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Coordination of cell behaviors during lumen remodeling in the zebrafish spinal neural tube

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Résumé de la thèse en français

Coordination des comportements cellulaires lors du remodelage de la lumière du tube neural chez le poisson-zèbre

Le développement de la moelle épinière a joué un rôle déterminant dans la compréhension du contrôle coordonné de la prolifération, de la différenciation et du patterning des progéniteurs neuronaux. Ces travaux ont permis de caractériser en détail les mécanismes moléculaires et cellulaires régissant la formation du tube neural, et en particulier le rôle central de la voie de signalisation Hedgehog (Hh) dans l'établissement de la spécification dorso-ventrale de la moelle épinière. Grâce à l'activité graduée de Sonic Hedgehog, un ligand de la voie Hh, émanant de la notochorde et de la plaque du plancher, des domaines de progéniteurs distincts sont spécifiés le long de l'axe dorso-ventral, chacun donnant naissance à des sous-types neuronaux définis. Cependant, alors que les programmes transcriptionnels et les cascades de signalisation ont été largement étudiés, le rôle du remodelage tissulaire et des forces mécaniques dans la neurogenèse de la moelle épinière en développement n'a pas été pleinement exploré.

À la fin de la neurogenèse, la lumière de la moelle épinière, où circule le liquide céphalo-rachidien, subit un remodelage radical. La hauteur de la lumière spinale est réduite pour former au niveau ventral le canal central, entouré de cellules souches quiescentes. Ce processus est conservé chez tous les vertébrés, chez les poissons comme chez les mammifères. Cette transition morphogénétique implique des comportements cellulaires actifs et coordonnés. Plus précisément, ce processus dépend de l'extrusion de la surface apicale des cellules progénitrices formant la bordure de la lumière et de l'extension des cellules gliales dorsales, appelées cellules de la plaque du toit, qui contribuent ensemble à remodeler l'architecture du tube neural. Malgré sa conservation et son importance probable pour le maintien à long terme de l'homéostasie et de la capacité régénérative de la moelle épinière, les mécanismes cellulaires et moléculaires contrôlant ce remodelage restent mal compris. Mon sujet de thèse porte sur comment ce remodelage est contrôlé et son importance pour le développement de la moelle épinière.

D'une part, j'ai utilisé le mutant *dzip1* chez le poisson-zèbre, qui ne parvient pas à réduire complètement la taille de la lumière, comme un outil génétique pour analyser les mécanismes sous-jacents à ce processus. Le mutant *dzip1* est dépourvu de cils primaires, des organites indispensables à la bonne transduction du signal Hh. J'ai étudié le comportement des cellules progénitrices associées à cette réduction inappropriée de la lumière dans ce contexte délétère. J'ai démontré que la signalisation Hh est altérée le long de l'axe dorso-ventral du tube neural du mutant *dzip1* et qu'elle a un impact direct sur le remodelage de la lumière. En étudiant les effecteurs de la signalisation Hh, les protéines Gli, j'ai identifié que l'équilibre entre les formes répressives et les formes actives de ces protéines est crucial pour établir un remodelage correct de la lumière du tube neural.

D'autre part, grâce à l'imagerie confocale *in vivo* à haute résolution associée à une analyse quantitative des images, j'ai décrit et étudié le couplage mécanique entre la réduction de la lumière et l'extension des cellules de la plaque du toit. Les données suggèrent que ces deux processus ne sont pas indépendants, mais qu'ils sont liés mécaniquement et fonctionnellement, ce qui soulève la possibilité que les forces générées lors de la réduction de la lumière puissent guider ou coordonner le comportement des cellules de la plaque du toit. En intégrant des approches génétiques et pharmacologiques, ce projet vise à affiner notre compréhension du développement du tube neural, afin d'inclure la morphogenèse et les régulations mécaniques dans les modèles actuels de développement de la moelle épinière.

Summary of the thesis in english

Coordination of cell behaviors during lumen remodeling in the zebrafish spinal neural tube

The development of the spinal cord has played a key role in understanding the coordinated regulation of the proliferation, patterning, and differentiation of neural progenitor cells. These works have led to a detailed characterization of the molecular and cellular mechanisms governing neural tube formation, and in particular the central role of the Hedgehog (Hh) signaling pathway in establishing the dorso-ventral patterning of the spinal cord. Through the graded activity of Sonic Hedgehog, a ligand of the Hh pathway, emanating from the notochord and the floor plate, distinct progenitor domains are specified along the dorso-ventral axis, each giving rise to defined neuronal subtypes. However, while transcriptional programs and signaling cascades have been extensively studied, the role of tissue remodeling and mechanical forces in the formation of the developing spinal cord has not been fully explored.

At the end of neurogenesis, the lumen of the spinal cord, where the cerebrospinal fluid circulates, undergoes a radical remodeling. The height of the spinal lumen is reduced to form the central canal at the ventral level, surrounded by quiescent stem cells. This process is conserved in all vertebrates, in fish as well as mammals. This morphogenetic transition involves active and coordinated cellular behaviors. More specifically, this process depends on the extrusion of the apical surface of progenitor cells forming the lumen border and the extension of dorsal glial cells, called roof plate cells, which together contribute to reshaping the architecture of the neural tube. Despite its conservation and likely importance for the long-term maintenance of homeostasis and the regenerative capacity of the spinal cord, the cellular and molecular mechanisms controlling this remodeling remain poorly understood. My thesis topic focuses on how this remodeling is controlled and its significance for spinal cord development.

First, I used the *dzip1* mutant in zebrafish, which fails to fully reduce the size of the lumen, as a genetic tool to explore the mechanisms underlying this process. The *dzip1* mutant lacks primary cilia, organelles that are essential for proper Hh signal

transduction. I have therefore studied the behavior of progenitor cells associated with this inappropriate reduction of the lumen in this deleterious context. I have demonstrated that Hh signaling is impaired along the dorso-ventral axis of the neural tube in the *dzip1* mutant and that it has a direct impact on lumen remodeling. By studying the effectors of Hh signaling, the Gli proteins, I have identified that the balance between the repressive and active forms of these proteins is crucial for establishing proper remodeling of the neural tube lumen.

Furthermore, using high-resolution *in vivo* confocal imaging combined with quantitative image analysis, I investigated the mechanical coupling between lumen reduction and the extension of roof plate cells. The data suggest that these two processes are not independent, but are mechanically and functionally linked, raising the possibility that the forces generated during lumen reduction could coordinate the behavior of roof plate cells. By integrating genetic and pharmacological approaches, this project aims to refine our understanding of neural tube development, incorporating morphogenesis and mechanical regulations into current models of spinal cord development.

Résumé de thèse vulgarisé pour le grand public en français

Comment notre moelle épinière prend-elle sa forme durant le développement ? C'est la question au cœur de ma thèse. Pour y répondre, j'ai étudié le poisson-zèbre : ses embryons sont transparents, se développent rapidement et sont facilement modifiables génétiquement. Grâce à la microscopie à haute résolution, j'ai observé en temps réel comment cellules et tissus se déforment pour donner naissance à la moelle épinière. En modifiant l'expression de certaines protéines ou en bloquant chimiquement certaines molécules, j'ai identifié des mécanismes qui orchestrent ces transformations. Mes résultats ont montré que deux tissus voisins interagissent mécaniquement pour se remodeler de façon coordonnée, sous l'influence d'une voie de signalisation appelée Shh. Comprendre les interactions et régulations à l'œuvre pendant le développement aide à déchiffrer les mécanismes fondamentaux qui façonnent la vie.

Simplified thesis summary for the general public in english

How does our spinal cord take shape during development? This is the central question of my thesis. To answer it, I study zebrafish: their embryos are transparent, develop rapidly, and are easily genetically modified. Using high-resolution microscopy, I observe in real time how cells and tissues reshape themselves to form the spinal cord. By modifying the expression of certain proteins or chemically blocking certain molecules, I identify the mechanisms that orchestrate these transformations. My results show that two neighboring tissues interact mechanically to remodel each other, under the influence of a signaling pathway called Shh. Understanding the interactions and regulatory processes at work during development helps us decipher the fundamental mechanisms that shape life.

INTRODUCTION

Understanding developmental processes has always been a challenge for biologists. How, from the fusion of two gametes, a complete multicellular organism emerges is a complex and fascinating question. A lot of different processes at molecular and tissue level have been discovered to be implicated during embryonic development. My PhD is focused on a 24 hour-long developmental process in zebrafish that implies cell deformation, signalling and cell-cell interaction. Studying this topic has enabled me to gain a deeper understanding of a wide range of biological concepts and knowledge. In the introduction, I will summarise numerous biological concepts, providing an overview of the current state of knowledge on the subject.

I. What is a lumen ?

A lumen is the inside space of a tube-shaped organ in the body. Blood vessels, intestines, lungs are examples of organs presenting a lumen filled with either blood, chyme or air respectively. These tubular organs acquire their shape during development; they are essential to compartmentalize and are vital for physiological exchange. Lumen formation occurs via diverse processes but in most cases it involves epithelial cells (Chung et Andrew, 2008).

I will start by briefly presenting epithelia and their characteristics, focusing on luminal epithelia.

A. An epithelium…

Lumens are often epithelial structures. An epithelium is a tissue made of polarized cells that line a surface and help divide the organism into compartments. Some epithelia cover the outside of the organism like the epidermis, while almost all metazoa contain internal hollow spaces or lumens, which are lined by a layer of epithelial cells (Datta, Bryant et Mostov, 2011). There are different types of epithelium depending on the shape and organisation of the cells composing it. We can classify them as squamous (large and flat cells), cuboidal (cells with similar height and width) or columnar (cells that are taller than they are wide) epithelium and each can be either a simple epithelium composed of

a monolayer or a stratified epithelium with multiple layers. Due to its elongated shape, the columnar epithelium can also be pseudostratified with a specific arrangement of nuclei apically or basally to their neighbors looking like different layers (Figure 1) (St Johnston et Sanson, 2011; Lemke et Nelson, 2021). The formation of a lumen from an epithelial structure is a process involving the remodeling of junctions, cell shape and cell organisation, in which numerous proteins and signalling molecules are implicated. (Bernascone, Hachimi et Martin-Belmonte, 2017).

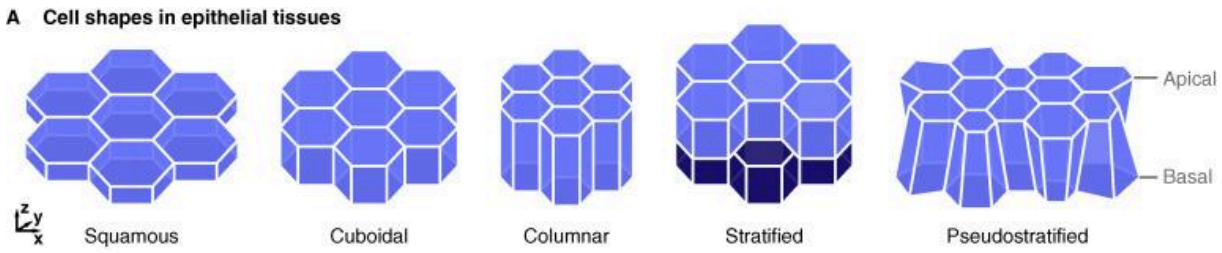

Figure 1. Schematic representation of typical cell shapes observed in epithelial tissues. *The classification of an epithelium depends on the shape of the cells that compose it as illustrated in this scheme. (From Lemke et Nelson, 2021)*

B. …with a specific polarity…

All epithelial cells are polarised and divided into three distinct domains. The apical domain, facing the outside of the tissue or the lumen, often contains specialized structures such as microvilli or cilia; the lateral domain adheres to the adjacent cells in the epithelium; and the basal domain contacts the basement membrane (Figure 2). All three domains can be recognized by their specific proteins localizing in each one. The apico-basal polarity of epithelia has been extensively studied but regulation of tissue-specific epithelium polarization is still under investigation.

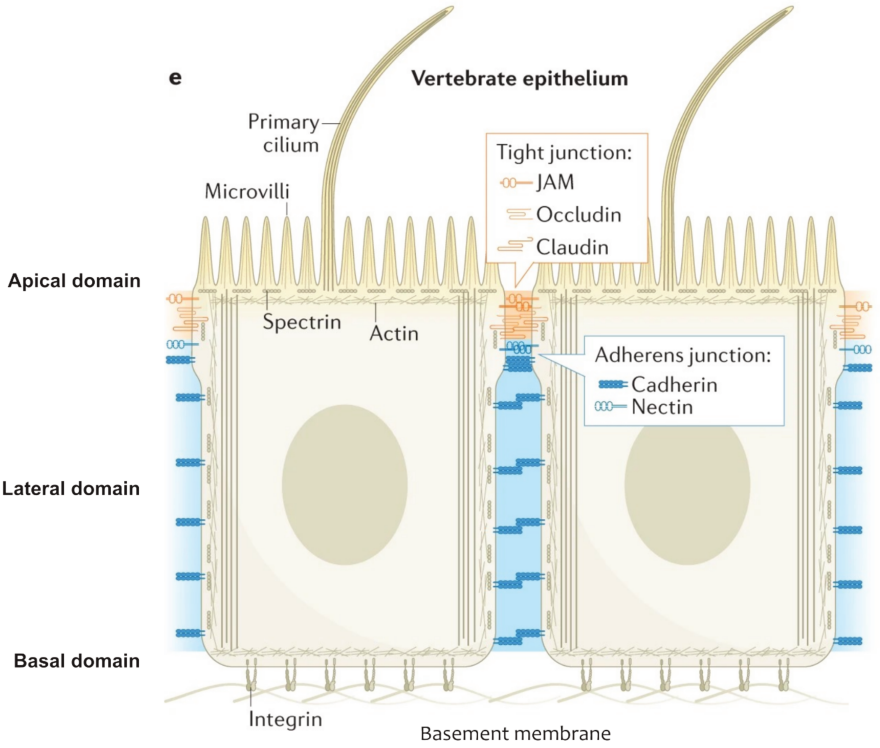

Figure 2. Structure of simple vertebrate epithelia. *Epithelium structures are formed with apical, lateral, basal domains. The apical domain features protrusions such as microvilli and cilia. Lateral domain is composed by tight and adherens junction and basal domain links the cells to the basement membrane by integrins. (Modified from Buckley et St Johnston, 2022)*

1. Apical polarity

The apical part of an epithelium secretes, senses and interacts with the environment that it faces. Signals from the outside can be transmitted via fluid (cerebrospinal fluid in the neural tube lumen, blood in blood vessels), vibration (inner ear) or gas (lung lumen) and are integrated by the apical domain of cells to transduce information (Shashikanth *et al.*, 2017).

Apical polarity emerges thanks to a reciprocal inhibition between the Par and Scribble complexes (Chen et Zhang, 2013). The apical side presents an enrichment of specific apical proteins forming the Par and Crumbs complexes, which initiate polarity; in their absence, polarity is disrupted. The presence of these proteins on the apical side allows for the structuring of the cell's apical region (Buckley et St Johnston, 2022). The Par complex is composed of Par6-aPKC-Par3 and the small GTPase Cdc42. One of the most important proteins of the apical region is atypical protein kinase C (aPKC). The exact

localization of all these proteins depends on the cell type and the context of observation. Par6-aPKC anchors to the apical side by binding to the transmembrane Crumbs protein. aPKC modulates the recruitment of proteins to the apical region by phosphorylation. Crumbs and aPKC mutants do not show apical polarity (St Johnston et Sanson, 2011). Overexpression of Crumbs in Drosophila endothelial epithelium increases the size of the apical domain (Rodriguez-Boulan et Macara, 2014). Crumbs proteins can be localized at the junctions forming the apical region but also within the microvilli found across the entire apical membrane (St Johnston et Sanson, 2011). To simplify, most observations describe the Crumbs complex forming a ring along the apical junctions between cells. This causes aPKC and Par6 proteins to exhibit the same pattern as they are linked to Crumbs. This specific localization of Crumbs complex defines an apical vertebrate marginal zone (VMZ, Figure 3) (Tan *et al.*, 2020). This localization also allows the Crumbs complex to interact with a lot of different proteins in the apical side but also the tight and adherent junctions on the lateral side.

In some contexts, apicolateral domain is defined as a transition zone in between apical and lateral domain. Par3 protein can be found in the apicolateral domain forming apical tight junction (Figure 3, Buckley et St Johnston, 2022). aPKC is known to repress lateral polarity proteins, such as Par3, from the apical site to ensure a proper cell-cell adhesion by enriching in junctional material.

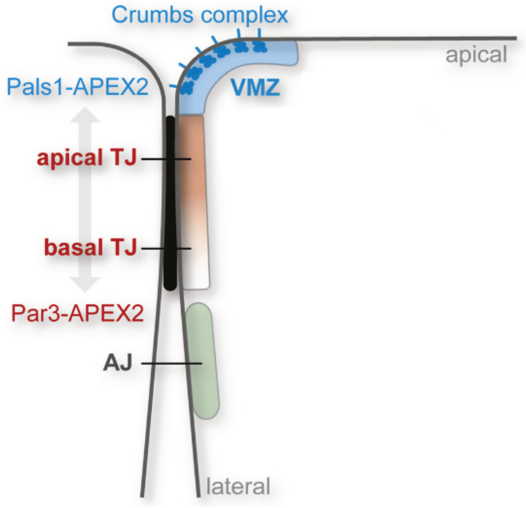

Figure 3. Apical-Lateral border organisation of polarity proteins in epithelial cells. *The apical-lateral border presents adherent junction (AJ), tight junction (TJ), and an apical vertebrate marginal zone (VMZ). All of these regions are defined thanks to specific molecules, the VMZ is defined by the Crumbs complex and TJ by Par3 protein. Graphical abstract modified from (Tan et al., 2020)*

2. Lateral polarity

Lateral junctions are essential to maintain epithelium and lumen integrity and impermeability. Cell-cell adhesion is composed of two main types of adhesion in vertebrates : the tight junction (TJ) and the adherent junction (AJ) (Figure 3). Near to the apical domain, the TJs, as their name refers to, are close links between cells and seal apical membrane connections essential for epithelium impermeability. In vertebrates, Par3 localises at the tight junction and binds the transmembrane proteins claudins and occulin forming them (Buckley et St Johnston, 2022). There are 27 mammalian claudin proteins; they can form homo- or heterodimers to link cells together (Baumholtz, Gupta et Ryan, 2017; Tsukita, Tanaka et Tamura, 2019). Claudins have been shown to be essential for morphogenesis and epithelial lumen formation. Their role during neural tube closure specifically in chicken has been extensively studied (Baumholtz *et al.*, 2017). Claudins bind cytoplasmic TJ proteins as the zonula occludens family (ZO-1, ZO-2, ZO-3) which link claudins to the actin cytoskeleton and Par complex (Figure 4).

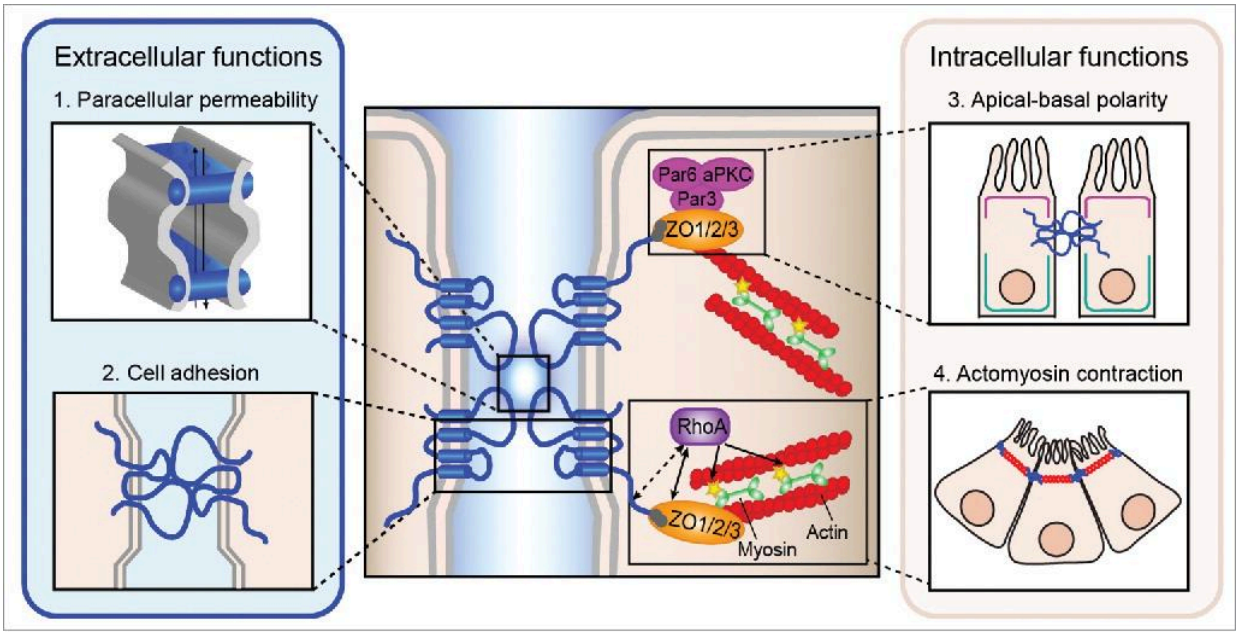

Figure 4 : Claudin's tight junction extracellular and intracellular functions. *Claudins regulate paracellular permeability by modulating ion and small molecule passage and cell adhesion through trans-interactions between adjacent cells. Intracellully, claudins bind to ZO-1/2/3 proteins to act on apical-basal polarity (Par6-Par3-aPKC) and actomyosin contraction via RhoA regulation of actin and myosin cables. (Baumholtz, Gupta et Ryan, 2017)*

Adherent junctions are more basal than tight junctions in vertebrates. The adherent junctions are formed by cadherins-catenin complexes (Harris et Tepass, 2010).

Cadherins are a large group of transmembrane proteins that bind as homodimers in a Calcium-dependent manner to link cells together, the homophilic binding has been demonstrated to be important to sort cell type (Gumbiner, 2005). On the cytoplasmic level they are known to bind mostly to the actin cytoskeleton thanks to its interaction with α-catenin but also to bind microtubules (Gumbiner, 2005; Harris et Tepass, 2010). The coupling of AJs to the cytoskeleton is essential for their function as the major mechanical link between cells. Adherent junctions and cadherins are essential for cell-cell adhesion and regulate cell rearrangement. Different papers present the response of cadherins to morphogen gradient, such as Shh and Wnt (Tsai et al., 2020; Ishitani, 2023). Both papers insist on the fact that Shh and Wnt by regulating cadherin production consolidate patterning robustness.

3. Basal polarity

Basal polarity is established by the Scribble complex, which includes Scribble, Discs large (Dlg), and Lethal giant larvae (LGL). This complex and the Par/Crumbs complexes mutually inhibit each other, creating two distinct cellular poles (St Johnston et Sanson, 2011; Buckley et St Johnston, 2022). Basal polarity also relies on integrin localization and interaction. Integrins are transmembrane proteins that connect the cell to the extracellular matrix (ECM), defining the basal membrane and linking to microtubules to regulate intracellular polarity. On the basal side, epithelial cells contact the ECM via integrins. This ECM-integrin connection enables cells to integrate external signals and sense environmental remodeling. The composition and stiffness of the ECM are crucial for tissue development, influencing cell differentiation, proliferation, and migration depending on the mechanical properties of the substrate (Xia *et al.*, 2023).

Epithelial cells can lose their polarity in specific contexts ; epithelial to mesenchymal transition (EMT) is one of the examples where they lose their apico-basal polarity and cell-cell adhesion to transit to mesenchymal cells which present migration properties. Some cancers are linked to epithelium apico-basal polarity loss. It is then really important to study polarity and epithelial structure to understand more the mechanism of cancer action (McCaffrey et Macara, 2011).

C. … that remodels.

Epithelial rearrangements are common. Cell movements within an epithelium are frequent, as are changes in shape. An epithelium can be enlarged or reduced due to multiple reasons : cell intercalation, cell division that will increase either the density or the size of the tissue or cell extrusion and cell death that will oppositely reduce density or size of the epithelium (Kuzmicz-Kowalska et Kicheva, 2021). On top of that, cell shape changes or cell rearrangements within the epithelium are important features. Apical junction remodeling is needed during epithelium rearrangements to maintain homeostasis and tissue integrity.

1. Epithelial growth and deformation

Adding cells is a common mechanism for tissue growth. Radial intercalation and cell division are two mechanisms that are commonly observed to induce tissue growth. Apical size increase also induces expansion of epithelia (Lemke et Nelson, 2021).

Cell division leads to the formation of two cells from one and increases the epithelium size, if the cell size does not shrink while dividing and if both cells maintain in the tissue. Oriented division along a specific axis allows expansion of the epithelium in one direction. Tissue growth in early development depends a lot on cellular division (Guillot et Lecuit, 2013).

Cell intercalation is described as local change of neighbors. We can distinguish two types of intercalation : lateral intercalation which is on-plane change of neighbors and radial intercalation which corresponds to off-plane cells introduced in-plane. Radial intercalation helps the growth of tissue by adding cells off-plane to the plane and increasing epithelium size at the same time (Figure 5). Adding cells in the middle of others remodels cell junctions and neighboring environments. It also induces growth of the epithelium, as adding cells expands the tissue size as shown in mammary gland tissue elongation or during Xenopus epiboly (Figure 5) (Stubbs *et al.*, 2006; Neumann *et al.*, 2018). Radial intercalation often transforms a multilayer epithelium to a bilayer or a monolayer epithelium (Szabó *et al.*, 2016; Neumann *et al.*, 2018).

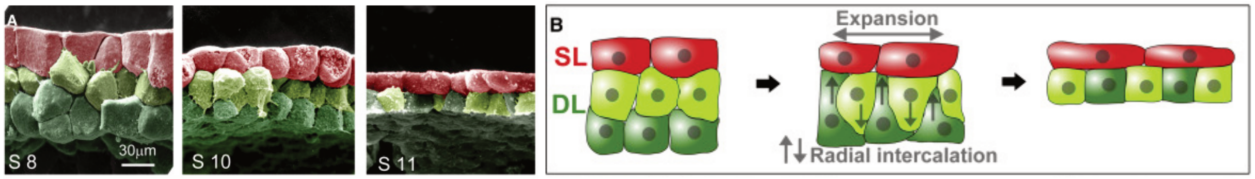

Figure 5. Radial Intercalation during Xenopus epiboly. *(A) Scanning electron microscope (SEM) images of blastocoel roof during epiboly in Xenopus embryos at stages 8, 10, and 11 showing radial intercalation. (B) Schematic illustration of the process of epiboly in Xenopus shown in (A). SL : superficial layer. DL : deep cell layer. (Szabó et al., 2016)*

Lateral intercalation, on the other hand, usually happens to reorganize a tissue. On-plane movements of cells like lateral intercalation implicate the transient formation of rosettes to allow change of neighbors (Figure 6). This reorganisation of the epithelium can deform it; lateral intercalation can induce a stretch in one specific direction and thus elongation of the tissue along one axis.

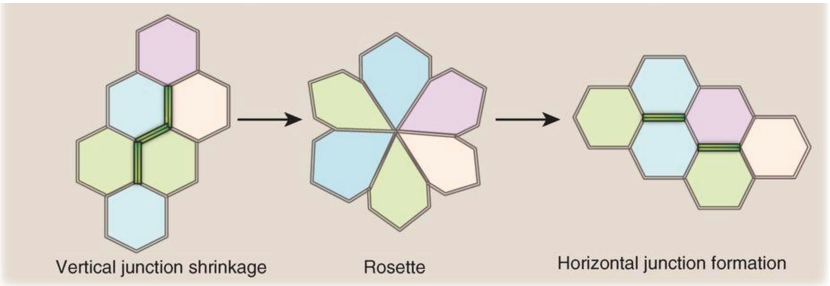

Figure 6. Epithelial remodeling by cell lateral intercalation*. Lateral intercalation remodeling is transiently forming rosettes to then exchange junction neighbors and leads to tissue elongation. (Guillot et Lecuit, 2013)*

The lateral and radial intercalation must depend on different processes but both reorganize cellular junction (Guillot et Lecuit, 2013; Szabó *et al.*, 2016). It has been published that the Par complex is implicated in radial intercalation by promoting centriole position and modulating microtubule stability (Werner *et al.*, 2014).

Overall, it is often a combination of these different processes that induces growth of an epithelium (Lemke et Nelson, 2021).

2. Cell suppression

On the other side, to reduce, shrink or maintain a luminal epithelium, cells can extrude, meaning that cells are pushed out from the epithelium inducing the reduction of the

structure. The apical surface of the extruded cell decreases and the neighboring cells compensate with their apical surface to form a new junction (Lemke et Nelson, 2021). Apoptosis or delamination may cause cell extrusion from an epithelial tissue.

If an epithelium has reached its final size but still pursues cell division, some cells have to leave the tissues to maintain the size and homeostasis of the epithelium. For example, in the mammalian gut epithelium, cells from the crypt divide and push the daughter cells into the epithelium to roll like in a treadmill inducing the cell that reaches the tip of the microvilli to extrude (Figure 7). This live extrusion phenomenon can also be found in the zebrafish fin (Guillot et Lecuit, 2013). Mechanical forces such as compaction can be involved in extrusion mechanisms (Figure 7). Otherwise, apoptotic cells can also be extruded. In both cases, apical rearrangements are needed to maintain epithelial integrity. Apoptotic cell extrusion and live cell extrusion present some specific regulation but happen in the same order.

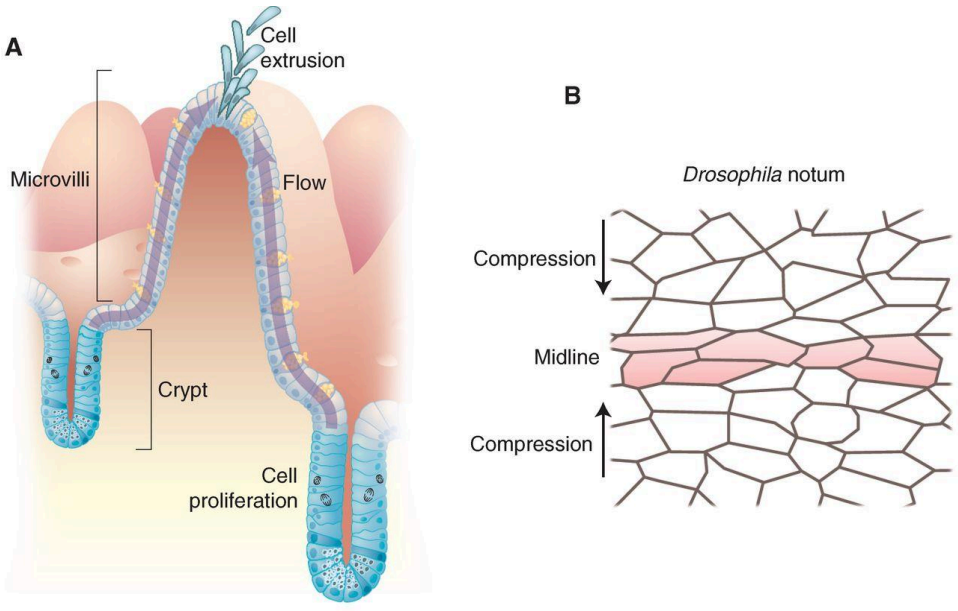

Figure 7. Cell extrusion examples. *a. schematic of mammalian gut epithelium rearrangement. b. drosophila notum epithelium cell extrusion, where pink cells in the middle are committed to extrude* [*(Guillot et Lecuit, 2013)*]

3. Lumen formation

Lumen morphogenesis is a form of epithelium reorganisation and involves a lot of cell rearrangements and polarization. Lumen formation can be induced by various ways, the

most characterized being wrapping, budding, cord hollowing, cell hollowing and cavitation (Figure 8) (Chung et Andrew, 2008; Baumholtz, Gupta et Ryan, 2017). Wrapping involves flat sheets of epithelial cells invaginating, with then more lateral cells fusion to form a closed tube. Budding begins with an existing epithelial tube where a group of cells migrates out and extends to form a lumen connected to the original tube. Cord and cell hollowing involve groups of mesenchymal cells condensing to form a single cell or multiple cell lumen. Cells undergo mesenchymal to epithelial transition to form the lumens that merge to generate a continuous epithelial tube. Finally, cavitation corresponds to condensation of mesenchymal cells to form a tubular structure. Cells in the center undergo apoptosis, while those on the periphery epithelialize resulting in formation of an epithelial tube. In each case, cells organize and form an epithelial structure around a cavity.

Once they are formed, lumens are still prompted to rearrange their epithelium, to change in size and organisation. The change of lumen shape or lumen size can involve a lot of different aspects. As all epithelium, rearrangement can be due to division, intercalation or extrusion.

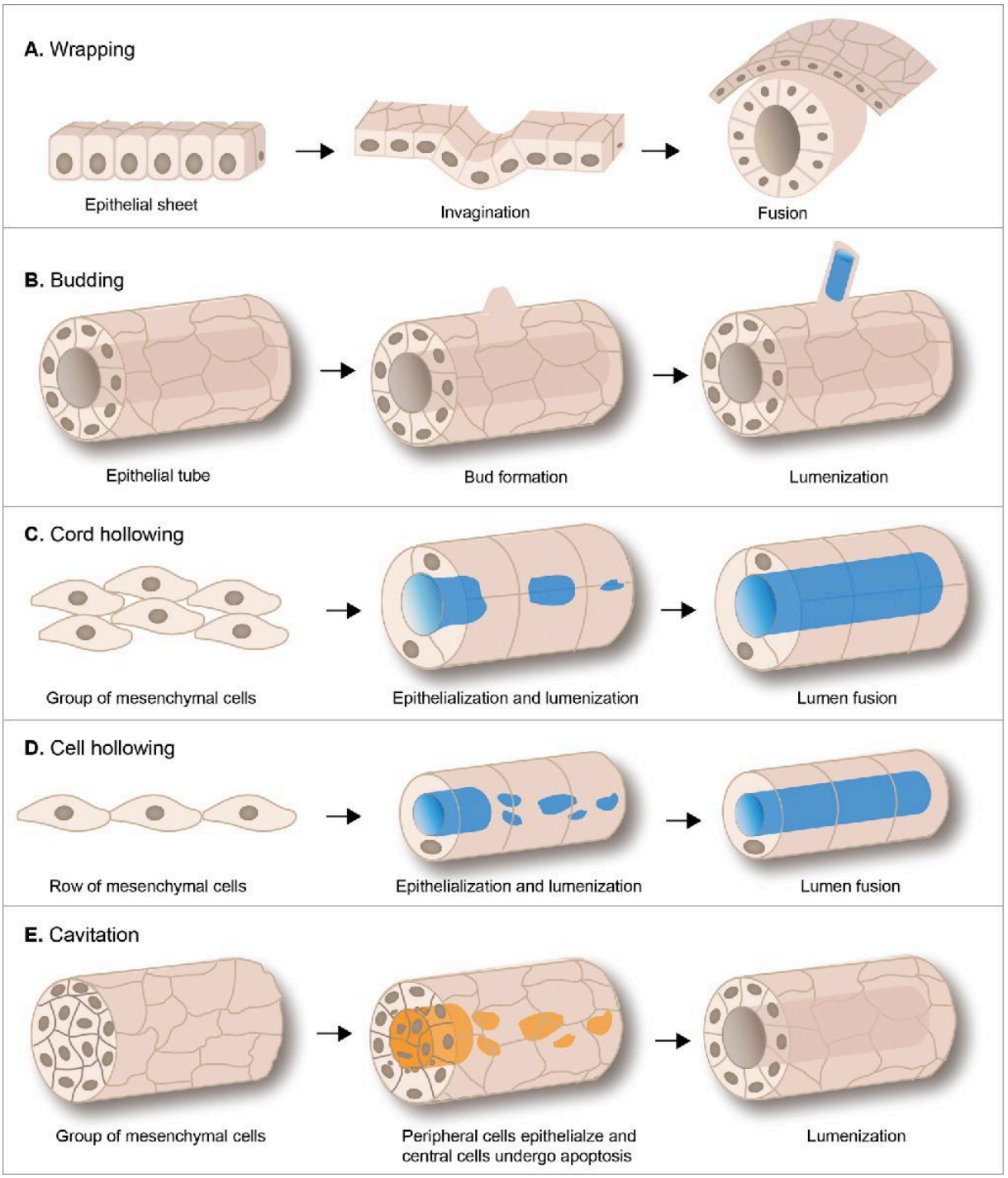

Figure 8. Mechanisms of epithelial tube formation*. Illustration of the 5 mechanisms that can give rise to epithelial tubes : wrapping (A), budding (B), cord hollowing (C), cell hollowing (D), cavitation (E). (Baumholtz, Gupta et Ryan, 2017)*

II. Neural tube development

At embryonic stages, all chordates share a dorsal cord filled with cerebrospinal fluid, called the neural tube, which is a pseudostratified epithelium (Figure 9) (Fame, Cortés-Campos et Sive, 2020; Adameyko, 2023). In vertebrates, the neural tube gives rise to the brain and spinal cord forming the central nervous system at the adult stage. The neural tube derives from the deformation of the neural plate. The mechanisms leading to the deformation of this organ vary among species (Araya *et al.*, 2016; Adameyko, 2023), and I will briefly detail them.

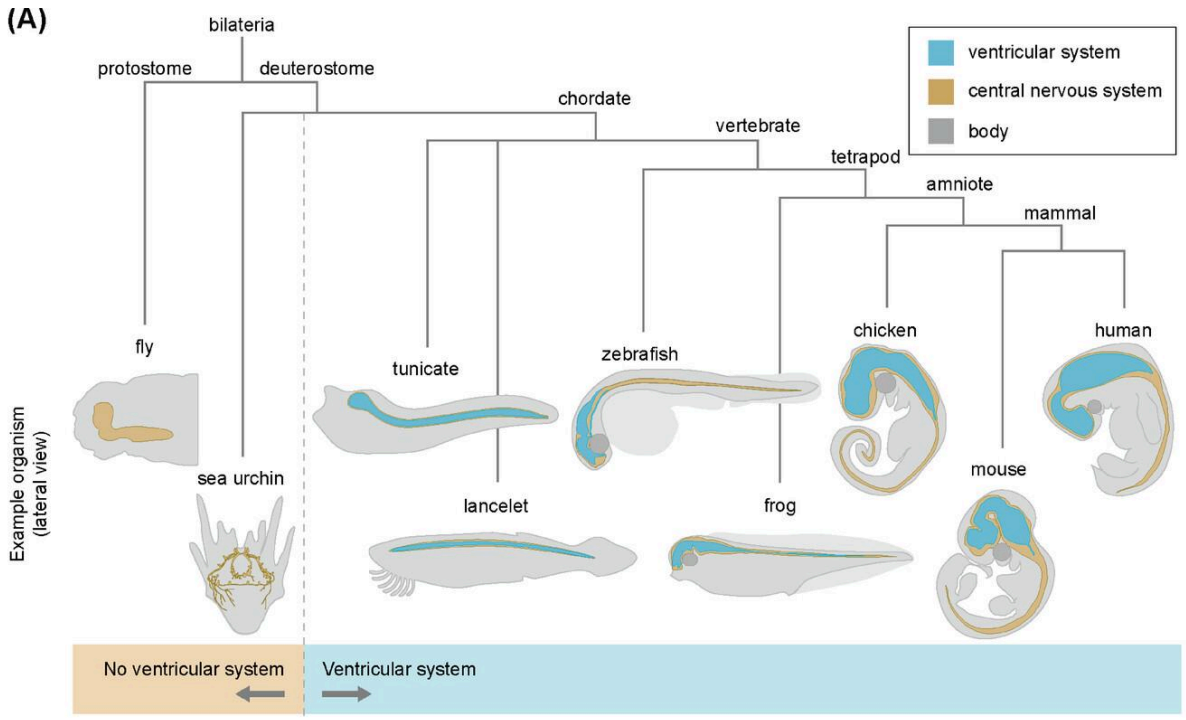

Figure 9. Phylogeny of bilaterian CNS and ventricular system organization. *The ventricular system in the central nervous system is a shared characteristic among chordates. (Fame, Cortés-Campos et Sive, 2020)*

A. Formation of the neural tube in different species

1. Primary and Secondary neurulation

Neurulation is a well studied phenomenon. It is the transformation process of the neural plate to form the neural tube. This process changes between species but all vertebrates start with a neural plate and form a neural tube (Araya *et al.*, 2016; Adameyko, 2023). Developmental biologists distinguish two types of neurulation in amniotes : the primary

neurulation corresponding to the first formation of the neural tube and the secondary neurulation describing the elongation of the neural tube in the most posterior/caudal region.

The neural plate is a monolayer epithelium or pseudoepithelium - depending on the species - derived from the ectoderm. Primary neurulation in amniote embryos happens by wrapping. The neural plate bends and allows the most lateral extremities of the epithelium to form the neural folds, which fuse to form the neural tube (Figure 10) (Martins-Green, 1988). In contrast, in zebrafish, neurulation happens by cavitation; invagination of the neural plate to form a tube, with two important intermediate structures, the neural keel and neural rod (Figure 10). (Schmitz, Papan et Campos-Ortega, 1993; Araya *et al.*, 2016; Adameyko, 2023).

Secondary neurulation in amniotes is the formation of the neural tube by cavitation in the caudal part of the embryo (Catala, 2021). During this process, mesenchymal cells organize to form a cord epithelium and create multiple lumen that further fuse to form the central lumen of the neural tube along the midline (Figure 10) (Araya *et al.*, 2016; Catala, 2021). This tube arising from secondary neurulation fuses with the neural tube formed by primary neurulation. Defects during either primary or secondary neurulation induce a set of anomalies called neural tube defects (NTDs), which occur approximately in 1 per 1,000 births worldwide (Copp et Greene, 2010; Chauhan *et al.*, 2024).

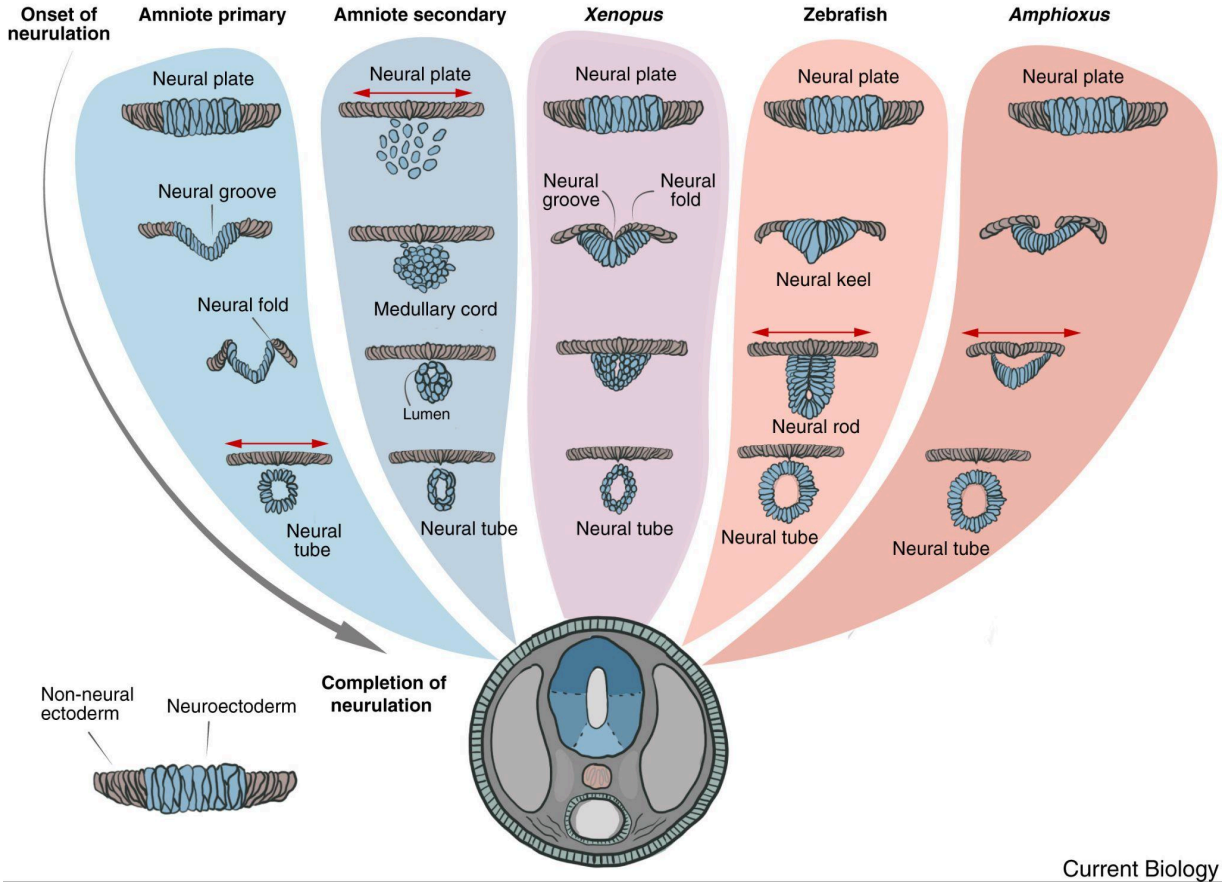

Figure 10. Primary and secondary neurulation in chordate groups. *(Adameyko, 2023)*

The neural tube goes from the anterior to posterior axis of the embryos in its dorsal part. In vertebrate embryos, the neural tube is patterned along its antero-posterior (AP) and dorso-ventral (DV) axes. Along the AP axis, two main structures will emerge : in the anterior part the future brain and in the posterior part the future spinal cord. Patterning along the antero-posterior axis of the spinal cord depends on the spatio-temporal activation of Hox genes, whose function sets up positional identity of neural progenitors and neurons (Hubert et Wellik, 2023). In the anterior part, the developing brain gets remodelled early into three main regions : the forebrain, the midbrain and the hindbrain, which then subdivide into several subregions and is patterned thanks to signalling centers called secondary organizers, which establish cell identity along the brain AP axis. The development of the brain is specific and differs from the spinal cord. Spinal cord lumen formation and DV patterning will be detailed below.

2. Lumen neuroepithelial structure

Both types of neurulation are forming a neural tube structure composed by different types of cells that are mainly radial glial cells after the initiation of neurogenesis. Radial glial cells are polarized cells organized into a pseudostratified epithelium. Their apical surfaces form the border of a lumen in the middle of the neural tube. Apical polarity proteins are thus essential for elaborating the neural tube lumen epithelium; mutants for cadherin, Par3, Par6, aPKC, Crumbs and other apical polarity proteins do not form a continuous neuroepithelium (Munson *et al.*, 2008; Symonds *et al.*, 2020). In amniotes, the bending of the neural plate epithelium maintains polarity while forming the neural tube whereas in zebrafish, epithelial-like cells of the neural plate are transitioning to epithelial and acquire polarity features (Tawk *et al.*, 2007; Araya *et al.*, 2016). In zebrafish, cells forming the transient neural rod, the structure before neural tube, divide with their nucleus aligned to the midline and form one daughter cell on each side of the midline (Figure 11). This mirror division, called C-division, is very characteristic of zebrafish neural tube formation (Tawk *et al.*, 2007; Araya *et al.*, 2016; Symonds *et al.*, 2020). It allows middle alignment of the apical surfaces of cells, followed later on by an antero-posterior shift leading to offset organisation of the apical surfaces of daughter cells (Figure 11) (Symonds *et al.*, 2020). In amniotes such as mice or chicken, a progenitor gives rise to two progenitors on one side of the neural tube (Saade *et al.*, 2013; Saade et Martí, 2025). Those specific division behaviors organize the neural tube lumen architecture.

