CONTEXT EFFECTS ON NONSENSE CODON SUPPRESSION IN *ESCHERICHIA COLI*

SHELDON I. FEINSTEIN[1] AND SIDNEY ALTMAN

Department of Biology, Yale University, New Haven, Connecticut 06520

Manuscript received June 30, 1977
Revised copy received September 12, 1977

ABSTRACT

The influence of mRNA context on nonsense codon suppression has been studied by suppression measurements at one site in the *Escherichia coli trpE* gene and at two sites in the *trpA* gene. The ratio of suppression efficiencies of amber and ochre codons at each site (homotopic pairs) has been compared using ochre suppressing derivatives of $tRNA_1^{Tyr}$. This ratio is independent of differential effects of the inserted amino acid on enzyme function. We have found that mRNA context can change the ratio of suppression efficiencies of homotopic nonsense codons at the three sites in the *trp* gene system over a ten-fold range. The causes of such variation, and, in particular the effect of certain adjacent nucleotides on nonsense codon suppression are considered.

THE efficiency with which nonsense codons can be suppressed in *E. coli* can be influenced by the identity of adjacent codons (SALSER 1969; SALSER, FLUCK and EPSTEIN 1970; YAHATA, OCADA and TSUGITA 1970; FLUCK, SALSER and EPSTEIN 1977; FEINSTEIN and ALTMAN 1977). To understand this phenomenon in detail, it is essential to know the nature of the coding sequences surrounding the suppressed nonsense codon. In certain bacteriophage T4 genes (lysozyme, *rII B* cistron) and at least two *E. coli* genes (*trpE*, *trpA*), sequence information is available adjacent to sites at which both amber and ochre nonsense mutants exist (Table 1). The availability of both ochre and amber nonsense codons at the same genetic site (a homotopic pair) allows a calculation of the ratio of the suppression efficiencies of these codons by an ochre-suppressing tRNA, which can wobble to suppress both ochre and amber codons. A ratio of suppression efficiencies is an indicator which is independent of the nature of the amino acid inserted by any one ochre-suppressing tRNA. An effect of mRNA context on suppression efficiency is apparent if this ratio changes at various genetic sites where homotopic mutants are available. Such changes have been observed in the T4 lysozyme gene and the *rII B* cistron (FEINSTEIN and ALTMAN 1977). We have now extended these studies to include sites in the *E. coli trp E* (anthranilate synthetase) and *trp A* (α subunit of tryptophan synthetase) genes where, perhaps, the most striking examples of mRNA context effects on suppression efficiency can

[1] Present address: Radiobiology Laboratories, Yale University School of Medicine, New Haven, Conn. 06510.

TABLE 1

Contexts of nonsense mutations in the tryptophan operon

	*trpE*9914 site*			*trpA*38 site†			*trpA*96 site‡		
Nonsense mutations	leu CU^{U}_{C}	UAA or UAG	leu CUG	arg CGC	UAA or UAG	glu GAA	glu GA^{A}_{G}	UAA or UAG	his CA^{U}_{C}
Wild type	leu CU^{U}_{C}	glu GAA	leu CUG	arg CGC	lys AAA	glu GAA	glu GA^{A}_{G}	gln CAA	his CA^{U}_{C}

* Sequence of the region containing the *trpE*9914 site is based upon a combination of nucleotide sequence analysis of messenger RNA and amino acid sequence analysis of protein in BRONSON, SQUIRES and YANOFSKY 1973.

† Sequence of the region containing the *trpA*38 site is based upon a combination of nucleotide sequence analysis of messenger RNA and amino acid sequence analysis of protein (YANOFSKY *et al.* 1967; YANOFSKY and HORN 1972; PLATT and YANOFSKY 1975).

‡ Sequence of the region containing the *trpA*96 site is inferred from the amino acid sequence analysis of protein (YANOFSKY *et al.* 1967; YANOFSKY and HORN 1972).

be found. The accumulated data from three gene systems show that certain nucleotides when found adjacent to the nonsense codons, may have systematic effects on nonsense-codon suppression.

MATERIALS AND METHODS

Strains of bacteriophage and bacteria

The $\phi 80$ bacteriophage strains used in this work are described in Table 2. The bacterial strains used are described in Table 3. Bacteriophage P1 *vir* and MS2 were a gift of K. B. Low, Yale University.

Sources of chemicals and reagents

Thiamine hydrochloride (B_1) and amino acids were purchased from Calbiochem. Lactose, IPTG†, ONPG†, pyridoxal phosphate, 5-methyltryptophan, chorismic acid (barium salt) and Coomassie Brilliant Blue G were purchased from Sigma Chemical Company. Bacto-tryptone, Bacto-yeast extract, and vitamin-free casamino acids were purchased from Difco. Agar was purchased from Difco or BBL (Becton, Dickinson). XG† was purchased from Schwarz/Mann or Sigma. Lactose-MaConkey plates were a gift of K. Low. All routine chemicals were of reagent grade.

Solutions and media

H plates, H top agar, M9 medium and B broth have been described (KATZ 1969). Minimal plates were prepared from M9 as described by RUSSELL *et al.* (1970). Sugars were added separately to a concentration of 2 grams per liter. L-amino acids were added as needed to a concentration of 20 mg/l or spread on the surface of the plate before use at 0.2 mg/plate. Thiamine hydrochloride was added as needed to a concentration of 5 mg/l and magnesium sulfate was added to a concentration of 0.0002 M. The minimal medium of VOGEL and BONNER (1956) was used in growing cells for use in assaying the enzymes of the tryptophan operon.

IPTG was dissolved in sterile distilled water at a concentration of 0.1 M. ONPG was dissolved at 0.013 M in 0.1 M Tris-HCl buffer, pH 7.0. XG was dissolved at 20 mg/ml in N,N'-dimethylformamide. XG indicator plates were prepared by overlaying an H-type plate with 0.01 ml of IPTG solution and 0.04 ml of XG solution mixed in 2.5 ml soft agar or H-type hard agar.

TABLE 2

Bacteriophage $\phi 80$ strains

Strain	Description	Anticodon of suppressor tRNA	5′ terminal sequence of suppressing tRNA	Source or reference
HA221	Carries su^+_{oc}-A2	U+UA	pGAU	ALTMAN, BRENNER and SMITH 1971
SI4061*	Revertant of HA221. Carries G2 mutation with su^+_{oc} and second non-suppressing tRNA	U+UA	pGGU	FEINSTEIN 1977
ts28	Carries su^+_3-A2	CUA	pGAU	SMITH *et al.* 1970
psu_3	Carries su^+_3-G2	CUA	pGGU	RUSSELL *et al.* 1970

* SI4061 was spontaneously induced from a nontemperature-sensitive suppressor strain derived from a temperature-sensitive suppressing lysogen of HA221.

† Abbreviations used: IPTG = isopropylthio-β-D-galactoside. ONPG = O-nitrophenyl-β-D-galactoside. XG = 5-bromo-4-chloro-3-indolyl-β-D-galactoside.

