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Antisuppression by mutations in elongation factor Tu

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Two slow-growing kirromycin-resistant *Escherichia coli* mutants with altered EF-Tu (Ap and Aa) were studied *in vivo* in strains with an inactive *tufB* gene. Mutant form Aa was isolated as an antisuppressor of the *tyrT*(Su3) nonsense suppressor, as described here. Ap, the *tufA* gene product of strain D2216 (from A. Parmeggiani), has previously been shown to give an increased GTPase activity. The slow cellular growth rates of both EF-Tu mutants are correlated with decreased translational elongation rates. Ap and Aa significantly decrease suppression levels of both nonsense and missense suppressor tRNAs [*tyrT*(Su3), *trpT*(Su9), *glyT*(SuAGA/G)], but have only little or no effect on misreading by wild-type tRNAs. A particular missense suppressor, *lysT*(SuAAA/G), which acts by virtue of partial mischarging as the result of an alteration in the amino acid stem, is not significantly affected by the EF-Tu mutations. The combination of *tufA*(Aa) and a *rpsD*12 ribosomal mutation is lethal at room temperature and the double-mutant strain has an elevated temperature optimum (42 °C) for growth rate, translation rate and nonsense suppression. Our data indicate an alterated interaction between Aa and the ribosome, consistent with our *in vitro* results.

We have previously shown that a mutant form of elongation factor Tu (EF-Tu), which is referred to as Ar [1] and gives increased translational error *in vitro* [2], has antagonistic effects on the phenotype of a *rpsL*(S12) ribosomal protein mutation [3]. Here, we have studied a new class of EF-Tu mutants in *Escherichia coli* with altered phenotypes with respect to growth rate and speed and accuracy of translation *in vivo*. We chose two slow-growing EF-Tu mutant (*tufA*) strains for these studies, bearing in mind that slow cellular growth has previously been correlated with increased translational accuracy of restrictive ribosomal mutants [4].

Both EF-Tu mutants give resistance to the antibiotic kirromycin in strains where the other gene coding for EF-Tu, *tufB* [5, 6] has been inactivated [7, 8]. Kirromycin resistance is normally a recessive characteristic, requiring for its expression the absence of the kirromycin-sensitive $tufA^+$ or $tufB^+$ products, As and Bs, respectively.

EF-Tu mutant protein Ap, the product of the *tufA* allele from strain D2216, has previously been characterised *in vitro* [9–14]. This mutant form of EF-Tu has been reported to display increased GTPase activity [12, 15]. This might lead to alterations in the accuracy of translation if the fidelity of protein synthesis is regulated by a mechanism involving EF-Tu-dependent hydrolysis of GTP on the ribosome [16–18]. The other mutant EF-Tu (Aa) was isolated in this laboratory as a slow-growing kirromycin-resistant strain exhibiting antisuppressor activity against a nonsense-suppressor tRNA (*tyrT*). A detailed *in vitro* characterisation of this mutant EF-Tu is presented in the accompanying paper [19].

We have here investigated the *in vivo* effects of mutations in EF-Tu (Aa and Ap) on decoding of nonsense and missense codons by suppressor tRNAs (nonsense and missense suppression). In addition, we have studied translational readthrough of stop codons in suppressor-tRNA-free strains (nonsense error).

It appears that the EF-Tu mutant forms Ap and Aa can act as nonsense and missense antisuppressors. In spite of their lowered elongation rates, Ap and Aa strains are essentially normal with respect to nonsense error formation *in vivo*.

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Abbreviations. EF-Tu, elongation factor Tu; EF-Ts, elongation factor Ts; iPrSGal, isopropyl-β-D-thiogalactopyranoside.

MATERIALS AND METHODS

Chemicals

Pyridoxal 5-phosphate, ribose 5-phosphate (sodium salt), indole, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, isopropyl-β-D-thiogalactopyranoside (iPrSGal) and *o*-nitrophenyl-β-D-galactopyranoside (NpGal) were purchased from Sigma Chemical Co; [^{14}C]tryptophan and [^{3}H]serine were from New England Nuclear. Kirromycin was a generous gift from Gist Brocades N.V., Delft, The Netherlands.

Construction of strains

E. coli K-12 strains used in this study are shown in Table 1. All strains were constructed by P1 transductions according to Miller [20]. The order of the genes in the *tufA* region is *zhd*-126::Tn10, *rpsL, tufA, aroE* and in the *tufB* region *metB, zij*-156::Tn10, *argH, glyT, tufB, rpoB*.

The kirromycin-resistant strain with *tufA*(Aa) was isolated as a spontaneous mutant from *E. coli* K-12 strain UI467 which harbours an inactive *tufB* gene and a *tyrT*(Su3) suppressor, as well as an F′ factor with an amber mutation in the *lacI* part of a fused *lacIlacZ* gene. Bacteria were grown in M9 minimal medium [20] supplemented with 0.4% glucose and required amino acids (0.4 mM) at 37 °C with vigorous shaking to mid-

Table 1. *Strains of* E. coli *K-12 used in this study*
Strain PM505 was generously provided by P. van der Meide [8]. D2216 by A. Parmeggiani [9], strains KL37, FTP4211, FTP3814 and FTP3703 by E. Murgola [20, 21] and strains XAc, XA103 and CDJ64 by J. Miller [19]. F′ factors carrying a *lacIlacZ* fusion with nonsense mutations in *lacI* have been described elsewhere [23]

De-signation	Chromosomal markers	Relevant characteristics
PM505	*tufB*::Mu, *rpoB*	As
D2216	*ampA, envA, proB, his, trp, rpsL, tufA, tufB*	Ap
KL37	*argH, Δ(tonBtrpAB), gly^{S}H, glyV55*	
UE125	as KL37 but *arg*$^+$, *glyT*	Su$^+$(AGA/G)
FTP4211	*Δ(tonBtrpAB), lysT*	Su$^+$(AAG/A)
FTP3814	*Δ(tonBtrpAB), nadA*::Tn10, *glyV55*	
FTP3703	*cysB, trpA2/F′ cys*$^+$, *trpA2*	
XAc	*Δ(lacproB), argE, ara, gyrA, rpoB, thi*	
UI538	as XAc but *arg*$^+$	AsBs
UI448	as UI1538 but *tufA*	AaBs
UI532	as UI538 but *tufA*	ApBs
UI465	as UI538 but *tufB*::Mu	As
UI483	as UI465 but *tufA*	Aa
UI534	as UI465 but *tufA*	Ap
UI336	as UI465 but *rpsL*282	AsSmR
UI564	as UI483 but *rpsL*282	AaSmR
XA103	*Δ(lacproB), argE, metB, ara, gyrA, rpoB, thi, tyrT*	
UI533	as XA103 but *arg*$^+$, *met*$^+$	Su3AsBs
UI433	as UI533 but *tufA*	Su3AaBs
UI512	as UI533 but *tufA*	Su3ApBs
UI467	as UI533 but *tufB*::Mu	Su3As
UI463	as UI467 but *tufA*	Su3Aa
UI514	as UI467 but *tufA*	Su3Ap
UI312	as UI467 but *rpsD*12	Su3AsD12
UI528	as UI463 but *rpsD*12	Su3AaD12
UI255	as UI467 but *rpsL*282	Su3AsSmR
UI541	as UI463 but *rpsL*282	Su3AaSmR
CDJ64	*Δ(lacproB), valR, gyrA, rpoB, thi, trpT*	Su9AsBs
UI464	as CDJ64 but *tufA*	Su9AaBs
UI511	as CDJ64 but *tufA*	Su9ApBs
UI468	as CDJ64 but *tufB*::Mu	Su9As
UI482	as UI468 but *tufA*	Su9Aa
UI513	as UI468 but *tufA*	Su9Ap
UI164	as KL37 but *arg*$^+$, *rpoB, glyT*	Su$^+$(AGA/G)AsBs
UI473	as KL37 but *rpo*$^+$, *tufB*::Mu	Su$^+$(AGA/G)As
UI478	as UI473 but *tufA*	Su$^+$(AGA/G)Aa
UI516	as UI473 but *tufA*	Su$^+$(AGA/G)Ap
UI346	as KL37 but *lysT*	Su$^+$(AAG/A)AsBs
UI347	as UI346 but *tufB*::Mu	Su$^+$(AAG/A)As
UI515	as UI347 but *tufA*	Su$^+$(AAG/A)Aa
UI521	as UI347 but *tufA*	Su$^+$(AAG/A)Ap

log phase and were plated on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside plates containing 0.4 mg/ml kirromycin. A kirromycin-resistant colony giving a light blue instead of a bright blue colour on the indicator plate, thus indicating antisuppression, was saved. P1 transductions confirmed that the antisuppression and kirromycin-resistance properties were closely linked.

The kirromycin-resistant *tufA*(Ap) mutation in D2216 was separated from the *rpsL* mutation by construction of an *aro*$^+$, *tufA*(Ap), *rpsL, zhd*-126::Tn10 intermediate strain. This strain was then used as a donor for generalised P1 transduction into an *aroE* recipient strain selecting for Aro$^+$ and screening for tetracycline (Tet-S) and streptomycin sensitivity. P1 lysates were made by growth on cells from several slow-growing colonies and used for transduction to a strain carrying *aroE, zhd*-126::Tn10, *tufB*::Mu. The presence of *tufA*(Ap) in one of the slow-growing derivatives was confirmed since Aro$^+$ transductants which were tetracycline-sensitive were also kirromycin-resistant at a high frequency.

The *tufA* region was introduced to *aroE*, *zhd*-126::Tn10 derivatives of KL37, XA and CDJ strains by selecting for Aro$^+$ and screening for the loss of tetracycline resistance as described in detail by Tapio and Isaksson [3]. The strains carrying only an active *tufA* gene (Aa or Ap) were constructed by introducing a *tufB*::Mu insertion [8]. In KL37 strains inactivation of *tufB* was achieved by selecting for Arg$^+$ and screening for rifampicin resistance. In the cases of XA and CDJ derivatives, they were first made *argH, zij*-156::Tn10, and subsequently the *tufB* allele was inactivated in a similar manner. Transductants carrying a mutated *tufA* were recognised by their kirromycin resistance after inactivation of *tufB*, since kirromycin resistance is a recessive property. This resistance property was later exchanged for kirromycin sensitivity, thus giving As derivatives lacking an active *tufB*.

