

PHD PROJECT DESCRIPTION

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

Project title: *Phospho-switch and CBC dynamics as triggers of programmed mRNA export release in plant generative cells*

1.1. Project goals

In *Larix decidua* microsporocytes, fully spliced mRNAs accumulate in the nucleus and Cajal bodies for 8–9 days before being suddenly released to the cytoplasm – yet the proteins forming the export machinery are already expressed at this stage. This argues for a regulatory switch acting on existing components, rather than for a missing component. This PhD project will test two candidate switches: (i) cytoplasmic-to-nuclear translocation of CBP20 as a rate-limiting step, suggested by our preliminary observation of stage-specific CBP20 nuclear depletion; (ii) phosphorylation status of SR adaptor proteins, which in metazoa must be dephosphorylated to recruit the export receptor. A third pillar links the system to a parallel context: (iii) heat-shock and osmotic-stress-induced poly(A) retention in vegetative cells, used as an orthogonal model to ask whether the same phospho-switch governs meiotic and stress retention. Goal: identify the molecular trigger that opens the export gate in plant cells..

1.2. Outline

The candidate will combine in-situ assays in synchronous larch microsporocytes with chemical biology in *Arabidopsis* protoplasts. Quantitative immunolabelling will map CBP20 abundance in nucleus versus cytoplasm at each of the five poly(A) RNA stages, while Proximity Ligation Assay (PLA) will quantify CBP20–CBP80, CBC–cap and CBC–ALY pairwise interactions in situ. SR-adaptor phospho-states will be profiled by phospho-peptide-enrichment LC-MS/MS on stage-resolved cell fractions, and stage-specific phospho-distributions confirmed in tissue by PLA with anti-phospho-RS antibodies. Pharmacological perturbation – SRPK/CLK kinase inhibitors and okadaic-acid-class phosphatase inhibitors – will be applied to *Arabidopsis* protoplasts to test whether export inhibition can be induced or reversed by manipulating the same switch.

1.3. Work plan

Year 1: anti-CBP20/CBP80 antibody validation; quantitative immunolabelling time-course across five poly(A) RNA stages; PLA pipeline setup. Years 1–2: phospho-peptide LC-MS/MS on retention vs export-

active microsporocyte fractions; mapping of phospho-sites on SR adaptors; PLA between phospho-SR and CBC. Years 2–3: poly(A) FISH; comparative phospho-profiling; test whether HSP70 mRNAs bypass the switch. Years 3–4: pharmacological perturbation in protoplasts; nuclear/cytoplasmic poly(A) ratios after kinase or phosphatase inhibitor treatment; integrative model linking CBP20 supply, SR phospho-state and export gating.

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* – 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. 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.