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Short Sequence-Paper

Cloning and sequencing of the *tuf* genes of *Streptomyces coelicolor* A3(2)

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Abstract

Two *tuf* genes are present in *Streptomyces coelicolor* A3(2), which have been cloned and sequenced. These genes show a high degree of nucleotide sequence identity to the *tuf1* and *tuf3* genes of *Streptomyces ramocissimus*: the *tuf1* genes are 94% identical, the *tuf3* genes 87%. *S. coelicolor tuf1* encodes a protein of 396 amino acids, while *tuf3* encodes a protein of 391 amino acids.

Keywords: Elongation factor Tu; Sequence comparison; Gene cloning; (*Streptomyces*)

One of the most abundant proteins in the bacterial cytoplasm is the *tuf*-encoded polypeptide chain elongation factor Tu (EF-Tu). Two *tuf* genes occur in *Escherichia coli*, encoding elongation factors EF-TuA and EF-TuB which differ only in their C-terminal amino acid [1,2]. The proteins occur in the bacterial cell in a 1:1 ratio [3], and are functionally indistinguishable.

Recently, we showed that in the kirromycin producer *Streptomyces ramocissimus* three *tuf* genes occur, which have been cloned and sequenced [4]. Analysis of these genes revealed that they are unexpectedly heterogeneous: *tuf1* and *tuf2* show 85% nucleotide sequence identity, whereas *tuf3* shows only 70% identity to *tuf1* and *tuf2*, which is in striking contrast to the much higher similarities found among *tuf* genes in other microorganisms [5,6]. EF-Tu1 has been shown to be a genuine elongation factor, but no physiological function could be revealed for the other two putative elongation factors. Hybridization analysis of other streptomycetes has shown that *Streptomyces coelicolor*

and *Streptomyces lividans*, genetically the best-characterised streptomycetes, have only two *tuf* genes.

Cloning of the *S. coelicolor tuf1* and *tuf3* genes. *S. coelicolor* presumably has two *tuf* genes, designated *tuf1* and *tuf3* by analogy to their homologues in *S. ramocissimus*. From Southern hybridization data it was concluded that *tuf1* could be cloned as an approx. 4.5 kb *Bam*HI fragment and *tuf3* as an approx. 10 kb *Bam*HI fragment [7]. *S. coelicolor* M145 total DNA was digested with *Bam*HI and separated electrophoretically on a 0.7% agarose gel in TAE buffer, whereupon fragments of the appropriate size were isolated from the gel.

For *tuf1*, these fragments were subcloned into *Bam*HI-digested pAT153 [8] and screened by hybridization of the *E. coli* colonies with the 244 bp *Sma*I fragment internal to the *S. ramocissimus tuf1* gene, which encodes most of the GTP-binding region. One positive signal was obtained, which appeared to contain a 4.3 kb insert, corresponding to the size expected on the basis of Southern hybridization data. This clone, which was designated pASCT1-1, contained the gene homologous to *S. ramocissimus tuf1*, as shown below.

For cloning of *tuf3*, fragments were cloned into pBR329 [9] and plasmid DNA was isolated from 600 colonies in pools of 24. The DNA was digested with

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*Bam*HI, subjected to agarose gel electrophoresis and blotted onto a Hybond-N nylon filter. Pools with the correct insert were identified on the basis of hybridization signals obtained with the 600 bp *Sal*I fragment from *S. ramocissimus tuf3*. After repeated colony purification and screening, one DNA preparation yielding

an unambiguous positive signal was obtained. *Bam*HI digestion proved the DNA to contain a 10.3 kb insert. The clone was designated pBSCT3–1. It was shown to contain the *tuf3* gene, as is demonstrated below.

