Elongation Factor-Ts Directly Facilitates the Formation and Disassembly of the Escherichia Coli Elongation Factor-Tu-GTP-Aminoacyl-tRNA Ternary Complex

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*Running Title: Dynamic properties of the EF-Tu-GTP-aminoacyl-tRNA ternary complex

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Background: Aminoacyl-tRNA (aa-tRNA) enters the ribosome in a ternary complex with the G-protein Elongation Factor Tu (EF-Tu) and GTP. Results: EF-Tu-GTP-aa-tRNA ternary complex formation and decay rates are accelerated in the presence of the nucleotide exchange factor Elongation Factor-Ts (EF-Ts).

Conclusion: EF-Ts directly facilitates the formation and disassociation of ternary complex.

Significance: This system demonstrates a novel function of EF-Ts.

SUMMARY

Aminoacyl-tRNA (aa-tRNA) enters the translating ribosome in a ternary complex with elongation factor Tu (EF-Tu) and GTP. Here, we describe bulk steady state and pre-steady state fluorescence methods that enabled us to quantitatively explore the kinetic features of Escherichia coli ternary complex formation and decay. The data obtained suggest both processes are controlled by a nucleotide-dependent, rate-determining conformational change in EF-Tu. Unexpectedly, we find that this conformational change is accelerated by elongation factor Ts (EF-Ts), the guanosine nucleotide exchange factor (GEF) for EF-Tu. Notably, EF-Ts attenuates EF-Tu’s affinity for GTP and destabilizes ternary complex in the presence of non-hydrolyzable GTP analogs. These results suggest that EF-Ts serves an unanticipated role in the cell of actively regulating the abundance and stability of ternary complex in a manner that contributes to rapid and faithful protein synthesis.

GTP hydrolyzing proteins (GTPase’s) play a central role in a vast array of biological systems (1-4). In bacteria, the three domain GTPase, EF-Tu - a member of the TRAFAC class of G-proteins (2) - chaperones the entry of aa-tRNA into the messenger RNA (mRNA) programmed ribosome during the process of protein synthesis (5-8). This multistep, and highly conserved process is a critical determinant of the mechanism of translational fidelity (9) and is exquisitely regulated by EF-Tu-catalyzed GTP hydrolysis while bound to the ribosome.

Efforts to understand how EF-Tu facilitates the process of aa-tRNA selection have depended critically on high-resolution structural information of active (EF-Tu·GTP) and inactive (EF-Tu·GDP) forms of EF-Tu. Towards this goal, atomic resolution structures of EF-Tu bound within its “ternary complex” with GTP and aa-tRNA, and bound to GDP have been solved (10-15). This work revealed that EF-Tu-GTP and EF-Tu-GDP exhibit markedly distinct conformations, characterized by “compact” and “extended”
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configurations, respectively. Related investigations showing that only EF-Tu-GTP binds aa-tRNA with high affinity (ca. nanomolar; nM) suggested that EF-Tu’s capacity to achieve its activated, compact form is a critical determinant of high-affinity aa-tRNA binding (16-20).

In its compact configuration, the three domains of EF-Tu (D1, D2 and D3) closely interact with each other and with the tRNA acceptor stem (Fig. 1A, 1B) (10,21). The high-affinity nature of the EF-Tu-GTP:aa-tRNA interaction is further buttressed by the formation of an amino acid binding pocket at the interface of domains 1 (also referred to as the G-domain) and 2 of the protein (20,22). There, histidine 66 (H66) within domain 1 stacks on the aminoacyl side chain linked to the 3'-hydroxyl group of the terminal adenosine residue (A76) of tRNA (20). In EF-Tu-GDP, domain 1 is distal to domains 2 and 3 (10), rationalizing why this form of the protein does not bind aa-tRNA (16,17,19). These findings suggest that compact and extended conformations of EF-Tu define active and inactive states of the G-protein, respectively, and that the active EF-Tu fold is somehow dependent on the terminal phosphate of GTP.

High-resolution structures of ternary complex bound to the ribosome have revealed that GTP hydrolysis leads directly to conformational changes within the GTP binding pocket (15). This region includes the so-called switch-1 (S1), switch-2 (S2) and P loop motifs that are conserved in all G-proteins (2). The S1 element is structurally linked to the amino acid binding pocket and engages the triphosphate moiety of the GTP nucleotide via a bridging magnesium ion (Mg$^{2+}$) (Fig. 1B). The S1 element becomes disordered following GTP hydrolysis on the ribosome (15) and adopts an alternative beta hairpin conformation in the EF-Tu-GDP structure (14). Collectively, these findings suggest that structural transitions within S1 likely contribute to the transition between GTP- and GDP-bound conformations of the protein and the aa-tRNA selection mechanism.

Ensemble steady state measurements of ternary complex (16,17,19,23-27), together with estimated intracellular concentrations of EF-Tu, aa-tRNA (ca. tens of micromolar) and GTP (ca. millimolar), suggest that most (>90%) aa-tRNA is complexed with EF-Tu in actively growing bacteria (24,28). However, the steady state concentration of ternary complex is strongly dependent on numerous biochemical processes, including the rates of ribosome-catalyzed protein synthesis, amino acid availability, and the relative intracellular concentrations of GTP and GDP. The concentration of ternary complex is also dependent on the activity of Elongation Factor-Ts (EF-Ts), the guanosine nucleotide exchange factor (GEF) for EF-Tu that is required to convert EF-Tu-GDP released from the ribosome into an EF-Tu-GTP form that is again competent for aa-tRNA binding. Notably, EF-Ts is also present at micromolar concentrations in the cell (29-31). EF-Tu has an approximately 60-fold higher affinity for GDP over GTP and a slow rate of spontaneous nucleotide exchange (28). Correspondingly, EF-Ts activity is essential for cellular growth (32,33) as it regulates ternary complex abundance in the cell and consequently the rates of protein synthesis.

The importance of normal EF-Tu functions to cellular growth is highlighted by the prevalence of chemically distinct antibiotics that alter EF-Tu activities (34-37). Thiolepton-class peptide antibiotics bind to the large ribosomal subunit GTPase-Activating Center (GAC) to disrupt ternary complex binding to the A site (38). GE2270A-class thiazolyl peptide antibiotics bind directly to EF-Tu at the domain 1/2 interface to prevent its interaction with aa-tRNA (36). Kilromycin binds at the domain 1/3 interface of EF-Tu to stall ternary complex on actively translating ribosomes (39,40-42) immediately after GTP hydrolysis by preventing conformational changes in EF-Tu required for its release from aa-tRNA. Thus, the dynamics of ternary complex formation and stability are critical for cellular viability. However, pre-steady state kinetic information regarding the rates of ternary complex formation and decay are currently lacking.

