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The Role of EF-Tu in the Expression of *tufA* and *tufB* Genes

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We have studied the regulation of the expression of *tufA* and *tufB*, the two genes encoding EF-Tu in *Escherichia coli*. To this aim we have determined the intracellular concentrations of EF-TuA and EF-TuB under varying growth conditions by an immunological assay in mutants of *E. coli* constructed for this purpose. The data show that in wild-type cells the expression of *tufA* and *tufB* is regulated coordinately. This coordination is not restricted to steady-state growth conditions but is maintained throughout the life cycle of the cells up till the stationary phase. The ratio in which the two genes are expressed, however, may vary among cells with different genetic constitutions. Neither complete elimination of EF-TuB from the cell (by insertion of bacteriophage Mu DNA into *tufB*) nor elevation of the intracellular EF-TuB concentration (by transformation with plasmids harbouring *tufB*) has any effect on the expression of *tufA*. A specific single-site mutation of *tufA*, however, rendering EF-TuA resistant to the antibiotic kirromycin, disturbs the coordinate expression of *tufA* and *tufB*, enhancing *tufB* expression exclusively. These results have been interpreted by assuming that in wild-type cells the EF-Tu protein itself is involved in the regulation of the expression of *tufB* and that the mutant species of EF-Tu has lost this capacity either partially or completely. In agreement with this hypothesis are experiments performed *in vitro* with a coupled transcription/translation system programmed with DNA from a plasmid harbouring the entire tRNA-*tufB* transcriptional unit as a template. They show that addition to this system of EF-Tu in concentrations 2–5% of the endogenous amount results in strong inhibition of EF-Tu synthesis.

We hypothesize that EF-Tu acts as an autogenous repressor, inhibiting *tufB* expression post-transcriptionally.

During protein biosynthesis in the bacterial cell, the elongation factor EF-Tu mediates the binding of aminoacyl-tRNA to the ribosomes [1,2]. It is of interest that the intracellular EF-Tu concentration is approximately equimolar to that of aminoacyl-tRNA [3]. This implies that EF-Tu is one of the most abundant proteins in the bacterial cell and exceeds in concentration the other elongation factors and the ribosomes by a factor of about 10 [3–6] (and this paper).

Another remarkable feature of EF-Tu is its encoding by two unlinked genes, distantly located on the *Escherichia coli* linkage map [7]. One of these genes, *tufA*, is positioned at 73 min and is the promoter-distal gene of the so-called *str* operon harbouring also the genes coding for the ribosomal proteins S12 and S7, and for the elongation factor G [8]. The other gene, *tufB*, lies near 88 min in the *rif* region [9] and is cotranscribed with four upstream tRNA genes [10,11]. The nucleotide sequences of *tufA* and *tufB* were found to differ at 13 positions only [12,13] and the corresponding gene products EF-TuA and EF-TuB are identical except for the C-terminal amino acid residue [14,15]. No functional differences between the two proteins have been reported [16,17]. Obviously the regulation of the expression of the two *tuf* genes is of considerable interest, the more so because *tufB* is cotranscribed with four tRNA genes [10,11,18]. The resultant transcript specifies both structural (tRNA and informational (mRNA) RNA which poses an interesting problem [10]. Studies on the expression of *tufA* and *tufB* are hampered by the great similarity in structure of EF-TuA and EF-TuB. To overcome this difficulty, advantage can be taken of structural differences caused by mutations, provided that these mutations do not alter the regulation of the expression of the two *tuf* genes. Reeh and Pedersen [19] thus studied the rates of synthesis of EF-TuA and EF-TuB which, as a result of an innocuous mutation in *tufB*, differed in isoelectric point. We have isolated a series of *E. coli* mutants, based on selection for resistance against the antibiotic kirromycin [20–23]. These mutants are altered in *tufA* and *tufB*.

Structural differences between the *tuf* gene products enables us to determine the intracellular concentrations of EF-TuA and EF-TuB, under various growth conditions. In addition, the cellular levels of EF-Ts and the ribosomes have been determined. These data obtained *in vivo* have been supplemented with experiments performed *in vitro* and together they strongly suggest that EF-Tu itself controls the expression of *tufB*, presumably at a post-transcriptional level.

Abbreviations. SDS, sodium dodecyl sulfate.

MATERIALS AND METHODS

Bacterial Growth Conditions

Bacterial strains used in this study are listed in Table 1. One-liter batch cultures were grown aerobically at 37 °C in three-liter conical flasks with rotary shaking (New Brunswick rotary shaker, 280 rev./min). To obtain exponential phase growth, fresh overnight cultures were diluted at least 100 times in fresh media and the bacterial mass was monitored as absorbance at 560 nm by using a Zeiss spectrophotometer with a 1-cm light path. The cells were harvested in the mid-log phase (A_{560} within 0.4–0.6). Cultures were grown in rich

medium (LC) which contains per liter: 10 g bactotrypton, 5 g yeast extract, 8 g sodium chloride, 1 mM Tris, 10 mM magnesium chloride, 20 mg thymine, 0.2% glucose; or in minimal medium (VB) containing per liter: 200 mg $MgSO_4 \cdot 7H_2O$, 2 g citric acid, 10 g K_2HPO_4, 3.5 g $Na(NH_4)HPO_4 \cdot 4H_2O$ and a mixture of $MnSO_4 \cdot H_2O$ (50 mg) and $FeSO_4 \cdot 7H_2O$ (50 mg), which was sterilized separately. In order to vary the growth rate VB medium was supplemented with different carbon sources. The growth rates (in doublings/h) of the various mutant and wild-type strains of Table 1 have been published previously [23]. Cells were rapidly cooled in ice and bacteria were harvested by centrifugation. The cells were washed twice with 0.85% sodium chloride and stored at −70 °C until use.

Preparation of Extracts and Ribosomes

Bacteria were resuspended in 5 vol. buffer A (20 mM Tris/HCl pH 7.6, 10 mM magnesium chloride, 20 mM ammonium chloride, 1 mM dithioerythritol, 0.1 mM phenylmethylsulphonyl fluoride, 15% glycerol and 10 µg/ml DNase). The cells were broken by ultrasonication (3-ml chilled suspensions were sonicated by means of 10 pulses of 30 s with 30-s intervals). The resulting suspension, which was called 'crude extract', was clarified by centrifugation at $30000 \times g$ for 30 min, yielding a 'low-speed' supernatant and a pellet. The latter was resuspended in the original volume buffer A and the procedure was repeated twice more, with the exception that sonication was reduced to four pulses. The 'low-speed' supernatants were collected and centrifuged at $100000 \times g$ for 3 h, yielding a 'high-speed' supernatant.

The ribosomal pellet was washed once (overnight at 4 °C) in 0.02 M Tris/HCl pH 7.6, 0.01 M $MgCl_2$, 1 M NH_4Cl, 1 mM dithioerythritol, 0.1 mM phenylmethylsulphonyl fluoride and 15% glycerol. After centrifugation the ribosomes were resuspended in buffer A and their contents were determined (see below).

Preparation of Elongation Factors

EF-Tu · GDP and EF-Ts were isolated as homogeneous proteins by affinity chromatography according to Jacobson and Rosenbusch [24].

