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Near-Cognate Peptidyl-tRNAs Promote +1 Programmed Translational Frameshifting in Yeast

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Summary

Translational frameshifting is a ubiquitous, if rare, form of alternative decoding in which ribosomes spontaneously shift reading frames during translation elongation. In studying +1 frameshifting in Ty retrotransposons of the yeast *S. cerevisiae*, we previously showed that unusual P site tRNAs induce frameshifting. The frameshift-inducing tRNAs we show here are near-cognates for the P site codon. Their abnormal decoding induces frameshifting in either of two ways: weak codon–anticodon pairing allows the tRNA to disengage from the mRNA and slip +1, or an unusual codon–anticodon structure interferes with cognate in-frame decoding allowing out-of-frame decoding in the A site. We draw parallels between this mechanism and a proposed mechanism of frameshift suppression by mutant tRNAs.

Introduction

Programmed frameshift sites are sequences in mRNAs that cause ribosomes to shift reading frames efficiently during translation (reviewed in Farabaugh, 1996; Gesteland and Atkins, 1996). Frameshifting at nonprogrammed sites occurs very infrequently, one estimate putting the rate below 3×10^{-5} per codon (Kurland, 1992). Programmed frameshifting occurs at rates from 1,000- to 10,000-fold higher. How programmed sites cause this drastic increase in frameshifting differs from one instance to another, but there are some general rules. The most general is that frameshifting occurs as a result of translational pausing. Ribosomes may pause when they encounter a poorly recognized codon or a downstream secondary structure like an RNA pseudoknot. The effect of the pause is to interfere with continued reading in the normal, or zero, frame. During the pause, the tRNA or tRNAs that occupy the ribosomal decoding site cause a shift in reading frame. The simplest way this can happen is for the mRNA-bound tRNA or tRNAs to transiently dissociate from the mRNA, rebinding to a codon in the shifted reading frame to which they make a minimum of two base pairs. Some programmed frameshifts appear to use a second mechanism that does not require tRNA slippage. A frameshift-inducing peptidyl-tRNA can induce out-of-frame reading by a cognate tRNA. In either mechanism, after acceptance of the aminoacyl-tRNA in the new reading frame translation continues normally.

The most common form of programmed frameshift is −1 simultaneous slippage frameshifting first found in eukaryotic viruses (reviewed in Farabaugh, 1996; Gesteland and Atkins, 1996). This event occurs when two tRNAs occupy the ribosome reading a sequence of the form X-XXY-YYZ (where X = G, A, U, or C; Y = A or U; and Z is species specific; for example, U-UUA-AAC). During a pause caused by a downstream secondary structure, usually a pseudoknot, the repetitive nature of the heptamer allows tRNAs decoding XXY-YYZ in the zero frame to shift to the left to XXX-YYY in the −1 frame. Although we know a great deal about the importance of the slipperiness of the heptameric frameshift site (see Brierley et al., 1992) and the structural requirements of the pseudoknot (Chen et al., 1996; Liphardt et al., 1999; Napthine et al., 1999), we know surprisingly little about the mechanism by which the pseudoknot induces −1 frameshifting. We do know that the pseudoknot does more than simply pause the ribosome (Tu et al., 1992; Somogyi et al., 1993), though what its second role may be is unclear.

Arguably, the fundamental mechanism underlying a second less ubiquitous type of event, +1 frameshifting, is better understood. In general, programmed +1 frameshifting occurs during slow recognition of a poorly recognized in-frame codon (reviewed by Farabaugh, 1996; Gesteland and Atkins, 1996). The first known and canonical example comes from the *prfB* gene of *E. coli*, which encodes peptide release factor 2 (RF2). Frameshifting in *prfB* is autogenously regulated by the rate of recognition of an in-frame UGA codon by RF2 (Craigen et al., 1985; Craigen and Caskey, 1986; Donly et al., 1990). Frameshifting appears to occur by slippage of peptidyl-$tRNA^{Leu}$ during a translational pause caused by slow recognition of the UGA when RF2 is limiting. Frameshifting results in increased synthesis of RF2, leading to reduced frameshifting. By replacing the UGA terminator by 29 different sense codons, Curran and Yarus (1989) showed that apparent slow decoding of sense codons could also stimulate frameshifting at the *prfB* site. The poor availability of aminoacyl-tRNAs cognate for these codons may cause a translational pause with the slippage-prone codon in the ribosomal A site. Interestingly, the effect of the pause is less pronounced in nonprogrammed frameshifts at UUU-Y sites, implying that frameshifting in these cases may occur in the ribosomal A site (Schwartz and Curran, 1997). Why this should differ from programmed events is unclear.

Slow decoding of an in-frame sense codon also stimulates programmed +1 frameshifting in expression of *Saccharomyces cerevisiae* retrotransposons Ty1 (Belcourt and Farabaugh, 1990) and Ty3 (Farabaugh et al., 1993). In each case, overexpressing the cognate tRNAs for a slowly recognized codon, AGG or AGU, reduces frameshifting to background levels. Deleting the gene encoding the AGG cognate, *HSX1*, forces slower decoding of AGG by the near-cognate $tRNA^{Arg}_{UCU}$ and stimulates frameshifting (Kawakami et al., 1993). Ty1 frameshifting

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closely resembles the *prfB* event, since during the translational pause, a peptidyl-tRNA^{Leu} bound immediately upstream of the pause codon slips +1 on the mRNA (Belcourt and Farabaugh, 1990). However, Ty3 frameshifting occurs by a then unexpected mechanism; during the translational pause, a peptidyl-tRNA^{Ala} stimulates out-of-frame binding of tRNA$^{Val}_{IAC}$ without itself slipping on the mRNA (Farabaugh et al., 1993).

In an effort to understand in detail the molecular mechanism underlying programmed +1 frameshifting in *S. cerevisiae*, we cataloged all of the codons that could promote the shift when placed in the ribosomal P site of the Ty3 frameshift site (Vimaladithan and Farabaugh, 1994). Somewhat surprisingly, we found that a total of 11 codons could stimulate frameshifting, some extremely efficiently, and that all of the other 53 stimulated frameshifting at much lower levels (Vimaladithan and Farabaugh, 1994). None of these frameshift-inducing codons is among the most commonly used codons, but most are not especially uncommon. One observation seemed particularly noteworthy. An AGG P site codon normally stimulates little frameshifting. However, in the absence of its cognate tRNA, it strongly stimulates frameshifting, implying that it is near-cognate tRNA$^{Arg}_{UCU}$ that stimulates the error. Modification of the wobble nucleotide of tRNA$^{Arg}_{UCU}$ to 5-methoxycarbonylmethyluridine (mcm^{5}U) weakens its pairing with AGG (Yokoyama et al., 1985). This situation resembles the strong stimulation of −1 programmed frameshifting at a site in the *dnaX* gene of *E. coli* caused by tRNA$^{Lys}_{UUU}$, which has 5-methylaminomethyl-2-thiouridine (mnm^{5}s^{2}U) at the wobble position (Tsuchihashi and Brown, 1992). In both cases, wobble modification weakens binding to the codon in the frameshift site and stimulates tRNA slippage. Given the ability of near-cognate decoding to stimulate +1 frameshifting, we wondered whether this was a general phenomenon; that is, does frameshifting induced by other codons in the P site depend on near-cognate decoding? The evidence presented here shows that in all cases +1 frameshifting occurs in *S. cerevisiae* because a near-cognate peptidyl-tRNA occupies the ribosomal P site during the translational pause. We will discuss how such noncanonical decoding induces efficient frameshift errors and describe how this effect is related to a recently proposed model of frameshift suppression by mutant tRNAs (Qian et al., 1998).

