Two distinct signaling pathways, involving Wnt signaling and polycystin, have been found to be critical for normal kidney development. Renal tubulogenesis requires the presence of certain Wnt proteins, whereas mutations in polycystin impede the terminal differentiation of renal tubular epithelial cells, causing the development of large cystic kidneys that characterize autosomal dominant polycystic kidney disease. Polycystin is an integral membrane protein, consisting of several extracellular motifs indicative of cell-cell and cell-matrix interactions, coupled through multiple transmembrane domains to a functionally active cytoplasmic domain. We report here that expression of the C-terminal cytoplasmic domain of polycystin stabilizes soluble endogenous β-catenin and stimulates TCF-dependent gene transcription in human embryonic kidney cells. Microinjection of the polycystin C-terminal cytoplasmic domain induces dorsalization in zebrafish. Our findings suggest that polycystin has the capacity to modulate Wnt signaling during renal development.

The kidney is widely used as a model system to study the intricacies of tissue induction underlying vertebrate organogenesis. Kidney development begins with the condensation of mesenchyme around the ureteric bud in response to a signal from the ureter. Through reciprocal interactions between the ureteric bud and the mesenchyme, the metanephric mesenchyme evolves into the tubular epithelium that, together with a glomerulus, constitutes the mature nephron of the mammalian kidney (reviewed in Ref. 1). Embryonic kidney induction is thought to require the presence of Wnts, a highly conserved family of developmentally important secreted signaling molecules involved in embryonic induction, generation of cell polarity, and the specification of cell fate (reviewed in Refs. 2 and 3). The proposed pathway for Wnt signaling involves the inhibition of glycogen synthase kinase (GSK)-3β and the consequent posttranslational stabilization of soluble β-catenin, leading to its accumulation in the cytoplasm and nucleus (reviewed in Ref. 3). In the nucleus, β-catenin interacts with members of the TCF/LEF family of transcription factors to regulate gene expression (4, 5). Certain Wnt family members appear to mediate renal morphogenesis. Wnt-1, a Wnt family member not expressed in the kidney, nevertheless induces metanephric mesenchyme to differentiate into glomerular and renal tubular epithelia (6), whereas contact with the metanephric mesenchyme maintains Wnt-11 expression at the tip of the ureteric bud (7). Mesenchymal expression of Wnt-4 is required for kidney tubulogenesis; Wnt-4−/− mice fail to form pretubular cell aggregates, a requisite stage of early tubule formation (8). Thus, in the developing kidney, Wnts appear to be involved in the reciprocal interactions between the ureteric bud and surrounding mesenchyme that enable the latter to differentiate into epithelial tubule cells.

Polycystin, the gene product of PKD1, is an integral membrane protein with 11 putative transmembrane helices, an N-terminal extracellular region that contains motifs characteristic of cell-cell and cell-matrix interactions, and a C-terminal cytoplasmic domain of 226 amino acids (9, 10). Mutations of polycystin account for the majority of patients with autosomal dominant polycystic kidney disease, a common hereditary disease (1:1,000) of slowly progressive epithelial cyst formation. Mice lacking polycystin die perinatally with massively enlarged cystic kidneys. Analysis of PKD1−/− mice indicates that polycystin is required during renal development for the elongation and maturation of tubular structures (11). Further functional characterization of polycystin and the genetic manipulation of full-length polycystin has been hindered by its complexity. To gain insight into the function of polycystin, we have begun to characterize the signaling pathways that are activated by the C-terminal domain of polycystin, and have recently demonstrated that the C-terminal 226 amino acids of polycystin trigger activation of the transcription factor AP-1 (12). This study demonstrates that the C-terminal cytoplasmic domain of polycystin activates Wnt signaling.

**EXPERIMENTAL PROCEDURES**

Reagents and Plasmids—MG132 (ProScript), lithium acetate (Sigma), cycloheximide (Sigma), and human epidermal growth factor (EGF) (Clonetics) were used at concentrations as indicated. The C-terminal domains of polycystin were expressed as membrane-bound fusion proteins using CD16-CD7 domains or the leader sequence of CD5 fused to the CH2 and CH3 domain of human IgG, followed by the transmembrane region of CD7 as described previously (12, 16). The AP-1 and Siamois promoter constructs have been recently described (12, 19). The His-tagged c-Jun and hemagglutinin (HA)-tagged ubiquitin were kindly provided by D. Bohmann, the HA-tagged GSK-3β by J.R. Woodgett, and the EGF receptor HER453 by A. Ullrich. The cDNAs encoding the CD16,7-polycystin fusions were cut with XhoI and inserted in frame into the SpeI site of the pX7T vector, a derivative of
pGEM4Z (Promega) and pSP64T. The GST-Axin 495–590 fusion protein contains amino acids 495 to 590 of mouse Axin fused to glutathione S-transferase (GST) and was generated by polymerase chain reaction, utilizing a myc-tagged full-length mouse axin as a template (kindly provided by F. Costantini).

**Western Blot Analysis**—HEK 293T cells seeded in 6-well plates were transiently transfected by the calcium phosphate method. After incubation for 24 h, cells were lysed in sample buffer, fractionated on SDS-PAGE, and electrophorobbed to polyvinylidene difluoride membrane (NEN Life Science Products). For the time course, cells were incubated with cycloheximide for the indicated durations after pretreatment with 40 μM MG132, 20 mM lithium acetate, or medium alone for 3 h as indicated. Cell lysates were immunoblotted with an anti-β-catenin antibody. In all experiments, coexpression of F.gfp was utilized to monitor transfection efficiency and equal loading. F.gfp was stable over the course of 8 h, and not affected by lithium, MG132, or polycystin. A representative Western blot analysis of F.gfp expression is shown at the bottom of the panel. c, the C-terminal domain of polycystin increases soluble but not membrane β-catenin levels. HEK 293T cells transiently transfected with CD16.7, CD16.7-PKD 1–226, or CD16.7-PKD 115–226 were lysed, anucleated, and fractionated at 100,000× g. Lysates of subcellular fractions were immunoblotted with an anti-β-catenin antibody (top). Amounts of cytoplasmic β-catenin (black bars) were quantified by densitometric tracing after normalizing for membraneous fractions (white bars) (bottom). d, c-Jun ubiquitination is reduced in the presence of C-terminal polycystin. Cell extracts of HEK 293T cells transiently cotransfected with CD16.7 control, CD16.7-PKD 115–226, His6-tagged c-Jun, HA-tagged ubiquitin constructs as indicated and incubated with 40 μM MG132 or medium alone for 3 h. His6-tagged c-Jun conjugates were purified by nickel-chelate affinity chromatography and immunoblotted with an anti-HA antibody to detect c-Jun-ubiquitin conjugates. The lower panel shows the same blot developed with a polyclonal antibody against c-Jun.

**Fig. 1. The C-terminal domain of polycystin increases β-catenin stability and inhibits c-Jun ubiquitination.** a, the C-terminal domain of polycystin increases β-catenin levels. Cell lysates of HEK 293T cells transiently cotransfected with Flag-tagged β-catenin, F.gfp, and CD16.7 or CD16.7-PKD 1–92 (controls), CD16.7-PKD 1–226, or CD16.7-PKD 115–226 were immunoblotted with an anti-Flag antibody. b, β-catenin is stabilized by MG132, lithium, and polycystin. HEK 293T cells were transiently transfected with control plasmids CD16.7 or CD16.7-PKD 115–226 and incubated with 40 μg/ml cycloheximide for 0, 1, 2, 4, 6, and 8 h after pretreatment with 40 μM MG132, 20 mM lithium acetate, or medium alone for 3 h as indicated. Cell lysates were immunoblotted with an anti-β-catenin antibody. In all experiments, coexpression of F.gfp was utilized to monitor transfection efficiency and equal loading. F.gfp was stable over the course of 8 h, and not affected by lithium, MG132, or polycystin. A representative Western blot analysis of F.gfp expression is shown at the bottom of the panel. c, the C-terminal domain of polycystin increases soluble but not membrane β-catenin levels. HEK 293T cells transiently transfected with CD16.7, CD16.7-PKD 1–226, or CD16.7-PKD 115–226 were lysed, anucleated, and fractionated at 100,000× g. Lysates of subcellular fractions were immunoblotted with an anti-β-catenin antibody (top). Amounts of cytoplasmic β-catenin (black bars) were quantified by densitometric tracing after normalizing for membraneous fractions (white bars) (bottom). d, c-Jun ubiquitination is reduced in the presence of C-terminal polycystin. Cell extracts of HEK 293T cells transiently cotransfected with CD16.7 control, CD16.7-PKD 115–226, His6-tagged c-Jun, HA-tagged ubiquitin constructs as indicated and incubated with 40 μM MG132 or medium alone for 3 h. His6-tagged c-Jun conjugates were purified by nickel-chelate affinity chromatography and immunoblotted with an anti-HA antibody to detect c-Jun-ubiquitin conjugates. The lower panel shows the same blot developed with a polyclonal antibody against c-Jun.
with M2 anti-Flag monoclonal antibody or anti-β-catenin followed by incubation with horseradish peroxidase-coupled sheep anti-mouse immunoglobulin (Dako). Imobilized antibodies were detected by chemiluminescence (Pierce).

