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Molecular basis of autosomal-dominant polycystic kidney disease

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Abstract. Autosomal-dominant polycystic kidney disease (ADPKD) is one of the most common monogenetic diseases in humans. The discovery that mutations in the *PKD1* and *PKD2* genes are responsible for ADPKD has sparked extensive research efforts into the physiological and pathogenetic role of polycystin-1 and polycystin-2, the proteins encoded by these two genes. While polycystin-1 may mediate the contact among cells or between cells and the extracellular matrix, a lot of evidence suggests that polycystin-2 represents an endoplasmic reticulum-bound cation channel. Cyst development has been compared to the growth of benign tumors and this view is highlighted by the model that a somatic mutation in addition to the germline mutation is responsible for cystogenesis (two-hit model of cyst formation). Since in vitro polycystin-1 and polycystin-2 interact through their COOH termini, the two proteins possibly act in a common pathway, which controls the width of renal tubules. The loss of one protein may lead to a disruption of this pathway and to the uncontrolled expansion of tubules. Our increasing knowledge of the molecular events in ADPKD has also started to be useful in designing novel diagnostic and therapeutic strategies.

Key words. *PKD1*; *PKD2*; polycystin-1; polycystin-2; ADPKD; polycystic kidney disease.

Introduction

Polycystic kidneys are encountered in an acquired and a hereditary form, and the latter can be transmitted in an autosomal-recessive (ARPKD) and an autosomal-dominant (ADPKD) fashion. Since the gene responsible for ARPKD has not yet been identified, the focus of this review will be the two genes mutated in patients with ADPKD, *PKD1* and *PKD2*. Findings in various animal models of PKD will also be discussed where appropriate. The cardinal finding in polycystic kidneys is the continuous formation of cysts, i.e., fluid-filled cavities lined by an epithelium. These cysts arise predominantly from proximal tubules and collecting ducts, as shown by microdissection and immunohistochemistry [1–6], although the preferred sites of cyst origin seem to differ among models [7]. ADPKD may eventually lead to chronic renal failure, with patients requiring dialysis and transplantation. Approximately 50% of ADPKD patients reach end-stage renal disease by the age of 60 [8–10], thus contributing to almost 10% of all cases of chronic renal failure [11–15]. The course of the disease apparently depends on the nature of the mutated gene, because patients with mutations in *PKD1* reach end-stage renal disease at an earlier age than those with mutations in *PKD2* [9, 10, 16–19]. Furthermore, both for *PKD1* [20] and for *PKD2* [21], a genotype-phenotype correlation,

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i.e., the correlation of a mild or severe prognosis with a certain mutation, has been suggested.

PKD1, the first gene identified in patients with ADPKD

Molecular genetics of *PKD1*

The finding that a region on chromosome 16 was linked to ADPKD was published in 1985 [22], but it took almost 10 years before the *PKD1* gene could be identified in this region [23]. Since then, a multitude of mutations have been described in polycystin-1, the protein product of *PKD1*, but no obvious mutational hot spots have become apparent so far [24].

Subsequent to the identification of human polycystin-1, related proteins have also been identified in the mouse [25], *Fugu* [26], and *Caenorhabditis elegans* [27]. The human *PKD1* gene is organized in 46 exons, which span ~52 kbp of DNA and are transcribed into an mRNA of ~14 kb. It lies very close to the *TSC2* gene in a tail-to-tail orientation, and so deletions affecting both genes have been described [28–30]. Mutations in *TSC2* are responsible for tuberous sclerosis, a syndrome which among other symptoms also presents with a comparatively mild form of polycystic kidney disease [31]. If, however, both *TSC2* and *PKD1* are mutated, pronounced polycystic kidney disease will develop [30]. A molecular explanation for this phenomenon may lie in the recent finding that tuberin, the protein product of the *TSC2* gene, is important for the trafficking of polycystin-1 from the Golgi apparatus to the plasma membrane. In cells lacking tuberin, polycystin-1 is retained in the Golgi compartment, and upon expression of exogenous tuberin in those cells, polycystin-1 is able to escape from the Golgi apparatus and is detected at the sites of cell-cell contact [32], where it may participate in a complex with E-cadherin [33–35].

