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Codon choice and gene expression: Synonymous codons differ in translational accuracy

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ABSTRACT **Ribosomes programmed by different synonymous codons also differ in discriminating among near-cognate aminoacylated tRNAs. In the initial step of the recognition reaction ribosomes programmed by UUC discriminate less well than ribosomes programmed by UUU against ternary complexes containing three types of Leu-tRNA, and ribosomes programmed by CUC discriminate less well than ribosomes programmed by CUU against ternary complexes containing Phe-tRNA. Furthermore, in the proofreading step ribosomes programmed by UUC discriminate less well than ribosomes programmed by UUU against two of three near-cognate Leu-tRNAs, and ribosomes programmed by CUC discriminate less well than ribosomes programmed by CUU against near-cognate Phe-tRNA. The codon-induced change in reaction rate with near-cognate ternary complexes is greater than that with cognate ternary complexes: the most efficient codon is, therefore, the least accurate. Because the efficient, but inaccurate, codon UUC is used preferentially in highly expressed mRNAs of *Escherichia coli*, maximization of translational accuracy apparently has not been significant in the evolution of this particular biased codon choice in *E. coli*.**

The ribosomes, tRNAs, and factors of the translational apparatus are usually thought devoid of intrinsic specificity because any specificity could interfere with accurate reading of mRNA. But surprisingly the translational apparatus shows a distinct specificity for certain codon–tRNA pairs. The translational apparatus of many organisms appears to prefer a distinct subset of possible codons in highly expressed genes (1–4). Explanations for this bias based on the existence of preferred structures for mRNAs are unconvincing, and attempts to explain the bias now focuses on the hypothesis that the disfavored codons are either less rapidly or less accurately read by the translational apparatus.

Evidence for the idea that disfavored codons are translated less rapidly includes the finding that the average *in vivo* translational-step times of highly expressed mRNAs increase when the mRNAs contain these codons (5) and that pause sites in translating several mRNAs associate with the disfavored codons (6). These findings have often been attributed to the fact that disfavored codons are generally translated by low-abundance tRNAs (7). However, we recently presented evidence that the ribosome works faster with certain codon–tRNA pairs (8). Thus, we showed *in vitro* that the phenylalanine codon UUC is more rapidly translated by Phe-tRNA than is the synonymous codon UUU. Results pointing to the same conclusion have been obtained *in vivo* by Curran and Yarus (29). That a favored codon is translated almost twice as fast as its disfavored synonym supports the idea that need for translational efficiency is one factor driving the evolution of codon bias in *Escherichia coli*.

Another factor possibly contributing to the evolution of codon bias is the need for accurate translation. The choice of asparagine codons may provide an example of this effect. Parker and his colleagues (9) have shown *in vivo* that the AAU codon is more likely to be mistranslated by Lys-tRNA than is AAC, and AAC is chosen >13 times as often as AAU to code for asparagine in highly expressed proteins in *E. coli*. However, Parker and Precup (10) have also presented a possible counterexample for the importance of accuracy in determining codon choice. At five phenylalanine codons in ornithine transcarbamoylase leucine is found more often at positions coded by UUC than at positions coded by UUU, although UUC is the favored codon for phenylalanine in highly expressed proteins. As stated by Parker and Precup (10) codon context may help determine the different accuracies of translation at UUU and UUC codons, and the results might also be affected if proteins with errors at some positions are degraded more rapidly than other proteins.

To study the reaction rate of ribosomes with near-cognate aminoacylated (aa)-tRNA ternary complexes, we recently modified an experimental system designed to measure the reaction rate of ribosomes programmed with defined, synonymous codons with cognate aa-tRNA ternary complexes. This system allowed us to determine accuracy of translation of synonymous codons without the complications of different contexts and product degradation and where errors could be assigned to the appropriate translation steps. In these initial experiments we measured (*i*) the accuracy of translation of UUU and UUC codons by several Leu-tRNAs and (*ii*) the accuracy of translation of the leucine codons CUU and CUC by Phe-tRNA and a Leu-tRNA.

