Isolation and characterization of cholangiocyte primary cilia

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Huang, Bing Q., Tatiana V. Masyuk, Melissa A. Muff, Pamela S. Tietz, Anatoliy I. Masyuk, and Nicholas F. LaRusso. Isolation and characterization of cholangiocyte primary cilia. Am J Physiol Gastrointest Liver Physiol 291: G500–G509, 2006; doi:10.1152/ajpgi.00064.2006.—Primary cilia are distinct organelles expressed by many vertebrate cells, including cholangiocytes; however, their functions remain obscure. To begin to explore the physiological role of these organelles in the liver, we described the morphology and structure of cholangiocyte cilia and developed new approaches for their isolation. Primary cilia were present only in bile ducts and were not observed in hepatocytes or in hepatic arterial or portal venous endothelial cells. Each cholangiocyte possesses a single cilium that extends from the apical membrane into the bile duct lumen. In addition, the length of the cilium was proportional to the bile duct diameter. We reproducibly isolated enriched fractions of cilia from normal rat and mouse cholangiocytes by two different approaches as assessed by scanning electron, transmission electron, and confocal microscopy. The purity of isolated ciliary fractions was further analyzed by Western blot analysis using acetylated tubulin as a ciliary marker and P2Y2 as a nonciliary cell membrane marker. These novel techniques produced enriched ciliary fractions of sufficient purity and quantity for light and electron microscopy and for biochemical analyses. They will permit further assessment of the role of primary cilia in normal and pathological conditions.

cilia isolation; fibrocystin; polycystins

Cilia are ubiquitous organelles found in most mammalian tissues. Structurally, they consist of a microtubule-based axoneme and a basal body. According to axonemal microtubule components, cilia are generally classified as primary or motile; basal bodies in both types of organelles are the same and consist of nine triplet microtubules. Primary cilia extend from a single parental centriole (i.e., basal body) of the diplosomal pair that relocates to a position beneath the cell membrane after mitosis. They have nine peripherally located microtubule pairs and are distinguished from motile counterparts by the absence of a central pair of microtubules and associated dynein arms, giving a 9+0 axonemal arrangement (6, 44, 45). The widespread presence and conserved structure of primary cilia suggest they are a highly organized, functional organelle that plays a significant role in cell physiology and pathophysiology. It has been suggested that primary cilia are involved in mechanosensation, chemosensation, and osmosenstation and cell cycle regulation. They also play a fundamental role in several other mammalian processes, including left-right axis determination, neurogenesis, and photoreceptor maintenance (2, 3, 29, 31, 34, 42). In kidney epithelia, primary cilia have been postulated to act as flow sensors responding to luminal flow-mediated bending by an increase in intracellular calcium with the involvement of two ciliary-associated proteins, polycystin 1 and polycystin 2 (32, 33). Furthermore, more than 450 proteins have been found to be ciliary associated by comparative genomics/proteomics approaches between ciliated and nonciliated organisms (25, 27, 34). Moreover, recent studies have confirmed the link between cilia and disease pathogenesis; ciliary abnormalities contribute to the development of polycystic kidney disease (PKD), nephrophenphisis, Senior-Loken (renal-retinal) syndrome, retinitis pigmentosa, anosmia, and laterality disturbances (1, 3, 6, 17, 19, 24, 26, 28, 42).

Cholangiocytes, the epithelial cells lining intrahepatic bile ducts, are important epithelia in the liver. We (12, 17, 19, 41) and others (9, 10, 13, 14, 21, 38, 46) have previously reported that cholangiocytes, like most other epithelial cells, possesses an individual cilium extending from the apical membrane. The strategic location of cilia in the bile duct lumen suggests a possible role in sensing and transducting mechanical and chemical signals. Moreover, our recent data have revealed a strong connection between cholangiocyte cilia and hepatic cystogenesis. Specifically, in an animal model of autosomal recessive PKD (ARPKD), the PCK rat, cilia are functionally and structurally abnormal; in contrast to normal, they are shorter, have bulbous extensions on the axonemal membrane, and do not express fibrocystin, the protein mutated in ARPKD (17, 19).

