

The role of wobble uridine modifications in +1 translational frameshifting in eukaryotes

Hasan Tükenmez^{1,†}, Hao Xu^{1,†}, Anders Esberg² and Anders S. Byström^{1,*}

¹Department of Molecular Biology, Umeå University, Umeå, 901 87, Sweden and ²Department of Odontology/Cariology, Umeå University, Umeå, 901 87, Sweden

Received August 05, 2015; Accepted August 06, 2015

ABSTRACT

In *Saccharomyces cerevisiae*, 11 out of 42 tRNA species contain 5-methoxycarbonylmethyl-2-thiouridine (mcm⁵s²U), 5-methoxycarbonylmethyluridine (mcm⁵U), 5-carbamoylmethyluridine (ncm⁵U) or 5-carbamoylmethyl-2'-O-methyluridine (ncm⁵Um) nucleosides in the anticodon at the wobble position (U₃₄). Earlier we showed that mutants unable to form the side chain at position 5 (ncm⁵ or mcm⁵) or lacking sulphur at position 2 (s²) of U₃₄ result in pleiotropic phenotypes, which are all suppressed by overexpression of hypomodified tRNAs. This observation suggests that the observed phenotypes are due to inefficient reading of cognate codons or an increased frameshifting. The latter may be caused by a ternary complex (aminoacyl-tRNA^{eEF1A}*GTP) with a modification deficient tRNA inefficiently being accepted to the ribosomal A-site and thereby allowing an increased peptidyl-tRNA slippage and thus a frameshift error. In this study, we have investigated the role of wobble uridine modifications in reading frame maintenance, using either the *Renilla*/*Firefly* luciferase bicistronic reporter system or a modified Ty1 frameshifting site in a *HIS4A::lacZ* reporter system. We here show that the presence of mcm⁵ and s² side groups at wobble uridines are important for reading frame maintenance and thus the aforementioned mutant phenotypes might partly be due to frameshift errors.

INTRODUCTION

Transfer of genetic information from mRNA into proteins is the most energy consuming process in the cell and the translation machinery needs to decode mRNAs with high efficiency and fidelity (1). Even though the translational machinery transfers the information in mRNA into protein with high fidelity, errors occur at a low frequency. Missense

errors are in most cases not harmful to the function of a protein, since such errors alter only one single amino acid, which will not interfere with the function or stability of the protein if they occur in non-critical positions. In contrast, processivity errors, like frameshift errors, are detrimental, since they completely change the amino acid sequence downstream of the frameshift site. Moreover, following such an error, the ribosome frequently encounters a stop codon in the new reading frame resulting in premature termination of translation. Accordingly, the frequency of frameshift errors is about 10-fold lower than the frequency of missense errors (1,2).

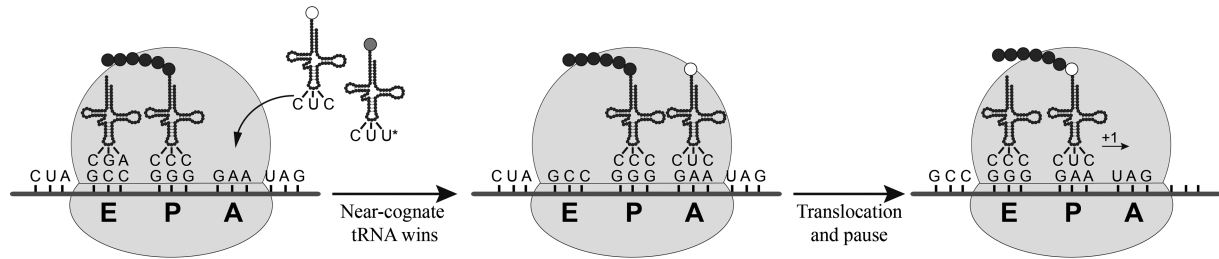
There are many examples where alterations in the tRNA structure, e.g. lack of a modified nucleoside, will affect the fidelity of reading frame maintenance (3,4). In bacteria, modified nucleosides of different chemical structures, present in different positions, and in different species of the tRNA all prevent frameshift errors (5,6). In eukaryotes, both wyosin (yW) and queosine (Q) in rabbit reticulocytes as well as other modified nucleosides present in the anticodon loop of eukaryotic tRNAs are important to maintain the reading frame (7,8). Synthesis of yW in yeast tRNA occurs in several steps and whereas fully modified yW has a low frequency of frameshifting, presence of any of the various intermediates in the synthesis of yW all increase frameshifting (9). Also, lack of either cyclic N6-threonylcarbamoyladenosine (ct⁶A) at position 37 or pseudouridine (Ψ) at position 38 and 39 in yeast tRNA increases +1 frameshifting (10–13). Relevant for this study, the modified wobble nucleoside 5-methylaminomethyl-2-thiouridine (mnm⁵s²U₃₄) present in bacterial tRNA specific for Gln, Lys and Glu, is important for proper reading frame maintenance (The wobble nucleoside is in position 34 of the tRNA and we denote such a nucleoside as N₃₄ where N is any nucleoside.) (6,14–17). Apparently, modification status both in bacteria and in eukaryotes is important for a proper reading frame maintenance (3,4).

A peptidyl-tRNA slippage model of how tRNA modification deficiency may induce frameshifting errors is well established (3,4,6,18–24). According to this model (Figure 1) modification deficient aminoacyl-tRNAs present

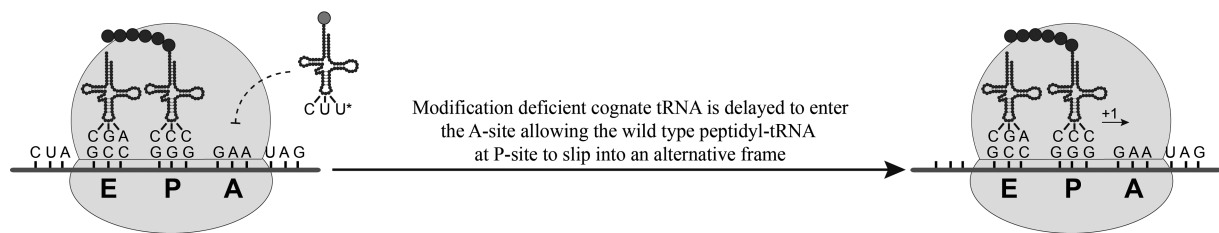
*To whom correspondence should be addressed. Tel: +46 90 7856764; Fax: +46 90 772630; Email: anders.bystrom@molbiol.umu.se

†These authors contributed equally to the paper as first authors.

A. A-site effect by modification deficient tRNA



B. A-site effect by modification deficient tRNA



C. P-site effect by modification deficient tRNA

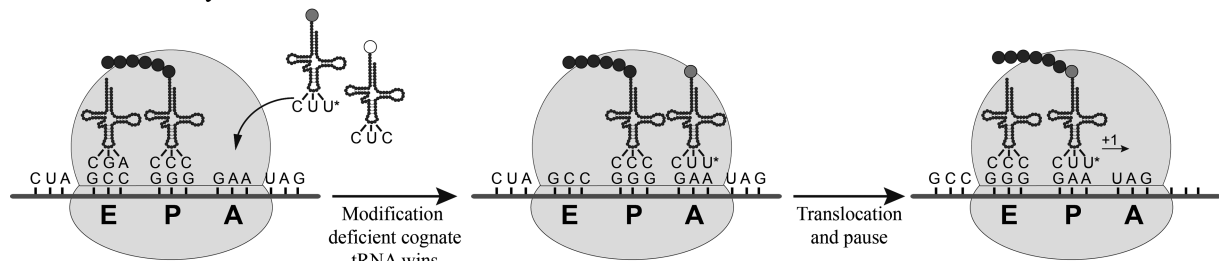


Figure 1. Dual-error frameshifting model. Modification deficient tRNAs can induce frameshifting by either an A- or a P-site effect, or a combination thereof. (A) Lack of wobble uridine modification reduces the efficiency of the ternary complex (aminoacyl-tRNA**eEF1A**GTP, here shorten as aminoacyl-tRNA) to be accepted to the A-site, allowing a near cognate aminoacyl-tRNA to be accepted in the A-site. After translocation to the P-site, the near cognate tRNA slips into an alternative reading frame, as it does not perfectly fit in the P-site. (B) Lack of wobble uridine modification reduces the efficiency of the cognate aminoacyl-tRNA to be accepted to the A-site, which induces a pause that allows the wild type peptidyl-tRNA to slip forward one nucleotide (denoted an A-site effect by modification deficiency, Figure 1B). When frameshifting is caused by a P-site effect, the hypomodified tRNA is efficiently accepted to the A-site, translocates to the P-site where its fit is not optimal why it slips into an alternative reading frame due to a reduced ribosomal grip (P-site effect by modification deficiency, Figure 1C) (3,6,20,21,23).

in a ternary complex, i.e. aminoacyl-tRNA**eEF1A**GTP (here shorten as aminoacyl-tRNA) induces frameshifts either by causing an A- or a P-site effect, or a combination thereof. Lack of modification causes a defect in the cognate aminoacyl-tRNA selection step (we denote such an error as an A-site effect by modification deficiency), allowing a ternary complex with a near cognate wild type aminoacyl-tRNA instead of a cognate aminoacyl-tRNA to be accepted in the A-site. After translocation to the P-site, the fit of the near cognate peptidyl-tRNA is not optimal why it slips one nucleotide forward (+1 frameshift) (Figure 1A). Alternatively, lack of a modified nucleoside reduces the efficiency by which a cognate aminoacyl-tRNA is accepted to the A-site, which induces a ribosomal pause allowing the wild type peptidyl-tRNA to slip forward one nucleotide (denoted an A-site effect by modification deficiency, Figure 1B). When frameshifting is caused by a P-site effect, the hypomodified tRNA is efficiently accepted to the A-site, translocates to the P-site where its fit is not optimal why it slips into an alternative reading frame due to a reduced ribosomal grip (P-site effect by modification deficiency, Figure 1C) (3,6,20,21,23).

