

# Are stop codons recognized by base triplets in the large ribosomal RNA subunit?

HAN LIANG,<sup>1</sup> LAURA F. LANDWEBER,<sup>2</sup> and JACQUES R. FRESCO<sup>3</sup>

Departments of Chemistry,<sup>1</sup> Ecology & Evolutionary Biology,<sup>2</sup> and Molecular Biology,<sup>3</sup> Princeton University, Princeton, New Jersey 08544, USA

## ABSTRACT

The precise mechanism of stop codon recognition in translation termination is still unclear. A previously published study by Ivanov and colleagues proposed a new model for stop codon recognition in which 3-nucleotide Ter-anticodons within the loops of hairpin helices 69 (domain IV) and 89 (domain V) in large ribosomal subunit (LSU) rRNA recognize stop codons to terminate protein translation in eubacteria and certain organelles. We evaluated this model by extensive bioinformatic analysis of stop codons and their putative corresponding Ter-anticodons across a much wider range of species, and found many cases for which it cannot explain the stop codon usage without requiring the involvement of one or more of the eight possible noncomplementary base pairs. Involvement of such base pairs may not be structurally or thermodynamically damaging to the model. However, if, according to the model, Ter-anticodon interaction with stop codons occurs within the ribosomal A-site, the structural stringency which that site imposes on sense codon-tRNA anticodon interaction should also extend to stop codon-Ter-anticodon interactions. Moreover, with Ter-tRNA in place of an aminoacyl-tRNA, for each of the various Ter-anticodons there is a sense codon that can interact with it preferentially by complementary and wobble base-pairing. Both these considerations considerably weaken the arguments put forth previously.

**Keywords:** translation termination; stop codon usage; stop codon recognition; putative rRNA anticodons; mitochondrial genetic code

## INTRODUCTION

Protein translation is a key cellular process. With protein sequences encoded in messenger RNA sequences, termination of the synthesis of any particular protein begins with the recognition of a stop codon in the message by some component(s) of the translation machinery. While the basic scheme for translation of the message has been known for many years, the precise way in which stop codon recognition occurs remains elusive (Nakamura and Ito 1998).

Recently, Ivanov et al. (2001) presented a new model for recognition of stop codons. They noted that hairpin helices 69 (domain IV) and 89 (domain V) in the rRNA of the large ribosomal subunit (LSU) of *Escherichia coli*, with 7-nucleotide (nt) hairpin loops (the same size as the anticodon loop of tRNA), contain CUA or UCA triplets in the middle of the loop, just like the anticodons in tRNA. These two triplets are antiparallel complements of the stop codons in *E. coli*, as if

they were their corresponding tRNA anticodons. For this reason, and because of the similarity between the anticodon stem and loop in tRNA and these two proposed ribosomal mimics, Ivanov et al. termed hairpins 69 and 89 termination tRNA1 (Ter-tRNA1) and termination tRNA2 (Ter-tRNA2), respectively. This terminology was introduced without any implication that tRNAs with stop-anticodons actually exist. Ivanov and colleagues conjectured that eubacteria and certain organelles (mitochondria and chloroplasts) recognize stop codons in mRNA by the so-called Ter-tRNA anticodons of the tRNA mimics within the LSU rRNA. Specifically, they proposed that Ter-tRNA1, containing “anticodon” CUA, recognizes both UAG and UAA stop codons, whereas Ter-tRNA2, containing “anticodon” UCA, recognizes UGA and UAA. This left the role of the protein release factors RF1 and RF2 to that of promoting the accuracy of stop codon-Ter-anticodon recognition. As supportive evidence for the generality of the model, Ivanov and colleagues noted that vertebrate mitochondria, which utilize two different stop codons, AGA and AGG, contain the corresponding stop Ter-anticodons, UCU and CCU, in the loops of hairpins 69 and 89 of the LSU rRNA. Armed with this example of a possible compensatory change, they implied that these mitochondria most likely conserve the Ter-tRNA mechanism to recognize their stop codons.

**Reprint requests to:** Jacques R. Fresco, Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA; e-mail: jfresco@molbio.princeton.edu; or Laura Landweber, Department of Ecology & Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA; e-mail: lfl@princeton.edu.