TABLE 3

Bacterial strains

A. Parental strains:

Strain	Sex	Markers	Source	Reference
CSH39	F′	*lac*YA536-am on F′, thi⁻	J. SADLER	MILLER 1972
W3110	F⁻	*trpR*⁻, *tna*⁻, *cysB*⁻	C. YANOFSKY	YANOFSKY and ITO 1966
W3110	F⁻	*trpE*9914-am	C. YANOFSKY	BRONSON, SQUIRES and YANOFSKY 1973
W3110	F⁻	*trpE*9914-oc	C. YANOFSKY	BRONSON, SQUIRES and YANOFSKY 1973
W3110	F⁻	*trpA*38-am	C. YANOFSKY	YANOFSKY and HORN 1972
W3110	F⁻	*trpA*38-oc	C. YANOFSKY	YANOFSKY and HORN 1972
W3110	F⁻	*trpA*96-am	C. YANOFSKY	YANOFSKY and HORN 1972
W3110	F⁻	*trpA*96-oc	C. YANOFSKY	YANOFSKY and HORN 1972
W3110	F′	*trp*A2/A2	C. YANOFSKY	WILSON and CRAWFORD 1965
W3110	F⁻	*trpB*8	C. YANOFSKY	HENNING *et al.* 1962

B. Newly constructed strains: All are *trpR*⁻, *tna*⁻, *lac*YA536-am. The construction of CT4000 is described in MATERIALS AND METHODS.

Strain designation		Markers	Prophage	How constructed
CT4000	F′	*cysB*⁻		Transfer from CSH39; strain homogenotization
CT4100	F′	*trpE*9914-am		P1 transduction into CT4000
CT4200	F′	*trpE*9914-oc		P1 transduction into CT4000
CT4160	F′	*trpE*9914-am	HA221 (su^+_{oc}-A2)	Lysogenization of CT4100
CT4170	F′	*trpE*9914-am	SI4061 (su^+_{oc}-G2)	Lysogenization of CT4100
CT4260	F′	*trpE*9914-oc	HA221 (su^+_{oc}-A2)	Lysogenization of CT4200
CT4270	F′	*trpE*9914-oc	SI4061 (su^+_{oc}-G2)	Lysogenization of CT4200
CT4300	F′	*trpA*38-am		P1 transduction into CT4000
CT4400	F′	*trpA*38-oc		P1 transduction into CT4000
CT4360	F′	*trpA*38-am	HA221 (su^+_{oc}-A2)	Lysogenization of CT4300
CT4370	F′	*trpA*38-am	SI4061 (su^+_{oc}-G2)	Lysogenization of CT4300
CT4460	F′	*trpA*38-oc	HA221 (su^+_{oc}-A2)	Lysogenization of CT4400
CT4470	F′	*trpA*38-oc	SI4061 (su^+_{oc}-G2)	Lysogenization of CT4400
CT4500	F′	*trpA*96-am		P1 transduction into CT4000
CT4600	F′	*trpA*96-oc		P1 transduction into CT4000
CT4560	F′	*trpA*96-am	HA221 (su^+_{oc}-A2)	Lysogenization of CT4500
CT4570	F′	*trpA*96-am	SI4061 (su^+_{oc}-G2)	Lysogenization of CT4500
CT4660	F′	*trpA*96-oc	HA221 (su^+_{oc}-A2)	Lysogenization of CT4600
CT4670	F′	*trpA*96-oc	SI4061 (su^+_{oc}-A2)	Lysogenization of CT4600
CT4700	F′	*trp*+		P1 transduction into CT4000

5-Methyltryptophan was added to minimal glucose plates lacking tryptophan at 100 μg/ml (MILLER 1972).

Construction of lysogens with derivatives of φ80-carrying suppressor genes

Bacterial strains carrying a *lac* amber mutation along with the appropriate tryptophan markers were streaked on XG indicator plates. After the plates had dried, approximately 10^9 of the

appropriate transducing phage (HA221 or SI4061) were spotted on the streak. Colonies showing blue color upon incubation were picked and purified by restreaking several times on XG indicator plates until relatively stable lysogens had been achieved.

Assays of β-galactosidase activity

The method used has been described by FEINSTEIN and ALTMAN (1977) for assays performed with extracts of lysogens.

Construction of strain CT4000

A *trpR*$^-$, *tna*$^-$, *cysB*$^-$ derivative of W3110 obtained from CHARLES YANOFSKY, Stanford University, was crossed with strain CSH39 (obtained from DR. JACK SADLER, University of Colorado), which carries an episome bearing a *lacZ* amber mutation, YA536. Cells carrying the episome were identified by testing for sensitivity to MS2 phage. One of these was screened for *lac*$^-$ colonies on *lac*$^-$ MaConkey plates. Such colonies arise by strain homogenotization. In spot tests with the φ80 transducing phages HA221 (su^+_{oc}-A2) and ts 28 (su^+_3-A2), which carry suppressor tRNA genes, these isolates could be transduced to a *lac*+ phenotype on XG indicator plates, thus showing that the lesion in the *lac* gene was an amber mutation. Further tests with one of these colonies showed that it could grow on 5-methyltryptophan plates, indicating the continued presence of the *trpR*$^-$ mutation. The new strain was called CT4000. It grew poorly, even in rich medium, unless supplemented with cystine.

P1 transductions and construction of suppressor lysogens

The general procedure used for P1 transduction of nonsense mutations in the tryptophan operon into strain CT4000 was that of LOW *et al.* (1971). Transductants were selected for the *cysB*+ phenotype and tested for tryptophan auxotrophy. Spot tests on minimal glucose plates were performed with psu3 (su^+_3-A2) or SI4061 (su^+_{oc}-G2) by selection on XG as described above or on minimal lactose plates. The intrinsic suppressor strength of each lysogen was monitored by assaying the β-galactosidase activity.

Assays of the enzymes of the tryptophan operon

Strains to be assayed for *trpE* or *trpA* activity were grown from a single colony of appropriate phenotype to saturation in the minimal medium of Vogel and Bonner, which was supplemented with 0.2% glucose and 10 μg/ml tryptophan. This culture was used as an inoculum for a 200 or 400 ml culture in the same medium supplemented with 0.2% glucose and 50 μg/ml tryptophan. The general procedure followed that of YANOFSKY and ITO (1966) for growth under conditions of repression. Cells were harvested in late log phase, washed once on 0.9% NaCl, concentrated 40–80 fold into 0.1 M Tris-HCl buffer, pH 7.8, sonicated, and centrifuged for 30 minutes at 30,000 × *g*. Supernatant liquids were kept on ice and assayed for the enzymes of the tryptophan operon.

Assays of anthranilate synthetase (the product of the *trpE* gene) were performed following the method described by CREIGHTON and YANOFSKY (1970). This involves the conversion of chorismate to anthranilate and is monitored by the increase in fluorescence using an Aminco-Bowman spectrophotometer, an activation wavelength of 314 nanometers and an emission wavelength of 390 nanometers. No excess of the D subunit was added, so that the assay was performed in two ways; using either glutamine as the amino donor or ammonium sulfate as the amino donor, which gives less activity but does not require an excess of the D subunit and is not inhibited by tryptophan. Wild-type and unsuppressed mutants were compared with the suppressed strains for activity.

