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# Mutants of elongation factor Tu promote ribosomal frameshifting and nonsense readthrough

**Diarmaid Hughes[1,2], John F.Atkins[3,4] and Shahla Thompson[1]**

[1]Department of Genetics, Trinity College, Dublin 2, Ireland, [2]Institute of Molecular Biology, The Biomedical Center, Box 590, S-751 24 Uppsala, Sweden, [3]Howard Hughes Medical Institute, Department of Human Genetics, University of Utah, Salt Lake City, UT 84132, USA and [4]Department of Biochemistry, University College, Cork, Ireland

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**This is the first report of ribosomal frameshifting promoted by mutants of the elongation factor Tu (EF-Tu). EF-Tu mutants can suppress both −1 and +1 frameshift mutations. The level of nonsense readthrough is also increased at some UGA (this paper) and UAG (Hughes, 1987) sites by these mutants. Suppression occurs when a mutant *tuf* allele is paired with a wild-type copy of the other *tuf* gene but is most efficient when both *tuf* genes are mutant. Frameshifting mediated by the *tuf* alleles studied, *tufA8* and *tufB103*, is not general; indeed most frameshift mutations are not suppressed. Several possible mechanisms by which mutant EF-Tu may cause frameshifting are discussed.**

*Key words:* ribosomal frameshifting/frameshift suppression/nonsense readthrough/mutated *tuf* genes/elongation factor Tu

## Introduction

The accuracy of protein synthesis during translational elongation is dependent on two selections: the selection of the correct amino acyl (aa)-tRNA on the codon-programmed ribosome and the selection of each correct successive codon on the message. The accuracy of the aa-tRNA selection is supported by a proof-reading mechanism involving the hydrolysis of GTP from the ternary complex of elongation factor Tu–aminoacyl tRNA–GTP (EF-Tu–aa-tRNA–GTP) (Hopfield, 19874; Ninio, 1975; Thompson and Stone, 1977; Ruusala *et al.*, 1982). The second selection, that of each correct successive codon, is potentially more problematic. Since the genetic code is punctuated, any reading frame error is transmitted distally down the length of the mRNA, usually creating an abortive translation event. Although the mechanisms controlling the accuracy of successive codon selection are uncertain, it has been suggested that the process must be more accurate than the aa-tRNA selection and therefore that it must also require proof-reading (Kurland and Ehrenberg, 1985).

The accuracy of aa-tRNA selection during translational elongation is altered by a large number of mutations affecting the structure of both the ribosome and tRNA species (Gorini, 1974; Smith, 1979). In addition it is found that nonsense read-through (Vijgenboom *et al.*, 1985; Hughes, 1987; this paper), and missense errors (Tapio and Kurland, 1986) are increased by mutants of EF-Tu. Accordingly, the data suggest a positive role for EF-Tu in modulating the accuracy of tRNA selection.

Mutants which reduce the accuracy of selection of successive codons have been isolated as external suppressors of frameshift mutants. With the exception of one rRNA mutant (Weiss-Brummer *et al.*, 1987), all mutants in which the origin of the suppression has been determined have altered tRNA (see Roth, 1981; Bossi and Smith, 1984; Winey *et al.*, 1986). This paper describes the finding, previously presented in thesis form (Hughes, 1984), that some mutant forms of EF-Tu in *Escherichia coli* and *Salmonella typhimurium* cause suppression of frameshift mutants. The data suggest that EF-Tu plays a direct or indirect role in normal reading frame maintenance.

