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Molecular cloning, nucleotide sequence and expression of the *tufB* gene encoding elongation factor Tu from *Thermus thermophilus* HB8

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The *tufB* gene encoding elongation factor Tu (EF-Tu) of *Thermus thermophilus* HB8 was cloned and expressed. Compared with the known *tufA* gene of *T. thermophilus*, nucleotide differences were found at 10 positions out of 1221 nucleotides, and amino acid substitutions were found at 4 positions out of 406 amino acids. The *tufB* product was 70.9% homologous to the corresponding sequence of the *tufB* product of *E. coli*. The G+C content of the third base of the codon in the *tufB* gene was 84.8% and G was especially preferred in this position.

Elongation factor Tu; *tufB* gene; *Thermus thermophilus* HB8; GTP-binding protein; Codon usage; Expression

1. INTRODUCTION

Elongation factor Tu (EF-Tu) which is one of the most important GTP-binding proteins that mediates the binding of aminoacyl-tRNA to the A site of ribosomes. EF-Tu of *E. coli* is encoded by two genes, *tufA* and *tufB*, which are closely related to each other [1–3]. Differences in the nucleotide sequences between the *tufA* and *tufB* genes are found at 13 positions out of 1185 nucleotides and the gene products, EF-TuA and EF-TuB, are identical except for one amino acid residue at the COOH-terminal. Recently, two *tuf* genes of *T. thermophilus* HB8 have also been identified by cross-hybridization with the *tufA* gene of *E. coli*, and one of the *tuf* genes, *tufA*, has been cloned [4]. In the *tufA* product, an additional peptide segment, Met^{183}–Gly^{192}, which consists predominantly of basic amino acids was found.

In this report, we describe the molecular cloning and sequence determination of the *tufB* gene. We also describe the expression of the gene in *E. coli* with the T7 expression system.

2. MATERIALS AND METHODS

2.1. *Cloning procedures*

The *tufB* gene of *T. thermophilus* is involved in a 4.5-kb *Bam*HI fragment of chromosomal DNA as described [4]. The fragments were run on a 0.7% agarose gel and the 4-kb to 5-kb fragments were electro-eluted. The fragments were then ligated to *Bam*HI-digested pBR322, and transformed into *E. coli* HB101. The transformants were screened by colony hybridization with the *Hin*cII–*Kpn*I 0.9-kb fragment of the plasmid pHBTU31 [4], which contains the *tufA* gene of *T. thermophilus*, as a probe. The fragment corresponds to the nucleotide sequence of 95 to 1017 of the *tufA* gene. Two positive colonies were obtained by colony hybridization. The plasmid DNA was isolated from the positive colony and was designated as pTUB322. The complete nucleotide sequence of both strands of the *tufB* gene was determined using 7-deaza-sequenase kit (United States Biochemical Corporation).

2.2. *Expression of the* T. thermophilus tufB *gene*

The *tufB* gene was cloned in an expression vector pAR2106 under control of the phage T7 promoter [5] as described below. The plasmid pTUB322 was digested with *Sac*I, and a 1.5-kb *Sac*I fragment containing the *tufB* gene was subcloned into *Sac*I-digested M13mp19. A *Nde*I site was introduced at the beginning of the *tufB* gene by site-directed mutagenesis. The plasmid then was digested with *Nde*I and *Hin*dIII and the fragment purified by agarose gel electrophoresis was ligated into the *Nde*I – *Hin*dIII site of the T7 expression vector pAR2106. The plasmid, designated as pTS2, was transformed into *E. coli* BL21 (DE3) [6]. The cells were cultured in YT medium with 0.13 mM ampicillin and induced by the addition of 0.5 mM isopropyl β-D-thiogalactopyranoside (IPTG). After 2.5 h of incubation, the cells were harvested and the lysates were analyzed by 12.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

2.3. *GTP-binding experiments*

The proteins separated by 12.5% SDS-PAGE were electroblotted onto nitrocellulose and the filters were preincubated in a buffer containing 50 mM sodium phosphate (pH 7.5), 0.5 mM $MgCl_2$, 100 mM NaCl and bovine serum albumin (1 mg/ml) overnight at 4°C. The filters were incubated in a buffer containing 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, and 0.3% Tween-20 with [α-^{32}P]GTP (0.74 MBq) for 90 min at room temperature and then were washed three times in the same buffer, air-dried, and exposed to film [7].

