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REGULATION OF THE EXPRESSION OF *tufA* AND *tufB*, THE TWO GENES CODING FOR THE ELONGATION FACTOR EF-Tu IN *ESCHERICHIA COLI*

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1. Introduction

The elongation factor EF-Tu has two important functions in the bacterial cell; it acts as a translational factor in protein synthesis [1,2] and as a subunit of phage RNA replicase [3]. Its intracellular abundance is surprisingly high [4–7]. *Escherichia coli* harbours two genes coding for EF-Tu: *tufA*, located at 73 min on the *E. coli* linkage map [8], lies in the so-called *str* region [9] as the distal-most gene of a 4-gene operon [10]; *tufB* lies in the *rif* region near 88 min [9] and is co-transcribed with 4 upstream tRNA genes [11,13]. The nucleotide sequences of *tufA* and *tufB* have been determined [14,15].

tufA and *tufB* are remarkably similar in nucleotide sequence and differences have been found at 13 positions only. Their corresponding gene products, EF-TuA and EF-TuB, are almost identical and differ only in the C-terminal amino acid [16,17]. The structure and the arrangement of the two *tuf* genes raise a number of genetic and regulatory problems. Here, we address the question of the regulation of their expression. We show that inactivation of *tufB* does not alter the expression of *tufA*. One single site mutation in *tufA*, however, has a drastic effect on the expression of *tufB*. This indicates that the EF-Tu protein exerts a regulatory function in *tufB* expression.

2. Materials and methods

2.1. *Materials*

EF-Tu · GDP and EF-Ts were isolated as homogeneous proteins by affinity chromatography according to [18]. Antibodies against EF-Tu were prepared as in [19]. For the preparation of anti-EF-Ts the procedure in [20] was used with some modifications (in preparation). The specificity of the antisera was checked with immuno-electrophoresis.

2.2. *Methods*

Cultures were grown in rich or in minimal medium. In order to vary the growth rate minimal medium was supplemented with different carbon sources (cf. table 1). For the preparation of 'crude extracts' cells were rapidly cooled in ice, harvested by centrifugation and broken by ultrasonication. The 'crude extracts' thus obtained were analyzed as such for EF-Tu and EF-Ts (see below). For preparation of ribosome-free supernatants the 'crude extract' was clarified by centrifugation at 30 000 × *g*. The supernatant (S-30) was centrifuged at 100 000 × *g*, yielding a ribosome-free supernatant (S-100). The ribosomal pellet was washed once in 1 M NH_4Cl, resuspended and the A_{260} of the suspension was measured to determine the cellular content of ribosomes [21].

Intracellular amounts of EF-Tu and EF-Ts were determined in 'crude extracts' by rocket immunoelectrophoresis based on the method in [22]; standard deviations were <5% (cf. fig.1).

Since our measurements yielded higher steady state EF-Tu and EF-Ts levels than reported by others [4–6,40] control experiments were performed (to be published elsewhere); they revealed that the immunological assay employed here, which makes fractionation of the ruptured cells superfluous, avoids losses of EF-Tu and other sources of errors and thus is preferable to other methods.

EF-TuA and EF-TuB species differing in isoelectric point were assayed separately by submitting S-100 preparations to isoelectric focusing on 6 mm cylindrical gels as in [23]. The gels were then rapidly frozen and

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Table 1
Growth rates of different strains of *Escherichia coli* K12 altered in *tufA* and/or *tufB* in different media

Strain	EF-Tu[c] symbols	Generation times (doublings/h)				
		LC	cas.a.a.	Glucose	Rhamnose	Acetate
LBE 1001	A_SB_S	n.d.[a]	2.30	0.99	0.68	0.24
LBE 2020	A_SB_O	2.25	1.50	0.89	0.52	0.30
LBE 2021	A_RB_O	1.95	1.16	0.63	0.43	n.g.[b]
PM 505	A_S	n.d.[a]	1.85	0.92	0.65	0.30
PM 455	A_R	2.11	1.37	0.88	0.48	n.g.[b]
PM 816	A_RB_S	1.98	1.39	0.89	0.63	0.36

[a] Not done; [b] no growth possible; [c] see table 2

Cells were grown in different media to obtain various generation times. The rich medium (LC) contains per liter: 10 g bactotrypton, 5 g yeast extract, 8 g NaCl, 10^{-3} M Tris, 10^{-2} M $MgCl_2$, 20 mg thymine, 0.2% glucose. The minimal medium (VB [41]) contains per liter: 200 mg $MgSO_4 \cdot 7\ H_2O$, 2 g citric acid, 10 g K_2HPO_4, 3.5 g $Na(NH_4)HPO_4 \cdot 4\ H_2O$, and a mixture of $MnSO_4 \cdot H_2O$ (50 mg) and $FeSO_4 \cdot 7\ H_2O$ (50 mg), which was sterilized separately. In order to vary the growth rate VB medium was supplemented with the carbon sources indicated in table 1 as cas.a.a. (casamino acids 0.5% + glucose 0.4%), glucose (glucose 0.4%), rhamnose (rhamnose 0.4%) and acetate (sodium acetate 1%).

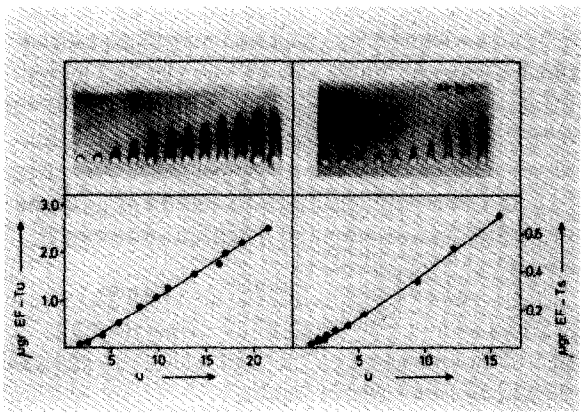

Fig.1. The standard curves for quantitative determinations of EF-Tu (left) and EF-Ts (right) by means of rocket immuno-electrophoresis. The curves were obtained by adding increasing amounts of EF-Tu · GDP (EF-Ts) to the wells (diam. 4.5 mm) of the gel. Pure EF-Tu · GDP was dissolved in a buffer containing Tris–HCl, 10 mM (pH 7.8), 10 mM magnesium acetate, 10 μM GDP, 0.1 mM phenylmethylsulphonyl fluoride, 1 mM dithioerythritol and 2 mg bovine serum albumin/ml. Pure EF-Ts was dissolved in the same buffer with a 4-fold excess of EF-Tu · GDP. The conditions for the formation of the immunoprecipitates (rockets) will be published elsewhere. The stained gels were photographed and the enlarged prints were used to cut out the areas under the rockets. The weights of the cuttings were plotted in arbitrary units (u) as a function of the amount of factor proteins.

cut into 1 mm slices which were subjected to rocket immuno-electrophoresis. Reference gels containing pure EF-Tu and S-100 extracts were run, fixed and stained for the exact location of EF-TuA and EF-TuB (fig.2). Cellular proteins were assayed as in [24]. The results were checked by amino acid analysis.

3. Results

3.1. *Outline of the experimental approach*

Regulation of *tufA* and *tufB* expression was studied by determining the intracellular contents of EF-TuA and EF-TuB in various mutant strains of *E. coli* under varying steady state growth conditions.

The mutant strains listed in table 2 were constructed for this purpose. They are all derived from the kirromycin-resistant mutant LBE 2012 which harbours a *tufA* coding for a kirromycin-resistant EF-TuA designated EF-TuA_R and a mutated *tufB* coding for an EF-TuB, sensitive to the antibiotic. The mutation in *tufB* of LBE 2012 is recessive with respect to that in *tufA* and the corresponding EF-TuB (designated EF-TuB_O) does not immobilize the ribosome on the messenger upon binding of kirromycin [25–27] as does wild-type EF-Tu [28].

