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# Effects of Surrounding Sequence on the Suppression of Nonsense Codons

JEFFREY H. MILLER† AND ALESSANDRA M. ALBERTINI‡

*Département de Biologie Moléculaire*
*Université de Genève, Geneva, Switzerland*

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Using a *lacI-Z* fusion system, we have determined the efficiency of suppression of nonsense codons in the *I* gene of *Escherichia coli* by assaying β-galactosidase activity. We examined the efficiency of four amber suppressors acting on 42 different amber (UAG) codons at known positions in the *I* gene, and the efficiency of a UAG suppressor at 14 different UGA codons. The largest effects were found with the amber suppressor *supE* (Su2), which displayed efficiencies that varied over a 35-fold range, and with the UGA suppressor, which displayed a 170-fold variation in efficiency. Certain UGA sites were so poorly suppressed (<0·2%) by the UGA suppressor that they were not originally detected as nonsense mutations. Suppression efficiency can be correlated with the sequence on the 3′ side of the codon being suppressed, and in many cases with the first base on the 3′ side. In general, codons followed by A or G are well suppressed, and codons followed by U or C are poorly suppressed. There are exceptions, however, since codons followed by CUG or CUC are well suppressed. Models explaining the effect of the surrounding sequence on suppression efficiency are considered in the Discussion and in the accompanying paper.

## 1. Introduction

Differences in the efficiency of suppression mediated by the same suppressor acting at different sites have been detected in a number of systems (Salser, 1969; Salser *et al.*, 1969; Yahata *et al.*, 1970; Akaboshi *et al.*, 1976; Colby *et al.*, 1976; Fluck *et al.*, 1977; Feinstein & Altman, 1977,1978; Fluck & Epstein, 1980), and have led to the idea that the surrounding sequence on the messenger RNA, or reading "context", plays a role in determining the efficiency of suppression. Bossi & Roth (1980) have determined the sequence change responsible for increasing the *supE*-mediated suppression at a particular amber (UAG) site. In order to elucidate general sequence patterns resulting in context effects, it would be desirable to compare the surrounding sequence and resulting suppression efficiencies for a large number of nonsense codons.

The *lacI* gene of *Eschericha coli* offers an excellent opportunity to examine

† Present address: Department of Biology, University of California, Los Angeles, Calif. 90024 U.S.A.
‡ Present address: Istituto di Genetica, Universita di Pavia, 27100, Pavia, Italy.



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context effects, since close to 90 nonsense mutations at known positions have been characterized (Coulondre & Miller, 1977; Miller *et al.*, 1978), and since the entire DNA sequence of the *lacI* gene has been determined (Farabaugh, 1978). This paper describes the construction of a system that employs the *lacI* nonsense mutations for measurements of suppressor activity. We present the results for a set of *E. coli* amber suppressors operating on 42 different amber codons, and for a UGA suppressor operating on 14 different UGA codons. The accompanying paper (Bossi, 1983) describes the efficiencies of four amber suppressors in *Salmonella typhimurium* operating on the 42 amber codons. These studies show that suppression efficiencies can vary by as much as 35-fold for amber suppression, and by 170-fold for UGA suppression, and that the efficiency of suppression can be correlated with the base sequence on the 3′ side of the codon being suppressed. Several models accounting for these effects are considered in the Discussion of this and the accompanying paper.

## 2. Experimental Design

In order to facilitate the measurement of suppression efficiencies, we have taken advantage of a deletion characterized by Müller-Hill and co-workers (Müller-Hill & Kania, 1974; Brake *et al.*, 1978), which fuses the end of the *I* gene to the beginning of the *Z* gene (see Fig. 1). Strains carrying this deletion (referred to here as *del.14*) synthesize a hybrid repressor–β-galactosidase molecule, which lacks the last four residues of *lac* repressor and the first 23 residues of β-galactosidase. The hybrid protein has normal β-galactosidase activity. The introduction of a nonsense mutation into the *I* portion of the fusion would result in the termination of translation before the *Z*-encoded portion of the protein is translated, and thus little or no β-galactosidase activity being produced. Suppression of the nonsense mutation by an appropriate suppressor would restore some level of β-galactosidase activity. The advantage of this system is that we can use the easy and precise β-galactosidase assay to measure the suppressed levels of nonsense mutations in the *I* gene.

