Dynamin Binding Protein (Tuba) Deficiency Inhibits Ciliogenesis and Nephrogenesis in Vitro and in Vivo*

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Dysfunction of renal primary cilia leads to polycystic kidney disease. We previously showed that the exocyst, a protein trafficking complex, is essential for ciliogenesis and regulated by multiple Rho and Rab family GTPases, such as Cdc42. Cdc42 deficiency resulted in a disruption of renal ciliogenesis and a polycystic kidney disease phenotype in zebrafish and mice. Here we investigate the role of Dynamin binding protein (also known as Tuba), a Cdc42-specific guanine nucleotide exchange factor, in ciliogenesis and nephrogenesis using Tuba knockdown as Tuba (tuba), a Cdc42-specific guanine nucleotide exchange factor, in ciliogenesis and nephrogenesis using Tuba knockdown Madin-Darby canine kidney cell cysts cultured in a collagen gel. Tuba depletion resulted in an absence of cilia, with impaired apical polarization and inhibition of hepatocyte growth factor-induced tubulogenesis in Tuba knockdown Madin-Darby canine kidney cell cysts cultured in a collagen gel. In zebrafish, tuba was expressed in multiple ciliated organs, and, accordingly, tuba start and splice site morphants showed various ciliary mutant phenotypes in these organs. Co-injection of tuba and cdc42 morpholinos at low doses, which alone had no effect, resulted in genetic synergy and led to abnormal kidney development with highly disorganized pronephric duct cilia. Morpholinos targeting two other guanine nucleotide exchange factors not known to be in the Cdc42/ciliogenesis pathway and a scrambled control morpholino showed no phenotypic effect. Given the molecular nature of Cdc42 and Tuba, our data indicate that Sec10 plays a key role in the regulation of ciliary protein trafficking and ciliogenesis. Importantly, an exocyst mutation was recently identified in a family with Joubert syndrome, a nephronophthisis form of PKD.

The Par complex, which consists of Par3, Par6, atypical PKC, and Cdc42, is localized to primary cilia and has been shown to be necessary for ciliogenesis (13, 14). Cdc42, an associated component of the Par complex, is a small GTPase. The exocyst is regulated by multiple Rho and Rab family GTPases, and it is known that, in yeast, Cdc42 regulates polarized exocytosis through interactions with the exocyst (15). Using MDCK cells, we established that Cdc42 co-localizes and interacts with Sec10 at the primary cilium and that knockdown of Cdc42 inhibits ciliogenesis in MDCK cells (16). Importantly, we investigated how Cdc42 participates in ciliogenesis and cooperates with the exocyst in ciliary membrane trafficking using two vertebrate models, zebrafish and mice. Knockdown of cdc42 produced a phenotype similar to sec10 knockdown in zebrafish, and cdc42 and sec10 showed a synergistic genetic interaction, suggesting that cdc42 and the exocyst act in the same pathway. Furthermore, Cdc42 kidney-specific knockout in mice resulted in PKD.

Primary cilia, cellular sensory organelles projecting from the apical membrane, are found on most eukaryotic epithelial cell types, including renal tubular epithelial cells, and contribute to development, tissue maintenance, and homeostasis by detecting diverse mechanical and chemical stimuli, including light, fluid flow, hormones, and growth factors (1, 2). Defective ciliogenesis interferes with the normal function of organs and leads to various human diseases, termed ciliopathies, which include polycystic kidney disease (PKD) (3). Dozens of ciliary proteins involved in ciliopathies have now been identified and studied (4). However, relatively little is known regarding how ciliary proteins are targeted and delivered to the cilium.

The exocyst, an evolutionarily conserved octameric protein complex comprised of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 (5, 6), has been shown to target and dock vesicles carrying membrane proteins from the trans-Golgi network to the plasma membrane (7). Sec10 is a core component of the exocyst complex and acts as a linker between transport vesicles and other exocyst components (8). Our previous studies showed that knockdown of Sec10 inhibited ciliogenesis, whereas overexpression of Sec10 increased ciliogenesis, cyst formation, and tubule morphogenesis in Madin-Darby canine kidney (MDCK) cells (9, 10). Furthermore, we demonstrated a biochemical interaction between Sec10 and major ciliary proteins, including polycystin-2, one of two genes mutated in autosomal dominant PKD (11). Sec10 knockdown in zebrafish phenocopied many aspects of polycystin-2 knockdown (11). These data indicate that Sec10 plays a key role in the regulation of ciliary protein trafficking and ciliogenesis. Importantly, an exocyst mutation was recently identified in a family with Joubert syndrome, a nephronophthisis form of PKD (12).

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2 The abbreviations used are: PKD, polycystic kidney disease; MDCK, Madin-Darby canine kidney; GEF, guanine nucleotide exchange factor; HGF, hepatocyte growth factor; MO, morpholino; hpf, hour(s) post-fertilization; dpf, day(s) post-fertilization; KD, knockdown.
with cystogenesis in distal tubules and collecting ducts and decreased ciliogenesis in cells surrounding cysts (17). Based on these data, we proposed that Cdc42 localizes the exocyst to primary cilia so that the exocyst can then target and dock vesicles carrying ciliary proteins.

The activities of Rho GTPases such as Cdc42 are regulated by guanine nucleotide exchange factors (GEFs) that exchange GDP for GTP (18). Tuba, a scaffold protein, plays a critical role as a GEF for Cdc42 in several intracellular processes associated with the actin and microtubule cytoskeleton (19–23). Our previous study demonstrated that knockdown of Cdc42 inhibited ciliogenesis in two-dimensional Transwell filter-grown MDCK cells (16). To investigate the hypothesis that Tuba plays a key role in ciliogenesis and cyst formation, we investigated Tuba function and its cellular mechanisms using MDCK knockdown cells grown to cysts in a three-dimensional collagen gel and Tuba knockdown in zebrafish.

**Experimental Procedures**

**Cell Culture**—Low-passage type II MDCK cells were obtained from Dr. K. Mostov (University of California San Francisco, San Francisco, CA). These cells were originally cloned by Daniel Louvard at the European Molecular Biology Laboratory and came to Keith Mostov via Karl Matlin. The Tuba knockdown stable cell line that we generated using a canine shRNA and a lentiviral vector delivery system was described and validated previously (16, 24). Cells were grown in modified Eagle’s minimal essential medium containing Eagle’s balanced salt solution and glutamine supplemented with 5% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin on plastic culture dishes. Some cells were also grown on Transwell 0.4-μm polycarbonate filters (3401, Corning Life Sciences) for a week. For cyst culture, Tuba knockdown and control cells were plated as single cells in a three-dimensional type I collagen matrix (66% collagen) as described previously (10) and were grown for 10–14 days. To induce tubulogenesis, cysts were treated with 10 ng/ml HGF for 1–3 days.

**Microinjection of Morpholinos and mRNA (tuba and cdc42)**—Wild-type zebrafish embryos were obtained from the University of Pennsylvania and the Medical University of South Carolina Zebrafish Cores, and all zebrafish experiments were approved by the Institutional Animal Care and Use Committees at the University of Pennsylvania, the Medical University of South Carolina, the Philadelphia Veterans Affairs Medical Center, and the Ralph H. Johnson Veterans Affairs Medical Center (Charleston, SC). For knockdown of tuba and cdc42, three antisense morpholino (MO) oligonucleotides that block translation or splicing were designed, targeting the ATG start codon or a splice donor site of exon 5 in tuba, and purchased from Gene Tools, LLC: tuba AUG MO (5’-AACCACGGACGCCCTCATGTTCCA-3’), tuba splice site MO (5’-AGCTGGGATT-TACAGACCTTGTTC-3’), and cdc42 AUG-MO (5’-CAACGACCTTGATCTGCAT-3’). Knockdown of two control GEFs, Obscurin and Fgdf5, was performed using the splice site morpholinos (25, 26). The scrambled control oligo purchased from Gene Tools was used as a negative control, and the total amount of MO injected under each condition was kept equivalent for the control MOs. The MOs were diluted with phenol red tracer (P0290, Sigma-Aldrich) at 0.05%. Final injection amounts were single doses of 3.4 ng of tuba MO or combined suboptimal doses of 1.7 ng of cdc42 MO and tuba MO per embryo. The pDNA3 and pEGFP-C1 vectors containing mouse Tuba full-length cDNA were given to us by Dr. Pietro De Camilli (Yale University, New Haven, CT) for rescue of the zebrafish tuba knockdown. Capped mouse Tuba full-length mRNA was synthesized using the mMessageMachine T7 kit as instructed by the manufacturer (AM1344, Ambicon). Finally, 70, 100, and 200 pg of capped Tuba mRNA were co-injected with 3.4 ng of tuba MO into one-cell stage embryos.

**Immunofluorescence Staining**—For immunofluorescence staining of MDCK cells grown on Transwell filters, the cells were directly fixed in 4% paraformaldehyde for 30 min at room temperature. The three-dimensional cysts grown in a collagen gel were incubated at 37 °C in collagenase solution to digest the collagen matrix before fixation in 4% paraformaldehyde. The fixed cells were permeabilized for 10 min at room temperature with 0.025% saponin in 1× PBS. After blocking with PBS buffer (0.025% saponin and 0.7% fish skin gelatin in 1× PBS), the cells were incubated with primary antibodies in the PBS buffer overnight at 4 °C and secondary antibodies overnight at 4 °C. After nuclear staining with DAPI, the cells were post-fixed in 4% paraformaldehyde and mounted with a mounting medium (71-00-16, KPL). To observe pronephric cilia in zebrafish, whole mount immunofluorescence was performed on 27 hpf zebrafish embryos based on a protocol published previously (27). Briefly, the samples fixed in 4% paraformaldehyde were permeabilized with prechilled acetone. After blocking with 10% normal donkey serum in PBDT buffer (PBS containing 1% dimethyl sulfoxide and 0.1% Tween 20), the samples were incubated with the primary and secondary antibody, each in the blocking buffer overnight at 4 °C. DAPI counterstaining was also performed. After washing with PBDT buffer containing 1% normal donkey serum and 0.1 M NaCl, the samples were mounted with glycerol.

**Western Blotting Analysis**—Whole embryo lysates were extracted from 2 dpf zebrafish as described previously (11). The protein samples were separated on NuPage 4–12% BisTris gels (NP0336, Novex) and then transferred to a nitrocellulose membrane (LC2000, Novex). The membranes were blocked with 5% nonfat dry milk in 1× PBS containing 0.1% Tween 20 and incubated with primary antibodies overnight at 4 °C. After washing with 1× PBS containing Tween 20, the membranes were incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. Finally, the membranes were exposed to a Western blotting chemiluminescence reagent (34080, Pierce), and developed on x-ray film.

**Antibodies**—The primary antibodies used in this study were mouse monoclonal anti-acetylated α-tubulin (T6793, Sigma-Aldrich), mouse anti-GP135 (a gift from G. Ojakian, State University of New York), rabbit polyclonal anti-β catenin (sc-7199, Santa Cruz Biotechnology), rabbit polyclonal anti-Tuba (a gift from Dr. Pietro De Camilli, Yale University), and mouse polyclonal anti-y tubulin (ab11316, Abcam). The secondary antibodies we used were donkey anti-mouse IgG Cy2, Alexa Fluor 555 phalloidin (A34055, Invitrogen), goat Alexa Fluor 488-conjugated anti-rabbit IgG (A21207, Life Technologies), donkey Alexa Fluor 594-conjugated anti-mouse IgG (A21203, Life
Tuba in Ciliogenesis and Nephrogenesis

Technologies), and goat anti-mouse/anti-rabbit HRP-conjugated secondary antibodies (1858413 for mouse and 1858415 for rabbit, Pierce).

Whole Mount in situ Hybridization in Zebrafish—Total RNA was extracted from zebrafish embryos at 1 dpf using the RNeasy Plus mini kit (74134, Qiagen) in accordance with the instructions of the manufacturer. cDNA was synthesized by reverse transcription using oligo(dT) primers and Multiscribe reverse transcriptase (4368814, Applied Biosystems). Digoxigenin-labeled sense and antisense RNA probes specific for tuba and myl7 (cardiac myosin light chain 7) were synthesized using the DIG-RNA labeling kit (11175025910, Roche) according to the instructions of the manufacturer. Whole mount in situ hybridization was performed as described previously (28, 29).

After rehydration and permeabilization using proteinase K (10 μg/ml), the RNA probes were hybridized to endogenous target mRNA overnight at 68 °C. The target hybridized RNA probes were recognized by anti-digoxigenin Fab fragment conjugated with alkaline phosphatase (11093274910, Roche), and visualized by BM Purple AP substrate precipitating solution (11442074001, Roche).

