Macromolecular mimicry in protein biosynthesis

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Elongation factor Tu (EF-Tu) is a G-protein which, in its active GTP conformation, protects and carries aminoacylated tRNAs (aa-tRNAs) to the ribosome during protein biosynthesis. EF-Tu consists of three structural domains of which the N-terminal domain consists of two special regions (switch I and switch II) which are structurally dependent on the type of the bound nucleotide. Structural studies of the complete functional cycle of EF-Tu reveal that it undergoes rather spectacular conformational changes when activated from the EF-Tu·GDP form to the EF-Tu·GTP form. In its active form, EF-Tu·GTP without much further structural change interacts with aa-tRNAs in the so-called ternary complex. The conformational changes of EF-Tu involve rearrangements of the secondary structures of both the switch I and switch II regions. As the switch II region forms part of the interface between domains 1 and 3, its structural rearrangement results in a very large change of the position of domain 1 relative to domains 2 and 3. The overall shape of the ternary complex is surprisingly similar to the overall shape of elongation factor G (EF-G). Thus, three domains of the protein EF-G seem to mimic the tRNA part of the ternary complex. This macromolecular mimicry has profound implications for the function of the elongation factors on the ribosome.

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Introduction

The elongation phase is a central part of the complicated process of protein biosynthesis during which a growing peptide is elongated according to the sequence of codons in the mRNA [1,2]. This is done in a cyclic manner involving the action of three elongation factors. An aa-tRNA first enters into a ternary complex with EF-Tu·GTP. This complex protects the aa-tRNA against hydrolysis and further catalyzes the interaction of the aa-tRNA with the ribosome at the ribosomal aa-tRNA site (the A site). If the codon exposed at the A site matches the anticodon of the aa-tRNA, the GTP on EF-Tu is hydrolyzed and inactive EF-Tu·GDP leaves the ribosome. EF-Tu·GDP is reactivated into EF-Tu·GTP by EF-Ts.

The ribosome at its peptidyl transferase center catalyzes the formation of a peptide bond between the amino acid on the aa-tRNA in the A site and the peptide chain attached to a tRNA at the ribosomal peptidyl-tRNA site (P site). The new peptide becomes bound to the tRNA in the A site, and a deacylated tRNA is now found in the P site. Translocation of tRNAs and mRNA relative to the ribosome now occurs, catalyzed by EF-G in its active GTP form. During this process, EF-G·GTP is hydrolyzed to EF-G·GDP, which leaves the ribosome. The deacylated tRNA is now at the exit site (E site) and peptidyl-tRNA at the P site. The ribosomal A site is empty and exposes the next codon along the mRNA. The ribosome is now ready for the next cycle of amino acid addition.

The general principles of bacterial protein biosynthesis as described above have been known for more than 20 years. During this time, many publications have dealt with biochemical results and derived functional models for this system. Structural information is available on several aa-tRNA synthetases, which catalyze the formation of the ester bond between an amino acid and a tRNA [3]. Structures of many ribosomal proteins are also known [4]. Recent reviews have summarized biochemical results and models for elongation factors [5–8], but it is only during the past few years that detailed structural information has become available for the elongation factor cycles.

Here, we describe structural information obtained on the EF-Tu cycle. The main emphasis will be on the conformational changes in EF-Tu during activation. Some detail will be given on the refolding of the short peptides of the switch regions and the effect of this on the internal organization of EF-Tu and on its interaction with aa-tRNA. The shapes of EF-Tu and EF-G will be compared, leading to the new concept of macromolecular mimicry and to new ideas about the functional details of the elongation phase of protein biosynthesis.

The structure of EF-Tu

EF-Tu was the first protein recognized as a G-protein, i.e. a protein using GTP/GDP as cofactors. The G-proteins are active when bound to GTP (they are sometimes referred to as molecular switches which are 'on' in their GTP form and 'off' in their GDP form). They are switched off by GTP hydrolysis, either by an intrinsic GTPase activity or by stimulation of a GTPase activity

during interaction with functional partners. A given protein is now easily recognized as a G-protein by five sets of consensus sequences, all of which are found in loops of a domain (the G-domain) binding the cofactors [9]. Several families of G-proteins have been found [10]. One family includes the heterotrimeric G-proteins involved in the transduction of external signals (hormones, light, odors etc.) into internal chemical signals such as cyclic AMP. Another includes the Ras-p21 proto-oncogene product, which is involved in the control of cell proliferation. Single-site mutations of Ras-p21 are found in almost all known cancer cells. Another family includes several of the protein factors involved in the translation process of protein biosynthesis.

