UNIVERSITY
OF WARMIA AND MAZURY
IN OLSZTYN

**dr hab. Elżbieta Łopieńska-Biernat M.Sc., PhD**
**University of Warmia and Mazury in Olsztyn**
**Faculty of Biology and Biotechnology**
**Department of Biochemistry**
**Oczapowskiego 1a Str., 10-719 Olsztyn POLAND**
**phone +48 89 523 48 83**
**fax. +48 89 535 20 15**
**Email: ela.lopienska@uwm.edu.pl**

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**Review of the doctoral dissertation by**

**Annasha Dutta M.Sc.**

**entitled**

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

The doctoral dissertation of Annasha Dutta, M.Sc was prepared in the Laboratory of Single Cell Analyses, Institute of Bioorganic Chemistry of the Polish Academy of Science in Poznań under the supervision of Paulina Jackowiak, PhD, DSc, Professor of ICHB PAN. This work was founded by the National Science Center, Poland, through a grant No 2019/35/B/NZ2/02658 awared to Dr. hab. Paulina Jackowiak.

On the basis of the dissertation, MSc Annasha Dutta is applying for a doctoral degree in the discipline of biological sciences. In the review below, I focused on evaluation criteria tailored to the requirements of these doctoral dissertations.

The research topic selected by the candidate is well-justified. Planarians, primitive triploblastic invertebrates with bilateral symmetry, have long served as classic model organisms for studying tissue repair and regeneration. Their remarkable ability to regenerate entire structures of the central nervous system, including the head ganglia, has fascinated researchers for over 240 years. Technological advances in molecular biology, especially single-cell RNA sequencing (scRNA-seq), have significantly advanced our understanding of the heterogeneity among planarian stem cells—known as neoblasts. These cells, the sole mitotically active somatic population, are responsible for generating all new cells during both tissue homeostasis and regeneration. Recent discoveries indicate that neoblasts are not a uniform population; rather, distinct subtypes exhibit different molecular characteristics and differential activation in response to injury. In parallel, increasing attention has been paid to small non-coding RNAs (sncRNAs), such as microRNAs (miRNAs) and PIWI-interacting RNAs (piRNAs), due to their regulatory roles in development and regeneration. miRNAs modulate gene expression mainly through mRNA destabilization or translational

repression. piRNAs, originally identified in the germline, are now known to be essential for stem cell function and genome integrity. Both types of sncRNAs are active during planarian regeneration, implicating them in injury response and cell fate regulation.

Furthermore, new classes of sncRNAs, including tRNA-derived fragments (tRFs), are being discovered. Previously thought to be mere degradation products, tRFs have now been linked to cellular proliferation, differentiation, and diseases such as diabetes. Their role in planarian regeneration remains largely unexplored. This dissertation addresses this knowledge gap comprehensively, underscoring the scientific merit and timeliness of the research.

**Major Achievements of the Doctoral Work:**

This doctoral research presents the first-ever comprehensive analysis of tRNA-derived fragments (tRFs) in planarians, shedding new light on their functional roles during regeneration. The findings strongly support previous research and establish the 5′ tRNA half Gly-GCC as a crucial small regulatory RNA involved in planarian regeneration.

Importantly, the study proposes that 5′ tRNA half Gly-GCC mediates central nervous system (CNS) development not only in *Schmidtea mediterranea* but also in Drosophila, indicating a conserved function across major invertebrate lineages. Remarkably, this regulatory role in CNS regeneration appears to be evolutionarily conserved in mammals as well.

It was demonstrated that disruption of homeostatic levels of 5′ tRNA half Gly-GCC, through RNAi-mediated knockdown of Smed ELAC2, impairs anterior regeneration and CNS formation in planarians. These findings underscore the essential role of this small RNA in nervous system repair and recovery.

This research is also the first to implicate Elac2 in the biogenesis of 5′ tRNA half Gly-GCC in planarians. Beyond its established functions in tRNA and mitochondrial transcript maturation, Elac2 was shown to play a direct role in the generation of this regulatory RNA, which is critical for both developmental and regenerative processes.

