1. PHD PROJECT DESCRIPTION

Project title:

Epitranscriptom - a new mechanism to control of gene expression in plants subjected to abiotic stress

1.1. Project goals

The aim of the project is study the function of N-6adenosine in mRNA metabolism with particular emphasis on the functional localisation in plant cells under hypoxic stress conditions

1.2. Outline

Epitranscriptome is a likely to be a new co-transcriptional process regulating the metabolism of transcripts based on the chemical modification of RNA nucleotides. N6-methyladenosine (m6A) is the most prevalent internal mRNA modification found in eukaryotes, and has received a burst of interest in recent years. Recently, it was shown that m6A is enzymatically added to and removed from mRNA molecules. If the presence of this modification is dynamic, it makes it potentially an important mechanism of transcriptome regulation. Although null mutations of 4 proteins formed in methylation complex in plants are embryonically lethal, the function of m6A in the regulation of the gene expression in plants is not clear (Růžička et al. 2017).

Recently, the role of functionally RNA in cell has been rediscovered. This phenomenon has been demonstrated in storing mature transcripts, separating them from ribosomes or accumulating them in structures in which they are degraded. During hypoxia stress mRNA is retained in the nucleus and accumulated in cytoplasmic stress granules (SGs). In the nucleus, hypoxia stress induces the retention of poly(A) RNA including mRNAs coding proteins not involved in the response to hypoxic conditions (Niedojadlo et al. 2014, Niedojadlo et al. 2016). This finding indicates that the nucleus is a storage site for those of mRNAs which are a in the response to hypoxia for used by the plants after being subjected to the hypoxic stress. Low O2 conditions may result in a strong inhibition of protein synthesis. This process saves ATP, which is reduced by 50% after two hours of hypoxia. The presence in the cytoplasm of SG rich in poly(A) RNA, is cellular expression of the inhibition of protein synthesis. The stress granules did not exhibit the presence of the translation initiation factors eIF2a, excluding translation occurrences in SG. This means that the location of mRNA in cells during hypoxia can be a strategic mechanism for coping with hypoxia stress, but the processes that regulate these phenomena are unknown. Preliminary data derived primarily from mammalian cells suggest the involvement of m6A in mRNA metabolism including export from the nucleus, mRNA stability and spatial control of translation.

Understanding the epitranscriptomic regulation of gene expressions responsible for an increase or reduction of tolerance to environmental conditions can help in the genetic modification of crop plants and contribute to an increase in yields.

1.3. Work plan

With the courtesy and cooperation of prof. Robert Frey (Faculty of Science from University of

Nottingham) we have come into possession of several A. thaliana mutants: 1) mta ABI3prom:MTA with adenosine methylation disorders after the second week after germination. 2) VIR and MTB (elements of methylation of adenosine complex) fused with GFP 3) PAP2-GFP protein being a marker of stress granules in hypoxia stress.

1) Localization and quantity measurement of poly(A) RNA and selected gene marker genes hypoxia in wt and mta ABI3prom: MTA A. thaliana.

2) Changes in the quantity and content of stress granules in a plant with a reduced degree of m6A with used double mutant A. thaliana PAP2-GFPx ABI3prom: MTA

3) Sequencing of mRNA after m6A-RNA immunoprecipitation for identified transcripts with m6A modification in wt and mta ABI3prom: MTA A. thaliana.

1.4. Literature

- **Niedojadło et al. PLoS One. 2014 Nov 4;9(11):e111780**
- **Niedojadło et al. RNA Biol. 2016 May 3;13(5):531-43**
- **Růžička et al. New Phytol. 2017 Jul;215(1):157-172**

1.5. Required initial knowledge and skills of the PhD candidate

A graduate of biology, biotechnology or related studies, interested in academic work in the domain of plant molecular biology. Experience in working with plant material, including A. thaliana, as well as the knowledge of techniques such as RNA isolation and analysis is welcome.

1.6. Expected development of the PhD candidate's knowledge and skills

During the doctoral thesis, full academic commitment, knowledge of the research topic, analysis of the obtained results and their presentation will be expected. The PhD student should develop and understanding and experience of using methods such as RNA isolation, RIP-seq, RNA-seq and in situ techniques.