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Evaluation of the PhD thesis of

Mgr. Juraj Sekereš

with the title

“Functional characterization of plant EXO 70 exocyst subunit isoforms and their membrane targeting mechanisms”

Herein please find my very positive evaluation of Juraj Sekereš PhD thesis. The work had clear focus on a single topic, the exocyst complex. A wide variety of methods were applied and apparently mastered by Mr. Sekers. The scientific output is enormous and the published work already well recognized by scientists worldwide. Therefore, I am clearly in favor of awarding the PhD to Mr. Sekereš.

The PhD thesis of Mr. Sekereš is submitted as a cumulative thesis consisting of a book chapter and two review articles as the introduction, four articles and two manuscripts as the result section and a further manuscript of a review as a discussion. I read the thesis with pleasure, although I was familiar with some of the work already as most of it has already been published in top plant biology journals.

The introduction starts with a book chapter, written predominantly by Mr. Sekereš, that gives a very interesting insight into the history of cell biology ranging from the 19th to the 21st century concluding with a few ideas about the future of cell biology. The article is well written and shows that Mr Sekereš is not only interested in current research but also in the history of science.

The second review, written predominantly by Mr. Sekereš as well, gives a perfect introduction of the function of signaling lipids especially in the context of cell polarity such as in pollen tubes. This is of relevance as these signaling lipids are important regulators of membrane trafficking and in this context also in the regulation of the exocyst complex. This exocyst complex is the main focus

of the concluding review to which Mr. Sekereš contributed equally with two further authors. This review describes mostly results from non-plant species highlighting the need for research on the plant exocyst complex.

Both reviews are important for people in the field and have been well cited. Once again the participation in the writing of two reviews shows that Mr. Sekereš is not only working on his own subject in the lab but is also strongly interested in the scientific background. It is also positive that Mr. Sekereš appears to be well acquainted with the non-plant literature of the exocyst field.

The results section starts with one of the two main works of Mr. Sekereš, a first author publication in *Plant physiology* from 2017 which has already been well cited for being out only for a short time. Here, he investigates the very large protein family (22! Members) of EXO70 subunits by expressing all of them in tobacco pollen tubes. Especially the isoforms A1 and A2 showed interesting localization at the apical plasma membrane. In addition overexpression phenotypes were observed in some cases, even though the work stops here without going any deeper. Still, this manuscript clearly identifies the most interesting isoforms for further deeper studies and one of these follow-up studies was already performed as outlined below. Therefore, this work is definitely a big step forward for or understanding of the function of the exocyst complex in pollen tube growth.

This publication is followed up by a manuscript on EXO70A1 that clearly was identified as one of the most interesting members of the protein family in the first publication. Here, Mr. Sekereš contributed equally as one of two main authors. This manuscript has high potential, as it shows for example the interaction of EXO70A1 with other subunits of the complex and anionic lipids. Furthermore, EXO70A1 seems to be crucial for correct targeting of the whole complex. It is very positive that most of the times several lines of evidence are presented as for example for the interaction with lipids. In addition, the protein is not only investigated as a whole, but also domains are identified that are important for interactions.

Beside these two main works, Mr. Sekereš contributed to further interesting studies also in the exocyst field. This displays his ability to work in a team and also on projects that are not in his main focus. Mr. Sekereš appears to be a specialist on microscopy and on EXO70 proteins and his work in this area important for the whole group.

Critical remarks:

Paper 4:

1. It should have been tested if the membrane invaginations are coming along with excessive pectin deposition.

Paper 5:

2. The language could be improved in some places and also PIP_2 should be written $PI(4,5)P_2$ instead to avoid confusion with $PI(3,5)P_2$

3. One could have tested if co-expression of AtPIP5K5 or better NtPIP5K6 and EXO70A1 results in additive effects for example on pectin secretion or the reduction of tube growth.

4. It should be clearly stated from which species the PI4P 5-kiase was taken.

5. The method for tryptic digest and LC-MS/MS is not properly described.

Questions:

Paper 3:

1. The exocyst is better explored in non-plant than in plant systems. However, a lot of progress has been made in the plant field. What are the most interesting recent results in the plant field and where are the most striking differences between plant and non-plant systems?

Paper 4:

2. Do you think that EXO70A1/A2 and B1 mark different zones for different types of exocytosis.

3. What could be transported out of the cell that is important for polar tip growth?

4. Could the plasma membrane invaginations be caused by excessive pectin secretion?

5. Would there be a possibility to overexpress dominant-negative isoforms of the exocyst subunits in tobacco pollen tubes to simulate a knockdown? What other possibilities can you envision for example to find out the function of EXO70B1?

Paper 5:

6. The localization of EXO70A1 is not altered in the *pip5k1 pip5k2* mutant. Why do you think that is, especially considering the results of the other experiments?

7. How can a plant survive without correct targeting of the exocyst complex? What is downstream of this complex and why is it important but not essential?

As a final conclusion, it can be said that few PhD student can show the number of publications as Mr. Sekers. Albeit a longer than usual period of time was need to complete the PhD.