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# Dramatic events in ciliate evolution: alteration of UAA and UAG termination codons to glutamine codons due to anticodon mutations in two *Tetrahymena* tRNAs$^{Gln}$

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**The three major glutamine tRNAs of *Tetrahymena thermophila* were isolated and their nucleotide sequences determined by post-labeling techniques. Two of these tRNAs$^{Gln}$ show unusual codon recognition: a previously isolated tRNA$^{Gln}_{UmUA}$ and a second species with CUA in the anticodon (tRNA$^{Gln}_{CUA}$). These two tRNAs recognize two of the three termination codons on natural mRNAs in a reticulocyte system. tRNA$^{Gln}_{UmUA}$ reads the UAA codon of α-globin mRNA and the UAG codon of tobacco mosaic virus (TMV) RNA, whereas tRNA$^{Gln}_{CUA}$ recognizes only UAG. This indicates that *Tetrahymena* uses UAA and UAG as glutamine codons and that UGA may be the only functional termination codon. A notable feature of these two tRNAs$^{Gln}$ is their unusually strong readthrough efficiency, e.g. purified tRNA$^{Gln}_{CUA}$ achieves complete readthrough over the UAG stop codon of TMV RNA. The third major tRNA$^{Gln}$ of *Tetrahymena* has a UmUG anticodon and presumably reads the two normal glutamine codons CAA and CAG. The sequence homology between tRNA$^{Gln}_{UmUG}$ and tRNA$^{Gln}_{UmUA}$ is 81%, whereas that between tRNA$^{Gln}_{CUA}$ and tRNA$^{Gln}_{UmUA}$ is 95%, indicating that the two unusual tRNAs$^{Gln}$ evolved from the normal tRNA$^{Gln}$ early in ciliate evolution. Possible events leading to an altered genetic code in ciliates are discussed.**

*Key words:* genetic code / glutamine tRNA / *Tetrahymena* / UAG and UAA suppression

## Introduction

We found previously that *Tetrahymena thermophila* contains a relatively large amount of cytoplasmic glutamine tRNA having UmUA as an anticodon which presumably recognizes the UAA termination codon (Kuchino *et al.*, 1985). In addition we isolated the corresponding tRNA gene from *Tetrahymena* macronuclear DNA and found that its nucleotide sequence exactly matched that of this glutamine tRNA (Kuchino *et al.*, 1985).

Recently several workers independently reported that in ciliate genes, such as the *Stylonichia* α-tubulin gene, *Paramecium* surface antigen genes and *Tetrahymena* histone H3 genes the stop codon UAA is present internally. Moreover, comparison of the structures of these genes with the amino acid sequences or amino acid compositions of the proteins corresponding or closely related to these genes suggested that this termination codon is read as glutamine or glutamic acid (Helftenbein, 1985; Caron and Meyer, 1985; Preer *et al.*, 1985; Horowitz and Gorovsky, 1985). These results are compatible with our finding of *Tetrahymena* tRNA$^{Gln}_{UmUA}$ and imply that the genetic code in these ciliates differs from the normal genetic code in using UAA as a glutamine codon.

In some of the reports mentioned above evidence is given that not only UAA but also UAG may not serve as a termination codon. According to the 'wobble' hypothesis, U in the first anticodon position of tRNA can recognize G as well as A (Crick, 1966). The question then arises whether tRNA$^{Gln}_{UmUA}$ can also interact with the UAG stop codon and if not, whether *Tetrahymena* contains another tRNA$^{Gln}$ with an unusual anticodon. To clarify these points we purified the three major glutamine tRNAs from *Tetrahymena* and determined their nucleotide sequences by post-labeling techniques. Furthermore we examined the codon recognition properties of these tRNAs by using an *in vitro* translation system and mRNAs with known termination codons, i.e. tobacco mosaic virus (TMV) RNA, which contains an internal UAG stop codon and α-globin mRNA which is terminated by a UAA stop codon.

In this report we show that besides tRNA$^{Gln}_{UmUA}$ and in addition to a normal tRNA$^{Gln}$ there is another tRNA$^{Gln}$ present in *Tetrahymena* which recognizes specifically the UAG termination codon. The evolution of these three tRNAs$^{Gln}$ and possible events leading to an altered genetic code during early ciliate evolution is discussed.