Assays of tryptophan synthetase activity followed the procedure of SMITH and YANOFSKY (1962) with the appropriate excesses of either α or β subunit being added. Extracts of strain B8 were used as a source of the α subunit of the tryptophan synthetase complex and extracts of strain A2/A2 were used as a source of the β subunit of the complex. The strains B8 and A2/A2 were supplied by CHARLES YANOFSKY (Stanford University). These cells were grown from a 1:100 dilution of a saturated B broth culture in the minimal medium of Vogel and Bonner,

supplemented with 0.5% glucose, 0.05% neutralized acid-hydrolyzed casein and 2.5 μg/ml indole. Cells were harvested in late log phase, washed once in 0.9% NaCl, concentrated about 400-fold into 0.1 M Tris-HCl buffer, pH 7.8, sonicated, centrifuged for 30 minutes at 144,000 × *g*, and the supernatant liquid was divided into small aliquots to be frozen at −15°. Each type was assayed in the presence of the other for activity in the assay of SMITH and YANOFSKY (1962) involving the measurement of the conversion of tryptophan to indole. The specific activity of the α extract was 2,000 units per gram of protein, while that of the β extract was about 600 units per gram of protein. Thawed extracts were not reused.

The uncombined β_2 subunit had a residual activity of about 2% of the $\alpha_2\beta_2$ activity. The formula used for calculating the true α activity is:

$$\alpha = \frac{\alpha' - 0.02\beta}{0.98}$$

where α = true α activity, β = β activity and α' = measured activity.

Protein concentrations were assayed by the method of BRADFORD (1976). The activity of the enzymes was normalized to the protein concentration.

Determination of generation times

Generation times of various tryptophan mutant strains and the appropriate wild type were determined using a Klett-Summerson colorimeter and sidearm flasks. Initial cell density was measured. Cells were then grown at 30° and readings of the optical density, using a No. 62 filter, were made at regular intervals. A blank of distilled water was employed to compensate for any drift of the colorimeter. Vogel and Bonner's medium supplemented with 0.2% glucose was used for wild type, mutants lysogenized with suppressor phage and nonsense mutants. Lysogenized mutants were also tested in medium supplemented with 2 μg/ml tryptophan and 0.2% glucose.

Cell-density readings *versus* time were plotted on semilog paper. The slope of the linear portion of the curve was measured in $\log_{10}$ units divided by hours. Generation time (in hours) was then determined by dividing the slope into $\log_{10} 2$.

RESULTS

General considerations: Measurement of suppression efficiencies were made with lysogens (or extracts of lysogens) constructed from the appropriate bacterial strains and φ80-transducing phage carrying the suppressor tRNA gene. In making lysogens with this phage, it has been found that different isolates can differ widely in their suppression efficiencies (FEINSTEIN and ALTMAN 1977; FEINSTEIN 1977). Therefore, the intrinsic suppressor strength of each lysogen used was quantitated by measuring suppression of an amber mutant in the structural gene for β-galactosidase (*lac* YA536-am) which was common to all the strains. As shown below, this number was used in the comparison of nonsense suppression in the *trpE* and *A* genes. Quantitation of suppression efficiencies of nonsense mutants in either the *trpE* or *trpA* genes was accomplished through measurements of growth rates in the absence of exogenous tryptophan or measurements of suppressed enzyme activity as was appropriate.

The assay for the *trpE* gene product involves the quantitation of the production of anthranilate from chorismate by fluorescence measurements (Figure 1). The assay is extremely sensitive and allows the detection of even very small amounts of suppressed enzyme activity. Therefore, at site E9914, both growth rate and enzyme activity were used to estimate suppression efficiencies. Either gluta-

mine or ammonium sulfate can be used as an amino group donor in the conversion of chorismate to anthranilate. Assays of the *trpE* gene product that use glutamine as the amino donor in the chorismate to anthranilate reaction require an excess of the *trpD* gene product (Figure 1). If ammonium sulfate is utilized as the amino donor instead, no excess of the *trpD* gene product is required; in addition, the reaction is then not subject to feedback inhibition by tryptophan as it is when glutamine is the amino donor. The use of ammonium sulfate to replace glutamine does, however, result in an approximately five1fold decrease in the efficiency of the reaction (ITO and YANOFSKY 1969).

A comparison of the results with glutamine and ammonium sulfate suggests that, except in the case of wild type, there is enough excess *trpD* gene product present in these extracts, so that it is not necessary to add it exogenously (Table 4C). The reaction in the presence of glutamine was sufficiently sensitive to quantitate even the enzyme activities of the unsuppressed nonsense mutations. For comparisons with wild type, the reaction with ammonium sulfate was employed.

The assay for tryptophan synthetase activity involves the conversion of indole to tryptophan in the presence of serine (Figure 1). This requires the combination of the α_2 (*trpA* gene product) and β_2 subunits; however, the β_2 subunit alone does have a residual activity of about 1–3% in this reaction (SMITH and YANOFSKY 1962). The particular amino acid being inserted by the suppressor is not a factor, since all α-CRM products are equally efficient in activating the β_2 complex to carry out this reaction (SOLL and BERG 1969). The method of quantitation involves the measurement of disappearance of indole. Measurement of the appearance of tryptophan, which would normally be more sensitive for small amounts of enzyme activity, are hampered by the presence of tryptophan in the crude extracts of the enzymes being assayed for activity.

The lack of sensitivity of the assay presents a problem in the quantitation of weak suppressors. An additional problem is presented by the residual activity of the β_2 subunit. Since the nonsense mutations are in the gene for the α subunit, small amounts of suppressed enzyme activity will be masked by the residual activity of the β_2 subunit, which is present at or near wild-type levels. Lysogens of HA221 (su^+_{oc}-A2) are weak suppressors, and suppressed enzyme activity in these lysogens could not readily be quantitated by the *trpA*38 and *trpA*96 sites because of these problems. However, growth rate of these lysogens in the absence of tryptophan was slower than that which they show in its presence, allowing the quantitation of differences in growth rate, which presumably reflect differences in suppression efficiency. Lysogens of SI4061 (su^+_{oc}-G2), on the other hand, show approximately equal growth rates whether or not tryptophan is supplied, but they produce enough suppressed enzyme activity to be quantitated with reasonable accuracy in the assay.

The trpE*9914 site*: The data concerning suppression at the homotopic pair located in the *trpE* gene at site 9914 are summarized in Table 4. The two lysogens of the homotopic pair at site 9914 in the *trpE* gene containing HA221 (su^+_{oc}-A2) (CT4160 and CT4260) give approximately equal amounts of β-galactosidase

