Autosomal dominant (ADPKD) and autosomal recessive (ARPKD) polycystic kidney disease are caused by mutations in *Pkd1*/*Pkd2* and *Pkhd1*, which encode polycystins (PCs) and fibrocystin/polyductin (FPC). Our recent study reported that a deficiency in FPC increases the severity of cystic disease in *Pkd2* mutants and down-regulates PC2 *in vivo*, but the precise molecular mechanism of these effects is unknown (Kim, I., Fu, Y., Hui, K., Moeckel, G., Mai, W., Li, C., Liang, D., Zhao, P., Ma, J., Chen, X.-Z., George, A. L., Jr., Coffey, R. J., Feng, Z. P., and Wu, G. (2008) *J. Am. Soc. Nephrol.* 19, 455–468). In this study, through the use of deletion and mutagenesis strategies, we identified a PC2-binding domain in the intracellular C terminus of FPC and an FPC-binding domain in the intracellular N terminus of PC2. These binding domains provide a molecular basis for the physical interaction between PC2 and FPC. In addition, we also found that physical interaction between the binding domains of PC2 and FPC is able to prevent down-regulation of PC2 induced by loss of FPC. *In vivo*, we generated a mouse model of ADPKD with hypomorphic *Pkd2* alleles (*Pkd2*<sup>12675sf/H11546</sup>) and show that PC2 down-regulation is accompanied by a phenotype similar to that of *Pkhd1<sup>-/-</sup>* mice. These findings demonstrate a common mechanism underlying cystogenesis in ADPKD and ARPKD and provide insight into the molecular relationship between PC2 and FPC.

**Autosomal dominant polycystic kidney disease (ADPKD)** is characterized by numerous fluid-filled, spherical renal cysts, and autosomal recessive polycystic kidney disease (ARPKD) is characterized by massive, spindle-shaped renal cysts (1, 2). The causal genes for both ADPKD and ARPKD have been identified (3–7). Mutations in *PKD1* and *PKD2*, which encode polycystins (PCs) and fibrocystin/polyductin (FPC), respectively, both lead to ADPKD with nearly identical clinical manifestations.

Mutations in *PKHD1*, which encodes fibrocystin/polyductin (FPC), lead to ARPKD (8–11).

*PKD1* has a 14-kb transcript and encodes PC1, a 4303-amino acid integral membrane protein with 11 putative transmembrane domains. PC1 is expressed in all tissues and organs of humans and mice (12). The N-terminal region contains an extracellular portion of >3000 amino acids, which is predicted to be a site of protein-protein or receptor-ligand interactions (2, 13). This region may also be released after cleavage at the GPS domain of PC1 and serve as a ligand for other proteins (14). The cytoplasmic C-terminal region of PC1 contains a putative coiled-coil domain that interacts with the *PKD2* gene product, PC2 (15).

*PKD2* has an ~5.4-kb transcript and encodes PC2, a 968-amino acid protein that is predicted to be an integral membrane protein with six putative transmembrane domains and intracellular N and C termini (4). PC2 is a receptor-operated, nonselective cation channel; it is also known as TRPP2, a member of the *trp* superfamily (16, 17).

*PKHD1* has a 16-kb transcript, contains at least 86 exons, and spans 470 kb on chromosome 6p12 (11). The longest open reading frame is predicted to be 66 exons and yields a 4074-amino acid type I membrane protein, FPC (5–7). The cytoplasmic C-terminal region of FPC is composed of 192 amino acids. FPC is predicted to be a membrane-associated, receptor-like protein. In addition, cleavage and release of its ectodomain into the renal tubular/duct lumen have been reported (5, 18).

Several recent reports have demonstrated functional and genetic relationships between FPC and the PCs (19–22). Wu *et al.* (19) have demonstrated that KIF3B, a motor subunit of the heterotrimer kinesin-2, is able to bridge FPC and PC2 bindings. The activity of the PC2 channel is significantly altered when the binding complex is disrupted. It has also been reported that FPC regulates mechanotransduced Ca<sup>2+</sup> responses, which may be induced by PC2 (21), and that loss of FPC down-regulates PC2 expression *in vivo* (22). In addition, loss of FPC worsens the cystic phenotype of *Pkd1* mutant mice, suggesting a genetic interaction between FPC and PC1 (20). These findings imply a strong functional relationship between FPC and PCs *in vivo*, although the underlying molecular mechanisms are not fully understood.