## Results

In both *E.coli* and *S.typhimurium*, EF-Tu is encoded by two unlinked genes, *tufA* and *tufB* (Jaskunas *et al.*, 1975; Furano, 1978; Hughes, 1986). Resistance to the antibiotic kirromycin (mocimycin) is conferred when both *tufA* and *tufB* are mutant (van de Klundert *et al.*, 1977; Hughes, 1986). The *Salmonella* strain TH89 carrying the EF-Tu mutations *tufA1* and *tufB101* is resistant to the antibiotic kirromycin while derivative strains, TH90 (*tufA1*, *tufB*$^+$) and TH131 (*tufA*$^+$ *tufB101*) are sensitive (Hughes, 1986). These strains also carry the −1 frameshift mutation *trpE91* (Atkins *et al.*, 1983) and the +1 frameshift mutation *hisG6609* (see Bossi *et al.*, 1983). Such frameshift mutations are suppressible by a variety of external suppressors. For example, *trpE91* is suppressible by *sufS*, *supK*, *hopR*, *hopE:sufR*, *sufY* and *sufW* (Riyasaty and Atkins, 1968; Atkins and Ryce, 1974; Hughes, 1984; B.Falahee and J.F.Atkins, in preparation; S.Thompson and J.F.Atkins, unpublished) while *hisG6609* is suppressed by *sufJ*, *sufT* and *sufU* (Kohno *et al.*, 1983; J.F.Atkins, unpublished; Hughes, 1984). Neither *trpE91* nor *hisG6609* are suppressed by *tufA1* or *tufB101*. We asked whether any other EF-Tu mutations could suppress either of these frameshift mutations.

### *Suppression of the frameshift mutation trpE91 by mutations in tufA and tufB*

The strain TH90 (*tufA1 tufB*$^+$) was used to select spontaneous kirromycin resistant mutants. These new mutations are expected to be alleles of the *tufB* gene. Of 120 mutants tested, 27 showed suppression of *trpE91*. In several independent selections ~20% of spontaneous kirromycin resistant mutants, selected in the presence of the non-suppressing *tufA1* allele, suppressed *trpE91*. In a parallel selection, the strain TH131 (*tufA*$^+$ *tufB101*) was used to select spontaneous kirromycin resistant mutants which were expected to be alles of *tufA*. In several independent selections 300 mutants were screened. Approximately 12% of spontaneous kirromycin resistant mutants selected in the presence of the non-suppressing *tufB101* allele suppressed *trpE91*. None of the kirromycin resistant mutants selected in TH90 or TH131 suppressed the other frameshift mutation *hisG6609*. These results suggest that some mutant forms of EF-Tu can cause frameshift mutant suppression but that this suppression is restricted to very specific combinations of mutations. We next examined the ability of specific *tufA* and *tufB* alleles to suppress the *trpE91* frameshift mutation.

### *tufA8 and tufB103 independently suppress trpE91*

One of the kirromycin resistant derivatives of TH90 was chosen

and its *tufB* allele designated *tufB103*. To confirm that suppression of *trpE91* in this strain was dependent on a mutation mapping in the *tufB* gene it was transduced with phage grown on a *tufB*$^+$ strain by selecting for a linked *argH*::Tn*10*. All kirromycin sensitive transductants (16/50) were TRP requiring, which is consistent with the function of *tufB103* as a suppressor of *trpE91*. The possibility that the suppression of *trpE91* and the kirromycin resistant phenotype might be due to separate mutations, one in *tufB* and the other in a linked suppressor gene, is made unlikely by (i) the high frequency with which suppressor Kir$^R$ phenotype is isolated, (ii) by the isolation of similar mutations mapping *tufA* and (iii) by the 100% cotransduction between *tufB103* and suppression of *trpE91* in many subsequent strain constructions.

The *tufB103* mutation was originally isolated and shown to suppress *trpE91* in the presence of *tufA1* which in itself has no detectable suppressor activity. To test whether *tufB103* can suppress *trpE91* in the presence of a wild-type *tufA* gene the strain ST100 *trpE91 tufB103* was constructed (see Materials and methods). This strain is kirromycin sensitive, but kirromycin resistant alleles can be isolated from it at high frequency, confirming the presence of *tufB103*. ST100 is TRP independent showing that *tufB103* can suppress *trpE91* in the presence of a wild-type *tufA* gene. The suppression of *trpE91* by *tufB103* in the presence of *tufA*$^+$ leads to a colony size of 1 mm on minimal medium after ~6 days.

