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REVIEW

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An ever-expanding story of cyst formation

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Abstract Autosomal-dominant polycystic kidney disease represents one of the most common monogenetic human disorders. The cloning of the *PKD1* and *PKD2* genes, which are mutated in far more than 90% of the patients affected by this disease, has generated high hopes for a quick understanding of the pathogenesis of cyst formation. However, these expectations have not yet been fulfilled, since the function of both polycystin-1 and polycystin-2, the two proteins encoded by *PKD1* and *PKD2*, still remains a puzzle. In this review, we will highlight some of the characteristics of polycystic kidney disease, briefly touch on polycystin-1, and then go on to describe recent results of experiments with polycystin-2, since the latter is the major focus of our work. We will discuss new evidence which suggests that autosomal-dominant polycystic kidney disease actually behaves recessively on a cellular level. Finally, a model will be presented that tries to explain the available data.

Key words Polycystic kidney disease · PKD1 · PKD2 · Polycystin-1 · Polycystin-2 · Two-hit hypothesis · Stop-signal hypothesis

Introduction

Hereditary polycystic kidney disease accounts for ~9% of all causes of end-stage renal disease (Lowrie and Hampers 1981), and it can follow an autosomal-recessive or an autosomal-dominant pattern of inheritance. Although autosomal-recessive polycystic kidney disease is typically diagnosed at a very early age and autosomal-dominant polycystic kidney disease in the 2nd or 3rd decade of life, the terms neonatal/infantile/juvenile polycystic kidney disease for the autosomal-recessive form and adult polycystic kidney disease for the autosomal-dominant form are obsolete, since there is no strict correlation between the pattern of inheritance and the time of diagnosis. At an estimated prevalence of ~1 in 800 (Dalgaard 1957), autosomal-dominant polycystic kidney disease is one of the most common monogenetic human disorders and will be the focus of this review; for a discussion of autosomal-recessive polycystic kidney disease, the reader is referred to another review (Zerres et al. 1998).

At least three genes have been made responsible for autosomal-dominant polycystic kidney disease, two of which have been cloned so far: *PKD1* and *PKD2*. The existence of a third gene, *PKD3*, has been postulated, but its location in the genome has not yet been determined (de Almeida et al. 1995; Bogdanova et al. 1995; Daoust et al. 1995; Turco et al. 1996). Mutations in the *PKD1* gene are found in ~80 to 85% of patients with autosomal-dominant polycystic kidney disease, whereas mutations in the *PKD2* gene occur in ~10 to 15% of all patients (Peters and Sandkuijl 1992; Roscoe et al. 1993). In addition to the disease caused by mutated *PKD1* and *PKD2* genes, mutations in two other genes are also known to result in renal cyst formation. Inactivation of the *TSC2* and *VHL* genes leads to tuberous sclerosis (Sampson 1996) and von Hippel-Lindau disease (Neumann and Zbar 1997), respectively, which, because of their more widespread symptoms, are not considered as autosomal-dominant polycystic kidney disease per se, although the patients affected by these syndromes also develop renal cysts and the diseases are inherited in an autosomal-dominant fashion. Since the *PKD1* gene is located in a tail-to-tail orientation immediately adjacent to the *TSC2* gene, it is a common finding that genomic deletions affect both genes (Brook-Carter et al. 1994; Longa et al. 1997; Sampson et al. 1997). The analysis of a large number of tuberous sclerosis patients suggests

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that severe polycystic kidney disease results from mutations affecting both *PKD1* and *TSC2*, whereas patients with only a mutated *TSC2* gene suffer from mild polycystic kidney disease (Sampson et al. 1997).

General features of autosomal-dominant polycystic kidney disease

Pathologists define cysts as cavities filled with liquid; in contrast to pseudocysts, which lack an epithelial lining, true cysts are lined by epithelial cells. Autosomal-dominant polycystic kidney disease is characterized by the lifelong development of cysts, which originate gradually from pre-existing structures, i.e., the tubular portion of the nephron and the collecting duct. Investigations with specific markers have demonstrated that cysts develop predominantly in the proximal tubule and collecting duct (Bachinsky et al. 1995; Devuyst et al. 1996; Faraggiana et al. 1985). The reasons for this particular distribution of cysts are unknown, but a preference for certain nephron segments and/or the collecting duct can also be seen in various animal models of polycystic kidney disease (Witzgall 1999). The course of the disease is slowly progressive, such that ~50% of the patients will reach end-stage renal disease by the age of 60 (Churchill et al. 1984; Gabow et al. 1992; Parfrey et al. 1990). In addition to the renal symptoms, patients with autosomal-dominant polycystic kidney disease also develop symptoms in other organs, mostly cysts in the liver and the pancreas, as well as aneurysms in cerebral arteries (Gabow 1993; Kaehny and Everson 1991).

The *PKD1* gene and polycystin-1

In 1985 the *PKD1* gene was localized on the short arm of chromosome 16 (Reeders et al. 1985), but it still took almost a decade until the gene was cloned (European Polycystic Kidney Disease Consortium 1994). Part of the reason for this long delay is the presence of several genes closely related to *PKD1*, which also makes the screening for mutations very difficult and tedious (European Polycystic Kidney Disease Consortium 1994).

The *PKD1* gene spans ~52 kbp and consists of 46 exons, it encodes a large, 4302-amino acid protein, with a M_r of 462 kDa, called polycystin-1 (American PKD1 Consortium 1995; Hughes et al. 1995; International Polycystic Kidney Disease Consortium 1995). A putative-signal peptide at the NH_2-terminus and several hydrophobic domains at the COOH-terminus suggest that polycystin-1 is an integral membrane protein. The predicted extracellular domain of polycystin-1 contains a number of domains such as a leucine-rich domain and so-called PKD repeats (Bateman and Sandford 1999; Hughes et al. 1995; International Polycystic Kidney Disease Consortium 1995; Ponting et al. 1999), which are also present in the orthologous proteins of mouse (Löhning et al. 1997) and *Fugu* (Sandford et al. 1997).