Each strain is designated by its serial number sup-

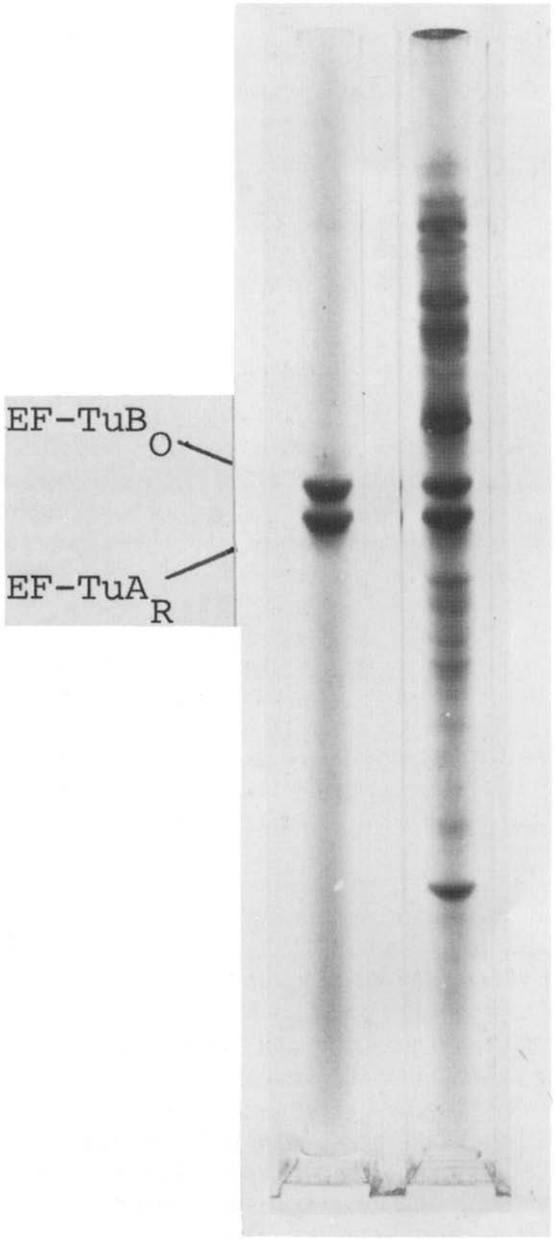

Fig.2. Isoelectric focusing of pure EF-Tu from strain LBE 2021, A_RB_O (gel A) and of an S-100 fraction from the same strain (gel B). Purified EF-Tu (20 μg) or S-100 protein (100 μg) were loaded on the gel and run as in [23]. Positions of EF-TuA_R and EF-TuB_O are indicated. EF-Tu from strain LBE 2021, A_RB_O was purified by means of affinity chromatography [18]. S-100 fraction was obtained from strain LBE 2021, A_RB_O cultured in rich medium (LC).

Table 2
Escherichia coli K12 strains used during this study

Strain	EF-Tu[a] symbols	Genotype	Phenotype[b]
LBE 1001	A_SB_S	Wild-type	
LBE 2020	A_SB_O	*tufB, rpoB*	Rif^r
LBE 2021	A_RB_O	*tufA, tufB, rpoB*	Kir^r, Rif^r
PM 505	A_S	*tufB::(Mu), rpoB*	Rif^r
PM 455	A_R	*tufA, tufB::(Mu), rpoB*	Kir^r, Rif^r
PM 816	A_RB_S	*tufA, fus*	Fus^r
LBE 2012	A_RB_O	*xyl, tufA, tufB*	Kir^r

[a] The designations A_S, A_R, B_S and B_O refer, respectively, to a wild-type *tufA* product, a kirromycin-resistant *tufA* product, a wild-type *tufB* product and an altered *tufB* product, the properties of which have been described in [25–27]

[b] Kir^r, kirromycin resistance; Rif^r, rifampicin resistance; Fus^r, fusidic acid resistance