Sequences of the inserts of pASCT1–1 and pBSCT3–1: nucleotide and amino acid comparisons. Sequencing of

1	CTCGAGCCGATGATGGCCGTCGAGGTCACACGCCCCGAGGACTACATGGGCGACGTCATCGGCGACATCAACTCC	75
	L E P M M A V E V T T P E D Y M G D V I G D I N S	
76	CGCCGTGGCCAGATCCAGGCCATGGAGGAGCGGATGGGTGCCCCGCTCGTGAAGGGCCTCGTGCCGCTGTCGGAG	150
	R R G Q I Q A M E E R M G A R V V K G L V P L S E	
151	ATGTTTCGGCTACGTCGGAGACCTCCGCGAGCAAGACGTCGGGTCGCGCAAGCTACTCGATGCAGTTTCGACTCCTAC	225
	M F G Y V G D L R S K T S G R A S Y S M Q F D S Y	
226	GCCGAGGTTCCCGGAACGTCGCGGAGGAGATCATCGCAAGGCCAAGGGCGAGTAACGGGCTACTCCGTTTAAC	300
	A E V P R N V A E E I I A K A K G E *	
301	GGACCCCGTTCTCACGCTTTAGGCTTGACCCCGAGCGCTGCATGGGGCATTCGCGCGTGAACCCGGTGAATGCC	375
376	CCCGGCACCCGGGCTTTCCAGCAAAGATCACCTGGCGCCGATGAGTAAGGGCTACAGAACCCTCCACAGGAGGA	450
451	CCCCAGTGGCGAAGGCGAAGTTCGAGCGGACTAAGCCGCACGTCAACATCGGCACCATCGGTCACATCGACCACG	525
	V A K A K F E R T K P H V N I G T I G H I D H G	
526	GTAAGACGACCTCAGGCGCCATTACCAAGGTGCTGCACGACGCGTACCCGACATCAACGAGGCGTCGGCGT	600
	K T T L T A A I T K V L H D A Y P D I N E A S A F	
601	TCGACCAGATCGACAAGGCTCCCGAAGAGCGCCAGCGCGTATCACCATCTCGATCGCGCACGTCGAGTACCAGA	675
	D Q I D K A P E E R Q R G I T I S I A H V E Y Q T	
676	CCGAGGCGCGTCACTACGCCACGTCGACTGCCCGGTACGCGGACTACATCAAGAACATGATCACGGGTGCCG	750
	E A R H Y A H V D C P G H A D Y I K N M I T G A A	
751	CGCAGATGGACGCGCCATCCTCGTGGTCGCGCCACCGACGCGCCGATGCGCGAGACCAAGGAGCACGTGCTCC	825
	Q M D G A I L V V A A T D G P M P Q T K E H V L L	
826	TGGCCCGCCAGGTCGGCGTTCCGTACATCGTGGTCGCGCTGAACAAGGCCGACATGGTGGACGACGAGGATCC	900
	A R Q V G V P Y I V V A L N K A D M V D D E E I L	
901	TGGAGCTCGTCGAGCTCGAGGTGCGTGAGCTCCTCTCCGAGTACGAGTTCCCGGGCGACGACGTCCCGTCGTCA	975
	E L V E L E V R E L L S E Y E F P G D D V P V V K	
976	AGGTCTCCGCTCTGAAGGCCCTCGAGGCGCACAGGAGTGGGGCAACTCGGTCTCGAGCTCATGAAGGCCGTGG	1050
	V S A L K A L E G D K E W G N S V L E L M K A V D	
1051	ACGAGGCCATCCCGAGCCGAGCGCGACGTCGACAAGCCGTTCTGATGCCGATCGAGGACGCTTCCACCATCA	1125
	E A I P E P E R D V D K P F L M P I E D V F T I T	
1126	CCGGTCGCGGTACGGTCGTACCGGCCGATCGAGCGTGGTGTCTCAAGGTCAACGAGACCGTCGACATCATCG	1200
	G R G T V V T G R I E R G V L K V N E T V D I I G	
1201	GCATCAAGACCGAGAAGACCACCACCGGTACCGGCATCGAGATGTTCCGCAAGCTCCTCGACGAGGGCCAGG	1275
	I K T E K T T T T V T G I E M F R K L L D E G Q A	
1276	CCGGTGAGAACGTCGGTCTGCTGCTTCGCGGCATCAAGCGCGAGGACGTCGAGCGCGGCCAGGTCATCATCAAGC	1350
	G E N V G L L L R G I K R E D V E R G Q V I I K P	
1351	CGGGCTCGGTACCCCCGACACCGAGTTTCGAGGCCAGGCCTACATCTGTGCAAGGACGAGGGTGGCCGTCACA	1425
	G S V T P H T E F E A Q A Y I L S K D E G G R H T	
1426	CCCCCTTCTTCAACAACCTACCGTCCGCGAGTTCTACTTCCGTACGACGGACGTGACCGGCGTCGTGACCTCCCCG	1500
	P F F N N Y R P Q F Y F R T T D V T G V V T L P E	
1501	AGGGCACCGAGATGGTCATGCCGGGTGACAACACCGAGATGAAGGTGGAGCTCATCCAGCCCGTCGCCATGGAAG	1575
	G T E M V M P G D N T E M K V E L I Q P V A M E E	
1576	AGGGCCTGAAGTTCGCCATCCGCGAGGGTGGCCGGACCGTGGGCGCGGCCAGGTCACCAAGATCAACAAGTAAC	1650
	G L K F A I R E G G R T V G A G Q V T K I N K *	
1651	TCCGCTTGCTTGTCGGTCGACCGACCTGACATGGGCTGATGCCTGAAGGGCCGTACGACTTCGGTCGTACGGGTC	1725
1726	CTTTCGCCATGTGCGGTGCGGTCCAGGCCGCTGAGGAAGTCGCCCTGCCAGAGCGCGCGGCCGTGCGCAGCCGG	1800
1801	GCCACCGCCTCTTCCCGCGGTCTCTGTGGCCGAGCCGCTTGGCCCCGCCACGAGGGCGGTGAAGAGTACGGCG	1875

