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Review of doctoral dissertation entitled "Investigating cytoplasmic polyadenylation and its role in gene regulation and physiology in *Caenorhabditis elegans*"

The PhD thesis written by Zuzanna Ewa Mackiewicz was prepared at the International Institute of Molecular and Cell Biology in Warsaw under the supervision of Prof. Andrzej Dziembowski and co-supervised by Dr. Vladyslava Liudkovska.

The presented thesis is in the form of an article-based dissertation. The thesis work comprises three published research articles of which one was peer-reviewed and published in the scientific journal *Science Advances* in 2022 (herein referred to as "Article 1"). The other two articles were published as pre-print in *bioRxiv* in 2024 (herein referred to as "Article 2" and "Article 3"). The contribution of Ms. Mackiewicz to "Article 1" is minor, which is also stated by her in the thesis. However, "Article 1" provides the scientific basis of her PhD research. Ms. Mackiewicz is the first author on "Article 2" and "Article 3" and is the person who primarily conducted the experiments. The thesis is comprised of an abstract in English and Polish language followed by a short introduction to the topic divided in subsections. The introduction could have been more accessible if supported by some illustrations. A summary of "article 1" is provided describing the main findings and is followed by description of the research objectives. Further, the thesis contains a result section also including unpublished results, a combined section for summary and future perspectives, methods (only for unpublished data), and list of references. As an appendix, the thesis contains the three research articles including statement of author contribution as well as a user manual for a data analysis tool developed by the PhD candidate. The author contribution statements are sufficient to evaluate the overall contribution of Ms. Mackiewicz for the published work.

The PhD work addresses the function of TENT-5 in *C. elegans*. TENT-5 is a member of the family of non-canonical poly(A) polymerases responsible for polyadenylation of transcripts

in the cytosol. The TENT5 family contains four proteins in mammals but has only one homolog in *C. elegans* making it a suitable model organism to decipher its function. The TENT5 family was recently described by the PhD student's lab, and Ms. Mackiewicz's work in *C. elegans* is largely based on previous work from the lab of the supervisor. The overall aim of the research was to characterize the role of TENT-5 in *C. elegans* and establish possible parallels to function of TENT5 family members in higher eukaryotes. Ms. Mackiewicz had four clearly stated specific research objectives: (i) to analyze poly(A) tail metabolism in *C. elegans* depended on the animal`s sex; (ii) to investigate potential redundancy with other poly(A) polymerases identified in *C. elegans*; (iii) to identify factors that determine the localization of TENT-5; and (iv) to characterize the function of the major TENT-5 targets. Main methods used throughout the studies were RNA sequencing and Nanopore Direct RNA sequencing, allowing the determination of the poly(A) length, including downstream bioinformatics analysis, preparation of *C. elegans* strains trough crossings and genetic manipulations including CRISPR/Cas9, standard bright field and fluorescent microscopy, and various behavioural assays to asses phenotypic changes of the different *C. elegans* strains. Familiar with the *C. elegans* work in the lab myself and the workload associated with the methodologies, the PhD candidate performed a large number of experiments with a high level of skill. The methods used are well-suited for the project and reflect the latest advances in the field. However, I would like to make a comment about the use of FUdR in lifespan experiments. The chemical FUdR should be avoided in lifespan experiments given the link between TENT-5 and innate immunity. FUdR is known to increase stress resistance in worms and might adversely affect the results in TENT-5 context. Methodologies described in “Article 2” and “Article 3” are sufficient to reproduce obtained results. The methodology part concerning the unpublished data is lacking information on primer sequences used for RT-qPCR and thermocycling conditions. Method for microscopy analysis should contain information on exposure time and processing of images if any was done. In general, the dissertation is carefully written with a minor problem in the labelling of the unpublished results in Figure 2; e.g. "RNAi *larp-5* mutant". RNAi does not cause a mutation in the classical genetic sense, so "mutant" should be omitted.

In summary, the results obtained established that TENT-5 has sex-specific roles with targets primarily important for mating in males. This was supported by the expression of TENT-5 in male specific tissues. The function of TENT-5 in *C. elegans* appears largely independent of the functions of other poly(A) tail polymerases, GLD-2 and GLD-4. Based on a targeted approach the protein LARP-5 was identified as potential regulator of TENT-5 activity. Lastly,

RNA sequencing analysis revealed that the most pronounced targets of TENT-5, NSPCs, might act upstream of the transcription factor DAF-16, which is a main player in longevity. I would like to commend Ms. Mackiewicz for her efforts in developing a bioinformatics tool that allowed her to compare her data sets. The code of the application is publicly available. The high-throughput results obtained and the various research tools developed provide a rich resource for further investigation. The discussion provided in the thesis along with the published work is adequate and provides further conceptual ideas to advance the research in the future.

Following scientific questions, I would like the PhD candidate to address during the denfense:

- How much of a difference in the poly(A) tail length significantly alters the stability of the transcript?
- What is the threshold in poly(A) tail length difference comparing wild type and *tent-5* mutant that would define the transcript as a target of TENT-5? Do you also take into account the total transcript levels when determining targets of poly(A) polymerases?
- Do you have any evidence that the protein levels of the proposed TENT-5 targets in males are altered in the *tent-5* mutant? I suppose that without changes in protein levels, it is unlikely that you would see altered physiology.
- Are the mutants of *tent-5*, *gld-2*, and *gld-4* loss-of-function mutants?
- Please comment on the statement page 13, which I find not precise enough: “However, I did not observe any transcripts behaving differentially depending on the presence…”. Did you compare the poly(A) length in *gld-4/tent-5* double mutant in parallel to the single mutants?
- How does differential polyadenylation correlate with differential expression (e.g. page 14 Figure 1J)?
- The structural similarity between NSPCs and INS peptides is intriguing. However, given the restricted localization of NSPCs it seems unlikely that they could affect DAF-16 localization throughout the worm, unless they are released and transported to other tissues. Was there a change in DAF-16 localization in the excretory gland cell?

Ms. Mackiewicz's findings are original and add to the knowledge of a recently discovered protein that is important for regulating RNA metabolism. In her work, Ms. Mackiewicz demonstrated very good knowledge in the discipline of biological sciences, the ability to logically approach a scientific question, and interdisciplinary skills in integrating large data sets.

I, the undersigned, hereby state that the doctoral dissertation of Zuzanna Ewa Mackiewicz meets the requirements specified in Article 187 of the Act of July 20, 2018 – Law on Higher Education and Science (c.t., Journal of Laws of 2024, item 1571, as amended). I hereby recommend to the Doctoral Committee of the International Institute of Molecular and Cell Biology in Warsaw to admit Zuzanna Ewa Mackiewicz to the subsequent stages of the procedure for the conferment of the doctoral degree in the field of natural sciences, in the discipline of biological sciences.

Ulrike Topf, PhD, DSc

Signed by / Podpisano przez:

Ulrike Topf
Instytut Biochemii i Biofizyki PAN

Date / Data: 2025-04-14 13:13