To explore the molecular relationships between these cystoproteins further, we studied the molecular interaction between PC2 and FPC. Through gene deletion and mutagenesis, we identified an FPC-binding domain (FBD) in the intracellular N terminus of PC2 and a PC2-binding domain (PC2BD) in the intracellular C terminus of FPC. This physical interaction
Molecular Interaction between FPC and PC2

prevents PC2 down-regulation induced by loss of FPC (22). Additionally, mice bearing a Neo-resistant cassette targeted to intron 2 of Pkd2, which leads to significant down-regulation of PC2, have a phenotype very similar to that of Pkd1 mutant mice. This model suggests that the formation of spherical cysts, commonly observed in ADPKD, and the spindle-shaped dilatation of tubules, commonly observed in ARPKD, are induced by a common mechanism. These findings further define the roles for PC2 and FPC in the pathogenesis of both ADPKD and ARPKD and reveal a cystogenic link between ARPKD and ADPKD.

EXPERIMENTAL PROCEDURES

Mouse Strains—We have previously reported the generation of the gene-targeted mouse model for Pkd1 (Pkd1−/−) (22). We have now generated a model in which a Cre-loxP/Frt-pyCre construct was introduced into a Neo cassette flanked by two FRT sites (Neolox), and two loxP sites were inserted to flank exon 3 of Pkd2 (see Fig. 4, A and B). We found 206 embryonic stem cell colonies resistant to G418; one (W2A4) was selected after PCR screening using a pair of outside-construct and cassette-based primers. This cell line was confirmed by Southern blot analysis and injected into C57BL/6 blastocysts at the University of Connecticut Health Center Gene Targeting and Transgenic Facility. All mouse lines used in this study have been backcrossed onto the Bl6/C57 congenic background.

Southern and Northern Blotting and Quantitative PCR—Southern analysis was used to genotype Pkd1 and Pkd2 mutant mice using our published approaches (22, 42). For Northern analysis, total RNA was isolated from embryos or kidneys using TRIzol reagent (Invitrogen) following the manufacturer’s instructions. A probe (838 bp of exons 3–6 of Pkd2) was labeled using the RadPrime DNA labeling system (Invitrogen) with [α-32P]dCTP (PerkinElmer Life Sciences) and was hybridized with total RNA blots (25 μg/lane). Images of 28 S rRNA bands in these same blots were used as a total RNA loading control.

Quantitative PCR was performed using the iCycler iQ real-time PCR detection system with the iQ SYBR Green Supermix kit (Bio-Rad). A pair of quantitative PCR primers was designed from the sequence of Pkd2 exon 6: 5′-GCC TGG TAC CCT CTT GGC AGT T-3′ (forward) and 5′-CAC GAC AAT CAC AAC ATC C-3′ (reverse).

Antibodies—Polyclonal and monoclonal antibodies against human FPC (including hAR-C2p) and antibodies against human PC2 (hPKD2-Cp and hPKD2-Cm1A11, formerly designated PKD2A11) were described in our previous studies (19, 23). A polyclonal antibody against the intracellular N terminus of PC2 (Arg50–Val226), hPKD2-Np, was generated, and anti-PC2 specificity was confirmed (supplemental Fig. 1). The following antibodies and staining materials were purchased: anti-acetylated α-tubulin, anti-γ-tubulin, anti-β-actin, anti-FLAG, and anti-hemagglutinin (HA) monoclonal antibodies (Sigma); fluorescein Lotus tetragonolobus lectin and fluorescein Dolichos biflorus agglutinin (Vector Laboratories); and fluorescein anti-Tamm-Horsfall glycoprotein (The Binding Site Ltd.).

