Mutagenesis of Glutamine 290 in *Escherichia coli* and Mitochondrial Elongation Factor Tu Affects Interactions with Mitochondrial Aminoacyl-tRNAs and GTPase Activity†

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ABSTRACT: Elongation factor Tu (EF-Tu) is responsible for the delivery of the aminoacyl-tRNAs (aa-tRNA) to the ribosome during protein synthesis. The primary sequence of domain II of EF-Tu is highly conserved. However, several residues thought to be important for aa-tRNA binding in this domain are not conserved between the mammalian mitochondrial and bacterial factors. One of these residues is located at position 290 (*Escherichia coli* numbering). Residue 290 is Gln in most of the prokaryotic factors but is conserved as Leu (L338) in the mammalian mitochondrial factors. This residue is in a loop contacting the switch II region of domain I in the GTP-bound structure. It also helps to form the binding pocket for the 5′ end of the aa-tRNA in the ternary complex. In the present work, Leu338 was mutated to Gln (L338Q) in EF-Tu_{mt}. The complementary mutation was created at the equivalent position in *E. coli* EF-Tu (Q290L). EF-Tu_{mt} L338Q functions as effectively as wild-type EF-Tu_{mt} in poly(U)-directed polymerization with both prokaryotic and mitochondrial substrates and in ternary complex formation assays with *E. coli* aa-tRNA. However, the L338Q mitochondrial variant has a reduced affinity for mitochondrial Phe-$tRNA^{Phe}$. *E. coli* EF-Tu Q290L is more active in poly(U)-directed polymerization with both mitochondrial and prokaryotic substrates and has a higher GTPase activity in both the absence and presence of ribosomes. Surprisingly, while *E. coli* EF-Tu Q290L is more active in polymerization with mitochondrial Phe-$tRNA^{Phe}$, this variant has low activity in the formation of a stable ternary complex with mitochondrial aa-tRNA.

Elongation factor Tu (EF-Tu)[1] is a guanine nucleotide-binding protein responsible for the delivery of aminoacyl-tRNA (aa-tRNA) to the ribosome during the elongation phase of protein synthesis. EF-Tu is inactive in its GDP-bound conformation. Elongation factor Ts (EF-Ts), a guanine nucleotide exchange factor, catalyzes the exchange of GDP for GTP on EF-Tu, promoting the formation of the active EF-Tu·GTP complex. EF-Tu·GTP binds aa-tRNA and delivers it to the A-site of mRNA-programmed ribosomes. Upon positive codon−anticodon interactions, GTP is hydrolyzed to GDP and EF-Tu·GDP is released, allowing the cycle to continue (*1*).

The primary sequence of EF-Tu is highly conserved throughout nature, and mammalian mitochondrial EF-Tu (EF-Tu_{mt}) is almost 60% identical to *Escherichia coli* EF-Tu (*2*). The crystal structures of EF-Tu_{mt} and *E. coli* EF-Tu show that the secondary and tertiary structures of the two factors are very similar (*3*, *4*). Previous studies have shown that the prokaryotic and mammalian mitochondrial factors function in similar manners in their respective translational systems. However, the binding constants that control the interactions between EF-Tu and its ligands differ considerably between *E. coli* EF-Tu and EF-Tu_{mt} (*5*, *6*).

The three-dimensional structures of *E. coli* EF-Tu and EF-Tu_{mt} can almost be superimposed upon each other. In contrast, the structures of their corresponding tRNAs are significantly different. Mitochondrial aa-tRNAs lack many of the invariant and semiinvariant nucleotides that stabilize the tertiary structures of other tRNAs (*7*). In addition, mitochondrial tRNAs have a higher A-U content than other tRNAs and frequently do not have the G-C-rich stem regions that stabilize most canonical tRNAs (*8*). The tertiary structures of mitochondrial tRNAs are predicted to be weaker than those of prokaryotic tRNAs, and in some cases, the mitochondrial tRNAs may form more of a boomerang-shape than the classic "L" shape (*9*). Both *E. coli* EF-Tu and EF-Tu_{mt} are able to form ternary complexes with mitochondrial aa-tRNAs. However, *E. coli* EF-Tu is less effective in delivering mitochondrial aa-tRNAs to the A-site of ribosomes than is EF-Tu_{mt} (*10*).

EF-Tu is composed of three domains. Domain I (residues 1−199, *E. coli* numbering) is the guanine nucleotide-binding domain. Domain II is composed of residues 209−299 and, along with domain I, forms the binding site for the amino acid and the 3′ end of the aa-tRNA (*1*, *4*). Domain III, residues 300−393, interacts with the extended acceptor-TΨC

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[1] Abbreviations: aa-tRNA, aminoacyl-tRNA; EF-Tu, elongation factor Tu (also referred to as EF1a); EF-Tu_{mt}, mitochondrial EF-Tu; EF-Ts, elongation factor Ts (also referred to as EF1b); IPTG, isopropyl thiogalactoside; GDPNP, guanosine 5′-(β,γ-imido)triphosphate.

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helix of the aa-tRNA. A portion of the 3′ acceptor stem and the 5′ end of the aa-tRNA are located in a pocket formed by all three domains of EF-Tu (*1*, *11*−*13*).

Most of the residues in EF-Tu that form the binding site for the aa-tRNA are highly conserved. However, there are several residues that are not conserved between the prokaryotic and mitochondrial factors. These residues may influence the interactions between EF-Tu_{mt} and its ligands and may also be essential for the delivery of mitochondrial aa-tRNA to the ribosome. One such residue, Leu338 in EF-Tu_{mt}, is present as Gln290 in *E. coli* EF-Tu. This residue is located in a region involved in aa-tRNA binding (*12*, *13*) and in the conformational changes occurring in EF-Tu upon GTP-binding (*14*, *15*). The current work investigates the effects of mutating Leu338 in EF-Tu_{mt} to Gln and of converting Gln290 in *E. coli* EF-Tu to Leu on the biological activities of these factors.

MATERIALS AND METHODS

Materials. Synthetic oligonucleotides were purchased from the Lineberger Comprehensive Cancer Research Center at the University of North Carolina at Chapel Hill. All enzymes used for cloning were obtained from New England Biolabs except Pfu high-fidelity polymerase, which was purchased from Stratagene. Radioactive materials were obtained from Perkin-Elmer Life Sciences Inc. Crude *E. coli* tRNA was from Boehringer Mannheim, and [^{14}C]Phe-$tRNA^{Phe}$ was prepared as described (*16*). Superase·In RNase inhibitor was purchased from Ambion. ScintiVerse II aqueous scintillation cocktail was obtained from Fisher Scientific. PEI−cellulose plates were acquired from Selecto Scientific. *E. coli* ribosomes were prepared from *E. coli* W (*10*, *17*). Elongation factor G (EF-G) was purified from *E. coli* as described (*17*). *E. coli* and mitochondrial EF-Ts were expressed in *E. coli* as histidine-tagged proteins and purified on Ni-NTA resin (*18*,*19*). Mitochondrial EF-G (*17*, *20*) and crude mitochondrial ribosomes (*21*) were purified from bovine liver. Purified mitochondrial ribosomes were prepared from crude ribosomes as described (*22*). Bovine mitochondrial tRNA was isolated from purified bovine mitoplasts (mitochondria from which the outer membrane has been removed) using the Qiagen RNA/DNA maxi kit. Mitochondrial $tRNA^{Phe}$ was aminoacylated with [^{14}C]Phe using human mitochondrial Phe-tRNA synthetase (*23*).

Cloning E. coli EF-Tu Q290L and EF-Tu_{mt} L338Q. E. coli EF-Tu Q290L was cloned using a His-tagged variant of the gene encoding *E. coli* EF-Tu inserted into pET24c(+) as a template (*19*). Following the Stratagene QuikChange site-directed mutagenesis protocol, the mutation was introduced using the synthetic oligonucleotides 5′-cgaacgtggtctggtactggctaagcc-3′ and 5′-tagccagtaccagaccacgttcgatttcttcacg-3′ as forward and reverse primers, respectively. Similarly, the L338Q variant was cloned using the gene encoding EF-Tu_{mt} inserted into pET24c(+) as a template (*2*) with the forward and reverse oligonucleotides 5′-ctgagacgtggccaggtcatggccaagccaggt-3′ and 5′-ttggccatgacctggccacgtctcaggtcctcccg-3′, respectively. The plasmids were transformed into *E. coli* DH5α cells, and the sequences were verified at the Automated DNA Sequencing Facility at the University of North Carolina at Chapel Hill.