Accuracy of translation is determined by two processes (11–14). In the first process, initial recognition, a ternary complex of aa-tRNA, polypeptide-chain elongation factor Tu (EFTu), and GTP is bound to the ribosome; GTP is then hydrolyzed or the ternary complex dissociates from the ribosome. Specificity in this step is measured by the apparent rate constant for GTP hydrolysis of the near-cognate aa-tRNA relative to the analogous rate constant for the cognate aa-tRNA. In the second process, proofreading, the aa-tRNA may be either incorporated into peptide or released from the ribosome. Specificity in this step is measured by the proofreading ratio—the number of ternary complexes that must react with the ribosome to incorporate a near-cognate aa-tRNA. The proofreading ratio for a cognate aa-tRNA with wild-type ribosomes always approaches 1 after correcting for the contribution of partially inactive ribosomes.

Our results support Parker's assertion that UUC, the preferred codon for phenylalanine in highly expressed genes of *E. coli*, is less accurately translated than UUU and show that any one of these Leu-tRNAs could account for this error. The lower accuracy of cytosine-terminating codons may be a common phenomenon, because the CUC codon for leucine is

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Abbreviations: aa-tRNA, aminoacylated tRNA; EFTu, polypeptide-chain elongation factor Tu; fMet-tRNA, formylmethionyl tRNA; k_{GTP}, apparent rate constant for GTP hydrolysis; RS, ribosomes programmed with mRNA and with fMet-tRNA in the P-site; GTPγS, guanosine 5′-[γ-thio]triphosphate.

also less accurately translated than CUU, its uridine-terminating synonym. Finally, our results show that the lower dissociation rate of near-cognate ternary complexes from the UUC-programmed A-site is a major contributor to the increased error frequency of this codon.

MATERIALS AND METHODS

Sources of tRNAs, radioactive amino acids, and $^{32}P_i$, have been described (13), except that $tRNA_4^{Leu}$ and $tRNA_5^{Leu}$ were purchased from Subriden RNA (Seattle). mRNAs prepared as described by Thomas *et al.* (8) have the general sequence GGGAGACCGGAAGCUUGGGCUGCAGGAGGAUUU-AAUCAUGXYZAAGAUCUCG, where XYZ is the codon to be located in the A-site by interactions between the ribosome and the Shine–Dalgarno sequence GGAGGA and between the initiator tRNA and AUG. aa-tRNAs, free ribosomes, mRNA-programmed ribosomes, and ternary complexes of EFTu·[γ-^{32}P]GTP·[^{3}H]aa-tRNAs were prepared and assayed according to Thompson and Dix (15) and Thomas *et al.* (8).

Kinetic studies of the reaction between ternary complexes and mRNA-programmed ribosomes involving C/UUC-programmed ribosomes were conducted at 5°C in the rapid-mixing apparatus described by Eccleston *et al.* (16). Five microliters of ternary complex was added to 15 μl of ribosome complex, and the reaction was stopped 2 sec–15 min later by adding 10 μl of EDTA (500 mM). Reactions involving C/UUU-programmed ribosomes were done by hand. Thirty-two and a half microliters of ternary complex was added to 97.5 μl of ribosome complex, and the reaction was stopped by withdrawing 10 μl between 10 sec and 80 min and adding 20 μl of EDTA (500 mM). Concentrations of ribosome complex were typically between 0.3 and 0.7 μM, and the concentrations of ternary complex were between 0.03–0.07 μM. Unless otherwise stated all reactions occurred at 5°C in 50 mM Tris·HCl, pH 7.2/65 mM KCl/50 mM NH_4Cl/1 mM dithiothreitol/5 mM $MgCl_2$ (final composition). Analysis of the reaction mixture has been described (15).

To calculate proofreading ratios (P_i/PT; where PT equals the extent of aa-tRNA incorporated into peptide), ribosome complexes were treated with excess ternary complex. Twenty-five microliters of ribosome complex ($\approx$0.3 μm) was mixed with 80 μl of ternary complex ($\approx$0.4 μM) at 5°C, and the reaction was stopped after 2 hr by adding 50 μl of EDTA (500 mM). Forty-five percent of the reaction mixture was then removed separately for analysis of GTP hydrolysis and peptide formation (15).

To block the A-site of UUC-programmed ribosomes and confirm that the GTPase reaction seen with near-cognate ternary complexes was due to discrimination at the A-site, we incubated 50 μl containing 0.7 μM UUC-programed ribosomes with 100 μl of buffer (50 mm Tris·HCl, pH 7.2/50 mM KCl/1 mM dithiothreitol/5 mM $MgCl_2$) or with 100 μl of buffer containing 0.9 μM EFTu·GTP·[^{3}H]Phe-tRNA for 5 min at 0°C. Fifteen-microliter aliquots of these reaction mixtures were mixed at 5°C with 35 μl of 0.25 μM EFTu·[γ-^{32}P]GTP·[^{3}H]Phe-tRNA complex to determine the fraction of A-sites blocked or with 5 μl of 0.25 μM EFTu·[γ-^{32}P]GTP·[^{3}H]Leu-$tRNA_2$ complex to measure their proofreading activity. When ribosome complexes were to be purified by ultracentrifugation, we incubated 50 μl containing 0.7 μM UUC-programmed ribosomes with 250 μl of buffer (50 mM Tris·HCl, pH 7.2/50 mM KCl/1 mM dithiothreitol/5 mM $MgCl_2$ or buffer containing 0.6 μM EFTu·GTP·[^{3}H]Phe-tRNA for 5 min at 0°C. These reaction mixtures were then purified by ultracentrifugation in 0.75-ml Eppendorf tubes, as described (17). The ribosome pellets were resuspended in 100 μl of the above buffer. Twenty-five microliters of these purified ribosomes were mixed with either 25 μl of 0.4 μM EFTu·[^{32}P]GTP·Phe-tRNA complex to determine the fraction of A-sites blocked, or with 25 μl of 0.3 μM EFTu·[^{32}P]GTP·Leu-$tRNA_2$ complex to determine their activity in proofreading.

EFTu·guanosine 5′-[γ-thio]triphosphate (GTPγS)·[^{3}H]Phe-tRNA containing either GTP[γ-^{35}S] or nonradioactive GTPγS was prepared and purified as described (18). In experiments to measure k_2, 30 μl of 0.3 μM phenylalanine ternary complex was added to 30 μl of either UUU- or UUC-programmed ribosomes (0.2 and 0.24 μM, respectively) at 5°C. In experiments to measure $k_2 + k_{-1}$, the reaction was started by mixing equal volumes of the phenylalanine ternary complex with either UUU- or UUC-ribosomes in a final volume of 30 μl at 5°C. After a 15-sec delay, a chase of 450 μl containing 15-fold excess of nonradioactive phenylalanine ternary complex was added. The reaction was stopped between 10 sec and 35 min by removing 5 μl (nonchase) or 40 μl (chase) and adding 40 μl of EDTA (500 mM). The extent of GTPγS hydrolysis was determined by analyzing the [^{35}S]thiophosphate formed as described (18). To determine k_1(GTPγS), 5 μl of UUC- or UUU-programmed ribosomes [0.2 and 0.15 μM, respectively] were mixed with 5 μl of 0.25 μM EFTu·GTPγS·[^{3}H]Phe-tRNA ternary complex at 5°C in the rapid-mixing apparatus. After times between 0.3 and 15 sec 20 μl of EFTu·GTPγS·[^{14}C]Phe-tRNA complex (0.47 μM) was added. To determine the extent of [^{3}H]Phe-tRNA bound to ribosomes in the initial incubation 24 μl of the mixture was removed and filtered through nitrocellulose (19) within 15 sec of the second addition of reagents.

