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HABILITATION THESIS REVIEWER'S REPORT

Masaryk University	
Applicant	Mgr. Petra Procházková Schrumpfová, Ph.D.
Habilitation thesis	Functions of Plant Proteins Associated with Telomeric Repeats and Telomerase
Reviewer	Prof. RNDr. František Marec, CSc.
Reviewer's home unit, institution	Biology Centre CAS, Institute of Entomology, České Budějovice

The habilitation thesis is based on a compilation of 18 scientific articles published in the period from 2004 to 2023. Of these, the applicant is the first author of 9 publications (in 4 of them also corresponding author) and corresponding author of 3 publications. It can therefore be stated that the applicant's contribution is significant in two thirds of the publications included. Her expertise is also reflected in the fact that 3 of the first-author publications are reviews summarizing the latest findings and advances in telomere research.

The entire topic of telomeres, telomeric repeats, telomerase and proteins associated with telomeric repeats is covered in detail in the habilitation thesis in about 60 pages, including references. This part of the thesis is logically organized into several sections and subsections covering all important aspects of telomere research. These include the structure and function of telomeres, telomere-associated proteins and their role, if known, interstitial telomeric sequences, the structure of telomerase and the mechanism of telomere maintenance, as well as the telomerase-independent mechanism of telomere maintenance, the so-called alternative lengthening of telomeres. The text is supplemented by 12 schematic illustrations that provide a better understanding of the relatively complex structures and mechanisms. Although this thesis focuses on plants, the applicant always provides general information and comparisons of findings on plants with other organisms, especially mammals including humans. Almost every (sub)section also presents results from the applicant's own publications or from publications of her colleagues, mostly obtained on the model species *Arabidopsis thaliana*, which further illustrates the outstanding contribution of her workplace to progress in plant telomere research.

While reading this thesis, I learned a lot about telomeric repeats and proteins in plants, which helped me clarify a number of questions about their function. Some information was completely new to me. For example, I was very intrigued by the information that the telomere repeat binding (TRB) proteins identified in *Arabidopsis thaliana*, AtTRB, have a similar affinity for binding typical plant and human telomeric motifs, which could facilitate adaptation to a change in divergent telomeric motifs. This information could guide research in arthropods where such a change has already occurred several times. Another very interesting piece of information for me was that many short ITSs, called *telo*-boxes, are localized in promoters of protein-coding genes, where they might be involved in transcriptional regulation of genes.

Final note: I would venture to disagree with the author's statement in the Conclusion that “the protein interactome associated with plant telomeres and telomerase is not as well-studied as the mammalian telomeric proteome”. Reading this thesis, I was quite surprised by the depth of knowledge in this field of research, which was achieved in plants with the significant contribution of the applicant and her workplace.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

(1) As mentioned on page 22, the TRFL (TRF-like) protein subfamily I is probably responsible for specific binding to plant telomeric DNA proteins in vitro. However, there is so far no functional evidence for its role at telomeres, which is surprising. What evidence is there that these proteins are actually telomeric proteins? Are they colocalized with plant telomeres?

(2) Do you have a hypothesis about the origin of the long interstitial telomeric sequences (ITSs) in plants? I know from the literature that the occurrence of ITSs can be correlated with evolutionary changes in karyotype, such as chromosome fusions, inversions, unequal crossovers, etc. We have previously mapped ITSs in some insects and our results suggest that ITSs reflect remnants of multiple chromosome fusions of ancestral chromosomes.

(3) In section 3.1. on telomerase (page 32), there is interesting information from Gomez et al. (2011) about the tendency for animal species smaller than 1 kg to have long telomeres and active telomerase, while species larger than 1 kg have short telomeres and suppressed telomerase activity, apparently in somatic tissues. Do you have a hypothesis or is there any hypothesis why this dependence on size or weight exists?

(4) Section 3.1.4 on telomerase assembly (page 38) states that “the enzyme telomerase is recruited to telomeres rather than simply encountering them by diffusion” because telomerase and telomeres are present in the nucleus in low abundance. That sounds logical, but as a non-protein person I cannot imagine how a telomere can recruit telomerase. Could you explain this to me?

(5) I like Figure 1 in the review by Procházková Schrumpfová et al. (2019). This figure illustrates very clearly the differences in telomerase activity in human and plant tissues. The lack of telomerase activity in mature sperm is not surprising, but in mature oocytes it surprises me a bit. Do you know what it looks like in developing spermatocytes and oocytes?

Conclusion

The habilitation thesis entitled “Functions of Plant Proteins Associated with Telomeric Repeats and Telomerase” by Petra Procházková Schrumpfová fulfils requirements expected of a habilitation thesis in the field of Genomics and Proteomics.

Date: 15 January 2024 Signature: