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Codon specificity of UGA suppressor $tRNA^{Trp}$ from *Escherichia coli*

(translational errors/conformational selection/wobble/nonsense suppressor)

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ABSTRACT A synthetic polyribonucleotide, poly(U_5,G), was used to study the codon specificity of wild-type and UGA suppressor $tRNA^{Trp}$ from *Escherichia coli*. Phe (UUU) incorporation directed by this synthetic messenger is reduced somewhat by omission from the incubation mixtures of Val (GUU), Leu (UUG), or Cys (UGU). In contrast, omission of Cys stimulates Trp incorporation, and this effect is much more pronounced with the UGA suppressor $tRNA^{Trp}$ than with wild-type tRNA. The apparent replacement of Cys by Trp is specific, because the omission of Val or Leu slightly inhibits Trp incorporation. These data suggest that the UGA suppressor $tRNA^{Trp}$ can translate codons of the form UGN (N is any ribonucleotide). In other words, the suppressor $tRNA^{Trp}$ translates codons that properly match two out of the three anticodon nucleotides.

An unusual mutant form of $tRNA^{Trp}$ that can translate the nonsense codon UGA as well as its normal codon UGG was described earlier by Hirsch (1) and Hirsch and Gold (2). This mutant tRNA was found, unexpectedly, to contain a normal anticodon loop, as well as a single base substitution in the D stem. Moreover, the mutant and the wild-type $tRNA^{Trp}$ could be shown to have indistinguishable affinities for both the UGG and UGA codons in the absence of the ribosome (3, 4). Because traditional views of tRNA selection during translation attribute all of the specificity of the interaction on the ribosome to the codon–anticodon interaction, the properties of this mutant tRNA were intially difficult to understand.

More recently, it has been suggested that tRNA selection on the ribosome involves both the anticodon and other domains in the tRNA molecule (5). Codon-dependent conformational changes of the tRNA are thought to position the anticodon as well as other sites on the tRNA in a three-dimensional configuration that is acceptable to the ribosomal amino acid acceptor (A) site. Such a view of tRNA selection accounts for the effects of D stem alterations on the codon specificity of the mutant tRNA as a change in the coupling between the codon–anticodon interaction and the conformation of the tRNA. In particular, this model suggests that such an alteration in the tRNA structure could permit a tRNA molecule to occupy the conformation favorable to A site binding when only two out of three of its anticodon bases are properly matched with the codon.

Accordingly, it might be anticipated that a mutant such as the $tRNA^{UGA}$ suppressor could translate the UGU and UGC codons as well as UGA and UGG. Both UGU and UGC code for cysteine. Therefore, we sought to determine whether or not the cysteine codon UGU can be translated by the UGA suppressor. Our results are that UGU can be translated and confirm this prediction of the conformational selection model.

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METHODS

Synthetic Polynucleotides. Poly(U,G) was prepared with polynucleotide phosphorylase (EC 2.7.7.8) in the usual manner (6). Initial substrate concentrations were 10 mM UDP and 1 mM GDP in 100 mM Tris·HCl, pH 9/4 mM $MgCl_2$. The product was precipitated with ethanol and purified on a column of Sephadex G-50 in 20 mM Tris·HCl, pH 8.0/5 mM $MgCl_2$. Base analysis was performed by hydrolysis in 0.3 M KOH and thin-layer chromatography of the products as described by Randerath and Randerath (7), using 1.6 M LiCl as solvent. A ratio for U to G of 5.2:1 was found for the product.

tRNA. Wild-type and UGA suppressor $tRNA^{Trp}$ were prepared from *Escherichia coli* as previously described (4). The unfractionated tRNA used in some experiments was oxidized with periodate (8), after being charged with Val, Leu, Cys, Phe, and Gly, using a mixture of aminoacyl-tRNA synthetases (9).

