Polycystin-2 Cation Channel Function Is under the Control of Microtubular Structures in Primary Cilia of Renal Epithelial Cells*

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Mutations in the gene encoding polycystin-2 (PC2) result in autosomal dominant polycystic kidney disease and defects in left-right asymmetry during embryogenesis. PC2 is a TRP-type Ca2+-permeable non-selective cation channel, which is expressed in kidney and other organs. PC2 is present and functional in microtubule-containing primary cilia of renal epithelial cells. However, no information is yet available as to whether PC2 interacts with microtubules. Here, we assessed the role of microtubular dynamics in regulating PC2 channel function in primary cilia. Isolated ciliary membranes from LLC-PK1 epithelial cells were reconstituted in a lipid bilayer system. The acute addition of the microtubular disrupter colchicine (15 μM) rapidly abolished, whereas the addition of the microtubular stabilizer paclitaxel (taxol, 15 μM) increased ciliary PC2 channel activity. The further addition of α-tubulin plus GTP also stimulated PC2 channel activity in ciliary membranes. However, α-tubulin and GTP had no effect on in vitro translated PC2. Using the yeast two-hybrid assay, we found that PC2 interacts with the microtubule-dependent motor kinesin-2 subunit KIF3A, a protein involved in polycystic kidney disease. The interaction occurred through the carboxyl terminus domain of both proteins, which was further confirmed by in vitro glutathione S-transferase pull-down and dot blot overlay assays. Co-immunoprecipitation experiments showed that PC2 and KIF3A are in the same complex in native HEK293, Madin-Darby canine kidney cells (MDCK), and LLC-PK1 cells. Immunofluorescent staining also showed substantial PC2 and KIF3A co-localization in primary cilia of renal epithelial cells. The data indicate that microtubular organization regulates PC2 function, which may explain, among others, the regulatory role of PC2 in the sensory function of primary cilia.

Autosomal dominant polycystic kidney disease is a common and lethal disease inherited as a dominant trait in humans, with an incidence of about one per 500–1000 in the population (1). The gene responsible for 85% of cases in autosomal dominant polycystic kidney disease is PKD1, encoding polycystin-1 (PC1), whereas most of the remaining cases are ascribed to mutations in the PKD2 gene, encoding polycystin-2 (PC2). PC2 is a TRP-type Ca2+-permeable non-selective cation channel that interacts with PC1 in a functional complex often implicated in a common signaling pathway. Recent studies demonstrated that actin cytoskeletal structures associate with PC2 (2–4) and modulate PC2 channel function (5, 6). We observed that PC2 anchors the actin bundling protein, α-actinin (6), which is implicated in cell adherence and proliferation (7). We also determined that the actin-severing protein, gelsolin, regulates PC2 channel function by remodeling cytoskeletal structures in a Ca2+-dependent manner (5). In comparison, whether PC2 has a connection to microtubules remains unknown. Nonetheless, recent evidence strongly suggests potential links between renal cystic diseases and microtubular structures, in particular, the microtubule-enriched organelle known as the primary cilium.

Interestingly, recent observations support the idea that autosomal dominant polycystic kidney disease cyst formation implicates specific structuralfunctional abnormalities in primary cilia of renal epithelial cells (8, 9). The primary cilium is a solitary, usually non-motile, organelle projecting from the apical surface of mammalian cells. The primary cilium is supported by an array of nine doublets of peripheral microtubules (9 + 0) known as an axoneme and a surrounding membrane (10, 11). Most renal tubular epithelial cells of the mammalian nephron, with the exception of intercalated cells in the collecting duct, express a single primary cilium, which protrudes into the tubular lumen of the nephron and responds to fluid flow or mechanical bending by eliciting cytosolic calcium signals. Thus, primary cilium play an important role in mechano-sensation (10, 11).

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5 The abbreviations used are: PC1, polycystin-1; PC2, polycystin-2; PC2N, amino terminus of PC2; GST, glutathione S-transferase; MDCK, Madin-Darby canine kidney cells; PBS, phosphate-buffered saline; MES, 4-morpholineethanesulfonic acid; MOPS, 4-morpholinepropanesulfonic acid; PKD, polycystic kidney disease; TRP, transient receptor potential; IMCD, inner medullary collecting duct.
Recent studies demonstrated that both PC1 and PC2 are expressed in renal primary cilia (9, 12). Further, renal epithelial cells expressing a dysfunctional PC1 or PC2 have morphologically normal cilia but an impaired calcium response to fluid flow, suggesting that the PC1/PC2 complex contributes to fluid-flow sensation by renal primary cilia (9).

Various forms of cystic kidney disease not directly linked to mutations in the PKD genes have been associated with microtubular-associated ciliary proteins (8, 13–16). Fibrocystin, encoded by PKHD1, whose mutations lead to autosomal recessive polycystic kidney disease, is present in primary cilia of renal epithelia (12, 13, 16, 17). The cpk gene, whose mutations also cause renal cystic disease, encodes cystin (13), a novel protein that is expressed and co-localizes with polaris in primary cilia of renal epithelial cells. Treatment of homozygous cpk/cpk mice with the microtubular stabilizer paclitaxel (taxol) (18) dramatically moderates progression of the disease. The Tg737 gene affected in the orpk PKD mouse encodes another ciliary protein, polaris, which localizes to the ciliary basal body and the microtubular axoneme (16).

Eukaryotic cilia are assembled and maintained by a process called intraflagellar (or intraciliary) transport in which the ciliary axoneme is assembled into large raft structures, the intraflagellar transport particles at the basal bodies (10, 20, 21). Intraflagellar transport rafts are then transported from the basal bodies to the distal tip of the primary cilium (21). Kinesin-2 and cytoplasmic dynein motor proteins mediate the anterograde and retrograde traffic of proteins along the axoneme, respectively. Kinesin-2 forms a heterotrimeric complex composed of two similar (but not identical) motor subunits KIF3A and KIF3B or KIF3C and a tail-associated non-motor accessory subunit KAP3 (22). Knock-out mice lacking either KIF3A or KIF3B fail to synthesize cilia in the embryonic node and exhibit randomization of left-right asymmetry and cystogenesis.
structural abnormalities of the neural tube, pericardium, brachial arches, and somites (22, 23). Interestingly, mice with either disrupted PC2, polaris, and KIF3A or disrupted KIF3B showed similar phenotypes, including defects in left-right asymmetry and other abnormalities during embryogenesis (15, 23, 24). Thus, herein, we hypothesized co-localization and functional regulation of the PC2 channel by microtubular structures of primary cilia. This interaction may be an important component of the novel sensory properties of the primary cilium.

Here, we determined that maneuvers that induce microtubular reorganization or stabilization activate, whereas disruption of endogenous microtubules inhibits, the channel activity of PC2 from the primary cilium of renal epithelial cells. Interestingly, however, tubulin itself was incapable of regulating the isolated PC2 channel, suggesting that immediate anchoring proteins mediate this interaction. In search for such molecular linkage, we identified a physical interaction between PC2 and KIF3A and observed their colocalization in primary cilia of renal epithelial cells. We further observed that the addition of exogenous KIF3A itself was sufficient to activate both ciliary and isolated PC2 channel activity. Thus, structural changes in microtubule-PC2 connections act as a regulatory mechanism of channel function and probably trafficking of the channel protein to the primary cilium of renal epithelial cells.

MATERIALS AND METHODS

Cell Culture—Several renal cell lines, including HEK293, MDCK, IMCD, and LLC-PK1 cells, were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, with or without 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere with 5% CO₂. Cells of fewer than 25 passages were cultured to full confluence before collection. For visualization of primary cilia, cells were typically grown for 3–7 days after confluence.

Reagents and Immunocytochemistry—Anti-PC2 mouse monoclonal 1A11, goat polyclonal G-20 (Santa Cruz Biotechnology, Santa Cruz, CA) (6), and rabbit polyclonal ZMD.28 antibody (Zymed Laboratories Inc., South San Francisco, CA) were used in this study. Rabbit polyclonal anti-KIF3A and mouse monoclonal anti-acetylated α-tubulin antibodies were purchased from Sigma Canada (Oakville, ON). Secondary antibodies included donkey anti-mouse/rabbit IgG, rabbit anti-goat IgG when conjugated with horseradish peroxidase (Chemicon International) for Western blotting or conjugated with fluorescein isothiocyanate or rhodamine (Chemicon International, Temecula, CA) for immunofluorescence. To immunolocalize PC2 and microtubular structures in primary cilia, cells were grown to confluence and fixed for 10 min in freshly prepared paraformaldehyde (4%) and 2% sucrose. Cells were rinsed (three times) with phosphate-buffered saline.
solution (PBS) and permeabilized with either 1% Triton X-100 or Nonidet P-40. Some cells were immunolabeled without permeabilization. Cells were blocked with 1% bovine serum albumin or Image-it (Molecular Probes) for 30 min prior to exposure to the primary antibody. Cells were immunolabeled with anti-acetylated α-tubulin antibody 2.8 μg/ml and/or anti-PC2 antibody ZMD.28 (0.2 μg/ml). For immuno-fluorescence microscopy, cells were seeded on poly-D-lysine-coated glass coverslips, fixed for 10 min at room temperature with 4% paraformaldehyde, washed twice with PBS, and permeabilized for 10 min in PBS containing 0.3% Triton X-100. After washing, cells were blocked in PBS containing 5% skim milk (1 h) and incubated for 2 h with primary antibodies in blocking buffer. Cells were then incubated with either fluorescein isothiocyanate-conjugated or rhodamine-conjugated secondary antibodies and mounted with VectaShield (Vector Laboratories, Burlingame, CA). Fluorescent images were captured with either a Zeiss 510 confocal laser scanning microscope or a motorized Olympus IX81 microscope connected to a CCD cooling RT SE6 monochrome camera (Diagnostic Instruments, Sterling Heights, MI). Final composite images were created using either Adobe Photoshop 5.5 or Image-Pro Plus 5.0 (Media Cybernetics Inc., Silver Spring, MD).

Isolation of Primary Cilia from LLC-PK1 Cells—Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, as reported (25). Primary cilia were isolated as follows. Cells were grown to confluence for 2–3 weeks. Confluent monolayers were scraped with Ca²⁺-free phosphate-buffered saline and centrifuged for 5 min at 52,000 g. The cell pellet was suspended in a high Ca²⁺ “deciliation” solution containing (in mM): 112 NaCl, 3.4 KCl, 10 CaCl₂, 2.4 NaHCO₃, 2 HEPES, pH 7.0. Resuspended cells were shaken for 10 min at 4 °C. Ciliary membranes were separated by centrifugation for 5 min at 7,700 g. The supernatant was loaded onto a 45% sucrose solution in high Ca²⁺ saline solution and centrifuged for 1 h at 100,000 g. The sucrose-supernatant interface band was collected and diluted nearly 10-fold and again centrifuged for 1 h at 100,000 g. The pellet was resuspended in normal saline solution adjusted to pH 7.0 and supplemented with 2.0 mM EGTA and 0.5 mM sucrose. The resuspended pellet was aliquoted and stored frozen at −80 °C until further use. Protein content was assessed by the method of Lowry (26). Ion channel reconstitution of ciliary membranes was conducted as reported (27) with some modifications. Briefly, isolated cilia were mixed and sonicated in the lipid mix (27) prior to reconstitution in the lipid bilayer chamber.

Microtubular Components for Functional Assays—α-Tubulin (Cytoskeleton, Denver, CO) was prepared in buffer containing (in mM): 2.0 Tris-Cl, 0.5 ATP, 0.2 CaCl₂, and 0.5 β-mercaptoethanol, pH 8.0. At the time of the experiment, GTP was added to the solution from a stock solution (100 mM) to reach a final concentration of 0.5 mM. Colchicine (Sigma) was dissolved...
in water and used at concentrations ranging from 1 to 15 \( \mu M \) in “trans” buffer solution (see below). Paclitaxel (taxol, 1 mg/ml, Sigma) was used at a 15 \( \mu M \) final concentration. Poly-His-tagged human KIF3AC (amino acids 407–702) was expressed in bacterial BL21 (DE3) cells induced by 0.1 mM isopropyl-\(-\)1-thio-\(\beta\)-galactopyranoside. The full-length human KIF3A (amino acids 1–702) was stably expressed in MDCK cells and purified using a modified tandem affinity purification.6

**Ion Channel Reconstitution**—Lipid bilayers were formed with a mixture of synthetic phospholipids (Avanti Polar Lipids, Birmingham, AL) in \( n \)-decane as reported (27). The lipid mixture was made of 1-palmitoyl-2-oleoyl phosphatidyl-choline and phosphatidyl-\(\beta\)-ethanolamine in a 7:3 ratio. The lipid solution (\( \sim 20–25 \) mg/ml) in \( n \)-decane was spread in the aperture of a polystyrene cuvette (CP13-150) of a bilayer chamber (model BCH-13, Warner Instruments Corp.). Both sides of the lipid bilayer were bathed with a solution containing MOPS-KOH, 10 mM, MES-KOH, 10 mM, pH 7.40, and 10–15 mM Ca\(^{2+}\). The final K\(^+\) concentration in the solution was \( \sim 15 \) mM. KCl was further added to the \( \text{cis} \) compartment to reach 150 mM.

**Data Acquisition and Analysis**—Electrical signals were obtained with a PC501A patch-clamp amplifier (Warner Instruments, Hamden, CT) with a 10 gigaohms feedback resistance. Output (voltage) signals were low pass-filtered at 700 Hz (\( \sim 3 \) db) with an eight-pole, Bessel type filter (Frequency Devices, Haverhill, MA). Signals were displayed on an oscilloscope. Single channel current tracings were further filtered for display purposes only. Unless otherwise stated, pCLAMP Version 5.5.1 (Axon Instruments, Foster City, CA) was used for data analysis, and Sigmaplot Version 2.0 (Jandel Scientific, Corte Madera, CA) was used for statistical analysis and graphics. Single channel conductances were calculated as reported (27). Mean currents were represented by \( I = N p_o \), where \( N \) is the total number of active channels, \( p_o \) is the average single channel current for the channel species, and \( p_o \) is the open probability of the open channel at a given holding potential. Most studies were normalized to \( N p_o \) by dividing the mean current by the maximal single channel current obtained at a given holding potential. Unless otherwise stated, statistical significance was obtained by unpaired Student’s test comparison of sample groups of similar size. Average data values were expressed as the mean \( \pm \) S.E. (n) under each condition, where \( n \) represents the total number of experiments analyzed. Statistical significance was accepted at \( p < 0.05 \) as calculated by paired Student’s test (28).

**Protein-Protein Interaction Assays**—To detect protein-protein interactions, the \textit{in vitro} glutathione S-transferase (GST) pull-down assay, dot blot overlay, yeast two-hybrid assay, and co-immunoprecipitation were conducted as recently described (4, 6).

**RESULTS**

**Microtubular Regulation of PC2 Function from Primary Cilia**—To assess a functional interaction between PC2 and microtubular structures, we explored the primary cilium of renal epithelial cells, where functional PC2 has recently been observed

\[\text{PC2} \quad \text{KIF3A} \quad \text{Merge}\]

**FIGURE 4. Co-localization of PC2 and KIF3A in primary cilia of renal epithelial cells.** The co-localization of KIF3A and PC2 was explored in confluent monolayers of renal epithelial cells, including MDCK, IMCD, and LLC-PK1. Epithelial cells were grown for 7 days after confluence and co-stained with goat anti-PC2 G-20 (green) and rabbit anti-KIF3A (red) antibodies. Nuclear labeling (4',6-diamidino-2-phenylindole, blue) was used as reference. The data indicate that the kinesin-2 subunit KIF3A co-localizes with PC2 in primary cilia of renal epithelial cells.

(29). PC2 was localized to the primary cilium of confluent LLC-PK1 cells (Fig. 1a) and co-localized with acetylated \( \alpha \)-tubulin (29). Primary cilia were isolated (see “Materials and Methods” and Fig. 1) (29), sonicated with lipids, and incorporated into a lipid bilayer reconstitution system. PC2-like cation channel activity was observed in 19 out of 19 reconstituted membranes, which were recognized by their large conductance, presence of substrates (Fig. 1c), and inhibition by amiloride and anti-PC2 antibodies (29). The addition of the microtubular disrupter colchicine (15 \( \mu M \)) inhibited by 93% (from 2.9 \( \pm \) 0.7 pA to 0.2 \( \pm \) 0.1 pA, \( n = 7 \), \( p < 0.01 \)) ciliary PC2 channel activity within a minute (Fig. 1, b and c), a time domain under which no spontaneous PC2 inactivation was observed. The addition of \( \alpha \)-tubulin (5 mg/ml) plus GTP (500 \( \mu M \)), in contrast, dramatically increased (from 0.4 \( \pm \) 0.2 pA to 7.9 \( \pm \) 2.3 pA, \( n = 4 \), \( p < 0.02 \)) ciliary PC2 channel activity (Fig. 2a, top). For these experiments, it was customary to “voltage-inactivate” PC2 function by switching the holding potential to negative values as reported previously (30) (Fig. 2a, middle and bottom). The effect of tubulin manifested as an increased open time of the channel in its maximal conductance (Fig. 2b). The addition of GTP alone, however, was without effect (Fig. 2, a–c).

**Effect of Tubulin on \textit{in Vitro} Translated PC2 Channel**—To assess whether the regulatory effect of changes in ciliary microtubular organization on PC2 channel from ciliary membranes was due to a direct binding between tubulin subunits and PC2, we utilized \textit{in vitro} translated PC2 channel protein as reported previously (30). The isolated protein was inserted into liposomes and fused into the lipid bilayer reconstitution chamber (Fig. 3). PC2 showed spontaneous single channel activity (30), which inactivated either spontaneously or by imposing a negative potential (data not shown). The addition of GTP alone (500 \( \mu M \)) or in combination with tubulin (15 \( \mu M \)), a maneuver that increases PC2 channel activity in ciliary membranes (Fig. 2), did

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not restore PC2 channel activity (Fig. 3). This suggests that intermediary proteins mediate the microtubular effect on PC2 channel function.

Co-localization of PC2 and KIF3A in Primary Cilia—Recent studies indicate that PC2 is present in both motile node cilia and non-motile primary cilia (12, 29). Nodal KIF3A is implicated in the generation of nodal flow. To determine the localization of endogenous PC2 and KIF3A, we performed double immunofluorescence assays in epithelial MDCK, IMCD, and LLC-PK1 cells. We found that both PC2 and KIF3A displayed enhanced staining in primary cilia in all cell lines examined (Fig. 4), indicating a co-distribution of the two proteins. In some cases, a triple labeling was conducted where anti-acetylated tubulin antibody was also used to identify primary cilia and its co-localization with both PC2 and KIF3A (data not shown).

Interaction of PC2 with KIF3A by Yeast Two-hybrid Experiments—In search for linking proteins that bridge the interaction between microtubules and the PC2 channel, we performed yeast two-hybrid screening using the carboxyl terminus of PC2 (amino acids 682–968, PC2C) as a bait. One of the PC2 interacting partners identified from human heart library was KIF3B, one of the two kinesin-2 motor subunits of kinesin-2.7 Given that the mammalian kinesin-2 possesses two similar motor subunits, KIF3A and KIF3B, we further explored whether KIF3A can also bind PC2 in a similar manner. Indeed, the corresponding region of KIF3A (amino acids 403–702, named KIF3AC) interacted with PC2C (Fig. 5). Further examination showed no interaction between KIF3AC and the corresponding amino terminus of PC2 (PC2N). To further explore and map the specific domains for the KIF3A-PC2 binding interaction, we next made a series of carboxyl-terminal truncation constructs of both PC2 and KIF3A. One-by-one pair assays identified the domains responsible for this association to be amino acids 872–927 of PC2 and amino acids 403–702 of KIF3A (Fig. 5, b and c).

Co-immunoprecipitation of Endogenous PC2 and KIF3A from Renal Epithelial Cells—To further determine whether PC2 interacts with KIF3A in vivo, we also performed co-immunoprecipitation experiments with native HEK293, MDCK, and LLC-PK1 cells (Fig. 6a). KIF3A was detected in the immunoprecipitates from all cells tested with the anti-PC2 (1A11) antibody but not in the control immunoprecipitates using non-immune mouse IgG (Fig. 6a, middle). Reciprocally, under the same experimental conditions, total protein extracts derived from the above renal

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cell lines incubated with the anti-KIF3A antibody resulted in the precipitation of PC2 (Fig. 6 bottom). These data demonstrate that PC2 interacts and forms a complex with kinesin-2 in vivo.

In Vitro Binding between the Carboxyl Termini of PC2 and KIF3A—To examine the interaction between PC2 and KIF3A, we also used a GST fusion protein affinity binding method. For this purpose, the PC2N and PC2C polypeptides were fused in-frame with a GST epitope, whereas the KIF3AC was tagged to a poly-histidine epitope. The constructs were expressed in the bacterial strain BL21 in the presence of 0.1 mM isopropyl-

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FIGURE 6. Biochemical binding assays between PC2 and KIF3A. a, the association between PC2 and KIF3A was explored by co-immunoprecipitation of either protein in MDCK and LLC-PK1 renal epithelial cells and HEK293 human embryonic renal cells. Top, total lysates (60 μg of each) of the three cell lines were resolved in 8% SDS-PAGE and visualized with either anti-KIF3A or anti-PC2 1A11 antibody. Middle, cell lysates were precipitated with the mouse anti-PC2 1A11 antibody (+) or non-immune mouse IgG (−). The precipitate was detected with an anti-KIF3A antibody. Bottom, reciprocal co-immunoprecipitations were also conducted where cell lysates from either cell line were precipitated with the anti-KIF3A antibody (+) or non-immune rabbit IgG (−). The precipitates were then visualized with the anti-PC2, 1A11, antibody. Molecular mass markers (in kDa) are shown.

b, a GST-pull-down assay confirmed the co-localization of KIF3A and PC2. Escherichia coli extracts expressing either one of the GST-tagged PC2 polypeptides (PC2N, PC2C) or GST alone were incubated with purified poly-His-tagged KIF3AC. Glutathione-agarose beads were used to precipitate GST-epitope-binding proteins. The resultant protein complexes were immunoblotted with a Poly-His antibody. c, in dot blot overlay assays, purified KIF3AC, bovine serum albumin (BSA), and PBS buffer (as negative controls) and GST-PC2C (antigen of the PC2 antibody 1A11 as positive control) were spotted on nitrocellulose membranes and incubated with total protein lysates of HEK293 and MDCK cells in a blocking buffer. After washes, the membranes were immunoblotted with 1A11.

Functional Effect of KIF3A on PC2 Channel Function—To determine whether KIF3A has a regulatory role in ciliary channel function, PC2 channels were first identified in isolated ciliary membranes reconstituted in a lipid bilayer system. Ciliary PC2 single channel activity was determined as shown in Figs. 1 and 2. Spontaneous channel activity increased after the addition of poly-His-KIF3AC (Fig. 7). Similar findings were obtained by exposure of ciliary PC2 to the full-length KIF3A protein obtained through a modified tandem affinity purifica-
tion from MDCK cells (data not shown). The data indicated that KIF3A stimulates PC2 channel activity previously inactivated spontaneously or by voltage changes (Fig. 7). To further demonstrate a direct effect of KIF3A on PC2 channel function, a similar experiment was conducted with tandem affinity purification (TAP)-purified PC2 in the absence of any other axonemal (microtubular) structures. The addition of KIF3AC (Fig. 8) or the full-length KIF3A protein (data not shown), but not KIF3B (Fig. 8a, top), increased PC2 channel activity reconstituted in the lipid bilayer system. Thus, our data demonstrate that KIF3A is not only the molecular link between microtubules and the PC2 channel but also a functional modulator of PC2.

**DISCUSSION**

In the present study, we determined that ciliary PC2 channel function is under control of the key component of the axonemal machinery, the ciliary microtubules. The addition of tubulin plus GTP was stimulatory, whereas treatment with colchicine inhibited PC2 channel activity in ciliary membranes from renal epithelial cells. Interestingly, in vitro translated PC2 protein had no such an effect. The encompassed evidence suggests a functional interaction between preformed microtubules and the PC2 channel. However, the data also indicate that microtubular tubulin does not directly interact with PC2, as we recently demonstrated for the actin cytoskeletal α-actinin (6). Rather, a structural complex involving motor proteins, such as the kinesin-2 components KIF3A and KIF3B, acts as a molecular link between PC2 and the microtubular cytoskeleton. We confirmed the presence of such a molecular complex, where the carboxyl terminus of PC2 directly associated with the carboxyl termini of KIF3A and KIF3B.7 This complex is endogenously present in various renal cell lines, including HEK293, MDCK, and LLC-PK1 cells, suggesting its widespread distribution and likely importance in the regulation of PC2 function. Interestingly, however, is the fact that KIF3A but not KIF3B was effective in regulating PC2 channel activity. Nonetheless, the findings suggest a novel feedback mechanism for PC2-microtubular interactions. PC2 is a Ca2+-permeable channel, whereas Ca2+ itself is a strong microtubular depolymerizing agent (31, 32). Thus, a likely scenario could be envisioned in which regulation of PC2 function in the ciliary compartment by kinesin-2 renders a functional channel complex, whose activity helps detach the channel from the axonemal machinery (Fig. 9). This interesting hypothesis will require further confirmation. However, the hypothesis is consistent with the possibility that PC2-mediated Ca2+ transport in the ciliary compartment may be an important contributing factor to the remodeling of its struc-

**FIGURE 8. Functional role of KIF3A on purified PC2 channel function.** a, PC2 channel function was observed in purified PC2, reconstituted in a lipid bilayer system. The addition of KIF3AC largely increased PC2 channel activity. Data are representative of five experiments. Inset, KIF3B, in contrast, was without stimulatory effect on the isolated protein. Data representative of three experiments. b, representative single channel currents before and after the addition of KIF3A (left). All-point histograms are shown on the right. KIF3A-activated PC2 cation channel activity indicates that PC2 spends more time in the maximal conductive state in the presence of the KIF3A subunit of the purified protein, demonstrating a functional link between this kinesin subunit and the isolated channel. c, left, the addition of KIF3A increased the mean PC2 current channel activity, determined as Np. Data are the mean ± S.E. of three paired experiments. Similar results were obtained by the addition of full-length KIF3A (data not shown). Right, the addition of KIF3B was without stimulatory effect on the purified PC2 channel function. However, the subsequent addition of KIF3A was effective in increasing PC2 channel activity even in the presence of KIF3B. Data are the mean ± S.E. of three paired experiments. The asterisk indicates statistical significance at p < 0.05 (n = 3).
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FIGURE 9. Schematic model of microtubular interaction with PC2 in primary cilia. The evidence in this report supports the following model: Cellular PC2 associates with kinesin-2 complexes by direct binding to KIF3A, and therefore, to microtubules. In the primary cilium, KIF3A regulates PC2 function whose activity likely detaches the channel from the axonemal machinery by the microtubular depolymerizing action of increased intra-ciliary Ca$^{2+}$, elicited by PC2 channel function. This interaction provides a molecular mechanism for microtubular regulation of PC2 channel activity, which in turn may help modulate ciliary properties such as length and function.

The data in this report also suggest that microtubular and actin cytoskeletal structures may show strong complementarity in the regulation of PC2. Either disruption of endogenous actin filaments with cytochalasin D (5) or disruption of microtubules with colchicine (this report) disrupts endogenous PC2 function, the only difference being their cellular location. The evidence does suggest distinct regulatory features by the cytoskeleton, depending on cell location and cell cycle, both of which modulate PC2 in ciliary membranes. This, in turn, suggests that a PC2-microtubule interaction may be a key signaling component that is defective in a number of cystic diseases.

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