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SURVEY AND SUMMARY

Misreading of termination codons in eukaryotes by natural nonsense suppressor tRNAs

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ABSTRACT

Translational stop codon readthrough provides a regulatory mechanism of gene expression that is extensively utilised by positive-sense ssRNA viruses. The misreading of termination codons is achieved by a variety of naturally occurring suppressor tRNAs whose structure and function is the subject of this survey. All of the nonsense suppressors characterised to date (with the exception of selenocysteine tRNA) are normal cellular tRNAs that are primarily needed for reading their cognate sense codons. As a consequence, recognition of stop codons by natural suppressor tRNAs necessitates unconventional base pairings in anticodon–codon interactions. A number of intrinsic features of the suppressor tRNA contributes to the ability to read non-cognate codons. Apart from anticodon–codon affinity, the extent of base modifications within or 3′ of the anticodon may up- or down-regulate the efficiency of suppression. In order to out-compete the polypeptide chain release factor an absolute prerequisite for the action of natural suppressor tRNAs is a suitable nucleotide context, preferentially at the 3′ side of the suppressed stop codon. Three major types of viral readthrough sites, based on similar sequences neighbouring the leaky stop codon, can be defined. It is discussed that not only RNA viruses, but also the eukaryotic host organism might gain some profit from cellular suppressor tRNAs.

INTRODUCTION

Regulation of gene expression is found at different stages, one of which operates at the level of termination in protein synthesis. Normally, termination of mRNA translation is signalled by the occurrence of any of the three nonsense (stop) codons UAG, UAA or UGA at the ribosomal A site. Protein release factors specifically bind to these codons (for which cognate tRNAs are not available) and subsequently mediate release of the nascent polypeptide chain from the ribosome. However, there are several processes known that can circumvent nonsense codons, for example ribosomal frameshifting and suppression by either mutated or natural cellular tRNAs. In the case of frameshifting, the reading frame is shifted in the 5′ or 3′ direction and as a consequence the suppressed stop codon is read as a sense codon (1–3). Mutated suppressor tRNAs carry an altered anticodon allowing the tRNA to misread a stop codon by normal base pair interactions. A bulk of chemically induced nonsense suppressors have been isolated in the past from *Escherichia coli* and *Saccharomyces cerevisiae* and employed in genetic and biochemical studies, but will not be the subject of this article.

Natural nonsense suppression, however, means the reading of stop codons as sense codons by normal cellular tRNAs which are called natural suppressors. This review describes primarily the structure and function of such tRNAs—with the exception of selenocysteine tRNA—in higher eukaryotes. It does not cover missense (i.e. the reading of sense codons by non-cognate tRNAs) and nonsense suppression in bacteria for which excellent reviews exist (4–6). Furthermore, this article deals with codon context effects, i.e. primary sequences and secondary structures in the vicinity of 'leaky' stop codons that influence the efficiency of suppression. A number of recent reports describing related topics are recommended to the reader (7–13).

IDENTIFICATION OF STOP CODON READTHROUGH

Translational readthrough provides a regulatory mechanism of gene expression by permitting the differential production of more than one polypeptide from a single gene. Notably, RNA viruses make use of this potential to expand the genetic information of their relatively small genomes (Fig. 1).

The first type of stop codon readthrough was detected in *E.coli* infected with RNA phage Qβ. It was shown that normal $tRNA^{Trp}$ with CCA anticodon stimulates readthrough over the UGA stop codon at the end of the coat protein cistron, resulting in an elongated coat protein that is essential for the formation of infective Qβ particles (14–16). It took more than a decade to detect natural suppressor tRNAs also in eukaryotic cells. Pelham (17) postulated the presence and 'leakiness' of a UAG codon at the end of the 126 kDa cistron in tobacco mosaic virus (TMV) RNA (Fig. 2), since he found stimulation of the *in vitro*

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Virus	Genus	"Leaky" termination codon	Readthrough product/ function
Enterobacteria phage Qβ	Allolevivirus	UGA	Coat protein extension; assembly
Murine leukemia virus (MuLV)	Gammaretrovirus	UAG	Reverse Transcriptase
Sindbis virus (SIN)	Alphavirus	UGA	Replicase
Tomato bushy stunt virus (TBSV)	Tombusvirus	UAG	Replicase
Carnation mottle virus (CarMV)	Carmovirus	UAG	Replicase
Tobacco necrosis virus (TNV)	Necrovirus	UAG	Replicase
Maize chlorotic mottle virus (MCMV)	Machlomovirus	UAG	Replicase
Barley yellow dwarf virus (BYDV)	Luteovirus	UAG	Coat protein extension: aphid transmission
Potato leafroll virus (PLRV)	Polerovirus	UAG	Coat protein extension; aphid transmission
Pea enation mosaic virus (PEMV)	Enamovirus		
RNA-1		UGA	Coat protein extension; aphid transmission
Tobacco mosaic virus (TMV)	Tobamovirus	UAG	Replicase
Tobacco rattle virus (TRV)	Tobravirus		
RNA-1		UGA	Replicase
Peanut clump virus (PCV)	Pecluvirus		
RNA-1		UGA	Replicase
Soil-borne wheat mosaic virus (SBWMV)	Furovirus		
RNA-1		UGA	Replicase
RNA-2		UGA	Coat protein extension; fungus transmission
Potato mop-top virus (PMTV)	Pomovirus		
RNA-1		UGA	Replicase
RNA-3		UAG	Coat protein extension
Beet soil-borne virus (BSBV)	Pomovirus		
RNA-1		UAA	Replicase
RNA-2		UAG	Coat protein extension
Broad bean necrosis virus (BBNV)	Pomovirus		
RNA-2		UAA	Coat protein extension
Beet necrotic yellow vein virus (BNYVV)	Benyvirus		
RNA-2		UAG	Coat protein extension; fungus transmission
Turnip yellow mosaic virus (TYMV)	Tymovirus	UAG	Replicase extension ?

Figure 1. Suppressible termination codons in positive sense ssRNA viruses, representing mostly type species from the corresponding genus. The classification of viruses is according to Pringle (104). Detailed information about sequences and function of readthrough products is listed in the legends to Figures 13 and 14.

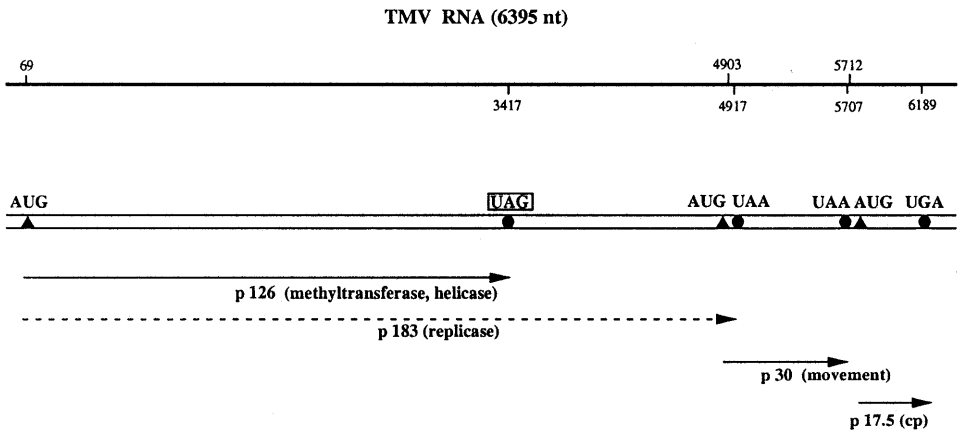

Figure 2. Schematic structure of TMV RNA. The 6.4 kb genomic ssRNA of TMV (18) is designated by a double horizontal line. The upper line indicates the locations of ORFs as determined from the sequence. The positions of initiation and termination codons are specified by closed triangles and circles, respectively. The proteins synthesised *in vivo* are shown as single lines with arrowheads, the 183 kDa readthrough product is indicated by a broken line. The 'leaky' UAG stop codon at the end of the 126 kDa cistron is boxed.

synthesis of the 183 kDa readthrough protein in the presence of yeast amber suppressor tRNA. The elucidation of the complete TMV RNA sequence confirmed the existence of an internal UAG stop codon (18). Moreover, the observation that the

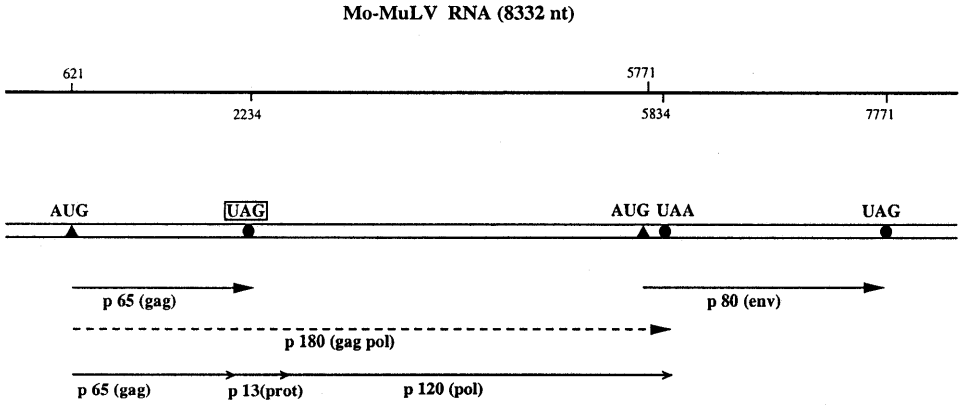

Figure 3. Schematic structure of Mo-MuLV RNA. The 8.3 kb genomic ssRNA of Mo-MuLV (105) is represented by a double line. The positions of initiation and termination codons are indicated by closed triangles and circles, respectively. The 'leaky' UAG codon is boxed. The two primary polypeptides of 65 and 80 kDa are indicated by single lines. The gag/pol readthrough product of 180 kDa is shown as a broken line. This polypeptide is subsequently cleaved into the gag protein, a protease of 13 kDa and the reverse transcriptase of 120 kDa.

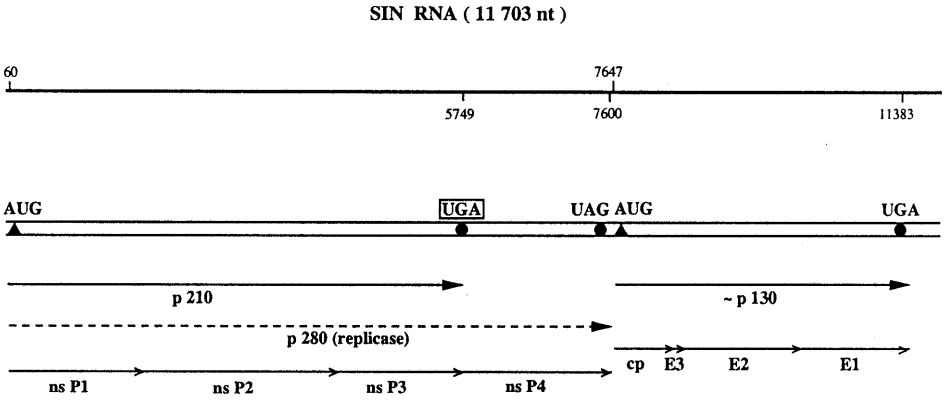

Figure 4. Schematic structure of sindbis virus (SIN) RNA. The 11.7 kb genomic ssRNA of SIN (106) is represented by a double line. The positions of initiation and termination codons are specified by closed triangles and circles, respectively. The translation of SIN non-structural (ns) proteins starts from the AUG codon at the beginning of nsP1 and proceeds to the UGA at the end of the nsP3 cistron. Partial readthrough of this 'leaky' stop codon yields the polypeptide of 280 kDa which is subsequently processed by proteolytic cleavage to produce the non-structural proteins nsP1 to nsP4.

183 kDa polypeptide was synthesised in TMV-infected tobacco protoplasts and tobacco leaves (19,20) supported the notion that the 'leaky' UAG codon is very likely suppressed by a cellular tobacco tRNA. The 126 kDa protein contains a putative methyltransferase and a helicase domain, whereas the 183 kDa readthrough product harbours the highly conserved GDD motif, responsible for replicase activity (10). Both polypeptides are essential for TMV multiplication (21).

The second well-known example for translational readthrough is found upon expression of the murine leukemia virus (MuLV) RNA in animal tissue. In MuLV-infected mouse cells the in-frame UAG stop codon at the end of the gag cistron is suppressed to yield a gag–pol fusion polypeptide of 180 kDa, which is subsequently cleaved into the gag protein, a protease of 13 kDa and the reverse transcriptase (Fig. 3). Thus, the readthrough product is the only source of reverse transcriptase, and consequently UAG suppression is necessary for normal multiplication of MuLV. Yoshinaka *et al.* (22) showed that the protease that overlaps the readthrough region contains glutamine inserted at the site of the UAG codon, indicating that a cellular $tRNA^{Gln}$ acts as a UAG suppressor. Many (but not all) members of the alphavirus genus contain a 'leaky' UGA stop codon within their genomic RNA separating the non-structural proteins nsP3 and nsP4 (Fig. 4). The nsP4 protein shares homologous amino acid sequences with the RNA-dependent RNA replicase of poliovirus and plant RNA viruses, and is supposed to contain the elongating activity of the sindbis virus replicase (23).

Besides TMV, a bulk of plant RNA viruses from at least 14 groups utilise stop codon readthrough to generate either a functional polymerase or an extended coat protein. The latter is important for the assembly of the virion and/or vector transmission. Of all known 'leaky' stop codons, UAA is by far the least frequently used. Some of the multicomponent viruses, such as beet soil-borne virus and potato mop-top virus contain diverse 'leaky' stop codons on different RNA species (Fig. 1).

To date, very few DNA viruses are known that employ nonsense suppression as a means of regulating gene expression.

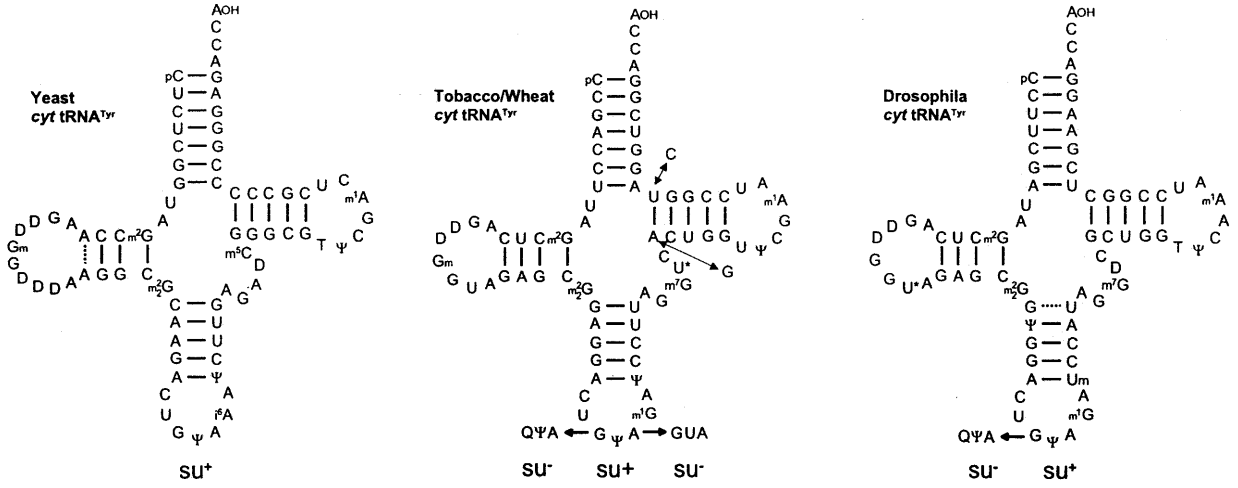

Figure 5. Nucleotide sequences of cytoplasmic (cyt) tRNAs^{Tyr} from *S.cerevisiae* (34), *Nicotiana rustica* (19), wheat germ (29) and *Drosophila melanogaster* (107). The tRNA^{Tyr} isoacceptors with GΨA anticodon have been shown to be active UAG suppressors *in vitro*, whereas the corresponding species with a GUA or QΨA anticodon are unable to misread this stop codon (19,27,29,39). U* (in position 20:A of *Drosophila* and 47 of plant tRNA^{Tyr}), acp^{3}U; m^{1}G, 1-methylguanosine; m^{2}G, N^2-methylguanosine; m$^2_2$G, N^2,N^2-dimethylguanosine; m^{7}G, 7-methylguanosine; m^{1}A, 1-methyladenosine; m^{5}C, 5-methylcytidine; Ψ, pseudouridine; T, ribosylthymine; Q, queuosine; D, dihydrouridine; Gm, 2′-O-methylguanosine; Um, 2′-O-methyluridine.

For instance, the 58 kDa virion protein of the human cytomegalovirus may be synthesised via translational readthrough of a UGA termination codon separating two open reading frames on the 1.6 kb late mRNA (24).

As mentioned above, the readthrough polypeptides have been shown in many cases to be essential for virus production. Furthermore, the ratio of the two synthesised products appears to be critical. Thus, the substitution of the 'leaky' UAG by a CAG glutamine codon in MuLV-RNA resulted in the inability to produce virus (25,26). An unbalanced synthesis of the 126 and 183 kDa polypeptides in TMV-infected tobacco plants also caused a low yield of progeny (21).

UAG/UAA SUPPRESSORS

Cytoplasmic tRNA^{Tyr}

The first eukaryotic natural UAG suppressor tRNA was isolated from tobacco leaves and adult *Drosophila* on the basis of its ability to promote readthrough over the 'leaky' UAG codon of TMV RNA in a messenger-dependent reticulocyte lysate and in *Xenopus* oocytes, respectively (19,27). The highly purified suppressor tRNA was sequenced by the fragment analysis of partially digested tRNA according to Stanley and Vassilenko (28). Its amino acid acceptance and anticodon sequence identified the UAG suppressor as cytoplasmic tRNA^{Tyr} with a GΨA anticodon (Fig. 5). Tobacco and wheat leaves contain only tRNA^{Tyr} isoacceptors with a GΨA anticodon, whereas ~85% of the tRNA^{Tyr} species from wheat germ and lupin seeds have a QΨA anticodon (29–31). Interestingly, tRNA^{Tyr} with a QΨA anticodon is totally unable to stimulate UAG readthrough (27,29,32). The highly modified Q nucleoside is synthesised as the free queuosine base (Q), which is then inserted into tRNA by a transglycosylase to replace guanine (33). Bacterial tRNAs are normally completely modified with respect to Q, whereas yeast is totally lacking Q-containing tRNAs (34). Yeast tRNA^{Tyr} with a GΨA anticodon is likewise able to suppress the TMV-specific UAG codon *in vitro* (H.Beier, unpublished result) although its nucleotide sequence differs considerably from plant and animal tRNAs^{Tyr} (Fig. 5).

Animal tRNAs exhibit a variable Q content depending on the developmental state. In differentiated liver only tRNA^{Tyr} with QΨA anticodon is present (35), but fetal liver, reticulocytes and tumour tissue contain significant amounts of tRNAs with G in place of Q in the first position of the anticodon (36,37). Adult *Drosophila* possess tRNA^{Tyr} isoacceptors with GΨA and a QΨA anticodon (27), and relevant changes in the Q content in tRNAs isolated from different ontogenetic stages of *Drosophila* have been observed (38).

