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Molecular Mechanisms of Axis Development: Insights from Cilia Biology

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Abstract Axis development is a critical biological process in the development of multicellular organisms, with cilia playing an important role. Cilia are small hair-like structures on the cell surface that are involved in various cellular activities, including signal transduction, cell movement, and sensing environmental signals. Studies have shown that cilia play a crucial regulatory role in the formation of the left-right and anterior-posterior axes by modulating morphogen gradients and signaling pathways such as Nodal, Wnt, and Hedgehog, thereby influencing gene expression and cell fate determination. This research systematically reviews the molecular mechanisms of cilia in axis development, exploring their specific roles in left-right asymmetry and anterior-posterior patterning, and analyzing related key genes and proteins. Additionally, the study introduces experimental models and techniques used in cilia research, discusses the impact of cilia dysfunction (ciliopathies) on developmental defects and their genetic basis. By summarizing current research progress and existing technical challenges, this research aims to provide guidance for future studies, promoting further breakthroughs in developmental biology and clinical treatment.

Keywords Axis development; Cilia; Signaling pathways; Left-right asymmetry; Ciliopathies

1 Introduction

Axis development is a fundamental process in embryogenesis that establishes the spatial organization of the body plan, including the formation of the anterior-posterior, dorsal-ventral, and left-right axes, which are crucial for the proper development of tissues and organs. In vertebrates, axis specification is initiated through the interaction of various signaling pathways, such as Wnt, TGF-β, and FGF, which regulate gene expression to define body axes (Ro et al., 2015), In zebrafish, axis specification begins during oogenesis and relies on the formation of cytoplasmic domains within the oocyte, essential for establishing the embryonic coordinate system (Fuentes et al., 2020), In plants, axis formation involves cytoskeletal reorganization and the establishment of the apical-basal axis during early embryogenesis (Ueda and Berger, 2019).

Cilia are microtubule-based organelles that project from the surface of most vertebrate cells and play a critical role in sensing and transmitting extracellular signals. Primary cilia, in particular, are involved in coordinating several key signaling pathways, such as Hedgehog (HH), Wnt, and TGF-β/BMP, which are essential for cell fate determination and tissue patterning during development, The Reissner fiber, a component of the cerebrospinal fluid, has been shown to be crucial for body axis morphogenesis in zebrafish, highlighting the role of cilia in axis development (Cantaut-Belarif et al., 2018), Defects in ciliary function can lead to a range of developmental disorders and diseases, collectively known as ciliopathies, affecting multiple tissues and organs (Anvarian et al., 2019).

This study will synthesize findings from various research to elucidate the molecular mechanisms by which cilia contribute to axis development. By integrating data from different studies, we aim to provide a comprehensive understanding of how ciliary signaling pathways regulate the establishment and patterning of body axes. The study will also explore the implications of ciliary dysfunction in developmental disorders and identify potential therapeutic targets for ciliopathies. Through this analysis, we hope to advance the current knowledge of cilia biology and its critical role in developmental processes.

2 Overview of Cilia Biology

2.1 Structure and types of cilia

Cilia are microtubule-based structures that extend from the surface of many eukaryotic cells. They are broadly classified into two types: motile and non-motile (primary) cilia. Motile cilia are typically found in large numbers on the cell surface and are responsible for generating fluid flow across epithelial surfaces, such as in the respiratory tract and the brain's ventricles (Bearce and Grimes, 2020; Thouvenin et al., 2020), Non-motile cilia, on the other hand, usually occur singly and function primarily as sensory organelles, detecting mechanical and chemical signals from the environment. The structure of cilia includes a core axoneme composed of microtubules arranged in a "9+2" pattern in motile cilia and a "9+0" pattern in primary cilia (Lee et al., 2015).

