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Review of the doctoral dissertation of Mr. Michał Antoni Mazur

1. Subject of the review

The doctoral dissertation of Mr. Michał Mazur, entitled *Exploring cytoplasmic polyadenylation: regulatory mechanisms affecting stability of endogenous and therapeutic mRNAs in hematopoietic cells*, was carried out under the supervision of Professor Andrzej Dziembowski (primary supervisor) and Dr. Monika Kusio-Kobiałka (auxiliary supervisor). The dissertation was submitted as part of the doctoral proceedings in biological sciences and conducted at the Laboratory of RNA Biology at the International Institute of Molecular and Cell Biology in Warsaw.

The dissertation examines how cytoplasmic polyadenylation by TENT5 enzymes stabilizes mRNAs in hematopoietic systems where transcription cannot compensate for RNA turnover. The work focuses on two biologically revealing contexts: the handling of SARS-CoV-2 mRNA vaccines by immune cells and the stabilization of endogenous transcripts during erythroblast differentiation. In these settings, the central aim was to determine how TENT5-mediated re-adenylation affects the stability and functional output of therapeutic mRNAs in macrophages and globin mRNAs in late erythroid progenitors, thereby defining the broader contribution of cytoplasmic polyadenylation to mRNA metabolism under transcription-limited conditions.

2. Assessment of originality and scientific contribution

The dissertation addresses a timely and significant problem in RNA biology: how cytoplasmic polyadenylation by TENT5 enzymes stabilizes mRNAs in systems where transcriptional input

is absent or severely restricted. This focus is both biologically compelling and medically relevant, and the candidate approaches it through two distinct hematopoietic contexts – macrophage uptake of therapeutic mRNA vaccines and globin mRNA regulation in late erythropoiesis.

The introductory chapter is broadly structured around cell biology foundations. It begins with an overview of hematopoiesis and immune cell function, then transitions to the biology of mRNA vaccines and the chemical modifications used to enhance their stability and translational performance. From there, it moves to principles of mRNA biogenesis and cytoplasmic metabolism, introducing the concept of cell type–specific regulation of RNA stability. This progression ultimately leads into the discussion of cytoplasmic polyadenylation and the role of the TENT5 family.

Conceptually, the introduction succeeds in laying out the biological background necessary to understand the work. However, the text would benefit from more careful editing. Several grammatical errors and vocabulary slips (e.g., misused connectives, inconsistent terminology, occasional misspellings) distract from an otherwise well-organized narrative. These issues do not undermine the scientific content, but they do detract from the professionalism of the written presentation.

The main scientific contribution of the dissertation is substantial. It is presented through two high-quality research papers – a published *Nature* study and a second manuscript under revision at *Nature Communications* – in which Mr. Michał Mazur is a second and first author, respectively. The work demonstrates that TENT5A/C-mediated re-adenylation stabilizes SARS-CoV-2 mRNA vaccines in macrophages, identifying lipid-associated macrophages as the dominant cell type responsible for vaccine retention and translation (accompanying unpublished data). In parallel, the dissertation reveals an unexpected ER-independent role for TENT5C in stabilizing cytoplasmic globin mRNAs during erythroblast differentiation, expanding the functional repertoire of TENT5 enzymes beyond previously known ER-tethered substrates.

Taken together, these findings represent a notable and original contribution to RNA biology. The dissertation advances our understanding of cytoplasmic polyadenylation in both therapeutic and endogenous settings, illustrating how TENT5-dependent stabilization mechanisms shape cellular function when transcription is not available as a buffer.

Paper #1 Krawczyk, P. S., **Mazur, M.,** Orzeł, W., Gewartowska, O., Jeleń, S., Antczak, W., Kasztelan, K., Brouze, A., Matylla-Kulińska, K., Gumińska, N., Tarkowski, B., Owczarek, E. P., Affek, K., Turowski, P., Tudek, A., Sroka, M., Śpiewla, T., Kusio-Kobiałka, M., Wesołowska,

A., […], Dziembowski, A., Mroczek, S. (2025). Re-adenylation by TENT5A enhances efficacy of SARS-CoV-2 mRNA vaccines. *Nature.* https://doi.org/10.1038/s41586-025-08842-1 and accompanying unpublished data.

The first publication included in the dissertation addresses a strikingly understudied question: how therapeutic mRNAs are metabolized inside the cells that take them up. Using direct RNA nanopore sequencing, the authors characterize individual mRNA vaccine molecules and reveal an unexpected regulatory layer governing their stability. They show that the Moderna mRNA-1273 vaccine carries a ~100-nt poly(A) tail appended with an mΨCmΨAG sequence that triggers rapid degradation in cell lines through removal of this modified tetranucleotide followed by CCR4–NOT–mediated deadenylation.

In medically relevant settings, most notably in macrophages, the behavior of the vaccine RNA is very different. Here, mRNA-1273 undergoes extensive re-adenylation by the TENT5A poly(A) polymerase, which can extend poly(A) tails to ~200 nt. This process was observed not only for mRNA-1273 but also for other synthetic mRNAs encoding ER-targeted proteins, including Zika and malaria antigens. The degree of re-adenylation depends strongly on spatial access to ER-associated TENT5A/C, explaining why the BNT162b2 vaccine shows comparatively weaker tail extension. Importantly, *in vivo* experiments demonstrate that loss of TENT5A reduces antigen-specific antibody production after mRNA vaccination, underscoring the functional relevance of this stabilization pathway.

Apart from the groundbreaking translational and molecular value, methodologically, the study is exceptionally strong. It integrates primary immune cells, tailored reporter constructs, inducible degradation systems (such as dTAG), RNAi models, and *in vivo* immunization coupled with full-length direct RNA sequencing and custom computational pipelines. The identification of lipid-associated macrophages (in the accompanying unpublished dataset) as the dominant vaccine-retaining cell type is intriguing and opens new avenues for understanding tissue-specific handling of therapeutic mRNA.

Scientifically, the work provides a clear conceptual advance: the stabilization of therapeutic mRNAs is not a passive consequence of their chemical design but an active, cell-type-dependent process mediated by TENT5A/C. The study establishes re-adenylation as a tunable determinant of vaccine efficacy and provides a roadmap for rational improvement of future mRNA therapeutics.

A few mechanistic points emerging from the data are especially thought-provoking, such as what ultimately terminates the initial wave of re-adenylation and shifts vaccine transcripts

toward degradation, and whether similar poly(A)-dependent mechanisms might influence viral mRNA metabolism during infection. These questions naturally extend from the present findings and highlight the depth and originality of the work. I propose to discuss these aspects during the PhD defence by the candidate.

Paper #2 **Mazur, M.,** Gumińska, N., Brouze, A., Cysewski, D., Mleczko-Sanecka, K., Niklewicz, M., Kusio-Kobiałka, M., & Dziembowski, A., Efficient globin production during terminal erythropoiesis depends on the synergistic action of TENT5C poly(A) polymerase and LARP4/5. Under review in *Nature Communications*.

This manuscript examines a biologically exceptional system: terminal erythropoiesis, where cells progressively shut down transcription and ultimately expel the nucleus, forcing them to rely entirely on residual cytoplasmic mRNAs. This creates a natural stress test for mRNA metabolism, and a setting where even subtle defects in deadenylation, translational control, or RNA protection become physiologically visible. Against this backdrop, the authors dissect the role of the cytoplasmic poly(A) polymerase TENT5C in maintaining globin mRNA stability.

Using a combination of TENT5C catalytic-dead knock-in mice, full knockouts, and comprehensive hematological and molecular profiling, the study demonstrates that loss of TENT5C activity results in microcytic, hypochromic anemia. The phenotype stems from progressive globin mRNA deadenylation during erythroblast maturation, culminating in a dramatic collapse of mRNA abundance in reticulocytes, precisely the stage at which new transcription is no longer possible. The work convincingly shows that TENT5C counteracts this erosion by re-adenylating globin transcripts, thereby sustaining hemoglobin synthesis during the final differentiation steps.