Another interesting attribute of tRNA^{Tyr} is the pseudouridine (Ψ35) at the second position of the anticodon, a unique feature of all eukaryotic cytoplasmic tRNAs^{Tyr}. An *in vitro* synthesised tRNA^{Tyr} with GUA instead of GΨA anticodon was unable to suppress the 'leaky' UAG codon of TMV RNA (39), indicating that the Ψ35 modification in the tRNA^{Tyr} anticodon is necessary for the unconventional codon reading. Pseudouridine can form a classical base pair with adenosine, but is more versatile in its hydrogen bonding interactions, possibly resulting in stabilisation of codon–anticodon interactions (40). Hence, there is a remarkable influence of base modifications on UAG suppression. The presence of the hypermodified Q at the first anticodon position of tRNA^{Tyr} prevents, whereas Ψ at the second anticodon position enhances, the unconventional base pairing (Fig. 5).

Plant cytoplasmic tRNA^{Tyr} with a GΨA anticodon is quite an efficient UAG suppressor. Under optimal conditions 10–30% of readthrough over the TMV-specific 'leaky' UAG are observed *in vitro* (19,39,41). The same tRNA^{Tyr} is able to read UAA, but not the UGA stop codon, albeit with lower efficiency (39). Although tRNA^{Tyr}(GΨA) is a very potent UAG suppressor *in vitro*, and very likely also *in vivo*, it cannot

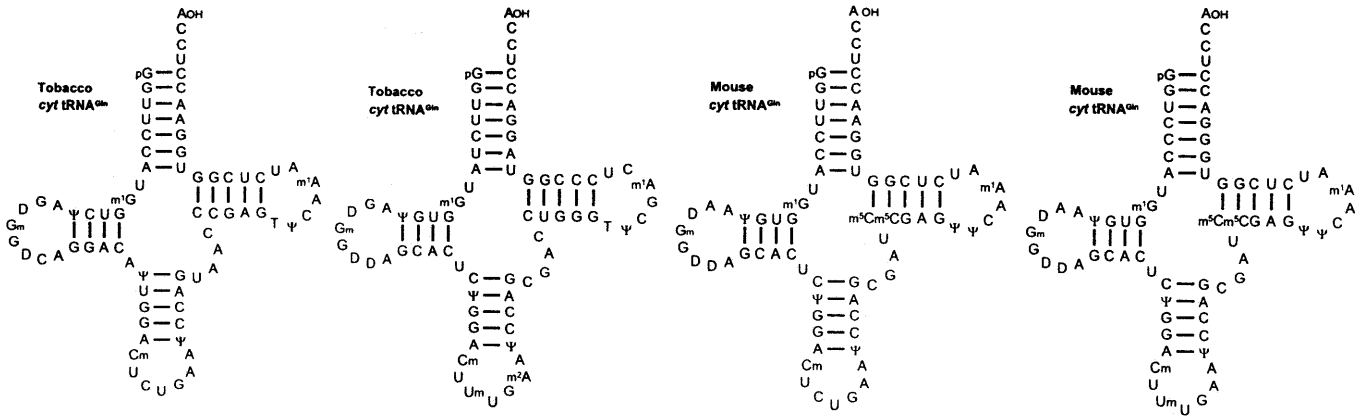

Figure 6. Nucleotide sequences of cytoplasmic (cyt) tRNAs^{Gln} from *N.rustica* (48) and mouse liver (46). All of these tRNA^{Gln} isoacceptors suppress either the UAG, UAA or both stop codons in a wheat germ (41,48) and in a reticulocyte lysate, respectively (46). A major tRNA^{Gln} with UmUG anticodon from *T.thermophila* is also a strong UAG/UAA suppressor (41,108). The modified nucleosides not listed in the Figure 5 legend are: m^2A, 2-methyladenosine; Cm, 2′-O-methylcytidine.

be regarded as a universal suppressor because of its limited presence in various tissues as outlined above.

Cytoplasmic tRNAs^{Gln}

The second class of eukaryotic UAG/UAA suppressors are cytoplasmic tRNAs^{Gln}. Two isoacceptors with a CUG or U*UG anticodon exist virtually in all pro- and eukaryotes (34). A number of observations had implied their putative ability to read UAG and/or UAA codons. For instance, in the yeast *S.cerevisiae*, transformation with high copy numbers of a tRNA^{Gln} isoacceptor with a CUG anticodon resulted in the suppression of a number of UAG mutations in the yeast genome (42–44). Likewise, Pure *et al.* (45) reported that overexpression of yeast tRNA^{Gln} with a UUG anticodon weakly suppressed internal UAA codons. Furthermore, glutamine is inserted at the site of the 'leaky' UAG codon in MuLV RNA (22).

Two cytoplasmic tRNA^{Gln} isoacceptors were isolated from mouse liver and tobacco leaves, and their sequences were determined (Fig. 6). The minor tRNA^{Gln} species from mouse liver with a UmUG anticodon was shown to stimulate readthrough over the 'leaky' UAG codon in TMV RNA in a reticulocyte lysate (46). Noticeably, the amount of this suppressor tRNA^{Gln} was greatly increased in NIH3T3 and Ehrlich ascites cells infected with Mo-MuLV, as reported by Kuchino *et al.* (46). In contrast, Feng *et al.* (47) observed equivalent amounts of the two tRNA^{Gln} isoacceptors in MuLV-infected and uninfected NIH3T3 cells. The reasons for this discrepancy are unknown.

Both cytoplasmic tRNA^{Gln} isoacceptors from tobacco stimulated readthrough over the TMV-specific UAG codon in a wheat germ extract partially depleted of endogenous tRNAs. In this system, *Nicotiana* tRNA^{Gln} with a UmUG anticodon was a less efficient UAG suppressor than tRNA^{Gln} with a CUG anticodon (48). One of the major tRNA^{Gln} isoacceptors from the unicellular ciliate *Tetrahymena thermophila* has a UmUG anticodon and comprises a high sequence similarity to its animal and plant counterparts (Fig. 6). This tRNA^{Gln} isoacceptor is a very potent UAG and UAA suppressor in wheat germ extract, provided appropriate amounts of a *Tetrahymena* synthetase preparation are added to the extract (41). Most of the sequenced cellular tRNA^{Gln} species carry an unmodified A residue immediately 3′ to the anticodon at position 37 (Fig. 6). Interaction of the two tRNA^{Gln} isoacceptors with UAG and/or UAA requires an unconventional G:U base pairing at the *third* anticodon position (see below). Presumably, an unmodified A adjacent to the anticodon facilitates non-canonical base pairing at the *third* anticodon position, since a number of reports have conversely shown that a hypermodified A, like i^6A (N^6-isopentenyladenosine) or ms^2i^6A (2-methylthio-N^6-isopentenyladenosine), impedes non-Watson–Crick interactions at this position (49–51).

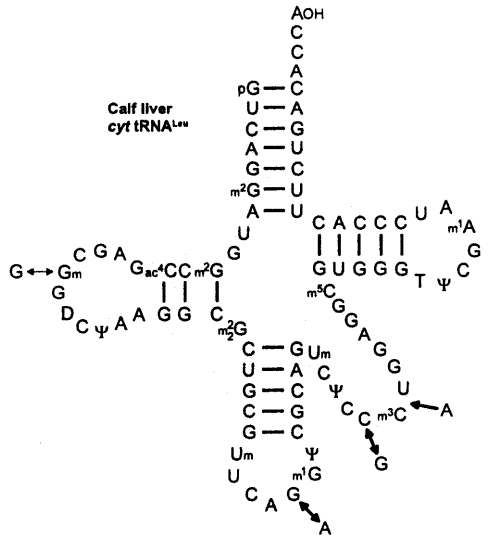

Figure 7. Nucleotide sequences of cytoplasmic (cyt) tRNAs^{Leu} from calf liver (52). The two tRNA^{Leu} isoacceptors have been shown to misread the TMV-specific 'leaky' UAG codon in a reticulocyte lysate. The modified nucleoside not listed in the Figure 5 legend is m^3C, 3-methylcytidine.

Cytoplasmic tRNAs^{Leu}

Two amber suppressor tRNAs have been isolated from calf liver that read the 'leaky' UAG codon of TMV RNA and RNA-2 of beet necrotic yellow vein virus (BNYVV) in a messenger-dependent reticulocyte lysate (52). They are neither tRNA^{Tyr} nor tRNA^{Gln} species, but tRNA^{Leu} isoacceptors of

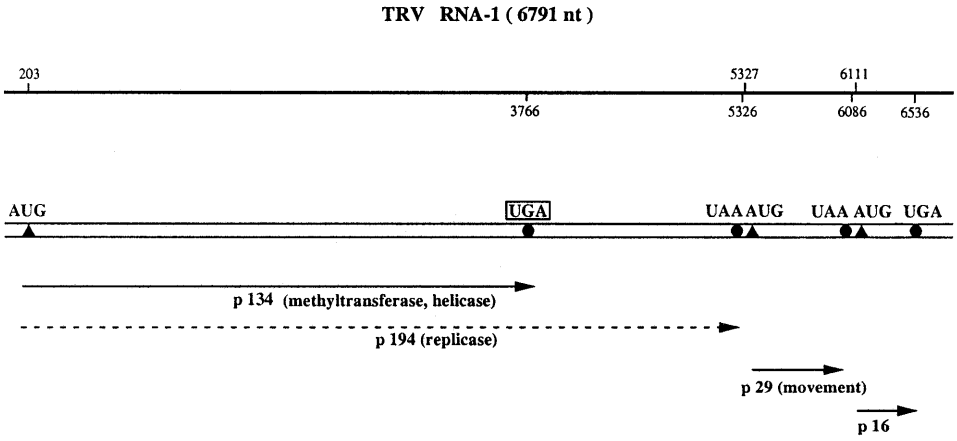

Figure 8. Schematic structure of TRV RNA-1. The 6.8 kb genomic ssRNA-1 of TRV (109) is designated by a double horizontal line. The positions of initiation and termination codons are indicated by closed triangles and circles, respectively. The first ORF codes for a non-structural protein of 134 kDa that is terminated by a UGA codon. Partial readthrough of this 'leaky' UGA stop codon yields the 194 kDa polypeptide (broken line).

