The Tricks of Ribosomal Elongation Factors**

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Elongation factors (EF) catalyze protein biosynthesis. Two classes of elongation factors can be differentiated: EF-Tu and EF-G in eubacteria and EF-1 and EF-2 in archaebacteria and eukaryotes. They can adopt at least two conformations; one when bound to GDP and another when bound to GTP. In addition, EF-Tu (EF-1) can have still other ligands.

The crystal structures of the various forms in which EF-Tu can appear[1-5] and those of EF-G with and without GDP[6, 7] have afforded exciting insights into the functions of elongation factors as well as into ribosomal mechanisms. The biggest surprise came from the crystal structure of the ternary complex aminoacyl-tRNA·EF-Tu·GTP.[4] Before we discuss the structures and some of the functional implications we will turn briefly to the process of protein biosynthesis.

In all cells ribosomes translate genetic information stored as nucleic acid (DNA or RNA) into proteins. The nucleic acids are composed of four building blocks, the nucleotides. A sequence of three nucleotides (codon) defines unequivocally one unit of a protein, an amino acid. Both nucleic acids and proteins are nonbranched molecules.

The information storage format is almost exclusively DNA; only some important virus families such as HIV store the protein sequences as RNA. To convert the information of a gene into a protein sequence, the corresponding region of the DNA is transcribed into an mRNA (transcription). The sum of all mRNAs of a cell describes the actual synthesis program for cell proteins, the central importance of which is evident—more than 99.9% of all enzymes are proteins. The synthesis program can be regulated and adjusted to changing conditions; that is the importance of this circumstantial transcription procedure: The storage medium, DNA, is very long-lived (many years), whereas the mRNA is short-lived, and thus can be easily regulated (half-life of seconds to minutes. One of the most stable mRNAs is that from globin in erythrocytes with a half-life of about 100 days.)

A ribosome "walks" in codon "steps" along an mRNA and synthesizes the corresponding protein. The ribosome cannot read the codons itself but makes use of transfer RNAs (tRNAs). tRNAs are L-shaped molecules that carry an amino acid at the end of their short arm and expose three nucleotides at the end of their long arm. The three nucleotides are complementary to the codon that codes for the corresponding amino acid. The exposed trinucleotide is therefore called an anticodon. The one-codon movement of the ribosome along the mRNA is called a translocation.

During elongation, the extension of the growing peptide chain amino acid by amino acid, there are at least two tRNAs on the ribosome. Before translocation the tRNAs are present at the A and P regions, respectively. "A" stands for aminoacyl-tRNA and "P" for peptidyl-tRNA (Fig. 1). After translocation the tRNAs are in the ribosomal P and E regions. "E" stands for exit, since the tRNA liberated from its acyl residue is released from this region of the ribosome during the subsequent occupation of the A region. The ribosomal states before (Pre) or after translocation (Post) are the main states of the active ribosome, which are separated by high energy barriers (about 80 to 90 kJ mol^{-1}[8]). The Post state is probably lower in energy than the Pre state. This is indicated by the fact that after peptide-bond formation incubation at 37 °C for a couple of minutes is sufficient to promote translocation from the Pre to the Post state in the absence of the translocation factor EF-G and GTP (spontaneous translocation[9]). In contrast, a reverse translocation has been never observed with an isolated Post state; however, a Post state can be converted into a Pre state in the presence of an A-region ligand such as *N*-acetyl-Phe-tRNA even in the absence of EF-Tu and GTP.

