Sequence and identification of the nucleotide binding site for the elongation factor Tu from Thermus thermophilus HB8

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ABSTRACT

Two structural genes for the Thermus thermophilus elongation factor Tu (tuf) were identified by cross-hybridization with the tufA gene from E.coli. The sequence of one of these tuf genes, localized on a 6.6 kb Bam HI fragment, was determined and confirmed by partial protein sequencing of an authentic elongation factor Tu from T.thermophilus HB8. Expression of this tuf gene in E.coli minicells provided a low amount of immuno-precipitable thermophilic EF-Tu. Affinity labeling of the T.thermophilus EF-Tu and sequence comparison with homologous proteins from other organisms were used to identify the guanosine-nucleotide binding domain.

INTRODUCTION

Elongation factor Tu (EF-Tu) from E.coli mediates the binding of aminoacyl-tRNA to programmed ribosomes (1) and plays a role in maintaining the fidelity of translation (2). Amino acid sequences were determined for the procaryotic elongation factors from E.coli (3, 4), yeast mitochondrion (5) chloroplasts of Euglena gracilis (6) as well as for several eucaryotic elongation factor Iα species (7, 8, 9). EF-Tu possesses some sequence homologies with eucaryotic elongation factor Iα and G-proteins mainly in the guanosine-nucleotide binding domain (10).

EF-Tu from T.thermophilus HB8 is temperature insensitive up to 65 °C (11) in contrast to EF-Tu from E.coli. It forms stable aa-tRNA•EF-Tu•GTP ternary complexes and is well suited for physical and biochemical investigations. We used this thermophilic EF-Tu to construct affinity columns for the isolation of aminoacyl-tRNAs (12) and for the investigation of aminoacyl-tRNA•EF-Tu•GTP interactions (13). In order to prepare sufficient quantities of the thermophilic elongation factor and its mutants for physical
studies the expression of its genes in *E. coli* was attempted. In this communication we present the sequence of one of the two *T. thermophilus* EF-Tu genes and the identification, by affinity labeling, of the nucleotide binding site in the protein.

**MATERIALS AND METHODS**

Enzymes, deoxynucleoside-5′-triphosphates and dideoxynucleosides-5′-triphosphates were obtained from Pharmacia (Uppala, Sweden), BRL (Eggenstein, FRG) and Boehringer (Mannheim, FRG). [\(^3\)H]GDP (11.3 Ci/m mole), [\(^{14}\)C]GTP (500 mCi/m mole), \(^{35}\)S-labeled methionine (1275 Ci/m mole) and 'Amplify' were purchased from Amersham-Buchler (Braunschweig, FRG). \(^{32}\)P-orthophosphate and \(^{32}\)P-labeled dATP (800 Ci/ m mole) were obtained from Du Pont/New England Nuclear (Bad Nauheim, FRG). \(^{32}\)P]GDP (1000 Ci/m mole) was prepared as described elsewhere (14). CM-Sepharose CL-6B and Sephadex LH-60 were from Pharmacia. NaIO\(_4\), NaBH\(_3\)(CN) and NaBH\(_4\) were from Serva (Heidelberg, FRG). Acrylamide and N,N′-methylenebisacrylamide were obtained from BRL. All other reagents were of analytical grade and purchased from Merck (Darmstadt, FRG).

The plasmid pPR 1 carrying the 1.0 kbp NruI/Hpal fragment of the *E. coli* tufA gene was obtained from Dr. L. Bosch (Leiden). *E. coli* strains RR1 and K11 were used for cloning experiments. The *E. coli* strain R312A which was used for minicell expression was from Dr. N. Schumann (Bayreuth).

For hybridization experiments 20 μg of *T. thermophilus* chromosomal DNA were digested with several restriction enzymes using 33 mM Tris-acetate pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT, 0.1 mg/ml BSA as incubation buffer, in a final volume of 60 μl. Cleavage was complete after 3 h at 37°C in the presence of 20 units of restriction enzyme. The resulting DNA fragments were separated by horizontal gel electrophoresis (1.5% agarose in 40 mM Tris-acetate pH 7.9, 25 mM sodium acetate, 0.5 mM EDTA) run at 3 V/cm for 4 h. Transfer of separated DNA to a nitrocellulose filter and hybridization to the \(^{32}\)P-labeled *E. coli* tufA fragment of pPR 1 were carried out as published elsewhere (15, 16).
For cloning the *T. thermophilus* chromosomal DNA, it was cleaved and separated as described above. 2 μg of isolated *T. thermophilus* DNA (Bam HI or Bgl II restriction fragments) was incubated with 1 μg BamHI cleaved, dephosphorylated pBR 322 DNA at 15°C, overnight. The ligation was performed in 50 mM Tris-HCl pH 8.0, 10 mM MgCl\(_2\), 20 mM DTT, with 10 units T4 DNA ligase in a total volume of 30 μl. Transformation was carried out according to the method of Inone and Curtiss (17). Isolated plasmid DNA of positive transformants was blotted onto nitrocellulose and hybridized with 32P-labeled *E. coli* tufA probe.

The subcloned 1.6 kbp SmaI fragment was sequenced in both directions using the Sanger M13/dideoxy method (18).

Minicell preparation, 35S-protein labeling in vivo, and gel electrophoresis of crude cell lysates were done as described elsewhere (19, 20).

EF-Tu·GDP was purified from *T. Thermophilus* cells, strain HB8, as described by Leberman et al. (21). Nucleotide-free EF-Tu was prepared as follows: Purified EF-Tu·GDP (30 mg in a volume of 2 ml) was diluted tenfold with 10 mM NH\(_4\)H\(_2\)PO\(_4\) pH 5.65, 5 M urea, 10 mM β-mercaptoethanol and 10 μM PMSF (buffer A). The pH was adjusted to 5.65 with acetic acid. The solution was then applied to an equilibrated (buffer A) CM-Sepharose CL-6B column (2 x 10 cm), and further washed with 50 ml of the same buffer. GDP-free EF-Tu was obtained by elution with buffer B (buffer A + 200 mM KCl, pH adjusted to 7.5). Urea was then removed by dialysis against labeling buffer (50 mM Na-borate, pH 7.5, 50 mM KCl and 10 mM MgCl\(_2\)). The protein had a GDP-binding activity of 21,000 U/mg. The [\(^3\)H]GDP activity was measured by the nitrocellulose membrane filter binding assay (11).

Affinity labeling with periodate oxidized GTP: 20 μM of nucleotide-free EF-Tu was incubated with 10 μM [\(^{14}\)C]GTP for 10 min at 37 °C in labeling buffer and dialyzed twice for two hours at 4 °C against the same buffer. Oxidation was performed in situ with 1 mM NaIO\(_4\) for one min at 37 °C. The reduction then followed by treatment with 20 mM NaBH\(_3\)(CN) for one min at 37 °C. The reaction was stopped by addition of 25 mM NaBH\(_4\), the mixture was desalted on a NAP-Column (Pharmacia), and lyophilized.
Photoaffinity labeling with $[^32P]GDP$: 20 μM nucleotide-free EF-Tu was incubated with 1 μM $[^32P]GDP$ for 10 min at 37 °C in 50 mM Tris/HCl pH 8.0, 100 mM NaCl, 5 mM MgCl$_2$ and 1 mM β-mercaptoethanol. 1 mM ATP was added and the reaction mixture was irradiated on ice with a laser beam at 257 nm using an argon ion laser (model 2000, Spectra Physics, Mountain View, USA) with a KDP crystal at an intensity of 5 mW/cm$^2$ for 5 min. The sample was desalted as described above.

Peptide analysis: A 500 μg sample of labeled EF-Tu was dissolved in 500 μl of 70 % formic acid containing 12.5 mg CNBr. After 24 hrs in the dark the mixture was diluted ten fold with water and lyophilized. Labeled peptides were analyzed by a modification of the slab gel system described by Swank and Munkres (22); 12.5 % acrylamide, 1.0 % N,N'-methylenebisacrylamide, 5 M urea, 0.1 % SDS, 0.1 M Na-phosphate, pH 6.8 with an upper gel (3 cm high) containing 8 % acrylamide, 0.064 % N,N'-methylenebisacrylamide in the same buffer. The gel was stained with Coomassie blue, treated for 30 min with 'Amplify', and dried. Labeled peptides were identified by autoradiography using Kodak XAR-5 film.

Sequencing of cyanogen bromide fragments was done as follows: 22 mg of EF-Tu were incubated with 220 mg CNBr in 22 ml of 0.1 N HCl for 48 hrs in the dark. After dilution with 200 ml of H$_2$O and lyophilisation the peptides were purified on a Sephadex LH-60 column (2 x 100 cm) according to Gerber et al. (23). Further purification was performed on reverse phase HPLC column (Vydac C$_4$, 300 Å, Macherey und Nagel, Düren, FRG). Sequencing of the large CNBr-fragments was carried out with a liquid phase sequencer.

