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Review of Ilkin Aygün Soyalp doctoral dissertation entitled "Mechanisms and developmental roles of XRN-2 mediated RNA regulation in *Caenorhabditis elegans*"

The PhD thesis written by Ilkin Aygün Soyalp was prepared at the Institute of Bioorganic Chemistry Polish Academy of Sciences in Poznan under the supervision of dr hab. Takashi Miki and co-supervised by dr hab. Agata Tyczewska.

The scientific aim of the work was to gain insights into the role of XRN-2 during *C. elegans* development. Since *xrn-2* is widely expressed in the worm tissues, the work focused on its role in the germline. The basis for the work are large datasets that were obtained by the supervisor dr hab. Takashi Miki and were partially published. This included a mutagenesis screen and identification of mutations by sequencing, an RNAi screen to find synthetic lethal genetic interaction partners of *xrn-2* as well as RNA sequencing and ChIP-seq experiment (published in Miki *et al.* Genes & Development, 2017). The description of the aim of the study (page 33) is however rather vague and fails to include that the primary datasets were previously produced and within the thesis work rather a candidate approach was taken. Only within the result/discussion section, the origin of the datasets was indicated. Therefore, I ask Ms. Soyalp that within the presentation during her defense she clearly states the objectives of her work and work not done by herself is consistently acknowledged and/ or the appropriate citation of published work is given.

XRN-2 is an evolutionarily conserved 5`-to-3`exoribonuclease. The protein is involved in transcription termination processes of various types of RNAs.

Within the first part of the results the newly generated conditional strain, *xrn-2ts*^{germ}, was characterized using microscopy. Animals of this strain could develop into adult worms but were largely infertile at restrictive temperature. The strain was used in the mutagenesis screen and four alleles within four different genes are presented that could rescue the sterility of *xrn-*

2ts^{germ}. Ms. Soyalp further characterized the phenotype of the four candidate genes (*dpy-10*, *ptr-6*, *osr-1*, C34C12.2) upon knockdown in the *xrn-2ts*^{germ} background. RNAi mediated knockdown resulted in fertile animals confirming the screening results. Quantification of these results were presented. Three of the suppressors (*dpy-10*, *ptr-6*, *osr-1*) were negative regulators of *gpdh-1* expression. Increased expression of *gpdh-1* upon knockdown of the candidate genes in wild-type worms was confirmed using quantitative PCR. The hypothesis that an increase in glycerol would rescue the sterile phenotype of *xrn-2ts*^{germ} could not be confirmed by the experiment performed. Further, the role of the largely uncharacterized gene C34C12.2 was investigated. Ms. Soyalp determined the proteins localization to be predominantly nuclear with a strong expression in the germline. The knockdown of a previously identified binding partner of C34C12.2, NRDE-2, also restored fertility in *xrn-2ts*^{germ} animals.

The second part of the results focused on the investigation of *puf-9* as a potential candidate exhibiting a synthetic lethal phenotype upon depletion in the *xrn-2ts* worms. Several phenotypes were mentioned, such as problems in body movement, blistering and molting defect. Further, PUF-9 protein distribution was examined using fluorescent microscopy, and consequences of *puf-9* mutations were characterized. The efforts are valuable since defects in germline development and function were previously not characterized. The part was concluded with the observation that a double mutant of *puf-9* and *xrn-2ts* showed increased brood size defect compared to the single mutants.

Part three of the result section concerns the molecular function of XRN-2 during mRNA degradation. The analysis was based on published RNA sequencing data and ChIP-seq data from Miki *et al.* Genes & Development, 2017. The candidate transcript *ceh-99* was chosen for the analysis. The analysis revealed that an element within *ceh-99* promotor region is responsible for the XRN-2-mediated repression of *ceh-99* transcript levels.

