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Functional Interaction Between Release Factor One and P-site Peptidyl-tRNA on the Ribosome

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Translation termination at UAG is influenced by the nature of the 5' flanking codon in *Escherichia coli*. Readthrough of the stop codon is always higher in a strain with mutant (*prfA1*) as compared to wild-type (*prfA*$^+$) release factor one (RF1). Isocodons, which differ in the last base and are decoded by the same tRNA species, affect termination at UAG differently in strains with mutant or wild-type RF1. No general preference of the last codon base to favour readthrough or termination can be found. The data suggest that RF1 is sensitive to the nature of the wobble base anticodon-codon interaction at the ribosomal peptidyl-tRNA binding site (P-site). For some isoaccepting P-site tRNAs ($tRNA_3^{Pro}$ *versus* $tRNA_2^{Pro}$, $tRNA_4^{Thr}$ *versus* $tRNA_{1,3}^{Thr}$) the effect is different on mutant and wild-type RF1, suggesting an interaction between RF1 at the aminoacyl-tRNA acceptor site (A-site) and the P-site tRNA itself. The glycine codons GGA ($tRNA_2^{Gly}$) and GGG ($tRNA_{2,3}^{Gly}$) at the ribosomal P-site are associated with an almost threefold higher readthrough of UAG than any of the other 42 codons tested, including the glycine codons GGU/C, in a strain with wild-type RF1. This differential response to the glycine codons is lost in the strain with the mutant form of RF1 since readthrough is increased to a similar high level for all four glycine codons. High α-helix propensity of the last amino acid residue at the C-terminal end of the nascent peptide is correlated with an increased termination at UAG. The effect is stronger on mutant compared to wild-type RF1. The data suggest that RF1-mediated termination at UAG is sensitive to the nature of the codon-anticodon interaction of the wobble base, the last amino acid residue of the nascent peptide chain, and the tRNA at the ribosomal P-site.

Keywords: release factor; translation termination; stop codon; readthrough; codon context

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Introduction

In *Escherichia coli*, the termination codons UAG and UGA are decoded by release factors one (RF1) and two (RF2), respectively; the third termination codon, UAA, is decoded by both these factors. For efficient termination, a third factor (RF3) is also needed (Milman *et al*., 1969; Mikuni *et al*., 1994; Grentzmann *et al*., 1994). Efficiency of the termination process is greatly dependent on the codon context (Bossi & Roth, 1983; Miller & Albertini, 1983; Stormo *et al*., 1986; Buckingham *et al*., 1990; Pedersen & Curran, 1991; Kopelowitz *et al*., 1992; Björnsson & Isaksson, 1993; Mottagui-Tabar *et al*., 1994, Björnsson *et al*., 1996). A codon context that gives inefficient termination leads to increased readthrough of the stop codon by some near-cognate tRNA, since the two reactions are competitive (Yarus & Curran, 1992). Increased readthrough is also obtained if the codon context has no direct effect on the termination reaction *per se*, but instead facilitates decoding of the stop codon by near-cognate or suppressor tRNA. Therefore, since readthrough is the result of decoding competition between the release factor and tRNA, it is difficult to evaluate the extent to which the two reactions are affected by the codon context (Rydén & Isaksson, 1984; Martin *et al*., 1988a,b; Pedersen & Curran, 1991).

The underlying molecular basis for stop codon context is not well understood. At the 3' side of the

Abbreviations used: A-site, aminoacyl-tRNA acceptor site; IgG, immunoglobulin G; P-site, peptidyl-tRNA binding site; RE, relative efficiency; RF, release factor; *T*, transmission.

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stop codon, the first base following the stop codon is of particular importance; this suggests that the signal for the termination factors includes this base in addition to the stop codon itself (Brown *et al*., 1990; Li & Rice, 1993; Arkov *et al*., 1993; Poole *et al*., 1995). Codons at the 5' side can affect termination in several ways. The determinant can be the codon itself, its decoding tRNA, or the nascent peptide it carries (Buckingham *et al*., 1990; Björnsson & Isaksson, 1993; Arkov *et al*., 1993; Mottagui-Tabar *et al*., 1994; Björnsson *et al*., 1996). If the 5' flanking codon is CGA (Arg), then the I·A anticodon-codon interaction at the wobble base of the peptidyl-tRNA binding site (P-site) tRNA has a negative effect on a suppressor tRNA that decodes the stop codon at the aminoacyl-tRNA acceptor site (A-site). A decreased readthrough is the result (Curran *et al*., 1995; Björnsson *et al*., 1996). Also, an altered interaction between a mutationally changed anticodon loop of the P-site tRNA and a nonsense suppressor tRNA that decodes at the A-site has been demonstrated (Smith & Yarus, 1989). Both these findings suggest that the codon context can affect the efficiency of the tRNA that competes with the termination factor at the stop codon (Pedersen & Curran, 1991; Yarus & Curran, 1992).

Recently, it has been shown that the last two C-terminal amino acid residues of the nascent peptide influence termination efficiency at UGA. According to these results, if the –2 amino acid residue at the C-terminal end of the nascent peptide is basic or hydrophilic, apparent termination at UGA is efficient. Termination is also increased if the –1 amino acid residue has a high α-helix or β-strand propensity. Thus, termination / readthrough seems to be dependent on both the structure of the C-terminal end of the nascent peptide and the nature of the codon or tRNA at the 5' side of the UGA codon. In the case of UGA readthrough, some data suggest that the tRNA-EF-Tu ternary complex is influenced (Mottagui-Tabar *et al*., 1994; Björnsson *et al*., 1996).

The sensitivity of a binding-defective mutant form of RF1 (R137P) to the UAG codon context is known to differ from that of normal RF1 (Rydén & Isaksson, 1984; Zhang *et al*., 1994). This suggests that the binding of release factor to the ribosome may also be affected by the codon context. Here we have varied the codon at the 5' side of UAG and determined readthrough of this stop codon in strains with normal or mutated RF1. We find that the mutant and normal RF1 in some cases are affected differently both by the codon / tRNA at the ribosomal P-site and by the corresponding amino acid residue. The results suggest that both the P-site tRNA itself, or its interaction with the codon, as well as the last amino acid residue of the nascent peptide, can influence RF1 efficiency during termination at UAG.

Results

Influence of the 5'-adjacent codon on RF1 efficiency

A semi-synthetic gene, S3A' (also referred to as Z or B'; Björnsson & Isaksson 1988, 1993), coding for three identical domains of an engineered B-domain of the antibody-binding protein A from *Staphylococcus aureus*, was used to determine the level of termination at UAG in *prfA*$^+$ and *prfA1* strains. The protein encoded by the assay gene is secreted into the growth medium as the result of a signal peptide (S) that precedes the first A-domain. The protein can be collected using affinity chromatography on a Sepharose-immunoglobulin G (IgG) column in one step. Stop codon contexts with a varied codon at the 5' side of UAG were inserted between the sequences coding for the second and third domains of this protein (Figure 1). Thus, termination gives a two-domain protein (15.1 kDa) whereas stop codon readthrough gives a complete three-domain product (23.6 kDa). The two proteins can be quantified with polyacrylamide gel electrophoresis and scanning of the protein bands in the gels. The relative amounts of the two protein products thus make it possible to quantify UAG termination / readthrough as a function of the varied –1 codon at the 5' side of the stop codon. Some representative gel results are shown in Figure 2.

Readthrough of UAG is caused by the *serU*(Su1) suppressor tRNA in competition with the RF1-promoted termination reaction. Therefore, any effect of codon context on readthrough values reflects an influence from the tRNA-dependent readthrough reaction, or the termination reaction, or both. Figure 2 and Table 1 show the results in the *prfA*$^+$ and *prfA1* strains for readthrough of UAG preceded by different codons at the 5' side (location –1). For the *prfA*$^+$ strain, readthrough values vary depending on the nature of this –1 codon. In several cases, two or

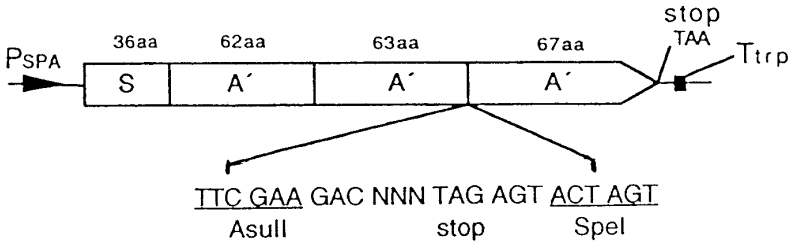

Figure 1. Principal construction of plasmids used in this study. S codes for a secretion signal peptide and A' is a gene segment that codes for a modified antibody-binding B-domain of protein A from *Staphylococcus aureus*. The *Asu*II-*Spe*I fragments with indicated varied codons at the –1 position relative to UAG were synthesised and inserted to obtain the various codon context plasmids.

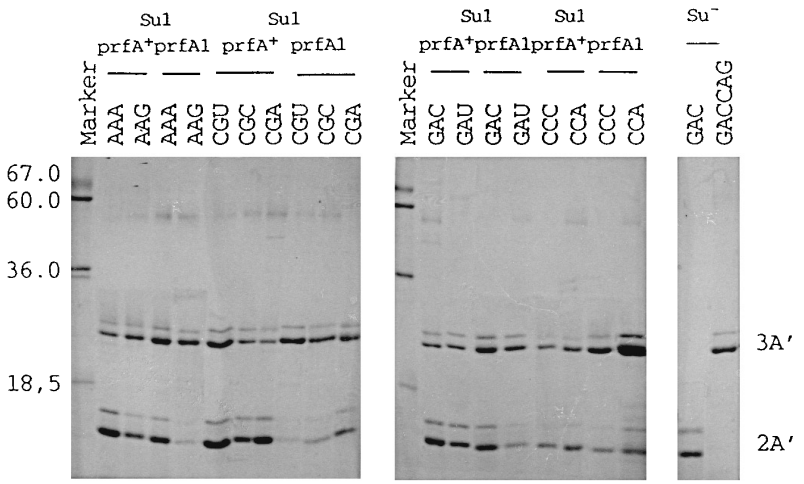

Figure 2. Protein samples from strains MRA21(*prfA*$^+$), MRA24-(*prfA1*), and the suppressor-free control strain MG1655 were collected on IgG-Sepharose mini-columns as described in Materials and Methods. The –1 codon relative to UAG is indicated. Samples were analysed using polyacrylamide gel electrophoresis as shown. The positions of the 3A' (readthrough product) and 2A' (termination product) bands are indicated. The last two lanes show analysis in MG1655 of GAC UAG and GAC CAG contexts, giving only 2A' or 3A' proteins, respectively. The minor bands above each of the two major products are proteins with incompletely processed signal sequence. These minor bands can be disregarded when termination/readthrough is determined by scanning, since transmission values are the same whether or not the contents of these minor bands are included in the calculation (Björnsson & Isaksson, 1993).

more codons of the same codon family (specifying the same amino acid) have been analysed. The data show that in most cases (Asp, Pro, Ser, Gln, Leu, Ala and Ile), readthrough is similar for all codons within the same family. However, for the arginine codons, CGA gives a significantly lower readthrough compared to the other codons. The glycine codons GGA and GGG are associated with higher readthrough than GGU and GGC. The lysine codon AAG gives a higher readthrough than the other lysine codon AAA in both the *prfA*$^+$ and *prfA1* strains.