To assess dynamic aspects of the E. coli EF-Tu-GTP:aa-tRNA ternary complex, here we describe a pre-steady state, fluorescence-based approach that reports on binding between EF-Tu and aa-tRNA. We use this signal to investigate rate-determining conformational changes that control EF-Tu’s high-affinity interactions with aa-tRNA. Remarkably, these investigations reveal that the dynamics of ternary complex formation and decay are markedly increased in the presence of EF-Ts. We conclude that EF-Ts directly
interacts with EF-Tu while bound aa-tRNA to regulate its affinity for GTP and aa-tRNA ligands. We speculate that EF-Ts regulates the stability and turnover of ternary complex by catalyzing rate-limiting conformational processes in EF-Tu’s nucleotide binding pocket that are responsible for aa-tRNA binding and release.

EXPERIMENTAL PROCEDURES

Purification of Elongation Factors – 6xHIS EF-Tu (tuFA) and EF-Ts (tsf) were expressed recombinantly in E.coli and purified by Ni-NTA affinity chromatography in the absence of magnesium as previously described (43). After cleavage, protein factors were further purified using Suprarex75 gel filtration chromatography equilibrated in buffer A (50 mM Tris-HCl pH 7.5, 100 mM NH4Cl, 10 mM MgCl2, 0.5 mM EDTA, 50 mM KCl, 1 mM DTT). EF-Tu-EF-Ts complexes were formed by adding EF-Tu and EF-Ts (1:1.1) in buffer A and incubating at 23°C for 15 minutes. The EF-Tu-EF-Ts complex was then isolated from unbound protein using Suprarex75 gel filtration chromatography equilibrated in buffer A. All factors were stored in buffer B (10 mM Tris-Cl, 1 mM MgCl2, 50 mM KCl, 1 mM DTT, 50% v/v glycerol) at -80°C until further use. Kirmomycin was purchased from Sigma and re-suspended in 100% DMSO to 1 mM and stored at -80°C until further use.

Purification and labeling of E. coli tRNA Phe – Wild-type E.coli tRNA Phe was purified from an RNase-deficient bacterial strain (MRE600) (44). Native tRNA Phe molecules were site-specifically labeled with the small-molecule organic fluorophore Cy3, via the naturally occurring modified nucleotide 3-(3-amino-3-carboxypropyl)uridine (acp3U) present at position 47 (43,45). tRNA molecules labeled in this fashion have been previously shown to retain wild-type activities in both aminoacylation and translation reactions (43,46). Purified tRNA Phe was aminoacylated by mixing 7.5 picomoles of labeled tRNA with 6 picomoles phenylalanyl-tRNA synthetase and 10 nanomoles of phenylalanine amino acid in a volume of 10 μL in buffer C (50 mM Tris-HCl pH 8, 20 mM KCl, 100 mM NH4Cl, 1 mM DTT, 2.5 mM ATP, 0.5 mM EDTA, 10 mM MgCl2). The sample was then incubated at 37°C for 10 minutes. Aminoacylated tRNA was used immediately without storage.

Nucleotide purification – Guanosine triphosphate (GTP), guanosine diphosphate (GDP), guanosine 5’-[β,γ-imido]triphosphate (GDPNP), and guanosine 5’-[γ-thio]triphosphate (GTPγS) were purchased from Sigma and further purified on a Tricorn Mono-Q 5/50 GL ion exchange column. 2/3-O-(N-methylanthraniloyl)-guanosine-5’-triphosphate (mant-GTP) was purchased from Jena Biosciences.

Fluorometer experiments – All fluorescent measurements were performed using a Photon Technology International fluorescence meter with a 532 nm high pass filter (LP03-532RS-25 RazorEdge by Semrock) on the emission side. All samples were analyzed in a 3 mL quartz cuvette with constant mixing at 23°C in buffer D (50 mM Hepes pH 7, 20 mM KCl, 100 mM NH4Cl, 1 mM DTT, 0.5 mM EDTA, 2.5 mM MgCl2) with either 10 μM GTP or 400 nM EF-Tu or EF-Tu-EF-Ts. Steady state measurements were made by manually adding EF-Tu or EF-Tu-EF-Ts to a solution of Phe-tRNA Phe (Cy3-acp3U47) while exciting at 532 nm and monitoring 565 nm (Cy3). The dissociation constant, K D, was determined by fitting the fluorescence data obtained to the equation (16):

$$ F/F_0 (T_u) = 1 + \frac{[F/F_0]_{\text{max}} - 1}{2c_t} C_t + T_u + K_D - \sqrt{(C_t + T_u + K_D)^2 - 4c_t T_u} $$

where F is the total fluorescence intensity, F 0 is the initial fluorescence intensity, C t is the total aa-tRNA concentration, and T u is the total EF-Tu concentration. These nucleotide titration experiments (Fig. 1D) yield ‘apparent’ K D’s. Identical factor titration experiments and fitting procedures (Fig. 1C) were performed to provide a qualitative comparison between data sets.

Pre-steady state measurements were made by manually adding saturating protein factor (400 nM EF-Tu or EF-Tu-EF-Ts) to 5 nM Phe-tRNA Phe (Cy3-acp3U47) and 10 μM GTP and monitoring fluorescence changes over time. Apparent rates, k app, were determined by fitting data to a single exponential (47).

Rapid stopped-flow experiments – All experiments were performed at 23°C in buffer D using an SX20 stopped-flow spectrometer from Applied Photophysics using a 550 nm long pass filter (OG550 by Schott). One injector port was
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loaded with protein factor pre-incubated with 2 mM GTP while the other injector port was loaded with 400 nM Phe-tRNA\textsubscript{Phe} (Cy3-acp\textsuperscript{3}U47). Concentrations reported in the main text are final concentrations after mixing.

**Ternary complex isolation by gel filtration chromatography** – Ternary complex was formed by adding EF-Tu or previously formed EF-Tu-EF-Ts complex to Phe-tRNA\textsubscript{Phe} (4:1) in buffer D with 10 μM GTP and incubating for 15 minutes at 37°C. Samples were purified using a Sephadex 75 gel filtration column on an Akta Purifier pre-equilibrated with buffer D either in absence or presence of GTP or GDPNP (10 μM).

