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Effects of the Nucleotide 3′ to an Amber Codon on Ribosomal Selection Rates of Suppressor tRNA and Release Factor-1

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Rates of ribosomal selection of both release factor 1 (RF1) and a suppressor tRNA (Su7C33) were studied at an amber codon at which the 3′ neighbor was permuted. Rates of RF1 selection vary 2·6-fold among contexts. The 3′ neighbor-dependent variation of RF1 action correlates very strongly with the non-random frequencies of 3′ neighbors at UAG terminators ($r = 0{\cdot}97$), which argues that the rate of RF1 selection is an important determinant 3′ neighbor choice at termination codons. The data are consistent with a model for RF1 selection in which RF1 makes a specific contact(s) to the 3′ neighbor and that this interaction is most favorable to uridylic acid.

Measured rates of Su7C33 selection vary fivefold among 3′ contexts. We also develop a method to calculate rates of selection for other suppressors, based on the assumption that rates of RF1 selection at each 3′ context can be generalized to other sites that have the same 3′ neighbor. Rates for various suppressors appear to vary from two- to fivefold depending on the 3′ neighbor. Generally, the rate of selection of suppressors at different contexts correlates with the stacking strength of the 3′ neighbor as measured *in vitro*.

The two- to fivefold range of 3′ neighbor effects on rate of aminoacyl-tRNA selection is greater than that previously observed within sets of codons read by the same tRNA. It is suggested that the choice of codons to achieve favorable contexts may be more important than the choice of a common codon at some message sites.

Keywords: context effects; translational rate; release factor; amber suppression; translational efficiency

1. Introduction

The message sequence surrounding a codon, its context, can affect the accuracy and efficiency of translation. For example, the positions of amber (Bossi & Roth, 1980; Bossi, 1983; Miller & Albertini, 1983) and missense (Murgola *et al.*, 1984) mutations within genes affects the probability of translation by suppressor tRNAs. Misreading of specific codons by normal tRNAs is also dependent on the positions of codons within genes (Parker & Precup, 1986; Rice *et al.*, 1986; Precup & Parker, 1987; Precup *et al.*, 1989). Observations that codons are found in non-random contexts in *Escherichia coli* genes and that patterns differ between highly and weakly expressed genes have led to the suggestion that context contributes to the level of gene expression (Yarus & Folley, 1985). In support of that idea is the observation that an artificial sequence in which eight codon contexts were chosen to represent preferences found in weakly expressed genes can reduce expression of a *lacZ* reporter gene by about 30% (Folley & Yarus, 1989). However, the mechanisms by which context affects the decoding process are not known.

The most extensive data on context effects come from studies of amber (UAG) suppression. Amber suppression efficiencies by each of four suppressor tRNAs vary over wide ranges among 42 amber sites in *lacI-Z* fusions (Miller & Albertini, 1983; Bossi, 1983). This variation was examined for systematic effects of the identities of neighboring nucleotides on suppression efficiency (Stormo *et al.*, 1986). The most significant nearby nucleotide is the one 3′ to UAG such that suppression efficiency is greatest at UAGG ≥ UAGA > UAGC > UAGU (Stormo *et al.*, 1986). Those observations suggest direct effects of the 3′ base on suppression efficiency, but cannot identify specific mechanisms. Because amber suppression must depend on the outcome of two

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mutually exclusive reactions, termination by release factor 1 (RF1†) and translation by an amber suppressor (Yarus *et al.*, 1986), the observed context-dependent pattern may be due to a complex combination of separate effects on these two factors. Here, we measure the effect of the 3′ neighbor nucleotide on rates of RF1 and suppressor tRNA selection at an amber codon to determine how changes in rates of factor action contribute to the context dependence of suppression efficiency.

2. Materials and Methods

(a) *Strains and plasmids*

All *lacZ* determinations were performed in MY649 (Δ*lac proXIII, ara, thi, recA56*; Curran & Yarus 1986). Permutations of the nucleotide following UAG for assays of rates of RF1 and suppressor tRNA selection were achieved either by site-directed mutagenesis of plac/RF-UAG (Curran & Yarus, 1988) using a described method (Curran & Yarus, 1986) or by cloning complementary mutagenic oligonucleotides between *Bam*HI and *Hin*dIII sites of pJC27 (Curran & Yarus, 1986). All mutants were confirmed by DNA sequencing as described (Curran & Yarus, 1988). Suppression efficiencies were measured with *lacZ* alleles encoded on plasmids described by Curran & Yarus (1987). Assayed strains carried either pRT33C, a tetracycline resistance plasmid that expresses the amber suppressor tRNA Su7-33C (Thompson *et al.*, 1982) or pOP203, the isogenic expression vector lacking a cloned suppressor gene (Fuller, 1982). Both plasmids are compatible with the chloramphenicol resistance plasmids encoding the various *RF-lacZ* fusions. Construction of pRPOLAC.1 is described in Fig. 1.

(b) lacZ *output determinations*

The assay is a modified version of that described (Curran & Yarus, 1986). β-Galactosidase units were determined as described (Curran & Yarus, 1987) and normalized to *lacZ* message levels, which are known to vary among *RF-lacZ* alleles due to polarity (Curran & Yarus, 1989). *lacZ* message levels were determined by an S_1 nuclease protection assay instead of a dot blot method used previously (Curran & Yarus, 1989). The S_1 protection assay is an improvement over dot blotting because it utilizes an internal standard for hybridizations and because it utilizes fewer steps that are sensitive to RNase contamination. The internal standard hybridizes to *rpoB* message, which is constant over a wide range of metabolic states (Dennis, 1977). The probe is a fusion fragment of *rpoB* to *lacZ* and is encoded within pRPOLAC.1 (Fig. 1). The polymerase chain reaction (PCR) (described below) is used to amplify and label a *lacZ-rpoB* fusion fragment for use as probe. Labeling the fragment by PCR ensures that the specific activity of the 2 probes within the fragment are constant among experiments. Total cellular RNA was prepared for probing by pelleting 1·5 ml portions from midlog cultures in a microfuge and lysing the pellets in 0·3 ml of a guanidinium isothiocyanate solution prepared as described by Maniatis *et al.* (1982). Lysates were extracted 3 times with phenol : chloroform (1 : 1) at 65°C to remove protein and DNA. Then probe was added in molar excess to total cellular RNA and the nucleic acids were then precipitated with ethanol. The pellet was resuspended, hybridized and treated with S_1 to digest unreactive probe as described by Curran & Stewart (1985). Hybridization products were separated by electrophoresis through a denaturing polyacrylamide gel. The radioactivity in the probe bands was determined by scintillation counting and 50 cts/min background was subtracted routinely from the raw data. Data in Tables 1 and 2 are averaged ratios (±S.E.M.) of *lacZ* cts/min to *rpoB* cts/min.

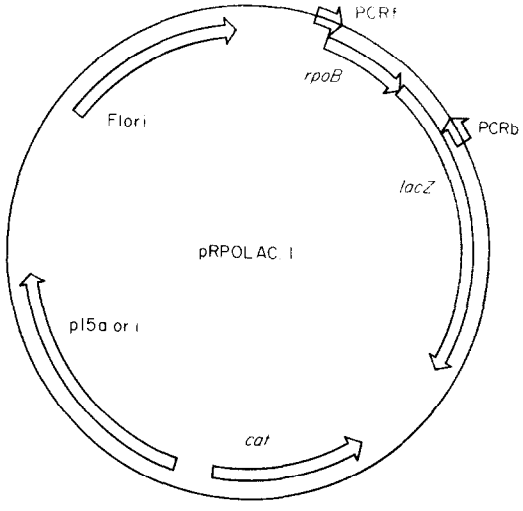

Figure 1. Construction of pRPOLAC.1. pRPOLAC.1 contains an internal fragment of *rpoB* fused to the distal third of *lacZ* and was constructed to provide a convenient source of the fusion fragment for probing the mRNA from those 2 genes. pRPOLAC.1 was constructed from pJC27 (Curran & Yarus, 1986) and a fragment from nucleotides 1009 to 1387 of *rpoB* (Post *et al.*, 1979), which was amplified from the *E. coli* chromosome by PCR. The PCR primers contained restriction sites for *Hin*dIII (5′ promoter proximal) and *Sac*I (promoter distal) for cloning into pJC27 at the *Hin*dIII site in the polylinker and the *Sac*I site at nucleotide 1979 of *lacZ*. The construction fuses 379 base-pairs of *rpoB* to the distal third of *lacZ*:

Promoter proximal *rpoB* PCR primer:
5′-CCAAGCTTCACCAACGATCTGGA-3′

Promoter distal *rpoB* PCR primer:
5′-GGAGCTCGGTTTTCCGCCATTTCG-3′

For labeling a fusion fragment for use as a hybridization probe, we use 2 PCR primers. PCRf primes synthesis in the clockwise direction and hybridizes beginning 23 nucleotides upstream from the *Hin*dIII site. PCRb primes counterclockwise synthesis and hybridizes from the point 204 nucleotides downstream from the *Sac*I site in the distal 3rd of *lacZ* (from nucleotide 2183 of *lacZ*):

PCRf: 5′-CAGCTATGACCATGATTACG-3′

PCRb: 5′-TGCTGCCAGGCGCTGATGTG-3′

(c) *Polymerase chain reactions*

PCR is carried out essentially as recommended by Perkin-Elmer, our source of heat-stable DNA polymerase. Reaction volumes were either 25 μl or 100 μl of aqueous solution under mineral oil. Reactions contained 0·1 μg template DNA/ml, 1 μM of each primer, 50 units amplitaq

† Abbreviations used: RF1, release factor 1; PCR, polymerase chain reaction.

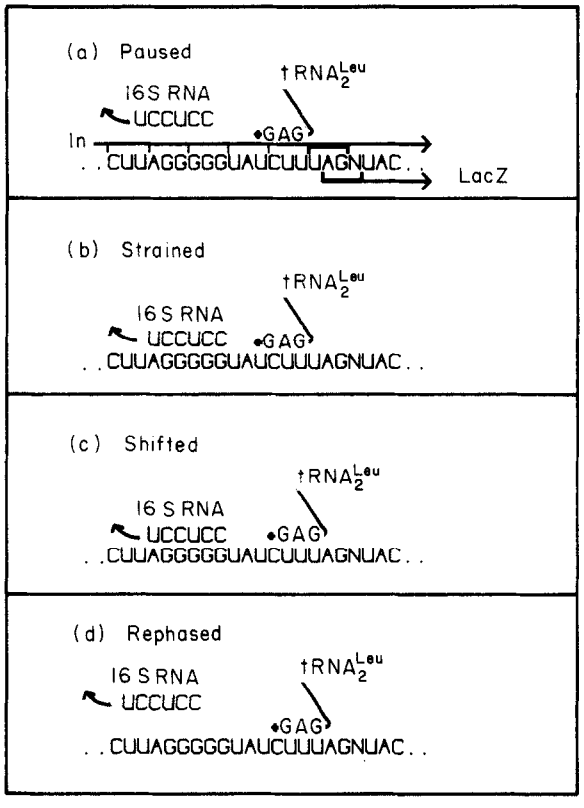

Figure 2. Frameshifting competes with translation of the amber codon. (a) $tRNA_2^{Leu}$ is paired to the CUU codon in the P site, while the UAG in the A site is unoccupied. At this point, the ribosome can frameshift as described in (b), (c) and (d). Alternatively, translation can be terminated by RF1 and, in Su^+ cells, the UAG may instead be translated by the suppressor, forcing translation to continue in an unproductive reading frame. Of these 3 reactions, frameshifting, termination and suppression, only frameshifting results in β-galactosidase synthesis. Because these reactions compete, the frequency of frameshifting depends on the relative rates of these reactions as described in Experimental Rationale. (b) The frameshift occurs when the Shine Dalgarno complement of 16 S rRNA, CCUCCU, base-pairs to the run of purines in the message. The short spacing between the Shine–Dalgarno interaction and the P site tRNA strains the message : ribosomal complex. (c) To relieve the strain, the P site tRNA shifts 1 nucleotide 3′ and then pairs to UUU. (d) Following the slippage, normal translation begins when the Shine–Dalgarno site is released. This mechanism has been described previously to explain frameshifting in alleles that have other codons in place of the UAG (Curran & Yarus, 1988, 1989).

polymerase (Perkin-Elmer)/ml. The reaction buffer contains 10 mM-Tris·HCl (pH 8·1), 50 mM-KCl, 1·5 mM $MgCl_2$, 0·001% gelatin, and if the amplified fragment is not to be ^{32}P-labeled each dNTP is used at 60 μM. For labeling reactions, the concentration of cold dCTP is reduced to 15 μM and [α-^{32}P]dCTP (3000 Ci/mmol) is used at 1·5 μM. Reactions are initiated by heating at 95 °C for 5 min and then cycled 25 to 30 times through incubations of 2 min duration at each temperature, 55 °C, 74 °C and 95 °C. Reactions are terminated after the 74 °C step of the last cycle, extracted twice with chloroform, precipitated with ethanol and resuspended in 10 mM-Tris·HCl, 1 mM-EDTA (pH 7·5).

3. Experimental Rationale

(a) *The basis of the assay for rate of RF1 and aminoacyl-tRNA selection*

We used the kinetic competition method of Curran & Yarus (1988, 1989) to measure, *in vivo*, the context dependence of rates of RF1 and tRNA action at an amber triplet. The basis of the assay is that, at an amber codon in *RF2-lacZ* fusions, ribosomes may either frameshift or participate in triplet translation (Fig. 2). In Su^- cells, triplet translation is by RF1, which terminates protein synthesis at UAG. In Su^+ cells, triplet translation can be by either RF1 or the suppressor tRNA. These fusions are constructed such that frameshifting is required for β-galactosidase synthesis. Therefore, the fraction of ribosomes that frameshift can be determined by measuring *lacZ* output.

Because frameshifting, termination and amber suppression are competing reactions at the amber codon, the fraction of ribosomes that frameshift (and therefore *lacZ* output) is dependent on the rates of these reactions. Below, we derive equations for calculating the rates of RF1 and suppressor tRNA action, relative to the rate of the frameshift, from measurements of *lacZ* output.

(b) *Measurements of* lacZ *output*

lacZ output is estimated from measurements of both β-galactosidase and *lacZ* mRNA level. *lacZ* output is taken as β-galactosidase units per unit *lacZ* message. The β-galactosidase assay is modified from that of Miller (1972) and has been described (Curran & Yarus, 1987). The measurements of *lacZ* message level are necessary because it can vary over a tenfold range among our set of *lacZ-RF* fusions (our unpublished results). These variations are probably due to polar effects triggered by termination of ribosomes that do not frameshift (Curran & Yarus, 1988). The RNA polymerase is more likely to terminate prematurely when not coupled to translating ribosomes (Platt, 1986).

lacZ mRNA levels are estimated from S_1 nuclease protection assays (see Materials and Methods). Messages from *lacZ* and *rpoB* are measured in the same assay and *lacZ* message is reported relative to the *rpoB* message, which has been shown to be a constant fraction of cellular RNA in various growth conditions (Dennis, 1977). Any physical measure of message level has the inherent uncertainty that chemically detected RNAs may not be functional. However, it seems reasonable that the levels of segments distal to the sites responsible for the polar effects are representative of levels of functional message. Therefore, we probed a segment of *lacZ* message that is about 2000 nucleotides downstream from the frameshift site (see the legend to Fig. 1) and over 1500 nucleotides distal to transcriptional terminators (Ruteshauser & Richardson, 1989) that are likely to be the sites of implementation of polar effects. Because it is far distal to those sites, we

assume that the level of the probed segment is a serviceable marker for functional *lacZ* message.

(c) *Formulae for calculations of rates of RF1 and suppressor tRNA selection, relative to the rate of the high-frequency frameshift, from* lacZ *output*

The relative rates of RF1 at UAG in each context are calculated from measurements of *lacZ* output in cells that do not contain an amber suppressor gene. At the in-phase amber codon in those cells, ribosomes may either frameshift or undergo RF1-catalyzed termination (Fig. 2). The fraction of ribosomes that frameshift (F) depends on the rates of RF1 selection and the rate of the shift such that:

$$F = \frac{R_s}{R_s + R_r}, \quad (1)$$

where R_s is the rate of the frameshift and R_r is the rate of RF1 (these formulae were first derived by Curran & Yarus (1988); however, there is an error in the unnumbered equation following equation (4) of that paper, and we reproduce the correct formulae here).

lacZ output depends on frameshifting; ribosomes that terminate (or undergo suppression in Su^+ cells) do not generate the β-galactosidase polypeptide. If we assume that the translational initiation frequency is constant among strains and that the activity of the pseudowild allele represents total ribosomal activity, then the fraction of ribosomes that frameshift (F) for each context allele can be determined:

$$F = \frac{\text{Output of the } \textit{RF2-lacZ} \text{ fusion in Su}^- \text{ cells}}{\text{Output of the pseudowild-type allele}}. \quad (2)$$

To determine the rate of RF1 action, relative to the rate of the frameshift, we rearrange equation (1) and substitute equation (2) for F:

$$\frac{R_r}{R_s} = \frac{\text{Output of the pseudowild-type allele}}{\text{Output of the } \textit{RF2-lacZ} \text{ fusion in Su}^- \text{ cells}} - 1. \quad (3)$$

Relative rates of suppressor selection were calculated from *lacZ* output measured for cells expressing a cloned suppressor tRNA gene. In these cells, F is determined by the rates of three common reactions at the amber codon: frameshifting, termination and suppression (Fig. 2). We therefore modify equation (1) to account for the rate of Su7C33 (R_t):

$$F = \frac{R_s}{R_s + R_r + R_t}. \quad (4)$$

R_t/R_s is calculated from a rearrangement of equation (4) with a substitution of equation (2) (modified for Su^+ cells) for F:

$$\frac{R_t}{R_s} = \frac{\text{Output of a pseudowild-type allele}}{\text{Output of the } \textit{RF2-lacZ} \text{ fusion in Su}^+ \text{ cells}} - \frac{R_r}{R_s} - 1. \quad (5)$$

R_t/R_s is determined from assays with Su cells and equation (3). This assay and modified versions have been used to detect differences in rates of aminoacyl-tRNA selection at all sense codons that begin with a pyrimidine (Curran & Yarus, 1989), various suppressors at an amber codon (Curran & Yarus, 1988), and due to changes in $tRNA^{Trp}$ concentration at the tryptophan codon (Curran & Yarus, 1989; J.F.C. & T. Howerton, unpublished results).

4. Results

(a) *Frameshift rate is a reliable standard for measurements of the context dependence of rate of RF1 selection*

lacZ output measurements and calculated relative rates of RF1 selection for the UAGN context alleles are in Table 1A. The relative rate of RF1 selection at UAG varies 2·6 fold among context alleles, being highest at UAGU > UAGG > UAGC > UAGA.

The identity of the nucleotide 3′ to natural terminators is non-random (Kohli & Grosjean, 1981; Brown *et al.*, 1990). For the 55 *E. coli* genes known to end with UAG, 21 are followed by U, 13 by G, 13 by C and 8 by A (from a sample of 862 genes without regard to expression level; Brown *et al.*, 1990). To determine whether our rate determinations correlate with this usage bias, we performed a linear regression between context-varied relative rates of RF1 selection and those terminator frequencies. The two data sets correlate very strongly, with $r = 0{\cdot}97$ (Fig. 3).

This high correlation between the context-dependent relative rate of RF1 selection and 3′ base frequency at natural terminators argues that changes in relative rate of RF1 selection are not spurious effects on frameshift rate because it is unlikely that permutation of the base four nucleotides 3′ to the shift site would fortuitously alter frameshift kinetics to give this high correlation. Instead, the high correlation argues that the effects on measured relative rate of RF1 selection are effects on RF1 action.

(b) *Measurements of rates of Su7C33, relative to the shift* (R_t/R_s)

Relative rates of Su7C33 selection were measured in cells expressing that tRNA from the expression vector pRT33C (Thompson *et al.*, 1982). Su7C33 is a U → C33 derivative of Su7 (Thompson *et al.*, 1982) and was chosen for study because of its well-characterized suppression efficiency (Thompson *et al.*, 1982; Yarus *et al.*, 1986; Curran & Yarus, 1986). Suppression efficiency is thought to depend on the relative rates of suppressor and release factor selection. Therefore, we could predict that Su7C33 would have rates of selection similar to those of RF1. Because calculations of relative rates of suppressor selection requires a term for the relative rate of RF1 (see Experimental Rationale), calculations using

Table 1

lacZ *output measurements and calculated relative rates of RF1 and suppressor tRNA selection at each context*

