Tuite, M.F. and von der Haar, T. (2016) Transfer RNA in Decoding and the Wobble Hypothesis. In *eLS*. John Wiley & Sons, Ltd: Chichester.

[DOI: 10.1002/9780470015902.a0001497.pub2]

Accepted for publication 12/7/16.

Transfer RNA in Decoding and the Wobble Hypothesis

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Translation of the genetic code stored in messenger RNA requires significantly fewer transfer RNAs (35-45) than there are codons (61, amino acid specifying). This is achieved through an increased flexibility in the allowable base-pair interactions between the messenger RNA and transfer RNA involving the third position of the codon and the first position of the corresponding anticodon. The rules governing this RNA:RNA interaction were originally summarized in Crick's 'wobble hypothesis'. Covalent modification of the first base of an anticodon of a transfer RNA can profoundly affect the degree of flexibility in its base pairing potential by either extending or restricting such interactions. Recent studies suggest that the rate at which a codon is processed by the ribosome is influenced by whether or not decoding of that codon is via wobble base interactions. Yet in spite of this flexibility and different rates of processing, decoding by transfer RNAs is achieved with considerable accuracy.

Keywords: wobble hypothesis; tRNA; anticodon; codon; mRNA decoding; codon recognition, base modification

Key Concepts:

- The genetic code is decoded via transient interactions between messenger RNA (mRNA) and a series of 'adaptor' RNA molecules called transfer RNAs (tRNAs).
- The mRNA–tRNA interaction occurs on the ribosome via the complementary base pairing between the three-base anticodon of the tRNA and the three-base codon in the mRNA.
- A greater degree of flexibility of allowable base-pair interactions between mRNA and tRNA allows most organisms to have far fewer tRNA species than there are codons.
- Specific non-Watson–Crick base-pair interactions occur between the third base of a codon and the first base of the anticodon of a tRNA during decoding, so called wobble.

- Wobble-base pairing enables the decoding of two or more codons by the same tRNA.
- Certain modified bases e.g. inosine (I) can extend or restrict the degree of flexibility in the range of mRNA:tRNA interactions.
- Codons that are decoded by wobble base interactions are processed at a slower rate in the ribosome.
- Certain antibiotics e.g. streptomycin can promote misreading at the wobble third position of a codon.
- The genetic code assignments are not universally conserved particularly in organellar genes e.g. mitochondrial genes.

Introduction

The genetic information stored in a newly synthesized messenger RNA (mRNA) molecule is decoded both efficiently and accurately via transient interactions between the mRNA and a series of 'adaptor' RNA molecules, the transfer RNAs (tRNAs). In an ordered series of decoding steps, amino acids covalently linked to the tRNAs are brought to the ribosome and assembled into the mRNA-directed polypeptide chain. The order in which the amino acids are delivered to the ribosome by the tRNAs is directed by the order of the codons in the translated region of the mRNA. The mRNA is translated one codon at a time during the ensuing elongation cycle with the incoming aminoacylated tRNA (aa-tRNA) being delivered to the ribosome by a protein elongation factor (EF-Tu in bacteria, eEF1A in eukaryotes).

The initial decoding step, i.e. the binding of the aa-tRNA to the mRNA, takes place at the ribosomal acceptor (A) site. Following transfer of the growing polypeptide chain to the aa-tRNA bound at the A site, the newly formed peptidyl-tRNA is then moved to a second ribosomal site (the peptidyl or P site) via a translocation-mediated step using a second protein elongation factor (EF-G in bacteria, eEF2 in eukaryotes) and requiring guanosine triphosphate (GTP) hydrolysis (Voorhees and Ramakrishnan, 2013). Movement from the ribosomal A site to the P site does not involve dissociation of the tRNA from its cognate codon on the mRNA. Following transfer of the peptidyl moiety to the next aa-tRNA to be delivered to the ribosomal A site, the now deacylated tRNA at the P site passes through a third site within the ribosome (the exit or E site) prior to final dissociation of the deacylated tRNA from the ribosome. Although the deacylated tRNA does remain in contact with the mRNA in the E site, it does so either transiently or possibly with reduced stability compared with the A and P site tRNA–mRNA interactions.

The specificity of the mRNA–tRNA interaction at the ribosomal A site is essentially driven via the complementary base pairing between the three-base anticodon of the tRNA and the cognate three-base codon in the mRNA (Figure 1). Such specificity, while depending to some extent on standard 'Watson–Crick' base pairing rules (i.e. U:A or G:C), shows a much greater degree of flexibility of allowable base-pair interactions than is seen between the complementary strands of, for example, the double-stranded DNA molecule. The outcome of this flexibility is that an organism does not have to encode a unique tRNA species for each of the 61 amino acid-specifying (sense) codons, with most organisms having between 35 and 45 different tRNA species. For example, the yeast *Saccharomyces cerevisiae* encodes 42 different tRNAs.

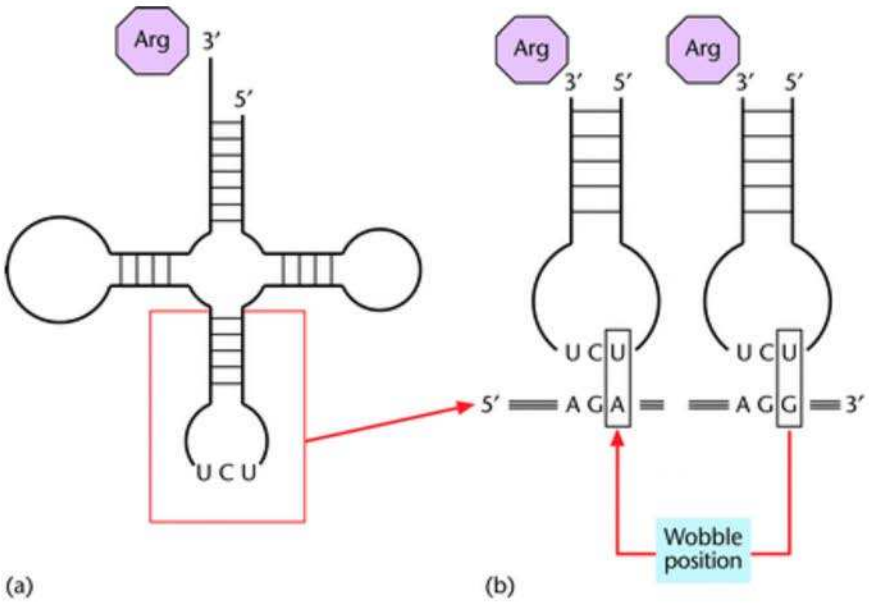

Figure 1. mRNA–tRNA interactions involve base pairing between the anticodon of the transfer RNA (tRNA) and the messenger RNA (mRNA) codon. (a) Standard depiction of the two dimensional 'clover-leaf' structure of a tRNA molecule. (b) An Arg-inserting tRNA with a UCU anticodon can translate both the AGA codon by standard base-pair interactions at all three positions, and the AGG codon by a non-Watson and Crick pairing in the third 'wobble' position of the codon.

The tRNA molecule, which is a single RNA chain of usually no more than 90 nucleotides in length, folds into a three-dimensional L-shaped structure containing a significant level of secondary and tertiary intramolecular interactions between bases through hydrogen bonding. The largest unpaired region of the molecule is the loop that contains the anticodon sequence (Figure 2). The three bases of the anticodon all point approximately in the same direction with their conformations being determined primarily through hydrophobic stacking interactions between the bases. Based on a standard numbering

system used for all tRNA molecules, the anticodon bases are numbered 5′-N^{34}, N^{35}, N^{36}-3′. The base immediately 5′ of the anticodon (N^{33}) is invariably a U and the tRNA chain forms the so-called 'U turn' between N^{33} and N^{34}, thereby presenting the three anticodon bases in such a way as to facilitate hydrogen bonding with the cognate codon during mRNA decoding at the ribosomal A site. The lack of tertiary hydrogen bonding linking the anticodon loop to the remainder of the tRNA molecule may allow the anticodon region to take up one or more alternative orientations during protein synthesis.

