Three *Tetrahymena* tRNA\(^{\text{Gln}}\) isoaceptors as tools for studying unorthodox codon recognition and codon context effects during protein synthesis *in vitro*

Christine Schüll and Hildburg Beier*
Institut für Biochemie, Bayerische Julius-Maximilians-Universität, Biozentrum, Am Hubland, D-97074 Würzburg, Germany

Received April 8, 1994; Revised and Accepted May 9, 1994

**ABSTRACT**

Three glutamine tRNA isoaceptors are known in *Tetrahymena thermophila*. One of these has the anticodon UmUG which reads the two normal glutamine codons CAA and CAG, whereas the two others with CUA and UmUA anticodons recognize UAG and UAA, respectively, which serve as termination codons in other organisms. We have employed these tRNA\(^{\text{Gln}}\)-isoaceptors as tools for studying unconventional base interactions in a mRNA- and tRNA-dependent wheat germ extract. We demonstrate here (i) that tRNA\(^{\text{Gln}}\)\(_{\text{UAU}}\) suppresses the UAA as well as the UAG stop codon, involving a single G:U wobble pair at the third anticodon position and two simultaneous wobble base pairings at the first and third position, respectively, and (ii) that tRNA\(^{\text{Gln}}\)\(_{\text{GUA}}\), in addition to its cognate codon UAG, reads the UAA stop codon which necessitates a C:A mispairing in the first anticodon position. These unorthodox base interactions take place in a codon context which favours readthrough in *tobacco mosaic virus* (TMV) or *tobacco rattle virus* (TRV) RNA, but are not observed in a context that terminates zein and globin protein synthesis. Furthermore, our data reveal that wobble or mispairing in the middle position of anticodon-codon interactions is precluded in either context. The suppressor activities of tRNAs\(^{\text{Gln}}\) are compared with those of other known naturally occurring suppressor tRNAs, i.e., tRNA\(^{\text{Ty}}\), tRNA\(^{\text{Tt}}\), and tRNA\(^{\text{CA}}\). Our results indicate that a 'leaky' context is neither restricted to a single stop codon nor to a distinct tRNA species.

**INTRODUCTION**

Transfer-RNAs which are able to suppress termination codons have been studied *in vivo* and *in vitro*. In the former case the activity of a suppressor tRNA has been assayed indirectly by measuring the amount of a gene product synthesized from a cistron carrying a nonsense codon mutation. These *in vivo* studies were mainly employed in the genetically well characterized *Escherichia coli* and *Saccharomyces cerevisiae* cells (1, 2). *In vitro* translation systems have been proven useful for the identification of a number of eukaryotic, naturally occurring suppressor-tRNAs (3-8). These tRNAs, with the exception of tRNA\(^{\text{Glu}}\)\(_{\text{CA}}\) (9), have in common that unconventional base pairings between the tRNA anticodon and the stop codon have to occur for the suppression of a termination codon.

Crick (10) proposed that the nucleotide at the first position of the anticodon, the wobble position, can make interactions with the nucleotide in the third position of the codon that are not allowed by the classical base pairing rules. On the other hand Crick (10) strictly excluded a number of interactions in the wobble position like purine-purine and pyrimidine-pyrimidine pairings. In order to overcome these restrictions for unorthodox base-pairings it has long been suspected that the translation of a stop codon by normal tRNAs may be influenced in some way by the nucleotides preceding or following the suppressed stop codon (11-16).

We have recently used a mRNA- and tRNA-depleted wheat germ extract for the identification and characterization of two plant suppressor tRNAs: Cytoplasmic (cyt) tRNA\(^{\text{UAG}}\)\(_{\text{AAU}}\) which promotes UAG suppression in *tobacco mosaic virus* (TMV) RNA (4, 16) and chloroplast (chl) tRNA\(^{\text{CA}}\)\(_{\text{CA}}\) which stimulates readthrough over a leaky UGA stop codon in *tobacco rattle virus* (TRV) RNA-1 (8). The tRNA-dependent extract has the advantage of allowing the unambiguous identification and characterization of any added suppressor tRNA.

