Transfer RNA modifications that alter +1 frameshifting in general fail to affect −1 frameshifting

JAUNIUS URBONAVIČIUS,[1,5] GUILLAUME STAHL,[2,3] JÉRÔME M.B. DURAND,[1] SAMIA N. BEN SALEM,[2] QIANG QIAN,[4] PHILIP J. FARABAUGH,[1,2] and GLENN R. BJÖRK[1]

[1]Department of Molecular Biology, Umeå University, SE-90 187 Umeå, Sweden
[2]Department of Biological Sciences and Program in Molecular and Cell Biology, University of Maryland Baltimore County, Baltimore, Maryland 21250, USA

ABSTRACT

Using mutants (*tgt, mnmA*(*asuE, trmU*), *mnmE*(*trmE*), *miaA, miaB, miaE, truA*(*hisT*), *truB*) of either *Escherichia coli* or *Salmonella enterica* serovar Typhimurium and the *trm5* mutant of *Saccharomyces cerevisiae*, we have analyzed the influence by the modified nucleosides Q34, mnm^5s^2U34, ms^2io^6A37, Ψ39, Ψ55, m^1G37, and yW37 on −1 frameshifts errors at various heptameric sequences, at which at least one codon is decoded by tRNAs having these modified nucleosides. The frequency of −1 frameshifting was the same in congenic strains only differing in the allelic state of the various tRNA modification genes. In fact, in one case (deficiency of mnm^5s^2U34), we observed a *reduced* ability of the undermodified tRNA to make a −1 frameshift error. These results are in sharp contrast to earlier observations that tRNA modification prevents +1 frameshifting suggesting that the mechanisms by which −1 and +1 frameshift errors occur are different. Possible mechanisms explaining these results are discussed.

Keywords: Translation, tRNA modification, nucleoside, frameshifting

INTRODUCTION

Many retroviruses express Gag-Pol or Gag-Pro-Pol proteins by coupling their translation from overlapping reading frames with −1 ribosomal frameshifts (Farabaugh 2000). The frameshift event occurs on a distinctive heptameric sequence of the general form X-XXY-YYZ, where X is any nucleotide, Y is A or U, and Z can be any nucleotide (Dinman et al. 1991; Jacks et al. 1988). From studies on the Rous sarcoma virus *gag-pol* frameshifting region, a pretranslocation "simultaneous slippage" model was proposed to explain the mechanism of frameshifting at such sequences (Jacks et al. 1988). According to this model, the frameshifting occurs before translocation and simultaneously by the two tRNAs present in the P- and A-sites. Later, an alternative hypothesis was presented that suggested that the frameshifting event occurs after translocation when the codons in the heptameric sequence occupy the E- and P-sites (Horsfield et al. 1995). Another type of −1 frameshifting at sites not containing this kind of heptameric sequences occurs when the ribosome is stalled at a "hungry codon" induced by, for example, aminoacyl-tRNA limitation (Weiss and Gallant 1983) or at nonsense codons (Weiss et al. 1987, 1990). The peptidyl-tRNA was proposed first to slip in the 5′ direction allowing the amino acid-tRNA in the A-site to decode the codon now at the newly formed frame by cognate interaction (Weiss et al. 1990; Yelverton et al. 1994; Barak et al. 1996).

The most energy-consuming process in the cell is translation of the genetic message transmitted via mRNA. In this process, it is pivotal for the ribosome to maintain the reading frame. Because the message lacks punctuations that would identify the reading frame, the ribosome cannot correct a reading frame error. Therefore, the translation machinery has evolved to reduce such errors to at least a magnitude lower in frequency than missense errors (Kurland et al. 1996). Transfer RNA is the molecule that decodes the message, and therefore it may be an important contributor to the reading frame maintenance. Changes in the tRNA structure, such as those induced by lack of a modified nucleoside, may therefore affect the reading frame mainte-

Reprint requests to: Glenn R. Björk, Department of Molecular Biology, Umeå University, SE-90 187 Umeå, Sweden; e-mail: Glenn.Bjork@molbiol.umu.se.

Present addresses: [3]Centre National de la Recherche Scientifique (CNRS), LMGM–IBCG, 118 route de Narbonne, 31062 Toulouse, France; [4]TeleCommunication System Inc., Annapolis, MD 21401, USA; [5]Laboratoire d'Enzymologie et Biochimie Structurals (LEBS), Centre National de la Recherche Scientifique (CNRS), Bldg. 34-1, Av de la Terasse, F-91 198 Gif-sur-Yvette, France

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nance. Modified nucleosides are derivatives of the four major nucleosides U, C, A, and G. At present, 81 different modified nucleosides have been characterized (Rozenski et al. 1999). Although they are present at different positions of the tRNA, most of them are present in the anticodon region and especially at positions 34 (wobble position) and 37 (3′ of and adjacent to the anticodon). Recently, we have demonstrated that the presence of several different modifications in the tRNA prevents +1 frameshifting (Urbonavičius et al. 2001). In that work, we also described a model explaining how frameshifting is promoted by hypomodified tRNA (see Fig. 1). This model has features in common with three of the above described models (Weiss et al. 1990; Horsfield et al. 1995; Barak et al. 1996) and with a recently described model to explain how +1 frameshifts occur (Stahl et al. 2002). We suggested that the same mechanism might also apply for −1 frameshifting errors. In the present work, we tested this hypothesis for several tRNA modifications. Surprisingly, our results demonstrate that in contrast to the +1 frameshifting (Urbonavičius et al. 2001), most tRNA modifications tested have no or in one case a slight *stimulatory* effect on −1 frameshifting. We suggest that in sharp contrast to the pivotal role of tRNA modification in preventing +1 frameshift errors, tRNA modification has no major role in preventing −1 frameshifting and that intrinsic features of the ribosome prevent this kind of errors.