Despite these findings, our understanding of the functions of the primary cilium and its role in responding to environmental stimuli are still poorly understood. Thus a detailed characterization of ciliary morphology under normal and pathological conditions, the development of approaches for the isolation of primary cilia, and an identification of ciliary-specific proteins are essential steps to further study the functions of these organelles.

In the present study, we provide a detailed multidimensional morphological description of cholangiocyte cilia and describe two novel approaches to isolate enriched ciliary fractions from cultured cholangiocytes.

MATERIALS AND METHODS

Animals, Isolated Bile Ducts, and Cultured Cells

Three-month-old (n = 5) normal rats were used for morphological study after approval by the Mayo Institutional Animal Care and Use Committee. Bile ducts were isolated from normal rats according to previously described procedures (36) and then cut longitudinally in half or obliquely for further processing. Simian virus 40-transformed normal mouse cholangiocytes (NMCs; a generous gift from Dr. Ueno)
and spontaneously immortalized normal rat cholangiocytes (NRCs) were grown in DMEM-F-12 culture medium as previously described (41).

**Cilia Isolation**

*Peel-off isolation.* Cilia were isolated from cultured NMCs or NRCs using a sandwich technique. Briefly, a 0.1% poly-L-lysine-coated coverslip was placed on the top of the cell monolayer and cultured medium was removed with a Pasteur pipette attached to a vacuum line positioned at the edges of the coverslip. At the same time, light pressure was applied on the top of the coverslip by placing a 20-mm-diameter rubber cork or by slightly pushing down with a finger for 20 s. The coverslip was then quickly lifted off from the cell monolayer with curved forceps. The samples were fixed for electron microscopy analysis or scraped off, centrifuged, and concentrated in sucrose density gradients as previously described (35) and then frozen for biochemical analysis.

*Slide pulling.* The exterior surface of a 6-cm-diameter petri dish cover was coated with 0.1% poly-L-lysine, dried before use, and then faced down and placed on the top of the NRCs or NMCs cultured in a larger petri dish (10 cm in diameter). The plates were shaken at 70–100 rpm for 5 min at room temperature. The supernatants were removed, vortexed for 1 min, and placed in an ice bath for 20–30 min to allow the debris to settle and then centrifuged in sucrose density gradient as previously described (35). Pellets were either fixed in 2% glutaraldehyde and then in 1% osmium tetroxide for electron microscopy analysis or used for biochemical analysis.

Fig. 1. Primary cilia in the whole rat liver. No visible cilia were observed in the portal vein (PV; A) or in the hepatic artery (HA; B). In the intrahepatic bile ducts (BDs; C and D), cilia were heterogeneous in length. Large BDs (C) have cilia ~2 times longer than small ones (D). Low-power (E) and high-power (F) micrographs show a typical cholangiocyte primary cilium growing out of a mother centriole (i.e., basal body) of a pair of mother-daughter centrioles. The transition fibers anchor the basal body to the plasma membrane and has been suggested to function as a docking site for ciliary-associated proteins. Scale bars = 10 µm in A and B, 1 µm in C and D, 5 µm in E, and 200 nm in F.
**Scanning and Transmission Electron Microscopy**

For scanning electron microscopy (SEM), livers were perfused with a mixture of 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 5–10 min, cut into small pieces (2–4 mm³), and then immersed in 2% phosphate-buffered glutaraldehyde for an additional 2 h.

Isolated bile ducts, NRCs, NMCs, or isolated cilia were directly immersed in 2% phosphate-buffered glutaraldehyde for 1 h, rinsed with phosphate buffer three times, and then postfixed in 1% osmium tetroxide for 1 h on ice. After fixation, the samples were rinsed in distilled water, dehydrated in serial ethanol, dried in a critical point dryer, sputter coated with gold-palladium, and examined using a Hitachi 4700 SEM.

For transmission electron microscopy (TEM), isolated cilia were first collected by centrifugation at 4,800 rpm. Bile ducts, NMCs, NRCs, or isolated cilia were fixed with 2% phosphate-buffered glutaraldehyde for 1 h and then postfixed in 1% osmium tetroxide for 1 h on ice. After dehydration with serial ethanol, the samples were infiltrated and embedded with Spurrs resin. Samples were examined using a Jeol 1200 TEM.

**Immunofluorescence**

Immunofluorescence microscopy was performed on isolated bile ducts, NRCs, NMCs, and isolated cilia. Cryosemithin sections of the bile duct were processed accordingly to a previously described protocol (20) with some modifications. Briefly, isolated bile ducts were fixed for 30 min with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), washed in PBS, cryoprotected by infiltration with 2.3 M sucrose in 0.1 M phosphate buffer, mounted on aluminum nails, and then frozen in liquid nitrogen. Semithin sections (0.2–0.5 μm) were cut on a Leica Ultracut microtome equipped with a FCS cryoattachment and collected on poly-L-lysine-coated slides for immunofluorescence microscopy.

NRCs, NMCs, and isolated cilia were rinsed in PBS and fixed in 4% paraformaldehyde for 30 min at room temperature. The specimens were permeabilized with 0.2% (vol/vol) Triton X-100 in PBS for 5 min.cryosemithin sections of the bile duct were prepared as described above. The specimens were permeabilized with 0.2% Triton X-100 and incubated with primary antibodies against the desired antigens. After primary antibody incubation, the samples were rinsed and incubated with secondary antibodies conjugated to fluorophores. The samples were then washed and mounted with mounting medium containing DAPI for nuclear staining. The images were captured using a confocal microscope.

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**Fig. 2. Primary cilia in isolated BDs.** A: scanning electron microscopy (SEM) image of a microdissected intrahepatic BD. B: each cholangiocyte possessed primary cilia that extended from the apical surface into the lumen. Transmission electron microscopy (TEM) images of longitudinal sections (C and D) and cross-sections (E) of cholangiocyte cilia showed a 9+0 axonemal structure. Transmission (F) and confocal (G) immunofluorescent microscopy of a cryosemithick cross-section of an isolated BD (F) and a whole mount of a split-open BD (G) stained with the ciliary marker anti-acetylated α-tubulin are also shown. Scale bars = 50 μm in A, 1 μm in B and C, 100 nm in D and E, and 10 μm in F and G. (E was reprinted with permission from Ref. 18.)
min and quenched in 0.01 M glycine for 10 min. Samples were then stained with the following antibodies: acetylated α-tubulin (1:200, Sigma-Aldrich), fibrocystin (1:100, a gift from Dr. Ward), polycystin 2 (1:200, Santa Cruz Biotechnology), and P2Y2 (1:200, Sigma-Aldrich). Fluorescently labeled specimens were observed using 1) a Zeiss Axiovert 100M inverted microscope equipped with an Apochromat ×100 oil-immersion objective and a LSM 510 confocal laser scanning microscope system with argon (488 nm) and HeNe (543 nm) lasers or 2) a Nikon fluorescent microscope with a DXM1200F camera. Images were prepared for publication using Adobe Photoshop software (Mountain View, CA).

Immunoblot Analysis

Ciliary pellets from three 10-cm petri dishes were pooled together, resuspended, and sonicated in 0.25 M sucrose containing 0.01% soybean trypsin inhibitor (Worthington Biochemical) and 0.1 mM PMSF. The remaining deciliated cells in the dishes were harvested and sonicated in 0.3 M sucrose containing the inhibitors mentioned above. The plasma membrane fraction from these cells was obtained by centrifugation at 200,000 g for 60 min on a discontinuous 1.3 M sucrose gradient.

The whole cell lysate, mixed plasma membrane, and isolated cilia fractions were solubilized, subjected to SDS-PAGE, and transferred to nitrocellulose membranes. After being blocked, the membranes were incubated overnight at 4°C with anti-acetylated tubulin (1:3,000, Sigma-Aldrich) and anti-P2Y2 receptor (1:500, Sigma-Aldrich) antibodies. The appropriate horseradish peroxidase-conjugated secondary antibodies were applied (1:2,000, Biosource), and bands were detected with the enhanced chemiluminescent plus detection system (Amersham). The bands were quantitated by densitometry using NIH Image software, and the values were graphed as fold increases or decreases compared with the whole cell lysate.

Statistical Analysis

All values are expressed as means ± SE. Statistical analysis was performed by Student’s t-test, and results were considered statistically different at P < 0.05.

RESULTS

Primary Cilia

Primary cilia in the whole liver. By SEM, no visible cilia were observed in hepatocytes or in the lumen of the portal vein and hepatic artery (Fig. 1, A and B). Primary cilia were present only in the intrahepatic bile ducts (Fig. 1, C and D). Along the biliary tree axis, cilia were heterogeneous in length. In the large bile ducts, they were approximately two times longer than in the small ones (7.35 ± 1.32 and 3.58 ± 1.12 μm, respectively, P < 0.05, n = 50). Primary cilia consist of two parts, a basal body and a membrane-bound ciliary axoneme (part of the organelle that extends from the distal end of the basal body). They arise from the oldest (mother) centriole, which acts as a basal body for the nucleation of axonemal microtubules during the G1 phase of the cell cycle (37, 40). In cholangiocytes, the basal body ranged in diameter from 0.143 to 0.257 μm and in length from 0.256 to 0.482 μm (n = 19). The basal body was situated adjacent to the trans-Golgi network and the nucleus and was anchored to the cell membrane by transition fibers (Fig. 1, E and F). This point of contact was considered as a boundary between the cell and ciliary membrane. Although the ciliary membrane appears to be a continuum of the cell plasma membrane, recent studies have suggested that they are both structurally and functionally different (6, 26, 29).

Primary cilia in isolated bile ducts. Primary cilia were clearly observed by SEM in the lumen of bile ducts isolated by

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Fig. 3. Primary cilia in cultured cholangiocytes. SEM micrographs show a cilium in normal rat cholangiocytes (NRCs; A) and normal mouse cholangiocytes (NMCs; B). C and D: confocal immunofluorescent image of cilia in NRCs (C; green) and merge image of immunofluorescent and transmission microscopy of cilia in NMCs (D; red) at 7 days after confluence. Scale bars = 1 μm in A and B and 10 μm in C and D. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI) (C; blue).

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Primary cilia in isolated bile ducts. Primary cilia were clearly observed by SEM in the lumen of bile ducts isolated by
microdissection from normal rats. Each cholangiocyte contained a single primary cilium on the apical membrane of ~7 μm in length (Fig. 2, A and B). TEM of longitudinal sections (Fig. 2, C and D) and cross-sections (Fig. 2E) clearly showed that the cholangiocyte cilia, like primary cilia in other tissues, were composed of microtubules and had a 9+0 axoneme structure, nine peripherally located microtubules that are doublets (Fig. 2E, inset), whereas the central microtubule pair is absent. Cilia were visualized using an antibody to acetylated α-tubulin, a known ciliary marker, and by transmission immunofluorescent (Fig. 2F) and confocal microscopy (Fig. 2G), which were found to project into the lumen of the bile duct from the apical membrane of cholangiocytes.

**Primary cilia in NRC and NMC cell lines.** In cultured NRCs (Fig. 3A) and NMCs (Fig. 3B), the cilia grew to the maximal length (~7–10 μm) at 7–10 days postconfluence. The presence of cilia in cultured cells was also confirmed by confocal (Fig. 3C) and transmission (Fig. 3D) immunofluorescent microscopy.