Histological Analysis—Zebrafish tissues were fixed in 4% paraformaldehyde overnight at 4 °C. After dehydration using a graded series of methanol/PBS, the samples were embedded in paraffin. The sections were cut at 5-μm thickness and stained with hematoxylin and eosin.

Imaging—All images were captured in tif format and processed in Adobe Photoshop CS5.1. For immunofluorescence, Transwell-cultured MDCK cells were imaged on an Olympus FV 101 confocal microscope with a ×600 water objective, and the MDCK cell cysts were imaged on a Leica SP5 confocal microscope with a ×600 oil objective. For histology, in situ hybridization, and phenotypic analysis of zebrafish, a Leica M205C light microscope and a Leica DFC 450 camera were used. For immunofluorescence of pronephric tubules, samples were imaged on an Olympus BX42 microscope.

Statistical Analysis—The height and width of MDCK cells grown on Transwell filters were measured using Image J, and the difference was compared between control and Tuba knockdown cells. Statistical significance was calculated by a paired, two-tailed Student’s t test. The ratio of the number of cells with and without cilia in a cyst was measured in a cross-section at the z position, where each cyst had a maximum diameter. For both control and Tuba knockdown groups, mean values obtained from at least 30 cysts were calculated as a percentage, and Student’s t test was used to determine statistical significance. The percentage of cysts initiating tubulogenesis was calculated from three replicate experiments with more than 50 cysts/replicate, and the significance was determined using χ² testing. For all tests, p < 0.01 was considered to represent statistically significant differences.

Results

Tuba Depletion Inhibits Ciliogenesis with No Gross Changes in Cell Polarity in Two-dimensional Transwell Filter-grown Cells—To determine how Tuba participates in renal ciliogenesis, control and Tuba knockdown (KD) MDCK cells that we generated previously (16) were grown on Transwell filters, and the cilia and cellular polarity marker proteins were identified by immunofluorescence. MDCK cells grown on two-dimensional Transwell filters differentiate into polarized columnar cells with an apical surface containing primary cilia and a distinct basolateral membrane. In this culture model, we found that classic primary cilia stained with a cilium marker, acetylated α-tubulin, were seen projecting from most of the control MDCK cells (Fig. 1A), whereas they were almost totally absent in Tuba knockdown cells (Fig. 1B). These data indicate that Tuba plays a key role in ciliogenesis in MDCK cells. To determine whether this might be an indirect effect because of changes in intracellular polarity, we examined basolateral (β-catenin) and apical (GP135) plasma membrane marker proteins. Both control and Tuba knockdown cells showed no gross alterations in cell polarity following Tuba depletion in two-dimensional culture (Fig. 1C–F). Tuba knockdown cells (Fig. 1H), compared with control cells (Fig. 1G), were more columnar, as indicated by their elongated cell shape, increased height, and nuclei located near the base of the cells, but narrower, with a decreased cell width, suggesting that Tuba knockdown cells may have increased basolateral and decreased apical membrane surface areas.

Tuba Depletion Results in Impaired Apical Polarization in Three-dimensional Collagen Gel Cysts—To investigate the role of Tuba in a more in vivo-like environment, we grew control and Tuba knockdown MDCK cells in a three-dimensional type I collagen gel matrix. In this culture system, normal MDCK cells proliferate and organize into polarized cysts with a hollow lumen. Control and Tuba knockdown cells displayed normal cystogenesis, forming a single lumen in the collagen gel, although cells in Tuba knockdown cysts either did not have cilia or had very short cilia (Fig. 2, A and B). Interestingly, we found that Tuba knockdown cysts displayed abnormal apical polarity by immunostaining with two apical markers, F-actin and GP135. The Tuba knockdown cysts showed a “double contour” F-actin staining. Actin staining is normally most prominent apically given the large amounts of actin in apical microvilli (Fig. 2, C and D). GP135, which is an apical plasma membrane protein, showed weak and diffuse staining in the Tuba knockdown cyst cells, consistent with a defect in apical polarity (Fig. 2, E and F). Basolateral polarity, as determined by β-catenin staining, appeared to be intact (Fig. 2, G and H). These results suggest that normal ciliogenesis may be necessary for the establishment of apical polarity in a three-dimensional system or, alternatively, that correct apical polarity is necessary for ciliogenesis.

Knockdown of Tuba Inhibits HGF-mediated Initiation of Tubulogenesis—Our previous studies showed that the exocyst and Cdc42 are centrally involved in tubulogenesis (10, 30). To determine whether Tuba is important for tubulogenesis, we investigated HGF-induced tubulogenesis of control and Tuba knockdown MDCK cysts in collagen gels by treatment with 10 ng/ml HGF, followed by staining for F-actin and DAPI. 24 h after HGF treatment, there were significantly more actin extensions, which is the first stage of tubulogenesis (31), at the level of the largest cyst diameter in control (Fig. 3A) versus Tuba knockdown MDCK cell cysts. (Fig. 3, B and C). Tuba is apparently not required for later stages of tubulogenesis because the ratio of
mature tubule formation was the same between control and Tuba knockdown cell cysts, assuming they had initiated similar numbers of actin extensions.

**Tuba Localizes to Ciliated Organs, and Depletion of Tuba Causes Phenotypic Defects in Ciliated Organs in Zebrafish**—In our previous studies, we showed that cdc42 and sec10 genetically interact and play a key role in ciliogenesis in zebrafish and mice (17). To determine whether Tuba also contributes to renal ciliogenesis, presumably as a Cdc42 GEF, we first needed to confirm that tuba expression is similar to cdc42 expression and that it includes the kidney. To identify the spatiotemporal expression pattern of tuba in zebrafish, we used whole mount in situ hybridization of zebrafish embryos from 1 hpf to 3 dpf using antisense tuba RNA probes. Although tuba was expressed prior to 1 dpf, tissue-specific expression was detected from 1 dpf in the brain and pronephric tubules (Fig. 4A). Finally, at 3 dpf, tuba expression expanded to the eyes and neuromasts, where mechanosensory hair cells exist, along the lateral line (Fig. 4B). This expression pattern was highly similar to that of cdc42 in zebrafish (17), with the exception of neuromast cells. These data demonstrate that tuba expression occurs in ciliated organs, including the kidney, during zebrafish development. After confirming tuba expression in the kidney, we knocked down tuba in zebrafish embryos using tuba start site (AUG) antisense morpholino oligonucleotides (tuba MO) that block initiation of translation, thereby targeting both maternal and zygotic transcripts. One cell-stage embryos were injected with 1.7 and 3.4 ng of tuba MO, and the scrambled control oligo was used at the same dose as a control morpholino. A reduction of Tuba protein expression was confirmed on Western blotting, indicating that the tuba AUG MO effectively inhibited tuba translation (Fig. 4C). We also knocked down Tuba protein in zebrafish embryos using tuba splice site antisense morpholino oligonucleotides (tuba splice site MO), which results in aberrant splicing of exon 5 and probable degradation (Fig. 4D).

Regarding the two Tuba bands seen on Western blotting analysis, other groups have also shown in both human and dog cells, Caco-2 (22) and MDCK (24), respectively, that there are two bands at the same molecular weight as ours representing Tuba on immunoblot. It is unclear which isoform is the active form. In Fig. 4, C and D, we show that both isoforms are knocked down following tuba start and splice site MO injections. The tuba morphants contained multiple phenotypic defects, beginning with a downward-curled tail at 2 dpf. Additional ciliary defects after 3 dpf included small eyes, pericardial edema, and hydrocephalus. These defects became more severe, leading to abdominal fluid accumulation at 5 dpf (Fig. 4E). The phenotypic severity increased in a dose-dependent manner for the tuba morphants, and a typical injection trial of 3.4 ng of tuba MO showed only 17% with a normal phenotype and 83% having a curly tail, small hydrocephalus, edema, or small eyes (n = 188). We then injected the tuba splice site morpholino and

**FIGURE 1. Tuba is required for ciliogenesis and cellular morphogenesis in two-dimensional Transwell filter-grown MDCK cells.** Control and Tuba knockdown MDCK cells were grown on Transwell filters for 10 days. A and B, primary cilia were labeled with an antibody against acetylated α-tubulin (red), and the basolateral membrane was visualized using an antibody to β-catenin (green). The cell nucleus was stained using DAPI (blue). Ciliogenesis was almost completely inhibited when Tuba was knocked down. Scale bar = 20 μm. C–F, distribution of basolateral (C and D, β-catenin, green) and apical (E and F, GP135, red) markers in control and Tuba knockdown cells. Scale bar = 10 μm. In two-dimensional Transwell filter-grown cells, apical and basolateral polarity were not grossly affected by Tuba knockdown. G and H, Tuba knockdown cells were significantly taller and narrower compared with control cells.

![Image](image-url)
found the same phenotype as we saw with the tuba start site MO. Uninjected wild-type \((n = 108)\) and injection control (scrambled control oligo groups, \(n = 41)\) zebrafish displayed few or no abnormal phenotypes (Fig. 4F). The morphant phenotype was rescued in a dose-dependent manner by mouse Tuba mRNA, which is resistant to the zebrafish tuba MO by virtue of a difference in primary base pair structure (Fig. 4F). To ensure specificity of the tuba MOs, we additionally performed a side-by-side morpholino analysis targeting two other GEFs, Obscurin and Fgd5, which are not known to be involved in the Cdc42-mediated ciliogenesis pathway (25, 26), in addition to the scrambled control MOs (Fig. 4F). The obscurin and fgd5 MOs successfully disrupted the normal transcripts at similar doses as we used for the tuba MO (3 and 6 ng); however, the morphants did not display the ciliary phenotypes found in tuba morphants. Indeed, the embryos appeared normal (Fig. 4, G and H). These controls make it highly unlikely that the tuba morphant phenotype is due to nonspecific off-target effects of the tuba MO. Moreover, we also found that tuba morphants have defects in cardiac laterality by labeling a cardiac marker gene, myl7, using whole mount in situ hybridization (Fig. 4I). Normal zebrafish development involves asymmetric left-right looping of the heart by 48 hpf (the ventricle toward the right and the atrium toward the left). The reversed left-right patterning of the heart and other organs is a common phenotypic defect caused by ciliary dysfunction (32–34). In this experi-

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**FIGURE 2.** Tuba depletion inhibits ciliogenesis and results in impaired apical polarization in MDCK cysts grown in a three-dimensional collagen gel. Control and Tuba KD MDCK cells were cultured in type I collagen gels for 10–14 days, and the middle of each cyst, as determined by the maximum diameter, was imaged by confocal microscopy. A, both control and Tuba knockdown cells formed single lumen cysts. However, primary cilia labeled with acetylated \(\alpha\)-tubulin (red) were either absent or severely stunted in Tuba knockdown cells. B, the percentage of the cells projecting primary cilia in a cyst was quantified. Approximately 80% and 20% of cells have primary cilia in control and Tuba knockdown cysts, respectively. Error bars represent mean ± S.D. C–F, Tuba knockdown cysts display abnormal apical polarity with double contour F-actin staining (arrows) and diffuse GP135 staining (red). G and H, basolateral polarity as determined by \(\beta\)-catenin appeared to be intact.
ment, 53% of *tuba* morphants (*n* = 15) showed reversed left-right looping of the heart, whereas 100% of wild-type zebrafish displayed a normal pattern (*n* = 34). These data indicate that Tuba/cilia are controllers of left-right patterning and that, following *tuba* knockdown (and the absence of cilia), cardiac laterality is random.

**Tuba Is Necessary for Kidney Development in Zebrafish**—To investigate the phenotypic defects in *tuba* morphant kidneys in more detail, histologic and immunofluorescence analyses were performed. H&E-stained sections of 5 dpf embryos showed hydrocephalus (Fig. 5A) as well as abnormal pronephric kidneys. Specifically, *tuba* morphants displayed morphologically disorganized and expanded glomeruli, although the pronephric ducts of the *tuba* morphants did not appear to be grossly different from wild-type embryos (Fig. 5, A and B). Our observation of inhibition of ciliogenesis in *tuba* knockdown MDCK cells (16) and the phenotypic ciliary defects found in *tuba* morphant zebrafish suggested that *tuba* morphants would have defects in renal ciliogenesis. To investigate this, we studied pronephric cilia in wild-type and *tuba* morphant embryos at 27 hpf using immunofluorescence. The renal cilia in the *tuba* morphants were highly disordered in both the medial and posterior pronephros (Fig. 5C). These results, showing severe morphological abnormalities of the kidney, including the glomeruli and pronephric cilia, indicate that *tuba* is essential for kidney development in zebrafish.