In the *prfA1* mutant, compared to the *prfA*$^+$ strain, higher readthrough of UAG is observed when the 5' codon is GAU, as compared to GAC (Asp). Of the proline codons, CCA promotes an exceptionally high readthrough in the mutant. Higher readthrough in the mutant strain, compared to the wild-type strain, is also associated with CGU, as compared to the other five arginine codons; similarly, CUG gives higher readthrough compared to the other leucine codon CUA. On the other hand, the glycine codons GGA/G seem to promote slightly lower readthrough than GGU/C in the mutant, which is contrary to what is observed for the *prfA*$^+$ strain. Thus, in several cases, readthrough in a *prfA1* strain is not affected by the 5' adjacent codon in the same manner as in the *prfA*$^+$ control strain. It appears that the mutated RF1 is more sensitive to the nature of the –1 codon than is its wild-type counterpart during termination at UAG. This difference in sensitivity of the two RF1 forms, which is associated with the codon at the 5'-side of UAG, is illustrated by the ratios in the last column of Table 1. The relative efficiency of the mutant RF1 is as low as 0.05 in the case of CCA, as compared to around 0.2 for most other cases, and up to 0.4 for the glycine codons GGA/G. The variation of these ratios suggests that part of the influence of the 5'-codon on readthrough is on the termination factor RF1 rather than on the competing EF-Tu ternary complex with the suppressor tRNA.

Some U/C-ending –1 codons within the same codon family are read by the same tRNA; Asp (GAU/C) and Arg (CGU/C) give a lower relative mutant RF1 efficiency (higher readthrough) when U instead of C is the third base of the codon (Tables 1 and 2). The effect of these two bases on the efficiency is similar in the cases of glycine (GGU/C) and isoleucine (AAU/C). The serine codon pair AGU/C represents the only case in which U, rather than C, is possibly associated with higher efficiency of the mutant RF1. Thus, in cases where the last base of the –1 codon is U or C, and these codons are decoded by the same tRNA, these two bases affect differently the mutant RF1 as compared to the wild-type enzyme. The data suggest that RF1 is sensitive to the nature of the third position codon-anticodon base-pair of the peptidyl-tRNA at the ribosomal P-site, and that the mutant RF1 is altered in this sensitivity.

Within some –1 codon families, examples can be found in which two codons are decoded by different isoaccepting tRNAs. For instance, the proline codons CCA and CCC are decoded by $tRNA_3^{Pro}$ and $tRNA_2^{Pro}$, respectively (Table 1). In this case, the decoding of UAG by the mutant RF1, as compared to the wild-type enzyme, is relatively less efficient with CCA than with CCC as the –1 codon. By contrast, for the threonine codon pair ACC/A, the C-ending codon is instead associated with a lower relative efficiency of the mutant RF1 than is the A-ending codon. As mentioned above, in the case of GGC/U *versus* GGA ($tRNA_3^{Gly}$ and $tRNA_2^{Gly}$, respectively), the apparent efficiency of mutant RF1 is similar for all codons. On the other hand, the wild-type RF1 is remarkably low in efficiency in the case of GGA/G, thus giving an extraordinarily high readthrough as compared to GGU/C. The isoleucine codons AUC/A have a

similar effect on termination, even though these codons are decoded by different tRNAs. Also the alanine codons GCA/C are decoded by different tRNAs and have similar effects on efficiency of the mutant RF1. There is one example in Table 1 of C/A-ending codons in the same codon family (CGC and CGA) that are decoded by the same isoacceptor tRNA ($tRNA_2^{Arg}$). In this case, the two codons give a similar effect on the relative efficiency of the mutant RF1, even though the transmission values for CGA are quite low for both wild-type and mutant RF1. Thus, C or A as the third base of the –1 codon does not by itself directly influence the relative efficiency of the mutant, as compared to wild-type, RF1. This suggests that in some cases (proline, threonine and glycine), it is instead the P-site tRNA that has this effect.

Effect of the last amino acid residue in the nascent peptide on termination at UAG

It has been shown that the C-terminal end of the nascent peptide has an effect on the apparent termination efficiency at UGA. For the last amino acid residue, high α-helix and β-strand propensity in natural secondary structures are correlated with

Table 1. Readthrough of UAG and relative efficiency of mutant RF1 at NNN UAG AGU codon contexts