Protein Expression and Purification. The plasmids carrying the genes encoding *E. coli* EF-Tu Q290L and EF-Tu_{mt} L338Q were transformed *E. coli* BL21(DE3) cells for expression. The cells were used to inoculate 2 L of 2×YT media containing 50 μg/mL kanamycin. The cell cultures were grown to mid-log phase at 37 °C with agitation and induced with 50 μM IPTG for 4 h. Cells carrying the overexpressed proteins were collected by centrifugation and resuspended in 40 mL of lysis solution [50 mM Tris-HCl (pH 7.6), 40 mM KCl, 7 mM $MgCl_2$, 10% glycerol, 7 mM β-mercaptoethanol, 0.1 mM phenylmethanesulfonyl fluoride, 0.03% egg white lysozyme, 0.1% Triton X-100, and 5 μg/mL DNase I]. The cells were incubated on ice for 10 min and sonicated on ice for 9 min (1 s bursts with 4 s cooling) at an output of 60 W. Extracts were centrifuged at 27000*g* for 30 min at 4 °C followed by ultracentrifugation at 100000*g* for 1 h at 4 °C. The factors were purified on Ni-NTA−agarose using buffers containing 10 μM GDP as described (*2*). Each variant factor was expressed and purified alongside the respective wild-type factor for direct comparison. Protein concentrations were determined by the Bradford method using bovine serum albumin as a standard (*24*).

Percentage of Active Molecules. Filter binding assays were used to measure the percentage of variant and wild-type *E. coli* EF-Tu active in binding GDP (*17*). Reaction mixtures (120 μL) contained 8.3−83 nM EF-Tu (as determined by Bradford assays) and a saturating level (0.4 μM) of [^{3}H]GDP. The percentage of EF-Tu active in binding GDP was determined from the point at which the factor was saturated with GDP after correcting for the background (retention of radiolabeled GDP in a minus EF-Tu control, less than 0.1 pmol). The binding of EF-Tu_{mt} to GDP is too weak to be measured in a filter binding assay, and the percentage of EF-Tu_{mt} capable of interacting with GDP was not determined.

The percentage of molecules capable of binding *E. coli* [^{14}C]Phe-$tRNA^{Phe}$ was determined for each wild-type and variant factor as described (*6*). A portion of prokaryotic or mitochondrial EF-Tu (0.2 μM, as determined by Bradford assays) was incubated with saturating amounts (0.8−1.2 μM) of *E. coli* [^{14}C]Phe-$tRNA^{Phe}$ in 50 μL assays, which were analyzed as described below. The values determined for the percentage of EF-Tu active in ternary complex formation were corrected for the background (the retention of label precipitated in a minus EF-Tu control at each aa-tRNA concentration, about 0.4 pmol). Unless otherwise indicated, input values of EF-Tu used in all assays are corrected for the percentage of EF-Tu molecules active in ternary complex formation.

Ternary Complex Formation. The abilities of wild-type and variant EF-Tu to form complexes with [^{14}C]Phe-$tRNA^{Phe}$ were determined basically as described previously (*6*). The indicated amount of EF-Tu in a buffer containing 10 μM GDP was incubated for either 15 min or 2 h at 37 °C in a reaction mixture (40 μL) containing 62.5 mM Tris-HCl (pH 7.8), 1.25 mM dithiothreitol, 85 mM KCl, 8.4 mM $MgCl_2$, 3.1 mM phosphoenolpyruvate, 0.625 unit of pyruvate kinase, and 0.625 mM GTP. Following preincubation, 0.38 μM *E. coli* or 0.1 μM mitochondrial [^{14}C]Phe-$tRNA^{Phe}$ was added in 10 μL, and the reaction was incubated for 15 min at 0 °C. RNase A (10 μg) was added to digest the free Phe-tRNA, and the reaction was incubated for an additional 30 s at 0 °C. The reaction was terminated by the addition of cold 5% trichloroacetic acid, and the precipitate was collected on a nitrocellulose membrane following a 10 min incubation on

ice. The amount of [^{14}C]Phe-tRNA^{Phe} remaining was used to determine the abilities of the factors to protect the aa-tRNA from hydrolysis (*25*, *26*). The percentage of molecules capable of binding aa-tRNA was verified in each ternary complex assay as described above. A blank representing the amount of label retained on the filter in the absence of EF-Tu (about 0.2 pmol for *E. coli* [^{14}C]Phe-tRNA^{Phe} and less than 0.1 pmol for mitochondrial [^{14}C]Phe-tRNA^{Phe}) has been subtracted from each value.

In Vitro Translation. Poly(U)-directed polymerization of [^{14}C]Phe was used to assay the activities of wild-type and variant proteins as described (*10*, *17*, *20*). Assays (100 μL) using 0.22 μM *E. coli* ribosomes and 0.35 μM *E. coli* [^{14}C]Phe-tRNA^{Phe} were incubated for 30 min at 37 °C in a mixture containing 50 mM Tris-HCl (pH 7.8), 1 mM dithiothreitol, 0.1 mM spermine, 80 mM KCl, 6 mM $MgCl_2$, 2.5 mM phosphoenolpyruvate, 0.5 unit of pyruvate kinase, 0.5 mM GTP, 0.125 mg/mL poly(U), 50 units of Superase·In, 24 units of *E. coli* EF-G, and 0.5−10 nM EF-Tu$_{mt}$ in the presence EF-Ts$_{mt}$ at a 1:1 (EF-Tu$_{mt}$:EF-Ts$_{mt}$) molar ratio or 0.5−5 nM *E. coli* EF-Tu. Reactions were terminated by the addition of 5% trichloroacetic acid and then incubated at 90 °C for 10 min. The precipitate was collected on nitrocellulose filter membranes, and the amount of [^{14}C]Phe incorporated was quantified using a liquid scintillation counter. The amount of ^{14}C label obtained was corrected for the blank (the amount of radioactivity retained on the filter in a minus EF-Tu control, approximately 0.5 pmol). Data were reproducible within ±5%.

In vitro translation assays using mitochondrial substrates were carried out as described above except that reaction mixtures contained 50 mM Tris-HCl (pH 7.8), 1 mM dithiothreitol, 0.1 mM spermine, 40 mM KCl, 7.5 mM $MgCl_2$, 2.5 mM phosphoenolpyruvate, 0.5 unit of pyruvate kinase, 0.5 mM GTP, 0.125 mg/mL poly(U), 50 units of Superase·In, 23 units of mitochondrial EF-G, 0.1 μM mitochondrial [^{14}C]Phe-tRNA^{Phe}, 16 units of purified mitochondrial ribosomes (where one unit is defined as the amount of ribosomes required to polymerize 1 pmol of [^{14}C]Phe using *E. coli* Phe-tRNA^{Phe} in 30 min at 37 °C in a total reaction volume of 100 μL), and 7.5−15 nM *E. coli* EF-Tu or 5−15 nM EF-Tu$_{mt}$ in the presence of EF-Ts at a 1:1 (EF-Tu$_{mt}$:EF-Ts$_{mt}$) or 5:1 (*E. coli* EF-Tu:*E. coli* EF-Ts) molar ratio (*17*, *20*).

A-Site Binding. The ability of variant and wild-type EF-Tu to deliver *E. coli* [^{14}C]Phe-tRNA^{Phe} to the A-site of *E. coli* ribosomes was determined as follows (*10*). Reaction mixtures (100 μL) containing 50 mM Tris-HCl (pH 7.8), 1 mM dithiothreitol, 0.1 mM spermine, 80 mM KCl, 6 mM $MgCl_2$, 0.5 mM guanosine 5′-(β,γ-imido)triphosphate (GDPNP), 0.125 mg/mL poly(U), 50 units of Superase·In, 0.38 μM purified *E. coli* ribosomes, 25 μg (10 μM) of crude *E. coli* tRNA, 0.1 μM *E. coli* [^{14}C]Phe-tRNA^{Phe}, and 10−55 nM *E. coli* EF-Tu or EF-Tu$_{mt}$ were incubated for 5 min at 37 °C. The quantity of [^{14}C]Phe-tRNA^{Phe} bound to the ribosome was determined using a filter binding assay and measured with a liquid scintillation counter (*10*). In addition, A-site binding assays using 2−10 nM wild-type and variant *E. coli* EF-Tu were performed as above except 0.5 mM GTP was used instead of GDPNP. All values were corrected for the retention of [^{14}C]Phe-tRNA^{Phe} in a minus EF-Tu control (about 0.8 pmol). Data were reproducible within ±5%.

