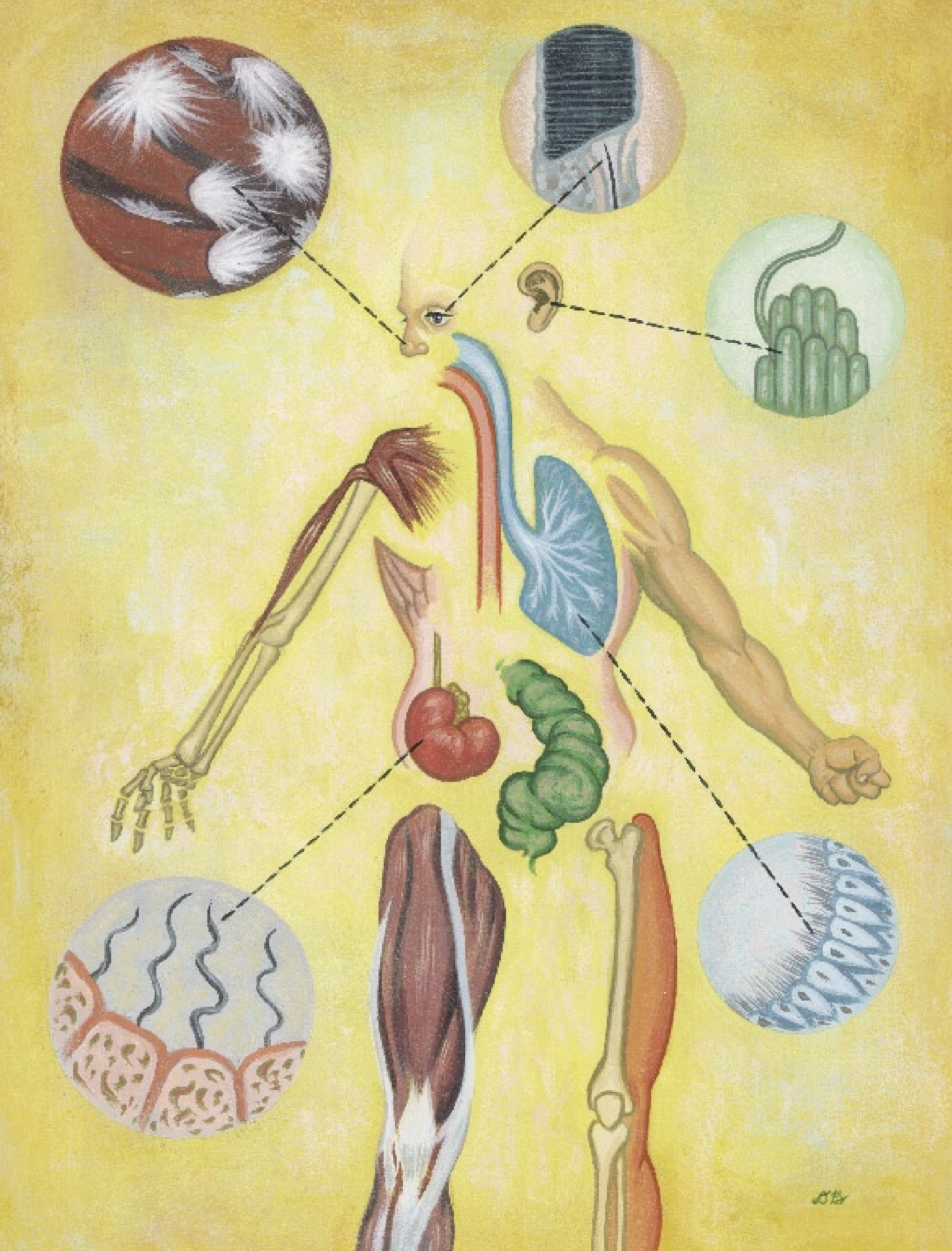


BY MARY BETH GARDINER

Illustration by David Brinley

The Importance of Being CiLiA

Once considered merely a vestige of evolution, cilia are in fact essential to many of the body's organs. As researchers learn more about cilia's role in disease, they're starting to pay this once-ignored organelle much more attention.