We have isolated a *tufA* mutation, *tufA8* which signficantly enhances *tufB103* suppression of *trpE91* (see Materials and methods). The strain, ST101 *trpE91 tufA8 tufB103*, grows to a colony size on minimal medium of 1 mm after ~4 days. *tufA8* was shown to map in the expected location at minute 71–72 by transduction with the linked marker *zbh-736*::Tn*10*. As predicted ~40% of Tet$^R$ transductants inheriting *tufA*$^+$ simultaneously became kirromycin sensitive and had reduced suppressor activity. We asked whether *tufA8* could suppress *trpE91* in the presence of a wild-type *tufB* gene by constructing ST102. ST102 has the genotype *trpE91 tufA8*, it is kirromycin sensitive and tryptophan independent, which indicates that suppression of *trpE91* by *tufA8* in the presence of a wild-type *tufB* gene is observed. Suppression by *tufA8* in ST102 allows a colony size of 1 mm on minimal medium after ~5 days.

We conclude that both *tufA8* and *tufB103* can suppress the frameshift mutation *trpE91*. Suppression of *trpE91* by *tufA8* is more efficient than suppression by *tufB103* but it is most efficient in strains carrying both *tufA8* and *tufB103*. Suppression does not require both genes for EF-Tu to be mutant.

In addition to causing frameshift suppression, each of the mutations, *tufA8* and *tufB103*, also causes a significant reduction in growth rate. The generation times (average of four independent experiments) measured in glucose tryptophan minimal medium with vigorous aeration are: the parental strain *trpE91 tufA*$^+$ *tufB*$^+$ (43.7 min); ST100 *trpE91 tufA8 tufB*$^+$ (49.0 min); ST102 *trpE91 tufA*$^+$ *tufB103* (48.7 min); ST101 *trpE91 tufA8 tufB103* (56.3 min). Thus the generation time for each of the strains with one *tuf* gene mutant is increased by ~12% while that of the *tufA8 tufB103* double mutant strain is increased by 28%.

*Specificity of frameshift suppression by tufA8 and tufB103*

To test the specificity of *tufA8* and *tufB103* mediated frameshift suppression, we introduced eight *trpE* frameshift mutations other than *trpE91* into ST104 (carrying *tufA8 tufB103*) and ST103 (carrying wild-type *tuf* genes). These eight mutations (Atkins *et al.*, 1983) are within 21 nucleotides of the suppressible *trpE91* mutation (Figure 1). They are of both signs and some are leaky. Only one of the eight *trpE* frameshift mutations, *trpE875*, was suppressed in the strain carrying *tufA8 tufB103*. However, *trpE875*

| | | |
|---|---|---|
| UUC CGU CUG UUA CAG GGA GUG GUG AAC | Wild-type | Suppression |
| UUU↓ CCG UCU GUU ACA GGG AGU GGU GAA | 871(L) | - |
| UUC ↓CCG UCU GUU ACA GGG AGU GGU GGA | 872 | - |
| UUC CGU ΔCU GUU ACA GGG AGU GGU GAA C | 874 | - |
| UUC CGU ΔUG CUU ACA GGG AGU GGU GAA C | 876 | - |
| UUC CGU CUG UUA ↓↓UAC AGG GAG UGG UGA | 879(L) | - |
| UUC CGU CUG UUA CAG ΔG GAG UGG UGA | 873(L) | - |
| UUC CGU CUG UUA CAG GGA GUG ΔG UGA | 875(91)* | + |
| -1 Frameshift sequence unknown | 877 | - |

**Fig. 1.** Wild-type *S.typhimurium trpE* base pair residues 379–405 (Yanofsky and van Cleemput, 1982). Sequences of *trpE* mutants are from Atkins *et al.* (1983). △ symbolizes deleted bases; ↓ denotes a base addition; (L) denotes leakiness; + and − indicate suppression or no suppression by the *tufA8, tufB103* alleles; **trpE91* and the independently isolated *trpE875* have the same sequence (B.P.Nichols, unpublished).

