Ciliary signaling goes down the tubes

James P. Calvet

Department of Biochemistry and Molecular Biology and the Kidney Institute, University of Kansas Medical Center, Kansas City, Kansas 66160, USA.
e-mail: jcalvet@kumc.edu

Published online 6 January 2002; doi:10.1038/ng1078

The focus of research on polycystic kidney disease (PKD) has recently shifted to the primary cilia of renal epithelial cells. A new study shows that the protein products of the genes mutated in PKD mediate mechanosensory calcium mobilization, suggesting that a disruption of fluid-flow sensing triggers abnormal cell proliferation and cyst growth.

PKD is an autosomal dominant disease that results from loss-of-function mutations in either *PKD1* or *PKD2* (ref. 1). The prevailing hypothesis is that these genes are akin to tumor-suppressor genes and thus require a second somatic hit to manifest the cellular phenotype—increased cell proliferation. The predominant pathology associated with this disease occurs in the kidneys, which become overwhelmed with fluid-filled, benign cystic tumors that are produced by abnormal renal tubular cell growth (Fig. 1). Although *PKD1* and *PKD2* are expressed widely in development and in many adult tissues, most organs of people with PKD seem normal. Some individuals, however, also suffer liver and pancreatic cysts, intestinal diverticula, cerebral aneurysms and heart valve defects.

PKD1 and *PKD2* encode polycystin-1 (PC1) and polycystin-2 (PC2), respectively, transmembrane proteins that directly interact with each other through cytosolic domains in the C termini of the two proteins. PC1 activates a number of signaling pathways and may be a receptor; PC2 is a transient receptor potential–related calcium channel. Although it is not yet clear how disruption of the polycystin protein complex gives rise to the abnormal cell proliferation phenotype, Surya Nauli and colleagues show in the accompanying article[2] that the polycystins are involved in sensing ciliary bending.

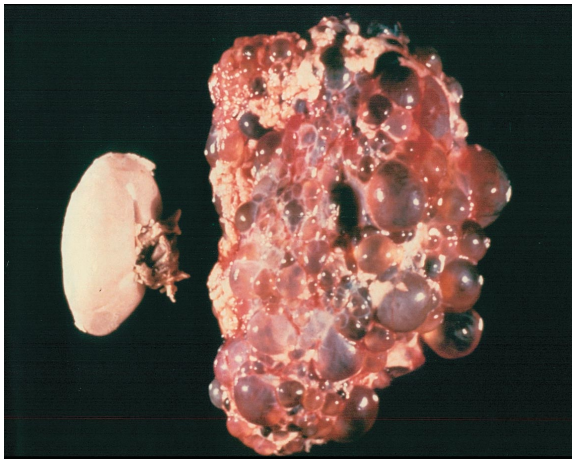

Fig. 1 End-stage human autosomal dominant polycystic kidney (right) compared with a normal kidney (left). Note the very large size of the polycystic kidney, due in part to the presence of numerous large, fluid-filled cysts. Photograph courtesy of A. Evan and J. McAteer.

Cilia everywhere!

Recent studies of a variety of animal models have identified a possible role for primary cilia in PKD. A number of groups have reported the involvement of genes associated with PKD in either the assembly or function of cilia in mice and *Caenorhabditis elegans* and of flagella in *Chlamydomonas*. The story began a number of years ago with the discovery of the mouse gene *TgN737Rpw* in an insertional mutagenesis screen. The original hypomorphic mutation was found to cause a recessive form of PKD in orpk mice, and the null mutation was found to cause embryonic lethality and an absence (or severe stunting) of cilia on ventral node epithelial cells[3], disrupting early morphological left–right axis determination[3]. The product of this gene, polaris, was localized in the axoneme and basal body of primary cilia and was required for ciliary assembly[4]. The spontaneously arising, recessive cpk mouse model for PKD was also linked with cilia. The gene *Cys1*, mutated in cpk mice, encodes cystin-1, which has now been localized in the axonemal region of primary cilia, partially overlapping with polaris[5].

Parallel work in *C. elegans* identified orthologs of *PKD1* (*lov-1*, for location of vulva) and *PKD2* (*pkd-2*), whose protein products were found specifically in the cilia of male sensory neurons[6] co-localizing with OSM-5, an ortholog of polaris[7,8] and IFT88, an intraflagellar transport protein of *Chlamydomonas*. Mutations in *lov-1* or *pkd-2* seemed to affect ciliary function, whereas the *osm-5* (*osm*, for osmotic avoidance) mutations affected ciliary structure. These discoveries prompted researchers to question whether the mammalian polycystins reside in the cilia of renal epithelial cells; this was then confirmed by several groups[9,10] and most recently by Nauli *et al.*[2] in the accompanying study.

Sensing lumenal flow rates

Nauli *et al.*[2] went on determine whether ciliary polycystins have a mechanosensory function by showing that fluid-flow ciliary bending can stimulate transient intracellular calcium increases in epithelial monolayers. These calcium transients were dependent on both extracellular and intracellular calcium pools, and did not occur in PC1-null cells or in cells treated with antibodies blocking PC1 or PC2. Thus, ciliary polycystins seem to be responsible for mechanosensory calcium signaling. How this elevated calcium affects cell function is still not known; it was speculated, however, that flow-sensing may regulate intratubular lumenal diameters and that a defect in this system might lead to compensatory growth of the tubular cells in a futile attempt to overcome a perceived lack of flow (Fig. 2). A possible candidate for a polycystin-regulated gene target is *CDKN1A*, encoding the cyclin-dependent kinase inhibitor p21. This gene has been shown to be upregulated by PC1 and PC2 in a JAK2–STAT1–dependent fashion, presumably to inhibit cell proliferation in the normal state, but when the polycystins are inactivated by mutation, cells are released from growth inhibition[11].

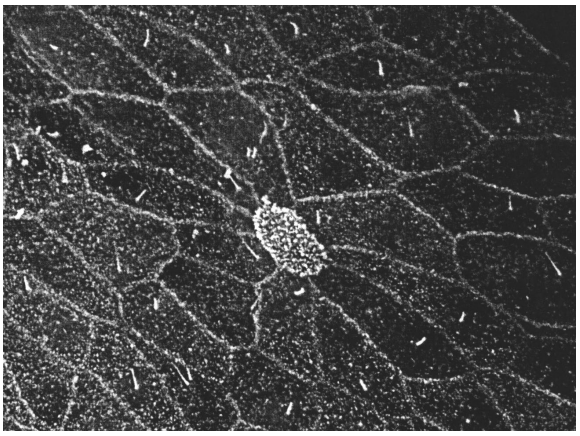

Fig. 2 Scanning electron micrograph of the inside of a collecting-duct cyst from a human autosomal dominant polycystic kidney. This region of the cyst wall shows a single intercalated cell in the center of the figure surrounded by principal cells, each with one (or several) primary cilia (white spikes). Although the cilia on these cells appear normal, they are presumably defective because of the mutation in *PKD1* or *PKD2*. Reprinted with permission from ref. 15.

Though this may seem to be a tidy explanation for the initiation of cyst growth, a connection between calcium mobilization and the JAK2–STAT1–p21 pathway is not immediately obvious.

Other functions?

A further complication is that a number of studies have localized PC1 and PC2 to lateral membrane junctional complexes, in particular to desmosomes, thus implicating the polycystins in cell–cell communication[12]. Furthermore, the bulk of PC2 is found in cytosolic membranes, probably those of the endoplasmic reticulum, and there is direct evidence that PC2 acts as a calcium-activated calcium-release channel requiring activation of the $InsP_3$ receptor[13]. The work by Nauli *et al.*[2], in contrast, argues that ciliary PC2 acts as a calcium-entry channel, triggering calcium release from intracellular stores in a ryanodine receptor–dependent, but $InsP_3$ receptor–independent process. Thus, there are knotty questions about where the polycystins function in the cell and what they are doing.

Integrating calcium signaling

One possible explanation for the above observations is that the polycystins do different things in different settings. For example, evidence is accumulating that PC1 is a G protein–coupled receptor[12], which can activate heterotrimeric G proteins and release Gβγ subunits that can then inhibit calcium-channel activity[14], among other activities[14]. Yet, the study by Nauli *et al.*[2] shows that the polycystin-dependent calcium increase does not require G-protein signaling. Thus, it is possible that the ciliary polycystins provide a calcium signal in response to mechanosensory stimulation, but that interpretation of this signal requires input from other polycystin-activated pathways and, ultimately, the integration of multiple signals into an appropriate cellular response. The transformation of a renal epithelial cell into a hyperproliferating (yet somewhat differentiated) cyst epithelial cell will probably involve changes in gene expression that may reflect aberrant calcium mobilization. If so, there will certainly be other affected pathways. Defining these pathways and their gene targets will be the next challenge. □

1. Igarashi, P. & Somlo, S. *J. Am. Soc. Nephrol.* **13**, 2384–2398 (2002).
2. Nauli, S.M. *et al. Nat. Genet.* **33** (2003); advance online publication, 6 January 2003 (doi:10.1038/ng1076).
3. Murcia, N.S. *et al. Development* **127**, 2347–2355 (2000).
4. Yoder, B.K. *et al*. Am. J. Physiol. Renal Physiol. **282**, F541–552 (2002).
5. Hou, X. *et al. J. Clin. Invest.* **109**, 533–540 (2002).
6. Barr, M.M. & Sternberg, P.W. *Nature* **401**, 386–389 (1999).
7. Qin, H., Rosenbaum, J.L. & Barr, M.M. *Curr. Biol.* **11**, 457–461 (2001).
8. Haycraft, C.J. *et al. Development* **128**, 1493–1505 (2001).
9. Pazour, G.J. *et al. Curr. Biol.* **12**, R378–380 (2002).
10. Yoder, B.K., Hou, X. & Guay-Woodford, L.M. *J. Am. Soc. Nephrol.* **13**, 2508–2516 (2002).
11. Bhunia, A.K. *et al. Cell* **109**, 157–168 (2002).
12. Scheffers, M.S. *et al. Hum. Mol. Genet.* **11**, 59–67 (2002).
13. Koulen, P. *et al. Nat. Cell Biol.* **4**, 191–197 (2002).
14. Delmas, P. *et al. J. Biol. Chem.* **277**, 11276–11283 (2002).
15. Schrier, R.W. & Gottschalk, C.W. (eds.) *Diseases of the Kidney* 6th edn. vol. I. 529 (Little Brown and Co., Boston, 1997).