Our major objective in the present study was to develop methods for primary cilia isolation. Two different approaches, peel off and slide pull, have been established to isolate cilia from cultured NMCs and NRCs. We have reported previously that cilia are not expressed in subconfluent and postconfluent (days 1–2) cultured cells. They appeared at day 3 of postconfluence and grew progressively up to days 7–10, when the majority of cells possessed cilia (18). Thus, for our isolation procedures, 7- to 10-day-old postconfluent cultured NRCs or NMCs were used.

**Isolation of Cholangiocyte Cilia**

**Isolation of cholangiocyte cilia by the peel-off technique.** The peel-off technique is depicted in Fig. 4A and described in detail in MATERIALS AND METHODS. Taking advantage of the poly-L-lysine-coated coverslips, we isolated cilia from the apical membrane of cultured NMCs (Fig. 4, B and C). The immunofluorescent confocal images in Fig. 4 show cells with a primary cilium visualized by anti-acetylated α-tubulin (B) and a cilium after isolation (C). We inspected isolated cilia by SEM and confirmed that they maintained their integrity very well (Fig. 4D). To assess the efficiency of cilia isolation, poly-L-lysine-coated coverslips with attached isolated cilia and cultured cells remaining after the procedures were stained with acetylated α-tubulin. We found that up to 70% of the cholangiocyte cilia were detached from the apical membrane of cultured cells (Fig. 4E). The high-power transmission electron micrographs in Fig. 4 show a cross-section (F) and longitudinal section (G) of an isolated cilium.

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![Diagram](image_url)

**Fig. 4.** Isolation of primary cilia by the peel-off technique. A: schematic illustration of the peel-off approach. A poly-L-lysine-precoated coverslip was placed on the top of cultured cells, pressed down, and then lifted up. Isolated cilia were attached to the coverslip. B and C: acetylated α-tubulin staining of a cilium before (B) and after isolation (C). D: SEM micrograph of isolated cilia. E: acetylated α-tubulin staining of cilia on poly-L-lysine-coated coverslips after isolation. F and G: TEM images of a cross-section (F) and longitudinal section (G) of cilia isolated and purified via centrifugation on a sucrose density gradient. Scale bars = 10 μm in B, C, and E, 1 μm in D, and 200 nm in F and G.
Isolation of cholangiocyte cilia by the slide-pull technique.

Details of the second approach for cilia isolation are described in MATERIALS AND METHODS and depicted in Fig. 5A. A small (6 cm) poly-L-lysine-coated petri dish was placed on the top of a cell monolayer growing in large petri dish (10 cm) and positioned in a sliding shaker. Cilia detached from the apical surface by generating pulling force were collected by centrifugation and used for further analysis (Fig. 5A). Immunofluorescent confocal microscopy (Fig. 5B) and TEM (Fig. 5, C and D) confirmed that we successfully isolated cilia and ciliary fragments by this approach.

Biochemical Characterization of Isolated Cilia

The protein content of isolated ciliary fractions was detected by pulling together pellets from three large (10 cm) petri dishes. The cilia fraction isolated by the peel-off technique contained about 35–130 μg total protein, whereas that obtained from the slide-pull technique contained about 150–270 μg total protein.

The purity of cilia isolated by both techniques was further analyzed by Western blot analysis. Three different cell fractions were used: 1) whole cell lysate, 2) mixed plasma membranes, and 3) isolated cilia. We found by immunofluorescence (Fig. 6A) that the P2Y2 receptor was not expressed in cilia. Thus the P2Y2 receptor served as a marker of the mixed plasma membrane fraction. Because it is well known that acetylated α-tubulin is a major component of the ciliary axoneme (Fig. 6B), we used it as a marker of the ciliary fraction. As shown in Fig. 7, in the ciliary fractions isolated by peel-off or slide-pull approaches, the content of acetylated α-tubulin was about 1.5- and 3-fold higher, respectively, than in the whole cell lysate fraction. A trace amount of acetylated tubulin was seen in the mixed plasma membrane fraction. In contrast, P2Y2 was about seven- to eightfold higher in the mixed membrane fraction than in the ciliary fraction isolated by the peel-off or slide-pull techniques (P < 0.05, n = 3).