Besides tRNA\(^{\text{Ty}}\) and tRNA\(^{\text{Tt}}\), eukaryotic tRNA\(^{\text{Gln}}\) is one of the best studied natural suppressor tRNAs. For instance, Yoshinaka et al. (17) showed that a viral protease encoded by Moloney murine leukemia virus (Mo-MuLV) is a readthrough product of the UAG stop codon between the gag and pol genes, with glutamine inserted at the site of the termination codon. It was suggested by Kuchino et al. (5) that tRNA\(^{\text{Gln}}\)\(_{\text{UAU}}\), whose synthesis is increased in NIH 3T3 cells infected with Mo-MuLV, is responsible for the suppression of the UAG codon in MuLV-RNA. In the yeast *Saccharomyces cerevisiae* the normal tRNAs\(^{\text{Gln}}\) with CUG and UUG anticodon have been shown to suppress UAG and UAA stop codons if the tRNA genes were

*To whom correspondence should be addressed*
expressed on multi-copy plasmids (18–20). All these interactions of tRNA\textsuperscript{Gln} with either the UAG or UAA codon require a wobble base pairing in the third anticodon position.

In order to study the influence of the codon context on mispairing and wobble pairing in any position of the anticodon-codon interaction we have selected the three well-known tRNA\textsuperscript{Gln} isoacceptors from \textit{T. thermophila} (21, 22): tRNA\textsuperscript{Gln}\textsubscript{UmUG} which recognizes the normal glutamine codons CAA and CAG and which has the potential to act as a weak suppressor tRNA, as well as tRNA\textsuperscript{Gln}\textsubscript{UmUA} which reads the UAG and UAA stop codon, respectively, by normal base pairs and can thus be regarded as strong suppressors. We show here that tRNAs\textsuperscript{Gln} can replace tRNA\textsuperscript{Glu} or tRNA\textsuperscript{Glu} in reading an appropriate stop codon by unconventional base pairing if this codon is placed into the TMV- or TRV-specific context.

\section*{MATERIALS AND METHODS}

\subsection*{Enzymes and reagents}

SP6 RNA polymerase, T4 polynucleotide kinase and RNase inhibitor from human placenta were from Boehringer, Mannheim. \[^{14}\text{C}\]Methylated proteins used as molecular weight markers, L-[^\text{35}\text{S}]methionine and L-[\text{U-}^{14}\text{C}]glutamine with specific activities of 37 TBq and 9.25 GBq/mmol, respectively, were obtained from Amersham. Untreated wheat germs were a gift from Synpharma GmbH, Augsburg.

\subsection*{Transcription \textit{in vitro}}

\textit{In vitro} transcription of the plasmids pSP65-TMV or pSP65-TRV and their derivatives with SP6 RNA polymerase was carried out as described by Zerfaß and Beier (8).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Mobility shift analyses of 5'-labelled tRNA\textsuperscript{Gln} from \textit{Tetrahymena thermophila}. The three purified \textit{Tetrahymena} tRNAs\textsuperscript{Gln} with UmUG (a), CU0 (b) and UmUO (c) anticodon were labelled at the 5'-end with \[^{\gamma-\text{32P}}\text{ATP and T4 polynucleotide kinase and subjected to controlled partial degradation with 50 mM NaHCO\textsubscript{3}, 0.5 mM EDTA (pH 9.0). Two-dimensional separation of the 5'-labelled fragments was carried out as described (44). First dimension: high voltage electrophoresis at pH 3.5 on cellulose acetate (from left to right); second dimension: homochromatography in a 30 mM KOH 'homomix' on DEAE-cellulose thin layer plates at 65°C (from bottom to top).}
\end{figure}

\section*{Fractionation and isolation of glutamine tRNAs from \textit{Tetrahymena thermophila}}

Preparation of unfractionated tRNA from cells of \textit{T. thermophila} was performed according to Kuchino \textit{et al.} (21). Total tRNA was loaded onto a BD-cellulose column and fractionated by elution with a linear gradient of 0.35 M NaCl (0.01 M MgCl\textsubscript{2}, 0.02 M NaOAc, pH 4.5) to 1 M NaCl in the same buffer, followed by an elution with 2 M NaCl/15% ethanol. The tRNA\textsuperscript{Gln}-specific fractions were further purified by electrophoresis in a native 10% polyacrylamide gel, pH 8.3 and then in a 10% denaturing polyacrylamide gel, pH 3.5.

