Volume 9 Number 4 1981 Nucleic Acids Research

UGA suppression by normal $tRNA^{Trp}$ in Escherichia coli: codon context effects

Hanna Engelberg-Kulka

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

Received 9 December 1980

ABSTRACT

The nucleotide sequences at the 3' side of in-phase UGA termination codons in mRNAs of various prokaryotic genes were re-examined. An adenine (A) residue is found to be adjacent to the 3' side of UGA in mRNAs which code for readthrough proteins by the suppression of UGA by normal Escherichia coli $tRNA^{Trp}$. It is suggested that the nature of the nucleotide following a UGA codon determines whether the UGA signals inefficiently or efficiently the termination of polypeptide chain synthesis: an A residue at this position permits the UGA readthrough process.

INTRODUCTION

The termination of polypeptide chain synthesis is signalled by three codons, UAG (amber), UAA (ochre), and UGA (opal). Termination can be suppressed by suppressor tRNAs which are able to translate one or more of the termination codons by inserting an amino acid at the corresponding point in the nascent polypeptide (1). In prokaryotes, amber and ochre suppression is caused by mutations leading to suppressor tRNAs: the base sequences of the tRNAs' anticodon is altered, permitting the formation of three base pairs with the UAA and/or UAG codons (2,3). This corroborates with standard anti-parallel base pairing between codon and anticodon: the first two positions of the codon are read by the anticodon strictly according to the rules of classic base pairing (A-U; G-C); the third position of the codon hydrogen-bonds with the first position of the anticodon (wobble position) according to the rules of the wobble hypothesis (4). On the other hand, with the exception of the $tRNA^{Arg}$ UGA suppressor of Escherichia coli (E. coli) phage T4 (5), UGA suppression cannot be explained by these rules of codon-anticodon recognition. UGA is suppressed at low frequency by the normal tryptophanyl-$tRNA^{Trp}$ ($tRNA^{Trp}$) of E. coli (6-10). This tRNA carries an anticodon, CCA, which can hydrogen-bond only to the first two nucleo-

tides in the UGA codon and not to the third, because the pairing A-C is not allowed even in the wobble position (4). UGA is also efficiently suppressed by a suppressor $tRNA^{Trp}$, induced by mutagenesis (6,11), which has no change in the nucleotide sequence of the anticodon CCA but rather a base change in the stem of the dihydrouracil loop (12). It was suggested (12) that this change, outside the anticodon sequence, enhances codon-anticodon recognition, leading to suppression of UGA by the suppressor mutant $tRNA^{Trp}$. The ability of the normal $tRNA^{Trp}$ of E. coli to misread the UGA codon has not yet been explained.

From recent studies on mRNA-tRNA recognition patterns, we can gain new insight into the problem: how can normal $tRNA^{Trp}$ suppress the UGA terminator codon. Results of several studies (13-20) indicate that codon recognition may be influenced by base sequences surrounding the codon in the mRNA. This influence was described as the effect of codon context on genetic code translation. Furthermore, there is direct evidence that the nucleotide adjacent to the 3' side of either the AUG initiation codon (21) or the UAG termination codon (22) has a role in determining the efficiency of translation. In this paper I ask whether the nucleotide sequence following UGA in the mRNA (codon context effect) can also explain the in vivo suppression of the UGA termination codon by normal E. coli $tRNA^{Trp}$.

RESULTS AND DISCUSSION

I re-examined the nucleotide sequences at the 3' side of in-phase UGA termination codons in mRNAs of various prokaryotic genes. Tables 1 and 2 include the sequenced prokaryotic mRNAs with in-phase UGA codons. This information was gathered from the literature published before August 1980. I divided the mRNAs into two groups: In the first group, the UGA codons are followed by an adenine (A) residue (Table 1); in the second group, the UGA codons are followed by a uracil (U), cytosine (C) or guanine (G) residue (Table 2). The mRNA of prokaryotic genes which specify a UGA readthrough protein are included in the first group, and I shall discuss them here.

The UGA readthrough protein whose in vivo synthesis has been best characterized is the minor capsid protein of E. coli RNA phage Qβ. The major Qβ capsid protein, the coat protein, and the minor capsid protein IIb, are translated from a common initiation site (7-9). The Qβ coat protein cistron is terminated by a UGA termination codon which is suppressed at low frequency by normal $tRNA^{Trp}$ of E. coli (10). As a result, translation may

Gene	UGA and following codon	The first in-phase stop codon after UGA[b]	References
Phage Qβ coat	UGAACX[a]	UAG (app. 200)	10
Phage λ gene O	UGAAAA	UAG (34)	23
Filamentous phages (fd, M13, f1) gene IX	UGAAAA	UAG (3)	24,25,26
E. coli trpR (aporepressor)	UGAAAG	UGA (15)[c]	27
E. coli trp leader	UGAAAC	UAA (8)	28
E. coli phe leader	UGAAUG	UAA (15)	29
Phage G4 gene C	UGAAAU	UAA (2)	30

Table 1. Prokaryotic gene mRNAs: UGA terminator codon followed by an adenine (A) residue. The A residue following UGA is underlined.