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The idea of stop codon recognition by a complementary triplet in the LSU rRNA does not conflict with the well-known “tripeptide anticodon” model, in which the tripeptide motifs of RF1 and RF2 are viewed as functionally equivalent to the tRNA anticodons and respond specifically to stop codons (Ito et al. 2000). As Ivanov et al. (2001) proposed, it is possible that RF1 and RF2 recognize the miniduplex formed by stop codon interaction with the anticodon of Ter-tRNA rather than the stop codon alone. Therefore, additional studies are required to determine whether the proposed Ter-tRNA anticodons are actually involved in stop codon recognition.

To test the Ivanov model, we examined a wide range of species for the relation between their stop codon usage and their potential Ter-anticodon sequences. Noticing particularly that many mitochondria and some eubacteria (e.g., Firmicutes) do not employ standard stop codons, we even considered possible alternative base pairs that might be compatible with the basic model of Ivanov et al. without requiring Watson–Crick stop codon-LSU Ter-anticodon complementarity.

## RESULTS

We first summarize our more extensive analysis of stop codons and potential Ter-anticodons, and then evaluate whether the base-pairing possibilities required by the model of Ivanov et al. are structurally and thermodynamically plausible and consistent with the specificity requirements of the model. We then consider several additional

observations that our data mining uncovered, and conclude with an overall evaluation of the Ter-tRNA model.

### Analysis of relevant sequences in mitochondrial rRNAs

The extensive variation in stop codon usage in mitochondria provides considerable opportunity to test the Ivanov model. We divided the mitochondrial LSU rRNAs (comparative RNA web, Cannone et al. 2002) into four groups according to stop codon usage. We considered only stop codon usage confirmed by gene annotation, and included cases with both standard and unusual stop codons.

Of the 242 mitochondrial species examined, 12 use the standard stop codons UAG, UAA, and UGA; and most of these have Ter-anticodons in hairpins 69 and 89 with non-complementary residues. For example, although the model predicts that hairpin 69 Ter-anticodons should recognize UAG/UAA and hairpin 89 Ter-anticodons should recognize UAA/UGA, 10 species have a putative Ter-anticodon in hairpin 69 of 5'-CUC-3' or 5'-CUA-3', whereas those in hairpin 89 include 5'-ACC-3', 5'-UCA-3', 5'-UCC-3', 5'-UCU-3', and 5'-CCC-3' (Table 1A). We therefore consider in the next section whether the implied noncomplementary base-pairing interactions are structurally and thermodynamically plausible.

We also found 73 species comprising a second group that uses only UAA and UAG as stop codons, since UGA is reassigned to tryptophan. Only hairpin 69 matters in this group, as it recognizes UAA and UAG according to

**TABLE 1.** Examples of noncomplementary base pairs required by the Ter-tRNA model in mitochondrial LSU rRNA loop sequences of hairpins 69 and 89 for (A) the standard stop codon group, (B) the UAR stop codon group, (C) the Vertebrate Mitochondria Stop Codon Group, and (D) two protists, *Thraustochytrium aureum* and *Scenedesmus obliquus*, with unique mitochondrial stop codon usage. Only hairpin 69 is considered in cases where UGA is a sense codon (B and C). Ter-anticodons are uppercase letters of loop sequences

Species	A					
	Ter-tRNA1 loop sequence	Hairpin 69		Hairpin 89		
		Stop codon UAG	Stop codon UAA	Ter-tRNA2 loop sequence	Stop codon UGA	Stop codon UAA
	Noncomplementary base pairs			Noncomplementary base pairs		
<i>Physarum polycephalum</i>	5'-aaCUAug-3'	—	A·C	5'-guACCua-3'	C·U A·A	C·U A·C A·A
<i>Chlamydomonas eugametos</i>	5'-aaCUCua-3'	C·U	C·U A·C	5'-guUCAua-3'	X	A·C
<i>Chlamydomonas reinhardtii</i>	5'-aaCUCua-3'	C·U	C·U A·C	5'-guUCCua-3'	X	C·U A·C
<i>Nephroselmis olivacea</i>	5'-aaCUCua-3'	C·U	C·U A·C	5'-gcUCUua-3'	X	U·U A·C
<i>Marchantia polymorpha</i>	5'-aaCUCua-3'	C·U	C·U A·C	5'-gcUCUua-3'	U·U	U·U A·C
<i>Triticum aestivum</i>	5'-aaCUCua-3'	C·U	C·U A·C	5'-gcUCUua-3'	U·U	U·U A·C
<i>Arabidopsis thaliana</i>	5'-aaCUCua-3'	C·U	C·U A·C	5'-gcUCUua-3'	U·U	U·U A·C
<i>Zea mays</i>	5'-aaCUCua-3'	C·U	C·U A·C	5'-gcUCUua-3'	U·U	U·U A·C
<i>Oenothera berteriana</i>	5'-aaCUCua-3'	C·U	C·U A·C	5'-gcUCUua-3'	U·U	U·U A·C
<i>Pilayella littoralis</i>	5'-aaCUAug-3'	—	A·C	5'-guCCCua-3'	C·U A·C	C·U A·C