GTP Hydrolysis. E. coli EF-Tu and its Q290L variant were tested for their ability to promote GTP hydrolysis (*27*). Reaction mixtures (100 μL) containing 50 mM Tris-HCl (pH 7.8), 1 mM dithiothreitol, 0.1 mM spermine, 80 mM KCl, 6 mM $MgCl_2$, 0.515 mM [γ-^{32}P]GTP (200 cpm/pmol), 0.125 mg/mL poly(U), 50 units of Superase·In, 0.38 μM purified *E. coli* ribosomes, 25 μg of crude *E. coli* tRNA, 0.1 μM *E. coli* [^{14}C]Phe-tRNA^{Phe}, and 0.075−0.15 μM *E. coli* EF-Tu in the presence of *E. coli* EF-Ts at a 5:1 (EF-Tu:EF-Ts) molar ratio were incubated for 5 min at 37 °C. Following incubation, the reactions were placed at 0 °C, and a 90 μL aliquot of each reaction was removed and mixed with 90 μL of 1 M $HClO_4$ and 3 mM NaH_2PO_4. Following centrifugation for 5 min at 8160*g*, 160 μL of the supernatant was removed and vortexed for 10 s with 640 μL of 6% activated charcoal in 1 M HCl. The mixture was centrifuged for 15 min at 2040*g*, and 600 μL of the supernatant was filtered through a glass pipet fitted with glass wool. A portion of the filtrate (200 μL) was added to 15 mL of ScintiVerse II aqueous scintillation cocktail, and the amount of radiolabeled P_i liberated in each assay was measured with a liquid scintillation counter. The values reported have been back-calculated to represent the original reaction mixture. The GTPase activity of each factor was corrected for the background (the amount of radiolabel detected in a minus EF-Tu control, approximately 16 pmol).

The intrinsic GTPase activities of wild-type and variant *E. coli* EF-Tu were determined as described above except reaction mixtures (100 μL) contained 0.04−0.2 μM EF-Tu, 50 mM Tris-HCl (pH 7.8), 1 mM dithiothreitol, 60 mM KCl, 6.5 mM $MgCl_2$, 0.515 mM [γ-^{32}P]GTP (about 200 cpm/pmol), 2.5 mM phosphoenolpyruvate, and 0.5 unit of pyruvate kinase. Alternatively, following the 5 min incubation at 37 °C, a portion (2 μL) of the reaction mixture was analyzed by thin-layer chromatography on PEI−cellulose in 0.75 M KH_2PO_4 (pH 3.5) containing 4 M urea, and the GTPase activities of EF-Tu were quantified using a phosphorimager. Assays that were analyzed by TLC used [γ-^{32}P]GTP at 10000−15000 cpm/pmol. The remainder of the sample in these assays was analyzed by charcoal extraction (see above) for comparison. The amount of radiolabeled γ-phosphate detected in each assay was corrected for the amount of radiolabel present in a minus EF-Tu control (about 20 pmol). To verify that a contaminating phosphatase activity was not responsible for the observed hydrolysis, ATP was added to the GTPase reaction at a concentration 100-fold greater than that of GTP, and reactions were supplemented with additional $MgCl_2$ as described (*28*). The presence of ATP did not significantly reduce the level of radiolabeled P_i, indicating that there was no contaminating phosphatase.

RESULTS

Analysis of the Important Residues in Domain II of EF-Tu. The amino acid sequence in domain II of EF-Tu is highly conserved (Figure 1). However, there are several potentially important residues that are not conserved when the prokaryotic and mitochondrial factors are compared. One interesting residue is Gln290 (*E. coli* numbering) found in most of the prokaryotic factors. This residue is conserved as Leu338 (full-length bovine EF-Tu$_{mt}$ numbering) in the mammalian mitochondrial factors. Residue 290 is occasion-

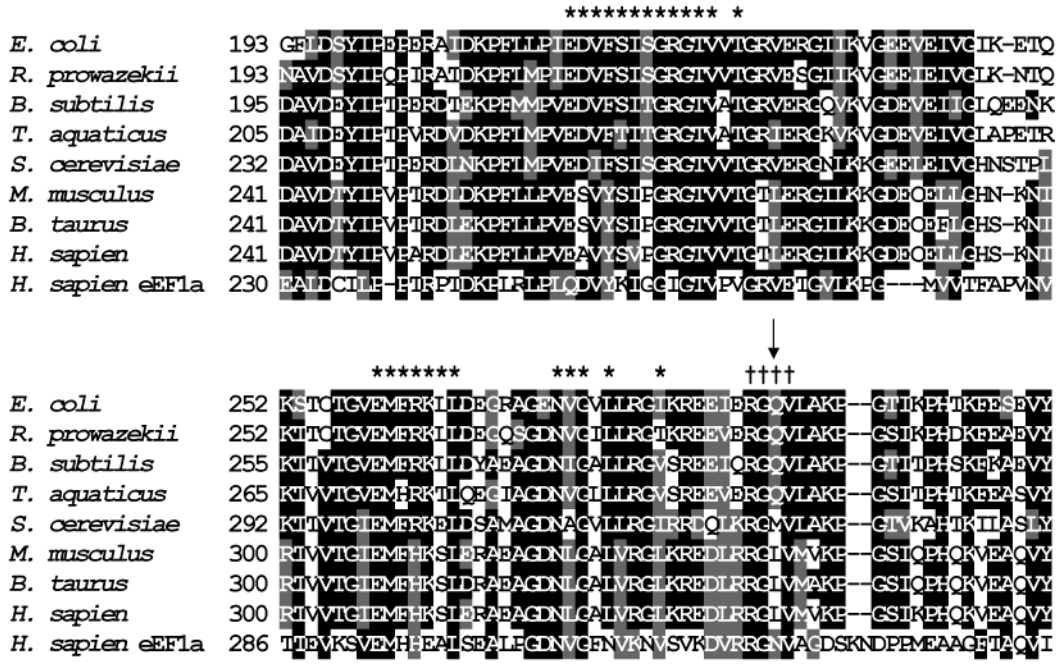

FIGURE 1: Primary sequence alignment of domain II of EF-Tu and eEF1A. The arrow indicates the residue under study. The * symbols specify residues involved in binding the 3′ acceptor stem region of the aa-tRNA. The † symbols identify residues involved in binding the 5′ end of the aa-tRNA. The accession numbers for each factor are as follows: *Escherichia coli* EF-Tu (*E. coli*), P02290; *Rickettsia prowazekii* EF-Tu (*R. prowazekii*), AJ235272; *Bacillus subtilis* EF-Tu (*B. subtilis*), P33166; *Thermus aquaticus* EF-Tu (*T. aquaticus*), S29293; *Saccharomyces cerevisiae* mitochondrial EF-Tu (*S. cerevisiae*), K00428; *Mus musculus* EF-Tu_{mt} (*M. musculus*), XM_133763; *Bos bovis* EF-Tu_{mt} (*B. bovis*), P49410; *Homo sapiens* EF-Tu_{mt} (*H. sapien*), BC010041; *Homo sapiens* cytoplasmic eEF1A (*H. sapien* eEF1a), P04720.

ally a Met in prokaryotic EF-Tu and in the EF-Tu_{mt} from the lower eukaryotes including yeast, *Caenorhabditis elegans*, and *Drosophila melanogaster*.

The only three-dimensional structure of EF-Tu_{mt} available to date is that of the bovine EF-Tu_{mt}·GDP complex (*3*). Overall, this structure is quite similar to that of the prokaryotic factors. Topology-based structural comparisons of domain II of EF-Tu_{mt} and *E. coli* EF-Tu complexed with GDP indicate an 88% match between the two factors with the root-mean-square deviation of 0.82 Å for all α-carbons in domain II (*3*, *4*, *29*). The backbone of domain II remains unchanged in the GDP, GDPNP, and ternary complex structures of *Thermus aquaticus* EF-Tu (the only factor for which all three structures have been solved) (*12*, *13*, *15*, *30*). Thus, it is reasonable to use the structure of the EF-Tu_{mt}·GDP complex as a model for the conformation of domain II in the GDPNP and ternary complexes of this factor.

In the crystal structures of the ternary complexes of *T. aquaticus* EF-Tu, the residue equivalent to Gln290 (Q302) is in a loop region of domain II that helps to form the binding pocket for the 5′ end of the aa-tRNA (*12*, *13*). Arg288 in this loop forms a salt bridge with the 5′-phosphate of the aa-tRNA in the ternary complex. This loop is tethered to the switch II region of domain I in the prokaryotic EF-Tu·GDPNP structure through interactions between Arg288 and Asn90, which in turn forms a H-bond to Ile62 in switch I (*14*, *15*). Thus, this loop is important in the interactions of the switch I and II regions of domain I with domain II and helps to connect domains II and III, acting as a unit, with domain I.