[a] This codon represents one of four possible threonine codons as derived from the amino acid sequence of this region (see text); the third nucleotide is unknown.

[b] The number in brackets represents the number of codons between UGA and the first in-phase termination codon following it.

[c] A third in-phase UGA follows this second in-phase UGA by 25 codons; there is an A residue on the 3' side of all three UGA termination codons.

proceed for about another 600 nucleotides, and is finally terminated at a double stop signal, UAGUAA, permitting the formation of a small amount of IIb protein during phage infection (41). The nucleotide sequence immediately following the coat cistron UGA terminator has not yet been sequenced; however, as can be derived from the partial amino acid sequence of IIb (10), the tryptophan residue which results from UGA suppression is followed by a threonine residue. Since all threonine codons start with the nucleotide A, it follows that an A residue is present at the 3' side of the UGA termination codon of the Qβ coat cistron (Table 1). Another characterized UGA readthrough protein is the λ O' protein which is the continuation of the O gene product of the E. coli DNA phage lambda (λ). The protein λ O' is produced in an in vitro λ DNA directed protein synthesizing system (42) and is presumably also synthesized in vivo (43). As in the case of the Qβ coat cistron, the UGA termination codon of the mRNA of the λ O gene is also followed by an A residue (Table 1).

We isolated a streptomycin-resistant E. coli mutant, designated LD1, which is temperature-sensitive for UGA suppression by normal $tRNA^{Trp}$ (43-47). When this mutant is infected by Qβ at high temperatures the UGA readthrough protein IIb is not synthesized and non-infectious particles are formed (45).

Gene	UGA and following codon	References
Phage ϕX174 genes A and A*	UGAGAA	31
Phage ϕX174 gene C	UGAGUC	31
Phage ϕX174 gene E	UGAUGU	31
Phage ϕX174 gene G	UGAGGU	31
Phage ϕX174 gene K	UGACGC	31
Phage G4 genes A and A*	UGAGGA	30
Phage G4 gene E	UGAUUU	30
Phage G4 gene K	UGACGC	30
Filamentous phages (fd,M13,f1) gene VII	UGAGUG	24,25,26
Phage λ gene cI	UGAUCG	32,33
Phage λ gene cII	UGAGGU	34,35
Phage λ gene xis	UGAGCG	36
E. coli trpB	UGAUGG	37
E. coli lacI (repressor)	UGAGCG	38
E. coli thr leader	UGACGC	39
E. coli transposon 3 (repressor)	UGAUAC	40

Table 2. Prokaryotic gene mRNAs: UGA terminator codons not followed by an adenine (A) residue. Only UGA termination codons not immediately followed by a second termination codon are included.

In addition, out of all phages tested by us so far, only the growth of Qβ, λ and the filamentous phages (fd, f1 and M13) is inhibited in the mutant at 42°C (43). Based on this selective inhibition in the mutant we suggested that like Qβ and λ also the filamentous phages specify a UGA readthrough protein (43). It is interesting that as in the case of Qβ coat cistron and λ O mRNA, the mRNA of gene IX of the filamentous phages also ends with a UGA codon followed by an A residue (Table 1).

I assume that the presence of the nucleotide A at the 3' side of UGA permits *in vivo* suppression of the UGA termination codons by normal $tRNA^{Trp}$, and that UGA codons not followed by an A residue are not involved in UGA readthrough. This assumption is supported by our experimental results with *E. coli* K12 single-stranded DNA phage ϕK. Phage ϕK (not yet sequenced) is of particular interest because of its similarity to *E. coli* single-stranded DNA phage ϕX174 (48) whose DNA is rich in genes (6 of 11) which use the UGA terminator (31). (We chose to work with phage ϕK because it grows on *E. coli* K12 while ϕX174 does not). Growth of ϕK at 42°C is unaffected in

E. coli mutant LD1 (43). This was surprising since we expected many phage øK genes to use the UGA terminator, as in the case of øX174 mRNAs. In none of the øX174 mRNAs, however, is there an A residue after the UGA terminator (Table 2). Although several øX174 genes ending with TGA specify multiple gene products in vivo, they have not been confirmed as UGA readthrough polypeptides; alternatively, these gene products may arise during translation due to multiple initiation sites (49).