Fig. 1. Nucleotide sequence of *tuf1* and flanking regions, which include the end of the *fus* gene. The deduced amino acid sequences of both *fus* (nt positions 1–282; encoding EF-G) and *tuf1* (nt positions 456–1649; encoding EF-Tu1) are given below the nucleotide sequence.

pASCT1–1 resulted in the identification of an ORF of 1194 bp, putatively encoding a 396 amino acid protein (Fig. 1). Sequencing of pBSCT3–1 identified an ORF of 1179 bp, corresponding to a protein of 391 amino acids (Fig. 2). The ORF located on pASCT1–1 is very similar to *S. ramocissimus tuf1*, and a second ORF is located upstream of it that very much resembles the *S. ramocissimus fus* gene, indicating its probable location in the so-called S12 operon, a location typical for the major *tuf* gene in all microorganisms [10]. Therefore, the gene was designated *tuf1*. Again on the basis of the very high similarity to its homologue in *S. ramocissimus*, the ORF identified on pBSCT3–1 was designated *tuf3*.

An amino acid alignment of the *tuf*-gene products

of *S. coelicolor*, *S. ramocissimus* and *E. coli* is shown in Fig. 3. From this alignment it follows that the *tuf1* and *tuf3* gene products contain the consensus sequences for GTP binding proteins [11] and show a perfect fit with the D-loop motif [12]. From alignments of both the *tuf*-gene nucleotide sequences and the amino acid sequences deduced thereof, identities were calculated (Table 1). The *tuf1* genes of *S. coelicolor* and *S. ramocissimus* are 94% identical, and the deduced amino acid sequences of their gene products (designated EF-Tu1) are 96% identical. The *tuf3* genes of these organisms are 87% identical, their gene products (designated EF-Tu3) sharing 91% identical amino acids. The low similarity of EF-Tu3 to EF-Tu1, the major EF-Tu in *Streptomyces*, is underlined by the fact that EF-Tu3

