

Polycystic kidney disease: Cell division without a c(l)ue?

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Polycystic kidneys are caused by an amazingly broad array of genetic mutations and manipulations. The ciliary hypothesis has evolved as the unifying concept of cystogenesis: cilia, bend by fluid flow, initiate a calcium influx that prevents cyst formation. The integrity of ciliary functions has been linked to the polycystic kidney disease gene products localizing to the cilium or the basal body/centrosome. Until recently, the signals and cellular programs located downstream of the ciliary-mediated calcium flux have remained elusive. Now, several reports point towards a role of the cilium or the basal body/centrosome complex in planar cell polarity, a pathway that orients cell in the plane of a tissue layer. First, *Inversin*, a protein mutated in nephronophthisis type II was found to act as a switch between the canonical and the noncanonical Wnt cascade, suggesting that β -catenin/TCF-dependent gene transcription has to be curtailed to allow normal tubular differentiation. Second, heterozygote deletions of Bardet-Biedl syndrome proteins affect neural tube closure and disrupt the cochlear stereociliary bundles, two typical planar cell polarity defects. Third, tubular epithelial cells undergo oriented cell division during tubular elongation, along the axis of the anterior-posterior axis of the nephron. Thus, the cilium or the basal body/centrosome complex may provide the spatial cues to position the centrosome and the mitotic spindle before the next cell division. Failure to communicate this spatial information may condemn the tubular epithelial cells to proliferate and to form cysts.

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NEPHRON FUNCTION AND ARCHITECTURE

Every day 150–180 liters of blood are filtered by the glomerulus. Driven by a pressure gradient and periodic muscle contractions, the filtrate then passes through the different segments of the tubules until it reaches the collecting duct system. During its journey through the tubules, the filtrate is processed by the reabsorptive and secretory functions of the distinct epithelial cell types lining the tubules. By the time, it reaches the collecting ducts, the daily volume has dropped to 1–2 liters. This ‘final’ urine shows high concentrations of salts, acids, and toxic waste products, contributing to the total body homeostasis.

It is clear that the functions of the tubular cells in each segment require a precisely defined structure not only at the single cell level but also at the tissue level. Therefore, one of the big challenges of the developing kidney is to assemble a perfect architecture of the nephron, the functional unit of the kidney. However, the formidable task to maintain a genetically defined tubular geometry does not end with birth. Renal ischemia and tubular necrosis can destroy substantial parts of the nephron, and decrease glomerular filtration to less than 10%. However, the regenerative process will reconstitute the tubular system precisely to its previous geometry. Tubular epithelial cells are polarized, that is they possess a topographically well-defined apical and basolateral side. In addition to this cell-autonomous polarity, tubular epithelial cells must be endowed with a second type of polarity that allows them to communicate with neighboring cells.

One heterogenous group of genetic disorders, polycystic kidney diseases (PKD), is characterized by an apparent loss of spatial orientation and intercellular communication. The consequence is dilatation of the renal tubules and cyst formation. The mutation of genes causing PKD results in defective tubules that either fail to form correctly, or lose their proper geometry (reviewed in Wilson¹ and Boletta and Germino²).

PKD TYPES

Autosomal dominant PKD (ADPKD) is the most common monogenic genetic disease, and affects one in 500–1000 humans.³ Approximately half of all affected patients develop end-stage renal disease in the fifth to sixth decade of life.

The *PKD1* and *PKD2* genes, responsible for ADPKD, encode polycystin-1, a large integral membrane protein with an unusual domain architecture, and polycystin-2. Polycystin-2 (TRPP2) is a member of the extended family of transient receptor potential (TRP) channels that is permeable for several cations, including calcium (reviewed by Delmas⁴). In autosomal-recessive PKD, the patients suffer from renal failure and liver fibrosis in early childhood. The mutated gene, *PKHD1*, encodes for fibrocystin, a type-I integral membrane protein with a large extracellular domain. Another autosomal recessive group of cystic kidney diseases is termed nephronophthisis (NPHP), and generally affects children between ages 1 and 20 years. Although the estimated prevalence is only 1 in 100 000, it is the most frequent genetic cause of chronic renal failure in children. Five different loci have been described so far that account for approximately 50% of all cases.⁵ Thus, it can be expected that several more NPHP genes will be identified. Apart from these diseases in which cystic kidneys associated with renal failure are the predominant clinical manifestation, renal cystic disease can also be found in pleiotropic diseases such as the Bardet-Biedl syndrome (BBS), Von Hippel-Lindau disease or the Meckel-Gruber Syndrome (see Wilson⁶ and Katsanis⁷ for more details).

CYSTS IN PKD

In ADPKD, the cysts arise as focal outpouches from all segments of the nephron. Cysts eventually separate from the parental nephron, and expand through proliferation and accumulation of cyst fluid. By contrast, in autosomal-recessive PKD the fusiform cysts develop as dilations of collecting ducts, and maintain contact with the parental nephron. In NPHP, the cysts are restricted to the corticomedullary border, and may be derived from collecting ducts and distal tubules. Unique histological features of NPHP are thickening of the basement membranes, and peritubular, inflammatory infiltration. NPHP kidneys are typically normal in size;⁶ however, patients with nephronophthisis type II (INVS) mutations have often enlarged kidneys, mimicking autosomal-recessive PKD or ADPKD.^{8,9}

PATHOGENESIS OF CYST FORMATION

Despite the different underlying mutations responsible for the monogenetic forms of PKD, it has been postulated that the molecular mechanisms causing cyst formation are similar, or at least, share a common final pathway. Several mechanisms have been suggested. However, they still remain poorly understood.

