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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. rer. Nat. Peter Walentek
Reviewer's home unit, institution	Internal Medicine IV (focus Nephrology), Department of Medicine, University Freiburg Medical Center

Dr. Harnos presented a habilitation thesis work delineating his and his laboratories research into the roles of the Planar Cell Polarity (PCP) signaling pathway and specifically into the roles of the family of Prickle proteins. PCP proteins establish and maintain directionality cues in tissues by asymmetric sorting and stabilizing PCP protein complexes along the axis of polarity. On one side of the cell, Frizzled/Dishevelled complexes are positioned, while at the other end Vangl/Prickeld complexes reside. These complexes then establish trans-cellular/membrane contacts to neighboring cells for polarity information propagation and maintenance. Starting by a historical contextualization, Dr. Harnos described that Prickel functions were proposed to act as mere stabilizers of PCP complexes (based on Drosphila studies), but his investigations (written up in a comprehensive review that includes reanalysis of available disease datasets and information) indicated that Prickle proteins might be more functionally involved in PCP establishment and consequently also in PCP-dependent developmental processes, including convergent extension, cell intercalation and apical constriction. The clinical data analysis further indicated clinically relevant functions for Prickled in human development, including congenital defects called neural tube defects (NTDs), in which tube morphogenesis and closure fails and leaves neural tissue exposed. PCP promotes neural tube formation via convergent extension and apical constrictions, two cellular processes reliant on Acto-myosin contractility. Mediation of contractility regulation via PCP was previously shown to depend on Frizzled/Dishevelled posterior cell complexes that activate Casein Kinase, which in turn removes an inhibitor of the Myosin motor, subsequently allowing for localized reduction of membrane size and increased mechanical forces (e.g. required for neural tube morphogenesis). In the second study provided in the habilitation, the Harnos lab has demonstrated that Prickled/Vangl anterior cell complexes also regulate Casein Kinase activity (via interaction), inhibitor removal (via degradation) and promote anterior membrane contractility in neurulation of the frog embryo. This established a new axis of PCP-dependent regulation of the process. In the third study provided, the Harnos lab uses proximity labeling and proteomics to investigate interaction partners of 3 human Prickle orthologs (1-3). They discovered that common as well as ortholog specific interacting proteins exist, that the less characterized Prickle 3 has a Vangl interaction domain and that Prickle 3 interacts with

an E3 ubiquitin ligase to regulate Vangl degradation. Hence, in absence of Prickle 3, Vangl is degraded and PCP information in the cell is impaired. Together, the work presented here demonstrates novel and surprising roles for Prickle proteins in the regulation of PCP, Acto-Myosin contractility and developmental morphogenesis. The publications included are of high quality, combining various biochemical, proteomics, imaging and functional assays, as well as investigations using frogs, fish and mammalian cell lines. They are clearly written, conclusive and really beautifully illustrated with great care. The Habilitation text is also clearly written and provides both necessary context as well as clearly defined hypotheses and understanding of the open questions in the field with relevance to human health. Overall, the work is very good and the results provide important new insights.

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

Statement in the habilitation:
“In vertebrates, PCP signaling functions in a more diverse biological setting than in *Drosophila*. It does not only control polarity within (epithelial) tissues but also regulates processes such as collective cell migration, individual cell motility, and axon guidance.”

Question 1:
Does Dr. Harnos imply that there is no involvement of PCP in these processes in drosophila or other non-vertebrates? If so, what are the arguments? If not, please clarify.

Statement in the habilitation:
“Interestingly, in Study II, we showed that Prickle2 can trigger ApCo via phosphorylation of MLC, thus showing its active role in ApCo and NT formation.”

Question 2:
Other studies have implicated PCP components, including Wnt ligand overexpression, in apical constriction. What does Dr. Harnos think of these studies? E.g. is there any reason why in his own work, he has not used Wnt ligand overexpression to induce premature apical constriction in early gastrula embryos? It seems like a quick assay over plain background, so it seems curious that these are not used more widely in the PCP field.

Statement in the habilitation:
“One important regulator is Celsr, which promotes remodeling of cell junctions and thereby enables cell intercalation (Nishimura et al., 2012).”

Question 3:
I was under the impression that Celsr is also a prerequisite for PCP component sorting? Is that not true? It is also required in other PCP processes, but is not asymmetrically localized in cells.

Statement in the habilitation:

“Thus, actomyosin contractility represents the principal mechanical output of PCP signaling during vertebrate neurulation.”

Question 4:
I would argue that the principle output of PCP is giving cells directionality and that contractility of actin is just one of the various downstream effects. How does Dr. Harnos see this and what would his main arguments pro and con be to reach his conclusion?

Conclusion

The habilitation thesis entitled “Prickle proteins in vertebrate neurulation and beyond” by Mgr. Jakub Harnoš, Ph.D. **fulfils** requirements expected of a habilitation thesis in the field of Developmental and Cell Biology. I congratulate Dr. Harnos to his insightful work and habilitation.

Date: Freiburg, 11.09.2026 Signature: