The exocyst acting through the primary cilium is necessary for renal ciliogenesis, cystogenesis, and tubulogenesis

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ABSTRACT

The exocyst is a highly conserved protein complex found in most eukaryotic cells and is associated with many functions, including protein translocation in the endoplasmic reticulum, vesicular basolateral targeting, and ciliogenesis in the kidney. To investigate the exocyst functions, here we exchanged proline for alanine in the highly conserved VxPx ciliary targeting motif of exocyst complex component 5 (EXOC5), a central exocyst protein, and generated stable EXOC5 ciliary targeting sequence-mutated (EXOC5CTS-m) Madin-Darby canine kidney (MDCK) cells. The EXOC5CTS-m protein was stable and could bind other members of the exocyst complex. Culturing stable control, EXOC5-overexpressing (OE), Exoc5-knockdown (KD), and EXOC5CTS-m MDCK cells on Transwell filters, we found that primary ciliogenesis is increased in EXOC5 OE cells and inhibited in Exoc5-KD and EXOC5CTS-m cells. Growing cells in collagen gels until the cyst stage, we noted that EXOC5-OE cells form mature cysts with single lumens more rapidly than control cysts, whereas Exoc5-KD and EXOC5CTS-m MDCK cells failed to form mature cysts. Adding hepatocyte growth factor (HGF) to induce tubulogenesis, we observed that EXOC5-OE cell cysts form tubules more efficiently than control MDCK cell cysts, EXOC5CTS-m MDCK cell cysts form significantly fewer tubules than control cell...
cysts, and Exoc5-KD cysts did not undergo tubulogenesis. Finally, we show that EXOC5 mRNA almost completely rescues the ciliary phenotypes in exoc5-mutant zebrafish, unlike the EXOC5CTS-m mRNA, which could not efficiently rescue the phenotypes. Taken together, these results indicate that the exocyst, acting through the primary cilium, is necessary for renal ciliogenesis, cystogenesis, and tubulogenesis.

INTRODUCTION

The exocyst is a highly-conserved eight-protein complex that was originally identified in a secretory screen in yeast by Novick, Field, and Schekman in 1980 (1). The eight homologous mammalian exocyst proteins were first identified in 1996 from rat brain (2). The exocyst is found in most cell types and has been linked by us and others to a wide variety of cellular processes, including: vesicular transport to the basolateral membrane (3,4), primary ciliogenesis in the kidney and eye (5-7), protein synthesis in the endoplasmic reticulum (8,9), and post-endocytic recycling (10).

Until recently, relatively little was known about the exocyst structure; therefore, it has been difficult to tease out the various functions of the exocyst. We previously showed that Exoc5 (aka Sec10) is a central component of the exocyst, linking Exoc6, which binds Rab8 (11), found on the surface of vesicles targeted by the exocyst, to the rest of the exocyst at the plasma membrane. In the absence of Exoc5, the exocyst complex disintegrates and is degraded, most likely via the proteasome (7). In 2017, the crystal structure of EXOC5 (12) was solved, and an in vivo three-dimensional (3D) integrative approach to the exocyst was performed (13). More recently, cryo-EM detailing the exocyst structure was reported (14). With the exocyst structure available, our goal here was to determine the role of the exocyst, and especially the central Exoc5 component, in renal primary ciliogenesis, and then, by extension, the role of the exocyst, and ciliogenesis, in cystogenesis and tubulogenesis.