(continued)

TABLE 1. Continued

B						
Species	Ter-tRNA1 loop sequence <sup>a</sup>	Alternate ter-anticodons <sup>b</sup>	Hairpin 69			
			UAA		Stop codon	
			Noncomplementary base pairs			
<i>Neurospora crassa</i>	5'-gUAAAg-3'	UAA/AAA	A-A/A-A			G-U A-A/G-A A-A
<i>Podospira anserina</i>	5'-aGCGUg-3'	GCG/CGU	A-G A-C	G-U/A-C	A-G U-U	G-G A-C U-G/A-G U-U
<i>Pecten maximus</i>	5'-aGCGUg-3'	GCG/CGU				"
<i>Ascaris suum</i>	5'-aGCGUg-3'	GCG/CGU				"
<i>Caenorhabditis elegans</i>	5'-aGCGUg-3'	GCG/CGU				"
<i>Onchocerca volvulus</i>	5'-aGCGUg-3'	GCG/CGU				"
<i>Meloidogyne hapla</i>	5'-aGCGUg-3'	GCG/CGU				"
<i>Meloidogyne javanica</i>	5'-aGCGUg-3'	GCG/CGU				"

C						
Species	Ter-tRNA1 loop sequence <sup>c</sup>	AGA	Hairpin 69			UAG
			Stop codon			
			New	AGA	UAA	
Noncomplementary base pairs						
<i>Raja radiata</i>	5'-auUAGg-3'	A-G	A-G	G-U	G-U A-G A-A	G-U A-G
<i>Rattus norvegicus</i>	5'-guAAAg-3'	A-A A-G	A-A	A-G	A-A G-U	G-U A-A A-G

D										
Species	Ter-tRNA1 loop sequence	Hairpin 69				Hairpin 89				
		Stop codon				Stop codon				
		UAG	UAA	UUA	UCA	UAA	UGA	UUA	UCA	
Noncomplementary base pairs										
<i>T. aureum</i>	5'-aaCUAua-3'	—	A-C	A-C U-U	X	5'-guUCAua-3'	A-C	—	C-U	X
<i>S. obliquus</i>	5'-aaCUCua-3'	X	A-C C-U	X	A-C C-U	5'-guUCCaa-3'	A-C C-U	C-U	X	C-C C-U

<sup>a</sup>Overlapping Ter-anticodons are shown in uppercase letters of loop sequences.  
<sup>b</sup>Two alternate Ter-anticodons are possible if at least one linker residue is allowed on both the 5' and 3' side of the loop, i.e., 1,2, 3, 4, 5,6.  
<sup>c</sup>New Ter-anticodons (nucleotides 3, 4, 5 in uppercase letters, corresponding to new stop codons AGR) overlap old ones (nucleotides 4, 5, 6, corresponding to old stop codons UAR).  
(—) No noncomplementary base pairs.  
(X) Stop codon absent from this species.

the model. Among these Ter-anticodons are base triplets that again imply noncomplementary base-pairing interactions. Also, in some species, hairpin 69 has a 6-nt instead of a 7-nt loop normally present in the anticodon loop of tRNA. This makes the location of the Ter-anticodon ambiguous. In tRNA, the anticodon loop contains 2 nt on either side of the anticodon triplet that presumably give the anticodon unrestricted interaction with the codon

triplet of the message. In a similar but restricted manner, we permit at least 1 nt in hairpin 69 to serve as a linker on both sides of the Ter-anticodon. Therefore, we consider two alternative triplets, either positions 2–4 or 3–5 of the 6-nt loop, counting the 5' position of the loop as nt 1. By this flexible criterion, there are still eight cases in which the stop codon-Ter-anticodon duplex cannot form by complementary base-pairing (Table 1B).

