Review

Various facets of vertebrate cilia: motility, signaling, and role in adult neurogenesis

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Abstract: Cilia are microtubule-based cellular organelles that are widely distributed in vertebrate tissues. They were first observed hundreds of years ago. Recent studies indicate that this small organelle plays important roles in numerous physiological phenomena, including tissue morphogenesis, signal transduction, determination of left-right asymmetry during development, and adult neurogenesis. Ciliopathies, syndromes resulting from a genetic disorder of cilial components, frequently have complex effects involving many organ systems, owing to the broad distribution of cilia in the body.

Keywords: cilia, ciliopathy, morphogenesis

Introduction

Cilia are small, largely external, cellular organelles that are involved in diverse biological processes. They were first observed on ciliated protozoa over 300 years ago by Dutch microscope maker Antoni van Leeuwenhoek. These hair-like structures protrude from the membrane of ciliates, and their primary role is in cell locomotion. In mammals, these "motile" cilia are located on the cells lining the brain ventricles and tracheal duct, and the female reproductive system; they are also present on sperm cells and at the embryonic ventral node. Dysfunction of the motile cilia can cause hydrocephalus, chronic airway disease, male infertility, and *situs inversus* (whole-body inversion of asymmetrical structures).[1)] Although this organelle has a long history, the exciting and rapid progress in understanding its physiological roles, which have been emerging over the past decade, have led to a renewed focus on cilia and ciliary function. In this article, we describe the function and structure of cilia. We then focus on the role of subventricular zone (SVZ) ependymal cilia in the migration of newborn neurons in the adult mammalian brain.

Cilia structure and ciliopathies

The cilia of eukaryotic cells are hair-like structures that extend from the cell surface. They are composed of microtubules and are classified according to their microtubule components and motility into four groups (9+2 motile, 9+2 immotile, 9+0 motile, and 9+0 immotile).[1)]

The axoneme of 9+2 motile cilia is composed of nine peripheral microtubule doublets and two central single microtubules (central pair) (Fig. 1). It also contains dynein arms, and radial spokes, which are important for motility. The dynein arms, which are bound to the ciliary doublet, enable the microtubules in the axonemes to slide in an ATPase-dependent reaction, which generates ciliary beating.[2),3)] The peripheral doublets extend from the basal body of the cilium, across the transition zone(Fig. 1), and reach almost to the tip of the cilium. The basal body is a special structure derived from the centriole, and is constructed of nine microtubule triplets without central singlets. A protrusion called the basal foot,

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Abbreviations: SVZ: subventricular zone; CSF: cerebrospinal fluid; RMS: rostral migratory stream; OB: olfactory bulb; PKD: polycystic kidney disease; Hh: Hedgehog; PCD: primary cilia dyskinesia; IFT: intraflagellar transport; PCP: planar cell polarity; KS: Kartagener's syndrome.

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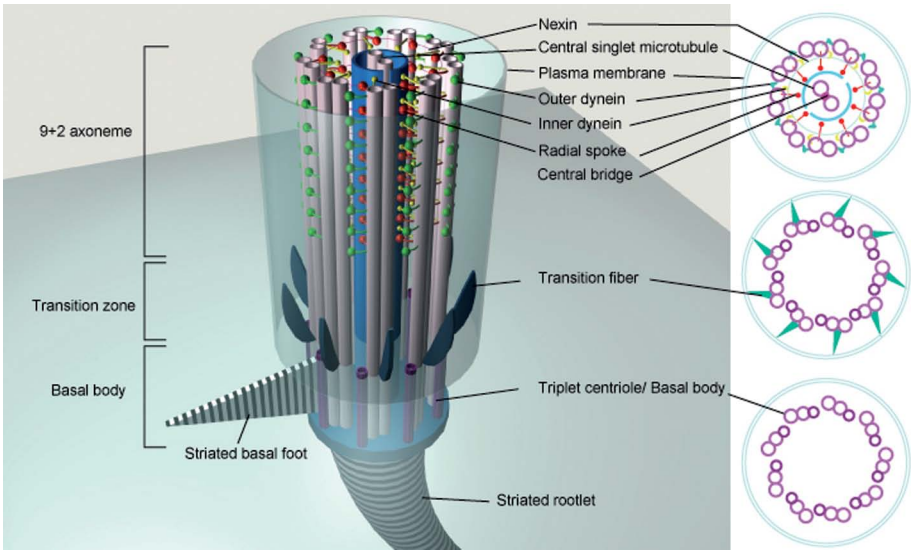


