Translational frameshifts induced by mutant species of the polypeptide-chain elongation factur-tu of escherichia-coli

Vijgenboom, E.; Bosch, L.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1989 by The American Society for Biochemistry and Molecular Biology, Inc.

Vol. 264, No. 22, Issue of August 5, pp. 13012-13017, 1989
Printed in U.S.A.

Translational Frameshifts Induced by Mutant Species of the Polypeptide Chain Elongation Factor Tu of *Escherichia coli**

(Received for publication, March 24, 1989)

Erik Vijgenboom and Leendert Bosch‡

From the Department of Biochemistry, Gorlaeus Laboratoria, Leiden University, 2333 CC Leiden, The Netherlands

Translational frameshifts, both +1 and −1, are promoted by mutations in *tufA* and *tufB*, the two genes encoding the polypeptide chain elongation factor (EF) Tu of *Escherichia coli*. Strains harboring the mutant EF-Tu(Ala^{375}→Thr) encoded by either *tufA* or *tufB* or by both, display a linear relationship between the frequency of frameshifting and the concentration of mutant EF-Tu, relative to the total amount of EF-Tu. A second mutant species, EF-TuB(Gly^{222}→Asp), also promotes frameshifting. The frequency is strikingly enhanced by the combined action of EF-TuA(Ala^{375}→Thr) and EF-TuB(Gly^{222}→Asp) and exceeds by far the total contribution of the two mutant EF-Tus studied separately. These observations raise the question whether the formation of each peptide bond under conditions that no frameshifting occurs also requires the combined action of two EF-Tu molecules, in this case not differing functionally.

The mechanism underlying the maintenance of the translational reading frame during polypeptide chain elongation is poorly understood. Although various members of the translational machinery are known to participate in each elongation step, their contribution, if any, to the correct readout of the three base-encoded messages has not always been assessed in detail. The role of two main interaction partners in this process, tRNA and mRNA, is illustrated by various features causing frameshifts such as "tRNA hops," "hungry codons," "shifty stops," and "shifty Shine and Dalgarno-like sequences" (Weiss *et al.*, 1988, a and b; Spanjaard and van Duin, 1988; Falahee *et al.*, 1988; Weiss *et al.*, 1987; Weiss and Gallant, 1983). Since a shift of the reading frame in general is abortive for translation, it is conceivable that the cell has much to invest in avoiding such an event (Kurland and Ehrenberg, 1985). Interestingly, the translation apparatus occasionally turns such an event to its own advantage and employs frameshifting to complete and regulate the synthesis of a correct protein. Telling examples are the synthesis of the polypeptide chain release factor RF2 of *Escherichia coli* (Craigen and Caskey, 1986; Curran and Yarus, 1988) and the synthesis of the *gag-pol* gene product of various retroviruses (Jacks *et al.*, 1988 and references therein).

A fruitful experimental approach of the frameshift phenomenon has been the study of extragenic and intragenic suppression. Most of the extragenic suppressors, frequently studied in *Salmonella typhimurium* and yeast, were tRNA species carrying an additional nucleotide in the anticodon loop (Bossi and Roth, 1981; Bossi and Smith, 1984; Cummins *et al.*, 1982) or a modification defect (Atkins and Ryce, 1974; Mendenhall *et al.*, 1987). In all cases, these suppressors affected either +1 or −1 frameshifts, but not both. They are thought to shift the reading frame by interference with the translocation step or by reading a quadruplet instead of a triplet. In *E. coli*, frameshift suppressors have been investigated in a few cases, the best studied examples being intragenic suppressors of bacteriophage T4 frameshift mutants (Crick *et al.*, 1967). More recently, extragenic suppressors have been described such as *thyA* mutants suppressing +1 frameshifts (Herrington *et al.*, 1986) and a *hopR* mutant suppressing −1 frameshifts (Weiss *et al.*, 1987; Falahee *et al.*, 1988).

Our recent finding (Vijgenboom *et al.*, 1985), that mutant species of the polypeptide chain elongation factor Tu from *E. coli* act as suppressors of nonsense mutations, prompted a study of their ability to shift reading frame. When the present study was almost completed, Hughes *et al.* (1987) reported suppression of frameshift mutations in kirromycin-resistant *S. typhimurium* strains. The suppressor mutations were mapped in the *tuf* genes. In this study, we show that both +1 and −1 frameshifts occur at moderate frequencies in *E. coli* strains producing mutant EF-Tu.[1] Strains, however, producing two different well characterized mutant EF-Tu species, encoded by *tufA* and *tufB*, respectively, display strongly enhanced levels of frameshifting. A similar synergistic action of these mutant EF-Tus was previously noted when studying nonsense suppression (Vijgenboom *et al.*, 1985).

MATERIALS AND METHODS

Strains—The *E. coli* K12 strains used in this study are listed in Table I. All strains are isogenic except for the *tufA* and *tufB* mutations and the markers used for strain constructions, *rpoB* and *fus*. Strains EV104, EV105, and EV114 were constructed with P_1 transduction (Miller, 1972). The donor strain, KMBL1164 (*supE*, *thi*, Δ*pro-lac*), was used to transduce the Δ*pro-lac* region to EV100, EV102, and EV110 (Vijgenboom and Bosch, 1987), respectively.

Plasmids—Frameshift mutations are indicated according to the convention established by Crick and Brenner (1967) and explained in Weiss *et al.* (1988a).

The plasmids pWS60 and pWS50 are generously provided by Dr. D. Court. All plasmids described in this paper are derivatives of pWS60 on which the gene for C_{II} is fused with a *Bam*HI linker to the coding sequence of *lacZ*. The gene fusion is under the control of the λ-P_L promoter. The fusion protein consists of the first 12 amino acids of C_{II} and all the amino acids of β-galactosidase except for the eight N-terminal amino acids. The plasmid pWS50 does not produce the fusion product due to an insertion of 20 bases in the C_{II} part of the fusion which results in a −1 frameshift mutation. The plasmids pWS60.1 and pWS60.7 were constructed by, respectively, filling and deleting the sticky ends of the *Bam*HI site at the fusion point

* This research was supported in part by Grant 0057 NL of the Commission of the European Communities, Biotechnology Action Programme (BAP). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence and reprint requests should be addressed.

[1] The abbreviation used is: EF, elongation factor.

TABLE I

Strain	Genotype	Reference
EV2	Δ(*pro-lac*)	Vijgenboom *et al.*, 1985
EV3	*tufB*[a] *rpoB* Δ(*pro-lac*)	Vijgenboom *et al.*, 1985
EV4	*tufB*::Mu *rpoB* Δ(*pro-lac*)	Vijgenboom *et al.*, 1985
EV5	*tufA*[b] *tufB*[a] Δ(*pro-lac*)	Vijgenboom *et al.*, 1985
EV8	*tufA*[b] *fus* Δ(*pro-lac*)	Vijgenboom *et al.*, 1985
EV9	*tufA*[b] *tufB*::Mu rpoB Δ(*pro-lac*)	Vijgenboom *et al.*, 1985
EV104	*tufA*[b] *tufB*[c] *rpoB fus* Δ(*pro-lac*)	This paper
EV105	*tufB*[c] *rpoB fus* Δ(*pro-lac*)	This paper
EV113	*tufA*[d] *tufB*::Mu *rpoB fus* Δ(*pro-lac*)	Vijgenboom and Bosch, 1987
EV114	*tufA*[d] *tufB*[a] *rpoB fus* Δ(*pro-lac*)	This paper

[a] Coding for EF-TuB (Gly^{222}→Asp).
[b] Coding for EF-TuA (Ala^{375}→Thr).
[c] Coding for EF-TuB (Ala^{375}→Thr).
[d] Coding for EF-TuA*.

(compare Fig. 1). With this procedure, both a +1 and a −1 frameshift mutant were expected, but DNA sequencing showed that GATCC instead of GATC was deleted in pWS60.7. Therefore, both pWS60.1 and pWS60.7 harbor a +1 frameshift mutation, but the context of both mutations is different. The other two frameshift mutations were made by respectively deleting and filling of the sticky ends of the *ClaI* site in the *lacZ* sequence of the fusion construct. The presence of the −1 and +1 frameshift mutation in pWS60.3 and pWS60.5, respectively, was confirmed by sequencing of the corresponding DNA fragment. The λ-P_L promoter on pWS60 and its derivatives is controlled by the temperature-sensitive repressor CI_{875} which is located on a second plasmid, pCI_{875}.

The plasmids overproducing either EF-TuA(Ala^{375}→Thr) or EF-TuB(Ala^{375}→Thr) were constructed by cloning the blunt-end fragments harboring the corresponding *tuf* gene, in the filled *Acc*I site about 1400 base pairs downstream of the repressor gene on pCI_{875}. Restriction analysis showed that the mutant *tufA* gene was cloned in the same direction as the λ-P_R promoter present on pCI_{875} and the mutant *tufB* gene in the opposite orientation. The expression of the *tuf* genes on these plasmids (pA_r and pB_r) was about equal as determined by rocket immunoelectrophoresis with antibodies raised against EF-Tu (van der Meide *et al.*, 1982).

Growth Conditions and β-Galactosidase Assay—Strains transformed with pCI_{875} and pWS60 or one of its derivatives were grown at 37 °C in a defined minimal medium (Vogel and Bonner, 1956) supplemented with 0.5% casamino acids, 0.5% glucose, 50 μg/ml kanamycin, and 50 μg/ml ampicillin. The growth temperature was experimentally determined to give the best reproducible results.

β-Galactosidase and total protein are assayed as described in Vijgenboom *et al.* (1985). The experiments were repeated at least three times with independently grown cultures.

RESULTS

Error-prone Mutant EF-Tus—The frequency of ribosomal frameshifting, induced by mutant species of EF-Tu, was determined in *E. coli* strains (Table II) transformed with plasmids harboring a frameshift mutation in a C_{II}-*lacZ* fusion (see Fig. 1 and "Materials and Methods"). Full length fusion products with β-galactosidase activity are formed upon ribosomal shifting to the correct reading frame. Three frameshift mutations are located in the C_{II} portion of the fusion: pWS50 (−1), pWS60.1 (+1), and pWS60.7 (+1), and two in the *lacZ* portion of the fusion: pWS60.3 (−1) and pWS60.5 (+1).