The strain carrying both *tufA*(Aa) and *rpsD*12 was made starting from a *rpsD*12 derivative of XAc lacking an active *tufB* gene. Into this strain, *tufA*(Aa), *zhd*-126::Tn10 was introduced by selecting for tetracycline resistance and screening for both kirromycin resistance and hypersensitivity for streptomycin, the latter property being related to the presence of *rpsD*12 [23]. To eliminate *zhd*-126::Tn10, a lysate was made from this derivative and was used to transduce a strain carrying only *aroE*. Selection was for Aro$^+$ and colonies were screened for tetracycline sensitivity and hypersensitivity to streptomycin. The presence of *tufA*(Aa) was shown by a subsequent transduction into an *aroE, zhd*-126::Tn10, *tufB*::Mu derivative of XA103. Selection was made for Aro$^+$. A high proportion of those transductants which were tetracycline sensitive and hypersensitive to streptomycin were also kirromycin resistant which verified the presence of *tufA*(Aa) in the donor strain.

The strain carrying a combination of *tufA*(Aa) and *rpsL*282 was made as described previously [3].

F′ factors with a fused *lacIlacZ* gene were introduced into the test strains by selecting for the Pro$^+$ phenotype associated with the plasmid. Rifampicin resistance was used as a counterselection against the F′-donor strain.

The strain constructions carrying *glyT*(AGA/G) [21] and *tufB*$^+$ were made by introducing *arg*$^+$, *glyT*(AGA/G), *rpoB* into KL37 by selecting for Arg$^+$ and screening for rifampicin resistance. The combination of *tufB*::Mu, *glyT*(AGA/G) was made by starting with a KL37 derivative which was *tufA, argH, zij*-156::Tn10, *tufB*::Mu, *rpo*$^+$ [3]. This strain was transduced using a donor strain which was *arg*$^+$, *glyT*(AGA/G), *rpoB*. Selection was for Arg$^+$ and colonies were screened for kirromycin resistance and rifampicin sensitivity. In all cases suppressor activity was recognised as a Trp$^+$ phenotype when an F′ factor carrying a missense mutation (AGA) in *trpA* was introduced.

The *lysT* missense suppressor [22] strains were made by first introducing a *nadA*::Tn10 allele into the recipient, selecting for tetracycline resistance together with nicotinamide auxotrophy. Such a strain was transduced with a P1 lysate carrying *lysT*(AAA/G) selecting for NadA$^+$. Transductants were screened for tetracycline resistance and suppressor ac-

Table 2. *Cellular growth and translational elongation rates of* tyrT-*(Su3) strains carrying different EF-Tu species*
Growth and elongation rate values are given as the average with standard error of four independent measurements. Relevant strains are UI533, UI467, UI463 and UI514

EF-Tu	Growth rate	Elongation rate
	doublings/h	amino acids/s
AsBs	1.62 ± 0.03	15.5 ± 1.0
As	1.53 ± 0.03	14.6 ± 0.9
Aa	1.09 ± 0.06	8.7 ± 0.6
Ap	0.91 ± 0.03	10.5 ± 0.4

tivity; i.e. a Trp^+ phenotype, when an F′ factor carrying a missence mutation (AAA) in *trpA* was introduced.

Determination of bacterial growth and elongation rates

Bacteria were grown in a M9 medium [20] supplemented with all amino acids (0.4 mM) and 0.4% glucose at 37 °C with vigorous shaking. Growth rates were determined as described previously [3].

Determinations of translation rates were made as described previously [23, 24] in a modified Luria broth medium [25] without glucose with strains listed in Table 2, which contained an inducible lactose operon on an F′ factor.

Determination of nonsense suppression and error

Cells with appropriate F′-*lacIlacZ* factors were grown to log phase in M9 minimal medium [20] containing all amino acids (0.4 mM) except proline [26]. Measurements of β-galactosidase activity resulting from nonsense codon readthrough, were performed as described by Miller [20], and elsewhere [3].

Determination of missense suppression

Tryptophan synthetase is a tetrameric enzyme composed of two α chains and a β-chain dimer. The α chain is encoded by *trpA* and the β chain by *trpB*. Each subunit catalyses its own half reaction; α the conversion of indoleglycerol phosphate to indole and β the conversion of indole to tryptophan. Each subunit can stimulate the other in its half reaction. It is important to note that α does not have to be in the active form in order to stimulate the β subunit [27]. Mutations in *trpA* are available which give an inactive protein. However, $tRNA^{Gly}$-derived missense suppressors which insert glycine at the mutant position give an active enzyme. Activity of the α subunit therefore serves as a measurement of missense suppression and was estimated by conversion of [^{3}H]serine to [^{3}H]tryptophan, based on the procedure of Mosteller et al. [28], modified by R. H. Buckingham and E. J. Murgola (personal communication).

