Unexpected Discoveries, Unsuspected Connections: The Case of the Primary Cilium and Kidney Disease

Greg Pazour

It took green algae, lab mice, and 40 years of basic research into an unexciting cell appendage to turn medical orthodoxy on its head. The unexciting cell appendage was the primary cilium, a small hair-like structure that protrudes, much like an antenna, from virtually every cell in the human body. Found in everything from one-celled eukaryotes to multicellular mammals, cilia come in two types—motile cilia and nonmoving "primary" cilia. Medicine had long recognized the importance of motile cilia (and structurally identical but typically larger flagella). For example, sperm propel themselves with motile flagellar tails. And cells lining our airways wave cilia to sweep out dust and foreign bodies. Yet primary cilia in organs like the kidneys didn't seem to do much of anything. Biologists decided that they were vestigial organelles, leftover evolutionary baggage like the appendix or wisdom teeth. Then in 2000, the green alga *Chlamydomonas* and a mutant mouse revealed that defective primary cilia were behind a deadly human disease, polycystic kidney disease (PKD).

The discovery that defective cilia are linked to PKD and photoreceptor degeneration was just the beginning of a scientific land rush to identify what are now known as "ciliopathies."

At first, it seemed a far-fetched connection. PKD is a genetic disorder that affects more than 600,000 Americans and 12.5 million people worldwide. Half of those diagnosed with PKD will progress to end-stage renal disease by the age of 60. PKD is characterized by the growth of multiple cysts in the epithelial tissue that lines the nephrons, the basic functional units of the kidney that filter blood and leave waste products behind to be expelled in urine. As cysts expand, the nephrons are destroyed and kidney function declines.

But what triggered cyst growth in PKD was a mystery. Genetics researchers had identified two major genes associated with the disorder by studying families with PKD. Yet no one knew what the PKD genes did or didn't do in kidney epithelial cells to cause cystic disease. It was then that cell biologists who had been studying flagella in *Chlamydomonas* posed their radical theory: Could PKD be caused by defective primary cilia?

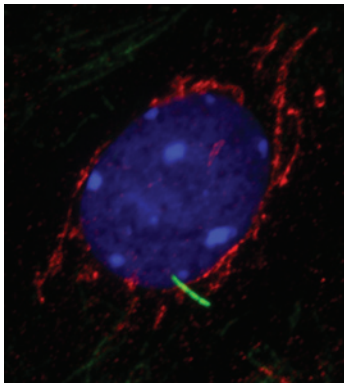
The Importance of Being Chlamy

Chlamydomonas, or "Chlamy" to its laboratory fans, is prized for the study of cilia because of its low cost, quick growth, and versatility for both genetic and biochemical studies. By breeding Chlamy mutants lacking in essential flagellar proteins, researchers identified an intraflagellar transport protein (IFT) that was absolutely necessary for building cilia in Chlamy. The researchers—Joel Rosenbaum of Yale University, George Witman and Greg Pazour of the University of Massachusetts Medical School at Worcester, and Doug Cole, now at the University of Idaho, Moscow—compared their Chlamy IFT protein to mouse and human proteins. They found a match to a gene in mouse called Tg737^{orpk} that causes PKD.

Pazour recalls, "We knew that kidneys had [primary] cilia and we thought that they must be important because they'd been around for almost two billion years. We knew from studies in Chlamy that it was not simple to build a cilium and felt that if they were vestigial organelles, they would have been lost by now." Mice carrying the Tg737^{orpk} mutation died shortly after birth from PKD. Pazour and his collaborators showed that these mice had defective primary cilia in their kidneys. This finding ended the speculation that primary cilia were vestigial organelles.

Yet this demonstration that mouse PKD—and probably human PKD as well—was caused by defective primary cilia was greeted with skepticism by medical experts. How could a nonfunctional organelle like a primary cilium

"I think this is one of the greatest examples of basic science having impact," says Peter Satir.



The primary cilium (green) projects from a wild-type epithelial kidney cell growing in cultured mouse tissue. Once dismissed as a useless evolutionary artifact, the primary cilium is now considered a prime factor behind inherited polycystic kidney disease (PKD). Image courtesy of John Follit and Greg Pazour.

control a complicated disorder like PKD? It took two years and more evidence for experts to concede that PKD was a disease of defective cilia.

Meantime, Pazour and colleagues widened the field beyond kidney cysts. They linked defective cilium transport to the degeneration of retinal photoreceptors. The outer segment of the photoreceptor, which is the light-sensing part, develops from a cilium. Massive amounts of transport are needed to build the outer segment and also to maintain it, because 10% of the discs are regenerated each day. It turns out that the same transport mechanism that builds a kidney cilium also builds an outer segment. Pazour and colleagues showed that the Tg737^{orpk} gene product, IFT88, which localizes to photoreceptor cilia in mouse retinal cells, is critical to regeneration. Knocking out IFT caused photoreceptor degeneration and led to blindness.

The scientific gold in a basic laboratory bench discovery like ciliopathy is in what it reveals about disease *mechanism,* that is, what exactly goes wrong in cells.