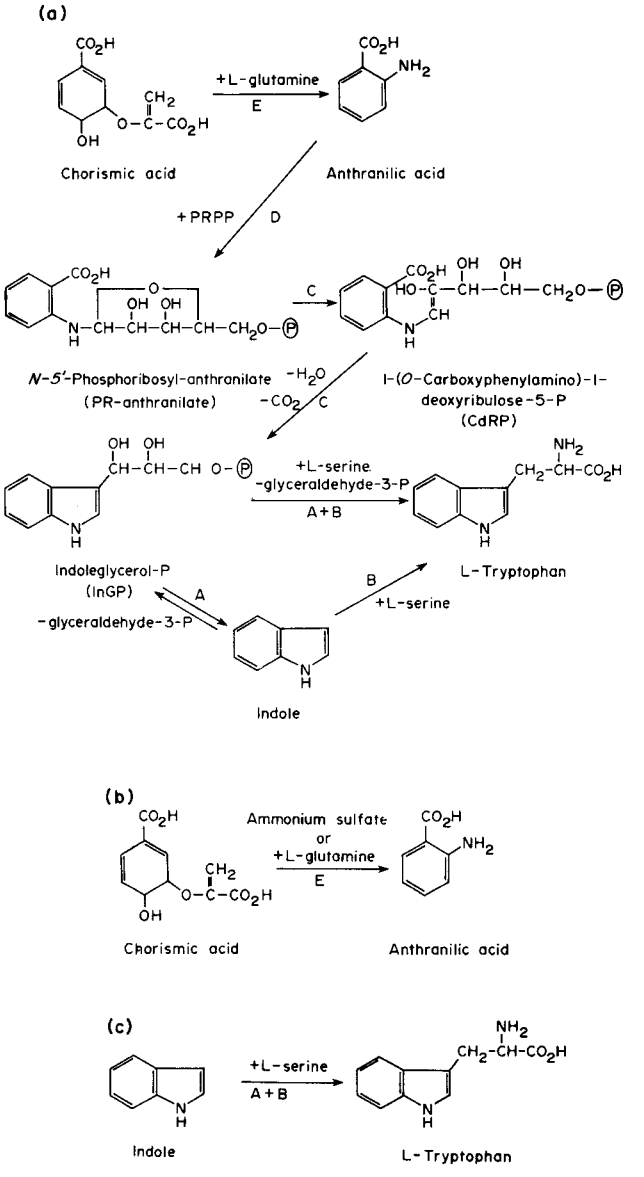

FIGURE 1.—Enzymes of the tryptophan operon. (Redrawn from CREIGHTON and YANOFSKY 1970).

(a) The tryptophan biosynthetic pathway: The lettering system follows the structural gene designations in *E. coli*. Thus, the enzymes catalyzing the various reactions are:

A, the α-subunit of tryptophan synthetase.

B, the β-subunit of tryptophan synthetase.

C, indole-3-glycerol phosphate synthetase and PR-anthranilate isomerase.

D, anthranilate-PR-transferase.

E, anthranilic acid synthetase.

(b) The reaction catalyzed by anthranilic acid synthetase: If glutamine is used as the amino donor, this reaction requires a complex of the anthranilic acid synthetase (E) and anthranilate-

TABLE 4

Suppression at site trpE*9914. Context:* $CUU\ UAA\ CUG$ / $C\ UAG$

A. β-Galactosidase activity:

Strain	Suppressed enzyme activity (O. D. units/(hr rxn × 10^8 cells/ml))
CT4160 (amber-HA221-*su*$^+_{oc}$-A2)	0.0065 (2)
CT4260 (ochre-HA221-*su*$^+_{oc}$-A2)	0.0066 (2)
CT4170 (amber-SI4061-*su*$^+_{oc}$-G2)	0.0446 (1)
CT4270 (ochre-SI4061-*su*$^+_{oc}$-G2)	0.0727 (1)

B. Doubling times:

Strain	Doubling time (hr) no tryptophan present	Doubling time (hr) tryptophan present
CT4100 (amber)	No growth	Not tested
CT4200 (ochre)	No growth	Not tested
CT4160 (amber-HA221-*su*$^+_{oc}$-A2)	3.55 (3)	2.99 (1)
CT4260 (ochre-HA221-*su*$^+_{oc}$-A2)	3.73 (3)	3.02 (1)
CT4170 (amber-SI4061-*su*$^+_{oc}$-G2)	3.19 (1)	3.05 (1)
CT4270 (ochre-SI4061-*su*$^+_{oc}$-G2)	3.01 (1)	3.03 (1)
CT4700 (wild type, *su*$^-$)	2.50 (1)	Not tested

C. *TrpE* Enzyme activity:

Strain	Suppressed *TrpE* activity with glutamine*	Suppressed *TrpE* activity with $(NH_4)_2SO_4$†	Percent wild type
CT4100 (amber)	1.1 (1)	Not detectable	
CT4200 (ochre)	0.91 (1)	Not detectable	
CT4160 (amber-HA221-*su*$^+_{oc}$-A2)	7.0 (2)	0.87 (2)	0.04
CT4260 (ochre-HA221-*su*$^+_{oc}$-A2)	7.3 (2)	0.94 (2)	0.04
CT4170 (amber-SI4061-*su*$^+_{oc}$-G2)	58.7 (2)	10.0 (2)	0.43
CT4270 (ochre-SI4061-*su*$^+_{oc}$-G2)	65.5 (2)	11.5 (2)	0.50
CT4700 (wild type, *su*$^-$)	6500 (1)	2300 (1)	100

* Glutamine was used as an amino donor.

† $(NH_4)_2SO_4$ was used as an amino donor.

Suppressed β-galactosidase activity, cell growth rate and *trpE* activity were measured as described in MATERIALS AND METHODS. Calculations of *trpE* activity units were based on measurement of an anthranilate standard. Percentages of wild type are based on results with strain CT4700, a *cys*+, *trp*+, *su*$^-$ derivative of CT4000. The number of times an experiment was performed is shown in parentheses. In cases where more than one experiment was performed, the numbers were averaged.

TrpE activity is presented in units/gram protein. Units are those of ITO, COX and YANOFSKY (1969).

PR-transferase (D). However, if ammonium sulfate is used as the amino donor, only anthranilic acid synthetase is required. Enzyme activity is assayed by the spectrophotoflurometric quantitation of the anthranilate that is produced.

(c) The reaction used to quantitate the enzyme activity of the α-subunit of tryptophan synthetase: All α-CRMs are known to activate the β_2-subunit equally in this reaction. The β_2-subunit itself is active, even when uncombined with α, at a level of 1–3% of the activity it shows when combined. Enzyme activity is quantitated by measuring the disappearance of indole using indole reagent (*p*-dimethylaminobenzaldehyde).

activity (Table 4A), indicating that the intrinsic amount of suppressor tRNA being made is the same in both cases. As shown in Table 4B, there is a small difference in the doubling time, with the amber member of the pair growing slightly faster in the absence of tryptophan. In the presence of tryptophan, the growth rate is not significantly different for the amber strain than for the ochre. The unsuppressed mutants fail to grow even after 48 hours of incubation. If the experiment is continued beyond this point, revertants overgrow the mutant cells. When enzyme activity is measured, the ochre lysogen HA221 (su^+_{oc}-A2) is found to give slightly more activity when tested with glutamine or with ammonium sulfate (Table 4C); however, the difference is again very small and not significant for our purposes.

Lysogens containing SI4061 (su^+_{oc}-G2), the nontemperature sensitive revertant of (su^+_{oc}-A2), were also constructed. In this case, however, the ochre member of the pair (CT4270) has somewhat more β-galactosidase activity than the amber-carrying lysogen (CT4170), indicating that it makes more suppressor tRNA molecules. Therefore it is not surprising to find that, in this case, the ochre lysogen (CT4270) shows both a slightly shorter doubling time (Table 4B) and slightly more enzyme activity (Table 4C) than its amber counterpart. With both suppressors, SI4061 (su^+_{oc}-G2) and HA221 (su^+_{oc}-A2) then, site E9914 is one at which no clear preference for the amber or ochre codons can be established. Rather both types of nonsense codon are suppressed with nearly equal efficiency by each of these two ochre suppressors.

