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Special Peptidyl-tRNA Molecules Can Promote Translational Frameshifting without Slippage

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Recently we described an unusual programmed +1 frameshift event in yeast retrotransposon Ty3. Frameshifting depends on the presence of peptidyl-tRNA $^{Ala}_{CGC}$ on the GCG codon in the ribosomal P site and on a translational pause stimulated by the slowly decoded AGU codon. Frameshifting occurs on the sequence GCG-AGU-U by out-of-frame binding of a valyl-tRNA to GUU without slippage of peptidyl-tRNA$^{Ala}_{CGC}$. This mechanism challenges the conventional understanding that frameshift efficiency must correlate with the ability of mRNA-bound tRNA to slip between cognate or near-cognate codons. Though frameshifting does not require slippery tRNAs, it does require special peptidyl-tRNAs. We show that overproducing a second isoacceptor whose anticodon had been changed to CGC eliminated frameshifting; peptidyl-tRNA$^{Ala}_{CGC}$ must have a special capacity to induce +1 frameshifting in the adjacent ribosomal A site. In order to identify other special peptidyl-tRNAs, we tested the ability of each of the other 63 codons to replace GCG in the P site. We found no correlation between the ability to stimulate +1 frameshifting and the ability of the cognate tRNA to slip on the mRNA—several codons predicted to slip efficiently do not stimulate frameshifting, while several predicted not to slip do stimulate frameshifting. By inducing a severe translational pause, we identified eight tRNAs capable of inducing measurable +1 frameshifting, only four of which are predicted to slip on the mRNA. We conclude that in *Saccharomyces cerevisiae*, special peptidyl-tRNAs can induce frameshifting dependent on some characteristic(s) other than the ability to slip on the mRNA.

Protein synthesis produces a low frequency of errors: both missense errors and processivity errors (those leading to premature termination). However, spontaneous frameshift errors are extremely infrequent (34). This means that the translational machinery must much more efficiently avoid changes in frame than it does other errors. Some genes have evolved sequences which manipulate this machinery to allow very high levels of frameshifting, with efficiencies from a few to nearly 100% (2, 22, 28). All such sequences, termed programmed frameshift sites, consist of two elements: the actual site at which the frame shifts, termed the recoding site (22), and sequences which increase the probability that the ribosome will slip, termed stimulators (2).

In all but one case, the recoding site is a sequence which allows slippage of one or more tRNAs between cognate or near-cognate codons and thus is termed a slippery site. Frameshifts occur in either the upstream, or leftward, direction (e.g., −1 frameshift) or in the downstream, or rightward direction (e.g., +1 frameshifting). In −1 simultaneous slippage frameshifting, common in retroviruses but found in other viruses and one chromosomal gene, the slippery site is a heptamer of the form X-XXY-YYZ. Frameshifting occurs by the simultaneous slippage of two tRNAs from XXY-YYZ to XXX-YYY (29, 46). Mutational changes which decrease the likelihood of the slip tend to decrease or eliminate frameshifting (8, 9, 15, 29). +1 frameshifting, by contrast, occurs while one tRNA engages the mRNA; in all known cases but one, this tRNA slips +1 to a cognate or near-cognate codon. Many other frameshift sites which have been studied confirm the correlation between frameshifting and tRNA slippage. This correlation was further strengthened by finding that among 32 sense codons in *Escherichia coli*, the efficiency of frameshifting is directly related to the ability of the cognate tRNA to slip +1 (14).

Stimulators of programmed frameshifting either increase the length of translational pausing at the recoding site or increase the rate at which recoding occurs. There are three types of stimulators which lengthen the pause: "hungry" codons, those decoded by limiting tRNAs (57); in-frame nonsense codons; and RNA secondary structures. Starving cells for appropriate amino acids has long been known to increase frameshifting at nonprogrammed frameshift sites; the lack of a particular aminoacyl-tRNA induces a translational pause, allowing frameshifting onto an overlapping codon (57). The retrotransposon Ty1 includes a programmed frameshift site stimulated by a naturally hungry codon, AGG, recognized by the naturally limiting tRNA$^{Arg}_{CCU}$ (5). In-frame nonsense codons also induce a translational pause, probably caused by slow recognition by peptide release factor (45). In the *prfB* gene of *Escherichia coli*, slow recognition of an in-frame UGA codon by the product of the gene, RF2, leads to frameshifting and production of RF2—an autogenous regulatory loop (11, 16). RNA secondary structures induce frameshifting by physically blocking the progression of the ribosome. Secondary structures, commonly pseudoknots, stimulate −1 simultaneous slippage frameshifts at least partly by inducing a transient translational pause (46, 48). These stimulators use different mechanisms, but they all enhance frameshifting by pausing elongation. The *prfB* gene provides the only characterized example of a stimulator which seems to directly increases the rate of shifting. In that gene a Shine-Dalgarno interaction (40) between a sequence upstream of the recoding site and the 16S rRNA probably stimulates frameshifting by decreasing the half-life of slipping, pushing the ribosome into the +1 reading frame (11, 13, 54–56, 59).

The yeast retrotransposon Ty3 challenges the correlation between tRNA slippage and frameshift efficiency since it promotes efficient frameshifting without tRNA slippage (21).

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Ty3 expresses a single RNA including two genes, *GAG3* and *POL3*, encoding structural and enzymatic activities, respectively, necessary for reverse transcription of the Ty3 RNA (23). As in retroviruses, the *POL3* gene is expressed as a GAG3-POL3 fusion (33). The fusion protein is expressed by +1 translational frameshifting within a 7-base sequence (21). Frameshifting occurs at the sequence GCG-AGU-U (shown as codons of the unshifted *GAG3* frame), which expresses Ala-Val by decoding the underlined codons. The shift occurs with peptidyl-tRNA^{Ala} bound in the ribosomal P site during a translational pause caused by the low availability of the cognate tRNA for the A site codon, AGU. The peptidyl-tRNA^{Ala} cannot slip onto the overlapping +1 codon, CGA, so frameshifting must occur without tRNA slippage. We concluded that valyl-tRNA^{Val} must bind to the +1 frame GUU codon out of frame without movement of peptidyl-tRNA^{Ala}. In this paper, we show that the ability of the GCG codon to stimulate frameshifting depends on recognition of the codon by its cognate tRNA. We also report the result of changing the P site GCG codon to all 63 possible codons in an effort to determine if this event is a special case or if frameshifting and tRNA slippage are indeed uncorrelated in *Saccharomyces cerevisiae*. Our results show that eight tRNAs can stimulate +1 frameshifting but that there is no correlation between the apparent slipperiness of these tRNAs and the efficiencies with which they stimulate frameshifting. We conclude that special peptidyl-tRNAs stimulate frameshifting independent of their ability to slip.

MATERIALS AND METHODS

Yeast strains, media, and general methods. The *S. cerevisiae* strains used in this work are the congenic pair of strains KK242 (*MATα ura3 leu2 trp1 his3*) and KK240 (*MATα ura3 leu2 trp1 his3 hsx::HIS3*); the *hsx::HIS3* mutation eliminates the gene for the AGG-decoding tRNA$^{Arg}_{CCU}$ (31, 32). DNA transformations of yeast strains were performed by the lithium acetate method (27). The activity of β-galactosidase expressed by transformants was determined as described earlier (18). Transformants were grown in SD (synthetic plus dextrose [glucose]) minimal media supplemented with the appropriate amino acids to allow selection for *URA3*$^+$-containing plasmids (39). Oligonucleotides were synthesized on a Biosearch Cyclone DNA synthesizer (Milligen) and purified by chromatography on Oligo-Pak columns (Milligen) according to the manufacturer's directions.

