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Review of Alicja Komur's Dissertation entitled "The C. elegans "hibernation": surviving cold through ferritin-mediated iron detoxification"

The PhD thesis of Alicja Komur was prepared in the Integrative Biology Team, Institute of Bioorganic Chemistry, Polish Academy of Sciences in Poznan under the supervision of Dr. Hab. Rafał Ciosk, prof. IBCh PAS and Dr. Daria Sobańska.

The scientific aim of the work was to investigate the mechanism underlying ferritin (FTN-1)-mediated cold protection during hibernation like state in *C. elegans*, based on the previous results of the Integrative Biology Team. First, Alicja Komur showed that *ftn-1* gene expression is upregulated, and FTN-1 protein is required for cold survival in the *ets-4* mutant. For these analyses, she prepared *ftn-1* overexpressing strains. Next, she confirmed that the ferroxidase activity of FTN-1, which is responsible for the detoxification of ions, is necessary for cold survival. Indeed, ferritin appeared to act as an antioxidant leading to a lower level of reactive oxygen species generated by cold. Furthermore, it changed the expression of other genes involved in the ROS response. In addition, the Author compared the regulation of *ftn-1* gene expression by transcription factors ETL-2 and HIF-1, in normal conditions and in cold. Finally, she described the contribution of RLE-1 and REGE-1 in *ets-4* expression during cold.

In terms of content, the research of the Ph.D. student is of high scientific value and provides new knowledge on the iron detoxification by ferritin during cold exposure to *C. elegans*. In my opinion, several other issues should be emphasized in this doctoral dissertation. The Author used many *C. elegans* lines, several of which she prepared by herself. She used different techniques of molecular biology to test her research hypotheses. She analyzed many pathways by which *C. elegans* respond to cold exposure, which undoubtedly enriched the research. Advanced Discussion is very interesting and touches, among others, many aspects of the role of ferritin in hibernation and hypothermia. The scientific background of the thesis (described in the Introduction and Discussion) is well-developed and supported by a long list of references. Considering all of these, in my opinion Alicja Komur is a mature scientist, familiar with the topic of her thesis.

In formal terms, the layout of this dissertation reflects the typical layout of diploma theses. It starts with the 'Introduction' part in which the Author describes the most important topics of her thesis, such as the hibernation process and its therapeutic utility, the use of *C. elegans* as a model organism, and ferritin and its involvement in cold survival. However, here I have to mention that the previous results of the group, published in *Nature Communications* 2022, which are highly connected with this thesis, are not described well. To understand the problem,

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I had to go into detail in the original publication. The aims of the project are listed in the second part. Then, we find the list of material and protocols of experiments performed in the project, prepared in a compact and very transparent way. The next part contains results of the experiment followed by Discussion with future perspectives. Finally, there are the final conclusions of the work and an impressive list of references, which includes almost 240 positions. Moreover, the thesis is supplemented by abstracts in Polish and English, a list of figures, tables, and abbreviations. The thesis is written in correct language, the sentences are clear, the conclusions are formulated correctly. The figures are well and carefully prepared. I found very few errors, which do not diminish the high scientific value of this work at all.

However, it would be grateful for providing additional comments during defense of the thesis, concerning as follows:

1. How would the Author explain the week correlation between the level of *ftn-1* mRNA and FTN-1::MYC protein? In Fig. 7B, we can see that the transcript level increases ~1000 times after 1 and 3 days of cold exposure, whereas in Fig. 7C we can barely see the band of FTN-1::MYC protein in the sample after 3 days of cold exposure. Therefore, in my opinion the statement "Above data suggests that the elevated levels of *ftn-1* mRNA in *ets-4*(-) mutants after cold exposure, corresponds to the increased FTN-1 protein levels" is overestimated. Considering that the protein is not synthesized at the same level as transcript, what can be the reasons of that?

