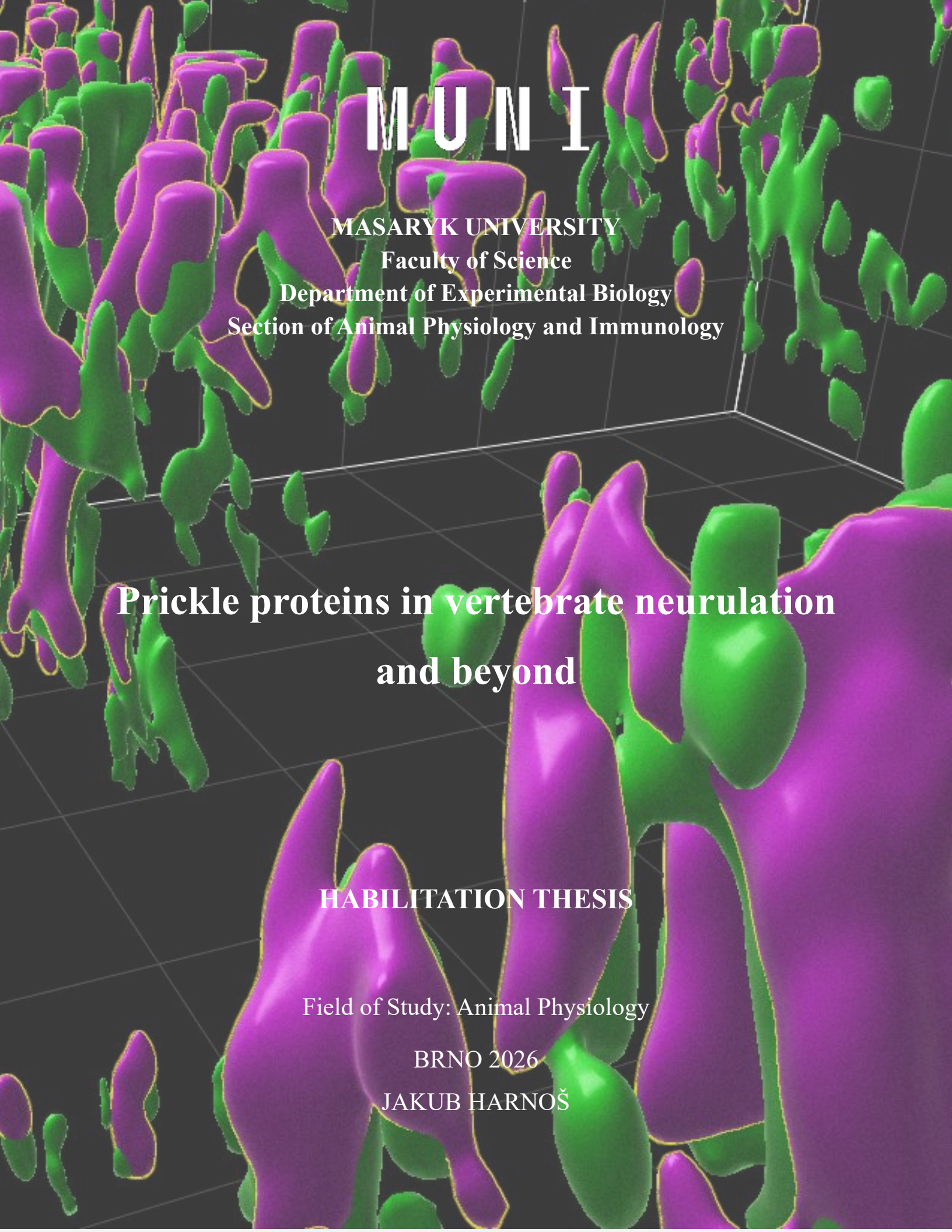

MASARYK UNIVERSITY
Faculty of Science
Department of Experimental Biology
Section of Animal Physiology and Immunology

Prickle proteins in vertebrate neurulation and beyond

HABILITATION THESIS

Field of Study: Animal Physiology

BRNO 2026

JAKUB HARNOŠ

Cover: Activated actomyosin localized above Prickle2 in the neural tube of Xenopus embryos

Dedicated to Pamela MVM, Sarah, and Jacob Jr. – the three key signaling pathways in my life.

Contents

List of abbreviations

AP	anteroposterior
ApCo	apical constriction
CE	convergent extension
CI	cell intercalation
CK1	Casein kinase 1
ML	mediolateral
MLC	Myosin light chain
NMII	non-muscle Myosin II
NT	neural tube
NP	neural plate
PCP	planar cell polarity
PTP	planar tissue polarity
pMLC	phosphorylated myosin light chain
WNT	Wingless/Integrated

COMMENTARY TO HABILITATION THESIS

Prickle proteins in vertebrate neurulation and beyond

Mgr. Jakub Harnoš, Ph.D.

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,

March 3, 2026

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.

Planar cell polarity (PCP) signaling

The PCP pathway represents the best characterized branch of non-canonical, or β-catenin-independent, WNT signaling (Butler & Wallingford, 2017; Gao, 2012; Humphries & Mlodzik, 2017; Qin et al., 2024). A defining feature of PCP signaling is its capacity to transmit polarized information between neighboring cells, which results in coordinated orientation of tissues within the plane of the epithelium (Butler & Wallingford, 2017; Davey & Moens, 2017; Zallen, 2007).

A brief history of PCP. The earliest observation of planar polarity can likely be traced back to Robert Hooke in the 17th century, who in his *Micrographia* described "conical bristles, all whose ends pointed backwards" on the bodies of insects (Hooke, 2003). However, a mechanistic understanding of this phenomenon emerged much later. In 1982, Gubb and García-Bellido identified genes controlling the orientation of wing hairs and sensory bristles in the cuticle of *Drosophila melanogaster* and introduced the term planar tissue polarity (PTP), which is now referred to as PCP (Gubb & García-Bellido, 1982; Y. Wang & Nathans, 2007).

Drosophila PCP system. The clear morphological readout provided by these wing hair and bristles enabled large-scale genetic screens in the fruit fly, which identified several so called 'core' PCP components, which regulate their formation through modulation of the actin cytoskeleton (Bastock et al., 2003; Goodrich & Strutt, 2011; Peng & Axelrod, 2012; Seifert & Mlodzik, 2007; Tree et al., 2002; Yang, 2012) (Adler, 2002, 2012). On the molecular level, these proteins cluster into distinct domains at opposite cell membranes, thereby dividing the cell cortex into two functional regions – a key hallmark of PCP. Specifically, at early pupal stages in *Drosophila melanogaster*, Frizzled is initially distributed symmetrically along the entire cell membrane. Subsequently, the cytoplasmic protein Dishevelled binds to Frizzled, and this complex forms a circumferential ring around the cell. Several hours after ring formation, the complex becomes enriched at the distal side of the cell, and later it is clearly restricted to the distal membrane (Axelrod, 2001). Thus, Dishevelled and Frizzled accumulate at the distal side, whereas the Prickle-Vangl complex is secondarily localized on the proximal side (Bastock et al., 2003; Strutt & Strutt, 2005). The establishment of proximal and distal cell sides is well illustrated in wing epithelial cells of the fruit fly, where only the distal complex, which contains Dishevelled and Frizzled, initiates hair outgrowth (**Fig. 1**) (Strutt & Strutt, 2008).

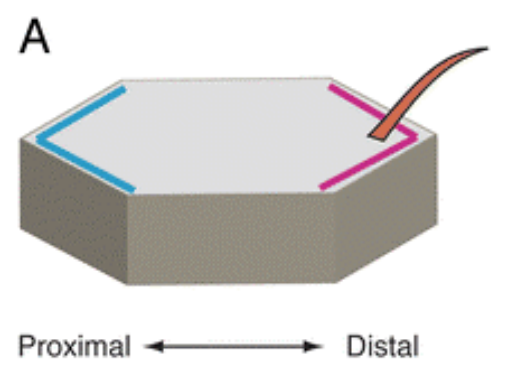

Figure 1. Schematic representation of planar cell polarity (PCP) protein complexes in wing cells of *Drosophila melanogaster*. Blue – Prickle/Vangl complex; pink – Dishevelled/Frizzled complex. Red indicates the wing hair. Dishevelled acts as a trigger of actin polymerization during hair formation. Proximal and distal orientation is determined according to the source of the Wnt ligand. Adapted from (Maung and Jenny, 2011).

Within this classical framework, Prickle was mainly regarded as a passive member of the Vangl-associated complex, required for maintenance of polarity, but not considered a direct regulator of cell behavior, or actomyosin remodeling in case of hair outgrowth (Maung & Jenny, 2011; McNeill, 2010; Simons & Mlodzik, 2008a). However, the transfer of this concept from invertebrate to vertebrate PCP systems, which happened in the following years, has had long-term impact. Thus, vertebrate PCP research has primarily concentrated on Vangl, Frizzled, Dishevelled, and their downstream small GTPases, largely inspired by the visible hair polarity phenotypes, whereas proteins such as Prickle were often viewed as secondary elements (Butler & Wallingford, 2017; Goodrich & Strutt, 2011). However, recent vertebrate studies presented in this thesis (as well as by other researchers) suggest that this simplified antagonistic model adopted from *Drosophila* is incomplete in vertebrates.