RESULTS

Short mRNAs of defined sequence were synthesized as described (8) by *in vitro* transcription of plasmids containing synthetic DNA coding for a ribosome binding site, an initiation codon, and the codon of interest. Ribosomes were bound to these mRNAs in the presence of initiation factors and formylmethionyl tRNA (fMet-tRNA), and the resulting complexes were treated with ternary complexes of EFTu, [^{3}H]aa-tRNA, and [γ-^{32}P]GTP (8). Progress of the GTPase and peptidyl transferase reactions was followed by stopping the reaction between 2 sec and 80 min later and analyzing for P_i and peptidyl-tRNA.

Because the GTPase reaction of these ribosomes with near-cognate ternary complexes is quite slow we performed two experiments to ensure that this reaction does, in fact, represent mistranslation of the phenylalanine codon in the ribosomal A-site followed by proofreading. In the first experiment, ribosomes programmed with the codon UUC (0.13 μM) were treated with excess phenylalanine ternary complex containing nonradioactive GTP (0.3 μM) before addition of the Leu2 ternary complex containing [γ-^{32}P]GTP (0.08 μM). These ribosomes had only 4% normal free A-sites, as shown by ribosomal ability to hydrolyze only 4% of normal GTP from additional phenylalanine ternary complex (30% of control ribosomes hydrolyzed GTP) and hydrolyze the GTP of the Leu2 ternary complexes at only 5% of the rate of control of UUC-programmed ribosomes. In the second experiment we isolated the ribosomes by centrifugation after the initial reaction with phenylalanine ternary complex and before testing ribosomal ability to proofread Leu2 ternary complexes. During centrifugation we believe some ribosomes lost the Phe-tRNA bound to their ribosomal A-site, because their ability to react with new phenylalanine ternary complex increased to 30% that of control UUC-programmed ribosomes that had not been treated with phenylalanine ternary complexes but had been centrifuged. Interestingly, these blocked ribosomes hydrolyze the GTP of Leu2 ternary complexes at 35% the rate of control ribosomes. Thus, the ability of phenylalanine ternary complex to prevent the reaction

with Leu2 ternary complexes parallels its ability to block the A-site to reaction with more phenylalanine ternary complex—a good indication that the reaction with Leu2 complexes also occurs at the ribosomal A-site.

Accuracy of Translation of UUU and UUC. Most translation errors probably involve mistaken identification of a single codon base (20), and pyrimidine seems more likely to be mistaken for another pyrimidine than for a purine. Therefore, to compare translation accuracy of the 5′ base of the codons UUU and UUC we treated ribosomes programmed by these codons with a ternary complex containing Leu-tRNA$_2$, which read codons CUU and CUC. Both reactions require formation of a G·U mispair at the 5′ position of the codon–anticodon complex.

The apparent second-order rate constant for GTP hydrolysis by UUC-programmed ribosomes with the Leu2 ternary complex is 8-fold higher than that of UUU-programmed ribosomes (Table 1). The proofreading ratio of the UUC-programmed ribosomes is one-third that of their UUU-programmed relatives. From these results ribosomes programmed with UUC are apparently more prone to errors than those programmed with UUU—both because they react more readily with near-cognate ternary complexes and because a greater proportion of the ternary complexes that react incorporate an amino acid into peptide.

The 3′, or wobble, nucleotide of the codon appears apt to make a different error, in which a pyrimidine is mistaken for a purine. Indeed, this causes the translational error characterized by Parker and his colleagues (9) as among the most frequent in *E. coli*. To compare the translation accuracy of the 3′ base in UUU and UUC, we challenged ribosomes programmed with these codons with ternary complexes of Leu-tRNA$_5$ and Leu-tRNA$_4$, which normally read UUA and UUG codons, respectively (22, 23) (for results, see Table 1).

