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The ciliary transitional zone and nephrocystins

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ABSTRACT

Loss of cilia and ciliary protein causes various abnormalities (called ciliopathy), including situs inversus, renal cystic diseases, polydactyly and dysgenesis of the nervous system. Renal cystic diseases are the most frequently observed symptoms in ciliopathies. Cilia are microtubule-based organelles with the following regions: a ciliary tip, shaft, transitional zone and basal body/mother centriole. Joubert syndrome (JBTS), Meckel Gruber syndrome (MKS) and Nephronophthisis (NPHP) are overlapping syndromes. Recent studies show that JBST and MKS responsible gene products are localized in the transitional zone of the cilia, where they function as a diffusion barrier, and control protein sorting and ciliary membrane composition. Nephrocystins are gene products of NPHP responsible genes, and at least 11 genes have been identified. Although some nephrocystins interact with JBST and MKS proteins, proteomic analysis suggests that they do not form a single complex. Localization analysis reveals that nephrocystins can be divided into two groups. Group I nephrocystins are localized in the transitional zone, whereas group II nephrocystins are localized in the Inv compartment. Homologs of group I nephrocystins, but not group II nephrocystins, have been reported in *C. reinhardtii* and *C. elegans*. In this review, we summarize the structure of the ciliary base of *C. reinhardtii*, *C. elegans* and mammalian primary cilia, and discuss function of nephrocystins. We also propose a new classification of nephrocystins.

1. Introduction

Cilia are tiny hair-like organelles extending from the cell surface (Fig. 1A). Each cilium has an axoneme, which is composed of a central pair of microtubules and nine outer doublet microtubules (Fig. 1B). Cilia that lack the central pair are called primary cilia. Longitudinally, a cilium can be divided into a ciliary tip, shaft, transitional zone and basal body/mother centriole (Wheatley, 1967). In addition to these sub-compartments, the primary cilium has a distinct molecular compartment within the proximal segment of ciliary shaft that had not been recognized by morphological examination. Since Inv/Nphp2 is accumulated in this region, this region is named the "Inv compartment" (Figs. 1 and 2) (Shiba et al., 2009). Primary cilia function as chemo-, mechano- and photo-sensors. Since ciliary function is versatile, loss of cilia and ciliary protein causes various abnormalities, including situs inversus, retinitis pigmentosa, renal cystic disease, polydactyly and dysgenesis of the nervous system (Hildebrandt et al., 2011). Abnormalities caused by ciliary defects, and defective ciliary proteins, are called ciliopathy. Among many abnormalities associated with ciliopathies, renal cystic diseases are the most common.

A renal cyst is a condition in which all or a part of the renal tubules are enlarged (Fig. 1C). Nephronophthisis (NPHP) is a recessive inherited renal cystic disease and the most common genetic cause of young adult or infantile end-stage renal diseases (ESRD) (Hildebrandt et al., 2009). Cysts commonly develop at the corticomedullary border of the kidneys. Eleven causative genes have been identified (Hildebrandt et al., 1997; Mollet et al., 2002; Olbrich et al., 2003; Otto et al., 2003, 2005, 2008, 2009, 2010; Sayer et al., 2006; Valente et al., 2006; Attanasio et al., 2007; Delous et al., 2007). Gene products of NPHP causative genes are collectively called naphrocystins. Causative genes for NPHP are also responsible for Joubert syndrome (JBTS), Senior–Loken syndrome (SLS), Meckel Gruber syndrome (MKS) and Bardet–Biedle syndrome (BBS) (Hurd and Hildebrandt, 2011). NPHP are classified according to the age of onset for ESRD: juvenile (NPHP1), infantile (NPHP2) and adolescent form (NPHP3) (Hildebrandt and Otto, 2000). However, it is becoming apparent that this phenotype–genotype correlation is not always preserved (Tory et al., 2009).

The connection between cilia and renal cystic diseases was first suggested in the tg737 mutant, in which an intraflagellar transporter, IFT88, is mutated (Pazour et al., 2000). Many causative genes for renal cystic diseases encode proteins localized in cilia or the basal

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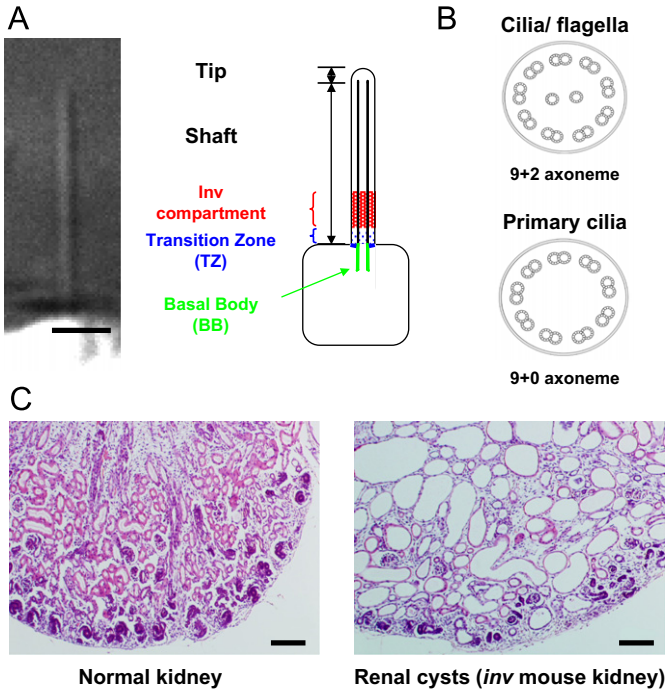