RESULTS

Slow growth of EF-Tu mutants is correlated with a decreased rate of translation in vivo

The growth and translational elongation rates of the wild-type and mutant EF-Tu strains are summarised in Table 2. Inactivation of the *tufB* gene, giving a strain with only the $tufA^+$ gene product (As), leads only to a slight decrease in cellular growth and translation rate. In contrast, among the strains carrying a non-functional *tufB* gene, the Aa and Ap mutants grow considerably more slowly than the corresponding As strain. The presence of the antibiotic kirromycin (0.1 mg/ml) had no effect on the growth of the resistant mutants whereas this concentration inhibits growth of sensitive strains (not shown).

To determine whether the decreased growth rates of the EF-Tu mutants are due to inefficient protein synthesis, the translational elongation rates were determined. This was carried out by measuring the time lag for appearance of β-galactosidase after induction with iPrSGal [23]. The elongation rate found for the wild-type strain (Table 2) is in good agreement with previous reports [24, 29]. In a mutant *tufA*(Aa) strain, the elongation rate was reduced by nearly 50%. Similarly, the strain with the mutant Ap shows a significantly decreased translational rate. Thus, decreased translational elongation rates correlate well with the slow growth rates of these mutants.

Table 3. *Nonsense suppression of UAG codons in strains carrying different EF-Tu species and the* tyrT*(Su3) suppressor*
All values are given as the average of four independent measurements. Chromosomal markers are indicated in Table 1 (see strains UI533, UI433, UI512, UI467, UI463 and UI514); the strains used for nonsense suppression and error measurements are F′-factor derivatives of these. Experimental error and assay conditions are described in Materials and Methods

EF-Tu	Suppression of UAG in *lacIlacZ* at position			
	84	117	189	220
	%			
AsBs	15	14	42	44
AaBs	8	9	23	23
ApBs	11	13	27	24
As	18	21	45	37
Aa	10	9	21	23
Ap	6	6	15	9

EF-Tu mutants show different effects on efficiency of suppressor tRNAs as compared to misreading by wild-type tRNAs

The correlation between decreased growth and translation rates of the EF-Tu mutants Aa and Ap indicates that the inefficiency of these factors has a general effect on translation. In order to investigate how these mutant proteins affect the efficiency of a particular tRNA species, their effects on nonsense suppression were studied using two different suppressor tRNAs. One of these suppressors results from a mutation in the anticodon sequence of *tyrT* tRNA making it complementary to the stop codon UAG [30]. The other tRNA suppressor carries a base change in the dihydrouridine stem of *trpT* tRNA which allows it to read UGA by wobbling [31].

Suppression of UAG codons at different positions in the *lacI* part of a fused *lacIlacZ* gene is presented in Table 3. In a strain with wild-type EF-Tu, the *tyrT*(Su3) suppressor competes effectively with release factors giving suppression values of more than 40% in some codon contexts. Both Aa and Ap decrease the efficiency of *tyrT* tRNA as compared to As, although the effects are not identical.

Table 4. *Nonsense suppression of UGA codons in strains carrying different EF-Tu species and the* trpT*(Su9) suppressor*
All values are given as the average of four independent measurements. Experimental details are as described in Table 3. Relevant strains are CDJ64, UI464, UI511, UI468, UI482 and UI513

EF-Tu	Suppression of UGA in *lacIlacZ* at position		
	189	220	289
	%		
AsBs	32	19	1.0
AaBs	14	6	1.1
ApBs	24	20	3.0
As	33	16	2.1
Aa	12	6	1.0
Ap	15	12	3.0

Table 5. *Nonsense error formation in strains carrying different EF-Tu species*
All values are given as the average of four independent measurements. Experimental details are explained in Table 3. Relevant strains are UI538, UI448, UI532, UI465, UI483 and UI534

EF-Tu	Error frequency in *lacIlacZ* at position (codon)					
	189 (UAG)	220 (UAG)	189 (UAA)	220 (UAA)	189 (UGA)	220 (UGA)
	%					
AsBs	0.12	0.16	0.26	0.19	0.57	0.62
AaBs	0.16	0.24	0.33	0.23	0.45	0.57
ApBs	0.07	0.18	0.25	0.15	2.59	2.45
As	0.30	0.18	0.16	0.10	0.40	0.70
Aa	0.10	0.20	0.17	0.12	0.35	0.76
Ap	0.12	0.10	0.18	0.09	2.46	2.68

A similar pattern is seen in the case of UGA suppression by *tyrT*(Su9) (Table 4). The mutant Aa decreases the rate of nonsense suppression by 2–3-fold, independently of the presence or absence of an active *tufB* gene. The effect of Ap on *trpT*(Su9) is significantly less prominent than observed on *tyrT*. The low suppression of UGA at position 289 is probably unaffected by Aa and Ap.

The effect of the mutant EF-Tu species on nonsense error formation, measured as read-through of nonsense codons in suppressor-tRNA-free strains, was also investigated. Mistranslation of UGA codons at two different positions is increased severalfold by Ap (Table 5) whereas the read-through of UAA and UAG is unaffected or slightly lowered. The increased mistranslation of UGA codons by Ap is unlikely to result from decreased efficiency of release factor(s) in this strain, since this would lead to a general enhancement of both error and suppression levels, which is not observed (compare Tables 4 and 5). In strains with mutant Aa, the nonsense error level is unaffected, with one exception. It should be noted that although the read-through level of UAA and UAG is low, it is not at the minimal level, since a further decrease in read-through of these codons can be seen in restrictive ribosomal mutants [4].