2. Having such a problem with the detection and functionality of MYC-tagged FTN-1, did the Author consider overexpression of a protein tagged at the C-terminus, or protein tagged by another tag? Alternatively, since "there is a possibility that endogenous FTN-1 can interfere with MYC-tagged FTN-1 and create non-functional heteropolymers", did the Author consider the overexpression of MYC-tagged FTN-1 in the background of *ftn-1(-)* mutant? Maybe, by this approach, she could avoid the effect of such 'negative autoregulation'.

3. In my opinion, the analysis shown in Figs. 11A and 11B should have been done for samples after 1 day of cold exposure, instead of 3 days. Now the results are very similar, because after 3 days of cold a similar level of *ftn-1* upregulation was observed in both samples, in wild type and *ets-4* mutant (according to Fig. 6C). A significant difference in *ftn-1* level in the *ets-4* mutant compared to the wild type is observed only after 1 day of cold exposure. Therefore, the analysis done after 1 day would be more informative, in terms of the identification of genes upregulated specifically by *ftn-1* overexpression due to the *ets-4* mutation.

4. According to Figs. 14A and 6C, in the wild type after 1 day of cold exposure compared to the wild type at 20°C, the level of *ets-4* and *ftn-1* mRNA increases significantly. ETS-4 is known to suppress the expression of *ftn-1,* how then would the Author explain the upregulation of the latter one in this condition?

5. In the thesis, I missed results on the level of *daf-16* and *pqm-1* mRNAs in the *est-4* mutant, moreover, the level of *ftn-1* mRNA should be checked in the *rle-1* and *rege-1* mutants, when we see the upregulation of *est-4* mRNA.

6. The authors showed that FTN-1 overexpression resulted in a reduced level of *sod-5* mRNA. I am wondering whether SOD-5 overexpression affects the expression of the *ftn-1* gene?

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Minor comments (no need to be commented during thesis)

1. In the graphs in Fig. 6, both the gene and mutant lines are described the same, *ftn-1* (compare Fig. 6C with Fig. 6E), which is misleading somehow. In Fig. 6E it should be *ftn-1 (-).*

2. The preparation of Plips-11::MYC::ftn-1::ftn-1 3´UTR; unc-119(+) and Plips-11::MYC::ftn-1::ftn-1 3´UTR;unc-119(+)] II.; ets-4(rrr16) X strains is not described.

3. In Fig. 12B, we cannot see bars corresponding to wild type samples, although they are mentioned in the text "Obtained results showed that the knockdown of *elt-2* led to the downregulation of *ftn-1* mRNA in the wild type (~0.6 fold) as well as in *ets-4*(-) mutants (~3.5 fold), only in cold conditions (Figure 12A). Interestingly, such an effect was not observed upon standard cultivation conditions of 20°C (Figure 12B)".

4. In Fig. 13A, I cannot see the signal for PQM-1. I hope the quality of image will be improved during defense of the thesis.

5. Two different shortcuts, MFN and MFRN, are explained the same as mitoferrin.

6. In the sentence 'FTH binds Fe2+ ions and rapidly transfers them to the ferroxidase center where ferric ions are oxidized to ferrous cations' (page 34) should be 'ferrous cations are oxidized to ferric ions'.

Summarizing, the research of Alicja Komur revealed a crucial role of ferritin in cold protection using a simple model organism, *C. elegans*. And since the described process exhibits high evolutionary conservation, the results of her studies may contribute to the improvement of therapeutic hypothermia application strategies with potential use, for example, in the treatment of patients with traumatic brain injury.

Based on the novelty of the findings of Alicja Komur, I conclude that the presented dissertation fulfils the requirements for a Ph.D. thesis and the statutory requirements necessary to obtain the Ph.D. degree (Prawo o Szkolnictwie Wyższym i Nauce, Art. 187, Dz.U.2022.574). Without any doubts, I recommend to the Scientific Council of the Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznan to admit Alicja Komur to further stages of the doctoral procedure.

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