3. RESULTS AND DISCUSSION

The 4.5-kb *Bam*HI fragment of *T. thermophilus* chromosomal DNA containing the tRNA-*tufB* operon and four tRNA genes followed by the *tuf*B gene has been reported before [8]. The nucleotide sequence of the *tufB* gene and its flanking region are shown in Fig. 1. Compared with the *tufA* gene, nucleotide dif-

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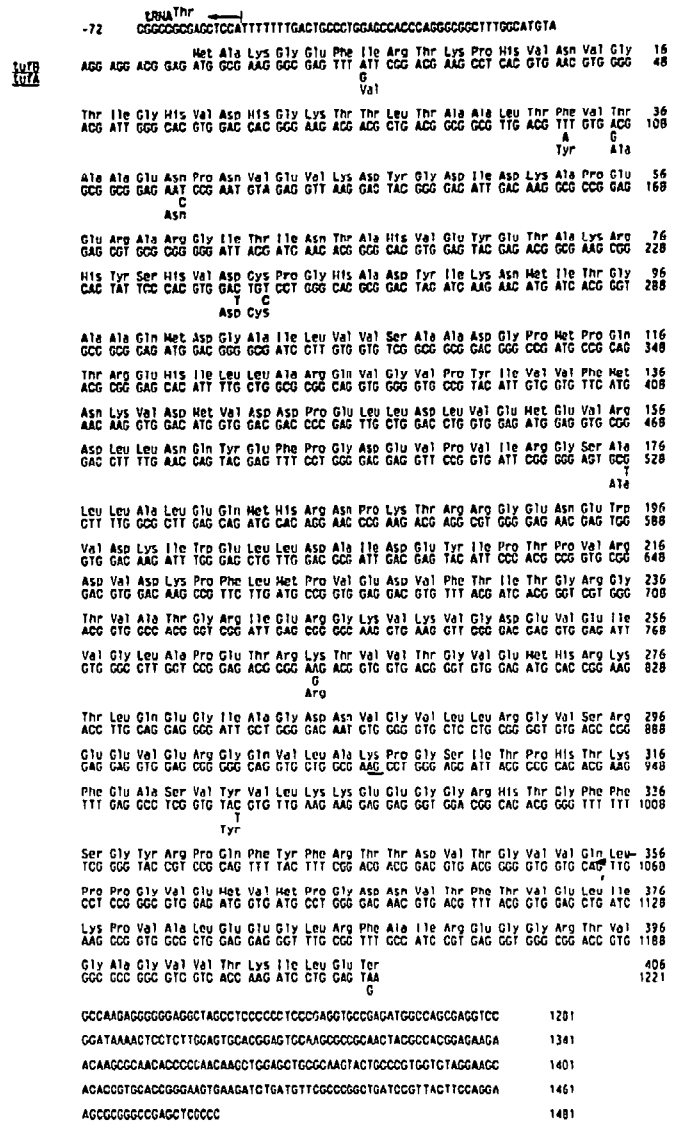

ferences were found in 10 positions out of 1221 nucleotides and amino acid changes were found at 4 positions out of 406 amino acids. The nucleotide sequence of the *tufB* gene was 65.4% homologous and deduced amino acid sequence of the *tufB* gene product was 70.9% homologous to the corresponding sequences of the *tufB* gene and the product of *E. coli*, respectively.

The codon usage of the *tufB* gene was summarized in Table I. The G + C content of *T. thermophilus* genomic DNA is 69%, which is extremely high in eubacteria. The overall G + C content of the *tufB* gene was 62.5% and that of the third base of the codon was 84.8%. As shown in Table II, G was especially preferred in the third base of the codon in the *tufB* gene as well as in the *tufA* gene. This feature differs from other genes [9–11], in which G and C are almost equally used in this position. Although high GC pressure was observed in the codons for almost all amino acid residues, U was exceptionally preferred in the third base of the codon for Phe and Ile residues in the *tufB* gene. Similar exceptions were found in the *tufA* gene. A possible reason for the unusual codon usage of Phe and Ile is as follows. *T. thermophilus* produces the restriction endonuclease *Tth*HB8I of which the recognition sequence is TCGA. If UUC or AUC is used as the codon for Phe or Ile, the TCGA sequence will appear; for instance, TTCGAN encoding Phe-Asp or Glu and ATCGAN encoding Ile-Asp or Glu (N = A, G, C and T). In such a case, UUU and AUU in the codon for Phe and Ile should be

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Fig. 1. The nucleotide sequence of the *tufB* gene of *T. thermophilus* and the deduced amino acid sequence. The reading frame starts at ATG (position 1) and terminates at TAA (position 1221). Nucleotide differences between the *tufB* and *tufA* [4] genes and amino acid changes are indicated.

