

ABSTRACT

Small non-coding RNAs (sncRNAs) are important regulators of gene expression, yet their functional interpretation in non-classical regeneration models is often constrained by incomplete reference resources. This dissertation aimed to develop and optimize bioinformatic approaches for sncRNA analysis in the planarian *Schmidtea mediterranea* and to use these approaches to clarify how perturbation of RNA metabolism influence regeneration.

First, an evidence-integrated, containerized genome annotation workflow (SmedAnno) was established to generate a reference annotation and to refine key loci of interest, including a corrected *Smed ELAC2* gene and corresponding transcript required for accurate mRNA quantification and design of sequence-dependent experiments. Next, RNA interference-mediated knockdown of *Smed ELAC2* (encoding a tRNA-processing ribonuclease) was used as a strategy to investigate the effects of alterations in the sncRNA pool on planarian regeneration.

Transcriptomic changes induced by *Smed ELAC2* silencing were profiled by bulk sncRNA and mRNA sequencing at 3 and 5 days post-amputation, and by time-resolved single-cell RNA sequencing. sncRNA analyses showed a selective, stage-dependent response concentrated in tRNA-derived fragments and miRNAs, with the strongest depletion observed for 5' tiRNA-Gly-GCC. Bulk mRNA analysis revealed modest transcriptional changes. Single-cell analyses localized *Smed ELAC2* expression to specific cell populations and indicated altered differentiation trajectory from broad neoblast stem cells toward selected lineage branches under knockdown. Complementary low-input profiling of sorted fractions and scRNA-seq-guided target-activity modeling indicated cell-type-biased regulatory potential of 5'-tiRNA-Gly-GCC, particularly in *PGRN*-positive parenchymal and phagocyte populations.

Together, this doctoral dissertation provides planarian-adapted reference resources and an integrative analysis framework for determining how specific changes in sncRNA accumulation affect lineage-resolved regeneration in planarians, highlighting 5' tiRNA-Gly-GCC as a regulatory molecule whose biogenesis is dependent on Smed ELAC2.

Keywords: *Schmidtea mediterranea*; regeneration; small non-coding RNA; tRNA-derived fragments; *ELAC2*; genome annotation; single-cell RNA-seq.