## Results

### *Detection of UAG and UAA readthrough activities in unfractionated tRNA from Tetrahymena*

The UAG and the UAA readthrough activities of total unfractionated tRNA were assayed in a rabbit reticulocyte lysate with TMV RNA or globin mRNA as messengers. Two high mol. wt proteins of 126 K and 183 K are synthesized *in vitro* when TMV RNA is translated. The 183-K protein is the product of a UAG readthrough at the end of the 5'-proximal cistron coding for the 126-K protein (Pelham, 1978; Goelet *et al.*, 1982) and its enhanced synthesis is correlated with the presence of a tRNA capable of recognizing this UAG termination codon (Beier *et al.*, 1984a). The globin mRNA preparation which we used in our experiments is a mixture of α- and β-globin mRNAs. α-Globin mRNA is terminated by a UAA codon, whereas β-globin mRNA is terminated by a UGA codon (Efstratiadis *et al.*, 1977; Heindell *et al.*, 1978). Recognition of any of these termination codons yields readthrough proteins which differ significantly in size from the major translation products and from each other. To verify whether the observed readthrough product was the α'- or β'-globin we routinely translated globin mRNA in parallel in the presence of yeast UAA and UGA suppressor tRNAs (as seen in Figure 3).

As shown in Figure 1A (lane c) a substantial amount of the UAG readthrough product of 183 K was produced when TMV RNA was used as the messenger in the presence of unfractionated *Tetrahymena* tRNA. Similarly, the UAA readthrough product (i.e. α'-globin) was visible — although to a lesser extent — when globin mRNA was translated *in vitro* (Figure 1B, lane c). Production of the 183-K protein and of α'-globin was complete-

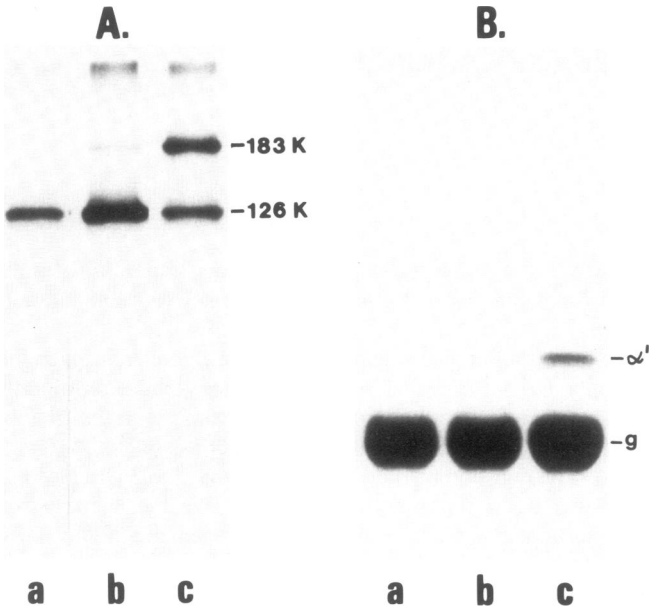


**Fig. 1.** *In vitro* translation of TMV RNA and globin mRNA in the presence of unfractionated *Tetrahymena* tRNA. TMV RNA (**A**) or globin mRNA (**B**) were translated in a messenger-dependent rabbit reticulocyte lysate as described in Materials and methods in the absence of added tRNA (**a**) or in the presence of 100 μg/ml of unfractionated *Tetrahymena* tRNA (**b,c**) and crude *Tetrahymena* aminoacyl-tRNA synthetase (**c**), respectively. [$^{35}$S]Methionine-labeled translation products were spearated on a 8% (**A**) or 15% (**B**) polyacrylamide gel and identified by fluorography. Numbers indicate mol. wts of the products. g = translation products of α- and β-globin mRNA; α′ = readthrough product of α-globin mRNA.

ly dependent on the simultaneous presence of crude aminoacyl-tRNA synthetase isolated from *Tetrahymena* cells, indicating that aminoacylation of the tRNAs responsible for the UAG and UAA readthrough does not take place in the reticulocyte lysate. However, it should be noted that addition of unfractionated *Tetrahymena* tRNA to the reticulocyte lysate leads to an increase of overall protein synthesis up to 50% even in the absence of *Tetrahymena* aminoacyl-tRNA synthetase (Figure 1A, lane b).

