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UGA suppression by $tRNA^{Trp}_{CmCA}$ occurs in diverse virus RNAs due to a limited influence of the codon context

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ABSTRACT

We have recently identified chloroplast and cytoplasmic $tRNA^{Trp}_{CmCA}$ as the first natural UGA suppressor tRNAs in plants. The interaction of these tRNAs with UGA involves a Cm:A mismatch at the first anticodon position. We show here that $tRNA^{Trp}_{CmCA}$ is incapable of misreading UAA and UAG codons *in vitro*, implying that unconventional base pairs are not tolerated in the middle anticodon position. Furthermore, we demonstrate that the ability of $tRNA^{Trp}_{CmCA}$ to promote UGA read-through depends on a quite simple codon context. Part of the sequence surrounding the leaky UGA stop codon in tobacco rattle virus RNA-1 was subcloned into a zein reporter gene and read-through efficiency was measured by translation of RNA transcripts in wheat germ extract. A number of mutations in the codons adjacent to the UGA were introduced by site-directed mutagenesis. It was found that single nucleotide exchanges at either side of the UGA had little effect on read-through efficiency. A pronounced influence on suppression by $tRNA^{Trp}_{CmCA}$ was seen only if 2 or 3 nt at the 3′-side of the UGA codon had been simultaneously replaced. As a consequence of the flexible codon context accepted by $tRNA^{Trp}_{CmCA}$, this tRNA is able to misread the UGA in a number of plant and animal viral RNAs that use translational read-through for expression of some of their genes.

INTRODUCTION

Many viral RNAs contain signals which promote read-through over internal termination codons by two completely distinct types of translational suppression. One of these is ribosomal frameshifting and the other is reading of an in-frame stop codon by a specific suppressor tRNA. These strategies allow the virus to regulate the synthesis of two related polypeptides which are needed in different amounts.

We have recently identified a number of natural suppressor tRNAs in plants: cytoplasmic $tRNA^{Tyr}_{G\Psi A}$ which promotes UAG suppression in tobacco mosaic virus (TMV) RNA (1–3), cytoplasmic as well as chloroplast $tRNA^{Trp}_{CmCA}$ and $tRNA^{Cys}_{GCA}$ respectively, which stimulate read-through over the leaky UGA stop codon in tobacco rattle virus (TRV) RNA-1 *in vitro* (4,5). Recognition of the corresponding termination codon by either of these tRNAs necessitates an unconventional base pairing in the wobble position. As a consequence, suppressor activity depends possibly on properties of the tRNA itself and on the nucleotides surrounding the leaky stop codon. Thus we have shown that base modifications in the first and second positions of the GΨA anticodon in $tRNA^{Tyr}$ have strong inhibitory or stimulating effects on UAG suppression (2,3).

In addition to features of the tRNA, the codon context plays an important role in translational suppression in both prokaryotic and eukaryotic systems. Suppression of the leaky UAG codon in TMV RNA depends strictly on the downstream 6 nt *in vivo* and *in vitro* (3,6,7), whereas in the case of UAG suppression in the RNA of murine leukemia virus (MuLV) a bipartite signal consisting of 8 nt downstream followed by a pseudoknot structure is necessary for efficient read-through (8,9).

In this report we have investigated the nucleotide context which determines suppression of the leaky UGA codon in TRV RNA-1 by tobacco $tRNA^{Trp}_{CmCA}$. We show here that the optimal codon context required by $tRNA^{Trp}$ is less stringent than that needed by $tRNA^{Tyr}_{G\Psi A}$ and that it involves mainly the downstream 3 nt. As a consequence a number of leaky UGA codons in quite different viral RNAs can be suppressed by $tRNA^{Trp}$.

MATERIALS AND METHODS

Enzymes and reagents

SP6 RNA polymerase, RNase A and RNase inhibitor from human placenta were from Boehringer (Mannheim, Germany). [^{14}C]Methylated proteins used as molecular weight markers and L-[^{35}S]methionine with a specific activity of 37 TBq/mmol were obtained from Amersham Buchler (Braunschweig, Germany).