The trpA*38 site*: Lysogens of the homotopic amber-ochre pair at the *trpA*38 site, whether made with HA221 (su^+_{oc}-A2) or with SI4061 (su^+_{oc}-G2) clearly suppress the ochre codon with greater efficiency. The HA221 (su^+_{oc}-A2) lysogen of the *trpA*38 ochre mutant, CT4460, has only one-third as much β-galactosidase activity as its amber counterpart (CT4360) (Table 5A), indicating that it contains a lower level of suppressor tRNA. Despite this, the suppressed ochre mutant CT4460 is found to double in the absence of tryptophan in 5.3 hours, while CT4360, containing both the amber mutant and the suppressor follows the pattern of the unsuppressed nonsense mutations in failing to display any growth at all under these conditions. In the presence of tryptophan, however, both strains exhibit approximately the same doubling time (Table 5B). Assays of enzyme activity in these strains are at the limits of sensitivity of the assay for tryptophan synthetase activity and not reliable.

Lysogens of the *trpA*38 homotopic amber-ochre pair made with the more efficient suppressor SI4061 (su^+_{oc}-G2) were found to have nearly equal amounts of β-galactosidase activity (Table 5C). However, the ochre lysogen (CT4470) has a slightly faster doubling time (in the absence of tryptophan than the amber member of the pair (CT4370) (Table 5B) and a much greater amount of enzyme activity that can be reliably measured (Table 5C). We conclude that site *trpA*38 is one at which both suppressors are more efficient on ochre rather than amber codons. The approximately six-fold difference in suppressed enzyme activity of the ochre codon (carried by CT4470) as compared to the amber codon (carried

TABLE 5

Suppresssion at site trpA *38. Context: CGC* $\overset{UAA}{UAG}$ *GAA*

A. β-Galactosidase activity:

Strain	Suppressed enzyme activity (O. D. units/(hr rxn × 10^8 cells/ml))
CT4360 (amber-HA221-su^+_{oc}-A2)	0.0060 (2)
CT4460 (ochre-HA221-su^+_{oc}-A2)	0.0021 (2)
CT4370 (amber-SI4061-su^+_{oc}-G2)	0.0717 (1)
CT4470 (ochre-SI4061-su^+_{oc}-G2)	0.0669 (1)

B. Doubling times:

Strain	Doubling time (hr) no tryptophan present	Doubling time (hr) tryptophan present
CT4300 (amber)	No growth (1)	Not tested
CT4400 (ochre)	No growth (1)	Not tested
CT4360 (amber-HA221-su^+_{oc}-A2)	No growth (2)	2.82 (1)
CT4460 (ochre-HA221-su^+_{oc}-A2)	5.32 (2)	2.89 (1)
CT4370 (amber-SI4061-su^+_{oc}-G2)	3.35 (2)	2.66 (1)
CT4470 (ochre-SI4061-su^+_{oc}-G2)	2.75 (2)	2.71 (1)
CT4700 (wild type, su^-)	2.50 (1)	Not tested

C. *TrpA* Enzyme activity:

Strain	Suppressed α activity as % β activity	Suppressed α activity/ gram protein*	Percent wild type
CT4300 (amber)	0.63	42.4	0.11 (2)
CT4400 (ochre)	0.67	31.8	0.08 (2)
CT4370 (amber-SI4061-su^+_{oc}-G2)	2.1	84.9	0.21 (2)
CT4470 (ochre-SI4061-su^+_{oc}-G2)	13.0	564	1.41 (2)
CT4700 (wild type, su^-)	107.4	40,000	100 (1)

* Units are those of SMITH and YANOFSKY 1962.

Suppressed β-galactosidase activity, cell growth rate and tryptophan synthetase activity for α and β subunits were measured as described in MATERIALS AND METHODS. Percentages of wild type are based on results with strain CT4700, a *cys*+, *trp*+, su^- derivative of strain CT4000. The number of times an experiment was performed is shown in parentheses. In cases where more than one experiment was performed, the numbers were averaged.

by CT4370) is the most decisive preference for ochre seen at any of the sites examined here or previously (FEINSTEIN and ALTMAN 1977).

The trpA*96 site*: The lysogens made with HA221 (su^+_{oc}-A2) of the homotopic amber and ochre mutants of the *trpA*96 site gave approximately equal amounts of suppressed β-galactosidase activity as shown in Table 6A. In the absence of tryptophan, the HA221 (su^+_{oc}-A2) lysogen of the ochre strain, CT4660, had a longer doubling time than the lysogen of the ochre strain, CT4660, had a a longer doubling time than the lysogen of the amber mutant with HA221 (su^+_{oc}-A2) CT4560 (Table 6C). No assay of enzyme activity was attempted with these lysogens, but it is apparent that this is a site at which the HA221-carried suppressor, su^+_{oc}-A2 favors amber.

TABLE 6

Suppression at site trpA*96. Context:* $GA^{A\ UAA}_{G\ UAG}CA^{U}_{C}$

A. β-Galactosidase activity:

Strain	Suppressed enzyme activity (O. D. units/(hr rxn × 10^8 cells/ml))
CT4560 (amber-HA221-su^+_{oc}-A2)	0.0106 (2)
CT4660 (ochre-HA221-su^+_{oc}-A2)	0.0104 (2)
CT4570 (amber-SI4061-su^+_{oc}-G2)	0.0295 (1)
CT4670 (ochre-SI4061-su^+_{oc}-G2)	0.0408 (1)

B. Doubling times

Strain	Doubling time (hr) no tryptophan present	Doubling time (hr) tryptophan present
CT4500 (amber)	No growth (1)	Not tested
CT4600 (ochre)	No growth (1)	Not tested
CT4560 (amber-HA221-su^+_{oc}-A2)	3.41 (3)	2.59 (2)
CT4660 (ochre-HA221-su^+_{oc}-A2)	6.72 (3)	2.57 (1)
CT4570 (amber-SI4061-su^+_{oc}-G2)	3.04 (1)	Not tested
CT4670 (ochre-SI4061-su^+_{oc}-G2)	2.75 (1)	Not tested
CT4700 (wild type, su^-)	2.50 (1)	Not tested

C. *TrpA* Enzyme activity:

Strain	Suppressed α activity as % β activity	Suppressed α activity/ gram protein*	Percent wild type
CT4500 (amber)	0.23	17.7	0.04 (1)
CT4600 (ochre)	No measurable α activity	—	—
CT4570 (amber-SI4061-su^+_{oc}-G2)	1.7	178	0.45 (1)
CT4670 (ochre-SI4061-su^+_{oc}-G2)	1.3	166	0.42 (1)
CT4700 (wild type, su^-)	107.4	40,000	100 (1)

* Units are those of SMITH and YANOFSKY 1962.

Suppressed β-galactosidase activity, cell growth rate and tryptophan synthetase activity for α and β subunits were measured as described in MATERIALS AND METHODS. Percentages of wild type are based on results with strain CT4700, a *cys*+, *trp*+, *su*− derivative of strain CT4000. The number of times an experiment was performed is shown in parentheses. In cases where more than one experiment was performed, the numbers were averaged.