Fig. 1. Anatomy of the 9+2 motile cilium. The axoneme, an array of nine microtubule doublets and two central singlets, is the core structure of the 9+2 motile cilium. The transition zone is a structure connecting the axoneme and the basal body. It converts the 9×2 axonemal doublet microtubules into the 9×3 triplet structure of the basal body. The transition fibers extend from the distal part of the basal body to the plasma membrane. The basal foot is a process that extends laterally from the basal body and is oriented in a consistent direction in polarized ciliated cells. The striated rootlet is a conical banded structure that extends from the proximal end of the basal body to the cell nucleus.

which extends from the lateral side of the basal body, indicates the direction in which the polarized cilium will beat. Below the basal body, there is a fibrillary compartment extending to the cell nucleus, called the striated rootlet. The rootlet is not essential for ciliogenesis or the formation of basal bodies, but it is necessary for the long-term stability of the cilia on photoreceptors.[4)]

Cilia perform a variety of functions, both by sensing signals from their surroundings and, often, by creating fluid flows. In the embryonic ventral node, which is located at the most posterior portion of the notochordal plate, 9+0 motile monocilia, called nodal cilia, generate the fluid flow that is necessary for the formation of the left-right asymmetry of the body.[5)–7)] The nodal cilia also sense FGFs, which trigger the secretion of vesicles carrying Sonic hedgehog and retinoid acid, which play critical roles in left-right determination.[5)] Asymmetric cilia-dependent fluid flow is also found in Kupffer's vesicle, a likely equivalent of the node, which determines left-right asymmetry in the medaka fish and the zebrafish.[6),7)]

A single 9+0 immotile, or "primary," cilium exists in almost every quiescent cell in the body, and some of these cilia can sense signals such as fluid flow or molecular components in their surroundings.[8),9)] The renal epithelial monocilia mediate the sensation of shear stress to activate the intracellular Ca^{2+} channel.[9),10)] The subsequent increase in intracellular Ca^{2+} is thought to influence numerous subcellular activities that are required for tissue morphogenesis. A defect in the mechanosensory monocilia, as occurs in polycystic kidney disease (PKD), for example, usually leads to abnormal renal cell proliferation and cyst formation.

Vertebrate primary cilia also play an essential role in the transduction of the Hedgehog (Hh) signal, which controls growth, cell-fate decisions, and morphogenesis during development. Several Hh signaling components, including Smoothened and Gli2/3, associate physically with the primary cilium. A defect in the intraflagellar transport (IFT) in cilia causes the loss of the primary cilium and defective Hh signaling. Cilia are also suggested to be associated with hedgehog-associated signaling particles. These data suggest that key steps of the Hh signaling pathway may occur within the cilium (Fig. 2).[1),5),11)–13)] Recent studies also indicate that a protein located in the ciliary basal body, Inversin, acts as a molecular switch between the canonical and non-canonical Wnt path-

