Translational Recoding and RNA Modifications

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Abstract

During protein synthesis, codons in mRNA are translated sequentially in frame on the ribosome following strict decoding rules. This process is usually very accurate. However, in some cases, recoding events occur at selected codons, leading to a high frequency of frameshifting or stop codon readthrough. The factors influencing these noncanonical decoding events are very diverse; among them are the codon usage and context, the presence of a stable mRNA secondary structure downstream of the decoding sites and the type and relative abundance of normally modified tRNA. Here, we discuss the role of certain modified nucleotides of tRNAs in a few cases of frameshifting and readthrough that occur in Bacteria and Eukarya. While in some cases the effect of a given modified nucleotide in a tRNA is to increase accuracy of the recoding process, in a few other cases the reverse has been observed. This review illustrates the power of using well characterized recoding systems, coupled with specific defects of RNA modification enzymes to assay for translational fidelity under *in vivo* conditions.

1 Introduction

1.1 Recoding events

During the complex translation elongation process, codons in mRNAs are translated on the ribosome by aminoacylated tRNAs following strict decoding rules. This process is usually very accurate, the average frequency of estimated miscoding being about 5.10^{-4} per codon or even less (Buckingham and Grosjean 1986; Kurland 1992; Kurland and Gallant 1996). However, over the last decade, data have demonstrated that reading the genetic code may be more flexible than initially anticipated and that in certain cases the frequency of unconventional decoding can be as high as 40% or even more (Grentzmann et al. 1998). These alternative readings of the genetic code have been called 'recoding' (Gesteland et al. 1992). This process corresponds to a subversion of normal decoding rules, leading to the synthesis of an unpredicted polypeptide carrying different biological functions. Such recoding phenomena comprise several translational events including readthrough of stop codons, frameshifting, and ribosomal hopping (Fig. 1, see also Baranov et al. 2002, 2003; Namy et al. 2004). Recoding events are always in competition with the standard decoding process and essentially depend on special

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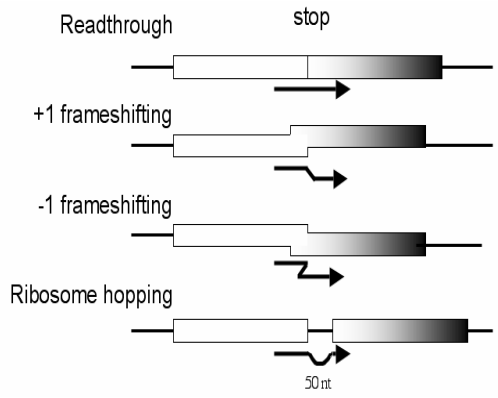


Fig. 1. Schematic representation of the different recoding events. During readthrough, a normal tRNA reads the stop codon, allowing protein synthesis to proceed to the next in-frame termination codon. Most of the +1 and -1 programmed translational frameshifting events also lead to the synthesis of an elongated protein, by escaping a stop codon. In the unique example of ribosome hopping (gene 60 of phage T4), the recoding event also ends with the by-pass of a stop codon. Recoding events are thus, most of the time, necessary to produce an elongated protein carrying new functional domains, such as reverse transcriptase activity in retroviruses and replicase activity in plant viruses.

sequences and structures on the mRNA ("recoding signal" *in cis*) as well as on *in trans* canonical components of the translation machinery. However, in the special case of recoding the UGA stop codon to selenocysteine or UAA codon to pyrrolysine, a specific non-canonical tRNA ($tRNA^{Ser\text{-}sec}$ or $tRNA^{Lys\text{-}Pyl}$) and also a special elongation factor and SECIS-binding protein or special lysyl-tRNA synthetase are needed (Hatfield and Gladyshev 2002; Driscoll and Copeland 2003; Blight et al. 2004). Beside these two last cases, translational recoding events are found mainly in small autonomous elements such as bacteriophages, viruses, or transposons, although the expression of a few cellular genes have been demonstrated to be controlled by this means (reviewed in Namy et al. 2004). In all cases, they allow the synthesis of two related polypeptides from the same mRNA, the one resulting from the recoding process being usually less abundant than the shorter polypeptide. This is typically the case in retroviruses where the GAG protein is synthesized by regular decoding while the polymerase domain is expressed as a GAG-POL fusion protein through a recoding event (Farabaugh 1996).

1.2 The stimulatory recoding signals

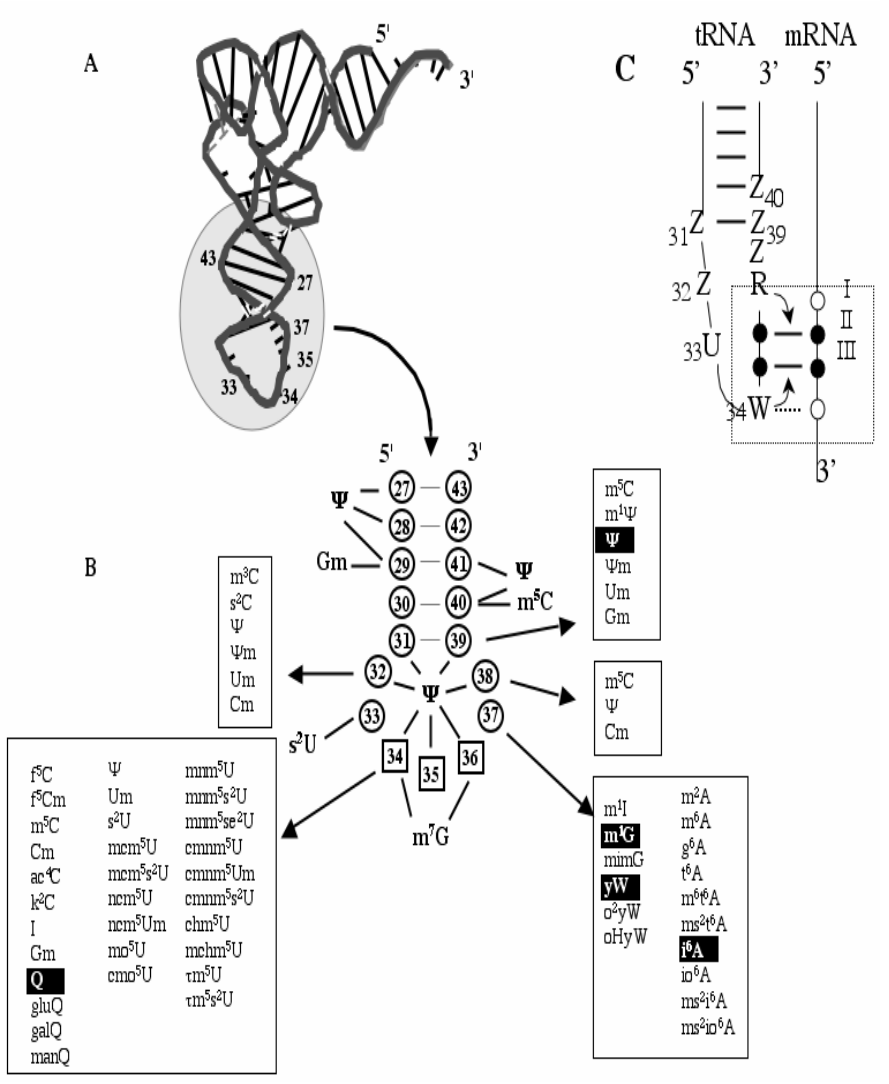
The efficiency of a recoding process depends on various elements of the translation machinery. The "cis-recoding signals" in an mRNA include a particular sequence where the recoding event takes place, which involves the ribosomal A- and/or P-site codon(s), and additional sequence information that is present upstream and/or downstream and increases the efficiency of the process ("stimula-

tory signals"). For example, the presence of either a pseudoknot or a stable stem-loop downstream of a shifty site strongly stimulates -1 frameshifting efficiency (Tzeng et al. 1992). Likewise, in prokaryotes (Bacteria and Archaea), the interactions between a Shine Dalgarno (SD) -like sequence located upstream of the frameshifting site and the rRNA serve as a stimulatory signal for -1 and +1 frameshifting events (Larsen et al. 1995; Marquez et al. 2004).

The "*trans*-recoding elements" include the type and availability of certain aminoacyl-tRNAs complexed with their GTP-elongation factor, the presence of fully active competing termination factors and possibly also some peculiar structural features of the rRNA and/or of the ribosome itself (Farabaugh 1996; Gesteland and Atkins 1996; Atkins et al. 2000). For example, the relative abundance of individual tRNA species, their decoding efficiency and/or their intrinsic capability to slip on certain mRNA sequences, are important factors that induce the ribosome-mRNA-tRNA machinery to occasionally bypass a stop codon or read an alternate frame. Since tRNA population (type and relative abundance of each individual normally modified isoacceptor) varies much between organisms, especially of the three domains of Life (reviewed in Marck and Grosjean 2002), the probability of recoding at certain mRNA sequences is usually species specific, although for -1 frameshifting and readthrough, some recoding signals can operate in heterologous species (Stahl et al. 1995; Cassan and Rousset 2001; Leger et al. 2004). Therefore, in a given organism, a 'subtle combination' of various *cis*- and *trans*-stimulatory signals can force the translation machinery to escape normal decoding rules, leading to readthrough or frameshifting phenomena. The occurrence and efficiency of such translational recoding processes ultimately depend on how these various *cis*- and *trans*-elements have co-evolved in order to work together in a synergistic way.

1.3 Modified nucleotides in RNA and decoding

An important distinctive structural feature of RNA (tRNA and rRNA) is the presence of a significant proportion of post-transcriptional modifications of nucleotides. Out of over 100 different structures reported to date, more than 80 modifications are present in tRNA and about 20 in rRNA (Sprinzl et al. 1998; Rozenski et al. 1999; McCloskey and Rozenski 2005). The pattern of modification (type and location) depends on the RNA molecule considered as well as on the organism or the organelle from which they originate from. In tRNA, the most characteristic and often hypermodified nucleotides are present in the anticodon loop and stem (positions 27-40; Fig. 2 part A and B). These modified nucleotides contribute to the built-in feature of the anticodon branch that ultimately determines the decoding properties (efficiency and accuracy) of the tRNA molecule during translation on the ribosome (reviewed in Agris 1996, 2004; Davis 1998). A majority of the hypermodified nucleotides (such as Q, mnm^5s^2U) are exclusively present in position 34 (the wobble base in the anticodon), while others (such as m^6t^6A, ms^2i^6A or the Y base) are exclusively present in position 37 (3'-adjacent to anticodon). More simple modified nucleotides (such as Ψ, Gm, Um, s^2C, m^3C or m^5C) are present



elsewhere in the anticodon branch. They are needed to modulate the flexibility and the preferential 3'-stacked conformation adopted by the anticodon loop when it binds to the complementary codon (extended anticodon theory, reviewed in Yarus 1982; Dao et al. 1994). In this context, the ubiquitous purine-37, especially its hypermodified derivatives, plays a major role in modulating the stability of the codon-anticodon interaction by a dangling end type of base stacking (see Fig. 2C, reviewed in Bubienko et al. 1983; Grosjean et al. 1998). Also, because most modified nucleotides-37 cannot base pair in a Watson-Crick mode, their presence 3'-adjacent to the anticodon restricts the tRNA to base pair to the in-frame codon-anticodon triplet pair, thus limiting (but not completely avoiding, see below) the

Fig. 2 (overleaf). Type and location of modified nucleotides in the anticodon stem and loop of tRNAs. Part A is a schematic representation of the three-dimensional architecture of tRNA. Numbering of nucleotide positions are those universally adopted. The anticodon nucleotides corresponding to positions 34, 35, and 36 are shown in square boxes in the anticodon hairpin representation (Part B). Symbols for modified nucleotides are those defined in Rozenski et al. (1999). The information is derived from the tRNA data bank of Sprinzl et al. (1998). Almost all the hypermodified nucleotides characterized so far occur exclusively in position 34 (the so –called wobble base) or in position 37 (3'-adjacent to the anticodon). In Part C, the 3' stacked conformation of the anticodon branch is schematically represented. Bases adjacent to the anticodon (denoted Z) are often modified (see part B) and play a role in the flexibility and hence the preferential conformation adopted by the anticodon when it binds to a complementary codon. The ribosomal milieu is also a major factor. Purine-37 (R), 3' of the anticodon is often hypermodified. It cannot base-pair with mRNA and plays a role in reading frame maintenance as well as in the stabilization of the base pair between the third base-36 of the anticodon and the first codon base (I), as schematized by an arrow. Certain modified bases such as t^6A, ms^2i^6A or Y base, are particularly efficient because of their stacking potential. The wobble base-34 (W) can form noncanonical base pair with the third base of the codon (III). It also stabilizes the pairing in the middle position of the codon-anticodon complex. Therefore, correct decoding may depend on a short double helix formed between the "two out of three" complementary codon-anticodon, "sandwiched" between stacked, but not necessarily complementary nucleotides present in their immediate context.

risk of frameshifting during translation (reviewed in Agris 2004). Likewise, the nucleotide at position 34 of the anticodon reads the third codon base, and it is the only anticodon position that allows a non-Watson-Crick, or wobble base pairing during a "normal" decoding process. The characteristic and often unique modified nucleotides in this position-34 functions to restrict or extend pairing (or base opposition) between anticodon base-34 and the third base of the codon and hence regulates the decoding pattern of individual isoacceptor tRNAs (Yokoyama and Nishimura 1995; Takai and Yokoyama 2003; see also the chapter in this volume by Suzuki). As for purine-37, the type of modification of nucleotide-34 (on the base and/or the ribose) can also modulate stacking interaction with the adjacent Watson-Crick middle base pair at position 35 of the anticodon and position 2 of the codon (Fig. 2C; reviewed in Grosjean et al. 1998).

It is noteworthy that the level of modification of certain modified nucleotides in tRNAs, especially in the anticodon branch such as Q34, ms^2i^6A37, or Y37, depends on cell growth or stress conditions, as well as on the availability of the cofactor(s) needed for enzymatic formation of the modified nucleotide during tRNA biogenesis. Depending on the influence of this modified nucleotide on the recoding process, its presence or absence in the tRNA may affect and possibly regulate the level of expression of the 'recoding-dependent' protein (discussed in Buck and Ames 1984; Persson 1993; Winkler 1998; Björk et al. 1999). An analogous situation exists for the expression of proteins from certain bacterial mRNAs by an 'attenuation-type' of regulation mechanism. In this last case, the presence or absence of certain modified nucleotides in tRNA (such as Ψ at positions 38-39-40 in $tRNA^{His}$ or ms^2i^6A37 in $tRNA^{Trp}$) determines whether the translation machinery

will pass through a row of several adjacent identical codons (for histidine or tryptophan respectively) and, thereby, produce or not a protein that is required to overcome the stress problem of the bacteria (reviewed in Landick et al. 1996).

1.4 Complexity of the decoding process within the ribosome

Today, three tRNA binding sites are accepted as a universal feature of ribosomes: an acceptor A-site where the aminoacyl-tRNA complexed with elongation factor-GTP checks the adequacy of codon-anticodon pairing, a peptidyl P-site 5' adjacent of the A-site codon where a peptidyl-tRNA is positioned, and an exit E-site where the previous peptidyl-tRNA now deacylated is waiting to escape the ribosome for recycling into a new round of aminocylation-aminoacyl transfer process (reviewed in Burkhardt et al. 1998). As indicated above, the modified nucleotides in tRNAs and especially in the anticodon branch are of utmost importance for the modulation (accuracy, efficiency, regulation) of codon-anticodon interaction within the protein synthesizing machinery as well as for maintenance of the correct reading frame during translation. However, the "normal" decoding process (Rodnina and Wintermeyer 2001; Noller et al. 2002; Ogle et al. 2003; Steitz and Moore 2003), does not depend solely on codon-anticodon interactions at the A-site, but also on codon-anticodon interactions at the P-site and possibly at the E-site (Schmeing et al. 2003). It also depends on many other factors of the translation machinery, such as the interactions of the tRNA with the rRNA which also contains many modified nucleotides, especially in the decoding as well as in the peptidyl centers (Decatur and Fournier 2002; Ofengand and Delcampo 2005; McCloskey and Rozenski 2005), and possibly on the interactions with certain ribosomal proteins as well as between the two adjacent tRNAs located in the A-site and P-side and/or in the P-site and the E- site of the ribosome (Nierhaus et al. 2000). The dynamic interplay of these diverse types of interactions between all partners of the ribosomal machinery along the translation process often makes it difficult to evaluate the effect of a single modified nucleotide in a tRNA (or rRNA) on one particular stage of the stepwise in-frame translation process. Whatever the mechanistic details of this "normal" ribosomal elongation process, the time taken by each individual step along the mRNA is of utmost importance for the accurate reading of the genetic code (Rodnina et al. 2000, 2002). Indeed, as illustrated below in section 2, most if not all of the many kinds of recoding (and occasionally premature termination events not discussed here), occur during so-called 'pauses' of the ribosome along the mRNA.

