New Developments in the Field of Cystic Kidney Diseases

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Abstract: For quite some time the field of polycystic kidney disease has led a life at the fringe of kidney research, but with the cloning of the *PKD1* and many other genes this situation has dramatically changed. Polycystic kidney disease often is a syndromic disease affecting a variety of organs in addition to the kidney. Most of the proteins involved in polycystic kidney disease have been localized to the primary cilium, an extension at the apical membrane of renal tubular epithelial cells, which may serve chemo- and mechanosensory functions. It is speculated that primary cilia and their associated proteins play a role in determining the proper tubular geometry.

INTRODUCTION

The metanephric kidney develops from two precursor tissues, the metanephrogenic mesenchyme and the ureteric bud, which give rise to hollow structures, the tubular portion of the nephron and the collecting ducts, respectively. Looking at the amazingly regular dimensions of the different structures this begs the question how their lumen is formed and how their diameter is determined (Figure **1**). A spectrum of diseases known as cystic kidney diseases may provide the solution to this fundamental problem since they are all characterized by the abnormal and continuous growth of the renal tubules and the collecting ducts. Cystic kidney diseases come in different flavors, with renal cysts observed in patients suffering from autosomal-dominant (ADPKD) and autosomal-recessive polycystic kidney disease (ARPKD), nephronophthisis, medullary cystic kidney disease, Bardet-Biedl syndrome and oro-facial-digital syndrome type I. Most of the genes mutated in those patients have been identified and except for the one mutated in medullary cystic kidney disease seem to point to a common pathogenetic pathway.

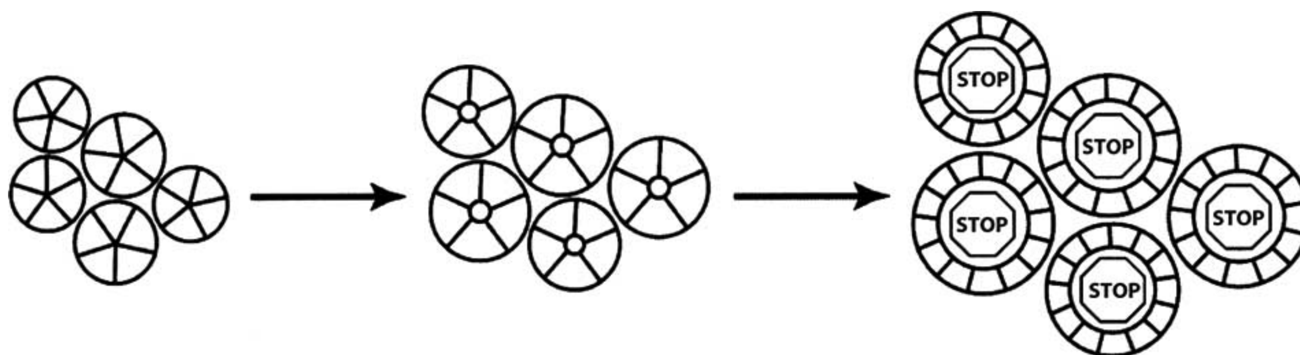

Figure 1. A schematic view of tubulogenesis. The tubular portion of the nephron develops from the metanephrogenic mesenchyme After the aggregation of mesenchymal cells, a lumen begins to form and expands until a sensor, possibly primary cilia, signals “stop”. Taken with permission from [118].

PKD1 AND *PKD2* ARE MUTATED IN PATIENTS WITH AUTOSOMAL-DOMINANT POLYCYSTIC KIDNEY DISEASE

ADPKD, one of the most common monogenetic diseases affecting mankind, has a prevalence that has been estimated at 1:1,000 or even higher [1,2]. By the age of 60, ~50% of the patients with ADPKD require renal replacement therapy, thus contributing to almost 10% of all cases of end-stage renal disease in the Western world [3-7]. Thanks to the efforts of several laboratories the identification of the *PKD1* gene was published in 1994 [8], and was soon followed by the identification of the *PKD2* gene [9]. A number of studies have shown that ~85% of patients with ADPKD suffer from mutations in the *PKD1* gene and ~15% of patients from mutations in the *PKD2* gene [10-13], but there may be very few patients who suffer from mutations in an as yet unidentified gene [14-17]. Although the symptoms in *PKD1* and *PKD2* patients are quite similar, the