The loop in which residue 290 is located is partially embedded in domain II and located near the interface between domains II and III (*3*, *4*, *12*, *13*). The regions surrounding Gln290 in *E. coli* EF-Tu and the corresponding Leu338 in EF-Tu_{mt} have several interesting differences arising in part from the different properties of Gln and Leu (Figure 2A,B) (*3*, *4*). In *E. coli* EF-Tu·GDP, the ϵ^1-oxygen of Gln290 participates in a H-bond with the backbone nitrogen of Glu287 while the amide nitrogen atom of Gln290 interacts with the carbonyl oxygen of Glu287 (Figure 2A). The carbonyl oxygen of Gln290 is stabilized by a H-bond to the amide nitrogen atom of Ile214 and the ϵ^2-nitrogen atom of Gln290 can form a H-bond with the carbonyl oxygen of Gly246 or to water.

As in *E. coli* EF-Tu, the amide nitrogen and carbonyl oxygen atoms of Leu338 in EF-Tu_{mt} interact with the carbonyl oxygen of Arg335 (the equivalent of Glu287 in *E. coli* EF-Tu) and the amide nitrogen of Val262 (*E. coli* EF-Tu Ile214), respectively (Figure 2B). However, Leu338 cannot form a H-bond to the backbone nitrogen of Arg335. Furthermore, there is no interaction between Leu338 and Gly294 (Gly246 in *E. coli* EF-Tu). Instead, Gly294 has two possible binding partners, Lys297 and Ser296 (*4*).

The lack of the interaction between Leu338 and Gly294 creates an alteration in the spatial orientation of several residues in EF-Tu_{mt} compared to those of *E. coli* EF-Tu. To illustrate the effects of these differences, the tertiary structure of EF-Tu_{mt}·GDP was superimposed onto that of *E. coli* EF-Tu·GDP (*3*, *4*, *29*), and the loop containing Leu338 from EF-Tu_{mt} (residues 327−340) was incorporated into the structure of *E. coli* EF-Tu. As illustrated in Figure 2C, Leu338 lies farther away from domain III than does Gln290 of *E. coli* EF-Tu. Thus, this residue may play a role in the correct formation of the interface between domains II and III. The L338Q mutation in EF-Tu_{mt} and the complementary Q290L mutation in *E. coli* EF-Tu were created to explore the potential role of the properties of these residues in the biological activities of EF-Tu.

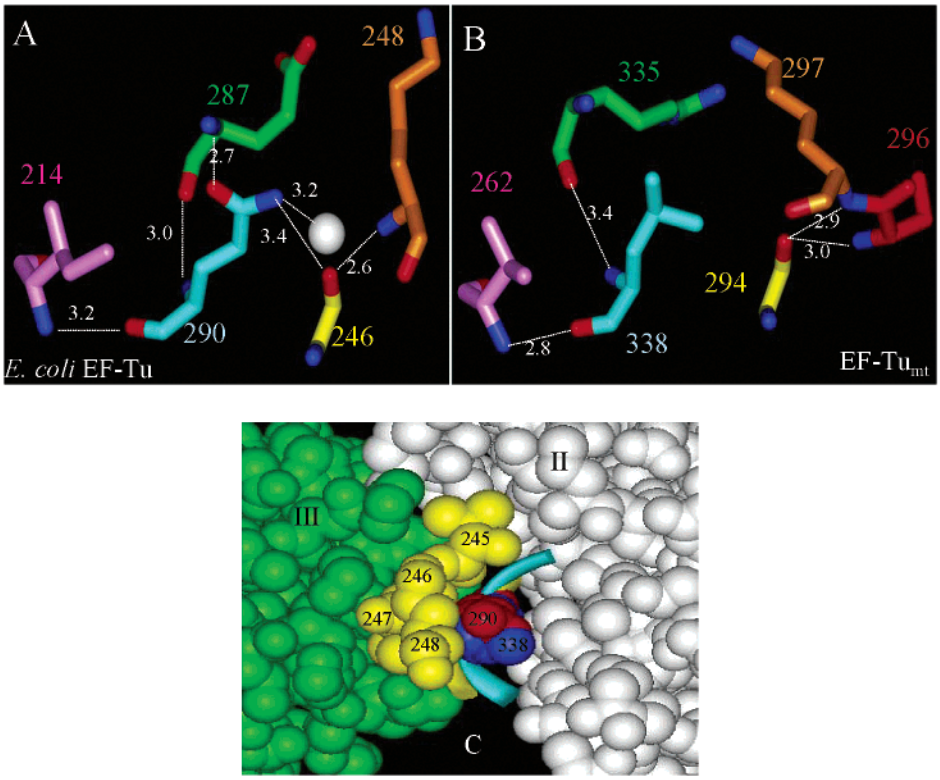

FIGURE 2: Interactions of residue 290 of *E. coli* EF-Tu and the equivalent residue 338 of EF-Tu$_{mt}$ with surrounding residues. Using the Insight II molecular modeling package (http://www.accelrys.com), the interactions between residue 290 (*E. coli* EF-Tu numbering) and the surrounding residues are displayed in both *E. coli* EF-Tu and EF-Tu$_{mt}$. The equivalent residues of *E. coli* EF-Tu and EF-Tu$_{mt}$ have the same colors. Nitrogen atoms are in blue and oxygen atoms in red. The potential H-bonds between atoms in each residue are characterized by dotted lines with distances indicated in angstroms. The coordinates for the *E. coli* EF-Tu and EF-Tu$_{mt}$ structures are given in PDB accession numbers 1EFC and 1D2E, respectively. (A) Gln290 is in cyan; Glu287 is in green; Gly246 is in yellow; Lys248 is in orange; Ile214 is in purple; the water molecule is in white. (B) Leu338 is in cyan; Arg335 is in green; Gly294 is in yellow; Lys297 is in orange; Val262 is in purple; Ser296 is in red. (C) Using the topological comparison program (TOP), the GDP-bound structure of *E. coli* EF-Tu was superimposed onto that of EF-Tu$_{mt}$ (*29*). Following the superposition, the PDB coordinates of the loop region surrounding Leu338 (residues 327−340) of EF-Tu$_{mt}$·GDP were spliced into the coordinates of *E. coli* EF-Tu and viewed using Insight II. The resulting structure of *E. coli* EF-Tu·GDP contains Gln290 as well as the equivalent residue Leu338 and the surrounding residues 327−340 of EF-Tu$_{mt}$. Depicted in panel C are Leu338 of EF-Tu$_{mt}$ in blue, residues 337 and 339 of EF-Tu$_{mt}$ in cyan (ribbon), Gln290 of *E. coli* EF-Tu in red, residues 289 and 291 of *E. coli* EF-Tu in yellow (ribbon), residues 245−248 of *E. coli* EF-Tu in yellow (space-filling model), a portion of domain II of *E. coli* EF-Tu in white, and a portion of domain III of *E. coli* EF-Tu in green.

Activities of EF-Tu$_{mt}$ L338Q and E. coli EF-Tu Q290L. Site-directed mutagenesis was used to prepare the L338Q variant of EF-Tu$_{mt}$ and the Q290L variant of *E. coli* EF-Tu. The variants were expressed in *E. coli* as His-tagged proteins, overproduced in soluble form, and purified to near homogeneity (data not shown). Both EF-Tu$_{mt}$ L338Q and *E. coli* EF-Tu Q290L are expressed at about 50% of the levels observed with their corresponding wild-type factors (data not shown).

An assessment of the effect of a mutation on the activities of EF-Tu requires information on the fraction of the expressed protein that is active since certain mutations can exert their effects primarily by affecting the percentage of the molecules that fold into an active conformation (*31*, *32*). Previous studies have shown that the concentration of molecules determined by Bradford assays is not indicative of the amount of EF-Tu that is able to interact with aa-tRNA (*6*). Typically, the percentage of wild-type EF-Tu$_{mt}$ active in ternary complex formation varies between 30% and 45% (*6*) while that of *E. coli* EF-Tu is 25−35% (*33*). Therefore, the percentage of each EF-Tu variant capable of forming ternary complexes was determined by examining the amount of ternary complex formed at saturating concentrations of *E. coli* Phe-tRNA^{Phe}. The percentage of EF-Tu$_{mt}$ L338Q active in ternary complex formation (40%) is similar to that of wild-type EF-Tu$_{mt}$ (45%; data not shown). In contrast, *E. coli* EF-Tu Q290L has only about 15% of the number of active molecules in ternary complex formation relative to that observed with wild-type *E. coli* EF-Tu (4.2% versus 30% for the wild-type factor; data not shown).

To examine whether the low percentage of active molecules in the *E. coli* EF-Tu Q290L preparations is also reflected in its ability to bind guanine nucleotides, the percentage of molecules active in binding [^{3}H]GDP was assayed for wild-type *E. coli* EF-Tu and the Q290L variant. As expected, approximately 85% of wild-type *E. coli* EF-Tu molecules are active in binding GDP (data not shown) (*33*). However, only about 13% of the *E. coli* EF-Tu Q290L is active in GDP binding (data not shown). Thus, overall *E. coli* EF-Tu Q290L has a low percentage of active molecules compared to the wild-type factor both in GDP binding and in ternary complex formation. These observations indicate that Gln290 in *E. coli* EF-Tu plays an important role in the ability of the factor to assume a biologically active conformation.

Poly(U)-Directed Polymerization with E. coli Phe-tRNA^{Phe}. The activities of EF-Tu$_{mt}$ L338Q and *E. coli* EF-Tu Q290L were tested in poly(U)-directed polymerization using *E. coli*

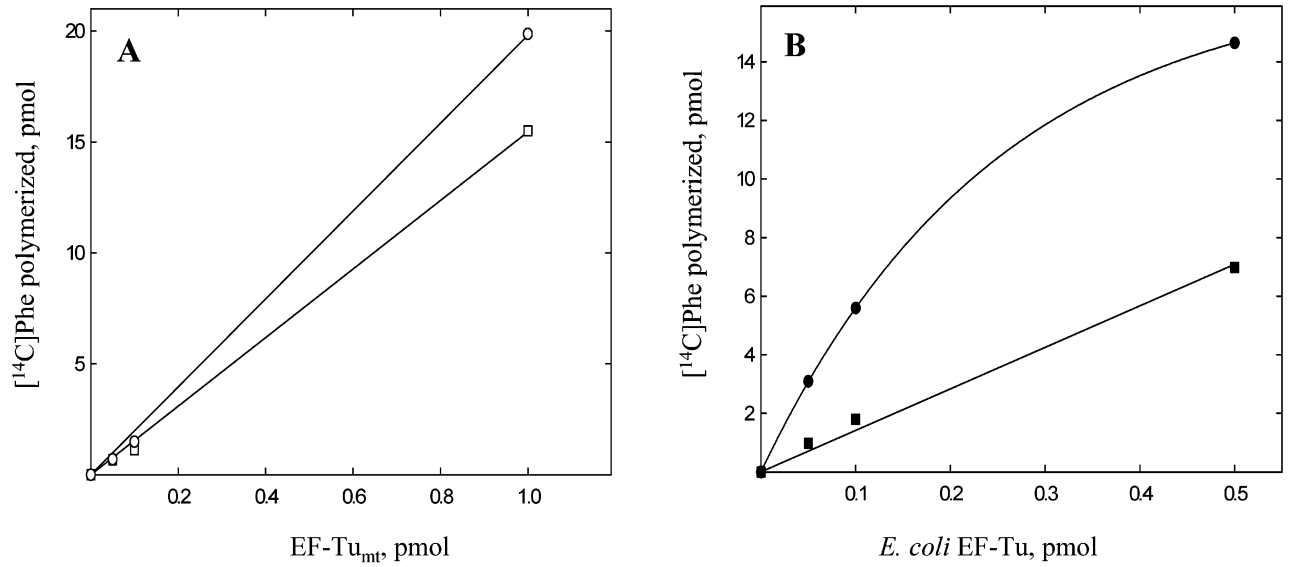

FIGURE 3: Poly(U)-directed polymerization with *E. coli* Phe-tRNA^{Phe} and *E. coli* ribosomes. Poly(U)-directed polymerization was used to assay the function of the wild-type and variant EF-Tu as described in Materials and Methods. Input values of EF-Tu were determined after correcting for the percentage of molecules active in ternary complex formation. (A) Wild-type EF-Tu$_{mt}$ (□) and EF-Tu$_{mt}$ L338Q (○) in the presence of mitochondrial EF-Ts. (B) Wild-type *E. coli* EF-Tu (■) and *E. coli* EF-Tu Q290L (●) in the absence of EF-Ts.

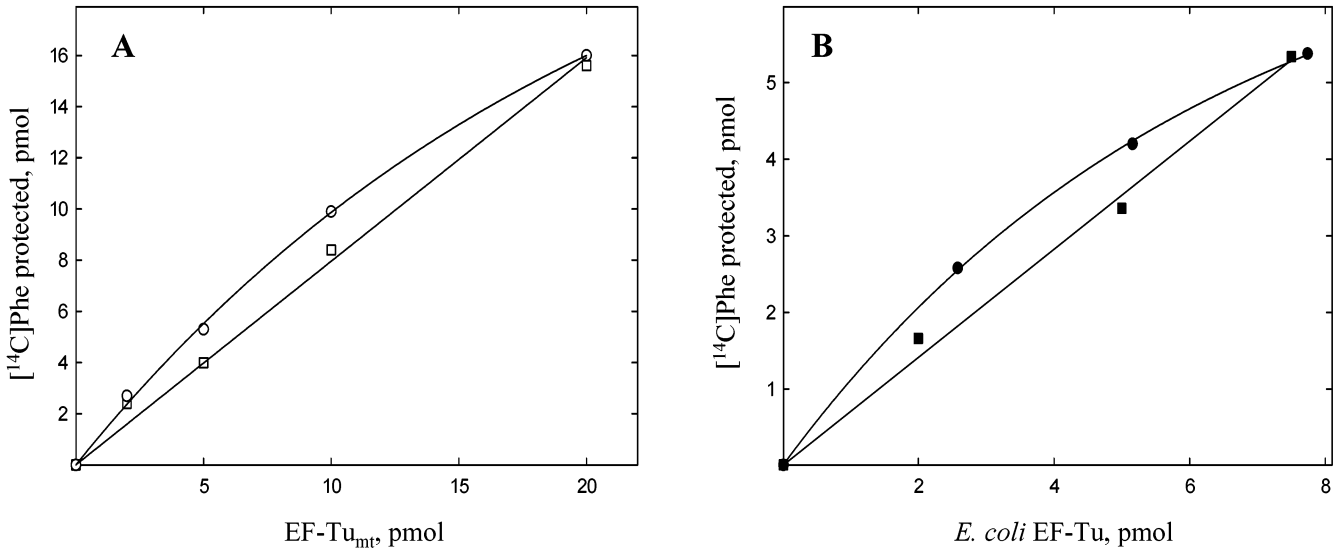

FIGURE 4: Ternary complex formation with *E. coli* Phe-tRNA^{Phe}. Ternary complex formation was assayed by examining the ability of EF-Tu to protect 0.38 μM *E. coli* Phe-tRNA^{Phe} from hydrolysis by RNase A as described in Materials and Methods. All values of EF-Tu reported are corrected for the percentage of active molecules. (A) 0.04−0.4 μM wild-type EF-Tu$_{mt}$ (□) and EF-Tu$_{mt}$ L338Q (○). (B) 0.04−0.15 μM wild-type *E. coli* EF-Tu (■) and 0.04−0.13 μM *E. coli* EF-Tu Q290L (●).

ribosomes and *E. coli* [^{14}C]Phe-tRNA^{Phe}. The activity of each variant was compared to the corresponding wild-type EF-Tu after the concentration of each factor was adjusted to reflect the percentage of molecules active in ternary complex formation. Thus, this assay is measuring the activities of the fraction of the mutated protein that has folded into an active conformation.

As indicated in Figure 3A, the activity of EF-Tu$_{mt}$ L338Q is similar to that of wild-type EF-Tu$_{mt}$. Similar results were obtained in both the presence and absence of EF-Ts$_{mt}$ (data not shown). Therefore, the L338Q mutation created in EF-Tu$_{mt}$ does not significantly affect the ability of the factor to function in in vitro protein synthesis. Surprisingly, *E. coli* EF-Tu Q290L is 3−5-fold more active in polymerization than wild-type *E. coli* EF-Tu (Figure 3B). A similar enhancement of activity was observed in both the presence (data not shown) and absence of EF-Ts. Thus, the Q290L mutation of *E. coli* EF-Tu alters the function of the factor in one or more of the steps involved in protein synthesis.