2.2 Cilia function in cellular processes

Cilia play crucial roles in various cellular processes, including fluid movement, signal transduction, and cellular orientation. In the central nervous system, motile cilia generate cerebrospinal fluid (CSF) flow (Figure 1), which is essential for brain development and the maintenance of the body axis (Cantaut-Belarif et al., 2018; Thouvenin et al., 2020), Cilia-driven CSF flow also facilitates the transport of signaling molecules, such as urotensin neuropeptides, which are critical for the straightening of the vertebrate body axis (Zhang et al., 2018), Additionally, cilia are involved in the establishment of planar cell polarity (PCP) in epithelial tissues, aligning cellular structures along a common axis (Chien et al., 2015; Chien et al., 2018), This alignment is crucial for the proper functioning of multiciliated cells in tissues such as the respiratory epithelium and the skin.

The study by Thouvenin et al. (2020) demonstrated the bidirectional velocity distribution of cerebrospinal fluid (CSF) flow in zebrafish embryos at 30 hours post-fertilization. This figure, through experimental measurements and numerical simulations, reveals the bidirectional flow pattern of CSF within the central canal, highlighting the influence of ciliary motion on fluid dynamics. This illustration provides important insights for studying how cilia regulate CSF flow during embryonic development.

2.3 Genetic regulation of cilia formation

The formation and maintenance of cilia are tightly regulated by a set of conserved genes and molecular pathways. Intraflagellar transport (IFT) proteins, such as IFT46, are essential for the assembly and function of cilia. Mutations in these genes can lead to various ciliopathies, characterized by defects in cilia structure and function (Lee et al., 2015), The V-ATPase accessory protein Atp6ap1b has also been shown to play a critical role in the development of ciliated organs by regulating proton flux and cytoplasmic pH, which are necessary for the proliferation of precursor cells that form cilia (Gokey et al., 2015), Furthermore, the planar cell polarity pathway and mechanical strain during embryonic development are key factors in determining cilia length, motility, and planar positioning, ensuring the proper function of cilia in processes such as left-right axis patterning (Chien et al., 2015; Chien et al., 2018; Xu, 2024).

Cilia are versatile organelles with diverse structural types and functions, ranging from fluid movement to signal transduction. Their formation and function are governed by a complex network of genetic and molecular mechanisms, highlighting their importance in cellular and developmental processes.

3 Molecular Mechanisms of Axis Development

3.1 Signaling pathways involved in axis formation

Axis development in vertebrates is orchestrated by a complex interplay of signaling pathways, including Wnt, TGF-β, and FGF pathways. These pathways are crucial for the establishment of the dorso-ventral (DV) and antero-posterior (AP) axes. The Wnt/β-catenin signaling pathway, activated by maternal determinants post-fertilization, plays a pivotal role in the formation of the Nieuwkoop center and subsequently the Spemann organizer, which are essential for DV and AP patterning (Carron and Shi, 2016), The TGF-β family, particularly Nodal and BMPs, are integral in DV patterning, with BMPs establishing gradients that define cellular fates along the DV axis (Bier and Robertis, 2015; Takebayashi-Suzuki and Suzuki, 2020), Additionally, the FGF pathway, along with retinoic acid (RA) and Wnt, contributes to the AP axis formation by creating gradients that pattern the trunk and posterior regions (Carron and Shi, 2016; Durston et al., 2019), The integration and fine-tuning of these signaling pathways ensure the correct establishment of the body plan (Roet al., 2015).