Immunostainings of cystic epithelial cells *in vitro* and *in vivo* for mitotic markers such as proliferating cell nuclear antigen, c-Myc, and Ki-67 suggest that cystic epithelia possess a high mitotic rate.^{10,11} There is also increased expression of growth factors such as epidermal growth factor and their receptors in ADPKD cysts.^{12,13} Some studies found that the Na⁺/K⁺-ATPase and the Na⁺/K⁺/2Cl⁻-symporter are sorted to the wrong surfaces of the tubular epithelial cells.¹⁴

This could lead to altered electrolyte transport, increased fluid secretion, and cyst expansion.¹⁵

SIGNALING IN PKD

The long list of potential signaling cascades triggered by polycystin-1 has left a confusing picture. In human embryonic kidney (HEK) 293T cells, the short carboxy-terminal domain of polycystin-1 activates AP-1 in a PKC- and c-Jun NH₂-terminal kinase-dependent manner.¹⁶ Polycystin-1 contains a G-protein motif that appears to bind and activate heterotrimeric G-proteins.^{17,18} More recently, AP-1 activation has been linked to the nuclear translocation of the C-terminal polycystin-1.¹⁹ Expression of the C-terminal polycystin-1 domain in Madin-Darby canine kidney cells triggers cell migration and branching morphogenesis that can be blocked by PKC inhibitors,²⁰ whereas expression of full-length polycystin-1 in Madin-Darby canine kidney cells induces cell cycle arrest at the G0/G1 phase with upregulation of p21 through activation of the Janus kinases-signal transducers and activators of transcription (JAK-STAT) pathway.²¹ A recent study suggests that PKD1 prevents proliferation of renal tubular epithelial cells through the induction of p53 and activation of c-Jun NH₂-terminal kinase.²² The importance of apoptosis has been highlighted in knockout mice, in which the inactivation of apoptosis inhibitors such as bcl-2 causes cystic kidney disease.²³ Apoptosis may also affect the normal tissue, and contribute to the loss of renal function in ADPKD.²⁴ A significant number of patients with tuberous sclerosis develop renal cysts owing to either TSC2, or (less often) TSC1 mutations. Both proteins form a heteromeric complex that inhibits mTOR, a kinase that controls cell growth, proliferation, and metabolism (reviewed in Inoki *et al.*²⁵). Two studies have now shown that inhibition of mTOR activity ameliorates cyst formation.^{26,27} mTOR is activated by AKT, a serine/threonine kinase that directly phosphorylates and inactivates TSC2.²⁸⁻³¹ AKT is a pro-oncogenic protein located downstream of growth factor receptors and generation of 3' phosphoinositides (PtdInsP3). One important inhibitor of AKT is the lipid phosphatase PTEN, which decreases the concentration of PtdInsP3. Thus, dysregulation of AKT, for example triggered by aberrant expression of growth factors and their accumulation in the cyst fluid, mislocalization of their receptors, and/or inhibition of PTEN function, can cause mTOR activation, and may represent a common final pathway of cystogenesis.

THE CILIARY HYPOTHESIS OF CYST FORMATION

The PKD field was revolutionized some years ago by a nearly forgotten organelle, the cilium.³² The cilium is a highly conserved appendage that projects from the surfaces of many cells (for a comprehensive list of publications on the occurrence of primary cilia, see <http://www.primary-cilium.co.uk/>) The essential structure of cilia consists of nine peripheral microtubule doublets, the axoneme, that emerges from the basal body (one of the two centrioles in a centrosome). The axoneme is surrounded by a membrane

lipid bilayer that is continuous with the plasma membrane. Based on whether the axoneme includes an additional central pair of microtubules, cilia are classified as '9 + 2' or '9 + 0' cilia. The '9 + 2' cilia contain additional dynein arms and are motile. In the mammalian kidney, the so-called primary cilium belongs to the group of immotile '9 + 0'-cilia. It is located on the apical surface of epithelial cells, and extends into the tubulus lumen (reviewed in Pazour³³). The first hint that cilia might be involved in the pathogenesis of PKD came from the *orpk* mouse model of PKD.³⁴ The *orpk* phenotype is caused by a hypomorphic allele of the *Tg737* gene, and is characterized by dilation of the proximal tubules, followed by cystic lesions in the collecting ducts. Targeted disruption of the *Tg737* gene causes growth arrest at mid-gestation, neural tube defects, and left-right abnormalities.³⁵ As randomization of the body axis is also observed in mice with motor protein KIF3B mutations,³⁶ the stunted cilia present on the ventral node in both mouse models pointed towards a unifying pathogenesis. Intraflagellar transport (IFT), powered by kinesin and dynein motor proteins, is an absolute prerequisite for normal flagellar biogenesis in *Chlamydomonas reinhardtii*, and requires several IFT proteins (reviewed in Rosenbaum³⁷ and Pazour *et al.*³⁸). *Chlamydomonas* with mutated IFT88, the homologue of *Tg737* (Polaris), fail to assemble flagella. Similarly, mice with defective *Tg737* not only lack cilia on the ventral node, but on tubular epithelial cells.³⁹ These findings prompted the hypothesis that IFT is important for both, flagella biogenesis and assembly of primary cilia in mammalian cells, and led to the ciliary hypothesis of PKD. Studies in *Caenorhabditis elegans* provided further support for his hypothesis. Consistent with the findings in *Chlamydomonas* and mice, the *C. elegans* homologue of IFT88/*Tg737*, *Osm-5*, localizes to the cilium base as well as the axoneme, and is essential for ciliogenesis.^{40,41} The *C. elegans* homologue of *PKD1* and *PKD2*, *lov-1* and *pkd-2* were the first cystoproteins to be identified in the cilia and cell bodies of male-specific sensory neurons, where they participate in a shared signaling pathway.⁴² *Lov-1* was originally identified in a screen designed for the isolation of genes specifically required for male sensory behaviors.⁴³ Male *C. elegans* execute an elaborate stereotyped program to search for the hermaphrodite vulva, insert the spicule, and release sperm.⁴⁴ In contrast to *osm-5*, *lov-1* or *pkd-2* mutations are not associated with structural abnormalities of the cilium.⁴² The mammalian polycystin-1, polycystin-2, polaris, and cystin were subsequently identified in the primary cilium of tubular epithelial cells.⁴⁵ Other PKD proteins followed, and the list of cilia-associated cystoproteins proteins still continues to grow (Table 1) (reviewed in Hildebrandt and Otto⁴⁶). More functional evidence for a critical role of cilia in the pathogenesis of PKD came from a retroviral-based insertional mutagenesis screen in zebrafish. Among the 11 isolates with pronephric cysts, seven genes encoded for ciliary functions or ciliogenesis.⁴⁷ Finally, the kidney-specific inactivation of the ciliary kinesin motor KIF3A in mice disrupted ciliogenesis, and led to cystic kidneys.⁴⁸

