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Evaluation of PhD thesis "FUNCTIONAL ANALYSIS OF SELECTED EXO70 EXOCYST SUBUNITS IN PLANTS" by Zdeňka Kubátová

(1) Overall assessment of the thesis

The dissertation has been carried out on the topic of leaf trichome development in the model plant Arabidopsis thaliana, with a focus on the exocyst subunit EXO70H4 and its role in secondary cell wall deposition. The ground-breaking work describes a characteristic cell wall structure, the Ortmannian Ring (OR), that separates basal and apical parts of the trichome cell and depends on the presence and correct localization of EXO70H4. The thesis further demonstrates the requirement of EXO70H4 for localized callose deposition in the apical branches of trichomes, their silification and the local accumulation of heavy metals. Finally, the thesis addresses the mechanisms underlying the differential targeting and polar localization of EXO70H4 and EXO70 paralogs by providing evidence for specific affinities to certain, asymmetrically distributed phospholipids of the plasma membrane.

The thesis is excellently written and provides a comprehensive introduction, clear scientific questions and research aims, as well as a critical and forward-looking discussion of the findings in context to the literature. The experiments were carefully designed and executed and the quality of the data, in particular of the imaging data, is excellent. In three original studies (two co-first, one first author paper) and one review article - a remarkable output for a PhD project - Ms Kubatova could demonstrate her scientific skills involving experimental design, mastering a range of techniques and the careful interpretation of results. As evident from her contributions to the three original papers, Ms Kubatova has taken on increasing responsibility and reached a mature level as a scientist.

In its nature, the thesis remains descriptive in several aspects, which, however, should not be seen as a weakness. Ms Kubatova has made a number of ground-breaking discoveries and has thus laid the foundations for multiple future projects on the molecular mechanisms underlying the exact role of EXO70H4 within the exocyst complex, polar protein targeting in trichomes, as well as the links between callose, silica and heavy metal deposition.

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(2) Specific critical comments

The thesis is generally phrased very carefully and acknowledges shortcomings or pitfalls. I am, however, not yet fully convinced that the presented data "clearly show the influence of local distribution of specific phospholipids on the recruitment of EXO70 paralogs" (pg. 32). I agree that both lipid distribution and EXO70H4 localization appear to be linked, but based on the phenotypes described in Kubatova et al. 2019 it remains unclear to me who regulates who.
A second criticism is more of a technical nature: while the microscopic data are of very high quality, I am missing quantifications and statistical analyses in several figures. I am convinced, a more quantitative approach could even further improve the already excellent work and would help to reveal informative details such as variability of phenotypes or to identify even subtle differences between mutant and control.

(3) Questions to the defendant (to be asked at the defense)

- Can you design an experiment that would provide direct evidence for the phospholipid-mediated recruitment of EXO70H4?
- How could you find out if the OR is cause or consequence of apical -basal polarity and how can you test whether it acts as diffusion barrier for PM proteins?
- How do your findings apply to other organisms? Please explain the differences between the OR, septum formation in yeast and tight junctions animal epithelia.
- Please explain functions of anionic phospholipids in protein targeting in polarized cells. Give an example where such lipids are involved in transient partitioning of the plasma membrane under changing environmental conditions.
- How would you identify interaction partners of EXO70H4 as well as other cargoes besides GSLs for this specific exocyst complex?
- Do you have an explanation for the patterned deposition of callose? Which other systems of regular patterning do you know in plants (or beyond) and how can they be explained (Turing systems)?

(4) Concluding statement

I have no doubt that the presented thesis is suitable for awarding a PhD title to Ms Kubatova.

Dr. Guido Grossmann