Antibodies against EF-Tu and EF-Ts: Preparation and Specificity

Antibodies raised against EF-Tu · GDP were prepared as described [25]. The antiserum was fractionated with 50% saturated ammonium sulphate and the pellet was taken up in a five-times-smaller volume. For the preparation of anti-(EF-Ts) the procedure of Carroll et al. [26] was used with some modifications. EF-Ts (about 90% pure) was separated from minor amounts of contaminants by electrophoresis in a polyacrylamide slab gel containing sodium dodecyl sulphate SDS. The proteins in the gel were fixed and stained in 40% methanol, 7% acetic acid (v/v) with 0.25% Coomassie brilliant blue R. The gel was destained by repeated washing in 40% methanol, 7% acetic acid. The stained band of EF-Ts was cut out and washed overnight at room temperature in 96% ethanol containing 0.1 M sodium acetate to remove SDS. The gel slices were swollen for 15 min in a sterile physiological salt solution. Slices containing about 50 µg of protein were homogenized in a total volume of 1 ml sterile physiological salt solution and injected subcutaneously in a rabbit (about 2.5 kg) over a period of five weeks (50 µg protein each week). One month after the last injection 500 µg pure EF-Ts dissolved in 10 mM Tris/HCl pH 7.4, 10 mM $MgCl_2$ and emulsified in an equal volume of complete Freund's adjuvant was given as a booster, 14 days later the rabbit was bled from the ear and the serum was collected by centrifugation. The antibodies were pelleted with 50% saturated ammonium sulphate, concentrated five times as described for anti(EF-Tu) and stored at −20 °C. The specificity of the antisera was studied with immunoelectrophoresis. When EF-Tu, prepared according to convential procedures [27], was used as an antigen the antibodies yielded two precipitin lines upon rocket immuno-electrophoresis of crude bacterial extracts. EF-Tu (at least 95% pure) added to the extracts specifically enhanced only one precipitin line. Elimination of contaminating antigens, undetectable with procedures other than immunoelectrophoresis, was achieved as described above for preparing antibodies against EF-Ts (SDS gel electrophoresis and injection of the stained band directly into the rabbit).

Isoelectric Focusing Procedures

The two EF-Tu species in high-speed supernatant preparations of LBE 2020,A_SB_O and LBE 2021,A_RB_O were separated by subjecting the preparations to isoelectric focusing on cylindrical gels of 6 mm diameter in perspex tubes. 50 − 100 µg of high-speed supernatant protein was loaded on the gel and run as described by O'Farrell et al. [28]. After electrophoresis the gels were rapidly frozen and stored at −20 °C. The frozen gels were then cut into 1-mm slices which were submitted to rocket immunoelectrophoresis as described below (compare also Fig. 1). The relative amounts of the two EF-Tu species were determined using the areas under the 'rockets' and a calibration curve as shown previously [23].

Reference gels containing pure EF-Tu were run, fixed and stained for the exact location of EF-TuA and EF-TuB. The yield of EF-Tu from the frozen and unfixed gels was at least 90% of the amount originally present in the high-speed supernatant fraction as determined by rocket immunoelectrophoresis.

Assay of the Content of EF-Tu and EF-Ts by Rocket Immunoelectrophoresis

The contents of EF-Tu and EF-Ts were determined in crude bacterial extracts by means of rocket immunoelectrophoresis [29]; 25 ml 1% agarose solution (55 °C) mixed with 100 µl anti-(EF-Tu) or anti-(EF-Ts) was poured on a prewarmed glass plate (8.2 × 20 cm) and polymerized at room temperature. The samples (20 µl crude extracts or 1-mm gel slices) were applied to wells which were punched into the agar at one side of the plate (see Fig. 1). The gel was connected with two 1-l buffer compartments containing 4.3 mM 5,5-diethylbarbituric acid and 20 mM sodium 5,5-diethylbarbiturate (pH 8.6) using 3MM Whatman filter strips. Electrophoresis was at 50 V (≈ 8 mA) and 4 °C for 17 h. The gels were covered with a sheet of filter paper and dried with warm air. Staining occurred in a solution containing 450 ml ethanol, 450 ml redistilled water, 100 ml acetic acid and 5 g Coomassie brilliant blue R (filtered before use) and destaining in the same solution without dye.

Assays for Ribosomes and Cellular Proteins

The content of ribosomes, isolated as described above, was determined from the A_{260} of the ribosomal pellet, as-

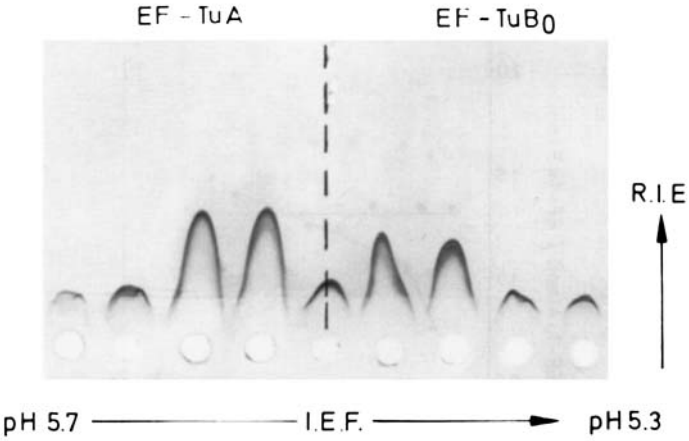

Fig. 1. *Determination of the relative amounts of EF-TuA and EF-TuB$_O$ in a high-speed supernatant extract of* E. coli *by means of isoelectric focusing (I.E.F.) and rocket immunoelectrophoresis (R.I.E.).* For experimental details see Materials and Methods

suming a molecular weight of 2.8×10^6 and an A_{260} of 14.4 for 1 mg/ml of ribosomes [30]. Cellular proteins were determined with the method of Bradford [31].

Construction of a Plasmid Bearing tufB$_O$

Escherichia coli K12 strain LBE 2012 harbouring pTuB$_1$ [32] was grown overnight at 37 °C in rich medium (broth) supplemented with 50 µg/ml ampicillin. Plasmid DNA was isolated using the cleared lysate technique of Birnboim and Doly [33]. *E. coli* cells from strain LBE 2020,A$_S$B$_O$, were prepared for transformation by the procedure of Lederberg and Cohen [34]. After transformation with pTuB$_1$ the transformants were selected for ampicillin resistance. The strain LBE 2020,A$_S$B$_O$, containing plasmid pTuB$_1$ was grown in rich medium (LC) in the presence of 50 µg/ml ampicillin to an A_{560} of about 0.1; 10 ml of the culture was sedimented and resuspended in 1 ml of a physiological salt solution. The suspension was irradiated (50 µJ). Cells were subsequently inoculated in 25 ml fresh medium (broth) containing 50 µg/ml ampicillin and grown overnight at 37 °C. Plasmid DNA was isolated from this culture and used for transformation of the kirromycin-resistant strain LBE 12021 (*RecA*$^-$). Colonies were selected for resistance to both ampicillin and kirromycin. (The selection was based on the inability of the plasmid encoded EF-TuB$_O$ to change the kirromycin-resistant phenotype into a sensitive one [32].) Three colonies were isolated and screened for their EF-TuB$_O$ content. All three showed an approximately, twofold increase in intracellular EF-TuB$_O$, confirming the presence of *tufB*$_O$ on plasmid pTuB$_O$.

RESULTS

In a preliminary communication [23] we reported on the cellular contents of EF-TuA and EF-TuB in various mutant and wild-type strains of *Escherichia coli* K12. Under steady-state growth conditions the intracellular levels of EF-Tu in wild-type cells were found to vary from 5% to 10% of the total bacterial protein (see Table 1). These values are higher than those reported by others [3,5]. The same holds true for the EF-Ts levels we report here (Table 1) [5,30,35]. Since the determination of the intracellular concentrations of these factor proteins forms the basis of the present investigation, it

Table 1. *Intracellular amounts of EF-Tu and EF-Ts in unrelated strains of* E. coli *at different growth rates*
The contents of EF-Tu and EF-Ts are expressed as percentages of total cellular protein. Cells were grown at 37 °C in rich medium (LC) or minimal medium (VB), supplemented with 0.4% glucose and 0.5% casamino acids (+ AA) or with 1% glucose (+ Glc). The analyses were performed as described under Materials and Methods

Strain	Type	Medium	Growth rate	EF-Tu content	EF-Ts content
			doublings/h	%	
NF 314	B	LC	2.7	8.9	1.05
		VB + AA	1.7	7.1	0.91
LBE 1001	K12	VB + AA	2.3	10.6	0.97
		VB + Glc	1.0	7.1	0.65
LBE 2020	K12	VB + AA	1.5	8.9	0.77
D 22	K12	LC	2.0	9.2	1.00

is necessary to establish first whether these differences are due to the assay employed, to the genetic background of the *E. coli* strains investigated or to both.

