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Pulling the Ribosome out of Frame by +1 at a Programmed Frameshift Site by Cognate Binding of Aminoacyl-tRNA

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Programmed translational frameshifts efficiently alter a translational reading frame by shifting the reading frame during translation. A +1 frameshift has two simultaneous requirements: a translational pause which occurs when either an inefficiently recognized sense or termination codon occupies the A site, and the presence of a special peptidyl-tRNA occupying the P site during the pause. The special nature of the peptidyl-tRNA reflects its ability either to slip +1 on the mRNA or to facilitate binding of an incoming aminoacyl-tRNA out of frame in the A site. This second mechanism suggested that in some cases the first +1 frame tRNA could have an active role in frameshifting. We found that overproducing this tRNA can drive frameshifting, surprisingly regardless of whether frameshifting occurs by peptidyl-tRNA slippage or out-of-frame binding of aminoacyl-tRNA. This finding suggests that in both cases, the shift in reading frame occurs coincident with formation of a cognate codon-anticodon interaction in the shifted frame.

Ribosomes normally faithfully maintain a particular reading frame when translating a gene. Errors in frame maintenance occur at rates of probably much less than 5×10^{-5} per codon (17). However, some genes have evolved sequences, termed programmed translational frameshift sites, which manipulate the process of translational frame maintenance to increase the probability of changing a translational frame as much as 10,000-fold. Programmed translational frameshifting occurs ubiquitously, if infrequently, among organisms from bacteria to higher eukaryotes. These mechanistically diverse events provide tools with which to probe the mechanism of frame maintenance during normal elongation. Frameshifting in the downstream, or rightward, direction (termed +1 frameshifts) occurs during an elongation pause caused by the presence of a slowly decoded ("hungry") codon, or a termination codon, in the ribosomal A site. Frameshifting occurs during this pause if a special peptidyl-tRNA, capable of inducing the shift, occupies the ribosomal P site (6, 24). The mechanism underlying the phenomenon of frameshifting is not clearly defined. It had been thought that programmed frameshifting depended on the ability of the peptidyl-tRNA to slip between cognate or near-cognate codons (6). Slippage need not be required, since in yeast cells there is no correlation between the ability of a peptidyl-tRNA to slip by +1 and the efficiency with which it induces +1 frameshifting (24). We do not know precisely how peptidyl-tRNAs could induce frameshifting without slippage, though it probably requires out-of-frame binding of the incoming aminoacyl-tRNA in the ribosomal A site. The ability to induce frameshifting in this manner depends on some special structural feature(s) of the frameshift-competent peptidyl-tRNAs, since replacing such a tRNA with a different isoacceptor eliminates frameshifting. Replacement can be done either by changing the codon to one which recruits a different tRNA (10) or by swapping anticodons between isoacceptors (24). Thus, mispairing of incoming aminoacyl-tRNA is apparently not forbidden during elongation, as experiments had suggested (5, 20). This sort of error probably occurs very infrequently during normal elongation (17), indicating that the translational apparatus must have evolved efficient means to eliminate them.

Translation efficiently avoids missense errors through a kinetic proofreading scheme (14, 18, 22). Cognate aminoacyl-tRNAs bind in the A site as a complex with elongation factor Tu (EF-Tu) and GTP. After binding, GTP is hydrolyzed to GDP, followed by a short pause before EF-Tu · GDP dissociates from the ribosome, quickly followed by peptide transfer and movement of the peptidyl-tRNA into the P site. Almost all cognate tRNA which enters the A site remains bound through peptide transfer. By contrast, kinetic proofreading rejects noncognate tRNAs in a two-stage process. First, noncognate aminoacyl-tRNA · EF-Tu · GTP complexes dissociate from the ribosome more rapidly than GTP can be hydrolyzed. Second, for those complexes which survive GTP hydrolysis, noncognate aminoacyl-tRNA again dissociates from the ribosome more quickly than does EF-Tu · GDP. The probability of selection of a noncognate aminoacyl-tRNA is very low, about 3×10^{-4} per codon, despite the fact that rejection of noncognate ternary complex or aminoacyl-tRNA occurs only about an order of magnitude faster than does GTP hydolysis and EF-Tu · GDP dissociation (23). The low error rate results from combining the two slightly improbable events in the two stages of kinetic proofreading. As a result, since the error rate depends on only a small magnitude difference in rates, small changes in any of these kinetic constants can cause much larger changes in the probability of error.

Much less is known about the mechanisms which preclude errors of frame maintenance. As with missense errors, frameshift errors are probably eliminated kinetically. This is clear for programmed frameshifts, which depend on an equally programmed pause in elongation. This pause allows sufficient time for a kinetically unlikely event to occur. In frameshifting dependent on tRNA slippage, the unlikely event is the slippage of one or two tRNAs onto cognate or near-cognate codons. The rate at which this occurs is low enough that during a normal elongation cycle, virtually no slippage can occur. However, as with missense errors, a small change in any of the kinetic

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constants can greatly increase the rate. In a simplified programmed frameshift, the kinetic constant modified is the rate of the competing in-frame event, either selection of an aminoacyl-tRNA (7) or recognition of a termination codon by peptide release factor (8). This pause does not change the microscopic rates of ternary complex selection; the probability of rejection of unconventional decoding events would remain unchanged. However, the many more attempts during an extended pause would result in a greater proportion of unconventional events surviving proofreading.

