EVALUATION OF THE PhD THESIS OF ROMAN SKOKAN**:**

The PhD Thesis of Mgr. Roman Skokan with the title '**The evolution of auxin homeostasis mechanisms'** (134 pages) will be evaluated in the following for content, methodology, bibliometric aspects. I attempt to give a concise and independent personal perspective, interpretation and criticism of the published work and discuss some future perspectives. The dissertation is a cumulative report and comprises **4 published papers**, where in two of them Roman Skokan serves either as first/join-first author. Another unpublished work is included, which will likely lead to a nice publication, too. The contributions of Mgr. Skokan are clearly defined. The **quality of original publications is clearly higher as usually reported** for a PhD Thesis (see bibliometric analysis) and therefor the publication record can be **evaluated as excellent**. This Thesis fulfills all requirement for a PhD Thesis according the strict rules of the Charles University in Prague.

Scientific content of the presented accepted publications

The central aim of the thesis is to understand the **evolutionary origin of phytohormone action**, in particular auxins. ***Kf*PIN** is a PM-localized auxin efflux carrier (Publication I, Skokan et al. 2019) when expressed in land plants, and localizes peripherally in *Klebsormidium,* a member of streptophyte green algae, related to land plants. Though *Kf*PIN did not behave identically to angiosperm PIN homologs in all aspects, the character of auxin transport into the apoplast was shown as a conserved feature of the PIN family in streptophytes. This is a very fruitful approach and brings new perspectives into this field of research; it has been known from earlier transcriptomic work, that phytohormone action is evident in different *Klebsormidium* sp. (e.g. Becker & Holzinger 2015, Comm Integr Biol DOI: 10.1080/19420889.2015.1059978; Hori et al. 2014, Nature Comm DOI: 10.1038/ncomms4978); but these authors relied on transcriptomic/genomic data, that give no direct evidence for the function of certain carriers; therefor studying the function of streptophyte derived PIN transporters is novel in the present research and therefor important for the understanding of these phytohormone carriers.

With the high number of available sequences, the conservation of these carrier proteins in streptophytes was studied (Publication II, Nishiyama et al. 2019). The ***Chara braunii*** *(Charophyceae)* genome project gave reason to investigate gene families known to be involved in auxin transport. More recently, detailed analysis of auxin transporter gene family's conservation in *Viridiplantae*, using available data from both chlorophyte and charophyte algae were undertaken. Relatedly, in a study focusing on two particular cysteine residues in *Arabidopsis* PIN2, these **residues were recognized to be conserved across land plants**, but NOT in the selected charophytes.

The evolutionary origin of auxin transport was nicely **summarized in a review article** (Publication III Vosolsobě et al. 2020), which appeared as invited publication in a special issue of J Exp Bot on Terrestrialization of streptophytes, edited by the reviewer in cooperation with H. Buschmann, Osnabrück Germany as an outcome of a Satellite Meeting of the Society of Experimental Biology in Sevilla Spain, 2019.

Challenges and resolutions during the research for this thesis

As pointed out the largest problem throughout these studies were found in the lack of established charophyte model organisms; there is an urgent need for such model organism which is increasingly recognized. There have been scares reports on molecular approaches in Desmids/Zygnematophyceae, but up to now only transient transformations work well in these organisms. The creation of model organisms that allow stable transformations are therefor of high priority. Previous attempts have been successful in the desmidiaceae – e.g. *Mougeotia scalaris*, *Closterium peracerosum-strigosum-littorale* Complex and *Penium margaritaceum*, as illustrated in the introduction of the unpublished research on

Closterium. Within the present study it was possible to create transformations by particle bombardment, however it was found difficult to get stable transformants.
From my perspective, the up to now unpublished research: '*In search for algal models: Auxin response in Closterium***'** is a very important approach. Ever since the nice work on transient transformations in *Mougeotia scalaris* published by Regensdorff et al. 2019 J Phycol Journa54, 840–849), I was hoping that these transformation experiments will continue in cooperation with H. Buschmann, which is apparently here the case. I see very high potential in these studies, as the authors managed to transform *Closterium* (previously stable transformants have been shown by Abe et al. 2011 (Plant and Cell Physiology 52:1676-85 DOI: 10.1093/pcp/pcr103), which will then allow to study mechanistically how the phytohormones act, and how their action may be different from higher plants; While a multicellular organism has much more complicated needs to orchestrate development and growth, also individual cells, as in the case of *Closterium* need to communicate their activities, eg. for sexual reproduction or in the event of sudden stresses etc. It can be expected that his work will be published in a good Journal and will then serve as a valuable contribution in search for individual model organisms from streptopyhte algae for future experimentation.
Possible Future directions and additional questions
The action of phytohormones has drastic effects not only on the communication of cells, but also intracellularly, as nicely pointed out by the experiments in *Closterium*. When it is possible to generate such strong malformations as elongated cells, a **drastic effect on the cell walls** is evident; by dissecting the different uptake/efflux mechanism of IAA, the precise and local effects on the cell walls and which components of the cell walls (e.g. cell wall remodeling proteins) are directly targeted can be studied. Moreover, the interaction of different phytohormones will be necessary to be studied; as in higher plants ABA and auxins work simplified as antagonists, these relations might be drastically different in green algae. Other possible questions might be: what is the role of phytohormones in interaction of different organisms like algae and fungi as found in the lichenizatioin process (e.g. Pichler et al. 2020, DOI: 10.1111/jpy.13032-19-279, where IAAs were abundantly present and exudated by all algal partners). These are just a few examples of potentially interesting questions, where the work from this dissertation gave the basis.
Summary of the evaluation of this PhD Thesis:
I) The formal analysis of the Publication organs (peer reviewed Journals) allows to judge the **very high quality** of the presented works: First-author: **Nature Plants** (IF= 13.3); Shared first author: **J. Exp. Bot** (IF=6.9); co-author on genome paper: **Cell** (IF=41.5); co-author: **Int. J. Mol. Sci.** (IF=5.9)

II) All of the mentioned Journals in the category Q1 of The Thompson Reuters Scientific Journal ranking list.

III) The contents analysis (see above) allows to conclude, that all raised research questions could be answered sufficiently and new and groundbreaking results were gained.

Due to this evaluation report, I suggest the **best available grade** (*summa cum laude*) for the presented PhD Thesis of Roman Skokan.

Assoc. Prof. Dr. Andreas Holzinger, University of Innsbruck, Aug 20, 2021