Although the basic structure of the G-domain is the same in all G-proteins, considerable variations do exist [9]. The domain has a central β-sheet of six strands, five of which are parallel, while one strand at one end of the sheet is antiparallel. The sheet is surrounded by five (or six) α-helices. In the loop between the first β-strand and the first α-helix is found a phosphate binding loop (Gly-X-X-X-X-Gly-Lys-Ser/Thr), which is also found in some ATP-binding proteins. The region between the first helix and the second β-strand is called the switch I region because its structure depends on the nature of the cofactor. It is also termed the 'effector loop', because in Ras-p21 this is a short loop interacting with the downstream effectors that interact with the activated form of Ras-p21. The effector loop varies considerably between the G-protein families; it is a complete domain in heterotrimeric G-proteins. It also contains the most variable sequence in the G-domain of EF-Tu (see Fig. 1). This region contains a threonine residue which is a ligand to a Mg^{2+} ion bound to GTP. The region containing the second α-helix and its surrounding loops is called the switch II region.

The conformational changes in EF-Tu

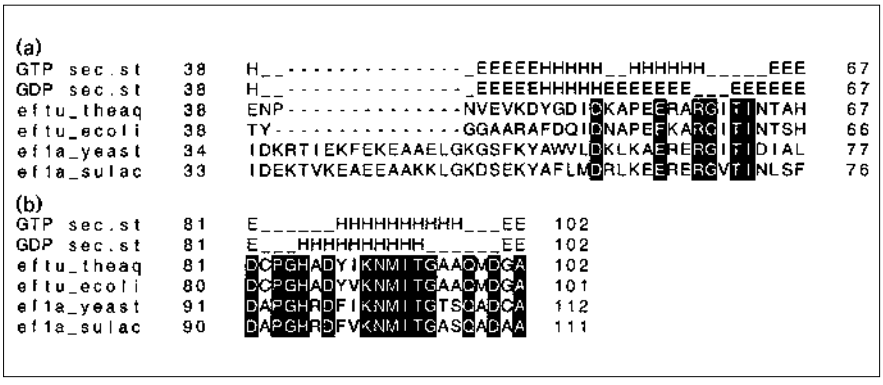
The following description of the structures of EF-Tu from *Thermus aquaticus* is based on two publications from our laboratory [11,12]. Similar structures have been determined in the laboratories of Hilgenfeld and Jurnak [13,14]. The two switch regions of EF-Tu undergo a spectacular rearrangement of their secondary and tertiary structure when EF-Tu is activated by EF-Ts (Fig. 2). The structure of the complex between EF-Tu and EF-Ts has been determined in the laboratory of Leberman [15]. In the switch I region, the first part (residues 40–51) remains constant and contains a short β-strand (residues 42–46) and a one-turn α-helix (residues 47–51). In EF-Tu·GDP, the second part (residues 52–64) forms a β-hairpin between two β-strands (residues 52–58 and 62–72) [12]. In EF-Tu·GTP, however, this second part has an α-helix (residues 54–59) and the following β-strand is shorter (residues 65–72) [11,13]. Thr62 seems to be an important part of this rearrangement, as it is conserved in EF-Tu (and indeed in all G-proteins) and as it is a ligand to Mg^{2+} in the GTP form, but displaced by about 12 Å in the GDP form [12]. Gly60 could introduce the flexibility into the region, allowing it to switch between the two very different structures [9].

In the switch II region, Gly84 is close to and directly influenced by the presence of the cofactor. In EF-Tu·GDP, the α-helix of this region is formed by residues 85–95 [12]. When GTP is introduced into the nucleotide-binding site, the peptide before Gly84 is flipped by ~150° so that the amide is pointing towards the γ-phosphate. This has the effect that the α-helix is shifted along the sequence (residues 88–98). Thereby, the axis of the helix is rotated by ~45° [11,13]. The flexibility of the peptide chain at Gly95 is important for this transformation [16]. As the helix forms part of the interface between domains 1 and 3 of EF-Tu, this rearrangement has a dramatic effect on the relative orientation of the domains of EF-Tu [11–13] (Fig. 2).

