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 × 10^10. 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-Tu-Ts complex (2). In *E. coli*, GDP 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-Ts$_{nm}$) (5, 6). The EF-Tu-Ts$_{nm}$ complex differs from the corresponding *E. coli* complex in that EF-Tu-Ts$_{nm}$ 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-Ts$_{nm}$ 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$_{nm}$ and EF-Ts$_{nm}$ have been cloned and sequenced from bovine and human liver (8, 9). Sequence analysis indicates that EF-Tu$_{nm}$ has 56% identity to *E. coli* EF-Tu while EF-Ts$_{nm}$ is less than 30% identical to *E. coli* EF-Ts. When EF-Ts$_{nm}$ is expressed and purified from *E. coli*, it forms a 1:1 complex with *E. coli* EF-Tu (EF-Tu$_{nm}$-EF-Ts$_{nm}$) (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-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$_{nm}$ 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 complex has also been determined (Fig. 1) (14). This structure indicates that EF-Ts 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$_{nm}$ binds to EF-Tu more tightly than does *E. coli* EF-Ts. This work attempts to determine the regions of the EF-Ts$_{nm}$ giving rise to its higher affinity for EF-Tu.
**EF-Tu**

**EF-Ts**

**N-Terminal**

**Subdomain N**

**Subdomain C**

**Dimerization**

**h1-h3**

**h4-h7**

**s1-s3**

**s4-s6**

**h8, h12**

**EF-Ts mt**

**FIG. 1. Three-dimensional representation of the structure of E. coli EF-Tu.** 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 with the N-terminal domain (h1–h3) at the top. Coordinates were kindly provided by Dr. T. Kawashima and are displayed using RASWIN.

**FIG. 2. General strategy for making chimeras between two proteins without the need to introduce new restriction sites.** A PCR fragment derived from the gene for the first protein (gene 1) is first generated using a normal 5' primer and a chimeric 3' primer. This 3' 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. This PCR reaction was performed with HindIII and XhoI and used to replace the HindIII to XhoI fragment of EF-Ts mt gene. The DNA fragment from this amplification reaction was purified by gel electrophoresis and cloned into the E. coli plasmid pET24C(+).

**MATERIALS AND METHODS**

**Construction of Deletion Mutants of EF-Ts**—Clones encoding His-tagged variants of E. coli EF-Ts and EF-Ts mt 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 by polymerase chain reaction (PCR) using primer ETsP4 (cgggatcccatatgcgtaagaccaaagaaggt) and C-ends (ccgctcgagagactgcttggacatcgcagc) and ETsD (cgggatcccatatgcgtaagaccaaagaaggt). The PCR fragment was digested with NdeI and XhoI and cloned into NdeI- and XhoI-digested pET24C(+), giving the construct pETsAN. To make a mutated form of EF-Ts mt lacking the N-terminal domain (residues 1 to 56), a fragment of EF-Ts mt gene containing the sequence encoding amino acid residues 57–283 was amplified by PCR using primer BMN-CN (cgggatccgatagacgatgctgctgacggc) and C-ends (cgggatcccatatgcgtaagaccaaagaaggt). The PCR fragment was digested with NdeI and XhoI and cloned into NdeI- and XhoI-digested pET24C(+), giving the construct pETsDAN.

**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 mt gene at nucleotide sequence position 342 by site-directed mutagenesis using the Chameleon™ Double-Stranded, Site-Directed Mutagenesis Kit (Stratagene). Primer MP15 (cttcctcccatgtacatatatgcaagtgaagc) 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 mt 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 (gggaagcttagctgctgacggc) and ETsP2 (cagctttact) was used for the mutagenesis. The PCR fragment was digested with NdeI and XhoI and cloned into NdeI- and XhoI-digested pET24C(+), giving the construct pETsDAN.