Plasmid construction. All plasmids used in this study are derivatives of pMB38 (5), a 2μm-*URA3*-based shuttle vector carrying a *lacZ* gene used to report expression dependent on +1 frameshifting. The plasmid carries a triple gene fusion. The yeast *HIS4* gene is fused to the *E. coli lacZ* gene through an intervening oligonucleotide which includes a potential +1 translational frameshift site. Translation initiates at the normal *HIS4* start site and proceeds into the frameshift site. Ribosomes which shift +1 then continue into *lacZ*, producing β-galactosidase, while ribosomes which do not shift terminate at an in-frame UGA codon immediately downstream. β-Galactosidase activity was determined as previously described (18). To determine the efficiency of frameshifting, we compared expression of the frameshift constructs to that of a construct, pMB38-Ty3FF (21), in which a single nucleotide within the Ty3 frameshift region was deleted, putting *lacZ* in frame with *HIS4*. Frameshift efficiency is defined as the ratio of expression of the frameshift to the frame fusion construct. Very efficient frameshifting cannot be accurately determined in this way because the efficient frameshift ceases to be rate limiting in expression of β-galactosidase. Some of the efficiencies reported here are at or above 100%, meaning that expression requiring frameshifting equaled expression which did not require frameshifting. We assume that some downstream step in elongation is rate limiting. We note that *lacZ* is a bacterial gene whose codon usage is not optimal for yeasts; decoding multiple suboptimal codons within the *lacZ* reading frame might be more rate limiting than a highly efficient frameshift event.

The object of one of the experiments reported here was to create a library of plasmids replacing the GCG codon of the Ty3 frameshift site (GCG-AGU-U) with all 63 other codons. To do this, we synthesized an oligonucleotide encompassing the frameshift site in which the three nucleotides corresponding to GCG were replaced with a mixture of random nucleotides (XXX-AGU-U). The oligonucleotide, 5′-GACCAATTA GATCTGAAGXXXAGTTCTAACCGA-3′ (the portion derived from Ty3 is underlined), introduces a *Bgl*II site (AG ATCT) immediately upstream of the frameshift site. It was used in a PCR along with a second oligonucleotide (3′ to *Sac*I [19]) which primes synthesis immediately downstream of the unique *Sac*I site in the *E. coli lacZ* gene. PCR was performed with, as a template, the plasmid pMB38-Ty3Δ2 (21), carrying a translational fusion between the Ty3 frameshift site and the *E. coli lacZ* gene. PCRs were carried out for 25 cycles, using the proofreading Vent polymerase (New England Biolabs) according to the manufacturer's directions to reduce errors during amplification. The PCR product was digested with *Bgl*II and *Sac*I and the fragment was inserted, replacing the analogous segment, between the *Bam*HI and *Sac*I sites, of pMB38-Ty3FF (21), screening for the lack of expression of β-galactosidase in *E. coli* caused by the presence of the Ty3 frameshift site which is inactive in *E. coli*. The frameshift region was sequenced to identify examples of each of the 64 possible codons. To avoid artifacts introduced by PCR, expression of β-galactosidase by two or three independent isolates of each construct was determined; no artifactual results were found.

We constructed versions of this library of 64 codon replacements in which the pause-inducing AGU-U sequence was replaced with the more efficient pause-inducing sequence AGG-C (5). The replacement was made by performing PCR on each of the 64 plasmids in the collection by using an oligonucleotide (oli 264; GCATCGGGTACCTCAAGATCG GTTAGGCCT) which is complementary to the region immediately downstream of the 64-fold redundant XXX codon. The mismatched bases which change AGU-U to AGG-C are underlined above; this PCR was performed with *Taq* polymerase (Promega), since the 3′→5′ proofreading exonuclease of Vent polymerase would tend to excise the last four nucleotides of the primer, including the two-nucleotide mismatch. PCRs, carried out for 25 cycles, were performed with oli 264 and the oligonucleotide Sal-upstream (5), which generates a fragment extending from a unique *Sal*I site, upstream of the promoter of the *HIS4::lacZ* fusion gene, through a unique *Kpn*I site immediately downstream of the frameshift site. The *Sal*I-*Kpn*I fragment produced was introduced, replacing the equivalent portion of pMB38-Ty3FF; was screened again for the lack of expression of β-galactosidase in *E. coli* and identified an example of each clone desired by DNA sequencing.

To test the effect of overexpressing peptidyl-tRNA^{Ala} isoacceptors on frameshifting, we cloned the tRNA$^{Ala}_{UGC}$ (which decodes GCA). The gene (GenBank accession number M10958) was isolated by PCR with oligonucleotide primers designed to excise the gene with an upstream *Sal*I and downstream *Xho*I site (relative to the direction of transcription of the gene). The upstream primer was designed to allow mutagenesis of the anticodon of the tRNA. The primer extended

from downstream of the gene to six nucleotides past the anticodon; at the position corresponding to the anticodon, equal quantities of two nucleotides were incorporated. The PCR product included both the wild-type $tRNA^{Ala}_{UGC}$ and a mutated form with the anticodon CGC, $tRNA^{Ala}_{CGC2}$. These genes were cloned into the unique *SalI* site upstream of the *his4::lacZ* reporter gene in a pMB38-derived plasmid as previously described (21).

RESULTS

Special peptidyl-tRNAs induce +1 frameshifting. +1 frameshifting in the Ty1 and Ty3 retrotransposons in *S. cerevisiae* occurs during a translational pause at a codon (termed a pause codon) recognized by either of two tRNAs: $tRNA^{Arg}_{CCU}$ for Ty1 (5) and $tRNA^{Ser}_{GCU}$ for Ty3 (21) (tRNAs are referred to by a superscript identifying their amino acid identify and a subscript identifying their anticodon, written 5′ to 3′). Frameshifting occurs by decoding of the +1 frame codon overlapping the pause codons. Frameshifting depends on the codon immediately upstream of the pause codon, since changes to this codon eliminate frameshifting, which during the induced translational pause binds a peptidyl-tRNA in the ribosomal P site (21). Thus, +1 frameshifting appears to depend on the identity of the peptidyl-tRNA occupying the ribosomal P site. If it is true that frameshifting depends on special peptidyl-tRNAs, then replacing the peptidyl-tRNA with a different isoacceptor would eliminate frameshifting.

