Abstract of Doctoral Dissertation

Degree requested Doctor of Life Science Applicant's name FIERRO Ludivine

Title of Doctoral Dissertation

Comprehensive studies of mRNA 3'UTR processing during zebrafish early development
ゼブラフィッシュ初期発生におけるmRNA の 3'UTR プロセシングの包括的研究

During the earliest stages of embryonic development, biological mechanisms are led almost exclusively by maternal mRNAs inherited from the oocyte. This thesis investigated post-transcriptional mechanisms regulating mRNA fate in zebrafish embryos, with a focus on 3' untranslated region (3'UTR) dynamics, alternative polyadenylation (APA), and the impact of mRNA processing on translation and microRNAs (miRNAs) in shaping the maternal-to-zygotic transition (MZT). The 3'UTR is a derminant region for its mRNAs' translational activation, localisation, as well as stability. *pou5f3* and *wnt8a* mRNAs, two important factors in zebrafish development, were shortened from the earliest stages of its zebrafish development. Poly(A) tail lengthening was observed in *pou5f3* and *wnt8a* at 4 hours post fertilization (hpf), pointing to a coordinated mechanism involving both tail dynamics and 3'UTR architecture. 3'UTR RNA sequencing of embryos revealed widespread mRNA shortening already prior to zygotic genome activation (ZGA), between 0 and 2 hours post-fertilisation (hpf) and an even more important activity from 2 hpf to 4 hpf. Thousands of transcripts, including core developmental regulators such as *pou5f3*, exhibited isoform shifts that are likely to impact translation by exposing or occluding regulatory motifs in the 3'UTR. These not only shortening, but also lengthening events correlated with dynamic APA regulation. Other types of mRNA processings could be widely observed, such as intronic polyadenylation (IPA). Peaks2UTR analysis, a program annotating unknown 3'UTR, could find over 1000 previously unknown 3'UTRs, and confirmed gene ontology (GO) results that transcripts tend to have more isoforms in the early development of zebrafish. Expression of *cpsf6* mRNA, a factor promoting proximal PAS use, rose during the first few hours of development. My results showed that Cpsf6 expression at 2–4 hpf aligns with the onset of 3'UTR lengthening, suggesting its mRNA length change effects on its translational activation. Similar results could be observed on *elavl1* and its translation rate. I also identified 26 novel miRNA candidates and 100 registered miRNAs in the early embryo, with predicted targets including *pou5f3*. My findings suggested regulatory parallels with other biological systems.Taken together, these point to a finely tuned regulatory landscape, in which 3'UTR processing, APA, and tail length dynamics coordinate the timely activation and translation of key mRNAs. This work refines our understanding of post-transcriptional control during early development and highlights zebrafish as a valuable model for exploring how mRNA architecture governs gene expression transitions.