On the surface of nearly every cell in the body is a slender protuberance called the primary cilium. Although ubiquitous, the primary cilium was long considered—with a few exceptions—to be a largely useless evolutionary vestige, destined to go the way of the tailbone and the wisdom tooth. But now we know that cilia are functioning organelles, essential to normal development and health. • Some cilia are rigid spikes that act as antennae, gathering sensory information for the cell from the surrounding environment. Other cilia are flexible and whip-like, capable of registering the surrounding fluid's flow and ebb. • Scientists have recently implicated malfunctioning cilia as factors in a number of diseases. One of the most devastating among them is the heritable Bardet-Biedl syndrome (BBS)—a rare disorder, involving multiple organ defects, that results in obesity, retinopathy, polydactyly (more than five digits on the hands or feet), kidney disease, and mental retardation, among other problems. HHMI investigator Val C. Sheffield at the University of Iowa Carver College of Medicine is among the scientists trying to get at the genetic origins of the disorder. So far, at least eight genes have been tied to BBS, several of which Sheffield's lab identified, and all of them have been linked to ciliary function. • But it was another malady affecting the kidney—polycystic kidney disease, or PKD—that fed the current surge of interest in cilia and what they do. It began when a group of scientists saw something in common between a mouse and a single-celled plant.

CRUCiAL RAiLWAYS

• **Primary cilia were first** described in 1898. For the next hundred years or so cell biologists largely ignored them, but microscopists continued to document their presence in the cells of most vertebrate organisms. It was generally believed that the nonmotile cilium was either a sensory organelle, because of its presence in the nose and eye, or that it no longer served any purpose. Understanding the role of the motile cilium and the flagellum (a structure nearly identical to the cilium) was easier. They provide movement, as in sperm and in the lungs and trachea of the respiratory tract.

In the 1990s, researchers began to understand more about the internal workings of cilia and flagella, and how cargo moves up and down the microtubular tracks within them. During such intraflagellar transport (IFT), large protein complexes are carried to the ciliary tip and then back to the cell body. "You can think of the IFT particle as the equivalent of railroad cars," says Gregory J. Pazour, a researcher at the University of Massachusetts Medical School. "It carries materials needed to build the cilia and returns with spent materials." Pazour suspects that the IFT particle, which is made up of at least 17 polypeptide subunits, may also carry signals—messages collected by various receptors embedded in the ciliary membrane—back from the tip.

During the late 1990s, Pazour partnered with Joel Rosenbaum at Yale University, Douglas Cole at the University of Idaho, and George Witman at the University of Massachusetts Medical School to purify and sequence subunits of the IFT particle isolated from the unicellular alga *Chlamydomonas*. In October 2000, the *Journal of Cell Biology* published their finding that one of the alga particle's subunits—termed IFT88, or polaris—is encoded by a gene that is homologous to the mouse and human gene *Tg737*. They observed that mutant *Chlamydomonas* lacking the *IFT88* gene are normal—except for the absence of flagella. And, it turns out, mice with defects in *Tg737* die shortly after birth from PKD.

The evidence suggested that IFT is important for primary cilia assembly and that defects in ciliagenesis in the kidney can lead to PKD. So the researchers got the defective mice, publicly available from the Oak Ridge National Laboratory, and looked at their kidneys.

"As we predicted," says Pazour, "the kidney cilia were aberrantly formed. This evidence laid to rest the idea that kidney cilia had no function—that they were vestigial organelles. That was pretty exciting for us."

Intraflagellar transport, known to be essential for the assembly and maintenance of cilium, may have far-reaching effects that researchers are only just beginning to discover.

POLARiZATION MATTeRS

• **Within our kidneys,** the dense winding ductwork that carries urine to the bladder is lined with cuboidal epithelial cells. In PKD, the kidney enlarges and fills with strangulating cysts thought to result from an overproliferation of those epithelial cells. Could it be that malfunctioning cilia on those cells play a causative role? It's beginning to look that way.

Ben Margolis, an HHMI alumni investigator at the University of Michigan, is a nephrologist who studies polarization of epithelial cells in the kidney. He's interested in knowing why those cells lining the ducts are specifically oriented so that the apical side, where the cilium is located, is exposed to urine flow while the opposite, or basolateral, side is exposed to blood.

"Cilia must point into the urine space," says Margolis. "The model is that urine flow bends the cilia, which send some kind of signal to tell the cell there's flow, and that this somehow inhibits cyst formation. Nobody is clear exactly how that happens."

Margolis became intrigued with the role of cilia in cyst formation through discussions with fellow University of Michigan researcher Friedhelm Hildebrandt, who studies a cystic disease in children called nephronophthisis, which is a much rarer condition than PKD but one that appears to be cilia-dependent.

Taking a closer look at one of their apical polarity proteins—called Crumbs—Margolis was surprised to see how it distributed itself. "It was on the apical surface and then it seemed to enter into the cilia," he says. "And it wasn't just *in* the cilium; it occurred in discrete punctate spots, which is consistent with the intraflagellar transport process in which seemingly large particles of material are moving along the cilium."

When Margolis knocked out the gene for Crumbs3 in cultured kidney cells, no cilia formed. And he found similar results with another family of proteins—called Par—that controls apical/basolateral polarity. Interestingly, Margolis points out, the *Crumbs1* gene (closely related to the *Crumbs3* gene) is linked to retinitis pigmentosa, a disease associated with defects in intraciliary transport. "We feel that the Crumbs protein can somehow regulate or have an important role in cilia formation," he says. "We're working to identify what that role is."