The homology of polycystin-1 to other proteins may shed some light on its function. When sperms of sea urchins bind to an egg, glycoproteins in the egg jelly trigger ion fluxes in the sperms, thus leading to the acrosome reaction (Darszon et al. 1988). REJ (*r*eceptor for *e*gg *j*elly), the receptor for these glycoproteins in the egg jelly, is a 210-kDa protein in the plasma membrane of sperms (Moy et al. 1996). Subsequently a related protein called PKDREJ was also found in human and mouse (Hughes et al. 1999; Veldhuisen et al. 1999). By Northern blot analysis, the PKDREJ mRNA was only detected in the testis, where it codes for a protein with a calculated molecular mass of 255 kDa (human) and 241 kDa (mouse). Both sea urchin REJ and PKDREJ contain a large domain of at least 700-amino acids length called the REJ module, which is also found in the putative extracellular domain of polycystin-1. It is possible therefore that the REJ module in polycystin-1 mediates binding to glycoproteins on neighboring cells or to the extracellular matrix.

A homologue of polycystin-1 has also been identified in *Caenorhabditis elegans*, where it is called LOV-1 (*l*ocation *o*f *v*ulva; Barr and Sternberg 1999). LOV-1 does not contain a REJ module but rather shares homology with polycystin-1 in a more COOH-terminal region, which also comprises the membrane-spanning domains. LOV-1 and PKD-2, the putative homologue of polycystin-2 in *C. elegans*, are both expressed in the same subset of neurons. A lack of LOV-1 leads to a defective mating behavior, but the precise function of LOV-1 and therefore the reason for the phenotype of the mutant worms is unknown (Barr and Sternberg 1999).

The characterization of the putative intracellular COOH-terminus has yielded the first hints about the mode of action of polycystin-1. This portion of polycystin-1 is able to activate the protein kinases C and JNK and the transcription factor AP-1 (Arnould et al. 1998), it modulates the activity of the monomeric G proteins Rac-1 and Cdc42 (Arnould et al. 1998) and of the trimeric G proteins G_i and G_o (Kim et al. 1999a; Parnell et al. 1998), and it also influences the Wnt-signal transduction cascade (Kim et al. 1999b). The location of the polycystin-1 protein in normal and polycystic kidneys, however, is very controversial (for review, see van Adelsberg 1999), such that an extrapolation of the in vitro data to the situation in vivo is limited.

The *PKD2* gene and polycystin-2

Structure of the *PKD2* gene and distribution of mutations

The *PKD2* gene is located on the long arm of chromosome 4 and was cloned by Somlo and associates in 1996 (Mochizuki et al. 1996). It consists of 15 exons and spans ~68 kbp of genomic DNA (Hayashi et al. 1997); the calculated molecular mass of the 968-amino acid polycystin-2 protein is 110 kDa. Hydropathy analysis of the protein predicts six transmembrane domains, similar

to what has been described for the α-subunits of voltage-gated Ca^{2+} and Na^{+} channels, and indeed polycystin-2 shows homology to some of those subunits and also to polycystin-1 (Mochizuki et al. 1996).

So far no clusters of mutations could be identified through the analysis of a number of pedigrees. The majority of known mutations are predicted to result in the synthesis of truncated proteins (Aguiari et al. 1999; Mochizuki et al. 1996; Pei et al. 1998a, 1998b; Reynolds et al. 1999; Torra et al. 1999a, 1999b; Veldhuisen et al. 1997; Viribay et al. 1997; Xenophontos et al. 1997), although some apparent missense mutations have also been described (Reynolds et al. 1999; Torra et al. 1999b; Veldhuisen et al. 1997).

Expression pattern of polycystin-2

The inactivation of the murine *Pkd1* (Lu et al. 1997) and *Pkd2* (Wu et al. 1998a) genes results in the development of polycystic kidneys, thus confirming the findings in human patients. The latter study has also provided the first solid evidence for the distribution of polycystin-2 in the kidney. It showed that in the normal mouse kidney the highest levels of polycystin-2 are expressed in the distal nephron segments and collecting ducts. In *Pkd2* knockout mice these are the regions of the kidney where the cysts develop, which correlates with the loss of polycystin-2 in the cyst wall epithelia. At a subcellular level, polycystin-2 appears to be sorted to the basal side of the cell. These findings in the mouse kidney were confirmed by a subsequent study (Markowitz et al. 1999); moreover a very similar distribution was demonstrated by us in rat and human kidneys, where polycystin-2 was very strongly expressed in the entire distal tubule and the connecting tubule (Obermüller et al. 1999). We, however, found no evidence for the expression of polycystin-2 in the macula densa. An immunohistochemical study conducted in human kidneys describes that polycystin-2 is predominantly expressed in the distal convoluted tubules and the collecting ducts, but the authors provide no evidence for the expression of polycystin-2 in the thick ascending limb. Moreover they describe an apical distribution in addition to a basal distribution (Ong et al. 1999b).

During nephron development in the mouse (Markowitz et al. 1999) and rat (Obermüller et al. 1999), no polycystin-2 immunoreactivity could be demonstrated in nephron precursors such as the aggregated metanephric mesenchyme and comma- and S-shaped bodies, whereas in human fetal kidneys already a faint expression of polycystin-2 in comma- and S-shaped bodies and a strong, but transient expression in proximal tubules was shown (Ong et al. 1999b).