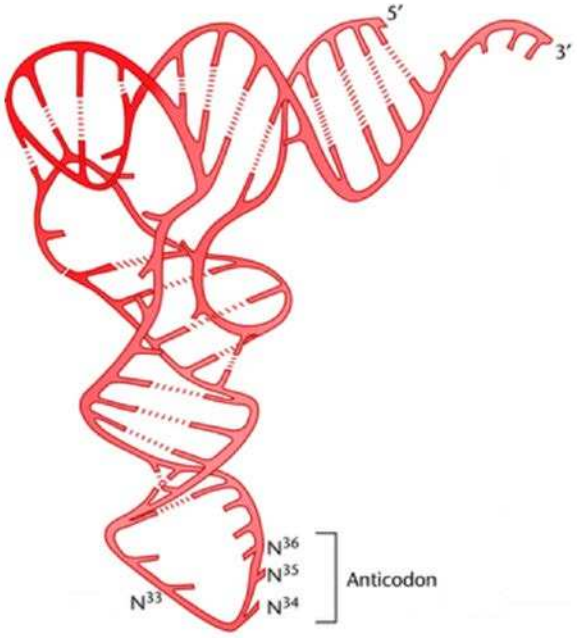

Figure 2. The three dimensional structure of a transfer RNA (tRNA) molecule. tRNA molecules take up an L-shaped structure due to a variety of intramolecular base interactions. The anticodon is present in a large unpaired region of the molecule with the three bases of the anticodon all pointing approximately in the same direction. The 'U turn' base (U^{33}) immediately adjacent to the anticodon is indicated.

Codon Assignments

Pioneering genetic and biochemical experiments in the 1960s revealed the nature of the triplet-based genetic code and assigned identities to each of the 61 sense codons (Nirenberg, 2014). These studies also confirmed that the remaining three codons (the nonsense codons UAA, UAG and UGA) are not translated by tRNAs, but rather act as polypeptide chain termination signals at the end of the translational reading frame. With only two exceptions (Met and Trp) each of the 20 amino acids is specified by at least two different codons with three amino acids (Arg, Ser and Leu) having six different codons.

This degeneracy in the genetic code is usually confined to the third base of the codon; for example, the four members of the codon family CCN (where N is any of the four bases U, A, C, G) all encode the amino acid Pro. There can, however, also be degeneracy within the first two bases as for the six-membered codon families of Arg, Ser and Leu; for example, Ser is encoded by the six codons CUN and UUA/G. For amino acids specified by two codons (e.g. Cys, Glu) the degenerate third base can either be a pyrimidine, U or C (e.g. Cys: UGU/C) or a purine, A or G (e.g. Glu: GAA/G). There is, therefore, an element of order in the make-up of the genetic code that has most likely evolved to ensure that the potential deleterious effects of mutations and/or mistranslation of sense codons by near cognate tRNAs is minimized.

The genetic code assignments are not universally conserved (Osawa *et al.*, 1992). In particular, genetic code variations in organellar genes – especially those of mitochondria – have now been described in most organisms except plants. Most striking is the use of nonsense codons as sense codons and *vice versa*; for example, in human mitochondrial genes, the nonsense codon UGA is decoded as a Trp codon while AGA and AGG act as chain termination codons. Genetic code variations in cytoplasmic mRNAs are much rarer with only two well-characterized examples: the decoding of the UGA codon as selenocysteine in several different mRNAs in a range of species (see below), and the decoding of the Leu codon CUG as a Ser codon in all mRNAs of some members of the fungal genus *Candida*. In most cases, the decoding of these genetic code variants involves a tRNA species with novel structural features. One further aberration in mRNA decoding can be found in certain bacterial species with extremely high or low G+C base content in their genomes where certain codons and/or their corresponding tRNAs have disappeared from the organism's genome. For example, in *Mycoplasma capricolum* neither the CGG (Arg) codon nor the $tRNA^{Arg}$ needed for its translation have yet been found (Andachi *et al.*, 1989).

The 'Wobble Hypothesis'

The early realization that there were many fewer tRNA species than there were codons, together with *in vitro* biochemical experiments showing that purified tRNA species could recognize two, sometimes three different – but related – codons led Francis Crick, in 1966, to formulate the 'Wobble Hypothesis' (Crick, 1966). In his hypothesis, Crick put forward the notion that specific non-Watson–Crick base-pair interactions could take place between the third base of a codon and the first base (N^{34}) of the anticodon of a tRNA during mRNA decoding. Only standard G:C and U:A pairings were, however, allowable at

the positions involving the N^{35} and N^{36} positions of the anticodon (Figure 1). Lagerkvist 1978 further suggested that where N^{35} and N^{36} of the anticodon were G or C and formed Watson and Crick pairs with the codon, the N^{34} base would not be required to form a stable base pair at the third position because the tRNA would be held in place by the two G:C pairs. However, where N^{35} and N^{36} are U or A, then base pairing involving the N^{34} base *would* become necessary because of the weaker nature of the two U:A pairs. This so-called 'two-out-of-three' hypothesis (Lagerkvist, 1978) is probably an oversimplification of the mRNA–tRNA interaction and a number of exceptions have been described.