Thus, in some cases, the modification deficiency reduces the rate of selection of the aminoacyl-tRNA (A-site effect) but also lack of the modification reduces the ribosomal grip in the P-site (P-site effect). Note, in all cases explained above, the error in reading frame maintenance is due to a peptidyl-tRNA slippage.

Modifications of uridines in the wobble position of tRNAs are frequent in all three domains of life. In *Saccharomyces cerevisiae*, there are 11 tRNA species having four related modified uridine nucleotides at wobble position (25–32). These modified nucleosides are 5-carbamoylmethyluridine (*ncm*⁵U₃₄) present in five (26,27,32), 5-carbamoylmethyl-2'-O-methyluridine (*ncm*⁵U₃₄m) present in one (25), 5-methoxycarbonylmethyluridine (*mcm*⁵U₃₄) present in two (29,30) and 5-methoxycarbonylmethyl-2-thiouridine (*mcm*⁵s²U₃₄) present in three tRNA species (Figures 2 and 3) (28,30,31).

The first step in the synthesis of the *mcm*⁵ and *ncm*⁵ groups of the uridine modifications mentioned above requires the six-subunit Elongator complex and its seven as-

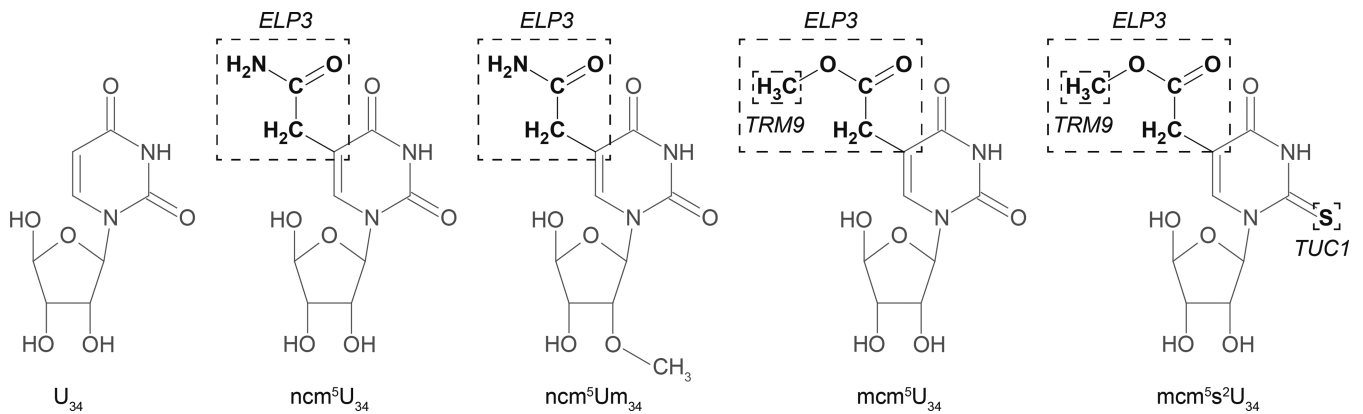


Figure 2. Chemical structures of uridine, 5-carbamoylmethyluridine (ncm^5U), 5-carbamoylmethyl-2'-O-methyluridine (ncm^5Um), 5-methoxycarbonylmethyluridine (mcm^5U) and 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2U) nucleosides. Each dotted box indicates the side group that is removed by mutating the indicated gene.

2nd 1st	U	C	A	G	3rd
U	Phe ● (10) Leu ● (7) ncm^5Um ● (10)	Ser ● (11) ● (3) ^a ncm^5U ○ ● (1) ^b	Tyr ● (8) Stop Stop	Cys ● (4) Stop Trp ● (6)	U C A G
C	Leu ● (1) ^a ● (3) U ●	Pro ● (2) ^a ● (10) ncm^5U ●	His ● (7) Gln ● (9) mcm^5s^2U ○ ● (1) ^b	Arg ● (6) ● (1) ^b	U C A G
A	Ile ● (13) ● (2) Ψ Met ● (4+5) ^c	Thr ● (11) ● (4) ncm^5U ○ ● (1) ^b	Asn ● (10) Lys ● (7) mcm^5s^2U ○ ● (14)	Ser ● (4) Arg ● (11) ncm^5U ● (1) ^a	U C A G
G	Val ● (14) ● (2) ^b ncm^5U ● (2) ^a	Ala ● (11) ● (5) ncm^5U	Asp ● (15) Glu ● (14) mcm^5s^2U ● (2) ^b	Gly ● (16) ● (3) ncm^5U ● (2) ^a	U C A G

Figure 3. The genetic code and decoding abilities of individual tRNA species. Circles and connecting lines indicate codons read by the same tRNA isoacceptor. Gray circles connected with a dashed line indicate that the tRNA species reads the codon only when it is overexpressed. The empty dashed circle for $tRNA_{IAC}^{Val}$ is shown only to indicate that this inosine containing tRNA species does not efficiently read the GUA codon. The nucleoside at the wobble position is given for the 13 wobble uridine containing tRNA species. Black and gray circles represent decoding abilities predicted by the wobble hypothesis, the revised wobble rules and the distribution of tRNA species. A gray circle indicates that the tRNA species is less likely to read the codon. The number of genes coding for a tRNA species is indicated next to the circle for the complementary codon. ^a The gene(s) encoding the tRNA is nonessential. ^b The gene(s) encoding the tRNA is essential. ^c Four genes code for $tRNA_{IAC}^{Met}$ and five for $tRNA_m^{Met}$. Copyright © American Society for Microbiology, [Molecular and Cellular Biology, 28, 2008, 3301–3312 and doi:10.1128/MCB.01542–07](26).

sociated proteins (Reviewed in Karlsborn et al. (33)). Mutations in any of the corresponding genes result in deficiency of these xm⁵-uridine modifications without affecting stability or aminoacylation of tRNA (26). These mutants also show strong pleiotropic phenotypes, such as defects in growth, transcription, chromatin remodelling, DNA repair and secretion (Reviewed in Karlsborn et al. (33)). All these phenotypes, except lack of xm⁵ side chains, are suppressed by overexpression of hypomodified tRNAs specific for Gln, Lys and Glu that in a wild type contains mcm⁵s²U₃₄ (34,35). It was concluded that lack of this wobble nucleoside reduces the efficiency to recognize the cognate codons for these tRNAs, which is compensated by an increased concentration of the modification deficient tRNA. Thus, the many different phenotypes of Elongator mutants are due to reduced efficiency in translating some key mRNAs encoding proteins important for manifesting a correct phenotype.

In bacteria, modified wobble uridines are important to prevent +1 frameshifting (6,36). In eukaryotes, only a limited study has been done, which focused on the influence of the esterified methyl group of mcm⁵U₃₄ in reading frame maintenance (37). However, no specific conclusion was made where the frameshift errors occur, since the frameshift window used was very large. Therefore, no extensive information of the role of modified wobble uridines in reading frame maintenance is available for eukaryotic tRNA. It was therefore important to investigate whether or not lack of the xm⁵U or mcm⁵s²U modifications are crucial for reading frame maintenance. Here, we show that presence of xm⁵- (x, any substitution) or s² side groups at wobble uridines in yeast is pivotal in maintaining the translational reading frame.

MATERIALS AND METHODS

Strains, media and genetic procedures

The source and genotypes of yeast strains used in this study are listed in Table 1. *E. coli* strain used was *DH5α* (Bethesda Research Laboratories). Yeast transformation (38), media and genetic procedures have been described previously (39).