### *UAG and UAA readthrough activities of fractionated Tetrahymena tRNA*

As a first step in identifying the tRNA species responsible for UAG and UAA readthrough, *Tetrahymena* tRNA was fractionated by BD-cellulose column chromatography. Since glutamine tRNAs are likely candidates for the tRNA species we were looking for, glutamine acceptor activity was determined after tRNA fractionation. As shown in Figure 2A, glutamine acceptor activity was roughly separated into three fractions (fractions I, II and III). The material of these fractions was pooled, precipitated with ethanol and tested in the *in vitro* translation assay with either TMV RNA (Figure 2B) or globin mRNA (Figure 2C) as messengers. All three fractions contained UAG suppressor activity as seen by the synthesis of the 183-K readthrough product (Figure 2B), whereas only fractions II and III contained UAA suppressor activity shown by the synthesis of α′-globin (Figure 2C). As reported previously (Kuchino *et al.*, 1985) fraction III contains glutamine tRNA with the UmUA anticodon. The observation that this fraction stimulates readthrough over the UAA and UAG codon is consistent with the assumption that $tRNA^{Gln}_{UmUA}$ may recognize both termination codons. On the other hand, fraction I, which shows no UAA readthrough activity and certainly does not contain $tRNA^{Gln}_{UmUA}$, stimulated the synthesis of the UAG readthrough product to a great extent

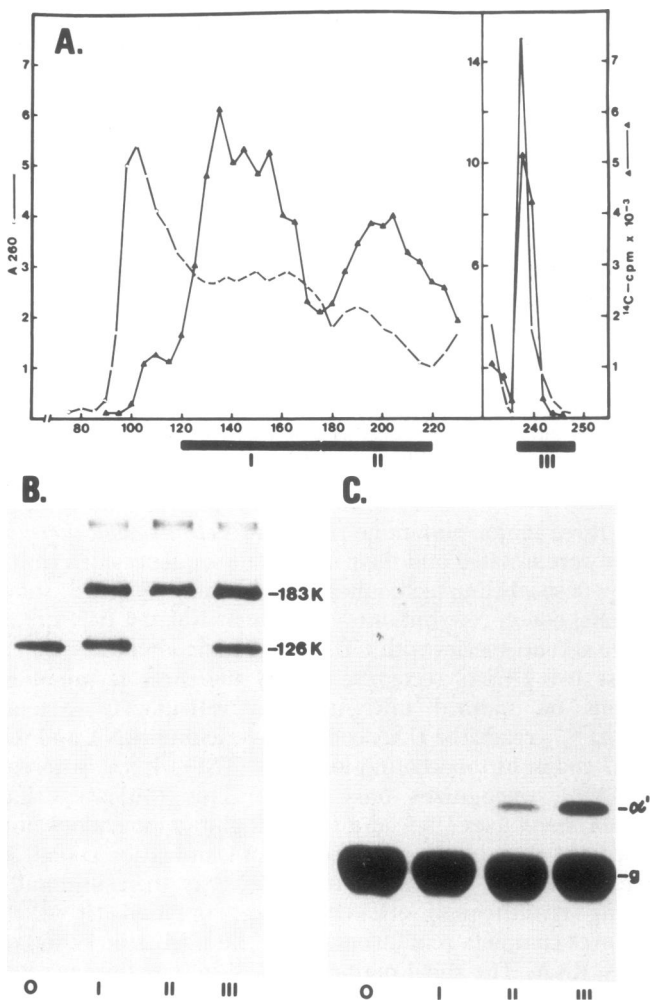


**Fig. 2.** Detection of UAG and UAA readthrough activity in the tRNAs fractionated by BD-cellulose column chromatography. 2000 $A_{260}$ units of *Tetrahymena* tRNA were fractionated by BD-cellulose column (1.5 × 80 cm) chromatography (**A**). Material was first eluted with a linear gradient of 0.3−1.2 M NaCl in 0.02 M NaOAc, pH 6.0 (total volume, 2 l). After 1.9 l of effluent had been collected (up to fraction 230), elution was continued with a second linear gradient of 1.2−2 M NaCl/20% ethanol in 0.02 M NaOAc, pH 6.0 (total volume, 1 l). The glutamine acceptor activity of each fraction was assayed by use of a crude *Tetrahymena* aminoacyl-tRNA synthetase preparation. The bars indicate the fractions which were pooled and used for further purification of glutamine tRNAs. The same fractions were assayed for readthrough activity in a reticulocyte lysate. **B** shows a fluorogram of [$^{35}$S]methionine-labeled proteins separated on a 8% polyacrylamide gel after *in vitro* translation of TMV RNA in the presence of 50 μg/ml tRNA of the fraction specified (0, no tRNA was added). The major translation product from TMV RNA (126-K protein) and the 183-K readthrough protein are visible. **C** shows a fluorogram of [$^{35}$S]methionine-labeled proteins separated on a 15% polyacrylamide gel after *in vitro* translation of globin mRNA. The conditions for *in vitro* translation were the same as in **B**. The major translation products (α- and β-globin) and the readthrough product of α-globin mRNA are marked as g and α′, respectively.