Assays of EF-Tu and EF-Ts by Rocket Immunoelectrophoresis: Recovery Studies

Intracellular concentrations of EF-Tu and EF-Ts were determined in crude bacterial extracts by means of rocket immunoelectrophoresis [29] as described in Materials and Methods. After fractionation of these extracts the following percentages of the original EF-Tu content (in crude extracts) were found in the cell wall/membrane fraction, the ribosomes and the ribosome-free supernatant, respectively: 2%, 3–5% and 75%. These data can be interpreted in two ways: (a) assays of crude bacterial extracts lead to overestimation of the EF-Tu content due to other components in these extracts; (b) extract fractionation causes losses due to proteolysis and/or adherence [36] to glassware and other equipment.

The first possibility was excluded in the following way. EF-Tu · GDP purified to homogeneity (cf. Materials and Methods) was supplemented with either of the following fractions: (a) combined cell wall/membrane fractions which had been washed with several detergents (see below) successively to remove EF-Tu; (b) high-speed supernatant fractions depleted of EF-Tu and/or EF-Ts by affinity chromatography [24]; (c) ribosomes washed with 1 M NH_4Cl. None of these fractions affected the EF-Tu assay quantitatively. Triton X-100 (1%), urea (9 M), sarkosyl (0.5%), sodium deoxycholate (0.08%), all used for washing the cell wall/membrane fractions, did not have any effect either. Exceptions were sodium dodecyl sulphate (1%) and high salt (> 500 mM NH_4Cl) which affected the results substantially. Urea and sarkosyl sharpened the precipitin line of the 'rocket'; EF-Ts had the opposite effect, but none of these three components affected the results quantitatively.

Similar experiments led to the same conclusion for EF-Ts. Addition of an excess of EF-Tu to pure EF-Ts resulted in a small change of the standard curve for EF-Ts but correction for this effect was made by adding a fourfold excess of pure EF-Tu to the samples (compare also [23]). We conclude from these experiments that fractionation of the bacterial extract causes losses of the elongation factor proteins, presumably due to proteolytic breakdown (in spite of the presence of 0.1 mM phenylmethylsulphonyl fluoride and 15% glycerol)

Table 2. *Strains of* E. coli *used during this study*
Strains LBE 1001, 2020, 2021, 2012, 2014, 2015, 2045 and PM 505, 455, 816 have been described previously [23,25]. For the introduction of the *RecA*$^-$ allele of strain KA 437 into strains LBE 2020 and LBE 2021, the bacteria were treated with trimethoprim to select Thy$^-$ cells as described by Miller [55]. Thy$^-$ cells were subsequently crossed with Hfr strain KA 273. Selection was for Thy$^+$ and screening for ultraviolet sensitivity. Strains D 22 and NF 314 were obtained from Drs A. Parmeggiani and A. V. Furano, respectively. The designations A_S, A_R, B_S and B_O for EF-Tu refer respectively to a wild-type *tufA* product, a kirromycin-resistant *tufA* product, a wild-type *tufB* product and an altered *tufB* product whose properties have been described previously [22,39,54]. In the phenotype description, Kir^r is kirromycin resistance; Rif^r is rifampicin resistance; Fus^r is fusidic acid resistance; Str^r is streptomycin resistance and UV^s is ultraviolet sensitivity

Strain	Type	EF-Tu symbols	Genotype	Phenotype
LBE 1001	K12	A_SB_S	wild-type	
LBE 2020	K12	A_SB_O	*tufB, rpoB*	Rif^r
LBE 2021	K12	A_RB_O	*tufA, tufB, rpoB*	Rif^r, Kir^r
PM 505	K12	A_S	*tufB::(Mu), rpoB*	Rif^r
PM 455	K12	A_R	*tufA, tufB::(Mu), rpoB*	Rif^r, Kir^r
PM 816	K12	A_RB_S	*tufA, fus*	Fus^r
LBE 12020	K12	A_SB_O	*tufB, rpoB, recA*$_{56}$	Rif^r, UV^s
LBE 12021	K12	A_RB_O	*tufA, tufB, rpoB, recA*$_{56}$	Rif^r, Kir^r, UV^s
LBE 2012	K12	A_RB_O	*xyl, tufA, tufB*	Kir^r
LBE 2014	K12	A_RB_O	*xyl, tufA, tufB, rpoB*	Kir^r, Rif^r
LBE 2015	K12	A_RB_O	*xyl, fus, tufA, tufB*	Kir^r, Fus^r
LBE 2045	K12	A_R	*cys-am, gal-am, his, mal, lam, fus, rpoB, tufA, tufB::(Mu)*	Kir^r, Fus^r, Rif^r
D 22	K12	A_SB_S	*envA, ampA, rpsL, his, proB, trp*	Str^r
KA 437	K12	A_SB_S	*thr, ilv, recA*$_{56}$ (Hfr)	UV^s
NF 314	B	A_SB_S	wild-type	

and/or adherence to glassware and other equipment. Our immunoassay, which is performed with total crude bacterial extracts, avoids these losses. The reproducibility of our measurements also illustrates the reliability of the immunoassay employed in the investigations described below (compare [23]).

EF-Tu and EF-Ts Contents of E. coli *Strains Differing in Their Genetic Constitution*

Several reports have shown that the intracellular amount of EF-Tu and EF-TS increases in proportion to the growth rate [3,5,6,23]. However, the absolute amount varies from one report to another [3,5,23]. Table 1 summarizes the intracellular amounts of EF-Tu in two unrelated K12 strains and one *E. coli* B strain (NF 314). It can be concluded that the amounts vary significantly between strains of *E. coli* K12 (D 22 and LBE 1001) and *E. coli* B (NF 314) normalized to comparable growth rates. Table 1 further shows that the EF-Ts content is more or less comparable in unrelated strains. It indicates that in these strains the molar ratio of EF-Tu to EF-Ts may vary and possibly also the ratio between EF-Tu and other components of the translational machinery.

Expression of tufA *and* tufB *under Various Growth Conditions*

In order to study the expression of *tufA* and *tufB* under various growth conditions we have constructed a number of

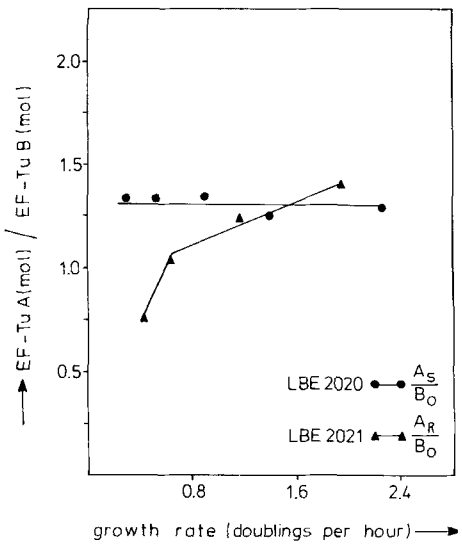

Fig. 2. *The ratios of EF-TuA$_S$/EF-TuB$_O$ (●—●) and EF-TuA$_R$/EF-TuB$_O$ (▲—▲) in cells from strains LBE 2020, A$_S$B$_O$ and LBE 2021, A$_R$B$_O$, respectively, at various growth rates.* For experimental details see Materials and Methods

mutants of *E. coli* altered in *tufA* and *tufB* [20,21,23]. These mutants are listed in Table 2. Some mutant strains produce an EF-TuB$_O$, which differs from the *tufA* product in isoelectric point by 0.1 [22]. This enables separation of *tufA* and *tufB* products present in ribosome-free supernatants by isoelectric focusing and analysis of the separated species by rocket immunoelectrophoresis. From the ratio of EF-TuA and EF-TuB concentrations and from the total EF-Tu content of the crude bacterial extract, the intracellular amounts of EF-TuA and EF-TuB can be calculated (cf. Materials and Methods and [23]). In this way a constant ratio of about 1.3 for the two EF-Tu species was found in cells from the strain LBE 2020,A$_S$B$_O$ over a wide range of steady-state growth rates (compare Fig. 2 and [23]).

