A Pattern Emerges

Diligent work yields the first branching diagram of the developing lung, a model that sets the stage for studying other branched organs.

THE THICK SCROLL IS MADE OF 29 PIECES OF PAPER TAPED END TO END. As HHMI investigator Mark Krasnow unrolls it in his office at Stanford University, an elaborate diagram looking something like a family tree reveals itself. But instead of chronicling births and deaths, marriages and children, it traces the lineage of thousands of branches of the mouse lung during its early development. ¶ This scroll—"our Torah of the lung," he calls it—represents the culmination of nearly a decade of painstaking work by Ross Metzger, a faculty fellow at the University of California, San Francisco, and a former student of Krasnow's. Armed with a fluorescence dissecting microscope and a lot of patience, Metzger examined hundreds of mouse embryo lungs at different stages of development to place them on a timeline.

"This is the first time anybody has constructed a branch lineage diagram of any organ in any animal," Krasnow says. The branching turned out to be stereotyped—that is, it follows a consistent, reproducible pattern. The model sets the stage for studying the development of other branched organs—the vascular system, kidneys, mammary glands—to learn if they also develop in a stereotyped way.

The finding settles a question that has perplexed scientists for at least a century. The branching in the lung seemed so complicated that some argued that it is a random process. Others believed that it follows mathematical rules, with branches simply splitting off from earlier ones in a repeated fashion, into ever smaller airways, creating geometrical patterns. The end of the process produces millions of tiny pockets called alveoli that allow gases to be exchanged with the blood.

Krasnow, whose lab had mapped the fruit fly tracheal system in detail over a decade ago, decided that the best way to answer the question was to map the mouse lung. Metzger might appear to have been an unusual pick for this daunting task: a philosophy major in college, he had no science background when he started working in Krasnow's lab nine years ago. He had enrolled at Stanford Medical School intending to pursue a career in psychiatry and approached Krasnow about gaining some lab experience.

But Metzger's medical studies and his scholarly research skills made him "the perfect person to set the whole project in motion," Krasnow says. "Because he was new to biological research, he had the freedom to think big and not feel constrained by the everyday challenges that sometimes sour people who have been working in the field a long time."

Metzger developed an antibody stain to make the airways glow bright green under the microscope. He studied embryonic mouse lungs from the 11th to the 15th day of development, a period when the first branches budding off the trachea bloom into thousands, filling out the shape of the lungs' five lobes.

"Even within the same litter of embryos, the lungs are not all at the same stage at the same time," Metzger says. "They may differ by a single branch." He stared at the eerie green airways under the microscope day after day, searching hundreds of specimens for these tiny differences in order to place

