**Abstract**

From the onset of zebrafish development both canonical and noncanonical Wnt signaling play major roles in a diverse array of developmental processes such as patterning, gastrulation, and neurulation making both pathways unarguably prime requests for normal development to occur. In this review, we will focus on their association with cilia and, in particular, how they regulate ciliogenesis and consequently how cilia may regulate them.

**Introduction**

Cilia are microtubule-based tail-like projections extending approximately 5–10 μm outward from the cell. Cilia are anchored to the cell via the cytoplasmic basal body, which acts as an interface in between. Typically, cilia are made up of nine microtubule doublets surrounding a central pair of single microtubules that altogether form a bundle called the axoneme (Fig. 1A). Proteins necessary for ciliogenesis must be transported up the cilium via a process known as intraflagella transport driven by kinesin motors that shuttle up and down the cilium. Generally, cilia exist in two flavors, a motile form whose primary function is to move various substances over and around the cell and an immotile form that serves as a sensory mechanism. The motile forms generate their movement via the sliding of their microtubule doublets. The mechanical constraints between the microtubule doublets, provided by dynein linkage arms and radial spokes, convert the sliding motion of the tubules into ciliary movement. The immotile form, on the other hand, serves as a mechanosensory organ that translates deflections of the cilia, caused for example by fluid shear, intracellularly. The earliest example of this process revealed that inducing cilia deflection in MDCK cells causes a release of intracellular calcium. The noncanonical Wnt pathway has been implicated both in ciliogenesis and in transmitting signals initiated from the cilia; however, its involvement in both these processes is radically different. In this review, we will discuss how Wnt signaling regulates various aspects of cilia along with the ciliary-associated proteins that have been shown to interact with it. We will also highlight a potential mechanism by which cilia can in turn regulate Wnt signaling.

**Ciliary-Associated Proteins**

To date, a number of ciliary-associated proteins have been linked to both canonical and noncanonical Wnt signaling; before we discuss these interactions, it is necessary to briefly introduce them and their locale. Mutations in Bardet-Biedl syndrome (BBS) genes give rise to a variety of ciliopathic symptoms, including mental retardation, renal failure, and obesity. Three of these genes, bbs1, 4, and mkks (bbs6), are all associated with the ciliary basal body (Fig. 1B). Another basal body–associated protein, Ofd1, is responsible for the X-linked disorder oral-facial-digital type I syndrome, and mutations in this gene lead to prenatal death in males, while females present a variety of symptoms such as polycystic kidney disease and brain malformations. Inturned and fuzzy were first described in Drosophila as being components of PCP signaling, similar to the vertebrate noncanonical Wnt pathway. Further, in *Xenopus* Inturned has been shown to localize, along with Dishevelled, in the vicinity of the ciliary basal body (Fig. 1B). The zebrafish gene *Duboraya* (*dub*), which was identified in a screen for zebrafish mutants with defective left/right patterning and is expressed in the tail of early embryos in a region where Kupffer’s vesicle resides (the prime coordinator of left/right asymmetry), has been implicated in the establishment of laterality and resides in the cytoplasm of ciliated cells where it is required to organize apical actin (Fig. 1B). While on the other hand, *inversin*, one of the genes mutated in the disease nephronophthisis, associates with β-tubulin-4, a constituent of the ciliary axoneme (Fig. 1B). The zebrafish homolog of the mammalian *lcc6* gene, *seahorse*, is widely expressed in ciliated tissues including Kupffer’s vesicle; however, it is not localized to the basal body or the cilia, and instead appears as cytoplasmic puncta (Fig. 1B).

**Noncanonical–Canonical Interactions**

Before addressing noncanonical Wnt’s involvement with cilia, we must first briefly look at the interactions that exist between canonical and noncanonical Wnt signaling as both ciliogenesis and cilia signal transduction highlight the cross...
talk that occurs between these two pathways. Previous findings have established that Dishevelled regulates both these pathways via distinct domains within its protein architecture.\textsuperscript{11} Dishevelled consists of three separate domains, DIX, PDZ, and DEP, and these dictate which of the Wnt pathways will be regulated. The PDZ domain appears to be the main switch between the two pathways and can bind a variety of different proteins, and depending on what is bound dictates which pathway is regulated. For canonical Wnt signaling, the DIX domain is crucial.\textsuperscript{12,13} It binds to Axin, thus inhibiting its involvement in targeting \(\beta\)-catenin for degradation.\textsuperscript{14,15} As such, \(\beta\)-catenin levels increase, and it is subsequently trafficked to the nucleus, thus activating canonical Wnt target genes (Fig. 1C).