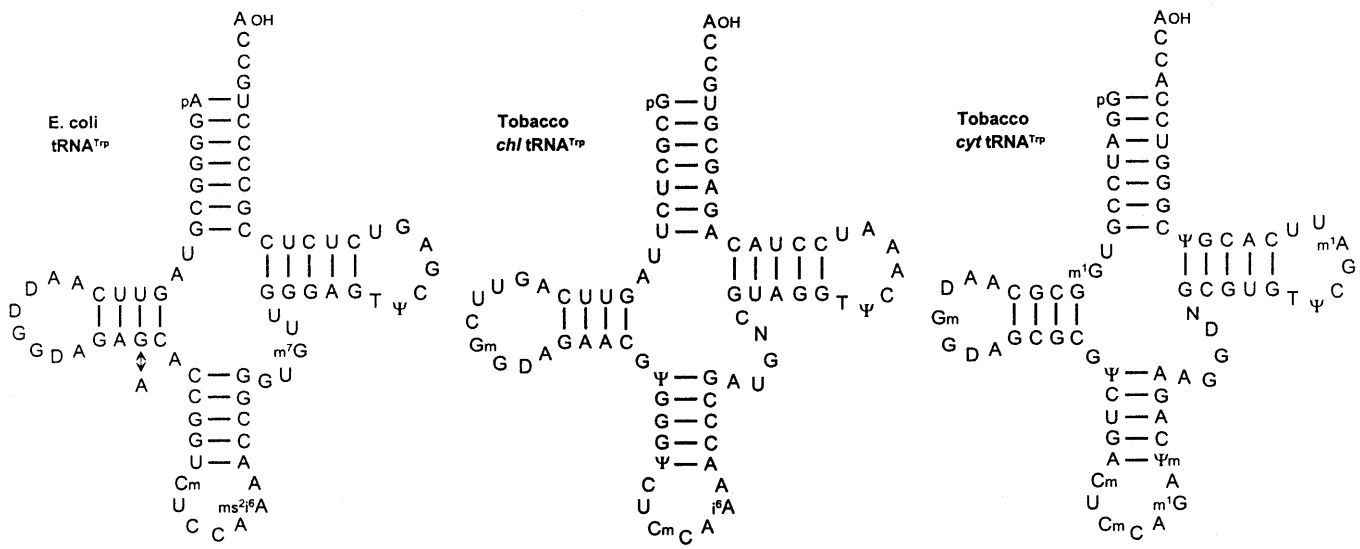

Figure 9. Nucleotide sequences of chloroplast (chl) and cytoplasmic (cyt) tRNAs^{Trp} from *N.rustica.* The two tRNA^{Trp} isoacceptors promote UGA readthrough preferentially in the TRV-specific codon context in wheat germ extract (54,88). For comparison, the sequences of wild-type tRNA^{Trp} from *E.coli* and a mutated derivative are also shown. The mutated tRNA^{Trp} contains a single nucleotide exchange (G→A) at position 24, and exhibits increased UGA suppressor activity as compared to the wild type (14). The modified nucleosides not listed in the Figure 5 legend are: i^6A, N^6-isopentenyladenosine; ms^2i^6A, 2-methylthio-N^6-isopentenyladenosine; Ψm, 2′-O-methylpseudouridine.

cytoplasmic origin with CAA and CAG anticodon (Fig. 7). The recognition of the UAG codon by these suppressors requires an unusual A:A pairing in the second, and also a G:U pairing in the third, position of the anticodon. It is not known whether the corresponding plant tRNA^{Leu} isoacceptors also comprise UAG suppressor activity *in vitro*.

UGA SUPPRESSORS

Chloroplast and cytoplasmic tRNAs^{Trp}

Readthrough over the 'leaky' UAG in TMV RNA was employed for the characterisation of virtually all eukaryotic UAG suppressors described above. In order to identify UGA suppressors, a second plant RNA virus was utilised in most cases. The tobravirus, tobacco rattle virus (TRV), has a bipartite genome. RNA-1 molecules contain the genetic information needed for RNA replication and intercellular movement, whereas RNA-2 specifies the coat protein. *In vitro* and *in vivo* translation studies have demonstrated that RNA-1 directs the synthesis of two polypeptides of 134 and 194 kDa (53). Expression of the 194 kDa replicase protein requires readthrough over a 'leaky' UGA stop codon at the end of the 134 kDa cistron (Fig. 8)

Two natural UGA suppressors were isolated from uninfected tobacco plants on the basis of their ability to promote readthrough over the 'leaky' UGA codon in TRV RNA-1 in a wheat germ extract (54). Their amino acid acceptance and nucleotide sequences identified the two UGA suppressors as chloroplast and cytoplasmic tryptophan-specific tRNAs with

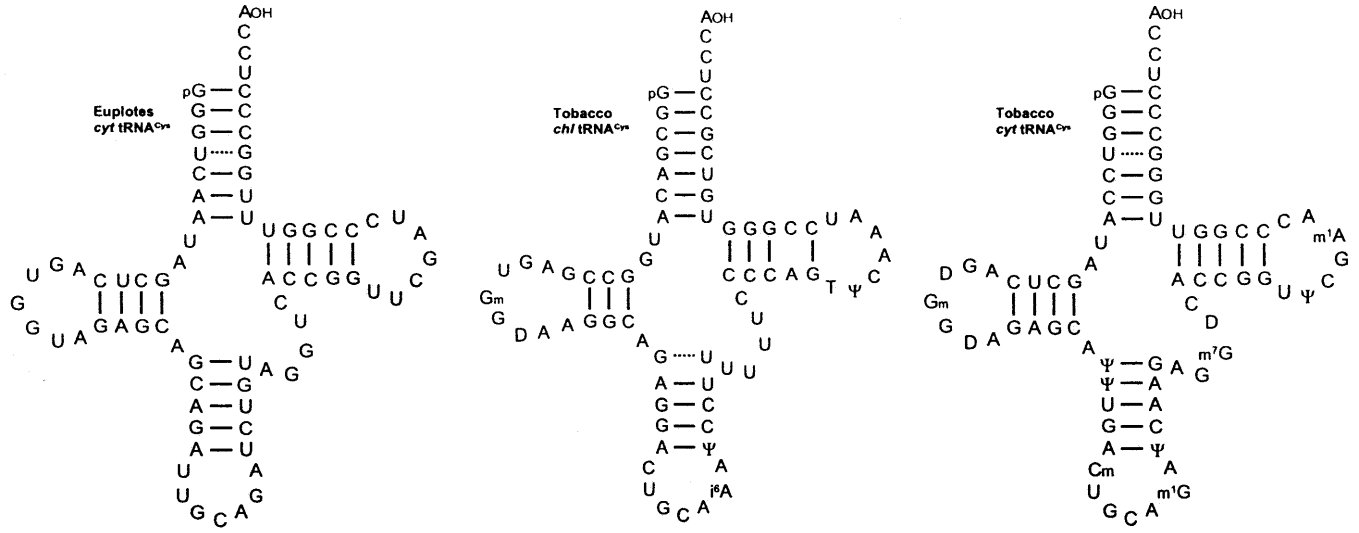

Figure 10. Nucleotide sequences of chloroplast (chl) and cytoplasmic (cyt) tRNAs^{Cys} from *N.rustica*. The two tRNA^{Cys} isoacceptors promote UGA readthrough in wheat germ extract (63). The sequence of tRNA^{Cys} from *E.octocarinatus* has been deduced from the known gene sequence contained within a cloned macronuclear DNA molecule. This tRNA^{Cys} isoacceptor presumably reads the in-frame UGA codons present in numerous gene-derived mRNAs in *Euplotes* cells (64).

the anticodon CmCA (Fig. 9). Unexpectedly, it was found that chloroplast tRNA^{Trp} suppresses the UGA codon more efficiently than cytoplasmic tRNA^{Trp}. It has been speculated that structural features and/or base modifications of the former contribute to higher suppression. Thus, a mutant strain from *E.coli* with increased UGA suppressor activity turned out to contain a tRNA^{Trp} species with an unaltered anticodon, but a single nucleotide exchange at position 24 leading to an A24:U11 instead of a G24:U11 base pair in the D-stem (14; Fig. 9). Presumably, the mutation at position 24 reduces the rate at which the ribosome rejects non-cognate tRNAs, more easily permitting unconventional base pairing (55,56). The chloroplast tRNA^{Trp} from tobacco contains the same A24:U11 base pair as the mutated *E.coli* tRNA^{Trp}, whereas cytoplasmic tRNA^{Trp} has a G:C pair at this postion (Fig. 9). It should be noted, however, that it is not known whether eukaryotic ribosomes express similar constraints. Another difference between the two tRNA^{Trp} isoacceptors lies in the nature of the modified nucleoside at position 37. Chloroplast tRNA^{Trp} carries i^{6}A or ms^{2}i^{6}A, whereas cytoplasmic tRNA^{Trp} has a m^{1}G (1-methylguanosine) at this position. It has been proposed that i^{6}A and its derivatives stabilise anticodon–codon interactions by increasing the stacking effect of the anticodon on neighbouring nucleotides, thus supporting non-canonical base interactions at the *first* anticodon position (57,58).

Given the high UGA suppressor activity of chloroplast tRNA^{Trp}, the question arises whether fidelity of chloroplast synthesis is impaired. However, close inspection of the 24 protein-coding genes in the *Nicotiana* chloroplast genome that terminate with a UGA codon, reveals that in ~50% of the cases the stop codon is flanked at the 3′ side by a second one within 0–3 codons (59) and in other cases it is embedded in an unfavourable codon context (see below), suggesting that deleterious effects are negligible.