My assumption is further supported by our recent studies on the regulation of trp operon enzymes in E. coli LD1 and in another mutant (LD2) temperature-sensitive for UGA suppression by normal $tRNA^{Trp}$ (47). We found that with an excess of tryptophan causing maximally repressing conditions in the parental strain Q13, at increased temperatures LD1 and LD2 exhibit derepression of the synthesis of trp operon enzymes. When our mutants are starved for tryptophan, causing maximally derepressed conditions, formation of trp enzymes is not affected by increased temperatures. Thus, in these mutants increased temperatures selectively affect repression of trp enzyme production, and not their synthesis per se. We used E. coli strains carrying a functional trpR gene, so these results may reflect the requirement of the UGA readthrough process for the synthesis of an active Trp aporepressor, the product of gene trpR. The trpR gene was recently sequenced (27); the trpR mRNA ends with the termination codon UGA. Downstream from this UGA codon, three additional in-phase termination codons are present: two UGA codons and one UAA codon which follow the first UGA by 16, 41 and 51 codons, respectively. The trpR gene, therefore, is potentially able to direct the synthesis of four polypeptides of which three are UGA readthrough products. Note that all three UGA codons are followed by an A residue (Table 1 and ref. 27). Since the active Trp aporepressor has been only partially purified (27,50), direct evidence is lacking that UGA readthrough products of the trpR gene are really synthesized. Based on our studies with E. coli mutants temperature-sensitive in UGA readthrough, together with the sequence data of the trpR gene, we assume that the fully active Trp aporepressor includes at least one UGA readthrough polypeptide. We suggested that the UGA readthrough process has a role in the autogenous regulation of the synthesis of the Trp aporepressor (47). In addition, an A residue adjacent to the 3' side of an in-phase UGA termination codon is also present in the leader RNAs of the trp and phe operons of E. coli, and in gene C of E. coli single-stranded DNA phage G4 (Table 1). We are presently looking for the involvement of UGA readthrough in the translation of these genes.

I suggest that the nature of the nucleotide adjacent to the 3' side of a UGA codon determines whether this codon signals the termination of polypeptide synthesis efficiently or inefficiently. In particular, the presence of an A residue adjacent to the 3' side of a UGA codon enables low frequency suppression of termination due to the readability of UGAA by normal $tRNA^{Trp}$ in E. coli (and probably also in other prokaryotic systems). I base my hypothesis on the following: 1) re-examination of the nucleotide sequences at the 3' side of in-phase UGA termination codons in mRNAs of various genes of prokaryotes; 2) the available information for the involvement of the UGA readthrough process in the translation of mRNAs where the UGA termination codon is followed by an A residue. This hypothesis is further supported by the recent elegant experiments of Bossi and Roth (22) showing that the reading efficiency of the UAG codon by amber suppressor tRNA supE of Salmonella typhimurium is significantly increased due to the alteration to A of the nucleotide following UAG. Also, Taniguchi and Weissmann showed (21) that the presence of an A residue at the 3' side of the initiation codon increases the ribosome binding capacity of the RNA phage Qβ coat cistron in the presence of fmet-$tRNA_f^{Met}$. The wild type base sequence in the initiation region of the coat cistron is AUGG. When mutations are induced in this region creating the sequences AUAG, AUGA and AUAA, the ribosome binding capacity changes to <0.1, 3.2, and 0.3 of the wild type capacity, respectively.

Several hypotheses have been proposed to explain the effect on the mRNA-tRNA recognition pattern of the nucleotide A adjacent to the 3' side of particular codons (21,22). The explanation of Taniguchi and Weissmann (21) for the recognition by fmet-$tRNA_f^{Met}$ of AUAA (double mutation in the initiation region of Qβ coat cistron) might also explain the low frequency recognition of UGAA by normal E. coli charged $tRNA^{Trp}$. Like AUA with the anticodon CAU of $tRNA_f^{Met}$, the codon UGA can hydrogen-bond to only two nucleotides of its anticodon CCA of $tRNA^{Trp}$. Invariantly adjacent to the 5' side of tRNA anticodons is a U residue (51) which can form an additional base pair, (A-U) with the A residue on the 3' side of the AUA or UGA codons. Since this base is separated from the other two by an unhydrogen-bonded pair, such an interaction would provide little stability in solution (21 and 52,53). It is more likely that the 3' side of the UGA codon increases the stacking energy of the codon-anticodon interaction, thus permitting the low frequency suppression of UGA by $tRNA^{Trp}$ (21 and 54).

In addition to its effect on normal E. coli $tRNA^{Trp}$, I also assume that an A residue following UGA may affect the readability of UGA by the

E. coli mutant suppressor $tRNA^{Trp}$ already discussed. The site at which UGA nonsense codons are formed by mutation would also affect the readability of UGA by a suppressor tRNA, since the appearance of an A residue would permit increased suppression.

That a normal $tRNA^{Trp}$ can misread the UGA termination codon provides a regulatory mechanism for gene expression: 1) It permits the production of more than one polypeptide in different amounts from a single gene; 2) as the conformation of mRNA is affected by the movement of the ribosome along its length (55-57), the occasional suppression of the UGA stop signal would also affect mRNA conformation. I suggest that this regulatory mechanism of prokaryotic genes is permitted by the presence of an A residue at the 3' side of an in-phase UGA codon. Such a codon context effect might also permit UGA readthrough of eukaryotic mRNAs. Unfortunately, with the little information available, it is difficult to draw analogy between eukaryotic and prokaryotic systems, especially because there are obvious differences between them. For example, nucleotide sequences in several parts of $tRNA^{Trp}$ are dissimilar in the two systems, in particular the anticodon of the $tRNA^{Trp}$ of eukaryotes is CmCA, while that of prokaryotes, at least in E. coli, is CCA (51). Though the differences in eukaryotic and prokaryotic systems may generate different rules for UGA readthrough context effects, nevertheless, the presence of a particular nucleotide at the 3' side of the UGA termination codon may be a regulatory factor in gene expression in both systems.

ACKNOWLEDGMENTS

I thank Dr. R. Schoulaker-Schwarz and F.R. Warshaw for reading the manuscript.

This research was supported by a grant from the United States - Israel Binational Science Foundation (BSF).

REFERENCES

1. Garen, A. (1968) Science 160, 149-159.
2. Korner, A.M., Feinstein, S.I. and Altman, S. (1979) in Transfer RNA, Altman, S. Ed., pp. 105-135. MIT Press, Cambridge, Massachusetts.
3. Smith, J.D. (1979) in Nonsense Mutations and tRNA Suppressors, Celis, J.E., and Smith, J.D., Eds, pp. 109-125, Academic Press.
4. Crick, F.H.C. (1966) J. Mol. Biol. 19, 548-555.
5. Kao, S-H., and McClain, W.H. (1977) J. Biol. Chem. 252, 8254-8257.
6. Hirsh, D., and Gold, L. (1971) J. Mol. Biol. 58, 459-468.

