Minireview

Structural information for explaining the molecular mechanism of protein biosynthesis

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Abstract Protein biosynthesis is controlled by a number of proteins external to the ribosome. Of these, extensive structural investigations have been performed on elongation factor-Tu and elongation factor-G. This now gives a rather complete structural picture of the functional cycle of elongation factor-Tu and especially of the elongation phase of protein biosynthesis. The discovery that three domains of elongation factor-G are structurally mimicking the amino-acylated tRNA in the ternary complex of elongation factor-Tu has been the basis of much discussion of the functional similarities and functional differences of elongation factor-Tu and elongation factor-G in their interactions with the ribosome. Elongation factor-G:GDP is now thought to leave the ribosome in a state ready for checking the codon-anticodon interaction of the aminoacyl-tRNA contained in the ternary complex of elongation factor-Tu. Elongation factor-G does this by mimicking the shape of the ternary complex. Other translation factors such as the initiation factor-2 and the release factor 1 or 2 are also thought to mimic tRNA. These observations raise questions concerning the possible evolution of G-proteins involved in protein biosynthesis.

Key words: Protein biosynthesis; RNA world; Macromolecular mimicry; Protein evolution; Elongation factor; Translation factor; Structure determination; Crystallography; Electron cryo-microscopy

1. Introduction

Protein biosynthesis is a central biological process in all living cells. It is one of the last steps in the transmission of genetic information stored in DNA on the basis of which useful active proteins are produced for maintaining the specific biological function of a given cell. Protein biosynthesis takes place on ribosomal particles where the genetic information transcribed into mRNA is translated into protein. The ribosome particle itself has been the subject for substantial scientific investigation for decades and much is therefore known about its function. Structural studies have recently provided results which are the prerequisite for obtaining detailed structural information on ribosomal subunits [1,2] and eventually also on the whole particle in various functional states within the next few years [3–7].

The process of synthesizing proteins on the ribosome is divided into three phases: initiation, elongation and termination. Both initiation and termination are special phases in that they are directed by specific codons, i.e. the start and stop codons of mRNA. In prokaryotic biosynthesis, which is the most studied, the initiation phase is controlled by a small number of initiation factors (IFs), IF-1, IF-2 and IF-3. Of these, IF-2 most likely forms a ternary complex with GTP and initiator $tRNA_f^{Met}$. All three factors are involved in assembling the initiation complex of initiation factors, initiator $tRNA_f^{Met}$, the ribosomal subunits and mRNA. Eukaryotic initiation is much more complex and involves a large number of eukaryotic initiation factors (eIFs) [8–10]. Termination is controlled by release factors (RFs) which have been extensively studies in recent years [11,12]. In prokaryotes, the stop codons are specifically recognized by RF1 and RF2, while a third factor, RF3, in complex with GTP stimulates the release of the fully synthesized protein from the ribosome. In eukaryotes, only two factors, eRF1 and eRF3, have been found.

The elongation phase has been the one phase most extensively studied, both functionally and structurally over the last 30 years [13]. It is controlled by three elongation factors (EFs). Elongation factor EF-Tu (EF-1α in eukaryotes) forms a ternary complex with GTP and amino-acylated tRNAs (aa-tRNAs), protects the amino acid ester bond against hydrolysis and carries the aa-tRNA to the ribosomal A-site for decoding of mRNA by codon-anticodon interactions. When correct codon-anticodon recognition occurs, GTP hydrolysis on EF-Tu is stimulated by the ribosome and EF-Tu:GDP is released. The nucleotide exchange factor EF-Ts (EF-1β in eukaryotes) converts EF-Tu:GDP into active EF-Tu:GTP. After a proofreading step, the aa-tRNA is brought into contact with the peptidyl-tRNA in the ribosomal P-site, where peptide bond formation is catalyzed adding one amino acid to the growing peptide. The last elongation factor EF-G (EF-2 in eukaryotes) in complex with GTP controls the translocation of tRNAs and mRNA on the ribosome.