Results

Some Frameshift-Stimulating Codons Lack Expected Cognate tRNAs

We showed that 11 of the 64 codons can stimulate frameshifting when they occur immediately preceding a pause-inducing codon (Vimaladithan and Farabaugh, 1994), but why do they stimulate the shift? The yeast genome sequence suggests a possible explanation. Independent searches of the *S. cerevisiae* genome sequence determined that the genome encodes 274 tRNA genes encoding 41 distinct elongator tRNAs and initiator tRNA^{Met} (el-Mabrouk and Lisacek, 1996; Percudani et al., 1997). Based on the tRNAs that were known before completion of the genome sequence, Guthrie and Abelson (1982) had predicted that there would be 45 elongator species but genes encoding cognate tRNAs for four

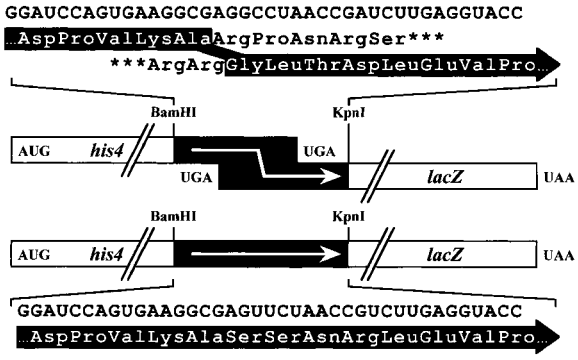

Figure 1. Frameshifting Assay

The structure of a representative frameshift reporter plasmid (pMB38-Ty3Δ2, top) and the in-frame control plasmid, pMB38-Ty3FF (bottom). In the frameshift reporter construct, translation begins at the *HIS4* initiator AUG and continues for 33 codons to the inserted programmed frameshift site, shown above. Ribosomes that frameshift continue into *lacZ* to express full-length β-galactosidase. In the in-frame control construct, ribosomes can continue directly into *lacZ* without frameshifting. The primary protein product of β-galactosidase from each plasmid is shown highlighted in white on black.

codons, CUG, CCG, CGA, and GCG, are absent from the genome. Significantly, these four codons each can induce efficient programmed +1 frameshifting. The lack of the predicted cognate tRNA implies that they are read by isoacceptors using a less than optimal wobble interaction, that is, a near-cognate tRNA. Is it possible that the mere fact of their obligate near-cognate decoding disposes them to induce frameshifting? Each of the other frameshift-inducing codons are recognized by low-abundance tRNAs suggesting that highly abundant near-cognate tRNAs might be able to compete to decode them as well and induce frameshifting.

If obligate near-cognate decoding induces frameshifting, then overexpressing a synthetic cognate tRNA for each of the four codons lacking one should reduce their ability to stimulate frameshifting. Cognate tRNA genes were made by altering the anticodons of genes encoding existing isoacceptors (using oligonucleotides shown in Table 5 as described in the Experimental Procedures). Each synthetic tRNA gene was cloned onto an expression plasmid carrying either of two *lacZ* reporter gene fusions (Figure 1). The first carries a *lacZ* reporter gene fusion, which expresses β-galactosidase via frameshifting at a programmed site with the synthetic tRNA's cognate codon as the P site codon (the codon occupied by peptidyl-tRNA when the frameshift occurs). The second is an in-frame reporter construct in which translation continues into *lacZ* without the need for frameshifting. The apparent frameshift efficiency is calculated as the ratio of expression from the frameshift expression plasmid to the in-frame control, assumed to allow 100% readthrough into *lacZ*. The effect of the synthetic cognate was determined by comparing frameshift efficiency with and without the synthetic tRNA gene. To maximize our ability to see the effect of expressing the cognate tRNAs for each frameshift-inducing codon, these experiments were done in a strain that maximally induces frameshifting, KK240. This strain lacks tRNA$^{Arg}_{CCU}$, the cognate tRNA for the pause-inducing AGG codon in the

Table 1. Overexpressing tRNAs Reduces Frameshifting on Their Cognate Codons

Frameshift Site[a]	Strain[b]	tRNA Overexpressed	Frameshift Efficiency, %[c]	
			− tRNA	+ tRNA
GCG-AGG-C	KK240	$tRNA^{Ala}_{CGC}$[d]	62 ± 6.5	7.9 ± 0.5
CCG-AGG-C	KK240	$tRNA^{Pro}_{CGG}$[d]	69 ± 3.5	5.0 ± 1.5
CUG-AGG-C	KK240	$tRNA^{Leu}_{CAG}$[d]	46 ± 2.0	2.6 ± 1.0
CGA-AGG-C	KK240	$tRNA^{Arg}_{UCG}$[d]	14 ± 1.0	2.6 ± 1.0
CUU-AGG-C	KK240	$tRNA^{Leu}_{GAG}$	69 ± 4.7	22 ± 5.3
CUC-AGG-C	KK240	$tRNA^{Leu}_{GAG}$	36 ± 3.0	1.6 ± 0.2
GGG-AGG-C	KK242	$tRNA^{Gly}_{CCC}$	18 ± 1.6	6.3 ± 0.3
CCU-AGG-C	KK240	$tRNA^{Pro}_{IGG}$	7.4 ± 0.3	4.6 ± 0.3
CCC-AGG-C	KK240	$tRNA^{Pro}_{IGG}$	7.1 ± 0.1	1.0 ± 0.02
GUG-AGG-C	KK240	$tRNA^{Val}_{CAC}$	5.5 ± 0.3	1.5 ± 0.05

[a] The sequences of the frameshift sites used (shown as P and A site codons).
[b] The yeast strain into which the plasmid constructs were introduced. KK240 lacks the gene encoding $tRNA^{Arg}_{CCU}$, and KK242 is the congenic wild type (see the Experimental Procedures for genotypes). Pausing at AGG is elongated in KK240 compared with KK242.
[c] The ratio of expression of β-galactosidase from a *lacZ* reporter requiring frameshifting at the site indicated to expression from an in-frame control, pMB38-Ty3FF.
[d] These are novel synthetic tRNAs constructed by modifying the anticodon of existing yeast tRNAs, as described in the Experimental Procedures.

programmed frameshift site. Especially slow decoding of AGG by $tRNA^{Arg}_{UCU}$ causes an elongated pause, drastically increasing apparent frameshift efficiency (Kawakami et al., 1993; Vimaladithan and Farabaugh, 1994).

As shown in Table 1 (lines 1–4), frameshifting on the codons GCG, CCG, CUG, and CGA is very efficient in this background, ranging from a high of 69% for CCG to a low of 14% for CGA. Introducing synthetic cognate tRNAs matching the codons in each case strongly reduced frameshift efficiency (compare columns 4 and 5). The effect was variable, the largest decrease being with CUG (18-fold from 46% to 2.6%). This result is entirely consistent with the idea that frameshifting requires near-cognate decoding. The residual level of frameshifting in the presence of the synthetic cognate tRNAs must result from continued reading by endogenous frameshift-inducing tRNA(s).

We performed three other types of experiments to test the hypothesized connection between near-cognate decoding and +1 frameshifting in *S. cerevisiae*. First, we overexpressed normal cognate tRNAs specific to certain frameshift-inducing codons or deleted the structural genes of some of these cognate tRNAs to force near-cognate decoding. If near-cognate decoding is a general cause of frameshifting, then overexpressing cognates tRNAs should reduce frameshifting, while eliminating them should stimulate frameshifting. Second, we overexpressed certain near-cognate tRNAs to impose near-cognate decoding. Again, under the hypothesis that overexpressing any frameshift-inducing isoacceptor should stimulate frameshifting. As described below, the results of each of these experiments proved consistent with the hypothesis.

