Role of Domains in *Escherichia coli* and Mammalian Mitochondrial Elongation Factor Ts in the Interaction with Elongation Factor Tu*

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Bovine mitochondrial elongation factor Ts (EF-Tsmt) stimulates the activity of *Escherichia coli* elongation factor Tu (EF-Tu). In contrast, *E. coli* EF-Ts is unable to stimulate mitochondrial EF-Tu. EF-Tsmt forms a tight complex with *E. coli* EF-Tu governed by an association constant of 8.6 × 10^10M. This value is 100-fold stronger than the binding constant for the formation of the *E. coli* EF-Tu-Ts complex. To test which domain of EF-Tsmt is important for its strong binding with EF-Tu, chimeras were made between *E. coli* EF-Ts and EF-Tsmt. Replacing the N-terminal domain of *E. coli* EF-Ts with that of EF-Tsmt increases its binding to *E. coli* EF-Tu 2-3-fold. Replacing the N-terminal domain of EF-Tsmt with the corresponding region of *E. coli* EF-Ts decreases its binding to *E. coli* EF-Tu 4-5-fold. A chimera consisting of the C-terminal half of *E. coli* EF-Ts and the N-terminal half of EF-Tsmt binds to *E. coli* EF-Tu as strongly as EF-Tsmt. A chimera in which Subdomain N of the core of EF-Ts is replaced by the corresponding region of EF-Tsmt binds *E. coli* EF-Tu 25-fold more tightly than *E. coli* EF-Ts. Thus, the higher strength of the interaction between EF-Tsmt and EF-Tu can be localized primarily to a single subdomain.

The classical model for the elongation cycle of protein biosynthesis was developed on observations made with *Escherichia coli* EF-Tu.1 EF-Tu forms a ternary complex with aminoacyl-tRNA and GTP that promotes the binding of the aminoacyl-tRNA with the A-site of the ribosome. EF-Tu then hydrolyzes the bound GTP and is released from the ribosome as an EF-Tu-GDP complex (1). A second elongation factor (EF-Ts) promotes the release of GDP, forming an intermediate EF-TsTu complex (2). In *E. coli*, GTP binds to the EF-Tu-Ts complex, promoting the release of EF-Ts and the formation of an EF-Tu-GTP complex. A new ternary complex can then form, and the cycle repeats. In *Thermus thermophilus*, a dimeric complex (EF-Tu-Ts)$_2$ occurs through the interaction of two EF-Tu molecules with a stable EF-Ts dimer (3). In contrast to the *E. coli* EF-Tu-Ts complex, the *T. thermophilus* complex is not dissociated to a significant extent by either GDP or GTP alone (4).

Mitochondrial EF-Tu and EF-Ts have been purified from bovine liver as a tightly associated complex (EF-Tu-Tsmt) (5, 6). The EF-Tu-Tsmt complex differs from the corresponding *E. coli* complex in that EF-Tu-Tsmt is not readily dissociated by guanine nucleotides (5, 6). Furthermore, no significant amounts of intermediates equivalent to EF-Tu-GTP or EF-Tu-GDP can be detected in the animal mitochondrial system. However, mammalian EF-Tu-Tsmt forms a ternary complex with GTP and aminoacyl-tRNA (7). The basic steps of the bacterial elongation cycle thus appear to be occurring in mammalian mitochondria. However, the equilibrium constants that govern the interaction of EF-Tu with EF-Ts and guanine nucleotides appear to be significantly different.

The cDNAs encoding EF-Tu mt and EF-Ts mt have been cloned and sequenced from bovine and human liver (8, 9). Sequence analysis indicates that EF-Tu mt has 56% identity to *E. coli* EF-Tu while EF-Ts mt is less than 30% identical to *E. coli* EF-Ts. When EF-Ts mt is expressed and purified from *E. coli*, it forms a 1:1 complex with *E. coli* EF-Tu (EF-Tu$/E$.T$^{mt}$) (9). This heterologous complex is very resistant to dissociation by guanine nucleotides even at high concentrations of GDP or GTP (10). This feature of the heterologous complex is quite reminiscent of the native EF-Tu-EF-Ts complex. Thus, it is apparently the nature of EF-Ts that determines the strength of its interaction with EF-Tu. However, it is not clear what features of EF-Ts mt modulate its tight interaction with EF-Tu.

The crystal structures of trypsin-modified *E. coli* EF-Tu-GDP and of *Thermus aquaticus* EF-Tu complexed with a nonhydrolyzable GTP analogue have been determined (11, 12). Analysis of these structures indicates that EF-Tu folds into three domains. Domain I encompasses about the first 200 residues and contains the guanine nucleotide binding site. Domains II and III are each ~100 residues long. All three domains are involved in binding aminoacyl-tRNA (13). The structure of the *E. coli* EF-Tu-Ts mt complex has also been determined (Fig. 1) (14). This structure indicates that EF-Ts mt consists of 4 structural modules: the N-terminal domain (residues 1–54); the core domain (residues 55–179 and 229–263); the dimerization domain (residues 180–228); and the C-terminal module (residues 264–282). The core domain is further divided into Subdomain N (residues 55–140) and Subdomain C (residues 141–179 and 229–263). The N-terminal domain, Subdomain N, and the C-terminal module interact with Domain I of EF-Tu, whereas Subdomain C interacts with Domain III of EF-Tu. EF-Ts mt binds to EF-Tu more tightly than does *E. coli* EF-Ts. This work attempts to determine the regions of the EF-Ts mt giving rise to its higher affinity for EF-Tu.

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1 The abbreviations used are: EF-Tu, *Escherichia coli* elongation factor Tu; EF-Tsmt, bovine mitochondrial elongation factor Ts; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis.

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EF-Ts

\[
\begin{align*}
\text{N-Terminal} & \quad \text{EF-Ts} \\
\text{Subdomain N} & \quad \text{h1-h3} \\
\text{Subdomain C} & \quad \text{h4-h7} \\
\text{Dimerization} & \quad \text{h8, h12} \\
\end{align*}
\]

FIG. 1. Three-dimensional representation of the structure of E. coli EF-Tu-Ts. The structure of EF-Tu is shown on the left with the domains labeled I, II, and III. EF-Ts is shown on the right. The N-terminal domain (h1–h3) at the top. Coordinates were kindly provided by Dr. T. Kawashima and are displayed using RASWIN.

MATERIALS AND METHODS

Construction of Deletion Mutants of EF-Ts—Clones encoding His-tagged variants of E. coli EF-Ts and EF-Ts<sub>m</sub> in pET24C(+) were prepared previously (9, 15). To make a mutant of E. coli EF-Ts lacking the N-terminal domain (residues 1 to 53), a fragment of the E. coli EF-Ts gene containing sequences encoding amino acid residues 54–282 was amplified bypolymerase chain reaction (PCR) using primer ETsP4 (cgctgctgatgagactgcttggacatcgcagc) and ETsAN (gggctccatagtaacggatggccggtcggc). The PCR fragment was digested with NdeI and HindIII and cloned into NdeI- and Xhol-digested pET24C(+), giving the construct pETsAN. To make a mutated form of EF-Ts<sub>m</sub> lacking the N-terminal domain (residues 1–56), a fragment of EF-Ts<sub>m</sub> gene containing the sequence encoding amino acid residues 57–283 was amplified by PCR using primer ETsP1 (gggaagcttcatatgaaattaccgcatccct) and ETsP2 (cgaagcttctgtacctataggttcggtcggc). The PCR fragment was digested with NdeI and HindIII and cloned into NdeI- and Xhol-digested pET24C(+), giving the construct pETsAN.

Preparation of Chimeras of E. coli and Mitochondrial EF-Ts—To make Chimeras I and II (see Fig. 5), a HindIII site was introduced into the EF-Ts<sub>m</sub> gene at nucleotide sequence position 342 by site-directed mutagenesis using the Chameleon<sup>TM</sup> Double-Stranded, Site-Directed Mutagenesis Kit (Stratagene). Primer MP15 (cttcctcccatgagcagctccgctgctgacggc) was used for the mutagenesis. The HindIII site is underlined and the mutated residues are shown in boldface. This mutation changes residue Arg-54 to Lys-54. In the human EF-Ts<sub>m</sub> gene, the amino acid residue at the same position is Lys-54. The N-terminal domain of E. coli EF-Ts from the N terminus to residue 49 was amplified by PCR using primer ETsP1 (cgctgctgatgagactgcttggacatcgcagc) and ETsAN and cloned into NdeI- and Xhol-digested pET24C(+), giving the construct pETsAN. EF-Ts was amplified by polymerase chain reaction (PCR) using primer ETsP4 and the PCR fragment prepared above as primers. This primer hybridizes on the 3′ side to gene 1 but has sequences at the 5′ end that are derived from the second gene (gene 2). PCR amplification results in the formation of a product that contains the desired portion of gene 1 with a short stretch of sequence complementary to gene 2 at the junction where the chimera is to be joined. This PCR fragment is used as a 5′ primer on gene 2 along with an appropriate 3′ primer in a second PCR reaction.

