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Review of the PhD Dissertation

Title: Loss of Function Studies of Synapse-enriched circRNAs in Neurons
Author: Ayça Olcay, MSc.
Supervisor: Dr hab. Monika Piwecka, Prof. IBCH
Institute: Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań

General evaluation

The doctoral dissertation entitled **"Loss of Function Studies of Synapse-enriched circRNAs in Neurons"** addresses an important and rapidly developing area of RNA biology, namely the functional role of circular RNAs (circRNAs) in neuronal systems and synaptic regulation. Circular RNAs represent a class of non-coding RNAs characterized by covalently closed structures produced by back-splicing events and have recently emerged as significant regulators of gene expression in the nervous system .

The dissertation focuses on circRNAs enriched in neuronal synapses and investigates their potential role in synaptogenesis and synaptic stability. Given the increasing evidence that circRNAs participate in neuronal plasticity and neurological disorders, the topic of this work is scientifically relevant and well aligned with current research trends in molecular neurobiology.

The thesis is written in English and consists of a comprehensive introduction, description of aims, materials and methods, results, discussion, and conclusions. The work is well structured and includes extensive methodological descriptions and numerous figures illustrating the obtained results.

Scientific achievements of the PhD candidate

An important aspect of the evaluation of a doctoral dissertation is the scientific activity of the candidate and her contribution to the research field. According to the information provided in the dissertation, the candidate is the author of one published scientific article related to the topic of circular RNAs in neuronal biology:

Olcay, A. *Circular RNAs as a novel player in synaptic transmission and neuropsychiatric disorders.* **Neuropsychopharmacology** (2025 Jul;50(8):1195-1196. doi: 10.1038/s41386-025-02121-3. Epub 2025 May 6; PMID: 40328919).

This article appeared in a well-recognized international journal in the field of neurobiology and neuropharmacology. In addition, the candidate is a co-author of a manuscript currently in preparation concerning the functional role of synapse-localized circRNAs in mouse cortical neurons.

The candidate has also presented the results of her research at international scientific meetings, including an oral presentation at the 8th European Synapse Meeting in Coimbra, as well as several poster presentations at international conferences. Her oral presentation was recognized by the organizers through selection as a speaker and the award of a travel grant.

Overall, the publication and conference activity of the candidate is relatively modest but remains consistent with the stage of doctoral training and fulfills the formal expectations typically associated with doctoral studies.

Introduction and literature review

The introductory section provides an overview of circRNA biology, including their biogenesis, expression patterns, and potential regulatory functions. The author discusses mechanisms of circRNA formation such as back-splicing and exon skipping and summarizes current knowledge on circRNA roles in neuronal development and synaptic function .

The literature review also includes examples of circRNAs implicated in neuronal signaling, synaptic plasticity, and neurological disorders. This section demonstrates that the author is familiar with the current literature and successfully places the presented research within the broader scientific context.

Overall, the introduction is well written and provides a clear rationale for studying synapse-enriched circRNAs.

Aims of the study

The main aim of the dissertation was to investigate the function of selected circRNAs enriched in synaptic compartments of neurons. Based on RNA-seq datasets, sixteen candidate circRNAs were initially selected, from which three circRNAs were prioritized for detailed functional studies:

- circGigyf2
- circDtnb
- circMyst4

The study sought to determine their expression patterns, validate their circular nature, and investigate their potential functional roles in neuronal cells through loss-of-function experiments.

The objectives of the project are clearly defined and logically follow from the current state of knowledge.

Methodological approach

The experimental approach applied in this dissertation is appropriate and includes a combination of molecular, biochemical, and computational methods.

Among the techniques used are:

- RNA-seq data analysis for candidate circRNA selection
- RT-qPCR analysis of circRNA and mRNA expression
- RNase R treatment to confirm circular RNA structure
- Sanger sequencing of back-splice junctions
- shRNA-mediated knockdown experiments
- immunofluorescence and protein expression analysis
- in silico prediction of RNA-binding protein interactions and structural features .

The experimental procedures are described in sufficient detail and appear technically sound. The use of multiple validation approaches strengthens the reliability of the results.

Results and scientific contribution

The author demonstrated that the investigated circRNAs are highly expressed in primary neurons compared to astrocytes and show developmental regulation in the mouse cortex. Importantly, functional knockdown experiments revealed that silencing circGigyf2 leads to significant changes in the expression of genes associated with synaptic organization.

In particular, the knockdown resulted in decreased expression of transcripts encoding key synaptic proteins such as:

- PSD-95 (Dlg4)
- SynCAM1 (Cadm1)
- Telencephalin (Icam5)

as well as reduced levels of the Synapsin-1 protein .

These findings suggest that circGigyf2 may play an important regulatory role in synaptic stability and synaptogenesis.