It has previously been shown that slow ribosomes can cause premature transcriptional termination [32]. In addition, the decreased ribosomal elongation rate may lead to an increased rate of messenger degradation, since messengers covered with ribosomes are to some extent protected from decay [33, 34]. Both increased polarity and messenger degradation may affect the phenotypes of the EF-Tu mutant strains. However, such effects would not influence suppression and error formation as studied here, since the suppression values are determined as ratios of enzyme activities in strains with the nonsense test mutation to the corresponding values from identical strains lacking the test mutation. Even if the mRNA pool is affected by the test mutation itself, the relative difference seen in suppression, when an EF-Tu mutant is compared to the control strain, gives relevant results for effects at the level of translation.

Table 6. *Missense suppression by* glyT*(SuAGA/G) and* lysT*(SuAAG/A) in strains with different EF-Tu species*
All values are given as the average of five independent experiments. The standard error is less than ± 10%. Experimental conditions and calculation of missense suppression levels are described in Materials and Methods

EF-Tu	tRNA	Codon	Position in *trpA*	
			211	234
			%	
AsBs	*glyT*	AGA	73	34
As	*glyT*	AGA	45	36
Aa	*glyT*	AGA	25	11
Ap	*glyT*	AGA	30	26
AsBs	*glyT*	AGG	16	15
As	*glyT*	AGG	20	12
Aa	*glyT*	AGG	5	5
Ap	*glyT*	AGG	14	14
AsBs	*lysT*	AAG	1.6	0.8
As	*lysT*	AAG	1.1	0.7
Aa	*lysT*	AAG	1.0	0.7
Ap	*lysT*	AAG	1.2	0.5
AsBs	*lysT*	AAA	1.1	0.6
As	*lysT*	AAA	0.8	0.6
Aa	*lysT*	AAA	0.8	0.6
Ap	*lysT*	AAA	1.0	0.4

It was recently reported that some mutants of EF-Tu promote both ribosomal frameshifting and nonsense codon read-through [35]. However, EF-Tu mutants Aa and Ap showed similar or only slightly decreased frameshift error rates compared to the wild-type strain (not shown).

EF-Tu mutants decrease the efficiency of a gly-T *missense suppressor*

In the case of nonsense suppression, the suppressor tRNAs are competing with release factors [36]. To show that EF-Tu mutants Aa and Ap directly affect translating tRNAs and do not interfere through changes in intracellular concentrations of release factors, the effects by the EF-Tu mutants on missense suppression were studied. The *glyT*(SuAGA/G) missense suppressor carries a mutated anticodon allowing it to read arginine codons [37]. The level of its suppression of mutant arginine codons in the *trpA* gene can be measured, since glycine, but not arginine, gives an active α-protein: one of the subunits of trypthophan synthetase. Missense suppression in strains carrying different *tufA* alleles was measured (Table 6). In agreement with the effect on nonsense sup-

pressors, EF-Tu mutant Aa decreases the efficiency of the *glyT* missense suppressor significantly in all cases, indicating a direct discrimination against the suppressor tRNA as compared to the cognate tRNA. On the other hand, the mutant Ap functions in a less prominent and more codon-context-dependent manner, since decreased suppression is not always seen.

The relative suppression values of AGA/G at position 211, as compared to position 234 in *trpA* for the control strain (AsBs), are in accordance with a previous report using a different method for suppression determination [38]. In agreement with other reports we find that, irrespective of the state of *tufA*, the efficiency of the *glyT* suppressor is decreased when the codon is read by wobbling (AGG) rather than by normal codon-anticodon interaction (AGA) [37, 38].

EF-Tu mutants do not interfere with a mutation in the amino acid stem of lysT-*derived tRNA*

Suppression by one *lysT*-derived missense suppressor results from a mutation in one of the two genes coding for $tRNA^{Lys}$, giving an altered amino acid stem (cytidine to uridine at position 70). This leads to a partial mischarging by alanine instead of lysine [22]. Since EF-Tu is believed to interact with tRNA in the amino acid stem [39], it is of interest to find out whether Aa or Ap are altered in their recognition of such a mutant tRNA. Similarly to the previous case with the *glyT* suppressor, missense suppression of the *trpA* mutations can be measured, since alanine insertion gives an active α protein, whereas insertion of lysine leads to an inactive product. Thus the *lysT* suppressor charged with alanine competes with the lysine-charged *lysT* suppressor as well as the wild-type Lys-$tRNA^{Lys}$. Table 6 shows that the suppression associated with *lysT*(AAA/G) is significantly lower than with *glyT*(SuAGA/G) and is dependent on the codon context [21]. The low efficiency of the former suppressor could possibly be a reflection of a low mischarging of the *lysT*(AAA/G) tRNA with alanine rather than a low translation activity of this tRNA. Suppression in the strain with only As appears to be somewhat decreased as compared to the AsBs wild type. However, the EF-Tu mutants Ap and Aa seem to recognise the *lysT* missense suppressor in a similar manner to the wild-type factor, since the suppression values are essentially unaffected. This indicates that there are no specific interactions between the mutational alterations in the amino acid stem of *lysT*(SuAAA/G) and the altered factors Ap and Aa.