The DNA fragment from this amplification reaction was purified by gel electrophoresis. A chimeric EF-Ts fragment was then amplified by using primer ETsP4 and the PCR fragment prepared above as primers with the E. coli EF-Ts gene in pET24C(+) as the template and the PCR conditions described above. This second DNA fragment was purified by gel electrophoresis, digested with NdeI and Xhol, and cloned into pET24C(+) to make Chimera III.

To make Chimera IV, a PCR fragment of the E. coli EF-Ts gene in pET24C from the N terminus to amino acid residue 148 was amplified by primer Vec-2 and MP24 (agagacaaacctagaacacetctcggggacgcaagc). This primer is a chimera in which the underlined sequence is derived from the E. coli EF-Ts gene while the sequence in boldface is derived from the EF-Ts<sub>m</sub> gene. The PCR conditions described above were used, and the amplified DNA was purified by gel electrophoresis. This PCR fragment and primer C-Xhol were then used as primers to amplify a chimeric EF-Ts fragment using the EF-Ts<sub>m</sub> DNA as template and the conditions described above. This second PCR fragment was purified by gel electrophoresis, digested with NdeI and Xhol, and cloned into...
The activities of E. coli and mitochondrial EF-Tu were examined as described under “Materials and Methods.” Reaction mixtures contained 7.3 μg of expressed E. coli EF-Tu (~80 pmol of active factor) and the indicated amounts of E. coli EF-Ts (EF-TsEco) or EF-Tsmt. Blanks representing the amount of GDP exchange carried out by EF-Tu alone during this incubation period (~7 pmol) have been subtracted from each value. No GDP binding could be detected in the absence of EF-Tu, indicating that the preparations of EF-Ts used here were free of EF-Tu. EF-Tsmt, EF-TsEco, and Chimera II to EF-Ts. Nearly 10-fold higher concentrations of EF-Ts following chromatography on nickel-nitrilotriacetic acid resins. E. coli EF-Ts was prepared free of E. coli EF-Tu by using buffers containing GDP and MgCl₂.

The abilities of EF-Tsmt and E. coli EF-Tu to stimulate GDP exchange and poly(U)-directed polymerization with E. coli EF-Tu were tested (Fig. 3 A and B). The purified EF-Tsmt stimulates guanine nucleotide exchange with E. coli EF-Tu and also stimulates the poly(U)-directed polymerization of phenylalanine (Fig. 3A). However, substantially higher levels of EF-Tsmt are required to achieve the same degree of stimulation observed with E. coli EF-Ts. Nearly 10-fold higher concentrations of EF-Tsmt are required to promote the same amount of nucleotide exchange obtained with E. coli EF-Ts (Fig. 3A). The activity of EF-Tsmt in stimulating poly(U)-directed polymerization...
tion with *E. coli* EF-Tu is ~25% of that obtained with *E. coli* EF-Ts (Fig. 3B).

EF-Tsmt stimulates the activities of both EF-Tu mt and *E. coli* EF-Tu in poly(U)-directed polymerization (Fig. 3, B and C). *E. coli* EF-Ts cannot stimulate the activity of EF-Tu mt (Fig. 3C). This observation indicates that *E. coli* EF-Ts may be unable to bind EF-Tu mt, or that it binds to EF-Tu mt much more weakly than GDP does. Alignment of the primary sequence of *E. coli* EF-Tu and EF-Tu mt indicates that these two factors are 56% identical. In addition, all of the residues in *E. coli* EF-Tu that are in contact with EF-Ts in the crystal structure are identical or are conservative replacements in EF-Tu mt. Thus, the failure of *E. coli* EF-Ts to stimulate the mitochondrial factor in translation is surprising.

As indicated above, higher levels of EF-Tsmt are required to produce the same degree of stimulation of *E. coli* EF-Tu observed with low levels of *E. coli* EF-Ts. Previous results have suggested that EF-Tsmt binds to *E. coli* EF-Tu more tightly than does *E. coli* EF-Ts (10). This idea is based on the observation that the heterologous complex EF-Tu Eco·Tsmt is not readily dissociated by guanine nucleotides while the homologous *E. coli* complex is. Thus, the lower activity of EF-Tsmt probably arises from its slow release from EF-Tu which reduces the rate of ternary complex formation. To test this idea, we have determined the approximate equilibrium association constant for the binding of EF-Tsmt to *E. coli* EF-Tu and have compared this value with that obtained with the homologous factor.

To determine the association constant for the binding of EF-Tsmt to *E. coli* EF-Tu, the $K_{obs}$ of the following reaction was measured as described under "Materials and Methods."

\[
E. coli\ EF-Tu \cdot GDP + EF-Ts_{mt} \rightleftharpoons EF-Tu_{mt} \cdot Ts_{mt} + GDP \quad (Eq. 4)
\]

The binding constant ($K_p$) of EF-Tsmt to *E. coli* EF-Tu was calculated from $K_{obs}$, and the binding constant of *E. coli* EF-Tu to GDP (3.3 × 10^8 M^{-1}) (Table I) as described by Miller and Weissbach (19). The value obtained (8.6 × 10^10) indicates that EF-Tsmt binds to *E. coli* EF-Tu quite tightly. The $K_p$ for *E. coli* EF-Ts was also measured, and the corresponding binding constant ($K_{p}$) was calculated (Table I). The $K_{p}$ of *E. coli* EF-Ts determined here (9 × 10^8 M^{-1}) is quite similar to the value of 5 × 10^8 M^{-1} obtained from the literature (19). Comparison of the $K_p$ of *E. coli* EF-Ts with the $K_{Ts}$ of EF-Tsmt indicates that EF-Tsmt binds to *E. coli* EF-Tu ~100 times more tightly than does *E. coli* EF-Ts.

Role of the N-terminal Domain of EF-Tsmt—Analysis of the protease sensitivity of EF-Ts (20) and the x-ray structure of the *E. coli* EF-Tu·Ts complex (14) shows that the N-terminal region of *E. coli* EF-Ts folds into an independent domain (Fig. 1). This region is essential for the ability of *E. coli* EF-Ts to stimulate guanine nucleotide exchange with EF-Tu (20). Sequence alignment indicates that there is significant homology between *E. coli* EF-Ts and EF-Tsmt in the N-terminal domain and it is likely that it will fold in a similar three-dimensional structure. It was, therefore, of interest to determine whether the N-terminal domain of EF-Tsmt was also important for its binding to EF-Tu or whether other interactions of this factor could compensate for the loss of this domain. To examine this question, N-terminal deletion mutants of *E. coli* EF-Ts and EF-Tsmt were constructed (Fig. 5). The EF-Ts deletion mutants were tested for their abilities to bind *E. coli* EF-Tu and to stimulate the activities of *E. coli* EF-Tu and EF-Tsmt in polymerization. The N-terminal deletion mutant of *E. coli* EF-Ts is unable to stimulate the activity of EF-Tu in guanine nucleotide exchange or in poly(U)-directed polymerization (data not shown). This observation is in agreement with previous results showing that a proteolytic derivative of *E. coli* EF-Ts lacking the N-terminal domain is unable to stimulate the activity of EF-Tu (20). The N-terminal deletion mutant of EF-Tsmt is unable to bind *E. coli* EF-Tu (data not shown). It is also inactive in stimulating the activity of either *E. coli* EF-Tu or EF-Tsmt (data not shown).

These data indicate that the N-terminal domain of EF-Tsmt, like that of *E. coli* EF-Ts, is important for its function in protein synthesis. These data, while indicating that the N-terminal domain is important for the interaction of EF-Tsmt with EF-Tu, do not provide any insight into whether this region plays a role in the stronger affinity for EF-Tu observed with EF-Tsmt.

**Predicted Secondary Structure of EF-Tsmt and Analysis of Chimeric Proteins between *E. coli* and Mitochondrial EF-Ts**—In the crystal structure of the *E. coli* EF-Tu·Ts complex (Fig. 1) (14), the N-terminal domain and Subdomain N of the core interact with Domain I of *E. coli* EF-Tu. Subdomain C of the core interacts with Domain III of *E. coli* EF-Tu. As indicated above, EF-Tsmt binds to *E. coli* EF-Tu more tightly than does *E. coli* EF-Ts. It has not yet been possible to make direct measurements of the binding constant of EF-Tsmt for EF-Tu mt. However, it is clear that guanine nucleotides cannot dissociate the EF-Tu·Tsmt complex (6). Thus, it is likely that EF-Tsmt will

