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The Busiest of All Ribosomal Assistants: Elongation Factor Tu

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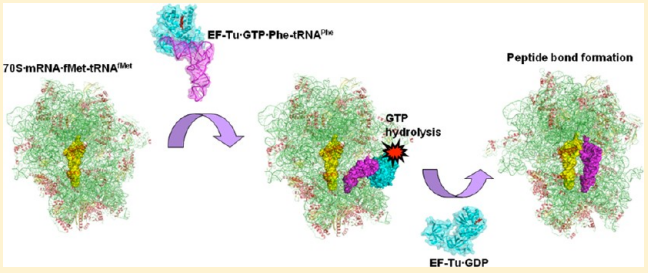
ABSTRACT: During translation, the nucleic acid language employed by genes is translated into the amino acid language used by proteins. The translator is the ribosome, while the dictionary employed is known as the genetic code. The genetic information is presented to the ribosome in the form of a mRNA, and tRNAs connect the two languages. Translation takes place in three steps: initiation, elongation, and termination. After a protein has been synthesized, the components of the translation apparatus are recycled. During each phase of translation, the ribosome collaborates with specific translation factors, which secure a proper balance between speed and fidelity. Notably, initiation, termination, and ribosomal recycling occur only once per protein produced during normal translation, while the elongation step is repeated a large number of times, corresponding to the number of amino acids constituting the protein of interest. In bacteria, elongation factor Tu plays a central role during the selection of the correct amino acids throughout the elongation phase of translation. Elongation factor Tu is the main subject of this review.



The ribosome is the key player in translation and is assisted by protein factors during all phases of translation. The ribosome has binding sites for mRNA and tRNAs and coordinates their interplay very accurately. The A site accepts incoming aminoacylated tRNAs; the P site carries a tRNA esterified to the growing polypeptide chain, while empty tRNAs are bound in the E site. The ribosome (70S in bacteria) is organized into two subunits: decoding takes place within the small subunit (30S), while formation of peptide bonds between amino acids is catalyzed by the large subunit (50S).

During the elongation phase of protein synthesis, amino acids coupled to their cognate tRNAs are selected in a stepwise manner on the basis of correct base pairing between the codon exposed in the A site and the anticodon of an incoming tRNA. In bacteria, the aa-tRNA is brought to the ribosome by translation elongation factor Tu (EF-Tu) as part of a ternary complex, EF-Tu·GTP·aa-tRNA. Several aa-tRNAs may be tested by EF-Tu before the recognition of a cognate tRNA. The correctly matched codon−anticodon minihelix induces a series of conformational changes causing GTP hydrolysis by EF-Tu. The resulting EF-Tu·GDP releases aa-tRNA into the ribosomal A site and dissociates from the ribosome. The amino acid of the newly delivered aa-tRNA is coupled to the growing polypeptide chain via peptide bond formation.

BASIC BIOCHEMISTRY OF EF-TU

Escherichia coli has two EF-Tu-encoding genes, *tufA* and *tufB*. Both genes give rise to 393-amino acid proteins, which are organized into three structural domains. EF-TuA and EF-TuB differ only at their C-terminal amino acid with no apparent biochemical consequence, and either of the *tuf* genes can be deleted without a loss of viability.[1] The amount of EF-Tu in the cell is equimolar to that of tRNA, while the total number of EF-Tu molecules is 8−14 times the number of ribosomes depending on growth conditions.[2]

EF-Tu binds GDP, GTP, and several other guanosine-containing polyphosphates (ppGpp, pppGpp, dGDP, GDPCP, and GDPNP) with high affinity, whereas nucleotides of other bases and GMP are bound very weakly. Removal of the Mg^{2+} cofactor reduces the affinity for guanine nucleotides by 3−4 orders of magnitude.[3] The affinity for GDP is approximately 100 times higher than for GTP. This difference in affinities is eliminated upon removal of domains 2 and 3,[4] indicating that these domains are important to the allosteric regulation of EF-Tu function. The spontaneous dissociation of GDP from EF-Tu is very slow (0.002 s^{-1}) and without physiological relevance. Thus, the reactivation of EF-Tu after amino acid delivery is stimulated by its guanine nucleotide exchange factor EF-Ts.[5] Guanine nucleotide exchange occurs via the formation of a labile EF-Tu·GDP·EF-Ts complex from which GDP dissociates (Figure 1, step 9). The resulting binary complex is stable but can bind GTP (or rebind GDP) and dissociate into EF-Tu·GTP upon the release of EF-Ts (Figure 1, step 10). The equilibrium is driven toward formation of the active form of EF-Tu by the high cellular concentration of GTP (0.9 mM vs 0.1 mM for GDP) and the subsequent formation of the ternary complex, EF-Tu·GTP·aa-tRNA (Figure 1, step 11).[5]

EF-Tu·GTP has a high affinity for aa-tRNA (K_d in the nanomolar range).[6] The affinities for different, correctly aminoacylated species of aa-tRNA vary by only 1 order of magnitude because of the phenomenon of thermodynamic compensation,[7] which implies that amino acids that make a

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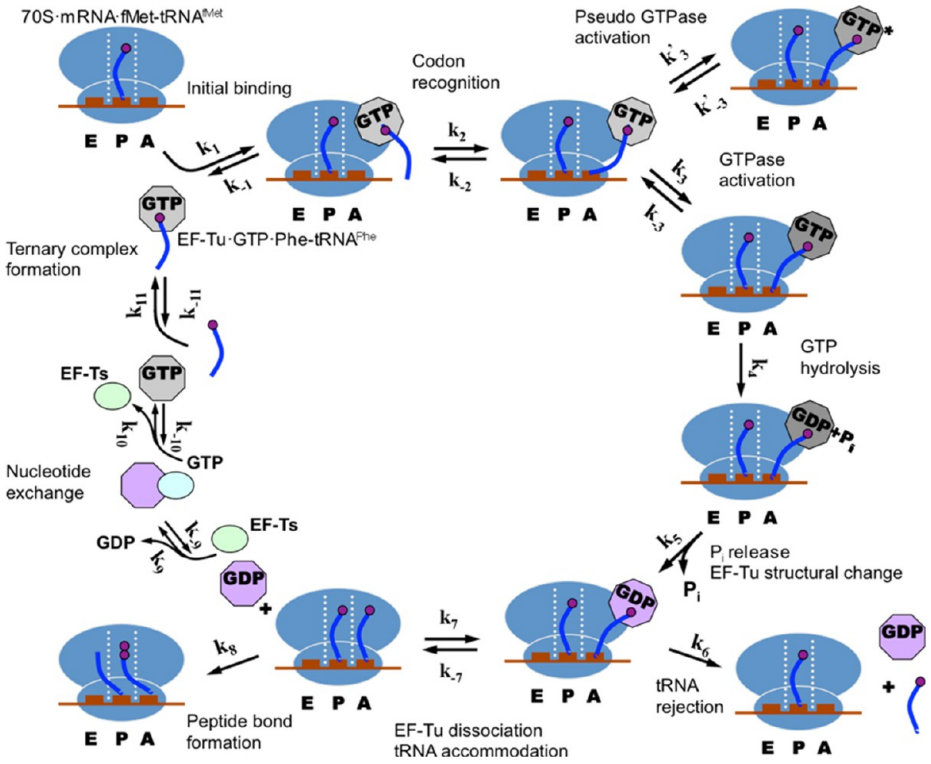

Figure 1. Functional cycle describing the kinetic steps of EF-Tu as defined by pre-steady state kinetics and/or single-molecule FRET studies. The kinetic constants define the following steps: k_1 and k_{-1}, initial binding of the ternary complex to the 70S initiation complex; k_2 and k_{-2}, codon recognition; k_3 and k_{-3}, GTPase activation; $k_{3'}$ and $k_{-3'}$, pseudo-GTPase activation; k_4, GTP hydrolysis; k_5, P_i release and subsequent EF-Tu conformational change; k_6, tRNA rejection; k_7 and k_{-7}, EF-Tu dissociation and aa-tRNA accommodation; k_8, peptide bond formation; k_9 and k_{-9}, binding of EF-Ts to EF-Tu·GDP and subsequent dissociation of GDP; k_{10} and k_{-10}, binding of GTP to EF-Tu and release of EF-Ts; k_{11} and k_{-11}, ternary complex formation. Different colors of EF-Tu represent distinct conformations. The kinetic steps were drawn according to ref 39, 40, and 43.

large contribution to the binding affinity for EF-Tu are bound to tRNAs with a smaller contribution to EF-Tu binding and vice versa. In this way, a uniform affinity of properly aminoacylated aa-tRNA for EF-Tu results with the purpose of ensuring an adequate rate of protein synthesis.[8] In contrast, misacylated aa-tRNAs display either significantly stronger or weaker binding to EF-Tu. A weakly binding aa-tRNA suffers from difficulties in being delivered to the ribosome at a proper frequency, while a strongly binding aa-tRNA will limit the rate of peptide bond formation because of its slow dissociation from EF-Tu after GTP hydrolysis.[9] In bacteria, the affinity of a tRNA for EF-Tu is adjusted via three T-stem base pairs, which interact with three amino acids in EF-Tu.[8]

The GTPase mechanism of EF-Tu and other G-binding proteins follows an in-line, S_N2 reaction pathway with inversion of configuration at the γ-phosphate.[10] The nature of the transition state is still a matter of dispute and may be anywhere between dissociative or associate depending on whether breaking of the linkage between the γ-phosphate and the GDP has already taken place when the nucleophilic water approaches or bond making to the nucleophile has taken place prior to bond breaking, respectively. The latter reaction pathway is characterized by a pentacoordinate transition state, in which the nucleophile and the leaving group occupy the apical positions of the trigonal bipyramid.

