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5′ Contexts of *Escherichia coli* and human termination codons are similar

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ABSTRACT

The nearest 5′ context of 2559 human stop codons was analysed in comparison with the same context of stop-like codons (UGG, UGC, UGU, CGA for UGA; CAA, UAU, UAC for UAA; and UGG, UAU, UAC, CAG for UAG). The non-random distribution of some nucleotides upstream of the stop codons was observed. For instance, uridine is over-represented in position –3 upstream of UAG. Several codons were shown to be over-represented immediately upstream of the stop codons: UUU(Phe), AGC(Ser), and the Lys and Ala codon families before UGA; AAG(Lys), GCG(Ala), and the Ser and Leu codon families before UAA; and UCA(Ser), AUG(Met), and the Phe codon family before UAG. In contrast, the Thr and Gly codon families were under-represented before UGA, while ACC(Thr) and the Gly codon family were under-represented before UAG and UAA respectively. In an earlier study, uridine was shown to be over-represented in position –3 before UGA in *Escherichia coli* [Arkov,A.L., Korolev,S.V. and Kisselev,L.L. (1993) *Nucleic Acids Res.*, 21, 2891–2897]. In that study, the codons for Lys, Phe and Ser were shown to be over-represented immediately upstream of *E.coli* stop codons. Consequently, *E.coli* and human termination codons have similar 5′ contexts. The present study suggests that the 5′ context of stop codons may modulate the efficiency of peptide chain termination and (or) stop codon readthrough in higher eukaryotes, and that the mechanisms of such a modulation in prokaryotes and higher eukaryotes may be very similar.

INTRODUCTION

Peptide chain termination, in the cytoplasm of mammalian cells, is directed by UAA, UAG and UGA stop codons and by two peptide chain release factors, eRF1 (1) and eRF3 (2). eRF1 functions in the termination at all three stop codons (1) and eRF3 stimulates eRF1 activity (2). In *Escherichia coli* there are three protein factors (RFs) that function in termination: RF1 works at UAA and UAG, RF2 at UAA and UGA [for reviews see (3,4)] and RF3 stimulates the activities of RF1 and RF2 (5,6).

It is known that the 5′ context of the stop codons plays an important role in modulation of nonsense suppression in *E.coli* (7–11). It was shown, for instance, that different Ser codons immediately upstream of UGA at codon position 234 in the *trpA* gene affect UGA suppression in different ways (10). Meanwhile, recent experiments indicate that the second to last amino acid in the nascent peptide can influence the efficiency of nonsense suppression (12). On the other hand, the 3′ context of stop codons has also been shown to affect the efficiency of nonsense suppression, in both bacteria (7,10,11,13–17) and mammals (18–20). Furthermore, the effect of codon context on the efficiency of suppression of nonsense codons by aminoglycoside antibiotics was also observed in both *E.coli* and human cells (21)

The search for efficient stop signals with a statistical approach has been done for different eukaryotic organisms (22–24). Although a non-random distribution of nucleotides has been reported for two positions immediately upstream of eukaryotic stop codons (22), no thorough investigation on the 5′ context has been done as yet. Much more attention has been paid to the 3′ context of stop codons (23,24). It has been noticed that, although eukaryotic taxa exhibit a purine bias at the nucleotide position immediately downstream of the termination codons (23,24), most of the eukaryotic groups also exhibit an appreciable frequency of U at the same position and all analysed eukaryotes exhibit a low frequency of C at this position (24). These data are consistent with experimental observations. Specifically, it was shown that the efficiency of UAG suppression in human cells varies such that UAGC>UAGG>UAGU>UAGA (18–20).

In the theoretical studies (23,24), either the expected values for the 5′ stop codon context were taken from a count of each nucleotide at a series of positions (23) or the 5′ context of stop codons was compared with that of codons adjacent to stop codons (24). In this study we have analysed the 5′ contexts of stop codons in human genes (3 nucleotide positions immediately upstream of the stop codons) and compared them with those of stop-like codons: UGG, UGC, UGU and CGA for UGA; CAA, UAU, UAC for UAA; and UGG, UAU, UAC and CAG for UAG. The distribution of codons immediately prior to the stop codons was compared with that of codons immediately upstream of the respective stop-like codons. We propose that this method of comparison should enable us to highlight specific 5′ context features of a stop codon that presumably make it efficient in termination and to eliminate aspects specific for a general codon

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type. The choice of the stop-like codons was justified in Materials and Methods (see also ref. 25). We have used this approach to analyse stop codon-specific 5′ contexts for *E.coli* genes (25). In the earlier study (25), we demonstrated the advantage of comparing the 5′ contexts of stop codons with those of stop-like codons. For example, we confirmed earlier data that C is over-represented in position –1 upstream of *E.coli* UGA codons (26), if compared with the total occurrence of C at the same position upstream of all *E.coli* codons. Nevertheless, from our data (25) it followed that the preference of C was also well manifested prior to UGA-like codons (UGG, UGC, UGU, as well as AGA and GGA). Moreover the difference between the frequency of C immediately upstream of UGA and that of C immediately upstream of UGG, UGC and UGU was not significant (25). Therefore we concluded that 'high C' in position –1 was the general feature of a certain codon type and was not peculiar to the UGA stop codon (25).

MATERIALS AND METHODS

Software

All programs were written in FORTRAN 77 and were run on a MicroVax 2 under VMS 4.7. The programs are available to the reader on request.

Codon context database

Human sequences were taken from the database of primate genes created by Wada *et al.* (27) for calculation of codon usage for each individual gene. Access to the database of primate genes was available by ftp server from DNA DATA BANK OF JAPAN (DDBJ). Originally primate sequences were obtained from the GenBank Genetic Sequence Data Bank (Release 69.0) (27) and were collected by Wada *et al.* in a file named as PRI.lig.69. This file contained complete primate coding DNA sequences (CDS) that were used for calculation of codon usage and listed in file PRI.SDR.69 (27). On the other hand, PRI.lig.69 included primate sequences that did not start with an initiator codon. Complete human CDSs listed in PRI.SDR.69 were extracted from PRI.lig.69 and used in this study. The database of human CDSs was composed of 2559 sequences. The total number of codons was 1 097 990.

Stop-like codons

The choice of stop-like codons was based on two criteria. The first criterion was a chemical similarity of stop-like codons with stop codons, where any single purine-purine or pyrimidine-pyrimidine substitution was permitted. By applying this criterion, UGG and CGA were chosen as UGA-like codons, CAA was selected as a UAA-like codon and UGG and CAG were UAG-like codons. The comparison of stop codon context with contexts of stop-like codons selected in this way was expected to identify stop-codon specific contexts and to eliminate features of a general codon type.

Stop-like codons selected according to the second criterion were: UGG, UGU and UGC for UGA and UAC and UAU for both UAA and UAG. Using this criterion, stop codon contexts were compared with the contexts of stop-like codons to indicate context features that presumably prevent readthrough of stop codons, thereby making them efficient stop signals. The second criterion was based on a proposed rule for codon reading, namely 'two out of three' (28). According to this rule only the first two nucleotides of the codon are important for codon–anticodon interaction and experiments supporting this have been done. For instance the $tRNA^{Val}$ anticodons U^*AC, GAC and IAC each could recognize all four Val codons *in vitro* (29). By this rule UGA may be read by $tRNA^{Trp}$ and by $tRNA^{Cys}$ [$tRNA^{Trp}$ normally recognizes UGG and $tRNA^{Cys}$ recognizes UG(U/C)]. Indeed it has been shown that wild-type $tRNA^{Trp}$ from *E.coli* can read the UGA *in vitro* (30). It is plausible to suggest that the natural 5′ context of UG(G/U/C) promotes polypeptide elongation at these codons. Therefore, we can expect that any frequent nucleotides (or codons) upstream of UG(G/U/C) serve as a tool to make elongation at these codons faster. Hence, we should see significantly less of those nucleotides or codons upstream of UGA codons, whose level of elongation (readthrough) has to be very low. A similar argument can be made for the analysis of UAA and UAG contexts based on the second criterion.

In summary, we have developed a relevant method for analysis of stop codon contexts, by comparing frequencies of nucleotides (or of codons) upstream of stop codons with those upstream of stop-like codons. By comparing 5′ contexts of stop codons with those of stop-like codons selected with the first criterion, we were able to focus on stop codon-specific features and to eliminate aspects specific for a general codon type. Using the second criterion, 5′ contexts of stop codons were compared with those of stop-like codons to indicate stop codon context features that presumably prevented the readthrough of stop codons. We considered nucleotides (or codons) as a 5′-context feature of an efficient stop codon if they were either over- or under-represented in comparison with the same nucleotides (or codons) at the same positions 5′ to all respective stop-like codons.