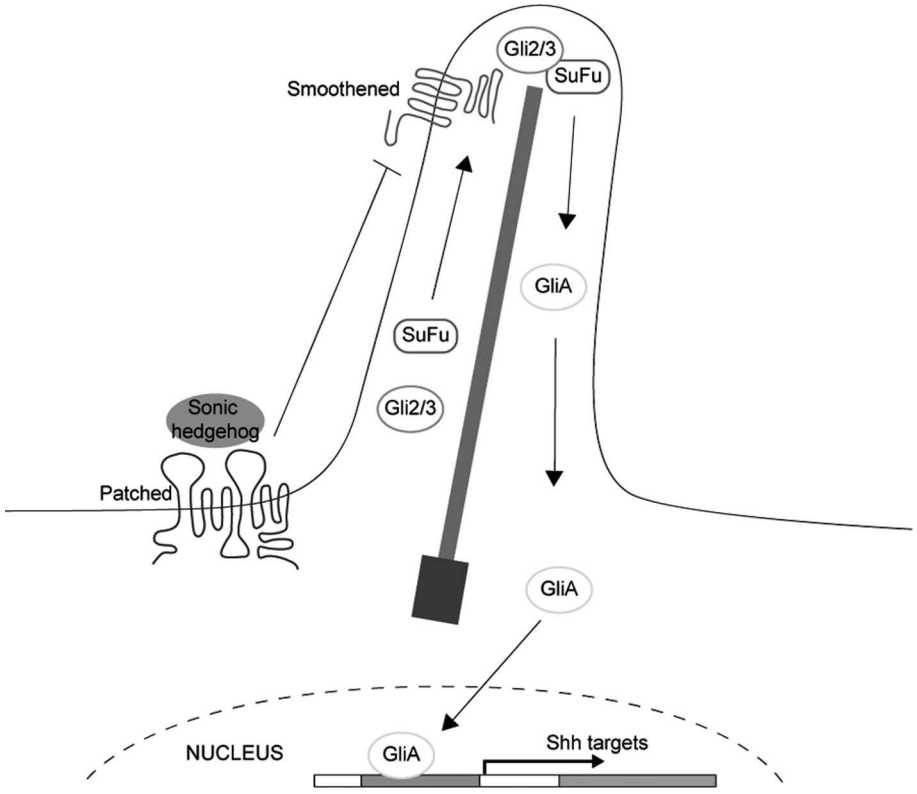


Fig. 2. Hedgehog signaling in primary cilia. The intraflagellar transport (IFT) machinery moves Hh proteins to their functional sites, and thus transduces the Hh signal. When the Hh ligand binds to its receptor Patched, the transcription factor Gli and suppressor of fused (SuFu) are transported by IFT to the cilium tip, where Smoothened interacts with SuFu and converts Gli to its active form (GliA). The GliA then enters the cell nucleus to turn on gene expression.

ways. Inversin interacts with cytosolic but not membrane-bound Dishevelled and promotes its degradation, thus inhibiting β-catenin signaling (Fig. 3).[14)]

Cilia are classified by their microtubule structure and motility: cilia composed of 9 peripheral doublets and 2 central singlets are "9+2 motile" or "9+2 immotile" cilia; those composed of 9 peripheral doublets without a central pair are "9+0 motile" (nodal cilia) and "9+0 immotile cilia" (other primary cilia) (Fig. 4). 9+2 immotile cilia are distributed in the hair cells of the inner ear and play important roles in auditory transduction.[15),16)] 9+2 motile ciliated cells overlie the surface of airways, oviducts, and brain ventricles, and transport mucus, the ovum, and cerebrospinal fluid (CSF), respectively. Although their function is still unclear, cilia with a 9+4 axoneme exist on the notochordal plate of the rabbit embryo.[17)] Since the cilia of the different groups share many common components, a defect in one structural protein usually leads to severe disorders (Table 1).

Defects in ciliary motility can cause ciliopathies. A defect in the directional beating of the SVZ ependymal cilia results in abnormal CSF flow and causes hydrocephalus in small animals such as mice. However, in humans the dysmotility of ependymal cilia, such as in Kartagener's syndrome (KS, also known as primary cilia dyskinesia, PCD) rarely causes hydrocephalus, but results in severe symptoms in the respiratory duct and inner ear.[18)–20)] The differences between humans and rodents are probably related to morphological differences, such as the diameter of the aqueduct. In human PCD patients, if hydrocephalus occurs, it is usually caused by congenital aqueduct closure (which occurs at a rate 83-fold higher than that of the general population).[2),21)–24)] The high incidence of congenital aqueduct closure indicates that a disturbance of ependymal flow due to dysmotile cilia may cause aqueduct stenosis, thus increasing the risk for hydrocephalus in humans.