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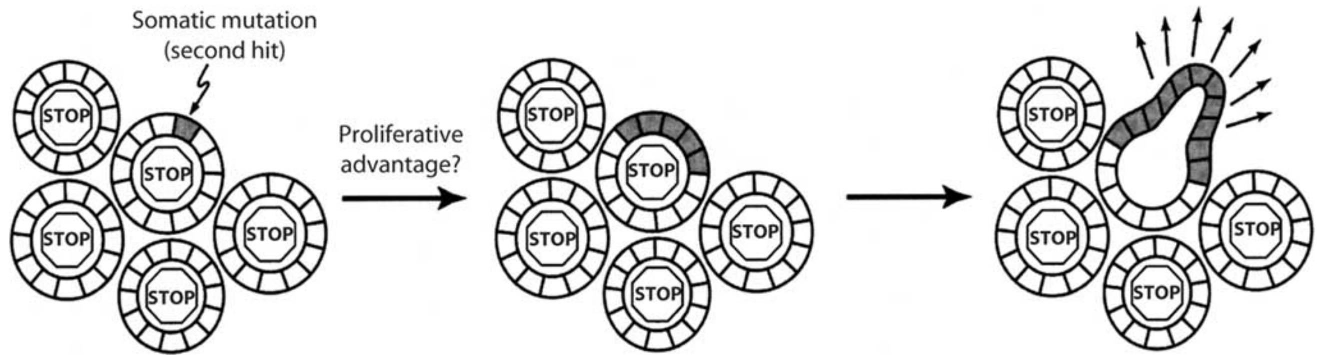

Figure 2. The formation of cysts—a loss of the lumen sensor? In patients with ADPKD there is considerable evidence that a somatic mutation triggers cyst formation. After an expansion of those cells with two mutant alleles of the polycystin proteins, the sensor measuring the width of the tubular lumen may no longer function properly and the "stop signal" is lost—cystogenesis begins. Taken with permission from [118].

course of the disease is probably more benign in the latter [13,18-22]. Cysts in patients with autosomal-dominant polycystic kidney disease develop focally (only in ~5% of the nephrons) and segmentally (even in the affected nephrons, they do not develop along the entire length of the nephron). This picture is reminiscent of tumors that develop due to mutations in tumor suppressor genes, and indeed somatic mutations ("a second hit") have been implicated in cyst formation (for review see [23]) (Figure **2**).

Polycystin-1

Polycystin-1, the gene encoded by the *PKD1* gene, consists of 4302 amino acids and contains a variety of potentially important domains [24-26]. Mutations have been described over virtually the entire length of the *PKD1* gene ([27] and http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html), which is organized into 46 exons and stretches out over ~52 kbp of genomic DNA. A careful topology analysis of the protein has provided evidence that it contains 11 membrane-spanning domains, that the NH_2-terminus is located extracellularly and that the COOH-terminus extends into the cytoplasm [28]. The significance of most of the motifs is unknown and only recently has preliminary evidence for the function of some domains begun to emerge. The REJ (receptor for egg jelly) motif has been named to reflect the fact that it was first identified in a protein that mediates the interaction of sea urchin sperm with glycoproteins in the egg jelly [29]. But it has also been described in polycystin-1 as well as PKDREJ, whose corresponding mRNA was only detected in the testis [30,31], and in two other sea urchin proteins, suREJ2 [32] and suREJ3 [33]. Immediately following the REJ domain, but still located extracellularly, lies the GPS (G protein-coupled receptor proteolytic site) motif, which has been demonstrated to serve as the recognition site for a so far unidentified protease also in polycystin-1. Interestingly, cleavage of polycystin-1 depends on the presence of an intact REJ domain, because an absent or mutated REJ domain results in very inefficient proteolysis. Even after cleavage, however, the NH_2-terminus of polycystin-1 is not shed but remains attached to the cell [34]. Wild-type polycystin-1 [35] and a COOH-terminal fragment [36] induce tubulogenesis in MDCK cells, a canine kidney cell line, when they are grown in three-dimensional collagen gels, but mutants lacking the COOH-terminus [35] or with inefficient cleavage of the NH_2-terminus do not [34].

In addition to the cleavage of the NH_2-terminus, the COOH-terminus of polycystin-1 also undergoes cleavage. Remarkably, this portion of the protein contains a functional nuclear targeting signal shown to facilitate translocation into the nucleus [37]. The synthesis of the COOH-terminus led to the activation of the AP-1 pathway, which was dependent on its nuclear accumulation as shown by constructs in which the nuclear targeting signal was deleted or the COOH-terminus of polycystin-1 was fused to a plasma membrane protein. Polycystin-2 was able to retain the COOH-terminus of polycystin-1 in the cytoplasm and thereby prevented the activation of the AP-1 pathway [37]. It is puzzling, however, that a plasma membrane-bound fusion protein between the extracellular portion of CD16, the membrane-spanning domain of CD7 and a slightly longer COOH-terminal fragment of polycystin-1 was able to activate AP-1 [38], and that a construct similar to the CD16-CD7 fusion protein activated AP-1 through trimeric G-proteins [39]. The question whether the cleavage and nuclear translocation of the COOH-terminus of polycystin-1 occurs constitutively or is subject to regulation was addressed by two experiments. In either setting, i.e. ureteral ligation (reduced tubular flow) and inactivation of the *Kif3a* gene (reduced mechanosensitivity due to the absence of primary cilia), settings in which the mechanical stimulation of tubular epithelial cells should be decreased, accumulation of the COOH-terminus in the nucleus increased [37].