is an independently arisen but sequentially identical mutation to *trpE91* (B.P.Nichols, personal communication). The leakiness of the mutations *trpE871*, *trpE873* and *trpE879* was marginally reduced in the *tufA8 tufB103* strains, but this effect may be due to reduction in growth rate caused by the *tuf* mutations. The absence of any suppression or enhancement of leakiness of these *trpE* mutations argues against these *tuf* alleles as non-specific suppressors. The lack of suppression of *trpE873*, which is also a −1 frameshift mutant, is particularly interesting because its reading frame sequence differs from that of the suppressible *trpE91* by only two adjacent codons (GAG UGG in *trpE873* and GGA GUG in *trpE91*).

As a more extensive test of *tufA8 tufB103* specificity we transduced 20 *his* operon frameshift mutations into *tufA*$^+$ *tufB*$^+$ and *tufA8 tufB103* carrying strains (see Materials and methods). Two of the mutants are −1 and not previously known to be externally suppressed. The other mutants are +1 and diverse in their known pattern of suppression (Table I). Five mutations, all +1 (*hisD3018, hisD3749* and its *S6, S7* and *S15* base substitution derivatives) are suppressed by *tufA8 tufB103, tufA8 tufB*$^+$ and *tufA*$^+$ *tufB103*. Suppression is weak because it takes 10–14 days to obtain a colony size of 1 mm on minimal medium with *tufA8 tufB103* and 2–4 days longer if either *tuf* gene is wild-type. The known nucleotide sequence of each of these +1 mutations and of some related but non-suppressed mutations (see Figure 2) suggests that the sequence CCCU may be important to be observed suppression. In addition to these five mutations the unsequenced +1 mutation *hisF3704* is very weakly suppressed. As all of these *his* frameshift mutants are non-leaky, we cannot rule out the possibility that some others are also weakly suppressed yet remain below the threshold of enzyme activity required for growth in the absence of HIS.

As a further test of the ability of *tufA8* and *tufB103* to supress frameshift mutations we have measured β-galactosidase enzyme activity in strains with an F′ factor carrying the *lacIZΔ14* fusion with +1 or −1 frameshift mutations at each of four positions in *lacI* (site 8,15,16 and 20; Calos and Miller, 1981). At one position (−1, site 8) *tufA8 tufB103* results in a 3-fold increase in the suppression level. The other seven frameshift mutations show small or insignificant increases in suppression with the *tufA8 tufB103* mutations (~10% for +1 site 8, −1 site 15, +1 site

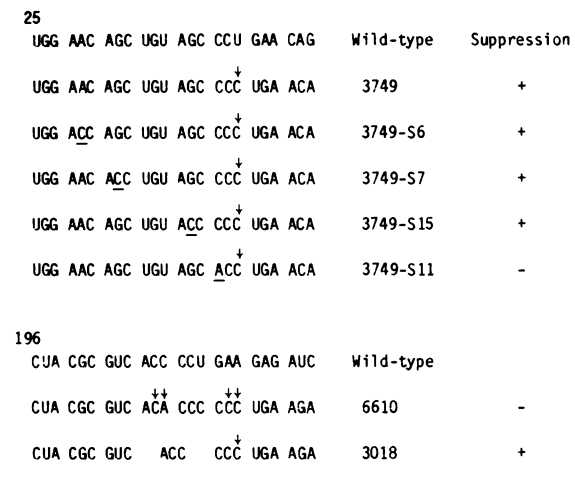


**Fig. 2.** Wild-type *S.typhimurium hisD* base pair residues 25–48 and 196–219 (Barnes and Husson, unpublished). Sequences of *hisD3749* and its derivatives are from Bossi and Roth (1981). Sequences of *hisD3018* and *hisD6610* are from Levin *et al.* (1982). + and − indicate suppression or no suppression by the *tufA8, tufB103* alleles; ↓ denotes a base addition. Base substitutions are underlined.