The data are somewhat more conflicting when it comes to the expression pattern of polycystin-2 in extrarenal tissues. Using the avidin-biotin-peroxidase technique, polycystin-2 was localized in several endocrine, mesenchymal, and epithelial tissues of the mouse (Markowitz et al. 1999). Except for the ducts of the paranasal sinuses, where polycystin-2 was located at the basal aspect of the epithelial cells, the staining was punctate cytoplasmic (Markowitz et al. 1999). By immunofluorescence and using the same antibody as in the study by Markowitz et al., we were able to unambiguously detect rat polycystin-2 in the adrenal gland, corneal epithelium, ovary, smooth muscle cells of arteries, and excretory ducts of salivary glands (Obermüller et al. 1999). The main discrepancies between the two studies were that in the mouse polycystin-2 was expressed in the retinal pig-

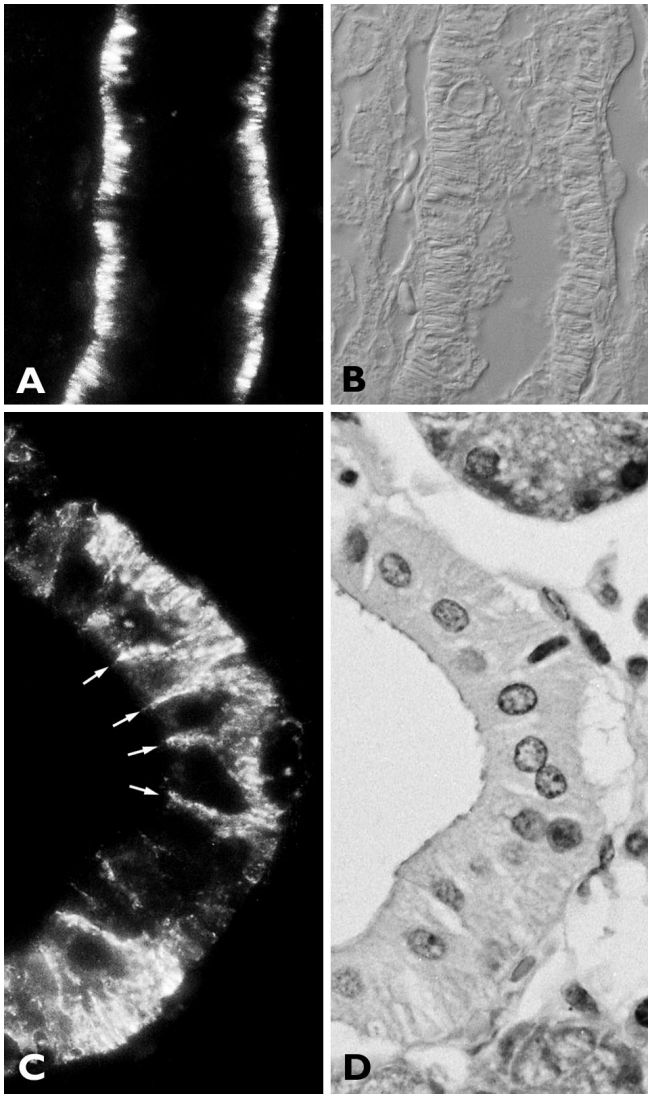

Fig. 1A–D Distribution of polycystin-2 in rat kidney and human submandibular gland. Paraffin sections of rat kidney and human salivary gland were incubated with a rabbit polyclonal antibody raised against the COOH-terminus of human polycystin-2 (Wu et al. 1998a). Specific staining was detected with a Cy3-coupled secondary antibody. The same sections are also shown as interference phase-contrast views (**B**) or after staining with H&E (**D**) in order to more easily discern the different structures. In the distal tubules of the kidney (**A**) and in the striated ducts of salivary glands (**C**) polycystin-2 is located at the basal and/or basolateral aspect of the epithelial cells. Lateral cell borders are highlighted by *arrows* (**C**). Taken with permission from Obermüller et al. 1999