Domain structure and possible functions of polycystin-1

Polycystin-1 represents a 4302-amino acid protein with a M_r of 462 kDa [36–38]. It is predicted to contain a signal peptide at the NH_2 terminus and several (up to 11) hydrophobic domains at the COOH terminus. Other characteristic motifs such as a leucine-rich domain, so-called PKD repeats, a C-type lectin domain, an LDL-A-like motif, and the REJ domain lie between the putative signal peptide and these proposed membrane-spanning domains [37, 38]. Hydropathy analysis of polycystin-1 in combination with its particular domain structure has been taken as evidence that polycystin-1 is located in the plasma membrane, that the NH_2 terminus extends into the extracellular space and that the COOH terminus extends into the cytoplasm, but so far no direct experimental evidence has been presented for these assumptions. The possibility to affinity-purify polycystin-1 from permanent cell lines [39] should help to answer some of these questions.

Two domains in particular, the REJ (receptor for egg jelly) motif and the PKD repeats, have attracted a lot of attention. The REJ motif was first described in a receptor which mediates the interaction of sea urchin sperm with glycoproteins in the egg jelly [40]. Upon binding of the sperm to the egg, a distinct pattern of ion fluxes results, which ultimately lead to the acrosome reaction [41]. A polycystin-1-related protein exists, PKDREJ, which also contains the REJ motif; since its corresponding mRNA was only detected in the testis [42, 43], it may be important for fertilization of the egg. Whether the REJ domain in polycystin-1 also binds to sugar moieties, and polycystin-1 therefore mediates the interaction with neighboring cells and/or the extracellular matrix, is unknown at this point. In this context, another motif in the NH_2 terminus of polycystin-1 should be mentioned, a C-type lectin domain, which may serve a related function, since a fusion protein of this domain with glutathione *S*-transferase is able to bind to various carbohydrate structures in vitro [44].

Polycystin-1 also contains a total of 16 PKD repeats, each being 80–90 amino acids long. Since these motifs assume an immunoglobulin-like fold [45], they have been proposed to represent cell adhesion receptors. In vitro studies have indeed demonstrated a homophilic interaction between PKD repeats and have provided evidence that PKD repeats mediate cell-cell contacts [46]. Furthermore, peptides derived from PKD repeats have been reported to be capable of inhibiting branching morphogenesis in renal organ culture [47]. Although these findings are very intriguing, they need to be reconciled with the fact that polycystin-1 may not be expressed in ureteric buds [48] and that early nephron development does not appear to be disturbed in *Pkd1* knockout mice [48–50].

So far, the function of polycystin-1 has remained a mystery. Much research effort has concentrated on its COOH terminus, since it has been suggested to extend into the cytoplasm and could therefore relay extracellular signals. This portion of polycystin-1 was shown to influence the activity of the monomeric G proteins, Rac-1 and Cdc42 [51], and of the trimeric G proteins, G_i and G_o [52, 53], it activates the protein kinases C and JNK and the transcription factor AP-1 [51], it modulates the Wnt signal transduction cascade [54], and it regulates a cation channel [55]. Although many if not all of these signaling pathways could play a role in cystogenesis, their precise contribution to cyst formation is not known. The COOH terminus of polycystin-1 is also serine- [56, 57] and tyrosine-phosphorylated [56, 58] under certain circumstances, and polycystin-1 may therefore serve as an important intermediate or end-point of a signal transduction cascade. Noteworthy in this context is that while the

growth of M-1 cells, a murine collecting duct cell line, is inhibited by cAMP, overexpression of the COOH terminus of polycystin-1 in those cells leads to a growth-promoting effect of cAMP [59].