\section*{Preparation of aminoacyl-tRNA synthetases}

A wheat germ aminoacyl-tRNA synthetase fraction (protein conc. = 15 mg/ml) was isolated from wheat embryos as described (8). Aminoacyl-tRNA synthetase from \textit{T. thermophila} (protein conc. = 11 mg/ml) was prepared according to Kuchino \textit{et al.} (21). A HeLa cytoplasmic S100 extract was used as a source for human aminoacyl-tRNA synthetase.

\section*{Aminoacylation of tRNAs}

Assay of glutamine acceptance of \textit{Tetrahymena} tRNA was performed in a reaction mixture containing 50 mM HEPES-KOH (pH 7.5), 5 mM Mg(OAc)\textsubscript{2}, 20 mM KOAc, 4 mM ATP, 3 mM dithiothreitol, 0.2 mM CTP, 74 Bq/\mu l of \[^{14}\text{C}\]labelled glutamine and appropriate amounts of tRNA and crude aminoacyl-tRNA synthetase. Incubation was for 20 min at 37°C.

\section*{In vitro translation in wheat germ extract}

A wheat germ cell-free extract depleted of endogenous mRNAs and tRNAs was prepared essentially as described by Pfitzinger \textit{et al.} (23). \textit{In vitro} translation was carried out in a total volume of 10 \mu l containing 35\% (v/v) wheat germ extract, 10\% (v/v) wheat germ initiation factor solution, 20 mM HEPES-KOH (pH 7.5), 2 mM dithiothreitol, 1 mM ATP, 20 \mu M GTP, 8 mM creatine phosphate, 40 \mu g/ml creatine phosphokinase, 50 \mu M spermine, 2.5 mM Mg(OAc)\textsubscript{2}, 135 mM KOAc, 1 U RNase inhibitor per \mu l, 70 \mu M each of 19 L-amino acids (without methionine), 17 kBq/\mu l \[^{35}\text{S}\]methionine and 50 \mu g/ml template RNA. The reaction mixture was incubated for 60 min at 30°C.

\section*{Analysis of translation products}

Proteins were analysed by electrophoresis in 15% polyacrylamide slab gels containing 0.1% SDS. Gels were fixed overnight, fluorographed and exposed to RX Fuji X-ray film at −80°C. The ratio of the protein products arising by termination and readthrough events, respectively, was determined by densitometric quantification of incorporated radioactive methionine using an Elscript 400 scanner. Appropriate corrections were made for the higher methionine content of readthrough products.

\section*{RESULTS}

\subsection*{Purification and identification of three tRNAs\textsuperscript{Gln} from \textit{Tetrahymena thermophila}}

Total tRNA from \textit{T. thermophila} was fractionated by BD-cellulose column chromatography as described by Hanyu \textit{et al.} (22). The tRNAs\textsuperscript{Gln} contained in the pooled fractions I, II and III were further purified by two successive polyacrylamide gel electrophoreses. The purity and identity of the isolated tRNAs\textsuperscript{Gln}
was confirmed by mobility shift analyses (Fig. 1). The three tRNA\textsuperscript{Gln} isoacceptors with the anticodons UmUG, CUA and UmUA differ characteristically from each other in two to three nucleotides in the 5' -region (22) thus allowing the unambiguous identification of the corresponding tRNA\textsuperscript{Gln} isoacceptor only by mobility shift analyses of 5' -end-labelled tRNA.

Two out of three Tetrahymena tRNA\textsuperscript{Gln} isoacceptors are charged with glutamine solely by the homologous aminoacyl-tRNA synthetase