Plasmid	NNN	tRNA	Readthrough (*T*)		Mutant RF1 efficiency (RE)
			prfA$^+$	*prfA1*	
pSP11	GAU	Asp1	0.32(±0.03)	4.0(±0.37)	0.08(±0.004)
pAB52	GAC	Asp1	0.23(±0.02)	1.1(±0.03)	0.21(±0.008)
pSP12	CCU	Pro2,3	0.45(±0.02)	4.6(±0.21)	0.10(±0.003)
pSP30	CCC	Pro2	0.49(±0.02)	3.0(±0.17)	0.16(±0.005)
pMF18	CCA	Pro3	0.43(±0.03)	9.0(±1.1)	0.05(±0.003)
pSP31	CCG	Pro1,3	0.52(±0.03)	4.3(±0.31)	0.12(±0.005)
pSP34	AGU	Ser3	0.25(±0.01)	2.1(±0.11)	0.12(±0.004)
pAB77	AGC	Ser3	0.32(±0.02)	4.0(±0.22)	0.08(±0.003)
pSP32	UCU	Ser1,5	0.43(±0.02)	4.0(±0.28)	0.11(±0.005)
pSP107	UCC	Ser5	0.45(±0.03)	2.9(±0.16)	0.16(±0.007)
pSP33	UCA	Ser1	0.39(±0.03)	3.2(±0.20)	0.12(±0.006)
pSP35	UCG	Ser1,2	0.28(±0.01)	2.0(±0.11)	0.14(±0.005)
pSP90	AGA	Arg4	0.43(±0.03)	1.5(±0.09)	0.29(±0.013)
pSP91	AGG	Arg4,5	0.43(±0.02)	1.9(±0.08)	0.23(±0.007)
pSP65	CGU	Arg2	0.47(±0.03)	7.3(±0.78)	0.06(±0.003)
pSP89	CGC	Arg2	0.39(±0.01)	1.6(±0.06)	0.24(±0.005)
pSP66	CGA	Arg2	0.19(±0.01)	0.96(±0.03)	0.20(±0.005)
pSP88	CGG	Arg3	0.47(±0.02)	1.7(±0.05)	0.28(±0.007)
pSP92	GGU	Gly3	0.56(±0.02)	4.6(±0.18)	0.12(±0.003)
pSP93	GGC	Gly3	0.59(±0.02)	4.0(±0.21)	0.15(±0.004)
pSP94	GGA	Gly2	1.5(±0.06)	3.6(±0.24)	0.42(±0.014)
pSP95	GGG	Gly1,2	1.3(±0.04)	3.4(±0.25)	0.40(±0.012)
pSP13	CAA	Gln1	0.30(±0.02)	1.4(±0.09)	0.22(±0.008)
pSP79	CAG	Gln1,2	0.27(±0.01)	1.8(±0.08)	0.15(±0.004)
pSP41	AAA	Lys	0.22(±0.01)	1.3(±0.06)	0.17(±0.004)
pSP81	AAG	Lys	0.52(±0.03)	2.9(±0.12)	0.18(±0.005)
pSP96	CUA	Leu3	0.35(±0.02)	1.3(±0.08)	0.26(±0.011)
pSP73	CUG	Leu1,3	0.49(±0.02)	3.6(±0.16)	0.14(±0.004)
pSP108	GCC	Ala2	0.41(±0.02)	1.9(±0.10)	0.22(±0.008)
pSP36	GCA	Ala1B	0.33(±0.02)	1.9(±0.12)	0.17(±0.008)
pUY91	AUU	Ilel	0.37(±0.02)	1.9(±0.12)	0.20(±0.007)
pSP111	AUC	Ilel	0.43(±0.03)	1.8(±0.10)	0.24(±0.011)
pSP109	AUA	Ile2	0.43(±0.02)	2.5(±0.14)	0.18(±0.006)
pSP76	ACC	Thr1,3	0.37(±0.02)	4.6(±0.32)	0.08(±0.003)
pSP97	ACA	Thr4	0.56(±0.03)	2.1(±0.14)	0.26(±0.009)
pSP14	GAG	Glu2	0.30(±0.01)	1.4(±0.04)	0.22(±0.005)
pSP72	UUC	Phe	0.41(±0.02)	4.0(±0.25)	0.10(±0.004)
pSP74	AUG	Metm	0.33(±0.01)	4.1(±0.28)	0.08(±0.002)
pSP75	GUG	Val1	0.28(±0.01)	2.0(±0.09)	0.14(±0.004)
pSP77	UAC	Tyr1,2	0.35(±0.02)	2.9(±0.11)	0.12(±0.004)
pSP78	CAC	His	0.43(±0.03)	3.0(±0.12)	0.14(±0.006)
pSP80	AAC	Asn	0.45(±0.02)	2.2(±0.14)	0.20(±0.008)
pSP83	UGC	Cys	0.52(±0.03)	2.6(±0.13)	0.20(±0.008)
pSP84	UGG	Trp	0.41(±0.02)	3.6(±0.20)	0.12(±0.004)

MRA21 (*prfA*$^+$) and MRA24 (*prfA1*) strains with the indicated –1 codon context plasmids (Figure 1) were grown and the plasmid-coded 2A' and 3A' proteins were separated and quantified as described in Materials and Methods. Readthrough (*T*) is given by the molar ratio *T* = [3A']/[2A'] and the indicated standard error of the mean is based on four to six determinations. Relative efficiency of the mutant RF1 (RE) is obtained by dividing the *T* value for the *prfA*$^+$ strain (MRA21) with the corresponding value for the *prfA1* strain (MRA24) and standard error is calculated as described in Materials and Methods. The –1 decoding tRNAs are indicated (Komine *et al*., 1990; Björk, 1996).