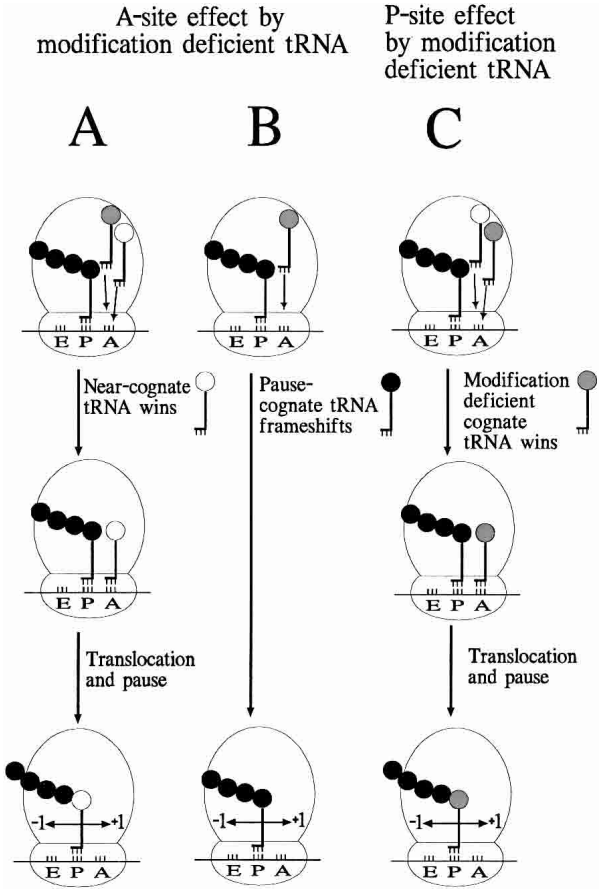

FIGURE 1. A dual-error model for frameshifting (Urbonavičius et al. 2001). (*A*) Hypomodified cognate tRNA is defective in the amino acid-tRNA selection step, thereby allowing a wild-type near-cognate tRNA to be accepted instead at the A-site. After a normal 3-nt translocation, the near-cognate tRNA slips into either −1 or +1 frame depending on the sequence of the mRNA. (*B*) The hypomodified tRNA is very slow in entering the A-site, inducing a pause, thereby allowing the wild-type tRNA in the P-site to frameshift into either −1 or +1 frame. (*C*) As in *A*, but the hypomodified cognate tRNA is accepted in the A-site and when residing in the P-site, the hypomodification induces slippage into the −1 or +1 frame.

RESULTS

The working model and the experimental systems used

Modification of tRNA may affect the accuracy of translation either by altering the rate tRNAs bind to the ribosome or by modulating the interaction of the tRNA•mRNA complex with the ribosome. Recruitment of each successive cognate, in-frame tRNA must occur in competition with all other tRNAs, including those that may decode out of frame; altering the rate of recruitment of cognate tRNA could change this competition to allow more frequent errant decoding. Alternatively, modification may modulate the interaction with the ribosome to ensure continued cognate, in-frame decoding by successive tRNAs. Figure 1 shows our model predicting how tRNA modification might influence reading frame maintenance. In all cases discussed below, the frameshift event occurs by peptidyl-tRNA slippage, although the identity of the tRNA that slips differs in each case. In the first case (Fig. 1A), hypomodification of a cognate tRNA might reduce its affinity for the codon in the ribosomal A-site. Because of its decreased rate of binding to the A-site, a wild-type near-cognate tRNA might successfully outcompete it, and be accepted into the A-site. After a normal 3-nt translocation, this near-cognate peptidyl-tRNA might not be able to interact optimally with the ribosomal P-site. Such nonoptimal P-site interaction has been suggested to reduce the efficiency of in-frame decoding leading to +1 frameshift errors (Belcourt and Farabaugh 1990; Urbonavičius et al. 2001). These errors are thought to result from slippage of peptidyl-tRNA prior to A-site recruitment. In principle, slippage in either the +1 or −1 direction might be possible, resulting in +1 or −1 frameshift errors. (P-site effect of near-cognate tRNA). In the second case (Fig. 1B), slow A-site recruitment of an undermodified cognate tRNA might induce frameshifting by causing a translational pause that allows a cognate wild-type peptidyl-tRNA in the P-site to slip either +1 or −1 (A-site effect by undermodified tRNA). Alternatively, in the third case, the defect of the undermodified cognate tRNA may not be in the A-site selection step, but rather undermodification may disrupt its interaction with the P-site after translocation (Fig. 1C). Undermodification might make the anticodon–codon interac-

tion less optimal, as with the interaction by a near-cognate tRNA, resulting in an increased frequency of frameshifting (P-site effect by undermodified tRNA). Of course, hypomodification may cause frameshifting by mediating both A-site and P-site effects. Thus, in contrast to the simultaneous slippage model (Jacks et al. 1988), this model does not require a simultaneous slippage of both A- and P-site-located tRNAs, and the frameshifting event occurs after translocation. Our model has features in common with the model presented by Horsfield et al. (1995) and by Gallant and coworkers (Weiss and Gallant 1986).

Recently, we have demonstrated that a common function of several modified nucleosides in tRNA is to prevent +1 frameshifting (Urbonavičius et al. 2001). In the present work, we have investigated the influence of various modified nucleosides on −1 frameshifting using several assay systems (Table 2, see below). To monitor −1 frameshifting in the bacteria *Escherichia coli* and *Salmonella enterica*, the HIV *gag-pol* programmed frameshifting site (pHIV-1; U-UUU-UUA), the mouse mammary tumor virus (MMTV) *gag-pro* programmed frameshifting site (A-AAA-AAC), and the nonprogrammed *argI* gene frameshifting site (G-UUU-UAU) were used. In these sequences, the first triplet denotes the codon in P-site, and the last triplet denotes the codon in A-site before the shift into the −1 frame has occurred according to the simultaneous slippage model (Jacks et al. 1988). In all cases, the *lacZ* gene is placed downstream from a short frameshifting window in such a way that the β-galactosidase activity is a direct measurement of the frequency by which the ribosome shifts to the −1 frame within this window (Table 1). Previously, both HIV and MMTV sequences were shown to stimulate frameshifting in *E. coli* (Weiss et al. 1989; Yelverton et al. 1994). Furthermore, in *E. coli*, a −1 frameshifting site in the *argI* gene was also demonstrated (Fu and Parker 1994). Therefore, these assay systems are suitable to test the influence of tRNA modifications on −1 frameshifting in bacteria. In the eukaryote *Saccharomyces cerevisiae*, the HIV *gag-pol* programmed frameshifting site (U-UUU-UUA) and the L-A virus frameshifting site (G-GGU-UUA) were tested. Both HIV and L-A sequences are prone to frameshift in *S. cerevisiae* (Stahl et al. 1995; Kurland et al. 1996). In this case, the activity of luciferase was the measurement of frequency by which the ribosome shifts frame.

According to our model (Fig. 1), lack of ms^2io^6A37 of, for example, the $tRNA^{Leu}_{cmnm5UmAA}$ may affect the A-site selection step of tRNAs at the HIV frameshifting sites (Table 2). This would stimulate a frameshifting event at the P-site, mediated by $tRNA^{Phe}_{GAA}$ (A-site effect by the undermodified tRNA; Urbonavičius et al. 2001). Alternatively, the frame-

TABLE 1. Plasmids and strains used

Plasmid	Sequence of the frameshifting window	Source/Reference
pHIV-1	AUG-AAA-AGC-UUA-GCU-AAU-UUU-UUA-GG-GGA-GAU-CUG-GCC-UUC-*lacZ*	Weiss et al. 1989
pMMTV1284	AUG-AAA-AGC-UUA-GCU-GAA-AAU-UCA-AAA-AAC-UU-GUA-AAG-GGG-*lacZ*	Weiss et al. 1989
pCFP3	AAUUC-AUG-UCC-GGG-UUU-UAU-CAC-AAG-CAU-UUC-AUC-AAA-UA(AGC-*lacZ*	Fu and Parker 1994
pAC1789	lacZ-CAG-GCU=AAU-UUU-UUA-GG-GGA-GA---*luc*	Stahl et al. 1995
pAC1790	lacZ-CAG-GCU-AAU-UUU-UUA-AGG-GGA-GA---*luc*	Stahl et al. 1995
pAC-LA	lacZ-UGG-CAG-CAG-GGU-UUA-GGA-GUG-GUA---*luc*	Bidou et al. 2000
pAC-LAFF	lacZ-UGG-CAG-CAG-GGU-UUA-AGG-AGU-GGU-A---*luc*	This work
Strain	**Relevant genotype or phenotype**	**Source/Reference**
S. typhymurium		
LT2	wild type	J. Roth, Utah
TT5866	*truA(hisT)*290::Tn*5*	J. Roth, Utah
GT 5880	*tgt*::Tn*10d*Cm	Urbonavičius et al. 2001
GT523	*miaA1*	Ericson and Björk 1986
GT2176	*miaB*2508::Tn*10d*Cm	Esberg and Björk 1995
GT3034	*miaE*2507::MudP	Persson and Björk 1993
GT4946	*miaA1*, *miaB*2508::Tn*10d*Cm	This work
E. coli		
TH98	Δ(*pro-lac*), *argE*(am), (val^R), *ilv*-135::Tn*10*, *nalA*, *rif*, *mnmE*+	Urbonavičius et al. 2001
TH99	Δ(*pro-lac*), *argE*(am), (val^R), *ilv*-135::Tn*10*, *nalA*, *rif*, *mnmE*	Urbonavičius et al. 2001
TH193	*ara*, Δ(*pro-lac*), *nalA*, *argE* (am), *rif*, *thi*, *mnmA*	Urbonavičius et al. 2001
TH194	*ara*, Δ(*pro-lac*), *nalA*, *argE* (am), *rif*, *thi*, *mnmA*+	Urbonavičius et al. 2001
GBEC384	=CSH41 Δ(*lac-pro*), *galE*	CSH laboratories
GRB1488	Δ(*lac-pro*), *galE*, *truA*(*hisT*)::*kan*	This work
GRB1490	Δ(*lac-pro*), *galE*, *truB*2422::mini-Tn*10*Cm	Urbonavičius et al. 2002
S. cerevisiae		
GBY15	*Mat a*, *his3*Δ*1*, *leu* Δ*0*, *met15* Δ*0*, *ura3* Δ*0*, *trm5*::*kanMX4*	Research Genetics
GBY18	*Mat a*, *his3*Δ*1*, *leu* Δ*0*, *met15* Δ*0*, *ura3* Δ*0*, *TRM5*	Research Genetics

TABLE 2. Assay systems used to monitor various tRNA modifications in −1 frameshifting

Site	Organism	Mutants used	Heptameric sequence[a]	tRNA in P-site	Modification tested in P-site tRNA	tRNA in A-site	Modification tested in A-site tRNA
HIV	*S. typh.*	*miaA*	U-UUU-UUA	$tRNA^{Phe}_{GAA}$	ms^2io^6- of ms^2io^6A37	$tRNA^{Leu}_{cmnm5UmAA}$	ms^2io^6- of ms^2io^6A37
HIV	*S. typh.*	*miaB*	U-UUU-UUA	$tRNA^{Phe}_{GAA}$	ms^2- of ms^2io^6A37	$tRNA^{Leu}_{cmnm5UmAA}$	ms^2- of ms^2io^6A37
HIV	*S. typh.*	*miaE*	U-UUU-UUA	$tRNA^{Phe}_{GAA}$	o^6- of ms^2io^6A37	$tRNA^{Leu}_{cmnm5UmAA}$	o^6- of ms^2io^6A37
HIV	*S. typh.*	*truA*	U-UUU-UUA	$tRNA^{Phe}_{GAA}$	Ψ39	$tRNA^{Leu}_{cmnm5UmAA}$	Ψ39
HIV	*E. coli*	*truB*	U-UUU-UUA	$tRNA^{Phe}_{GAA}$	Ψ55	$tRNA^{Leu}_{cmnm5UmAA}$	Ψ55
argI	*S. typh.*	*miaA, miaB*	G-UUU-UAU	$tRNA^{Phe}_{GmAA}$	ms^2io^6- of ms^2io^6A37	$tRNA^{Tyr}_{QUA}$	ms^2io^6- of ms^2io^6A37
argI	*S. typh.*	*tgt*	G-UUU-UAU	$tRNA^{Phe}_{GmAA}$	None	$tRNA^{Tyr}_{QUA}$	Q34
MMTV	*E. coli*	*mnmA*	A-AAA-AAC	$tRNA^{Lys}_{mnmn5s2UUU}$	s^2- of mnm^5s^2U34	$tRNA^{Asn}_{QUU}$	None
MMTV	*E. coli*	*mnmE*	A-AAA-AAC	$tRNA^{Lys}_{mnmn5s2UUU}$	mnm^5- of mnm^5s^2U34	$tRNA^{Asn}_{QUU}$	None
MMTV	*S. typh.*	*tgt*	A-AAA-AAC	$tRNA^{Lys}_{mnmn5s2UUU}$	None	$tRNA^{Asn}_{QUU}$	Q34
MMTV	*E. coli*	*truA*	A-AAA-AAC	$tRNA^{Lys}_{mnmn5s2UUU}$	Ψ39	$tRNA^{Asn}_{QUU}$	Ψ39
MMTV	*E. coli*	*truB*	A-AAA-AAC	$tRNA^{Lys}_{mnmn5s2UUU}$	Ψ55	$tRNA^{Asn}_{QUU}$	Ψ55
HIV	*S. cerev.*	*trm5*	U-UUU-UUA	$tRNA^{Phe}_{GmAA}$	Y-base	$tRNA^{Leu}_{U?AA}$	m^1G37
L-A	*S. cerev.*	*trm5*	G-GGU-UUA	$tRNA^{Gly}_{U?CC}$	None	$tRNA^{Leu}_{U?AA}$	m^1G37

[a]The first triplet denotes the codon in the P-site and the next triplet the codon in the A-site, if the frameshifting occurred as postulated by the "simultaneous slippage model."

shifting can be stimulated by ms^2io^6A37 of, for example the $tRNA^{Phe}_{GAA}$ reading the UUU codon at the P-site, provided that the ribosome makes a pause at the A-site (P-site effect by the undermodified tRNA). Because the modified nucleoside ms^2io^6A37 is present in both of these tRNA, the effect monitored may be either caused by an A- or a P-site effect of the undermodified tRNA or a combined effect. Using the *argI* site (G-UUU-UAU) and the *tgt* mutant, which blocks formation of queuosine, we monitored specifically the influence of Q34 deficiency in $tRNA^{Tyr}_{QUA}$ on reading the UAU codon in this sequence. Table 2 summarizes the assay systems used and indicates which modification was tested using the various mutants defective in tRNA modification. The assay systems, which are present on plasmids, were introduced into congenic strains of either *E. coli*, *S. enterica*, or *S. cerevisiae*, only differing in the allelic state of genes involved in the synthesis of various modified nucleosides. The frameshifting frequencies were compared with those in the wild type.

Influence on −1 frameshifting by modification in position 34 (the wobble position)

Role of Q34

The hypermodified base 7-(((4.5-cis-dihydroxy-2-cyclopenten-1-yl)-amino) methyl)-7-deazaguanosine or queuosine (Q) is present in position 34 in tRNAs specific for Tyr, His, Asn, and Asp, encoding UAU/C, CAU/C, AAU/C, and GAU/C, respectively. In a *tgt* mutant, these tRNAs have an unmodified G34 instead of Q34. Of the −1 frameshifting sites available, the presence of G34 instead of Q34 may influence the A-site selection of $tRNA^{Asn}_{QUU}$ at the A-AAA-AAC (pMMTV1284) or the A-site selection of $tRNA^{Tyr}_{QUA}$ at the G-UUU-UAU (pCF3). To test this hypothesis, we introduced either plasmid pMMTV1284 or plasmid pCFP3 into the wild type and the *tgt* mutant, respectively, and measured the level of frameshifting. No difference in −1 frameshifting was observed between wild type and the *tgt* mutant, suggesting that the A-site selection of $tRNA^{Asn}_{QUU}$ or $tRNA^{Tyr}_{QUA}$ was not affected by Q34 (Fig. 2A).

Role of mnm^5s^2U34

The modified nucleoside 5-methylaminomethyl-2-thio-uridine (mnm^5s^2U) is present in position 34 in tRNAs specific for Gln, Lys, and Glu codons, which are part of the split codon boxes; that is, a codon box in which the four codons encode two amino acids. These tRNAs read codons CAA/G (Gln), AAA/G (Lys), and GAA/G (Glu). To test how the mnm^5- or the s^2- groups influence −1 frameshifting in the P-site by affecting the interaction of $tRNA^{Lys}_{mnm5s2UUU}$ with the AAA codon, we introduced plasmid MMTV1284 (A-AAA-AAC) into wild type and *mnmE* (containing s^2U34 instead of mnm^5s^2U34) or *mnmA* (containing mnm^5U34) mutants, and measured the level of frameshifting. Lack of the mnm^5-group, as in the *mnmE* mutant, reduced the rate of −1 frameshifting 1.7-fold, whereas lack of the s^2-group, as in the *mnmA* mutant, did not affect the frequency of −1 frameshifting (Fig. 2B).

Influence on −1 frameshifting by modification in position 37

Role of ms^2io^637

The modified nucleoside 2-(methylthio-N^6-isopentenyl) adenosine ms^2i^6A37 is present in all *E. coli* tRNAs reading codons starting with U except $tRNA^{Ser\ I,\ V}_{GGA}$ (Grosjean et al.