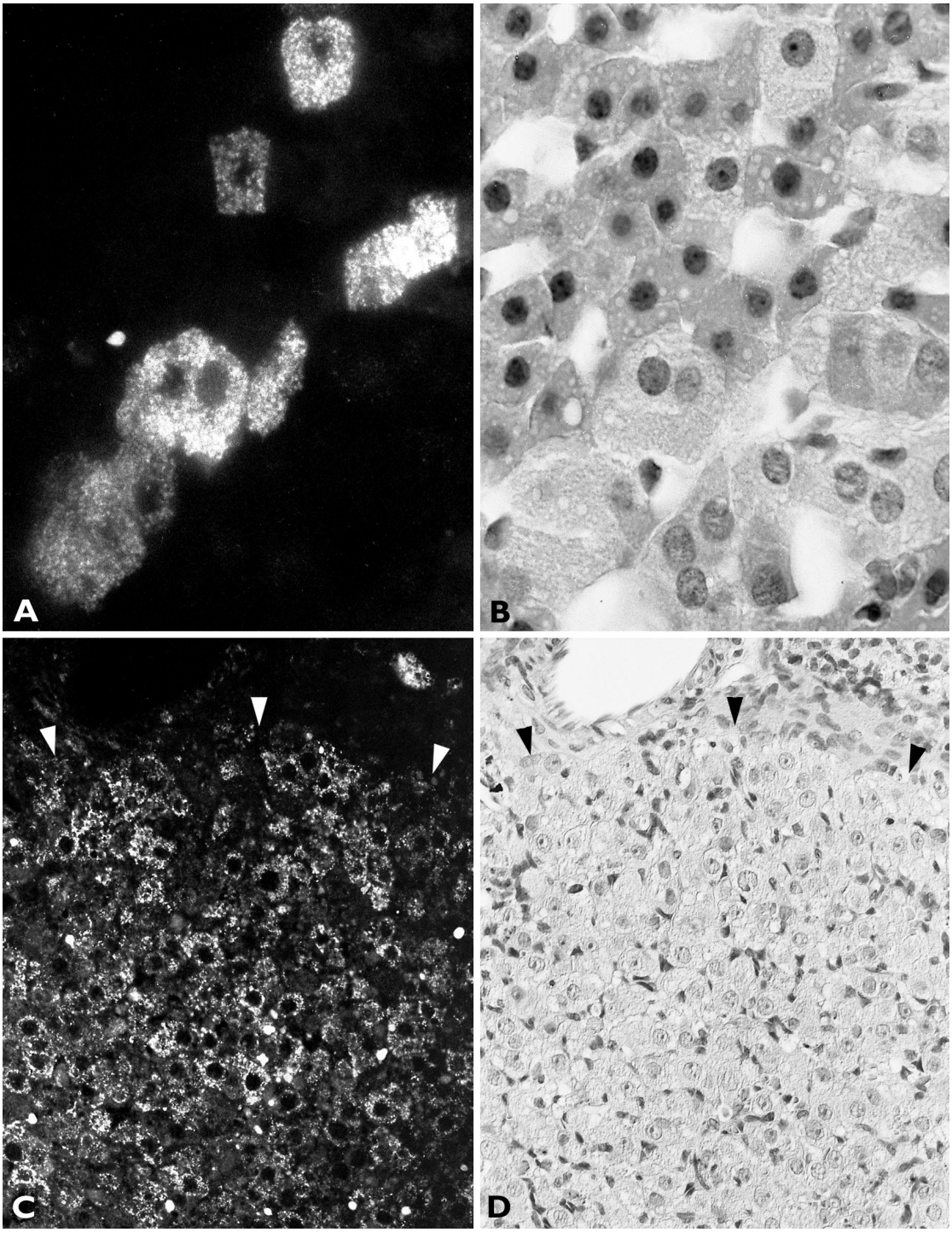

Fig. 2A–D Distribution of polycystin-2 in rat adrenal gland and ovary. Paraffin sections of rat adrenal gland and ovary were incubated with a rabbit polyclonal antibody raised against the COOH-terminus of human polycystin-2 (Wu et al. 1998a). Specific staining was detected with a Cy3-coupled secondary antibody. A distinct punctate cytoplasmic distribution of polycystin-2 can be seen in the cortex of the adrenal gland (**A**) and in the corpus luteum of the ovary (**C**). The same sections were also stained with H&E in order to more easily discern the different structures (**B, D**). The polycystin-2-expressing cells in the adrenal cortex contain a lighter cytoplasm and a more euchromatic nucleus (**B**). In the ovary only the granulosa cells express polycystin-2, but the smaller fibroblasts do not (**C**). *Arrowheads* in **C** and **D** mark the border between the corpus luteum and the surrounding stroma. Taken with permission from (Obermüller et al. 1999)

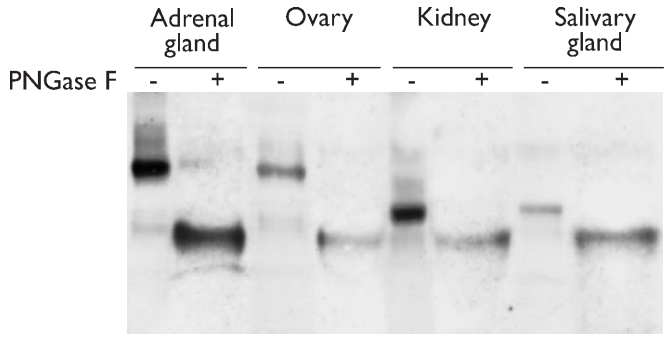

Fig. 3 The polycystin-2 protein is differentially *N*-glycosylated in rat adrenal gland, ovary, kidney and salivary gland. Western blot analysis of membrane protein preparations with a polyclonal anti-polycystin-2 antibody (Wu et al. 1998a) showed a higher mobility of polycystin-2 isolated from the kidney and salivary gland compared to polycystin-2 isolated from the adrenal gland and ovary. Treatment with peptide: *N*-glycosidase F (*PNGase F*), which cleaves off *N*-linked sugar moieties, resulted in the equal mobility of polycystin-2, indicating a different degree of *N*-glycosylation in the various organs

ment epithelium but not in the salivary glands (Markowitz et al. 1999), whereas, in the rat, polycystin-2 was detected in the salivary glands and the corneal epithelium but not in the retinal pigment epithelium (we have also shown the expression of polycystin-2 in excretory ducts of salivary glands in human specimens; Obermüller et al. 1999). Both studies, however, demonstrated that only in certain organs, that is, the kidney (mouse and rat), salivary glands (rat and human), and ducts of the paranasal sinuses (mouse), was polycystin-2 located in the basal compartment of the cells (Fig. 1); otherwise a punctate cytoplasmic distribution was noted (Fig. 2). Somewhat surprisingly the polycystin-2 protein in the kidney and salivary gland is less heavily *N*-glycosylated than in the adrenal gland and the ovary (Fig. 3; Obermüller et al. 1999). Again quite a different distribution pattern was determined for the human polycystin-2 protein using a different antibody (Ong et al. 1999b). The reason for those discrepancies between the different studies are not clear at the moment, but they may be due to species differences, the use of different antibodies, or immunohistochemical techniques.