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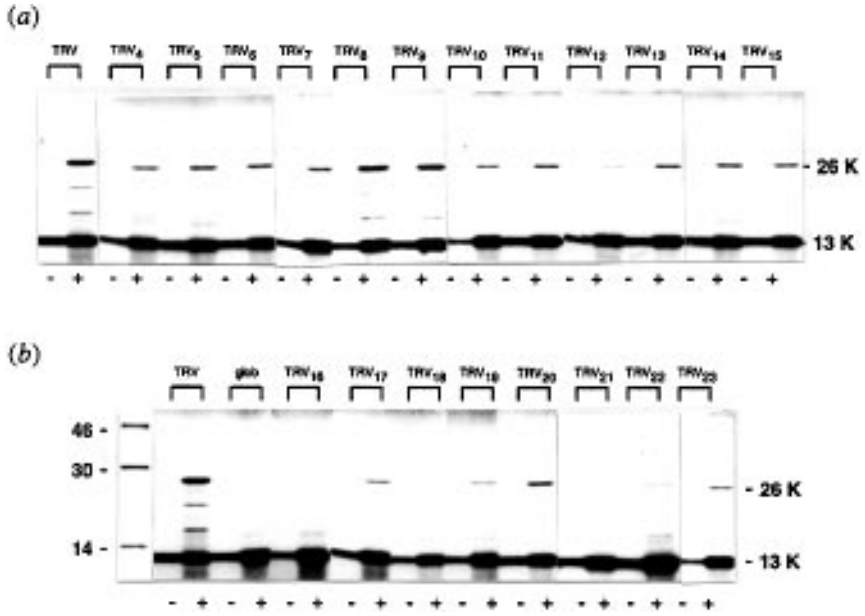

Figure 1. Read-through over the UGA stop codon in mutated transcripts derived from pSP65-TRV or pSP65-globin by *Nicotiana* chl $tRNA^{Trp}_{CmCA}$. *In vitro* synthesized transcripts carrying either single (**a**) or multiple (**b**) mutations in the TRV-specific read-through region 5′ or 3′ of the UGA codon (indicated in Table 1) were translated in wheat germ extract in the absence (–) or presence (+) of 50 µg/ml partially purified chl $tRNA^{Trp}_{CmCA}$. The addition of this tRNA fraction to the tRNA-depleted wheat germ extract leads to an overall stimulation of translation activity. The [^{35}S]methionine-labelled proteins were separated on a 15% denaturing gel. Molecular weight markers are shown at the left. The major translation product of 13 kDa and the read-through protein of 26 kDa are indicated at the right.

Untreated wheat germ was a gift from Synpharma GmbH (Augsburg, Germany).

Plasmids

The recombinant plasmid pSP65-ML1 carries a zein gene from maize seedlings (10) cloned into the *Bam*HI and *Pst*I sites of pSP65 vector DNA. The expression vectors pSP65-TRV, pSP65-TMV_3 and pSP65-globin are described elsewhere (4,11). For construction of the vectors pSP65-PEMV and pSP65-SINV the two synthetic oligonucleotides 5′-GTAGTTGCAAACCAGGC-CTCCCTCTGAGGGGACGACTTTCTGCCAGCGC-3′ and 5′-GTAGTTGCAAACCAGACTGAATACTGACTAACCGGGT-TTCTGCCAGCG-3′ were used to replace the TAG and the surrounding codons present in the zein gene by TGA and the corresponding nucleotides flanking the leaky TGA codon in RNA-1 of pea enation mosaic virus (12) and in sindbis virus RNA (13) respectively.

Isolation of plant and yeast UGA suppressor tRNAs

Chloroplast (chl) and cytoplasmic (cyt) $tRNA^{Trp}_{CmCA}$ were isolated from leaves of *Nicotiana rustica*. Total tRNA was fractionated on a BD-cellulose column and $tRNA^{Trp}$-enriched fractions were further purified by gel electrophoresis in polyacrylamide gels as described (4). Cyt $tRNA^{Trp}_{CmCA}$ was identified by aminoacylation with [^{3}H]tryptophan using a crude synthetase preparation from wheat germ and chl $tRNA^{Trp}_{CmCA}$ was detected in individual fractions by RNA dot blot analysis with a specific probe (5). Yeast UGA suppressor tRNA was prepared from the *Schizosaccharomyces pombe* strain sup3-5h$^-$ and partially purified by BD-cellulose chromatography.

Transcription *in vitro*

In vitro transcription of pSP65 plasmid DNAs by SP6 RNA polymerase was essentially carried out as described by Zerfaß and Beier (3).