**TABLE I**

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</tr>
<tr>
<td>EF-Tsmt</td>
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<td>8.6 ± 1.0 × 10¹⁰</td>
</tr>
<tr>
<td>Chimera I</td>
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<td>2.2 ± 0.6 × 10¹⁰</td>
</tr>
<tr>
<td>Chimera II</td>
<td>59 ± 0.2</td>
<td>1.9 ± 0.2 × 10¹⁰</td>
</tr>
<tr>
<td>Chimera III</td>
<td>308 ± 62</td>
<td>1.0 ± 0.2 × 10¹¹</td>
</tr>
<tr>
<td>Chimera V</td>
<td>67 ± 12</td>
<td>2.2 ± 0.4 × 10¹⁰</td>
</tr>
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**FIG. 4. Secondary structure of EF-Ts.** The secondary structure of *E. coli* EF-Ts is based on the crystal structure (14). The secondary structure of EF-Tsmt is based on multiple sequence alignments using the Pileup program in GCG and the Clustalw program followed by manual adjustment of the alignment. aa, amino acids. 

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EF-Ts and several secondary structure prediction helix h10 appear to be present. Finally, the C-terminal module region(s) might be important for the strong interaction of EF-Ts is predicted to begin with and EF-Tsmt (Fig. 5, Chimeras I and II). These two chimeric constructs.

FIG. 5. Schematic representation of E. coli EF-Ts, EF-Tsmt, and the chimeric constructs. EF-TsmtΔN contains amino acid residues 54 to the C terminus of E. coli EF-Ts. EF-TsmtΔN contains amino acid residues 57 to the C terminus of EF-Tsmt. Chimera I contains amino acid residues of EF-Tsmt from the N terminus to residue 54 and amino acid residues of E. coli EF-Ts from residue 57 to the C terminus. Chimera II contains amino acid residues of E. coli EF-Ts from the N terminus to residue 49 and amino acid residues of EF-Tsmt from residue 55 to the C terminus. Chimera III contains amino acid residues of EF-Tsmt from the N terminus to residue 162 and amino acid residues of E. coli EF-Ts from residue 149 to the C terminus. Chimera IV contains amino acid residues of E. coli EF-Ts from the N terminus to residue 148 and amino acid residues of EF-Tsmt from residue 163 to the C terminus. Chimera V contains residues 1–49 and 149–283 from E. coli EF-Ts and residues 55 to 162 from EF-Tsmt.

To localize the region giving EF-Tsmt its stronger affinity for EF-Tu, equilibrium association constants were determined as described above for the normal proteins. For these experiments, E. coli EF-Ts and Chimera I were prepared free of EF-Tu by the use of buffers containing GDP during the preparation of the factors. EF-Tsmt and Chimera II were prepared by the denaturation of the EF-Tu/E. coli EF-Ts complex (Fig. 1). However, the three-stranded β-sheet structure in Subdomain N forms an interface with the guanine nucleotides and aminoacyl-tRNA present in the extract. Chimera I which carries the N-terminal domain of EF-Tsmt binds E. coli EF-Tu much more tightly than does E. coli EF-Ts but it somewhat less tightly than EF-Tsmt (Fig. 6, lane 4).

To obtain more quantitative measurements of the affinity of these chimeras for E. coli EF-Tu, equilibrium association constants were determined as described above for the normal proteins. For these experiments, E. coli EF-Ts and Chimera I were prepared free of EF-Tu by the use of buffers containing GDP during the preparation of the factors. EF-Tsmt and Chimera II were prepared by the denaturation of the EF-Tu/E. coli EF-Ts complex followed by renaturation of the EF-Tsmt (10). Analysis of these preparations on SDS-PAGE indicated that they were free of EF-Tu (data not shown). As indicated in Table I, replacing the N-terminal domain of E. coli EF-Ts with that of EF-Tsmt (Chimera I) increases the binding constant for EF-Tu ~2–3-fold. This observation suggests that this region of EF-Tsmt has a small effect on the strength of the interaction with EF-Tu. In the complementary construct (Chimera II), replacing the N-terminal domain of EF-Tsmt with that of E. coli EF-Ts decreases the binding constant of EF-Tsmt to E. coli EF-Tu ~4–5-fold. Chimera II which is predominantly derived from EF-Tsmt still binds EF-Tu ~20-fold more tightly than does E. coli EF-Ts. These observations indicate that the strength of the interaction observed with EF-Tsmt is governed primarily by sequences from h4 to the C terminus with a small contribution from sequences in the N-terminal domain.

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Fig. 7. Stimulation of guanine nucleotide exchange with E. coli EF-Tu.

