

## EMBO MEMBER'S REVIEW

# How translational accuracy influences reading frame maintenance

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**Most missense errors have little effect on protein function, since they only exchange one amino acid for another. However, processivity errors, frameshifting or premature termination result in a synthesis of an incomplete peptide. There may be a connection between missense and processivity errors, since processivity errors now appear to result from a second error occurring after recruitment of an errant aminoacyl-tRNA, either spontaneous dissociation causing premature termination or translational frameshifting. This is clearest in programmed translational frameshifting where the mRNA programs errant reading by a near-cognate tRNA; this error promotes a second frameshifting error (a dual-error model of frameshifting). The same mechanism can explain frameshifting by suppressor tRNAs, even those with expanded anticodon loops. The previous model that suppressor tRNAs induce quadruplet translocation now appears incorrect for most, and perhaps for all of them. We suggest that the 'spontaneous' tRNA-induced frameshifting and 'programmed' mRNA-induced frameshifting use the same mechanism, although the frequency of frameshifting is very different. This new model of frameshifting suggests that the tRNA is not acting as the yardstick to measure out the length of the translocation step. Rather, the translocation of 3 nucleotides may be an inherent feature of the ribosome.**

*Keywords:* dual-error model/frameshift/missense errors/translation/yardstick model

## Introduction

How does the ribosome manage to decode a messenger RNA reading successive adjacent triplet codons without the use of some kind of internal punctuation to identify the reading frame? Even before the triplet nature of the code had been established, Crick *et al.* (1961) suggested that the protein must be expressed by 'starting at a fixed point and working along the sequence of bases three at a time'. Only the starting and ending points of translation are encoded in the RNA, and the ribosome is somehow able to faithfully maintain the reading frame recognizing only adjacent triplets. The fact that the anticodon of the tRNA consists of three nucleotides complementary to the codon (Holley, 1965) suggested that the tRNA may measure out the codon using the anticodon as a yardstick. The later identification of

frameshift suppressor tRNAs that appeared to use an expanded 4-nucleotide anticodon to read a 4-nucleotide codon (reviewed by Atkins *et al.*, 1991) reinforced the idea that the length of the anticodon determines step size during translation. Yet although the yardstick model is approaching its 40th anniversary we still have an inadequate description of molecular mechanisms that allow the ribosome to choose faithfully only adjacent triplets.

## Types of translational errors

The process of translation elongation is a complex one, and therefore there are potentially many ways the process can go awry. Formally, there are two possible kinds of elongation errors: missense errors, which result in the substitution of one amino acid for another (termination codon readthrough is a special case of this type), and processivity errors, which cause premature termination. We actually know a great deal about missense errors—the types of errors that occur, the stage of the translation cycle during which they occur and the identity of many factors that modulate the frequency of such errors. The consensus of the data is that missense errors occur during aminoacyl-tRNA selection either because of reading by mischarged cognate-tRNAs or by a non-cognate tRNA. Misacylation is outside the scope of this review, but it is subject to its own error-correction mechanisms (reviewed by Jakubowski and Goldman, 1992). Surprisingly, the mechanism of translational missense error correction is still controversial. One concept is that the ribosome distinguishes between correct and incorrect tRNAs by the kinetics of their associating with and dissociating from the ribosome, a process termed kinetic proofreading (reviewed by Thompson, 1988). In this model, cognate tRNAs bind irreversibly to the ribosome, but non-cognates are rejected at either of two successive discrimination steps, before and after GTP hydrolysis by elongation factor-1A (EF-1A, formerly termed in eukaryotes as eEF-1 $\alpha$  and in bacteria as EF-Tu). An alternative view states that discrimination is accomplished by an allosteric interaction between tRNAs in the Exit, or E-site, and the A-site that reduces the rate of binding of incorrect tRNAs to the A-site (Nierhaus, 1990). Of course, the two concepts need not be in conflict, as recently pointed out by Czworkowski and Moore (1996). What is important about both models is that the concentration of cognate-tRNA relative to all other incorrect tRNAs is an important determinant of translational accuracy. Perhaps this is why there is no more than a ~10-fold difference in concentration between the most and least abundant tRNAs (Ikemura and Ozeki, 1983; Inokuchi and Yamao, 1995); a greater difference might lead to incorrect decoding of the codons read by non-abundant tRNAs. In fact, missense errors are stimulated by amino acid starvation, consistent with the

idea that competition between cognate and non-cognate aminoacyl-tRNAs determines the frequency of errors.

Most missense errors are not harmful, since most amino acid substitutions do not eliminate protein function, in sharp contrast to processivity errors which result in a truncated and usually non-functional protein (Kurland *et al.*, 1996). Processivity errors are of two types: premature termination of translation, and translational frameshifting. Of these, premature termination is far more common (Kurland *et al.*, 1996) and almost always occurs by a process called ribosome editing (Menninger, 1977) in which the peptidyl-tRNA spontaneously dissociates from the ribosome ('drop-off'). The model suggests that drop-off occurs when erroneously decoding non-cognate peptidyl-tRNAs dissociate from the P-site because of their weak interaction with the mRNA. In support of this concept, ribosome editing is stimulated by amino acid starvation (Caplan and Menninger, 1979). Premature termination could also occur if peptide release factor were to inaccurately recognize a sense codon as a terminator, but recent work has shown that such false stops constitute an insignificant proportion of processivity errors (Jørgensen *et al.*, 1993).

The second type of error, translational frameshifting, affects processivity because it precludes completion of the nascent peptide chain in the normal reading frame and because ribosomes usually encounter a termination codon rather soon in the shifted frame. But like false stops, frameshift errors are very infrequent, probably much less frequent than  $10^{-5}$  per codon (Kurland, 1979, 1992). As will be shown below, frameshift errors, like missense errors, may occur subsequent to selection of near or non-cognate tRNA in the A-site.

### Frameshift mutations and tRNA suppressors

The genetic analysis of reading frame maintenance is founded on analysis of mutants that disrupt the reading frame by deleting or inserting single nucleotides. Exposure to the acridine mutagens proflavin or ICR-191 induced classical frameshift mutations, insertions or deletions within runs of G-C (Calos and Miller, 1981; Skopek and Hutchinson, 1984). External suppressors of 1 bp deletions (-1 frameshifts) were rare, but suppressors of +1 insertion mutations were easily identified. The two most common classes of +1 frameshift suppressors were mutant forms of tRNA<sup>Pro</sup> or tRNA<sup>Gly</sup> isoacceptors (reviewed by Atkins *et al.*, 1991). Most of these tRNAs have expanded anticodon loops, many having a single nucleotide inserted into the anticodon. Mutants of tRNA<sup>Gly</sup>, suppressing at GGN sites (where N could be any nucleotide), had an extra C in their NCC anticodon (Riddle and Carbon, 1973; Gaber and Culbertson, 1982). Similarly, tRNA<sup>Pro</sup> mutant suppressors of CCCN sites had an extra G added to an NGG anticodon (Sroga *et al.*, 1992; J.N.Li and G.R.Björk, unpublished).

The structure of these mutant tRNAs suggested a simple and elegant hypothesis to explain frameshift suppression: a 4-base anticodon could base pair with a 4-base codon, causing quadruplet translocation and thereby shifting the reading frame +1 as a result (Figure 1). Surprisingly, certain mutant tRNAs suppress without the need to form a fourth base pair with the mRNA. In particular, for *sufJ*

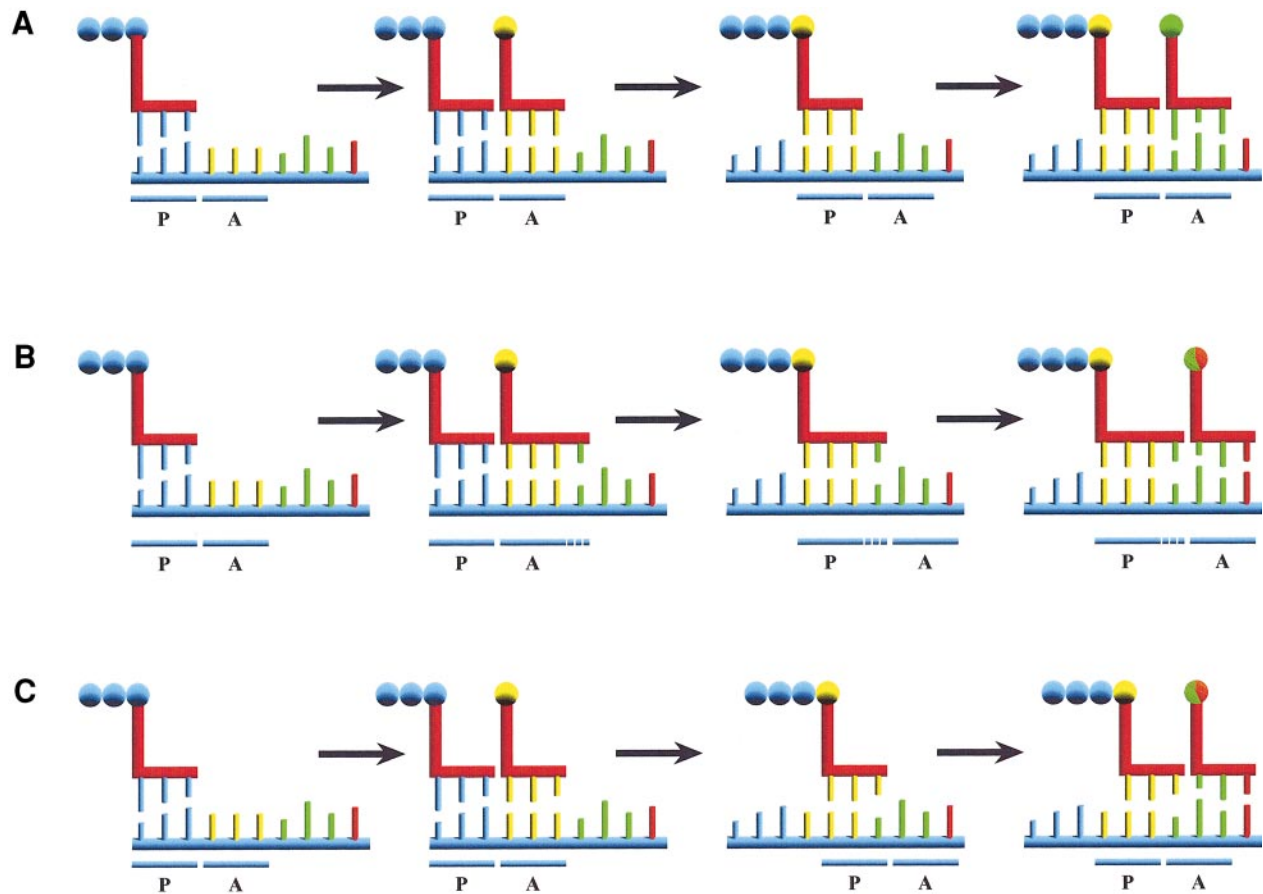
suppressors in *Salmonella typhimurium* (Bossi and Roth, 1981) and SUF16 suppressors of *Saccharomyces cerevisiae* (Gaber and Culbertson, 1984), suppression is nearly insensitive to the nature of the first base of the presumptive 4-nucleotide anticodon. Bossi and Smith (1984) proposed a modification of the classical suppression model in which the extended anticodon sterically interferes with reading the adjacent in-frame codon by the next tRNA without the need for base pairing. The model maintains a 4-nucleotide translocation and thereby a yardstick role of the tRNA. By inference, the 3-base repeat of normal translocation would be dictated by the normal sized anticodon loop using a 3-nucleotide yardstick. Some frameshift suppressor tRNAs actually have normal anticodon loops containing 3-nucleotide anticodons, but are altered within the body of the tRNA (Hüttenhofer *et al.*, 1990; Sroga *et al.*, 1992; Qian and Björk, 1997). It is unclear how the yardstick role of the anticodon is modified in these tRNAs.