1	GGTACCGCTCTCGAACGGCCGTTCCATAAAAAACCATTCGACGTGCGACGAAGCGTCGGCGATGATCTGTCTCAT	75
76	GTTCCGGTACGCCCTTCCACCTCGCAGCATCCCGGTCGCGGATGCCCGAAGGCTGCCGTCGCCCACCTTACCCGC	150
151	CGCAGTCGACGGCGCCCGAAGCTGACCTCCCGGATCGTCCGGCGGACCCCGCAGGGGAGGGTCGGCCGGCCC	225
226	CAGGGGTCCCCACCACCGCCACGAGGCTTCGAGGTACCGCCATGTCCAAGACGGCGTACGTCGCGACCAACCG	300
	M S K T A Y V R T K P	
301	CATCTGAACATCGGCACGATGGGCCATGTGACACACGGCAAGACCACCTGACCGCCGCATCACCAAGGTCCTC	375
	H L N I G T M G H V D H G K T T L T A A I T K V L	
376	GCCGAGCGCGGGCCCGCAGCACACCCAGTACGTTTCGTTTCGACCGCATCGACCGCGCCCGGAGGAGGCGCG	450
	A E R G A G S T T Q Y V S F D R I D R A P E E A A	
451	CGCGGCATCACCATCAACATCGCGCAGTCGAGTACGAAACCGACACCCGGCACTACGCCCACGTCGACATGCCC	525
	R G I T I N I A H V E Y E T D T R H Y A H V D M P	
526	GGCCACGCGGACTACGTCAAGAACATGGTCACCGGCGCCCGCCAGCTCGACGGGGCGATCCTCGTCGTATCCGCG	600
	G H A D Y V K N M V T G A A Q L D G A I L V V S A	
601	CTGGACGGGATCATGCGCAGACCGCGAACACGTGCTGCTCGCCCGCAGGTGGGCGTCGACCACATCGTGGTC	675
	L D G I M P Q T A E H V L L A R Q V G V D H I V V	
676	GCGCTCAACAAGGCCGACGCGGGTGACGAGGAGCTGACCGACCTGGTCGAGCTGGAGGTGCGCGAACTGCTCACC	750
	A L N K A D A G D E E L T D L V E L E V R E L L T	
751	GCGCACGGCTACGGCGGCGACGCCGTGCCCGTCGTACGGGTCTCGGGGCTGAAGGCCCTGGAGGGCGACCCGCGG	825
	A H G Y G G D A V P V V R V S G L K A L E G D P R	
826	TGGACGGCCTCCGTCGAGGCGTGTCTGACGCGCGTGACACGTACGTGCCCATGCCCGAGCGGTACCTGGACGCG	900
	W T A S V E A L L D A V D T Y V P M P E R Y L D A	
901	CCGTTCTGTGTCGGGTGGAGAAGCTGCTCACCATACCGCGCGCGCACCGTCGTACCGCGCCGTCGAGCCG	975
	P F L L P V E N V L T I T G R G T V V T G A V E P	
976	GGCACCGTGCCTGCGCGACCGGGTCGAGGTGCTCGGGGCGTCCGTCGAGACGGTCGTACCGCGCCTGGAGACC	1050
	G T V R V G D R V E V L G A S V E T V V T G L E T	
1051	TTCGCAAGCCGATGGAGGAGCGCAGGCCGGGACAACGTGGCGTGTGCTGCGCGGGGTCGCCCGCGACACG	1125
	F G K P M E E A Q A G D N V A L L L R G V A R D T	
1126	GTGCGCCGCGGGCAGGTGGTCGCGCACCCGCGCAGCGTCGTCCCGCGCGGCGTTTCCGGGCGGGGTGTACGTG	1200
	V R R G Q V V A A P G S V V P A R R F R A R V Y V	
1201	CTCTCGGCGCGCAGGGCGGCGCTCGACACCGCTCACCACCGGATACCGCGCGAGTTCTACATCCGCACCGCC	1275
	L S A R E G G R S T P L T T G Y R P Q F Y I R T A	
1276	GACGTGGTCGCGACGTGGACCTCGGCGAGGAGCCGTCGCGCGGGGACACCGTCACCATGACGGTCGAG	1350
	D V V G D V D L G E E A V A R P G D T V T M T V E	
1351	CTGGGACGGGACGTGCGCGTGGAGACGGGGCTCGGCTTCGCGATCCGCGAGGGCGGTCGACCGTGGGGGCGGG	1425
	L G R D V P L E T G L G F A I R E G G R T V G A G	
1426	ACCGTGACCGCGGTGGAGTGAGCGCGCCGGGTGAGTGGGCGGGCTGAGGGC	1478
	T V T A V E *	

Fig. 2. Nucleotide sequence of *tuf3* and flanking sequences. The deduced amino acids sequence of *tuf3* is given below the nucleotide sequence.