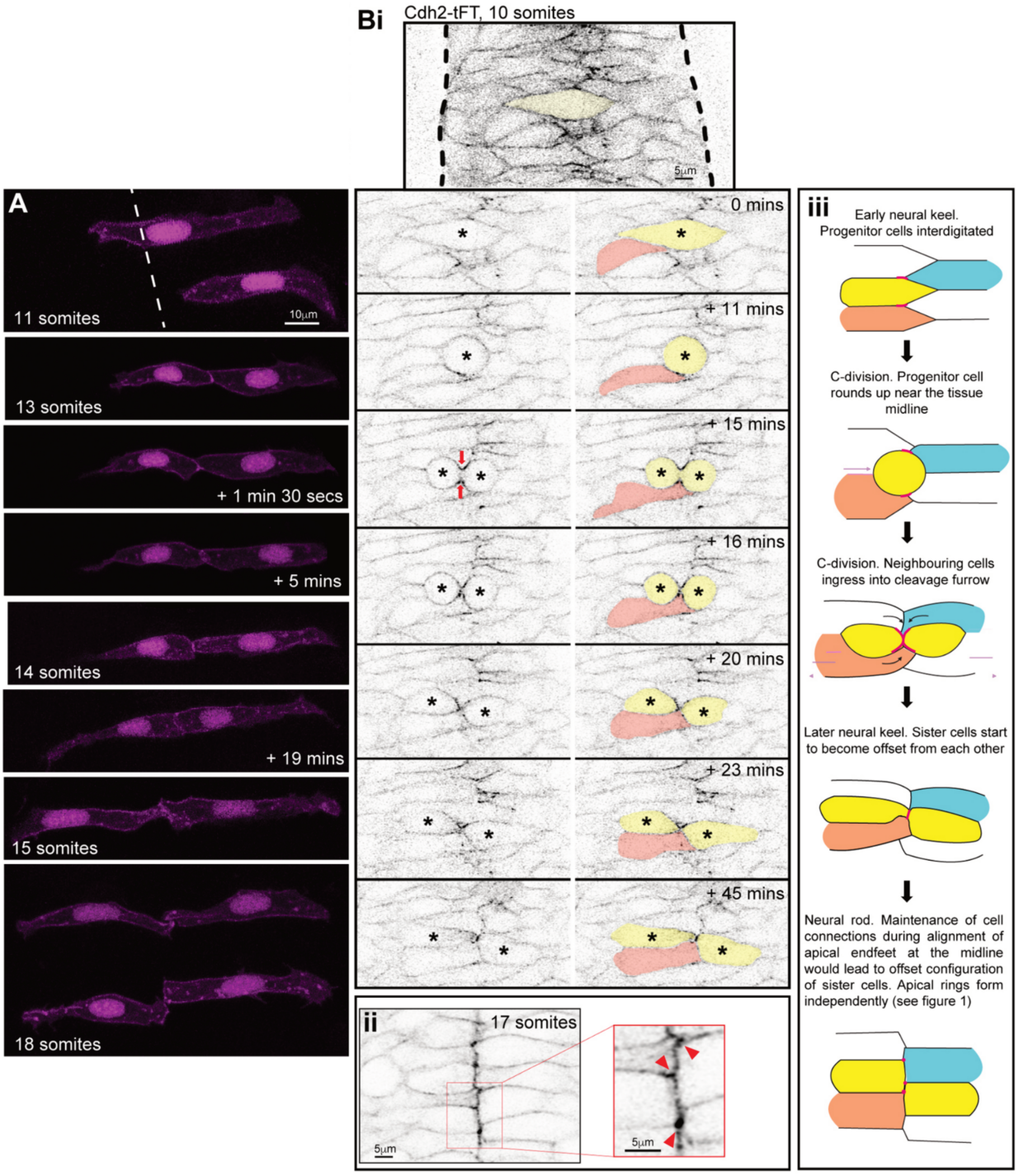

Figure 11. Formation and organisation of zebrafish neural tube midline*. Live-image and scheme of C-division of radial glial cells followed by daughter cells apical offset. A. Images from time-lapse movie projection in dorsal orientation of neuroepithelial cells in the hindbrain from 11 to 18 somites during C-division in wild-type embryos. Membrane and nuclei are mosaically labelled in magenta. At 18 somites we can observe that C-division results in pairs of sister cells attached across the tissue midline. B. Single z-planes from a timelapse movie of a C-division (yellow cell) starting at the 10-somite stage, from a Cdh2-tFT transgenic embryo. The neighbouring cell (pink cell) enters the cleavage furrow (red arrows) and connects the apical region of the daughter cell resulting from C-division, thereby creating an offset alignment of the*

apical surfaces along the midline. (Bii) 5 µm projection of 17-somite stage neural rod from a Cdh2-tFT transgenic embryo. Endfeet are aligned along a centrally located midline and présent apical offset. Cdh2-GFP is upregulated along the midline (red arrowheads in the magnified region). (Biii) Model depicting the co-ingression of neighbouring cells into the cleavage furrow during C-division (yellow cells) and the subsequent offsetting of sister cells from each other, which precedes apical ring formation, based on images similar to those in Bi. Red lines and dots represent high levels of Cdh2 associated with the dividing cell. (Symonds et al., 2020)

The lumen, corresponding to the central canal at later stages in the spinal cord and the ventricles in the brain, is created by neurulation. This structure is maintained at the adult stage and is the site of cerebrospinal fluid circulation (CSF). The CSF is essential for the development, protection, and homeostasis of the central nervous system. It is secreted by the neuroepithelium at early stages and by the choroid plexus at later stages of development and circulates in the brain ventricles and the spinal cord central canal but also in between the skull and the brain (Fame, Cortés-Campos et Sive, 2020). The CSF is well-known as a mechanical barrier that absorbs shocks and protects neural tissues. It has also a critical role as a medium for the transport of signaling molecules, hormones, growth factors, and morphogens. During neurogenesis, its composition evolves and supports the proliferation, differentiation, and migration of neural progenitor cells, thereby influencing brain morphogenesis and the establishment of neural circuits. Additionally, the CSF contributes to the regulation of adult physiological processes such as sleep, underscoring its vital functions throughout both developmental and adult stages (Czarniak *et al.*, 2023).

Zebrafish embryos allow live imaging tracking of cell and tissue organisation. In zebrafish, after the closure of the neural tube, the lumen organisation is asymmetric. In the hindbrain, the dorsal part is inflated while in the ventral part the progenitor apical domains of each hemisphere are attached to each-other via cadherin proteins (Guo *et al.*, 2018; Symonds *et al.*, 2020). Oppositely, in the spinal cord, the ventral part is inflated and allows CSF circulation while in the dorsal part the apical surface of progenitors remain close to each other (Thouvenin *et al.*, 2020). However, Thouvenin and al. described the presence of a smaller inflated region at the top of the dorsal part of the spinal cord, with a little CSF circulation (Thouvenin *et al.*, 2020). In mouse and chicken, no live imaging of the process has been reported; but the current knowledge, based on

sections of the neural tube, is an open lumen along the dorso-ventral axis with CSF circulation.

My PhD project is focused on spinal cord morphogenesis. In the following part, I will describe events happening in the trunk of embryos corresponding to the part of the neural tube that gives rise to the spinal cord at the adult stage. To start the description of the neural tube system, I will detail the different cell types composing the neural tube lumen epithelium. Because the neural tube is composed of many different cell types, I am describing only cells forming the lumen border and lumen composition by itself.

B. Which cells form the embryonic lumen border of the neural tube ?

At early stages, the neural tube is mainly composed of neural progenitors which are radial glial cells. Radial glial cells are recognised as morphologically, biochemically and functionally distinct from other neural cell types. They display an apical–basal polarity with on the apical side a ventricular attachment and on the basal side an end-foot anchored to the pial surface (Barry, Pakan et McDermott, 2014).

1. Neural progenitors

Neural progenitor cells are ciliated radial glial cells, forming the neuroepithelium surrounding the ventricular zone of the neural tube, called lumen in the spinal neural tube. They present a cilium on their apical surface bathing into the CSF of the lumen. They represent the progenitor pool giving rise to neurons and glial cells (oligodendrocytes and astrocytes) and serve as guides for migration of neurons from the ventricular surface to their final positions in the central nervous system (Ferent, Zaidi et Francis, 2020; Jurisch-Yaksi, Yaksi et Kizil, 2020). CSF circulating factors and secreted molecules such as morphogens and growth factors but also extracellular matrix (ECM) proteins impact progenitor behaviors by regulating their proliferation, differentiation or maintenance (Sagner et Briscoe, 2019). Neural progenitor proliferation and differentiation is a stereotyped process. While dividing, progenitors undergo interkinetic nuclear migration (INM), whereby the nucleus moves from apical to basal and back to the apical region of the pseudostratified epithelium depending on the stage of the cell cycle, with mitosis occurring close to the apical surface (Figure 12) (Saade et Martí, 2025). This specific oscillation of nuclear position is important for cell rearrangement in

the crowded neuroepithelial tissue. A lot of cytoskeleton reorganisation occurs during cell division and INM, including a retraction of cilia during centrosome duplication in S phase. During neurogenesis, progenitors undergo three main types of divisions: progenitor-progenitor (PP), progenitor-neuron (PN) or neuron-neuron (NN) divisions. In textbooks it seems that at early stages the pool of progenitors amplifies via PP division; then during neurogenesis these are replaced by PN divisions, which produce neurons while maintaining the pool of progenitors, while at the end of neurogenesis more NN divisions are observed (Saade *et al.*, 2013; Kuzmicz-Kowalska et Kicheva, 2021; Mida *et al.*, 2026). After neurogenesis, some neural progenitors persist and continue dividing, no longer differentiating into neurons but into glial cells. This phenomenon, called gliogenesis, gives rise to the two main glial cell types of the spinal cord: astrocytes and oligodendrocytes (Neely et Lyons, 2021). Glial cells are non-neuronal components of the CNS that outnumber neurons in many regions and have been shown to be essential for a wide range of functions, including metabolic support, maintenance of ion and neurotransmitter homeostasis, myelination of axons, formation and refinement of synapses, and immune surveillance of neural tissue (He et Sun, 2007; Neely et Lyons, 2021).

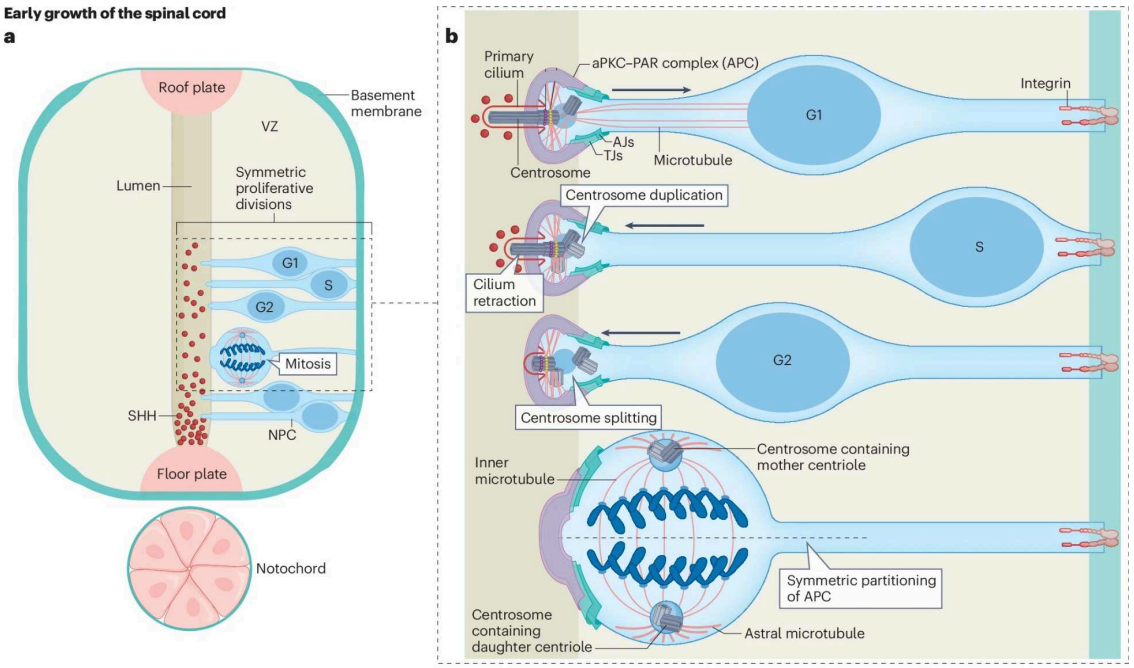

Figure 12. Steps of the cell cycle of epithelial cells in the developing spinal cord*. Scheme of cell division and interkinetic nuclear migration (INM) in the trunk neural tube with description of microtubule and cilia reorganisation. Modified from (Saade et Martí, 2025)*

At the end of the production of all CNS cells, some neural progenitors persist beyond embryonic stages as quiescent radial glial cells (Becker, Becker et Hugnot, 2018). At the adult stage, the cells forming the final spinal cord lumen, the central canal, are ependymal cells - ciliated cells lining the ventricles of the central nervous system. (Becker, Becker et Hugnot, 2018; Jurisch-Yaksi, Yaksi et Kizil, 2020; Xie et Li, 2024). In zebrafish, after injury the spinal cord recovers its motor function in 2 weeks after recruiting the ependymal stem cell niche (Ribeiro *et al.*, 2017; Becker, Becker et Hugnot, 2018). Ependymal cells present different properties in vertebrates after a spinal cord lesion; in mammals, ependymal cells are able to proliferate but only regenerate glial cells while regeneration of neurons and complete recovery is observed in axolotl or zebrafish (Becker, Becker et Hugnot, 2018). Investigating central canal cells during development helps to better understand the adult behavior of ependymal cells after injury and the pathways differently recruited in healing or complete regeneration (Spassky *et al.*, 2005; Becker, Becker et Hugnot, 2018; Rodrigo Albors *et al.*, 2023).

a. Dorso-ventral patterning in the neural tube

As described above, during development, neural progenitors first proliferate to expand the progenitor pool and increase tissue size, and then differentiate, in an organized manner, into the wide variety of neuronal cell types forming the central nervous system. (Kuzmicz-Kowalska et Kicheva, 2021; Mida *et al.*, 2026). The specification, differentiation and proliferation of progenitors has been well studied and depends on the fine regulation of morphogen diffusion and signalling pathways along the dorso-ventral axis of the neural tube such as the Hedgehog (Hh), Wnt and Bone Morphogenetic Protein (BMP) pathways (Sagner et Briscoe, 2019; Kuzmicz-Kowalska et Kicheva, 2021). These signals are secreted by floor and roof plate cells from the ventral and dorsal region, respectively (see below) and spread to form a concentration gradient across a tissue. Progenitor cells interpret their position by sensing the local concentration of this molecule, triggering different gene expression programs depending on whether they are close to or far from the source. The combination of these signals induces the specification and then the differentiation of progenitors into different types of motor neurons in the ventral part and sensory neurons in the dorsal part of the neural tube (Sagner et Briscoe, 2019). The different domains are determined thanks to the

graded signals that generate sharp, reproducible boundaries between cell fates along the DV axis of the neural tube, also called French Flag Model. The differentiation of progenitors is regulated medio-laterally : the cell body of progenitors localized in the medial part of the neural tube moves laterally following the interkinetic movement to divide, then the newborn neuron detaches and migrates in the lateral part of the neural tube (Saade et Martí, 2025). This process creates a layer of progenitors in the center of the neural tube, called the ventricular zone (VZ), surrounded by a layer of differentiated neurons called the mantle zone (MZ) (Figure 13). Before progenitors start to differentiate, the neural tube is composed of subpopulations of progenitors expressing basic-helix–loop–helix (bHLH) families of transcription factors (TFs) determining the DV identities. In the ventral part five progenitor domains are characterized : p3, pMN, p2, p1, p0; and six in the dorsal part : dP1, dP2, dP3, dP4, dP5, dP6 (Le Dréau et Martí, 2012). All progenitors expressed Sox2 on top of the specific TFs that predisposed their identity; dorsal progenitors express Pax7, Olig3 or Lbx1 while ventral progenitors express Nkx2.2, Nkx6.1, Nkx6.2 or Olig2 (Figure 13). Each progenitor domain differentiates to specific subsets of neurons forming along the dorso-ventral and medio-lateral axis restricted progenitors and differentiated neurons population domain (Figure 13). This stereotyped progenitor pattern is controlled by the activity of a combination of signaling pathways along the dorso-ventral axis of the neural tube that I will present in the following parts. In addition to this spatial control, the timing of pathway activity is equally important, allowing a single progenitor domain to sequentially adopt different fates. This temporal dimension allows a single progenitor domain to sequentially generate different cell types: the ventral pMN domain, for instance, first produces motor neurons during neurogenesis before switching to the production of oligodendrocyte precursor cells (OPCs) during gliogenesis (Danesin *et al.*, 2006). This ability of a single domain to change fate over time illustrates the dynamic nature of neural tube development, in which spatial and temporal patterning act together to diversify the cell types of the central nervous system (Ge *et al.*, 2023).

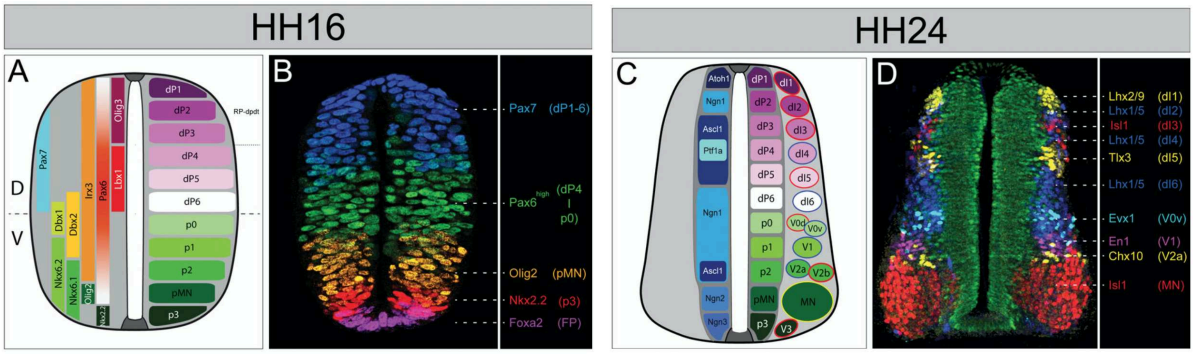

Figure 13. Neural tube identities at different stages*. Schematic representation of a transversal section of a chick neural tube at different stages with photographic reconstruction of immunostainings. The subpopulation of progenitors are conserved in vertebrates. (Le Dréau et Martí, 2012)*

b. The Notch pathway regulates progenitor behavior

Notch is known to be important to regulate proliferation, survival, self-renewal and differentiation of neural stem cells (Gaiano et Fishell, 2002; Lathia, Mattson et Cheng, 2008). Its main role is to maintain a proliferative progenitor state; in absence of Notch, progenitors differentiate. Notch-Delta regulation needs cell-cell interaction, contrary to other signaling pathways for which ligands can diffuse in the tissue. In vertebrates, four paralogs of the notch receptor gene (Notch 1–4) and five ligands (Jagged 1 and 2; Delta 1, 2 and 3) have been identified. Both receptor and ligand are membrane bound proteins expressed by neural progenitors (Kopan et Ilagan, 2009). When one of the membrane ligands binds to the Notch receptor of a neighboring cell, it activates multiple cleavages of the Notch receptor (Figure 14a). The cleavage by the γ-secretase complex releases the Notch intracellular domain (NICD), which translocates to the nucleus and associates with the transcription factor CSL complex and and the co-activator MAM (Figure 14a) (Bray, 2016). The NICD-CSL-MAM complex activates the transcription of target genes, most notably the Hes family of transcriptional repressors, leading to inhibit the expression of a set of differentiation genes like Neurogenin (Figure 14b). Thus, the Notch-receiving cell remains a progenitor, while the cell expressing the ligand differentiates into a neuron. This process is governed by a mechanism known as lateral inhibition, in which stochastic fluctuations in Delta ligand expression among an initially equivalent population of progenitor cells confer a competitive advantage to certain cells. A cell expressing higher levels of Delta activates Notch signaling in its neighbors, which in turn represses Delta expression in those adjacent cells. This lateral negative feedback

loop self-amplifies until a stable binary state is reached: the "winning" cell maintains high Delta and low Notch activity and commits to a neuronal fate, while its neighbors, locked in a high Notch and low Delta state, are prevented from differentiation (Figure 14b). This contact mechanism ensures that only a subset of progenitor cells differentiate into neurons, while others remain in a proliferative state (Figure 14b). To be more specific, the duration and the level of Notch signalling seems to control cell fate specification in certain neurones types (Shin *et al.*, 2007; Jacobs *et al.*, 2022).

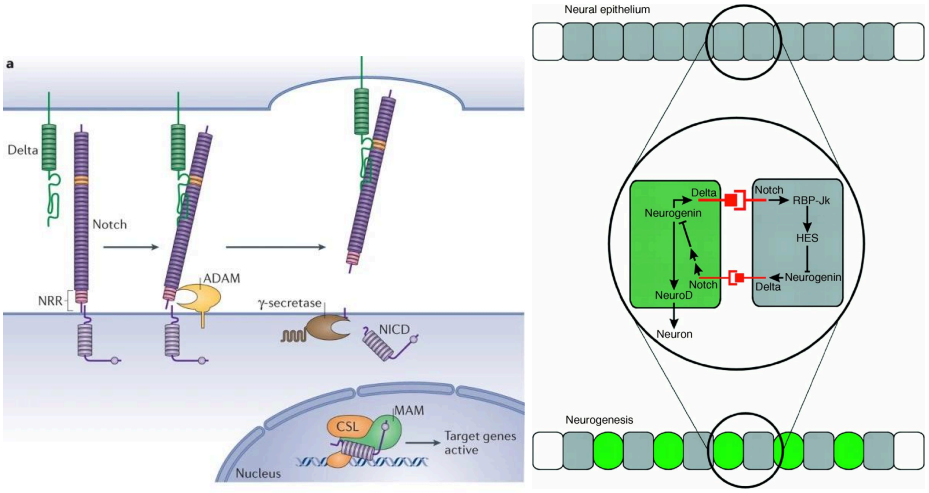

Figure 14. Notch signaling pathway in progenitor cells. *On the left, summary of canonical Notch ligands : Delta (green) binding to Notch receptors (purple). Notch receptors undergo two cleavage events, the first in the extracellular part by ADAM that detaches Notch receptors from the cell and the second by γ-secretase that releases the Notch intracellular domain (NICD). Once inside the nucleus, NICD binds to CSL (CBF1–Suppressor of Hairless–LAG1) and MAM (Mastermind) to activate the transcription of target genes (Bray, 2016). On the right, schematic representation of the Notch signaling between two neural cells after progenitor division. A cell expressing higher levels of Delta activates Notch signaling in its neighbors, which in turn represses Delta expression in those adjacent cells. Cells expressing the pro-differentiated gene Neurogenin will increase its expression of Delta ligands that reinforce Neurogenin expression. The neighboring cell which thanks to Delta-Notch interaction will promote hes gene expression that inhibits neurogenin expression and promotes the progenitor identity (Tanabe et Jessell, 1996).*

In the neural tube, studies highlight the importance of Notch to mediate spinal cord morphogenesis. Indeed Mindbomb1 zebrafish mutants (mib1-/-), defective for an E3-ubiquitin ligase that regulates Notch activation, present no apical polarity organisation of the spinal progenitors normally lining the lumen (Sharma *et al.*, 2019). The authors conclude that Notch activity is important for progenitor maintenance as well as for the establishment of neuroepithelial tissue architecture in the neural tube.

They propose that Notch might act upstream of apical polarity complexes, given the failure of upregulation of Crumbs 1 and 2 but also of Par6 proteins in *mib1* mutant.

Aligning with its role in progenitor maintenance, the Notch pathway has been shown to be important for neuronal delamination. When progenitors differentiate into neurons, the newborn neurons detach their apical domains, a process known as delamination (Figure 15). In chicken, the Notch signaling pathway is active in all progenitors of the neural tube and its inhibition is critical for neurons to commit to differentiation (Baek *et al.*, 2018). Asymmetric division, that generates a progenitor and a neuron, differently segregates apical polarity complexes and Notch activity in the two daughter cells leading to the delamination and differentiation of one of them (Alexandre *et al.*, 2010; Das et Storey, 2012; Moore et Alexandre, 2020). The apical surface of the differentiating progenitor reduces and then decreases apical junction protein level, such as N-cadherin and the Par complex, in order to delaminate (Das et Storey, 2014b). Consistent with its early role in neuroepithelium polarisation, it has been suggested that Notch regulates this decrease in apical surface and the delamination of cells from the neuroepithelium of the neural tube. The delamination process is still under a lot of investigation. In the chicken embryo, it has been observed that during delamination, neuronal progenitors undergo apical abscission and cut out the apical part containing primary cilia (Das et Storey, 2014b; Tozer et Morin, 2014; Toro-Tapia et Das, 2020; Saade et Martí, 2025).

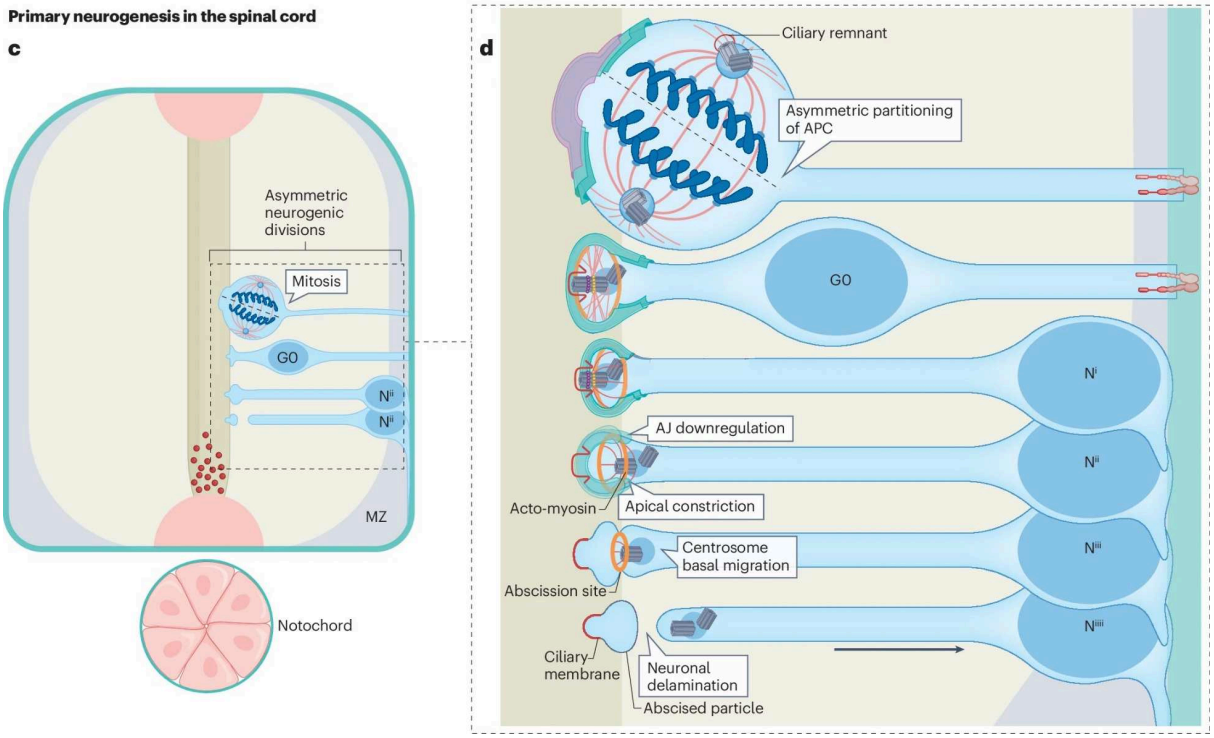

Figure 15. Delamination of a cell committed to differentiate in neuron in the spinal neural tube. *Scheme of asymmetric division during neurogenesis inducing neuronal delamination of the new born neurons (Saade et Martí, 2025)*

Neural progenitors constitute the vast majority of cells lining the lumen of the neural tube at early developmental stages, positioning them as central players in neural tube development. Through their capacity to self-renew and differentiate, and by integrating opposing morphogen gradients, notably Shh from the floor plate and BMP/Wnt signals from the roof plate, neural progenitors generate most of the cellular diversity of the central nervous system in a spatially and temporally coordinated manner. In the following section, I will present the two other specialized cell populations that define the dorsoventral boundaries of the neural tube lumen: the roof plate cells and the floor plate cells.

2. Roof plate cells

a. Description of the roof plate cells

On the dorsal part, the neural tube is formed transiently by pre-migratory neural crest cells and roof plate cells. The roof plate cells are the polarised cells forming the dorsal midline of the neural tube after neural crest cell migration. Neural crest cell migration

has been extensively studied. This pool of cells migrates early during development to give rise to many different cell types, including the peripheral nervous system (Theveneau et Mayor, 2012; Szabó et Mayor, 2018; Rekler et Kalcheim, 2021; Shinozuka et Takada, 2021). In avian, migratory neural crest precursors are predefined with early fate restrictions excluding roof plate cells (Krispin *et al.*, 2010), while in mammals, neural crest cells seem multipotent, and the same population of cells gives the migratory neural crest cells and the roof plate cells that stay in the dorsal midline of the neural tube (Baggiolini *et al.*, 2015; Kalcheim et Douarin, 1986). Roof plate cells are radial glial cells that scaffold the neural tube and secrete morphogens (Chizhikov et Millen, 2005). They are polarised cells: their apical region forms the roof of the neural tube lumen and their basal part corresponds to the ceiling of the neural tube. This cell population is present all along the antero-posterior axis of the neural tube at the dorsal midline and is conserved in vertebrates. At the adult stage, the roof plate cells are mainly radial glial cells and maintain their scaffolding role (Becker, Becker et Hugnot, 2018; Rekler et Kalcheim, 2021).

b. The role of roof plate cells in dorsal patterning through BMP and Wnt pathways

Dorsal neural tube formation requires various signals necessary for cell type specification, proliferation and differentiation, from neural crest to neurons (Lee et Jessell, 1999). These processes depend both on the exposure time and on the intensity of the signal received by the cells. Roof plate cells are essential for patterning the dorsal part of the neural tube because they secrete BMP and Wnt ligands (Bond, Bhalala et Kessler, 2012). Genetic ablation of roof plate cells using Gdf7-DTA mice leads to an absence of dI1 to dI3 specification, corresponding to the loss of the more dorsal interneurons (Figure 16) (Lee, Dietrich et Jessell, 2000). Gdf7 is a BMP family member and Gdf7 null mouse embryos lose dI1 interneurons, demonstrating the major role of roof plate cells and BMP signalling in dorsal patterning (Lee, Mendelsohn et Jessell, 1998). Dreher mutant mice, which are mutated in the *Lmx1a* gene, fail to develop a roof plate tissue, and present a defect of dI1 interneuron differentiation and a general defect of specification of dorsal cell fates in the CNS (Figure 16) (Millonig, Millen et Hatten, 2000; Millen, Millonig et Hatten, 2004). Meanwhile, ectopic expression of Lmx1a in

chicken induces ectopic roof plate cell differentiation and ectopic induction of dI1 interneurons but not dI2 and dI3 interneurons (Figure 16) (Chizhikov et Millen, 2004). Lmx1a is a target of BMP signalling, showing an essential role of BMP to induce roof plate cell specification by regulating Lmx1a expression (Chizhikov et Millen, 2004). BMP signaling thus has a two-step function in dorsal patterning: First, the secretion of BMP ligands by the ectoderm leads to roof plate cell specification; then, roof plate cells themselves secrete BMP ligands that diffuse as a gradient in a spatio-temporal manner to allow dorsal patterning (Tozer *et al.*, 2013; Rekler et Kalcheim, 2021).

Wnt signalling is also an important regulator of dorsal neural tube patterning. The Wnt ligands Wnt1 and Wnt3a are expressed in the dorsal midline region by roof plate cells in both mouse and chick developing spinal cord (Hollyday, McMahon et McMahon, 1995; Megason et McMahon, 2002; Chizhikov et Millen, 2005). *Wnt1/Wnt3a* double mutant mouse embryos display a severe reduction in the number of dI1-3 dorsal interneurons accompanied by an increased number of more ventrally located interneurons (Muroyama *et al.*, 2002). These results suggest that through the Wnt pathway roof plate cells are responsible for the proliferation and specification of the dorsal neural tube (Muroyama *et al.*, 2002).

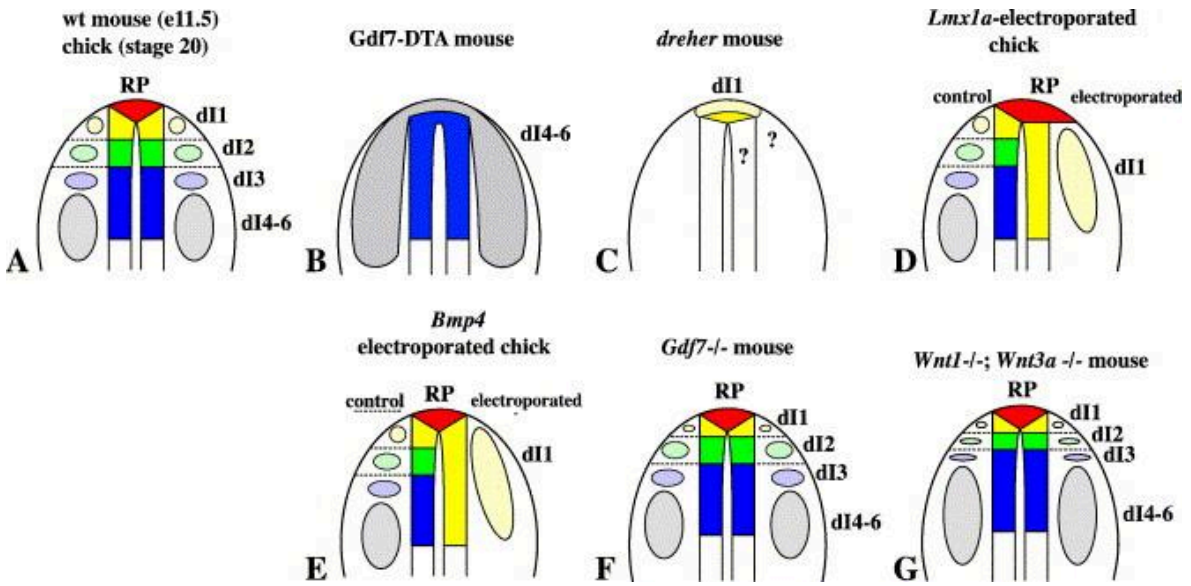

Figure 17. Dorsal patterning in various conditions in chicken and mice. *A. Schematic representation of wild type mouse and chick neural tube with : roof plate (red), Math1-expressing domain (yellow), Ngn1/2-expressing domain (green), Mash1 -expressing domain (blue) and 6 classes of dorsal interneurons (dI1–6) are shown. B-G Schematic representation of dorsal neural tube patterning in : B. Gdf7-DTA mouse, C.*

dreher mouse, which both lack the roof plate. D. Lmx1a/b overexpression electroporated chick, E. Bmp4/7 electroporated chick, F. Gdf7-/- mouse and G. Wnt1/Wnt3a double mutant mouse (Chizhikov et Millen, 2005)

BMP is known to regulate the Wnt pathway during dorsal neural tube patterning (Chesnutt *et al.*, 2004; Le Dréau et Martí, 2012; Manzari-Tavakoli *et al.*, 2022). Coordination of both pathways is mandatory for the proper organisation of the dorsal neural tube (Zechner *et al.*, 2007; Lehr *et al.*, 2025). Roof plate cells, by secreting BMPs and Wnts, are key elements of neural tube patterning and morphogenesis. BMP signaling, acting through SMAD transcription factors, promotes the specification of dorsal interneuron progenitor domains (dI1–dI6), while Wnt signaling, acting through the canonical β-catenin pathway, regulates both dorsal cell fate specification and the proliferation of neural progenitors (Figure 17) (Le Dréau et Martí, 2012). Together, these two pathways form an integrated dorsal signaling center that is essential for the patterning of the dorsal spinal cord. Their presence in the dorsal part of the neural tube is crucial for differentiation of the dorsal part and for maintaining tissue integrity (Caspary et Anderson, 2003; Chizhikov et Millen, 2005; Ventriglia et Kalcheim, 2024).

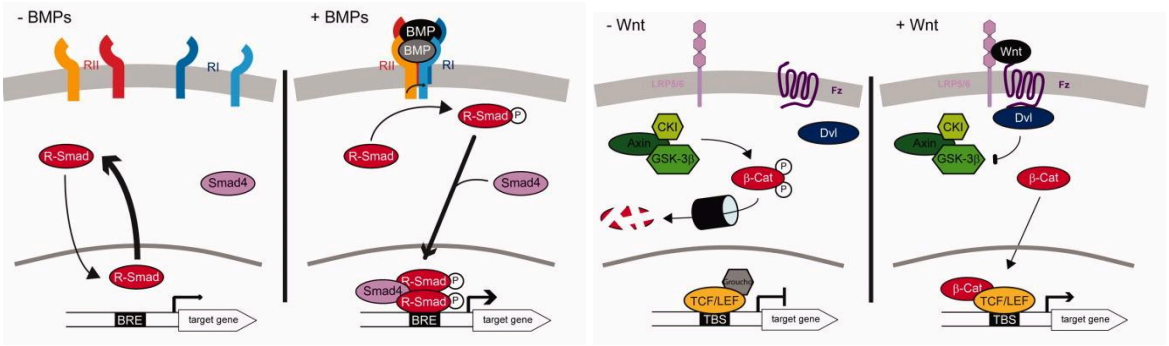

Figure 17. BMP and Wnt signalling pathways. *On the left, BMP signalling pathway. Without BMP ligands, receptor-regulated Smads (R-Smads) localize in the cytoplasm, limiting their transcriptional activity. BMP ligands promote the formation of a complex of transmembrane kinase formed with Type-2 receptors (RII) and Type-1 receptors (RI) that phosphorylate R-Smads. Phosphorylated R-Smad interacts with Smad4, the complex translocates to the nucleus and binds to the BMP-responsive elements (BRE) in target gene promoters, and modulates their expression in cooperation with transcriptional cofactors. On the right, the canonical Wnt pathway. Without Wnt ligands, Axin, CKI, and GSK-3β phosphorylate β-catenin (β-Cat), targeting it for proteasomal degradation. TCF/LEF transcription factors then bind TBS-containing promoters and recruit repressors such as Groucho, silencing target genes. When Wnt binds to Frizzled (Fz) receptors and LRP5/6 co-receptors, Dishevelled (Dvl) is recruited and blocks β-catenin phosphorylation, allowing its nuclear translocation. Nuclear β-catenin interacts with TCF to form a complex that activates target gene expression. (Le Dréau et Martí, 2012)*

3. Floor plate cells

a. Description of the floor plate cells

On the ventral part of the neural tube, the floor plate cells are glial cells aligned along the ventral midline. These cells are essential for vertebrate nervous system development because they specify cellular identity of the ventral neural tube and are essential for axon guidance (Placzek et Briscoe, 2005; Yu, McGlynn et Matise, 2013). The Sonic hedgehog (Shh) and Nodal pathways are required for proper floor plate induction but depending on the species some variation occurs (Odenthal *et al.*, 2000; Placzek et Briscoe, 2005). Floor plate cells secreted Shh after receiving high doses of Shh from the notochord, the cord structure below the neural tube. We can distinguish two groups of floor plate cells: the medial floor plate cells in the midline of the neural tube, that remain scaffolding glial cells until adulthood, and lateral floor plate cells on each side of the medial floor plate, which are neuronal progenitors (Odenthal *et al.*, 2000; Strähle *et al.*, 2004; Ribes *et al.*, 2010). Some studies also present differences in the identity of the floor plate cells along the antero-posterior axis (Placzek et Briscoe, 2005).

b. Organisation of ventral patterning, role of HH pathway

Neural tube ventral patterning depends on the Hedgehog (Hh) pathway (Ribes et Briscoe, 2009). The most important ligand of the Hh pathway in vertebrate neural development is Sonic Hedgehog (Shh), and in many studies the Hh pathway is referred to as the Shh pathway. The ventral secretion of Shh first by the notochord and then by the floor plate creates a Shh gradient from the ventral to the dorsal part of the neural tube. In amniotes, Shh from the notochord is necessary to specify the floor plate and to trigger *Shh* gene expression in floor plate cells. Then, Shh diffusion is responsible for all motor neuron specification depending on the concentration and the time of contact with the morphogen (Le Dréau et Martí, 2012; Sagner et Briscoe, 2019). Hh signalling is transduced through primary cilia of neuronal progenitors, a microtubule-based organelle present on the apical surface of most radial glial cells of the neural tube, which I will detail later (Andreu-Cervera, Catala et Schneider-Maunoury, 2021). Shh and cilia proteins have been demonstrated to be essential for patterning. When the Shh pathway or cilia proteins are deficient, various defects in ventral cell type specification are observed (Figure 18). Indeed, in absence of Shh or Smoothened (Smo), the

transmembrane activator of Shh signalling, the dorsal domain is extended to the ventral part and all ventral identity is lost, whereas when Ptch1 or SuFu are defective, which inhibit Shh pathway activity, an extended ventral identity and a loss of the dorsal one are observed in the neural tube (Figure 18). Then, depending on the cilia proteins that are mutated, we can observe variation on the dorso-ventral identity along the neural tube (Figure 18).

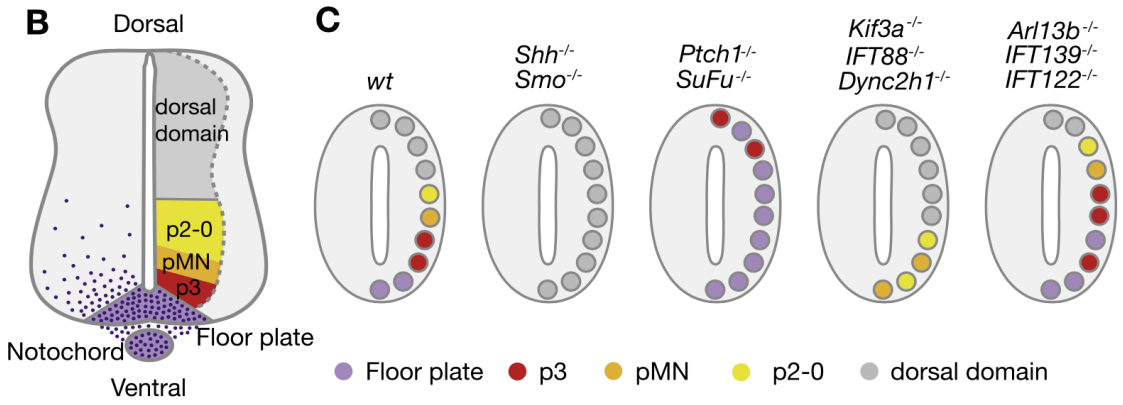

Figure 18 : HH signalling and dorso-ventral patterning in the neural tube in wt and mutants conditions. *B. Dorsoventral patterning in wild-type embryos. C. Effects of disrupting Hedgehog pathway components or cilia-related genes on neural tube ventral patterning. In the absence of Shh or Smoothened (Smo), the transmembrane protein required for Shh signal transduction, the dorsal progenitor domain expands ventrally and all ventral cell identities are lost. Loss of Patched1 (Ptch1) or Suppressor of Fused (SuFu), two negative regulators of the Shh pathway, results in an expansion of ventral progenitor domains at the expense of dorsal identity. Mutants affecting anterograde intraflagellar transport (Kif3a^{-}/$^{-}$, IFT88^{-}/$^{-}$, Dync2h1^{-}/$^{-}$) exhibit a partial reduction in the ventral domains while other ciliary mutants (Arl13b^{-}/$^{-}$, IFT139^{-}/$^{-}$, IFT122^{-}/$^{-}$) exhibit ectopic expansions of certain ventral domains [(Andreu-Cervera, Catala et Schneider-Maunoury, 2021)]*

III. The cilium, an important organelle during neural tube development

As presented in the above chapters, cells forming the lumen are ciliated cells (Sturrock, 1980). They extend either a primary cilium like progenitor cells or a motile cilium as zebrafish floor plate cells. As for roof plate cells, electron microscopy images have reported the presence of a cilium or at least of a basal body at the tip of the roof plate cells in the area close to the lumen but the function of this cilium has not been explored (Sturrock, 1980; Shinozuka *et al.*, 2019).

Cilia are extensions from the cell membrane. They are composed of a core of 9 microtubules doublets, and contain multiple specific proteins localizing at the ciliary membrane or inside of cilia. Cilia are built from the basal body and are subdivided into a transition zone proximally and an axoneme distally (Figure 19) (Ishikawa et Marshall, 2017). The basal body is a modified centriole made of nine microtubules triplets, linking the cilium to the cytoskeleton. The transition zone, where microtubule doublets are linked to the membrane via Y links and a "ciliary necklace", acts as a gate for controlling protein entry into, and exit from, the cilium. Many different proteins are circulating in the cilia along the axoneme, mainly thanks to intraflagellar transport (IFT) (Figure 19) (Ishikawa et Marshall, 2017).

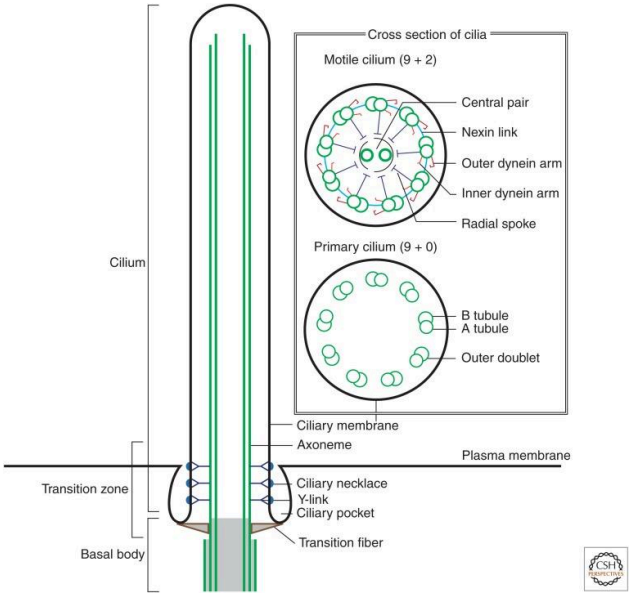

Figure 19. Schematic of the architecture of cilia. *The cross sections present the difference between motile and primary cilia. (Ishikawa et Marshall, 2017)*

We can distinguish two types of cilia : primary and motile. Primary cilia are, in general, composed of 9 microtubule doublets forming the axoneme (Figure 19). Primary cilia mediate signaling pathways such as the Shh pathway and are essential for their fine regulation (Andreu-Cervera, Catala et Schneider-Maunoury, 2021). They are known as sensory organelles that sense both chemical and physical signals (Johnson, Fair et Hoey, 2021). Motile cilia have a 9+2 structure: they also have 9 doublets of microtubules forming the axoneme and one doublet of microtubules in the middle (Figure 19) (Ishikawa et Marshall, 2017). As the name indicates, motile cilia have a motility functions, regulating mainly flow circulation as well as cell/organism motility (Bearce et Grimes, 2021; Xie *et al.*, 2023). Studies present cilia as a modulator of multiple signals integrating mechanical and chemical cues in the same organelle.

A. Motile cilia are important for flow circulation

Motile cilia are motile thanks to the dynein arm motors that are present in their axonemes. The motile cilia are present in many epithelial tissues on the lumen size to make fluid flow or generate the force for locomotion in motile cells such as spermatozoids or paramecia. In the neural tube of zebrafish, chickens and mice, floor plate cells are mono-ciliated cells (Kramer-Zucker *et al.*, 2005; Borovina *et al.*, 2010; Cruz *et al.*, 2010). In zebrafish, floor plate cilia are located on the cell apical surface facing the lumen and are motile. The flow generated by motile cilia of the floor plate cells is essential to create cerebrospinal fluid circulation in the central canal - the final cavities of the neural tube (Kramer-Zucker *et al.*, 2005; Thouvenin *et al.*, 2020; Donati *et al.*, 2021). Thouvenin et al., (2020) analyzed central canal inflation in zebrafish neural tubes. Using different ciliary mutants, they measured the bidirectional flow of the cerebrospinal fluid and demonstrated that motile cilia, localized in the ventral part of the central canal, are essential to induce cerebrospinal fluid flow circulation. In their study, they observed in all motile cilia mutants a smaller inflation of the central canal, meaning a smaller diameter of fluid circulation in the central canal of mutant neural tubes. In zebrafish, motile cilia of the floor plate have been then demonstrated to be important for the development of a straight axis and also for the formation of the flow circulating in all the ventricles of the central nervous system (Ringers, Olstad et Jurisch-Yaksi, 2020; Xie *et al.*, 2023). Their absence or defect of motility has been linked to scoliosis in zebrafish

(Xie *et al.*, 2023). Floor plate motile cilia are two to three times longer than primary cilia of neural progenitors. Foxj1 transcription factors have been demonstrated to be sufficient to generate motile cilia in different organs of zebrafish embryos and have been associated with the production of longer cilia in mouse and chicken floor plate cells (Yu *et al.*, 2008; Cruz *et al.*, 2010). Foxj1a has been reported as a master gene of motile cilia and also regulates floor plate cell response to Shh activity by interfering with effectors of the pathway (Cruz *et al.*, 2010). Motility of floor plate cells motile cilia has been observed in zebrafish but still need further investigations in other species (Ringers, Olstad et Jurisch-Yaksi, 2020; Kramer-Zucker *et al.*, 2005; Cantaut-Belarif *et al.*, 2018).

B. Primary cilia are essential for Hh signaling

Primary cilia are sensory organelles and are necessary for Hh signalling. Several transmembrane proteins involved in Hh signalling circulate along the primary cilia membrane to get activated or repressed. In the absence of the Shh ligand, the transmembrane receptor Patched (Ptc) localizes at or around the ciliary membrane and inhibits the translocation of the transmembrane protein Smoothened (Smo) to the ciliary membrane. In these conditions, cAMP concentration in the cilium is high and activates cAMP-dependent Protein Kinase A (PKA); This leads to the phosphorylation and ubiquitination of Gli-full length proteins Gli2 and Gli3, the effectors of the Hh pathway, that are then partially processed by the proteasome to produce the repressor forms of Gli transcription factors (GliR) (Figure 20) (Andreu-Cervera, Catala et Schneider-Maunoury, 2021). Gli3 forms a particularly potent repressor. In contrast, when Shh binds to Ptc, Smo inhibition is relieved, allowing its translocation to the primary cilium and inhibition of PKA activity. Gli-full length is then converted to Gli activator form (GliA) by poorly understood mechanisms (Figure 20). Gli2 forms a potent Gli activator in mice; This results in the expression of downstream target genes such as *Ptc1*, *Ptc2*, and *Gli1*. The ventral origin of Shh establishes a ventral-to-dorsal gradient of Gli activity, crucial for early signalling in the neural tube (Ribes et Briscoe, 2009). Indeed, Gli activator up-regulates ventral target genes such as Nkx6.1 and Olig2 that are both transcription factors inducing motor neuron differentiation (Sander *et al.*, 2000).

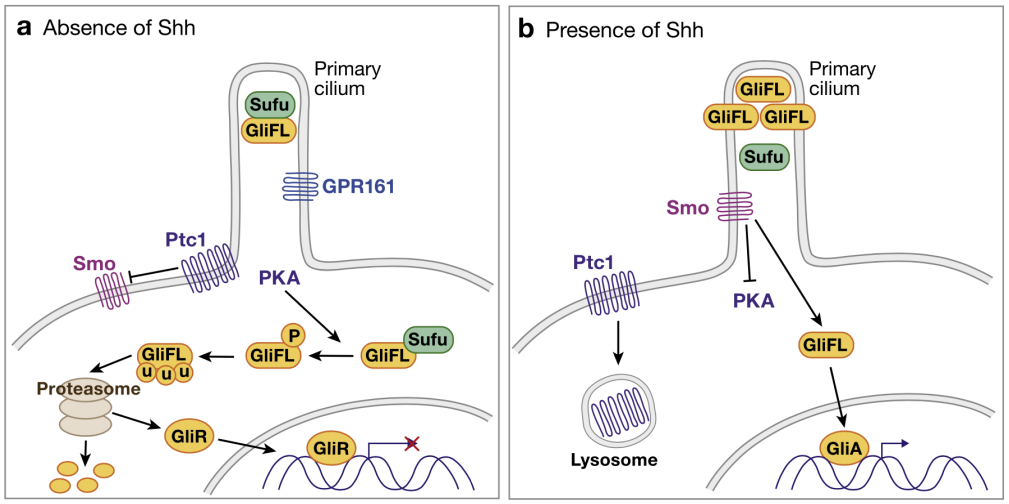

Figure 20. Hh pathway regulation by cilia. *In absence of the ligand Sonic Hedgehog (Shh), the transmembrane receptor Patched (Ptc) inhibits the activity of the transmembrane protein Smoothened (Smo). This allows priming phosphorylation of the Gli full length transcription factors (GliFL) by the Protein Kinase A (PKA) in the cytoplasm, thereby sending phosphorylated Gli to undergo a partial degradation by the proteasome machinery. These truncated forms of (GliR) can bind to Gli-binding sites (GBS)-containing promoters and act as transcriptional repressors of target genes of the Shh-Gli pathway. Binding of Shh to Ptc can relieve its repression on Smo, thus allowing Smo to inhibit PKA, which avoids Gli truncation. GliFL can thereby act as transcriptional activator (Gli-A) of target genes. [(Andreu-Cervera, Catala et Schneider-Maunoury, 2021)]*

Importantly, cilia are required for both the repression of the HH pathway in the absence of ligand, and the activation of the pathway in the presence of Shh ligand; thus, cilia loss leads to a low-level, basal activity of the pathway, and different phenotypes are seen in regions that normally encounter high or low levels of SHH signaling (for a review, see Andreu-Cervera et al 2021).