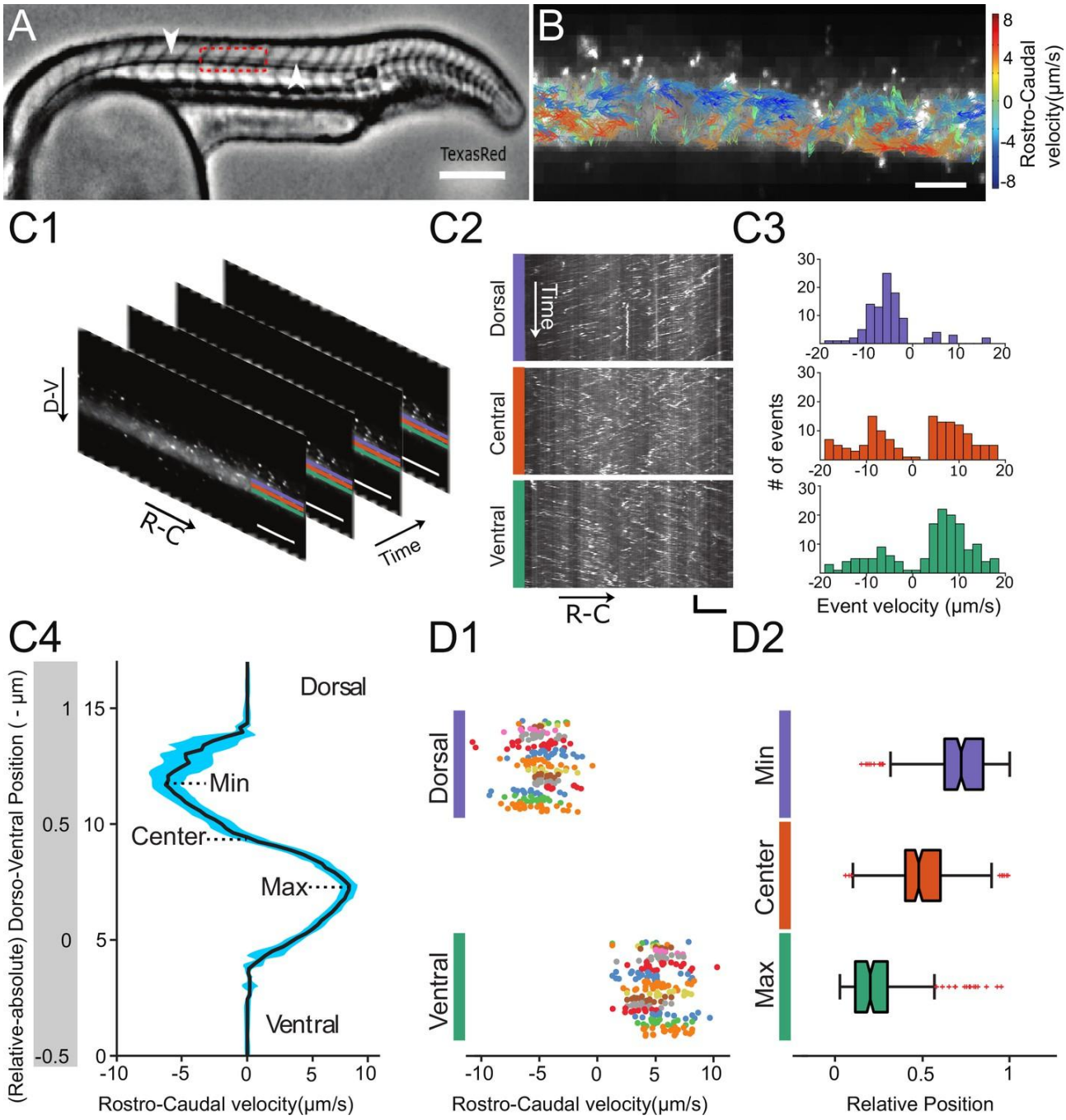

Figure 1 Automated CSF flow analysis in the central canal quantifies the bidirectional velocity profile in 30 hpf embryos (Adopted from Thouvenin et al., 2020)

Image caption: (A) A 30 hpf zebrafish embryo injected with Texas Red dye, marking all fluid-filled cavities, such as ventricles, central canal (CC, white arrows), and blood vessels. (B) Instantaneous velocities of 20 nm fluorescent particles measured within the central canal using a particle tracking velocimetry (PTV) algorithm, with arrow length, direction, and color indicating particle velocity (ranging from -8 to 8 μm/s). (C1-C4) Display of fluorescent particle motion trajectories and velocity distribution in the central canal at different dorsal-ventral (D-V) positions, generating a velocity profile. (D1-D2) Extreme velocity values measured in 110 zebrafish embryos, showing the minimum and maximum speeds and their relative D-V positions in the central canal (Adopted from Thouvenin et al., 2020)

3.2 Role of morphogens

Morphogens are signaling molecules that form concentration gradients and provide positional information to cells, guiding their fate during embryonic development. BMPs, a subset of the TGF-β family, are key morphogens in DV patterning, establishing distinct cellular domains through their gradients (Bier and Robertis, 2015), In the AP

axis, morphogens such as Wnt, FGF, and RA play significant roles. The inhibition of Wnt and BMP signals is necessary for the formation of anterior neural tissue, while gradients of Wnt, FGF, and RA pattern the posterior regions (Carron and Shi, 2016; Takebayashi-Suzuki and Suzuki, 2020), The interplay between these morphogens and their antagonists, such as Chordin and Noggin, further refines the patterning process, ensuring precise spatial and temporal gene expression (Bier and Robertis, 2015; Tingler et al., 2022).

