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Opinion

Cilia as Wnt signaling organelles

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Cilia and Wnt signaling have a complex relationship, wherein Wnt regulates cilia and, conversely, cilia may affect Wnt signaling. Recently, it was shown that Wnt receptors are present in flagella, primary cilia, and multicilia, where they transmit an intraciliary signal that is independent of β-catenin. Intraciliary Wnt signaling promotes ciliogenesis, affecting male fertility, adipogenesis, and mucociliary clearance. Wnt also stimulates the beating of motile cilia, highlighting that these nanomotors, too, are chemosensory. Intraciliary Wnt signaling employs a Wnt–protein phosphatase 1 (PP1) signaling axis, involving the canonical Wnt pathway's inhibition of glycogen synthase kinase 3 (GSK3) to repress PP1 activity. Collectively, these findings support that cilia are Wnt signaling organelles, with implications for ciliopathies and cancer.

Highlights

Cilia are Wnt signaling organelles. The ciliary membrane harbors Wnt receptors that transmit a signal across the axonemal membrane. The intraciliary Wnt signaling cascade employs the upstream cascade of canonical Wnt pathway to inhibit glycogen synthase kinase 3.

Intraciliary Wnt signaling is β-catenin independent and instead engages a conserved Wnt–protein phosphatase 1 axis.

Intraciliary Wnt signaling stimulates ciliogenesis and ciliary beating. It promotes maturation of sperm flagella, primary cilium formation in mouse preadipocytes, and mucociliary membrane function in *Xenopus* embryonic epidermis.

Diverse cilium classes (primary cilia, motile cilia, flagella) feature intraciliary Wnt signaling but not all ciliated cell types do, indicating context dependence.

Wnt signaling and cilia

Cilia are membrane-bound, microtubule-based organelles that play crucial roles in the generation of movement [motile cilia, **flagella** (see Glossary)] and transduction in cell signaling pathways (**primary cilia**). Dysfunction in cilium formation or function gives rise to a group of syndromes referred to as ciliopathies [1]. Primary cilia are prominent signaling hubs for growth factors including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), Notch, and Hedgehog (Hh) [2–4]. Missing from this impressive list was, until recently, one growth factor family of paramount importance, Wnt, which plays a pivotal role in regulating numerous processes during development and disease, notably in embryonic and adult stem cells [5,6]. Recent studies indicate that not only are primary cilia **Wnt signaling** organelles but this extends to motile cilia as well, including sperm flagella and ciliary bundles of mucociliary membranes (**intraciliary Wnt signaling**) [7–9].

This opinion article focuses on intraciliary Wnt signaling and its role in the regulation of cilium biogenesis and function. It only touches on the connections of Wnt pathways with various ciliary structures such as the **basal body**, transition zone (TZ), and cilium pocket, among others. We refer the reader interested in these and related topics concerning the controversial role of cilia in the regulation of Wnt signaling [3,10,11] and their interaction with Wnt–planar cell polarity (PCP) (noncanonical) signaling [12] to these reviews.

Cilia as signaling organelles

Motile cilia and primary cilia are the two basic classes of cilia and they are found on the surfaces of many cell types in various organisms, even unicellular. Primary cilia are solitary and extend from the surface of most mammalian cells. They serve as sensory organelles, detecting extracellular signals and relaying them to the cell's interior. Motile cilia are typically multiciliary bundles and exert a coordinated beating motion that propels fluids and particles along surfaces (**multicilia**). **Multiciliated cells (MCCs)** are found in the respiratory tract and reproductive organs. They help to move mucus and other substances, enabling mucociliary clearance of debris and pathogens in the respiratory tract and uterine egg transportation during reproduction. Sperm flagella are a specialized type of motile cilia that have evolved to propel sperm cells through fluid

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environments, such as the female reproductive tract, to reach and fertilize eggs. Dysfunction in cilium formation or function gives rise to a group of syndromes called ciliopathies [1]. This group of genetic disorders can manifest in a wide range of symptoms and affect multiple organ systems (e.g., kidney cysts, vision and hearing impairment, respiratory illnesses).

Ciliogenesis, the growth and development of cilia, depends on a dynamic equilibrium of ciliary assembly and disassembly (ciliogenesis). Nucleating ciliogenesis are centrioles, which give rise to basal bodies that attach to the cortex and prompt the extension of the cilium. Cilium assembly involves the entry of cytoplasmic or membrane proteins from the non-ciliary plasma membrane into the cilium via the cilium base, and the selective import of ciliary proteins through the TZ. The TZ acts as a diffusion barrier and gatekeeper, allowing proteins to enter and exit the cilium to maintain the specific composition of proteins and lipids, including of the ciliary membrane. While molecules under 40 kDa freely diffuse through the TZ, translocation of larger molecules into cilia involves specific mechanisms, including lipid acetylation, late endosomal pathway targeting, and directed transport facilitated by **ciliary targeting sequences (CTSs)** [13].

Within the cilium, the intraflagellar transport (IFT) machinery involving IFT-A, IFT-B, and BBSome protein complexes facilitates the movement of proteins along the ciliary axoneme [14–16]. The IFT machinery operates from the TZ to the cilium tip using a kinesin motor complex for anterograde transport and a dynein motor complex for retrograde transport [17,18]. When proteins fail to return from the cilium tip, they can be shed in the form of extracellular vesicles called ciliary ectosomes [18,19]. Cilia typically assemble during the G1 or G0 phase of the cell cycle and disappear around mitosis, by a continual balance between assembly and disassembly that controls cilium length.

Primary cilia play crucial roles in the transmission of growth factor signaling pathways. The ciliary membrane harbors transmembrane receptors that enable the cell to respond to external stimuli and growth factors including Hh, PDGF, EGF, IGF, and Notch. These cues are transmitted to regulatory proteins at the basal body, the TZ, and distal regions of the primary cilium, which in turn control intracellular signaling cascades [2–4]. Unlike these chemosensory antennae, motile cilia are generally considered signaling-inert nanomotors. However, there are precedents for motile cilia bearing signaling receptors (e.g., human airway epithelial and kidney cells) and exhibiting responses to environmental cues and morphogens like Hh, suggesting they may also function as signaling organelles [20,21].

