# THE HEART OF THE MATTER

**CECILIA LO STALKS THE MOLECULES THAT SHAPE A VITAL ORGAN**

**BY SHARON TREGASKIS**

**PHOTOGRAPH BY STEPH HOOTON**

In the adult human, healthy function of the respiratory tract relies, among other things, on the rhythmic wave action of hair-like cilia lining the airways. Their coordinated beat, like fields of grain undulating in the wind, clears excess mucus and airborne contaminants from the lungs. In the fallopian tubes, cilia usher mature eggs from the ovaries to the uterus. In the ear, their dance transmits vibrations to auditory nerves that we ultimately register as sound. In the brain cavity, they circulate cerebrospinal fluid and prevent overaccumulations that might compress the skull's precious cargo. Kids in grammar school learn about cilia in tandem with the dangers of smoking: Nicotine temporarily paralyzes our pulmonary street sweepers—and smoker's cough results from their return to work after a few cigarette-free hours.

First described by microscopists more than a century ago, cilia appear in nearly every cell type of the vertebrate organism and in such simple organisms as mollusks and algae. Their ubiquity—even in organ systems where wave action seems irrelevant—once led biologists to dismiss the organelle as a vestige of evolution. That attitude reflects oversight or, perhaps, lack of vision. Previously, scientists could observe the cilia in action only in single-cell organisms, or within the first few hours after the death of more complex creatures. In the past decade, however, scientists armed with advanced imaging equipment and an enhanced view of their microscopic targets—alive and at work—have reconceptualized the cilia as elaborate microsensors, scanning their environment and generating and responding to the molecular signals that convey information from cell to cell, maintain cellular homeostasis, and even pattern the growing embryo.

Research scientist Cecilia Lo, soon to be founding chair of the University of Pittsburgh School of Medicine's new Department of Developmental Biology, has spent the past 30 years investigating the mechanisms of communication between cells in the developing embryo. Her quest: to understand how congenital heart defects emerge during gestation.

Like origami, much of embryonic development depends on the influence of patterning: Fold, turn, divide, repeat, and the neural tube yields a brain and spinal cord, the protean heart splits into quadrants and migrates to the left of the chest, four limb buds differentiate into a pair of arms and a pair of legs.

Most recently, Lo has begun to home in on the role of cilial motion during the first few weeks following conception in the formation of the cardiovascular system.

"You never know where the discoveries will come from that will benefit a patient, whether it's developing diagnostics, medication, or some other therapeutic tool," says Lo, who heads the National Heart, Lung, and Blood Institute's Developmental Biology and Genetics Center in Bethesda, Md., as well as its Laboratory of Developmental Biology. She set out to catalogue all of the genes responsible for congenital heart defects in mice. In the process, her lab revealed a link to a syndrome known as primary ciliary dyskinesia (PCD) that affects the respiratory system in humans. Lo now heads a study at Children's National Medical Center to investigate the relationship between PCD and heart disease, a project she plans to continue at Children's Hospital of Pittsburgh of UPMC when she arrives in Pittsburgh this July.

"When basic science impinges on clinical medicine, it behooves us to really pursue it and bring that science to benefit the clinical population, whether it's diagnostic or therapeutic," she says. "I'm excited that we are at that boundary."

Much like seating assignments at a state dinner, the relative arrangement of organs in the chest and abdomen of a human embryo matters enormously: heart to the left, liver to the right, stomach and spleen to the left, gall bladder to the right.

Occasionally, however, the whole assemblage develops in mirror image, a condition known as *situs inversus totalis*. Providing that the organs are otherwise normally conformed, the condition may go undetected for a lifetime and even supply an elegant plot twist, as in *Dr. No*. In that story, the fictional James Bond lives another day when a would-be assassin misses the spy's right-sided heart by a few vital inches.

Far more worrisome, however, and associated with myriad congenital deformities, is the class of deformities Lo studies known as *situs ambiguous*, or heterotaxy. In heterotaxy, some organs are flipped while others occupy their standard location. Ultimately, the problem boils down to bad plumbing. With the organs themselves out of place, the valves, veins, arteries, and chambers of the heart that normally move oxygenated and deoxygenated blood in discrete circulatory loops develop with quirks and malformations that can be fatal.

Many genes are implicated in heterotaxy, which comes in multiple iterations of varying severity. Even so, the condition is rare—fewer than 2 in 10,000 live births involve cardiac defects associated with left-right asymmetry malformations of any kind, and a diagnosis generally means a fast track to the operating room. Although milder cases often end happily, some infants with heterotaxy weather the open-heart surgery only to spend the rest of their lives tethered to a ventilator. Worse, others undergo multiple operations and still die before their first birthday. Because no one has catalogued all of the genes involved or detailed the specific conformation of organs each mutation yields, pediatric cardiologists have lacked reliable indicators to predict a baby's odds before wielding a scalpel, leaving parents and doctors with little clarity about how to proceed.

"Cecilia has a big vision," says longtime collaborator Linda Leatherbury, a pediatric cardiologist at Children's National Medical Center who oversees imaging of Lo's mice and conducts the study on children with heterotaxy and other congenital heart diseases.

"It's not like she wants to work on one model, one specific defect, for the rest of her career. She wants to find *all* of the genes that cause congenital heart disease."

If Lo succeeds, one day clinicians will be able to test for specific mutations associated with heterotaxy and other complex congenital heart diseases and help parents understand the treatment options for their babies.

In its facility at the National Institutes of Health, Lo's team injects mice with a chemical that causes random genetic mutations, then uses echocardiography to identify fetal mice with heart defects. The team details the genome of every single affected mouse, then compares it with the genome of a healthy mouse to narrow in on anomalies in the mutated genome. In 2007, *The Journal of Clinical Investigation* (*JCI*) published the team's description of a recessive lesion in Dnahc5—an analog to the genetic glitch that in humans causes primary ciliary dyskinesia.

"That was a big surprise, because usually when you think of patients with PCD, it's generally thought that [the] heart is normal," says Lo. "No one really thought of PCD as a disease that could cause structural heart disease. We initially identified this mouse model as a mutant with complex structural heart disease. When we identified the gene, it was a complete surprise."

Stanford University surgical intern Serena Tan was first author of the *JCI* paper. At the time, she was a second-year Duke University medical student with 12 months of funding from the Sarnoff Foundation.

"Dr. Lo was committed to giving me a project," says Tan, whose first project ate up six months and terminated in a dead end. "She gave me the most exciting project going on. She's very hands-on, oriented to detail, and very thorough in her investigation. [In her lab], you can't just be swept away by current trends. My paper, I think, was published partly because no one has made such a detailed study on the phenomenon."

People with PCD have compromised cilial function in their respiratory tracts. In time, mucus and bacteria accumulate in the lung, leading to recurrent infection and compromised pulmonary function. Often the damage is so profound that by the time they reach early adulthood, lung transplantation is the only viable treatment. Clinicians already knew that some kids with PCD have *situs inversus totalis*.

Based on the *JCI* findings, Lo hypothesized that infants with heterotaxy whose open-heart surgery leaves them ventilator dependent have undiagnosed PCD—"respiratory complications in such patients are unfortunately attributed to the heart disease," she says.

The genetic link also validated Lo's hypothesis: She suggested that at the earliest stages of embryonic development, ciliary-wave action programs the pattern and formation of the cardiovascular system. When the cilia don't work properly, the organs form out of place. Effectively, PCD is a secondary outcome of the same genetic anomaly.

In a swank, wood-paneled conference room on the seventh floor of the flagship National Institutes of Health facility that houses her laboratory, Lo gathers with her research team for their weekly journal club meeting. On this late winter morning, a junior associate presents a pair of 2008 *Science* papers investigating the role of three proteins implicated in embryonic development and in ciliary action. Lo sits quietly, a can of diet cola on the table in front of her, flipping between pages of the papers and intent on the slides of data projected on the screen at the front of the room. Finally, as the colleague describes the authors' conclusions, Lo breaks in. "The point is to show specificity," Lo says, glancing again at the manuscript in her hands and back to the screen, "but they haven't done the control. I'd like to see a parallel where they didn't get the result with the original sequence."

The critique sparks a minidebate between the dozen grad students, research techs, and fellows in the room about how the authors could have done better. The study examines the response of the proteins to antibodies, and part of the problem, says Lo, is that the best antibody to serve as a control in this case isn't widely available—its creator has closely guarded distribution. Soon the session is over, but Lo has made her point: Claims by scientists aren't the same as scientific proof from a well-designed experiment, and collaboration can mean the difference between a so-so study and one with the power to convince.

"I send Cecilia a paper to read, and she'll say, 'You haven't proved this,' or 'You haven't done that.' Likewise, she'll take criticism," says Vanderbilt University Medical Center chief of pediatric cardiology Scott Baldwin, who investigates vascular formation in mammalian embryos and worked with Lo when both were on the University of Pennsylvania faculty in the '90s. "She'll often send her work to say, 'What do you think of this? Are we missing something?' It goes both ways."