1.5 Testing the roles of modified nucleotides of RNA in recoding

Since recoding corresponds to an exhausted propensity to make translational errors, it constitutes a choice target to check the effect of RNA modifications on the decoding capacities of the cell. Taking advantage of the complete sequence of many genomes now available, including those of the bacterium *Escherichia coli*

and the yeast *Saccharomyces cerevisiae,* together with the powerful genetic tools available to create and study *E. coli* or yeast mutants, a systematic investigation at identifying the role of particular modified nucleotides of tRNA in recoding events is now possible. The almost complete set of genes encoding tRNA modification enzymes in both *E. coli* and *S. cerevisiae* is now being determined (see Bujnicki et al. 2004; De Crécy-Lagard 2004 and the chapter in this volume by Johansson and Bystrom). The fact that deletion/inactivation of most of the genes encoding tRNA modification enzymes usually does not affect cell growth, at least when tested individually, strongly suggests that their roles concern mainly fine tuning of the tRNA molecules, rather than drastic defects of an essential function of the corresponding modification enzymes and/or of the undermodified tRNA molecules (discussed in Hopper and Phizicky 2003). Moreover, the use of mutants, associated with various reporter systems (natural or synthetic), allows a precise quantification of recoding events in *E. coli* and *S. cerevisiae* (Stahl et al. 1995; Grentzmann et al. 1998; Paul et al. 2001; Harger and Dinman 2003).

This experimental approach permits in principle the analysis of the decoding potential of cells in which a single modification enzyme is inactivated or absent. However, this powerful approach suffers several drawbacks: i) modification enzymes are usually not specific of a single isoacceptor tRNA nor of a single position in different tRNA species (Motorin and Grosjean 1999). In other words, when an effect is observed, it can be difficult (or even impossible) to determine which one(s) of the undermodified isoacceptor tRNA species is (are) involved in the recoding process, or which of the several positions where the modified nucleotide is normally present is (are) critical. To overcome this problem, one should preferentially test those recoding events that depend only on a single, or at least a limited number of isoacceptor tRNA with a well known pattern of modified nucleotides; ii) deletion/inactivation of a gene coding for a given modification enzyme may lead to pleiotropic effects depending on how the enzyme is interconnected with the expression of other cellular processes, including the activities of other tRNA modification enzymes. This is the case of enzymes belonging to enzymatic metabolons leading to the formation of several hypermodified bases in tRNAs, usually present in positions 34 (wobble position) and 37 (5'-adjacent to the anticodon, see above). While no evidence of such interconnectivity has yet been demonstrated between RNA modification enzymes catalyzing reactions at different locations in a tRNA molecule, this possibility cannot be ruled out and should be kept in mind; iii) lack of a given modified nucleotide in a tRNA may also lead to subtle changes in the multiple functions of the tRNA molecules, such as passage through the nuclear pore in eukaryotic cells (Grosshans et al. 2001), and specific interactions with aminoacyl-tRNA synthetases (reviewed in Giegé et al. 1998; Beuning and Musier-Forsyth 1999) or with various factors (Forster et al. 1993; Huang et al. 2005). It can also lead the undermodified tRNA to become a target for the surveillance system and be selectively degraded by the exosome machinery (Kadaba et al. 2004; see also the chapter in this volume by Anderson and Droogmans,). Carefully designed controls have to be performed to test whether the undermodified tRNA remains stable enough in the cell and continues to fulfill its role in mRNA translation; iv) some modification enzymes could be involved in another function

than tRNA modification, such as a "chaperone-like" activity during rRNA biosynthesis (Lafontaine et al. 1998) In this latter case, knowledge of which is (are) the essential amino acid(s) of the active site of the enzyme makes it possible to design specific mutations resulting in the production of stable proteins devoid of the RNA modification activity but still endowed with other function(s).

Despite the many difficulties encountered in attributing a specific function to a given modified nucleotide in the tRNA of *E. coli* or yeast strains defective in one of the several tRNA modification enzymes, interesting results concerning the role of tRNA modifications in recoding events have recently been published, mostly with *S. cerevisiae* and *E. coli* as model systems. In this review, we have excluded most of the abundant data concerning the role of modified nucleotides in natural spontaneous (non-programmed) readthrough, as well as those involving mutated suppressor tRNAs or abnormal termination factors (for details concerning this aspect of decoding, consult excellent reviews by Björk 1995; Murgola 1985, 1995; Curran 1998; Agris 2004).

2 Influence of modified tRNA nucleotides in frameshifting

2.1 Programmed +1 frameshifting in bacteria

In bacteria, the case of the *prfB* gene of *E. coli,* encoding the release factor 2 (RF2), which recognizes the UAA and UGA codons, is the best understood phenomenon (Craigen and Caskey 1986). The *prfB* open reading frame is interrupted by a UGA stop codon 26 codons downstream of the initiation codon. However, the coding sequence corresponding to the long C-terminal part of the protein continues immediately in the +1 reading frame, for a further 340 codons (Fig. 3). This recoding event is used as an autoregulatory mechanism controlling the abundance of full length RF2 in the cell (Craigen and Caskey 1986; Adamski et al. 1993). In the presence of high RF2 concentrations, the competition between termination and frameshifting is changed in favor of termination, leading to a decrease of the RF2 concentration in the cell. When RF2 becomes limiting, frameshifting begins to dominate, thus, increasing cellular RF2 level.

Frameshifting occurs at the slippery heptanucleotide sequence CUU.UGA.C (the zero frame is represented) located at the junction of the zero and +1 ORFs and allows expression of the active RF2 protein. The frameshifting event requires the peptidyl-$tRNA^{Leu}$ (anticodon $^{5'}GAG^{3'}$ with an m^1G_{37} 3' adjacent to the anticodon), which normally decodes the zero frame leu-codon CUU in the P-site of the ribosome, now to slip by a single base toward the 3' end and to miscode the +1 frame Phe-codon UUU. It also involves an unorthodox G*U base pair between the third anticodon base of the peptidyl-$tRNA^{Leu}$ and the first base of the new +1 Phe-codon UUU. This +1 frameshift process depends on a Shine-Dalgarno-like (SD) sequence positioned upstream of the slippery sequence, which interferes with the tRNA in the E-site (see below). The transient interaction between the 16S rRNA

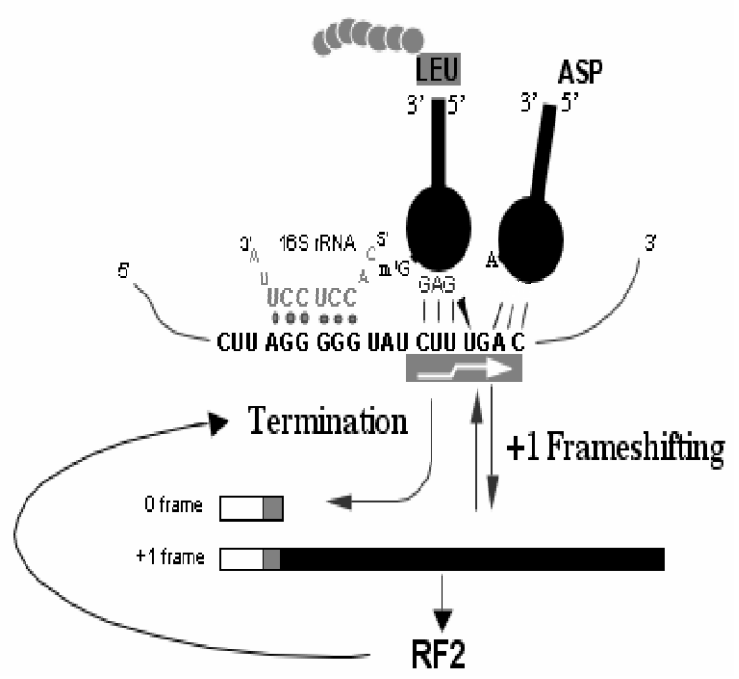


Fig. 3. RF2 +1 frameshifting in *E. coli*. An UGA stop codon interrupts the *prfB* gene at position 26. In the presence of a limiting concentration of RF2 protein, a pause of the ribosome is induced. In association with the Shine-Dalgarno-like sequence which increases the probability that the E-site tRNA will be ejected from the ribosome, the pause allows the leucine $tRNA_{GAG}$ (carrying the m^1G modification at position 37) to slip one nucleotide upstream. During the next step of elongation the Asp-$tRNA_{GUC}$ (not modified at position 37) is incorporated in the +1 frame leading to a +1 frameshifting event that allows the synthesis of full length RF2. If RF2 is not in limiting amounts, it recognizes the UGA termination codon and protein synthesis stops. This represents an elegant autoregulatory mechanism controlling the abundance of RF2 in a large number of bacteria.