This review will concentrate mostly on the structural studies of EF-Tu performed for some years in our laboratory. However, the elongation phase has been studied in other laboratories as well and all of this work now provides a rather complete picture of most of the major functional steps in the elongation phase of protein biosynthesis.

2. Elongation factor Tu

The functional cycle of EF-Tu is depicted in Fig. 1. EF-Tu was the first GTP-binding protein (G-protein) to be structurally investigated. The first structural details of the GDP-binding domain (G-domain) of EF-Tu:GDP from *Escherichia coli*

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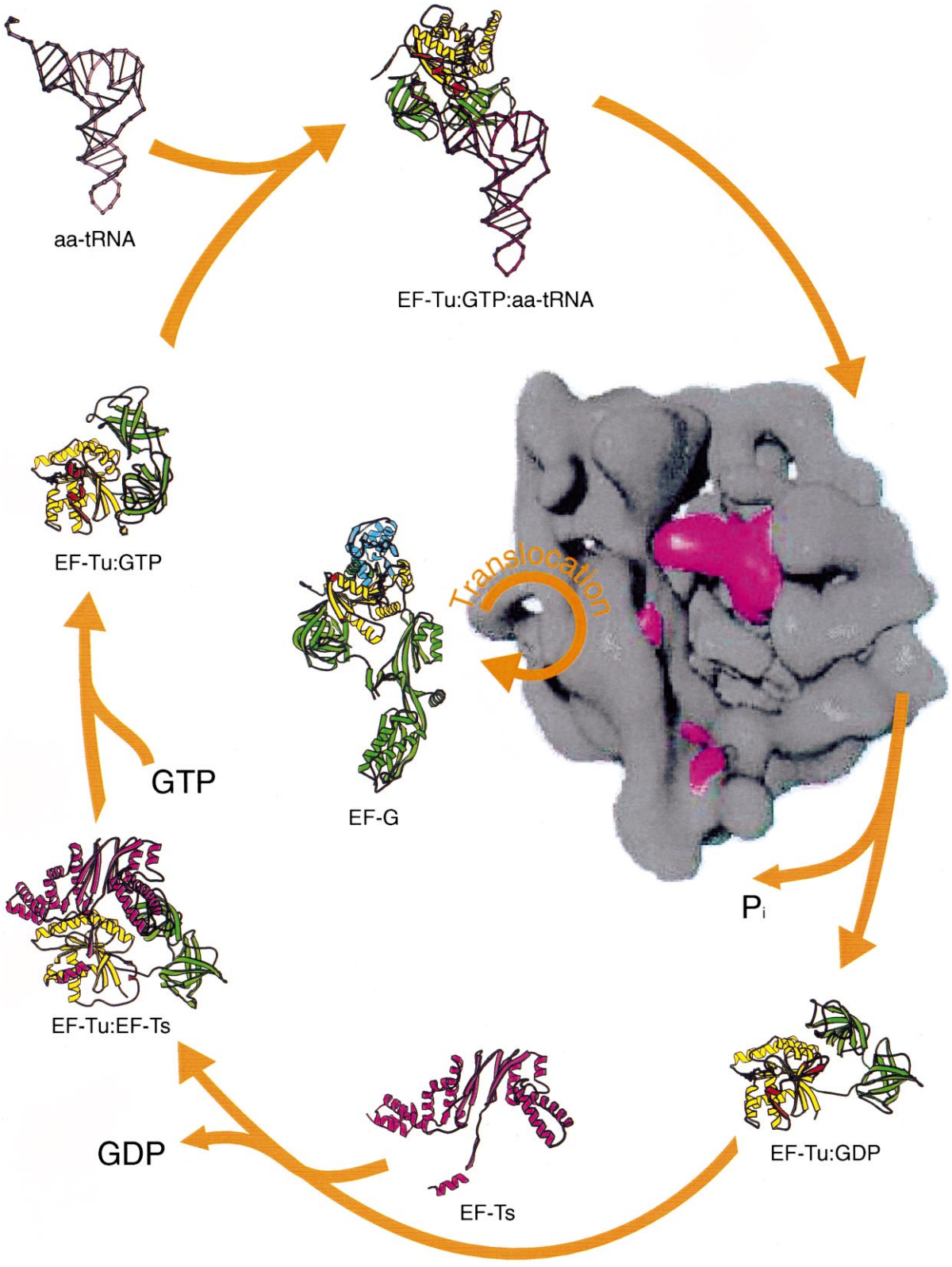