**tuba and cdc42 Genetically Interact for Ciliogenesis**—Tuba is a GEF of Cdc42 during formation of the cytoskeleton and cell polarization (19, 20, 22, 24), and most morphological and histological defects observed in *tuba* morphants were also detected in *cdc42* morphants (17). Therefore, we investigated a genetic interaction between *tuba* and *cdc42*. We first identified suboptimal doses of each morpholino that caused few or no phenotypic defects alone, which were 1.7 ng of both *tuba* MO and *cdc42* MO. Next, we co-injected suboptimal doses of the two morpholinos at the one-cell stage, and the phenotypes were examined at 5 dpf. When each morpholino was injected alone, only 7.4% and 3.8% of *tuba* and *cdc42* morphant embryos, respectively, showed defective phenotypes (Fig. 6A). However, co-injection of 1.7 ng of *tuba* and *cdc42* morpholinos revealed a curved tail, hydrocephalus, small eyes, glomerular edema, or abdominal fluid accumulation in 68% of the embryos (*p* < 0.001) (Fig. 6A). This proportion was much higher than expected based on the results of single morpholino injections (Fig. 6B) and suggests that these genes act in the same pathway. In addition, medial and posterior pronephric duct cilia in the double MOs were also disordered (Fig. 6C). Together, these results strongly support the idea that *tuba* plays a central role, through *cdc42*, in ciliogenesis and kidney development in zebrafish.

**Discussion**

Tuba was first identified as a scaffold protein that binds to numerous actin-regulatory proteins. Salazar *et al.* (19) demonstrated that Tuba has a remarkably high expression pattern in the kidney and that it can promote F-actin nucleation and recruitment via direct interaction with Wiskott-Aldrich syndrome protein, dynamin-1, Cdc42, and other actin-regulatory proteins. Other studies also showed that Tuba regulates the geometrical configuration of tight and adherens junctions in Caco-2 cells (22) and that Tuba participates in the normal lumenogenesis of epithelial cell cysts by regulating spindle orientation (23, 24). Importantly, all of these intracellular signaling pathways involve Cdc42 activation. Based on these findings, we hypothesized that Tuba is also centrally involved in renal cilio-
genesis and nephrogenesis via regulation of Cdc42 activation and investigated it here using both *in vitro* and *in vivo* systems.

We first investigated ciliogenesis and cell polarity in MDCK cells because ciliogenesis has been found to be associated with the establishment of apical cell polarity by direct or indirect interactions between polarity protein complexes, including the Par complex (13, 14, 35). In particular, Cdc42, the effector of Tuba, directly binds to Par6, leading to phosphorylation of atypical PKC targets, which initiates epithelial cell polarity (36–39). These reports suggest that intact cell polarity regulated by Cdc42 is required for ciliogenesis. In this study, we found impaired apical polarization and an absence of cilia in Tuba knockdown MDCK cysts grown in collagen gels. Interestingly, Tuba knockdown MDCK cells grown on two-dimensional...
Transwell filters showed no gross signs of abnormal polarity. Multiple studies, however, have demonstrated that cell polarity can be different in cells grown as a monolayer on two-dimensional filters compared with cells grown in three-dimensional matrix (14–40–43). For example, galectin-3 is secreted apically in two-dimensional monolayers (42, 44) but basolaterally in three-dimensional cysts (43, 45). Another study showed that dominant negative expression of Rac1 caused an inversion of cell polarity in MDCK cell cysts in three-dimensional culture, whereas it had no effect on cell polarity in two-dimensional culture (40).

We also showed that loss of Tuba had no effects on cyst lumen formation in a type I collagen gel despite ciliogenesis and apical polarity defects. Overall, the morphogenetic defects seen in Tuba knockdown cysts were mild compared with Cdc42 knockdown, which involved markedly abnormal cyst formation along with inverted polarity (30, 46). Our results also differed from previous studies showing that disturbance of cell polarity caused perturbations in lumen formation using a three-dimensional Matrigel culture system (24, 41, 47). There are several possible explanations. First, although Tuba is a Cdc42-specific GEF, other GEFs, including Intersectin 2, Fgd1, and Frabin, also have been shown to regulate Cdc42 activity in intracellular signaling, actin polymerization, cell cycle regulation, and extracellular matrix remodeling (48–50). Of these GEFs, Intersectin 2 is expected to have similar, and possibly overlapping, functions with Tuba. Its structural/molecular features, including direct interaction with Wiskott-Aldrich syndrome protein and Cdc42 in actin polymerization and subapical localization in MDCK cells, are analogous to Tuba (51, 52), and a functional human genomic screen for modulators of ciliogenesis identified Intersectin 2 as a positive regulator of ciliogenesis (53). Recently, Rodriguez-Fraticelli et al. (52) demonstrated that Intersectin 2 controls mitotic spindle orientation and normal cyst formation through Cdc42 in MDCK cells. Thus, we cannot exclude the possibility that Tuba is not the only Cdc42 GEF involved in ciliogenesis, cystogenesis, and tubulogenesis. Second, in epithelial cyst morphogenesis, external cues from the extracellular matrix have important roles in cyst formation. Different extracellular matrix compositions could affect cyst formation and tubulogenesis differently in MDCK three-dimensional culture models (54–58).

Type I collagen gels have a vastly different protein composition compared with another widely used gel matrix, Matrigel, derived from Engelbreth-Holm-Swarm mouse sarcoma cells. In addition, it has been shown that MDCK cells grown in Matrigel form single-lumen cysts when stimulated with HGF, whereas they undergo tubulogenesis following HGF stimulation in type I collagen gels (55). Because

