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Review of the PhD thesis by Mikołaj Sokołowski

Triggers of the thiolation cascade - biochemical and structural analysis of sulfur transfer in the tRNA modification pathway

The Doctoral Thesis of Mikołaj Sokołowski, entitled **Triggers of the thiolation cascade - biochemical and structural analysis of sulfur transfer in the tRNA modification pathway** was carried out under the supervision of dr hab. Sebastian Glatt at the Max Planck Laboratory of Małopolska Center of Biotechnology, Jagiellonian University and focused on thiolation cascade, an enzymatic pathway involved in the modification of tRNA. While the identity of the enzymes and individual steps of the thiolation cascade are known, how the specificity of the sulfur transfer is achieved and the ways in which involved enzymes avoid deleterious off-target effects, remain elusive. Taking advantage of earlier studies from the Glatt lab, in his PhD Thesis, Mikołaj Sokołowski tackles these fundamental biological questions. Specifically, Mikołaj takes advantage of recent developments in single particle cryo-EM to structurally interrogate the full-length Uba4/Urm1 complex and provides insights into how Uba4 thiocarboxylates Urm1. Thanks to *in vitro* reconstitution of the thiocarboxylation of Urm1 Mikołaj was able to probe the regulatory mechanisms involved in all stages of the Uba4/Urm1 reaction cycle, including binding, adenylation, thioesterification, thiocarboxylation, acyl-disulfide cleavage and release of activated Urm1 from Uba4. Further, Mikołaj managed to obtain soluble Ncs2 and Ncs6 proteins and show that they form a high affinity, heterodimeric Ncs2/6 complex which he further scrutinized by extensive crystallization trials. PhD thesis of Mikołaj Sokołowski provides important contributions to our understanding of the mechanism of sulfur transfer in

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a single Uba4/Urm1 reaction cycle and represents the first biochemical characterization of the Ncs2/6 complex. As such, this Thesis brings original and very relevant data and represents significant advancement in the field.

Layout of the Thesis is clear and consists of several standard sections: Abstract (both Polish and English version), followed by a Table of contents which is very much useful since the Author decided not to number the sections of the Thesis. After that comprehensive and clearly written Introduction section follows. Here Mikołaj describes the state of the knowledge in field as pertaining to his project and ends the section explaining clinical relevancy of his work. The Introduction section is followed by Aims of the Thesis. Here Mikołaj clearly lays out the void in the current knowledge and the aims of his work. Comprehensive Materials and Method sections follows. Results and Discussion sections are clearly written and lead the reader to the Highlights and Conclusions sections followed by References. Overall, I find this work easy to read with only minor mistakes. Figures are clear, well-described, clearly convey the message and emphasize the amount of work performed by Mikołaj during the PhD studies. This mature style of writing and data presentation shows that during his doctoral studies Mikołaj acquired necessary knowledge and technical expertise in biochemistry and structural biology.

I find this Doctoral Dissertation impressive for several reasons. First, it tackles a fundamental question in the field and delivers number of significand discoveries. Second, while the idea beyond experimental work could be thought of as simple or straightforward, the number of experimental results necessary to understand the innerworkings of thiolation cascade, clearly shows the complexity of the system Mikołaj was interrogating. This is exemplified by extensive the Methods section (pages 41- 52). It is clear that in order to put this dissertation together Mikołaj had to master a wide spectrum of experimental techniques. Starting from protein expression and purification, mutagenesis, through very well-designed biochemical experiments the work presented in this Thesis produces very valuable scientific findings but also shows proper attention to details. In addition to biochemical and biophysical analysis, Mikołaj also performed structural studies using single particle cryo electron microscopy to interrogate the full-length ChtUba4/Urm1 complex. While resolution of obtained density map is indeed lower than expected form the state-of the- art single-particle cryo-EM, it provided some hints about distinct conformations that could be present in the ChtUba4/Urm1 complex. Furthermore, Mikołaj performed comprehensive crystallization trails for Ncs2/6 complex.

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Here, Table 24 highlights hard work that was necessary to drive this project. Overall, the amount of work and the quality of data produced by Mikołaj are highly commendable. It is my belief that the training Mikołaj received during his PhD studies will allow him to take on any scientific project that he chooses in the future.

In conclusion, the work described in this PhD Thesis is of high standards. Studies presented by Mikołaj are highly relevant and advanced our knowledge in the field. Therefore, I believe that PhD Thesis by Mikołaj Sokołowski fulfils the requirements for the title of Doctor of Philosophy based on Dz.U. z 2020 r. poz. 85 z późn. zm., and I recommend that Mikołaj Sokołowski is admitted to further stages of the doctoral dissertation proceedings. In addition, because of high quality of work presented here, I recommend that this Thesis should receive appropriate award/distinction (if allowed by the Doctoral School regulations).

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