Identification of Cilia-Associated Protein in Isolated Cilia

To further prove that isolated cilia are a useful system in which to examine the expression of proteins that potentially may be involved in ciliary functions, we analyzed several of them previously identified by us (18, 19) or by others (3, 24, 29) as ciliary associated. By immunofluorescent confocal microscopy, polycystin 1 (Fig. 8, A–C), polycystin 2 (Fig. 8, D–F), and fibrocystin (Fig. 8, G–I) were shown to be expressed on the isolated peel-off (Fig. 8, A–C) or slide-pull (Fig. 8, D–I) cholangiocyte cilia colocalized with acetylated α-tubulin.

DISCUSSION

In the present study, we 1) described the detailed morphology and ultrastructure of cholangiocyte primary cilia in the whole rat liver, isolated intrahepatic bile ducts, and cultured rat and mouse cholangiocytes; 2) observed that cholangiocyte cilia are heterogeneous in length along the biliary tree axis (i.e., longer in large vs. small bile ducts); 3) described two novel approaches for isolating primary cilia from cultured cells; 4) provided morphological and biochemical evidence of the relative purity of isolated ciliary fractions using SEM, TEM, and confocal microscopy and Western blot analysis; 5) proved that the yield of isolated cilia is sufficient to perform biochemical analysis; and 6) presented evidence showing that isolated cilia are an excellent source for the detection of ciliary-associated proteins.

Primary cilia were discovered in 1898 in kidney epithelia (43). They were first found in bile ducts in 1963 (9), and their
Fig. 6. Expression of acetylated α-tubulin and P2Y2 in NMCs. A: P2Y2 (red) was not expressed in cilia of cultured cholangiocytes. B: acetylated α-tubulin (green), a known ciliary marker, mainly localized to cilia. C: nuclei were stained with DAPI. D: merged image of triple staining of P2Y2, acetylated α-tubulin, and nuclei. Scale bars = 10 μm in A–D.

Fig. 7. A: Western blots of the whole cell lysate fraction, mixed plasma membrane (MPM) fraction, and ciliary fraction isolated by peel-off (left) and slide-pull (right) techniques. Acetylated α-tubulin was used as a ciliary marker and P2Y2 was used as a MPM marker. B: densitometric analysis showing that acetylated tubulin was enriched in the ciliary fraction, whereas P2Y2 was enriched in the MPM fraction. P < 0.05, n = 3.
presence in cholangiocytes has been mentioned several times over a period of decades in several reports (9, 10, 12–14, 16, 17, 21, 38, 41, 46). However, in none of these studies were cholangiocyte cilia characterized in detail. In this study, we described the ultrastructure of cholangiocyte primary cilia in different model systems using SEM, TEM, and confocal microscopy. Our data showing that one cilium per cholangiocyte was present in the bile ducts and that no cilia were found in the portal vein, hepatic artery, or hepatocytes are consistent with previously published reports on the liver ultrastructure in normal rats (9, 10, 13, 14, 21, 38, 46). However, the presence of cilia in bile canaliculi has been described in larval lamprey (30). In humans with primary biliary cirrhosis, primary cilia were observed in the bile canaliculi of the periportal area (47).

For the first time, we have shown that primary cilia are heterogeneous in length along the intrahepatic biliary tree. Because it is well-known that the intrahepatic biliary tree is heterogeneous along its axis with regard to bile duct diameter, protein expression, and responses to proliferative stimuli, it is possible that cilia function differently in small and large bile ducts and that they express different proteins. Interestingly, in the brain, the mean cilium length varies significantly, ranging from 2.1 to 9.4 μm across 23 regions of the central nervous system (8). Because cholangiocyte cell lines derived from different portions of the biliary tree have been developed (39), we can now, using these cell lines and cilia isolation techniques, explore differences in ciliary protein expression along the biliary tree axis.

