Nucleic Acids Research, Vol. 19, No. 19 **5281–5283**

Ribosomes containing the C1054-deletion mutation in *E.coli* 16S rRNA act as suppressors at all three nonsense codons

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Received July 8, 1991; Revised and Accepted September 12, 1991

ABSTRACT

It was established some time ago that the deletion of base C1054 in *E.coli* 16S rRNA specifically affects UGA-dependent termination of translation. Based on this observation, a model for the termination event was proposed in which the UGA nonsense codon on the mRNA base-pairs with a complementary motif in 'helix 34' of the 16S rRNA, thus potentially providing a recognition signal for the binding of the release factor. This model has been re-examined here and evidence is presented which demonstrates that ribosomes containing the C1054Δ mutation enhance the activity of suppressors of both UAG and UAA termination codons introduced into the host. The results do not support the nonsense codon-16S rRNA base pairing model, and rather imply a more general involvement of 'helix 34' in the translation termination reactions.

INTRODUCTION

The events at termination are somewhat of an enigma: stalling of 70S particles at one of the three termination codons (UAA, UAG, UGA) is accompanied by the interaction of protein factors which then results in the release of the nascent peptide. The discovery that the deletion of base C1054 (C1054Δ) in 16S rRNA could effect UGA nonsense suppression [1], provided the first suggestion that the rRNA is directly involved in the termination event. Base C1054 is part of helix 34 (numbering according to [2]) which contains two tandem 5'-UCA-3' triplets (1199–1204), complementary to the 5'-UGA-3' triplet termination codon (Figure 1). This region of the rRNA has been proposed to base pair directly with the UGA stop codon, and, by coaxial stacking, to form a helical element which was suggested to be the recognition signal for release factor 2 (RF-2) mediated termination [3,4].

This model has been re-examined and we now provide evidence that the deletion of base C1054 in 16S rRNA causes the ribosome to act as a 'super suppressor' by enhancing the activities of suppressors introduced into the host.

In vivo levels of UGA 'leakiness' indicate that there is weak suppressor activity attributed to a tRNA, accounting for the 3–5% level of readthrough [5;6]. In contrast, termination at UAA and UAG is much more stringent [5;6], there being no apparent equivalent misreading by a normal tRNA. Any mutation in the rRNA which effects nonsense suppression, such as the deletion of base C1054, still requires a tRNA to be present in order to decode the nonsense codon, thereby allowing the ribosome to resume the translation elongation cycle. That such a natural, weak suppressor tRNA is uniquely available for the UGA nonsense codon could account for detection of the apparently UGA-specific activity attributable to the mutant ribosomes. We thus reasoned that when these mutant ribosomes are presented with the opportunity to continue the elongation cycle following an encounter with either UAA or UAG nonsense codons (by the addition of suppressor tRNAs), they might also more efficiently cause suppression of these stop codons. This was indeed what we found and this observation in combination with absence of an obvious equivalent Watson–Crick base-pairing opportunity for the UAA and UAG stop codons in the vicinity of the C1054Δ mutation in helix 34, disfavours the proposed base-pairing model for translation-termination [3;4].

MATERIALS AND METHODS

Strains and Plasmids

The C1054Δ mutation was expressed from derivatives of either plasmid pKK3535 (pkkC1054Δ) which contains the complete *rrnB* operon including the natural promoters P_1P_2 [7], or from plasmid pNO2680 (pNOC1054Δ) which contains the *rrnB* transcriptional unit under control of the lambda P_L promoter [8]. Conditional expression from this promoter is achieved in the presence of the thermal sensitive repressor CI_{857}, whose inactivation at 42°C allows transcription to proceed.

Stop codon suppression analysis was achieved *in vivo* using a system in which ribosomes must translate through a termination triplet in order to synthesize functional β-galactosidase. The test strains available were: DEV1-*lacZ*UAG and DEV14-*lacZ*UAA and DEV15-*lacZ*UGA.

Construction of suppressor strains

The UAG-specific suppressor *supD* was obtained from WM902 (*ara, (lac-pro)*Δ, *gyrA*, *supD*, *thi*) and transduced by T4 bacteriophage in to the DEV1-*lacZ*UAG [9]. Positive transductants were selected on M9 minimal media containing lactose as the sole carbon source. Transductants free of the T4 bacteriophage were obtained by growth in the presence of antisera raised against T4 bacteriophage (a gift from Walter Messer). For DEV14-*lacZ* UAA, a UAA-suppressor was obtained

spontaneously by selecting for growth on M9 minimal media containing lactose. The suppressor strains were further analyzed for their ability to ferment lactose during growth on MacConkey agar indicator plates.