**Subcellular Fractionation**—HEK 293T cells seeded in 10-cm plates were transiently transfected with the indicated constructs. After incubation for 24 h, cells were homogenized in 0.5 ml of 250 mM sucrose, 10 mM HEPES (pH 7.4), 2 mM MgCl₂, 1 mM EGTA, 0.5 mM EDTA, 2 mM Na₃VO₄, containing 4 μg/ml phenylmethylsulfonyl fluoride, and protease inhibitor mixture (Boehringer Mannheim). After two successive centrifugations at 1,000 g for 10 min at 4 °C, the anucleated supernatant was centrifuged at 100,000 g for 1 h. The supernatant (S₁₀₀ soluble fraction) was removed and the pellet (P₁₀₀ membrane fraction) was resuspended in 0.1 ml of solubilization buffer (homogenization buffer containing 1% Triton X-100 and 1% SDS). After determination of protein concentrations by the Bio-Rad DC protein assay, subcellular fractions containing equal amounts of protein were concentrated by acetone precipitation, eluted by heating in SDS-PAGE sample buffer, and subjected to SDS-PAGE and immunoblot analysis using an anti-β-catenin monoclonal antibody (Santa Cruz Biotechnology, Inc.).

**Ubiquitination Assay**—HEK 293T cells seeded in 10-cm plates were transiently transfected with the indicated constructs. After incubation for 24 h, cells were incubated with MG132 or medium alone for 3 h, lysed in 6 M guanidinium-HCl, 0.1 M Na₂HPO₄/NaH₂PO₄ (pH 8.0), 5 mM imidazole, and sonicated for 1 min. After centrifugation for 15 min at 4 °C, the His₆-tagged c-Jun conjugates were precipitated from the cleared lysate with Ni²⁺-NTA-agarose (Qiagen) for 2 h at room temperature. Complexes were washed with 8 M urea, 0.1 M Tris, pH 8.0, and resuspended in sample buffer. Proteins were fractionated on SDS-PAGE, electroblotted to polyvinylidene difluoride membrane, and immunostained with rabbit polyclonal anti-α-HA serum (Santa Cruz), followed by incubation with horseradish peroxidase-coupled anti-rabbit immunoglobulin (Amersham Pharmacia Biotech). Immobilized antibodies were detected by chemiluminescence (Pierce).

**In Vitro Binding Assay**—[³⁵S]Methionine-labeled GSK-3β was generated in a 40-μl reaction mix, using the Promega TNT system following the instructions of the manufacturer. 10-μl of the reaction mix was then incubated with 2 μg of GST-PKD 115–226 or GST-Axin 485–590, and immobilized on glutathione-Sepharose in the presence of 450 μl reaction mix.
tion buffer (50 mM potassium phosphate, pH 7.5, 150 mM KCl, 1 mM MgCl₂, 10% (v/v) glycerol, 1% Triton X-100, and protease inhibitors). The reaction mix was incubated for 2 h, washed three times in reaction buffer, and separated on a 10% SDS acrylamide gel. Radiolabeled GSK-3β was detected by autoradiography. The amount of bound GSK-3β was compared with 5% of labeled input protein equivalent to 0.5 µl of the labeling mix.

In Vivo Coimmunoprecipitation—HEK 293T cells were transiently transfected with 5 µg of plasmid encoding HA-tagged GSK-3β and 5 µg of plasmids encoding slg.7, slg.7-PKD 115–226 (16), or myc-Axin (kindly provided by F. Costantini) by the calcium phosphate method. After incubation for 24 h, cells were washed twice with phosphate-buffered saline and then lysed in 1 ml of 1% Triton X-100, 0.5% Nonidet P-40 buffer containing 150 mM KCl, 20 mM Tris-HCl, pH 7.5, 1 mM EDTA, and protease inhibitors. Cell lysates containing equal amounts of total protein were incubated for 4 h at 4 °C with 30 µl of protein G-Sepharose beads (Amersham Pharmacia Biotech); the myc-Axin containing lysate was additionally incubated with 5 µg of anti-mouse monoclonal antibody (Calbiochem). The beads were washed extensively with lysis buffer, and bound proteins were fractionated by 10% SDS-PAGE. Western blot analysis was performed with anti-HA rabbit polyclonal antibody (Santa Cruz) followed by incubation with horseradish peroxidase-coupled donkey anti-rabbit immunoglobulin (Amersham Pharmacia Biotech). Immobilized antibodies were detected by chemiluminescence (Pierce).

GSK-3 Kinase Assay—HEK 293T cells were transfected with plasmids encoding CD16.7-PKD 115–226 or control CD16.7 as indicated. Total DNA amount was titrated to 10 µg with control vector. Cells from one 10-cm dish were lysed 24 h after transfection in 1 ml of cold lysis buffer containing 1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM EGTA, 1 mM Na₃VO₄, and a protease inhibitor mixture (Boehringer Mannheim). After centrifugation for 15 min at 4 °C, GSK-3β was immunoprecipitated from the cleared lysate with 5 µg of monoclonal anti-GSK-3β antibody (Transduction Laboratories) for 2 h at 4 °C. Immune complexes were immobilized by adding 40 µl of Gamma-Bind-Sepharose (Amersham Pharmacia Biotech) and washed twice with 800 µl of lysis buffer. During the second wash, 200 µl of resuspended beads were removed for Western blot analysis. Immunoprecipitates were washed with 500 µl of kinase reaction buffer (20 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 5 mM dithiothreitol) and resuspended in 50 µl of kinase reaction buffer containing 50 µM of cAMP-responsive element binding phosphopeptide (NEB). The assay was carried out in the presence of 20 µM unlabeled ATP and 10 mM MgCl₂ (γ-32P)ATP for 30 min at 30 °C. A 25-µl aliquot was applied to a phosphocellulose membrane spin column (Pierce), washed with 500 µl of 75 mM phosphoric acid, and assayed by scintillation spectrophotometry. GSK-3β immunoprecipitates, removed before the kinase assay, were analyzed by SDS-PAGE and Western blot, using the anti-GSK-3β monoclonal antibody in combination with a goat anti-mouse horseradish peroxidase antibody (Dako) and enhanced chemiluminescence (Pierce).

Luciferase Assay—HEK 293T cells seeded in 12-well plates were transiently transfected with a luciferase reporter construct, a β-galactosidase expression vector (kindly provided by C. Cepko), and vectors directing the expression of CD16.7 fusión proteins, Wnt signaling components, and the EGF receptor HER453 as indicated. Total DNA amount was 3 µg/well. Cells were serum starved for 24 h, harvested in cold phosphate-buffered saline, and lysed in 100 µl of reporter lysis buffer (Promega) for 15 min at room temperature. Lysates were centrifuged at 14,000 rpm for 3 min to remove insoluble material. Luciferase activity was determined using a commercial assay system (Promega) following the manufacturer’s instructions, and normalized for β-galactosidase activity to correct for the transfection efficiency. EGF at 20 ng/ml was added for 8 h before the assay.

Embryo Microinjection—Zebrafish embryos were injected at the 1–2 cell stage with 10–20 pg of either DNA expression vectors or capped mRNA. For DNA expression vector studies, embryos were injected with CD16.7-PKD 115–226 or the control vector CD16.7 in 200 mM KCl and 0.1% phenol red as a tracer. Capped mRNA for injection was synthesized using a MessageMachine kit (Ambion) and T7 polymerase. RNA was injected in a solution of 0.1% phenol red. Embryos were examined during epiboly, 5–10 h post-fertilization (hpf), and at 24 h.

RESULTS
Polycystin Stabilizes Soluble β-Catenin—Recent studies indicate that Wnt signal transduction involves the inhibition of GSK-3β activity, leading to a posttranslational stabilization of soluble β-catenin that is commonly used as an indicator for Wnt signaling. We found that the C-terminal cytoplasmic domain of polycystin significantly elevated total β-catenin steady-state protein levels (Fig. 1a) in HEK 293T cells that were transiently cotransfected with a Flag-tagged β-catenin plasmid together with cDNA encoding the C-terminal cytoplasmic tail of polycystin, CD16.7-PKD 1–226. The C-terminal domain of polycystin was fused to the extracellular domain of CD16 and the transmembrane domain of CD7, forming a heterologous integral membrane protein. This approach has been used to delineate effector functions and protein-protein interactions of various receptor cytoplasmic domains by targeting them to the plasma membrane (14–16). The accumulation of β-catenin was further localized to the C-terminal 113 amino acids of polycystin, whereas two controls, CD16.7, a construct with a stop codon shortly after the transmembrane domain and CD16.7-PKD 1–92, a construct containing the N-terminal 92 amino acids of the C-terminal cytoplasmic domain of polycystin, had no detectable effect on β-catenin levels. As a positive control, exposure to lithium, a known inhibitor of GSK-3β, stabilized β-catenin in CD16.7-transfected HEK 293T cells. Cells were cotransfected with a constant amount of plasmid DNA encoding Tag-flagged green fluorescent protein (EGFP) to control for loading and transfection efficiency.

To demonstrate that the accumulation of β-catenin was mediated through the stabilization of β-catenin, we examined the effect of polycystin on the half-life of endogenous β-catenin...
while inhibiting protein synthesis. HEK 293T cells were transfected with a control plasmid CD16.7 or the construct CD16.7-PKD 115–226 and treated with the protein synthesis inhibitor cycloheximide (Fig. 1b). HEK 293T cells transfected with CD16.7 showed a gradual decline of β-catenin protein levels over the course of 8 h. β-catenin is subject to ubiquitin-dependent proteasome degradation, a process downstream of GSK-3β that can be retarded by inhibiting ubiquitination or the proteasome (19). Preincubation of CD16.7-transfected cells with lithium, an inhibitor of GSK-3β and ubiquitination, or MG132, a proteasome inhibitor, prolonged the half-life of β-catenin. A similar prolongation of β-catenin half-life was seen in HEK 293T cells transfected with CD16.7-PKD 115–226. To determine whether polycystin increased the cytoplasmic fraction of β-catenin, transfected HEK 293T cells were fractionated with CD16.7 showed a gradual decline of β-catenin protein levels over the course of 8 h. β-catenin is subject to ubiquitin-dependent proteasome degradation, a process downstream of GSK-3β that can be retarded by inhibiting ubiquitination or the proteasome (19). Preincubation of CD16.7-transfected cells with lithium, an inhibitor of GSK-3β and ubiquitination, or MG132, a proteasome inhibitor, prolonged the half-life of β-catenin. A similar prolongation of β-catenin half-life was seen in HEK 293T cells transfected with CD16.7-PKD 115–226. To determine whether polycystin increased the cytoplasmic fraction of β-catenin, transfected HEK 293T cells were fractionated with lithium, an inhibitor of GSK-3β and ubiquitination, or MG132, a proteasome inhibitor, prolonged the half-life of β-catenin. 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The inhibition of GSK activity is comparable to that reported for Wg in mouse fibroblasts, which is sufficient to promote Wnt signaling (23), and to the inhibitory effect of insulin-like growth factor-1 or insulin in muscle cells (24).

**Polycystin Induces TCF-dependent Gene Transcription**—To establish that the inhibition of GSK-3β activity by polycystin functionally couples to downstream events of Wnt signaling, the formation of transcriptional active β-catenin/TCF complexes was assayed by cotransfecting a Xenopus Siamois luciferase reporter construct (−833pSiaLuc) into HEK 293T cells. The promoter of the dorsalizing homeobox gene Siamois is a direct target for the β-catenin/TCF complex (25). Both CD16.7-PKD 1–226 and CD16.7-PKD 115–226, but not CD16.7-PKD 1–92, activated the Siamois promoter 10–12-fold (Fig. 3a). In contrast, CD16.7-PKD 1–226 and CD16.7-PKD 1–92, but not CD16.7-PKD 115–226 induced AP-1-dependent transcription (Fig. 3b). Wnt-dependent activation of the Siamois promoter requires the most proximal TCF-binding site (13). A truncated Siamois promoter construct (−245pSiaLuc) with a mutation of the proximal activating TCF-binding site showed significantly decreased responsiveness to polycystin (Fig. 4b) compared with the unmutated, truncated Siamois promoter (Fig. 4a), indicating that polycystin-mediated activation of the Siamois promoter depends upon the binding of β-catenin/TCF complexes. The differences in signaling mediated between polycystin and EGF are demonstrated in Fig. 4c. EGF is a GSK-3 inhibitor that does not activate Wnt signaling. EGF stimulation of HEK 293T cells expressing EGF receptor did not activate the Siamois, but activated the AP-1 promoter 3–4-fold. In contrast, polycystin mediated a 10–12-fold induction of both AP-1 and Siamois promoter constructs. Thus, unlike growth factors such as EGF, which inhibit GSK-3β without activating the Wnt signaling pathway, polycystin both inhibits GSK-3β and activates Wnt signaling, a signaling pattern resembling that of canonical Wnt signaling molecules. Furthermore, polycystin augmented the activity of four Wnt signaling components, XWnt8, XDsh, rFz2, and β-catenin (Fig. 5).

**Polycystin Dorsalizes Zebrafish Embryos**—Previous work has shown that Wnt signaling is involved in specifying dorsal anterior fates and antagonizing signals specifying ventral posterior cell fates in the early embryonic axis (26–28). In zebrafish, β-catenin induces an ectopic embryonic axis, whereas lithium produces hyperdorsalization (29, 30). To examine whether polycystin has a similar activity during early zebrafish development, embryos were injected at the 1–2 cell stage with either the control DNA expression vector CD16.7 or CD16.7-PKD 115–226. Control injected embryos showed no morphological abnormalities at any stage of development indicating that expression of the CD16.7 fusion protein does not disrupt normal development (Fig. 6a). In contrast, a marked dorsalizing effect of CD16.7-PKD 115–226 DNA was observed in injected embryos examined at 24 hpf (Fig. 6b). Embryos formed relatively normal head and brain tissue but invariably showed defects in posterior trunk and tail development. Injections of DNA encoding the same portion of the polycystin C terminus resulted in similarly dorsalized embryos (Fig. 6c). In the most extreme cases the embryo failed to complete epiboly resulting in a constriction around the yolk cell and an exposed yolk cell mass at 24 hpf (Fig. 6c). Trunk muscle and pronephric duct development was detectable although the form and organization of these tissues was disrupted (data not shown). In the least severely affected embryos, the tail formed, but defects in the development of ventral tissue just posterior to the yolk extension were always observed (Fig. 6d). The results indicate that when ectopically expressed in the context of early zebrafish development, the polycystin C terminus can have hyperdorsalizing effects, similar to that seen with GSK-3β inhibition by lithium.