Mutant EF-Tu Aa has a temperature-dependent compensatory effect on a rpsD *mutation*

Ribosomes with an altered S4 protein (*rpsD*) show a characteristic increase in the translational error level *in vivo* [24]. Furthermore, the mutant ribosomes show a decreased ability to proofread *in vitro* [40]. To analyse whether the mutation in Aa has any deleterious effect on ribosomes with an altered S4 protein, a strain carrying both a *tufA*(Aa) and an *rpsD*12 mutation was made. The combination of the two mutations appeared to be lethal to the cell at temperatures below 30 °C. Therefore, a systematic study of the effects of temperature on growth and translation rates was made, using this double mutant (Fig. 1).

The temperature sensitivity of the *rpsD*12 strain has previously been shown to result from the lowered affinity of S4 for its binding site on ribosomal RNA, leading to a defect in the ribosome assembly [41]. However, as can be seen in Fig. 1, defective assembly is not the only effect of high temperature since, even though the translation rate is essentially normal at 37 °C, it is decreased by the *rpsD*12 mutation at elevated temperatures. The decreased growth and translation rate associated with *rpsD*12 at 42 °C is to some extent counteracted by introducing *tufA*(Aa) into the strain, since the double mutant has optima for growth and translation rates at this temperature.

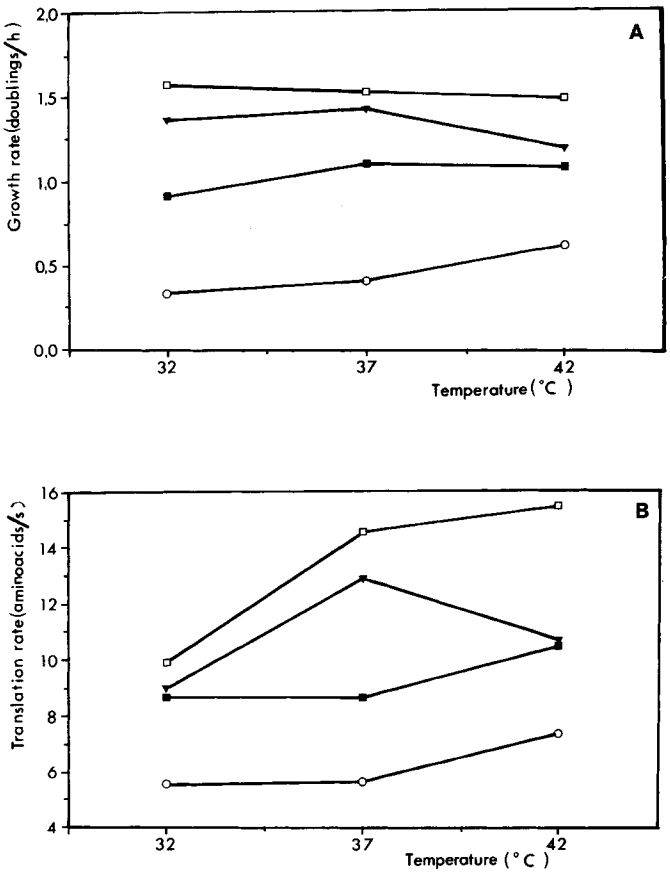

Fig. 1. *Cellular growth rates (A) and translation rates (B) were measured as a function of temperature in strains carrying EF-Tu species As (wild-type product) or Aa (mutant product) together with wild type ribosomes (D⁺) or* rpsD *mutant ribosomes (D12).* AsD^+ (□, UI467); AsD12 (▼, UI312); AaD^+ (■, UI463) and AaD12 (○, UI528). Chromosomal markers of the strains are indicated in Table 1. Growth conditions and calculations are described in Materials and Methods. All values are the average of at least four different experiments

To look for a possible correlation between the growth and translation rates and the level of nonsense suppression, we measured the efficiency of the *tyrT*(Su3) suppressor at the same temperatures using the strains described in Fig. 1. The data are summarised in Fig. 2. Strains carrying a wild-type EF-Tu or EF-Tu mutant Aa with wild-type ribosomes show a decrease in nonsense suppression as a function of temperature. In contrast, the *tufA*, *rpsD*12 double-mutant strain shows an increase in nonsense suppression when the temperature is raised. This effect is independent of the codon context, although it is more pronounced at the 'leaky' contexts at positions 189 and 220. Thus, there is a compensatory effect at high temperature by Aa on the phenotype of the *rpsD*12 mutation with regard to rates of growth, translation, and nonsense suppression. This may indicate a temperature-de-