Measurement of β-Galactosidase activity

β-galactosidase measurements were performed according to Miller [10]. Transformants were grown in 1×A medium (10.5g $K_2HPO_4.3H_2O$, 4.5g KH_2PO_4, 1g $(NH_4)_2SO_4$, 0.5g Na citrate.$2H_2O$) supplemented with 20μg/ml B1, 10^{-3} $MgSO_4$ and 0.4% glucose, at 28°C until mid-logarithmic phase and their suppressor activities were measured following a 15 minute pulse at 42°C and a subsequent incubation for 100 minutes at 37°C, to permit assembly and accumulation of the pNO2680 plasmid encoded ribosomes.

Whole-cell assays were carried out in a final volume of 1.0ml Z-buffer at 28°C and begun following the addition of 200μl o-nitro-phenyl-β-D galactopyranoside (4mg ml^{-1}). The reaction was terminated following the addition of 500μl 1M Na_2CO_3. The activity was measured as β-gal units = $[10^3 \times [A_{420} - (1.75 \times A_{500})] / [OD_{600} \times \text{time (min)} \times \text{vol. (ml)}]$.

RESULTS

Transformation of suppressor strains

The existence of a natural, weak suppressor activity exclusively for suppression at UGA nonsense codons [5;6] is believed to account for the apparent UGA-specific suppressor activity attributable to ribosomes containing the C1054Δ mutation [1]. Since no equivalent suppressor activity for either UAA or UAG nonsense codons exists [5;6], this would prevent the detection of suppressor activity attributable to mutations in the rRNA. Accordingly, suppressors were introduced in to the reporter strains DEV1-*lacZ* UAG and DEV14-*lacZ* UAA (materials and methods).

Derivatives of the two parental plasmids pKK3535 and pNO2680 harbouring the C1054Δ mutation (pKKC1054Δ and pNOC1054Δ, respectively) were used to transform the test strains: DEV1-*lac*ZUAG, DEV14-*lac*ZUAA and DEV15-*lac*ZUGA, and in addition, the derivatives of DEV1-*lac*ZUAG and DEV14-*lac*ZUAA carrying the suppressor tRNAs. Transformants could readily be obtained in the DEV-*lacZ* strains that lacked the additional suppressor tRNA. However, transformants could not be obtained in the DEV-*lac*Z strains containing the suppressors with the plasmid pKKC1054Δ. Apparently, constitutive expression of the mutation from the P_1P_2 promoters present on pKK3535, in combination with suppressor activity was lethal to the host. Transformants could only be obtained under conditions that ensured no expression of the mutation. Accordingly, competent cells containing the temperature sensitive repressor CI_{857} were prepared at 28°C to ensure the presence of functional repressor molecules and transformed by pNOC1054Δ.

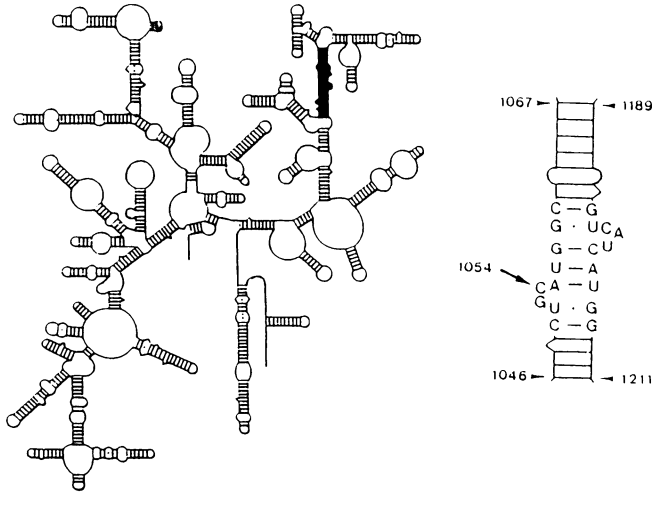

Figure 1. Secondary structure model of *E.coli* 16S rRNA [12]. The shaded region indicates the position of the sequences that encompass helix 34, enlarged on the right hand side.

Expression of the C1054Δ mutation caused enhanced read through of all three nonsense codons

Conditional expression of the helix 34 mutation from pNOC1054Δ was necessary in order to analyze the ability of the mutant ribosomes to enhance the readthrough activity of the UA-G and UAA nonsense suppressors. This was achieved *in vivo* by exploiting the system in which ribosomes must translate through a stop codon in order to synthesize functional β-galactosidase [9]. The levels of enzyme activity in each of the strains reflects the level of readthrough activity. Transformants of the wild type plasmid pNO2680 were used as a control to estimate the levels of activity both in the presence and absence of the suppressors. A summary of the data for nonsense suppression activities in transformants of the DEV-*lac*Z strains both in the presence and absence of additional suppressors is presented in Table I. We were able to re-confirm the previous results demonstrating that in the absence of suppressors for either UAA or UAG nonsense codons, only negligible levels of β-galactosidase activity were detected in the DEV-*lacZ* strains and that the presence of the C1054Δ mutation did not altered this observation [4].

