COMMENTARY TO HABILITATION THESIS

Prickle proteins in vertebrate neurulation and beyond

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Prickle proteins are evolutionarily conserved molecular components exclusively associated with planar cell polarity (PCP) signaling. This signaling pathway provides directional and positional cues to eukaryotic cells along the plane of an epithelial sheet, orthogonal to both the apicobasal and left-right axes. Through pioneering studies in the fruit fly *Drosophila*, we learned that PCP signaling is manifested by the spatial segregation of two protein complexes, namely Prickle/Vangl and Frizzled/Dishevelled. While Vangl, Frizzled, and Dishevelled proteins have been extensively studied, Prickle has been largely neglected.

This likely happened because of two main reasons. First, the Frizzled/Dishevelled complex was found as the main driver of bristle and hair formation in *Drosophila*. Second, the original phenotype of *Prickle*$^{-/-}$ flies did not look very dramatic. It showed "only" a few misaligned bristles and hairs, and therefore, was not considered biologically exciting at that time. This early view influenced the field and led to a long-term underestimation of Prickle proteins in vertebrates. As a result, their roles in vertebrate development and disease are still not fully understood. In particular, the function of Prickle proteins during neurulation – the first step in central nervous system formation – has remained insufficiently explored.

Neurulation is a complex morphogenetic event driven by a set of coordinated cell movements and cell shape changes, all mediated by the actomyosin contractile apparatus. Failure of this process leads to neural tube (NT) defects, which belong among the most frequent and clinically relevant congenital malformations in humans. Genetic mutations of Prickle have been associated with NT defects across multiple vertebrate species, including frogs, fish, chicken, mouse, and human. How is this possible? Well, Prickle has been repeatedly implicated in regulating these processes, yet the exact underlying molecular mechanisms remain incompletely defined. By integrating molecular, cellular, and tissue-level aspects – which is a key feature of my

work – the studies presented here aim to demonstrate that planar polarity is translated through the Prickle protein family into coordinated mechanical activity during vertebrate development.

This thesis is structured into three main parts. In the first part (pages 5-9), I summarize the theoretical framework necessary to understand PCP signaling, with a specific focus on Prickle proteins. In the second part (p. 10-24), a substantial portion of this thesis is devoted to general principles of neurulation, toward the specific molecular properties of Prickle family members in NT formation and defects. In the last part (p. 25-33), I present and discuss in more detail three original research articles that form the backbone of this work. These studies provide new mechanistic insight into how Prickle proteins regulate morphogenetic processes in vertebrates, particularly during neurulation.

Taken together, this habilitation thesis aims not only to summarize the current state of knowledge but also to redefine how we view the role of Prickle proteins in vertebrate morphogenesis.

I wish the reader an engaging reading experience.

In Brno,

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Jakub *"James"* Harnoš

List of included research papers and my specific contribution:

[1] Radaszkiewicz, K. A., Sulcova, M., Kohoutkova, E., & **Harnos, J.** (2024).
The role of prickle proteins in vertebrate development and pathology.
Molecular and cellular biochemistry, *479*(5), 1199–1221. https://doi.org/10.1007/s11010-023-04787-z

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
N.A.	**100**	**60**	**100**

Key conceptual contributions: Shift of paradigm – Prickle is not passive, but active and paralog-specific regulator in vertebrates.

[2] Novotna, S., Maia, L. A., Radaszkiewicz, K. A., Roudnicky, P., & **Harnos, J.** (2024).
Linking planar polarity signaling to actomyosin contractility during vertebrate neurulation.
Open biology, *14*(11), 240251. https://doi.org/10.1098/rsob.240251

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
5	**95**	**90**	**100**

Key conceptual contributions: Prickle2 actively drives neurulation – links PCP to actomyosin and pMLC in vivo.

[3] Radaszkiewicz, K. A., Radaszkiewicz, T. W., Kolářová, P., Paclíková, P., Gömöryová, K., Novotná, Š., Maia, L. A., Číhalová, T., Le, Y., Bárta, T., Hanáková, K., Hýsková, A., Tripsianes, K., Zdráhal, Z., Winkler, C., & **Harnos, J**. (2025).
PRICKLE3 protects VANGL proteins from CK1-mediated phosphorylation and RNF43-mediated degradation.
Communications biology, *9*(1), 142. https://doi.org/10.1038/s42003-025-09422-9

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
5	**95**	**85**	**85**

Key conceptual contributions: Prickle3 stabilizes Vangl – controls CK1/RNF43-dependent receptor degradation.