Fig. 1. The fFunctional cycle of elongation factor EF-Tu. At the top is the ternary complex of Phe-tRNA:EF-Tu:GDPNP with Phe-tRNA, at the top left entering the complex (PDB 1ttt [29]). Going round clockwise is the ribosome particle (not to scale), re-drawn after Stark et al. [3]. Next is the EF-Tu:GDP after release from the ribosome (PDB 1tui [18]). At the bottom is EF-Ts entering the complex of EF-Tu:EF-Ts [25] for nucleotide exchange. Finally, the active EF-Tu:GDPNP is found ready for interacting with aa-tRNA (PDB 1eft [22]). In the middle is shown EF-G:GDP (PDB 1dar [35]), which in its active form translocates tRNAs on the ribosome. The structural cartoons are drawn using MOLSCRIPT [56].

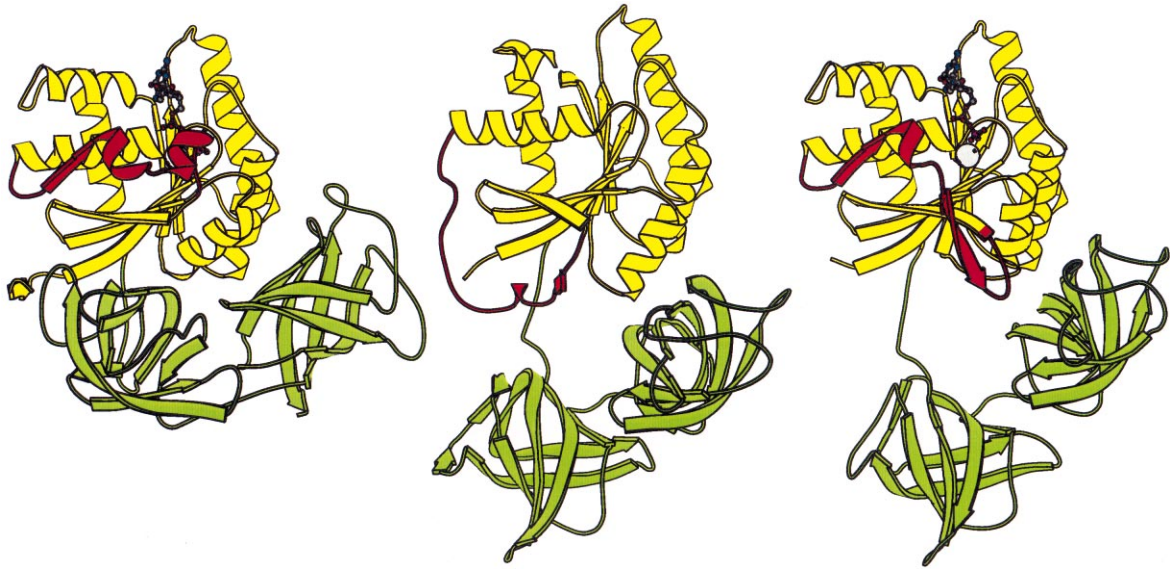

Fig. 2. Comparison of various functional states of EF-Tu. At the left is EF-Tu:GDPNP (PDB 1eft [22]), in the middle, EF-Tu as found in the EF-Tu:EF-Ts complex [25] and at the right, EF-Tu:GDP (PDB 1tui [18]). Domain 1 is yellow and domains 2 and 3 are green [28]. Nucleotides are shown as ball and stick model. Notice the structural changes of switch region I shown in red. The figure has been drawn using MOLSCRIPT [56].