We had previously shown that frameshifting in the Ty3 retrotransposon occurs with GCG in the ribosomal P site (21). Single base changes in the GCG codon eliminated frameshifting; only the GCG→CCG mutation had no effect. In particular, mutating GCG to GCA eliminated frameshifting, possibly because $tRNA^{Ala}_{UGC}$ (decoding GCA) lacks some special feature present within $tRNA^{Ala}_{CGC}$ (decoding GCG). To test this hypothesis, we performed an anticodon swap between the two isoacceptors. A strict test of this hypothesis would be to change the anticodon of $tRNA^{Ala}_{CGC}$ to UGC; this should allow recognition of the GCA codon by the putative frameshift-competent tRNA, causing efficient frameshifting on GCA-AGU-U. Unfortunately, the $tRNA^{Ala}_{CGC}$ has not been identified (we presume its anticodon is CGC by analogy to other tRNAs decoding G-ending codons). We therefore performed the next best test of the hypothesis, changing the anticodon of $tRNA^{Ala}_{UGC}$ to CGC; overexpression of this tRNA would result in decoding of GCG by the putative frameshift-incompetent tRNA, eliminating frameshifting on GCG-AGU-U. By PCR mutagenesis, we introduced the CGC anticodon into $tRNA^{Ala}_{UGC}$ to create a tRNA we termed $tRNA^{Ala}_{CGC2}$. The swapped tRNA should decode GCG, but otherwise is identical to $tRNA^{Ala}_{UGC}$. If $tRNA^{Ala}_{UGC}$ cannot induce frameshifting, then neither should the swapped tRNA. We introduced the gene for either $tRNA^{Ala}_{UGC}$ or $tRNA^{Ala}_{CGC2}$ onto multicopy *lacZ* fusion frameshift reporter plasmids carrying either the normal frameshift site, GCG-AGU-U, or the frameshift-incompetent site, GCA-AGU-U, and measured the efficiency of frameshifting by using a convenient *lacZ* reporter system as described in Materials and Methods. As shown in Fig. 1, overproducing the frameshift-incompetent $tRNA^{Ala}_{UGC}$ had no significant effect on the low level of frameshifting at its cognate codon, GCA, but reduced frameshifting at GCG twofold. After switching the anticodon on this tRNA to CGC, overproducing it still had no effect on frameshifting at GCA but now reduced frameshifting at GCG 10-fold. This result confirms that the ability of GCG in the P site to induce frameshifting depends on its being recognized by $tRNA^{Ala}_{CGC}$. Because of the predicted low abundance of $tRNA^{Ala}_{CGC}$ (25), the ribosome would preferentially bind the overproduced tRNA. Ribosomes binding this tRNA must not be able to perform +1 frameshifting. The twofold reduction caused by overproducing $tRNA^{Ala}_{UGC}$ probably reflects inefficient decoding of GCG by this near-cognate tRNA, partially suppressing frameshifting. We conclude that the sequence of the peptidyl-tRNA decoding GCG is responsible for inducing frameshifting and therefore that efficient +1 frameshifting depends on special peptidyl-tRNAs.

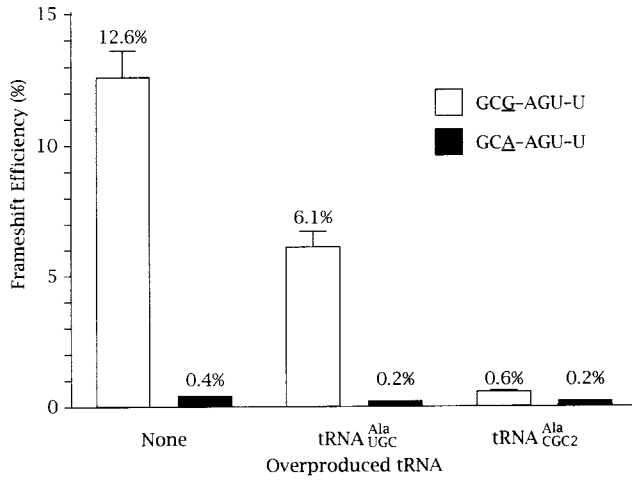

FIG. 1. Overproducing anticodon-swap tRNAs. We tested the effect on frameshift efficiency of overproducing two forms of the frameshift-incompetent GCA-decoding $tRNA^{Ala}$: one with the normal GCA-recognizing anticodon, $tRNA^{Ala}_{UGC}$, and one in which the anticodon was replaced with a GCG-recognizing anticodon, $tRNA^{Ala}_{CGC2}$. The graph plots the efficiency of frameshifting (defined in Materials and Methods) on two sites, the frameshift-competent GCG-AGU-U, and the frameshift incompetent GCA-AGU-U, in a wild-type background (normal) and when either $tRNA^{Ala}_{UGC}$ or $tRNA^{Ala}_{CGC2}$ was overproduced. The strain used for this experiment was 387-1D; this strain induces higher levels of frameshifting at an AGU pause codon than the nonisogenic strains KK240 and KK242 (see Table 1), perhaps because the AGU-decoding $tRNA^{Ser}_{ICU}$ is present at a more limiting concentration. The error bars show the standard error of the means of the measurements.

The ability to efficiently stimulate frameshifting does not correlate with the tendency of a tRNA to slip +1. Two codons were known to substitute for the GCG codon of the Ty3 frameshift site: CUU, the P site codon of the Ty1 site (5), and CCG, the codon created by the one mutation of GCG which does not reduce frameshifting (21). The three frameshift-stimulating codons are decoded by three tRNAs: $tRNA^{Ala}_{CGC}$ (CGC), $tRNA^{Leu}_{UAG}$ (CUU), and $tRNA^{Pro}_{CGG}$ (CCG). But are these the only tRNAs which can promote +1 frameshifting? To determine how many P site codons, and thus which tRNAs, stimulate frameshifting, we replaced GCG with all 63 other codons. These constructs, described in Materials and Methods, include the region of the Ty3 frameshift site from one codon upstream of the GCG through the 13 nucleotides following the AGU codon. Included in this region is the Ty3 context, a downstream stimulator of +1 frameshifting which increases efficiency 7.5-fold (21).

The majority of the P site codon replacements virtually eliminate frameshifting. Of 64 mutations, 61 result in frameshift efficiencies of below 2.4%. All of these constructs promoted very low level frameshifting, averaging 0.42% (standard deviation [SD], 0.16%). This level of frameshifting is about

TABLE 1. Frameshifting efficiencies with 64 P site codons

Amino acid	Codon	Stability[a]	Frameshift efficiency (%)[b]		
			AGU-U in KK242[c]	AGG-C in KK242	AGG-C in KK240[d]
Phe	UUU	−4	0.5	0.6	1.4
	UUC	−1	0.3	0.3	0.3
Leu	UUA	−2	0.4	0.1	0.2
	UUG	0	0.3	0.2	0.7
	CUU	−5	8.2	37	110
	CUC	−2	1.2	2.3	45
	CUA	−1	0.3	0.2	0.7
	CUG	−1	0.2	7.6	39
Ile	AUU	−2	0.4	0.8	1.0
	AUC	1	0.5	0.8	1.0
	AUA	0	0.4	0.2	0.4
Met	AUG	2	0.4	0.2	0.3
Val	GUU	−2	0.4	0.5	1.4
	GUC	1	0.4	0.3	1.6
	GUA	0	0.5	0.3	1.0
	GUG	2	0.6	1.0	5.5
Ser	UCU	0	0.6	0.4	0.6
	UCC	−2	0.2	0.4	0.9
	UCA	0	0.4	0.1	0.8
	UCG	2	0.5	0.4	1.3
Pro	CCU	−2	0.5	0.2	7.0
	CCC	−4	0.6	0.2	5.0
	CCA	−2	0.4	0.2	0.5
	CCG	0	5.0	17	90
Thr	ACU	0	0.3	0.2	1.4
	ACC	−2	0.4	0.6	1.3
	ACA	0	0.3	0.5	0.7
	ACG	2	0.5	0.8	1.4
Ala	GCU	1	0.5	0.5	1.4
	GCC	−1	0.5	0.7	1.2
	GCA	1	0.4	0.6	1.6
	GCG	3	3.3	15	100
Tyr	UAU	−1	0.4	0.1	0.9
	UAC	0	0.5	0.5	0.6
Stop	UAA	*	0.2	0.2	0.7
Stop	UAG	*	0.0	0.1	0.5
His	CAU	−1	0.4	0.9	1.4
	CAC	0	0.3	0.1	0.7
Gln	CAA	−4	0.5	0.1	0.7
	CAG	0	0.4	0.1	0.5
Asn	AAU	−3	0.5	0.3	0.7
	AAC	−2	0.4	0.2	0.5
Lys	AAA	−6	0.6	0.6	0.7
	AAG	−2	0.3	0.2	0.5
Asp	GAU	−1	0.5	0.5	1.5
	GAC	0	0.3	0.3	0.9
Glu	GAA	−4	0.3	0.5	1.9
	GAG	0	0.4	0.4	0.8
Cys	UGU	0	0.3	0.2	1.0
	UGC	1	0.4	0.1	0.3
Stop	UGA	*	0.4	0.6	1.0
Trp	UGG	−2	0.3	0.3	0.7
Arg	CGU	0	0.2	0.2	0.6
	CGC	1	0.5	0.5	1.3
	CGA	−1	0.8	1.0	10
	CGG	−2	0.4	0.6	2.7
Ser	AGU	−1	0.4	0.1	0.7
	AGC	0	0.2	0.3	1.2
Arg	AGA	−2	0.4	0.5	0.5
	AGG	−3	0.5	0.3	45
Gly	GGU	−2	0.5	0.5	1.8
	GGC	−1	0.5	0.8	1.3
	GGA	−3	0.4	0.4	2.2
	GGG	−4	2.1	10	110

