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A New Model for Phenotypic Suppression of Frameshift Mutations by Mutant tRNAs

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Summary

According to the prevailing model, frameshift-suppressing tRNAs with an extra nucleotide in the anticodon loop suppress +1 frameshift mutations by recognizing a four-base codon and promoting quadruplet translocation. We present three sets of experiments that suggest a general alternative to this model. First, base modification should actually block such a four-base interaction by two classical frameshift suppressors. Second, for one *Salmonella* suppressor tRNA, it is not mutant tRNA but a structurally normal near cognate that causes the +1 shift in-frame. Finally, frameshifting occurs in competition with normal decoding of the next in-frame codon, consistent with an event that occurs in the ribosomal P site after the translocation step. These results suggest an alternative model involving peptidyl-tRNA slippage at the classical CCC-N and GGG-N frameshift suppression sites.

Introduction

Ribosomes read messenger RNAs in sequential nonoverlapping triplet codons without the need for any punctuation to identify the reading frame (Crick et al., 1961). 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. This concept was strengthened with the isolation of the first classical +1 frameshift suppressors (Riyasaty and Atkins, 1968; Riddle and Roth, 1970; Yourno and Tanemura, 1970), and the later demonstration that one of these, *sufD42* in *Salmonella typhimurium*, introduces an additional C in the anticodon of one of the $tRNA^{Gly}$ isoacceptors (Riddle and Carbon, 1973). The additional C enlarges the region of the anticodon from 5′-CCC-3′ to 5′-CCCC-3′. Similar suppressors were isolated in *Saccharomyces cerevisiae* (reviewed in Culbertson et al., 1990). These mutant tRNAs suppress frameshift mutations in which an extra G has been inserted into a GGN codon, creating a GGGN codon. Similarly, frameshift-suppressor derivatives of $tRNA^{Pro}$ isoacceptors in *S. typhimurium* and *S. cerevisiae* were isolated that suppress CCN-to-CCCN frameshift mutations, and these mutant tRNAs have an extra G in the anticodon loop (Culbertson et al., 1990; Sroga et al., 1992; J.-n. L. and G. R. B., unpublished data). The structure of these mutant tRNAs suggested a simple and elegant hypothesis to explain frameshift suppression: a four-base anticodon could base-pair with a four-base codon, thereby shifting the reading frame +1 as a result (Figure 1A). This mechanism is generally accepted, though it has never been explicitly demonstrated. Surprisingly, certain mutant tRNAs suppress without the need to form a fourth base pair with the mRNA. In particular, for *sufJ* and *sufG* suppressors in *S. typhimurium* (Bossi and Roth, 1981), and the SUF16 suppressor of *S. cerevisiae* (Gaber and Culbertson, 1984), suppression is nearly insensitive to the nature of the first base of the presumptive 4 nt 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, maintaining a 4 nt translocation and thereby the yardstick role of the tRNA without the need for base pairing at the fourth position of the presumptive 4 nt codon. However, some tRNA suppressors have normal anticodon loops but are altered within the body of the tRNA (Hüttenhofer et al., 1990; Sroga et al., 1992; Qian and Björk, 1997a). It is unclear how the yardstick role of the anticodon is modified in these tRNAs.

In this paper, we present data that invalidates the classical model of frameshift suppression derived from analysis of frameshift-suppressing mutations affecting $tRNA^{Pro}_{GGG}$ and $tRNA^{Pro}_{CGG}$ from *S. typhimurium* and $tRNA^{Gly}_{CCC}$ and $tRNA^{Pro}_{IGG}$ from *S. cerevisiae*. First, we show that the tRNAs encoded by two classic frameshift suppressors, *sufB2* ($tRNA^{Pro}_{GGG}$ derivative) and *sufA6* ($tRNA^{Pro}_{CGG}$) derivative), are modified in such a way that they are incapable of making a 4 bp codon–anticodon interaction with the mRNA, but instead must read a 3 nt codon. Second, by manipulating in vivo tRNA modification, we show that in the presence of a frameshift-suppressor form of $tRNA^{Pro}_{GGG}$ it is not this tRNA, but rather the structurally normal near-cognate $tRNA^{Pro}_{cmo5UGG}$ that decodes the suppression site, causing the +1 shift in-frame. Third, frameshifting by these and several other $tRNA^{Pro}$ and $tRNA^{Gly}$ suppressors all occur in competition with normal decoding of the next in-frame codon, showing that shifting only occurs after the Gly or Pro codon moves to the P site. These results are not consistent with the classical model of four-base translocation mediated by suppressor tRNAs with an oversized anticodon loop. Instead, we propose a new model in which frameshifting results from peptidyl-tRNA slippage at the classically defined CCCN and GGGN suppression sites (Figure 1B).

Results

Classical Frameshift Suppressors with an 8 nt Anticodon Loop have a 3 nt Anticodon Bordered by U33 and 1-Methylguanosine at Position 37 (m^1G37)

Frameshift suppressors of $tRNA^{Pro}$ isoacceptors, *sufA6* ($tRNA^{Pro}_{CGG}$) and *sufB2* ($tRNA^{Pro}_{GGG}$) (Riddle and Roth, 1972a,

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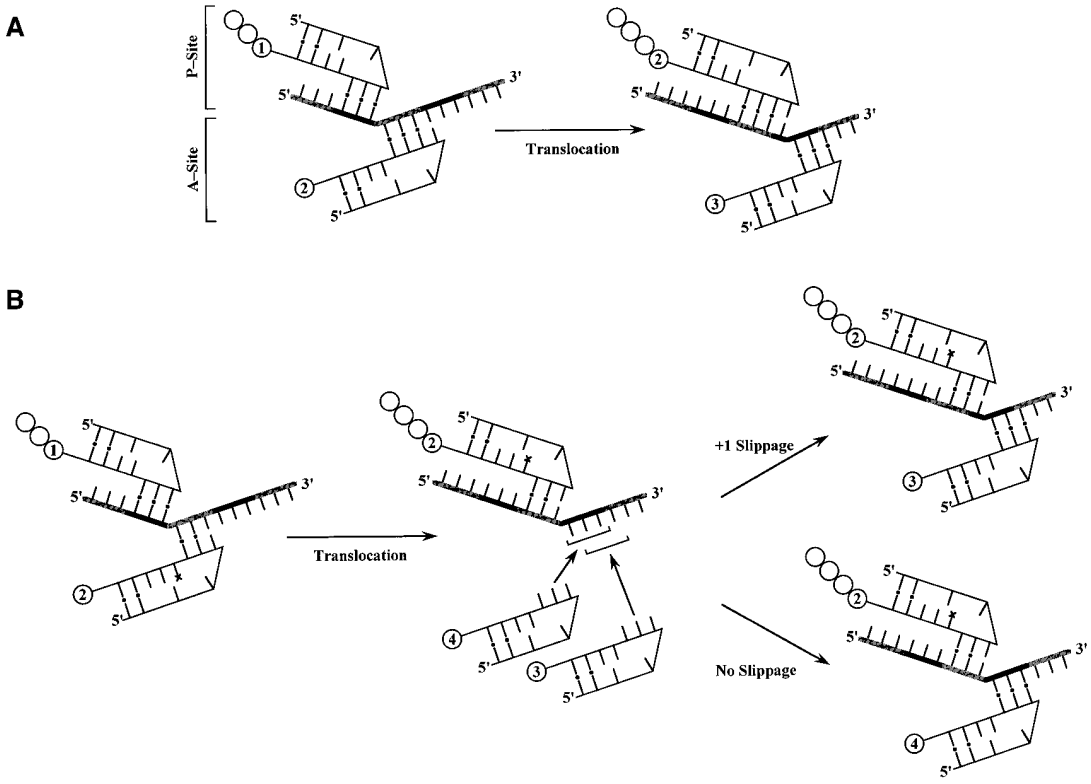


Figure 1. Models for Frameshift Suppression

(A) The prevailing model. The suppressor tRNA (number 2) recognizes a 4 nt codon in the A site (left). When it translocates to the P site (right), it interferes with recognition of the next in-frame codon, promoting reading of the next +1 frame codon (tRNA number 3). The tRNAs are cartooned to reflect the apparent arrangement of the A- and P-site tRNAs (Agrawal et al., 1996). The region of the anticodon loop is shown, showing bases (bars) that must pair (dots) both within the tRNA and with the mRNA.

(B) A new model. The suppressor tRNA (number 2) recognizes a 3 nt codon, making at least 2 base pairs in the A site (left). After translocation into the P site (middle) it slips +1 (right), allowing recognition of the next +1 frame codon (tRNA number 3). Slippage competes with in-frame decoding (tRNA number 4). The position corresponding to the 1meG in $tRNA^{Pro}$ is denoted with an X to indicate its inability to base-pair with the mRNA.

1972b) each encode a tRNA with an extra G in the anticodon loop (Sroga et al., 1992; J.-n. L. and G. R. B., unpublished data), creating a putative expanded 4 nt anticodon, 5′-CGGG-3′ and 5′-GGGG-3′, respectively. The wild-type forms of these tRNAs have m^1G in position 37 (adjacent and 3′ of the anticodon) (Kuchino et al., 1984), which cannot form a Watson-Crick base pair with the mRNA (Newmark and Cantor, 1968). It is not clear a priori which nucleotide in the expanded anticodon the modifying enzyme would recognize and modify, the G immediately 3′ of the expanded anticodon (corresponding to the extra nucleotide between positions 37 and 38, G37A in Figure 2A), or the G immediately adjacent to the normal 3 nt anticodon (G37 in Figure 2A). The position of the modification is critical and determines the 3′ border of the anticodon.

To determine the position of m^1G in the frameshift-suppressor forms of these tRNAs, we performed primer extension analysis on tRNAs purified to homogeneity from various strains (diagrammed in Figure 2B). The primer is complementary to the region immediately 3′ to the anticodon, ending at the base complementary to the last nucleotide in the anticodon stem, position 39 of the tRNA. The presence of m^1G37 in the tRNA blocks primer extension since it cannot base-pair with C, producing a modification-dependent cDNA product extending to the position 1 nt short of the modified base in the tRNA as cartooned in Figure 2B. Figure 2C shows that a primer complementary up to position 39 (lane 4) is extended 1 nt on *sufA*$^+$ $tRNA^{Pro}_{CGG}$ (lane 1) or *sufB*$^+$ $tRNA^{Pro}_{GGG}$ (lane 5). This termination product is eliminated when the tRNA is purified from a strain lacking m^1G since it lacks the modified base (lane 3); on this unmodified template, primer extension can continue to the end of the tRNA (data not shown). These data confirm the known position of the methylated base at position 37. The prevailing model for frameshifting would predict that the modification in the expanded anticodons of the suppressor tRNAs would occur at the same base, leading to the same 1 nt extended product. Contrary to expectations, in the case of both the *sufA6* (lane 2) and *sufB2* tRNA (lane 6), the primer is actually extended an additional nucleotide, demonstrating that the modified base in these tRNAs is within the putative expanded anticodon. (An anomalous band about halfway between the primer and the first stop visible in lanes 1 and 5 is

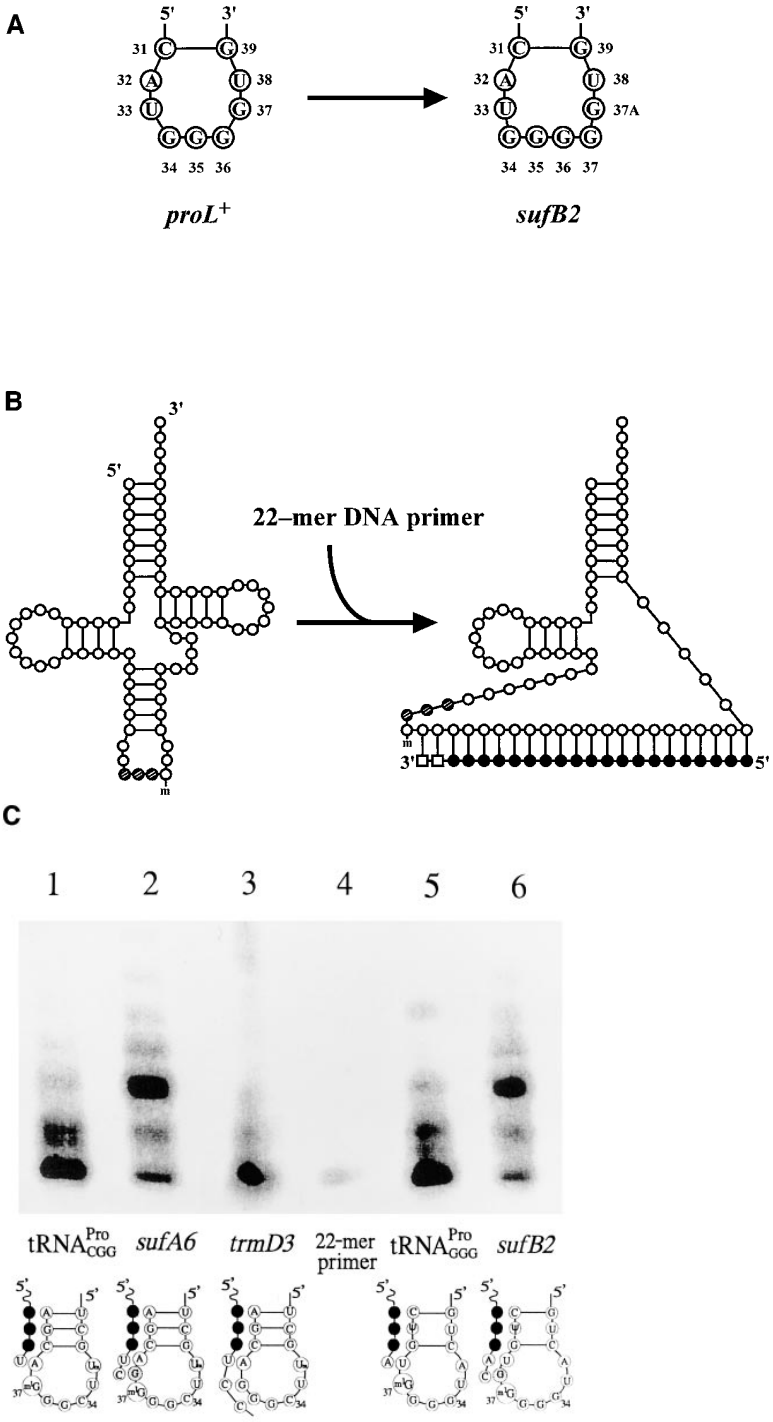


Figure 2. Location of m^1G in Wild-Type and Suppressor Forms of $tRNA^{Pro}$

(A) Numbering system for expanded anticodon loops. The additional base is placed between G37 and U38, and numbered G37A. The normal 3 nt anticodon corresponds to G34-G35-G36.

(B) The position of the 1meG modification is identified by primer extension with a 22 nt primer. The primer (closed circles) is shown base-pairing to the complementary portion of the tRNA. The position of the methyl group on position 1 of G blocks addition of more than one nucleotide onto the primer (open square).

(C) Primer extension results for $tRNA^{Pro}_{CGG}$ from *sufA*$^+$ *trmD*$^+$ (lane 1), *sufA6 trmD*$^+$ (lane 2) and *sufA*$^+$ *trmD3* (lane 3) strains, and $tRNA^{Pro}_{GGG}$ from *sufB*$^+$ *trmD*$^+$ (lane 5), and *sufB2 trmD*$^+$ (lane 6) strains. Lane 4 is the untreated primer. The location of the termination site on each wild-type and suppressor tRNA, and their deduced structures are diagrammed below lanes 1–3 and 5 and 6; the dash at the end of the *trmD3* extended product indicates that extension continues to the end of the tRNA on that molecule (data not shown).

caused by a contaminant in the primer, since upon longer exposure this band is also visible in lane 4 [primer alone] as well as in lanes 2, 3, and 6 [data not shown].)

Thus, the insertion of an extra G in *sufA6* and *sufB2* tRNAs can be thought of as being 3′ of the modified nucleotide m^1G37 (Figures 2B and 2C). These frameshift-suppressor tRNAs therefore have a normal 3 nt anticodon bordered on the 5′ side by the universal U33 and on the 3′ side by the m^1G37, precluding any quadruplet interaction between these mutated tRNAs and the mRNA. Most importantly, the modified G residue is predicted under the classical model to pair with the first base of the 4 nt codon. The inability to base-pair at this position would make the tRNA incapable of distinguishing between suppressible CCCN sites and the related, but nonsuppressible, UCCN, ACCN, and GCCN sites (Mathison and Culbertson, 1985; Q. Q. and G. R. B., unpublished data). Moreover, though *sufB2* tRNA retains an anticodon that is cognate for the CCC codon at the frameshift site, the *sufA6* anticodon is CGG, so it can

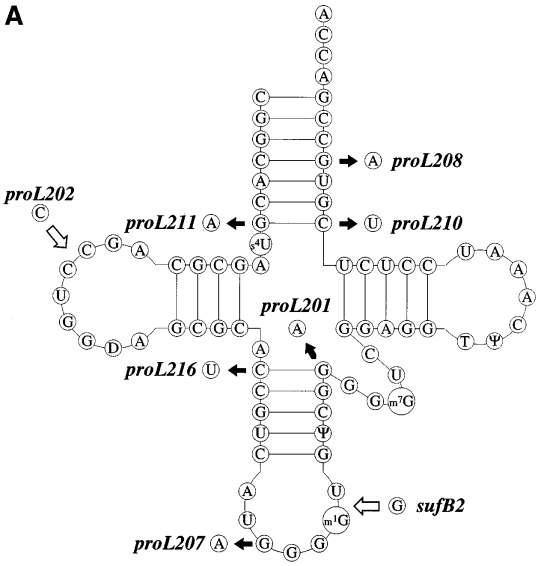


Figure 3. Suppression by a Structurally Normal Near Cognate in *proL* Mutant Strains

(A) The *proL*-encoded $tRNA^{Pro}_{GGG}$ and the various mutants that induce +1 frameshifting. The position of each mutation and alteration is shown on the wild-type sequence of the mature tRNA (Qian and Björk, 1997b). Open arrows indicate a nucleotide insertion, and closed arrows a nucleotide replacement.

(B) Effect of lack of cmo^5U34 modification on frameshifting. Frameshifting was measured in a wild-type strain, and those carrying various $tRNA^{Pro}$ frameshift-suppressor mutations, either in the absence of cmo^5U modification (*aroD* strain lacking chorismic acid, closed bars) or the presence of modification (*aroD*$^+$, open bars). The data are expressed as the ratio of β-galactosidase to β-lactamase activity ("ratio(galactosidase/lactamase)"), a measure of relative expression dependent on frameshifting, as described in Experimental Procedures.

actually form only 2 bp with CCC. Clearly, neither tRNA could promote frameshifting by the classical model.

Mutant Forms of $tRNA^{Pro}_{GGG}$ Induce Frameshifting by Allowing Near-Cognate Decoding by the Wild-Type Form of $tRNA^{Pro}_{cmo5UGG}$

S. typhimurium encodes three proline isoacceptors: $tRNA^{Pro}_{GGG}$, which reads CCU and CCC; $tRNA^{Pro}_{CGG}$, which reads CCG; and $tRNA^{Pro}_{cmo5UGG}$, which reads CCA, CCG, and CCU. The presence of the oxyacetic acid group at the position 5 of U in the wobble position (cmo^5U34) extends the coding capacity of U to pair with U in addition to A and G (Yokoyama et al., 1985). However, in the absence of a competing cognate, tRNAs with this modification must also be able to extend decoding to C, since an *Escherichia coli* mutant deleted for the gene encoding the minor GCC-decoding $tRNA^{Ala}_{GGC}$ and possessing only $tRNA^{Ala}_{cmo5UGC}$, is viable (Gabriel et al., 1996). The fact that cmo^5U cannot pair with C (Yokoyama et al., 1985, and references therein) suggests that a cmo^5U-containing tRNA may recognize C-ending codons by two-out-of-three decoding (Lagerkvist, 1978).

We have recently isolated several mutations in the *proL* gene in *S. typhimurium* encoding $tRNA^{Pro}_{GGG}$ that phenotypically suppress +1 frameshift mutations at the P site (Qian and Björk, 1997a). One of the mutants (*proL207*) changes the wobble base G34 to A34 (Figure 3A); unlike most such tRNAs, the A34 is not modified to

inosine (Q. Q. and G. R. B. unpublished data). The change should make the tRNA unable to read the codon CCC, since A-C wobble pairing is not permitted, leaving the codon without a true cognate tRNA. This raises the question of which tRNA in this strain decodes CCC to induce frameshifting, the mutant form or perhaps the near-cognate $tRNA^{Pro}_{cmo5UGG}$. An *aroD* mutation blocks production of chorismic acid, which is required for cmo^5 modification (Björk, 1980; Hagervall et al., 1990), so that in an *aroD* strain the wobble U of $tRNA^{Pro}_{cmo5UGG}$ remains unmodified. Figure 3B shows that the absence of cmo^5U modification, in an *aroD* strain, reduced the frameshifting activity of the *proL207* mutant to the level of the nonsuppressing parental strain. Since *aroD* has no effect on $tRNA^{Pro}_{GGG}$, this result suggests that in fact it is not the mutant tRNA encoded by the *proL* that decodes CCC and induces frameshifting, but rather that frameshifting occurs as a result of decoding of CCC by the near-cognate $tRNA^{Pro}_{cmo5UGG}$. As in the case of $tRNA^{Ala}_{cmo5UGC}$, the lack of a true cognate tRNA apparently allows the cmo^5U-containing $tRNA^{Pro}$ to read CCC. Two-out-of-three base pairing is thought to cause efficient +1 programmed frameshifting by allowing slippage of the peptidyl-tRNA (Vimaladithan and Farabaugh, 1994). This result suggests that frameshift suppression can occur by a similar event caused by near-cognate decoding.

Since the *proL207* mutation eliminates the cognate tRNA for CCC, it was possible that this effect was peculiar to that tRNA. We tested whether the absence of cmo^5 modification affected frameshifting induced by several other mutant forms of the same tRNA, all of which retain the normal GGG anticodon. The mutations are not limited to the anticodon loop, but map to each of the stems and loops of the tRNA except the TψC stem and loop (Figure 3A). Though each of these tRNAs retains a normal GGG anticodon, we found again that frameshifting stimulated by each was reduced to wild-type levels in the presence of *aroD* (Figure 3B), suggesting that in each of these cases as well it is the near cognate that stimulates frameshifting. Apparently, each of these mutations reduces the efficiency of decoding by the tRNA sufficiently that the near cognate can compete effectively for the CCC codon.

Finally, we tested whether cmo^5 modification had any effect on the two classical frameshift suppressors *sufB2*, which is allelic to the *proL* suppressors and affects $tRNA^{Pro}_{GGG}$, and *sufA6*, which affects the near-cognate $tRNA^{Pro}_{CGG}$. In the case of *sufB2* as with all of the other mutants affecting $tRNA^{Pro}_{GGG}$, lack of cmo^5-modification reduced frameshifting to wild-type levels, arguing that even for this tRNA near cognate, decoding by $tRNA^{Pro}_{cmo5UGG}$ is responsible for frameshifting. The effect of *aroD* on *sufA6* was much less, reducing frameshifting about 2-fold. This result shows that at least a substantial fraction of frameshifting in this strain is independent of $tRNA^{Pro}_{cmo5UGG}$. Since we cannot exclude indirect effects of the lack of modification, such as a change in competition for the codon CCG read by both $tRNA^{Pro}_{CGG}$ and $tRNA^{Pro}_{cmo5UGG}$, it may be that near-cognate decoding does not contribute to frameshifting by *sufA6*.