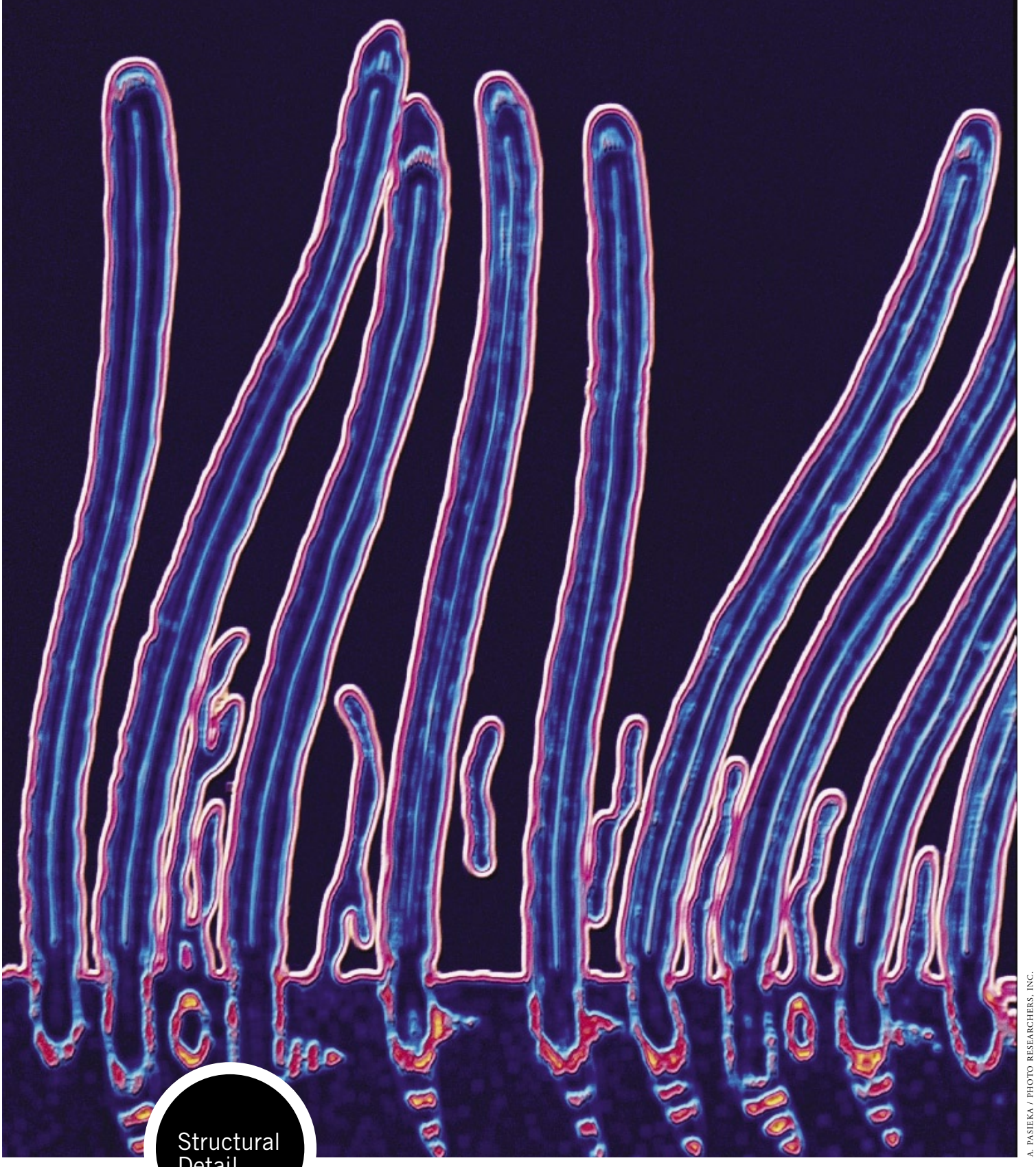
PINPOiNTING THE GeNES

• **Iowa researcher Sheffield** first started studying the genetics of BBS in 1993. Analyzing the DNA from three large families of Bedouin Arabs with BBS-like symptoms, Sheffield's lab came up with a surprising result: Each of the families mapped to a different place in the genome. "That told us this was a genetically heterogeneous disorder, which other groups' work has since confirmed," says Sheffield. "To make a long story short, eight genes at eight different loci have been identified, and five of those came out of our laboratory."

He thinks the number of BBS genes won't stop at eight, and the search continues in his and other labs. Meanwhile, there is the question of what unifying disease mechanism might link all these genes. By applying the power of bioinformatics, scientists have identified at least one common thread: cilia.

Sheffield's lab and others compared the sequences of human BBS genes to genome sequences from other organisms—from algae to higher plants, insects, fish, and mice—in search of similarities. They found that BBS genes are conserved in organisms with cilia but not in nonciliated organisms.

Charles S. Zuker, an HHMI investigator at the University of California, San Diego, was one of the first to carry



A. PASIEKA / PHOTO RESEARCHERS, INC.

Structural Detail

Inside cilia and flagella is a microtubule-based cytoskeleton called the axoneme. The axoneme of primary cilia typically has a ring of nine outer microtubule doublets (called a 9+0 axoneme), and the axoneme of a motile cilium has two central microtubule doublets in addition to the nine outer doublets (called a 9+2 axoneme). The axonemal cytoskeleton acts as scaffolding for various protein complexes and provides binding sites for molecular motor proteins, such as kinesin II, that help carry proteins up and down the microtubules. • At the base of the cilium is the microtubule organizing center, also called a basal body, which is created as the centriole (a microtubular structure essential to cell division) migrates to the surface. The transition zone between basal body and axoneme serves as a docking station for intraflagellar transport and motor proteins. • Cilia and basal bodies have been implicated directly in a number of developmental processes, including left-right asymmetry, heart development, maintenance of the renal epithelium, respiratory function, electrolyte balance in the cerebrospinal fluid, and reproductive fecundity.

out a whole-genome subtraction study. Zuker's postdoc, Tomer Avidor-Reis, crafted the analysis, which identified nearly 200 conserved ciliary genes encoding both known and candidate ciliary proteins. Using the fruit fly *Drosophila* as a model system, the lab then investigated the function of some of the candidate proteins, at least two of which were suspected to derive from BBS genes.

Another comparative study, this one led by Susan K. Dutcher, a researcher at Washington University School of Medicine, looked at the genes and proteins of humans, *Chlamydomonas*, and a weedy plant. The researchers subtracted all genes found in the weed from the combined genomes of humans and algae, leaving them with a set of 688 genes found exclusively in organisms with cilia or flagella. From this set, they identified a novel BBS gene, *BBS5*, and showed that the protein product from that gene localizes to the "basal body"—the point, underlying the cilium, from which its microtubule railways originate—in the mouse and in the worm *Caenorhabditis elegans*.

