

The Nature of the Nucleotide at the 5' Side of the tRNA Su9 Anticodon Affects UGA Suppression in *Escherichia coli*

Ronit GOLDMAN-LEVI, Juné KOPELOWITZ, and Hanna ENGELBERG-KULKA*

Department of Molecular Biology, Hebrew University-Hadassah Medical School, Jerusalem 91010, Israel

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Abstract

In *Escherichia coli* a UGA codon can be efficiently suppressed by a suppressor tRNA^{Trp} called Su9. Here, we show that the level of UGA suppression is determined by the nature of the nucleotide at the 5' side of the anticodon of the suppressor (position 33). UGA suppression occurs when a pyrimidine residue is located in position 33 of the tRNA, and suppression is more efficient with a U than with a C in this position. On the other hand, when a purine residue is located at this position UGA suppression is extremely low. These results show that in the case of tRNA Su9, the UGA codon context effect does not require base pairing between the nucleotide at the 3' side of the codon and the 5' side of the anticodon.

Key words: genetic code, nonsense suppression, tRNA, *Escherichia coli*

1. Introduction

In *Escherichia coli*, a normal tRNA^{Trp} can suppress the UGA stop codon at low frequency¹⁻⁴ through a mechanism called UGA readthrough.^{5,6} A suppressor tRNA^{Trp}, induced by mutagenesis⁷ and now called Su9,⁸ can suppress UGA even more efficiently. Usually, nonsense suppressors induced by mutagenesis carry a mutation in the anticodon; the Su9 suppressor is unique because instead it carries a mutation in the stem of the dihydrouracil loop.¹ Su9 carries the anticodon CCA of the wild type tRNA^{Trp}; it can hydrogen-bond to the first two nucleotides of the UGA codon only, and not to the third because the base pair C-A is not allowed even in the wobble position.⁹ It was originally suggested that the change in the stem of the dihydrouracil loop, outside of the anticodon sequence, may increase UGA suppression by enhancing the stability of the codon-anticodon interaction.¹ However, later experimental results indicated that the enhanced UGA suppressor activity of Su9 could be explained by parameter independent of the codon-anticodon interaction itself,¹⁰ probably by reduced dissociation of the tRNA from the ribosome.¹¹

Codon context also influences the level of UGA readthrough by normal tRNA^{Trp}, UGA suppression by Su9, and also the efficiency of nonsense suppressors, studied mostly in *E. coli* and *Salmonella typhimurium*. The nucleotide at the 3' side of the codon has an important role in nonsense suppression. Generally, a purine at this location permits greater suppression than does a

pyrimidine.¹²⁻¹⁸ In the case of UGA suppression by Su9, this effect was first shown by Miller and Albertini¹⁶ who examined TGA codons in different contexts at various sites of a gene. Recently, to study the effect of codon context on UGA suppression by Su9, we built constructs into which we inserted TGA codons with different contexts, always located at the same site of the gene. We found that the effect of the nature of the nucleotide at the 3' side of the UGA codon is more complex than reported earlier: it positively influences the level of UGA suppression in the order A > G > C > U. This pattern of context effect is not seen at either extremely low or extremely high levels of UGA suppression.¹⁹

In all elongator tRNAs, including the UGA suppressor tRNA^{Trp} Su9, the U residue at position 33 at the 5' side of the anticodon is one of the most conserved,^{8,20} implicating this nucleotide in UGA suppression and probably in translation in general. Here we have studied its role in UGA suppression by replacing this U residue at position 33 in Su9 by C, G or A.