In vitro studies have added another twist to the intracellular distribution of polycystin-2. Transfection studies with the human embryonic kidney 293 cell line and the porcine kidney epithelial cell line LLC-PK_1 demonstrated a strictly cytoplasmic distribution of the full-length polycystin-2 protein (Fig. 4; Cai et al. 1999). Moreover full-length polycystin-2 was sensitive to endoglycosidase H digestion and cosegregated with calnexin after ultracentrifugation in a linear iodixanol gradient, thus strong-

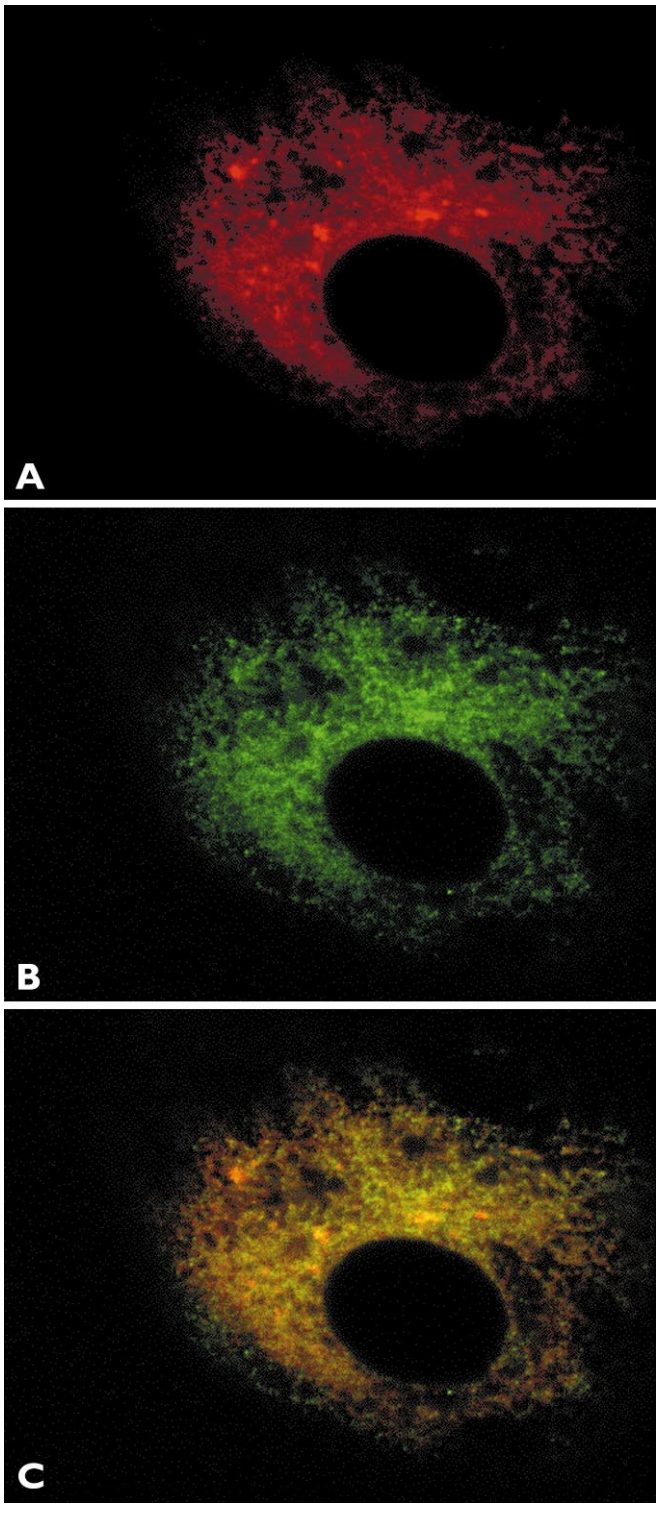

Fig. 4A–C Polycystin-2 is located in the endoplasmic reticulum. The porcine kidney epithelial cell line LLC-PK_1 was stably transfected with the human PKD2 cDNA, which was modified to encode a full-length polycystin-2 fusion protein with a 9-amino acid-hemagglutinin epitope at the COOH-terminus. Double-immunofluorescence staining with a monoclonal antibody against the hemagglutinin epitope (**A**) and a polyclonal antibody against Sec61 β (**B**), a marker for the endoplasmic reticulum, demonstrates an overlap between the two proteins (**C**), thus indicating that polycystin-2 is associated with the endoplasmic reticulum. We cannot rule out the possibility that the overexpression of polycystin-2 leads to a missorting of the protein, but we have found virtually identical distribution patterns in a number of other cell lines from very different origins

ly suggesting that polycystin-2 is an endoplasmic reticulum-associated protein (Cai et al. 1999). Removal of the COOH-terminal portion of polycystin-2 resulted in the integration of a portion of polycystin-2 into the plasma membrane, which was accompanied by its resistance to treatment with endoglycosidase H. The region in polycystin-2 responsible for the retention in the endoplasmic reticulum could be narrowed down to a 34-amino acid domain comprising residues 787 through 820 (Cai et al. 1999). This domain may therefore represent the interface for the interaction with a protein "X," which holds polycystin-2 back in the endoplasmic reticulum.

