Nek8 Mutation Causes Overexpression of Galectin-1, Sorcin, and Vimentin and Accumulation of the Major Urinary Protein in Renal Cysts of jck Mice*

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The jck murine model, which results from a double point mutation in the nek8 gene, has been used to study the mechanism of autosomal recessive polycystic kidney disease (ARPKD). The renal proteome of jck mice was characterized by two-dimensional gel electrophoresis combined with mass spectrometry (MALDI-TOF/TOF). Four newly identified proteins were found to accumulate in the kidneys of jck mice with polycystic kidney disease (PKD) compared with their wild-type littermates. The proteins galectin-1, sorcin, and vimentin were found to be induced 9-, 9-, and 25-fold, respectively, in the PKD proteome relative to the wild type. The identity of these proteins was established by peptide mass fingerprinting and de novo MS/MS sequencing of selected peptides. Up-regulation of these three proteins may be due to the nek8 mutation, and their function may be related to the signaling and structural processes in the primary cilium. Additionally a series of protein isoforms observed only in the ARPKD kidney was identified as the major urinary protein (MUP). Peptide sequencing demonstrated that the isoforms MUP1, MUP2, and MUP6 are contained in this series. The MUP series showed a number of male-specific isoforms and a phosphorylation of the entire series with an increasing degree of phosphorylation of the acidic isoforms. In addition, the MUP series was localized to the cyst fluid of PKD mice, and a cellular mislocalization of galectin-1, sorcin, and vimentin in PKD tubular epithelial cells was shown. The abnormal and extremely high accumulation of the MUPS in the ARPKD kidney may be linked to a defect in protein transport and secretion. The discovery of these proteins will provide new information on the molecular and cellular processes associated with the mechanism of ARPKD. Molecular & Cellular Proteomics 4:1009–1020, 2005.

Polycystic kidney disease (PKD)1 is an important heritable disease in humans and is caused by the accumulation of fluid-filled cysts in the kidneys and other organs. The renal cysts originate from a single layer of epithelial cells lining the nephrons and renal collecting ducts. Cells from cystogenic epithelium have a higher rate of cellular proliferation and are less differentiated than normal collecting duct cells. The autosomal dominant form of the disease (ADPKD) occurs at a rate of 1 in 500–1,000 individuals, whereas the autosomal recessive form (ARPKD) occurs at a rate of 1 in 6,000–40,000 live births (1). Mutations in the genes PKD1 or PKD2 are responsible for ADPKD, whereas the gene PKHD1 is mutated in ARPKD. The proteins encoded by the PKD1 and PKD2 genes, polycystin-1 and polycystin-2, are both integral membrane proteins and associate to function as receptors or cation channels, affecting cytosolic Ca2+ concentration. The protein fibrocytin/polyductin, encoded by the gene PKHD1, is also a membrane protein, and although its function is unknown, its large extracellular domain suggests receptor activity (1). The proteins polycystin-1 and polycystin-2, as well as fibrocytin/polyductin, have been localized to the primary cilia, an organelle associated with the basal bodies and predominantly observed in epithelial cells, and have been found to interact directly with each other to form a complex (2–4).

A number of murine models have been developed to study the mechanism of PKD. These models were generated by spontaneous mutation or gene-targeted engineering and closely resemble human PKD with regards to cyst morphology, cyst localization, and disease progression (5). Several models resemble ADPKD, characterized by the formation of cysts originating from most parts of the nephron, whereas other models resemble ARPKD with cyst formation predominantly originating in the collecting duct. The proteins mutated in several murine models have been identified, specifically the protein cystin in the cpk model, Bicaudal C in the bpk and jcpk models, polaris in the orpk model, inversin in the inv model, and Nek1 in the kat model. Most of the murine PKD models, .