3.3 Gene expression patterns

The establishment of body axes is closely linked to the regulation of gene expression patterns. The expression of Hox genes, which are regulated by morphogen gradients, is crucial for AP patterning. These genes exhibit collinear expression patterns that correspond to their positions along the body axis, with early expressed Hox genes defining anterior regions and later expressed genes defining posterior regions (Carron and Shi, 2016; Durston et al., 2019), Additionally, the expression of transcription factors such as Pitx2 and Southpaw is essential for left-right (LR) axis determination, with their asymmetric expression being regulated by cilia-driven fluid flow and signaling pathways like PCP and JNK (Derrick et al., 2022; Tingler et al., 2022), The precise regulation of these gene expression patterns ensures the correct development of the vertebrate body plan.

4 Cilia and Left-Right Axis Development

4.1 Role of cilia in left-right asymmetry

Cilia play a pivotal role in establishing left-right (L-R) asymmetry during embryonic development. The initial breaking of L-R symmetry is controlled by motile cilia that generate a leftward fluid flow within the left-right organizer (LRO) (Little and Norris, 2020; Hamada, 2020), This cilia-driven flow is crucial for the asymmetric expression of genes such as Nodal, which is restricted to the left side of the lateral plate mesoderm (LPM) (Grimes et al., 2016; Grimes and Burdine, 2017). In vertebrates, this process is essential for the correct positioning and morphogenesis of internal organs (Hamada, 2020).

4.2 Mechanisms of left-right patterning

The mechanisms underlying L-R patterning involve a complex interplay of genetic and cellular processes. The motile cilia in the LRO create a directional fluid flow that is detected by crown cells, which in turn activate the Nodal signaling pathway on the left side (Grimes et al., 2016), This asymmetric gene expression is further propagated by the Nodal-Pitx2 pathway, which influences lateralized cell differentiation and organogenesis (Grimes and Burdine, 2017; Hamada, 2020), Additionally, planar cell polarity (PCP) signaling and the JNK gene family have been implicated in the proper functioning and orientation of nodal cilia, further contributing to L-R asymmetry (Derrick et al., 2022).

4.3 Key genes and proteins

4.3.1 Nodal signaling

Nodal signaling is a critical determinant of L-R asymmetry. The asymmetric expression of Nodal in the LPM is initiated by cilia-driven fluid flow and is essential for the subsequent activation of downstream targets such as Pitx2 (Grimes and Burdine, 2017; Hamada, 2020), Nodal signaling is tightly regulated to ensure it is restricted to the left side, a process that involves various genetic interactions, including those with the polycystin-encoding genes PKD1L1 and PKD2 (Grimes et al., 2016).

4.3.2 Lefty and Pitx2

Lefty and Pitx2 are key downstream effectors of the Nodal signaling pathway. Lefty acts as a feedback inhibitor to restrict the range of Nodal signaling, ensuring that it remains confined to the left side (Grimes and Burdine, 2017). Pitx2, on the other hand, is a transcription factor that mediates the effects of Nodal signaling, driving the asymmetric development of organs (Grimes and Burdine, 2017; Hamada, 2020), The precise regulation of these genes is crucial for the correct establishment of L-R asymmetry.

4.3.3 Dynein and kinesin motor proteins

Dynein and kinesin motor proteins are essential for the function and movement of cilia. Dynein, in particular, is involved in the generation of ciliary movement, which is necessary for the creation of the leftward fluid flow in

the LRO, Inhibition of dynein function disrupts ciliary movement and consequently the establishment of L-R asymmetry (Yamada et al., 2019), Kinesin motor proteins also play a role in the transport of signaling molecules within cilia, contributing to the overall process of L-R patterning (Zhu et al., 2019).