Since the polycystin-2 protein isolated from the kidney was still sensitive to a digest with endoglycosidase H (Cai et al. 1999), it has to be assumed that, despite its basal distribution, polycystin-2 is still located in the endoplasmic reticulum of the kidney and possibly also the salivary gland. We would propose therefore that, in all organs investigated, polycystin-2 does not leave the endoplasmic reticulum, and that, similarly to what has been described for other proteins (Benallal and Anner 1994), the different degrees of *N*-glycosylation are organ-specific. Ultrastructural studies on the organization of the endoplasmic reticulum have shown that it extends deep down into the lateral interdigitations of renal distal tubular cells, where it appears to wrap around the mitochondria and comes to lie between them and the plasma membrane (Bergeron and Thiéry 1981; Bergeron et al. 1987). It therefore is possible that the specific location of polycystin-2 in the basal compartment serves to fulfill a very distinct function in this particular cell type.

The putative function of polycystin-2

The genetic analysis of several model organisms such as *Caenorhabditis elegans* or *Drosophila melanogaster* has provided ample evidence that mutations in proteins which are part of the same signaling complex or even interact with each other lead to very similar phenotypes. This notion led to the question of whether polycystin-1 and polycystin-2 might also interact with each other, and that indeed proved to be the case (Qian et al. 1997; Tsiokas et al. 1997). Both reports also provide evidence that polycystin-2 is able to form homodimers through a domain distinct from that required for the heterodimerization with polycystin-1, whereas in the case of polycystin-1 a homophilic interaction was demonstrated only by one study in vitro (Qian et al. 1997) but not by the other (Tsiokas et al. 1997).

Although polycystin-2 shows some homology to α-subunits of voltage-activated calcium channels, no evidence has been reported yet to support the hypothesis that polycystin-2 can modulate Ca^{2+} currents. Recently, however, two cDNAs were cloned that encode polycystin-2-related proteins, the first of which is called PKD2L (Veldhuisen et al. 1999; Wu et al. 1998b) or PKDL (Nomura et al. 1998), and the second has been named PKD2L2 (Veldhuisen et al. 1999). PKD2L/PKDL also contains six putative transmembrane domains and shares ~50% identity with polycystin-2. Microinjection of RNA encoding PKD2L/PKDL into *Xenopus* oocytes resulted in increased cation fluxes with a large unitary conductance of 137 pS across the oocyte plasma membrane (Chen et al. 1999). The channel activity associated with PKD2L/PKDL was rather nonspecific, since this protein was permeable to both monovalent cations such as Na^+ and K^+ and divalent cations such as Ca^{2+} and Ba^{2+}. Furthermore PKD2L/PKDL was subject to regulation by Ca^{2+}, which might be mediated by a potential Ca^{2+}-binding EF-hand at the COOH-terminus of the protein (Chen et al. 1999). The reason why no channel activity associated with polycystin-2 has been described yet could be due to the fact that polycystin-2 resides in an intracellular compartment such as the endoplasmic reticulum or that the experiments were conducted in an inappropriate physiological environment where other necessary subunits were lacking. In this context it has to be mentioned that polycystin-2 can also associate with a member of the transient receptor potential channels, TRPC1 (Tsiokas et al. 1999). However, in the case of PKD2L/PKDL, the channel activity was not increased by pretreatment of oocytes with thapsigargin (Chen et al. 1999), which is one of the hallmarks of the TRP channels (Holda et al. 1998; Zhu and Birnbaumer 1998).

Possible mechanisms of cystogenesis

The two-hit hypothesis

A typical pathological description of a polycystic kidney reads that "the kidney contains thousands of cysts". Such a number sounds quite impressive, but still poses a serious problem. There are about a million nephrons per kidney, and some nephrons may even give rise to several cysts, so there should be "millions of cysts" if every single nephron was affected. The simplest explanation for such a phenomenon would be that all the nephrons suffer from the same basic defect, i.e., a mutation on one of the chromosomes 16 in the case of *PKD1* or one of the chromosomes 4 in the case of *PKD2*, but that there are other regulatory factors that influence cystogenesis. The strength of these modulating effects may be described according to a normal distribution, so that only the nephrons "on the far left" of the bell-shaped curve become cystic if the regulatory factors exert a protective effect, and only the nephrons "on the far right" become cystic if the modulating factors act in a cyst-promoting fashion (Fig. 5A).

Another model that has attracted a lot of attention recently is the two-hit model of cystogenesis (Fig. 5B). The analysis of individual cysts both in the case of *PKD1* (Brasier and Henske 1997; Koptides et al. 1998; Qian et al. 1996; Watnick et al. 1998) and in the case of *PKD2* (Koptides et al. 1999; Pei et al. 1999; Torra et al. 1999a) revealed somatic mutations in the allele, which is not mutated in the germline. Since one mutated allele is