Polycystin-2

With a structure of 15 exons [40], the human *PKD2* gene is much less complex than the *PKD1*

gene. As in the case of *PKD1*, mutations have been found along the whole *PKD2* gene ([41] and http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html). *PKD2* encodes polycystin-2, a 968-amino acid protein with 6 transmembrane domains, whose NH_2- and COOH-termini are predicted to extend into the cytoplasm. Sequence comparisons indicated already early on that polycystin-2 functions as an ion channel and indeed this has turned out to be the case [42-46]. Polycystin-2 and the related proteins polycystin-2L [47] and mucolipin [48] are rather non-selective cation channels with a large conductivity (for a compilation of the relevant experiments see a recent review [49]). There is good evidence that *PKD2* is strongly expressed in the distal portion of the nephron, where it was detected in the basal aspect of the tubular cells [50-52], but there is a lot of controversy regarding the cellular compartment in which polycystin-2 is located. Two not necessarily mutually exclusive models state that polycystin-2 is retained in the endoplasmic reticulum or alternatively reaches the plasma membrane. A battery of different techniques and models has been employed to support either hypothesis.

The exogenous polycystin-2 protein in transfected HEK 293, MDCK, LLC-PK_1 and HeLa cells was distributed in a reticular fashion and co-localized with a marker protein of the endoplasmic reticulum [45,53]. These immunocytochemical findings have been supported biochemically. *N*-glycosylated proteins on their way from the endoplasmic reticulum to the plasma membrane are usually modified in the Golgi apparatus such that the sugar residues cannot be removed any more by the enzyme endoglycosidase H. Indeed polycystin-2 produced in HEK 293, LLC-PK_1 and HeLa cells is *N*-glycosylated and still sensitive to treatment with endoglycosidase H [53,54]. After density gradient centrifugations of homogenates from the same cells polycystin-2 was found to co-migrate with markers of the endoplasmic reticulum [53]. By surface biotinylation no evidence for polycystin-2 in the plasma membrane could be obtained [53]. The results in cell lines have been confirmed in experiments using kidney tissue. Both in human kidney using sensitivity to endoglycosidase H [53] and in murine kidney using a combination of density gradient centrifugation and sensitivity to endoglycosidase H [45], polycystin-2 was localized to the endoplasmic reticulum.

There is, however, conflicting evidence as well. Dependent on the cellular context and whether endogenous or exogenous polycystin-2 protein was examined, it was possible to detect polycystin-2 in the plasma membrane. In contrast to exogenous polycystin-2 protein, which was detected in an intracellular compartment of MDCK [55] and mIMCD3 cells [46] by immunocytochemistry, endogenous polycystin-2 protein was found both in an intracellular compartment and in the plasma membrane (in particular cell-cell contacts) of MDCK and mIMCD3 cells by immunocytochemistry [46,55], density gradient centrifugation [55] and surface biotinylation [46]. However, the polycystin-2 protein in LLC-PK_1 cells was found almost exclusively in intracellular membranes by immunocytochemistry [46,55]. This latter result together with the following suggests that the cellular environment has an important influence on the trafficking of polycystin-2. Without additional polycystin-1, polycystin-2 was located in an intracellular compartment of CHO cells by immunocytochemistry, whereas in its presence it reached the plasma membrane [42]. This is a somewhat surprising observation considering the fact that polycystin-2 is able to prevent the COOH-terminus of polycystin-1 from moving into the nucleus (see above). Polycystin-1 and polycystin-2 interact through their respective COOH-termini [56,57], suggesting the hypothesis that polycystin-1 masks the retention signal of polycystin-2 for the endoplasmic reticulum by direct interaction or by influencing the folding of polycystin-2. Studies with cell lines established from transgenic mice containing a human *PKD1* gene indicate that the situation is not as straightforward. Because of the presence of the human polycystin-1 protein it would have been predicted that the murine polycystin-2 protein is predominantly present in the plasma membrane, which is not the case. By immunocytochemistry and density gradient centrifugation the majority of polycystin-2 was detected in the endoplasmic reticulum of these cell lines (by the latter technique this was also demonstrated for human kidney), but some of the protein also seemed to be present in the plasma membrane. Somewhat surprisingly, all polycystin-2 in the cell lines and in human kidney was sensitive to treatment with endoglycosidase H [58].

A deeper molecular and cell biological understanding of the trafficking of polycystin-2 will likely resolve the controversy regarding its site of action. There is general agreement that the COOH-terminus of polycystin-2 contains a retention signal for the endoplasmic reticulum [53]. A two-hybrid screen with this portion of the protein resulted in the identification of a novel protein called PIGEA-14 (polycystin-2 interactor, Golgi- and endoplasmic reticulum-associated protein with a molecular weight of 14 kDa) [54]. The human *PGEA1* gene on chromosome 22q12-13, which encodes PIGEA-14, is organized into 5 exons. It encodes a soluble protein of 126 amino acids in length, whose signature motif is a coiled-coil domain extending from amino acids 69 to 109. Co-expression of the PIGEA-14 and PKD2 cDNAs in LLC-PK_1 and HeLa cells results in a redistribution of both proteins from the endoplasmic reticulum to a compartment positive for TGN46, a marker of the trans-Golgi network. It therefore appears that PIGEA-14 aids in the escape of polycystin-2 from the endoplasmic reticulum, possibly through its association with GM130, which was identified as an interacting partner in a second two-hybrid screen using PIGEA-14 as the bait [54]. This would suggest that polycystin-2 is able to reach the *cis* side of the Golgi apparatus but under normal