Wnt/GSK3 signaling

The Wnt signaling pathway is a complex network of signaling molecules and receptors that plays critical roles in development, tissue homeostasis, and disease (Wnt signaling) [5,6]. The pathway is traditionally divided into two main branches: the canonical Wnt/β-catenin pathway and the noncanonical Wnt/PCP pathway [6,12]. However, the Wnt/β-catenin cascade is more aptly classified as part of a more general Wnt/GSK3 signaling pathway, which has multiple branches, Wnt/β-catenin being one of them (Figure 1).

Wnt/β-catenin signaling (a.k.a. the canonical Wnt pathway), is the best-characterized Wnt cascade. In the absence of Wnt ligands, cytoplasmic β-catenin is targeted for degradation by a complex called the destruction complex, which comprises Axin, adenomatous polyposis coli (APC), GSK3, and casein kinase 1α (CK1α) [22]. This complex phosphorylates β-catenin, marking it for proteasomal degradation [22]. When Wnt ligands bind to and form a ternary complex with Frizzled (FZD) and low-density lipoprotein receptor-related protein 5/6 (LRP5/6) receptors, LRP6 becomes phosphorylated by CK1γ and GSK3 and recruits Dishevelled (DVL) and Axin scaffold proteins to form (likely phase separated) signalosomes [23–25]. A key result of signalosome

Glossary

Basal bodies: centriole-like structures in the cytoplasm of ciliated cells that form the base of each cilium.
Ciliary beat frequency (CBF): the frequency at which cilia or flagella undergo rhythmic, coordinated beating or movement. CBF is a crucial parameter that influences the function of cilia in various physiological processes, particularly in the movement of fluids and particles along surfaces lined with ciliated cells. CBF is measured in beats per second (Hz).
Ciliary targeting sequence (CTS): a specific amino acid motif found in proteins that are destined for localization to cilia. The CTS serves as a signal directing the transport and proper positioning of proteins >40 kDa within the ciliary compartment.
Ciliogenesis: the dynamic and reversible process whereby cilia are formed and developed, involving their assembly, elongation, and maturation.
Flagella: whip-like appendages found on the surface of some cells that are involved in locomotion. These structures are primarily responsible for the movement of cells such as bacteria, protozoa, and spermatozoa through fluid environments.
Intraciliary Wnt signaling: Wnt signaling occurring within the cilium, transmitted by Wnt receptors (LRP6, Fz) located in the ciliary membrane and by intraciliary GSK3 inhibition (Figure 2).
Multicilia: comprise motile ciliary bundles that line surfaces of cells where they generate fluid flow through coordinated beating (i.e., in the respiratory or reproductive tract).
Multiciliated cells (MCCs): specialized cells characterized by the presence of multiple motile cilia on their surface. MCC ciliary bundles beat in a coordinated manner. These cells play essential roles in moving fluids across epithelial surfaces.
Primary cilia: sensory cell appendages of somatic cells that transduce molecular signals from the environment.
Wnt signaling: intercelluar signaling by Wnt glycoproteins involves highly conserved signaling pathways that proceed by a variety of cellular downstream cascades that regulate key events in embryogenesis, adult homeostasis, and disease.

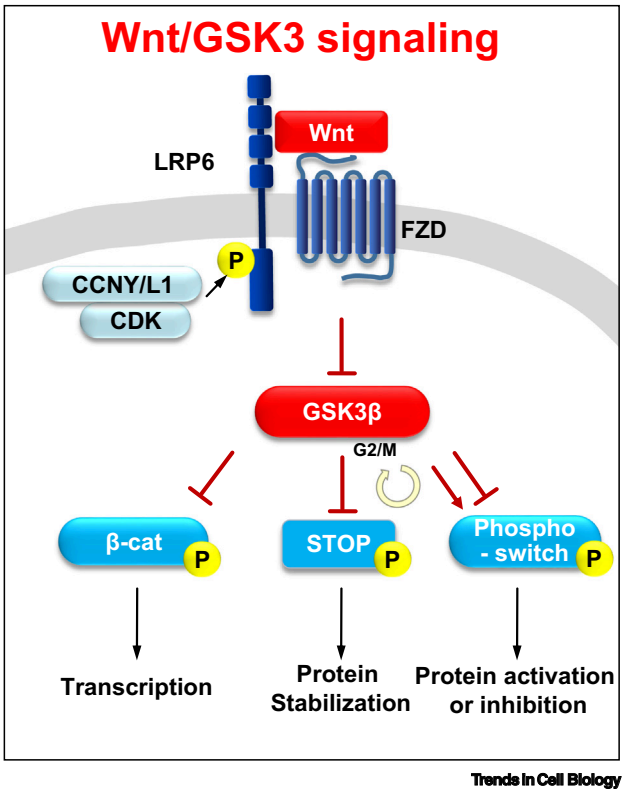

Figure 1. Wnt/glycogen synthase kinase 3 (GSK3) signaling. Wnt ligand binding and the consequent inhibition of GSK3 activity can result in: (i) stabilization of β-catenin and enhanced transcription; (ii) stabilization of Wnt/stabilization of proteins (STOP) target proteins; or (iii) dephosphorylation-triggered activation or inhibition of GSK3 target protein activity [e.g., TSC2, septin 4, protein phosphatase 1 regulatory inhibitor subunit 2 (PPP1R2)] (see main text). Abbreviations: CCNY/L1, cyclin Y-like 1; CDK, cyclin-dependent kinase; FZD, Frizzled; LRP6, low-density lipoprotein receptor-related protein 6.

formation is the inhibition of GSK3, which derepresses β-catenin, allowing it to accumulate, translocate to the nucleus, and activate target gene transcription [24–27]. The Wnt/PCP pathway also transduces through FZD but employs ROR1,2 receptors instead of LRP5/6 [28]. As the name implies, Wnt/PCP signaling regulates processes related to cell and tissue polarity as well as morphogenesis, and it is linked to cilia [12]. Among Wnt/β-catenin target genes is a master regulator of ciliogenesis, the transcription factor *Foxj1* [29]. There are multiple links between cilia and Wnt/β-catenin signaling (Box 1).

Box 1. Wnt/β-catenin signaling and cilia

There are multiple relationships between cilia and Wnt/β-catenin signaling. First, primary cilia may exert an inhibitory influence on Wnt/β-catenin signaling, although this is disputed [3,10,11]. Conversely, ciliary proteins, in particular the IFT-A complex, are required for canonical Wnt/β-catenin signaling, while the cilium as a structure seems not [12]. Second, several signal-transducing proteins in the Wnt pathway, including DVL, FZD, Inversin, and GSK3, localize to the cilium [46–51]. Third, Wnt/β-catenin signaling stimulates motile cilium formation transcriptionally. In mouse tracheal cells and *Xenopus* embryos, for example, it triggers the differentiation of multiciliated cells, thereby influencing motile cilium formation indirectly through changes in cell fate [71,72]. In a more direct mode, Wnt signaling induces ciliogenesis directly through Foxj1, a transcriptional master regulator, as observed in zebrafish Kupffer's vesicle and ependymal layer, as well as *Xenopus* epidermis [73–77].

For ciliogenesis of primary cilia, the role of Wnt/β-catenin signaling is less clear. WNT3A induces primary cilium formation in RPE1 and breast cancer cells [78], but in mouse neocortex and NIH3T3 cells hyperactivation of Wnt/β-catenin signaling due to impaired APC function inhibits, rather than promotes, primary cilium formation [48,79]. Moreover, neither WNT3A treatment nor pharmacological Wnt inhibition affects primary cilium formation in NIH3T3, HEK293, and RPE1 cells [64]. Similarly, in HEK293 cells, deletion of DVL1/2/3 or LRP5/6 has little effect on ciliogenesis [64]. While the requirement for Wnt/β-catenin signaling in ciliogenesis may vary between different cell types, the reasons behind the discrepancies in RPE1 cells [64,78] are unclear. Taking these findings together, transcriptional Wnt/β-catenin signaling exerts a positive influence mostly on motile ciliogenesis.

Historically, only β-catenin was considered a relevant GSK3 target in Wnt signaling. However, the Wnt/GSK3 cascade regulates the phosphorylation of many proteins, protecting them from GSK3 phosphorylation and proteasomal degradation in a Wnt-dependent manner [30]. This has been termed the Wnt–stabilization of proteins (Wnt/STOP) pathway and it peaks during the G2/M phase of the cell cycle [31]. Key effectors of this mitotic Wnt/STOP signaling are cyclin Y (CCNY) and CCNY-like 1 (CCNYL1), conserved cyclins that, together with their cyclin-dependent kinases (CDKs) 14 and 16, phosphorylate and activate the Wnt co-receptor LRP6. Since CCNY/CCNYL1 are mitotic cyclins, their activity promotes a surge of Wnt signaling and GSK3 inhibition during the G2/M phase of the cell cycle [32]. Hence, in dividing cells, Wnt/STOP signaling is most active during mitosis, when cells are transcriptionally silent. Mitotic Wnt/STOP signaling stabilizes proteins during cell division, promoting cell growth and cell cycle progression of daughter cells [31]. Concordantly, several Wnt components functionally associate with centrosomes, kinetochores, and the spindle during mitosis [33–35]. CCNY/CCNYL1 depletion selectively inhibits Wnt/STOP signaling while leaving Wnt/β-catenin signaling intact in several cell types [36,37], although in mammary gland progenitor conditional *Ccny/l1* mutants, β-catenin overexpression rescues certain abnormalities [38].

The Wnt/STOP pathway is involved in germ cell regulation [7,36], neurogenesis [37], chromosome segregation [39,40], and cancer cell proliferation [41–44]. However, Wnt/GSK3 signaling not only prevents the degradation of GSK3 substrates as in Wnt/STOP, it can also modulate their activity (Figure 1) [22]. For example, in a Wnt-dependent phospho-switch, GSK3 phosphorylates TSC2 to regulate the mTOR pathway [45]. As detailed below, it is these β-catenin-independent Wnt/GSK3 functions that occur prominently in intraciliary Wnt signaling.

Intraciliary Wnt/GSK3 signaling regulates biogenesis and activity of cilia

Multiple Wnt effectors, including DVL, FZD, GSK3, components of the destruction complex, and β-catenin, associate with primary cilium catenin [46–51]. Notably, DVL1,3, FZD2, and GSK3 are found in the ciliary axoneme, suggesting the presence of intraciliary Wnt signaling [49–51]. Three studies have now demonstrated that sperm flagella, motile multicilia, and primary cilia all exhibit intraciliary Wnt signaling [7–9].

Intraciliary Wnt signaling in sperm flagella

The first direct evidence for intraciliary Wnt signaling came through work on the maturation of mouse spermatozoa, highly specialized cells that are propelled by a unique motile cilium: the flagellum. In mammals, testicular spermatozoa acquire their motility and fertilization competence only during a week-long maturation process when they pass through and receive maturation signals from the epididymis. At this stage, spermatozoa already contain a highly condensed nucleus and their response is purely post-transcriptional and post-translational (i.e., involving only the modification of existing proteins). The initial observation was that mouse mutants for the Lrp6 kinase regulator *Ccnyl1* are male sterile, with immotile and malformed spermatozoa due to failure of sperm maturation [7]. Analysis showed that Wnt ligands released from the epididymis activate Lrp6 in the spermatozoan membrane that is phospho-primed by Ccnyl1 to inhibit GSK3β. The inhibition of GSK3β then promotes spermatozoa maturation in three different ways. First, it engages Wnt/STOP signaling to globally reduce protein degradation. Second, GSK3β inhibition prevents septin 4 phosphorylation, which is necessary to establish a membrane diffusion barrier in the sperm tail. Third, GSK3 inhibition inhibits PP1 (Figure 2), and this Wnt–PP1 signaling axis is also employed in other cilia types (see below). PP1 is not the direct GSK3 target; instead, PP1 regulatory inhibitor subunit 2 (PPP1R2) is an inhibitor of PP1 that becomes activated by GSK3 phosphorylation. PP1 plays a critical role in inhibiting mammalian sperm motility by dephosphorylating certain proteins involved in the motility process [52]. Blocking PP1 is akin to lifting a brake