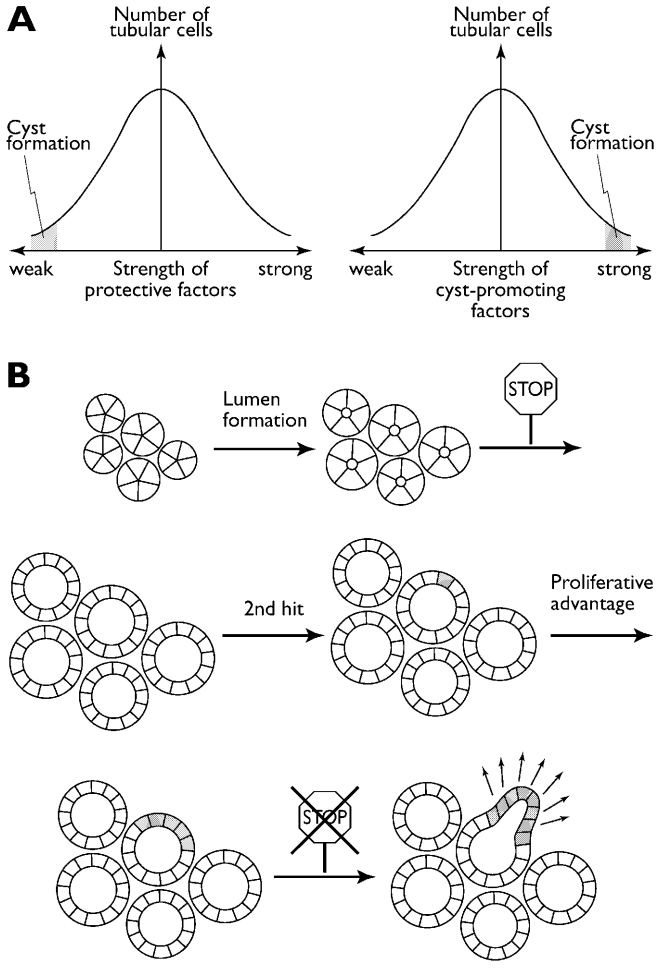

Fig. 5A,B Possible mechanisms of cystogenesis. **A** Stochastic model of cyst formation. It is likely that a number of proteins have an effect on the development of cysts, some of which protect against cyst formation, whereas others are cyst-promoting. If one assumes that the strengths of these factors are distributed in a bell-shaped fashion (e.g. the number of adhesion molecules, cytoskeleton-associated proteins, pro- and anti-apoptotic proteins in a given cell), one could argue that although all tubular cells suffer from the same germline mutation, only a subset of them will be prone to cyst formation, i.e., those which are least protected or those with the strongest influence of cyst-promoting factors. **B** Combination of the two-hit model and the stop-signal hypothesis of cyst formation. The epithelial structures of the nephron originate from mesenchymal precursor cells. After mesenchymal aggregates have formed, a lumen begins to develop until a certain diameter is reached, at which point a so-far hypothetical sensor acts as a stop-signal and lumen formation comes to a halt. All the cells in the kidney of a patient with autosomal-dominant polycystic kidney disease already contain one allele with a germline mutation of either *PKD1* or *PKD2*. A somatic mutation affects the second allele of any given tubular cell (indicated by shading), which may convey a certain proliferative advantage to such a cell. The inactivation of both alleles also disables the sensor, which is required to register the width of the lumen, so that the tubule begins to enlarge further and a cyst forms

already present in every cell throughout the kidney, the inactivation of the second allele would result in the complete loss of polycystin-1 and polycystin-2 activity, respectively, in a subset of tubular epithelial cells. On a cellular level, autosomal-dominant polycystic kidney disease could therefore be considered as acting in an autosomal-recessive fashion. The two-hit model is supported by findings in the *Pkd1* and *Pkd2* knockout mice. Mice in which only one *Pkd2* allele has been inactivated do not develop polycystic kidneys, whereas cysts accumulate rapidly in *Pkd2* (–/–) mice (Wu et al. 1998a). In a second, more complex *Pkd2* knockout model, a mutant exon was integrated adjacent to the wild-type exon. Intragenic recombination between the mutant and wild-type sequences leads to the loss of the wild-type allele and to the development of polycystic kidneys. Using an anti-polycystin-2 antibody, it was demonstrated that, in these polycystic kidneys, cyst-lining cells did not express the polycystin-2 protein any longer (Wu et al. 1998a). In heterozygous *Pkd1* (+/–) knockout mice, cysts do develop, but only after a prolonged period of time, and it is not known whether a mutation has occurred in the wild-type *Pkd1* allele in the cyst-lining epithelial cells (Lu et al. 1999). In contrast, homozygous *Pkd1* (–/–) knockout mice already have polycystic kidneys in utero (Lu et al. 1997).

Although the two-hit model represents an attractive hypothesis, a few questions still remain. Again keeping in mind the fact that a human kidney contains in the order of one million nephrons, thousands of cysts do not appear to be very much for an autosomal-dominant disease; but, in order for so many cysts to develop, quite a large number of second hits appear to be required. It is likely that in a given nephron a somatic hit will only affect very few (or only a single) cells. Assuming a cell-autonomous mechanism of cyst formation, the mutated cells somehow have to displace their neighbors until a small patch of mutated cells has formed and the tubule begins to enlarge. Although tubular cells are able to enter the cell cycle again, the basal mitotic rate is low (Witzgall et al. 1994) and the resident tubular cells should not be easily displaced, but cells with a second hit may have a proliferative advantage over cells with only a germline mutation. So far no estimates have been presented as to what frequency a second hit would have to occur at for cysts to develop at such a rate. Moreover a mutated second allele has not been detected in 100% of all cysts examined. This could be due to technical problems, but one should also consider the possibility that a mutation in the second allele arises by chance after a cyst has developed and therefore represents an epiphenomenon. A very unexpected explanation may come from the recent finding of *trans*-heterozygosity in renal cysts of a patient with a germline mutation in the *PKD1* gene. Whereas some cyst-lining cells contained a somatic mutation in the second *PKD1* allele, epithelial cells from other cysts contained a mutated *PKD2* allele (Koptides et al. 2000). Since polycystin-1 and polycystin-2 have been shown to form heteromeric complexes (Qian et al. 1997; Tsiokas et al. 1997), reduced amounts of both polycystin-1 and polycystin-2 may therefore suffice to cause cystic expansion of tubules.