Recent work has brought this long accepted model into question (Qian *et al.*, 1998). The data show that some suppressor tRNAs are incapable of reading a 4-nucleotide anticodon, apparently invalidating the yardstick model. But then how do mutant tRNAs shift reading? The answer to this question suggests both a connection to the seemingly unrelated phenomenon of programmed translational frameshifting, and a different solution to the corollary problem of how normal tRNAs maintain the 3-nucleotide reading frame of translation.

### Programmed frameshifting

Programmed frameshifting, reading frame changes caused by mRNA sequences, have generally been considered special cases, quite distinct from 'spontaneous' frameshift errors or frameshifting caused by mutant tRNAs. Partly, this was because programmed frameshifting could be so efficient, approaching 100% (Farabaugh, 1996, 1997), compared with the much lower frequency of spontaneous frameshift errors ( $<10^{-5}$  per codon) or frameshift suppression by mutant tRNAs (a few percent). In addition, programmed frameshift sites include special sequence features that are not observed at spontaneous frameshifting sites. The dogma of the field has been that these special features cause a change in the rules of translation that allows a normally very unlikely event to occur frequently.

Two programmed +1 frameshifts provide examples for which the mechanism of shifting has been revealed in detail. These events are the autoregulatory synthesis of the bacterial release factor 2 (RF2) encoded by the *prfB* gene in *Escherichia coli*, and frameshift-dependent expression of a retroviral *pol* gene analog in the yeast Ty (reviewed in Farabaugh, 1996). Frameshifting in *prfB* depends on three stimulatory features: a 4-nucleotide slippery sequence, an in-frame stop codon (UGA) and a Shine-Dalgarno interaction site upstream of the frameshift site (Weiss *et al.*, 1987, 1988). A ribosome initiating on the *prfB* messenger translates until it encounters the UGA codon in the A-site. The ability to recognize the UGA regulates frameshifting (Craigie and Caskey, 1986; Donly *et al.*, 1990). If the level of RF2 is sufficiently high, it will efficiently recognize the UGA codon, and promote



**Fig. 1.** (A) The process of elongation. The translation cycle begins with a peptidyl-tRNA (anticodon bases shown in blue) bound to the mRNA in the P-site and an empty A-site (codon bases in yellow). An aa-tRNA (yellow anticodon bases) is accepted in the A-site. Peptide transfer and a 3-nucleotide translocation, which moves the aa-tRNA into the P-site, presents the next codon (green) in the A-site, to which the next aa-tRNA binds. (B) The previous model of +1 frameshift suppression by a tRNA with an extra nucleotide in the anticodon loop (symbolized as having an anticodon with three yellow and one green base). After peptide transfer to the suppressor tRNA the anticodon forces quadruplet translocation presenting a shifted anticodon in the A-site. A tRNA reading in the +1 frame (green/red) is then accepted in the +1 frame. (C) New dual-error model of frameshift induction. In this model the suppressor aa-tRNA (yellow anticodon) reads the mRNA by forming only two base pairs; the wobble bases do not pair (shown as a large gap between the bases). The yellow tRNA can be a normal near-cognate tRNA in those cases in which the mutated tRNA is out-competed in the selection at the A-site, or it can be an undermodified or otherwise altered cognate tRNA. After a normal 3-nucleotide translocation moves it into the P-site the yellow tRNA slips +1 on the mRNA. This places a codon in the shifted frame (green/red) into the A-site which is then recognized by a cognate aa-tRNA. A similar model could be drawn showing slippage in the -1 direction provided the tRNA can base pair to the -1 shifted codon.

translation termination; if the level is too low, termination will occur slowly enough to allow slippage of the peptidyl-tRNA<sup>Leu</sup> from CUU to UUU causing a +1 frameshift. Frameshifting allows the ribosome to complete synthesis of RF2, eventually leading to efficient termination at the UGA. So frameshifting occurs by slippage of peptidyl-tRNA during a translational pause caused by poor recognition of the codon in the ribosomal A-site. Frameshifting in the yeast Ty1 element is remarkably similar (Belcourt and Farabaugh, 1990). In this case the ribosome pauses with a poorly-decoded AGG codon in the A-site. Ribosomal pausing at this codon allows the peptidyl-tRNA<sup>Leu</sup> to slip forward one nucleotide, from CUU to UUA, which results in +1 frameshifting.

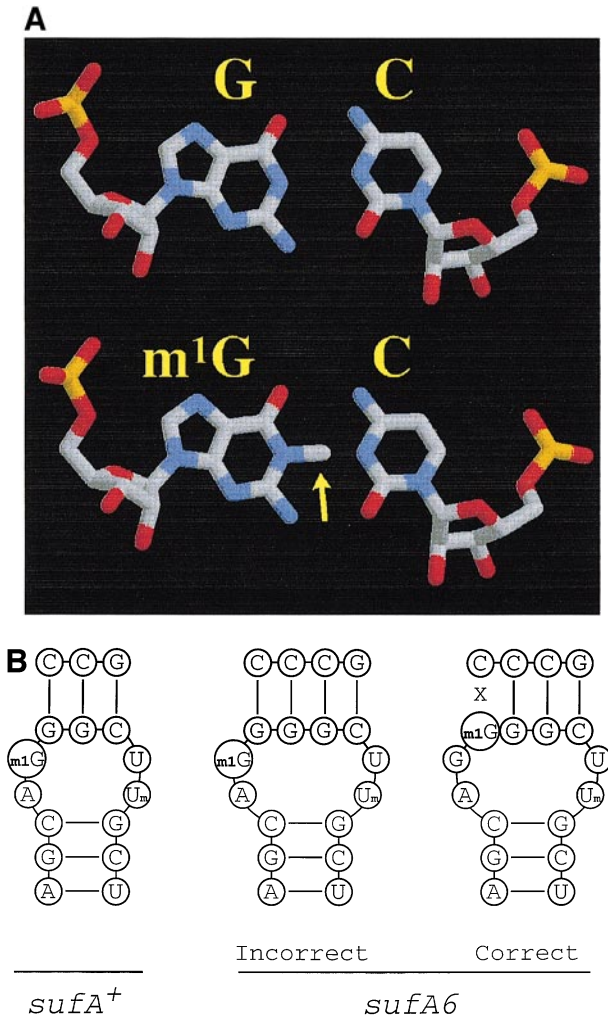
The common features of these two events, pausing and tRNA slippage, were thought to be universal aspects of programmed frameshifts until recently. However, analysis of +1 frameshifting in the Ty3 retrotransposon has shown that slippage is not essential for programmed frameshifting (Farabaugh *et al.*, 1993). Frameshifting in Ty3 does occur when the ribosome pauses with a poorly decoded codon,

AGU, in the A-site. However, the P-site codon, GCG, could not allow slippage of peptidyl-tRNA<sup>Ala</sup>. Rather, the peptidyl-tRNA appears to stimulate a different error, recruitment in the A-site of an aminoacyl-tRNA<sup>Val</sup> in the +1 frame. Recruitment out of frame depends on an abnormal feature of the peptidyl-tRNA and apparently not on any special feature of the tRNA<sup>Val</sup> (Vimaladithan and Farabaugh, 1994).

These three events imply a general mechanism of +1 programmed frameshifting. During a translational pause induced by poor recognition of the A-site codon, the P-site codon induces an error in frame, either by itself slipping +1 or by inducing recruitment of a codon out of frame in the A-site. Below we will present a unifying model for frameshifting which can explain spontaneous frameshifting, frameshifting induced by mutant tRNAs and programmed frameshifting.

### A dual-error model for frameshift suppression

The structures of frameshift suppressor tRNAs have been inferred in most cases from the sequence of the gene



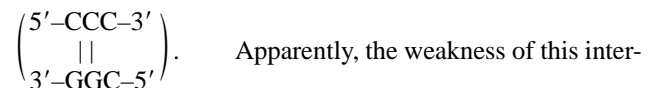
**Fig. 2.** (A) Comparison of normal Watson–Crick base pairing of a G–C (upper) to a similar structure involving 1-methylguanosine, m<sup>1</sup>G (lower). The presence of the methyl group (indicated by arrow) sterically interferes with pairing (lower panel). (B) The sequence of *sufa*<sup>+</sup> (*proK*<sup>+</sup>) tRNA to the left and the two possible alternative placements of the m<sup>1</sup>G in the *sufa6* +1 frameshift suppressor tRNA. Modification of the fourth of four guanosines (labeled ‘incorrect’) would have allowed formation of the putative 4 bp codon–anticodon interaction. Modification at the correct position (Qian *et al.*, 1998) precludes this 4 bp interaction, invalidating the former model of frameshift suppression, as described in the text.

encoding the tRNA. Of course, this information does not reveal how the tRNA might be post-transcriptionally modified, and modification can have a profound effect on the function of the tRNA. Recent work by Qian *et al.* (1998) on the modification of suppressor forms of tRNA<sup>Pro</sup> in *S.typhimurium* has shown that these tRNAs are modified in a way that precludes their reading a 4-nucleotide anticodon. In all tRNA<sup>Pro</sup> isoacceptors the guanosine (G) immediately 3' to the anticodon is always modified to 1-methylguanosine (m<sup>1</sup>G; see Figure 2A). This base is part of a short run of guanosine nucleotides that is lengthened by one in the frameshift suppressor. Since m<sup>1</sup>G modification interferes with the formation of G–C basepairs with the mRNA (Newmark and Cantor, 1968), the exact position of the modification in the suppressor tRNA is crucial to how the tRNA may interact with the messenger. The quadruplet translocation model would

predict that in the suppressor form the last G in the run is modified to m<sup>1</sup>G, allowing the other four to act as the predicted expanded anticodon. However, as illustrated in Figure 2B, the actual position of modification is the fourth in the run of five in all suppressor forms of both tRNA<sup>Pro</sup> isoacceptors in *S.typhimurium* (Qian *et al.*, 1998). This modification precludes formation of a 4 base pair codon–anticodon interaction. Importantly, the modified base is the one predicted to interact with the first position of the anticodon, the position that discriminates between the suppressible site (CCCN) and non-suppressible sites (UCCN, ACCN and GCCN). Since the modification would render the tRNA incapable of distinguishing between suppressible and non-suppressible sites, this observation fundamentally invalidates the quadruplet translocation model for all of the tRNA<sup>Pro</sup> isoacceptors in *S.typhimurium*. The only other identified frameshift suppressor forms of tRNA<sup>Pro</sup> are from the yeast *S.cerevisiae*; the wild type yeast tRNAs are modified to m<sup>1</sup>G in the identical position as in *S.typhimurium*. Although it has not been tested yet, it seems likely that the yeast suppressor tRNA<sup>Pro</sup> species are modified as are the bacterial tRNAs.

The presence of the modified base in bacterial tRNA<sup>Pro</sup> frameshift suppressors is the strongest evidence against the quadruplet translocation model. Under the quadruplet translocation model (Figure 1B) frameshifting results from the 4 bp interaction in the A-site; the frameshift occurs when peptidyl-tRNA translocates to the P-site with normal reading of the first +1 shifted codon. In both *S.typhimurium* and *S.cerevisiae*, however, a transient translational pause caused by slow recognition of the zero frame codon immediately following the frameshift site strongly stimulates frameshifting (Qian *et al.*, 1998). This implies that while the frameshift site occupies the ribosomal P-site a slow event must occur that causes frameshifting. In programmed +1 frameshifting efficient slippage of peptidyl-tRNA depends on slow decoding of the next zero frame codon (Curran and Yarus, 1989; Belcourt and Farabaugh, 1990; Farabaugh *et al.*, 1993), suggesting that the slow event during suppression may also be peptidyl-tRNA slippage.

Surprisingly, 1-methylguanosine modification actually has the effect of forcing suppressing tRNAs to read by a two-out-of-three mechanism. One suppressor, *sufa6*, is a mutated form of tRNA<sup>Pro</sup><sub>CGG</sub> with an expanded anticodon. However, given the presence of m<sup>1</sup>G this tRNA must read the CCC codon using a CCG anticodon, meaning it can only form two base pairs with the mRNA



action predisposes the peptidyl-tRNA to slip +1 in the P-site. What is more surprising is that suppression in the presence of a mutant form of tRNA<sup>Pro</sup><sub>GGG</sub>, encoded by *suB2*, actually occurs because of decoding of CCC by the highly abundant, structurally normal near-cognate isoacceptor, tRNA<sup>Pro</sup><sub>cmo5UGG</sub>. Near-cognate reading is revealed by the fact that suppression in a *suB2* strain depends on wobble modification of tRNA<sup>Pro</sup><sub>cmo5UGG</sub>. Again, frameshifting must result from the weakness of the interaction of this tRNA with a CCC codon.

These results are consistent with a dual-error model (Figure 1C). A shortage of the cognate tRNA causes the

ribosome to pause with an empty A-site. During the pause, a near-cognate tRNA is accepted in the A-site (first error). After a normal 3-nucleotide translocation, because of its weak aberrant anticodon–codon interaction the near-cognate peptidyl-tRNA in the P-site shifts either to the left (–1 frameshift) or to the right (+1 frameshift) (second error). Instead of a normal near-cognate tRNA, an under-modified or in other way altered cognate tRNA may also be prone to slippage in the P-site in a way similar to the near-cognate tRNA.

It is possible to explain any of the several types of frameshift suppressors using this same model including both +1 and –1 frameshift suppressor tRNAs (Qian *et al.*, 1998). The previous model for frameshift suppressors did not have the same universal applicability. The explanatory power of the model, reducing all of the frameshifting phenomena to a single mechanism, is perhaps the strongest argument for its adoption. However, the details of the model have yet to be tested fully so it should be considered provisional only.

### In general do +1 frameshifts occur in the P-site?

Formally, +1 frameshift errors could originate in either of three ways: in the P-site by transient tRNA unpairing followed by repairing on an overlapping codon ('slipping'); in the A-site by illegal recruitment of an aminoacyl-tRNA in an incorrect reading frame ('misframing'); or during translocation of peptidyl-tRNA from the A- to the P-site ('mistranslocation'). Clearly misframing would have to occur when the ribosomal A-site is available to be filled by aminoacyl-tRNA as the EF-1A ternary complex. But when would slipping be likely to occur? Again, from a formal perspective the time during the elongation cycle when slipping should be most likely is when only a single tRNA interacts with the mRNA. According to the hybrid-sites model for translational elongation (Moazed and Noller, 1989), after translocation only the peptidyl-tRNA basepairs with the mRNA; at all other steps of the elongation cycle two ribosome-bound tRNAs engage the mRNA. A single tRNA is responsible for maintaining translational frame at this stage. The inherent instability of a single relative to a dual tRNA–mRNA complex has suggested the potential for frame errors by mistranslocation (e.g. Wilson and Noller, 1998). Translocation occurs by movement of the peptidyl-tRNA into the P-site, concomitant with movement of deacyl-tRNA to the E-site and release from the mRNA. The instability of a single tRNA–mRNA complex suggests the possibility that translocation itself might directly cause frameshifting.

