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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	name and surname, including academic degrees
Reviewer's home unit, institution	Max Planck Center for Physics and Medicine, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany

Dr. Jakub Harnoš's habilitation thesis, "Prickle proteins in vertebrate neurulation and beyond," examines how members of the Prickle protein family function within planar cell polarity (PCP) signalling, with particular emphasis on their role in vertebrate neural tube formation. The central argument challenges the classical view, largely derived from *Drosophila*, that Prickle proteins are relatively passive components of PCP complexes. Instead, the thesis proposes that vertebrate Prickle proteins are active, paralog-specific regulators that connect cellular polarity to cytoskeletal mechanics and the dynamic organization of PCP signalling complexes.

The thesis first reviews PCP signalling, Prickle1–4, and the cellular mechanisms underlying neurulation, particularly apical constriction, convergent extension and cell intercalation. These processes require spatially coordinated actomyosin contractility, and the thesis asks how Prickle proteins contribute to translating PCP information into these mechanical cellular behaviours.

The experimental and conceptual core consists of three studies. Study I provides a systematic overview of vertebrate Prickle proteins and argues that the different paralogs have evolutionarily conserved but distinct functions in development and disease. It identifies major unresolved questions concerning paralog specificity, cytoskeletal coupling and regulation of PCP-complex stability.

Study II provides the principal mechanistic link between PCP and tissue mechanics. Using *Xenopus* neurulation, the work shows that Prickle2 regulates actomyosin contractility and apical constriction. Both depletion and overexpression of Prickle2 disturb coordinated morphogenetic behaviour. Mechanistically, the thesis proposes a pathway involving CK1ε-dependent degradation of Rap1GAP2, Rap1 activity and phosphorylation of myosin light chain (pMLC), thereby connecting PCP signalling to non-muscle myosin II activation and mechanical force production.

Study III extends this concept from mechanical output to regulation of the PCP machinery itself. It identifies a role for Prickle3 in stabilizing VANGL proteins, showing that Prickle3 protects VANGL from CK1-mediated phosphorylation and RNF43-mediated degradation. Thus, Prickle

proteins not only influence downstream cytoskeletal behaviour but also regulate the stability and dynamics of core PCP receptor complexes.

Overall, the thesis proposes a broader model in which Prickle proteins actively couple planar polarity to morphogenesis at two complementary levels: by controlling actomyosin-dependent mechanical activity and by regulating the stability of PCP complexes. This provides a mechanistic framework for understanding how PCP signalling remains functional during highly dynamic processes such as vertebrate neurulation and potentially helps explain how disruption of Prickle-dependent mechanisms contributes to neural tube defects.

The thesis concludes by pointing toward an additional, less explored direction: proximity data suggest that some Prickle isoforms may associate with mitochondria, raising the possibility that PCP signalling could coordinate cytoskeletal mechanics with cellular metabolism and energy production.

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

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1. Your thesis argues for a shift from viewing Prickle as a passive component of PCP to an active regulator of morphogenesis. What, in your view, is the strongest experimental evidence that Prickle is instructive rather than simply permissive for PCP-dependent actomyosin organization?
2. In Study II, both depletion and overexpression of Prickle2 disrupt neurulation. How do you interpret this bidirectional phenotype mechanistically? Does it suggest that the absolute amount of Prickle2 is important, or rather its spatial distribution, stoichiometry within PCP complexes, or dynamics?
3. You propose a Prickle2–CK1ε–Rap1GAP2–Rap1–pMLC mechanism connecting PCP to actomyosin contractility. What experiment would you consider the most decisive test that this pathway is responsible for the spatially polarized contractility required for neurulation, rather than simply changing global actomyosin activity?
4. The thesis emphasizes paralog-specific functions of Prickle1, Prickle2 and Prickle3. What determines that specificity? Do you think it primarily comes from expression patterns, distinct binding partners, subcellular localization, or intrinsic differences in the proteins themselves, and how would you experimentally separate these possibilities?
5. Study III suggests that PRICKLE3 protects VANGL from excessive CK1-dependent phosphorylation and RNF43-mediated degradation. Yet VANGL phosphorylation is also required for normal PCP function and trafficking. How do you reconcile these observations? Could Prickle3 therefore act as a quantitative “buffer” that defines an optimal range of VANGL phosphorylation rather than simply inhibiting it?
6. If you had to formulate one unified mechanistic model from the habilitation, how would you connect the Prickle2-dependent regulation of actomyosin in Study II with the Prickle3-dependent regulation of VANGL stability in Study III? Are these genuinely different paralog-specific functions, or could they represent different manifestations of a common role of Prickle proteins in controlling the dynamics of PCP complexes?

Conclusion

The habilitation thesis entitled “Prickle proteins in vertebrate neurulation and beyond” by Jakub Harnoš **fulfils** requirements expected of a habilitation thesis in the field of biology.

Date: 07.09.2026 Signature: Prof. Jakub Sedzinski