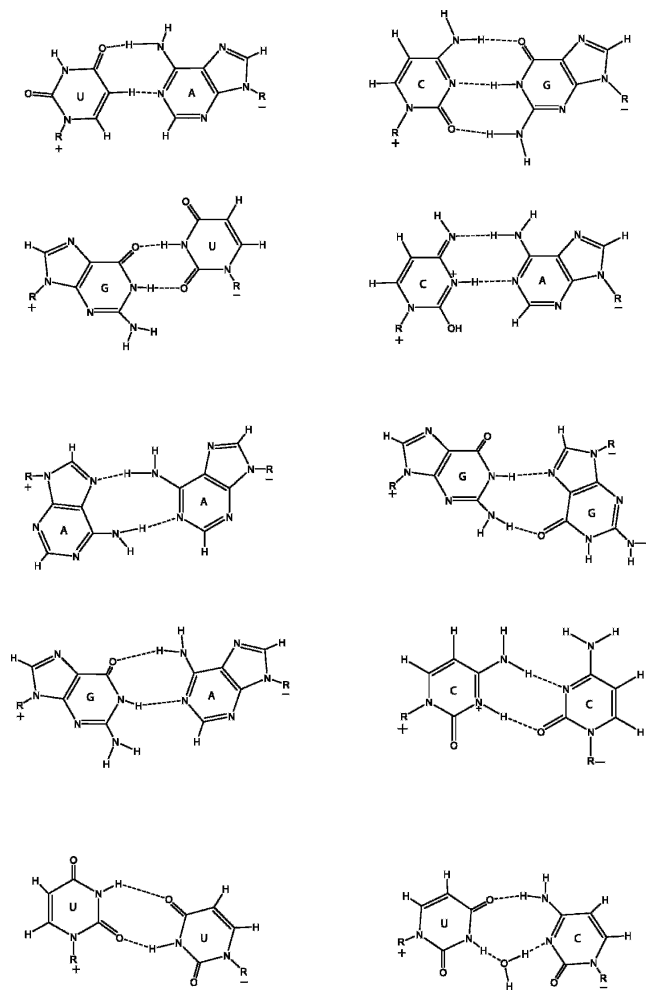
Another 155 species comprise a Vertebrate Mitochondria Stop Codon Group using UAG, UAA, AGA, and AGG as stop codons. In Ivanov et al. (2001), vertebrate mitochondria were taken to indicate strong support for their model because the “new” AGG and AGA stop codons match CCU or UCU Ter-anticodons in hairpin 69, whereas triplets CUA or CUG at the 3' end of the hairpin loop are complementary to the “old” stop codons UAG and UAA. This implies that one linker residue was deleted from the 3' end of the loop, reducing the loop size to 6 nt. However, our search uncovered two exceptions (Table 1C) in *Raja radiata* and *Rattus norvegicus* that imply noncomplementary base pairs. Ivanov and colleagues also explained the presence of the Ter-anticodon UUU, positioned at nt 3–5 in hairpin 69 of fish mitochondrial LSU rRNA, by noting that the AGG and AGA stop codons are absent from these species. However, we found several closely related species (Table 2) with the same sequence in the loop of hairpin 69, that nevertheless do use AGA or AGG as stop codons. This would require G-U base pairs in the second or both the second and third codon positions.

Two protists, the stramenopile *Thraustochytrium aureum* and green algae *Scenedesmus obliquus*, with “new” stop codons UUA and UCA, respectively, also require noncomplementary base-pairing with the corresponding Ter-anticodons at either hairpin site (Table 1D). Although a majority of genes in both species use new stop codons, the Ter-anticodons cannot pair conventionally with the two “old” stop codons in these cases.

In sum, we found that the Ter-tRNA model requires noncomplementary base pairs for each stop codon group. Therefore, we further tested the model by evaluating the feasibility of noncomplementary base pairs within the context of the ribosome.

### Analysis of noncomplementary base pairs

The four canonical bases conceivably give rise to 10 unique base pairs (Fig. 1). The cases cited above require every



**FIGURE 1.** Proposed hydrogen bonding schemes for the eight non-complementary antiparallel base pairs required if the stop codons used by different species interact with putative Ter-anticodons according to the model of Ivanov et al. (2001). Although there is more than one possible antiparallel arrangement for some base pairs, we show the arrangement that appears to be most compatible with the two complementary base pairs. All of these arrangements have been experimentally observed in rRNA or tRNA, except for the hemiprotonated C-C<sup>+</sup> pair. The Watson-Crick base pairs are shown on top for reference.

**TABLE 2.** Exceptions in the Vertebrate Mitochondrial Stop Codon Group that use AGA and AGG in addition to the UAA and UAG stop codons

Species	Number of atypical stop codons in genome	
	AGA	AGG
<i>Mustelus manazo</i>	0	1
<i>Gadus morhua</i>	2	0
<i>Neoceratodus forsteri</i>	1	0
<i>Lepidosiren paradoxa</i>	1	0
<i>Protopterus dolloi</i>	0	1
<i>Petromyzon marinus</i>	6	0

The species shown all contain the sequence 5'-auUUUg-3' in hairpin 69, with UUU representing the Ter-anticodon for new stop codons UAR, requiring G-U wobble pairs at second and third stop codon positions.

pairwise combination in at least one species for the Ter-tRNA model to hold. Consequently, we considered how the eight noncanonical antiparallel base pairs, including one wobble (G-U/U-G), one other purine-pyrimidine (A-C/C-A), three purine-purine (A-A, G-G, A-G/G-A), and three pyrimidine-pyrimidine (U-U, U-C/C-U, C-C) pairs could contribute to stop-codon-Ter-anticodon specificity.

By all accounts, the two complementary base pairs are dominant: they are the most stable, and readily conform to a common helical array. The other eight base pairs have all been experimentally observed in crystal structures, either within or adjacent to antiparallel complementary duplexes (summarized in Nagaswamy et al. 2002). Each noncomplementary pair can form at least two hydrogen bonds in pairing arrangements,

most of which have nearly standard glycosyl bond separation distances (Fig. 1; Donohue 1956). The question then is whether a variety of these base pairs can form three-base pair minihelices, stabilized within the ribosome A-site.

#### *Wobble G-U/U-G combination*

This antiparallel base pair is well known from standard codon-anticodon interactions, so it is the most easily accepted. It occurs in helices in solution (Strazewski et al. 1999) and in RNA duplexes bounded by complementary base pairs (Holbrook et al. 1991; Cruse et al. 1994) and has a glycosyl bond separation similar to that of a complementary base pair.

#### *A-C/C-A combination*

This antiparallel base pair requires protonation of either the A or the C residue (Topal and Fresco 1976a; Hunter et al. 1986a; Gao and Patel 1987; Purrello et al. 1993). It is possible that RF2 could promote A-C base pair formation with concurrent protonation of the C residue to form a wobble-type pair between stop codon and Ter-anticodon. The significantly higher  $pK_a$  value of C (4.3) than of A (3.4) makes it more likely that the C residue is protonated (Fig. 1). In fact, a base pair with  $C^+$  is isosteric with the standard wobble U-G base pair (Topal and Fresco 1976a). In addition, the macromolecular crowding inside the cell (Zimmerman 1993; J.R. Fresco, M. Ulasli, S. Kukreti, and O. Amosova, in prep.) makes such protonation seem especially likely.

Many Firmicute species have CCA at the Ter-anticodon sites, which require two sequential A-C pairs to recognize the UAA stop codon, however, which raises the energetic cost in a three-base pair miniduplex. Nevertheless, binding by a protein can drive the equilibrium distribution toward protonation of C residues very significantly.

#### *Purine-purine combinations*

All three antiparallel combinations, A-A, G-G, and A-G/G-A, have two hydrogen bonds and no protonated bases. Whereas a pair of purines in the *anti* configuration about the glycosyl bond requires a glycosyl bond separation  $\sim 2.4$  Å longer than a complementary base pair, a pair with one base oriented *syn* and the other *anti* maintains the standard separation distance for a complementary pair, although there may be some distortion of the dihedral angles of the glycosyl bonds (Topal and Fresco 1976b; Patel et al. 1984; Hunter et al. 1986b; Dolinnaya et al. 1997; Nissen et al. 2001; Battle and Doudna 2002; Dolinnaya and Fresco 2003). The cost of forming such a base pair with a *syn*-oriented purine residue is hardly more than a kcal/mol (Topal and Fresco 1976b).