"Though none of the precise functions of the BBS gene products are known, it is clear that cilia dysfunction is involved in some of the phenotypic characteristics," says Sheffield. Male patients have infertility problems, for example, and in the BBS mouse model, sperm are missing flagella. Another phenotype of BBS, blindness, is also cilia-dependent. Humans and mice with BBS see fine at birth, but in time the photoreceptor cells within the retina degenerate. A key component of photoreceptors, it turns out, is a structure called a connecting cilium.

"What we think is going on is that there is abnormal transport—intraciliary transport—related to the connecting cilia," says Sheffield. "That eventually leads to the dysfunction of those photoreceptor cells and ultimately to their death."

SECReTS OF CHuBBY MiCE

• **Intraflagellar transport,** a process now known to be essential for the assembly and maintenance of the cilium, may have far-reaching effects that researchers are only just beginning to discover. What if, for example, IFT is responsible for delivering receptor proteins to the membrane that encases the cilium? The University of Massachusetts' Pazour, who sees the cilium as a sensory antenna, says he is putting a lot of energy into learning how proteins are localized to the ciliary membrane and whether any of the IFT proteins play a role. "We're searching for targeting sequences in those proteins," he says, "looking for the 'address' that directs them to the ciliary membrane. If we can get the address, maybe we can work backwards from that point."

Ciliary membrane proteins also play into Susan Dutcher's favorite working model for why obesity occurs in BBS patients and BBS mouse models, although she's quick to say the work hasn't gotten very far.

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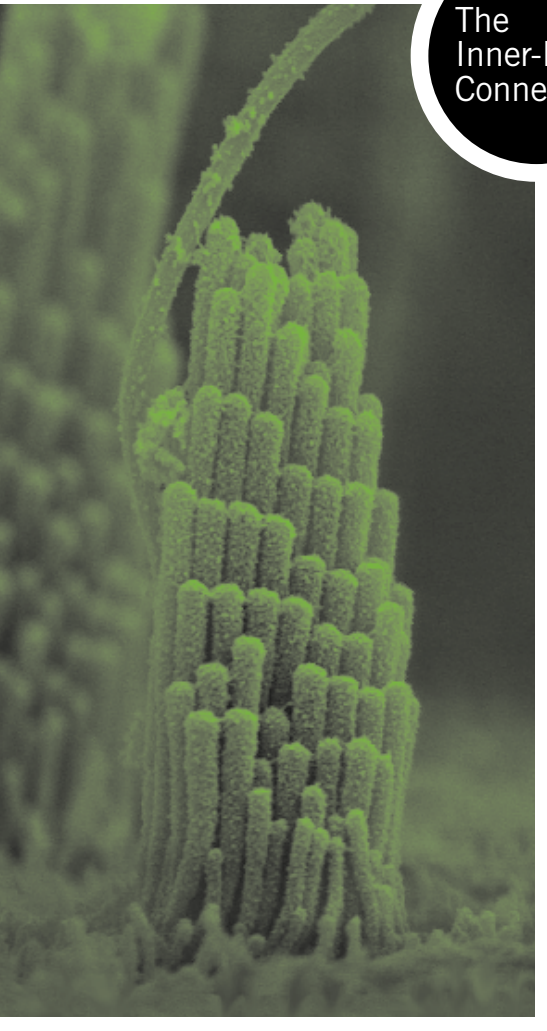