Further computational analyses indicated the presence of a conserved sequence region within circGigyf2 and predicted interactions with RNA-binding proteins such as FMRP and TDP-43, suggesting a possible post-transcriptional regulatory mechanism.

The results therefore contribute to expanding the current understanding of circRNA functions in neurons and provide new evidence linking synapse-enriched circRNAs with regulation of synaptic gene expression.

Critical remarks

Despite the overall high quality of the dissertation and the interesting results presented, several aspects of the study could benefit from further clarification or experimental development.

Functional validation: The knockdown experiments performed in this study suggest that circGigyf2 may influence the expression of several genes associated with synaptic organization, including Dlg4, Cadm1, and Icam5. While these results are intriguing and indicate a potential regulatory role of circGigyf2 in synaptic biology, the functional consequences of these transcriptional changes remain somewhat indirect. Additional experimental approaches, such as electrophysiological measurements, analysis of synapse morphology, or quantitative assessment of synaptic density, could further strengthen the conclusions regarding the impact of circGigyf2 on neuronal function and synaptic plasticity.

Mechanistic insight: The dissertation proposes that circGigyf2 may interact with RNA-binding proteins such as FMRP and TDP-43, which are well-known regulators of neuronal RNA metabolism and synaptic translation. However, these interactions are currently based mainly on computational predictions and bioinformatic analyses. Experimental validation of such interactions—for example through RNA pull-down assays, CLIP-based approaches, or co-immunoprecipitation experiments—would provide stronger support for the proposed regulatory mechanism and would significantly enhance the mechanistic interpretation of the results.

Relationship between circRNAs and host gene expression: Since circRNAs are generated from precursor transcripts of protein-coding genes, an important question concerns the relationship between circRNA expression and the expression of their corresponding linear transcripts. Although this issue is briefly addressed in the dissertation, a more detailed analysis or discussion of the potential regulatory interplay between circRNAs and their host genes would be valuable. In particular, it would be interesting to consider whether the observed phenotypes could result from indirect effects related to host gene regulation.

In connection with the points raised above, I would like to ask the doctoral candidate to address the following issues during the public defense of the dissertation:

1. How does the candidate envision the molecular mechanism by which circGigyf2 regulates synaptic gene expression, and what experimental strategies would be most appropriate to test this mechanism (to be done in the future)?
2. To what extent could the observed changes in gene expression be explained by effects on the host gene Gigyf2, rather than by circRNA-specific regulatory activity?
3. What future experimental approaches would be most promising to determine the functional role of circGigyf2 in synapse formation and neuronal physiology?

These comments do not diminish the scientific value of the presented work, but rather indicate potential directions for future studies and provide topics for further discussion during the doctoral defense.

Although the experimental scope of the study is somewhat limited and the proposed mechanisms require further experimental validation, the dissertation presents original data and addresses a relevant biological problem. The work demonstrates the candidate's ability to design and perform independent research and therefore fulfills the requirements for a doctoral dissertation in the field of molecular biology.

Formal aspects of the dissertation

The thesis is well organized and written in clear scientific language. Figures and tables are generally well prepared and facilitate understanding of the presented results.

The bibliography is extensive and includes relevant and up-to-date publications in the field of RNA biology and neurobiology.

Conclusion

In summary, the dissertation by Ayça Olcay presents original research that significantly contributes to the understanding of circular RNA function in neuronal systems. The study identifies circGigyf2 as a potential regulator of synaptic gene expression and provides a solid experimental foundation for further investigations into circRNA-mediated regulation in neurons.

The work demonstrates that the doctoral candidate possesses the ability to design and conduct independent scientific research and to interpret experimental results critically.

Final recommendation

Taking into account the scientific value of the presented results and the overall quality of the dissertation, I conclude that the thesis meets the requirements for a doctoral dissertation as specified in the regulations governing doctoral proceedings at the Institute of Bioorganic Chemistry, Polish Academy of Sciences .

Therefore, I recommend that the dissertation be accepted and that Ms. Ayça Olcay, MSc., be admitted to the public defense of her doctoral thesis.

Przedstawiona do recenzji rozprawa doktorska spełnia warunki określone w Ustawie z dnia 20 lipca 2018 roku prawo o szkolnictwie wyższym i nauce (Dz.U. z 2018 r. poz. 1668 ze zm.) oraz w Sposobie postępowania w sprawie nadania stopnia doktora w Instytucie Chemii Bioorganicznej PAN w Poznaniu (uchwała Rady Naukowej ICHB PAN nr 40/2025/Internet/RN_140 z dnia 18 marca 2025 r.) i wnioskuję do Rady Naukowej Instytutu Chemii Bioorganicznej PAN o dopuszczenie ***mgr Ayça Olcay*** *do dalszych etapów postępowania o nadanie stopnia doktora.*

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