Even more puzzling is the fact that, in human polycystic kidneys, polycystin immunoreactivity has still been

observed in cyst wall epithelia (Geng et al. 1996; Griffin et al. 1996; Ibraghimov-Beskrovnaya et al. 1997; Ong et al. 1999a, 1999b; Peters et al. 1996; Ward et al. 1996). A trivial explanation for this discrepancy would be a lack of specificity of the anti-polycystin antibodies used in those studies, but then we also have to question the findings in non-cystic kidneys and the extrarenal expression pattern of the polycystins. Alternatively the persistence of polycystin immunoreactivity could be explained by the occurrence of mostly missense mutations in the second allele, since the change of only one amino acid probably will not alter the epitope recognized by the antibody. The vast majority of germline mutations, however, are predicted to result in the synthesis of truncated proteins, so why should the somatic mutations preferentially be missense mutations? Indeed in the case of *PKD2*, the somatic mutations are not missense mutations, but rather nonsense mutations and frameshifts (Koptides et al. 1999; Pei et al. 1999; Torra et al. 1999a). Clearly the immunohistochemical staining pattern will have to be correlated with the molecular nature of the germline and somatic mutations before this discrepancy can be resolved.

The stop-signal hypothesis

The structures of the kidney are derived from at least two different precursor tissues: the metanephrogenic mesenchyme gives rise to the nephrons, and the ureteric bud develops into the collecting ducts (Saxén 1987). In other words, the epithelial cells of the renal tubules develop from mesenchymal cells, which aggregate upon induction by the invading ureteric bud, and after a mesenchymal-to-epithelial transformation form a tubular structure with a lumen. Looking at the incredibly uniform profiles of any given nephron segment on a kidney section, one has to wonder how the formation of the lumen is controlled. We postulate that there is a sensor, which somehow tells the epithelial cells that enough lumen has been established and to stop expanding into the surrounding interstitium ("stop-signal hypothesis" of cyst formation; Witzgall 1999). If that sensor fails, the expansion continues and cysts form (Fig. 5B). What might such a sensor look like? Obvious candidates for such a sensor are cell-cell and cell-matrix contacts, and indeed there is precedence in so far as the inactivation of genes coding for proteins such as tensin (Lo et al. 1997) and Rho GDIα (Togawa et al. 1999), which are associated with cell-matrix contacts, leads to the development of polycystic kidneys. Whether polycystin-1 and polycystin-2 are involved in the formation of cell-cell and/or cell-matrix contacts remains unclear at present. Whereas in some studies polycystin-1 has been found in cell-cell contacts (Huan and van Adelsberg 1999; Ibraghimov-Beskrovnaya et al. 1997; Peters et al. 1999), another study has localized polycystin-1 in cell-matrix contacts (Wilson et al. 1999), and, in the case of polycystin-2, the results of experiments in order to determine its ultrastructural location have not yet been published.

The stop-signal hypothesis integrates many of the models proposed for the pathomechanims of cystogenesis. One hypothesis argues that tubular cells are arrested during differentiation and that this developmental arrest leads to cyst formation. In many studies of polycystic kidneys from human patients and several animal models, a variety of nephron segment-specific markers have been used in order to determine the origin of cysts, and only in a minority of cysts were those markers absent (Witzgall 1999). Our own investigation of the Han:SPRD (*cy*/+) rat model has demonstrated that the loss of differentiation markers only occurs at advanced stages of cyst formation (Obermüller et al. 1997). Therefore we do not believe that the development of tubules is affected per se, but rather that the primary defect lies in the control of lumen formation, and a dedifferentiation of fully differentiated tubules takes place after cysts have formed.

Another phenomenon observed in polycystic kidneys is an increased proliferation rate (Nakamura et al. 1993; Ramasubbu et al. 1998) and expression of mRNAs coding for proto-oncoproteins (Cowley et al. 1987, 1991; Harding et al. 1992). Furthermore the introduction of oncogenic proteins into transgenic mice has resulted in polycystic kidney disease (Kelley et al. 1991; MacKay et al. 1987; Schaffner et al. 1993; Trudel et al. 1991). These observations have resulted in the hypothesis that an imbalance between cell death and cell survival plays a role in cyst formation. If the stop-signal for lumen formation is provided by cell-cell contacts, the apparent increased cell proliferation in cyst-lining epithelial cells can be easily explained by a loss of cell contact inhibition. The increased rate of apoptosis (Winyard et al. 1996; Woo 1995), on the other hand, could be due to anoikis if cell-matrix contacts serve as a sensor. Anoikis is defined as the phenomenon whereby many cells grow in an anchorage-dependent fashion and succumb to apoptosis when they detach from the underlying matrix (Ruoslahti and Reed 1994). The apoptotic cells would have to be replaced by adjacent surviving cells, thus again leading to increased cell proliferation.

One popular model of cyst formation argues that a reversal of cell polarity results in cyst formation (Wilson 1997). Transporters and channels would not be sorted to the correct portion of the plasma membrane any longer, so that instead of reabsorbing the tubular fluid the epithelial cells would secrete more fluid into the lumen. It is generally assumed that cell-cell contacts play a critical role in the maintenance of cell polarity (Cereijido et al. 1998); if they serve as the stop-signal of lumen formation and become dysfunctional in cyst wall epithelia, a loss of cell polarity would be the result.

Conclusions

The cloning of the *PKD1* and *PKD2* genes has opened a new era in polycystic kidney research. A steadily growing number of mutations have been identified, but so far no mutational hot spots or genotype-phenotype correla-

tions have been established. Although mutations in the second allele could be demonstrated in some cysts, it is not clear at this point whether autosomal-dominant polycystic kidney disease develops in a recessive fashion on the cellular level. The available data concerning the location and function of polycystin-1 and polycystin-2 are still very preliminary. Clearly a lot more work needs to be done in order to better understand the pathogenesis of cyst development in the kidney.

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