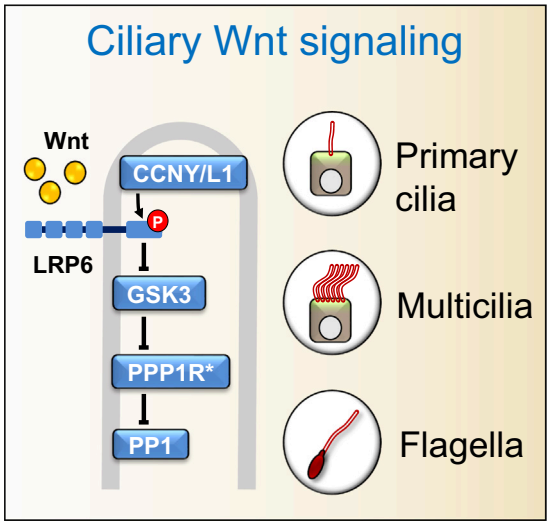

Figure 2. A conserved ciliary Wnt–protein phosphatase 1 (PP1) signaling axis. Primary cilia [9], multicilia [11], and flagella [10] share a distinct ciliary Wnt signaling axis. The PP1 regulatory subunit may differ between primary (PPP1R2) and motile (PPP1R11) multicilia. The role of other Wnt pathway components commonly acting upstream of glycogen synthase kinase 3 (GSK3) [e.g., Frizzled (FZD) receptors, Dishevelled (DVL), Axin, adenomatous polyposis coli (APC), cyclin-dependent kinase 14–16 (CDK14–16)] in ciliary Wnt–PP1 signaling is less well characterized and they are omitted for clarity. Abbreviations: CCNY/L1, cyclin Y-like 1; LRP6, low-density lipoprotein receptor-related protein 6.

on sperm motility, and this effect can be mimicked artificially by using PP1 inhibitors like okadaic acid (OA). OA induces the motility of spermatozoa, emphasizing the possibility of manipulating sperm movement by interfering with PP1 activity. Thus, post-transcriptional Wnt/GSK3β signaling orchestrates a multipronged response that mediates flagellum maturation. Supporting these findings, the Ccnyl1 partner kinase Cdk16 [53] is also essential for sperm morphogenesis [54]. Of note, it appears counterintuitive that flagellum maturation in postmitotic spermatozoa depends on the mitotic cyclin Ccnyl1. However, there is mounting evidence that quiescent cells redeploy cyclins and CDKs to control motile ciliogenesis [54–57], possibly reflecting commonalities in the regulation of centrioles during ciliogenesis and the cell cycle [58]. In conclusion, Wnt signaling directly promotes the maturation of a highly specialized motile cilium, the flagellum, and it does so via post-transcriptional Wnt/GSK3β signaling.

Intraciliary Wnt signaling in primary cilia

Ccnyl1 and its homolog *Ccny* have overlapping roles in development [38]. Given the role of *Ccnyl1* in sperm and flagellum maturation, we sought ciliary defects in mice double mutant for *Ccnyl1* and its close homolog *Ccny* (collectively *Ccny/l1*) [8]. Mouse *Ccny/l1* double mutants are embryonic lethal and display forebrain abnormalities, including exencephaly [37,38]. Neural tube closure defects such as exencephaly are frequently associated with primary cilium abnormalities and *Ccny/l1* embryos show reduced cilium numbers, not only in forebrain progenitors but also in kidney proximal tubules [8]. Primary cilia are also required to promote the differentiation of adipocyte progenitor cells into multiple adipose tissue types via intraciliary IGF-1 receptor and FFAR4/GPR120 signaling [59]. Concordantly, reduced adipose tissue development observed in single-mutant $Ccny^{-/-}$ mice relates to ciliogenesis defects of adipocyte progenitors [8].

Both *in vivo* and in HEK293T cells, LRP6 receptors and CCNYl1 and CCNY are found in the axoneme of primary cilia. HEK293T double mutants of either *CCNY/L1* or *LRP5/6* show reduced ciliogenesis, which is partially rescued by pharmacological inhibition of GSK3β but not by overexpression of constitutively active β-catenin. LRP6 contains a CTS with an AQ motif [60] in its extracellular domain, mutation of which impairs its ciliary localization. CTS mutation also abrogates the ability of LRP6 to rescue ciliogenesis defects in *LRP6* mutants, without compromising its ability to transduce Wnt/β-catenin signaling. Thus, intraciliary and non-intraciliary Wnt/LRP6/GSK3β

signaling are functionally separable. Looking downstream, Wnt engages the same GSK3β–PPP1R2–PP1 signaling axis in primary cilia as it does in sperm flagella (Figure 2). Ppp1r2 locates to cilia in the forebrain and kidney and its GSK3-dependent phosphorylation is increased in *Ccny/l1* mutant mice. Moreover, PPP1R2 depletion induces ciliogenesis defects while pharmacological inhibition of PP1 rescues ciliogenesis defects in *CCNY/L1* mutant cells. Collectively, these results indicate that primary cilia are Wnt signaling organelles that transduce a distinct Wnt–PP1 response that promotes ciliogenesis.