Mouse models for *Pkd1*

Several knockout and transgenic mice have been established to better understand the function of polycystin-1. In the first *Pkd1* knockout mouse generated, exon 34 was replaced by the selection cassette [49]. This resulted in the appearance of renal and pancreatic but not hepatic cysts. The predicted Mendelian ratio among the offspring was maintained until embryonic day 18.5, but only a few *Pkd1* (–/–) mice survived to term. The second *Pkd1* knockout mouse was generated by deleting parts of exons 43 and 45 and all of exon 44 [50]. As in the first *Pkd1* knockout mouse, cysts developed in the kidneys and the pancreas, but not in the liver. However, none of these (–/–) mice survived beyond embryonic day 15.5, death probably being due to vascular leakage and hemorrhage. An even earlier embryonic lethality was reported when exons 17–21 of the *Pkd1* gene were deleted [48]. In this third model, *Pkd1* (–/–) mice died between embryonic day 13.5–14.5, again probably because of edema formation and hemorrhage. Renal cyst formation in these *Pkd1* (–/–) mice was mentioned, but whether cysts developed in the pancreas and the liver was not reported. Furthermore, they displayed cardiac and skeletal defects, which were apparently not present in the other two *Pkd1* knockout models. Finally, transgenic mouse lines were established, which contained multiple copies of a P1-derived artificial chromosome with the human wild-type *PKD1* gene [60]. Almost 40% of transgenic mice developed renal (mostly glomerular) cysts and 16% developed hepatic cysts. While these studies indicate that the lack of polycystin-1 leads to renal cyst formation, the variable phenotypes are somewhat puzzling. One possible explanation could be that the mutated genes in the *Pkd1* knockout mice do not represent null alleles and that the expression of the mutant proteins interferes with essential cellular functions. Depending on the type of mutant protein generated, only certain tissues may be affected and the phenotype may be more or less severe. The result of the transgenic experiment [60] further suggests that the expression level of polycystin-1 may also be an important parameter, although one cannot be sure that the human and murine polycystin-1 proteins are functionally identical. That the human protein acts like a mutant protein in the mouse cannot be ruled out.

Distribution of polycystin-1

Despite much research effort, the organ distribution, expression pattern in the kidney, subcellular location, and membrane topology of polycystin-1 are still a matter of debate. A number of monoclonal and polyclonal antibodies against polycystin-1 have been raised to determine its expression pattern, resulting in partly overlapping, partly discrepant results [for a review see ref. 61]. The recent description of the *Pkd1* knockout mouse, in which exons 17–21 of *Pkd1* were deleted and a *LacZ* gene was inserted instead, should help to answer some of the open questions [48]. In this knockout mouse, β-galactosidase activity was absent in the ureteric buds and the early stages of nephron development such as comma- and S-shaped bodies, whereas low levels of β-galactosidase activity were detected in collecting ducts and throughout the fully differentiated nephron. Consistent with the appearance of hepatic and pancreatic cysts and the observation of cerebral aneurysms and cardiac valve defects in ADPKD patients, β-galactosidase activity was also observed in bile ducts and pancreatic ducts as well as in the cardiovascular system. However, no association between polycystic kidney disease and skeletal abnormalities has yet been reported; it is therefore somewhat surprising that β-galactosidase activity was high in cartilage and these *Pkd1* knockout mice also suffer from bone defects [48].

Mutations in the *PKD2* gene are the secondmost common cause of ADPKD

Polycystin-2 is a member of a large protein family

While the *PKD1* gene is mutated in ~85% of patients with ADPKD, the majority of the remaining patients suffer from mutations in the *PKD2* gene [18, 62–64], which was identified 2 years after the cloning of *PKD1* [65]. Although *PKD2* consists of only 15 exons, it spans ~68 kbp of genomic DNA, i.e. it is longer than *PKD1* [66]. By hydropathy analysis, six transmembrane domains are predicted for polycystin-2 [65], the protein product of the *PKD2* gene, with both the NH_2 and COOH termini extending into the cytoplasm, but experimental evidence for this topology is sparse. An antibody directed against the NH_2 terminus of polycystin-2 failed to stain non-permeabilized transfected cells [67], thus supporting the assumption that this portion of polycystin-2 is located in the cytoplasm. Since the initial description of polycystin-2, three other related proteins have been identified. The first has been named polycystin-2L [43, 68] and polycystin-L [69], the second, polycystin-2L2 [43], and the third, mucolipin [70, 71] and mucolipidin [72]. While the first two proteins are ~45% identical to polycystin-2, mucolip(id)in only shows ~20% identity to polycystin-2. All three related proteins are predicted to contain six transmembrane domains and together with polycystin-2 form a subfamily of hexahelical integral membrane proteins.