Cilia are integral to the establishment of L-R asymmetry through their role in generating directional fluid flow and facilitating the asymmetric expression of key signaling molecules. The interplay of various genetic and molecular mechanisms ensures the precise regulation of this process, which is essential for proper organ development and function.

5 Cilia and Anterior-Posterior Axis Development

primary cilia are essential for the proper development of the anterior-posterior axis by mediating key signaling pathways and interacting with morphogen gradients. The dynamic and coordinated output of ciliary signaling is crucial for the spatial and temporal regulation of gene expression during embryogenesis, highlighting the importance of cilia in developmental processes.

5.1 Involvement of cilia in anterior-posterior patterning

Cilia play a crucial role in the development of the anterior-posterior (AP) axis by mediating the signaling pathways that are essential for the spatial and temporal regulation of gene expression during embryogenesis. Primary cilia, which are immotile and project from the surface of most vertebrate cells, act as sensory organelles that detect and transmit extracellular signals to regulate cellular processes critical for AP patterning (Anvarian et al., 2019; Kopinke et al., 2020), Mutations in ciliary trafficking genes can differentially affect Sonic Hedgehog (Shh)-dependent neural tube patterning along the AP axis, highlighting the importance of cilia in this developmental process (Legué and Liem, 2020).

5.2 Key signaling pathways

Several key signaling pathways are coordinated by primary cilia to control AP axis development. These include the Hedgehog (Hh), Wnt, fibroblast growth factor (FGF), and bone morphogenetic protein (BMP) pathways. The canonical Hh pathway, for instance, is a well-established ciliary signaling system that regulates cell fate and tissue homeostasis (Anvarian et al., 2019; Kopinke et al., 2020), Additionally, the Wnt and FGF pathways are critical for posteriorizing signals, while BMP signaling is involved in ventralizing signals along the AP axis (Tuazon and Mullins, 2015; Carron and Shi, 2016).

5.3 Interaction with morphogen gradients

Cilia interact with morphogen gradients to facilitate the precise patterning of the AP axis. These interactions are essential for the proper spatial distribution of signaling molecules and the subsequent activation of downstream genetic programs.

5.3.1 Wnt signaling

The Wnt signaling pathway is integral to AP axis patterning, with primary cilia playing a role in modulating Wnt activity. In planarians, for example, Wnt signaling establishes anterior versus posterior pole identities, and cilia are involved in the regulation of this pathway (Bonar et al., 2022), Additionally, the inhibition of Wnt signaling is necessary for the formation of anterior neural tissue during vertebrate development (Carron and Shi, 2016).

5.3.2 Hedgehog pathway

The Hedgehog (Hh) pathway is closely associated with cilia, as the majority of Hh signaling components are localized within the cilium-centrosome complex. Cilia are required for both the repression and activation of Hh signaling, which is crucial for the patterning of various tissues along the AP axis (Kopinke et al., 2020), For instance, proper ciliary assembly is critical for restricting Hh activity during early eye development, ensuring the correct formation of optic structures (Burnett et al., 2017).

5.3.3 FGF and BMP pathways

The FGF and BMP pathways also interact with cilia to influence AP patterning. FGF signaling, which acts downstream of Hh signaling, is necessary for anterior mesoderm morphogenesis during gastrulation (Guzzetta et

al., 2020), BMP signaling, on the other hand, is involved in ventralizing signals that contribute to AP axis patterning (Tuazon and Mullins, 2015), The integration of these pathways through ciliary signaling ensures the coordinated development of the AP axis.

