

Reviewer's Dissertation Thesis Report

Masaryk University	
Faculty of Science	
Doctoral Study Field	Life Sciences/Bio-omics
Student	<i>Ing. Tomáš Janovič</i>
Dissertation Thesis Title	<i>Human shelterin assembly and transcription regulation</i>
Reviewer	Mgr. Veronika Altmannová, Ph.D.
Reviewer's Workplace	Max-Planck Institute, Tübingen, Germany

The thesis of Ing. Tomáš Janovič is focusing on the study of the human telomere complex called shelterin, which is necessary to protect telomeres from DNA repair mechanisms. The dysfunction of this protein complex has serious consequences for the human organism and can cause, for example, premature aging or cancer. Thus, detailed elucidation of the function of shelterin is not only a key step towards understanding the mechanism of telomere protection, but can also be used for potential drug development.

Overall, the thesis is well written, and logically structured. A comprehensive theoretical introduction focuses on telomeres and the function of shelterin, with a detailed description of the current knowledge of the individual subunits of this complex. The candidate's ability to carry out scientific review, critically analyse relevant material and to compile the various information in a professional and comprehensible way is further demonstrated by his co-authorship of two review articles included in this thesis. One of these articles is devoted to the main topic of candidate's scientific work, i.e. telomeres and the shelterin complex. The second article focuses on completely different research area and gives a reflective view on the very current topic of oncoviruses, their regulation at the transcriptional level and potential use for the development of anticancer drugs or vaccines against viral diseases.

The theoretical introduction is followed by a summary of the clearly specified objectives of the thesis and a description of the key methods used during the research, which

in my opinion facilitates a better understanding and interpretation of the results of candidate's scientific work. The scientific results from two attached research publications on which the candidate is first author or co-author are summarised and commented on in a separate chapter. Since his first-author paper was published in 2019, the candidate further reflects his own data and the latest findings from telomeres research in the "Discussion" chapter and sheds light on the possible future directions. The thesis only lacks the supplementary data to the scientific articles, which I think would be useful to include for a comprehensive overview of his work.

The candidate's contribution to all published papers, which is described in one of the chapters, clearly demonstrates his versatility and ability to perform a wide range of biochemical, biophysical and microscopic techniques. From my point of view, the essential technique that helped the candidate to achieve the stated scientific goals was fluorescence cross-correlation spectroscopy (FCCS), which allows the study of fluorescently labelled proteins at the single molecule level. The candidate was able to fully master this challenging technique and apply it to the study of the shelterin complex formation. He managed to provide the first description of the interaction of shelterin subunits using the single-molecule technique, which demonstrates the uniqueness of his work. He also used this technique to investigate the effect of deuterated water on detection of different fluorescent dyes in various samples. In general, fluorescent labels are widely used in scientific research and improving fluorescence detection, especially under *in vivo* conditions, can thus significantly improve the study of cellular processes.

I have the following questions for the discussion:

- 1) In the theoretical introduction (page 37) you mention that there are two variants of TIN2 protein - variant S and variant L. What is the difference between these variants in terms of e.g. structure or function? Which of these variants did you use in your experiments?
- 2) On page 43, you state that shelterin is in cells most of the time disassembled. How is the formation of a functional shelterin complex in cells activated?
- 3) In Figure 3 (Janovič et al. 2019 JMB) you show that TRF2 does not affect the binding of the TIN2-TRF1 complex to DNA. Considering that TRF2, like TRF1, has the ability to bind DNA, wouldn't you expect a rather additive effect leading to increased DNA binding? Have you also tried to test the DNA binding of the TRF1-TIN2-TPP1-TRF2 complex on the same DNA substrate?
- 4) You mention several times in your thesis that TPP1 protein is the weakest point in shelterin formation due to its lowest binding affinity to TIN2 when compared to the interactions between other subunits of shelterin (see Figure 3 in the review by Veverka et al. 2019). Since the results published in your first-author paper clearly show that this binding is an essential step to form the TRF1-TIN2-TPP1-TRF2 complex, is it possible that the interaction between TPP1 and TIN2 proteins in cells is stimulated by some additional factor that would increase the binding affinity of these two proteins?

5) Based on your experience, what are the biggest advantages and disadvantages of fluorescence cross-correlation spectroscopy in comparison to other methods used to study protein interactions? What other method would you choose to confirm the results that TPP1 changes the conformation of TIN2 to allow binding of TRF2 and subsequent formation of the TRF1-TIN2-TPP1-TRF2 complex?

In summary, Tomáš has written an outstanding thesis and he has clearly demonstrated the ability to perform independent and creative scientific work, explain his ideas and present the results in a clear manner to the scientific community. I recommend the thesis for defence without any hesitation and wish the author all the best for his future career.

Conclusion

In the dissertation thesis, the student has demonstrated his/her creative abilities in the relevant research area:

YES ☒

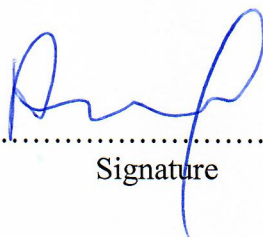
NO ☐

I am recommending the dissertation thesis for defence:

YES ☒

NO ☐

Date 18.4.2022


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Signature