Overexpressing Normal Cognate tRNAs of Other Frameshift-Inducing Codons

One or two structural genes encode cognate isoacceptors for six other frameshift-inducing codons: $tRNA^{Leu}_{GAG}$ for CUU and CUC, $tRNA^{Pro}_{IGG}$ for CCU and CCC, $tRNA^{Val}_{CAC}$ for GUG, and $tRNA^{Gly}_{CCC}$ for GGG (Sprinzl et al., 1996; Percudani et al., 1997). Since the abundance of tRNAs in yeast is directly related to gene number (Percudani et al., 1997) and highly abundant tRNAs often are encoded by over ten gene copies, these tRNAs would be expected to be low abundance. Data is only available for $tRNA^{Val}_{CAC}$, which is one of the lowest abundance tRNAs in yeast (Ikemura, 1985). Further, the putative cognate for CUU and CUC, $tRNA^{Leu}_{GAG}$ may actually be partially defective since a universal nucleotide in the tRNA, U_{33}, has been replaced by cytidine. Although early work showed that altering U_{33} had no effect on tRNA function in vitro (Bare et al., 1983), subsequently data showed that such mutations strongly reduce tRNA function in vivo (Ayer and Yarus, 1986). More recent work showed that C_{33} $tRNA^{Phe}$ binds to poly(U)-programmed 30S ribosomes with about 15-fold lower affinity (Ashraf et al., 1999). Perhaps the low abundance or poor decoding ability of these tRNAs allows near-cognate decoding by another much more abundant isoacceptor, therefore promoting frameshifting.

We inserted a copy of each tRNA gene as above into a frameshift–reporter *lacZ* fusion plasmid carrying a frameshift site consisting of its cognate codon followed by the AGG pause codon (e.g., CCU-AGG for $tRNA^{Pro}_{IGG}$). The reporter plasmids are present at about four copies per genome (data not shown). Since tRNA abundance is directly related to gene number (Percudani et al., 1997), increasing the gene number by introducing plasmid-encoded copies should correspondingly increase the concentration of tRNAs in vivo. RNA blotting experiments show that this is roughly the case (data not shown). The striking result of this analysis as predicted by the near-cognate decoding hypothesis is that overexpressing the cognate tRNA in every case reduced the efficiency of frameshifting (Table 1, lines 5–10). In nearly every case, the reduction was quite substantial, up to 22-fold on the sequence CUC-AGG-C. No significant change in frameshift efficiency resulted when the unusual C_{33} base of $tRNA^{Leu}_{GAG}$ was changed to U_{33} (data not shown) implying, contrary to expectation, that the base may not significantly affect the tRNA's decoding ability. In one case, with $tRNA^{Pro}_{IGG}$ and CCU-AGG-C, the reduction was less than 2-fold (Table 1, line 8), though this is still quite statistically significant. Since frameshifting was reduced to different extents on two codons read by the

Table 2. Deleting the Structural Genes for tRNAs Induces Frameshifting on Their Cognate Codons

Strain	Relevant Genotype[a]	Frameshift Site[b]	Frameshift Efficiency, %[b]
UPF7	*tV(CAC)D tV(CAC)H*	GUG-AGG-C	2.2 ± 0.1
UPX12-1B	*tv(cac)d::kanR tV(CAC)H*	GUG-AGG-C	2.7 ± 0.1
UPX14-1A	*tV(CAC)D tv(cac)h::kanR*	GUG-AGG-C	2.1 ± 0.2
UPX19-3C	*tv(cac)d::kanR tv(cac)h::kanR*	GUG-AGG-C	6.0 ± 0.3
UPF7	*SUF3 SUF5*	GGG-AGG-C	9.2 ± 0.2
UPF76	*suf3::kanR SUF5*	GGG-AGG-C	17 ± 4.3
UPF78	*SUF3 suf5::kanR*	GGG-AGG-C	13 ± 2.2
UPX20-16A	*suf3::kanR suf5::kanR*	GGG-AGG-C	26 ± 1.2

[a] The yeast strain into which the plasmid constructs were introduced. The two genes encoding $tRNA^{Val}_{CAC}$ are referred to using their systematic names. V stands for the amino acid specificity, CAC for the anticodon, D or H for the chromosome (Chr. IV or VIII) on which the gene resides, and *kanR* for the gene used in the disruption of locus.
[b] As in Table 1.

same isoacceptor, CCU and CCC, the effect is not an intrinsic effect of the tRNA. We suspect that the difference reflects a difference in the strength of the codon–anticodon interaction. An I·C base pair has normal Watson/Crick geometry, but an I·U base pair does not, suggesting that recognition of CCC by $tRNA^{Pro}_{IGG}$ using an I·C wobble may be more efficient than of CCU using I·U (Yokoyama and Nishimura, 1995). Thus, $tRNA^{Pro}_{IGG}$ should compete against a presumed near-cognate tRNA more effectively on CCC than on CCU, as observed.

Deleting Genes Encoding Cognate tRNAs Strongly Stimulates Frameshifting

If overexpressing cognate tRNAs reduces frameshifting by reducing the probability of near-cognate decoding, then any effect tending to reduce the rate of cognate reading should have the opposite effect, stimulating frameshifting. Deleting the structural genes encoding tRNAs cognate for frameshift-inducing codons should stimulate frameshifting since the codons would then be obliged to be decoded by a near-cognate tRNA. As described above, we had already performed such an experiment by deleting the gene encoding the AGG-decoding $tRNA^{Arg}_{CCU}$ to force decoding of AGG by the near-cognate $tRNA^{Arg}_{UCU}$. In the presence of cognate $tRNA^{Arg}_{CCU}$, AGG stimulated frameshifting very weakly, 0.3%, while in the absence of the tRNA it increased to 45% (Vimaladithan and Farabaugh, 1994).

We attempted to delete the duplicated structural genes encoding the GGG cognate $tRNA^{Gly}_{CCC}$ and the GUG cognate $tRNA^{Val}_{CAC}$. The PCR-based deletion method used (Güldener et al., 1996) involves replacing the gene with a bacterial kan^R gene, conferring resistance to the antibiotic G-418 in yeast (see the Experimental Procedures). Insertions into each of the two genes for each isoacceptor were created in congenic strains of opposite mating type. Among the meiotic progeny produced from diploids formed from two such strains were strains lacking both genes. Such doubly deleted strains could be identified in meiotic tetrads in which resistance to G-418 segregated in a 2:2 fashion.

The results were qualitatively similar for deletions of the structural genes for $tRNA^{Gly}_{CCC}$ (*SUF3* and *SUF5*) and $tRNA^{Val}_{CAC}$ [identified by the systematic names used in the *Saccharomyces* Genome Database, *tV(CAC)D* and *tV(CAC)H*]. In each case, deletion of both of the tRNA structural genes stimulated frameshifting on the cognate codon about 3-fold, from 2.2% to 6.0% for $tRNA^{Val}_{CAC}$ (compare lines 1 and 4 of Table 2) and from 9.2% to 26% for $tRNA^{Gly}_{CCC}$ (compare lines 5 and 8 of Table 2). Single deletions of either *tV(CAC)* gene had little or no effect (see lines 2 and 3), but deletions of the *SUF3* or *SUF5* stimulated frameshifting about 2-fold (see lines 6 and 7). Since reducing the availability of cognate tRNA (for $tRNA^{Gly}_{CCC}$) or eliminating all cognate tRNA (for both isoacceptors) stimulated frameshifting, we can conclude that the presence of a low abundance cognate isoacceptor for the GGG and GUG codons actually reduces frameshifting. These data are clearly also compatible with the hypothesis that frameshifting depends on near-cognate decoding.

Overexpression of Near-Cognate tRNAs Stimulates +1 Frameshifting

A last test of the near-cognate decoding model would be to show that overexpression of a particular tRNA stimulates frameshifting on its near-cognate codon. We have tested the ability of near-cognate tRNAs to induce frameshifting stimulated by proline, glycine, and valine codons (CCU, CCC, CCG, GGG, and GUG) by overexpressing specific isoacceptors.

Yeast only encodes two proline isoacceptors, $tRNA^{Pro}_{IGG}$ and $tRNA^{Pro}_{UGG}$, so the only near-cognate tRNA for the codons CCU and CCC is $tRNA^{Pro}_{UGG}$, while both $tRNA^{Pro}_{IGG}$ and $tRNA^{Pro}_{UGG}$ are near-cognates for CCG. To test if overexpressing $tRNA^{Pro}_{UGG}$ stimulates frameshifting on these codons, we cloned a structural gene $tRNA^{Pro}_{UGG}$ onto *lacZ* reporter plasmids carrying the CCU-AGG-C, CCC-AGG-C, or CCG-AGG-C frameshift sites, as well as one carrying the control in-frame reporter gene. Overexpression had little effect on the CCG construct, as shown in Table 3 (line 3), but increasing the availability of $tRNA^{Pro}_{UGG}$ stimulated frameshifting about 3- to 4-fold at either CCC or CCU (Table 3, lines 1 and 2). The same overexpression had the opposite effect on the cognate codon for $tRNA^{Pro}_{UGG}$, CCA, reducing its already very low frameshifting efficiency 2-fold, from 1.0% to 0.6%. These data are consistent with the idea that near-cognate $tRNA^{Pro}_{UGG}$ can compete successfully against the cognate $tRNA^{Pro}_{IGG}$ to read the codons CCU and CCC and that when it does it can promote +1 frameshifting. The lack of a large effect on the CCG site does not mean that the tRNA is

Table 3. Overexpressing tRNAs Can Induce Frameshifting on Their Near-Cognate Codons

Frameshift Site[a]	Strain[a]	tRNA Overexpressed	Frameshift Efficiency, %[a] − tRNA	+ tRNA
CCU-AGG-C	KK240	$tRNA^{Pro}_{UGG}$	7.4 ± 0.3	27 ± 3
CCC-AGG-C	KK240	$tRNA^{Pro}_{UGG}$	7.1 ± 0.1	20 ± 0.7
CCG-AGG-C	KK242	$tRNA^{Pro}_{UGG}$	25 ± 4.7	31 ± 2.6
GGG-AGG-C	KK242	$tRNA^{Gly}_{UCC}$	18 ± 1.6	37 ± 1.0
GGG-AGG-C	KK242	$tRNA^{Gly}_{GCC}$	18 ± 1.6	41 ± 3.0
GUG-AGG-C	KK242	$tRNA^{Val}_{UAC}$	3.0 ± 0.13	9.0 ± 0.12

[a] As in Table 1.

Table 4. Overexpressing Near-Cognate $tRNA^{Arg}_{CCG}$ Reduces Frameshifting on CGA

Frameshift Site[a]	Strain[a]	tRNA Overexpressed	Gene Copies per Plasmid[b]	Frameshift Efficiency, %[a]
CGA-AGG-C	KK240	none	na	18 ± 0.9
CGA-AGG-C	KK240	$tRNA^{Arg}_{ICG}$	1	14 ± 4.0
CGA-AGG-C	KK240	$tRNA^{Arg}_{ICG}$	2	15 ± 1.6
CGA-AGG-C	KK240	$tRNA^{Arg}_{ICG}$	4	18 ± 2.3
CGA-AGG-C	KK240	$tRNA^{Arg}_{CCG}$	1	10 ± 0.01
CGA-AGG-C	KK240	$tRNA^{Arg}_{CCG}$	2	12 ± 0.9
CGA-AGG-C	KK240	$tRNA^{Arg}_{CCG}$	4	2.8 ± 0.03

[a] As in Table 1.
[b] Copies of the gene encoding each tRNA were inserted singly or in tandem multimers as described in the Experimental Procedures.

not responsible for frameshifting on that codon. Rather, $tRNA^{Pro}_{UGG}$ may normally decode CCG, without competition by any other tRNA. In that case, overexpression should have no effect on the probability that $tRNA^{Pro}_{UGG}$ would read CCG and, thus, no effect on the frequency that it induces frameshifting.

We also tested the ability of near-cognate $tRNA^{Gly}_{GCC}$ and $tRNA^{Gly}_{UCC}$ to induce frameshifting on GGG again cloning copies of the genes into a *lacZ* reporter construct carrying the GGG-AGG-C frameshift site and into an in-frame control plasmid. The frameshift efficiency at this site in the presence of either overexpressed tRNA was about 2-fold higher than in the presence of the normal level of the tRNA (Table 3, lines 4 and 5) showing that near-cognate decoding of GGG by either tRNA induces frameshifting. Finally, we tested near-cognate decoding of GUG. Overexpressing near-cognate $tRNA^{Val}_{UAC}$ in the same way stimulated frameshifting on a GUG-AGG-C frameshift site about 3-fold (Table 3, line 6). The quoted efficiencies of frameshifting in the absence of the overproduced tRNAs is higher in Table 1 because the strain used in those experiments carried a deletion of the *HSX1* gene encoding $tRNA^{Arg}_{CCU}$ while the strain used for the experiments reported in Table 3 did not. In each of the cases tested, improving the probability of near-cognate decoding stimulates frameshifting, consistent with the near-cognate decoding hypothesis.

Not All Near-Cognate tRNAs Induce Frameshifting

Given the example of the $tRNA^{Gly}$ family, in which both near-cognate tRNAs stimulate frameshifting on GGG, it is possible that any near-cognate peptidyl-tRNA would stimulate +1 frameshifting. Alternatively, it may be that only certain near-cognate tRNAs can induce frameshifting; forcing near-cognate decoding by other isoacceptors might actually reduce frameshifting. In testing the effect of overexpressed near-cognates of the remaining frameshift-inducing codons, GCG and CGA, we found evidence that some near-cognate interactions do not induce frameshifting.

We isolated copies of the genes encoding near-cognate tRNAs for GCG ($tRNA^{Ala}_{UGC}$) and CGA ($tRNA^{Arg}_{ICG}$ and $tRNA^{Arg}_{CCG}$). Increasing the gene dosage of the wild-type $tRNA^{Ala}_{UGC}$ from five to about nine copies per cell caused a 2-fold reduction in frameshifting induced by a GCG codon, from 13% to 6.1%, as reported before (Vimaladithan and Farabaugh, 1994). When the anticodon was changed to CGC, cognate for GCG, frameshifting declined 21-fold (to 0.6%). Clearly, if an oversupply of $tRNA^{Ala}_{UGC}$ tends to reduce the efficiency of frameshifting, near-cognate decoding of GCG by that tRNA does not induce frameshifting. In addition, since we have shown that the GCG is decoded as Ala (Farabaugh et al., 1993) and *S. cerevisiae* encodes only one other isoacceptor, the extremely abundant $tRNA^{Ala}_{IGC}$, these data indirectly implicate that isoacceptor as the one that stimulates frameshifting at GCG.