20 and −1 site 20; 40% for +1 site 15 and −1 site 16; 75% for +1 site 16). The large potential frameshifting windows for each of these mutations does not allow a simple correlation between particular short messenger sequences and frameshifting. These results show that mutant EF-Tu mediated frameshifting is quantitatively different at different sites, and support our inference from the *trpE* and *hisD* results above, that suppression shows some sequence specificity.

*Suppression of nonsense mutations in his operon by tufA8 tufB103*

It has been shown that the *tufAr tufBo* mutations in *E.coli* can act as nonsense suppressors (Vijgenboom *et al.*, 1985). Accordingly, we asked whether *tufA8* and *tufB103*, which show specificity in their frameshift suppression spectra, could also suppress nonsense mutations. We transduced 19 nonsense mutations in the *his* operon (9 UGA, 5 UAG, 5 UAA) into *tufA*$^+$ *tufB*$^+$ and *tufA8 tufB103* carrying strains (see Materials and methods). The nonsense mutations tested were, UGA: *hisA3715, hisB278, hisB2442, hisB6484, hisC3714, hisF3717, hisG200, hisG3720, hisI570*; UAG: *hisC50, hisC364, hisC446, hisC544, hisC881*; UAA: *hisC117, hisC151, hisC342, hisC354, hisC502* (Whitfield *et al.*, 1966; Roth, 1970). We find that seven of the nine UGA mutations are strongly suppressed (a colony size of 1 mm in 1½–4 days). No suppression of *hisG200* or *hisI570* or of any of the UAG or UAA mutations was observed. We then transduced some of the UGA mutations into TH139 (*tufA8 tufB*$^+$) and TH140 (*tufA*$^+$ *tufB103*) and again we observed UGA supression (a colony size of 1 mm in 3–7 days). Thus *tufA8* and *tufB103* either together or in combination with a wild-type copy of the other *tuf* gene can suppress *his* UGA mutations efficiently to allow growth in the absence of HIS. Recently Hughes (1987) has shown that *tufA8* and *tufB103* independently suppress both UGA and UAG mutations in the *lacI* gene in a site specific manner.

## Discussion

We find that mutant species of EF-Tu act as frameshift mutant suppressors. This implies that wild-type EF-Tu plays a role, direct or indirect, in reading frame maintenance. This might reflect the situation that EF-Tu has an important role in the initial positioning of the incoming tRNA. Thus, van Noort *et al.* (1982, 1986) suggest that EF-Tu from *E.coli* has two tRNA binding sites. Apparently the second site is revealed on contact of the ternary complex with the ribosome, and Kraal *et al.* (1983) have proposed that the second site may be involved in positioning the incoming tRNA with respect to the peptidyl tRNA. If this model is correct, it is then possible that the *tufA8* and *tufB103* mutations studied here are affecting the second site and thus may show imprecision in the relative positioning of tRNAs and consequently increase the basal level of frameshifting.

Another possible explanation of our results is that a perturbation of the aa-tRNA selection role of EF-Tu increases the level of missense interactions. We have measured, in an optimized *in vitro* translation system, the missense error rates supported by EF-Tu purified from our strains. When either $tRNA_2^{Leu}$ or $tRNA_4^{Leu}$ is the non-cognate tRNA, competing with $tRNA^{Phe}$ for poly(U), the missense error rate supported by EF-Tu from *tufA8 tufB103* is ~4-fold higher than that with wild-type EF-Tu. EF-Tu purified from strains with one wild-type and one mutant *tuf* gene, *tufA8 tufB*$^+$ and *tufA*$^+$ *tufB103*, supports a missense error rate intermediate between that of the wild-type and the double mutant. This shows, at least *in vitro*, that these EF-Tu species cause missense errors. These *in vitro* experiments will be reported in detail later (D.Hughes and C.G.Kurland, in preparation). As pointed out by Kurland (1979) mismatched codon–anticodon interactions may well involve altered tRNA conformation which in turn could affect the positioning of the incoming tRNA and lead to frameshifting. This sort of error coupling has been observed by Weiss and Gallant (1986), Bruce *et al.* (1986). Further, we have shown here that these mutant forms of EF-Tu that function as frameshift suppressors are also moderately efficient UGA suppressors. The mutant EF-Tu suppressible +1 and −1 frameshift mutations studied here are each closely followed by a UGA terminator. Although there is no evidence to link the frameshift and nonsense suppression, formally *tufA8* and *tufB103* can be viewed as allowing missense errors at UGA sites and a frameshifting mechanism related to mismatched interactions would apply to each UGA site.

