

Review of the dissertation of Keerthiraju Ethiraju Ravichandran

entitled

tRNA modifications in human diseases -

Structural and functional consequences of Urmylation in response to Oxidative stress

Oxidative stress is caused by an imbalance between the production and accumulation of reactive oxygen species in cells and the detoxification capacity of the biological system to neutralize these reactive products. It imposes a number of consequences on cell function, including the refinement of translation rates as a result of the modification of RNA thiolation levels by the urmylation pathway. Interestingly, urmylation has also been shown to act as a modifier of proteins under oxidative stress, and the enzymes of this pathway are susceptible to it. This study aimed to investigate posttranslational modification - urmylation in response to oxidative stress. The topic undertaken by the Ph.D. candidate is relevant and timely, as it may help elucidate urmylation mechanisms and cellular role in the stress response, especially given that this modification is believed to be the mother of all ubiquitin-like systems in eukaryotes.

The work has a correct layout and structure of the content division. It is well written, and the number of unclear formulations or grammatical errors low. The theoretical introduction lacked an in-depth analysis of the evolution of pathways using UBL protein labeling, although the PhD candidate makes it clear that Uba4 and Urm1 E1-UBL conjugation represent the most ancient UBL-E1 system among eukaryotes. The broader description of the Elongator complex, crucial for carboxymethyl modification of uridine as well as its interplay with the Uba4/Urm1 thiolation pathway, was also missing.

The experimental part of the dissertation is extensive and undoubtedly requires a great deal of work and skill in biophysics and biochemistry. The Ph.D. candidate exhaustively accomplished the goal of the work, which was to understand the basis of recognition, adenylation, and thiocarboxylation of Urm1 as well as to develop an in vitro system to study urmylation. The experiments were planned carefully, using the current state of knowledge, and performed using cutting-edge techniques. The doctoral student seems confident in the issues studied, reflected in the level of knowledge presented in the dissertation. The inclusion of diagrams facilitates the analysis of the results obtained.

The results of the dissertation formed the basis of three published original manuscripts, including one in the prestigious EMBO Journal with the Ph.D. candidate as the first author and a patent. In addition, the Ph.D. candidate has also participated in other research projects that have resulted in publications, received several awards, and presented his research at international conferences.

During the defense, I would like to ask the Doctoral Student to discuss the following issues:

1. How is the Urm1 molecule with a thiocarboxylate group released from Uba4?
2. What the expected differences are between the Uba4C202K-Urm1 complex with covalently attached Urm1 compared to the wild-type complex with non-covalent interactions.
3. Could the Ph.D. candidate comment on whether he also pursued the identification of serine and threonine residues urmylated in Uba4 (mass spectrometry analysis), as was later found to be the case for modifications on these residues in ScAhp?
5. How the Urm1 turnover is regulated?
6. Does oxidative stress limit the sulfur delivery function for tRNA thiolation by Urm1?
7. Why the persulfidated cysteine is followed by covalent coupling of the C-terminus of Urm1 to the corresponding lysine/serine/threonine acceptor residue? Could it be a byproduct of cysteine persulfidation? What is the function of these modifications on K/S/T? Does a cross-talk occur between other modifications for these positions (e.g., ubiquitylation)?

I highly appreciate the substantive quality of the dissertation of Keerthiraju Ethiraju Ravichandran. It fully meets the requirements of Article 187. paragraphs 1. and 2. of the Law of 20.07.2018. Law on Higher Education and Science (Journal of Laws 2018 item 1668 as amended) in terms of the doctoral student's broad general knowledge, ability to conduct scientific work independently, as well as the subject of the dissertation, which is the original solution of a scientific problem using the results of own research. The dissertation presented to me for evaluation describes scientific research at a high technical and substantive level. It represents an important contribution to the understanding of the mechanisms urmylation. I believe that the dissertation meets the conditions set forth in the Act on Scientific Degrees and Academic Title and on Degrees and Title in the Arts. I request that Keerthiraju Ethiraju Ravichandran be admitted to the further stages of the doctoral program. At the same time, taking into account the high substantive level of the dissertation and the Ph.D. candidate's publication success, I request that the dissertation be honored with an appropriate award.

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