Fig. 1. Basic structures of primary cilia and renal cysts. (A) Renal primary cilia from the side (left panel). Primary cilia are structurally divided into the ciliary tip, shaft, transitional zone (TZ, green) and basal body (BB, blue). The Inv compartment (red) is localized at the proximal part of the ciliary shaft. The compartment is characterized as molecular accumulated region of Inv/Nphp2. Scale bar=2.5 μm. (B) Cross-section diagrams of cilia/flagella and primary cilia. Cilia/flagella are composed of a central pair of microtubules and nine outer doublet microtubules (9+2 axoneme), whereas primary cilia lack a central pair (9+0 axoneme). (C) Histological sections of a normal (left) and an *inv* cystic kidney mutant (right) at 1 week of age. Tissue sections were stained with hematoxylin and eosin. Scale bar=100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

body/centriole (Torres et al., 2007). Although many cyst-proteins that are localized in the cilium/centriole have been identified, their roles in renal cilia are still obscure. It has been proposed that a transitional zone, at the base of the cilium establishes a barrier between it and the cytoplasm, and that MKS and JBTS proteins are molecular components of the barrier (Garcia-Gonzalo et al., 2011). The ciliary base has a distinct structure and most nephrocystins (gene products of nephronophthisis) are localized in the base of cilia. Nephrocystins have been reported to interact with MKS and JBTS proteins (Garcia-Gonzalo et al., 2011; Sang et al., 2011). Thus, nephrocystins, interacting with MKS and JBTS, are presumed to function as a diffusion barrier at the transitional zone. This idea explains overlapping phenotypes seen in MKS, JBTS and NPHP. This review summarizes the structure of the ciliary base and discusses the diversity of nephrocystins. We try to divide nephrocystins into their intra-ciliary localizations, and propose a new classification of nephrocystins.

2. Structure of the ciliary base and its function

Eukaryotic cilia/flagella are highly conserved organelles. Their basic structure consists of nine peripheral doublets in the axoneme (Fig. 1B). The central portion of the axoneme is variable. Primary cilia, including mammalian renal cilia, lack the central pair of microtubules (9+0 axoneme) (Fig. 1B), which differs from the 9+2 axoneme in *Chlamydomonas* flagella (Fig. 2A) (Ringo, 1967; Wheatley, 1967). *C. elegans* sensory cilia contain 9 outer doublets and a variable number of inner single microtubules (Chalfie and Thomson, 1982) (Fig. 2A, right).

The transitional zone is the area between the ciliary shaft and basal body. The ciliary base structure has been best studied in *C. reinhardtii* (Ringo, 1967). In the transition zone of its cilia, the central pair of microtubules is lost, and triplet microtubules of the basal body change to the doublet microtubules in the axoneme (Fig. 2A middle). Although considerable variation among species and even tissues exists in this region, two common structures, Y-links/champagne glass structures and transitional fiber, are observed. The Y-links/champagne glass structures connect the peripheral doublets and ciliary membrane (Ringo, 1967; Gilula and Satir, 1972; Perkins et al., 1986; Gluenz et al., 2010). The transition fibers tether the distal portion of the basal body to the plasma membrane. The transition fiber is probably derived from the distal appendages of the mature mother centriole (Rohatgi and Snell, 2010). *C. elegans* lacks a classical basal body at the base of cilia (Fig. 2C). Initially, the *C. elegans* transition zone was thought to be analogous to the mammalian basal body (Perkins et al., 1986). However, recent molecular biological studies suggest that the basal body of *C. elegans* lacks discernable microtubules and may consist mainly of transition fibers (Williams et al., 2011).

The transition zone is defined by electron microscopic analysis. However, the transitional region is conventionally determined as the "gap" between acetylated α-tubulin staining (an axoneme marker) and γ-tubulin staining (a basal body marker) (Fig. 2B and C). Proteins accumulated in the region are called transition zone proteins.

At the transition zone of *Chlamydomonas* flagella, in addition to the 9 peripheral microtubule doublets, two cylindrical structures are present in the central area (Ringo, 1967). The cylindrical structures are not observed at the transition zone of mammalian primary cilia (Gluenz et al., 2010; Blair et al., 2011). In the region where Y-links/champagne glass structures exist, freeze-fracture electron microscopy has revealed the presence of the ciliary necklace (Fig. 2A) (Gilula and Satir, 1972). The ciliary necklace consists of several parallel strands of membrane particles that encircle the ciliary shaft.

Outer dense fiber 2 (Odf2) and centrosomal protein 164 kDa (Cep164) are localized at the distal appendage of the centriole and are essential for ciliogenesis in mammalian cells (Ishikawa et al., 2005; Graser et al., 2007). Immunoelectron microscopy indicates that *Chlamydomonas* IFT52 is associated with the periphery of the transitional fibers (Deane et al., 2001). This suggests that the transitional fibers form a docking complex for the IFT particles destined for the cilia/flagellum. Rosenbaum and Witman proposed that the transitional fibers form a pore complex similar to the nuclear pore (Rosenbaum and Witman, 2002). Several studies support the hypothesis that the transitional zone functions as a gate or a barrier for selective protein sorting in mammalian cells. Hu et al. reported that Sept2, which is localized between distal ends of the basal body and the proximal end of the axoneme, functions as a part of the diffusion barrier at the base of primary cilia (Hu et al., 2010). Furthermore, mammalian MKS and JBTS proteins, Tctn1, Tctn2 Cc2d2a and Tmem67, localize at the transition zone and regulate not only ciliary assembly but also ciliary membrane composition in mouse embryonic fibroblasts (Garcia-Gonzalo et al., 2011). MKS and JBTS responsible gene products appear to cooperate to establish a diffusion barrier or a ciliary gate in protein transportation at the transition zone.

3. Nephrocystins

All examined nephrocystins are localized in primary cilia, the basal body and/or centrioles (Otto et al., 2003, 2005, 2008, 2009, 2010;