These data strongly argue that frameshift-suppressor mutations affect $tRNA^{Pro}_{GGG}$ function by reducing its ability to recognize its cognate codon CCC, allowing decoding by the much more abundant near-cognate $tRNA^{Pro}_{cmo5UGG}$. This tRNA can make only two base pairs with CCC, which appears to predispose it to induce frameshifting. Remember that when the *sufA6* suppressor reads CCC, it also makes only two base pairs, since its 3 nt anticodon CGG cannot pair at the wobble position. There is no way to accomodate these data under the classical model of frameshift suppression. First, we find that frameshifting does not even require the direct participation of the mutated tRNA. The fact that the *sufB2* tRNA has an expanded anticodon appears to only result in its inefficient decoding of its cognate codon. The model in which the tRNA directly induces quadruplet decoding is therefore clearly invalid for this tRNA. Second, we find that other tRNAs that have normal 7 nt anticodon loops stimulate frameshifting by the same mechanism. It is possible that this is the general mechanism for frameshift stimulation by such tRNAs. Third, we find a striking similarity between the way near-cognate decoding by a structurally normal $tRNA^{Pro}_{cmo5UGG}$ induces frameshifting and the mechanism used by a classical frameshift suppressor, *sufA6*. These data invalidate the prevailing model for the $tRNA^{Pro}$ suppressors in *S. typhimurium*.

Frameshift Suppression Occurs in Competition with In-Frame Decoding of the Next Codon

The involvement of a structurally normal tRNA in suppression suggests a parallel with programmed frameshifting in which special mRNA sequences stimulate frameshifting by normal tRNAs (reviewed in Farabaugh, 1996a, 1996b). Programmed +1 frameshifts occur when a shift-inducing peptidyl-tRNA occupies the P site, and a poorly recognized codon occupies the A site (Farabaugh, 1996a, 1996b). The poorly recognized codon presumably induces a translational pause stimulating frameshifting. If suppression by *sufB2* resembles programmed frameshifting, i.e., it occurs at the P site, it should also be sensitive to the rate of decoding of the next in-frame codon at the A site. In *S. typhimurium*, the rate of recognition of a UAA termination codon can be slowed using either a temperature-sensitive form of the UAA-specific peptide release factor, RF1, or accelerated by overproducing the wild-type factor. Increasing the rate at which RF1 recognizes a following UAA stop codon reduces frameshifting stimulated by all mutations in *proL* (including *sufB2* [Qian and Björk, 1997a]) and in *proK* (*sufA6*, see Figure 4A), while decreasing it has the opposite effect for *sufB2* (Qian and Björk, 1997a). Similarly, the rate of recognition of UAC codons is reduced 90% in *Salmonella* strains carrying the mutation *miaA1* that reduces ms^2io^6 modification of A37 of its cognate $tRNA^{Tyr}_{QUA}$ (Li et al., 1997). Reducing the rate of recognition of a following UAC codon in the presence of the *miaA1* mutation increased frameshifting both for *sufB2* (Qian and Björk, 1997a) and *sufA6* (Figure 4B). Thus, both classical suppressors *sufB2* and *sufA6* in *S. typhimurium* stimulate frameshifting in competition with normal decoding of the next in-frame codon.

To test the generality of this observation, we measured the effect of translational pausing at the codon following the suppression sites of the classical $tRNA^{Pro}$ and $tRNA^{Gly}$ suppressors in *S. cerevisiae*. In yeast, programmed +1 frameshifting at a site derived from the

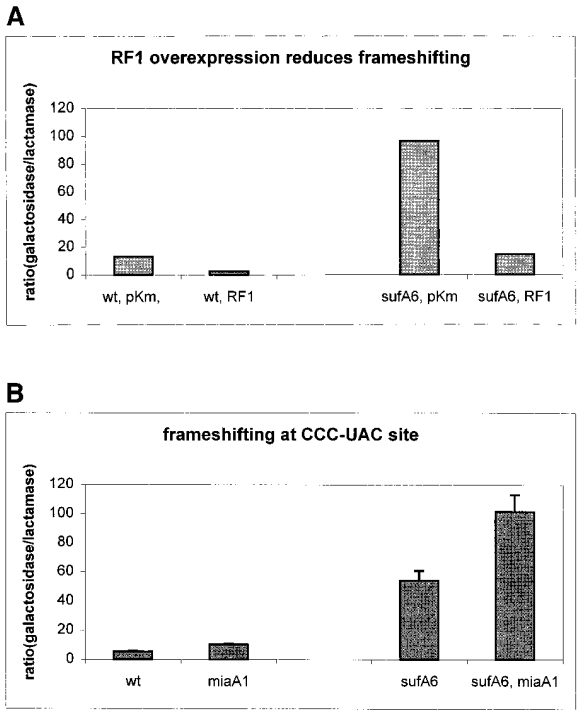


Figure 4. *sufA6*-Mediated Frameshifting Occurs at P Site

(A) Overexpressing RF1 reduces frameshifting at 5′-CCC-UAA-3′ site, measured as described in Figure 3 and Experimental Procedures. Plasmid pFJU335 (*lacI*$^+$, *prfA*$^+$) or pKm66EH (*lacI*$^+$) was introduced (Qian and Björk, 1997b). Cells were than harvested after IPTG induction for one and a half generations, which induces expression of *prfA*. Under these conditions, the growth rate was not reduced. The range of variation is less than 10%.

(B) The *miaA1* mutation, which abolishes the formation of ms^{2}io^{6}A of the UAC cognate (Ericson and Bjork, 1986), increases frameshifting at 5′-CCC-UAC-3′ site. The values are the average of three independent determinations, with error ranges indicated.

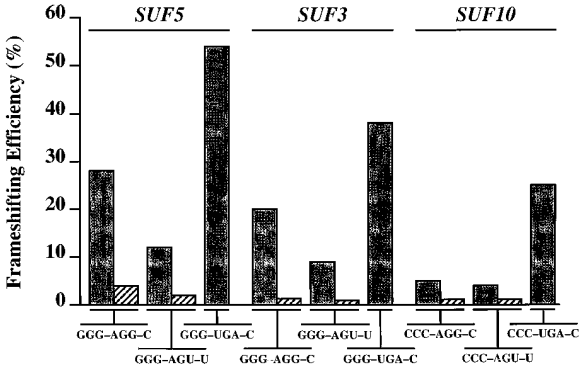


Figure 5. Frameshift Suppression in *S. cerevisiae* Stimulated by Translational Pausing

The frameshift efficiency at each of several programmed frameshift sites involving a sense pause codon (AGG or AGU) or a terminator pause codon (UGA) are shown (closed bars). In the case of the sense codons, the effect of overexpressing their cognate tRNA is shown (paired hatched bars). Frameshifting is expressed as the ratio (in percentage) of activity of β-galactosidase expressed dependent on frameshifting to that expressed without the need for frameshifting ("Frameshift Efficiency (%)"), as described in Experimental Procedures.

retrotransposon Ty3 (Farabaugh et al., 1993b) depends on the rate of recognition of the next codon, a poorly decoded sense codon or a poorly recognized termination codon. We can reduce pausing at a sense codon, and thus reduce programmed frameshifting at the preceding codon, by overexpressing the cognate tRNA that reads the poorly decoded codon (Belcourt and Farabaugh, 1990; Farabaugh et al., 1993b). We tested whether frameshifting induced by classical frameshift suppressors were also sensitive to the rate of decoding of the next in-frame codon. Figure 5 shows that they are. The efficiency of frameshifting varies with the identity of the next in-frame codon; frameshifting is the greatest with a poorly recognized in-frame UGA termination codon, next greatest with a very poorly recognized AGG codon, and least with a less poorly recognized AGU codon. In addition, overexpression of the cognates for AGG ($tRNA^{Arg}_{CCU}$) and AGU ($tRNA^{Ser}_{GCU}$), which reduces pausing (Belcourt and Farabaugh, 1990; Farabaugh et al., 1993b), reduced suppression virtually to background levels.

The effect of the rate of decoding of the next in-frame codon also cannot be accomodated to the classical model of frameshift suppression. That model proposes that the mutant tRNA causes quadruplet translocation of the tRNA·mRNA complex, so that the next normal frame codon is not displayed in the ribosomal A site. In that case, the relative ability to recognize that codon should be irrelevant to the efficiency of frameshifting. All three types of experiments presented here are fundamentally inconsistent with the prevailing model of frameshift suppression by mutant tRNAs, suggesting that the model should be abandoned and replaced by a model that can explain these new data.

Discussion

Frameshift-suppressor mutations were first discovered thirty years ago, and the fact that they involve an expansion of the anticodon loop has been recognized for 25 years. The presumption has always been that decoding by the mutated isoacceptor causes the frameshift, though this cannot be proven from the protein sequence alone. Indeed, *sufB2*-mediated frameshifting does result in insertion of proline at a CCC-U site (Yourno and Tanemura, 1970), but it is only an assumption that the *sufB2* tRNA is the responsible decoding species. The classical model for frameshift suppression explained suppression with such elegance and simplicity that it was readily accepted, and has never been seriously challenged. It is a cornerstone of molecular genetics since it helped demonstrate that reading frame is established by the size of the anticodon. It appears in textbooks as a demonstration of this fact. It has been accepted though, of course, it has not been possible to definitively demonstrate its correctness.

Here, we have presented evidence that fundamentally challenges the model, showing that some classical frameshift-suppressor tRNAs do not function as originally described and implying that perhaps none do. Most particularly, frameshift-suppressor forms of $tRNA^{Pro}$ in *S. typhimurium* cannot base-pair with a 4 nt codon.

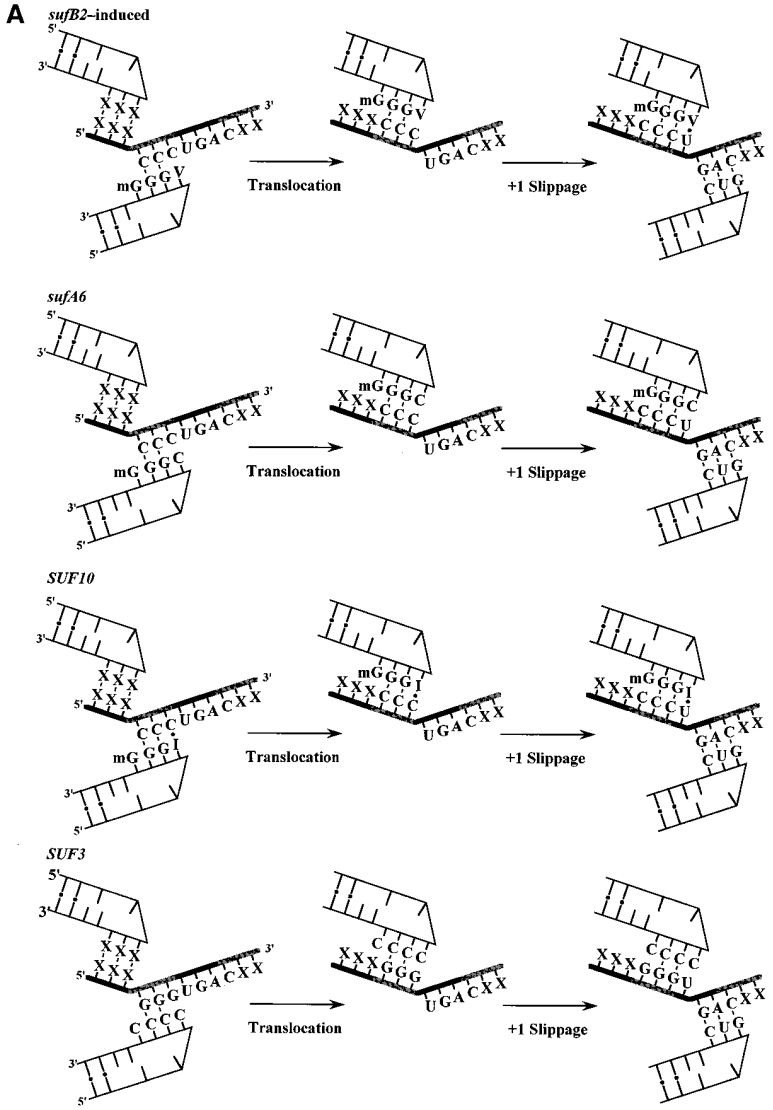
Modification of position 37 to 1-methylguanosine (m^1G) precludes Watson/Crick base pairing with the first base of the putative expanded codon, which is required to distinguish between nonsynonymous codons. The inability to form a base pair at this position fundamentally challenges the idea of a 4 nt codon. It should be emphasized that previous evidence had suggested that it is unnecessary for the decoding tRNA to base-pair with the fourth nucleotide of the expanded codon (Bossi and Roth, 1981; Gaber and Culbertson, 1984). However, the inability to pair with the first base of the proposed expanded codon, while still being able to distinguish between nonsynonymous codons, cannot be reconciled with the classical model of frameshift suppression.

Frameshift suppression by these classical $tRNA^{Pro}$ suppressors actually occurs by near-cognate, or two-out-of-three decoding. In the case of mutant forms of *proL*, for example *sufB2*, frameshifting occurs because of near-cognate decoding by the structurally normal $tRNA^{Pro}_{cmo5UGG}$. Frameshift suppression in a *sufB2* strain (carrying the mutant form of $tRNA^{Pro}_{GGG}$) depends on cmo^5U modification of the near-cognate major isoacceptor, $tRNA^{Pro}_{cmo5UGG}$. This suggests that reading by this normal near cognate actually induces frameshifting. Presumably, an expanded anticodon loop reduces the ability of the *sufB2* form of $tRNA^{Pro}_{GGG}$ to decode, allowing near-cognate recognition by the much more abundant $tRNA^{Pro}_{cmo5UGG}$. This tRNA is structurally normal, so it must recognize a 3 nt codon, though since the wobble cmo^5U cannot pair with C (Yokoyama et al., 1985 and references therein) it can form only two base pairs with the codon. Such pairing can stimulate +1 and −1 frameshifting at programmed frameshift sites because the weakness of this near-cognate interaction allows peptidyl-tRNA to slip efficiently (Farabaugh, 1996a, 1996b). The structure of the second $tRNA^{Pro}$ suppressor *sufA6* suggests that even it must decode by forming only two base pairs with CCC. It carries a normal CGG anticodon in the context of an expanded anticodon loop; this anticodon can only form two G·C base pairs with CCC rather than the three or four required by the prevailing model (Roth, 1981; Bossi and Smith, 1984). Clearly, two-out-of-three decoding by the *sufA6* tRNA, and by wild-type $tRNA^{Pro}_{cmo5UGG}$ in the presence of the *sufB2* mutation is inconsistent with the prevailing model of suppression.