RESULTS

Site-directed mutagenesis of the human EXOC5 ciliary targeting sequence leads to a stable protein that can bind other exocyst complex proteins. EXOC5 contains a VxPx ciliary targeting sequence that is highly-conserved from yeast to humans (Fig. 1A). We analyzed solvent accessibility of the Val666, Ala667, and Pro668 residues of EXOC5 represented by the 5h11 structure (12). Solvent-accessible surface areas were 37, 49, and 52 Å² and relative accessibilities were 32, 73, and 50%, respectively. Thus, all three residues are exposed to solvent and are available for binding, though proline to a greater degree than valine (Fig. 1B). We, therefore, performed site-directed mutagenesis of EXOC5-myc cDNA in a pcDNA3 vector, mutating the cytosine at position 2002 to a guanine (cca to gca), leading to alanine being translated instead of proline. Successful site-directed mutagenesis was confirmed by sequencing the full cDNA transcript (Fig. 1C). The pcDNA3 vector containing the human EXOC5-myc mutated ciliary targeting sequence (EXOC5CTS-m) was transfected into MDCK cells and stable cell lines generated. Three clonal cell lines expressing human EXOC5CTS-m were identified (G5, G7, and G9) using an antibody we made against human EXOC5 (7), and an antibody against the myc epitope tag (Fig. 1D). Using our anti-EXOC5 antibody, we show that the A1 MDCK cells have approximately two-fold higher expression of Exoc5 than untransfected MDCK cells (transfected EXOC5-myc is the top band in Fig. 1D, and is of equal intensity to the bottom native Exoc5 band, and to the band in the untransfected MDCK cells). Based on the intensity of the bands stained with the anti-myc antibody, clone G5 expressed the human EXOC5CTS-m protein to a similar degree as A1 MDCK cells stably expressing wild-type human EXOC5-myc that we previously generated and used in
multiple studies (4,7,15,16) (Fig. 1D). We then performed co-immunoprecipitation (co-IP), using antibody against the myc epitope tag, and found that human EXOC5CTS-m protein was able to bind another exocyst component, Exoc4 (Fig. 1E).

**Mutation of the EXOC5 ciliary targeting sequence inhibits ciliogenesis.** c2002g mutagenesis of the ciliary targeting sequence did not inhibit cell growth, or the time it took cells to reach confluence (Fig. 2A; data not shown); however, ciliogenesis was inhibited. We previously reported, and reconfirm here, that EXOC5 OE MDCK cells (A1) have longer cilia, and Exoc5 KD cells have shorter, or absent, cilia, compared to control MDCK cells (7). We also show here that EXOC5-myc OE cells have longer (Figs. 2B,D) and more abundant cilia (Figs. 2B,C), compared to the usual 25% ciliation of MDCK cells. EXOC5CTS-m cells (G5 clone) have fewer ciliated cells compared to control MDCK cells. The decrease in ciliogenesis in EXOC5CTS-m cells, was, in fact, very similar to what we found in Exoc5 KD cells (Figs. 2B,C), though the length of the primary cilia were not significantly different from control MDCK cell cilia (Figs. 2B,D).

**Mutation of the EXOC5 ciliary targeting sequence inhibits cystogenesis and tubulogenesis.** We previously showed in 3D collagen gel culture that EXOC5 OE MDCK cells formed mature single lumen cysts more rapidly, and that Exoc5 KD MDCK cells formed cysts more slowly, and were unable to form a proper lumen (7), compared with control MDCK cells. Here, we show that mutation of the Exoc5 ciliary targeting sequence in MDCK cells also prevents cystogenesis, similar to what we found in Exoc5 KD cells (Fig. 3A). These data support the idea that the inhibition of cystogenesis in MDCK cells following Exoc5 KD is due to the exocyst failing to act at the primary cilium.

We also previously showed that EXOC5 OE cell cysts experience enhanced tubulogenesis when exposed to hepatocyte growth factor (HGF) (4). We, therefore, added 10 ng/mL HGF to the media of control, EXOC5 OE, Exoc5 KD, and EXOC5CTS-m MDCK cell cysts, and found that EXOC5 OE cell cysts had enhanced tubulogenesis, while Exoc5 KD and EXOC5CTS-m MDCK cell cysts exhibited impaired tubulogenesis following induction with HGF. Specifically, mature tubules containing lumens projecting from the cysts were not observed in Exoc5 KD cell cysts, and significantly fewer mature tubules were seen in EXOC5CTS-m MDCK cell cysts, compared to control MDCK cell cysts, induced with HGF (Fig. 3B). These data support the idea that Exoc5, acting at the primary cilium, is also centrally involved in renal tubulogenesis.

**Mutation of the EXOC5 ciliary targeting sequence prevents rescue of exoc5 mutant zebrafish.** We recently showed multiple ciliary phenotypes, including in the kidney and heart, in exoc5 morphant (5,17) and mutant (6) zebrafish (Fig. 4A). We also found decreased levels of Exoc4 protein in exoc5 morphant and mutant zebrafish. Furthermore, phosphorylated (active) Mob1 (pMob1), of the Hippo pathway, was found in exoc5 mutant zebrafish. This suggests that the Hippo pathway, involved in organogenesis, is activated following loss of the exocyst (Fig. 4B). Injection of WT EXOC5 mRNA rescued exoc5 mutant zebrafish in a dose-dependent manner; however, EXOC5CTS-m mRNA was unable to efficiently rescue the ciliary phenotypes (Fig. 4C), and there was no rescue with mRNA for retinol binding protein receptor 2 (Rbpr2), a gene not known to interact with the exocyst or primary cilia (18). We also injected EXOC5 mRNA and EXOC5CTS-m mRNA into wildtype embryos and there was no phenotypic effect (n=82 embryos, data not shown).
DISCUSSION