Because the *I*-encoded portion of the fused protein is dispensible for β-galactosidase activity, most or all single amino acid substitutions should be tolerated without interfering with the activity of the enzyme. Although it is

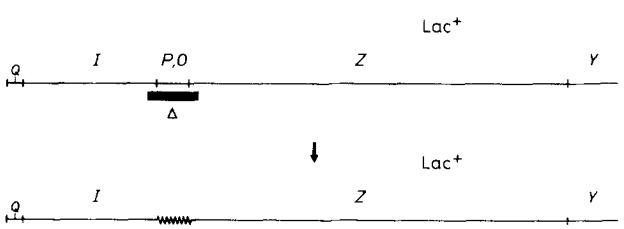


FIG. 1. The deletion *del.14* characterized by Müller-Hill & Kania (1974; see also Brake *et al.*, 1978) fuses the *Z* gene to the *I* gene. The resulting hybrid repressor–β-galactosidase protein still retains normal β-galactosidase activity (see the text).

possible to envision certain substitutions that lower activity by inhibiting proper folding and thus inviting degradation by cellular proteases, the data from the study presented here argue that this happens only very rarely, if ever (see Discussion). In any case, one can always consider separately the examples in which the wild-type amino acid is inserted by the respective suppressor. These examples are sufficiently numerous to allow us to determine independently the sequence pattern responsible for context effects.

A second source of interference could be background translation resulting either from reinitiation of translation after the nonsense site, or from non-specific readthrough past the nonsense block. In either case, the background levels can be controlled for by determining the β-galactosidase levels in an $Su^-$ strain.

## 3. Materials and Methods

### (a) *Media*

All media and buffers are as described by Miller (1972). Galactosides are as described by Schmeissner *et al.* (1977*a*).

### (b) *Enzyme assays*

β-Galactosidase assays are as described by Miller (1972). Exponential cultures were grown in minimal A medium supplemented with methionine. Multiple determinations were made in each case.

### (c) *Strain constructions*

We constructed fusion derivatives carrying different nonsense mutations as follows. We first prepared strain LF32 (see also Table 1) by crossing the *I-Z* fusion deletion (*del.14*;

TABLE 1

*Bacterial strains used in this study*

| Strain | Sex | Genetic markers |
|---|---|---|
| P90C | $F^-$ | *ara Δ(lacproB) thi* |
| S90C | $F^-$ | *ara Δ(lacproB) thi strA* |
| XA101 | $F^-$ | *ara Δ(lacproB) nalA metB argE-am rif supD thi* |
| XA102 | $F^-$ | *ara Δ(lacproB) nalA metB argE-am rif supE thi* |
| XA103 | $F^-$ | *ara Δ(lacproB) nalA metB argE-am rif supF thi* |
| XA105 | $F^-$ | *ara Δ(lacproB) nalA metB argE-am rif supG thi* |
| XA10B | $F^-$ | *ara Δ(lacproB) nalA metB argE-am rif supB thi* |
| XA10C | $F^-$ | *ara Δ(lacproB) nalA metB argE-am rif supB thi* |
| XA96 | $F^-$ | *ara Δ(lacproB) nalA argE-am rif thi sup6* |
| BMH 14-7156 | F′*lacproB* ($I^Q$, *del.14*) | *Δ(lacproB) thi* (from B. Müller-Hill) |
| X7800 | $F^-$ | *ara* $val^r$ *Δ(lacproB) galE strA thi* ($\phi$80d*lac*$^+$) |
| X7832 | $F^-$ | Derivative of X7800 carrying a *tonB* deletion extending into the *lacI* gene on the $\phi$80d*lac* prophage |
| LF32 | $F^+$ *kan*$^r$ | Derivative of X7832 carrying the *del.14 I-Z* fusion deletion |
| CAJ64 | $F^-$ | *Δ(lac)* (λ) *sup9 thi* (from S. Brenner) $val^r$ *Δ(lacproB) nal rif* |
| CDJ64 | $F^-$ | *sup9 thi* carries UGA suppressor of CAJ64 (from D. Ganem) |

For further details see Coulondre & Miller (1977). SupJ encodes $Su_6$, the leucine-inserting amber repressor.