Fig. 8. Confocal microscopy showing the colocalization of acetylated α-tubulin, a ciliary marker, with the following tree ciliary-associated proteins in isolated cilia: polycystin 1 (A–C), polycystin 2 (D–F), and fibrocystin (G–I). A, D, and G: acetylated α-tubulin (green). B, E, and H: polycystin 1, polycystin 2, and fibrocystin, respectively (red). C, F, and I: merged images. Scale bars = 10 μm in A–I.
Approaches for primary cilia isolation offer a suitable model to identify proteins important for ciliary function. Different deciliation procedures using chemicals and detergent treatments, extreme pH, and calcium shock have been developed for the isolation of motile cilia in a variety of organisms (4, 11, 22, 23). Surprisingly, these techniques have not been useful for the isolation of primary cilia, perhaps because they do not produce sufficient yields of cilia for biochemical analysis. For example, Tetrahymena thermophila, an excellent source of prokaryotic cilia, contains 400–600 cilia/cell, providing a large amount of starting material for cilia isolation (4). To the best of our knowledge, there is only one report of primary cilia isolation. Using porcine kidney epithelial cells, ciliary membranes were isolated by exposure to a high-calcium solution and concentrated on a sucrose density gradient. Isolated cilia were used to detect the presence of functional channels in the ciliary membrane (35).

In this study, we developed two separate but complementary approaches for primary cilia isolation. Cholangiocyte cilia, isolated using the peel-off technique, directly attach to poly-L-lysine-coated coverslips. The isolated cilia are intact and well-preserved. This approach, however, also has drawbacks. First, in some cases, not only primary cilia but also portions of the apical membrane were detached. Second, the resulting yield of isolated cilia (35–150 μg) was relatively low for biochemical analysis; and, finally, some manual skills were required. The second approach for cilia isolation, the slide-pull technique, required brief mechanical agitation followed by centrifugation. By this approach, we were able to obtain a large quantity (150–270 μg) of ciliary protein. Both deciliation approaches described in this study are efficient for the preparation of the isolated ciliary fraction. The peel-off approach, because less quantity of protein is obtained, is more suitable for confocal immunofluorescent microscopy to study the expression of ciliary-associated proteins. The slide-pull technique is more suitable for biochemical analyses because larger amounts of protein can be obtained; however, it also can be used for confocal microscopy. Indeed, we prefer the slide-pull technique for most studies.

It has been shown that molecules in bile through unknown mechanisms can influence the function of cholangiocytes (5, 7, 15). The strategic location of primary cilia on the apical membrane of cholangiocyte suggests that they may play an important role in these processes. However, until recently, the functions of these organelles in the liver have remained obscure. The first insights into the function of cholangiocyte cilia have been generated in the PCK rat, an animal model of ARPKD, suggesting that they contribute to liver pathology. Fibrocystin, the protein responsible for the development of disease, is not expressed in cilia under pathological conditions, resulting in an abnormal and malformed ciliary structure (17, 19). Moreover, our recent data demonstrate that cholangiocyte primary cilia function as mechanosensors, responding to changes in luminal flow by activation/inhibition of second messengers such as intracellular calcium or cAMP with the involvement of two ciliary-associated proteins, polycystin 1 and polycystin 2 (16). In the present study, using ciliary fractions isolated by both approaches, we confirmed the previous findings made by us in cholangiocytes (18, 19) and by others in renal epithelia (3, 24, 29), showing that fibrocystin, polycystin 1, and polycystin 2 all localize to cilia.

Thus these novel techniques allowing the isolation of primary cilia will permit further study of ciliary localization of different proteins and continue to elucidate their role in both normal and pathological conditions.

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