

# Elongation factor G with effector loop from elongation factor Tu is inactive in translocation

Alexander Kolesnikov, Anatoly Gudkov\*

*Institute of Protein Research, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia*

Received 11 November 2001; revised 19 November 2001; accepted 21 November 2001

First published online 25 January 2002

Edited by Lev Kisselev

**Abstract** Elongation factors Tu and G (EF-Tu and EF-G) alternately interact with the ribosome during the elongation phase of protein biosynthesis. The function of both factors depends on GTP binding, and the factors are ascribed to a superfamily of G-proteins. All G-proteins contain the effector loop, a structural element that is important for the protein's interaction with its target molecule. In this study the effector loop of EF-G was replaced by the loop taken from EF-Tu. The EF-G with EF-Tu loop has markedly decreased GTPase activity and did not catalyze translocation. We conclude that these loops are not functionally interchangeable since the factors interact with different states of the ribosome. © 2002 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

**Key words:** Ribosome; Elongation factor G; Translocation; GTP hydrolysis

## 1. Introduction

Elongation factors Tu and G (EF-Tu, EF-G) participate in the elongation cycle during protein synthesis on ribosomes. The ternary complex EF-Tu with aminoacyl-tRNA and GTP is formed in solution and after that interacts with the ribosome in a codon-dependent way. After GTP hydrolysis, EF-Tu dissociates from the ribosome in complex with GDP leaving the aminoacylated tRNA on the ribosome, which reacts with ribosome-bound peptidyl-tRNA. The reaction of transpeptidation leads to the formation of a new peptide bond. As a result, the ribosome is now in the pretranslocation state. The interaction of EF-G and GTP with the ribosome stimulates translocation, i.e. transfer of the peptidyl-tRNA from one ribosomal site to another with concurrent movement of the messenger RNA by one codon. After GTP hydrolysis, EF-G dissociates from the ribosome and the elongation cycle is repeated (reviewed in [1]). Hence, the activity of both factors depends on binding and hydrolysis of GTP. Thus, the elongation factors are ascribed to a broad family of GTP-binding proteins (such as Ras-p21, transducin, ADP-ribosylating factor ARF-1 and others [2,3]). Moreover, both factors have GTP-binding domains with structural elements common to all G-proteins, namely the phosphate-binding loop, the consensus motif DxxG (a part of the switch II region) and the effector loop [3]. These structural elements are important

for nucleotide binding, and their conformations depend on GTP or GDP bound to the factors. The effector loop structure is known for EF-Tu complexed either with GDP or GTP (for review see [3]). Significant changes in the EF-Tu molecule occur both in the spatial arrangement of its domains [3] and in the secondary structure of the effector loop (Fig. 1) as a result of GTP binding and its hydrolysis. Unfortunately it was impossible to establish unambiguously the conformation of the effector loop in EF-G by crystallographic studies, probably because of its different conformation in the crystals [4].

The amino acid composition of effector loops varies in different G-proteins [3]. This can be explained by the fact that the loops interact with different target molecules. In our case, however, both proteins, EF-Tu and EF-G, interact with the same target, the ribosome. For the reasons mentioned above it may be proposed that a particular conformation of the effector loop of both EF-Tu and EF-G complexed with GTP is important for interaction of the factors with the ribosome in the post- and pretranslocation states, respectively. To study the functional role of the effector loop in the interaction of EF-G with the ribosome, we have constructed mutated *Thermus thermophilus* EF-G containing the effector loop from *Thermus aquaticus* EF-Tu and investigated the function of the mutated protein.

