The role of alternative EF-G's in translation regulation during antibiotic biosynthesis in Myxococcus xanthus

The basis of life of every living cell is hierarchical management of genetic information, which is stored in DNA, and provides instructions for the cell's functioning. The use of this information requires many coordinated actions, including the rewriting of the information contained in DNA into RNA in the process of transcription. Then, RNA acts as an intermediary in the management of information in the cell. In the final stages, the data contained in RNA is transformed, where the language of four nucleotides is translated into the language of twenty amino acids in the form of a functional protein. Protein biosynthesis, also called translation, is a fascinating process with a ribosome at its heart. Thus, the ribosome transforms genetic information into a functional system manifested as a protein, which is the fundamental molecule conditioning the functioning of all living organisms.

The ribosome consists of two subunits, each built of RNA and proteins, which together form a functional whole. The ribosome function is supported by a number of factors. In addition to a set of conserved and necessary for the functioning of translation: initiation, elongation and, release and recycling factors, evolution has selected for a number of proteins that can help maintain and preserve translation under stress conditions, including nutrient deficiency or presence of antibiotics. Specialized translation factors help protein biosynthesis machinery respond to aberrations in bacterial growth conditions. Translation factors that respond to antibiotic stress play a special role – they are ***ribosome protecting proteins* (*RPP*)** known to help maintain uninterrupted translation under conditions where antibiotics that block ribosome function are present. The best known example is a TetM protein found in *Enterococcus faecalis*. This protein removes the tetracycline antibiotic from the ribosome. Another known example is a FusB protein, which protects the ribosome from the action of the antibiotic fusidic acid, enabling translation in the presence of this compound. Such factors belong to the group of antibiotic resistance genes and are often located on bacterial mobile genetic elements and on plasmids. Antibiotic resistance is a naturally occurring phenomenon associated with the biosynthesis of antibiotics that provides manufacturers with self-defence processes. However, antibiotic resistance genes are a source of multi-drug resistance in bacteria that cause incurable bacterial infections.

The interest of my research group are translation factors responding to antibiotic stress. In this project, we will explore *Myxococcus xanthus* DK 1622 – a bacterium commonly found in soil, known for its large genome and secondary metabolite production, including antibiotics. Interestingly, bacteria belonging to this group of microorganisms are predators feeding on other microorganisms, including pathogenic bacteria from the Gram-negative and Gram-positive groups and on yeast. Our initial analyses revealed multiplication of the elongation factors EF-G and EF-Tu – proteins essential for the translation process. Interestingly, some of the genes we identified, are located adjacent the putative biosynthetic gene cluster for the thiopeptide antibiotic. The group of thiopeptide antibiotics is known to inhibit translation mechanisms. Class I of thiopeptides (e.g. thiostrepton) blocks the function of basic ribosomal factors: EF-G, EF-Tu. In this project, we plan to investigate whether the EF-G and EF-Tu paralogs identified here act as classic translational elongation factors, or rather act as ribosome protecting proteins by removing the thiopeptide antibiotic from the ribosome, thereby becoming thiopeptide resistance factors.

The latest generation of high-throughput technologies will be used in this project, i.e. RNA sequencing, supported by research in the field of biochemistry and structural biology. Research will focus on the regulation of gene expression during antibiotic biosynthesis, and in particular the role of the elongation factor EF-G paralogs in the regulation of biosynthesis of thiopeptide antibiotics. The results will allow not only for a better understanding of the protein biosynthesis process itself, but also for understanding the mechanisms of regulation of gene expression from the level of the translation apparatus. Moreover, the methods used in this project will allow the development of fast and comprehensive approach to search and analyse biosynthetic gene clusters for antibiotics, which will make a major contribution to the field of research into antibiotic discovery