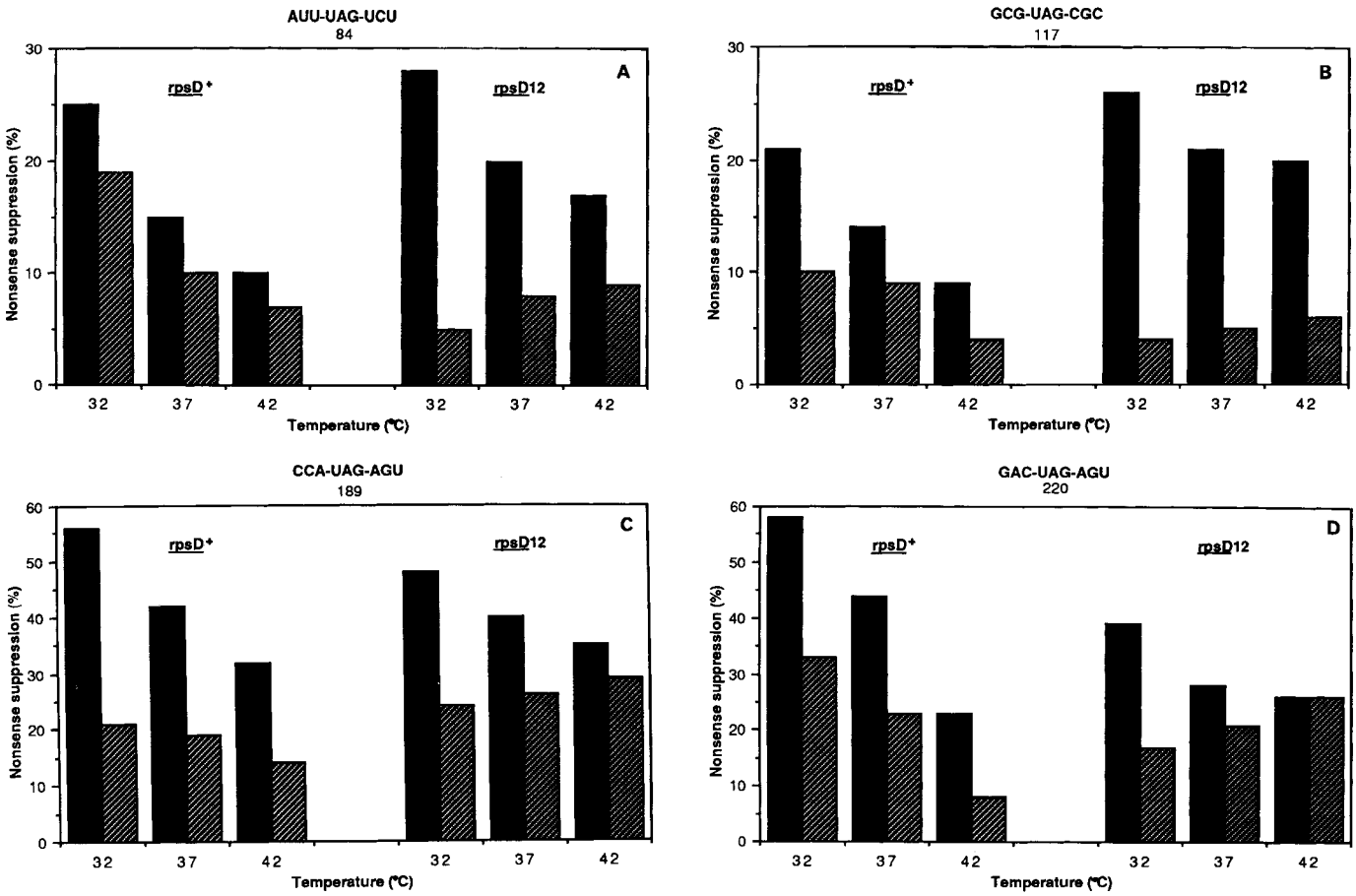

Fig. 2. *Nonsense suppression in* tyrT*(Su3) strains measured as read-through of UAG codons in the* lacI *part of a fused* lacIlacZ *gene at different temperatures.* The suppression rates in strains with EF-Tu As (wild-type product) and with EF-Tu Aa (mutant product) are shown as black columns and shaded columns, respectively. In the left-hand side the strains harbour genes for wild-type ribosomes (*rpsD*$^+$) and in the right panel mutated ribosomes (*rpsD*12) as indicated in the figure. The locations and contexts of UAG codons are shown. The strains, experimental conditions and standard errors are described in Fig. 1, Table 3 and Materials and Methods. All values are the average of four independent measurements

Table 7. *Bacterial growth and nonsense suppression rates of* tyrT*(Su3) strains and nonsense error rates in Su*$^-$ *strains carrying different EF-Tu species and ribosomal alleles*

All values are the average four independent measurements. The error frequencies were measured at UGA codons since they give significantly higher β-galactosidase activities than UAG codons. The experimental details are described in Table 3. Relevant strains are UI465, UI483, UI336, UI564, UI467, UI463, UI255 and UI541

EF-Tu	Ribosomal genotype	Growth rate	Suppression of *lacIlacZ* (UAG) at position		Error frequency in *lacIlacZ* (UGA) at position	
			189	220	189	220
		doubling/h	%			
As	*rpsL*$^+$	1.53 ± 0.03	48	37	0.40	0.70
Aa	*rpsL*$^+$	1.09 ± 0.06	21	23	0.35	0.76
As	*rpsL*282	1.30 ± 0.02	57	51	0.045	0.089
Aa	*rpsL*282	0.85 ± 0.04	28	26	0.038	0.090

pendent conformational change in the mutant elongation factor which allows it to function better at elevated temperatures with the mutant ribosomes.