Two tRNAs decode the CGN Arg codons: $tRNA^{Arg}_{ICG}$ and $tRNA^{Arg}_{CCG}$. Even introducing 16 extra copies per genome of the gene for $tRNA^{Arg}_{ICG}$ (four plasmids, each carrying four genes), there was no decrease in frameshifting at CGA (Table 4). However, extra copies of the gene encoding $tRNA^{Arg}_{CCG}$ progressively reduced frameshifting from 18% to 2.8% (Table 4, compare line 1 and lines 5–7). Again, the fact that overexpressing $tRNA^{Arg}_{CCG}$ reduces frameshifting indicates that it does not induce frameshifting on CGA. The lack of an effect by $tRNA^{Arg}_{ICG}$ as with $tRNA^{Pro}_{UGG}$ on CCG described above does not mean that the tRNA is irrelevant but rather that it is the normal decoder of CGA, as expected, though it inefficiently induces frameshifting when it reads the codon.

Discussion

These data challenge assumptions about how alternative translational events occur. The natural assumption when looking at coding sequences is to suppose that cognate tRNAs decode all codons. If a region induces frameshifting, it must be the cognate tRNAs that do cause the effect. The data presented here show that in the yeast *S. cerevisiae* +1 frameshifting results from reading by other than cognate tRNAs. Direct experiments implicate near-cognate tRNAs in inducing the frame error at programmed sites.

Several experimental tests validate the connection between frameshifting and near-cognate decoding of the last zero frame codon. First, expressing tRNAs that are true cognate tRNAs of the codons, whether natural or artificial, reduces frameshifting. Second, deleting genes encoding isoacceptors stimulates frameshifting on their cognate codon. Third, frameshift efficiency on certain codons increased as a result of overexpressing near-cognate tRNAs.

Some of the experiments show evidence of a dose–response effect, increased effects with increasing amounts of tRNA. For example, deleting either *SUF3* or

SUF5 resulted in frameshifting intermediate between the wild-type and the doubly deleted strain (Table 2). Also, overexpressing $tRNA^{Arg}_{CCG}$ to intermediate levels caused an intermediate level of frameshifting at CGA-AGG-C (Table 4). These data show that we can observe an appropriate dose–response effect. This effect is not observed in all cases. For example, deletion of either of the genes encoding $tRNA^{Val}_{CAC}$ had little or no effect on frameshifting on GUG-AGG-C, but deleting both caused a large increase (Table 2). In this case, the level of frameshifting in the presence of cognate $tRNA^{Val}_{CAC}$ is at the background level (2%). We cannot prove, but suspect, that this low level of apparent frameshifting may result from a different cause than near-cognate decoding since all 53 codons that fail to induce efficient frameshifting still induce about 1% frameshifting. Deleting one $tRNA^{Val}_{CAC}$ gene had little effect, indicating that in this strain background near-cognate $tRNA^{Val}_{UAC}$ could not compete well enough against the cognate to induce frameshifting on GUG. This makes the effect all-or-nothing for inducing frameshifting.

We previously showed that the rate of decoding of certain codons can influence frameshift efficiency by overexpressing (Belcourt and Farabaugh, 1990; Farabaugh et al., 1993; Pande et al., 1995) or eliminating (Vimaladithan and Farabaugh, 1994) particular isoacceptors. The effects shown in this work cannot be explained using such a timing model. Suppose hypothetically that slow recognition of the last codon decoded before the frameshift in some way stimulated frameshifting. In that case, overexpressing or underexpressing their cognate tRNA should have the results seen in this paper. However, under this model we would predict that overexpressing near-cognate tRNAs, were they able to decode the codons, should accelerate the rate of decoding and therefore should also decrease frameshift efficiency. The fact that near-cognate tRNAs can have the opposite effect invalidates this model. Second, we know that the last zero frame codon occupies the ribosomal P site during frameshifting because altering the rate of A site recognition of the next in-frame codon (Belcourt and Farabaugh, 1990; Farabaugh et al., 1993) or of the first +1 frame codon (Pande et al., 1995) alters frameshift efficiency. Thus, when the frameshift takes place, the peptidyl-tRNA has already been selected, so the rate of its recognition in the previous cycle could not be directly relevant. A model invoking an effect of rate of recognition would require the ribosome to "remember" the duration of the previous step in elongation, and we cannot envision such a model. If we exclude that slow recognition of the last zero frame codon is the reason for increased frameshifting, it must be the abnormal nature of the codon–anticodon interaction that induces frameshifting.

Our previous work identified two types of frameshift, one in which peptidyl-tRNA slips +1 during a translational pause caused by poor recognition of the next in-frame codon (Belcourt and Farabaugh, 1990) and a second in which frameshifting occurs without slippage by out-of-frame binding of aminoacyl-tRNA in the ribosomal A site (Farabaugh et al., 1993). Eight of the eleven codons known to induce significant +1 frameshifting in yeast appear to do so by allowing peptidyl-tRNA slippage since they can form at least 2 bp with the shifted codon. Data from a large number of frameshift systems, both +1 and −1, suggest that after slippage tRNAs must make at least two Watson/Crick base pairs with the mRNA (reviewed in Farabaugh, 1996). This type of pairing is not possible for the codons GCG, CGA, and GUG. For these tRNAs, frameshifting probably occurs without slippage by errant recruitment of the next tRNA in the +1 reading frame (Farabaugh et al., 1993). Previously, we had proposed that slippage would be impossible on CCG codons as well (Farabaugh et al., 1993). That prediction was mistaken because it was based on the prediction that the codon would be read by a putative cognate $tRNA^{Pro}_{CGG}$, which could not slip, rather than by the near-cognate $tRNA^{Pro}_{UGG}$, which can.

We can explain all +1 programmed frameshifting in *S. cerevisiae* as arising from either of two causes. First, frameshifting can result from a weak interaction of the peptidyl-tRNA in which the wobble nucleotides either do not hydrogen bond or do so very weakly. Such a weak interaction is predicted for each of the slippage-prone tRNAs. During frameshifting, each tRNA juxtaposes a U in the wobble position (usually modified) with a U, C, or G in the mRNA. Two pyrimidines (U·U or C·U) cannot form a Watson-Crick base pair, implying that the tRNA makes only two pairs with the mRNA using the two-out-of-three mechanism first proposed by Lagerkvist (1978). If they do pair, they would require an abnormal non-Watson/Crick geometry (reviewed by Yokoyama and Nishimura, 1995). Even U·G pairs are predicted to be unstable. Wobble uridines are often modified at the 5 position to destabilize pairing to bases other than A (Björk, 1992; Yokoyama and Nishimura, 1995). Strong pairing between U and G, common only in bacteria, actually requires the stabilizing modification uridine 5-oxyacetic acid (cmo^5U), which is absent in eukaryotes (Björk, 1995; Yokoyama and Nishimura, 1995). This form of noncanonical base pairing would stimulate slippage by allowing transient dissociation of peptidyl-tRNA from the mRNA. Obvious precedents exist for weak pairing stimulating slippage both −1 (Tsuchihashi and Brown, 1992) and +1 (Kawakami et al., 1993), though in the past the emphasis has been on the ability of the tRNA to interact successfully in the shifted frame to promote slippage (i.e., Curran, 1993). The necessity of near-cognate decoding in yeast in +1 frameshifting suggests that where cognates are used, for example in −1 frameshifting in yeast and other systems (reviewed in Farabaugh, 1996), other aspects of the frameshift site must overcome the barrier to slippage imposed by cognate decoding.