Müller-Hill & Kania, 1974) into a strain carrying a *tonB-lacI* deletion in a *galE* background (X7832; Table 1). To accomplish this, we transferred the F'*lacproB-del.14* episome from strain BMH 14-7156 to X7832, and then grew the resulting partial merodiploid overnight and plated on medium containing phenyl-β,D-galactoside (Pgal), glucose, and 5-bromo-4-chloro-3-indolyl-β,D-galactoside (Xgal), which selects for $Z^-$ and $GalT^-$ or $GalK^-$ strains. The $Z^-$ colonies were recognized by their white color. $Z^-$ colonies arising from recombination of the fusion deletion into the chromosome were 20-fold more frequent (on the average) than spontaneous $Z^-$ colonies. The resulting strain carries 2 partial deletions of the *I* region. The *tonB-trp* deletion (*Δ32*), which eliminates the *I* promoter and the first half of the *I* gene, and the fusion deletion, *del.14* (Fig. 1). The F'*lacproB* episome was cured by introducing an F factor carrying a kanamycin resistance marker. The resulting strain, LF32, is now $Pro^-$, and can be a recipient for any F'*lacproB* episome, since the $Pro^+$ selection can be employed.

F'*lacproB* episomes carrying different nonsense mutations were introduced into LF32. These heterodiploids were then used as donors to transfer the episome to different suppressor strains that were deleted for *lacproB* and which were known to restore the $I^+$ phenotype in response to the specific nonsense mutation. The mating mixtures were plated on medium containing nalidixic acid (to select against the donor), methionine, and Xgal. Most of the recipient suppressor strains received the episomes carrying the original nonsense mutation and gave rise to white colonies. Strains receiving episomes carrying the fusion formed blue colonies. In cases where the episome carried both the fusion and the nonsense site, blue colonies were formed. (The *Δ32* deletion prevents or reduces the loss of the nonsense site due to recombination). Nonsense mutation derivatives of the fusion strain were verified by mapping against different deletions.

For nonsense sites expected to yield levels of suppression that were very low, a second procedure was employed. This involved mainly the UGA mutations. Here the heterodiploids (LF32 and the F'*lacproB* episome carrying the UGA mutation) were plated on medium containing Pgal, glucose, and Xgal, and $Z^-$ colonies (most of which resulted from crossing the fusion deletion onto the episome in combination with the nonsense site) were recognized by their white color. The resulting episome was then transferred to different $Su^+$ and $Su^-$ strains and examined for its response to specific suppressors. The mutations were also mapped against different deletions.

## 4. Results

### (a) *Suppression of UAG mutations*

We constructed F'*lacproB* episomes carrying each respective amber mutation and the fusion deletion *del.14* (see Materials and Methods for details of the constructions). The $I^Q$ promoter mutation (Müller-Hill *et al.*, 1968) is present in each case. Table 2 lists the amber sites employed in this study. β-Galactosidase assays were performed on strains harboring a chromosomal *lacproB* deletion and containing the episome described above, and carrying different suppressor (*sup*) alleles (see Table 1 and Materials and Methods). The results of the β-galactosidase assays are presented in Table 3, where the amber sites have been arranged according to the codon on the 3′ side of the nonsense site.

### (b) *Suppression of UGA mutations*

#### (i) *Previously described UGA sites*

Table 4 describes the ten characterized UGA mutations in *lacI*. We constructed fusion derivatives of the strains carrying the respective mutations, as described in

TABLE 2

*Amber sites employed in combination with the* lacI-Z *fusion*

| Site | Coding position | Wild-type residue | Site | Coding position | Wild-type residue |
|---|---|---|---|---|---|
| *A5* | 18 | Glutamine | *O9* | 54 | Glutamine |
| *A6* | 26 | Glutamine | *O10* | 55 | Glutamine |
| *A9* | 60 | Glutamine | *O11* | 78 | Glutamine |
| *A10* | 61 | Serine | *O12* | 84 | Lysine |
| *A11* | 62 | Leucine | *O13* | 89 | Glutamine |
| *A13* | 97 | Serine | *O14* | 100 | Glutamic acid |
| *A15* | 131 | Glutamine | *O15* | 105 | Glutamic acid |
| *A16* | 153 | Glutamine | *O17* | 117 | Glutamine |
| *A17* | 172 | Glutamic acid | *O18* | 126 | Tyrosine |
| *A18* | 177 | Leucine | *O19* | 137 | Glutamic acid |
| *A19* | 180 | Glutamine | *O21* | 181 | Glutamine |
| *A20* | 193 | Serine | *O23* | 204 | Tyrosine |
| *A23* | 211 | Glutamine | *O24* | 209 | Glutamine |
| *A24* | 220 | Tryptophan | *O25* | 215 | Glutamic acid |
| *A26* | 248 | Glutamine | *O27* | 227 | Glutamine |
| *A27* | 259 | Glutamic acid | *O28* | 228 | Glutamine |
| *A29* | 273 | Tyrosine | *O34* | 298 | Glutamine |
| *A30* | 282 | Tyrosine | *O35* | 306 | Glutamine |
| *A31* | 291 | Glutamine | *O36* | 322 | Serine |
| *A33* | 309 | Glutamine | *X3* | 90 | Leucine |
| *A34* | 311 | Glutamine | *X15* | 293 | Phenylalanine |

