DC5: Identification and functional analysis of new factors and regulatory mechanisms of mitochondrial antisense RNA surveillance

Host institution: Institute of Biochemistry and Biophysics, Polish Academy of Science, Warsaw, Poland.

Supervisor: Dr. Roman Szczesny

Co-Supervisors: Dr. Carlo Vascotto, Institute of Hematology and Transfusion Medicine, Department of Experimental Hematology, Warsaw, Poland (Academic); Dr. Thomas Frischmuth, baseclick GmbH, Neuried, Germany (Industrial).

Project description: Expression of the human mitochondrial genome is an extreme example of spurious transcription. The majority of mitochondrial DNA-templated transcripts are antisense, non-coding RNAs, which are swiftly degraded after synthesis. Consequently, the levels of mitochondrial non-coding RNAs are kept at a very low level. So far, the mitochondrial degradosome, a complex of RNA helicase SUV3 and phosphorylase PNPase, has been shown to be a key player in the surveillance of mitochondrial non-coding RNAs (mt-ncRNA).

The function of the degradosome is supported by an RNA binding protein GRSF1 and an oligoribonuclease REXO2. GRSF1 melts stable G-quadruplex structures present in some mt-ncRNAs. REXO2, in turn, is responsible for removal of short RNAs, which are end products of degradosome-mediated mtRNA decay. However, the high efficiency of removal of mt-ncRNA suggests the existence of additional factors involved in surveillance of these RNA species. For example, an endoribonuclease-dependent processing of antisense transcripts would lead to the generation of additional entry sites for the degradosome, which degrades its substrates in an exoribonucleolytic manner.

The posttranslational modifications (PTMs) of proteins play an important role in the regulation of many biological processes. Interestingly, while global analyses of posttranslational modifications of human proteins suggest that the components of the mitochondrial degradosome and REXO2 can undergo such modifications, nothing is known about their functional importance.

In this project, we aim to identify new factors involved in mt-ncRNA regulation. We also aim to explore the role of PTMs in the regulation of mitochondrial antisense transcripts surveillance mechanisms. To this end, we will perform a targeted siRNA screening test to identify genes whose silencing results in the upregulation of mitochondrial antisense transcripts. We will also conduct a comprehensive mass spectrometry-based analysis of PTMs present in proteins involved in mtRNA metabolism. Subsequently, we will investigate the functional importance of identified PTMs. The proposed research project will allow to build a broader picture of proteins involved in the surveillance of mitochondrial antisense RNAs. We will also learn about potential PTMs-dependent regulation of mt-ncRNA.

Host laboratory: Dr. Szczesny laboratory is interested in the mechanisms that control the quality, quantity, and processing of RNAs that originate from expression of the mitochondrial and nuclear genomes in humans (SzczesnyLab). Our main goal is to decipher a molecular

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machinery that is responsible for RNA surveillance. We also investigate the mechanisms that maintain and regulate expression of the mitochondrial genome and the ways in which they respond and adapt the cell to different stress conditions. The laboratory is located in one of the best research institutions in Poland (IBB PAS). The home institute is localized at the Ochota Research Campus, where other life sciences institutes are placed. We have direct access to all necessary equipment, such as the automated liquid handling workstation, fluorescent microscopes for automated quantitative imaging, as well as the genome-wide and targeted siRNA libraries. The host Institute is very well equipped and provides access to a wide-range of modern methods, such as NGS or quantitative and qualitative proteomics. The Institute anticipates great benefits from the internationalization of its community. We understand that moving to and living in a foreign country, with a different culture, may be associated with some difficulties. Thus, in order to overcome the relocation barriers, the Welcome IBB Center was established that helps out the foreigners to mitigate these difficulties (Welcome IBB Center).

Secondments: This project is carried out in strong collaboration with the following groups, and visits to their laboratories are expected during the project. A willingness to travel and spend time abroad is therefore essential:

- Dr. Carlo Vascotto, Institute of Hematology and Transfusion Medicine, Warsaw, Poland;
- Dr. Thomas Frischmuth, baseclick GmbH, Neuried, Germany.

Eligibility conditions

- Master's degree, medical doctor or equivalent in the field of exact sciences, natural sciences, medical sciences or related disciplines
- The successful candidate will need to be enrolled at the Doctoral School of Molecular Biology and Biological Chemistry IBB PAS (Doctoral School IBB PAS)

Required Skills

- Passion for science, love of experimental research, creativity.
- Good interpersonal skills, willingness to learn, and the ability to work both in a team and independently.
- At least one year of experience in experimental research (molecular and/or cellular biology).
- Interest in various life science disciplines would be an advantage.
- Any experience in imaging, RNA biology, *in vitro* cell culture, next-generation sequencing, and involvement in studies on mitochondrial biology will be an advantage.
- Proficiency in written and spoken English.

Enquiries

- For general information about the MITGEST Doctoral Network visit the visit the project website (www.mitgest.eu) or send an email to (info@mitgest.eu).
- For additional information on this project please contact Dr. Roman Szczęsny (rszczesny@ibb.waw.pl).

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How to apply

To complete your online application, visit the MITGEST recruitment web page (https://www.mitgest.eu/open-positions/).

Application deadline

The closing date for applications is **November 15th 2022**.

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