The extracellular fluid flow generated by motile

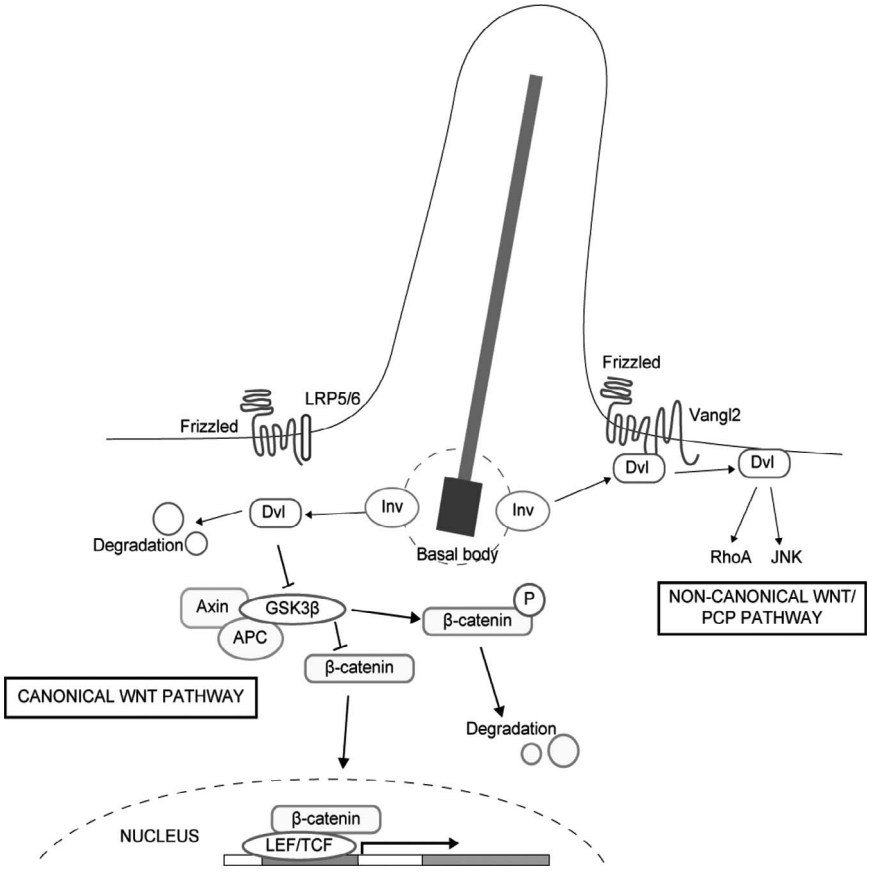


Fig. 3. Inversin functions as a switch between the canonical Wnt and non-canonical Wnt/PCP pathways. Inversin (Inv) is localized to the basal body, where it can interact with cytoplasmic but not membranous Dishevelled (Dvl) and promote its degradation. The degradation of cytoplasmic Dishevelled blocks transduction of the canonical Wnt pathway. Membrane-bound Dishevelled then switches on the non-canonical Wnt/PCP signaling.[14)]

cilia has important roles during embryonic development. In mammals, the 9+0 motile monocilia at the surface of the embryonic ventral node move to generate fluid flow. Unlike other motile cilia, such as ependymal cilia, which beat back and forth, the nodal cilia move with a tilted and clockwise rotation that generates a leftward extra-embryonic fluid flow (nodal flow), which is required for the establishment of the left-right asymmetry of the body (Fig. 4).[25)–29)] About 50% of KS patients develop *situs inversus totalis*, in which the organs are reversed in a ''mirror-image'' pattern from that of normal individuals, which is thought to result from the absence of nodal ciliary rotation and failure of the leftward fluid flow. These patients mainly suffer from chronic respiratory infections due to the impaired motility of their respiratory epithelial cilia,[18),30)] and from male infertility due to sperm immotility.

Ciliary polarity

The function of motile cilia requires that they beat coordinately in the same direction on each cell within a region of tissue. Within a tissue, most cells display several types of polarization. Some cell types with oriented motile cilia are polarized in a plane orthogonal to the epithelial apical-basal axis and generate directional fluid flow. In vertebrate embryos, monocilia are tilted posteriorly and generate a localized net leftward fluid flow over the surface of the ventral node.[28),29)] This polarity is generally referred to as planar cell polarity (PCP). The signaling pathway that regulates the establishment of PCP was originally identified in *Drosophila,* and is evolutionarily conserved in vertebrates.[31)] Most of the core PCP genes, including Frizzled, Strabismus (Stbm, also known as Vang), and Dishevelled, were

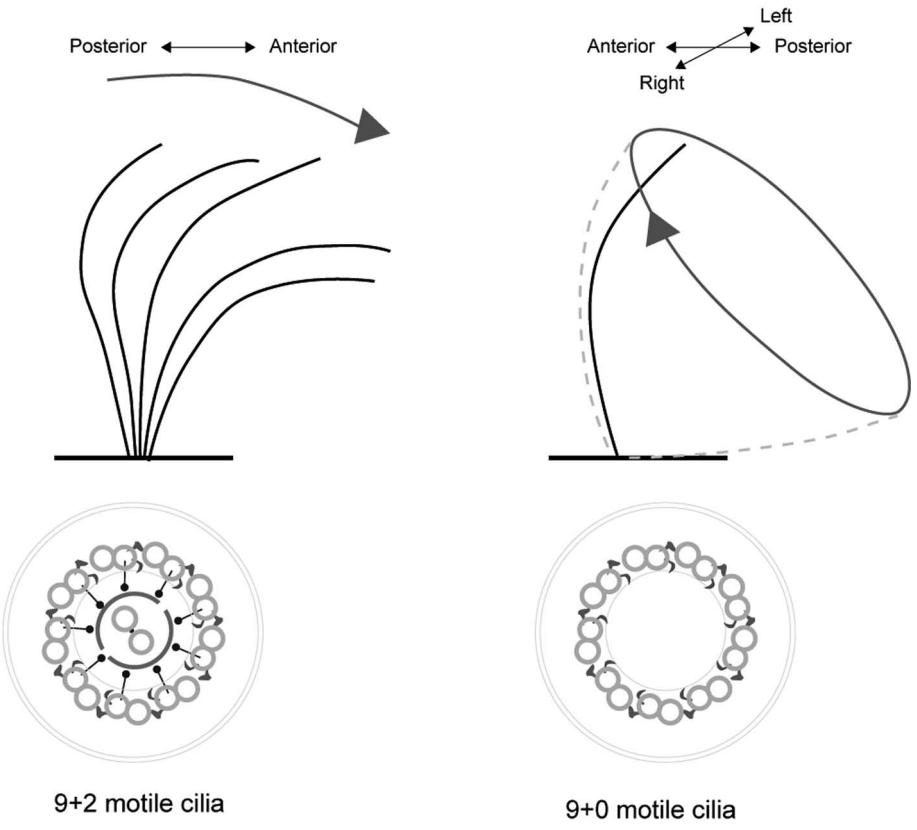


Fig. 4. Movement patterns of 9+2 motile cilia and 9+0 motile cilia. The 9+2 motile cilia are found in the lateral ventricle, respiratory ducts, female reproductive system, and sperm. 9+0 motile monocilia are located at the surface of the embryonic ventral node and rotate quickly to create a smooth leftward flow, which determines the left-right asymmetry of the animal body. The generation of the leftward nodal flow is dependent on the posterior tilt of the rotating cilia. Motile cilia beat coordinately along the anterior-posterior axis and generate a directional fluid flow, a phenomenon known as planar cell polarity. The sperm flagellum is a specialized 9+2 structure that gives sperm cells their motility.

discovered in genetic studies in *Drosophila*, in which PCP signals regulate the orientation of hairs on the wings and abdomen and the arrangement of ommatidia in the compound eyes.

Current models propose that in *Drosophila* epithelial tissues, neighboring cells communicate PCP signals through PCP proteins that are distributed asymmetrically between cells (Fig. 5A).[32),33)] PCP signaling, which is also called non-canonical Wnt signaling, is activated by the binding of the extracellular ligands Wnt11/5a to the Frizzled family membrane receptors. The intracellular PCP effector Dishevelled binds to Frizzled and/or Stbm and transduces signals through the RhoA and/or JNK signaling cascade to control tissue polarity. Dishevelled can also be bound by Prickle to antagonize the Frizzled/PCP signaling (Fig. 6).[34),35)] The seven-pass transmembrane protein Flamingo (Fmi) mediates the membrane localization of Stbm and Frizzled.[33)]

In mouse, mutants in the PCP protein Vangl2 (also known as Strabismus1) exhibit an open neural tube, defective convergent extension, and disorganized kinocilia in the organ of Corti (Fig. 5B).[36)–39)] Moreover, recent studies indicated that a mouse Frizzled orthologue, Fz6, controls hair patterning in mice (Fig. 5C). In the mouse embryonic epidermal basal layer and hair germ, the PCP proteins Vangl2 and an Fmi homologue, Celsr1, show anterior-posterior polarization, as reported in *Drosophila* models, and a defect in these proteins leads to the loss of the global asymmetry of hair follicles.[40),41)] These findings indicate that PCP genes play important roles during PCP establishment in both invertebrates and vertebrates. Although the relationship be-

Table 1. Congenital human ciliopathies[22),52),53)]

Disease	Gene	Cellular Function	Protein Location
Almström syndrome[54),55)]	ALMS1	Ciliogenesis	Basal body
Hypersecretory lungs			
Retinitis			
Obesity			
Diabetes mellitus			
Senior-Loken syndrome	NPHP1	Uncertain	Cilia, basal body
(type 1, 4, 5, 6)[56)–59)]	NPHP4		
Nephronophthisis	NPHP5/IQCB1		
Progressive eye disease	NPHP6/CEP290		
Polycystic kidney disease	PKD1	Mechanosensing	Cilia
(PKD)[60)]	PKD2		
Urinary tract infections	PKHD1	Unknown	Cilia, basal body
Liver and pancreatic cysts			
Primary cilia dyskinesia	DNAH5	Ciliary motility	Outer dynein arms
(PCD), also known as	DNAl1	Ciliary motility	Outer dynein arms
immotile cilia syndrome, or			
Kartagener's syndrome[61),62)]			
Bronchiectasis			
Chronic sinusitis			
Infertility			
Situs inversus			
Bardet-Biedl syndrome[63)–68)]	BBS1-12	Ciliogenesis	Basal body,
Obesity			IFT complex
Retinal degeneration			
Polycystic kidney			
Meckel-Gruber	Cep290	Unknown	Basal body
syndrome[69),70)]	MKS1	Ciliogenesis	Basal body
Brain malformation	MKS3	Ciliogenesis	Ciliary membrane
Polydactyly			
Polycystic kidney			
Oral-Facial-Digital	OFD1	Ciliogenesis	Basal body
syndrome[71)]			
Craniofacial abnormality			
Polydactyly			
Polycystic kidney			
Nephronophthisis	NPHP1-9	Uncertain	Axoneme, basal body
Retinitis pigmentosa[72)]	RPGR	Retinal transport	Basal body
Situs inversus[73)]	DNAH11	Ciliary motility	Dynein arms
Neural tube defect[74)]	VANGL1	Uncertain	Uncertain
Spinal dysraphisms			

tween the polarity of motile cilia and the PCP pathway is still unclear, one study showed that Vangl2 is localized to the basal part and transition zone of the motile cilia of airway epithelial cells,[38)] suggesting that Vangl2 might also influence the polarization of motile cilia. A recent study showed that, in the mucociliary epithelia of the *Xenopus laevis* embryo, Dishevelled signaling governs both the apical docking and the planar polarization of the basal bodies during the ciliogenesis of motile cilia.[42)]