All sites originally derived from wild-type as ochre mutations are listed as *O1*, *O2*. . .etc. These sites were converted to UAG with 2-aminopurine (see Coulondre & Miller, 1977).

Materials and Methods. We utilized the *sup9* UGA suppressor derived from stain CJ64 (Sambrook *et al.*, 1967; Hirsch, 1971). The results of β-galactosidase assays in $Su9^+$ and $Su9^-$ strains are recorded in Table 4. It can be seen that, whereas some UGA sites, such as *U5*, are very poorly suppressed (yielding 0·15 to 0·3% suppression), other sites such as *U6* and *U10* are suppressed at 20 to 30% efficiency. Moreover, there is a background suppression in $Su9^-$ strains that is as high as 3% in some cases. This background (see Discussion) is virtually eliminated in the *strA* strain employed here (Table 4).

(ii) *"Hidden" UGA mutations*

An extensive search for UGA mutations in *lacI* (Coulondre & Miller, 1977) detected the ten sites listed in Table 4. However, the elucidation of the DNA sequence of the *I* gene (Farabaugh, 1978) indicated that we had failed to detect six UGA mutations that could be generated by a single base change in the portion of the gene encoding the first 330 residues of the repressor. Four of these positions (6, 146, 185 and 286) are encoded by UUA on the mRNA, or TTA on the DNA, and can be converted to UGA *via* a T → G transversion. Since this transversion is stimulated by *mutT* (Cox & Yanofsky, 1967), we had expected to find several different UGA mutations among *mutT*-induced UGA sites. However, among 50 independently generated UGA mutations induced by *mutT*, only mutations at