The intrinsic GTPase activity of EF-Tu is extremely slow compared to most other G-binding proteins ($<10^{-5}$ s^{-1}).[11] In comparison, the physiological rate of protein synthesis is 10−20 amino acids/s. The key effector of EF-Tu's GTPase activity is the programmed ribosome, which causes a 10^7-fold increase in activity.[12] The mechanism of intrinsic GTP hydrolysis by EF-Tu was heavily debated in the last half of the 1980s and the beginning of the 1990s,[13] but from the mid-1990s, the focus has been on the mechanism of GTP hydrolysis as it occurs on the ribosome during translation (see below).

EF-Tu can be targeted by four classes of antibiotics via two different mechanisms: (i) pulvomycin and GE2270A hinder formation of the ternary complex and might stabilize the EF-Tu·EF-Ts complex,[14] while (ii) kirromycin and enacyloxin IIa lock EF-Tu·GDP on the ribosome after delivery of aa-tRNA.[15]

STRUCTURAL STUDIES OF EF-TU OFF THE RIBOSOME

EF-Tu is the most abundant protein in the bacterial cell occurring in amounts of up to 5% and was therefore an amenable target for X-ray crystallography structural studies in the beginning of the 1970s. All available crystal structures of EF-Tu complexes off the ribosome are reported in Table 1. The first studies were conducted on crystals containing a truncated form of EF-Tu lacking part of the effector region (see below), which was generated during crystallization or by mild trypsin treatment. The molecule was found to consist of three domains arranged around a striking hole. The tight domain turned out to bind GDP/GTP and was later named domain 1 or the G-domain. The G-domain was soon after recognized as a common building block responsible for GDP/GTP binding in all G-binding proteins.[16] The G-domain of EF-Tu contains consensus sequences I [GxxxxGK(S/T); *E. coli* EF-Tu G_{18}HVDHGKT$_{25}$, "P-loop"], II (DxxG; *E. coli* EF-Tu D_{80}CPG$_{83}$), and III (NKxD; *E. coli* EF-Tu N_{135}KCD$_{138}$), as

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Table 1. Available Crystal Structure of EF-Tu Complexes off the Ribosome

source of EF-Tu	complex	resolution (Å)	PDB entry
E. coli	EF-Tu·GDP	2.05	1EFC
E. coli	EF-Tu·GDP	3.4	2FX3
E. coli	EF-Tu·GDP	2.5	1DG1
Thermus aquaticus	EF-Tu·GDP	2.7	1TUI
Bos taurus, mitochondria	EF-Tu·GDP	1.94	1D2E
Sulfolobus solfataricus	EF1-α·GDP	1.8	1SKQ
S. solfataricus	EF1-α·GDP	1.8	1JNY
Thermus thermophilus	EF-Tu·GDP·methylkirromycin	2.0	1HA3
E. coli	EF-Tu·GDP·thiocillin·GE2270	2.35	1D8T
E. coli	EF-Tu·GDP·tetracycline	2.12	2HCJ
E. coli	EF-Tu·GDP·tetracycline	2.8	2HDN
E. coli	EF-Tu·GDP·LFF571	2.7	3U2Q
T. thermophilus	EF-Tu·EF-Ts	3.0	1A1P
E. coli	EF-Tu·EF-Ts	2.5	1EFU
B. taurus, mitochondria	EF-Tu·EF-Ts	2.2	1XB2
T. thermophilus	EF-Tu·GDPNP	1.7	1EXM
T. aquaticus	EF-Tu·GDPNP	2.5	1EFT
T. thermophilus	EF-Tu·GDPNP·pulvomycin	1.4	2C78
T. thermophilus	EF-Tu·GDPNP·thiocillin·GE2270	1.6	2C77
E. coli	EF-Tu·GDPNP·enacyloxin IIa	2.3	2BVN
E. coli	EF-Tu·GDPNP·kirromycin·Phe-tRNA^{Phe}	3.3	1OB2
T. aquaticus	EF-Tu·GDPNP·Phe-tRNA^{Phe}	2.7	1TTT
T. aquaticus	EF-Tu·GDPNP·Cys-tRNA^{Cys}	2.6	1B23
T. aquaticus	EF-Tu·GDPNP·enacyloxin IIa·Phe-tRNA^{Phe}	3.1	1OB5
Aeropyrum pernix	EF1-α·GTP·Pelota	2.3	3AGJ
E. coli	EF-Tu·EF-Ts·β-subunit	2.5	3MMP
E. coli	EF-Tu·EF-Ts·β-subunit form I	2.8	3AGP
E. coli	EF-Tu·EF-Ts·β-subunit form II	3.2	3AGQ
E. coli	EF-Tu·EF-Ts·β-subunit; initiation stage; contains RNA template and 3′-dGTP	2.6	3AVT
E. coli	EF-Tu·EF-Ts·β-subunit; 7-nucleotide stage; contains 12-nucleotide RNA template and 7-nucleotide RNA product	2.9	3AVU
E. coli	EF-Tu·EF-Ts·β-subunit; 8-nucleotide stage; contains 12-nucleotide RNA template and 8-nucleotide RNA product	3.1	3AVV
E. coli	EF-Tu·EF-Ts·β-subunit; 9-nucleotide stage; contains 12-nucleotide RNA template, 8-nucleotide RNA product and 3′-dGTP	2.6	3AVW
E. coli	EF-Tu·EF-Ts·β-subunit; 10-nucleotide stage; contains 13-nucleotide RNA template, 9-nucleotide RNA product, and 3′-dGTP	2.4	3AVX
E. coli	EF-Tu·EF-Ts·β-subunit; 14-nucleotide stage; contains 18-nucleotide RNA template, 13-nucleotide RNA product, and 3′-dCTP	2.6	3AVY

well as the less conserved SAL motif (*E. coli* EF-Tu $S_{173}AL_{175}$). Consensus sequences I and II together with the effector region (*E. coli* EF-Tu G_{41}–T_{64}; named by homology with the ras p21 region interacting with effectors), a Mg^{2+} ion and water molecules coordinate the phosphate groups of the nucleotide, while consensus sequence III and the SAL motif interact with the guanine base (Figure 2C).[16]

In 1993, the structures of intact EF-Tu from two thermophilic bacteria appeared in their active conformation bound to the nonhydrolyzable GTP analogue, GDPNP.[17,18] The structures revealed a dramatic conformational change in domain 1 relative to domains 2 and 3 (compare panels A and B of Figure 2), leading to the disappearance of the central hole seen in the inactive form of EF-Tu from *E. coli*.[19] The origin of the rearrangement appeared to be in the switch I (*E. coli* EF-Tu D_{51}–T_{64}) and switch II (*E. coli* EF-Tu G_{83}–A_{95}) regions (Figure 2C). Still, the question of whether the conformational rearrangement was functionally relevant or only caused by the fact that the structures originated from EF-Tu of different species or related to the absence or presence of an intact switch I region remained. This discussion was not put to an end until 1996, when structures of intact EF-Tu·GDP from *Thermus aquaticus* and *E. coli* confirmed the significance of the previously observed domain rearrangement.[20,21]