Vertebrate PCP. 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 (Čada & Bryja, 2022; Goodrich & Strutt, 2011; Koca et al., 2022; Wansleeben & Meijlink, 2011). In these contexts, planar polarity is not static. It must be repeatedly established and adjusted as cells rearrange neighbors and experience mechanical forces, as in case of vertebrate neurulation (Butler & Wallingford, 2017, 2018; Shindo et al., 2019; Wallingford, 2004).

This dynamic environment has important mechanistic consequences. PCP signaling in vertebrates must couple polarity information with cytoskeletal remodeling, membrane trafficking, and controlled protein turnover, as shown for example on Vangl (Feng et al., 2022). Asymmetric

protein localization alone does not sufficiently explain persistent directional behavior. Instead, PCP output relies on localized activation of actomyosin contractility, modulation of junctional tension, and coordinated collective movement (Merks et al., 2018). Disturbances in vertebrate PCP signaling result in developmental abnormalities. These include NT defects and craniofacial malformations, and are also associated with skeletal and neurological disorders (Cai & Shi, 2014a; Gentzel & Schambony, 2017; Radaszkiewicz et al., 2024). In addition, altered PCP signaling has been implicated in cancer progression, where it contributes to metastatic spread and therapy resistance (Humphries & Mlodzik, 2018; VanderVorst et al., 2018). Therefore, a key unresolved question in vertebrate PCP signaling has been whether Prickle proteins act only as polarity stabilizers or whether they directly and actively regulate cytoskeletal dynamics and morphogenetic behavior – a question addressed in the following chapter(s).

Prickle protein family

Functionally, Prickle is a cytoplasmic protein without known enzymatic activity and represents an essential component of the PCP pathway (Radaszkiewicz et al., 2024). For its activity, Prickle associates with the transmembrane protein Vangl, leading to accumulation of Vangl-Prickle complexes at the plasma membrane (Bastock et al., 2003; Jenny, 2003; Radaszkiewicz et al., 2024; Song et al., 2025). In parallel, Prickle negatively regulates other PCP components, including the cytoplasmic protein Dishevelled (Jenny, 2003; Tree et al., 2002) and its transmembrane partner Frizzled (Schulte & Bryja, 2007; Warrington et al., 2017).

A brief history of Prickle. Prickle was first identified in *Drosophila* in the 1940s and was named according to a mutant phenotype characterized by disoriented thoracic bristles, described as “irregularly erected and whorled, giving a prickle effect “ (Radaszkiewicz et al., 2024). In the 1980s, this phenotype was connected to defective PCP signaling responsible for the formation and orientation of surface body structures (Adler, 2012). Because the prickle mutant in flies was not lethal, it may have been initially considered less critical for invertebrate signaling. However, vertebrate PCP signaling occurs in a substantially different biological context. In addition to passive role in epithelial polarity, vertebrate Prickle is actively involved in complex and dynamic processes such as neural tube formation, organogenesis, and cell migration (Butler & Wallingford, 2017). This increased complexity is reflected by gene expansion: the single invertebrate Prickle

gene has four paralogs in vertebrates, Prickle1-4 (PRICKLE1-4 in humans), identified in the late 1990s and early 2000 (Fisher et al., 1997; Katoh & Katoh, 2003; Nishikawa & Kawamoto, 2015; Ossipova et al., 2015; Teufel et al., 2005).

Structure of Prickle proteins. In short, all four members of vertebrate Prickle family share two main conserved elements – the N-terminal PET domain and several LIM domains – together with a couple of short motives such as VBMs (**Fig. 2**).

PET domain. The PET (Prickle, Espinas, Testin) domain comprises approximately 110 amino acids forming α-helical structures (Sala et al., 2017; Sweede et al., 2008a). It mediates protein–protein interactions and participates in signal transduction (Sala et al., 2017). In Prickle proteins, the PET domain contributes to interaction with other PCP components, including Dishevelled (Gubb et al., 1999; Tree et al., 2002) and supports Prickle membrane association, which is further influenced by LIM domains (Sweede et al., 2008b). On the other hand, it has been suggested recently that PET domain can also inhibit LIM domains in Prickle in actin recruitment (Bejar-Padilla et al., 2026).

LIM domain. The LIM domain is a very conserved cysteine-rich module of about 60 amino acids found in many LIM proteins (Anderson et al., 2021; Blum et al., 2025; Kadrmas & Beckerle, 2004a). It consists of two zinc fingers and functions mainly as an adaptor for protein–protein interactions (Kadrmas & Beckerle, 2004b). Vertebrate Prickle1-3 contain three LIM domains, while Prickle4 has two (Radaszkiewicz et al., 2024). Multiple LIM domains increase structural flexibility and allow interaction with several binding partners (Dawid et al., 1998).

VBMs. The VBMs (Vangl-binding motives) are a short conserved sequence responsible for binding of Prickle to the intracellular region of Vangl, which anchors the complex at the plasma membrane and is required for proper PCP complex assembly and signaling. It was thanks to recent cryo-EM data (Song et al., 2025; Zhang et al., 2025) that redefined the previously proposed VBD (Jenny, 2003) into two shorter VBMs in Prickle1, which are enriched in negatively charged residues (**Fig. 2**). In Study III, we demonstrated that Prickle3 also contains functional VBMs, which were not shown previously. This evidence led to discover a new functional phenotype in Vangl protein stability.

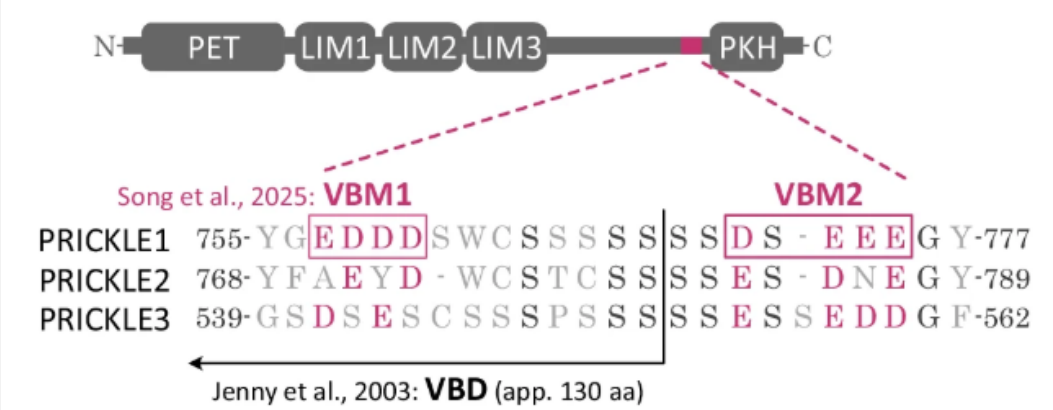

Figure 2. Multiple sequence alignment of PRICKLE1–3 in the region corresponding to the VANGL-binding domain (VBD) and two VANGL-binding motifs (VBMs). The alignment reveals that PRICKLE3 also contains conserved regions. Amino acid positions are indicated by numbers. The schematic above illustrates the domain organization of PRICKLE proteins, including the PET domain, three LIM domains, a central intrinsically disordered region, and the Prickle homology (PKH) domain.

Beyond PET, LIM, and VBMs elements, all Prickle paralogs contain a central IDR which can represent up to 50% of the protein length. Due to this large unstructured region, it is likely that no complete 3D structure of Prickle paralogs has yet been resolved, since intrinsically disordered regions lack stable tertiary structure under physiological conditions. At the same time, these regions are often enriched in PTMs, particularly phosphorylation (Iakoucheva, 2004). Consistent with this, several kinases – including Nemo (Collu et al., 2018) or Misshapen-like kinase 1 (MINK1) (Daulat et al., 2012) have been reported to interact with and functionally modify Prickle proteins. In Studies II-III, we demonstrated for the first time that CK1δ/ε, one of the key kinases regulating PCP components (Janovska et al., 2018; Kaucká et al., 2013), uniquely phosphorylates Prickle2 and Prickle3 proteins. However, the exact functional output of these phosphorylation events waits for future detailed explanation.

A PKH domain is sometimes mentioned at the extreme C-terminus of Prickle proteins by some authors (Radaszkiewicz et al., 2024). However, its functional significance in signaling is not clearly defined and requires further investigation.

This overview of the PCP system and the Prickle protein family provide the necessary molecular background for Studies I-III. In the following chapter, I will focus on vertebrate neurulation, which represents the second main topic of this habilitation thesis.