Codon–Anticodon Interactions

The ability to match two codons to a single anticodon, which is at the heart of wobble-decoding, requires a degree of flexibility in the nucleotides at the so-called 'wobble position'. Since bases in the ribosomal A-site are spatially constrained by the structure of the ribosome, this flexibility relies primarily on an ability of the N34 base of the anticodon to change its location relative to the third codon base. The fact that the N^{34} base of the tRNA is located in the tertiary structure of the tRNA at the end of the five-base stack containing the anticodon just before the U turn of the anticodon loop (see Figure 2) means that there is less constraint placed on its movement compared with the N^{35}/N^{36} bases. In addition, the N^{36} base – the 3′ base of the anticodon – is usually adjacent to a modified (and therefore bulky) purine base which may further reduce the flexibility of pairing involving N^{36} and perhaps even the N^{35} base.

The discovery that a number of tRNAs able to recognize three different codons have the modified base inosine (I) at position N^{34} rather than one of the four standard bases, allowed for a further development of the concept of third base wobbling. Crick thus derived a set of rules that could account for the degeneracy of the genetic code and the multicodon recognition properties of many tRNAs (Table 1). Central to this hypothesis was the ability of G^{34} to pair with either U or C (with the 2-amino group of the G preventing the formation of a G:A base pair), and U^{34} to pair with either A or G (Figure 3).

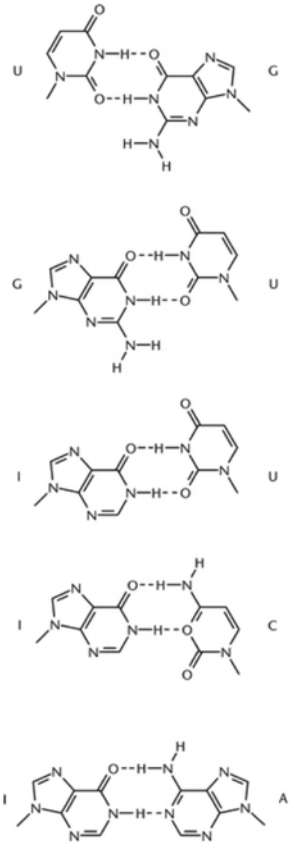

Figure 3. Wobble base-pair interactions that occur between the first base of the anticodon (left) and the third base of the codon (right).

Inosine, which is generated post-transcriptionally by deamination of A, can pair with any one of the three bases A, C or U (Figure 3). Such an expanded base-pairing behaviour can be explained by the absence of the 2-amino group. Thus, according to the wobble hypothesis, only tRNAs with an I^{34} base in their anticodon can pair with three different codons, while no single tRNA species should be able to pair with all four members of a codon family. Interestingly, the base-pairing properties of I are more close to those of G than the base from which it is derived (i.e. A).

The base pairings permitted by the wobble rules are those that give ribose–ribose distances that are close to those of standard Watson and Crick base pairs. For this reason, purine–purine and pyrimidine–pyrimidine pairs are not allowed.

Modified bases and codon recognition

Although the basic concept of the formation of wobble pairs as proposed by Crick remains valid, there are now numerous examples of tRNAs whose codon-recognition properties do not conform with the original rules. The single most important reason for a need to revise the original wobble rules has been the realization that I is not the only modified base that can be found at the N^{34} wobble base position of tRNAs. At this position, such modified bases can have base-pairing properties that are different from the bases from which they are chemically derived, usually restricting rather than expanding the base-pairing possibilities. While much of the pioneering work on codon assignment was undertaken in the bacterium *Escherichia coli*, the modified base I – which featured in Crick's original wobble rules – is only found in one bacterial tRNA species, a $tRNA^{Arg}$ (Curran, 1995). However, I is present in a relatively large number of different eukaryotic tRNAs, particularly those that recognize members of a four-codon family.