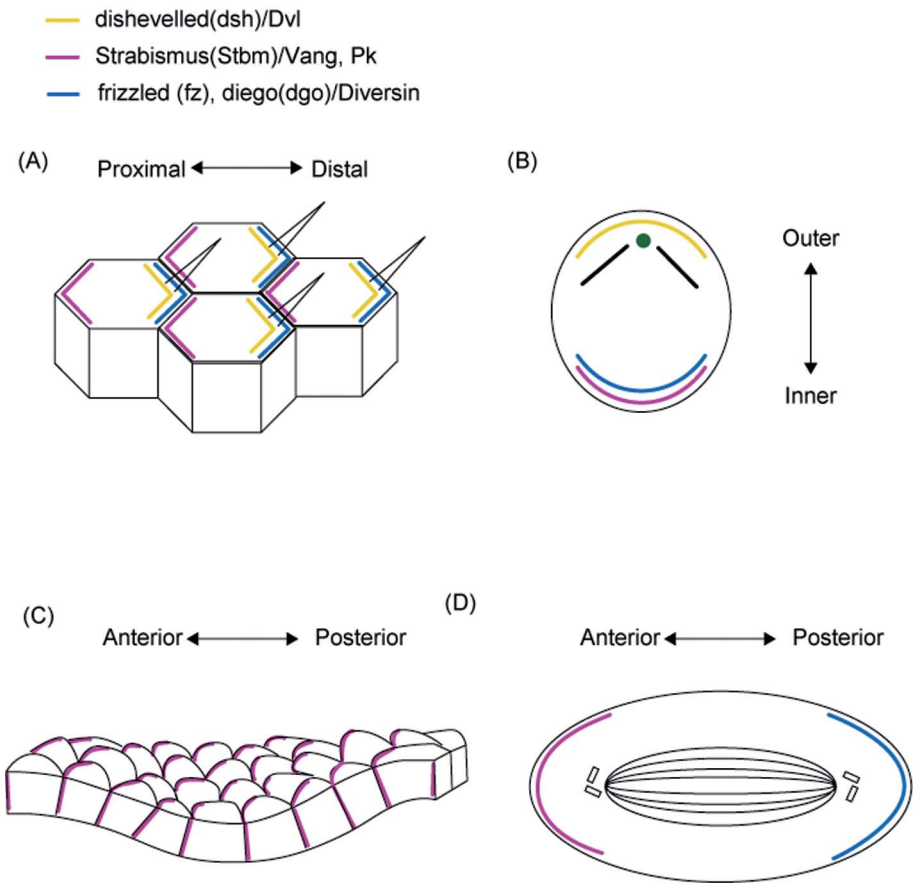


Fig. 5. Distribution of the core PCP factors in *Drosophila melanogaster* and vertebrate cells. (A) Schematic of the core PCP (planar cell polarity) protein localization in *Drosophila melanogaster* wing cells (A) and mouse sensory hair cells in the cochlea (B). Vangl2 is polarized along the anterior-posterior (A-P) axis in the epidermal basal layer of embryonic mouse skin (C). The asymmetric PCP protein distribution orients the *D. melanogaster* sensory organ precursor (SOP) cell division (D). Colored lines indicate the location of core PCP proteins. Yellow: Dishevelled; purple: Vang/Vangl2 and Prickle; blue: Frizzled and Diego/Diversin.

Moreover, 9+2 motile cilia not only cause fluid flow, but may also sense signals from the flow (e.g., shear stress) to determine their orientation.[43),44)] Recent studies showed that ultrastructural defects in the central pair apparatus of motile cilia cause disturbances in both ciliary beating and polarization.[18),21),45)] Similarly, in PCD patients, a deficiency of the central pair of 9+2 motile cilia results in the impairment of cilia motility and the loss of ciliary orientation.[18)] These observations suggest that motile cilia might sense the extracellular fluid flow during ciliogenesis.

Neurogenesis in the subventricular zone

Until recently, it was believed that new neurons could not be generated in the adult mammalian brain. However, recent studies have shown that in restricted areas, new neurons are born throughout adulthood. The first evidence of postnatal neurogenesis in a rodent brain was obtained in 1965, and by the 1990's it had become generally accepted that adult neurogenesis indeed occurs in mammals, including humans.[46),47)] Neural stem cells are found in the dentate gyrus of the hippocampus and in the subventricular zone (SVZ) of the lateral ventricles, where they divide to self-renew and to generate new neurons throughout life.