Chimeras III and IV were prepared using chimeric primers and two rounds of PCR. This strategy, which is outlined in Fig. 2, permits the preparation of chimeric proteins at any position without the need to introduce new restriction sites. To make Chimera III, a portion of EF-Ts mt gene from the N terminus to amino acid residue 162 in the mature form of this factor (9) was amplified by PCR using primer Vec-2 (taggggaattgagcggataac), derived from the pET24C(+) vector, and primer MP25 (taggggaattgagcggataac), derived from E. coli EF-Ts mt gene. This primer is in itself a chimeric piece of DNA. The sequence shown in boldface is derived from the EF-Ts mt gene, while the underlined sequence is from the E. coli EF-Ts gene. DNA was amplified from 100 ng of plasmid DNA carrying the EF-Ts mt gene in a 100-μl reaction mixture containing 100 pmol of each primer and 2.5 units of Taq polymerase. PCR was carried out for 20 cycles (94 °C for 1 min, 55 °C for 2 min, and 72 °C for 2 min).
FIG. 3. Stimulation of the activities of E. coli and mitochondrial EF-Tu by E. coli Ts and EF-Tsmt. In A, the stimulation of GDP exchange with E. coli EF-Tu was 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 exchanged 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. B, stimulation of the activity of E. coli EF-Tu in poly(U)-directed polymerization by E. coli EF-Ts (EF-TsEco) and EF-Tsmt. Reaction mixtures contained 1 pmol of expressed E. coli EF-Tu and the indicated amount of EF-Ts. Blanks representing the amount of polymerization catalyzed by EF-Tu alone (~7 pmol) have been subtracted from each value. C, stimulation of the activity of EF-Tsmt in polymerization by E. coli EF-Ts (EF-TsEco) and EF-Tsmt. Reaction mixtures contained 3 pmol of expressed EF-Tsmt and the indicated amount of EF-Ts. Blanks representing the amount of polymerization obtained with EF-Tu alone (~2.3 pmol) have been subtracted from each value.

EF-Tu · GDP + EF-Ts ⇄ EF-Tu · Ts + GDP (Eq. 1)

\[ K_{obs} = \frac{[EF-Tu \cdot Ts][GDP]}{[EF-Tu \cdot GDP][EF-Ts]} \] (Eq. 2)

\[ K_{obs} \] was calculated in each case and used to determine the binding constant (\( K_{obs} \)) for the reaction

\[ EF-Tu + EF-Ts ⇄ EF-Tu · Ts \] (Eq. 2)

using the relationship

\[ K_{obs} = K_{GDP} \cdot K_{Ts} \] (Eq. 3)

\( K_{GDP} \) is the binding constant of E. coli EF-Tu to GDP (3.3 × 10^8 M^-1) (19). The E. coli EF-Tu used for these experiments was ∼50% active based on its ability to bind GDP. EF-Ts was estimated to be fully active based on the percentage of EF-Tsmt that could bind EF-Tu.

RESULTS

Interaction of E. coli and Mitochondrial EF-Ts with E. coli and Mitochondrial EF-Tu—When mitochondrial EF-Tsmt is expressed in E. coli as a His-tagged protein, it forms a 1:1 complex with E. coli EF-Tu (9). Free EF-Tsmt can be purified by denaturing this complex and then allowing renaturation of the EF-Tsmt (10). E. coli EF-Ts has also been overexpressed in E. coli and purified as a His-tagged protein (15). When no GDP is added to the isolation buffers, a small amount of E. coli EF-Tu co-purifies with the E. coli EF-Ts following chromatography on nickel-nitritolactic acid resin. E. coli EF-Ts was purified by using buffers containing GDP and MgCl2.

The abilities of EF-Tsmt and E. coli EF-Ts 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 (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 polymeriza-
tion with E. coli EF-Tu is ~25% of that obtained with E. coli EF-Ts (Fig. 3B).

EF-Tsmt binds to EF-Tu in poly(U)-directed polymerization (Fig. 3, B and C). 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 homologous complex EF-TuEc-Tsmt is 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 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.”

\[ E. coli \text{EF-Tu} \cdot \text{GDP} + \text{EF-Tsmt} \leftrightarrow \text{EF-TuEco} \cdot \text{Tsmt} + \text{GDP} \quad (\text{Eq. 4}) \]

The binding constant (K_{b}) 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 \times 10^8 M^{-1}) (Table I) as described by Miller and Weissbach (19). The value obtained (8.6 \times 10^{10}) indicates that EF-Tsmt binds to E. coli EF-Tu quite tightly. The K_{b} for E. coli EF-Ts was also measured, and the corresponding binding constants (K_{p}) was calculated (Table I). The K_{p} of E. coli EF-Ts determined here (9 \times 10^{8} M^{-1}) is quite similar to the value of 5 \times 10^{8} M^{-1} obtained from the literature (19). Comparison the K_{p} of E. coli EF-Ts with the K_{b} 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-TuTs complex (14) shows 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 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-TuTs 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. However, it is clear that guanine nucleotides cannot dissociate the EF-TuTsmt complex (6). Thus, it is likely that EF-Tsmt will