Expression pattern and intracellular distribution of polycystin-2

Most of the evidence collected so far indicates that in the kidney, polycystin-2 is located in the basal compartment of tubular cells, particularly the thick ascending limb, distal convoluted tubule, and connecting tubule [34, 73, 74] (fig. 1), although alternative data have also been presented [75]. A basal staining pattern for polycystin-2 has also been demonstrated in the striated ducts of salivary glands [74] and the ducts of paranasal sinuses [73], but in all other organs, polycystin-2 is distributed in a punctate or reticular pattern in the cytoplasm. The different intracellular distribution appears to be reflected by an organ-specific glycosylation pattern in so far as polycystin-2 is more heavily *N*-glycosylated in the adrenal gland and ovary than in the kidney and salivary gland [74, 76]. A cytoplasmic reticular or punctate distribution of polycystin-2 has also been observed in many cell lines, which together with biochemical analyses localizes polycystin-2 to the endoplasmic reticulum [67, 77] (fig. 2). The endogenous protein in the kidney is sensitive to digestion with endoglycosidase H, which argues for its location in the endoplasmic reticulum of renal tubules as well [67]. To correlate the basal distribution pattern of polycystin-2 in the distal nephron by immunohistochemistry with the biochemical evidence for the location of polycystin-2 in the endoplasmic reticulum, the special arrangement of the endoplasmic reticulum in tubular cells has to be considered. Distal tubular cells are connected by extensive lateral interdigitations, which contain many mitochondria. The endoplasmic reticulum lies between the mitochondria, which are arranged with their longitudinal axis parallel to the apical-to-basal axis of the cell and the plasma membrane [78, 79]. Such a complex relationship between adjacent cells, mitochondria, the endoplasmic reticulum, and the plasma membrane must be carefully maintained, and the peculiar distribution of polycystin-2 in the kidney may also tell us something about its function.

Functional characteristics of polycystin-2

The homology of polycystin-2 to α subunits of voltage-activated calcium channels and the interaction of polycystin-2 with TRPC1 [80], a member of the transient receptor potential channels, has led to great efforts to

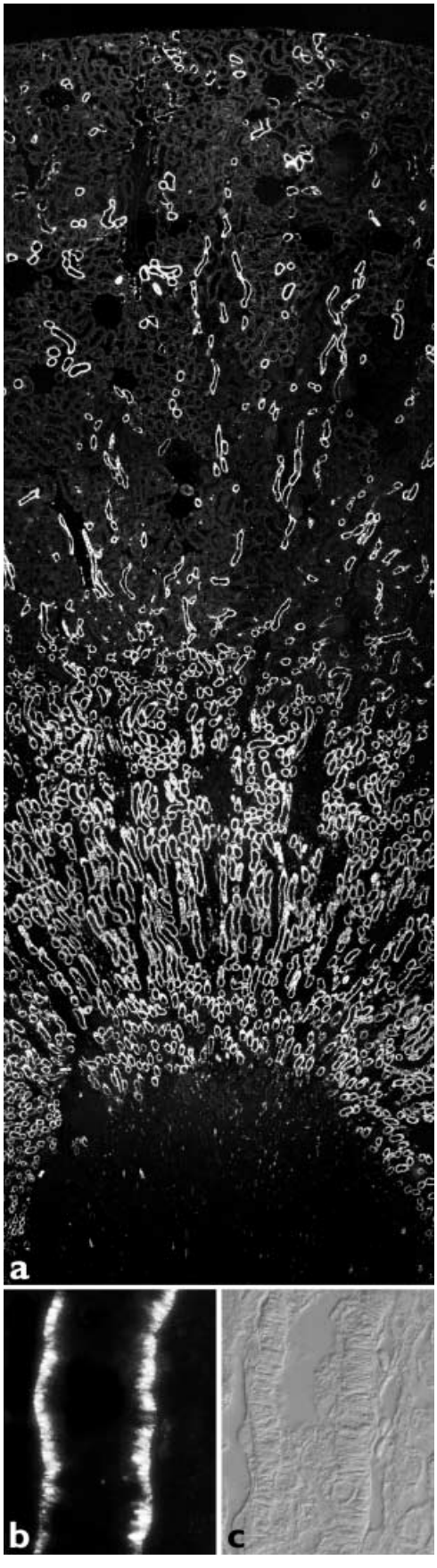

Figure 1. Distribution of polycystin-2 in the kidney. Immunofluorescence staining of an adult rat kidney with a rabbit polyclonal anti-polycystin-2 antibody [123] demonstrates that tubular profiles with the strongest immunoreactivity are present from the cortex through the inner stripe, which already hints at the distal nephron as the predominant site of expression (*a*). A higher magnification of a distal tubule shows that polycystin-2 is located in the basal compartment of tubular cells (immunofluorescence in *b*, interference-phase contrast in *c*). Taken with permission from Obermüller et al. [74].