Second, frameshifting can result when an abnormal codon–anticodon peptidyl-tRNA interaction deforms the structure of the tRNA–mRNA complex in the P site and interferes with proper recognition by cognate aminoacyl-tRNAs in the A site. In this case, frameshifting occurs when the ribosome erroneously accepts an out-of-frame cognate aminoacyl-tRNA. In two such cases, a purine–purine wobble pair induces frameshifting ($tRNA^{Ala}_{IGC}$ pairing with GCG and $tRNA^{Arg}_{ICG}$ with CGA). Forming such a pair requires a deformation of the standard Watson/Crick geometry, which may destabilize the codon–anticodon complex (Lim and Venclovas, 1992; Lim, 1995; Yokoyama and Nishimura, 1995). In *E. coli*, a bulky A·I wobble pair in the P site can interfere with cognate

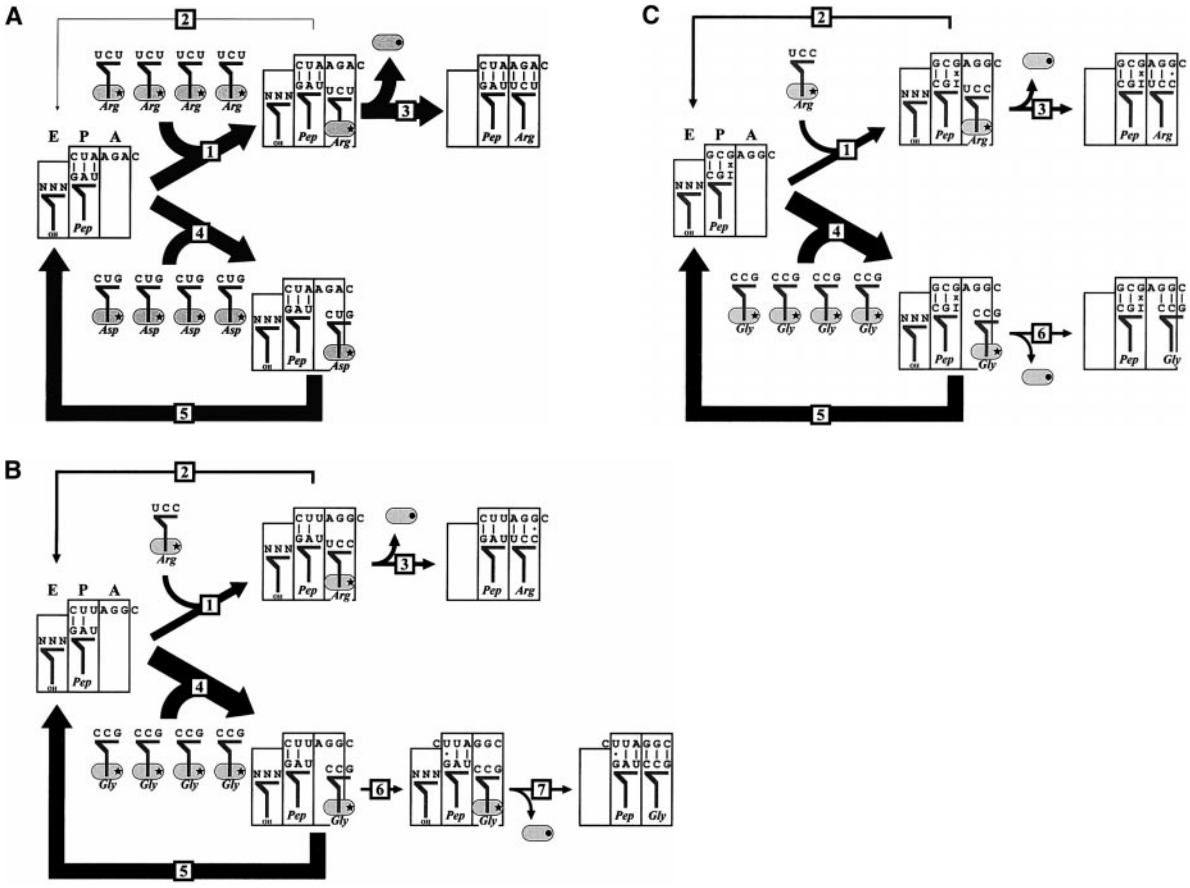

Figure 2. Two Frameshifting Mechanisms

(A) Normal translation.

(B) Slippage-based frameshifting.

(C) Non-slippage-based frameshifting.

See text for a description of the mechanisms. The figures depict in rough fashion the relative rates of competing processes at normal sites and programmed frameshifting sites. The tRNAs are depicted in complex with eEF-1A (oval) and GTP (star). GDP is cartooned as a black circle. Groups of three boxes indicate the three ribosomal decoding sites E (exit), P (peptidyl), and A (aminoacyl). Watson/Crick pairing is indicated by a vertical line, wobble pairing by a dot, and a purine–purine clash by a small letter X.

recognition of the next codon by a nonsense suppressor tRNA (Curran, 1995; Björnsson et al., 1996). This effect could explain how bulky purine–purine P site wobble pairing stimulates +1 frameshifting in *S. cerevisiae* since aminoacyl-tRNAs cognate for the zero and +1 frame codons compete for binding to the A site at the frameshift site (Pande et al., 1995). By reducing the efficiency of in-frame cognate decoding, this effect would indirectly increase the probability of out-of-frame recognition and, therefore, of frameshifting. It is also possible that the unusual pairing actually directly promotes out-of-frame recognition as well, again stimulating frameshifting.

A purine–purine wobble pair is not strictly necessary for frameshifting by out-of-frame recognition since peptidyl-$tRNA^{Val}_{UAC}$ binding to GUG appears to cause the same error without a purine–purine wobble interaction. The wobble nucleotide of $tRNA^{Val}_{UAC}$ is 5-carbamoylmethyluridine (ncm^5U) (Sprinzl et al., 1998), a modification thought to reduce the ability of the tRNA to pair with any nucleotide other than adenosine (Berman et al., 1978; Glasser et al., 1992). This suggests that normal Watson/Crick wobble pairing in the P site precludes out-of-frame binding in the A site and that some non-Watson/Crick pairs may interfere with this effect. An $ncm^5U{\cdot}G$ pair appears to do so, though other non-Watson/Crick pairs do not. For example, near-cognate recognition of GCG by $tRNA^{Ala}_{UGC}$ or CGA by $tRNA^{Arg}_{CCG}$ does not induce frameshifting. An A·C pair can form with nearly Watson/Crick geometry and has been shown to occur during codon recognition (see the discussion by Yokoyama and Nishimura, 1995, and references therein). Our previous work suggested that slippage and nonslippage +1 frameshifting share a common dependence on acceptance of out-of-frame aminoacyl-tRNAs in the A site (Pande et al., 1995). The fact that weak wobble pairing by $tRNA^{Val}_{UAC}$ can stimulate nonslippage frameshifting suggests that weak pairing may have two functions in slippage frameshifting: transiently stabilizing entry of cognate out-of-frame aminoacyl-tRNA in the A site and allowing disengagement and slippage of peptidyl-tRNA during this transient occupation.