Ternary Complex Formation with E. coli Phe-tRNA^{Phe}. Hydrolysis protection assays used to determine the percentage of active molecules indicate that both the mitochondrial and *E. coli* EF-Tu variants are able to form ternary complexes with *E. coli* Phe-tRNA^{Phe}. To obtain a more quantitative measure of the abilities of the variants to bind aa-tRNA, assays were carried out in the presence of limiting concentrations of Phe-tRNA^{Phe}. Both EF-Tu$_{mt}$ L338Q and *E. coli* EF-Tu Q290L are able to protect *E. coli* Phe-tRNA^{Phe} from hydrolysis as well as their respective wild-type factors (Figure 4). Therefore, although the residue at position 290 helps to form the binding pocket for the 5′ end of the aa-tRNA, the mutations introduced do not disrupt or enhance

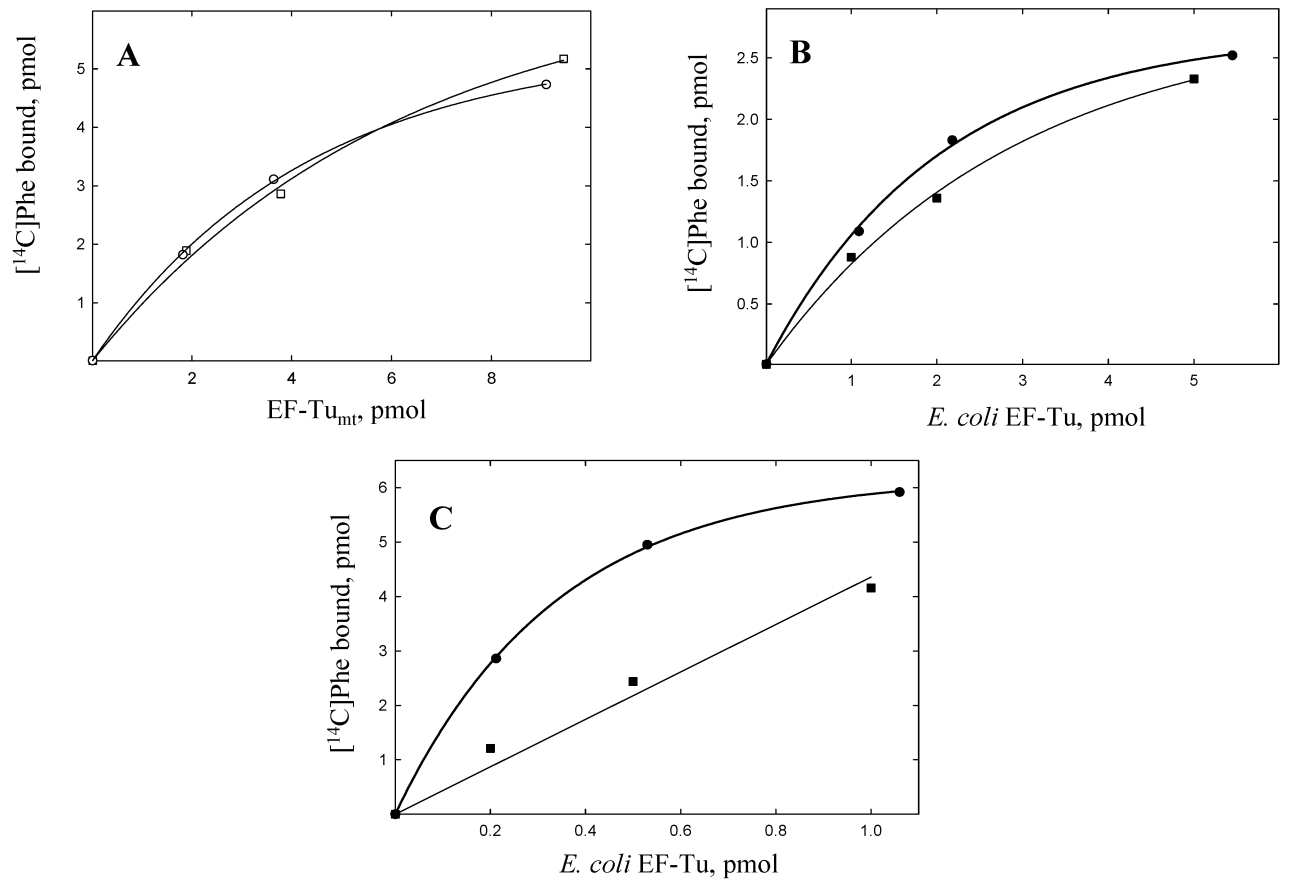

FIGURE 5: Binding of *E. coli* Phe-tRNA^{Phe} to the A-site of *E. coli* ribosomes. To determine the ability of wild-type and variant EF-Tu to deliver *E. coli* [^{14}C]Phe-tRNA^{Phe} to the A-site of the ribosome, A-site binding assays using the nonhydrolyzable analogue of GTP, GDPNP, were performed as described in Materials and Methods. In addition, A-site binding assays were carried out in the presence of GTP instead of GDPNP to monitor the turnover of the wild-type and variant *E. coli* factors. The picomoles of EF-Tu indicated are corrected for the percentage of EF-Tu molecules active in ternary complex formation. (A) Wild-type EF-Tu$_{mt}$ (□) and EF-Tu$_{mt}$ L338Q (○) with GDPNP. (B) Wild-type *E. coli* EF-Tu (■) and *E. coli* EF-Tu Q290L (●) with GDPNP. (C) Wild-type *E. coli* EF-Tu (■) and *E. coli* EF-Tu Q290L (●) with GTP.

the binding of *E. coli* Phe-tRNA^{Phe} to either factor. This assay also indicates that the increased activity of *E. coli* EF-Tu Q290L in poly(U)-directed polymerization is not due to an altered interaction between the variant and aa-tRNA.

A-Site Binding. The observation (Figure 3) that the EF-Tu$_{mt}$ L338Q variant is active in polymerization suggests that it is capable of delivering *E. coli* Phe-tRNA^{Phe} to the A-site of *E. coli* ribosomes. This question was tested directly in an assay using GDPNP, a nonhydrolyzable analogue of GTP, to provide a direct measure of A-site binding. The EF-Tu·GDPNP complex is able to bind to and deliver aa-tRNA to the ribosome. However, following codon−anticodon recognition, hydrolysis of GDPNP cannot occur, and thus, EF-Tu·GDPNP remains bound to the aa-tRNA and EF-Tu is unable to dissociate from the ribosome. In A-site binding assays with GDPNP, the activity of the mitochondrial variant was essentially identical to that of wild-type EF-Tu$_{mt}$ (Figure 5A). This observation is in agreement with the activity of EF-Tu$_{mt}$ L338Q in polymerization.

E. coli EF-Tu Q290L was shown to be more active in polymerization than its wild-type counterpart (Figure 3B) and thus is clearly able to deliver *E. coli* Phe-tRNA^{Phe} to the A-site of *E. coli* ribosomes. However, A-site binding assays indicate that *E. coli* EF-Tu Q290L functions similarly to wild-type *E. coli* EF-Tu in A-site binding in the presence of excess GDPNP (Figure 5B). This observation indicates that the higher activity of *E. coli* EF-Tu Q290L in poly(U)-directed polymerization is not due to an altered ability of the variant to deliver Phe-tRNA^{Phe} to the ribosome. Thus, the higher activity of *E. coli* EF-Tu Q290L may arise from a more rapid turnover and higher catalytic activity of the variant factor. To test this possibility directly, A-site binding assays were performed in the presence of GTP instead of GDPNP. *E. coli* EF-Tu Q290L is more active than wild-type *E. coli* EF-Tu under these assay conditions (Figure 5C). The increase in activity observed (about 3-fold) is quite similar to that seen with the Q290L variant in poly(U)-directed polymerization (about 4-fold) (Figure 3B). The fact that the prokaryotic variant functions similarly to wild-type *E. coli* EF-Tu in A-site binding with GDPNP but shows increased activity with GTP suggests that it may catalyze GTP hydrolysis more rapidly or that the EF-Tu·GDP complex formed with the Q290L variant dissociates more rapidly from the ribosome. Since the dissociation of the EF-Tu·GDP complex is the slowest step involving EF-Tu in the A-site binding pathway, it may be the main point effected by the Q290L mutation (*34*).