In vitro translation in wheat germ extract

A wheat germ cell-free extract depleted of endogenous mRNAs and tRNAs was prepared according to Pfitzinger *et al.* (14). *In vitro* translation was performed for 1 h at 30°C in the presence of 10 MBq/ml [^{35}S]methionine in a total volume of 10 µl containing 25% (v/v) wheat germ extract and 10% (v/v) wheat germ initiation factor solution as described recently (5).

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 Fuji RX X-ray films at –80°C for the appropriate times, avoiding overexposure of individual films. The ratio of the protein products derived from termination and read-through events respectively was determined by densitometric quantification of incorporated radioactive methionine using a LKB UltraScan XL® laser densitometer. Appropriate corrections were made for the higher methionine content of the read-through products.

RESULTS

Single nucleotide exchanges surrounding the leaky UGA codon in TRV RNA-1 have little effect on the efficiency of suppression by $tRNA^{Trp}_{CmCA}$

In order to characterize the parameters influencing the efficiency of UGA read-through by tobacco $tRNA^{Trp}_{CmCA}$, we have inserted the UGA codon and six codons flanking this stop codon in TRV RNA-1 into a zein gene from maize and have investigated the expression of the full-length zein protein of 26 kDa in a tRNA-depleted wheat germ extract. We have already shown that this short sequence, consisting of only seven authentic codons, is sufficient for stimulating read-through up to 30% *in vitro* (4,5). This observation ruled out the possibility that secondary structures upstream or downstream of the UGA codon have any major influence on suppression by $tRNA^{Trp}_{CmCA}$ and made it plausible to assume that only nucleotides in the immediate neighbourhood of the UGA codon are components of the codon context. We have selected chloroplast rather than cytoplasmic $tRNA^{Trp}_{CmCA}$ for our studies since the former is a more active UGA suppressor (4,5), thus facilitating quantitative analyses.

An inspection of plant virus RNAs which harbour similar UGA read-through sites reveals that they have in common only an A residue at the 5′- and a CGG codon at the 3′-side of the internal UGA codon (5). Consequently, we started our studies by introducing substitutions at each of the 4 nt positions in the original pSP65-TRV construct. The derivatives of pSP65-TRV indicating the performed changes are listed in Table 1. Transcripts containing these mutations were produced *in vitro* by SP6 RNA polymerase, translated in wheat germ extract and the proteins were analysed by SDS–PAGE. Most single base exchanges had only minor effects on the efficiency of UGA read-through, as can be seen in Figure 1a and Table 1. Replacement of the A residue at the 5′-side by G or U or of the C residue at the 3′-side by A or U had virtually no effect, whereas a C at the 5′- and a G at the 3′-side of the UGA codon decreased the read-through efficiency relative to the unmutated sequence to 44 and 26% respectively. Substitutions of the two G residues at the second and third downstream positions each led to a relative read-through activity of ~30%. It should be pointed out that the valine codon at the second position upstream of the UGA is GUC in the original TRV sequence and that due to a mutagenesis artefact some mutants carry a GUA codon at this position (Table 1). However, we found that the corresponding single nucleotide exchange had no effect on the suppression efficiency.

Table 1. Read-through over the UGA stop codon by *Nicotiana* chl $tRNA^{Trp}_{CmCA}$ as a function of surrounding nucleotides