(Figure 2A). These results imply that *Tetrahymena* possesses another tRNA which is able to recognize the UAG stop codon.

### *UAG and UAA readthrough activities of purified Tetrahymena glutamine tRNAs*

The glutamine tRNAs present in fractions I, II and III were further purified by RPC-5 column chromatography and polyacrylamide gel electrophoresis. During the fractionation we monitored the glutamine acceptor and readthrough activities of each fraction. The results showed that each fraction contained one major

glutamine tRNA species and also small amounts of isoacceptors corresponding to the major species of neighbouring fractions, indicating that the three glutamine tRNA species were not completely separated by the first BD-cellulose column chromatography (Figure 2). Several other minor glutamine tRNA species were also isolated during the fractionation, but their yield was very low and preliminary analyses of their nucleotide sequences indicated that they were undermodified variants of the major glutamine tRNAs. Thus, three major glutamine tRNAs were isolated and designated as $tRNA^{Gln}_{UmUG}$ (from fraction I), $tRNA^{Gln}_{CUA}$ (from fraction II) and $tRNA^{Gln}_{UmUA}$ (from fraction III), according to their anticodon structures reported later.

These three purified glutamine tRNAs were tested for their ability to recognize UAG and UAA codons in the *in vitro* translation system. As shown in Figure 3, $tRNA^{Gln}_{UmUA}$ was found to interact with both codons, UAG and UAA, whereas $tRNA^{Gln}_{CUA}$ recognized only UAG, while $tRNA^{Gln}_{UmUG}$ had no readthrough activity at all. Thus, the earlier observations of UAG readthrough activity of fraction I tRNA and UAA readthrough activity of fraction II tRNA (Figure 2) were probably due to contaminations of fraction I with $tRNA^{Gln}_{CUA}$ and of fraction II with $tRNA^{Gln}_{UmUA}$.

According to the 'wobble' hypothesis it was not very surprising that $tRNA^{Gln}_{UmUA}$, which reads the UAA codon on globin mRNA, can also misread the UAG codon on TMV RNA (Figure 3A, lane c). It remains obscure, however, whether $tRNA^{Gln}_{UmUA}$ can also interact with the UAA stop codon which follows the UAG codon on TMV RNA (Goelet *et al.*, 1982). Since the next stop codon in phase (UGA) occurs only six codons downstream, a putative readthrough product would not be resolved from the 183 kd protein on an 8% SDS polyacrylamide gel. If concentrations >25 μg/ml of $tRNA^{Gln}_{UmUA}$ were used in the translation mixture, the amounts of both the 126 kd and the 183 kd polypeptides decreased and a number of smaller products appeared instead. This suggests that perhaps readthrough over the UAA codon had occurred, but that the synthesized product with an elongated C-terminus was easily degraded because of an unstable tertiary structure.

The detection of a second *Tetrahymena* glutamine tRNA with unusual codon recognition properties, i.e. $tRNA^{Gln}_{CUA}$, was rather unexpected. The UAG readthrough activity of this glutamine tRNA is extremely high, showing a 183-K : (126 K + 183 K) ratio of 0.95 as compared with a ratio of 0.3−0.4 shown by a natural UAG suppressor tRNA ($tRNA^{Tyr}_{G\Psi A}$) isolated from tobacco and wheat leaves (Beier *et al.*, 1984a, 1984b) and a *bona fide* suppressor $tRNA^{Tyr}_{C\Psi A}$ from yeast (Bienz and Kubli, 1981).