Plasmid constructions

Plasmid pJD375 contains a *Renilla/Firefly* luciferase bicistronic reporter system (40). To introduce various frameshifting windows between the luciferase genes, a BamHI-XhoI fragment from plasmid pJD375 containing the *Firefly* luciferase gene was cloned into corresponding sites of YCp50, generating plasmid YCp50-*Firefly*. Two complementary oligonucleotides carrying various frameshifting windows (see Supplementary Table S1) were annealed into the BamHI and SacI sites of YCp50-*Firefly*. The newly constructed plasmids were digested with restriction enzymes (BamHI and XhoI) and fragments containing the frameshifting sites linked to the *Firefly* luciferase gene were cloned back into the corresponding sites of pJD375 restoring the bicistronic reporter system with the frameshifting window.

Plasmids pMB38-9mer (FF and WT) contain a *HIS4A::lacZ* reporter cassette. In pMB38-9merFF (in-frame control construct), the *lacZ* gene is in 0 frame, while

in pMB38-9merWT (test construct), the *lacZ* gene is in +1 frame (Figure 4B) (41). These plasmids were used as templates for PCR oligonucleotide directed mutagenesis to alter the Ty1 sequence (CTT-AGG-C) (Figure 4B and Supplementary Table S2).

For the overexpression of the Lys-tRNA encoded by the *tK(UUU)L* gene, we first introduced SphI and NheI restriction sites to plasmids pMB38-9mer (FF and WT) carrying the 'CUU-AAA-C' sequence by PCR oligonucleotide directed mutagenesis. Oligonucleotides used were 5'-GGTGTCTCGGGGCGCATGCATGACCCAGTCAC-3' and 5'-AGAGTGCACCATATGCGGTGTGAGCTAGCGCACAGATGCG-3'. The *tK(UUU)L* gene was amplified from strain UMY2067 by using oligonucleotides 5' AAAAGCATGCCGGTAGAGTCTTCTTGGTC-3' and 5' AAAAGCTAGCCGGTAAGAGAGAAACCTCCA-3' and cloned between SphI and NheI sites of these plasmids.

Dual-luciferase assays

Three individual transformants of each dual luciferase assay construct (biological replicates) were grown at 30°C in synthetic complete (SC)-Ura medium to an optical density at 600 nm (OD₆₀₀) of 0.5. For each transformant triplicate samples (technical replicates) of 10 μl cells were collected and kept at -80°C. The luciferase assays were performed according to the instructions of Dual-Luciferase Reporter Assay System (Promega, Catalog No. E1960). The luciferase activities were determined in a white 96-well plate (Thermo Scientific, #436111) using a TECAN infinite 200 luminometer. The levels of +1 frameshifting (%) were determined by normalization of each biological test replicate with the average of the three biological replicates of the in-frame control. Each value of the biological replicates was determined by taking the median of the three technical replicates. The significant differences between wild type and mutant were determined by two-tail *t*-test.

β-galactosidase assays

Three transformants of each Ty1 assay construct (biological replicates) were grown in SC-Ura to OD₆₀₀ ≈ 0.5 and 20 OD₆₀₀-units were collected and kept at -20°C. For each transformant, β-galactosidase measurements were done three times (technical replicates). β-galactosidase activities were determined as described previously (39). Values of the biological replicates were determined by taking the median of the technical replicates. The levels of +1 frameshifting (%) were determined by normalization of each biological test replicate with the average of the three biological replicates of the in-frame control. The significant differences between wild type and mutant were determined by two-tail *t*-test.

RESULTS AND DISCUSSION

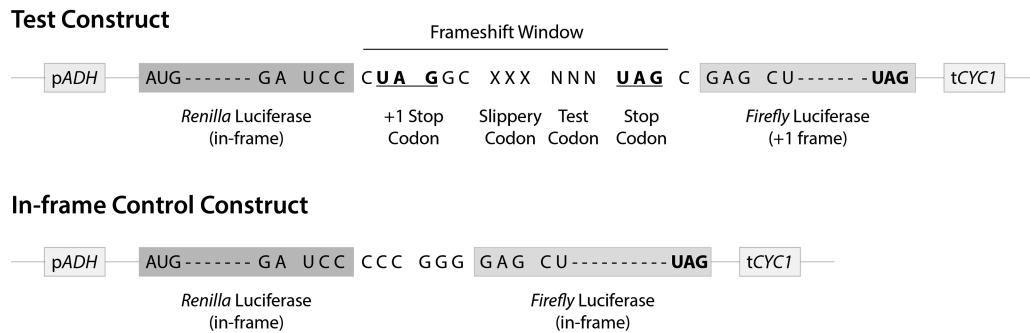
Assay system

To analyze the role of wobble uridine modifications ncm⁵U, ncm⁵Um, mcm⁵U or mcm⁵s²U in reading frame maintenance, we used defined yeast mutants unable to form the s²

Table 1. Yeast strains used in this study

Strains	Genotype	Source
W303-1A	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15</i>	(52)
UMY3269	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 elp3::KanMX4</i>	(30)
UMY3164	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 tuc1::TRP1</i>	(48)
UMY3267	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 trm9::KanMX4</i>	(53)

A. Dual-Luciferase Assay System



B. Ty1 Assay System

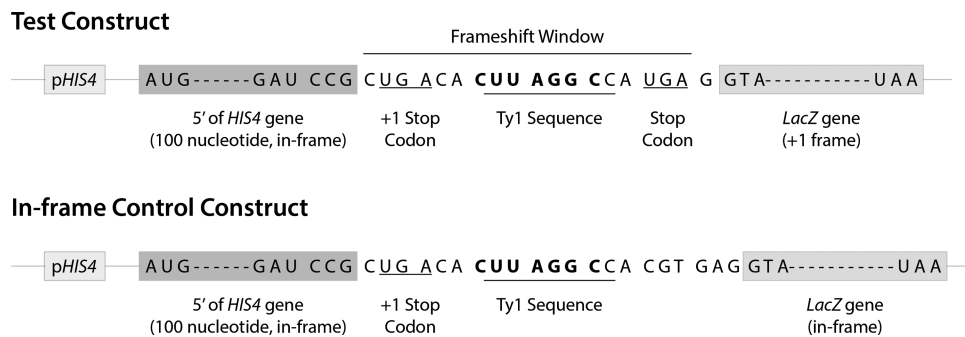


Figure 4. (A) Schematic drawing of the dual-luciferase assay system. Transcription of the genes encoding the *Renilla*- and *Firefly*-luciferase is under the *ADH1* promoter and terminated by *CYC1* terminator. Frameshift sites were cloned between the luciferase genes and expression of the *Firefly* luciferase gene requires +1 frameshifting. The frameshifting site is as follows: XXX-slippy site, NNN-assay codon and UAG-stop codon (all in-frame). An upstream stop codon (UAG) was placed in the +1 frame to eliminate frameshifting events occurring before the assay site. The frame of the different luciferase genes is indicated. In the in-frame control construct, *Renilla*- and *Firefly*-luciferase genes are in-frame. (B) Schematic drawing of the Ty1 assay system. Transcription from *HIS4* promoter generates a transcript containing the first 100 nucleotides of the *HIS4* gene in the in-frame and the *lacZ* gene of *Escherichia coli* in the +1 frame. Expression of the *lacZ* gene is dependent on a +1 ribosomal frameshift event taking place within Ty1 sequence. An upstream stop codon (UGA) was placed in the +1 reading frame to eliminate frameshifting events occurring before the assay site. In the in-frame control construct, the first 100 nucleotides of the *HIS4* gene and *lacZ* gene are in-frame.

group (*tuc1*Δ; also denoted as *ncs6*Δ), the *ncm*⁵ or *mcm*⁵ groups (*elp3*Δ) or the esterified methyl group (*trm9*Δ) of the *mcm*⁵ side chain.