## 2. Materials and methods

Mutagenesis was carried out by PCR technique according to the procedure described in [5]. The first DNA fragment was obtained with the help of the following oligonucleotides: 5'-CGGTGGTGCATATGCGGTCAAGGTAG-3' (contains the site for *NdeI* enzyme) and 5'-GTCGATATCGCCGTAGTCTTTGACCTCGCCGATCTT-3' (encodes a part of the EF-G loop and *EcoRV* site). Primers 5'-GCGAATTCATTGACCCTTGATGAGC-3' with an *EcoRI* site and 5'-ACGGCGATATCGACAAAGCTCCGGAGGAGCGGGAGC-3' (encodes another part of the loop and *EcoRV* site) were used to obtain the second fragment. The restriction sites are underlined and the nucleotides of the effector loop are given in bold. Both DNA fragments were treated either with *NdeI* and *EcoRV* or with *EcoRV* and *EcoRI* endonucleases, and were simultaneously ligated into plasmid pET11c digested with *NdeI* and *EcoRI*. Since the mutated EF-G retained high thermostability, the protein from the cell extract was isolated using heat treatment for coagulation of *Escherichia coli* proteins [5]. The functional tests were carried out as described earlier [5].

## 3. Results and discussion

The mutated factor G (EF-Gmod) was obtained as a result of mutagenesis of the *fus* gene following its expression in *E. coli*. The mutant protein contains a part of the EF-Tu effector loop (segment 45–55, Fig. 1) instead of amino acid

\*Corresponding author. Fax: (7)-095-924 04 93.  
E-mail address: gudkov@vega.protres.ru (A. Gudkov).

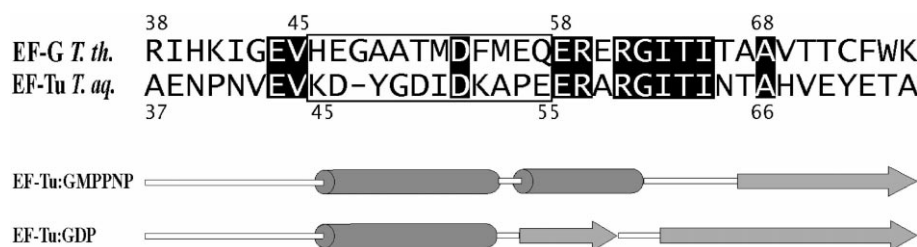


Fig. 1. Amino acid sequences of the effector loops from *T. aquaticus* EF-Tu and *T. thermophilus* EF-G. Identical residues are on a black background. The residues that were exchanged are in the rectangle. The scheme of secondary structure changes in the EF-Tu effector loop is given underneath (cylinders represent  $\alpha$ -helices and arrows represent  $\beta$ -strands).

sequence 46–57 of EF-G. Considering identical amino acids in the loops (Fig. 1, black background) and the fact that glutamic acid at position 58 has been found in most EF-Tu species [6], we can say that the mutant EF-G now contains the effector loop from EF-Tu (residues 43–63, Fig. 1).

The ribosome-dependent GTPase activity of EF-Gmod demonstrates that the protein interacts with the ribosome (Fig. 2). Since fusidic acid prevents dissociation of the factor from the ribosome after one round of GTP hydrolysis [7], this interaction was also confirmed by inhibition of GTP hydrolysis by the antibiotic. According to titration of GTPase reaction with fusidic acid, the half-inhibition concentrations of fusidic acid were 7 and 11  $\mu$ M for the wild-type EF-G and the mutated protein, respectively. However, the GTPase activity of EF-Gmod was about two times lower than that of the wild-type EF-G (Fig. 2). At the same time, the EF-Gmod was not active in the poly(U)-directed translation system (Fig. 3), and virtually did not stimulate translocation in the ribosome (puromycin test, Fig. 4). Hence, the intact effector loop of EF-G should be present to promote translocation activity of the factor. The integrity of the effector loop of EF-Tu and its Glu56 and Arg59 residues are also important for an effective function of the factor [8]. The effector loops of both factors have some identical amino acid residues (Fig. 1); however, the complete function of EF-Gmod is severely impaired. This fact might reflect different conformations of the effector loops when the factors are bound to the specific ribosomal state.