### Figures

**Figure 4. Tuba is expressed in ciliated organs, and Tuba knockdown results in abnormal phenotypes.** A and B, whole mount in situ hybridization of zebrafish embryos at 1 and 3 dpf with antisense RNA tuba probes. Tuba is expressed in the brain, eye, pronephric duct (arrows), and neuromasts (arrowheads). Scale bar = 0.5 mm. C, down-regulation of Tuba expression was confirmed at the protein level by Western blotting at 2 dpf, following injection of tuba start site antisense morpholinos (tuba MOs). Scrambled MOs, at the same dose as the tuba MOs, were used as controls in side-by-side experiments. γ Tubulin was used as a loading control. D, down-regulation of Tuba expression was confirmed at the protein level by Western blotting at 2 dpf following injection of tuba splice site antisense morpholinos (tuba splice site MOs). Scrambled MOs were used as controls in side-by-side experiments. γ Tubulin was used as a loading control. E, phenotype of wild-type, injection control (with phenol red), and tuba MO zebrafish embryos at 5 dpf. Defects from the tuba MOs include a curved tail, hydrocephalus (arrowhead), small eyes with pericardial edema (asterisk), and abdominal fluid accumulation (arrows). Scale bar = 5 mm. F, the bar graph represents the percentage of abnormal phenotypes caused by tuba knockdown. There was an increase in abnormal tuba morphant phenotypes in a dose-dependent manner as well as increasing rescue with co-injection of increasing amounts of mouse Tuba mRNA, which is resistant to the zebrafish morpholino because of a difference in primary base pair structure. Injection of a scrambled control MO had no phenotypic effect. *p < 0.001. C, Obscurin, a GEF for RhoA, was inhibited using 3 ng of a splice site morpholino. This caused exon skipping but no specific phenotype. H, injection of 6 ng of fgf5 morpholino generated an abnormal transcript that included an additional intron, but, again, the morphants did not display phenotypic defects. I, whole mount in situ hybridization of control and tuba MOs for cardiac myl7 at 2 dpf. Reversed left-right looping of the heart is shown in the tuba morphants. v, ventricle; a, atrium.

**Figure 5. Tuba depletion causes abnormal renal development in zebrafish.** A, H&E staining of fixed sections showed a normal compact glomerulus in the control (left panel), whereas tuba MOs displayed morphologically disorganized and expanded glomeruli (arrows) at 5 dpf. B, pronephric ducts of control and tuba MOs were labeled with cdh17 by in situ hybridization at 3 dpf. Both control and tuba MOs developed renal tubules without any obvious abnormalities. PCT, proximal convoluted tubule; DT, distal tubule. Scale bar = 0.2 mm. C, medial and posterior pronephric duct cilia were visualized with acetylated α-tubulin (green) at 27 hpf. The renal cilia of tuba MO embryos were highly disordered.
Tuba in Ciliogenesis and Nephrogenesis

FIGURE 6. Tuba genetically interacts with cdc42 in zebrafish. A, phenotypic analysis of Tuba and cdc42 double-knockdown zebrafish at 5 dpf. A single injection of 1.7 ng of each morpholino did not result in an abnormal phenotype. However, co-injection of these two morpholinos at the same dose resulted in severe phenotypes, including a curved tail (arrowhead), small eyes (asterisk), and abdominal fluid accumulation (arrows). Scale bar = 1 mm. B, abnormal phenotypes in each injection group were quantified. Double morphants resulted in a dramatic increase in abnormal phenotypes. Three separate trials and a total of 515 embryos were examined. C, immunofluorescence of renal tubule cell cilia in controls and morphants showed that normal ciliogenesis is disrupted when low doses of both Tuba and cdc42 MOs were injected together compared with either of the two MOs alone at 27 hpf.

Type I collagen gels have been considered to be the optimal three-dimensional matrix for tubulogenesis in various cell studies (10, 59–61), we investigated both cyst formation and tubulogenesis in the same extracellular environment using type I collagen. Finally, we showed that Tuba is centrally involved in ciliogenesis and tubulogenesis as well as the establishment of apical polarity in MDCK cysts. This study does not give a definitive answer to the question of whether normal Tuba function and ciliogenesis are necessary for the establishment of apical polarity or, alternatively, whether correct apical polarity is necessary for ciliogenesis in the three-dimensional culture system. Nevertheless, we favor the first possibility because cell polarity was grossly intact in the two-dimensional filter-grown Tuba knockdown MDCK cells, but primary cilia did not form.

Our in vitro findings were confirmed in vivo using zebrafish. Zebrafish have been extensively used for studies of ciliogenesis and kidney development (62–64). The zebrafish kidney pronephros is remarkably simple but functionally conserved (65). Using zebrafish, we show that Tuba is expressed in multiple ciliated organs and that Tuba morphant zebrafish display various ciliary phenotypes. All of these phenotypes are known to be associated with ciliogenesis defects in zebrafish (32, 66–68) and correlated quite closely with the Tuba expression pattern detected by in situ hybridization. Morphogenetic defects of glomeruli and pronephric cilia are representative defects caused by loss of pkd2 or invs, major causative genes of autosomal dominant PKD and nephronophthisis, respectively, in humans (11, 34). Inversion of left-right asymmetry, such as inherited situs inversus, which includes defects in cardiac laterality, is also associated with defective cilium formation (69, 70). Kupffer’s vesicle in zebrafish is a ciliated tissue regulating left-right asymmetry (71). Cilia in these organs secrete an extra-cellular directional fluid flow and convert this physical stimulation to intracellular signaling to generate asymmetry (72). All of the defects observed in our Tuba morphants suggest that Tuba is an essential factor for ciliogenesis and kidney development in zebrafish. The fact that Tuba start and splice site MOs showed similar phenotypic defects and that the MOs for two other GEFS not known to be in the cdc42/ciliogenesis pathway, Obscurin and Fgd5, as well as a scrambled control MO showed no effect at equivalent dosages, indicates that the Tuba morphant phenotype was specific and not an off-target effect.

In the analysis of Tuba morphants, we found that Tuba knockdown phenocopies most aspects of cdc42 knockdown (17) and that Tuba and cdc42 genetically interact in zebrafish, supporting our hypothesis that Tuba regulates ciliogenesis via Cdc42. Interestingly, the curly tail phenotype displayed in the Tuba but not in the cdc42 morphants was not significantly increased by co-injection of both morpholinos, although the proportion and severity of all the other ciliary defects were dramatically increased. Tuba is expressed in neuromasts of both the anterior lateral line and posterior lateral line, a location in which cdc42 expression was not detected, and the downward-curled tail phenotype was seen mainly in Tuba morphants but not in cdc42 morphants. The downward-curled tail has been associated with ciliary dysfunction in various ciliated organs, including neuromasts (73, 74). Aquatic vertebrates have a sensory system, lateral line, comprised of mechanosensory organs called neuromasts that consist of a core of hair cells surrounded by support cells and mantle cells. Thus, this observation raises the possibility that Tuba may have an important role as a GEF of another small GTPase besides Cdc42 in zebrafish neuromasts.