A particularly compelling aspect of the study is the identification of a transient but functionally essential cooperation between TENT5C and the RNA-binding proteins LARP4 and LARP5. Proteomic experiments (including TurboID-based proximity labeling) reveal that LARP4/5 protect globin poly(A) tails and facilitate TENT5C-mediated re-adenylation. Knockdowns of LARP4/5 mimics (to the varying degree) the globin mRNA destabilization seen in TENT5C-deficient cells. Particularly, LARP5 emerges as a main regulator with not fully delineated effect on the levels of TENT5C. This points to a finely tuned regulatory circuit that balances deadenylation and re-adenylation during erythroid maturation.

The study also uncovers an additional layer of regulation: TENT5C is highly unstable and is rapidly degraded by the CNOT4 E3 ubiquitin ligase associated with the CCR4–NOT deadenylase complex. This finding reinforces the idea that globin mRNA stability arises from

the interplay between stabilizing (TENT5C-LARP4/5) and destabilizing (CCR4–NOT–CNOT4) forces.

From a broader perspective, erythroid differentiation is intrinsically susceptible to disruptions in RNA metabolism, and this study offers a compelling mechanistic explanation for how poly(A)-tail maintenance safeguards globin production in a transcriptionally silent environment. One aspect that warrants deeper discussion is the observation that both erythroid and megakaryocytic lineages are compromised in TENT5C-deficient mice. While the manuscript attributes this primarily to a pro-inflammatory milieu, an alternative, and equally plausible, interpretation is dysfunction arising at the level of early multipotent progenitors, such as the multipotent progenitor (MPP2) compartment.

Another intriguing observation is the capacity of fetal and young animals to compensate for anemia through robust extramedullary erythropoiesis in the spleen, paradoxically accompanied by elevated red blood cell counts in TEMT5C-defective animals. This adaptive response raises longer-term questions: how sustainable is this compensation with aging, and does chronic reliance on stress erythropoiesis impose cumulative strain on the hematopoietic system? Persistent proliferative pressure could, in principle, predispose to bone marrow exhaustion or even malignant transformation. These broader physiological implications make the phenotype all the more compelling and worthy of future investigation.

Overall, this manuscript is a strong, technically sophisticated piece of work. It integrates challenging primary cell systems with high-throughput approaches such as Oxford Nanopore Technology direct RNA and cDNA sequencing and TurboID proteomics. The study significantly advances our understanding of how re-adenylation contributes to erythroid homeostasis and reveals previously unrecognized regulatory partners that determine TENT5C function *in vivo*.

3. Methodological Evaluation

The dissertation employs a strong combination of approaches, integrating primary cell systems, *in vivo* models, and sophisticated molecular assays to dissect RNA metabolism, with a particular focus on mRNA re-adenylation by TENT5 enzymes. The experimental design is robust, combining genetic models (knockouts, catalytic mutants, knock-ins), tailored reporter constructs, and cutting-edge technologies such as direct RNA sequencing (Oxford Nanopore) and proteomic proximity labeling (TurboID) to probe both the dynamics of poly(A) tails and protein–RNA interactions.

This methodological breadth allows for mechanistic insights into mRNA stabilization at unprecedented resolution, while simultaneously demonstrating translational relevance. In

particular, the work on SARS-CoV-2 mRNA vaccines provides *in vivo* validation that TENT5A-mediated re-adenylation enhances antigen production and antibody responses, highlighting a principle that is likely applicable to other therapeutic mRNAs. Overall, the methodological approach is rigorous, innovative, and well-suited to the research questions addressed, positioning the dissertation at the forefront of both basic and applied RNA biology.