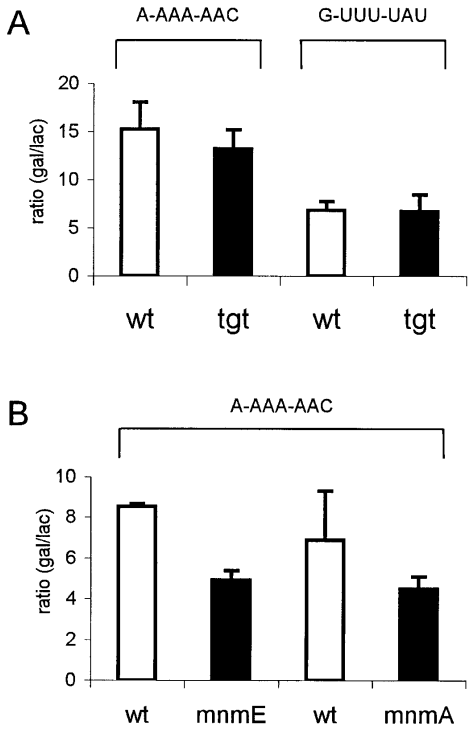

FIGURE 2. Influence of the Q34 and mnm^5s^2U34 on frameshifting at A-AAA-AAC and G-UUU-UAU sites. (*A*) In the *tgt* mutant, the $tRNA^{Lys}_{mnm5s2UUU}$, reading AAA codon, is fully modified, whereas $tRNA^{Asn}_{QUU}$, reading AAC codon, contains G34; the $tRNA^{Phe}_{GmAA}$, reading UUU codon, is fully modified, whereas $tRNA^{Tyr}_{QUA}$, reading UAU codon, contains G34. (*B*) In the *mnmE* mutant, AAA codon is read by $tRNA^{Lys}_{mnm5s2UUU}$, containing s^2U34, whereas in the *mnmA* mutant, this tRNA contains mnm^5U34; $tRNA^{Asn}_{QUU}$, reading AAC codon, is fully modified in both mutants. The frequency of frameshifting is expressed as the β-galactosidase activity normalized to the β-lactamase activity normalized encoded by the *bla* gene present on the same plasmid as that encoding *lacZ* gene.

1985). In *S. enterica*, the hydroxylated derivative ms^2io^6A37 is present in the corresponding tRNAs. Three *S. enterica* genes involved in the synthesis of ms^2io^6A37 have been identified: *miaA* (Ericson and Björk 1986), *miaB* (Esberg and Björk 1995), and *miaE* (Persson and Björk 1993). The *miaA* mutant contains A37 instead of ms^2io^6A37 in its tRNA, whereas the *miaB* mutant tRNA lacks the ms^2-group and therefore contains mainly i^6A37 and a small amount of io^6A37 (Esberg and Björk 1995). The *miaE* mutant lacks the hydroxyl-group and tRNA contains ms^2i^6A37 instead of ms^2io^6A37. We tested how the ms^2io^6- (as in *miaA*), ms^2- (as in *miaB*), and o^6- (as in *miaE*) groups influence the −1 frameshifting of $tRNA^{Leu}_{cmnm5UmAA}$, $tRNA^{Tyr}_{QUA}$, or $tRNA^{Phe}_{GAA}$ using the HIV (U-UUU-UUA) or the *argI* (G-UUU-UAU) frameshifting sites. We introduced the plasmids containing these sites into wild type and the *miaA*, *miaB*, and *miaE* mutants. We also introduced the plasmid containing the *argI* frameshifting site into wild type and the *miaA-miaB* double mutant, containing A37 instead of ms^2io^6A37. We measured the level of −1 frameshifting, and no difference between wild type and any of the mutants was observed (Fig. 3A).

Role of m^1G37 and yW37

The Trm5p protein is involved in the formation of modified nucleosides m^1G and wybutosine (yW; nucleoside of the Y-base) at position 37 in a subset of tRNAs in yeast *S. cerevisiae*. The tricyclic nucleoside yW is present only in $tRNA^{Phe}_{GmAA}$, and the first step in its synthesis is the formation of m^1G37 (Droogmans and Grosjean 1987). We have earlier shown that the *trm5* mutant is devoid of yW and has an unmodified G37 in its $tRNA^{Phe}_{GAA}$ (Björk et al. 2001). The m^1G in position 37 is present in several tRNA species, including $tRNA^{Leu}_{U?AA}$. The *trm5* mutant of *S. cerevisiae* contains G37 in those tRNAs.

