

## PHD PROJECT DESCRIPTION

(4000 characters max., including the aims and work plan to be published online)

**Project title:** *Cis-motifs and RNA structures that target transcripts to plant Cajal bodies*

### 1.1. Project goals

Nuclear mRNA retention in Cajal bodies (CBs) represents a poorly understood layer of post-transcriptional gene regulation. This PhD project aims to decode the molecular signatures that target specific transcripts to CBs in European larch (*Larix decidua*) microsporocytes. The candidate will pursue four interconnected objectives: (i) Identify cis-regulatory elements enriched in CB-retained transcripts, with focus on terminal introns, exon-intron boundaries, and 5' UTR regions; (ii) Map RNA-protein interactions using RIP-seq: coilin, Sm proteins (SmB, SmD1, SmD3), PHAX (snRNA export adaptor), and WRAP53/TCAB1 (CB integrity factor); (iii) Profile poly(A) tail dynamics using Nanopore sequencing; (iv) Characterise the interplay between mRNA retention and snRNA/scaRNA maturation. The goal is to establish the first integrated map linking cis-motifs, poly(A) status, protein-RNA interactions and snRNA/scaRNA biology in plant CB-mediated mRNA retention.

### 1.2. Outline

The project exploits the five-month meiotic prophase of *Larix decidua* microsporocytes, where up to 41% of nuclear poly(A) RNA accumulates in Cajal bodies in a five-stage retention-release cycle. Laser-capture microdissection of nucleoplasm and CBs combined with multi-platform sequencing (Illumina RNA-seq for mRNAs/snRNAs/scaRNAs, Nanopore for poly(A) lengths) will reveal transcript distributions. RIP-seq targeting coilin, Sm proteins, PHAX and WRAP53 will identify physically associated transcripts. Bioinformatics will focus on terminal introns, exon-intron flanks ( $\pm 50$  nt), 5' UTRs, and scaRNA-like motifs. Validation: smFISH for mRNAs + FISH for snRNAs (U1-U6) to assess co-localisation with CB markers.

### 1.3. Work plan

Years 1-2: LCM of CB and nucleoplasm fractions (5 stages  $\times$  3 replicates); Illumina RNA-seq; Nanopore poly(A) profiling; snRNA/scaRNA quantification. Years 1-3: Cis-motif discovery in terminal introns, exon-intron flanks, 5'UTRs using MEME-Suite; RNAfold secondary structure prediction; ML classifier for CB retention. Years 2-3: RIP-seq for coilin, SmB/D1/D3, PHAX, WRAP53 at 3 retention stages; integration with motif data. Years 3-4: smFISH + snRNA FISH validation; fluorescent reporters with mutated motifs; test snRNA depletion effects.

### 1.4. Literature

1. Rudzka M et al. (2022) Plant Cell 34: 2404 – nuclear retention via Cajal bodies. 2. Taliansky M et al. (2023) Plant Cell – Cajal body review. 3. Ohno M et al. (2002) Cell 101: 187 – PHAX in snRNA export. 4. Mahmoudi S et al. (2010) PLoS Biol – WRAP53 in CB formation. 5. Kiss T (2004) J Cell Sci – snRNP biogenesis. 6. Hyjek-Składanowska M et al. (2020) Plant J – Sm proteins in mRNPs. 7. Matinahmadi A et al. (2025) Plant Comm – P-bodies review.

### 1.5. Required initial knowledge and skills of the PhD candidate

Solid background in molecular biology, RNA handling. Basic bioinformatics. Experience with fluorescence microscopy and/or NGS library preparation. Good manual skills for LCM. Collaborative team player with good English.

- 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

Training in: (a) LCM, RIP-seq, Nanopore sequencing; (b) snRNA/scaRNA biology; (c) bioinformatics for motif discovery and ML; (d) smFISH/snRNA FISH microscopy. Expertise in nuclear RNA quality control and condensate biology. Preparation for academic or biotech careers.

- **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.