

**Review of thesis entitled “Investigating cytoplasmic polyadenylation and its role in gene regulation and physiology in *Caenorhabditis elegans*” by Zuzanna Ewa Mackiewicz**

**Reviewer: Dr. Oliver Hobert, Professor of Biological Sciences, Columbia University, New York**

Zuzanna Mackiewicz has undertaken extensive studies aimed at a better understanding of the molecular and physiological functions of a protein, TENT-5, involved in cytoplasmic polyadenylation. Elucidation the function of this protein represents an interesting and important question. The thesis is lucidly and concisely written. It provides a good, scholarly introduction to the problem. The thesis is original, rich in data and thoughtfully presented.

Zuzanna joined her thesis laboratory and begun her thesis work by rounding up work in her advisor’s lab which had found that genetic elimination of *C. elegans* TENT-5, a component cytoplasmic polyadenylation complex, affects polyadenylation of two sets of potentially related proteins – immunity-related proteins and secreted protein of the NSPC family. Zuzanna made an important contribution to this study by showing that *tent-5* mutants have an impaired immune-response and an impaired lifespan. These contributions earned her authorship on a paper published in *Science Advances*.

The next more independent part of her thesis work lay in asking an intriguing question –

are there potential sexual dimorphisms in poly-adenylation. This is an intriguing question because studies on sex-specificity are rare, yet there are indications in the literature that at least life-span regulation (and perhaps also immunity) may be regulated in sexually dimorphic manner. Zuzanna made two intriguing observations: First, there a sex-specific transcripts, apparently enriched in male-specific structures, that display a longer poly-A length and, second, this increased length genetically requires *tent-5*. Zuzanna undertook extensive test to find a physiological read-out of these changes, particularly relating to male mating and fecundity, but any defects have so far eluded identification. Zuzanna is to be commended for venturing into these assays and trying hard to elucidate physiological functions. She has written up these finding in a manuscript, posted on bioRxiv.

In the next major component of her thesis work, Zuzanna undertook a careful, detailed and comprehensive analysis of one prominent set of TENT-5 target genes, the small secreted NSPC peptides. The poly-adenylation of their encoding mRNA is controlled by TENT-5. Zuzanna finds that these NSPC proteins are exclusively expressed in a single cell, the excretory gland cell. Zuzanna then pursued two rigorous approaches to assess their functional significance. First, she has set up a system to genetically ablate the excretory gland cell, using a light-inducible cell killing agent (mini-SOG), that she specifically expresses in the excretory gland cell. She undertook substantial and careful efforts to set up the proper conditions for cell ablations and succeeds indeed in killing these cells. She has been using a wide range of potential physiological as well as molecular read-outs of deleting the excretory gland cell, but did not observe any effects.

Zuzanna then went on to delete all 18 nspc genes using CRISPR/Cas9 genome engineering, an impressive feat that attests to Zuzanna's dedication. She subjected the mutant strain to again wide range of assays, finding no appreciable phenotypic consequences. She did discover, however, there there is a potential link between the NSPC proteins and insulin signaling. This link is suggested by a range of similar targets, as well as a potential similarity of NSPC proteins with insulins. Genetic mutant analysis did, however, not provide additional support of an intersection of these pathways. The apparent lack of phenotypes are not an indication of these proteins (or the excretory gland cells) not doing anything important; they simply attest to the fact that this cell and its secreted proteins have functions that are not readily measurable under the unnatural laboratory conditions in which nematodes are commonly reared. These results were also written up in a complete manuscript.

In addition to the work written up in manuscripts, Zuzanna also explored other aspects of *tent-5* function, in particular potential redundancies with other ncPAPs encoded in the *C. elegans* genome. She found no such redundancies. My recommendation would be to wrap this work into either of her two bioRxiv manuscripts. Lastly, Zuzanna has also developed a number of useful tools for high-throughput data analysis. This reveals an impressive range of skills.

In summary, I have been very much impressed by the wide ranges of techniques used not only in this part of her study, but her entire thesis, ranging from molecular biology to

cell biology to animal physiology. I have been equally much impressed by the rigor, care and patience that Zuzanna has displayed throughout her thesis work. It is not always easy to try to find effects of experimental manipulations, not find much, but then not giving up and continue to try to find effects. The tenacity is highly commendable.

I will finish by commenting on a few other general aspects of the thesis. The thesis is well structured with a scholarly introduction, followed by succinct summaries of each major component of her thesis work, each of which written up in manuscript formats. The final concluding chapter is a little short, but I do not believe this to be a problem because each manuscript already contained reasonable discussion sections. Her citation of literature is also comprehensive. Overall, this doctoral dissertation demonstrates the candidate's theoretical knowledge in the discipline of biological sciences and the ability to conduct independent scientific work.

I have also been asked to assess the practical application of her research. While there are no immediate medically relevant applications, Zuzanna's studies have advanced our basic understanding of basic biochemical and cellular processes, which lays the groundwork of a better understanding of human biology as well.

Laslty, I, the undersigned, hereby state that the doctoral dissertation of Zuzanna 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 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.

A handwritten signature in blue ink, appearing to read "Oliver Hobert". The signature is fluid and cursive, with a long horizontal stroke at the end.

Professor of Biological Sciences, Columbia University, New York

March 17<sup>th</sup>, 2025