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Review of the Nour-el-hana Abbassi's doctoral dissertation

Doctoral dissertation entitled "Biochemical and structural characterization of the catalytic subunit of Elongator complex" was prepared by mgr Nour-el-hana Abbassi under the supervision of dr hab. Sebastian Glatt. The work was submitted to the Biological Sciences Council of the Jagiellonian University.

The main goal of the work presented in the thesis was to characterize structurally and functionally proteins Elp123 from the Elongator complex (with a focus on Elp3), responsible for the first step of uridine34-tRNA modification with 5-carbonylmethyl moiety. Results presented in this thesis are novel, partially published by the author et al. and strongly related to other publications from the Glatt group.

Dissertation is written in English. The content of the dissertation is consistent with the subject indicated in the title. The layout of the thesis is appropriate and correct for this format. The work is divided into a number of chapters: *Abstract*, *Introduction*, *Aims of the work*, *Methods*, *Materials*, *Results*, *Discussion*. The author used an extensive bibliography referring to 139 scientific articles. The author uses language and terminology appropriate for this format. The table of contents is prepared correctly. The work is clear and factual. The text is provided with 50 figures and 2 Appendices. The figures are carefully prepared, the descriptions on them are clear but the numbering of Figure legends appear to be incorrect for many of them. The bibliography was appropriately used in the text and appropriately used to discuss the results.

The author begins the *Introduction* with a concise description of the protein biosynthesis process and the role of tRNA in this process. She describes tRNA structure and modifications of nucleotides of the tRNAs. Author summarises current knowledge about the Elongator complex presenting known structural and functional details. In the last paragraph of the *Introduction*, she describes known mutations in Elongator complex related to human pathologies, including neurodegenerative disorders and cancers.

In the next chapter, the author describes *Aims of the work* focusing on Elp3 protein, including tasks related to studies on (1) Elp3 – tRNA interaction, (2) tRNA triggered Elp3-mediated Acetyl-CoA (ACO) hydrolysis, (3) differences/similarities of the Elp3 activity of the proteins originating from yeast, archaea and human. She is drawing hypothesis and briefly describes expected results.

In the *Methods* chapter, the author describes the protocols she used to carry out her research tasks. The preparation of Elp3 constructs, including mutants, for heterologous expression in bacterial and insect systems, purification of recombinant proteins using affinity purification, preparations of tRNAs are

described. Short protocols for the methods used in the analysis of Elp3-tRNA interactions are also presented. In the last part of the chapter, the author describes protocols for structural analyses, including modelling and Cryo-EM.

In the *Materials* chapter, the author provides lists of reagents and equipment used in the experiments.

Results are divided into two sections: (1) characteristics of viral, bacterial, archaeal Elp3 in comparison to yeast Elp3, (2) work on human Elongator complex.

