not induce any detectable structural perturbations in the 16S rRNA fine structure (Figure 5). In summary, we have not been able to identify structural abnormalities of the mutated ribosomes.

In contrast to our conclusions based on the structural data, the phenotypic analysis revealed that each of the three mutants exhibited individual characteristics: the 1054A transformants grew in a manner similar to wild-type plasmid containing cells; the 1054U mutants had a slightly increased generation time (close to the limit of resolution of the assay), whereas 1054G transformants grew 46% more slowly by comparison to transformants containing the wild-type plasmid (Table 1). The decreased growth rate was consistent with the reduced ability of mutant 1054G 70S particles to terminate at UGA stop codons *in vivo* (Figure 4). It is pertinent to note here, that initiation and elongation events could not have been affected by the mutation, since functionally active β-galactosidase was synthesized. The exclusive suppression specificity for UGA termination codons was also characteristic of the 1054 deletion mutant (2). Therefore, this provides additional experimental support for the functional participation of that rRNA region in UGA dependent translational termination. However, a comparison of the extent of suppression activity demonstrates that the deletion of base 1054 is 4 to 5 times more effective than the 1054G transversion mutant (Göringer, manuscript submitted). This leaves us with the interesting result that although cytidine 1054 is a highly conserved nucleotide in small subunit rRNA (32), an adenine or uracil base at the same position is tolerated by the cells. By contrast, a guanosine nucleotide is incompatible with proper UGA dependent termination, but to a lesser extend when compared to the deletion of base 1054. It is interesting to note that of all three mutants, the 1054G mutation exhibits the most pronounced phenotypic effect and showed the lowest incorporation level into 70S particles. This observation is consistent with other rRNA mutations that are deleterious to their host (34, 35) and is indicative of of a gene dosage effect.

All three mutant 70S ribosomes showed an identical *in vitro* association with RF-2 release factor protein, that was indistinguishable from wild-type ribosomes. Therefore, the UGA suppression does not reflect the failure of the binding of RF-2 factor. We envisage a scenario in which termination factor association and stop codon recognition are two separate and independent events. This observation fully supports that of Lang et al. (4) who proposed that stop codon decoding requires a rRNA component of the ribosome.

Whilst helix 34 is recognized as being a dynamic and flexible secondary structure element (7), the steady state conformation of the helices 34 in the three mutant 16S rRNA molecules were identical (Figure 5). Therefore, the UGA suppression phenotypes of the 1054G and 1054 deletion mutants can be explained by the loss or impairment of molecular flexibility during a defined step of the termination reaction, such as stop codon binding. The substitution or deletion of a single nucleotide can result in a dramatic change in the dynamic properties of a structural motif which as a consequence is able to trigger the equilibrium between defined, functionally dependent structural intermediates. We believe this to be the case for the two UGA suppressor mutants in helix 34. Indeed, helix 34 is the only extended helical element in small subunit rRNA that contains highly conserved nucleotides (32) which is an additional argument that this domain might switch into different (partly single stranded) conformations during specific stages of the translation event.

In summary, the data present a preliminary view of how the structure-function correlation of helix 34 in 16S rRNA may contribute towards UGA dependent termination and demonstrate a requirement for structural flexibility of that rRNA motif. Furthermore, the data provide evidence that the first reaction of the termination cycle can be defined as involving at least two steps: stop codon decoding by the 16S rRNA and release factor binding to the ribosome.

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