Vertebrate neurulation

The formation of the central nervous system is one of the most mechanically and organizationally complex events during vertebrate development. The generation of the neural tube (NT) represents the first step of this process, which later leads to the formation of the brain and spinal cord (**Fig. 3, red box**) (Zhou et al., 2024). Neurulation is therefore a key event in early vertebrate embryogenesis and has been intensively studied for more than 150 years (Holmdahl, 1933; Schroeder, 1970; Vijayraghavan & Davidson, 2017a). It is also important to emphasize that correct neurulation is required not only for NT formation, but also for the proper development of other body structures, such as the urogenital tract (Hong et al., 2021).

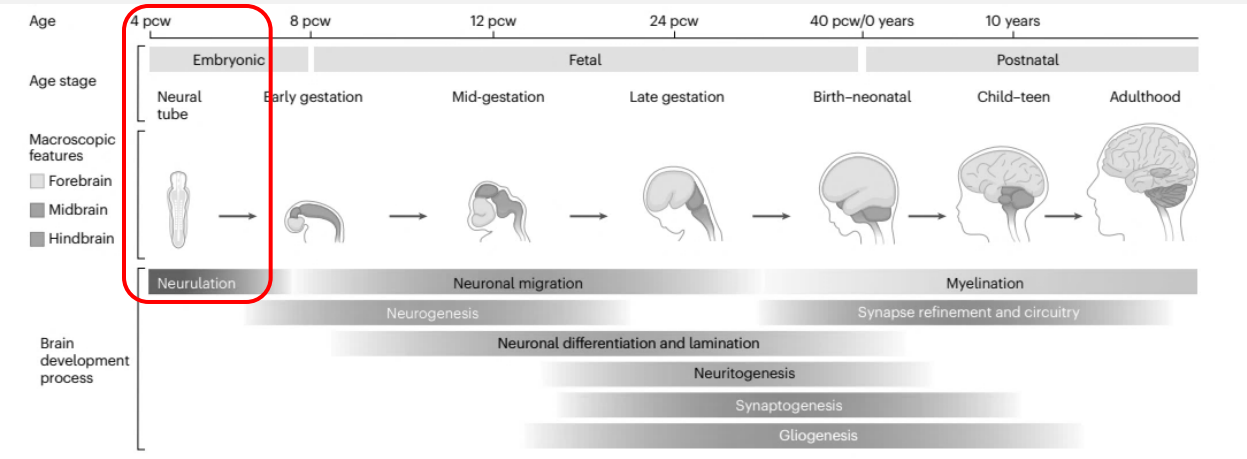

Figure 3. Schematic overview of major developmental stages of the human brain, including early neural tube formation and later postnatal maturation processes. The timeline and brain size are not shown to scale. Adopted from Zhou et al., 2024.

Neurulation can be divided into two types: primary and secondary. Primary neurulation is evolutionarily older and represents the original mechanism of NT formation within the *Chordata* group (Handrigan, 2003). It is generally accepted that primary neurulation is driven by a combination of intrinsic forces within the neural plate and extrinsic forces coming from the surrounding nonneural ectoderm (Colas & Schoenwolf, 2001; J. Smith, 1997). In contrast, secondary neurulation occurs through condensation of mesenchymal cells in the tailbud region beneath the surface ectoderm, forming a solid cord-like structure. A central lumen subsequently develops within this compact cord, resulting in the formation of the NT (Keller, Shih, Sater, et al., 1992; Schoenwolf, 1979; J. Smith, 1997; Smith & Schoenwolf, 1989). Based on morphology,

primary and secondary neurulation can be distinguished by several characteristic features. In this work, we focus exclusively on primary neurulation.

Primary neurulation is a highly complex and precisely regulated process, controlled by signals originating from the embryo itself. In very simplified terms, it can be described in four main steps: neural plate formation, shaping of the neural plate (NP), generation of neural folds and bending of the NP, and finally closure of the neural groove through fusion of the neural folds (**Fig. 4**) (Colas & Schoenwolf, 2001).

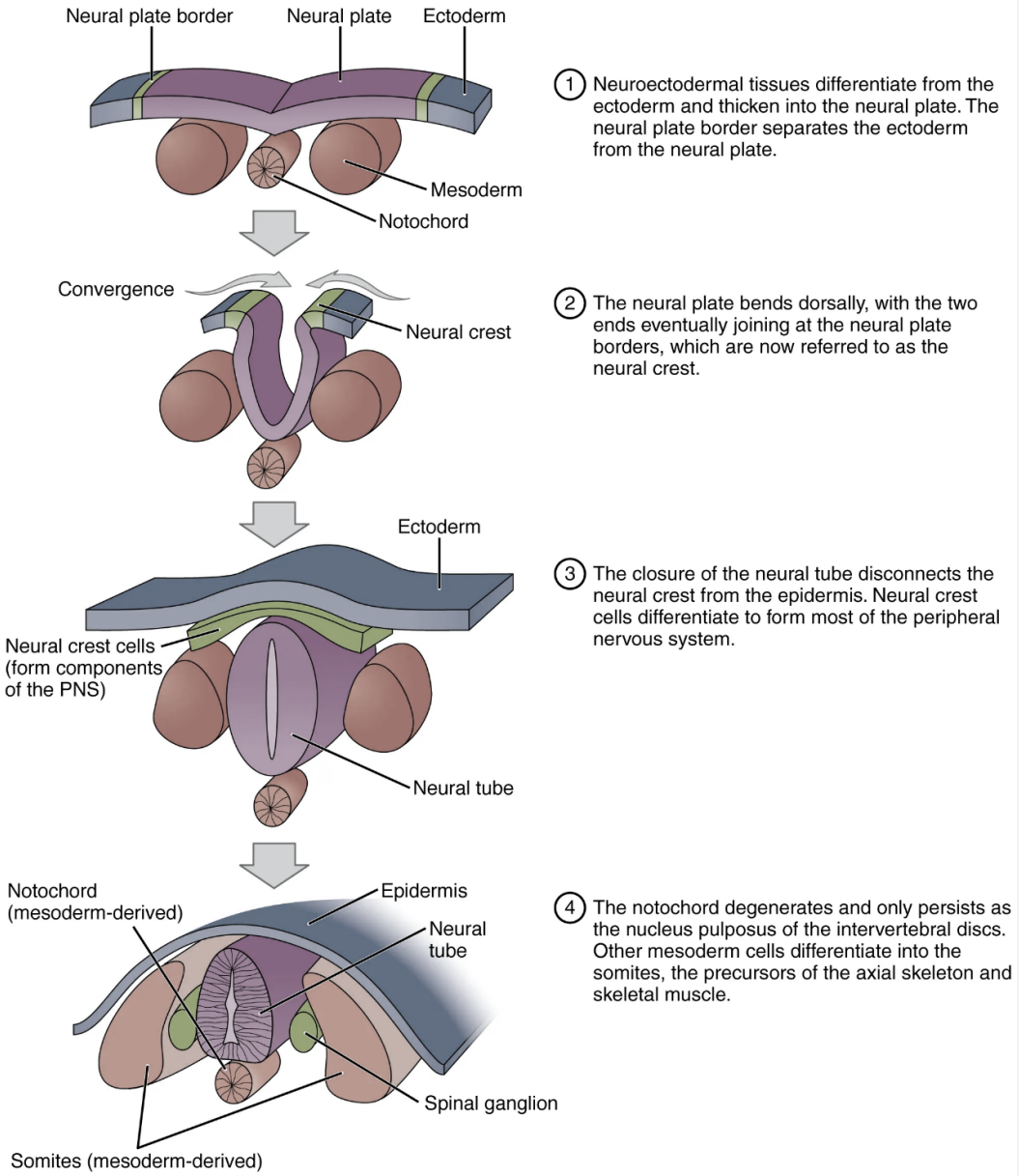

Figure 4. The embryonic process of neurulation establishes the rudiments of the future central nervous system and skeleton (adopted from ISBN-13: 978-1-951693-42-8).

For the NP to bend, three hinge points must be established: one median hinge point at the dorsal midline and two dorsolateral hinge points. Their formation depends on apical constriction, during which cells change from columnar to wedge-like shape due to apical narrowing (apical constriction – see below) (Martin & Goldstein, 2014; Sawyer et al., 2010; Schroeder, 1973). The strongest apical constriction defines the hinge points (J. L. Smith & Schoenwolf, 1989). Because cell–cell junctions remain intact and connect actin filaments between neighboring cells, the tissue bends as a whole part (Martin & Goldstein, 2014). This bending moves the neural folds towards each other and enables their fusion. In vertebrates, fusion involves formation of actin-rich protrusions that connect cells across the neural groove (Nikolopoulou et al., 2017). These protrusions called filopodia and lamellipodia support adhesion and merging of the neural folds, leading to closure of the neural groove and formation of NT (Rolo et al., 2016).

From a mechanistic view, neurulation represents coordinated cellular behaviors that must be regulated in space and time. NT formation requires collective cell movements and tissue deformations across large cell populations, often described as stage-dependent processes during neurulation (shown for *Xenopus* in **Fig. 5**) (Davidson & Keller, 1999; Vijayraghavan & Davidson, 2017; Ybot-Gonzalez et al., 2007).