In the first part of the *Results*, the author begins with comparison of the Efp3 protein sequences originating from Human, Mouse, yeast *Saccharomyces cerevisiae*, archaea *Methanocaldococcus infernus*, bacteria *Dehalococcoides mccarty* and six Elp3's sequences from viruses. Sequence alignment, topology of proteins and phylogeny of Elp3 proteins is described. Author shows that the core of Elp3's is in some extend similar between all testes organisms, except for N-termini which display no similarities neither in length nor in amino acid sequences. Next, author describes her attempts to express and purify mentioned above Elp3 proteins. She succeeds to obtain soluble archaeal *M. infernus Min*Elp3 and bacterial *D. mccarty Dmc*Elp3 using bacterial expression system. Elp3 protein within the Elp123 complexes catalyses addition of 5-carbonylmethyl to U34 of tRNA (cm^5U_{34}). Since Elp3 protein consists of two functional domains, an S-Adenosyl-methionine (SAM) domain and a lysine acetyltransferase (KAT) domain containing ACO binding pocket, the author tests the role of the ACO binding loop. She checks tRNA-activated ACO hydrolysis and finds that out of two tested proteins: *Min*Elp3 and *Dmc*Elp3, only *MinElp3* shows activity *in vitro*. Therefore, she then focuses on archaeal Elp3. Since the full length *Min*Elp3 does not crystalize, the author prepares various truncations of the protein. However, a reader of this thesis can only guess how the truncations were prepared based on the Figure 17, which should be numbered 15 (Sequence Alignment of Elp3 N-termini). Numbering mistakes of labels of the deletion strains within the text makes it difficult to follow the results. Author obtains crystals for truncated versions of the *Min*Elp3 protein and could solve the structures at resolution ranging from 1.9 – 2.1 Å. Resolved structures confirms high structural conservation with known structures of Elp3 from bacteria and yeast. In the next steps, author aimed to obtain tRNA-*Min*Elp3 crystal structure. She tested Full length *Min*Elp3, N-terminal truncated *Min*Elp3 and N-termini by itself for the ability to bind tRNA. At first, in the electrophoresis mobility shift assay (EMSA) she shows that all three variants have ability to interact with tRNA. To gain more details, author uses microscale thermophoresis (MST) to determine K_D for *Min*Elp3 and its N-terminal truncated variants. She then demonstrates that N-terminal sequence is important for tRNA binding. In addition, tRNA triggered Acetyl-CoA (ACO) hydrolysis is abolished when N-terminus is truncated. Revealing the importance of the N-terminus to tRNA bonding and ACO hydrolysis, author generates chimeric Elp3 proteins with the *Min*Elp3 core and N-termini originating from Human and yeast Elp3. She shows that tRNA binding is restored, compared to N-terminus lacking *Min*Elp3, however, ACO hydrolysis activity is not recovered. Author implies the role of N-terminus in a tRNA recognition and an enzyme activity. She then checks the specificity of the tRNA recognition by Elp3 protein and shows that a presence of the 3'CCA of the tRNA enhances its affinity to the Elp3 protein. Moreover, she shows that despite being initially recognized as a histone acetyltransferase, Elp3 does not Histon H3-tail-originating peptide. Next, author aims to characterise *Min*Elp3-mediated ACO hydrolysis. Based on the sequence alignment, she prepares *Min*Elp3 mutants with substitution of some selected

amino acids. While tRNA binding is not affected in such mutants, even with ACO loop deleted, the ACO hydrolysis is abolished or strongly diminished in single mutants: K150A, K266A, Q461A or Y517A. ITC measurements shows that *Min*Elp3K150A and K266A retains some ACO binding propensity, while mutations Q461A or Y517A abolishes ACO binding. The first part of the *Results* section is finished with comparison of structural models of viral Elp3 proteins.

In the second part of the *Results*, the author studies proteins from Human Elongator complex. She successfully expresses and purifies *Hs*Elp123 complex using insect cell expression system. It is shown that *Hs*Elp123 can bind various tested tRNAs with varying K_Ds but not peptide substrates. All tested tRNAs induce ACO hydrolysis to a various extend, not corelating with K_D. These findings suggest that *Hs*Elp3 display specificity towards tRNAs. In the next steps, author is testing *Hs*Elp123 mutans identified in patients with neurodegenerative disease: carrying amino acids substitution K815T in Elp1 subunit of the Elongator complex. Mutation has no influence on the structural integrity of Elp123 complex, however affinity to tRNA is decreased and ACO hydrolysis less efficient.