Though clearly frameshifting occurs as a result of the presence of a special peptidyl-tRNA, this and a translational pause may not be the only requirements. Frame disruption requires the insertion of an aminoacyl-tRNA out of frame. Do aminoacyl-tRNAs have an active role in frameshifting, or do they participate only passively, decoding the +1 frame codon when directed by the ribosome? Here we show that the tRNA which decodes the first +1 codon actively pulls the ribosome into the +1 frame.

MATERIALS AND METHODS

Yeast strains, media, and general methods. The *Saccharomyces cerevisiae* strain used in this work is 387-1D (α *his4Δ38 ura3-52 trp1-289 HOL1-1*). DNA transformations of yeast cells were performed by the lithium acetate method (16). The activity of β-galactosidase expressed by transformants was determined as described earlier (9). Transformants were grown in SD (synthetic plus dextrose) minimal medium supplemented with the appropriate amino acids to allow selection for *URA3*$^+$-containing plasmids (19). Oligonucleotides were synthesized on a Biosearch Cyclone DNA synthesizer (Milligen) and purified by chromatography on Oligo-Pak columns (Milligen) according to manufacturer's directions.

Plasmid construction. All plasmids used in this study are derivatives of pMB38 (1), 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 *Escherichia 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 Ty3 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 described previously (9). To determine the efficiency of frameshifting, we compared expression of the frameshift constructs with that of a construct, pMB38-Ty3FF (10), in which a single nucleotide within the frameshift region was deleted, putting *lacZ* in frame with *HIS4*. Frameshift efficiency is defined as the ratio of expression of the frameshift to expression of the frame fusion construct.

To determine what sequences could substitute for the pause codon of the Ty3 frameshift site, we constructed a library of plasmids in which the four nucleotides downstream of the frameshift-inducing GCG codon were randomized (GCG-XXX-X). The library was constructed in a plasmid derived from pMB38. The construction of alternative frameshift sites within this type of vector by PCR has been described (10) and involves replacing a portion of the plasmid by a fragment of DNA generated by PCR which incorporates the desired changes. The library was constructed by randomizing the sequence immediately distal to the GCG codon by incorporating equal amounts of all three nucleotides when synthesizing the PCR primer. The resulting plasmid places the randomized sequence in the context of a minimal frameshift site derived from the Ty3 retrotransposon frameshift site, Ty3Δ2 (10), which includes three nucleotides upstream of the GCG P-site codon and 18 bp downstream of it. Included in the downstream region is a 14-nucleotide sequence, termed the Ty3 context, which stimulates frameshifting 7.5-fold when present.

The library was introduced into yeast cells by transformation, and 1,600 transformants were screened by using a microtiter plate-based β-galactosidase assay. The procedure was a modification of our normal assay (9), adjusting reactions downward by approximately fivefold. The assay was quantitated on a Bio-tek EL311 automated microplate reader.

Cloning of $tRNA^{Ser}_{CGA}$ and anticodon swap mutagenesis. The gene encoding $tRNA^{Ser}_{CGA}$, decoding UCG, is a single-copy gene in *S. cerevisiae* (11). A clone of the gene (Genbank accession number K00571) was obtained by PCR. Oligonucleotide primers were designed to excise the entire sequenced region flanked by *Sal*I sites. The PCR product was digested with *Sal*I and inserted into the unique *Sal*I site of the pMB38-derived plasmid vector as described for the $tRNA^{Ser}_{GCU}$ gene (10). We mutagenized $tRNA^{Ser}_{CGA}$, changing its anticodon to GCU (creating a $tRNA^{Ser}_{GCU2}$ gene) to test whether the stimulation of frameshifting by overexpression of $tRNA^{Ser}_{GCU}$ depended on the primary sequence of that tRNA. A primer was designed to change the anticodon of the $tRNA^{Ser}_{CGA}$ gene. The bottom-strand primer extended from a unique *Ban*II site, located 27 bp downstream of the anticodon, to 8 bp upstream of the anticodon; the primer changed the sequence of the anticodon from CGA to GCU.

RESULTS

In *S. cerevisiae*, only three P-site codons (CUU, GCG, and CCG) efficiently induce +1 frameshifting, although a lengthened translational pause identifies five more tRNAs which can induce frameshifting (24). This finding in itself suggests that special P-site tRNAs promote efficient frameshifting. Several of these tRNAs clearly induce frameshifting without slipping on the mRNA. This fact was unexpected, since the ability to slip has been considered a necessary feature of frameshift sites, and mere inspection of the RNA sequence to identify sequences with a propensity to induce slippage can locate potential frameshift sites. Those tRNAs which cannot slip on the mRNA must induce frameshifting by promoting out-of-frame binding of incoming aminoacyl-tRNA. In either case, whether it occurs by peptidyl-tRNA slippage or by out-of-frame binding of incoming aminoacyl-tRNA, a peptidyl-tRNA present in the ribosomal P site during a translational pause actively promotes frameshifting.