Context allele	β-Gal. units	*lacZ* mRNA level	*lacZ* output	R_r/R_s
A. lacZ *output measurements in the Su⁻ strain and calculated* R_r/R_s				
UAGA	3468±89	1·5±0·13	2330±215	0·9±0·1
UAGG	2543±115	1·3±0·1	1947±150	1·3±0·1
UAGU	2716±66	2·0±0·2	1372±165	2·3±0·3
UAGC	5798±207	2·8±0·2	2078±139	1·1±0·1
Context allele	β-Gal. units	*lacZ* mRNA level	*lacZ* output	R_t/R_s
B. lacZ *output measurements in the Su⁺ strain and calculated* R_t/R_s				
UAGA	1505±101	1·8±0·13	821±81	3·5±0·5
UAGG	1607±61	1·5±0·1	1057±82	1·9±0·2
UAGU	1712±126	1·9±0·1	881±80	1·8±0·25
UAGC	4092±136	2·6±0·2	1558±123	0·7±0·1

Context allele shows the amber codon at the frameshift site and its 3′ neighbor (Fig. 2). (Su⁺) indicates that cells contain pRT33C (Thompson *et al.*, 1982), which encodes Su7C33; (Su⁻) indicates that the cells lack pRT33C. β-Gal. units are β-galactosidase units obtained as described (Curran & Yarus, 1987). The values presented here are the averages of at least 19 assays ± S.E.M. *lacZ* mRNA level is the average *lacZ* specific cts/min divided by that for *rpoB* ± S.E.M. for at least 8 hybridization assays for these specific mRNAs (Materials & Methods). *lacZ* output is β-galactosidase units divided by *lacZ* mRNA level (Experimental Rationale). R_r/R_s (see Table 3A) is the rate of RF1 selection relative to the rate of frameshift; R_t/R_s (see Table 3B) is the rate of aminoacyl-tRNA selection, relative to the rate of the frameshift. These values are calculated using eqn (3) and eqn (5) from Experimental Rationale, respectively. Output of the pJC27 pseudowild-type allele (Curran & Yarus, 1986) is 4479 and is the average of many assays in Su⁺ and Su⁻ strains. Because calculations of R_t/R_s include those from R_r/R_s, the former measurements are necessarily less well determined. Accordingly, error measurements on estimates of R_r/R_s are carried through calculations of R_t/R_s.

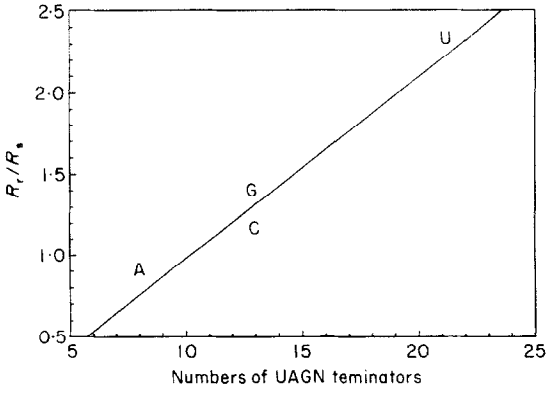

Figure 3. Relative rates of RF1 selection plotted *versus* UAGN terminator usage in *E. coli*. Relative rates of RF1 selection (R_r/R_s) values are from Table 1A. Of the 862 termination sites analyzed by Brown *et al.* (1990), there are 21 UAGU, 13 UAGG, 13 UAGC and 8 UAGA. The linear correlation coefficient (r) for this plot is 0·97.

factors with similar rates were expected to be less sensitive to measurement errors.

lacZ output measurements for each context allele and calculated rates of Su7C33 action at each context are listed in Table 1B. Relative rates of aminoacyl-tRNA selection for Su7C33 varies five-fold in the order UAGA > UAGG = UAGU > UAGC.

(c) *Measurements of the context dependence of suppression efficiency for Su7C33*

Suppression efficiencies for Su7C33 at each 3′ context were determined using two sets of four *lacZ* alleles (Curran & Yarus, 1987). In the first set, the glutamine codon (CAG) at position 366 is converted to UAG and the first nucleotide of codon 367 is permuted. This set was used to measure the context dependence of suppression efficiency. The second set has the wild-type CAG at codon 366, but is permuted at the first nucleotide in codon 367. This set was used to normalize for effects that the amino acid changes at position 367 apparently have on β-galactosidase activity (about 2·5-fold among alleles, Table 2). The nucleotides that flank UAGN (and CAGN) are identical with those in the constructs used for the rate measurements partially to control context effects by other message nucleotides. We also measured *lacZ* message levels of the various alleles to normalize differential polar effects.

The suppression efficiencies vary over a twofold range in the order UAGA = UAGG > UAGC > UAGU (Table 2). This order is the same pattern as is observed for four other suppressors studied by Bossi (1983) and Miller & Albertini (1983).

Table 2

lacZ *output measurements and* SE *for Su7C33*

Context allele pairs	β-Gal. units	*lacZ* mRNA level	*lacZ* output	Suppression efficiency
UAG A	3044±168	4·0±0·2	754±54	0·33±0·03
CAG A	10,053±259	4·4±0·14	2302±91	
UAG G	5304±329	4·4±0·5	1205±155	0·32±0·05
CAG G	14,652±771	3·8±0·4	3825±409	
UAG U	930±59	3·2±0·25	288±29	0·15±0·02
CAG U	5177±237	2·7±0·14	1920±131	
UAG C	1172±48	3·5±0·3	338±35	0·21±0·03
CAG C	6002±392	3·8±0·3	1593±162	

Context allele pairs are the amber site and the corresponding pseudowild-type control for suppression efficiency determinations. The amber codon is at codon 366 of *lacZ*; the sequence of the amber codon and flanking triplets is: GGU UAG NUC. Suppression efficiency is the fraction of UAG N allele activity, relative to the corresponding CAG N control allele. All other columns are defined in the legend to Table 1. All output determinations include a minimum of 5 β-galactosidase and *lacZ* message level measurements.

5. Discussion

We have estimated effects of 3′ message context on the rates of RF1 and aminoacyl-tRNA selection for an amber suppressor at an amber codon *in vivo*. Rates of RF1 and aminoacyl-tRNA selection are measured relative to the rate of a high-frequency frameshift from *E. coli* RF2 (see Experimental Rationale; Craigen *et al.*, 1985; Weiss *et al.*, 1987, 1988; Curran & Yarus, 1988). Rates of selection of both RF1 and a suppressor tRNA (Su7(33) vary several-fold depending on the 3′ neighbor.

(a) *The frameshift is a reliable standard for comparisons of rates of factor selection at the amber codon*

Use of the frameshift as a standard for comparison of rates assumes that the rate of frameshift is unaffected by the sequence changes at the 3′ neighbor of UAG. The frameshift occurs when the petidyl-tRNA paired to the CUU codon upstream from UAG dissociates and then binds at the overlapping triplet including the U of the UAG codon (UUU, Fig. 2). The frameshift kinetic parameters cannot be directly measured, but several arguments support the assumption of constancy among these alleles. First, using alleles in which the codon at the amber site was varied (Curran & Yarus, 1989), it was possible to compare directly rate constants calculated for five different codons using the frameshift as a standard for the rates measured using programmed ribosomes *in vitro* (Thomas *et al.*, 1988). A strong correlation between rates determined by the two methods argues strongly that the shift is a reliable standard for determining relative rates of aminoacyl-tRNA selection at codons (Curran & Yarus, 1989). In the same study, using a collection of alleles that have all 29 sense codons that begin with U or C in place of the amber codon, we showed that frameshift kinetics are not systematically correlated with the identities of the nucleotides that make up the codon at that position (Curran & Yarus, 1989). Because nucleotides within the codon whose first nucleotide participates directly in the frameshift are not critical to frameshift kinetics, our assumption that the next downstream nucleotide does not affect shift rate seems reasonable. Finally, the strong correlation between the rate of RF1 at different contexts and the frequency of natural UAGN termination sites (Fig. 3) also supports the idea that changes in frameshifting frequency are due to specific effects on translation at the amber codon and not to spurious effects on the frameshift.

