Interaction between a mutant release factor one and P-site peptidyl-tRNA is influenced by the identity of the two bases downstream of the stop codon UAG

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Abstract Termination efficiency of a mutant form of RF (release facor) 1, as compared to the wild-type enzyme, is influenced by the P-site peptidyl-tRNA if the termination signal is UAGA. This effect is weaker at the stronger termination signal UAGU. Similarly, low efficiency of the mutant RF1, together with certain peptidyl-tRNAs, can be increased by changing the second base of the 3′-flanking codon from C to G. The data suggest that the mutant RF1 interacts with the P-site peptidyl-tRNA in conjunction with the context at the 3′-side of the termination codon.

Key words: Translation termination; Codon context; P-site tRNA; *Escherichia coli*

1. Introduction

The efficiency of translation termination is influenced by the codon context, defined by the sequence surrounding the termination codon. Information on codon context effects has been obtained from genetic studies of nonsense codon suppression [1–7] and statistical analysis of sequence data banks. The usage of a stop codon and its context in genes is non-random and the patterns differ between highly and poorly expressed genes in *Escherichia coli* [8–10]. The context effect is mediated by one or more of the determinants of the A-site pocket: the mRNA sequence per se, the P-site tRNA or the C-terminal end of the nascent peptide [11]. Termination efficiency is the result of the competition between termination mediated by release factors (RFs) and elongation (read-through) mediated by a near-cognate tRNA or by a suppressor tRNA in complex with elongation factor Tu-GTP (EFTu-GTP) [12].

In a prokaryote like *E. coli*, RF1 recognizes the UAG and UAA stop signals, while RF2 responds to UGA and UAA. RF3 stimulates the activity of both factors without being essential for cell viability (reviewed in [13]). RF1 and RF2 activities have shown to be sensitive to the codon context [14–16]. The efficiency of RF to terminate at each stop signal varies widely depending on the identity of the fourth base, with U and A being the most and least favorable bases for termination, respectively [7,16]. The different efficiencies of termination at different four base stop signals were found to correlate well with their frequencies of usage in *E. coli*, suggesting that certain nonsense codon contexts are selected for efficient RF function [9,16,17]. The identity of the base at the 3′-side (position +4) of the stop codon affects strongly translation termination, suggesting that the stop signal is a tetranucleotide sequence rather than just the stop codon triplet [7,9,18]. Also the second base (position +5) following the stop codon could be of importance for termination [19,20]. Evidence has been provided that the four base stop signal is recognized directly by the RF [21,22] even though the involvement of 16S rRNA in stop codon recognition has been considered [23–26].

The sequence at the 5′-side of the stop codon is important for translation termination [6,9,27–29]. It has been demonstrated that an interaction can occur on the ribosome between the P-site tRNA and a suppressor tRNA at the A-site [30]. Furthermore, the third base pair of the P-site codon-anticodon complex may interact with a tRNA in the A-site [29,31–34]. Also, certain peptidyl-tRNAs have been shown to promote translational frame-shifting [35]. This suggests that the P-site peptidyl-tRNA may play a role in A-site conformation and activity. It has also been shown that the last amino acids of the nascent peptide influence translation termination and correlations between physical properties of these amino acid residues and efficiency of termination have been found [6,28,29,36,37].

Previously, we have analyzed the 5′-context effect on RF1 efficiency at the UAGA termination signal. We found that a mutant form of RF1 [14,38] is altered in its sensitivity to the last amino acid in the nascent peptide. It has also been found that certain iso-codons at the 5′-side of UAGA affect the efficiency of the mutant RF1 differently, even though they encode the same amino acid. An interaction between RF1 and the P-site tRNA or the P-site codon-anticodon complex, rather than an interaction with the mRNA sequence per se, has been suggested to explain these results [29,34]. In this report, we show that the sensitivity of this mutant RF1 to the 5′-context is modulated by the two bases immediately following the UAG stop codon.

2. Materials and methods

2.1. Strains and media

E. coli strains used in this study are MRA21 ((Δ*lacproAB*) *serU* (*supD*) *rph*) [39] and MRA24 ((Δ*lacproAB*) *prfA1 serU* (*supD*) *rph*) [29]. The two strains are derivatives of MG1655 [40,41]. A strain carrying the *prfA1* mutation has a temperature sensitive (Ts) phenotype and shows enhanced misreading of the stop codons UAG and UAA [14].

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Abbreviations: RF, release factor; EFTu, elongation factor Tu; Ts, temperature sensitive

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2.2. Translation assay system

The *E. coli* translation assay system S3A′ was used to measure UAG read-through. Read-through of an internal stop codon in this test gene gave a full-length 3A′-protein. Termination at the internal stop codon gave a shorter 2A′-protein. Other details of the assay procedure for determination of the in vivo apparent efficiency of RF1 have been described previously ([28,29,42] and references therein).

