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Programmed translational –1 frameshifting on hexanucleotide motifs and the wobble properties of tRNAs

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Programmed –1 ribosomal frameshifting, involving tRNA re-pairing from an AAG codon to an AAA codon, has been reported to occur at the sequences CGA AAG and CAA AAG. In this study, using the recoding region of insertion sequence IS*3*, we have investigated the influence on frameshifting in *Escherichia coli* of the first codon of this type of motif by changing it to all other NNA codons. Two classes of NNA codons were distinguished, depending on whether they favor or limit frameshifting. Their degree of shiftiness is correlated with wobble propensity, and base 34 modification, of their decoding tRNAs. A more flexible anticodon loop very likely makes the tRNAs with extended wobble more prone to liberate the third codon base, A, for re-pairing of tRNA^{Lys} in the –1 frame.

Keywords: frameshifting/hexanucleotide/insertion sequences/tRNA modification/wobble

Introduction

The expression of a minority of genes in probably all organisms involves a proportion of ribosomes shifting reading frame at specific sites. In some cases the function of this programmed event is regulatory and in others the synthesis of two products, with different C-termini, is the important consequence (Farabaugh, 1997; Atkins *et al*., 2001). Examples of the former often implicate +1 frameshifting, whereas many of the latter involve –1 frameshifting. Beside its role in gene expression, frameshifting elicits interest because of what it reveals about the functioning of the translational molecular machine, especially in view of the recent advances in our understanding of the ribosome at the atomic level (Ogle *et al*., 2001, 2002, 2003; Yusupova *et al*., 2001; Noller *et al*., 2002; Valle *et al*., 2002; Gao *et al*., 2003). In particular, frameshifting brings into light the intricacies of the relation between a tRNA, its codon and the ribosome, as illustrated by the present work, and also raises the question of the maintenance of the translational reading frame (Farabaugh and Björk, 1999; Atkins *et al*., 2000). All known cases of –1 ribosomal frameshifting involve dissociation of codon: anticodon pairing followed by anticodon re-pairing to mRNA at an overlapping –1 frame codon. Early work with frameshift mutant leakiness and synthetic constructs focused on low frequency dissociation and re-pairing events involving a single tRNA anticodon (Weiss *et al*., 1987; Gallant and Lindsley, 1992). The high frequency programmed frameshifting events involved in decoding potato virus M (Gramstat *et al*., 1994), bacterial insertion sequences IS*3* (Sekine *et al*. 1994) and IS*1222* (N.Mejlhede, P.Licznar, M.F.Prère, N.Wills, R.F.Gesteland, J.Atkins and O.Fayet, in preparation) and that associated with decoding the *Bacillus subtilis* cytidine deaminase gene (*cdd*) (Mejlhede *et al*., 1999) have been considered in these terms. However, the great majority of known programmed –1 frameshifting involves re-pairing by tandem tRNAs at heptanucleotide sequences. Tandem slippage was discovered by Jacks and Varmus (1988) in their studies on the frameshifting required for retroviral gene expression and has since been found mostly in the decoding of viruses from diverse sources and in bacterial programmed frameshifting. Searches for additional cases of frameshifting were therefore concentrated on the characteristic heptanucleotide motifs for tandem re-pairing with little attention to single re-pairing possibilities.

The frameshifting that occurs in decoding *B.subtilis cdd* is 16% efficient. The intrinsic level of frameshifting at its A AAG shift site is 1.5%; as originally shown in *Escherichia coli*, tRNA^{Lys} (anticodon 3′-UUmnm^{5}s^{2}U-5′, where mnm^{5}s^{2}U is 5-methylaminomethyl-2-thiouridine) is prone to shift –1 from AAG to AAA (Weiss *et al*., 1989; Tsuchihashi and Brown, 1992). A Shine–Dalgarno-like sequence within the coding sequence nine bases 5′ of the shift site acts to stimulate –1 frameshifting 10.6-fold (Mejlhede *et al*., 1999). Analogous stimulatory effects of nearby 5′ internal Shine–Dalgarno sequences are known for tandem –1 frameshifting (Larsen *et al*., 1994; Rettberg *et al*., 1999). The identity of the codon, CGA, upstream of the AAG is crucial for high efficiency frameshifting, but not the base 5′ of it, leading to the hypothesis of a hexameric shift site (Mejlhede *et al*., 1999). The anticodon of the CGA-decoding tRNA^{Arg} (3′-GCI-5′) contains inosine, I. Previous studies have shown very inefficient A:I pairing *in vivo* (Curran, 1995; Carter *et al*., 1997). It was therefore suggested that apposition of the purine inosine in the anticodon with the purine A of the *cdd* CGA codon does not permit strong pairing and would frequently result in the liberation of the third codon base, thereby allowing re-pairing of tRNA^{Lys} from AAG to AAA.

Decoding of a bacterial transposable element, insertion sequence IS*1222* (Steibl and Lewecke, 1995), also uses –1

frameshifting at a CGA AAG hexamer. Frameshifting is required for synthesis of the transposase, and so for transposition, of IS*1222* (N.Mejlhede, P.Licznar, M.F.Prère, N.Wills, R.F.Gesteland, J.Atkins and O.Fayet, in preparation). This recoding event, occurring at a frequency of ~7%, is stimulated by a weaker 5′ Shine–Dalgarno sequence than in *cdd*, but has a 3′ stimulatory stem–loop sequence. A stimulatory 3′ stem–loop is also not unique to this type of shift site, as it is known for several cases of bacterial dual-slippage frameshift regions including that for synthesis of a DNA polymerase component encoded by the *E.coli dnaX* gene (Larsen *et al*., 1997). In another insertion sequence, IS*3*, an A AAG frameshift site is associated with a pseudoknot as 3′ stimulator (there is no 5′ stimulatory SD sequence) and the frequency of frameshifting was reported to be 6% (Sekine *et al*., 1994). In this example the two upstream nucleotides are CA, which gives a CAA AAG hexamer. However, the role in frameshift modulation of the CAA codon was not determined in the IS*3* context, nor had it been tested within the IS*1222* recoding signal. Possible different mechanistic consequences of the two types of sequences were examined in the current study.

The present work also determines whether NNA codons other than CGA, in the sequence NNA AAG, are decoded by tRNAs that liberate the third codon base, A, permitting realignement of $tRNA^{Lys}$ in the –1 frame. The incidence of the nucleotide 5′ of the NNA codon and the effect of the modification status of anticodon base 34 of the NNA-decoding tRNA were also analyzed. Two versions of a model for single re-pairing frameshifting are presented.

Results

Members of the IS*3* family of insertion sequences have two partially overlapping open reading frames, *orfA* and *orfB*, with –1 ribosomal frameshifting at a specific site in the overlap region yielding an OrfAB transframe protein with transposase function (Mahillon and Chandler, 1998). IS*3* itself, the archetype of the family, and IS*1222* have this gene organization (Figure 1A). Their *orfA* gene encodes a protein containing a predicted α-helix–turn–α-helix motif, as well as a leucine-zipper motif, and their *orfB* gene encodes a protein with a domain characteristic of retroviral integrases and IS*3* family transposases (Mahillon and Chandler, 1998). So far in IS elements, the OrfB polypeptide has only been found to be important for transposition activity when fused to the OrfA protein (Polard *et al*., 1992). In the *orfA–orfB* overlap region of both IS*3* and IS*1222*, frameshifting presumably occurs by re-alignement of one $tRNA^{Lys}$ on the A AAG sequence. To elucidate the exact role of the upstream codon in each IS, we cloned both frameshift regions into a reporter plasmid and changed the upstream codon of their respective hexamer to all 13 other $N_1N_2A_3$ sense codons (diagrammed in Figure 1A). In addition, we investigated the incidence of the nucleotide on the 5′ side of the hexamer (nucleotide N_0). In one set, N_0 was different from N_1 to prevent re-pairing of the $N_1N_2A_3$-decoding tRNA, and in the other set it was identical to N_1 in order to allow re-pairing of at least the third anticodon base (tRNA nucleotide 36). Since identical results were found with IS*3* and IS*1222*, only the IS*3* results are presented below.

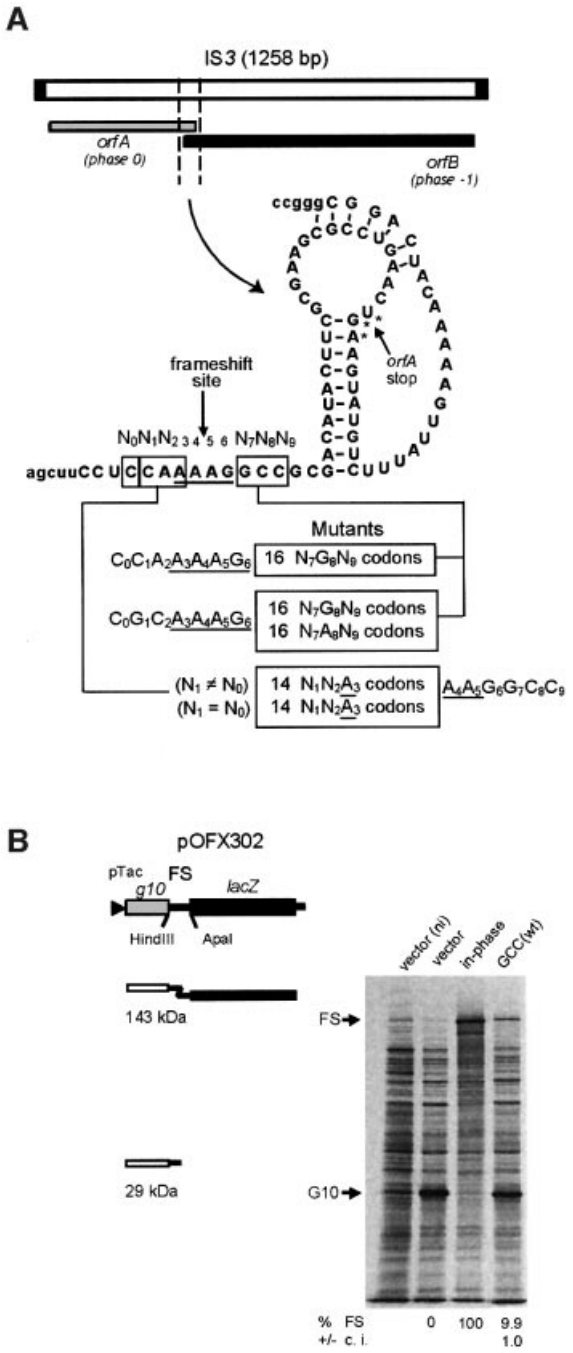

Fig. 1. The IS*3* frameshift region, its various derivatives and the plasmid reporter system. (**A**) The segment of IS*3* shown and the derived mutants were cloned in the pOFX302 plasmid. (**B**) Frameshifting efficiency was determined by protein labeling with [^{35}S]methionine. The results obtained with two control strains (one with an in-phase construct, giving the theoretical 100% frameshifting value, and the other containing the vector plasmid, 0% frameshifting value), and with the IS*3* 'wild type' region (wt) are shown. One culture of the vector-containing strain was labeled in the absence of IPTG (ni). The position of the product from normal translation (G10) or frameshifting (FS) is indicated. The calculated level of frameshifting and the 95% confidence interval (c.i.) are indicated below the relevant lanes. In the natural IS3 frameshift region an AUG codon (frame –1) overlapping the UGA stop codon of *orfA* (frame 0) is used to initiate synthesis of the OrfB protein. This AUG codon was changed to CUG in order to prevent initiation without interfering with frameshifting (Sekine *et al*., 1994).

For reasons discussed in the next section, we also analyzed in the case of IS*3* the effect on frameshifting of the codon 3′ to the A AAG shift site (nucleotides $N_7N_8N_9$ in Figure 1A).

To study IS*3* frameshifting, the 81-nucleotide segment shown in Figure 1A was inserted between, and fused to, two genes. The end of *orfA* is in-frame with gene *10* of phage T7 and the beginning of *orfB* is in-frame with the *lacZ* coding sequence on a plasmid-borne construct (Figure 1B; Rettberg *et al*., 1999). Quantitation of the G10-OrfA′-OrfB′-LacZ transframe product (FS in Figure 1B) and G10-OrfA′ (G10 in Figure 1B) products was performed by *in vivo* protein pulse labeling followed by PAGE separation or by β-galactosidase assay.

Frameshifting occurs while the AAG codon is in the ribosomal A-site

To gain evidence concerning the ribosomal site at which the frameshift occurs with the IS*3* motif, the GCC codon 3′ to the C CAA AAG wild-type sequence was changed to all 16 possible $N_7G_8N_9$ codons (Figure 1A); in another set of constructs, a different shift site was used, C GCA AAG, and the 3′ codon was changed to the 32 possible $N_7(A/G)_8N_9$ codons. Slow-to-decode codons, especially stop codons, in the ribosomal A-site can stimulate non-programmed (i.e. low level) –1 frameshifting of peptidyl-tRNA in the P-site, if upstream re-pairing is possible (Weiss *et al*., 1987; Gallant and Lindsley, 1992). Consequently, if a 3′ stop or rare (e.g. AGG or AGA in *E.coli*) codon has a stimulatory effect, this indicates P-site slippage, whereas absence of an effect suggests that frameshifting occurred while the shifty motif was in the A-site.

Figure 2A and B shows the results of the analysis carried out on the wild-type and mutant IS*3* signals, respectively. Interestingly, in this set of 48 $N_7(A/G)_8N_9$ constructs the absolute level of frameshifting varies with the 3′ context. In Figure 2A, for example, there is a 2-fold difference between GGA and GGC or a 3-fold factor between GGC and CGG. In Figure 2B, β-galactosidase assay was used to measure frameshifting. Even if levels of frameshifting thus measured are lower than with the pulse-labeling method (see Materials and methods), significant differences also exist between constructs (e.g 2.5-fold between GAA and CAG). In a recent study we observed a similar effect of the 3′ context with the four heptameric X XXA AAG dual slippage motifs (Bertrand *et al*., 2002). The statistical analysis of nearly 200 mutants showed that the first nucleotide after the motif has the primary effect on frameshifting, with, in order of decreasing efficiency, U > C > A > G. Our interpretation was that when the AAG slippery codon enter the A-site, there is a competition between standard decoding and –1 frameshifting the outcome of which could be in part determined by the stacking of the next nucleotide of the message on the AAG codon–anticodon helix. Purines, having a higher stacking potential than pyrimidines, would therefore tend to limit frameshifting (see discussion in Bertrand *et al*., 2002). Comparison of the mean value after grouping of the constructs according to the identity of the first nucleotide of the 3′ codon (Figure 2) demonstrates that, for the IS*3* shift site also, pyrimidines in this position generally results in a higher level of frameshifting than when it is a purine.

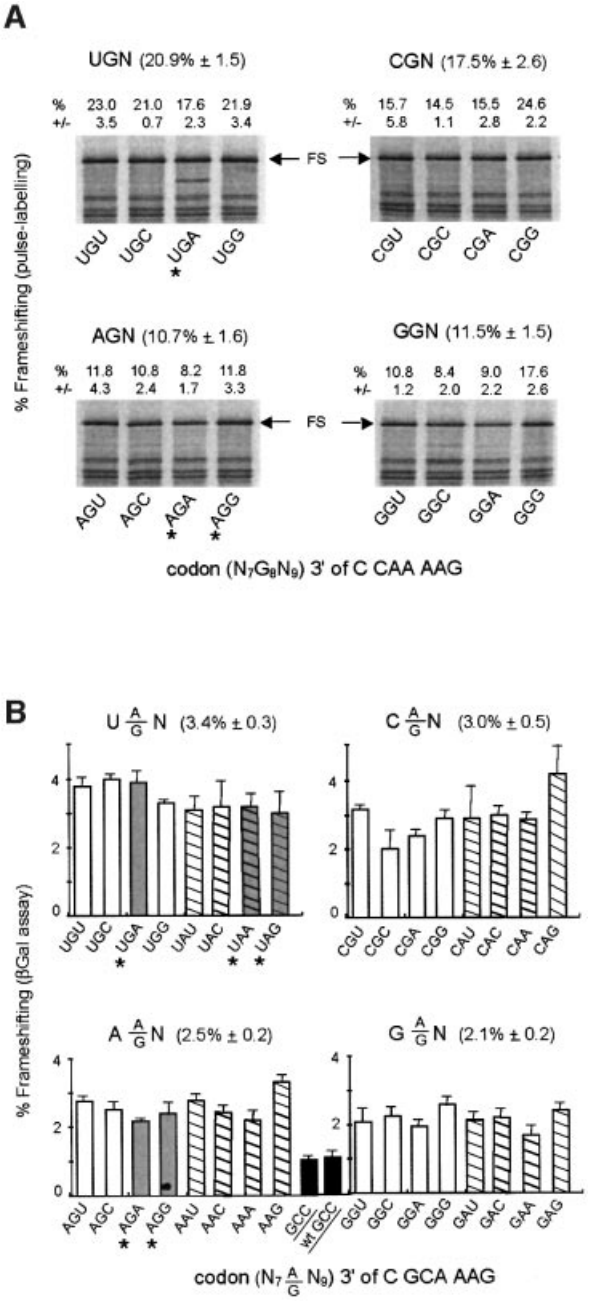

Fig. 2. Effect on frameshifting of variants of the codon 3′ of the IS*3* shift site. The mutations indicated in Figure 1A were introduced on the 3′side (nucleotides $N_7N_8N_9$) of the A AAG shift site and the modified frameshift regions were cloned into the pOFX302 reporter plasmid. (**A**) Summary of the results obtained by pulse-labeling of C CAA AAG $N_7G_8N_9$ constructs. (**B**) Results obtained by performing β-galactosidase assays with C GCA AAG $N_7(G/A)_8N_9$ constructs. The value for both motifs with GCC as 3′ codon is also given (wt stands for C CAA AAG).

However, exceptions to the rule do exist (e.g. CGG or GGG in Figure 2A are higher than their three relatives), suggesting that the second and third 3′ nucleotides can also influence frameshifting.

Whatever the real cause(s) of the observed 3′ context effect is, and more importantly in view of the initial question concerning the position of the AAG-decoding tRNA, the results presented in Figure 2 show clearly that the levels of frameshifting do not significantly vary

between the eight constructs with a 3′ stop, or rare, codon (marked with an asterisk in Figure 2A and B) and most of their related sense codon constructs. There is no increase in frameshifting caused by the presence of a 3′ stop or rare codon. Therefore, in the context of the IS*3* recoding region, frameshifting of $tRNA^{Lys}$ from AAG to AAA is most likely initiated while AAG is in the A-site.

First codon of the hexanucleotide shift site

$tRNA^{Lys}$ re-pairing to mRNA at a cognate codon requires that the last base of the previous codon be A. Previous partial mutational analysis of the *cdd* signal suggested a strong influence on recoding of the identity of the NNA codon, with CGA apparently being the most shift prone (Mejlhede *et al*., 1999). To investigate this question more systematically, CAA was substituted in the IS*3* recoding region by all other NNA codons except for UAA and UGA that would be in-frame stop codons. The 5′ nucleotide, N_0, was also changed as indicated to preclude its involvement in Watson–Crick pairing with a tRNA attempting to re-pair to mRNA at the overlapping –1 frame codon. The results presented in Figure 3A show clearly that the level of frameshifting is strongly influenced by the identity of the first two nucleotides of the NNA AAG hexamer and that several codons are equal to, or better, than CGA. The 14 NNA codons can be separated into two classes, the ones that lead to 'low' frameshifting and those that give 'high' frameshifting. Within each class, there is a notable amount of variation indicative of an extra layer of idiosyncratic behavior. For example, GUA and AGA are respectively remarkably higher and lower than the others. Codons UCA and ACA first appeared as intermediate, but not overlapping with any in the low category (>95% confidence level).

The decoding properties of the wobble base (Crick, 1966; Yokoyama and Nishimura, 1995) of the cognate tRNAs for NNA codons are given in Figure 3B. With the exception of GGA, the NNA codons which give a low level of frameshifting belong to split codon boxes. These codons are decoded by tRNAs with a 'restricted' wobble capacity, i.e. they have a 3′-$N_{36}N_{35}U_{34}$-5′ anticodon (except $tRNA^{Ile}$, which has a 3′-UAC-5′ anticodon) that read NNA and NNG codons only (or AUA only for $tRNA^{Ile}$). In contrast, the NNA codons associated with high frameshifting come from four-codon family boxes. Their respective tRNAs also have a 3′-$N_{36}N_{35}U_{34}$-5′ anticodon, except $tRNA^{Arg}$ 3′-GCI-5′, but can read three codons, those ending with A, G and U (or C, U and A for $tRNA^{Arg}$ 3′-GCI-5′). All the NNA-specific tRNAs have a modified anticodon base U_{34} (or C_{34} to k^2C in $tRNA^{Ile}$, or A_{34} to I in $tRNA^{Arg}$) and the type of U_{34} modification is clearly correlated with 'low' or 'high' shiftiness (Figure 3B). The tRNAs which have an xm^5 type modification (i.e. mnm^5, $cmnm^5$ and mnm^5s^2) restrict frameshifting, whereas those having a modification of the xo^5 type allow more frameshifting to occur.

Possible upstream re-pairing for $tRNA^{NNA}$

A second set of 14 constructs was generated by changing the 5′ N_0 nucleotide to one identical to the first of the NNA codon. The consequences in terms of re-pairing in the –1 frame vary according to each $tRNA/N_0N_1N_2A_3$ pair (Figure 3C). In four cases a consensus heptameric X

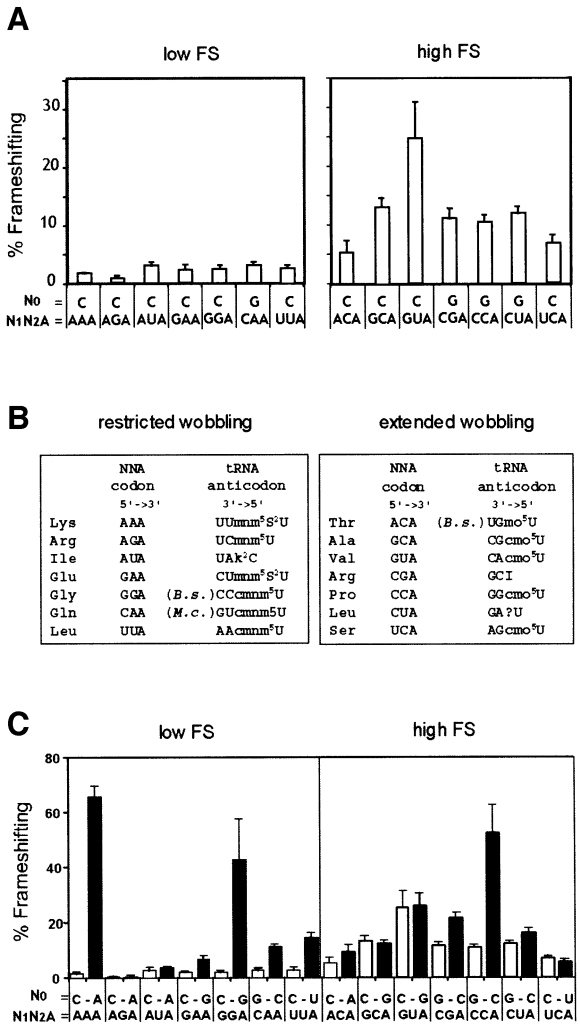

Fig. 3. Effect on frameshifting of $N_0N_1N_2A$ variants: correlation with wobble properties and modification of base 34 of the N_1N_2A-decoding tRNA (**A** and **B**), and variants where N_0 is identical to N_1 (**C**). In (A) and (C), frameshift efficiencies were measured by quantitation of [^{35}S]methionine labeled products. The error bars indicate the 95% confidence interval. In (B), the sequences of the anticodons of the *E.coli* NNA-decoding tRNAs including modifications of base 34 are shown. The modifications are abbreviated as follows: 5-methylaminomethyluridine (mnm^5U), 5-methylaminomethyl-2-thiouridine (mnm^5s^2U), 5-caboxymethylaminomethyluridine (cmnm5U), 5-methoxyuridine (mo^5U), uridine-5-oxyacetic acid (cmo^5U), inosine (I) and lysidine (k^2C). Three anticodon sequences are not from *E.coli* but from *Bacillus subtilis* (B.s.) or *Mycoplasma capricolum* (M.c.) as indicated.

XXY YYZ site for tandem slippage is generated, allowing cognate (A AAA AAG, G GGA AAG) or near cognate (C CCA AAG and U UUA AAG) interaction of P-site $tRNA^{NNA}$ in the –1 phase. Accordingly, frameshifting is greatly stimulated from 6- to nearly 50-fold. For the 10 other NNA codons, the outcome is variable. In two cases, G GAA and C CAA, there is a 4-fold stimulation perhaps related to Watson–Crick pairing of tRNA bases 34 and 36 with the –1 frame codon (middle base 35 would form either an acceptable U·G pair or a less favorable U·C pair). Two others have a 2-fold increase (A ACA and C CGA) and the six remaining cases are not affected; for all eight, Watson–Crick pairing is limited to the interaction between N_0 and anticodon base 36. From this we conclude that providing $tRNA^{NNA}$ with an opportunity to re-pair in a

cognate or near-cognate manner in the –1 phase increases frameshifting efficiency. Restricting pairing to the first position, N_0, of the codon in the new frame has no, or only a marginal, positive effect on frameshifting which must then proceed via re-pairing of the AAG-decoding tRNA only.

Role of tRNA base U_{34} modification

The correlation between base U_{34} modification and frameshifting propensity, as well as many data suggesting that U_{34} modification may contribute to the wobble property of the tRNAs, prompted us to investigate the effect of mutations affecting specifically the xo^5 or the xm^5 modification. Inactivation of the *aroD* gene prevents the formation of cmo^5U (Björk, 1995). Inactivation of *mnmA* and *mnmE*, respectively, precludes replacement of o^2 by s^2 and insertion of the mnm^5 group (Björk, 1995). Two subsets of the NNA AAG constructs, three 'low' and three 'high' frameshifters, were tested in the three modification-deficient mutants (Figure 4); in the chosen constructs, the N_0 nucleotide does not allow upstream re-pairing. The *aroD* mutation did not appear to have any significant effect on frameshifting modulation by the two classes of NNA codons. This indicates that the cmo^5 modification is not what makes the tRNAs with extended wobbling more shift prone. In contrast, the *mnmE* and, more clearly, the *mnmA* mutations led to reduced frameshifting frequency, especially in the case of the 'high' frameshifting NNA codons, for which there is a 2- to 4-fold reduction. With these two mutants, the modification deficiencies affect not only the 'low' frameshifting NNA-decoding tRNAs, but also the downstream $tRNA^{Lys}$, the one that shifts from the 0 to the –1 frame. So in the case of 'high' frameshifting NNA codons, the assay in the *mnm* mutants reveals the importance of modification for the frameshifting capacity of $tRNA^{Lys}$. Obviously, both *mnmA* and *mnmE* alter this capacity, the former more than the latter.

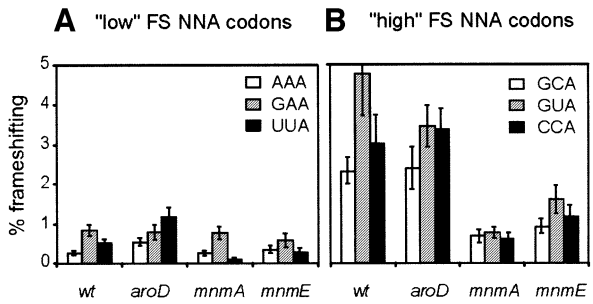

Fig. 4. Frameshifting in modification-deficient mutants. Six NNA mutants (three low frameshifters and three high) were introduced into isogenic wild-type, *aroD*, *mnmA* and *mnmE* strains. Frameshifting was estimated by measuring the β-galactosidase activity of the resulting strains (in the absence of IPTG). The construct in which *g10* and *lacZ* are in the same frame (in-phase construct in Figure 1) served to define the 100% reference activity and a construct in which the A AAG sequence was mutated to a non-slippery one (G AAA) was used to determine the background level.

Discussion

P-site pairing maintained or irreversibly disrupted in hexanucleotide shifting

Influence of a stop codon (or rare codon) on –1 frameshifting is evidence that disruption of codon:anticodon base-pairing and re-pairing in a new frame occurs in the P-site. The observed lack of influence of a stop or rare codon placed immediately 3′ of the CAA AAG or GCA AAG hexanucleotide shift site is interpreted to mean that CAA or GCA, and by extension any other NNA codon, is in the ribosomal P-site and AAG is in the A-site when frameshifting occurs. This is comparable to classical heptanucleotide frameshifting on X XXY YYZ sequences, where the XXY and YYZ 0 frame codons are in the P- and A-site, respectively (Jacks *et al*., 1988; Weiss *et al*., 1989; Harger *et al*., 2002).

An interesting feature of the results is the involvement of hexanucleotide, rather than heptanucleotide, shift sites for the lesser, but still significantly efficient, –1 frameshifting studied. This hexanucleotide frameshifting likely involves the same mRNA movement as in tandem slippage in –1 heptanucleotide frameshifting (Jacks *et al*., 1988; Weiss *et al*., 1989; Harger *et al*., 2002). The difference is that in hexanucleotide frameshifting there is no re-pairing of the P-site tRNA to mRNA. In one model of hexanucleotide frameshifting, outlined in Figure 5A, there is dissociation of pairing in the P-site without re-pairing to mRNA. Lack of involvement of P-site re-pairing is quite plausible, since peptidyl-transfer can sometimes be carried out, *in vitro* and *in vivo*, in the absence of codon–anticodon interaction (Yusupova *et al*., 1986; Atkins *et al*., 2001; A.J.Herr, N.M.Wills, C.Nelson, R.F.Gesteland and J.F.Atkins, in preparation). Our data show that –1 frameshifting is more efficient when there is limited P-site re-pairing potential on non-standard heptamers (e.g. C CAA AAG or G GAA AAG; see Figure 3), provided that two conditions are met: N_0 and N_1 have to be identical and at least one other Watson–Crick pair exists between the tRNA and the –1 frame $N_0N_1N_2$ codon.

An alternative model for hexanucleotide –1 frameshifting is that P-site pairing is partially maintained, detachment of only anticodon base 34 from the third codon base is involved allowing $tRNA^{Lys}$ to re-pair in the –1 frame (Figure 5B). Retention of codon pairing by P-site anticodon bases 36 and 35 would require not only a change of the relative positions of anticodon bases 35 and 34, but also a large change in position within the P-site of the whole anticodon to permit pairing between anticodon base 36 of A-site $tRNA^{Lys}$ and what was the third codon base of the P-site. It is unlikely that $tRNA^{Lys}$ initially pairs in the –1 frame with AAA in the sequence A AAG since the AAG lysine codon is required for efficient frameshifting.

A-site tRNA

Both models require re-pairing of the A-site tRNA in the –1 frame. The frame change could then happen before and/or after GTP hydrolysis and EF-Tu release, after codon recognition, during the second-half of the 'initial selection' steps or at the onset of the following 'accommodation' step, as defined by Rodnina and Wintermeyer (2001). It possibly occurs after the correct codon–anticodon interaction in the A-site triggers adoption of a 'closed' conformation by the 30S subunit (Ogle *et al*., 2002, 2003). Once accepted-in, the A-site tRNA, still in the A/T hybrid state, with or without EF-Tu attached, must have more

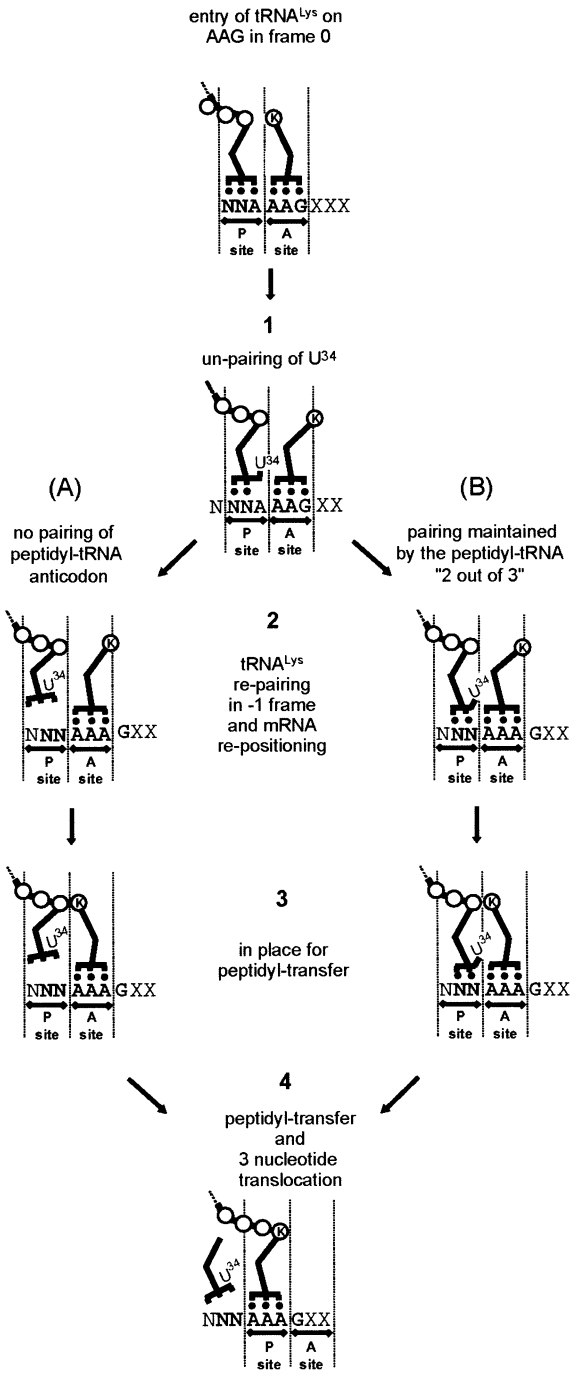

Fig. 5. Models for frameshifting on hexameric motifs. The $N_1N_2A_3$ AAG hexamer is normally read as $N_1N_2A_3$ and $A_4A_5G_6$ in frame 0 (top). Base 34 of the $N_1N_2A_3$-decoding tRNA disengages from pairing with A_3 (stage 1). A-site $tRNA^{Lys}$ re-pairs on the –1 frame $A_3A_4A_5$ codon with re-positioning of the mRNA (stage 2). In (**A**), the P-site anticodon irreversibly dissociates from the mRNA whereas in (**B**), anticodon bases 36 and 35 maintain pairing. Accommodation is then completed (stage 3), bringing the two tRNAs and the mRNA in the configuration seen in crystallized complexes (Yusupova *et al*., 2001) allowing peptidyl-transfer and standard three-base translocation establishing the change in frame (stage 4).

leeway: it can disengage, re-pair in the –1 frame and stay there, especially in the case of *E.coli* $tRNA^{Lys}$, which has a stronger interaction with AAA than with AAG (Lustig *et al*., 1981; Yokoyama and Nishimura, 1995). Re-adjustment by one nucleotide of the mRNA position, brought along by the 3′ pseudoknot (Plant *et al*., 2003), probably occurs at that time (stage 2 in Figure 5). The A-site tRNA eventually moves to reach the A/A state; this requires a large movement (56 Å) of its CCA end and the repositioning by 9 Å of anticodon base 34, accompanied by a rotation around the phosphodiester bond between the P and A codons (Noller *et al*., 2002). Locking in the P/P and A/A states of the two tRNAs and their anticodons probably makes the change in frame irreversible. There is, then, a kink in the message between the P and A codons (stage 3 in Figure 5) and the phosphate group of base 1401 of 16S RNA is wedged between the last and the first bases of each codon (Ogle *et al*., 2001; Yusupova *et al*., 2001). What is unusual here is that the P-site codon should contain at most two paired bases. Re-pairing of the A-site tRNA in the –1 frame and mRNA movement resets the reading frame. Peptidyl transfer can now take place and be followed by a normal three-base translocation (stage 4 in Figure 5).

This model of re-alignment of aminoacyl $tRNA^{Lys}$ occurring prior to translocation derives from the one originally proposed for tandem –1 slippage (Jacks *et al*., 1988) and very recently refined and justified by Harger *et al*. (2002). However, for both tandem –1 slippage (Weiss *et al*., 1989; Atkins and Gesteland, 1995) and the P-site pairing model presented here, another proposal invokes slippage after transpeptidation, and perhaps during translocation resulting effectively in a two-base translocation. Arguments against this version were presented in detail by Harger *et al*. (2002). What we would like to add, in view of the three-dimensional model for tRNAs movements outlined by Noller *et al*. (2002), is that directly after transpeptidation the tRNAs are still in the same 'locked' configuration, and are therefore unlikely to change frame. Translocation *per se* is, together with peptidyl-transfer, the fundamental function of the ribosome, and as such it probably is the most constrained one. Again it is difficult to envision the tRNAs and the mRNA being loose at this critical stage. The advantage of frameshifting occurring at the accommodation step is that it does not tamper with the strict three-base translocation mechanism.

P-site tRNA

In both versions of the model, anticodon base 34 of the P-site tRNA has the ability (to different extents depending on the tRNA) of un-pairing from the third codon base. In the version where P-site pairing is irreversibly disrupted, there is no necessary tRNA distortion. However, in the version where anticodon bases 35 and 36 maintain pairing, disruption of the anticodon base 34 interaction is most likely due to its flipping out of the anticodon stack. tRNAs are known to possess a large degree of structural flexibility. Recent studies by cryo-electron microscopy suggest that the aminoacyl-tRNA could participate actively in the accommodation step via conformational changes in its anticodon arm (Stark *et al*., 2002; Valle *et al*., 2002). At a more refined level, molecular dynamics studies indicates that the anticodon loop (as well as the acceptor arm) is potentially a region with a large amplitude mobility (Matsumoto *et al*., 1999). NMR analyses of synthetic anticodon regions derived from a few tRNAs

give an even more precise idea of the degree of mobility of individual nucleotides in the anticodon loop and of the effect of modification of bases 34 and 37 in particular (Clore *et al*., 1984; Schweisguth and Moore, 1997; Sundaram *et al*., 2000; Cabello-Villegas *et al*., 2002). Even if most, in their fully modified form, adopt, in solution, a classical 3′-stacked loop configuration, with a U-turn between nucleotide 33 and 34, anticodon bases 34, 35 and 36 are still fairly mobile. In one case, *E.coli* $tRNA^{Phe}$, the loop is reduced to bases 34 to 36 even when base 37 is modified as it is *in vivo*, suggesting that some anticodon loops may adopt, in solution, a conformation differing from the classical one (Cabello-Villegas *et al*., 2002). However, two missing pseudouridine modifications (U32 and U39) in the analyzed anticodon stem–loop may be in part responsible for this unorthodox configuration. In the case of $tRNA^{Lys}$, absence of modification also lead to a pseudo tri-loop anticodon, and addition of the modifications ($t6A_{37}$, $mnm^5s^2U_{34}$, ψU_{39}) bring the structure to the standard 7-nucleotide loop, which, however, remains flexible (Durant and Davis, 1999; Sundaram *et al*., 2000). It therefore appears that the s^2 and mnm^5 modifications increase the rigidity of the anticodon loop, in particular by strongly shifting the ribose conformation toward the C3′-endo form, and thus allowing reading of A- and G-ending codons only (restricted wobble). In contrast the cmo^5, and related modification of U_{34}, have been proposed to tilt the balance in a more moderate manner. The C2′-endo form predominates but the C3′-endo form is also present and the end result is more flexibility. This allows interaction of U_{34} with G- or U-ending codons (extended wobble), when in the C2′-endo configuration, and also with A-ending codons, when in the C3′-endo form (Yokoyama *et al*., 1985; Yokoyama and Nishimura, 1995). Thus these data, linking anticodon base flexibility and wobble capacity, are in agreement with our finding that there are two types tRNAs as judged by their effect on frameshifting on NNA AAG hexamers. We can now reformulate our conclusion and say that tRNAs with a more flexible base 34 (xmo^5U in six cases and I in one; see Figure 3) are more frameshift-promoting than those with less flexibility at that position (xm^5U in six cases and k^2C in one). At the molecular level, the C2′/C3′-endo interconversion may be what temporarily brings base 34 out of pairing with the third base of NNA codons (or what causes all three bases of $tRNA^{NNA}$ anticodon to disengage, according to the alternate scenario). To conclude, it appears that tRNAs anticodons are not extremely rigid and that there are probably large differences among them from that point of view. Such flexibility may well allow transitory un-pairing of base 34 (and perhaps of bases 35 and 36 also), especially since there is not a close monitoring of the codon–anticodon interaction in the P-site (Ogle *et al*., 2001).

Modification of tRNA base 34

The apparent correlation between frameshifting, wobbling and the modification pattern of base U_{34} (Figure 3) led us to examine frameshifting in modification-deficient mutants, with the hope it would provide a new window for assessing the function of modification at that position in the anticodon (Figure 4). This hope was only partly fulfilled. Absence of the cmo^5 modification did not change the incidence on frameshifting of the relevant NNA-tRNAs. This means that the flexibility of base U_{34} is still the same without the cmo^5 group. The result was not entirely unexpected in view of the higher mobility displayed by that nucleotide when it is non-modified. This higher mobility expands wobbling further, since unmodified U_{34} recognizes codons ending with any nucleotide, at least *in vitro* (Yokoyama and Nishimura, 1995). Thus, the cmo^5 modification is not the cause of U_{34} mobility, it probably limits it to prevent pairing with C; it rather is, like frameshifting stimulation, a consequence of structural properties of the anticodon region shared by one class of NNA-decoding tRNAs (Grosjean *et al*., 1996). In contrast, absence of either the s^2 or mnm^5 modifications had a negative effect on frameshifting on the most efficient NNA AAG motifs. There, it was the A-site $tRNA^{Lys}$ that was affected by the mutation. A known effect of s^2, and of mnm^5 to a lesser extent, is to favor pairing of $tRNA^{Lys}$ on AAA over AAG by increasing the rigidity of the anticodon (Yokoyama *et al*., 1985; Yokoyama and Nishimura, 1995). In the absence of one or other modification, anticodon base 34 is more flexible, allowing easier adjustment for proper pairing with G. This makes re-pairing from AAG to AAA energetically less advantageous and therefore frameshifting becomes less frequent.

The finding of significant levels of frameshifting at multiple hexanucleotide sequences has substantial relevance for ongoing searches to discover where programmed frameshifting is utilized for gene expression. While utilization by the IS elements mentioned above provide some initial examples, the generality of this form of recoding remains to be determined.

Materials and methods

Bacterial strains and growth conditions

The *E.coli* K12 strain JS238 [MC1061, *araD* Δ*(ara leu) galU galK hsdS rpsL* Δ*(lacIOPZYA)X74 malP::lacIQ srlC::*Tn*10 recA1*] was used for all cloning experiments.

Strains with mutations in tRNA modification genes were provided by Professor G.Björk: TH194 (*aroD*$^+$, *mnmA*$^+$, *mnmE*$^+$), GRB2162 (*aroD*), TH193 (*mnmA*) and TH99 (*mnmE*) (Urbonavicius *et al*., 2001). These strains were transformed with plasmid pAP2-*lacI*^{Q} (P.Polard, unpublished) before introduction of the various pOFX302-based frameshift constructions. This plasmid, being based on a p15A replicon, is compatible with pBR322 derivatives and carries a kanamycin resistance gene as well as the *lacI*^{Q} gene, which ensure a tight control of the Tac promoter carried by pOFX302. Bacteria were grown in LB medium (Sambrook *et al*., 1989) or, for protein labeling, in MOPS medium (Neidhardt *et al*., 1974) supplemented with glucose (0.5%), thiamine (2 mg/l) and all amino acids at 50 μg/ml each (except methionine, tryptophan and tyrosine). Rambach agar plates (Merck) were used to identify clones expressing β-galactosidase. Ampicillin (40 μg/ml) plus oxacillin (200 μg/ml), and kanamycin (25 μg/ml) were added when necessary.

DNA techniques and quantitation of radioactive macromolecules

Plasmid DNA was prepared using the Qiaprep or Qiagen-tip100 systems as indicated by the supplier (Qiagen). Restriction enzymes, T4 polynucleotide kinase and T4 DNA ligase were from New England Biolabs. Ampli*Taq* DNA polymerase and the Amplicycle sequencing kit were from Applera. Cloning, transformation, agarose gel electrophoresis, and sequencing gels were carried out according to standard procedures (Sambrook *et al*., 1989). Radioactive products ([γ-^{33}P]ATP for DNA sequencing and [^{35}S]methionine for *in vivo* protein labeling) were obtained from Amersham. The Fuji X BAS1000 phosphoimager and the

PCBas software were used for the quantitative analysis of electrophoresis gels in which ^{35}S-labeled proteins were separated.