Neurogenesis occurs continuously in the SVZ of postnatal rodent brains. The slowly dividing neural stem cells (type B cells) self-replicate and differentiate into transit-amplifying cells (type C cells) (Fig. 7B). The type C cells proliferate rapidly to give rise

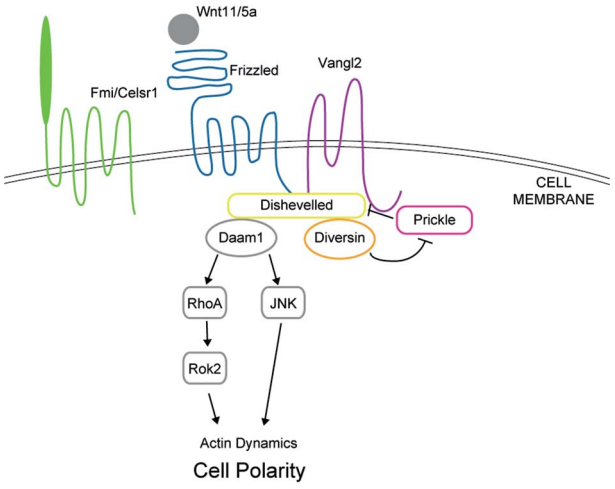


Fig. 6. The planar cell polarity pathway (PCP pathway, non-canonical pathway) cascade. The extracellular ligands (Wnt11/5a, etc.) bind to the membrane protein/receptor (Frizzled, Vangl2, Celsr1), then activate the PCP pathway and influence the dynamics of the actin cytoskeleton, thus controlling cell polarity. Core PCP proteins (in color): Frizzled, Vangl2, Fmi/Celsr1, Diversin, Dishevelled, and Prickle. PCP effectors (gray): Daam1, RhoA, Rok2, and JNK.

to neuroblasts (type A cells).[47] The newborn neurons in the SVZ attach to one another and form elongated cell aggregates called "chains," in which cells migrate tangentially. These neuroblasts then enter a highly restricted route termed the "rostral migratory stream" (RMS) and migrate ~8 mm (in mice) anteriorly toward the olfactory bulb (OB). Once the chain of migrating neuroblasts reaches the OB, the cells detach and begin to migrate radially to either the deep granular layer or the superficial periglomerular layer of the bulb, and differentiate into GABA-containing and dopaminergic local circuit interneurons (Fig. 7A).[48] The regulatory mechanisms underlying such long-distance, directional, and high-speed (about 100 μm/hr) neuronal migration in the mature brain are still unclear.

We recently showed that the migrational direction of newborn neurons corresponds to the direction of CSF flow generated by the SVZ ependymal cilia.[49] It is possible that the CSF flow affects the distribution of various proteins, and directs the formation of anterior-posterior concentration gradients in the SVZ, which are important for the control of neuroblast migration. A secreted protein, Slit, is a chemorepulsive molecule that plays an important role in directing the extension and branching of developing sensory axons by regulating the cytoskeletal distribution of the growth cone, via its binding of the Robo receptor. In mutant mice with defective cilia, the concentration gradient of Slit is disturbed, and neuroblast migration occurs in an irregular direction. These results suggest that the CSF flow generated by the polarized ependymal cilia is important for neuronal migration in the adult brain.

Although the ependymal cilia beat coordinately and generate directional fluid flow in adult mammalian brain ventricles, it is still unclear how this polarity is determined. Recent studies indicate that both PCP signaling and fluid flow play important roles in the ciliary polarity of the developing *Xenopus larval* skin.[42),44] These mechanisms may also be involved in the coordinated ciliary beating of SVZ ependymal cells. Ependymal cells are transformed from radial glial cells at the late embryonic stage and generate motile cilia postnatally.[50] Since CSF is produced by the choroid plexus, circulated in the lateral ventricles, and absorbed in the anterior part of ventricles, there is fluid flow in the lateral ventricle during the period of differentiation of ependymal cells. These findings raise the possibility that CSF flow is involved in the polarization of the ependymal cilia during development.

Conclusion and discussion

The motile cilia/flagella are complicated cellular organelles that link various physiological functions—even some that precede conception—from the motility of sperm to respiratory tract clearance. In addition, our studies have suggested that the coordinated beating of ependymal cilia plays important roles in neuronal migration and adult neurogenesis. A recent study indicated that the ependymal cells retain their radial glial potential in the adult forebrain, and they can respond to ischemia by giving rise to neurons.[50),51]

Clarification of the molecular mechanisms of the polarization of cilia may help us not only to understand the mechanisms of ciliary development, but also to develop new therapeutic strategies for treating ciliary disorders. Furthermore, elucidating the involvement of ciliary functions in adult neurogenesis and neuronal migration may lead to new ideas for regeneration therapies for the injured adult brain.