**GTP exchange assay** – Experiments were performed at room temperature in buffer D under conditions >10-fold above the $K_D$ for ternary complex formation to ensure that all EF-Tu present in the reaction is bound to tRNA (400 nM Phe-tRNA\textsubscript{Phe}, 5 nM Phe-tRNA\textsubscript{Phe} (Cy3-acp\textsuperscript{3}U47), 400 nM EF-Tu or EF-Tu-EF-Ts, 10 µM mant-GTP). Complex formation was monitored by tracking the fluorescence of the Cy3 and mant fluorophore over time. Measurements of Cy3 and mant fluorescence were made by switching detection modes from 532 ex./565 em. to 280 ex./440 em., respectively (28). The mant fluorescence signal was processed using the Savitzky-Golay method in OriginLab8 with a window of 9 points (48).

**RESULTS**

The process of ternary complex formation minimally proceeds through the following sequence of biochemical steps (49,50):

\[
\begin{align*}
& \frac{k_{1+}}{k_{1-}} \quad (1) \quad EF-Tu \cdot GDP + EF-Ts \leftrightarrow EF-Tu \cdot EF-Ts + GDP \\
& \frac{k_{2+}}{k_{2-}} \quad (2) \quad EF-Tu \cdot EF-Ts + GTP \leftrightarrow EF-Tu \cdot GTP + EF-Ts \\
& \frac{k_{3+}}{k_{3-}} \quad (3) \quad EF-Tu \cdot GTP + aatRNA\textsuperscript{aa} \leftrightarrow EF-Tu \cdot GTTP \cdot aatRNA\textsuperscript{aa}
\end{align*}
\]

Here, equations (eqs) 1 and 2 define the GEF activities of EF-Ts, where EF-Ts functions to displace GDP from EF-Tu and facilitate EF-Tu’s association with GTP (18,28,51,52). In this minimal reaction scheme, the EF-Tu-GDP-EF-Ts complex implicit in eq. 1 exists only transiently (k. $= 125 s^{-1}$) (28). Likewise, the EF-Tu-GTP-EF-Ts species implicit in eq. 2 is also transient in nature (k. $= 60 s^{-1}$) (28). The rates of ternary complex formation and dissociation (eq. 3 in this reaction scheme) have been largely inferred from an array of steady state investigations (16,17,19,23,25,26). Direct pre-steady state information, however, is currently lacking. In particular, little is presently known about how this reaction proceeds in the presence of EF-Ts, as it natively occurs in the cell.

**Steady state measurements of ternary complex formation** – Following procedures analogous to those previously described using Phe-tRNA\textsubscript{Phe} fluorescently labeled at the 4-thiouridine residue at position 8 (s\textsuperscript{4}U8) (16), we first attempted to determine the apparent affinity of EF-Tu for Cy3-acp\textsuperscript{3}U47-labeled Phe-tRNA\textsubscript{Phe} using a steady-state approach. Here, the fluorescence intensity of the Cy3 fluorophore linked to tRNA was tracked as a function of EF-Tu concentration. Titrations were performed by addition of either EF-Tu-GTP, where EF-Tu was pre-incubated with 10 µM GTP, or an EF-Tu-EF-Ts complex, to a reaction mixture containing 5 nM Phe-tRNA\textsubscript{Phe} (Cy3-acp\textsuperscript{3}U47) and 10 µM GTP at room temperature (23°C).

Under these experimental conditions, both measurements resulted in an EF-Tu-dependent increase in Cy3 fluorescence intensity that plateaued at approximately 30% above baseline (Fig. 1C). Such changes are speculated to arise from an environment-specific increase in Cy3 quantum yield upon ternary complex formation, stemming from reductions in solvent-mediated, non-radiative relaxation pathways and/or cis-trans isomerization rates (53,54). Consistent with previous studies (16) and the determinants of ternary complex formation as outlined in eq. 3, the observed increase in Cy3 fluorescence intensity was strictly dependent upon the presence of GTP as well as the acylation of tRNA\textsubscript{Phe} with the phenylalanine (Phe) amino acid (Fig. 1C). The apparent equilibrium dissociation constant for the ternary complex interaction was estimated by fitting the raw data to the reaction scheme derived for relative fluorescence measurements (Experimental Procedures). In agreement with previously described steady-state measurements of this type, a high affinity interaction was observed both in the absence ($K_D$ $\sim 47 \pm 3.1$ nM) and presence of EF-Ts ($K_D$ $\sim 12.6 \pm 1.1$ nM) (Table 1) (16,17,19,20,23,55,56). These findings suggest that the observed changes in Cy3 fluorophore
intensity specifically report on a GTP-dependent interaction of EF-Tu with the acylated acceptor stem of Phe-tRNA\(^{\text{Phe}}\). They also suggest that EF-Ts substantially increases the apparent affinity of this interaction.

The observed disparity between EF-Tu and EF-Tu·EF-Ts affinities for aa-tRNA could not be explained by differences in EF-Tu activities (Fig. 2). Activity was determined by titrating either EF-Tu or EF-Tu·EF-Ts into a solution of 400 nM Phe-tRNA\(^{\text{Phe}}\) (Cy3-acp\(^1\)U47) and 10 µM GTP. For 100% active reagents, a linear increase in signal is expected followed by a sharp inflection at a protein concentration equal to the concentration of Phe-tRNA\(^{\text{Phe}}\) present in the reaction (57). In our experiments such an inflection was observed at ~500 nM factor indicating that EF-Tu is ~80% active both in the absence and presence of EF-Ts.

Analogous steady state measurements were next conducted to assess the nucleotide-dependence of ternary complex formation. To do so, experiments were performed by titrating GTP into a solution of 5 nM Phe-tRNA\(^{\text{Phe}}\) (Cy3-acp\(^1\)U47) in the presence of saturating concentrations of either nucleotide-free EF-Tu or EF-Tu·EF-Ts complex, both at 500 nM (>10-fold above their apparent \(K_D\)). For both systems, an approximately 30% increase in Cy3 fluorescence intensity was observed at elevated GTP concentrations (Fig. 1D). For the experiments with EF-Tu alone, fitting procedures (Experimental Procedures) revealed an apparent affinity of \(K_D\) ~195 ± 12 nM, while experiments performed with EF-Tu·EF-Ts revealed that the apparent affinity was approximately 3.5-fold weaker (\(K_D\) ~685 ± 35 nM) (Fig. 1D). These data suggest that additional complexities exist in the ternary complex formation reaction beyond those delineated by the reaction scheme of eq. 3. Such findings could be explained if ternary complex is in a dynamic equilibrium and that EF-Ts alters this exchange process by specifically modulating the nucleotide’s affinity for EF-Tu.