were elucidated in 1985 [14,15] and were soon after used to formulate a model for the ras p21 protein [16]. Four years later, it was shown that this model was essentially correct [17]. The structural information now includes the crystal structures of EF-Tu:GDP from *Thermus aquaticus* [18] and from *E. coli* [18–20], crystal structures of EF-Tu:GDPNP, where GDPNP is a non-hydrolyzable analogue of GTP, from *Thermus thermophilus* [21] and from *T. aquaticus* [22] and models of EF-Tu:GDP and EF-Tu:GTP from *Bacillus stearothermophilus* [23] based on the crystal structures. The crystal structures of EF-Tu:EF-Ts have been determined both from *E. coli* [24,25] and from *T. thermophilus* [26]. Recently, a solution structure of a fragment of human EF-1β has been determined revealing that this eukaryotic EF is structurally related to EF-Ts [27], but surprisingly even closely related to domains 3 and 5 of EF-G (see later).

From all this structural information, it is known that EF-Tu consists of three structural domains. Domain 1 (the G-domain) of about 200 amino acid residues is binding GDP/GTP, is a typical nucleotide-binding domain having a central β-sheet surrounded by α-helices and is found to be similar in other G-proteins [28]. Domains 2 and 3 of about 100 residues each are both β-barrels and are in all known structures held together as one structural unit by strong interdomain interactions. The structures also reveal that EF-Tu is extraordinary in the sense that the two structural units (domain 1 is one and domains 2 and 3 together is the other) have large variations in their relative orientations (Fig. 2). The large difference between the EF-Tu:GDP and EF-Tu:GTP can be explained by the difference in structure of two regions on the G-domain called switch I and switch II. Switch I changes from a β-hairpin in EF-Tu:GDP to a short α-helix in EF-Tu:GTP. In switch II, the α-helix is shifted along the sequence by approximately four residues, thereby rotating the axes of the helix by about 45°. Since this helix (termed helix B) is part of the interface between domains 1 and 3, this explains the large conformational change of EF-Tu upon its activation. The change in helix B is directly coupled to the introduction of a γ-phosphate in the nucleotide-binding site, because this phosphate induces an almost 180° peptide flip at a conserved Gly just prior to the α-helix.

The large conformational change must involve a temporary dissociation between domains 1 and 3 [22]. Such a dissociation is precisely seen in the EF-Tu:EF-Ts complex [24,25], where domains 2 and 3 have yet again a third orientation relative to domain 1 (Fig. 2). Although this is seen to be more similar to the orientation found in EF-Tu:GDP, it is obvious that the complex formation allows the two switch regions to assume any one of the two structures found in EF-Tu:GDP and EF-Tu:GDPNP. Such information gives the impression that EF-Tu:GDP is a very flexible complex, whose structure can be influenced by many external factors. This is supported by the crystallographic observation that in this structure, the temperature factors of domain 2 are generally higher than those of the other two domains. EF-Tu:GTP seems to be in a much more fixed conformation ready to bind aa-tRNAs.

The structure of the ternary complex of *T. aquaticus* EF-Tu, GDPNP and yeast Phe-tRNA [29] showed that the two major parts in the complex are not much changed upon binding to each other. The CCA-Phe end of Phe-tRNA in the complex is bent relative to that of the free tRNA and lies in a narrow cleft between domains 1 and 2, where the amino acid pocket is also found. The amino acid ester is held by the backbone of a loop on the surface of domain 2 and the terminal A-base snuggles neatly into a narrow cleft with hydrophobic amino acids on one side and on the other side in a stacking configuration with a conserved Glu. There is a rather non-specific interaction between a surface of domain 3 with one side of the T-stem helix of Phe-tRNA. The anticodon is pointing away from EF-Tu, thus creating a very elongated complex. Recently, the structure of a second ternary complex of *T. aquaticus* EF-Tu, GDPNP and *E. coli* Cys-tRNA was determined [30]. The structure is overall very similar to the first ternary complex, but reveals the specific features of the tRNA struc-