TABLE 3

*β-Galactosidase assays*

| Amber site | mRNA sequence | β-Galactosidase activity (% wild-type) | | | | |
|---|---|---|---|---|---|---|
| | | Su1 | Su2 | Su3 | Su6 | Su⁻ |
| *O12* | AUU UAG UCU | 7·7 | 2·3 | 13·8 | 13·8 | 0·04 |
| *A27* | ACC UAG UCC | 15·3 | 3·0 | 31·8 | 70·0 | |
| *A9* | AAA UAG UCG | 9·5 | 2·5 | 32·5 | 37·5 | 2·2 |
| *A10* | CAG UAG UUG | 14·6 | 3·8 | 21·4 | 49·4 | 2·2 |
| *O17* | GCG UAG CGC | 6·3 | 0·8 | 10·6 | 30·4 | |
| *X15* | GAU UAG CGC | 5·5 | 0·7 | 20·9 | 32·5 | |
| *O25* | GCG UAG CGG | 7·8 | 1·8 | 13·8 | 29·9 | |
| *O14* | GUA UAG CGA | 8·8 | 2·8 | 16·7 | 39·1 | |
| *O18* | AAC UAG CCG | 12·6 | 1·7 | 31·8 | 60·6 | |
| *A23* | AUU UAG CCG | 14·7 | 4·2 | 29·8 | 46·0 | |
| *A19* | CAC UAG CAA | 21·1 | 3·8 | 26·9 | 67·8 | |
| *O27* | UUU UAG CAA | 12·8 | 2·5 | 24·2 | 41·1 | |
| *O9* | GCA UAG CAA | 18·2 | 5·1 | 28·1 | 53·1 | 1·2 |
| *O13* | GAU UAG CUG | 26·0 | 11·3 | 46·4 | 57·8 | |
| *O10* | CAA UAG CUG | 28·0 | 12·3 | 36·2 | 47·7 | 1·3 |
| *O36* | GUC UAG CUG | 14·8 | 10·3 | 21·7 | 40·6 | 4·0 |
| *A11* | UCG UAG CUG | 19·5 | 8·1 | 30·3 | 60·3 | |
| *O35* | CUG UAG CUC | 17·1 | 7·5 | 23·5 | 55·1 | |
| *O23* | AAA UAG CUC | 22·8 | 11·5 | 24·0 | 51·6 | |
| *O15* | GUC UAG GCC | 18·4 | 8·3 | 32·6 | 46·1 | |
| *A6* | AAC UAG GCU | 23·1 | 12·9 | 24·8 | 32·1 | 0·83 |
| *O19* | GUG UAG GCU | 19·7 | 9·3 | 36·7 | 55·6 | |
| *A34* | GGC UAG GCG | 23·8 | 8·3 | 34·1 | 64·0 | |
| *A20* | GUC UAG GCG | 22·8 | 12·3 | 42·8 | 72·9 | |
| *A33* | UCU UAG GGC | 19·7 | 10·6 | 28·4 | 61·7 | |
| *X3* | CAA UAG GGU | 36·3 | 18·4 | 55·7 | 73·6 | |
| *A18* | GCU UAG GGU | 16·4 | 14·2 | 45·8 | 57·9 | |
| *O30* | GGA UAG GAC | 21·3 | 10·6 | 37·5 | 53·6 | |
| *A15* | GAC UAG GAU | 36·7 | 13·8 | 55·4 | 66·7 | |
| *A31* | AAA UAG GAU | 19·5 | 12·1 | 54·9 | 54·8 | |
| *O11* | UCG UAG AUU | 34·8 | 18·0 | 52·3 | 60·0 | |
| *O24* | AAU UAG AUU | 29·5 | 20·7 | 52·5 | 57·0 | |
| *O21* | CAG UAG AUC | 26·6 | 14·6 | 33·5 | 71·6 | |
| *A30* | UGU UAG AUC | 53·8 | 25·6 | 66·7 | 69·5 | |
| *A5* | UAU UAG ACC | 35·4 | 9·4 | 46·8 | 58·0 | |
| *O28* | CAA UAG ACC | 31·3 | 10·1 | 62·0 | 79·6 | |
| *O34* | GGG UAG ACC | 22·7 | 9·2 | 45·4 | 57·4 | |
| *A16* | GAC UAG ACA | 21·1 | 9·6 | 42·6 | 61·7 | |
| *A24* | GAC UAG AGU | 22·2 | 10·8 | 45·8 | 59·0 | |
| *A13* | GUG UAG AUG | 32·9 | 19·3 | 53·1 | 73·7 | |
| *A26* | GAU UAG AUG | 25·4 | 17·8 | 100 | 100 | |

All assays were carried out at 37°C and were determined in duplicate and, in most cases, in several different experiments. Values are given as the percentage of the wild-type fusion in the respective suppressor strains. Su1, 2, 3, and 6 values are from the strains XA101, XA102, XA103 and XA96, respectively (see Table 1). The wild-type controls averaged 120 units, using the units of Miller (1972).

the UUA codon at position 189 were detected (Coulondre & Miller, 1977). The four other UGA sites might have escaped detection due either to a low efficiency of suppression and/or the failure of tryptophan to substitute for leucine at the respective site in the protein. We therefore decided to examine the *mutT*-induced

TABLE 4

*β-Galactosidase assays of strains with UGA mutations*

| UGA site | Coding position | mRNA sequence | β-Galactosidase activity (% wild-type) Su⁺ (UGA) | Su⁻ (Str$^s$) | Su⁻ (Str$^r$) |
|---|---|---|---|---|---|
| *U1* | 101 | ACG UGA AGC | 5·3 | 0·27 | 0·01 |
| *U2* | 140 | GCC UGA ACU | 5·9 | 0·05 | 0·01 |
| *U3* | 107 | GCC UGA AAA | 5·9 | 0·18 | 0·08 |
| *U4* | 189 | CCA UGA AGU | 17·3 | 1·8 | 0·04 |
| *U5* | 201 | GGC UGA CAU | 0·38 | 0·05 | 0·02 |
| *U6* | 220 | GAC UGA AGU | 12·7 | 1·29 | 0·17 |
| *U7* | 280 | AGC UGA UGU | 0·78 | 0·28 | 0·25 |
| *U8* | *281* | UCA UGA UAU | 0·92 | 0·29 | 0·26 |
| *U9* | 322 | | — | — | — |
| *U10* | 168 | GAC UGA CUG | 27·6 | 2·75 | 0·04 |

For further details see Tables 1 and 3. P90C and S90C were used as Su⁻ control strains.