RESULTS
Chromosomal DNA from *T.thermophilus* HB8 was isolated and digested with Bam HI or Bgl II restriction endonucleases. The fragments were separated by electrophoresis and hybridized to an *E.coli* tuf A probe. Bam HI fragments of 6.6 and 4.5 kbp could be hybridized to *E.coli* tufA-DNA while Bgl II fragments of 9.0 kbp and 6.6 kbp provided a positive signal (fig. 1). This indicates the presence of two EF-Tu genes in *T.thermophilus*, a situation similar to *E.coli* (24). The two 6.6 kbp restriction fragments
were cloned into pBR 322 using the Bam HI site. Attempts to clone the 4.5 kbp and 9.0 kbp fragments containing the putative second *T. thermophilus* tuf gene were not successful. In fig. 2 the restriction map of the cloned DNA fragments is shown. The smallest region providing a positive hybridization signal with *E. coli* tuf A is located on a 1.6 kbp Sma I restriction fragment. This DNA was sequenced using the M13/Sanger sequencing system. The entire sequence is presented in fig. 3. The *T. thermophilus* tuf1 gene codes for a protein of molecular weight 44600 D. It is 70% homologous to the *E. coli* EF-Tu on the protein level.
Fig. 3. Sequence of the tufl gene of *T. thermophilus*. The cyanogen bromide fragments CB1-CB12 were identified by protein sequencing (■) and deduced from the positions of methionine residues. CB1 and CB4 indicated by thick lines were labeled by GTP and photolabeled by GDP, respectively. The putative Shine-Dalgarno sequence is indicated.
Table 1. Nucleotide composition of the codons used in the EF-Tu genes of *T. thermophilus* tufl (TT) and *E. coli* tufA (EC) in percent.

<table>
<thead>
<tr>
<th>Nucl.</th>
<th>total</th>
<th>1st letter</th>
<th>2nd letter</th>
<th>3rd letter</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>TT</td>
<td>TT</td>
</tr>
<tr>
<td>G</td>
<td>41.8</td>
<td>44.3</td>
<td>17.8</td>
<td>63.3</td>
</tr>
<tr>
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<td>25.1</td>
<td>30.5</td>
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<td>10.1</td>
<td>31.2</td>
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<td>25.9</td>
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The nucleotide composition in the codons of the thermophilic tufl gene is summarized in table 1 and compared to the tufA gene of *E. coli*. As expected, the G+C content in the thermophilic gene is higher than that in *E. coli*. This difference is much more pronounced in the third letter of the codons. Furthermore, in the case of the *T. thermophilus* gene G is an especially preferred nucleotide in this position. The structural gene is preceded by a purine-rich, putative ribosomal binding sequence which continues up to the ATG protein initiation site. The codon usage in *T. thermophilus* is considerably different from that in *E. coli* (table 2).

Table 2. Comparison of the codon usage in the *T. thermophilus* tufl (TT) and *E. coli* tufA (EC) genes.