We report two principle findings here, both of which are novel and important for our understanding of ciliogenesis, cystogenesis, and tubulogenesis. First, we show that the EXOC5 VxPx motif is necessary for the generation of primary cilia, though not for Exoc5 protein stability, or the ability to bind other exocyst complex members. This is supported by the structural data showing that proline (and valine) are on the outer surface of EXOC5, available for binding (12-14). Interestingly, while several other exocyst complex members also have VxPx motifs (Exoc1, Exoc3, Exoc6, Exoc7, and Exoc8), mutation of the VxPx ciliary targeting sequence in EXOC5 alone was sufficient to prevent ciliogenesis. This is consistent with our previous data showing that knockdown of Exoc4 and Exoc7 did not result in loss of other members of the exocyst complex or changes in ciliogenesis, cystogenesis, or tubulogenesis, while loss of Exoc5 did result in loss of other exocyst proteins and changes in ciliogenesis, cystogenesis, and tubulogenesis ((7) and Fig. 4B). These data demonstrate the centrality of Exoc5 to the function of the exocyst and primary ciliogenesis, and the role of primary cilia in cystogenesis and tubulogenesis. How cilia are involved in cystogenesis and tubulogenesis is not known, and is an area ripe for further study. We also showed, similar to what we previously reported (6), that the Hippo pathway was activated in exoc5 mutant zebrafish. We and others have linked alterations in the Hippo pathway to abnormal ciliogenesis and cystogenesis (6,17,20), and activation of the Hippo pathway has been shown to control organ development (20,21). Activation of Mob1, of the Hippo pathway, could help explain the ciliary phenotypes that we reported in exoc5 mutant zebrafish, and might be an important area for therapeutic investigation, as small-molecule modulators of the Hippo pathway have already been approved by the Food and Drug Administration (22).

Finally, there is the question of how the exocyst can be involved in so many different cellular processes. We, and others, have shown that small GTPases from the Rab (23), Arf (10,17), Rho (24-26), and Ral (27-30) families regulate the exocyst. We...
hypothesize that the different small GTPases, found at different locations in the cell, give the exocyst specificity of function. We have shown using cell culture, zebrafish, and kidney-specific knockout mice that Cdc42, a Rho family member, is found at the primary cilium and regulates the exocyst (25). Likewise, Tuba, a ciliary Cdc42 guanine nucleotide exchange factor (GEF), regulates the exocyst and is also necessary for proper ciliogenesis, cystogenesis, and tubulogenesis (31,32). We have similarly shown that Arl13b, an Arf family member, in its GTP form regulates the exocyst, arl13b and cdc42 genetically interact in zebrafish, and knockout of Arl13b in mice leads to renal cystogenesis, which phenocopies mice surviving for 30 days after kidney-specific knockout of Exoc5 (17). The fact that multiple small GTPases seem to regulate the exocyst at the primary cilium, suggests that the exocyst, in addition to trafficking vesicles to the primary cilium, may have other function(s) in the primary cilium (e.g. secretion of small extracellular vesicles).

In summary, we show here for the first time that the exocyst acting through the primary cilium is necessary for renal ciliogenesis, cystogenesis, and tubulogenesis (Fig. 5). Given our studies showing that the Exoc5 is necessary, in both zebrafish and mice, for renal (7) and photoreceptor (6) ciliogenesis, these results may be applicable to a wide-variety of organs and species.

MATERIALS AND METHODS

Materials. All chemicals, unless stated otherwise, were cell culture grade and purchased from Sigma-Aldrich.

Animal approval. All experiments on zebrafish were approved by the Institutional Animal Care and Use Committee (IACUC) of the Medical University of South Carolina and/or the Ralph H. Johnson VAMC.

