1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title: The role of nuclear biomolecular condensates in posttranscriptional regulation of gene expression.

1.1 Project goals

In addition to canonical membrane-bound organelles, eukaryotic cells contain numerous membrane-less compartments. In recent years, there has been increasing evidence for the involvement of membrane-less organelles in various cellular processes, including the stress response and the regulation of gene expression. These supramolecular assemblies are composed of proteins, nucleic acids, and other molecular components. However, questions have remained about their formation, purpose, and how their physical characteristics contribute to biological function (Molliex 2015).

Liquid-liquid phase separation (LLPS) is a fundamental organizational principle in cells and serves as another mechanism for achieving internal organization. Specific molecules with distinct physical and chemical properties preferentially self-associate, leading to the formation of biomolecular condensates. These phase-separated domains exist in the nucleoplasm or cytoplasm and are formed through weak and highly multivalent interactions. In the project presented for evaluation, our aim is to investigate the role of both nuclear biomolecular condensates (Cajal bodies, CBs) and cytoplasmic biomolecular condensates (P-bodies, S bodies) in the posttranscriptional regulation of protein-coding genes (Hyjek-Składanowaska et al. 2020; Taliansky et al. 2023).

The project focuses on the newly discovered role of Cajal bodies (CBs) in the accumulation of pre-mRNA. Increasing evidence in recent years suggests the involvement of membrane-less organelles in various cellular processes, including the stress response, the regulation of gene expression, and the control of signal transduction. Our previous studies on larch microsporocytes have revealed the accumulation of mRNAs in Cajal bodies (CBs) during the diplotene stage. The accumulation of poly(A) RNA in these bodies represents a phenomenon not previously described in the literature.

The accumulation of mRNA in CBs is preceded by a high level of RNA synthesis. Newly synthesized transcripts accumulate in the nucleus. Subsequently, mRNA retention takes place within CBs when the transcription level is low, preceding mRNA transport to the cytoplasm and subsequent translation. These findings have led us to hypothesize that Cajal bodies are nuclear domains involved in the regulation of mRNA expression, particularly through translation delays. Retention and subsequent removal of introns from pre-mRNA play a role in translation regulation during fast, posttranscriptionally controlled spermatogenesis in Marsilea vestita (Boothby 2013). A similar mechanism of gene expression regulation has also been observed in meiotically dividing yeast cells and during oogenesis in Xenopus (Halpern 2015). In our research, we aim to examine whether a similar mechanism of translation regulation exists in the male generative cells of larch and whether Cajal bodies are the sites of transcript accumulation with retained introns.

1.2 Outline

The research material will consist of larch microsporocytes in the diplotene stage. These cells are characterized by high metabolic activity and periodic retention of poly(A) RNA in the nucleus. CBs, nucleoplasm, and cytoplasm will be isolated from cells in different stages, and poly(A) RNA will be extracted using a similar method. The isolated transcripts will then undergo sequencing and functional analysis. The sequencing results will enable the design of molecular probes for tracking the process of retaining specific mRNAs in situ within larch microsporocytes. To confirm that the retention of mRNAs in the nucleus of microsporocytes leads to delayed translation, cytoplasmic proteins will be isolated from microsporocytes and subjected to proteomic analysis.
The results will provide a vast amount of data regarding the involvement of CBs in the regulation of gene expression through mRNA retention and post-transcriptional splicing. This research will yield pioneering insights into the mechanisms of post-transcriptional regulation of gene expression through transcript retention in the cell nucleus in higher plants. Moreover, it will significantly expand our current understanding of the spatial organization of post-transcriptional gene expression regulation processes within cells, including the functionality and transport of such transcripts 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:

•perform transcriptome analysis of larch microsporocytes during different stages of cyclic flow of poly(A) RNA in the cells,
•analyze the transcriptome of Cajal bodies isolated from larch microsporocytes to find transcripts with retained introns,
•determine which genes are transcribed before poly(A) RNA accumulation in the CBs,
•determine the dynamics of the synthesis and transport of mRNA transcripts that are accumulated in the CBs,
•examine if the transcripts detected in the CBs occur in functional complexes with splicing factors whose presence in the CBs has been shown in our previous study,
•study the level of protein synthesis.
The study will use the following methods: CB isolation, RNAseq analysis of both microsporocytes and CBs, immunofluorescence, FISH, fluorescent in vivo hybridization, microinjection, advanced bioimaging techniques such as FRET, FISH TSA and ELF97 systems, PLA method, in situ detection with many single-labeled probes (Stellaris), in situ detection of transcription and translation, super-resolution microscopy (Bhat et a. 2020; Gonzalo et al. 2022; Stępień et al. 2022).

1.4 Literature

1. Bhat SS, Bielewicz D, Grzelak N, Gulanicz T, Bodi Z, Yu X, Anderson SJ, Szewc L, Bajczyk M, Dolata J, Smolinski DJ, Gregory BD, Fray RG, Jarmolowski A, Szweykowska-Kulinska Z (2020).

mRNA adenosine methylase (MTA) deposits m6A on pri-miRNAs to modulate miRNA biogenesis in *Arabidopsis thaliana*. **PNAS** DOI: 10.1073/pnas.2003733117
2. 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.
3. Gonzalo L, Tossolini I, Gulanicz T, Cambiagno DA, Kasprowicz-Maluski A, Smolinski DJ, Mammarella MF, Ariel FD, Marquardt S, Szweykowska-Kulinska Z, Jarmolowski A, Manavella PA (2022). R-loops at microRNA encoding loci promote co-transcriptional processing of pri-miRNAs in plants. **Nature Plants** 8: 402-418. DOI: 10.1038/s41477-022-01125-x.
4. Hyjek-Skladanowska M, Bajczyk M, Gołembiewski M, Nuc P, Kołowerzo-Lubnau A, Jarmołowski A, Smoliński DJ (2020). Core spliceosomal Sm proteins as constituents of cytoplasmic mRNPs in plants. **The Plant Journal** DOI: 10.1111/TPJ.14792
5. 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.
6. Molliex A, Temirov J, Lee J, Coughlin M, Kanagaraj AP, Kim HJ, Mittag T, Taylor JP. (2015). Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. **Cell** 163, 123–133. (doi:10.1016/j.cell. 2015.09.015)
7. Rudzka M, Wróblewska-Ankiewicz P, Majewska K, Hyjek-Składanowska M, Gołębiewski M, Sikora M, Smoliński DJ, Kołowerzo-Lubnau (2022) Functional nuclear retention of pre-mRNA involving Cajal bodies during meiotic prophase in European larch (Larix decidua). A **The Plant Cell** 34 (6): 2404–2423
8. Stepien A, Dolata J, Gulanicz T, Bielewicz D, Bajczyk M, Smolinski DJ, Szweykowska-Kulinska Z, Jarmolowski A (2022). Chromatin-associated microprocessor assembly is regulated by PRP40, the U1 snRNP auxiliary protein**. Plant Cell** DOI: 10.1093/plcell/koac278
9. Taliansky M, Love AJ, Kołowerzo-Lubnau A, Smoliński DJ (2023). Cajal bodies – evolutionarily conserved nuclear biomolecular condensates with properties unique to plants. **The Plant Cell 10.1093/plcell/koad140**

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.