As the group disperses to go off to their labs and computers, Lo stops to ask staff scientist Biswanath Chatterjee about the status of a litter of mutated mice in the team's animal facility, a 15-minute walk across the NIH campus.

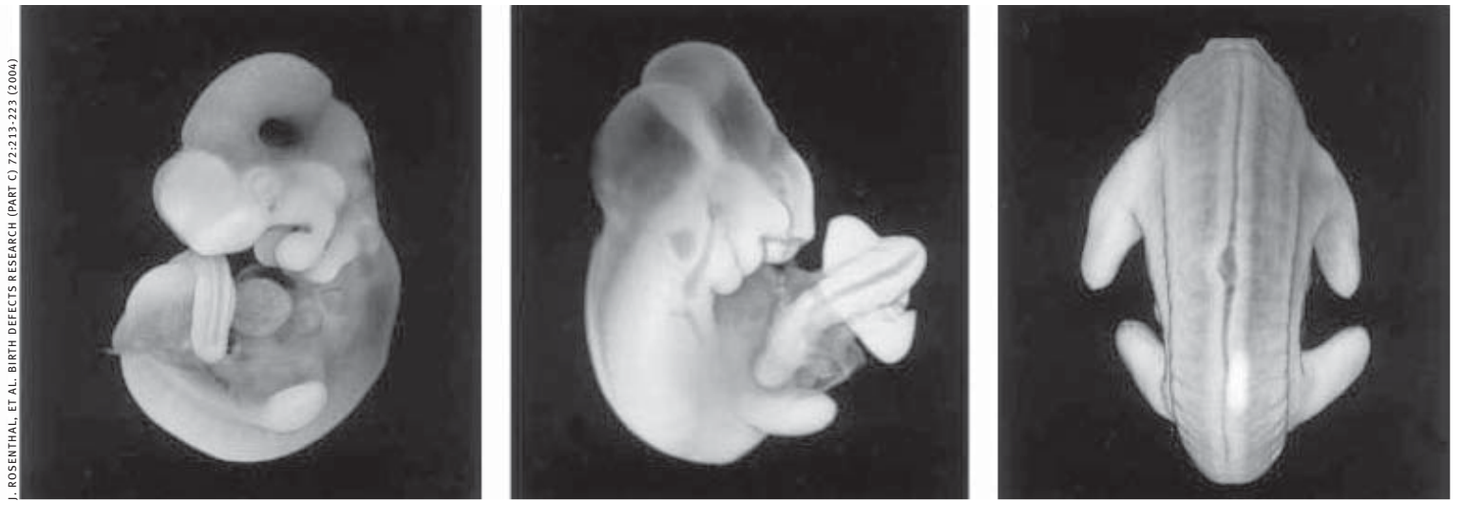
Chatterjee has known Lo since he spent four years as a postdoctoral fellow in her lab at Penn in the late '80s.

"Science is in her blood," says Chatterjee, noting that he'll often receive a series of e-mails from Lo as she reviews literature from home, long after her family has gone to bed. "Sometimes the e-mails just keep coming—1 o'clock, even 2 o'clock at night, and the next at 5 o'clock in the morning. She really enjoys this thing."

Lo's enthusiasm drives the lab forward, says Chatterjee, and she balances rigorous expectations with acknowledgment that not everyone can maintain her pace. She also makes a point, he says, of cultivating and protecting the junior scientists in her group. Late last year, a researcher called Lo after a postdoc presented the group's preliminary identification, using massive-parallel DNA sequencing, of a heterotaxy-inducing mutation of the gene Megf8. The other scientist was also working on Megf8, he said, and if Lo's group would hold their findings for a year, the two groups could collaborate and then publish simultaneously. Lo called a meeting of the 18 scientists working with her and the postdoc and presented the opportunity. Everyone had a chance to voice an opinion, says Chatterjee, a member of the team. "She protected everyone who was working on this project."

There were too many questions about the value of the collaboration for her group, says Lo, and the team opted to stay the course. Their findings appeared in the March 3, 2009 issue of the *Proceedings of the National Academy of Sciences*.