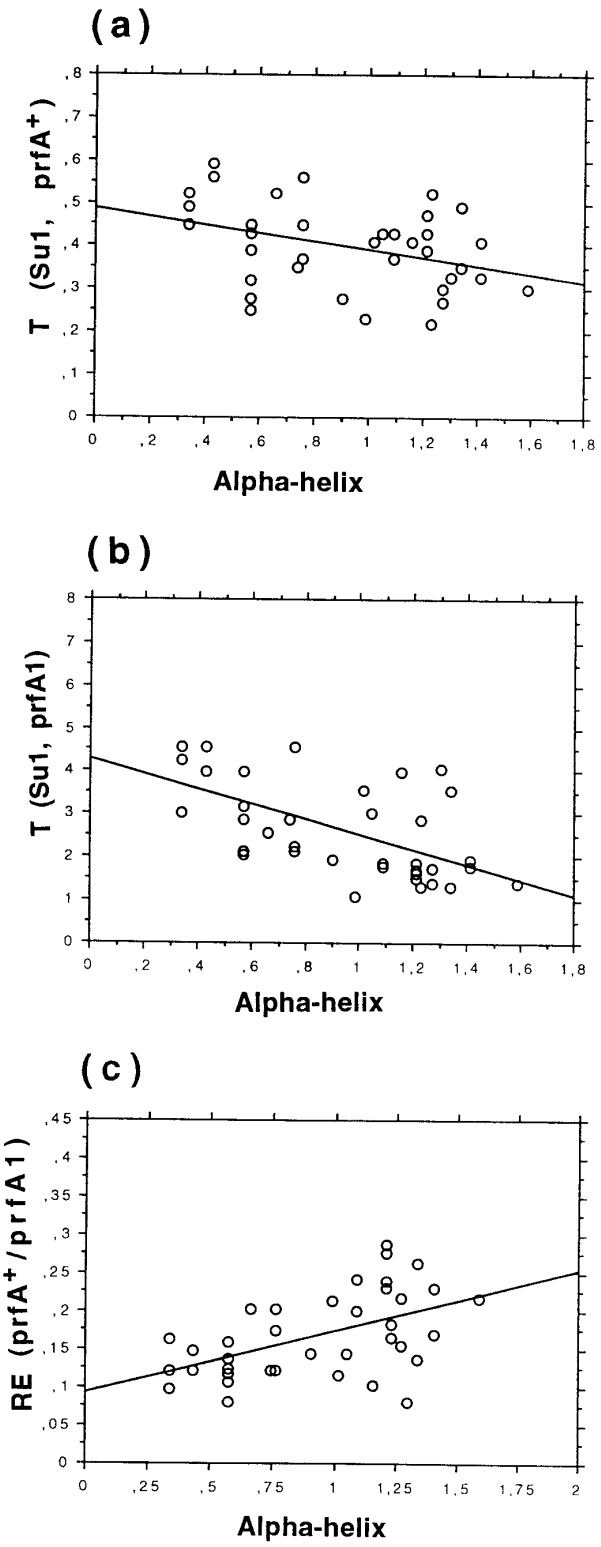

Figure 3. Transmission values (*T*) for UAG with various amino acids corresponding to the –1 codon, plotted against the α-helix propensity for the corresponding amino acid residue in natural protein structures (Williams *et al*., 1987; Creighton 1993). Readthrough is caused by the *serU*(Su1) suppressor tRNA in strains with RF1 coded by *prfA*$^+$ or*prfA1*, as indicated. Efficiency values (RE) are obtained by dividing *T* values for the *prfA*$^+$ strain with the corresponding values for the *prfA1* strain. Transmission values (*T*) plotted against α-helix propensity of the –1 amino acid residue in natural proteins: (a), strain MRA21 (*prfA*$^+$, $r = -0.36$, $p = 0.03$); (b), strain MRA24 (*prfA1*, $r = -0.58$, $p = <0.0001$); (c), ratios of transmission values (MRA21/MRA24) plotted against the α-helix propensity of the –1 amino acid residue ($r = 0.525$, $p = 0.0006$).

efficient termination. Amino acid residues that participate in protein turns instead favour readthrough (Björnsson *et al*., 1996). It is possible that termination at UAG is also dependent on the properties of the C-terminal end of the nascent peptide. To investigate this possibility, *T*-values (readthrough) were plotted against different properties of the C-terminal amino acid residue corresponding to the codon at the 5'-side of UAG (Williams *et al*., 1987; Creighton, 1993). Figure 3 shows that termination in the *prfA1* strain is significantly increased if the last amino acid residue in the nascent peptide has a high preference for α-helical structures. The effect is barely significant in the *prfA*$^+$. The selective effect on mutant, but not wild-type, RF1 is revealed if the relative efficiency of the mutant RF1 (RE) is plotted against α-helical propensity of the last amino acid residue in the nascent peptide (Figure 3(c)). A weak correlation between the amino acid residue propensity in turn II structures and termination at UAG can also be found but in this case the effect is not differential when the *prfA*$^+$ and *prfA1* strains (Figure 4(a) and (b)), are compared (Figure 4(c)).

Discussion

The results presented here show that termination at the UAG stop codon is dependent on the neighbouring codon at the 5' side. These effects are different in *prfA1* and *prfA*$^+$ strains, with mutant and wild-type RF1, respectively. In both strains, readthrough is promoted by the same *serU*(Su1) suppressor tRNA. The difference when the two strains are compared should therefore be caused by the alteration in the mutant form of RF1, and not by the suppressor *serU*(Su1) tRNA that competes with RF1 during UAG decoding in both strains. This suppressor tRNA is relatively insensitive to the codon context, probably as the result of a large variable loop (Curran *et al*., 1995). The effect of the 5'-neighbouring codon on RF1 action could either be direct through the codon itself, or indirect *via* the P-site-bound tRNA and its associated nascent peptide.

Lack of influence by the P-site codon itself on RF1

The wild-type RF1 is affected in a similar manner if GAU/C (Asp), which are both read by the same $tRNA_1^{Asp}$, are the P-site codons. However, the mutant RF1 is almost three times less efficient if UAG is preceded by GAU, as compared to GAC, at the same location. Similar results are obtained for