To prepare *Hs*Elp123 for structural analyses, author tests various ACO derivatives, such as Desulfo-CoA, Ethyl-CoA, and CoA, to get the most stable complex with tRNA. CryoEM attempts with Elp123-tRNAGlu-DCA (and ECA) resultes in three variants of the complex, with and without tRNA and DCA at the resolution ranging between 3.8 and4.3 Å. Author focuses on a structure depicting full Elp123 with tRNA, DCA. She compares obtained results with those known for different organisms and already published. Author shows that despite low sequence similarities between organisms, structural features and binding motifs responsible for binding of the tRNA are preserved. Author tests motifs located within the N-terminus of the Elp3 and selected Arginines of the Elp3 located in the vicinity of the U33 and U34 of the ASL to determine their involvement in tRNA binding and ACO hydrolysis. She confirms their role in the activity of Elp3. Author builds an atomic model of Elp3 interaction with ASL showing U33 pointing toward the KAT domain and U34 pointing the iron-sulphur cluster in the rSAM domain. Author shows a series of measurements demonstrating that U33 is responsible for triggering ACO hydrolysis and as a consequence, modification of the U34. In the next step, author analyses a DCA binding to the *Hs*Elp3. Highly conserved residues are tested. Author prepares a series of mutans and measures tRNA binding and ACO hydrolysis to show the involvement of the selected amino acids in this reaction. She then tests the potential role of the lysine Lys280 and Lys316 to transfer the hydrolysed acetyl group across the domain. Mass spectrometry analyses shows that both residues can be acetylated but since some other Lysine residues are also acetylated, the results are considered inconclusive. Mutation of K280A and Tyrosines located in the vicinity of the K280 showes reduced ACO binding suggesting and important role of these residues in U34 modification. In the last paragraph of the *Results*, author describes testing of the pathogenic Elp3 variants and discusses correlation of the mutation with the structure and function.

In *Discussion*, the author considers the role of the N-terminus in tRNA binding by the Elp3. She emphasises lack of amino acids sequence conservation and depicts the model based on the structure recognition. She then discusses the series of events necessary for Elp3 to modify tRNA – tRNA binding, ACO binding and hydrolysis, role of the U33. Author compares her own and published data for various organisms. She finishes this chapter by discussing the structural advanced she manages to obtain in this study.

In *Summary*, author shows that (I) tRNA binding by Elp3 is dependent on N-terminus, (II) Elp3 binds tRNA but not proteins or peptides, (III) a role of U33 in the modification of the U34, (IV) structural details of the ACO binding and hydrolysis, (IV) suggests the possible pathway of ACO hydrolysis and transfer to cm^5U moiety.

Critical Remarks:

- Thesis lacks proofreading. There are many numbering mistakes for the figures as well as mistaken mutants numbering (for example page 47), colours not matching the calculated numbers (Fig 20A at page 52). Which makes the thesis difficult to the reader to follow.
- Is K_D for $tRNA^{Ser}_{UGA}$(CCA) correctly calculated based on the graph Fig 242, which is in fact Fig 22 at page 54?
- Why is *Dmc*-tRNA used for assay with *Min*Elp3 instead of *Min*-tRNA?
- ITC results for *Min*Elp3 wt + ACO could have been included in Fig 25.
- Was crosslinking used to stabilize structure for Cryo-EM?
- How is allocated local resolution within the complexes?
- In the *Materials* chapter, the lists the sequences of DNA primers used to prepare constructs for expression of recombinant proteins, their mutants, plasmids used for research, bacterial strains used for protein expression and buffers used for protein purification and assays are missing. Even if some of these information is included in the *Methods* section.
- Crystallization is not described in *Methods* section.
- Names of the organisms should be written in *italic*.

Despite some critical remarks to the content and preparation of the dissertation, I grade this work *very good* and would suggest a *distinction*. The author correctly plans experiments and can apply trouble shutting protocols. Mgr Abbassi clearly put in a lot of work into this research. Experiments required preparations of various mutants, DNA manipulations, protein expression and purification. The results are novel, solid and a continuation of the scientific issues studied in the Glatt's laboratory. They will certainly be published in a very good journal. Mgr Abbassi already has shown a solid track record of very good publications.

Niniejszą pracę oceniam *bardzo dobrze* oraz wnioskuję o *wyróżnienie*. Stwierdzam, że praca doktorska Pani mgr Nour-el-hana Abbassi spełnia warunki określone w artykule 187 Ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2018r. poz. 1668 z późn. zm.). Zwracam się do Rady Dyscypliny Nauk biologicznych Uniwersytetu Jagiellońskiego o dopuszczenie Pani mgr Nour-el-hana Abbassi do dalszych etapów postępowania o nadanie stopnia doktora w dziedzinie nauk ścisłych i przyrodniczych w dyscyplinie nauk biologicznych.

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