Finally, both in bacteria and in yeast, frameshift suppression occurs in competition with normal decoding of the next in-frame codon (see Qian and Björk, 1997a, Figures 4 and 5). We have shown that this is true for the classical $tRNA^{Pro}$ and $tRNA^{Gly}$ suppressors. Slow recognition of the next in-frame codon, either a poorly recognized sense or termination codon, stimulates frameshifting; overexpressing the cognate tRNA or peptide release factor reverses this stimulation. The effect is not limited to these suppressors since Bossi et al. (1983) showed that mutating the *hisT* gene, encoding the enzyme that catalyzes formation of pseudouridine (ψ) at positions 38–40 of tRNAs (Cortese et al., 1974), increased the *sufJ*-mediated suppression. However, the tRNA encoded by *sufJ*, $tRNA^{Thr}_{GGU}$, does not have ψ in either of these positions (Sprinzl et al., 1996). The *hisT* effect is specific for a single site at which the next codon that would be read if translation continued in-frame is CUG (Bossi et al., 1983). This codon is read in *Salmonella* by $tRNA^{Leu}_{CAG}$, which has ψ at positions 38 and 40 (Sprinzl et al., 1996). Significantly, *hisT* reduces by 70% the rate of A-site selection of a CUG codon by this tRNA (Li et al., 1997), consistent with the idea that pausing at the next in-frame codon also stimulates suppression by *sufJ*. The prevailing model proposes that the suppressor tRNA acts in the A site, reading a 4 nt codon, and causes quadruplet translocation, moving a +1 frame codon into the ribosomal P site (Roth, 1981; Bossi and Smith, 1984). In this model, the overlapping in-frame codon should not be available for decoding in the A site after translocation. Bossi et al. (1983) recognized that the *hisT* data implied competition occurring in the A site while the suppressor tRNA occupies the P site, and suggested that the presumed expanded anticodon might compete with recognition of the next in-frame codon. This suggestion is difficult to accomodate to the prevailing model without suggesting that the nature of the peptidyl-tRNA·mRNA interaction must be dynamic, and that a structural change may occur in competition with continued normal decoding.

We propose an alternative model that explicitly involves such a dynamic event in the P site, and that provides a unified explanation for all forms of phenotypic frameshift suppression. The model is based on similarities between frameshift suppression and programmed frameshifting. It proposes that suppression results from +1 slippage of a peptidyl-tRNA (Figure 1B). As in programmed +1 frameshifting, suppression results from a stochastic competition between continued in-frame reading, and slippage into the +1 frame. The efficiency of suppression should depend on the ability of the peptidyl-tRNA to slip +1, and the length of a translational pause at the next in-frame codon. Since tRNAs making only two base pairs with the mRNA are more prone to slippage, they should be intrinsically more likely to cause frameshifting, as we have found. The model also predicts that the rate of decoding of the next in-frame codon would modulate suppression since slippage is stochastic. The longer the ribosome pauses at the suppression site, the more slippage should occur, as we have also found.

The new model does not explicitly explain the role of the extra base in the anticodon loop. If the extra base does not expand the anticodon, how does it influence frame maintenance? In general, there are two possible explanations. First, the extra nucleotide may influence the ability of the mutant aminoacyl-tRNA to compete against the normal cognate tRNA in the A site. Alternatively, it may predispose the peptidyl-tRNA to slip. In fact, both ideas seem to be correct. The *sufA6* form of $tRNA^{Pro}_{CGG}$ suppresses at CCCN sites under the model by reading the CCC by two-out-of-three decoding $\left(\begin{smallmatrix}5'\text{-CCC-}3'\\ \quad\;|\;|\\ 3'\text{-GGC-}5'\end{smallmatrix}\right)$. Formally, the extra anticodon loop nucleotide must promote this interaction since it is the only difference between the suppressor tRNA and the wild type that cannot suppress, though it could pair in the same way. How it promotes the interaction is unclear. The ribosome probably monitors the correctness of codon·anticodon pairing based on its structure. One can imagine that the additional nucleotide makes the near-cognate codon·anticodon structure more similar to a normal



cognate structure, allowing it to be accepted in the A site more efficiently than a normal near cognate. A corollary would be that the additional nucleotide might make the structure of the mutant cognate complex less like a normal cognate, causing the mutant cognate to be accepted less readily in the A site. This effect would explain the fact that the *sufB2* mutation allows decoding by near-cognate $tRNA^{Pro}_{cmo5UGG}$; the less efficient *sufB2* cognate would be less able to compete with the near cognate for CCC.

Most frameshift suppressors are dominant, arguing that they are gain-of-function mutations. A mutation like *sufB2*, if it causes a loss-of-function, should be recessive. The original characterization of *sufB2* showed that it was dominant to a single copy of $sufB^+$ carried on an F′ episome (Riddle and Roth, 1972a). Later, chromosomal *sufB2* was found to be recessive to $sufB^+$ carried on a multicopy plasmid (Sroga et al., 1992). The assay for suppression was for loss of the His^- phenotype of mutations in histidine biosynthetic genes, a very sensitive assay. Even very low efficiency frameshifting could result in a His^+ phenotype. The assay appears much more sensitive than the *lacZ* reporter system used here since *sufB2* is able to weakly suppress the His^- phenotype of *hisD3018* in the absence of cmo^5U modification of near-cognate $tRNA^{Pro}_{cmo5UGG}$ (Q. Q. and G. R. B., unpublished data), even though the *lacZ* assay shows no frameshifting. Apparently, *sufB2* is in fact weakly dominant because the mutant tRNA can itself induce frameshift suppression, though most suppression results form reading by $tRNA^{Pro}_{cmo5UGG}$. The equivalent mutations affecting the CCC cognate $tRNA^{Pro}_{IGG}$ in yeast (SUF2 and SUF10, Cummins et al., 1980) and the GGG cognates $tRNA^{Gly}_{CCC}$ in yeast (SUF3 and SUF5, Cummins et al., 1980) and *Salmonella* (sufD2, Riddle and Carbon, 1973) are actually much more strongly dominant, suggesting that they do not suppress by allowing near-cognate decoding, but themselves cause suppression, as does the dominant *sufA6* suppressor (Figure 3B).

Since none of the wild-type forms of these cognate tRNAs cause suppression, expansion of the anticodon loop nucleotide must induce suppression. Since these suppressor tRNAs are still able to make a normal cognate 3 bp interaction with the mRNA, perhaps the role

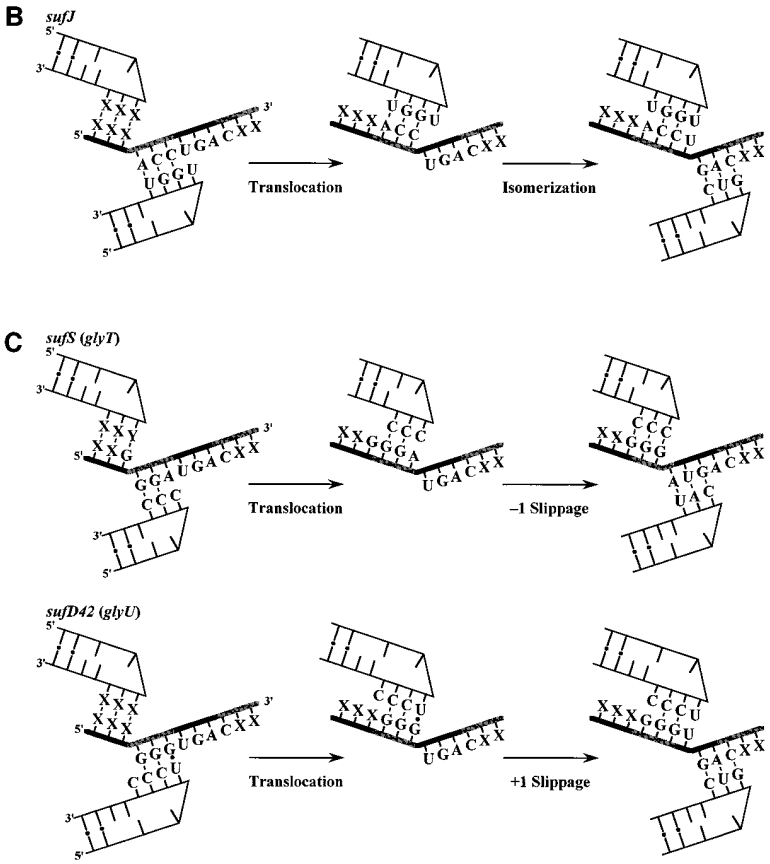


Figure 6. Predicted Frameshift Mechanism for Eight Frameshift Suppressors

(A) Mechanism for the classical $tRNA^{Pro}$ suppressors of *S. typhimurium*, *sufB2*, and *sufA6*, and for the classical $tRNA^{Gly}$ suppressors, *SUF3* and *SUF16* of *S. cerevisiae*.