and such a SD-like sequence in the mRNA slows down progression of the ribosome on the mRNA, allowing the decoding machinery to better sensor the availability of RF2. When RF2 is in low abundance, the peptidyl-$tRNA^{Leu}$ has more time (better chance) to slip rightward to the +1 phe codon UUU (Fig. 3). Urbonavicius and collaborators (2001) directly demonstrated the role of modifications in position 37 in improving reading frame maintenance on frameshifting sites derived from *prfB*, using *E. coli* and *Salmonella typhimurium* mutants deficient in m^1G37 or ms^2io^6A37 modifications. The same study also demonstrated the role of modifications at position 34 (Q34, mnm^5s^2-U34) and Ψ38-40 on maintenance of the reading frame.

Recently, premature release of the E-site tRNA from the ribosome has been demonstrated to be coupled with high-level +1 frameshifting at the *prfB* gene (Marquez et al. 2004). This study showed that in an *in vitro* reconstituted system,

the presence of the E-site tRNA can prevent the +1 frameshifting event. Indeed, when the internal UGA is at the A-site, the SD-like sequence is separated from the peptidyl-tRNA at the P-site by only two nucleotides and thus competes with the first nucleotide of the E-site codon. The result is a steric clash between the SD-antiSD of the 16S rRNA and codon-anticodon interaction at the E-site. This situation allows an easier release of the E-site tRNA and consequently favors the subsequent frameshifting event. This study demonstrates for the first time the importance of the E-site tRNA for reading frame maintenance. As for the tRNA in the A- and P-sites, it remains to be determined whether the stability of the E-site tRNA also depends on its modification status, and hence leading to a modulation of frameshifting efficiency, at least in Bacteria.

2.2 Programmed +1 frameshifting in Eukarya

In eukaryotes, several genes have been reported to be expressed through a programmed +1 frameshifting event (reviewed in Farabaugh 1996; Namy et al. 2004). Transposon Ty1 and Ty3 frameshifting, together with the ornithine decarboxylase antizyme of higher eukaryotes are probably the best studied examples. In all these cases, a "hungry" codon in the A-site due to limitation in the abundance or decoding capacity of aminoacyl-tRNAs or a severe functional defect in the tRNA (Gallant and Lindsley 1992) is responsible for the ribosomal pause, and favors the probability of a slippage at a specific heptanucleotide sequence of the peptidyl-tRNA from the P-site into the +1 out of frame codon. Depending on the cooperation of various stimulating elements, the efficiency of this recoding process in *S. cerevisiae* ranges from a few percent to 90%. These elements (see also section 1.2) are: the peculiar mRNA slippery sequence, the decoding property of the corresponding tRNAs and the presence and/or absence of certain modified nucleotides in these tRNAs (see below).

Two models have been proposed to explain such high level of Ty1 frameshifting. In the first model (Stahl et al. 2001), the peptidyl-tRNA^{Leu} (UAG), originally located at the P-site Leu-CUU codon, was proposed to bind the out-of-frame +1 Leu-UUA codon by a unorthodox base pairing (a G-U within the third anticodon base and the first codon base, see Fig. 4). This noncanonical phenomenon has a certain probability to occur, because: i) the normal in-frame AGG codon corresponds to a slow decoding tRNA^{Arg}(anticodon U*CU, with t^{6}A37 and Ψ39, where U* stands for mcm^{5}U), thus inducing a pause of the ribosome traveling along the mRNA; ii) the overlapping +1 codon GGC corresponds to an abundant and efficient tRNA^{Gly} (GCC with A37Ψ38), able to make three GC pairs; iii) naturally occurring tRNA^{Leu}(UAG with m^{1}G37 and Ψ39), in contrast with all other U34-containing tRNAs in *S. cerevisiae,* has an unmodified U34 in the wobble position of anticodon and is able to recognize all six leucine codons (including UUA), at least *in vitro* (Weissenbach et al. 1977).

In the second slightly different alternative model (Hansen et al. 2003), the same peptidyl-tRNA^{Leu} is proposed to slip on the mRNA without fully pairing with the

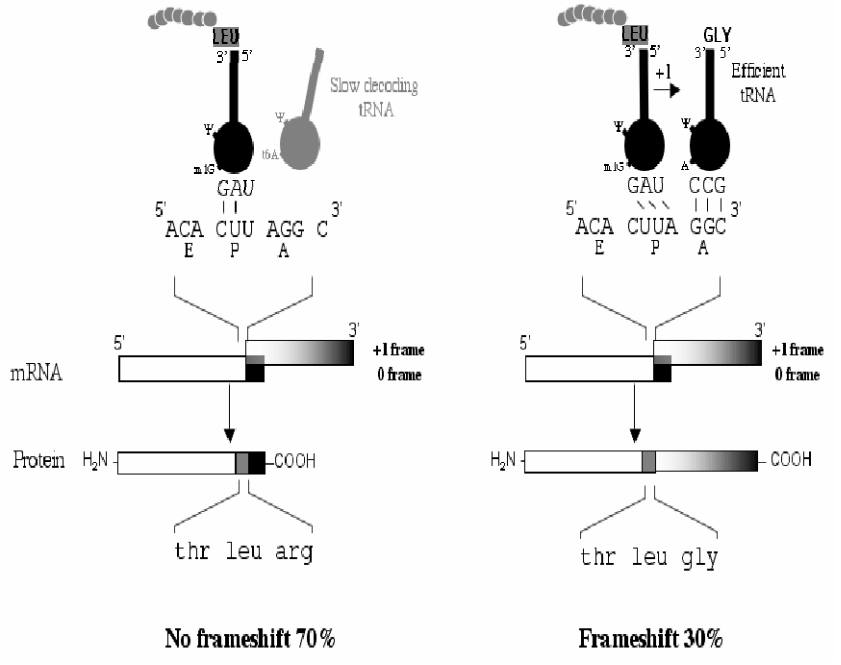


Fig. 4. Ty1 +1 frameshifting in *S. cerevisiae*. The leucine-tRNA$_{UAG}$ in the P-site can re-pair in the +1 position. This event depends on the low availability and decoding capacity (slow decoding) of the arginine tRNA$_{U*CU}$ (where U* stands for mcm^{5}U34). The polypeptide synthesized after frameshifting carries the polymerase activity, whereas the short polypeptide has a GAG-like function. The efficiencies of the two events (normal decoding and +1 frameshifting) are indicated in %. Pseudouridine (Ψ) are in position 39 in tRNA^{Leu} and tRNA^{Arg}, but in position 38 in tRNA^{Gly}.

new +1 codon, the important thing still remains the ability of the incoming abundant tRNA^{Gly}(GGC with A37Ψ38) to outcompete with the inefficient tRNA^{Arg}(U*CU, t^{6}A37) for the decoding of the +1 codon instead of the in-frame codon. Whatever the exact mechanism is, the common important features for most programmed +1 frameshifting in Eukarya are an unstable codon/anticodon base pairing in the P-site together with a peptidyl-tRNA having a special ability to slip, a codon pausing the ribosome in the A-site due to the existence of an inefficient or low abundant decoding tRNA and a G/C rich codon in the +1 overlapping frame corresponding to an abundant tRNA.

In this context, one can expect some modified nucleotides in the anticodon branch of the tRNA (and possibly in the decoding site of the rRNA; Decatur and Fournier 2002) to influence in one way or another the efficiency of the recoding process. Recently, we have shown that the absence of pseudouridine (Ψ) at position 38 or 39 in the anticodon arm of yeast tRNAs decreases frameshifting efficiency at the Ty1 site. Indeed, disruption of the *PUS3* gene, coding for tRNA (Ψ38/Ψ39) pseudouridine synthase in *S. cerevisiae*, or point mutation in the *PUS3*

gene that inactivates the gene product, leads to an almost twofold reduction of frameshifting at the Ty1 slippery sequence (Lecointe et al. 2002). Obviously, Ψ39-containing $tRNA^{Leu}$(UAG) is more adapted to "recoding" at the Ty1 slippery sequence, probably by making a more stable codon-anticodon interaction in the out-of-frame codon than the $tRNA^{Leu}$ lacking of Ψ39 (Davis et al. 1998; Yarian et al. 1999).