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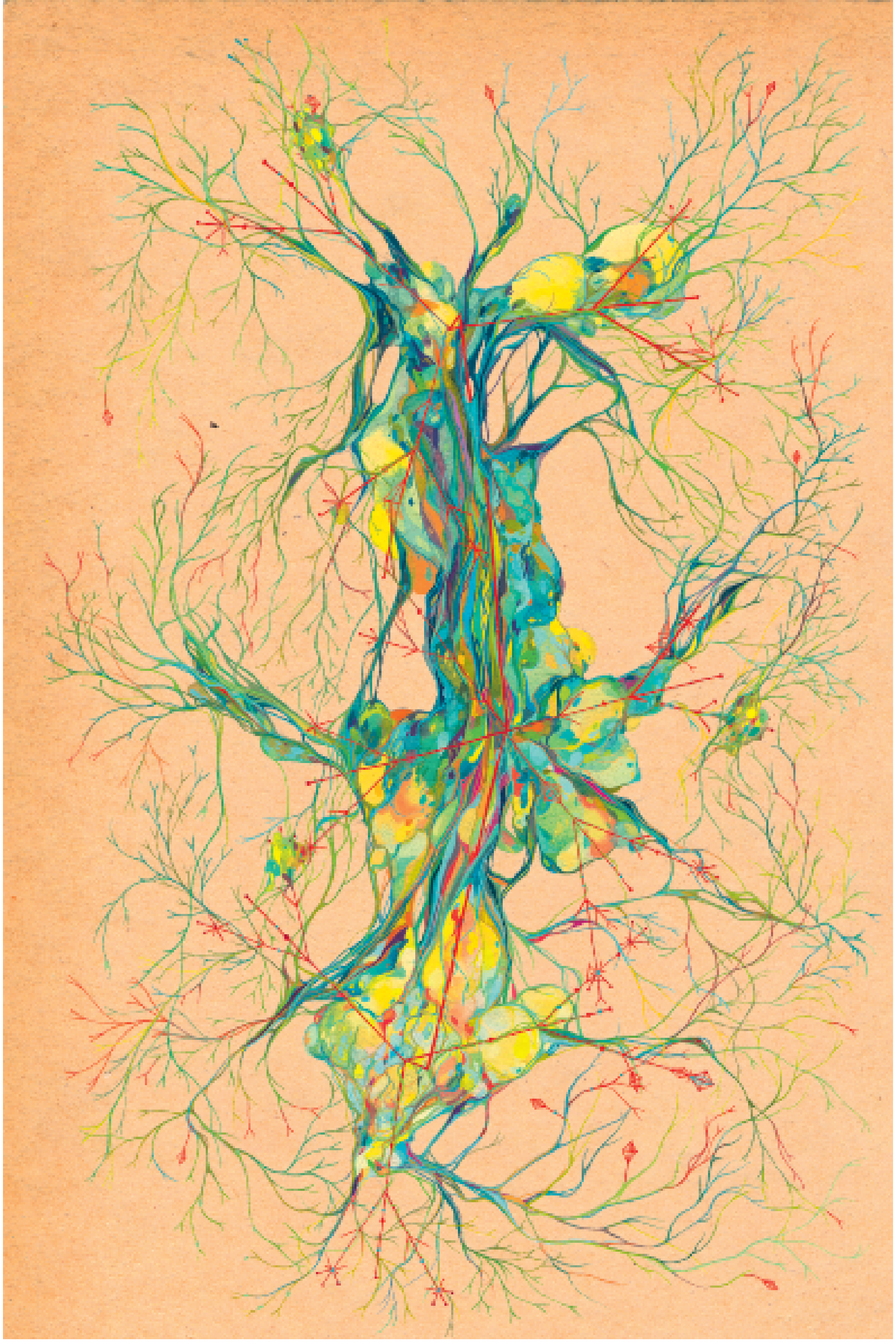

Jacob Magraw-Mickelson

the lungs in a developmental sequence. ("It does help that they're beautiful," Metzger says.) Gradually, over many years of work, the branching pattern started to emerge, and its elegance was startling.

Metzger and Krasnow deduced that the complex network of airways arises from just three simple types of branching. During domain branching, airways sprout in rows from the sides of an existing branch like bristles on a bottlebrush. But the domains don't grow haphazardly; the branches start from the near end and move toward the far end within each domain, with new domains spiraling around the circumference. Domain branching sets out a scaffold for each lobe of the lung, determining its overall shape.

From these domains, the airways branch in two different ways. During planar bifurcation, an airway forks into two in the plane of the original branch, helping to form the thin edges of the lobes. During orthogonal bifurcation, the airway splits perpendicular to the plane, which helps form the surfaces of the lobes and fill out their shape. The researchers describe the process in the June 5, 2008, issue of *Nature*.

As if in a computer program, each of these types of branching forms a "subroutine" that gets called on at predictable stages. "What we'd like to do now is start assigning genes and gene products to translate the computational model into a genetic and molecular model," Krasnow says. Their strategy is to look at mice with mutations in genes known to be expressed in the lungs. Before, such mutants might not have shown obvious lung defects, but perhaps the defects were so subtle that they escaped notice. Metzger is also working with another group to do computer simulation of the branching using the rules discovered in mapping the normal mouse lung.

"We're hoping that understanding the process in such detail will not only reveal interesting developmental principles but will also lead to novel types of treatments for lung disease," Krasnow says. Premature infants often have problems with lung function, and studies have found that differences in lung development might predispose people to respiratory disease later in life. The scientists' Torah of the lung took a decade to write; now, scroll in hand, Metzger and Krasnow say they have enough to fuel their research for decades to come.

■ –CORINNA WU