Table 1 | Cystoproteins

Gene	Protein	Subcellular localization
PKD1	Polycystin-1	Cilia, basolateral PM
PKD2	Polycystin-2	Cilia, ER, basolateral PM
PKHD1	Polyductin, Fibrocystin	Cilia
NPHP1	Nephrocystin-1	Cilia, focal contacts, transition zone
NPHP2/inv5	Inversin	Cilia, basolateral PM, spindle poles, midbody
NPHP3	Nephrocystin-3	NA
NPHP4	Nephrocystin-4	NA
NPHP5	Nephrocystin-5	Cilia
cys1	Cystin	Cilia
TgN737	Polaris/Tg737	Cilia, basal bodies
Nek1	Nek1	Centrosomes
Nek8	Nek8	Cilia
bcl-2	bcl-2	ER, mitochondria
c-myc	c-myc	Nucleus
Beta-catenin	beta-catenin	Basolateral PM, nucleus
kif3a	Kinesin 3a	Cilia
bicc1	BicaudalC	NA
Pontin	Pontin	NA
Qilin	Qilin	NA
CHE-13	Hippi	Cilia
BBS1-8	BBS1-8	Cilia, basal bodies
MKS1	MKS1	NA
MKS3	Meckelin	NA
TSC1+2	hamartin/tuberin	Apical PM, centrosomes, cytoplasm

BBS, Bardet-Biedl syndrome; MKS, Meckel-Gruber syndrome; NPHP, nephronophthisis; PKD, polycystic kidney disease; PM, plasma membrane.

THE FUNCTION OF RENAL CILIA

The cilia of tubular epithelial cells have been implicated in mechano-sensation. When cilia are stimulated by laminar flow or a micropipette, a calcium flux into the cell occurs potentially through the cation-permeable ion channel polycystin-2/TRPP2 located in the cilium.⁴⁹ These observations suggest that cilia in the tubulus lumen are stimulated by the ultrafiltrate passing through the tubular system. The zebrafish *pronephros* possesses motile cilia, and cilia-driven fluid flow is required for normal organogenesis; loss of fluid flow by disruption of cilia function results in pronephros distension and cyst formation.⁵⁰ The cilia in the human kidney are typically nonmotile. However, in the fetal kidney and under pathological conditions, bundles of 9 + 2 cilia have been detected.^{51,52} Based on these findings, it has been proposed that cilia may be motile during early kidney development.⁵⁰ Recent data point to the exciting possibility that cilia constitute an essential platform where signaling processes are initiated. For example, the platelet-derived growth factor receptor and the Hedgehog receptor Smoothened localize to the cilium, and in both cases, the ciliary localization seems to be required for signal transduction.⁵³⁻⁵⁵ In Hedgehog signaling, the absence of functional cilia leads to the defective processing of the full-length Gli3 transcription factor to the truncated repressor form suggesting a role for the cilium as a site for assembly and processing of signaling complexes.⁵⁵

Taken together, exploring the function of cilia in the kidney remains an active area of research with many unanswered questions. How the cilium together with its

relative the centrosome could regulate cell cycle and cell division events is particularly fascinating, and will be discussed here in more detail.

CILIA AND THE CELL CYCLE

The ciliary axoneme is anchored to the basal body, a microtubule-based structure that emerges from one of the two centrioles that together constitute the centrosome. The presence of a cilium is closely related to the differentiation of the cell and establishment of polarity; typically, ciliated cells are in the G0 phase of the cell cycle. Before cells enter mitosis, cilia are absorbed. Following the absorption, the basal body converts back into a centriole. After centriole duplication, the two centrosomes (i.e. four centrioles) form the poles of the spindle apparatus. Shortly after mitosis, the centrosome containing two centrioles migrates towards the apical membrane, where the mother centriole gives rise to the basal body, providing the base for ciliary assembly.^{56–59} If cilia communicate information that keeps cells in their differentiated G0 state, disturbances of cilia function could lead to increased proliferation and PKD. A direct molecular link between cilia and the cell cycle in tubular epithelial cells, however, is still lacking.