Several reports indicate that tRNA^{Trp} isoacceptors with UGA suppressor activity are also present in higher vertebrates. A tRNA^{Trp} was purified from rabbit reticulocytes and was shown to efficiently suppress the UGA codon at the end of the β-globin gene *in vitro* (60). However, the sequence of this tRNA was not determined and, thus, the possibility cannot be ruled out that the suppressor was in fact mitochondrial tRNA^{Trp} with a U*CA anticodon, the only tRNA^{Trp} species encoded by the rabbit mitochondrial genome (34), and not cytoplasmic tRNA^{Trp} with a CCA anticodon. Furthermore, a tRNA^{Trp} with a CmCA anticodon was isolated from avian sarcoma virus virions whose sequence and nucleoside modifications were identical to cytoplasmic tRNA^{Trp} of uninfected cells, yet the former was a more active UGA suppressor *in vitro* as compared to its cellular counterpart (61). The reason for this unusual observation is yet unknown. The authors speculate that two conformations of avian tRNA^{Trp} may exist that influence the biological activity of the tRNA.

Chloroplast and cytoplasmic tRNAs^{Cys}

A number of observations made the existence of cysteine-accepting UGA suppressors very likely. Feng *et al.* (62) demonstrated that replacement of the 'leaky' UAG by a UGA stop codon in MuLV RNA (Fig. 3) directs the incorporation of cysteine in addition to tryptophan and arginine at the corresponding position of the readthrough product upon translation in a reticulocyte lysate.

Chloroplast and cytoplasmic tRNA^{Cys} isoacceptors with a GCA anticodon (Fig. 10) were isolated from uninfected tobacco leaves on the basis of aminoacylation assays with [^{35}S]cysteine in the presence of an unfractionated wheat germ aminoacyl-tRNA synthetase preparation. Subsequently, the readthrough activity of the two isoacceptors was studied in wheat germ extract programmed with *in vitro* synthesised transcripts containing the 'leaky' UGA region of TRV RNA-1 (Fig. 8). Chloroplast tRNA^{Cys} turned out to suppress the UGA in the TRV as well as in the TMV codon context more efficiently than the cytoplasmic counterpart (63), an observation already

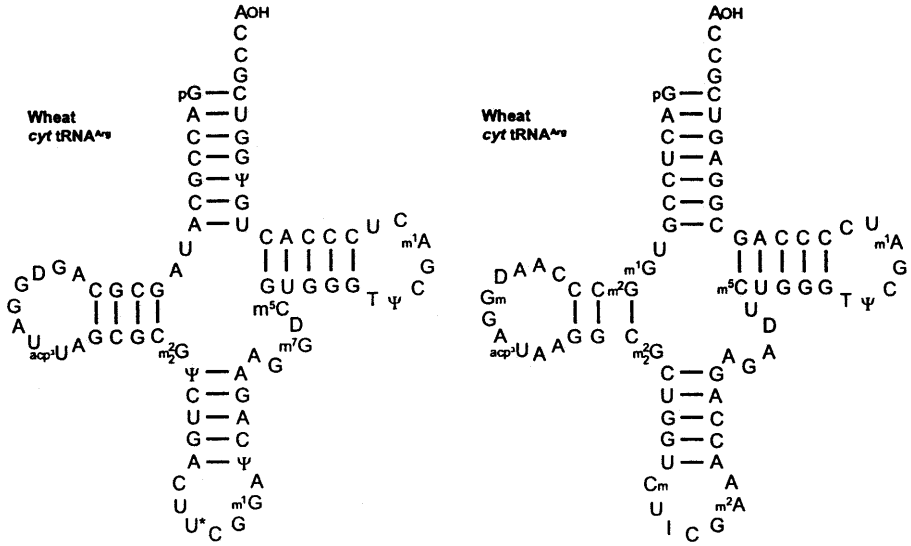

Figure 11. Nucleotide sequences of cytoplasmic (cyt) tRNAs^{Arg} from wheat germ. The tRNA^{Arg} with U*CG and to a minor extent tRNA^{Arg} with the ICG anticodon promote UGA readthrough preferentially in the PEMV-specific codon context in a wheat germ extract (70). U* (in position 34 of tRNA^{Arg}), mcm^{5}U (5-methoxy-carbonylmethyluridine) and/or mcm^{5}s^{2}U (5-methoxy-carbonylmethyl-2-thiouridine); I, inosine.

made for chloroplast tRNA^{Trp} (see above). No data are available about putative UGA suppressor activity of cytoplasmic tRNA^{Cys} from animal cells. However, as deduced from the overall sequence homology of tRNA^{Cys} gene sequences in the mouse and human genome with the corresponding plant genes (64) it is reasonable to assume that animal tRNAs^{Cys} also have the potential to act as UGA suppressors.

An interesting situation applies for the unicellular ciliate *Euplotes octocarinatus*. In this organism deviations from the universal genetic code have been described. The UGA stop codon is translated as cysteine within coding regions and does not function as a termination codon in protein synthesis (65). Presumably, only a single tRNA^{Cys} isoacceptor exists in *Euplotes* cells which contains a GCA anticodon like all known tRNAs^{Cys} species (64; Fig. 10). Plant tRNAs^{Cys} have turned out to be relatively inefficient UGA suppressors *in vitro* (63). One major argument in favour of a more active UGA-decoding *Euplotes* tRNA^{Cys}(GCA) is the existence of a release factor in this organism that has lost the capability to bind to UGA codons (66,67), so that competition between the latter and tRNA^{Cys} is greatly reduced.

Cytoplasmic tRNAs^{Arg}

The third class of UGA suppressors has been exclusively characterised in plants on the tRNA level. The six arginine codons CGN and AGR are read by four isoacceptors in *E.coli* and *S.cerevisiae*, and by five isoacceptors in the cytosol of higher eukaryotes. Of these five isoacceptors, tRNA^{Arg} with a U*CG anticodon is not found in *E.coli* and yeast (68,69). It is noteworthy that it is just this isoacceptor that is a potent UGA suppressor. The major tRNA^{Arg} species have been isolated from wheat germ. *In vitro* translation of transcripts containing the UGA codon in the context of TRV RNA-1 (Fig. 8) in wheat germ extract in the presence of either of the wheat tRNA^{Arg} isoacceptors revealed that mainly tRNA^{Arg} with U*CG, and to a lesser extent tRNA^{Arg} with ICG anticodon (Fig. 11), stimulated UGA readthrough. Moreover, tRNA^{Arg} with a U*CG anticodon was found to also suppress the 'leaky' UGA codon in the pea enation mosaic and sindbis virus context (70). Studies of Feng *et al.* (62) and Chittum *et al.* (71) indicate that tRNAs^{Arg} are also potential UGA suppressors in animals. They identified the amino acid arginine (among others) inserted at the site of UGA in the MuLV-specific context and at the UGA terminating the β-globin cistron upon translation in rabbit reticulocytes *in vitro* and *in vivo*

Sindbis virus RNA contains an in-frame UGA termination codon in the region separating the non-structural proteins nsP3 and nsP4 (Fig. 4). Readthrough over this UGA codon has been observed in cultured cells of chicken, human and insect origin (23). Remarkably, in the closely related semliki forest virus (SFV) there is no UGA, but instead a CGA arginine codon at this position (72), suggesting that possibly the 'leaky' UGA in sindbis virus RNA is recognised preferentially by tRNA^{Arg}(U*CG), which routinely reads the CGA arginine codon. Li and Rice (73) have presented indirect evidence that tryptophan (and not arginine) is incorporated at the site of the 'leaky' UGA in RNA transcripts containing the sindbis virus-specific readthrough region upon *in vitro* translation in a rabbit reticulocyte lysate. In this connection it should be emphasised that reticulocytes represent a highly specialised type of cells in which the nuclei, and to some extent also the mitochondria, are disintegrated, resulting in the accumulation of mitochondrial tRNA^{Trp} with U*CA anticodon in the cytosol. This tRNA isoacceptor utilises normal base interactions to read UGA and consequently may easily out-compete any other natural suppressor.

UNCONVENTIONAL BASE INTERACTONS

Crick (74) had postulated that G:U, U:G and I:C/U/A base pairs at the first anticodon position (also called the 'wobble' position) would not affect the fidelity of protein synthesis, due to the degeneracy of the genetic code. Later, some restrictions from this rule were observed in different organisms. For

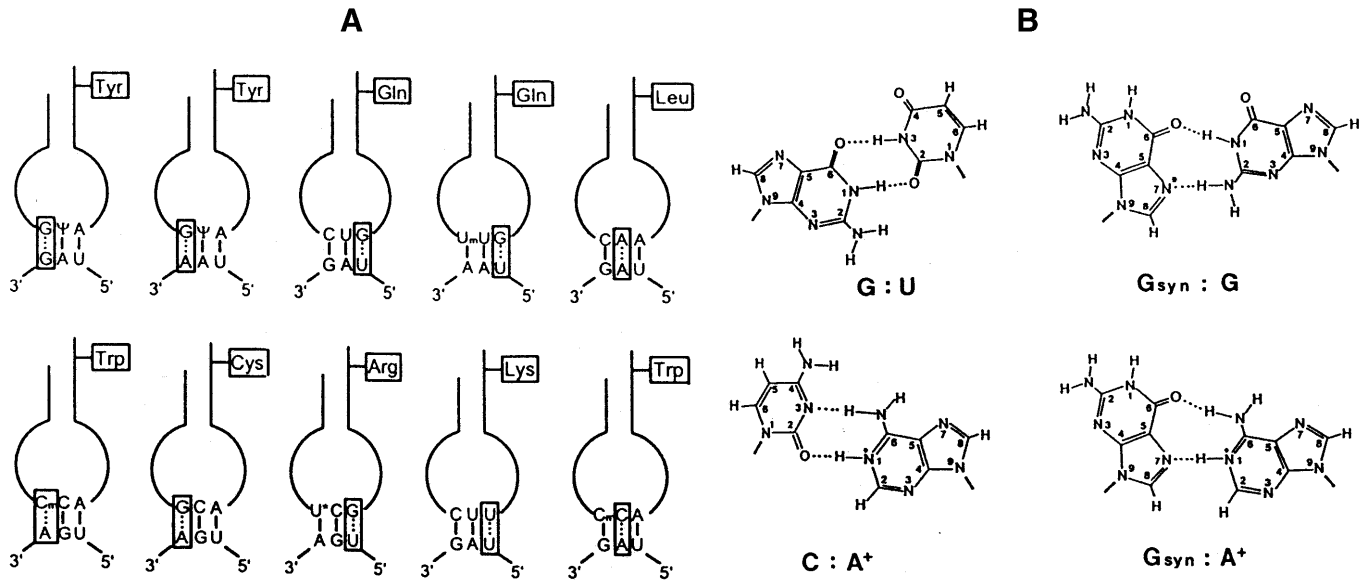

Figure 12. Unconventional base interactions involved in the misreading of termination codons by natural suppressor tRNAs. (**A**) Schematic presentation of putative anticodon–codon interactions by eukaryotic suppressor tRNAs. With the exception of $tRNA^{Lys}$(CUU) and $tRNA^{Trp}$(CmCA) misreading the UAG codon, all types of non-canonical codon reading by means of an appropriate suppressor tRNA have been demonstrated directly *in vitro* (19,39,48,52,54,63,70,89). In the case of the two former examples, incorporation of lysine and tryptophan (in addition to tyrosine) has been elucidated by amino acid sequence analysis of the translation protein produced by readthrough of an in-frame UAG codon within the yeast Ste6 gene (78). The $tRNA^{Gln}$ isoacceptors with a UmUG anticodon from *Tetrahymena*, *Nicotiana* and mouse liver are able to also read UAG besides UAA (41,46,48). (**B**) Hypothetical interactions between non-complementary bases. In purine–purine mismatches, the nucleoside of the anticodon is presented in the *syn* configuration (isomerisation about the glycosyl bond of the nucleotide). The space-filling cyclopentene-diol side chain of the queuine base is attached to guanine at the indicated position (*) via a C–C bond, thus impairing the proposed G–G interaction (39). The adenosine in G:A and C:A mismatches is shown in the protonated form.

instance, in the yeast *Schizosaccharomyces pombe,* nonsense suppression has been shown to be strictly codon specific. The ability of ochre suppressors with a U*UA anticodon (where U* is mcm^5U or mcm^5s^2U) to read only UAA and not the UAG stop codon was attributed to the modification present at the first anticodon position, which obviously reduces the interaction with a G residue (75).

In all types of stop codon recognition by natural suppressor tRNAs non-canonical base pairing occurs mainly at the first or the third anticodon position. Thus, the misreading of UAG or UAA by $tRNA^{Tyr}$(GΨA) and of the UGA stop codon by $tRNA^{Cys}$(GCA) involves a G:G and G:A base interaction, respectively, whereas the reading of UGA by $tRNA^{Trp}$(CmCA) depends upon a Cm:A mismatch at the same position. On the other hand, G:U base pairs are required at the third anticodon position in the unconventional codon reading by $tRNAs^{Gln}$ and $tRNAs^{Arg}$ (Fig. 12A). The $tRNA^{Leu}$ isoacceptor with a CAA anticodon (Fig. 7) that is capable of suppressing the 'leaky' UAG codon of TMV RNA (52) necessitates an unusual A:A interaction at the second anticodon position. A few examples exist in prokaryotes in which unconventional base pairing has been reported in the middle position of the anticodon (76). Strigini and Brickman (77) have shown that *E.coli* $tRNA^{Trp}$(CCA) misreads *in vivo* not only UGA (14) but also the UAA codon, requiring two C:A base pairs in the latter case. Likewise, yeast cytoplasmic $tRNA^{Trp}$ appears to be able to suppress internal UAG codons *in vivo*, as demonstrated indirectly by amino acid sequence analysis of the translation product produced by UAG readthrough (78). The comprehensive study by Fearon *et al.* (78) further revealed that the UAG codon—placed within a favourable nucleotide context within the yeast Ste6 gene—directed not only the incorporation of tyrosine and tryptophan, but also of lysine. Misreading of UAG by $tRNA^{Lys}$ with a CUU anticodon requires an unorthodox U:U interaction at the third anticodon position (Fig. 12A). Readthrough over UAA and UGA codons—placed at identical sites as the UAG codon—has also been observed *in vivo*, but the nature of the incorporated amino acids has not been elucidated.

The mechanism of this unorthodox codon reading is not clear. Based on investigations by Topal and Fresco (79), an increasing number of non-Watson–Crick base pairs have been identified in DNA and RNA helices, some of which were proven by thermodynamic and/or X-ray crystallographic analyses (80–83). Such unconventional base pairs can be formed provided the nucleosides assume minor conformations (*syn*) and/or minor tautomeric or protonated forms, accompanied often by a slight displacement of the glycosidic bond as in the classical G:U pair (Fig. 12B). Whether these non-Watson–Crick pairs also occur in anticodon–codon interactions remains to be resolved. However, as mentioned above, $tRNA^{Tyr}$ with the hypermodified nucleoside Q at the first anticodon position does not promote UAG readthrough (27,29,32), emphasising that the guanosine at this position must play a selective role in stop codon suppression (Fig. 12B).

CODON CONTEXT EFFECTS

It has been reported by many groups that translational readthrough of a termination codon is affected by the nucleotide

A

Virus	Genus	Readthrough region sequence	Readthrough product	References/ accession no.
		G T Q * Q L Q		
TMV-U1	Tobamovirus	GGA-ACA-CAA-**UAG**-CAA-UUA-CAG	Replicase	(18)
		G S R * Q L Q		
TMGMV	-"-	GGU-AGU-AGA-**UAG**-CAA-UUA-CAG	-"-	NC 001556
		G I L * Q L Q		
ORSV-Cy	-"-	GGG-AUC-UUA-**UAG**-CAA-UUA-CAG	-"-	X80053
		G T Q * Q L Q		
CRMV	-"-	GGU-ACC-CAA-**UAG**-CAA-UUA-CAG	-"-	U30944
		G V Q * Q L Q		
TVCV	-"-	GGG-GUC-CAA-**UAG**-CAA-UUA-CAG	-"-	NC 001873
		P T K * Q L M		
CGMMV	-"-	CCU-ACC-AAA-**UAG**-CAA-UUA-AUG	-"-	AB015146
BNYVV		P G Q * Q L A		
RNA-2	Benyvirus	CCC-GGA-CAA-**UAG**-CAA-UUA-GCU	Cp extension	AF197547
BBNV		P T A * Q L T		
RNA-2	Pomovirus	CCG-ACA-GCA-**UAA**-CAA-UUA-ACG	-"-	D86637
PMTV		A G A * Q L T		
RNA-3	-"-	GCU-GGU-GCA-**UAG**-CAA-UUA-ACC	-"-	D16193
BSBV		W V E * Q S T		
RNA-2	-"-	UGG-GUU-GAA-**UAG**-CAA-UCA-ACU	-"-	U64512
BVQ		T G S * Q S I		
RNA-2	-"-	ACC-GGC-UCA-**UAG**-CAA-UCA-AUU	-"-	AJ223597
		Y V Q * Q S A		
TYMV	Tymovirus	UAC-GUC-CAA-**UAG**-CAA-UCA-GCC	-"-	NC 001509

B

Virus	Genus	Readthrough region sequence	Readthrough product	References/ accession no.
TRV		T V L * R F R		
RNA-1	Tobravirus	ACC-GUC-UUA-**UGA**-CGG-UUU-CGG	Replicase	(109)
PepRSV		A A L * R C R		
RNA-1	-"-	GCU-GCC-UUA-**UGA**-CGG-UGU-CGG	-"-	L23972
PEBV		A M K * R C R		
RNA-1	-"-	GCU-AUG-AAA-**UGA**-CGG-UGU-CGG	-"-	X14006
PCV		Q T K * R F G		
RNA-1	Pecluvirus	CAG-ACC-AAA-**UGA**-CGG-UUU-GGG	-"-	X78602
SBWMV		L T K * R F G		
RNA-1	Furovirus	CUU-ACU-AAA-**UGA**-CGG-UUU-GGG	-"-	L07937
SBWMV		G S S * R D G		
RNA-2	-"-	GGU-UCG-AGU-**UGA**-CGG-GAC-GGC	Cp extension	L07938
CWMV		F D K * R F G		
RNA-1	-"-	UUC-GAC-AAA-**UGA**-CGG-UUU-GGG	Replicase	AJ012005
CWMV		G S S * R D G		
RNA-2	-"-	GGU-UCG-AGU-**UGA**-CGG-GAU-GGC	Cp extension	AJ012006
OGSV		N Q K * R F G		
RNA-1	-"-	AAU-CAG-AAA-**UGA**-CGG-UUU-GGG	Replicase	AJ132578
OGSV		G S A * R G G		
RNA-2	-"-	GGU-AGU-GCC-**UGA**-CGG-GGC-GGC	Cp extension	AJ132579
BBNV		G P K * R C G		
RNA-1	Pomovirus	GGU-CCU-AAA-**UGA**-CGG-UGU-GGG	Replicase	D86636
PMTV		G V K * R Q A		
RNA-1	-"-	GGU-GUG-AAA-**UGA**-CGC-CAG-GCG	-"-	AJ238607
BSBV		S T Q * R C G		
RNA-1	-"-	AGU-ACU-CAA-**UAA**-CGG-UGU-GGG	-"-	Z97873
BVQ		S V Q * R F G		
RNA-1	-"-	UCU-GUU-CAA-**UAA**-CGG-UUU-GGG	-"-	AJ223596

sequences surrounding the 'leaky' stop codon in the mRNA of prokaryotic and eukaryotic species (84–86). The codon context can simply consist of only 1–6 nt at the 3′ side of the suppressed stop codon or may involve more complex signals like stem–loop or pseudoknot structures.