4. Structure and style of the dissertation

The dissertation presents a coherent progression from general principles of hematopoietic cell biology to specific mechanisms of mRNA regulation, effectively linking organismal and cellular contexts to molecular processes. The chapters are organized to highlight both the physiological relevance of the systems studied and the mechanistic insights gained, particularly regarding TENT5-mediated mRNA re-adenylation. Detailed descriptions of experimental designs, primary cell models, *in vivo* studies, and high-throughput assays allow the reader to appreciate the rigor and scope of the work.

Nevertheless, the presentation occasionally suffers from avoidable issues, including typographical inconsistencies, uneven formatting, lack of referencing the figures in introduction, and minor language errors. While these do not compromise the scientific quality, they can impede readability and reduce the overall polish of the dissertation. Careful proofreading and typesetting would have strengthened the clarity and professional presentation of an otherwise outstanding piece of research.

5. Original publications

The dissertation is built around two major research articles: one published in *Nature* and a second under revision in *Nature Communications* at the time of submission. This publication record speaks for itself and reflects the high scientific quality, originality, and relevance of the work to the RNA biology community. Beyond these cornerstone studies, the doctoral candidate has also contributed as a co-author to **three** additional peer-reviewed publications, further demonstrating a consistent level of research productivity.

6. Specific comments

This doctoral dissertation represents a coherent, intellectually engaging, and methodologically rigorous body of research. The publication of the main findings in leading journals of molecular and cellular biology underscores the high quality of the work, leaving little room for substantive

critique. At the same time, I would be interested in the candidate's perspective on several points, which I suggest for discussion during the defence following the presentation of the dissertation's key results:

- Both manuscripts highlight the impact of TENT5-mediated re-adenylation in contexts of minimal *de novo* transcription. To what extent might these findings be generalizable to other transcriptionally silent systems, such as the maternal-to-zygotic transition? What is currently known about the roles of TENT5 or related enzymes in these contexts?
- Are there population-level differences in TENT5A or TENT5C expression or activity in humans, and could such variability contribute to differential responses to mRNA vaccines? Could targeting mRNA vaccines more specifically to the endoplasmic reticulum enhance re-adenylation efficiency?
- In the analysis of poly(A) tail length dynamics in endogenous transcripts following mRNA-1273 vaccination, there appear to be distinct waves of re-adenylation (e.g., early for "cell activation" versus later for "translation"; Manuscript #1, Fig. 3a) that coincide with differential expression of TENT5 family members. How might cis- and trans-acting regulatory features of the mRNAs contribute to this temporal pattern?
- Single-cell profiling of infiltrating cells after mRNA-1273 or BNT162b2 vaccination shows that lipid-associated macrophages (LAMs) accumulate the most vaccine mRNA and over time express increased levels of TENT5C with comparatively stable TENT5A. The paper does not directly compare the effects of TENT5A versus TENT5C knockdowns. Could there be cell-type specificity in the functions of these two enzymes? (Figure 5)
- How can the apparently low stability of TENT5C be reconciled with the requirement for high levels of this enzyme in transcriptionally inactive erythroblasts and reticulocytes to maintain globin mRNA stability?
- What are the potential determinants of substrate specificity and functional differences between LARP4 and LARP5 in their interactions with TENT5C? How might these differences shape the stabilization of particular mRNAs during erythropoiesis?

These questions are solely intended to guide and stimulate the scientific discussion during the defence.

7. Concluding remarks

In summary, the doctoral dissertation by Mr. Michał Mazur presents an original solution to a scientific problem and has been prepared at a high substantive and methodological level. It meets all the requirements specified in Article 187, paragraph 1 of the Act of 20 July 2018 - Law on Higher Education and Science (Journal of Laws 2018, item 1668, as amended).

In view of the scientific quality of the dissertation and the strength of its contributions to our understanding of post-transcriptional regulation at both molecular and physiological levels, I strongly recommend the thesis to be **accepted** for the subsequent stages of the doctoral procedure. Moreover, given the exceptional merit of the work and its demonstrable impact, I support awarding the dissertation a **distinction**.

With best regards,

Maciej Cieśla
2025-11-30

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