Proteomic analysis of centrosomes revealed that apart from structural components, several kinases and phosphatases involved in cell cycle progression and cytokinesis localize to the centrosome.^{60–62} In *Chlamydomonas*, the *FA2* gene was identified in a genetic screen for mutants defective in deflagellation.⁶³ Deflagellation is an evolutionary conserved cellular response to stress.⁶⁴ Fa2p is essential for calcium-activated axonemal microtubule severing during deflagellation. In addition, deletion of Fa2p leads to a delay at the G2/M transition.⁶³ Fa2p is a member of the NIMA-related kinase family, which currently comprises at least 11 genes, and are termed Neks in mammals (the NIMA-related expressed kinases). Although Nek1 deficiency leads to PKD in mice, targeted knockdown of Nek8 causes pronephric cysts in zebrafish.^{65,66} Recently, it was reported that Nek1 localizes to the centrosome, and Nek8 to the proximal region of the primary cilium.⁶⁷

Another candidate for a molecular link between cilia and the cell cycle is Inversin. INVS was originally identified in the OVE210 transgenic mouse (*inv/inv*-mice) with *situs inversus*, cystic kidney disease, liver abnormalities, and death owing to renal failure shortly after birth. Mutations of INVS causes nephronophthisis type II, the infantile NPHP characterized by PKD and *situs inversus*.⁸ The gene product Inversin contains 1065 amino acids with 16 tandem ankyrin repeats, two nuclear localization signals, two calmodulin-binding IQ domains, a conserved lysine-rich central domain, and two destruction or D-boxes. The first D-box of Inversin interacts with subunit 2 of the anaphase-promoting complex (APC2).⁶⁸ The APC/C is an E3 ubiquitin ligase that regulates cell cycle progression by selectively degrading checkpoint proteins such as cyclin B. An Inversin-specific antibody revealed a dynamic expression pattern throughout the cell

cycle in Madin–Darby canine kidney cells.⁶⁸ In polarized interphase cells, Inversin localized to primary cilia, basal bodies, the nucleus, and the cell–cell borders but in metaphase and anaphase Inversin localized to the spindle poles.^{68–70} The APC/C has been localized to the centrosome as well, and is involved in the proteasomal degradation of centrosomal proteins such as Nek2a.^{71–73} Interestingly, BBS8 bears similarity to yeast *cdc23*, a member of the APC/C.⁷⁴ BBS8 is one of eight proteins mutated in BBS.⁷⁴ In BBS8 patients manifests PKD together with *situs inversus* like nephronophthisis type II patients.⁷⁴ Furthermore, the specific localization of BBS8 to the basal body and its interaction with PCM1, a pericentriolar marker, raise the possibility that it either participates in ciliogenesis, or mediates communication between the cilium and APC/C-mediated cell cycle control.⁷⁴ It will be interesting to test whether Inversin and BBS8 act in a common pathway. As the centrosomes represent a cellular organelle shared by mitotic spindles and cilia, it is conceivable that they integrate cilia-sensed signals into cellular programs that affect proliferation and differentiation.

CILIA AND WNT SIGNALING

Apart from the observation that the bending of renal cilia increases intracellular calcium, it is virtually unknown what downstream signaling events are triggered, and how defective cilia signaling contributes to the pathogenesis of PKD. As cyst formation in PKD commences in embryonic development, developmental signaling pathways need to be considered when exploring ciliary function. One important event in mammalian kidney development is the invasion of the ureteric bud epithelium into the metanephric mesenchyme. The mesenchyme is subsequently induced to form S- and comma-shaped epithelial tubes that are precursors of the proximal parts of the nephron including the glomerulus. The tubes eventually fuse with the distal branches of the ureteric bud to generate the architecture of a functional nephron. Although many signaling pathways are involved in these processes, the Wnt signaling pathway is particularly important in the induction of the metanephric mesenchyme. In mice deficient of Wnt-4, mesenchymal-to-epithelial transdifferentiation does not occur, leading to renal aplasia.⁷⁵ Wnt-4 has been shown to act downstream of another Wnt, Wnt-9b, in this process.⁷⁶ Wnt-11 is also important in the early steps of renal development, regulating proliferation and ureteric bud branching.^{75,77} Apart from Wnt ligands, other members of the Wnt signaling cascade have been detected in the kidney, but their functional roles remain unclear. Soluble Wnt molecules bind to their cognate Frizzled receptors, leading to the activation of intracellular molecules such as Dishevelled. Dishevelled is located at a decisive branch point, and either activates the canonical β -catenin-dependent Wnt pathway, or the noncanonical Wnt or planar cell polarity (PCP) pathway (see Figure 1).⁷⁸ Unopposed canonical Wnt signaling during renal development causes PKD. This was demonstrated by transgenic overexpression of an activated