Transcript	Sequence of the read-through region	Relative read-through (%)
pSP65-TRV	-ACC-GUC-UUA-UGA-CGG-UUU-CGG-	100
pSP65-TRV_4	-ACC-GUC-UUG-UGA-CGG-UUU-CGG-	72
pSP65-TRV_5	-ACC-GUA-UUC-UGA-CGG-UUU-CGG-	44
pSP65-TRV_6	-ACC-GUC-UUU-UGA-CGG-UUU-CGG-	78
pSP65-TRV_7	-ACC-GUA-UUA-UGA-GGG-UUU-CGG-	26
pSP65-TRV_8	-ACC-GUA-UUA-UGA-AGG-UUU-CGG-	88
pSP65-TRV_9	-ACC-GUA-UUA-UGA-UGG-UUU-CGG-	94
pSP65-TRV_{10}	-ACC-GUA-UUA-UGA-CAG-UUU-CGG-	29
pSP65-TRV_{11}	-ACC-GUA-UUA-UGA-CCG-UUU-CGG-	30
pSP65-TRV_{12}	-ACC-GUA-UUA-UGA-CUG-UUU-CGG-	28
pSP65-TRV_{13}	-ACC-GUC-UUA-UGA-CGA-UUU-CGG-	25
pSP65-TRV_{14}	-ACC-GUA-UUA-UGA-CGC-UUU-CGG-	33
pSP65-TRV_{15}	-ACC-GUA-UUA-UGA-CGU-UUU-CGG-	35
pSP65-globin	-AAA-UAC-CAC-UGA-GAU-CUU-UUU-	0
pSP65-TRV_{16}	-ACC-GUA-UUA-UGA-GAU-CUU-UUU-	3
pSP65-TRV_{17}	-AAA-UAC-CAC-UGA-CGG-UUU-CGG-	24
pSP65-TRV_{18}	-ACC-GUA-CAC-UGA-GAU-UUU-CGG-	1
pSP65-TRV_{19}	-ACC-GUA-UUA-UGA-GAU-UUU-CGG-	10
pSP65-TRV_{20}	-ACC-GUC-CAC-UGA-CGG-UUU-CGG-	35
pSP65-TRV_{21}	-ACC-GUC-UAC-UGA-GAG-UUU-CGG-	5
pSP65-TRV_{22}	-ACC-GUA-UUA-UGA-GAG-UUU-CGG-	12
pSP65-TRV_{23}	-ACC-GUC-UUC-UGA-GGG-UUU-CGG-	22
pSP65-globin	-AAA-UAC-CAC-UGA-GAU-CUU-UUU-	0
pSP65-$globin_4$	-AAA-UAC-CAA-UGA-CAU-CUU-UUU-	7
pSP65-$globin_5$	-AAA-UAC-CAC-UGA-CGU-CUU-UUU-	5
pSP65-$globin_6$	-AAA-UAC-CUA-UGA-CGU-CUU-UUU-	8
pSP65-$globin_7$	-AAA-UAC-UUA-UGA-CGG-CUU-UUU-	10

Compilation of data shown partially in Figure 1. Read-through activity was defined as 100% for translation of the pSP65-TRV transcript, containing the unmutated read-through region from TRV RNA-1. Substituted nucleotides differing from the pSP65-TRV and pSP65-globin transcript respectively are underlined.

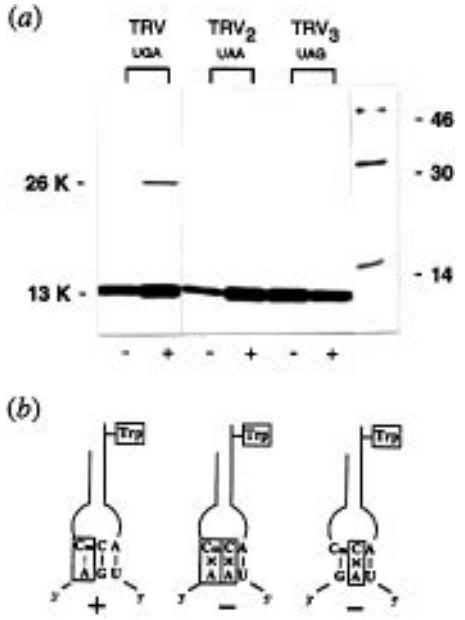

Figure 2. Ability of *Nicotiana* $tRNA^{Trp}_{CmCA}$ to read UGA but not the UAA or UAG codons. (**a**) The *in vitro* synthesized transcripts pSP65-TRV, pSP65-TRV_2 and pSP65-TRV_3, carrying a UGA, UAA and UAG stop codon respectively in the TRV codon context (11), were translated in wheat germ extract in the absence (–) or presence (+) of 30 µg/ml highly purified *Nicotiana* chl $tRNA^{Trp}_{CmCA}$. (**b**) Schematic presentation of data shown in (a). The unorthodox Cm:A interaction has been observed *in vitro* at the first anticodon position, but has not been detected in the middle anticodon position.

