Subject: Evaluation of the PhD thesis work by Mgr. Matouš GLANC, entitled: Mechanisms of establishment and maintenance of PIN polarity in Arabidopsis

This thesis addresses mechanisms controlling subcellular distribution of members of a plant-specific PIN-FORMED (PIN) family of auxin transport proteins, aiming at unraveling pathways controlling polar targeting to distinct plasma membrane domains. Polarity is essential for virtually every aspect of plant development and numerous studies demonstrated mechanistic links between directional protein targeting and polarity establishment, associated with tissue/organ development. The subject of this thesis work therefore can be considered an extremely important topic in the plant sciences.

This monography is divided into distinct sections: Summary, Introduction, Results, Discussion, Conclusions, References and Appendix.

Introduction
Provides a clear and succinct introduction into the topic. In particular, Matouš methodically presents and evaluates findings from earlier studies and integrates them into a comprehensive picture of our current state-of-knowledge on regulation of PIN polarity establishment and maintenance. Specifically, known molecular players involved are introduced and their respective function is accurately explained.

Results
This section is divided into 4 sub-chapters describing major results obtained as a part of this thesis work. A central part of this thesis work dealt with the question, as to how PIN polarity might be (re-)established upon completion of cytokinesis. By generation of a set of sophisticated tools and techniques, Matouš studied polarity acquisition of root-specific PIN2. These experiments revealed, PIN2 polar targeting to the apical domain of post-cytokinetic root meristem epidermis
cells in dependence of WAG1 protein kinase. PIN2 phosphorylation by WAG1, thus might act as an essential switch in the regulation of polarity acquisition. Notably, basal-to-apical sorting processes as well as key components of the cytoskeleton are seemingly dispensable for PIN2 polarity acquisition in cells that have gone through cytokinesis. Perhaps the most intriguing aspect of this study is evidence for cell-intrinsic cues, governing PIN2 polarity establishment uncoupled from signaling events influenced by proximal cell (files).

Another sub-chapter addresses the function of so-called MAB4/MEL proteins that were suggested to function as PIN sorting/polarity determinants in some earlier studies. During his thesis, Matouš observed peculiar crosstalk between PINs and MAB4/MEL proteins. Specifically, whilst the latter are seemingly contributing to PIN polarity maintenance they appear dispensable for PIN polarity establishment. In fact, genetic and molecular analysis revealed that PINs themselves are required for MAP4/MEL targeting to the plasma membrane. This somewhat unexpected type of interaction seemingly depends on the phosphorylation status of PINs, uncovering a so-far unknown mechanism in the control of polarity acquisition in higher plants.

In yet another subchapter Matouš summarizes on his contributions to the characterization of molecular interactors of Clathrin Light Chain proteins. Two of these interactors, exhibiting quite some similarity to auxilins found in animals, were found to decisively influence the endocytic sorting of plasma membrane proteins in plant cells. Specifically, overexpression of AUXILIN-LIKE 1/2 was found to block this particular step in the sorting of PIN proteins. Further, polarity defects associated with variations in AUXILIN-LIKE expression, strongly support a scenario, in which clathrin-dependent endocytic sorting decisively contributes to the regulation of polar protein distribution at the plasma membrane.

Both, work on AUXILIN-LIKE 1/2 as well as analysis of PIN2 polarity establishment after cytokinesis, have been published in the meantime, and the corresponding articles are therefore part of this thesis section. Work on the roles of cytoskeletal components during PIN2 polarity acquisition, and experiments describing crosstalk between MAB4/MELs and PIN proteins are provided as manuscripts.

A brief Discussion section recapitulates and highlights key findings of this thesis work, trying to implement these results into existing models and concepts for polarity establishment in plant cells. Based on his observations, Matouš suggests the existence of some elusive 'Master Polarity Regulator', analogous to the situation in fungi/animals, conceptually acting upstream of cellular sorting events guiding polarly distributed membrane proteins to their sub-cellular domains. I am really looking forward to seeing this working hypothesis being tested by Matouš during the next steps of his scientific career.
Finally, the Appendix section lists additional published research from the Friml lab, with Matouš listed as a co-author.

This thesis work is of outstanding scientific quality, addressing some key issues in plant molecular biology, in general, and in the field of plant cell biology, in particular. Experimental approaches described within this thesis work employ state-of-the-art methodology, and are flawless with respect to experimental design, controls and interpretation. With this highly innovative and pioneering work, it will now be possible to address central questions of polarity establishment in higher plants. Clearly, Matouš’s work described in this thesis represents a major step forward in our understanding of basic cellular processes in plants, which is further underlined by a highly impressive list of publications, already at this very early stage of his scientific career.

Please feel free to contact me, should you like to discuss Matouš’s thesis work in further detail. I would be very happy to expand on my recommendation and wish Matouš all the best with his next steps in his very promising scientific career.

Best Regards!

Assoc. Prof. Mag. Dr. Christian Luschnig