Sc EF-Tu1	VAKAKFERTK	PHVNIGTIGH	IDHGKTTTLTA	AITKVLHDAY	PD.INEASAF	49
Sr EF-Tu1					.L TP	49
Sc EF-Tu3	MS TAYV	L M V		AERG	AGSTTQYVS	50
Sr EF-Tu3	MS TAYV	L M V		AERG	SGT...FVP	47
Sr EF-Tu2	Q			RF	.L PFTP	49
Ec EF-TuA	S E	V V		T AKT	GG...A R	47
Consensus	V-K-KF-RTK	PHVNIGTIGH	-DHGKTTTLTA	AITKVL----	-----F	
Sc EF-Tu1	DQIDKAPEER	QRGITISIAH	VEYQTEARHY	AHVDCPGHAD	YIKNMITGAA	99
Sr EF-Tu1	N					99
Sc EF-Tu3	R R A A	N	E DT	M	V V	100
Sr EF-Tu3	R R A A	N	E DT	M	V V	97
Sr EF-Tu2						99
Ec EF-TuA	N K A	NTS	D PT		V	97
Consensus	D-ID-APEE-	-RGITI-IAH	VEY-T--RHY	AHVDCPGHAD	Y-KNMITGAA	
Sc EF-Tu1	QMDGAILVVA	ATDGPMPQTK	EHVLLARQVG	VPYIVVALNK	ADMVDDEEIL	149
Sr EF-Tu1					M	149
Sc EF-Tu3	L	S L I A		DH	.AG LT	149
Sr EF-Tu3	L	S L I A		DH	.AG LT	146
Sr EF-Tu2					T	149
Ec EF-TuA			R I G	I F	C L	147
Consensus	QMDGAILVVA	ATDGPMPQT-	EHVLLARQVG	VPYIVVALNK	ADMVDDEE--	
Sc EF-Tu1	ELVELEVREL	LSEYEFPGDD	VPVVKVSALK	ALEGDKKEWGN	SVLELMKAVD	199
Sr EF-Tu1			L R	AQ TQ	D	199
Sc EF-Tu3	D	TAHGYG A	R G	PR TA	EA LD	199
Sr EF-Tu3	D	D HGYG G A	R G	PK TA	IEA LD	196
Sr EF-Tu2		T		PR TR	LD	199
Ec EF-TuA	M	Q D	T I RG	A EA KI	AGFL	197
Consensus	ELVELEVREL	LSEY-FPGDD	-PVVRVSALK	ALEGD--WT-	SVL-L--AVD	
Sc EF-Tu1	EAIPEPERDV	DKPFLMPIED	VFTITGRGTV	VTGRIERGV	KVNETVDIIG	249
Sr EF-Tu1	S					249
Sc EF-Tu3	TYV M YL	A L V N L		AV P TV	R GDR EVL	249
Sr EF-Tu3	TYV M Y	A L V N L		AV	TV R GNR EVL	246
Sr EF-Tu2	FV V	R			T N TE E	249
Ec EF-TuA	SY AI	L S S		V II	G E E V	247
Consensus	---PEPER-V	D-PFL-PIED	VFTITGRGTV	VTGR-ERG--	-V---VEI-G	
Sc EF-Tu1	IKTEKTTTV	TGIEMFRKLL	DEGQAGENVG	LLLRGIKRED	VERGQVIKIP	299
Sr EF-Tu1						299
Sc EF-Tu3	ASV ... V	L T G PM E A	D A	VA DT	R VAA	296
Sr EF-Tu3	AGL ... V	L T G PM A	D A	VP DA	R H VAA	293
Sr EF-Tu2	HEQR R		R	V Q	V R	299
Ec EF-TuA	. TQKS C	V	R	V	E I LA	296
Consensus	I--E---TTV	TG-EMFRKLL	DEGQAGENVG	LLLRG-KRE-	VERGQV---P	
Sc EF-Tu1	GSVTPHTEFE	AQAYILSKDE	GGRHTPPFNN	YRPQFYFRIT	DVTGVVTLPE	349
Sr EF-Tu1					H	349
Sc EF-Tu3	V ARR R	RV V AR	S LTG	I A	V D D G	346
Sr EF-Tu3	V RSR S	V V AR	T VTSG	I A	V D D G	343
Sr EF-Tu2	Q		E		K	349
Ec EF-TuA	TIK K	SEV	KG		TIE	346
Consensus	GSV-PHT-FE	AQ-YILSKDE	GGRHTPPF--	YRPQFYFRIT	DVTG-V-LPE	
Sc EF-Tu1	GTEMVMPGDN	TEMKVELIQP	VAMEEGLKFA	IREGGRTVGA	GQVTKINK	397
Sr EF-Tu1		R			V	397
Sc EF-Tu3	E.AVAR	T VT T	GRD PL T G		T AVE.	392
Sr EF-Tu3	V.GVAR	ET VS I	GRE PL P G		T ALV.	389
Sr EF-Tu2		A H Q	I		R V	397
Ec EF-TuA	V	IK V T H	I DD R		V A VLG	394
Consensus	G-EMVMPGDN	--M-VELI-P	VAME-GL-FA	IREGGRTVGA	G-VT----	

Table 1

Nucleotide sequence identities between the *tuf* genes of *S. coelicolor*, *S. ramocissimus* and *E. coli* (above the diagonal) and amino acid identities between their deduced gene products (below the diagonal)

	Sc 1	Sc 3	Sr 1	Sr 2	Sr 3	Ec A
Sc <i>tuf1</i> , EF-Tu1		69	94	84	70	71
Sc <i>tuf3</i> , EF-Tu3	63		69	70	87	61
Sr <i>tuf1</i> , EF-Tu1	96	63		85	70	71
Sr <i>tuf2</i> , EF-Tu2	89	64	88		70	69
Sr <i>tuf3</i> , EF-Tu3	64	91	65	64		63
Ec <i>tufA</i> , EF-TuA	75	60	74	72	60	

All values are given in percentages. Abbreviations: Sc, *S. coelicolor*; Sr, *S. ramocissimus*; and Ec, *E. coli*. Alignments were done with the program 'Gap' [13].

shows almost as much amino acid identity with *E. coli* EF-Tu as with *S. coelicolor* EF-Tu1.

A surprising observation is that the nucleotide sequences of *tuf1* and *tuf3* are more homologous to each other than the deduced amino acid sequences (69% versus 63%), which is also observed for the *S. ramocissimus* *tuf1* and *tuf3* genes and their gene products (Table 1). This is due to nucleotide substitutions in the *tuf3* gene at the first and second codon position rather than the third ('wobble') position, so that many nucleotide substitutions lead to amino acid substitutions. Still, amino acids in the GTP-binding domains are conserved, suggesting that the *tuf3* gene product also belongs to the family of GTP-binding proteins.

Since it has been demonstrated for *S. ramocissimus* EF-Tu1 that it functions as an elongation factor [4] we

assume on the basis of the very high similarity of the proteins that such is also the case for *S. coelicolor* EF-Tu1 and no further investigation to address this point has been performed. The role of EF-Tu3 is unclear and is presently being investigated in our laboratory.

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Fig. 3. Amino acid alignment of *Streptomyces* EF-Tus and *E. coli* EF-TuA. Abbreviations: Ec, *E. coli*; Sc, *S. coelicolor*; Sr, *S. ramocissimus*. Numbers at the right of the figure refer to the amino acid positions. A consensus (grey shaded) is given when the amino acid alignment shows more than three identical amino acids. The amino acids (of Sc EF-Tu1) that constitute the GTP binding consensus sequence are shown in bold face.