Zebrafish husbandry. Adult zebrafish were maintained and raised in an Aquatic Habitats recirculating water system (Tecniplast) in a 14:10-hour light-dark cycle. The exoc5 mutant line was purchased from the Zebrafish International Resource Center (ZIRC, exoc5-sa23168). The exoc5 C377T point-nonsense mutation was verified by PCR and direct sequencing of both strands in heterozygote adults and mutant larve progeny. Genomic DNA from clipped fins, or whole 3.5 dpf zebrafish, was extracted in 50 µL 1x lysis buffer (10 mM Tris-HCl pH 8.0, 50 mM KCl, 0.3% Tween 20, 0.3% NP40), denatured at 98 °C for 10 minutes, digested at 55 °C for 6 hours with 10 µg/mL proteinase K, and the reaction was stopped at 98 °C for 10 minutes. The PCR primers were: forward primer, 5’-CTATATAGACATGGAGCGGCAAT-3’; reverse primer: 5’-CCAACAATTCTCCTACCTTCC-3’.

Sequencing was performed by Genewiz (South Plainfield, NJ, U.S.A.) with the forward PCR primer.

Immunofluorescence confocal microscopy. Cells were grown on Transwell filters and fixed with 4% paraformaldehyde for 15 minutes at room temperature, permeablized for 15 minutes at 37 °C with 0.025% saponin in phosphate buffered saline containing 0.7% fish skin gelatin (PFS buffer), and incubated with primary antibodies overnight at 4 °C, and secondary antibodies for 1 hour at room temperature.

Cysts grown in collagen gel were fixed with 4% paraformaldehyde for 30 minutes at room temperature after digesting in collagenase (100 U/ml; Sigma, St. Louis, MO) for 10 minutes at 37 °C as previously described (33). The cysts were blocked and permeablized with PFS buffer for 30 minutes at room temperature, and stained with DAPI and Alexa Fluor 555 phalloidin for 10-20 hours at 4 °C. Cells or cysts were postfixed with 4% paraformaldehyde and mounted with mounting medium (Kirkegaard & Perry Laboratories). 100 cysts or aggregations of cells were assessed to determine lumen formation for each cell line, and the experiment was repeated three times.

To induce tubulogenesis, HGF (a gift from Genentech) was added to the medium...
bathing the cysts on days 11, 12, and 13 at 10 ng/mL concentration. 100 cysts or aggregations of cells were assessed at the area of greatest diameter to determine tubule formation for each cell line, and the experiment was repeated three times.

Images were acquired on a confocal microscope (Leica TCS SP5) with the accompanying software (both from Leica, Inc.), using an HCX PL APO 63X/1.4-0.6 OIL objective to detect fluorochromes of cilia, and an HCX PL APO 20X/0.70 DRY CS objective to detect fluorochromes of cysts and tubules.

Co-immunoprecipitation (Co-IP) and Western blot analysis. MDCK type II cells grown on 10-cm dishes were collected on ice in a lysis buffer containing 20 mM HEPES, pH 7.4, 120 mM NaCl, 1 mM EDTA, 1% IGEPAL CA-630, 0.02% NaN3, 0.2% Trasylol, and proteinase inhibitor cocktail (1:1000) and then centrifuged at 14,000 rpm for 20 minutes at 4°C. The soluble supernatants were incubated overnight at 4°C with the anti-myc antibody (Cell Signaling Technology, Inc) at a concentration of 1 µl/mL. Immunocomplexes were then precipitated with Protein A/G Agarose (Pierce). The immunocomplexes were washed five times with lysis buffer, eluted by boiling in SDS-polyacrylamide gel electrophoresis sample buffer, and then subjected to immunoblot analysis. The immunocomplex was blotted with a rabbit polyclonal anti-EXOC5 antibody that we generated (7), and a mouse anti-EXOC4 antibody (Enzo Life Sciences). Blots were developed by enhanced chemiluminescence (Thermo Scientific).

Exoc5 mutant mRNA rescue experiments. For rescue experiments of zebrafish exoc5 mutants, capped and polyadenylated mRNA of wild-type (WT) EXOC5, ciliary targeting sequence-mutated human EXOC5, and retinol binding protein receptor 2 (Rbpr2) (18) mRNA was synthesized in vitro using the mMESSAGE mMACHINE kit (Ambion). Two doses of EXOC5 WT and mutant (low: 150 pg or high: 250 pg), or 250 pg of Rbpr2, mRNA were injected, using a Sutter Instruments microinjector, into 100 embryos at the one-cell stage. At 3.5 dpf, twelve randomly selected larvae, individually genotyped by direct sequencing, were imaged as outlined above.