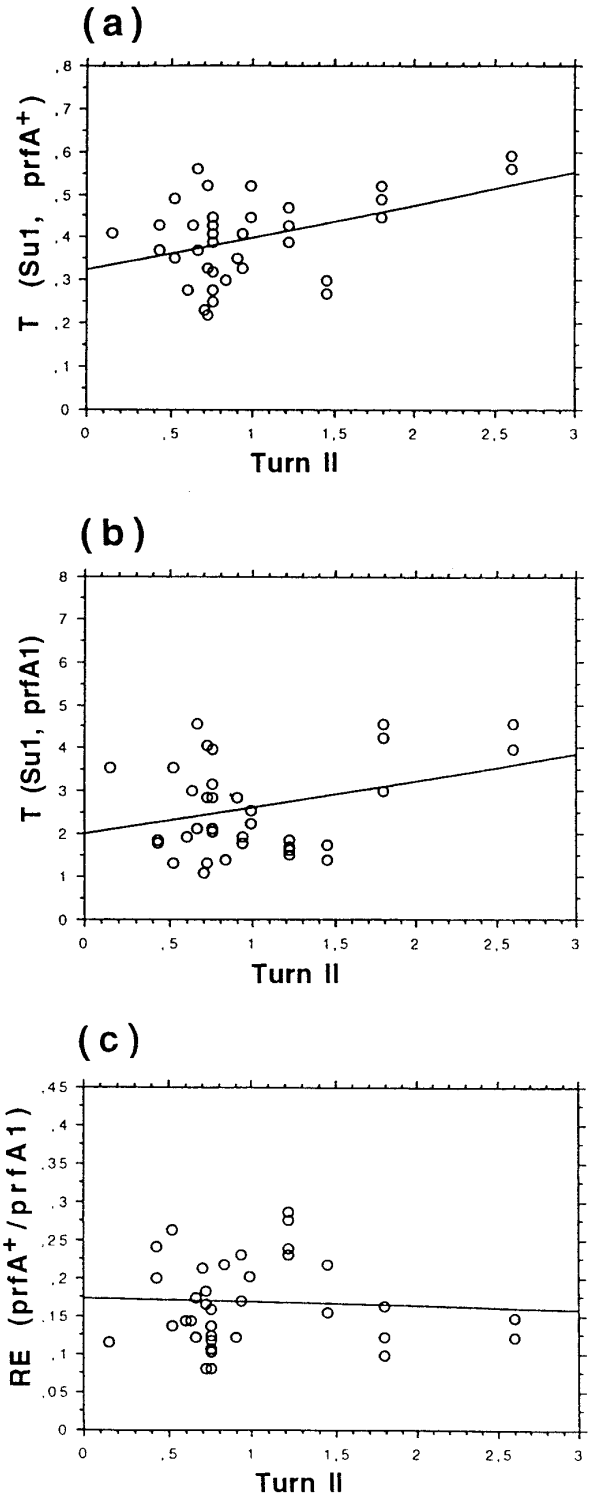

Figure 4. Transmission values (*T*) for UAG with various amino acids corresponding to the –1 codon, plotted against the turn II propensity for the corresponding amino acid residue in natural proteins (Williams *et al*., 1987; Creighton 1993): (a), strain MRA21 (*prfA*$^+$, $r = 0.44$, $p = 0.005$); (b), strain MRA24 (*prfA1*, $r = 0.31$, $p = 0.06$); (c), ratios of transmission values (MRA21/MRA24) plotted against the turn II propensity of the –1 amino acid residue ($r = -0.05$, $p = 0.77$).

the arginine codons CGU/C, which are both decoded by $tRNA_2^{Arg}$. Thus, in these two cases it seems that U, compared to C, as the last base of the P-site codon has a negative effect on the efficiency of the mutant, but not on that of the wild-type RF1. However, this is not a general effect since, for the GGU/C glycine codons ($tRNA_3^{Gly}$), as well as the AUU/C isoleucine codons ($tRNA_1^{Ile}$), U or C as the third base of the –1 codon is associated with a similar relative efficiency of the mutant RF1. Furthermore, for the serine codons AGU/C ($tRNA_3^{Ser}$), a slightly lower efficiency of the mutant RF1 is seen with C, compared to U, as the last P-site codon base. Thus, there is no consistent effect of the last base of the P-site codon itself on the relative efficiency of the mutant, as compared to wild-type, RF1.

Influence of the P-site tRNA on RF1 efficiency

Several codon pairs in the same codon family that end with C/A have been tested. The arginine codons CGC/A are read by the same $tRNA_2^{Arg}$, which carries an I-base as the anticodon wobble base. In this case, the effects of A or C on the efficiency of mutant or wild-type RF1 are quite similar, even though A gives very low readthrough values in both the *prfA*$^+$ and *prfA1* strains. This suggests that the I·A anticodon-codon base-pair, in contrast to I·C and I·U as discussed above, does not affect the A-site binding of mutant or wild-type RF1 differently. Instead, the data support the model that the P-site I·A pair has a negative effect on tRNA binding at the A site (Curran, 1995; Björnsson *et al*., 1996), thus counteracting readthrough. The proline codon CCA, which is read by $tRNA_3^{Pro}$, is associated with a low relative efficiency for the mutant RF1, compared to CCC, which is read by $tRNA_2^{Pro}$. By contrast, for the threonine codons ACA/C, which are decoded by $tRNA_4^{Thr}$ and $tRNA_{1,3}^{Thr}$, respectively, the C-ending codon is associated with low mutant RF1 efficiency. Other A- or C-ending codon pairs (UCA/C (serine), AUA/C (isoleucine) and GCA/C (alanine)) promote similar effects on the mutant RF1, even though the A- and C-ending codons are decoded by different isoaccepting tRNAs. Altogether, the simplest explanation of these results is that A or C as the third base of the –1 codon does not by itself affect efficiency of the mutant RF1. Instead, the data suggest that in the cases of $tRNA_3^{Pro}$ *versus* $tRNA_2^{Pro}$ and $tRNA_4^{Thr}$ *versus* $tRNA_{1,3}^{Thr}$ it is the tRNA at the P-site that interacts differently with mutant and wild-type RF1 at the A-site.

A compilation of termination efficiency data in cases where the P-site codon ends with U or C, as well as structures of the P-site tRNA anticodons, is presented in Table 2. An inspection of the presence of modified bases at positions 34 and 37 of the P-site tRNA reveals that $tRNA_2^{Arg}$ and $tRNA_1^{Asp}$ are modified at base 34 (I and Q, respectively) and these are the two tRNAs that promote different efficiency of the mutant RF1 when the 5' flanking codon ends with U or C. In the other cases, when

Table 2. Readthrough of UAG at NNU/C UAG AGU contexts

		P-site anticodon base					
tRNA	P-site codon	34	35	36	37	Relative efficiency	Ratio
Arg2	CGU	I	C	G	m^2A	0.06	4.0
	CGC					0.24	
Asp1	GAU	Q	U	C	m^2A	0.08	2.6
	GAC					0.21	
Pro2,3	CCU	G,cmo^5U	G	G	m^1G	0.10	1.6
Pro2	CCC	G	G	G	m^1G	0.16	
Ser5,1	UCU	G,cmo^5U	G	A	ms^2i^6A	0.11	1.5
Ser5	UCC	G	G	A	A	0.16	
Gly3	GGU	G	C	C	A	0.12	1.3
	GGC					0.15	
Ile1	AUU	G	A	U	t^6A	0.20	1.2
	AUC					0.24	
Ser3	AGU	G	C	U	t^6A	0.12	0.67
	AGC					0.08	