Background levels of frameshifting in the wild type strain EV2, transformed with these plasmids, differ 1 order of magnitude (Table II). Whether these differences are due to frameshift dependence on codon context or to differences in specific β-galactosidase activities of the various fusion products cannot be decided. In Table III, all background levels were arbitrarily set at one, and the measured β-galactosidase activities of the mutant EF-Tu strains were normalized to the activities of the wild type strain, transformed with the plasmid harboring the same frameshift mutation.

Frameshifting was measured in *E. coli* strains with mutations in either one of the *tuf* genes or in both. These mutations were obtained previously by selection for kirromycin resistance (van de Klundert *et al.*, 1978) or by homologous recombination (Vijgenboom and Bosch, 1987). Of the two mutant EF-TuA species, $EF\text{-}TuA_r$ has alanine-375 replaced by thre-

TABLE II

Strain	Frameshift construct					
	pWS60[a] (wild type)	pWS50 (−1)	pWS60.7 (+1)	pWS60.1 (+1)	pWS60.3 (−1)	pWS60.5 (+1)
		%			%	
EV2 (AB)[b]	100	0.02	0.005	0.01	0.001	0.005

[a] The β-galactosidase activity is expressed as the percentage of the wild type construct, pWS60.
[b] The capital letters in parentheses indicate the EF-Tus. Wild type = A and B. Mutation at amino acid position 375, Ala→Thr = A_r and B_r. Mutation at amino acid position 222, Gly→Asp = B_o. Mutation at unknown position = A*.

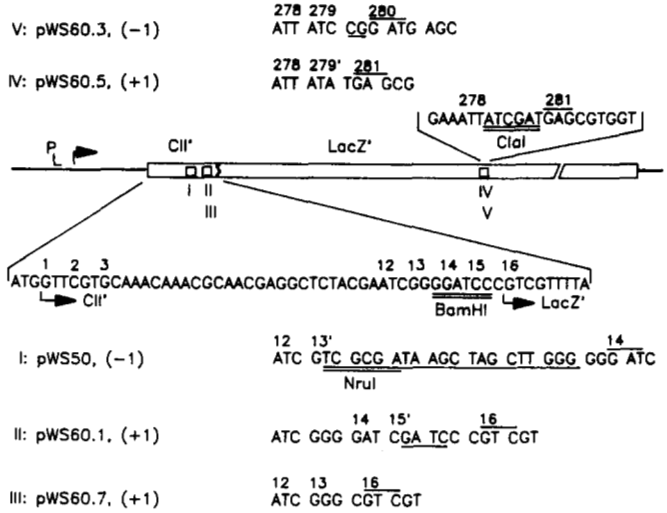


FIG. 1. **Frameshift mutations in pWS60.** The wild type sequence of the fusion C_{II}/lacZ is drawn in the *middle* of the figure. Derivatives harboring a frameshift mutation in the C_{II} part of the fusion are in the *lower part* of the figure, and those with a frameshift mutation in the *lacZ* part of the fusion in the *upper part*. Insertions, due to the construction of the frameshift mutation, are *underlined*, and relevant restriction sites are *double underlined*. The codon for the first amino acid in the correct frame 3′ of the frameshift mutation is indicated with the corresponding *amino acid number* and a *line* above the sequence. The numbering is relative to the start codon (0) for the frameshift mutations in the C_{II} part (No. 16 = No. 9 of the wild type *lacZ* sequence) and according to *lacZ* for the mutations in pWS60.3 and pWS60.5. Those amino acids indicated with a prime are the first amino acids changed due to the frameshift mutation.

TABLE III

Strain[a]	Frameshift constructs[b] pWS50 (−1)	pWS60.7 (+1)	pWS60.1 (+1)	pWS60.3 (−1)	pWS60.5 (+1)
EV2 (AB)[c]	1	1	1	1	1
EV5 (A_rB_o)	12	77	43	13	27
EV8 (A_rB)	1	3	2	1	2
EV3 (AB_o)	1	3	3	2	2
EV4 (A)	1	2	1	2	1
EV9 (A_r)	1	4	5	ND[d]	ND
EV114 (A^*B_o)	8	24	36	ND	ND
EV113 (A*)	2	2	1	ND	ND

[a] See Footnote *b*, Table II.

[b] Frameshift constructs are described under "Materials and Methods," the frameshift mutation is indicated in parentheses.

[c] The β-galactosidase activity is normalized to the wild type strain, EV2, transformed with the same frameshift construct.

[d] ND, not determined.

onine, and EF-TuA* has an unknown amino acid substitution. One of the two mutant EF-TuB species (EF-TuB_o) has aspartic acid substituted for glycine-222, the other (EF-TuB_r) is identical with EF-TuA_r (except for the difference in C terminus also found in wild type EF-TuA and EF-TuB). In some strains, *tufB* has been inactivated by insertion of the bacteriophage Mu (compare Table I).