circumstances is immediately returned to the endoplasmic reticulum. What proteins may be involved in the Golgi-to-endoplasmic reticulum transport, and why does polycystin-2 get stuck in a TGN46-positive compartment? At least part of an answer to those questions lies in the interaction between polycystin-2 on the one side and PACS-1 and PACS-2 (phosphofurin acidic cluster sorting proteins) on the other. Both PACS proteins bind to the acidic cluster of polycystin-2, an interaction that is increased by the casein kinase 2-dependent phosphorylation of S812 contained therein [59]. The synthesis of a dominant-negative mutant of PACS-2 leads to the translocation of polycystin-2 to the Golgi apparatus, and the added synthesis of a dominant-negative mutant of PACS-1 leads to trafficking of polycystin-2 from the Golgi apparatus to the plasma membrane. These remarkable observations may be explained by the association of PACS-2 with COPI and of PACS-1 with AP-1 and AP-3, coat complexes required for the transport of vesicles from the Golgi apparatus to the endoplasmic reticulum and from the plasma membrane to the trans-Golgi network, respectively [59]. There is some controversy, however, regarding the location of a S812A mutant polycystin-2 protein that cannot be phosphorylated by casein kinase 2 any longer. In one publication the S812A mutant was reported to remain in the endoplasmic reticulum [60], whereas in another it reached the plasma membrane [59]; possibly the cellular environment (LLC-PK_1 *vs* COS cells) may explain this difference.

There is an additional twist to the trafficking of polycystin-2. As outlined below, many of the cystic kidney disease-associated proteins, among them polycystin-2, have been localized to primary cilia. Since it is generally believed that the endoplasmic reticulum does not extend into primary cilia [61,62], polycystin-2 would have to reach the plasma membrane at least in the case of the primary cilium (Figure **3**). The fact that primary cilia are not elaborated by every cell line in culture and that they constitute only a small portion of the cell surface would explain the fact that polycystin-2 was not detected in the plasma membrane by resistance to Endo H, density gradient centrifugation and surface biotinylation.

AUTOSOMAL-RECESSIVE POLYCYSTIC KIDNEY DISEASE

There are no published studies on the prevalence of ARPKD, but the heterozygosity frequency probably lies at ~1:70 [63]. In contrast to the autosomal-dominant form, the recessive disease typically affects children, of which between 9% and 24% die in the first year of life [63]. The cloning of the mutated *PKHD1* gene was reported in 2002 [64-66] and opens the opportunity towards a better