The structures of EF-Tu bound to GDPNP reveal a water molecule positioned approximately 3.3 Å from the γ-phosphate of GTP that may potentially act as the nucleophilic water during GTP hydrolysis.[17,18] The water molecule is shielded from bulk solvent by the so-called "hydrophobic gate" comprised of the side chains of residues Val20 and Ile60 situated in the P-loop and switch I region, respectively. The water molecule needs to be activated by a general base and moved closer to the γ-phosphate to perform the hydrolytic attack leading to GTP hydrolysis. His84 of the switch II region was found to be the most likely candidate to cause activation of the water molecule. However, considerable rearrangements would be required for His84 to be positioned correctly for hydrolytic attack. The ribosome was suggested to induce the necessary structural change.[18]

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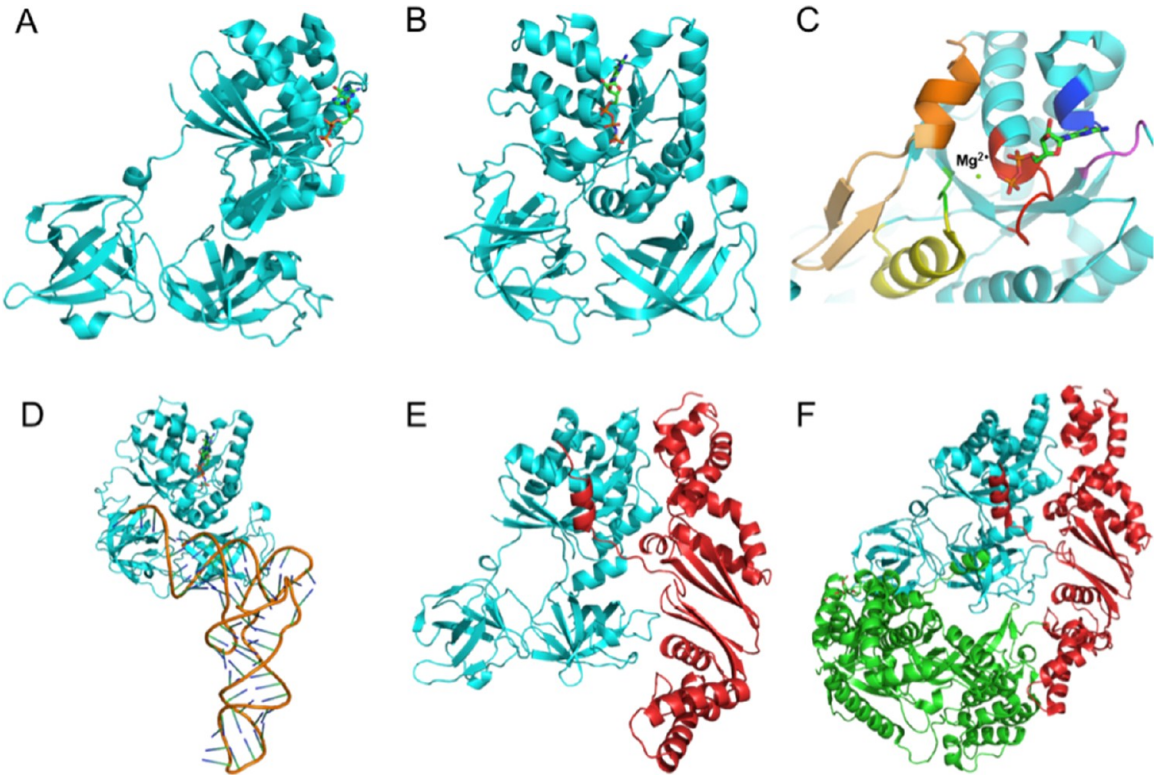

Figure 2. Structures of various EF-Tu complexes. (A) EF-Tu in its inactive, GDP-bound conformation (PDB entry 1DG1). (B) EF-Tu in the active, GTP-bound conformation (PDB entry 1EXM). (C) Close-up of the guanine nucleotide-binding pocket of EF-Tu·GDP (PDB entry 1DG1). Color-coded regions: consensus sequences I (red), II (green), and III (magenta), SAL motif (blue), effector region (orange and light orange), switch I (light orange), and switch II (yellow). (D) EF-Tu·GDPNP·Phe-tRNA^{Phe} ternary complex (PDB entry 1TTT). (E) EF-Tu in a complex with EF-Ts (PDB entry 1EFU). (F) EF-Tu in a complex with EF-Ts and the β-subunit constituting the Qβ replicase core complex (PDB entry 3MMP). The following color code is applied: red for EF-Ts, cyan for EF-Tu, and green for the β-subunit. All figures were prepared with PyMol.

Another important breakthrough in the structural studies of EF-Tu occurred when the structure of the ternary complex, EF-Tu·GDPNP·Phe-tRNA^{Phe} (Figure 2D), was determined in 1995.[22] The aminoacylated CCA-3′ end binds in a pocket formed between domains 1 and 2 of EF-Tu. The acceptor stem contacts the switch regions, while the T stem binds to domain 3. A later structure of the ternary complex containing Cys-tRNA^{Cys} suggested it was possible that the mode of aa-tRNA binding observed in the first structure was universal.[23]

In 1996, the structures of the complex between EF-Tu and EF-Ts from two different species became available (Figure 2E).[24,25] EF-Ts was found to bind across domains 1 and 3 of EF-Tu. In particular, a conserved phenylalanine residue from EF-Ts, Phe81, attracted attention because of its intrusion between two histidines, His84 and His118, in domain 1 of EF-Tu. A comparison of the crystal structures of EF-Tu·GDP and EF-Tu·EF-Ts suggested that the displacement of His118 would release the β-phosphate of GDP, while the displacement of His84 in the switch II region would disrupt the Mg^{2+} binding site. Finally, a movement of helix D was predicted to loosen the binding of the ribose and the guanine base.

In the past few years, the structures of different complexes containing antibiotics have been determined.[26] Enacyloxin and kirromycin were found to bind at the interface between domains 1 and 3, thereby locking the two domains in a conformation similar to the active one despite the nature of the bound nucleotide. Consequently, EF-Tu does not dissociate from the ribosome and aa-tRNA after GTP hydrolysis, thereby causing a "traffic jam" of ribosomes on the mRNA. The binding sites of pulvomycin and GE2270 overlap with the site used for binding of the 3′ end of aa-tRNA, thereby preventing its binding. In addition, pulvomycin is in contact with the amino acids normally engaged in the binding of the 5′ end of the tRNA.

When the RNA virus Qβ infects bacteria, the β-subunit encoded by the virus hijacks the host proteins EF-Tu, EF-Ts, and ribosomal protein S1 to accomplish genome replication.[27] The β-subunit is an RNA-dependent RNA polymerase, and the EF-Tu·EF-Ts complex is important for the initiation of amplification of the bacteriophage genome. Specifically, EF-Tu plays a role in template recognition and binding. Recently, the crystal structure of the Qβ replicase core complex composed of the β-subunit in complex with EF-Tu·EF-Ts has been determined at a resolution of 2.5 Å (Figure 2F).[28,29] The structure of the EF-Tu·EF-Ts unit within the Qβ replicase complex is identical to the previously determined structures of the isolated EF-Tu·EF-Ts complex (see above). Notably, two α-helices belonging to the C-terminal region of the β-subunit are placed in the proximity of parts of EF-Tu with important roles during translation: the first α-helix interacts directly with the CCA-binding pocket of EF-Tu, while the second is placed in the proximity of the switch regions of EF-Tu. Very recently, six structures of the Qβ replicase core complex bound to various RNAs representing template and product strands during different stages of replication from initiation to elongation were published.[30] When the double-stranded RNA consisting of the template and a nascent product RNA reaches a length of approximately 10 bp, the 3′ end of the template

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appears to enter an exit channel between the C-terminal region of the β-subunit and domains 2 and 3 of EF-Tu. In this way, EF-Tu may assist the β-subunit in peeling the product strand off the template strand during the elongation phase of replication. Thereby, exponential amplification of the bacteriophage genome is allowed, because both template and product will be released in their single-stranded form and be able to act as new templates in the next round of replication.[28]

FUNCTIONAL STUDIES OF EF-TU ON THE RIBOSOME

Since the 1960s, EF-Tu has been subjected to extensive biochemical studies aimed at deducing different aspects of the factor's multifunctionality during translation. Most recently, pre-steady state kinetic studies[31] and single-molecule techniques[32] have allowed the resolution of the elongation phase into a number of discrete steps (Figure 1) via the application of different fluorescent and radioactive probes.