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**Table I**

<table>
<thead>
<tr>
<th>EF-Ts used</th>
<th>K_{obs} (M)</th>
<th>K_{b} (M^{-1})</th>
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<tbody>
<tr>
<td>E. coli EF-Ts</td>
<td>2.7 ± 0.4</td>
<td>8.9 ± 1.3 \times 10^{8}</td>
</tr>
<tr>
<td>EF-Tsmt</td>
<td>260 ± 29</td>
<td>8.6 ± 1.0 \times 10^{10}</td>
</tr>
<tr>
<td>Chimera I</td>
<td>6.8 ± 1.9</td>
<td>2.2 ± 0.6 \times 10^{9}</td>
</tr>
<tr>
<td>Chimera II</td>
<td>59 ± 0.2</td>
<td>1.9 ± 0.2 \times 10^{10}</td>
</tr>
<tr>
<td>Chimera III</td>
<td>308 ± 62</td>
<td>1.0 ± 0.2 \times 10^{11}</td>
</tr>
<tr>
<td>Chimera V</td>
<td>67 ± 12</td>
<td>2.2 ± 0.4 \times 10^{10}</td>
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also have a high affinity for EF-Tu mt. To help determine which region(s) might be important for the strong interaction of EF-Ts mt with EF-Tu, an analysis of the possible structure of EF-Ts mt was carried out using the 3-dimensional structure of E. coli EF-Ts and several secondary structure prediction programs as a guide (Fig. 4). The overall lengths of E. coli EF-Ts and the mature form of EF-Ts mt are the same (9). However, the two factors appear to have several significant differences in their overall structure. EF-Ts mt aligns well with E. coli EF-Ts in the N-terminal domain, and this region of the protein most likely folds into 3 helices as observed for the E. coli factor. The first 2 strands of β-sheet in Subdomain N and helices h4 and h5 are also predicted to be present in EF-Ts mt. However, Subdomain N is interrupted by an insertion of ~20 amino acids. The precise position of this insertion is difficult to predict since the two proteins show very little primary sequence homology in this region making the alignment difficult. The remainder of Subdomain N including helices h6 and h7 and the final strand of the β-sheet (s3) are all predicted to be present. Subdomain C is predicted to begin with β-strand s4 which is followed by an insertion of ~12 residues before strands s5 and s6 and helices h8 and h12 are found. The dimerization domain (helices h9, h10, and h11) which is involved in contacts between two EF-Ts molecules in the crystal structure of the EF-TuTs complex is largely missing from EF-Ts mt. Only portions of what may be helix h10 appear to be present. Finally, the C-terminal module (h13) present in E. coli EF-Ts is missing in EF-Ts mt.

The secondary structural analysis described above provided a good tool for the analysis of what regions of EF-Ts mt are important for the tight binding to EF-Tu observed with EF-Ts mt. Based on this analysis, a series of chimeric proteins consisting of portions of E. coli EF-Ts and EF-Ts mt were constructed (Fig. 5). Chimeras were first constructed by exchanging the N-terminal domains (h1 through h3) of E. coli EF-Ts and EF-Ts mt (Fig. 5, Chimeras I and II). These two chimeric proteins were expressed in E. coli as His-tagged proteins and purified by chromatography on nickel-nitrilotriacetic acid columns. In addition, His-tagged forms of EF-Ts mt and E. coli EF-Ts were also expressed and purified. All of these proteins were initially purified from cell extracts in buffers without GDP but containing MgCl2. The purified proteins were analyzed by SDS-PAGE followed by Coomassie Blue staining (Fig. 6).