The structure of the ternary complex

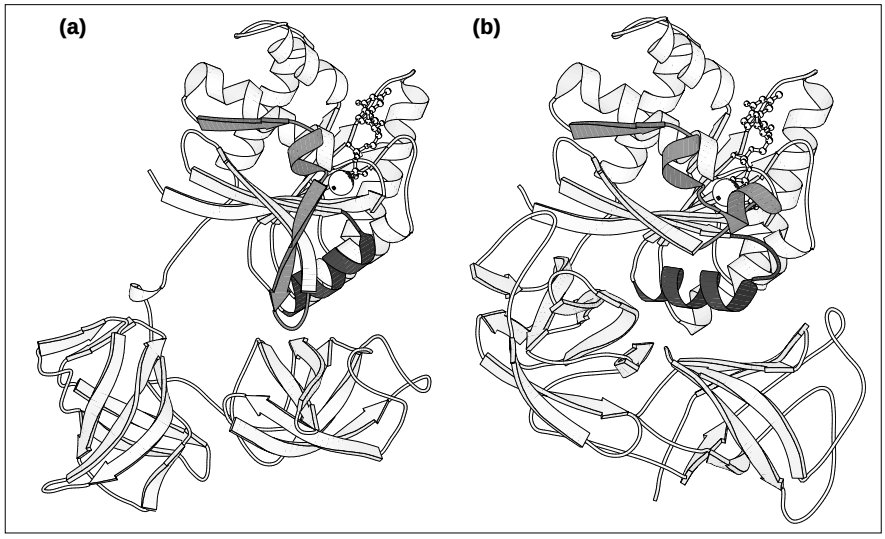
The structure of the ternary complex of EF-Tu·GTP from *Thermus aquaticus* and phenylalanyl-tRNA (Phe-tRNA)

Figure 1



Sequence alignments of regions of EF-Tu. Excerpts of a large alignment of all known sequences of prokaryotic EF-Tu and eukaryotic EF-1α are shown. The alignment has been performed with the sequence alignment editor ALMA [30]. Sequences of (a) switch I and (b) switch II regions of EF-Tu from the bacterium *Thermus aquaticus* (theaq), *Escherichia coli* (ecoli), yeast, and the archaebacterium *Sulpholobus acidocaldarius* (sulac). Secondary structure assignments (H, α-helix; E, β-strand) are from the crystal structures of *T. aquaticus* EF-Tu·GTP (GTP sec.st) and EF-Tu·GDP (GDP sec.st). Residues conserved in all known EF-Tu sequences are marked.

Figure 2

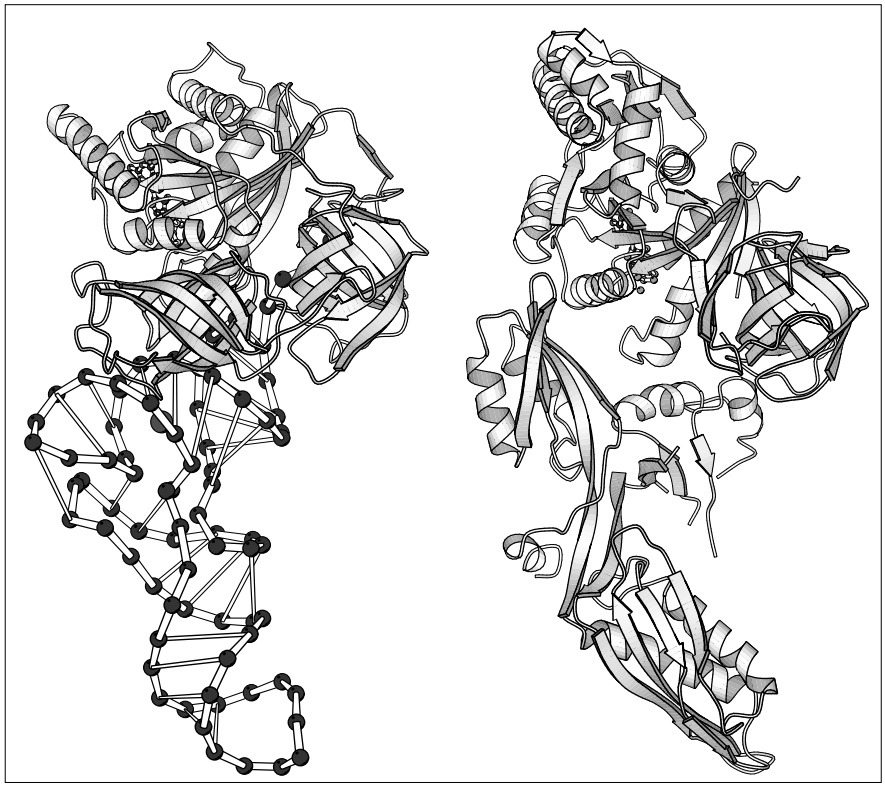


Structural comparisons of EF-Tu·GDP and EF-Tu·GTP. (a) The structure of *T. aquaticus* EF-Tu·GDP. The figure is produced by the program MOLSCRIPT [31]. Domain 1 is at the top, domain 2 is to the left at the bottom, and domain 3 to the right. The nucleotide is shown as a ball-and-stick model. The Mg^{2+} ion is shown as a larger ball. Switch region I is shown in grey shade, and switch region II in dark shade. (b) The structure of *T. aquaticus* EF-Tu·GTP.

from yeast has been determined in our laboratory [17]. The structures of the two components in the complex are virtually identical to those of the individual free molecules [11,18]. The ternary complex is very elongated, with the anticodon of Phe-tRNA pointing away from EF-Tu (Fig. 3). Three areas of Phe-tRNA are in close contact with EF-Tu: one side of the T-stem helix, the 5′-phosphate, and the CCA-end with the attached amino acid.

The T-stem helix is mainly in contact with domain 3 of EF-Tu. The contact area is rather unspecific. It is not surprising that the contact area on the tRNA is composed of

Figure 3



Macromolecular mimicry of the ternary complex and EF-G. The ternary complex is to the left and EF-G is to the right. The figure is produced by the program MOLSCRIPT [31]. EF-Tu·GTP is seen in a view turned relative to Figure 2 by about 180° around a vertical axis. The two structures are aligned on domains 1 and 2 by the program O [32]. For EF-G, the domain 3, which is not fully determined, is just below domain 2. Domain 5 is to the left of domain 3, while the elongated domain 4 is at the bottom.

only the backbone phosphates and riboses, as EF-Tu has to recognize all tRNAs. It is somewhat more surprising that the amino acid residues in the contact area of domain 3 of EF-Tu are not conserved. This points to the possibility that this contact could be weak. The two remaining contact areas are formed only in the activated EF-Tu·GTP. The 5′-phosphate and ribose are tucked into a small depression formed by the contacting corners of all three domains of EF-Tu. Part of the depression is formed by the α-helix of switch region I which is found only in EF-Tu·GTP. The phosphate forms a salt bridge to the conserved Arg300 of domain 2, while the ribose is fixed by the conserved residues Lys90 and Asn91 of the helix in switch region II. The CCA-end is found in a narrow channel between domains 1 and 2. The 3′-terminal A base and the amino acid Phe are bound in two separate binding pockets. The pocket for the A base is found between two protruding loops of domain 2 [11]. On one loop are found three hydrophobic residues of which two, Ile231 and Val237, are completely conserved. On the other are Glu271, which forms a stacking interaction with the base, and Arg274, which is in contact with the 3′-phosphate. Glu271 also forms a hydrogen bond with the 2′-OH of the terminal ribose. The amino acid ester is formed with the 3′-OH of this ribose. Its free amino group is fixed by hydrogen bonds to backbone atoms of EF-Tu. The amino acid sidechain of Phe is stacked on the sidechain of His67, which for a long time has been known to be part of the amino acid binding site of EF-Tu [19].

It is thus evident from the structure of the ternary complex that only EF-Tu·GTP is able to form a stable binding surface for aa-tRNAs. It is less obvious, though, why deacylated tRNA has low affinity for EF-Tu·GTP. The reason for this could be that only when tRNA is aminoacylated will the amino acid ester find its pocket by which the subsequent binding of the A base provides additional binding affinity [20]. Another possibility is that the aminoacylation by itself alters the structure of the CCA-end of tRNA.

Macromolecular mimicry

When the structure of the ternary complex was determined a very surprising observation was made. The overall shape of the ternary complex is very similar to the shape of the structure of EF-G·GDP (Fig. 3) [17,21–23]. Domains 3, 4, and 5 of EF-G seem to mimic the tRNA part of the ternary complex. This new concept, where part of a protein is mimicking the structure and possibly also the function of an RNA, has profound implications for ideas on the basic features of the elongation cycle [24,25].

At first it is puzzling that it is the active form of EF-Tu (in the form of the ternary complex) which is similar to the inactive form of EF-G. However, both are involved with the post-translocational part of the elongation cycle [24]. The structure of EF-G·GTP, as yet unknown, is most likely only slightly different from EF-G·GDP, perhaps by only ~10 Å at the tip of domain 4 [20,25,26]. In any case, the recent finding that the ribosomally induced GTPase activity of EF-G precedes the translocation of the ribosome [27] makes it very plausible that a conformational change of EF-G drives the translocation in a mechanical way [28] by physically chasing the newly formed peptidyl-tRNA out of the ribosomal A site. During this process, EF-G·GDP could shape a binding pocket for the ternary complex [25,25].

It is now very likely that all G-proteins involved in the translation of mRNA share a common GTPase activating center on the ribosome [17]. It remains to be seen to what extent other protein factors also exhibit macromolecular mimicry of RNA. It has been shown that all of these seem to have domains similar to domain 2 of EF-Tu [29].

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