INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS
POLISH ACADEMY OF SCIENCES

1. **Research Unit:** Laboratory of Translatomics
2. **Supervisor:** dr hab. Agata Starosta
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4. **Project title (English):**
   The role of alternative EF-G's in translation regulation during antibiotic biosynthesis in *Myxococcus xanthus*
5. **Project title (Polish):**
   Rola alternatywnych EF-G w regulacji translacji podczas biosyntezy antybiotyków u *Myxococcus xanthus*
6. **Description of the project (up to 500 words):**

Translation is performed by the ribosome and associated factors. Besides the set of conserved and essential translation factors including initiation, elongation and recycling factors, evolution selected for a range of proteins which can help preserve and sustain translational machinery during stress conditions including starvation or antibiotic stress. Specialized translation factors are known to help protein biosynthesis machinery to respond to aberrations of bacterial growth conditions. Many factors involved in the response to non-optimal growth conditions are well conserved within the bacterial kingdom. A group of specialized translation factors – Ribosomal Protection Proteins (RPPs) – is known to aid uninterrupted translation when antibiotics targeting the ribosome are present. The best known examples are TetM/TetO from *Enterococcus faecalis* – an ortholog of elongation factor EF-G – which can dislodge tetracycline off the ribosome or FusB/FusC which bind to EF-G trapped on the ribosome by the antibiotic fusidic acid and promote dissociation of EF-G, thereby enabling translation to continue and conferring resistance to fusidic acid.

The interest of my research group are translation factors responding to antibiotic stress. Curiously, my initial analyses of the genome of *Myxococcus xanthus* DK 1622 have revealed triple copy of the gene encoding elongation factor EF-G (varying amino acid sequences). Interestingly, two of these genes are located adjacent to two distinct putative biosynthetic gene clusters encoding thiopeptides. Strikingly, class I of thiopeptide group of antibiotics (thiostrepton) targets translation by binding to the large ribosomal subunit spanning the N-terminal domain of protein L11 to H43/44 leading to disturbance of the GTPase centre. Subsequently, the action of initiation factor IF2, elongation factors EF-G and EF-Tu is inhibited. It is unknown whether paralogs of EF-G identified here function as elongation factors and are indispensable for the general translation or rather act as ribosome protective proteins dislodging thiopeptide bound to the ribosome. In this project, we plan to (I) investigate the role of alternative elongation factors EF-Gs in translation and (II) their possible interplay with thiopeptide antibiotics biosynthesis regulation, inhibitory action and resistance.

We will apply a combination of in silico bioinformatical analyses of the genome, genes and proteins and next generation sequencing including analyses of transcriptome (RNAseq) and translatome (Ribosome profiling – RIBOseq) to identify genes regulated by the action of the investigated factors. We will also determine when each of the genes encoding EF-G is activated, while using targeted RIBOseq we will identify which mRNAs are translated with the assistance of each of the paralogue. We will take advantage of the unique life style of *M. xanthus*, which may include vegetative growth, sporulation, fruiting body formation or predation, and screen for conditions where antibiotics are produced. These techniques will allow us to identify a detailed timeline for the antibiotic production and regulation. Analyses *in vivo* will be complemented by in vitro investigations of the role of the paralogs in translation, sensitivity to antibiotics and their ability to protect translation from inhibitory action of antimicrobials. Lastly, together with Dr. Jean-Paul Armache, we will determine the atomic model of the factors bound to the ribosome using Cryo-EM.

7. **References related to conducted /planned research (maximum 3):**

Jenner et al. PNAS 2013 10.1073/pnas.1216691110
Cox et al. PNAS 2012 10.1073/pnas.1117275109
Arenz et al. PNAS 2015 10.1073/pnas.1501775112

8. **Scholarship amount (net):** please contact the project supervisor.