Under these conditions, EF-Ts mt is purified as a 1:1 complex with E. coli EF-Tu (Fig. 6, lane 1). In contrast, E. coli EF-Ts binds to E. coli EF-Tu much less tightly, and only ~1 mol of EF-Tu is present for every 20 mol of E. coli EF-Ts (Fig. 6, lane 2). The weaker binding of E. coli EF-Ts prevents it from competing effectively for EF-Tu with the guanine nucleotides and aminoacyl-tRNA present in the extract. Chimera I which carries the N-terminal domain of EF-Ts mt binds E. coli EF-Tu ~2–3-fold better than E. coli EF-Ts (Fig. 6, lane 3). It is isolated with a higher ratio of EF-Tu present in the preparations (~1:10) than observed with E. coli EF-Ts. This observation suggests that the N-terminal domain has a small effect on increasing the affinity of EF-Ts for EF-Tu. Chimera II binds to E. coli EF-Tu much more tightly than does E. coli EF-Ts but it is somewhat less tightly than EF-Ts mt (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-Ts mt and Chimera II were prepared by the denaturation of the EF-TuEcoTs mt complex followed by renaturation of the EF-Ts mt (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-Ts mt (Chimera I) increases the binding constant for EF-Tu ~2–3-fold. This observation suggests that this region of EF-Ts mt 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-Ts mt with that of E. coli EF-Ts decreases the binding constant of EF-Ts mt to E. coli EF-Tu ~4–5-fold. Chimera II which is predominantly derived from EF-Ts mt 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-Ts mt is governed primarily by sequences from h4 to the C terminus with a small contribution from sequences in the N-terminal domain.

To localize the region giving EF-Ts mt its stronger affinity for EF-Tu more closely, two more chimeras were prepared (Fig. 5, Chimeras III and IV). The preparation of these chimeras was based on the observation that the N-terminal half (the N-terminal domain and Subdomain N, h1 through s3) and the C-terminal half (from s4 to the C terminus) of E. coli EF-Ts fold somewhat independently in the crystal structure of the E. coli EF-TuTs complex (Fig. 1). However, the three-stranded β-sheet structure in Subdomain N forms an interface with the three-stranded sheet in Subdomain C (Fig. 1) and these chime-
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-Tsmt in polymerization by the chimeras. A, reaction mixtures contained 3 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-Tsmt and the indicated amount of EF-Ts. Blanks representing the amount of polymerization catalyzed by EF-Tsmt 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 NH2-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 s3, 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 co-purifies 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. In contrast, EF-Tsmt 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 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. Chimera II, in which the N-terminal domain of E. coli EF-Ts has replaced that in EF-Tsmt, 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-Ts

The activity of Chimera III in stimulating GDP exchange is about the same as that observed with EF-Ts

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-Ts

This assay is probably somewhat similar to that of EF-Ts

*E. coli* EF-Tu (14). Some of these residues are conserved in EF-Ts

while others are primarily conservative replacements. These latter residues in EF-Ts

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-Ts

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-Ts

contributes to the stronger interaction observed between EF-Ts

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-Ts

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-Ts

do not contribute to the stronger interaction of EF-Ts

with *E. coli* EF-Tu. However, as indicated in Fig. 4, Subdomain N of the core of EF-Ts

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-Ts

and EF-Tu.

**Decreased Tightness of Binding between EF-Ts

Increases Its Ability to Stimulate the Activity of EF-Tu**—Although EF-

Ts

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-

Ts

The reaction of *E. coli* EF-Ts in stimulating guanine nucleotide exchange can be described as

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

*E. coli* EF-Ts is recycled by the dissociation of the EF-Tu-Ts complex by GDP or GTP. When EF-Ts

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-Ts

in the reaction low. The small turnover number may explain the low activity of EF-Ts

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 a somewhat higher relative activity of EF-Ts

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-Ts

**—Despite the 56% identity in sequence between *E. coli* EF-Tu and EF-Ts

*E. coli* EF-Ts is unable to stimulate the activity of EF-Ts

in polymerization. This result suggests that *E. coli* EF-Ts does not interact very well with EF-Tu

or that the interaction occurring fails to result in effective nucleotide exchange. Unfortunately, the poor binding of guanine nucleotides to EF-Tu

does not allow a direct measure of the exchange reaction. Since Chimera II is able to stimulate the activity of EF-Tu

in polymerization, 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-Ts

to *E. coli* EF-Tu—The data presented here show that EF-Tu

binds to *E. coli* EF-Tu

more tightly than *E. coli* EF-Ts. Analysis of the chimeric proteins indicates that sequences 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-Ts

while others are primarily conservative replacements. These
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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