By and large, the results obtained using these two types of techniques are in agreement with each other. The elongation cycle is subsectioned into the three processes of aa-tRNA selection, peptide bond formation, and translocation, which are managed by EF-Tu, 23S rRNA of the large ribosomal subunit assisted by the 3′ terminal 2′-OH group of P site-bound tRNA,[33] and EF-G,[34] respectively. The decoding process has so far been further divided into initial binding of the ternary complex, codon−anticodon recognition, GTPase activation, GTP hydrolysis, P_i release, and A site accommodation of the aa-tRNA (Figure 1). These steps play a vital role in ensuring a high fidelity when the anticodons of incoming aa-tRNAs are tested against the codon exposed in the ribosomal A site.

All cognate tRNA anticodons display very similar affinities for the A site as a result of post-transcriptional modifications in the anticodon regions. The binding of a cognate versus a noncognate anticodon can be easily distinguished in this manner, while near-cognate tRNAs with one mismatched base pair of the three possible exhibit a challenge during decoding. EF-Tu plays a central role in weeding out the near-cognate tRNAs.

Initial binding is a reversible reaction (Figure 1, step 1), which is independent of the codon exposed in the A site. Accordingly, the ribosomal site of initial binding is not overlapping with the A site.[35] Also, the subsequent step of codon recognition is reversible (Figure 1, step 2) but may lead to GTPase activation (Figure 1, step 3) if a proper codon−anticodon interaction is established. GTPase activation depends on a rate-limiting conformational change, which is transmitted via the tRNA and parts of the ribosome to the G-domain of EF-Tu (see below). GTPase activation is considerably faster (approximately 100−1000 times) for cognate tRNA species than for near-cognate species,[36−38] and near-cognate as well as noncognate ternary complexes may be rejected at this point without triggering GTP hydrolysis. Single-molecule FRET studies indicate that the ribosome with a bound ternary complex attempts to reach the GTPase-activated state several times via the reversible sampling of a pseudo-GTPase-activated state (Figure 1, step 3′). The sampling occurs more frequently for a cognate ribosome complex than for a near-cognate complex and with a higher success rate.[39] The cognate conformational changes associated with codon−anticodon recognition cause the proper positioning of the catalytic machinery and allow the chemical step of GTP hydrolysis (Figure 1, step 4). The cleaved-off γ-phosphate is then released whereupon the conformational switch of EF-Tu occurs (Figure 1, step 5). Dissociation of P_i appears to depend on the mobility of the switch 2 region and limits the rate of the structural rearrangement of EF-Tu into its inactive GDP-bound conformation.[40] As a consequence of the structural transition, the factor looses its affinity for the aa-tRNA and the ribosome and dissociates (Figure 1, step 7). The aa-tRNA is thereby set free to fully accommodate in the ribosomal A site, whereafter peptide bond formation can occur (Figure 1, step 8). This step, like the step of GTPase activation, is accelerated in the case of cognate tRNAs.[36] Alternatively, the aa-tRNA may be rejected at this point (Figure 1, step 6). Thus, the forward rate constants of GTPase activation and tRNA accommodation are selectively increased for cognate tRNAs relative to those for near-cognate ones via the mechanism of "induced fit". In summary, the sequence of events during decoding defines two possibilities of aa-tRNA selection occurring on the basis of their different stabilities on the ribosome.[41] The first possibility, termed "initial selection", occurs before GTP hydrolysis (Figure 1, reverse of step 2), while the second option occurs after and is known as "proofreading" (Figure 1, step 6). According to the current model of elongation, the irreversible nature of GTP hydrolysis separates the two selection steps, and their overall effect on accuracy is therefore a product of the individual error frequencies. Recent single-molecule FRET studies, however, indicate that the assumed strict separation of the two selective events by GTP hydrolysis may be an oversimplification of the actual steps occurring during tRNA selection on the ribosome. Most likely, fluctuations of aa-tRNA molecules also contribute to the accuracy of translation during both initial selection and proofreading.[42−44] This is in accordance with the proposed role of the tRNA molecule as a transmitter of signals in the decoding center of the 30S subunit to regions of the 50S subunit involved in GTPase activation and peptide bond formation,[45] respectively. Frequent excursions of the aa-tRNA positioned within the A site can be detected on the ribosome both before and after GTP hydrolysis with the possible aim of aligning ternary complexes and rRNA (rRNA) for GTP hydrolysis and peptide bond formation to occur.[42−44] Notably, cognate aa-tRNAs have been observed to make more frequent attempts than near-cognate ones to reach the accommodated state before GTP hydrolysis. These studies imply that GTP hydrolysis does not strictly partition the two selection steps; i.e., accommodation-like transitions can occur before GTP hydrolysis, while codon recognition-like excursions can take place after GTP hydrolysis. The precise role of tRNA fluctuations during selection is still unclear but may be a means by which the ternary complex searches for and establishes stabilizing contacts with the ribosome.

In vivo, the error frequency during decoding appears to vary among different codons (ranging from 10^{-3} to 10^{-4}) and is mainly determined by tRNA competition.[46] After translocation, erroneous decoding results in mismatched codon−anticodon pairs in the P and/or E site. Such mismatches have been reported to stimulate premature release of the peptide chain on sense codons as a mechanism of reducing the number of malfunctioning proteins in the cell.[47]

Apart from the vital role of EF-Tu in preventing missense reading of the genetic message, EF-Tu also helps in the prevention of translational read-through at stop codons as well as frameshifting. The responsible mechanisms, however, are poorly understood.[48]

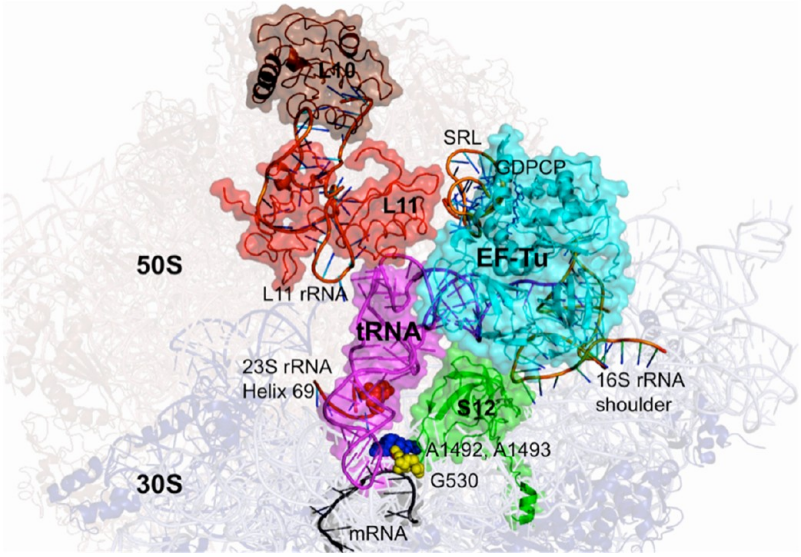

Figure 3. Structure of the EF-Tu·GDPCP·Trp-tRNA^{Trp} ternary complex bound to the 70S ribosome. Elements of the ribosome that make important contributions to binding of the complex are emphasized. EF-Tu and the tRNA are colored cyan and magenta, respectively. The figure was prepared with PyMol using PDB entries 2XQE and 2XQD. The structure does not contain density for any of the four copies of L12. However, L10 is indicated, which constitutes the platform for the anchoring of copies of L12.