In Crick's original set of rules the assumption was made that – with the exception of I – the N^{34} base was either A, G, C or U. With well over 12000 tRNA molecules sequenced to date (Jühling et al. 2009), most strikingly, we now know that U^{34} is only present in tRNAs from mitochondria, chloroplasts and *Mycoplasma* species. Furthermore such U^{34}-containing tRNAs are able to recognize all four members of a codon family, i.e. U^{34} is able to form a base pair with each of the four bases at the third 'wobble' position of the codon, although recognition of C by U^{34} is at much lower efficiency than with the other three non-modified bases. Yet this is not the only exception. Unmodified A^{34} has been found in only two tRNA species; a $tRNA^{Arg}$ in yeast mitochondria and a $tRNA^{Thr}$ in various *Mycoplasma* species and such tRNAs can recognize all four members of a codon family (Sibler *et al.*, 1986).

In contrast to the situation with A^{34}/U^{34}, nonmodified G^{34} and C^{34} are found in many different tRNA species in all groups of organisms although modified forms of both bases do occur in tRNAs. For example, queuosine (Q) or a Q derivative is found at N^{34} of tRNAs that decode NAY (Y = either C or U) codons in bacteria and most eukaryotes apart from yeast, and 2′-o-methylcytidine (Cm) is found at position N^{34} in most prokaryotic and eukaryotic $tRNA^{Trp}$ and UUG-decoding $tRNA^{Leu}$ species. Cm is also present in some eukaryotic elongator $tRNA^{Met}$.

Restricted wobble

The presence of modified bases in a tRNA molecule at the N^{34} position (and to a lesser extent N^{37} adjacent to the anticodon) can have profound effects on the codon-recognition properties of the tRNA. In particular, modifications of U^{34} can reduce the potential for non-Watson and Crick base pairs at the wobble position, so-called 'restricted wobble'.

There are a number of well-characterized examples where a modification to U^{34} in the anticodon of tRNA restricts the codon-recognition properties of that tRNA. For example, modification of the U^{34} to a 5′-methyl-2-thiouridine derivative, as is the case in tRNAs decoding Gln, Lys and Glu codons in many if not all prokaryotic and eukaryotic species, restricts codon recognition to codons ending in A. This restriction prevents misrecognition of members of the respective codon families ending in U or C. In a two-codon family (such as those for Gln, Lys and Glu) such decoding would result in mistranslation, e.g. a $tRNA^{Glu}$ reading an Asp codon. The modification of U^{34} leads to increased conformational rigidity of the wobble base and this in turn prevents, or significantly reduces, non-Watson and Crick base-pair interactions that usually require conformational flexibility.

C^{34} modifications are much less frequently found in tRNAs than U^{34} modifications and do not appear to have as profound effect on the codon-recognition properties of a tRNA. However, one exception to this is the L^{34} (lysidine) modified base, a C modification found only in a minor $tRNA^{Ile}$ in bacteria and plant mitochondria. The presence of L^{34} at the wobble base position in the anticodon dramatically alters the base-pairing properties of the base; it recognizes A instead of G, a rare example of a complete switch in base-pairing specificity. In the absence of this modification, the tRNA with C^{34} would decode the AUG (Met) codon and would be acylated with Met. Thus, this single base modification also prevents (mis)acylation of the $tRNA^{Ile}$ with Met (Muramatsu *et al.*, 1988).

Modified bases are also present in some tRNAs at the positions within the anticodon loop of a tRNA but are not part of the anticodon sequence. In particular, certain modification of N^{37} – the base located immediately 3′ of the anticodon – may influence codon recognition by stabilizing the relatively weak U:A base pairs that occur outside the wobble position, for example, where the codon is UNN.

As a consequence of the recognition of the importance of modified bases in altering the codon-recognition properties of a tRNA during mRNA decoding, the set of wobble rules originally developed by Crick 1966 has now been modified and expanded (Table 1). In

most cases studied, the influence of the modification of the N^{34} base alters the conformational properties of the wobble base, thus highlighting the importance of base flexibility in the wobble position during mRNA decoding.

Although the 'expanded wobble rules' account for almost all known tRNA decoding behaviour it is likely that exceptions will still be found. Any tRNA modification that influences the overall tertiary structure of the anticodon loop may influence the base-pairing properties at position N^{34}. There is no reason why such changes could not occur outside the anticodon arm of the tRNA given the extensive tertiary intramolecular interactions that occur in tRNAs.