[a] Predicted stability of peptidyl-tRNAs after slipping +1 on the indicated codon, as defined in Materials and Methods.
[b] As defined in Materials and Methods.
[c] KK242 (*HSX1*) encodes normal amounts of the AGG-decoding $tRNA^{Arg}_{CCU}$.
[d] KK240 is congenic with KK242 except for a mutation (*hsx1*) which eliminates expression of $tRNA^{Arg}_{CCU}$.

10^2-fold above the rate of spontaneous frameshifting during elongation, 5×10^{-5} per codon (34). Apparently the presence of a strong pause codon, AGU, along with the Ty3 context, stimulates frameshifting by any codon to far above the spontaneous rate. However, the three codons previously known to induce +1 frameshifting, CUU, GCG, and CCG, promoted much higher levels of frameshifting, ranging from 3.3 to 8.2% (Table 1, column AGU-U in KK242).

It is surprising that no codons other than CUU, GCG, and CCG promoted efficient frameshifting. It is particularly notable that certain codons one might predict to enable +1 tRNA slippage do not promote frameshifting. For example, the sequence AAA-AGU-U, which should allow slippage between overlapping AAA codons, allowed only the background level of frameshifting. Similarly, replacing AAA with UUU, CCC, or GGG allowed little frameshifting. This result confirms our previous conclusion that +1 frameshifting in *S. cerevisiae* does not correlate with the ability of a peptidyl-tRNA to slip.

In *E. coli* there is a strong exponential relation between frameshift frequency induced by a particular codon and its predicted ability to slip +1, as determined by the estimated stability of its cognate tRNA when shifted +1 (14). The stability of a codon-anticodon interaction is not easy to determine, since the thermodynamic parameters derived from normal RNA-RNA helices are not necessarily relevant. Curran (14) approximated these stabilities by assigning values for individual base interactions, from −2, for strongly stabilizing interactions, to +2, for strongly destabilizing ones. The values should not be confused with free energy values but are useful in ranking stabilities of codon-anticodon interactions. To approximate the stability of a codon-anticodon pair, one sums the contribution of each of the three interactions of base pairs. We have used Curran's index to quantify the relation between frameshifting and tRNA slippage in *S. cerevisiae*. The natural logarithm of frameshift efficiency for each of the 64 codon replacement mutants which we constructed was plotted against the predicted +1 shifted stability for each cognate tRNA (Fig. 2). The plot clearly shows a cluster of points which correspond to the codons which promote 2.4% frameshifting (GGG) or less, and three points clearly falling outside that cluster which correspond to the codons CUU, GCG, and CCG. The plot itself suggests that the latter three codons are different from the codons which fall within the main cluster. However, the abilities of these codons to stimulate frameshifting do not correlate with the abilities of their cognates tRNAs to slip +1; in fact, $tRNA^{Ala}_{CGC}$ is predicted to slip the least efficiently of all yeast tRNAs.

We conclude that the ability of a tRNAs to slip +1 does not determine its ability to induce frameshifting. This may seem counterintuitive; clearly a tRNA which is able to slip onto the

shifted frame should enhance frameshifting, as described for many systems. Our results do not argue that the ability to slip is irrelevant. In fact, by comparing the ability of the frameshift-prone $tRNA^{Leu}_{UAG}$ to promote frameshifting on its four cognate codons—CUU, CUC, CUA, and CUG—we see a direct relationship between increasing stability in the shifted frame and the natural logarithim of frameshift efficiency. The values for the four cognate codons are shown in Fig. 3. These points closely fit a straight line, indicating a strong correlation between propensity to slip and frameshift efficiency. So, for a single frameshift-prone tRNA, increasing the probability of slippage increases frameshift efficiency. The ability to slip is not sufficient, since several tRNAs predicted to slip nearly as well as $tRNA^{Leu}_{UAG}$ promote much less frameshifting (e.g., those which decode AAA, GGG, GAA, CCC, UUU, and CAA). The tRNAs which recognize CUU, GCG, and CCG must have some other feature which gives them the special ability to frameshift.

Prolonged translational pauses identify additional frameshift-inducing peptidyl-tRNAs. Two factors influence frameshift efficiency, the rate at which out-of-frame decoding occurs and the length of the translational pause. During the pause induced by the hungry AGU codon in the Ty3 site, the tRNAs decoding CUU, GCG, and CCG stimulate +1 frameshifting at a frequency detectable above the background. Are these the only tRNAs which can stimulate frameshifting, or is the pause induced by AGU insufficient to allow detection of tRNAs which induce frameshifting less efficiently? The wide variation in frameshifting stimulated by $tRNA^{Leu}_{UAG}$ on the CUX codons shows that during a given translational pause a tRNA can appear to stimulate frameshifting, or not, depending on differences in the intrinsic rate of frameshifting. Perhaps, then, by extending the translational pause one would discover other tRNAs which stimulate frameshifting too inefficiently to be detectable with a shorter pause. We know that an AGG codon acting as the pause codon induces more frameshifting than does AGU (5). In addition, the gene for the AGG cognate $tRNA^{Arg}_{CCU}$ is not essential; it can be deleted without affecting cell viability (32). Cells lacking this tRNA experience a much greater frequency of frameshifting at CUU-AGG-C (31). In the strain lacking $tRNA^{Arg}_{CCU}$, AGG codons must be inefficiently decoded by a near-cognate, presumably $tRNA^{Arg}_{UCU}$, the AGA

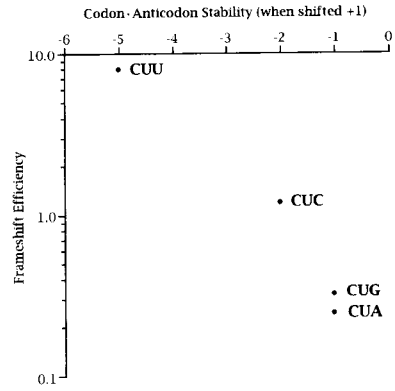

FIG. 3. Frameshift on the CUX family of codons by $tRNA^{Leu}_{UAG}$ does correlate with peptidyl-tRNA slipperiness. The relation between slipperiness and frameshift efficiency of the CUX family of codons, all decoded by $tRNA^{Leu}_{UAG}$, is plotted as in Fig. 2.

cognate. By changing the pause codon in our library of 64 P site codon constructs from AGU to AGG, we could analyze the effect of prolonged pausing on all yeast tRNAs, either a moderately lengthened pause caused by AGG in the wild type or a greatly lengthened pause caused by AGG in the deletion strain.