A comparison of sequences from a number of plant and animal viral RNAs that harbour a 'leaky' stop codon reveals that they have similar sequences around the stop codon, preferentially at the 3′ side. These readthrough regions can be classified into three groups according to the sequence homology. The type I readthrough region is found exclusively in plant viral RNAs of quite unrelated origin (Fig. 13A). These viruses share the six nucleotides CAA UYA at the 3′ side of the suppressed UAG or UAA codon. A number of nucleotide exchanges within this conserved sequence—embedded in a zein pseudogene—were introduced by site-directed mutagenesis. It was found that changes at each position completely abolished or greatly reduced UAG suppression by

C

Virus	Genus	Readthrough region sequence	Readthrough product	References/ accession no.
		G V K * G G L		
TBSV	Tombusvirus	GGU-GUC-AAA-**UAG**-GGA-GGC-CUA	Replicase	NC 001554
		G V K * G G L		
CNV	- " -	GGU-GUG-AAA-**UAG**-GGA-GGC-CUA	- " -	NC 001469
		F P K * G G L		
CarMV	Carmovirus	UUU-CCC-AAA-**UAG**-GGG-GGC-CUG	- " -	X02986
		Y H K * G G L		
SCV	- " -	UAC-CAC-AAA-**UAG**-GGG-GGC-CUA	- " -	NC 001780
		F S N * G G L		
JINRV	- " -	UUC-UCC-AAC-**UAG**-GGG-GGU-CUC	- " -	NC 002187
		F V R * G C L		
TCV	- " -	UUU-GUC-CGC-**UAG**-GGG-UGC-UUG	- " -	M22445
		L V N * G C L		
MNSV	- " -	UUG-GUC-AAC-**UAG**-GGG-UGC-CUG	- " -	NC 001504
		R S K * G C L		
TNV-A	Necrovirus	CGG-UCC-AAA-**UAG**-GGG-UGC-CUU	- " -	X58455
		E L K * G C L		
MCMV	Machlomovirus	GAG-UUG-AAA-**UAG**-GGG-UGU-CUU	- " -	X14736
		A S L * G D D		
PEMV RNA-1	Enamovirus	GCC-UCC-CUC-**UGA**-GGG-GAC-GAC	Cp extension	(110)
		T A K * V D S		
BYDV (PAV)	Luteovirus	ACG-GCC-AAA-**UAG**-GUA-GAC-UCC	Cp extension	NC 002160
		N P K * V D E		
BWYV	- " -	AAC-CCC-AAA-**UAG**-GUA-GAC-GAG	- " -	X13063
		N P K * V D K		
BMYV	- " -	AAU-CCG-AAA-**UAG**-GUA-GAC-AAG	- " -	X83110
		N P K * V D S		
PLRV	Polerovirus	AAC-CCC-AAA-**UAG**-GUA-GAC-UCC	- " -	NC 001747

Figure 13. (Opposite and above) Plant viral readthrough sites. (**A**) Type I: TMV, tobacco mosaic virus; TMGMV, tobacco mild green mosaic virus; ORSV-Cy, odontoglossum ringspot tobamovirus; CRMV, chinese rape mosaic virus; TVCV, turnip vein clearing virus; CGMMV, cucumber green mottle mosaic virus; BNYVV, beet necrotic yellow vein virus; BBNV, broad bean necrosis virus; PMTV, potato mop-top furovirus; BSBV, beet soil-borne virus; BVQ, beet virus Q; TYMV, turnip yellow mosaic virus. (**B**) Type II: TRV, tobacco rattle virus; PepRSV, pepper ringspot virus; PEBV, pea early-browning virus; PCV, peanut clump virus; SBWMV, soil-borne wheat mosaic virus; CWMV, chinese wheat mosaic virus; OGSV, oat golden stripe virus. (**C**) Type III: TBSV, tomato bushy stunt virus; CNV, cucumber necrosis virus; CarMV, carnation mottle virus; SCV, saguaro cactus virus; JINRV, japanese iris necrotic ring virus; TCV, turnip crinkle virus; MNSV, melon necrotic spot virus; TNV, tobacco necrosis virus; MCMV, maize chlorotic mottle virus; PEMV, pea enation mosaic virus; BYDV, barley yellow dwarf virus; BWYV, beet western yellows virus; BMYV, beet mild yellowing virus; PLRV, potato leafroll virus. The luteoviruses and PLRV are separated by a horizontal line from the other type III viruses because their stop codons are followed by a valine instead of a glycine codon.

$tRNA^{Tyr}$(GΨA) in a mRNA- and tRNA-depleted wheat germ extract (39), indicating that this tRNA species needs a very specific codon context for its suppressor activity. The hexanucleotide motif is apparently effectual in quite different systems. Thus, *in vitro* transcripts containing the TMV-specific readthrough region were translated in a messenger-dependent rabbit reticulocyte lysate and the importance of all 6 nt was shown by measuring UAG suppression via a chloramphenicol acetyltransferase reporter gene (87). Skuzeski *et al.* (88) had previously come to the same conclusion by studying the transient expression of a β-glucuronidase reporter gene containing part of the TMV-specific readthrough region in tobacco protoplasts. The effects on suppression of all possible single-base mutations in the hexanucleotide motif indicated that the consensus sequence of the form CAR YYA confers leakiness to all three stop codons.

Readthrough regions of type II are found in plant and animal RNA viruses (Figs 13B and 14A). They have in common either a CGG in almost all plant virus and some alphavirus RNAs, or a CUA codon at the 3′ side of the suppressed UGA (rarely UAA) codon. Extensive mutational analyses of these triplets provided evidence that 1–3 nt are sufficient to stimulate UGA readthrough. Thus, it has been found that single nucleotide exchanges of either of the three positions in the CGG codon adjacent to the 'leaky' UGA in TRV RNA-1 (Fig. 13B) had only marginal effects on UGA suppression by $tRNA^{Trp}$(CmCA) upon translation in a wheat germ extract, whereas a pronounced influence on UGA readthrough was only seen if 2 or 3 nt were replaced simultaneously (89). As a consequence of the more flexible codon context accepted by $tRNA^{Trp}$(CmCA), as compared to $tRNA^{Tyr}$(GΨA), this tRNA species is able to misread the UGA in nucleotide environments other than the TRV-specific codon context, like those of tobacco mosaic and sindbis virus and to a minor extent that of pea enation mosaic virus RNA (89; Figs 13 and 14). Similarly, it was established that cytoplasmic $tRNA^{Arg}$(U*CG) is capable of reading the UGA in quite different contexts, best of all in the pea enation mosaic and sindbis virus contexts (70). Although natural suppressor tRNAs differ in their efficiency to recognise 'leaky' stop codons in various codon contexts, none of them are capable of misreading 'genuine' stop codons at the end of open reading frames. For instance, the three natural UGA suppressors, $tRNA^{Trp}$(CmCA), $tRNA^{Arg}$(U*CG) and $tRNA^{Cys}$(GCA), are absolutely incompetent for stimulating readthrough over the UGA at the end of the β-globin cistron in plant and animal *in vitro* systems (54,63,70,89).

A

Virus	Genus	Readthrough region sequence	Readthrough product	References/ accession no.
		T E Y * L T G		
SIN	Alphavirus	ACU-GAA-UAC-**UGA**-CUA-ACC-GGG	Replicase	(106)
		T S A * L D R		
MID	- " -	ACG-UCA-GCA-**UGA**-CUA-GAC-CGG	- " -	J02246
		D Q F * L S R		
RRV	- " -	GAC-CAA-UUC-**UGA**-CUA-AGC-AGA	- " -	NC 001544
		Q Q Q * L F D		
VEEV	- " -	CAA-CAA-CAA-**UGA**-CGG-UUU-GAU	- " -	NC 001449
		H S N * R Y E		
EEEV	- " -	CAC-UCG-AAU-**UGA**-CGG-UAC-GAA	- " -	U01034
		D V L R L G R		
SFV	- " -	GAC-GUC-CUG-**CGA**-CUA-GGC-CGC	- " -	(71)
		E E L R L D R		
ONNV	- " -	GAA-GAG-UUA-**CGA**-CUA-GAC-AGA	- " -	NC 001512

B

Virus	Genus	Readthrough region sequence	Readthrough product	References/ accession nol.
		L D D * G G Q	Reverse	
Mo-MuLV	Gammaretrovirus	CUA-GAU-GAC-**UAG**-GGA-GGU-CAG	transcriptase	(105)
		L D D * G G Q		
AKV	- " -	UUA-GAC-GAC-**UAG**-GGG-GGU-CAG	- " -	(111)
		D S E * G C Q		
BaEV	- " -	GAC-AGC-GAA-**UAG**-GGG-UGU-CAG	- " -	D10032
		E L Q * G R Q		
SNV	- " -	GAA-UUA-CAA-**UAG**-GGC-CGU-CAG	- " -	(112)
		L E D * E S Q		
FeLV	- " -	UUA-GAA-GAU-**UAG**-GAG-AGU-CAG	- " -	NC 001940
		L D N * G S Q		
GaLV	- " -	CUA-GAU-AAC-**UAG**-GGG-AGU-CAG	- " -	NC 001885
		Y P A * D P I		
WDSV	Epsilonretrovirus	UAU-CCU-GCA-**UAG**-GAU-CCA-AUU	- " -	NC 001867

Figure 14. Animal viral readthrough sites. (**A**) Type II: SIN, sindbis virus; MID, middelburg virus; RRV, ross river virus; VEEV, venezuelan equine encephalitis virus; EEEV, eastern equine encephalitis virus; SFV, semliki forest virus; ONNV, O'nyong-nyong virus. The two alphaviruses SFV and ONNV do not contain 'leaky' UGA stop codons but instead contain a CGA arginine codon at this position. (**B**) Type III: Mo-MuLV, moloney murine leukemia virus; AKV, AKV murine leukemia virus; BaEV, baboon endogenous virus; SNV, spleen necrosis virus; FeLV, feline leukemia virus; GaLV, gibbon ape leukemia virus; WDSV, walleye dermal sarcoma virus. The epsilonretrovirus WDSV is separated by a horizontal line from the other type III viruses because its UAG stop codon is followed by an aspartate instead of a glycine codon.