C. Cilia mutants and defects in morphogenesis of the neural tube

Motile and primary cilia are composed of mostly the same proteins, but some specificity exists between the two. Mutation of ciliary proteins can then induce motile or primary cilia defects, or both. The composition of cilia varies upon time and tissues, the recruitment of some specific proteins or complex is then controlled and the effects of their loss can affect only specific tissues or organs. Cilia mutation has been discovered in a high variety of human diseases, collectively called ciliopathies (Andreu-Cervera, Catala

et Schneider-Maunoury, 2021). To understand more about these human diseases, researchers study the effect of ciliary gene mutations in other organisms.

In zebrafish, many ciliary mutants present a defect of axis curvature, which is in most cases a curled down phenotype. This body axis curvature has been linked to motile cilia and flow circulation defects in the CNS (Bearce et Grimes, 2021). Cantaut-Belarif and al. (2018) present in the ciliary mutant a defect of formation of a specific fiber called the Reissner fiber, bathed in the middle of the central canal and spanning the length of the CNS from its production and assembly site in the anterior diencephalon to its disassembly at the posterior extremity of the spinal cord. This fiber was described in 1969 by Kohno by electron microscopy and recent research shows that straight axis morphogenesis requires cilia-dependent assembly of the Reissner fiber (Bearce et Grimes, 2021).

As previously presented, mutations in ciliary genes lead to ventral patterning defects. In mice, cilia loss induces a complete loss of Shh target gene expression, such as *Ptch1* and *Gli1,* whereas in zebrafish ciliary mutants present tissue-dependent effects. *MZoval (Maternal-Zygotic)* mutant embryos - ift88 mutation - and *iguana* embryos - dzip1 mutation - show a loss of high Hh pathway activity in the neural tube but a higher Hh pathway activity in somites (Sekimizu *et al.*, 2004; Wolff *et al.*, 2004; Huang et Schier, 2009; Borovina et Ciruna, 2013). Indeed, Sekimizu et al. describe an overall lower Gli activity in *iguana* mutant leading to a lower Hh activity in the neural tube but a higher HH activity in somites. The tissue-specific response to Hh pathway dysregulation has also been observed in other mutants. Ben et al. generated zebrafish *MZta3* mutants, talipd 3 mutants, is a protein necessary for ciliogenesis, and observed expanded domains of *en1* (*engrailed1)* and *ptc2* expression in the somites, indicative of a higher Hh activity whereas in the neural tube they described a decrease of *ptc2* expression and an almost complete depletion of *nkx2.2*-expressing domain in ventral neural tube (Ben *et al.*, 2011).

During my PhD, I have studied zebrafish ciliary mutants and more specifically I focused my study on *iguana*/dzip1^{ts294e} mutants. The *iguana* mutant (dzip1^{ts294e}) (Kim *et al.*, 2010) is defective for the Dzip1 protein. This protein localises to the basal body of cilium and its absence causes defects in primary ciliogenesis (Kim *et al.*, 2010; Tay *et al.*, 2010).

Indeed, *iguana* mutants present a complete loss of primary cilia and a partial loss of motile cilia (Tay *et al.*, 2010). At 28hpf a complete loss of motile cilia in floor plate cells and a reduced number in Kupffer's vesicle were observed; However, a reappearance at 48hpf and a full recovery at 5 dpf of motile cilia was observed in *iguana* mutant zebrafish embryos (Kim *et al.*, 2010). This suggests that Dzip1 function is required specifically for the formation of primary cilia and is not essential for motile cilia formation.

A conserved regulator role of ciliogenesis for Dzip1 in metazoa has been reported. In planarian, Dzip1 localizes at the basal body of cilia and its inhibition leads to the loss of cilia (Glazer *et al.*, 2010). This activity in cilia biology has evolved in vertebrates to be essential for proper Hh signaling. Different papers suggest a role for Dzip1 in the regulation of Hh, via distinct mechanisms. One suggests that Dzip1 sequestrates Gli3, the repressor of the Hh pathway, to the cytoplasm to avoid its activity, while another one shows that Dzip1 stabilizes Spop, an ubiquitin ligase necessary for Gli turnover (Schwend *et al.*, 2013; Wang *et al.*, 2013).

IV. Gli proteins, the effectors of the Shh pathway

A. Gli proteins are transcription factors

Gli proteins (Gli1, Gli2 and Gli3) are the main effectors of the canonical Hh pathway. Requirement for Gli proteins varies between species. In vertebrates, Gli proteins have the potential to be either transcriptional activators or repressors of the Hh pathway. The regulation of the formation of activator or repressor forms depends on Shh concentration (Figure 21). Gli proteins are transcribed as Gli full length (Gli-FL), an inactive form, that can be cleaved by the proteasome into a transcriptional repressor form, GliR, or phosphorylated into a transcriptional activator form, GliA (Figure 21). Only Gli2 and Gli3 present a repressor domain, while Gli1 is a mandatory activator (Figure 22). In mice, *Gli1* expression depends only on Hh activity. It is one of the main targets when the pathway is activated. Thus, Gli1 amplifies the Shh signal (Bai *et al.*, 2002). Gli2 has been identified as the main activator of the Hh pathway and Gli3 as the main repressor. Indeed, loss of function of Gli3 leads to higher Hh activity while Gli2 inactivation leads to the loss of Hh activity (Bai *et al.*, 2002; Persson *et al.*, 2002; Niewiadomski *et al.*, 2019). In zebrafish, the regulation of Gli proteins varies from the one observed in mammals. In zebrafish, Gli1 acts as the main activator of Hh pathway and Gli3 as the main repressor, while Gli2 (encoded by two orthologous genes, *gli2a* and *gli2b*) can be processed either into an activator or into a repressor form (Huang et Schier, 2009). A major difference is that, in zebrafish, *gli1* expression presents a basal activity independent of its activation by the Hh pathway and thus independent of cilia (Karlstrom *et al.*, 2003; Huang et Schier, 2009). As demonstrated in some studies, these main roles are general trends, and Gli3 has been demonstrated to act as an activator at early stages in the zebrafish ventral spinal cord (Tyurina *et al.*, 2005).

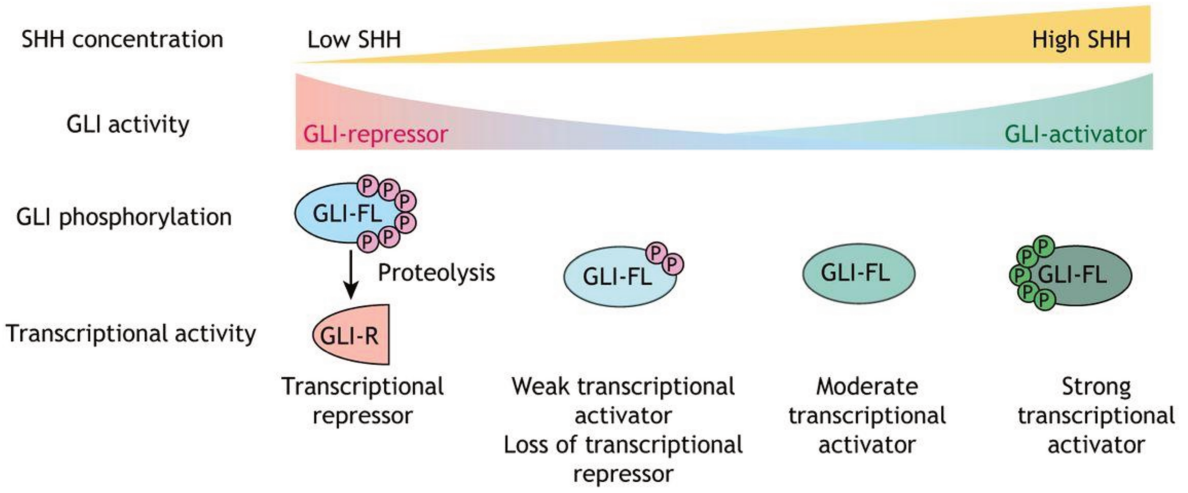

Figure 21. Gli activity depends on Shh concentration. *Post-translational modifications of Gli full length (Gli-FL) induce a change of activity of the Gli proteins. When Gli-FL is fully phosphorylated, it undergoes proteolytic processing into its repressor form (GLI-R), leading to suppression of target genes. Progressive dephosphorylation prevents GLI-R generation and progressively enhances transcriptional activation. GLI activator function is further associated with a distinct hyper-phosphorylation event on different sites that promotes activation (Kong, Siebold et Rohatgi, 2019).*

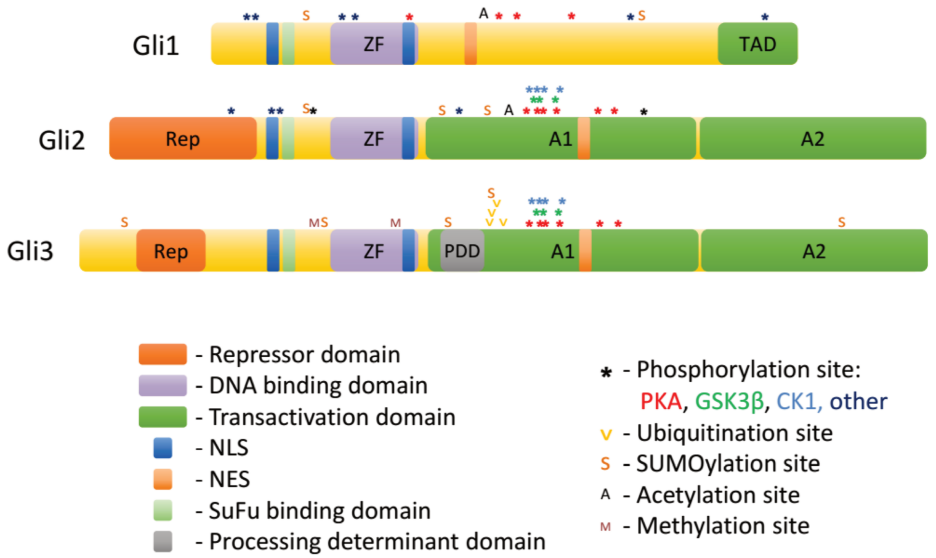

Figure 22. Gli protein domains in mammals. *Gli proteins (Gli1, Gli2, Gli3) representations of specific domains and posttranslational modifications* [*(Niewiadomski et al., 2019)*]

The Hh signalling gradient in the neural tube leads to form two opposite gradients of Gli proteins (Figure 21) (Dessaud, McMahon et Briscoe, 2008; Ribes et Briscoe, 2009). As presented above, the presence of Shh in the ventral part induces the activation of the Gli activator forms while the absence of Shh in the dorsal neural tube leads to the processing of Gli proteins into repressor forms, due to the regulation through cilia

(Persson *et al.*, 2002). Patterning regulation by the Shh pathway depends mainly on Gli balance along the dorso-ventral axis. Gli activator function is essential for ventral neuron induction such as V3 interneurons and motor neurons, and Hh-regulated gene expression in the zebrafish spinal cord (Vanderlaan *et al.*, 2005). Gli3 protein inhibits Hh activity in the dorsal part and prevents motor differentiation (Persson *et al.*, 2002). In the mouse double Shh;Gli3 mutant, the loss of ventral patterning observed in Shh simple mutants is rescued (Litingtung et Chiang, 2000).

All of those studies present the complex but important Gli balance along the dorso-ventral axis and its role to induce the patterning of all the ventral part of the neural tube (Dessaud, McMahon et Briscoe, 2008; Ribes et Briscoe, 2009). Each Gli protein contributes to generating a gradient of Gli activity that is proportional to the level of Shh signal transduction along the dorso-ventral axis. The specific contribution of each Gli protein to the gradient depends on its intrinsic transcriptional regulatory activity, its expression level, and its post-translational regulation by Shh signaling.

B. Gli proteins at the junctions between pathways

Gli proteins are Hh pathway effectors but a number of studies present a link of Gli proteins with different pathways such as Notch, BMP or Wnt. Shh, BMP and Wnt ligands form gradients and as the french flag model presented, the amount of morphogens a cell receives can dictate its behavior. It is a composed action of high or low doses of signal and time of exposure that matters (Dessaud, McMahon et Briscoe, 2008; Ribes et Briscoe, 2009). Moreover, because the spinal cord presents opposite gradients of these signals, we can assume that the combination of complex regulation of pathways along the DV axis produces such an organized and robust DV pattern in vertebrates. I am exploring now and presenting literature about the crosstalk between those major pathways and the Shh pathway through Gli proteins, focusing mainly on the spinal cord.

1. Notch - Shh crosstalk

In chicken, zebrafish and mice, several studies suggested a cross-talk between the Shh and Notch pathways during spinal cord patterning. It has been demonstrated that Notch activation induces the accumulation of Smo to the primary cilia, while Notch inhibition

causes Ptc1 enrichment at cilia (Kong *et al.*, 2015; Stasiulewicz *et al.*, 2015). Notch has been shown to modulate Gli balance and to regulate the expression of all the Gli proteins and Gli activity in zebrafish spinal cord. (Jacobs et Huang, 2019). Activation and inactivation of the Notch pathway modify the responses of neural progenitor cells to Shh (Jacobs *et al.*, 2022). Activation of Notch signalling enhances the response of neural progenitor cells to Shh, while inactivation of Notch signalling compromises ventral fate specification which depends on Shh activity (Jacobs *et al.*, 2022). Altogether, the results suggest that Notch signalling regulates Hh response of progenitor cells by modulating the localisation of key Hh pathway components at primary cilia and modulating Gli balance. It seems that not only Notch regulates the Hh pathway but also Hh regulates Notch, presenting a Notch-Hh crosstalk in vertebrates (Jacobs et Huang, 2021; Kumar *et al.*, 2021). Gli2 has been reported to transcriptionally regulate the *Hes1* gene, which encodes an effector of the Notch pathway, in mouse retinal progenitor cells (Wall *et al.*, 2009). The Hh target Nkx6.1 has been suggested to regulate the expression of Notch ligands along the dorso-ventral axis of the neural tube (Marklund *et al.*, 2010; Jacobs et Huang, 2021). The interaction between the Notch and Hh pathways seems to happen at different levels of each pathway (Figure 23) (Jacobs et Huang, 2021; Kumar *et al.*, 2021).

In other tissues, like epidermal cells and hematopoietic stem and progenitor cells, cilia have been shown to regulate Notch activity (Ezratty *et al.*, 2011; Liu *et al.*, 2019). Cancer research has investigated a lot of developmental signaling pathways, and particularly the crosstalk between the Hedgehog and Notch pathways with growing evidence suggesting that their interactions play important roles in tumor initiation and progression (Citarella *et al.*, 2023).

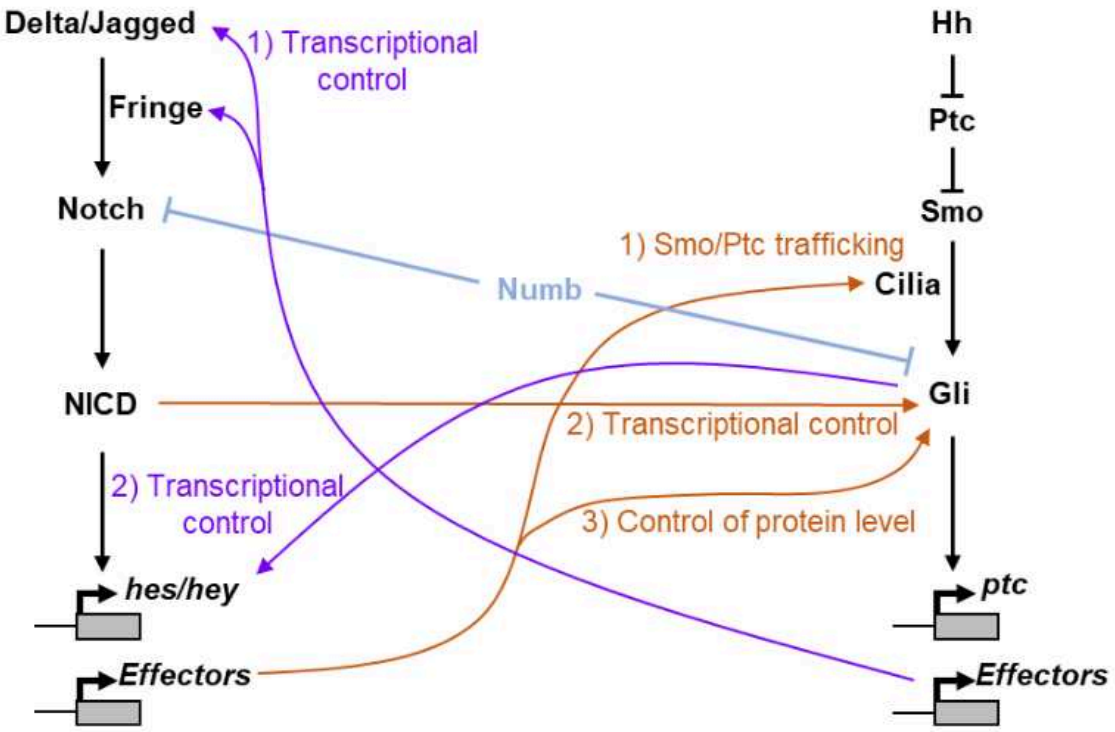

Figure 23. Crosstalk between Notch and Hedgehog (Hh) signalling. *Interactions between the Hedgehog and Notch pathways are represented by coloured arrows indicating the direction of regulation, with indication of the level at which each regulation occurs, transcriptional or protein level. (Jacobs et Huang, 2021).*

2. Shh - Wnt crosstalk

Shh and Wnt signaling pathways play crucial, often antagonistic, roles in the patterning of the vertebrate neural tube along the DV axis. Wnt has been shown to restrict and regulate Shh activity (Ulloa et Martí, 2010). Alvarez-Medina et al. indicated in chicken a role for the Wnt canonical pathway in DV patterning of the spinal cord independent of Bmp activity. They concluded that Wnt signalling positively controls the expression of *Gli3*, the main repressor of the Shh pathway, to restrict Shh activity in the dorsal neural tube. Indeed, they demonstrated that gain and loss of β-catenin/TCF function, the main effectors of the Wnt pathway, in chick embryos also directly regulate Gli3 expression (Figure 24) (Alvarez-Medina *et al.*, 2008). This result has been also found in other models and confirmed that *Gli3* expression is regulated by β-catenin/TCF activity (Lei *et al.*, 2006; Hasenpusch-Theil *et al.*, 2012). A negative crosstalk comes from the fact that, in turn, Gli3R can directly bind to β-catenin and inhibit its activity (Ulloa, Itasaki et Briscoe, 2007). Gli3, by acting as a transcriptional repressor, restricts the graded Shh/Gli ventral

activity to properly pattern the spinal cord (Lei *et al.*, 2006; Alvarez-Medina *et al.*, 2008). In the absence of Wnt activity, like in Wnt1/Wnt3a double mutant mouse embryos, a decreased Gli3 expression was observed (Muroyama *et al.*, 2002). Thus, dorso-ventral patterning of the neural tube depends on Wnt inhibition of Shh through the β-catenin/TCF activity regulating Gli3. Shh effector Gli1 has been suspected to activate Wnt signal and interact with β-catenin in cancer conditions (Li *et al.*, 2007; Kumar *et al.*, 2021).

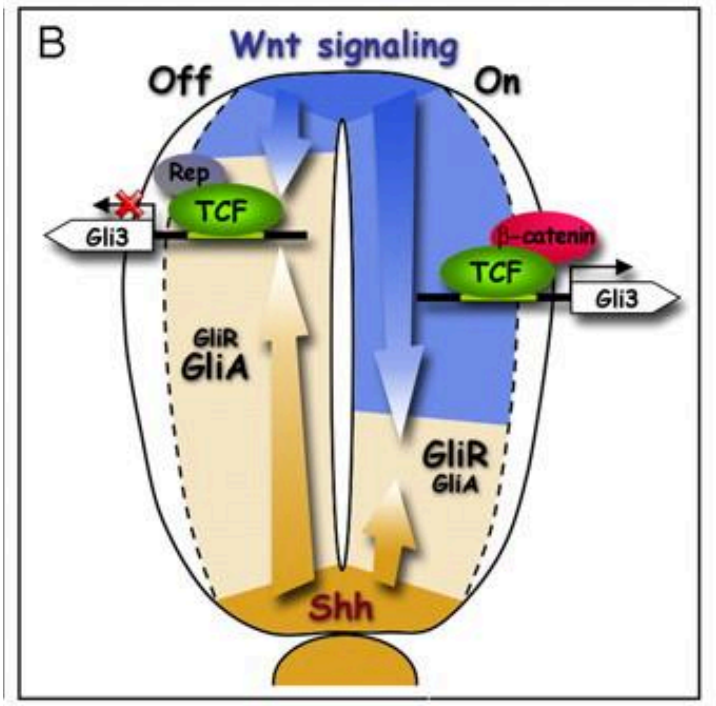

Figure 24. Wnt and Shh signalling interaction in the neural tube. *Schematic representation antagonistic activities of Shh and Wnt signalling pathways modelling dorsoventral patterning of the spinal cord (Alvarez-Medina et al., 2008).*

Overall, Shh interacts with Notch and Wnt pathways but reviews present interaction between Notch and Wnt (Figure 25) (Kumar *et al.*, 2021). Most of the studies presenting those interactions are cancer related but it is likely that the neural tube, which concentrates all of these signals, also depends on cross-regulation of the three pathways.

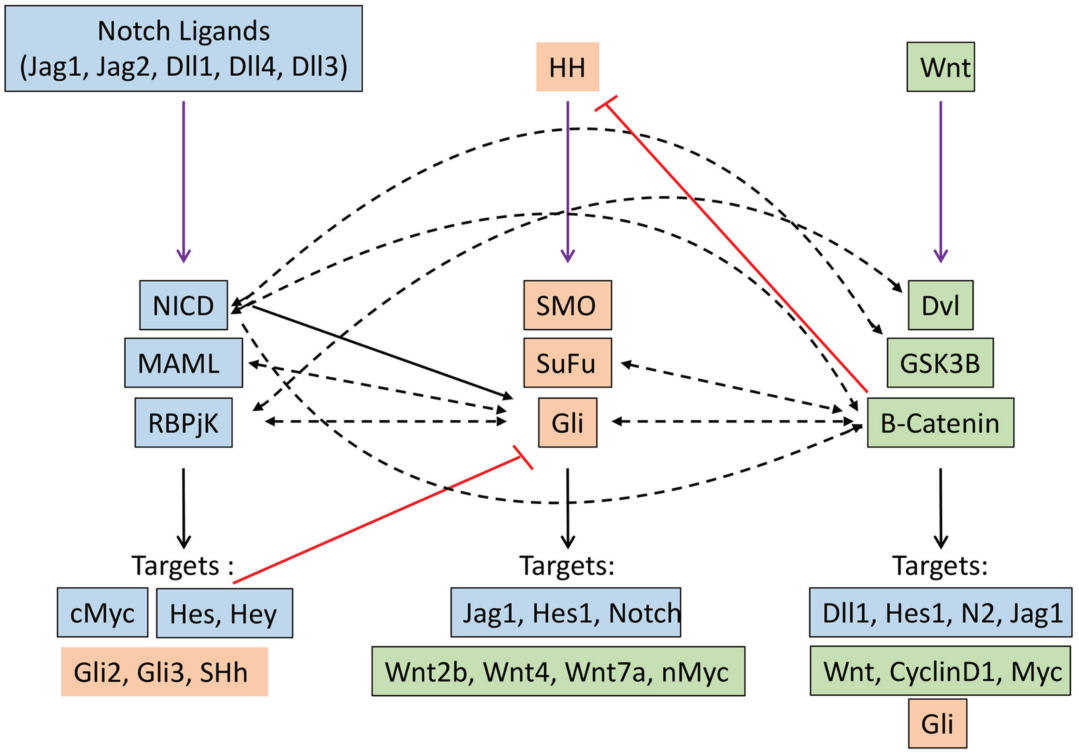

Figure 25. Crosstalk between Notch (blue), Hedgehog (orange) and Wnt (green) signaling pathways in cancer. *Black arrow indicates transcriptional regulation. Dashed black arrows indicate direct interactions. Red lines indicate inhibitory regulation. (Kumar et al., 2021).*

3. BMP - Shh crosstalk

BMP and Shh signals have antagonistic functions in controlling cell fate along the dorsoventral axis of the neural tube (Liem, Jessell et Briscoe, 2000; Le Dréau et Martí, 2012). Shh is known to induce ventral neuronal types, whereas BMP, along with Wnt, are known to regulate dorsal fate of the neural tube. Opposite gradients of Shh and BMP are formed along the D-V axis of the neural tube by mutual inhibition of those signals (Manzari-Tavakoli *et al.*, 2022). Shh signaling actively represses the dorsalizing effect of BMP signaling, thereby promoting ventral patterning in the ventral neural tube. Conversely, elevated BMP levels inhibit Shh ventralization induction, which is essential for the differentiation of motor neurons (Bond, Bhalala et Kessler, 2012). Published data present a regulatory link between Smad transcription factors, the effectors of BMP signalling, and Gli proteins, the effectors of Shh signalling. These works suggest that Smad decreases Gli activator forms but increases the Gli repressor form expression (Liu, Massagué et Altaba., 1998; Maurya *et al.*, 2011; Daoud *et al.*, 2014; Guo *et al.*, 2024). Arl13b mutant mice, a cilia protein important for Shh signaling, present a defect of

dorsal patterning with BMP misregulation (Horner et Caspary, 2011). These data suggest that proper GliA/R balance along the DV axis is necessary for proper dorsal patterning and BMP activity.

In conclusion, numerous papers present a link between Gli protein and Shh, Notch, BMP and Wnt. Gli proteins appear to be a crucial point of interaction between fundamental developmental pathways in the neural tube. In the following part I will describe the process that I am studying during my PhD, the remodeling of the lumen.

V. Neural tube lumen remodeling

A. First descriptions of lumen remodeling

As presented above, following neural tube closure, progenitors attached to the lumen give rise to spinal neurons and glial cells in a tightly regulated process. At the end of this neurogenesis phase, a particular morphogenetic phenomenon is observed : the reduction of the spinal lumen height down to a small ventral canal. In 1883, Barnes studied the reduction of the central canal in the pig spinal neural tube (Barnes, 1883). At this time, a few scientists had already observed the central canal reduction : Kolliker in 1861, Balfour and Foster in chick embryos in 1874 or even Loewe describing nervous systems in mammals and humans in 1880. To be more specific, Kolliker says that the *"primitive canal undergoes a gradual atrophy from the dorsal edge ventralwards, and that its obliteration is due to the increase in size of the posterior columns"*. Balfour then described in 1881 that, *"the walls of the canal coalesce dorsally, and that the coalescence then proceeds ventralwards, so that finally it reduces the canal to a minute tube formed of the ventral part of the original canal".* They all described a drastic reduction of the initial lumen down to a ventral central canal. Barnes measured the central canal reduction and tried to decipher between the divergent opinion about a dorsal or ventral collapse to form the central canal. He observed at different antero-posterior levels of the CNS the "dorsal collapse" of the central canal, that we referred to as lumen reduction, together with the elongation of specific dorsal cells called at this time Burdach's column (Barnes, 1883).

A century later, in 1988, Böhme et al. characterized from 25 days of gestation to birth what he called *"obliteration"* corresponding to the central canal reduction in cats (Bohme, 1988). He defined the process as *"a coalescence of the opposing walls or a filling of the lumen with cells."* He measured the deformation of all tissues forming the neural tube at different stages of gestation (Figure 26) : neural tube height increase (a), roof plate cells elongation (b), lumen retraction (d) and floor plate little increase in height (c).

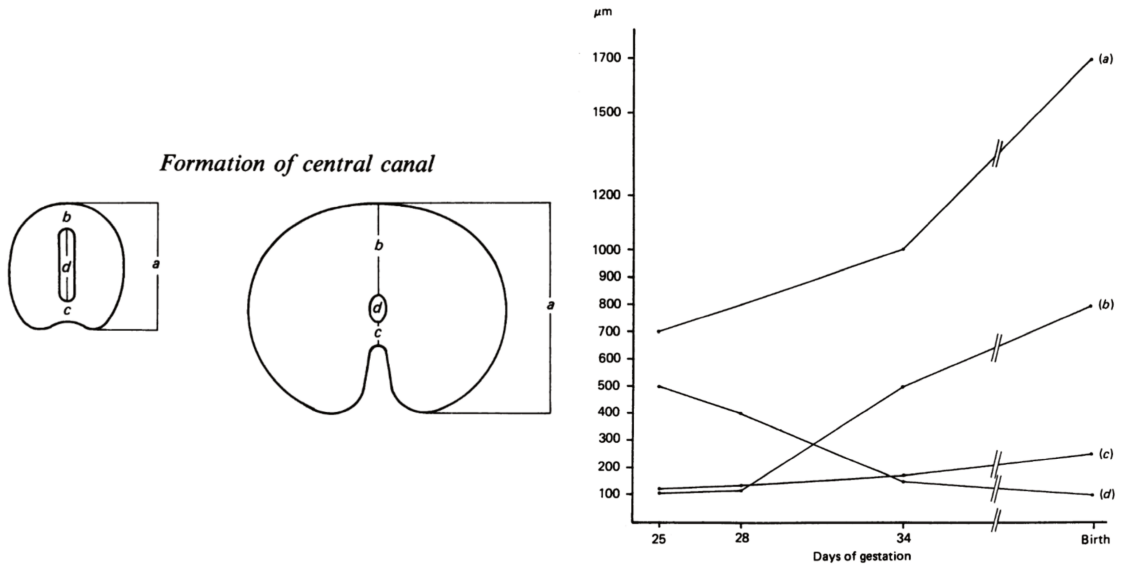

Figure 26. Neural tube lumen remodeling in cats from 25 days of gestation to birth. *The scheme represents the organisation of the neural tube and the graph below reports measurement of each component of the neural tube height. a : neural tube, b: roof plate cells, c : floor plate cells, d : lumen. (Bohme, 1988)*

A few years before, Sturrock in 1980 used electron microscopy to describe in mice the evolution of the epithelial layer organisation of cells surrounding the central canal of the neural tube between E10 and P150 (Sturrock, 1980). He described the roof and floor plate cells elongation around the central canal while all progenitors differentiate.

Since Böhme's paper, the central canal reduction that we call lumen reduction has been observed in many vertebrate species : mice (Sturrock, 1980; Xing *et al.*, 2018; Cañizares *et al.*, 2020; Tait *et al.*, 2020; Shinozuka et Takada, 2021; Ventriglia et Kalcheim, 2024), zebrafish (Hudish, Blasky et Appel, 2013; Kondrychyn *et al.*, 2013; Ribeiro *et al.*, 2017), rat (Ševc, Daxnerová et Miklošová, 2009), chicken (Tait *et al.*, 2020), or even camel (Elmonem, Mohamed et Aly, 2007). These studies complement each other to build a rudimentary understanding of the lumen remodeling that I will describe in the following part. This dynamic event takes more or less time depending on the species; in mice it takes 6 days (from E12.5 to E18.5), in zebrafish the remodeling happens in 24 hours (from 48hpf to 72hpf).

B. Regulation of lumen remodeling

All the above-mentioned studies describe that the reduction of the lumen height to form the central canal is concomitant to the roof plate cell elongation in the dorsal part. Before remodeling, the lumen represents 80% of the neural tube height while at the end of the remodeling, the central canal represents 20% of the neural tube height in the ventral part (Bohme, 1988; Kondrychyn *et al.*, 2013; Shinozuka et Takada, 2021; Ventriglia et Kalcheim, 2024). The central canal is maintained at the adult stage allowing cerebrospinal fluid circulation essential for the CNS physiology. Vladimir Korzh suggested that neurulation should be redefined. Instead of calling the end of neurulation the formation of the neural tube he suggested to include the lumen remodeling as the last step of neurulation (Korzh, 2014). He argues that after neural tube closure, many signals and processes are active in the neural tube whereas lumen remodeling is the last step in the morphogenesis of the neural tube. He suggested to discriminate between the early neurulation corresponding to the neural tube closure and the late neurulation taking in account all the patterning, proliferation and lumen remodeling processes. In the following section, I will describe all the knowledge we have on the lumen remodelling process separating the lumen reduction by focusing on neuronal progenitor behaviors and the roof plate cells elongation.

1. Progenitor behavior during lumen remodeling

After dorsal closure, the apical surfaces of neural tube progenitors are forming the lumen border. During the neurogenic phase, neuronal progenitor daughter cells that are committed to differentiate into neurons delaminate from the ventricular surface, while daughters committed to remain progenitors continue to form the lumen border. The lumen remodeling has been proposed to be the consequence of excessive delamination of dorsal ventricular progenitors, which thereby lose their apical attachment to the lumen (Cañizares *et al.*, 2020; Tait *et al.*, 2020). To characterise the nature of the progenitors that delaminate during lumen retraction, studies mainly relied on their molecular identity. Before remodeling, all progenitors forming the lumen border are characterised by Sox2 expression, and acquired different identities according to their DV position, with specific genes expressed, as mentioned before. After remodeling, the final central canal is formed by progenitor cells that keep their Sox2+ identity, although they

are quiescent (Hudish *et al.*, 2016; Ribeiro *et al.*, 2017; Cañizares *et al.*, 2020). No specific difference in the molecular identity of the progenitors has been reported between mouse and zebrafish at the end of remodeling. They mostly express Nkx6.1+, reflecting their ventral origin, with a subgroup of Pax6+ cells (Hudish *et al.*, 2016; Becker, Becker et Hugnot, 2018; Cañizares *et al.*, 2020; Tait *et al.*, 2020). At embryonic and adult stages, all cells express Foxj1+, reflecting the probable presence of motile cilia characteristic of ependymal cells lining the spinal cord and cerebral ventricles, allowing CSF circulation (Ribeiro *et al.*, 2017; Becker, Becker et Hugnot, 2018; Cañizares *et al.*, 2020). The maintenance of only ventral markers suggests a loss of most dorsal progenitors in the formation of the final central canal.

Some vertebrates such as zebrafish can regenerate, it has been shown that motility and sensitivity can be restored after spinal injury while in mammals glia scarring is the only mechanism observed. Most of the cells forming the central canal in adult zebrafish are still radial glial cells and present a proliferative and regenerative potential forming the stem cells niche (Ribeiro *et al.*, 2017; Becker, Becker et Hugnot, 2018; Jurisch-Yaksi, Yaksi et Kizil, 2020). While in mice a subtype of ependymal cells has been reported to be potential neural stem cell niche recruited after injury to partially repaired (Spassky *et al.*, 2005; Cañizares *et al.*, 2020; Jurisch-Yaksi, Yaksi et Kizil, 2020; Deng *et al.*, 2023).

Cañizares et al. showed that ventricular layer remodeling begins not long after the switch from neurogenesis to gliogenesis in the developing spinal cord (Cañizares *et al.*, 2020). They observed that this switch coincides with increased delamination of dorsal ventricular progenitors, leading to the "dorsal collapse", which we refer to as lumen reduction. This delamination is accompanied by an increased exit from the cell cycle, characterised by a decrease in the proportion of cells in S/G2/M phases in favour of G0/G1, and this occurs in the absence of any detectable apoptosis within the ventricular layer. At the molecular level, the authors report an increase in BMP signalling response, by the ventral expansion of phosphorylated SMAD1/5 detection from E13.5 onwards, at the same time as a progressive decline in Shh signalling in this same region, revealed by the downregulation of *Shh* and its receptor *Ptch1* expression. These observations are consistent with the reduction in the dorsal extent of Shh and Gli activity at the time of lumen remodeling in zebrafish, as described by Jacobs and Huang in 2019 (Jacobs et

Huang, 2019). Taken together, these data suggest that progenitor delamination at the end of neurogenesis, associated with a change of BMP and Shh signalling, constitutes the central mechanism driving the progressive reduction of the ventricular lumen.

2. Implication of Shh signaling and apical polarity in lumen remodeling

Recent studies have highlighted the critical role of Shh signaling and apical polarity proteins in lumen remodeling.

First, Hudish et al. demonstrated that the zebrafish microRNA miR-219 promotes lumen reduction by inhibiting the mRNA translation of Par3 and Prkci, which control the apical polarity of progenitors forming the lumen of the neural tube, thereby promoting cell delamination (Hudish, Blasky et Appel, 2013; Hudish *et al.*, 2016). Indeed, inhibiting miR-219 activity with a morpholino led to an incomplete lumen retraction by maintaining a higher number of progenitors (Sox2+ cells) in the spinal ventricular zone. Complementing this, Tait et al. studied lumen remodeling in mice and chicken embryos and described a concentration of adhesion molecules forming the lumen border, ZO-1, aPKC, Crumbs2, Par3, F-actin, except in the most dorsal progenitors close to the roof plate cells (Tait *et al.*, 2020). They suggested that roof plate cells down-regulate apical polarity proteins in dorsal progenitors, specifically transmembrane Crumbs2, inducing their delamination. They describe a ratchet model, whereby the local secretion of Crumbs2 by roof plate cells would promote the delamination of the most dorsal progenitors that receive this signal. This dorsal delamination would trigger the roof plate cells to elongate to the next progenitor, which would then receive the Crb2S signal and, in turn, delaminate (Tait *et al.*, 2020).

Second, regarding Shh signaling, Ribeiro et al. described, using Foxj1a-MO knockdown, a delay of lumen remodeling together with an increase of ptch2 expression, a target of Shh activity (Ribeiro *et al.*, 2017). They suggest that the lumen remodeling defect is a consequence of extended progenitor proliferation controlled by Shh activity. Building on this, Hudish et al. further demonstrated that miR-219 specifically inhibits Shh signaling along the dorsoventral axis (Hudish *et al.*, 2016). In miR-219 MO-mediated knockdown experiments, they observed extended dorsal expression of olig2, ptch2, and higher levels of gli1 mRNA, indicating a slightly broader response to Shh signaling along the

dorsoventral axis of the neural tube. Importantly, the MO-mediated increase of lumen size can be reverted by repressing Shh signaling through cyclopamine treatment, indicating that miR-219 partly promotes lumen reduction by repressing Shh signaling. They performed an early overexpression of Shh leading to a defect in lumen reduction. Of note, their manipulation of Shh signaling is made at early stages, when it is known to have important consequences on DV patterning: these early changes in patterning might lead to confusion in the interpretation of their results.

Taken together, these findings allow to propose a model whereby lumen retraction is initiated by the down-regulation of apical domain determinants by miR-219, which in turn modifies the cell response to Shh signaling to promote their delamination (Hudish, Blasky et Appel, 2013; Hudish *et al.*, 2016). Overall, these studies present Shh activity and apical polarity as regulators of progenitor delamination, controlling the neural tube lumen remodeling process.

3. Roof plate cells secretion might regulate lumen remodeling

Roof plate cells have been studied primarily for their role in morphogen production and, consequently, for their role in the control of proliferation and differentiation of dorsal neurons, as well as for their importance in tissue organisation and axon guidance (Rekler et Kalcheim, 2021). While lumen reduces to form the central canal, roof plate cells elongate 3 times their initial size in zebrafish and seven times in cats (Figure 26). Few studies reviewed this elongation and dug into the mechanisms regulating it (Kondrychyn *et al.*, 2013; Shinozuka et Takada, 2021; Ventriglia et Kalcheim, 2024).

In zebrafish, Kondrychyn et al. obtained by enhancer trap the SqET33 transgenic line expressing GFP in roof plate cells (Kondrychyn *et al.*, 2013). We can observe the expression of GFP from 24hpf in the most dorsal part of the CNS from the anterior to the posterior part of the embryo, making this line an amazing tool to follow roof plate cells during development and analyse changes in their shape in a targeted manner, as well as to observe their behaviour under different conditions. As the GFP gene has been inserted near the Zic6 locus in this line, the authors investigate the potential role of Zic6 in roof plate elongation. While Zic6 protein is specific to zebrafish, the Zic protein family of transcription factors is involved in central nervous system development in many

vertebrates. Using Zic6-MO, the authors observed locally some roof plate cells that did not elongate; they therefore assumed that Zic6 regulates the roof plate cells' cytoskeleton. However, this result is not very strong, based on one set of morpholino injections with little further characterisation.

Kondrychyn explored various methods for disrupting the elongation of roof plate cells. They observed disruption of the lumen remodeling in a few conditions : injecting Rock inhibitor at high dose in the CSF, they showed a disruption of roof plate extension, pointing towards a mechanism that requires active involvement of the acto-myosin cytoskeleton downstream Rock activity. Looking at mib1 and pard6 mutants, two mutants that prevent the early neurulation processes to form a continuous neural tube with a lumen, they did not observe elongation of the roof or floor plate cells at the end of remodeling. This suggests some coordination between the polarisation of progenitors, lumen remodeling and roof plate elongation, although it does not point towards a precise mechanism (Kondrychyn *et al.*, 2013; Sharma *et al.*, 2019).

Other studies suggest that roof plate cells initiate lumen remodeling (Shinozuka *et al.*, 2019; Tait *et al.*, 2020). Shinozuka et al., suggest that the dorso-ventral extension of the roof plate may change the range and distribution of morphogens it produces. This displacement of local expression of molecules could impact the behavior of progenitors that are in contact or nearby roof plate cells source of secretion. By blocking specifically Wnt secretion by roof plate cells, dorsal progenitor cells are losing Wnt activity. This specific inhibition induces less elongated roof plate cells and a higher lumen. Their work suggested that Wnt secreted by roof plate cells, while they are elongating, activated Wnt/B-cat activity (reported by Axin2 and TOPGAL) in progenitor cells with a more important effect on dorsal progenitors that are near the source of secretion. However, since Wnt together with BMP also regulates the cell fate choice and proliferation of dorsal progenitors, it is difficult to disentangle the specific requirement of Wnt signaling in the roof plate extension process.

Altogether, while several molecular players have been implicated in roof plate elongation, including the acto-myosin cytoskeleton downstream of Rock activity, apico-basal polarity of progenitors, and Wnt secretion, the mechanisms governing this

process remain largely unclear. A fundamental question remains unanswered: do roof plate cells elongate by generating their own internal forces, analogous to a growing axon, or do they respond to external mechanical forces, potentially generated by the progressive reduction of the lumen? So far, no experiment has allowed to discriminate between these two hypotheses(Tait *et al.*, 2020; Shinozuka et Takada, 2021; Ventriglia et Kalcheim, 2024). Addressing this question is an essential prerequisite to understand how roof plate elongation and lumen remodeling are coordinated during spinal cord development.

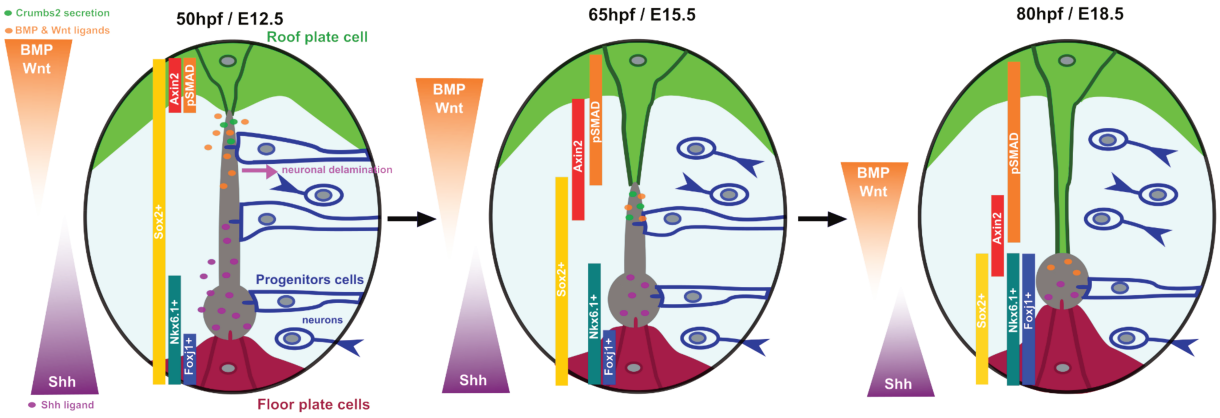

Figure 27. Scheme of lumen remodeling interactions in vertebrates across developmental time. *Three successive stages are represented (50hpf/E12.5, 65hpf/E15.5, 80hpf/E18.5) illustrating the progressive remodeling of the neural tube lumen. Lumen remodeling involves progenitor delamination (pink arrow, blue cells), lumen reduction (gray; the lumen is represented with an inflated portion in the ventral part as in zebrafish), roof plate cell elongation (green) and floor plate minor elongation (red). The neural tube is patterned by two opposing morphogenetic gradients: a dorsal BMP/Wnt gradient (orange) and a ventral Shh gradient (purple). BMP & Wnt ligands are shown as orange dots, Shh ligands as pink dots, and Crumbs2 secretion as green dots. Axin2 and pSMAD represent Wnt and BMP active domains. Identity along the dorso-ventral axis is indicated by colored bars representing the expression of: Sox2+ (progenitor domain), Nkx6.1+ (intermediate/ventral progenitor domain), Foxj1+ (motile cilia, first in the floor plate, later in ependymal cells). Over time, these domains progressively narrow and shift ventrally, reflecting the contraction of the progenitor pool and the maturation of the tissue.*

C. Open questions

In summary, lumen remodeling is a conserved mechanism in vertebrates that is initiated at the end of neurogenesis. As outlined throughout this introduction, neural tube cells integrate a wide range of signals governing differentiation, proliferation, and cell

behavior. The main objective of my PhD project is to elucidate the regulatory mechanisms underlying neural tube lumen remodeling, using the zebrafish embryo as a model system. Despite recent progress, the cellular and molecular bases of this process remain poorly understood. My work focuses on two interconnected phenomena: the coordinated deformation of roof plate cells and the delamination of progenitor cells. A central question is whether progenitor delamination is driven by intrinsic signals, linked to differentiation state or cell cycle exit, or whether it is triggered by extrinsic cues from the surrounding tissue. Beyond biochemical signaling, we also asked whether mechanical forces contribute to the remodeling process. In particular, we sought to determine whether roof plate elongation is an active, cell-autonomous process or a passive response to neighboring cell movements, and whether it is the delaminating progenitors that pull on the roof plate, or the elongating roof plate that pushes progenitors out of the epithelium.

Building on evidence suggesting a role for Sonic Hedgehog (Shh) signaling in lumen remodeling, we investigated cilia mutants and demonstrated the involvement of Dzip1, a protein of the ciliary basal body, as well as the importance of the balance between Gli transcriptional effectors along the dorso-ventral axis, in regulating neural tube lumen remodeling.

RESULTS

I. Research article - Spinal lumen remodeling under the control of Gli signaling mechanically drives roof plate cells extension

In the following section, you will find the article that summarizes the data and results from my thesis. This article is currently under review by the journal *Development*.

Detail participation of authors in the paper :

- Conceptualization: AM (equal), SSM (support), PLB (equal),
- Data curation: AM (lead), AMDZ (support), SSM (support), PLB (support),
- Formal analysis: AM (lead), AMDZ (support), PLB (support),
- Funding acquisition: PH (support), SSM (equal), PLB (equal),
- Investigation: AM (lead), AMDZ (support), IA (support), AE (support), PLB (support)
- Methodology: AM (lead), IA (support), AE (support), KMK (support), PH (support), SSM (support), PLB (support)
- Project administration : SSM (equal), PLB (equal)
- Resources: AM (equal), IA (support), AE (support), KMK (support), PH (support), SSM (equal), PLB (equal)
- Software: AM (support), PLB (lead)
- Supervision: SSM (support), PLB (lead)
- Visualization: AM (equal), PLB (equal)
- Writing – original draft: AM (equal), PLB (equal)
- Writing – review & editing: AM (equal), PH (support), SSM (support), PLB (equal)

Graphical abstract

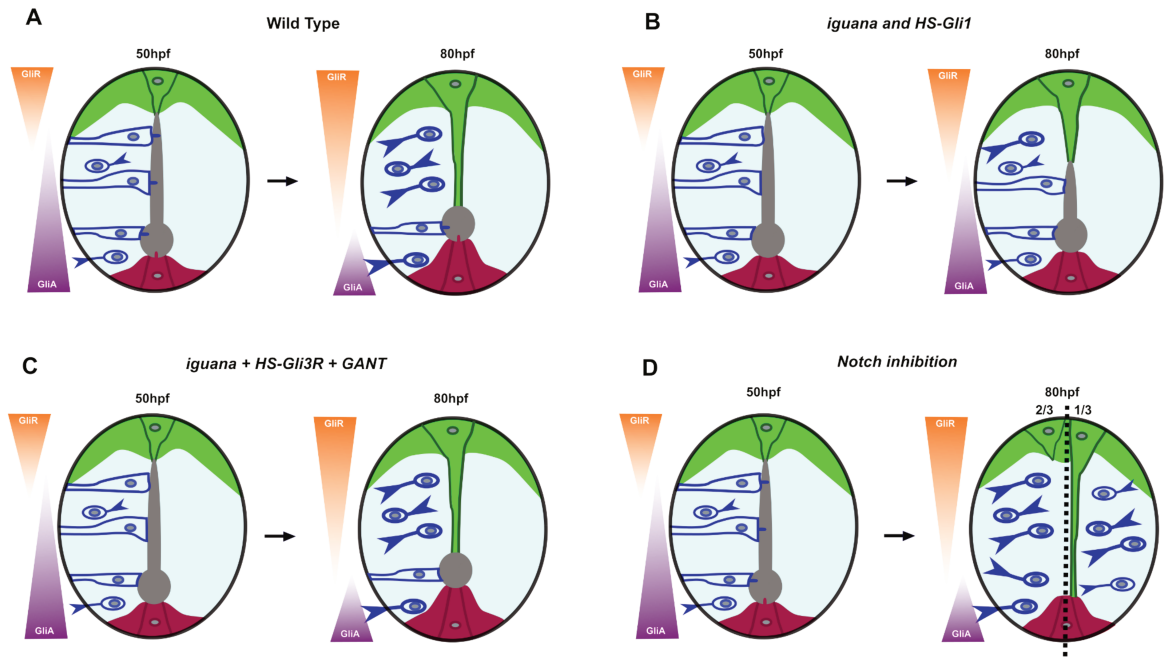

Figure 28. Graphical abstract of the main result of my PhD. *Each panel represents a schematic cross-section of the developing neural tube lumen remodeling in zebrafish from 50hpf to 80hpf, subjected to a dual morphogenetic gradient: the GliR gradient (repressive form of Gli, in orange, dorsal domain) and the GliA gradient (activating form of Gli, in purple, ventral domain). A. Lumen remodeling in wild type condition : roof plate cells (green) elongate while progenitors (blue) delaminate inducing lumen (gray) reduce and floor plate small (red) elongate. The remodeling is accompanied by a change of GliA/GliR balance, GliR expands ventrally while GliA is reduced in the ventral part. B. Lumen remodeling in iguana mutants and Gli1 overexpression (HS-Gli1) condition. The lumen remodelling is impaired and Gli1 activity is maintained in the neural tube, the GliA/GliR ratio along the DV axis is shifted dorsally. C. Overexpression of Gli3R and inhibition of Gli1 (by GANT) in iguana mutants rescue the lumen remodeling defect by readapting the GliA/GliR balance. D. Lumen remodelling in Notch inhibition context. The progenitors delaminates all and the roof plate cells elongate but retract to the dorsal part affecting losing the attachment to the progenitors.*

Spinal lumen remodeling under the control of Gli signaling mechanically drives roof plate cells extension

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ABSTRACT

Morphogenesis often requires different cell types to coordinate their behaviors for an harmonious developpement. How these different cell type behaviors are synchronized within and between tissues remains one of the important questions to fully understand morphogenesis. We used the zebrafish developing spinal cord to study this question. At later stages of neurogenesis, the lumen of the neural tube remodels, by reducing its height dramatically to form the persisting ventral central canal, a morphogenetic process conserved in vertebrates. By combining genetics, cell signaling manipulation with antagonist drugs and high-resolution in vivo live imaging, we better characterised the dynamics and control of this remodeling process. We showed that the lumen retraction depends on Gli activity regulation, a downstream effector of the Shh morphogen signal. We further established that the lumen retraction is instrumental in the cellular elongation of spinal roof plate cells, a population that forms the ceiling of the spinal cord lumen. Our work therefore establishes that the Gli transcriptional regulators under the control of long-range morphogen Shh control lumen retraction and that this retraction is a key driver of the roof plate cells extension.