2. Materials and Methods

2.1. Bacterial strains and plasmid derivatives

The strains of *E. coli* used in this work, MC4100 and its derivative YN3230, carrying a mutation in the *prfB1*-gene have been previously described.¹⁹

Plasmids pMR1(CTGAA), pMR1(CTGAG), pMR1(CTGAC) and pMR1(CTGAT), pMR1(CTAGA) and pJK1 were constructed as previously described.¹⁹ Here, we designated pJK1 as pJK1Su9(U33). In this study, by site-specific mutagenesis, we replaced the T residue at the 5' side of the CCA anticodon in the Su9

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* To whom correspondence should be addressed. Tel. +972-2-758250, Fax. +972-2-784010

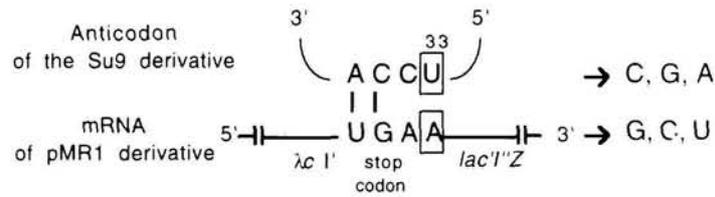


Figure 1. Base pairing of the anticodon of the UGA suppressor tRNA^{Trp} and the UGA stop codon in the mRNA. The boxed U residue on the 5' side of the anticodon (position 33) is the nucleotide normally present in tRNA^{Trp} Su9. Below is the mRNA of the plasmid pMR1 derivative which carries the fused genes $\lambda cI'$ - $lac'I''Z$.¹⁹ At the junction of these genes is a UGA stop codon: Suppression of this stop codon leads to β -galactosidase activity. The boxed A residue on the 3' side of the UGA codon is the nucleotide in pMR1(TGAA)mRNA. To the right are indicated the different replacements carried out in this study. Standard base pairing between codon and anticodon is indicated by solid lines.

gene of pJK1Su9(U33) to C, G or A (Fig. 1). The resulting plasmids are called pJK1Su9(C33), pJK1Su9(G33) and pJK1Su9(A33). pMR1 derivatives and pJK1 derivatives are compatible plasmids.

2.2. Molecular cloning

All recombinant DNA manipulations were carried out by standard procedures.²¹ Point mutations in *Su9* were generated using synthetic oligonucleotides in site-specific mutagenesis reactions using the Amersham (England) kit for M13 site-specific mutagenesis.

2.3. Determination of the level of UGA suppression

The level of UGA suppression was determined by the percentage of β -galactosidase activity of the pMR1 derivative (carrying a TGA at the junction of the fused genes $\lambda cI'$ - $lac'I''Z$) relative to that of pMR1 (no TGA at this position). We removed the small background of about 2.5% translation, probably resulting from reinitiation of translation after the UGA by subtracting the level of β -galactosidase activity directed by a pMR1 derivative, pMR1(CTAGA), carrying a TAG codon instead of a TGA codon at the junction of $\lambda cI'$ - $lac'I''Z$. The numbers in Fig. 2 represent the averages of the results of at least five experiments.

3. Results and Discussion

We wished to study the influence on UGA suppression of the nucleotide immediately adjacent to the 5' side of the Su9 anticodon, normally a U (Fig. 1). Would replacing the nucleotides normally present affect the level of UGA suppression? To investigate this question, we constructed plasmids pJK1Su9(C33), pJK1Su9(G33), and pJK1Su9(A33) which are derivatives of pJK1Su9(U33). The UGA suppressor activity directed by each of these plasmids was studied by measuring its effect on the expression of *lac'Z* directed by derivatives of pMR1. In these derivatives, we inserted a TGA codon, followed