Mutant EF-Tu might also suppress frameshift mutations indirectly by disturbing the normal functioning EF-G. EF-G plays a central but poorly understood role in translocation (Spirin, 1985). Both EF-G and EF-Tu bind to the same or overlapping sites on the ribosome (reviewed by Liljas, 1982). This raises the possibility that some mutants of EF-Tu with altered ribosomal binding could perturb the kinetics of EF-G interactions with the ribosome and thus increase the probability of an abnormal translocation event.

Provocatively *tufA8* and *tufB103* appear to exhibit specificity in the messenger sequences at which they cause frameshifting. Within the *trpE* gene only one sequence, that of the −1 mutant *trpE91*, is detectably suppressed among eight variants of a 21-bp sequence (Figure 1). Among 20 frameshift mutations of largely +1 sign scattered in the *his* operon (Table I) only six are detectably suppressed [four of these have an identical +1 mutation but different base substitutions close by (Figure 2)]. In addition we have measured β-galactosidase enzyme activity to quantify suppression of eight frameshift mutations of both plus and minus signs in the *lacI* part of a *lacIZ* fusion. There is a 3-fold increase in suppression of one −1 mutation but only small or insignificant increases for the other seven frameshift mutations. We conclude that *tufA8* and *tufB103* act preferentially at a limited number of message sequences to cause frameshifting of either sign.

No unifying pattern concerning the nature of message se-

**Table I.** Frameshift mutations in the *his* operon tested for suppression by *tufA8 tufB103*

| | Sign | Suppressible by: |
|---|---|---|
| *hisC2126* | −1 | − |
| *hisC2259* | +1 | *sufA* |
| *hisC3072* | +1 | *sufD,E,F* |
| *hisC3734* | +1 | *sufA,B,C* |
| *hisC3736* | +1 | *sufD,E,F* |
| *hisC3737* | +1 | *sufA,B,C* |
| *hisD3018* | +1 | *sufA,B,J,M* |
| *hisD3052* | −1 | − |
| *hisD3749* | +1 | *sufA,B,C* |
| *hisD3749-S6* | +1 | *sufA,B,C,J* |
| *hisD3758-S7* | +1 | *sufA,B,C,J* |
| *hisD3749-S11* | +1 | *sufJ* |
| *hisD3749-S15* | +1 | *sufA,B,C,J* |
| *hisD6580* | +1 | *sufG,J* |
| *hisD6610* | +1 | *sufA,B,M* |
| *hisF2118* | +1 | *sufD* |
| *hisF2439* | +1 | *sufD* |
| *hisF3044* | +1 | *sufD* |
| *hisF3704* | +1 | *sufD,E* |
| *hisG6609* | +1 | *sufJ,T,U* |

Data from Riddle and Roth (1970), Bossi and Roth (1981), Kohno *et al.* (1983), Hughes (1984), J.F.Atkins (unpublished).