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1 The abbreviations used are: PKD, polycystic kidney disease; ADPKD, autosomal dominant polycystic kidney disease; ARMS, amplification refractory mutation system; ARPKD, autosomal recessive polycystic kidney disease; jck, juvenile cystic kidney; MUP, major urinary protein; NEK, NIMA-related kinase; NIMA, Never In Mitosis, gene A; PKA, protein kinase A; WT, wild-type; 2D, two-dimensional; DAPI, 4',6-diamidino-2-phenylindole dihydrochloride; PKHD, polycystic kidney and hepatic disease.
including cpk, bpk, orpk, and inv, resemble human ARPKD in phenotype (5). Several of the proteins associated with PKD disease in mice, including cystin, polars, and inv, have been localized to the primary cilium (5, 6). A double point mutation in the nek8 gene has been found to cause cystic kidney disease in the juvenile cystic kidney (jck) murine model, which resembles human ARPKD genetically (7). A related member of the highly conserved phylogenetic group of Nek proteins, Nek Fa2p, has recently been localized to the primary cilium in mouse kidney cells (8).

The jck murine model of cystic disease closely parallels the phenotype of human ARPKD in that it is a juvenile form of PKD and in cyst formation originating in the collecting ducts. We studied homozygous jck mice (PKD) relative to their wild-type (WT) littermates using a proteomic approach. The proteins galectin-1, sorcin, and vimentin were found to be up-regulated in the kidneys of PKD mice and may be functionally linked to the polycystin/fibrocystin network. A series of MUP isoforms was also found to be extremely up-regulated in PKD kidneys, which may be linked to abnormal secretion due to cell polarity defects in tubular epithelial cells. The mechanisms causing the accumulation of these four proteins in cystic kidneys may lead to new insights into the molecular and cellular pathology of ARPKD.

EXPERIMENTAL PROCEDURES

Animals and Genotyping—The jck mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Heterozygous jck breeder pairs that are phenotypically normal were raised, and their homozygous offspring (PKD and WT) were used in this study. Tetraprimer amplification refractory mutation system (ARMS)-PCR was used for genotyping jck mice with DNA extracted from tail clippings according to a method developed recently (9). The following primers were used based on the mutation at nucleotide 1346 (G to T) in the nek8 transcript: outer forward primer, AAGTACTGGAAGCTTCCTTCCCCAGTG; inner forward primer, TCTAGCCCACCATTGTAGAAGCCTGTGG; outer reverse primer, GGCCACGSGAGGTAGTACAGGTATAGG; inner reverse primer, AGGCCACCTCAACATTCTCAGTCA. Touchdown PCR was used to amplify allele-specific nek8 cDNA as follows: initial melting: 94 °C for 4 min; 2 cycles: 94 °C for 45 s, 68 °C for 45 s, 72 °C for 1 min; 2 cycles: 94 °C for 45 s, 67 °C for 45 s, 72 °C for 1 min; 2 cycles: 94 °C for 45 s, 66 °C for 45 s, 72 °C for 1 min; 2 cycles: 94 °C for 45 s, 65 °C for 45 s, 72 °C for 1 min; final extension: 72 °C for 10 min. PCR products were separated on 1.5% agarose gels and distinguished based on allele-specific size with 255 bp for the PKD mutant (H11002/H11002) and 220 bp for the WT (H11001/H11001). Heterozygous mice (H11001/H11002) show both PCR products. A 420-bp product is also formed due to the annealing of the outer forward and reverse primers (Fig. 1).

