Prof. dr hab. Bartosz Jan Płachno
Instytut Botaniki, Wydział Biologii
Uniwersytet Jagielloński w Krakowie

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Review of the doctoral dissertation by Mr. Arash Matinahmadi, M.A.
entitled "*Post-Transcriptional Regulation of mRNA Mediated by Decapping and Degradation Proteins in the Cytoplasm and Processing Bodies of Larix decidua*"

General description

The doctoral dissertation of Mr Arash Matinahmadi was prepared at the Department of Cellular and Molecular Biology under the supervision of Professor Dariusz Jan Smoliński (Nicolaus Copernicus University in Toruń) and Professor Mehrdad Hashemi (Tehran University of Medical Sciences). The thesis is presented in the form of a monograph and comprises the following sections: abstracts in Polish and English, introduction, hypotheses and objectives, materials and methods, results, discussion, conclusions, perspectives, references, and supplementary materials. Several chapters are further subdivided into clearly defined subsections. The dissertation also includes detailed information on research funding and a list of publications authored or co-authored by the doctoral candidate.

The dissertation consists of 174 pages and contains 35 figures, including diagrams, tables, and high-quality photographic plates. The reference list comprises 232 literature items, reflecting a thorough engagement with both classical and contemporary studies relevant to the subject matter.

From a formal standpoint, the dissertation has been prepared with considerable care. The scientific language is generally precise and appropriate for an academic work of this level, although minor stylistic and syntactic improvements could be made in some passages. Occasional inconsistencies occur in the formatting of Latin names, particularly with respect to the use of italics and capitalization in the text and reference list. Importantly, the scientific documentation, especially the confocal microscopy images, is of exceptionally high quality and significantly enhances the clarity and credibility of the presented results.

It should also be emphasized that the doctoral candidate has successfully obtained research funding from multiple sources, demonstrating both initiative and the ability to compete effectively for financial support.

Scientific and substantive value of the dissertation

The research topic addressed in this dissertation is timely, original, and of clear scientific

importance. Processing bodies (P-bodies) constitute a central component of cytoplasmic RNA metabolism, being involved in mRNA decapping, degradation, translational repression, and cellular stress responses. Despite extensive research in model organisms such as Arabidopsis thaliana and yeast, many aspects of P-body function, dynamics, and developmental relevance in plants remain insufficiently understood.

In the introduction, the doctoral candidate provides a broad and well-structured overview of post-transcriptional gene regulation, mRNA turnover pathways, and RNA granules, with particular emphasis on P-bodies. The literature review is comprehensive and demonstrates a solid command of the field. Key conceptual frameworks and unresolved questions are clearly identified. Nevertheless, the introduction would benefit from a more extensive discussion of plant species other than *Arabidopsis thaliana* in which P-bodies have been investigated, thereby placing the chosen experimental system in a broader comparative context.

The candidate formulated three principal research objectives:

(1) to determine whether P-bodies form and function as active sites of mRNA decapping and degradation during the five-stage poly(A) cycle, with particular attention to the contribution of decapping to late-stage poly(A) shortening;

(2) to quantify changes in P-body abundance, size, and protein composition across developmental stages;

(3) to identify transcript populations associated with individual decapping components using RNA immunoprecipitation followed by sequencing, thus generating candidate substrates for further functional analyses.

These aims are clearly articulated, logically derived from the current state of knowledge, and scientifically well justified.

European larch (*Larix decidua* Mill.) was selected as the research model. The choice of microsporocytes of this species as a research model should be considered particularly good, both on the characteristics of the microsporocytes themselves and due to the long-standing expertise of the host research group (Department of Cellular and Molecular Biology) in studying this system. The applied research methodology is appropriate for the stated aims and based on modern techniques of molecular and cellular biology. The candidate employs, among others: confocal microscopy, immunocytochemical localization of P-body components, quantitative image analysis, experimental approaches allowing assessment of P-body dynamics. The methodological descriptions are detailed and enable reproducibility. The technical execution of imaging-based analyses is of a high standard and reflects solid experimental competence. The results are presented in a clear and logically organized manner, supported by

well-prepared figures and quantitative analyses.

The candidate's most important achievements:

- The candidate clearly demonstrated that P-bodies form and operate under physiological developmental conditions in *Larix decidua* microsporocytes, and are not restricted to stress-induced responses. Thus P-bodies are not stress granules here. He introduces an original five-stage model of the poly(A) RNA cycle and demonstrates its close temporal coupling with cytoplasmic mRNA decapping and degradation processes.
- He showed the dynamics of P-bodies and that P-body size classes are functionally relevant.
- Quantitative colocalization analyses and dynamic imaging demonstrated that P-bodies represent the main cytoplasmic hubs for decapping-dependent mRNA decay in the investigated developmental system.
- The candidate established that proteins involved in mRNA turnover are recruited to P-bodies in a reproducible, stage-specific order, allowing the proposal of a temporal framework for P-body function during diplotene.
- Based on imaging and biochemical evidence, the candidate proposed a stable decapping core composed of DCP5, DCP2, and EDC4, supported by more dynamic, stage-dependent components of the mRNA decay machinery.
- The application of RIP-seq enabled the identification of shared and protein-specific sets of target transcripts, indicating specific cellular pathways regulated at the post-transcriptional level.

I believe that the doctoral **candidate solved the original research problem** he set for herself. In addition, he demonstrated scientific maturity, the ability to work in a team, mastered both the skills of a molecular biologist and working with confocal microscopy, and was able to obtain funding for her research. Furthermore, the candidate is aware of what further research directions should be pursued in order to fully understand the role of P-bodies, which speaks very well of the candidate as a future independent researcher.

While reading the dissertation, several questions came to mind:

- In which plant species other than *Arabidopsis thaliana* have P-bodies been studied?
- Have different classes of P-bodies been found in *Arabidopsis thaliana*, and do they have a similar structure to those in *Larix*?

- Does the candidate not believe that the presence of P-bodies and dynamics should be checked in *Larix* in somatic cells, e.g., needle mesophyll and cells preparing for mitotic divisions?

In conclusion, I find that the doctoral dissertation of Mr. Arash Matinahmadi meets the conditions set forth for doctoral dissertations in accordance with the requirements specified in Article 187(1)-(2) and Article 190(3) of the Act of July 20, 2018. Law on Higher Education and Science (Journal of Laws 2024, item 1571) and I request that the esteemed Council of Biological Sciences at Nicolaus Copernicus University in Toruń admit the doctoral student to the next stages of the doctoral program.