In a previous report we had shown that recognition of the UAG and UAA stop codon by Tetrahymena tRNA\textsuperscript{Gln} and tRNA\textsuperscript{Gln}_{UmUA}, respectively, in a rabbit reticulocyte lysate was dependent on the simultaneous presence of an aminoacyl-tRNA synthetase preparation from Tetrahymena cells (22), indicating that rabbit glutamine synthetase does not recognize these two tRNA\textsuperscript{Gln} isoacceptors as substrates. We have now realized that glutamine synthetase from wheat germ is also unable to charge these two isoacceptors with glutamine. Surprisingly, the third Tetrahymena tRNA\textsuperscript{Gln} isoacceptor with UmUG anticodon is charged by wheat germ synthetase with an efficiency of about 50% as compared with the homologous synthetase (Table 1). The same differential glutamine charging activities were observed with a S100 HeLa synthetase preparation (not shown). Although the bases in E.coli tRNA\textsuperscript{Gln} that are recognized by glutaminytRNA synthetase have been completely elucidated (24), they are not yet known in eukaryotic tRNAs\textsuperscript{Gln}. Therefore we can only speculate about the features of tRNA\textsuperscript{Gln}_{CUA} and tRNA\textsuperscript{Gln}_{UmUA} which make them unsuitable substrates for plant and mammalian glutaminyl synthetase. Major differences between these two tRNA\textsuperscript{Gln} isoacceptors and all other sequenced tRNAs\textsuperscript{Gln} or tRNA\textsuperscript{Gln} genes (25) reside in positions 9, 12 and 36 which are A residues in tRNA\textsuperscript{Gln}_{CUA} and tRNA\textsuperscript{Gln}_{UmUA} and G residues in all other tRNAs\textsuperscript{Gln} including Tetrahymena tRNA\textsuperscript{Gln}_{UmUG}.

Evidence that an unusual wobble base pairing in the third anticodon position is accomplished by tRNA\textsuperscript{Gln}_{UmUG}

For our studies we used a set of SP6 RNA polymerase-generated zein mRNA transcripts into which all three stop codons had been inserted in either the TMV- or the TRV-specific context (Fig. 2). The two viral readthrough regions comprising only seven codons have been shown to be sufficient to stimulate UAG readthrough by cytoplasmic (cyt) tRNA\textsuperscript{Gln}_{CUA} (16) and UGA readthrough by chloroplast (chl) tRNA\textsuperscript{Gln}_{UmUG}, respectively (8).

We first examined the effect of Tetrahymena aminoacyl-tRNA synthetase on the suppression efficiencies displayed by all three tRNA\textsuperscript{Gln} isoacceptors. Figure 3 (lanes c - f) shows that tRNA\textsuperscript{Gln}_{CUA} and tRNA\textsuperscript{Gln}_{UmUA}, respectively, read the two UAG stop codons in the transcript pSP65-TRV\textsubscript{2} (Fig. 2) only if Tetrahymena synthetase had been added to the wheat germ extract. However, tRNA\textsuperscript{Gln}_{UmUG} suppresses the UAA stop codon in the transcript pSP65-TRV\textsubscript{2} — albeit inefficiently — in the presence as well as in the absence of added Tetrahymena synthetase as revealed by the synthesis of the 26K readthrough protein in both cases (Fig. 3, lanes a, b). These results mimic the data obtained in our \textit{in vitro} aminoacylation assays (Table 1) and exclude the possibility that tRNAs\textsuperscript{Gln} with CUA and UmUA anticodon are mischarged to a measurable extent in plant extracts.

Translation of the transcripts pSP65-TMV or pSP65-TMV\textsubscript{2} in the presence of tRNA\textsuperscript{Gln}_{UmUG} resulted in the synthesis of low

Figure 2. Structures of expression vector pSP65-ML1 and its derivatives. A 1.2 kb fragment from Zea mays DNA, harbouring a zein gene (45) was cloned into the BamHI and PvuI sites of the SP6 RNA polymerase-specific vector pSP65 (8). The coding region of the zein gene is indicated by a hatched box, the flanking sequences originating from the Zea mays genome are marked by thick lines. The constructs pSP65-TMV, pSP65-TMV\textsubscript{2} and pSP65-TMV\textsubscript{3} contain a TAG, TAA or TGA stop codon and a total of six codons flanking the leaky UAG in TMV RNA (46). The constructs pSP65-TRV\textsubscript{1}, pSP65-TRV\textsubscript{2} and pSP65-TRV\textsubscript{3} contain a TAG, TAA or TGA stop codon and a total of six codons flanking the leaky UAG in RNA-1 of TRV (47). The constructs pSP65-glob carry a TGA stop codon and six codons flanking the UGA at the end of b-globin mRNA (48). \textit{In vitro} translation of the run-off transcript results in the synthesis of a 13K termination protein and — in the presence of appropriate suppressor tRNAs — in the production of one or two readthrough proteins of 26 and 28K.

