# Post-Transcriptional Regulation of mRNA Mediated by Decapping and Degradation Proteins in the Cytoplasm and Processing Bodies of *Larix Decidua*


## Abstract

**Importance:** Post-transcriptional mRNA turnover is a central regulator of gene expression during development, determining which transcripts are available for translation and when. Yet the cellular sites and mechanistic timing of decay pathways as modes of post-transcriptional regulation (most notably decapping-dependent 5′→3′ decay) remain insufficiently defined in a developmental context. *Larix decidua* microsporocytes provide a natural model; during a prolonged diplotene (several months), they execute reproducible, cyclical bursts of transcription and a clear five-stage poly(A) RNA cycle, thereby creating a temporal window to link mRNA fate to developmentally programmed events.

**Research gap:** Many studies have characterized P-bodies and decapping machinery in non-plant systems or outside defined developmental programmes; far less is known about whether visible P-bodies assemble and actively mediate decapping-dependent decay during plant development, and how quantitative changes in P-body architecture relate to substrate load. In *Larix decidua* microsporocytes, the poly(A) cycle continues with a conspicuous late-stage (stage 5) reduction of cytoplasmic polyadenylated RNA, but the mechanistic contribution of P-body–centered decapping (DCP2, DCP5, EDC4/VCS, and LSM4) and downstream 5′→3′ exonucleolysis (XRN4) has not been tested. This gap prevents confident linkage of spatial RNP dynamics and transcriptome-level outcomes during meiosis.

**Objectives:** The thesis therefore pursued three coordinated aims: (1) to determine whether P-bodies form and act as sites of decapping/decay during the five-stage poly(A) cycle and specifically whether decapping contributes to the late-stage poly(A) decline, (2) to quantify how P-body metrics (number per cell, mean volume and volume-class distribution) and protein composition (DCP5, DCP2, EDC4, LSM4, XRN4) change across stages, and (3) to identify sets of transcript associated with individual decapping components by RNA-immunoprecipitation sequencing (RIP-seq), thereby generating candidate substrates for functional tests. These objectives were formulated to move beyond descriptive imaging toward mechanistic inference and target discovery.

**Methodology:** To connect space, time, and molecules, stage-resolved confocal microscopy was used to measure cytoplasmic poly(A) RNA and distributions of DCP5, DCP2, EDC4/VCS,

LSM4, and XRN4, alongside P-body counts, and size classes across stages 1-5 were combined. Biochemical P-body enrichment and co-immunoprecipitation validated protein interactions, while RIP-seq captured RNAs associated with DCP2, DCP5, EDC4, and LSM4.

**Key findings:** Visible P-bodies assemble during the diplotene and are dynamically regulated across the poly(A) cycle: mid-sized P-bodies (0.3-1.2 $\mu m^3$) constitute the dominant functional class throughout, whereas larger P-bodies (1.2-6 $\mu m^3$) become more prominent as catalytic factors engage. Protein recruitment follows a reproducible temporal order, organizational/pre-decapping factors DCP5 and LSM4 peak earlier (stage 3), the catalytic decapping module (DCP2 and EDC4/VCS) engages at stage 4, and XRN4 colocalization rises at stages 4-5, identifying stage 4 as the control/peak activity point and stage 5 as the resolution/cleanup phase. Co-immunoprecipitation supports a candidate DCP5/DCP2/EDC4 core module, whereas LSM4 and XRN4 exhibit more dynamic or downstream behaviour. Finally, RIP-seq recovered 413 mRNA sequences (216 annotated mRNAs), comprising shared and protein-specific sets enriched for mitochondrial, chloroplast, and membrane-associated functions, thereby delivering concrete candidate substrates for follow-up tests.

**Implications:** Taken together, these results define a supply-responsive, staged programme of cytoplasmic mRNA turnover in *Larix decidua* microsporocytes, in which P-bodies serve as principal reaction hubs coordinating decapping-dependent 5′→3′ decay during meiosis. The work fills a key gap by demonstrating P-body activity within a defined developmental programme, presenting a quantitative imaging/molecular pipeline and a validated RIP-seq resource. While spatiotemporal overlap implicates decapping-mediated 5′→3′ decay in the late-stage poly(A) RNA decline in the deplotene phase of *Larix decidua* microsporocytes, alternative pathways (deadenylation/exosome, transcriptional downregulation) are also likely to contribute; accordingly, the thesis establishes the experimental roadmap and molecular entries needed to distinguish these routes.