



NATURE'S WAY

— ALLAN C. SPRADLING —

ALLAN C. SPRADLING'S AIM IS TO SEE THE BIG PICTURE, TO FOCUS ESPECIALLY ON FUNDAMENTAL PROCESSES IN BIOLOGY, AND TO EXPLOIT NATURE'S OWN WAYS IN ORDER TO ADVANCE SCIENCE.

*The HHMI investigator and his colleagues blend genetics with cellular anatomy to develop new tools for genetic analysis. Along the way, they have made remarkable discoveries in the fruit fly *Drosophila* about stem cells and the surrounding tissue, called a niche, that supports them. When we spoke with Spradling in his lab at the Carnegie Institution of Washington in Baltimore, he offered these observations.*

Dedifferentiation may be used normally to maintain and repair tissues. There has been a great deal of success in taking an undifferentiated cell and directing its differentiation into a particular cell type. But the challenge remains to make those cells do something useful. We've started to look at the process of dedifferentiation, which is thought to take place normally in the body as part of some wound-healing and repair processes. Dedifferentiation may be a useful approach to the end goal of medically oriented stem-cell research, which is to correct adult degenerative conditions in a valuable way.

One of the problems in studying dedifferentiation has been the lack of an accessible system to study how it works. Postdoc Toshie Kai devised a method that takes germline stem cells [from which egg or sperm cells are derived], causes them to differentiate to the 8-cell stage, and then reverts them back at 100-percent efficiency. We've used these events as an assay to look for genes that turn off when the stem-cell state is lost and that come back when the stem-cell state returns. This assay has given us some new genetic handles on stem-cell regulation. But the pathways that stop development and reverse its course are still not understood.

We try to ask cells to do what they already know how to do. In approaching problems, we have attempted to use an existing biological mechanism to attain our goals, especially when developing new tools. For example, during the last 2 years, another very talented postdoc in the lab, Michael Buszczak, has been fusing genes to green fluorescent protein

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For protein crystals, researchers typically solve the phase problem by infusing them with heavy metal ions to act as landmarks because they bind to the protein in predictable ways. Then, the phases from the metal ions can be used to estimate the phases for the protein atoms. In principle, a similar approach should work for RNA crystals, but because RNA is negatively charged it binds metals much more nonspecifically than protein does. The result would be an overabundance of landmarks that would obscure the RNA's structural information. "I definitely had many, many sleepless nights," worrying whether the problem was unsolvable and wondering if it was wise to pursue such a big gamble, says Doudna.

Eventually, one of Doudna's first graduate students, Jamie Cate (who years later would become Doudna's husband) suggested soaking the RNA crystals in a chemical called osmium hexamine. The compound binds only to a specific RNA base pair sequence and is chemically similar to a fully hydrated magnesium ion—which means, Doudna says, that it is "a fairly bulky kind of ion that wouldn't wedge itself into too many sites." Doudna recalls being in the lab at 3 a.m. when the "molecule was, in effect, emerging out of the computer screen. It was an incredible moment when we could very clearly see the helices of the RNA." The image revealed how the RNA molecule was folded into domains that suggested how the ribozyme's catalytic active site might form.

This work, which earned Doudna the Waterman award in 2000, led her lab and others to solve structures of RNA-protein complexes in general and of the ribosome (the cell's protein-synthesis site) in particular. Like Anseth, though, Doudna thought solving a structure was just a starting point for her lab's projects. "The structure suggested maybe this is how it binds to something. Or maybe this is how it interacts with a protein. Then we can design experiments to test those ideas," she says.

Recently, Doudna's lab has been investigating an RNA, found in the human hepatitis C virus, that directs the ribosome of infected cells to start making viral proteins. "Do these RNAs have a defined structure they are using to hijack the ribosome?" Doudna asks her students.

The question is part of a line of inquiry that Doudna wants her students to understand. She advises them to ask,

at each stage of scientific research, "What is the biggest, most important question I can address?"

Doudna's healthy attitude flows in part from some of the balancing influences in her life—her 2-year-old son Andrew, retreats in Napa Valley wine country, and vinyasa yoga. She also draws inspiration from Rosalind Franklin (the essential but largely unheralded collaborator of James Watson and Francis Crick on the DNA double-helix discovery), who she describes as "a maverick who was trying to do something very hard and very interesting."

BIG SCIENCE

The "maverick" label might apply equally to Anseth, Amon, and Doudna. Their respective scientific breakthroughs have transformed the way we engineer tissue repair in the body, the way we order the events of cell division, and the way we view RNA structures. Their individual Waterman awards strongly underscore the importance of their findings.

Keys to their successes as scientists, however, transcend their curricula vitae. Any fledgling scientist would do well to take note that Doudna and Anseth both took huge leaps of faith in their post-doctoral work, which gave their fields new technologies. Anseth and Amon, colleagues say, can sort through a flood of ideas to find the best experiment to do next. All three women have stuck with a general line of inquiry they started in graduate school.

Big science, these researchers understand, requires hard work and taking risks. Each has applied the \$500,000 that the Waterman award carries to push into new areas—Doudna pursued the hepatitis project and RNA's role in targeting proteins to cell membranes, Amon's lab will explore cells with incorrect numbers of chromosomes, and Anseth might choose to address cancer or the retina (she hasn't yet decided). Given their track records, it's safe to expect each will continue to make her mark in research and scientific knowledge. ■

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(GFP), *in vivo*, on a large scale by taking advantage of the biology of *Drosophila* transposable elements [bits of DNA that can move from place to place in an organism's genome]. About 2,000 fruit fly strains have been produced so far in

which a different gene is fused to GFP. Since each of these GFP-fusion proteins is produced using the endogenous gene's normal control circuits, they are likely to reveal the gene's normal pattern of expression. There is also an excellent chance of seeing the normal tissue distribution and subcellular location of the gene product. A large collection of such strains will allow us to map the cellular structure of *Drosophila* tissues at the single-cell level and to identify genes involved in many biological processes—including stem cells. Eventually, it may be possible to insert even more sophisticated reporters—for example, those that fluoresce when certain signals are sent or when a cell activates other internal processes.

We understand relatively little of what goes on in multicellular organisms at the level of molecular processes within specific, individual cells. Not too many years ago, the general feeling was that insects didn't have stem cells. Turns out that it's not that way at all. And now, in some quarters, you'd think that biology is practically all figured out: We just need computer models and we'll understand the whole thing. In reality, we suspect that one could take virtually any tissue, even tissues that have been studied for a long time, and find new cell types and new interactions that are important and unexpected. For example, we found a new epithelial stem cell within the germline stem cell niche. It produces a small set of somatic cells that interact with early germ cells. We suspect that the interactions between these cells and early differentiating germ cells are among the most critical in determining whether reversion happens or development continues. We didn't even know these cells existed, and now we see that they are maintained by their own set of stem cells, and that both sets of stem cells and their progeny signal back and forth. It's a small example of a type of analysis that remains to be done with many metazoan tissues. ■

- Cori Vanchieri -

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