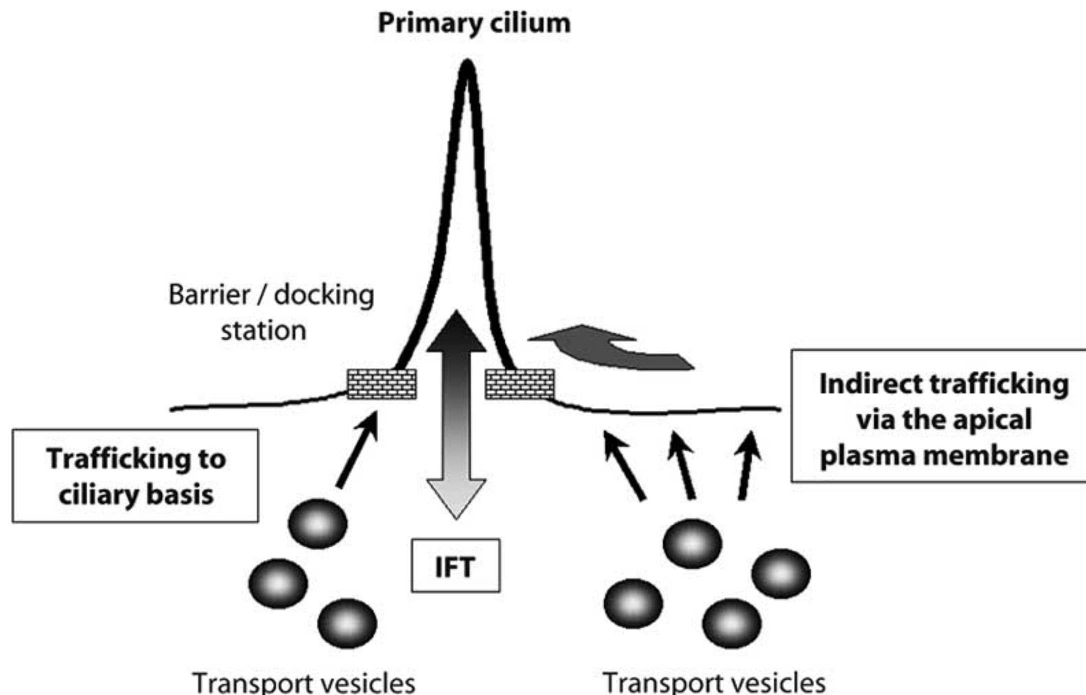

Figure 3. Models for the trafficking of ciliary proteins. Proteins may traffick to primary cilia via three different pathways. Soluble and non-integral membrane proteins may reach the basal body and move up and down the primary cilium by intraflagellar transport (IFT). No vesicles have been observed in the flagella of *Chlamydomonas* [61,62] (although there is contrasting evidence in vertebrate cells [143]) and integral membrane proteins therefore should reach and be retained in the plasma membrane of the primary cilium through a different pathway. This could either be through the neighboring plasma membrane (depicted on the right) or by going through a docking station at the base of the cilium that also serves as a diffusion barrier against the adjacent membrane. There is indeed recent evidence that components of the exocyst complex are located at the ciliary base [144]. Taken with permission from [145].

understanding of the pathogenetic events in this disease. Due to the large size of the protein, which has been named fibrocystin, polyductin and tigmin, and due to extensive alternative splicing [64,67,68], the characterization of the protein poses a formidable problem. Fibrocystin/polyductin/tigmin contains up to 4,074 amino acids and is predicted to be an integral membrane protein with a single membrane-spanning domain. The vast majority of the protein supposedly extends into the extracellular space, which together with the presence of several characteristic domains suggests that fibrocystin/polyductin/tigmin mediates cell-cell and/or cell-matrix contacts. Mutations have been described over virtually the entire length of the *PKHD1* gene. There does appear to be a genotype-phenotype correlation in so far as patients with missense mutations show a more benign course of the disease [69-72]. By immunohistochemistry, fibrocystin/polyductin/tigmin has been localized to primary cilia [68,73-75], which fits well with the finding that primary cilia are shortened and malformed in the *pck* rat, a rodent model with a spontaneous mutation in the *Pkhd1* gene [73]. It is remarkable that two recent publications already provide evidence on the regulation of the *Pkhd1* gene in the mouse. The inactivation of the *Tcf2* gene, which encodes the transcription factor Hnf1β, leads to renal cyst formation. By several assays Hnf1β was shown to bind to the promoter of the *Pkhd1* and also to that of the *Pkd2*, *Umod* (the gene encoding Uromodulin/Tamm-Horsfall protein, see below) and *Ttc10* (the gene encoding Polaris, see below) genes, thus giving a strong hint how cysts develop in this model [76,77]

THE NEPHROCYSTINS

At a birth prevalence of ~1:80,000 nephronophthisis is a rare disease [78]. It is inherited in an autosomal-recessive fashion and consists of several subtypes. Patients suffering from nephronophthisis typically form renal cysts at the cortico-medullary border. On the light microscopic level a pronounced tubulointerstitial fibrosis and both a thickening and a fragmentation of the tubular basement membrane can be observed [79,79a]. End-stage renal disease usually develops in the first two decades of life, although the course of the disease depends on what genes are mutated [80]. Thus far, positional cloning has identified five mutated genes. Identification of *NPHP-1*, the gene encoding nephrocystin-1, was reported in 1997 [81,82]. *NPHP-1* is mutated in ~85% of patients with nephronophthisis. Nephrocystin-1 has been localized to different cellular compartments. In early publications nephrocystin-1 was reported to associate with $p130^{Cas}$, tensin and Pyk2 [83,84], proteins typically associated with focal adhesions. Somewhat surprisingly, however, nephrocystin-1 and $p130^{Cas}$ co-localized at sites of cell-cell contacts [83], to which the former is targeted by virtue of a conserved domain at its COOH-terminus [85]. The interaction of nephrocystin-1 with filamins [85], a family of actin-associated proteins, is consistent with its location at cell contacts. In later publications, however, nephrocystin-1 has also been found to interact with β-tubulin, which in turn is consistent with the second site it has been found at—primary cilia [86].

The *NPHP2* gene turned out to be the human orthologue of the murine *Invs* gene [86]. The *Invs* mutant strain of mice, which in addition to polycystic kidneys also present with *situs inversus*, was first described in 1993. This strain resulted from the random integration of a transgene into the *Invs* locus [87]. The corresponding protein, inversin, contains 16 ankyrin repeats at its NH_2-terminus [88,89] and was localized to centrosomes and primary cilia [86,90,91], which fits well with the observation that inversin associates with the anaphase promoting complex protein Apc2 [90] and with tubulin [92]. It is remarkable that the inactivation of the *Apc2* gene in mice also results in polycystic kidney disease [93]. As in the case of nephrocystin-1, nephrocystin-2 was found at cell-cell contacts as well, where it is part of a complex with catenins and cadherins [94].