Interestingly, deletion of the *PUS4* gene involved in pseudouridine formation at positions 55, as well as of the *TRM4* gene involved in 5-methylcytosine formation at positions 48 of $tRNA^{Leu}$(UAG) have no noticeable effect on Ty1 +1 frameshifting (Lecointe et al. 2002). Thus, efficiency of +1 programmed frameshifting depends on selected modified nucleotides, which in the present case are located at the end of the anticodon arm.

2.3 Programmed -1 frameshifting in Bacteria and Eukarya

Among recoding events, -1 programmed frameshifting is probably the most frequently encountered. It is found in eukaryotes, bacteria, viruses (mostly retroviruses), phages, and also in transposable elements (IS). In viruses, programmed frameshifting is generally used to express the viral polyprotein Gag-Pol. However, a few cellular genes both in prokaryotes and eukaryotes are known to use a -1 frameshifting event for their expression (Baranov et al. 2002; Namy et al. 2004).

The -1 type of frameshifting appears to occur either by tandem or single tRNA slippage depending on the tRNAs and the slippery sequence involved (Napthine et al. 2003). One of the major determinants influencing the efficiency of -1 frameshifting is the presence of a stimulatory signal located downstream or upstream of the slippery codons. This *cis*-acting element acts at least in part by slowing ribosome progression along the mRNA, allowing a better chance for the tandem tRNAs to slip backwards, depending of course on the adequacy of the new -1 mRNA hexanucleotide sequence for base pairing with tRNA anticodons (Jacks et al. 1988). This could be either an upstream Shine-Dalgarno-like sequence (as in +1 frameshifting, see section 2.1 above) or a downstream secondary structure, which could be either a stem loop or more often a pseudoknot (see Fig. 5 for a typical -1 frameshifting site). The influence of the stacking potential of the base immediately adjacent to the base 3' of tandem shift codons on -1 ribosomal frameshifting has also been demonstrated, thus indicating that the stability (lifetime) of the codon-anticodon interaction in the new -1 frame is an important parameter (Bertrand et al. 2002).

Beside these mRNA structural determinants in *cis*, the question of the involvement of only certain types of tRNAs as being able to shift backward and also the role of certain modified nucleotides in these tRNAs have been controversial. First, soon after the discovery of the -1 translational frameshifting sites in various mammalian retroviruses, such as Human Immunodeficiency virus, Human T-cell lymphotrophic virus 1, and Bovine Leukemia virus, a correlation was made with the observation that aminoacyl-tRNAs required to translate the slippery sequence

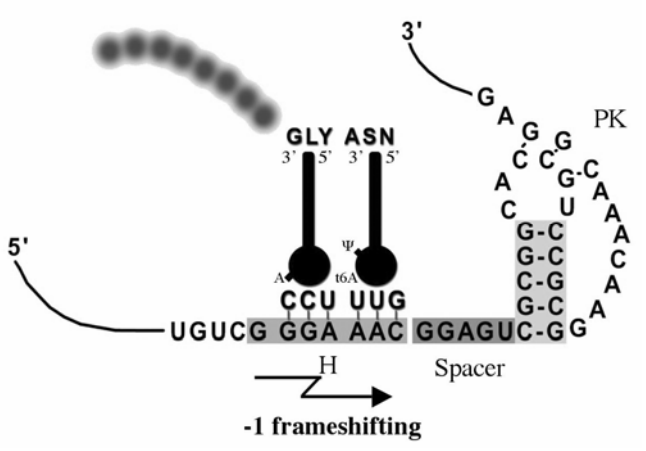


Fig. 5. -1 frameshifting site from Beet western yellows virus (BWYV). A shifty heptamer (H) of the form X.XXY.YYZ allows tandem slippage of the tRNAs in the P- and A-sites. The special nature of the heptamer sequence allows "re-pairing" of the two tRNAs in the -1 frame. Although the heptamer sequence is the causative element, a downstream stimulatory secondary structure, in general a pseudoknot (PK) is necessary to reach a high efficiency of frameshifting. The length and sequence of the region between the pseudoknot and the slippery sequence (spacer) is also a critical parameter to achieve a maximal level of frameshifting.

were always hypomodified in infected cells, i.e. the degree of modification of certain nucleotides in tRNAs, such as Q34 in several tRNAs and of Y37 in $tRNA^{Phe}$ was severely reduced (Hatfield et al. 1989). However, several laboratories have analyzed the role of the Q-containing tRNAs and the conclusion was that when the asparaginyl-tRNA (anticodon QUU and t^6A37) decodes the A-site codon AAC the presence of Q34 and consequently the presence of the precursor G34 in the mammal tRNA has no significant effect on -1 frameshifting at the slippery sequence U.UUA.AAC, either *in vitro* or in mammalian cells (Cassan et al. 1994; Reil et al. 1994; Marczinke et al. 2000; Carlson et al. 2000).

Second, using a rabbit reticulocyte lysate and the sequences A.AAU.UUU and U7 as model systems, Hatfield and collaborators (Carlson et al. 2001) demonstrated that rabbit reticulocyte $tRNA^{Phe}$ (anticodon GmAA and Y37) bearing a m^1G37 instead of the hypermodified Y-base-37 (wybutosine-37) stimulates -1 frameshifting threefold. No such effect was observed with the U7 slippery sequence under the same experimental conditions. However, when $tRNA^{Phe}$(GmAA) from the yeast *S. cerevisiae* was tested *in vivo* on another slippery sequence U.UUU.UUA (instead of A.AAU.UUU as above) and in a yeast *trm5* mutant defective in the formation of m^1G37 (m^1G37 is the first biosynthetic step of Y37 formation), no significant difference in the level of frameshifting was observed as compared with the same analyses performed using the wild type strain (Urbonavicius et al. 2003). If we assume that the two types of test systems are comparable, the result may indicate that a slippery undermodified tRNA in the P-site (as

in the *in vitro* reticulocyte experiment) or in the A-site (as in the *S. cerevisiae* yeast system) can make a difference. Moreover the absence of effect observed by Carlson et al. (2001) with the U7 frameshifting sequence, as tested in the *in vitro* reticulocyte test system, would indicate that undermethylated tRNAs located in the A-site can neutralize/counteract the effect of the same undermethylated $tRNA^{Phe}$ in the P-site, probably because the global stability/life time of the tandem $tRNA^{Phe}$ bound in the -1 frame of the slippery U7 sequence is too low.

In bacteria, one of the most frequently -1 frameshift prone sequence found is U.UUA.$AA^{A}/_{G}$ (nucleotides involved with tRNA pairing are underlined). It involves a specific $tRNA^{Lys}$ bearing an anticodon U*UU flanked with t^6A37(U* stands for 5'-methylaminomethyl-2-thio-uridine-34) complementary to codons $AA^{A}/_{G}$ in the A-decoding site. Among the *E. coli* mutants that affect the activity of the different enzymes involved in the multistep formation of this hypermodified uridine-34, only *mnmE* (*trmE*) catalyzing the early step of the methylaminomethyl-group (mnm) on C5 of U-34 has an effect on -1 frameshifting efficiency. In this mutant, in which, the $tRNA^{Lys}$ harbors only the thiolated uridine (s^2U34, instead of the fully modified mnm^5s^2U34), -1 frameshifting at the U.UUA.$AA^{A}/_{G}$ slippery sequence is stimulated twofold (Brierley et al. 1997). However, an independent recent report indicates that a twofold decrease in -1 frameshifting efficiency is observed when the same *mnmE* mutant *E. coli* strain is used to test another slippery sequence A.AAA.AAC, where the $tRNA^{Lys}$ is located in the P-decoding site (Urbonavicius et al. 2003). Again, the apparent discrepancy between the two sets of data may result from the fact that in one case the $tRNA^{Lys}$(U*UU.t^6A) reading the AAA codon, was initially located in the ribosomal A-site while in the second case the same $tRNA^{Lys}$ was initially present in the P-site. This illustrates the fact that different requirements are involved in codon/anticodon base pairing depending on whether the concerned tRNA is present in the ribosomal A-site or the P-site. Unfortunately, no data has been reported with A7 and the *E. coli* hypomodified $tRNA^{Lys}$ in order to verify, as in the U7 sequence tested above with undermodified $tRNA^{Phe}$, whether the presence of the same hypomodified tRNA in both the P- and the A-sites would neutralize/counteract the effect observed in each individual situation.