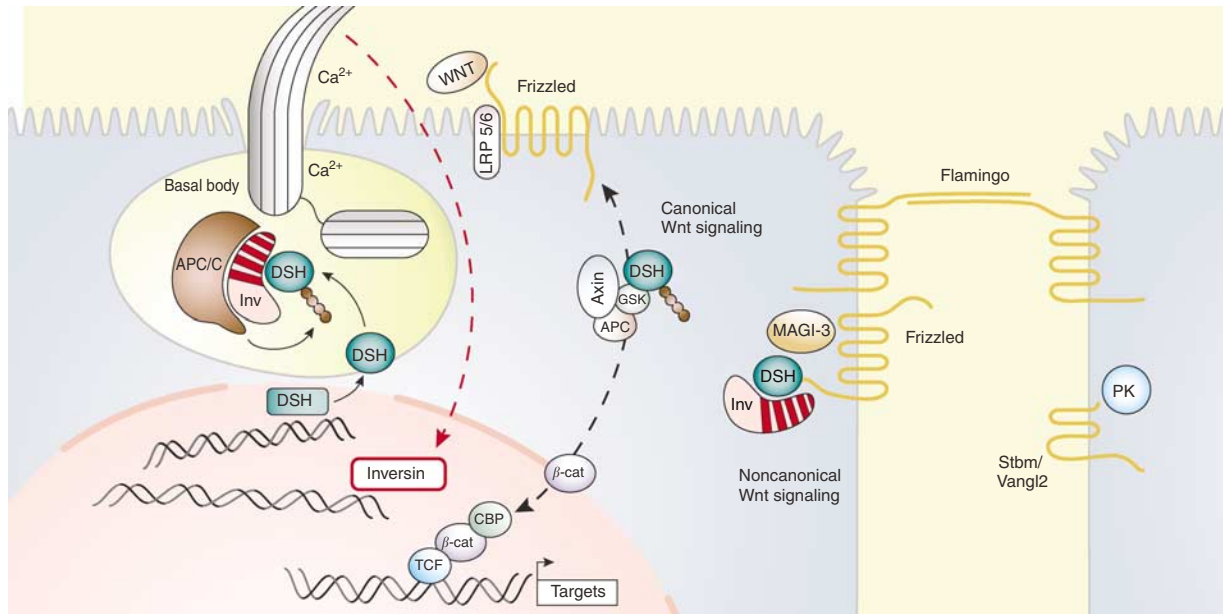


Figure 1 | Inversin signaling. Depicted is a ciliated tubular epithelial cell. Urine flow bends the cilium, initiating a calcium influx. This calcium influx is amplified by release of calcium from internal storages, resulting in an increased expression of inversin (red arrow). Inversin localizes to the centrosome and the plasma membrane; inversin (Inv) interacts with cytoplasmic Dishevelled (DSH), and targets Dishevelled for APC/C-dependent ubiquitination and degradation. Soluble Wnt molecules interact with members of the Frizzled family of 7-transmembrane proteins and the Wnt coreceptor LRP5/6. Induction of the canonical Wnt signaling pathway encompasses the recruitment of Dishevelled to Frizzled, inhibition of GSK-3 β (GSK), and stabilization of β -catenin (β -cat), which translocates to the nucleus to induce TCF-dependent transcription of Wnt target genes. In the presence of inversin, cytoplasmic Dishevelled levels are reduced, and not available for canonical Wnt signaling. In contrast, inversin does not affect the recruitment of Dishevelled to the plasma membrane, where it is available for noncanonical Wnt signaling. In the *Drosophila* wing, Frizzled and Dishevelled are found at the distal plasma membrane, whereas strabismus (Vangl2) and prickle (pk) are found at the proximal plasma membrane. Flamingo localizes to both membranes. MAGI-3, membrane associated guanylate kinase with inverted orientation; CBP, cAMP response element binding protein (CREB)-binding protein.

form of β -catenin in mice.⁷⁹ The cystic epithelia displayed increased proliferation and apoptosis rates. In addition, the kidneys of these mice also revealed a delay in tubular maturation with a persistent appearance of S- and comma-shaped bodies at the renal cortex.⁷⁹

Based on a similar renal histology found in *inv/inv*-mice, a potential role of Inversin in Wnt signaling was explored in a recent study.⁸⁰ Using reporter genes in cultured cells and Dishevelled-mediated double-axis formation in micro-injected *Xenopus laevis* embryos as assays, an inhibitory role of Inversin in canonical Wnt signaling upstream of the β -catenin degradation complex was established. Inversin interacts with Dishevelled and decreases its half-life, possibly, by linking Dishevelled to the APC/C. The first D-box of Inversin is recognized by the APC/C. A patient mutation of Inversin (L493S), which destroys this D-box, does no longer target Dishevelled for degradation, suggesting that inversin-mediated Dishevelled degradation plays an important role during normal kidney development.^{8,80} Consistent with a role in noncanonical Wnt signaling, Inversin is required for convergent extension movements in *Xenopus* gastrulation and elongation of *Xenopus* animal caps. In zebrafish, Inversin knockdown by antisense morpholino-oligonucleotides causes pronephric cysts. This phenotype can be rescued by Diversin, another ankyrin-repeat protein that inhibits canonical Wnt

signaling, while facilitating noncanonical Wnt pathways.^{80,81} This result suggests that cyst formation in the absence of Inversin is caused by unopposed Wnt signaling during permissive periods of renal development. The reversal of cyst formation by Diversin supports a role for Inversin in noncanonical Wnt signaling during renal development.

What could be regulating the switch from canonical to noncanonical Wnt signaling during renal development? Clearly, the onset of glomerular filtration and production of primary urine marks a new stage in the development of a nephron, exposing tubular epithelial cells for the first time to shear stress and bending of their primary cilium. Interestingly, a flow rate equivalent to urine flow upregulated Inversin expression in a kidney cell line growing in a flow chamber. The flow-induced expression of Inversin was accompanied by a modest reduction of β -catenin levels.⁸⁰ It remains to be determined whether this model system adequately reflects the *in vivo* situation. The BATgal-mouse, which allows visualization of β -catenin-dependent Wnt signaling, may provide more insight in the spatial and temporal activation of the canonical Wnt signaling during renal development. In the BATgal-mouse, a striking difference in Wnt activation in the tip versus the trunk of the ureteric bud was observed.⁸² Tip cells that display a migratory phenotype to allow invasion of the ureteric bud into the metanephric mesenchyme have

high levels of Wnt activation, whereas trunk cells, forming the tubular lumen, have low levels. Although highly speculative, this difference could reflect a difference in exposure to flow occurring in the tubes of the early nephron. Based on old studies from the 1950s, urine production begins remarkably early in embryogenesis, long before fluid and electrolyte balance is required for homeostasis.⁸³ It is therefore tempting to speculate that urine flow terminates canonical Wnt signaling to facilitate β -catenin-independent Wnt pathways.

PCP SIGNALING

Originally, the noncanonical Wnt pathway (also called tissue polarity or PCP pathway; hereafter referred to as PCP pathway) was discovered in *Drosophila* where all adult cuticular structures show PCP features.⁸⁴ In addition to the apico-basolateral polarity of epithelial tissues, PCP describes a second polarity axis in the plane of the epithelium. In the *Drosophila* wing, for example, each cell in the epithelium orients itself with respect to the proximo-distal axis by developing a distally pointing actin-based hair.⁸⁴ Apart from regulating convergent extension movements during embryo gastrulation, prominent vertebrate examples of PCP signaling include body hair orientation, neural tube closure as well as the organization of the stereocilia bundles of the inner ear epithelium (see Figure 2). Also the unidirectional beating of cilia in the respiratory epithelium or the oviduct have been discussed to involve aspects of PCP. The role of PCP in the kidney remains to be determined. It has been proposed that a planar orientation plays a role during renal development when newly formed tubules undergo progressive lengthening by directed cell migration and cell division (see Figure 3).⁸⁵ Maintenance of tubular geometry at later stages of development could also require PCP signaling with tubular flow and ciliary bending serving as a positional cue for each epithelial cell.