This model predicts that the ternary complex formation, and thus the observed fluorescence intensity change, will be sensitive to the precise nature of the EF-Tu-GTP interaction. To examine this hypothesis, identical experiments were repeated with non-hydrolyzable GTP analogs: guanosine 5′-[\(\beta,\gamma\text{-imido}\)]triphosphate (GDPNP) and guanosine 5′-[\(\gamma\text{-thio}\)]triphosphate (GTP\(\gamma\)S). As anticipated by the model, the extent of ternary complex formation was significantly lower in the presence of non-hydrolyzable GTP analogs (Fig. 1D; Table 1). For each analogue, this trend was exacerbated in the presence of EF-Ts. These findings suggest that the observed fluorescence change reports on conformational events in the system that are sensitive to chemical and/or structural features of the nucleotide’s \(\gamma\)-phosphate constituent. Inspection of the ternary complex structure (Fig. 1A, 1B) suggests that such conformational changes likely entail the formation of contacts between the S1 helix and the \(\gamma\)-phosphate of GTP.

**Pre-steady state measurements of ternary complex formation** – In order to obtain a deeper understanding of the kinetic parameters underpinning eq. 3, we performed pre-steady state measurements to monitor ternary complex formation in real time. Data were obtained by rapidly adding saturating (400 nM final) concentrations of EF-Tu or EF-Tu·EF-Ts complex to a solution of 5 nM Phe-tRNA\(^{\text{Phe}}\) (Cy3-acp\(^1\)U47) and 10 µM GTP. Consistent with our steady state measurements, the addition of EF-Tu in resulted in a ~30% increase in Cy3 fluorescence intensity that reached steady state within seconds (Fig. 3A) and remained stable over several hours (data not shown). Fitting the time course of the fluorescence intensity change to an equation for pre-steady state reactions of this kind (Experimental Procedures), revealed an apparent association rate, \(k_{\text{app,1}}\), of 0.38 s\(^{-1}\) for EF-Tu and 0.51 s\(^{-1}\) for the EF-Tu·EF-Ts complex (Fig. 3B). The factor concentration-dependence of \(k_{\text{app,1}}\) revealed that the maximum rates of ternary complex formation for both systems at 10 µM GTP saturated at approximately similar levels: ~0.5 s\(^{-1}\) for EF-Tu; 0.55 s\(^{-1}\) for the EF-Tu·EF-Ts complex.

Further insights into the on- and off-rate kinetics underpinning ternary complex formation and dissociation reactions were obtained by inspecting how \(k_{\text{app,1}}\) changed at low factor concentrations, where the concentration-dependence is linear. The on-rate of the ternary complex formation, determined by setting the slope of \(k_{\text{app,1}}\) to \(k_{\text{off}}/(1 + k_{\text{off}}/k_{\text{app,1}})\) (47), revealed that the on-rate in the presence of 10 µM GTP is approximately 1.2 ± 0.07 µM\(^{-1}\) s\(^{-1}\) and 1.7 ± 0.1 µM\(^{-1}\) s\(^{-1}\) for EF-Tu and the EF-Tu·EF-Ts complex, respectively (Fig. 3B, inset), in close agreement
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with published values (17). A preliminary estimate of the factor off-rates, defined by the y-intercept, revealed that EF-Tu dissociates slowly from aa-tRNA (on the order of 0.05 s⁻¹). These values are in good agreement with both our own (Table 1), and previous affinity measurements (20).

In order to directly examine the dissociation kinetics of ternary complex, pre-steady state measurements were performed in which a 10-fold molar excess of GDP (100 µM) was added to ternary complex, pre-formed with 10 µM GTP and 400 nM of either EF-Tu or EF-Tu-EF-Ts. In line with the notion that EF-Tu binds tightly to aa-tRNA, the addition of GDP to the pre-formed ternary complex caused a slow decrease in Cy3 fluorescence, returning the system to baseline intensity at a rate, \( k_{app,2} \), of approximately 0.015 s⁻¹ (Fig. 3C). This rate was independent of factor (Fig. 3D) and GTP concentrations (Fig. 3E), and consistent with our off-rate estimates (Fig. 3B).

Identical experiments performed with 400 nM EF-Tu-EF-Ts showed a similar overall reduction in fluorescence intensity upon GDP addition but at an approximately 20-fold faster decay rate (\( k_{app,2} \sim 0.3 \text{ s}^{-1} \)) (Fig. 3C). Notably, the apparent rate of GDP-induced ternary complex dissociation, \( k_{app,2} \), increased linearly as a function of EF-Tu-EF-Ts concentration from approximately 0.03 s⁻¹, extrapolated to 0 nM EF-Ts, up to ~0.5 s⁻¹ at the highest concentration tested (1 µM) (Fig. 3D). The accelerated rate of GDP-induced ternary complex dissociation in the presence of EF-Ts was observed to be independent of GTP concentration (Fig. 3E). Although the concentration of free EF-Ts (not bound to EF-Tu) in these experiments is difficult to estimate, it is anticipated to be substantially lower than EF-Tu-EF-Ts concentration given the known \( K_D \) of the EF-Tu-EF-Ts interaction (3 nM) (28). We conclude that EF-Ts plays a direct role in ternary complex dissociation in the presence of GDP and that the recognition process occurs at a rate that is likely to significantly exceed 15 µM⁻¹s⁻¹ (determined from the slope of the line shown in Figure 3D).

To examine the physical basis of the observed dissociation reaction coordinate, identical experiments were performed in the presence of kirromycin, an antibiotic that directly binds EF-Tu at the interface between domains 1 and 3 (21). As anticipated from its known propensity to prevent EF-Tu dissociation from aa-tRNA (39-41), in the presence of kirromycin ternary complex was observed to be strongly resistant to GDP-induced dissociation, even in the presence of EF-Ts (Fig. 3F). This finding is consistent with a model in which EF-Tu’s dissociation from aa-tRNA proceeds via rearrangements at the interface between domains 1 and 3 of EF-Tu.

To address the physiological relevance of these findings, we performed pre-steady state measurements of ternary complex formation at near cellular concentrations of protein factors (0.2-15 µM), aa-tRNA (0.2 µM) and GTP (1 mM) using stopped-flow instrumentation. Here, EF-Tu and EF-Tu-EF-Ts were pre-incubated with GTP. As previously shown (Fig. 3), the signal obtained was strictly dependent on the aminoacyl moiety of Phe-tRNA^{Phe} and GTP (data not shown). At these concentrations, the apparent rate of ternary complex formation for EF-Tu alone plateaued at a rate of ~20 s⁻¹ (Fig. 4), whereas in the presence of EF-Ts, the rate of ternary complex formation increased to ~85 s⁻¹. These distinct asymptotic values suggest that EF-Ts accelerates the rate-determining step of ternary complex formation by directly facilitating the process of EF-Tu-GTP loading onto Phe-tRNA^{Phe}. Under these conditions, ternary complex dissociation was also observed to be accelerated by the presence of EF-Ts (Fig. 3D). These findings suggest that our findings are relevant to ternary complex dynamics as they occur in the cell.

