Candidate's name and surname: **Nour-el-hana Abbassi**

PhD Thesis Title: **Biochemical and structural characterizations of the catalytic subunit of Elongator complex**

Thesis Supervisor: **Dr hab. Sebastian Glatt**

Reviewer: **Dr hab. Magdalena Wołoszyńska, associate professor**

THESIS EVALUATION

Scientific merit of the thesis and originality of the research

The Elongator complex has been identified almost 25 years ago in yeast and not much later in multicellular eukaryotes, while the catalytic Elp3 subunit of the complex has been found in archaea, bacteria and viruses. Over the years we got used to the fact that the structure of the Elongator complex and the Elp3 sequence were commonly described in research and review articles as highly conserved. Although it is true, still some surprising differences between N-termini of Elp3 proteins from various domains of life and viruses existed and were finally spotted by Nour-el-hana Abbassi. She cleverly explored this issue in the first part of the thesis devoted to the non-eukaryotic Elp3 proteins, mainly archaeal Elp3. The straightforward experiments proved the essential role of the N-terminus length and identity for tRNA binding and acetyl-coenzyme A (ACO) hydrolysis – the molecular events required for the best known function of the Elongator complex which is modification of some tRNAs. The research also explained that Elp3 recognizes substrate via mechanism based on tRNA shape rather than nucleotide sequence and defined amino acid residues crucial for ACO binding and hydrolysis. Consequently, at the end of the part I the author proposed the convincing model presenting novel and sound details of Elp3-tRNA-ACO interaction and order of events leading to tRNA modification where tRNA binding occurs before ACO enters the KAT domain.

I remembered the words of Sir Alfred Hitchcock while reading the second part of the thesis: "A good film should start with an earthquake and be followed by rising tension". Similarly, the results related to the human Elongator complex and described in the second part of the thesis gradually created a more and more complete picture of the tRNA interaction with the

complex. Some important details are still missing: the author did not observe the 5′-carboxymethyluridine generation or the fully assembled Elongator complex in her experimental set-up, the data related to acetylation of the lysine residues in the catalytic center also leave some doubts. Nour-el-hana Abassi proved her scientific maturity and critically evaluated the weak points of the research. Taking into account all these reservations, she proposed the answer to one of the most intriguing questions about Elongator – how does the acetyl group make the relatively long way from the hydrolyzed ACO molecule to the wobble U_{34} of tRNA? The high-quality cryo-EM structures of Elp123 subcomplex captured at various stages of the reaction in combination with biochemical assays allowed the author to reconstruct the course of the tRNA modification at the level of full atomic model of the Elp3 catalytic center and tRNA anticodon and stem loop. The coordinated action of the U_{33}, basic arginine residues and alternating lysine-tyrosine residues, acting in a series of the acetylation and deacetylation reactions, make possible the transfer of the acetyl group.

In addition to the high originality and scientific soundness of this fundamental research, the work also has a strong application because some mutations in the human genes encoding subunits of the Elongator complex result in pathogenic variants. The expression system constructed by the author appeared to serve as a very useful tool for production and analysis of the pathogenic Elp1 and Elp3 variants.

Considering that the findings presented in the thesis were mainly obtained under the *in vitro* conditions, I would like to discuss some issues during the defense:

1. As the Elp3 of archaea and human Elongator complex recognize and bind tRNAs based on their structure and interact with all analyzed tRNA types including those which do not undergo cm^5 modification, what would be the consequences of such a mode of action for translation speed in the cell? Is it possible that interaction with non-modifiable tRNAs could slow-down or even inhibit the Elp3 activity? According to the proposed models, the tRNA molecules bound by Elp3 are released after the ACO hydrolysis and U_{34} modification. Would non-modifiable tRNAs be released without acetylation?

2. The author writes that Elp123 *selectively* binds to tRNA substrates (pg. 82). How exactly should this selectivity be understood? Taking into account that tRNA recognition is structure based, how possible is it that the complex could interact with other nucleic acids, for instance microRNAs? Similar dual activities of tRNA modifying enzymes are known, e.g. in the case of the tRNA pseudouridine synthase, TruB1.

Substantial merit of the thesis

The research is introduced with the state-of-the-art description based on well selected literature giving the overview of protein translation, tRNA structure and modifications and finally zooming in on the Elongator complex. The aims of the work and hypotheses are presented in the separate short chapter and are rather clearly explained except the penultimate hypothesis relating to the efficiency differences expected between eukaryotic and archaeal Elp3. I have an impression that this hypothesis was not addressed in the thesis as clearly as the remaining ones. The research was performed with well selected methods and, what makes this research especially sound, experiments were accompanied by appropriate controls. The data is in general well presented, I appreciate clear introductions to experiments explaining their purpose and main rudiments of the applied techniques. My small criticism goes to insufficiently structured paragraphs. For example, paragraph Characterization of MinElp3-tRNA interaction (Part I) could be subdivided to better display the important results relating to N-terminus-tRNA interaction on one hand and substrate specificity on the other. Both parts of the Results chapter are followed by summaries given as the short text and visualized as very informative figures.

Layout and register

The thesis follows the classical layout starting with well written abstracts in English and polish, followed by the table of contents and list of abbreviations which help to navigate and understand the text. Methods are explained rather briefly although in most cases provide sufficient detail. Materials are described in a separate chapter, which I find a good idea.

The thesis is written with clarity additionally supported by numerous very well designed figures. The illustrations or panels of illustrations are simple and make reasonable use of colors. I appreciate a lot that most relevant information is actually placed in the main body of the figure and there is no need to look for them in the legends.

Critical notes

1. There is some confusion about the expression yield of HsElp123 in the insect cells. In pg. 63 the high value of 300 µg/l is indicated while in page 68 the yield is only 50-100 µg/l.

2. In fig. 24A the tRNA binding of several MinElp3 variants is shown but the wild type binding is not presented and therefore I find it a bit risky to conclude that mutations present in the Elp3 variants do not affect their ability to bind tRNA.

3. In the Method chapter the sequences of primers used in the PCR assays are not provided.

4. I have noticed several editorial issues: in the Table of contents some page numbers are missing, majority of figures have wrong numbers, in pg. 47 five truncated variants of the

*Min*Elp3 should be mentioned but instead the name of the same variant is repeated, the Kd values shown in the fig. 32B for the wild type and K815T Elp123 subcomplex (400 and 657 nM) are different from values mentioned in the text (50 and 150 nM).

Final grade

The PhD thesis written by Nour-el-hana-Abbassi is a highly significant contribution to understanding of the Elp3 activity and tRNA modifications but also in general to most fundamental mechanisms of enzymatic catalysis. Considering the scientific value of this work but also the other publications co-authored by the student, her active participation in several conferences and workshops as well as involvement in coaching masters students, I am convinced that Nour-el-hana Abbassi is ready to obtain her PhD.

I, hereby, declare that the reviewed PhD thesis entitled "Biochemical and structural characterizations of the catalytic subunit of Elongator complex" by Nour-el-hana Abbassi meets the criteria under art. 187 of Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended), and request that the Research Discipline Council of Biological Sciences of the Jagiellonian University in Cracow accepts Mohib Abdullah for further stages of doctoral proceedings in the field of exact and biological sciences, in the discipline of biological sciences.

Zaświadczam, że przedstawiona mi do recenzji rozprawa doktorska pt.: „Biochemiczna i strukturalna charakterystyka katalitycznej podjednostki kompleksu Elongatora" napisana przez 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 2018 r. poz. 1668 z późn. zm.). Proszę Radę Dyscypliny Nauk Biologicznych Uniwersytetu Jagiellońskiego o dopuszczenie mgr Nour-el-hana Abbassi do dalszych etapów postępowania o nadanie stopnia doktora w dziedzinie nauk ścisłych i przyrodniczych w dyscyplinie nauki biologiczne.

Considering the excellence of the results presented in the thesis, the huge scope of work and especially its impact on the field, I, hereby, request that the thesis is accepted with distinctions.

Wroclaw, 27.07.2023 ....

date Reviewer's signature