Statistical analysis of the 5′-codon context

Deviation of the observed values from the expected ones was estimated by Chi squared (χ^2) criterion as described earlier (25). As a rule, the significance level was either $P<0.01$ or $P<0.005$. The χ^2 was not calculated if an expected value was ≤ 2.

RESULTS

Nucleotide distribution in the three positions upstream of stop codons

Table 1 indicates that G is under-represented upstream of UAA in positions –2 and –3 and U is over-represented before UAG in position –3. On the other hand, nucleotide frequencies in the three positions 5′ to UGA are not significantly different from those in the same positions 5′ to one (or more) UGA-like codons (Table 1).

Non-random usage at the last sense codon

UUU (Phe), AGC (Ser), and the Lys and Ala codon families are over-represented upstream of UGA (Table 2A). AAG (Lys), GCG (Ala), and the Ser and Leu codon families are over-represented upstream of UAA (Table 2C). UCA (Ser), AUG (Met), and the Phe codons are over-represented before UAG (Table 2E). The Gly codon family is under-represented prior to UGA and UAA (Table 2B and D). The Thr codon family is under-represented upstream of UGA (Table 2B) and ACC (Thr) is under-represented upstream of UAG (Table 2F).

Table 1. The χ^2 values for each nucleotide in the three positions prior to stop codons compared with the expected values from the same positions 5′ to the stop-like codons

The context, prior to

		UGA						UAA						UAG					
cod	pos	A	C	G	U	[χ^2]	cod	A	C	G	U	[χ^2]	cod	A	C	G	U	[χ^2]
ugg	-3	0	3	0	1	4	caa	3	1	*6**	4	**14**	ugg	1	6	3	**13**	**23**
	-2	**13**	5	3	0	**21**		2	0	*15*	2	**19**		3	0	*9*	**23**	**35**
	-1	1	6	2	6	**15**		0	0	1	1	2		3	*11*	1	**40**	**55**
ugc	-3	0	2	2	0	4	uau	1	0	*15*	**16**	**32**	uau	0	1	*10*	**32**	**43**
	-2	**11**	2	3	1	**17**		2	0	*16*	2	**20**		8	6	5	**9**	**28**
	-1	**13**	*27*	4	**34**	**78**		2	4	0	2	8		2	1	1	0	4
ugu	-3	6	**16**	3	2	**27**	uac	0	0	*10*	8	**18**	uac	0	1	6	**20**	**27**
	-2	4	0	0	5	9		2	1	*28*	7	**38**		8	7	*12*	**17**	**44**
	-1	6	1	2	0	9		**42**	*44*	0	**43**	**129**		5	*13*	0	**17**	**35**
cga	-3	0	1	2	0	3							cag	4	*10*	6	**24**	**44**
	-2	**16**	0	2	*9*	**27**								1	1	7	4	13
	-1	2	4	**19**	*32*	**57**								4	0	*9*	**14**	**27**

The χ^2 values for each nucleotide are bold-typed if the nucleotide frequency is significantly higher than expected. If the nucleotide frequency is significantly lower than expected the χ^2 values are in italics (significance level $P<0.005$, one degree of freedom).
The total χ^2 values [χ^2] are estimated with three degrees of freedom (bold-typed if $P<0.005$). cod, stop-like codons. pos, position number.
* $P < 0.02$

DISCUSSION

The non-random distribution of certain nucleotides and codons prior to stop codons in human genes has been revealed in this work. In principle, codon usage at the last sense codon position might be influenced by: (i) the necessity of achieving efficient hydrolysis of peptidyl-tRNA at stop codons; (ii) the need to decrease readthrough of a stop codon by normal tRNAs; (iii) the requirement for sufficient stability of the C-terminus of the given protein (or of the whole molecule). Efficient peptide chain termination implies fast hydrolysis of peptidyl-tRNA and slow readthrough of a stop codon. No significant deviations from the expected values in the usage of the majority of codons were noticed in this and in the similar study for *E.coli* (25). However, codons for a few amino acids exhibited significantly different occurrences (see below).

Over- and under-represented codons prior to human stop codons fall into three groups: (i) significant increase (or decrease) in codon usage can be visualized only for a whole codon set for a particular amino acid, but not for any one of the member codons; (ii) significant increase (or decrease) is seen for a whole codon set for a given amino acid as well as for one or more of the member codons; (iii) an over-represented (or an under-represented) codon does not change significantly the frequency for the respective amino acid. The examples of over- and under-represented codons for each of these groups are given below.

Group 1. The Lys codon family is over-represented and the Gly codon family is under-represented prior to UGA (Table 2A and B).