Lysogens of the mutants at this site were also made with the phage SI4061 (su^+_{oc}-G2). As shown in Table 6A, there is more suppressed β-galactosidase activity in the ochre member of the pair, CT4670, than in its amber counterpart, CT4570. The difference seen in doubling times in the absence of tryptophan (Table 6B) may not be significant as the variation in measurements of doubling times is about 5%. An examination of tryptophan synthetase activity (Table 6C) indicates that both the CT4670 and CT4570 lysogens produce approximately equal amounts of this suppressed enzymatic activity. Since the ochre lysogen (CT4670) has more intrinsic suppressor tRNA as judged by the greater amount

of β-galactosidase activity it produces, one might conclude that the amber codon is somewhat better suppressed at this site than the ochre codon by the SI4061 (su^+_{oc}-G2) suppressor. However, it is difficult to know exactly how to use the measurements of intrinsic suppressor strength for accurate strain comparison. (The β-galactosidase active protein is a tetramer, whereas the subunit composition of the *trpE* gene functional unit is unknown and the *trpA* gene product functions in an $\alpha_2\beta_2$ complex.)

DISCUSSION

A different suppression pattern of homotopic ochre and amber mutations is observed at each of three sites in the tryptophan operon. Each site occurs in a different context of adjacent nucleotides. Previously, it was found that the presence of CAA following the nonsense codon resulted in the favoring of the amber codon by all ochre suppressors tested (FEINSTEIN and ALTMAN 1977). Data gathered at the *trpA*96 site indicate that CA^U_C has a similar effect. However, CUG following the nonsense codon at site *trpE*9914 results in approximately equal suppression of amber and ochre codons. Thus, more nucleotides than just a C following the nonsense codon are involved in eliciting the greater suppression efficiency of the amber codon. At the *trpA*38 site, GAA follows the nonsense codon and ochre is decisively favored.

The strongest suppressor used has an absolute efficiency of 1–2% when compared to wild-type level *trpA* enzymatic levels in the strain CT4700. This calculation of suppression efficiency is a minimum one since, among other factors, it does not take into account the possible deleterious effect of the suppressor tRNA on general cell metabolism. Nevertheless, 2% of wild-type enzymatic activity in the *trpA* gene is sufficient to give a growth rate close to that of wild type. A detailed discussion of suppression patterns exhibited by derivatives of $tRNA^{Tyr}_1$ is given in FEINSTEIN and ALTMAN (1977).

The nature of the nucleotides preceding the nonsense codon may also be important in governing suppression efficiency. However, there is direct evidence in the *rII B* cistron that most ochre suppressors are relatively unaffected by a change in the nucleotides preceding the nonsense codon (FEINSTEIN and ALTMAN 1977).

Of course, in our studies we have measured the effects on codon-anticodon pairing at the 3′ end of the codon (the wobble position). It seems reasonable that the nucleotides nearest this site would have the most influence on selection of the standard pairing needed for suppressing ochre codons or the wobble pairing required for suppressing amber codons. Consistent with this notion are the results of a review of the data we presented here and those previously published (Table 7), which show that all ochre suppressors tested (with one possible exception; see Table 7E) favor the amber codon at sites followed by CAX. Furthermore the ochre suppressors psu^+_2 and su^+_B both favor the ochre codon at sites at which A follows the nonsense codon. Su^+_{oc}-A2 sometimes favors amber at these latter sites.

There is increasing evidence that nucleotide context can exert an influence on the translation of messenger RNA. (YAHATA, OCADA and TSUGITA 1970; COMER,

GUTHRIE and MCCLAIN 1974; COMER, Foss and MCCLAIN 1975; COLBY, SCHEDL and GUTHRIE 1976; AKABOSHI, INOUYE and TSUGITA 1976; ZILBERSTEIN *et al.* 1976; FLUCK, SALSER and EPSTEIN 1977). The studies described here and in FEINSTEIN and ALTMAN (1977) confirm the effect of mRNA context on codon-anticodon interaction and attempt to relate the particular sequences surrounding the nonsense codons to the suppression pattern found with various nonsense suppressors (Table 7). Obviously, these genetic studies are only a beginning in understanding the workings of this phenomenon, how it is brought about, and how it might function in translational control.

There are several ways in which interactions other than the standard codon-anticodon pairing could influence suppression patterns. First, the nucleotides adjacent to the codon could interact with the codon itself or other nearby nucleotides in the tRNA through hydrogen bonding, stacking or steric interference. Such interactions would be expected to have the greatest effect on the nucleotides nearest to the codon and anticodon. Nucleotide modification would be quite likely to affect such interactions, and according to this scheme differences in modification could explain differences between the suppression patterns of different tRNAs (ALTMAN 1976; COLBY, SCHEDL and GUTHRIE 1976).

Steric interactions between two tRNAs on the ribosome at the same time during protein synthesis (BRIMACOMBE *et al.* 1976) and binding to adjacent codons, must also be considered. These interactions may vary depending upon the shape, size and base composition of the tRNA as well as the conformation it takes upon binding to the messenger RNA on the ribosome. It is possible that the binding of the incoming suppressor tRNA might be constrained by the presence of the tRNA that is bound by the preceding codon. Similarly, if the suppressor tRNA that pairs with the nonsense codon being examined is hindering the binding of the incoming tRNA that would normally pair with the next codon, translation will be slowed down. Such a delay could give an advantage to the release factor in its competition with the tRNA (review by TATE and CASKEY 1974), thereby increasing the chances of an incomplete polypeptide being released.

Although these two schemes might be expected to have some special effects on systems where a nonsense suppressor is being studied, they are generally applicable to translation at any codon. Translation of nonsense codons in particular might be affected by competition between release factors and suppressor tRNA (review by TATE and CASKEY 1974). mRNA context could affect the pairing of release factors with the nonsense codon. Changes in this process caused by context differences may be the cause of the variation in suppression patterns seen at different sites. FLUCK, SALSER and EPSTEIN (1977) suggest that this is the main cause of such variation, arguing on theoretical grounds that otherwise the variation observed is so large that if it were true of translation in general, the rate of the process would have to vary over at least a thirty-fold range. However, our results (FEINSTEIN and ALTMAN 1977), which indicate that different suppressor tRNAs can vary widely in their suppression efficiencies at the same site, require an alternative explanation. Similarly, ZILBERSTEIN *et al.* (1976) have found that in extracts of interferon-treated cells, certain codons can be translated only by a