The elongation factors lower the activation barrier separating the two main states of the ribosome and thus dramatically accelerate protein synthesis by more than 10^4-fold. In this way they resemble enzymes, which also lower the activation energy of a reaction and lead to enormous acceleration factors of 10^6 to 10^{12}. But enzymes accelerate a reaction only until equilibrium is reached; in other words, one enzyme can catalyze both the forward and the back reaction. An elongation factor can do more: it accelerates a reaction and determines the direction of reaction as well. For example, EF-Tu catalyzes the Post → Pre transition and EF-G the Pre → Post transition. For this reason there are two elongation factors, one for each direction of reaction. Only the higher fungi such as yeast or *Candida albicans* require a third factor, EF-3, which is an E-region factor and essential for protein synthesis.[10]

Both universal elongation factors EF-Tu and EF-G are prototypes of the large superfamily of the G-proteins which regulate important processes of the cell. G-proteins are GTPases and pass through a basic GTPase cycle.[11] They can bind GTP and GDP and thus assume an "ON" and "OFF" conformation. In the "ON" conformation (GTP is bound) a G-protein can bind a protein or a complex and trigger a distinct reaction. Then the GTPase center of the G-protein is activated, the terminal phosphate residue is cleaved such that GDP is now bound, and the G-protein adopts an "OFF" conformation and dissociates from the protein or complex. A new cycle begins after the GDP has been regenerated to GTP.

Figure 1 shows the GTPase cycles of EF-G and EF-Tu which bind in the "ON" conformation to the ribosome. EF-G follows the most simple scheme possible for a G-protein. The EF-G·GTP complex binds to a ribosome which is in the Pre state and which carries the peptidyl-tRNA in the A region after peptidyl transfer. EF-G lowers the activation energy between the Pre and Post states of the ribosome (Fig. 1). Since the Post state is apparently lower in energy, the ribosome assumes this state

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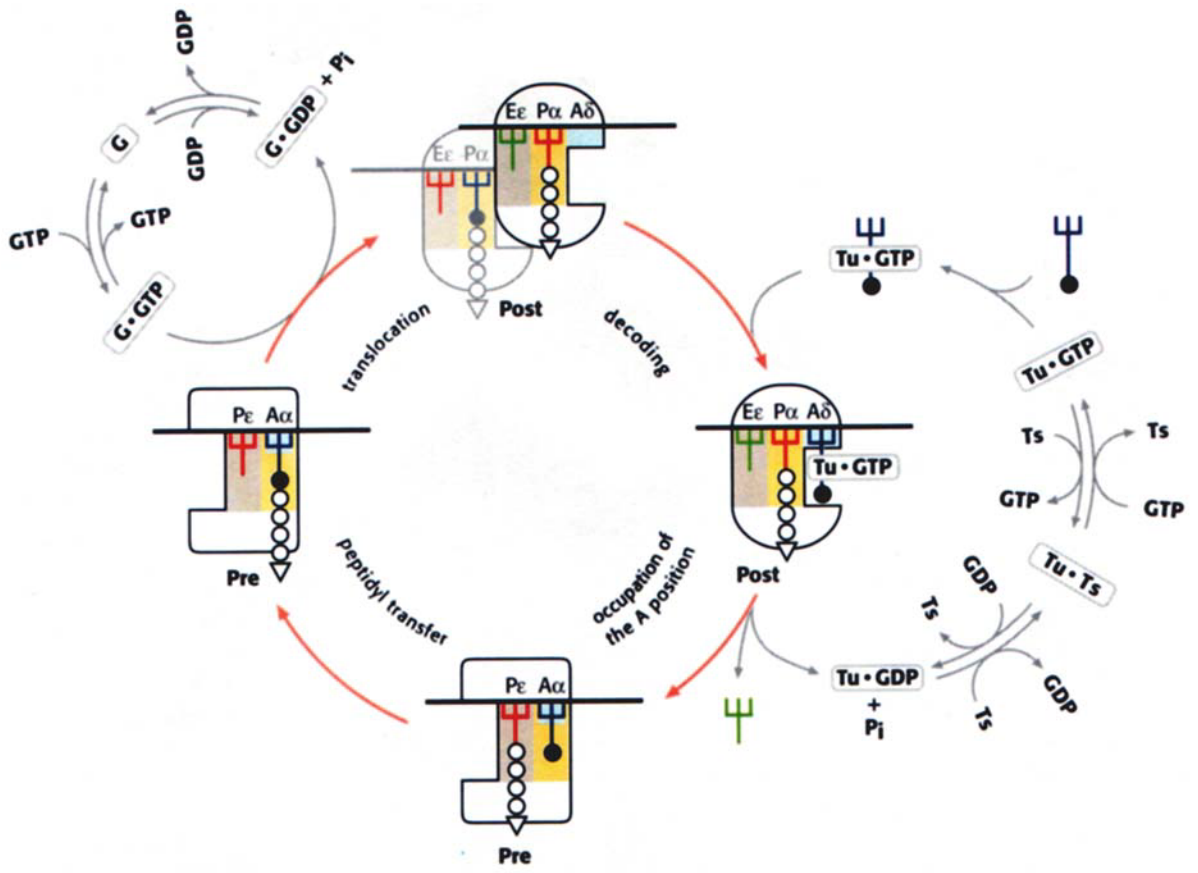