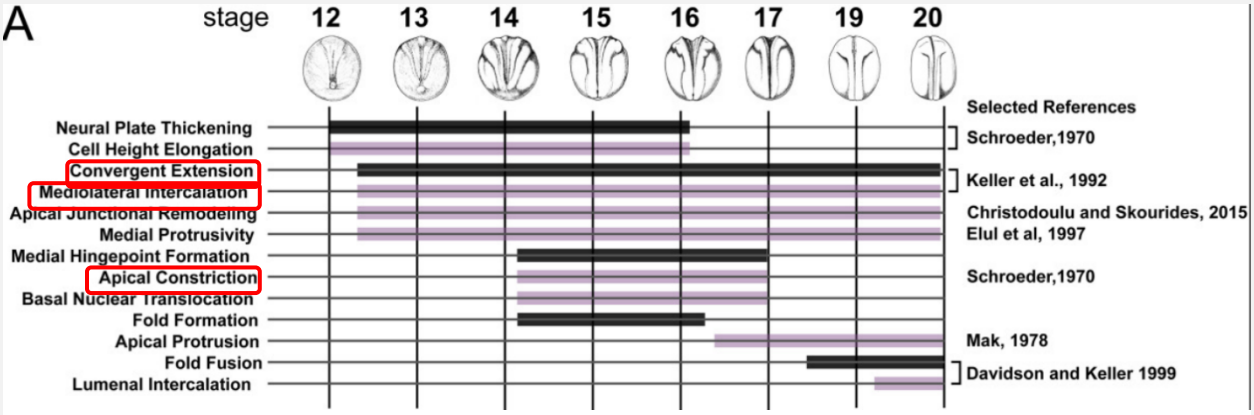

Figure 5. Schematic representation of the cellular and tissue-level changes occurring during neurulation in *Xenopus laevis*. The diagram illustrates when key processes begin, how long they persist, and when they end, including mediolateral intercalation, apical constriction, convergent extension (red boxes). Black bars indicate stage-dependent tissue deformation processes, while purple bars represent underlying cell behaviors over time. NT – neural tube; NF – developmental stages according to Nieuwkoop and Faber. Adapted from (Vijayraghavan and Davidson, 2017).

The key processes of vertebrate neurulation include apical constriction, convergent extension, and cellular (or mediolateral) interaction.

Apical constriction (ApCo)

ApCo is a morphogenetic mechanism that supports epithelial folding in many developmental situations, including NT formation (Martin & Goldstein, 2014; Sawyer et al., 2010; Schroeder, 1973). ApCo is defined as reduction of the apical domain size (Martin & Goldstein, 2014; Sawyer et al., 2010; Schroeder, 1973). The principle of ApCo is a rapid contraction of the actomyosin network at the apical side of the cell – during neurulation, this corresponds to the side that will later face the interior of the NT (**Fig. 6**). This contraction often occurs in cycles, where actomyosin contracts and then relaxes again, resembling a pulsed behavior (Martin et al., 2009).

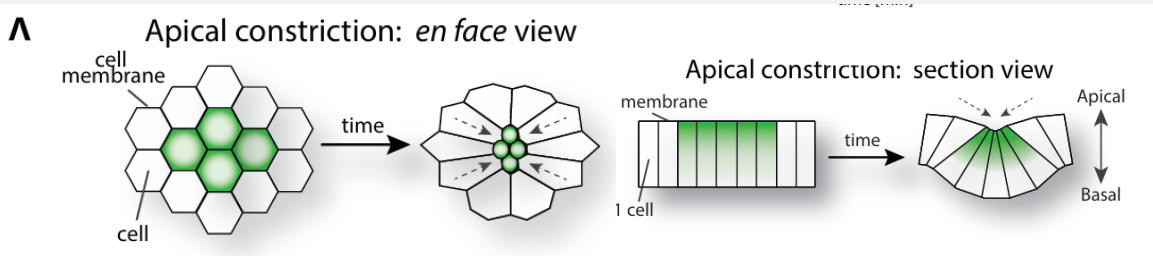

Figure 6. Schematic representation of apical constriction. The left panel shows cells in top (en face) view, and the right panel shows a cross-sectional view. Constricting cells are shown in green.

In vertebrates, coordinated ApCo is highly heterogeneous and asynchronous when individual cells are analyzed. During folding of a whole epithelium, such as the neuroectoderm, cells display considerable variability in apical domain size, shape, and orientation (Baldwin et al., 2022; Christodoulou & Skourides, 2023; Colas & Schoenwolf, 2001; Smith & Schoenwolf, 1989; Suzuki et al., 2017). This contrasts with ApCo in invertebrates, where the process is often regulated by specialized signaling pathways (Benton et al., 2019; Costa et al., 1994; Martin & Goldstein, 2014). Such anisotropy of actomyosin contractility is important for maintaining NT length during folding. Anisotropic actomyosin contractions were also described in the neuroectoderm at early neurulation stages, when convergent extension (CE) elongates the neuroectoderm along the AP axis by shrinking mediolaterally (ML)-oriented junctions and elongating AP-oriented junctions (Baldwin et al., 2022; Christodoulou & Skourides, 2023; Matsuda & Sokol, 2025). Therefore, regulation likely requires a unifying signal acting across the tissue, rather than independent control at the level of single cells. Planar cell polarity is a strong candidate for such regulation.

Only a limited number of proteins have so far been shown to directly induce apical constriction. One example is Shroom (Haigo et al., 2003a; Hildebrand & Soriano, 1999), which was proposed to promote apical constriction by recruiting the ROCK kinase (McGreevy et al., 2015; Mohan et al., 2012; Nishimura & Takeichi, 2008). Expanding the list of proteins that can trigger ApCo would be beneficial for future studies; therefore, see the following chapter.

ApCo is mediated by the actomyosin contractile apparatus (Hildebrand, 2005; Hildebrand & Soriano, 1999; Martin & Goldstein, 2014; Sawyer et al., 2010; Schroeder, 1973) , which consists of a network of actin filaments and non-muscle Myosin II (NMII) together with associated molecules. Proper *in vivo* function of the contractile machinery depends mainly on phosphorylation of NMII (regulatory) light chain (further referred as MLC) (Getz et al., 2010; Kaneko-Kawano et al., 2012; Moussavi et al., 1993; Sakurada et al., 1998; Sun et al., 2020). 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.

Convergent extension (CE)

CE is a morphogenetic process in which cells within a tissue rearrange to narrow and extend the body axes (**Fig. 7**). It represents a form of collective cell movement, where cells located at the tissue margins move toward the center while the tissue elongates in the same direction (Keller & Sutherland, 2020). CE is characterized by exchange of positions between neighboring cells within a single layer (Keller & Sutherland, 2020). As a result, the embryo becomes narrower along the mediolateral axis and simultaneously longer along the anteroposterior axis, which is essential for proper tissue elongation (Keller & Sutherland, 2020; Sutherland et al., 2020). Due to this elongation of both tissue and embryo, convergent extension is considered one of the key steps in NT formation. This is a crucial mechanism for neural tube closure and body axis elongation, as it extends the length of the body axis and reduces the distance between the adjacent neural folds, leading eventually to their fusion (Tada & Heisenberg, 2012). Disruption of convergent extension results in neural plate widening and neural tube closure defects, a phenotype consistently observed in PCP mutants (Wallingford & Harland, 2002; Ybot-Gonzalez et al., 2007).

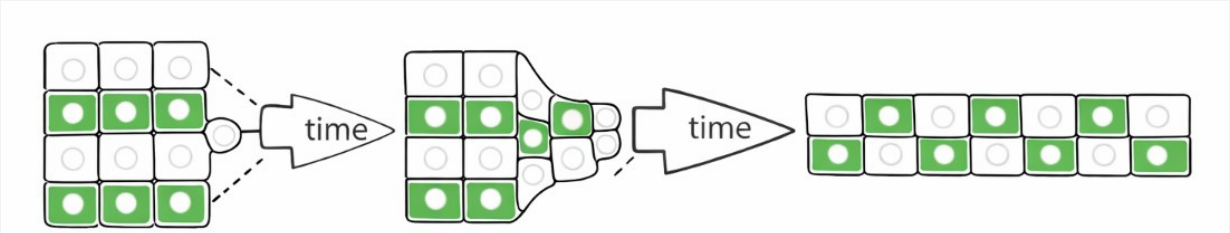

Figure 7. Schematic representation of convergent extension (CE). Cells progressively intercalate mediolaterally, leading to tissue narrowing (convergence) and simultaneous elongation along the perpendicular axis (extension) over time.