On the cellular level, PCP is necessary for the subcellular localization of signaling complexes that drive morphogenetic processes such as the asymmetric organization of the actin cytoskeleton. Based mostly on genetic studies, PCP proteins fall into two classes: An upstream cassette of transmembrane proteins, providing an extrinsic spatial cue at the cell surface (Fat, Dachous, Four-jointed); and downstream components that interpret the cue and establish asymmetry by localizing to different ends of the cell (Frizzled, Dishevelled, Flamingo, Prickle, Strabismus and Diego, the fly orthologue of Diversin). In *Drosophila* wing cells, for example, Frizzled, Dishevelled, and Diego localize to the distal cell-cell boundaries, whereas Prickle and Strabismus localize proximally.⁸⁴ Asymmetry in one cell is transmitted to the neighboring cell by a feedback loop which amplifies small imbalances of Frizzled activity.⁸⁶ However, at present, it is unclear how initial asymmetry is established. Selective stabilization and degradation is conceivable, but also selective transport. In the case of Frizzled, it was recently shown that intracellular particles that contain green fluorescent protein (GFP)-tagged Frizzled move preferentially toward distal cell boundaries before asymmetry is established.⁸⁷ Frizzled-GFP

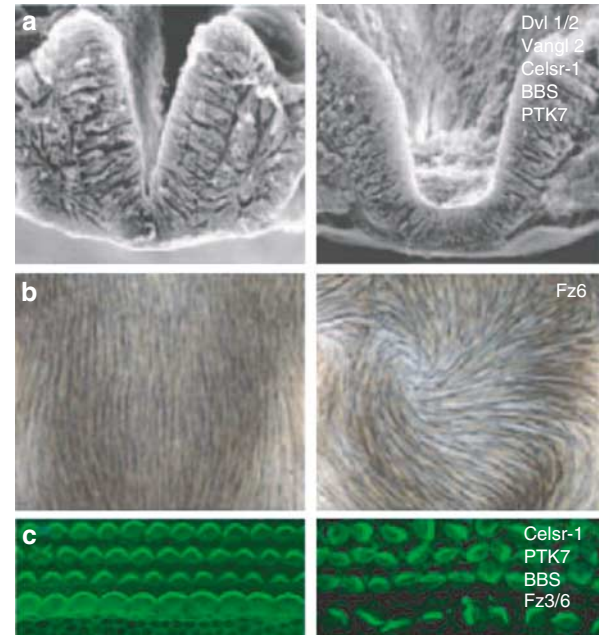


Figure 2 | PCP in mammals. (a) During neurulation (occurring around E8.5 in the mouse), convergent extension movements facilitate medial movement of neural folds. In the absence of PCP signaling, convergent extension fails and so does neural tube closure. Dvl1/2 (Dishevelled1/2), Vangl2 (Strabismus), Celsr-1 (Flamingo), protein tyrosine kinase 7 (PTK7), and the BBS proteins are PCP proteins that have been implicated in this process (courtesy of Dr Copp). (b) Body hair patterning is disorganized in Fz6 $-/-$ mice. This phenotype is similar to the wing hair phenotype in *Drosophila* (courtesy of Dr Nathans). (c) Cochlear hair cells display a regular arrangement of V-shaped stereocilia bundles which is distorted in defective PCP signaling. So far this has been shown for Fz 3/6 Celsr-1, PTK7, and BBS mutant mice, respectively (courtesy of Dr Kelley).

seems to travel along a proximal-distal oriented microtubular web close to the apical surface. Interestingly, in this web the number of plus end-distal microtubules was higher than the number of plus end-proximal microtubules. This suggests that there must be upstream mechanisms that organize the microtubular cytoskeleton.⁸⁷ A recent study revealed an unexpected role for PCP signaling in microtubular organization and ciliogenesis.⁸⁸ Deletion of Inturned (*in*) or Fuzzy (*fy*) cause PCP defects without affecting the polarized distribution of the core PCP proteins, placing *in* and *fy* as effectors of the core PCP complex downstream of Dishevelled (Figure 3). The combined PCP and Hedgehog signaling defects in *in/fy*-deficient *Xenopus* uncovered a defective ciliogenesis as the unifying pathogenesis: Inturned and Fuzzy control the assembly of an apical actin network essential for the orientation of ciliary microtubules. Thus, PCP signaling controls ciliogenesis, whereas the CBC complex may in turn provide spatial cues to instruct the PCP complex. In the kidney, for example, tubular flow might aid in 'shaping' the microtubular web and/or actin cytoskeleton either through pure shear stress and/or ciliary bending, and affect the transport of PCP proteins such as Frizzled.