Nucleotide binding assays were carried out as described under "Materials and Methods" and contained 7.3 μg of expressed E. coli EF-Tu (~80 pmol of active factor) and the indicated amount of E. coli EF-Ts, Chimera I or Chimera II (A) and EF-Tsmt or Chimera III (B). For Chimera V, the EF-Tu, Chimera V complex (3–9 pmol) was used directly in the GDP exchange assay. The amount of GDP binding obtained with this complex alone was very small (<1 pmol). Blanks representing the amount of GDP exchange carried out by EF-Tu alone during this incubation period (~7 pmol) have been subtracted from each value.

Fig. 8. Stimulation of the activities of E. coli EF-Tu and EF-Tu<sub>mt</sub> in polymerization by the chimeras. A, reaction mixtures contained 1 pmol of expressed E. coli EF-Tu and the indicated amounts of EF-Ts or chimera. Blanks representing the amount of polymerization catalyzed by EF-Tu alone (~4 pmol) have been subtracted from each value. B, reaction mixtures contained 3 pmol of expressed EF-Tu<sub>mt</sub> and the indicated amount of EF-Ts. Blanks representing the amount of polymerization catalyzed by EF-Tu alone (~2–3 pmol) have been subtracted from each value.

 ras must be able to form this structure to fold correctly. Chimera III consists of the N-terminal domain and Subdomain N of EF-Tsmt with the C-terminal half of E. coli EF-Ts while Chimera IV is the reverse construct (Fig. 5). Chimera III was purified as a 1:1 complex with E. coli EF-Tu (Fig. 6, lane 5). This observation suggests that it has folded correctly and that the determinants for the strong interaction between EF-Tsmt and EF-Tu reside in the NH<sub>2</sub>-terminal half of the protein. The association constant for the binding of Chimera III to EF-Tu (Table I) indicated that this chimera binds to EF-Tu as strongly as does the native EF-Tsmt. This observation indicates that all of the stronger binding between EF-Tsmt and EF-Tu arises from the N-terminal domain and Subdomain N of EF-Tsmt. This region of EF-Ts is in contact with Domain I of EF-Tu. These data also indicate that the C-terminal half of EF-Tsmt does not contribute significantly to the ability of EF-Tsmt to bind EF-Tu more tightly than E. coli EF-Ts.

The reciprocal construct (Chimera IV, Fig. 5) was unable to bind E. coli EF-Tu (Fig. 6, lane 6) and probably folds incorrectly. This observation suggests that the interface between Subdomain N and Subdomain C does not form correctly in this construct. Chimera IV is expressed well in E. coli and does not appear to form inclusion bodies or to be readily degraded. Hence, it is reasonable to suggest that Chimera IV has significant structure but that certain features crucial to its interaction with EF-Tu are not correctly positioned. A detailed examination of the β-sheet interface indicates that sequence differences in s6 affect the interaction of s6 with s8, possibly resulting in a distortion of the interface between the β-sheets.

The data obtained with Chimeras I, II, and III suggest that sequences within Subdomain N of EF-Tsmt are primarily responsible for the stronger affinity of this factor for EF-Tu. To confirm this idea, an additional chimera was prepared (Chimera V, Fig. 5) in which Subdomain N of E. coli EF-Ts was replaced with that of EF-Tsmt. When Chimera V is prepared from E. coli, it copurifies with considerable amounts of EF-Tu as does EF-Tsmt (Fig. 6, lane 7). This chimera is longer than the others and migrates on SDS-PAGE at a higher molecular weight than the other chimeras, due to the insertion present in Subdomain N of EF-Tsmt. The association constant for the binding of Chimera V to EF-Tu (Table I) was ~25-fold higher than that of E. coli EF-Ts. This observation agrees with the idea that sequences in Subdomain N of EF-Tsmt are primarily responsible for the stronger interaction of this factor with EF-Tu. Since there is an insertion of ~20 amino acids in this region, contacts between one or more of these residues may be occurring between EF-Tsmt and Domain I of EF-Tu.

Stimulation of the Activity of EF-Tu by EF-Ts and Its Chimeras—As indicated in Fig. 3, E. coli EF-Ts is active with its endogenous factor but not with EF-Tsmt<sub>mt</sub>. In contrast, EF-Tsmt<sub>mt</sub> can stimulate the activities of both bacterial and mitochondrial EF-Tu, although it is less efficient than E. coli EF-Ts in stimulating the activity of E. coli EF-Tu. The activities of the chimeric EF-Ts proteins in stimulating guanine nucleotide exchange with E. coli EF-Tu were tested. As indicated in Fig. 7A, replacing the N-terminal domain of E. coli EF-Ts with that of EF-Tsmt<sub>mt</sub> in Chimera I results in a factor that has the same activity as E. coli EF-Ts in promoting guanine nucleotide exchange. This level of activity, like that of E. coli EF-Ts, is ~10-fold higher than the activity observed with EF-Tsmt<sub>mt</sub>. Chimera II, in which the N-terminal domain of E. coli EF-Ts has replaced that in EF-Tsmt<sub>mt</sub>, has about one-half of the activity...
observed with *E. coli* EF-Ts (Fig. 7A). This chimera has 3–4-fold higher activity than that seen with EF-Tsmt. The activity of Chimera III in stimulating GDP exchange is about the same as that observed with EF-Tsmt (Fig. 7B). This chimera has the entire N-terminal half of EF-Tsmt, and binds to EF-Tu as tightly as EF-Tsmt. Chimera IV is not active in stimulating GDP exchange as would be expected from its apparent inability to bind EF-Tu. The activity of Chimera V is slightly higher than that of EF-Tsmt, but significantly lower than *E. coli* EF-Ts. Overall, these results indicate that EF-Tsmt and the chimeras that bind to EF-Tu more tightly have lower activities in promoting guanine nucleotide exchange.

The activities of the chimeras in stimulating the activity of *E. coli* EF-Tu in polymerization were also tested (Fig. 8A). In this assay, as in the GDP exchange assay, EF-Tsmt is less active than *E. coli* EF-Ts (Figs. 3 and 8A). The activity of Chimera I is very similar to that of *E. coli* EF-Ts. The activity of Chimera II is only slightly lower than that of *E. coli* EF-Ts and much higher than that of EF-Tsmt. This assay is probably somewhat less sensitive to changes in the affinity of EF-Ts for EF-Tu, since the formation of the EF-Tu-Ts complex is coupled to the subsequent very favorable formation of the ternary complex. This coupling might tend to offset the stronger interaction between EF-Tu and EF-Ts to some extent. The activity of Chimera III is about the same as that observed with EF-Tsmt. Since this chimera has the same affinity for EF-Tu as does EF-Tsmt, this result is to be expected. These data further indicate that replacing the N-terminal domain of EF-Tsmt (Chimera II) increases the activity of EF-Tsmt in stimulating the activity of *E. coli* EF-Tu, while replacing the C-terminal half of EF-Tsmt does not appear to affect the activity of EF-Tsmt.

As shown in Fig. 3C, *E. coli* EF-Ts is not able to stimulate the activity of EF-Tu mt in polymerization. The activities of the chimeras in stimulating EF-Tsmt in polymerization were tested (Fig. 8B). Replacing the N-terminal domain of *E. coli* EF-Ts with the corresponding region of EF-Tsmt (Chimera I) does not restore activity, indicating that the interaction between Domain I of EF-Tu and the N-terminal domain of EF-Ts is not responsible for the lack of activity observed with *E. coli* EF-Ts. In agreement with this idea is the observation that Chimera II, in which the N-terminal domain of EF-Tsmt is replaced by the corresponding region from *E. coli* EF-Ts, is quite comparable with that of EF-Tsmt (Fig. 8B). Chimera III, in which the C-terminal half of EF-Tsmt has been replaced by that from *E. coli* EF-Ts, has very little or no activity in stimulating EF-Tsmt in polymerization. Chimera V could not be tested in this assay since it could not be refolded into an active conformation following denaturation of the EF-TuGDP-Chimera V complex. These data indicate that *E. coli* EF-Ts cannot stimulate the activity of EF-Tsmt because of a failure of the C-terminal half of this factor to interact correctly with Domain III of EF-Tsmt. This observation is surprising since residues in EF-Tu making contact with Subdomain-C of EF-Ts in the crystal structure have been conserved in the mitochondrial factor.

**DISCUSSION**

The N-terminal Domain and Subdomain N Determine the Tight Binding of EF-Tsmt to *E. coli* EF-Tu—The data presented here show that EF-Tsmt binds to *E. coli* EF-Tu ~100-fold more tightly than *E. coli* EF-Ts. Analysis of the chimeric proteins indicates that residues in Subdomain N of the core of EF-Ts are primarily responsible for the difference in the tightness of binding observed while the N-terminal domain also makes a small contribution. Six residues in the N-terminal domain of *E. coli* EF-Ts make contact with residues in Domain I of *E. coli* EF-Tu (14). Some of these residues are conserved in EF-Tsmt while others are primarily conservative replacements. These latter residues in EF-Tsmt may account for the small effect on binding contributed by the N-terminal domain.