Analysis of amino acid accessibility in the EXOC5. The protein structure was loaded from the PDB database, PDB ID 5h11 (12), and protonated with the ICM-Browser (34). Solvent-accessible surface areas of side chains of Val666, Ala667 and Pro668 residues were calculated using solvent probe radius 1.4 Å as implemented in the ICM-Browser. Relative accessibility was calculated using maximal values derived from Gly-X-Gly tripeptides (35). An amino acid residue was considered as solvent-accessible if its accessibility was more than 30%, which is the mean accessibility in proteins (35).
Site-directed mutagenesis. To inhibit the ciliary targeting sequence VxPx in human EXOC5, the QuikChange Site-Directed Mutagenesis Kit (Stratagene, #200518) was used for in vitro site-directed mutagenesis of proline 668 to alanine. The primers were:

5’
CTTCTGGTAGTTGCCGCAGATAATTT
AAAGCAAGTCTGC 3’

5’
GCAGACTTGCTTTAAATTATCTGCGG
CAACTACCAGAAG 3’

Plasmid pcDNA3-hEXOC5 was used as template. Sequencing confirmed the specific amino acid change.

Statistics
Cilia length: z-series confocal images were used to reconstruct a 3D cilia image using IMARIS software (V7.2, Bitplane), and the cilia number and individual cilia length were then quantified.

Cysts: 100 cysts were randomly identified for each cell line, and the number of cysts with single lumens, multiple lumens, and no lumens were determined.

Tubules: 100 cysts treated with 10 ng/mL HGF were randomly identified for each cell line, and the number of tubules with lumens were counted at the area of greatest cyst diameter.

The Student’s t-test was applied in order to determine the difference in ciliogenesis, cystogenesis and tubulogenesis between control and EXOC5-perturbed MDCK cell lines. All statistical tests were two-sided and unpaired, and expressed as the means and standard deviations. Statistical significance was defined as p<0.05. Data analysis was performed using Microsoft Excel software.

Conflict of interest: The authors have declared that there are no conflicts of interest.

Author contributions:
X.Z. and J.H.L. designed the research studies and wrote the manuscript. G.P.L., Y.S., Y.D., X.Z., conducted experiments and acquired data. D.F., L.G., D.I, D.N., G.P.L., B.R., R.A.N., S.C.B., and J.H.L. analyzed and interpreted the data, and reviewed the manuscript.