Physical isolation of the dynamic ternary complex – To directly test the hypothesis that ternary complex formation and decay is in dynamic equilibrium, we set out to examine its stability by gel filtration chromatography. Here, a strong prediction of the dynamic equilibrium model is that ternary complex will dissociate when efficiently separated from GTP. For these experiments, a single solution of ternary complex was prepared using saturating concentrations of GTP, EF-Tu (or EF-Tu-EF-Ts) and Phe-tRNA^{Phe} (Cy3-acp^3U47) (10 µM, 12 µM and 3 µM, respectively). Portions of this reaction were subjected to gel filtration experiments, first in the absence, and then in the presence of GTP in the running buffer, while monitoring the elution time of Cy3-labeled Phe-tRNA^{Phe} by UV absorbance at 550 nm (Experimental Procedures). Both in the absence and presence of EF-Ts, detection of the Cy3-labeled Phe-tRNA^{Phe} ternary complex, eluting...
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at the predicted molecular weight of ~75,000Da, was maximized when GTP was included in the running buffer (Fig. 5). Consistent with the highly active nature of the components used in our investigations >90% of Cy3-labeled Phe-tRNA_{Phe} was found in a ternary complex under these conditions. When GTP was not included in the mobile phase, the amount of tRNA detected in ternary complex was significantly reduced in the absence of EF-Ts and completely abolished in the presence of EF-Ts. Analogous experiments conducted with 10 µM GDPNP in the mobile phase showed only ~5% of the total tRNA in ternary complex (Fig. 5). These findings provide compelling evidence in support of the dynamic nature of ternary complex. They also support a model in which ternary complex dissociation proceeds through sequential conformational events in EF-Tu that first enable GTP dissociation and then aa-tRNA release. Direct evidence that EF-Ts facilitates ternary complex dynamics — Our previous data indicate that EF-Ts accelerates the disassembly of ternary complex upon GDP addition. To differentiate between a model where EF-Ts only breaks down ternary complex in response to GDP addition or where EF-Ts is continually facilitating ternary complex formation and decay, we set out to directly monitor the nucleotide exchange process on EF-Tu using the fluorescent GTP analogue (10 µM), 2′/3′-O- (N-methyl-anthraniloyl)-guanosine-5′-triphosphate (mant-GTP) under conditions where ternary complex appears to be stable for extended periods (as seen in Fig. 3A). To ensure that the majority of EF-Tu is bound to aa-tRNA at steady state, ternary complex was formed at component concentrations >10-fold the apparent K_D (400 nM EF-Tu or EF-Tu-EF-Ts and 400 nM Phe-tRNA_{Phe}, 5 nM Phe-tRNA_{Phe} (Cy3-3′U47) (Fig. 2; Experimental Procedures). This experimental setup allowed us to monitor ternary complex formation by two distinct spectroscopic means in the same experiment: a change in Cy3 fluorescence intensity (Figs. 1, 3 and 4) and FRET between tryptophan 184 in EF-Tu and the mant moiety of the mant-GTP nucleotide (28). As expected for a reaction in which the mant-GTP nucleotide binds to EF-Tu, addition of the EF-Tu-EF-Ts complex to Phe-tRNA_{Phe} and mant-GTP resulted in a rapid increase in mant-GTP fluorescence intensity (Fig. 6, region II). Consistent with ternary complex formation, Cy3 fluorescence intensity also increased during this reaction.

To directly monitor the process of nucleotide exchange, a 10-fold molar excess of unlabeled GTP was added to the same reaction while monitoring mant-GTP fluorescence (Fig. 6, region III). Here, the mant-GTP signal rapidly returned to baseline (k_\text{turnover} ~0.6 ± 0.03 s\(^{-1}\)), while examination of the Cy3 fluorescence intensity showed that ternary complex remained intact (Fig. 6, region III). A slight increase in Cy3 fluorescence was also observed likely reflecting a subtle change in ternary complex stability in the presence of unlabeled GTP. In similar experiments using only EF-Tu the rate of the mant-GTP exchange was greatly reduced (k_\text{turnover} ~0.005 ± 4 x 10^{5} s\(^{-1}\), data not shown). These experiments demonstrate that the EF-Ts activities observed are not in response to an insult (such as GDP) but are instead actively occurring in the presence of GTP, where ternary complex appears stable. Inclusion of kirromycin in the experiment effectively blocked the mant-GTP nucleotide exchange process (data not shown). This finding suggests that rearrangements at the interface of domains 1 and 3 of EF-Tu are required for the exchange process.

DISCUSSION

In the present investigation, we have developed a means to explore the kinetic and thermodynamic properties of the E. coli ternary complex EF-Tu-GTP-Phe-tRNA_{Phe} using bulk fluorescence methods. Akin to earlier steady-state measurements of ternary complex formation (16), the fluorescence-based assays described here are based on changes in relative fluorescence intensity. However, unlike earlier work, the approach we describe is based on the environmentally sensitive Cy3 fluorophore (58,59) at a distinct site of tRNA attachment, the naturally occurring modified nucleotide, 3-(3-amino-3-carboxypropyl) uridine (acp^3U) present at position 47 in E. coli tRNA_{Phe}. While this modification is not as ubiquituous as the 4-thiouridine (s^4U) residue utilized previously, tRNA molecules modified at this site have been shown to be fully functional in all aspects of the translation process including aminoacylation, tRNA selection and translocation (43,46,60). The data presented show that the functionality of acp^3U-labeled tRNA_{Phe} also
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extends to ternary complex formation. In agreement with prior fluorescence investigations of ternary complex formation obtained under steady state conditions (16), we show that the formation of ternary complex strictly depends on the GTP nucleotide and the aminoacyl moiety of aa-tRNA. We also show that the EF-Tu-GTP-Phe-tRNA\textsuperscript{Phe} complex forms a high-affinity (nM K\textsubscript{D}) interaction (Fig. 1C) (17,19,20,23,25,26).