Figure 2 contrasts models of normal translation (Figure 2A) and each of the mechanisms of frameshifting,

Table 5. Oligonucleotides

Number	tRNA	Type[a]	Site[b]	Sequence[c]
oli 918	$tRNA^{Leu}_{GAG}$ C_{33}	G	XhoI	gtacctcgagTGGTTCGGACACACCTC
oli 919			SalI	gtacgtcgACAAGGAGCCACGTATGAATAC
oli 1090	$tRNA^{Leu}_{GAG}$ U_{33}	G	XhoI	aggcctcgagCAGATGAATTGGTACTCTGGCCGAGTGGTCTAAGGCGTCAGGTTGAGG
40 D				CCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGA
oli 900	$tRNA^{Leu}_{CAG}$	S	XhoI	ccggctcgagCTTCCAACATACAATGGGAGTTTGGCCGAGTGGTTTAAGGCGTCAGAT TCAGGTGGATTTAACCTC
oli 901			XhoI	aattctcgagAAAAAGCAAAAAATAATGAGAGCTAAGGGATTCGAACCCTTGCATCCGA AGATATCAGAGATTTTAGAGGTTAAATCCACC
oli 1091	$tRNA^{Val}_{UAC}$	G	XhoI	aggcgcatgcctcgagTGGGAAACATTGCATAATCACTTCCGT
oli 1092			XhoI	ctggtctcgagCTGTCCTTTGAATTGCAGGCATAACTTG
oli 1015	$tRNA^{Val}_{CAC}$	G	XhoI	ccaactcgagTTACTGAGTACTGTGGTTGATATGATTATGT
oli 1014			XhoI	aaggctcgagTCTGTTCAAACACTATCGCCCTTAAGTGTC
oli 741	$tRNA^{Arg}_{ICG}$	G	XhoI	ggcctcgagGCTTTTATTCACAATGGAACCCAACAATTATTTCAAAA
oli 742			XhoI	ggcctcgagCCAATTGATCTGTTAACTGTACTTTACTACTTATACTA
oli 743	$tRNA^{Arg}_{CCG}$	G	XhoI	ggcctcgagAGTTTTATACCTCTCTTATATAAGCACAGGAAGGTCCA
oli 744			XhoI	ggcctcgagCCTCTAGCTACTGATTTTCAGAAAAAAAAAAAAGAA
oli 802	$tRNA^{Pro}_{IGG}$	S	SphI/XhoI	ggccgcatgcGGGCGTGTGGTCTAGAGGTATGATTCTCGCTTAGGGTGCGGGAG GTCCCGGGTTCGAGTCCCGGCTCGCCCCCATTTTTTTTTTTTctcgagaccg
oli 807			XhoI	cggtctcgagAAAAAAAAAAAATG
oli 957	$tRNA^{Pro}_{UGG}$	G[d]	XhoI	ggaactcgagAAGCCAATTGGTGCGGCAATTGAT
oli 956			NarI	cattggcgccGCGGGGTGAGATAGTGCTAGTGATCCGTA
oli 550	$tRNA^{Gly}_{CCC}$	S	XhoI/XhoI	ggcctcgagACGCGCAAGTGGTTCAGTGGTTAGAATTTATGCTTGGGAAGCATGA GGCCCGGGTTCGATTCCCGGCTTGCGCATTTTTTTTTTTTctcgagaccg
oli 558			XhoI	cggtctcgagAAAAAAAAAAAATG
oli 553	$tRNA^{Gly}_{UCC}$	S	XhoI/XhoI	ggcctcgagCCGGGCGGTTAGTGTAGTGGTTATCATCCCACCCTXCCAAGGTGGG GACACGGGTTCGATTCTCGTACCGCTCATTTTTTTTTTTTctcgagccgg
oli 627			XhoI	ccggctcgagAAAAAAAAAAAATG
oli 554	$tRNA^{Gly}_{GCC}$	S	XhoI/XhoI	ggcctcgagAAGCGCAAGTGGTTTAGTGGTAAAATCCAACGTTGCCATCGTTGGG CCCCCGGTTCGATTCCGGGCTTGCGCAATTTTTTTTTTTctcgagccgg
oli 555			XhoI	ccggctcgagAAAAAAAAAAATT

[a] The type of clone: G, genomic; S, synthetic.
[b] The restriction endonuclease sites used to clone the gene. Some oligonucleotides used to create synthetic tRNAs include sites both upstream and downstream of the tRNA gene, in which case two enzymes are noted.
[c] The sequence of each oligonucleotide presented in 5′ to 3′ format. The lower case letters correspond to sequences introducing flanking restriction sites. Four nucleotide tails facilitated restriction digestion.
[d] These primers amplified the *TRN1* gene, one of three encoding $tRNA^{Pro}_{UGG}$. All other genomic clones were of single-copy genes.

slippage-dependent (Figure 2B) and nonslippage frameshifting (Figure 2C). As an example of normal decoding, Figure 2A cartoons the events occurring on a nonframeshifting site, CUA-AGA-C. With a cognate peptidyl-tRNA in the P site, an abundant aminoacyl-tRNA in complex with eEF-1A and GTP enters the A site (step 1). The complex rarely dissociates (step 2) but, rather, is accepted when eEF-1A-GDP dissociates from the ribosome (step 3). In a competing reaction, a cognate tRNA for the +1 shifted codon enters the A site (step 4) but is virtually always rejected (step 5). Slippage-dependent frameshifting occurs when a near-cognate tRNA occupies the P site (shown with peptidyl-$tRNA^{Leu}_{UAG}$ decoding CUU). The low abundance of the ternary complex cognate for the A site codon causes it to be slowly recognized (Figure 2B, step 1). A poor codon–anticodon interaction in the P site may cause the cognate aminoacyl-tRNA to be rejected more than normal (we have no direct evidence to support this point) (step 2), though we think that most would still be accepted (step 3). In the competing reaction, the abundant +1 frame cognate ternary complex rapidly enters the A site (step 4) but is still mostly rejected (step 5). The weak codon–anticodon interaction of the peptidyl-tRNA allows it to sometimes slip +1 (step 6), leading to acceptance of the tRNA in the +1 frame, which causes the frameshift. Slippage is cartooned as occurring while the +1 frame ternary complex transiently occupies the A site, or perhaps the T site at which ternary complex initially docks with the ribosome (Wilson and Noller, 1998), as previously suggested by Pande et al. (1995), though this mechanism remains hypothetical. Nonslippage frameshifting is very similar to slippage-dependent frameshifting except in step 6. Figure 2C cartoons the effect of the purine–purine clash of $tRNA^{Ala}_{IGC}$ on GCG. This clash we hypothesize stabilizes the out-of-frame

cognate ternary complex, increasing the probability that the ribosome would accept it (Figure 2C, step 6).

The proposed frameshift mechanisms strongly resemble a new mechanism of frameshift suppression by mutant tRNAs (Qian et al., 1998) proposed to replace the long-standing quadruplet translocation model (reviewed by Roth, 1981). The older model suggested that frameshift suppressor tRNAs read a 4 nt codon using a 4 nt anticodon. It has increasingly become clear that frameshift suppressor tRNAs need not cause shifting by reading a 4 nt codon (for a review, see Atkins et al., 1991). Based on analysis of frameshift suppression in *S. typhimurium* and *S. cerevisiae*, Qian et al. (1998) offered an alternative model in which suppressor tRNAs induce near-cognate decoding allowing +1 slippage of peptidyl-tRNAs at the suppression site. We propose an identical mechanism to explain programmed +1 frameshifting induced by mRNA contexts, though resulting from a different cause. The Qian et al. (1998) model proposes that mutant tRNAs during frameshift suppression read the suppressible codon using a noncanonical two-out-of-three interaction. The weak interaction would allow the peptidyl-tRNA to slip +1 before the next in-frame codon can be decoded, causing +1 frameshifting. Programmed +1 frameshifting in yeast appears in most cases to result from slippage of a similarly weak peptidyl-tRNA because of the inability of a true cognate tRNA to compete effectively enough for the codon to preclude noncanonical decoding. In one and perhaps two cases, frameshift suppression results from an identical cause. The suppressor *sufB2* induces +1 frameshifting at CCCN sites by recognizing CCC so poorly that the near cognate $tRNA^{Pro}_{cmo5UGG}$ decodes it instead (Qian et al., 1998). This tRNA reads by two-out-of-three decoding since the wobble base, cmo^5U pairs very poorly or not at all with C. Frameshifting occurs when this normal near-cognate slips +1 from CCC to CCN.