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Figure 1
Figure 1: Site-directed mutagenesis of the ciliary targeting sequence in human EXOC5 cDNA results in a stable protein that can bind other exocyst components. (A) The EXOC5 VxPx ciliary targeting sequence is highly-conserved from yeast to humans. (B) The VxPx ciliary targeting sequence in the EXOC5 3D protein model shows that proline (and to a lesser degree valine) are on the outside of the EXOC5 protein and, hence, are available for binding. The right panel demonstrates the solvent-accessible surface of EXOC5 in the 5h11 structure. The protein is shown as backbone trace (magenta), and the molecular surface obtained with a spherical water probe with a radius of 1.4 Å (white). Three residues Val666, Ala667 and Pro668 are shown by balls-and-sticks. The contribution of these residues to the molecular surface is marked by the green color. (C) Site-directed mutagenesis of cytosine at position 2002, results in a guanine substitution (cca to gca), which leads to alanine being translated instead of a proline. (D) EXOC5CTS-m protein is stable as determined by Western blot. Indeed, lysates from the three stable clonal EXOC5CTS-m cell lines (G5, G7, G9) show more mutated EXOC5 protein than endogenous Exoc5 protein found in control MDCK cells. The amount of mutated EXOC5 protein, especially in clone G5, is similar to the amount of EXOC5 protein that we found in EXOC5 OE cells that we previously generated (clone A1). The mutated and control EXOC5 proteins likely run slower on the gel due to the additional amino acids found in the myc epitope tag. Confirmation of the presence of human EXOC5 protein is demonstrated by staining using 9E10 antibody against the myc epitope tag. (E) Immunoprecipitation using antibody against the myc epitope tag of the EXOC5CTS-m protein, shows that EXOC5CTS-m co-immunoprecipitates endogenous Exoc4.
Figure 2: Mutation of the EXOC5 ciliary targeting sequence inhibits ciliogenesis. (A) Control, EXOC5-overexpressing (OE), Exoc5 knockdown (KD), and EXOC5CTS-m stable cell lines were grown on Transwell filters to confluency. All cell lines reached confluency at the same time, as determined by a fluid maintenance test (36). The Transwell filters were fixed, and the cells stained with nuclear DAPI (blue color), and acetylated α-tubulin antibody against ciliary axonemes (red color). (B) Higher magnification image of control, EXOC5 OE, Exoc5 KD, and EXOC5CTS-m MDCK cells stained with DAPI (blue color), and acetylated α-tubulin antibody (red color). (C) Quantitation of the percent of cells that have primary cilia shows that EXOC5 OE cells are more ciliated, while Exoc5 KD and EXOC5CTS-m are less ciliated, than control MDCK cells. (D) Quantitation of ciliary length shows longer cilia in EXOC5 OE, compared to control, MDCK cells. N=700 cells counted for each cell line, with the experiment repeated three times.
Figure 3: Mutation of the EXOC5 ciliary targeting sequence inhibits cystogenesis and tubulogenesis. (A) Control, EXOC5-overexpressing (OE), Exoc5 knockdown (KD), and EXOC5CTS-m cysts were grown from single cells in 3D collagen gels until the cyst stage. At day 10 the collagen gels were fixed and stained with nuclear DAPI (blue color), and Alexa Fluor 555 phalloidin that stains F-actin (red color). More mature single lumen cysts were found in EXOC5 OE cell cysts, compared to control cysts. In Exoc5 KD and EXOC5CTS-m cell cysts, no mature single lumen cysts were seen, and many “cysts” had no lumen at all. (B) Following addition of hepatocyte growth factor (HGF), MDCK cell cysts are induced to form tubules. Three days following the addition of HGF, more mature tubules (containing lumens) were seen in EXOC5 OE, compared to control, MDCK cell cysts. No tubules were seen in Exoc5 KD cell cysts, and fewer tubules were seen in EXOC5CTS-m, compared to control, MDCK cell cysts. For both A and B, N=100 cysts (or aggregations of cells) were counted for each cell line, with the experiment repeated three times.
Figure 4. Exoc5 mutant zebrafish display ciliopathy phenotypes and cannot be efficiently rescued by EXOC5 ciliary targeting sequence-mutated mRNA. (A) Lateral view of representative wild-type (exoc5+/+) and exoc5 homozygous mutant (exoc5−/−) zebrafish at 3.5 dpf. Exoc5 mutants showed cilia defects, including pericardial edema (arrowhead indicates pericardium), small eyes, and a curved tail. Scale bar=0.276 mm for all the zebrafish images. (B) By Western blot analysis, Exoc5 protein, normalized to glyceraldehyde-3-phosphate dehydrogenase (Gapdh) (a housekeeping protein), was almost undetectable in exoc5 mutant larvae, and Exoc4 protein, normalized to Gapdh, was also significantly decreased, when compared to WT siblings. The ratio of phosphorylated (active) Mob1 (pMob1) to total Mob1 (tMob1) was increased in the exoc5 mutant zebrafish. Quantification is shown below and next to the Western blot. y-axis=arbitrary units. (C) Injection of wild-type human EXOC5 mRNA rescued the exoc5 mutant phenotypes in zebrafish in a dose dependant manner, while human EXOC5 mRNA with a mutated ciliary targeting sequence (VxPx to VxAx) was unable to efficiently rescue the phenotypes, and mRNA for retinol binding protein receptor 2 (Rbpr2), a gene not known to interact with the exocyst or primary cilia, showed no rescue of exoc5−/− zebrafish (designated “Mock mRNA”). N=50 zebrafish for WT.EXOC5 mRNA injections, 60 zebrafish for the ciliary targeting sequence mutant EXOC5 human (Mut-h.EXOC5) mRNA, and 60 zebrafish for the Rbpr2 mRNA. All of the zebrafish were genotyped.
Figure 5. Model for how the exocyst is involved in ciliogenesis, and subsequently cystogenesis, tubulogenesis, and organogenesis. Genes are transcribed into mRNA in the nucleus, and mRNA is translated into proteins in the endoplasmic reticulum. Proteins destined for the primary cilium are packaged in vesicles in the trans-Golgi network, and trafficked to the primary cilium by the exocyst complex. Exoc5 is a central exocyst member as it links Exoc6 (bound to the vesicle via Rab8) and the rest of the exocyst complex. Primary cilia are necessary for generating cysts and tubules, which in turn, are necessary for generating the kidney, and involve the Hippo pathway.
The exocyst acting through the primary cilium is necessary for renal ciliogenesis, cystogenesis, and tubulogenesis
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