1. PHD PROJECT DESCRIPTION

Project title: Role of Cajal bodies in nuclear retention and posttranscriptional modifications of mRNA in plants.

1.1 Project goals

The life course of mRNA begins with transcription by RNA Pol II, splicing, and processing 5' and 3' ends, which generally occur at the nuclear sites of transcription, and ends with cytoplasmic translation and degradation. mRNAs are thought to predominantly reside in the cytoplasm for the majority of their lifetime (Halpern. et al. 2015). However, in many cell types, have been observed that a significant portion of polyadenylated transcripts (approx. 30%) are nuclear retained and undetected in the cytoplasm. In recent years, there were reports concerning both animals and plants, revealing that the proteins-coding transcripts may be retained in cell nucleus for most of their lifetime (e.g. Halpern et al. 2015). Recent studies on cells of the male fern *Marsilea vestita* showed that retention and subsequent removal of introns from pre-mRNA regulates the translation pattern during post-transcriptionally regulated spermatogenesis (Boothby et al. 2013). As sequencing analyzes revealed, during the mRNA splicing one intron was usually left, generally not exceeding 100 nt. This intron is removed post-transcriptionally, when the cell is in a stage of development, when the protein encoded in the mRNA is needed. This process is a functional mechanism that prevents premature translation of proteins required during a specific stages of the development of transcriptionally silent gametes.

So far, the only domain related with the accumulation of polyadenylated transcripts in animal cells were nuclear speckles. However, the transcripts accumulated in the speckles do not appear to be transported to the cytoplasm. In the larch microsporocytes during diplotene, lasting about 5 months in this species, large quantities of polyadenylated RNA are synthesized (Kołowerzo-Lubnau et al. 2015, Hyjek et al. 2015). In the mid-diplotene we observed that poly(A) RNA is accumulated in Cajal bodies (CBs) (Kołowerzo et al. 2009). We also showed that the poly(A) RNAs accumulated in CBs encode proteins - i.e. are mRNAs. These include housekeeping gene transcripts (Smoliński and Kołowerzo 2012).

In the light of studies on the newly-understood mechanism of gene expression regulation by nuclear retention of mRNAs we want to check: (1) which transcripts are retained in CBs, (2) whether the transcripts stored in the CBs are mature or immature, (3) whether CBs are solely a place of retained mRNAs accumulation or they can be a domain where post-transcriptional modifications of mRNAs take place leading to inhibition of their export to the cytoplasm.

1.2 Outline

The research material will constitute larch microsporocytes in the diplotene stage. These cells are characterized by high metabolic activity and periodic retention of poly (A) RNA in the nucleus. From the cells in the individual stages will be isolated CBs, nucleoplasm and cytoplasm, from which poly (A) RNA will be isolated in an analogous manner. Isolated transcripts will be sequenced and subjected to functional analysis. Based on the results of sequencing, it will be possible to design molecular probes that will be used to trace the process, retention of selected mRNAs in microsporocytes larch in situ. To verify that mRNAs retention in the nucleus of microsporocytes is to delay the translation there will be cytoplasmic protein isolated from microsporocytes and proteomics analysis will be performed.

The results will give a vast body of data on the involvement of the CBs in the regulation of gene expression by mRNAs retention, (2) post-transcriptional splicing. The results of this research will give pioneering reports on the mechanisms of posttranscriptional regulation of gene expression through the retention of transcripts in the cell nucleus in higher plants. In addition, it will significantly expand the existing knowledge about the spatial organization of processes of posttranscriptional regulation of gene expression occurring within a cell, and whether such transcripts are fully functional and transported to the cytoplasm.

1.3 Work Plan

Due to the fact that the Cajal bodies seem to be the domain associated with the retention and subsequent export of poly(A) RNA into the cytoplasm, in this project we want to explain which mechanism/or mechanisms are responsible for the retention of poly(A) RNA in CB and determine which genes are regulated by nuclear retention of the mRNAs and identify the processes which may be regulated through this mechanism.

To test these assumptions we have planned the following research tasks:

1. Identification of retained polyadenylated transcripts in CB and nucleoplasm
2. Identification of the retention mechanisms of polyadenylated transcripts within CB
3. Dynamics of mRNA transport in the nucleus
4. Checking, if the retained transcripts are translated

1.4 Literature

Boothby TC, Zipper RS, van der Weele CM, Wolniak SM. 2013. Removal of retained introns regulates translation in the rapidly developing gametophyte of Marsilea vestita. Dev Cell. 24:517-529.

Halpern KB, Caspi I, Lemze D, Levy M, Landen S, Elinav E, Ulitsky I, Itzkovitz S. 2015. Nuclear Retention of mRNA in Mammalian Tissues. Cell Reports 13, 2653-2662.

Hyjek M, Wojciechowska N, Rudzka M, Kołowerzo-Lubnau A, Smoliński DJ. 2015. Spatial regulation of cytoplasmic snRNP assembly at the cellular level. J Exp Bot. 66:7019-7030.

Kołowerzo A, Smoliński DJ, Bednarska E. 2009. Poly(A) RNA a New component of Cajal bodies. Protoplasma, 236:13-19.

Kołowerzo-Lubnau A, Świdziński M, Niedojadło J, Bednarska-Kozakiewicz E, Smolinski DJ. 2015. Transcriptional activity of larch microsporocytes during diplotene with emphasis on the diffuse stage of meiosis. PLoS One. 2015;10(4):e0125647.

Smoliński DJ, Kołowerzo A. 2012. mRNA accumulation in the Cajal bodies of the diplotene larch microsporocyte. Chromosoma 121: 37-48.

1.5 Required initial knowledge and skills of the PhD candidat

- Should love the research (internal motivation will help to overcome the many obstacles that will surely come).
- "flexibility" and "thinking outside the box"
- The ability to work in a team and ability to communicate with the audience
- Analytical thinking.
- Have a little bit of luck (the ability to think positively).
- Skill and willingness to learn new things.
- Knowledge and understanding of cell biology, biochemistry and molecular biology.
- Recognising research problems and critical thinking.
- Skill to ask for help when needed.

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

- **Project management:** the ability to plan and organize the project as well as delegating and negotiating tasks among project members.
- **Perseverance:** the drive and determination to continue and finish a project.
- **Supervising and coaching:** the ability to transfer knowledge and inspire others.