As is apparent from Table III, the level of frameshifting is raised maximally in strains carrying mutations in both *tuf* genes: EV(A_rB_o) and EV114(A^*B_o). Strains with a mutant and a wild type EF-Tu such as EV8(A_rB) and EV3(AB_o) display levels of frameshifting only slightly higher than that of the wild type strain. That EF-TuA_R is error-prone, indeed, is shown more significantly by inactivation of the wild type *tufB* gene. The β-galactosidase of strain EV9(A_r) is approximately 2 to 5 times higher than that of strain EV4(A). EF-TuA* of strain EV113(A*) also promotes frameshifting above the background level of strain EV4(A). The contribution of EF-TuB_o to frameshifting cannot be studied separately, since inactivation of *tufA* is lethal to the cell[2] and the procedure successfully applied to transfer the mutation, Ala^{375}→Thr to *tufB* or *tufA* (Vijgenboom and Bosch, 1987), failed in transferring the mutation Gly^{222}→Asp to the chromosomal *tufA*.

A notable finding presented in Table III is that the frameshift levels caused by the single *tuf* gene mutations of the strains EV8(A_rB) and EV3(AB_o) are not additive in the double mutant EV5(A_rB_o). The same, most likely, is true for the frameshifts measured in the strain EV114(A^*B_o), although the combination of this mutant *tufA* with wild type *tufB* has not been studied. This synergism between the mutant species of EF-TuA and EF-TuB raises interesting mechanistic questions (see "Discussion"). It is also noteworthy that combinations of EF-TuB_o with EF-TuA_r or with EF-TuA* suppress both +1 and −1 frameshift mutations. Preference for suppression of +1 frameshifts is suggested by the data obtained with frameshift mutations in the C_{II} portion of the C_{II}-*lacZ* fusion. The data may be biased, however, by the different codon contexts of the mutations. An indication for the effect of codon context may be derived from the differences in suppression of +1 frameshift mutations at position 14 of the C_{II}-*lacZ* fusion (pWS60.1 and pWS60.7).

Frameshifting Is Dependent on the Mutant EF-Tu Concentration—The extent of frameshifting, induced by the error-prone A_r mutation of *tufA*, is decreased when the cell harbors in addition a wild type *tufB*, as can be concluded from the β-galactosidase activities of strains EV8(A_rB) and EV9(A_r)

[2] E. Vijgenboom, A. Talens, and L. Bosch, manuscript submitted for publication.

TABLE IV

Strain	Frameshift construct[a] Relative concentration [EF-Tu Ala^{375}→Thr]	pWS50 (−1)	pWS60.1 (+1)
	%		
EV105 (AB_r)	25	1	2
EV8 (A_rB)	35	1	2
EV2 (AB) pA_r	50	2	3
EV9 (A_r)	60	1	5
EV4 (A) pA_r	65	3	7
EV4 (A) pB_r	70	4	6
EV105 (AB_r) pA_r	95	3	10
EV9 (A_r) pB_r	150	6	22
EV9 (A_r) pA_r	160	5	15
EV104 (A_rB_r)	100	3	16
EV104 (A_rB_r) pA_r	220	9	35
EV5 (A_rB_o)	35	12	47
EV5 (A_rB_o) pA_r	115	10	36

[a] The β-galactosidase activity is normalized to the wild type strain EV_2 transformed with pW550 or pW560.1.

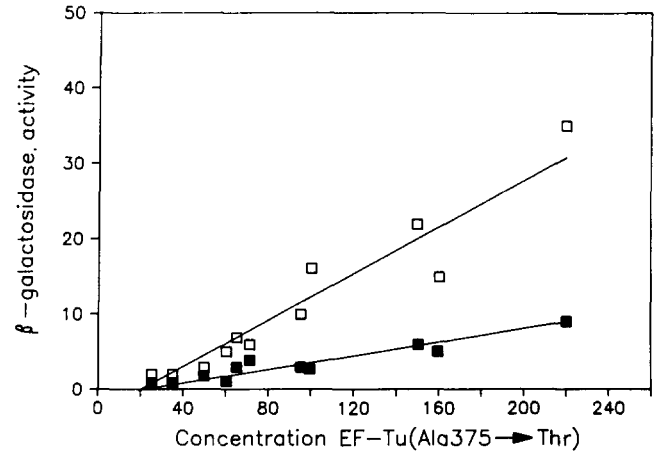


FIG. 2. The β-galactosidase activity is expressed in arbitrary units, and the relative concentration EF-Tu(Ala^{375}→Thr) (EF-TuA_r + EF-TuB_r) is calculated with the formula:

$$\frac{[\text{EF-Tu}(Ala^{375}\rightarrow\text{Thr})]}{[\text{total EF-Tu}]} \times \frac{[\text{EF-Tu}(Ala^{375}\rightarrow\text{Thr})]}{[\text{total EF-Tu of EV104}]} \times 100\%$$

Strains transformed with pWS50 are indicated with ■ and transformants of pWS60.1 with □ (compare Table IV).