Does incoming aminoacyl-tRNA participate only passively in frameshifting, decoding the +1 frame codon when it is presented in the ribosomal A site, or does it actively participate, drawing the ribosome into the +1 frame? A simple model for frameshifting would propose that during a translational pause at a hungry codon, the presence of a frameshift-inducing peptidyl-tRNA in the ribosomal P site would cause an alteration in the ribosome such that the A site is no longer occupied by the next in-frame codon, but instead the +1 frame codon is presented there. In canonical frameshifting dependent on tRNA slippage, one imagines that during the translational pause, the peptidyl-tRNA slips along the mRNA (perhaps it is better to say that the mRNA slips on the tRNA), presenting the +1 frame codon as the next in-frame codon. In such a model, the first +1 frame aminoacyl-tRNA would function as it does during a normal elongation cycle, binding to the codon presented in the A site. One can imagine a more complex model in which the tRNA which decodes the first +1 frame codon participates actively. For example, the shift into the +1 frame might require that this tRNA be present in the ribosomal A site before the shift. If this were true, then the energy required for the shift would be recovered in the form of the three base pairs formed between the tRNA and its cognate +1 frame codon. The probability of shifting in this model would depend on the availability of the cognate aminoacyl-tRNA. Reducing the concentration of that tRNA would decrease its rate of selection; during a pause of finite duration, decreasing the selection rate would result in a decreased likelihood of shifting.

Identification of all sequences which can induce a translational pause sufficient to promote +1 translational frameshifting. A simple test of these models then would involve testing the effect of overproducing the +1 frame tRNA, looking for an effect on frameshift efficiency. We had available two frameshift sites derived from Ty1, CUU-AGG-C (1), and Ty3, GCG-AGU-U (10). In each case, each of the first +1 frame codons, GGC and GUU, respectively, is decoded by an abundant tRNA, its gene present in multiple dispersed copies in the yeast genome (12). These frameshift sites could not be used to test the models since it would be difficult to overproduce significantly an already abundant tRNA. We therefore chose to attempt to identify other +1 frameshift sites by selecting among random sequences for those which would promote frameshifting. We constructed a library of *lacZ* frameshift re-

TABLE 1. Seven possible pause sequences in *S. cerevisiae*

Sequence	No. of times obtained	Frameshifting (%)	+1 frame codon
AGG-C	1	31	GGC
AGU-C	4	14	GUC
AGU-U	2	15	GUU
UGG-C	1	19	GGC
UAA-C	1	12	AAC
UGA-C	3	37	GAC
UAG-C	0[a]	30	AGC

[a] A site with this sequence was not obtained in this screen but was constructed independently.

TABLE 2. Restriction of the seventh base in frameshift sites

Frameshift site	Frameshift efficiency (%)			
	C[a]	A	G	U
GCG-AGU[b]	15	4.5	ND[c]	15
CUU-AGG[d]	43	0.8	0.9	0.7
GCG-UGG	19	0.3	0.5	ND
GCG-UAA	12	0.8	1.4	0.9
GCG-UAG	30	0.7	0.3	0.4
GCG-UGA	37	0.7	ND	0.8

[a] Seventh nucleotide.
[b] Data from reference 10.
[c] ND, not determined.
[d] Constructs lack the Ty3 context sequence; data from reference 1.

porter plasmids bearing four randomized nucleotides immediately downstream from a frameshift-inducing P-site codon, GCG-XXX-X. The library was introduced into yeast cells by transformation, and 1,600 transformants were screened individually for the ability to promote significant levels of frameshifting. Forty-two colonies that appeared to induce more than 2% frameshifting were obtained; a second screen reduced this number to 26. These plasmids were rescued into *E. coli* (13), and the frameshift regions were sequenced to identify the four bases present.

Frameshifting well above the 2% minimum occurred with only six sequences (Table 1). Four of these introduced a sense codon into the A-site codon, either AGU-U, AGU-C, AGG-C, or UGG-C. Two others introduced stop codons, UAA-C and UGA-C. The pause sequences generally confirm our understanding of the mechanism of +1 frameshifting, specifically, that frameshifting occurs when a hungry or termination codon occupies the ribosomal A site. For example, both AGG and AGU are recognized by rare tRNAs, the products of single genes in *S. cerevisiae*. For both AGG and AGU, we have demonstrated that frameshifting occurs as a result of limiting amounts of the tRNAs being available by showing that overproducing the cognate tRNAs drastically reduces frameshift efficiency (1, 10). However, UGG is not rare, since it is present at about half the concentration of the most abundant isoacceptors (15). Clearly, the concentration of this tRNA alone cannot explain its ability to induce frameshifting. However, even an abundant tRNA can be limiting for translation, for example, if it is poorly aminoacylated or competes poorly for EF-1α, thus lowering its concentration in ternary complexes. Since the rate of codon recognition depends on the concentration of ternary complex (22), this should tend to lengthen the pause at the cognate codon. Alternatively, the rate at which a ternary complex enters an empty A site may depend on a structure in the tRNA, causing some codons to be slowly decoded even with abundant ternary complex. This effect could be an intrinsic feature or could result from interactions with the P-site tRNA (20). We know of no evidence which supports any of these models.

We had previously found that changes to the nucleotide immediately downstream of the pause codon would eliminate frameshifting. In the case of the Ty1 site, the pause codon AGG must be followed by C. In Ty3, the AGU pause codon must be followed by either U or C. The collection that we identified in this screen implied a similar restriction for the codons UGG, UAA, and UGA. To determine if the fourth nucleotide was critical in these cases as well, we constructed a collection of plasmids randomizing the position 3′ to these five pause codons. We also tested if the third nonsense codon, UAG, could stimulate frameshifting and if it required a neighboring nucleotide. As shown in Table 2, all three of the nonsense codons and UGG stimulate frameshifting, and all four require the same 3′ neighboring nucleotide, C. We have therefore identified seven possible pause sequences in *S. cerevisiae*: AGU-U, AGU-C, AGG-C, UGG-C, UAA-C, UAG-C, and UGA-C.

