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Review of the PhD thesis of Annasha Dutta
entitled
"Small non-coding RNAs in regeneration of *Schmidtea mediterrranea*"

The PhD thesis of Annasha Dutta has been prepared in the Laboratory of Single Cell Analyses of the Institute of Bioorganic Chemistry Polish Academy of Sciences, under the supervision of dr hab. Paulina Jackowiak, prof. IChB PAN. The main focus of the thesis is the role of small non-coding RNAs in the process of regeneration in planarians *Schmidtea mediterranea*. To achieve that the PhD candidate silenced RNAse Elac2, which is involved in non-coding RNA processing, and monitored the effects of the silencing on various aspects of regeneration process, including non-coding RNA levels. The topic of the thesis fits perfectly into the scope of expertise of the PhD supervisor, prof. Paulina Jackowiak, because the studies on non-coding RNAs, in particular tRNA fragments, have been an important focus of her research, and her laboratory has also been consistently developing studies on the regeneration process of *Schmidtea mediterranea* planarians.

The introduction to the PhD thesis is a very thorough explanation of most important processes involved in regeneration, and molecules involved in them, which are essential to understand the rationale and the context of the aims of the PhD thesis. Ms. Annasha Dutta clearly explained the general differences between embryonic development and regeneration processes, which was followed by the description of the different mechanisms of regeneration in animals, and the function of polyploid cells in the regeneration processes. A special emphasis was placed on the role of non-coding RNAs

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in regeneration, with focus in particular on miRNAs, piRNAs, and tRNA derived fragments (tRFs). The introduction concludes with a thorough description of the planarian organism, Schmidtea mediterranea, used in this PhD project. This includes information on their life cycle and the reasons why they are useful as regeneration models. The PhD candidate described in detail the anatomy of Schmidtea mediterranea and its cellular composition as these properties were important for planning the experimental work and analyzing the data presented in the PhD thesis. Finally Ms. Annasha Dutta included a very extensive description of the regeneration process itself and reviewed the information on various kinds of small non-coding RNAs, which role in this process has already been suggested. The information on the different types of miRNAs, piRNAs, and tRFs is presented in the tables, which helps in clear presentation of these extensive data. Overall, the introduction presents all the necessary information that introduces the reader to the topics covered in the PhD dissertation. The introduction is presented using a clear, logical language, and it is illustrated using simple, carefully prepared figures, which emphasize the most important points.

The results section has been split into four well defined chapters. The first one focuses on finding a way in which non-coding RNA levels could be perturbed during the process of regeneration in the studied planarian, so as to assess the outcomes of changed levels of ncRNAs on the regeneration process. For that purpose Ms. Annasha Dutta firstly reviewed available information on RNases present in this organisms, which have already been found to affect the fates of ncRNAs, and which homologs have important function in humans. This allowed her to select four RNases, which expression was increased in neoblasts or differentiated cells, and which she then silenced using RNAi technology. The most profound phenotypic effects during 10-day monitoring of a regeneration process were observed for Smed ELAC2 RNase.

In the second chapter Ms. Annasha Dutta described extensive results of her studies on the impact of the silencing of Smed ELAC2 RNase on the process of regeneration. She observed effects both at the level of phenotypical and anatomical changes and at cellular level. The most striking phenotype was the delay of eye

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development with 70% of regenerating tail fragments displaying eyeless or cyclopian phenotype at around day 5 of regeneration, which normalized at day 10. At anatomical level these changes correlated with poorer development of nerve cords. This study was followed by flow cytometer analysis of populations of cells obtained from Smed ELAC2 silenced organisms, wild type organisms and GFP control organisms. The main conclusion from these studies was that at the same phase of regeneration process, at which the phenotypical effects occurred, there was also an increase in the level of nuclei in multinucleated cells. These cells were further analyzed leading to the definition of true planarian multinucleated cells (MuNs), and to the definition of subpopulations within this class. The studies presented in this chapter very carefully and critically analyzed the outcomes of Smed ELAC2 RNase silencing leading to better understanding of the process of regeneration in this model organism.

The third and fourth chapters of the PhD thesis of Ms. Annasha Dutta concern the analysis of transcriptomic changes in planarians with silenced expression of Smed ELAC2. The former chapter describes the conclusions of transcriptomic analyses, while the latter describes the functional analysis of one identified tRNA fragment derived from tRNA-Gly-GCC. These data allowed validating the sequence of Smed ELAC2 and confirming the role of this RNase in the maturation of mitochondrial tRNAs. The most important conclusion was that during the regeneration process the main fraction of small regulatory RNAs that were affected were tRFs. Most of tRFs that were downregulated were 5′ tRNA halves, mostly derived from tRNA-Gly-GCC. Further functional analyses suggested that a regulatory target of this tRNA half could be a gene encoding a receptor-type tyrosine phosphatase. In the discussion section the PhD candidate further explored possible ways in which the changes in the levels of the 5′ half of tRNA-Gly-GCC could result in changed phenotypes observed during regeneration of planarians with silenced Elac2 RNase. I would like to ask the PhD candidate to show during the public defense how the 5′ tRNA-Gly-GCC is predicted to base pair with the proposed target mRNA? Are other 5′ tRNA halves detected during the study also predicted to interact with this mRNA?

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To summarize, the PhD dissertation prepared by Ms. Annasha Dutta explores important observations related to the role of non-coding RNAs, which levels were perturbed by silencing Elac2 RNase, in regeneration of model organism Schmidtea mediterranea. The studies showed that silencing of expression of this RNAse resulted in specific phenotypic changes, and also led to identification of multinucleated cells, which nucleation levels increase upon silencing of Smed ELAC2. Ms. Annasha Dutta discovered a potential role of a specific tRNA fragment as a regulatory RNA linking the silencing of Elac2 RNase with defects in the regeneration process. In this study the PhD candidate used a very wide range of molecular biology methods to thoroughly analyze the outcomes of Elac2 silencing from phenotype to transcriptomic changes. The thesis presents important discoveries, which provide new insights into the regulation of regeneration process in model planarians. The thesis has been written in a very logical way, in which each next experimental step is explained in the context of the previous results. The comprehensive approach to problem exploration, data analysis, and logical presentation of results show that Ms. Annasha Dutta is already an experienced researcher, who will successfully tackle further tasks in her scientific career.

The dissertation being the subject of the review fulfills the conditions laid down in the Act of July 20, 2018, The Law on Higher Education and Science (Journal of Laws 2018, item 1668 as amended), the Act of July 3, 2018, Provisions Introducing the Act – The Law on Higher Education and Science (Journal of Laws 2018, item 1669 as amended), and The Rules of Proceeding in the Matter of Awarding the Doctoral Degree in the Institute of Bioorganic Chemistry PAS (Resolution of the Scientific Board of IBCH PAS No. 28/2024/Internet of March 20, 2024) and I recommend that the Scientific Board of the Institute of Bioorganic Chemistry PAS allows it to further steps in PhD defense process.

Sincerely,

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