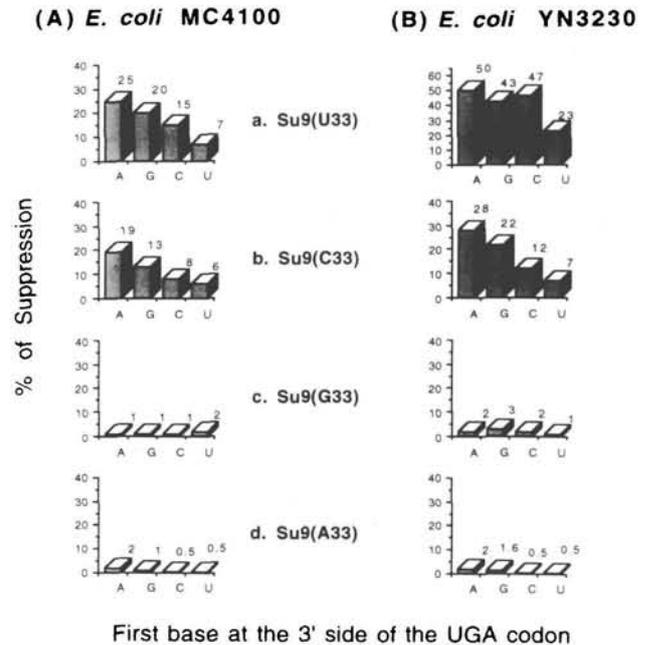


Figure 2. Replacing the U residue at position 33 of Su9 tRNA^{Trp} by C, G or A affects both the level of UGA suppression and the codon context effect. *E. coli* strains MC4100 (A) or YN3230 (B) were transformed first by pMR1 or by one of its derivatives pMR1(CTGAAA), pMR1(CTGAGG), pMR1(CTGACC) and pMR1(CTGATT). Then they were transformed by either pJK1Su9(U33)[a], pJK1Su9(C33)[b], pJK1Su9(G33)[c], or pJK1Su9(A33)[d]. Freshly doubly transformed cells were grown overnight on YT plates at 37°C and then for one generation in YT liquid medium at 37°C, and the level of β -galactosidase activity was determined. Suppression of the UGA mutation by the UGA suppressor Su9 results in the synthesis of β -galactosidase. In both of our host strains, 100% β -galactosidase activity as directed by pMR1 was 6000 units.

by one of the nucleotides A, G, C or T at its 3' end, at the junction of the fused genes $\lambda cI'$ - $lac'I''Z$.¹⁹ These plasmids were called pMR1(CTGAAA), pMR1(CTGAGG), pMR1(CTGACC), and pMR1(CTGATT); they express β -galactosidase activity only when the UGA codon is sup-

pressed. Using the 16 possible combinations of these two sets of plasmids (pMR1 and pJK1 derivatives), we examined the effect on UGA suppression by measuring the β -galactosidase activity in two *E. coli* host strains: MC4100 and its derivative YN3230 (Fig. 2). Strain YN3230 carries a mutation in the *prfB1* gene in the gene coding for the protein release factor 2 (RF2) and therefore probably increases the level of UGA suppression.^{19,22,23} For each combination we examined the efficiency of suppression and the pattern of the codon context effect.

The cells were transformed first by a pMR1 derivative and then by the pJK1 derivative. It was necessary to use freshly transformed cells. As shown in Fig. 2 in both MC4100 and YN3230, UGA is suppressed when there is a pyrimidine residue at position 33 of Su9. Furthermore, a U in this position permits greater UGA suppression than does a C (compare Figs. 2Aa and 2Ab, and Figs. 2Ba and 2Bb). On the other hand, UGA suppression is not seen at all, or is extremely low, when a purine residue (G or A) is located at this position (Figs. 2Ac and 2Ad, Figs. 2Bc and 2Bd). The reduction in UGA suppression by the Su9 derivatives carrying a purine residue at position 33 is most pronounced in strain YN3230 where the maximum level of UGA suppression by Su9(U33) is about 50% (Fig. 2Ba) and the highest level, by Su9(G33) or Su9(A33), is only about 2–3% (Figs. 2Bc and 2Bd).

The inability of both Su9(G33) and of Su9(A33) to suppress UGA may be explained by the possible interference by a nucleotide with a shape and/or size of a purine with the codon-anticodon interaction. It is not yet clear which characteristic of a U residue at this position of Su9 permits the highest efficiency of UGA suppression, nor do we yet understand its particular role in translation in general.