The other three sequences introduce termination codons into the A site during frameshifting. These sites resemble slippery stops identified in *E. coli* (25). Slow recognition of the termination codon by release factor induces a translational pause similar to that induced by a hungry codon (21). Efficient induction of frameshifting depends on inefficient recognition by release factor. Apparently release factor efficiency is determined by the nucleotide 3′ to the termination codon (3, 4). Consistent with this hypothesis, we found a strict requirement for C immediately 3′ to each stop codon; in fact, we have found that substituting C with any other nucleotide eliminates frameshifting. These same tetranucleotides, UAA-C, UAG-C, and UGA-C, occur infrequently at the ends of yeast genes; only one highly expressed gene terminates with UAA-C, and none use UAG-C or UGA-C (2, 3). Apparently, these sequences are particularly slowly recognized by release factor, leading to a protracted pause which enables frameshifting to occur.

Out-of-frame decoding of the first +1 frame codon pulls the ribosome into the shifted frame. We have identified seven possible +1 frameshift sites: GCG-AGG-C, GCG-AGU-U, GCG-AGU-C, GCG-UGG-C, GCG-UAA-C, GCG-UGA-C, and GCG-UAG-C (the first +1 frame codon is underlined in each case). In only one of these cases is the +1 frame codon decoded by a rare tRNA, GCG-UAG-C. This frameshift site could be used to test the effect of availability of the +1 frame tRNA on frameshift efficiency. The codon AGC is decoded by $tRNA^{Ser}_{GCU}$. Changing the frameshift site from GCG-UAG-C to GCG-UAG-U eliminates frameshifting, though $tRNA^{Ser}_{GCU}$ also decodes AGU. The sequence GCG-UAG-U might fail to stimulate frameshifting because peptide release factor recognizes the sequence UAG-U more efficiently than it does UAG-C, eliminating the required translational pause (although UAG-U is as underrepresented among true terminators, as is UAG-C) (2, 3). Alternatively, it may fail because $tRNA^{Ser}_{GCU}$ decodes AGU less efficiently than it does AGC, eliminating a hypothetical pulling effect of decoding the first +1 frame codon.

We tested the effect of overexpressing $tRNA^{Ser}_{GCU}$ or an irrelevant seryl-tRNA, $tRNA^{Ser}_{CGA}$ (which decodes UCG), on frameshift efficiency at several +1 frameshift sites: the Ty1 site, CUU-AGG-C; GCG-UAG-C; GCG-UAG-U; and the Ty3 site, GCG-AGU-U (Fig. 1). The results of this experiment are diagrammed in Fig. 2. Frameshifting on the Ty1 site, CUU-AGG-C, was unaffected by expression of either tRNA, as expected. Since as we have shown, frameshifting on the Ty3 site

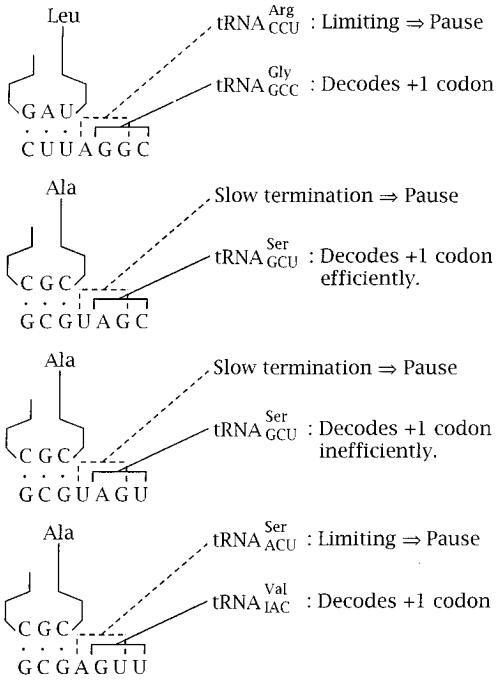

FIG. 1. Testing the pulling effect of the +1 frame tRNA. The cartoons show how each of four sites is decoded. A tRNA is represented binding to the first codon of the frameshift site. The 0 and +1 frame codons are bracketed; a dashed bracket identifies each pause codon, and an undashed bracket identifies the +1 frame codon. The effect of each 0 and +1 frame codon on frameshifting is indicated.

depends on the slow decoding of AGU by $tRNA^{Ser}_{GCU}$ (10), overexpression of $tRNA^{Ser}_{GCU}$ virtually eliminated frameshifting on this site; the irrelevant seryl-$tRNA^{Ser}_{CGA}$ had no effect. This result confirms the overexpression of $tRNA^{Ser}_{GCU}$ and shows that its effect is anticodon specific. By contrast, overexpressing $tRNA^{Ser}_{GCU}$ caused a dramatic increase in frameshifting on both GCG-UAG-C and GCG-UAG-U, while $tRNA^{Ser}_{CGA}$ had no effect. This result in itself demonstrates that the concentration of $tRNA^{Ser}_{GCU}$ has a rate-limiting effect on frameshifting when its cognate codon is the first +1 frame codon. Note that the *lacZ* fusion gene includes 35 AGU and AGC codons. Since overproducing $tRNA^{Ser}_{GCU}$ had no effect on the CUU-AGG-C control, normal decoding of these codons, either individually or in aggregate, is not rate limiting in elongation. Therefore, the fact that decoding of AGU or AGC as the first +1 frame codon is rate limiting suggests that that decoding event is unlike a normal elongation event. Thus, these data do not support the hypothesis that decoding of the first +1 frame codon occurs as during a normal cycle. Rather, decoding of this codon accelerates the rate-limiting step in *lacZ* translation, the +1 frameshift event.