6 Experimental Models and Techniques

6.1 Model organisms in cilia research

Model organisms have played a crucial role in advancing our understanding of cilia biology and their role in body axis development. The ciliate *Tetrahymena thermophila* has been a key model for studying intracellular patterns along the anteroposterior axis. *Zebrafish* research has provided insights into the role of cilia-driven cerebrospinal fluid flow in body axis morphogenesis, showing that cilia promote the formation of the Reissner fiber, which is essential for correct body axis development (Cantaut-Belarif et al., 2018; Zhang et al., 2018). It was found that mutations in the scospondin gene lead to impaired Reissner fiber formation, resulting in curled phenotypes in larvae. This phenomenon is evident early in development and shows significant differences among various mutants, highlighting the critical role of the Reissner fiber in spinal cord development (Figure 2). Additionally, a network of highly conserved kinases has been discovered to regulate organelle positioning by creating cortical inhibition zones (Cole and Gaertig, 2022). In vertebrates, mouse models have been pivotal in elucidating the role of planar cell polarity (PCP) genes, such as Vangl1 and Vangl2, in breaking left-right symmetry by controlling cilia positioning (Song et al., 2010). The medaka fish has also been used to study the role of the LIM protein Ajuba in ciliogenesis and left-right axis determination, emphasizing its important function in the cells lining the Kupffer's vesicle (Nagai et al., 2010).

6.2 Genetic and molecular tools

Genetic and molecular tools have been crucial in dissecting the mechanisms of cilia function and axis development. Mutational analyses in Tetrahymena have revealed genes whose mutations cause organelle positioning defects, providing insights into the molecular underpinnings of anterior-posterior patterning (Cole and Gaertig, 2022, In mice, the removal of Vangl1 and Vangl2 has demonstrated the necessity of PCP in interpreting anterior-posterior patterning information and linking it to left-right asymmetry (Song et al., 2010), Knockdown experiments in medaka have shown that Ajuba is essential for ciliogenesis and left-right axis determination, with its absence leading to randomized organ asymmetries (Nagai et al., 2010), In zebrafish, the mutation of the scospondin gene has shown that the Reissner fiber is critical for body axis morphogenesis, linking cilia function to the control of body axis curvature (Cantaut-Belarif et al., 2018), Additionally, the knockdown of ift46 in zebrafish has revealed its essential role in ciliary development, with defects leading to multiple ciliopathies (Lee et al., 2015).

6.3 Imaging and visualization techniques

Imaging and visualization techniques have been indispensable in studying cilia and their role in axis development. Classical microsurgical experiments in large ciliates, such as Stentor, have provided foundational observations that have been interpreted in light of recent molecular findings in Tetrahymena (Cole and Gaertig, 2022), In Xenopus, the use of imaging techniques has shown that mechanical strain during gastrulation plays a major role in determining the global axis of planar polarity in ciliated epithelia (Chien et al., 2015), Advanced imaging in zebrafish has demonstrated how cilia-driven cerebrospinal fluid flow directs the expression of urotensin neuropeptides, which are crucial for straightening the vertebrate body axis (Zhang et al., 2018), These techniques have also been used to visualize the Reissner fiber and its role in body axis morphogenesis, providing a deeper understanding of the interplay between cilia and cerebrospinal fluid flow (Cantaut-Belarif et al., 2018), Furthermore, imaging studies in zebrafish mutants have elucidated the role of motile cilia and cerebrospinal fluid flow in axial morphogenesis and spinal straightness, offering insights into conditions such as idiopathic scoliosis (Bearce and Grimes, 2020).

7 Disorders of Cilia Function

7.1 Ciliopathies and developmental defects

Ciliopathies are a group of genetic disorders caused by defects in the structure or function of cilia, which are microtubule-based organelles protruding from the cell surface. These disorders are often pleiotropic, affecting

multiple organ systems and leading to a wide range of developmental defects. For instance, defects in the intraflagellar transport protein IFT46 result in various ciliopathies, including kidney cysts, pericardial edema, and ventral axis curvature, as observed in zebrafish and mouse models, Primary cilia play crucial roles in signaling pathways such as Sonic hedgehog (Shh) and Wnt, which are essential for brain development and other organ systems. Defects in these pathways due to ciliary dysfunction can lead to severe developmental anomalies, including brain malformations and primary microcephaly (Wheway et al., 2019).