Strains LBE 2012 and 1001 have been described in [39]. LBE 2020 was obtained by P1 transduction using LBE 2014 [39] as donor and LBE 1001 as recipient. Selection was for rifampicin resistance and screening for EF-TuB_O according to [25]. LBE 2021 was obtained by P1 transduction using LBE 2014 as donor and LBE 2020 as recipient. Selection was for kirromycin resistance. Strain PM 505 was derived from strain LBE 2045 [39] by P1 transduction using LBE 2045 as donor and LBE 1001 as recipient. Selection was for rifampicin resistance and screening for Mu-production. Strain PM 455 was constructed by P1 transduction using PM 505 as recipient and LBE 2012 as donor. Selection was for kirromycin resistance. PM 816 was obtained by P1 transduction using LBE 1001 as recipient and LBE 2015 [39] as donor. Selection was for fusidic acid resistance. To ensure the presence of the mutated *tufA* (A_R) gene in strain PM 816, a second P1 transduction was performed with LBE 2012 as donor and PM 816 as recipient. Selection was for kirromycin resistance

plemented with the corresponding EF-Tu symbols (tables 1,2). Since it is essential to work with *E. coli* strains which are isogenic except for the *tuf* genes under study we transduced *tuf* genes of LBE 2012, A_RB_O and LBE 2045, A_R into the wild-type strain LBE 1001, A_SB_S.

To determine the intracellular amounts of EF-Tu (and EF-Ts) rocket immuno-electrophoresis of total crude bacterial extracts was used (to be published elsewhere). For the determination of the relative amounts of EF-TuA and EF-TuB advantage was taken of the fact that EF-TuB_O differs in isoelectric point from wild-type EF-Tu and EF-TuA_R by 0.1 pH unit [25]. This enabled separation of the two EF-Tu species present in ribosome-free supernatants by isoelec-

Fig.3

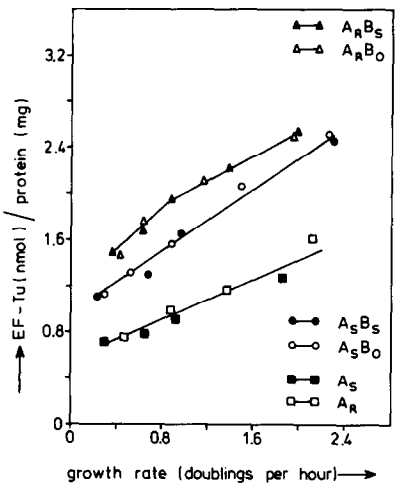

Fig.4

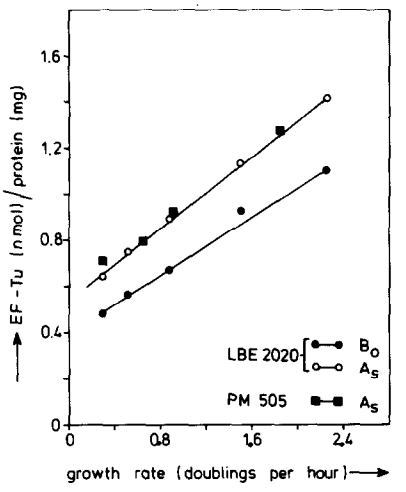

Fig.5

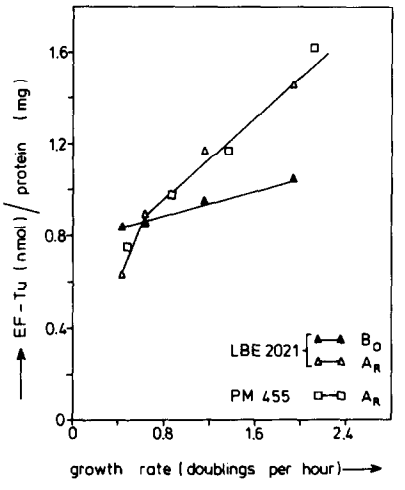

Fig.3. Cellular amounts (nmol) of EF-Tu in various strains of *E. coli* altered in *tufA* and/or *tufB* at different growth rates. The content of EF-Tu was determined in crude bacterial extracts by means of rocket immuno-electrophoresis (section 2). The EF-Tu concentration is plotted against growth rate. The growth rates were varied by culturing the cells in media with different carbon sources (table 1). The EF-Tu symbols refer to the strains mentioned in table 2.

Fig.4. The cellular amounts (nmol) of EF-TuA_S and EF-TuB_O in crude extracts of strain LBE 2020, A_SB_O cultured at different growth rates. The total cellular amount (nmol) of EF-Tu from strain PM 505, A_S is also indicated. Isoelectric focusing and rocket immuno-electrophoresis as in section 2 were used for the quantitative determination of each protein. For growth conditions compare legend to fig.3.

Fig.5. The cellular amounts (nmol) of EF-TuA_R and EF-TuB_O in crude extracts of strain LBE 2021, A_RB_O cultured at different growth rates. The total cellular amount (nmol) of EF-Tu from strain PM 455, A_R is also presented. For further experimental details see section 2 and fig.3,4.

tric focusing and analysis of the separated species by rocket immuno-electrophoresis. The ratios thus obtained for ribosome-free supernatants and the total EF-Tu contents of crude bacterial extracts were used to calculate the intracellular amounts of EF-TuA and EF-TuB.

3.2. *Inactivation of* tufB *does not affect the expression of* tufA

The cellular EF-Tu content (nmol EF-Tu/mg crude extract protein) varies linearly with the growth rate under steady state growth conditions for most of the strains studied (fig.3), in agreement with [4,6,7,12,29]. A slight deviation from this behaviour was found for strains LBE 2021, A_RB_O and PM 816, A_RB_S. The EF-Tu contents of cells with a wild type *tufA* gene and a non-functional *tufB* (PM 505, A_S) is reduced by 43% as compared to wild-type cells (LBE 1001, A_SB_S) and strain LBE 2020, A_SB_O. This reduction is the same for all growth rates studied. Interestingly the intracellular content of the single *tufA* gene product: EF-TuA_S from strain PM 505, A_S is identical to that of cells from LBE 2020, A_SB_O at comparable growth rates in (fig.4).

Apparently the cells from PM 505, A_S do not compensate for the loss of the *tufB* gene by an increased expression of the *tufA* gene. A similar conclusion emerges when the EF-TuA_R content of cells from PM 455, A_R is compared to that of LBE 2021, A_RB_O cells (fig.5); exactly the same levels of EF-TuA_R are found in both strains at comparable growth rates.

These experiments therefore demonstrate that inactivation of *tufB* does not affect the expression of *tufA* at growth rates varying from ~0.4–2.0 doublings/h. A single site mutation in *tufB* as present in LBE 2020, A_SB_O does not influence the expression of *tufA* either. This is demonstrated by the data in fig.3 showing that the total amounts of EF-Tu in cells from LBE 1001, A_SB_S are identical to those of cells from LBE 2020, A_SB_O.

3.3. *A specific single site mutation of* tufA *disturbs the coordinate expression of* tufA *and* tufB

Cells from LBE 2020, A_SB_O display a coordinate expression of *tufA* and *tufB*, confirming [31]. This can be deduced from fig.4, which illustrates that EF-TuA_S and EF-TuB_O occur in these cells in a constant molar ratio of 1.3 at all growth rates studied. Since the B_O mutation of *tufB* has no apparent effect on the expression of *tufA* (see above) and the total EF-Tu level of LBE 2020, A_SB_O is identical to that of wild-type cells from LBE 1001, A_SB_S this coordinate expression of the two *tuf* genes most likely also occurs in the latter cells.

