dr hab. Andrzej Dziembowski Warszawa, dn. 3.02.2021

EVALUATION OF RESEARCH, DIDACTIC and ORGANISATIONAL ACHIEVEMENTS of Dr. Sebastian Glatt

The review of the habilitation thesis according to recommendations of Rada Doskonałości Naukowej:

1. basic information about the candidate, including:

a. The date of the doctoral degree and the name of the organizational unit in which the degree was conferred;

See Autoreferat

b. information as to whether the candidate has previously applied for the conferment of the degree of doktor habilitowany, including, as far as it is evident from the case file, information on the course and termination of previous proceedings;

To my knowledge, no

c. scientific and professional work history (workplace, positions held);

See Autoreferat

2. information on the applicable laws as of the date of initiation of the habilitation procedure under review, including the applicable evaluation criteria.

Art, 221 para 14 of the Act of 20 July 2018. - Law on Higher Education and Science (Journal of Laws of 2018., item 1668 as amended).

3. Informacje ocenianych osiągnięciach naukowych wedle rekomendacji Rady Doskonałości Naukowej :

a. the title of the research achievement

Structural and functional characterization of eukaryotic tRNA modification complexes

b. scientometric data, such as the cumulative Impact Factor, the cumulative Ministerial points, the number of citations, and the Hirsch Index

See Autoreferat, I did not conduct independent analyses, including the addition of ministerial credits before and after Ph.D. of Sebastian Glatt.

c. information on the number of scientific publications, monographs, chapters in monographs authored or co-authored by the candidate,

See Autoreferat

d. information on the major journals in which the candidate has published his/her scholarly work;

See Autoreferat and below

e. Information on whether the candidate played a leading role within the production of co-authored scientific papers;

See Autoreferat; in my opinion, the role was sufficient

f. evaluation of the candidate's indicated research achievement, including whether it represents a significant contribution to the development of a specific scientific discipline;

After his Ph.D., the main research interest of Dr. Glatt was the mechanism of chemical modifications of tRNA molecules. The field of RNA modifications, known as epitansicroptomis, is rapidly growing and attracting a lot of attention from researchers. In the case of tRNA, modifications are needed for the stability of the tertiary structure and increase the accuracy of the amino acids decoding during the protein production.

Dr. Glatt was focusing on the so-called elongator complex, an evolutionarily conserved, large, and complex macromolecular assembly essential for the generation of carboxymethylated uridine (cm5U) in the tRNA wobble position. Subsequently, this elongator dependent modification is transformed into 5-methoxycarbonylmethyl 5-carbamoylmethyl, or 5-methoxy-carbonyl-methyl-2-thio by other enzymatic cascades. The structure and the mechanism of action of the elongator are unexpectedly complex with unusual chemistry, and dr Glatt, together with his collaborations was instrumental, for its understanding. Briefly, the eucaryotic elongator complex is composed of six subunits (Elp1 to Elp6) essential for tRNA modification *in vivo*. Elp123 (620 kDa) forms a catalytic subcomplex, while the Elp456 (230 kDa) forms a ring-shaped supporting subcomplex. Moreover, the elongator complex needs Kti11/Kti13 cofactor. Elp3 is the catalytic subunit with lysine acetyltransferase (KAT) and radical S-adenosyl methionine (SAM) domains. These two different activities together carry out initial cm5U formation in a hydroxyl radical dependent manner. Actually, Elp456 subcomplex is an ATPase, but that actively supports the substrate biding rather than uridine modification itself

Over the last 9 years, there are about 15 publications about elongator complex, of which dr Glatt is a co-author. The majority of them are published in prestigious journals and present crystal or cryEM structures of sub-assemblies in different states. The structural data are very nicely supported by biochemical, biophysical, and functional data. They represent state of the art in both their experimental work and conceptual adance.

Three research papers are presented as a main achievement for the habilitation and are briefly described below:

1) "Structure of the Kti11/Kti13 heterodimer and its double role in modifications of tRNA and eukaryotic elongation factor 2." Glatt S, Zabel R, Vonkova I, Kumar A, Netz DJ, Pierik AJ, Rybin V, Lill R, Gavin AC, Balbach J, Breunig KD, Müller CW. Structure. 2015 Jan 6;23(1):149-160.

In this publication, Dr Glatt, together with his collaborators, solved the structure of Kti11/Kti13 heterodimer, which is a cofactor of the elongator complex and an essential component of the pathway leading to the diphtamide modification of conserved histidine residue of translation elongation factor 2. Both RNA base modifications by the elongator complex and amino acid modification in diphtamide pathwy require iron-sulfur cluster. The structure of Kti11/Kti13 heterodimer explains how this complex can play a role as an electron donor.

2) “The Elongator subunit Elp3 is a non-canonical tRNA acetyltransferase.” Lin TY, Abbassi NEH, Zakrzewski K, Chramiec-Głąbik A, Jemioła-Rzemińska M, Różycki J, Glatt S. Nat Commun. 2019 Feb 7;10(1):625. 2.