Finding that near-cognate decoding stimulates two quite different phenomena that result in +1 frameshifting suggests a reappraisal of the meaning of redundant decoding. Why do cells encode multiple isoaccepting tRNA species? Commonly, both eukaryotes and prokaryotes express three distinct tRNAs to encode 4-fold redundant codon families. These isoacceptors are often modified to restrict reading to one or at most two codons. This pattern is not required since in plastids it is common for all four codons to be read by a single tRNA with unmodified uridine in the wobble position. Why then does the cytoplasmic translation system use a much more complicated system of tRNAs? The common explanations include enhanced kinetic or recognition effects during aminoacylation, discrimination in split codon boxes (those encoding two amino acids), and enhanced kinetics of decoding (reviewed in Björk, 1995). This work suggests that an important function of modification may be to reduce decoding by near-cognate isoacceptor since they are prone to a very serious frameshifting error when they move to the P site. Previous explanations of frameshift enhancement by hypomodified tRNAs have focused on their potential to form enlarged anticodons, for example, stimulation of frameshifting by lack of 1-methylguanosine in $tRNA^{Pro}$ in *S. typhimurium* (Björk et al., 1989; Hagervall et al., 1993). Probably, these effects result from inappropriate near-cognate recognition followed by peptidyl-tRNA slippage (Qian and Björk, 1997a, 1997b). Perhaps the translational apparatus evolved to its current structure driven more by the need to limit frameshifting resulting from weak codon–anticodon interactions than from any other cause.

Experimental Procedures

Frameshift Reporter Plasmids

The efficiency of frameshifting is estimated indirectly using a pair of plasmids in which expression of the *E. coli* gene *lacZ* depends on translation initiating in an upstream truncated yeast gene, *HIS4* (Farabaugh et al., 1993). In both plasmids, translation initiates at the normal *HIS4* codon and continues through the first 33 codons of the gene. At this point, an oligonucleotide derived from the programmed +1 frameshift site of the retrotransposon Ty3 has been inserted. In one plasmid (pMB38-Ty3FF), translation continuing in the normal reading frame can proceed directly into *lacZ*, resulting in expression of the *lacZ* product, β-galactosidase (in-frame). In the second, translation must shift reading frames +1 in order to express β-galactosidase (pMB38-Ty3Δ2). This plasmid carries a BamHI–KpnI fragment with the wild-type +1 frameshift site from the overlap between the Ty3 *GAG3* and *POL3* genes (GGATCCAGTGAAG*GC GAGTT*CTAACCGATCTTGAGGTACC [frameshift site italicized]). The efficiency of frameshifting is calculated as the ratio of expression of β-galactosidase from pMB38-Ty3Δ2 to that from pMB38-Ty3FF. We constructed 63 variants of pMB38-Ty3Δ2 by replacing the frameshift-inducing GCG codon with each of the other 63 codons (Vimaladithan and Farabaugh, 1994). The experiments reported here involve some of these constructs. In each case, the GCG-AGU-U sequence is replaced by a different frameshift signal, as indicated in the text. Frameshift induction by each codon was quantitated in the same manner.

Cloning tRNA Genes

To overexpress certain tRNA isoacceptors, we inserted a copy of their structural gene into the frameshift reporter plasmid using unique sites upstream of the promoter driving the *lacZ* reporter gene but downstream of the *URA3* gene used as a selectable marker in transformation. See Table 5 for the sequences of the oligonucleotides used in constructing the fragments carrying the tRNA genes. DNA fragments carrying the genes were constructed in one of two ways. Genomic copies of the genes were isolated by the polymerase chain reaction using DNA primers complementary to sequences far upstream and downstream of the gene. The primers incorporated restriction endonuclease cleavage sites for the enzymes XhoI, SalI, or NarI. Restriction endonuclease cleaved fragments were inserted into a polylinker located between *URA3* and the reporter gene's promoter. The XhoI and SalI ends were inserted into a unique SalI site using compatible 5′ ends, and the NarI ends were inserted into a unique NarI site. All of the constructs were confirmed by DNA sequencing, including a complete sequence of the tRNA structural gene. Some of the genes do not exist as genomic copies, so synthetic genes were required. These were constructed using mutually priming oligonucleotides, in the case of $tRNA^{Leu}_{CAG}$, or by priming synthesis of the bottom strand of a gene-length oligonucleotide using a short primer. Again, the primers incorporated restriction XhoI, SalI, or SphI endonuclease cleavage sites and cloned into a unique SalI (for XhoI and SalI) or SphI site in the polylinker. The primary transcription products from tRNA genes usually include a short sequence upstream of the gene and part of an oligoU tail at the termination site (Wolin and Matera, 1999). We placed the restriction cleavage sites so that they would not be included in the primary transcript in case they might interfere with posttranscriptional processing. Construction of synthetic tRNA genes was performed using the mutually primed DNA synthesis procedure as described (Ausubel et al., 1991), except that the double-stranded DNA products created by extension of the oligonucleotides were purified using the MERmaid system (BIO 101) as directed by the manufacturer. The system purifies short double-stranded DNAs away from contaminating single-stranded primers. The products were digested with the relevant

restriction enzymes and inserted into linearized plasmids using standard procedures.

To test the importance of the unusual C_{33} base found in $tRNA^{Leu}_{GAG}$, we changed the sequence of the tRNA to replace C_{33} with the canonical U_{33} found in nearly all other tRNAs (Sprinzl et al., 1998). Table 5 gives the sequence of the oligonucleotide used to make this change, oli1090, which incorporates a T corresponding to nucleotide 33 or the tRNA. A polymerase chain reaction was performed with this oligonucleotide and the oligonucleotide 40D (Table 5) using the plasmid carrying the wild-type $tRNA^{Leu}_{GAG}$ as template. 40D is complementary to codons 46–59 of the *lacZ* gene located 0.8 kb downstream. The DNA product was digested with XhoI (a unique site in oli1090) and KpnI, a unique site at the 5′ end of the *lacZ* gene in the reporter and used to replace the corresponding region of the fusion reporter plasmid between the unique SalI and KpnI sites.

Constructing Gene Deletions

Genes encoding $tRNA^{Gly}_{CCC}$ and $tRNA^{Val}_{CAC}$ were deleted using the method of Güldner et al. (1996). Oligonucleotides consisting of the 60 nt immediately upstream or downstream of the gene to be deleted fused to 20 nt sequences immediately flanking the site of insertion of the *kan*^{R} gene of the plasmid pUG6 (Güldener et al., 1996). A polymerase chain reaction with these oligonucleotides on pUG6 created a fragment that when introduced into yeast would insert into the genome replacing the tRNA structural gene. Transformants selected on rich medium supplemented with 200 μg/ml G418 (Geneticin, Sigma) were restreaked on the same medium. DNA minipreps were prepared from each putative deletion strain (Hoffman and Winston, 1987), and the presence of the deletion was tested by PCR using one primer within the *kan*^{R} gene and one in the flanking yeast DNA.

β-Galactosidase Assay

The frameshift reporter and frame fusion control plasmids were introduced into strains KK242 (*MAT*α *ura3 leu2 trp1 his3*) or KK240 (*MAT*α *ura3 leu2 trp1 his3 hsx::HIS3*) as indicated in the text, using the method of Ito et al. (1983). The mutation *hsx1::HIS3* inactivates the sole structural gene for the arginine-decoding $tRNA^{Arg}_{CCU}$ (Kawakami et al., 1992, 1993). Triplicate β-galactosidase assays of six independent transformants were conducted as described (Belcourt and Farabaugh, 1990). Units of β-galactosidase are in nanomoles of orthonitrophenyl-β-D-galactopyranoside cleaved per minute per milligram protein. The standard error of the mean of the reported values was less than 10% in each case. Variations in expression levels do not result from differences in transcriptional efficiency or plasmid copy number for the plasmids used in this study (Belcourt and Farabaugh, 1990).

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