Since we have shown earlier that another mutant EF-Tu (Ar) compensates aa *rpsL*(S12) mutation [3], we combined *tufA*(Aa) with this ribosomal mutation. The growth rate of such a double-mutant strain is slightly decreased compared to growth rates of strains with only one of the mutations present (Table 7). Furthermore, Aa acts as an antisuppressor, without decreasing the translational nonsense error level, in the same manner in a *rpsL*282 strain as in a strain with wild-type ribosomes. Thus, EF-Tu (Aa), which partly compensates the

*rpsD*12(S4) mutation, does not compensate the *rpsL*282(S12) mutation.

DISCUSSION

In this study we have used two kinds of competition assays *in vivo* to measure the effect of EF-Tu mutations on translational rate and accuracy. In nonsence suppression and nonsence error experiments, ternary complexes compete with release factors. The nonsense codon read-through experiments clearly imply that the mutationally altered EF-Tu forms Aa and Ap decrease the efficiency of nonsense suppressor tRNAs (Tables 3, 4). On the other hand, *in vitro* results in the accompanying paper [19] indicate that the association rate (k_{cat}/K_M) of Aa ternary complexes to the ribosomal A-site is reduced about threefold. This may explain the reduction in the *in vivo* elongation rate (Table 2), as well as in nonsense suppression (Table 3). Nonsense errors, in contrast, remain as in the wild-type factor or increase in the cases of Aa or Ap, respectively (Table 5); this indicates a more complex phenotype for these mutants than simply a general reduction in k_{cat}/K_M. It is thus conceivable that the reduced affinity for ribosomes of mutant ternary complexes is not uniform to all aminoacyl-tRNAs, but rather varies in an isoacceptor-dependent manner. Alternatively, mutant EF-Tu could be defective in some reaction at the ribosome which is specific for cognate-reading tRNAs but does not affect misreading tRNAs. Consequently, this would mean that the translational kinetics of cognate tRNAs differ basically from that of misreading tRNAs.

In the missense suppression assay, EF-Tu, complexed with missense-suppressor tRNA, competes with a ternary complex carrying a cognate wild-type tRNA. The decreased missense suppression levels seen in Aa and Ap strains may indicate that a ternary complex carrying only one mutation (*tufA*) is more efficient than the one with two mutations (*tufA*, *glyT*) in a competitive situation.

There is, *a priori*, the possibility that a change in EF-Tu may perturb its interaction with EF-Ts. This, in turn, could lead to a pile-up of EF-Tu · GDP and a corresponding reduction in the level of ternary complex. We have tested whether the antisuppressing effect of Aa can be complemented by increased levels of EF-Ts in the cell. The introduction of a plasmid with a cloned gene for EF-Ts (*tsf*$^+$) into strains harbouring *tufA*(Aa) did not alter the level of antisuppression (not shown). This result is in perfect accordance with the observation that EF-Tu Aa exhibits normal kinetics in its interaction with EF-Ts *in vitro* [19].

EF-Tu Ap has been previously characterised *in vitro* concerning some of its physicochemical properties. It displays a high intrinsic GTPase activity, in the absence of any other component of protein synthesis [12], which is further stimulated by ribosomes and Phe-tRNA^{Phe} at low magnesium concentrations (6 mM). Significantly, the presence of a poly(U) messenger is not necessary for the spontaneous GTPase reaction on the ribosome by Ap, whereas poly(U) is strictly required for stimulation of GTPase activity of wild-type EF-Tu [11]. If we assume that the hydrolysis of EF-Tu · GTP is normally triggered by some ribosomal component(s) together with the codon-anticodon interaction, it is possible that the mutant Ap has, to some degree, lost this coupling to codon recognition, and this leads to premature GTP hydrolysis. Such uncoupling could decrease the efficiency of elongation if, e.g. an increased number of ribosomes are occupied with ternary complexes incapable of making peptide bonds. Further experiments will be necessary to clarify this point concerning Ap. In any case, our results showing decreased translational rate as well as cellular growth rate are in line with known physicochemical properties of Ap.

We have shown that mutations in EF-Tu can be isolated which decrease nonsense suppression without lowering the translational nonsense error level *in vivo* or missense error *in vitro* [19]. The opposite phenomenon is also known; mutant ribosomes altered in protein S4 (*rpsD*12) or the addition of streptomycin can promote translational misreading without necessarily increasing nonsense suppression in a correlated manner [24, 38, 42]. We have previously shown that a mutant form of EF-Tu (Ar), which increases translational error *in vitro* and *in vivo*, counteracts both the hyperaccurate phenotype and decreased growth rate exhibited by a S12 (*rpsL*) ribosomal mutant [3]. It is interesting in this context that one of the EF-Tu mutants analysed here (Aa) does not compensate the same *rpsL* mutation. Instead, it partly compensates the *rpsD*12 mutation with respect to both growth rate, translational step time as well as nonsense suppression in a temperature-dependent manner. Taken together, mutations in EF-Tu and ribosomal proteins S4 and S12 affect each other in an allele-specific manner suggesting a very close functional interaction between EF-Tu and these ribosomal proteins, corroborated by structural studies of ribosomes and EF-Tu–ribosome complexes [43, 44].

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