This paper describes the structure of bacterial Elp3 with one of the substrates essential for the elongator complex dependent cm5U formation, an acetyl-CoA analog. The presented structural data, together with series of biochemical experiments and previous knowledge, allowed the authors to propose how Elp3 undergoes structural rearrangement during tRNA modification contributing to our understanding of the reaction cycle.

3) "Molecular basis of tRNA recognition by the Elongator complex." Dauden MI, Jaciuk M, Weis F, Lin TY, Kleindienst C, Abbassi NEH, Khatter H, Krutyhołowa R, Breunig KD, Kosinski J, Müller CW, Glatt S. Sci Adv. 2019 Jul 10;5(7):eaaw2326. doi: 10.1126/sciadv.aaw2326

This publication describes the high resolutions cryoEM structures of tRNA bound and apo structures of the yeast elongator complexes. This work is instrumental in understanding the structural rearrangements of the complex upon binding of substrates. It also, together with the prior knowledge, allowed the authors to propose a model of the catalytic cycle. The catalytic subunit is responsible for two individual reactions in parallel. One leads to the formation of 5’-deoxyadenosyl radical (5’dA•) in the rSAM domain with the help of 4Fe4S cluster as an electron donor. At the same time, upon incoming tRNA substrate acetyltransferase domain activates the acetyl group. tRNA binding is proposed to triggers acetyl-CoA hydrolysis, and the acetyl group is transferred into the close proximity of 5’-dA• and the U34 uridine. Then it can get converted into acetyl radical and cm5U is formed. In sum, this work solved many questions regarding the elongator complex mechanism of action.

Surprisingly several publications in which the candidate had a prominent role and provided data essential for understanding the elongator complex action are not a part of the main achievement, which is somehow inconsistent.

For instance, publication: "Structural basis for tRNA modification by Elp3 from Dehalococcoides mccartyi" Glatt S, Zabel R, Kolaj-Robin O, Onuma OF, Baudin F, Graziadei A, Taverniti V, Lin TY, Baymann F, Seraphin B, Breunig KD, Müller CW. Nat Struct Mol Biol. 2016 Sep;23(9):794-802. is essential for underspending the chemistry of the elongator reactions.

The first structure of ELP456 structure is also not listed "The Elongator subcomplex Elp456 is a hexameric RecA-like ATPase"Glatt S, Létoquart J, Faux C, Taylor NM, Séraphin B, Müller CW. Nat Struct Mol Biol. 2012 Feb 19;19(3):314-20. as well as a first publication describing the overall architecture of the whole complex "Architecture of the yeast Elongator complex." Dauden MI, Kosinski J, Kolaj-Robin O, Desfosses A, Ori A, Faux C, Hoffmann NA, Onuma OF, Breunig KD, B; EMBO Rep. 2017 Feb;18(2):264-279.

This remark does not diminish my enthusiasm about Sebastian Glatt's application for the habilitation degree since I assume lack of this paper in the main achievement is related to often faced difficulties in gathering co-authors' formal statements regarding individual contributions, an essential part of the documents provident at the time of application. However, to fully appreciate and summarize the work of the applicant, all those papers should be taken into consideration and summarized.

In conclusion, the scientific achievements of the candidate are outstanding and prove his scientific maturity.

g. information on the applicant's fulfillment of the criterion of demonstrating significant scientific or artistic activity;

The scientific career of Dr. Glatt can be described as exemplary. Studies and a PhD program at one research center, then a post-doctoral fellowship at another, and then start of an independent research position at yet another.

h. information about the didactic, organizational, and popularization achievements of the candidate for the habilitation degree

Since Dr. Glatt established his independent research group, Sebastian Glatt was not just involved in conducting research but also contributed significantly to developing research capabilities in his host institution and the whole country. Dr. Glatt's main organizational achievement is the establishment of the national Electron Microscopy facility. Thanks to the initiative of many researchers in Poland, the modern infrastructure of cryoEM microscopy was established. Dr. Glatt we the main executor here, and he is a head of this facility. What is important, he also obtained independent funding, a Foundation for Polish Science TEAM Tech core facility grant for this purpose. This facility not only provides access to this equipment essential for modern structural biology but also helps the researchers to process the data and gain knowledge.

Besides this Dr. Glatt is also a deputy director of his host institute. Moreover, he supervised many students and post-docs.

General Conclusion

The scientific and organizational achievements of the candidate are clearly outstanding, which was recently independently confirmed by the award of European Research Council consolidation grant in which dr Glatt is planning to continue his studies of tRNA modifications Thus, taking into account the scientific, organizational, and didactic achievements and the overall scientific output of Dr. Sebastian Glatt, the criteria for candidates for the degree of doctor of habilitation are met. I, therefore, request that Habilitantka be admitted to the next stages of the habilitation procedure. Moreover, because of Dr. Glatt's work's exceptionally high quality, I recommend a distinction or appropriate award.

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