#### *Pyrimidine-pyrimidine combinations*

Antiparallel U-U and U-C base pairs have been observed in rRNA (Ban et al. 2000; Carter et al. 2000). Two crystallographic studies of RNA duplexes with U-C pairs bounded

by complementary base pairs suggest that an interbase water bridge between the endocyclic N3 atoms of the U and C residues in the minor groove widens the glycosyl bond separation to approximately that of a complementary base pair (Holbrook et al. 1991). Such a water bridge could possibly have a comparable effect on the U-U and hemiprotonated C-C<sup>+</sup> pairs (Fig. 1) that also form at neutral pH.

### **Other relevant considerations**

While evaluating hairpin 69 and 89 sequences regarding stop codon usage, we made a number of additional observations that seem to question the validity of the Ter-tRNA model.

#### *Absence of hairpin 69*

The very short LSU rRNAs of kinetoplastid mitochondria lack hairpin 69. This leaves no apparent way to signal protein termination within the framework of the model.

#### *Secondary structure variability in the regions of hairpins 69 and 89*

Ivanov et al.'s model was inspired by the observation that hairpins 69 and 89 in *E. coli*, together with their neighboring sequences, mimic the cloverleaf secondary structure of tRNA. However, we found the size of the loops of these hairpins quite variable. For example, the loops of hairpin 89 in the mitochondrial LSU rRNA of kinetoplastids are usually only 4 nt, whereas that of *Onchocerca volvulus* are 8 nt. Moreover, in many species the secondary structures around these hairpins differ significantly from that of a tRNA cloverleaf. For hairpin 89, there are no neighboring hairpins mimicking the TΨC and DHU hairpins. Also, hairpin 70, which Ivanov and colleagues designated the TΨC-stem-loop mimic (in *E. coli*) of the tRNA-like cloverleaf centered around hairpin 69, is only 3 nt in most other bacteria; in some species, e.g., the mitochondria of *Loligo bleekeri*, the size of hairpin 68 (the putative DHU stem-loop mimic) is much smaller than that in *E. coli*. Whether these variations are significant is unknown.

#### *Apparent absence of conservation of Ter-anticodon sequences*

If Ter-anticodons in hairpin 69 pair with the dominant UAA and UAG stop codons, one might expect them to be under strong selective constraint to maintain their most favorably pairing sequences. However, in very closely related *Plasmodium* species that contain fragmented mitochondrial LSU rRNAs (Gillespie et al. 1999), the Ter-anticodons lie on a conserved fragment but are very diverse in sequence (Table 3), implying lack of constraint in this region.

#### *Ter-anticodon sequences in Firmicute LSU rRNAs*

In some Firmicutes, the UGA stop codon is reassigned to tryptophan. We divided the 71 Firmicute species in the

**TABLE 3.** Variation among closely related species of *Plasmodium* in Ter-tRNA1 anticodons of hairpin 69 in mitochondrial LSU rRNA

Species	Hairpin 69
	Ter-tRNA1 loop sequence <sup>a</sup>
<i>P. berghei</i>	5'-auCAUaa-3'
<i>P. chabaudi</i>	5'-auUCUaa-3'
<i>P. falciparum</i>	5'-auUUUaa-3'
<i>P. vivax</i>	5'-auCGUaa-3'
<i>P. yoelii</i>	5'-auCAUaa-3'

<sup>a</sup>Ter-anticodons are shown in uppercase letters.

rRNA database into two major groups, according to their UGA usage. We designate the 65 species that use UGA as stop, the Standard Codon Group, and the six remaining species that use UGA as tryptophan, the W Codon Group. Within the Standard Codon Group, 68% have CCA at the putative Ter-anticodon site of Ter-tRNA2, 29% have UCA, and 3% have a quadruplet Ter-anticodon, either UCCA or UCAA; within the W Codon Group, 100% have UCA at the putative Ter-anticodon site.