Very little is known about the other 3 genes that are mutated in nephronophthisis patients. The *NPHP3* gene is also mutated in the *pcy*/*pcy* mouse [95], a spontaneous mouse model of polycystic kidney disease [96,97]. It has not been reported where nephrocystin-3 is located, although its interaction with nephrocystin-1 [95] suggests that it is also located in primary cilia and at sites of cell-cell contact. Nephrocystin-4 (*syn.* nephroretinin) is a novel protein without homology to other known proteins [98,99], but it also associates with nephrocystin-1 [98,100] and $p130^{Cas}$, Pyk2 and α-tubulin [100]. Not surprisingly, nephrocystin-4 was detected in primary cilia and at sites of cell-cell contact [100]. Finally, nephrocystin-5 does not interact with the other known nephrocystins and Bardet-Biedl proteins (see below), but rather with calmodulin and RPGR, a protein mutated in patients with retinitis pigmentosa. Nonetheless, nephrocystin-5 is also located in primary cilia [101].

MEDULLARY CYSTIC KIDNEY DISEASE

Patients suffering from medullary cystic kidney disease present with very similar symptoms to those suffering from nephronophthisis, but medullary cystic kidney disease is inherited in an autosomal-dominant fashion and end-stage renal disease typically develops at the onset of the third decade of life [80]. Two loci have been identified in patients with medullary cystic kidney disease, but so far only one gene has been cloned. Whereas the *MCKD1* gene has not been identified yet, the *MCKD2* locus was found to be identical with the *UMOD* gene (first reported in [102]), which encodes Uromodulin (*syn.* Tamm-Horsfall protein), a GPI-anchored protein

confined to the thick ascending limb of the kidney [103]. How mutations in Uromodulin lead to renal cyst formation is a complete puzzle at present.

BARDET-BIEDL PROTEINS

Bardet-Biedl syndrome is a complex disease both phenotypically and genetically, with a prevalence that obviously very much depends on the population investigated and ranges from 0.7 to 7.4:100,000 [78]. Cardinal features of the patients are retinal dystrophy, obesity, polydactyly and renal abnormalities. The syndrome is caused by mutations in at least 8 different genes that have been identified since the year 2000. *BBS6* was the first gene to be cloned [104-106], but its sequence just as that of *BBS2* [107], *BBS4* [108], *BBS1* [109] and *BBS7* [110] provided only very limited clues regarding the pathogenesis of the disease. Although the primary sequence of the BBS8 protein also gave little insight into its function [111], it was localized to centrosomes and the basal body of primary cilia in mammalian cells. Indeed, the orthologues of the *BBS1*, *BBS2*, *BBS7* and *BBS8* genes in the nematode *Caenorhabditis elegans* were all exclusively expressed in ciliated neurons [111], the respective protein products are located in cilia and undergo intraflagellar transport [112]. No orthologue for *BBS6* has been identified *in C. elegans*, but its protein product has meanwhile been localized to the centrosome of mammalian cells [113]. Mutant worms lacking a functional *bbs-8* gene elaborated shorter cilia. *bbs-8* and *bbs-7* mutant worms also showed defects in intraflagellar transport [112], and similar results were also obtained for the BBS3 and BBS5 proteins. The *BBS3* gene encodes the ARL6 protein, a member of the Ras superfamily of monomeric G-proteins. In *C. elegans*, *bbs-3* is expressed in ciliated neurons and its protein product undergoes intraflagellar transport [114]. The *BBS5* gene was identified by comparative genomics, it encodes a novel protein whose orthologue in *C. elegans* is exclusively expressed in ciliated neurons. Knockdown of the nematode *bbs-5* gene resulted in a partial or complete loss of flagella [115]. These first intriguing findings in *C. elegans* have been expanded by knockout experiments in mice. Whereas no kidney abnormalities were reported after the inactivation of the *Bbs4* gene [116], polycystic kidneys were observed in *Bbs2* knockout mice [117].

MECHANISM OF CYST FORMATION: THE EMERGING ROLE OF PRIMARY CILIA

The mechanism underlying cyst formation still poses a mystery. Several scenarios such as dysregulated cell proliferation and apoptosis, missorting of membrane proteins and defects in the extracellular matrix have been put forward to explain cystogenesis, but none has found general acceptance [118]. Over the last few years an impressive amount of cirumstantial evidence has implicated primary cilia in the development of cysts. In contrast to kinocilia with their (9 x 2) + 2 arrangement of microtubules, primary cilia lack the central pair of microtubules and therefore save for one exception (primary cilia in the primitive node) are felt to be immotile. Although primary cilia have been found in many different tissues, among them the kidney [119-122], they were considered an evolutionary relict and consequently were neglected for a long time. Some of the first evidence that primary cilia could be important arose from the finding that the orthologues of polycystin-1 and polycystin-2 in *C. elegans* are located in the ciliated endings of sensory neurons [123]. The same is true for the orthologues of the Bardet-Biedl proteins (see above) and of Polaris [124], the product of a gene mutated in a murine model of polycystic kidney disease [125]. Those results in the nematode were confirmed and expanded in mammalian cells by showing that polycystin-1 [126], polycystin-2 [126,127], Polaris [126,128,129], fibrocystin/polyductin/tigmin (see above), most of the nephrocystins (see above), the BBS proteins (see above), OFD1 [130], a protein mutated in patients with oral-facial-digital syndrome, which is also associated with polycystic kidney disease [131], and cystin [126,129], which is mutated in a spontaneous model of polycystic kidney disease in the mouse [129], were also located in primary cilia. This poses the question whether primary cilia are affected in polycystic kidney disease. The data support a definitive “yes” (e.g. [73,91,132,133]). How might those proteins affect ciliary structure and function? Cultured cells respond to the bending of primary cilia with an increase in $[Ca^{2+}]_i$ [134], a response which may depend at least in some circumstances on intact polycystin-1 and -2 proteins [135]. While the role of polycystin-2 can be easily understood as a cation channel (see above), the roles of other proteins in cilia are probably divergent. Polaris, for example, has been implicated in intraflagellar transport and ciliary growth [124,136]. It is remarkable that the kidney-specific loss of a microtubule-associated motor protein, Kif3a, in the mouse results in polycystic kidney disease [137]. There are obviously additional genes that we have just become aware of and which may also figure prominently in cystic kidney disease [138].