*Nucleotide sequences of the three Tetrahymena glutamine tRNAs*

The nucleotide sequences of the three major glutamine tRNAs were determined by post-labeling techniques and their cloverleaf structures are shown in Figure 4. The sequence of $tRNA^{Gln}_{UmUA}$ has recently been published by Kuchino *et al.* (1985) and is shown for comparison. The normal $tRNA^{Gln}$ which presumably recognizes the two glutamine codons CAA and CAG has a UmUG anticodon. The sequence homology between $tRNA^{Gln}_{UmUG}$ and $tRNA^{Gln}_{UmUA}$ is 81%, whereas $tRNA^{Gln}_{UmUA}$ differs from $tRNA^{Gln}_{CUA}$ in only four nucleotides, resulting in a homology of 95%.

## Discussion

This work clearly demonstrates that *Tetrahymena* contains two glutamine tRNAs with unusual codon recognition: a previously isolated species with UmUA in the anticodon ($tRNA^{Gln}_{UmUA}$) and a second species with CUA in the anticodon ($tRNA^{Gln}_{CUA}$). These

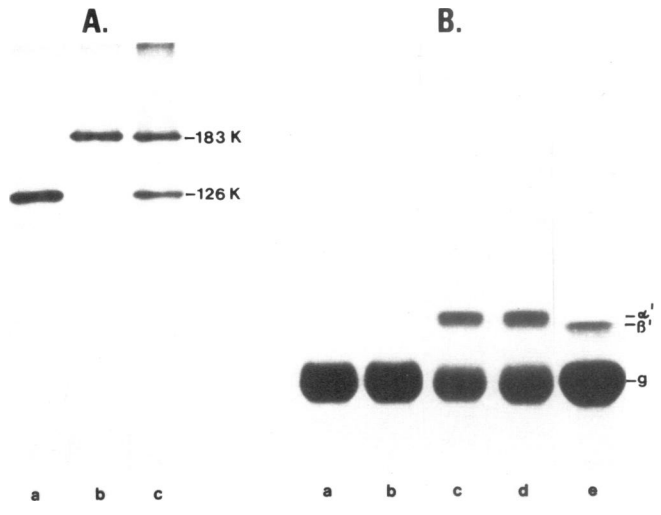


**Fig. 3.** Abilities of purified *Tetrahymena* glutamine tRNAs to recognize UAG and UAA codons *in vitro*. **Panel A** shows the translation of TMV RNA in the presence of 25 μg/ml of $tRNA^{Gln}_{UmUG}$ (**a**), $tRNA^{Gln}_{CUA}$ (**b**) and $tRNA^{Gln}_{UmUA}$ (**c**). **Panel B** shows the *in vitro* translation of globin mRNA in the presence of 25 μg/ml of $tRNA^{Gln}_{UmUG}$ (**a**), $tRNA^{Gln}_{CUA}$ (**b**) and $tRNA^{Gln}_{UmUA}$ (**c**), in parallel with 50 μg/ml of UAA yeast suppressor tRNA (**d**) and UGA yeast suppressor tRNA (**e**). The readthrough products of α- and β-globin mRNA are marked α′ and β′, respectively.

two glutamine tRNAs recognize ochre and amber nonsense codons on natural mRNAs in a reticulocyte cell-free protein-synthesizing system. $tRNA^{Gln}_{UmUA}$ interacts with UAA and UAG, while $tRNA^{Gln}_{CUA}$ recognizes only UAG.

The codon recognition pattern of $tRNA^{Gln}_{UmUA}$ is in principle consistent with the 'wobble' hypothesis: unmodified U in the first position of the anticodon is supposed to interact with A as well as with G (Crick, 1966). However, it is not clear whether the fact that this tRNA reads UAA and UAG codons means that Um acts completely like an unmodified U, i.e., whether or not the ribose methylation increases the interaction with A over G as suggested for base-modified U (McCloskey and Nishimura, 1977). The observation that there is only one tRNA in *Tetrahymena*, namely $tRNA^{Gln}_{UmUG}$, for the classical glutamine codons CAA and CAG favours the first assumption. It should be noted in this connection that these $tRNAs^{Gln}$ are the first tRNAs known to have Um in the first position of the anticodon. The normal $tRNA^{Gln}$ ($tRNA^{Gln}_{UmUG}$) does not misread the UAA and UAG codons as has been reported for a case of UAA suppression in yeast (Pure *et al.*, 1985) and thus it is only used for the recognition of the two standard glutamine codons.

