Why Nature introduced selenium to wobble nucleosides in transfer RNA?

1. Research objectives/ research hypothesis

The tRNA molecules contain more than 100 of post-transcriptionally modified nucleosides, and a majority of them is located in the wobble position of the anticodon loop. They contribute to the regulation of gene expression by restricting, expanding, or altering the decoding properties of the tRNAs, and modulate protein translation rates in a highly dynamic manner. Sulfur-modified uridines are present in tRNA in all three domains of life (Bacteria, Archaea and Eukarya), but only in bacteria they are accompanied by their selenium-containing analogs and by *S*-geranylated derivatives. It has been proposed that *in vivo* these bacteria-specific modified uridines are synthesized from their 2-thio precursors in two independent reactions, yet catalyzed by the same enzyme, the tRNA 2-selenouridine synthase (SelU or MnmH). Of note, our recent studies suggest that the SelU-catalyzed selenation and geranylation reactions are not unrelated, but are executed in a linear arrangement, so the product of geranylation constitutes the substrate for selenation. While the function of sulfur-modified uridines in translation process is partially understood, the function of selenium- as well as geranyl-modified uridines in the protein translation is elusive. Moreover, differences in function between seleno- and thio-nucleosides may also originate from different redox chemistry of both elements.

The main goal of the Project is to answer the questions:

1.Why Nature developed so complex enzymatic apparatus to introduce selenium-modified nucleosides into bacterial tRNAs at the expense of their thio-precursors?

2. More specifically, what is(are) the function(s) of 2-seleno-uridines and *S*-geranyl-2-thiouridines in translation process?

3. How oxidative stress and other stress conditions alter the profile of the sulfur, selenium and geranyl modifications in *Escherichia coli* specific tRNAs?

2. Research project methodology

We will elucidate experimentally and theoretically the tautomeric structures of 5-substituted 2-selenouridines (R5Se2U) and their affinity to the A and G complements in the codon-anticodon context. The results of physico-chemical experiments will be checked against data obtained from computational calculations. The set of sulfur-, selenium- and geranyl-containing nucleosides will be synthesized and an analytical approach will be developed for assessment of the abundance of S/Se-modified nucleosides in the tRNA pool in *E. coli*. The *in vitro* differences in oxidative damage of R5S2U-RNA and R5Se2U-RNA will be analyzed RP-HPLC/LC-MS. The landscape of sulfur-, selenium- and *S*-geranyl-modified units in the wild type and genetically modified bacteria (*E. coli*) will be analyzed by Q-TOF MS/MS approach, and used for interpretation of function of Se2U/geS2U modifications in various stress conditions. The influence of R5Se2U-tRNA on the "codon usage" (NNA vs NNG codons) will be assessed and the affinity of the S/Se/Sge -ASL models to the ribosome will be analyzed.

3. Expected impact of the research project on the development of science

The proposed research program is challenging for several reasons. We will focus on tRNAs containing nucleosides bearing a selenium atom, for which limited structural and biological data have been published so far. Specifically, the project will allow to learn on the structure and tautomeric forms of the selenium-modified nucleosides, as well as on the differences in their binding affinity towards adenosine and guanosine units present at the 3'-position of the synonymous codons. These differences are crucial for the fidelity of the codon-anticodon recognition and for precise tuning of the protein synthesis. Our profound experience in the synthesis of modified nucleosides and in site-specific positioning of them in RNA oligomers, will be profitable for generation of a set of sulfur-, geranyl- and selenium-modified nucleosides (present in *Escherichia coli*), which will have practical application not only in our Project, but also in search for the correlation of the observed functions of transfer RNAs with the relative content of particular modifications. The dynamics of changes, being induced in *E. coli* by certain genetic manipulations or selected stress conditions (e.g. oxidative stress), will help to define the role of sulfur-, selenium- and geranyl-containing modifications in regulation of gene expression (the codon usage). Our interdisciplinary project will provide a platform for experiments in organic chemistry, biophysics, theoretical modeling, as well as in molecular and cell biology, focused on transfer RNAs containing sulfur-, geranyl- and selenium-containing modifications. The results of the Project will be published in 4-5 papers of the world wide Journals from JCR list and discussed at scientific conferences (IRT, GRC, FEBS meetings, etc.)