ÉCOLE NORMALE SUPÉRIEURE DE LYON
15 parvis René-Descartes
BP 7000, 69342 Lyon cedex 07
Tel. +33 (0)4 37 37 60 00
Fax +33 (0)4 37 37 60 60
www.ens-lyon.fr

REPRODUCTION ET DEVELOPPEMENT DES PLANTES
46 Allée d'Italie
Bâtiment M5,
69364 Lyon cedex 07
http://www.ens-lyon.fr/RDP

JAILLAIS YVON
Equipe Signalisation Cellulaire et Endocytose (SiCE)
N° de téléphone 04 72 72 86 09
N° de fax 04 72 72 86 00
yvon.jaillais@ens-lyon.fr
http://www.ens-lyon.fr/RDP/SiCE

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Report on Juraj Sekeres thesis manuscript in order to obtain a PhD degree at the Faculty of Science of Charles University, Prague.

Juraj Sekeres did his graduate studies under the supervision of Dr Martin Potocky, in the Laboratory of Cell Biology from the Institute of Experimental Botany and Charles University, Prague. The aim of the laboratory is to understand the role of trafficking complexes and lipids in membrane organization in plants, including vesicular trafficking, cell polarity, development and environmental interactions. In this framework, Martin Potocky's group focuses on the interconnection between anionic phospholipids and the exocyst complex, an evolutionary conserved octameric trafficking complex involved in exocytosis and cell polarity. Juraj Sekeres work focused on one subunit of the exocyst complex, the EXO70 subunit, using two plant model systems: the *Arabidopsis thaliana* sporophyte and the growing pollen tube of *Nicotiana tabacum*.

The document is presented in three main chapters, including an introduction, a results sections and a discussion/conclusion. Each section is constituted of either published or soon to be submitted papers (3 in the introduction, 6 in the results section, and 1 for the discussion), including 3 review/perspective papers and 2 original research papers signed as first/co-first author by Juraj Sekeres.

The three papers of the introduction are extremely rich and introduce the topic of the thesis from a broad perspective, ranging from a historical perspective on the concept of a cell (paper 1), to the interplay between proteins and lipids for plant polarity across various scales (paper 2) and the connections between the exocyst, membranes and the cytoskeleton across eukaryotes (paper 3).

The experimental work focusses on the mechanisms that target the exocyst complex to various plasma membrane domains (or intracellular compartments). In particular, Juraj Sekeres investigated the localization and targeting determinant of one subunit of this complex, EXO70. EXO70 is a critical subunit of the exocyst complex in yeast and mammals, which is notably involved in plasma membrane targeting of the entire complex, via interaction with the phosphoinositide $PI(4,5)P_2$. In these organisms, EXO70 is a single gene, but in plants, there are many EXO70 isoforms, suggesting that each isoform could provide specificity in targeting the exocyst complex to various membrane domains. This hypothesis is tested in the first experimental paper of the thesis (Sekeres as first authors), using *Nicotiana tabacum* growing pollen tube as an experimental model. The main conclusions of this study are as follow:

- Different EXO70 members have distinct localization in pollen tube such as distinct PM area at the tip of pollen tube (EXO70A1/A2), the shank of pollen tube (EXO70B1), the inverted cone of secretory vesicles (EXO70E1b, EXO70C1a), the

cytosol or even the nucleus. This suggest that EXO70 isoforms have sequence specific localization determinant.

- Only pollen expressed EXO70s show distinct localization pattern, while homologues normally expressed in sporophytic tissues do not, when heterologously expressed in pollen. This suggest that EXO70 isoforms have cell type specific localization.
- Overexpression studies suggest that different EXO70 isoforms might have distinct functions in growing pollen tube.
- Certain EXO70 isoforms have mutually exclusive localization pattern, but localize, at least in part, in specialized PM domain enriched in certain anionic phospholipids (PA, $PI(4,5)P_2$) or endocytic components (dynamins).

The idea that some EXO70 isoforms could rely on the combination of particular acidic phospholipids for membrane targeting is then tested in the second paper of the experimental result section (Sekeres as cofirst authors). This paper focus on one EXO70 isoform, EXO70A1, that is a major expressed EXO70 isoform, and for which the corresponding single mutant has very strong developmental phenotypes in Arabidopsis. The main conclusions of this second study are as follow:

- The plant exocyst is composed of two modules that are loosely connected, notably by EXO70A1.
- EXO70A1 is required to target the core exocyst to the plasma membrane, but not to the cell plate.
- EXO70A1 interact with anionic phospholipids *in vitro* and this interaction is required for localization and function *in vivo*.
- EXO70A1 localization at the tip of pollen tube is regulated by PA/PIP2 combination, while its localization at the plasma membrane of root epidermal cell (outer polar domain) is dependent on PA/PI4P combination.

In the remaining papers, Juraj Sekeres is not a main author and contributed either genetic material and/or experimental expertise (notably in microscopy). These chapters mainly deal with the functional characterization of certain EXO70 paralogs in *Arabidopsis thaliana* in various developmental context.

The discussion is again presented as a review paper that will soon be submitted and signed by Juraj Sekeres as first author. This paper discusses the functional diversity of EXO70 isoforms in plants and in particular emphasis on how they could be differentially targeted to various membrane domains for specific function.

It is clear from the very first page that Juraj Sekeres is a first-rate investigator and thinker, extremely dedicated and excited about science. The document is very well written and illustrated throughout the manuscript. The number of publication is highly impressive and the fact that Juraj Sekeres signed so many of them as first authors (five in total) is a testament to his writing and experimental skills, as well as ability for independent thinking. Furthermore, it is obvious that Juraj has integrated a vast amount of literature on broad topics connected to eukaryotic cell biology. I was also quite impressed by the diversity of experimental approaches that Juraj used during his PhD, ranging from the use of several experimental systems, genetics, developmental biology, high-end cell biology, biochemistry, proteomic and molecular modeling.

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To conclude, Juraj Sekeres PhD document is among the top manuscripts that I had to evaluate. It leaves no doubt that he has acquired the necessary skills to be awarded his degree and to become a successful and independent scientist in the future.

Specific questions regarding the thesis:

- What are the implication of EXO70 diversification in plants? and what does that tell us on the similarities and differences of cell polarity regulation between plants and animals?
- To what extent EXO70 function(s) are linked to the exocyst complex and/or are independent of it?
- Can the candidate speculate on the molecular mechanisms mediating isoform-specific EXO70 differential targeting? What experimental system(s) could be used to tackle this question?
- Why EXO70 isoform need to be studied in their native cellular environments? and what does this observation tells us about the mechanisms of EXO70 targeting?
- What are the possible mechanisms used to form lipid domains at the PM? both in term of macrodomains and micro/nano-domains.
- Overexpression studies in pollen tube suggest a role for PI(4,5)P2 and PA in EXO70A1 PM targeting, but this is rather indirect. How would the candidate address this in a more direct way?
- Was there any dominant-negative effect upon EXO70A1$^{5K>E}$ overexpression?

Dr Yvon Jaillais