**DISCUSSION**

Autosomal dominant polycystic kidney disease, the leading genetic cause of renal failure, is primarily caused by mutations in polycystin. Polycystin is required for normal tubulogenesis during renal development, but its precise physiologic function is not yet defined. Studies of PKD1−/− mutant mice indicate that early stages of tubule morphogenesis, such as nephrogenic condensation and epithelialization, remain intact (11). Expression of polycystin can be detected at E14 in the ureteric bud, and subsequently in the S- and Comma-shapes of the condensing mesenchyme (31–37). Cyst formation begins at embryonic day 15.5 (E15.5) in proximal tubules and progresses rapidly to replace the entire renal parenchyma. Wnt-4 is also required for in...
renal tubulogenesis during this time; at E15.5 the mesenchyme of Wnt-4→→ mice fails to aggregate and differentiate, lacking pretubular aggregates and more developed tubules (8). Kidneys of Wnt-4→→ mice are growth retarded at E15.5 and agenic at E18.5, consisting of undifferentiated mesenchyme interspersed with branches of collecting duct epithelium. In the developing kidney, the expression pattern of polycystin appears to overlap with at least two known Wnt family members, Wnt-11, expressed in the ureteric bud (38), and Wnt-4, expressed in mesenchymal aggregates undergoing epithelial transition (8). Both spatially and temporally, Wnt and polycystin localization and signaling events coincide, supporting the hypothesis that polycystin may modulate Wnt signaling. Polycystin augmented the transcriptional activity of XWnt8, XDsh, Rfp2, and β-catenin, indicating that polycystin may serve to reinforce or maintain Wnt signaling during critical stages of renal tubulogenesis. The combinatorial interaction of Wnt signaling with other signaling pathways promotes functions that are different from the function of Wnt molecules alone (reviewed in Ref. 2). Although Wnt family members induce mesenchymal-epithelial conversion, cross-talk with polycystin may be required at a later stage to maintain tubular structure, polarity, and integrity. It is particularly interesting that β-catenin, a transcriptional mediator of Wnt signaling, recently has been reported to regulate the reorganization of renal epithelial cell aggregates into tubules (39).

Ectopic expression of many Wnt pathway components upstream of β-catenin can be mimicked by the overexpression of β-catenin in a variety of systems, suggesting that the role of certain Wnt family members is to increase levels of soluble β-catenin (40). Genetic and biochemical studies indicate that secreted Wnts, through the family of frizzled receptors, activate disheveled and inhibit glycogen synthase kinase, to stabilize β-catenin and facilitate transcription of certain target genes (reviewed in Refs. 2 and 3). In the absence of Wnt signaling, cytoplasmic β-catenin is rapidly degraded in mammalian cells by a process involving the adenomatous polyposis coli tumor suppressor protein, ubiquitination, and proteasome (19, 41). Inhibition of the ubiquitination-proteasome pathway or overexpression of its β-catenin results in cytoplasmic accumulation of β-catenin and its translocation into the nucleus (42). A modest inhibition of GSK-3β activity appears sufficient to transduce Wnt signaling, raising the possibility that only a Wnt-sensitive subcellular GSK-3β pool is able to regulate the stability of β-catenin (reviewed in Ref. 3). Our data suggests that expression of the C-terminal domain of polycystin stabilizes soluble β-catenin and increases the amount of transcriptionally active β-catenin. Although GSK-3β activity was reduced in the presence of polycystin, we were unable to detect a direct interaction of the C terminus of polycystin with GSK-3β in vitro or in vivo, indicating that polycystin does not directly inhibit GSK-3β kinase activity through a physical interaction.

A critical role for GSK-3β in early vertebrate pattern formation has been clearly established in dorsoventral axis formation (43–45). Lithium, an inhibitor of GSK-3β, dorsalizes zebrafish and Xenopus (30, 46), β-catenin induces induction of a complete secondary body axis in both species (29, 40), and some upstream components of the Wnt-signaling pathway induce an ectopic embryonic axis in Xenopus (reviewed in Ref. 3). A marked dorsalizing effect of the terminal 112 amino acids of polycystin was observed in zebrafish embryos. The polycystin phenotype strikingly resembles the exaggerated dorsi-anterior structures resulting from lithium treatment, indicating that polycystin may similarly inhibit GSK-3β during zebrafish development (30).

The ligand of polycystin remains unidentified; hence, it is still unknown how extracellular events may influence the capacity of polycystin to modulate Wnt signaling. We speculate that ligand-receptor interactions governing the activity of the C-terminal portion of polycystin will be found to influence cytoplasmic-signaling cascades during critical stages of renal tubulogenesis.

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