Figure 3. Effect of Tetrahymena aminoacyl-tRNA synthetase on stop coding reading in wheat germ extract by Tetrahymena tRNA\textsuperscript{Gln}. \textit{In vitro} transcripts derived from pSP65-TRV\textsubscript{2} (lanes a, b) and pSP65-TRV\textsubscript{3} (lanes c - f) as illustrated in Fig. 2 were synthesized by SP6 RNA polymerase and translated in wheat germ extract. Tetrahymena tRNA\textsuperscript{Gln}_{UmUG} (lanes a, b), tRNA\textsuperscript{Gln}_{CUA} (lanes c, d) and tRNA\textsuperscript{Gln}_{UmUA} (lanes e, f) were added to the translation mixture at concentrations of 50 \(\mu\text{g}/\text{mL}\) and incubation was carried out either in the absence (lanes a, c, e) or in the presence of crude Tetrahymena synthetase (final dilution 1:200). The termination product of 13K and the two readthrough proteins of 26K and 28K are indicated at the right side of the gel.
amounts of the 26K readthrough protein (Fig. 4, lanes a, b). The second readthrough protein of 28K could not be detected, indicating that the first stop codon, which in these transcripts is UAG or UAA in the TMV context, is suppressed and the second UAG stop codon terminating the zein cistron is not (Fig. 2). The readthrough activity obtained was 5.5% with pSP65-TMV2 and 8% with pSP65-TMV. About the same suppressor activities were measured when the UAG and UAA codons had been placed in the TRV context (Fig. 3, lane b). The recognition of the UAA and UAG stop codons by tRNA^Gln^UmUG signifies that a single wobble at the third and a double wobble base pairing at the first and the third anticodon position have occurred.

As expected we found that *Tetrahymena* tRNA^Gln^UmUA efficiently (up to 60%) recognizes the first UAG stop codon in the TRV context as indicated by the synthesis of the 26K protein and is also able to read the second UAG in the zein context as manifested by the synthesis of the 28K protein (Fig. 4, lane c). Surprisingly, the same tRNA can also recognize the UAA stop codon if it is placed in the TRV (Fig. 4, lane f) or in the TMV context (Fig. 5, lane c). If the UAA stop codon in the middle of the transcript is suppressed, then translation continues to the UAG stop codon at the end of the zein cistron which is normally decoded by tRNA^Gln^UmUA as shown above. Accordingly only the occurrence of the second readthrough product of 28K is observed.

The third tRNA^Gln^ with UmUA anticodon reads the UAA stop codon in the TRV context with an efficiency of up to 70% (Fig. 4, lane c, d, g, h, i and m). A protein of ~22K was detected in some assays when readthrough over the first stop codon had occurred (e.g., Fig. 4, lanes e, i and k). The amount and size of this product varied depending on the source of wheat germ extract and suppressor tRNA used in the assay. It may be a degradation product of an unstable readthrough protein or a premature termination product.

**Mispairing in the first position of the anticodon depends on the context but not on the tRNA species**

We have recently characterized two natural suppressor tRNAs in plants: tRNA^Cya^ which reads the UAG stop codon in TMV RNA (4, 16) and tRNA^Cyc^ which suppresses the UGA stop codon in TRV RNA-1 (8). An unconventional base pairing at the first anticodon position is a necessity for the stop codon reading in both types of anticodon-codon interactions. As shown above, tRNA^Glu^ is able to interact with a UAA stop codon if this is either placed in the TMV- or TRV-specific context. A C:A mismatch is required for this interaction. Fig. 5 shows a comparison of stop codon reading by these three different suppressor tRNAs. Tobacco tRNA^Cya^ and tobacco tRNA^Cyc^ recognize their natural stop codons UAG and UGA in the TMV and TRV context, respectively, with about the same efficiencies of 25 to 30% (Fig. 5, lanes b, c) whereas *Tetrahymena* tRNA^Gln^ reads the UAA stop codon in either the TMV or TRV context only with an efficiency of 5 to 10% (Fig. 4, lane f; Fig. 5, lane a).

