

Review of the PhD Thesis

“Modulation of the endogenous MBNL1 expression via RNA activation (RNAa) as a novel therapeutic approach towards myotonic dystrophy type 1 (DM1)”

Author: Nikola Musiała-Kierklo

Supervisor: Prof. UPP dr hab. inż. Ewa StępniaK Konieczna

Institution: Adam Mickiewicz University Poznań / Poznań University of Life Sciences

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General assessment

The doctoral dissertation entitled “*Modulation of the endogenous MBNL1 expression via RNA activation (RNAa) as a novel therapeutic approach towards myotonic dystrophy type 1 (DM1)*” addresses a medically important and scientifically relevant topic in the field of molecular biology, RNA therapeutics, and neuromuscular disorders. The thesis focuses on the development and mechanistic characterization of a novel strategy aimed at upregulating endogenous MBNL1 expression using small activating RNAs (saRNAs) in the context of myotonic dystrophy type 1 (DM1).

The dissertation is based on two publications published in *Nucleic Acids Research*, including one original research article and one review paper. It is accompanied by an introduction, clearly defined aims, interesting preliminary data, as well as general conclusions and discussion. Since the publications are multi-authored, Nikola Musiała-Kierklo’s significant contribution to the work is clearly demonstrated. Together, these studies form a coherent scientific achievement combining broad theoretical expertise with innovative experimental investigation.

DM1 is a severe multisystemic disorder for which no effective causal therapy is currently available. The pathogenic mechanism involving sequestration of MBNL proteins by toxic transcripts containing expanded CUG repeats has been extensively studied. However, therapeutic restoration of MBNL activity remains a major challenge. In this context, the dissertation proposes an innovative and well-justified approach based on RNA activation (RNAa), targeting endogenous regulation of the MBNL1 gene rather than exogenous overexpression.

The dissertation demonstrates scientific maturity, methodological rigor, and critical interpretation of the obtained results. The work is logically structured, clearly written, and supported by up-to-date literature. The results presented in the thesis substantially contribute to the understanding of RNAa-mediated gene regulation and its therapeutic potential in DM1.

Scientific value and originality

The dissertation is based on two thematically coherent and scientifically valuable publications:

1. Musiała Kierklo N, Konieczny P, Plewka P, Jasiok A, StępniaK Konieczna E. *MBNL proteins in health, disease, and therapeutic applications. Nucleic Acids Research*, 2026.
2. Musiała Kierklo N, Plewka P, Jasiok A, StępniaK Konieczna E. *Promoter-targeted small RNA duplexes increase MBNL1 transcription and mitigate myotonic dystrophy-associated spliceopathy. Nucleic Acids Research*, 2025.

The first publication is a comprehensive and well-structured review article addressing the biology of MBNL proteins, their roles in health and disease, and potential therapeutic applications. The manuscript demonstrates a deep understanding of RNA biology, regulation of alternative splicing, and molecular

mechanisms underlying myotonic dystrophy. Importantly, the review critically summarizes current therapeutic strategies targeting MBNL dysfunction, thereby providing a strong conceptual framework for the experimental part of the doctoral thesis.

The second publication constitutes the core original research achievement of the dissertation. In this study, the Author demonstrates that small duplex RNAs targeting the genomic locus of *MBNL1* can effectively increase endogenous *MBNL1* transcription and partially reverse DM1-associated spliceopathy. This strategy, previously applied in the context of other diseases, is known as RNA activation using small activating RNA duplexes (saRNAs). The work combines mechanistic insight with translational relevance and represents an important contribution to the field of RNA therapeutics.

Particularly noteworthy are:

- the identification and validation of saRNAs targeting the P2 promoter of *MBNL1*;
- the demonstration of transcriptional activation of endogenous *MBNL1* at the pre-mRNA, mRNA, and protein levels;
- evidence that moderate endogenous upregulation is sufficient to improve alternative splicing defects associated with DM1;
- mechanistic studies indicating AGO2-dependent recruitment and involvement of RNAa pathway components;
- analysis of transcription factors MEIS1 and MEIS2 in saRNA-mediated activation;
- careful evaluation of specificity and possible off-target effects.