Ribosomes programmed with UUC are again the most prone to mistranslation during initial recognition. The k_{GTP} values for the reaction of these ribosomes with ternary complexes of Leu-tRNA$_4$ and Leu-tRNA$_5$ are, respectively, 2-fold and 17-fold higher than the analogous rate constants for ribosomes programmed with UUU. Ribosomes programmed with UUC are also less able to proofread Leu-tRNA$_5$ because their proofreading ratio is one-half that of UUU-programmed ribosomes. However, ribosomes programmed with the UUU codon are more likely than ribosomes programmed with a UUC codon to err during proofreading of Leu-tRNA$_4$. This exception to the rule that ribosomes programmed with UUC are less accurate than those programmed with UUU is discussed below.

Accuracy of Translation of CUU and CUC. These results indicate that during recognition of a synonymous pair of codons differing only in having uridine or cytidine as their 3′ base, the codon with a 3′ cytidine is more apt to be mistranslated. To explore the generality of this conclusion, we conducted a similar series of experiments to determine which of the two leucine codons, CUU or CUC, is more likely to be mistranslated.

The translation accuracy of the 5′ base of these codons was determined by treating programmed ribosomes with phenylalanine ternary complexes, which normally read UUU and UUC codons. In both cases the error involves the formation of a C·A pair at the 5′ position of the codon–anticodon complex. As judged by the apparent second-order rate constant for GTP hydrolysis, ribosomes programmed with CUC make errors of initial recognition more often than do ribosomes programmed with CUU. k_{GTP} for the reaction of CUC-programmed ribosomes with the near-cognate phenylalanine ternary complex is 8-fold greater than k_{GTP} for CUU-programmed ribosomes (Table 2). Also, as judged by their proofreading ratio, CUC-programmed ribosomes are less accurate than CUU-programmed ribosomes in discriminating against Phe-tRNA (Table 2).

The accuracy of translation of the wobble base of the codons CUU and CUC was determined by the reaction of programmed ribosomes with ternary complexes of Leu-tRNA$_1$. Although technically not a translational error because the correct amino acid is incorporated, this reaction shows all the characteristics of a translational error in that a nonstandard base pair (U·C or C·C) must be formed by the 3′ nucleotide of the codon. As such, it compares interestingly with the true misreading of UUU and UUC codons by tRNAs (tRNA$_4^{Leu}$ and tRNA$_5^{Leu}$) that also require nonstandard base pairs.

When the cognate, but mispaired, Leu1 ternary complex misreads the 3′ base of the codon, k_{GTP} for CUC-programmed ribosomes is 4-fold greater than the k_{GTP} of CUU-programmed ribosomes. However, in a second exception to the rule that codons terminating in cytidine are less accurate than their synonyms ending in uridine, CUC-programmed ribosomes have a proofreading ratio with Leu-tRNA$_1$ ternary complexes twice that of CUU-programmed ribosomes (see below).

Elementary Rate Constants k_1 and k_{-1} differ for UUC and UUU-Programmed Ribosomes. Ribosomes programmed with UUC generally react faster with both cognate (8) and with near-cognate ternary complexes (Table 1). The apparent rate constant k_{GTP} for both reactions is a composite of three elementary rate constants k_1, k_{-1}, and k_2 (Fig. 1). To determine which rate constant contributes to the increased reactivity of UUC-programmed ribosomes, we measured the elementary rate constants k_1, k_{-1}, and k_2 for the reaction of UUC- and UUU-programmed ribosomes with ternary complexes of Phe-tRNA, EFTu, and the GTP analog, GTPγS. The use of the GTPγS for GTP slows cleavage of the pyrophosphate bond and allows k_2 to be directly determined. At the same time, stabilization of the ribosome–ternary complex complex to hydrolysis allows k_{-1} to be determined from the rate at which a ternary complex dissociates from the ribosome (18).