(Table III). The absolute amount of EF-TuA_r in both types of cells is the same, but the total EF-Tu concentration of EV8(A_rB) is twice that of EV9(A_r). This prompted the study of the relationship between the frequency of frameshifting and the cellular concentration of EF-Tu(Ala^{375}→Thr) (EF-TuA_r plus EF-TuB_r) relative to the total EF-Tu concentration. Elevation of the mutant species concentration was achieved either by constructing a strain in which both *tufA* and *tufB* encode EF-Tu(Ala^{375}→Thr) (Vijgenboom and Bosch, 1987) or by transforming cells with plasmids harboring the mutant *tuf* gene (see "Materials and Methods"). The β-galactosidase activities of the various strains are presented in Table IV and are plotted against the relative cellular concentration of mutant EF-Tu in Fig. 2. As can be seen in this figure, cells respond with a linear increase in both +1 (□) and −1 (■) frameshifts upon raising their EF-Tu(Ala→Thr) concentration. Effects of EF-TuA_r or EF-TuB_r are identical as can be concluded, among others, from the levels of frameshifting in either pA_r or pB_r transformants of EV4(A) and EV9(A_r) (Table IV). This is in agreement with previous reports that EF-TuA and EF-TuB do not differ in the interaction of their

ternary complexes with the ribosome (Miller *et al.*, 1978) or in their ability to sustain protein synthesis.[2]

A striking exception to the strains displaying the frameshifting pattern shown in Table IV and Fig. 2 is the strain EV5(A_rB_o). As shown in Table III, frameshifting in this strain exceeds by far that in all other strains studied here. Raising the EF-TuA_r concentration of this strain by transformation with pA_r lowers the level of frameshifting (Table IV) in contrast to the increased frameshifting seen upon raising the EF-TuA_r concentration of cells lacking EF-TuB_o. The consequence of the raised EF-Tu(Ala^{375}→Thr) concentration is a reduction in the relative concentration EF-TuB_o participating in protein synthesis. As a result, frameshifting drops to the level of pA_r transformants of the EV104(A_rB_r) strain since the synergistic effect of the combination: EF-Tu(Gly^{222}→Asp)/EF-Tu(Ala^{375}→Thr) is now strongly reduced (compare the last four lines of Table IV).

DISCUSSION

Shifty EF-Tus—The results of the present paper show that mutant species of EF-Tu from *E. coli* induce frameshifting at moderate frequencies. A similar phenomenon has been reported by Hughes *et al.* (1987) for mutant EF-Tu from *S. typhimurium* and by Sandbaker and Culbertson (1980) for mutant EF-1α. These findings suggest that wild type EF-Tu plays an important role in the maintenance of the correct reading frame during translation of the mRNA. Since mutant EF-Tus also promote ribosomal readthrough of nonsense codons (Vijgenboom *et al.*, 1985; Hughes, 1987; Hughes *et al.*, 1987) and missense errors (Tapio and Kurland, 1986), a more general function of EF-Tu in regulating translational accuracy may be assumed.

EF-TuA(Ala^{375}→Thr), competent in sustaining *in vivo* polypeptide synthesis, is error-prone. This can be concluded from the frequency of frameshifting in a strain producing EF-TuA_r as the sole EF-Tu species (strain EV9(A_r), Table III). Previously, we did not find readthrough of stop codons to be increased in this strain (Vijgenboom *et al.*, 1985). Studying the same mutant EF-Tu *in vitro*, Tapio and Kurland (1986) measured an increased missense error.

Elevation of EF-Tu(Ala^{375}→Thr) in *E. coli* cells (either EF-TuA_r or EF-TuB_r or both) revealed a linear relationship between the frequency of frameshifting and the concentration of mutant EF-Tu relative to the total EF-Tu population (Fig. 2). No plateau level of frameshifting was observed, even at relative mutant EF-Tu concentrations up to 220% (in pA_r transformants of EV104(A_rB_r); see Table IV). This may be ascribed to the lowered affinity of the mutant EF-Tu·GTP for aminoacyl-tRNA (Sam, 1983; van der Meide *et al.*, 1983; Tapio and Kurland, 1986), although the relatively small difference in binding constants measured *in vitro* may not fully account for the data measured *in vivo*. The conclusion from Fig. 2 is that the frequency of frameshifting depends on the concentration of ternary complexes containing EF-Tu(Ala^{375}→Thr) in the cell. *In vitro* studies of the function of these ternary complexes in protein synthesis (Duisterwinkel *et al.*, 1981; van der Meide *et al.*, 1981) or *in vivo* studies with cells producing EF-TuA_r only (van der Meide *et al.*, 1982, 1983) yield a ready explanation for the role of EF-TuA_r in frameshifting. Suboptimal concentrations of ternary complexes and translational errors can explain the lower growth rates of strains EV9(A_r) and EV104(A_rB_r), as compared to those of the wild type strains EV4(A) and EV2(AB) (data not shown).

The error-prone character of EF-TuB(Gly^{222}→Asp) cannot be determined separately, since *tufA* cannot be inactivated,[2] and attempts to isolate strains with EF-Tu(Gly^{222}→Asp) as the sole *tuf* gene product failed (Vijgenboom and Bosch, 1987). Frameshifting was therefore studied in strains that harbor EF-TuB_o in combination with mutant or wild type EF-TuA. EV3(AB_o) cells are not telling much since their frameshift frequency exceeds only slightly the background level. That of strains EV5(A_rB_o) and EV114(A^*B_o), however, clearly demonstrates the error-prone behavior of EF-TuB_o since frameshifting at that level cannot be ascribed to EF-TuA_r or EF-TuA* alone (Table III, Table IV, and Fig. 2). Again, as in the case of EF-TuA_r, neither *in vivo* nor *in vitro* data regarding the participation of EF-TuB_o in protein synthesis lend themselves to an interpretation of the functioning of EF-TuB_o in frameshifting. *In vitro* translation experiments with poly(U) and purified EF-TuB_o showed a reduced misincorporation of Leu at high Mg^{2+} concentrations as compared to that with wild type EF-Tu. Furthermore, *in vitro* experiments suggested a defective GTPase for EF-TuB_o, which was ascribed primarily to an anomalous interaction of the ternary complex with the ribosome (Swart *et al.*, 1987). At low Mg^{2+} concentrations, EF-TuB_o appeared to be unable to sustain poly(U)-directed polypeptide synthesis (Swart *et al.*, 1987; Tapio and Kurland, 1986), but further *in vitro* translation experiments using natural messengers have to be awaited.

EF-TuA* has been poorly characterized, both structurally and functionally. It sustains protein synthesis *in vivo* (Vijgenboom and Bosch, 1987), and we have to conclude from the results that this mutant EF-Tu is error-prone (compare strains EV113(A*) and EV114(A^*B_o) in Table III).

Synergistic Action of Two Different Mutant EF-Tu Species—The synergistic effect on frameshifting exerted by the combinations EF-TuA_r/EF-TuB_o (EV5) and EF-TuA*/EF-TuB_o (EV114) is striking. Since the frameshifting caused by EF-TuB_o in cells, harboring this mutant species as the sole EF-Tu, cannot be determined, we cannot exclude that the low frequency observed with the EF-TuB_o/wild type EF-Tu (EV3) combination is due to an antagonistic action of the wild type species. If so, the synergism recorded in Table III is only apparent, and the high frequency observed with the double mutant combinations has to be ascribed to a high intrinsic frameshifting capacity of EF-TuB_o, exceeding by far that of EF-TuA_r. In that case, wild type EF-Tu interacts with EF-TuB_o, thus reducing the extent of frameshifting. Alternatively, each of the mutant EF-Tus has a low intrinsic frameshifting capacity, but when co-existing in the cell, they exert a genuine synergistic effect. Also in that case one has to assume interactions of the two EF-Tus during the frameshift event. If frameshifting occurs within one single translational step, which is likely, this may have implications for the mechanism of translation under normal conditions when perturbation of the reading frame does not occur.

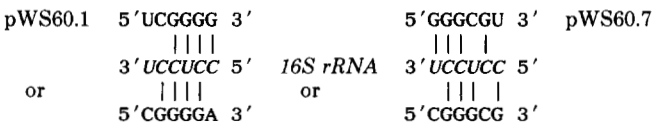
The frequency of frameshifting is strongly reduced by the introduction into EV5(A_rB_o) cells of plasmid-borne EF-TuA_r, in contrast to the increase in frameshifting observed upon introduction of EF-TuA_r into cells lacking EF-TuB_o. Due to the anomalous interaction of EF-TuB_o with ribosomes (Swart *et al.*, 1987), ternary complexes containing EF-TuA_r may effectively compete with ternary complexes containing EF-TuB_o for ribosomal binding sites. Raising the EF-TuA_r concentration will then cause a relatively pronounced reduction in the participation of EF-TuB_o in protein synthesis and thus in the synergism of the two mutant EF-Tu species.

We also found synergism with EF-TuA_r and EF-TuB_o when studying nonsense readthrough in the same strains (Vijgenboom *et al.*, 1985). Frameshift and nonsense suppression studies performed with EF-Tu mutants in *S. typhimurium*,

with unknown amino acid substitutions, did not reveal any synergism (Hughes *et al.*, 1987). While confirming synergism in nonsense suppression by EF-TuA$_r$ and EF-TuB$_o$ in strain EV5, Tapio and Isaksson (1987) noticed that the synergism disappeared when studies were performed in a different genetic environment. We conclude that a synergistic effect, if any, of two different species of mutant EF-Tu in promoting translational errors may become apparent depending on the nature of the mutations and on the genetic constitution of the cell. The linear relationship between the relative concentration in the cell of EF-Tu(Ala^{375}→Thr) and the frequency of frameshifting (Fig. 2) showing that no synergism becomes apparent under these conditions, may indicate that synergism requires the combined action of two different mutant EF-Tu molecules.