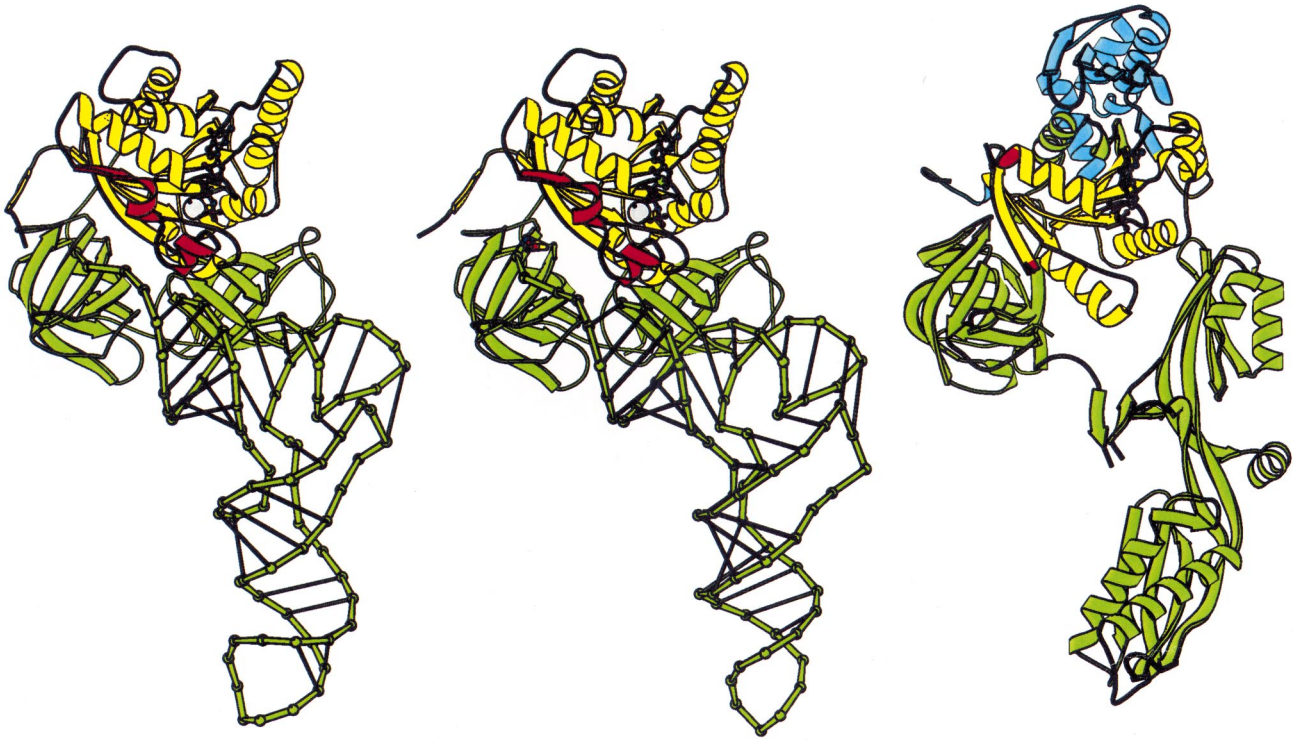

Fig. 3. Comparison of ternary complexes of EF-Tu with EF-G. At the left is Phe-tRNA:EF-Tu:GDPNP (PDB 1ttt [29]), in the middle is Cys-tRNA:EF-Tu:GDPNP (PDB 1b23 [30]) and to the right is EF-G:GDP (PDB 1dar [35]). The colouring scheme is similar to the one used in Fig. 2. The special inserted α-helical domain in domain 1 of EF-G is blue, while tRNAs and domains 3, 4 and 5 of EF-G are green. The figure has been drawn using MOLSCRIPT [56].

ture. The ternary complex has also been shown to have the same structure in solution as in the crystals [31].