Furthermore, overexpression of the SMESG000048842.1 gene, encoding a receptor-type tyrosine phosphatase, was found to inactivate Rho GTPases and impair cytoskeletal remodeling, ultimately reducing cell migration. The physiological levels of 5′ tRNA half Gly-GCC appear to repress the expression of this gene, thereby promoting cell migration—a key component of regeneration. Notably, 5′ tRNA half Gly-GCC binds to its target within the coding sequence (CDS) of the phosphatase gene.

The implications of Smed ELAC2 knockdown extend to human health, linking planarian findings to Mendelian disorders such as COXPD-17, associated with ELAC2 dysfunction and mitochondrial abnormalities.

Finally, an innovative methodology combining imaging flow cytometry with traditional FACS was developed to overcome the limitations of microscopy and conventional flow cytometry. This approach enabled the analysis of multinucleated cells (MuNs), opening new avenues for cellular studies in regeneration biology.

**Comments, questions and discussion points**

I have a few questions to PhD student for clarification and present below a few minor remarks to the substantive part of the dissertation.

- Page 91, section 5.2.3 cites Dutta et al. under revision should be listed as unpublished in the references.
- The statistical analysis is appropriate; however, it would benefit from an explicit explanation of the p-value threshold (e.g., $p < 0.05$), and clarification of the meaning of asterisks denoting significance (e.g., *, **, ***) in figures such as 8, 9, and 10.
- Bioinformatics analysis by Msc Anastasia Zaremba (page 103), it is understandable, we work in teams, and that our research is about, but I could not find a submitted paper in references. PhD student should provide bioinformatics analysis in the Materials and Methods section.
- page 105, editor's error in numbering of figures 22 and 23 B, C in text.

Finally, I would like to ask the PhD student, **what is your opinion on the advantages and disadvantages, but also on the question of which of these methods is better for regeneration (is it possible to judge this) and why: stem cells and organoids or non-mammalian models of regeneration differentiation?**

The results obtained confirm that the objectives of the research were successfully met. The work is distinguished by a well-considered choice of experimental methods, robust conclusions, and thoughtful selection of follow-up procedures. Notably, the emergence of new scientific questions is a hallmark of high-quality experimental research, reflecting the scientific maturity and critical thinking of the doctoral student. These open questions provide valuable directions for future exploration.

In this context, one particularly intriguing question arises: **Which of the proposed directions for further research do you consider most promising or worthy of pursuit?** For example: identification of RNA-binding proteins that associate with 5′ tRNA half Gly-GCC or functional characterization of

SMESG000048842.1, the receptor-type tyrosine phosphatase gene or others mentioned in Discussion. Each of these avenues could yield insights with broader relevance beyond the model organism, especially in understanding post-transcriptional regulation, cell plasticity, and regeneration. **Which of these directions do you find most compelling—and why?**

I greatly appreciate the dissertation of Annasha Dutta, MSc. The results obtained are undoubtedly valuable and add considerably to our knowledge of the sncRNA *S. mediterianian*. In my opinion, this study establishes a previously unrecognized molecular pathway — mediated by ELAC2-generated 5′ tRNA halves and their downstream targets — that controls crucial aspects of planarian regeneration. Moreover, the identification and characterization of multinucleated cells (MuNs) as stable, probable progenitor cells open a new dimension for understanding cell type dynamics in regeneration. Taken together, these results provide a solid framework for future research into both basic regeneration biology and translational applications in regenerative medicine.

**Summary and conclusion**

In conclusion, I state that the doctoral dissertation submitted for assessment contributes significant values to Science in the field of sncRNA in regeneration of the *Schmidtea mediterranea* and meets all the requirements specified in the Higher Education and Science Act of July 20, 2018 (Polish Journal of Laws of 2018, item 1668, as amended) and in the procedure for awarding the doctoral degree at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznań (Resolution of the Scientific Council of the Institute of Bioorganic Chemistry of the Polish Academy of Sciences no. 40/2025/Internet/RN_140 of March 18, 2025) and I request the Scientific Council of the Institute of Bioorganic Chemistry of the Polish Academy of Sciences to admit Annasha Dutta to the further stages of the doctoral program.

UNIWERSYTET WARMIŃSKO-MAZURSKI
w Olsztynie
WYDZIAŁ BIOLOGII i BIOTECHNOLOGII
Katedra Biochemii
10-957 Olsztyn-Kortowo, ul. Oczapowskiego 1A
tel. 89 523 39 90

Elżbieta Łopieńska-Biernat M.Sc., PhD, associate professor