Plant Science and Biodiversity Centre

SLOVAK ACADEMY OF SCIENCES

PLANT SCIENCE & BIODIVERSITY CENTRE SAS

Nitra, September 9, 2024

To whom it may concern:

Report on the doctoral thesis of Mgr. Jan Martínek
'SPECIFIC FUNCTIONS OF ARP2/3 COMPLEX IN PLANTS'

I enjoyed reading the doctoral thesis of a Ph.D. candidate, Mgr. Jan Martínek, from the Department of Experimental Plant Biology, Faculty of Science, Charles University, supervised by Dr. Kateřina Schwarzerová. This Ph.D. study aimed to gain new insight into the function of the ARP2/3 complex and its different subunits and further elucidate their role in different aspects of plant biology, from pavement cell lobe formation to pollen tubes to autophagy. I consider this work both highly ambitious and technically challenging. The findings presented in this thesis represent a significant step forward in understanding this important multifunctional protein complex and regulation of actin cytoskeleton. The candidate published several important articles as a coauthor; he published one article in *Nature Plants* as a first author, in which he demonstrated the role of ARP2/3 in pexophagy, and one manuscript is in preparation.

The thesis is divided into standard parts: an introduction, aims, and summaries of the four papers published, plus a manuscript in preparation, discussion, and conclusion. The published scientific articles and manuscripts in preparation are included as attachments. My comments on separate items of the thesis:

Introduction: The introduction covers three main topics: the actin cytoskeleton, its regulation and biological roles, general aspects of the ARP2/3 complex, and the role of the ARP2/3 complex in plants. Although I consider the scope and details of the introduction adequate, I expected more figures. For instance, one paragraph deals with methods for actin localization in plant cells; at this place, including pictures of actin staining would be very informative.

Aims: The four aims of the thesis were ambitious, very clearly stated, and, in the end, were successfully achieved.

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Summarization of the included work: This part summarizes each paper's main results and provides a short discussion. Article 1 demonstrated the binding of the ARPC2A subunit to microtubules. Article 2 showed the labour division and genetic interaction between two types of actin nucleation factors in plants and their role in pavement cell lobe formation. In article 3, via CRISPR/Cas9 mutational analysis, the authors demonstrated the distinct roles of ARPC1 and ARPC3 subunits. The most significant paper of the candidate was published in *Nature Plants*, which shows that the ARP2/3 complex associates with peroxisomes to participate in plant pexophagy. Finally, a manuscript in preparation describes the role of ARP2/3 in pollen tube formation and the effect on pectin distribution in pollen tube cell walls of *arpc3* and *arpc5* mutations.

Discussion and conclusion: I praise the discussion as well-drafted and comprehensive. It integrates the candidate's work and published knowledge, explains some observations, and provides an inductive summary and conclusion. It covers some important aspects, such as the different functionality of subunits, the role of ARP2/3 in cell wall remodeling, and membrane dynamics, including endocytosis. However, I think the candidate could take some liberty to outline his vision and what he sees as the required steps to elucidate the unresolved questions. Including some integrative schemes of the known roles of the ARP2/3 complex in plants would also be an added value.

I have a couple of questions I would like to ask the candidate during the oral defense:

1) Article 1: The authors demonstrated that the ARPC2 subunit interacts with microtubules. Can you speculate about the possible relevance of this "cytoskeleton crosstalk"? specifically for cell wall-driven morphogenesis?

2) The precise mechanism of lobe formation in epidermal pavement cells is seemingly complex and not fully resolved. In 2020, Haas et al. presented a controversial model based exclusively on cell wall activities—differential formation of pectin nanofilaments. How would you integrate observations on ARP2/3 presented in this thesis and of Haas et al. 2020 in one model?

3) Article 2 shows that mutants in two types of actin nucleation factors in plants, formins and the ARP2/3 complex, exhibit opposite severe defects in pavement cell lobe formation; however, the rosette leaves of the respective mutants appear relatively normal. A more philosophical question: Why are epidermal pavement cells lobed?

4) Unlike mutants in all other complex subunits, the *arpc3* mutant has only a mild phenotype in vegetative organs. Besides the already mentioned possibility of retained functionality of a partial complex, can one speculate there is some kind of genetic redundancy involved? Or would you rule it out completely?

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5) In the last manuscript in preparation, the authors proposed that the changes in the pectin distribution in *arpc3* and *arpc5* mutant pollen tubes are due to compromised endocytosis of PMEIs at the subapical region. Is it possible that observed phenotypes and the effect on pectin distribution are due to the altered positioning and trafficking of some pollen-specific homogalacturonan synthesizing galactouronosyl transferases (GAUTs)?

6) Article 4 showed the involvement of the ARP2/3 complex in pexophagy, but what is missing is an exact mechanistic explanation of how it is established. What kind of experiments would the candidate perform to elucidate the mechanism?

7) What is the difference between the 2F4 and OGA488 probes?

8) Can new observations on ARP2/3 from plants be somehow extrapolated into animal biology? For instance, how can it be relevant to a cell wall-like extracellular matrix in some animal cells or peroxisome degradation in animal cells?

In conclusion, I consider this doctoral thesis to be of high quality, both in formal aspects and scientific content. It reaches high international standards. Despite a few minor shortcomings, I can endorse it for oral defense.

Sincerely Yours,

Mgr. Jozef Mravec, PhD

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