|       | Gly ** | Glu * | Asp ** | Val * | Ala ** | Glu ** | Gln ** | Asp ** | His ** | Leu ** | Pro ** | Leu ** | Pro ** | Glu ** | Gln ** | Asp ** | His ** | Leu ** | Pro ** | Glu ** | Gln ** | Asp ** | His ** | Leu ** | Pro ** | Glu ** | Gln ** | Asp ** |
|-------|--------|-------|--------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|       | GGG    | GGA   | GGT    | GCC    | GAG    | GAT    | GAC    | GTG    | GAA    | GAT    | GAC    | GTC    | GCC    | GCA    | GCT    | GCC    | GCA    | GCT    | GCC    | GCA    | GCT    | GCC    | GCA    | GCT    | GCC    | GCA    | GCT    | GCC    |
| TT    | 24     | 1     | 8      | 7      | 37     | 1      | 23     | 45     | 1      | 4      | 2      | 19     | 3      | 5      | 21     | 7      | 30     | 4      | 20     | 4      | 10     | 24     | 8      | 5      | 13     | 1      |
| EC    | 1      | 19    | 21     | 7      | 30     | 4      | 20     | 4      | 10     | 24     | 8      | 5      | 13     | 1      |
|       |        |       |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Arg **| Ser    | Lys * | Arg    | Ser    | Lys    | Arg    | Ser    | Lys    | Leu    | His    | Leu    | His    | Leu    | His    | Leu    | His    | Leu    | His    | Leu    | His    | Leu    | His    | Leu    | His    | Leu    |
|       | AGA    | AGT   | AGA    | AGT    | AGA    | AGG    | AGT    | AGA    | AGG    | AGT    | AGA    | AGG    | AGT    | AGA    | AGG    | AGT    | AGA    | AGG    | AGT    | AGA    | AGG    | AGT    | AGA    |
| TT    | 3      | 1     | 2      | 20     | 2      | 9      | 12     | 13     | 8      | 29     | 8      | 29     | 8      | 29     | 8      | 29     | 8      | 29     | 8      | 29     | 8      | 29     | 8      | 29     |
| EC    | -      | -     | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      |
| Trp **| Cys    | Tyr    | Leu    | Phe    | Ser    | Trp    | Cys    | Tyr    | Leu    | Phe    | Ser    | Trp    | Cys    | Tyr    | Leu    | Phe    | Ser    | Trp    | Cys    | Tyr    | Leu    |
|       | TGG    | TGA    | TGG    | TAG    | TAA    | TTA    | TGG    | TGA    | TGG    | TGA    | TGG    | TGA    | TGG    | TGA    | TGG    | TGA    | TGG    | TGA    | TGG    | TGA    | TGG    | TGA    | TGG    |
| TT    | 2      | 1     | 1      | 3      | 8      | 11     | 10     | 2      | 3      | 1      | 13     | 1      | 7      | 3      | 1      | 13     | 1      | 7      | 3      | 1      | 13     | 1      | 7      | 3      | 1      |
| EC    | 1      | 1     | 2      | 1      | 2      | 8      | -      | 1      | 13     | -      | 7      | 3      | 1      | 13     | 1      | 7      | 3      | 1      | 13     | 1      | 7      | 3      | 1      | 13     | 1      |
| Arg **| Ser    | Lys    | Arg    | Ser    | Lys    | Arg    | Ser    | Lys    | Leu    | His    | Leu    | His    | Leu    | His    | Leu    | His    | Leu    | His    | Leu    | His    | Leu    | His    | Leu    | His    | Leu    |
|       | CGG    | CGA    | CGT    | CGC    | CAG    | CAT    | CAC    | CTG    | CTA    | CTT    | CTC    | CCG    | CCA    | CTT    | CTC    | CCG    | CCA    | CTT    | CTC    | CCG    | CCA    | CTT    | CTC    | CCG    |
| TT    | 19     | -      | 5      | -      | 8      | -      | 12     | 10     | 5      | 1      | 15     | -      | 6      | 2      | -      | 19     | -      | 1      | 19     | -      | 1      | 19     | -      | 1      |
| EC    | -      | 21     | 2      | 8      | -      | 1      | 10     | 27     | -      | 1      | -      | 19     | -      | 1      | 2      | -      | 19     | -      | 1      | 19     | -      | 1      | 19     | -      | 1      |

9269
Fig. 4. Comparison of the tight domain of the EF-Tu sequences from *T. thermophilus* (TT), *E. coli* EF-TuA (EC), *S. cerevisiae* mitochondrion (SC) and *Euglena gracilis* chloroplast (EG). Regions of homology are framed. Amino acids of the putative GDP binding domain are underlined. The cys-81 which have all elongation factors Tu in common is marked by an asterisk.

For example the lysine codon AAG is used twenty times in the *T. thermophilus* EF-Tu gene but AAA is absent. On the other hand, in the *E. coli* tufA gene the codon AAG is used only five times whereas AAA appears eighteen times. The only exceptions to the preference for G and C at position 3 of the codon exists for phenylalanine and isoleucine. In these cases T is preferred over C in the third position. This is in accordance to the observation that in *T. thermophilus* codons with a C in the third position are generally avoided (table 2).

The protein sequence of *T. thermophilus* elongation factor Tu is highly homologous to the *E. coli* elongation factor. In fig. 4 the sequences of elongation factor Tu from *T. thermophilus*, *E. coli* (3, 4), *Saccharomyces cerevisiae* mitochondrion (5) and *Euglena gracilis* chloroplasts (6) are compared. Identical regions were found in the sequences of all four proteins. In general the four proteins show a high degree of homology especially in the guano-
sine nucleotide binding domain. Regions where the four sequences are not homologous are confined mostly to loops. A remarkable feature of the *T.thermophilus* elongation factor Tu are ten amino acids in the region 181 - 190. This sequence is absent in *E.coli* and is partially absent in yeast mitochondrial EF-Tu. In *Euglena gracilis* chloroplast EF-Tu this sequence is present and contains several basic amino acids as is the case with *T.thermophilus*. By comparing the amino acid sequences there is no obvious structural feature which could be attributed to the thermostability of *T.thermophilus* EF-Tu. In contrast to an earlier work which reported the presence of a disulfide bridge (11) we identified only one cysteine residue in the protein.