We had shown previously that a special feature of sequences of some peptidyl-tRNAs allows them to promote frameshifting. It is possible that the aminoacyl-tRNAs which promote frameshifting also are special, having a structure which allows them to bind out of frame. Alternatively, the tRNAs may have no special feature, frameshift efficiency depending on the rate at which they can be recruited to the ribosomal A site. We showed that the structure of the peptidyl-tRNA was necessary by mutating a $tRNA^{Ala}_{UGC}$ (specific for GCA) which does not promote frameshifting, introducing the CGC anticodon (specific for GCG). Overproducing the mutated tRNA eliminated frameshifting, demonstrating that some structure(s) of $tRNA^{Ala}_{CGC}$ promotes frameshifting after a GCG codon (24). Similarly, we mutated $tRNA^{Ser}_{CGA}$, introducing the anticodon GCU to create $tRNA^{Ser}_{GCU2}$. If a structure of the normal $tRNA^{Ser}_{GCU}$, absent from $tRNA^{Ser}_{GCU2}$, were required to allow out-of-frame decoding of AGU and AGC, then overproducing $tRNA^{Ser}_{GCU2}$ would have no effect on frameshifting on GCG-UAG-U or GCG-UAG-C. As shown in Fig. 2, however, overproducing $tRNA^{Ser}_{GCU2}$ also greatly stimulated frameshifting in both cases. This result suggests that the primary sequence of $tRNA^{Ser}_{GCU}$ is not critical for its pulling effect, though we cannot eliminate the possibility that $tRNA^{Ser}_{CGA}$ shares this capacity.

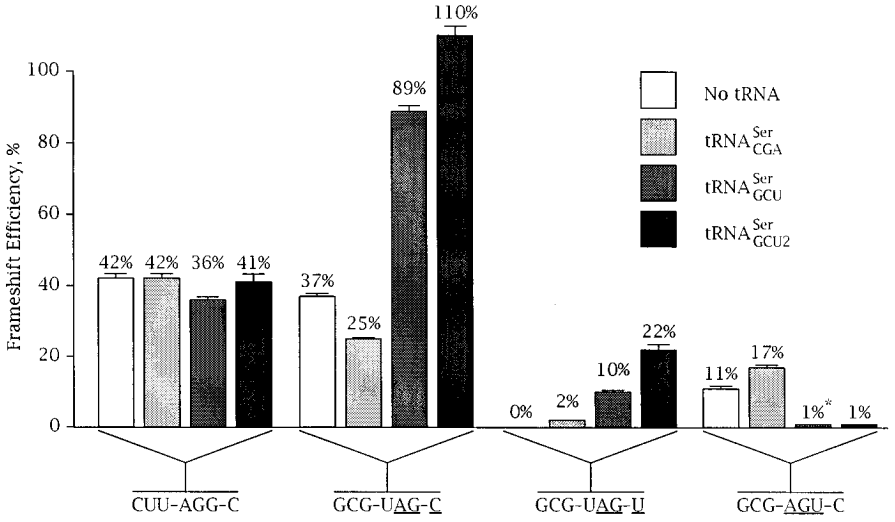

FIG. 2. Out-of-frame recognition of the +1 frame by an aminoacyl-tRNA pulls the ribosome into the shifted frame. The graphs represent the frameshift efficiencies (expressed relative to a control in which β-galactosidase expression does not require frameshifting) on four frameshift sites, CUU-AGG-C, GCG-UAG-C, GCG-UAG-U, and GCG-AGU-C. The columns represent expression in the presence of overexpressed $tRNA^{Ser}_{CGA}$ (which decodes UCG), $tRNA^{Ser}_{GCU}$ (decoding AGU and AGC), and $tRNA^{Ser}_{GCU2}$, as indicated by the key to symbols. $tRNA^{Ser}_{GCU2}$ is the $tRNA^{Ser}_{CGA}$ with its anticodon changed to GCU; it includes 25 base changes from wild-type $tRNA^{Ser}_{GCU}$. The column represented as the effect of overexpressing $tRNA^{Ser}_{GCU}$ on expression of GCG-AGU-C (asterisk) actually involved GCG-AGU-U, which promotes frameshifting at an equivalent efficiency (5).

