Small non-coding RNAs in regeneration of *Schmidtea mediterranea*

Annasha Dutta

Abstract

Planarians like *Schmidtea mediterranea* are flatworms known for their extraordinary regenerative abilities and hence have been the subject of scientific study for over two centuries. Recent discoveries have unveiled multiple cellular and molecular mechanisms that drive this process. However, many questions remain open, particularly concerning the role of small non-coding RNAs (ncRNAs), a group of molecules that includes some of the most significant regulators of metazoan gene expression. Considering that the *S. mediterranea* shares homologous genes with humans, including those that are associated with Mendelian diseases when disrupted, a deeper understanding of the molecular mechanisms in this organism can provide universal insights.

The major objective of this study was to identify and characterize small ncRNAs involved in *S. mediterranea* regeneration, with particular focus on the RNA fragments – molecules derived from mature constitutive ncRNAs. To achieve this goal, the research focused on developing a strategy to disrupt the pool of small ncRNA in regenerating planarians and assess the impact of this disruption across multiple physiological levels. Accordingly, the ncRNA-processing ribonuclease **Smed Elac2** was stably depleted in an asexual strain of *S. mediterranea*, and the resulting effects were examined at phenotypic, anatomical, cellular, gene expression, and small ncRNA levels.

Following *Smed ELAC2* knockdown a delay in eye regeneration and abnormal ventral nerve cord development was observed at the phenotypic and anatomic levels, respectively. The cellular-level analysis of *Smed ELAC2* knockdown worms led to the discovery of multinucleated cells (**MuNs**) in planarians. While the established differentiative cell populations remained unaffected, *Smed ELAC2* disruption resulted in a progressive increase in the nuclearity of MuNs during regeneration. In-depth characterization of MuNs using imaging flow cytometry and fluorescence-activated cell sorting (FACS), revealed them to be large homeostatic cells with a potential epidermal progenitor-like identity. Differential gene expression and gene ontology analyses upon *Smed ELAC2* knockdown identified key dysregulated processes, including the overall negative regulation of cell proliferation and migratory pathways, developmental processes, and synaptic transmission—crucial elements in the regeneration context. This study identified a small non-coding RNA essential for proper

regeneration timing: the **5′ tRNA half Gly-GCC**, which was drastically reduced following *Smed ELAC2* knockdown. This reduction highlighted a previously unknown role of Smed Elac2 in the production of 5′ tRNA half Gly-GCC. Functional *in vivo* studies further uncovered that this small ncRNA silenced the expression of a receptor-type tyrosine phosphatase gene. The overexpression of this gene upon *Smed ELAC2* knockdown appeared to be linked to the increase in nuclearity of MuNs at the cellular level and the negative regulation of cell migratory pathways at the gene expression level.

In summary, the mechanistic link between Smed Elac2 and 5′ tRNA half Gly-GCC, the functional significance of 5′ tRNA half Gly-GCC, and the identification of MuNs provide new insights in planarian regeneration and open avenues for further research of the conserved role of small ncRNA across diverse biological contexts.