Fig. 1. The ribosomal elongation cycle (middle) and the GTPase cycles of the elongation factors EF-Tu (abbreviated as Tu, right) and EF-G (abbreviated as G, left). The elongation cycle is shown along the lines of the α–ε model, according to which the two tRNAs on the ribosome are bound to a movable domain of the ribosome. This movable domain contains two binding sites α and ε. The binding sites expose the tRNAs at the A and P regions of the ribosome in the Pre state and carries the tRNAs to the P and E regions, respectively, during translocation, thus establishing the Post state. In addition the decoding center δ is fixed at the A region; it overlaps with the α site in the the Pre state but stands alone in the Post state.

and now carries the peptidyl-tRNA at the P region and the deacylated tRNA at the E region. After the ribosome activates the GTPase center by means of an unknown mechanism, the resulting EF-G·GDP assumes the "OFF" conformation and dissociates from the ribosome. The activation barrier is raised again and keeps the ribosome stable in the Post state.

EF-G has a very low affinity to both GTP and GDP (2.7×10^4 M^{-1}[12] and 2.5×10^5 M^{-1},[13] respectively) so that the actual concentrations of GTP and GDP in the cell (ca. 1 mM and 0.1 mM, respectively[14]) are sufficient to replace GDP by GTP without the help of an additional factor, thus enabling the next round of an EF-G cycle.

The situation with EF-Tu is more complicated since EF-Tu·GTP alone cannot bind to the ribosome. It must first bind to an aminoacyl-tRNA and then form the ternary complex aminoacyl-tRNA·EF-Tu·GTP. This is a consequence of the second essential task of EF-Tu besides catalyzing the Post → Pre transition, namely to carry an aminoacyl-tRNA to the ribosome.

At this point, the X-ray crystal structure of the ternary complex (Fig. 2A[4]) is quite surprising. The ribosome is thought to use more than once the codon–anticodon interaction (proofreading) in order to achieve the accuracy of about one error per 1000 incorporated amino acids. Proposed proofreading mechanisms assume that EF-Tu uses the energy released during the hydrolysis of GTP for driving the proofreading process.[15] However, the crystal structure of the ternary complex demonstrates that EF-Tu binds the aminoacyl-tRNA at the short arm of the tRNA about 50 Å away from the anticodon (Fig. 2A), and thus EF-Tu hardly has a chance to interfere with the mechanism of codon–anticodon interaction. This conclusion is in accord with the results of recent investigations,[16] which suggest that the recognition (initial binding) of a correct codon–anticodon interaction in the ribosomal decoding center δ raises sufficient energy to explain the accuracy of 1:1000 of protein synthesis without the assumption of a proofreading mechanism. This explanation significantly simplifies the hypothesis for the selection of the correct ternary complex.