We had expected a more pronounced influence of the nucleotides adjacent to the stop codon because: (i) suppression of the internal UAG codon in TMV RNA strictly depends on the six downstream codons, as mentioned above; and (ii) we had recently shown that tobacco $tRNA^{Trp}_{CmCA}$ does not promote read-through over the UGA stop codon at the end of the β-globin cistron (4), indicating that the β-globin sequence contains signals which do not support UGA suppression by $tRNA^{Trp}_{CmCA}$. In order to pinpoint these inhibitory elements we exchanged the six codons surrounding the UGA in TRV RNA-1 for the corresponding globin-specific codons. This construct was called pSP65-globin (Table 1 and Fig. 3). Translation in the wheat germ extract of the pSP65-globin transcript confirmed that the UGA in the globin context was not recognized at all by $tRNA^{Trp}_{CmCA}$. We then successively substituted TRV-specific codons or nucleotides with globin-specific sequences and examined their effects on read-through activity. These results, which are presented in Figure 1b and Table 1, indicate a complex influence of nucleotides at the 3′- as well as the 5′-side of the UGA codon, but clearly show that the downstream nucleotides have a stronger inhibitory effect than the upstream nucleotides. For instance, replacing the upstream codons in the pSP65-TRV transcript by the corresponding globin codons (i.e. pSP65-TRV_{17}) resulted in a relative read-through activity of 24%, whereas a similar exchange at the 3′-side (i.e. pSP65-TRV_{16}) reduced suppression to 3%. The same cooperative inhibitory effects on UGA suppression by globin-specific downstream and upstream nucleotides was principally observed in the mutated transcripts pSP65-TRV_{18} to pSP65-TRV_{23}, containing 3, 2 or 1 mutated nt at either side of the UGA codon. The results further showed that the transcript with only the dinucleotide GA at the 3′-side of the UGA (i.e. pSP65-TRV_{22}) expressed a relative read-through activity of 12%, which was distinctly lower than any activity measured upon single nucleotide substitutions (Fig. 1a and b and Table 1).

In a third series of mutational analyses we basically introduced TRV-specific nucleotides into the pSP65-globin construct in order to create a transcript which would allow efficient read-through. However, even the presence of the TRV-specific UUA codon at the 5′-side and the CGG codon at the 3′-side of the UGA in the pSP65-$globin_7$ transcript permitted only 10% read-through as compared with the pSP65-TRV transcript (Table 1), indicating that the sequence around the UGA in β-globin mRNA contains further inhibitory elements in addition to the adjacent nucleotides.

UGA but not the UAA or UAG stop codon are recognized by *Nicotiana* $tRNA^{Trp}$

We have previously demonstrated that $tRNA^{Tyr}$ with a GΨA anticodon is not only capable of recognizing the UAG but also the UAA codon in the TMV context (3). In order to study the capability of $tRNA^{Trp}_{CmCA}$ to interact with any of the other two stop codons in addition to UGA, we used two templates in which the original UGA in the pSP65-TRV construct was replaced by either UAA or UAG. These clones are called pSP65-TRV_2 and pSP65-TRV_3 respectively (11). The two corresponding *in vitro* transcripts were translated in wheat germ extract in the presence of highly purified chl $tRNA^{Trp}_{CmCA}$. As can be seen in Figure 2, neither stop codon was suppressed at all by $tRNA^{Trp}_{CmCA}$, indicating the exclusive nature of $tRNA^{Trp}_{CmCA}$ as a UGA suppressor.

The leaky UGA stop codon in different plant and animal viral RNAs is suppressed by $tRNA^{Trp}_{CmCA}$

Unexpectedly we have found that a number of single nucleotide exchanges in the immediate vicinity of the UGA codon in TRV RNA-1 do not have a considerable effect on the ability of $tRNA^{Trp}$ to suppress the UGA codon. This observation prompted us to study the suppression of leaky UGA codons in viral RNAs which are surrounded by quite different nucleotides as compared with TRV. The only well-known plant virus with a dissimilar UGA read-through sequence in its RNA-1 is pea enation mosaic virus (PEMV). Among animal viruses alphaviruses use UGA read-through to express their RNA polymerase (i.e. nsP4). From these we have selected sindbis virus (SINV) for our studies, since it is the only alphavirus for which the influence of the codon context on UGA read-through has yet been investigated (15). The read-through sequences of PEMV and SINV, consisting of a total of seven codons, were cloned into the pSP65-ML1 construct as shown in Figure 3. It should be pointed out here that the 6 nt located 3′ to the leaky UGA codon in Venezuelan equine encephalitis virus (VEEV) RNA, another member of the alphaviruses, are identical to those flanking the UGA in TRV RNA-1, i.e. CGG-UUU (16). The *in vitro* synthesized transcripts were translated in wheat germ extract in the presence of chl $tRNA^{Trp}_{CmCA}$ in parallel with the pSP65-TRV and pSP65-TMV_3 transcripts, the latter containing a UGA codon in the TMV context (5). The estimated absolute read-through activities were 24, 23, 18 and 6% for the transcripts containing a UGA codon in the TRV, TMV, SINV and PEMV contexts respectively (Fig. 4a),