"non-suppressible" mutations (Miller & Schmeissner, 1979) that map in the region corresponding to the undetected UGA sites.

The detailed gene–protein map of the *I* gene enables us to position mutations within five base-pairs or less on the *I* DNA sequence (Schmeissner *et al.*, 1977*a*,*b*; Miller *et al.*, 1978). Out of 88 non-suppressible mutations induced by *mutT*, one maps in the same small interval covering position 6, five in the same interval as

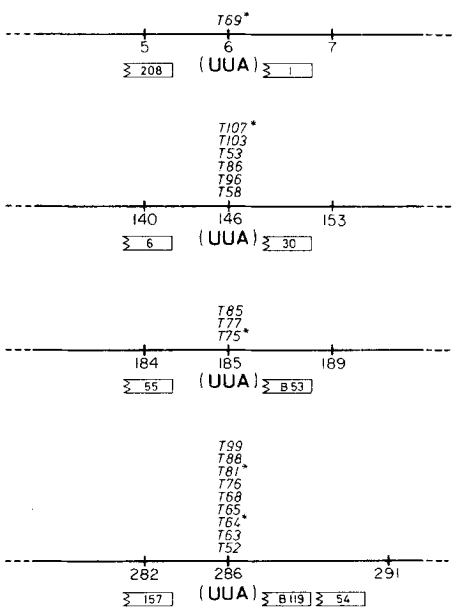


FIG. 2. The map position of *mutT*-induced mutations that occur at or near coding positions for UUA triplets. The A·T → C·G transversion can result in UGA mutations at these respective sites. The asterisks indicate sequence mutations that result from the creation of a UGA codon (see the text).

TABLE 5

*Position of six* mutT*-derived* I⁻ *mutations, as determined by DNA sequencing*

| Mutation | Coding position | Base change |
|---|---|---|
| *T69* | 6 | TTA → TGA |
| *T107* | 146 | TTA → TGA |
| *T75* | 185 | TTA → TGA |
| *T64, T81* | 286 | TTA → TGA |
| *T63* | 286 | TTA → TTC |

position 146, three in the same interval as position 185, and nine in the same interval as position 286 (see Fig. 2; and Schmeissner, 1978). We therefore sequenced one mutation from each cluster and also attempted to recover fusion derivatives of the putative UGA mutations, as depicted in Figure 1. Table 5 shows the results. Indeed, *T69*, *T107*, *T75* and *T64* are UGA mutations resulting from a T → G transversion in the second base of the codon at positions 6, 146, 185 and 286, respectively. (One of the mutations, *T63*, was caused by the same transversion at the third base in the codon at position 286, leading to a leucine to phenylalanine change at the respective position in the protein.)

We constructed UGA fusion derivatives for *T69*, *T107*, *T75* and *T64*. Table 6 depicts the β-galactosidase assays of the new UGA mutations in strains with and without the UGA suppressor.

## 5. Discussion

### (a) *Evaluation of the system*

We have used the *lacI* system to examine the effects of surrounding base sequence on UAG suppression at 42 different sites and on UGA suppression at 14 different sites. We have taken advantage of the properties of fusion strains in which the *I* and *Z* gene products are synthesized as a chimeric protein, under the control of the *I* promoter, as a result of a deletion that eliminates the *lac* control region (Müller-Hill & Kania, 1974; Brake *et al.*, 1978). Introduction of nonsense mutations into the *I* gene portion of the fusion results in termination of protein synthesis and

TABLE 6

*β-Galactosidase levels in fusion strains carrying the UGA mutations described in Table 5*

| UGA site | Coding position | mRNA sequence | β-Galactosidase activity (% wild-type) Su⁺ (UGA) | Su⁻ (Str$^s$) | Su⁻ (Str$^r$) |
|---|---|---|---|---|---|
| *T69* | 6 | ACG UGA UAC | 0·36 | 0·07 | 0·02 |
| *T107* | 146 | GCG UGA UUU | 0·17 | 0·01 | 0·01 |
| *T75* | 185 | CUG UGA GCG | 0·80 | 0·02 | 0·02 |
| *T64* | 286 | CCG UGA ACC | 8·9 | 0·44 | 0·02 |

loss of the *Z*-encoded β-galactosidase activity, which can be partially restored by suppression of the nonsense mutation. In principle, this system should afford an excellent opportunity to study quantitatively different levels of suppression. There are, however, two sources of error that must be controlled for; namely, translational reinitiation and lowered β-galactosidase activity.