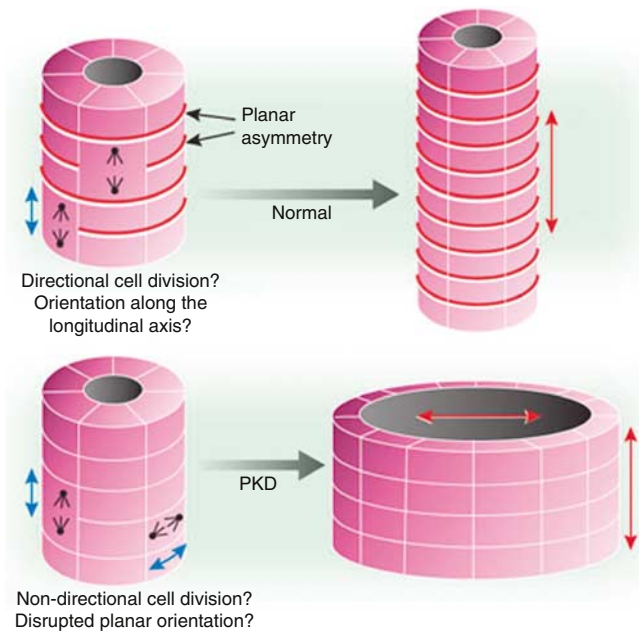


Figure 3 | PCP defects in the developing nephron causes cyst formation. When tubules elongate during kidney development, tubular cells undergo massive cell proliferation. The cell division axis of the proliferating tubular epithelial cells is oriented along the tubular axis. Tubular lengthening occurs without any increase in tubular diameter. PCP signaling has been implicated in the regulation of the cell division axis. Tubular flow occurring within the lumen of the early nephron could be one of the signals that instructs the PCP machinery to define the tubular axis. Defective sensing of the tubular flow or defective PCP signaling could lead to misoriented cell division and, eventually, to cyst formation (courtesy of Dr Germino).

PCP SIGNALING AND THE BBS

The BBS is a pleiotropic disorder characterized by retinal dystrophy, obesity, polydactyly, renal cystic dysplasia, reproductive tract abnormalities, *situs inversus*, and cognitive impairment. BBS is characterized by an interesting genetic heterogeneity, even allowing triallelic inheritance traits.⁸⁹ So far mutations have been identified in eight genes including *BBS1*, *BBS2*, *ARL6/BBS3*, *BBS4*, *BBS5*, *MKKS/BBS6*, *BBS7* and, as discussed above, *TTC8/BBS8*.⁹⁰ Based on a number of studies, it has been proposed that the BBS phenotype is owing to a defect in the assembly or function of cilia and/or basal bodies.⁷⁴ In two comparative genomics studies, for example, all known *BBS* orthologs were detected among genes that are present exclusively in ciliated organisms.^{91,92} The precise function of *BBS* proteins remains unknown. However, *BBS4* (and *BBS8*) interacts with *PCM1*, a component of the pericentriolar material, and may be involved in the loading of cargo from the centrosomes onto dynein-motored microtubular transport carriers.⁹³ The observation that *BBS6* $-/-$ mice also suffer from the neural tube defect craniorachischisis, a typical PCP phenotype, led Ross *et al.*⁹⁴ to test whether *BBS* proteins function in the PCP pathway. Cochlear stereocilia bundles in both, *BBS6* and *BBS4* ($-/-$) mice displayed abnormalities reminiscent of PCP defects. Furthermore, the double heterozygotes of the

PCP protein *Vangl2* (*Trilobite* in zebrafish, *Strabismus* in *Drosophila*) and *BBS1* and *BBS6*, respectively, revealed significant genetic interactions among these proteins. In a similar fashion, the knockdown of both *Vangl2/Trilobite* and *BBS4* in zebrafish resulted in a convergent extension impairment. Interestingly, *Vangl2* localizes to the basal bodies and cilia emanating from the epithelial apical surface.⁹⁴ Taken together, these findings strongly support the emerging link between PCP and the CBC complex.

Another pleiotropic disease that includes PKD and neural tube defects is the Meckel-Gruber syndrome. The antenatal presentation is sometimes difficult to distinguish from the BBS, although the symptoms are often more severe, resulting in early death of the infants.⁹⁵ Three loci have been described so far, and for two of these loci, the mutant genes have been identified very recently.^{96,97} *MKS1* encodes a member of the CBC proteome, and *MKS3* or *meckelin* for a seven-pass transmembrane receptor. Interestingly, the latter protein shares a similar domain structure with *Frizzled* including the CRD domain in the ectodomain that is supposed to bind Wnt ligands. Although at this point there is no experimental evidence, it is very well possible that these proteins are part of a signaling network that could link PCP with the CBC complex.

ORIENTED CELL DIVISION

A rather obscure aspect of the ciliary hypothesis is that the cystoproteins seem to localize to different parts of the CBC complex. *BBS* proteins mainly localize to the basal bodies. *Polaris* can be found in the ciliary shaft and the very tip of the cilium.^{93,55} Dependent on the cell cycle stage, *Inversin* localizes to the cilium and also to the spindle poles.⁶⁸ Therefore, a difficult task will be to delineate the contributions of each component of the CBC complex to the pathogenesis of PKD.

Centrosomes are required for axonal versus somatodendritic polarity in neurons.⁹⁸ After the last mitosis in neuronal differentiation, centrosomes are positioned to the area where the axon will form. In fact, the plane of mitotic division seems to define this location. It is very likely that the centrosomes in the post-mitotic neuron are already instructed where to place the axon before cell division. A role for centrosomes in defining cell polarity is also emerging from other studies. It has long been known in plant cells that centrosomes are not essential for assembly and organization of the mitotic spindle. Following the destruction of the centrosome by laser beam irradiation in mammalian cells, a mitotic spindle is formed, chromosomes are separated, but the cells undergo abnormal cytokinesis, and fail to exit the G1 phase of the cell cycle. One very interesting aspect of acentrosomal spindles is the lack of astral microtubules (microtubules that link centrosomes to the cell cortex). Astral microtubules are important for the correct positioning of the spindle during mitosis, and for the establishment of the spindle axis.⁹⁹ In the *C. elegans* embryo, the first cell division of the zygote is characterized by an asymmetric polarity axis. This axis is defined by the asymmetric distribution of *PAR* proteins in the respective

anterior and posterior cortical regions of the *C. elegans* embryo. Laser ablation of the centrosome before establishment of polarity does not prevent mitosis but the establishment of a polarity axis.¹⁰⁰ In polarized renal epithelial cells, components of the PAR complex (Par3/Par6/aPKC) localize to tight junctions, but have recently been discovered in the cilium, and found to regulate ciliogenesis.¹⁰¹ These polarity proteins bind kinesin-2, and interact with microtubules in a PKC-dependent fashion. Although PAR proteins may regulate microtubule-dependent trafficking and/or axonemal stability to facilitate cilia assembly, these observations could also suggest that the cilium plays a role in directing the transport of PAR proteins to the apical/basolateral junction of epithelial cells.