3. Results and discussion

The termination efficiency is the outcome of the competition between a suppressor tRNA (read-through product) and the RF (termination product). To be able to determine the factors that influence the RF, we compare a strain with a mutant RF1 to the wild-type strain. An altered read-through ratio when different contexts are compared in the two strains indicates an altered relative termination efficiency for the mutant RF1. Any effect on the suppressor tRNA will be canceled out since it is present in both strains.

Some 5′-flanking codons which have been analyzed earlier together with the UAGA stop signal [29] were re-evaluated here with the more efficient UAGU stop signal [7,16].

The results presented in Table 1 show that, for most 5′-flanking codons, read-through (transmission) is lower with UAGU than with UAGA, as expected, both for the wild-type strain and the strain with a mutated RF1. The activity of the mutant RF1 is about 20% of the activity for the wild-type enzyme, as represented by the RE values in Table 1. However, it can be seen that when UAGA is together with the upstream codons CCA, CGU, AUG and AGC, the relative efficiency of the mutant RF1, as compared to wild-type RF1, is notably lower than for other 5′-flanking codons [29]. The negative effects on the mutant RF1 that are associated with the first three of these upstream codons are less pronounced together with UAGU than with UAGA. AGC as the 5′-codon provides an exception since the low apparent efficiency of mutant RF1 persists even if the stop signal is UAGU. For other upstream codons, the effect on the efficiency of the mutant RF1 obtained by changing the stop signal from UAGA to UAGU is negligible in most cases or possibly even slightly positive. Thus, effects of the upstream codons on the apparent relative efficiency of the mutated RF1 at UAG are dependent on the nature of the extended stop signal, i.e. on the first base at the 3′-side of the stop codon. If the relative efficiency values for the mutant RF1 at UAGA are compared to those at UAGU (Table 1, last column), it can be seen that the apparent efficiency of the mutant RF1 is particularly lowered by changing the stop signal from UAGU to UAGA if the 5′-neighboring codons are CCA, CGU, AUG and perhaps also AGU.

The 5′-codons CCA, CGU, AUG and AGU are decoded by $tRNA^{Pro3}$, $tRNA^{Arg2}$, $tRNA^{Met}$ and $tRNA^{Ser3}$, respectively. For $tRNA^{Met}$, the whole molecule, the codon-anticodon interaction or the amino acid can be the determinant. It is not possible to distinguish between these three possibilities since there is only one codon for methionine. The effects of the proline codons CCA and CCC on the relative efficiency of mutant RF1 are different, suggesting that the amino acid is not the determinant for the observed differential effect. Instead, since CCC is decoded by $tRNA^{Pro2}$ and CCA by $tRNA^{Pro3}$, it is likely that the negative effect associated with CCA is caused by $tRNA^{Pro3}$, in the P-site, interacting with the mutated RF1 in the A-site. The wobble base of the codon is not likely to give the effect, since no general differential effect can be seen when C- and A-ending codons are compared. Together with P-site $tRNA^{Arg2}$, which can read three codons

Table 1
Cooperativity of 5′-context and 3′-neighboring base on the RF1 efficiency

Codon	tRNA	NNN UAG AGU			NNN UAG UGU			Efficiency ratio (RE_A/RE_U)
		Transmission		RE_A	Transmission		RE_U	
		prfA$^+$	*prfA1*		*prfA*$^+$	*prfA1*		
CCC	Pro-2	0.49	3.0	0.16	0.18	1.5	0.12	1.3
CCA	Pro-3	0.43	9.0	0.05	0.25	1.8	0.14	0.36
CGU	Arg-2	0.47	7.3	0.06	0.15	1.3	0.12	0.5
CGC	Arg-2	0.39	1.6	0.24	0.11	0.54	0.20	1.2
CGA	Arg-2	0.19	0.96	0.20	0.06	0.43	0.14	1.4
AGU	Ser-3	0.15	2.1	0.12	0.15	0.67	0.22	0.55
AGC	Ser-3	0.32	4.0	0.08	0.14	1.6	0.09	0.89
AAA	Lys	0.22	1.3	0.17	0.11	0.72	0.15	1.1
AAG	Lys	0.52	2.9	0.18	0.14	0.52	0.27	0.67
AUU	Ile-1	0.37	1.9	0.20	0.10	0.67	0.15	1.3
AUC	Ile-1	0.43	1.8	0.24	0.18	0.72	0.25	0.96
AUA	Ile-2	0.43	2.5	0.18	0.20	0.92	0.22	0.82
GGU	Gly-3	0.56	4.6	0.12	0.23	1.5	0.15	0.8
GGC	Gly-3	0.59	4.0	0.15	0.25	1.1	0.23	0.65
GGA	Gly-2	1.5	3.6	0.42	0.61	1.9	0.33	1.3
GGG	Gly-2, -1	1.3	3.4	0.40	0.54	1.8	0.30	1.3
GAG	Glu	0.30	1.4	0.22	0.20	0.67	0.30	0.73
AUG	Met	0.33	4.1	0.08	0.16	0.72	0.22	0.36

Codons at the 5′-side of UAG, decoded by indicated tRNAs, were combined with the 3′-flanking codons AGU or UGU. The transmission value represents the molar ratio between the amount of read-through protein (3A′-) product divided by the amount of shorter protein (2A′-) that is formed as the result of the termination event [29]. Thus, low transmission values represent efficient termination.