The UGA codon and the nucleotides flanking this stop codon in sindbis virus RNA were subcloned into a heterologous sequence and readthrough efficiency was measured by cell-free translation of RNA transcripts in a rabbit reticulocyte lysate. Mutagenesis of residues in the CUA triplet downstream of the suppressed UGA (Fig. 14A) revealed that only the replacement of the cytidine by any of the other three nucleotides drastically reduced the readthrough efficiency (73).

A more complex signal is required for efficient readthrough of the internal UAG of MuLV RNA located between the *gag* and *pol* coding regions (Fig. 3). The role of the nucleotide context was investigated in a rabbit reticulocyte lysate and the results indicated that readthrough is mediated by at least 50 nt on the 3′ side of the UAG codon (90–92). Within this region, a purine-rich spacer sequence consisting of 8 nt immediately following the UAG and a pseudoknot structure spanning the next 49 nt have been found to be essential for MuLV-specific UAG suppression (Fig. 15). The linear octanucleotide sequence of this bipartite signal is conserved in diverse gammaretroviral RNAs, representing type III of the listed readthrough regions (Fig. 14B). Alteration of 6 conserved nt within this motif by mutational analyses either eliminated or significantly reduced UAG suppression (91). Likewise, all of these viruses can form similar pseudoknot structures on the 3′ side of their UAG codons. Substitutions of several conserved nucleotides in loop 2 of the MuLV-specific pseudoknot resulted in detrimental effects on UAG readthrough *in vitro*, showing the importance of structural elements for efficient suppression (92).

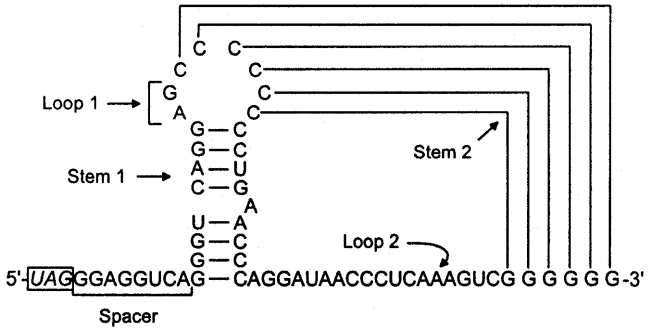

Figure 15. Proposed secondary and pseudoknot structures in the vicinity of the 'leaky' UAG codon in Mo-MuLV RNA. The bipartite signal involved in efficient UAG readthrough consists of 8 nt (underlined) immediately at the 3′ side of the UAG and a stem–loop structure, which can form a pseudoknot with downstream G residues (91,92).

Several plant RNA viruses, among them members of the carmovirus genus, exhibit a conserved octanucleotide sequence at the 3′ side of the suppressed UAG (or UGA) codon that is highly homologous to the corresponding motif in MuLV RNA (Figs 13C and 14B). However, a pseudoknot structure downstream of this sequence that is similar to the one present in retroviruses has not been detected (93), so it remains open whether these plant viruses utilise a similar strategy, i.e. a bipartite signal for stop codon suppression. Barley yellow dwarf virus (BYDV), from the luteovirus group, utilises stop codon suppression to produce an extended coat protein which becomes part of the virion. Analysis of the *cis*-acting sequences required for UAG readtrough in oat protoplasts, wheat germ extract and in reticulocyte lysate revealed two essential regions at the 3′ side of the stop codon: a C-rich region in the vicinity of the UAG, and another more distal sequence located ~700 nt downstream. It is speculated that the distal element interacts with the C-rich region by long-distance base pairing (94).

An influence of sequences immediately upstream of the termination codon on the efficiency of translational readthrough has been observed in the yeast *S.cerevisiae* (95,96), but appears to be of minor importance in plant and animal systems (73,87,89).

The precise molecular mechanism explaining the influence of downstream nucleotides and secondary structures on stop codon readthrough is still obscure. It has been suggested that the major forces involve (i) tRNA selection through stabilisation of the A-site tRNA:mRNA interaction by stacking effects; (ii) interaction between the stop codon and the rRNA; and (iii) interaction between the stop codon and the polypeptide chain release factor (85). Clearly, the competition between the suppressor tRNA and the release factor is of great significance. It has been proposed that the latter binds, in fact, to a tetranucleotide sequence (97). While the preferred termination signal differs in prokaryotic and eukaryotic species, a common feature appears to be a strong bias against a cytidine residue following a termination codon in all organisms (98,99), implying that any termination codon followed by a C is a weak stop codon. Consistent with this assumption is the presence of a C residue at the 3′ side of all readthrough regions of type I and type II (Figs 13A and B and 14A). The nucleotide following the stop codon at the 3′ side of type III readthrough regions is a G residue, which is the most favoured nucleotide at this position for efficient termination in the eukaryotic tetranucleotide stop signal (99), suggesting that readthrough by natural suppressor tRNAs is impaired. Possibly as a consequence of this unfavourable tetranucleotide signal, putative secondary structures beyond this position at the 3′ side are required for efficient readthrough as is the case for the UAG suppression in MuLV and BYDV RNA (92,94).

All three types of codon context sequences stimulating efficient stop codon readthrough *in vivo* and *in vitro* are not restricted to the suppression of a single stop codon. For example, UAG, UAA and UGA codons in the TMV-specific context (type I) can be suppressed in tobacco protoplasts (88), rabbit reticulocyte lysate (87) and wheat germ extract (39,41); UGA as well as UAA and UAG codons in the sindbis-specific context (type II) are suppressed in cultured cells of animal origin (23); and all three termination codons in the MuLV-specific context are effective in reticulocyte lysate and infected cells (100). Furthermore, a particular stop codon is not read by a single suppressor tRNA isoacceptor. Thus, it has been shown that the 'leaky' UGA codon in the TRV-specific context (Fig. 13B) is recognised *in vitro* by tobacco $tRNA^{Trp}$(CmCA), wheat $tRNA^{Arg}$(U*CG) and tobacco $tRNA^{Cys}$(GCA), albeit with quite different efficiencies (54,63,70). Similarly, the 'leaky' UAG within TMV RNA is read by tobacco and wheat $tRNA^{Tyr}$(GΨA), tobacco $tRNA^{Gln}$(CUG), tobacco and mouse $tRNA^{Gln}$(UmUG) and calf $tRNA^{Leu}$(CAA/CAG) in different *in vitro* systems (19,29,46,48,52).

CONCLUSION

The dogma of the non-ambiguity of the genetic code is shattered by the observation that a stop codon can have two or more meanings. Thus, the UGA codon may mediate termination of polypeptide synthesis or trigger the incorporation of tryptophan, arginine or cysteine. Likewise, the UAG termination codon may provoke the incorporation of tyrosine, glutamine or leucine. The misreading of termination codons is achieved by a variety of naturally occurring suppressor tRNAs. All of the nonsense suppressors characterised to date (with the exception of selenocysteine tRNA) are normal cellular tRNAs that are primarily needed for reading their cognate sense codons. As a consequence, recognition of stop codons by suppressor tRNAs necessitates unconventional base pairings in anticodon–codon interactions (Fig. 12).

Direct methods for the characterisation of potential suppressor tRNAs involve the purification of a specific suppressor tRNA from any organism or tissue, followed by examination of its suppressor activity in an *in vitro* system programmed with RNA transcripts that contain defined viral stop codon readthrough regions (19,48,52,54,63,70). Indirect procedures for the detection of presumable suppressor activities utilise the isolation of readthrough proteins from suitable systems and identification of the amino acid(s) incorporated at the stop codon site by protein sequencing. This allows the prediction of the nature of the putative tRNA species involved in the suppression event (62,71,78). In most cases the same natural suppressor tRNAs have been identified by direct and indirect means, with two exceptions. The incorporation of lysine in response to an internal UAG codon within the yeast Ste6 gene (78) and of serine at the UGA terminating the β-globin mRNA (71) suggests the existence of further suppressors not yet characterised in detail.

The observation that more than one natural UAG/UAA and UGA suppressor exist in eukaryotes raises the question whether misreading of a stop codon by suppressor tRNAs is regulated or not. One factor that influences readthrough activity is simply the abundance of a specific tRNA suppressor in a given tissue. This is most obvious for the distribution of $tRNA^{Tyr}$(GΨA) versus $tRNA^{Tyr}$(QΨA) [the latter not being a UAG suppressor (Fig. 5)], in various plant and animal tissues. In addition to the available amount, a number of intrinsic features of the suppressor tRNA contribute to the ability to read non-cognate codons. Apart from anticodon–codon affinity, the degree of base modifications within the anticodon or in the vicinity of the anticodon may up- or down-regulate the efficiency of misreading.

In order to out-compete the polypeptide chain release factor for their common target, an absolute prerequisite for the action

of natural suppressor tRNA is a suitable nucleotide context, preferentially at the 3′ side of the suppressed stop codon. This context may consist of only 3–6 downstream nt, or may involve more complex signals like stem–loop and pseudoknot structures.

Without doubt, a bulk of RNA viruses rely on stop codon readthrough to express part of their genetic information (Fig. 1). Hence, the question arises whether the eukaryotic host organisms also profit from their tRNAs that read termination codons and, thus, facilitate the multiplication of viral pathogens or, in other words, whether these natural suppressor tRNAs have any essential or at least beneficial effect on cellular biosynthesis. One of the very few examples of a natural cellular readthrough protein is the rabbit β-globin readthrough polypeptide that has been identified in reticulocyte lysate by immunoblotting (71,101). This β′-globin is generated by readthrough over the 'genuine' UGA stop codon at the end of the β-globin cistron (flanked by a G at the 3′ side), but it is unknown whether it has an essential function. However, an increasing number of reports have been published recently indicating that natural suppressor tRNAs have apparently corrected deleterious nonsense mutations within open reading frames of genes from the human and yeast genome *in vivo* (e.g. 44,78,102,103). Although this is a mostly unpredictable and inefficient event, the low synthesis of a full-length product might be sufficient in special cases to ensure viability of the organism.

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