Intercalation during CE can proceed through two main mechanisms. The first involves cellular protrusions and is referred to as the crawling mode (Shindo, 2018). Through protrusions, a cell can insert itself between neighboring cells, which leads to their rearrangement within the plane (Keller, Shih, & Domingo, 1992). These protrusions are polarized, and cell movement is accompanied by oscillatory contraction of F-actin filaments (Kim & Davidson, 2011). The second mechanism of intercalation is based on remodeling of cell–cell junctions. Importantly, active adhesive junctions between cells are required for this process. If cells are only pressed together due to spatial limitation but do not share proper intercellular junctions, intercalation cannot be initiated (Rauzi, 2020). These two mechanisms are not strictly separated and can overlap. For example, during mouse neurulation, cells intercalate simultaneously through protrusive activity and remodeling of apical junctions (Williams et al., 2014).

Cell intercalation (CI)

Cell intercalation represents an important topological transformation contributing to tissue morphogenesis, tissue homeostasis, and pathological conditions such as cancer cell invasion. In recent years, many studies have focused on understanding the fundamental mechanisms controlling this process. Cells frequently use protrusions to insert between neighboring cells, leading to changes in cell neighbor relationships (Clément, 2024; Rauzi, 2020; Walck-Shannon & Hardin, 2014).

In simple epithelial tissues, composed of a single layer of densely packed prism-shaped cells, topology changes occur while preserving apical–basal polarity and maintaining an intact

epithelial layer (**Fig. 8**). Medio-lateral intercalation in simple epithelia is therefore an example of both robustness and plasticity. In these tissues, cells typically use a combination of mechanisms to enable topological transformation at both apical and basal sides (Clément, 2024; Rauzi, 2020; Walck-Shannon & Hardin, 2014).

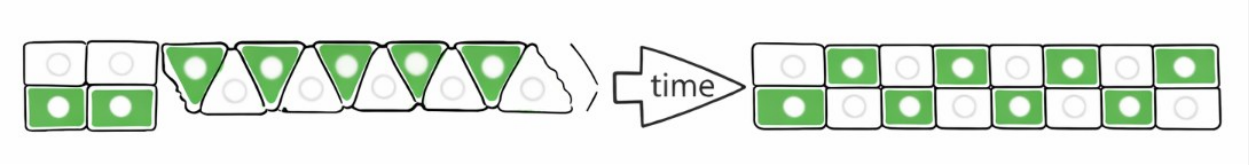

Figure 8. Schematic illustration of cell intercalation (CI). Individual cells insert between neighboring cells, resulting in rearrangement of cell positions and formation of an elongated cellular array**.** Intercalation can involve even one cell.

Remodeling of cell junctions is fundamentally driven by contraction of the actin cytoskeleton, which is highly enriched in these regions. As will be discussed below in more detail, kinases that regulate cytoskeletal dynamics can play essential roles in these processes. One important regulator is Celsr, which promotes remodeling of cell junctions and thereby enables cell intercalation (Nishimura et al., 2012).

Last but not least, intercalation differs from CE in that it does not necessarily have a defined global direction. Not all intercalation events result in convergent extension; intercalation can occur at the cellular level without producing a large-scale tissue elongation, as it happens in case of radial cell intercalation (Ossipova et al., 2015).

Actomyosin as a molecular driver of cell movements

Actomyosin-mediated contractility represents a conserved mechanism generating mechanical force in living cells and driving tissue morphogenesis (Murrell et al., 2015). While this system is well characterized in striated and smooth muscle, its regulation by developmental signaling pathways in non-muscle cells remains less understood (Cowan et al., 2022).

Actomyosin consists of actin filaments, non-muscle Myosin II (NMII), and associated regulatory proteins. This cytoskeletal system is considered an evolutionary prerequisite for eukaryotic cell organization (Cavalier-Smith, 2002). In non-muscle cells, NMII forms bipolar filaments that slide actin filaments relative to each other, thereby generating contractile force and

altering cell shape (Cowan et al., 2022b) (Shutova et al., 2012). These forces are transmitted across tissues through intercellular junctions, particularly adherens junctions (Martin, 2010; Martin & Goldstein, 2014b). Polymerization of G-actin into F-actin contributes to cell shape regulation (Ulferts et al., 2021), although the major contractile force arises from NMII activity (Quintin et al., 2008). Activation of NMII depends on phosphorylation of the regulatory light chain (MLC), especially at Ser19 (Vicente-Manzanares et al., 2009). Phosphorylation of MLC at this amino acid (pMLC; sometimes referred as Thr18/Ser19) regulates NMII conformation and activity, which is essential for force generation (**Fig. 9**) (Getz et al., 2010; Kaneko-Kawano et al., 2012; Moussavi et al., 1993; Sakurada et al., 1998; Sun et al., 2020).

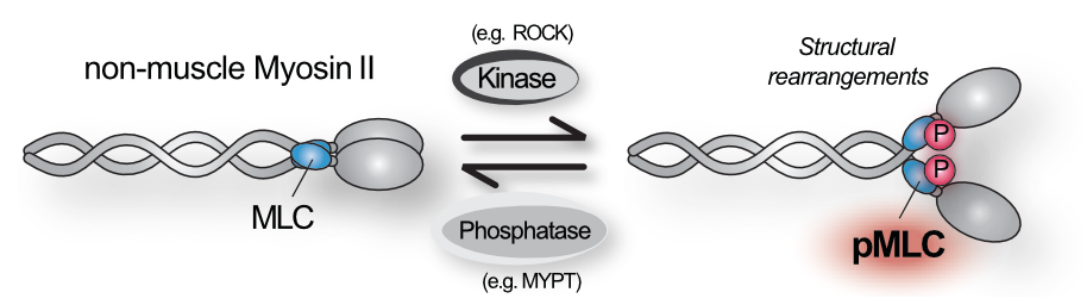

Figure 9. Myosin II activation leads to the actomyosin contractility. This process is tightly regulated by phosphorylation of MLC (pMLC) by a specific kinase (e.g. ROCK) and dephosphorylation by a phosphatase (e.g. MYPT).

PCP proteins (Dishevelled, Prickle, Vangl, Frizzled) do not directly phosphorylate MLC. Kinases such as MLCK and ROCK catalyze this phosphorylation (Vicente-Manzanares et al., 2009). Thus, PCP downstream effectors such as small GTPases Rho (Habas et al., 2001), Rac (Habas et al., 2003), and Rap1 (our Study II) mediate this process. ROCK inhibition disrupts mediolateral cell migration and results in neural tube defects (Engelhardt et al., 2022; Escuin et al., 2015), demonstrating that Rho-kinase functions as a critical effector linking polarity signaling to contractile mechanics. Thus, actomyosin contractility represents the principal mechanical output of PCP signaling during vertebrate neurulation. The remaining question is how individual PCP components, including Prickle proteins, modulate this contractile machinery in vivo.

Now, we will find out more about how Prickle is connected to individual cell movements, mentioned in this chapter.

Molecular links between Prickle and neurulation

Although multiple signaling pathways contribute to NT formation, PCP signaling is recognized as a central regulatory pathway coordinating these events in vertebrates (Goodrich & Strutt, 2011; Seifert & Mlodzik, 2007; Wallingford & Harland, 2002). Genetic studies demonstrated that NT closure is directly controlled by this signaling pathway (Butler & Wallingford, 2017; Cai & Shi, 2014b; Humphries et al., 2020; Simons & Mlodzik, 2008). Specifically, disruption of core PCP components leads to defects in convergent extension, widening of the neural plate, and failure of neural tube closure in multiple vertebrate species, including frog, fish, chicken, mouse, and human (Brouns & Van Straaten, 2005; De Marco et al., 2013; Radaszkiewicz et al., 2024; Wallingford & Harland, 2002; Ybot-Gonzalez et al., 2007). These phenotypes clearly show that PCP signaling is essential during vertebrate neurulation and represents a direct connection between molecular polarity and large-scale tissue morphogenesis.

A major open question in early neurulation research is how planar polarity information is converted into localized cytoskeletal activity. While PCP signaling aligns cells within the tissue plane, the molecular mechanisms connecting asymmetric PCP complexes to actomyosin activation are still not fully clarified. Classical models highlight Dishevelled-mediated activation of Rho GTPase signaling; however, these models do not completely explain the variability and strength of neurulation phenotypes observed in vivo. Specifically, it has been proposed that the posterior PCP complex containing Dishevelled and the formin protein Daam1 (Habas et al., 2001; McGreevy et al., 2015; Nishimura et al., 2012) activates RhoA GTPase, which subsequently stimulates NMII activity. Nevertheless, the main experimental evidence supporting this model originates from tissue culture cells lacking proper PCP organization (Nishimura et al., 2012), and direct in vivo confirmation has remained limited. In addition, this model focuses primarily on one cellular side, where the Frizzled/Dishevelled complex is localized. The reported co-localization of Prickle proteins with NMII (Butler & Wallingford, 2018; Newman-Smith et al., 2015), defective NTC after depletion of endogenous *Prickle2* (Butler & Wallingford, 2018), and our shown here indicate that the *Drosophila*-based model requires reconsideration in vertebrates.