The ribosomal +1 frameshifting assay system used contains a *Renilla* luciferase (*R-luc*)/ *Firefly* luciferase (*F-luc*) bicistronic reporter system (Figure 4A) (see Material and Methods) (40). This bicistronic mRNA synthesizes a two domain protein with the indicated enzymatic activities. To analyze a +1 frameshift event a sequence is introduced between these two cistrons in such a way that translation of *R-luc* is in the 0 frame and the *F-luc* is in the +1 frame (Figure 4A). To obtain *F-luc* activity the ribosome must shift into the +1 frame before entering the *F-luc* gene. The inserted sequence between the *R-luc* and the *F-luc* reporter genes consists of a slippy codon (XXX) at which the peptidyl-

tRNA will slip, the codon to be assayed for A-site selection (NNN), followed by a stop codon in zero frame (UAG) (Figure 4A). To terminate all ribosomes that have accidentally slipped into the +1 frame upstream the slippy codon, a stop codon was inserted in the +1 frame just a few nucleotides upstream the slippy codon (See Figure 4). Thus, to obtain *F-luc* activity a +1 frameshift must occur at the +1 frameshift sequence upstream of the stop codon in the zero frame. This construct results in a very short frameshifting window between the upstream stop codon in the +1 frame and the downstream in-frame stop codon. The slippy codon is determined individually for different assay sites in order to optimize the slippage of the peptidyl tRNA at the P-site. We chose UUU, CCC or GGG codons as the slippy codons (Supplementary Figure S1 and Table

2 and Supplementary Table S1). Codon UUU is decoded by tRNA^{Phe}_{GAA}, which has the wobble nucleoside Gm₃₄ (42), and its structure is not affected by the *elp3*, *tuc1* or *trm9* mutations. Codon CCC is read by the I₃₄ (inosine) containing tRNA^{Pro}_{IGG} and the mcm⁵U₃₄ containing tRNA^{Pro}_{mcm⁵UGG} and the slippery codon GGG is read by the mcm⁵U₃₄ containing tRNA^{Gly}_{mcm⁵UCC} and the C₃₄ containing tRNA^{Gly}_{CCC} (Figure 3) (26,30,43). Note that the structures of the mcm⁵U containing tRNA^{Pro}_{mcm⁵UGG} reading the slippery codon CCC and the mcm⁵U₃₄ containing tRNA^{Gly}_{mcm⁵UCC} reading the slippery codon GGG are affected by the *elp3* mutation and might therefore obscure the monitoring of an A-site effect at these test codons. These issues will be addressed below. As a control, we used a construct carrying the *R-luc* and *F-luc* genes in-frame. By dividing the ratio of *F-luc*/*R-luc* activities generated from the frameshifting construct with the ratio of activities from the *F-luc*/*R-luc* in-frame control, the level of frameshifting was revealed. Using these reporter systems, the level of frameshifting for specific tRNA isoacceptors was investigated in the presence or absence of s², mcm⁵, mcm⁵s² groups or the esterified methyl group of the mcm⁵ side chain at U₃₄.

In the bacterial system, modification deficiency of aminoacyl-tRNA in the ternary complex causes in most cases a slow entry of it to the A-site and thereby induces a peptidyl-tRNA slippage (Figure 1A and B) (6). Therefore, we suspected that in the cases below where we observed an effect on the frequency of frameshifting in the modification deficient mutants, it would primarily be due to an A-site effect, i.e. slow entry of the ternary complex containing aminoacyl-tRNA cognate to the test codon allowing a peptidyl-tRNA interacting with the slippery codon XXX to slip (Figure 1A and B). In the constructs used, all have a UAG stop codon just after the test codon NNN (i.e. the sequence is in zero frame -XXX-NNN-UAG). Translational termination in yeast is controlled by two interacting protein chain release factors, eRF1 and eRF3. Whereas eRF1 recognizes all three stop codons, binds to ribosomal A-site, and promotes hydrolysis of the P-site located peptidyl-tRNA, eRF3 stimulates the termination activity of eRF1 (Reviewed in Kisselev and Buckingham (44)). A poor eRF1 binding to the UAG in the A-site may induce slippage by the modification deficient peptidyl-tRNA from cognate NNN codon to NN-U codon. Therefore, the +1 frameshifting observed using the luciferase assay may be caused by either an A-site or a P-site effect or both. Note that, in all these cases the error occurs in the P-site (either by the tRNA reading the slippery codon or a tRNA cognate to the test codon). Although the luciferase system used by us is unable to distinguish between an A- or a P-site effect caused by modification deficiency, it is still a valuable method to address whether or not modification is important for maintaining the reading frame. To address specifically if modification deficiency induces an A- or a P-site effect, we used the Ty1 system, which is explained below.

Role of xm⁵U or mcm⁵s²U nucleosides in reading frame maintenance

In yeast, there are 11 tRNA species having mcm⁵s²U₃₄, mcm⁵U₃₄, ncm⁵U₃₄ or ncm⁵U₃₄m nucleosides at wobble position (Figures 2 and 3). The role of these modified uridines was analyzed for ribosomal +1 frameshifting using the *Renilla*/*Firefly* luciferase bicistronic reporter system described in the previous section.

In an *elp3* mutant these tRNA species are missing the mcm⁵ and mcm⁵s² groups at wobble position (U₃₄) (26,45). The role of the ncm⁵ and mcm⁵ groups present in these tRNAs in reading frame maintenance was investigated in a wild type and in an *elp3* mutant strain using cognate or near cognate codons as test codons. Lack of the mcm⁵ side chain in tRNA^{Arg}_{mcm⁵UCU}, tRNA^{Gly}_{mcm⁵UCC}, tRNA^{Lys}_{mcm⁵s²UUU} and tRNA^{Glu}_{mcm⁵s²UUC} resulted in significantly higher levels of +1 frameshifting with either A-ending cognate or G-ending near cognate codons (Table 2 and Supplementary Figure S1). However, absence of the mcm⁵ group in tRNA^{Gln}_{mcm⁵s²UUG} did not have any significant effect on reading frame maintenance for the Gln codons CAA or CAG (Table 2). Lack of the ncm⁵ group of U₃₄ in tRNA^{Val}_{ncm⁵UAC} and tRNA^{Ser}_{ncm⁵UGA} resulted in an increased level of +1 frameshifting with near cognate Val codon GUG or cognate Ser codon UCA. In contrast, absence of the ncm⁵ group of U₃₄ in tRNA^{Pro}_{ncm⁵UGG} resulted in a decreased level of +1 frameshifting with near cognate Pro codon CCG. Lack of the ncm⁵ group of U₃₄ of the remaining tRNAs did not cause a significant difference in levels of +1 frameshifting (Table 2 and Supplementary Figure S1). We conclude that in the xm⁵U and mcm⁵s²U tRNA isoacceptors, the mcm⁵ group plays a more vital role than the ncm⁵ group in reading frame maintenance.

Codon CCC that can be read by ncm⁵U₃₄ containing tRNA^{Pro}_{ncm⁵UGG} is used as a slippery codon upstream next to Gln-, Lys-, Arg-, Gly- and Thr- test codons (Table 2 and Supplementary Figure S1). Thus, the ncm⁵U₃₄ present in the potential peptidyl-Pro-tRNA might influence the ribosomal grip in the P-site and thus influence the slippage. To test directly the influence of the ncm⁵ group in Pro-tRNA in peptidyl-Pro-tRNA slippage, we used a construct -UUU-CCC-UAG- in the luciferase system. The stop codon UAG is in the zero frame just after the Pro codon CCC. Since eukaryotic release factor 1 (eRF1) acts in the A-site (Reviewed in Kisselev and Buckingham (44)) a possible +1 frameshift by Pro-tRNA lacking ncm⁵U would occur in the P-site. However, no significant +1 frameshifting was observed when the CCC codon was just upstream the stop codon and thus in the P-site (Table 2 and Supplementary Figure S1). We conclude that the ncm⁵ group in Pro-tRNA does not increase peptidyl-tRNA slippage at the slippery codon CCC.

The slippery codon GGG is decoded by both C₃₄ containing tRNA^{Gly}_{CCC} and mcm⁵U₃₄ containing tRNA^{Gly}_{mcm⁵UCC}. The structure of the latter tRNA is affected by the *elp3* mutation and this tRNA reads the GGG codon very inefficiently compared to the cognate tRNA^{Gly}_{CCC} (26). Therefore, in the *elp3* mutant it is not likely that tRNA^{Gly}_{mcm⁵UCC} lack-