Western Blotting and Immunoprecipitation—Western analyses and immunoprecipitation experiments were performed using protocols similar to those described in our previous study (23). To avoid nonspecific binding in co-immunoprecipitation (co-IP), 0.05–3 m NaCl was used to wash the IP reaction. The entire intracellular termini and the deletion constructs of FPC and PC2 were constructed in FLAG-tagged and HA-tagged pCMV expression vectors (40); their names and descriptions are listed in Fig. 1A and 2A. The FLAG-tagged entire intracellular C terminus of PC2 was designated PC2CT-F (amino acids 682–968).

Histology and Immunofluorescence Staining—Detailed procedures for histology and immunofluorescence were published previously (23). For microscopic analysis, images were obtained using a Zeiss Axioplan 2IE research microscope system with 4×, 10×, 20×, and 40× objectives.

Cell Lines and Mouse Kidney Primary Epithelial Cell Cultures—All cell lines used in this study were cultured under previously described conditions (30). The isolation and culture of primary renal epithelial cells from 2-month-old Pkd1−/−and wild-type littermates were described in our previous study (22).

Retrovirus Generation and Infection—The LZRS-ms-GFP system (a gift from Dr. Albert B. Reynolds, Vanderbilt University) was used to generate FPC fragment retroviruses (FCT-H and FCT1-H) and to transduce Pkd1−/−cell pools. Cells were transduced by the retroviruses and collected after 1 day of culture for Western analysis (41).

Statistics—All biochemical assays were repeated two to three times. Statistical analysis was performed where appropriate using Student’s t test or one-way analysis of variance followed by Tukey’s multiple comparison test. Differences with p values <0.05 were considered statistically significant.

RESULTS

Identification of an FBD in the Intracellular N Terminus of PC2—Recently, evidence for a physical interaction between the intracellular N terminus of PC2 and the C terminus of FPC was reported (22), but the precise nature of this interaction is unknown. Here, we applied a deletion-based strategy to identify the domains involved in the interaction between PC2 and FPC. First, we generated both FLAG- and HA-tagged constructs (FCT-F and FCT-H) that contain the entire intracellular portion of FPC. We also produced a series of HA-tagged deletion constructs of the intracellular N terminus of PC2 (Fig. 1A). We used two HA-tagged halves of the N terminus of PC2 (PC2NTa-H and PC2NTb-H) to perform co-IP experiments with the FLAG-tagged C terminus of FPC (FPC-F) cotransfected into HEK293 cells. Co-IP of the entire PC2 N terminus (PC2NT-H, HA-tagged) with FPC-F was used as a positive control (22). Positive immunoreactivity was observed for the positive control and the PC2NTa-H fragment, but not for the PC2NTb-H fragment (Fig. 1B), suggesting that the distal portion of the N terminus of PC2 (PC2NTb) is not involved in the FPC-PC2 interaction. To confirm, we singly transfected the same HA-tagged constructs and attempted to co-immunoprecipitate each construct with endogenous FPC using a polyclonal antibody specific for the intracellular C terminus of FPC (hAR-C2p) (23). These experiments confirmed that the proximal portion of the N terminus of PC2 (PC2NTa), but not the distal portion (PC2NTb), is involved in the binding between FPC and PC2 (Fig. 1C).
To determine the minimum FBD of PC2, we generated a series of HA-tagged deletion constructs (PC2NTc–f-H) derived from PC2NTa (Fig. 1A) for co-IP with FPC-F. Positive immunoreactivity was observed for the positive control (PC2NT-H), PC2NTa-H, PC2NTc-H, and PC2NTe-H, but not for the PC2NTf-H fragment (Fig. 1, A and D). These data suggest that the amino acid fragment within the PC2NTe construct contains the FBD of PC2. Conversely, we used anti-HA antibody for co-IP and anti-FLAG antibody for Western detection and obtained the same results (data not shown). To confirm these results, we singly transfected the same HA-tagged constructs and attempted to co-immunoprecipitate each construct with

FIGURE 1. Identification of the FBD in the intracellular N terminus of PC2. A, diagram of HA- and FLAG-tagged N-terminal fragments of PC2 and intracellular C-terminal fragments of FPC. Amino acid (a.a.) numbers defining each fragment are provided in parentheses. Co-IP results with FCT constructs are indicated with “+” for positive binding and “−” for no evidence of binding. B, co-IP assays of FCT-F with PC2NT-H, PC2NTa-H, or PC2NTb-H. Western blots show the level of cotransfected protein detected by anti-FLAG (left panel) or anti-HA (middle panel) antibody. The right panel shows co-IP using anti-FLAG antibody for IP and anti-HA antibody for Western detection. Immunoreactivity indicates that PC2NT-H and PC2NTa-H immunoprecipitated with FCT-F, C, co-IP using anti-FPC polyclonal antibody (hAR-C2p) for IP and anti-HA antibody for Western detection. The left panel shows a Western blot of the level of expression of PC2NT fragments detected by anti-HA antibody. The right panel shows hAR-C2p pulldown of endogenous FPC detected with anti-HA antibody. Immunoreactivity indicates that PC2NT-H and PC2NTa-H immunoprecipitated with endogenous FPC. IgG was used as a negative control for pulldown. D, co-IP of FCT-F with PC2NT-H, PC2NTa-H, PC2NTc-H, PC2NTe-H, or PC2NTf-H. Western blots show the level of cotransfected protein detected by anti-HA (lower panel) or anti-FLAG (middle panel) antibody. The upper panel shows co-IP using anti-FLAG antibody for IP and anti-HA antibody for Western detection. Immunoreactivity indicates that PC2NT-H, PC2NTa-H, PC2NTc-H, and PC2NTe-H immunoprecipitated with FCT-H, but PC2NTf-H did not. E, co-IP using hAR-C2p for IP and anti-HA antibody for Western detection produces results similar to those in D. F, alignment of human and mouse PC2NTc. Mutagenized PC2NT constructs (human (h) PC2NTcMut1 and PC2NTcMut2) were generated from PC2NTc. Human PC2NTc-H, a candidate FBD, is shown for reference. m, mouse. G, co-IP of FCT-F with PC2NTc-H, PC2NTcMut1-H, or PC2NTcMut2-H. Western blots show the levels of cotransfected protein detected by anti-FLAG (upper left panel) or anti-HA (lower left panel) antibody. The right panel shows co-IP using anti-FLAG antibody for IP and anti-HA antibody for Western detection. Immunoreactivity indicates that PC2NT-H and PC2NTc-H immunoprecipitated with FCT-F, but PC2NTcMut1-H or PC2NTcMut2-H did not. H, diagram of PC2NT-F and the construct PC2NTf–F, which has a candidate FBD deleted in-frame. I, co-IP of FCT-H with PC2NT-F or PC2NTf–F. Western blots show the levels of cotransfected protein by anti-HA antibody (left panel), anti-FLAG antibody (middle panel), or an IgG negative control (middle panel). The right panel shows co-IP using anti-HA antibody for IP and anti-FLAG antibody for Western detection. Immunoreactivity indicates that PC2NT-H immunoprecipitated with FCT-H, but the deletion construct missing the FBD did not.
Together, these results indicate that Asp90–Arg139 of PC2, the candidate binding domain for FPC, Arg90–Arg139, was PC2NTc-H construct, placing two mutations within the FBD candidate PC2BD binds FPC. We generated two mutagenic clones using the construct harbors a mutation that replaces Ser122 with alanine (S122A), selected because Ser122 is a predicted protein kinase phosphorylation site (4). The PC2NTcMut1-H construct harbors an R124A mutation, selected because the positively charged arginine may regulate the spatial conformation of the FBD. Immunoblotting using hPKD2-Np for IP and anti-HA antibody for Western detection. Immunoreactivity indicates that PC2NT-F immunoprecipitated with PC2NT-F, but the mutagenized FCTMut-H did not. Western blots show the level of cotransfected protein detected by anti-HA (upper right panel) and anti-FLAG (lower right panel) antibodies. D, co-IP using anti-PC2 polyclonal antibody (hPKD2-Np) for IP and anti-HA antibody for Western blotting. Immunoreactivity indicates that FCT1-H, FCT4-H, FCT7-H, FCT6-H, FCT3-H, or FCT5-H was not precipitated with PC2NT-F, and the mutagenized FCTMut-H did not. Immunoreactivity was observed for the positive control and the FCT1-H fragment, but not for the FCT2-H fragment or the negative controls (Fig. 2B). These data suggest that the distal half of the C terminus of PC2 (FCT2-H) is not involved in the FPC–PC2 interaction.