SOME (CRITICAL) THOUGHTS ON PRIMARY CILIA, IN PARTICULAR THEIR ROLE AS MECHANO- AND CHEMOSENSORS

The evidence implicating abnormal primary cilia structure and function in the pathogenesis of polycystic kidney disease has almost become overwhelming in the recent past. But virtually all the evidence has been indirect so far. Many of the proteins discussed in this review article have not only been localized to primary cilia but also to other cellular compartments. It therefore remains to be formally shown that the lack of those proteins only or

primarily affects the function of primary cilia and no other cellular functions. For example, the Kif3a protein has a known function as a general microtubule-associated transport protein, such that it is likely that a number of other cellular functions are affected when Kif3a expression is absent. The knockout of the *Pkd2* gene in the mouse leads to *situs inversus*, possibly due to a defect of primary cilia located in the primitive node [133,139]. But so far no evidence has been presented that *situs inversus* also develops in the *Pkd1* knockout mouse although both proteins interact with each other [56,57]. It is also puzzling that the introduction of a wild-type Polaris cDNA into mice defective for Polaris prevents the development of *situs inversus*, but not of cyst formation. Furthermore the length of primary cilia and the formation of cysts did not correlate [140]. And finally it should also be pointed out that the *PKD2* gene is not uniformly expressed throughout the kidney but that its strongest expression is found in the distal tubule [50-52]. Primary cilia, however, are present in the whole nephron and in collecting ducts, which would suggest that polycystin-2 is not or not at equally high levels necessary for all primary cilia to function properly.

Two mechanisms have been put forward by which tubular dimensions may be measured based on the function of primary cilia. One hypothesis resorts to the putative mechanosensory role of primary cilia, while the other stresses their chemosensory role. There has been increasing evidence that fluid flow causes an increase in $[Ca^{2+}]_i$ and that this increase is mediated by primary cilia [134,135]. Assuming a constant delivery of fluid, the velocity of the fluid will be low in a tubule with a wide lumen and high in a tubule with a narrow lumen (Figure **4**). Thus the velocity with which the filtrate flows through the tubule may serve to indicate the diameter of the tubular lumen. If the velocity is too high, the tubule will expand further, if the flow rate is appropriate or too slow, the tubule will stop expanding. Although such a scenario appears very reasonable and straightforward, a few caveats shall be mentioned. Of course the mechanosensory model will only work if a constant volume of primary filtrate will be delivered by the glomerulus to the tubule, which probably is not the case. Another problem arises from the fact that ~99% of the primary filtrate is reabsorbed along the tubule and that the reabsorption rate in the collecting duct varies depending on the homeostatic requirements of the body. In other words, the velocity in any given nephron segment and the collecting ducts may vary considerably and will not only depend on the tubular dimensions. Let us look at the proximal tubule to illustrate the point. In the proximal tubule alone ~two-thirds of the primary filtrate is reabsorbed. In order to compensate for this loss of fluid from the tubular lumen, the set point for the desired velocity would have to vary along the length of the proximal tubule, which of course is possible but somehow hard to imagine. Furthermore, in the case of the proximal tubule we are also faced with an anatomical difficulty because the primary cilium is buried in the brush border [140], making it difficult to envision how the cilium can freely respond to mechanical forces. Evidence has been presented that the brush border of the proximal tubule serves a mechanosensory role [141], which would imply that primary cilia are not general mechanosensors.

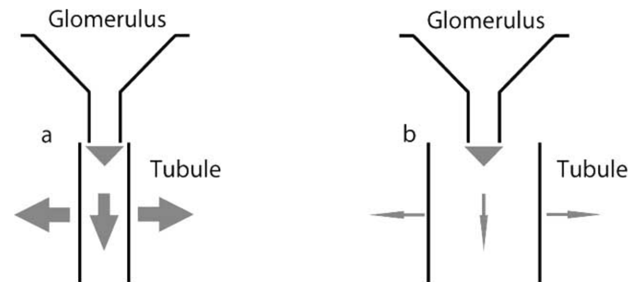

Figure 4. Mechanosensory control of lumen geometry. Assuming a constant flow of primary filtrate (arrow head) from the glomerulus to the tubule, the width of the tubular lumen can be measured through the velocity of the tubular fluid. (a) In the case of too narrow a lumen, the velocity will be high and the tubule will still expand. (b) Once the tubular diameter is about to reach its proper dimension, the velocity will decrease and tubular expansion will cease. The problems inherent in this model are discussed in the text.