Expression of *T.thermophilus* EF-Tu was achieved by transferring pLS 601 and pLS 652 carrying the 6.6 kb BglII and the 6.6 kb BamHI fragment, respectively, to an *E.coli* minicell producing strain. In case of pLS 601 EF-Tu was identified as a radioactive protein band comigrating with the *T.thermophilus* EF-Tu sample (fig. 5, lane 3). This band showed a positive reaction with anti EF-Tu GDP antibodies from rabbit (data not shown). Three additional proteins were expressed from pLS 601.

No expression occurs using pLS 652 suggesting that the promoter region is situated at least 1.5 kbp upstream from the *T.thermophilus* tufl gene.

In order to identify the nucleotide binding domain of EF-Tu from *T.thermophilus* and especially to clarify the role of the insertion of the additional 10 amino acids (residues 181-190) in the protein, we performed affinity labeling experiments. Elongation factor Tu was therefore prepared in a nucleotide-free form and charged with radioactive guanosine-5'-diphosphate or guanosine-5'-triphosphate. In one experiment EF-Tu*[^14]C]GTP was oxidized by sodium periodate and subsequently reduced with sodium cyanoborohydride. After cyanogen bromide cleavage of the labeled protein the radioactive fragment was identified by autoradiography (fig. 6). Assignment of cyanogen bromide fragments was performed by their partial sequencing in a liquid phase sequenator. GTPox1 binds specifically to a region containing the 10.1 kD cyanogen bromide fragment CB1 (fig. 3). This part of the elongation factor Tu originates from the N-terminus and contains a so called
glycine loop (residues 20-28) which was suggested to be a part of the GDP binding domain in the *E. coli* protein (25). In another affinity labeling experiment the nucleotide-free EF-Tu was charged with [\(^{32}\)P]GDP and the complex irradiated with a laser. In this case the 5.0 KD fragment CB4 (fig. 3) was predominantly labeled by a guanosine nucleotide. This fragment contains a part of *T. thermophilus* EF-Tu which corresponds to the helical domain F of the *E. coli* protein (25). Eight of ten aminoacids comprising the extra loop (residues 181-190) which is not found in *E. coli* EF-Tu is localized at the N-terminus of this fragment.

The results of both affinity labeling experiments are consistent with placing the extra loop in the vicinity of the GDP-binding site in *T. thermophilus*.

DISCUSSION

The elongation factor Tu from *T. thermophilus* has a molecular weight of 44,600 D, only slightly higher than the EF-Tu from *E. coli*. Determination of the molecular weight by SDS-PAGE pro-
Fig. 6. Affinity labeling of the GDP/GTP-binding site of EF-Tu.

EF-Tu was labeled by [\textsuperscript{35}S]GTP (A) and photolabeled by [\textsuperscript{32}P]GDP (B). Cyanogen bromide fragments were separated by SDS-urea-polyacrylamide gel electrophoresis (lane 1 and 3). The gel was dried and analysed by autoradiography (lane 2 and 4). The 10.1 kD and the 5.0 kD cyanogen bromide fragments correspond to CB1 and CB4 in fig. 3. For details see 'materials and methods'.

vides a considerably higher value of 51,000 D. This apparent difference is not as large as that found for the E. coli EF-Tu (26), probably due to the more lipophilic character of the thermophilic protein.

A typical feature of proteins from thermophilic bacteria is their low cysteine content (27). Correspondingly, the T. thermophilus EF-Tu has only one cysteine residue compared to three in the E. coli and B. stearothermophilus elongation factors Tu (28). The cysteine present in the T. thermophilus EF-Tu corresponds to residue 81 in the homologous E. coli protein. This cysteine is conserved in all procaryotic elongation factors Tu and can be specifically labeled with N-tosyl-L-phenylalanylchloromethane (28). This modified EF-Tu interacts with guanosine nucleotides as
does the native protein but binds the aminoacyl-tRNA poorly. Therefore cys-81 is probably involved in the binding of aminoacyl-tRNA to EF-Tu·GTP (28). This hypothesis is supported by the fact that the sequence corresponding to residues 79 - 88 of the \textit{T.thermophilus} EF-Tu is identical for all known procaryotic factors Tu.