It was of interest therefore to know whether cells maintain this EF-TuA/EF-TuB ratio when grown under non-steady-state conditions. To this aim cells of LBE 2020,A$_S$B$_O$ were grown to various cell densities in minimal medium (VB) supplemented with 0.5% casamino acids and 0.4% glucose. At various stages of growth their EF-TuA/EF-TuB ratio was monitored (compare Fig. 3). As is evident from Table 3, this ratio remained unaltered from early log phase (stage A) till in the stationary phase (stage F). Only when the cells were kept for another 12 h under the same conditions (stage G) was the ratio significantly increased. By then, however, cells showed substantial losses in viability as judged by a 60% drop in their ability to form colonies when plated on solid agar in rich medium (Table 3). These data show that the expression of *tufA* and *tufB* is coordinately regulated not only under varying conditions of steady-state growth but also in other stages of growth.

Previously [23], we found that the total EF-Tu contents of wild-type cells of the strain LBE 1001,A$_S$B$_S$ were identical to those of LBE 2020,A$_S$B$_O$ under all growth conditions studied. This suggests that expression of the two *tuf* genes in LBE 1001,A$_S$B$_S$ is also regulated at a constant ratio of 1.3 and that the specific point mutation of *tufB* (*tufB$_O$*) does not affect the coordination in the expression of *tufA* and *tufB*. Below we shall see that cells with a specific point mutation in *tufA* have lost this coordination completely.

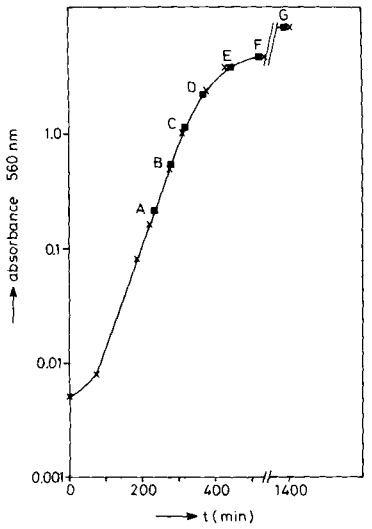

Fig. 3. *Growth curve of cells from LBE 2020,A_SB_O.* At different times (A–G) samples were taken from the culture and analyzed for the ratio EF-TuA_S/EF-TuB_O (see Table 3). For experimental details see the legend to Table 3 and Materials and Methods

Table 3. *Effects of the growth stage on the ratio of EF-TuA and EF-TuB in* E. coli *K12*
Cells of strain LBE 2020,A_SB_O were added to 3-l conical flasks containing 1 l minimal medium (VB) supplemented with 0.5% casamino acids and 0.4% glucose. Bacteria were incubated at 37 °C for 24 h under rotational shaking (280 rev./min). At the times indicated (A, B, etc., see Fig. 3) cells were harvested and assayed for the intracellular ratio of EF-TuA_S and EF-TuB_O. The number of viable cells was determined by plate assay. The total number of cells was determined by mixing the cells at a proper dilution with prewarmed agarose (1%). The mixture was put on a slide and dried with warm air. The slide was coloured with Coomassie brilliant blue. The fixed and coloured bacteria were subsequently counted with the aid of a conventional microscope. The intracellular ratio of EF-TuA_S and EF-TuB_O was determined as described under Materials and Methods

Designations from Fig. 3	Growth	Surviving fraction	Intracellular ratio of EF-TuA_S/ EF-TuB_O
	A_{560}	%	
A	0.22	100	1.3
B	0.53	100	1.3
C	1.10	100	1.3
D	2.38	100	1.3
E	3.90	100	1.3
F	4.95	100	1.3
G	8.80	42	1.8

Table 4. *Intracellular amounts of EF-TuA and EF-Ts in cells of* E. coli *K12 with two active* tuf *genes (LBE 2020) and in cells in which the* tufB *gene is inactivated by the insertion of bacteriophage Mu (PM 505)*
The growth conditions and analyses of EF-TuA, total EF-Tu and EF-Ts are described in detail under Materials and Methods. The contents are expressed as percentages of total cellular protein

Strain	Medium	Growth rate	Total EF-Tu content	EF-TuA content	EF-Ts content
		doublings/h	%		
LBE 2020,A_SB_O	LC	2.25	10.6	6.0	1.00
LBE 2020,A_SB_O	VB + AA	1.50	8.9	5.1	0.77
LBE 2020,A_SB_O	VB + Glc	0.89	6.7	3.8	0.54
LBE 2020,A_SB_O	VB + rhamnose	0.52	5.6	3.2	0.46
PM 505,A_S	VB + AA	1.85	5.4	5.4	0.88
PM 505,A_S	VB + Glc	0.92	3.9	3.9	0.60
PM 505,A_S	VB + rhamnose	0.65	3.4	3.4	0.46
PM 505,A_S	VB + acetate	0.30	3.1	3.1	0.31

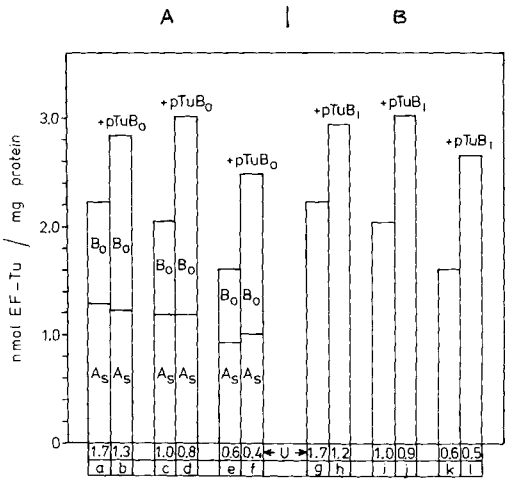

Fig. 4. *(A) Intracellular amounts of EF-TuA in cells of LBE 12020,A_SB_O before (lanes a, c and e) and after transformation with $pTuB_O$ (lanes b, d and f). (B) Total amounts of EF-Tu in cells of LBE 12020,A_SB_O before (lanes g, i and k) and after transformation with $pTuB_I$ (lanes h, j and l).* For further experimental details see Materials and Methods

Neither an Enhanced nor a Reduced Intracellular Level of EF-Tu Affects the Expression of tufA

The intracellular level of EF-TuB can be modulated in two ways. Insertion of bacteriophage Mu-DNA into *tufB* leads to inactivation of the latter gene and to a complete elimination of EF-TuB from the cell [23]. Transformation of the cell with a plasmid harbouring *tufB* on the other hand results in elevated levels of EF-TuB.