"The human element is really important," she says. "It's not just my career, and it's not just the science. People's lives and careers are at stake. It's my job to do the best



J. ROSENTHAL, ET AL. BIRTH DEFECTS RESEARCH (PART C) 72:213-223 (2004)

**Lo and her colleagues can't afford to capture only a fraction of the information needed for heart-defect research, which is what they get with 2-D images. Using a technique known as EFIC, they've created digital, 3-D atlases of both human and mouse embryonic development that allow researchers to view samples from any vantage point, including cross-sections of the heart. The fetal mice above were each imaged at a different stage of maturity and are shown from different angles.**

I can for the people in my lab."

Beyond performing their wide-ranging genetic analyses, Lo and her team have also refined and automated the technology to generate a pair of three-dimensional online atlases of human and mouse embryonic development based on a technique known as episcopic fluorescence image capture (EFIC), now a cornerstone of Lo's documentation of complex structural heart defects. Using EFIC, the group creates 3-D, digitized renderings that can be viewed in any plane.

EFIC provides an elegant work-around to a basic problem in conventional histology, in which thin slices of paraffin-embedded samples go under the microscope. Of course, the heart functions and develops in three dimensions; when sectioned into thin slices, critical information gets obscured or destroyed. And though each slice retains its integrity in two dimensions, stretching and compression caused by the slicing itself makes a 3-D reassembled composite impossible. Structural anomalies aren't replicated across specimens, and Lo's team can't afford to capture only a fraction of the information needed from each heart. Using EFIC, they image the block face that remains, instead of the slice they've removed.

Premed student Rajeev Samtani joined Lo's team in 2007 as a freshman in biological engineering at George Washington University, in D.C. Lo's use of EFIC captured Samtani's imagination, and today the 20-year-old has developed some expertise in the technique.

"Dr. Lo is the best mentor I've ever had," says the Maryland native. "It may take twice as long to do a gross necropsy with me, but Dr. Lo stops every second to make sure I know what she's doing—what she's imaging. Some mentors just give you a protocol to follow, but not Dr. Lo. She's always teaching. And if I run into a problem, she has 30 different solutions to try."

This spring, Samtani assisted a cardiology fellow developing a digital video of human cardiac development. "Viewers, other scientific researchers and clinicians, can go through the entire embryo," he explains. "But it may be hard to orient." The challenge is easy to get around in still images. A simple compass icon does the trick. Samtani suggested that incorporating a comparable strategy into the video would enhance its value. "Dr. Lo said, 'Go for it,' and now we're trying to label everything, and we've got some new software. If I have an idea and voice my opinion, everyone in the lab, especially Dr. Lo, is so open."

More recently, a late-night e-mail exchange with Lo about the possibility of combining EFIC with confocal microscopy (which allows scientists to create a sharper image by illuminating one point of a specimen at a time) had Samtani's adrenaline running high. He barely slept. "I was so excited to get to work," he says.

For Lo, the give-and-take of study design and execution is precisely what makes science a rewarding pursuit. "If you can't discuss what you have," she says, "you're missing a big part of the fun of doing science.

"I feel like you can go a lot further in your work, faster, by sharing resources, information, working with others. To me, being generous with your colleagues is a good thing. Ultimately, it's a win-win."

At Pitt, junior developmental biologists populate multiple departments throughout the health sciences. Lo's job will be to provide direction, says Arthur S. Levine, dean of the School of Medicine and senior vice chancellor for the health sciences.

"What's been needed in my view has been a senior developmental biologist of wide repute and recognition to bring together and coalesce the theme, crystallizing what we already have," says Levine. "Adding Cecilia to the mix will accelerate our momentum—give it shape and form and visibility."

At Pitt, Lo will have a joint appointment in pediatrics, and the proximity of her lab in the John G. Rangos Research Center to the children's hospital (next door instead of a 25-minute drive away, as in Bethesda) will promote the synergy she craves. She has already begun exploring partnerships with Pitt pediatricians who treat congenital cardiovascular and renal disease.

"This offers a whole new area of research to what we've traditionally studied," says Steven Webber, medical director of pediatric heart and heart-lung transplantation and an associate professor of pediatrics whose work with heterotaxic infants makes him particularly interested in Lo's arrival.

"From an intellectual standpoint on our end, clearly [Lo's appointment] broadens the scope of what we do."

Lo's first recruitment effort, to bring a stem cell biologist into the fold, got a boost this winter when President Barack Obama rescinded the Bush Administration's funding restrictions on embryonic stem cell research.

"I hope that in building the department we can take an approach where you have basic scientists pursuing very fundamental issues related to how development is regulated and a translational component with investigators trying to relate those findings to disease processes in children," she says. "I really feel like the time is right to integrate the two. I don't think you can do one or the other alone." ■