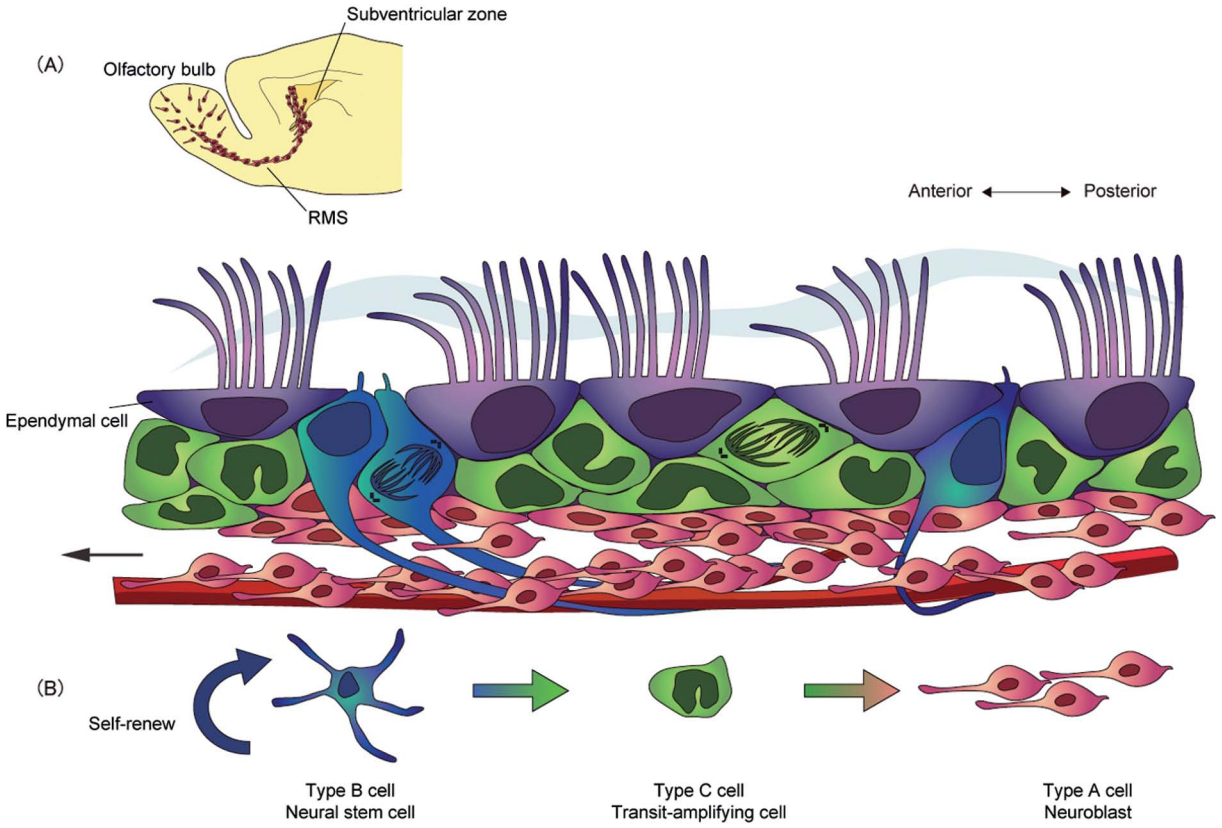


Fig. 7. Neuronal migration in the adult rodent brain. (A) Neurogenesis occurs continuously in the postnatal rodent brain. In the subventricular zone (SVZ), neural stem cells (type B cells, blue) generate migratory neuroblasts (type A cells, red) via highly proliferative transit-amplifying cells (type C cells, green). The ciliated ependymal cells (type E cells, purple) line the surface of the ventricle. Neuroblasts migrate along a restricted pathway, the rostral migratory stream (RMS), toward the olfactory bulb (OB). Once the chain of migrating neuroblasts reaches the OB, the cells detach and disperse to either the deep granular layer or the superficial periglomerular layer, and differentiate into interneurons. The cerebrospinal fluid (CSF) flow, created by the beating of polarized ependymal cilia, and the concentration gradient of Slit protein are necessary to orient neuroblast migration. (B) The lineage of SVZ cells.

Acknowledgments

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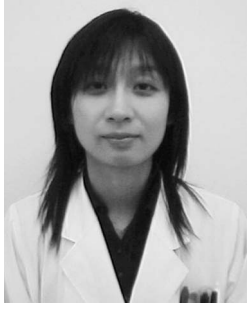
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