The identification of a major glutamine tRNA which exclusively reads the UAG codon ($tRNA^{Gln}_{CUA}$) was a real surprise. Internal UAG codons have been found in two ciliate genes sequenced so far (Caron and Meyer, 1985; Preer *et al.*, 1985) and these findings could partly explain the necessity for this tRNA. But why did a second glutamine tRNA evolve in *Tetrahymena* when another tRNA ($tRNA^{Gln}_{UmUA}$) existed which is able to read the UAG codon? A possible explanation would be that in fact $tRNA^{Gln}_{UmUA}$ does not recognize UAG codons on *Tetrahymena* mRNAs as efficiently as the UAG codon on TMV RNA which is known to be a rather 'leaky' stop codon (Pelham, 1978; Bienz and Kubli, 1981; Beier *et al.*, 1984a).

Thus, the occurrence of a second tRNA with the ability to recognize the UAG codon would have been of some advantage for the organism. But whatever the reason for their appearance,

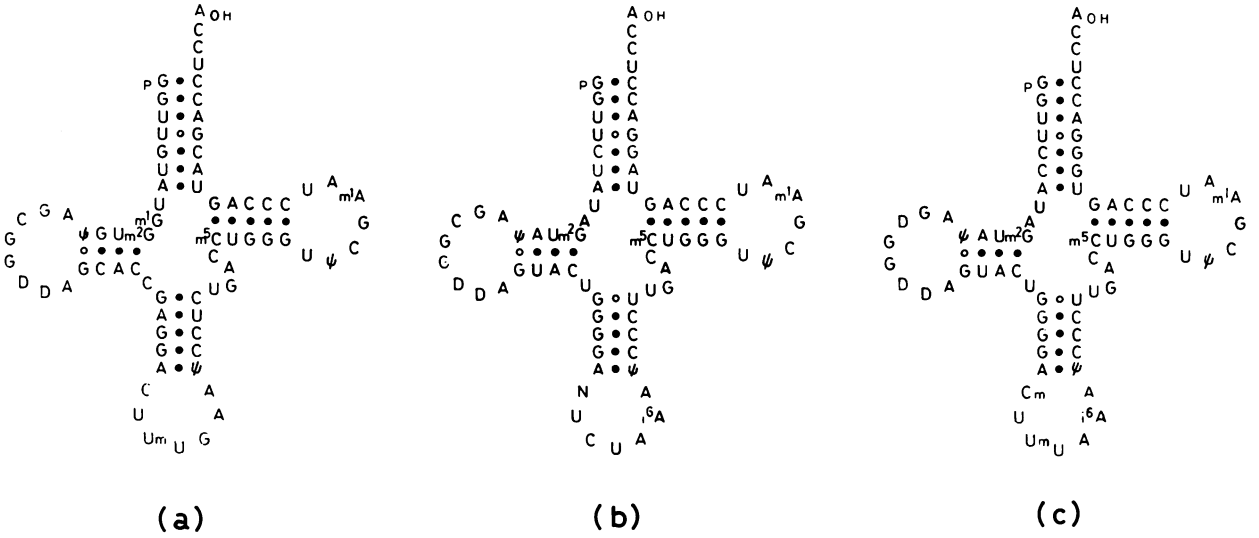


**Fig. 4.** Cloverleaf structures of the three glutamine tRNAs from *T. thermophila*. (a) $tRNA^{Gln}_{UmUG}$, (b) $tRNA^{Gln}_{CUA}$, (c) $tRNA^{Gln}_{UmUA}$. N in position 32 of sequence (b) is probably a derivative of C.

the fact remains that *Tetrahymena* contains two glutamine tRNAs which can read the two stop codons UAA and/or UAG as glutamine codons. This is consistent with a previous proposal that UAA and UAG are not used as termination codons in ciliates (Caron and Meyer, 1985; Preer *et al.*, 1985). The only codon for which no tRNA has yet been found is UGA, which is in fact the only termination codon identified in all ciliate genes investigated so far (Kaine and Spear, 1982; Bannon *et al.*, 1984; Helftenbein, 1985). Finally it should be pointed out that higher eukaryotes seem to use only one release factor for all three termination codons (Beaudet and Caskey, 1971). It will be of interest to find out how the *Tetrahymena* release factor, which certainly exists, and which should recognize UGA only, differs from its known eukaryotic counterpart.

