Candidate's name and surname: **Marta Smejda**

PhD Thesis Title: **Molecular characterization of human KTI12**

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 doctoral dissertation of Marta Smejda describes precise and elegant research devoted to characterize the human *Kluyveromyces lactis* Toxin Insensitive 12 (KTI12) protein as an interactor of the Elongator complex and to compare its functions to structurally similar O-phosphoseryl-tRNA kinase (PSTK). Although Kti12 has been studied in detail in yeast, this thesis presents the first characterization of the human orthologue which points to the strong originality of this work. The importance of the studies is further enhanced by potential therapeutic application of the results. Malfunctions of the Elongator complex are associated with fatal neurodegenerative or tumor diseases but due to its structural features, Elongator itself is not a promising therapy target. The research presented in the reviewed thesis suggests that KTI12 would be a better candidate.

The thesis proves that in human cells Elongator is the major interactor of the KTI12 protein which seems to be present mainly or exclusively in cytoplasm and supports the complex in its main function of tRNA wobble uridine modification. These findings, although expected in the light of the earlier research, are extremely well confirmed. However, the most challenging and scientifically exciting aspect of the research is analysis of the KTI12 interactome. Diverse experimental and bioinformatics approaches were combined to identify proteins stably or transiently interacting with or just proximate to KTI12. Consequently, the intracellular position

of KTI12 and its proteomic environment was pictured including the KTI12-Elongator-interactors orientation.

Structural similarity between KTI12 and PSTK was known and intriguing already for some time but potential biological relevance of this resemblance could only be properly evaluated in the organism like humans, where both proteins are active. Potential role of Elongator as regulator of two tRNA related pathways was appealing. The thesis of Marta Smejda verifies these speculations and convincingly shows functional independence of PSTK and the KTI12 – Elongator duo: "Same but different" as we can learn from the title of the article enclosing most of the data presented in the thesis. I can imagine that initially this finding was disappointing for the PhD candidate but luckily, and cleverly, she could see it from a different point of view - as a favorable circumstance for the use of KTI12 as a therapy target.

Considering that this research, which was published last year, was the first report related to the human KTI12, it should be highly appreciated how much the single PhD thesis contributes to the state-of-the art. These results suggest potential novel roles for KTI12 and Elongator and provide a very good starting point and solid basis for the upcoming research. The significant potential of the studies described by Marta Smejda to trigger further research is just another evidence of the high quality and novelty of her findings.

Some of the possible opportunities for the future studies are indicated by the discovered KTI12 interactors including particularly interesting proteins involved in a vesicle-mediated transport, autophagy, RNA metabolism or cell migration. The analysis of the direct and indirect interactors of KTI12 is thorough and comprehensive. At this point I would like to ask the first question to the candidate: I am curious whether author considers the set of interactors just as an evidence for diverse and unrelated processes regulated by KTI12-Elongator team, or whether is there any chance to translate the revealed protein-protein interaction network to biological pathways interrelated and synchronized by KTI12-Elongator?

As written in the thesis, the intention of the author is to continue her work in aim to develop a method allowing inhibition of the KTI12 activity leading to decreased Elongator performance in the cancer cells. My concern is raised by unaffected migration of cells with knocked-down or overexpressed KTI12 while ELP3 silencing has a negative effect. These two results are inconsistent in the context of clearly essential KTI12 role in the tRNA modification by Elongator. To explain this contradiction, author suggests in Discussion that Elongator is involved in cell migration via its alternative, and tRNA unrelated, activity. Is this explanation plausible regarding that not only Elongator but also other enzymes of the same tRNA

modification pathway are upregulated in the cancer cells correlating with their tumorigenicity and migration potential? Another explanation discussed in the thesis assumes that KTI12, although silenced and present at low levels, may still successfully fulfil the role of Elongator interactor during tRNA modification. Therefore, I wonder whether KTI12 is indeed such a promising target for the potential anti-cancer therapy designed to reduce Elongator activity. I would be happy to discuss this issue during the defense.