In summary, this study is the first report demonstrating that Tuba plays a critical role in ciliogenesis and nephrogenesis,
most likely via Cdc42 activation. Together with our previous finding that the exocyst and polycystin-2 no longer localized at the primary cilium or ciliary region in Cdc42 and Tuba knock-down MDCK cells (16), we suggest that Tuba activates Cdc42 in the ciliary region and that activated Cdc42 then localizes the exocyst, which, in turn, targets and docks vesicles carrying ciliary proteins, such as polycystin-2 (Fig. 7). Given the importance of ciliary function in a multitude of ciliopathies, further investigation of Tuba function in mice as well as analysis of other Cdc42 GEFs involved in ciliogenesis should help us to identify the mechanisms of ciliary assembly governed by the exocyst and result in the discovery of novel therapeutic targets.

**Author Contributions**—J. I. B. performed the experiments, analyzed the data, and wrote the paper. S. Y. C. and X. Z. performed the experiments and reviewed the manuscript. S. H. Kwon and S. H. Kim reviewed and edited the manuscript. J. H. L. conceived and designed the study, provided guidance, and edited the manuscript.

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**References**
1. Gerdes, J. M., Davis, E. E., and Katsanis, N. (2009) The vertebrate primary cilium in development, homeostasis, and disease. *Cell* 137, 32–45
2. Badano, J. L., Mitsuma, N., Beales, P. L., and Katsanis, N. (2006) The ciliopathies: an emerging class of human genetic disorders. *Annu. Rev. Genomics Hum. Genet.* 7, 125–148
3. Sun, Z., Amsterdam, A., Pazour, G. J., Cole, D. G., Miller, M. S., and Hopkins, N. (2004) A genetic screen in zebrafish identifies cilia genes as a principal cause of cystic kidney. *Development* 131, 4085–4093
4. Gherman, A., Davis, E. E., and Katsanis, N. (2006) The ciliary proteome database: an integrated community resource for the genetic and functional dissection of cilia. *Nat. Genet.* 38, 961–962
5. Guo, W., Grant, A., and Novick, P. (1999) Exo84p is an exocyst protein essential for secretion. *J. Biol. Chem.* 274, 23558–23564
6. Liu, J., and Guo, W. (2012) The exocyst complex in exocytosis and cell migration. *Protoplasma* 249, 587–597
7. Lipschutz, J. H., and Mostov, K. E. (2002) Exocytosis: the many masters of the exocyst. *Curr. Biol.* 12, R212–214
8. Guo, W., Roth, D., Walch-Solimena, C., and Novick, P. (1999) The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. *EMBO J.* 18, 1071–1080
9. Zuo, X., Guo, W., and Lipschutz, J. H. (2009) The exocyst protein Sec10 is necessary for primary cilogenesis and cystogenesis in vitro. *Mol. Biol. Cell* 20, 2522–2529
10. Lipschutz, J. H., Guo, W., O’Brien, L. E., Nguyen, Y. H., Novick, P., and Mostov, K. E. (2000) Exocyst is involved in cilogenesis and tubulogenesis and acts by modulating synthesis and delivery of basolateral plasma membrane and secretory proteins. *Mol. Biol. Cell* 11, 4259–4275
11. Fogelgren, B., Lin, S. Y., Zuo, X., Jaffe, K. M., Park, K. M., Reichert, R. J., Bell, P. D., Burdine, R. D., and Lipschutz, J. H. (2011) The exocyst protein Sec10 interacts with Polycystin-2 and knockdown causes PKD-phenotypes. *PLoS Genet.* 7, e1001361
12. Dixon-Salazar, T. J., Silhavy, J. L., Udpa, N., Schroth, J., Bielas, S., Schaffer, A. E., Olvera, J., Bafna, V., Zaki, M. S., Abdel-Salam, G. H., Mansour, L. A., Selim, L., Abdel-Hasid, S., Marzouki, N., Ben-Omar, T., Al-Saana, N. A., Sonmez, F. M., Celep, F., Azam, M., Hill, K. J., Collazo, A., Fenstermaker, A. G., Novarino, G., Akizu, N., Garimella, K. V., Sougnez, C., Russ, C., Gabriel, S. B., and Gleeson, J. G. (2012) Exome sequencing can improve diagnosis and alter patient management. *Sci. Transl. Med.* 4, 138ra78
Tuba in Ciliogenesis and Nephrogenesis

13. Fan, S., Hurd, T. W., Liu, C. J., Straight, S. W., Weimbs, T., Hurd, E. A., Domino, S. E., and Margolis, B. (2004) Polarity proteins control ciliogenesis via kinesin motor interactions. *Curr. Biol.* 14, 1451–1461

14. Sfakianos, J., Togawa, A., Maday, S., Hull, M., Pypaert, M., Cestra, G., Kwiatkowski, A., Salazar, M. A., Butler, M. H., and De Camilli, P. (2004) Cdc42 mediates Brn-induced spindling anastrosis through Fmn3-driven assembly of endothelial filopodia in zebrafish. *Dev. Cell* 10, 102–112

15. Raeker, M. O., Bieniek, A. N., Ryan, A. S., Tsai, H. J., Zahn, K. M., and Mostov, K. E. (2010) Targeted deletion of the zebrafish obscurin A Rho-GEF domain affects heart, skeletal muscle and brain development. *Dev. Biol.* 337, 432–443

16. Choi, S. Y., Chacon-Heszele, M. F., Huang, L., McKenna, S., Wilson, F. P., Zuo, X., and Lipschutz, J. H. (2013) Cdc42 deficiency causes ciliary abnormalities and cystic kidneys. *Am. J. Soc. Nephrol.* 24, 1435–1450

17. O'Brien, L. E., Jou, T. S., Pollack, A. L., Zhang, Q., Hansen, S. H., Yurchenco, P., and Mostov, K. E. (2001) Rac1 orients epithelial apical polarity through effects on basolateral laminin assembly. *Nat. Rev. Mol. Cell Biol.* 15, 225–242

18. Salazar, M. A., Kwiatkowski, A., Yin, Y., Du, Q., Chen, X., Zheng, Z., Balsbaugh, J. L., Maitra, S., Shabanowitz, J., Hunt, D. F., and Macara, I. G. (2010) Par3 controls epithelial spindle orientation by aPKC-mediated phosphorylation of apical PIns. *Curr. Biol.* 20, 1809–1818