Table I demonstrates that the actual levels of β-galactosidase activity in the presence of the chromosomally encoded suppressors and the wild-type ribosomes (transformants of pNO2680) was dependent upon both the efficiency of the suppressor itself and the codon context, as has been previously reported [11]. In the presence of the C1054Δ mutation, there was an increase in the levels of readthrough of all three nonsense codons. For the UAG and UAA nonsense codons, this was reflected by increases in the

Table I. Levels of suppression activity observed *in vivo* in the presence of the C1054Δ mutation.

HOST	PLASMID pNO2680	PLASMID pNOC1054Δ	%*
DEV1-*lac*ZUAG	1.6	1.2	–
DEV1-*lac*ZUAG/*sup*	163	907	556
DEV14-*lac*ZUAA	<0.5	<0.5	–
DEV14-*lac*ZUAA/*sup*	407	933	230
DEV15-lacZUGA	21	57	271

Termination codon suppression analysis in the presence of the C1054Δ mutation *in vivo*. The levels of synthesis of β-galactosidase activity in the DEV-*lacZ* strains containing suppressor activity (DEV-*lacZ/sup*) and in the absence of additional suppressor activity (DEV-*lacZ*) were measured according to Miller [10] and reflect the average of at least three independent experiments. The difference between the wild type and mutant values is expressed as a percentage (%*). Values in the absence of IPTG were subtracted. Experimental error was always within 20%.

levels of β-galactosidase synthesized of between 2.3 and 5.5 fold. These results were closely similar to the level previously demonstrated for the UGA suppression activity in DEV15-*lacZ* UGA [4], which we also re-confirmed (Table I).

DISCUSSION

The specificity of the C1054Δ mutation for suppressing the UGA-dependent termination event [1] has been re-examined and we here provide evidence that this mutation in fact caused the ribosome to act as a 'super suppressor' by enhancing the activities of tRNA suppressors.

In the DEV-*lacZ* strains, synthesis of functional β-galactosidase requires suppression of the internal nonsense mutation and continued elongation of the nascent peptide. In the absence of a suppressor tRNA, the mutant ribosomes will either remain in contact with the messenger or will at some point in time dissociate without having the opportunity to complete the synthesis of the enzyme. Therefore, we reasoned that if these mutant ribosomes were presented with the chance to continue the elongation cycle following an encounter with either UAA or UAG nonsense codons, (by the addition of suppressor tRNAs), they would enhance the suppression event since translation termination is altered. *In vitro* studies have shown that 70S ribosomes isolated from transformants containing the C1054Δ mutation exhibit a reduction in the relative apparent association of RF-2 [4].

The lethal effect on the host strains caused by the combination of suppressor activity for either UAA or UAG and ribosomes containing the C1054Δ mutation, indicated that the mutation had an effect on RF1 activity, in addition to the RF2 activity previously reported [1]. The lethality was presumably due to an intolerable level of readthrough of termination codons on mRNAs, resulting in the synthesis of aberrant proteins. Therefore, conditional expression of the C1054Δ mutation from pNOC1054Δ was required for further analysis of the affect of this mutation on the suppressor tRNAs. This was demonstrated by monitoring the levels of β-galactosidase synthesized in the DEV-*lacZ* strains. The results presented in Table I show that in the presence of the mutant ribosomes, there was a 2.3 to 5.5 fold increase in the level of suppression activities at both UAG and UAA nonsense codons in the *lacZ* transcripts. These results were consistent with the levels of UGA-dependent suppression observed originally in DEV15-*lacZ* UGA [4]. That the stop codons are located at varying positions within the *lacZ* messenger (DEV1-*lacZ*105UAG and DEV14-*lacZ*659UAA [9]) and different tRNA species must be involved in order to recognise the nonsense codons, this supports the notion that the affect is indeed a general phenomenon attributable to the translation-termination event rather than being unique to a particular codon context.

Since ribosomes carrying the C1054Δ mutation enhance the activities of suppressors specific for all three nonsense codons (Table I), this would indicate that the mutation had an affect on *both* RF-1 and RF-2 activities. Thus it would appear that helix 34 plays a more universal role in defining a termination domain.

ACKNOWLEDGEMENTS

We would like to thank Uli Göringer for the plasmids pKKC1054Δ and pNOC1054Δ and Walter Messer for the T4 bacteriophage and suppressor containing strains. Special thanks go to Richard Brimacombe for helpful comments and critical reading of the manuscript.

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