Translation *In Vitro*. Ribosomes were prepared as previously described from *E. coli* MRE 600 (10) and washed in 10 mM Tris·HCl, pH 7.5/10 mM $MgCl_2$/60 mM NH_4Cl/7 mM 2-mercaptoethanol. Supernatant proteins were prepared and freed from nucleic acids by chromatography on DEAE-cellulose (11). The *in vitro* translation was conducted in 60 mM Tris·HCl, pH 7.8/100 mM NH_4Cl/11 mM $MgCl_2$/1 mM ATP/0.4 mM GTP/4 mM phospho*enol*pyruvate/1 mM dithiothreitol/0.1 mM leucovorin/0.1 mM methionine, and contained per incubation (50 μl): 0.75 A_{260} unit of tRNA, 0.025 A_{260} unit of wild-type or suppressor $tRNA^{Trp}$, 1 μg of pyruvate kinase, 50 μg of supernatant proteins, 1.2 A_{260} unit of ribosomes, 0.11 A_{260} unit of poly(U_5,G), and 50 nmol of each amino acid except tryptophan, which was 10 nmol. (One A_{260} unit is the amount of material that gives an A_{260} of 1.0 when dissolved in 1 ml and the light path is 1 cm.) The incubations were for 30 min at 37° and were stopped by the addition of 1.5 ml of 10% trichloroacetic acid. In incubations containing labeled tryptophan, the precipitates were sedimented by centrifugation and resuspended in 1.5 ml of 10% trichloroacetic acid. This reduced background radioactivity. The acid precipitates were heated for 15 min at 90°, cooled, and collected on Whatman GF/C filters, washed with 10% trichloroacetic acid, dried, and assayed for radioactivity by liquid scintillation counting.

RESULTS

Characterization of the polynucleotide messenger

A random polymer was synthesized with a high ratio of U to G so that the G-containing codons in any reading frame would be predominantly UUG (Leu), UGU (Cys), and GUU (Val), with relatively few codons containing two Gs. Such a polymer, synthesized by polynucleotide phosphorylase from a mixture of UDP and GDP (10:1) was shown by base analysis of an alkaline hydrolysate to have a U-to-G ratio of 5.2:1.

Table 1. Amino acid incorporation directed by poly(U_5,G)

Labeled amno acid	Codon(s) present	Calculated relative abundance	Poly(U_5,G) pmol	Poly(U_5,G) % Phe	Poly(U) pmol	Poly(U) % Phe
Phe	UUU	100	157	100	417	100
Val	GUU, GUG	23	23.5	15	—	—
Leu	UUG	19	55	35	61	15
Cys	UGU	19	42	27	—	—
Gly	GUU, GGG	4.4	8	5	—	—
Trp	UGG	3.7	5.1	3.2	—	—

The *in vitro* system was as described in *Methods* and contained, in addition to other components, 0.11 A_{260} unit of poly(U_5,G) or 0.06 A_{260} unit of poly(U); 50 nmol each of Phe, Val, Leu, Cys, and Gly; and 10 nmol of Trp. One ^{14}C-labeled amino acid at a time was added. The calculated relative abundance of codons shown assumes a random sequence of U and G in the polymer. A — indicates incorporation was not significant (<5 pmol).

The polymer was further characterized by examining the incorporation of each amino acid corresponding to codons containing only U and G. The results (Table 1) show that the relative incorporation of these amino acids is approximately as expected for a messenger of random sequence and overall composition of 5.2 U/1 G. In fact, the sequence is probably not random because G incorporation is favored over U incorporation and the composition of the dinucleotide substrate pool must therefore change during synthesis of the polynucleotide. The high incorporation of Leu is probably due to misreading of UUU codons; Leu is the only amino acid present in the system that is misread significantly with poly(U) (Table 1).

Misreading of codons by Trp-tRNA

The effect of omitting amino acids was examined for the incorporation of Phe, which is the predominant amino acid in the product and serves as a measure of a total amino acid polymerization. The results (Table 2) show that omission of Leu, Val, or Cys does not greatly reduce the amount of Phe polymerized. Several factors may contribute to this. Thus, in the absence of the charged cognate tRNA, the corresponding codon might be read by noncognate tRNA sufficiently fast for this step not to be rate limiting. It is also possible that the cognate tRNA is mischarged under these conditions to an extent that is small but sufficient to permit elongation to continue. Finally, it is conceivable that the ribosomes detach from the message at nonreadable codons and reinitiate translation. Nevertheless, these data show that the omission of other amino acids did not stimulate Phe incorporation.

The possibility of misincorporation of Trp in response to the codons UUG, UGU, and GUU was investigated by leaving out of the *in vitro* translation system, one at a time, the corresponding amino acids, Leu, Cys, and Val. The tRNA used in the incubations was either unfractionated, untreated tRNA from UGA nonsuppressor bacteria or a mixture of tRNAs in which $tRNA^{Trp}$ had been selectively inactivated by periodate oxidation. These tRNAs were supplemented with either wild-type or suppressor $tRNA^{Trp}$. It can be seen (Table 2) that omission of Val or Leu from the incubation produces no significant increase in the amount of Trp incorporated into material precipitable by hot trichloroacetic acid. This indicates that neither codon UUG nor GUU can be read by Trp-tRNA at a detectable level in the system. However, omission of Cys increased Trp incorporation by 93%in the presence of the suppressor tRNA and by 28% in the presence of wild-type tRNA (see Table 2).

