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Ilkin Aygun Soyalp PhD thesis was prepared in the lnstitute 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 aim of the thesis was to understand the function of XRN-2, 5'-3' exoribonuclease in animal development. Describing the role of post-transcriptional regulators of gene expression in development is fundamental, as increasing number of these factors is found to be misregulated in human conditions. PhD candidate chose *C.elegans* as her model organism, which is a suitable model to address her question, given the well established techniques, short and well tractable development and evolutionary conservation of proteins involved in gene expression regulation.

The project builds upon previous work published by the supervisor of the PhD candidate. Specifically, dr hab. Takashi Miki created a conditional, temperature-sensitive *C.elegans* model of XRN2 (*xrn2-2ts*), and showed that XRN2 is a key regulator of embryogenesis and fertility in worms. He found that XRN2 regulates gene expression by modulating levels of different kinds of RNAs, such as miRNA or pre-mRNAs. Ilkin's work directly builds upon Miki's work and aims at, firstly: identifying genetic suppressors of sterility and secondly: discovering genes that cause synthetic lethality in the context of loss of XRN2.

Initial work described in Ilkin's thesis reproduces Miki's previous finding, i.e. observation that transition of temperature sensitive worms from 20°C to 26°C results in sterility. The genetic suppressor screen, also previously performed by dr hab. Miki, using EMS, led to identification of 4 alleles that restore fertility in the *xrn2-2ts* worms at 26°C, i.e. *dpy-10, osr-1, ptr-6* and *C34C12.2*. All these identified genes carried point mutations. Ilkin hypothesised that these mutations were LOF and therefore performed RNA-i mediated knockdown of each of the identified genes. These experiments indeed confirmed that the four alleles counteract XRN2 function during germline development. Ilkin states that the extensive exploration of literature leads her onto proposing that 3 out of 4 studied alleles regulate *gpdh-1* promoter. She shows that upon knockdown of those 3 genes, the levels of *gpdh-1* mRNA are greatly elevated. This leads Ilkin to suggesting that increased *gpdh-1* expression results in the accumulation of glycerol that subsequently restores the fertility. At this stage, the function of GPDH-1 or glycerol in the context of fertility is unclear to the reader. What happens upon knockdown of *gpdh-1* allele? Does it lead to worms' sterility? What are the levels of GPDH-1 in germ cells vs somatic cells? These questions are important to be addressed to distinguish a cause from effect in her findings.

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In parallel, in the search of the roles of identified genetic suppressors, Ilkin explores the role of C34C12.2 protein. She demonstrates that amongst various cell types, C34C12.2 can be found in the nucleoli of germ cells. Her literature search and homology analysis reveals some interesting connections to gene silencing and RNAi pathway, including its interaction with NRDE-2. She shows that depleting NRDE-2 itself in the context of temperature sensitive XRN2 mutants, restores worms' fertility. The mutation identified in *C34C12.2* localises to splice site. How many exons does this gene have? Is the mutation proposed to result in the NMD-mediated degradation of the transcript or perhaps intron retention? What is the proposed role of C34C12.2-NRDE-2 complex and how could this be tested?

In the second part of her thesis, Ilkin again builds upon the result of dr hab. Miki, where he undertook an RNAi screen encompassing 500 RNA-regulating factors at 20°C. This screen led to the identification of 17 potential synthetic lethality partners of *xrn-2*. Ilkin focuses on PUF domain-containing proteins and specifically on *puf-9*. Ilkin goes onto characterising the consequences of loss of *puf-9* in worms. She references Nolde et al., subsequently reproduces their work, but also expands on this study, presenting the consequences of *puf-9* loss on germline development. This observation prompted Ilkin to identify synthetic lethality partners of *puf-9,* that reveals two other members of the family, *puf-3* and *puf-8*.