INTRODUCTION

Morphogenesis is a tightly controlled process during metazoan development. The shape of growing tissues during development depends on a small set of cell behaviours that are controlled by cell-cell signaling pathways (Lecuit and Le Goff, 2007). Within a tissue, we start to better understand how biochemical and mechanical signals control precisely morphogenesis, like for example in lumen formation (Chan and Hiiragi, 2020). Yet, how two tissues made of different cell types that remodel within the same organ coordinate their morphogenesis is poorly understood and crucial to investigate.

To study the coordination of different cell types remodeling concomitantly, we focus on the vertebrate spinal neural tube. The neural tube is formed by radial glia, most of which are neuroepithelial progenitors that proliferate and differentiate to establish the entire diversity of cell types of the adult central nervous system (CNS) (Saade and Martí, 2025). The apical surfaces of radial glial cells surround a cavity also called lumen in the center of the neural tube, in which cerebrospinal fluid (CSF) circulates in the inflated part. Radial glia forming the neural tube of the CNS present primary cilia that bathe into the CSF (Kramer-Zucker et al., 2005). Cilia are essential in development ; they have a central role in orchestrating many sensory and signalling events of the cell (Andreu-Cervera et al., 2021; Bloodgood, 2009). In the CNS, primary cilia are essential for the differentiation and patterning of neural progenitors, whereas motile cilia are required for CSF circulation in brain ventricles and the spinal cord central canal (Kramer-Zucker et al., 2005; Ringers et al., 2019; Thouvenin et al., 2020). Cilia are therefore prime candidates to explore how cell signaling participates in coordination during vertebrate neural tube morphogenesis.

Shh is an important signaling pathway to control the proliferation and the patterning of differentiation of neural progenitors (Sagner and Briscoe, 2019). Shh morphogen is produced ventrally by floor plate cells and notochord and diffuses as gradient from ventral to dorsal in the neural tube (Andreu-Cervera et al., 2021). This Shh gradient is forming a Gli (glioma-associated oncogene) activity gradient along the dorso-ventral axis, that will regulate a series of target genes expression, including *patched-2* (*ptc2*). Gli transcriptional factors are the effectors of the signaling pathway, balancing between the Gli activator form (GliA) and the Gli repressor form (GliR) (Andreu-Cervera et al., 2021). The processing of these two forms requires the presence of a primary cilium. In zebrafish, mutants that lack primary cilia from early stage of development lose their Gli activity gradient, but maintain a residual low Gli1 activity - the activated form in zebrafish - that extends dorso-ventrally more than normal (Ben et al., 2011; Huang and Schier, 2009). Notch is another cell-cell signaling pathway that is crucial for neural progenitors to maintain both self-renewal and their apical domains

forming most of the embryonic lumen border (Sharma et al., 2019). How these signals are involved in late morphogenesis events is not known.

In vertebrates, the CNS emerges from the embryonic neural tube, a pseudostratified epithelial structure with an internal lumen. At late stages of embryogenesis, the spinal cord central canal forms through a specific remodeling of the neural tube, namely the ventral retraction of the primitive lumen. This remodeling, sometimes called “dorsal collapse”, is conserved among vertebrates and has been observed in zebrafish, chicken, mouse, rat but also cat or camel (Böhme, 1988; Cañizares et al., 2020; Elmonem et al., 2007; Kondrychyn et al., 2013; Ribeiro et al., 2017; Ševc et al., 2009; Shinozuka and Takada, 2021; Sturrock, 1981; Tait et al., 2020). In the zebrafish, the primitive lumen extends over 80% of the total height of the neural tube, although only the most ventral part is inflated by the circulating CSF (Figure S1C) (Thouvenin et al., 2020). Between 2 and 3 days of development, this primitive lumen shrinks to the ventral part of the neural tube to form the central canal (Figure 1 A,B) (Ribeiro et al., 2017). Lumen remodeling is the scene of various concomitant cellular rearrangements : dorsal lateral progenitors (also called dVL for “dorsal ventricular layer”) stop contributing to the formation of the lumen border by detaching their apical surfaces (a process sometimes called “cell attrition”), while the roof plate cells that form the dorsal midline of the neural tube (also called dmRG, for dorso-medial radial glia), adapt to this reduction and elongate ventrally to several times their initial size (Böhme, 1988; Kondrychyn et al., 2013; Shinozuka et al., 2019; Tait et al., 2020). The lateral progenitors and dorsal roof plate cells thus remodel in a coordinated manner during this period. Tait et al. (2020) proposed a model, in mouse and chicken, where roof plate cells promote the loss of apical polarity of the most dorsal lateral progenitors forming the lumen by secreting CRB2S molecules, leading to their delamination and the reduction of lumen height.

To improve our understanding of behaviour coordination of various cell types within an organ, we decided to use the zebrafish embryo spinal cord remodeling as a model to perform live confocal imaging and functional studies. We sought to better understand how cell-cell signaling regulates the spinal lumen retraction and its coordination with roof plate cells elongation.

We first turned our attention to cilia as an important relay in multiple signaling pathways. By studying different zebrafish mutants defective in cilia functions, we demonstrated that cilia absence does not directly impact lumen remodeling but that *dzip1* mutant embryos (*iguana*) - encoding the Dzip1 protein located at the ciliary basal body - present a defect of lumen remodelling. While characterising the *iguana* mutant phenotype, we observed an expansion of *ptc2* expression, a well known target of the Gli activity in this mutant. Our findings demonstrate that Gli activity needs to be shut down for dorsal

progenitors to withdraw from the lumen border, hence leading to lumen retraction. Indeed, modulation of Gli activity in embryos impacted lumen remodeling. Over-expression of the Gli activator reproduced the *iguana* defect of lumen retraction while over-expression of Gli repressor and inhibition of Gli activator rescued the *iguana* phenotype. By artificially accelerating progenitor extrusion via blocking Notch signaling, we uncover a mechanical coupling between the retracting lumen border and roof plate cell extension. We therefore demonstrate that progenitor cells extrusion from the lumen border under Gli control is the driver of lumen retraction and, via mechanical coupling, of roof plate cell extension.

MATERIALS AND METHODS

Zebrafish strains

All zebrafish strains used in this study were maintained and raised under standard conditions. Animals were raised at 28.5℃ under a 14/10 light/dark cycle until the start of the experiment. Eggs were collected an hour after mating and maintained at 28.5 ℃ in E3 medium (2011 Cold Spring Harbor Laboratory Press). All procedures were performed on zebrafish embryos between 30hpf and 95hpf. All experiments were performed on Danio rerio embryos of AB, Tüpfel long fin (TL) and nacre background. The transgenic strains used were: *TgKI(tjp1a-eGFP)*^{pd1252}, *TgKi(tjp1a-tdTomato)*^{pd1224} (Levic et al., 2021), *Et(krt4:EGFP)*^{sqet33} (Kondrychyn et al., 2013), *Tg(hsp70l:GFP-gli1)*^{a4594} (Huang and Schier, 2009), *TgBAC(ptc2:Kaede)*^{a4596} (Huang et al., 2012), *Tg(hsp:Gli3R-EGFP)*^{ca121} (see below). The *traf3ip1*$^{tp49d/tp49d}$, referred to as *elipsa* mutant (Driever et al., 1996), foxj1a$^{nw2/nw2}$, referred to as *foxj1a* mutant (Thouvenin et al., 2020), *dnaaf1*$^{tm317b/tm317b}$, referred to as *dnaaf* mutant (Brand et al., 1996; Van Rooijen et al., 2008), and *dzip1*$^{ts294e/ts294e}$, referred as *iguana* mutant (Brand et al., 1996) mutant strains were maintained as heterozygotes, and homozygous embryos were generated by intercrossing heterozygous carriers.

Building of the *Tg(hsp:Gli3R-EGFP)*^{ca121} transgenic line

To generate the hsp:Gli3R-EGFP construct, a C-terminally truncated zebrafish gli3 gene fused to EGFP (Huang and Schier, 2009) was cloned downstream of the hsp70 promoter. To establish a stable transgenic line, 40 pg of hsp:Gli3R-EGFP plasmid DNA was co-injected with 40 pg of tol2 transposase mRNA into wild-type embryos at the one-cell stage. Positive transgenic lines were identified by screening for EGFP expression in F1 embryos following heat shock.

Genotyping

Homozygous ciliary mutant embryos for *elipsa, dnaaf1, foxj1a* and *iguana* were identified by observation of curled down embryos after crossing, leading to identify adult heterozygous zebrafish. For *dnaaf1* and *foxj1a*, primers were also designed to identify the mutated loci in adult zebrafish. DNA of anesthetised adult fish were extracted using fin clipping. The fin piece was then digested for 3 hours at 55 °C in 100ul of fin clip medium (100mM de Tris-Hcl pH7,5, 1mM EDTA, 250mM NaCl, 0,2%SDS) + 1ul of PKA (0,1µg/µl). Amplification of the genomic DNA were performed using the following primers for *foxj1a*, forward-mutation 5’ -TCTCCATCCTCAACGCCAGG- 3’ and reverse 5’ -ACTTCGTCGGGACAGTGTCC- 3’ and *dnaaf1* forward 5’ -GCAAGCTTTGCACGCTTAATGTCTC- 3’ reverse-mutation 5’ -GTCACAAACATTCTCCAGTGTT- 3’. For hsp:Gli3R-EGFP and hsp:EGFP-Gli1 transgenic lines were genotyped by eGFP PCR detection using the following primers : forward 5’ -GCATGGACGAGCTGTACAAG- 3’ and reverse 5’ -TGAACAGCTCCTCGCCCTTG- 3’

Immunostaining

Embryos were manually dechorionated and anesthetized using 0.4% tricaine (MS 222, E10521, Sigma) for 2 minutes prior to fixation. All the following steps are done with agitation. Embryos were then fixed over-night in 4% PFA 1,5% saccharose or 4h at room temperature (RT), rinsed 5 times for 15 mins in 1X PBS. Embryos were blocked at least 1h at room temperature in a solution containing bovine serum albumin (BSA, 2mg/ml), 0.7% Triton, 1% DMSO, and 10% normal goat serum (NGS). Embryos were incubated with primary antibodies 48h at 4 ℃ in a solution containing BSA (2mg/mL), 0.5% Triton, 1% DMSO, and 1% NGS and subsequently washed four times during one hour in a PBS 1X 0.5% Triton. Secondary antibodies were added overnight at 4 ℃ in a solution containing 0.5% Triton, 1% DMSO, and 1% NGS. Embryos were then washed 4 times for 15mins using 1X PBS 0.5% Triton. The yolk and the head of the zebrafish embryo were removed, and the trunk was mounted laterally in Vectashield Antifade Mounting Medium (Clinisciences, H1000). The following dilutions of primary antibodies were used: chicken IgY anti-GFP 1:250 (AVES Lab), mouse IgG1k anti-ZO-1 1:250 (Zymed-Invitrogen), mouse IgG2b anti-Acetylated-tubulin 1:300 (Sigma), mouse IgG1k anti-GT335 (AdipoGen LifeSciences), rabbit polyclonal anti-Sox2 (GeneTex), mouse monoclonal anti-HuC/HuD (Invitrogen LifeTechnologies), mouse IgG1 anti-Nkx6.1 1:150 (DSHB), phalloidin alexa 568 1:400 (Invitrogen) and DAPI 1:1000. All secondary antibodies were purchased from Molecular Probes and The Jackson Laboratory and used at 1:200.

For HuC staining, to improve antibodies penetration, embryos were cut in two at the cloaca level after PFA fixation and incubated 7mins in acetone at -20°C before following the usual protocol.

Image acquisition

Fixed embryos were imaged on a Zeiss LSM 980 Upright Confocal microscope with 63X Oil magnification. The images were all acquired at a fixed rostro-caudal position, at the level of the cloaca in the dorsal part. We acquired Z-stacks of the neural tube to obtain a 3D stack acquisition in a lateral view, except for HuC stainings where the embryos were mounted head down in agarose to acquire transverse sections. Images were then processed using Fiji.

For live imaging, embryos at the appropriate stage were manually dechorionated, anaesthetised with 0.4% tricaine and then embedded in 0.5% low melting agarose in 1X E3 medium on 1% Agarose E3 mould. Movies were recorded on a Zeiss Spinning Disk upright confocal microscope using a 40X water lens, one image every 15 minutes were taken.

Quantification of the different neural tube heights

All images were treated with Fiji software. We used digital reslicing to reconstruct a series of transverse sections of the neural tube. We determined manually the height of all regions composing the neural tube (neural tube height, roof plate length, lumen height, floor plate length) using Fiji straight line measure, using usually at least 3 sections per embryo that represents internal diversity. The limit of each domain was determined by the intercept between the dorso-ventral axis and a perpendicular line at the level of the extremity of the signal delineating the neural tube and the lumen. For Sox2 and Nkx6.1 stainings, the same was done with a perpendicular line marking the limit of the expression domain. Prism software (GraphPad) or Matlab (MathWorks) were then used to compute the heights of the different regions of interest using the bottom of the neural tube as a reference at different stages (hpf) and condition (mutant vs control), plot them and compute statistical comparisons.

Specificities for quantification of live imaging movies

After measuring the different neural tube heights on images from live time-lapse movies, we used Matlab to plot them and measure the evolution of lumen height, and fit a linear curve to calculate the speed of lumen retraction. In the case of the *iguana* movies, we noticed that the control embryo had a lower speed of retraction when compared to a fixed sibling raised at 28°5C. This was due to a colder temperature (around 24°C) in the microscope room. We therefore corrected the time step of this experiment so the live control embryos fit the fixed controls. This correction was confirmed by the fact that after this correction, the *iguana* homozygote mutants imaged in the same dish had the same speed of lumen retraction as fixed embryos raised at 28°5C.

Cilia density counting and normalisation

Acetylated tubulin and GT-335 antibodies were used to stain cilia. Cilia density was calculated by counting the number of cilia in 100x100 pixels square in each image. All data were normalized by the means of controls.

Segmentation

To quantify the area and elongation of the apical surface of the lumen cells we used a combination of MATLAB software algorithm (The MathWorks) and Fiji analysis. Due to technical limitations, only the most ventral cells, at the level of the inflation of the lumen, were amenable to a Z-projection compatible with a segmentation. First we created one stack image of the apical surface of the progenitors (using either max or average projection) on Fiji. These images were then segmented using MATLAB script (Bardet et al., 2013). Finally

quantification of the area and cells elongation of each cell were performed thanks to MorphoLib plugin (https://github.com/ijpb/MorphoLibJ)

Counting apical surface of progenitors forming the lumen

To determine the number of progenitor apical surfaces contributing to the lumen on one side of the border along the dorso-ventral axis, we used Z-stack of ZO-1 or phalloidin staining on fixed embryos at different stages. The delineation of the apical junction belt by ZO-1 or phalloidin allowed us to determine the outline of each progenitor apical surface, while moving in 3D through the Z-stack. We counted 3 positions per embryo along the rostro-caudal axis of the neural tube. The mean of these three counts is the mean of the number of apical surfaces forming the lumen.

Cerebrospinal fluid (CSF) labelling

49hpf, 54hpf, 74hpf embryos were manually dechorionated, mounted in 0.5% low-melting point agarose in the lateral position covered by E3 medium 0.4% tricaine to anaesthetize the embryos. A few nanoliters containing Rhodamine Dextran 10 000 MW (D1817, Invitrogen) at 5 mg/ml diluted in E3 medium were injected in the pericardiac chamber in order to label CSF. Following the injection, we waited one hour before performing experiments to allow the particles to be transported down the central canal. We acquire live embryos to access the height of the circulating flow in control vs mutant embryos.

PHotoconvertile REporter of Signalling History (PHRESH) analysis

All fluorescent imaging was carried out using the Zeiss LSM980 Upright confocal microscope and the Zeiss software. Photoconversion was carried out using the 405 nm laser with a 20x water-dipping objective. ptc2:Kaede embryos at the appropriate stages were anaesthetised with 0.4% tricaine and then embedded in 0.5% low melting agarose. To achieve complete conversion over a large area, a rectangular area of 1000 by 300 pixels was converted by scanning the area with 50% 405 nm laser at 200 ms per pixel. Following confirmation of Kaede-red expression by acquisition, embryos were recovered in E3 medium for 20hours post-conversion before imaging again the same region. Appropriate imaging parameters were established using the unconverted region as a reference to avoid over or under exposure of the Kaede-green signal. Cross-sections were generated using Fiji-ImageJ software (Schindelin et al., 2012) to create a 3D reconstruction of the image, then ‘resliced’ to yield transverse views of the spinal cord.

To generate PHRESH signalling profiles from the reconstructed transverse views along the dorso-ventral axis, three boxes were drawn directly through average projections of transverse sections stacks of the spinal canal and the fluorescent intensity of Kaede-green was measured along long axis of the box. Measurements were taken at three different

positions of the neural tube, and the average of the three boxes was presented in the graph. Distance in µm were normalised per the neural tube height to allow comparison between fish with slight variations of the total neural tube height, and interpolation used to replot the profile with the same distance step for all embryos. These profiles were generated using Fiji-ImageJ software then graphically represented using GraphPad.

Drug Treatment

Embryos at the appropriate stage were treated with LY-411575 (Sigma, 25µM), GANT-61 (Sigma, 80µM) or DMSO in E3 medium. For the live LY-411575 experiment, the drug was added 30 minutes before the beginning of acquisition. For the fixed experiment, the drug-treated larvae were kept in the dark at 28°C until the time of fixation.

Heat Shock experiments

To induce expression from the heat shock promoter, embryos at the relevant stage were placed in a 2 ml tube with 1ml of E3 medium in a water bath set to 37,5 ℃ for 1hr. For hsp:EGFP-Gli1 experiment, embryos were placed at 28°C from 0hpf to 30hpf, then at 25°C from 30hpf for 18hours to have embryos at 40hpf stage at 9am. Two heat shocks were then performed, one at 40hpf stage and the other at 50hpf stage. Embryos were transferred back into E3 medium in a petri dish and recovered at 28 ℃ until 70hpf stage for fixation. Between the two heat shocks, embryos were placed back to E3 medium at 28°C. For hsp:Gli3R-EGFP, one heat shock was performed at 50hpf. After heat shock, embryos were transferred back into E3 medium in a petri dish and recovered at 28 ℃ until 70hpf for fixation. For drug treatment after heat shock, embryos were transferred directly from the heat shock to E3 medium containing the appropriate drug.

Statistics

Statistical details related to sample size and p values, are reported in the figure legends. All statistics were performed using Prism or Matlab. In the figure panels, asterisks denote the statistical significance calculated using the appropriate test (stated for each test in the legends): * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; **** = $p<0.0001$; ns = $p>0.05$.

RESULTS

1. Cilia are dispensable during lumen remodeling, but Dzip1/*iguana* function is required

We first explored whether cilia were involved in cell signaling required to control spinal remodeling coordination. We noticed that the inflated ventral part of the lumen seems to coincide with the final border of the central canal, and we thought that circulating CSF ventrally might be a cue to determine the upper limit for lumen border retraction (Figure 1A-D, Figure S1C compare 55 hours post fertilization (hpf) and 75hpf, (Ribeiro et al., 2017). We therefore focused on mutants affecting cilia motility only, since they do not properly inflate the ventral circulating portion of the lumen early on (Thouvenin et al., 2020). We analysed two mutants defective for cilia motility : *foxj1a* (*foxj1a*$^{nw2/nw2}$) (Thouvenin et al., 2020), which affects a master transcription factor driving cilia motility, and *dnaaf1,* (dnaaf1$^{tm317b/tm317b}$) (Brand et al., 1996) that encodes dynein axonemal assembly factor 1, involved in cilia motility. We did not observe defects of lumen border height during (55hpf) and at the end (75hpf) of lumen remodeling in the *foxj1a* and *dnaaf1* mutants (Figure 1E-F and 1E'-F' for quantification). This indicates that CSF circulation and motile cilia are dispensable for proper lumen border remodeling in the zebrafish spinal cord.

We then focused our attention on mutants with defects in primary cilia, known to be important relays in different signaling pathways during neurogenesis (Andreu-Cervera et al., 2021). We studied the *elipsa* mutant (*traf*^{3ip1}) (Driever et al., 1996; Bizet et al., 2015) and the *iguana* mutant (*dzip1*^{ts294e}) (Kim et al., 2010). In the *elipsa* mutant embryo, that affects a protein involved in the intraflagellar transport (IFT) and leads to loss of both motile and primary cilia (Omori et al., 2008), we observed a normal lumen height at the two timepoints we analysed (Figure 1G, 1G'). As this mutant lacks all cilia at 55hpf - the onset of lumen remodeling (Omori et al., 2008) (Figure S1A-B), this indicates that cilia are dispensable at the onset of and during lumen retraction.

Contrary to the other mutants we studied, *iguana* mutant embryos presented a defect in lumen remodeling. The *iguana* mutant (*dzip1*^{ts294e}) is defective for the Dzip1 protein that localises to the basal body of cilium, causing defects in primary ciliogenesis (Kim et al., 2010). At 55hpf, the lumen border of *iguana* mutants was already 28% higher than that of control siblings (Figure 1H-H'). This difference was further increased at the end of remodeling at 75hpf, as the lumen border was on average two times higher in *iguana* embryos than in controls with a higher variability of lumen height in iguana embryos (Figure 1H-H', see also Figure S2A, dispersion at 75hpf). We therefore concluded that Dzip1 is required for spinal lumen remodeling. Contrary to *elipsa*, the *iguana* mutant loses most of its primary cilia at an

early stage, and exhibits a change in Gli activity regulation that other ciliary mutants don't have (30hpf - Figure S1A-B) (Huang and Schier, 2009; Kim et al., 2010; Omori et al., 2008).

Moreover, previous works showed the role of motile cilia in central canal inflation (Thouvenin et al., 2020). We therefore compared central canal inflation in *iguana and elipsa* mutants. We observed a decrease in the diameter of the inflated part of the canal in both ciliary mutants at all stages, further demonstrating that canal inflation has no impact on lumen remodeling and retraction (Figure S1C-E). Altogether, our work points towards a specific requirement for the *dzip1* gene in lumen border remodeling.

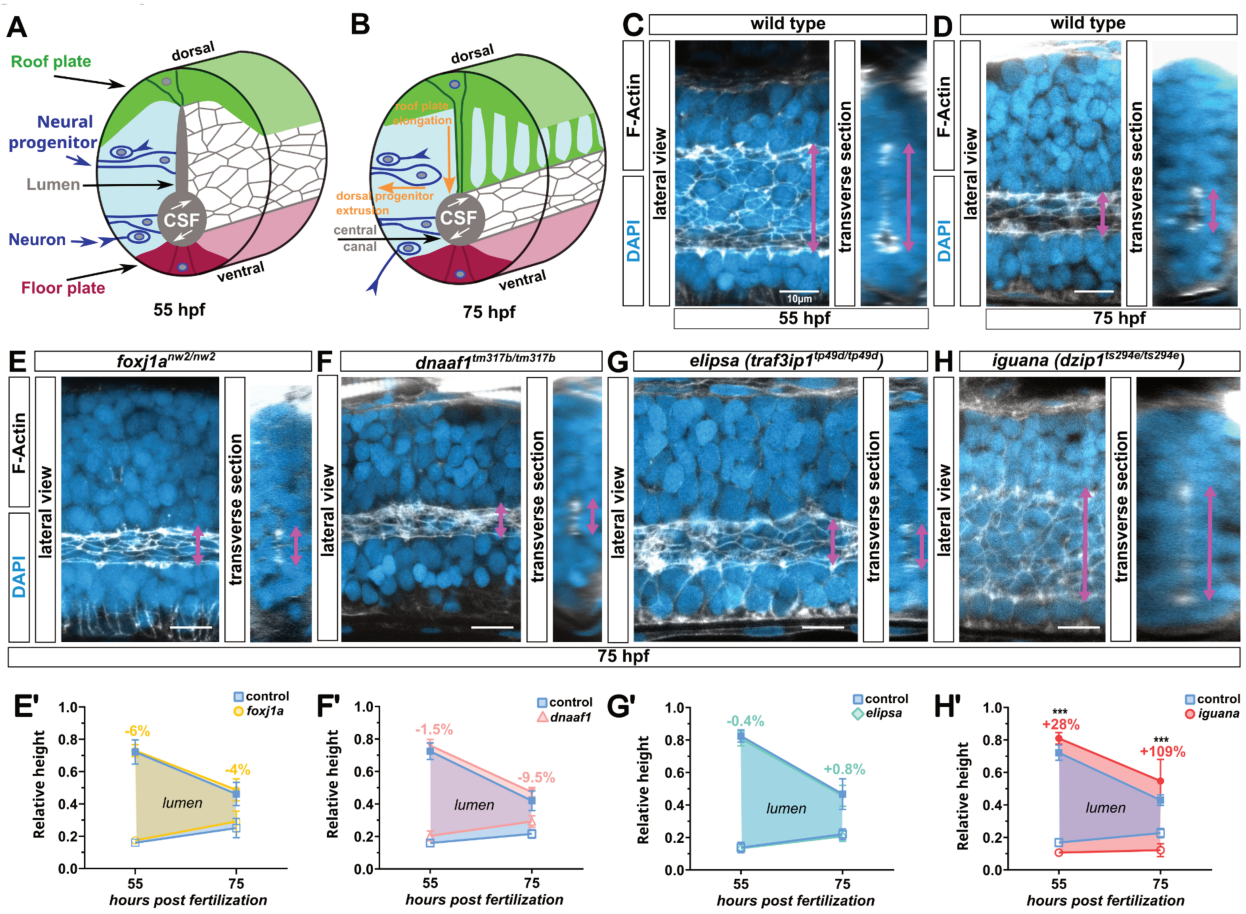

Figure 1: Lumen remodeling is not impaired in the absence of cilia, but is altered in iguana mutants. ***A.*** *3-D diagram of the neural tube at 55hpf depicting the full extent of the spinal lumen formed by the lateral progenitors apical surfaces (blue). It is connected dorsally to the roof plate (green) and ventrally to the floor plate (purple).* ***B.*** *3-D diagram of the neural tube at 75hpf depicting the spinal central canal after remodelling. Orange arrows represent the typical cell behaviours associated with lumen height reduction : progenitor extrusion and roof plate elongation.* ***C-H.*** *Confocal images of F-actin delineating the neural progenitors apical surfaces forming the lumen (phalloidin staining, white) and of the neural tube nuclei (DAPI in blue) in zebrafish embryonic spinal cords at different hours post-fertilisation (hpf). The pink arrows indicate the dorso-ventral extent of the lumen.* ***C.*** *Neural tube at 55hpf, the lumen is still extended dorsally.* ***D.*** *Neural tube at 75hpf, lumen has been remodelled to form the central canal in the ventral part of the neural tube.* ***E, F, G, H*** *Confocal images*

of a 75hpf spinal neural tube of foxj1a (E), dnaaf1 (F), elipsa (G) and iguana (H) mutants. ***E', F', G', H'.*** *Graphical representation of the relative lumen height at 55hpf and 75hpf comparing control siblings (blue) to foxj1a homozygous mutants (E', yellow, n=14 at 55hpf, n=19 at 75hpf), dnaaf1 (F', pink, n=14 at 55hpf, n=21 at 75hpf), elipsa (G', green, n=10 at 55hpf, n=19 at 75hpf) and iguana (H', red, n=13 at 55hpf, n=32 at 75hpf). Relative lumen height is highlighted with a coloured area, while the floor plate and roof plate relative heights are below and above coloured area respectively. Percentages represent the relative lumen heights difference between the mutant and the control. *** = p < 0.0001.*

2. Lumen remodeling in *iguana* presents changes in apical surface shape and behavior of progenitors

To better understand the lumen border height difference between *iguana* and control sibling embryos, we looked at the dynamics of lumen remodelling. We used a double transgenic line expressing GFP specifically in roof plate cells cytoplasm (Et(krt4:eGFP)^{sqEt33}, Kondrychyn et al., 2013), together with a fusion of the tight junction protein ZO-1 with tdTomato, to outline the apical surfaces of the neural progenitors forming the lumen border (*TgKi(tjp1a-tdTomato)^{pd1224}*, Levic et al., 2021). We followed lumen border remodeling by real-time confocal imaging. Control siblings exhibited a regular lumen border retraction between 55hpf and 75hpf (Figure 2A, blue curve in B for quantification ; average retraction speed close to 1 µm/h, see Movie S1 and Figure S2A-B). Spinal cords of *iguana* mutants displayed normal proportions of lumen border height at the beginning of our real-time experiments (50hpf, Figure 2A'-B and Figure S2B-D). However, the retraction of the lumen border was slower (Figure 2A', red curve in B for quantification ; average retraction speed around 0.5µm/h, see figure S2B). These observations are consistent with our time course experiments on fixed embryos (from 35hpf to 95hpf, Figure S2A-C) (Ribeiro et al., 2017). Despite a slightly shorter neural tube in *iguana* mutants (Figure S1E'), the lumen border absolute height and its proportion of the neural tube were significantly higher in *iguana* compared to wild-type siblings from 55hpf onward (see quantifications on Figure S2C). The lumen border of *iguana* mutants never retracted to the proportion observed in wild-type until as late as 95hpf, when remodeling was long finished in the control siblings (Figure S2A-D). Note that the progression of the roof plate extension was also slower in the *iguana* mutants compared to control siblings (Figure 2A-A', 2B), supporting a coupling between the remodeling of these tissues (see below, Figure 4). These results confirm that Dzip1 is required for proper control of lumen border retraction.

As the spinal lumen border is composed of the apical surfaces of neural progenitors, we reasoned that a higher border in *iguana* could be the consequence of an increased number of apical surfaces or of higher apical surfaces, or both (Figure 2C). We counted the

number of progenitor apical surfaces contributing to the lumen border from 50hpf to 95hpf in both *iguana* embryos and control siblings (Figure 2D). While there was no difference at 50hpf, before the onset of lumen retraction, *iguana* mutant lumen borders rapidly displayed an excess of 1 to 2 progenitor apical surfaces forming the lumen border (Figure 2D). We also noticed a larger of Sox2+ domain along the spinal lumen from 60hpf onward (Figure S2E-H), suggesting that the *iguana* mutant has indeed an excess of polarised neural progenitors persisting in time. We then evaluated the shape of the apical surfaces of the most ventral progenitors, that eventually persist to form the central canal (Figure 2E). From 60hpf to 80hpf, apical surfaces of *iguana* mutants consistently displayed a greater height/length ratio (Figure 2F), meaning they were less elongated along the rosto-caudal axis but the apical surface did not present any change in their area quantification (Figure 2G).

The defects in lumen border height in *iguana* mutants can therefore be explained by an increased number of progenitor apical surfaces forming the lumen and by a greater height-to-length ratio of these surfaces (Figure 2D, 2F, 2G). Dzip1 is hence required to regulate both the shape of apical surfaces and the number of progenitors contributing to the lumen border.

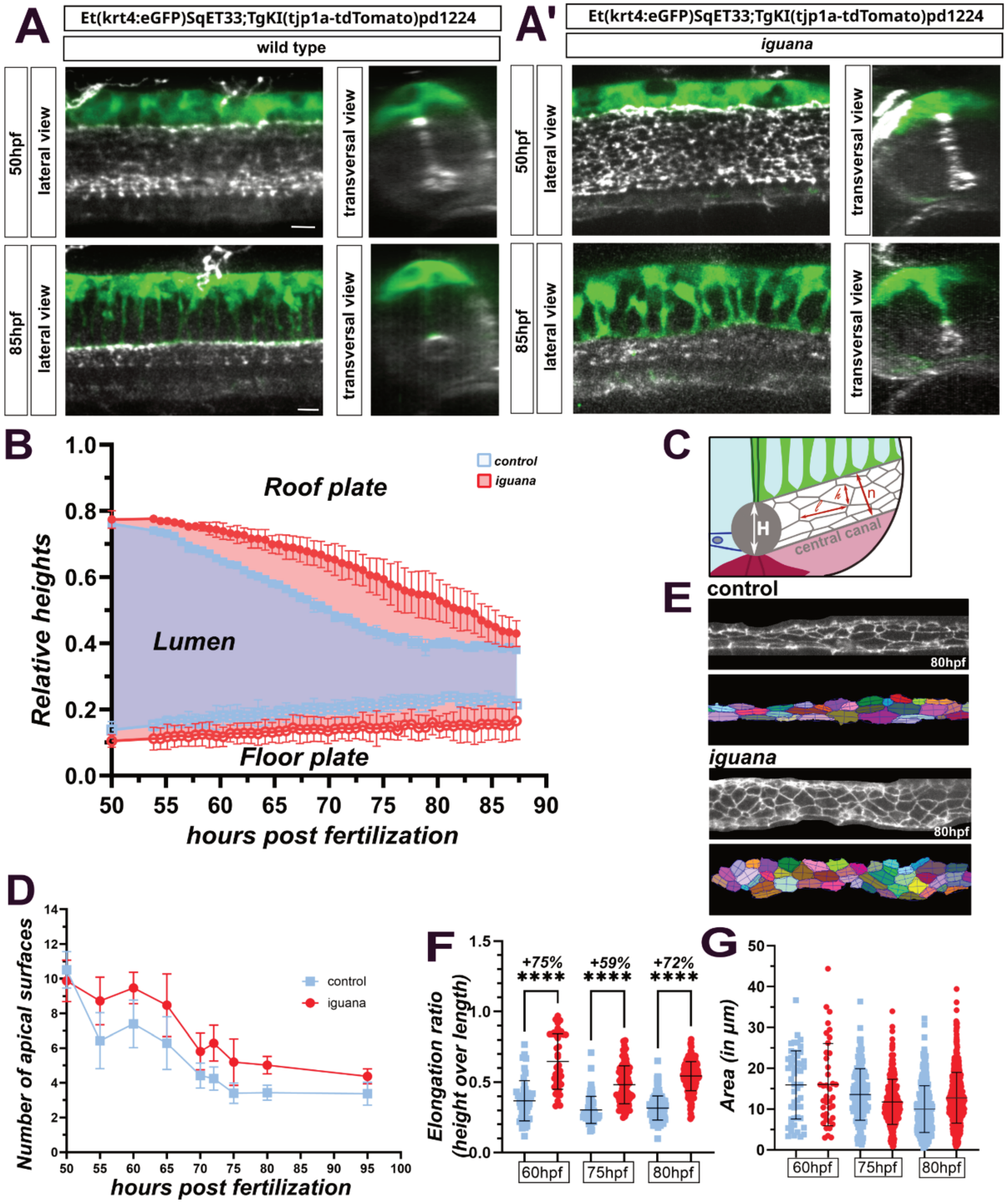

Figure 2. Iguana defect* is due to an increased number of progenitors contributing to the lumen together with a change in their apical surface shape. *A-A'. *Lateral and transversal views of live Et(krt4:eGFP)^{SqET33};TgKI(tjp1a-tdTomato)^{pd1224} embryos at 50hpf and 85hpf of control (A) and iguana mutant (A'). **B.** Quantification of lumen remodelling live in control (blue, n=2) and iguana embryos (red, n=8) from 50hpf to 87hpf. **C.** Scheme of central canal, presenting the height of the lumen, H, dependent on the number of progenitors composing the lumen, n, and the height of their apical surface, h. **D.** Number of progenitor apical surfaces, outlined by ZO-1 staining, composing the lumen during remodeling from 50hpf to 95hpf in control (blue, n=13 at 50hpf, n=11 at 55hpf, n=8 at 60hpf, n=14 at 65hpf, n=6 at 70hpf, n=15 at 72hpf, n=16 at 75hpf, n=4 at 80hpf, n=4 at 95hpf) and iguana (red, n=9 at 50hpf and 55hpf, n=12 at 60hpf, n=8 at 65hpf, n=13 at 70hpf, n=18 at 72hpf, n=23 at*

75hpf, n=5 at 80hpf, n=4 at 95hpf). ***E.*** *Example of segmentation of apical surfaces of lumen-forming progenitors at 80hpf in control and iguana.* ***F.*** *Elongation ratio (height/length) of the progenitors apical surfaces and* ***G.*** *Area in µm of the progenitors apical surfaces forming the lumen in iguana (red) and control siblings (blue) at 60hpf (n=1 for control and iguana), 75hpf (n=1 for control and n=3 for iguana) and 80hpf (n=4 for control and iguana).*

3. Gli activity at the onset of lumen remodeling controls the final height of the central canal

As shown previously (Figure 1), we have excluded a direct requirement for cilia during the lumen border remodeling process itself. However, previous observations in *iguana* mutants reported a dorsal shift of medium-range Shh targets like Olig2 (Jacobs and Huang, 2019) that is thought to depend on Gli1 activity (Huang and Schier, 2009). This prompted us to carefully assess the Gli activity distribution along the dorso-ventral axis of the neural tube of *iguana* mutants at the time of lumen remodeling. To that end, we used the TgBAC-(ptch2:Kaede)^{a4596} reporter line (Huang et al., 2012), as ptch2 is a direct target of Gli activity.

By photoconverting Kaede of the TgBAC-(ptch2:Kaede)^{a4596} reporter line at 55hpf in *iguana* and control siblings, the neo-synthetised Kaede pattern at 75hpf reflects the dorso-ventral gradient of Gli activity during the lumen retraction process (Figure 3A) (Jacobs and Huang, 2019). Ventrally, we observed a decrease in the maximal Gli activity in *iguana* compared to controls (Figure 3B, 3B'), as expected in the absence of the primary cilium that is required for maximal Gli activation (Huang and Schier, 2009; Jacobs and Huang, 2019). More dorsally, we observed an increased medial activation of the Gli activity in *iguana* mutant when compared to controls that display a very low level of fluorescence (Figure 3B, 3B'). This strongly suggests that the population of dorsal progenitors that continued to participate abnormally in the lumen border formation in *iguana* has a higher Gli activity than dorsal progenitors in control siblings (Figure 3B, 3B').

Since the early gradient of Gli activity is known to set the ventral identities of progenitors (Jacobs and Huang, 2019), we wondered if this dorsal shift of Gli activity observed in late development was associated with a dorsal shift of the ventral identities of progenitors. To that end, we immunostained embryos at different stages for Nkx6.1, a marker for ventral progenitor identity (Sander et al., 2000), in *iguana* mutants. When compared to siblings, the higher limit of the Nkx6.1+ domain was only slightly shifted dorsally (Figure S3). Noticeably, the most dorsal progenitors characterised by the absence of Nkx6.1 expression, represented a significant part of the abnormally high lumen border of *iguana* embryos at 72hpf (more than 3 times higher in *iguana* than in control, Figure S3). This indicates that the

dorsal cells that persisted in *iguana* dorsal lumen border did not change their identity for Nkx6.1+ ventral progenitors, suggesting the higher lumen height in *iguana* is not the result of simple cell fate change of dorsal to ventral progenitors.

We therefore hypothesised that the absence of Dzip1 in *iguana* mutants leads to abnormally high Gli activity in these dorsal progenitor cells, preventing them from exiting the lumen. Accordingly, we predicted that inducing an ectopic Gli activation at late embryonic stages would prevent lumen retraction. We used a transgenic line over-expressing the activator Gli1 under the control of the heat-shock promoter Tg(hsp70l:GFP-gli1)^{a4594} (Huang et al., 2012). By triggering the induction of Gli1 expression from 40hpf onwards, we observed a defect in lumen retraction : the lumen height at 75hpf of Gli1 expressing embryo was on average 36% higher than heat-shocked controls devoid of Tg(hsp70l:GFP-gli1)^{a4594} (Figure 3C-D). Of note, this late induction of Gli activity did not significantly modify the range of ventral Nkx6.1 expression but did induce a change in the number of progenitor apical surfaces composing the lumen and their apical height-to-length ratio (Figure 3E-G). Hence, Gli1 overactivation before the onset of lumen retraction phenocopies the progenitor behaviour defects of *iguana* mutants. This reinforces our conclusion that, independently of their dorso-ventral identity, a late activation of Gli1 in dorsal progenitors prevents their exit from the lumen and their elongation along the rostro-caudal axis.

We also predicted that reducing the general level of Gli activity in *iguana* mutants should rescue lumen retraction defects. To do so, we first used a heat-shock inducible Gli3R, one of the repressive forms of the Shh pathway, Tg(hsp:Gli3R-EGFP)^{ca121}. By triggering the expression of Gli3R just before the onset of lumen remodeling, at 50hpf, we restored a lumen retraction in *iguana* closer to that of the wild-type at 75hpf (Figure 3I-J). Gli3R over-expression from 50hpf onwards had no effect on the wild-type siblings, and did not affect the Nkx6.1 pattern at this late stage where patterning is probably already set (Figure 3M-N, and table S1 for statistical tests). We also submitted *iguana* clutches to pharmacological inhibition of Gli activity with GANT-61, Gli antagonist 61, that interferes with DNA binding of the Gli activator forms (Büttner et al., 2012; Lauth et al., 2007). When treated from 32hpf onwards, the *iguana* mutant lumen was shorter at 75hpf than their counterparts exposed to DMSO (Figure 3K, 3M, and table S1 for statistical tests). Again, this treatment had no effect on lumen border height in wild-type siblings (Figure 3M), nor on Nkx6.1 dorsal border position (Figure 3N, and table S1 for statistical tests). These two experiments present partial rescue of *iguana* phenotype while combining them led to a better rescue of the *iguana* lumen retraction defect (Figure 3L, 3M, 3N, and table S1 for statistical tests). These results indicated that the defects in lumen retraction in *iguana* mutants are caused, at least partially, by an increased Gli activity in dorsal progenitors that form the lumen border. Altogether, our work suggests that the decrease in Gli activity in dorsal progenitors at the onset of spinal

remodeling is required for controlling the withdrawal of their apical surface from the lumen, and the shape of the apical surfaces of persisting ventral progenitors.

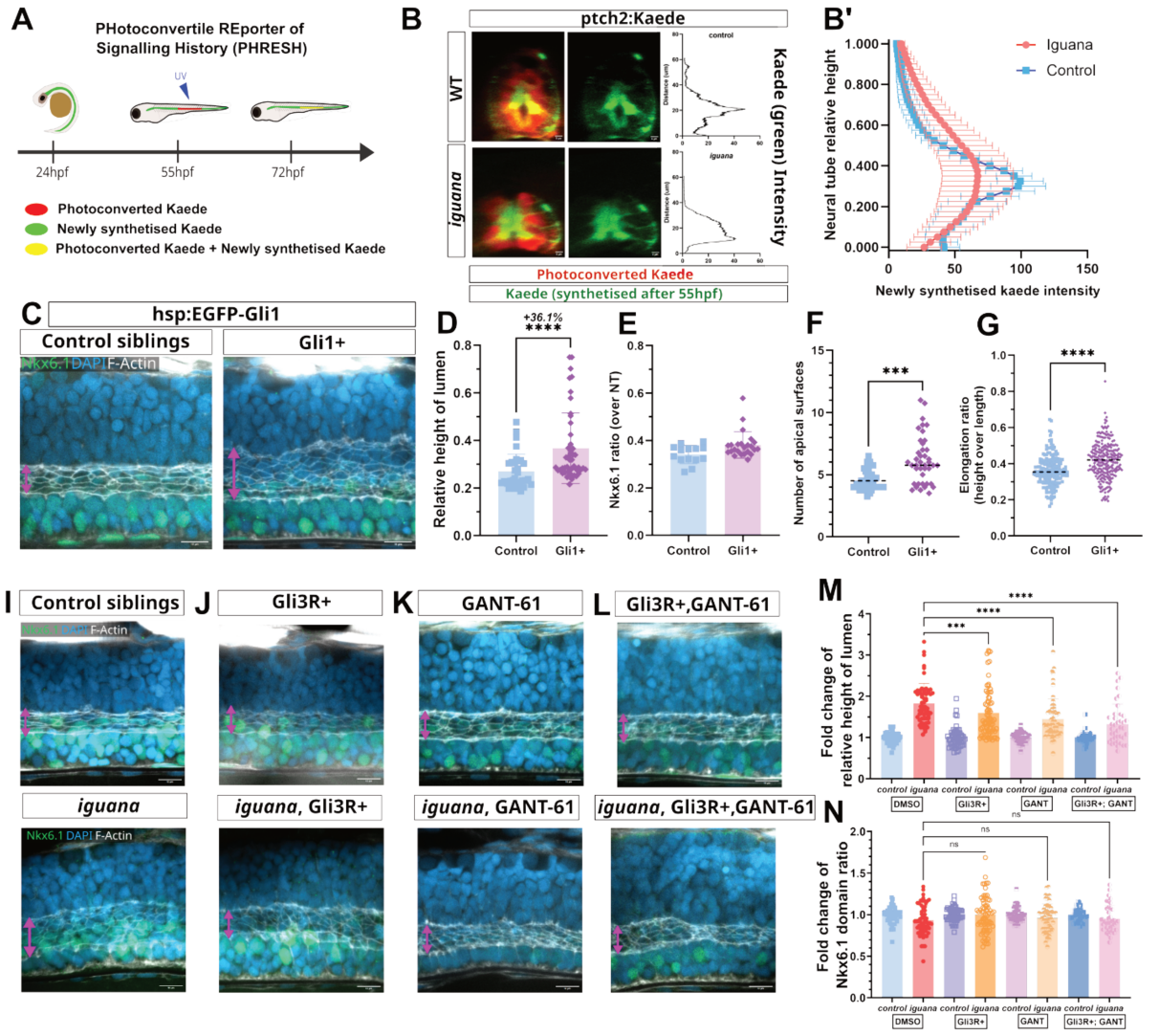

Figure 3. Gli activity is controlling lumen remodelling. ***A.*** *Schematic representation of the experimental design Gli activity is measured using a ptch2:Kaede reporter line. A section of the spinal cord above the yolk extension was photoconverted by the UV light at 55hpf and the fluorescent profile was analysed at 72hpf. We can identify cells that have either new signalling response after photoconversion (green), continued response from before and after photoconversion (yellow), or have ended signalling response before photoconversion (red).* ***B.*** *Transverse views of confocal projections of slices of the spinal cord where we photoconverted with an example of graphical signalling profiles from the dorsoventral axis on the green channel, presenting Kaede produce after photoconversion.* ***B'*** *Global Hh signalling dynamics by PHRESH analysis. Assessment of Gli activity in control (blue) or iguana (red) embryos along the dorso-ventral axis of the neural tube.* ***C.*** *Confocal image of the lumen (F-actin in white) within the neural tube (DAPI in blue) of 75hpf Tg(hsp70l:GFP-gli1)^{a4594} embryos heat-shocked at 40hpf and 50hpf inducing Gli1 over-expression.* ***D.*** *Relative lumen height (lumen height over total neural tube height) in controls (blue, n=32) and Gli1 over-expressing embryos (purple, n=46).* ***E.*** *Nkx6.1 expression in neural tube (height of Nkx6.1+ domain over total height of the neural*

tube). ***F.*** *Number of progenitor apical surfaces.* ***G.*** *Elongation ratio (height over length) in control siblings (bleu) and Gli1-overexpressed (purple) embryos.* ***I-J-K-L.*** *Confocal image of the lumen (white) within the neural tube (blue) in 75hpf Tg(hsp:Gli3R-EGFP)^{ca121} embryos heat shocked at 50hpf to over-express Gli3R (J) or treated at 32hpf with GANT-61 80µM (K) or with both over-expression of Gli3R + GANT-61 treated embryos (L). Pink double arrows represent lumen height.* ***M.*** *Graphical representation of lumen size in controls (blue for control n=11 and red for iguana n=10) and Gli3R over-expressing embryos (purple for control n=19 and orange for iguana n=22), GANT-61 treated embryos (light purple for control n=15 and light orange for iguana n=13) and GANT-61 treated embryos (dark blue for control n=19 and pink for iguana n=22).* ***N.*** *Graphical representation of Nkx6.1 expression in controls (blue for control n=11 and red for iguana n=10) and Gli3R over-expressing embryos (purple for control n=19 and orange for iguana n=22), GANT-61 treated embryos (light purple for control n=15 and light orange for iguana n=13) and GANT-61 treated embryos (dark blue for control n=19 and pink for iguana n=22). For M and N graphs, each data was normalized by the mean of their relative control condition.*

4. The spinal lumen retraction drives roof plate cells extension

Although described in many species, the physical mechanism that coordinates roof plate extension with lumen retraction is not known (Shinozuka and Takada, 2021; Tait et al., 2020). While lumen retracted, the roof plate cells elongated in a coordinated manner. We noticed a slowing down of the roof plate extension concomitant to the defective lumen retraction in *iguana* mutants compared to siblings (GFP signal in green on Figure 2, Figure S2). The coordination between tissues suggests a coupling. To further explore this interaction we sought to accelerate lumen remodeling. Since the lumen border is mostly formed by the apical surfaces of the lateral neural progenitors, we decided to decrease Notch signaling, known to be required for the apical polarisation of neural progenitors from the beginning of lumen formation (Kondrychyn et al., 2013; Sharma et al., 2019). By adding a drug that blocks Notch signaling (LY-411575, a γ-secretase inhibitor) at the onset of lumen remodeling (50hpf), we were able to let the lumen form normally and then determine the effect of a synchronised loss of progenitors apical polarity during lumen remodeling (Figure 4).