Typical changes are apparent comparing the amino acid composition of homologous proteins from thermophilic and mesophilic bacteria. For instance in thermophilic proteins glutamic acid is preferentially used in place of aspartic acid and arginine is preferred over lysine (29). These changes result from an increased usage of codons with a high G+C content in thermophilic bacteria.

In the case of EF-Tu from \textit{T.thermophilus} there is a preference for valine (38 in \textit{E.coli} and 52 in \textit{T.thermophilus}) over isoleucine (29 in \textit{E.coli} and 21 in \textit{T.thermophilus}). This is related to the base composition of the codons for these amino acids. \textit{T.thermophilus} uses the GTG valine codon very often whereas the ATA, ATC and ATT codons for isoleucine are avoided. The evolutionary pressure towards codons with a high G+C content is obvious for almost all codons used in the \textit{T.thermophilus} EF-Tu gene. A high G+C content is most pronounced in the first and third codon letter. This phenomenon was also observed for the moderate thermophilic \textit{B.stearothermophilus} genes (30, 31) - in a less pronounced manner - and confirmed for the gene of isopropylmalate dehydrogenase in \textit{T.thermophilus} (32), where a very high G+C content (89\%) in the third letter was found. A remarkable exception for the preference of codons with a high G+C content in thermophilic genes is found in the codons for phenylalanine and isoleucine. In the \textit{T.thermophilus} tuf\textsubscript{I} gene the phenylalanine codon TTT is used ten times and the codon TTC only three times, whereas an opposite ratio of 1 to 13 is found in the \textit{E.coli} tufA gene. Similarly the codon ATT is preferred over ATC for isoleucine in the thermophilic protein in contrast to the mesophilic variant. For unknown reasons in the thermophilic \textit{tuf} gene the TTT (ATT) codon has an advantage over the TTC (ATC) codon. However, this is not the case in the \textit{T.thermophilus} isopropylmalate dehydrogenase gene. Parker and Precup recently reported that leucine is misincorporated with a higher frequency at UUC than UUU in
E.coli (33), a finding which may reflect the stability of codon : anticodon interactions involving UUN codons. As an alternative explanation the differences in codon usage for phenylalanine in E.coli and _T. thermophilus_ tuf genes could be due to different concentrations of tRNA isoacceptors in both bacteria. However, in _E.coli_ as well as in _T. thermophilus_ only a tRNA^Phe_ with an anticodon GAA was identified (K. Watanabe, M. Sprinzl, unpublished). A possible explanation for the unusual phenylalanine and isoleucine codon usage would be the different distribution of the respective codons in the two _T. thermophilus_ tuf genes. In such a case, the unusual UUU and AUU codons could have a regulatory function in expression of the different tuf genes. The tuf2 gene of _T. thermophilus_ has to be sequenced to test this possibility.

The _T. thermophilus_ EF-Tu gene has a long open reading frame in the complementary DNA strand. Whether this is connected to the fact that in highly expressed genes the codons complementary to nonsense codons are extremely rare (34) or this arrangement is important for a regulatory principle connected with an anti-sense mRNA remains to be clarified.

Expression of _T. thermophilus_ EF-Tu in _E.coli_ is possible but not efficient. There are several factors which could negatively influence the expression. A toxic effect of the thermophilic EF-Tu on the translation system of _E.coli_ can be excluded since other proteins encoded on the plasmid pLS 601 (e.g. B-lactamase) are normally expressed. Comparison of the translation products of pBR 322 and pLS 601 indicates the additional expression of four proteins. If the tuf1 gene of _T. thermophilus_ is a part of a polycistronic mRNA, homologous to the _E.coli_ str mRNA (35), these translational products could correspond to the _T. thermophilus_ variants of the _E.coli_ EF-G, EF-Tu and ribosomal proteins S7 and S12. An identical gene organization exists for _Bacillus stearothermophilus_ (M. Kimura, personal communication). Since the two putative S7 and S12 proteins (17 kD and 22 kD) are expressed to an appreciable amount in the minicell system a misfunction of their _T. thermophilus_ promoter in _E.coli_ is not likely. The long ribosomal binding sequence in _T. thermophilus_ as compared to _E.coli_ (36) may be a reason for the low expression rate of _T. thermophilus_.
philus EF-Tu in E.coli. Work is in progress to clarify these open questions.

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