The Mechanism of Ribosomal Frameshifting—Several causes of ribosomal frameshifting have been reported in the literature. Frameshifts can be induced by Shine and Dalgarno-like sequences in the coding region of a gene (Weiss *et al.*, 1988b). Pausing at codons that are translated by minor tRNAs or tRNAs that are temporarily at low concentration (Spanjaard and van Duin, 1988; Weiss *et al.*, 1988a) can result in a shift of the reading frame. Shifty stop codons or "ribosomal jumps" (Weiss *et al.*, 1987) have been shown to be involved.

In order to see whether one or more of these mechanisms play a role in the phenomena presently described, the frameshift window for each of the five frameshift constructs has been depicted in Fig. 3. The constructs pWS60.1 and pWS60.5, carrying a +1 frameshift mutation in the C$_{II}$ part of the fusion, share the first 13 codons of their frameshift windows. The difference in frameshift suppression observed with these constructs, therefore, has to be related to the sequence downstream of these 13 codons. The nucleotide sequences overlapping codons 12 to 14 of both windows display complementarity to the nucleotide sequence 1535–1540 (5′CCUCCU 3′) near the 3′-end of 16 S rRNA:



That base-pairing, as depicted above, may lead to ribosomal frameshifting, has been demonstrated by Weiss *et al.* (1988b) for certain nucleotide stretches of the mRNA. Here, the effect of the interaction on frameshifting may be enhanced by the presence of mutant EF-Tu. Such a mechanism cannot explain the frameshifting observed with the constructs pWS50, pWS60.3, and pWS60.5, however, as an inspection of their window sequences shows.

In the constructs pWS60.3 and pWS60.5, the change of the reading frame has to occur close to the mutation site, located in the middle of the *lacZ* coding region, since preservation of the β-galactosidase activity does not permit extensive alterations of the primary structure. This restricts the number of possible frameshifts with these constructs and makes the combined action of a minor tRNA and mutant EF-Tu unlikely. Frameshift at a shifty stop codon is excluded in the case of pWS60.3 since the stop codon in the 0 frame is too far away from the frameshift mutation (130 base pairs) and thus would lead to loss of enzymic activity.

In conclusion, no common mechanism underlying frameshifting in all constructs studied here can be offered. The possibility of mutant EF-Tu, inducing shifts at random along the messenger chain, both to the +1 and −1 frame, may be envisaged leaving beside some preference for sites in certain codon contexts.

The recent finding (Moazed *et al.*, 1988) that EF-Tu and EF-G both interact with a common region on the 23 S rRNA may indicate that mutant EF-Tu perturbs a proper translocation of the ribosome along the messenger, resulting in an alteration of the translational reading frame. Whatever the mechanism of such a perturbation is, it has to account for the present finding that the cooperation between two EF-Tu molecules can enhance the frameshifting disproportionately.

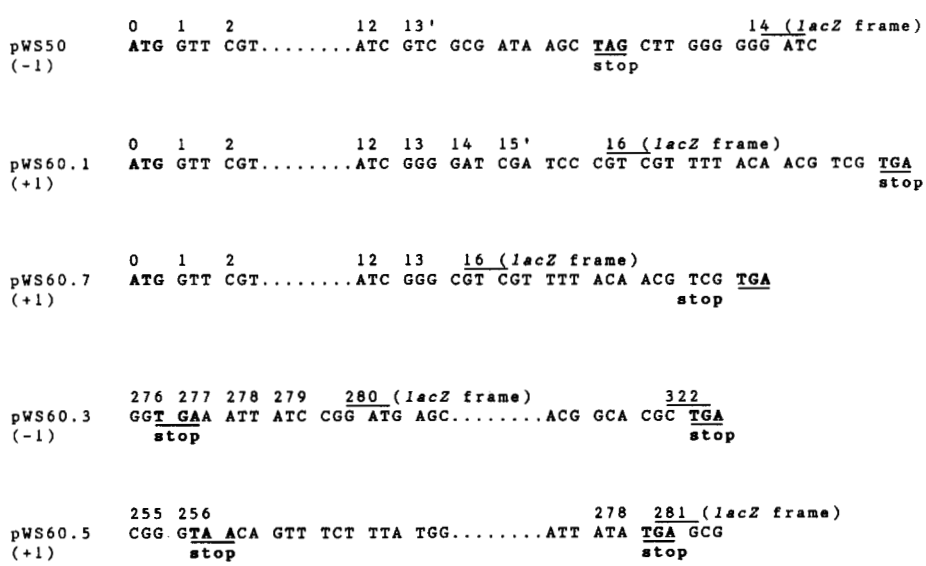


FIG. 3. **Frameshift "windows."** The reading frame translated by the ribosomes before frameshifting is defined as the 0 frame. The 5′ border of the window is the initiation codon (pWS50, pWS60.1, and pWS60.7) or the first stop codon upstream of the frameshift mutation in the +1 or −1 reading frame (pWS60.3 and pWS60.5). The 3′ border is the first stop codon in the 0 frame downstream of the frameshift mutation. The start codon and stop codons delimiting the available sequence for frameshifting are in *boldface*. Stop codons are also *underlined*. The numbering of the amino acids is explained in the legend to Fig. 1. The correct reading frame 3′ of the frameshift mutation is indicated with a *horizontal line* immediately above the sequence and the corresponding amino acid number.