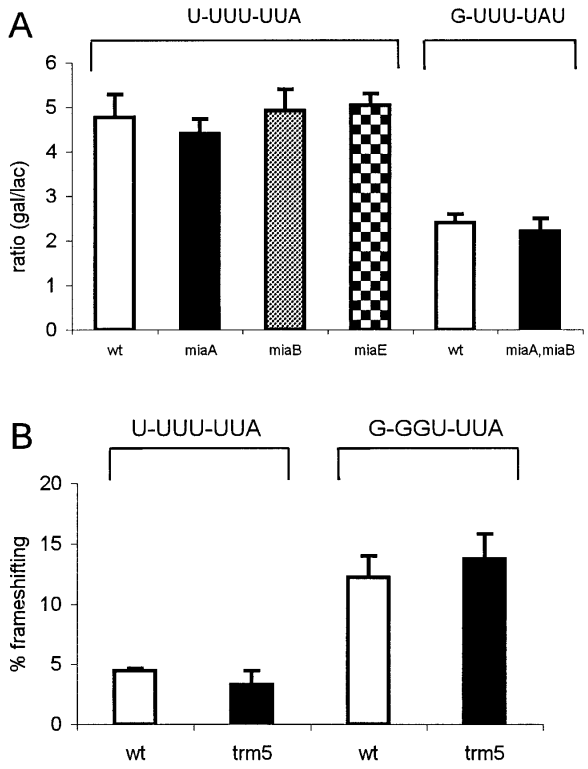

FIGURE 3. Influence of ms^2io^6A37, yW37, and m^1G37 on frameshifting at U-UUU-UUA, G-UUU-UAU, and G-GGU-UUA sites. (*A*) In the *miaA* mutant, both UUU and UUA codons at slippery sites are read by tRNAs containing A37. In the *miaB* mutant, those tRNAs contain io^6A37, and the *miaE* mutant, they contain ms^2i^6A37. In the double *miaA-miaB* mutant, both UUU and UAU codons are read by tRNAs containing A37. The frequency of frameshifting is expressed as the β-galactosidase activity normalized to the β-lactamase activity normalized encoded by the *bla* gene present on the same plasmid as that encoding *lacZ* gene. (*B*) In the *trm5* mutant, $tRNA^{Phe}_{GmAA}$, reading the UUU codon, contains G37 instead of yW37; $tRNA^{Leu}_{U?AA}$, reading the UUA codon, contains G37 instead of m^1G37, and $tRNA^{Gly}_{U?CC}$, reading the GGU codon, contains m^1G37. The frequency of frameshifting is expressed as the luciferase/β-galactosidase ratio of a test construct (pAC1789 or pAC-LA) normalized to the ratio obtained with the in-frame control (pAC1790 or pAC-LAFF).

To test how m^1G37 influences reading frame maintenance by affecting A-site selection of $tRNA^{Leu}_{U?AA}$, we introduced the L-A site (G-GGU-UUA) into wild type and the *trm5* mutant, and measured the level of frameshifting. We found no difference in frameshifting (Fig. 3B). Thus, lack of m^1G37 in $tRNA^{Leu}_{U?AA}$ had no influence on −1 frameshifting compared to the fully modified tRNA. The combined deficiency of yW in $tRNA^{Phe}_{GmAA}$ and of m^1G37 in $tRNA^{Leu}_{U?AA}$ had no impact on −1 frameshifting when monitored at the HIV-site (U-UUU-UUA) (Fig. 3B).

Influence on −1 frameshifting by other modifications outside the anticodon

Role of Ψ39

The modified nucleoside pseudouridine (Ψ) is present in the anticodon stem and loop of many bacterial tRNAs, including $tRNA^{Leu}_{cmnm5UmAA}$, $tRNA^{Asn}_{QUU}$, $tRNA^{Phe}_{GAA}$, and $tRNA^{Lys}_{mnm5s2UUU}$. The *truA* (*hisT*) gene product catalyzes the formation of Ψ in position 39 in those tRNA species. To test how Ψ39 influences reading frame maintenance by affecting A-site selection of $tRNA^{Leu}_{cmnm5UmAA}$ and/or P-site codon–anticodon interaction of the $tRNA^{Phe}_{GAA}$, the HIV frameshifting site (U-UUU-UUA) was introduced into wild-type strain and the *truA* mutant, and the level of frameshifting was measured. We found no difference in −1 frameshifting between the wild type and the *truA* mutant (Fig. 4A). To test how Ψ39 influences reading frame maintenance by affecting A-site selection of $tRNA^{Asn}_{QUU}$ and/or P-site codon–anticodon interaction of $tRNA^{Lys}_{mnm5s2UUU}$, the MMTV frameshifting site (A-AAA-AAC) was introduced into wild type and the *truA* mutant, and the level of frameshifting was measured. We observed no difference in −1 frameshifting between these strains, as in the case of pHIV-1 (Fig. 4A). Thus, Ψ39 did not influence −1 frameshifting in any case.

Role of Ψ55

The modified nucleoside Ψ55 is part of the TΨC loop and thus is present in all tRNA species of *E. coli* and *S. typhimurium*. To test whether Ψ55 influences frameshifting, either the HIV frameshifting site (U-UUU-UUA) or the MMTV frameshifting site (A-AAA-AAC) was introduced into wild type and the *truB* mutant, and the level of frameshifting was measured. We found no influence of Ψ55 on the frameshifting at both these frameshifting sites (Fig. 4B).

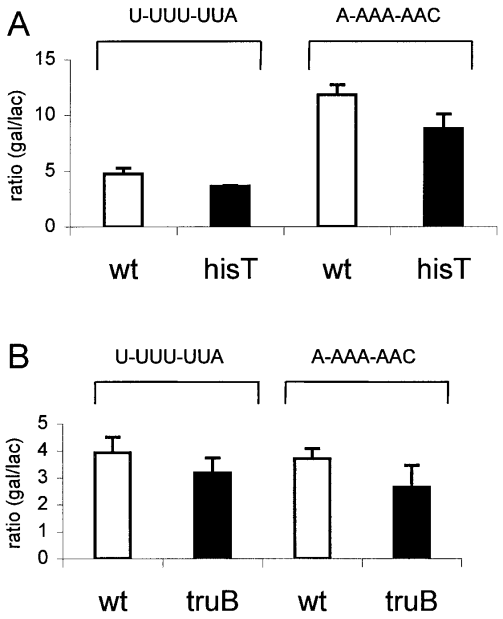

FIGURE 4. Influence of Ψ39 and Ψ55 on frameshifting at U-UUU-UUA and A-AAA-AAC sites. (*A*) In the *truA* mutant, all tRNAs, reading codons at slippery sites, contain U39 instead of Ψ39. (*B*) In the *truB* mutant, all tRNAs, reading codons at slippery sites, contain U55 instead of Ψ55. The frequency of frameshifting is expressed as the β-galactosidase activity normalized to the β-lactamase activity normalized encoded by the *bla* gene present on the same plasmid as that encoding the *lacZ* gene.