Reinitiation of translation can occur after chain termination (Sarabhai & Brenner, 1967; Zipser *et al.*, 1970; Platt *et al.*, 1972; Napoli *et al.*, 1981) and three different reinitiation points in the beginning of *lacI* have been described (Files *et al.*, 1974). Efficient reinitiation can interfere with attempts to measure suppression, since the β-galactosidase levels may reflect, in part, the activity of translational reinitiation fragments. For any given nonsense site, the β-galactosidase levels in an $Su^-$ strain (a $Str^r$, $Su^-$ strain for UGA sites) represent the maximum background due to reinitiation of translation. Several of the UAG sites and one UGA site activate weak translation restarts. The resulting levels (5% or less of the wild-type controls) have been subtracted from the suppressed β-galactosidase values, as indicated in Tables 3 and 4.

Comparison of suppressed β-galactosidase levels with wild-type enzyme levels is valid only if the assumption is correct that amino acid substitutions in the *I* portion of the hybrid protein do not lower the β-galactosidase activity of the fusion protein, either by directly affecting the protein itself, or by allowing protein degradation during synthesis. This objection does not apply to strains with very high levels of suppressed β-galactosidase, or to situations in which the amino acid inserted by the suppressor is identical to that present in the wild-type. Among the amber sites, the most pronounced context effects occur with *supE*, which results in the insertion of glutamine in response to the amber codon. The 15 amber sites derived from glutamine codons provided a representative sample of the context effects found with *supE*, in a situation where the wild-type amino acid is inserted. Similarly, the UGA sites *U5* and *U6*, which show a large difference in efficiency (35×) of suppression by the tryptophan-inserting UGA suppressor, are both derived from codons specifying tryptophan in the wild-type protein. Therefore, the main conclusions drawn from this study are not affected by the possible change in activity of β-galactosidase. In any case, examination of the large series of substitutions generated by amber suppression in fusion derivatives (Tables 3 and 4) reveals no tendency of substitutions that are known to cause inactive repressors (Miller *et al.*, 1979) to lower the β-galactosidase levels of the fused protein.

### (b) *Evaluation of results*

Analysis of Tables 3, 4 and 6 shows that suppression of nonsense codons occurs at widely varying efficiencies. The strongest effects are seen with the *supE* amber suppressor and with a UGA suppressor. Suppression efficiencies vary over a 35-fold range with *supE* and over a 170-fold range with the UGA suppressor. The amber suppressors *supD*, *supF* and *supJ* (Su6) operate at efficiencies differing by seven- to eight-fold. The two cases where suppression approaches 100% (*supF* and *supJ* at site *A26*) are noteworthy and are considered below.

In general, the efficiency of suppression is site specific; that is, nonsense sites that

TABLE 7

*β-Galactosidase levels in different suppressed fusion derivatives*

| Nonsense site | mRNA sequence | β-Galactosidase activity (% wild-type) Su$^+$ (UGA) | Su2 |
|---|---|---|---|
| *T69* | ACG UGA UAC | 0·36 | |
| *U10* | ACG UGA CUG | 27·6 | |
| *X15* | GAU UAG CGC | | 0·7 |
| *O13* | GAU UAG CUG | | 11·3 |
| *A26* | GAU UAG AUG | | 11·8 |
| *O18* | AAC UAG CCG | | 1·7 |
| *A6* | AAC UAG GCC | | 8·8 |
| *O12* | AAA UAG UCU | | 2·3 |
| *A9* | AAA UAG UUG | | 2·5 |
| *O23* | AAA UAG CUC | | 11·5 |
| *A31* | AAA UAG GAU | | 9·3 |