Long term real-time confocal imaging allowed us to describe the whole remodeling process in DMSO-treated control embryos, starting from a fully extended lumen border dorso-ventrally and a small dorsal roof plate (50hpf, Figure 4A). We observed that the roof plate cells extended dynamic processes concomitantly to lumen retraction, with their endfeet continuously forming a dorsal ceiling of the lumen along the rostro-caudal axis, consistently with previous observations (Kondrychyn et al., 2013 and Figure S2). This remodeling stopped around 75hpf, when the central canal persisted ventrally (Figure 4A''). As described previously, by measuring the dorso-ventral height of the different tissues, we were able to

assess a remodeling speed of lumen roughly 1µm/h between 50 and 75hpf (Figure 2B, Figure S2B, Figure 4B see Figure S5A and Material and methods for details). We then compared with embryos treated with LY-411575 from the beginning of our time-lapse imaging (49hpf – Figure 4C), and we observed as anticipated an acceleration of lumen retraction, including the loss of the most ventral apical surfaces that normally persist (compare Figure 4A' and C'). We evaluated that the lumen retraction was more than three times faster (from ~1.25 µm/h in DMSO-treated to ~4 µm/h in LY-treated, Figure 4B and 4D, Figure S5A). The roof plate cell extension presented the same acceleration of lumen retraction and proceeded faster than normal for the first 10 hours of filming (Figure 4C', D-E). We then observed two types of behaviour : (i) the majority (66%, 203 cells out of 307, in 13 embryos) of roof plate processes retracted dorsally (Figure 4C'', 4D), following a loss of contact with the apical material of the fastly retracting lumen border, giving rise to a middle spinal space without a lumen (polarised progenitor cells were mostly replaced by extra differentiated neurons as a result of Notch inhibition; Figure S5E'-G). (ii) Some roof plate processes (33%) kept a constant attachment to the apical surfaces of the fastly retracting lumen, eventually forming a contact with the floor plate cells that appeared to be resistant to Notch signal loss (Figure 4C", 4E, S5E"). In both cases, we observed a decrease of Sox2+ cells number and an increase of HuC+ cells number meaning a loss of progenitors due to LY-411575 treatment (Figure S4F-G).

We reasoned that the retraction following the loss of contact with apical surfaces forming the lumen might be the result of the roof plate processes "springing back" after losing the force that extended them. Indeed, we noticed that the floor plate behaviour was modified upon LY-411575 treatment. In the wild-type controls, the floor plate cells exhibited a minimal change of height compared to the roof plate cells (Figure 4A-B). However, upon acceleration of the lumen retraction by Notch inhibition, the floor plate cells were abnormally stretched, which could reflect an increased traction force exerted by the fastly retracting lumen (compare movie 2, and Figure 4 B, D and E for quantification). Consistently, the floor plate cells also changed their height in movement resembling a relaxation when the majority of roof plate cells detached from the lumen around 59hpf (Figure S4D-E for quantification). Altogether, our results argue that the lumen retraction is generating the forces that drive roof plate cells extension through a mechanical coupling between the two tissues.

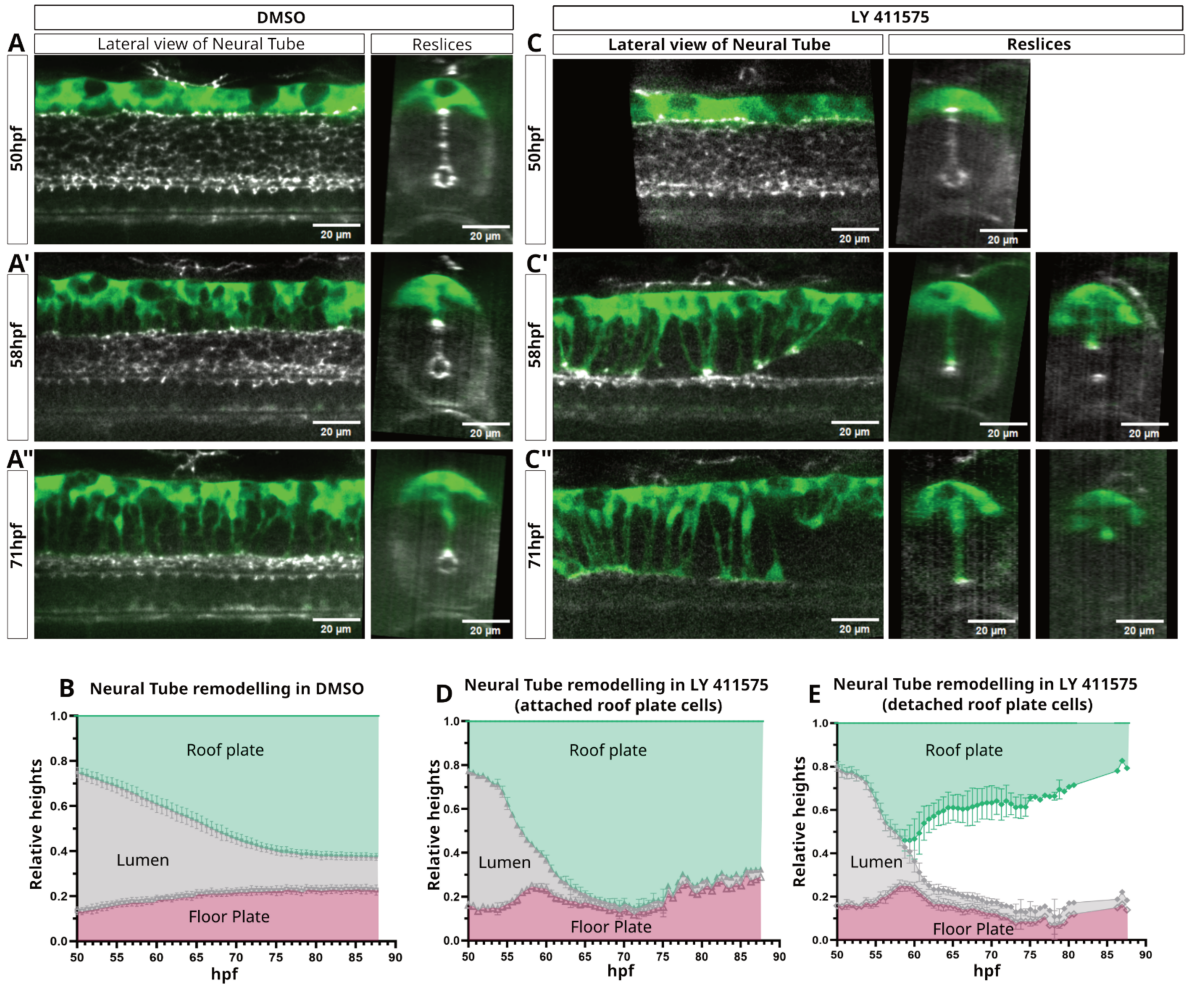

Figure 4. Neural tube remodeling dynamics after treatment inhibiting Notch*. Movie images of the neural tube of transgenic control embryos* Et(krt4:eGFP)^{sqEt33}*, TgKi(tjp1a-tdTomato)^{pd1224} treated with 0.05% DMSO (A, A', A') and embryos treated with LY-411575 (25µM), a Notch inhibitor (C, C', C") at 50hpf, 60hpf and 75hpf, lateral and transverse views. Two distinct behaviours are observed in the RP cell population, with some cells detaching from the lumen and others remaining attached to the lumen (C''). Graphical representation of the movie with analysis of the size of the different tissues making up the neural tube, RP cells in green, lumen in grey, FP cells in purple in control embryos (B, n = 10 embryos) or embryos treated with LY 411575 (D, E, n = 6 embryos). Separate analyses of the two RP cell behaviours, RP cells that remain attached to the lumen (D) and RP cells that detach from the lumen (E). Lumen borders retract faster in LY condition and RP cells elongate at the speed of lumen border retraction. We can observe a slight deformation of the FP around 57hpf, when RP cells start to detach from the lumen border.*

DISCUSSION

In this work, we showed that Gli activity regulation is central for the retraction of the spinal lumen and that this retraction is the driving force of roof plate cell elongation. It illustrates the importance of fine-tuned coordination between cell behaviours in the control of morphogenetic processes involving different cell types to achieve the harmonious development of a tissue.

Cilia are not required for lumen border remodeling, despite their importance in spinal development

We observed that the lumen border remodeling proceeded normally without cilia, even in ciliary mutants that had an impact on lumen inflation (Figure 1 and S1) (Thouvenin et al., 2020). This suggests that the size of the inflated ventral part of the lumen where the CSF circulates does not control the final height of the central canal that results from lumen remodeling. Our results with the *foxj1a* mutant failed to reproduce the morpholino phenotype targeting *foxj1a* mRNA, which displayed a transitory delay in lumen retraction (Ribeiro et al., 2017), maybe due to technical differences in our respective approaches. Our results with *foxj1a* mutants are consistent with the other mutants we used to deplete motile (*dnaaf1*) or all (*elipsa*) cilia, comforting us in our conclusion that neither motile nor primary cilia are directly required for lumen remodeling.

We noted that *dnaaf1* mutants displayed an abnormal elongation of the floor plate domain inducing a slight dorsal shift of the central canal (Figure 1F'), as it has been observed in *foxj1a* morphants in Ribeiro et al. 2017. This suggests that motile cilia are not necessary to control lumen border retraction but might be implicated in the proper position of the canal dorso-ventrally by controlling either the elongation of floor plate cells or the differentiation of neurons deriving from ventral progenitors.

Dzip1 acts upstream Gli activity in lumen retraction control

The *iguana* mutant is an exception among the ciliary mutants we observed, as its lumen border failed to fully retract (Figure 1). While manipulating *iguana* embryos, we noticed a minor but reproducible deformation of their whole neural tube. The *iguana* mutants presented a neural tube with a slightly shorter dorso-ventral axis while slightly larger in medio-lateral axis (Figure S1C-E-E'). We think that this small neural tube deformation is the consequence of a general change in the trunk mechanical properties, as it was more prominent when we mounted live embryos in agarose for live imaging compared to fixed samples. This could be a consequence of the role of cilia in somite development (Huang and Schier, 2009). Several pieces of argument prompted us to decouple this general change of embryonic shape from the specific lumen retraction defects we studied : (1) The neural tube deformation precedes a significant lumen border height difference between *iguana* and

controls (Figure S1, before 50hpf). (2) The lumen border in *iguana* is higher than in control when measured in absolute height (µm on Figure S2), the opposite of what would be expected in a shorter neural tube. (3) We also observed a slightly shorter neural tube in the *elipsa* mutant, but no lumen retraction defect was observed (Figure 1 and S1). (4) When we mimicked the *iguana* phenotype with an overactivation of Gli, we observed a change of lumen border height independently of an effect on neural height (Figure 3C).

Our PHRESH experiment in the *iguana* spinal cord results suggest a change in the dorso-ventral gradient of Gli at the time of lumen remodeling (Figure 3B-B'), where a low-level Gli activity is progressing more dorsally in the absence of Dzip1. The rescue experiments showing that reducing Gli activity restores a shorter lumen in *iguana* mutants confirms that the phenotype we observed is a consequence of an abnormal activity of Gli preventing lumen border retraction. Our results therefore demonstrate a role of Dzip1 in regulating the Gli activity gradient for controlling spinal lumen remodeling.

Dzip1 regulates the Gli activity gradient at the onset of lumen remodeling

Dzip1 is required for the early maintenance of primary cilia, which are known to be crucial to control the post-translational modifications of the Gli proteins to control the balance between activator and inhibitor forms (Andreu-Cervera et al., 2021). In the zebrafish embryo muscles and neural tube, it has been suggested that the early absence of cilia prevents the maximal Gli activation, while promoting a larger domain of low Gli activity, via the transcriptional regulation of Gli1 (Huang and Schier, 2009). This dorsal extension of low activity might in part rely on the absence of Gli repression dorsally, as cilia are also essential for the processing of Gli repressor forms (Andreu-Cervera et al., 2021). Our PHRESH experiment (Figure 3A-C) indicates that this persists between 2 and 3 days post fertilization (dpf) when the lumen remodeling takes place. However, the absence of lumen retraction defects in the *elipsa* mutant indicates a specific requirement for Dzip1 in regulating Gli.

This difference might be due to a specific role of Dzip1 in zebrafish ciliogenesis. The major difference of the *iguana* mutant compared to other ciliary mutants is the earlier loss of primary cilia in the *iguana* mutant due to a minimal maternal contribution, as supported by previous works and ours (Figure S1) (Omori et al., 2008; D'Gama et al., 2024). One possibility is that the loss of primary cilia at early stages of development triggers an aberrant Gli gradient regulation that later persists until the stage of lumen remodeling, due to the ability of Gli1 to act on its own promoter (Jacob and Briscoe, 2003), therefore perpetuating a defect caused by early loss of cilia in iguana.

Alternatively, we cannot exclude that Dzip1 is required in setting the Gli activity gradient independently of its role in ciliogenesis. One report described a dual role of Dzip1 in regulating the Gli activity in HEK293, including via direct interaction between Dzip1 and Gli3, preventing this repressor form of Gli to localise to the nucleus (Wang et al., 2013).

Regardless of the mechanism, our results show that Dzip1 specifically controls Gli gradient activity in late developmental stages to control the spinal lumen remodeling.

A new role for the spinal Gli activity gradient in regulating morphogenesis

While Gli activity was already known to regulate proliferation and patterning of the spinal progenitor cells (Sagner and Briscoe, 2019), our work proposes that the regulation of Gli activity is also essential for lumen retraction control. This control appears largely independent of the early role of Gli in setting the upper limit of Nkx6.1 expression, suggesting that most of our manipulation of the Gli gradient did not significantly affect patterning the differentiation of neural progenitor cells.

It was recently reported that the extent of the Gli activity domain in the zebrafish embryo spinal cord decreases ventrally from 55hpf to 72hpf, a period that coincides with the lumen remodeling (Jacobs and Huang, 2019). In addition, Canizares et al. (2020) observed in mice neural tube a decline of Shh activity concomitant with central canal retraction. Our different experiments in the zebrafish *iguana* mutant and with the ectopic expression of Gli activator all show that an excess of Gli activity prevents lumen retraction. We therefore propose that the gradual decrease of Gli activity in the most dorsal progenitors is a prerequisite for their disengagement of the lumen border, independently of a change of cell fate. Interestingly, this suggests that progenitor proliferation, patterning and cell behaviour are under the control of the same gradient, allowing synchronised cell cycle exit, differentiation and morphogenesis downstream of the same Gli effectors.

Mechanisms downstream of Gli controlling lumen retraction

Shh signaling has already been suggested to control neural tube morphogenesis in the past, namely by regulating the apical domain size in the ventral midline of the folding hindbrain in mice (Brooks et al., 2020, 2025). Interestingly, the apical area of progenitors forming the lumen in zebrafish is not affected when we manipulate Gli activity, but its height-to-length ratio is (Figure 2), arguing for different mechanisms downstream of Shh signaling in these different systems. Although slightly different, these two mechanical changes regulated by Gli activity are commonly dependent on a contractile acto-myosin network (Lecuit and Le Goff, 2007). It suggests that effectors downstream of Gli signaling can regulate the mechanical properties of this acto-myosin network of epithelial progenitor cells.

The precise mechanism for the disengagement of dorsal progenitors from the lumen border, as well as the downstream effectors of Gli controlling this behaviour, are not known and will be the subject of further studies. Noticeably, when we experimentally decreased Gli activity in *iguana* mutants to rescue their lumen border phenotype, we never observed the extrusion of ventral progenitor that form the final central canal, as it is the case when we

inhibited Notch signaling (compare Figure 3J-K-L and Figure 4). This suggests that dorsal and ventral progenitors regulate the engagement of their apical domain into the lumen border differently. It is consistent with the observation in different species that the final central canal is formed by a majority of Nkx6.1 positive progenitors, that correspond to the most ventral progenitor (Figure S3) (Cañizares et al., 2020; Fu et al., 2003; Ribeiro et al., 2017; Tait et al., 2020). The signaling pathway(s) affected by Gli activity in the control of progenitor engagement in the lumen are not known. One hypothesis is that it converges with Notch signaling at some level, as Notch is a very important signal for maintaining apical polarisation of spinal progenitors (Sharma et al., 2019), and Shh and Notch are interacting at different levels by cross-regulation (Jacobs and Huang, 2021).

During early neurogenesis, it has been shown that polarised radial glia cells that engage into neural (or oligodendrocyte lineage) differentiation extrude from the lumen, sometimes by severing their apical domain, as shown in chicken embryos (Das and Storey, 2014; Saade and Martí, 2025). However, self-renewal of polarised progenitors maintains the extent of the lumen along the dorso-ventral axis during most of the neurogenesis period. One can hypothesise that the same extrusion behaviour is recapitulated at the end of neurogenesis by terminating dorsal progenitors and contributes to lumen border size reduction.

To explain this lumen reduction, a previous study suggested that during neurogenesis, the most ventral progenitors can disengage from the lumen through terminal differentiation and that more dorsal progenitors replenish the ventral population of polarized progenitors (Ravanelli and Appel, 2015). On the opposite, another study has proposed a model whereby the most dorsal radial glia cells delaminate in an ordered manner at the end of neurogenesis, giving rise to the lumen retraction that resembles a ratchet (Tait et al., 2020). A more stochastic process can also be envisioned. To date, our results do not allow us to decide the precise position and order of the disengagement of polarized progenitors in the remodeling zebrafish spinal lumen. The dynamics of this cell behaviour leading to spinal lumen retraction in our zebrafish model will require further studies, to fully characterise the link between individual cell extrusion and global lumen retraction.

In the literature, the microRNA miR-219 has been involved in the control of the size of the lumen. When blocked by a morpholino, the lumen retraction failed (Hudish et al., 2016, 2013). This miR-219 is targeting a series of genes encoding proteins required for apical polarisation, like Prcki and Pard3, suggesting that its expression is required at the end of neurogenesis for progenitors withdrawal from the lumen border (Hudish et al., 2013). These works identified Shh signaling as a regulator of lumen retraction (Hudish et al., 2016), consistently with our own results. However, all the manipulation of the Shh were performed

from early developmental stages, which suggests that they might change the whole patterning of the spinal cord progenitor. In our case, we manipulated Gli activity later in development, and always monitored that the ventral limit of Nkx6.1 - a marker for ventral progenitors - was not massively shifted dorsally (Figure S3). Therefore, the precise link between miR-219, apical domain regulation and Gli activity needs to be reexamined in the future. Nevertheless, together with these previous works, our results lead us to propose that the decrease of the Gli activity in the dorsal part of the spinal cord is required to promote the dorsal progenitors behaviour associated with apical domain disengagement from the lumen border, leading to its retraction.

Retraction of the lumen is the driving mechanical force leading to roof plate cells extension

As mentioned in introduction, the concomitance of the roof plate cell extension with lumen retraction was already well described in the past (Böhme, 1988; Cañizares et al., 2020; Kondrychyn et al., 2013; Ribeiro et al., 2017; Ševc et al., 2009; Shinozuka and Takada, 2021; Tait et al., 2020). However, the driving force of this remodeling process remained an open question (Shinozuka and Takada, 2021; Tait et al., 2020).

By manipulating Notch signaling at the beginning of lumen remodeling, we managed to accelerate lumen border retraction, and showed that the roof plate cells were accelerating their extension. This argues for the lumen border retraction being the pulling force onto the roof plate cells, which are prone to deformation in response to this stress. Our conclusions are comforted by a series of observations : (1) previous reports showed that the roof plate (and floor plate) cells do not lose their radial glia identity when Notch was blocked (Kondrychyn et al., 2013; Sharma et al., 2019); it is therefore unlikely that our LY-411575 treatment modified the roof plate cells behaviour directly. This is further confirmed by the fact that the lumen retraction speed upon LY-411575 treatment is constant, independently of whether roof plate cells remained attached to the lumen or recoiled back (Figure 4). We therefore conclude that Notch inhibition primarily affects lumen border retraction, and that the roof plate responds to this increased pulling force via mechanical coupling. (2) This hypothesis is further reinforced by the behaviour of roof plate cells that failed to keep their attachment to the lumen border. At this stage, we have no evidence that can explain why some cells behaved this way, whereas others managed to keep a constant attachment to the lumen border. However, once detached, these roof plate cells retracted their extension in a way that resembles a spring recoiling back, arguing for the fact that they experienced tension from the lumen retraction. This could be further examined in the future with the help biophysical modeling. (3) Although floor plate cells seemed resistant to large deformations, we noticed that they slightly increased their height in response to lumen retraction acceleration, arguing for a pulling force being applied symmetrically on both extremities of the lumen. This slight deformation is also released when a large fraction of roof plate cells

recoiled, as if this detachment of roof plate cells was liberating the excessive force applied by the retracting lumen onto the floor plate. The molecular nature of the mechanical attachment between the roof plate and the lumen, and the biophysical properties allowing roof plate cells to elongate cellular extension will be the scope of further studies.

It has been proposed that, at least in chicken and mouse, roof plate cells secrete a soluble version of the Crumbs2 protein that promotes apical disengagement of the dorsal progenitors (Tait et al., 2020). This is compatible with our observations in the zebrafish embryo, in a model that would suggest that a secretion from the roof plate participates in the lumen retraction that in return, exerts a pulling force on the roof plate cells to trigger their elongation.

Altogether, our work offers new evidence of the importance of mechanical coupling in a morphogenesis process involving several cell types. Interestingly, the zebrafish spinal cord remodeling allowed us to describe quantitatively the effect of this mechanical coupling together with refining our understanding of the signaling regulation of neural progenitor behaviours by Gli. Our system opens new avenues to describe and model the detailed control of morphogenesis that is required for harmonious spinal cord development in vertebrates.

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Movies Legendes

Movie 1. Lumen remodelling in a typical control embryo.

This movie presents images of a live time-lapse confocal acquisition from 50hpf until 85hpf of the spinal neural tube of a non-mutant transgenic embryo : Et(krt4:eGFP)^{sqEt33}*;TgKi(tjp1a-tdTomato)^{pd1224} . Roof plate cells are shown in green by cytoplasmic EGFP inserted in the sqEt33 locus and the apical surfaces of progenitor cells are outlined in gray by a fusion of the junctional protein ZO-1 fused to tdTomato. A stack of images was acquired every 15 minutes, average projections are shown.*

Movie 2. Lumen remodelling in a typical* iguana *embryo.

This movie presents images of a live time-lapse confocal acquisition from 50hpf until 85hpf of the spinal neural tube of an iguana mutant transgenic embryo : Et(krt4:eGFP)^{sqEt33}*;TgKi(tjp1a-tdTomato)^{pd1224} . Roof plate cells are shown in green by cytoplasmic EGFP inserted in the sqEt33 locus and the apical surfaces of progenitor cells are outlined in gray by a fusion of the junctional protein ZO-1 fused to tdTomato. A stack of images was acquired every 15 minutes, average projections are shown. Notice the higher lumen at the end of the process and the slower extension of roof plate cells.*

Movie 3. Lumen remodelling in a typical control embryo treated with DMSO.

This movie presents images of a live time-lapse confocal acquisition from 50hpf until 75hpf of the spinal neural tube of a transgenic embryo treated with 0.05% DMSO : Et(krt4:eGFP)^{sqEt33}*;TgKi(tjp1a-tdTomato)^{pd1224} . Roof plate cells are shown in green by cytoplasmic EGFP inserted in the sqEt33 locus and the apical surfaces of progenitor cells are outlined in gray by a fusion of the junctional protein ZO-1 fused to tdTomato. A stack of images was acquired every 15 minutes, average projections are shown.*

Movie 4. Lumen remodelling in a typical control embryo treated with LY-411575.

This movie presents images of a live time-lapse confocal acquisition from 50hpf until 75hpf of the spinal neural tube of a transgenic embryo treated with LY-411575 at 25µM : Et(krt4:eGFP)^{sqEt33}*;TgKi(tjp1a-tdTomato)^{pd1224} . Roof plate cells are shown in green by cytoplasmic EGFP inserted in the sqEt33 locus and the apical surfaces of progenitor cells are outlined in gray by a fusion of the junctional protein ZO-1 fused to tdTomato. A stack of images was acquired every 15 minutes, average projections are shown. Notice the faster retraction of the lumen, the concomitant acceleration of roof plate cells elongation and the loss of contact of some roof plate cells with the lumen border accompanied by a dorsal retraction of their extension.*

Supplementary Figures

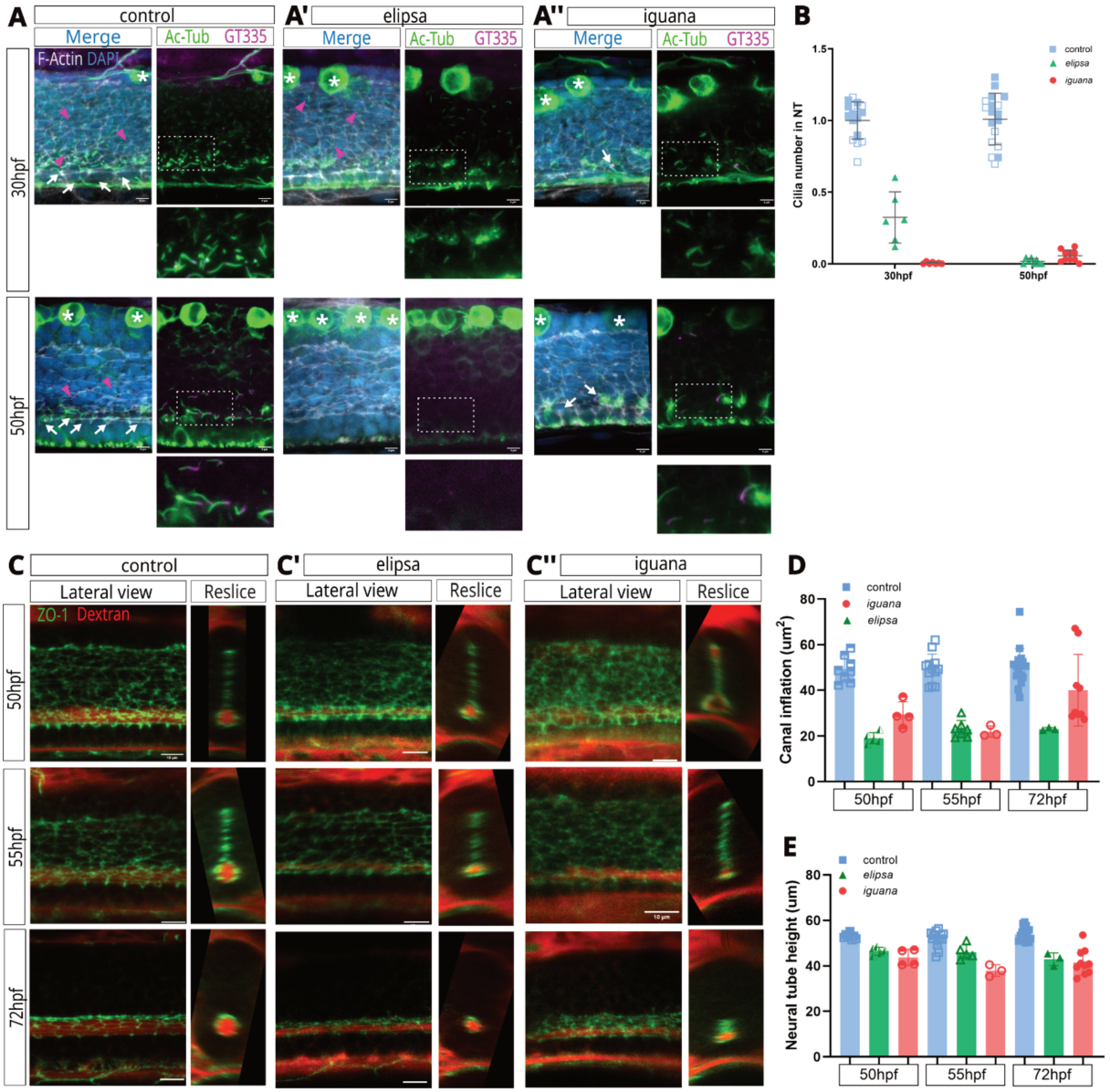

Figure S1. Comparison of phenotypes between ciliary mutants iguana and elipsa. A. *Confocal images of immunostainings for cilia axonemes (ac-tub for acetylated tubulin – green) and basal bodies (GT335 for glutamylated-tubulin, magenta), shown on lateral views of the spinal cord recognized by its dense nuclear DAPI staining (blue). Note that the large dorsal Rohon Beard neurons have a strong cytoplasmic ac-tub signal independent of cilia (asterisk). In control siblings at 30 and 50hpf (n=16 and n=17), we observed a widespread distribution of short primary cilia along the dorso-ventral axis (pink arrowheads on left panels), and a specific ventral enrichment in longer cilia that correspond to the motile cilia (white arrows on left panels)* (Borovina et al., 2010). ***B.*** *Quantification of the relative cilia density on the different mutant conditions at 30 and 50hpf - quantifications are plotted in reference to the control number of cilia. We observe a 75 % decrease in the cilia density in elipsa at 30hpf, probably reflecting a maternal contribution of elipsa mRNA (n=6, pink arrowhead on upper middle panel)* (Omori et al., 2008). *On the contrary, the iguana cilia density is close to zero with only a few*

ventral motile cilia persist (n=7, white arrow in top right panel), consistent with previous results (Huang and Schier, 2009)*. At 50hpf, at the onset of lumen remodeling, elipsa mutants were completely devoid of cilia (n=8, lower middle panel) while in iguana we could detect no primary cilia and a few ventral motile cilia, sometimes together with aberrant bundles of tubulin-based protrusion (n=10, white arrow in lower right panel).* ***C.*** *Confocal imaging of live embryos where the apical sides of progenitor cells forming the lumen is labelled by ZO1-GFP (green) and the circulation cavity of the lumen is filled with a TexasRed-Dextran (red - see* (Thouvenin et al., 2020)*. The Dextran also fills extracellular spaces around the neural tube. Typical lateral views of spinal cords at 50, 55 and 75hpf are shown, together with transverse views reconstructed from the confocal stacks. These images were used to measure neural tube height, canal height and canal inflation.* ***D-D'.*** *Canal inflation measurement in mutants and control siblings. On the transverse images of the spinal cord at the three time points, the diameter of the lumen circulating canal was measured and represented as bar plots for the elipsa mutants (D - green, n=5 at 50hpf, n=5 at 55hpf, n=3 at 72hpf) or iguana mutants (D' - red, n=4 at 50hpf, n=3 at 55hpf, n=10 at 72hpf) and their siblings (in blue, n=8 at 50hpf, n=14 at 55hpf, n=17 at 72hpf). Consistent with previous works showing the role of motile cilia in lumen inflation* (Thouvenin et al., 2020) *we observed a decrease in the diameter of the two ciliary mutants at all stages.* ***E-E'.*** *We also measured and plotted the total height of the neural tube in the same genetic conditions and time points, and we noted a small consistent decrease of the neural tube height in the two mutants elipsa (E) and iguana (E'). We therefore chose to normalise most of our height measurement in ciliary mutants by the total height of the neural tube to compensate for these small variations.*

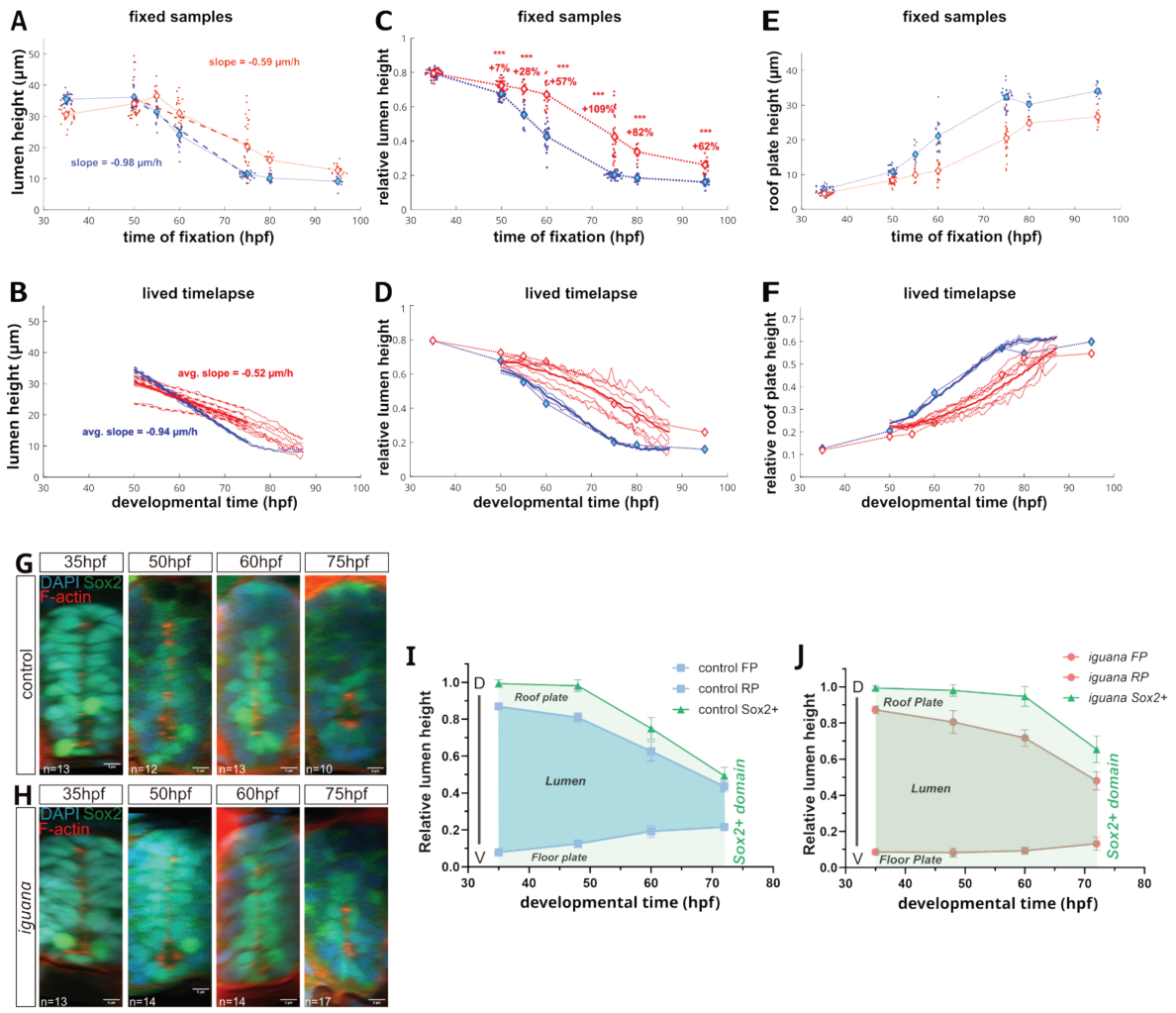

***Figure S2**. **Quantification of lumen height lived and fixed and Sox2+ cells in iguana. A.** Raw data in µm of lumen height along time in fixed control (blue) and iguana (red) embryos. Linear fits between 55 and 75hpf are shown as dashed lines, their slope representing the speed of retraction. **B.** Raw data live timelapse in µm of lumen height over time, in control (blue) and iguana (red) embryos. Linear fits between 55 and 75hpf are shown as bold lines, their slope representing the speed of retraction. **C.** Relative lumen height (lumen height over total neural tube height) in iguana (red) and control siblings (blue).The percentage of increase in the iguana mutant is indicated above each timepoint. **D.** Relative lumen height of live timelapse and fixed embryos with corrected time for live, iguana (red) and control siblings (blue). **E.** Raw data in µm of roof plate height along time in fixed control (blue) and iguana (red) embryos. **F.** Relative roof plate height (roof plate height over total neural tube height) in iguana (red) and control siblings (blue).The percentage of increase in the iguana mutant is indicated above each timepoint. Large dots represent the mean of fixed data and the dashed line the average of each genotype, while dotted lines are lumen retraction of each embryo in the live experiment. The mean of the control and embryos lumen retraction is in bold line. A good agreement between fixed and live experiments is found. A-F For fixed embryos, at 35 hpf, n=28 controls and n= 25 iguana ; at 50hpf, n=25 controls and n= 26 iguana; at 55 n=11controls and n= 10 iguana ; at 60 hpf, n=22 controls and n= 22 iguana ; at 75 hpf n= 30 controls and n= 27 iguana : at 80 hpf, n=12 controls and n= 11 iguana ;*

at 95 hpf, n=15 controls and n= 16 iguana. For live timelapse movies, n=2 controls and n=8 iguana. ***G-H.*** *Sox2 expression in control* ***(G)*** *and iguana* ***(H)*** *neural tube at 35hpf, 50hpf, 60hpf, 75hpf. Sox2 expression is in green, F-actin (stained with phalloidin, red) and nucleus (stained with DAPI, blue).* ***I-J.*** *Quantification of Sox2 expression domain height along the neural tube dorso-ventral axis in control* ***(I)*** *and iguana* ***(J)****. Graphs represent each part of the neural tube (floor plate, lumen, roof plate) within their relative height to the neural tube. Lumen height is highlighted in blue (control) or red (iguana) and the Sox2+ domain is highlighted in green along the dorso-ventral axis. We can observe a persistence of progenitors Sox2+ cells in iguana in the dorsal region.*

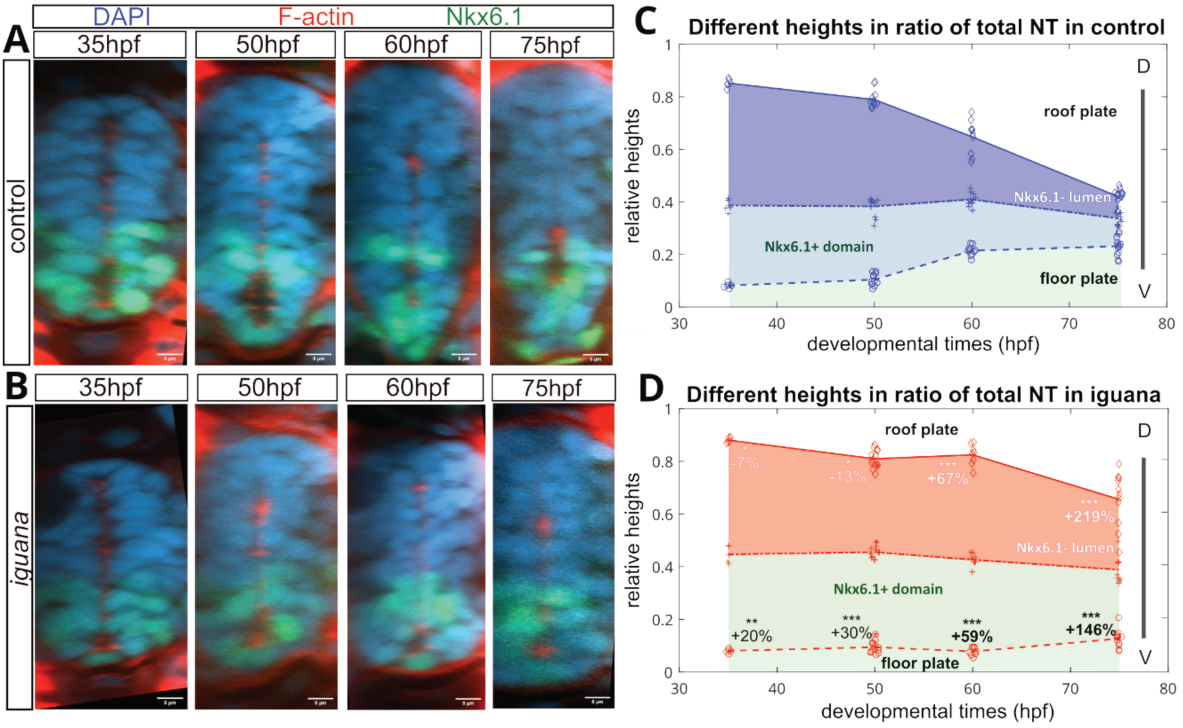

Figure S3. Nkx6.1 expression in control and iguana neural tube A-B. *Confocal images of neural tube (at the level of the cloaca) at 35hpf, 50hpf, 60hpf, 75hpf. Nkx6.1 expression is in green, F-actin (stained with phalloidin) is in red and nuclei (stained with DAPI) are in blue.* ***C-D.*** *Quantification of the Nkx6.1 expression domain along the neural tube dorso-ventral axis in control (n=6 at 35hpf, n=12 at 50hpf, n=10 at 60hpf, n=13 at 75hpf) and iguana (n=5 at 35hpf, n=13 at 50hpf, n=8 at 60hpf, n=11 at 75hpf). Graphs represent each part of the neural tube (floor plate, lumen, roof plate) within their relative height to the neural tube. We highlight the lumen part and distinguish the Nkx6.1- domain of the lumen with dark color and Nkx6.1+ domain of the lumen in light color. Percentage represents the difference between control and iguana at each stage for either the Nkx6.1- or Nkx6.1+ domain. See* (Sander et al., 2000)

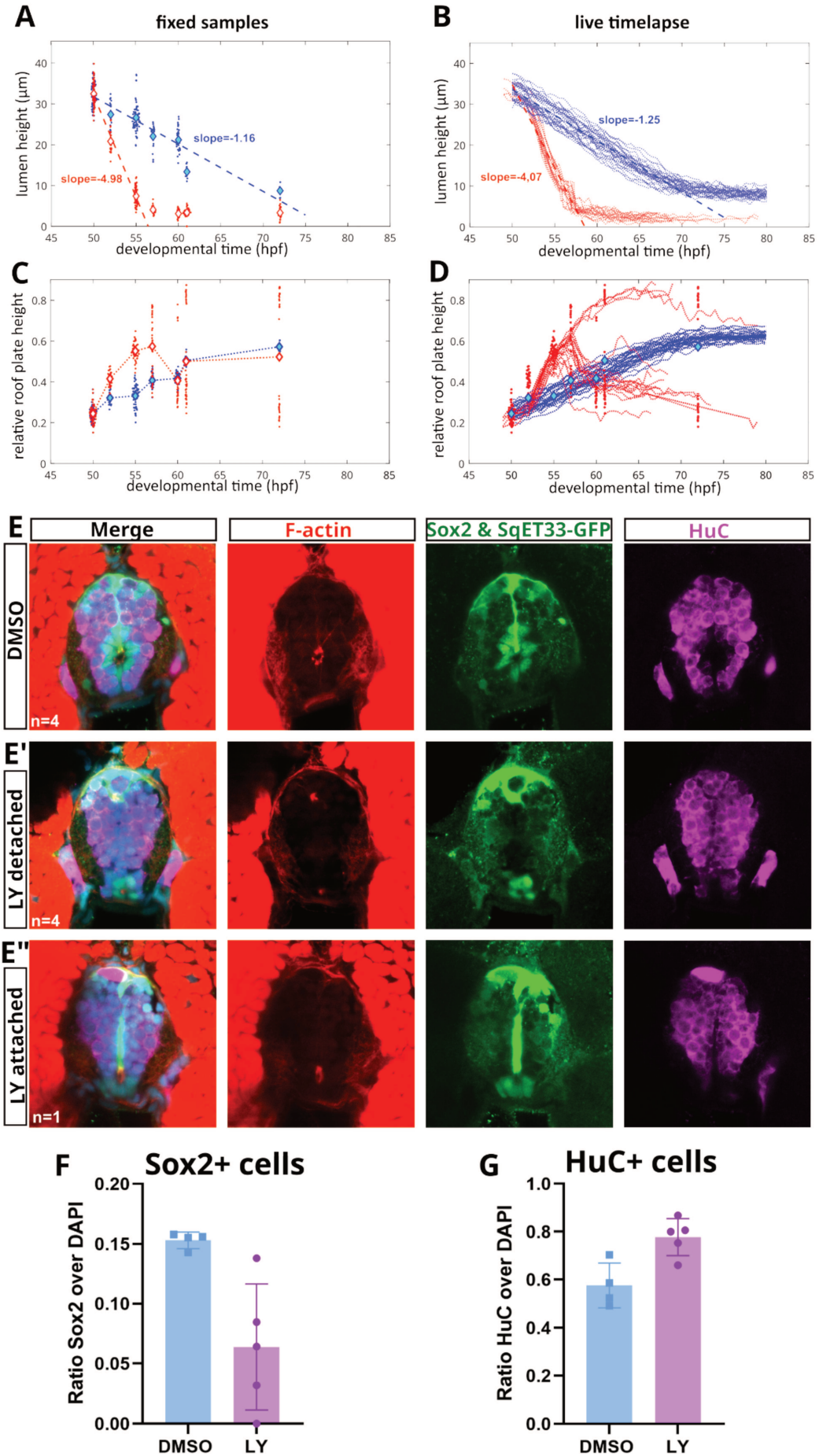
A
fixed samples
lumen height (µm)
slope=-1.16
slope=-4.98
developmental time (hpf)
B
live timelapse
lumen height (µm)
slope=-1.25
slope=-4,07
developmental time (hpf)
C
relative roof plate height
developmental time (hpf)
D
relative roof plate height
developmental time (hpf)
E
Merge
F-actin
Sox2 & SqET33-GFP
HuC
DMSO
n=4
E'
LY detached
n=4
E''
LY attached
n=1
F
Sox2+ cells
Ratio Sox2 over DAPI
DMSO
LY
G
HuC+ cells
Ratio HuC over DAPI
DMSO
LY

Figure S4. LY411575 induces progenitor differentiation. A. *Lumen height in µm, measured in fixed embryos treated by DMSO (blue) or LY-411575 (25µM, red) from 50hpf to 72hpf.* ***B.*** *Lumen height in µm, measured in live embryos treated by DMSO (blue) or LY-411575 (25µM, red) from 50hpf to 80hpf, one image is taken every 15 min.* ***C.*** *Relative height of roof plate in the neural tube, measured in fixed embryos treated by DMSO (blue) or LY-411575 (25 µM, red) from 50hpf to 72hpf.* ***D.*** *Relative height of roof plate in the neural tube, measured in live embryos treated by DMSO (blue) or LY-411575 (25 µM, red) from 50hpf to 80hpf, one image is taken every 15min. A-D. Fixed embryos : 50 hpf, n= 55 slices of DMSO and 55 slices of LY-411575 ; 55 hpf, n= 21 slices of DMSO and 30 slices of LY-411575 ; 55 hpf, n= 62 slices of DMSO and 63 slices of LY-411575 ; 57 hpf, n= 21 slices of DMSO and 32 slices of LY-411575 ; 60 hpf, n= 33 slices of DMSO and 25 slices of LY-411575 ; 61 hpf, n= 12 slices of DMSO and 43 slices of LY-411575 ; 72 hpf, n= 9 slices of DMSO and 31 slices of LY-411575. Live imaging : n=19 slices in 6 embryos from DMSO, n=30 in 10 embryos in LY-411575.* ***E-E'-E".*** *Confocal images of neural tube of 72hpf* Et(krt4:eGFP)^{sqEt33} *zebrafish embryos in DMSO (E, n=4) or treated with LY411575 (25 µM, n=5) from 50hpf to 72hpf (E'-E"). Roof plate cells are GFP+ in green in the dorsal part of the neural tube and the extension is along the spinal midline. The central canal is stained with phalloidin (F-actin, red), progenitor cells are Sox2 (green) and differentiated cells HuC (far red). In LY411575 embryos, two distinct behaviours are observed in the RP cell population, some RP are detaching from the lumen (E') and others remaining attached to the lumen (E").* ***F.*** *Ratio of Sox2+ cells over DAPI+ cells in the neural tube of DMSO and LY-411575 embryos.* ***G.*** *Ratio of HuC+ cells over DAPI cells in DMSO and LY-411575 conditions. In both cases, we observed a reduced number of Sox2+ cells (F) and an increase of HuC+ cells (G).*

Table1			
Lumen Height comparison	Mean diff,	Summary	Adjusted P Value
control DMSO vs. iguana DMSO	-0,1677	****	<0,0001
control DMSO vs. control HS-GFP	0,009004	ns	0,9984
control DMSO vs. control GANT	-0,01597	ns	0,9577
control DMSO vs. control GANT HS-GFP	0,01302	ns	0,9951
iguana DMSO vs. iguana HS-GFP	0,05812	**	0,0012
iguana DMSO vs. iguana GANT	0,04272	ns	0,068
iguana DMSO vs. iguana GANT HS-GFP	0,1197	****	<0,0001
control HS-GFP vs. iguana HS-GFP	-0,1186	****	<0,0001
control HS-GFP vs. control GANT	-0,02498	ns	0,7471
control HS-GFP vs. control GANT HS-GFP	0,004012	ns	>0,9999
iguana HS-GFP vs. iguana GANT	-0,01539	ns	0,9756
iguana HS-GFP vs. iguana GANT HS-GFP	0,06163	**	0,0054
control GANT vs. iguana GANT	-0,109	****	<0,0001
control GANT vs. control GANT HS-GFP	0,02899	ns	0,6855
iguana GANT vs. iguana GANT HS-GFP	0,07702	***	0,0002
control GANT HS-GFP vs. iguana GANT HS-GFP	-0,06098	**	0,0089
Nkx6.1 domain comparison	Mean diff,	Summary	Adjusted P Value
control DMSO vs. iguana DMSO	0,02311	ns	0,1448
control DMSO vs. control HS-GFP	0,006577	ns	0,9971
control DMSO vs. control GANT	-0,01683	ns	0,6631
control DMSO vs. control GANT HS-GFP	-0,02344	ns	0,4539
iguana DMSO vs. iguana HS-GFP	-0,004275	ns	0,9998
iguana DMSO vs. iguana GANT	-0,02746	ns	0,086
iguana DMSO vs. iguana GANT HS-GFP	-0,02786	ns	0,2107
control HS-GFP vs. iguana HS-GFP	0,01226	ns	0,8757
control HS-GFP vs. control GANT	-0,02341	ns	0,3163
control HS-GFP vs. control GANT HS-GFP	-0,03002	ns	0,1561
iguana HS-GFP vs. iguana GANT	-0,02318	ns	0,3302
iguana HS-GFP vs. iguana GANT HS-GFP	-0,02359	ns	0,4043
control GANT vs. iguana GANT	0,01248	ns	0,8888
control GANT vs. control GANT HS-GFP	-0,006613	ns	0,999
iguana GANT vs. iguana GANT HS-GFP	-0,0004044	ns	>0,9999
control GANT HS-GFP vs. iguana GANT HS-GFP	0,01869	ns	0,719

Table S1. *Tables of statistics for Tg(hsp:Gli3R-EGFP)^{ca121} treated with GANT-61 experiment, a two-way ANOVA were performed with multiple comparisons. Statistics are done with GraphPad (Prism). Mean difference, summary of significance and adjusted p-value of each comparison is shown in those tables. On the left, the table of lumen height comparison between each condition of the experiment and on the right the Nkx6.1 domain analysis. ns = p>0.05; * = p<0.05; ** = p<0.01; *** = p<0.001; **** = p<0.0001.*

BIBLIOGRAPHY

Andreu-Cervera, A., Catala, M., Schneider-Maunoury, S., 2021. Cilia, ciliopathies and hedgehog-related forebrain developmental disorders. Neurobiol. Dis. 150, 105236. https://doi.org/10.1016/j.nbd.2020.105236

Bardet, P.-L., Guirao, B., Paoletti, C., Serman, F., Léopold, V., Bosveld, F., Goya, Y., Mirouse, V., Graner, F., Bellaïche, Y., 2013. PTEN Controls Junction Lengthening and Stability during Cell Rearrangement in Epithelial Tissue. Dev. Cell 25, 534–546. https://doi.org/10.1016/j.devcel.2013.04.020

Ben, J., Elworthy, S., Ng, A.S.M., van Eeden, F., Ingham, P.W., 2011. Targeted mutation of the talpid3 gene in zebrafish reveals its conserved requirement for ciliogenesis and Hedgehog signalling across the vertebrates. Development 138, 4969–4978. https://doi.org/10.1242/dev.070862

Bizet, A.A., Becker-Heck, A., Ryan, R., Weber, K., Filhol, E., Krug, P., Halbritter, J., Delous, M., Lasbennes, M.-C., Linghu, B., Oakeley, E.J., Zarhrate, M., Nitschké, P., Garfa-Traore, M., Serluca, F., Yang, F., Bouwmeester, T., Pinson, L., Cassuto, E., Dubot, P., Elshakhs, N.A.S., Sahel, J.A., Salomon, R., Drummond, I.A., Gubler, M.-C., Antignac, C., Chibout, S., Szustakowski, J.D., Hildebrandt, F., Lorentzen, E., Sailer, A.W., Benmerah, A., Saint-Mezard, P., Saunier, S., 2015. Mutations in TRAF3IP1/IFT54 reveal a new role for IFT proteins in microtubule stabilization. Nat. Commun. 6, 8666. https://doi.org/10.1038/ncomms9666

Bloodgood, R., 2009. From Central to Rudimentary to Primary: The History of an Underappreciated Organelle Whose Time Has Come.The Primary Cilium, in: Methods in Cell Biology. Academic Press, pp. 2–52. https://doi.org/10.1016/S0091-679X(08)94001-2

Böhme, G., 1988. Formation of the central canal and dorsal glial septum in the spinal cord of the domestic cat. J. Anat. 159, 37–47.