7. Horiuchi, K., Webster, R.E., and Matsuhashi, S. (1971) Virology 45, 429-439.
8. Moore, C.H., Farron, F., Bohnert, D., and Weissmann, C. (1971) Nature New Biol. 234, 204-206.
9. Weiner, A.M., and Weber, K. (1971) Nature New Biol. 234, 206-209.
10. Weiner, A.M., and Weber, K. (1973) J. Mol. Biol. 80, 837-855.
11. Chan, T-S., Webster, R.E., and Zinder, N.D. (1971) J. Mol. Biol. 56, 101-116.
12. Hirsh, D. (1971) J. Mol. Biol. 58, 439-458.
13. Salser, W. (1969) Molec. gen. Genet. 105, 125-130.
14. Yahata, H., Ocada, Y. and Tsugita, A. (1970) Molec. gen. Genet. 106, 208-212.
15. Akaboshi, E., Inouye, M., and Tsugita, A. (1976) Molec. gen. Genet. 149, 1-4.
16. Colby, D.S., Schedl, P., and Guthrie, C. (1976) Cell 9, 449-463.
17. Feinstein, S.I., and Altman, S. (1977) J. Mol. Biol. 112, 453-470.
18. Fluck, M.M., Salser, W. and Epstein, R.H. (1977) Molec. gen. Genet. 151, 137-149.
19. Feinstein, S.I., and Altman, S. (1979) Genetics 88, 201-219.
20. Fluck, M.M., and Epstein, R.H. (1980) Molec. gen. Genet. 177, 615-627.
21. Taniguchi, T., and Weissmann, C. (1978) J. Mol. Biol. 118, 535-565.
22. Bossi, L., and Roth, J.R. (1980) Nature 286, 123-127.
23. Scherer, G. (1978) Nucleic Acid Res. 5, 3141-3155.
24. Beck, E., Sommer, R., Auerswald, E.A., Kurz, Ch. Zink, B., Osterburg, G., Schaller, H., Sugimoto, K., Sagisaki, H., Okamoto, T., and Takanami, M. (1978) Nucleic Acid Res. 5, 4495-4503.
25. Huselbos, T., and Schoenmakers, J.G.G. (1978) Nucleic Acid Res. 5, 4677-4698.
26. Hill, D.F., and Petersen, G.B. (1980) J. of Virol. 34, 40-50.
27. Singleton, C.K., Roeder, W.D., Bogosian, G., Somerville, R.L., and Weith, H.L. (1980) Nucleic Acid Res. 8, 1551-1560.
28. Bertrand, K., Korn, L., Lee, F., Platt, T., Squires, C.L., Squires, C., and Yanofsky, C. (1975) Science 189, 22-26.
29. Zurawski, G., Brown, K., Killingly, D., and Yanofsky, C. (1978) Proc. Nat. Acad. Sci. USA 75, 4271-4275.
30. Godson, G.N., Barrell, B.G., Staden, R., and Fiddes, J. (1978) Nature 276, 236-247.
31. Sanger, F., Coulson, A.R., Friedmann, T., Air, G.M., Barrell, B.G. Brown, N.L., Fiddes, J.C., Hutchison III, C.A., Slocombe, P.M., and Smith, M. (1978) J. Mol. Biol. 125, 225-246.
32. Ptashne, M., Beckman, K., Humayun, M.Z., Jeffery, A., Maurer, R., Meyer, B., and Sauer, T. (1976) Science 194, 156-161.
33. Humayun, A. (1977) Nucleic Acid Res. 4, 2137-2143.
34. Rosenberg, M., Court, D., Shimatake, H., Brady, C., and Wulff, D.L. (1978) Nature 272, 414-423.
35. Schwarz, E., Scherer, G., Hobom, G., and Kössell, H. (1978) Nature 272, 410-414.
36. Davies, R.W. (1980) Nucleic Acid Res. 8, 1765-1782.
37. Platt, T., and Yanofsky, C. (1975) Proc. Nat. Acad. Sci. USA 72, 2399-2403.
38. Dickson, R.C., Abelson, J., Barnes, W.M., and Reznikoff, W.S. (1975) Science 187, 27-35.
39. Gardner, J. (1979) Proc. Nat. Acad. Sci. USA 76, 1706-1710.
40. Heffron, F., McCarthy, B.J., Ohtsubo, H., and Ohtsubo, E. (1979) Cell 18, 1153-1163.
41. Weissmann, C., Billeter, M.A., Goodman, H.M., Hindley, J., and Weber, H. (1973) Annu. Rev. Biochem. 42, 303-328.

42. Yates, J.L., Gette, W.R., Furth, M.E., and Nomura, M. (1977) Proc. Nat. Acad. Sci. USA 74, 689-693.
43. Engelberg-Kulka, H., Dekel. L., and Israeli-Reches, M., and Belfort, M. (1979) Molec. gen. Genet. 170, 155-159.
44. Engelberg-Kulka, H., Dekel, L., and Israeli-Reches, M. (1977) J. Virol. 21, 1-6.
45. Engelberg-Kulka, H., Israeli-Reches, M., Dekel, L., and Friedmann, A. (1979) J. Virol. 23, 1107-1117.
46. Zeevi, M., Daniel, V., and Engelberg-Kulka, H. (1979) Molec. gen. Genet. 170, 149-153.
47. Engelberg-Kulka, H., Dekel, L., and Israeli-Reches, M. (1981) Biochem. Biophys. Res. Commun. (In press).
48. Taketo, A. (1976) Molec. gen. Genet. 148, 25-29.
49. Pollock, T.J., Tessman, I., and Tessman, E.S. (1978) Nature 274, 34-37.
50. Squires, C.L., Lee, F.D., and Yanofsky, C. (1975) J. Mol. Biol. 92, 93-111.
51. Sprinzl, M., Grueter, F., Spelzhaus, H., and Gauss, D.H. (1980) Nucleic Acid Res. 8, r1-r22.
52. Gralla, J., and Crothers, D.M. (1973) J. Mol. Biol. 73, 497-511.
53. Tinoco, I., Jr, Borer, P.N., Dengler, B., Levine, M.D., Ulenbeck, O.C., Crothers, D.M., and Gralla, J. (1973) Nature New Biol. 246, 40-41.
54. Grosjean, H., Söll, D.G., and Crothers, D.M. (1976) J. Mol. Biol. 103, 499-519.
55. Lee, F., and Yanofsky, C. (1977) Proc. Nat. Acad. Sci. USA 74, 4365-4369.
56. Barnes, W.M. (1978) Proc. Nat. Acad. Sci. USA 75, 4281-4285.
57. Keller, E.B., and Calvo, J.M. (1979) Proc. Nat. Acad. Sci. USA 76, 6186-6190.