We also assume that our alleles have a constant translational initiation frequency. This assumption is reasonable because the single-base mutations that distinguish the context alleles are at least 20 nucleotides downstream from the initiation footprint (40 nucleotides downstream from the AUG; initiation sites have been reviewed by Gold (1988)). Furthermore, in none of the context alleles is the site of the context mutations predicted to participate in secondary structures (unpublished results) that might inhibit initiation (DeSmit & Van Duin, 1990). Therefore, we expect that initiation frequency is essentially constant among these alleles.

(b) *Nucleotide 3′ to natural terminators may be selected for speed of release factor selection*

The high correlation (Fig. 3, linear $r > 0{\cdot}97$) between the relative rate of RF1 selection and the frequency of UAGN at natural termination sites supports the suggestion of Brown *et al.* (1990) that the non-random 3′ neighbor usage at natural terminators is due to an interaction between that nucleotide and release factors. We suggest that interactions that increase the rate are preferable, possibly because increased rates of release factor selection decrease the probability of readthrough by aminoacyl-tRNA.

(c) *RF1 may make specific contacts to the 3′ neighbor*

Interactions between RF1 and the 3′ nucleotide could conceivably be either indirect, for example by base stacking onto the codon, or could be direct contacts between RF1 and the 3′ nucleotide. The order of RF1 selection rates at UAGN is not explained by increased rates resulting from increased (or decreased) base stacking strength between the 3′ neighbor and the codon. Purines are expected to stack more strongly than pyrimidines (Saenger, 1984), but rates are not assorted by the heterocycle class of the 3′ neighbor.

We suggest that RF1 makes direct contacts to the fourth message nucleotide and that contacts are most favorable to uridylic acid, which causes UAGU to select RF1 about twofold faster than the other UAGN sites (Table 1A). Uridylate has an exocyclic keto group and an imino hydrogen atom at positions 4 and 3 (Saenger, 1984), respectively, which could participate in hydrogen bonding or van der Waals' contacts to RF1. These functional groups distinguish uridylic acid from the other nucleotides.

(d) *Use of relative rates of RF1 selection to calculate relative rates of selection of other amber suppressors as a function of 3′ nucleotide context*

Amber suppressors generally show similar patterns of context sensitivity (Bossi, 1983; Miller & Albertini, 1983; Stormo *et al.*, 1986), which suggests that the mechanisms of context effects are general. However, it has not previously been possible to distinguish between effects on RF1 and tRNA. If we assume that our observed 3′ nucleotide effects on RF1 selection can be generalized to other sites, then it is possible for the first time to factor out the contribution of RF1 to suppression efficiency. Using that assumption, we will estimate the rates of selection of the suppressors (Su1, Su2, Su3, Su6) studied by Miller & Albertini (1983) as a function of 3′

Table 3

Linear correlation coefficients between (1/SE) − 1 for five suppressors at all 3′ contexts and either $(R_r/R_s)/(R_t/R_s)$ *or* R_r/R_s

UAGN Group	Suppressor Su1	Su2	Su3	Su6	Su7C33
A. *(1/SE) − 1 at UAG sites, grouped by 3′ neighbor*					
UAGA	2·5 (29)	6·5 (13)	0·97 (51)	0·51 (66)	2 (33)
UAGG	3·5 (22)	7·9 (11)	1·6 (3·8)	0·81 (55)	1·13 (32)
UAGU	8·2 (11)	34·8 (3)	3·5 (22)	2·3 (30)	5·7 (15)
UAGC	7 (13)	38·2 (3)	3·4 (23)	1·2 (45)	3·8 (21)

Estimated R_t/R_s	Suppressor Su1	Su2	Su3	Su6	Su7C33
B. *Estimated relative rates of aminoacyl-tRNA selection for each suppressor at each 3′ context using equation (7)*					
UAGA	0·36	0·14	0·93	1·8	0·45
UAGG	0·37	0·16	0·81	1·6	1·2
UAGU	0·28	0·1	0·66	1	0·4
UAGC	0·16	0·03	0·35	0·9	0·29

For Su1, Su2, Su3 and Su6, average $(1/SE)-1$ are calculated as described in Discussion. $(1/SE)-1$ for Su7C33 was calculated from the suppression efficiency measured at a single site (Table 2). Numbers in parentheses are the average suppression efficiency (for clarity, represented as percentages rather than fractions) calculated from averaged $(1/SE)-1$.

We used eqn (7), the R_t/R_s data from Table 1A and the $(1/SE)-1$ data from A to estimate R_t/R_s for each suppressor as a function of the 3′ neighbor.

context for use in understanding the mechanism for those effects on suppressor selection.

It is predicted (1st equation in the Appendix of Yarus *et al.*, 1986) that suppression efficiency depends on the rates of RF1 and suppressor tRNA selection such that:

$$SE = \frac{R_t}{R_t + R_r}, \qquad (6)$$

where SE is suppression efficiency and R_t and R_r are the relative rates of aminoacyl-tRNA and RF1 selection, respectively. We rearrange equation (6) so that we can calculate the rates of aminoacyl-tRNA selection using Miller & Albertini's reported suppression efficiency data and our measured rates of RF1 selection:

$$R_t/R_s = \frac{R_r/R_s}{[(1/SE)-1)]}, \qquad (7)$$

where the factor R_s (rate of the frameshift) is included in the rate terms to denote that our rates for RF1 selection are relative to the rate of the shift. In this analysis, therefore, we assume that the rate of the shift is a reliable kinetic standard (see above).

For each amber suppressor, Miller & Albertini (1983) measured suppression efficiency at 42 sites in the *lacI* portion of *lacI-Z* fusions. For each suppressor, we averaged suppression efficiency data grouped by message sites according to the base 3′ to the amber codon. Because suppression efficiency is not linear with respect to rates of tRNA and RF1 selection (see eqn (6)), the suppression efficiency data were transformed to $(1/SE)-1$ prior to averaging within groups. If arithmetic averages of suppression efficiency are used in equation (7), the rates of aminoacyl-tRNA selection will be overestimated.