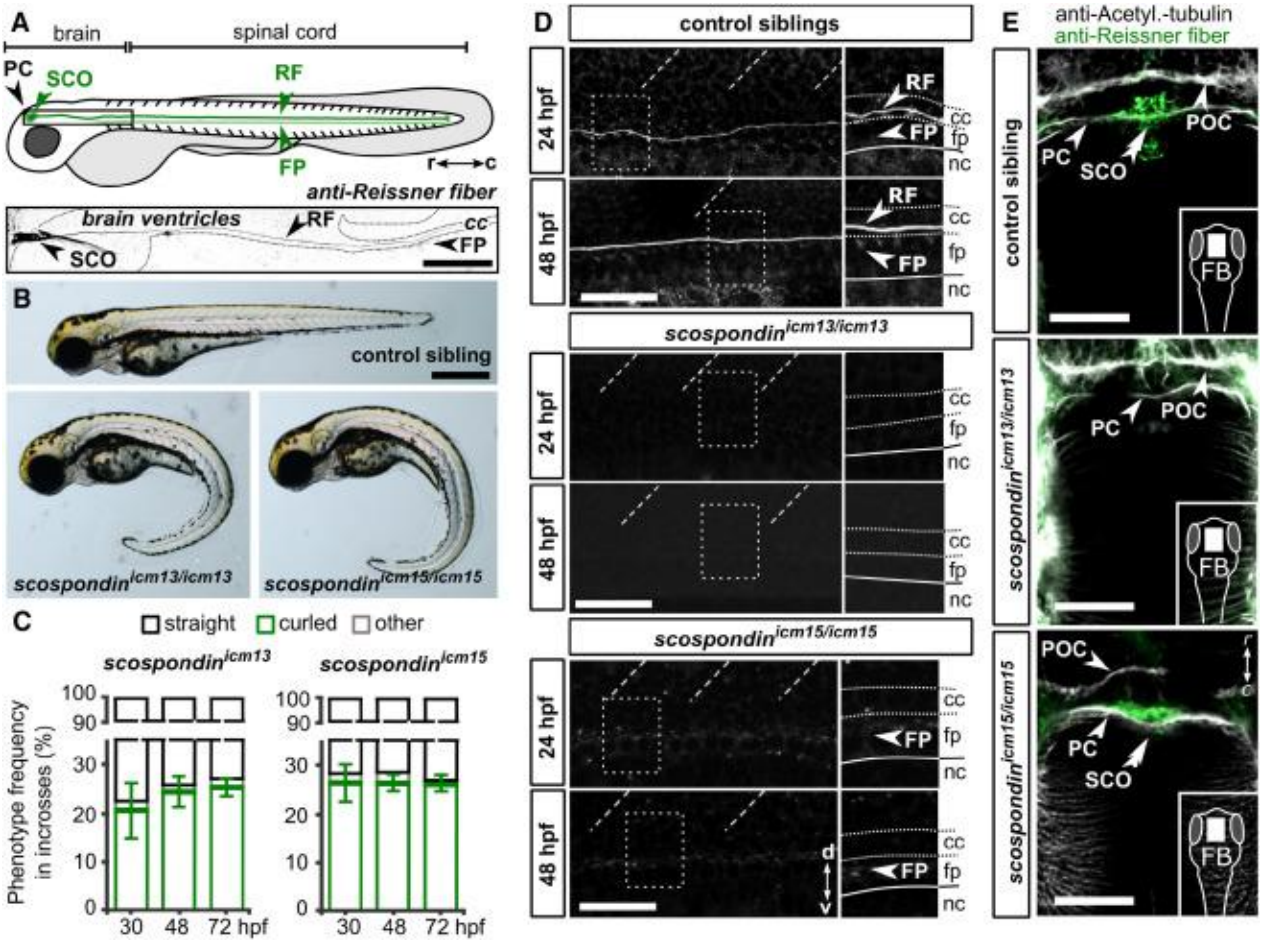

Figure 2 Mutations in scospondin Lead to the Absence of the Reissner Fiber and Defects in Body Axis Formation (Adopted from Cantaut-Belarif et al., 2018)