Our results confirmed our previous results on the effect of the level of UGA suppression on the codon context effect.¹⁹ There, and here, the level of UGA suppression was increased by the presence of the *prfB1* mutation which increases the chances for a UGA to be translated by incapacitating the release factor so that it cannot compete with the suppressor tRNA. Here, we found that both the level of UGA suppression and the manifestation of the UGA context effect can be modulated by changing the nucleotide at the 5' side of the anticodon. When this nucleotide is a G or an A, UGA suppression was so low that there was no measurable-codon context effect. When the nucleotide is a U or a C, however, the modulation is pronounced; this is especially clear in the host YN3230 where there is a greater level of suppression. When the base is a U, suppression is high and no context effect is seen. However, when the base is a C, the level of suppression is down-regulated and the codon context effect is manifested again.

Crystallographic studies of various tRNAs have revealed that the U residue at position 33 is not always

oriented in precisely the same way; nevertheless, it connects the anticodon stacked along the 3' side of the loop with the pyrimidine at position 32 stacked on the 5' side of the loop.²⁴ The unique position of this U residue in the anticodon loop structure and its remarkably conserved nature suggest that it is essential for translation.^{13,25,26} Our results here on the UGA suppressor Su9, and the previous studies on the UAG suppressor Su7 of Yarus and co-workers²⁷ are in agreement with this assumption, although the nucleotide C can replace U to some extent. On the other hand, in the case of yeast UAG suppressor tRNA^{Tyr} it was shown that the nature of the nucleotide at position 33 does not affect UAG suppression.²⁴ It is not yet established whether the translational role of the U residue in position 33 is a general phenomenon, or whether it is limited to some specific tRNAs, such as tRNA^{Trp} of which Su9 and Su7 are variants.

Several models have been proposed to explain the influence on nonsense suppression of the nucleotide directly adjacent to the 3' side of the codon.^{13,17,28,29,30} The hypothesis, originally suggested by Taniguchi & Weissman,²⁹ implicated a role for the U residue, invariably at position 33, adjacent to the 5' side of tRNA anticodon; this U residue could base pair with the purine residue at the 3' side of the codon, forming a U-A or U-G pair. However, the possibility of such base pairing was ruled out by direct experimentation on the UAG suppressor variant tRNA^{Trp} Su7-UAG,¹⁷ and here by us for the UGA suppressor variant Su9. As shown in Figs. 2Aa, 2Ab and 2Bb, the effect on UGA suppression of the nature of the nucleotide adjacent to the 3' side of the UGA codon is in the order A > G > C > U. (As described above, UGA suppression is extremely low when a purine is located at this position, and therefore, the effect of a purine on UGA codon context cannot be studied). The possibility for base pairing between the nucleotide at the 3' side of the codon and that at the 5' side of the anticodon should change the effect of the nature of the purine at the 3' side of the UGA from A > G by Su9(U33) to G > A by the mutated Su9(C33). Since this does not happen, we favor another hypothesis originally suggested by Grosjean et al.²⁸ and further developed by Ayer and Yarus.¹⁷ This hypothesis suggests that a purine at the 3' side of the codon would increase the stability of the codon-anticodon interaction. This would be analogous to the "dangling-end effect" on the stability of pairing of short duplexes in solution;^{31,32} it follows that the nucleotide at the 3' side of the codon would be the most effective in the stabilizing role, and that a purine would stabilize better than a pyrimidine. We found previously¹⁹ and again here, that at least in UGA suppression by Su9 the effect of the nature of the nucleotide at the 3' side of the UGA is more complex than just purine > pyrimidine: The nucleotide in this position influences the level of UGA suppression in the order A > G > C > U. Such a

hierarchy is not seen in the "dangling-end effect" on the stability of pairing of short duplexes in solution, for which only purines and pyrimidines can be differentiated.^{31,32} This is probably because the effect of the 3' nucleotide on the mRNA-tRNA interaction is more complicated than is the base pairing of short duplexes in solution. Alternatively, it may be that the suppressor tRNA^{Trp} Su9, with its mutation in the stem of the dihydrouracil loop (rather than in the anticodon) and which can base pair only to the first two nucleotides of the UGA codon, simply represents a unique case of mRNA-tRNA interaction.

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