Since the W Codon Group no longer uses UGA as stop, but nevertheless has 5'-UCA-3' at the Ter-anticodon site, the possibility for UGA stop codon-UCA Ter-anticodon interaction via three complementary base pairs still exists. Therefore, if the model is valid, there would seem to be a high risk of misreading UGA in these organisms. However, as Ivanov et al. (2001) remark, Trp-tRNA would always win in competition with Ter-tRNA, suppressing misreading of UGA as stop, because the process of codon recognition is much faster in elongation than in termination (Parker 1989). It is also possible that an unknown mechanism blocks binding between the stop codon and Ter-anticodon site in the W Codon Group.

*Cross-linking does not seem to place the putative Ter-anticodons in close proximity to the termination site*

A cross-linking study by Bulygin et al. (2003) tested whether the LSU rRNA of mammalian ribosomes could recognize a stop codon. Because the stop codon at the ribosomal A site cross-links to the 40S (small) subunit and not to the LSU after mild UV irradiation, those authors concluded that the model does not seem relevant in such species. However, there was no direct comparison of Ter-anticodons and stop codons.

## DISCUSSION

Although the structural and thermodynamic aspects of noncomplementary base-pairing may be compatible with the qualitative notion of stop codon-Ter-anticodon interaction, there are strong reasons to be skeptical of the Ter-tRNA model. For one thing, studies in *E. coli* (Ito et al.

2000; Klaholz et al. 2003) indicate that tripeptide sequences of release factors RF1 and RF2 directly interact in some unknown way with stop codons in the ribosomal A site. The direct role of these release factors in stop codon recognition is not well understood. But the question raised by the Ivanov model is whether those release factors recognize the stop codons by themselves or in the context of stop codon-Ter-anticodon three-base pair minihelices. In contrast to the claim by Ivanov and colleagues that the model only requires A-C in addition to complementary and G-U wobble pairs, our study reveals a requirement for all eight noncomplementary base pairs, implying that all combinations of these pairs be able to form stable but stereochemically varied minihelices within the confines of the ribosome.

A serious problem arises if such interaction occurs in the ribosomal A-site, which imposes striking structural stringency on the sense codon-tRNA anticodon interaction to maintain fidelity of protein synthesis (Parker 1989), and only allows complementary base pairs in the first and second codon positions (Yusupov et al. 2001). If stop codon-Ter-anticodon interaction occurs within the same site, it is difficult to understand why the same machinery should tolerate so many noncomplementary bases pairs, even at first and second nucleotide positions of the stop codon. Also, with Ter-tRNA in place of aminoacyl tRNA, it seems reasonable to expect that each of the Ter-anticodons ought to interact preferentially by complementary and wobble base-pairing with a particular sense codon in elongation, which could lead to a high risk of premature termination, although the downstream context of true stop codon would be lacking. And even if, as Ivanov and coworkers suggested, RF-1 and RF-2 do not interact directly with the stop codon alone, but instead recognize the minihelix between Ter-anticodon and stop codon, our findings suggest that the structural variability due to the range of noncomplementary base pairs in the minihelix would be too great to be a source of specificity. Since one would expect release factors to share a common cavity for sensing and stabilizing all stop codon-Ter-anticodon minihelices, the fact that the structures of these minihelices is so varied makes that possibility seem very unlikely.

Taken together, these considerations cast considerable doubt on the Ter-tRNA model, which we therefore consider highly speculative. Substantial experimental evidence is needed to lend the model credibility.

## MATERIALS AND METHODS

Mitochondrial and bacterial 23S rRNA sequence alignments were downloaded from the comparative RNA Web site ([www.rna.icmb.utexas.edu](http://www.rna.icmb.utexas.edu)). After removing redundant sequences, there were 240 mitochondrial and 71 Firmicute sequences in the study. Sequences in hairpins 69 and 89 (Ivanov et al. 2001) of each species were then extracted from the sequence alignment by a PERL script. For those rRNAs with characterized secondary structures, the loop sequences were confirmed.

GenBank annotation of stop codon usage was further confirmed with information from the codon usage database (<http://www.kazusa.or.jp/codon/>) (Nakamura et al. 2000). To be conservative, data for species whose stop codon usage is ambiguous [either because of a lack of cDNA data support, or because a stop codon is absent from the available data (if not all full genomes)] were excluded from the analysis.

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