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Report on
The PhD Thesis

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

by

Anamika Ashok Rawat

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Anamika Rawat aimed to characterize exocyst complex in growth and development of moss *Physcomitrella patens.* Characterization of exocyst in plants presents a long term research programme in laboratory of her supervisor. The topic of PhD thesis offers original new way to analyze the role of exocyst complex in model moss *Physcomitrella patens* where expertise and experimental approaches used for study of exocyst in higher plants were successfully applied.

PhD thesis with 116 pages of the text is structured in a standard way to the part of Introduction, Aims of the Project, Results, Discussion and Conclusion. Chapter Introduction provides surrey and critical assessment of related work. Aims of the project are described clearly. In the Results analysis, design, implementation and interpretation of own data are provided. Critical assessment of own work is provided in Discussion and the data are discussed in detail with recent published data. The PhD thesis is written in good English which reflects advanced language skills.

The first part of the Results provides the data on evolution of the land plant exocyst complexes. Detailed advanced database search showed evolution of exocyst subunits and provides data on diversity of exocyst variants among land plants.

Next set of experiments aimed to characterize exocyst subunit EXO70.3d. Generation of EXO70.3d knock-out mutants and detailed phenotype analyses provided the data on role of EXO70.3d in growth and development of *Physcomitrella patens.* Results of phylogenetic and phenotypic analysis including auxin sensitivity test and detailed analyses of cell wall formation and cytokinesis pointed to an essential role of EXO70.3d in completion of the moss life cycle. Characterization of EXO70.3d function was not an easy task because of a functional redundancy of distinct Exo70 paralogues. Data were successfully published with Anemika Rawat being the first author.

Next aim was to characterize the phenotype of *Ppsec6* mutants and to determine role of PpSEC6 in *Physcomitrella patens* cell morphogenesis. Partial functional conservation of SEC6 exocyst subunit was shown using the full disruption of *PpSEC6* by targeted gene replacement or

mutants with partial deletion at the C´-terminus of the *PpSEC6* and by complementation with PpSEC6 and AtSEC6 cDNA. Phenotypic characteristics suggested that there are also moss specific molecular features of SEC6 which are necessary for life cycle to be finished. The PpSEC3 exocyst subunit is expressed mainly in sporophytes and its function in sporophyte development was shown using PpSEC3a and PpSEC3b knock out mutants and double mutant. Delayed response to mechanical stress of PpSec3 and conservation of interaction of SEC3 N-terminaly located PH-domain with phospholipids in moss PpSEC3A were shown.

My questions or comments:

Your data suggested role of exocyst in auxin signalling in *Physcomitrella patens*. Are you going to progress further in characterizing participation of exocyst in PIN recycling using tools you have developed?

Will you please summarize what are moss specific features of PpSEC6 observed in your experiments ?

As mentioned in Discussion PpSec6 mutants showed low response or resistance to cytokinins, what molecular mechanisms do you expect to be behind?

Minor comments:

Cells of Sec6 knock-out mutants showed severe cytokinetic defects, stopped dividing and necrosis was observed. Cell death as a consequence of aberrant cell division should be also mentioned.

Though it is clear from the context the term Yeast used repeatedly in the text of Introduction should be specify to S. cerevisiae or S. pombe.

In summary experimental data obtained during PhD studies contributed to understanding of subunits of function of Exocyst in moss *Physcomitrella patens*. Anemika Rawat has authored two full journal papers and two manuscripts which clearly demonstrate her research potential. Anemika Rawat is a student of demonstrated achievement and high potential, who is able to conduct independent research. I fully expect that she will continue at a good level research in her future scientific career. I recommend the PhD Thesis of Anemika Rawat to be submitted for defence.

Prague September 2nd

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