Cystin, Cilia, and Cysts: Unraveling Trafficking Determinants

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The past 10 yr has seen an exponential increase in research into a previously often ignored organelle, the primary cilium. This is largely as a result of the discovery that ciliary dysfunction underlies many human inheritable disorders, termed ciliopathies.[1] These include polycystic kidney disease (PKD), nephronophthisis, and retinal degeneration. Cilia are microtubule-based organelles whose core components, the intraflagella transport proteins, are highly conserved evolutionarily from single-celled *Chlamydomonas* through to higher vertebrates, including humans.[2] They are found on many different cell types, and their function varies greatly depending on the cell type from which they emanate.

In renal epithelia, primary cilia protrude from the apical surface into the lumen of the nephron. Here, they are involved in the detection of fluid flow along the nephron. This is thought to occur by flow-induced bending of the cilium, which triggers calcium transients, an effect mediated through the polycystin 1/2 channel complex, both of the components of which are mutated in autosomal dominant PKD.[3,4]

Through the use of comparative genomics and proteomics, much has been learned about the gamut of proteins necessary for cilia formation and function; however, although it is well established that kinesins and dyneins mediate the trafficking of intraflagella transport rafts within the ciliary axoneme, much less is known about the mechanisms involved in the sorting and trafficking of cilia-destined proteins before entry into the cilia. Although there is a large body of evidence describing peptide motifs that direct the delivery of proteins to specific subcellular destinations, identification of ciliary trafficking determinants has proved somewhat elusive. Arguably the best understood mechanistically is the VxPx motif found in polycystin 2, CNGB1b, and rhodopsin that is necessary for targeting of these proteins to renal cilia, olfactory cilia, and photoreceptor outer segments, respectively.[5–7] In photoreceptors, this motif binds the small G-protein, Arf4, and in conjunction with Rab11, FIP3, and ASAP1 promotes budding of ciliary-destined cargo from the transgolgi network.[7]

In this issue of *JASN*, Tao *et al.*[8] identify a novel ciliary trafficking determinant in the cystoprotein cystin that furthers our understanding of how proteins are selectively targeted to the cilium. This group previously identified cystin as the gene disrupted in the cpk (congenital polycystic kidney) mouse model of autosomal recessive PKD.[9] Of particular interest is the previous observation that the cystin protein localizes to renal cilia[3]; however, little was known about the function of this protein or the mechanism by which it traffics to the cilium.

Sequence and domain analyses of cystin yield no sequence homology to other proteins, although it is predicted to be myristoylated at the amino-terminus. Tao *et al.*[8] test this hypothesis by *in vivo* labeling and indeed prove this is the case. Furthermore, they demonstrate this modification is necessary for association of cystin with membrane microdomains/lipid rafts. This later observation is of particular interest because the cilium is enriched in the raft-associated gangliosides GM1 and GM3 as well as the cholesterol-binding protein prominin1.[10] Furthermore, polycystin-1 has been demonstrated to co-fractionate with lipid raft markers.[11] In fact, proteomic analyses of cilia from *Chlamydomonas* revealed multiple myristoylated proteins are present in cilia, suggesting that association of proteins with lipid rafts may represent a prerequisite for entry of a subset of proteins into the cilium.[12]

Indeed, studies of the flagella in trypanosome demonstrate the necessity of myristoylation for entry of a number of flagella-localized proteins to the flagella. Of particular interest is the observation that detergent extraction of trypanosome flagella reveals detergent-resistant membrane patches approximating the size of intraflagella transport particles.[13] This finding suggests transmembrane proteins traffic in the cilium through their clustering into membrane microdomains coupled to core intraflagella transport machinery; however, Tao *et al.*[8] demonstrate that myristoylation is necessary but not sufficient to target cystin to the cilium, indicating a second trafficking determinant is required.

Through use of deletional analyses, the authors identify a short peptide sequence (AxEGG) that when fused to a myristoylated non–cilia-localized protein is able to traffic to the cilium. Furthermore, mutation of the sequence prevented localization of this protein to the cilium. The authors examined various cilia proteomes but were unable to detect this motif in other cilia proteins; however, further mutational analysis of individual residues and surrounding sequence may reveal a broader consensus sequence that can be applicable to other cilia proteins. It will also be of interest to examine whether the AxEGG motif, like the VxP motif of rhodopsin, also interacts with specific signaling modules necessary for formation of a cystin-containing cargo complex destined for delivery to the cilium.

What still remains unclear is whether all proteins require a ciliary localization signal within their sequence. This scenario seems unlikely, with a simpler model being the assembly of

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multiprotein cilia-destined cargo within the cell. Then through association of an adaptor molecule containing a cilia-targeting motif, perhaps such as cystin, multiple proteins deliver to the cilium. Further complexity in this model arises from the fact that not all proteins constitutively localize to cilia but require an external stimulus that promotes cilia entry. A good example of this is Hedgehog signaling. It was recently shown upon binding of Hedgehog to its receptor Patched, the protein Smoothened is targeted to the cilium[14]; therefore, multiple targeting motifs and adaptors likely regulate ciliary entry of proteins either in a constitutive manner or in response to a specific cue. Understanding these targeting signals will be key as we continue to unravel the mysteries of the cilia.

DISCLOSURES

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See related article, "Cystin Localizes to Primary Cilia *via* Membrane Microdomains and a Targeting Motif," on pages 2570–2580.

It's about Time: Extending our Understanding of Cardiovascular Risk from Chronic Kidney Disease

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It is widely accepted that chronic kidney disease (CKD) associates with accelerated cardiovascular disease and a higher rate of death than would occur otherwise.[1] These associations are based on studies of patients who had low renal function identified at a single point in time or, at most, two measurements separated by 3 mo to confirm chronicity. When caring for patients with CKD, we, too, generally estimate risk on the basis of single time point measurements of renal function. For example, a 65-yr-old man with a GFR of 80 ml/min per 1.73 m^2 has an estimated risk for death of 12% during the subsequent 5 yr; however, if his GFR is 40 ml/min per 1.73 m^2, then this risk is at least doubled.[1]

In this issue of *JASN*, Shlipak *et al.*[2] determined whether changes in kidney function during the first 7 yr of the Cardiovascular Health Study associated with increased cardiovascular risk during the subsequent 8 yr. The authors compared 1083 community-dwelling, ambulatory older adults with rapid declines in kidney function to 3295 adults without rapid decline. At baseline, participants were an average age of 72 yr, and 14% had diabetes. The incidence of cardiovascular disease was significantly higher in individuals with rapid declines in kidney function (defined as an annual decline in cystatin C–based eGFR >3 ml/min per 1.73 m^2), even after multivariable adjustment for demographics, aver-

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