Evaluation report

Alicja Komur presented a PhD thesis entitled 'The *C. elegans* 'hibernation': surviving cold through ferritin-mediated iron detoxification'. The thesis was scientifically novel, with sufficient solid scientific data presented to enable the candidate to be qualified for a PhD. The PhD mentor is Prof. Rafal Ciosk.

The thesis has focused on a novel and previous unexplored field in *C. elegans*. After the introduction of the small roundworm *C. elegans* to the biological laboratory by the deceased Nobel Laureate Prof. Sidney Brenner in the 1960s, there has been broad applications of using *C. elegans* in exploring different scientific questions: e.g., mechanisms of ageing, reproduction, and neurodegeneration, among others. While the lives are classified into ectotherms and endotherms, some of the endotherms can developed a self-protective state called torpor that occurs on a daily or seasonal basis (a.k.a., hibernation). However, whether there is 'hibernation' (or hibernation-like behaviour) in worms, and if yes, how worms regulate hibernation, are largely unexplored. This thesis focuses on the exploration of a 'hibernation-like status' in worms growing in cold environment, a totally new and important topic for exploration. Komur investigated protective role of FTN-1 (ferritin) in cold protection.

Materials, methods, experimental design, and statistics. The roundworm C. elegans is the major material used in the studies. Different methods, such RNAi, imaging, RNA-seq, sequence analysis, etc, were used for the experiments. For most of the experiments (if not all), two-three biological repeats were performed with the right statistical methods used for data analysis.

Novel and solid conclusions were made based on the data collected. Major discoveries include: only overexpression of *ftn-1*, but not *ftn-2*, enhanced cold survival of *ets-4* mutants; cold environments induced transcriptional upregulation of *ftn-1*; the *ftn-1* overexpression enhanced cold survival, but not lifespan; cold environment increased oxidative stress (e.g, higher ROS) leading to worm death while this condition was alleviated by anti-oxidant treatment. Additionally, RLE-1 and REGE-1 are involved in cold response via ETS-4.

Potential applications: studies by Komur have enabled us a deeper understanding of a hibernation-like behaviour in worms; molecular mechanisms, such as the involvement of FTN-1, RLE-1, and REGE-1, were unveiled. These discoveries may provide clues on understanding the mechanisms of hibernation in mammals.

While the thesis is well-written, addressing below small concerns will improve the quality of the paper:

1. As Fe2+ is a major topic in the thesis, it could be useful to include the background of Fe2+ and cell death, ferroptosis. Ferroptosis-inducing factors reduce glutathione peroxidase resulting in a decrease in antioxidant capacity and accumulation of lipid reactive oxygen species (ROS) in cells, ultimately leading to oxidative cell death. Could cold environment-caused worm death be at least partially attributed to ferroptosis? The author should at least bring this topic in the Discussion (future perspective) part.
2. Figs. Many of the introductory figures (e.g., Figs. 1-3) were from published papers. The author should consider to take time and draw some of them by himself/herself and make an update. This will increase the novelty and 'first-hand' of this thesis.
3. Fig. 6E: statistic resutls should be added. This suggestion also applies to other curve figures.

Postal address:
Evandro F. Fang
Akershus University Hospital, EpiGen, PB 1000, 1478 Lørenskog.
https://www.med.uio.no/klinmed/english/people/aca/evandrof/index.html
Lab web: https://evandrofanglab.com/

4. Fig. 8E: How about the lifespan of other FTN-1 overexpressed worm strain(s)? Does FTN-1 overexpression show protection at higher temperature (like 25 C)? The author has no need to do additional experiments if without time permits, but it is instructed to discuss such possibilities in the 'Discussion' part.

This dissertation being the subject of the review fulfils 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. 99/2022/Internet of June 9, 2022), and I recommend that the Scientific Board of the Institute of Bioorganic Chemistry PAS allows it to further steps in PhD defense process.

Based on the novelty of the thesis, a 'Distinction' is highly recommended.

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https://www.med.uio.no/klinmed/english/people/aca/evandrof/index.html
Lab web: https://evandrofanglab.com/