2-D Electrophoresis—Kidneys were collected at 9 weeks of age from homozygous PKD and WT littermates and snap frozen in liquid nitrogen after removing the papilla. For initial comparison of protein patterns in kidneys from PKD mice and WT littermates, proteins were extracted by homogenization of the tissue with RIPA buffer (50 mM Tris HCl, pH 7.5, 150 mM NaCl, 2% Triton X-100, 1% CHAPS, 1 mmp tablet Complete protease inhibitors (Roche Applied Science), 200 mM NaF, 200 mM Na2VO3) in a glass homogenizer on ice. The homogenate was centrifuged at 19,000 x g at 4°C for 5 min, and protein in the supernatant was precipitated with 3 volumes of acetone for 30 min at −20°C. Protein was resuspended in UT buffer (7 m urea, 2 m thiourea, 2% CHAPS, 15 mM dithioerythritol, 0.1% ampholytes pH
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3–10 (Amersham Biosciences) and quantified using the 2D Quant assay (Amersham Biosciences). IPG dry strips (24 cm, pH 3–10 nonlinear, Amersham Biosciences) were rehydrated with 450 μl of sample containing 1.5 mg of protein, and isoelectric focusing was carried out in an IPGphor unit for 64,000 V-h (Amersham Biosciences). IPG strips were frozen at −80 °C and equilibrated for 10 min in equilibration buffer (375 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS) containing 100 mM DTT and for another 10 min in equilibration buffer containing 250 mM iodoacetamide. They were then loaded on 25 × 20-cm slab gels (11% polyacrylamide), and proteins were separated according to molecular weight in an Ettan-DaltSix unit (Amersham Biosciences). Gels were stained with colloidal Coomassie Blue containing 20% trichloroacetic acid (10), imaged with an Epson 1680 densitometer, and analyzed using Delta2D software (Decodon, Greifswald, Germany).

For micromanage electrophoresis, kidneys were homogenized in sample solubilizing solution containing C7 detergent, reduced with tributylphosphine, and alkylated with acrylamide monomer (Proteome Systems, Woburn, MA). The cyst fluid was extracted from the renal cortex and medulla by finely mincing the tissue with razor blades, a process that would not disrupt cell membranes, and collecting the supernatant after centrifugation. Cyst fluid samples were mixed with C7 sample solubilizing solution prior to electrophoresis. The protein concentration in the cyst fluid was determined with the bicinchoninic assay (Pierce) and measured with the 2D Quant assay for all C7 detergent-containing samples. All samples were applied to 18-cm narrow range (pH 4.5–5.5) IPG strips (Amersham Biosciences) by dehydration loading. Proteins were separated according to their isoelectric point in the IEF II cell (Bio-Rad) for 100,000 V-h and subsequently frozen at −80 °C. The thawed IPG strips were equilibrated in ProteomIQ equilibration buffer (Proteome Systems) for 2 × 10 min and loaded on 16 × 16-cm slab gels (13% acrylamide). Second dimension electrophoresis was carried out on the Proteon II xi cell (Bio-Rad), and the gels were stained with colloidal Coomassie Blue or Pro-Q Diamond phosphoprotein stain (Molecular Probes, Eugene, OR). The induced spots were quantified based on their absolute spot volume relative to the combined spot volume of five consistently expressed spots selected as a base line for each gel.

**RESULTS**

Analysis of the PKD Kidney Proteome—Total protein from kidneys of PKD and WT mice was resolved by two-dimension electrophoresis. An initial characterization of the total protein between pH 3 and 10 showed that the most dramatic changes, with three replicates, occurred in the acidic region (Fig. 2). Hence this region was the focus of further detailed analysis. Differential analysis showed a series of strongly overexpressed spots present in the PKD kidney and nearly absent in the corresponding WT kidney. In addition, three distinct spots were highly up-regulated in the PKD kidney (Fig. 3, A and B). These spots were identified by a combination of peptide mass fingerprinting and MS/MS peptide sequencing as the series of major urinary protein (MUP), galectin-1, sorcin, and vimentin. The up-regulation of galectin-1, sorcin, and vimentin in the PKD kidney relative to the WT model was quantified with n = 3 (Fig. 3C). The spot volume was used in gel quantification, and up-regulation was expressed relative to the spot volume of designated internal standard spots. Five spots that were consistently expressed in PKD and WT mice (Fig. 3, A and B, arrows) were designated as internal standards and used as a base line in each gel. Galectin-1 and sorcin were both up-regulated 9-fold, whereas vimentin showed a 25-fold induction relative to the WT. A quantification of the up-regulation of the MUPs in the PKD kidney was not done due to the near absence of corresponding MUP spots in the WT kidney.