Table 4 shows the effect of inactivation of *tufB* on the expression of *tufA*. In these experiments the EF-TuA content of cells from the strain LBE 2020,A_SB_O was compared with that of cells from the strain PM 505,A_S under varying nutritional conditions. The latter strain harbours a *tufB* inactivated by Mu-DNA insertion. Restriction analysis demonstrated [37] that the insertion had taken place in the C-terminal part of the gene, thus leaving the regulatory elements of *tufB* intact. As a result of this inactivation the drop in total EF-Tu level is constant under all environmental conditions (cf. Table 4; [23]) and the EF-TuA contents of the two types of cells are essentially the same at comparable growth rates. It may thus be concluded that inactivation of *tufB* resulting in a substantial loss of total intracellular EF-Tu (about 40%) does not alter the expression of *tufA*.

Alteration of *tufA* expression does not occur either when the EF-Tu level is raised by transformation with plasmids harbouring *tufB*. Fig. 4 illustrates this phenomenon for cells of the strains LBE 12020,A_SB_O which have been transformed with the plasmid $pTuB_O$. As can be seen in this figure, both parental cells and transformants produce the same amount of EF-TuA (lower parts of the bars) despite the fact that the

Table 5. *Intracellular amounts of total EF-Tu and EF-Ts before (−p) and after transformation with pTuB$_1$ (+p)*
Cells were grown at 37 °C in different media to vary the generation time. Transformed cells were grown in the presence of 50 μg/ml ampicillin. The contents are expressed as percentage of total cellular protein

Strain	Medium	Growth rate		EF-Tu content		EF-Ts content	
		+p	−p	+p	−p	+p	−p
		doubling/h		%			
LBE 12020,A$_S$B$_O$	LC	1.2	1.7	12.6	9.5	0.97	0.97
LBE 12020,A$_S$B$_O$	VB + AA	0.9	1.0	12.9	8.8	0.91	0.94
LBE 12020,A$_S$B$_O$	VB + Glc	0.5	0.6	11.4	6.9	0.66	0.63

total EF-Tu content is elevated substantially. The plasmid pTuB$_O$ is identical to the plasmid pTuB$_1$ described by Miyajima and Kaziro [32], except that the *tufB* gene codes for EF-TuB$_O$ (see Materials and Methods). This plasmid contains the entire tRNA-*tufB* transcription unit [32].

Cells from LBE 12020,A$_S$B$_O$ transformed with either pTuB$_1$ or pTuB$_O$ produce the same amounts of total EF-Tu when growing in the same media (compare Fig. 4). Transformation with pTuB$_1$ does not alter the intracellular concentrations of EF-Ts as can be concluded from Table 5. This argues against the possibility that changes in growth rates, which are observed after transformation with pTuB$_1$ or pTuB$_O$ have affected in any way the EF-TuA contents of the cells. We conclude that neither reduction nor elevation of the intracellular level of EF-Tu influences the expression of *tufA*.

The Effect of a Specific Single-Site Mutation of tufA *on the Expression of* tufB, tsf *and Ribosomal Genes*

Fig. 2 illustrates the ratio of EF-TuA and EF-TuB in strains LBE 2020,A$_S$B$_O$ and LBE 2021,A$_R$B$_O$ at various growth rates. As mentioned above, EF-TuA$_S$ and EF-TuB$_O$ of the former strain occur in a constant molar ratio of 1.3 at all growth rates studied. In striking contrast the ratio of EF-TuA$_R$ and EF-TuB$_O$ of strain LBE 2021,A$_R$B$_O$ is not constant but varies with the growth rate.

Previously [23], we showed that this is mainly due to an enhanced expression of *tufB*, which becomes more pronounced at lower growth rates. Apparently the coordination in expression of *tufA* and *tufB* which is characteristic for wild-type cells (and cells of the strain LBE 2020,A$_S$B$_O$) is completely lost in cells (LBE 2021,A$_R$B$_O$) harbouring the specific single-site mutation in *tufA* which renders the EF-TuA product resistant to kirromycin.

In order to investigate whether this mutation exerts any effect on the levels of other proteins of the translational machinery, we determined the intracellular contents of EF-Ts and of the ribosomes of a number of mutant and wild-type strains under varying growth conditions. As is apparent from Fig. 5 the EF-Ts contents of all strains studied display identical growth dependencies. Raising the generation time from 0.3 to 2.4 doublings/h results in a fourfold increase in EF-Ts content. Similar findings were obtained for the ribosomes so that ratio EF-Ts/ribosome remains constant over the entire range of growth parameters (Fig. 6). Similar results have been described for wild-type cells by other investigators [3, 4, 30, 36]. The present findings that the EF-Ts contents of

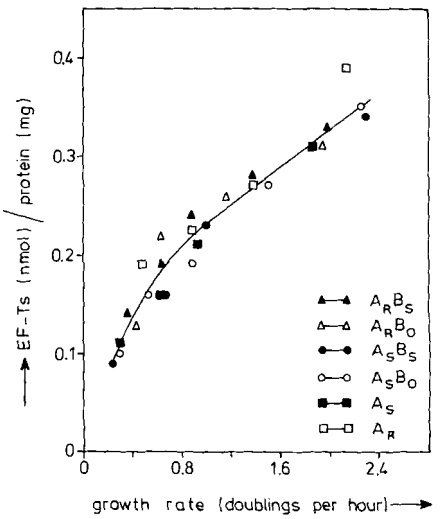

Fig. 5. *The intracellular amounts of EF-Ts in different strains of* E. coli *K12 at various growth rates.* The EF-Tu symbols in the figure refer to the strains mentioned in Table 2. For further experimental details see Materials and Methods

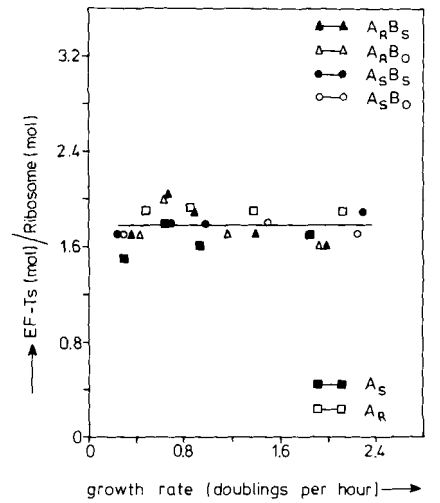

Fig. 6. *The molar ratio of EF-Ts/ribosome in different strains of* E. coli *K12 at various growth rates.* For symbols and further experimental details see legend to Fig. 5 and Materials and Methods

all strains studied are the same (Fig. 5) and that the EF-Ts/ribosome ratio is constant and independent of the generation time (Fig. 6) strongly suggest that the mutations in *tufA* and/or *tufB* do not affect the expression of *tsf* and ribosomal genes.

