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Review of Susheel Sagar Bhat Ph.D. thesis entitled " N6 Methyladenosine (m<sup>6</sup>A) and its writer mRNA adenosine methylase (MTA) are required for proper miRNA biogenesis in *Arabidopsis thaliana*."

The dissertation presented for review by Susheel Sagar Bhat was performed under the supervision of Prof. Zofia Szweykowska-Kulinska and dr Dawid Bielewicz at the Institute of Molecular Biology and Biotechnology of the Faculty of Biology of Adam Mickiewicz University in Poznan.

The regulation of gene expression at the posttranscriptional level is complex and involves many proteins and assemblies. One of the most exciting aspects of this process is the regulation of mRNA stability by small RNAs called microRNAs (miRNAs). These are short about 20 nucleotide fragments of RNAs, which, by interacting with target mRNAs, regulate their stability and translation. Another very interesting aspect of posttranscriptional regulation are chemical modifications of RNA molecules such as the deposition of methyl groups into bases, known as epitranscriptomics. This dissertation focuses on the connection between the RNA N6 Methyladenosine (M6A) formation and miRNA biogenesis.

### **Evaluation of the results obtained**

M6A is the best-studied epitranscriptomics modification of mRNA molecules or, more broadly speaking RNA species transcribed by the RNA polymerase II. In recent years it was shown that this modification is evolutionary conserved and affects several aspects of mRNA metabolism. This study is based on the hypothesis that M6A may also affect miRNA biogenesis in a model plant species, *Arabidopsis thaliana*.

To study the connection between the miRNA metabolism and M6A, Susheel Bahtr used a viable hypomorphic mutant of the catalytic subunit of the M6A methylating complex, MTA. RNA-seq experiments, combined with comprehensive RT-qPCR analysis, revealed that several miRNAs are downregulated in MTA mutant. This was accompanied by the accumulation of precursor molecules, which was a very strong indication that M6A modification affects miRNA biogenesis. Immunoprecipitation experiments with anti-M6A antibody provided evidence for the methylation of miRNA precursors suggesting a direct involvement of this modification in the pathway. Indeed, experiments performed by collaboration partners revealed that M6A modification affects secondary structures formed by the miRNA precursors enhancing their stability, which should accelerate there



processing by DCL. This gives a mechanistic explanation for the observed accumulation of miRNA precursors in the MTA mutant.

Then the Author asked how the methylating complex is recruited to miRNA primary transcripts. The protein-protein interaction analysis based on yeast-two-hybrid, immunoprecipitations, and microscopic studies revealed that the methylation complex interacts with the miRNA processing machinery as well as other proteins connected to co-transcriptional processing of RNA. This is a solid suggestion that M6A is deposited co-transcriptionally, which in turn recruits miRNA processing machinery.

In the final part of the results sections, the Author is looking for physiological consequences of deregulated miRNA biogenesis in MTA mutant. Experiments based on transient transfection of *Nicotiana bentamina* leaves revealed that M6A regulated miR393b biogenesis plays a role in the regulation auxin response, which is one of the physiologically relevant signaling pathways in plants.

Generally, the results presented in the dissertation are very interesting from a scientific point of view and broaden our knowledge about miRNA biology and the role of epitranscriptomics modifications in plants. The experiments are well-planned, and appropriate controls are performed. The Author used a variety of different experimental technics and approaches, thanks to which this dissertation provides a deep insight into the phenomenon studied. The story is complete as it presents an observation, provides the mechanism and its physiological consequences. This is very rarely seen in PhD dissertations. It should be noticed that a large fraction of the results presented in the thesis is already published in the prestigious journal, *Proceedings of the National Academy of Sciences of the United States of America*, where it undergone a comprehensive peer-review (This was a direct submission without a sponsor). Moreover, although the paper was published just a month ago, it was already cited 5 times. This proves that the results described in this outstanding dissertation are timely and interesting to the broad scientific community.

Although I have a very good opinion about this thesis, there are some issues that can be discussed. First of all, the exact positions of M6A modifications in miRNA precursors were not determined, which slightly diminishes the trustability of the results. I would be happy to hear some discussion of this aspect during the defense. The Author used various approaches to study interactions involving M6A depositing complex. The list of potential interactions is quite long. Are all those proteins real, physiologically relevant, interaction partners?

#### **Assessment of the dissertation itself.**

This dissertation has a classical layout and consists of an introduction, a chapter describing methods, results, and discussion. The bibliography contains 150 citations. The work is written in clear, understandable English language.

In the introduction, the Author introduces to the reader to the subject of the studies describing miRNA biogenesis and epitranscriptomics regulation in plants in the context of the knowledge acquired using other model systems. It is very well written.

The Materials and Methods section is sufficiently comprehensive and concise at the same time, and I have no critical comments to it.

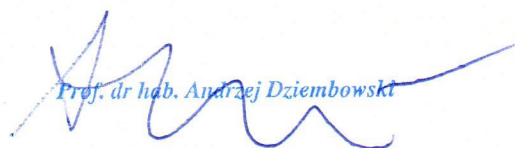
The results are described in a very clear and logical way. The figures are also very well prepared and informative.

The discussion of the obtained results is comprehensive. It proves that the PhD student is able not only to plan the experiments properly and apply appropriate techniques to their execution but also to interpret the experimental data correctly, compare it with the results obtained by others and propose the probable mechanism of the examined phenomena. What is important, the discussion is written thoughtfully and takes into account the current knowledge in the field, which indicates the scientific maturity of the PhD student.

### Conclusions

I conclude that the dissertation of Susheel Sagar Bhat entitled "N6 Methyladenosine (m<sup>6</sup>A) and its writer mRNA adenosine methylase (MTA) are required for proper miRNA biogenesis in *Arabidopsis thaliana*." meets the requirements for a doctoral thesis.

I, therefore, recommend proceeding to the further stages of the doctoral defense. Taking into consideration the exceptionally high quality of the dissertation reviewed, I propose to award the Autor.



Prof. dr hab. Andrzej Dziembowski