Work done in the worm *C. elegans* argues for a chemosensory role of cilia [123,124,142], which leads us to the question how the tubular diameter could be determined via a chemical signal. Simply speaking, the concentration of a given amount of any substance will depend on the volume it is dissolved in. Therefore tubular dimensions may be measured by determining the concentration of a molecule in the filtrate. So far it is purely hypothetical whether such a substance exists but it is worth thinking about the characteristics such a substance "X" should have (Figure **5**). Its concentration should only depend on the tubular dimension and not on the reabsorption processes along the length of the nephron and the collecting duct. It is therefore unlikely that molecule "X" reaches the tubule from the blood, because the concentration of "X" in the tubule will depend on its serum concentration and its filtration coefficient and not on the tubular dimensions. If a substance that reaches the tubule from the blood is reabsorbed in the same fashion along the nephron as water, its tubular concentration will not change nor depend on the tubular dimensions, but rather on its serum concentration. If a substance, which reaches the tubule from the blood, is not reabsorbed, its concentration will increase along the nephron. Therefore it has to be postulated that "X" is produced at a constant rate in the kidney, ideally the podocytes, to ensure that its concentration only depends on the tubular dimensions. Furthermore, the tubular concentration of "X" should not vary along the length of the

nephron and the collecting duct, i.e. it should be independent of the reabsorption process. In order to fulfil this condition, "X" should behave like water. Although at first sight those demands appear rather high, they are not unreasonable and would be much better suited to determine the tubular diameter than a mechanical stimulus.

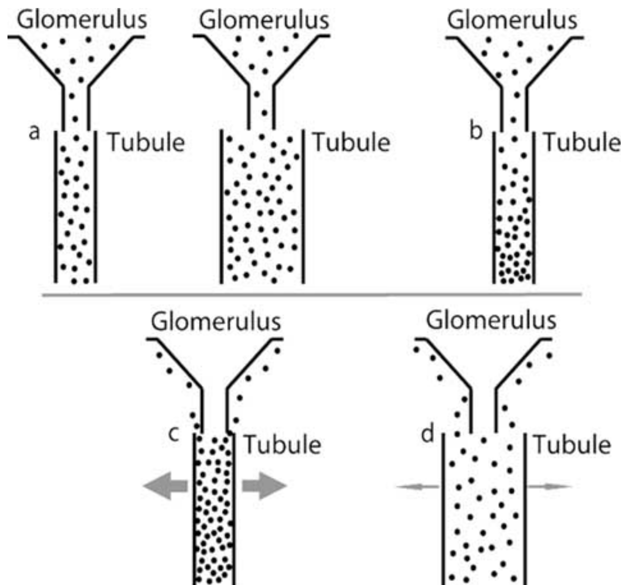

Figure 5. Chemosensory control of lumen geometry. The width of the tubular lumen could also be determined through the concentration of a substance "X" (depicted as full circles), which should be produced in the kidney itself because then its concentration will depend on the tubular geometry. The first two models (a and b) are therefore unlikely to work. (a) If "X" reaches the tubule from the blood and is reabsorbed to the same extent as water, its concentration will be independent of the reabsorption processes and will not vary along the length of the nephron (in this case the filtration coefficient of "X" has been assumed as 1, i.e. its concentration in the blood and in the primary filtrate are identical). However, it would not be suitable as a parameter to measure the tubular dimension because its tubular concentration would be determined by its plasma concentration and will be no different in a tubule with a narrow (left) or a wide lumen (right). (b) If "X" reaches the tubule from the blood and is not reabsorbed to the same extent as water, its concentration will increase along the length of the nephron and it again could not be used as a parameter to determine tubular geometry. (c, d) If "X" is produced at a constant rate in the kidney itself (e.g. as depicted here in the glomerulus), it can be used as a parameter to determine the tubular dimensions. The concentration of "X" will be high if it distributes in a small volume (c) and it will be low if it distributes in a large volume (d).

PERSPECTIVES

The problem of geometry is one of the fundamental issues still unresolved in biology: How is the diameter of blood vessels, of the tracheo-bronchial tree or any other system of ductal structures determined (why is the aorta so wide and the capillaries so narrow), or how does the liver know to what extent to regrow after partial hepatectomy? At least in the case of the renal tubules, the family of cystic kidney diseases may give us important insights into this fundamental problem, although it is certainly possible that the respective proteins have nothing do with determining the dimensions of the renal tubules. All of a sudden the kidney has entered the limelight of basic research in cell biology and promises to teach us some very important lessons.

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