An important question is therefore whether Prickle is expressed in the early neuroectoderm. From the six basic model organisms used in the field of developmental biology (Lania et al., 2025), *Xenopus* represents a highly suitable vertebrate model for studying neurulation (Matsuda & Sokol,

2021). Its advantages include accessible biochemical tools, precise targeting of protein expression, external embryo development, and strong similarities of NT development to mammals (Matsuda & Sokol, 2021). The relevance of *Xenopus* findings to human embryogenesis, particularly in the context of NT defects, further supports its importance (Wallingford, 2019; Wallingford et al., 2013). The *Xenopus* neural ectoderm is also a well-established model for studying planar polarized vertebrate tissues (Butler & Wallingford, 2018; Wang, 2012) and has been essential for analyzing actomyosin contractility in vivo (Haigo et al., 2003b). Moreover, *Xenopus* neurulation more closely resembles amniote primary neurulation than, for example, zebrafish (**Fig. 10**), making it a highly relevant system for studying these processes (Harrington et al., 2009). Studies using model organisms such as *Xenopus* have significantly contributed to understanding the molecular mechanisms underlying human NT defects (Harrington et al., 2009).

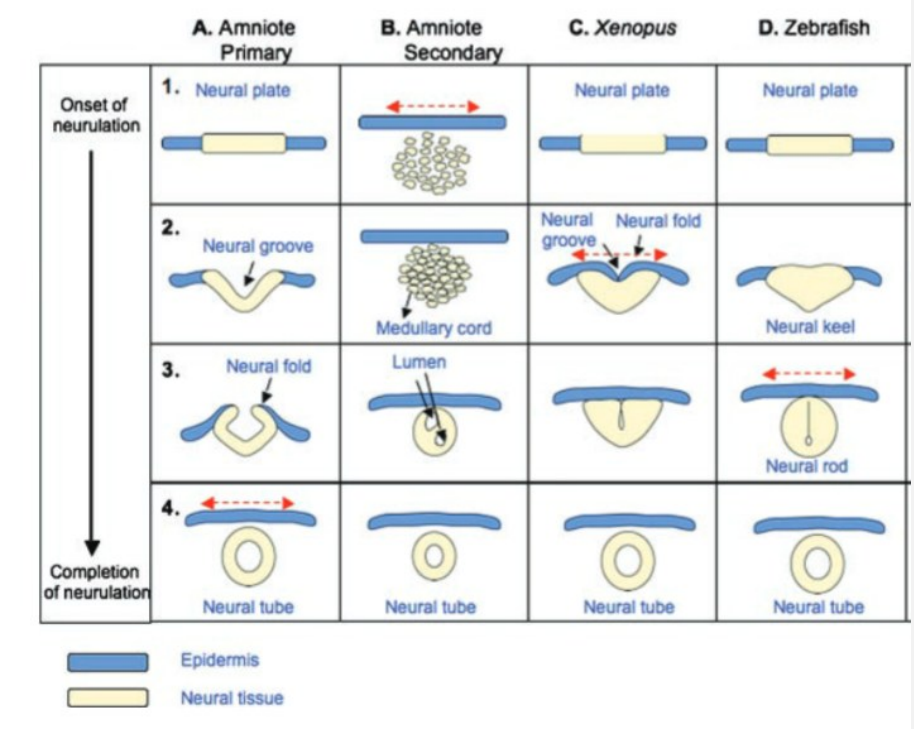

Fig. 10. Variations of neurulation at a morphological level. Primary neurulation (A) and secondary neurulation (B) in amniotes, neurulation in *Xenopus* (C), and zebrafish (D). Blue represents the epidermis, and yellow, the neural tissue. Red double arrowheads indicate epidermal fusion. Numbers 1–4 indicate different stages of neurulation. Adopted from Harrington et al., 2009.

Well, are Prickle proteins expressed in developing of *Xenopus* then? In *Xenopus* embryos, endogenous Prickle1 is strongly expressed during embryogenesis in the posterior neural ectoderm and remains present throughout the neurula stage (Takeuchi et al., 2003; Wallingford et al., 2002), suggesting a role in neurulation. Expression analysis indicates that *Xenopus* Prickle2 is also present at the neurula stage (Session et al., 2016). In contrast, Prickle3 expression at this stage appears rather weaker (Session et al., 2016). During neurulation, Prickle2 colocalizes with Vangl2 at the

anterior cell borders of the neural plate at NF stage 13 (Ossipova et al., 2015), reflecting active PCP signaling. This colocalization becomes stronger at shrinking cell–cell junctions during neural tube closure (Butler & Wallingford, 2018), further supporting an active role of Prickle proteins in neurulation, although Prickle3 may also contribute (see our Study III).

In the following sections, I discuss the role of individual Prickle proteins in three main cellular behaviors during neurulation: apical constriction (ApCo), convergent extension (CE), and cell intercalation (CI).

Prickle and ApCo

The role of Prickle in ApCo remains relatively understudied, despite ApCo being one of the most important morphogenetic processes during neurulation. A recent preprint from 2025 (Wang et al., 2025) addressed this question for Prickle1. Using high-resolution quantitative live imaging in transgenic quail embryos, the authors showed that PRICKLE1 is enriched at the apical cortex of medial cells, where it promotes actomyosin accumulation and apical constriction.

However, in Study II (published in the end of 2024, see below), my group provided the first direct in vivo evidence demonstrating the involvement of Prickle2 in ApCo, together with activation of pMLC. The participation of Prickle3 in ApCo is currently not supported (see Table 1 below) and appears unlikely, as it is not expressed in the neural plate (Harnos lab data, not published; www.xenbase.org).

For these reasons, Study II was important in demonstrating that Prickle2, which represents the predominant Prickle variant during neurulation, is directly involved in ApCo. Moreover, we identified the precise molecular mechanism linking Prickle2 to actomyosin regulation during vertebrate neurulation (see Study II below).

Prickle and CE

It is well established that PCP signaling is essential for coordinated cell polarity and force generation during CE of the neural plate in vertebrate gastrulation (Davey & Moens, 2017).

Neuroepithelial progenitors display planar polarized localization of PCP proteins during CE, with the polarity axis oriented perpendicular to the direction of convergence (Davey & Moens, 2017). Prickle and Vangl localize together at apical anterior cell junctions in the neural plate (**Fig. 11**) (Ossipova et al., 2015). Their membrane localization places them in an optimal position to regulate polarized actomyosin contractility and membrane shortening.

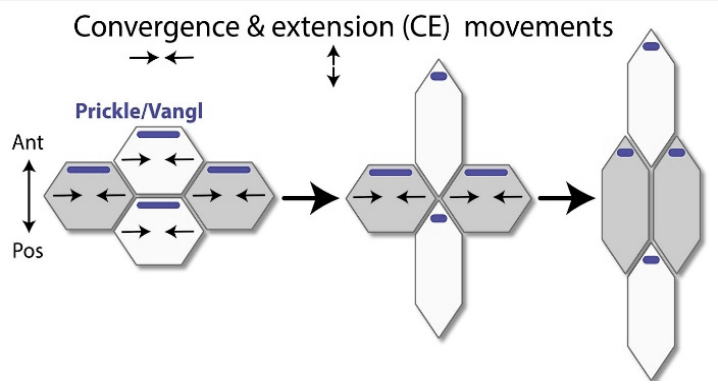

Figure 11. Asymmetric localization of Prickle/Vangl drives collective neuroepithelial CE movements during neurulation.

In zebrafish, Prickle1 regulates gastrulation CE movements and neuronal migration (Carreira-Barbosa et al., 2003). Loss of Pk1 function causes defective CE, enhances silberblick (slb)/wnt11 and pipetail (Ppt)/wnt5 phenotypes, and interferes with Wnt11-mediated rescue of slb. Gain-of-function of Pk1 also inhibits convergent extension, likely through downregulation of Dsh and inhibition of Fz7-dependent membrane localization of Dsh. Genetic interaction between pk1 and trilobite (tri)/strabismus contributes to caudal migration of cranial motor neurons and CE. These findings suggest that during zebrafish gastrulation, Pk1 interacts with noncanonical Wnt signaling to regulate CE, but does not function as a simple linear component (Carreira-Barbosa et al., 2003).

Quantitative live imaging in *Xenopus* neural plate epithelium (Butler & Wallingford, 2018) revealed asymmetric enrichment of PCP proteins and a strong correlation between PCP localization and actomyosin-driven junction contraction. Turnover of junctional PCP proteins correlated with contractile behavior, and these dynamics were disrupted when PCP signaling was altered. Together, these findings demonstrate a close relationship between PCP protein localization, actomyosin assembly, and polarized junction shrinking during CE.

Overall, these data indicate that Prickle functions as an active regulator, rather than a passive component, during CE in vertebrate neurulation.