On the ribosome, the ternary complex is blocked by the antibiotic kirromycin, such that even after the GTP on EF-Tu has been hydrolyzed, the EF-Tu:GDP remains bound, most likely still in a complex with aa-tRNA. Crystals have been obtained of such a quaternary complex [32] and the structure determination is underway. The position on the ribosome of the kirromycin-blocked ternary complex has been revealed by cryo-electron microscopy (cryo-EM) [4]. This shows the ternary complex at the entrance to the interface of the two ribosomal subunits with the anticodon of aa-tRNA pointing deep into the interior of the ribosome. The G-domain of EF-Tu is found very close to the proposed position of the GTPase center of the 50S subunit while domain 2 is in close contact with the 30S subunit. When comparing the position of tRNA in this structure with that of the tRNA in the A-site [3,6], it is obvious that the tRNA must turn around the anticodon helix in order for the CCA-aa end to reach the A-site and the peptidyl-transferase center of the ribosome.

3. EF-G and macromolecular mimicry

The structures of the nucleotide-free EF-G [33] and of EF-G:GDP from *T. thermophilus* [34,35] have been determined. The structures show that EF-G has five domains. Domains 1 and 2 are very similar to domains 1 and 2 of EF-Tu apart from the fact that EF-G has a small all helical insertion in domain 1. However, the relative orientation of these domains in inactive EF-G:GDP are the same as in active EF-Tu:GDPNP. Domains 3 and 5 have folds which are similar to those of a number of ribosomal proteins [36,37]. Domain 4 is very elongated, is pointing away from the rest of the molecule and has an unusual fold [33,34].

When the structure of the ternary complex of EF-Tu was compared to that of EF-G:GDP [29,34], it became obvious that the shape of domains 3, 4 and 5 of EF-G together are mimicking the shape of aa-tRNA (Fig. 3). Thus, the two factors which are controlling the elongation phase of the protein biosynthesis present themselves to the ribosome in very similar overall shapes. The structure of EF-G:GTP is not known at present and it could conceivably be somewhat different from that of EF-G:GDP [38,39]. Nevertheless, the three C-terminal domains of EF-G most likely still mimic tRNA and the fact that EF-G:GDP is similar in the overall shape to that of the ternary complex clearly points to the possibility that EF-G:GDP on leaving the ribosome is re-shaping its surroundings so that they form a site suitable for checking incoming aa-tRNAs for codon-anticodon interactions [40–42]. The part of EF-G that is mimicking tRNA suggests that the function of EF-G in translocation is to chase the newly synthesized peptidyl-tRNA out of the A-site and into the P-site [43].

This view is supported by cryo-EM observations of EF-G on the ribosome [7]. EF-G, like EF-Tu, is blocked on the ribosome by an antibiotic. In the cryo-EM experiment, EF-G:GTP was blocked by fusidic acid and the protein is seen as occupying a site very similar to the one for the ternary complex. At least domains 1 and 2 of EF-G are seen to contact the 50S and 30S subunits, respectively, in the same mannner as EF-Tu. This is not trivial since all G-proteins of translation, i.e. also IF-2 and RF3, have been shown to have domains similar to domains 1 and 2 of EF-Tu and EF-G [44]. These therefore can also be expected to have an initial contact

with the ribosome similar to the ones observed for EF-Tu and EF-G. The fact that the tRNA mimicking part of EF-G in the cryo-EM result is not as deep into the ribosome by about 10 Å compared with the aa-tRNA of the ternary complex indicates that the conformation of EF-G:GTP is also different from that of EF-G:GDP by a similar amount [39], suggesting that on GTP hydrolysis, the tRNA mimic of EF-G moves into the A-site on the 30S subunit.