Unexpectedly however, we observed that the apparent affinity of ternary complex formation is significantly enhanced in the presence of EF-Ts, the GEF for EF-Tu (K\textsubscript{D} ~12 nM vs. ~47 nM; Table 1). These findings could not be rationalized based on trivial differences in EF-Tu activities in the absence and presence of EF-Ts (e.g. that 80% of the EF-Tu preparation was bound to GDP; Fig. 2). Moreover, the propensity of EF-Ts to decrease the efficiency with which ternary complex was formed in the presence of non-hydrolyzable GTP analogues also suggested an unanticipated function of EF-Ts as a fidelity determinant of ternary complex formation and stability (Fig. 1D). In striking contrast to contemporary models in which EF-Ts serves only as the GEF for EF-Tu (61,62), these findings collectively led us to examine whether EF-Ts functions to regulate ternary complex stability in a direct manner through modulation of the nucleotide binding site.

In line with previous investigations using yeast components (63), pre-steady state measurements obtained under conditions designed to approximate the cellular concentrations of aa-tRNA, GTP and EF-Tu suggested that the bacterial ternary complex forms via a rate-limiting conformational event in EF-Tu that occurs at a rate of ~20 s\textsuperscript{-1}. However, in the presence of cellular concentrations of EF-Ts, ternary complex formation was observed to proceed at a rate 4-times faster (~85 s\textsuperscript{-1}) (Fig. 4). As the fluorescence signal detected here depends exquisitely on the presence of the aminoacyl moiety and the nature of the \gamma-phosphate of the GTP nucleotide, this conformational change likely reports on the ordering of S1, S2 and P loop elements in the G domain of EF-Tu, the structural scaffold of the nucleotide binding pocket (Fig. 1A, 1B).

A trivial explanation for the observed disparity in rates would be that EF-Ts drives ternary complex formation by accelerating nucleotide loading (eq. 1 and 2). However, in this case the apparent rates of ternary complex formation would eventually plateau at the same maximum value (~85 s\textsuperscript{-1}). Instead, two distinct maximum rates were observed. Moreover, these experiments were performed following pre-incubation of EF-Tu and the EF-Tu-EF-Ts complex with saturating concentrations of GTP (1 mM). We conclude that EF-Ts directly facilitates ternary complex formation by accelerating EF-Tu-GTP loading onto aa-tRNA. Indeed, direct interactions between EF-Ts and ternary complex have been suggested previously (18,64,65). Such insights suggest that the established model of ternary complex formation (eq. 1-3 above) needs revision. Thus, although the direct binding of EF-Tu-GTP to aa-tRNA is feasible (eq. 3) (17,19,20,22,66-68), our findings suggest that the kinetically favored pathway for ternary complex formation proceeds via the direct binding of an EF-Tu-GTP-EF-Ts complex to aa-tRNA (Fig. 7).

While previous investigations have shown that the EF-Tu-GTP-EF-Ts complex is labile, dissociating at a rate of 60 s\textsuperscript{-1} (28), at cellular concentrations of EF-Ts (ca. 10 μM) (29-31), the rate of EF-Ts re-binding to EF-Tu-GTP is estimated to be on the order of 300 s\textsuperscript{-1} (28). These considerations predict that the EF-Tu-GTP-EF-Ts complex may be relatively abundant in the cell. Based on these insights, we conclude that EF-Ts directly facilitates ternary complex formation and disassembly by lowering the effective activation barrier for a rate-determining conformational change in EF-Tu that is required for the protein to fully engage aa-tRNA and GTP ligands (Fig. 7). Our nucleotide binding studies suggest that ordering of the switch I helix in EF-Tu plays a critical role in this process (Fig. 1D). We speculate that EF-Ts influences the folding of the switch I helix in a manner that modulates this commitment step. Further experiments will be required to delineate precisely how EF-Ts impacts the reaction coordinate for ternary complex formation and disassembly.

These previously unanticipated functions of EF-Ts may provide several advantages to the cell. First, the observed capacity to increase the rate of ternary complex formation may increase the maximum rate of translation that can be achieved in the cell. Second, under steady state conditions, EF-Ts’s capacity to facilitate nucleotide turnover may allow a means of translational control under
changing cellular conditions. For instance, under conditions of rapid growth where the concentration of GTP (900 µM) is in great excess over GDP (100 µM), EF-Ts ensures that ternary complex is abundant. However, during stress where the GTP/GDP ratio drops, EF-Ts may enable an energy neutral means of lowering the cellular concentrations of ternary complex and thus the rate of translation. The inferred capacity of EF-Ts to directly act on the EF-Tu·GDP·aa-tRNA complex also has potentially important implications for the mechanism of translation. Indeed, an EF-Tu·GDP·aa-tRNA complex is formed on the ribosome during every step of the elongation cycle of protein synthesis. Therefore, during active growth, the concentration of EF-Tu·GDP·aa-tRNA approaches the concentration of ribosomes in the cell (ca. 10 µM). Notably, the concentrations of EF-Ts and ribosomes have been shown to exhibit a 1:1 stoichiometry over a range of cellular conditions (29-31). To our knowledge, the kinetic impact of EF-Ts on the rates and fidelities of aa-tRNA selection have yet to be explored. However, experiments performed in the absence of EF-Ts suggest that the departure of EF-Tu·GDP from the ribosome is rate determining to the process (ca. 2-4 s⁻¹) (67,68).

The structure of the EF-Tu·GTP-EF-Ts complex is not presently known but is speculated to adopt a conformation that is distinct from EF-Tu·GTP, EF-Tu·GDP and the EF-Tu-EF-Ts complex (28). Structural modeling suggests that an EF-Tu·GTP-EF-Ts complex would be sterically permitted to bind the acceptor stem of aa-tRNA if it adopts a conformation that is globally similar to that of the EF-Tu-EF-Ts complex (Fig. 8). Functional investigations of the QBeta replicase, an RNA-dependent RNA polymerase that contains an EF-Tu-EF-Ts complex, are consistent with this model, as the template recognition in this system has been attributed to the direct binding of the EF-Tu-EF-Ts component of QBeta replicase to a primer region that contains a single-stranded 3′-CCA overhang (69). We therefore speculate that initial interactions between the EF-Tu·GTP-EF-Ts and aa-tRNA may occur in a manner akin to what is observed for QBeta replicase - and more recently for archaeal translation initiation factor-2 (70) - wherein domains 1 and 2 of EF-Tu bind the 3′-CCA end of aa-tRNA. Alternatively, initial interactions could occur via the direct binding of domains 2 and 3 of EF-Tu in complex with EF-Ts to the acceptor stem helix (Fig. 8). Irrespective of these considerations, we propose that the rate-determining conformational change in EF-Tu that gives rise to the high affinity ternary complex interaction initiates with the compaction of the G domain onto domains 2 and 3 and the aa-tRNA acceptor stem. This conformational change would terminate with the formation of the interaction network linking the amino acid to the switch domains of EF-Tu and the γ-phosphate of GTP, followed by the release of EF-Ts (Figs. 1 and 8).

