Review of the Ph.D. thesis of Anamika Rawat:

"Role of the exocyst complex in growth and development of moss *Physcomitrella patens"*

Reading the Ph.D. Thesis of Anamika Rawat was a pleasant task. It is a very coherent piece that focuses on the role of the exocyst complex in cell biology and development of the model moss *Physcomitrella patens*. The thesis is of a very high quality; it is clearly written and contains an extensive but focused introduction, four experimental chapters, and a concluding discussion. Two of the experimental chapters have been published as journal articles in Frontiers in Plant Science and New Phytologist, and two manuscripts are ready to be published. Anamika Rawat is the leading author of two of the manuscripts, proving her crucial contribution to the presented work. It is, however, a pity that her contribution to the other two papers is not clearly specified. The presented work contains a lot of novel experimental material that Anamika Rawat had to create, and from my own experience with the moss model system I know how daunting a task creating knockout and transgenic moss lines is – unlike in the community of the Arabidopsis model system, one has to build everything from scratch when working with moss. She conducted a very careful and honest phenotypic characterization of her mutant lines, and described the results in a comprehensible way using appropriate statistical tools. Some of the images of the dividing moss cells are simply astonishing; illustrating that Anamika Rawat mastered the live imaging techniques. The results and findings are extensively discussed and placed into the context of contemporary literature. I appreciate that methods sections in the experimental chapters are very detailed indeed and precise. The results themselves are a crucial contribution to the cell biology of moss and to understanding the role of the exocyst complex in cell biology and development of plants. Therefore I enthusiastically recommend that Anamika Rawat is awarded a Ph.D. title based on the submitted thesis.

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In Kierling, Austria, 12th September 2017

Comments and discussion questions to the individual parts of the thesis

P3: In the introduction, the advantages of the moss model are listed. I would like to hear your opinion what are the downsides of this model system.

P4: is the Figure original, or a reference to the source is missing? The arrows and arrowheads mentioned in the legend are missing in the figure.

P5: “mosses rely completely on tip growth in their development”, but later in the thesis (p33) it is stated that the leaflet cells grow by diffuse growth. Is it the case?

P7 and elsewhere: The role of the exocyst and of exocytosis in polarity is stressed. In my opinion, while establishing and keeping polarity requires functional exocytosis, exocytosis is crucial not only for polarity, but is essential to keep the plasma membrane as such functional, and virtually all processes happening at the PM require exocytosis at some point.

P27 and further: the supplementary figures for this paper are not included, neither are the supplemental videos. Even though readers can find them online, I think they should have been part of the thesis.

This paper features really beautiful images of the WT and mutant lines. What I really miss though is a transcriptional reporter of EXO70.3d.

P31: the EXO70.3 clade contains only one Arabidopsis EXO70 – EXO70G1. What is the phenotype of the exo70G1 mutant in Arabidopsis? Are there any similarities to that of the moss exo70.3d?

P35: the fact that auxin application is unable to force the exo70.3d mutant chloronema into the caulonema cell fate is really interesting; the question is to what extent the filaments are unable *to look like* caulonema due to a lack of rapid tip growth. I do not think that the lack of caulonema appearance is due to a problem in PIN protein trafficking, because the mutants in the PM PIN proteins in moss switch to the caulonema fate extremely rapidly because of the lack of auxin transport out of the chloronema cells (Viaene et al., 2014).

P44 and further: Again, I think the paper really misses a SEC6 transcriptional reporter, and a native-promoter driven GFP-tagged SEC6 protein, that would be used to complement the mutant phenotype. One should not judge the strength of the SEC6 signal in different tissues of the moss body, because this is likely caused simply by the activity of the promoter used (p 50, 54). Maybe immunolocalization of SEC6 using the anti AtSec6 antibody could overcome this limitation? The SEC6-GFP shows only cytosolic localization. Have you tried to examine the PM localization using a high-resolution objective and a spinning disc or TIRF microscope?

This manuscript refers to supplemental movies, but these are not available in the printed thesis. A CD with the movies could be included to solve this issue.

P47 and figure S1d: it is not clear to me which of the bands correspond to the truncated version of SEC6.

P48 and figure 3: the images of the unfinished cell plates are just amazing. Can you comment on why the problem appears only in certain cell plates? What makes them different from the ones where cytokinesis appears more or less ok?

P52: the phenotype of the exo70.3d was more wt-like in ow temperature. Have you tested low temperature on the sec6 mutants?

P80 and further: as in the previous papers, I think a reporter that would show where and when the SEC3s are expressed is missing, as well as the complementation with the SEC3 GFP. The results with SEC3 binding to phospholipids is interesting, but misses further context in the manuscript.

I also think that the chapter "literature" is missing for the manuscript nr.4, there are several references missing in the final "literature" on p109.

P82 and figure 1: how was the sec3ab mutant selected? Figure 1 claims that both of the knockout constructs harbor the nptII cassette; I guess that is a mistake in the figure?

P83: the sec3 mutant protoplasts have troubles regenerating filaments and sometimes create "a tiny mass of callus". Did you have a look at the cell plates in these structures?

P83: What is known about the gravity sensing and response in the unicellular filament? That seems as a fascinating example of cell polarity.

I offer my copy of the thesis where some typographical errors are highlighted.