The noncanonical Wnt pathway utilizes the DEP domain of Dishevelled, which binds to and is involved in the activation of the small GTPase Rac.\textsuperscript{16} Further, it would also appear that the DEP domain is necessary for the translocation of Dishevelled to the plasma membrane following Frizzled activation.\textsuperscript{12} Mutant dishevelled RNA, which encodes protein lacking the DEP domain (Dvl-DEP), has proved an invaluable tool to researchers, allowing them to inhibit noncanonical signaling while leaving the canonical pathway unaffected. This has uncovered numerous roles for dishevelled/noncanonical Wnt signaling in a variety of developmental processes such as gastrulation, organogenesis, and neurulation.\textsuperscript{16–18}

Similarly, diversin, a close relative of the Drosophila planar cell polarity component diego, can also serve to regulate both pathways. As we have already mentioned, canonical Wnt signaling relies on the stability of the downstream effector \(\beta\)-catenin, which is regulated by a multiprotein degradation complex. Diversin negatively regulates this pathway by recruiting Casein kinase1e to the complex and thus promoting the degradation of \(\beta\)-catenin. In zebrafish, the canonical Wnt pathway is required very early in development for the formation of the organizer and embryonic axis. Consequently, overexpression of diversin leads to ventralization of embryos and a loss of goosecoid gene expression in the organizer. Morpholino-mediated knockdown on the other hand has the opposite effect, resulting in strongly dorsalized embryos and an expansion of goosecoid expression.\textsuperscript{19} As far as noncanonical Wnt signaling is concerned, diversin acts as a potentiator. Diversin directly interacts with Dishevelled via binding of the former’s ankyrin repeat domain to the latter’s DEP domain. Injection of diversin RNA, which encodes a protein lacking the ankyrin repeat domain, produces cardia bifida, a similar phenotype to that induced by Dvl-DEP expression. Further, wild-type diversin RNA is also able to rescue \textit{wnt11}5 morphant

\begin{figure}
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\caption{(A) Cilia structure. Cilia consist of the axoneme that is anchored to the cell via the basal body. The axoneme is comprised of nine microtubule doublets surrounding a central pair of single microtubules. The constraints between the dynein arms, radial spokes, and microtubules convert the sliding motion of the microtubules into ciliary movement. (B) Ciliogenesis. Dishevelled (Dvl) is involved in regulating the actin cytoskeleton necessary for ciliogenesis. Inturned (Int) colocalizes with Dvl at the basal body where they ensure correct basal body docking. Duboraya (Dub) is also involved in regulating the actin cytoskeleton and is phosphorylated in a frizzled (Fz)-dependent manner. BBS proteins are also involved in ciliogenesis and are localized to the basal body. Similarly, OFD1 (OFD) also associates with the ciliary basal body, while the zebrafish lrrc6 homolog Seahorse (Sea) appears as cytoplasmic puncta. Inversin (Inv) associates with the ciliary axoneme. (C) Cilia signaling. In undeflected cilia the canonical Wnt pathway is activated as Dvl binds to axin (Ax), inhibiting its involvement with \(\beta\)-catenin (\(\beta\text{Cat}\)) degradation. Consequently, \(\beta\text{Cat}\) levels increase, leading to activation of canonical Wnt target genes. Upon deflection, inversin (Inv) expression increases. This leads to the cytoplasmic breakdown of Dvl and shuts down the canonical Wnt pathway. Simultaneously, Inv also permits the build up of Dvl at the plasma membrane where it can form a complex with trilobite (Tri) and prickle (Pk) leading to activation of the noncanonical Wnt pathway.}
\end{figure}
embryos, again indicating that it is downstream of Wnt in the noncanonical pathway.20

**Ciliogenesis**

For normal ciliogenesis to occur, there needs to be extensive rearrangements of the actin cytoskeleton, a process that can be regulated by the noncanonical Wnt pathway. To date, several proteins involved in ciliogenesis have now been linked to the noncanonical Wnt pathway, including BBS proteins (a disorder associated with ciliary disfunction), inturned, fuzzy, ofd1, and duboraya. Knockdown of dub produces a similar phenotype to that seen in frizzled2 (fz2) morphants, a component of noncanonical Wnt signaling. Both these morphants display defects in left/right patterning of the heart and other visceral organs and also display gastrulation-associated convergent extension (CE) defects. Circulating fluid flow generated by cilia within Kupffer’s vesicle is responsible for initiating left/right patterning.21,22 This flow can be analyzed directly using fluorescently labeled beads that, in a normal situation, will rotate in a counter clockwise fashion. However, dub and fz2 morphants have no flow at all within Kupffer’s vesicle. Clser examination reveals that fewer cilia are present within the vesicle and those that are appearing shorter. A synergy exists between dub and fz2, and it would appear that Fz2 actually directly phosphorylates Dub (Fig. 1B). Confirmation of noncanonical Wnt signaling involving in this process is provided by the observation that expression of Dvl-PDZ RNA also produces a similar phenotype. A disruption of the actin cytoskeleton at the apical surface of Kupffer’s vesicle cells appears to be the primary defect associated with defective ciliogenesis found in these morphants.8

Inturned and fuzzy are core PCP effectors in Drosophila.5,6 In Xenopus, Xint/Xfuz morphants develop with CE defects accompanied by perturbed Hedgehog signaling, a process reliant on correct cilia function.23 Closer examination of Xint/Xfuz morphants reveals that they have no cilia within the ventral neural plate. Although microtubules are present, they do not form cilia, indicating a failure in the correct organization necessary to construct functional cilia. Orientation and elongation of ciliary microtubules depend on the basal ciliary apparatus, which itself is regulated by the actin cytoskeleton, and this actin network is disrupted in Xint/Xfuz morphants.24 It seems that Dishevelled colocalizes, along with Inturned, at the basal body where they are both required to regulate RhoA activity to ensure that the basal body docks correctly (Fig. 1B). Once this process has occurred, both Dishevelled and RhoA are again required to establish the planar polarization necessary for ciliary beating.7

OFD1, a gene mutated in oral-facial-digital type I syndrome, has also been shown to be necessary for proper ciliary function. Knockdown of OFD1 in zebrafish results in a wide range of phenotypes associated with ciliary disfunction.24 As we have seen previously with duboraya, OFD1 knockdown results in a disruption of flow within Kupffer’s vesicle leading to laterality defects such as reversed cardiac jogging/looping. Closer examination reveals that the cilia within Kupffer’s vesicle are disrupted. Further, OFD1 morphants also display a CE phenotype similar to noncanonical Wnt morphants embryos. In fact, coinjection of suboptimal concentrations of OFD1 with either wnt11 or vang-like 2/vangl2 morpholinos leads to an enhanced CE phenotype. Exactly how OFD1 and the noncanonical Wnt pathway interact remains to be determined, but it appears that there are two possibilities. First, like duboraya, there could be a direct interaction between the noncanonical pathway and OFD1 during ciliogenesis. Second, as will be discussed later, correct ciliary function is required upstream of the noncanonical pathway to activate it. Thus, disrupted ciliogenesis caused by OFD1 knockdown may impair noncanonical Wnt signaling.24

**BBS genes encode ciliary proteins that have also been shown to interact with noncanonical Wnt genes.** Mice knockouts for BBS genes show similar developmental defects (neural tube defects and disrupted cochlear stereo ciliary bundles) to noncanonical Wnt knockouts such as vangl2 (trilobite). Further, in crosses between these two strains result in an enhanced phenotype, indicating a genetic cooperation. In zebrafish, knockdown of BBS4 in trilobite mutants enhances the trilobite CE phenotype, again showing that a genetic interaction exists between BBS genes and noncanonical Wnt signaling.25 As is the case with dishevelled, both Vangl2 (trilobite) and BBS proteins colocalize to the basal body in ciliated cells. Taken together, these data suggest a role for noncanonical Wnt signaling in the organization of the actin cytoskeleton necessary for the correct positioning of the basal body required for subsequent ciliogenesis to occur. More recently, a concerted analysis of bbs1/4 and mkks (bbs6) has been conducted in the zebrafish.26 Morpholino-mediated knockdown of any of the three results in CE defects. Further, this phenotype is actually enhanced when bbs1/4 and mkks are individually knocked down on a wnt11 or wnt5b mutant background. This suggests that they act synergistically with noncanonical Wnt signaling, when impaired, leads to the observed CE defects. Interestingly, the canonical Wnt pathway appears to be upregulated in all three morphants, raising the possibility that bbs genes may facilitate a switch between noncanonical and canonical Wnt signaling. Analysis of mammalian cells utilizing the well-established TOP/FOP luciferase assay shows that disruption of either of these genes does indeed lead to upregulation in canonical Wnt signaling. Further, these results are recapitulated when intraflagella transport is disrupted via silencing of kif3a (an axonemal-specific kinesin motor), suggesting that the observed increase in canonical Wnt signaling is due to loss of ciliary function.26

**Cilia Signaling**

Cilia are also able to transduce mechanical signals intracellularly, and emerging data suggest that this can be achieved via the noncanonical Wnt pathway. Nephronophthisis type II is an autosomal recessive disease caused by mutations of the ciliary protein Inversin and is characterized by widespread renal cysts, situs inversus, and renal failure. Knockdown of inversin in zebrafish not only results in kidney cysts, a hallmark of defective ciliary function in mammals, but also leads to left/right heart patterning defects, while in Xenopus inversin regulates CE during gastrulation.27 In a normal situation Inversin targets cytoplasmic Dishevelled for breakdown that leads to inhibition of canonical Wnt signaling, but, as indicated earlier, dishevelled is a key component of both canonical and noncanonical Wnt signaling. It would appear then that despite Inversin’s role in cytoplasmic Dishevelled degradation, it also permits its accumulation at the plasma membrane where it can bind to and form a complex with both
Trilobite and Prickle and thus also regulate noncanonical Wnt signaling (Fig. 1C). Further, the other previously described link between the two Wnt pathways, diversin, can actually rescue the kidney cysts of inversin morphants, presumably by increasing β-catenin degradation. As mentioned earlier, fluid passing over MDCK cells results in cilia-mediated intracellular calcium release. This same process also results in an increase in inversin expression and a consequent decrease in canonical Wnt signaling. If this model were correct, then this mechanical stimulus would also lead to accumulation of Dishevelled at the plasma membrane and induction of the noncanonical Wnt pathway.

A subsequent study in zebrafish has helped to further substantiate this model. Seahorse encodes a zebrafish homolog of the mammalian lrcc6 gene and was identified in a large-scale insertional mutagenesis screen for cystic kidney mutants. Seahorse is widely expressed in ciliated tissues including Kupffer’s vesicle; however, it is not localized to the basal body or the cilia, and instead appears as cytoplasmic puncta. Along with kidney cysts seahorse mutants also show defective left/right patterning, a phenotype also associated with perturbed noncanonical Wnt signaling. However, the surprise comes with the finding that seahorse does not affect ciliogenesis or cilia function, indicating that it is downstream of cilia signaling. As we have seen with both inversin and diversin, it would appear that seahorse could also antagonize the canonical pathway and potentiate the noncanonical Wnt pathway, and once again we also find that it too could interact with dishevelled. It would seem then that not only is noncanonical Wnt signaling required for ciliogenesis, but also, upon completion of this process, it is employed once more to transduce the mechanical signals initiated by cilia deflection.

Conclusion

In this review, we have highlighted some recent data establishing a link between cilia and the noncanonical Wnt pathway. Cilia are now beginning to be recognized as an important organelle with many different diseases being linked to dysfunctional cilia. Further investigation of these conditions has revealed that the noncanonical Wnt pathway is also a key component for correct cilia function. Indeed, many of the phenotypes associated with defective cilia such as situs inversus (left/right patterning), neural tube defects, and disrupted kidney function are also present when noncanonical Wnt signaling is perturbed. The exciting prospect that is beginning to emerge here is that noncanonical Wnt signaling appears to be involved in two separate processes both of which are necessary for correct cilia function. First, by regulating the actin cytoskeleton, the noncanonical Wnt pathway plays a key role in the formation of the cilia. Second, it would seem that cilia also utilize the noncanonical Wnt pathway for signal transduction. While the details of these mechanisms are still to be unraveled, this seems to be a wonderful example of a biological switch that transduces a mechanical stimulus intracellularly by turning off the canonical Wnt pathway and turning on the noncanonical Wnt pathway.

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