**DISCUSSION**

As a consequence of the deviation from the ‘universal’ genetic code, three tRNA^Gln^ isoacceptors exist in the ciliate *Tetrahymena thermophila*: tRNA^Gln^UmUG decodes the normal glutamine codons CAG andCAA, whereas tRNA^Gln^CuUA and tRNA^Gln^UmUA read the classical UAG and UAA termination

**Figure 4. In vitro translation in wheat germ extract of different transcripts harbouring either of the three stop codons in the presence of *Tetrahymena* tRNA^Gln^**

In vitro transcripts derived from pSP65-TMV (lane a), pSP65-TMV2 (lane b), pSP65-TMV3 (lane c), pSP65-TRV2 (lanes d, e, i), pSP65-TRV2 (lanes f, k), pSP65-TRV (lanes g, l) and pSP65-glob (lanes h, m) as indicated in Fig. 2 were translated in wheat germ extract in the presence of tRNA^Gln^UmUG (lanes a–d), tRNA^Gln^CuUA (lanes e–h) and tRNA^Gln^UmUA (lanes i–m). *Tetrahymena* aminoacyl-tRNA synthetase was added to each translation mixture. The stop codons indicated at the top of the fluorograms refer to the first stop codon present in the transcripts. ^14C^-methylated protein standards ranging in size from 14.3 to 97 kDa are shown at the left side.

**Figure 5. Stop codon misreading by three tRNAs of different origin. In vitro translation in wheat germ extract of different transcripts derived from pSP65-TMV2 (lane a), pSP65-TRV (lane b) and pSP65-TMV (lane c) were translated in wheat germ extract in the presence of either *Tetrahymena* cyt tRNA^Gln^UmUG (lane a), *Nicotiana* chi tRNA^Gln^Cyc (lanes b) or *Nicotiana* cyt tRNA^Gln^Cya (lane c). The characterization of *Nicotiana* tRNA^Cyc^ and tRNA^Cya^ has been reported elsewhere (4, 8, 16).
codons, respectively, as glutamine codons (22, 26). All three isoacceptors are charged with glutamine by *Tetrahymena* aminoacyl-tRNA synthetase with about the same efficiency (Table 1). Hence they represent a defined set of closely related isoacceptors the use of which is not impaired by inefficient charging or mischarging with a non-cognate amino acid (27) as long as the homologous synthetase is present (Fig. 3).

We tested each particular tRNA for its capacity to read each of the three termination codons in a tRNA-dependent wheat germ extract. The stop codons had been placed either in a context that favoured readthrough by tRNA$_{UA}^{Gln}$ over the UAG in TMV-RNA (16) or in a context that allowed suppression of the UGA in TRV RNA-1 by tRNA$_{CA}^{Gln}$ (8). These studies revealed a number of exceptional codon recognition properties of tRNAs$^{Gln}$ which are summarized together with other observations made in our laboratory in Figure 6.

### Wobble base pairing at the first and third anticodon position

Crick (10) had postulated that, for instance, G:U and U:G base pairs at the first anticodon position would not affect the fidelity of protein synthesis due to the degeneracy of the genetic code. In accordance with this assumption we have found that tRNA$_{UmUG}^{Gln}$ also reads the UAG codon with high efficiency besides its cognate codon UAA independent of the nature of surrounding nucleotides (Fig. 4, lane i). However, the reading of UAG by tRNA$_{UmUG}^{Gln}$ is exceptional in so far as in the yeasts *S. cerevisiae* and *S. pombe* nonsense suppression has been shown to be strictly codon-specific (2, 28, 29). The restriction of ochre suppressors with U$^*$UA anticodon (U$^*$ = mcm$^5$U or mcm$^5$S$^5$U) to read only UAA stop codons was attributed to the modification present at the first anticodon position which reduces the interaction with a G residue. The 2'-O-methyluridine (Um) at position 34 is found only in eukaryotic tRNAs$^{Gln}$ (25) and contributes to strict codon recognition through its conformational rigidity (30), but obviously allows wobble base pairing.

The ability of *Tetrahymena* tRNA$_{UmUG}^{Gln}$ to suppress UAA and UAG stop codons in wheat germ extract (Fig. 4, lanes a, b) indicates that wobble base pairing has occurred at the third anticodon position. This is forbidden by the normal wobble rules (10). Overexpression of the yeast tRNA$_{UmUG}^{Gln}$ gene coding for tRNA$_{UmUG}^{Gln}$ in *S. cerevisiae* resulted in the partial suppression of UAA stop codons (18). These *in vitro* data match essentially our results obtained *in vivo*. However, Pure et al. (18) could not detect UAG suppression by overexpression of this gene. Recognition of the UAG stop codon by tRNA$_{UmUG}^{Gln}$ involves two simultaneous wobble base pairings in the first and third anticodon position and consequently is dependent on a number of restrictions as discussed below.