Bases 34 to 37 in tRNAs (Björk, 1995) that decode the NNU/C codons at the –1 position relative to UAG are shown. Values for the relative efficiency of the mutant RF1 (RE) are taken from Table 1. The effect of U or C as the third base of the anticodon on the relative efficiency of the mutant RF1 is the ratio (last column) obtained by dividing the RE value for the codon ending with C by the corresponding value for the codon ending with U.

mutant and wild-type RF1 are affected to a similar extent by U and C, the anticodon wobble base is unmodified. The modified nucleotide Q34 in $tRNA_1^{Asp}$ is a hyper-modified G base that restricts recognition of the tRNA to the cognate GAC and GAU aspartic acid codons (Valle & Morch, 1988). It has also been shown that the Q-containing $tRNA_1^{Asp}$ favours binding to GAU compared to GAC (Björk, 1995; Yokoyama & Nishimura, 1995). In the case of the I-base, the C·I pair is more stable than the U·I pair (Curran, 1995). Thus, I or Q at position 34 in the anticodon of the P-site tRNA ($tRNA_2^{Arg}$ and $tRNA_1^{Asp}$) affects the anticodon-codon interaction. This suggests that, for these P-site tRNAs, the different effect on the mutant *versus* wild-type RF1 efficiency is also caused by the P-site codon-anticodon interaction rather than by the U/C-ending codon base alone. It is possible that the weaker-binding mutant RF1 becomes more sensitive to certain P-site codon-anticodon complexes if the anticodon wobble base is modified, even though other factors could certainly also be involved. Both the $tRNA_2^{Arg}$ and $tRNA_1^{Asp}$ carry the m^2A modification at position 37. However, this base is also modified in the other U/C-decoding P-site tRNAs. Therefore, no obvious correlation can be found between the modification pattern of base 37 and the relative efficiency of mutant RF1.

The –1 glycine codons GGA/G are associated with uniquely high UAG readthrough (low termination) values in the *prfA*$^+$ strain, since for GGA/G they are almost three times higher than for any other tested codon, including the other glycine codons GGU/C. By contrast, in the *prfA1* strain all readthrough values are increased and those associated with the glycine codons are similar to each other. This finding suggests that GGA/G as 5' neighbouring codons to UAG are associated with a uniquely low efficiency for wild-type RF1. This sensitivity is eliminated by the mutational alteration at position 137 in the mutant RF1, which is coded by the *prfA1* allele (Zhang *et al*., 1994). It is not possible to conclude from the data presented here whether the GGU/C ($tRNA_3^{Gly}$) codons themselves, as compared to GGA ($tRNA_2^{Gly}$) or GGG ($tRNA_1^{Gly}$ and $tRNA_2^{Gly}$), influence RF1 efficiency, or whether the effects originate from their decoding tRNAs. However, we have reason to believe that the $tRNA_2^{Gly}$ is one of the determinants involved (Zhang *et al*., unpublished observation). This remarkable effect on termination associated with the GGA/G P-site codons is probably not specific for UAG and RF1, since it was earlier found that termination at GGA/G UGA is less efficient than at GGU/C UGA by RF2 in a *prfB*$^+$ strain (Björnsson *et al*., 1996). It is possible that termination factor RF3, which interacts with both RF1 and RF2, is involved in the unusual response associated with the P-site glycine codons.

Influence of the C-terminal end of the nascent peptide on RF1 efficiency

It has been shown recently that readthrough of UGA is low if the last amino acid residue at the C-terminal end of the nascent peptide has a high propensity for ordered structures (α-helix and β-strand). Readthrough is high if the –1 amino acid residue participates in protein turns (Björnsson *et al*., 1996). Here we find that amino acid residues that participate in turn II structures are associated with a slightly higher readthrough of UAG in both the *prfA*$^+$ and *prfA1* strains. The influence is similar in both strains; this could suggest an effect on the readthrough reaction mediated by the suppressor tRNA rather than on the termination reaction mediated by RF1. It was not possible to demonstrate any correlation between UAG readthrough and the participation of the –1 amino acid residue in β-strands or other types of protein turns, or to its

volume (results not shown). However, our results show that efficiency of termination at UAG can be correlated to a high α-helix propensity of the last amino acid residue of the nascent peptide in the *prfA1* and possibly also the *prfA*$^+$ strains (Figure 3). Since this effect is more pronounced on the mutant than on the wild-type RF1, this suggests an influence on the release factor itself. Thus, turn II and α-helix-participating amino acid residues may affect the readthrough and termination reactions differently.

The increased readthrough of UAG in *prfA1* strains (Rydén & Isaksson, 1984) is probably the result of a decreased affinity for the stop-codon-programmed ribosome (Zhang *et al*., 1994). If the α-helix propensity of the last amino acid residue in the nascent peptide is correlated with efficiency of RF1 binding, then the effect on a weakly binding mutant factor should be more pronounced than on the wild-type enzyme, as is observed. The result implies that the nascent peptide effect is of particular importance for inefficient termination by the mutant enzyme at UAG. This is in line with the finding that the nascent peptide effect is important for termination at the weak stop signal UGAA (Björnsson *et al*., 1996).

It is known that a UAAA stop signal, which is recognized by both RF1 and RF2, is preferentially decoded by RF1 (Martin *et al*., 1988a,b). By comparing the nascent peptide effects on UAG and UGA decoding by RF1 and RF2, described here and elsewhere (Mottagui-Tabar *et al*., 1994; Björnsson *et al*., 1996), it is worth noting that the effect on termination at UAG is much smaller than at UGA in a strain with wild-type release factors. On the other hand, the effect on wild-type RF2 and mutant RF1 seems to be similar. Together, the data suggest that wild-type RF1 binds more efficiently than wild-type RF2 to a stop-codon-programmed ribosome, and that a strong effect by the –1 amino acid residue in the nascent peptide is correlated with a low efficiency of release factor binding to the ribosome.