The question, that has accompanied me from the beginning of reading the Results chapter, relates to the HEK293T and BJ cell lines used in the *in vivo* experiments: why were these particular cell lines selected? In Discussion, the author considers the possibility that activity of KTI12 may be cell-specific and suggests neuronal precursor cells as potential alternatives. I would like to ask: which features of the HEK293T and BJ cells could prevent observation of KTI12 influence on the cellular processes analyzed in the thesis, and what would be the criteria to make a better choice in future?

Substantial merit of the thesis

The whole thesis is written with high clarity, has logical structure and seems to follow the rule: minimum words – maximum information. Theoretical background presented in the Introduction is accurately selected and ordered allowing the reader to smoothly pass from general aspects of protein translation to specific and detailed information related to the Elongator complex. Although research hypotheses are not strongly pronounced, I do not see it as the major weakness since the aims of the thesis are very well formulated. The methodical toolset applied in the presented research is impressively wide, especially when it comes to assays addressing protein-protein interactions. Described technics are not only challenging and informative but also give insight to interactions of diverse nature. As written by the author, the thesis describes results which pave the way for further research related to human KTI12 and as such it should bring a very solid, clear and even instructive base for the future experimental work. This aspect is successfully addressed by detailed description of used materials and performed protocols. The results are very well presented providing the reasoning behind each experiment, performed controls and high quality documentation of obtained data. I was very satisfied with discussion which proves erudition of the candidate and courage of critical thinking about own results in the context of research done by the other authors. The final conclusions are very well formulated but it was probably not difficult considering logical experimental design and high quality of the obtained conclusive results.

Layout and register

Thesis has a generally typical layout which has been very well applied to present the data. I would like to specially appreciate the clear language which is one of the main strengths of this thesis. I have also noticed how much the visual aspect of data presentation contributes to clarity of the message. There is a series of experiments which were performed in parallel for KTI12 and PSTK which could easily lead to confusion of the reader. However, the blue (KTI12) – purple (PSTK) color code applied to respective tables and figures makes it quite easy to recognize and distinguish the two datasets.

Critical notes

In the Polish version of Abstract fungal Kti12 is phrased as "grzybiczy Kti12". I would suggest to avoid this adjective which refers rather to fungal infection but not to the object, in this case protein, present in fungal cells. In general the thesis is very well written in terms of formal aspects but occasionally the abbreviated protein names are not explained while used for the first time, for example KTI12 or Cytosolic Thiouridylase 1/2 (CTU1/CTU2). Although Methods are very well described, as an enthusiast of quantitative analyses of nucleic acids, I noticed that description of the real-time PCR does not provide description related to the raw data analysis (how the Ct values were translated to quantitative differences between the samples). Figure 16 presents ATP hydrolysis compared between KTI12 and PSTK. Was there any statistical analysis of the data performed and is it possible to conclude about the statistical significance of the results? In Fig. 22 Western Blot with anti-MINDY-3 antibodies of the Co-IP samples visualizes two weak bands in the case of the N-terminal domain of KTI12 which look like very much different than bands obtained for the wild type or K14A mutant. This difference is not addressed in the results description.

Final grade

The PhD thesis written by Marta Smejda is highly original, presents important and sound data with potential to significantly influence the research of human KTI12 and Elongator. This work proves scientific maturity of candidate and shows that she is ready to continue her fascinating work as Doctor of Philosophy.

I, hereby, declare that the reviewed PhD thesis by **Marta Smejda** meets the criteria pursuant to art. 13.1 of Act of 14 March 2003 on Academic Degrees and Academic Title and Title in the Arts (O.J. no 65 item 595 as amended) and request that the Research Discipline Council of Biological Sciences of the Jagiellonian University in Kraków accepts **Marta Smejda** for further stages of doctoral proceedings.

Considering the value of the research presented in the PhD thesis of Marta Smejda, quality of the data, novelty of results and applied methods as well as the impact on the field, I, hereby, request that the thesis is accepted with distinctions.

Wroclaw, 18.02.2002 .....

date Reviewer's signature