The importance of the hypermodified uridine-34 in preventing tRNA slippage during the elongation process in *E. coli* was also demonstrated by Brégeon et al. (2001). Using the artificial frameshifting site GAG.AGA.G within the β-galactosidase ORF expressed in *E. coli*, coupled with mutagenesis experiments, it was beautifully demonstrated that characteristic +2 frameshifting events (apparent -1 frameshift) occurred in mutants affecting the genes coding for one or two of the several enzymes involved in the formation of methylaminomethyl group of U34 in $tRNA^{Glu}$(U*UC.m^2A37, where U* stands for mnm^5s^2U34), namely *gidA* and *mnmE*.

Last but not least, while most studies have concerned only de/recoding properties of tRNA in either the A- or the P-decoding site within a given slippery sequence, recent results from our laboratory indicate the importance of at least one modified nucleotide in the anticodon branch of a tRNA located in the E-site during -1 frameshifting (Bekaert and Rousset 2005). Based on a clear-cut correlation

between the existence of an efficient -1 frameshifting event and the presence of a tRNA carrying a Ψ at position 39 in the P-site of the decoding cassette, we experimentally verified with a *PUS3* deleted yeast strain that the presence of the precursor U39 instead of Ψ39 in the tRNA, decreases by a factor of 2 the efficiency of -1 frameshifting event of several slippery sequences, as compared with the situation in a wild type yeast strain. This observation is reminiscent of the role of the tRNA in the E-site in +1 frameshifting of the *E. coli prfB* gene, although the mechanism could be different (see above in section 2.1 and Marquez et al. 2004). Whatever the molecular basis of these phenomena, discussions of earlier studies considering only the identity of the tRNAs in the A- and P-sites of the ribosome to interpret -1 and +1 frameshifting should be reconsidered.

3 Modified nucleotides in tRNA also affect stop codon readthrough efficiency

When a stop codon is presented in the ribosomal A-site of the ribosome, specific release factors bind to the ribosome and trigger the hydrolysis of the peptidyl chain of the peptidyl-tRNA that is present in the P-site. The termination process is usually very accurate, the probability of a readthrough event being estimated to be as low as 0.005 - 0.001% in both Bacteria and Eukarya. The machinery that determines such an efficient process is rather complex (for reviews see Murgola et al. 2000; Wilson et al. 2000; Bertram et al. 2001). Again, as discussed above for frameshifting, a combination of several factors in *cis* and in *trans* of the stop codon (readthrough signals) can considerably influence the accuracy of the termination process and enhance the propensity of a given stop codon to be read (in fact miscoded) by a normal elongator tRNA instead of the expected release factor. Among these factors are the stop codon itself, UGA being more 'leaky' than UAG, which is less efficient than UAA (Lovett et al. 1991; Manuvakhova et al. 2000; Bidou et al. 2004), the surrounding nucleotide context, up to 6 nucleotides downstream and 2 nucleotides upstream of the stop codon (Bonetti et al. 1995; Bertram et al. 2001; Namy et al. 2001; Harrell et al. 2002; Tork et al. 2004) and the presence (type and abundance) of tRNAs able to decode a given stop codon (Chittum et al. 1998). The ability of these natural suppressor tRNAs to compete with the release factor by reading a stop codon depends very much also on its modified nucleotide content, especially in their anticodon branch (Beier and Grimm 2001). After stop codon readthrough translation continues in the original reading frame and results in synthesis of a longer protein with potentially new biochemical properties. Programmed readthrough of stop codons is used by a number of plant viruses to express their replicase domain in the form of a fusion protein (Beier and Grimm 2001).

Again, as described above for viral frameshifting, most of the stop codon readthrough events described are found in viruses infecting eukaryotic cells and are used to control gene expression (Blum et al. 1989; Feng et al. 1992; Zerfass and Beier 1992a; Li and Rice 1993). Several examples of stop codon readthrough

events have been recently identified in eukaryotic cellular genes, and with the growing number of fully sequenced genomes that become available, more examples are expected to be identified (reviewed in Namy et al. 2004). In bacteria, excluding the very efficient selenocysteine and pyrolysine incorporation in response to a stop codon, no obvious programmed stop codon readthrough has been identified to date.

In Eukaryotes, several naturally occurring cytoplasmic tRNAs have been shown to recognize stop codons involved in programmed translational readthrough events (see references below). In all cases, stop codon recognition implies non-orthodox base pairing between the second or the third base of the anticodon and the first or second base of the codon. The probability of such miscoding is highly dependent on the presence or absence of modified nucleotides in the anticodon (first and/or second position) and/or in position 37, adjacent to the third base-36 of the anticodon:

i. Eukaryotic $tRNA^{Tyr}$ normally decodes exclusively $UA^{C}/_{U}$ codons, except in certain cells where it can also efficiently read both the UAG and UAA stop codons, despite a GxG or GxA clash between the first wobble base of the anticodon and the third base of the codon. This phenomenon, originally discovered by studying translation of the Tobacco mosaic virus (TMV) was observed later also in many other plant viruses for which the expression of the polymerase domain depends on efficient readthrough of a characteristic stop codon (Beier et al. 1984). The anticodon $tRNA^{Tyr}$ of tobacco and wheat leaves is GΨA with m^1G37, whereas in $tRNA^{Tyr}$ of wheat germ, the anticodon is doubly modified into QΨA with m^1G37. Interestingly, only $tRNA^{Tyr}$ (GΨA with m^1G37) from tobacco and wheat leaves can efficiently translate the stop codon of TMV *in vitro*, but not $tRNA^{Tyr}$(QΨA) from wheat germ. Likewise, natural $tRNA^{Tyr}$ of *S. cerevisiae* (anticodon GΨA), that is naturally devoid of Q34 but contains Ψ35 in the middle of the anticodon and i^6A37 instead of m^1G37, was shown to be an efficient suppressor of the TMV UAG stop codon, while the same $tRNA^{Tyr}$ in which the GΨA anticodon was replaced by unmodified GUA, became incompetent for UAG stop codon reading in the TMV context (Zerfass and Beier 1992b). These results clearly indicate that the Ψ35 modification is a major determinant for $tRNA^{Tyr}$ to suppress the UAG stop codon, and that the Q modification in position 34 of the same tRNA counteracts the property of Ψ35.

ii. Eukaryotic cytoplasmic $tRNA^{Gln}$ are known to be able to suppress UAG and UAA stop codons either *in vitro* or *in vivo* (Pure et al. 1985; Kuchino et al. 1987; Kuchino and Muramatsu 1996; Hoja et al. 1998; Namy et al. 2002). Two isoacceptors with either CUG or UmUG anticodons are found in mammalian cells (Sprinzl et al. 1998). These $tRNA^{Gln}$ can each read one of the two UAG/UAA stop codons, thus, including a noncanonical G*U wobble base pairing between the third anticodon base (position 36) and the first codon base. It is noteworthy that the sequenced $tRNA^{Gln}$ from mouse and tobacco carry an unmodified A in position 37 (instead of the usual m^1G37 adjacent to a G36), which is believed to favor such unconventional G*U wob-

ble base-pair as stated above (Weissenbach and Grosjean 1981). Moreover, one of the two $tRNA^{Gln}$ isoacceptors harbors a 2'*O*-methyl ribose at position 34 which was demonstrated to strengthen base pairing between codon and anticodon (Satoh et al. 2000).