We use the averaged $(1/SE)-1$ values (Table 3A) and equation (7) to estimate the relative rate of aminoacyl-tRNA selection for each suppressor at each 3′ context (Table 3B). We also calculate relative rates for Su7C33 using the suppression efficiency data from Table 2. For all suppressors, the relative rate of aminoacyl-tRNA selection is lowest at the C context and highest at one or both of the purine contexts. The calculated relative rates for the various suppressors are compared by linear regression to the relative rate of aminoacyl-tRNA selection measured at each context for Su7C33 using the frameshift competition assay (data from Table 1B). The four suppressors used by Miller & Albertini (1983) give linear correlation coefficients ranging from 0·73 to 0·92 (see the legend to Fig. 4), which argues that the context dependence of the relative rate of RF1 selection and the relative rate of aminoacyl-tRNA selection as measured for Su7C33 are generally, though not precisely, applicable to the averaged $(1/SE)-1$ for those suppressors.

(e) *The 3′ nucleotide effects on the relative rate of aminoacyl-tRNA selection are correlated with base stacking strength*

The relative rate of aminoacyl-tRNA selection estimates vary among contexts for each suppressor. The relative rate of aminoacyl-tRNA selection depends on rate of association of the aminoacyl-tRNA : elongation factor-Tu complex with the ribosome (Dix *et al.*, 1986; Thomas *et al.*, 1988) and on resistance to dissociation of the formed aminoacyl-tRNA : ribosomal complexes (Thompson *et al.*, 1986; Ruusala *et al.*, 1982). The 3′ neighbor could conceivably influence rates of binding and/or dissociation of aminoacyl-tRNA with the ribosome by either base stacking or by forming a fourth base-pair with the tRNA.

It was previously suggested that the increased suppression efficiency observed for UAG at 3′ purine contexts might result from increased tRNA activity due to favorable stacking between the 3′ purines and the codon : anticodon complex (Ayer & Yarus, 1986). However, because the relative rate of RF1 selection is low at UAGA and high at UAGU, the correlation between 3′ purines and increased suppression efficiency may be due in part to context effects on the relative rate of RF1 selection. To gauge the significance of base stacking between the amber codon and the 3′ neighbor on rate of tRNA selection, we compared the relative rate of aminoacyl-tRNA selection at each context with the base stacking strength as measured by changes in stability of RNA duplexes caused by a 3′ unpaired

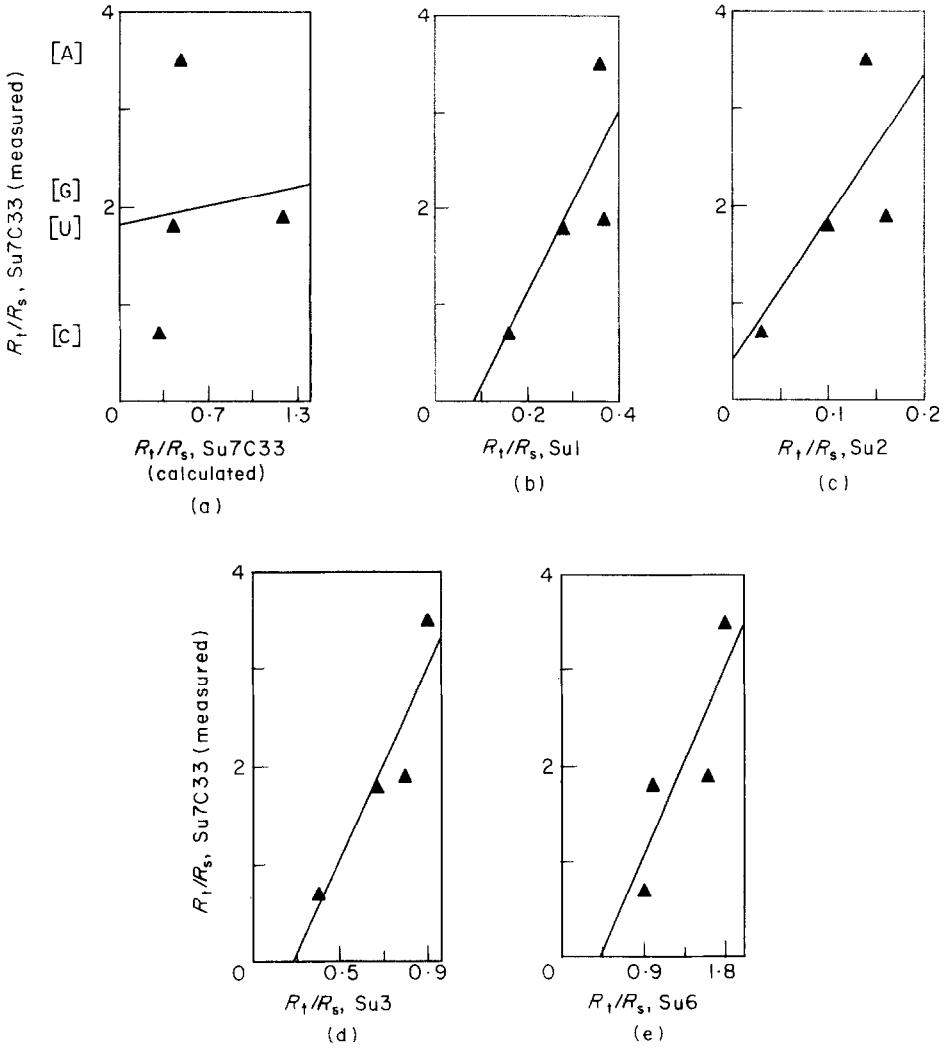

Figure 4. Calculated rates of aminoacyl-tRNA selection for various suppressors plotted *versus* that measured for Su7C33. Measured relative rates of aminoacyl-tRNA selection (R_t/R_s) for Su7C33 are from Table 1B and are plotted against calculated R_t/R_s (Table 3B) for (a) Su7C33 (linear $r = 0{\cdot}12$). (b) Su1 ($r = 0{\cdot}8$). (c) Su2 ($r = 0{\cdot}73$). (d) Su3 ($r = 0{\cdot}92$). (e) Su6 ($r = 0{\cdot}83$).

nucleotide (3′ dangling end). We used the data from Freier *et al.* (1986) for dangling ends on duplexes that have a 3′ terminal G : C pair, which is the base-pair at the wobble (3′) of an amber codon : suppressor complex. Those plots are shown in Figure 5.

All of the suppressors have context-varied relative rates of aminoacyl-tRNA selection that correlate with 3′ dangling end base stacking strength (Fig. 5), which argues that stacking between the 3′ neighbor and the amber codon : suppressor complex does contribute to rates of suppressor selection. The correlation is lowest for the relative rate of aminoacyl-tRNA selection measured for Su7C33 using the frameshift assay ($r = 0{\cdot}54$; see the legend to Fig. 5). This may be due to our measuring the relative rate of aminoacyl-tRNA selection at a single site. Effects at other neighboring positions may affect the relative rate of aminoacyl-tRNA selection; those effects are best controlled for the suppressors from the Miller & Albertini study because effects by other neighbors are normalized by averaging at many sites.