Prickle and CI

In *Xenopus* prechordal mesoderm, diffusely localized cytoplasmic Prickle1 increases cortical F-actin levels and regulates cortical tension upstream of casein kinase II (Huang & Winklbauer, 2022). Diffuse and punctate Prickle1 show distinct effects on cortical F-actin, suggesting its multiple regulatory modes in cell intercalations.

A recent study (Matsuda & Sokol, 2025) showed that Prickle2 regulates apical junction remodeling and tissue fluidity during vertebrate neurulation. Prickle2 increases tissue fluidity by promoting apical junction remodeling in *Xenopus* embryos, mediated by a conserved Ser/Thr-rich region. This effect requires Rac1 and is associated with increased dynamics of C-cadherin and tricellular junctions. Prickle2 depletion results in accumulation of mediolaterally oriented intercalations, whereas overexpression promotes elongation along the anteroposterior axis.

Similarly, Prickle3 was shown to regulate radial intercalation in *Xenopus* ectoderm (Ossipova et al., 2015). Prickle complexes are enriched at the apical domain of intercalating multiciliated cells and are required for their intercalatory behavior. PCP signaling is therefore essential for cell intercalation during vertebrate morphogenesis.

All data discussed in this chapter are summarized in Table 1 below.

	Prickle1	**Prickle2**	**Prickle3**
Apical constriction (ApCo)	Wang et al., bioRvix, 2025	*This habilitation work, study II*	Not shown
Convergent extension (CE)	Takeuchi et al., Curr Biol, 2003.; Carreira-Barbosa et al., Dev, 2003.	Butler & Wallingford, eLife, 2018.	Not shown
Cell intercalation (CI)	Huang, Y., J Cell Biol, 2022.	Matsuda & Sokol, J Cell Biol, 2025.	Ossipova et al., Dev Biol, 2015.

Prickle and NT defects

Successful neurulation requires coordination at multiple levels, from molecular mechanisms to cellular behavior and tissue organization. In addition, environmental influences also contribute to this process (Bhandari & Thada, 2025; Copp et al., 2013). Disruption at any of these levels can result in NT defects, which represent common and clinically significant congenital malformations in humans (Copp & Greene, 2010). NT defects occur in approximately 1:2,000 births in Western countries, and in some reports up to 1 in 1,000 live births (Avagliano et al., 2019). They account for about 28% of all congenital malformations (**Fig. 12, left**) (Hassan, 2021). Intriguingly, congenital malformations in infants and young children cause more deaths than heart attacks, diabetes, and strokes combined in adults (Wallingford, 2019) (**Fig. 12, right**). Despite this, these numbers are not often discussed in public or scientific debates (Wallingford, 2019).

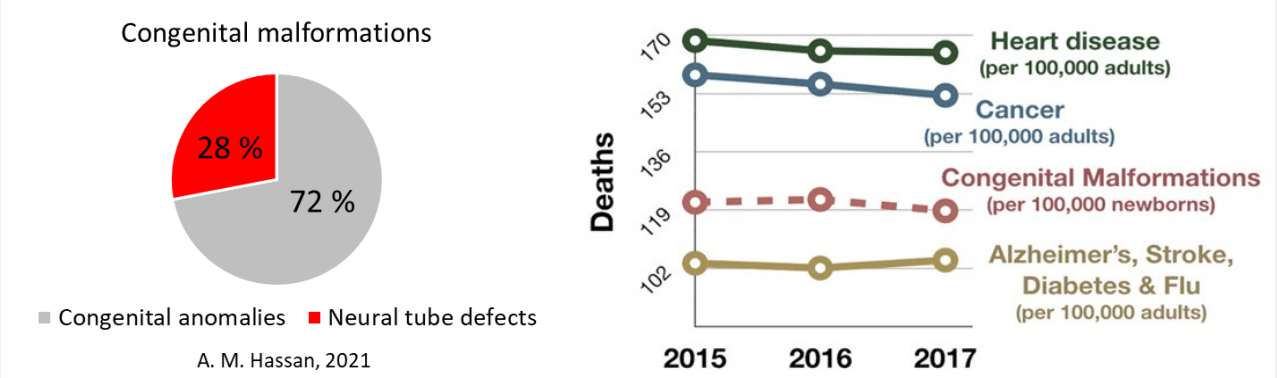

Figure 12. Left: Neural tube (NT) defects account for approximately 28% of all congenital malformations. Adopted from Hassan, 2021.
Right: Congenital malformations cause more deaths in children than Alzheimer's, stroke, diabetes, and flu combined cause in adults. Adopted from Wallingford, 2019.

To reduce the incidence of NT defects, only limited preventive strategies are available. Folic acid supplementation is one of the most established approaches and significantly lowers NT defects risk (Berry et al., 1999; Czeizel & Dudás, 1992; Quinn et al., 2024). However, the precise molecular mechanism of its protective effect remains unclear. Current hypotheses suggest roles in nucleotide synthesis, protection against bacterial liposaccharides, methylation reactions, and/or metabolism of cysteine (Cao, Xie, & Zhang 2022; D'Souza & Glazier 2022; Zhao et al., 2014). Further studies are needed to clarify how folic acid supports proper neurulation and to identify additional molecular pathways that could be targeted to further reduce the incidence of NT defects, including emerging regulators such as Prickle proteins.

Prickle proteins have been shown to be involved in several forms of NT defects, particularly *spina bifida*. *Spina bifida* refers to a group of NT defects affecting the spine along its length. It is defined by an opening in the vertebral column where meninges, spinal cord, or both can be exposed (Copp et al., 2015; Hassan et al., 2022). When the defect is covered by skin, it is termed *spina bifida occulta*. Such cases may remain asymptomatic, and affected individuals can live without major interventions (Alruwaili & Das, 2024). In the USA, the prevalence of spina bifida was 3.48 cases per 10,000 live births in 2016–2020 (Stallings et al., 2024).

Genetic studies suggest that PRICKLE genes directly contribute to *spina bifida*, as rare heterozygous missense mutations in human PRICKLE1 were identified in approximately 0.8% of 810 patients (Bosoi et al., 2011). In addition, both gain-of-function and loss-of-function of Prickle1 in *Xenopus* severely disrupted gastrulation and produced *spina bifida* embryos (Takeuchi et al., 2003). PRICKLE2 has been also proposed as a potential modifier of *spina bifida* risk in humans (Wen et al., 2010), although its role has been more extensively studied in the context of adult brain development and neurological disorders (Yang et al., 2025).

Together, these findings indicate that PRICKLE proteins are clinically relevant regulators of neurulation and warrant further investigation in the context of congenital malformations.

Now, after introducing the basic principles of PCP signaling, the Prickle protein family, vertebrate neurulation and NT defects, the reader is prepared to better understand the main concepts presented in my Studies I–III.

Study I

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

This review on Prickle paralogs in vertebrates forms the conceptual basis of the present habilitation thesis. To the best of my knowledge, it is the first focused review dedicated specifically to Prickle proteins since their discovery in the 1980s. Earlier reviews on PCP signaling mainly concentrated on core receptors such as Vangl and Frizzled, on cytoplasmic Dishevelled, or on downstream GTPase signaling. In those works, Prickle was usually mentioned only briefly and mostly in the context of *Drosophila* models.

Here, we first summarized the historical background of Prickle research, including the identification of all four vertebrate paralogs. We then performed a phylogenetic analysis demonstrating that orthologs across species are more closely related to each other than paralogs within the same species. For example, human PRICKLE1 is structurally and functionally closer to Prickle1 in *Mus* or *Xenopus* than to PRICKLE2 in human. This observation suggests that each paralog has an evolutionarily conserved and distinct function within vertebrates. In this part, we also critically evaluated subcellular localization patterns and the degree of sequence conservation, pointing to important functional links.

In the second section, we discussed the roles of Prickle proteins during vertebrate development. Evidence shows that Prickle actively participates in neurulation, body axis elongation, and organogenesis, including development of the eye, inner ear, heart, lungs, liver, kidney, and renal system.

The final section addressed pathological conditions. We summarized data linking Prickle proteins to cancer as well as non-cancer disorders, including progressive myoclonus epilepsy syndrome, autism spectrum disorders, and Alzheimer's disease. In the Future perspectives section, we identified several mechanistic gaps, such as limited understanding of paralog-specific functions, insufficient insight into cytoskeletal coupling, and incomplete knowledge about

regulation of PCP complex stability. These unresolved questions directly motivated the experimental work presented in the following publications.

The main contribution of this review is a critical re-evaluation of Prickle function in vertebrates, with special emphasis on human paralogs. By systematically comparing *Drosophila* and vertebrate findings, we show that the traditional view of Prickle as a passive polarity stabilizer originates mainly from static epithelial systems. Such an interpretation appears insufficient when applied to dynamic vertebrate morphogenesis.

Study II

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

This study examined the relationship between PCP signaling and actomyosin-dependent mechanics during vertebrate neurulation. Using proteomic and microscopy approaches, we demonstrated that PCP proteins are directly connected to cytoskeletal organization and force production, rather than functioning only as polarity-maintaining components. Our main aim was to define a novel molecular link between PCP-mediated signaling and the actomyosin contractility network. We focused on Prickle2 and analyzed its interaction with actomyosin.