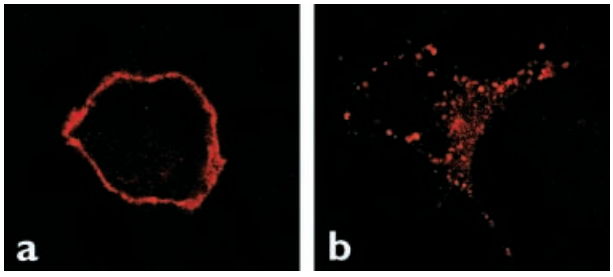

Figure 2. The COOH terminus of polycystin-2 contains an endoplasmic reticulum retention signal. COS-7 cells were transiently transfected with expression plasmids encoding a truncated CD8 protein (*a*) or a fusion protein between CD8 and the COOH terminus of human polycystin-2 (*b*). Two days after transfection, cells were stained with a monoclonal anti-CD8 antibody and positive cells were visualized by confocal microscopy. While the CD8 protein reaches the plasma membrane (*a*), the CD8/polycystin-2 fusion protein is retained inside the cell (*b*).

demonstrate channel activity for polycystin-2 as well. Transient expression of polycystin-2L/polycystin-L, one of the proteins closely related to polycystin-2, in *Xenopus* oocytes resulted in the appearance of a rather non-selective cation channel with a very large unitary conductance [81], thus further fueling the assumption that polycystin-2 can conduct cations. Three recent reports indeed provide experimental evidence for the role of polycystin-2 as a cation channel. Employing transient expression of both polycystin-1 and polycystin-2, the authors of the first publication reported the novel appearance of a non-selective, outwardly rectifying cation channel in the plasma membrane of CHO cells. This channel was approximately six times more permeable to Ca^{2+} than to Na^{+} and Cs^{+}, and was furthermore inhibited by Ca^{2+}, La^{3+}, and niflumic acid [82]. When polycystin-2 was expressed alone, no novel channel activity could be recorded, which correlated well with its intracellular location. In the absence of polycystin-1, polycystin-2 remained in the endoplasmic reticulum, whereas in its presence it was able to reach the plasma membrane [82]. In a second report, evidence was presented that polycystin-2 is located in the apical plasma membrane of human syncytiotrophoblast cells, where it is responsible for a non-selective cation conductance [83]. This initial observation was corroborated by the expression of polycystin-2 in Sf9 insect cells and by the incorporation of polycystin-2 into lipid bilayers. As described in the first publication [82], polycystin-2 channel activity could be inhibited by Ca^{2+} and La^{3+} and furthermore by Gd^{3+} and amiloride [83]. The trafficking of polycystin-2 to the plasma membrane of Sf9 insect cells is quite remarkable, since in all cell lines investigated so far, polycystin-2 was detected in the endoplasmic reticulum. The insect cells possibly produced so much polycystin-2 that not all of it could be retained in the endoplasmic reticulum, or the retention signal was not recognized by the insect cells. In a third study, *Xenopus* oocytes were employed to examine the channel properties of polycystin-2 [84]. Using proteasome inhibitors and chemical chaperones, the authors succeeded in translocating polycystin-2 to the plasma membrane in a substantial portion of the injected oocytes, thus creating a novel non-selective cation conductance, which was activated by the addition of 1 µM Ca^{2+} and inhibited by La^{3+} and millimolar concentrations of Ca^{2+} [84]. In contrast to one of the previous studies [82], the truncation mutant polycystin-2 R742X still exhibited channel activity [84, 85].