(f) *A fourth base-pair is unlikely to form between tRNA and the 3′ neighbor*

The highest relative rates of aminoacyl-tRNA selection calculated for the suppressor assayed by Miller & Albertini (1983) are observed at 3′ purine contexts. Though those results are suggestive of an effect by base stacking (above), they are also consistent with a base-pair interaction between those message nucleotides and the uridine 5′ to the anti-codon. However, Ayer & Yarus (1986) have concluded that the context effect does not require a fourth base-pair. In that study, suppression efficiency did not correlate with the potential for base-pairing when all possible tRNA and message nucleotide pairings at this fourth position were systematically tested. In addition, several studies of tRNA function on ribosomes (Matzke *et al.*, 1980; Yarus *et*

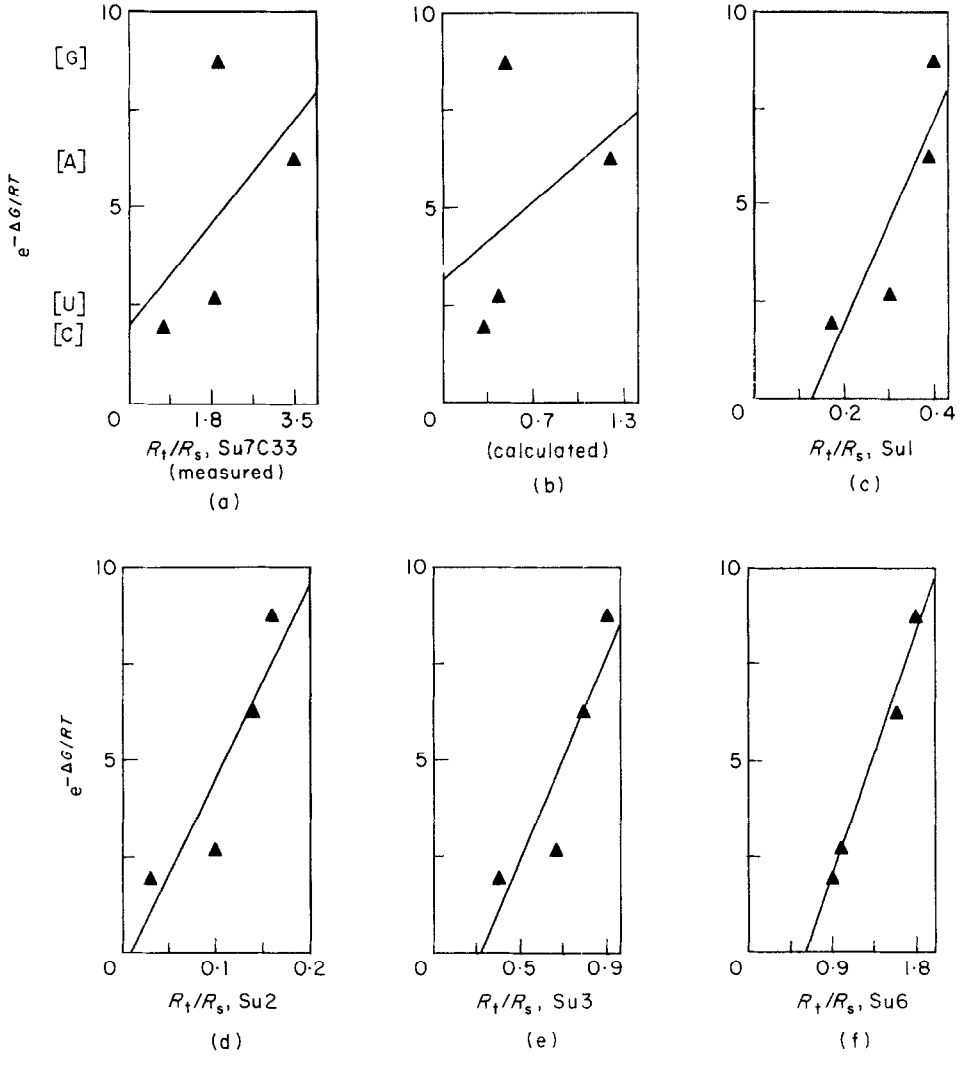

Figure 5. Relative rates of aminoacyl-tRNA selection for various suppressors plotted *versus* stacking stability of 3′ neighbors. Measured R_t/R_s values for Su7C33 are those determined using the frameshift competition assay from Table 1B. All other R_t/R_s values are those calculated with eqn (7) and suppression efficiency data from Table 3B. Values, $e^{-\Delta G/RT}$, for the free energy changes estimated for each 3′ dangling end onto an RNA duplex with a 3′ G·C pair measured by Freier *et al.* (1986) were regressed against R_t/R_s for each context for (a) Su7C33, measured (linear $r = 0{\cdot}54$), (b) Su7C33, calculated ($r = 0{\cdot}87$), (c) Sul ($r = 0{\cdot}88$), (d) Su2 ($r = 0{\cdot}9$), (e) Su3 ($r = 0{\cdot}78$), (f) Su6 ($r = 0{\cdot}89$).

al., 1986; Curran & Yarus, 1987) support a model for active tRNA structure like that observed in tRNA crystals in which the base 5′ to the anticodon is separated from the anticodon by a kink in the sugar-phosphate backbone of the tRNA and is thus not available for direct base-pair interaction with mRNA (Quigley & Rich, 1976; Holbrook *et al.*, 1978).

(g) *Selection of codons to achieve optimal contexts may drive codon choice at some sites*

Rates of aminoacyl-tRNA selection for all sense codons that begin with U or C were measured in one context (Curran & Yarus, 1989). Rates vary 25-fold within that set and rate is correlated with codon usage, which suggests that rate may be important to codon choice (Curran & Yarus, 1989). The rates of aminoacyl-tRNA selection at codons within six sets of codons read by the same tRNA vary by factors of 2 or less. For five of these six sets, codon usage is biased in favor of the fastest codon; the sixth set (UGU/C) shows no usage preference. Because the 3′ nucleotide can cause rates of aminoacyl-tRNA selection to vary by factors that are greater than that observed within these isocoding sets (2 to 5-fold, Table 3 *versus* $\leq$ 2-fold), context-dependent changes in rate might be more important to codon choice than use of a common codon at some message sites.

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References

Ayer, D. & Yarus, M. (1986). The context effect does not require a fourth base pair. *Science*, **231**, 393–395.

Bossi, L. (1983). Context effects: translation of UAG codon by suppressor tRNA is affected by the sequence following UAG in the message. *J. Mol. Biol.* **164**, 73–87.

Bossi, L. & Roth, J. (1980). The influence of context on genetic code translation. *Nature (London)*, **286**, 123–127.

Brown, C. M., Stockwell, P. A., Trotman, C. N. A. & Tate, W. P. (1990). The signal for the termination of protein synthesis in procaryotes. *Nucl. Acids Res.* **18**, 2079–2086.

Craigen, W. J., Cook, R. G., Tate, W. P. & Caskey, C. T. (1985). Bacterial peptide chain release factors: conserved primary structure and possible frameshift regulation of release factor 2. *Proc. Nat. Acad. Sci., U.S.A.* **82**, 3616–3620.

Curran, J. F. & Stewart, C. R. (1985). Transcription of *Bacillus subtilis* plasmid pBD64 and expression of bacteriophage SP01 genes cloned therein. *Virology*, **142**, 98–111.