Table 1. Rate constants and proofreading ratios for the reaction of UUU/C-programmed ribosomes with near-cognate ternary complexes

Codon	aa-tRNA	Anti-codon	[Mg^{2+}], mM	T, °C	k_{GTP} × 10^{-4}·M^{-1}·sec^{-1}	P_i/PT
UUU	Leu2	GAG	5	5	0.7	271
UUC	Leu2	GAG	5	5	5.1	94
UUU	Leu2	GAG	5	25	17	ND
UUC	Leu2	GAG	5	25	100	ND
UUU	Leu2	GAG	10	5	1.1	ND
UUC	Leu2	GAG	10	5	7.5	ND
UUU	Leu4	NAA	5	5	2.5	97
UUC	Leu4	NAA	5	5	5.5	159
UUU	Leu5	NAA	5	5	1.0	55
UUC	Leu5	NAA	5	5	20	28

P_i, extent of GTP hydrolysis; PT, extent of aa-tRNA incorporated into peptide; N (in NAA), unidentified nucleotide (21); T, temperature.

Table 2. Rate constants and proofreading ratios for the reaction of CUU/C-programmed ribosomes with near-cognate ternary complexes

Codon	aa-tRNA	Anti-codon	[Mg^{2+}], mM	T, °C	k_{GTP} × 10^{-4}·M^{-1}·sec^{-1}	P_i/PT
CUU	Phe	GAA	5	5	0.7	95
CUC	Phe	GAA	5	5	5.5	60
CUU	Leu1	CAG	5	5	4.0	65
CUC	Leu1	CAG	5	5	15.0	130

PT, extent of aa-tRNA incorporated into peptide.

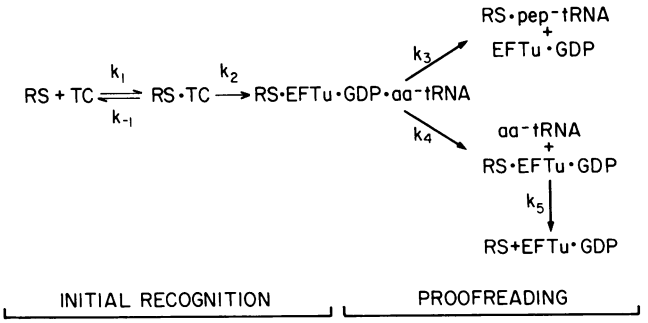

FIG. 1. Mechanism for aa-tRNA binding to ribosomes. RS, ribosomes programmed with mRNA and with fMet-tRNA in the P-site. TC, ternary complex of EFTu·GTP·aa-tRNA.

We found the rates of GTPγS cleavage by UUU- and UUC-programmed ribosomes virtually indistinguishable, indicating that differences in k_2 do not explain the different reactivities of these two ribosome sets (Table 3). Instead, the different reactivities appear to be associated with the noncovalent steps of the reaction: the Phe-tRNA·EFTu·GTPγS ternary complex binds (k_1) 1.7-fold faster to UUC-programmed ribosomes and dissociates (k_{-1}) 1.8-fold slower from these ribosomes (Table 3).

DISCUSSION

Misreading of the Codon 5′ Base. The data reported in Tables 1 and 2 indicate that near-cognate ternary complexes that misread the 5′ base of the codon react more rapidly with ribosomes programmed with codons terminating in cytidine than with ribosomes programmed by codons terminating in uridine. Data reported previously showed that cognate ternary complexes also react faster with ribosomes programmed by codons terminating in cytidine (8). These results imply that the strength of cognate and near-cognate codon–anticodon interactions in the ribosome–ternary complex complex is enhanced by a C·G base pair at the wobble position. Near-cognate aa-tRNAs that misread the 5′ base of the codon are also less likely to be eliminated by proofreading on ribosomes programmed by codons terminating in cytidine than on ribosomes programmed by codons terminating in uridine. This result implies that the stabilizing effect of a C·G pair over a U·G pair is also apparent in the RS·aa-tRNA·EFTu·GDP complex that precedes proofreading. That this effect is found in both cognate and near-cognate pairs and in the initial recognition and pre-proofreading complexes argues that it reflects a structural feature of the ribosome–codon complex independent of the precise nature of the tRNA. As discussed (8), this property may not even require the ribosome because Labuda and co-workers (24, 25) have shown that cognate trinucleotides ending in cytidine and uridine differ in their ability to bind and induce conformational changes in tRNAs.

The codon-related differences associated with reaction of a near-cognate ternary complex are generally greater than those associated with reaction of a cognate ternary complex. An analysis of the individual rate constants for the reaction between ribosomes and ternary complexes shows that this is due to the C·G base pair reducing the value of k_{-1} as well as increasing the value of k_1. The codon that performs most efficiently in initial recognition is, therefore, the codon that is translated least accurately. This finding represents a further example of the inverse correlation, noted earlier (15, 26), between efficiency and accuracy in translation.

Choice of the most efficient, rather than the most accurate, codon in highly expressed mRNAs indicates that for the UUU/C codon pair maximization of translational accuracy has not determined codon choice. In contrast, Parker's results indicate that maximization of accuracy does play a role in codon choice at the AAU/C codon pair. If, as seems possible, AAU is the least efficient of these two codons, some codon biases would reflect optimization for accuracy, whereas other codon biases reflect optimization for speed. Why different factors determine codon choice at different positions in the mRNA is not clear.

Misreading of the 3′ Base of the Codon. Near-cognate ternary complexes that misread the 3′ base of the codon also show a higher rate of GTP hydrolysis with ribosomes programmed with codons terminating in cytidine. In at least one case (Leu1 ternary complexes reacting with CUU- and CUC-programmed ribosomes) the higher rate of GTP hydrolysis associates with a C·C rather than a U·C base pair in the wobble position. However, in proofreading, no simple relationship exists between the type of mispairing and complex stability, for, in two cases, it is the ribosome programmed by a uridine-terminating codon that is least accurate in proofreading. For the Leu1 ternary complex reacting with CUU- and CUC-programmed ribosomes, a U·C pair seems to stabilize the complex more than C·C. This reversal of specificity between initial recognition and proofreading probably indicates the existence of a subtle difference between the codon–anticodon interactions in the RS·aa-tRNA·EFTu·GTP and RS·aa-tRNA·EFTu·GDP complexes.

Comparison of the misreading of UUU- and UUC-programmed ribosomes by Leu4 ternary complex, and of CUU- and CUC-programmed ribosomes by Leu1 ternary complex is instructive in that the former event will lead to an error in translation, whereas the latter will not. Previous results have suggested that the wobble nucleotide plays a reduced role in the codon–anticodon interaction when the first two nucleotides of the codon suffice to specify the appropriate amino acid (27). However, we find that wobble base recognition is just as strong in the latter case as in the former. Ribosomes programmed with CUU and CUC discriminate as strongly against $tRNA_1^{Leu}$ as ribosomes programmed with UUU and UUC discriminate against $tRNA_4^{Leu}$ and $tRNA_5^{Leu}$. Clearly all three bases, and not two of three (28), determine the specificity of translation in both cases.

It is worth noting that the translational error levels seen with ribosomes programmed with these synthetic mRNAs are much lower than those seen previously with ribosomes programmed with poly(U) (13–15). However, a direct comparison between these error frequencies is difficult because, in addition to the mRNA differences, the earlier experiments used ribosomes that had been initiated with high Mg^{2+} concentrations and acetyl Phe-tRNA rather than initiation factors and fMet-tRNA. Part of the decreased accuracy of the poly(U)-programmed ribosomes comes from the high [Mg^{2+}] method of programming because (unpublished work) we have demonstrated that poly(U)-programmed ribosomes initiated using initiation factors and acetyl Phe-tRNA have error levels 4-fold lower than poly(U)-programmed ribosomes initiated at high [Mg^{2+}]. However, their error rate is still 12-fold greater than ribosomes initiated on the UUU-containing synthetic mRNA with initiation factors. We conclude that either the context of the codon or the species of peptidyl-tRNA in the

Table 3. Rate constants for the reaction of UUU/C-programmed ribosomes with EFTu·GTPγS·Phe-tRNA complexes

Codon	aa-tRNA	GTPγS k_2, sec^{-1}	GTPγS k_{-1}, sec^{-1}	GTPγS k_1, $\times 10^{-6} \cdot M^{-1} \cdot sec^{-1}$	k_{GTP}* $\times 10^{-6}$, $M^{-1} \cdot sec^{-1}$
UUU	Phe	0.0039	0.0150	2.9	2.8
UUC	Phe	0.0037	0.0085	5.4	4.5

*From ref. 8.

ribosomal P-site can greatly influence accuracy of translation.