Group 2. The over-represented Ala codon family before UGA, the over-represented families of Ser and Leu codons 5′ to UAA and over-represented Phe codons prior to UAG are the examples of this group (Table 2A, C and E). We also showed the paucity of Thr codons and Gly codons 5′ to UGA and UAA respectively (Table 2B and D). In this group the preference or paucity is well manifested not only for the whole codon set for an amino acid but also for one or more of the member codons. For instance, GCU (Ala) is over-represented 5′ to UGA and GGC (Gly) is under-represented prior to UAA (Table 2A and D).

Group 3. Over-represented AAG (Lys) and GCG (Ala) upstream of UAA fall in this group (Table 2C). It has been noticed, that AAG (Lys) is the abundant triplet immediately upstream of termination codons for vertebrates and invertebrates (24). Over-represented UUU (Phe) and AGC (Ser) upstream of UGA as well as over-represented UCA (Ser) and under-represented ACC (Thr) before UAG also belong to this group (Table 2A, E and F).

The 5′ codon contexts of termination codons for *E.coli* and humans are compared in Table 3. It is seen that Lys codons are over-represented upstream of UAA in *E.coli* whereas the Lys codon AAG and the Lys codon family are over-represented 5′ to UAA and UGA respectively in human cells. The families of Phe and Ser codons are over-represented upstream of UGA in *E.coli* and, quite remarkably, Phe codons are over-represented before UAG and the Ser codon family is over-represented upstream of UAA in humans. Moreover, AGC (Ser) and UCA (Ser) are

Table 2. Non-randomness in usage of the last sense codon prior to stop codons

Codon(s)/Amino Acid	Statistics										
A. Over-represented codons prior to UGA											
	Obs	Exp*	χ^2	Exp UGG	χ^2	Exp UGU	χ^2	Exp UGC	χ^2	Exp CGA	χ^2
UUU/Phe	25	19.4	1.6	11.5	**15.8**	13.7	**9.2**	7.0	**46.6**	15.1	**6.6****
AGC/Ser	52	23.1	**36.1**	33.8	**9.8**	36.4	**6.7****	32.7	**11.3**	22.9	**37.0**
GCU/Ala	35	23.3	**5.9****	14.7	**27.9**	15.1	**26.3**	11.4	**48.6**	19.7	**11.9**
All/Ala	120	86.9	**12.6**	77.4	**23.4**	75.4	**26.3**	80.0	**20.0**	73.7	**29.1**
All/Lys	102	70.2	**14.4**	68.6	**16.3**	77.4	**7.9****	57.1	**35.4**	65.9	**19.8**
B. Under-represented codons prior to UGA											
ACC/Thr	8	27.8	*14.1*	46.4	*31.8*	43.1	*28.6*	58.1	*43.2*	25.7	*12.2*
All/Thr	31	68.8	*20.8*	81.2	*31.0*	83.7	*33.1*	94.8	*43.0*	63.9	*16.9*
All/Gly	41	88.2	*25.3*	77.5	*17.2*	64.5	*8.6*	79.5	*18.6*	85.4	*23.1*
C. Over-represented codons prior to UAA.											
	Obs	Exp*	χ^2	Exp UAC	χ^2	Exp UAU	χ^2	Exp CAA	χ^2		
UCA/Ser	16	7.3	**10.3**	2.9	**60.8**	6.1	**16.2**	6.8	**12.5**		
All/Ser	79	55.9	**9.6**	60.0	**6.0****	53.9	**11.7**	53.8	**11.8**		
UUA/Leu	22	4.1	**78.3**	3.0	**122.4**	4.7	**64.4**	6.9	**32.8**		
All/Leu	102	73.0	**11.6**	64.8	**21.4**	65.0	**21.1**	77.4	**7.8****		
AAG/Lys	41	26.7	**7.6****	24.3	**11.5**	26.5	**8.0**	23.0	**14.0**		
GCG/Ala	10	5.6	**3.6**	2.5	**23.1**	2.1	**30.2**	2.0	-		
D. Under-represented codons prior to UAA											
GGC/Gly	4	19.3	*12.1*	36.2	*28.6*	24.2	*16.8*	15.0	*8.1*		
All/Gly	14	54.5	*30.1*	59.1	*34.3*	56.2	*31.6*	50.9	*26.8*		
E. Over-represented codons prior to UAG											
	Obs	Exp*	χ^2	Exp UAC	χ^2	Exp UAU	χ^2	Exp CAG	χ^2	Exp UGG	χ^2
UCA/Ser	14	5.5	**13.3**	2.1	**66.3**	4.5	**19.7**	4.0	**25.1**	4.0	**24.7**
AUG/Met	23	12.7	**8.4**	9.5	**19.0**	13.5	**6.7****	10.9	**13.4**	11.9	**10.4**
UUU/Phe	18	9.0	**9.1**	5.1	**32.4**	9.2	**8.3**	5.2	**31.6**	5.3	**30.3**
UUC/Phe	35	12.6	**40.0**	20.6	**10.2**	15.2	**25.8**	19.5	**12.3**	17.0	**19.2**
All/Phe	53	21.5	**46.0**	25.7	**29.1**	24.5	**33.3**	24.7	**32.4**	22.3	**42.3**
F. Under-represented codons prior to UAG											
ACC/Thr	1	12.8	*10.9*	19.7	*17.7*	15.6	*13.6*	15.0	*13.0*	21.4	*19.5*