A notable feature of the two glutamine tRNAs is their strong readthrough activity. Even with the unfractionated tRNA, extensive formation of the 183-K readthrough protein was observed when TMV RNA was used as a messenger in the *in vitro* translation system (Figure 1A). When purified $tRNA^{Gln}_{CUA}$ was added, readthrough over the UAG codon in TMV RNA was complete and no 126-K protein was found (Figure 3A). This strong readthrough activity has not been observed with natural eukaryotic suppressor tRNAs such as the UAG suppressor tRNA identified in *Drosophila*, tobacco or wheat, which is in fact a normal tyrosine tRNA with an undermodified G$\psi$A anticodon (Bienz and Kubli, 1981; Beier *et al.*, 1984a, 1984b). This $tRNA^{Tyr}_{G\psi A}$ cannot interact with the UAG codon via classical base pairing and is therefore a weaker suppressor tRNA.

We postulate that, whenever TMV RNA or $\alpha$- and $\beta$-globin mRNA produce their respective readthrough proteins in an *in vitro* assay in the presence of unfractionated tRNA from any organism to such an extent as seen in Figure 1, the genetic code of this organism must deviate from the classical genetic code with respect to the use of the three termiantion codons.

The sequence homology between *Tetrahymena* $tRNA^{Gln}_{UmUA}$ and $tRNA^{Gln}_{CUA}$ is much higher than that between them and the normal $tRNA^{Gln}_{UmUG}$ (Figure 4). Therefore, conceivably one of the suppressor tRNAs (probably $tRNA^{Gln}_{UmUA}$) has evolved from normal glutamine tRNA ($tRNA^{Gln}_{UmUG}$) and then $tRNA^{Gln}_{CUA}$ derived from $tRNA^{Gln}_{UmUA}$ to strengthen recognition of the UAG codon in *Tetrahymena*.

It is tempting to speculate why and how the use of UAA and UAG codons as glutamine codons has evolved in ciliates. Because of many unique features this group of organisms probably branched off in a very early stage of eukaryote evolution. Since all eubacteria and eukaryotes use UAA, UAG and UGA as termination codons (with the exception of mitochondria and mycoplasma) it is more likely that the ciliates lost two of these codons in their evolution rather than that these termination codons were acquired in the other organisms by convergent evolution.

Although other mechanisms are possible and have been discussed recently (Horowitz and Gorovsky, 1985), we can also imagine that the stop codons UAA and UAG were rarely used during prociliate evolution and that weak suppressor tRNAs existed for these codons very early. Spontaneous point mutations creating UAA and UAG codons within protein genes would then not have been lethal for those prociliates. Consequently, these prociliates would thus have been protected against the deleterious effects of such mutations. Once those early suppressor tRNAs acquired mutated anticodons for perfect UAA and UAG recognition, respectively, the use of these codons as amino acid codons became fixed.

This raises the question whether primitive organisms existed, or yet exist without any termination codons at all, i.e. which terminate their proteins by 'runoff' synthesis.

## Materials and methods

*TMV RNA, globin mRNA and yeast suppressor tRNAs*

TMV RNA was extracted from purified virus (vulgare strain) by phenol extraction. Globin mRNA, isolated from rabbit reticulocytes, was a gift from Dr. H.J.Gross. The yeast suppressor strains used for the isolation of suppressor tRNAs were obtained from the stock collection of Dr. J.Kohli. Total tRNAs were prepared from the *Schizosaccharomyces pombe* strains, sup 3-5 $h^-$, which contains an opal suppressor $tRNA^{Ser}$, and strain sup 3-18 $h^-$, which contains an ochre suppressor $tRNA^{Ser}$, respectively. The suppressor tRNAs were each partially purified by BD-cellulose column chromatography. The two yeast suppressor tRNAs were eluted after switching from the first gradient of 0.35−1 M NaCl to the second gradient of 1−2 M NaCl, containing 20% ethanol.

*Tetrahymena tRNA and aminoacyl-tRNA synthetase*

Unfractionated tRNA and aminoacyl-tRNA synthetase from *Tetrahymena* were isolated from the cells of *T. thermophila* mating type IV as described previously

(Kuchino *et al.*, 1985). Fractionation of tRNAs by BD-cellulose column chromatography, RPC-5 column chromatography and polyacrylamide gel electrophoresis was carried out according to reported procedures (Nishimura, 1971; Nishimura and Kuchino, 1983).

*Translation in vitro*

Translation in a nuclease-treated reticulocyte lysate was performed as described (Pelham and Jackson, 1976; Beier *et al.*, 1984a) except that the lysate was not supplemented with calf liver tRNA. TMV RNA and globin mRNA were added at concentrations of 100 μg/ml and 25 μg/ml, respectively. When the suppressor activity of *Tetrahymena* tRNA was assayed, crude *Tetrahymena* aminoacyl-tRNA synthetase was added to the translation mixture at a concentration which would not inhibit overall protein synthesis (final dilution 1:200). The reaction mixture was incubated at 30°C for 60 min.

*Analysis of translation products*

Proteins were analysed by gel electrophoresis in 8% and 15% SDS polyacrylamide gel slabs (Laemmli, 1970). Translation mixtures containing globin mRNA specific proteins were first diluted 1:10 in dissociation buffer before being applied to a 15% gel in order to reduce the high amount of unlabeled globin present in the *in vitro* translation mixture. Gels were fixed overnight, fluorographed as described by Laskey and Mills (1975) and exposed to RX Fuji-X-Ray films for 1–2 h if TMV RNA or for 2 days if globin mRNA had been translated *in vitro*.

*Sequencing of tRNA*

The primary structures of tRNAs were determined by the post-labeling procedure described previously (Nishimura and Kuchino, 1983).

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## References

Bannon,G.A., Bowen,J.K., Yao,M.C. and Gorovsky,M.A. (1984) *Nucleic Acids Res.*, **12**, 1961–1975.
Beaudet,A.L. and Caskey,C.T. (1971) *Proc. Natl. Acad. Sci, USA*, **68**, 619–624.
Beier,H., Barciszewska,M., Krupp,G., Mitnacht,R. and Gross,H.J. (1984a) *EMBO J.*, **3**, 351–356.
Beier,H., Barciszewska,M. and Sickinger,H.D. (1984b) *EMBO J.*, **3**, 1091–1096.
Bienz,M. and Kubli,E. (1981) *Nature*, **294**, 188–190.
Caron,F. and Meyer,E. (1985) *Nature*, **314**, 185–188.
Crick,F.H.C. (1966) *J. Mol. Biol.*, **19**, 548–555.
Efstratiadis,A., Kafatos,F.C. and Maniatis,T. (1977) *Cell*, **10**, 571–585.
Goelet,P., Lomonossoff,G.P., Butler,P.J.G., Akam,M.E., Gait,M. and Karn,J. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 5818–5822.
Heindell,H.C., Liu,A., Paddock,G.V., Studnicka,G.M. and Salser,W.A. (1978) *Cell*, **15**, 43–54.
Helftenbein,E. (1985) *Nucleic Acids Res.*, **13**, 415–433.
Horowitz,S. and Gorovsky,M.A. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 2452–2455.
Kaine,B.P. and Spear,B.B. (1982) *Nature*, **295**, 430–432.
Kuchino,Y., Hanyu,N., Tashiro,F. and Nishimura,S. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 4758–4762.
Laemmli,U.K. (1970) *Nature*, **227**, 680–685.
Laskey,R.A. and Mills,A.D. (1975) *Eur. J. Biochem.*, **56**, 335–341.
McCloskey,J.A. and Nishimura,S. (1977) *Accounts Chem. Res.*, **10**, 403–410.
Nishimura,S. (1971) In Cantoni,G.L. and Davies,D.R. (eds) *Procedures in Nucleic Acid Research*. Harper & Row, NY, Vol. **2**, pp. 542–564.
Nishimura,S., and Kuchino,Y. (1983) In Weissman,S.M. (ed), *Methods of DNA and RNA Sequencing*. Praeger, NY, pp. 235–260.
Pelham,H.R.B. (1978) *Nature*, **272**, 469–471.
Pelham,H.R.B. and Jackson,R.J. (1976) *Eur. J. Biochem.*, **67**, 247–256.
Preer,J.R. Jr., Preer,L.B., Rudman,B.M. and Barnett,A.J. (1985) *Nature*, **314**, 188–190.
Pure,G.A., Robinson,G.W., Naumovski,L. and Friedberg,E.C. (1985) *J. Mol. Biol.*, **183**, 31–42.

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