

PHD PROJECT DESCRIPTION

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

Project title: *RNA-binding regulators of selective mRNA release and export from plant Cajal bodies*

1.1. Project goals

Selective mRNA retention in Cajal bodies (CBs) requires trans-acting RNA-binding proteins that recognise retention signals. This PhD project will identify "gatekeeper" proteins controlling mRNA traffic in European larch microsporocytes, emphasising snRNA/scaRNA metabolism proteins. Objectives: (i) RIP-seq targeting SMN complex (SMN, Gemin2/3/5/7), PABPs (PABP, PABPN1), PHAX, and WRAP53 to identify associated transcripts; (ii) Map cis-elements in 3' UTRs, terminal exons and splice site flanks, including scaRNA-like motifs; (iii) TurboID-coilin and TurboID-SMN proximity proteomics to build gatekeeper interactome; (iv) Test whether snRNA modification status (2'-O-Me, pseudouridylation) couples to mRNA retention timing. Goal: define protein machinery linking snRNA biogenesis to mRNA retention/export control.

1.2. Outline

The project uses *Larix decidua* microsporocytes with their five-stage retention-release cycle. RIP-seq with anti-SMN, anti-Gemin, anti-PHAX, anti-WRAP53, anti-PABP antibodies will identify associated mRNAs and snRNAs. Proximity proteomics (TurboID-coilin, TurboID-SMN) will map the CB gatekeeper interactome, focusing on scaRNA-associated proteins (fibrillarin, dyskerin). Compartment-resolved RNA-seq and Nanopore will reveal modification status. Functional validation: morpholino knockdown monitored by MERFISH (50-plex mRNA) + snRNA FISH + polysome profiling.

1.3. Work plan

Years 1-2: Validate antibodies; RIP-seq for SMN/Gemin/PHAX/WRAP53/PABPs at 3 retention stages; identify bound mRNAs, snRNAs, scaRNAs. Years 1-3: LCM + RNA-seq; Nanopore for RNA modifications; cis-motif discovery in 3'UTRs and splice flanks; search for box H/ACA and C/D motifs. Years 2-3: TurboID-coilin and TurboID-SMN proximity labelling; DIA-PASEF MS; identify fibrillarin, dyskerin and other scaRNA-associated proteins. Years 3-4: Morpholino knockdown of SMN, PHAX, WRAP53; MERFISH + snRNA FISH; RiboMeth-seq; polysome profiling; integrative model.

1.4. Literature

1. Rudzka M et al. (2022) *Plant Cell* 34: 2404 – nuclear retention. 2. Taliansky M et al. (2023) *Plant Cell* – Cajal bodies. 3. Pellizzoni L et al. (2002) *Science* 298: 1775 – SMN in snRNP assembly. 4. Mahmoudi S et al. (2010) *PLoS Biol* –

SZKOŁA DOKTORSKA NAUK ŚCISŁYCH I PRZYRODNICZYCH "ACADEMIA SCIENTIARUM THORUNIENSIS"

UNIwersytet Mikołaja Kopernika w Toruniu

Collegium Humanisticum, ul. Bojarskiego 1, 87-100 Toruń, Polska

tel. +48 56 611 33 21, NIP: 879-017-72-91, REGON: 000001324

e-mail: ast@umk.pl, strona internetowa: www.phd.umk.pl/ast/

WRAP53. 5. Kiss T (2004) J Cell Sci – snRNP biogenesis. 6. Darzacq X et al. (2002) EMBO J – scaRNAs. 7. Matinahmadi A et al. (2025) Plant Comm – P-bodies.

1.5. Required initial knowledge and skills of the PhD candidate

Strong motivation for experimental work combining molecular biology, proteomics and microscopy. Background in biochemistry, protein methods (Western, cloning). MS-based proteomics or RNA-protein interaction experience advantageous. Good manual skills for proximity labelling. 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) RIP-seq for multiple RBPs; (b) TurboID proximity proteomics with DIA-PASEF MS; (c) snRNA modification detection; (d) morpholino knockdown; (e) MERFISH + snRNA FISH. Expertise in snRNA biogenesis–mRNA metabolism coupling in nuclear condensates. Preparation for careers in RNA biology, proteomics or condensate biophysics.

- **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.