### Table 1. Amino acid acceptor activity of *Tetrahymena thermophila* tRNAs$^{Gln}$

<table>
<thead>
<tr>
<th>tRNA$^a$</th>
<th>Source of synthetase</th>
<th>Gln acceptance (pmol/A$_{260}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tRNA$_{UmUG}$</td>
<td><em>T. thermophila</em></td>
<td>265</td>
</tr>
<tr>
<td>tRNA$_{CUA}$</td>
<td>Wheat germ</td>
<td>141</td>
</tr>
<tr>
<td>tRNA$_{UmUA}$</td>
<td><em>T. thermophila</em></td>
<td>226</td>
</tr>
<tr>
<td>tRNA$_{CUA}$</td>
<td>Wheat germ</td>
<td>0</td>
</tr>
<tr>
<td>tRNA$_{UmUA}$</td>
<td><em>T. thermophila</em></td>
<td>260</td>
</tr>
<tr>
<td>tRNA$_{CUA}$</td>
<td>Wheat germ</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ The amounts of tRNA used in the aminoacylation experiments were less than 0.01 A$_{260}$.

An obvious feature of *Tetrahymena* tRNA$_{UmUG}^{Gln}$ is the presence of an unmodified A residue immediately 3' to the anticodon at position 37 (22). Jukes (31) originally proposed that a hypermodified nucleotide at this position (i.e., m$^5$A or m$^6$S$^5$A) found in many tRNAs that read codons beginning with U or A serves to maintain fidelity of protein synthesis by preventing wobble base pairing in the third anticodon position. Indeed there are many reports which support the notion that the hypermodified A residue stabilizes the anticodon-codon interaction and increases the accuracy of codon reading (32–35). Thus, the unmodified A at position 37 in tRNA$_{UmUG}^{Gln}$ appears to contribute to the unconventional base pairing displayed by this tRNA.

### Mispairing at the first position of the anticodon

It was first shown in *Escherichia coli* that wild-type tRNA$_{Glu}^{Gln}$ can misread the UGA stop codon at the end of the coat protein cistron in phage Q8 RNA (36, 37). We have previously isolated chloroplast and cytoplasmic tRNA$_{CA}^{Gln}$ from *Nicotiana* and demonstrated that both are UGA suppressors albeit to a different extent (8). Furthermore we have characterized *Nicotiana* cyt tRNA$_{CA}^{Gln}$ as being capable to suppress the UAG and UAA stop codon, respectively, in a TMV-specific context (16). We now

---

**Figure 6.** Schematic presentation of data shown in Figs. 4 and 5. (A) Wobble base pairing in the first and/or third position of the anticodon; (B) Mispairing in the first position of the anticodon; (C) Inability of wobble or mispairing in the middle position of the anticodon. The tRNA$^{Gln}$ with the anticodon U$^*$GA (U$^*$ = cm$^5$U) has been isolated from *Nicotiana rustica* as described by Teichmann et al. (49). The base pairs studied here are indicated by boxes: (−−) refer to classical or authentic wobble base pairs; (−⋯) indicate mispairings or wobble base pairs in the third anticodon position as observed *in vitro*; (X) refer to unorthodox pairings which do not contribute to anticodon-codon interactions in our studies.
present evidence that the UAA codon placed in the same context can also be recognized by either tRNA\textsubscript{Gln}\textsuperscript{UAG} as discussed above, as well as by tRNA\textsubscript{Gln}\textsuperscript{UUA} (Fig. 5, lane a). The interaction of the latter tRNA with UAA involves a C:A mismatch at the first anticodon position comparable with the Cm:A mispairing occurring in tRNA\textsubscript{Gln}\textsuperscript{CAG}/UGA codon interaction. However, in the latter case a C:G base pair in the middle position apparently stabilizes the unorthodox codon reading as does the Y:A base pair at the same position in tRNA\textsubscript{Gln} (16). Both tRNAs exhibit relatively high suppressor activities of about 30%, whereas the ability of tRNA\textsubscript{Gln} to read the UAA codon amounts to about 10% only (Fig. 5). It should be noted in this context that a derivative of yeast tRNA\textsuperscript{Gln} with the anticodon CUA has been shown to translate CAG glutamine codons, involving a C:A mispairing at the third anticodon position, at a low level in vivo (38).