are efficiently or poorly suppressed are usually affected in a similar manner by the other suppressors tested here (although *subJ* represents a partial exception). The largest context effects are a function of the sequence on the 3′ side of the codon being suppressed. The effects depend mainly on the nature of the codon on the 3′ side of the nonsense site, and in many cases possibly only on the first base on the 3′ side of the nonsense codon. Table 7 shows the lack of correlation between suppression efficiencies and the *preceding* codon. In the four cases shown, significant differences in suppression efficiencies occur despite identical preceding codons. Considering Tables 3 and 4, it is apparent that when the codons on the 3′ side of the nonsense site begin with A or G, the suppression efficiencies are high, whereas when the codons begins with U or C, suppression is usually low. The codons CUC and CUG represent obvious exceptions, however, since high-level suppression occurs when a nonsense codon is followed by either of these two codons. This is particularly evident for CUG, which is represented by four examples in this study and which promotes very efficient suppression of the UGA codon *U10*. There are other differences within the groups of codons (arranged in Tables 3 and 4 according to the starting base), but the variations are smaller and we have chosen not to deal with them here.

We can outline a number of possible explanations for the context effects observed here. For a detailed discussion of these and other models, please see the accompanying paper (Bossi, 1983 and references therein). (1). Based on the assumption that the observed suppression efficiency ultimately results from a competition between translation termination factors and suppressor tRNAs, it is conceivable that: (a) the operation of the translation termination factor is affected by the sequence on the 3′ side of the nonsense codon. Although this possiblity is not excluded by the data presented here, the finding that certain ochre suppressors may not follow the same rules when operating on these amber codons (Bossi & Roth, unpublished results) leads one to consider the explanations mentioned below.

(b) The suppressor tRNA interacts with the sequence on the 3′ side of the nonsense codon by making a fourth base-pair. The finding that all known tRNAs have a U at the relevant position (on the 5′ side of the anti-codon) fits with the fact that suppression is efficient when A and (to a lesser extent) G are on the 3′ side of the nonsense codon and, in general, suppression is inefficient when the following base is a U or C. This hypothesis cannot explain all of the data, however, since when C is part of a CUC or CUG codon, suppression of the preceding nonsense site is very efficient.

(2) Suppression efficiencies might reflect tRNA–tRNA interactions independent of the competition with chain terminating factors. This hypothesis might be invoked to explain the CUC and CUG exceptions, or else might even include all of the codons, since tRNAs that read codons beginning with the same base may have similar modified bases on the 3′ side of the anti-codon (Nishimura, 1972; see Bossi, 1983).

(3) Superimposed on either of the above mechanisms is the possible modulating effect of mRNA secondary structure. Site *A26* is suppressed at 100% efficiency by Su3 and Su6. Although this might seem only a small difference from the average of 50 to 60% for these suppressors at sites followed by an A residue, one can also consider that these same values reflect a change from an average of 40 to 50% termination to an undetectable level in the case of *A26*. Perhaps it is more appropriate to consider the ratio of suppression to termination, which for Su3 and Su6 is close to 1 : 1 for sites followed by A. This ratio increases to 10 : 1 or greater at site *A26*. One possibility is that the secondary structure around site *A26* affects suppression efficiency. Figure 3 shows a plausible secondary structure for the portion of the mRNA near *A26*. One might imagine that certain structures inhibit release factor action on nonsense sites, thus increasing the observed suppression efficiency. It remains for future experiments to test the effect of potential secondary structures by altering specific base-pairs that would promote or hinder specific structures.

It remains to be seen whether the efficiency of translation itself is context dependent, resulting from tRNA–tRNA interactions, and what role if any, context effects play in gene control and in gene evolution. The results in Table 4 show that,

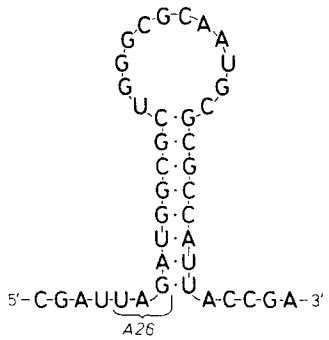


FIG. 3. A potential secondary structure involving the mRNA sequence surrounding the amber site *A26*.

at least for UGA terminators, context effects can account for different levels of proteins resulting from the natural readthrough of the UGA codons. Such proteins were first observed for bacteriophage Qβ (Horiuchi *et al.*, 1971; Moore *et al.*, 1971; Weiner & Weber, 1971) and were subsequently detected for bacteriophage lambda (Yates *et al.*, 1977) and for filamentous phages (Engelberg-Kulka *et al.*, 1979). (See the Discussion by Bossi, 1983.)

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