Intraciliary Wnt signaling in MCCs

Widely considered signaling-inert nanomotors, motile multicilia in human airway epithelia actually express chemosensory bitter receptors. These receptors localize in the axoneme and on exposure to bitter compounds trigger a Ca^{2+} influx, thereby stimulating the **ciliary beat frequency (CBF)** [61]. Moreover, the Hh signaling cascade is active in motile cilia, where a noncanonical signaling cascade is activated that reduces intracellular cAMP levels and slows ciliary beating [21]. So, if a Wnt–PP1 cascade functions in flagella and primary cilia, what about motile cilia in MCCs?

To investigate the possibility of intraciliary Wnt signaling in motile cilia, we analyzed the mucociliary epidermis of *Xenopus tropicalis* embryos, a widely used model akin to human airway epithelia [62]. The *Xenopus* mucociliary epidermis develops after neurulation, with postmitotic MCCs and multiciliary bundles beating in a polarized direction to generate fluid flow from the head to the tail of the embryo that removes pathogens and aids oxygenation. In MCCs, Ccny and Lrp6 reside in the axoneme, and knockdown of *ccny/l1* or *lrp6* reduces cilium numbers and impairs their morphology [9]. This effect on ciliogenesis is independent of either cell fate change or *β-catenin*. Instead, again, the Wnt–PP1 signaling axis operates in MCCs to promote ciliogenesis (Figure 2), but with Ppp1r11 in place of Ppp1r2 as the inhibitory PP1 regulatory subunit. Like sperm flagella and primary cilia, MCC cilia are Wnt signaling organelles. (i) They carry the Lrp6 receptor in the axoneme, which becomes phosphorylated on Wnt treatment. (ii) Unlike wild-type Lrp6, a CTS mutant Lrp6 fails to rescue ciliogenesis defects in Lrp6 knockdown embryos while it transmits normal Wnt/β-catenin signaling. (iii) A GFP-based ciliary GSK3 biosensor responds within 3 min to Wnt stimulation, showing axonemal puncta.

Different from sperm flagella and primary cilia in the mouse, *Xenopus* MCCs employ Ppp1r11 in place of Ppp1r2 as the inhibitory PP1 regulatory subunit and Gsk3b phospho-target. There are ~200 PP1Rs in the human genome that regulate substrate specificity, allosteric regulation, and subcellular compartmentalization of the large PP1 catalytic subunit (PP1C)[i]. Ppp1r2 and Ppp1r11 belong to the large group of inhibitory PPP1R proteins. Most PPP1Rs have not been thoroughly investigated and their physiological roles are unclear. We note that yet another PP1 inhibitor, Ppp1r35, is a centrosomal protein that is required for the formation of mouse nodal cilia [63]. This suggests that PPP1Rs may be a hotspot for GSK3-controlled ciliogenesis and that different members confer cell- and tissue specificity as well as target different ciliary PP1 substrates.

Wnt signaling not only regulates MCC ciliogenesis but Wnt treatment also enhances the CBF, in both in *Xenopus* embryos and human airway epithelia. Moreover, Wnt treatment improves ciliary function in *Xenopus* knockdown embryos for genes homologous to human ciliopathy models (*ccdc108*, male infertility; *gas2l2*, primary ciliary dyskinesia). Thus, intraciliary Wnt signaling is required for motile ciliogenesis, increases CBF, and improves ciliopathy-related CBF defects. The findings also imply that motile cilia may possess much richer chemosensory abilities than is commonly believed.

Concluding remarks

Studies in sperm flagella, primary cilia, and MCCs of mucocilia converge on the key conclusion that cilia are Wnt-signaling organelles that employ a conserved Wnt–PP1 axis to promote ciliogenesis. Thus, Wnts join a number of other growth factors that transmit a signal within cilia, notably Hh. Importantly, intraciliary Wnt signaling does not operate in all ciliated cells (e.g., LRP5/6 deficiency does not lead to ciliary defects in RPE1 cells or HEK293 cells), indicating context dependence [64]. Even in cells that do employ intraciliary Wnt signaling, the requirement is not absolute since Wnt receptor deficiency only reduces, and does not abolish, ciliogenesis. Since at the heart of the intraciliary Wnt–PP1 axis is GSK3 inhibition, it is conceivable that other signals known to decommission GSK3 also regulate ciliogenesis (e.g., growth factors that transduce through PI3K/AKT). Localization of GSK3 to cilia and its ability to inhibit ciliogenesis is evolutionarily conserved down to *Chlamydomonas*, a green alga that has no Wnts [65–69] but where pharmacological inhibition of GSK3 rapidly induces a long cilium phenotype [65]. Wnt/GSK3 signaling regulates not only the activity of PP1 but also the stability of proteins (Wnt/STOP) and, extrapolating from the situation in flagella, probably also modulates the assembly of ciliary scaffold proteins such as septins. Thus, GSK3 inhibition orchestrates a ciliogenesis program and enhances the beating of motile cilia. Intraciliary Wnt/GSK3 signaling is part of a multipronged Wnt-driven ciliogenesis program since Wnts also promote motile cilium formation via transcriptional β-catenin signaling.

Box 2. A practical guide to intraciliary Wnt signaling

Studying intraciliary Wnt signaling and distinguishing it from canonical Wnt signaling is challenging due to their shared upstream components. To implicate intraciliary Wnt signaling in a cellular response of interest (ROI) and distinguish it from β-catenin signaling, the following criteria can be used.

Detection of phospho-LRP6 in the cilium

Detection of phospho-LRP6 (i.e., Wnt activated) in the ciliary axoneme using commercially available antibodies is indicative of intraciliary Wnt signaling. However, due to the low abundance of LRP6, this may require LRP6 overexpression.

Okadaic acid treatment

To promote ciliogenesis, intraciliary Wnt signaling employs a Wnt–PP1 signaling axis where PP1 is inhibited (Figure 2). PP1 can be pharmacologically inhibited by, for example, OA treatment. Thus, if a ROI is also induced by OA or, better still, if inhibition of the ROI by a Wnt signaling inhibitor (e.g., DKK1, FRZB) can be rescued by OA, it is an indication for intraciliary Wnt signaling.