**Table II.** Bacterial strains used in this study

| | |
|---|---|
| *trpE91* | |
| *trpE91* | *sufS602* |
| Th89 | *hisT1504 his01242 hisG6609 trpE91 argH1823*::Tn*10* *rpoB tufA1 tufB101* |
| TH90 | *hisT1504 his01242 hisG6609 trpE91 argH1823*::Tn*10 rpoB tufA1* |
| TH91 | *hisT1504 his01242 hisG6609 trpE91 rpoB tufA1 tufB1023* |
| TH131 | *hisT1504 his01242 hisG6609 trpE91 zbn-736*::Tn*10 tufB101* |
| TH136 | *trpE91 hisΔ644 zee-1*::Tn*10* |
| TH138 | *trpE91 hisΔ644 zee-1*::Tn*10 tufA8 tufB103* |
| TH139 | *trpE01 hisΔ644 zee-1*::Tn*10 A8* |
| TH140 | *trpE921 hisΔ644 zee-1*::Tn*10 tufB103* |
| ST100 | *trpE92 tufB103* |
| ST101 | *trpE91 tufA8 tufB103* |
| ST102 | *trpE91 tufA8* |
| ST103 | *trp-2451*::Tn*10* (from *trpE91*) |
| ST104 | *trp-2451*::Tn*10 tufA8 tufB103* |

quences crucial to frameshifting is yet evident. For example, the present data show that while *trpE91* is suppressed, *trpE873* is not, although their reading frames differ only in the two codons prior to the termination codon (Figure 1). This result appears to restrict the −1 suppression site mediated by *tufA8* and *tufB103* to the sequence GGA GUG UGA. In the *his* operon, two clusters of +1 mutants ~170 bp apart which are suppressed by *tufA8 tufB103* share the sequence AGC/ACC CCC UGA (Figure 2). Suppression is abolished by changing either the CCC or the upstream ACC.

Since EF-Tu is the carrier of all elongating tRNAs to the ribosome, the observation of specificity of suppression by its mutant forms is intriguing. As shown here and in Hughes (1987) *tufA8* and *tufB103* are 'specific' in the choice of message sequences (contexts) at which they cause frameshifting and nonsense suppression, but non-specific in so far as they promote both +1 and −1 frameshifting and UGA and UAG nonsense suppression. Here we consider some factors which could lead to sequence specific suppression. Mutations in EF-Tu might alter either the accuracy of aa-tRNA selection on the ribosome (Tapio and Kurland, 1986) or alter the rate of ternary complex formation (Louie *et al.*, 1984). If these effects were most pronounced on a subset of aa-tRNA species it would generate a sequence specific pattern of suppression. Alternatively, codon context effects, involving the sequences surrounding a codon, or specific tRNA−tRNA interactions, might limit the sequences at which mutant EF-Tu mediated suppression is detectable. Our data on nonsense suppression by *tufA8* and *tufB103* show that is is subject to strong context effects (Hughes, 1987).

The mutations, *tufA8* and *tufB103*, are approximately additive in their effect on frameshift and nonsense suppression (see also Hughes, 1987). We do not find the synergism which Vijgenboom *et al.* (1985) find for nonsense suppression by the *tufAr* and *tufBo* alleles in *E.coli*. We have, however, isolated kirromycin resistant mutants mapping at the *tufB* locus in *E.coli* that suppress the frameshift mutation *trpE91* in the presence of a wild-type *tufA* gene (Hughes, 1984). This rules out the possibility that there is some significant difference between *S.typhimurium* and *E.coli* resulting in a requirement for synergism in one species.