Accordingly it is probable that the correctness of the codon–anticodon interaction is checked at the decoding center δ without the participation of EF-Tu. In this view the considerable binding energy of EF-Tu and the aminoacyl-tRNA outside the anticodon region is exploited to drive the transition to the pretranslocational state only after the decoding has been completed successfully. It is generally accepted that replicases and trancriptases, which synthesize DNA and RNA, respectively, probe the stereochemoical correctness of base pairing in the course of nucleic acid synthesis and that these enzymes achieve an accuracy of better than 1:10000 without proofreading.[17]

The biggest surprise is that the X-ray crystal structures of the ternary complex and EF-G·GDP are very similar (cf. Figs. 2A

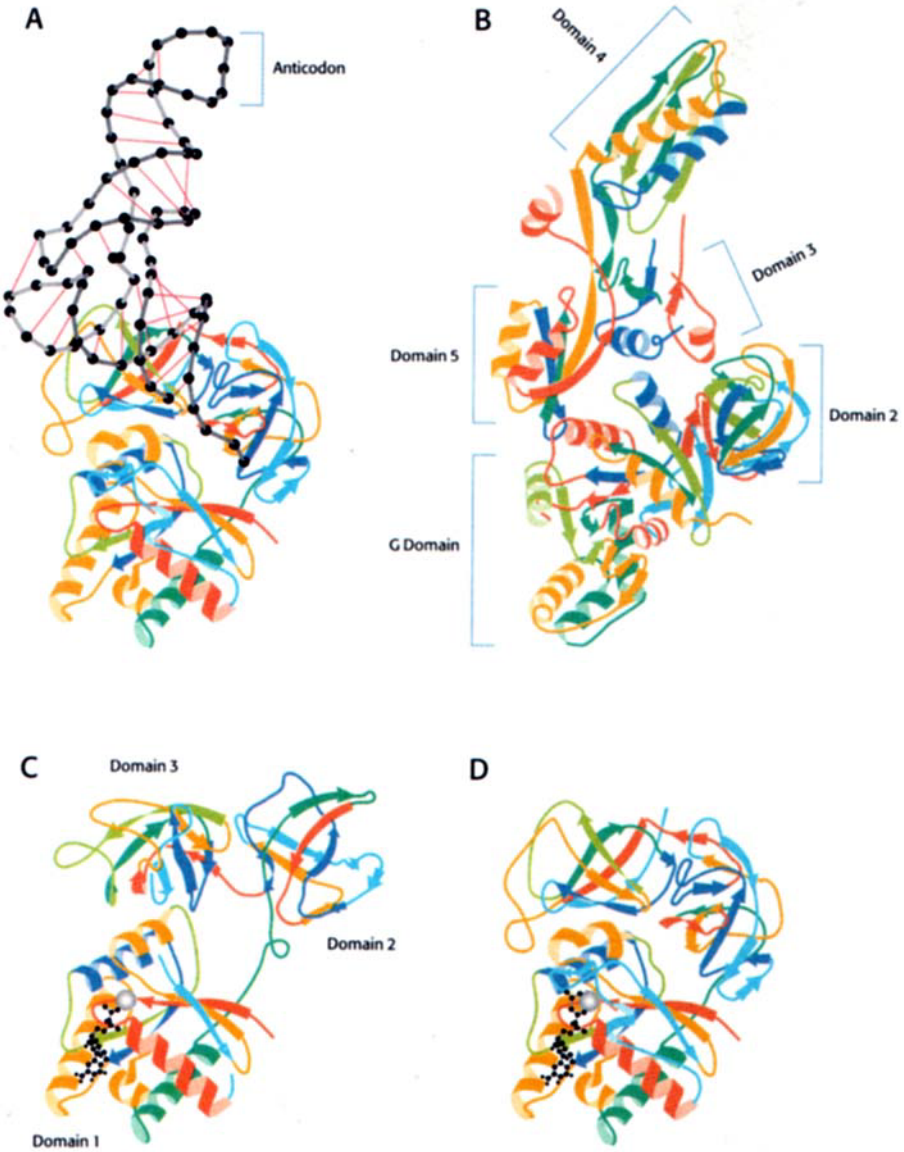

Fig. 2. X-ray crystal stuctures of elongation factors. A: Ternary complex Phe-tRNA·EF-Tu·GTP, adapted from refs. [4, 21]. B: EF-G from *T. thermophilus*, adapted from refs. [6, 7]. C and D: EF-Tu·GDP and EF-Tu·GTP, respectively. The *E. coli* sequence of EF-Tu has been modeled in analogy to the structure of EF-Tu·GTP from *Thermus thermophilus* [2]. GDP and GTP are represented as black ball-and-stick models. The Mg^{2+} ion important for the GTP/GDP binding is shown as a gray sphere; adapted from ref. [21].

and B)! The EF-G structure shows five domains (Fig. 2B); domains three to five imitate the structure of the tRNA component of the ternary complex! This is a surprising example of molecular mimicry. The ribosome model shown in Figure 1 suggests the following interpretation:[18] After EF-G·GTP has established the Post state of the ribosome, the domains of EF-G mimicking a tRNA bind to the vacant decoding center. This simple action prevents a reverse translocation of the ribosome into the Pre state as long as the presence of EF-G on the ribosome keeps the energy barrier between the two ribozome states low. After the hydrolysis of GTP, EF-G·GDP dissociates from the ribosome, the activation energy is raised again, and the ribosome is held in the Post state until the next ternary complex has finished decoding.

After EF-Tu has triggered the Pre state of the ribozyme, the ribosome activates the GTPase center of EF-Tu, probably by a similar mechanism to that in the case of EF-G. The resulting EF-Tu·GDP adopts the "OFF" conformation and dissociates from the ribosome. The structure of the "OFF" conformation of EF-Tu·GDP is more open than that of EF-Tu·GTP (cf. Figs. 2C and 2B). Upon cleavage of GTP, domains 2 and 3 move away from domain 1 as a block, and domain 2 departs from domain 1 by astonishing 40 Å.