Borovina, A., Superina, S., Voskas, D., Ciruna, B., 2010. Vangl2 directs the posterior tilting and asymmetric localization of motile primary cilia. Nat. Cell Biol. 12, 407–412. https://doi.org/10.1038/ncb2042

Brand, M., Heisenberg, C.-P., Warga, R.M., Pelegri, F., Karlstrom, R.O., Beuchle, D., Picker, A., Jiang, Y.-J., Furutani-Seiki, M., Eeden, F.J.M.V., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D.A., Kelsh, R.N., Mullins, M.C., Odenthal, J., Nüsslein-Volhard, C., 1996. Mutations affecting development of the midline and general body shape during zebrafish embryogenesis. Development 123, 129–142. https://doi.org/10.1242/dev.123.1.129

Brooks, E.R., Hwang, S.-H., White, K.A., Mukhopadhyay, S., 2025. GPR161–GLI3 repressor signaling at cilia directs apical constriction and cell fate during cranial neural tube closure. Development 152, dev205171. https://doi.org/10.1242/dev.205171

Brooks, E.R., Islam, M.T., Anderson, K.V., Zallen, J.A., 2020. Sonic hedgehog signaling directs patterned cell remodeling during cranial neural tube closure. eLife 9, e60234. https://doi.org/10.7554/eLife.60234

Büttner, A., Busch, W., Klüver, N., Giannis, A., Scholz, S., 2012. Transcriptional responses of zebrafish embryos exposed to potential sonic hedgehog pathway interfering compounds deviate from expression profiles of cyclopamine. Reprod. Toxicol. 33, 254–263. https://doi.org/10.1016/j.reprotox.2011.12.010

Cañizares, M.A., Albors, A.R., Singer, G., Suttie, N., Gorkic, M., Felts, P., Storey, K.G., 2020. Multiple steps characterise ventricular layer attrition to form the ependymal cell lining of the adult mouse spinal cord central canal. J. Anat. 236, 334–350. https://doi.org/10.1111/joa.13094

Chan, C.J., Hiiragi, T., 2020. Integration of luminal pressure and signalling in tissue self-organization. Dev. Camb. 147, 1–10. https://doi.org/10.1242/dev.181297

Das, R.M., Storey, K.G., 2014. Apical Abscission Alters Cell Polarity and Dismantles the Primary Cilium During Neurogenesis. Science 343, 200–204. https://doi.org/10.1126/science.1247521

D'Gama, P.P., Jeong, I., Nygård, A.M., Trinh, A.-T., Yaksi, E., Jurisch-Yaksi, N., 2024. Ciliogenesis defects after neurulation impact brain development and neuronal activity in larval zebrafish. iScience 27, 110078. https://doi.org/10.1016/j.isci.2024.110078

Driever, W., Solnica-Krezel, L., Schier, A.F., Neuhauss, S.C.F., Malicki, J., Stemple, D.L., Stainier, D.Y.R., Zwartkruis, F., Abdelilah, S., Rangini, Z., Belak, J., Boggs, C., 1996. A genetic screen for mutations affecting embryogenesis in zebrafish. Development 123, 37–46. https://doi.org/10.1242/dev.123.1.37

Elmonem, M.E.A., Mohamed, S.A., Aly, K.H., 2007. Early Embryonic Development of the Camel Lumbar Spinal Cord Segment. Anat. Histol. Embryol. 36, 43–46. https://doi.org/10.1111/j.1439-0264.2006.00716.x

Fu, H., Qi, Y., Tan, M., Cai, J., Hu, X., Liu, Z., Jensen, J., Qiu, M., 2003. Molecular mapping of the origin of postnatal spinal cord ependymal cells: Evidence that adult ependymal cells are derived from Nkx6.1+ ventral neural progenitor cells. J. Comp. Neurol. 456, 237–244. https://doi.org/10.1002/cne.10481

Huang, P., Schier, A.F., 2009. Dampened Hedgehog signaling but normal Wnt signaling in zebrafish without cilia. Development 136, 3089–3098. https://doi.org/10.1242/dev.041343

Huang, P., Xiong, F., Megason, S.G., Schier, A.F., 2012. Attenuation of Notch and Hedgehog Signaling Is Required for Fate Specification in the Spinal Cord. PLoS Genet. 8, e1002762. https://doi.org/10.1371/journal.pgen.1002762

Hudish, L.I., Blasky, A.J., Appel, B., 2013. miR-219 Regulates Neural Precursor Differentiation by Direct Inhibition of Apical Par Polarity Proteins. Dev. Cell 27, 387–398. https://doi.org/10.1016/j.devcel.2013.10.015

Hudish, L.I., Galati, D.F., Ravanelli, A.M., Pearson, C.G., Huang, P., Appel, B., 2016. *miR-219* regulates neural progenitors by dampening apical Par protein-dependent Hedgehog signaling. Development dev.137844. https://doi.org/10.1242/dev.137844

Jacob, J., Briscoe, J., 2003. Gli proteins and the control of spinal-cord patterning. EMBO Rep. 4, 761–765. https://doi.org/10.1038/sj.embor.embor896

Jacobs, C.T., Huang, P., 2021. Complex crosstalk of Notch and Hedgehog signalling during the development of the central nervous system. Cell. Mol. Life Sci. 78, 635–644. https://doi.org/10.1007/s00018-020-03627-3

Jacobs, C.T., Huang, P., 2019. Notch signalling maintains Hedgehog responsiveness via a Gli-dependent mechanism during spinal cord patterning in zebrafish. eLife 8, 1–24. https://doi.org/10.7554/elife.49252

Kim, H.R., Richardson, J., van Eeden, F., Ingham, P.W., 2010. Gli2a protein localization reveals a role for Iguana/DZIP1 in primary ciliogenesis and a dependence of Hedgehog signal transduction on primary cilia in the zebrafish. BMC Biol. 8, 65. https://doi.org/10.1186/1741-7007-8-65

Kondrychyn, I., Teh, C., Sin, M., Korzh, V., 2013. Stretching Morphogenesis of the Roof Plate and Formation of the Central Canal. PLoS ONE 8, e56219. https://doi.org/10.1371/journal.pone.0056219

Kramer-Zucker, A.G., Olale, F., Haycraft, C.J., Yoder, B.K., Schier, A.F., Drummond, I.A., 2005. Cilia-driven fluid flow in the zebrafish pronephros, brain and Kupffer's vesicle is required for normal organogenesis. Development 132, 1907–1921. https://doi.org/10.1242/dev.01772

Lauth, M., Bergström, Å., Shimokawa, T., Toftgård, R., 2007. Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists. Proc. Natl. Acad. Sci. 104, 8455–8460. https://doi.org/10.1073/pnas.0609699104

Lecuit, T., Le Goff, L., 2007. Orchestrating size and shape during morphogenesis. Nature 450, 189–192. https://doi.org/10.1038/nature06304

Levic, D.S., Yamaguchi, N., Wang, S., Knaut, H., Bagnat, M., 2021. Knock-in tagging in zebrafish facilitated by insertion into non-coding regions. Dev. Camb. 148. https://doi.org/10.1242/DEV.199994

Omori, Y., Zhao, C., Saras, A., Mukhopadhyay, S., Kim, W., Furukawa, T., Sengupta, P., Veraksa, A., Malicki, J., 2008. elipsa is an early determinant of ciliogenesis that links

the IFT particle to membrane-associated small GTPase Rab8. Nat. Cell Biol. 10, 437–444. https://doi.org/10.1038/ncb1706

Ravanelli, A.M., Appel, B., 2015. Motor neurons and oligodendrocytes arise from distinct cell lineages by progenitor recruitment. Genes Dev. 29, 2504–2515. https://doi.org/10.1101/gad.271312.115

Ribeiro, A., Monteiro, J.F., Certal, A.C., Cristovão, A.M., Saúde, L., 2017. Foxj1a is expressed in ependymal precursors, controls central canal position and is activated in new ependymal cells during regeneration in zebrafish. Open Biol. 7, 170139. https://doi.org/10.1098/rsob.170139

Ringers, C., Olstad, E., Jurisch-Yaksi, N., 2019. The role of motile cilia in the development and physiology of the nervous system. Phil Trans Roy Soc B This Issue.

Saade, M., Martí, E., 2025. Early spinal cord development: from neural tube formation to neurogenesis. Nat. Rev. Neurosci. 26, 195–213. https://doi.org/10.1038/s41583-025-00906-5

Sagner, A., Briscoe, J., 2019. Establishing neuronal diversity in the spinal cord: a time and a place. Development 146, dev182154. https://doi.org/10.1242/dev.182154

Sander, M., Paydar, S., Ericson, J., Briscoe, J., Berber, E., German, M., Jessell, T.M., Rubenstein, J.L.R., 2000. Ventral neural patterning by *Nkx* homeobox genes: *Nkx6.1* controls somatic motor neuron and ventral interneuron fates. Genes Dev. 14, 2134–2139. https://doi.org/10.1101/gad.820400

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019

Ševc, J., Daxnerová, Z., Miklošová, M., 2009. Role of Radial Glia in Transformation of the Primitive Lumen to the Central Canal in the Developing Rat Spinal Cord. Cell. Mol. Neurobiol. 29, 927–936. https://doi.org/10.1007/s10571-009-9377-3

Sharma, P., Saraswathy, V.M., Xiang, L., Fürthauer, M., 2019. Notch-mediated inhibition of neurogenesis is required for zebrafish spinal cord morphogenesis. Sci. Rep. 9, 9958. https://doi.org/10.1038/s41598-019-46067-1

Shinozuka, T., Takada, R., Yoshida, S., Yonemura, S., Takada, S., 2019. Wnt produced by stretched roof-plate cells is required for the promotion of cell proliferation around the central canal of the spinal cord. Dev. Camb. 146, dev159343. https://doi.org/10.1242/dev.159343

Shinozuka, T., Takada, S., 2021. Morphological and Functional Changes of Roof Plate Cells in Spinal Cord Development. J. Dev. Biol. 9, 30. https://doi.org/10.3390/jdb9030030

Sturrock, R.R., 1981. An electron microscopic study of the development of the ependyma of the central canal of the mouse spinal cord. J. Anat. 132, 119–136.

Tait, C.M., Chinnaiya, K., Manning, E., Murtaza, M., Ashton, J.-P., Furley, N., Hill, C.J., Alves, C.H., Wijnholds, J., Erdmann, K.S., Furley, A., Rashbass, P., Das, R.M., Storey, K.G., Placzek, M., 2020. Crumbs2 mediates ventricular layer remodelling to form the spinal cord central canal. PLOS Biol. 18, e3000470. https://doi.org/10.1371/journal.pbio.3000470

Thouvenin, O., Keiser, L., Cantaut-Belarif, Y., Carbo-Tano, M., Verweij, F., Jurisch-Yaksi, N., Bardet, P.-L., van Niel, G., Gallaire, F., Wyart, C., 2020. Origin and role of the cerebrospinal fluid bidirectional flow in the central canal. eLife 9, e47699. https://doi.org/10.7554/eLife.47699

Van Rooijen, E., Giles, R.H., Voest, E.E., Van Rooijen, C., Schulte-Merker, S., Van Eeden, F.J., 2008. LRRC50, a Conserved Ciliary Protein Implicated in Polycystic Kidney Disease. J. Am. Soc. Nephrol. 19, 1128–1138. https://doi.org/10.1681/ASN.2007080917

Wang, C., Low, W.-C., Liu, A., Wang, B., 2013. Centrosomal Protein DZIP1 Regulates Hedgehog Signaling by Promoting Cytoplasmic Retention of Transcription Factor GLI3 and Affecting Ciliogenesis*. J. Biol. Chem. 288, 29518–29529. https://doi.org/10.1074/jbc.M113.492066

II. Supplementary results

A. Notch inhibition effect in *iguana*

As Notch target genes are important regulators of the attachment of progenitors to the lumen, and knowing that Gli can in some cellular contexts regulate positively some Notch target genes (Jacobs et Huang, 2021), I wonder if Gli activity was bypassing Notch requirement to maintain the attachment of dorsal progenitors into the lumen. If so, these extra dorsal progenitors would become insensitive to Notch inhibition. I treated the *iguana* and controlled sibling embryos at 50hpf with 50µM of LY 411575 Notch inhibitor. As already shown in our manuscript, my results indicated that the Notch is essential for keeping wild-type progenitor cells in the border of the final central canal at 70hpf. As also described before, at 70hpf *iguana* DMSO treated embryos present a defect of lumen retraction compared to their control siblings. When treated with LY 411575, lumen in *iguana* embryos was completely lost. This suggests that the Gli balance defects of *iguana* do not act directly on Notch targets, but downstream (Jacobs et al 2019) and/or independently of Notch signaling pathway.

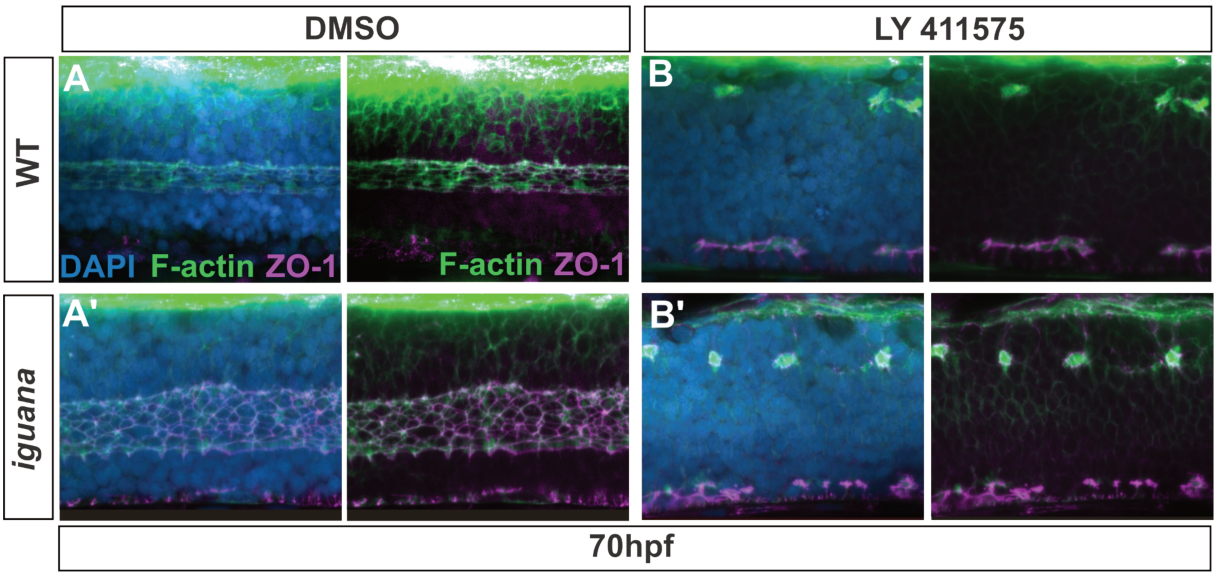

Figure 29. Neural tube remodeling dynamics after treatment inhibiting Notch in iguana. *Confocal images of the neural tube lateral view of control (A) and iguana (A') mutant embryos treated with 0.05% DMSO (A, A') or treated with LY-411575 (50µM), a Notch inhibitor (B, B') at 75hpf. Lumen borders are labelled with phalloidin and ZO-1 staining.*

B. Crosstalk between roof plate cells and progenitors

Tait et al., demonstrated that a truncated form of Crumbs2 secreted by the roof plate cells can promote dorsal progenitors forming the lumen border to delaminate (Tait *et al.*, 2020). Our data suggest that progenitors behavior leads to lumen retraction and exerts pulling force onto roof plate cells to trigger their elongation thanks to a mechanical coupling. This is not contradicting the former suggestion of a secreted protein from the roof plate contributing to the detachment of progenitors from the lumen border. A reciprocal crosstalk between progenitors and roof plate cells might be necessary to finely coordinate their behavior. Using a transgenic zebrafish line in which all roof plate cells are labeled with GFP (Et(krt4:EGFP)sqET33) while some expressed on top the nitroreductase protein (NTR, TgKI(krt4:KALTA4); Tg(UAS:NTR-TagRFPT-UTR)y362), I was able to track roof plate cells ablation and visualised their behaviors. NTR is a well known and well used enzyme that kills cells where it's expressed and active (Johnson *et al.*, 2016). To activate it, I added Nifurpurinol (NFP), a pharmacological component that has been shown to be more efficient in zebrafish than metronidazole, the commonly used drug to activate NTR (Bergemann *et al.*, 2018). In our setting I added the drug at the onset of lumen remodeling to estimate the effect of roof plate cells loss. I tried at 30hpf to see if ablating the roof plate cells before would impact the remodeling but at 50hpf and 72hpf in those conditions the roof plate seems to compensate the early loss of cells (data not shown). Focusing on the roof plate loss during lumen remodeling I exposed the embryos to NFP (1,5uM) at 50hpf and fixed the embryos at 70hpf (Figure 30A). In WT embryos, roof plate cells are forming a continuous line with their end-foot on top of the lumen, forming the ceiling of the lumen. All roof plate cells are then connected and during lumen remodeling this end-foot connection moves coordinately.

Within NTR-expressing embryos treated with NFP, we distinguished three distinct cellular behaviors. First, some roof plate cells expressing the UAS:NTR-RFP transgene did not appear to undergo cell death at 72hpf, and their presence had no detectable effect on lumen morphology or neighboring cell behavior (Figure 30A-B). Second, when a single roof plate cell was ablated, neighboring roof plate cells compensated by maintaining a continuous end-foot connection with each other and with the lumen border, but lumen retraction can be impacted in some cases. These two observations

highlight the robustness and compensatory capacity of the roof plate epithelium at the individual cell level and give first cues about their important role for lumen reduction. Third, when a cluster of adjacent roof plate cells was degraded, a local and more severe phenotype emerged: the nearby roof panel cells were unable to stretch, and the continuous end-foot connection they normally form at the lumen border became bent and deformed (Figure 30A). At the location where a deformation is observed, phalloidin staining of F-actin, which concentrates at the apical surface of progenitors forming the lumen border, revealed that progenitors remained anchored at the lumen border beneath the dying roof plate cells, failing to delaminate locally (Figure 30A). This local maintenance of progenitors at the lumen border spatially correlates with the position of ablated roof plate cells, suggesting that roof plate cells are locally required to trigger progenitor delamination. Consistent with this, lumen height measurements at 72 hpf showed that NTR-expressing embryos treated with NFP displayed a significantly larger lumen compared to both DMSO treated NTR-expressing controls and NFP-treated wild-type embryos, which were indistinguishable from each other (Figure 30B). This indicates that the lumen reduction defect is specifically caused by roof plate cell ablation during the remodeling period, and is not an artifact of drug treatment or transgene expression. We can correlate progenitors maintenance and roof plate cell degradation.

To follow the dynamics of roof plate cells loss and the deformation at the tissue level, I filmed live the process from 50hpf, when I put the NFP drug to induce apoptosis of the roof plate cells to 96hpf (Figure C-C''). During the 48 hours of development 71% of roof plate cells expressing UAS:NTR transgene are dying (81/131 cells, in 12 movies). The elongation of roof plate cells is less continuous and their end-foot connection bent where roof plate cells are dying at 72hpf (Figure C'). At 96hpf, the roof plate elongation is complete but gaps at the level of the end-foot connection of roof plate cells appeared where the roof plate cells are dying. At the tissue level, roof plate cells elongated and compensated for the lack of the once degraded. The gap might be a consequence of late degradation of roof plate cells. Comparison between early and later loss of roof plate cells in the formation of gaps is needed to evaluate this. Beyond the number of cells lost, a collective compensation by the other cells in the roof maintains the lumen remodelling process complete.

Taken together, these preliminary results indicate that roof plate cells are locally and transiently required to promote progenitor delamination and lumen retraction. When individual cells are lost, neighboring cells compensate and remodeling proceeds normally. However, when a group of cells is simultaneously ablated, local progenitor delamination is impaired, causing a transient delay in lumen reduction that is ultimately rescued by the collective behavior of the remaining roof plate population. These data support a model in which lumen remodeling relies on a reciprocal and local crosstalk between roof plate cells and progenitors: roof plate cells promote progenitor delamination from the lumen border, which in turn drives lumen retraction and mechanically stimulates roof plate elongation. This self-regulating collective process ensures robust and complete remodeling even in the face of local cellular perturbations. Since these data are preliminary data, additional quantification and repeat experiments will be necessary to fully establish the significance of these observations.

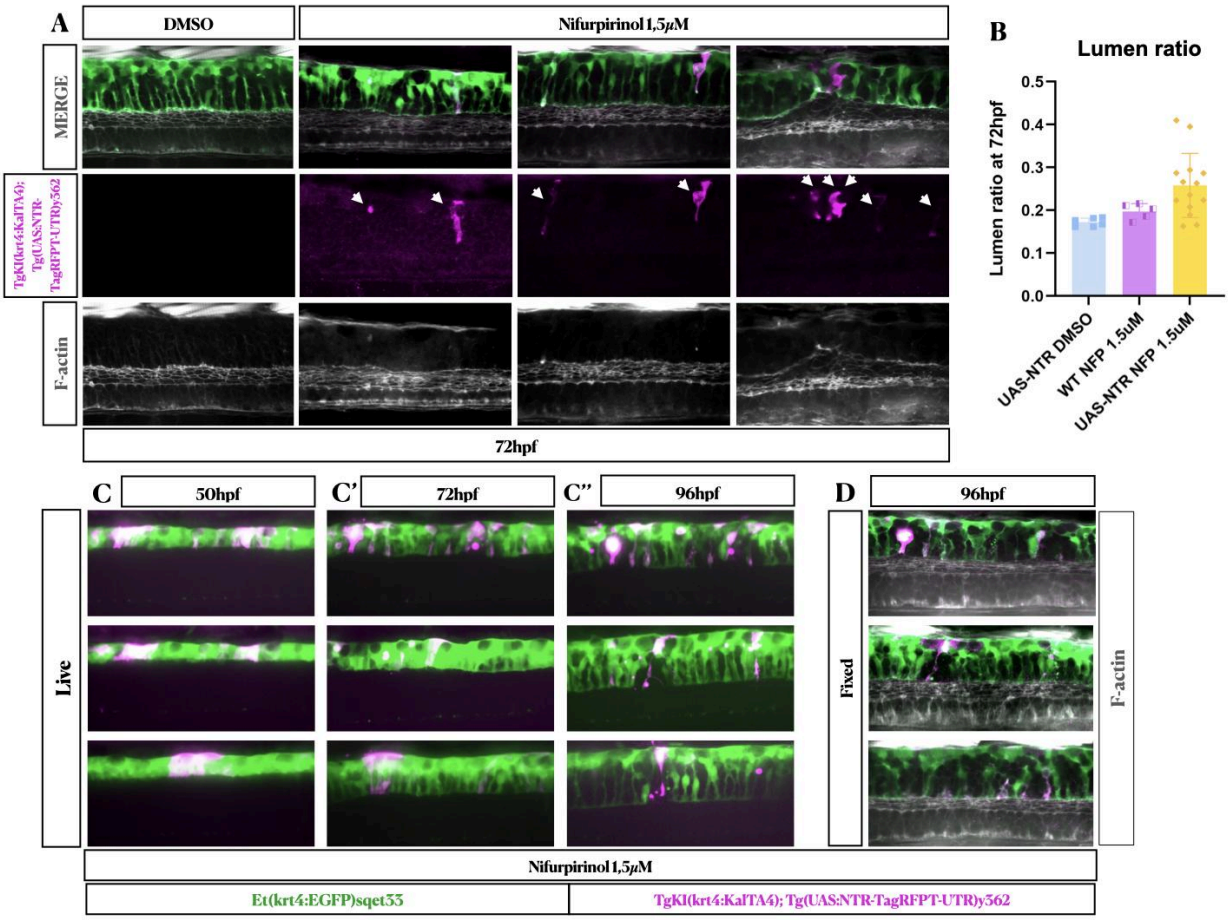

Figure 30 : Ablation of roof plate cells disrupts lumen remodeling in zebrafish neural tube. *(A) Confocal images of the zebrafish neural tube at 72 hpf, treated with DMSO (control) or Nifurpirinol (NFP, 1.5 µM). Embryos carry the transgenic line TgKI(krt4:KalTA4);Tg(UAS:NTR-TagRFPT-UTR)y362, driving expression of nitroreductase (NTR) fused to the TagRFPT fluorochrome (magenta). Filamentous actin (F-actin) is shown in grey. MERGE panels represent the overlay of the green channel (Et(krt4:EGFP)sqet33) and the magenta channel. White arrowheads indicate NTR-positive cells showing induced or enhanced signals upon Nifurpirinol treatment. Due to experimental issues, we do not detect UAS:NTR-RFP cells in the DMSO treated embryos. (B) Quantification of the lumen ratio at 72 hpf across three experimental groups: UAS-NTR larvae treated with DMSO (blue), wild-type (WT) larvae treated with NFP 1.5 µM (purple), and UAS-NTR larvae treated with NFP 1.5 µM (yellow). Bars represent mean ± standard deviation. Individual dots correspond to values per embryos. (C–C'') Live images of TgKI(krt4:KalTA4);Tg(UAS:NTR-TagRFPT-UTR)y362 embryos treated with NFP 1.5 µM, at 50 hpf. (C) 72 hpf (C'), and 96 hpf (C''). The time course shows the progressive appearance of TagRFPT-positive cells (magenta) over development.(D) Fixed embryos at 96 hpf after NFP 1.5 µM treatment, showing the localization of NTR-TagRFPT-positive cells (magenta) and F-actin staining (grey), alongside the EGFP signal (green). Green: Et(krt4:EGFP)sqet33; magenta: TgKI(krt4:KalTA4);Tg(UAS:NTR-TagRFPT-UTR)y362. hpf: hours post-fertilization; NFP: Nifurpirinol; NTR: nitroreductase.*

DISCUSSION AND PERSPECTIVES

My PhD project has established the role of Gli balance in the remodeling of the neural tube lumen. However, this project brings up a lot of new questions. Firstly, the study of the cilia-deficient *iguana* mutant allowed us to identify a link between defective Gli balance and lumen reduction. As this phenotype has not been observed in other cilia-deficient mutants, I will discuss the specific characteristics of this mutant that could explain this difference, as well as the data still to be explored in this mutant to better understand the regulation of the lumen remodeling. Secondly, the remodeling of the lumen appears to be divided into three key stages: the initiation of remodeling; the mechanisms regulating the reduction of lumen size; and, finally, the retention of certain progenitor cells to form the final central canal. I will briefly outline the potential reasons why the lumen suddenly begins to narrow. I will then discuss possible upstream and downstream regulations of Gli proteins, which govern the behaviour of progenitor cells and induce their delamination from the lumen border. Finally, it would be interesting to study more in detail the specific properties that distinguish the delaminating progenitors from those that maintain their apical polarity to form the central canal. Having explored individual behaviour, remodeling occurs at the tissue level, and it is important to discuss this at that scale as well. The behaviour of progenitor cells during remodeling raises few questions as: which progenitor cells delaminate, and is there a predefined order to their delamination during remodeling? In this context, the coupling and coordination with roof plate cells is a key aspect. I will therefore discuss some open questions of research that remain to precisely identify the mechanisms of mechanical and chemical coupling between roof plate cells and progenitor cells, based on the existing literature and the most likely hypotheses of interaction. Finally, the collective behaviour of roof plate cells under constraints allows us to consider the coordination of this group of cells at the tissue level. Ultimately, the deformation of roof and floor plate cells differs, and it would be interesting to determine whether these are intrinsic characteristics of the two cell types or whether the differing properties of the tissue along the dorso-ventral axis explain their distinct behaviour.

I. Why is the study of neural tube morphogenesis important ?

The neural tube is a crucial organ in vertebrate development, and its formation is well conserved across species. As presented in the introduction, the neural tube gives rise to the diversity of neurons and glial cells following a stereotypic organisation in response to different morphogen gradients and complex signaling pathway interactions. This complex relationship between molecular signals, via morphogen diffusion, cell-cell interaction and molecular integration is essential to understand tissue development. Investigation of morphogenesis and mechanical interactions in neural tube development improves our understanding of the fundamental mechanisms that govern early embryonic development.

In mice, the progenitor cells forming the lumen at embryonic stages start to differentiate from E14-E16 into ependymal cells, which are epithelial ciliated cells lining the spinal cord central canal and cerebral ventricles (Spassky *et al.*, 2005; Deng *et al.*, 2023). In zebrafish, most of the cells forming the CNS ventricles in adult remain ciliated radial glial cells which present a proliferative and regenerative potential, forming a stem cells niche (Ribeiro *et al.*, 2017; Becker, Becker et Hugnot, 2018; Jurisch-Yaksi, Yaksi et Kizil, 2020), while in mice the ability of ependymal cells to form a neural stem cell niche is more controversial (Spassky *et al.*, 2005; Cañizares *et al.*, 2020). During the last decade several papers tried to decipher how cells that persist around the adult spinal central canal contribute to regeneration. Some vertebrates such as the zebrafish, axolotl and lizard can regenerate organs and it has been shown that motor movement and sensitivity can be restored in those animals after spinal injury, while in humans glia scarring is the only mechanism taking place (Becker, Becker et Hugnot, 2018). The lizard tail is well known to regenerate completely after an injury. Remarkably, their neural tube patterning during embryonic development is well conserved with other vertebrates. During tail regeneration, the production of ventral embryonic neural tube-like structures is observed while dorsal structures are absent (Lozito et al., 2021). Studying neural tube development allows us to understand more specifically the pathways and molecules involved in the regeneration process. Understanding the different cell behaviors

between ventral and dorsal neural tube during development could highlight important mechanisms of regenerative regulation.

Lumen remodeling during development has never been specifically investigated to determine why it is important for development. After neural tube closure, the neuroepithelium formed by the apical surface of progenitor cells is essential for neurogenesis. Later in development, the neuroepithelium surface is reduced and lumen height decreases. The phenomenon of lumen reduction through the reduction of this neuroepithelium is conserved in many vertebrates, but its function remains largely mysterious.

The remodeling of the lumen could be a passive consequence of other mechanisms. At the end of neurogenesis, it is thought that dorsal progenitor cells differentiate and lose their apico-basal polarity while delaminating, reducing the number of progenitor cells that form the lumen (Tait *et al.*, 2020). Only a small group of progenitor cells remains around the inflated portion of the canal. Therefore, the lumen could mostly shrink as a passive consequence of neurogenesis termination and the only progenitors that maintain would be the ones in contact with the circulating CSF, forming at the end the final central canal and allowing CSF circulation. The elongation of the roof plate would allow maintaining a tight sealing of the lumen.

Lumen remodeling could also have more active functions in development, for example in axon guidance and the establishment of the spinal sensory circuitry. One study has shown that the absence of roof plate cells, as seen in Dreher mice that carry null mutations of the *Lmx1a* gene, leads to defects in peripheral neuronal axon elongation (Kridsada *et al.*, 2018). Indeed, axon guidance can depend on morphogen gradients via their non-canonical pathways (Yam et Charron, 2013). Roof plate cell extension displaces the source of BMP and Wnt secretion ventrally and, as has been reported, Shh activity is reduced dorsoventrally during lumen remodeling (Jacobs et Huang, 2019; Shinozuka *et al.*, 2019; Cañizares *et al.*, 2020). The Shh, BMP and Wnt modifications during lumen remodeling seem therefore to be necessary for proper axon guidance along the neural tube. In addition, roof plate cells are recognized as scaffolding glial cells and it has been

reported in birds that elongated roof plate cells inhibit axons from crossing the midline in the trunk (Rekler et Kalcheim, 2021).

Moreover, it has been shown that Wnt secretion is important for the development of blood vessels. The colonization of the central nervous system by blood vessels appears to depend on the attraction by Wnt (Sebo *et al.*, 2025). The elongation of the roof plate at the end of neurogenesis increases the extent of Wnt secretion ventrally, which could be a good way to attract in different places the blood vessels. This hypothesis could be tested experimentally with zebrafish models where roof plate cells elongation are impacted like, HS-Gli1 or LY 411575 condition. In Hs-Gli1, the roof plate cells are less elongated due to an overexpression of Gli1, while in LY411575, a Notch inhibitor, the roof plate cells extension is sped up. In both conditions, analyzing blood vessels colonisation within the central nervous system would enable us to assess the role of the roof plate cells elongation in blood vessel development.

In any case, the study of central nervous system morphogenesis is crucial for the understanding of various diseases and the global understanding of biological processes. The lumen remodeling as I have studied during my PhD is a largely conserved phenomenon in vertebrates and is important for the development of the central canal and the central nervous system. In the following part I will discuss my results and suggest potential investigations to better understand the regulation of lumen remodeling.

II. How does the loss of Dzip1 function affect dorsal neural progenitor behavior and Gli signalling?

A. Why did we study the *iguana* mutant ?

The *iguana* mutant is the only one among the various ciliary mutants we studied to exhibit a defect in lumen retraction during lumen remodeling. In fact, other ciliary mutants we examined such as *dnaaf*, *elipsa*, and *foxj1* mutants, show no defects in

remodeling although they are all affected in cilia function. This suggests that the phenotype observed in *iguana* is not a general consequence of ciliary loss, but may instead reflect a more specific role of Dzip1 in this process.

The *iguana* mutant is known to exhibit early ciliary absence, a similar feature to what is observed in maternal-zygotic IFT88 mutants (also known as *MZoval*), where both maternal and zygotic contributions of the gene are abolished (Huang et Schier, 2009). We demonstrated, using the PHRESH reported system, that in *iguana* embryos, Gli activity maximal amplitude is decreased ventrally but a lower activity spanned more dorsally compared to controls. This dorsoventral imbalance in Hedgehog signaling output highlights a complex role of Dzip1 in modulating Gli activity. One hypothesis is that the early loss of cilia prevents the establishment of Gli balance along the dorso-ventral axis by the Shh morphogen gradient. Instead, a basal expression of Gli1 independent of the Shh ligand, specific to zebrafish, leads to a lower but broader Gli activity along the dorso-ventral axis (Huang et Schier, 2009).

To determine whether the failure of lumen retraction is truly specific to *iguana* or whether it can be reproduced by other severe ciliary mutations, we could examine other genetic models, notably the *MZoval* mutant. This maternal-zygotic mutant results from the complete loss of function of the *oval* gene, which encodes the IFT88 protein, an essential component of the intraflagellar transport (IFT) machinery required for cilium formation (Borovina et Ciruna, 2013). Since the persistence of maternal mRNA in *oval* zygotic mutants still allows for partial cilium formation at early stages, maternal-zygotic (*MZoval*) mutants were generated to produce embryos completely devoid of cilia (Huang et Schier, 2009). This model thus constitutes an interesting tool for studying the consequences of a total absence of cilia in zebrafish. *MZoval* mutants exhibit reduced Hedgehog pathway activity but a dorsally-expanded domain of medium-level Hh targets such as Olig2 which appears similar to the phenotype we and others described in *iguana*. Comparing this mutant with *iguana* would therefore allow me to dissociate the effects of early and total ciliary loss from the potential cilium-independent functions of Dzip1 (see below), and to determine whether the lumen retraction phenotype observed in *iguana* reflects a functional specificity of this protein or a more general consequence of the absence of cilia during early developmental stages.

B. Potential role of Dzip1 in regulating Gli independently of cilia

Dzip1 is a basal body protein shown to be essential for ciliogenesis. In the absence of Dzip1, a lack of cilia in multiple organs has been reported. Cilia are essential for regulating the Gli activity but as far as we know, I cannot exclude that Dzip1 is required in setting the Gli activity gradient independently of its role in ciliogenesis. One report described a dual role of Dzip1 in regulating the Gli activity in HEK293, including via direct interaction between Dzip1 and Gli3, preventing Gli3R, the repressor form of Gli3, to migrate to the nucleus (Wang *et al.*, 2013). Moreover, Dzip1 has been demonstrated to be important for Gli turnover independently of cilia by stabilizing Spop (Speckle-type POZ protein), a protein promoting proteasomal degradation of Gli transcription factors (Schwend *et al.*, 2013). Whether or not the defect of lumen remodeling in *iguana* mutant is cilia dependent, both studies report an impact of Dzip1 on the Gli balance, assuring that Gli regulation depends on Dzip1. Regardless of the mechanism, my results show that Dzip1 specifically controls the Gli activity gradient in late developmental stages to control the spinal lumen remodeling. Deciphering what regulates the Gli gradient and what the Gli gradient regulates to modulate lumen remodeling are the questions addressed in the following parts of the discussion.

C. Are dorsal sensory progenitors maintained in *iguana* ?

I have shown that in *iguana* mutant, progenitor cells retain their apical surface forming the lumen in the dorsal region; these progenitor cells are not ventral progenitors, as they are Nkx6.1-negative. As *iguana* presents an expanded GliA activity in the dorsal part of the neural tube, it would be interesting to determine the identity of the dorsal progenitor cells that maintain their apical surface in *iguana*. To that end, we could describe all progenitor identities in *iguana* by immunostaining at an early stage of development in the neural tube. If the progenitors maintained in *iguana* present dorsal identity we should then verify if some dorsal interneurons domain is expanded. As the dorsal neural tube gives rise mainly to sensory neurons, deciphering if *iguana* presents more sensory neurons or not and if sensory functions are impaired in this mutant could help us to determine the biological importance of the lumen remodeling process. As *iguana* has a modified gradient of Gli activity from an early timepoint that might lead to complex

identity phenotypes difficult to interpret, doing the same characterisation of dorsal specification in the late hs-Gli1 experiment could yield clearer results.

III. What initiates lumen remodeling?

After neural tube closure a first phase of division occurs, during which progenitor cells proliferate. This phenomenon has been well described in chicken and mouse, where it is combined with an increase in neural tube size, while in zebrafish no significant size increase has been reported at this stage (Saade *et al.*, 2013; Kicheva *et al.*, 2014; Kuzmicz-Kowalska et Kicheva, 2021; Kicheva et Briscoe, 2023). During proliferation, the pool of progenitor cells increases and eventually the cells start to differentiate to produce neurons: this is called neurogenesis. During neurogenesis, the newborn neurons delaminate from the lumen but during a certain period, the lumen size is maintained independently of the neuron delamination rate. At the end of neurogenesis, lumen remodeling occurs : the lumen reduces to the ventral part of the neural tube while roof plate cells elongate (Cañizares *et al.*, 2020). An undetermined shift of behavior of progenitor cells is happening and the regulation of this specific shift has not been fully identified.

A. Shift of cell division mode

In chick embryos, Saade et al. observed a shift during neurogenesis from asymmetric divisions (giving rise to one progenitor and one neuron -PN) to symmetric divisions (giving rise to two neurons - NN) in the ventral neural tube, dependent on Shh activity and on a reduction of the cell cycle (Saade *et al.*, 2013). An increase of symmetric divisions would lead to an increase in the delamination rate and thus to a decrease of the lumen border size. The authors did not look at lumen remodeling while assessing the division analysis. It could be interesting to look at the division characteristics along the complete dorso-ventral axis of the neural tube during remodeling. A current PhD student in the lab is trying to assess the differentiation rate of the progenitors before and during lumen remodeling in the dorsal and ventral parts of the neural tube to decipher if the differentiation rate and the increase in symmetric NN divisions is related to lumen

reduction. The perfect way to answer this question would be to label each progenitor individually and live track their differentiation with neuronal markers. If remodeling is linked to the cell division mode, the prediction is that before remodeling most of the progenitors would undergo asymmetric divisions (PN), while during remodeling they would undergo symmetric divisions (NN). This experiment would help us to determine whether a real link between cell division mode, differentiation and lumen reduction exists.

B. Spatio-temporal shift of Notch and Shh signalling

At the end of neurogenesis in the zebrafish neural tube, Jacobs et al. observed a sharp decrease of Shh and Notch activity, reported by *ptc2* and *her12* expression respectively (Jacobs et Huang, 2019). The activity of the two pathways decreased in intensity and reduced to the ventral part of the neural tube during the stages of lumen remodeling (from 48hpf to 72hpf). The authors demonstrated that inhibition of Notch activity in the zebrafish neural tube led to decreased Shh activity. Also, an ectopic activation of Notch leads to an increase and expansion of Shh activity. Both mechanisms seem to be independent of cilia but regulated at the level of Gli proteins. The authors also showed that inhibiting Hh activity by cyclopamine treatment does not impact Notch activity in the neural tube. They conclude that Notch acts on Shh signaling by regulating Gli activity (Figure 31) (Jacobs et Huang, 2019). These observations have been made from 20hpf to 30hpf in zebrafish spinal cord, i.e. 24 hours before lumen remodeling. Knowing that the neural tube is a dynamic structure, we should verify if the relationship between Shh and Notch is similar at the time of remodeling, during the 40hpf to 72hpf period.

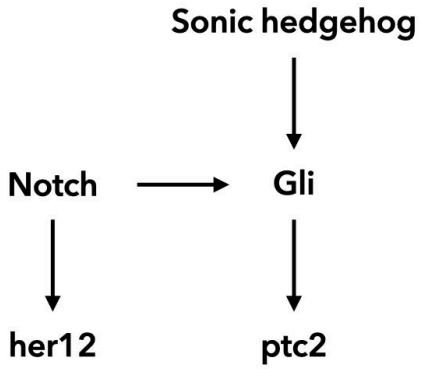

Figure 31. Regulation of Gli by Notch. *Adapted from(Jacobs et Huang, 2019). Arrows represent activation relationships between the indicated elements.*

Notch inhibition has been shown to be essential for neural tube progenitor delamination (Baek *et al.*, 2018). Thus, a spatio-temporal shift of Notch signalling to the ventral part of the neural tube leading to a following decrease of Gli activity could be a mechanism initiating and controlling lumen reduction. To verify this hypothesis, it would be interesting to overactivate Notch activity by driving the expression of NICD specifically in progenitor cells just before lumen remodeling (around 50hpf) to observe if the lumen remodeling is delayed or even stopped. As observed with heat-shock Gli experiments (HS-Gli), the time window of Gli activity is important. Indeed, some experiments of HS-Gli1 did not show any variation of lumen height, depending on the timing of heat-shock. The temporal control of lumen remodeling seems essential. Conducting experiments involving the overexpression or inactivation of Notch and Shh at different timepoints before lumen remodeling would be a good way to identify the key timepoints at which the reduction in Notch and Shh signaling is important to induce lumen remodeling.

IV. What regulates progenitor delamination during lumen remodeling ?

I demonstrated that the involvement of progenitor cells in the formation of the lumen border depends on Gli activity. The lumen reduction process might depend on delamination of progenitors forming the lumen. The delamination process has been linked to differentiation of neurons to allow their displacement to their final position in the CNS (Kawaue *et al.*, 2019; Kawaguchi, 2021). The delamination process of newborn neurons in the spinal cord is still an incompletely defined mechanism. In the present chapter, I will summarize the current mechanisms reported to be implicated in lumen remodeling or known to regulate delamination of neuronal cells in the process of differentiation and address how they could act upstream or downstream of Gli regulation during lumen remodeling.

A. What could be upstream of Gli ?

Hudish et al. demonstrate that miR-219 promotes lumen remodeling by inhibiting Pard3 and Prkci and downregulating Shh signaling in the neural tube. Indeed, as described in the introduction, by adding cyclopamine (a Shh inhibitor) while inhibiting miR-219, the lumen remodeling process is rescued (Hudish *et al.*, 2016) miR-219 would thus be upstream of Gli activity and negatively control it to regulate lumen remodeling. The authors demonstrate that this regulation occurs through a decrease of Par polarity proteins, which then decreases the progenitor cell response to Shh promoting cell cycle exit and loss of neural progenitor characteristics (Figure 32) (Hudish, Blasky et Appel, 2013; Hudish *et al.*, 2016).

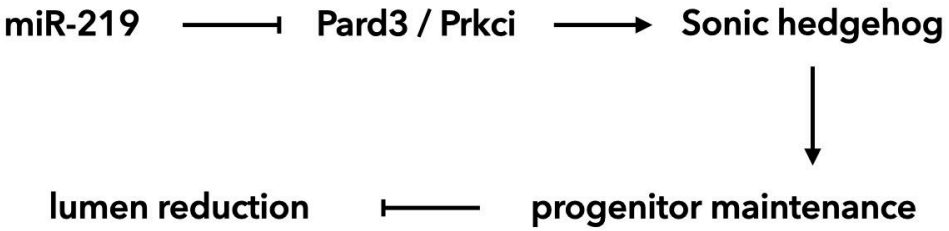

Figure 32. Regulation of lumen remodeling by miR-219 *adapted from.(Hudish, Blasky et Appel, 2013; Hudish et al., 2016) results. Arrows represent activation (simple arrow) or repression (flat-headed arrow) relationships between the indicated elements.*

In their discussion, Hudish et al suggest that Pard3 might regulate Shh activity through Notch signaling. The Notch signalling pathway has been suggested to regulate Shh activity through the modulation of Gli transcription factors (Jacobs et Huang, 2019). Based on this, I propose that apical polarity proteins could act upstream of Notch, such that a reduction in apical polarity would lead to decreased Notch activity, which in turn would reduce Gli activity and ultimately promote lumen reduction (Figure 33). To test this hypothesis, apical polarity proteins such as Par3 or aPKC could be manipulated in a temporally controlled manner, followed by quantification of Notch and Shh transcriptional targets and lumen height measurements.

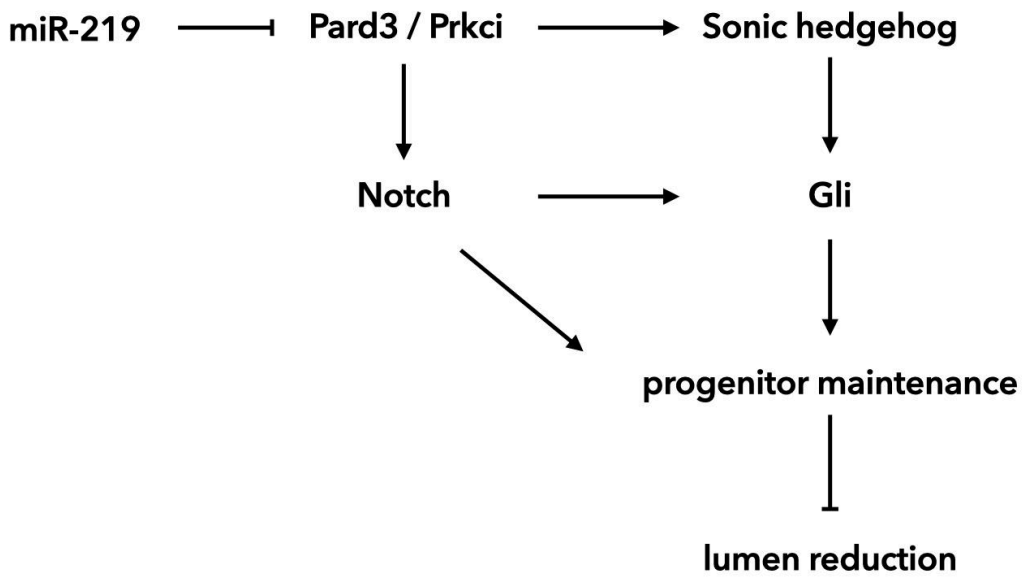

Figure 33. Schematic representation of my hypothesis of upstream regulation of Gli during the lumen remodeling process. *Arrows represent activation (simple arrow) or repression (flat-headed arrow) relationships between the indicated elements.*

B. What could be the downstream targets of Gli activity ?

By demonstrating a link between Gli activity and lumen reduction, I hypothesise that Gli influences the delamination of progenitor cells. Study has reported that the decrease of N-cadherin (N-cad) levels is a key step in the delamination process (Kasioulis, Das et Storey, 2017). This reduction of N-cad has been shown to be regulated by numerous mechanisms. In the following section, I detail the mechanisms that could be downstream of Gli and act on the decrease of cadherin at the apical surface to induce delamination.