## Materials and methods

*Bacterial and phage strains*

The bacterial strains used in this study are listed in Table II. All *S.typhimurium* strains are derived from LT-2. Our laboratory collections of *his* and Tn*10* bearing strains were originally from one of the following: J.R.Roth (University of Utah), P.E.Hartman (Johns Hopkins University), B.N.Ames (University of California), K.E.Sanderson (University of Calgary). The high frequency generalized transducing bacteriophage P22 mutant HT105/1, int 201 (see Sanderson and Roth, 1983) and KB1-int1 (McIntire, 1974) were used for transductions in *S.typhimurium*. The construction of isogenic *S.typhimurium* strains was as follows: *tufB103* was isolated by selecting for kirromycin resistance in the strain TH90 containing the *tufA1* mutation described by Hughes (1986) followed by screening for suppression of *trpE91*. *tufB103* was transduced into the *trpE91* background via its linkage to *argH*::Tn*10* to make ST100. *tufA8* was isolated in this strain by selection for kirromycin resistance to give ST101. Twenty-nine kirromycin resistant derivatives of ST100 were isolated. Four of these showed significant enhancement of *trpE91* suppression while the other 25 had marginally enhanced suppression. One of the four showing enhanced suppression of *trpE91* was designated ST101 and its *tufA* allele, *tufA8*. *tufA8* itself is shown in Results to suppress *trpE91* thus accounting for the more efficient suppression. The marginal enhancement of suppression in the other 25 strains is a phenomenon we observe when suppressor alleles of *tufA* and *tufB* are paired with most non-suppressor kirromycin resistant alleles of the other *tuf* gene. This is probably due to the impaired activity of the kirromycin resistant non-suppressing *tuf* allele, alternatively it may reflect very weak suppressor activity by these alleles which we do not detect when these alleles are paired with the wild-type copy of the other *tuf* gene. A *tufB*$^+$ derivative, ST102, of the *tufA8 tufB103* containing strain ST101, was made by transduction with the linked *argH*::Tn*10* marker, and subsequent elimination of Tn*10*. The evidence that *tufA8* and *tufB103* encode mutant species of EF-Tu is (i) their genetic map and location and kirromycin resistant phenotype (Hughes, 1986; this paper) and (ii) *in vitro* translation assays which show that EF-Tu purified from *tufA8 tufB103* mutant strains supports a higher missense error rate than wild-type EF-Tu (D.Hughes and C.G.Kurland, in preparation). The construction of isogenic *S.typhimurium* strains carrying *trpE* mutations other than *trpE91* (Atkins *et al.*, 1983) was as follows: *trpE91* and ST101 (both grow normally on anthranilic acid, ANT) were transduced with P22HT grown on TT1333, *trp-2451*::Tn*10* (confers TRP requirement not satisfied by ANT) to give ST103 and ST104. Tet$^R$ transductants which had recombined out *trpE91* were identified when loss of Tn*10* conferred full prototrophy on the strains. Any *trpE* mutation can be transduced into these *trp-2451*::Tn*10* strains by selecting transductants on ANT and subsequently screening for ANT requiring transductants. Isogenic *S.typhimurium* strains carrying frameshift and nonsense mutations in the *his* operon were constructed as follows: *trpE91*, ST100, ST101 and ST102 were tranduced with P22 on *hisΔ644, zee-1*::Tn*10* (Tn*10* linked to *his*). Tet$^R$ HIS enquiring strains were retained. Mutations in the histidine biosynthetic pathway other than *hisD*$^-$ can be transduced into these strains by selection on histidinol (HOL). Mutations in the

*hisD* gene were introduced by first linking them to *zee-1*::Tn*10* and then transducing for tetracyline resistance and screening for HIS requirement.

*Media*

Luria broth and Vogel and Bonner salts supplemented with 0.2% glucose (Davis *et al.*, 1980) were used as liquid media. Solid media contained 1.5% agar (DIFCO). Kirromycin resistance was checked on LC plates (Van der Klundert *et al.*, 1978) containing 2 mM EDTA. Where appropriate, media contained tetracyline, 10 μg/ml; streptomycin, 100 μg/ml; kirromycin, 100–250 μg/ml.

*Determination of suppression*

Suppression of mutations in the *trpE* gene or the genes of the *his* operon was determined by streaking the strains for single colonies on minimal media lacking TRP or HIS as appropriate and incubating at 37°C. Absence of suppression is defined here as lack of visible growth (or increase in leakiness) under these conditions. In some cases we may not detect suppression if the resulting enzyme levels are insufficient to support growth. However, some of the *trpE* mutations used in this study are leaky and allow slow growth in the absence of tryptophan (Atkins *et al.*, 1983). The measure of the relative efficiency of suppression we have used in this study is the colony size on minimal media lacking TRP or HIS as a function of incubation time at 37°C. β-galactosidase enzyme activity was measured as described by Miller (1972).

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