Curran, J. F. & Yarus, M. (1986). Base substitutions in the anticodon arm do not degrade the accuracy of reading frame maintenance. *Proc. Nat. Acad. Sci., U.S.A.* **83**, 6538–6542.

Curran, J. F. & Yarus, M. (1987). Reading frame selection and transfer RNA anticodon loop stacking. *Science*, **238**, 1545–1550.

Curran, J. F. & Yarus, M. (1988). Use of tRNA suppressors to probe regulation of *Escherichia coli* release factor 2. *J. Mol. Biol.* **203**, 75–83.

Curran, J. F. & Yarus, M. (1989). Rates of aminoacyl-tRNA selection at 29 sense codons *in vivo*. *J. Mol. Biol.* **209**, 65–77.

Dennis, P. P. (1977). Transcription patterns of adjacent segments on the chromosome of *Escherichia coli* containing genes coding for four 50 S ribosomal proteins and the beta and beta' subunits of RNA polymerase. *J. Mol. Biol.* **115**, 603–625.

DeSmit, M. H. & Van Duin, J. (1990). Control of prokaryotic translational initiation by mRNA secondary structure. *Progr. Nucl. Acids Res.* **38**, 1–35.

Dix, D. B., Wittenberg, W. L., Uhlenbeck, O. C. & Thompson, R. C. (1986). Effect of replacing uridine 33 in Yeast $tRNA^{Phe}$ on the reaction with ribosomes. *J. Biol. Chem.* **261**, 10112–10118.

Folley, L. S. & Yarus, M. (1989). Codon contexts from weakly expressed genes reduce expression *in vivo*. *J. Mol. Biol.* **209**, 359–378.

Freier, S. M., Kierzek, R., Jaeger, J. A., Sigimoto, N., Caruthers, M. H., Neilson, T. & Turner, D. H. (1986). Improved free energy parameters for predictions of RNA duplex stability. *Proc. Nat. Acad. Sci., U.S.A.* **83**, 9373–9377.

Fuller, F. (1982). A family of cloning vectors containing the *lacUV5* promoter. *Gene*, **19**, 43–54.

Gold, L. (1988). Posttranscriptional regulatory mechanisms in *Escherichia coli*. *Annu. Rev. Biochem.* **57**, 199–223.

Holbrook, S. R., Sussman, J. L., Warrant, R. W. & Kim, S. H. (1978). Crystal structure of yeast phenylalanine transfer RNA. II. Structural features and functional implications. *J. Mol. Biol.* **123**, 631–660.

Kohli, J. & Grosjean, H. (1981). Usage of the three termination codons: compilation and analysis of the known eukaryotic and prokaryotic translation termination sequences. *Mol. Gen. Genet.* **182**, 430–439.

Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982). *Molecular cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Matzke, A. J. M., Barta, A. & Kuechler, E. (1980). Mechanism of translocation: relative arrangement of tRNA and mRNA on the ribosome. *Proc. Nat. Acad. Sci., U.S.A.* **77**, 5110–5114.

Miller, J. W. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Miller, J. H. & Albertini, A. M. (1983). Effects of surrounding sequence on the suppression of nonsense codons. *J. Mol. Biol.* **164**, 59–71.

Murgola, E. J., Pagel, F. T. & Hijazi, K. A. (1984). Codon context effects in missense suppression. *J. Mol. Biol.* **175**, 19–27.

Parker, J. & Precup, J. (1986). Mistranslation during phenylalanine starvation. *Mol. Gen. Genet.* **204**, 70–74.

Platt, T. (1986). Transcription termination and the regulation of gene expression. *Annu Rev. Biochem.* **55**, 339–372.

Post, L. E., Strycharz, G. D., Nomura, M., Lewis, H. & Dennis, P. P. (1979). Nucleotide sequence of the ribosomal protein gene cluster adjacent to the gene for RNA polymerase subunit beta in *Escherichia coli*. *Proc. Nat. Acad. Sci., U.S.A.* **76**, 1697–1701.

Precup, J. & Parker, J. (1987). Missense misreading of asparagine codons as a function of codon identity and context. *J. Biol. Chem.* **262**, 11351–11355.

Precup, J., Ulrich, A. K., Roopnarine, O. & Parker, J. (1989). Context specific misreading of phenylalanine codons. *Mol. Gen. Genet.* **218**, 397–401.

Quigley, G. J. & Rich, A. (1976). Structural domains of transfer RNA molecules. *Science*, **194**, 796–806.

Rice, J. B., Seyer, J. J. & Reeve, J. N. (1986). Identification of sites of cysteine misincorporation during in vivo synthesis of bacteriophage T7 0·3 protein. *Biochim. Biophys. Acta*, **867**, 57–66.

Ruteshauser, E. C. & Richardson, J. P. (1989). Identification and characterization of transcriptional termination sites in the *E. coli lacZ* gene. *J. Mol. Biol.* **208**, 23–43.

Ruusala, T., Eherenberg, M. & Kurland, C. G. (1982). Is there proofreading during polypeptide synthesis? *EMBO J.* **1**, 741–745.

Saenger, W. (1984). *Principles of Nucleic Acid Structure*. Springer-Verlag, New York.

Stormo, G. D., Schneider, T. D. & Gold, L. (1986). Quantitative analysis of the functional relationship between nucleotide sequence and functional activity. *Nucl. Acids Res.* **14**, 6661–6679.

Thomas, L. K., Dix, D. B. & Thompson, R. C. (1988). Codon choice and gene expression: synonymous codons differ in their ability to direct aa-tRNA binding to ribosomes *in vitro*. *Proc. Nat. Acad. Sci., U.S.A.* **85**, 4242–4246.

Thompson, R. C., Cline, S. W. & Yarus, M. (1982). Site directed mutagenesis of the anticodon region: the "universal U" is not essential to tRNA synthesis and function. In *Interaction of Translational and Transcriptional Controls in the Regulation of Gene Expression* (Grunberg-Manago, M. & Safer, B., eds), pp. 189–202, Elsevier, New York.

Thompson, R. C., Dix, D. B. & Karim, A. R. (1986). The reaction of ribosomes with elongation factor Tu-GTP complexes. *J. Biol. Chem.* **261**, 4868–4874.

Weiss, R. B., Dunn, D. M., Atkins, J. F. & Gesteland, R. F. (1987). Slippery runs, shifty stops, backwards steps and forward hops. *Cold Spring Harbor Symp. Quant. Biol.* **52**, 687–693.

Weiss, R. B., Dunn, D. M., Dahlberg, A. E., Atkins, J. F.

& Gesteland, R. F. (1988). Reading frame switch caused by base-pair formation between the 3′ end of 16 S rRNA and the mRNA during elongation of protein synthesis in *Escherichia coli*. *EMBO J.* **7**, 1503–1507.

Yarus, M. & Folley, L. S. (1985). Sense codons are found in specific contexts. *J. Mol. Biol.* **182**, 529–540.

Yarus, M., Cline, S. W., Wier, P., Breeden, L. & Thompson, R. C. (1986). Actions of the anticodon arm in translation on the phenotypes of RNA mutants. *J. Mol. Biol.* **192**, 235–255.

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