19. Choi, S. Y., Chacon-Heszele, M. F., Huang, L., McKenna, S., Wilson, F. P., Zuo, X., and Lipschutz, J. H. (2013) Cdc42 deficiency causes ciliary abnormal-
Aza-Blanc, P., and Gleeson, J. G. (2010) Functional genomic screen for modulators of ciliogenesis and cilium length. Nature 464, 1048–1051
54. Santos, O. F., and Nigam, S. K. (1993) HGF-induced tubulogenesis and branching of epithelial cells is modulated by extracellular matrix and TGF-β. Dev. Biol. 160, 293–302
55. Sakurai, H., and Nigam, S. K. (1998) In vitro branching tubulogenesis: implications for developmental and cystic disorders, nephron number, renal repair, and nephron engineering. Kidney Int. 54, 14–26
56. Gudjonsson, T., Rønnov-Jessen, L., Villadsen, R., Rank, F., Bissell, M. J., and Petersen, O. W. (2002) Normal and tumor-derived myoepithelial cells differ in their ability to interact with luminal breast epithelial cells for polarity and basement membrane deposition. J. Cell Sci. 115, 39–50
57. Yu, W., Shewan, A. M., Brakeman, P., Eastburn, D. J., Datta, A., Bryant, D. M., Fan, Q. W., Weiss, W. A., Zegers, M. M., and Mostov, K. E. (2008) ERK and MMPs sequentially regulate disassembly of renal functional genomics for ciliogenesis and cilium length. Front Biosci. 13, 1866–1880
58. Marszalek, J. R., Ruiz-Lozano, P., Roberts, E., Chien, K. R., and Goldstein, L. S. (1999) Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. Proc. Natl. Acad. Sci. U.S.A. 96, 5043–5048
59. O’Brien, L. E., Tang, K., Kats, E. S., Schutz-Geschwender, A., Lipschutz, D. M., Fan, Q. W., Weiss, W. A., Zegers, M. M., and Mostov, K. E. (2005) β1-integrin orients epithelial polarity via Rac1 and laminin. Mol. Biol. Cell 16, 433–445
60. O’Brien, L. E., Zegers, M. M., and Mostov, K. E. (2002) Opinion: Building epithelial architecture: insights from three-dimensional culture models. Nat. Rev. Mol. Cell Biol. 3, 531–537
61. Cheng, H. Y., Lin, Y. Y., Chen, J. Y., Shen, K. F., Lin, W. L., Liao, H. K., Chen, Y. J., Liu, C. H., Pang, V. F., and Jou, T. S. (2005) Molecular identification of canine podocalyxin-like protein 1 as a renal tubulogenic regulator. J. Am. Soc. Nephrol. 16, 1612–1622
62. Lu, Q., Insinna, C., Ott, C., Stauffer, J., Pintado, P. A., Rahajeng, J., Baxa, U., Walia, V., Cuenca, A., Hwang, Y. S., Daar, I. O., Lopes, S., Lippincott-Schwartz, J., Jackson, P. K., Caplan, S., and Westlake, C. J. (2015) Early steps in primary cilium assembly require EHD1/EHD3-dependent ciliary vesicle formation. Nat. Cell Biol. 17, 228–240
63. Clément, A., Solnica-Krezel, L., and Gould, K. L. (2011) The Cdc14B phosphatase contributes to ciliogenesis in zebrafish. Development 138, 291–302
64. Wingert, R. A., Selleck, R., Yu, J., Song, H. D., Chen, Z., Song, A., Zhou, Y., Thise, B., Thise, C., McMahon, A. P., and Davidson, A. J. (2007) The cdx genes and retinoic acid control the positioning and segmentation of the zebrafish pronephros. PLoS Genet. 3, 1922–1938
65. Wingert, R. A., and Davidson, A. J. (2008) The zebrafish pronephros: a model to study nephron segmentation. Kidney Int. 73, 1120–1127
66. Tobin, J. L., and Beales, P. L. (2008) Restoration of renal function in zebrafish models of ciliopathies. Pediatr. Nephrol. 23, 2095–2099
67. Gerdes, J. M., Liu, Y., Zaghoul, N. A., Leitch, C. C., Lawson, S. S., Kato, M., Beachy, P. A., Beales, P. L., DeMartino, G. N., Fisher, S., Badano, J. L., and Katsanis, N. (2007) Disruption of the basal body compromises pro- ternal function and perturbs intracellular Wnt response. Nat. Genet. 39, 1350–1360
68. Wessely, O., and Obara, T. (2008) Fish and frogs: models for vertebrate cilia signaling, Front Biosci. 13, 1866–1880
69. Marszalek, J. R., Ruiz-Lozano, P., Roberts, E., Chien, K. R., and Goldstein, L. S. (1999) Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. Proc. Natl. Acad. Sci. U.S.A. 96, 5043–5048
70. Pennekamp, P., Menci, T., Dworniczak, B., and Hamada, H. (2015) Situs inversus and ciliary abnormalities: 20 years later, what is the connection? Cilia 4, 1
71. Essner, J. J., Yogan, K. J., Wagner, M. K., Tabin, C. J., Yost, H. J., and Brueckner, M. (2012) Conserved function for embryonic nodal cilia. Nature 418, 37–38
72. Kramer-Zucker, A. G., Olale, F., Haycraft, C. J., Yoder, B. K., Schier, A. F., and Drummond, I. A. (2005) Cilia-driven fluid flow in the zebrafish pronephros, brain and Kupffer’s vesicle is required for normal organogenesis. Development 132, 1907–1921
73. Zhao, C., Omori, Y., Brodowska, K., Kovach, P., and Malicki, J. (2012) Kinesin-2 family in vertebrate ciliogenesis. Proc. Natl. Acad. Sci. U.S.A. 109, 2388–2393
74. Jurisch-Yaksi, N., Rose, A. J., Lu, H., Raemaekers, T., Munck, S., Baetsen, P., Baerts, V., Vermeire, W., Scales, S. J., Verleyen, D., Vandepoele, R., Tylzanowski, P., Yaksi, E., de Ravel, T., Yost, H. J., Froyen, G., Arrington, C. B., and Annaert, W. (2013) Rer1p maintains ciliary length and signaling by regulating γ-secretase activity and FoxJ1a levels. J. Cell Biol. 200, 709–720