

Overall Statement

The exocyst complex is highly conserved platform essential for polarized secretion, polarity and morphogenesis in eukaryotes. After its discovery in yeast, numerous studies have been performed in fungal and animal cells. The group around Viktor Žárský and Fatima Cvrčková has discovered and characterization of the exocyst complex in plants. These three PhD Thesis represent further significant advancement in our understanding of this critical evolutionary conserved octameric complex coordinating targeting, tethering and polarized secretion of secretory vesicles in cells of Arabidopsis. Hana Toupalová has analyzed the AtSEC15b subunit and its interaction partner AtRABA4a, Edita Janková Drdová has studied roles of the exocyst in the polar auxin transport, and Matyáš Fendrych has focused on interactions of the exocyst complex with the plant cytoskeleton and impacts of these interactions on the plant cytokinesis and plant morphogenesis.

Review Report to the Thesis by Mgr. Matyáš Fendrych:

The interface between secretory pathway and cytoskeleton – the exocyst tethering complex and cortical cytoskeleton in plant cell morphogenesis

This Thesis has three main goals – to characterize plant formins and their roles in interconnecting cortical cytoskeleton with the plasma membrane, to investigate possible role of exocyst complex in plant cytokinesis, and to visualize individual subunits of the plant exocyst complex using high resolution microscopy. The core of his Thesis is provided by two published papers. In the first one, Matyáš Fendrych has joint first authorship with Michael Deeks on paper published in the Journal of Cell Science on plant formin AtFH4. The second one is the Plant Cell paper dealing with roles of the exocyst complex during plant cytokinesis, especially in the formation of cytokinetic cell plates. Both these papers represent some sort of landmark papers in these

particular fields of the plant cell biology. In the Journal of Cell Science paper, overexpressed of AtFH4 accumulated at ER and allowed co-alignment of microtubules with ER. Moreover, FH1 and FH2 domains, which are conserved in plants, nucleate F-actin; allowing AtFH4 to integrate microtubules and actin cytoskeleton with ER membranes. In the Plant Cell paper, Matyáš Fendrich is the first author and he also performed the most important experiments and investigations reported by this excellent paper. T-DNA insertional mutant *exo84b-2* was shown to have cytokinetic defects with leaf cells having numerous cell wall stubs. In addition, epidermis leaf cells showed often accumulations of vesicles containing fibrous electron-dense materials. Stably transformed EXO84b-GFP seedlings allowed analysis of cytokinetic cells which revealed that EXO84b-GFP signal was enriched within early cell plates. After completion of cytokinesis, EXO84b-GFP remained associated with young cell wall for some time. Detailed analysis of other exocyst subunits showed that also SEC6, SEC8, SEC15e, and EXO70A1 are enriched with the cytokinetic cell plate. Analysis of another viable exocyst mutant *exo70a1* documented that also this mutant has defective cell plate assembly. Intriguingly, larger portion of the aberrant cell plates in *exo70a1* cells showed empty spaces in their centres.

The final part of this Thesis deals with visualization of exocyst complex dynamics at the plasma membrane. This part is presented in form of shorter manuscript, obviously submitted (accepted). In this analysis, high resolution VAEM (Variable Angle Epifluorescence Microscopy) has been used to analyse dynamics of exocyst subunits localizations. This is promising approach to track individual secretory vesicles in their phases of approaching to/tethering at the plasma membrane. SEC6, SEC8, SEC15e, and EXO70A1 have been colocalizing together with vesicle markers at distinct spots, representing presumably individual vesicles. Experiments of cytoskeletal drugs showed that while the short-term cytoskeleton disruptions do not affect exocyst localizations, the long-term actin depolymerization resulted in changes of exocyst polarity and dynamics at the plasma membrane. The PhD Thesis is completed with short but very informative Discussion.

This PhD Thesis reports breakthrough cutting-edge data. It certainly fulfills all the relevant criteria for such Thesis and I am rather happy to recommend acceptance of this PhD Thesis.

Súhlasím.

František Baluška