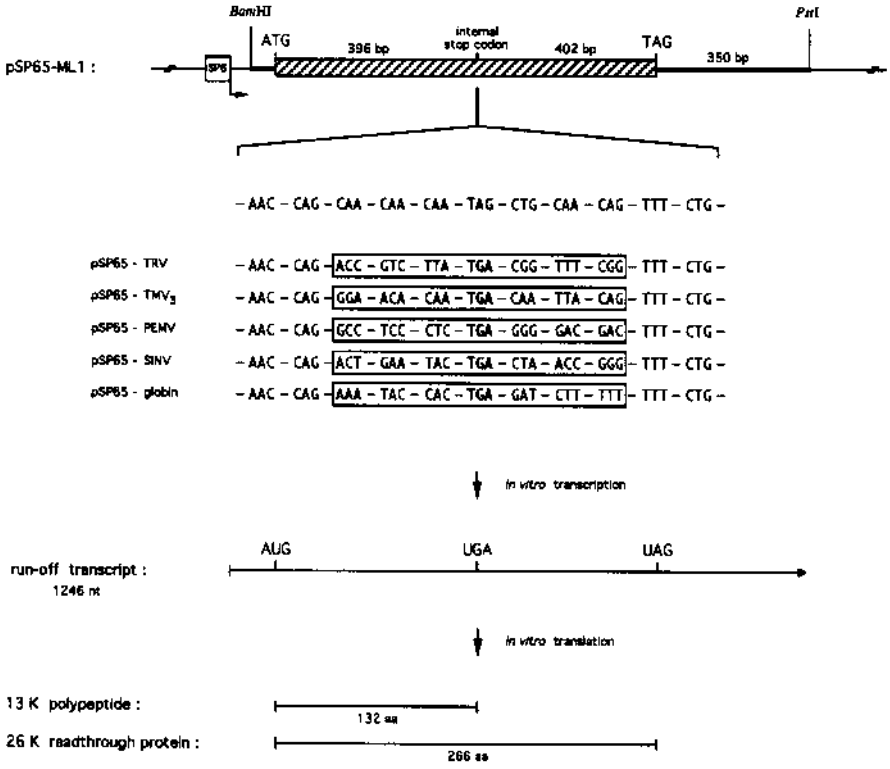

Figure 3. Structures of expression vectors pSP65-ML1 and its derivatives carrying read-through regions from different virus RNAs. A 1.2 kb fragment from *Zea mays* DNA, harbouring a zein gene, was cloned into the *Bam*HI and *Pst*I sites of the SP6 RNA polymerase-specific vector pSP65 (3). The transcripts generated by the constructs pSP65-TRV to pSP65-globin carry a total of six codons flanking the leaky stop codon in TRV, TMV, PEMV and SINV RNA respectively and six codons neighbouring the UGA codon at the end of β-globin mRNA. *In vitro* translation of the run-off transcripts results in the synthesis of a 13 kDa termination protein and, in the presence of an appropriate UGA suppressor, in the production of a 26 kDa read-through protein.

i.e. all four viral contexts are permissive for UGA read-through by $tRNA^{Trp}_{CmCA}$, albeit to a different extent. Yeast UGA suppressor $tRNA^{Ser}_{U*CA}$ derived from chemical mutagenesis reads the UGA codon in the TRV, TMV and PEMV contexts with about the same efficiencies of 69, 58 and 71% respectively (Fig. 4b). In contrast with $tRNA^{Trp}_{CmCA}$, the yeast suppressor is also able to suppress the UGA codon in the globin context with an efficiency of 25%.

DISCUSSION

Expression of the viral replicase domain in the RNA of TMV and TRV respectively is mediated by translational read-through of either an in-frame UAG or a UGA termination codon. We have recently identified cyt $tRNA^{Tyr}_{GΨA}$ as the first natural suppressor tRNA in plants that is capable of reading the UAG and UAA codons in a TMV-specific context (3). Furthermore we have discovered that chl and cyt $tRNA^{Trp}_{CmCA}$ are able to suppress the UGA codon in a TRV-specific context (4). Here we have shown that $tRNA^{Trp}_{CmCA}$ is incapable of misreading UAG or UAA codons (Fig. 2), thus indicating its nature as an exclusive UGA suppressor. The interaction of $tRNA^{Trp}_{CmCA}$ with UAA or UAG would have required either a single C:A mispair at the middle position or two adjacent C:A mispairs in the anticodon–codon complex, which obviously are not tolerated in our *in vitro* translation system. These results are in accordance with earlier observations which showed that all kinds of unconventional base pairs in the middle anticodon position appear to be prohibited (11).

It has been reported by many groups that translational read-through of a termination codon is affected by the nucleotide sequence surrounding the stop codon in the mRNA of prokaryotic and eukaryotic species (17–22). We have concentrated our efforts on analysing the influence of codon context effects on the read-through activity of specific natural suppressor tRNAs *in vitro*. For this purpose we have chosen a wheat germ extract depleted of endogenous mRNA and tRNAs that allowed us to study the influence of read-through sequences on the action of a particular tRNA. Thus we have ascertained that the 6 nt downstream of the UAG codon in the TMV context are involved in permitting efficient suppression by $tRNA^{Tyr}_{GΨA}$ (3). Skuzeski *et al.* (6) had previously come to the same conclusion by studying the transient expression of a β-glucuronidase reporter gene containing part of the TMV-specific read-through sequence *in vivo*.

In the present report we have examined the signals that determine UGA read-through by tobacco $tRNA^{Trp}_{CmCA}$. The TRV-specific read-through sequence that was inserted into a zein gene consisted of only seven codons, including the UGA codon (Fig. 3). The UGA in the corresponding *in vitro* transcript pSP65-TRV was by-passed up to 25% (Figs 1 and 4), implying that complex signals like stem–loop or pseudoknot structures are not involved in the read-through process. This is reflected in the absence of defined stem–loop structures downstream of the UGA codon in TRV RNA-1 (23).

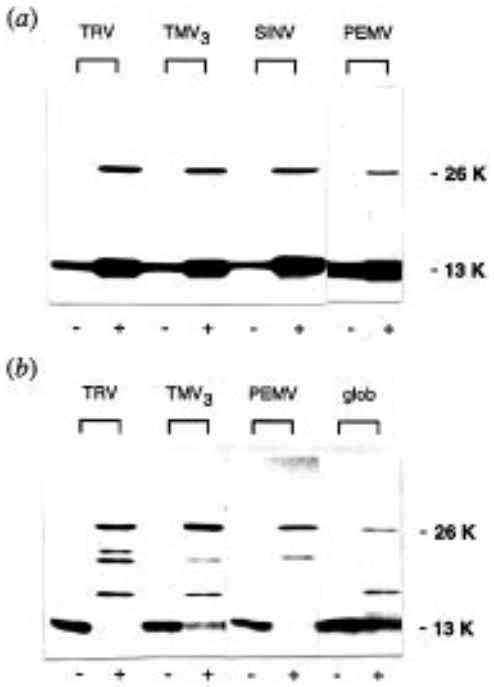

Figure 4. Suppression of the UGA stop codon in transcripts containing different viral read-through regions. *In vitro* synthesized transcripts carrying part of the read-through regions contained in TRV, TMV, SINV and PEMV RNA (see Fig. 3) were translated in wheat germ extract in the absence (–) and presence (+) of either 50 µg/ml chl $tRNA^{Trp}_{CmCA}$ from *Nicotiana* (**a**) or 50 µg/ml UGA suppressor $tRNA^{Ser}_{U*CA}$ from *S.pombe* (**b**). The major translation product of 13 kDa and the read-through polypeptide of 26 kDa are indicated at the right.

Mutations upstream of the UGA had either little effect or reduced read-through activity by a factor of three, as seen in the transcript pSP65-TRV_{20}, in which the original UUA had been replaced by a CAC codon (Table 1). A generally accepted model for the role of upstream sequences in the termination or translational read-through process has not yet emerged. Recently the identities of the codons and amino acids immediately in front of a stop codon in *Escherichia coli* mRNAs were shown to be non-random. In particular, amino acids like lysine and glutamine were common at the 5′-side of stop codons (24), suggesting a selection for basic amino acids near the C-terminus of proteins. Another study proposed that the rate of stop codon suppression correlated with the nature of the amino acid encoded by the tRNA present in the E site of *E.coli* ribosomes (25). The highest UGA read-through was measured if the second to last amino acid in the nascent polypeptide was any acidic amino acid, whereas a basic amino acid at this position did not promote stop codon read-through, irrespective of the nature of the codon or its decoding tRNA present in the A site. It has further been suggested that the 5′ context is mediated by a direct tRNA–tRNA interaction between the tRNA residing in the P site and the suppressor tRNA entering the A site (26).

Nucleotides immediately 3′ of the UGA codon appear to have a major effect on suppression by $tRNA^{Trp}_{CmCA}$, as had been established for UAG suppression by $tRNA^{Tyr}_{G\Psi A}$. However, in contrast with the very specific nucleotide sequence CAR-YYA apparently required for efficient UAG suppression *in vivo* and *in vitro* (3,6), we have found that single nucleotide exchanges at either of the three downstream positions had only marginal effects on UGA suppression by $tRNA^{Trp}_{CmCA}$. For instance, replacement of the C residue at the 3′-side of the UAG in the TMV context to a G or A residue reduced the relative read-through activities in wheat germ extract to 1.5 and 4% respectively (3; C. Schüll and H. Beier, unpublished), whereas the corresponding changes in the pSP65-TRV transcript resulted in relative UGA suppression activities of 26 and 88% (Table 1). Single base changes in the CGG arginine codon at the 3′-side of UGA subsequently resulted in various alterations of the amino acid identity, i.e. the occurrence of a UGG tryptophan or a CCG proline codon (Table 1). Since none of these changes had a dramatic effect on UGA suppression, the possibility can be ruled out that a $tRNA^{Arg}$ species is favoured at the 3′-side of the leaky UGA codon. A pronounced influence on UGA suppression was seen only if 2 or 3 nt had been simultaneously replaced, as in the two transcripts pSP65-TRV_{19} and pSP65-TRV_{22} (Table 1).

At the moment we cannot explain why the nucleotides downstream of the UAG and UGA codons facilitate read-through to a different extent. It has been proposed that 3′ codon effects may depend on interaction between the stop codon and the rRNA (27) and/or the polypeptide chain release factor (24,28). Without doubt, one has to consider competition between the release factor that specifically recognizes each of the three termination codons and the suppressor tRNA occupying the A site of the ribosome. It has been noticed that the nucleotide following known termination codons is clearly non-random. This observation led to the proposal that the release factor may in fact recognize a tetranucleotide signal (24,28,29). While the preferred termination signal differs in prokaryotic and eukaryotic species and may vary in highly expressed genes, a common feature appears to be a strong bias against a cytidine residue following a termination codon in all species, including plants (30,31), implying that UAG-C or UGA-C are weak stop signals. Consistent with this assumption is the presence of a C residue at the 3′-side of all stop codons which are efficiently suppressed (3,4,15; Figs 3 and 4).

Li and Rice (15) have examined the influence of neighbouring nucleotides on suppression of the UGA codon in the SINV context in reticulocyte lysate. They found that the CUA codon at the 3′-side was sufficient for efficient translational read-through and that mutations at the second or third residue had little effect, but that replacement of the C residue immediately downstream of the UGA to U, A or G resulted in an almost complete loss of read-through activity. This is unlike our observations made in wheat germ extract (Table 1) and might possibly be due to the particular translation system chosen for the corresponding studies. It should be noted in this connection that all three codons arising by first position mutations of the CUA codon, i.e. UUA, AUA and GUA happen to be codons that are not used in either α- or β-globin mRNA (32) and consequently the analogous tRNA isoacceptors might be under-represented in reticulocyte lysate.

Due to the flexible codon context accepted by $tRNA^{Trp}_{CmCA}$, this tRNA is able of by-passing the UGA codon existing in quite different codon contexts, as in TRV, TMV or SINV RNAs. Interestingly, the UGA in the PEMV context, whose suppression in RNA-1 of PEMV yields an extended coat protein (12), is also recognized by $tRNA^{Trp}_{CmCA}$ (Fig. 4a). The UGA in the PEMV context is followed by a G residue at the 3′-side (Fig. 3), the most unfavourable nucleoside tolerated by $tRNA^{Trp}_{CmCA}$ at this position

(Table 1), yet read-through activities of up to 6% were measured *in vitro* (Fig. 4a).

Although we have employed chl $tRNA^{Trp}_{CmCA}$ in most of our studies, we have confirmed that cyt $tRNA^{Trp}_{CmCA}$ in principle expresses similar features in being able to recognize quite different viral codon contexts (not shown). Since cyt $tRNAs^{Trp}$ have almost identical nucleotide sequences in plants and animals (33) and exist only as a single isoacceptor in diverse tissues and cells, $tRNA^{Trp}_{CmCA}$ clearly has the potential of a universal efficient UGA suppressor in all eukaryotic species.

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