I have following specific questions to the results part:

1. Figure 11A: It is indicated that the experiment was performed in wild-type worms. Was this experiment also performed in *xrn-2ts*^{germ} mutant, which would be a better approach to link increase in *gpdh-1* expression with rescue of the sterility in the conditional mutant? Do *xrn-2ts*^{germ} have less *gpdh-1* expression compared with N2 worms and/ or are they more sensitive to hyperosmotic stress?
2. Figure 11C: The addition of glycerol seemed not to affect the phenotype of *xrn-2ts*^{germ}.

What were the expectations for such an experiment? How would you interpret the actual result? How the worms would take up the glycerol?

3. *S. cerevisiae* Net1 was found to have sequence similarities to C34C12.2. What is the connection between Net1 and NRDE-2-containing complex that was investigated? Based on the results obtained could you speculate how the decrease in functional NRDE-2-containing complex would restore germline function in the *xrn-2ts*^{germ} strain?
4. Figure 15A: Quantification of the phenotypes would substantiate the findings.
5. Figure 20: A wild-type control is missing in this experiment. Having such a control would allow to judge if the effect of the combination of the loss of both genes is additive. Are any additional preliminary experiments available analyzing the phenotype of *puf-9* knockdown or deletion in *xrn-2ts*^{germ} strain? Such observations could consolidate the hypothesis that indeed *xrn-2* and *puf-9* share a function within the germline.
6. Figure 24A/B: Has the expression of *gfp* from the constructs 1, 2, 4, and 6 been tested? Would you expect to have increased *gfp* expression upon *xrn-2* RNAi from the constructs that lack the gene body? Did the "in-depth examination of the *xrn-2ts* RNA sequencing data" reveal other transcripts with a similar mechanism then *ceh-99*? Could you elaborate on the potential mechanism itself? Is XRN-2 recruiting RNAPII to the promotor region as proposed for XDT genes?

The Results/Discussion section contains several formal concerns. These are listed below and should be re-evaluated:

7. Figure 6: panel A and panel B are switched (minor issue);
8. Figure 7: Images for WT at 20°C and 26°C were previously published in Miki *et al.*, PLOS Genetics, 2016; Image for strain *xrn-2ts* at 26°C was previously published in Miki *et al.*, 2017. For all three images correct citations are missing in the figure legend;
9. Figure 9: Images from Figure 7 reused in addition to comment above;
10. Figure 16A: Image wt mock reused from Figure 15; Image *puf-9::gfp*+*xrn-2* RNAi is shown in Figure 15 as *xrn-2* RNAi in wt;
11. Figure 17A. Images reused from Figure 15;
12. Figure 21: How the data were processed to obtain the heat map, transcript levels, and snapshot of RNA sequencing? No information were given in the method section;
13. The origin of the plasmids and worm strains should be clearly indicated in the Material

sections including who generated the plasmid/ strain if it was not previously published or commercially obtained;

14. Description on page 100 should be in the introduction.

Initially, the thesis has a classical structure containing an abstract, introduction, a separate materials section and methods section. The results and discussion were presented in a combined chapter, followed by a section with main conclusions and a list of references. The thesis also includes a list of abbreviations, figures and tables. In my opinion, very little discussion on the obtained results was presented and no further scientific questions or experiments were suggested.

Finally, the text of the thesis would have been more appealing if it had been written in a simpler style. Although formally there are few errors in the English language, a clearer and simpler description would have avoided over-interpretation of the data.

As outlined above, there are several shortcomings in the scientific work and presentation of the written thesis that need to be addressed during the formal defence of the thesis.

However, Ms. Soyalp work presents some original findings advancing the knowledge about XRN-2 during organismal development. Moreover, Ms. Soyalp published two articles, one experimental paper and one review, which are connected to here PhD work. She is the first author on both publications.

Thus, the dissertation being the subject of the review fulfills 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. 59/2023/Internet of March 29, 2023) and I recommend that the Scientific Board of the Institute of Bioorganic Chemistry PAS allows it to further steps in PhD defense process.

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