DISCUSSION

We have earlier shown that a common function for several tRNA modifications is to improve the reading frame maintenance by *preventing* +1 frameshift (Björk et al. 1989; Hagervall et al. 1993; Li et al. 1997; Urbonavičius et al. 2001). Contrary to this observation, here we show that several different modified nucleosides *do not influence or only slightly increase* the frequency of −1 frameshifting (summarized in Table 3). Taken together, these observations suggest that the mechanism by which tRNA modification exerts its effect on the reading frame maintenance is different for shifts of the reading frame in the 5′ (−1) and the 3′ (+1) direction.

The Q34 deficiency in $tRNA^{Asn}_{QUU}$ acting at the slippery sequence A-AAA-AAC did not influence the −1 frameshifting (Fig. 2A), as was observed for +1 frameshifting (Urbonavičius et al. 2001). Similarly, the absence of Q34 in $tRNA^{Asn}$ did not influence the level of −1 frameshifting at U-UUA-AAC/U sites in the cos-cells and in vitro using the rabbit reticulocyte lysate (RRL) system (Marczinke et al. 2000). Using the same in vitro system, the level of −1 frameshifting at the A-AAA-AAC site only increased 1.5-fold when the Q-deficient $tRNA^{Asn}_{GUU}$ from yeast was added to the lysate (Carlson et al. 2001). When the effect by Q34 was analyzed in vivo using a Q34 deficient mutant of *E. coli*, frameshifting by $tRNA^{Leu}_{U?AA}$ decreased 2-fold at the U-UUA-AAC site (i.e., when $tRNA^{Leu}_{U?AA}$ interacted with the AAC codon) and increased 2-fold at the U-UUA-AAU site (i.e., when $tRNA^{Leu}_{U?AA}$ interacted with the AAU codon; Brierley et al. 1997). The difference between those and our (Fig. 2A) results may be explained by presence of different tRNAs ($tRNA^{Leu}_{cmnm5UmAA}$ versus $tRNA^{Lys}_{mnm5s2UUU}$ or $tRNA^{Phe}_{GAA}$) in

TABLE 3. Comparison of the effect of tRNA modification on −1 and +1 frameshifting

Modification tested	Codon tested	Increased −1 fs		Increased +1 fs	
		P-site	A-site	P-site	A-site
Q34	UAU	ND	No	No	Yes[a]
Q34	AAC	ND	No	ND	ND
s^2- of mnm^5s^2U34	AAA	No	ND	Yes[a]	Yes[a]
mnm^5- of mnm^5s^2U34	AAA	No[b]	ND	Yes[a]	Yes[a]
ms^2io^6- of ms^2io^6A37	UUU	No	ND	Yes[a]	No[c]
ms^2- of ms^2io^6A37	UUU	No	ND	Yes[a]	No[c]
o^6- of ms^2io^6A37	UUU	No	ND	Yes[a]	ND
ms^2io^6- of ms^2io^6A37	UUA	ND	No	ND	ND
ms^2- of ms^2io^6A37	UUA	ND	No	ND	ND
o^6- of ms^2io^6A37	UUA	ND	No	ND	ND
ms^2io^6- of ms^2io^6A37	UAU	ND	No	Yes[a]	Yes[c]
Y-base	UUU	No	ND	ND	ND
m^1G37	UUA	ND	No	ND	ND
Ψ39	UUU	No	ND	No[d]	ND
Ψ39	UUA	ND	No	ND	ND
Ψ39	AAA	No	ND	ND	ND
Ψ39	AAC	ND	No	ND	ND
Ψ55	UUU	No	ND	No[e]	ND
Ψ55	UUA	ND	No	ND	ND
Ψ55	AAA	No	ND	ND	No[e]
Ψ55	AAC	ND	No	ND	ND

(ND) Not determined.
[a]Adapted from Urbonavičius et al. (2001).
[b]Reduced level of frameshifting was observed.
[c]Adapted from Li et al. (1997).
[d]Adapted from Qian (1997).
[e]Adapted from Urbonavičius et al. (2002).

the P-site. Apparently, Q34 in $tRNA^{Asn}_{QUU}$ interacting with the AAC codon slightly stimulates −1 frameshift errors, whereas when interacting with the AAU codon, it slightly prevents −1 frameshifts. Therefore, presence of Q34 in the tRNA stimulates −1 frameshifting when it interacts with C, whereas it prevents the same error if interacting with U as the third nucleoside of the codon. Similarly, when monitoring +1 frameshifting, Q34 prevented such errors when interacting with a U-ending codon (Urbonavičius et al. 2001). Q34 deficiency of $tRNA^{His}$ does not influence −1 frameshifting on the sequence U UUC AUA in vivo in *E. coli* (Masucci et al. 2002), consistent with our observation and that of Brierly et al. (1997) that Q34 does not influence −1 frameshifting. Because in most cases no effect on −1 frameshift that could be attributed to Q was observed and if an effect was observed, it was small, these results show that Q34 has no major role in preventing −1 frameshifting.

Lack of either the mnm^5- or the s^2-group of mnm^5s^2U34 increases the frequency of +1 frameshifting for the $tRNA^{Lys}_{mnm5s2UUU}$ independently if the codons AAA/G are in the A- or in the P-site (Urbonavičius et al. 2001). In contrast, frequency of −1 frameshifting decreased at the A-AAA-AAC site when $tRNA^{Lys}_{mnm5s2UUU}$ was lacking the mnm^5-group, whereas lack of the s^2-group did not make any difference (Fig. 2B). Similarly, in *E. coli*, the s^2- or the mnm^5-group each has very little or no influence on −1 frameshifting at a U-UUA-AAG site, and the s^2-group has no influence on −1 frameshifting at the U-UUA-AAA site (Brierley et al. 1997). However, lack of the mnm^5-group increases the −1 frameshifting at the slippery U-UUA-AAA sites twofold (Brierley et al. 1997). Thus, contrary to the large impact of mnm^5s^2U34 to prevent +1 shifts, its role in −1 frameshifting is minor, and, in fact, in some cases the presence of it *increases* the −1 frameshift error.

Transfer RNA from all organisms frequently contains modified nucleosides at position 37 (3′ and adjacent to the anticodon). Two such modified nucleosides are ms^2io^6A37 and m^1G37, which both prevent +1 frameshift errors (Björk et al. 1989; Hagervall et al. 1993; Li et al. 1997; Urbonavičius et al. 2001). However, ms^2io^6A37 did not influence −1 frameshifting (Fig. 3A) at any sequence tested. We found also no effect of m^1G37 in yeast $tRNA^{Leu}_{U?AA}$ on −1 frameshifts (Fig. 3B). Thus, whereas m^1G37 and ms^2io^6A37 are very important in preventing +1 frameshifts, they apparently have no role in preventing −1 frameshifts.

The highly complex tricyclic modified nucleoside wybutosine (yW) is present at position 37 only in eukaryotic $tRNA^{Phe}_{GmAA}$. Using the rabbit reticulocyte lysate (RRL) in vitro translation system, a threefold increase in −1 frameshifting at the A-AAU-UUU site was attributed to the exchange of yW37 to m^1G37 in $tRNA^{Phe}_{GmAA}$, reading UUU in the A-site (Carlson et al. 1999, 2001). However, the level of −1 frameshifting at U-UUU-UUU/C sites, which have codons read by $tRNA^{Phe}_{GmAA}$ both in P- and A-sites, was the same using yW or m^1G37 containing $tRNA^{Phe}_{GmAA}$. Similarly, we observed in vivo no influence by yW37 on −1 frameshifting at the U-UUU-UUA site (Fig. 3B), which has UUU in the P-site, similarly to the U-UUU-UUU/C sequence. Thus, lack of yW37 of $tRNA^{Phe}_{GmAA}$ decoding UUU in the P-site did not influence −1 frameshifting either in vivo or in vitro. Because lack of yW37 of $tRNA^{Phe}_{GmAA}$ increased −1 frameshifting at A-AAU-UUU but not at U-UUU-UUU/C (Carlson et al. 1999, 2001), the yW37-mediated effect on $tRNA^{Phe}_{GmAA}$ decoding UUU in the A-site may be sensitive to which tRNA is occupying the P-site (peptidyl-$tRNA^{Lys}$ versus peptidyl-$tRNA^{Phe}$).

Taken together, whereas modifications such as ms^2io^6A37 and m^1G37 at position 37 have a profound influence on preventing +1 frameshifting, these modifications and yW37 have no or only a minor effect on −1 frameshift errors.

According to our model (Fig. 1; Urbonavičius et al. 2001; Stahl et al. 2002), +1 frameshifts occur after translocation and by slippage of the P-site tRNA when the A-site is empty. According to the simultaneous slippage model (Jacks et al. 1988), −1 frameshifts occur after the A-site is filled, by simultaneous slippage of the tRNAs present in both A- and P-sites, that is prior to translocation. In general, our results (Table 3) and those of others (Hagervall et al. 1993; Brierley et al. 1997; Carlson et al. 1999; Marczinke et al. 2000) sug-

gest that tRNA modifications have no or only a minor effect on −1 frameshifts. In fact, in some cases, the presence of the modified nucleoside actually stimulates −1 frameshifting (Fig. 2B) in sharp contrast to earlier observations that the presence of several modifications in tRNA prevents +1 frameshifts (Urbonavičius et al. 2001). These results suggest that −1 and +1 frameshifting occur by distinct mechanisms. It is known that +1 frameshifting is quite sensitive to the rate of recognition of the codon in the empty A-site, for example, because of slow selection of mutant tRNAs (Li et al. 1997; Qian and Björk 1997; Urbonavičius et al. 2001) or low concentration of cognate tRNA (Belcourt and Farabaugh 1990; Farabaugh et al. 1993). Such slow A-site decoding increases the probability of a forward shift of the reading frame. In contrast, a −1 slippage by the peptidyl-tRNA would depend on a movement of the apparently strongly bound deacylated tRNA in the E-site (for a review, see Ramakrishnan 2002), suggesting that such a peptidyl-tRNA slippage is unlikely. According to the simultaneous slippage model, the E-site is empty at the time of slippage. Still, both tRNAs interact with various parts of the ribosome, making the presence of modified nucleoside of minor importance to inhibit a simultaneous slippage in the 5′ direction. Perhaps interactions between the two tRNAs, the mRNA, and the ribosome during −1 simultaneous slippage frameshifting are epistatic to small structural changes in the tRNA, such as those induced by hypomodification. Such considerations could explain our inability to show an effect of hypomodification on −1 frameshifting and why tRNA modification is more important to prevent +1 than −1 frameshift errors. Our results are therefore consistent with the predictions of the simultaneous slippage model (Jacks et al. 1988).

MATERIALS AND METHODS

Plasmids, strains, and growth conditions

Plasmids and strains, which were constructed by standard genetic methods (Davis et al. 1980), are listed in Table 1. *S. enterica* serovar Typhimurium strains harboring various plasmids were grown at 37°C in the rich medium NB + AV + ADE, Difco nutrient broth (0.8%; Difco Laboratories) supplemented with the aromatic amino acids (A), aromatic vitamins (V), and adenine (ADE; Davis et al. 1980). Strains of *E. coli* were grown at 37°C in LB medium (Bertani 1951). Strains of *S. cerevisiae* were grown at 30°C in SD medium supplemented with appropriate amino acids or uracil (Adams et al. 1997).

Determination of the β-galactosidase, β-lactamase, and luciferase activity

In bacteria, β-galactosidase and β-lactamase activity and the frequency of frameshifting were measured as described earlier (Hagervall et al. 1993; Li et al. 1997). In yeast, β-galactosidase and luciferase activities and the frequency of frameshifting were measured as described previously (Stahl et al. 1995). Statistical variation was calculated using the *t* test with two tails. A difference in the frequency of frameshifting was considered significant when $p < 0.05$.

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