Report on doctoral dissertation by Mateusz Kwiatkowski

Thesis title: "Phosphodiesterases in higher plants – a missing link in cyclic nucleotide signal transduction"

This thesis reports on the design and implementation of methodology to identify proteins capable of incorporating phosphodiesterase enzymatic activity in plants. This is a highly important topic as until recently, such proteins have not been identified in plants and as the candidate argues, these proteins form an essential "off" component of intracellular cyclic nucleotide signalling.

The thesis begins with an excellent introduction to the topic. This introduction describes cyclic nucleotide signalling in plants and how use of precise measurement techniques such as mass spectrometry was required to overcome a general scepticism surrounding roles of these signalling molecules. The introduction then lays out the need for enzymes such as phosphodiesterases that break the cyclic bonds to act as "off" switches to return cyclic nucleotide signals to a ground or neutral state and assist in plant homeostasis. The introduction thoroughly includes early reports of phosphodiesterase activity in plant extracts to the recent discovery of a cGMP dependent phosphodiesterase in the model plant Arabidopsis. The introduction thus lays out the essential background and the knowledge gaps that are to be addressed in the aims of the study.

The outcomes of the aims are described in two published articles with the candidate as the first author that are appended at the end of the thesis. Surprisingly to the reader, a discussion intervenes before the abstract and these articles. I chose to read the articles before the discussion and conclusion.

The candidate should be congratulated on these two published articles that add considerably to the body of knowledge in cyclic nucleotide research. I only briefly comment on these articles as they have undergone the peer review process. The first article Kwiatkowski *et al*. 2021 in Computational and Structural Biotechnology describes how a tandem bioinformatic and biochemical approach can be used to reveal new moonlighting functions in proteins – in this case Arabidopsis K^+ uptake permease (AtKUP5). In the article, the bioinformatic identification of putative phosphodiesterase and calmodulin binding sites was used to create hypotheses that were elegantly tested using bioanalytical and molecular techniques adding to the known phosphodiesterase molecules in Arabidopsis. The second article Kwiatkowski *et al*. 2021 in the International Journal of Molecular Sciences makes use of alternate bioinformatic searches to explore the presence of phosphodiesterases in monocots using Brachypodium as an example and identifying a homologue of AtCN-PDE that is then functionally characterised. Identifying and characterising this phosphodiesterase activity, permitted the establishment of an extended search motif for phosphodiesterases that will be of considerable value in identifying these hidden cryptic regions in plant proteins.

The discussion summarises these strategies and the basis of developing the structural homology models to investigate feasibility of substrate and allosteric binding also shown in the publications and relates the findings back to existing data and how the candidate has extended knowledge. It is interesting that many other phosphodiesterases were identified in Brachypodium and one wonders what other functions these molecules have (known and or annotated/predicted) and how they may contribute to plant growth and development. This

matter is left hanging in the thesis but it should be touched on – perhaps in a section following the conclusions as these proteins could direct future hypothesis driven research. Another point that should be touched on is how the proteins in Brachypodium relate to those found in cereal crop plants and can they be possibly used to help identify analogues.

In summary, the candidate has developed a strong thesis about a very topical problem in plant biology and made important and original additions to the existing body of knowledge in this succinct and valuable dissertation. On the basis of this thesis, I have no hesitation in recommending the passing of this dissertation for the PhD degree.

Helen R Irving

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Below are some suggested minor revisions.

1. Include some subheadings to guide the reader in the introduction and discussion
2. Consider a sentence or two on role of dinucleotides in signalling in the introduction
3. Moonlight should read moonlighting (p9)
4. Check thesis for minor spelling errors (e.g. incompletes p 10, leucin p 16)

5. Consider incorporating summary diagrams in discussion to summarise key findings and to elaborate on rationale of future work.
6. How do phosphodiesterases in Brachypodium relate to yet to be identified phosphodiesterases in cereal crop plants?