Title of the Thesis: *„Dynamics of Cajal bodies' molecular composition as regulators of mRNA retention".*

Research problem: Gene expression in eukaryotic cells results from a complex network of processes that extend far beyond transcription initiation. Although transcription constitutes the starting point, the ultimate level and timing of protein synthesis are determined by multiple mechanisms acting also at the post-transcriptional level. These mechanisms allow the cell to flexibly manage its transcript pool, establishing buffering systems that enable adaptation to fluctuating conditions and dynamic physiological needs. They include, among others, the regulation of RNA export, mRNA translation, pre-mRNA and mRNA degradation, as well as control of protein activity itself. One such regulatory mechanism is nuclear retention of poly(A)RNA, which delays the utilization of mature transcripts and thereby fine-tunes gene expression.

Studies on microsporocytes of European larch (*Larix decidua* Mill.) indicate that mRNA retention is a global phenomenon tightly associated with the presence of Cajal bodies (CBs). This represents the first evidence suggesting the involvement of these nuclear domains in transcript fate control in plants. Preliminary analyses revealed that many of the mRNAs accumulated within CBs retain intronic sequences, suggesting intron retention as a key component of this regulatory mechanism. This is particularly relevant in cells exhibiting pulsatile transcriptional activity, where nuclear retention of transcripts allows their translation to be shifted in time and synchronized with the metabolic needs of the cell, even during periods of reduced transcriptional output. Although the exact mechanism of nuclear retention involving Cajal bodies remains unclear, elucidating this process constitutes an important research challenge. Advancing knowledge in this area may shed new light on the role of these nuclear structures in plant gene expression regulation and provide essential insights into the mechanisms underpinning meiotic progression and gametophyte development.

Aim of the study: The aim of this work was to identify transcripts characterized by intron retention and to investigate the molecular composition of Cajal bodies at successive stages of the poly(A)RNA synthesis and export cycle. The results presented here constitute

the first description of CBs involvement in nuclear RNA retention in plants. The observed accumulation of intron-retaining transcripts within CBs points to a strong link between the presence of introns and the localization of pre-mRNAs in these nuclear bodies. This highlights the significant role of CBs in post-transcriptional gene regulation, which may be crucial for proper cell division and the formation of a mature, functional microspore.

Results: It was demonstrated that in microsporocytes of European larch, characterized by pulsatile transcriptional activity, individual mRNAs exhibit distinct dynamics of nuclear export. Such heterogeneity may serve as a precise regulatory mechanism controlling the timing of protein synthesis, thereby enabling efficient management of the transcript pool in response to changes in gene expression. Bioinformatic analyses, supported by microscopic observations, showed that most transcripts accumulated in CBs contain retained introns, which are ultimately removed through splicing. Among these transcripts are mRNAs encoding components of the transcriptional and splicing machineries, whose retention may modulate the maturation of other mRNAs and contribute to their transient nuclear accumulation. Furthermore, the analysis of changes in the molecular composition of Cajal bodies during the cycle revealed that their function evolves over time. At the beginning of the cycle, CBs accumulate mature U snRNP complexes, which are primarily employed in co-transcriptional splicing. By contrast, at the end of the cycle they serve mainly as sites of snRNA accumulation, preparing these molecules for export from the nucleus to the cytoplasm. The snRNA stored in this manner constitutes a reservoir that is subsequently utilized at the onset of the next cycle, during the following pulse of transcriptional activity.

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