Possible mechanisms of cyst formation

The stop-signal hypothesis of cyst formation

The tubular structures in the kidney originate from two different precursor tissues. Whereas the nephrons are derived from the metanephrogenic mesenchyme, the ureteric bud gives rise to the collecting ducts [86]. Differentiation of the metanephrogenic mesenchyme in particular is a remarkable achievement, because a mesenchymal has to transform into an epithelial structure, i.e., a lumen has to form (fig. 3a). Looking at the rather uniform and characteristic dimensions of any given nephron segment, one has to assume that this process is tightly regulated. There must be a signal which tells the cells to initiate lumen formation, i.e., to loosen cell-cell contacts at their apical plasma membranes, and another mediator, which conveys a stop signal once enough lumen is established (how tubular geometry is sensed by the cells is completely unknown). The expanding tubules have to degrade the surrounding extracellular matrix, and indeed they have been shown to synthesize matrix metalloproteinases [87, 88], key enzymes for the breakdown of matrix components [89, 90]. Along these lines, cystogenesis can be considered as a process, in which tubular cells are lacking this stop signal due to mutations in polycystin-1 and polycystin-2, and continue to expand [76]. Such an assumption is supported by our recent finding that cyst-lining epithelial cells express matrix metalloproteinase 14, a key membrane-bound metalloproteinase, and that treatment of (*cy*/+) rats, a rodent model of ADPKD, with a metalloproteinase inhibitor leads to decreased cyst formation [91]. Secondly, if only the stop signal is lacking, then tubular differentiation per se should not be affected, but dedifferentiation of cyst-lining cells should occur after cyst formation has begun. The fact that nephrogenesis is not affected in the *Pkd1* knockout mice [48–50] together with our results in (*cy*/+) rats support this point of view, since the loss of tubular markers in (*cy*/+) rats is a secondary and not a primary event [92].

What might be the role of the polycystins in transmitting a signal stopping lumen formation in tubular cells? Polycystin-1 and polycystin-2 have been shown to interact

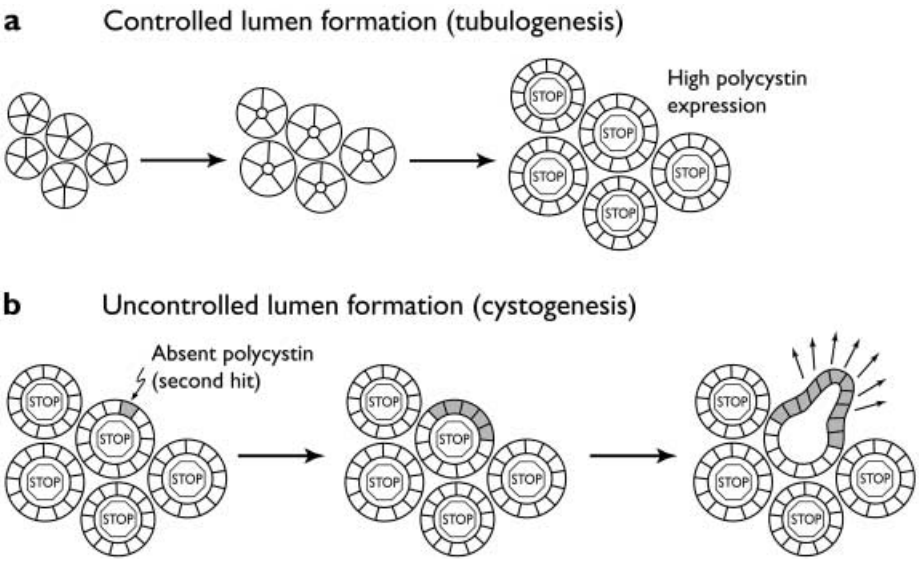

Figure 3. Model for tubulogenesis and cyst formation. (*a*) Renal tubules originate from mesenchymal precursor cells, i.e., the metanephrogenic mesenchyme. Upon induction by the ureteric bud, the mesenchymal cells aggregate and begin to form a lumen. At a certain point, the tubular cells have to receive a so far unknown stop signal and then lumen formation arrests. Most of the available evidence shows that the expression of polycystin-2 is highest in fully developed tubules, and polycystin-2 may therefore be a component of the sensor which measures the width of the lumen, or part of the stop signal. (*b*) Patients with autosomal-dominant polycystic kidney disease carry a germline mutation in one of the *PKD* genes. At any given time, a somatic mutation may occur in the second allele of the affected *PKD* gene, thus creating a tubular cell (shaded) without functional polycystin protein. This cell may have a proliferative advantage, so that it slowly displaces the cells in which no second hit has taken place. Since the stop signal mechanism is no longer functional, lumen formation starts again and a cyst begins to develop.

through their respective COOH termini [93, 94], and while such an interaction usually takes place between two proteins located in the same intracellular compartment, there is also precedent from the analysis of the interaction between the ryanodine receptor, IP_3-receptor, and TRP channels that even two integral membrane proteins in different cellular membranes can interact [95–97]. Polycystin-1 could therefore very well be located in the plasma membrane and participate in cell-cell contacts, whereas polycystin-2 serves as an endoplasmic reticulum-bound calcium channel. Due to the close apposition of the plasma membrane and the endoplasmic reticulum in the distal tubule [78, 79], such a scenario can be easily envisioned. In such a model, polycystin-1 as a component of cell-cell contacts would sense the width of the tubular lumen, and the signal would be transduced via polycystin-2 and local Ca^{2+} currents. The disruption of cell-cell contacts would explain the increased mitotic index (loss of cell contact inhibition) in cyst wall epithelia [98–100] and the loss of polarity observed under certain circumstances [101]. Of further note is that transgenic mice expressing a constitutively active β-catenin mutant develop polycystic kidney disease [102, 103]. Experimental support for the role of polycystin-1 in these processes stems from a recent publication, which demonstrated that expression of exogenous polycystin-1 in MDCK cells, a well-differentiated canine renal epithelial cell line, inhibits cell proliferation and shifts the cells away from cyst formation toward tubulogenesis [104]. Furthermore, exogenous polycystin-1 protected the cells from apoptosis upon serum withdrawal [104], which correlates well with the fact of increased apoptosis in polycystic kidneys [99, 105, 106]. Not only cell-cell, but also cell-matrix contacts may be involved in maintaining tubular geometry, because the inactivation of the genes coding for tensin [107] and Rho GDIα [108] leads to cyst development. Furthermore, nephrocystin, one of the proteins mutated in juvenile nephronophthisis type 1, another renal cystic disease, associates with tensin, $p130^{Cas}$ and Pyk2 [109, 110]. All those proteins have been associated with focal adhesions, so a mutation in nephrocystin may lead to dysfunctional cell-matrix contacts in tubular cells, thus rendering the cells unable to measure the width of the tubular lumen and leading to continued tubular expansion.

The two-hit hypothesis of cyst formation

One puzzling observation in polycystic kidney disease remains the focal nature of cyst formation. Out of the $\sim 1 \times 10^6$ nephrons in a human kidney, only an estimated 5% develop cysts. This phenomenon resembles the growth characteristics of tumors, which are caused by mutations in tumor suppressor genes. Similar to Knudson's two-hit hypothesis, which states that patients with those tumors suffer from a germline mutation in one

allele of a certain tumor suppressor gene and tumors develop in those cells in which the second allele is inactivated by a somatic hit [111], second hits have also been observed in cyst wall epithelia (fig. 3b). However, neither for *PKD1* [112–116] nor for *PKD2* [117–119] have mutations been described in all cysts investigated. While this result could be due to the challenging techniques necessary to detect a somatic hit, other mechanisms such as trans-heterozygosity may also be responsible. Trans-heterozygosity describes the fact that in renal cysts of patients with a germline mutation in the *PKD1* gene, somatic mutations in *PKD2* have occurred and vice versa [120, 121]. Results from gene inactivation experiments in mice add further support for the two-hit model of cyst formation. *Pkd1* (+/–) knockout mice show a small number of cysts after a rather long observation period [48, 122], and somatic intragenic inactivation has been made responsible for cyst development in a more complex *Pkd2* knockout mouse model [123]. In the latter case, no polycystin-2 immunoreactivity was detected in the cyst-lining epithelial cells, thus indicating the loss of both *Pkd2* alleles [123]. It would be interesting to examine whether spontaneous renal cysts, which are not an unusual finding in ultrasound investigations, also develop as a result of mutations in the *PKD1* and *PKD2* genes.

Several open questions still remain concerning the two-hit hypothesis of cystogenesis. For one, some authors were not able to detect trans-heterozygosity in their samples of polycystic kidneys [113, 119]. Furthermore it is puzzling, that many cyst-lining epithelial cells still show immunoreactivity for the respective polycystin protein [75, 124–129]. In the case of *PKD2,* there is no indication that somatic mutations are preferentially missense mutations, which would probably preserve the immunoreactivity of the polycystin-2 protein [130]. Nonsense mutations, insertions, and deletions, which are commonly observed as germline and somatic mutations, will lead to a loss of polycystin-2 expression and therefore polycystin-2 should no longer be detected by immunohistochemistry.

We also need to consider why there is a dominant and a recessive pattern of inheritance. If the two-hit hypothesis is correct and a somatic mutation has to occur in the second allele of a *PKD* gene, such a mechanism should in principle also be possible in the case of a gene mutated in patients with ARPKD. Does that mean that two germline mutations have to be present in the genes causing the autosomal-recessive forms, because a somatic mutation is unlikely to occur in those genes? Why might the *PKD1* and *PKD2* genes be so much more susceptible to somatic mutations? In the case of the human *PKD1* gene, a polypyrimidine tract has been made responsible for its high somatic mutation rate, but this particular sequence has not been found in the murine *Pkd1* gene [131] or the *PKD2* gene, making it somewhat more difficult to explain the appearance of cysts in heterozygous *Pkd1* knockout mice and cyst formation when polycystin-2 is involved.

The use of molecular findings in the diagnosis and treatment of polycystic kidney disease

Clusterin as a possible urinary marker for polycystic kidney disease

A cheap and easy assay, which could be employed both to screen for patients with polycystic kidney disease and to monitor the course of the disease, would be very useful. Previous publications have reported that polycystic kidneys produce clusterin, a heterodimeric glycoprotein induced after several types of injury [92, 132–134]. We have established an ELISA which can be used to monitor the urinary excretion of clusterin, and have shown that male (*cy*/+) rats, which represent a widely used model for autosomal-dominant polycystic kidney disease, excrete increased amounts of clusterin in comparison to age-matched wild-type rats [S. Hidaka, B. Kränzlin, N. Gretz, R. Witzgall, unpublished data]. Such an assay or modifications thereof may also prove convenient to determine the efficacy of a therapy against polycystic kidney disease.

Novel forms of treatment for patients with polycystic kidney disease

Several therapeutic strategies have been pursued to prevent or slow down cyst formation [for recent reviews see refs. 135, 136]. Ideally, these strategies should be designed according to the defects observed in polycystic kidneys and they should be applicable to a large number of patients. One very promising strategy is based on the finding that transgenic mice overexpressing transforming growth factor (TGF)-α develop polycystic kidneys [137] and that a mouse line, which expresses a mutant epidermal growth factor (EGF) receptor with decreased tyrosine kinase activity, is markedly protected against renal cyst formation [138]. Furthermore, a compound which inhibits autophosphorylation of the EGF receptor can reduce cyst formation in vitro [139]. Subsequent studies in the *bpk*/*bpk* mouse model of ARPKD have indeed demonstrated that a specific inhibitor of the EGF receptor [140] and an inhibitor of TACE [141], the enzyme releasing mature TGF-α from its membrane-bound form, decrease the rate of renal cyst formation. The attraction of such an approach also stems from the fact that at least in some models of polycystic kidney disease, members of the EGF receptor family are relocated from the basal to the apical side of cyst-lining epithelial cells [138, 142–145]. It may therefore be possible to use a dosage of these pharmaceuticals which does not result in any systemic side effects, but which may still be benefi-

cial in the kidney, because that is where they reach a therapeutic threshold upon being concentrated in the urine.

Cyst formation has been likened to the growth of benign tumors [146]. Such a comparison is supported by the finding that drugs which are targeted against the EGF receptor inhibit cyst development, and by our finding that cyst-lining epithelial cells produce a crucial membrane-bound matrix metalloproteinase, MMP-14 [91]. Similar to tumors, cysts have to degrade the surrounding extracellular matrix in order to expand. When (*cy*/+) rats were treated with the synthetic metalloproteinase inhibitor batimastat, cyst formation was significantly inhibited [91]. Synthetic metalloproteinase inhibitors are being used in several trials against neoplastic and inflammatory diseases and could therefore also be employed against polycystic kidney disease.

Of course, the animal studies described above can only be the beginning of our attempts to exploit novel molecular findings. We have to learn much more about the molecular pathogenesis of polycystic kidney disease to improve our therapeutic strategies, and while some approaches may work in animals, they may not be easily transferred into a clinical setting [147]. Perhaps the ideal therapy would be to get at the root of the problem and use gene therapy to express the respective wild-type polycystin protein or even replace the mutated by the wild-type *PKD* gene, but obviously this can only be a long-term goal.

Conclusions

The molecular and genetic dissection of polycystic kidney disease has turned out to be an extremely interesting topic in renal biology, but the genes and proteins involved are still holding onto many of their secrets. With several more polycystic kidney disease genes remaining to be identified in human patients and in rodents, these molecules will continue to draw increasing interest from scientists inside and outside the field of kidney research.

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