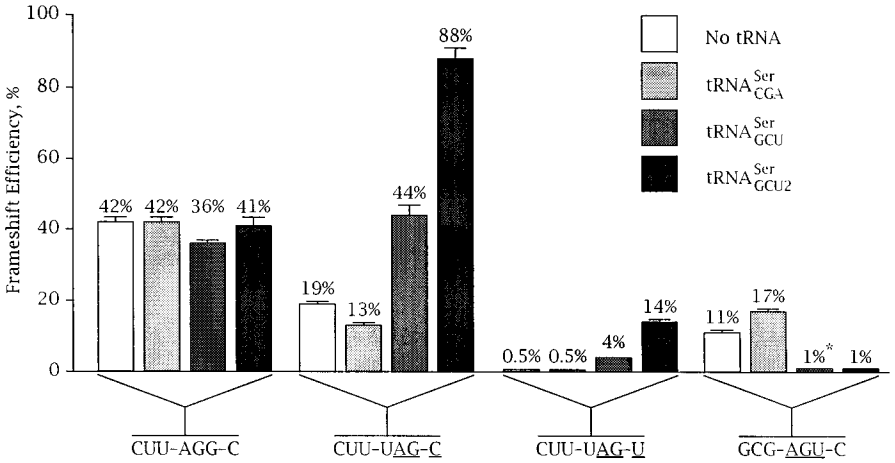

FIG. 3. Decoding the first +1 frame codon stimulates frameshifting by tRNA slippage. The effect of overexpression of $tRNA^{Ser}_{CGA}$, $tRNA^{Ser}_{GCU}$, and $tRNA^{Ser}_{GCU2}$ on peptidyl-tRNA slippage-dependent frameshifting on the frameshift sites CUU-UAG-C and CUU-UAG-U is graphed as in Fig. 2.

Aminoacyl-tRNA also participates in frameshifting which depends on slippage of peptidyl $tRNA^{Leu}_{UAG}$. We have shown that the ability of the special peptidyl-$tRNA^{Ala}_{CGC}$ to direct out-of-frame binding of an aminoacyl-tRNA requires the active participation of that incoming tRNA. This suggests a model in which the peptidyl-tRNA directs the misalignment of the tRNA in the ribosomal A site, shifting the reading frame by +1. The model which we described for +1 frameshifting on the Ty1 site differs in several respects from this model (1). First, frameshifting on the Ty1 site, CUU-AGG-C, depends on slippage of $tRNA^{Leu}_{UAG}$ from CUU +1 to UUA (1, 24). Second, that slippage occurs during a translational pause caused by the limited availability of $tRNA^{Arg}_{CCU}$, which deocodes AGG. We proposed a model for this event in which peptidyl-tRNA slippage occurs during the pause caused by the hungry AGG codon and thus when the A site was empty. Given that the incoming aminoacyl-tRNA corresponding to the first +1 frame codon drives frameshifting after a GCG codon, could the single tRNA slippage model for Ty1 frameshifting be wrong, and frameshifting occur when the A site is occupied by the first +1 frame tRNA? If this were true, then overexpression of this tRNA would drive frameshifting just as with frameshifting by out-of-frame binding.

To test this hypothesis, we introduced a frameshift reporter construct carrying the frameshift site CUU-UAG-C or CUU-UAG-U, an analog of the constructs described above. As with the constructs described above, frameshifting at the UAG-C pause site was much more efficient than frameshifting at UAG-U (Fig. 3). However, we found that overexpressing $tRNA^{Ser}_{GCU}$ increased +1 frameshifting on both of these constructs. Frameshifting at CUU-UAG-C increased 2.3-fold, while frameshifting at CUU-UAG-U increased 8-fold, from only 0.5%, indistinguishable from background expression, to a level approximately equal to normal frameshifting at CUU-UAG-C. We also tested the effect of $tRNA^{Ser}_{GCU2}$, the mutated form of $tRNA^{Ser}_{CGA}$ carrying the anticodon GCU to enable it to recognize AGU and AGC. Overproducing this tRNA had a larger effect, increasing frameshifting at CUU-UAG-C nearly 7-fold and frameshifting at CUU-AGU-U 28-fold. The greater effect may reflect the fact that the $tRNA^{Ser}_{CGA}$ gene is more actively transcribed in vivo, causing greater overproduction. Once again, this result clearly shows that frameshifting depends on the availability of the +1 frame cognate aminoacyl-tRNA. This cannot be understood in the context of the simple model of Ty1 frameshifting. Though frameshifting occurs before $tRNA^{Arg}_{CCU}$ can enter and decode the 0 frame codon, AGG, it may not occur with the A site empty. The efficiency of frameshifting depending on the concentration of the +1 frame $tRNA^{Ser}_{GCU}$ strongly suggests that that tRNA must enter the A site in order for slippage to occur. This is most consistent with a model in which slippage of peptidyl-$tRNA^{Leu}_{UAG}$ occurs after aminoacyl-$tRNA^{Ser}_{GCU}$ enters the A site. Part of the energy which drives the slippage may derive from the ability to form three base pairs between $tRNA^{Ser}_{GCU}$ and the +1 frame AGC or AGU codon. We note the similarity between this model and the simultaneous slippage model for −1 frameshifting; in that model, frameshifting occurs by simultaneous slippage of two ribosome-bound tRNAs and depends on the ability to form cognate or near-cognate base-pairing interactions in the shifted frame. The difference between that model and the one that we propose here for +1 frameshifting is that the tRNA in the A site of the ribosome is selected by its ability to bind to the out-of-frame codon, rather than the last in-frame codon, and therefore should be recruited to the ribosome through a nonstandard mechanism.