The observed frequencies (Obs), the expected frequencies (Exp) and χ^2 values are listed for codons.
The expected values are taken from the codon distribution immediately prior to a stop-like codon.
A significant increase in positions before stop-codons is bold-typed, a decrease, in italics (significance level $P<0.005$). The deviations from expected values were estimated with one degree of freedom.
*The expected values were calculated from the average codon usage.
**$P<0.02$.

over-represented before UGA and UAG respectively in humans. Furthermore UUU (Phe) is over-represented 5′ to UGA in humans. The Thr codon family is under-represented upstream of both UAA and UGA in *E.coli* whereas in humans the family of Thr codons is under-represented before UGA and the Thr codon ACC is under-represented prior to UAG. It is clear from these kinds of data that human and *E.coli* mRNAs tend to utilize at the last codon position the codons for Lys, Phe and Ser. On the other hand, both human and *E.coli* mRNAs tend to avoid Thr codons upstream of UGA (see Table 3). From the analysis of nucleotide distribution in three positions upstream of stop codons in both *E.coli* (25) and human CDSs (Table 1) it follows that U is over-represented in position –3 upstream of UGA in *E.coli* (25) and upstream of UAG in humans (Table 1, this study). At the same time, there are some features peculiar to the context of human stop codons as for example, the paucity of Gly codons upstream of UAA and UGA (Table 3).

Table 3. The over- and under-represented codons upstream of stop codons in *E.coli* and humans

E.coli	Humans
A. Over-represented codons prior to UAA	
AAA(Lys)	AAG (Lys)
AAG(Lys)	UCA(Ser)
Lys codon family	Ser codon family
	UUA(Leu)
	Leu codon family
	GCG (Ala)
B. Over-represented codons prior to UGA	
UUC(Phe)	UUU(Phe)
Phe codon family	AGC(Ser)
UCC(Ser)	GCU(Ala)
Ser codon family	Ala codon family
	Lys codon family
C. Over-represented codons prior to UAG	
ND*	UUU(Phe)
	UUC(Phe)
	Phe codon family
	UCA(Ser)
	AUG(Met)
D. Under-represented codons prior to UAA	
Thr codon family	GGC(Gly)
Pro codon family	Gly codon family
E. Under-represented codons prior to UGA	Gly codon family
Thr codon family	Thr codon family
	ACC(Thr)
F. Under-represented codons prior to UAG	
ND*	ACC(Thr)

The data for *E. coli* 5′ context were taken from ref. 25.
*Not determined due to the low number of sequences (25).

A single polypeptide, eRF1, recognizes all three stop codons in eukaryotic organisms (1). It has been proposed (23), that eRF1 recognizes a tetranucleotide, i.e. the stop codon and the first downstream nucleotide. Consistent with this hypothesis, recent *in*

vivo and *in vitro* experiments suggest that mammalian termination is modulated by the base immediately downstream of the stop codon (31). For *E.coli* the base following a stop codon has been shown to affect the selection rates of release factors in an *in vivo* termination/frameshift assay (32,33). On the other hand the selection rates of an amber suppressor tRNA and the $tRNA^{Trp}$ were shown to be affected by the first downstream nucleotide after UAG (32) and UGG (33) respectively. On the basis of our context analysis of *E.coli* (25) and human stop codons we can speculate that the immediate 5′ specific context of stop codons may modulate the efficiency of peptide chain termination and (or) the efficiency of stop codon readthrough in both prokaryotes and higher eukaryotes. The mechanism of such a modulation may be very similar among living organisms from *E.coli* to humans.

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