Regulation of the Expression of tufA *and* tufB *versus that of* tsf *and Ribosomal Genes*

The data presented in Fig. 7 show that at growth rates exceeding 1.0 doubling/h the molar ratio EF-Tu/EF-Ts remains virtually constant. This is in agreement with other investigations [3, 4, 36]. Fig. 7 shows that it holds true for cells with one active *tuf* gene (PM 505,A$_S$) and for cells with two active *tuf* genes (LBE 2020,A$_S$B$_O$, LBE 1001,A$_S$B$_S$). At lower growth rates, however, this ratio rapidly increases. It also applied to the separated gene products EF-TuA$_S$ and EF-TuB$_O$ from strain LBE 2020,A$_S$B$_O$ and for reasons mentioned above to the EF-TuA$_S$ and EF-TuB$_S$ species from wild-type strain

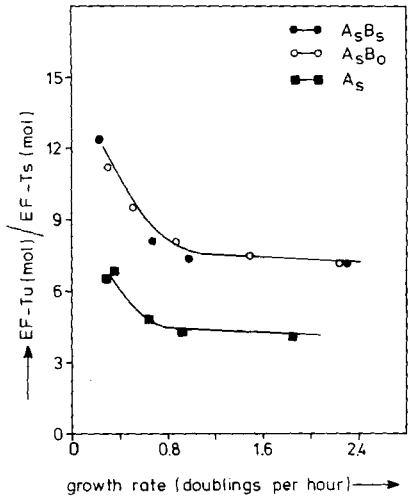

Fig. 7. *The molar ratio of EF-Tu/EF-Ts in three strains of* E. coli *at various growth rates.* For symbols and further experimental details see legend of Fig. 5 and Materials and Methods

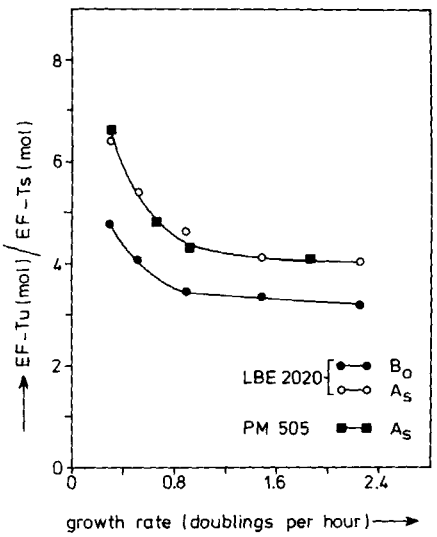

Fig. 8. *The molar ratio of EF-TuA$_S$ and EF-TuB$_O$ versus EF-Ts in cells of strain LBE 2020,A$_S$B$_O$ at various growth rates.* The ratio of total EF-Tu of strain PM 505,A$_S$ versus EF-Ts is also presented. For experimental details and symbols compare Materials and Methods and legend to Fig. 5

LBE 1001,A_SB_S. It means that at growth rates above 1.0 doubling/h the expression of *tufA*, *tufB*, *tsf* and the ribosomal genes is coordinately regulated. At lower growth rates this coordination persits for *tufA* and *tufB* (Fig. 8) but the expression of *tsf* and the ribosomal genes drops faster than that of the *tuf* genes (Fig. 5 and 6).

Synthesis of EF-Tu in vitro *in a Coupled Transcription/Translation System is Suppressed by Addition of EF-Tu*

Above we have seen that a specific point mutation of *tufA* causes an enhancement of *tufB* expression, thus upsetting the coordination in the expression of the two *tuf* genes. This observation suggested a direct involvement of a product of the mutated *tufA* in the expression of *tufB*. In order to study such a direct control function of EF-Tu we have added in-

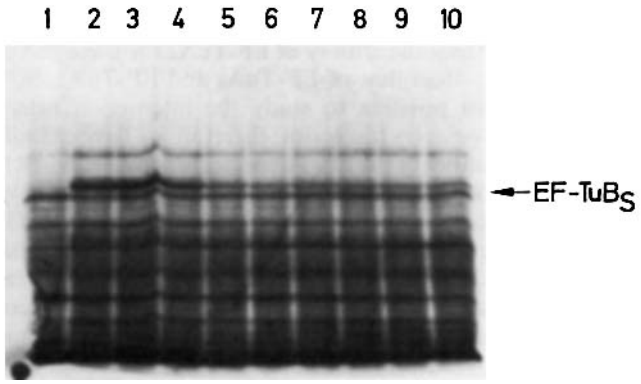

Fig. 9. *The effect of increasing amounts of EF-Tu on synthesis of EF-Tu* in vitro *in a cell-free coupled transcription/translation system programmed with DNA from pTuB$_1$.* The reaction mixtures contained in a final volume of 50 µl: 50 mM Tris/acetate (pH 8.2), 100 mM potassium acetate, 40 mM ammonium acetate, 14 mM magnesium acetate, 1.5 mM dithioerythritol, 0.75 mM each of GTP, UTP, CTP, 3 mM ATP, 10 mM phospho*enol*pyruvate, 1.0 µg pyruvate kinase, 1.6% (w/w) poly(ethylene glycol) 6000, 4 pmol of [^{35}S]methionine (special activity 1195 Ci/mmol) and 0.1 mM each of other 19 amino acids, 0.088 mM cAMP, 10 µg leucoverine, 45 µg low-speed supernatant proteins, prepared as described by Zubay [56] and 1 µg pTuB$_1$ as template. The components were mixed and incubated for 45 min at 37 °C. After incubation the reaction mixtures were supplemented with 1.0 ml trichloroacetic acid (5%) and heated for 5 min at 90 °C. The precipitates were collected by centrifugation and washed with trichloroacetic acid (5%). The washing procedure was subsequently repeated twice with 1.0 ml ethanol. The dried pellets were dissolved in a buffer containing $NaDodSO_4$ (2%), 2-mercaptoethanol (5%), glycerol (10%) and Tris/HCl (45 mM) pH 8.2, and heated for 5 min at 90 °C. The samples were submitted to electrophoresis on SDS/10% polyacrylamide gels. In the experiment of lane 1 no pTuB$_1$ DNA was present in contrast to all other experiments. Lanes 2–10 represent experiments in which 0, 2, 5, 10, 20, 30, 40, 50 and 60 pmol EF-Tu was added, respectively. The position of EF-Tu is indicated by an arrow

creasing amounts of this protein to a cell-free coupled transcription/translation system using plasmid DNA (pTuB$_1$) as a template. As can be seen in Fig. 9, this addition suppresses the synthesis of EF-Tu considerably (compare for instance lanes 2 and 5) without affecting the formation of other proteins in this system. In parallel experiments with template DNA derived from a plasmid harbouring *tufA* (pTuA$_1$, compare [38]), no suppression of EF-Tu synthesis was observed (Fig. 10). This selective effect of the EF-Tu protein on EF-TuB synthesis occurs already upon addition of 5–10 pmol of EF-Tu which is about 2–5% of the EF-Tu proteins endogenously present in the cell-free extract. Since most (more than 80%) of the endogenous EF-Tu is complexed with aminoacyl-tRNA [57], this suppression is due to an increase in EF-Tu not taken up in a ternary complex (see also Discussion).

EF-TuA$_R$ · GTP has a Lowered Affinity for Aminoacyl-tRNA

The observation that the specific mutation of *tufA* has different consequences for the expression of *tufA* and *tufB in vivo* and the selective action of EF-Tu on *tufB* expression *in vitro* may be correlated with the different organization of the *tuf* genes in two distinct transcription units. The fact that *tufB* is cotranscribed with four upstream tRNA genes [11, 18] raises the possibility that the EF-Tu protein can influence the expression of *tufB* exclusively by binding one or more of the tRNA elements of the primary transcript of the *tufB*

transcription unit. If so, the experiments described above would indicate that the affinity of EF-TuA_R for these putative targets is lower than that of EF-TuA_S and EF-TuB_O. So far it has not been possible to study the interaction between EF-Tu and the primary transcript; therefore we have examined the relative affinities of the EF-Tu species for aminoacyl-tRNA using the nitrocellulose filter technique previously employed [38]. Ternary complexes were formed between EF-Tu · GTP and a mixture of aminoacyl-tRNAs. The reaction mixtures were filtered through nitrocellulose filters and the filtrates were supplemented with ethanol. Precipitates were collected and, after resuspending, submitted to isoelectric focusing in a pH gradient (pH 5–7). This analysis takes advantage of the fact that the ternary complexes pass through the filters whereas unbound EF-Tu · GTP is retained. Fig. 11 A shows the analyses of the ternary complexes formed when the aminoacyl-tRNAs were incubated with a mixture of EF-TuA_S · GTP and EF-TuB_O · GTP, and Fig. 11 B that of the ternary complexes formed with EF-TuA_R · GTP and EF-TuB_O · GTP. In both experiments EF-TuB_O acted as an internal standard. The ratio between aminoacyl-tRNA and EF-Tu varied from 0.5 to 5.0 in lanes 3–7 of Fig. 11 A and from 0.2 to 5.0 in lanes 3–9 of Fig. 11 B. As a control, aminoacyl-tRNA was omitted from the reaction mixture. In these cases (lanes 2 of Fig. 11 A and B) no EF-Tu appeared in the filtrate. The ratios between the EF-TuA and EF-TuB species in the original reaction mixtures were determined by isoelectric focusing of these mixtures prior to millipore filtration (lanes 1 of Fig. 11 A and B). From the analysis of Fig. 11 A it can be concluded that the ratio between EF-TuA_S and EF-TuB_O in the ternary complexes remained essentially the same when the reaction was performed with increasing amounts of aminoacyl-tRNA. This is brought out more clearly in Table 6 which gives the ratios based on scanning profiles of the isoelectric focusing gels.

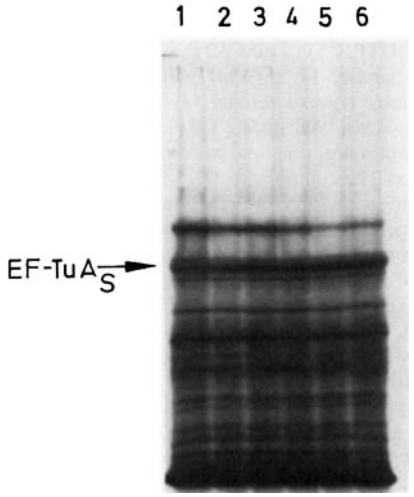

Fig. 10. *The effect of increasing amounts of EF-Tu on synthesis of EF-Tu* in vitro *in a cell-free coupled transcription/translation system programmed with DNA from pTuA_1.* The experimental conditions were as described in the legend to Fig. 9. Lanes 1–6 represent experiments in which 0, 2, 5, 10, 20 and 60 pmol EF-Tu was added, respectively. The position of EF-Tu is indicated by an arrow

In contrast, the relative amounts of EF-TuA_R and EF-TuB_O appearing in the ternary complexes varied considerably with the aminoacyl-tRNA/EF-Tu · GTP ratios in the reaction mixtures. EF-TuA_R was almost entirely absent in the ternary complex formed at a low input of aminoacyl-tRNA and the preponderance of EF-TuB_O persisted at aminoacyl-tRNA/EF-Tu · GTP ratios up to 5.0. These data suggest a reduced affinity of EF-TuA_R · GTP for aminoacyl-tRNA as compared to that of EF-TuA_S · GTP and EF-TuB_O · GTP.

It has been reported [1] that ternary complexes tend to dissociate when passed through millipore filters. The possibility exists, therefore, that this tendency of complexes containing EF-TuA_R is higher than that of the complexes containing EF-TuA_S or EF-TuB_O. Such a tendency would not display a dependence on the aminoacyl-tRNA/EF-Tu · GTP ratio, however. It can explain why the EF-TuA_R/EF-TuB_O ratio in the ternary complexes does not reach the value of 1.3 even at high inputs of aminoacyl-tRNA (Table 6).

We conclude that EF-TuA_R · GTP binds aminoacyl-tRNA less efficiently than wild-type EF-Tu · GTP or EF-TuB_O · GTP. This conclusion has recently been confirmed (to be published) by the aminoacyl-tRNA ester protection

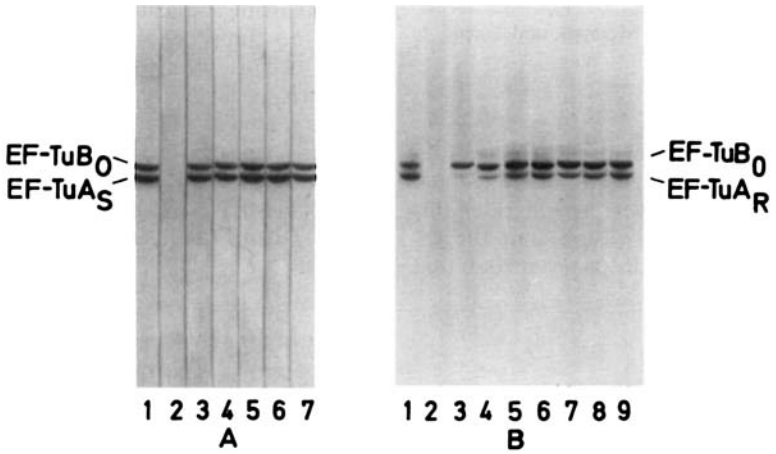

Fig. 11. *Analysis of ternary complexes formed from aminoacyl-tRNA and EF-Tu · GTP isolated from strains LBE 2020,A_SB_O (A) and LBE 2021,A_RB_O (B).* Reaction mixtures (0.5 ml) contained 50 mM Tris/HCl pH 7.5, 10 mM NH_4Cl, 10 mM $MgCl_2$, 100 mM KCl, 4 mM 2-mercaptoethanol, 5 mM phospho*enol*pyruvate, 2 µg pyruvate kinase, 1.4 nmol EF-Tu · GTP and varying amounts of aminoacyl-tRNA (more than 90% charged). The preparation of aminoacyl-tRNA was similar to that described by Duisterwinkel et al. [39], using a commercial tRNA preparation from *E. coli*. After incubation at 0 °C for 10 min the reaction mixtures were passed through millipore filters and the ternary complexes in the filtrates were analyzed by isoelectric focusing as described in the text. For further experimental details see also Materials and Methods and Duisterwinkel et al. [39]

Table 6. *Analyses of the EF-Tu species in ternary complexes formed from aminoacyl-tRNA and various mutant species of EF-Tu · GTP*
For experimental details see Duisterwinkel et al. [39], Fig. 1 and the text

EF-Tu species in reaction mixture	Ratio aminoacyl-tRNA/EF-Tu · GTP in reaction mixture	Ratio EF-TuA/EF-TuB in ternary complex
	mol/mol	
$EF\text{-}TuA_S$ + $EF\text{-}TuB_O$	0.5	1.4
	1.0	1.3
	2.0	1.3
	3.0	1.4
	5.0	1.3
$EF\text{-}TuA_R$ + $EF\text{-}TuB_O$	0.2	0.1
	0.5	0.3
	0.8	0.3
	1.0	0.5
	2.0	0.4
	3.0	0.6
	5.0	0.7

procedure [40]. Evidently the present data do not inform us about the relative affinities of the EF-Tu species for different aminoacyl-tRNAs.

DISCUSSION

Coordinate Regulation of the Expression of tufA *and* tufB

The determinations of the intracellular concentrations of EF-TuA and EF-TuB in wild-type cells described in this paper clearly demonstrate that the expression of the two EF-Tu encoding genes, which are distantly located on the *Escherichia coli* chromosome and are positioned in two different transcription units, is regulated coordinately. This coordination is not restricted to steady-state growth conditions but is maintained throughout the life cycle of the cells up till the stationary phase (cf. Fig. 3 and Table 3). Previously Reeh and Pedersen [19] found a constant ratio between the synthesis rates of EF-TuA and EF-TuB at all generation times studied. These authors studied cells from the *E. coli* strain HAK 88 [41], which harbours an innocuous mutation in *tufB*. Somewhat striking is their finding that the ratio of the intracellular levels of EF-TuA and EF-TuB is 2.5, clearly different from the ratio of 1.3–1.4 found in our strains. Apparently strains with a different genetic background can differ in the ratio in which the two *tuf* genes are expressed.