Table 2. Effect of the omission of different amino acids on phenylalanine or tryptophan incorporation directed by poly(U_5,G)

Omission	Phe incorporation, pmol	Trp incorporated, pmol Wild type	Trp incorporated, pmol Suppressor
None	467	13.1	12.3
Val	397	11.1	13.4
Leu	419	11.6	11.8
Cys	336	16.8	23.8
Trp	409		

The incubation mixtures (50 μl) contained 0.11 A_{260} unit of poly(U_5,G), 50 nmol each of Phe (either unlabeled or labeled with ^{14}C, 19.9 mCi/nmol), Val, Leu, Cys, and Gly (except as noted); and 10 nmol of Trp (unlabeled or labeled with ^{14}C, 52 mCi/nmol). Total tRNA (0.75 A_{260} unit) from wild-type cells, containing 12 pmol of $tRNA^{Trp}$, was supplemented with pure wild-type $tRNA^{Trp}$ or suppressor $tRNA^{Trp}$ (42 pmol). The background values observed in the absence of messenger (3.2 pmol of Trp or 6.5 pmol of Phe) have been subtracted. The stimulation of Trp incorporation is calculated as the difference of Trp incorporation in the absence of Cys and that with no omissions. The relative magnitude of this effect varied (see text). Phe incorporation was monitored in the presence of wild-type tRNA.

Average values for the increase of Trp incorporation on omitting Cys were 156% (over seven experiments) for suppressor tRNA and 21% (over four experiments) for wild-type tRNA. Because the difference in incorporation depends on which of the two tRNA species is present, the increased incorporation in the absence of Cys cannot easily be attributed to mischarging of $tRNA^{Cys}$ by Trp. These data suggest that the UGU codon can be translated as Trp and that such misreading is much more probable (*ca.* 7 times) with the suppressor tRNA than with the wild-type tRNA.

The data in Table 2 enable us to calculate the proportion of UGU codons that can be translated as Trp. In the presence of Cys the amount of Trp incorporated is consistent with the amount of UGG codons expected in the polymer. When Cys is omitted, there is an increase in Trp incorporation with suppressor tRNA in different experiments that corresponds to a range of mistranslation of 13–45% of the Cys codons as Trp. Thus, in the presence or absence of Cys, the suppressor tRNA is a relatively inefficient translator of UGU. What is important, however, is that, just as in the case of translating the UGA codon (2), the suppressor is only quantitatively more efficient at translating UGU than is the wild-type form of $tRNA^{Trp}$. Thus, the D stem alteration in the mutant has enhanced an ambiguity already present in the wild-type tRNA, but expressed only in the absence of Cys.

DISCUSSION

The present data suggest that $Trp\text{-}tRNA_{UGA}$ can translate UGU to a substantially greater extent than can wild-type Trp-tRNA. In contrast, the mutant tRNA is unable to translate the GUU and UUG codons to an appreciable extent. Such results indicate that the mutant tRNA is not simply "stickier" than wild-type tRNA, but is selectively responding to codons of the form UGN (N being any ribonucleotide).

It would appear that the alteration of the D stem in the $Trp\text{-}tRNA_{UGA}$ has enhanced an aberrant form of wobble in this tRNA. Accordingly, these data confirm the notion that the structure of the tRNA at positions distant from the anticodon influences the acceptability of tRNA to the mRNA-programmed ribosome. Such a conclusion is equivalent to confirmation of a fundamental assumption of the conformational

selection model (5). In addition, this interpretation is consistent with the notion that wobble at the third position with wild-type tRNAs is also a consequence of the conformational constraints attending the normal tRNA–ribosome interaction.

Finally, we note that the $tRNA_{UGA}$ suppressor is a weak translator of the UGU codon. Indeed, it is only quantitatively more effective at this than wild-type $Trp\text{-}tRNA^{Trp}$. However, the cognate $Cys\text{-}tRNA^{Cys}$ translates this codon so much more effectively than the suppressor that tryptophan insertion at this codon is not detectable in the presence of cysteine. This is precisely the property required of a nonsense suppressor. Thus, the suppressor is selected by virtue of its ability to translate a codon for which there is normally no corresponding tRNA. However, it must be sufficiently inefficient as a mistranslator so that other related codons are not translated to a significant extent. Otherwise, the organism would run the risk of being killed by the efficient function of the suppressor at sense codons.

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