(B) Mechanism for the low-efficiency suppressors *sufJ*.

(C) Mechanism for −1 (*sufS*) and +1 (*sufD42*) suppression by $tRNA^{Gly}$ suppressors. In each case, the figure shows pairing between the mRNA and the anticodon loop of the tRNA, progressing from initial pairing in the ribosomal A site (left), through movement of the suppressor tRNA into the P site (middle), to slippage or isomerization (right). The mRNA is shown paired with tRNAs in the P site (above) and A site (below); the anticodon loops of each of the tRNAs are shown with the potentially base-pairing bases labeled. Normal Watson/Crick pairing is denoted by bars, canonical wobble pairing by dots.

of expansion for these tRNAs is to promote a different event. Possibly, expansion directly stimulates the event causing frameshifting, which the model proposes is slippage by the peptidyl-tRNA once it moves into the ribosomal P site. In effect, the expansion of the anticodon loop may weaken the interaction between these tRNAs and the mRNA, just as the lack of base pairing in the wobble position does for a near-cognate suppressor tRNA. So the extra nucleotide could have at least two roles: promoting near-cognate decoding, and stimulating slippage of peptidyl-tRNA. For suppressors affecting near-cognate tRNAs expansion could have both roles, while for cognates only the slippage effect may stimulate frameshifting.

One obvious objection to our new model is that a mutant $tRNA^{Pro}$ invariably only suppresses sites including a CCC-N sequence, shown in the normal reading frame, and not sequences such as CCG-N (Mathison and Culbertson, 1985; Q. Q. and G. R. B., unpublished data). This has implied that they must recognize the third base by a G-C pair. The lack of pairing in the third position by suppressor tRNAs that use two-out-of-three decoding would seem inconsistent with the observation. However, the need for the peptidyl-$tRNA^{Pro}$ to slip +1 explains the third base, since only a CCC-N sequence would allow formation of at least two base pairs in the shifted frame, which is necessary for +1 slippage.

An attractive aspect of the new model is its ability to explain all frameshift-suppressor tRNAs, including several not originally explained by the prevailing model. Figure 6 shows how the new model can explain the several types of frameshift suppressors that have been identified in both bacteria and yeast. In a *sufB2* strain, the near-cognate $tRNA^{Pro}_{cmo5UGG}$ can decode CCC in the A site forming two base pairs, and translocate into the P site where it undergoes +1 slippage (Figure 6A). A similar mechanism can be proposed for other $tRNA^{Pro}$ suppressors, diagrammed for the *sufA6* suppressor and the yeast suppressor *SUF10*, and for $tRNA^{Gly}$ suppressors, diagrammed for the *SUF3* suppressor of yeast (Figure 6A). In each case, the anticodon used would consist of the three nucleotides immediately 5′ to the conserved kink after U33, the location of the anticodon in a normal tRNA. In each case, frameshifting would occur because this anticodon dissociates from the mRNA, and repairs with the codon overlapping in the +1 frame.

The *sufJ*, *sufT*, and *proK* and *su7* suppressors have a slightly different structure (Bossi and Smith, 1984; Curran and Yarus, 1987; Tuohy et al., 1992). They have an extra nucleotide inserted between U33 and the three bases that form the anticodon that can pair to the zero frame codon at the suppression site. These suppressors must induce frameshifting by a slightly different mechanism (Figure 6B shows this mechanism for the *sufJ* suppressor). We assume that the suppressors form a 3 bp codon·anticodon complex that is shifted 1 nt on the tRNA. The structure in the A site would not be normal since pairing in the A site is to a shifted anticodon, as

Table 1. Strains and Plasmids

	Genotype	Source
Plasmids	*Salmonella typhimurum*	
	pTHF15(CCC-UAA-A)	Hagervall et al., 1993
	pTHF54(CCC-UAC-A)	Hagervall et al., 1993
	pKM66EH(*lac1*$^+$)	Qian and Björk, 1997b
	pFJU335(*lac1*$^+$, *prfA*$^+$)	Jörgensen et al., 1993
Plasmids	*Saccharomyces cerevisiae*	
	pMB38-Ty3FF	Farabaugh et al., 1993b
	pMB38-Ty3-GGGAGGC	Vimaladithan and Farabaugh, 1994
	pMB38-Ty3-GGGAGUU	Vimaladithan and Farabaugh, 1994
	pMB38-Ty3-CCCAGGC	Vimaladithan and Farabaug, 1994
	pMB38-Ty3-CCCAGUU	Vimaladithan and Farabaugh, 1994
	pMB38-Ty3-GGGUGAC	This study
	pMB38-Ty3-CCCUGAC	This study
Strains	*Salmonella typhimurum*	
LT2	Wild type	
TR936	*hisO1242 hisD3018 sufB2*	Riddle and Roth, 1972b
GT875	*trmD3*	Björk et al., 1989
TR1457	*hisO1242 hisD3749 sufA6*	Riddle and Roth, 1972b
GT1380	*hisO1242 hisC3737 proL201 zxx-2502::Tn10*	Srogo et al., 1992
GT1480	*hisO1242 hisC3737 proL202 zxx-2502::Tn10*	Qian and Björk, 1997b
GT2920	*hisO1242 hisD3749 proL208 zef-2502::Tn10*	Qian and Björk, 1997b
GT2922	*hisO1242 hisD3749 proL210 zef-2502::Tn10*	Qian and Björk, 1997b
GT3022	*hisO1242 hisD3749 proL211 zef-2502::Tn10*	Qian and Björk, 1997b
GT3027	*hisO1242 hisD3749 proL216 zef-2502::Tn10*	Qian and Björk, 1997b
GT3674	*hisO1242 hisD3749 proK207 zef-201::Tn10del16del17Cm*	This study
GT4517	*hisO1242 hisD3749 aroD-553::Tn10 zef-2516::Tn10dCm*	This study
GT484	*hisO1242 hisD3018 sufB2 aroD553::Tn10*	This study
GT483	*hisO1242 hisD3749 sufA6 aroD553::Tn10*	This study
GT4571	*hisO1242 hisC3737 proL201 zef-2502::Tn10 aroD-549::Tn5*	This study
GT4572	*hisO1242 hisD3737 proL202 zef-2502::Tn10 aroD-549::Tn5*	This study
GT4518	*hisO1242 hisD3749 aroD-553::Tn10 proL207 zef-2516::Tn10dCm*	This study
GT4574	*hisO1242 hisD3749 proL208 zef-2502::Tn10 aroD-549::Tn5*	This study
GT4576	*hisO1242 hisD3749 proL210 zef-2502::Tn10 aroD-549::Tn5*	This study
GT4577	*hisO1242 hisD3749 proL211 zef-2502::Tn10 aroD-549::Tn5*	This study
GT4582	*hisO1242 hisD3749 proL216 zef-2502::Tn10 aroD-549::Tn5*	This study
GT563	*hisO1242 hisD3749 sufA6 zjc-u8::Tn10 miaA1*	This study
Strains	*Saccharomyces cerevisiae*	
UPX32-3B	*leu2-3 ura3-52 trp1Δ63 his3 SUF5*	This study
UPX33-5A	*leu2-3 ura3-52 trp1Δ63 his3 SUF3*	This study
UPX-37-3A	*leu2-3 ura3-52 trp1Δ63 his3 SUF10*	This study

shown in Figure 6B. Shifted pairing might be expected to be inefficient, and indeed, these suppressors are very weak. When these tRNAs translocate to the P site, the codon·anticodon complex could isomerize with the mRNA slipping by one base to shift the A site +1 on the mRNA (note in Figure 6B how the fourth nucleotide, a U, moves from the A to the P site during isomerization). Note that also in these cases frameshifting should be stimulated by a translational pause, which has been shown to be the case for *sufJ* (Bossi et al., 1983). The main difference between this mechanism and that described above is that the tRNA does not dissociate from the mRNA, but simply moves the equivalent of 1 nt in the P site. This movement may place the tRNA in the P site closer to its normal position.