iii. Likewise, two natural $tRNA^{Trp}$ suppressors of the UGA stop codon, with a CmCA anticodon have been isolated from plants, one cytoplasmic and one from the chloroplast. The cytoplasmic tRNA carries an m^1G at position 37, whereas its chloroplast counterpart has either an i^6A or ms^2i^6A derivative at position 37 (Beier and Grimm 2001). It has been shown that both the 2' *O*-methylribose and isopentenyl derivative of A37 stabilize codon/anticodon interactions (Houssier and Grosjean 1985; Satoh et al. 2000; reviewed in Grosjean et al. 1998), thus, allowing the unconventional CmxA base pairing between the first wobble anticodon position and the third codon base. Interestingly, in vertebrate reticulocytes, β-globin is naturally extended beyond its UGA stop codon by multiple suppressions and translational reading gaps. Identification of the amino acids in response of UGA have shown the presence of serine, tryptophan, cysteine, and arginine (Chittum et al. 1998). Also, three peptides result from translational reading gaps as they lack an amino acid or amino acids corresponding to UGA and/or one or two of the immediate downstream codons. Clearly, bypass of a stop codon may involve several "natural" tRNA suppressors, some of which are probably better suppressors than others, depending in part on the sequence of their anticodon but also on their modified nucleotide content. Figure 6A illustrates the case where the stop codon UAG in a context similar to the TMV readthrough site has been shown to be misread in *S. cerevisiae* by tRNA isoacceptors corresponding to Tyr, Lys, and Trp, yet with different efficiencies (Fearon et al. 1994).

iv. So far, only in plants has a cytoplasmic suppressor $tRNA^{Arg}$ bearing the anticodon U*CG (where U* stands for mcm^5U and/or mcm^5s^2U) been identified as efficient natural UAG suppressor in the PEMV (Pea enation mosaic virus) context in a wheat germ extract (Baum and Beier 1998). Readthrough of the UGA codon of Sindbis virus has been observed in cultured cells of chicken, human, and insect. The possibility exists that the $tRNA^{Arg}$ (U*CG, where U* stands for mcm^5U and/or mcm^5s^2U) which is present in these cells is responsible of the UGA readthrough; however, direct evidence is still lacking (Takkinen 1986; Li and Rice 1989).

v. The presence or the absence of a modified nucleotide not only in the anticodon, but also in another position of the tRNA molecule can control suppressor efficiency. We have recently shown that lack of pseudouridinylation at positions 38 or 39 of the anticodon branch decreases readthrough efficiency of stop codons in *S. cerevisiae*. Indeed, deletion of the *PUS3* gene responsible for the formation of pseudouridines at these positions, affects readthrough of all three stop codons placed in the TMV context. Because all three known natural suppressors of stop codons in *S. cerevisiae*, i.e. $tRNA^{Trp}$(CmCA.A37), $tRNA^{Tyr}$(GΨA.i^6A37) and $tRNA^{Lys}$(CUU.t^6A37) (Fig. 6A and 6B; Fearon et al. 1994) harbor a pseudouridine at position 39, it was

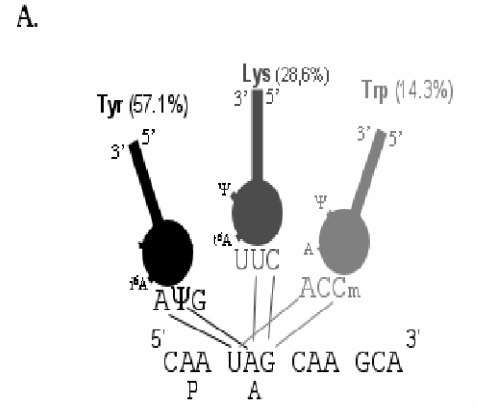
concluded that this modification improves the decoding efficiency of stop codons, probably because the Ψ39-containing tRNA allows a stronger interaction between codon and the anticodon (Davis et al. 1998; Yarian et al. 1999), a situation that is particularly important for miscoding at stop codons by natural tRNAs.

vi. Among various types of recoding events that occur at a stop codon, cotranslational incorporation of selenocysteine in response to UGA is certainly the most spectacular and the best studied one. It requires a specialized $tRNA^{SerSec}$ bearing a U*mCA.i^6A37 anticodon complementary to UGA (where U* stands for mcm^5U34 in mammalian tRNA and a nonmodified U34 in *E. coli*), a specific elongation factor and a protein that recognizes a secondary structure in the mRNA designated SECIS, located downstream of the UGA codon. Altogether, these *cis*- and *trans*- factors drive efficient incorporation of selenocysteine by the $tRNA^{SerSec}$ at the UGA stop codon (for review see Walczak et al. 1996; Tujebajeva et al. 2000; Hatfield and Gladyshev 2002; Driscoll and Copeland 2003). Interestingly, even though the anticodon of $tRNA^{SerSec}$ is strictly complementary to the UGA stop codon (as a true suppressor tRNA), both the isopentenyl group on adenosine-37 (i^6A37) and the 2'*O*-methyl group on U34 were nevertheless demonstrated to be important for efficient reading of the UGA within the recoding cassette (Warner et al. 2000; Jameson and Diamond 2004). The possibility also exists that the level of 2'-*O*-methylation of U34 in response to a specific metabolic stress, such as limitation of available free selenium or selenium derivatives, plays a role in regulating the expression of a group of important cellular selenocysteine-containing proteins (Jameson and Diamond 2004).

In the chapter by Rubio and Alfonzo of this volume, there is another interesting case of UGA stop codon recoding that occurs in mitochondria of the trypanosome *Leishmania tarentolae*. In this case, a nuclear encoded $tRNA^{Trp}$ (anticodon CCA.i^6A37) is first imported into the mitochondria where a fraction of the imported tRNA (about 50%) becomes modified at positions 32 and 33 into 2' *O*-methyl- and 2-thiolated uridine derivatives respectively, before C34 at the anticodon is edited into U34 and subsequently modified again into 2' *O*-methyl-U34. The resulting multi-step modified/edited $tRNA^{Trp}$ (with the modified anticodon UmCA) is now a true UGA suppressor, making canonical Watson-Crick base pairs between codon and anticodon, exactly as with $tRNA^{SerSec}$ involved in selenocysteine incorporation where the 2' *O*-methylation of U34 also plays a major role in the efficiency of decoding (see above).

4 Conclusions and Perspectives

Codon recognition by tRNAs plays a central role in decoding the genetic message. This process occurs on the ribosome and involves many steps and components of the translation machinery. Obviously, complexity is required to maintain high



B.

LEU	SER	TYR	TRP
m^5cAA	CGA	GΨA	CmCA
		GLN CUG	
		LYS CUU	
		GLU CUC	

Fig. 6. Stop codon readthrough. Part A: Several natural suppressor tRNAs are able to read the UAG stop codon. Among them, three have been identified by protein sequencing in *S. cerevisiae*, using the CAA.UAG.CAA.GCA readthrough context, similar to the well known TMV readthrough site (stop codon is underlined). Relative proportion of each of the amino acids identified is indicated in brackets. A common feature of all these tRNAs is the presence of a mismatch (noncanonical base pairing) in the codon/anticodon base pair (GxG, UxU or CxA), as well as the presence of a Ψ residue at position 39. The absence of this Ψ39 reduces the efficiency of readthrough in a yeast system (see text). In part B, are shown the anticodon of natural tRNAs of *S. cerevisiae* that can theoretically make a "genetically incorrect" mismatch with one of the bases of the UAG stop codon. These bases are indicated in bold and underlined. Grey boxes correspond to the anticodons of tRNAs that were demonstrated to induce readthrough in a TMV context (Part A).

fidelity and efficiency of the decoding process. In the present review, we summarized what is known about the role of modified nucleotides in tRNA involved in 'recoding' processes (mainly frameshifting and stop codon readthrough). These recoding events can be very efficient and have been selected and optimized during evolution. Comparative studies of how most codons in mRNAs are faithfully de-

coded in frame while in certain contexts selected codons are efficiently recoded (miscoded) should light on the molecular basis of translational accuracy. It may also help identify the elements within tRNAs and rRNAs that allow a ribosome either to shift or to bypass a termination codon in organisms of the three domains of Life (Bacteria, Archaea, and Eukarya).

4.1 Decoding rules of recoding process are special

The main difference between a highly efficient recoding process at specific recoding signals and a highly efficient decoding event during translation of mRNA is that the rules underlying the interactions between a codon and an anticodon on the ribosome are different. According to the genetic code, interactions between codon and anticodon during normal mRNA translation, involve strict Watson-Crick base-pairing between the two first bases of the codon and the two last bases of the anticodon, and only the first base of the anticodon can accommodate (wobble) with a different third base of the codon following the so-called "wobble rules" (Crick 1966; reviewed in Agris 2004). In contrast, recoding phenomena involving natural suppressor tRNAs often imply noncanonical base pairing, usually between the first base of the codon and the third base of anticodon (in addition or not to normal wobbling base pairing on the other side of the codon-anticodon interaction) or, as in very few cases, a unique mismatch in the middle of the codon-anticodon pair. This fundamental difference may explain why some features, such as the presence or absence of a given modified nucleotide in the anticodon of a tRNA, may differently affect the efficiency of the normal decoding process as estimated in 'classic genetic experiments' and the efficiency of the recoding process.

4.2 *Trans*-recoding elements are complex and difficult to identify

To date much effort to understand the mechanism of recoding events has concentrated on the elucidation of the *cis*-elements that direct the ribosome to bypass a stop codon, or to shift out of the normal decoding frame. This led to the identification of several types of recoding and stimulatory signals such as slippery sequences, codon context, and secondary structures downstream of the recoding cassette as well as upstream SD-like sequences in Bacteria. In all systems studied so far, a pause during decoding is a critical element of recoding efficiency (Farabaugh 1996).

Identification of the *trans*-acting elements that stimulate the same recoding events has been more challenging. In a given cell, elements that may affect de/recoding are: i) in the ribosome itself, with the rRNA containing many characteristic modified nucleotides, mainly in the decoding- and the peptidyl-sites; ii) in specific normally modified isoacceptor tRNAs (type and abundance); iii) in proteins and/or specific factors acting during the frameshifting or termination process. As far as RNA modification is concerned, sequencing tRNAs and rRNAs from many organisms has revealed their complexity and variability (type, location). De-

tailed biochemical and genetic analyses have revealed a modulation of the level of certain modifications in tRNAs in response to physiological stresses or biochemical constraints. In other words, a given nucleotide in an RNA (tRNA or rRNA) is not necessarily fully modified, as may appear from inspection of RNA modification databanks. Such information is, however, difficult to obtain and even impossible unless specific analyses designed to address this important question are presented. Only in few cases, has this type of analysis been done (Persson 1993; Yu et al. 1997; Björk and Rasmuson 1998; Winkler 1998).

Also, ribosomes and translation factors from organisms of each of the three domains of Life are not identical and important differences exist. The majority of these *trans*-elements are therefore species-, sometimes cell-specific and not necessarily interchangeable in reconstituted *in vitro* and *in vivo* heterologous systems. However, several good model systems (natural or synthetic with artificial reporter mRNA) have been developed to study various aspects of the recoding process in Bacteria (*E. coli* or *S. typhimurium*) and Eukarya (mammalian cells in culture or *S. cerevisiae*) transformed or not with ad hoc plasmids. The use of rabbit reticulocyte lysates and wheat germ extracts has also been instrumental in developing tools to study recoding processes. Using these tools, it has been shown that well-characterized recoding systems, that are efficient in the homologous translation system, when transposed to another type of cell (such as between yeast and bacteria), no longer work or at least with much lower efficiency. This is the case of the Ty1 frameshifting site that is completely inefficient in mammalian cells (Stahl et al. 1995). Therefore, what we know from one type of cell may not necessarily apply to another type of cell.

However, in other cases, recoding signals from one organism work equally well in heterologous systems. Although, it is not obligatory that the same precise mechanism be involved in different species, this implies that the major determinants of recoding have been conserved during evolution. Indeed, the readthrough signal of TMV, initially characterized in plant cells, works also in mouse cells and even better in yeast (Skuzeski et al. 1991; Stahl et al. 1995; Cassan and Rousset 2001) and similarly, at least some retroviral -1 frameshift sites also act in *E. coli* (Horsfield et al. 1995; Leger at al. 2004).

At a given recoding signal for frameshifting or stop codon readthrough, different alternative or even competitive mechanism(s) may exist. In the few cases analyzed in which a stop codon was recoded into a natural amino acid, it was shown that multisuppression phenomena exist, albeit with different relative efficiencies, one of the natural 'suppressor' tRNAs being always more efficient than the other(s) (Feng et al. 1989; Fearon et al. 1994; Chittum et al. 1998; see also Fig. 6A). Likewise, during codon reading, competition probably occurs between different tRNAs able to misread a given codon. Therefore, when a tRNA usually playing a major role in recoding becomes less efficient because of a defect of its modified nucleotide content, or because of a parameter that affects its cellular concentration, then alternate natural suppressor tRNA(s) may take over recoding, thereby confusing the final interpretation of the data.

Precise identification of tRNA(s) involved in each of the recoding events identified so far is no easy task. Only in few cases, has the precise *trans*-acting

tRNA(s) responsible for a recoding event been identified. Hopefully, due to the rapid development of crystallization techniques allowing a detailed molecular view at a few angstrom resolution of the ribosome complexed with tRNA in the A-, P- and/or E-site(s) associated with a fragment of mRNA, one can dream that the 3D-structure of a ribosome stalled at a characteristic recoding signal will soon be solved. This might reveal some important clues of how a miscoding-type of recoding process versus a normal correct decoding process occur on the ribosome. A first step in this direction has been reached recently by Yusupov and collaborators, showing how an mRNA regulatory domain upstream of a coding region can regulate the progression of the ribosome along the mRNA (Yusupova et al. 2001; Noller et al. 2002; M Yusupov, personal communication).

4.3 Role of modified nucleotides in both tRNA and rRNA

As is clear from the data reviewed above, the presence or absence of a given modified base in a tRNA may either stimulate or reduce the efficiency of recoding. This apparently contradictory dual effect depends on the system considered and the position of the shifty/miscoding tRNA not only in the A- site and/or P-site but also in the E-site of the ribosome. Indeed, recent studies point to the importance of a fully modified tRNA in the E-site for -1 frameshifting (Bekaert and Rousset 2005). To have a clear view of how tRNA modification influences various recoding events, much more work must be done. Indeed, most information available on the role of a modified nucleotides in tRNA concerns “normal” decoding processes and not recoding events, which, as stated above, are two different (although related) processes. In these studies, reporter systems used in a given biological model system should be carefully designed to address precise questions. The comparison of the results obtained with different reporter systems as well as in different biological systems should be made with much caution. Discrepancies in the scientific literature probably result in part from different reporters used in different laboratories.

As a number of nucleotides are modified in each tRNA, it may well be that a defect in only one modified nucleotide in a tRNA is not sufficient to observe a measurable effect. The possibility exists that when combined with other modifications, located at other sites on the tRNA molecule, a more pronounced effect would be observed. Although most of the modifications that have been shown to affect recoding are located at or near the anticodon loop, more distant modifications within the tRNA molecule could also be important.

Thanks to the progress that has been made concerning the structure of prokaryotic and eukaryotic ribosomes, the precise localization of modified sites in rRNAs demonstrated that most of these sites are located in important functional regions of the ribosome (Decatur and Fournier 2002; Ofengand 2002). In particular, in *S. cerevisiae*, 9 modified nucleotides are found near the A-, P- or E-site in the small subunit and 16 in the large subunit of the ribosome. These are likely candidates for a potential role in decoding accuracy. Since most single rRNA modifications appear to be dispensable for ribosome function, experimental studies on the effects

of rRNA modifications are likely to be feasible. The same tools that have been used to analyze tRNA modifications can now be used to study the role of rRNA modifications in recoding and decoding accuracy. Recent results demonstrate that the lack of U2552 methylation in the 23S subunit of *rrmJ*-deficient *E. coli* strains, leads to a decrease in programmed +1 and -1 translational frameshifing and in readthrough of UAA and UGA stop codons. This suggests that the interaction between aminoacyl-tRNA and U2552 is involved in the selection of the correct tRNA at the ribosomal A (Widerak et al. 2005).

Finally, another possibility is that some effects might be dependent on the specific association of tRNA modifications with modifications of the rRNA. Associating tRNA and rRNA modification deficiencies in the same cell might reveal unexpected synergy between the role of tRNA and rRNA in decoding.

Last but not least, to date, evidence for a functional recoding process in Archaea is very limited. The only case reported so far concerns a potential programmed -1 frameshifting in α-1-fucosidase of *Sulfolobus solfataricus* (Cobucci-Ponzano et al. 2003). More systematic exploration of recoding in organisms of this third domain of Life, in particular the importance of modified nucleotides in both tRNA and rRNA (see e.g. Edmonds et al. 1991; McCloskey et al. 2001), may reveal additional parameters and/or mechanisms of recoding, as well as possibly reveal the evolutionary origin of recoding processes that are common to organisms of the three biological domains.

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Data bases

http://recode.genetics.utah.edu/
http://medstat.med.utah.edu/RNAmods
http://www.unibareuth.de/departments/biochimie/rna
http://medstat.med.utah.edu/SSUmods/
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http://mbcr.bcm.tmc.edu/smallRNA/smallrna.html