Table 2. Available Cryo-EM and Crystal Structures of EF-Tu Complexes on the Ribosome

source of EF-Tu	complex	resolution (Å)	PDB, EMDB entries
	Crystal Structures		
T. thermophilus	EF-Tu·GDP·kirromycin·Thr-tRNA^{Thr}·70S	3.6	2WRN, 2WRO, 2WRQ, 2WRR
T. thermophilus	EF-Tu·GDPCP·Trp-tRNA^{Trp}·70S	3.2	2XQE, 2XQD
T. thermophilus	EF-Tu·GDP·kirromycin·Trp-tRNA^{Trp}·70S (cognate)	3.1	2Y10, 2Y11, 2Y18, 2Y19
T. thermophilus	EF-Tu·GDP·kirromycin·Trp-tRNA^{Trp}(G24A)·70S (cognate)	3.1	2Y14, 2Y15, 2Y16, 2Y17
T. thermophilus	EF-Tu·GDP·kirromycin·Trp-tRNA^{Trp}(G24A)·70S (near-cognate)	3.1	2Y0Y, 2Y0Z, 2Y12, 2Y13
T. thermophilus	EF-Tu·GDP·kirromycin·Trp-tRNA^{Trp}(A9C)·70S (near-cognate)	3.1	2Y0U, 2Y0V, 2Y0W, 2Y0X
	Cryo-EM Structures		
E. coli	EF-Tu·GDP·kirromycin·Phe-tRNA^{Phe}·70S	16.8	1LS2, 1045
E. coli	EF-Tu·GDP·kirromycin·Phe-tRNA^{Phe}·70S	13	1MJ1, 1004
E. coli	EF-Tu·GDP·kirromycin·Phe-tRNA^{Phe}·70S	9	1QZD, 1055, 1056
E. coli	EF-Tu·GDP·kirromycin·Phe-tRNA^{Phe}·70S	6.7	3FIH, 3FIK, 5036
T. thermophilus	EF-Tu·GDP·kirromycin·Phe-tRNA^{Phe}·70S	6.4	3FIC, 3FIN, 5030
E. coli	EF-Tu·GDP·kirromycin·Leu-tRNA^{Leu}·70S	12	3EQ4, 1564
E. coli	EF-Tu·GDP·kirromycin·Trp-tRNA^{Trp}·70S	9	3EQ3, 1565
E. coli	EF-Tu·GDP·kirromycin·Trp-tRNA^{Trp}·70S (cognate)	8.25	3IZU, 3IZW, 1849
E. coli	EF-Tu·GDP·kirromycin·Trp-tRNA^{Trp}·70S (near-cognate)	13.2	3IZT, 3IZV, 1850
T. thermophilus	EF-Tu·GDP·kirromycin·tmRNA·70S·SmpB	13	1ZC8, 1122
T. thermophilus	EF-Tu·GDP·kirromycin·tmRNA·70S·(SmpB)$_2$·S$_1$	13	1311, 1312

STRUCTURAL STUDIES OF EF-TU ON THE RIBOSOME

For the past four decades, researchers have been struggling to obtain structural information about the ribosome by using cryo-electron microscopy (cryo-EM), neutron scattering, and X-ray crystallography techniques. The lack of sophisticated tools and difficulties in getting a high-quality sample postponed the discovery until 2000, when the first high-resolution structures of the 30S[49] and 50S[50] subunits were reported. Already, one year later, the structure of the 70S complex containing mRNA and tRNAs bound in the A, P, and E sites was published at 5.5 Å resolution.[51]

X-ray studies of the 30S subunit during decoding have shed light on how highly conserved bases in 16S rRNA [G530, A1492, and A1493 (Figure 3)] monitor the formation of correct Watson−Crick base pairs at the first and second codon positions via formation of hydrogen bonds within the minor groove of a correctly formed base pair, which displays a characteristic stereochemical geometry.[52] Furthermore, the establishment of a cognate codon−anticodon minihelix in the decoding center induces a characteristic rotation of the head and shoulder domains of the 30S subunit termed "domain closure". This is a prerequisite for the subsequent steps in the tRNA selection process involving EF-Tu.

The binding of EF-Tu·GTP·aa-tRNA to the ribosome is transient but can be stabilized in the presence of antibiotics and/or a nonhydrolyzable GTP analogue, which has allowed structural studies of the ribosome-bound complex by cryo-EM or X-ray crystallography. The available structures of EF-Tu complexes bound to the ribosome are summarized in Table 2. The first view of the ternary complex on the ribosome was

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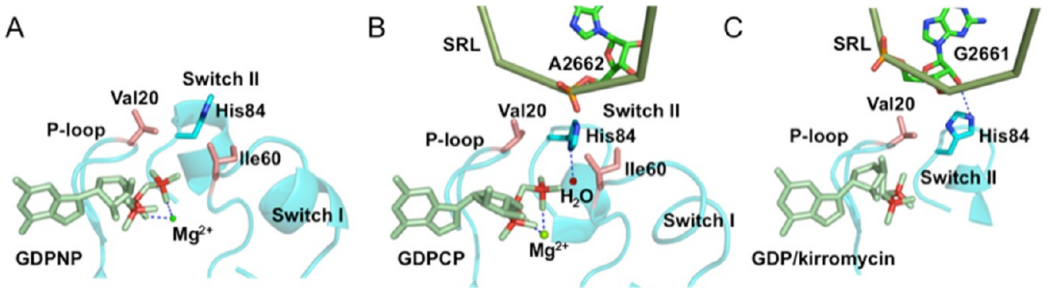

Figure 4. Snapshots of the GTP binding site of EF-Tu illustrating the structural changes accompanying GTPase activation and GTP hydrolysis. (A) Structure of the isolated ternary complex, EF-Tu·GDPNP·Phe-tRNA^{Phe} (PDB entry 1TTT), illustrating the GTPase incompetent conformation with a closed hydrophobic gate (Val20 and Ile60) and the catalytic His84 pointing away from the γ-phosphate. (B) Structure of the GTPase-activated state of EF-Tu·GDPCP·Trp-tRNA^{Trp} bound on the 70S ribosome (PDB entries 2XQE and 2XQD). One wing of the hydrophobic gate has opened, and His84 has been positioned for activation of the catalytic water molecule by interaction with the conserved A2662 of the sarcin–ricin loop (SRL). (C) Posthydrolytic state illustrated by the structure of EF-Tu·GDP·kirromycin·Trp-tRNA^{Trp} on the 70S ribosome (PDB entries 2WRN and 2WRO). After P_i dissociation, His84 rotates away from the GDP molecule and is stabilized in this position by G2661 of the SRL. The switch I region becomes disordered and is not visible in the structure.

reported in 1997,[53] where cryo-EM showed an aa-tRNA molecule with an aminoacyl end bound to EF-Tu and an anticodon end interacting with the decoding center of the small ribosomal subunit. The conformation was suggested to correspond to an A/T state, where testing of the codon–anticodon match is taking place. Later, studies at higher resolutions revealed that the occupation of this state required the tRNA to bend and twist to allow its simultaneous binding of the mRNA codon and EF-Tu.[54] Evidence has been obtained indicating that this is a universal mechanism of aa-tRNA testing,[55] and EF-Tu seems to ensure that all aa-tRNAs are presented in the same way to the ribosome.

The first encounter between the 70S ribosome and EF-Tu·GTP·aa-tRNA is supposed to occur via the "stalk" of the 50S subunit, which is a flexible protrusion consisting of four copies of L12 proteins (*E. coli*) bound as dimers to protein L10 close to L11 (Figure 3).[56,57] Subsequently, the long, unstructured hinge of L12 as well as the flexible connection between L10 and L12 may allow the transfer of the ternary complex to its ribosomal binding site. On the ribosome, the EF-Tu binding site consists of the sarcin–ricin loop (SRL) of the 23S rRNA, the shoulder region of 16S rRNA, the L11 protein, and associated rRNA as well as protein S12. These sites of interaction are shared with the tRNA, which furthermore interacts with the decoding center as well as C1914 of helix 69 in 23S rRNA (Figure 3).[54]

All structures of the ternary complex bound on the ribosome have been carefully examined with the aim of understanding how the sensing of a correctly proportioned codon–anticodon minihelix by the decoding center of the 30S subunit can be faithfully relayed to the GTPase site of EF-Tu approximately 80 Å away. For a long time, however, the only successful strategy for arresting EF-Tu on the ribosome was the inclusion of kirromycin during structure analysis (Table 2), whereby EF-Tu was captured in its posthydrolytic state leaving interpretations of the mechanism of GTPase activation speculative. Very recently, a more convincing reconstruction of the structural rearrangements responsible for transmitting the signal of cognate codon–anticodon interaction to the GTPase center of EF-Tu has been presented on the basis of the crystal structure of EF-Tu·GDPCP·Trp-tRNA^{Trp} bound to the 70S ribosome in the presence of the antibiotic paramomycin.[58] Paramomycin causes decoding errors by facilitating the conformational changes leading to GTP hydrolysis by EF-Tu.