Table I

Codon usage in the *tufB* gene of *T. thermophilus*. The numbers in parentheses represent codon usage in the *tufB* gene of *E. coli* [3]

Phe	UUU	11	(1)	Ser	UCU	–	(7)	Tyr	UAU	1	(1)	Cys	UGU	1	(1)
	UUC	2	(13)		UCC	1	(3)		UAC	9	(9)		UGC	–	(2)
Leu	UUA	–	(–)		UCA	–	(–)	Ter	UAA	1	(1)	Ter	UGA	–	(–)
	UUG	11	(–)		UCG	3	(–)		UAG	–	(–)	Trp	UGG	2	(1)
Leu	CUU	5	(1)	Pro	CCU	6	(–)	His	CAU	–	(2)	Arg	CGU	5	(20)
	CUC	1	(–)		CCC	2	(–)		CAC	12	(9)		CGC	–	(3)
	CUA	–	(–)		CCA	–	(1)	Gln	CAA	–	(–)		CGA	–	(–)
	CUG	10	(27)		CCG	15	(19)		CAG	9	(8)		CGG	19	(–)
Ile	AUU	14	(3)	Thr	ACU	–	(12)	Asn	AAU	3	(–)	Ser	AGU	1	(–)
	AUC	8	(26)		ACC	3	(15)		AAC	8	(7)		AGC	2	(1)
	AUA	–	(–)		ACA	–	(2)	Lys	AAA	–	(17)	Arg	AGA	–	(–)
Met	AUG	12	(10)		ACG	29	(1)		AAG	21	(6)		AGG	2	(–)
Val	GUU	3	(22)	Ala	GCU	2	(11)	Asp	GAU	–	(4)	Gly	GGU	8	(19)
	GUC	2	(1)		GCC	5	(1)		GAC	24	(20)		GGC	6	(20)
	GUA	1	(11)		GCA	–	(6)	Glu	GAA	–	(30)		GGA	1	(–)
	GUG	45	(3)		GCG	20	(9)		GAG	37	(7)		GGG	24	(1)

Table II

Nucleotide compositions of the third base of the codon in *T. thermophilus* genes [4,9,10,11] (%)

	A	G	C	U
tufA	**0.5**	**63.6**	**20.9**	**15.0**
tufB	**0.5**	**63.9**	**20.9**	**14.7**
leuB	5.3	46.1	43.0	5.6
trpE	2.6	46.3	48.1	3.0
trpG	3.5	43.1	49.0	4.4
rpsL	2.2	47.1	47.1	3.6
rpsG	3.2	48.4	45.9	2.5
fus	0.9	41.0	55.4	2.7

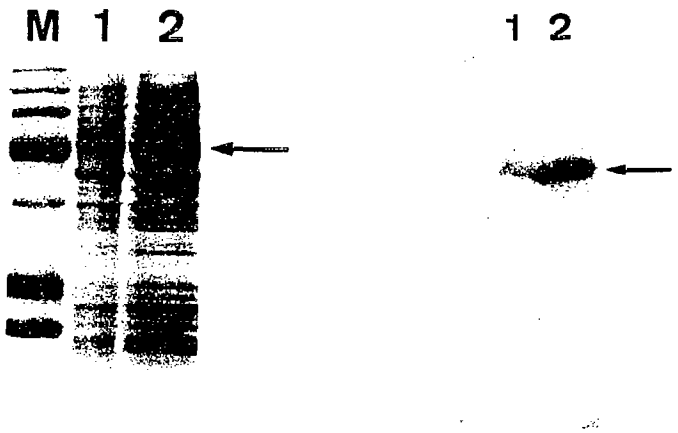

Fig. 2. Expression and GTP-binding experiments of the *tufB* gene product. A. 12.5% SDS-PAGE of the cell lysates of *E. coli* BL21 harbouring pTS2. The extracts were prepared from cells that were incubated in the absence (lane 1) and presence (lane 2) of IPTG. Lane M shows molecular weight markers. After electrophoresis the gel was stained with Coomassie brilliant blue. The protein marked by the arrow was induced with IPTG. B. GTP-binding experiments. Cells harbouring pTS2 were incubated in the absence (lane 1) and presence (lane 2) of IPTG. After 12.5% SDS-PAGE of the cell lysates, filters electroblotted with proteins were hybridized with [α-^{32}P]GTP. The band in lane 2 shows the position of migration of the protein marked by the arrow in Fig. 2A.

preferentially used. This explanation is applicable to 4 out of 25 cases for the use of UUU and AUU codons in the *tufB* gene. Major reasons for the unusual codon usage would be still hidden.

The cell lysates of *E. coli* BL21 harbouring the plasmid pTS2 were analyzed by 12.5% SDS-PAGE as shown in Fig. 2A. Compared to non-induced cells (Fig. 2A, lane 1), a major protein band of M_r 44 000 was produced in the presence of IPTG (Fig. 2A, lane 2). It is in good agreement with a calculated molecular weight of EF-Tu of *T. thermophilus tufB* gene (44 781 Da). To determine GTP-binding ability of the expressed protein, nitrocellulose filters electroblotted with the cell lysates were hybridized with [α-^{32}P]GTP. As indicated in Fig. 2B (lane 2), the expressed EF-Tu bound to labeled GTP.

Purification of the expressed EF-Tu is in progress. The present work will make it possible to investigate this interesting gene and protein further.

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