The sequence adjacent to the P site codon of each of the 64 was changed from AGU-U to AGG-C, as described in Materials and Methods. These XXX-AGG-C constructs were introduced by transformation into two congenic strains with (KK242), or without (KK240) the gene encoding $tRNA^{Arg}_{CCU}$. In KK242, 59 codons induce little frameshifting, averaging 0.41% (SD 0.35%). Five constructs stimulated much higher levels of frameshifting. These included those involving the codons CUU, GCG, and CCG. Two other codons, GGG and CUG, stimulated frameshifting nearly as efficiently as did GCG (Table 1, column AGG-C in KK242, and Fig. 4), though both had been much less efficient with the AGU pause (Fig. 2). The codon CUG is decoded by $tRNA^{Leu}_{UAG}$, the tRNA which decodes CUU, a codon already known to cause frameshifting, while GGG is decoded by $tRNA^{Gly}_{CCC}$. This result identifies the GGG cognate $tRNA^{Gly}_{CCC}$ as a fourth frameshift-competent

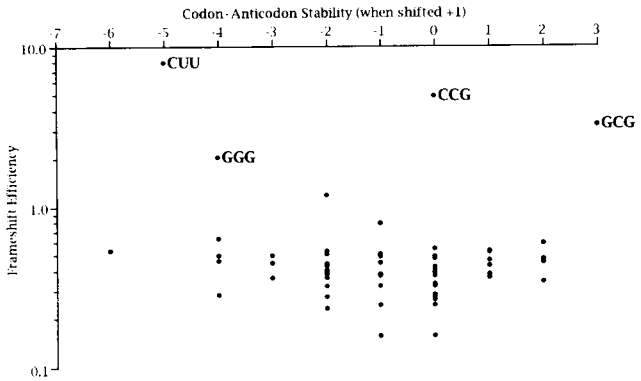

FIG. 2. Frameshift frequency and slipperiness of the peptidyl-tRNA are not correlated in +1 frameshifting in *S. cerevisiae*. Values of frameshift efficiency for all 64 P site codons are plotted versus the predicted codon-anticodon stability after a +1 slip of the peptidyl-tRNA (as defined in Materials and Methods). The frameshift efficiencies are for a frameshift site XXX-AGU-U in the wild-type strain, KK242.

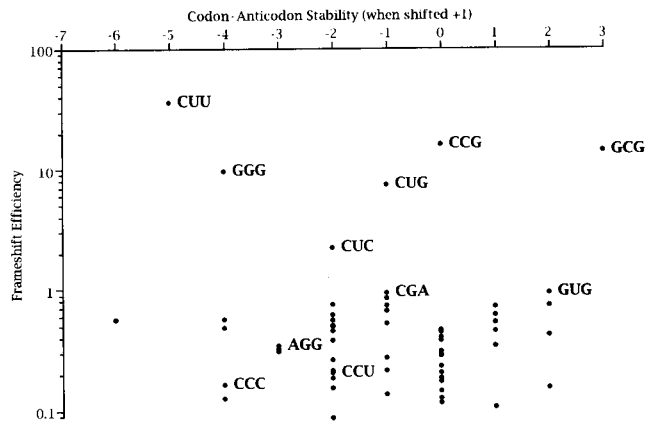

FIG. 4. The longer pause at an AGG codon identifies six frameshift-competent codons. The relation between slipperiness and frameshift efficiency of the 64 XXX-AGG-C frameshift sites in the strain KK242 is plotted as in Fig. 2.

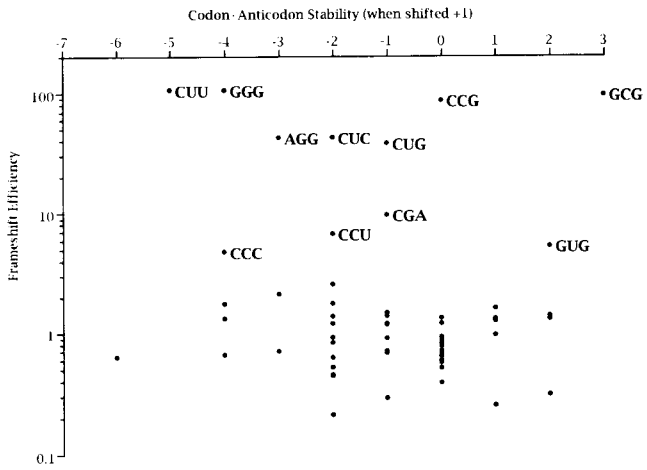

FIG. 5. An extended pause at an AGG codon in the absence of the AGG-decoding $tRNA^{Arg}_{CCU}$ identifies 11 frameshift-competent codons. Shown is a plot as in Fig. 4 of the XXX-AGG-C frameshift sites in KK240 (*hsx1*::*HIS3*) deficient for $tRNA^{Arg}_{CCU}$.

tRNA. Presumably the longer pause induced by the AGG pause codon increased the rate of frameshifting stimulated by this tRNA to measurable levels.

Deletion of the $tRNA^{Arg}_{CCU}$ gene in KK240, which should cause a more protracted pause, generated a qualitatively different profile of frameshift efficiency (Table 1, column AGG-C in KK240, and Fig. 5). In this case, 53 of the codons still induced only low levels of frameshifting, averaging 0.95% (SD, 0.53%). As we have seen before (31), eliminating $tRNA^{Arg}_{CCU}$ greatly stimulated frameshifting on CUU-AGG-C. Similar increases were observed with constructs involving the P site codons GCG, CCG, and GGG. However, three additional codons, AGG, CUC, and CUG, stimulated very high efficiency frameshifting in KK240, and four others, CCC, CCU, CGA, and GUG, induced levels significantly above those of the other 52 codons. Clearly, the seven codons inducing efficiencies of greater than 39% are different than the vast majority of codons. The lower efficiencies stimulated by the four other codons are still 7 to 17 SD above the mean of the remaining 52 codons, so the increased frameshifting on these codons is also statistically significant.

The conditions imposed in this strain are highly unusual; the lack of $tRNA^{Arg}_{CCU}$ probably produces an protracted pause in translation at the AGG pause codon. It is perhaps more surprising that 53 codons fail to stimulate frameshifting than that 11 codons do. The fact that in *S. cerevisiae*, even during such a protracted pause, most codons do not allow significant shifting suggests that during elongation frameshifting is greatly disfavored and that the tRNAs which do not stimulate frameshifting differ in kind from the 11 tRNAs which do.

Five tRNAs decode the six additional codons which induced +1 frameshifting during a prolonged pause: $tRNA^{Leu}_{UAG}$ (CUC), $tRNA^{Arg}_{UCU}$ (AGG), $tRNA^{Pro}_{IGG}$ (CCU and CCC), $tRNA^{Arg}_{UCG}$ (CGA), and $tRNA^{Val}_{CAC}$ (GUG). Two of these, as well as those decoding GCG, GGG, and CCG, are specific to only one codon. Three of them, $tRNA^{Pro}_{IGG}$, $tRNA^{Leu}_{UAG}$, and $tRNA^{Arg}_{UCU}$, recognize multiple codons. $tRNA^{Pro}_{IGG}$ induces equivalent rates of frameshifting on both CCU and CCC. Since $tRNA^{Pro}_{IGG}$ is predicted to slip much more efficiently on CCC than on CCU, these data do not support the idea that slippage by this tRNA induces frameshifting. By contrast, the efficiency of frameshifting by $tRNA^{Leu}_{UAG}$ on the CUX codons varies by a total of 157-fold from CUU (110%) to CUA (0.7%). In the wild-type strain, KK242, the ability of this tRNA to induce frameshifting correlates with its predicted ability to slip +1. During a prolonged pause this still holds; slippage should occur most easily at the CUU codon and less efficiently at CUA, CUG, and CUC. The pattern of frameshift expression roughly matches expectation, with CUU efficiently stimulating frameshifting, CUG and CUC stimulating less efficiently, and CUA promoting only low-level frameshifting. $tRNA^{Arg}_{UCU}$ stimulates frameshifting very efficiently on its near-cognate codon, AGG, but allows only background levels on its cognate AGA. In this case slippage may occur as a result of instability of the codon-anticodon pair in the normal reading frame; the lower stability would increase the likelihood that the tRNA would dissociate from the 0 frame codon and reassociate with the +1 frame codon. In at least these two cases, frameshifting probably occurs by tRNA slippage.

DISCUSSION

It has been commonly assumed that programmed frameshifting involves slippage of tRNAs along the mRNA (22). In fact, until our description of the Ty3 retrotransposon system, all programmed frameshift sites have included a slippery sequence which allows one or two tRNAs to slip between cognate or near-cognate codons. Indeed, the presence of such a sequence often has identified frameshift sites. The involvement of tRNA slippage has been repeatedly confirmed by mutagenesis; mutants which reduce the ability of tRNA to slip tend to reduce frameshift efficiency. In addition, Curran found a close relationship between the ability to slip and the ability to stimulate frameshifting (14).

The relationship between frameshift efficiency of codons and slipperiness of their cognate codon provides a simple explanation of the mechanism of frameshifting. The obligate translational pause with the slippery tRNA-mRNA complex in the decoding site allows sufficient time for a significant proportion of the tRNAs to slip. The slip itself could be entirely stochastic, its rate determined by the energy of interaction between the anticodon and the codon in the shifted frame. The only special requirements of a frameshift site then would be the ability to promote a sufficiently long pause and the presence of a slip-prone tRNA.

This simple picture, at least for *S. cerevisiae*, cannot be accurate, given our characterization of the Ty3 retrotransposon frameshift, which occurs without tRNA slippage (21). We showed that frameshifting in Ty3 occurs when the ribosome encounters the sequence GCG-AGU-U. The shift occurs with peptidyl-$tRNA^{Ala}_{CGC}$ bound to the GCG codon in the ribosomal P site during a translational pause caused by the slow decoding of the AGU codon in the A site. Since $tRNA^{Ala}_{CGC}$ cannot slip +1 onto the overlapping CGA codon, this shift must occur without tRNA slippage. If Ty3 frameshifting does not rely on the presence of a slip-prone tRNA, what stimulates the shift in frame? We can imagine two mechanisms. $tRNA^{Ala}_{CGC}$ could, by its structure, induce misreading in the A site, allowing decoding out of frame by +1. Alternatively, the structure of the tRNA may be irrelevant, and an abnormal conformation of the mRNA, or of the codon-anticodon pairs, could cause the incoming aminoacyl-tRNA to bind to the +1 frame codon.

We show here that it is the structure of $tRNA^{Ala}_{CGC}$ which stimulates frameshifting. We had shown that replacing the P site codon GCG with GCA eliminates frameshifting (21). The frameshift incompetence of the GCG→GCA mutant could reflect the inability of the GCA-decoding $tRNA^{Ala}_{UGC}$ to position an incoming aminoacyl-tRNA in the +1 reading frame.

Under this hypothesis, some structure(s) present in $tRNA^{Ala}_{CGC}$, but absent from $tRNA^{Ala}_{UGC}$, allows it to misdirect aminoacyl-tRNA onto the +1 frame codon. Replacing $tRNA^{Ala}_{CGC}$ with the frameshift-incompetent $tRNA^{Ala}_{UGC}$ would eliminate frameshifting. We created a mutant form of $tRNA^{Ala}_{UGC}$ carrying the GCG-specific anticodon. Overexpressing this tRNA inhibited frameshifting on the wild-type frameshift site, demonstrating that frameshifting requires a special structure specific to $tRNA^{Ala}_{CGC}$ and lacking in $tRNA^{Ala}_{UGC}$, and eliminating any model explaining frameshifting as a result of an unusual conformation of the mRNA.

It has long been recognized that structural alterations to tRNAs generate frameshift suppressors (reviewed in (3)). One class of mutations introduce an extra nucleotide into the anticodon loop, allowing quadruplet decoding, and thus efficient +1 frameshifting. A second class allows doublet decoding, and thus −1 frameshifting; these suppressors do not arise by deletion of a base from the anticodon loop, but from changes elsewhere within the tRNA. These frameshift suppressors provide a paradigm for how altering the structure of tRNAs can induce frameshifting. Even normal tRNAs can, under appropriate conditions, induce frameshifting. In vitro experiments demonstrated that excess amounts of two *E. coli* tRNAs induce −1 frameshifting (1). An anticodon swap of the sort we have described here demonstrated that in contrast to our own results, the anticodon alone, and no other part of the tRNA, is necessary and sufficient to induce frameshifting (10).

These results cannot provide an explanation for frameshifting induction by peptidyl-$tRNA^{Ala}_{CGC}$ in *S. cerevisiae* since the unusual structure of an aminoacyl-tRNA causes noncanonical decoding. Experiments demonstrating +1 frameshifting at specific sites during amino acid deprivation provide a more direct analog (57). Evidence suggests that frameshifting in this case requires a specific peptidyl-tRNA to occupy the P site of the ribosome during a starvation-induced translational pause (38). As in the case of peptidyl-$tRNA^{Ala}_{CGC}$, frameshifting must occur without peptidyl-tRNA slippage (38, 57). The evidence does not prove that the structure of a special peptidyl-tRNA induces misframing, but given our results, that conclusion seems likely. The similarity between these results and our own suggests that this phenomenon is a general one, rather than a peculiarity of either system.

Is $tRNA^{Ala}_{CGC}$ unique in its ability to promote an aberrant decoding event, or can other yeast tRNAs also induce misframing? We found that a total of eight tRNAs can stimulate frameshifting significantly above background levels and that among these tRNAs no correlation exists between frameshift efficiency and the ability of the peptidyl-tRNA to slip +1. On the basis of other systems, we had expected that codons which created redundant runs of nucleotides (GGG-A, CCC-A, UUU-A, and AAA-A) would allow frameshifting. The first two of these do, but the latter two do not. In addition, several codons which do not create slippery sequences (e.g., GCG-A, CCG-A, and GUG-A) do promote frameshifting. In fact, in a previous study (5) we were surprised to find that the sequence UUU-A did not promote frameshifting and suggested that there might be some unknown special feature of the CUU decoding $tRNA^{Leu}_{UAG}$ which allowed it to stimulate frameshifting.

Curran developed a system to approximate the ability of a tRNA to slip +1 based on summing the effect of the three codon-anticodon base interactions (14). We have adapted that metric for use with the yeast tRNAs, as described in Materials and Methods, and used it to compare frameshift efficiency of each of the 64 codons with its predicted slipperiness. As shown in Fig. 5, there is no correlation between this rough measure of tRNA slipperiness and frameshift efficiency. A total of 53 of the codons, from those predicted to slip very efficiently (AAA; score, −6) to very inefficiently (AUG, ACG, and UCG; score, +2), show very low levels of frameshift expression. The 11 codons which show significantly more frameshift expression vary from those predicted to efficiently slip (CUU, GGG, and CCC; score, −5 to −4) to those which should very inefficiently slip (CCG, GUG, and GCG; score, 0 to +3). We conclude from these data that in *S. cerevisiae* no necessary correlation exists between potential for tRNA slippage and the efficiency of +1 frameshifting.