In the final part of Ilkin's thesis, she aims at understanding mechanistically the effect of xrn2 inactivation on RNA metabolism. She refers to Miki's RNAseq data. This data shows that depletion of *xrn2* leads to upregulation of RNAs due to transcription read-through. There was a subset of RNAs which are downregulated and Ilkin suggests that this is due to RNAPII head-on collisions. This is an interesting hypothesis, but there is no experimental evidence to suggest this. Could Ilkin suggest experiments that could help in measuring such phenomena? For example, is increased ubiqutination of RNAPII observed in this context? What other consequences, in addition to gene expression changes, would be expected if *xrn2* depletion led to RNAPII collisions? It is not clear if this data was analysed by Ilkin or comes from Miki's published work.

Ilkin identifies an interesting candidate in the RNAseq data that she decides to follow up, it is *ceh-99* which as she confirms by RT-qPCR is downregulated by XRN2. She observes that XRN2 leads to premature RNAPII termination. By deleting different region upstream of *ceh-99* gene, Ilkin tests the role of transcription read-through of these genes in *ceh-99* upregulation, showing that this mechanism is not involved. Instead, she finds that *ceh-99* has two alternative TSS, and proposes that only one of them is targeted by XRN-2 for degradation. The loss of *xrn2* leads to accumulation of TC1 transposon encoded within intron 1 of *ceh-99*. How is TC1 expression measured? Can expression of the transposon be distinguished from intron retention in this context? Where is the promoter of TC1 located? Overall this is perhaps the strongest part of the thesis as offers more mechanistic explanation of XRN2-mediated changes in gene expression.

The thesis is written in the clear, scientific manner. It is well structured. The introduction introduces the reader to the subject, starting from the general concept of RNAses, through explanation of the XRN2 protein function in RNA degradation and transcription termination. This is followed by the description of her model organism. Materials and methods section is strong - very detailed and well written, which should allow reproducing the experiments.

<u>However, there are parts of thesis that could be improved:</u>

- Abstract is too detailed for a scientific abstract. The candidate should aim for 250- 500 words.

- In some sections of the thesis newer references could be used. For example, the candidate discusses the function of XRN2 in transcription termination, but fails to mention the recent work from Bentley's where using a range of genomic approaches they confirm XRN2's role in limiting RNAPII transcription by inducing premature termination near 5′ ends of genes.

- There are full paragraphs of thesis that should be shortened to one sentence (e.g. last paragraph on page 111, p112 etc.). These are sections where candidate is trying to add the significance to her findings or questions she is addressing, but this can only be achieved by providing a context, why addressing the question is important. Stating "Such insights (…) have the potential to advance our understanding (…) with implications spanning various fields, from molecular biology to potential applications in therapeutics" may well be correct, but is meaningless without context. In her defence, this is a work-in-progress for myself and many in the scientific community.

- Along the same lines: essential detail is often missing to substantiate candidate claims. For example, we read that "it would be essential to assert the functions of XRN2 as in doing so we could develop therapeutics". Therefore a broader discussion on the role of XRN2 and other ribonuclease in disease is missing. Is human XRN2 involved in disease?

Specific corrections that are recommended

- Page 56 - "To dissecting" should be changed to "To dissect"

- Page 73 - what is "general RNAs", does the candidate mean "total RNA"?

- Figure 6 - A and B sections are swapped

Despite all this comments, Ilkin's thesis has been well-designed and carefully executed and highlights the role of post-transcriptional gene expression regulation in animal development.

Przedstawiona do recenzji rozprawa doktorska spełnia warunki określone w Ustawie z dnia 20 lipca 2018 roku prawo o szkolnictwie wyższym i nauce (Dz.U. z 2018 r. poz. 1668 ze zm.), Ustawie z dnia 3 lipca 2018 r. Przepisy wprowadzające ustawę – Prawo o szkolnictwie wyższym i nauce (Dz.U. z 2018 r. poz. 1669 ze zm.) oraz w Sposobie postępowania w sprawie nadania stopnia doktora w Instytucie Chemii Bioorganicznej PAN w Poznaniu (uchwała Rady Naukowej ICHB PAN nr 56/2023/ Internet z dnia 29 marca 2023 r.) i wnioskuję do Rady Naukowej Instytutu Chemii Bioorganicznej PAN o dopuszczenie [imie i nazwisko kandydata] do dalszych etapów postępowania o nadanie stopnia doktora.