Bardet-Biedl Syndrome

The discovery that defective cilia are linked to PKD and photoreceptor degeneration was just the beginning of a scientific land rush to identify what are now known as "ciliopathies." Researchers found links between defective cilia and a wide range of disorders such as heart malformations, male infertility, reversed left–right organ symmetry, and extra digits. The list continues to grow. Cilium defects are now linked to a raft of rare genetic conditions including Bardet-Biedl syndrome (BBS), a suite of devastating birth defects that can leave BBS children with impaired kidney function, reduced mental capacity, and uncontrollable obesity. Some BBS children born with normal vision lose their night vision by the age of eight and go completely blind within a decade. In studying these rare genetic disorders, researchers are always looking for unexpected connections to more common disorders. The link to obesity in BBS has already led to work on the role of cilia in signaling the feeling of satiation and to speculation about their role in addiction.

"I think this is one of the greatest examples of basic science having impact," says Peter Satir, a pioneer in flagellar research who now works on ciliopathies at the Albert Einstein School of Medicine in the Bronx. "Ten years ago, if you said to people that I have [government] funding to work on a single-cell green alga because I think it's interesting and I think it might have impact some day on human health, you would have been awarded one of those Golden Fleece awards."

The scientific gold in a basic laboratory bench discovery like ciliopathy is in what it reveals about disease *mechanism,* that is, what exactly goes wrong in cells. Once they have a good idea of the mechanism, drug researchers can scour chemical libraries for substances that might rescue lost function or at least slow down a degenerative condition like PKD where most carriers have few symptoms of cystic kidney failure until middle age.

But moving from bench to bedside can be a tortuous process. The ciliopathy field is nine years old and its insights into mechanism have yet to be "translated" into clinical treatments for people. But they are coming closer, especially for treatment of PKD, a disease with great human impact, believes Philip Beales of University College London and the Great Ormond Street Hospital for Children. According to Beales, the same basic laboratory approaches that revealed ciliopathies can also speed up the search for new medicines, sometimes in old bottles. Alongside the alga and the mouse, Beales has recruited a new model organism to the search, *Danio rerio*, a transparent, freshwater tropical minnow, more commonly known as the zebrafish.

A Model Fish

Beales created a zebrafish model of cystic kidney disease by using antisense morphilinos, short oligonucleotide molecules that can precisely switch off any gene in the fish's genome. The evidence now points to ciliopathies as a breakdown in cell signaling, with the cilium acting like a cellular flagpole sticking out from the cell. The IFT-related proteins are needed to hoist receptor molecules into place on the cilium to send or receive messages from other cells. Beales scoured the literature for drugs that affect intracellular signaling pathways, looking especially closely at immune suppressants. Commonly used to dampen tissue rejection after organ transplantation, one of these drugs was already approved by the U.S. Food and Drug Administration as safe for human use. Promising drug candidates in Beales' zebrafish experiments could thus go to clinical testing quickly. Beales theorized that immune suppressants would turn

off defective cell signaling and keep PKD kidney cells from proliferating into cysts.

With their see-through bodies, the PKD zebrafish quickly revealed the impact of each drug, allowing the Beales lab to scan 10 drugs in a matter of weeks. The results were surprising. One of the oldest immune suppressing drugs, rapamycin, stopped cyst formation in its tracks, rescuing kidney function in the PKD zebrafish. While Beales continues his exploration of cilium-related signaling pathways in his fish, other researchers have already tested rapamycin in mouse PKD models to great effect. "It could be just a few years before this goes into human trials," says Beales "but it does show how the fish provide a rapid means to assess existing drugs or potentially to screen whole chemical compound libraries." The therapeutic idea is to slow down PKD, says Beales. "If we can intervene in these pathways (at an early stage), we could slow or even possibly prevent the cysts from developing. The thing about renal disease in the most common form of PKD is that progression is insidious, sometimes occurring over 20–30 years. If you could slow it down, perhaps you could give people another 10 years of high-quality life. That would be quite an achievement."

Basic research can change the therapeutic game, says Satir, who sees the cilium connection revealing new dimensions in heart development, cell migration, and addiction. In his own work with Søren Christensen of the University of Denmark, Satir is exploring yet another role for the primary cilium as a "cellular GPS," directing wound-closing cells into position. A decade ago, a researcher suggesting such a role for an unexciting cell appendage would have been laughed at. Satir remembers, "When Joel [Rosenbaum], Greg [Pazour], and George [Witman] first presented this work, for two years they had kidney people pushing them to back down, saying this was ridiculous. The experts all knew the key ingredient in PKD and it wasn't the cilium. And then it became clear that it was the cilium. Then everyone in kidney research went wild over this."

Where this unexciting cell appendage will take cell biology and clinical medicine next is anybody's guess. "That's what makes it so exciting to be in this field now," says Beales. ■

—John Fleischman

"The experts all knew the key ingredient in PKD and it wasn't the cilium. And then it became clear that it was the cilium. Then everyone in kidney research went wild over this."