The main contribution of this work is the identification of a direct functional connection between Prickle-containing PCP complexes and actomyosin regulation in the morphogenetically active *Xenopus* neural plate. We show that both over-expression and depletion of Prickle2 disturb coordinated cell movements and tissue mechanics typical for neurulation. These results provide a mechanistic explanation for classical PCP-associated neurulation defects. Our data challenge simplified PCP models in which cytoskeletal regulation is mainly attributed to the Frizzled-Dishevelled branch, as proposed by *Drosophila* studies, and instead support a more integrated model. We propose that PCP proteins influence actomyosin contractility through small Rho GTPase Rap1-dependent phosphorylation of myosin light chain (pMLC), a key molecular indicator of non-muscle Myosin II activation, mediated by CK1ε-dependent degradation of Rap1GAP2 (**Fig. 13**). Overall, this study positions Prickle proteins as key mediators that convert planar polarity information into mechanical output, bridging molecular polarity cues with tissue-level morphogenetic behavior during neurulation. This concept represents a central pillar of the scientific argument developed throughout this habilitation thesis.

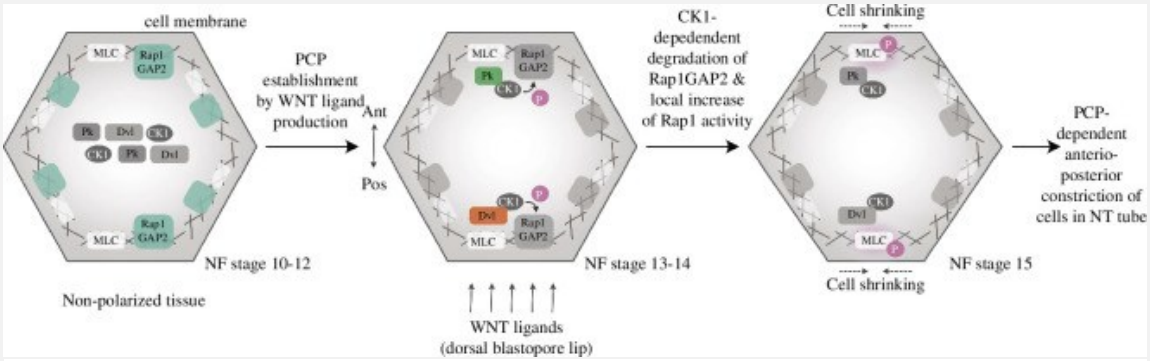

Figure 13. Schematic illustration of Prickle2 function during neurulation, highlighting its role in regulating apical constriction (ApCo).

Study III

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

Although Prickle1 and Prickle2 isoforms have received considerable attention, Prickle3 has remained less characterized and was often considered of minor importance. We did not fully agree with this assumption, as our initial experiments in human cell lines already indicated that Prickle3 shows distinct behavior compared to other family members.

In this study, we were the first to apply proximity biotinylation (miniTurboID) to analyze the interactomes of human PRICKLE1-3. This approach confirmed several previously reported interactors and, importantly, identified new candidates. Regarding subcellular distribution and paralog specificity, our proximity data showed that PRICKLE1-3 interactors are largely localized at the cell periphery, centrosomes, endomembrane compartments, and mitochondrial matrix. However, further work is required to clarify whether and how PRICKLE paralogs functionally communicate with mitochondria (see **Future perspectives**).

The study subsequently focused on Prickle3, a vertebrate paralog that had not been fully incorporated into canonical PCP models, mainly due to the reported absence of a Vangl-binding motif, VBM. Our data provide mechanistic evidence that Prickle3 regulates the stability of PCP receptor complexes by controlling Vangl1/2 protein turnover. We demonstrated that Prickle3 interferes with CK1 kinase-dependent phosphorylation and RNF43 ubiquitin ligase-mediated degradation of Vangl proteins, thereby regulating their abundance and signaling capacity at the plasma membrane. Importantly, we also identified a VBM (Vangl-binding motif) in PRICKLE3, which had previously not been recognized (**Fig. 14**).

These findings represent a conceptual advance in vertebrate PCP signaling. Classical models primarily focus on asymmetric protein localization, while regulated protein stability has received less attention. By identifying Prickle3-dependent control of Vangl turnover, we introduce

protein stability as a central regulatory layer of PCP signaling. This mechanism is particularly relevant in dynamic tissues, such as the *Xenopus* neural plate, where we experimentally demonstrated its importance.

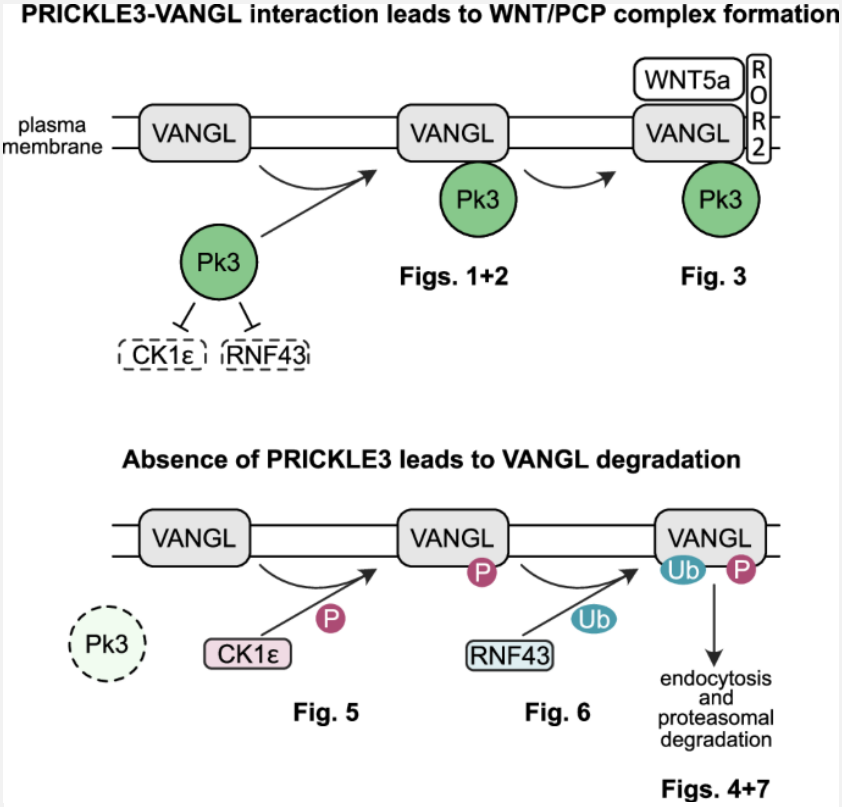

Figure 14. Schematic illustration of how Prickle3 stabilizes Vangl, for example during neurulation.

Our results also argue against simple functional redundancy within the Prickle family. Furthermore, the data suggest that Prickle3 may influence RNF43 activity, revealing a new regulatory axis within the non-canonical WNT pathway. Given that RNF43 is an E3 ubiquitin ligase with important roles in development and tumorigenesis, this connection may have broader pathological relevance.

Concluding synthesis

This thesis presents three publications. The first study summarizes current knowledge on Prickle1-4 and identifies key unresolved mechanistic questions. The second study demonstrates that Prickle2 links PCP signaling to cytoskeletal regulation and mechanical output during *Xenopus* neurulation. The third publication uncovers the role of Prickle3 in controlling PCP receptor stability and signaling dynamics in human cells and the *Xenopus* neural plate. Together, these studies establish Prickle proteins as active regulators rather than passive polarity stabilizers in vertebrate PCP systems such as neurulation.

Vertebrate neurulation is characterized by continuous tissue remodeling, junctional reorganization, and fluctuating mechanical forces. Under such conditions, signaling complexes must be dynamically regulated through protein turnover, trafficking, and post-translational modifications. In our Studies I-III, we showed that these processes are actively regulated by Prickle proteins and that they are relevant during neurulation. These features expose limitations of simplified PCP models derived from relatively static epithelial systems such as *Drosophila*.

Our data strongly support a model in which Prickle-containing complexes spatially organize and directly coordinate actomyosin activity, while stabilizing PCP receptor assemblies. By linking polarity information to cytoskeletal control, Prickle proteins enable robust morphogenesis under mechanically demanding conditions. This view creates a basis for further mechanistic work on polarity-dependent tissue organization in vertebrates, with a possibility to shed light on devastated conditions such as NT defects in humans.

Future perspectives

Based on our proximity data in Study III, certain Prickle isoforms have been found in mitochondria. This raises an important question of whether and how PCP proteins coordinate cytoskeletal remodeling with cellular energy production, and whether polarity cues directly influence mitochondrial and metabolic responses during development and in the context of actomyosin rearrangements. Addressing these issues in *Xenopus* embryos represents a major future direction of the Harnos lab.

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