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1. Post, L. E., Strycharz, G. D., Nomura, M., Lewis, H. & Dennis, P. P. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1697–1701.
2. Bennetzen, J. L. & Hall, B. D. (1982) *J. Biol. Chem.* **257,** 3026–3031.
3. Grosjean, H. & Fiers, W. (1982) *Gene* **18,** 199–209.
4. de Boer, H. A. & Kastelein, R. A. (1986) in *Maximizing Gene Expression*, eds. Reznikoff, W. & Gold, L. (Butterworth, Stoneham, MA), pp. 225–285.
5. Pederson, S. (1984) *EMBO J.* **3,** 2895–2898.
6. Varenne, S., Knibiehler, M., Cavard, D., Morlon, J. & Lazdunski, C. (1982) *J. Mol. Biol.* **159,** 57–70.
7. Ikemura, T. (1981) *J. Mol. Biol.* **146,** 1–21.
8. Thomas, L. K., Dix, D. B. & Thompson, R. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4242–4246.
9. Parker, J., Johnston, T. C. Borgia, P. T., Holtz, G., Remaut, E. & Fiers, W. (1983) *J. Biol. Chem.* **258,** 10007–10012.
10. Parker, J. & Precup, J. (1986) *Mol. Gen. Genet.* **204,** 70–74.
11. Hopfield, J. J. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4135–4139.
12. Thompson, R. C. & Stone, P. J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 198–202.
13. Thompson, R. C., Dix, D. B., Gerson, R. B. & Karim, A. M. (1981) *J. Biol. Chem.* **256,** 81–86.
14. Ruusala, T., Ehrenberg, M. & Kurland, C. G. (1982) *EMBO J.* **1,** 741–745.
15. Thompson, R. C. & Dix, D. B. (1982) *J. Biol. Chem.* **257,** 6677–6682.
16. Eccleston, J. F., Messerschmidt, R. G. & Yates, D. W. (1980) *Anal. Biochem.* **106,** 73–77.
17. Dix, D. B., Wittenberg, W. L., Uhlenbeck, O. C. & Thompson, R. C. (1986) *J. Biol. Chem.* **261,** 10112–10118.
18. Karim, A. M. & Thompson, R. C. (1986) *J. Biol. Chem.* **261,** 3238–3243.
19. Nirenberg, M. & Leder, P. (1964) *Science* **145,** 1399–1407.
20. Davies, J., Jones, D. S. & Khorana, H. G. (1966) *J. Mol. Chem.* **18,** 48–57.
21. Yanaizumi, Z., Kuchino, Y., Harada, F., Nishimura, S., & McCloskey, J. A. (1980) *J. Biol. Chem.* **255,** 2220–2225.
22. Blank, H. U. & Soll, D. (1971) *J. Biol. Chem.* **246.** 4947–4950.
23. Natale, P. O. & Elilat, D. (1976) *Nucleic Acids Res.* **3,** 917–931.
24. Labuda, D. & Porschke, D. (1983) *J. Mol. Biol.* **167,** 205–209.
25. Labuda, D., Striker, G. & Porschke, D. (1984) *J. Mol. Biol.* **174,** 587–604.
26. Thompson, R. C. & Karim, A. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4922–4926.
27. Parker, J. & Friesen, J. D. (1980) *Mol. Gen. Genet.* **177,** 439–445.
28. Lagerkvist, U. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1759–1762.
29. Curran, J. F. & Yarus, M. (1989) *J. Mol. Biol.* **209,** in press.