The relative efficiency (RE) given for the mutated RF1 is the ratio between termination efficiencies for the mutant and wild-type strains as reflected by the respective transmission values. Thus, low RE values represent a low relative efficiency of the mutant RF1. RE values for NNN UAG AGU have been published earlier [29] and are reprinted with the permission from the journal. The S.E.M. for transmission determinations and RE is $<12\%$.

Table 2
Sensitivity of mutant RF1 to UGU or UCU as codon on the 3′-side of UAG

P-site	tRNA	Relative efficiency of mutant RF1	
Codon		NNN UAG UGUa	NNN UAG UCU
AUU	Ile-1	0.15	0.07
AUC	Ile-1	0.25	0.19
AUA	Ile-2	0.22	0.17
AUG	Met	0.22	0.13
CCA	Pro-3	0.14	0.15

Codons at the 5′-side of UAG, decoded by indicated tRNAs, were combined with the 3′-flanking codons UGU or UCU. The S.E.M. = < 10%. Calculation of the relative efficiency is described in Table 1.
[a]Values for relative efficiencies are taken from Table 1.

CGU, CGC and CGA, the relative efficiency of the mutated RF1 at UAGA is lower with CGU as a 5′-flanking codon as compared to the CGC and CGA (Table 1). This can be compared to the $tRNA^{Gly3}$ which reads the two codons GGU and GGC. For these two upstream codons, the relative efficiency of the mutated RF1 is similar. Thus, there is no general correlation between the third base of the P-site codon being a U or C and the effect on the efficiency of the mutated RF1. Instead, the structure of the P-site tRNA is likely to be a determinant also in the case of $tRNA^{Arg2}$.

For $tRNA^{Ser3}$, the apparent efficiency of the mutant RF1 is low if AGU is preceding UAGA, but higher if the stop signal is UAGU. Such a difference is not seen if the 5′-codon is AGC since the termination efficiency is low both for UAGA and UAGU. Since AGU and AGC are decoded by the same tRNA, the results point to an effect of the codon-anticodon interaction, in conjunction with the nature of the extended stop signal, on the relative efficiency of the mutated RF1. It is notable that $tRNA^{Ser3}$ has a large extra arm [43]. Perhaps this large arm together with the anticodon interaction with AGC disturbs the mutant RF1 in a manner that cannot be compensated for by a U- instead of an A-residue, following the UAG codon.

It has been observed that the termination efficiency of RF2 can be affected by the second base of the codon that follows the stop codon itself [19]. Therefore, we decided to test a few codons at position −1 upstream of UAG, followed by UGU or UCU as the downstream codons. The reason for the choice of UCU was based on theoretical data from an analysis of the entire *E. coli* that UAA followed by UGU is much less common than when followed by UCU, in the genome [20]. Even though only a few combinations were analyzed, it appears from the results presented in Table 2 that UGU as the 3′-flanking codon is associated with a higher relative efficiency of the mutant RF1 than UCU together with most, but not all, of the analyzed 5′-neighboring codons. These data suggest that the mutant and wild-type RF1 also can be different in sensitivity to the middle base of the 3′-flanking codon and that this sensitivity is affected by the nature of the codon at the 5′-side of UAG.

RF binding to the mRNA is determined by a four or five base stop signal [7,16,19]. The relative efficiency of RF1 to terminate at UAGN in vivo is: UAGU > UAGG > UAGC > UAGA [16] and in vitro, UAGU > UAGC [44]. Our results are in line with this ranking. However, we have also found that if the weak stop signal is UAGA, the termination efficiency can be influenced by the P-site peptidyl-tRNA. Several different determinants may contribute to this effect: the C-terminal end of the nascent peptide, the P-site codon-anticodon interaction, in particular the wobble position [29] and possibly other parts of the P-site tRNA that could interact with the RF, for example the extra arm [45]. Furthermore, as described here, the P-site codon-anticodon duplex can influence the efficiency of the mutant RF1 at the ribosomal A-site differently, depending on the fourth base of the stop signal. In addition, the second base of the 3′-flanking codon also can have an influence on termination, since a change from UGU to UCU, together with certain 5′-flanking codons, gives a changed apparent efficiency of the mutant RF1, as compared to the wild-type enzyme.

The mutant RF1 analyzed here is the result of a single amino acid substitution (R137P) and it is impaired in ribosome binding. This impediment can be suppressed by a mutationally altered L7/L12 [38]. It has been suggested that the fourth base of the stop signal influences the conformation of the stop codon and thereby its interaction with RF at the ribosomal A-site [7]. RF1 may also interact directly with the last uridylic acid residue in the UAGU stop signal through hydrogen-bonding, giving an increased termination efficiency [17]. The data presented here suggest that such RF interactions with bases following the stop codon itself could be influenced by the peptidyl-tRNA at the ribosomal P-site.

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