After the initial binding of the ternary complex by the L7–L12 stalk, subtle rotations of domain 1 of EF-Tu with respect to domains 2 and 3 allow the factor to adopt different aa-tRNA structures and present them to the ribosome in a uniform manner.[59] Next, the codon–anticodon interaction is subjected to testing, causing the tRNA to bend and twist. The structural change in the tRNA induces the "closed" conformation of the 30S subunit that moves the 16S rRNA toward EF-Tu. This results in structural changes in two loops of domain 2 of EF-Tu (see below), which are thereby brought into contact with the 16S rRNA shoulder.[54] Also, contacts between EF-Tu and tRNA change as a consequence of the 30S closure. Notably, the interaction between the switch II region of EF-Tu containing the catalytic His84 and the 5′ end of tRNA is altered, while the contact between the switch I region of EF-Tu containing one-half of the hydrophobic gate (Ile60) and the tRNA 3′ end is abolished. The residues of the hydrophobic gate undergo only small changes, which may not be critical for GTP hydrolysis contrary to previous predictions.[58] The series of conformational changes mentioned above are concluded by an ordering of His84 into its catalytic conformation by the phosphate residue of A2662 of the SRL in 23S rRNA (compare panels A and B of Figure 4). The essential role of A2662 explains the toxicity of α-sarcin, which cleaves the phosphodiester bond containing the phosphate group activating His84. α-Sarcin acts on both prokaryotic and eukaryotic ribosomes, suggesting that the mechanism of GTPase activation involving A2662 may be common to all GTPases involved in ribosomal protein synthesis. His84 has been suggested to serve as a general base that deprotonates the catalytic water molecule to facilitate its nucleophilic attack of the γ-phosphate of GTP. After GTP hydrolysis, the cleaved-off γ-phosphate is released, leading to disorder of the switch I region and a rotation of His84 into its inactive state, which appears to be stabilized via an interaction with the 2′-OH group of G2661 of the SRL (Figure 4C).[54] The ability of His84 to act as a general base during ribosome-induced GTP hydrolysis by EF-Tu has recently been questioned.[60,61] However, the importance of His84 seems unequivocal,[62,63] yet the precise mechanism of action needs to be defined via biochemical analysis and further structural studies. In particular, the pH dependency of the GTPase reaction needs to be carefully studied. Also, the role of the ribosomal L7–L12 protein during the decoding process remains unclear; e.g., the reason for its ability to stimulate

the GTPase activity of EF-Tu by 2500-fold is not yet apparent.[64]

Very recently, cryo-EM studies have shed new light on the tRNA selection process by comparing the binding modes of near-cognate and cognate ternary complexes in the presence of kirromycin.[65] The two types of tRNAs were shown to bind to the A/T site in two distinct conformations with different geometries with respect to their interaction with EF-Tu. The acceptor arm seems to be more flexible for the near-cognate tRNA, which gives rise to changes in the structure of EF-Tu. Notably, the hydrophobic gate appears to be less open in the near-cognate ternary complex, while the switch II region containing the catalytic His84 seems to be more flexible because of the absence of a clearly defined acceptor arm. These changes along with changes in the positions of elements of the GTPase-associated center within the 50S subunit as well as the head and shoulder domains of the 30S subunit may explain the reduced rate of GTPase activation for the near-cognate ternary complex.

■ STRUCTURE–FUNCTION STUDIES OF EF-TU

The structures of EF-Tu described above serve as excellent starting points for structure–function studies via engineered mutants. In this way, the following issues of EF-Tu functionality have been addressed: guanine nucleotide binding and exchange, GTP hydrolysis, conformational switching, tRNA binding, and ribosome binding.

With respect to the mechanism of guanine nucleotide exhange, the roles of EF-Tu residues His84[66] and His118[67] as well as residues in helix D[68] have been studied. In addition, the functions of residues Asp80 and Phe81 of EF-Ts have been investigated.[69] Among the residues mutated, no single side chain can account for the 60000-fold acceleration of nucleotide exchange achieved by the action of EF-Ts, and the mechanism appears to be more complex than first predicted on the basis of structural studies (see above).

Deduction of the mechanism of GTP hydrolysis off and on the ribosome has been the goal of another extensive series of point mutations of EF-Tu. The most dramatic effect was observed upon mutation of His84 to alanine, which resulted in a 10^5-fold decrease in the rate of ribosome-stimulated GTP hydrolysis,[62] in accordance with the most recent structural studies of EF-Tu on the ribosome.[58]

Pre-steady state kinetic studies of EF-Tu mutants affected at glycine residues providing flexibility around the switch II region have revealed the functional reason behind the strict conservation of these residues. The C-terminal glycine at position 94 appears to control P_i release[40] as well as conformational switching,[70] while the N-terminal glycine at postion 83 plays a role during GTP hydrolysis[70] by coordinating the catalytic water molecule and allowing the structural transition required for GTPase activation.[58]

Recently, mutations identified via classical selection schemes have attracted new attention because of novel structural studies of EF-Tu on the ribosome.[54] EF-TuB_0 was identified on the basis of resistance toward kirromycin and results of the Gly222Asp substitution in the *tufB* gene.[71] EF-TuB_0 binds kirromycin but leaves the ribosome in a complex with the antibiotic (i.e., the phenotype is recessive). In addition, EF-TuB_0 is deficient in ribosome-stimulated GTP hydrolysis indicative of problems in transmitting codon–anticodon recognition to the GTPase center. The deficiency can be rescued by high concentrations of Mg^{2+}.[72] Inspection of the X-ray structure of the kirromycin-stalled ternary complex on the ribosome explains the need for flexibility at this position.[54] Gly222 is positioned in one of the two domain 2 loops (residues 219–226) that undergo structural rearrangements upon domain closure of the 30S subunit. Most likely, the introduction of an aspartate affects the structural transition and disables the interaction with the backbone of 16S rRNA via electrostatic repulsion. Mg^{2+} concentrations of >10 mM may screen the negative charge of the aspartate side chain and thereby rescue the defective signal transmission. Similarly, the restriction of flexibility in the second domain 2 loop subjected to conformational changes on the ribosome (residues 256–273) by mutation of Gly280 to valine severely affects the productive interaction between the ternary complex and the ribosome.[73]

■ REMAINING CHALLENGES

The abundant structural information about components of the translation apparatus has been like a treasure chest for scientists with an interest in the translation process. Most astonishing are the structures of several ribosomal complexes. The solution of the structure of the ribosome may serve as an ideal example to the scientific community showing how a combination of crazy ideas, patience, technical developments, timely coincidences, and hard work may pave the way to reaching even the most unbelievable goals.

The structures of an almost complete set of stable EF-Tu complexes have provided tremendous insight into the mechanism of decoding the genetic message by fuelling an amazing number of functional studies, yet a number of functionally relevant intermediates such as the complexes formed upon initial binding and codon recognition as well as the transition state of GTP hydrolysis are not amenable to structural studies because of their transient nature. Contrary to a number of other GTP-binding proteins, fluoroaluminates do not mimic the γ-phosphoryl transfer of GTP in EF-Tu,[53] and an alternative transition state analogue has not been reported yet for EF-Tu; however, mutant forms may be helpful. Another approach to obtaining information about the dynamic aspects of the decoding process could be to identify short-lived intermediates and relate these to functional events. In particular, the sequence of structural changes resulting from cognate codon–anticodon interaction that ultimately leads to GTP hydrolysis by EF-Tu remains to be clearly delineated with specific attention to differences between transitions evoked by noncognate, near-cognate, and cognate ternary complexes.

The usage of single-molecule techniques in the studies of translation is relatively new and probably still in its infancy with respect to labeling, immobilization, and resolution in time and space. Such techniques have not yet been applied directly to EF-Tu but may in the future become feasible and result in a more complete picture regarding the role of EF-Tu during early decoding events as well as tRNA accommodation.[74]

The ribosome is the target of ~50% of all medically relevant antibiotics, which act by interference with decoding, blockage of translocation, inhibition of peptide bond formation, or hindrance of the progression of growing peptide chains.[75] However, the development of resistance among pathogenic bacteria is becoming a growing problem, and new targets need to be explored via structure-based drug design and/or functional assays for lead compound discovery. EF-Tu represents a poorly exploited drug target of potential value in

the treatment of infections in humans caused by Gram-negative as well as Gram-positive bacteria.

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ABBREVIATIONS

mRNA, messenger RNA; tRNA, transfer RNA; aa-tRNA, aminoacyl-tRNA; EF-Tu, elongation factor Tu; rRNA, ribosomal RNA; PDB, Protein Data Bank; SRL, sarcin−ricin loop; FRET, fluorescence resonance energy transfer.

REFERENCES

(1) Zuurmond, A., Rundlöf, A., and Kraal, B. (1999) Either of the chromosomal tuf genes of *E. coli* K-12 can be deleted without loss of cell viability. *Mol. Gen. Genet. 260*, 603−607.

(2) Furano, A. (1975) Content of elongation factor Tu in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A. 72*, 4780.

(3) Miller, D., and Weissbach, H. (1977) Factors involved in the transfer of aminoacyl-tRNA to the ribosome. In *Molecular Mechanisms in Protein Biosynthesis* (Weissbach, H., and Petska, S., Eds.) pp 323−373, Academic Press, New York.

(4) Parmeggiani, A., Swart, G., Mortensen, K., Jensen, M., Clark, B., Dente, L., and Cortese, R. (1987) Properties of a genetically engineered G domain of elongation factor Tu. *Proc. Natl. Acad. Sci. U.S.A. 84*, 3141−3145.

(5) Gromadski, K., Wieden, H., and Rodnina, M. (2002) Kinetic mechanism of elongation factor Ts-catalyzed nucleotide exchange in elongation factor Tu. *Biochemistry 41*, 162−169.

(6) Louie, A., and Jurnak, F. (1985) Kinetic studies of *Escherichia coli* elongation factor Tu−guanosine 5′-triphosphate−aminoacyl-tRNA complexes. *Biochemistry 24*, 6433−6439.

(7) LaRiviere, F., Wolfson, A., and Uhlenbeck, O. (2001) Uniform binding of aminoacyl-tRNAs to elongation factor Tu by thermodynamic compensation. *Science 294*, 165−168.

(8) Schrader, J., and Uhlenbeck, O. (2011) Is the sequence-specific binding of aminoacyl-tRNAs by EF-Tu universal among bacteria? *Nucleic Acids Res. 39*, 1229−1234.

(9) Schrader, J., Chapman, S., and Uhlenbeck, O. (2011) Tuning the affinity of aminoacyl-tRNA to elongation factor Tu for optimal decoding. *Proc. Natl. Acad. Sci. U.S.A. 108*, 5215−5220.

(10) Eccleston, J., and Webb, M. (1982) Characterization of the GTPase reaction of elongation factor Tu. Determination of the stereochemical course in the presence of antibiotic X5108. *J. Biol. Chem. 257*, 5046−5049.

(11) Parmeggiani, A., and Sander, G. (1981) Properties and regulation of the GTPase activities of elongation factors Tu and G, and of initiation factor 2. *Mol. Cell. Biochem. 35*, 129−158.

(12) Pape, T., Wintermeyer, W., and Rodnina, M. (1998) Complete kinetic mechanism of elongation factor Tu-dependent binding of aminoacyl-tRNA to the A-stie of the *E. coli* ribosome. *EMBO J. 17*, 7490−7497.

(13) Hilgenfeld, R. (1995) How do the GTPases really work? *Nat. Struct. Biol. 2*, 3−6.

(14) Parmeggiani, A., Krab, I., Okamura, S., Nielsen, R., Nyborg, J., and Nissen, P. (2006) Structural basis of the action of pulvomycin and GE2270 A on elongation factor Tu. *Biochemistry 45*, 6846−6857.

(15) Parmeggiani, A., Krab, I., Watanabe, T., Nielsen, R., Dahlberg, C., Nyborg, J., and Nissen, P. (2006) Enacyloxin IIa pinpoints a binding pocket of elongation factor Tu for development of novel antibiotics. *J. Biol. Chem. 281*, 2893−2900.

(16) Kjeldgaard, M., Nyborg, J., and Clark, B. (1996) The GTP binding motif: Variations on a theme. *FASEB J. 10*, 1347−1368.

(17) Kjeldgaard, M., Nissen, P., Thirup, S., and Nyborg, J. (1993) The crystal structure of elongation factor EF-Tu from *Thermus aquaticus* in the GTP conformation. *Structure 1*, 35−50.

(18) Berchtold, H., Reshetnikova, L., Reiser, C., Schirmer, N., Sprinzl, M., and Hilgenfeld, R. (1993) Crystal structure of active elongation factor Tu reveals major domain rearrangements. *Nature 365*, 126−132.

(19) Kjeldgaard, M., and Nyborg, J. (1992) Refined structure of elongation factor EF-Tu from *Escherichia coli*. *J. Mol. Biol. 23*, 721−742.

(20) Polekhina, G., Thirup, S., Kjeldgaard, M., Nissen, P., Lippmann, C., and Nyborg, J. (1996) Helix unwinding in the effector region of elongation factor EF-Tu-GDP. *Structure 4*, 1141−1151.

(21) Abel, K., Yoder, M., Hilgenfeld, R., and Jurnak, F. (1996) An a to b conformational switch in EF-Tu. *Structure 4*, 1153−1159.

(22) Nissen, P., Kjeldgaard, M., Thirup, S., Polekhina, G., Reshetnikova, L., Clark, B., and Nyborg, J. (1995) Crystal structure of the ternary complex of Phe-tRNAPhe, EF-Tu and a GTP analog. *Science 270*, 1464−1472.

(23) Nissen, P., Thirup, S., Kjeldgaard, M., and Nyborg, J. (1999) The crystal structure of Cys-tRNACys−EF-Tu−GDPNP reveals general and specific features in the ternary complex and in tRNA. *Structure 7*, 143−156.

(24) Kawashima, T., Berthet-Colominas, C., Wulff, M., Cusack, S., and Leberman, R. (1996) The structure of the *Escherichia coli* EF-Tu·EF-Ts complex at 2.5 Å resolution. *Nature 379*, 511−518.

(25) Wang, Y., Jiang, Y., Meyering-Voss, M., Sprinzl, M., and Sigler, P. (1997) Crystal structure of the EF-Tu·EF-Ts complex from *Thermus thermophilus*. *Nat. Struct. Biol. 4*, 650−656.

(26) Parmeggiani, A., and Nissen, P. (2006) Elongation factor Tu-targeted antibiotics: Four different structures, two mechanisms of action. *FEBS Lett. 580*, 4576−4581.

(27) Brown, D., and Gold, L. (1996) RNA replication by Qβ replicase: A working model. *Proc. Natl. Acad. Sci. U.S.A. 93*, 11558−11562.

(28) Kidmose, R., Vasiliev, N., Chetverin, A., Andersen, G., and Knudsen, C. (2010) Structure of the Qβ replicase, and RNA-dependent RNA polymerase consisting of viral and host proteins. *Proc. Natl. Acad. Sci. U.S.A. 107*, 10884−10889.

(29) Takeshita, D., and Tomita, K. (2010) Assembly of Qβ viral RNA polymerase with host translation elongation factors EF-Tu and -Ts. *Proc. Natl. Acad. Sci. U.S.A. 107*, 15733−15738.

(30) Takeshita, D., and Tomita, K. (2012) Molecular basis for RNA polymerization by Qβ replicase. *Nat. Struct. Mol. Biol. 19*, 229−237.

(31) Rodnina, M., and Wintermeyer, W. (2001) Fidelity of aminoacyl-tRNA selection on the ribosome: Kinetic and structural mechanisms. *Annu. Rev. Biochem. 70*, 415−435.

(32) Blanchard, S. (2009) Single-molecule observations of ribosome function. *Curr. Opin. Struct. Biol. 19*, 103−109.

(33) Zaher, H., Shaw, J., Strobel, S., and Green, R. (2011) The 2′-OH group of the peptidyl-tRNA stabilizes an active conformation of the ribosomal PTC. *EMBO J. 30*, 2445−2453.

(34) Rodnina, M., and Wintermeyer, W. (2011) The ribosome as a molecular machine: The mechanism of tRNA-mRNA movement in translocation. *Biochem. Soc. Trans. 39*, 658−662.

(35) Rodnina, M., Pape, T., Fricke, R., Kuhn, L., and Wintermeyer, W. (1996) Initial binding of the elongation factor Tu·GTP·aminoacyl-tRNA complex preceding codon recognition on the ribosome. *J. Biol. Chem. 271*, 646−652.

(36) Pape, T., Wintermeyer, W., and Rodnina, M. (1999) Induced fit in initial selection and proofreading of aminoacyl-tRNA on the ribosome. *EMBO J. 18*, 3800−3807.
(37) Gromadski, K., and Rodnina, M. (2004) Kinetic determinants of high-fidelity tRNA discrimination on the ribosome. *Mol. Cell 13*, 191−200.
(38) Gromadski, K., Daviter, T., and Rodnina, M. (2006) A uniform response to mismatches in codon-anticodon complexes ensures ribosomal fidelity. *Mol. Cell 21*, 369−377.
(39) Lee, T.-H., Blanchard, S., Kim, H., Puglisi, J., and Chu, S. (2007) The role of fluctuations in tRNA selection by the ribosome. *Proc. Natl. Acad. Sci. U.S.A. 104*, 13661−13665.
(40) Kothe, U., and Rodnina, M. (2006) Delayed release of inorganic phosphate from elongation factor Tu following GTP hydrolysis on the ribosome. *Biochemistry 45*, 12767−12774.
(41) Rodnina, M., Gromadski, K., Kothe, U., and Wieden, H. (2005) Recognition and selection of tRNA in translation. *FBES Lett. 579*, 938942.
(42) Blanchard, S., Gonzalez, R. Jr., Kim, H., Chu, S., and Puglisi, J. (2004) tRNA selection and kinetic proofreading in translation. *Nat. Struct. Biol. 11*, 1008−1014.
(43) Geggier, P., Dave, R., Feldman, M., Terry, D., Altman, R., Munro, J., and Blanchard, S. (2010) Conformational sampling of aminoacyl-tRNA during selection on the bacterial ribosome. *J. Mol. Biol. 399*, 576−595.
(44) Mishra, P., Qureshi, M., Ren, W., and Lee, T.-H. (2010) Codon-dependent tRNA fluctuations monitored with fluorescence polarization. *Biophys. J. 99*, 3849−3858.
(45) Jenner, L., Demeshkina, N., Yusupova, G., and Yusupov, M. (2010) Structural rearrangements of the ribosome at the tRNA proofreading step. *Nat. Struct. Mol. Biol. 17*, 1072−1078.
(46) Kramer, E., and Farabaugh, P. (2007) The frequency of translational misreading errors in *E. coli* is largely determined by tRNA competition. *RNA 13*, 87−96.
(47) Zaher, H., and Green, R. (2009) Quality control by the ribosome following peptide bond formation. *Nature 457*, 161−166.
(48) Atkins, J., and Björk, G. (2009) A gripping tale of ribosomal frameshifting: Extragenic suppressors of frameshift mutations spotlight P-site realignment. *Microbiol. Mol. Biol. Rev. 73*, 178−210.
(49) Wimberly, B., Brodersen, D., Clemons, W. J., Morgan-Warren, R., Carter, A., Vonrhein, C., Hartsch, T., and Ramakrishnan, V. (2000) Structure of the 30S ribosomal subunit. *Nature 407*, 327−339.
(50) Ban, N., Nissen, P., Hansen, J., Moore, P., and Steitz, T. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science 289*, 905−920.
(51) Yusupov, M., Yusupova, G., Baucom, A., Lieberman, K., Earnest, T., Cate, J., and Noller, H. (2001) Crystal structure of the ribosome at 5.5 Å resolution. *Science 292*, 883−896.
(52) Ogle, J., and Ramakrishnan, V. (2005) Structural insights into translational fidelity. *Annu. Rev. Biochem. 74*, 129−177.
(53) Stark, H., Rodnina, M., Rinke-Appel, J., Brimacombe, R., Wintermeyer, W., and van Heel, M. (1997) Visualization of elongation factor Tu on the *Escherichia coli* ribosome. *Nature 389*, 403−406.
(54) Schmeing, T., Voorhees, R., Kelley, A., Gao, Y.-G., Murphy, F. IV, Weir, J., and Ramakrishnan, V. (2009) The crystal structure of the ribosome bound to EF-Tu and aminoacyl-tRNA. *Science 326*, 688−694.
(55) Li, W., Agirrezabala, X., Lei, J., Bouakaz, L., Brunelle, J. L., Ortiz-Meoz, R. F., Green, R., Sanyal, S., Ehrenberg, M., and Frank, J. (2008) Recognition of aminoacyl-tRNA: A common molecular mechanism revealed by cryo-EM. *EMBO J. 27*, 3322−3331.
(56) Kothe, U., Wieden, H., Mohr, D., and Rodnina, M. (2004) Interaction of helix D of elongation factor Tu with helices 4 and 5 of protein L7/L12 on the ribosome. *J. Mol. Biol. 336*, 1011−1021.
(57) Diaconu, M., Kothe, U., Schlünzen, F., Fisher, N., Harms, J., Tonevitsky, A., Stark, H., Rodnina, M., and Wahl, M. (2005) Structural basis for the function of the ribosomal L7/L12 stalk in factor binding and GTPase activation. *Cell 121*, 991−1004.
(58) Voorhees, R., Schmeing, T., Kelley, A., and Ramakrishnan, V. (2010) The mechanism for activation of GTP hydrolysis on the ribosome. *Science 330*, 835−838.
(59) Schmeing, T., Voorhees, R., Kelley, A., and Ramakrishnan, V. (2011) How mutations in tRNA distant from the anticodon affect the fidelity of decoding. *Nat. Struct. Mol. Biol. 18*, 432−437.
(60) Liljas, A., Ehrenberg, M., and Åqvist, J. (2011) Comment on "The mechanism for activation of GTP hydrolysis on the ribosome". *Science 333*, 37a.
(61) Adamczyk, A., and Warshel, A. (2011) Converting structural information into an allosteric-energy-based picture for elongation factor Tu activation by the ribosome. *Proc. Natl. Acad. Sci. U.S.A. 108*, 9827−9832.
(62) Daviter, T., Wieden, H., and Rodnina, M. (2003) Essential role of histidine 84 in elongation factor Tu for the chemical step of GTP hydrolysis on the ribosome. *J. Mol. Biol. 332*, 689−699.
(63) Voorhees, R., Schmeing, T., Kelley, A., and Ramakrishnan, V. (2011) Response to comment on "The mechanism for activation of GTP hydrolysis on the ribosome". *Science 333*, 37b.
(64) Mohr, D., Wintermeyer, W., and Rodnina, M. (2002) GTPase activation of elongation factors Tu and G on the ribosome. *Biochemistry 41*, 12520−12528.
(65) Agirrezabala, X., Schreiner, E., Trabuco, L., Lei, J., Ortiz-Meoz, R. F., Schulten, K., Green, R., and Frank, J. (2011) Structural insights into cognate versus near-cognate discrimination during decoding. *EMBO J. 30*, 1497−1507.
(66) Schümmer, T., Gromadski, K., and Rodnina, M. (2007) Mechanism of EF-Ts-catalyzed guanine nucleotide exchange in EF-Tu: Contribution of interactions mediated by helix B of EF-Tu. *Biochemistry 46*, 4977−4989.
(67) Dahl, L., Wieden, H., Rodnina, M., and Knudsen, C. (2006) The importance of P-loop and domain movements in EF-Tu for guanine nucleotide exchange. *J. Biol. Chem. 281*, 21139−21146.
(68) Wieden, H., Gromadski, K., Rodnin, D., and Rodnina, M. (2002) Mechanism of elongation factor (EF)-Ts-catalyzed nucleotide exchange in EF-Tu. Contribution of contacts at the guanine base. *J. Biol. Chem. 277*, 6032−6036.
(69) Zhang, Y., Li, X., and Spremulli, L. (1996) Role of the conserved aspartate and phenylalanine residues in prokaryotic and mitochondrial elongation factor Ts in guanine nucleotide exchange. *FEBS Lett. 391*, 330−332.
(70) Knudsen, C., Wieden, H., and Rodnina, M. (2001) The importance of structural transitions of the switch II region for the functions of elongation factor Tu on the ribosome. *J. Biol. Chem. 276*, 22183−22190.
(71) Vijgenboom, E., and Bosch, L. (1989) Translational frameshifts induced by mutant species of the polypeptide chain elongation factor Tu of *Escherichia coli*. *J. Biol. Chem. 264*, 13012−13017.
(72) Vorstenbosch, E., Pape, T., Rodnina, M., Kraal, B., and Wintermeyer, W. (1996) The G222D mutation in elongation factor Tu inhibits the codon-induced conformational changes leading to GTPase activation on the ribosome. *EMBO J. 15*, 6766−6774.
(73) Tubulekas, I., and Hughes, D. (1993) A single amino acid substitution in elongation factor Tu disrupts interaction between the ternary and the ribosome. *J. Bacteriol. 175*, 240−250.
(74) Perla-Kajan, J., Lin, X., Cooperman, B., Goldman, E., Jakubowski, H., Knudsen, C., and Mandecki, W. (2010) Properties of *Escherichia coli* EF-Tu mutants designed for fluorescence resonance energy transfer from tRNA molecules. *Protein Eng., Des. Sel. 23*, 129−136.
(75) Wilson, D. (2009) The A−Z of bacterial translation inhibitors. *Crit. Rev. Biochem. Mol. Biol. 44*, 393−433.

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