β-Catenin independence

Canonical Wnt signaling involves β-catenin nuclear translocation. If a cellular response requires LRP6 and GSK3 inhibition but lacks β-catenin involvement (e.g., tested by siRNA treatment), it suggests Wnt/STOP or intraciliary Wnt signaling. Conversely, if the ROI is sensitive to CCNY or CCNYL1 deficiency, it suggests Wnt/STOP or intraciliary Wnt signaling. Wnt/β-catenin signaling generally shows low sensitivity to CCNY/CCNYL1 deficiency.

Monitoring a ciliary GSK3 biosensor

A ciliary biosensor is available [30], which features GSK3 phospho-degrons in a fusion protein between the ciliary transmembrane protein Arl13b and GFP. In the absence of Wnt, GFP fluorescence is low due to its proteasomal degradation. On intraciliary Wnt signaling, GSK3 is inhibited and the biosensor is stabilized, leading to fluorescence increase. Sensitivity issues currently limit this approach to MCCs, while detection in primary cilia is challenging.

LRP6 CTS mutant rescue

LRP6 is the common co-receptor in canonical Wnt signaling, Wnt/STOP, and intraciliary Wnt signaling. The use of a LRP6 mutated in its CTS is a powerful way to invoke intraciliary Wnt signaling in a ROI.

- Begin by testing for LRP6 dependency using the commercially available LRP6 antagonist DKK1.
- In case of DKK1 sensitivity, confirm using available LRP6-deficient cells or generate a LRP6-deficient cell line by genome editing.
- Test whether the LRP6-deficient cell line also shows impaired ROI. If the ROI is rescued by provision of wild-type but not by CTS mutant LRP6, this indicates intraciliary Wnt signaling.

Outstanding questions

What are the molecular mechanisms underlying intraciliary Wnt signaling; that is, what are the ciliary Wnt/GSK3 target proteins that modulate ciliary biogenesis and beating?

Is there crosstalk between Wnt and other ciliary signaling cascades (e.g., Hh, receptor tyrosine kinase receptor pathways)?

Is there an interplay between ciliary and either Wnt/β-catenin or Wnt/PCP signaling?

Does ciliary Wnt signaling regulate cellular functions beyond cilia?

Which Wnt ligands trigger motile ciliary Wnt signaling in mucociliary membranes? What is the role of the periciliary liquid layer in preventing ligands from diffusing away into the extracellular milieu?

Which organs exhibit ciliary Wnt signaling and what physiological roles does it play in them?

Does Wnt–PP1 signaling offer possibilities for therapeutic interventions in ciliopathy, infertility, or cancer?

What are key future research topics (see Outstanding questions)? At the cellular level, it will be important to define the full spectrum of Wnt/GSK3 downstream targets and functions in the cilium as well as the crosstalk with other ciliary signaling pathways such as Hh. At the organismic level, we are only beginning to understand the physiological role of intraciliary Wnt signaling. The challenge will be to deconvolute ciliary from canonical Wnt signaling. Separation-of-function mutants, such as *LRP6* CTS mutants, will be precious in this context (Box 2). With regard to future translational opportunities, the central role of GSK3 and PP1 inhibition in intraciliary Wnt signaling raises the possibility of modulating their enzymatic activity for interventions in ciliopathies and respiratory disease. Moreover, several cilium-mediated signaling pathways are oncogenic, raising the question of whether some oncogenic Wnt signaling proceeds through cilia. Along those lines, loss of cilia enhances protumorigenic WNT/β-catenin signaling, and is itself sufficient to drive metastatic melanoma [70]. The emerging conceptual framework of intraciliary Wnt signaling will guide these endeavors.

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Declaration of interests

F.D.S. is a current employee of Octapharma Biopharmaceuticals. The other authors have no interests to declare.