An absolute prerequisite for all types of unconventional base pairings mentioned above is a favourable codon context. That is, all anticodon-codon interactions shown in Fig. 6A and B, with the exception of tRNA\textsubscript{Gln}\textsuperscript{UUA}, which decodes UAG, were detected only when the corresponding stop codon had been placed in either the TMV- or TRV-specific context (Fig. 2). Neither the UAG stop codon at the end of the zein cistron nor the UGA stop codon at the end of the \( \beta \)-globin cistron was recognized by any of the weak suppressors (Fig. 4). Moreover, we have shown earlier that tRNA\textsubscript{Glu} and tRNA\textsubscript{Gln} respectively, cannot read the UAA stop codon at the end of the \( \alpha \)-globin cistron (22).

The precise molecular mechanism by which neighbouring nucleotides influence the fidelity of translation is still obscure (15, 39, 40). It is, however, generally accepted that only weak suppressors which require an unorthodox base pairing at any position of the anticodon are dependent on a favourable codon context, whereas strong suppressors are not. This notion was first proposed by Miller and Albertini (13) and Bossi (14) on the basis of studies performed in \( E. \) coli and our data support this rule for the misreading of eukaryotic tRNAs in a plant extract. Furthermore, our results demonstrate that a leaky context supporting stop codon readthrough is not restricted to the suppression of a single stop codon, an observation made also by Feng et al. (41) who exchanged the leaky UAG codon in murine leukemia virus RNA to UAA or UGA, nor is it used exclusively by only one type of suppressor tRNA. Thus tRNA\textsuperscript{Gln} have the potential to suppress UAG and UAA stop codons in the TMV- and TRV-specific context with about the same efficiencies. Similarly, tRNA\textsubscript{Glu} reads UGA in the TMV context with about 60 to 70% efficiency as compared with the TRV context, whereas tRNA\textsubscript{Glu} decodes the UAG in the TRV context only with an efficiency of about 20% as compared with the TMV context (unpublished observation), indicating some restrictions on the tRNA level.

Wobble or mispairing at the middle position of the anticodon is not allowed

None of the three \textit{Tetrahymena} tRNA\textsuperscript{Gln} isoacceptors decoded the UGA stop codon, neither in the leaky TMV- and TRV-specific context, respectively, nor in the globin context (Fig. 4). This type of interaction would have needed a single U:G wobble base pairing in the middle position of the anticodon in case of tRNA\textsubscript{Gln} (Fig. 6C) or additionally other unconventionally interactions. The inability of all three tRNA\textsuperscript{Gln} isoacceptors to read the UGA stop codon supports the notion that UGA serves as a strong and unique signal for translation termination in \textit{Tetrahymena} (22).

There are a few cases known in which unconventional base pairings at the middle anticodon position, mostly at very low levels, have been reported (6, 42, 43). We have found no evidence for the occurrence of this type of mispairing in our in vitro system. Neither tRNA\textsuperscript{Gln} nor any other suppressor tRNAs examined for its ability to read stop codons displayed this particular feature. For instance, \textit{Nicotiana} tRNA\textsubscript{Glu} and \textit{Nicotiana} tRNA\textsubscript{Gln} turned out to be unable to read the UAG and UGA stop codon, respectively, placed in the TRV context (C. Urban and H. B., unpublished), which would require a single mismatch in the middle position of the anticodon in both cases (Fig. 6C). Consequently, our data clearly indicate that — although wobble and mispairings can occur at the first and third position in anticodon-codon interactions to a measurable extent — unorthodox base pairings in the middle position is at the best minimized to a very low level.

ACKNOWLEDGEMENTS

We thank Dr Karin Zerfaß (Heidelberg) for providing clones pSP65-TMV, pSP65-TRV and its derivatives and Christiane Fingerhut for the construction of clone pSP65-glob. We are grateful to Carsten Urban and Armin Nafl for a gift of \textit{Nicotiana} chi tRNA\textsubscript{Glu} and cyt tRNA\textsubscript{Gln}, respectively. This work was supported by a grant from the Deutsche Forschungsgemeinschaft to H.B.

REFERENCES