**Protein Identification by Mass Spectrometry**—Peptide se-
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quencing was based on the MS/MS fragmentation spectra of a tryptic peak selected from the MS spectrum of each protein. The fragmentation of the peak $m/z$ 1486, found in the MS spectrum of all the spots in the MUP series, showed the presence of two co-eluting peptides (Fig. 4A). The peptides FAQLCEEHGLR and DGETFQLMGLYGR were identified based on the $y$-ion series with supporting sequence information from the $b$- and $a$-ion series. The presence of specific amino acids in these sequences was also confirmed by their characteristic immonium ions (Fig. 4A). The identification of these peptides allowed a designation of specific MUP isoforms as many variants are documented in the literature (11). These peptides are both found only in isoforms MUP1, MUP2, and MUP6, which differ in sequence only by a few single nucleotide polymorphisms.

The identification of the proteins sorcin, vimentin, and galectin-1 was also based on MS/MS sequencing of selected tryptic peptides. The protein sorcin was identified based on a complete $y$-ion series and supporting $b$- and $a$-ions corresponding to the peptide QHFISFDSDR (data not shown). The protein vimentin was identified based on the fragmentation of the tryptic peak $m/z$ 2216, yielding the complete series of $y$-ions corresponding to the sequence EENFALEAANYQDT-IGR (Fig. 4B). In addition, the sequence information obtained from this tryptic peptide demonstrates the capabilities of mass spectrometry for high molecular weight peptide sequencing. The identification of galectin-1 was based on the fragmentation of the tryptic peak $m/z$ 1500, which showed a complete $y$-ion and partial $b$- and $a$-ion series corresponding to the sequence DSNNLCLHFNP (data not shown). Although there are more than 10 mouse galectin isoforms, this peptide sequence is only found in galectin-1.

Additional confirmation of the identification of the spot series as major urinary protein was obtained from peptide mass fingerprinting based on the tryptic profile observed in the MS spectrum. All spots in the series were identified with a molecular weight ($M_r$) and isoelectric point (pI) corresponding to their position on 2D gels (Table I). In addition, the high Mascot score (173) and extensive sequence coverage (57%) demonstrate a high level of confidence in the protein identification. The number of matched peptides in the MS spectrum (ten)
and the number of these peptides used for sequencing (four) as well as the accession number are also indicated. The identification of galectin-1, sorcin, and vimentin was also supported by peptide mass fingerprinting information, which includes matching Mr and pI as well as high Mascot scores and sequence coverage (Table I).

Analysis of the MUP Isoforms—A comparison of the MUP series accumulated in the kidneys of female and male mice showed significant differences in the number of spots. A series of seven MUP spots (1–7) was found in the kidneys of both sexes and showed similar spot intensity. However, six highly expressed MUP isoforms (a–f) were observed only in the kidneys of male mice (Fig. 5, A and B). This abundance and variance of the MUP series suggest possible post-translational modifications of the protein. A phosphoprotein stain showed that the entire MUP series was phosphorylated to a
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**TABLE I**

| Protein   | M<sub>r</sub> | pl  | Score | Coverage | Peptides (MS) | Peptides (MS/MS) | Accession number |
|-----------|---------------|-----|-------|----------|---------------|------------------|-----------------|
| MUP       | 20,660        | 5.04| 173   | 57       | 10            | 4                | gi|47059037        |
| Galectin-1| 15,138        | 5.32| 119   | 41       | 6             | 3                | gi|193442          |
| Sorcin    | 20,542        | 5.11| 82    | 37       | 7             | 1                | gi|13385076        |
| Vimentin  | 51,590        | 4.96| 182   | 37       | 21            | 2                | gi|2078001         |

**Identification of MUP, galectin-1, sorcin, and vimentin by peptide mass fingerprinting**

Mascot search parameters were as follows: mass tolerance, 50 ppm; variable modification, oxidation of methionine; species, *Mus musculus*; data base, NCBInr.

**Fig. 5.** The overexpression of several MUP isoforms in the PKD kidney is sex-specific. A series of seven spots (1–7) is observed in the kidney of both female (A) and male (B) PKD mice. An additional six isoforms (a–f) are highly expressed only in the kidney of male PKD mice. C, quantification of the degree of phosphorylation of the MUP series. The main MUP series (1–7) as well as additional isoforms are phosphorylated (inset) as indicated by the Pro-Q Diamond phosphoprotein stain. The more acidic MUP isoforms show a higher level of phosphorylation based on the ratio of phosphorylated versus total protein spot intensity.

