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Annex No. 10 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University	
Applicant	Mgr. Jakub Harnoš, Ph.D.
Habilitation thesis	Prickle proteins in vertebrate neurulation and beyond
Reviewer	Dr Elias H Barriga, PhD
Reviewer's home unit, institution	Excellence Cluster Physics of Life (PoL) at Tu Dresden, Dresden, Germany.

The habilitation thesis Prickle proteins in vertebrate neurulation and beyond addresses the functions of Prickle proteins in vertebrate planar cell polarity signaling, with particular emphasis on neural tube formation. It combines an integrating commentary with three published papers: a review of vertebrate Prickle proteins, an experimental study linking planar polarity signaling to actomyosin contractility during neurulation, and an experimental study of PRICKLE3-dependent control of VANGL protein stability. The central proposition is clear and coherent, stating that in vertebrates, Prickle proteins should be understood as active and paralog-specific regulators of morphogenesis rather than only as passive components that stabilize polarity complexes.

The introductory synthesis gives the reader the necessary background in planar cell polarity, the structure and known functions of the Prickle family, and the principal cell behaviors that drive vertebrate neurulation. The discussion of apical constriction, convergent extension, cell intercalation, and actomyosin mechanics provides an effective bridge between molecular signaling and tissue morphogenesis. The thesis also places the work in a medically relevant context by discussing neural tube defects, while recognizing that mechanistic findings in model systems cannot by themselves establish clinical causation. This distinction was very much appreciated.

Study I, provides the conceptual foundation. It assembles a broad and previously fragmented literature on PRICKLE1-4, compares developmental and pathological functions, and identifies gaps in the understanding of paralog specificity, cytoskeletal coupling, and the turnover of planar cell polarity complexes. Its main value for the habilitation thesis is not a single experimental result, but the formulation of a research program that is pursued in the two subsequent studies.

Study II, supplies the strongest connection to neurulation. Using a combination of tools in cultured cells, and Xenopus embryos, the study shows that Prickle2 and Dishevelled2 associate with MLC9 and regulate localized myosin light-chain phosphorylation. The proposed Pk2/Dvl2-CK1ε-Rap1GAP2-Rap1-pMLC pathway offers a plausible mechanism by which planar polarity information can be converted into contractile output. The combination of loss-of-function, overexpression, localization, interaction, and phenotypic data makes this a good contribution to the field.

Study III, in a way, extends the argument from cytoskeletal output to receptor-complex stability. The work uses proximity labeling, biochemical assays, inducible and genome-edited human cell lines, and Xenopus material to show that PRICKLE3 protects VANGL1 and VANGL2 from CK1-dependent phosphorylation and RNF43-mediated degradation. The identification of a functional VANGL-binding motif in PRICKLE3 and the comparison with PRICKLE1 support genuine paralog specificity. This study therefore adds regulated protein turnover to the mechanisms through which Prickle proteins can control planar cell polarity signaling.

The three publications are thematically well connected and appear in peer-reviewed journals. The stated contribution record shows that the applicant was responsible for the research direction and supervision of Studies I to III. Although most experimental work was performed by collaborators and supervised researchers, the reported intellectual leadership, formulation of the research questions, and integration of the results demonstrate the scientific independence expected at habilitation level.

The thesis is logically organized, readable, and supported by useful schematics and an extensive bibliography. A final language and copy-editing pass would improve occasional colloquial phrasing, minor grammatical errors, and inconsistencies in terminology and citation formatting. These issues do not affect the scientific substance. Overall, the submitted work demonstrates a strong command of the field, presents original and coherent contributions to vertebrate planar cell polarity research, and documents the applicant's capacity to define and lead an independent research program.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

Beside the above-mentioned points, several points deserve discussion at the defence. In Study II, the evidence supports a functional link between planar polarity proteins and pMLC, but not every step of the proposed signaling chain has been established causally in the neural plate. Colocalization, protein interaction, pMLC levels, and neural tube phenotypes should be distinguished from direct measurements of force and from epistasis within the proposed pathway. In Study III, the evidence for PRICKLE3-dependent VANGL stability is convincing, whereas the functional state of the accumulated VANGL complexes and their direct activation of non-canonical WNT or planar cell polarity output remain as an open end. The thesis occasionally states these models more strongly than the available evidence warrants. Its relevance to human neural tube defects should likewise be presented as mechanistic and hypothesis-generating rather than as an immediate translational conclusion– as in the introductory part.

Some specific questions are:

1. Which experiment or sequence of experiments would most rigorously establish causality along the proposed Prickle2-CK1ε-Rap1GAP2-Rap1-pMLC pathway in the Xenopus neural plate? Please describe the rescue or epistasis tests and the quantitative mechanical readouts that would distinguish direct pathway control from secondary effects of altered morphogenesis.

2. Study III shows that PRICKLE3 stabilizes predominantly non-phosphorylated VANGL while limiting CK1-mediated VANGL phosphorylation, although VANGL phosphorylation has also been associated with activation and membrane trafficking. How do you reconcile these findings, and which direct assay of planar cell polarity output would determine whether the PRICKLE3-stabilized complexes are signaling-competent?

3. The thesis argues for paralog-specific Prickle functions, yet expression, redundancy, and tissue context remain incompletely resolved. What experimental strategy would you use to define the physiological division of labor among Prickle1-4 during neurulation, particularly where Prickle3 expression is low, and which observations would falsify the proposed paralog-specific model?

4. In the same vein, would Xenopus knockdown would be suitable for the dissection of redundancy and other paralog related effects? Would mutant approaches be more suitable? If so, why and what systems would the applicant use?

5. Final comment on future plans and what new avenues the applicant foresees in their research, teaching and self-administration vision(s). In other words, what is beyond “Prickle proteins in vertebrate neurulation and beyond”? The topic is interesting but can be limited considering a long-term tenure.

Conclusion

I do not have a large n number to compare with others at the same University and career stage, but from what I see, the habilitation thesis entitled "Prickle proteins in vertebrate neurulation and beyond" and achievements presented by Mgr. Jakub Harnoš, Ph.D. **fulfils** the requirements expected for a habilitation in the field of Animal Physiology.

Date: 08 September 2026 Signature: Elias Barriga Digitally signed by Elias Barriga Date: 2026.09.08 20:21:35 +02'00'