Is translocation an inherently error-prone event? It appears not to be. All of the available data on +1 frameshifting argue that frameshifting occurs only after a normal translocation event. Pausing by the ribosome after translocation, with an empty A-site, strongly stimulates frameshifting both +1 programmed frameshifting (mRNA-directed) and frameshift suppression (tRNA-directed). However, the data are consistent with the idea that +1 frame errors occur while only one tRNA pairs with the mRNA, validating the concept that this is a critical step in frame maintenance. The fact that translocation is not error-prone must constrain models to describe how

this process is accomplished. As discussed by Wilson and Noller (1998), the lack of errors implies that ribosomal components must stabilize the codon–anticodon complex during translocation. Yet once transposed into the P-site, the peptidyl-tRNA–mRNA complex appears too weak to preclude frame errors from occurring during a translational pause. Indeed, Dabrowski *et al.* (1995) have shown that the anticodon of the P-site tRNA is involved in fewer interactions than is the anticodon of the A-site tRNA, suggesting that the tRNA–mRNA interaction may be weaker in the P-site than in the A-site.

Wilson and Noller (1998) point out that the stability of the tRNA–mRNA complex must depend on the stabilizing effect of the ribosomal decoding sites, and that the exchange of peptidyl-tRNA during translocation (from the P/A to the P-sites in the hybrid-sites model) might cause an exchange of stabilizing contacts. How does the ribosome manage this exchange without causing frameshift errors? We would argue that the lack of frame errors at this stage implies that EF-2 (formerly called EF-G) stimulates movement of the peptidyl-tRNA without disrupting those stabilizing contacts, or by processively substituting each of one set of contacts for another. An attractive hypothesis is that complete realignment of the peptidyl-tRNA into a canonical P-site only occurs when cognate aminoacyl-tRNA is deposited in the A-site by EF-1A (which involves movement of the aminoacyl-tRNA from the T to the A-site). The existence of two tRNA interactions at this step might stabilize the frame sufficiently to allow substitution of stabilizing contacts.

### What causes frameshift errors?

What is the significance of this new model of frameshift suppression on the larger question of frame maintenance in general? One clear implication of the suppressor analysis is that frameshifting is strongly stimulated by near-cognate decoding, that is decoding by an isoacceptor that makes a less than optimal wobble interaction with the mRNA. The example of suppression by a structurally normal near cognate tRNA in the *sufB2* strain of *S.typhimurium* clearly shows that near-cognate decoding can stimulate frame errors. Moreover, overproduction of the same near-cognate tRNA induces frameshifting at the same sites suppressed by *sufB2* (discussed in O'Connor, 1998). Some programmed frameshifts are also stimulated by near-cognate decoding. The first example comes from the *dnaX* gene of *E.coli*, which encodes alternative forms of a subunit of DNA polymerase III. Frameshifting results in the expression of a C-terminally truncated form of the protein (Blinkowa and Walker, 1990; Flower and McHenry, 1990; Tsuchihashi and Kornberg, 1990) and occurs on a slippery heptameric sequence A-AAA-AAG, two tRNAs simultaneously slipping –1 from AAA-AAG to AAA-AAA (Tsuchihashi and Brown, 1992). The unusually high efficiency of this site partly results from the near-cognate recognition of the AAG codon by a tRNA with a modified U in the wobble position which restricts the ability of tRNA to decode AAG. Expressing a tRNA that recognizes AAG in a completely cognate fashion reduced frameshifting on the site. The weakness of the interaction apparently predisposes the ribosome to frameshift.

A second example of near cognate decoding in frame-

shifting comes from +1 frameshifting in the Ty element family of retrotransposons in the yeast *S.cerevisiae*. Ty elements express homologues of retroviral Gag and Pol genes, with Pol expressed as a Gag–Pol fusion by +1 frameshifting. Frameshifting in Ty1 occurs by slippage of a peptidyl-tRNA<sup>Leu</sup><sub>UAG</sub> from CUU to UUA (Belcourt and Farabaugh, 1990). The wobble base of tRNA<sup>Leu</sup><sub>UAG</sub> is an unmodified U, which apparently allows it to read the codons CUU, CUC and CUG by a two-out-of-three mechanism, base pairing in only the first and second positions of the codon (Weissenbach *et al.*, 1977; Randerath *et al.*, 1979). The extremely weak interaction between peptidyl-tRNA<sup>Leu</sup><sub>UAG</sub> and CUU predisposes the ribosome to slip +1 (Vimaladithan and Farabaugh, 1994). Furthermore, replacing the CUU codon with an AGG codon allows frameshifting to occur efficiently only if the single gene encoding the AGG cognate tRNA<sup>Arg</sup><sub>CCU</sub> is deleted, forcing near-cognate decoding of AGG (Vimaladithan and Farabaugh, 1994).

