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Supervisor's statement

Doctoral thesis entitled "Identification and characterization of flagellar tip proteins in *Trypanosoma brucei*" by Mgr. Hana Pavlisková

Mgr. Hana Pavlisková belongs to the very first generation of my PhD students, and hence also to very first members of my laboratory. This meant a significant delay for her experimental work, as first Hanka had to help me setting up the laboratory. In this task Hanka was very instrumental, as she is an orderly person and feels responsible for the laboratory, readily participating in housekeeping and maintenance.

The flagellar tip is an essential region of the organelle but it has been little understood as a limited number of tip-specific proteins have been known. Hanka's main project focused on identification and subsequent characterization of flagellar tip proteins in *Trypanosoma brucei*, the first flagellated organism with known cellular localization of nearly all proteins encoded in its genome. Initially, the number of flagellar tip proteins was unclear with me expecting a maximum of 25 proteins. However, as the project proceeded more and more tip proteins were identified, totalling nearly 80 proteins. This is a substantial number for characterization even in such highly experimentally tractable organism as *T. brucei*. Yet, Hanka was very dedicated to the project wanting to include all of these proteins in her study and experimentally finish the entire project to be able to include the results in her PhD thesis. Such a large-scale project required a methodical attitude (for example over one hundred cell lines were generated and 50 depletion cell lines were characterized in a complex way) and Hanka turned out to be the right person. In addition to her being systematic she is a very diligent scientist and the quality and reproducibility of data produced by her are superb. Thanks to this, the project yielded numerous exciting findings, which for the first time revealed the true complexity of the flagellar tip in a eukaryote, the extent of its evolutionary conservation, novel functions of some of the proteins in respect to the axonemal construction, as well as highlighted the role of the flagellum in morphogenesis of a trypanosome cell. Finally, one of the proteins appears to have an essential role in the life cycle of the parasite, initiating an entirely new project in the laboratory, the results of which are not included in the thesis.

Importantly, Hanka was also involved in several other projects, which introduced important reagents and approaches facilitating her main project but also used by a wider community. Hanka is the first author of the publication introducing the PCR only based overexpression approach, which will be critical for the future characterization of biochemical activities of the identified tip proteins. She is the second author of the publication describing changes to the flagellar length and morphology of trypanosome cells post cytokinesis and a co-author of the publication establishing a powerful super-resolution approach known as expansion microscopy for studies of kinetoplastid cells.

Towards the end of her PhD Hanka became rather independent in designing and performing experiments and analysis of results. Moreover, she has an excellent knowledge of literature related to her projects, enjoys thinking about how her results fit into what has been known in the field and is in general passionate about science.

To summarize, during her doctoral studies Hanka demonstrated that she became an independent scientist, capable of designing experiments, obtaining data of a very high quality, performing data analysis, interpretation of results in the context of the current knowledge in the field, and preparation of data for scientific publications. The results obtained during her PhD formed a part of several publications with another manuscript containing novel and exciting data getting close to submission. For these reasons I strongly recommend Mgr. Hana Pavlisková for the PhD degree.



Mgr. Vladimír Varga, PhD

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