Table 2. Influence of tRNA modifications $mcm^5s^2U_{34}$, mcm^5U_{34} , ncm^5U_{34} and ncm^5U_{34m} on reading frame maintenance based on data using the *Renilla/Firefly* luciferase bicistronic reporter system

tRNA Assayed	Slippery Codon	Test Codon	tRNA modifications at position 34				Frameshift (fold to WT) ^a						
			WT	<i>elp3Δ</i>	<i>trm9Δ</i>	<i>tuc1Δ</i>	<i>elp3Δ</i>	<i>trm9Δ</i>	<i>tuc1Δ</i>				
tRNA ^{Gln} _{<i>mcm</i>⁵<i>s</i>²UUG}	CCC	CAA	<i>mcm</i> ⁵ <i>s</i> ² U	<i>s</i> ² U ^c	<i>ncm</i> ⁵ <i>s</i> ² U and <i>cm</i> ⁵ <i>s</i> ² U ^d	<i>mcm</i> ⁵ U	1.2	0.8*	0.9				
	CCC	CAG					0.9	0.9	0.8*				
tRNA ^{Lys} _{<i>mcm</i>⁵<i>s</i>²UUU}	CCC	AAA					2.2**	1.2	1.8*				
	CCC	AAG					2.3**	1.2	1.7**				
tRNA ^{Glu} _{<i>mcm</i>⁵<i>s</i>²UUC}	GGG	GAA					3.0**	1.6	1.7*				
	GGG	GAG					1.8**	1.4	1.5*				
tRNA ^{Arg} _{<i>mcm</i>⁵UCU}	CCC	AGA					<i>mcm</i> ⁵ U	U	<i>ncm</i> ⁵ U and <i>cm</i> ⁵ U ^d	<i>mcm</i> ⁵ U	2.0**	1.0	n.a.
	CCC	AGG									2.3**	1.1	
tRNA ^{Gly} _{<i>mcm</i>⁵UCC}	CCC	GGA									2.9**	1.3	
	CCC	GGG									1.8*	1.1	
tRNA ^{Val} _{<i>ncm</i>⁵UAC}	GGG	GUA	<i>ncm</i> ⁵ U	U	<i>ncm</i> ⁵ U	<i>ncm</i> ⁵ U	1.6	n.a.	n.a.				
	GGG	GUG					1.5*						
tRNA ^{Ser} _{<i>ncm</i>⁵UGA}	UUU	UCA					1.8*						
	UUU	UCG					1.3						
tRNA ^{Ala} _{<i>ncm</i>⁵UGC}	GGG	GCA					0.9						
	GGG	GCG					1.5						
tRNA ^{Leu} _{<i>ncm</i>⁵UAA}	UUU	UUA					1.2						
	UUU	CCA					0.9						
tRNA ^{Pro} _{<i>ncm</i>⁵UGG}	UUU	CCC					0.9						
	UUU	CCG					0.7**						
	UUU	CCU					1.0						
	UUU	CCU					1.0						
tRNA ^{Thr} _{<i>ncm</i>⁵UGU}	CCC	ACA					1.1						
	UUU	ACA					1.0						
	CCC	ACG					0.9						
	UUU	ACG					0.9						
tRNA ^{Tyr} _{<i>GΨA</i>} ^b	UUU	UAC	G	G	G	G	1.0	0.8	0.7				

^aBold indicate significant difference in frameshifting levels between indicated mutant and wild type as determined by two-tail *t*-test. (*) indicates $P < 0.05$ and (**) indicates $P < 0.01$.

^btRNA^{Tyr}_{*GΨA*} has an unmodified G nucleoside at wobble position and its structure is not affected by the *elp3*, *trm9* or *tuc1* mutations, thus it is used as a negative control (54).

^cIn an *elp3* mutant, levels of *s*² group is reduced (55–57).

^dIn a *trm9* mutant, tRNA^{Arg}_{*mcm*⁵UCU} and tRNA^{Glu}_{*mcm*⁵*s*²UUC} contain a mixture of *ncm*⁵U/*cm*⁵U and *ncm*⁵*s*²U/*cm*⁵*s*²U nucleosides, respectively (53). n.a., not applicable.

ing the *mcm*⁵ side chain at U₃₄ will out-compete the efficiently decoding cognate tRNA^{Gly}_{CCC} at GGG codons. Consequently, the observed +1 frameshifting for tRNA^{Glu}_{*mcm*⁵*s*²UUC} and tRNA^{Val}_{*ncm*⁵UAC} (Table 2) is most likely not caused by slippage of an unmodified peptidyl-tRNA^{Gly}_{*mcm*⁵UCC} but rather a poor A-site entry by tRNA^{Glu}_{*mcm*⁵*s*²UUC} and tRNA^{Val}_{*ncm*⁵UAC}, respectively.

The formation of the esterified methyl group of *mcm*⁵U, which is the last step in the synthesis of the *mcm*⁵ side chain,

is catalyzed by the dimeric Trm9/Trm112 protein complex (46,47).

The influence of the esterified methyl group of the *mcm*⁵U₃₄ and *mcm*⁵*s*²U₃₄ nucleoside in reading frame maintenance was investigated in a wild type and in a *trm9* mutant strain using cognate or near cognate codons as test codons (Table 2). Lack of the esterified methyl group of the *mcm*⁵ side chain of U₃₄ in tRNA^{Gln}_{*mcm*⁵*s*²UUG} resulted in significantly decreased +1 frameshifting at the Gln codon CAA (Table 2 and Supplementary Figure S1). There were

no significant differences in the levels of frameshifting between wild type and *trm9* mutant in the remaining test constructs (Table 2 and Supplementary Figure S1). We conclude that presence or absence of the esterified methyl group in the mcm^5 side chain only seem to play a minor role in +1 frameshifting.

In a *tuc1* mutant, the s^2 group of mcm^5s^2U at the wobble position (U_{34}) is absent in $tRNA_{mcm^5s^2UUG}^{Gln}$, $tRNA_{mcm^5s^2UUU}^{Lys}$ and $tRNA_{mcm^5s^2UUC}^{Glu}$ (48). The role of the s^2 group present in these tRNAs in reading frame maintenance was investigated in a wild type and a *tuc1* mutant strain using cognate or near cognate codons as test codons (Table 2). Absence of the s^2 group in $tRNA_{mcm^5s^2UUU}^{Lys}$ and $tRNA_{mcm^5s^2UUC}^{Glu}$ resulted in significantly higher levels of +1 frameshifting with either A-ending cognate or G-ending near cognate codons (Table 2 and Supplementary Figure S1). However, lack of the s^2 group in $tRNA_{mcm^5s^2UUG}^{Gln}$ resulted in significantly decreased +1 frameshifting with the near cognate CAG (Table 2 and Supplementary Figure S1).

In the cases stated above, Gln- and Pro-tRNAs showed reduced levels of +1 frameshifting due to lack of esterified methyl, s^2 or ncm^5 groups (Table 2). This reduced level of frameshifting might be surprising but similar observations were noted earlier. In bacteria the Gln-, Lys- and Glu-tRNA contain as wobble nucleoside the mnm^5s^2U , which is structurally related to the mcm^5s^2U present in the corresponding yeast tRNAs. Lack of either the mnm^5 side chain or the sulphur at position 2 reduced frameshifting similarly as noted by us for the two aforementioned cases (15,16). Although these results seems counterintuitively strange, one has to remember that the structure of the different tRNA species is optimized and in fact has evolved to have similar decoding activity, which is obtained partly due to modification of it (49). Therefore, a modification may improve the activity of one tRNA whereas it might reduce the activity of another tRNA species (See discussion of this issue in Björk and Hagervall (4)). From such considerations, one would expect that when measuring a specific activity of a tRNA, like influencing reading frame maintenance, a modification might improve or reduce the fidelity of it.

The frameshifting error occurs by peptidyl-tRNA slippage

A key feature of the peptidyl-tRNA slippage model is that the error in reading frame maintenance, induced either by an A- or a P-site effect due to modification deficient tRNA, occurs in the P-site by peptidyl-tRNA slippage. There are two ways to establish if the frameshift errors occur in the ribosomal A- or P-site. Either one determines the amino acid sequence of the frameshift peptide covering the frameshift window or by overexpressing the tRNA cognate to the A-site codon. In the latter case, if the frameshift error occurs due to an A-site effect, such overexpression would decrease the frameshift error, since it reduces the ribosomal pause and thereby reduces the ability of the peptidyl-tRNA to slip forward. We chose the latter method, since this approach is relevant for this study, as such a treatment also suppresses all the pleiotropic phenotypes induced by a mutation in, e.g. the *ELP3* gene. Thus, the strong pleiotropic phenotypes ob-

served in an *elp3* mutant might be due, at least partly, to errors in reading frame maintenance of some key mRNAs.

As stated in the description of the assay system, the dual-luciferase assay system is not designed to clarify the difference between an A- or a P-site effect caused by modification deficiency, we decided to use Ty1 assay system to address this question. The expression of the *TYB* gene of yeast Ty retrotransposon requires a ribosomal +1 frameshift event caused by a peptidyl-tRNA slippage (41). Only a seven nucleotide sequence CUU-AGG-C is required for the +1 frameshift event to occur and thus only two tRNA species— $tRNA_{UAG}^{Leu}$ and $tRNA_{CCU}^{Arg}$ —are participating in this event. In the yeast strain used, the availability of $tRNA_{CCU}^{Arg}$ is low resulting in a low rate of ribosomal A-site selection, which induces a slippage by $tRNA_{UAG}^{Leu}$ at the CUU P-site codon into the +1 frame (UU-A) (41). Therefore, we decided to use an altered version of the Ty1 +1 frameshift system to study whether or not the +1 frameshifting caused by lack of the mcm^5 side chain in the *R-luc-F-luc* system, is due to a peptidyl-tRNA slippage. We altered the 'CUU-AGG-C' +1 frameshift site by changing the Arg codon (AGG) into either a Lys codon AAA decoded by $tRNA_{mcm^5s^2UUU}^{Lys}$ or an Arg codon AGA decoded by $tRNA_{mcm^5UCU}^{Arg}$ to test whether or not lack of mcm^5 side chain of these tRNAs induce +1 frameshifting (Table 3). If the hypomodified tRNA is inefficiently accepted to the A-site in an *elp3* mutant, the AAA (Lys) and/or AGA (Arg) test codons will act similarly as codons decoded by the low available $tRNA_{CCU}^{Arg}$ resulting in a slow entry to the A-site by the ternary complex containing the unmodified tRNA. If so, the $tRNA_{UAG}^{Leu}$ in the P-site will slip into the +1 frame (from cognate CUU to non-cognate UU-A). All alterations of the Ty1 sequence were made in the *HIS4A::lacZ* frameshift reporter plasmid (see Materials and Methods) (Figure 4B). The levels of frameshifting were calculated by dividing the β -galactosidase values generated from the test construct with the values from the in-frame control construct. Table 3 shows that for the 'CUU-AAA-C' Lys codon test construct, lack of mcm^5 side group in the mcm^5s^2U nucleoside of $tRNA_{mcm^5s^2UUU}^{Lys}$ resulted in 10-fold increased +1 frameshifting in the *elp3* mutant compared to wild type. In contrast, for the 'CUU-AGA-C' Arg codon test construct, lack of mcm^5 side group in $tRNA_{mcm^5UCU}^{Arg}$ did not increase frameshifting in the *elp3* mutant compared to the wild type (Table 3). Thus, similar to the results obtained by the luciferase system lack of the mcm^5 group of $tRNA_{mcm^5s^2UUU}^{Lys}$ induced increased +1 frameshifting. Although we observed an increased frameshifting for mcm^5 deficient $tRNA_{mcm^5UCU}^{Arg}$ in the luciferase assay system (Table 2), this was not the case using the Ty1 assay system (Table 3).

To analyze whether lack of the mcm^5 group of $tRNA_{mcm^5s^2UUU}^{Lys}$ could induce +1 frameshifting due to a P-site effect by modification deficiency, we placed a Lys codon AAA instead of the CUU codon in the Ty1 assay system and varied the following codon. The concentration of a tRNA species is proportional to the number of the corre-

Table 3. Influence of *mcm*⁵ side chain on reading frame maintenance based on data using the *HIS4A::TyI::lacZ* reporter system

Ty1 Frameshift Site	tRNA decoding the codon and Its Gene Copy Number		Normalized Frameshift Rates (%)		Frameshift Ratios <i>elp3</i> Δ/WT
	at the P-site	at the A-site	WT	<i>elp3</i> Δ	
CUU-AGA-C	tRNA ^{Leu} _{UAG} , (3 ^a)	tRNA ^{Arg} _{<i>mcm</i>⁵UCU} , (11 ^a)	0.142 ± 0.066	0.114 ± 0.020	0.80 ^d
CUU-AAA-C	tRNA ^{Leu} _{UAG} , (3 ^a)	tRNA ^{Lys} _{<i>mcm</i>⁵s²UUU} , (7 ^a)	0.191 ± 0.092	1.907 ± 0.787	9.99^c
CUU-AAA-C	tRNA ^{Leu} _{UAG} , (3 ^a)	tRNA ^{Lys} _{<i>mcm</i>⁵s²UUU} , (7 ^a + 4 ^b)	0.076 ± 0.013	0.239 ± 0.020	3.14^c
AAA-AGG-C	tRNA ^{Lys} _{<i>mcm</i>⁵s²UUU} , (7 ^a)	tRNA ^{Arg} _{CCU} , (1 ^a)	1.203 ± 0.558	0.892 ± 0.225	0.74 ^d
AAA-CGT-C	tRNA ^{Lys} _{<i>mcm</i>⁵s²UUU} , (7 ^a)	tRNA ^{Arg} _{ACG} , (6 ^a)	0.053 ± 0.021	0.039 ± 0.010	0.74 ^d
AAA-ATT-C	tRNA ^{Lys} _{<i>mcm</i>⁵s²UUU} , (7 ^a)	tRNA ^{Ile} _{AAU} , (13 ^a)	0.023 ± 0.008	0.025 ± 0.009	1.07 ^d

^aGenomic copy numbers of the tRNA genes.

^bCopy number of pMB38-9merWT/9merFF plasmid carrying the *tK(UUU)L* gene encoding tRNA^{Lys}_{*mcm*⁵s²UUU} (41,58).

^cDifference in frameshifting between *elp3* mutant and wild type was significant as determined by two-tail *t*-test ($P < 0.02$).

^dDifference in frameshifting between *elp3* mutant and wild type was not significant as determined by two-tail *t*-test ($P > 0.05$).

sponding tRNA genes in the yeast genome (50). Accordingly, by placing different codons in the A-site, the concentration of the corresponding tRNAs in the cell reading this codon is changed and thereby the efficiency of reading the A-site codon is altered. Thus, to test for a possible P-site effect induced by a lack of the *mcm*⁵ group of *mcm*⁵s²U in Lys-tRNA we placed an Arg codon AGG read by the rare cognate tRNA^{Arg}_{CCU} (1 genomic copy) and the near cognate tRNA^{Arg}_{*mcm*⁵UCU} (11 genomic copies) after the Lys codon AAA. In an *elp3* mutant tRNA^{Arg}_{CCU} is essential, demonstrating that *mcm*⁵ group of the near cognate Arg-tRNA is required for efficient reading of the Arg codon AGG (26). Therefore, in an *elp3* mutant, a situation is generated where the AGG codon is read slowly since it is read mainly by the rare cognate tRNA^{Arg}_{CCU} and inefficiently by the more abundant modification deficient near cognate tRNA^{Arg}_{*mcm*⁵UCU}. Such a condition would allow tRNA^{Lys}_{*mcm*⁵s²UUU} at the P-site to slip to the +1 translational frame. Furthermore, we made test constructs to increase the rate of A-site selection by introducing either an Ile codon AUU decoded by cognate tRNA^{Ile}_{AAU} present in 13 genomic copies or an Arg codon CGU decoded by cognate tRNA^{Arg}_{ACG} present in 6 genomic copies after Lys codon AAA (Table 3). By varying concentration of the potential A-site coding tRNAs from 1 genomic copy to 13 genomic copies, we did not observe any significant difference in the levels of +1 frameshifting between wild type and *elp3* mutant (Table 3). Apparently, the possible peptidyl-tRNA^{Lys}_{*mcm*⁵s²UUU} slippage is not sensitive to the rate of A-site selection suggesting that lack of *mcm*⁵s²U does not cause any P-site effect and thus an increased peptidyl-tRNA slippage.

If the frameshifting event occurring at the modified Ty1 site 'CUU-AAA-C' was caused by a slow entry of the ternary complex containing the hypomodified tRNA^{Lys}_{*mcm*⁵s²UUU} causing a peptidyl-tRNA^{Leu}_{UAG} slippage to +1 translational frame, an elevated level of the hypomod-

ified tRNA^{Lys}_{*mcm*⁵s²UUU} should increase the rate of A-site selection and thereby reducing +1 frameshifting (Figure 1B). We therefore cloned the *tK(UUU)L* gene, which encodes tRNA^{Lys}_{*mcm*⁵s²UUU} into either plasmid pMB38-9merWT (test construct, containing the CUU-AAA-C frameshift site) and pMB38-9merFF (corresponding in-frame control construct, Figure 4 and Supplementary Table S2). Thus, the plasmids harbor both the tRNA gene and the β-galactosidase gene with either a frameshift site or an in-frame control. The plasmid encoded *tK(UUU)L* gene results in overexpression of tRNA^{Lys}_{*mcm*⁵s²UUU} and concomitantly reduced the levels of +1 frameshifting in the *elp3* mutant from 10- to 3-fold compared to wild type (Table 3). This data strongly suggest that the +1 frameshifting event at 'CUU-AAA-C' Lys codon test construct occurs by peptidyl-tRNA^{Leu}_{UAG} slippage due to an A-site effect caused by a slow entry of the hypomodified tRNA^{Lys}_{*mcm*⁵s²UUU}. As was suggested earlier by us (34,48) and confirmed by Rezgui et al. (51), the major function of the *mcm*⁵s²U₃₄ nucleoside in Lys-tRNA is to improve the reading of the cognate codon. Thus, *mcm*⁵s²U₃₄ deficiency results in slow decoding and reduced translation elongation rate but also, as shown here, induces +1 frameshifting by reducing the rate of A-site selection.