In striking contrast to EF-G, the factor EF-Tu binds GDP with high affinity ($6 \times 10^8\ M^{-1}$) and GTP with ten times less affinity (which is still relatively high).[19] The consequence is that the concentrations of GDP and GTP in the cell do not influence the binding of the guanosine nucleotides to EF-Tu. Therefore, the special factor EF-Ts exists which regenerates EF-Tu·GTP from EF-Tu·GDP; this factor binds to EF-Tu·GDP and mediates the replacement of GDP with GTP.

The X-ray crystal structure of EF-Tu·EF-Ts[5] demonstrates that the complex is not a heterodimer as expected but rather a tetramer. In the tetramer both EF-Ts units have distinct contact areas, whereas the EF-Tu molecules hardly touch each other. Accordingly, the structure of the tetramer is best described as EF-Tu·$(EF\text{-}Ts)_2$·EF-Tu. The motif Thr-Asp-Phe-Val82, which is conserved in all structures of EF-Ts known, plays a dominant role in the replacement of GDP. The amino acids Asp80-Phe81 press into the GDP binding site and displace three amino acids of EF-Tu, which stabilize two H_2O molecules. Both water molecules are coordinated to a Mg^{2+} ion which is essential for the affinity of Ef-Tu to GDP. The disruption of the Mg^{2+} binding site causes the loss of GDP, which now can be replaced by GTP. These reactions are reversible, but for effective protein synthesis GDP must be replaced by GTP and not vice versa. The direction of the GTPase cycle (Fig. 1) is determined by the practically irreversible formation of the ternary complex, which removes EF-Tu·GTP from the equilibrium reactions.[20]

The X-ray crystal structures discussed here have afforded surprising insights into the functions of elongation factors and of G-proteins in general. The strucures shed some light on ribosomal mechanisms such as decoding and give an exciting example of molecular mimicry. Yet the ribosome, the most complicated complex in the cell, is not well understood. Exactly how the ribosome activates the GTPase centers of the elongation factors and by which mechanisms elongation fac-

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tors lower the activation barriers separating ribosome states is not known.

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