Non-programmed –1 frameshifting may also be stimulated by near-cognate decoding. Frameshifting in the –1 direction occurs at GGA codons in strains carrying mutated forms of its cognate tRNA<sup>Gly</sup><sub>UCC</sub>, encoded by the *glyT* gene of *S.typhimurium* (O'Mahony *et al.*, 1989). We have argued (Qian *et al.*, 1998) that frameshifting at GGA may occur because of near-cognate decoding of GGA by tRNA<sup>Gly</sup><sub>UCC</sub>. In fact, a recent paper by O'Connor (1998) shows that merely overproducing tRNA<sup>Gly</sup><sub>UCC</sub> causes frameshifting on GGA, consistent with the near-cognate decoding model. Though O'Connor argues that frameshifting may occur by aberrant doublet decoding of GGA in the A-site the data are equally consistent with triplet recognition of GGA, without wobble pairing, followed by –1 slippage in the P-site. The two models could be distinguished by testing the effect of slow recognition of the next in-frame codon on frameshifting at GGA. If frameshifting occurs by doublet decoding there should be no effect, if there should be by slippage.

These data demonstrate that two-out-of-three decoding by peptidyl-tRNA can predispose it to slipping +1 on the mRNA and suggest that a similar mechanism may account for –1 frameshifting. It is attractive to propose that a similar type of error might be at the root of spontaneous frameshift errors in the absence of special mRNA sequences or mutant tRNAs. The best model system for spontaneous frameshifting comes from the work of Gallant and his colleagues (reviewed in Gallant and Lindsley, 1993). Gallant's laboratory has been interested in how amino acid starvation stimulates frameshifting. The original work using the *rIIB* cistron of bacteriophage T4 identified several sites especially prone to shifting during starvation for either lysine or tryptophan (Weiss and Gallant, 1986). Later work has pinpointed the site of the frameshift and determined the rules that govern frameshifting in either the –1 ('leftward') or +1 ('rightward') directions. An *rIIB* –1 frameshift site consists of a lysine codon (AAG) preceded by the sequence U-UUC (shown in codons of the normal, or zero frame). Amino acid starvation appears to cause a translation pause during which the peptidyl-tRNA<sup>Phe</sup> slips –1 from UUC to UUU (Barak *et al.*, 1996). The identity of the pause codon is not important since it can be replaced by an isoleucine codon, though the starvation must match the identity of the codon used (Barak *et al.*, 1996). The +1 site consists of an AAG lysine codon

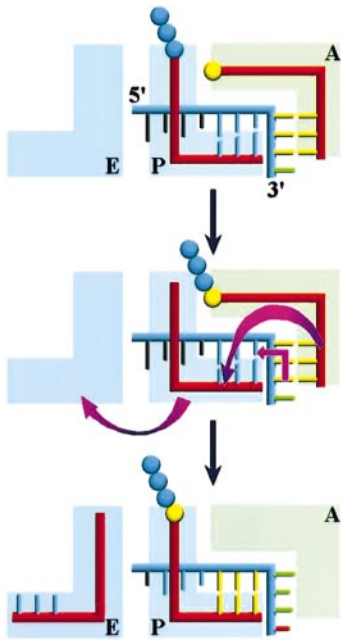
preceded by a GCC alanine; the lysine codon again presumably provides a translational pause.

Peter *et al.* (1992) argue that frameshifting results from out-of-frame decoding of an AGC serine codon overlapping the AAG, but it is possible to explain it by near-cognate decoding. The GCC codon that causes +1 frameshifting has a near-cognate tRNA<sup>Ala</sup><sub>cmo5UGC</sub> which Gabriel *et al.* (1996) have shown can decode GCC. Since tRNA<sup>Ala</sup><sub>cmo5UGC</sub> is the major tRNA<sup>Ala</sup> isoacceptor (Inokuchi and Yamao, 1995) it may out-compete the minor cognate tRNA<sup>Ala</sup><sub>GCC</sub>. Thereafter this near-cognate tRNA<sup>Ala</sup><sub>cmo5UGC</sub> could slip +1 on the GCC-AAG frameshift site and still form the essential two base pairs in the shifted frame since the wobble uridine-5-oxyacetic acid (cmo5U) can base pair with adenosine. Peter *et al.* (1992) suggested that slippage on GCC was unlikely with cognate tRNA<sup>Ala</sup><sub>GCC</sub>, which can make only one base pair on the +1 shifted codon CCA. It should be possible to demonstrate that near-cognate decoding of GCC causes frameshifting as Qian *et al.* (1998) did for frameshifting at CCCN sites by the *suFB2* suppressor. If the model is correct then lack of cmo5U modification should reduce or eliminate frameshifting at the GCC-AAG site and if it is incorrect there should be no effect.

It is more difficult to rationalize near-cognate decoding with –1 frameshifting at U-UUC. Peter *et al.* (1992) showed that UUC is decoded as phenylalanine prior to the frameshift, and the UUY Phe codons have only one isoacceptor. Therefore, near-cognate decoding is not possible. However, the strains used by Gallant and his colleagues always include a *relA* marker. It is formally possible that in a *relA* strain the interaction between even a cognate tRNA and its codon is weakened sufficiently to allow frameshifting.

### Implications of the suppression model for our understanding of frame maintenance

With the demonstration that translation consists of reading successive adjacent 3-nucleotide codons, the question became what mechanism determined the repetitive 3-nucleotide movement of the ribosome on the mRNA. Holley's demonstration that tRNAs have 3-nucleotide anticodons complementary to 3-nucleotide codons suggested that the length of the anticodon–codon interaction might determine the translocation step size. But now we see that there is not a direct relationship between codon–anticodon interaction and translocation step size. Far from quadruplet anticodons causing +1 frameshifting by quadruplet translocation, tRNAs making only 2 bp with the mRNA actually stimulate +1 frameshifting apparently by peptidyl-tRNA slippage (Qian *et al.*, 1998). We have proposed that a near cognate tRNA<sup>Gly</sup><sub>CCC</sub>, which also would make a 2 bp interaction with the mRNA, may actually cause the opposite effect on GGA codons, –1 frameshifting by slippage in the leftward direction. The difference between the two events appears to be the ability of the tRNA to form at least 2 bp with the codon in the shifted reading frame. In both cases, and in the case of tRNAs with expanded anticodon loops, the peptidyl-tRNA appears to undergo a normal 3-nucleotide translocation with post-translocation slippage causing frameshifting. It seems the ribosome is incapable of promoting translocation of other



**Fig. 3.** Proposed structural model for the 3-nucleotide translocation step. The figure shows the complex rotational movement that Wilson and Noller (1998) have proposed occurs during translocation of the peptidyl-tRNA to the P-site of the ribosome, as described in the text. Top, the P- and A-sites are occupied by the peptidyl-tRNA (blue anticodon) and aa-tRNA (yellow anticodon). Middle, peptide transfer creates a deacyl-tRNA (blue) and peptidyl-tRNA (yellow). Bottom, the two tRNAs undergo rotational movements (indicated by the curved arrows in the middle panel) moving them to the E- and P-sites. The rotation is accompanied by movement of the mRNA 3 nucleotides (indicated by the angled arrow in the middle panel). The placement of the E-site tRNA is controversial and this is only one of several possible placements.

than 3 nucleotides, regardless of the structure of the anticodon loop.

Wilson and Noller (1998) have argued that translocation involves two rotational movements of the peptidyl-tRNA: a  $60^\circ$  rotation about an axis perpendicular to the anticodon arm and centered near the anticodon and a  $60^\circ$  rotation along the axis of the anticodon arm. After this movement the codon bound by the peptidyl-tRNA has moved 3 nucleotides and a free codon occupies the A-site. Translocation occurs without altering the conformation of the nucleotides in the anticodon loop (Matzke *et al.*, 1980) so the movement of the mRNA can not result from any intrinsic change in the tRNA. Rather, it must result from the movement in space of the tRNA itself. An attractive hypothesis to explain the 3-nucleotide step size of translocation is to suppose that the two rotational movements of the tRNA cause the anticodon to move the precise distance necessary to place the next triplet into the ribosomal A-site. This hypothesis is illustrated in Figure 3, though the two  $60^\circ$  rotations are represented as  $90^\circ$  and  $180^\circ$  rotations in the cartoon for clarity, so as to maintain the planar nature of the representation. As can be seen in Figure 3, a simple rotational translocation (in the sense of movement) of the tRNA can cause a precise movement of the mRNA attached to it. This hypothesis is completely consistent with 3-nucleotide translocation by tRNAs bound to the mRNA by either 2 or 3 base pairs.

Of course, the distance of mRNA movement must have

evolved along with the code. It is clear that at least a 3-nucleotide codon is necessary to allow the specification of 20 amino acids and termination, but perhaps the selection of a 3-nucleotide code was equally driven by thermodynamic considerations. Analysis of frameshifting indicates that when a tRNA forms fewer than 3 base pairs with the mRNA, the interaction is insufficiently stable to preclude realignment of the tRNA in the P-site. The normal 3 bp interaction is just stable enough that slippage occurs very infrequently, even at sites involving repeating nucleotides (e.g. CCCC). There could be many reasons why a 4-nucleotide code was not adopted: an insufficient need to encode more amino acids; too stable a tRNA-mRNA interaction (irreversibility); or perhaps the impossibility of forming a longer codon-anticodon complex. In the context of the 3-nucleotide code it is clear that expanding the anticodon does disrupt translational reading frame though not by forcing recognition of a larger codon.

The fact that near-cognate decoding can greatly degrade the ability to maintain translational reading frame implies an important connection between missense decoding and frameshifting (Kurland, 1979; Tucker *et al.*, 1989). Data clearly implicating near-cognate decoding in frameshifting is still relatively sparse. However, the effect is very striking. The clear implication of the data is that if ribosomes were to frequently accept near-cognate tRNAs in preference to cognates they should allow a much higher frequency of translational frameshifting. This effect may be enough to explain the elaborate mechanisms that the translational apparatus has evolved to restrict decoding to cognate interactions. Modification of tRNAs, for example, may restrict aberrant decoding, even by near-cognate isoacceptors, to avoid the resulting frameshift errors rather than to modulate the rate or accuracy of translation elongation. Many examples exist in which frameshifting was induced by lack of tRNA modification.

The tRNA yardstick model should probably be abandoned as irrelevant to our understanding of translation. This will be a difficult transition since the model is so venerable, having been first proposed over 25 years ago. A correct conception of the mechanism of frame maintenance now appears much more complicated. It involves a specific choreography of tRNAs on the ribosome and the formation of accurate 3 bp interactions between the tRNAs and the mRNA to generate the 3-nucleotide repetitive step to the translational dance. The mechanisms that force correct selection of aminoacyl-tRNAs and impose the specific movements of the tRNAs within the ribosome remain to be discovered.

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