This finding raises the question of whether such a cooperation is restricted to frameshift events in the mutated cell, or that each peptide bond formation in the wild type cell also requires the combined action of two EF-Tu molecules, which in the latter case are functionally identical. This possibility, forwarded earlier (Vijgenboom *et al.*, 1985), may imply that the lack of a profound insight into the mechanism of polypeptide chain elongation forms one of the major obstacles to an understanding of the mechanism of frameshifting promoted by mutant species of EF-Tu.

Acknowledgment—Our thanks are due to Dr. L. Nilsson for fruitful discussions and critical reading of the manuscript.

REFERENCES

Atkins, J. F., and Ryce, S. (1974) *Nature* **249,** 527–530
Bossi, L., and Roth, J. R. (1981) *Cell* **25,** 489–496
Bossi, L., and Smith, D. M. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 6105–6109
Craigen, W. J., and Caskey, C. T. (1986) *Nature* **322,** 273–275
Crick, F. H. C., and Brenner, S. (1967) *J. Mol. Biol.* **26,** 361–363
Crick, F. H. C., Barnett, L., Brenner, S., and Watts-Tobin, R. J. (1967) *Nature* **192,** 1227–1232
Cummins, C. M., Donahue, T. F., and Culbertson, M. R. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 3565–3569
Curran, J. F., and Yarus, M. (1988) *J. Mol. Biol.* **203,** 75–83
Duisterwinkel, F. J., de Graaf, J. M., Schretlen, P. J. M., Kraal, B., and Bosch, L. (1981) *Eur. J. Biochem.* **117,** 7–12
Falahee, M. B., Weiss, R. B., O'Connor, M., Doonan, S., Gesteland, R. F., and Atkins, J. F. (1988) *J. Biol. Chem.* **263,** 18099–18103
Herrington, M. B., Kohli, A., and Faraci, M. (1986) *Genetics* **114,** 705–716
Hughes, D. (1987) *J. Mol. Biol.* **197,**
Hughes, D., Atkins, J. F., and Thompson, S. (1987) *EMBO J.* **6,** 4235–4239
Jacks, T., Madhani, H. D., Masiarz, F. R., and Varmus, H. E. (1988) *Cell* **55,** 447–458
Kurland, C. G., and Ehrenberg, M. (1985) *Q. Rev. Biophys.* **18,** 423–450
Mendenhall, M. D., Leeds, P., Fen, H., Mathison, L., Zwick, M., Sleiziz, C., and Culbertson, M. R. (1987) *J. Mol. Biol.* **194,** 41–58
Miller, D. L., Nagarkatti, S., Laursen, R. A., Parker, J., and Friesen, J. D. (1978) *Mol. & Gen. Genet.* **159,** 57–62
Miller, J. H. (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Moazed, D., Robertson, J. M., and Noller, H. F. (1988) *Nature* **334,** 362–364
Sam, T. (1983) *Physico-chemical Studies on Bacterial Elongation Factor EF-Tu.* Ph.D. thesis, Leiden University, The Netherlands
Sandbaker, M. G., and Culbertson, M. R. (1988) *Genetics* **120,** 923–939
Spanjaard, R. A., and van Duin, J. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 7967–7971
Swart, G. W. M., Parmeggiani, A., Kraal, B., and Bosch, L. (1987) *Biochemistry* **26,** 2047–2054
Tapio, S., and Isaksson, L. A. (1988) *Biochimie* **70,** 273–281
Tapio, S., and Kurland, C. G. (1986) *Mol. & Gen. Genet.* **205,** 186–188
van de Klundert, J. A. M., van der Meide, P. H., van de Putte, P., and Bosch, L. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **75,** 4470–4473
van der Meide, P. H., Duisterwinkel, F. J., de Graaf, J. M., Kraal, B., and Bosch, L. (1981) *Eur. J. Biochem.* **117,** 1–6
van der Meide, P. H., Vijgenboom, E., Dicke, M., and Bosch, L. (1982) *FEBS Lett.* **139,** 325–330
van der Meide, P. H., Vijgenboom, E., Talens, A., and Bosch, L. (1983) *Eur. J. Biochem.* **130,** 397–407
Vijgenboom, E., and Bosch, L. (1987) *Biochimie* **69,** 1021–1030
Vijgenboom, E., Vink, T., Kraal, B., and Bosch, L. (1985) *EMBO J.* **4,** 1049–1052
Vogel, H., and Bonner (1956) *J. Biol. Chem.* **218,** 97–106
Weiss, R. B., and Gallant, J. (1983) *Nature* **302,** 389–393
Weiss, R. B., Dunn, D. M., Atkins, J. F., and Gesteland, R. F. (1987) *Cold Spring Harbor Symp. Quant. Biol.* **52,** 678–693
Weiss, R. B., Lindsley, D., Falahee, M. B., and Gallant, J. (1988a), *J. Mol. Biol.* **203,** 403–410
Weiss, R. B., Dunn, D. M., Dahlberg, A. E., Atkins, J. F., and Gesteland, R. F. (1988b) *EMBO J.* **7,** 1503–1507