However, the specific single site mutation of *tufA*, rendering the EF-TuA product resistant to the antibiotic kirromycin, disturbs this coordinate expression completely as is evident from the data presented in fig.5. Fig.5 reveals that the EF-TuA_R/EF-TuB_O ratio of strain LBE 2021, A_RB_O decreases upon lowering of the growth rate, whereas the ratio of EF-TuA_S/EF-TuB_O of strain LBE 2020, A_SB_O remains constant under these conditions. This difference can be ascribed almost exclusively to an enhanced expression of *tufB* which becomes more pronounced at lower growth rates. This enhanced expression of *tufB* also becomes apparent in the total EF-Tu levels of LBE 2021, A_RB_O and PM 816, A_RB_S as presented in fig.3. They are significantly increased as compared to those of LBE 2020, A_SB_O and LBE 1001, A_SB_S, both harbouring wild-type *tufA*.

It may be noted that the EF-TuA_R contents of LBE 2021, A_RB_O and of PM 455, A_R (fig.5) slightly but significantly exceed those of EF-TuA_S of LBE 2020, A_SB_O and PM 505, A_S (fig.4) at growth rates >1.0 doubling/h. This also contributes to the elevated EF-Tu levels of LBE 2021, A_RB_O and PM 816, A_RB_S (fig.3) but this effect of the mutation of *tufA* is relatively small and it remains to be seen whether this is a direct or an indirect effect on the expression of *tufA*.

4. Discussion

In wild-type cells the expression of *tufA* and *tufB* is regulated coordinately (fig.4, [31]). Remarkably the expression of *tufA* remains unchanged upon complete inactivation of *tufB* by the insertion of the bacteriophage Mu. Apparently the expression of *tufA* is independent of that of *tufB*. On the contrary, in [30] it was reported that cells lacking a functional *tufB*, due to Mu insertion, compensate for the loss of the latter gene by increasing the expression of *tufA*. A strain (KB 31) originally constructed in our laboratory and comparable to PM 505, A_S (table 2) was used [30]. The conclusion in [30] is unwarranted, since it was based on comparisons of *tuf* mRNA levels of non-isogenic strains.

A most significant finding of this investigation is that the coordinate expression of *tufA* and *tufB* breaks down completely by a specific single site mutation of *tufA*, rendering EF-TuA resistant to kirromycin. We cannot exclude the possibility that the effect of the *tufA* mutation on the expression of *tufB* is indirect. This seems less likely, however, since the enhancement of *tufB* expression, which becomes more pronounced at lower growth rates, is specific in the sense that the expression of *tsf* (coding for EF-Ts) and that of the ribosomal genes is not affected by the *tufA* mutation under all environmental conditions (in preparation). Therefore, our data are more readily explained by a direct involvement of a product of the mutated *tufA* gene on the expression of *tufB*.

The *tufA* mutation is due to a G → A transition resulting in a replacement of alanine by threonine at position 375 of the EF-TuA chian [32]. This replacement could be directly responsible for the enhanced expression of *tufB*, and would imply that the EF-Tu protein itself exerts an autogenous control function in *tufB* expression. One can only speculate about the level at which EF-Tu might exert this regulatory function. In [33] it was reported that the complex EF-Tu · EF-Ts stimulates transcription of rRNA genes and a control function of EF-Tu in regulating *tufB* transcription may be considered. However, in [34,35] no specific effect of EF-Tu on rRNA synthesis or on RNA polymerase activity was detected. An autogenous control of the expression of ribosomal protein genes has been demonstrated at the level of translation (review [36]).

Certain key ribosomal proteins (r-proteins) act as negative feedback regulators, inhibiting the translation of mRNA coding for themselves and for certain other r-proteins in the same transcription unit. In the case of S4, S7 and S8, structural homologies have been reported to exist between the binding sites on 16 S rRNA and the target sites on the mRNAs coding for the respective r-proteins [37,38]. The possibility that EF-Tu controls the expression of *tufB* by affecting the translation of *tufB* mRNA therefore deserves serious consideration.

The fact that *tufB* is cotranscribed with 4 upstream tRNA genes may provide the primary transcript with one or more target sites for EF-Tu binding which are structurally homologous to aminoacyl-tRNA, the binding partner of EF-Tu · GTP during the elongation cycle. Our recent experiments (to be published elsewhere), using plasmids harbouring *tufA* or *tufB*, support this model.

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