Differential processing of wobble-decoded codons by the ribosome

While wobble-base pairing enables the decoding of two or more codons by the same tRNA, the processing of these codons in the ribosome is not identical. The differences in kinetics of this step has been studied in detail for a small number of codon:tRNA combinations, including for $tRNA^{Ala}_{CGU}$ (Kothe and Rodnina 2007). The ribosome processes the decoding of ACG, the Watson-Crick pairing codon for this tRNA, with faster forward rate constants than decoding of GCC, the wobble-pairing codon for the same tRNA. Moreover, while the Watson-Crick paired codon leads to successful peptidyl transfer with a high probability, the wobble-base paired codon leads to tRNA release rather than peptidyl transfer with a probability of about 50%, based on the rate constants measured *in vitro*. Because of the frequent erroneous release of the correct tRNA on wobble-decoded codons, sampling of the tRNA pool requires more time on average and wobble-decoded codons are therefore usually decoded more slowly than Watson-Crick decoded ones. These findings were confirmed in vivo by analysing ribosomal footprinting data, and demonstrating that footprints are detected more frequently on wobble-decoded codons than on Watson-Crick decoded ones. The slower processing of wobble-decoded codons likely explains the observation that in most organisms Watson-Crick-decoded codons are preferred over wobble-decoded ones in terms of codon usage.

The differential processing the two types of codon connect wobble-base pairing to translational control of gene expression and to biological pathways. For example, changes in the methylation state of the wobble base of $tRNA^{Leu}_{CAA}$ are linked to an upregulation of one of the two genes encoding the ribosomal protein RPL22, with a higher content of TTG leucine codons during the oxidative stress response in baker's yeast, and this was required for an optimal adaptation to the stress (Chan et al 2012).

Another study showed that U34 modifications are required to prevent the formation of toxic protein aggregates in yeast and worms (Nedialkova and Leidel 2015).

Accuracy in Decoding

In spite of the flexibility of the codon–anticodon interaction in mRNA decoding, each codon is accurately decoded by the correct aa-tRNA. Nevertheless, there are ways in which the accuracy of this decoding can be subverted, particularly where nonsense codons are involved.

Maintaining accuracy of the mRNA–tRNA interaction

mRNA decoding by aa-tRNAs is a remarkably accurate process with reported sense codon misreading rates of the order of 10^{-4}–10^{-5} in both prokaryotes and eukaryotes, i.e. one incorrect amino acid inserted for every 10 000–100 000 codons translated (Parker, 1992). This degree of accuracy is all the more remarkable given the relatively weak binding affinity between tRNA and its cognate codon in solution and the low level of discrimination between members of a four-codon family XXN. The specificity of mRNA decoding at the A site is controlled primarily by the ribosome itself, but translation factors may also have an influence, particularly the factor that delivers the aa-tRNA to the A site, namely EF-Tu/eEF1A. Any mismatched aa-tRNA must be removed from the A site before the bound amino acid participates in the next peptide bond to be formed during polypeptide chain elongation. There is evidence that such mismatched aa-tRNAs dissociate more rapidly from the ribosome by a factor of 5–10 times compared with a correctly matched aa-tRNA (Thompson *et al.*, 1981).

A number of antibiotics are able to perturb the accuracy of mRNA decoding at the A site to increase the rates of mistranslation (i.e. acceptance of mismatched tRNAs) by 1–2 orders of magnitude. Particularly effective in this context are the antibiotics streptomycin in bacteria and paromomycin in eukaryotic cells. Streptomycin appears to promote misreading at the wobble third position of the codon involving U and C. Such error-inducing antibiotics mediate their effects by binding to the ribosome either through one or more specific ribosomal proteins or ribosomal RNA. By isolating mutants that are resistant to these antibiotics one is able to identify those ribosomal proteins that play a role in controlling the accuracy of mRNA decoding. For example, such studies in *Escherichia coli* have identified three proteins in the small subunit of the ribosome,

namely S4, S5 and S12, as being important for maintaining the accuracy of mRNA decoding.

Translating nonsense codons as sense codons

Cells do not normally have tRNAs that can efficiently translate one or other of the three stop codons. Such stop codons are recognized by a protein release factor (RF) leading to termination of polypeptide chain elongation and release of the completed polypeptide chain from the ribosome. Yet stop codons are related to a number of sense codons by a single base; for example, UGA, stop, UGG, Trp; or UAG, stop, CAG, Gln. It is therefore to be expected that tRNAs, able to translate a stop codon, can be generated by single base mutations in the anticodon of a new cognate tRNA. For example, the *trp*T suppressor mutation of *E. coli* arises through a U to C substitution at N^{34} in the UGG-decoding $tRNA^{Trp}$. Because of the wobble rules of decoding, however, the mutant suppressor $tRNA^{Trp}$ would be expected to be able also to translate the UGG codon and thus this mutational event should not be lethal to the cell. However, since no viable haploid *E. coli* cell carrying the *trp*T mutation has been described, this would suggest that the degree of wobble at the third base position is insufficient to ensure a significant level of UGG translation. A further mutant nonsense suppressor derivative of the same UGG-decoding $tRNA^{Trp}$, with a mutation outside the anticodon sequence (G^{24} to A^{24}) is also able to translate both the UGA and UGG codons, indicating that the tertiary structure of a tRNA also plays an important role in maintaining the specificity of wobble interactions involving the third position of a codon. This mutant is viable as a haploid. In addition, the wild-type UGG-decoding $tRNA^{Trp}$ is able to translate UGA codons, albeit with very low efficiency (1–3%) (Hirsh and Gold, 1971) compared with the mutant tRNAs (Figure 4). Thus, a study of the decoding properties of nonsense suppressor tRNAs has provided further insights into the flexibility of the codon–anticodon interaction.

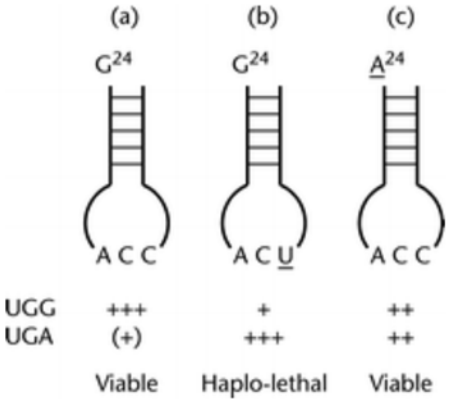

Figure 4. Translation of the UGA codon in *Escherichia coli* by a Trp-inserting tRNA. The relative, approximate efficiencies of UGG/UGA decoding by (a) the wild-type transfer RNA (tRNA), (b) a nonsense suppressor mutant of the tRNA in which C^{34} has been replaced by U^{34}; and (c) a novel suppressor variant of the tRNA with G^{24} replaced by A^{24}.

Accurate mRNA decoding by tRNA involves, in principle, straightforward RNA–RNA interactions mediated by base pairing between codon and anticodon bases. The flexibility introduced into this interaction by the wobble base, while reducing the numbers of tRNAs required by a cell to decode the 61 sense codons, nevertheless does not subvert the accuracy of decoding. In fact, such flexibility may ensure that minor miscodings are not detrimental to the cell and may also provide a means for the continued evolution of the codon assignments as exemplified by the cases of nonsense codons being decoded as sense.

Glossary

Aminoacyl-tRNA (aa-tRNA)
A transfer RNA (tRNA) charged with the appropriate amino acid which is esterified to the 3-OH of the 3′-terminal adenosine residue of the tRNA.

Anticodon
The triplet of ribonucleotides within a transfer RNA (tRNA) molecule that base pair directly with a codon in the messenger RNA (mRNA).

Codon
A triplet of ribonucleotides which code for a single amino acid.

Isoacceptor tRNA
Two or more tRNAs that are charged with the same amino acid.

Nonsense codons
Codons which do not specify an amino acid but, rather, signal the end of the region of a messenger RNA (mRNA) to be decoded.

Ribosomal profiling
A method that identifies all messenger RNA (mRNA) molecules that are being actively translated by ribosomes in a cell at a given moment in time.

Transfer RNA (tRNA)
A family of small nucleic acids that mediate the translation of a messenger RNA (mRNA) molecule into the amino acid sequence of the encoded polypeptide chain.

Watson and Crick base pairing

Hydrogen bonding between A (adenine) and T (thymine) or U (uracil) and G (guanine) and C (cytosine).

Wobble

A nonstandard base-pair interaction between the third nucleotide of a codon (the wobble base) and the first nucleotide of the anticodon.

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Table 1. The wobble rules, taking into account the influence of base modifications at the N^{34} wobble base

N^{34}	Old rules	New rules
U	A,G	A,G,U, (C)
e.g. mcm^5U etc	–	A, (G)
e.g. $mcmo^5U$ etc	–	U,A,G
G	C,U	C,U
A	U	A,G,U,C
e.g. I	A,C,U	A,C,U
C	G	G
e.g. k^2C/L	–	A

The 'old rules' are those originally proposed by Crick 1966. The 'new rules' take into account the known effects of base modifications at position N^{34} on codon recognition.