4. Other translation factors

Much less structural information has been obtained on the other translation factors. However, structures are known for IF-1 from *E. coli* [45] and of the N- and C-terminal fragments of IF-3 [46]. Many attempts have been made to crystallize IF-2 or fragments thereof, but until now with very little success. It has been attempted to localize tRNA mimicking domains within IF-2 by comparing sequence multiple alignments of this factor with alignments of EF-Tu and EF-G [47], but the results are not convincing. Some structural information has however been obtained of the much more complicated process of initiation in eukaryotes [8–10]. The structure of eIF4E, which binds the cap-structure of eukaryotic mRNA, is known [48] and reveals a specific recognition of the terminal m7Gppp with some similarity to the recognition of GDP/GTP in G-proteins. The structure of eIF-5A from *Pyrobaculum aerophilum* has also recently been determined [49].

No structural information has as yet been obtained for the RFs. It is however generally believed that RF1 and RF2 (eRF1) are tRNA mimics [29,41,50], as they have to recognize specifically the stop codons and because their function can be suppressed by suppressor tRNAs. The RF3 (eRF3) is a G-protein and thus believed to be similar to EF-Tu, also in the sense that it can be isolated as a complex with RF1 (or RF2). This is supported by extensive work on comparison of amino acid sequences and molecular genetics studies aimed at determining the interactions between RF3 and RF1 [50]. It is still not completely clear whether the final release of protein from the ribosome also involves the action of EF-G as a translocase or whether the RF3:RF1 alone can act both as a ternary complex bringing water into the peptidyl transferase center and as a translocase.

5. Conclusions

The way that EF-G is mimicking the ternary complex (or vice versa), as a citation from a lecture by P. Moore, 'ought to tell us something about the ribosomal function. However, it is not easy to grasp what it is telling us'. The similarity in overall shape is striking and posesses the question whether there is some evolutionary relationship between the two [29,39–41,51], although protein mimicking RNA is an evolutionary event which has not been dealt with in many evolutionary theories. That proteins have evolved from ancestral proteins is now generally accepted and treated in text books. Even cases of convergent evolution are evident. That RNAs are mimicking tRNAs is also widely accepted [52,53]. But have some proteins evolved to replace the function of ancient RNAs by mimicking their shapes?

If this were the situation, one place to look for it is certainly the ribosomal machinery of the protein biosynthesis or indeed the biological processes of replication, transcription and translation, where at present, proteins and RNAs are seen to work together so intimately. The fundamental concept thus postulates that in ancient evolutionary times, these basic biological processes were based exclusively on RNA, of which some acted as ribozymes. It seems logical enough that a gigantic step forward in evolution could be reached by utilizing the much more versatile physical, chemical and structural properties of the 20 amino acids adopted by nature as compared to the four nucleotides of RNA (and DNA). The logic is somewhat more strained by assuming that ribozymes evolved to synthesize amino acids, although peptide bond formation on the ribosome seems to involve RNA-based catalysis. If an RNA-based machinery had evolved to provide protein synthesis, it is again quite logical that some RNAs were replaced by proteins and that these, at least as a first evolutionary attempt, were mimicking already evolved RNAs. Is the observable fact that the three C-terminal domains of EF-G are so similar to tRNA an example of such an early successful attempt of a protein to mimic RNA?

If one wants to speculate as far as possible, one could examine the recently determined structure of the ribozyme of the group I intron [54,55], especially with respect to the way that RNA helical structures are packed together and the way that they interact. This is very reminiscent of the way that domain 3 of EF-Tu, which has a dimension and a shape of a short piece of RNA helix, recognizes the T-stem helix of tRNA. More importantly perhaps, domain 2 and ribosomal protein L14 [37] also have the shapes of RNA helices. Is it thus a trait of evolution that RNA evolved to recognize RNA, that protein replaced some RNA by mimicking and by recognizing RNA and that proteins eventually evolved to recognize other proteins?

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