EF-TuA and EF-TuB differ in their COOH-terminal amino acid residues, glycine occupying the terminal position in EF-TuA and serine that in EF-TuB [14, 15]. Structural analyses of EF-Tu species derived from different strains were recently performed in our laboratory [42]. They showed, in agreement with the above conclusion, that the glycine/serine ratio at the COOH termini of EF-Tu differed from strain to strain. The data of Table 1 of the present paper demonstrate that the total EF-Tu contents of cells with a different genetic constitution but growing at comparable rates can also vary considerably. All these results have the important implication that studies of the regulation of the expression of *tuf* genes, which are based on intracellular levels of EF-Tu, can only be performed with strains which are virtually isogenic.

Recently Young and Furano [37] reported that inactivation of *tufB* by insertion of bacteriophage Mu is compensated for by an increased expression of *tufA*. Their conclusion was based on a comparison between *E. coli* K12 cells (KB 31,A_S) a strain originally constructed in our laboratory and comparable to PM 505,A_S and cells from an *E. coli* B strain (NF 314,A_SB_S). Our results clearly demonstrate that this conclusion is unwarranted.

Expression of tsf *and Ribosomal Genes*

The expression of *tufA* and *tufB* is also regulated coordinately with that of *tsf* (the gene encoding EF-Ts) and the ribosomal genes. This coordination is restricted, however, to a certain range of growth rates. Below 1 doubling/h it breaks down (cf. Fig. 6–8). Under the latter conditions *tsf* and the ribosomal genes are still expressed at a constant ratio but their expression drops faster than that of the *tuf* genes when growth declines. The underlying mechanism of this uncoupling is unknown. Similar observations concerning the intracellular concentrations of EF-Ts, the ribosomes and total EF-Tu have been made by various authors [3–6, 19, 30, 35, 37].

Two Distinct Mechanisms Control the Expression of tufA *and* tufB

Two major findings emerging from the present investigation have a bearing on the mechanisms controlling the expression of *tufA* and *tufB*, respectively. A specific single-site mutation of *tufA*, rendering EF-TuA resistant to the antibiotic kirromycin, disturbs the coordinate expression of *tufA* and *tufB*, enhancing *tufB* expression exclusively (compare Fig. 2 and [23]). Second, complete inactivation of *tufB* by insertion of bacteriophage Mu DNA, or elevation of the *tufB* dosage by transformation with plasmids harbouring *tufB* leaves the expression of *tufA* unaltered (Fig. 4, Table 4 and [23]). These results demonstrate that the expression of *tufA* is independent of that of *tufB* but that the expression of *tufB* does depend on that of *tufA*.

During steady-state growth, two distinct mechanisms apparently control the expression of *tufA* and *tufB*. It may be recalled that Reeh et al. [43] have previously demonstrated that the synthesis of EF-TuA and EF-TuB in the strain HAK 88 responds differently to starvation for charged valyl-tRNA in $relA^+$ cells. This indicates that under non-steady-state conditions the molecular mechanisms regulating *tufA* and *tufB* expression are also different.

EF-Tu Itself Is Involved in the Regulation of the Expression of tufB

The finding that a specific mutation of *tufA* affects the expression of *tufB*, strongly suggests a direct involvement of a product of the mutated *tufA* gene in the expression of *tufB*. It may be assumed therefore that the EF-Tu protein itself exerts a control function in *tufB* expression. Support for this assumption is lent by the suppression *in vitro* by EF-Tu of *tufB* expression in a DNA-dependent coupled transcription/translation system. It may imply that EF-Tu, not complexed with aminoacyl-tRNA, acts as a repressor and that the mutant species $EF\text{-}TuA_R$ has lost this function partially or even completely. This would explain why cells harbouring $tufA_R$ and $tufB_O$ show an enhanced expression of the latter gene particularly at low growth rates. Under the latter conditions the degree of tRNA aminoacylation may be relatively low and the proportion of free EF-Tu molecules not taken up in ternary complexes relatively high. Competition for amino-

acyl-tRNA will then occur between EF-TuA_R · GTP and EF-TuB_O · GTP, a competition which will be decided, according to the results of Fig. 11 and Table 6, in favour of EF-TuB_O · GTP. This means that the major part of free EF-Tu · GTP is EF-TuA_R · GTP, a poor repressor. The expression of *tufB* will then be enhanced, particularly under restrictive nutritional conditions, i.e. at lower growth rates.

The effect of the *tufA* mutation on *tufB* expression seems to be rather specific. This can be concluded from our finding that the expression of *tsf* and that of the ribosomal genes are not affected by the mutation (cf. Fig. 5 and 6). Despite this specificity the effect of the *tufA* mutation could be an indirect one. As a consequence of the lowered affinity of EF-TuA_R · GTP for aminoacyl-tRNA (cf. Fig. 11), for instance, the pool of free aminoacyl-tRNA not complexed with EF-Tu · GTP may be enlarged. It free aminoacyl-tRNA stimulates the expression of *tufB* this would explain the results obtained. So far, however, no evidence exists for such a stimulatory function of aminoacyl-tRNA. It seems therefore that the results of the present investigations and those of a previous one [23] are most readily explained by postulating a direct regulatory role of EF-Tu in the expression of *tufB*.

Our recent experiments [23, 44] (and following paper) with plasmids harbouring either *tufA* or *tufB* support this concept. Furthermore Miyajima and Kaziro [32] reported that in cells transformed with a multicopy plasmid bearing the entire tRNA-*tufB* operon, the rate of EF-Tu synthesis was only marginally increased. They suggested a post-transcriptional control mechanism which limits overproduction of EF-TuB. Also the results of Gausing [45] are in agreement with our model. She reported that *tufB* expression in a *tufA*-defective strain is stimulated. On the other hand, the investigations of Zengel and Lindahl failed to reveal any evidence for autogenous regulation of EF-TuB [46]. They studied the synthesis of EF-Tu in cells which had accumulated this protein after transformation with plasmids harbouring *tufA*. No effect on the synthesis of total EF-Tu was observed.

EF-Tu as an Autogenous Repressor

If EF-Tu is directly involved in the regulation of the expression of *tufB* the question may be asked at which level EF-Tu exerts such a regulatory function. Although an action at the level of *tufB* transcription can be envisaged (compare [47]), a post-transcriptional role deserves serious consideration. The results of the present investigation are clearly reminiscent of recent observations concerning the ribosomal protein S4 [48]. A specific mutation of *rpsD*, the gene encoding S4, was shown to stimulate the expression of a set of ribosomal protein genes *in vivo*. Experiments both *in vivo* and *in vitro* [49, 50] indicated that S4 acts as an autogenous repressor of a number of protein genes present in one transcription unit. This repression occurs at the level of translation. Other key ribosomal proteins have also been found to act as negative feedback regulators inhibiting the translation of mRNA coding for themselves and for certain other ribosomal proteins in the same transcription unit. 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 ribosomal proteins [50, 51].

We have previously [23] suggested that EF-Tu controls the expression of *tufB* post-transcriptionally by binding to the tRNA elements of the primary transcript of the tRNA-*tufB* transcription unit. Although at present nothing is known regarding such a binding of EF-Tu, both high-frequency NMR [52] and binding studies with N^{α}-tosyl-phenylalanine chloromethyl ketone [53] have shown that EF-Tu is able to interact with non-aminoacylated-tRNA. The present observation that EF-TuA_R has a reduced affinity for aminoacyl-tRNA lends suggestive support to the idea that the binding of EF-TuA_R to the tRNA targets on the primary transcript is also impaired and causes the enhanced expression of *tufB*. This may imply that autogenous repression of *tufB* by EF-Tu also interferes with the processing of the primary transcript affecting the intracellular concentration of certain specific tRNA species.

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