Resource

[i]www.genenames.org/data/genegroup/#!/group/694

References

1. Hildebrandt, F. *et al.* (2011) Ciliopathies. *N. Engl. J. Med.* 364, 1533–1543
2. Hilgendorf, K.I. *et al.* (2016) The primary cilium as a cellular receiver: organizing ciliary GPCR signaling. *Curr. Opin. Cell Biol.* 39, 84–92
3. Anvarian, Z. *et al.* (2019) Cellular signalling by primary cilia in development, organ function and disease. *Nat. Rev. Nephrol.* 15, 199–219
4. Nachury, M.V. and Mick, D.U. (2019) Establishing and regulating the composition of cilia for signal transduction. *Nat. Rev. Mol. Cell Biol.* 20, 389–405
5. Gordon, M.D. and Nusse, R. (2006) Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. *J. Biol. Chem.* 281, 22429–22433
6. Niehrs, C. (2012) The complex world of WNT receptor signalling. *Nat. Rev. Mol. Cell Biol.* 13, 767–779
7. Koch, S. *et al.* (2015) Post-transcriptional Wnt signaling governs epididymal sperm maturation. *Cell* 163, 1225–1236
8. Zhang, K. *et al.* (2023) Primary cilia are WNT-transducing organelles whose biogenesis is controlled by a WNT–PP1 axis. *Dev. Cell* 58, 139–154.e8
9. Seidl, C. *et al.* (2023) Mucociliary Wnt signaling promotes cilia biogenesis and beating. *Nat. Commun.* 14, 1259
10. Oh, E.C. and Katsanis, N. (2013) Context-dependent regulation of Wnt signaling through the primary cilium. *J. Am. Soc. Nephrol.* 24, 10–18
11. Lee, K.H. (2020) Involvement of Wnt signaling in primary cilia assembly and disassembly. *FEBS J.* 287, 5027–5038
12. Vuong, L.T. and Mlodzik, M. (2023) The complex relationship of Wnt-signaling pathways and cilia. *Curr. Top. Dev. Biol.* 155, 95–125
13. Long, H. and Huang, K. (2019) Transport of ciliary membrane proteins. *Front. Cell Dev. Biol.* 7, 381
14. Jordan, M.A. and Pigino, G. (2021) The structural basis of intraflagellar transport at a glance. *J. Cell Sci.* 134, jcs247163
15. Lechtreck, K.F. (2015) IFT–cargo interactions and protein transport in cilia. *Trends Biochem. Sci.* 40, 765–778
16. Taschner, M. and Lorentzen, E. (2016) The intraflagellar transport machinery. *Cold Spring Harb. Perspect. Biol.* 8, a028092
17. Klena, N. and Pigino, G. (2022) Structural biology of cilia and intraflagellar transport. *Annu. Rev. Cell Dev. Biol.* 38, 103–123
18. Nager, A.R. *et al.* (2017) An actin network dispatches ciliary GPCRs into extracellular vesicles to modulate signaling. *Cell* 168, 252–263
19. Volz, A.K. *et al.* (2021) Bardet–Biedl syndrome proteins modulate the release of bioactive extracellular vesicles. *Nat. Commun.* 12, 5671
20. Jain, R. *et al.* (2012) Sensory functions of motile cilia and implication for bronchiectasis. *Front. Biosci. (Schol. Ed.)* 4, 1088–1098
21. Mao, S. *et al.* (2018) Motile cilia of human airway epithelia contain Hedgehog signaling components that mediate noncanonical hedgehog signaling. *Proc. Natl. Acad. Sci. U. S. A.* 115, 1370–1375
22. Li, C. *et al.* (2023) When you come to a fork in the road, take it: Wnt signaling activates multiple pathways through the APC/Axin/GSK-3 complex. *Cells* 12, 2256
23. Bilic, J. *et al.* (2007) Wnt induces LRP6 signalosomes and promotes Dishevelled-dependent LRP6 phosphorylation. *Science* 316, 1619–1622
24. Davidson, G. *et al.* (2005) Casein kinase 1 gamma couples Wnt receptor activation to cytoplasmic signal transduction. *Nature* 438, 867–872
25. Niehrs, C. and Shen, J. (2010) Regulation of Lrp6 phosphorylation. *Cell. Mol. Life Sci.* 67, 2551–2562
26. Kikuchi, A. *et al.* (2009) Selective activation mechanisms of Wnt signaling pathways. *Trends Cell Biol.* 19, 119–129
27. Zeng, X. *et al.* (2005) A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. *Nature* 438, 873–877
28. Humphries, A.C. and Mlodzik, M. (2018) From instruction to output: Wnt/PCP signaling in development and cancer. *Curr. Opin. Cell Biol.* 51, 110–116
29. Yu, X. *et al.* (2008) Foxj1 transcription factors are master regulators of the motile ciliogenic program. *Nat. Genet.* 40, 1445–1453
30. Taelman, V.F. *et al.* (2010) Wnt signaling requires sequestration of glycogen synthase kinase 3 inside multivesicular endosomes. *Cell* 143, 1136–1148