EF-G lacking domain 4 is highly active in ribosome-dependent multiple rounds of GTP hydrolysis but does not stimulate translocation [9]. Moreover, conformation distortion of

the loop at the distal end of EF-G domain 4 by insertion of a few amino acids considerably decreases the translocation activity of the factor [10]. Deletion of domain 3 leads to considerably decreased GTPase activity and complete loss of translocation ability of EF-G [5], despite the presence of the intact domain 4, which is important for translocation [9]. On the grounds of the above data we suggest that to catalyze translocation EF-G should interact with the ribosome in a conformation when all its domains occupy definite positions in the pretranslocated ribosome. Apparently, this can be realized by exact mutual arrangements of EF-G domains in the ribosomal complex with EF-G and GTP (or its uncleavable analog). In such a situation, different conformations of EF-Tu and EF-G effector loops may be important in alternative interaction of the factors with post- and pretranslocated states of the ribosomes, respectively. This suggestion is in agreement with the fact that the spatial shape of the aminoacyl-tRNA\*EF-Tu\*GTP ternary complex is similar to the shape of the binary complex of EF-G with GDP (molecular mimicry) [11].

Slow translocation occurs in the ribosome spontaneously (without EF-G) and is strongly stimulated by EF-G with an uncleavable analog (for example, GMPPCP (guanylyl-methylene diphosphonate)) [1]. Moreover, effective and repetitive translocation can be obtained on the matrix-bound poly(U)-containing ribosomes with the presynthesized polypeptide (polyphenylalanyl-tRNA) after the addition of EF-G with GMPPCP [12]. Hence, translocation can be achieved without coupled GTP hydrolysis either spontaneously or as a result of EF-G attachment with an uncleavable GTP analog to the

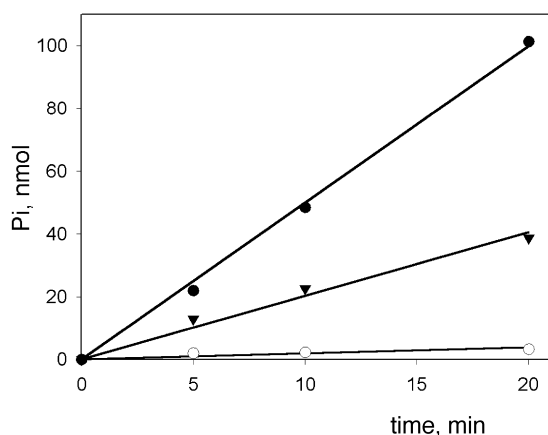


Fig. 2. Kinetics of ribosome-dependent GTP hydrolysis: (○) without EF-G; (●) with addition of intact EF-G; (▼) in the presence of EF-Gmod.

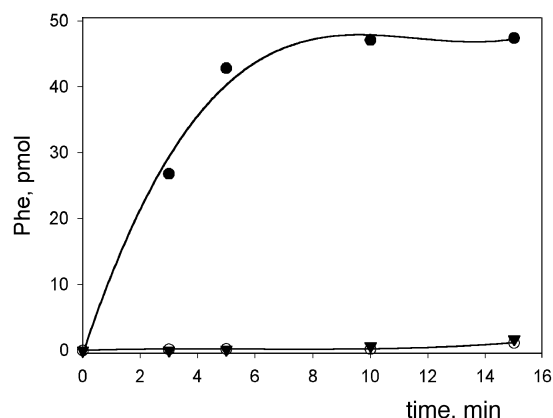


Fig. 3. Polyphenylalanine synthesis in poly(U)-directed cell-free system: (○) without EF-G; (●) in the presence of intact EF-G; (▼) with addition of EF-Gmod.

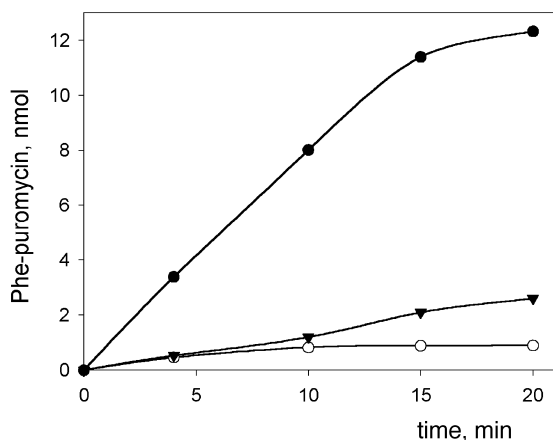


Fig. 4. The yield of Phe-puromycin in the reaction of puromycin with pretranslocated ribosomes promoted by the factors: (○) control without EF-G; (●) in the presence of intact EF-G; (▼) with addition of EF-Gmod.

pretranslocated ribosome. The pretranslocated state in the ribosome arises after transpeptidation reaction (when a new peptide bond is formed and deacetylated tRNA appears) and it is considered to be a metastable state [13]. Hence, it can be concluded that correct and high complementary attachment of EF-G and GTP (or with its uncleavable analog) to this ribosomal state is necessary to catalyze translocation. Free energy is gained as a result of the high-affinity attachment of EF-G complexed with GTP to the ribosome; this new complex is thought to be analogous to the enzyme–substrate complex (for details see review [13]). GTP hydrolysis in the complex decreases the EF-G affinity with the ribosome, and this leads to dissociation of the factor. In other words, as Spirin suggested in 1988 [13], the exergonic reaction of GTP hydrolysis is necessary in order to compensate the free-energy gained during the complex formation between the ribosome and the factor. After GTP hydrolysis and EF-G dissociation the ribosome is in the posttranslocated state, which has a high affinity

to the aminoacyl-tRNA\*EF-Tu\*GTP ternary complex, and the next elongation cycle can be started. In these events the effector loops play an important role in the alternative and specific interactions of the factors with the ribosome. Hence, in general words, the EF-G function consists in that it catalyzes the transformation of non-covalent bonds in the ribosome, i.e. the transfer of peptidyl-tRNA from one ribosomal site to another.

**Acknowledgements:** The authors are grateful to Prof. A.S. Spirin for discussions and critical reading of the manuscript. This work was supported by Grant 99-04-48231 from the Russian Foundation for Basic Research.

## References

- [1] Spirin, A.S. (1999) Ribosomes, Kluwer Academic Publishers/Plenum Press, New York.
- [2] Bourne, H.R., Sanders, D.A. and McCormick, F. (1991) *Nature* 349, 117–127.
- [3] Kjeldgaard, M., Nyborg, J. and Clark, B.F.C. (1996) *FASEB J.* 10, 1347–1368.
- [4] Laurberg, M., Kristensen, O., Martemyanov, K., Gudkov, A.T., Nagaev, I., Hughes, D. and Liljas, A. (2000) *J. Mol. Biol.* 303, 593–603.
- [5] Martemyanov, K.A. and Gudkov, A.T. (2000) *J. Biol. Chem.* 275, 35820–35824.
- [6] Nissen, P., Thirup, S., Kjeldgaard, M. and Nyborg, J. (1999) *Structure* 7, 143–156.
- [7] Willie, G.R., Richman, N., Godtfredsen, W.O. and Bodley, J.W. (1975) *Biochemistry* 14, 1713–1717.
- [8] Zeidler, W., Schirmer, N.K., Egle, C., Ribeiro, S., Kreutzer, R. and Sprinzl, M. (1996) *Eur. J. Biochem.* 239, 265–271.
- [9] Martemyanov, K.A. and Gudkov, A.T. (1999) *FEBS Lett.* 452, 155–159.
- [10] Martemyanov, K.A., Yarinin, A.S., Liljas, A. and Gudkov, A.T. (1998) *FEBS Lett.* 434, 205–208.
- [11] Clark, B.F.C., Thirup, S., Kjeldgaard, M. and Nyborg, J. (1999) *FEBS Lett.* 452, 41–46.
- [12] Belitsina, N.V., Glukhova, M.A. and Spirin, A.S. (1976) *J. Mol. Biol.* 108, 609–613.
- [13] Spirin, A.S. (1988) in: *The Roots of Modern Biochemistry* (Kleinkauf, H., von Döhren, H. and Jaenicke, R., Eds.), pp. 512–533, Walter de Gruyter, Berlin.