Analysis of the Cyst Fluid of PKD Kidneys—The analysis of the proteome of the cyst fluid of PKD mice showed the presence of the MUP series, whereas the proteome of the extracellular fluid of WT kidneys did not show a detectable trace of the MUPs (Fig. 6, A and B). The entire MUP series was observed in the cyst fluid, including the male-specific isoforms shown in Fig. 6A, to an abundance comparable to that of the renal cell proteome. The distinct compartmentalization of galectin-1, sorcin, and vimentin in the cyst fluid could not be established. No protein or low abundance spots were observed in their expected positions, and no consistent pattern in the proteome of the cyst fluid of PKD relative to WT mice was apparent for these proteins (Fig. 6, boxes). The cyst fluid collected from the PKD kidneys ranged from 40–250 µl, whereas 3–10 µl of extracellular fluid were collected from WT kidneys (Fig. 6C). Both the cyst fluid and the extracellular fluid from WT kidneys were found to contain a significant amount of protein, ranging between 40–50 µg/µl for the PKD kidneys (2–13 mg of total protein) and 70–90 µg/µl for the WT kidneys (300–700 µg of total protein) (Fig. 6D).

**DISCUSSION**

The *jck* murine model of polycystic kidney disease was used to characterize the renal proteome of mice with PKD and their WT littermates, which were genotyped using a novel method to identify a *nek8* single point mutation (Fig. 1). The total protein in PKD and WT mice was analyzed on broad range gels (pH 3–10), and it was found that the most dramatic changes occurred in the acidic region (Fig. 2). A strong up-regulation of three proteins with possible functional roles in the mechanism of PKD, galectin-1, sorcin, and vimentin, was shown in an initial characterization of the renal proteome of *jck* mice. Furthermore an abnormal accumulation to extremely high levels of a series of MUP isoforms was found in the
kidneys of mice with PKD, whereas the MUPs were virtually absent from the kidneys of their WT littermates (Fig. 3). The utilization of C7-containing sample solubilizing buffer did not yield an increased amount of membrane/hydrophobic proteins relative to the buffer utilized for total protein extraction, which contained a mixture of Triton X-100 and CHAPS. Additionally it was found that electrophoresis using C7 buffer did not reduce the amount of streaking artifacts in the basic pH region during first dimension electrophoresis (data not shown). The induced proteins were unambiguously identified based on both peptide mass fingerprinting and de novo sequencing of selected peptides using a MALDI-TOF/TOF approach (Fig. 4 and Table I).

The PKD phenotype in jck mice is caused by a mutation in...
the nek8 kinase gene, a highly conserved member of the Never In Mitosis, gene A (NIMA)-related kinases (NEKs). NEK kinases are associated with the microtubule-organizing centers (centrosomes) at the base of primary cilia and hence regulate microtubule dynamics and are required for G2/M cell cycle progression (12). The centrosomal location of these kinases connects them to ciliary proteins, including microtubule proteins and the polycystin/fibrocystin complex. In the jck murine model, an abnormal interaction between NEK8 and the polycystin complex may give rise to PKD by disrupting microtubule dynamics, the mitotic spindle checkpoint, and the cytoskeleton. The proteins galectin-1, sorcin, and vimentin may participate in the signaling network associated with the primary cilium, and their up-regulation may be due to compensatory or consequential mechanisms associated with the loss of NEK8 function.

The up-regulated proteins identified in this study may be involved in the progression of polycystic kidney disease through a variety of pathways. The proteins vimentin and sorcin may associate directly with polycystin/fibrocystin in the primary cilia to form an osmo- and mechanosensing complex (Fig. 8). Extracellular galectin-1 may also be involved in the formation of such a complex. Downstream cell signaling from this complex may involve Ca\(^{2+}\) release or several developmental and osmosensing pathways (Fig. 8, box). Alterations in signal transduction may affect tubulogenesis, leading to defects in cell polarity and cystogenesis. The accumulation of the MUPs in the kidneys is directly linked to cystogenesis because MUPs are mainly present in cyst fluid, whereas an accumulation of vimentin may lead to tubular epithelial-mesenchymal trans-differentiation.

