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Annex No. 10 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

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. Ing. Petr Smýkal, Ph.D.
Reviewer's home unit, institution	Department of Botany, Faculty of Science, Palacky University, Olomouc, Czech Republic

The topic of the thesis focuses on the ends of the chromosomes, named telomeric DNA repeats, protective ends, very specific sequences whose integrity is important for cell divisions, complete chromosome replication, chromosome localization and meiotic chromosome pairing and life altogether and in more details to the proteins associated to these repeats.

The thesis is based on selected 18 publications where the applicant is the first or co-author, focused on the study of telomeric repeats and interacting proteins in plants. It clearly shows the growing knowledge through the quality and frequency of publications. It profits from a longer time focus on the topic. Moreover, the quality of the work is demonstrated by the number and quality of citations including leading journals. Many of these papers have high IF and are influential in the field of cell biology and plant sciences. Besides the members of the home lab including the former mentor prof. J. Fajkus, there is a list of leading national and international scientists involved in the studies, illustrating the skill of scientific communication and collaboration, both essentials for nowadays science. The team at MU and CEITEC led by prof. J. Fajkus, has been working on plant telomeres for over 20 years and has become world experts. Petra Procházková Schrumpfová has demonstrated that although she grew up in this environment, she was able to find her path and further contribute to this field.

All this knowledge, contributed significantly to our understanding of the function and maintenance of plant telomeres and the function of associated proteins, comparably less studied than in animals. On the other hand, plants offer more diverse scenarios, and this is also true in the telomeres and the associated machinery. Perhaps because they are sessile, plants have evolved robust DNA repair mechanisms and a high tolerance to genome instability. This resilience is visible in *Arabidopsis* mutants devoid of telomerase or proteins implicated in

chromosome end protection. Thus telomere-related mutations that are lethal in mammalian cells and even yeast can be viable in *Arabidopsis* and other plants. In contrast to the telomere sequences, the telomere proteins evolve rapidly and the thesis provides a detailed description of all protein families.

To investigate the interactions, there is a broad range of employed biochemical and molecular techniques, such as protein expression and purification, electrophoretic mobility shift assay, yeast two-hybrid assay, co-immunoprecipitation, and more sophisticated ones as bimolecular fluorescence complementation, tandem affinity purification, chromatin immunoprecipitation and of course the analysis of DNA sequences too. Moreover, in the course of projects applicant has been involved in various other methods including the characterization of protein localization using microscopic techniques, the investigation of plant gametogenesis, and bioinformatic analysis. The latter led to the development of a new software tool, Gene RegulatOry ELEMents (GOLEM), to precisely localize the distribution of telo-boxes motives in the vicinity of the Transcription Start Site (TSS) and Translation Start Site (ATG). All these characterize the methodological skills used to approach the given topic from various angles.

The recognition of the applicant´s knowledge can be seen by being one of the main organizers of the successful event - Czech Plant Nucleus Workshop in 2021, which put together over 80 researchers and students working in the field of plant nuclear and chromosome biology in the Czech Republic.

The habilitation thesis is written on 66 pages, including extensive literature (14 pages). I must say that it is a bit pitty that the applicant has not expressed a bit more general view on the importance of telomere during the evolution and including being speculative, as in my view habilitation provides a unique opportunity for this.

Altogether, the thesis clearly demonstrates the knowledge of the topic and through her scientific work, Dr. Petra Procházková Schrumpfová has proved that is an excellent plant molecular biologist. In the presented body of work, she has confirmed that is able to (i) work scientifically at an excellent level even when solving methodologically very complicated problems, (ii) to process the results of his work and publish them effectively.

Reviewer's questions for the habilitation thesis defence

- Telomere length shortens with age. Progressive shortening of telomeres leads to senescence, apoptosis, or oncogenic transformation of somatic cells, affecting the health and lifespan of an individual. On the other hand, plants are often referred as a kind of immortal organism, living for millennia or even perhaps "indefinitely" when propagated in vitro. How are the telomeres and their maintenance mechanism under these circumstances?

- What about the effect of distant hybridization and often linked polyploidy occurrence in plants? How does this affect the function of telomeres and respective binding proteins? Is this affected by the age of the event? Furthermore, what about the comparison of plants differing in genome size and/or chromosome numbers? The smaller 2n=4 versus giants of 2n=1260.

- It was shown that in mammals, telomere shortening correlates with between-species differences in lifespan. What about in plants? When comparing annual and perennial species, perhaps even within the genera. What about the very long-lived plants, like sequoia? Do they have different telomeres when young compared to old ones?

- Similar question, inspired by some of the applicant's work. How it is in the case of lower plants, e.g bryophytes and ferns, how they manage telomers and associated proteins? Especially when compared sporophyte and gametophyte.

- Nowadays, one of the hot topics in biology, in general, is the influence of stress on the organism and cell. What about the effect of stress on telomere, telomerase and associated proteins?

Conclusion

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

In Olomouc 25 January 2024 Signature: