



Review Report on a Dissertation Thesis

Author: MSc. Filip Knop

Name: Non-enzymatic roles of kinases and phosphatases – the case of MTMR9 and AAK1

The submitted Dissertation by Mr. Filip Knop aims to study the role of non-enzymatic functions of the MTMR9 pseudophosphatase and SEL-5/AAK1 kinase in relation to Wnt signaling pathway.

The thesis is the extended version of the work, the main points of which were in part published in the *Experimental Cell Research* journal (2020) and in its majority submitted to *bioRxiv* (April 2024). This yet unpublished manuscript represents the major portion of the thesis dedicated to the work on the worm *C. elegans*. According to the latest news, this work currently made it to the second round of revisions in *eLife*.

Formally, the thesis is written in excellent English. The many names of all the mentioned genes/proteins are tediously well formatted. Formally, I have no concerns about the submitted Thesis. My major concern is the stylistic part of it.

Negative points:

As a non-specialist in the field of kinases and phosphatases, I must admit having hard times reading the details of each kinase or phosphatase. I do agree that this information is important to know in the context of the work. At the same time, such details unfortunately refrain the reader from paying attention to it. I strongly believe these details are correct, but I am simply not eligible to evaluate them.

What I would like to suggest to the author in this context is refraining from all the details of what all the various functions of all the various kinases and phosphatases are and concentrate on better presentation of the studied systems and phenomena. Or, alternatively, to add more general chapters that would allow the reader to jump back in after being in the details-limbo. For example, nowhere in the introduction could I find an information on characteristics of *C. elegans* QL.d or excretory cell lineages, which are mentioned already in the abstract and which form major study systems of the second manuscript. Another example: maybe I missed it, but nowhere could I find a general introduction of ER-to-Golgi trafficking features and mechanisms (terms like ERES, ERGIC, retromer), again, crucial for the Thesis and the attached papers and later evaluated in the discussion part of the Thesis.

Nowhere could I find, what the constituent major and minor aims of the submitted Thesis are!

Another example is the abstract. Abstracts are usually written at the end the whole writing process. However, it is read as the first (!) text part by the reviewers and should represent the reason why the reader should continue with reading. Therefore, it should be as simple as possible, ideally without any details and with a general evaluation of why the work is important. Unfortunately, this last part is completely missing from the abstract of this Thesis.



I understand that in case of the Dissertation these points are, after all, not of a major concern, however, if the author wants to stay in science, a better (more general) introduction to any scientific text is desired and would help in the general understanding and appreciation of the work been done, or (even more importantly) comprehension by reviewers during evaluation processes of submitted grants and prospective manuscripts.

Positive points:

While I had hard times reading the introductory parts, going through the attached papers and the discussion part of the Thesis was fun. The text is logically aligned, understandable, and discussed thoroughly, and it is a pity that the introduction part does not correspond to the discussion part.

I hail the indicated contribution by the author to the *bioRxiv* manuscript because the work must have been immense, not only generating and characterizing the lines, but also performing the general molecular biology, CRISPR/Cas9, RNAi, protein analysis, and imaging.

Also, I really like the topic of regulation of the excretory cell extension. I believe this is a wonderfully tractable system for further evaluating the permissive and restrictive signals for cell behavior.

Overall decision

Despite my criticism on the way of presentation of the project, I overall advocate for evaluating this work positively. If no formal obstacles exist, I propound, upon successful defense, awarding Mr. Filip Knop the Ph.D. Title.

Prague, 14 May 2024

Mgr. Vladimír Soukup, Ph.D.

Reviewer questions:

- 1) What is the fate of the *C. elegans* QL.d cell lineage? What are its predecessors, its descendants and to what tissues does this cell population contribute?
- 2) Molecular regulation of excretory cell extension in *C. elegans* is an interesting phenomenon. How is it possible that *lin-44/Wnt* and *lin-17/Fzd* versus *cwn-2/Wnt* and *cfz-2/Fzd* mutants have completely opposing phenotypes? How to explain the supposedly completely divergent roles in regulating the length of the excretory cell extensions despite affecting the same loci?
- 3) Could you speculate on the possible mechanism that could play role in the switch between the initiation of the secretory cell growth and its length regulation?
- 4) Are there other worm species with either shorter or longer excretory canals that could help study the underlying molecular mechanisms from a comparative point of view?