Finally, the model can explain *glyT* −1 frameshift suppressors—the suppressor mutations are termed *sufS* though they occur in the *glyT* gene (Figure 6C). These are the only known class of −1 suppressors (O'Mahony et al., 1989). *glyT* is the only gene encoding $tRNA^{Gly}_{UCC}$, the GGA cognate tRNA. A tRNA in which the normal UCC anticodon is replaced by CCC causes −1 frameshifting at N-GGA sites, but about 2-fold more efficiently at G-GGA. This tRNA can make only 2 bp with GGA; since *glyT* is the only gene encoding $tRNA^{Gly}_{UCC}$, there would be no competition by a cognate tRNA, so the near cognate could decode efficiently. Once the tRNA translocated to the P site, it could slip −1 onto GGG, forming a 3 bp codon·anticodon complex. This model strongly resembles the +1 suppression model. Interestingly, different mutants of $tRNA^{Gly}$ cause +1 frameshifting (for example, *sufD42*, Figure 6C). In this case, the tRNA has an expanded 8 nt anticodon loop. The new model again predicts that it decodes a GGG codon with a 3 nt anticodon, and slips +1 in the P site after translocation. The fact that +1 suppressors do not cause suppression at −1 frameshift sites implies that their expanded anticodon constrains slippage to occur in the +1 direction. The structurally normal *glyT* −1 suppressor tRNA, on the other hand, is constrained to slip −1 by the lack of available pairing to the +1 frame codon. So the different suppression patterns of the two tRNAs can be explained by pairing interactions, and the effect of the expanded anticodon loop.

Some of the *glyT* suppressors have normal anticodon loops retaining the UCC anticodon, but have mutations elsewhere in the molecule. These suppressors are similar to the *proL* +1 suppressors of *Salmonella* (Qian and

Björk, 1997a, 1997b). All of these suppressors are recessive, suggesting that they are loss-of-function mutations. We suggest that each mutation reduces the ability of the tRNA to decode its cognate codon, allowing near-cognate decoding, for the *proL* mutations by $tRNA^{Pro}_{cmo5UGG}$ as we have shown (Figure 3B), and for the *glyT* (*sufS*) suppressors by the near-cognate $tRNA^{Gly}_{CCC}$. Decoding by this near cognate could promote −1 frameshifting as described above for a $tRNA^{Gly}_{UCC}$ mutated to have a CCC anticodon. Pagel et al. (1992) purported to demonstrate that −1 frameshifting by the *glyT* suppressors requires the mutant form of $tRNA^{Gly}_{UCC}$, which would seem to invalidate this argument. However, what the paper actually demonstrates is that frameshifting only occurs when a cognate form of $tRNA^{Gly}_{UCC}$ is not present. In every case, suppression is abolished when a tRNA with anticodon UCC is present in vivo, encoded either by a wild-type form of *glyT*, or by a mutant *glyV55*, a mutation that introduces the UCC anticodon into $tRNA^{Gly}_{GCC}$ (Carbon et al., 1970). In effect, they demonstrated the necessity of mutant forms of $tRNA^{Gly}_{UCC}$ to suppression, but not their sufficiency. These data are in fact also consistent with the new model.

While it is not possible to definitively invalidate the classical quadruplet translocation model for all suppressors with expanded anticodon loops, the data here invalidate that model for the classical suppressor forms of $tRNA^{Pro}$ isoacceptors in *S. typhimurium*. An alternative model to explain frameshifting in the presence of these suppressors can explain frameshift suppression by all of the classical frameshift +1 suppressors, and can be extended to explain suppression by other mutant tRNAs that do not have expanded anticodon loops, and even −1 suppressors. Since this model provides a unified explanation for all phenotypic suppression, we consider it to be preferable to the classical model, and suggest that it be considered a provisional general explanation pending more definitive proof.

Experimental Procedures

Strains and Materials

The *S. typhimurium* and *S. cerevisiae* strains used are listed in Table 1. ONPG was from Sigma (St. Louis, MO), and Nitrocefin from Oxide (UK). Transductions with P22 HT105 (int-201, Schmieger, 1972) were performed as described (Davis et al., 1980). For genetic experiments, medium E was used (Vogel and Bonner, 1956). Rich medium for *S. typhimurium* strains was NB+AV+ADE (NAA) medium (Difco nutrient broth [0.8%], Difco Laboratories, Detroit, MI) supplemented with 0.5 M NaCl, tyrosine, tryptophan, phenylalanine, 2,3-dihydroxybenzoate, p-hydroxybenzoate, p-aminobenzoate, and adenine. All supplements were provided at the concentration recommended (Davis et al., 1980).

S. cerevisiae strains were transformed by the method of Gietz et al. (1992). Transformants were selected and grown in SD medium supplemented with required amino acids but lacking uracil, to select for the transforming plasmid (Rose et al., 1990).

Determining the Efficiency of Translational Frameshifting

Translational frameshifting was assayed in *S. typhimurium* and in *S. cerevisiae* using rather different methods. For the bacterium *S. typhimurium*, the approach involves measuring the level of β-galactosidase activity expressed from a construct containing a suppressible frameshift mutation, normalizing the expression in the various strain backgrounds using the level of β-lactamase expressed from the plasmid-encoded *bla* gene to control for variation in plasmid copy number and general translational efficiency. These assays were performed as described (Hagervall et al., 1993; Qian and Björk, 1997b). For the yeast *S. cerevisiae*, we used a programmed frameshifting system derived from the retrotransposon Ty3 (Farabaugh et al., 1993b). The efficiency of frameshifting was estimated by comparing the level of β-galactosidase expressed from a construct in which its expression required +1 frameshifting at the programmed site (frameshift construct) to the level expressed from a control plasmid in which the gene was expressed without the need for frameshifting (frame-fusion construct). The efficiency is expressed as the ratio of the activity expressed from the frameshift construct to that of the frame-fusion given as a percentage. The assays were performed in triplicate as described (Farabaugh et al., 1993a).

Determining the Position of m^1G Using Primer Extension

Strains LT2 (WT), TR1457 (*sufA6*), TR936 (*sufB2*), and GT875 (*trmD3*) were grown in NAA medium at 37°C (LT2, TR1457, and TR936) or 42°C (GT875); incubation of GT875 at 42°C was done to eliminate all m^1G modification. Bulk tRNAs were prepared according to Buck et al. (1983). Wild-type and mutant forms of $tRNA^{Pro}_{CGG}$ and $tRNA^{Pro}_{GGG}$ species were purified to homogeneity by hybridizing to a dynal-labeled oligonucleotide complementary to the anticodon region of each tRNA (Morl and Schmelzer, 1993; Qian and Björk, 1997b). Primer extension was performed using DNA oligonucleotides end-labeled with [γ-^{32}P]-ATP complementary to nucleotides 39–60 of $tRNA^{Pro}_{CGG}$ (5′-AUUCGAACCUCCGACCCCUUCG-3′) and $tRNA^{Pro}_{GGG}$ (5′-AUUUGAACCUCCGACCCCCGAC-3′). Extension products were separated by electrophoresis through a 12% acrylamide, 8.3 M urea gel.

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