TABLE 7

Correlation of reading context with suppression pattern of ochre suppressors

Gene-Site	Suppressors tested	Context of nonsense mutation
A. Sites at which all tested suppressors favor amber:		
rII–X655	su^+_B, su^+_C, su^+_{oc}-A2, su^+_{oc}-A2 (non-ts)*	$\frac{\text{UAA}}{\text{UAG}}$ CAA
lysozyme-eL1–1a	psu^+_2, su^+_B, su^+_{oc}-A2	CUX or UPPu $\frac{\text{UAA}}{\text{UAG}}$ CAA
*trpA*96	su^+_{oc}-A2	GAA_{G} $\frac{\text{UAA}}{\text{UAG}}$ $\text{CA}^{\text{U}}_{\text{C}}$
B. Sites at which all tested suppressors favor ochre:		
rII–N24*	su^+_B, su^+_C, su^+_{oc}-A2, su^+_{oc}-A2 (non-ts)*	CAA $\frac{\text{UAA}}{\text{UAG}}$
*trpA*38	su^+_{oc}-A2, su^+_{oc}-G2	CGC $\frac{\text{UAA}}{\text{UAG}}$ GAA
C. Sites at which suppressors other than su^+-A2 favor ochre:		
rII–N24	psu^+_2, su^+_B	UGG $\frac{\text{UAA}}{\text{UAG}}$
lysozyme-eL2–2a	psu^+_2, su^+_B	CAA $\frac{\text{UAA}}{\text{UAG}}$ AAPu
lysozyme-eL3–3a	psu^+_2, su^+_B	UUPy $\frac{\text{UAA}}{\text{UAG}}$ AUG
lysozyme-eL5–5a	psu^+_2, su^+_B	AAPy $\frac{\text{UAA}}{\text{UAG}}$ ACX
D. Sites at which su^+-A2 favors amber while others favor ochre:		
rII–N24	su^+_{oc}-A2	UGG $\frac{\text{UAA}}{\text{UAG}}$
lysozyme-eL2–2a	su^+_{oc}-A2	CAA $\frac{\text{UAA}}{\text{UAG}}$ AAPu
lysozyme-eL5–5a	su^+_{oc}-A2	AAPy $\frac{\text{UAA}}{\text{UAG}}$ ACX
E. Sites at which no clear preference could be demonstrated:		
lysozyme-eL3–3a	su^+_{oc}-A2	UUPy $\frac{\text{UAA}}{\text{UAG}}$ AUG
*trpE*9914	su^+_{oc}-A2, su^+_{oc}-G2	CUU_{C} $\frac{\text{UAA}}{\text{UAG}}$ CUG
*trpA*96†	su^+_{oc}-G2	GAA_{G} $\frac{\text{UAA}}{\text{UAG}}$ $\text{CA}^{\text{U}}_{\text{C}}$

The suppressor preferences shown in this table are based on data from this paper and FEINSTEIN and ALTMAN (1977).

* non-ts = nontemperature sensitive derivative.

† Based on intrinsic suppressor strength measurements, the data for this case seem to indicate a preference for the amber codon. But since quantitative comparison of intrinsic suppressor strength in the two suppressor strains is difficult (see RESULTS) we have included this case in part E rather than part A of the table.

a particularly minor iso-accepting species of leucine tRNA, although the major species recognizes the very same codon. Furthermore, the wide variation in the contexts of natural termination signals (Table 8) and the finding that these natural signals can be suppressed with an efficiency of the same order of magnitude as nonsense codons internal to the translated portion of the messenger RNA (REMAULT and FIERS 1972; ATKINS and GESTELAND 1975), support the notion that more than just differences in release-factor affinity must be involved in context-mediated variations of suppression efficiencies.

TABLE 8

Reading contexts of natural termination signals

Termination signal(s) and context	Where found
A. Ambers:	
ser arg UCU AGA *UAG* AGC CCU	MS2 A or maturation protein*
ser lys UCU AAA *UAG* AGC CCU	R17 A or maturation protein*
pro arg CCU CGG *UAG* CUG ACC	MS2 replicase protein†
B. Ochres:	
val met met ser GUG AUG *UAA*UG UCU	End D-start J protein of ϕX174‡
arg lys ACG AAA *UAA* CCG UCA	Probable end of H protein of ϕX174‡
gln phe CAA UUU *UAA* UUG CAG	End J protein gene of ϕX174‡
tyr arg UAC CGU *UAA* GCU GGA	End α-hemoglobin gene of *H. sapiens*§
C. UGAs:	
ala tyr GCX UAP *UGA* ACX $^{\text{UUPu}}_{\text{CUX}}$	End coat protein of Qβ‖
gly lys GGA AAA *UGA* GAA AAU	End A protein of ϕX174‡
lys val AAG GAG *UGA* UGU AAU‡	End E protein gene of ϕX174‡
leu lys CUU AAG *UGA* GGU GAU	End G protein gene of ϕX174‡
gln ile met glu CAA AUG *UGA*UG GAA	End B-start A protein of *E. coli* tryptophan operon¶
D. Ochre-amber:	
ile tyr AUG UAC *UAA* *UAG* ACG CCG	End coat protein of MS2**

Termination signal(s) and context	Where found
ile tyr AUC UAC *UAA UAG* AUG CCG	End coat protein of R17*
ile tyr AUC UAC *UAA UAG* A$^{U}_{C}$G CCG	End coat protein of f2*
E. UGA-ochre: thr ser ACU UCG *UGA UAA* AAG AUU	End F protein of ϕX174‡
F. UGA-UGA: tyr phe UAU UUC *UGA UGA* GUC CAA	End B protein of ϕX174‡

* RNA sequence summarized in BARRELL and CLARK 1974.
† RNA sequence determined by VANDERBERGHE, MIN JOU and FIERS 1975.
‡ Based on DNA sequences determined by SANGER *et al.* 1977.
The sequence following the terminator of the E protein is also involved in the termination of the D protein and the beginning of the J protein as follows:

start J
v a l.m e t end D
l y s.v a l.end e
A A G G A G U G A U G U A A U G U C U

§ RNA sequence inferred from amino acid variants by CLEGG *et al.* 1974.
‖ RNA sequence inferred from sequence of read-through protein in WEINER and WEBER 1973.
¶ RNA sequence determined by PLATT and YANOFSKY 1975.
** RNA sequence found in MIN JOU *et al.* 1972.

We thank MARILYN NEWMUIS and LOIS ATKINS for technical assistance. We especially acknowledge the suggestions and criticism offered by K. BROOKS LOW. We are indebted to C. YANOFSKY for providing strains and advice concerning their use. S.I.F. was supported by a Public Health Service predoctoral training grant and the research was supported by Public Health Service Grant GM 19422 to S.A.

LITERATURE CITED

AKABOSHI, E., M. INOUYE and A. TSUGITA, 1976 Effect of neighboring nucleotide sequences on suppression efficiency in amber mutants of T4 phage lysozyme. Molec. Gen. Genet. **149:** 1–4.

ALTMAN, S., 1976 A modified uridine in the anticodon of *E. coli* $tRNA_1^{Tyr}su_{oc}^+$. Nucl. Acids Res. **3:** 441–448.

ALTMAN, S., S. BRENNER and J. D. SMITH, 1971 Identification of an ochre-suppressing anticodon. J. Mol. Biol. **56:** 195–197.

ATKINS, J. F. and R. F. GESTELAND, 1975 The synthetase gene of the RNA phages R17, MS2 and f2 has a single UAG terminator codon. Molec. Gen. Genet. **139:** 19–31.

BARRELL, B. G. and B. F. C. CLARK, 1974 *Handbook of Nucleic Acid Sequences.* Joynson-Bruvvers, Oxford, England.

BRADFORD, M. M., 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:** 248–254.

BRIMACOMBE, R., K. H. NIERHAUS, R. A. GARRETT and H. G. WITTMAN, 1976 The ribosome of *Escherichia coli.* Prog. Nucl. Acid Res. Mol. Biol. **18:** 1–44.

BRONSON, M. J., C. SQUIRES and C. YANOFSKY, 1973 Nucleotide sequences from tryptophan messenger RNA of *Escherichia coli*: The sequence corresponding to the amino-terminal region of the first polypeptide specified by the operon. Proc. Nat. Acad. Sci., U.S. **70:** 2335–2339.

CLEGG, J. B., D. J. WEATHERALL, I. CONTROPOLOU-GRIVA, K. CAROUTSOS, P. POUNGOURAS and H. TSEVRENIS, 1974 Haemoglobin Icaria, a new chain-terminaiton mutant which causes α-thalassaemia. Nature **251:** 245–247.

COLBY, D. S., P. SCHEDL and C. GUTHRIE, 1976 A functional requirement for modification of the wobble nucleotide in the anticodon of a T4 suppressor tRNA. Cell **9:** 449–463.

COMER, M. M., K. Foss and W. H. MCCLAIN, 1975 A mutation of the wobble nucleotide of a bacteriophage T4 transfer RNA. J. Mol. Biol. **99:** 283–293.

COMER, M. M., C. GUTHRIE and W. H. MCCLAIN, 1974 An ochre suppressor of bacteriophage T4 that is associated with a transfer RNA. J. Mol. Biol. **90:** 665–676.

CREIGHTON, T. E. and C. YANOFSKY, 1970 Chorismate to tryptophan (*Escherichia coli*)-anthranilate synthetase, PR transferase, PRA isomerase, InGP synthetase, tryptophan synthetase. pp. 365–380. In: *Methods in Enzymology*. Vol. XVIIA. Edited by H. TABOR and C. W. TABOR. Academic Press, New York.

FEINSTEIN, S. I., 1977 Suppression properties of an ochre suppressor derived from *Escherichia coli* $tRNA_1^{Tyr}$. Ph.D. Thesis, Yale University.

FEINSTEIN, S. I. and S. ALTMAN, 1977 Coding properties of an ochre-suppressing derivative of *Escherichia coli* $tRNA_1^{Tyr}$. J. Mol. Biol. **112:** 453–470.

FLUCK, M. M., W. SALSER and R. H. EPSTEIN, 1977 The influence of the reading context upon the suppression of nonsense codons. Molec. Gen. Genet. **151:** 137–149.

HENNING, U., D. R. HELINSKI, F. C. CHAO and C. YANOFSKY, 1962 The A protein of the tryptophan synthetase of *Escherichia coli*. J. Biol. Chem. **237:** 1523–1530.

ITO, J., E. C. Cox and C. YANOFSKY, 1969 Anthranilate synthetase an enzyme specified by the tryptophan operon of *Escherichia coli*: Purification and characterization of component I. J. Bacteriol. **97:** 725–733.

ITO, J. and C. YANOFSKY, 1969 Anthranilate synthetase, an enzyme specified by the tryptophan operon of *Escherichia coli*: Comparative studies on the complex and the subunits. J. Bacteriol. **97:** 734–742.

KATZ, E., 1969 Genetic studies in the rII region of bacteriophage T4. D. Phil. Thesis, Cambridge University, Cambridge, England.

LOW, K. B., F. GATES, T. GOLDSTEIN and D. SÖLL, 1971 Isolation and partial characterization of temperature-sensitive *Escherichia coli* mutants with altered leucyl- and seryl-transfer ribonucleic acid synthetases. J. Bacteriol. **108:** 742–750.

MILLER, J. H., 1972 *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MIN JOU, W., G. HAEGMAN, M. YSEBAERT and W. FIERS, 1972 Nucleotide sequence of the gene coding for the bacteriophage MS2 coat protein. Nature **237:** 82–88.

PLATT, T. and C. YANOFSKY, 1975 An intercistronic region and ribosome-binding site in bacterial messenger RNA. Proc. Nat. Acad. Sci. U.S. **72:** 2399–2403.

REMAUT, E. and W. FIERS, 1972 Studies on the bacteriophage MS2. XVI. The termination signal of the A protein cistron. J. Mol. Biol. **71:** 243–261.

RUSSELL, R. L., J. N. ABELSON, A. LANDY, M. L. GEFTER, S. BRENNER and J. D. SMITH, 1970 Duplicate genes for tyrosine transfer RNA in *Escherichia coli*. J. Mol. Biol. **47:** 1–13.

SALSER, W., 1969 The influence of the reading context upon the suppression of nonsense codons. Molec. Gen. Genet. **105:** 125–130.

SALSER, W., M. FLUCK and R. EPSTEIN, 1970 The influence of the reading context upon the suppression of nonsense codons, III. Cold Spring Harbor Symp. Quant. Biol. **34:** 513–520.

SANGER, F., G. M. AIR, B. G. BARRELL, N. L. BROWN, A. R. COULSON, J. C. FIDDES, C. A. HUTCHISON, III, P. M. SLOCOMBE and M. SMITH, 1977 Nucleotide sequence of bacteriophage ϕX174 DNA. Nature **265:** 687–695.

SMITH, J. D., L. BARNETT, S. BRENNER and R. L. RUSSELL, 1970 More mutant tyrosine transfer ribonucleic acids. J. Mol. Biol. **54:** 1–14.

SMITH, O. H. and C. YANOFSKY, 1962 Enzymes involved in the biosynthesis of tryptophan. pp. 794–806. In: *Methods in Enzymology*. Vol. V. Edited by S. P. COLOWICK and N. O. KAPLAN. Academic Press, New York.

SOLL, L. and P. BERG, 1969 Recessive lethals: A new class of nonsense suppressors in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S. **63:** 392–399.

TATE, W. P. and C. T. CASKEY, 1974 The mechanism of peptide chain termination. Molec. Cell. Biochem. **5:** 115–126.

VANDERBERGHE, A., W. MIN JOU and W. FIERS, 1975 3′-Terminal nucleotide sequence (n = 361) of bacteriophage MS2 RNA. Proc. Nat. Acad. Sci. U.S. **72:** 2559–2565.

VOGEL, H. J. and D. M. BONNER, 1956 Acetylornithinase of *Escherichia coli*: Partial purification and some properties. J. Biol. Chem. **218:** 97–106.

WEINER, A. M. and K. WEBER, 1973 A single UGA codon functions as a natural termination signal in the coliphage Qβ coat protein cistron. J. Mol. Biol. **80:** 837–855.

WILSON, D. A. and I. P. CRAWFORD, 1965 Purification and properties of the B component of *Escherichia coli* tryptophan synthetase. J. Biol. Chem. **240:** 4801–4808.

YAHATA, H., Y. OCADA and A. TSUGITA, 1970 Adjacent effect on suppression efficiency. II. Study on ochre and amber mutants of T4 phage lysozyme. Molec. Gen. Genet. **106:** 208–212.

YANOFSKY, C., G. R. DRAPEAU, J. R. GUEST and B. C. CARLTON, 1967 The complete amino acid sequence of the tryptophan synthetase A protein (α subunit) and its colinear relationship with the genetic map of the A gene. Proc. Nat. Acad. Sci. U.S. **57:** 296–298.

YANOFSKY, C. and V. HORN, 1972 Tryptophan synthetase α chain positions affected by mutations near the ends of the genetic map of *trpA* of *Escherichia coli*. J. Biol. Chem. **247:** 4494–4498.

YANOFSKY, C. and J. ITO, 1966 Nonsense codons and polarity in the tryptophan operon. J. Mol. Biol. **21:** 313–334.

ZILBERSTEIN, A., B. DUDOCK, H. BERISSI and M. REVEL, 1976 Control of messenger RNA translation by minor species of leucyl-transfer RNA in extracts from interferon-treated L cells. J. Mol. Biol. **108:** 43–54.

Corresponding editor: F. SHERMAN