Image caption: (A) The Reissner fiber (RF) is localized in the posterior ventricles of the brain and the spinal central canal, formed by SCO-spondin secreted from the sub-commissural organ (SCO) and floor plate (FP). (B) At 72 hours post-fertilization (hpf), scospondinicm13/icm13 and scospondinicm15/icm15 mutant larvae exhibit a curled-down phenotype. (C) The proportion of curled-down phenotype over developmental time in scospondin mutant alleles is shown. (D) Immunostaining at 24 hpf and 48 hpf reveals the condition of the Reissner fiber in the spinal cord of both mutants and control groups. (E) Immunostaining of the forebrain at 48 hpf shows the presence of Reissner fiber material in scospondinicm15/icm15 mutants but not in scospondinicm13/icm13 mutants (Adopted from Cantaut-Belarif et al., 2018)

7.2 Genetic basis of ciliopathies

The genetic basis of ciliopathies is highly complex, involving mutations in over 150 different genes that affect cilia structure and function. Advances in genetic analysis and whole genome sequencing (WGS) have significantly enhanced our understanding of the molecular mechanisms underlying these disorders. The 100 000 Genomes Project, for example, aims to improve diagnosis and care for individuals with rare diseases, including ciliopathies, by utilizing WGS to identify causative mutations , This project highlights the potential of large-scale genomic initiatives to uncover the genetic underpinnings of ciliopathies and pave the way for personalized medical interventions.

7.3 Clinical implications and therapies

The clinical implications of ciliopathies are profound, given their impact on multiple organ systems and their association with severe developmental and degenerative diseases. Understanding the molecular mechanisms of cilia function and the genetic basis of ciliopathies is crucial for developing targeted therapies. Current research is focused on elucidating the roles of primary cilia in signaling pathways and cell cycle progression, which are critical for early patterning, neurogenesis, and neuronal maturation (Youn and Han, 2018), Therapeutic strategies may include gene therapy to correct defective genes, pharmacological interventions to modulate signaling pathways, and regenerative medicine approaches to repair damaged tissues. The ongoing advancements in genomics and cell biology hold promise for improving the diagnosis, treatment, and management of ciliopathies in the future (Sreekumar and Norris, 2019; Wheway et al., 2019).

8 Current Challenges and Future Directions

One of the primary technical challenges in studying cilia biology and its role in axis development is the complexity of ciliary structures and their dynamic nature. The intricate architecture of cilia, including the basal body and axoneme, requires advanced imaging techniques for detailed visualization and analysis. High-resolution microscopy and live-cell imaging are essential but often limited by technical constraints and the need for specialized equipment, Additionally, genetic manipulation of ciliary components, such as IFT46, poses significant challenges due to the essential roles these proteins play in various cellular processes, making it difficult to generate viable knockout models .

Another methodological challenge is the need for precise and controlled experimental conditions to study the effects of mechanical strain and fluid flow on cilia function. For instance, the role of mechanical strain in determining cilia length, motility, and planar position in the left-right organizer (LRO) requires sophisticated setups to apply and measure strain accurately (Chien et al., 2015; Chien et al., 2018), Similarly, studying cerebrospinal fluid (CSF) flow and its impact on body axis morphogenesis necessitates advanced fluid dynamics modeling and in vivo imaging techniques (Cantaut-Belarif et al., 2018; Zhang et al., 2018).

Despite significant progress, several critical questions remain unanswered in the field of axis development. One major question is the precise molecular mechanisms by which cilia-driven fluid flow translates into downstream signaling events that influence axis formation. While it is known that cilia-driven CSF flow can induce the expression of neuropeptides like urotensin, the exact pathways and intermediate steps involved in this process are not fully understood.

Future research should prioritize the development of advanced imaging and genetic tools to overcome current technical limitations. Enhancing the resolution and capabilities of live-cell imaging techniques will allow for more detailed studies of ciliary dynamics and their role in axis development, Creating more sophisticated genetic models, including conditional knockouts and tissue-specific manipulations, will help elucidate the functions of specific ciliary components, Additionally, further research should focus on the molecular pathways linking cilia-driven fluid flow to axis development and explore the role of mechanical forces in cilia function and axis patterning.

Interdisciplinary approaches combining developmental biology, biophysics, and computational modeling will provide a more comprehensive understanding of these fundamental processes and pave the way for potential therapeutic interventions for cilia-related disorders.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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