Plasmids constructions

Mutants of the IS*3* (or IS*1222*) frameshift region were cloned into the reporter plasmid pOFX302 described by Rettberg *et al*. (1999). In one set the second and third nucleotides of the C CAA AAG sequence containing the IS*3* shift site was changed to all possible sequences (except TA and TG, to avoid in-frame stop codon); the first base was changed to G when the second was C. In a second set of 14 constructions, the first base was made identical to the second. Two control plasmids were also generated, in one the C CAA AAG was changed to C CAG AAA, to prevent frameshifting (0% frameshifting construct) and in the other a base was added to C CAA AAA G in order to set *g10* and *lacZ* in the same phase (100% frameshifting construct).

Measurement of frameshifting frequency

Frameshifting frequency was determined by *in vivo* protein pulse labeling with [^{35}S]methionine on four independent clones for each construct, following a previously described protocol (Rettberg *et al*., 1999; Bertrand *et al*., 2002). To calculate the frequency of frameshifting, the fraction of the total radioactivity present in the relevant band was divided by the corresponding value obtained for the in-phase control. Precision was assessed by calculation of the 95% confidence interval.

In some experiments frameshifting was estimated by measuring β-galactosidase activity. For each strain, four to eight tubes containing 0.5 ml of LB (supplemented with kanamycin, ampicillin and oxacillin) were inoculated with independent clones and incubated overnight at 37°C. After a 1/5 dilution in LB, the absorbance at 600 nm of each culture was measured on 125 µl in a 96 flat-bottomed wells microplate (optical path of 0.38 cm) with a spectramax 340PC spectrophotometer (Molecular Devices). The diluted cultures (0.5 ml) were adjusted to 1× Z* buffer [Z buffer from Miller (1992), supplemented with 0.005% SDS, 1 mg/ml BSA and 10 mM DTT instead of β-mercaptoethanol] and treated for 10 min at 0°C with 10 µl of CHCL3. Assays were prepared in 96-well microplates. A volume of extract depending on the activity was completed to 200 µl with Z* buffer and 50 µl of 4 mg/ml ONPG were added. Absorbance was read at 420 nm each minute over a 30 min period with a Spectramax 340PC spectrophotometer. In order to be directly comparable to those obtained with the classical protocol of Miller (1992), our specific activities were calculated for a volume of extract of 125 µl and for an OD_{600} of 1. As in the pulse-labeling experiments, the in-phase control served as 100% reference and precision was assessed by determining the 95% confidence interval.

Note that absolute levels of frameshifting measured that way are about nine times lower than those obtained by pulse-labeling of the same constructs. This is likely due to underestimation of the 100% value in the case of pulse-labeling (induction of the strong pTac promoter probably saturates the protein synthesis capacity). However, both methods gave identical results in terms of relative activities of the various constructs.

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References

Atkins,J.F. and Gesteland,R.F. (1995) Discontinuous triplet decoding with or without re-pairing by peptidyl tRNA. In Söll,D. and RajBhandary,U.L. (eds), *tRNA: Structure, Biosynthesis and Function*. ASM Press, Washington, DC, pp. 471–490.

Atkins,J.F., Herr,A., Massire,C., O'Connor,M., Ivanov,I. and Gesteland, R.F. (2000) Poking a hole in the sanctity of the triplet code: inferences for framing. In Garrett,R.A., Douthwaite,S.R., Liljas,A., Matheson, A.T., Moore,P.B. and Noller,H.F. (eds), *The Ribosome: Structure, Function, Antibiotics and Cellular Interactions*. ASM Press, Washington, DC, pp. 369–383.

Atkins,J.F. *et al*. (2001) Over-riding standard decoding: implication of recoding for ribosome function and enrichment of gene expression. In *Cold Spring Harbor Symposium on Quantitative Biology, Vol. LXVI, The Ribosome*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 217–232.

Bertrand,C., Prère,M.F., Gesteland,R.F., Atkins,J.F. and Fayet,O. (2002) Influence of the stacking potential of the base 3′ of tandem shift codons on –1 ribosomal frameshifting used for gene expression. *RNA*, **8**, 16–28

Björk,G.R. (1995) Biosynthesis and function of modified nucleosides in tRNA. In Söll,D. and RajBhandary,U.L. (eds), *tRNA: Structure, Biosynthesis and Function*. ASM Press, Washington, DC, pp. 165–205.

Brierley,I., Jenner,A.J. and Inglis,S.C. (1992) Mutational analysis of the "slippery-sequence" component of a coronavirus ribosomal frameshifting signal. *J. Mol. Biol.*, **227**, 463–479.

Cabello-Villegas,J., Winkler,M.E. and Nikonowicz,E.P. (2002) Solution conformations of unmodified and A(37)N(6)-dimethylallyl modified anticodon stem–loops of *Escherichia coli* tRNA(Phe). *J. Mol. Biol.*, **319**, 1015–1034.

Carter,R.J., Baeyens,K.J., SantaLucia,J., Turner,D.H. and Holbrook,S.R. (1997) The crystal structure of an RNA oligomer incorporating tandem adenosine-inosine mismatches. *Nucleic Acids Res.*, **25**, 4117–4122.

Chandler,M. and Fayet,O. (1993) Translational frameshifting in the control of transposition in bacteria. *Mol. Microbiol.*, **7**, 497–503.

Clore,G.M., Gronenborn,A.M., Piper,E.A., McLaughlin,L.W., Graeser,E. and van Boom,J.H. (1984) The solution structure of a RNA pentadecamer comprising the anticodon loop and stem of yeast $tRNA^{Phe}$. A 500 MHz 1H-n.m.r. study. *Biochem. J.*, **221**, 737–751.

Crick,F.H.C. (1966) Codon–anticodon pairing: the wobble hypothesis. *J. Mol. Biol.*, **19**, 548–545.

Curran,J.F. (1995) Decoding with the A:I wobble pair is inefficient. *Nucleic Acids Res.*, **23**, 683–688.

Durant,P.C. and Davis,D.R. (1999) Stabilization of the anticodon stem-loop of $tRNA^{Lys,3}$ by an A^{+}–C base-pair and by pseudouridine. *J. Mol. Biol.*, **285**, 115–131.

Farabaugh,P.J. (1997) *Programmed Alternative Reading of the Genetic Code*. R.G.Landes Company, Austin, TX.

Farabaugh,P.J. and Björk,G.R. (1999) How translational accuracy influences reading frame maintenance. *EMBO J.*, **18**, 1427–1434.

Gallant,J.A. and Lindsley,D. (1992) Leftward ribosome frameshifting at a hungry codon. *J. Mol. Biol.*, **223**, 31–40.

Gao,H. *et al*. (2003) Study of the structural dynamics of the *E. coli* 70S ribosome using real-space refinement. *Cell*, **113**, 789–801.

Gramstat,A., Prüfer,D. and Rohde,W. (1994) The nucleic acid-binding zinc finger protein of potato virus M is translated by internal initiation as well as by ribosomal frameshifting involving a novel mechanism of P-site slippage. *Nucleic Acids Res.*, **22**, 3911–3917.

Grosjean,H., Edqvist,J., Straby,K.B. and Giegé,R. (1996) Enzymatic formation of modified nucleosides in tRNA: dependence on tRNA architecture. *J. Mol. Biol.*, **255**, 67–85.

Harger,J.W., Meskauskas,A. and Dinman,J.D. (2002) An "integrated model" of programmed ribosomal frameshifting. *Trends Biochem. Sci.*, **27**, 448–454.

Jacks,T., Madhani,H.D., Masiarz,F.R. and Varmus,H.E. (1988) Signals for ribosomal frameshifting in the Rous Sarcoma Virus *gag-pol* region. *Cell*, **55**, 447–458.

Larsen,B., Wills,N.M., Gesteland,R.F. and Atkins,J.F. (1994) rRNA–mRNA base pairing stimulates a programmed –1 ribosomal frameshift. *J. Bacteriol.*, **176**, 6842–6851.

Larsen,B., Gesteland,R.F. and Atkins,J.F. (1997) Structural probing and mutagenic analysis of the stem–loop required for *Escherichia coli dnaX* ribosomal frameshifting: Programmed efficiency of 50%. *J. Mol. Biol.*, **271**, 47–60.

Lustig,F., Elias,P., Axberg,T., Samuelsson,T., Tittawella,I. and Lagerkvist,U. (1981) Codon reading and translational error. Reading of the glutamine and lysine codons during protein synthesis *in vitro*. *J. Biol. Chem.*, **256**, 2635–2643.

Mahillon,J. and Chandler,M. (1998) Insertion sequences. *Microbiol. Mol. Biol. Rev.*, **62**, 725–774.

Matsumoto,A., Tomimoto,M. and Go,N. (1999) Dynamical structure of transfer RNA studied by normal mode analysis. *Eur. Biophys. J.*, **28**, 369–379.

Mejlhede,N., Atkins,J.F. and Neuhard,J. (1999) Ribosomal frameshifting

during decoding of *Bacillus subtilis cdd* occurs at the sequence CGA AAG. *J. Bacteriol.*, **181**, 2930–2937.
Miller,J.H. (1992) *A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for Escherichia coli and Related Bacteria*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Neidhardt,F.C., Bloch,P.L. and Smith,D.F. (1974) Culture medium for enterobacteria. *J. Bacteriol.*, **119**, 736–747.
Noller,H.F., Yusupov,M.M., Yusupova,G.Z., Baucom,A. and Cate,J.H. (2002) Translocation of tRNA during protein synthesis. *FEBS Lett.*, **514**, 11–16.
Ogle,J.M., Brodersen,D.E., Clemons,W.M.,Jr, Tarry,M.J., Carter,A.P. and Ramakrishnan,V. (2001) Recognition of cognate transfer RNA by the 30S ribosomal subunit. *Science*, **292**, 897–902.
Ogle,J.M., Murphy,F.K., Tarry,M.J. and Ramakrishnan,V. (2002) Selection of tRNA by the ribosome requires a transition from an open to a closed form. *Cell*, **111**, 721–732.
Ogle,J.M., Carter,A.P. and Ramakrishnan,V. (2003) Insights into the decoding mechanism from recent ribosome structures. *Trends Biochem. Sci.*, **28**, 259–266.
Plant,E.P., Jacobs,K.L., Harger,J.W., Meskauskas,A., Jacobs,J.L., Baxter,J.L., Petrov,A.N. and Dinman,J.D. (2003) The 9-Å solution: How mRNA pseudoknots promote efficient programmed –1 ribosomal frameshifting. *RNA*, **9**, 168–174.
Polard,P., Prère,M.F., Chandler,M. and Fayet,O. (1991) Programmed translational frameshifting and initiation at an AUU codon in gene expression of bacterial insertion sequence IS*911*. *J. Mol. Biol.*, **222**, 465–477.
Polard,P., Prère,M.F., Fayet,O. and Chandler,M. (1992) Transposase-induced excision and circularization of the bacterial insertion sequence IS*911*. *EMBO J.*, **11**, 5079–5090.
Rettberg,C.C., Prère,M.F., Gesteland,R.F., Atkins,J.F. and Fayet,O. (1999) A three-way junction and constituent stem–loops as the stimulator for programmed –1 frameshifting in bacterial insertion sequence IS*911*. *J. Mol. Biol.*, **286**, 1365–1378.
Rodnina,M. and Wintermeyer,W. (2001) Fidelity of aminoacyl-tRNA selection on the ribosome: kinetic and structural mechanisms. *Annu. Rev. Biochem.*, **70**, 415–435.
Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Schweisguth,D.C. and Moore,P.B. (1997) On the conformation of the anticodon loops of initiator and elongator methionine tRNAs. *J. Mol. Biol.*, **267**, 505–519.
Sekine,Y., Eisaki,N. and Ohtsubo,E. (1994) Translational control in production of transposase and in transposition of insertion sequence IS*3*. *J. Mol. Biol.*, **235**, 1406–1420.
Stark,H., Rodnina,M.V., Wieden,H.J., Zemlin,F., Wintermeyer,W. and van Heel,M. (2002) Ribosome interactions of aminoacyl-tRNA and elongation factor Tu in the codon-recognition complex. *Nat. Struct. Biol.*, **9**, 849–854.
Steibl,H. and Lewecke,F. (1995) IS*1222*: analysis and distribution of a new insertion sequence in *Enterobacter agglomerans* 339. *Gene*, **156**, 37–42.
Sundaram,M., Durant,P.C. and Davis,D. (2000) Hypermodified nucleosides in the anticodon of $tRNA^{Lys}$ stabilize a canonical U-turn structure. *Biochemistry*, **39**, 12575–12584.
Tsuchihashi,Z. and Brown,P.O. (1992) Sequence requirements for efficient translational frameshifting in the *Escherichia coli dnaX* gene and the role of an unstable interaction between $tRNA^{Lys}$ and an AAG lysine codon. *Genes Dev.*, **6**, 511–519.
Urbonavicius,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.
Valle,M., Sengupta,J., Swami,N.K., Grassucci,R.A., Burkhardt,N., Nierhaus,K.H., Agrawal,R.K. and Frank,J. (2002) Cryo-EM reveals an active role for aminoacyl-tRNA in the accommodation process. *EMBO J.*, **21**, 3557–3567.
Weiss,R.B., Dunn,D.M., Atkins,J.F. and Gesteland,R.F. (1987) Slippery runs, shifty stops, backward steps and forward hops: –2, –1, +1, +2, +5 and +6 ribosomal frameshifting. In *Cold Spring Harbor Symposium on Quantitative Biology*, Vol. LII. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 687–693.
Weiss,R.B., Dunn,D.M., Shuh,M., Atkins,J.F. and Gesteland,R.F. (1989) *E. coli* ribosomes re-phase on retroviral frameshift signals at rates ranging from 2 to 50 percent. *New Biol.*, **1**, 159–169
Yokoyama,S. and Nishimura,S. (1995) Modified nucleosides and codon recognition. In Söll,D. and RajBhandary,U.L. (eds), *tRNA: Structure, Biosynthesis and Function*. ASM Press, Washington, DC, pp. 207–223.
Yokoyama,S., Watanabe,T., Murod,K., Ishikura,H., Yamaizumi,Z., Nishimura,S. and Miyazawa,T. (1985) Molecular mechanism of codon recognition by tRNA species with modified uridine in the first position of the anticodon. *Proc. Natl Acad. Sci. USA*, **82**, 4905–4909.
Yusupova,G.Z., Belitsina,N.V. and Spirin,A.S. (1986) Template-free ribosomal synthesis of polypeptides from aminoacyl-tRNA. Polyphenylalanine synthesis from phenylalanine-$tRNA^{Lys}$. *FEBS Lett.*, **206**, 142–146.
Yusupova,G.Z., Yusupov,M.M., Cate,J.H. and Noller,H.F. (2001) The path of messenger RNA through the ribosome. *Cell*, **106**, 233–241.

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