It has been shown that the *cat-86* leader pentapeptide can inhibit translation termination *in vitro* by blocking peptidyl-tRNA hydrolysis (Moffat *et al*., 1994). It is possible that the –1 amino acid residue in the nascent peptide also has an effect on this step during termination at UAG and UGA, even though it would be less dramatic. The result would be a delayed peptide release in connection with a longer pausing of the release-factor-binding ribosome at the stop codon.

The results presented here show that mutant and wild-type RF1 respond differently to changes of the upstream codon at the ribosomal P-site. This different response is likely to be due to the P-site tRNA or to the anticodon-codon complex and to a changed interaction with the last amino acid residue in the nascent peptide. The simplest explanation for these findings is a direct physical interaction between RF1 and the peptidyl-tRNA-codon complex at the ribosomal P-site during translation termination at UAG.

Materials and Methods

Strains

The bacterial strains used in this study are listed in Table 3. For the construction of strain MRA24, a P1vir lysate made on strain US468 (*zcg-174*::Tn*10*, Δ(*trpABtonB*)) was used to transduce MRA21, with selection for tetracycline resistance. Transductants were screened for Trp$^-$; one such transductant was retained (MRA23). A P1vir lysate grown on strain US486 (*prfA1*) was used to transduce MRA23, with selection for Trp$^+$. The resulting tetracycline-sensitive transductants were screened for temperature sensitivity (Ts) at 42°C; one such clone was retained (MRA24).

Assay system and plasmid construction

The *E. coli* translational assay system S3A' was used (Björnsson & Isaksson, 1988, 1993). Recombinant DNA techniques were performed according to standard procedures (Sambrook *et al*., 1989). All test sequences containing a different codon upstream of UAG were cloned using the *Spe*I and *Asu*II sites in the linker region of plasmid pAB7 (Figure 1; Björnsson & Isaksson, 1993). Oligonucleotides were either synthesised by using a Clone plus DNA synthesizer (Millipore) or bought from Pharmacia. A QIAEX gel extraction kit (Qiagen) was used to purify restriction fragments from a 1% (w/v) agarose gel (Sigma). Strain MG1655 was used as the recipient for transformation of recombinant plasmids. Plasmid DNA was prepared by using a Wizard™ miniprep kit (Promega). All sequences inserted in the vector were verified using the chain-termination DNA sequencing method (Sequenase kit version 2.0 from USB) with [α-^{35}S]dATP from Amersham. At least 50 bases flanking the insertion sequence were determined. The sequence primer, ABPO2 (Björnsson & Isaksson, 1993), is complementary to the unique sequence at the 3'-end of the S3A' gene.

Table 3. Strains used in this study

Name	Genotype	Reference/source
MG1655	*rph*	Bachmann (1987); Jensen (1993)
MRA21	Δ(*lacproAB*) *serU*(*supD*)	Rydén-Aulin *et al*. (1993)
MRA23	Δ(*lacproAB*) *zcg-174*::Tn*10* Δ(*trpABtonB*) *serU(supD)*	This study
MRA24	Δ(*lacproAB*) *prfA1 serU*(*supD*) *ara*Δ(*lacproAB*) *zcg-174*::Tn*10*	This study
US468	*ara*Δ(*lacproAB*) *zcg-174*::Tn*10*Δ(*trpABtonB*) *gyrA*(*nalA*) *argE*(UAG) *thi*	This laboratory
US486	*ara* Δ(*lacproAB*) *prfA1 gyrA*(*nalA*) *argE*(UAG) *thi*	This laboratory

Analysis of A'-protein and calculation of the mutant RF1 efficiency

The strains MRA21 (*prfA*$^+$) and MRA24 (*prfA1*), which harbour wild-type and mutant forms of RF1, respectively, were transformed with the codon context plasmid constructs. Strains to be tested were grown at 37°C in 20 ml of M9 minimal medium (Miller, 1972), supplemented with 100 μg/ml ampicillin, 0.2% (w/v) glucose, 0.01 μg/ml thiamine, 22.4 μg/ml uracil and all amino acids at the recommended concentrations (Neidhardt *et al*., 1974). Growth was stopped at mid-log phase by cooling on ice. The assay procedure was essentially as described previously (Björnsson & Isaksson, 1993). The cooled culture was centrifuged at 3000 ***g*** at 4°C for ten minutes and the A' -protein was isolated from the cell periplasm and the supernatant of the growth medium using an IgG-Sepharose 6FF columm (Pharmacia). The protein samples were then analysed by SDS-PAGE. Following fixing and staining, the gels were scanned using a laser scanner. The transmission value (T) for UAG readthrough is calculated as the molar amount of three-domain (3A') protein, divided by the two-domain (2A') protein, as obtained by scanning the gels. Transmission is a linear function which describes the relative efficiency of RF1 in its competition with the suppressor tRNA that gives the readthrough. This function can be used to record changes both at low and high readthrough, in contrast to suppression values which approach 100% (1.00) asymptotically as readthrough becomes high, thus losing linearity (Björnsson & Isaksson, 1993; Mottagui-Tabar *et al*., 1994, 1996; Curran *et al*., 1995; Pedersen & Curran, 1991; Smith & Yarus, 1989). The relative efficiency (RE) of the mutant RF1 is the T-value of MRA21 (*prfA*$^+$) divided by the corresponding value for MRA24 (*prfA1*). All T-values are averages of four to six independent measurements, giving a standard error of the mean as indicated. Standard error for the RE values are calculated by an error propagation function according to:

$$S_{RE} = (s_a^2 T_b^2 / n_a T_a^4 + s_b^2 / n_b T_a^2)^{1/2}$$

where T_a and T_b are the mean transmission values for the *prfA1* and *prfA*$^+$ strains, respectively, s_a and s_b are the standard deviation values associated with T_a and T_b, and n_a and n_b represent the number of determinations used to obtain T_a and T_b.

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