The remarkable difference between these data and those of Curran for *E. coli* (14) suggests that translation in *S. cerevisiae* might differ in some fundamental way from translation in *E. coli*. The nature of that difference is unclear. Alternatively, the difference may arise out of differences in the system used to measure frameshifting. In each case the core frameshift site, the so-called recoding site (22), is flanked by a context element which stimulates the efficiency of frameshifting. In the gene used by Curran, *prfB*, which encodes peptide release factor −2, that element is a site which mimics the Shine-Dalgarno mRNA-rRNA interaction site used by bacterial ribosomes to locate translational initiation sites (11, 13, 55, 59). Stimulation of frameshifting by this site depends on the ability to form the mRNA-rRNA duplex (55). This interaction probably applies a force which pushes the mRNA along the ribosome in a direction which would cause +1 slippage of the peptidyl-tRNA occupying the P site. With this system it is reasonable that frameshift efficiency would depend on the ability of the P site tRNA to slip, consistent with Curran's results.

In our case the region immediately distal to the recoding site stimulates frameshifting about 7.5-fold (21). It is conceivable that this context acts to promote out-of-frame binding of incoming aminoacyl-tRNA and has no effect on tRNA slippage. In that case, one might expect that frameshifting stimulated by the context would not correlate with the ability of the peptidyl-tRNA to slip on the mRNA, in agreement with our results. Whether the difference between the results reported here for the yeast system and those reported by Curran for the *E. coli* system reflect fundamental differences in translation or are artifacts of the systems used to monitor frameshifting cannot be determined from the available data.

This conclusion does not mean that the ability to slip is irrelevant. For a given tRNA, the ability to slip may critically control the probability of frameshifting. One way to test this is to look for codon-specific effects on frameshifting with a tRNA which decodes multiple codons. The data set includes two examples of such tRNAs, $tRNA^{Leu}_{UAG}$ and $tRNA^{Pro}_{IGG}$. $tRNA^{Leu}_{UAG}$ decodes all four members of the CUX codon family. Frameshifting stimulated by this tRNA varies up to 167-fold; in the experiment whose results are depicted in Fig. 4, frameshifting on CUU (37%) is 167-fold more efficient than on CUA (0.22%). Frameshifting on the two other codons is intermediate (CUC, 2.3%; CUG, 7.6%). tRNA slippage is predicted to be greatest on CUU-A, less on CUC-A, and least on CUG-A and CUA-A, agreeing roughly with the observations. This suggests that frameshift efficiency by $tRNA^{Leu}_{UAG}$ roughly correlates with its ability to slip. In the case of $tRNA^{Pro}_{IGG}$, which decodes both CCU and CCC, although slippage on CCC-A is predicted to occur more rapidly than on CCU-A, frameshifting on the two codons is nearly identical, with CCU-A actually slightly more efficient than CCC-A (Fig. 4). This result suggests that slippage and frameshifting are not correlated for $tRNA^{Pro}_{IGG}$.

Our observations present a paradox. On the one hand, frameshifting appears unrelated to tRNA slippage. Several tRNAs which should be able to slip +1 during a translational pause apparently do not do so since they cannot induce

frameshifting, while others which cannot slip do induce frameshifting. On the other hand, slippage does appear to occur during frameshifting on the CUX family of codons, decoded by $tRNA^{Leu}_{UAG}$. Is slippage by this tRNA a special case, with slippage by other tRNAs for some reason prevented? We note that $tRNA^{Leu}_{UAG}$ is unusual in its ability to decode all four codons of the CUX family. tRNAs which decode entire codon families almost invariably have an unmodified U as the wobble base (4, 7, 24). The unusual ability of these tRNAs to decode four codons has been explained by invoking unusual U · U and U · C wobble pairs in addition to the normal U · A and U · G pairs; modification of U (U*) probably precludes such noncanonical U · pyrimidine pairing, restricting pairing to purines (24). An alternate explanation suggested that the tRNAs recognize codons by a two of three base pairing mechanism, with a U in the wobble position because it is least likely to clash with the nucleotide in the wobble position of a codon (7, 35).

Either of these models could explain an unusual tendency of this tRNA to slip +1. Either noncanonical recognition of the CUX codons or the lack of base pairing at the wobble position could weaken the interaction between the tRNA and its cognate codons in the A site. The weakness of this interaction in the A site could allow slippage disallowed for other tRNAs to occur with $tRNA^{Leu}_{UAG}$. The only CUX codon which fails to promote frameshifting even during a prolonged translational pause is CUA. The tRNA should recognize CUA as a normal cognate ($^{5'\text{-CUA-}3'}_{3'\text{-GAU-}5'}$). This interaction should have normal stability, so if unstable binding in the A site is required, CUA should not induce frameshifting as observed. Why then should CUG allow frameshifting when base pairing to this codon involves the normal U · G wobble pairing? Perhaps the explanation relates to the fact that in every case in which the sequence of the tRNA is known, tRNAs which recognize G in the wobble position either have C or a modified U (U*) as the antiwobble base (44). U modification in these cases tends to enhance U* · G base pairing. The need for this modification argues that wobble pairing between an unmodified U and G might be much weaker than the normal U* · G wobble. Alternatively, some feature of $tRNA^{Leu}_{UAG}$ responsible for relaxing its discrimination between codons may further weaken the U · G interaction.

The concept that weak base pairing in the A site would lead to tRNA slippage is supported by a second example. We found that the sequence AGG-AGG-C stimulated only background levels of frameshifting, but frameshift efficiency increased 140-fold in the strain lacking the AGG-decoding $tRNA^{Arg}_{CCU}$. In *E. coli*, high-level frameshifting occurs at tandem rare AGG or AGA codons (42, 43). Tandem rare codons are predicted to create a protracted translational pause (52); presumably during such a pause at either AGG-AGG or AGA-AGA, peptidyl-$tRNA^{Arg}$ slips +1, though this has not been directly demonstrated. As we have seen, $tRNA^{Arg}_{CCU}$ is also rare in *S. cerevisiae*, yet tandem AGG codons do not normally stimulate frameshifting. A protracted pause induced by competition for a rare tRNA cannot explain the drastic increase in frameshifting at AGG-AGG-C. First, since its normal cognate tRNA is absent, AGG would be decoded by the near-cognate $tRNA^{Arg}_{UCU}$, which is abundant. Second, changing the frameshift site to AGA-AGG-C, which are still tandem codons decoded by $tRNA^{Arg}_{UCU}$, eliminates frameshifting. Third, tandem codons are not necessary for frameshifting since the sequence AGG-AGU-U stimulates frameshifting with an efficiency of 4.9% in KK240 in which the AGG is decoded by the near-cognate $tRNA^{Arg}_{UCU}$ (data not shown), about 10-fold higher than the efficiency in KK242 with which AGG is decoded by cognate $tRNA^{Arg}_{CCU}$. If not tandem rare codons, what does cause the increase in frameshifting on AGG-AGG-C? We presume the reason is that AGG is recognized by the noncognate $tRNA^{Arg}_{UCU}$. The wobble U in this tRNA is modified (5-methoxycarbonylmethyluridine, mcm5U) to restrict pairing to mcm5U · A, and not mcm5U · G (58). Recognition of AGG by $tRNA^{Arg}_{UCU}$ would require the formation of the unstable mcm5U · G wobble pair, rather than the C · G pair formed when it is recognized by $tRNA^{Arg}_{CCU}$. Changing the frameshift site to AGA-AGG-C eliminates the unconventional codon-anticodon interaction on the AGA codon and reduces frameshifting 106-fold. These data support the conclusion that the unconventional mcm5U · G pair is responsible for the increase in efficiency.

The unusual circumstances required to cause tRNA slippage stand as counterexamples underscoring the fact that tRNA slippage normally occurs only inefficiently during +1 frameshifting in *S. cerevisiae*. This is in striking contrast to the common occurrence of tRNA slippage in *E. coli* (14). It could be that in *S. cerevisiae*, tRNA slippage is inhibited because the interaction between peptidyl-tRNA and the message is stronger than in *E. coli*. The strength of codon-anticodon interactions depends in part on tRNA modification. For example, in tRNAs whose anticodon ends in U or A, modification of the position immediately 3′ to the anticodon, position 37, serves to stabilize the A · U wobble by increasing stacking energy in the complex (6). Differences in modification are unlikely to cause the difference in slippage, since both yeast and *E. coli* tRNAs include such stabilizing modifications. Alternatively, the difference in strength of interaction may reflect a difference not in the tRNA but in the ribosomes themselves. It may be that the codon-anticodon interaction in the yeast P site is stronger than in the bacterial P site, inhibiting peptidyl-tRNA slippage.

How could special peptidyl-tRNAs stimulate frameshifting without slipping? We can imagine three ways in which special peptidyl-tRNAs could stimulate frameshifting without slipping. The first model posits partial occlusion of the ribosomal A site by the frameshift-stimulating peptidyl-tRNAs, in the P site. The second imagines that special peptidyl-tRNAs may stabilize the interaction of noncognate tRNAs in the A site. The third model argues that if the average peptidyl-tRNA tends to dissociate from the ribosome during a translational pause, leading to the high rate of spontaneous abortion of elongation (34), the special property of the peptidyl-tRNA may be that it dissociates more slowly from the ribosome.

Arguably the simplest model is that peptidyl-tRNA stimulates frameshifting by interfering with normal binding of aminoacyl-tRNA in the ribosomal A site. The interaction between successive tRNAs in the ribosomal decoding sites, A and P sites, defines the 3-nucleotide translational step size, the codon (3, 12, 41). This probably means that the incoming EF-Tu–GTP–aminoacyl-tRNA complex (EF-1α–GTP–aminoacyl-tRNA, in eukaryotes) binds to a site on the ribosome which is partly defined by the already bound peptidyl-tRNA. Part of that positioning depends on the close apposition of the anticodon loop of the incoming aminoacyl-tRNA with the peptidyl-tRNA in the P site. However, an additional interaction between the P site-bound peptidyl-tRNA and the EF-Tu complex may aid in positioning the incoming aminoacyl-tRNA in the A site. The EF-Tu complex has two distinct binding for tRNAs: one the classical binding site for aminoacyl-tRNA, and a second binding site for peptidyl-tRNA which is induced by binding to the ribosome (49). The ribosome-bound interaction with peptidyl-tRNA activates the EF-Tu GTPase, the first step in acceptance of cognate aminoacyl-tRNA into the A site (50). Since the EF-Tu complex apparently includes two molecules of the elongation factor (17, 53), it is possible that the peptidyl-tRNA binding site resides on the second EF-Tu, possibly at the

same site which binds aminoacyl-tRNA on the other molecule, though cross-linking experiments indicate that the two binding sites are topologically distinct (51). The structure of the frameshift-prone peptidyl-tRNA, then, by binding to the incoming EF-Tu complex could misposition the aminoacyl-tRNA in the A site leading to out-of-frame binding and frameshifting.

A second model proposes that peptidyl-tRNAs promote frameshifting by stabilizing the transient binding of noncognate tRNAs in the A-site. Programmed frameshifts can be thought of as amplified errors, whose probability of occurring is increased by the existence of a prolonged translational pause (20). Since the ribosome excludes errors in elongation by kinetic proofreading (26, 37), programmed frameshift sites could induce noncanonical decoding by evading this proofreading. How would this evasion work? Kinetic proofreading depends on two short timing steps imposed by EF-Tu (47). The EF-Tu–GTP–aminoacyl-tRNA complex enters the ribosomal A site, but GTP is not hydrolyzed for a short period. Cognate and noncognate tRNA complexes bind the ribosome with the same kinetics, but the rate of dissociation of noncognate complexes is much faster than the rate of GTP hydrolysis. After hydrolysis, EF-Tu–GDP does not dissociate from the ribosome for a short period. Cognate complexes essentially never dissociate at this stage, but again, dissociation of noncognate complex is much faster than dissociation of EF-Tu from the tRNA. The low frequency of misincorporation errors depends on setting the EF-Tu timing step long enough so that noncognate tRNAs are much less likely to be accepted than are cognates. Programmed +1 frameshifting evades this proofreading, dependent on two effects. First, the rate of cognate decoding is reduced, resulting in a translational pause. As a result, the competition between cognate and noncognate outcomes is less skewed toward the cognate. Second, the structure of special peptidyl-tRNAs promotes the noncanonical outcome. One way that this could be achieved, in the context of kinetic proofreading, would be to stabilize transient binding of noncognate tRNAs, increasing the probability that they would survive the timing step and be accepted by the proofreading process.

By noncognate, we mean a tRNA which does not match the codon in the A site: in Ty3, the AGU pause codon. But in order to promote frameshifting, the noncognate accepted would have to be the tRNA which recognizes the overlapping +1 frame GUU codon, $tRNA^{Val}_{IAC}$. Acceptance of a random noncognate tRNA would simply lead to misincorporation at the hungry AGU codon. The model, to explain the ability of the ribosome to efficiently move into the +1 frame, must propose that accepting $tRNA^{Val}_{IAC}$ into the A site depends on its cognate recognition of the +1 frame codon. This model proposes an active role for $tRNA^{Val}_{IAC}$, pulling the ribosome into the shifted frame. This contrasts with models in which the aminoacyl-tRNA passively participates by filling the A site after the ribosome shifts +1. Since, in the active model, the probability of frameshifting depends on the rate the tRNA enters the A site, increasing the concentration of aminoacyl-tRNA should drive increased frameshifting.

An alternative method to evade kinetic proofreading would be to lengthen the translation pause associated with frameshifting. Kinetic proofreading can only operate after the EF-Tu–GTP–aminoacyl-tRNA complex enters the A site. Since nearly all cognate tRNAs entering are accepted, and noncognates are rarely accepted, the success of kinetic proofreading ultimately depends on the rate at which cognate aminoacyl-tRNA enters the ribosome. However, for programmed +1 frameshift sites, the cognate tRNA is limiting and enters the decoding site very slowly. Reducing the rate of cognate decoding allows normally improbable noncanonical events to occur at a measurable frequency. But for these events to occur they must also be more probable than other alternative events. Most errors leading to premature termination of elongation probably occur by peptidyl-tRNA dissociation (36) rather than by either release factor-dependent false stops at sense codons or translational frameshifting (30, 34). This suggests that dissociation may be the likeliest outcome during a translational pause at a random codon, much more likely than frameshifting. Translational frameshifting may only occur in cases in which the peptidyl-tRNA does not dissociate rapidly enough. The special property of those tRNAs which stimulate frameshifting may then be that they reduce the rate of peptidyl-tRNA dissociation, indirectly increasing frameshifting by increasing the translational pause with the A site empty.

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