In direct support of a model in which EF-Ts can physically associate with EF-Tu while it is bound to aa-tRNA, we find that EF-Ts actively dissociates ternary complex in the presence of GDP (Fig. 3C, D). Ternary complex dissociation was inhibited by kirromycin (Fig. 3F), suggesting that the dissociation pathway likely entails reorganization of the interface of domains 1, 2 and 3 in EF-Tu prior to EF-Tu release in a manner that mirrors the association pathway.

Importantly, precedence for direct interactions between GEFs and activated G-proteins exists in signal transduction cascades involving other small G-proteins, which are regulatory in nature. In yeast, the GEF Scd1 chaperones its activated G-protein, Cdc42, to its effector Shk1 via interactions with Scd2 (71). GEFs for the monomeric G-protein Ral, RalGDS and Rlf, interact with the GTP-bound state of upstream G-proteins Ras and Rap through Ras-association domains (3,72). Effector-bound conformational changes of G-proteins have also been previously described. For instance, the monomeric G-protein, Ras, has been shown to bind to its effector protein, Raf, in a two-step process termed “dynamic triggering” (73). NMR studies have also revealed that switch 1 of Ras-GDPNP, is dynamic, exhibiting at least two conformations in the Raf-bound state (73,74). Thus, the findings presented here likely reflect a more general capacity of GEFs to facilitate G-protein loading onto their effector substrates. In addition to accelerating signaling processes, such interactions may also serve to influence the choice of downstream effectors. Future experiments testing these hypotheses are warranted and will need to be explored using approaches that are amenable to probing the complexity and range of dynamics that are likely to be found.
REFERENCES


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FOOTNOTES

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2The abbreviations used in this manuscript are: EF-Tu, elongation factor Tu, EF-Ts, elongation factor Ts, aa-tRNA, aminoacyl-transfer RNA, GEF, guanosine nucleotide exchange factor, GTPγS, guanosine 5′-[γ-thio]triphosphate, GDPNP, guanosine 5′-[β,γ-imido]triphosphate, and mant-GTP, 2,7′-O-(N-methyl-anthraniloyl)-guanosine-5′-triphosphate.

FIGURE LEGENDS

FIGURE 1. Ternary complex structure and steady-state measurements of ternary complex formation. A) Structure of ternary complex stabilized by the antibiotic kirromycin (pdb accession 1OB2): E. coli EF-Tu (blue) bound to GDPNP (carbon atoms (grey), nitrogen (blue), oxygen (red), and phosphorous atoms (orange)), complexed with S. cerevisiae Phe-tRNA^Phe^ (wheat). Domains of EF-Tu are represented as D1, D2, and D3. For simplicity, kirromycin is not shown. B) Specific functional elements are highlighted: switch-1 (S1) (red), switch-2 (S2) (orange), histidine 66 (H66) (green), and the Phe amino acid (purple). C) The affinity of EF-Tu for aminoacyl-tRNA was determined by titrating EF-Tu (open diamonds) or EF-Tu·EF-Ts (closed squares) into a solution of Cy3-labeled Phe-tRNA^Phe^ and 10 µM GTP. An identical titration of EF-Tu was performed with either deacylated tRNA^Phe^ (circles) or in the absence of GTP (blue squares). D) The apparent nucleotide affinity was measured by titrating GTP into a cuvette containing Phe-tRNA^Phe^ (Cy3-acp^3^U47) and EF-Tu (open diamonds) or EF-Tu·EF-Ts (closed diamonds). Identical titration experiments of GDPNP with EF-Tu (open triangles) and EF-Tu·EF-Ts (closed triangles) and GTPγS with EF-Tu (open squares) or EF-Tu·EF-Ts (closed squares) are shown. Error bars represent the standard error of 3 separate experiments. Estimates of the apparent K_D were obtained by fitting the titration data as described in Experimental Procedures. Data points were splined for clarity.

FIGURE 2. EF-Tu is highly active in both the absence and presence of EF-Ts. The fraction of active EF-Tu molecules present in our protein preparation was determined by titrating EF-Tu (open squares) or EF-Tu·EF-Ts (closed diamonds) into a solution of 400 nM Phe-tRNA^Phe^ (Cy3-acp^3^U47) in the presence of 10 µM GTP. Linear fits of the initial and final ten data points of these two experiments intercept at 517 nM and 496 nM for EF-Tu and EF-Tu·EF-Ts, respectively, indicating that EF-Tu is ~ 80% active in the absence and presence of EF-Ts.

FIGURE 3. Pre-steady state measurements of ternary complex formation and dissociation: dependence on factor concentration. A) The time-dependent response in fluorescence intensity observed upon addition of saturating amounts (400 nM) of either EF-Tu (blue) or EF-Tu·EF-Ts (black) to Cy3-labeled Phe-tRNA^Phe^ (5 nM) and GTP (10 µM) or EF-Tu·EF-Ts in the absence of GTP (grey). Fitting the data (See Experimental Procedures) provided a quantitative measure of the apparent rate of ternary complex formation, k_app,1. A focused plot of the formation process is
also shown (inset). B) Measurements of $k_{app,1}$ as a function of either EF-Tu (open diamonds) or EF-Tu·EF-Ts (closed squares). Inset shows the linear fits of early factor titration data points. C) The time-dependent response in fluorescence intensity observed upon addition of saturating amounts of GDP (100 μM) to ternary complex pre-formed as described in A) with either EF-Tu (blue) or EF-Tu·EF-Ts (black). Identical experiments performed using ADP (100 μM) (grey). D) Fitting to a single exponential function provided a quantitative measure of the off-rate of ternary complex formation, $k_{app,2}$ as a function of either EF-Tu (open diamonds) or EF-Tu·EF-Ts (closed squares) as described in C). E) Similar disassociation experiments were performed at varying GDP concentrations. Addition of saturating GDP to ternary complex pre-formed with an excess of EF-Tu (open diamonds) or EF-Tu·EF-Ts (closed squares) and 5 nM Phe-tRNA$^{Phe}$ in the presence of GTP (50 nM – 500 μM). Apparent decay rates, $k_{app,2}$, were estimated by fitting to a single exponential. F) GDP (100 μM) was delivered to ternary complex preformed with EF-Tu·EF-Ts in the absence (black) or presence (tan) of kirromycin. The rate of GDP mediated dissociation in the absence of kirromycin ($k_{app,2} = 0.28 \text{ s}^{-1}$) was found to be 14 times faster than in the presence of kirromycin ($k_{app,2} = 0.02 \text{ s}^{-1}$). Error bars represent the standard error from 3 independent experiments.