The obtained results substantially broaden the potential applications of RNAa-based therapeutic strategies and provide convincing evidence that controlled activation of endogenous *MBNL1* may represent a safer and more physiologically relevant alternative to exogenous overexpression approaches. The research findings were published in *Nucleic Acids Research*, a highly recognized journal in the field of molecular biology and nucleic acid research, and have already undergone a rigorous peer-review process, further confirming the scientific quality and international relevance of the presented work.

Methodological assessment

The research was conducted using a broad spectrum of modern molecular biology techniques appropriate for the objectives of the study. The Author demonstrates proficiency in experimental methodology and proper interpretation of the obtained results.

The methodological approach appears comprehensive and carefully planned. The experiments include:

- design and validation of promoter-targeting saRNAs,
- gene expression analyses at the pre-mRNA, mRNA, and protein levels,
- alternative splicing assays,
- mechanistic studies related to AGO2 and RNA polymerase II recruitment,
- investigation of transcription factor involvement,

- evaluation of off-target effects.

The obtained results are presented coherently and critically discussed in the context of current literature.

Formal assessment

The dissertation is carefully prepared and fulfills the formal requirements for a doctoral thesis. The text is clear, well organized, and written in appropriate scientific language. Figures and descriptions are informative and facilitate understanding of the presented data.

The bibliography is extensive and includes relevant contemporary literature related to RNA biology, DM1 pathogenesis, and RNA-based therapeutic strategies.

Critical remarks

Although the dissertation is of very high quality, several issues could be discussed further:

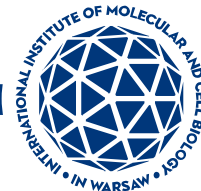
1. Although the molecular mechanisms underlying RNAa-mediated transcriptional activation remain less comprehensively characterized than those of more established gene-activation systems, such as dCas9-mediated CRISPR activation, the presented study provides important mechanistic insight into saRNA function. The Author demonstrates AGO2-dependent recruitment and involvement of RNAa-associated components in *MBNL1* activation, thereby substantially strengthening the mechanistic aspect of the work. Nevertheless, the precise molecular determinants governing saRNA specificity, efficiency, and long-term regulatory effects remain incompletely understood. Therefore, it would be interesting to hear a broader discussion during the defense on how the proposed mechanism compares with other endogenous gene-activation strategies and what challenges may still limit the therapeutic translation of RNAa-based approaches.
2. While approximately twofold activation of endogenous *MBNL1* appears biologically relevant, it would be interesting to discuss further the threshold of *MBNL1* restoration required for clinically meaningful improvement.
3. Future long-term studies evaluating the stability and safety of saRNA-mediated activation would further strengthen the translational significance of the proposed approach.

These comments, however, do not diminish the overall scientific value of the dissertation.

Final conclusion

In conclusion, I assess the doctoral dissertation of Ms. Nikola Musiała-Kierklo very positively. The thesis presents original, scientifically valuable, and methodologically sound research that substantially contributes to the field of RNA therapeutics and molecular mechanisms underlying myotonic dystrophy type 1.

The Author demonstrated the ability to independently design and conduct advanced scientific research, critically analyze experimental data, and formulate well-supported conclusions. Importantly, the presented findings may contribute to the future development of endogenous gene-activation strategies for neuromuscular disorders.



Therefore, I state that the dissertation fulfills the requirements for a doctoral thesis specified in the applicable regulations, and I recommend that Ms. Nikola Musiała-Kierklo be admitted to the subsequent stages of the doctoral procedure.

Considering the high scientific quality, originality, and potential translational significance of the presented research, the dissertation may also be considered for distinction.

Reviewer:

Prof. Andrzej Dziembowski

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Laboratory of RNA Biology

International Institute of Molecular and Cell Biology in Warsaw

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Department of Genetics & Biotechnology

Warsaw University