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**EF-Tu PROTEIN DOMAINS
Functions and Thermostability**

PhD thesis

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Summary of PhD Thesis

The bacterial elongation factor Tu (EF-Tu) has been extensively studied for decades as it plays a key role in protein biosynthesis. It is a model, multifunctional GTP-protein. This protein is also in the centre of interest as a possible target for new antibiotics. Moreover, the high homology in structure and function makes EF-Tu proteins suitable for the studies of evolutionary relationships between organisms and for elucidation of the structural features of adaptation to various living conditions. Furthermore, since all known EF-Tu proteins are composed of three distinct domains, they can also serve as suitable models for the understanding of domain organization in proteins.

One of the main research projects of the Department of Gene Expression at the Institute of Molecular Genetics AS CR, where I did my PhD studies, was the study of the primary structure, transcription regulation and functions of bacterial elongation factors Tu from Gram positive thermophilic bacterium *Bacillus stearothermophilus* and from Gram negative mesophilic bacterium *Escherichia coli*.

In this work, we focused on the structure-function relationships between EF-Tu proteins and their domains. The domain effect had been before our studies tested mainly by truncated EF-Tu forms lacking one or two domains. In contrast, we decided to study the properties of individual domains within full-length three-domain EF-Tu proteins (by domain chimerization approach) in comparison to isolated domain 1 (G-domain).

I focused on two topics:

1. Evaluation of the effect of individual domains of EF-Tu proteins from *E. coli* and *B. stearothermophilus* on their basic functions, namely GDP/GTP binding, GTPase activity and on the thermostability.

We showed that (i) *B. stearothermophilus* EF-Tu and *B. stearothermophilus* G-domain bound GDP and GTP with differential affinities in nanomolar and submicromolar ranges, respectively. These affinities were fully comparable with those of *E. coli* EF-Tu. In contrast, the *E. coli* G-domain did not display the differential affinity for GDP and GTP, typical for intact EF-Tus, and bound both nucleotides with much lower, micromolar affinities. Therefore, in *E. coli*, all three domains were required for the high and differential affinity for GDP and GTP, a physiological feature of bacterial EF-Tus. In contrast, the *B. stearothermophilus* G-domain itself already possessed the high and differential affinity for GDP and GTP.

(ii) The isolated catalytic G-domain of both EF-Tus displayed similar GTPase activities at their optimal temperatures. However, noncatalytic domains 2+3 of the EF-Tus influenced

the GTPase activity of G-domains differently, depending on the domain origin. *E. coli* domains 2+3 suppressed the GTPase activity of the *E. coli* G-domain, whereas those of *B. stearrowthermophilus* EF-Tu stimulated the *B. stearrowthermophilus* G-domain GTPase.

(iii) We demonstrated that the overall thermostability level of either EF-Tu was the result of cooperative interactions between the G-domain and domains 2+3. It appeared that the G-domains set up a basal level of the thermostability of both EF-Tus. Domains 2+3 contributed by further stabilization of α -helical regions of G-domains. This in turn, allowed the G-domains to function at temperatures corresponding to growth temperature optima of respective bacteria.

2. Characterization of thermostability elements of *B. stearrowthermophilus* G-domain by comparative analysis with the *B. subtilis* G-domain.

We demonstrated that the N-terminal 12 amino acid residues play a key role in the thermostability of the G-domain. Our experiments further suggested that the thermostabilizing effect of the N-terminus could be mediated by stabilizing the functionally important effector region. The effect of the N-terminus was also significant for the thermostability of the full-length EF-Tu.

Our results contribute to the understanding of domain arrangement of ubiquitously occurring proteins elongation factors Tu. By a systematic analysis, we investigated the effect of the domains on EF-Tu functions and thermostability. The results obtained during my PhD studies were presented in five papers, in several oral presentations and in numerous posters at international conferences.