1. Apical constriction

In *iguana* and under conditions of Gli1 overexpression, we observed that the shape of the apical surface of the most ventral progenitors, which persist to form the central canal, was altered. Their apical surfaces were less elongated along the rostral-caudal axis, with no change in their surface area. Recent studies suggest a link between Shh and Gli3 repressor and apical constriction in the neuroepithelium (Brooks *et al.*, 2020, 2025). The apical surface of neuroepithelial cells has been demonstrated to be regulated by Shh signaling and Gli3 proteins during neural tube closure and in the hindbrain ventricular zone. This regulation follows a precise spatial pattern along the mediolateral axis: cells

receiving high levels of Shh signalling at the midline remain flat and apically expanded, whereas lateral cells, where Shh activity is low and Gli3R is predominant, undergo coordinated apical constriction (Brooks *et al.*, 2020, 2025). This patterned cell remodelling is driven by the apical organisation of actomyosin, specifically, the assembly of F-actin and phosphorylated myosin II cables in lateral cells. When Shh signalling is ectopically elevated in these cells, actomyosin cables become disorganised and apical constriction fails, ultimately preventing neural fold elevation (Brooks *et al.*, 2020). We could suggest that during lumen remodeling Gli3R in the dorsal part of the neural tube could trigger the apical delamination of the progenitors by promoting apical constriction.

Taken together, these findings suggest that Gli transcription factors may differentially regulate apical constriction in neural progenitors: while Gli1 activity would promote maintenance of the apical surface, Gli3R would instead reduce N-cadherin expression and thereby favour progenitor delamination. Since N-cadherin at adherens junctions physically anchors the apical actomyosin network through its association with α-catenin and F-actin, a reduction in N-cadherin levels would be expected to weaken this anchorage and thereby impair the transmission of actomyosin-generated contractile forces, ultimately disrupting apical constriction (Harris et Tepass, 2010). Consistent with this model, our heat-shock experiments and analysis of *iguana* mutants both appear to show a disorganisation of the progenitor apical surface, which could reflect a reduction in N-cadherin in adherent junction. Analyzing at high resolution N-cadherin of progenitor apical surface comparing *iguana*, Gli3R and Gli1 overexpression would be a way to assess N-cadherin variation in these conditions.

2. Differentiation

Our data demonstrate that neural tube lumen remodeling is under the control of Gli activity. Changing Gli balance in the neural tube at the onset of lumen remodeling impacts progenitor behaviors. Gli proteins are already known to be important for proliferation and specification in the neural tube acting downstream of the Hh morphogen pathway to regulate it. It seems that the same signals are used for multiple actions regulating progenitor patterning, behavior but also morphogenesis according to my work, accessing a robust way of synchronizing distinct behaviors.

Recently, Das et al. described the apical abscission and the dismantlement of cilia during delamination followed by an inactivation of the canonical Shh activity (Figure 34) (Das et Storey, 2014b). Their work among others helps to determine that N-cadherin decreases at the onset of differentiation and induces apical abscission (Figure 34) (Das et Storey, 2014b, 2014a; Kasioulis, Das et Storey, 2017; Kawaguchi, 2021). Neurogenin 2 (Neurog2) has been reported to decrease N-cadherin proteins inducing cell delamination (Rousso *et al.*, 2012; Kasioulis, Das et Storey, 2017).

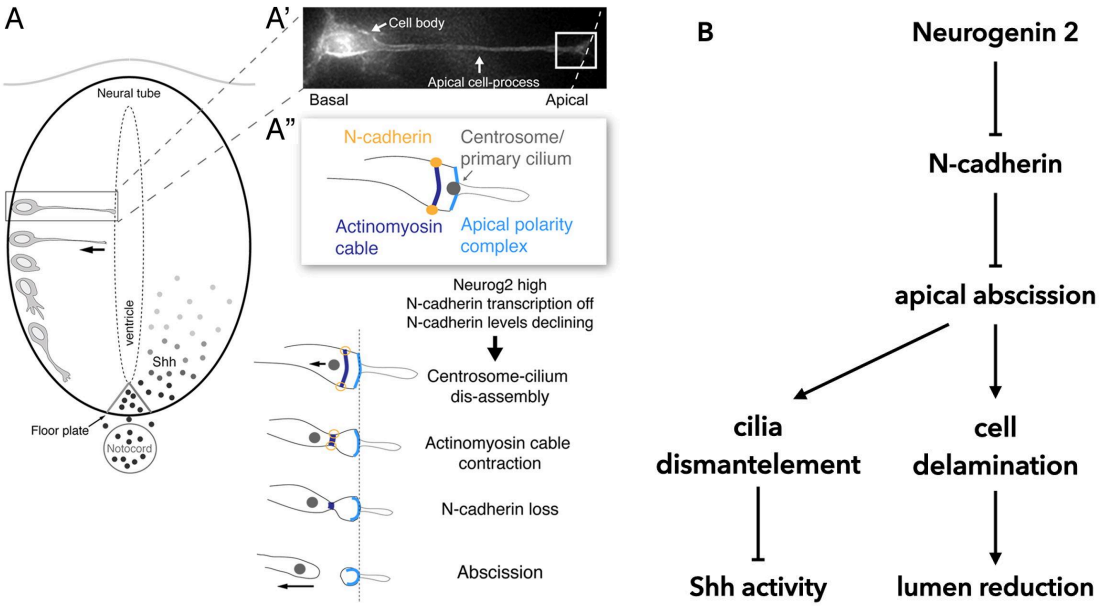

Figure 34. Regulation of neuronal apical abscission in the neural tube. *A. Schematic of sequence of events taking place as a cell differentiates into a neuron in the early neural tube from (Das et Storey, 2014a). B. Schematic representation of the relationship between neurogenin2 and lumen reduction. Arrows represent activation (simple arrow) or repression (flat-headed arrow) relationships between the indicated elements.*

Without cilia, Shh signaling is shutting down in the cells allowing cell cycle exit. Given that active Shh signaling through Gli transcription factors is known to maintain progenitor identity and prevent premature neurogenesis, and that Notch signaling through its downstream effectors Hes1/Her5 directly represses the transcription of proneural genes (Shimojo, Ohtsuka et Kageyama, 2011), it is tempting to speculate that both pathways converge to inhibit Neurog2 expression in cycling progenitors, thereby creating a regulatory switch in which the simultaneous downregulation of Gli activity would release Neurog2 from repression and allow commitment to neuronal

differentiation (Figure 35). Neurog2 has been demonstrated to be regulated by BMP in the dorsal neural tube and seems to be activated all along the dorso-ventral axis, with specific enhancer regions regulating each domain of expression including Pax6, Nkx2.2 or Olig2 (Scardigli *et al.*, 2001; Simmons *et al.*, 2001; Ota et Ito, 2006; Sagner *et al.*, 2018). Reviews have reported the essential role of Neurog2 in the differentiation of various types of neurons (Vaid et Huttner, 2020; Hulme *et al.*, 2022). Indeed, Neurog2 acts as a master proneural transcription factor that drives commitment to the neuronal fate through multiple mechanisms: it inhibits glial fate, induces a cascade of pan-neuronal genes, promotes cell cycle exit, and promotes neuronal migration (Hulme *et al.*, 2022). Neurog2 could therefore be the central factor that initiates delamination in differentiating neurons by decreasing N-cadherin expression. It would then be interesting to describe lumen remodeling in Neurog2 null mutants or to inhibit Neurog2 expression specifically during lumen remodeling. The specific activation of Neurog2 along the DV axis of the neural tube by different enhancer regions regulated by known transcription factors has been described in mice (Scardigli *et al.*, 2001; Simmons *et al.*, 2001). Blocking these enhancers individually during lumen remodeling would help decipher which progenitor populations are preferentially delaminating. Since Neurogenin family genes are expressed across the entire DV axis of the neural tube, the mechanisms of delamination could be regulated by different proteins in different domains. Further experiments to determine whether a common protein regulates cell delamination across domains would therefore be required. Investigating the direct or indirect transcriptional relationship between Gli proteins and Neurog2 in zebrafish, notably by analyzing Neurog2 expression in Gli loss- or gain-of-function mutants during neural tube development, would provide a powerful *in vivo* framework to test this hypothesis.

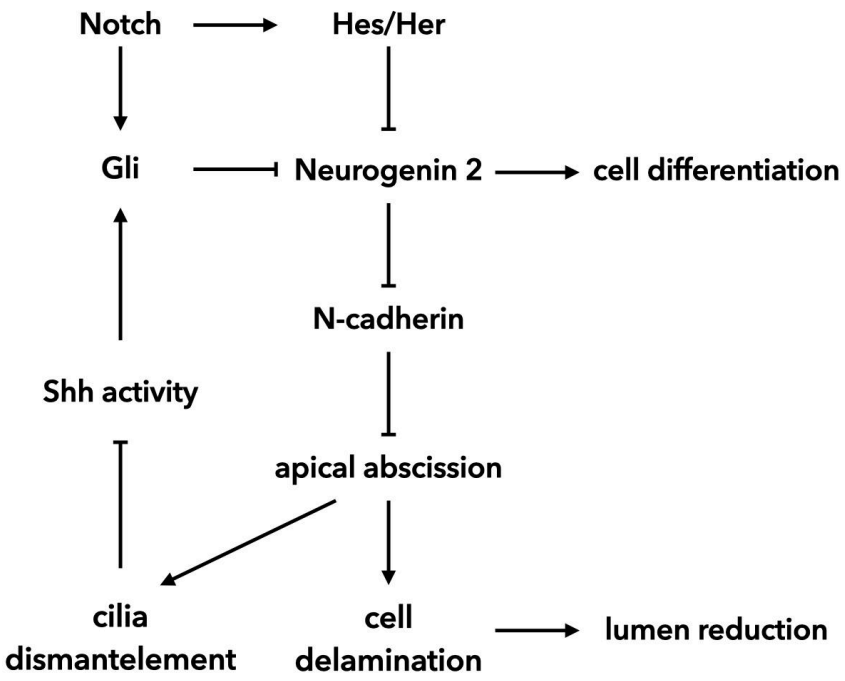

Figure 35. Schematic representation of my hypothesis of lumen remodeling regulation by Neurogenin2. *Arrows represent activation (simple arrow) or repression (flat-headed arrow) relationships between the indicated elements.*

3. Lzts1, a positive regulator of delamination

Delamination could also be independent of differentiation. Indeed, an interesting paper presents that Lzts1 (Leucine-Zipper Tumor Suppressor 1) regulates neuronal delamination in the chicken trunk neural tube under the control of HoxB (Wilmerding *et al.*, 2021). The authors show that overexpression of Lzts1 (by electroporation) induces delamination of progenitors without differentiation: indeed they observed that half of the cells that have delaminated are Sox2+ cells. In addition, by loss of function of Lzts1 using ShRNA, they observed cells positive for Tuj1 and HuC (two neuronal markers) maintaining their apical attachment to the lumen border. This study thus uncovers a mechanism to uncouple differentiation and delamination of the spinal neural tube progenitors, by interfering with Lzts1.

Looking in the literature, Lzts1 has been described as a master regulator of delamination in the neocortex (Kawaue *et al.*, 2019) Lzts1 appears to modulate the cytoskeleton,

including both actomyosin and microtubules but also adherent junction at the apical endfeet of radial glial cells. Kawaue et al. demonstrate that Lzts1 acts downstream of Neurog2 without impacting differentiation, only the delamination process by downregulating N-cadherin (Figure 36). Lzts1 expression has been negatively correlated with hes1 expression in radial glial cells, suggesting an implication of Notch signaling pathway.

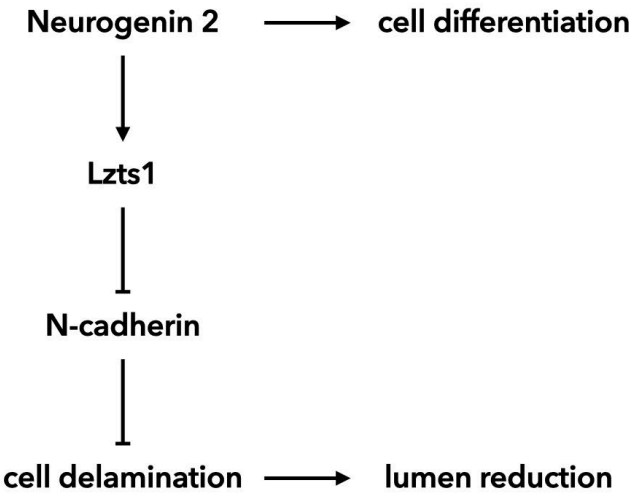

Figure 36. Schematic representation of the hypothesis regarding the relationship between Neurogenin2 and Lzts1 in lumen reduction. *Arrows represent activation (simple arrow) or repression (flat-headed arrow) relationships between the indicated elements.*

Most of the studies performed have identified a role of Lzst1 in cancer. Lzts1 has been demonstrated to regulate cell cycle by lowering CyclinB/Cdk1 (Vecchione, Croce et Baldassarre, 2007). Moreover, all the studies presented above are done in mice or chicken embryos, Lzts1 function has not been described in zebrafish. A sequence encoding a similar protein as Lzts1 in zebrafish exists but has never been investigated in zebrafish (zfin.org). We could first analyze the expression of this gene in zebrafish to determine if it is compatible with an implication during nervous system development. If so, examination of the phenotype of Lzts1 loss and gain of function would be a good start to appreciate the potential conserved mechanism compared to chicken. To test whether Lzts1 regulates progenitor delamination during lumen remodeling, specific activation using optogenetic or heat-shock of Lzts1 in the neural tube would be a good way to see if this maintains, as in chicken, differentiated neurons attached to the lumen and if it blocks lumen reduction. To specifically inhibit Lzts1 in the neural tube, the creation of a

Lzts1-GFP fusion to the endogenous gene line would be necessary to then use the deGradFP approaches, that address the GFP and the protein linked to it to the proteasome to degrade it (Caussinus, Kanca et Affolter, 2012).

Related to the Lzts1 phenotype observed in chicken, a similar phenotype has been reported by modifying Foxp-family genes (Rousso *et al.*, 2012). This study shows that Foxp2 and Foxp4 are important for regulating the cytoarchitecture of neuroepithelial progenitors. Foxp4 expression downregulates N-cadherin and leads to delamination of ventral neural tube progenitors. Inactivation of Foxp2 and Foxp4 function impairs progenitors differentiation and delamination from the neuroepithelium. The authors demonstrate that Foxp proteins play an opposite role compared to Sox2 that maintains N-cadherin and maintains progenitors (Figure 37). Knowing its effect on differentiation, Foxp proteins might act upstream or in parallel with Lzts1.

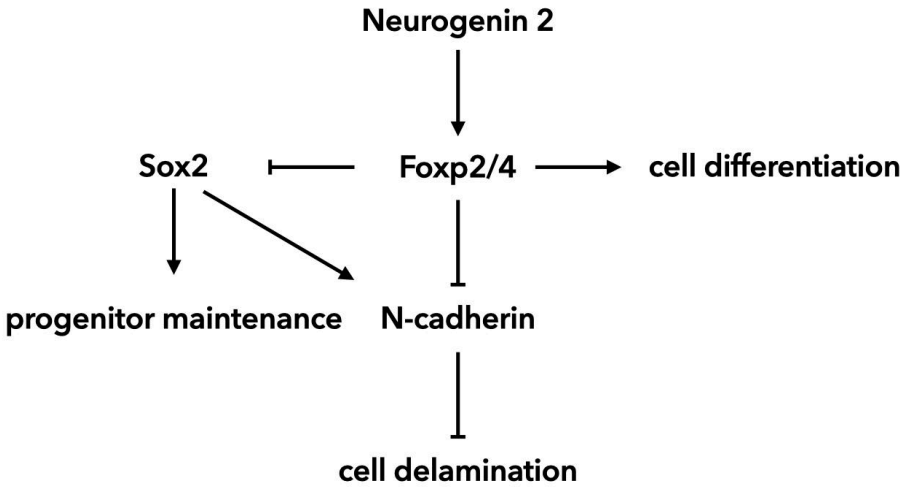

Figure 37. Schematic representation of the hypothesis regarding the relationship between Neurogenin2 and Foxp2/4 in lumen reduction. *Arrows represent activation (simple arrow) or repression (flat-headed arrow) relationships between the indicated elements.*

All the data reported suggests that the delamination process might still require a lot more investigation to describe all the participants of the regulation in the spinal cord and their interactions.

V. What differentiates the progenitors that form the final central canal from the progenitors that stop contributing?

Strikingly, in all experimental conditions where Gli balance was disturbed, whether by cyclopamine treatment (Hudish *et al.*, 2016), GANT61 treatment, or Gli3 repressor overexpression, the final central canal never became smaller than in control embryos, and ventral progenitors never detached from the lumen. This strongly suggests that Gli signalling is not the regulator of the final maintenance of progenitors contributing to the central canal, and that other molecular determinants must govern this process. One compelling candidate is Notch signalling. Indeed, the only condition in our experiments where progenitors forming the central canal lost their apical polarity and delaminated was upon Notch inhibition by LY411575 treatment; including in *iguana* mutants treated with LY (Figure 29). Under this condition, all progenitors progressively lost their apico-basal polarity in an ordered manner, with only roof plate and floor plate cells retaining polarity after 10 hours of treatment, highlighting the central role of active Notch signalling in the maintenance of the progenitor pool forming the central canal.

A critical observation that may explain this selective maintenance is that, at the end of remodeling, most progenitors lining the central canal are Nkx6.1-positive, a finding conserved across both mouse and zebrafish (Ribeiro *et al.*, 2017, and our data; Tait *et al.*, 2020). I suggest that the Nkx6.1 identity might reflect an intrinsic predetermination that distinguishes progenitors that will persist from those that delaminate. Published data demonstrated in the mouse ventral neural tube that the dorsoventral progenitor identity domains defined by Shh targets like Nkx6.1, Dbx1, and Dbx2, spatially segregate Notch ligand expression: Dll1 overlaps with the Nkx6.1 and Dbx1 domains, while Jag1 overlaps with the Dbx2 domain (excluding the Dbx1-Dbx2 common domain) (Figure 38) (Marklund *et al.*, 2010).

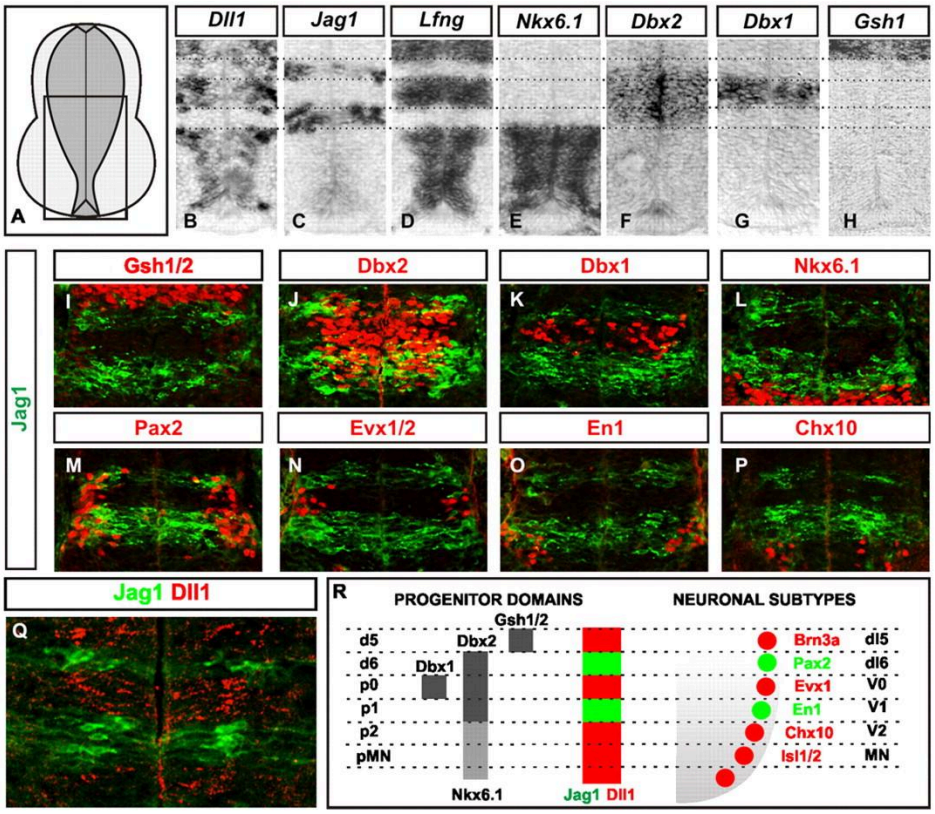

Figure 38. Notch ligand expression in the ventral neural tube*. A. Schematic representation of a cross-section of the neural tube, showing the region studied in panels B-H. B-H. Distribution of Dll1, Jag1, and Lfng mRNAs compared to that of homeodomain (HD) transcription factors in cross-sections of chicken spinal cord at embryonic day 3.5. I–P. Expression of the Jag1 protein in relation to that of HD transcription factors and the indicated neuronal subtype-specific markers, in sections of the mouse spinal cord intermediate region at embryonic stages E10.5–E11. Q. Immunohistochemistry illustrating the complementary expression of Dll1 and Jag1 in the chicken spinal cord at embryonic day 3.5. R. Schematic diagram showing the expression of Jag1 and Dll1 relative to progenitor domains in the developing chicken spinal cord. (Marklund et al., 2010)*

Since Dll1 has been shown to promote neuronal differentiation and delamination through Notch activation, notably via cis- and trans-interactions (Baek *et al.*, 2018), its restriction to the Nkx6.1 domain may define a boundary where progenitors respond differently to remodeling signals. I therefore hypothesise that the establishment of ventral identity domains (Nkx6.1/Dbx2) induces a spatially defined Notch ligand pattern, which in turn creates a differential Notch response among progenitors, determining which cells are competent to resist delaminate during remodeling. To directly test whether the maintenance of the progenitor pool depends on these ligand expression domains, a key experiment would be to manipulate Dll1 or Jag1 expression at

the onset of remodeling and determine whether the boundary of the persisting progenitor pool shifts.

VI. Is there a predefined order of progenitor delamination ?

Several models have been proposed to explain progenitor delamination during lumen remodeling. Tait et al suggest a “ratchet model” where the most dorsal progenitor cells in contact with roof plate cells delaminate in an ordered manner giving rise to the lumen retraction that resembles a ratchet (Tait *et al.*, 2020). This model suggests that the decrease of progenitor apical surface, during delamination, pulls on roof plate cells to elongate them and connect them to the following progenitor. Others suggested that, during neurogenesis, ventral progenitors differentiate and delaminate while more dorsal progenitors slide down to replace them, being induced to express ventral markers like Nkx6.1 as they get closer to the Shh source (Ravanelli et Appel, 2015). Indeed, it has been demonstrated in mice that some early dorsal progenitors forming the lumen are participating in the central canal postnatally (Xing *et al.*, 2018). Moreover, a more stochastic process can also be envisioned.

Our results from the LY411575 experiment suggest a predefined order for progenitor delamination. In this condition where LY411575 forces, by inhibiting Notch signal, progenitors to differentiate, they did not all differentiate at the same time, thereby creating a general collapse of the lumen as sometimes observed in other systems (Baek *et al.*, 2018). The complete loss of the apical marker ZO-1 takes approximately 10 hours while we can still observe the maintenance of neuroepithelium integrity. This 10 hours delay from the first to the last progenitor cell to delaminate might result from cell cycle step heterogeneity in the cells forming the lumen. It is thought that cells that delaminate have exited cell cycle after mitosis, in G0 (Saade et Martí, 2025). The time that all cells forming the neural tube epithelium to complete to this step of the cell cycle might be responsible for a variable offset in the delamination timing of progenitor cells. None of our results allows us to determine the precise order of the disengagement of polarized progenitors in the remodeling zebrafish spinal lumen. The dynamics of this cell

behaviour leading to spinal lumen retraction in our zebrafish model will require further studies, to fully characterise the link between individual cell delamination and global lumen retraction.

Preliminary data from the lab and the multiple lumen remodeling movie analyzed make me think that progenitor delamination occurs all along the dorso-ventral axis but I do not exclude a predominance of dorsal delamination. Looking carefully at the ZO-1-tdTomato movie staining during remodeling, I can appreciate delamination of progenitors at all levels of the dorso-ventral axis. However, the apposition of the two apical surfaces of the lumen border dorsally, and the bleaching of ZO-1-tdTomato signals over time makes the proper analysis complicated. To decipher this question of delamination order and from where the cells forming the final central canal are coming, one would have to perform live cell tracking during lumen retraction. A PhD student in the lab has already started to investigate the question of individual progenitor behaviors during remodeling. Using a mosaic membrane labelling of isolated progenitors forming the lumen (GFAP:Gal4,UAS:lyn-RFP), she already described, during remodeling, dorsal progenitors sliding down to the ventral part, suggesting that ventral delamination participates to the lumen reduction. Pursuing these acquisitions could give us an overview of the delamination patterns of progenitors during the remodeling.

More investigations would be required to answer this question properly. Expression of a photoconvertible fluorescent protein in all glial cells bordering the lumen border of the neural tube by using GFAP:Gal4;UAS:Kaede zebrafish line would allow us to follow in live the proportion of delamination along time in the ventral versus the dorsal part by photoconverting one half of the lumen border at the beginning of remodeling. This experiment will also give us information about the participation of dorsal cells to the final central canal by reporting at the end of the remodeling the fluorescent composition of the final central canal. If by photoconverting the dorsal part we observe red fluorescence cell, corresponding to photoconverted Kaede, around the final ventral central canal this would suggest that progenitor cells forming the dorsal part of the lumen before remodeling are contributing to the lumen at the end of remodeling. Moreover, by photoconverting more specific areas of the lumen border we could describe more accurately the contribution of each part of the lumen to the formation of

the central canal. Combining all data from different experiments analysing the delamination rate of each dorso-vetral domain would help to discriminate if the dorso-ventral delamination is precisely ordered from dorsal to ventral or if random delamination occurs.

VII. How do roof plate cells and dorsal progenitors interact during lumen remodeling?

A. Progenitors as motors of the lumen remodeling pulling on roof plate cells

We concluded from our experiments that the behaviour of progenitors forming the lumen border is driving the remodeling. Delamination of progenitor cells decreases lumen size. As presented in the article, Notch inhibition, by LY 411575 drug treatment that impacts only the progenitors behavior, modifies the lumen remodeling speed. In response to that, roof plate cells extend faster following the accelerated speed of lumen reduction. The progenitors therefore seem to drive the remodeling process.

Although the drug can theoretically affect any cell type, there is evidence in the literature that the roof and floor plate have a very low Notch activity (Baek *et al.*, 2018). In zebrafish, this is further confirmed as mutants that lack Notch activity (as Minbomb1 mutant) or progenitors do not form a neuroepithelium lumen in the center of the neural tube. In this mutant, the roof plate cells are specified and express the glia marker GFAP, but never elongate as in WT (Kondrychyn *et al.*, 2013; Sharma *et al.*, 2019). This suggests that loss of progenitor identity abrogates the roof plate cell - progenitor connection and the elongation of the roof plate cells. This could argue in favor of a pulling force from the progenitors on the roof plate cells to induce their elongation by mechanical coupling.

To discriminate if the roof plate cells are indeed under tension, laser ablation is a good tool. Unfortunately, because of the thickness of the zebrafish embryos and the position in the middle of the embryo of the neural tube, laser ablation failed in our system when I tried it.

B. Potential adhesion molecules linking roof plate cells and dorsal progenitors

The neural tube epithelium is maintained thanks to different types of cell-cell adhesions : progenitor-progenitor, roof plate-progenitors, roof plate-roof plate, floor plate-progenitors and floor plate-floor plate. During lumen remodeling, most of these adhesions are challenged and remodeled while cells change their neighbors. One can wonder if the same molecules regulate the different adhesions, or if there are specific molecules for each different combination of cell-cell adhesions.

I propose an important role of Claudins in all of the physical couplings of cells forming the neural tube lumen border. Claudins are adherent proteins located at the membranes (Tsukita, Tanaka et Tamura, 2019). Several publications suggest the importance of the Claudin proteins in the maintenance of lumen integrity and neural tube closure - specifically in chick embryos (Baumholtz *et al.*, 2017; Baumholtz, Gupta et Ryan, 2017). In zebrafish, more than 30 claudin proteins exist. Looking at single-cell RNAseq online data (daniocell.nichd.nih.gov), I could find that cluster of roof plate cells (*glia.28* - gdf7+/lmx1a+) and radial glial progenitors (multiple clusters of cell) clusters displayed a high expression of claudin 5a (cldn5a) for lateral progenitor cells and claudin 7a (cldn7a) for roof plate cells.

Claudin5a is known to be important for blood-brain barrier and immunostaining in zebrafish confirms its localization at the apical surface of most progenitors in the neural tube (Zhang *et al.*, 2010). Zhang et al. demonstrate that Cldn5a colocalized with aPKC in zebrafish neuroepithelium progenitors forming the lumen. This could suggest that Cldn5a is important for progenitor-progenitor adhesion. Using *mok* mutant (*mpp5a*-/- embryos), an apical polarity protein, they observed a loss of Cldn5a, aPKC and ZO-1 apical localization in the neural tube meanwhile in *cldn5a* morphants, aPKC or ZO-1 do not show any variation but cerebral-ventricular barrier decreases upon loss of *cldn5a*. Following these results, Claudin5a localization at the apical membrane depends on apical polarity, is important to regulate lumen neuroepithelium permeability and might not be important for the progenitor-progenitor adhesion.

On the other hand, one paper observed that Claudin7 is important for self renewal of intestinal epithelial crypt cells in mice by regulating the Wnt pathway (Xing *et al.*, 2020). In *cldn7* mutant mice the authors observed lower self renewal, defect of differentiation and Wnt/β-catenin activity. Given that cldn7 regulates both Wnt signaling and epithelial differentiation in the intestinal crypt, we reasoned it might play a similar role in the dorsal neuroepithelium, where Wnt signaling is also a key regulator of progenitor identity. Wnt ligands are secreted by the roof plate cells and induce Wnt activity in the dorsal progenitors during the remodeling process (Shinozuka *et al.*, 2019). As *cldn7* is expressed in roof plate cells, it is therefore a strong candidate to activate Wnt signaling and to promote the differentiation and delamination of dorsal progenitor cells during lumen remodeling.

This hypothesis is further supported by evidence linking claudin 7 loss to epithelial-to-mesenchymal transition (EMT) in various epithelial cancer contexts (Bhat *et al.*, 2015; Wang *et al.*, 2019). Both EMT and neuronal differentiation share a key cellular event: the exit of cells from an epithelial sheet. EMT induces the extrusion of cells from an epithelial tissue and neuronal differentiation induces the delamination of cells from the neuroepithelium. Since *cldn7a* appears to be involved in both processes in other biological contexts, it represents a compelling candidate for regulating progenitor delamination from the dorsal neural tube. Furthermore, as a tight junction protein, *cldn7a* is well positioned to modulate the cell-cell adhesion interfaces that are likely remodeled during this process, including progenitor–roof plate and roof plate–roof plate contacts. We are currently generating *cldn7a* mutants to directly test whether *cldn7a* regulates progenitor delamination or cell adhesion at these specific interfaces.

Claudins form heterophilic adhesions, thus claudin 5 and claudin 7 could be the two specific proteins linking progenitors and roof plate cells together. To study this possibility, high resolution microscopy to identify potential colocalization of claudin 5 and claudin 7 would be a good start. Inhibition of Claudins during lumen remodeling using time-controlled mutants or broad-spectrum claudin inhibitor (C-terminal domain of C. perfringens enterotoxin, C-CPE) would be another way to decipher Claudins implication during lumen remodeling.

C. Crosstalk between roof plate cells and lumen progenitors

Targeted ablation of roof plate cells during lumen remodeling transiently blocks progenitor delamination, revealing a functional interdependence between these two cell populations. As proposed by Tait et al., roof plate cells initiate progenitor delamination through the secretion of specific signaling molecules, including a secreted form of Crumbs2 (Tait *et al.*, 2020). To test this hypothesis, we used a UAS:NTR (nitroreductase) strategy, in which the NTR enzyme is specifically expressed in roof plate cells and selectively degrades them upon drug administration (Nifurpiriol, NFP) at the onset of lumen remodeling. In regions where multiple roof plate cells were lost, we observed a local arrest of lumen reduction, which was accompanied by a failure of adjacent progenitors to delaminate. This spatial and temporal correlation strongly supports the existence of a bidirectional crosstalk between roof plate cells and lumen progenitors. In this model, roof plate cells would act upstream by secreting molecular cues that trigger progenitor delamination, while delaminating progenitors, in turn, would exert a mechanical pulling force on roof plate cells, promoting their elongation and contributing to the progressive remodeling of the lumen. Together, these findings suggest that lumen morphogenesis is not a cell-autonomous process but rather emerges from the coordinated biochemical and mechanical interplay between roof plate cells and neighboring progenitors.

D. Collective behavior of roof plate cells

The Notch inhibition experiment revealed two distinct behaviours among roof plate cells in response to accelerated lumen reduction. Two thirds of the roof plate cells lost their attachment to the lumen progenitors and recoiled to the dorsal part of the neural tube, while the remaining third stayed attached to the shrinking lumen and, ultimately, to the floor plate cells. Strikingly, this population demonstrated a remarkable deformation capacity, extending up to four times their original size within 10 hours. Together, these observations suggest that roof plate cells are not passive during lumen reduction, but display heterogeneous and highly dynamic mechanical responses, highlighting the critical role of Notch signalling in regulating their attachment and morphological plasticity.

Following numerous quantifications across some twenty films, I observed that, in the majority of cases, roof plate cells exhibited collective behaviour regarding whether or not they maintained their adhesion to the progenitors. Indeed, before detaching, the apical end-feet of the roof cells clustered together. These clusters consisted of a minimum of two cells up to nine cells, forming a point of apical material concentration (ZO-1 in live movies, AJ F-actin on fixed samples with phalloidin) at the junction between these roof plate endfeet. Similarly, cells that maintained their elongation exhibited a slight clustering movement before becoming individualised again (Figure 39).

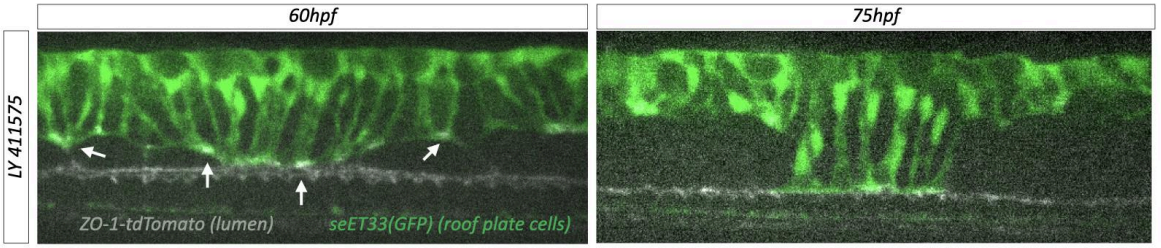

Figure 39. Collective behavior of roof plate cells. *Time-lapse images of the neural tube in embryos treated with LY 411572, a Notch inhibitor 60 hpf, and 75 hpf, showing lateral views. Two distinct behaviors are observed in the roof cell population: some cells detach from the lumen, while others remain attached to the lumen at 75hpf. In both cases, the roof plate cells group their end-feet together by group of 2 to 9 cells before detaching or maintaining their attachment. White arrows are pointing roof plate cells end-foot clusters.*

This collective behaviour of the roof plate cells suggests a regulation at the tissue level rather than at the cellular level. This is further supported by the behaviour of the floor plate cells under Notch inhibition (LY411575). Floor plate cells undergo increasing deformation during lumen accelerated reduction, until the moment when part of the roof plate cells detach, after which floor plate cell size decreases, consistent with a mechanical recoil following tension release. We do not observe any local difference of floor plate cells deformation along the antero-posterior axis depending on whether the roof plate cells are attached or detached. If roof plate cell behaviour were regulated autonomously, we would expect spatially heterogeneous mechanical responses in the floor plate. The absence of such local variation instead points to a tissue-level coordination, in which mechanical tension is distributed globally rather than cell-to-cell

Collective behavior and interaction of roof plate cells are appreciated also in the UAS:NTR experiment where some roof plate cells are killed at the onset of lumen

remodeling. We can appreciate that the death of a single roof plate cell is not sufficient to induce a phenotype, probably because the collective action of the others compensate for this loss. In contrast, the death of a group of roof plate cells abrogates lumen reduction. These preliminary data suggest a collective behavior even in their potential role of delamination instigator.

Mechanics appears to be an essential regulator of lumen remodelling (Navis et Nelson, 2016). The behaviour of roof plate cells during Notch inhibition, where cells either remain stretched and anchored or abruptly detach and recoil, strongly resembles that of a system under mechanical tension. I therefore propose that lumen remodeling is governed by a mechanical tension at the tissue level. Understanding how individual cell properties, such as adhesion and deformability, contribute to or are constrained by collective mechanical properties will be essential to explain how tissue architecture is actively remodeled during neural tube development.

VIII. What determines the differential extension of the roof and floor plate cells ?

As described all over this PhD manuscript, I mainly focused my research on progenitors and roof plate cells behavior during lumen remodeling. These two types of cells present the most striking behavior, while the last component of the lumen epithelium, the floor plate cells, change their shape less. In zebrafish, the floor plate cells elongate twice its height while roof plate cells elongate three to four times their initial size. This asymmetric stretching of cells in the dorsal and ventral part of the neural tube is common to all vertebrates (Barnes, 1883; Bohme, 1988; Kondrychyn *et al.*, 2013; Ribeiro *et al.*, 2017; Shinozuka et Takada, 2021). The roof and floor plate cells behave differently during lumen remodeling. Vladimir Korzh suggested that this asymmetry could be due to the ECM and to differences of stiffness along the dorso-ventral axis (Korzh, 2014). It has been recently published that the mouse neural tube presents a dorso-ventral gradient of stiffness, with a higher stiffness in the ventral part than in the dorsal part (Cheng *et al.*, 2023). The rigidity of the environment could explain the difference of cell extension behavior between floor plate cells and roof plate cells. It has been shown that axons of neurons extend more on soft substrates than on stiff substrates (Fox, 2008;

Martínez *et al.*, 2022; Nemoz-Billet *et al.*, 2024). A difference of stiffness along the dorso-ventral axis could be one of the explanations of asymmetric elongation of roof plate cells and floor plate cells. Analyzing zebrafish neural tube stiffness using atomic force microscopy would allow us to verify if the pattern of dorso-ventral gradient of stiffness is conserved compared to mice.

Korzh suggested that the asymmetric stretching difference of roof plate cells and floor plate cells could be due to collagen molecules (Korzh, 2014). At this time it was known that at least six subunits of collagen were expressed by the floor plate cells (col2a1a, col8a1a, col9a2, col11a1a, col14a1a, col27a1a; Zebrafish Information Network, ZFIN; and data not shown from Korzh) and only two by roof plate cells (col15a1b and collagen-like col7al1, Zebrafish Information Network, ZFIN). Comparing data from 2014 and now, I could find on daniocell that the six subunits suggested by Korzh correlate with the cluster that has other floor plate identity markers in the single-cell RNAseq data. For the roof plate cells, col7a1l expression correlates at early stages (from 14hpf to 46hpf) and col15a1b do not correlate with roof plate cells but retinal glial cells during development. Looking on daniocell database other subunits of collagen correlate positively with roof plate cells during the remodelling process : *col4a5, col4a6, col18a1a and col28a2a*. Both subunits of collagen, *col4a5* and *col4a6,* expressions were found to correlate positively with *GFAP*, *cldn7a* and *vimentin* which are highly expressed in roof plate cells. Type IV Collagens compose the basement membrane of epithelial tissue. Both col4a5 and col4a6 mutants present axogenesis defects of granule cells in the cerebellum and retinal ganglion cells (Takeuchi *et al.*, 2015). Investigating roof plate cell elongation in col4a5 and col4a6 mutants or double mutants would be a way to see possible links between basement membrane collagen and roof plate deformation. Analyzing the extracellular matrix composition of the neural tube could help understanding differential behaviors of the different cell types composing the neural epithelium lumen along the dorso-ventral axis.

On top of that, Shh has been shown to promote collagen production. In the zebrafish myotome, *col15a1* expression depends on Hh/Gli signaling (Guillon, Bretaud et Ruggiero, 2016). The Shh gradient could regulate collagen and matrix proteins/molecule production or sequestration and induce gradients of stiffness along the dorso-ventral

axis. As illustrated in figure 40, dorso-ventral gradient of Shh could induce a DV stiffness gradient by promoting collagen expression and modulate cell elongation properties of roof and floor plate cells due to this gradient.

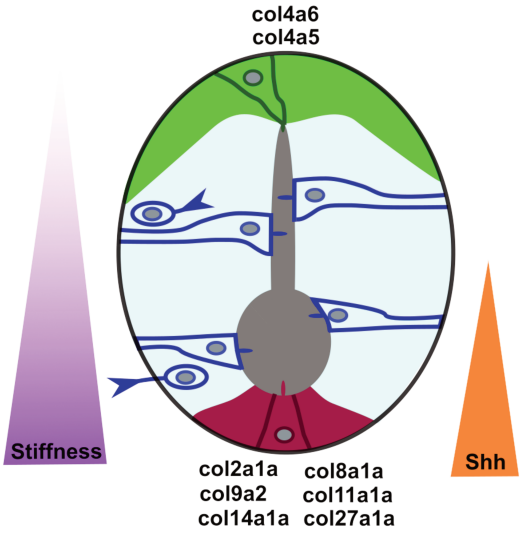

Figure 40. Hypothesis of stiffness regulation of roof and floor plate cells elongation during lumen remodeling. *The schematic represents a transverse section of the neural tube at a late stage of lumen remodeling. The neural tube is patterned by two opposing morphogenetic gradients: a dorsal BMP/Wnt gradient (purple, left) and a ventral Shh gradient (orange, right). The roof plate (green, dorsal) and floor plate (dark red, ventral) serve as dorsal and ventral signaling centers, respectively. Col4a5 and Col4a6, two type IV collagen isoforms, are specifically expressed at the dorsal midline, in close association with the roof plate cells and the apical surface of the ventricular zone. We hypothesize that their localized deposition modulates the stiffness of the extracellular matrix at the dorsal pole of the lumen, thereby creating a mechanical constraint that drives roof plate cell elongation and contributes to lumen remodeling. The reduced expression or absence of equivalent collagen isoforms at the ventral pole may explain the comparatively minor elongation of floor plate cells during this process. Blue cells represent delaminating neurons migrating laterally following progenitor cell exit from the ventricular zone. The gray structure represents the remodeling lumen, with its inflated portion displaced ventrally as remodeling progresses.*

CONCLUSION

The remodeling of the neural tube lumen represents a critical morphogenetic transition in spinal cord development. My thesis has provided new insights into the cellular and molecular mechanisms governing this process, integrating signalling, cell behaviour, and mechanical regulation into a more complete model.

Using the zebrafish *dzip1* mutant as a genetic tool, I established that the balance between the repressor and activator forms of Gli proteins, the downstream effectors of the Hedgehog signalling pathway, is a key determinant of proper lumen remodeling. The loss of primary cilia in this mutant disrupts Hedgehog signal transduction along the dorso-ventral axis, ultimately impairing the coordinated delamination of progenitor cells from the lumen border. This finding positions the Gli balance, on top of its known early role in patterning, as a regulator of the morphogenetic behaviour of neural progenitors at the time of lumen reduction. Importantly, the incomplete lumen reduction, a specific phenotype observed in the *dzip1* mutant, distinguishes it from other cilia-deficient models, suggesting that particular molecular or cellular features of this mutant context amplify the consequences of impaired Gli activity on tissue architecture.

Beyond molecular regulation, this work has uncovered the importance of mechanical coupling in orchestrating lumen remodeling at the tissue scale. High-resolution *in vivo* confocal imaging combined with quantitative analysis revealed that lumen reduction and roof plate cell extension are physically and functionally linked processes. These two cellular behaviours appear to be coordinated, suggesting that forces generated during lumen reduction may instruct or reinforce the elongation of roof plate cells. Regulation at the cellular level, as well as tissue mechanics, adds a new dimension to our understanding of neural tube morphogenesis.

Taken together, these results support a model in which the remodeling of the spinal cord lumen is a multi-scale process, integrating upstream Hedgehog signalling through Gli proteins, the active delamination of progenitor cells, and the mechanically coupled behaviour of roof plate cells. The conservation of this transition across vertebrates underscores its fundamental importance, not only for the proper formation of the

central canal, but likely for the long-term maintenance of the spinal cord stem cell niche and its regenerative capacity.

Several important questions remain open. The order of delaminating progenitor cells, the nature of the signals that trigger the onset of remodeling, the precise interaction of Gli activity with other signals (Notch, BMP, Wnt…) and other cell processes (cell polarity, cell cycle, differentiation…) and the molecular basis distinguishing delaminating progenitors from those retained to form the central canal all require further investigation. Similarly, a deeper characterisation of the mechanical and chemical interactions between roof plate cells and progenitor cells will be essential to fully decode the tissue-level coordination of this process. Finally, the differential deformation observed between roof and floor plate cells invites further inquiry into whether these reflect intrinsic cell-type differences or emergent properties of the tissue along the dorso-ventral axis.

BIBLIOGRAPHY

Adameyko, I. (2023) « Evolutionary origin of the neural tube in basal deuterostomes », *Current Biology*, 33(8), p. R319‑R331. Disponible sur: https://doi.org/10.1016/j.cub.2023.03.045.

Alexandre, P. *et al.* (2010) « Neurons derive from the more apical daughter in asymmetric divisions in the zebrafish neural tube », *Nature Neuroscience*, 13(6), p. 673‑679. Disponible sur: https://doi.org/10.1038/nn.2547.

Alvarez-Medina, R. *et al.* (2008) « Wnt canonical pathway restricts graded Shh/Gli patterning activity through the regulation of Gli3 expression », *Development*, 135(2), p. 237‑247. Disponible sur: https://doi.org/10.1242/dev.012054.

Andreu-Cervera, A., Catala, M. et Schneider-Maunoury, S. (2021) « Cilia, ciliopathies and hedgehog-related forebrain developmental disorders », *Neurobiology of Disease*, 150, p. 105236. Disponible sur: https://doi.org/10.1016/j.nbd.2020.105236.

Araya, C. *et al.* (2016) « Coordinating cell and tissue behavior during zebrafish neural tube morphogenesis », *Developmental Dynamics*, 245(3), p. 197‑208. Disponible sur: https://doi.org/10.1002/dvdy.24304.

Baek, C. *et al.* (2018) « Mib1 prevents Notch Cis-inhibition to defer differentiation and preserve neuroepithelial integrity during neural delamination », *PLOS Biology*. Édité par M. Bronner, 16(4), p. e2004162. Disponible sur: https://doi.org/10.1371/journal.pbio.2004162.

Baggiolini, A. *et al.* (2015) « Premigratory and Migratory Neural Crest Cells Are Multipotent In Vivo », *Cell Stem Cell*, 16(3), p. 314‑322. Disponible sur: https://doi.org/10.1016/j.stem.2015.02.017.

Bai, C.B. *et al.* (2002) « Gli2, but not Gli1, is required for initial Shh signaling and ectopic activation of the Shh pathway », *Development*, 129(20), p. 4753‑4761. Disponible sur: https://doi.org/10.1242/dev.129.20.4753.

Barnes, W. (1883) « On the Development of the Posterior Fissure of the Spinal Cord, and the Reduction of the Central Canal, in the Pig », *Proceedings of the American Academy of Arts and Sciences*, 19, p. 97. Disponible sur: https://doi.org/10.2307/25138722.

Barry, D.S., Pakan, J.M.P. et McDermott, K.W. (2014) « Radial glial cells: Key organisers in CNS development », *The International Journal of Biochemistry & Cell Biology*, 46, p. 76‑79. Disponible sur: https://doi.org/10.1016/j.biocel.2013.11.013.

Baumholtz, A.I. *et al.* (2017) « Claudins are essential for cell shape changes and convergent extension movements during neural tube closure », *Developmental Biology*, 428(1), p. 25‑38. Disponible sur: https://doi.org/10.1016/j.ydbio.2017.05.013.

Baumholtz, A.I., Gupta, I.R. et Ryan, A.K. (2017) « Claudins in morphogenesis: Forming an epithelial tube », *Tissue Barriers*, 5(4), p. e1361899. Disponible sur: https://doi.org/10.1080/21688370.2017.1361899.

Bearce, E.A. et Grimes, D.T. (2021) « On being the right shape: Roles for motile cilia and cerebrospinal fluid flow in body and spine morphology », *Seminars in Cell & Developmental Biology*, 110, p. 104‑112. Disponible sur: https://doi.org/10.1016/j.semcdb.2020.07.005.

Becker, C.G., Becker, T. et Hugnot, J.-P. (2018) « The spinal ependymal zone as a source of endogenous repair cells across vertebrates », *Progress in Neurobiology*, 170, p. 67‑80. Disponible sur: https://doi.org/10.1016/j.pneurobio.2018.04.002.

Ben, J. *et al.* (2011) « Targeted mutation of the talpid3 gene in zebrafish reveals its conserved requirement for ciliogenesis and Hedgehog signalling across the vertebrates », *Development*, 138(22), p. 4969‑4978. Disponible sur: https://doi.org/10.1242/dev.070862.

Bergemann, D. *et al.* (2018) « Nifurpirinol: A more potent and reliable substrate compared to metronidazole for nitroreductase-mediated cell ablations », *Wound Repair and Regeneration*, 26(2), p. 238‑244. Disponible sur: https://doi.org/10.1111/wrr.12633.

Bernascone, I., Hachimi, M. et Martin-Belmonte, F. (2017) « Signaling Networks in Epithelial Tube Formation », *Cold Spring Harbor Perspectives in Biology*, 9(12), p. a027946. Disponible sur: https://doi.org/10.1101/cshperspect.a027946.

Bhat, A.A. *et al.* (2015) « Claudin-7 expression induces mesenchymal to epithelial transformation (MET) to inhibit colon tumorigenesis », *Oncogene*, 34(35), p. 4570‑4580. Disponible sur: https://doi.org/10.1038/onc.2014.385.

Bohme, G. (1988) « Formation of the central canal and dorsal glial septum in the spinal cord of the domestic cat ».

