

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 Fendrich 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 Edita Janková Drdová:

The secretory vesicles tethering complex exocyst and the auxin transport polarization

In her PhD Thesis, Edita Janková Drdová provided manuscript entitled '*The Arabidopsis exocyst complex regulates the recycling of PIN auxin efflux carriers and polar auxin transport*' in which she is the first authors, accompanied by 11 co-authors. Moreover, she included into her PhD Thesis reprints of three published papers (from 2003, 2008, 2010) in which she acts as a co-author. Obviously, her manuscript represent the most important part of the Thesis and, therefore, I will focus on this part of her Thesis in my report. This manuscript is well written and organized, and one can expect that it will be accepted (or perhaps already is accepted) in one of the best journal of plant sciences. The story is based on the *exo70A1* mutant line which is shown to be affected specifically in the rootward transport of auxin. Edita Janková Drdová

has used DR-5:GUS line to monitor endogenous auxin distribution after its local application at the root-shoot junction. Roots of the *exo70A1* mutant line accumulate smaller amount of PIN2-GFP into mobile BFA-induced compartments which do not colocalize with VHA1:GFP signal. Moreover, the PIN2 recycling was markedly slower in the *exo70A1* mutant line and also PIN2 transcytosis after longer (12 h) BFA exposures has not been observed. In addition, Edita Janková Drdová demonstrated that also recycling of the brassinosteroid receptor BRI1 is significantly slowed down. In conclusion, EXO70A1 exocyst subunit is important for the constitutive recycling of plasma membrane proteins and transcytosis of recycling PINs.

Finally, possible interacting partners for the EXO70A1 exocyst subunit, relevant for the rootward auxin transport, have been searched for and ROP-GTPase interacting protein ICR1 has been identified as a very strong candidate. Using the GFP-specific antibody, EXO70A1 coimmunoprecipitated with ICR1:GFP, but not with BRI1:GFP. Importantly in this respect, ICR1 was reported to interact also with SEC3 in previous studies and ICR1 is known to control PIN localization and auxin transport. Localization of EXO70A1 at the plasma membrane has been shown to require specific phosphoinositides (PIs) as fungal-based inhibitor of PI kinases wortmannin released not only EXO70A1 but also SEC8 and SEC10 from the plasma membrane at 66 μ M concentration applied for 90 minutes. I find this result very exciting and, together with the impact on lateral root cap separation, worth of further experimental studies.

In the final part of her PhD Thesis, Edita Janková Drdová has discussed comprehensively all these exciting new data with other papers on which she acted as co-author, as well as with other relevant literature. It is little bit pity that she do not stick here to the rootward/shootward terminology but uses apical/basal terminology (according to Jürgens/Friml); which makes harder to follow her nice discussion. Nevertheless, this is only minor point and this PhD Thesis certainly

fulfills all the relevant criteria for such Thesis and I am happy to recommend acceptance of this nice PhD Thesis.

Súhlasím.

František Baluška