The galectins are a family of highly conserved carbohydrate-binding proteins that may occur in extracellular locations as well as in the nucleus and cytosol (13). Galectin-1 binds to cell surface glycoproteins with \(\beta\)-galactose residues in the glycan side chains (14). This important regulatory protein modulates cell-cell and cell-matrix interactions, cellular adhesion signaling pathways, cell proliferation, and apoptosis (15). This lectin is normally expressed at early stages of kidney development where it modulates tissue organization, and its expression is markedly down-regulated in healthy adult kidneys (13, 14). These processes are all affected in the course of PKD, suggesting that galectins may be important in the molecular pathology of the disease.

Galectin-1 is most similar to galectin-3, which has recently been shown to accumulate in the human ARPKD and the murine cpk models of cystic disease (15). Galectin-3 also plays a role in the determination of cell polarity in collecting duct epithelium (16). The role of galectin-1 in PKD has not been characterized to date. However, the subcellular localization of the polycystins at lateral borders of polarized kidney cells and of fibrocystin in the collecting ducts makes these proteins accessible to interactions with galectin-3 or -1 (15). The interaction of the polycystin/fibrocystin complex with extracellular galectin-1 may transduce signals affecting downstream tubulogenesis and cystogenesis (Fig. 8). Galectin-1 and galectin-3 are also involved in pre-mRNA splicing, and galectin-1 may be involved in pre-mRNA splicing of PKHD1, the gene mutated in human ARPKD (17).

The polycystin/fibrocystin complex in the primary cilia may also associate with sorcin and vimentin. The protein sorcin has been shown to interact with Ca\(^{2+}\) channels such as the ryanodine receptor Ca\(^{2+}\) channels in cardiomyocytes. This protein has been characterized as an endogenous inhibitor of
smooth endoplasmic reticulum Ca\(^{2+}\) release and has been shown to depress Ca\(^{2+}\) transients in cardiomyocytes (18, 19). Sorcin is phosphorylated by protein kinase A (PKA) and may be critical in maintaining intracellular Ca\(^{2+}\) homeostasis (18). Polycystin-2 and other transient receptor potential channels function as Ca\(^{2+}\)-permeable nonselective cation channels and are of prime significance for the molecular mechanisms underlying PKD (20). The influx of Ca\(^{2+}\) due to the polycystin-1/poly cystin-2 interaction in the primary cilium has been implicated in a downstream signaling cascade involving the activation of the ryanodine receptor in the endoplasmic reticulum (21). Renal sorcin has not been characterized, and its up-regulation in a model of ARPKD suggests that it may be a novel and critical regulator of Ca\(^{2+}\) signaling via the polycystin complex. Downstream Ca\(^{2+}\) signaling from this complex affects PKA activity, which may affect tubulogenesis and cystogenesis pathways. Feedback loops based on the phosphorylation of sorcin by PKA and of NEK8 by CDC2 may reinforce the mechanism of PKD (Fig. 8).

The intermediate filament protein vimentin has recently been shown to interact with the cytoplasmic portion of polycystin-1 in kidney epithelial cells, directly linking polycystin-1 to the intermediate filament network (22). Hence vimentin associates with the polycystin complex in an important interaction that may affect cell-cell adhesion junctions and polycystin-mediated cell signaling pathways (Fig. 8). The intracellular portion of polycystin-1 also interacts with important signaling molecules, such as 14-3-3, G-proteins, and regulator of G-protein signaling 7, and hence participates in activating protein 1-dependent gene transcription via the c-Jun NH\(_2\)-terminal kinase/mitogen-activated protein kinase pathway and T cell factor-mediated gene transcription via the Wnt/\(\beta\)-catenin pathway (22). These signaling processes have a direct impact on cell proliferation/apoptosis and affect tubulogenesis and cyst formation (Fig. 8, box). Vimentin accumulation during cystogenesis may also act as a feedback loop reinforcing these mechanisms and may lead to tubular epithelial-mesenchymal trans-differentiation for which it has been used as an in vitro marker (23).