To determine the minimum PC2BD of PC2, we generated a series of HA-tagged deletion constructs (FCT3–7-H) within the intracellular C terminus of FPC (Fig. 2A) for co-IP with PC2NT-F. Positive immunoreactivity was observed for the positive control (FCT-H), FCT3-H, FCT4-H, and FCT5-H, but not for the FCT6-H or FCT7-H fragment (Fig. 2, A and C). These data suggest that FCT5-H is the minimum construct for the PC2BD of PC2. To confirm these results, we singly transfected the same HA-tagged constructs and attempted to co-immunoprecipitate each construct with endogenous PC2 using a polyclonal antibody specific for the intracellular C terminus of PC2 (hPKD2-Np) (Fig. 2D). Because the N terminus of PC2 bound to FCT5-H but not to FCT6-H or FCT7-H, Glu303–Glu306 may function as a PC2BD.

Once again, we used a mutagenic strategy to verify that the candidate PC2BD binds PC2. We generated a construct (FCTMut-H) that harbors an R3931A missense mutation (Fig. 2F). Because the N terminus of PC2 bound to FCT5-H but not to FCT6-H or FCT7-H, Glu303–Glu306 may function as a PC2BD.

Determination of a PC2BD in the Intracellular Portion of FPC—Once the FBD of the intracellular N terminus of PC2 was determined, we used a similar strategy to identify the PC2BD of the intracellular C terminus of FPC. We used two HA-tagged halves of the C terminus of FPC (FCT1-H and FCT2-H) (Fig. 2A) to perform co-IP experiments with the FLAG-tagged N terminus of PC2 (PCNT-F) cotransfected into HEK293 cells. Co-IP of the entire FPC C terminus (FCT-H, HA-tagged) with PC2NT-F was used as a positive control. Cotransfection of the FLAG-tagged intracellular C terminus of PC2 (PC2CT-F) with FCT-H, as well as other single transfactions, was used as a negative control (Fig. 2B). Positive immunoreactivity was observed for the positive control and the FCT1-H fragment, but not for the FCT2-H fragment or the negative controls (Fig. 2B). These data suggest that the distal half of the C terminus of PC2 (FCT2-H) is not involved in the FPC–PC2 interaction.

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FIGURE 3. Binding of FPC to PC2 prevents down-regulation of PC2 expression induced by loss of FPC. A, shown is a Western blot of duplicate protein lysates from wild-type (WT) and Pkd1−/− renal epithelial cell pools in primary culture. Compared with wild-type cells, a significant down-regulation of PC2, detected with the hPKD2-Cm1A11 monoclonal antibody, was observed in Pkd1−/− cells. Anti-β-tubulin antibody was used as a control for protein loading. B, shown is a Western blot of protein lysates from the same primary cultured cell pools shown in A, but the Pkd1−/− cell pool was retrovirally transduced with FCT-H, leading to a restoration of PC2 expression. C, shown is a Western blot similar to that in B, but the Pkd1−/− cell pool was retrovirally transduced with FCT1-H, also leading to a restoration of PC2 expression. D–F, all Western assays (A–C) were repeated three times, and normalized quantitative analyses were performed by densitometry. Error bars indicate the means ± S.D. *p < 0.05 compared with the other group(s).

FIGURE 4. Generation and characterization of mice with a hypomorphic allele at the Pkd2 locus. A, a schematic representation of the gene-targeting strategy is shown. The partial genomic map shows Pkd2 exons 3–6. The Pkd2nf3 targeting vector was inserted into intron 2 of Pkd2 by homologous recombination, B, BglII; X, XbaI, T, tail-biopsy DNA from mutant mice with the germ line-targeted mutation in Pkd2 were digested with XbaI and BglII and then hybridized with probes A and B, respectively. In the XbaI digestion, the expected 4-kb wild-type band was observed in wild-type (WT) and Pkd2 heterozygous mice. A mutant 2-kb band was observed in Pkd2 heterozygous and homozygous mice. In the BglII digestion, the expected 15-kb wild-type band and a mutant 6.5-kb band were observed. C, quantitative reverse transcription-PCR analysis was performed using total adult kidney RNA from wild-type and mutant mice. Pkd2 mRNA levels in Pkd2nf3/+/ mice were reduced to one-third of wild-type levels. Error bars indicate the means ± S.D. A, a Northern probe (42) was used to detect Pkd2 mRNA levels in adult kidneys to confirm the above findings (upper panel). The 28 S rRNA was used as a total RNA loading control (lower panel). D, shown is the Western blot detection of PC2, hPKD2-Cm1A11 was used to detect immunoreactive PC2 in the adult kidneys of wild-type, Pkd2−/−, and Pkd2nf3/+/ mice. PC2 expression was significantly reduced in Pkd2nf3/+/ kidneys compared with wild-type littermate kidneys, reflecting the reduced levels of Pkd2 mRNA.