31. Acebron, S.P. *et al.* (2014) Mitotic Wnt signaling promotes protein stabilization and regulates cell size. *Mol. Cell* 54, 663–674
32. Davidson, G. *et al.* (2009) Cell cycle control of Wnt receptor activation. *Dev. Cell* 17, 788–799
33. Kikuchi, K. *et al.* (2010) Dishevelled, a Wnt signalling component, is involved in mitotic progression in cooperation with Plk1. *EMBO J.* 29, 3470–3483
34. Hadjihannas, M.V. *et al.* (2006) Aberrant Wnt/β-catenin signaling can induce chromosomal instability in colon cancer. *Proc. Natl. Acad. Sci. U. S. A.* 103, 10747–10752
35. Fumoto, K. *et al.* (2006) GSK-3β-regulated interaction of BICD with dynein is involved in microtubule anchorage at centrosome. *EMBO J.* 25, 5670–5682
36. Huang, Y.L. *et al.* (2015) Maternal Wnt/STOP signaling promotes cell division during early *Xenopus* embryogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 112, 5732–5737
37. Da Silva, F. *et al.* (2021) Mitotic WNT signalling orchestrates neurogenesis in the developing neocortex. *EMBO J.* 40, e108041
38. Zeng, L. *et al.* (2016) Essential roles of cyclin Y-like 1 and cyclin Y in dividing Wnt-responsive mammary stem/progenitor cells. *PLoS Genet.* 12, e1006055
39. Stolz, A. *et al.* (2015) Wnt-mediated protein stabilization ensures proper mitotic microtubule assembly and chromosome segregation. *EMBO Rep.* 16, 490–499
40. Bufe, A. *et al.* (2021) Wnt signaling recruits KIF2A to the spindle to ensure chromosome congression and alignment during mitosis. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2108145118
41. Hinze, L. *et al.* (2019) Synthetic lethality of Wnt pathway activation and asparaginase in drug-resistant acute leukemias. *Cancer Cell* 35, 664–676
42. Madan, B. *et al.* (2018) Temporal dynamics of Wnt-dependent transcriptome reveal an oncogenic Wnt/MYC/ribosome axis. *J. Clin. Invest.* 128, 5620–5633
43. Loxha, L. *et al.* (2023) GSK3α regulates temporally dynamic changes in ribosomal proteins upon amino acid starvation in cancer cells. *Int. J. Mol. Sci.* 24, 13260
44. Demagny, H. and De Robertis, E.M. (2016) Point mutations in the tumor suppressor Smad4/DPC4 enhance its phosphorylation by GSK3 and reversibly inactivate TGF-β signaling. *Mol. Cell. Oncol.* 3, e1025181
45. Inoki, K. *et al.* (2006) TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell* 126, 955–968
46. Gupta, G.D. *et al.* (2015) A dynamic protein interaction landscape of the human centrosome–cilium interface. *Cell* 163, 1484–1499
47. Lancaster, M.A. *et al.* (2011) Subcellular spatial regulation of canonical Wnt signalling at the primary cilium. *Nat. Cell Biol.* 13, 700–707
48. Nakagawa, N. *et al.* (2017) APC sets the Wnt tone necessary for cerebral cortical progenitor development. *Genes Dev.* 31, 1679–1692
49. Stypulkowski, E. *et al.* (2021) Rab8 attenuates Wnt signaling and is required for mesenchymal differentiation into adipocytes. *J. Biol. Chem.* 296, 100488
50. Thoma, C.R. *et al.* (2007) pVHL and GSK3β are components of a primary cilium-maintenance signalling network. *Nat. Cell Biol.* 9, 588–595
51. Veland, I.R. *et al.* (2013) Inversin/Nephrocystin-2 is required for fibroblast polarity and directional cell migration. *PLoS One* 8, e60193
52. Vijayaraghavan, S. *et al.* (1996) Sperm motility development in the epididymis is associated with decreased glycogen synthase kinase-3 and protein phosphatase 1 activity. *Biol. Reprod.* 54, 709–718
53. Zi, Z. *et al.* (2015) CCNYL1, but not CCNY, cooperates with CDK16 to regulate spermatogenesis in mouse. *PLoS Genet.* 11, e1005485
54. Mikolcevic, P. *et al.* (2012) Cyclin-dependent kinase 16/PCTAIRE kinase 1 is activated by cyclin Y and is essential for spermatogenesis. *Mol. Cell. Biol.* 32, 868–879
55. Vladar, E.K. *et al.* (2018) Cyclin-dependent kinase control of motile ciliogenesis. *Elife* 7, e36375
56. Al Jord, A. *et al.* (2017) Calibrated mitotic oscillator drives motile ciliogenesis. *Science* 358, 803–806
57. Tam, L.W. *et al.* (2007) A CDK-related kinase regulates the length and assembly of flagella in *Chlamydomonas*. *J. Cell Biol.* 176, 819–829
58. Al Jord, A. *et al.* (2019) Motile ciliogenesis and the mitotic prism. *Biol. Cell.* 111, 199–212
59. Scamfer, S.R. *et al.* (2022) Ciliary control of adipocyte progenitor cell fate regulates energy storage. *Front. Cell Dev. Biol.* 10, 1083372
60. Berbari, N.F. *et al.* (2008) Identification of ciliary localization sequences within the third intracellular loop of G protein-coupled receptors. *Mol. Biol. Cell* 19, 1540–1547
61. Shah, A.S. *et al.* (2009) Motile cilia of human airway epithelia are chemosensory. *Science* 325, 1131–1134
62. Walentek, P. (2021) *Xenopus* epidermal and endodermal epithelia as models for mucociliary epithelial evolution, disease, and metaplasia. *Genesis* 59, e23406
63. Archambault, D. *et al.* (2020) Protein phosphatase 1 regulatory subunit 35 is required for ciliogenesis, notochord morphogenesis, and cell-cycle progression during murine development. *Dev. Biol.* 465, 1–10
64. Bernatik, O. *et al.* (2021) Primary cilia formation does not rely on WNT/β-catenin signaling. *Front. Cell Dev. Biol.* 9, 623753
65. Wilson, N.F. and Lefebvre, P.A. (2004) Regulation of flagellar assembly by glycogen synthase kinase 3 in *Chlamydomonas reinhardtii*. *Eukaryot. Cell* 3, 1307–1319
66. Ou, Y. *et al.* (2012) Targeting of CRMP-2 to the primary cilium is modulated by GSK-3β. *PLoS One* 7, e48773
67. Yuan, S. *et al.* (2012) Target-of-rapamycin complex 1 (Torc1) signaling modulates cilia size and function through protein synthesis regulation. *Proc. Natl. Acad. Sci. U. S. A.* 109, 2021–2026
68. Nakakura, T. *et al.* (2015) The elongation of primary cilia via the acetylation of α-tubulin by the treatment with lithium chloride in human fibroblast KD cells. *Med. Mol. Morphol.* 48, 44–53
69. Thompson, C.L. *et al.* (2016) Lithium chloride modulates chondrocyte primary cilia and inhibits Hedgehog signaling. *FASEB J.* 30, 716–726
70. Zingg, D. *et al.* (2018) EZH2-mediated primary cilium deconstruction drives metastatic melanoma formation. *Cancer Cell* 34, 69–84
71. Brechbuhl, H.M. *et al.* (2011) β-Catenin dosage is a critical determinant of tracheal basal cell fate determination. *Am. J. Pathol.* 179, 367–379
72. Huang, Y.L. and Niehrs, C. (2014) Polarized Wnt signaling regulates ectodermal cell fate in *Xenopus*. *Dev. Cell* 29, 250–257
73. Caron, A. *et al.* (2012) Wnt/β-catenin signaling directly regulates Foxj1 expression and ciliogenesis in zebrafish Kupffer's vesicle. *Development* 139, 514–524
74. Walentek, P. *et al.* (2015) ATP4a is required for development and function of the *Xenopus* mucociliary epidermis – a potential model to study proton pump inhibitor-associated pneumonia. *Dev. Biol.* 408, 292–304
75. Walentek, P. *et al.* (2012) ATP4a is required for Wnt-dependent Foxj1 expression and leftward flow in *Xenopus* left-right development. *Cell Rep.* 1, 516–527
76. Walentek, P. *et al.* (2015) ATP4 and ciliation in the neuroectoderm and endoderm of *Xenopus* embryos and tadpoles. *Data Brief* 4, 22–31
77. Zhang, J. *et al.* (2020) Wnt–PLC–IP_3–connexin–Ca^{2+} axis maintains ependymal motile cilia in zebrafish spinal cord. *Nat. Commun.* 11, 1860
78. Kyun, M.L. *et al.* (2020) Wnt3a stimulation promotes primary ciliogenesis through β-catenin phosphorylation-induced reorganization of centriolar satellites. *Cell Rep.* 30, 1447–1462
79. Song, L. *et al.* (2014) N-terminal truncation mutations of adenomatous polyposis coli are associated with primary cilia defects. *Int. J. Biochem. Cell Biol.* 55, 79–86