CONCLUSION

Among the tRNA isoacceptors having xm⁵U₃₄ or xm⁵s²U₃₄ wobble uridine nucleosides, only Lys- and Gln-tRNAs has been investigated for +1 frameshifting in both bacteria and yeast. The modified wobble nucleoside 5-methoxycarbonylmethyl-2-thiouridine (*mcm*⁵s²U₃₄) present in yeast tRNAs specific for Gln, Lys and Glu has a chemically related form, 5-methylaminomethyl-2-thiouridine (*mnm*⁵s²U₃₄) present in the corresponding bacterial tRNAs. In bacteria, lack of the *mnm*⁵ group in Gln-tRNA results in increased +1 frameshifting at both cognate (CAA) and near cognate (CAG) codons, whereas

absence of the s^2 group results in +1 frameshifting only at the cognate (CAA) codon (6). In contrast, lack of mcm^5 or s^2 groups in yeast Gln-tRNA does not result in increased +1 frameshifting at either CAA or CAG codons. Instead, absence of the s^2 group results in reduced +1 frameshifting at the CAG codon. In bacteria, lack of mnm^5 or s^2 groups in Lys-tRNA cause increased +1 frameshifting at both cognate (AAA) and near cognate (AAG) codons by A- and P-site effects (6). In yeast, we also observed an increased +1 frameshifting due to lack of mcm^5 or s^2 groups of Lys-tRNA at AAA and AAG codons. However, we show that +1 frameshifting at the cognate (AAA) codon is induced by an A-site effect, not a P-site effect.

It has been shown that presence of modified nucleosides in tRNAs are required for tuning the decoding activity in order to maintain uniformity in translation (49). An *in vitro* study in yeast showed that presence of the mcm^5 and s^2 groups of Lys-tRNA are required for efficient A-site binding (51). Consistent with these observations, our *in vivo* studies show that presence of the mcm^5 group of Lys-tRNA promotes its entry to ribosomal A-site and thereby avoids +1 frameshift errors. Thus, wobble uridine modifications are required to optimize the function of tRNAs and thereby promote a proper reading frame maintenance.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENT

We acknowledge Prof. G. R. Björk, Dr. M. Johansson and Dr. S. Tuck for valuable comments on the manuscript.

FUNDING

Swedish Cancer Foundation [13 0301]; Swedish Research Council [621-2012-3576]; Karin and Harald Silvanders Foundation [223-2808-12 to A.S.B.]; Carl Tryggers Foundation [to G.R.B.]. Fund for open access charge: Swedish Cancer Foundation [13 0301]; Swedish Research Council [621-2012-3576]; Karin and Harald Silvanders Foundation [223-2808-12 to A.S.B.]; Carl Tryggers Foundation [to G.R.B.]. *Conflict of interest statement.* None declared.

REFERENCES

- Kurland, C.G. (1992) Translational accuracy and the fitness of bacteria. *Annu. Rev. Genet.*, **26**, 29–50.
- Parker, J. (1989) Errors and alternatives in reading the universal genetic code. *Microbiol. Rev.*, **53**, 273–298.
- Atkins, J.F. and Björk, G.R. (2009) A gripping tale of ribosomal frameshifting: extragenic suppressors of frameshift mutations spotlight P-site realignment. *Microbiol. Mol. Biol. Rev.: MMBR*, **73**, 178–210.
- Björk, G.R. and Hagervall, T.G. (2014) Transfer RNA Modification: Presence, Synthesis, and Function. *EcoSal Plus*, doi:10.1128/ecosalplus.ESP-0007-2013.
- Björk, G.R., Wikström, P.M. and Byström, A.S. (1989) Prevention of translational frameshifting by the modified nucleoside 1-methylguanosine. *Science*, **244**, 986–989.
- Urbonavičius, J., Qian, Q., Durand, J.M., Hagervall, T.G. and Björk, G.R. (2001) Improvement of reading frame maintenance is a common function for several tRNA modifications. *EMBO J.*, **20**, 4863–4873.
- Carlson, B.A., Kwon, S.Y., Chamorro, M., Oroszlan, S., Hatfield, D.L. and Lee, B.J. (1999) Transfer RNA modification status influences retroviral ribosomal frameshifting. *Virology*, **255**, 2–8.
- Carlson, B.A., Mushinski, J.F., Henderson, D.W., Kwon, S.Y., Crain, P.F., Lee, B.J. and Hatfield, D.L. (2001) 1-Methylguanosine in place of Y base at position 37 in phenylalanine tRNA is responsible for its shiftiness in retroviral ribosomal frameshifting. *Virology*, **279**, 130–135.
- Waas, W.F., Druzina, Z., Hanan, M. and Schimmel, P. (2007) Role of a tRNA base modification and its precursors in frameshifting in eukaryotes. *J. Biol. Chem.*, **282**, 26026–26034.
- Lecointe, F., Namy, O., Hatin, I., Simos, G., Rousset, J.P. and Grosjean, H. (2002) Lack of pseudouridine 38/39 in the anticodon arm of yeast cytoplasmic tRNA decreases *in vivo* recoding efficiency. *J. Biol. Chem.*, **277**, 30445–30453.
- Lin, C.A., Ellis, S.R. and True, H.L. (2010) The Sua5 protein is essential for normal translational regulation in yeast. *Mol. Cell. Biol.*, **30**, 354–363.
- El Yacoubi, B., Hatin, I., Deutsch, C., Kahveci, T., Rousset, J.P., Iwata-Reuyl, D., Murzin, A.G. and de Crécy-Lagard, V. (2011) A role for the universal Kae1/Qri7/YgjD (COG0533) family in tRNA modification. *EMBO J.*, **30**, 882–893.
- Miyauchi, K., Kimura, S. and Suzuki, T. (2013) A cyclic form of N6-threonylcarbamoyladenine as a widely distributed tRNA hypermodification. *Nat. Chem. Biol.*, **9**, 105–111.
- Brierley, I., Meredith, M.R., Bloys, A.J. and Hagervall, T.G. (1997) Expression of a coronavirus ribosomal frameshift signal in *Escherichia coli*: influence of tRNA anticodon modification on frameshifting. *J. Mol. Biol.*, **270**, 360–373.
- Liczner, P., Mejlhede, N., Prère, M.F., Wills, N., Gesteland, R.F., Atkins, J.F. and Fayet, O. (2003) Programmed translational -1 frameshifting on hexanucleotide motifs and the wobble properties of tRNAs. *EMBO J.*, **22**, 4770–4778.
- Urbonavičius, J., Stahl, G., Durand, J.M., Ben Salem, S.N., Qian, Q., Farabaugh, P.J. and Björk, G.R. (2003) Transfer RNA modifications that alter +1 frameshifting in general fail to affect -1 frameshifting. *RNA*, **9**, 760–768.
- Maynard, N.D., Macklin, D.N., Kirkegaard, K. and Covert, M.W. (2012) Competing pathways control host resistance to virus via tRNA modification and programmed ribosomal frameshifting. *Mol. Syst. Biol.*, **8**, 567–579.
- Farabaugh, P.J. (1996) Programmed translational frameshifting. *Microbiol. Rev.*, **60**, 103–134.
- Farabaugh, P.J. (1996) Programmed translational frameshifting. *Annu. Rev. Genet.*, **30**, 507–528.
- Björk, G.R., Durand, J.M., Hagervall, T.G., Leipuvienė, R., Lundgren, H.K., Nilsson, K., Chen, P., Qian, Q. and Urbonavičius, J. (1999) Transfer RNA modification: influence on translational frameshifting and metabolism. *FEBS Lett.*, **452**, 47–51.
- Farabaugh, P.J. and Björk, G.R. (1999) How translational accuracy influences reading frame maintenance. *EMBO J.*, **18**, 1427–1434.
- Gallant, J., Lindsley, D. and Masucci, J. (2000) The unbearable lightness of peptidyl-tRNA. *Ribosome: Struct. Func. Antibiot. Cell. Interact.*, 385–396.
- Näsvall, S.J., Nilsson, K. and Björk, G.R. (2009) The ribosomal grip of the peptidyl-tRNA is critical for reading frame maintenance. *J. Mol. Biol.*, **385**, 350–367.
- Jäger, G., Nilsson, K. and Björk, G.R. (2013) The phenotype of many independently isolated +1 frameshift suppressor mutants supports a pivotal role of the P-site in reading frame maintenance. *PLoS One*, **8**, e60246.
- Glasser, A.L., El Adlouni, C., Keith, G., Sochacka, E., Malkiewicz, A., Santos, M., Tuite, M.F. and Desgrès, J. (1992) Presence and coding properties of 2'-O-methyl-5-carbamoylmethyluridine (ncm5Um) in the wobble position of the anticodon of tRNA(Leu) (U*AA) from brewer's yeast. *FEBS Lett.*, **314**, 381–385.
- Johansson, M.J., Esberg, A., Huang, B., Björk, G.R. and Byström, A.S. (2008) Eukaryotic wobble uridine modifications promote a functionally redundant decoding system. *Mol. Cell. Biol.*, **28**, 3301–3312.
- Keith, G., Desgrès, J., Pochart, P., Heyman, T., Kuo, K.C. and Gehrke, C.W. (1990) Eukaryotic tRNAs(Pro): primary structure of the anticodon loop; presence of 5-carbamoylmethyluridine or inosine

- as the first nucleoside of the anticodon. *Biochim. Biophys. Acta.*, **1049**, 255–260.
28. Kobayashi, T., Irie, T., Yoshida, M., Takeishi, K. and Ukita, T. (1974) The primary structure of yeast glutamic acid tRNA specific to the GAA codon. *Biochim. Biophys. Acta.*, **366**, 168–181.
 29. Kuntzel, B., Weissenbach, J., Wolff, R.E., Tumaitis-Kennedy, T.D., Lane, B.G. and Dirheimer, G. (1975) Presence of the methylester of 5-carboxymethyl uridine in the wobble position of the anticodon of tRNA^{III} Arg from brewer's yeast. *Biochimie*, **57**, 61–70.
 30. Lu, J., Huang, B., Esberg, A., Johansson, M.J. and Byström, A.S. (2005) The *Kluyveromyces lactis* γ -toxin targets tRNA anticodons. *RNA*, **11**, 1648–1654.
 31. Smith, C.J., Teh, H.S., Ley, A.N. and D'Obrenan, P. (1973) The nucleotide sequences and coding properties of the major and minor lysine transfer ribonucleic acids from the haploid yeast *Saccharomyces cerevisiae* S288C. *J. Biol. Chem.*, **248**, 4475–4485.
 32. Yamamoto, N., Yamaizumi, Z., Yokoyama, S., Miyazawa, T. and Nishimura, S. (1985) Modified nucleoside, 5-carbamoylmethyluridine, located in the first position of the anticodon of yeast valine tRNA. *J. Biochem. (Tokyo)*, **97**, 361–364.
 33. Karlsborn, T., Tükenmez, H., Mahmud, A.K., Xu, F., Xu, H. and Byström, A.S. (2014) Elongator, a conserved complex required for wobble uridine modifications in Eukaryotes. *RNA Biol.*, **11**, 1519–1528.
 34. Esberg, A., Huang, B., Johansson, M.J.O. and Byström, A.S. (2006) Elevated Levels of Two tRNA Species Bypass the Requirement for Elongator Complex in Transcription and Exocytosis. *Mol. Cell.*, **24**, 139–148.
 35. Chen, C., Huang, B., Eliasson, M., Rydén, P. and Byström, A.S. (2011) Elongator complex influences telomeric gene silencing and DNA damage response by its role in wobble uridine tRNA modification. *PLoS Genet.*, **7**, e1002258.
 36. Näsvall, S.J., Chen, P. and Björk, G.R. (2004) The modified wobble nucleoside uridine-5-oxyacetic acid in tRNA^{Pro}(cmo5UGG) promotes reading of all four proline codons in vivo. *RNA*, **10**, 1662–1673.
 37. Patil, A., Chan, C.T., Dyavaiah, M., Rooney, J.P., Dedon, P.C. and Begley, T.J. (2012) Translational infidelity-induced protein stress results from a deficiency in Trm9-catalyzed tRNA modifications. *RNA Biol.*, **9**, 990–1001.
 38. Gietz, D., St Jean, A., Woods, R.A. and Schiestl, R.H. (1992) Improved method for high efficiency transformation of intact yeast cells. *Nucleic Acids Res.*, **20**, 1425.
 39. Burke, D., Dawson, D. and Stearns, T. (2000) *Methods in yeast genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
 40. Harger, J.W. and Dinman, J.D. (2003) An in vivo dual-luciferase assay system for studying translational recoding in the yeast *Saccharomyces cerevisiae*. *RNA*, **9**, 1019–1024.
 41. Belcourt, M.F. and Farabaugh, P.J. (1990) Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site. *Cell*, **62**, 339–352.
 42. Rajbhandary, U.L., Chang, S.H., Stuart, A., Faulkner, R.D., Hoskinson, R.M. and Khorana, H.G. (1967) Studies on polynucleotides, Ixviii the primary structure of yeast phenylalanine transfer RNA. *Proc. Natl. Acad. Sci. U.S.A.*, **57**, 751–758.
 43. Jühling, F., Mörl, M., Hartmann, R.K., Sprinzl, M., Stadler, P.F. and Pütz, J. (2009) tRNAdb 2009: compilation of tRNA sequences and tRNA genes. *Nucleic Acids Res.*, **37**, D159–D162.
 44. Kisselev, L.L. and Buckingham, R.H. (2000) Translational termination comes of age. *Trends Biochem. Sci.*, **25**, 561–566.
 45. Huang, B., Johansson, M.J.O. and Byström, A.S. (2005) An early step in wobble uridine tRNA modification requires the Elongator complex. *RNA*, **11**, 424–436.
 46. Kalthor, H.R. and Clarke, S. (2003) Novel methyltransferase for modified uridine residues at the wobble position of tRNA. *Mol. Cell Biol.*, **23**, 9283–9292.
 47. Mazauric, M.H., Dirick, L., Purushothaman, S.K., Björk, G.R. and Lapeyre, B. (2010) Trm112p is a 15-kDa zinc finger protein essential for the activity of two tRNA and one protein methyltransferases in yeast. *J. Biol. Chem.*, **285**, 18505–18515.
 48. Björk, G.R., Huang, B., Persson, O.P. and Byström, A.S. (2007) A conserved modified wobble nucleoside (mcm5s2U) in lysyl-tRNA is required for viability in yeast. *RNA*, **13**, 1245–1255.
 49. Fahlman, R.P., Dale, T. and Uhlenbeck, O.C. (2004) Uniform binding of aminoacylated transfer RNAs to the ribosomal A and P sites. *Mol. Cell.*, **16**, 799–805.
 50. Percudani, R., Pavesi, A. and Ottonello, S. (1997) Transfer RNA gene redundancy and translational selection in *Saccharomyces cerevisiae*. *J. Mol. Biol.*, **268**, 322–330.
 51. Rezgui, V.A., Tyagi, K., Ranjan, N., Konevega, A.L., Mittelstaet, J., Rodnina, M.V., Peter, M. and Pedrioli, P.G. (2013) tRNA t^{KUUU}, t^{QUUG}, and t^{EUUC} wobble position modifications fine-tune protein translation by promoting ribosome A-site binding. *Proc. Natl. Acad. Sci. U.S.A.*, **110**, 12289–12294.
 52. Fiorentini, P., Huang, K.N., Tishkoff, D.X., Kolodner, R.D. and Symington, L.S. (1997) Exonuclease I of *Saccharomyces cerevisiae* functions in mitotic recombination in vivo and in vitro. *Mol. Cell Biol.*, **17**, 2764–2773.
 53. Chen, C., Huang, B., Anderson, J.T. and Byström, A.S. (2011) Unexpected accumulation of mcm(5)U and mcm(5)S(2)U in a trm9 mutant suggests an additional step in the synthesis of mcm(5)U and mcm(5)S(2)U. *PLoS One*, **6**, e20783.
 54. Madison, J.T. and Kung, H.K. (1967) Large oligonucleotides isolated from yeast tyrosine transfer ribonucleic acid after partial digestion with ribonuclease T1. *J. Biol. Chem.*, **242**, 1324–1330.
 55. Nakai, Y., Nakai, M. and Hayashi, H. (2008) Thio-modification of yeast cytosolic tRNA requires a ubiquitin-related system that resembles bacterial sulfur transfer systems. *J. Biol. Chem.*, **283**, 27469–27476.
 56. Leidel, S., Pedrioli, P.G., Bucher, T., Brost, R., Costanzo, M., Schmidt, A., Aebersold, R., Boone, C., Hofmann, K. and Peter, M. (2009) Ubiquitin-related modifier Urm1 acts as a sulphur carrier in thiolation of eukaryotic transfer RNA. *Nature*, **458**, 228–232.
 57. Noma, A., Sakaguchi, Y. and Suzuki, T. (2009) Mechanistic characterization of the sulfur-relay system for eukaryotic 2-thiouridine biogenesis at tRNA wobble positions. *Nucleic Acids Res.*, **37**, 1335–1352.
 58. Farabaugh, P., Liao, X.B., Belcourt, M., Zhao, H., Kapakos, J. and Clare, J. (1989) Enhancer and silencerlike sites within the transcribed portion of a Ty2 transposable element of *Saccharomyces cerevisiae*. *Mol. Cell Biol.*, **9**, 4824–4834.