GTP Hydrolysis. The effect of the Q290L mutation on the GTPase activity of *E. coli* EF-Tu in the presence of mRNA-programmed ribosomes was compared directly to that of the wild-type factor. As shown in Figure 6A, *E. coli* EF-Tu Q290L has up to a 10-fold increase in GTPase activity

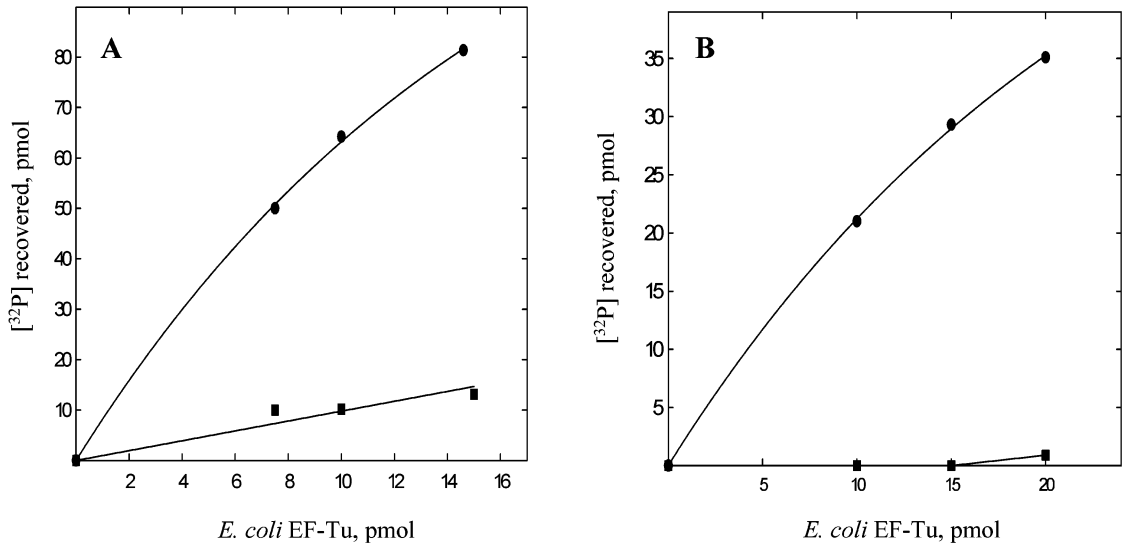

FIGURE 6: GTP hydrolysis with *E. coli* EF-Tu. The ability of *E. coli* EF-Tu Q290L to liberate P_i from [γ-^{32}P]GTP was monitored and compared to that of wild-type *E. coli* EF-Tu. Input values for EF-Tu have been corrected for the percentage of molecules capable of binding aa-tRNA. The quantity of radiolabeled P_i obtained in all assays was corrected for the background (the presence of radioactivity in a minus EF-Tu control, approximately 16 pmol for charcoal extractions and 20 pmol with thin-layer chromatography). (A) Wild-type *E. coli* EF-Tu (■) and *E. coli* EF-Tu Q290L (●) in the presence of mRNA-programmed ribosomes. The release of radiolabeled P_i was analyzed by charcoal extractions. (B) Wild-type *E. coli* EF-Tu (■) and *E. coli* EF-Tu Q290L (●) in the absence of ribosomes. The release of radiolabeled P_i was measured by thin-layer chromatography.

over wild-type *E. coli* EF-Tu. One explanation for this observation is that the Q290L variant hydrolyzes GTP more rapidly than the wild-type factor. However, such an increase would not be expected to enhance its activity in protein synthesis since GTP hydrolysis is not rate limiting for this process. Rather, the enhanced GTP hydrolysis observed may arise from a more rapid release of the *E. coli* EF-Tu·GDP Q290L complex from the ribosome and the subsequent rapid reuse of this EF-Tu variant.

EF-Tu-mediated GTP hydrolysis in the absence of ribosomes or antibiotics is remarkably slow (*27*). However, since the Q290L variant of *E. coli* EF-Tu shows increased catalytic activity in ribosome-stimulated GTP hydrolysis, the intrinsic GTPase activity of the prokaryotic variant was examined. As indicated in Figure 6B, *E. coli* EF-Tu Q290L hydrolyzes GTP in the absence of ribosomes at a substantial level, while no GTP hydrolysis can be observed under similar conditions with the wild-type factor. Thus, the intrinsic GTPase activity of the *E. coli* variant is significantly enhanced by the Q290L mutation.

Poly(U)-Directed Polymerization with Mitochondrial Phe-tRNA^{Phe}. Protein biosynthesis in mitochondria is much slower than that in *E. coli* (*35*). The lower activity of the mitochondrial translation system is also reflected in in vitro translation assays using mitochondrial substrates. The low activity observed in mitochondria may be due in part to the noncanonical mitochondrial tRNAs (*8*). To determine if both the mitochondrial and prokaryotic variants are able to function in protein synthesis with mitochondrial aa-tRNAs, poly(U)-directed polymerization was carried out using mitochondrial [^{14}C]Phe-tRNA^{Phe} and mitochondrial ribosomes. All assays were carried out in the presence of the appropriate EF-Ts to stimulate guanine nucleotide exchange and increase the activity of EF-Tu.

The activity of EF-Tu$_{mt}$ L338Q in poly(U)-directed polymerization is similar to that of the wild-type factor (Figure 7A). Thus, a Leu residue at position 338 in EF-Tu$_{mt}$ is not essential for the use of mitochondrial Phe-tRNA^{Phe}. In contrast, in poly(U)-directed polymerization with mitochondrial Phe-tRNA^{Phe}, *E. coli* EF-Tu Q290L has a higher activity compared to the wild-type factor (Figure 7B). *E. coli* EF-Tu is much less active in polymerization assays using mitochondrial Phe-tRNA^{Phe} and mitochondrial ribosomes than EF-Tu$_{mt}$ (note the scale of the *y*-axis of Figure 7A compared to that of Figure 7B). The Q290L mutation apparently increases the activity of the prokaryotic factor to levels comparable to that observed with EF-Tu$_{mt}$. However, the effect is clearly not specific to its activity in the mitochondrial translation system since a similar observation is seen with *E. coli* Phe-tRNA^{Phe} and *E. coli* ribosomes (Figure 3B). Here again, the increased activity in polymerization probably arises from the rapid dissociation of the EF-Tu·GDP Q290L variant from the ribosome, allowing it to recycle more effectively.

Ternary Complex Formation with Mitochondrial Phe-tRNA^{Phe}. Mammalian mitochondrial tRNAs often have weaker structures than canonical tRNAs, and many lack interactions that stabilize the three-dimensional structures of other tRNAs. To assess whether the residue at position 290 influences the binding of mitochondrial aa-tRNAs to either factor, the abilities of EF-Tu$_{mt}$ L338Q and *E. coli* EF-Tu Q290L to protect mitochondrial [^{14}C]Phe-tRNA^{Phe} from hydrolysis by RNase A were investigated. These assays were carried out in the presence of limiting amounts of mitochondrial Phe-tRNA^{Phe}, and the input values for EF-Tu were corrected for the percentage of molecules capable of forming a ternary complex with *E. coli* Phe-tRNA^{Phe}. In such assays, EF-Tu$_{mt}$ L338Q shows a significantly reduced ability to bind mitochondrial Phe-tRNA^{Phe} compared to wild-type EF-Tu$_{mt}$ (Figure 8). *E. coli* EF-Tu Q290L also appears to be deficient in ternary complex formation with the mitochondrial aa-tRNA (Figure 8), again suggesting that this residue is important for allowing the bacterial factor to form a stable ternary complex with mitochondrial aa-tRNA.

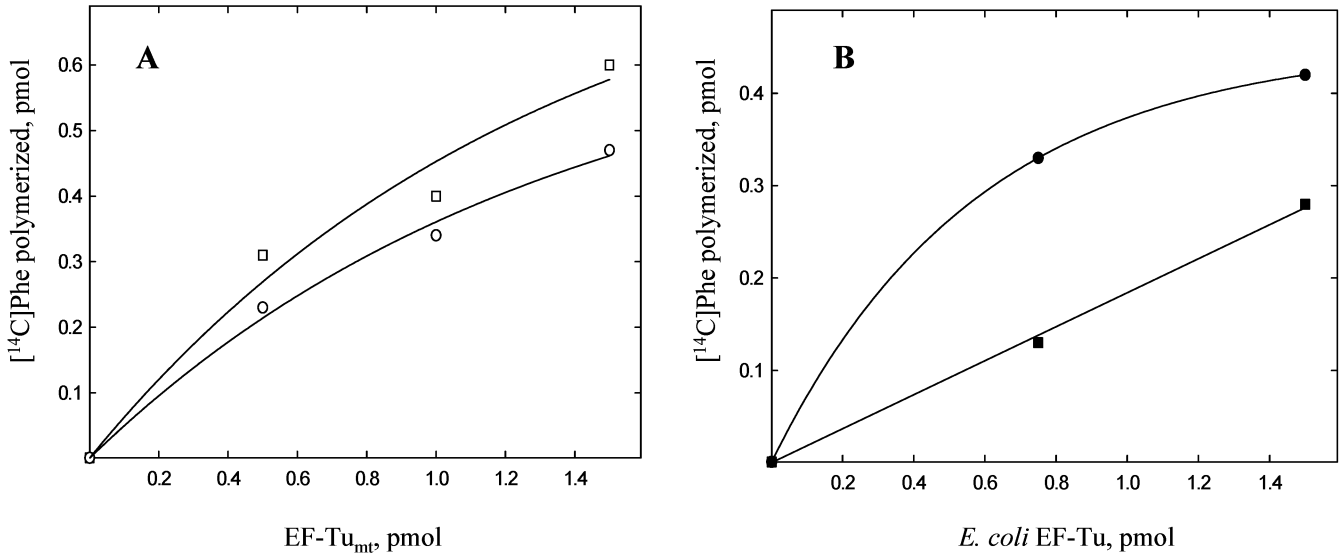

FIGURE 7: Poly(U)-directed polymerization with mitochondrial Phe-tRNA^{Phe} and mitochondrial ribosomes. Poly(U)-directed polymerization of Phe was used to assay the function of wild-type and variant EF-Tu with mitochondrial ribosomes and Phe-tRNA^{Phe} as described in Materials and Methods. The percentage of EF-Tu molecules active in ternary complex formation was used to determine the input values for each factor. (A) Wild-type EF-Tu$_{mt}$ (□) and EF-Tu$_{mt}$ L338Q (○). (B) Wild-type *E. coli* EF-Tu (■) and *E. coli* EF-Tu Q290L (●).

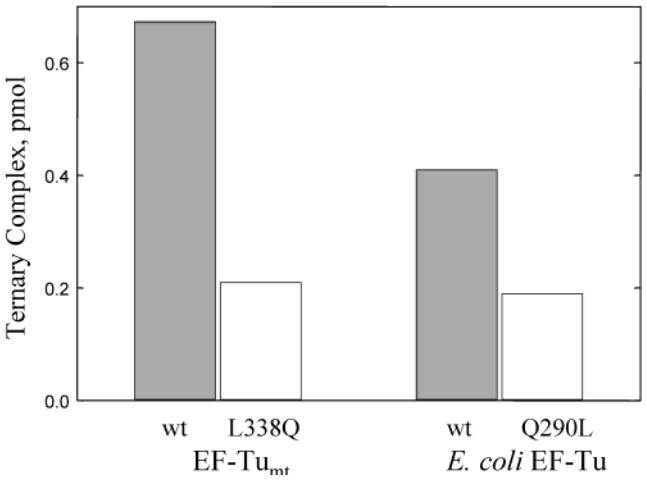

FIGURE 8: Ternary complex formation with mitochondrial Phe-tRNA^{Phe}. Ternary complex formation was assayed by examining the ability of 5 pmol of EF-Tu to protect 0.1 μM mitochondrial [^{14}C]Phe-tRNA^{Phe} from hydrolysis by RNase A as described in Materials and Methods.

DISCUSSION

Preparations of the *E. coli* EF-Tu Q290L variant contain significantly fewer active molecules than preparations of the prokaryotic wild-type factor. The model of the structure of *E. coli* EF-Tu containing Leu338 of EF-Tu$_{mt}$ (Figure 2C) suggests that the Q290L mutation disrupts the H-bonds between the ϵ^1-oxygen and ϵ^2-nitrogen atoms of residue 290 and the adjacent residues (Figure 2A). In addition, it is likely that the mutation alters the interface between domains II and III. These structural changes apparently affect the folding or stability of the prokaryotic variant.

Unlike *E. coli* EF-Tu Q290L, the replacement of Leu for Gln at position 338 in EF-Tu$_{mt}$ does not involve the loss of any H-bonds. Thus, EF-Tu$_{mt}$ can accommodate the L338Q mutation without any significant loss in the percentage of active molecules. However, solvent accessibility must be accommodated in the mitochondrial variant to allow the H-bonds necessary to satisfy the ϵ^1-oxygen and ϵ^2-nitrogen atoms of the incorporated Gln. It is likely that the mutated residue is oriented to allow a H-bond between the ϵ^2-nitrogen atom of Gln338 in EF-Tu$_{mt}$ L338Q and a water molecule. However, it is unclear what stabilizes the ϵ^1-oxygen atom of the inserted Gln338 residue in EF-Tu$_{mt}$ L338Q.

Two interesting alterations in the properties of *E. coli* EF-Tu are observed in the Q290L variant: the higher intrinsic GTPase activity of the mutated protein and its more rapid dissociation from the ribosome. Wild-type EF-Tu has a low intrinsic GTPase activity and, as is characteristic of guanine nucleotide-binding proteins, requires a GTPase-activating protein (GAP) to be functionally active. In the EF-Tu cycle, the ribosome is viewed as acting as the GAP, triggering GTP hydrolysis following cognate codon−anticodon interactions. EF-Tu·GDP has a low affinity for both the aa-tRNA and the ribosome, and as a result, the complex releases the aa-tRNA and dissociates from the ribosome, allowing the aa-tRNA to be accommodated in the ribosomal A-site. Much remains to be learned about how the signal from the codon−anticodon interaction is transmitted to the GTPase center in domain I of EF-Tu. Previous studies have indicated that the aa-tRNA plays a crucial role in transmitting the signal from cognate codon−anticodon interactions in the decoding center to the GTPase center of the ternary complex on the ribosome (*36*, *37*). Interactions of domain I of EF-Tu with the 50S subunit then facilitate the hydrolysis step. The signal is likely to be transmitted through domain II since mutations in this domain such as G222D near the 3′ end of the aa-tRNA are inefficient in triggering the GTPase activity of EF-Tu (*38*). The loop in which Gln290 is located contacts the 5′ end of the aminoacyl-tRNA, and it is logical to postulate that any signal from codon−anticodon interactions may also involve conformational changes in this region that are transmitted to the G-domain through contacts between this loop and the switch II region in domain I (*39*).

E. coli EF-Tu Q290L has an elevated GTPase activity even in the absence of ribosomes. This observation suggests that the replacement of Gln for Leu in this region alters contacts between domain II and the G-domain, resulting in a

conformation in domain I that hydrolyzes GTP spontaneously. This mutant may mimic in some ways the conformational changes that normally occur upon cognate codon−anticodon interactions. Indeed, *E. coli* EF-Tu Q290L may be too poised for GTP hydrolysis, making the variant more error-prone and increasing the rate of misincorporation of near cognate amino acids into the nascent peptide chain.

The heightened activity of the prokaryotic variant in GTP hydrolysis would result in the more rapid accommodation of the aa-tRNA into the ribosomal A-site. However, as stated previously, the dissociation of EF-Tu from the ribosome is the rate-limiting step in the ribosomal cycle for EF-Tu (*34*). Thus, the increased ability to stimulate poly(U)-directed polymerization by *E. coli* EF-Tu Q290L probably arises from an increased rate of dissociation from ribosomes following GTP hydrolysis (Figures 3B and 7B). Cryo-EM structures of the ternary complex docked onto the ribosome reveal that domain II of EF-Tu interacts with the 16S rRNA of the small subunit (*40*). In addition, mutations such as G222D and G280A in EF-Tu that affect the ribosome-dependent functions of the factor implicate domain II in ribosome binding (*38*, *41*). Gly280 is located on the surface of domain II and is a likely point of contact between EF-Tu and the 30S subunit. This residue is connected to the loop containing Gln290 through a short coiled segment. Alterations in the structure and organization of the Gln290 loop are likely to be transmitted to the surface of domain II around residue 280, resulting in subtle conformational changes in this region of the protein. These changes are the most likely cause of the reduced interaction of EF-Tu with the ribosome and the more rapid dissociation of *E. coli* EF-Tu Q290L from the ribosome.

At first glance, the replacement of Leu338 with Gln in the mitochondrial factor appears to have little effect on the activity of this factor. However, a striking result reported in this work is the observation that Leu338 is important for the interaction of EF-Tu_{mt} with mitochondrial Phe-$tRNA^{Phe}$ (Figure 8). As mentioned previously, this residue is part of a loop that contains the highly conserved Arg288, which serves to stabilize the interaction of the 5′ end of the aa-tRNA with EF-Tu·GTP (*39*). No crystal structure of the mitochondrial ternary complex is available; thus the interactions between EF-Tu_{mt} and mitochondrial aa-tRNAs are not known. However, it is apparent from results reported here that Leu338 provides stability to the ternary complex involving mitochondrial Phe-$tRNA^{Phe}$ and EF-Tu_{mt}.

Surprisingly, although the variants were impaired in their ability to form ternary complexes with mitochondrial Phe-$tRNA^{Phe}$, they were both able to sustain poly(U)-directed polymerization as well as (or better than, in the case of the Q290L variant) their respective wild-type factors (Figure 8). Previous studies have shown that the initial docking of the EF-Tu onto the ribosome occurs rapidly following ternary complex formation (*34*). Both the mitochondrial and the prokaryotic variants are sufficiently able to form ternary complexes with the mitochondrial aa-tRNA to allow their delivery to the A-site of the ribosome. However, unlike the wild-type factors, the variants are unable to maintain the prolonged interactions with the aa-tRNA that are necessary when monitoring stable ternary complex formation.

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