Oriented cell division is the result of a correctly positioned spindle axis. A number of studies in different model organisms have placed the spindle axis orientation under the control of PCP pathway. In *Drosophila*, the size of the final wing and the shape of bristle mechanosensory organs are determined by oriented cell division requiring Frizzled and Fat and Dachshous, respectively.^{102,103} In zebrafish, Dishevelled, Strabismus, and the noncanonical Wnt11 are involved in oriented cell division during gastrulation.¹⁰⁴ Another study using the zebrafish model recently provided mechanistic insight into this process. Similar to gastrulation, neuroepithelial cells undergoing neurulation use convergent extension movements to intercalate in a polarized fashion. At the same time this accounts for closure of the neural tube folds. Apart from migrating, cells also have to divide during this process. By cell tracing and real-time imaging experiments in the *Vangl2/Trilobite* mutant, Ciruna *et al.*¹⁰⁵ found that the PCP pathway is necessary to re-establish cell polarity that is transiently lost during mitotic cell division and to reintegrate newly formed daughter cells in to the tissue. Importantly, inhibition of cell division rescued neurulation defects in almost all *Vangl2/Trilobite* mutant embryos.¹⁰⁵ It was also shown that the anterior localization of GFP-Pk depends on *Vangl2/Stbm*. This asymmetry was lost completely during cell division and then adopted again by the daughter cells.¹⁰⁵ These findings support a fundamental role for PCP in cell morphogenesis during cell division by regulating the asymmetric distribution of signaling complexes.

ORIENTED CELL DIVISION IN RENAL DEVELOPMENT AND REGENERATION

Can these findings in other systems be applied to renal development? When tubules elongate during renal development, tubular cells undergo massive cell proliferation.¹⁰⁶ Remarkably, this massive proliferation lengthens tubules almost without any increase of tubular diameter. Also regeneration after ischemia and tubular necrosis imposes a need for cell division in the fully differentiated nephron. In either case, spatially controlled division of regenerating tubular cells is required to ensure nephron integrity. Dividing cells have to maintain an apico-basal polarity in order to seal the disrupted epithelium, restore intercellular communica-

tion, and stop dividing as soon as the tubular geometry of an intact nephron is achieved.

When tubular cells divide, their spindle axis is parallel with the tubular axis.¹⁰⁶ In rodent models for PKD such as the HNF1 β -deficient mouse and the *pcy* rat, however, the spindle orientation is disturbed. Importantly, this deviation is present before cyst formation, excluding a possible secondary phenomenon. The *pcy* rat harbors a mutation in the *PKHD1* gene affecting the CBC-associated fibrocystin. HNF1 β is a transcription factor that is involved in regulating the expression of various CBC-associated cystoproteins.^{107,108}

On the basis of these considerations, we would like to propose that tubular epithelial cells are endowed with a pre-determined cellular program that controls the orientation of cell division relative to the neighboring cells. This program can be modulated by mechanosensory input or positional cues, for example, originating from bent cilia. In PKD, this program is defective and leads to cyst formation. Cyst formation may occur during development, but also during regeneration.

CONCLUSIONS AND PERSPECTIVES

A number of open questions remain. PKD is a very heterogenous disorder and this review focuses only on a few possible pathogenetic pathways. To establish defective PCP signaling as a major process in the etiology of PKD, further experimental evidence will be required. First, it will be interesting to determine whether PCP-gene deficient animals suffer from PKD and, vice versa, whether PKD-associated genes other than Inversin and the BBS proteins act in the PCP pathway. Second, the precise role of cystoproteins in the different stages of the cell cycle needs to be explored. Cystoproteins mainly reside at the plasma membrane, or in the cilium. How can they control cell cycle and cell division events taking place in the cytoplasm? One function could include the organization of astral microtubules, which anchor spindle poles to protein complexes at the plasma membrane. Interestingly, adherens junctions, which contain polycystin-1 and polycystin-2, could directly influence the ability of cells to exercise asymmetric cell division.^{109,110} Another report suggests that the centrosome may process information transmitted from focal adhesions.¹¹¹ Furthermore, it has to be considered that the cilium and the centrosome are part of a versatile microtubular network that during mitosis gives rise to the spindle apparatus. Cilia might sense stimuli in the cell environment to regulate the checkpoint that gives the signal to enter mitosis. Importantly, spindle poles have to be formed at the right place in the cell to prevent a misoriented spindle axis. The correct positioning of the spindle axis might involve cystoproteins like Inversin that have the capability to localize to the cilium, the centrosome and the plasma membrane. The demands after mitosis are equally challenging. The centrosome represents a spatial hallmark for axon formation after mitosis in neurons. Thus, in epithelial cells the centrosome might

provide a spatial cue mandatory to reintegrate daughter cells into a polarized epithelium.

After all, tubular epithelial cells are social beings with a sense for integrity. To unravel the machineries and the signaling networks involved to generate a functioning nephron, composed of many different cell types and capable of regenerating after damage, represents a formidable challenge for kidney researchers around the world. The different PKD syndromes provide helpful hints to understand how inborn errors in the architectural plan of the kidney result in fatal cyst formation.

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