DISCUSSION

Cognate recognition of the first +1 frame codon drives two types of +1 frameshifts. Frameshifting by out-of-frame binding of incoming aminoacyl-tRNA is not a special case, specific to the Ty3 frameshift system. We have shown that of the approximately 45 tRNAs in *S. cerevisiae*, 8 stimulate levels of frameshifting significantly above background when introduced into the Ty3 context (24). Of these tRNAs, four are very unlikely to undergo slippage on the messenger and must promote frameshifting by out-of-frame binding of aminoacyl-tRNA. If these special peptidyl-tRNAs do not slip on the messenger, how do they shift the ribosome into the +1 reading frame? There are essentially two ways in which this can be done. First, the peptidyl-tRNAs could manipulate the ribosome, moving the +1 frame codon into the ribosomal A site. In the simplest version of this model, the translational pause allows sufficient time for a significant proportion of paused ribosomes to isomerize in this way. Recognition of the +1 frame codon would then proceed as normal, by binding of the cognate aminoacyl-tRNA · EF-Tu · GTP ternary complex. The frequency of frameshifting, in this model, would depend en-

tirely on the rate of isomerization and should be no more sensitive to a limiting concentration of ternary complex than would any other elongation step. A second model posits an active role for ternary complex in shifting the ribosome. In the simplest version of this model, the special peptidyl-tRNA does not manipulate the ribosome but rather alters the way ternary complex interacts with the ribosome. Interaction between the ribosome-bound peptidyl-tRNA and the incoming ternary complex would tend to misposition the aminoacyl-tRNA onto the +1 frame codon. Peptide transfer to this out-of-frame tRNA would shift the ribosome into the +1 frame for continued elongation. The frequency of frameshifting in this model would depend on the rate of recognition of the +1 frame codon, which, since it involves an improbable, nonstandard recruitment of a specific aminoacyl-tRNA, would be sensitive to limitations in availability of that tRNA.

The data presented here clearly support the second of these models. A limiting concentration of aminoacyl-tRNA decoding the first +1 frame codon can measurably decrease the efficiency of +1 frameshifting. The two models described above correspond to two extreme views of the mechanism. In fact, a mechanism falling somewhere between the two views would be equally consistent with the experimental data. For example, binding of a ternary complex could occur during a transient isomerization of the A site brought about by peptidyl-tRNA. In this case, the transient nature of the isomerization would make decoding the +1 shifted codon sensitive to the concentration of the cognate ternary complex.

We should note that the frameshift site used to test the role of the first +1 frame tRNA differs in a significant way from the wild-type Ty3 site in that the pause codon is a stop codon rather than a poorly recognized sense codon. Frameshifting on the Ty3 site is stimulated by the in-frame AGU pause codon and a 15-nucleotide downstream context; the context stimulates frameshifting 7.5-fold (10). Changing the sense pause codon to a stop codon eliminates stimulation by the context. Conceivably the presence of the context could eliminate the dependence of frameshifting on the concentration of the +1 frame codon. More likely, however, the basic mechanism would remain the same, with the context merely increasing the efficiency of the process.

Surprisingly, we found that the +1 frame tRNA also drives frameshifting dependent on tRNA slippage. Our previous data had shown that frameshifting on the Ty1 site, CUU-AGG-C, occurs when peptidyl-$tRNA^{Leu}_{UAG}$ is bound to its cognate codon, CUU, during a translational pause caused by the low availability of the AGG-specific $tRNA^{Arg}_{CCU}$ (1). We proposed that this shift requires slippage of $tRNA^{Leu}_{UAG}$ from CUU onto the overlapping near-cognate leucine codon UUA. This conclusion was based on two facts. First, in vitro $tRNA^{Leu}_{UAG}$ can recognize all six leucine codons (26), indicating that this tRNA could slip from CUU onto UUA; second, overexpressing a competing $tRNA^{Leu}$ with the anticodon AAG, predicted not to slip, eliminated frameshifting (1). In addition, among mutants of the Ty1 site, frameshifting efficiency correlated with the strength of codon-anticodon interaction after +1 shifting (1, 24). Our model proposed that peptidyl-$tRNA^{Leu}_{UAG}$ slipped +1 while the the ribosomal A site was empty. In this model, decoding of the first +1 frame codon would then occur as a normal elongation step. A similar model was proposed for the +1 frameshift at the *prfB* gene of *E. coli*, in which tRNA slippage was envisaged to occur in competition with recognition of a nonsense codon in the 0 frame; after tRNA slippage, the presence of a new codon in the A site allows rapid decoding by the first +1 frame tRNA (8). However, we show here that a limiting concentration of the +1 frame ternary complex restricts frameshifting by tRNA slippage, indicating an active role for the first +1 frame tRNA in slippage. In the simplest model for this effect, slippage would occur with the +1 frame-specific ternary complex in the ribosomal A site. Slippage would then lead to a normal cognate interaction in the A site, providing extra energy to drive the shift. Again, these data do not exclude an alternate model in which peptidyl-$tRNA^{Leu}_{UAG}$ transiently slips into the +1 reading frame, with binding of cognate aminoacyl-tRNA to the +1 frame codon in the A site, trapping the ribosome in the shifted frame.

While we can only speculate on the biochemical mechanisms underlying the phenomena that we have described, the data do support a general model for +1 frameshifting at a hungry codon. Frameshifting occurs during a translational pause and requires two things to occur during that pause: a frameshift-competent tRNA must occupy the P site of the ribosome, and the tRNA which decodes the first +1 frame codon must transiently enter the ribosomal A site. With a "slippery" peptidyl-tRNA in the P site, transient entry of the tRNA allows slippage into the +1 frame. Frameshift-competent peptidyl-tRNAs which cannot slip promote the out-of-frame binding of this tRNA. During a pause of sufficient duration these events can occur, though they are very unlikely during the normally more rapid elongation step. As with missense errors, avoiding errors in frame maintenance would depend on kinetic constants greatly favoring normal in-frame decoding. Though a translation system cannot be designed to absolutely exclude these errors without violating the laws of thermodynamics, errors occur infrequently if kinetic differences are large enough, as in vitro analysis suggests they are. However, the programmed +1 frameshift sites which we have studied would slow the kinetics of normal decoding so that the probability of a noncanonical outcome approaches that of in-frame reading. Experiments are now in progress to determine what biochemical mechanisms govern both programmed frameshifting and normal maintenance of frame.

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REFERENCES

1. **Belcourt, M. F., and P. J. Farabaugh.** 1990. Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site. Cell **62:**339–352.
2. **Brown, C. M., M. E. Dalphin, P. A. Stockwell, and W. P. Tate.** 1993. The translational termination signal database. Nucleic Acids Res. **21:**3119–3123.
3. **Brown, C. M., P. A. Stockwell, C. N. A. Trotman, and W. P. Tate.** 1990. Sequence analysis suggests that tetra-nucleotides signal the termination of protein synthesis in eukaryotes. Nucleic Acids Res. **18:**6339–6345.
4. **Brown, C. M., P. A. Stockwell, C. N. A. Trotman, and W. P. Tate.** 1990. The signal for the termination of protein synthesis in procaryotes. Nucleic Acids Res. **18:**2079–2086.
5. **Curran, J., and M. Yarus.** 1987. Reading frame selection and transfer RNA anticodon loop stacking. Science **238:**1545–1550.
6. **Curran, J. F.** 1993. Analysis of effects of tRNA: message stability on frameshift frequency at the *Escherichia coli* RF2 programmed frameshift site. Nucleic Acids Res. **21:**1837–1843.
7. **Curran, J. F., and M. Yarus.** 1989. Rates of aminoacyl-tRNA selection at 29 sense codons *in vivo*. J. Mol. Biol. **209:**65–77.
8. **Donly, B. C., C. D. Edgar, F. M. Adamski, and W. P. Tate.** 1990. Frameshift autoregulation in the gene for *Escherichia coli* release factor 2: partly functional mutants result in frameshift enhancement. Nucleic Acids Res. **18:** 6517–6522.
9. **Farabaugh, P., X.-B. Liao, M. Belcourt, H. Zhao, J. Kapakos, and J. Clare.** 1989. Enhancer and silencerlike sites within the transcribed portion of a Ty2 transposable element of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**4824–4834.
10. **Farabaugh, P. J., H. Zhao, and A. Vimaladithan.** 1993. A novel programed frameshift expresses the *POL3* gene of retrotransposon Ty3 of yeast: frame-

shifting without tRNA slippage. Cell **74:**93–103.
11. **Guthrie, C., and J. Abelson.** 1982. Organization and expression of tRNA genes in *Saccharomyces cerevisiae*, p. 487–528. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Hinnebusch, A. G., and S. W. Liebman.** 1991. Protein synthesis and translational control in *Saccharomyces cerevisiae*, p. 627–735. *In* J. R. Broach, J. R. Pringle, and E. W. Jones (ed.), The molecular biology of the yeast *Saccharomyces*, vol. 1. Genome dynamics, protein synthesis, and energetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Hoffman, C. S., and F. Winston.** 1987. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. Gene **57:**267–272.
14. **Hopfield, J.** 1974. Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity. Proc. Natl. Acad. Sci. USA **71:**4135–4139.
15. **Ikemura, T.** 1982. Correlation between the abundance of yeast transfer RNAs and the occurrence of the respective codons in protein genes. J. Mol. Biol. **158:**573–597.
16. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:**163–168.
17. **Kurland, C. G.** 1992. Translational accuracy and the fitness of bacteria. Annu. Rev. Genet. **26:**29–50.
18. **Ninio, J.** 1975. Kinetic amplification of enzyme discrimination. Biochimie **57:** 587–595.
19. **Rose, M., F. Winston, and P. Hieter.** 1990. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
20. **Smith, D., and M. Yarus.** 1989. tRNA-tRNA interactions within cellular ribosomes. Proc. Natl. Acad. Sci. USA **86:**4397–4401.
21. **Tate, W. P., and C. M. Brown.** 1992. Translational termination: "stop" for protein synthesis or "pause" for regulation of gene expression. Biochemistry **31:**2443–2450.
22. **Thompson, R.** 1988. EFTu provides an internal kinetic standard for translational accuracy. Trends Biochem. Sci. **13:**91–93.
23. **Thompson, R. C., and D. B. Dix.** 1982. Accuracy of protein biosynthesis. A kinetic study of the reaction of poly(U)-programmed ribosomes with a leucyl-$tRNA_2$-elongation factor Tu-GTP complex. J. Biol. Chem. **257:**6677–6682.
24. **Vimaladithan, A., and P. J. Farabaugh.** 1994. Special peptidyl-tRNA molecules can promote translational frameshifting without slippage. Mol. Cell. Biol. **14:**8107–8116.
25. **Weiss, R. B., D. M. Dunn, J. F. Atkins, and R. F. Gesteland.** 1987. Slippery runs, shifty stops, backward steps, and forward hops: −2, −1, +1, +2, +5, and +6 ribosomal frameshifting. Cold Spring Harbor Symp. Quant. Biol. **52:**687–693.
26. **Weissenbach, J., G. Dirheimer, R. Falcoff, J. Sanceau, and E. Falcoff.** 1977. Yeast $tRNA^{Leu}$ (anticodon U-A-G) translates all six leucine codons in extracts from interferon treated cells. FEBS Lett. **82:**71–76.