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Review of the PhD thesis by Nour-el-hana Abbassi

Biochemical and structural characterizations of the catalytic subunit of Elongator complex

The Doctoral Thesis of **Nour-el-hana Abbassi**, titled **"Biochemical and structural characterizations of the catalytic subunit of Elongator complex"** was conducted under the supervision of Dr. hab. Sebastian Glatt at the Max Planck Laboratory of Małopolska Center of Biotechnology, Jagiellonian University. The work presented in this Thesis focuses on understanding the structural aspects of the Elp3-tRNA complex formation and the role of Elp3 within the context of Elp123 and the complete Elongator complex. In my opinion, Nour-el-hana work is significant for several reasons. First, the basic science aspect. The study of complex macromolecular structures like Elongator contributes to our fundamental understanding of cellular biology and molecular processes. Second, biological significance. Elongator is a key macromolecular complex involved in the modification of transfer RNAs (tRNAs), particularly in the conversion of U_{34} to cm^5U_{34}. These modified tRNAs play a fundamental role in maintaining the plasticity and decoding ability of tRNAs during the protein synthesis process. Third, disease relevance and translational insights. Elongator has been implicated in various diseases, including neurodegenerative disorders and cancers. A better understanding of its structure and function can shed light on the molecular mechanisms underlying these diseases, potentially leading to novel therapeutic strategies.

Overall, the organization of the Thesis is clear and follows a logical sequence. The Thesis comprises several sections, including an Abstract (in both Polish and English versions), a Table

of Contents, List of Publications, and Abbreviations. Following that, there is a comprehensive Introduction section. Here, Nour-el-hana describes the state of knowledge in the field and explains the clinical relevance of her work. The Introduction section is followed by well-defined "Aims of this work," which I particularly appreciate. It is explicit and well-written, with the hypothesis clearly laid out. Comprehensive Method and separate Materials sections follow. These sections are particularly rich in information and, in my opinion, highlight the hard work that was necessary to drive this PhD project. Results and Discussion sections follow. In the first section of the Thesis, Nour-el-hana presents data that contributed to the structural and functional characterization of DmcElp3 and MinElp3, while also explaining why she focused on MinElp3 in the next stage of her research. She conducted an extensive crystallization campaign for both enzymes, and many of those attempts proved unsuccessful. Finally, she managed to obtain several crystals of truncated MinElp3, enabling her to solve the structures of monomeric MinElp3. This, in turn, allowed Nour-el-hana to draw the conclusion that Elp3s from bacteria, archaea, and yeast are structurally conserved. Regrettably, the structures did not contain the expected iron-sulfur cluster. Next, Nour-el-hana aimed to obtain the structure of the MinElp3-tRNA complex. She used Electrophoresis Mobility Shift Assay (EMSA) and MST to interrogate tRNA-MinElp3 interactions to select the best binders and set up crystallization trials. Despite extensive efforts she was unable to obtain crystals. In the next step Nour-el-hana conducted biochemical characterizations of MinElp3-mediated ACO hydrolysis. Concluding the first part of the Thesis, she demonstrated that Elp3 utilizes both its N-terminus and active site to bind to tRNA. Importantly, Nour-el-hana showed that the substrate selection is determined by the shape of the tRNA molecule rather than specific sequence patterns and not all bound tRNAs trigger ACO hydrolysis of Elp3. These findings are effectively illustrated in the proposed diagram, which outlines the various steps involved in the Elp3-mediated tRNA modification process, found on page 59. In the second part of the Thesis, Nour-el-hana delves into the characterization of the eukaryotic Elongator complex, aiming to unravel its biochemical, biophysical, and structural properties. She begins by providing a detailed account of her approach to constructing the human Elongator complex (ELP123) for overexpression in insect cells, ensuring the precise stoichiometry of each subunit. On the other hand, her description of the purification process for the ELP123 subcomplex is somewhat vague and she swiftly transitions to the examination of biochemical and biophysical aspects, including tRNA binding

and the activity of tRNA-induced ACO hydrolysis. I find the next section of the Thesis, titled "Case study of the ELP1K815T23 mutant detected in patients with neurodegenerative disease" to be somewhat out of place. In my view, it would be easier to read if that section was moved towards the end of the Thesis, perhaps to the section where Nour-el-hana describes the biochemical characterizations of pathogenic ELP3 variants. In the next section, she describes her efforts to prepare the complex and solve the structure of the core Elongator using cryo-EM. She successfully obtained the cryo-EM structures of ELP123-tRNAGlu-DCA and ELP123-tRNAGlu-ECA but focuses on the results of ELP123-tRNAGlu-DCA (as both are similar). Given the high quality of the tRNA-bound human ELP123 map, she was able to identify several residues interacting with the ASL of tRNA and tested them in tRNA binding and enzymatic ACO hydrolysis activity assays, confirming their importance in tRNA binding and/or downstream activity. Additionally, thanks to the high-resolution of tRNA-bound structure of HsELP123, Nour-el-hana could confidently construct the atomic model of ASL. This model reveals that the universally conserved U_{33} is oriented toward the KAT domain, while U_{34} faces the iron-sulfur cluster in the rSAM domain. These structural insights, coupled with the subsequent biochemical results presented by Nour-el-hana, represent the first data that implicates U_{33} in the triggering U_{34} modifications. Next, based on structural and biochemical data, Nour-el-hana investigates residues critical for ligand binding and acetyl transfer. This part of the Thesis concludes with Summary Part II, which highlights the most important findings. While the Results section is sometimes spotty and a bit hard to follow, the Discussion part of the Thesis is easier to read. The extensive list of References covers pages 93 to 111. The Thesis is rounded out by the List of Figures, Appendix, List of Scientific Achievements, and Acknowledgements. I must emphasize that I am genuinely impressed by the content of the List of Scientific Achievements section. It shows how active Nour-el-hana was during her PhD. She listed participation in 12 conferences and workshops, supervision of Master Students, and serious involvement in scientific broadcasting. To conclude this section, the Thesis contains excellent scientific content, however, I did come across a number of spelling mistakes (for instance, p. 47, "the transactions of *Min*Elp3 N-terminus', or p. 69, "The prepared cryo grids were screened using a Glacios microscopy", just to name a few). Mistakes also included some formatting issues. For instance, in the Table of Contents (pages 7 and 8 displaying "ERROR! BOOKMARK NOT DEFINED"). There are multiple places where the font changes in a single word or some unfortunate statements

that make me guess what Nour-el-hana wanted to convey. The figures are clear, mostly well-described, effectively conveying the message, and emphasizing the substantial research efforts carried out by Nour-el-hana during her PhD. However, there are some issues with the numbering of figures (for instance, p.53, Figure 231, p.54, Figure 242, and so on), as well as errors in the descriptions of certain figures (e.g., p. 70, "Form1p2"). Unfortunately, this continues thought the Thesis. While these mistakes are distracting, they do not diminish the overall high quality of the scientific content presented in the Thesis.

The results described by Nour-el-hana Abbassi in her PhD Thesis have been published in two international, peer-reviewed journals: *Nature Communications* (DOI: 10.3390/ijms21218209), where Nour-el-hana is a second author, and *J Hum Genet* (DOI: 10.1038/s10038-023-01135-3), where she is a co-first author. Another publication, which includes the results described in this Thesis is currently in preparation (with Nour-el-hana as the co-first author,). It should also be noted that during her PhD, Nour-el-hana Abbassi contributed as an author to four additional high-quality publications published in *Sci Adv* (DOI: 10.1126/sciadv.aaw2326), *Int J Mol Sci* (DOI: 10.1126/sciadv.aaw23263), *EMBO Mol Med* (DOI: 10.15252/emmm.202115608), and *Nucleic Acid Research* (DOI: 10.1126/sciadv.aaw2326). These achievements are truly remarkable.

In summary, the work presented in this Thesis upholds high standards, with some parts having already been published in prestigious international peer-reviewed journals. Additionally, Nour-el-hana has proven to be a highly effective team player, as evidenced by her contributions to four additional publications. I firmly believe that throughout her PhD, Nour-el-hana has acquired significant expertise in Biochemistry and Structural Biologist. Her studies are highly relevant and have significantly advanced our understanding in the field. Most notably, her work lays the groundwork for future investigations into the mechanisms of action of the Elongator complex, which has implications not only for fundamental scientific research but also for potential applications in fields related to human health and disease.

In conclusion, I firmly believe that Nour-el-hana Abbassi's PhD Thesis meets all the requirements for the title of Doctor of Philosophy, as stipulated in Dz.U. z 2018 r. poz. 1668 z

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późn. zm. Therefore, I recommend that she is admitted to the subsequent stages of the doctoral dissertation proceedings. Furthermore, owing to the high quality of the work presented in this Thesis, I suggest that it be considered for an appropriate distinction, if permitted by the Doctoral School regulations.

Podsumowując, jestem przekonany, że praca doktorska Nour-el-hana Abbassi spełnia wszystkie warunki określone w artykule 187 ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz.U. z 2018 r. poz. 1668 z późn. zm.). Dlatego też rekomenduję, aby Pani Nour-el-hana Abbassi została dopuszczona do kolejnych etapów postępowania doktorskiego. Ponadto, ze względu na wysoką jakość pracy przedstawionej w tej rozprawie, rekomenduję rozważenie możliwości przyznania wyróżnienia, jeśli pozwala na to regulamin Szkoły Doktorskiej.

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