In contrast to galectin-1, sorcin, and vimentin, the MUPs do not appear functionally linked to the polycystin/fibrocystin complex. The MUPs are normally synthesized in the liver, secreted into the circulation, filtered at renal glomeruli, and excreted in urine (11). Normal mice synthesize a large amount of MUPs, consisting of 5–10% of total liver mRNA in male mice and 1–2% in female mice. The MUP gene family consists of 35–40 highly homologous genes and pseudogenes, and MUP synthesis is under the control of different developmental and hormonal stimuli (11). MUPs function in binding and transport of small hydrophobic molecules and are involved in pheromone transport, detoxification, inflammatory responses, and animal behavior (24). Although the MUPs are members of the highly conserved lipocalin family, there is no known close functional analog of the rodent MUPs in humans. The most closely related human lipocalins are the odorant-binding proteins (25).

The abnormal accumulation of the MUPs in the PKD kidney may not result from increased renal synthesis, as for galectin-1, sorcin, and vimentin, but instead from a defect in their normal secretion pathway. In addition to abnormal ion and water transport, ARPKD may thus be characterized by abnormal protein transport across the renal tubular epithelium. The polarity defects of tubular epithelial cells associated with PKD may cause the abnormal secretion and retention of these proteins in the renal cysts. Furthermore the accumulation of the MUPs in the cysts may cause an increase in colloidal osmotic pressure, leading to further cystogenesis in a reinforcing mechanism. Significantly the cyst fluid collected from PKD mice contained the MUP series as observed in the renal proteome, whereas the equivalent extracellular fluid collected from WT mice did not show any detectable traces of these proteins (Fig. 6). The high protein concentration in the extracellular fluid of WT mice may originate from plasma proteins because the WT kidney is highly vascularized (Fig. 6). Immunohistochemical data suggest that all three proteins are mis-localized in PKD epithelium with an abnormal presence in the nucleus, cytoplasm, and possibly cystic epithelium, whereas their localization is limited to the basolateral membrane in WT cells (Fig. 7).

The multiple protein spots observed in the MUP series suggest a variety of post-translational modifications that affect their isoelectric point and, to a lesser extent, their molecular weight. The synthesis of these proteins is higher in male than female mice, and accordingly a number of sex-specific isoforms were observed in male mice only that may be related to their physiological function. Post-translational modifications are important in ligand recognition and signal transduction. The MUPs have been characterized as glycosylated proteins with a complex heterogeneity of the N-linked oligosaccharides (26). A phosphoprotein-specific stain also showed that the entire MUP series was phosphorylated with an increasing degree of phosphorylation of the more acidic isoforms, corresponding to their shift in isoelectric point (Fig. 5). The characterization of the post-translational modifications of the MUPs may provide important clues regarding substrate recognition by defective transporters in the ARPKD mechanism.

The discovery of three up-regulated proteins in the ARPKD kidney that can be functionally linked to the polycystin/fibrocystin signaling complex is a significant first stage study that may provide important new information leading to a better understanding of the molecular and cellular pathology of ARPKD. Galectin-1 is a signaling molecule involved in a variety of cellular proliferation, apoptosis, and adhesion processes, all of which are disrupted in PKD. The function of sorcin in the modulation of intracellular Ca\(^{2+}\) may be a critical and previously unknown component of the signaling involved in PKD. The direct association of vimentin with polycystin-1...
also shows that this protein may play a vital role in cytoskeletal function and possibly additional pathways. In addition, the high accumulation of an unrelated group of proteins, the MUPs, in ARPKD kidneys and specifically in the cyst fluid suggests that additional processes such as protein secretion and transport may be disrupted in ARPKD. The up-regulation of the MUPs and of galectin-1, sorcin, and vimentin in renal epithelial cells suggests changes in vital signaling pathways linked to abnormal mechano- and osmosensing in the primary cilium that lead to cystogenesis and ARPKD.

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