In the interactions between Domain I of *E. coli* EF-Tu and Subdomain N of the core of *E. coli* EF-Ts, four residues (Asp-80, Phe-81, Ile-125, and Gly-126) of *E. coli* EF-Ts are directly involved. The only residue in EF-Tsmt that is different in this group is the conservative replacement of Leu-151 for the corresponding residue Ile-125 in *E. coli* EF-Ts. Since Ile-125 of *E. coli* EF-Ts makes a backbone contact with EF-Tu, it is unlikely that Leu-151 of EF-Tsmt contributes to the stronger interaction observed between EF-Tsmt and EF-Tu. D80A and F81A mutants of *E. coli* EF-Ts bind to EF-Tu much more weakly than does wild type EF-Ts. However, the corresponding D84A and F85A mutants of EF-Tsmt still bind to *E. coli* EF-Tu as tightly as does the wild-type factor (15). These results suggest that Asp-84 and Phe-85 of EF-Tsmt do not contribute to the stronger interaction of EF-Tsmt with *E. coli* EF-Tu. However, as indicated in Fig. 4, Subdomain N of the core of EF-Tsmt has an insertion of ~20 residues. Although the exact position of this insertion is difficult to assess, it is likely that these residues account for the stronger interaction between EF-Tsmt and EF-Tu.

**Deincreased Tightness of Binding between EF-Tsmt Increases Its Ability to Stimulate the Activity of EF-Tu—** Although EF-Tsmt interacts with *E. coli* EF-Tu very well, it is significantly less active than *E. coli* EF-Ts in stimulating the activity of *E. coli* EF-Tu. One explanation for the lower activity observed is that the strong binding actually inhibits the activity of EF-Tsmt. The reaction of *E. coli* EF-Ts in stimulating guanine nucleotide exchange can be described as

\[
\text{EF-Tu} \cdot \text{GDP} + \text{EF-Ts} \rightleftharpoons [\text{EF-Tu} \cdot \text{Ts} \cdot \text{GDP}] \\
\leftarrow [\text{EF-Tu} \cdot \text{Ts} + \text{GDP} \quad \text{(Eq. 5)}
\]

*E. coli* EF-Ts is recycled by the dissociation of the EF-Tu-Ts complex by GDP or GTP. When EF-Tsmt forms a tight complex with *E. coli* EF-Tu, the dissociation of the complex by GDP or GTP is very slow, making the turnover number of EF-Tsmt in the reaction low. The small turnover number may explain the low activity of EF-Tsmt in stimulating GDP exchange. In the polymerization assay, the availability of Phe-tRNA probably pulls the reaction toward the formation of the ternary complex and increases the turnover of EF-Ts, which results in the somewhat higher relative activity of EF-Tsmt in the polymerization assay. In agreement with this idea is the observation that the chimeras that bind EF-Tu less tightly generally have higher activities than those that bind EF-Tu more tightly.

**Interactions of *E. coli* EF-Ts and Chimeras with EF-Tu mt—** Despite the 56% identity in sequence between *E. coli* EF-Tu and EF-Tsmt, *E. coli* EF-Ts is unable to stimulate the activity of EF-Tu mt in polymerization. This result suggests that *E. coli* EF-Ts does not interact very well with EF-Tu mt, or that the interaction occurring fails to result in effective nucleotide exchange. Unfortunately, the poor binding of guanine nucleotides to EF-Tu mt does not allow a direct measure of the exchange reaction. Since Chimera II is able to stimulate the activity of EF-Tsmt, the N-terminal domain of *E. coli* EF-Ts probably interacts with EF-Tu mt, quite well. The low activities of Chimeras I and III with EF-Tu mt suggest that the C-terminal half of *E. coli* EF-Ts does not form a good interaction with Domain III of EF-Tu mt. This observation is unexpected since the residues in EF-Tu making contact with Subdomain C of EF-Ts in the crystal structure have been conserved in EF-Tu mt. On the other hand, the alignment of the sequences of EF-Ts from a number of organisms shows that the C-terminal half of EF-Ts is not as conserved as the N-terminal half. *E. coli* EF-Ts has an extra α-helix (h13) at the C-terminal terminus compared with
EF-Ts<sub>mt</sub>. In addition, *E. coli* EF-Ts has a dimerization domain that is not present in EF-Ts<sub>mt</sub>. These structural differences may lead to the inability of *E. coli* EF-Ts to stimulate the activity of EF-Tu<sub>mt</sub>.

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Role of Domains in *Escherichia coli* and Mammalian Mitochondrial Elongation Factor Ts in the Interaction with Elongation Factor Tu

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