Pkd2nf3 kidneys compared with wild-type littermate kidneys (22) to determine whether loss of FPC reduces PC2 expression in vivo (22), but the mechanism by which this occurs remains unknown. On the basis of the findings described above, we hypothesized that the physical interaction between the C terminus of FPC and the N terminus of PC2 is involved in the regulation of PC2 expression. First, we used primary cultures of renal epithelial cells derived from 2-month-old wild-type and Pkd1−/− kidneys (22) to determine whether loss of FPC reduces PC2 expression in vitro. Western analysis using the anti-PC2 antibody hPKD2-Cm1A11 confirmed that loss of FPC reduced PC2 protein levels (Fig. 3, A and D).

To test our hypothesis, we retrovirally transduced Pkd1−/− cells in primary culture with FCT-H, which contains the entire intracellular C terminus of FPC (Fig. 2A). These transduced cells demonstrated a restored expression of PC2 (Fig. 3, B and E), suggesting that physical interaction between the C terminus of FPC and the N terminus of PC2 prevents the down-regulation of PC2 induced by loss of FPC. To confirm this finding, we retrovirally transduced Pkd1−/− cells with FCT1-H, which contains the PC2BD, and obtained similar results (Fig. 3, C and F). These results indicate that the interaction of FPC and PC2 prevents the down-regulation of PC2 induced by loss of FPC.

In Vivo Down-regulation of PC2 Recapitulates Cystic Phenotypes of Pkd1 Knock-out Mice—To functionally validate the molecular interaction between FPC and PC2, we used a mouse line bearing a hypomorphic Pkd2 allele, in which a Neo-resistant cassette targeted to intron 2 of Pkd2 significantly down-regulates PC2 expression by a position effect (Fig. 4, A and B). The homozygous hypomorphic alleles in Pkd2 (Pkd2nf3/−/−) decreased Pkd2 mRNA and PC2 expression to one-third of normal levels (Fig. 4, C–E). Because Pkd2nf3/−/− mice retain some PC2 expression (Fig. 4E), they were able to escape embryonic lethality and reach adulthood (Table 1). However, 15% of the mutant pups experienced growth retardation (Fig. 5A, panel a). In addition, 10% exhibited severe dilatation of the common bile duct (Fig. 5A, panel b).

During 12 months of observation, some Pkd2nf3/−/− mice developed gross cystic phenotypes and lesions in the kidneys and liver similar in
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The severity of cysts and the age at disease onset varied among individual Pkd2<sup>nf3<sup> allele versus f<sup> mice (<sup>Fig. 5A, panel c versus d</sup> and panel e versus f<sup>). The onset varied among individual Pkd2<sup>nf3<sup> mice had microscopic cyst formation/tubular dilatation by 6 months, whereas all others developed marked cystic phenotypes in the kidneys until 12 months (<sup>Fig. 5B, panels e</sup> and f<sup>). Cystic or dilated ducts were also observed in the liver (<sup>Fig. 5, A, panel e, and B, panel g</sup> and pancreas (<sup>Fig. 5B, panel h</sup>) of Pkd2<sup>nf3<sup> mice. These phenotypes closely mimic those of Pkd1<sup>−/−<sup> mice, including massive, fusiform, dilated renal tubules (<sup>Fig. 5B, panel e versus i</sup> and panel f versus j<sup>); dilation of hepatic central veins with a patchy, periporal, lymphocytic infiltrate (panel g versus k<sup>); and dilation of pancreatic ducts (panel h versus l<sup>). The fact that down-regulation of Pkd2 induces the ARPKD-like phenotypes in vivo provides strong evidence that FPC and PC2 are functionally linked and that the down-regulation of either results in similar cystic phenotypes in the kidneys.

### DISCUSSION

Although the genetic and functional relationships between the ADPKD and ARPKD causal gene products (PCs and FPC, respectively) have been reported (19–22), the precise molecular mechanisms and consequences of the interaction between the two cystoproteins remain largely unknown (24). Here, we used deletion and mutagenesis strategies to identify a PC2BD in the intracellular C terminus of FPC and an FBD in the intracellular N terminus of PC2. These results provide a molecular basis for the physical interaction between PC2 and FPC.

ADPKD is characterized by focal, spherical renal cysts, whereas ARPKD is characterized by numerous spindle-shaped ectasias of renal tubules (1, 2). Although it has been shown that FPC and PC2 colocalize to the same subcellular organelles (23, 25, 26) and that they are functionally linked (19–22), how a common pathogenesis could lead to such different morphomorphology (ADPKD and ARPKD) remains unclear. In theory, ADPKD cysts are generally considered to arise from loss of heterozygosity (27, 28), in which a single renal epithelial cell that carries an inherited mutant allele experiences a random, somatic “second hit” at the other allele. This leads to loss of PC1 or PC2 at the cellular level and triggers downstream signaling that leads to abnormal proliferation and apoptosis, as well as aberrant cell-cell contacts and epithelial polarity (29–31), culminating in the formation of a focal cyst. In ARPKD,
however, the congenital presence of defects in both Pkd1 alleles results in all renal epithelia being affected by loss of FPC. Therefore, the renal tubule ectasia characteristic of ARPKD appears throughout the nephron at the onset. In our Pkd2\(^{G316D/316D}\) mice, PC2 expression is universally down-regulated in all nephron segments of the kidneys. In this case, PC2 expression falls below a threshold required for normal function, tubule malformation can occur throughout the nephron. The fact that our Pkd2\(^{G316D/316D}\) mice present a histologic appearance similar to that of the kidneys of Pkd1\(^{−/−}\) mice suggests a common molecular mechanism underlying the cystogenesis of ADPKD and ARPKD. Our Pkd2\(^{G316D/316D}\) mice are able to serve as a novel mouse model to study cyst morphogenesis in human polycystic kidney disease.

Our recent study indicates that loss of FPC down-regulates PC2 expression in vivo but that Pkd2 mRNA levels remain unchanged (22), suggesting that FPC expression may be essential for PC2 protein stability. A recent report suggests that TAZ (transcriptional co-activator with PDZ-binding motif) physiologically interacts with the intracellular C terminus of PC2 and regulates PC2 stability via an SCF\(^{−\mathrm{TRCP}}\) E3 ligase pathway in vivo (32). Taz\(^{−/−}\) mice develop cystic kidneys and emphysema, suggesting that TAZ may play a role in the pathophysiology of polycystic kidney disease (33). The SCF\(^{−\mathrm{TRCP}}\) E3 ligase complex is thought to be involved in ubiquitination and degradation of many cytosolic proteins (34); it may therefore modulate PC2 stability. On the basis of these findings, we investigated whether physical interaction between FPC and PC2 prevents down-regulation of PC2. Given that cells transduced with the FBD-containing construct are able to restore normal PC2 expression, we speculate that FPC regulates PC2 expression by preventing TAZ-mediated degradation.

The clinical features of ARPKD are widely variable among affected individuals (35, 36). Approximately 50% of ARPKD patients have disease onset as neonates (37), and the mortality is 30–50% for patients affected during the neonatal period. Among patients with ARPKD that survive beyond the first year, 75% reach age 5, and only half of these patients develop end-stage renal disease (36–38). In addition, some ARPKD patients also experience systemic hypertension (36), portal hypertension (37), and suggestions and Dr. Caiying Guo (University of Connecticut) for suggestions and Dr. Caiying Guo (University of Connecticut) for molecular interaction between FPC and PC2

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