FIGURE 4. EF-Ts accelerates the rate-determining step in EF-Tu binding to aa-tRNA. Ternary complex was formed by rapid mixing of 200 nM Phe-tRNA$^{Phe}$ (Cy3-acp$^3$U47) with EF-Tu or EF-Tu·EF-Ts pre-incubated in the presence of 1 mM GTP. Time-courses of complex formation were fit to a single exponential and apparent rates plotted as a function of EF-Tu (open diamonds) or EF-Tu·EF-Ts (closed squares) concentration. Under these conditions, the apparent rate of complex formation observed asymptotically approached ~20 s$^{-1}$ for EF-Tu and ~85 s$^{-1}$ for EF-Tu·EF-Ts.

FIGURE 5. Physical isolation of ternary complex. Ternary complex, formed in the presence of saturating concentrations of EF-Tu (circles) or EF-Tu·EF-Ts (diamonds), Cy3-labeled Phe-tRNA$^{Phe}$ and GTP (Experimental Procedures), was fractionated over a Superdex-75 gel filtration column in the presence (black) or absence (red) of GTP (10 μM) in the running buffer. Absorbance was recorded at 550 nm, and 260 nm to specifically track the elution times of “unbound” and “ternary complex” bound Cy3-labeled Phe-tRNA$^{Phe}$ as indicated. This was repeated with GDPNP in the reaction and running buffer (blue triangles).

FIGURE 6. EF-Ts directly facilitates ternary complex turnover. Region I) The relative fluorescence intensities Cy3 (green, right axis) and mant (black, left axis) obtained from a solution containing 400 nM Phe-tRNA$^{Phe}$ (unlabeled), 5 nM Phe-tRNA$^{Phe}$ (Cy3-acp$^3$U47) and 10 μM mant-GTP in the absence of factor. Region II) The increase in mant fluorescence intensity (black, left axis) and Cy3 fluorescence intensity (green, right axis) resulting from addition of 400 nM EF-Tu·EF-Ts to the mixture. Region III) Addition of saturating amounts of unlabeled GTP (100 μM) resulted in a rapid decrease ($k_{turnover} = 0.6 \pm 0.03 \text{ s}^{-1}$) in the mant signal (black, left axis), while the Cy3 signal (green, right axis) exhibited a small increase in intensity.

FIGURE 7. Ternary complex formation and disassembly can occur via two distinct pathways. In both pathways, the binary complex EF-Tu·EF-Ts binds GTP forming an EF-Tu-GTP·EF-Ts complex. In pathway 1, this species directly binds aa-tRNA forming a quaternary complex of EF-Tu-GTP·EF-Ts·aa-tRNA, which decays to the EF-Tu-GTP·aa-tRNA ternary complex following EF-Ts dissociation. In pathway 2, EF-Ts dissociates from the EF-Tu-GTP·EF-Ts complex allowing EF-Tu·GTP to bind aa-tRNA directly to form ternary complex.

FIGURE 8. Hypothetical EF-Tu·GTP·EF-Ts·Phe-tRNA$^{Phe}$ quaternary complex. A) A top-down perspective of the EF-Tu·GTP·Phe-tRNA$^{Phe}$ ternary complex (pdb accession code 1OB2)
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showing EF-Tu (blue) bound to Phe-tRNA^{Phe} (wheat) and GDPNP (green spheres). Here, the center panel shows an “extended” conformation (purple) of EF-Tu as it is observed in the *E.coli* EF-Tu-EF-Ts crystal structure (pdb accession code 1EFU), where domains 2 and 3 of both EF-Tu structures are superimposed (N, Cα, CO, O RMSD = 0.886Å). The right panel highlights the position of EF-Ts (red) in a quaternary complex if its interactions with EF-Tu are identical to those observed in the *E.coli* EF-Tu-EF-Ts crystal structure. This model reveals only minor steric clashes between EF-Ts and the D-stem of the tRNA. The blue arrow indicates the hypothesized motions of EF-Tu’s G domain during ternary complex formation and decay. B) Identical structures as in A) from the perspective of looking down the axis of the tRNA acceptor stem. Structures were analyzed and rendered in PyMol.
TABLE 1: Equilibrium dissociation constant of ternary complex. Ternary complex was formed by titrating either Factor or Nucleotide in the absence or presence of EF-Ts.

<table>
<thead>
<tr>
<th>Dissociation Const.</th>
<th>EF-Ts (-)</th>
<th>EF-Ts (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K_d (nM)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor</td>
<td>47 ± 3.1</td>
<td>12.6 ± 1.1</td>
</tr>
<tr>
<td>GTP</td>
<td>195 ± 25</td>
<td>685 ± 35</td>
</tr>
<tr>
<td>GDPNP</td>
<td>7000 ± 100</td>
<td>9270 ± 105</td>
</tr>
<tr>
<td>GDPγS</td>
<td>240 ± 18</td>
<td>490 ± 41</td>
</tr>
</tbody>
</table>

TABLE 2: Summary of kinetic parameters of ternary complex. Kinetic descriptors of ternary complex were determined from the apparent rates of ternary complex formation at 10 μM GTP.

<table>
<thead>
<tr>
<th>Kinetic Parameters</th>
<th>(^*k_{on} (\text{μM}^{-1} \text{s}^{-1}))</th>
<th>(^*k_{off} (\text{s}^{-1}))</th>
<th>(^*k_{\text{turnover}} (\text{s}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF-Tu</td>
<td>1.2 ± 0.07</td>
<td>0.05 ± 0.004</td>
<td>0.005 ± 4 × 10^{-5}</td>
</tr>
<tr>
<td>EF-Tu·EF-Ts</td>
<td>1.7 ± 0.1</td>
<td>0.07 ± 0.006</td>
<td>0.6 ± 0.03</td>
</tr>
</tbody>
</table>

*Values are obtained from data presented in Fig. 3.
†Values are obtained from data presented in Fig. 6.
Figure 1
Figure 2
Dynamic properties of the EF-Tu-GTP-aminoacyl-tRNA ternary complex

Figure 3
Figure 4
Dynamic properties of the EF-Tu-GTP-aminoacyl-tRNA ternary complex

Figure 5
Figure 6
Figure 7

Dynamic properties of the EF-Tu-GTP-aminoacyl-tRNA ternary complex
Dynamic properties of the EF-Tu-GTP-aminoacyl-tRNA ternary complex

Figure 8
Elongation Factor-Ts Directly Facilitates the Formation and Disassembly of the
Escherichia Coli Elongation Factor-Tu-GTP-Aminoacyl-tRNA Ternary Complex
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