PHOTO COURTESY OF BECHARA KACHAR/NIH; COLORIZED SEM

The Inner-Ear Connection

In mammals, sound is detected by hair cells of the snail-shaped cochlea, which form a long, spiraling ribbon of sensory epithelium. At birth, each hair cell has a single true cilium, called the kinocilium, and each kinocilium is associated with 100 to 200 stereocilia—appendages that bundle together and jut from the top surface of each hair cell into the cochlear fluid. Stereocilia are not true cilia—they are instead microvillae, based on the structural protein actin, with no tubulin (the protein that makes up microtubules) present. Stereocilia vary in height, becoming progressively shorter the farther they are from the long kinocilium at the edge of the bundle. • Sometime after birth, the kinocilium is lost from the hair cell in the mammalian cochlea. Although the precise role of the kinocilium remains unclear, it is believed essential to proper development of the stereocilia. Adding to the mystery is the fact that the kinocilium persists in other parts of the ear and in lower vertebrates. • "We know it's not necessary for [signal] transduction, but we can't say why it survives elsewhere in the body," says HHMI investigator A. James Hudspeth, a researcher at the Rockefeller University who has been studying signal transduction in hair cells for decades. "The only hint we have is that when the hair bundle is formed, the kinocilium develops first, and it always moves to one edge of the cell. It seems to set up the axis along which the stereocilia are subsequently polarized. But the evidence for that is still circumstantial." • Signal transduction in hair cells involves movement of the stereocilia in the direction of the kinocilium—or rather, where the kinocilium was. The mechanical action of the cilia bending somehow triggers a signal necessary to hearing. The long-held suspicion is that spring-like "tip links" extending between adjacent stereocilia physically tug open ion channels when hair bundles are deflected by sound or movement. Hudspeth and several other HHMI investigators are determined to figure out how that happens. • David P. Corey, an HHMI investigator at Harvard Medical School, has identified a protein at the tips of stereocilia—called TRPA1—that is a candidate for the mechanically sensitive channel in hair cells. "Currently, we're carrying out a number of tests, including making a knockout mouse, to see whether it's doing what we think it's doing," he reports. • Corey says TRPA1 is very similar in structure to the nompC (no mechanoreceptor potential, type C) protein discovered in the laboratory of HHMI investigator Charles S. Zuker at the University of California, San Diego. In 2000, Zuker identified the protein, which is also a member of the

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"When you look at these kids with BBS and you look at Val Sheffield's BBS4 mouse, it looks like they are probably overeaters, that they lose their appetite control," says Dutcher. "One of the things we're doing is to ask whether the leptin receptor, which is involved in appetite control, is actually localized to ciliary membranes." If that is the case, she reasons, if the cilia don't get built, the leptin receptor isn't present and the "hunger satisfied" message is never received.

Meanwhile, the "tubby" (*tub*) genes tantalize the Dutcher lab. Tubby is a mouse mutant discovered almost 15 years ago that displays some of the same characteristics—obesity and retinopathy, primary among them—as seen in BBS. Yet the role of the tubby genes in the overweight mouse remains unclear. *Chlamydomonas* has three tubby genes, and through its comparative genomics study, the lab found that two of the three tubby genes encode ciliary proteins. Now, the researchers are asking what those proteins do in the alga and how in the world that function might relate to what happens in mammals.

Sheffield, who is a physician as well as a researcher, is also very interested in understanding cilia's contribution to his chubby mice. "It's intriguing, really," he says. "None of us would have thought that cilia would be involved in something like obesity. I'm still trying to figure out how that one happened." ■

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[THE IMPORTANCE OF BEING CILIA, INNER-EAR CONNECTION SIDEBAR]

TRP family of ion channels and is now called TRPN1, in the sensory bristle of the fruit fly *Drosophila*. Interestingly, the bristle's composition is that of a true cilium: tubulin-based rather than actin-based. Corey has found TRPA1 in mouse hair cell kinocilia as well, perhaps suggesting further evolutionary conservation across species. ● Teresa Nicolson, an HHMI investigator at Oregon Health & Science University, studies mutant zebrafish that swim in circles—a classic sign of inner-ear dysfunction. When she learned of Zuker's finding in the fly bristle, she thought it might be the same transduction channel as the one in hair cells. She was excited to find a version of the TRPN1 channel expressed in zebrafish hair cells. ● When her lab knocked out the gene in zebrafish, they saw deafness and balance defects in the mutants. In addition, electrical potential in the channels disappeared. "The results suggest that the TRPN1 channel could be the transduction channel in zebrafish hair cells," says Nicolson. "Now we are trying to show it is actually in the right place to be doing that job." ● Nicolson's lab also has a bead on the tip link protein. Her candidate, cadherin 23, has a very long extracellular domain that could serve to physically link the stereocilia tips. Labeling experiments have localized cadherin 23 to the tips of the bundles, and mutants lacking the protein have no tip links. They are also deaf.