Bond, A.M., Bhalala, O.G. et Kessler, J.A. (2012) « The dynamic role of bone morphogenetic proteins in neural stem cell fate and maturation », *Developmental Neurobiology*, 72(7), p. 1068‑1084. Disponible sur: https://doi.org/10.1002/dneu.22022.

Borovina, A. *et al.* (2010) « Vangl2 directs the posterior tilting and asymmetric localization of motile primary cilia », *Nature Cell Biology*, 12(4), p. 407‑412. Disponible sur: https://doi.org/10.1038/ncb2042.

Borovina, A. et Ciruna, B. (2013) « IFT88 Plays a Cilia- and PCP-Independent Role in Controlling Oriented Cell Divisions during Vertebrate Embryonic Development », *Cell Reports*, 5(1), p. 37‑43. Disponible sur: https://doi.org/10.1016/j.celrep.2013.08.043.

Bray, S.J. (2016) « Notch signalling in context », *Nature Reviews Molecular Cell Biology*, 17(11), p. 722‑735. Disponible sur: https://doi.org/10.1038/nrm.2016.94.

Brooks, E.R. *et al.* (2020) « Sonic hedgehog signaling directs patterned cell remodeling during cranial neural tube closure », *eLife*. Édité par J.F. Reiter et M.E. Bronner, 9, p. e60234. Disponible sur: https://doi.org/10.7554/eLife.60234.

Brooks, E.R. *et al.* (2025) « GPR161–GLI3 repressor signaling at cilia directs apical constriction and cell fate during cranial neural tube closure », *Development*, 152(24), p. dev205171. Disponible sur: https://doi.org/10.1242/dev.205171.

Buckley, C.E. et St Johnston, D. (2022) « Apical–basal polarity and the control of epithelial form and function », *Nature Reviews Molecular Cell Biology*, 23(8), p. 559‑577. Disponible sur: https://doi.org/10.1038/s41580-022-00465-y.

Cañizares, M.A. *et al.* (2020) « Multiple steps characterise ventricular layer attrition to form the ependymal cell lining of the adult mouse spinal cord central canal », *Journal of Anatomy*, 236(2), p. 334‑350. Disponible sur: https://doi.org/10.1111/joa.13094.

Cantaut-Belarif, Y. *et al.* (2018) « The Reissner Fiber in the Cerebrospinal Fluid Controls Morphogenesis of the Body Axis », *Current Biology*, 28(15), p. 2479-2486.e4. Disponible sur: https://doi.org/10.1016/j.cub.2018.05.079.

Caspary, T. et Anderson, K.V. (2003) « Patterning cell types in the dorsal spinal cord: what the mouse mutants say », *Nature Reviews Neuroscience*, 4(4), p. 289‑297. Disponible sur: https://doi.org/10.1038/nrn1073.

Catala, M. (2021) « Overview of Secondary Neurulation », *Journal of Korean Neurosurgical Society*, 64(3), p. 346‑358. Disponible sur: https://doi.org/10.3340/jkns.2020.0362.

Caussinus, E., Kanca, O. et Affolter, M. (2012) « Fluorescent fusion protein knockout mediated by anti-GFP nanobody », *Nature Structural & Molecular Biology*, 19(1), p. 117‑121. Disponible sur: https://doi.org/10.1038/nsmb.2180.

Chauhan, D. *et al.* (2024) « Tracking the Global Burden of Neural Tube Defects and Assessing Disparities Across World Health Organization Regions: A Scoping Literature Review », *Neurosurgery*, 95(5), p. 963‑975. Disponible sur: https://doi.org/10.1227/neu.0000000000002996.

Chen, J. et Zhang, M. (2013) « The Par3/Par6/aPKC complex and epithelial cell polarity », *Experimental Cell Research*, 319(10), p. 1357‑1364. Disponible sur: https://doi.org/10.1016/j.yexcr.2013.03.021.

Cheng, C. *et al.* (2023) « Yap controls notochord formation and neural tube patterning by integrating mechanotransduction with FoxA2 and Shh expression », *Science Advances*, 9(24), p. eadf6927. Disponible sur: https://doi.org/10.1126/sciadv.adf6927.

Chesnutt, C. *et al.* (2004) « Coordinate regulation of neural tube patterning and proliferation by TGFβ and WNT activity », *Developmental Biology*, 274(2), p. 334‑347. Disponible sur: https://doi.org/10.1016/j.ydbio.2004.07.019.

Chizhikov, V.V. et Millen, K.J. (2004) « Control of roof plate formation by Lmx1a in the developing spinal cord », *Development*, 131(11), p. 2693‑2705. Disponible sur: https://doi.org/10.1242/dev.01139.

Chizhikov, V.V. et Millen, K.J. (2005) « Roof plate-dependent patterning of the vertebrate dorsal central nervous system », *Developmental Biology*, 277(2), p. 287‑295. Disponible sur: https://doi.org/10.1016/j.ydbio.2004.10.011.

Chung, S. et Andrew, D.J. (2008) « The formation of epithelial tubes », *Journal of Cell Science*, 121(21), p. 3501‑3504. Disponible sur: https://doi.org/10.1242/jcs.037887.

Citarella, A. *et al.* (2023) « Hedgehog-GLI and Notch Pathways Sustain Chemoresistance and Invasiveness in Colorectal Cancer and Their Inhibition Restores Chemotherapy Efficacy », *Cancers*, 15(5), p. 1471. Disponible sur: https://doi.org/10.3390/cancers15051471.

Copp, A.J. et Greene, N.D. (2010) « Genetics and development of neural tube defects », *The Journal of Pathology*, 220(2), p. 217‑230. Disponible sur: https://doi.org/10.1002/path.2643.

Cruz, C. *et al.* (2010) « Foxj1 regulates floor plate cilia architecture and modifies the response of cells to sonic hedgehog signalling », *Development*, 137(24), p. 4271‑4282. Disponible sur: https://doi.org/10.1242/dev.051714.

Czarniak, N. *et al.* (2023) « Cerebrospinal Fluid–Basic Concepts Review », *Biomedicines*, 11(5), p. 1461. Disponible sur: https://doi.org/10.3390/biomedicines11051461.

Danesin, C. *et al.* (2006) « Ventral Neural Progenitors Switch toward an Oligodendroglial Fate in Response to Increased Sonic Hedgehog (Shh) Activity: Involvement of Sulfatase 1 in Modulating Shh Signaling in the Ventral Spinal Cord », *The Journal of Neuroscience*, 26(19), p. 5037‑5048. Disponible sur: https://doi.org/10.1523/JNEUROSCI.0715-06.2006.

Daoud, G. *et al.* (2014) « BMP-mediated induction of GATA4/5/6 blocks somitic responsiveness to SHH », *Development*, 141(20), p. 3978‑3987. Disponible sur: https://doi.org/10.1242/dev.111906.

Das, R.M. et Storey, K.G. (2012) « Mitotic spindle orientation can direct cell fate and bias Notch activity in chick neural tube », *EMBO reports*, 13(5), p. 448‑454. Disponible sur: https://doi.org/10.1038/embor.2012.42.

Das, R.M. et Storey, K.G. (2014a) « Apical abscission, a novel cell biological mechanism regulating neurogenesis », *Neurogenesis*, 1(1), p. e29555. Disponible sur: https://doi.org/10.4161/neur.29555.

Das, R.M. et Storey, K.G. (2014b) « Apical Abscission Alters Cell Polarity and Dismantles the Primary Cilium During Neurogenesis », *Science*, 343(6167), p. 200‑204. Disponible sur: https://doi.org/10.1126/science.1247521.

Datta, A., Bryant, D.M. et Mostov, K.E. (2011) « Molecular Regulation of Lumen Morphogenesis », *Current Biology*, 21(3), p. R126‑R136. Disponible sur: https://doi.org/10.1016/j.cub.2010.12.003.

Deng, S. *et al.* (2023) « Roles of Ependymal Cells in the Physiology and Pathology of the Central Nervous System », *Aging and Disease*, 14(2), p. 468‑483. Disponible sur: https://doi.org/10.14336/AD.2022.0826-1.

Dessaud, E., McMahon, A.P. et Briscoe, J. (2008) « Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen-regulated transcriptional network », *Development*, 135(15), p. 2489‑2503. Disponible sur: https://doi.org/10.1242/dev.009324.

Donati, A. *et al.* (2021) « Planar polarization of cilia in the zebrafish floor-plate involves Par3-mediated posterior localization of highly motile basal bodies », *Development*, 148(13), p. dev196386. Disponible sur: https://doi.org/10.1242/dev.196386.

Elmonem, M.E.A., Mohamed, S.A. et Aly, K.H. (2007) « Early Embryonic Development of the Camel Lumbar Spinal Cord Segment », *Anatomia, Histologia, Embryologia*, 36(1), p. 43‑46. Disponible sur: https://doi.org/10.1111/j.1439-0264.2006.00716.x.

Ezratty, E.J. *et al.* (2011) « A Role for the Primary Cilium in Notch Signaling and Epidermal Differentiation during Skin Development », *Cell*, 145(7), p. 1129‑1141. Disponible sur: https://doi.org/10.1016/j.cell.2011.05.030.

Fame, R.M., Cortés-Campos, C. et Sive, H.L. (2020) « Brain Ventricular System and Cerebrospinal Fluid Development and Function: Light at the End of the Tube: A Primer with Latest Insights », *BioEssays*, 42(3), p. 1900186. Disponible sur: https://doi.org/10.1002/bies.201900186.

Ferent, J., Zaidi, D. et Francis, F. (2020) « Extracellular Control of Radial Glia Proliferation and Scaffolding During Cortical Development and Pathology », *Frontiers in Cell and Developmental Biology*, 8, p. 578341. Disponible sur: https://doi.org/10.3389/fcell.2020.578341.

Fox, M.A. (2008) « Novel roles for collagens in wiring the vertebrate nervous system », *Current Opinion in Cell Biology*, 20(5), p. 508‑513. Disponible sur: https://doi.org/10.1016/j.ceb.2008.05.003.

Gaiano, N. et Fishell, G. (2002) « The Role of Notch in Promoting Glial and Neural Stem Cell Fates », *Annual Review of Neuroscience*, 25(1), p. 471‑490. Disponible sur: https://doi.org/10.1146/annurev.neuro.25.030702.130823.

Ge, M. *et al.* (2023) « A Spacetime Odyssey of Neural Progenitors to Generate Neuronal Diversity », *Neuroscience Bulletin*, 39(4), p. 645‑658. Disponible sur: https://doi.org/10.1007/s12264-022-00956-0.

Glazer, A.M. *et al.* (2010) « The Zn Finger protein Iguana impacts Hedgehog signaling by promoting ciliogenesis », *Developmental Biology*, 337(1), p. 148‑156. Disponible sur: https://doi.org/10.1016/j.ydbio.2009.10.025.

Guillon, E., Bretaud, S. et Ruggiero, F. (2016) « Slow Muscle Precursors Lay Down a Collagen XV Matrix Fingerprint to Guide Motor Axon Navigation », *Journal of Neuroscience*, 36(9), p. 2663‑2676. Disponible sur: https://doi.org/10.1523/JNEUROSCI.2847-15.2016.

Guillot, C. et Lecuit, T. (2013) « Mechanics of Epithelial Tissue Homeostasis and Morphogenesis », *Science*, 340(6137), p. 1185‑1189. Disponible sur: https://doi.org/10.1126/science.1235249.

Gumbiner, B.M. (2005) « Regulation of cadherin-mediated adhesion in morphogenesis », *Nature Reviews Molecular Cell Biology*, 6(8), p. 622‑634. Disponible sur: https://doi.org/10.1038/nrm1699.

Guo, C. *et al.* (2018) « Apical Cell-Cell Adhesions Reconcile Symmetry and Asymmetry in Zebrafish Neurulation », *iScience*, 3, p. 63‑85. Disponible sur: https://doi.org/10.1016/j.isci.2018.04.007.

Guo, H. *et al.* (2024) « Smad4 regulates TGF-β1-mediated hedgehog activation to promote epithelial-to-mesenchymal transition in pancreatic cancer cells by suppressing Gli1 activity », *Computational and Structural Biotechnology Journal*, 23, p. 1189‑1200. Disponible sur: https://doi.org/10.1016/j.csbj.2024.03.010.

Harris, T.J.C. et Tepass, U. (2010) « Adherens junctions: from molecules to morphogenesis », *Nature Reviews Molecular Cell Biology*, 11(7), p. 502‑514. Disponible sur: https://doi.org/10.1038/nrm2927.

Hasenpusch-Theil, K. *et al.* (2012) « Transcriptional Analysis of Gli3 Mutants Identifies Wnt Target Genes in the Developing Hippocampus », *Cerebral Cortex*, 22(12), p. 2878‑2893. Disponible sur: https://doi.org/10.1093/cercor/bhr365.

He, F. et Sun, Y.E. (2007) « Glial cells more than support cells? », *The International Journal of Biochemistry & Cell Biology*, 39(4), p. 661‑665. Disponible sur: https://doi.org/10.1016/j.biocel.2006.10.022.

Hollyday, M., McMahon, J.A. et McMahon, A.P. (1995) « *Wnt* expression patterns in chick embryo nervous system », *Mechanisms of Development*, 52(1), p. 9‑25. Disponible sur: https://doi.org/10.1016/0925-4773(95)00385-E.

Horner, V.L. et Caspary, T. (2011) « Disrupted dorsal neural tube BMP signaling in the cilia mutant *Arl13b*hnn stems from abnormal Shh signaling », *Developmental Biology*, 355(1), p. 43‑54. Disponible sur: https://doi.org/10.1016/j.ydbio.2011.04.019.

Huang, P. et Schier, A.F. (2009) « Dampened Hedgehog signaling but normal Wnt signaling in zebrafish without cilia », *Development*, 136(18), p. 3089‑3098. Disponible sur: https://doi.org/10.1242/dev.041343.

Hubert, K.A. et Wellik, D.M. (2023) « Hox genes in development and beyond », *Development*, 150(1), p. dev192476. Disponible sur: https://doi.org/10.1242/dev.192476.

Hudish, L.I. *et al.* (2016) « *miR-219* regulates neural progenitors by dampening apical Par protein-dependent Hedgehog signaling », *Development*, p. dev.137844. Disponible sur: https://doi.org/10.1242/dev.137844.

Hudish, L.I., Blasky, A.J. et Appel, B. (2013) « miR-219 Regulates Neural Precursor Differentiation by Direct Inhibition of Apical Par Polarity Proteins », *Developmental Cell*, 27(4), p. 387‑398. Disponible sur: https://doi.org/10.1016/j.devcel.2013.10.015.

Hulme, A.J. *et al.* (2022) « Making neurons, made easy: The use of Neurogenin-2 in neuronal differentiation », *Stem Cell Reports*, 17(1), p. 14‑34. Disponible sur: https://doi.org/10.1016/j.stemcr.2021.11.015.

Ishikawa, H. et Marshall, W.F. (2017) « Intraflagellar Transport and Ciliary Dynamics », *Cold Spring Harbor Perspectives in Biology*, 9(3), p. a021998. Disponible sur: https://doi.org/10.1101/cshperspect.a021998.

Ishitani, T. (2023) « Cadherin-linked morphogen gradient actualizes robust tissue patterning », *Current Opinion in Cell Biology*, 85, p. 102275. Disponible sur: https://doi.org/10.1016/j.ceb.2023.102275.

Jacobs, C.T. *et al.* (2022) « Temporal cell fate determination in the spinal cord is mediated by the duration of Notch signalling », *Developmental Biology*, 489, p. 1‑13. Disponible sur: https://doi.org/10.1016/j.ydbio.2022.05.010.

Jacobs, C.T. et Huang, P. (2019) « Notch signalling maintains Hedgehog responsiveness via a Gli-dependent mechanism during spinal cord patterning in zebrafish », *eLife*, 8, p. 1‑24. Disponible sur: https://doi.org/10.7554/elife.49252.

Jacobs, C.T. et Huang, P. (2021) « Complex crosstalk of Notch and Hedgehog signalling during the development of the central nervous system », *Cellular and Molecular Life Sciences*, 78(2), p. 635‑644. Disponible sur: https://doi.org/10.1007/s00018-020-03627-3.

Johnson, G.P., Fair, S. et Hoey, D.A. (2021) « Primary cilium-mediated MSC mechanotransduction is dependent on Gpr161 regulation of hedgehog signalling », *Bone*, 145, p. 115846. Disponible sur: https://doi.org/10.1016/j.bone.2021.115846.

Johnson, K. *et al.* (2016) « Gfap-positive radial glial cells are an essential progenitor population for later-born neurons and glia in the zebrafish spinal cord », *Glia*, 64(7), p. 1170‑1189. Disponible sur: https://doi.org/10.1002/glia.22990.

Jurisch-Yaksi, N., Yaksi, E. et Kizil, C. (2020) « Radial glia in the zebrafish brain: Functional, structural, and physiological comparison with the mammalian glia », *Glia*, 68(12), p. 2451‑2470. Disponible sur: https://doi.org/10.1002/glia.23849.

Kalcheim, C. et Douarin, N.M.L. (1986) « Requirement of a Neural Tube Signal for the Differentiation of Neural Crest Ceils into Dorsal Root Ganglia ».

Karlstrom, R.O. *et al.* (2003) « Genetic analysis of zebrafish gli1 and gli2 reveals divergent requirements for gli genes in vertebrate development », *Development*, 130(8), p. 1549‑1564. Disponible sur: https://doi.org/10.1242/dev.00364.

Kasioulis, I., Das, R.M. et Storey, K.G. (2017) « Inter-dependent apical microtubule and actin dynamics orchestrate centrosome retention and neuronal delamination », *eLife*. Édité par D.J. Solecki, 6, p. e26215. Disponible sur: https://doi.org/10.7554/eLife.26215.

Kawaguchi, A. (2021) « Neuronal Delamination and Outer Radial Glia Generation in Neocortical Development », *Frontiers in Cell and Developmental Biology*, 8. Disponible sur: https://doi.org/10.3389/fcell.2020.623573.

Kawaue, T. *et al.* (2019) « Lzts1 controls both neuronal delamination and outer radial glial-like cell generation during mammalian cerebral development », *Nature Communications*, 10(1), p. 2780. Disponible sur: https://doi.org/10.1038/s41467-019-10730-y.

Kicheva, A. *et al.* (2014) « Coordination of progenitor specification and growth in mouse and chick spinal cord », *Science*, 345(6204), p. 1254927. Disponible sur: https://doi.org/10.1126/science.1254927.

Kicheva, A. et Briscoe, J. (2023) « Control of Tissue Development by Morphogens », *Annual Review of Cell and Developmental Biology*, 39(Volume 39, 2023), p. 91‑121. Disponible sur: https://doi.org/10.1146/annurev-cellbio-020823-011522.

Kim, H.R. *et al.* (2010) « Gli2a protein localization reveals a role for Iguana/DZIP1 in primary ciliogenesis and a dependence of Hedgehog signal transduction on primary cilia in the zebrafish », *BMC Biology*, 8(1), p. 65. Disponible sur: https://doi.org/10.1186/1741-7007-8-65.

Kondrychyn, I. *et al.* (2013) « Stretching Morphogenesis of the Roof Plate and Formation of the Central Canal », *PLoS ONE*, 8(2), p. e56219. Disponible sur: https://doi.org/10.1371/journal.pone.0056219.

Kong, J.H. *et al.* (2015) « Notch Activity Modulates the Responsiveness of Neural Progenitors to Sonic Hedgehog Signaling », *Developmental Cell*, 33(4), p. 373‑387. Disponible sur: https://doi.org/10.1016/j.devcel.2015.03.005.

Kong, J.H., Siebold, C. et Rohatgi, R. (2019) « Biochemical mechanisms of vertebrate hedgehog signaling », *Development*, 146(10), p. dev166892. Disponible sur: https://doi.org/10.1242/dev.166892.

Kopan, R. et Ilagan, Ma.X.G. (2009) « The Canonical Notch Signaling Pathway: Unfolding the Activation Mechanism », *Cell*, 137(2), p. 216‑233. Disponible sur: https://doi.org/10.1016/j.cell.2009.03.045.

Korzh, V. (2014) « Stretching cell morphogenesis during late neurulation and mild neural tube defects », *Development, Growth & Differentiation*, 56(6), p. 425‑433. Disponible sur: https://doi.org/10.1111/dgd.12143.

Kramer-Zucker, A.G. *et al.* (2005) « Cilia-driven fluid flow in the zebrafish pronephros, brain and Kupffer's vesicle is required for normal organogenesis », *Development*, 132(8), p. 1907‑1921. Disponible sur: https://doi.org/10.1242/dev.01772.

Kridsada, K. *et al.* (2018) « Roof Plate-Derived Radial Glial-like Cells Support Developmental Growth of Rapidly Adapting Mechanoreceptor Ascending Axons », *Cell Reports*, 23(10), p. 2928‑2941. Disponible sur: https://doi.org/10.1016/j.celrep.2018.05.025.

Krispin, S. *et al.* (2010) « Evidence for a dynamic spatiotemporal fate map and early fate restrictions of premigratory avian neural crest », *Development*, 137(4), p. 585‑595. Disponible sur: https://doi.org/10.1242/dev.041509.

Kumar, V. *et al.* (2021) « The Role of Notch, Hedgehog, and Wnt Signaling Pathways in the Resistance of Tumors to Anticancer Therapies », *Frontiers in Cell and Developmental Biology*, 9, p. 650772. Disponible sur: https://doi.org/10.3389/fcell.2021.650772.

Kuzmicz-Kowalska, K. et Kicheva, A. (2021) « Regulation of size and scale in vertebrate spinal cord development », *WIREs Developmental Biology*, 10(3), p. e383. Disponible sur: https://doi.org/10.1002/wdev.383.

Lathia, J.D., Mattson, M.P. et Cheng, A. (2008) « Notch: from neural development to neurological disorders », *Journal of Neurochemistry*, 107(6), p. 1471‑1481. Disponible sur: https://doi.org/10.1111/j.1471-4159.2008.05715.x.

Le Dréau, G. et Martí, E. (2012) « Dorsal-ventral patterning of the neural tube: a tale of three signals », *Developmental Neurobiology*, 72(12), p. 1471‑1481. Disponible sur: https://doi.org/10.1002/dneu.22015.

Lee, K.J., Dietrich, P. et Jessell, T.M. (2000) « Genetic ablation reveals that the roof plate is essential for dorsal interneuron specification », *Nature*, 403(6771), p. 734‑740. Disponible sur: https://doi.org/10.1038/35001507.

Lee, K.J. et Jessell, T.M. (1999) « THE SPECIFICATION OF DORSAL CELL FATES IN THE VERTEBRATE CENTRAL NERVOUS SYSTEM », *Annual Review of Neuroscience*, 22(1), p. 261‑294. Disponible sur: https://doi.org/10.1146/annurev.neuro.22.1.261.

Lee, K.J., Mendelsohn, M. et Jessell, T.M. (1998) « Neuronal patterning by BMPs: a requirement for GDF7 in the generation of a discrete class of commissural interneurons in the mouse spinal cord », *Genes & Development*, 12(21), p. 3394‑3407. Disponible sur: https://doi.org/10.1101/gad.12.21.3394.

Lehr, S. *et al.* (2025) « Self-organized pattern formation in the developing mouse neural tube by a temporal relay of BMP signaling », *Developmental Cell*, 60(4), p. 567-580.e14. Disponible sur: https://doi.org/10.1016/j.devcel.2024.10.024.

Lei, Q. *et al.* (2006) « Wnt Signaling Inhibitors Regulate the Transcriptional Response to Morphogenetic Shh-Gli Signaling in the Neural Tube », *Developmental Cell*, 11(3), p. 325‑337. Disponible sur: https://doi.org/10.1016/j.devcel.2006.06.013.

Lemke, S.B. et Nelson, C.M. (2021) « Dynamic changes in epithelial cell packing during tissue morphogenesis », *Current Biology*, 31(18), p. R1098‑R1110. Disponible sur: https://doi.org/10.1016/j.cub.2021.07.078.

Li, X. *et al.* (2007) « Gli1 acts through Snail and E-cadherin to promote nuclear signaling by β-catenin », *Oncogene*, 26(31), p. 4489‑4498. Disponible sur: https://doi.org/10.1038/sj.onc.1210241.

Liem, K.F., Jr, Jessell, T.M. et Briscoe, J. (2000) « Regulation of the neural patterning activity of sonic hedgehog by secreted BMP inhibitors expressed by notochord and somites », *Development*, 127(22), p. 4855‑4866. Disponible sur: https://doi.org/10.1242/dev.127.22.4855.

Litingtung, Y. et Chiang, C. (2000) « Specification of ventral neuron types is mediated by an antagonistic interaction between Shh and Gli3 », *Nature Neuroscience*, 3(10), p. 979‑985. Disponible sur: https://doi.org/10.1038/79916.

Liu, F., Massagué, J. et Altaba., A.R. i (1998) « Carboxy-terminally truncated Gli3 proteins associate with Smads », *Nature Genetics*, 20(4), p. 325‑326. Disponible sur: https://doi.org/10.1038/3793.

Liu, Z. *et al.* (2019) « Primary cilia regulate hematopoietic stem and progenitor cell specification through Notch signaling in zebrafish », *Nature Communications*, 10(1), p. 1839. Disponible sur: https://doi.org/10.1038/s41467-019-09403-7.

Lozito, T.P. *et al.* (2021) « Introducing dorsoventral patterning in adult regenerating lizard tails with gene-edited embryonic neural stem cells », *Nature Communications*, 12(1), p. 6010. Disponible sur: https://doi.org/10.1038/s41467-021-26321-9.

Manzari-Tavakoli, A. *et al.* (2022) « The Cross-Talks Among Bone Morphogenetic Protein (BMP) Signaling and Other Prominent Pathways Involved in Neural Differentiation », *Frontiers in Molecular Neuroscience*, 15. Disponible sur: https://doi.org/10.3389/fnmol.2022.827275.

Marklund, U. *et al.* (2010) « Domain-specific control of neurogenesis achieved through patterned regulation of Notch ligand expression », *Development*, 137(3), p. 437‑445. Disponible sur: https://doi.org/10.1242/dev.036806.

Martínez, G.F. *et al.* (2022) « Extracellular matrix stiffness negatively affects axon elongation, growth cone area and F-actin levels in a collagen type I 3D culture », *Journal of Tissue Engineering and Regenerative Medicine*, 16(2), p. 151‑162. Disponible sur: https://doi.org/10.1002/term.3269.

Martins-Green, M. (1988) « Origin of the dorsal surface of the neural tube by progressive delamination of epidermal ectoderm and neuroepithelium: implications for neurulation and neural tube defects », *Development*, 103(4), p. 687‑706. Disponible sur: https://doi.org/10.1242/dev.103.4.687.

Maurya, A.K. *et al.* (2011) « Integration of Hedgehog and BMP signalling by the engrailed2a gene in the zebrafish myotome », *Development*, 138(4), p. 755‑765. Disponible sur: https://doi.org/10.1242/dev.062521.

McCaffrey, L.M. et Macara, I.G. (2011) « Epithelial organization, cell polarity and tumorigenesis », *Trends in Cell Biology*, 21(12), p. 727‑735. Disponible sur: https://doi.org/10.1016/j.tcb.2011.06.005.

Megason, S.G. et McMahon, A.P. (2002) « A mitogen gradient of dorsal midline Wnts organizes growth in the CNS », *Development*, 129(9), p. 2087‑2098. Disponible sur: https://doi.org/10.1242/dev.129.9.2087.

Mida, B. *et al.* (2026) « A low-level Cdkn1c/p57kip2 expression in spinal progenitors drives the transition from proliferative to neurogenic modes of division », *EMBO Reports*, 27(2), p. 433‑470. Disponible sur: https://doi.org/10.1038/s44319-025-00653-9.

Millen, K.J., Millonig, J.H. et Hatten, M.E. (2004) « Roof plate and dorsal spinal cord dl1 interneuron development in the dreher mutant mouse », *Developmental Biology*, 270(2), p. 382‑392. Disponible sur: https://doi.org/10.1016/j.ydbio.2004.03.008.

Millonig, J.H., Millen, K.J. et Hatten, M.E. (2000) « The mouse Dreher gene Lmx1a controls formation of the roof plate in the vertebrate CNS », *Nature*, 403(6771), p. 764‑769. Disponible sur: https://doi.org/10.1038/35001573.

Moore, R. et Alexandre, P. (2020) « Delta-Notch Signaling: The Long and the Short of a Neuron's Influence on Progenitor Fates », *Journal of Developmental Biology*, 8(2), p. 8. Disponible sur: https://doi.org/10.3390/jdb8020008.

Munson, C. *et al.* (2008) « Regulation of neurocoel morphogenesis by Pard6γb », *Developmental Biology*, 324(1), p. 41‑54. Disponible sur: https://doi.org/10.1016/j.ydbio.2008.08.033.

Muroyama, Y. *et al.* (2002) « Wnt signaling plays an essential role in neuronal specification of the dorsal spinal cord », *Genes & Development*, 16(5), p. 548‑553. Disponible sur: https://doi.org/10.1101/gad.937102.

Navis, A. et Nelson, C.M. (2016) « Pulling together: Tissue-generated forces that drive lumen morphogenesis », *Seminars in Cell & Developmental Biology*, 55, p. 139‑147. Disponible sur: https://doi.org/10.1016/j.semcdb.2016.01.002.

Neely, S.A. et Lyons, D.A. (2021) « Insights Into Central Nervous System Glial Cell Formation and Function From Zebrafish », *Frontiers in Cell and Developmental Biology*, 9. Disponible sur: https://doi.org/10.3389/fcell.2021.754606.

Nemoz-Billet, L. *et al.* (2024) « Dual topologies of myotomal collagen XV and Tenascin C act in concert to guide and shape developing motor axons », *Proceedings of the National Academy of Sciences*, 121(13), p. e2314588121. Disponible sur: https://doi.org/10.1073/pnas.2314588121.

Neumann, N.M. *et al.* (2018) « Coordination of Receptor Tyrosine Kinase Signaling and Interfacial Tension Dynamics Drives Radial Intercalation and Tube Elongation », *Developmental Cell*, 45(1), p. 67-82.e6. Disponible sur: https://doi.org/10.1016/j.devcel.2018.03.011.

Niewiadomski, P. *et al.* (2019) « Gli Proteins: Regulation in Development and Cancer », *Cells*, 8(2), p. 147. Disponible sur: https://doi.org/10.3390/cells8020147.

Odenthal, J. *et al.* (2000) « Two Distinct Cell Populations in the Floor Plate of the Zebrafish Are Induced by Different Pathways », *Developmental Biology*, 219(2), p. 350‑363. Disponible sur: https://doi.org/10.1006/dbio.1999.9589.

Ota, M. et Ito, K. (2006) « BMP and FGF-2 regulate neurogenin-2 expression and the differentiation of sensory neurons and glia ». Disponible sur: https://doi.org/10.1002/dvdy.20673.

Persson, M. *et al.* (2002) « Dorsal-ventral patterning of the spinal cord requires Gli3 transcriptional repressor activity », *Genes & Development*, 16(22), p. 2865‑2878. Disponible sur: https://doi.org/10.1101/gad.243402.

Placzek, M. et Briscoe, J. (2005) « The floor plate: multiple cells, multiple signals », *Nature Reviews Neuroscience*, 6(3), p. 230‑240. Disponible sur: https://doi.org/10.1038/nrn1628.

Ravanelli, A.M. et Appel, B. (2015) « Motor neurons and oligodendrocytes arise from distinct cell lineages by progenitor recruitment », *Genes & Development*, 29(23), p. 2504‑2515. Disponible sur: https://doi.org/10.1101/gad.271312.115.

Rekler, D. et Kalcheim, C. (2021) « From Neural Crest to Definitive Roof Plate: The Dynamic Behavior of the Dorsal Neural Tube », *International Journal of Molecular Sciences*, 22(8), p. 3911. Disponible sur: https://doi.org/10.3390/ijms22083911.

Ribeiro, A. *et al.* (2017) « Foxj1a is expressed in ependymal precursors, controls central canal position and is activated in new ependymal cells during regeneration in zebrafish », *Open Biology*, 7(11), p. 170139. Disponible sur: https://doi.org/10.1098/rsob.170139.

Ribes, V. *et al.* (2010) « Distinct Sonic Hedgehog signaling dynamics specify floor plate and ventral neuronal progenitors in the vertebrate neural tube », *Genes & Development*, 24(11), p. 1186‑1200. Disponible sur: https://doi.org/10.1101/gad.559910.

Ribes, V. et Briscoe, J. (2009) « Establishing and Interpreting Graded Sonic Hedgehog Signaling during Vertebrate Neural Tube Patterning: The Role of Negative Feedback », *Cold Spring Harbor Perspectives in Biology*, 1(2), p. a002014‑a002014. Disponible sur: https://doi.org/10.1101/cshperspect.a002014.

Ringers, C., Olstad, E.W. et Jurisch-Yaksi, N. (2020) « The role of motile cilia in the development and physiology of the nervous system », *Philosophical Transactions of the Royal Society B: Biological Sciences*, 375(1792), p. 20190156. Disponible sur: https://doi.org/10.1098/rstb.2019.0156.

Rodrigo Albors, A. *et al.* (2023) « An ependymal cell census identifies heterogeneous and ongoing cell maturation in the adult mouse spinal cord that changes dynamically on injury », *Developmental Cell*, 58(3), p. 239-255.e10. Disponible sur: https://doi.org/10.1016/j.devcel.2023.01.003.

Rodriguez-Boulan, E. et Macara, I.G. (2014) « Organization and execution of the epithelial polarity programme », *Nature Reviews Molecular Cell Biology*, 15(4), p. 225‑242. Disponible sur: https://doi.org/10.1038/nrm3775.

Rousso, D.L. *et al.* (2012) « Foxp-Mediated Suppression of N-Cadherin Regulates Neuroepithelial Character and Progenitor Maintenance in the CNS », *Neuron*, 74(2), p. 314‑330. Disponible sur: https://doi.org/10.1016/j.neuron.2012.02.024.

Saade, M. *et al.* (2013) « Sonic Hedgehog Signaling Switches the Mode of Division in the Developing Nervous System », *Cell Reports*, 4(3), p. 492‑503. Disponible sur: https://doi.org/10.1016/j.celrep.2013.06.038.

Saade, M. et Martí, E. (2025) « Early spinal cord development: from neural tube formation to neurogenesis », *Nature Reviews Neuroscience*, 26(4), p. 195‑213. Disponible sur: https://doi.org/10.1038/s41583-025-00906-5.

Sagner, A. *et al.* (2018) « Olig2 and Hes regulatory dynamics during motor neuron differentiation revealed by single cell transcriptomics », *PLOS Biology*, 16(2), p. e2003127. Disponible sur: https://doi.org/10.1371/journal.pbio.2003127.

Sagner, A. et Briscoe, J. (2019) « Establishing neuronal diversity in the spinal cord: a time and a place », *Development*, 146(22), p. dev182154. Disponible sur: https://doi.org/10.1242/dev.182154.

Sander, M. *et al.* (2000) « Ventral neural patterning by *Nkx* homeobox genes: *Nkx6.1* controls somatic motor neuron and ventral interneuron fates », *Genes & Development*, 14(17), p. 2134‑2139. Disponible sur: https://doi.org/10.1101/gad.820400.

Scardigli, R. *et al.* (2001) « Crossregulation between Neurogenin2 and Pathways Specifying Neuronal Identity in the Spinal Cord », *Neuron*, 31(2), p. 203‑217. Disponible sur: https://doi.org/10.1016/S0896-6273(01)00358-0.

Schmitz, B., Papan, C. et Campos-Ortega, J.A. (1993) « Neurulation in the anterior trunk region of the zebrafish Brachydanio rerio », *Roux's Archives of Developmental Biology*, 202(5), p. 250‑259. Disponible sur: https://doi.org/10.1007/BF00363214.

Schwend, T. *et al.* (2013) « Stabilization of Speckle-type POZ Protein (Spop) by Daz Interacting Protein 1 (Dzip1) Is Essential for Gli Turnover and the Proper Output of Hedgehog Signaling* », *Journal of Biological Chemistry*, 288(45), p. 32809‑32820. Disponible sur: https://doi.org/10.1074/jbc.M113.512962.

Sebo, D.J. *et al.* (2025) « Activation of Wnt/β-catenin in neural progenitor cells regulates blood–brain barrier development and promotes neuroinflammation », *Scientific Reports*, 15(1), p. 3496. Disponible sur: https://doi.org/10.1038/s41598-025-85784-8.

Sekimizu, K. *et al.* (2004) « The zebrafish *iguana* locus encodes Dzip1, a novel zinc-finger protein required for proper regulation of Hedgehog signaling », *Development*, 131(11), p. 2521‑2532. Disponible sur: https://doi.org/10.1242/dev.01059.

Ševc, J., Daxnerová, Z. et Miklošová, M. (2009) « Role of Radial Glia in Transformation of the Primitive Lumen to the Central Canal in the Developing Rat Spinal Cord », *Cellular and Molecular Neurobiology*, 29(6), p. 927‑936. Disponible sur: https://doi.org/10.1007/s10571-009-9377-3.

Sharma, P. *et al.* (2019) « Notch-mediated inhibition of neurogenesis is required for zebrafish spinal cord morphogenesis », *Scientific Reports*, 9(1), p. 9958. Disponible sur: https://doi.org/10.1038/s41598-019-46067-1.

Shashikanth, N. *et al.* (2017) « Epithelial Organization: The Gut and Beyond », in R. Terjung (éd.) *Comprehensive Physiology*. 1re éd. Wiley, p. 1497‑1518. Disponible sur: https://doi.org/10.1002/cphy.c170003.

Shimojo, H., Ohtsuka, T. et Kageyama, R. (2011) « Dynamic Expression of Notch Signaling Genes in Neural Stem/Progenitor Cells », *Frontiers in Neuroscience*, 5. Disponible sur: https://doi.org/10.3389/fnins.2011.00078.

Shin, J. *et al.* (2007) « Notch signaling regulates neural precursor allocation and binary neuronal fate decisions in zebrafish », *Development*, 134(10), p. 1911‑1920. Disponible sur: https://doi.org/10.1242/dev.001602.

Shinozuka, T. *et al.* (2019) « Wnt produced by stretched roof-plate cells is required for the promotion of cell proliferation around the central canal of the spinal cord », *Development (Cambridge)*, 146(2), p. dev159343. Disponible sur: https://doi.org/10.1242/dev.159343.

Shinozuka, T. et Takada, S. (2021) « Morphological and Functional Changes of Roof Plate Cells in Spinal Cord Development », *Journal of Developmental Biology*, 9(3), p. 30. Disponible sur: https://doi.org/10.3390/jdb9030030.

Simmons, A.D. *et al.* (2001) « *Neurogenin2* Expression in Ventral and Dorsal Spinal Neural Tube Progenitor Cells Is Regulated by Distinct Enhancers », *Developmental Biology*, 229(2), p. 327‑339. Disponible sur: https://doi.org/10.1006/dbio.2000.9984.

Spassky, N. *et al.* (2005) « Adult Ependymal Cells Are Postmitotic and Are Derived from Radial Glial Cells during Embryogenesis », *The Journal of Neuroscience*, 25(1), p. 10‑18. Disponible sur: https://doi.org/10.1523/JNEUROSCI.1108-04.2005.

St Johnston, D. et Sanson, B. (2011) « Epithelial polarity and morphogenesis », *Current Opinion in Cell Biology*, 23(5), p. 540‑546. Disponible sur: https://doi.org/10.1016/j.ceb.2011.07.005.

Stasiulewicz, M. *et al.* (2015) « A conserved role for Notch signaling in priming the cellular response to Shh through ciliary localisation of the key Shh transducer Smo », *Development*, 142(13), p. 2291‑2303. Disponible sur: https://doi.org/10.1242/dev.125237.

Strähle, U. *et al.* (2004) « Vertebrate floor-plate specification: variations on common themes », *Trends in Genetics*, 20(3), p. 155‑162. Disponible sur: https://doi.org/10.1016/j.tig.2004.01.002.

Stubbs, J.L. *et al.* (2006) « Radial intercalation of ciliated cells during *Xenopus* skin development », *Development*, 133(13), p. 2507‑2515. Disponible sur: https://doi.org/10.1242/dev.02417.

Sturrock, R.R. (1980) « An electron microscopic study of the development of the ependyma of the central canal of the mouse spinal cord ».

Symonds, A.C. *et al.* (2020) « Coordinated assembly and release of adhesions builds apical junctional belts during *de novo* polarisation of an epithelial tube », *Development*, 147(24), p. dev191494. Disponible sur: https://doi.org/10.1242/dev.191494.

Szabó, A. *et al.* (2016) « The Molecular Basis of Radial Intercalation during Tissue Spreading in Early Development », *Developmental Cell*, 37(3), p. 213‑225. Disponible sur: https://doi.org/10.1016/j.devcel.2016.04.008.

Szabó, A. et Mayor, R. (2018) « Mechanisms of Neural Crest Migration », *Annual Review of Genetics*, 52(1), p. 43‑63. Disponible sur: https://doi.org/10.1146/annurev-genet-120417-031559.

Tait, C.M. *et al.* (2020) « Crumbs2 mediates ventricular layer remodelling to form the spinal cord central canal », *PLOS Biology*. Édité par C. Becker, 18(3), p. e3000470. Disponible sur: https://doi.org/10.1371/journal.pbio.3000470.

Takeuchi, M. *et al.* (2015) « Type IV Collagen Controls the Axogenesis of Cerebellar Granule Cells by Regulating Basement Membrane Integrity in Zebrafish », *PLOS Genetics*, 11(10), p. e1005587. Disponible sur: https://doi.org/10.1371/journal.pgen.1005587.

Tan, B. *et al.* (2020) « The Mammalian Crumbs Complex Defines a Distinct Polarity Domain Apical of Epithelial Tight Junctions », *Current Biology*, 30(14), p. 2791-2804.e6. Disponible sur: https://doi.org/10.1016/j.cub.2020.05.032.

Tanabe, Y. et Jessell, T.M. (1996) « Diversity and Pattern in the Developing Spinal Cord », *Science*, 274(5290), p. 1115‑1123. Disponible sur: https://doi.org/10.1126/science.274.5290.1115.

Tawk, M. *et al.* (2007) « A mirror-symmetric cell division that orchestrates neuroepithelial morphogenesis », *Nature*, 446(7137), p. 797‑800. Disponible sur: https://doi.org/10.1038/nature05722.

Tay, S.Y. *et al.* (2010) « The iguana/DZIP1 protein is a novel component of the ciliogenic pathway essential for axonemal biogenesis », *Developmental Dynamics*, 239(2), p. 527‑534. Disponible sur: https://doi.org/10.1002/dvdy.22199.

Theveneau, E. et Mayor, R. (2012) « Neural crest delamination and migration: From epithelium-to-mesenchyme transition to collective cell migration », *Developmental Biology*, 366(1), p. 34‑54. Disponible sur: https://doi.org/10.1016/j.ydbio.2011.12.041.

Thouvenin, O. *et al.* (2020) « Origin and role of the cerebrospinal fluid bidirectional flow in the central canal », *eLife*, 9, p. e47699. Disponible sur: https://doi.org/10.7554/eLife.47699.

Toro-Tapia, G. et Das, R.M. (2020) « Primary cilium remodeling mediates a cell signaling switch in differentiating neurons », *Science Advances*, 6(21), p. eabb0601. Disponible sur: https://doi.org/10.1126/sciadv.abb0601.

Tozer, S. *et al.* (2013) « Temporal control of BMP signalling determines neuronal subtype identity in the dorsal neural tube », *Development*, 140(7), p. 1467‑1474. Disponible sur: https://doi.org/10.1242/dev.090118.

Tozer, S. et Morin, X. (2014) « Young Neurons Sever Ties to the Parental Niche », *Science*, 343(6167), p. 146‑147. Disponible sur: https://doi.org/10.1126/science.1249497.

Tsai, T.Y.-C. *et al.* (2020) « An adhesion code ensures robust pattern formation during tissue morphogenesis », *Science*, 370(6512), p. 113‑116. Disponible sur: https://doi.org/10.1126/science.aba6637.

Tsukita, S., Tanaka, H. et Tamura, A. (2019) « The Claudins: From Tight Junctions to Biological Systems », *Trends in Biochemical Sciences*, 44(2), p. 141‑152. Disponible sur: https://doi.org/10.1016/j.tibs.2018.09.008.

Tyurina, O.V. *et al.* (2005) « Zebrafish Gli3 functions as both an activator and a repressor in Hedgehog signaling », *Developmental Biology*, 277(2), p. 537‑556. Disponible sur: https://doi.org/10.1016/j.ydbio.2004.10.003.

Ulloa, F., Itasaki, N. et Briscoe, J. (2007) « Inhibitory Gli3 Activity Negatively Regulates Wnt/β-Catenin Signaling », *Current Biology*, 17(6), p. 545‑550. Disponible sur: https://doi.org/10.1016/j.cub.2007.01.062.

Ulloa, F. et Martí, E. (2010) « Wnt won the war: Antagonistic role of Wnt over Shh controls dorso-ventral patterning of the vertebrate neural tube », *Developmental Dynamics*, 239(1), p. 69‑76. Disponible sur: https://doi.org/10.1002/dvdy.22058.

Vaid, S. et Huttner, W.B. (2020) « Transcriptional Regulators and Human-Specific/Primate-Specific Genes in Neocortical Neurogenesis », *International Journal of Molecular Sciences*, 21(13), p. 4614. Disponible sur: https://doi.org/10.3390/ijms21134614.

Vanderlaan, G. *et al.* (2005) « Gli function is essential for motor neuron induction in zebrafish », *Developmental Biology*, 282(2), p. 550‑570. Disponible sur: https://doi.org/10.1016/j.ydbio.2005.04.010.

Vecchione, A., Croce, C.M. et Baldassarre, G. (2007) « Fez1/Lzts1 a new mitotic regulator implicated in cancer development », *Cell Division*, 2(1), p. 24. Disponible sur: https://doi.org/10.1186/1747-1028-2-24.

Ventriglia, S. et Kalcheim, C. (2024) « From neural tube to spinal cord: The dynamic journey of the dorsal neuroepithelium », *Developmental Biology*, 511, p. 26‑38. Disponible sur: https://doi.org/10.1016/j.ydbio.2024.04.001.

Wall, D.S. *et al.* (2009) « Progenitor cell proliferation in the retina is dependent on Notch-independent Sonic hedgehog/Hes1 activity », *Journal of Cell Biology*, 184(1), p. 101‑112. Disponible sur: https://doi.org/10.1083/jcb.200805155.

Wang, C. *et al.* (2013) « Centrosomal Protein DZIP1 Regulates Hedgehog Signaling by Promoting Cytoplasmic Retention of Transcription Factor GLI3 and Affecting Ciliogenesis * », *Journal of Biological Chemistry*, 288(41), p. 29518‑29529. Disponible sur: https://doi.org/10.1074/jbc.M113.492066.

Wang, K. *et al.* (2019) « Claudin-7 downregulation induces metastasis and invasion in colorectal cancer via the promotion of epithelial-mesenchymal transition », *Biochemical and Biophysical Research Communications*, 508(3), p. 797‑804. Disponible sur: https://doi.org/10.1016/j.bbrc.2018.10.049.

Werner, M.E. *et al.* (2014) « Radial intercalation is regulated by the Par complex and the microtubule-stabilizing protein CLAMP/Spef1 », *Journal of Cell Biology*, 206(3), p. 367‑376. Disponible sur: https://doi.org/10.1083/jcb.201312045.

Wilmerding, A. *et al.* (2021) « HoxB genes regulate neuronal delamination in the trunk neural tube by controlling the expression of Lzts1 », *Development*, 148(4), p. dev195404. Disponible sur: https://doi.org/10.1242/dev.195404.

Wolff, C. *et al.* (2004) « iguana encodes a novel zinc-finger protein with coiled-coil domains essential for Hedgehog signal transduction in the zebrafish embryo », *GENES & DEVELOPMENT*, 18, p. 1565‑1576.

Xia, M. *et al.* (2023) « Varying mechanical forces drive sensory epithelium formation », *Science Advances*, 9(44), p. eadf2664. Disponible sur: https://doi.org/10.1126/sciadv.adf2664.

Xie, H. *et al.* (2023) « Ependymal polarity defects coupled with disorganized ciliary beating drive abnormal cerebrospinal fluid flow and spine curvature in zebrafish », *PLOS Biology*. Édité par D. Wachten, 21(3), p. e3002008. Disponible sur: https://doi.org/10.1371/journal.pbio.3002008.

Xie, S. et Li, F. (2024) « Ependymal cells: roles in central nervous system infections and therapeutic application », *Journal of Neuroinflammation*, 21(1), p. 255. Disponible sur: https://doi.org/10.1186/s12974-024-03240-2.

Xing, L. *et al.* (2018) « Wnt/β-catenin signaling regulates ependymal cell development and adult homeostasis », *Proceedings of the National Academy of Sciences*, 115(26). Disponible sur: https://doi.org/10.1073/pnas.1803297115.

Xing, T. *et al.* (2020) « Tight Junction Protein Claudin-7 Is Essential for Intestinal Epithelial Stem Cell Self-Renewal and Differentiation », *Cellular and Molecular Gastroenterology and Hepatology*, 9(4), p. 641‑659. Disponible sur: https://doi.org/10.1016/j.jcmgh.2019.12.005.

Yam, P.T. et Charron, F. (2013) « Signaling mechanisms of non-conventional axon guidance cues: the Shh, BMP and Wnt morphogens », *Current Opinion in Neurobiology*, 23(6), p. 965‑973. Disponible sur: https://doi.org/10.1016/j.conb.2013.09.002.

Yu, K., McGlynn, S. et Matise, M.P. (2013) « Floor plate-derived sonic hedgehog regulates glial and ependymal cell fates in the developing spinal cord », *Development*, 140(7), p. 1594‑1604. Disponible sur: https://doi.org/10.1242/dev.090845.

Yu, X. *et al.* (2008) « Foxj1 transcription factors are master regulators of the motile ciliogenic program », *Nature Genetics*, 40(12), p. 1445‑1453. Disponible sur: https://doi.org/10.1038/ng.263.

Zechner, D. *et al.* (2007) « Bmp and Wnt/β-catenin signals control expression of the transcription factor Olig3 and the specification of spinal cord neurons », *Developmental Biology*, 303(1), p. 181‑190. Disponible sur: https://doi.org/10.1016/j.ydbio.2006.10.045.

Zhang, J. *et al.* (2010) « Establishment of a neuroepithelial barrier by Claudin5a is essential for zebrafish brain ventricular lumen expansion », *Proceedings of the National Academy of Sciences*, 107(4), p. 1425‑1430. Disponible sur: https://doi.org/10.1073/pnas.0911996107.