THE UNSUNG HEROES OF CRISPR

The soaring popularity of gene editing has made celebrities of the principal investigators who pioneered the field — but their graduate students and postdocs are often overlooked.

BY HEIDI LEDFORD

When Blake Wiedenheft started studying microbes, his work was both remote and obscure.

He spent his PhD sampling hot springs in Yellowstone National Park, then created artificial versions in the laboratory to study the microorganisms that lived in the inhospitable water. "We wanted to understand how life could survive in boiling acid," he says.

Over time, Wiedenheft became more interested in how microbes fend off viruses. He read around, and came across a peculiar bacterial immune system called CRISPR. In 2007, he approached Jennifer Doudna, a molecular biologist at the University of California, Berkeley, and found that she shared his interest. Join the lab, she said — and he did. Over the next five years, he studied the structure and biochemistry of CRISPR systems, landing a first-author publication in *Nature*[1].

Today, CRISPR is a household name for molecular biologists around the world. Researchers have eagerly co-opted the system to insert or delete DNA sequences in genomes across all kingdoms of life. CRISPR is being used to generate a new breed of genetically modified crops and may one day treat human genetic diseases. Doudna and other principal investigators involved in the seminal work have become scientific celebrities: they are profiled in major newspapers, star in documentaries and are rumoured to be contenders for a Nobel prize. "When I came to the lab, I was the only person studying CRISPR," Wiedenheft says. "When I left the lab, almost everyone was studying it."

Wiedenheft, however, has hardly achieved the same fame as his mentor — and nor have the other students and postdocs who toiled at the bench to make CRISPR genome editing a reality. They certainly reap benefits from their work: support and reflected glory from their supervisors, as well as expertise in a coveted technique. But some also face a difficult transition to becoming independent scientists as they try to establish themselves in a hypercompetitive field.

For Wiedenheft, the key to survival has been seclusion. When he struck out from Doudna's lab, he opted for a return to Montana State University in Bozeman, where he did his PhD, over an offer from a larger, better-known institution. "At the end of the day, the opportunities for solitude and being outdoors make me more creative and a better scientist," he says. But like other young scientists who graduate from powerhouse labs, he can't help but wonder how different life might have been if accolades in biomedical science were given to the first authors on a paper, rather than the last. Now and then, he admits, he doesn't feel quite appreciated enough. "Some days it matters, some days it doesn't."

AN EDITED HISTORY

The history of CRISPR–Cas9 gene editing has become a subject of fierce debate and a bitter, high-stakes patent battle. Researchers and institutes have been jostling aggressively to make sure that they are credited for their share of the work in everything from academic papers to news stories. "I get a lot of phone calls from lawyers about what I did and when," Wiedenheft says.

In January, Eric Lander, president of the Broad Institute of MIT and Harvard in Cambridge, Massachusetts, tossed into this minefield a historical portrait called 'The Heroes of CRISPR'[2]. It was instantly controversial. Some said that it marginalized the contributions of certain researchers, and they questioned the decision to publish the article without a conflict-of-interest statement noting that the Broad Institute is embroiled in a patent dispute that hinges on determining who invented CRISPR–Cas9 gene editing.

But for George Church, a geneticist at Harvard Medical School in Boston, Massachusetts, who is also a pioneer in the field, it was particularly painful to see statements attributing key discoveries to him rather than his postdocs and graduate students. "Eric said my name too many times," Church says.

Lander says that there was no intended slight in the 'Heroes' story. He was mindful that there were dozens of other co-authors on the key papers, "But I couldn't figure out how to collect and tell their stories within a nine-page article." If anything, he adds, the article widened the CRISPR spotlight: most discussion up to that point had focused on 3 major contributors to the field, whereas his piece featured 17 major players and acknowledged that there were many others.

Any lack of attention to CRISPR's junior discoverers comes despite fervent advocacy on the part of their advisers. Junior investigators in the Church lab praise their leader's unwavering support, along with the unique intellectual environment he has fostered in the lab. Doudna is a fierce champion of the scientists she has mentored. "It's really important for junior investigators to get the credit they deserve," she says. "They really drive the scientific enterprise." What is more, academic papers often set out each author's contribution to the work.

But those details often get lost simply because, broadly speaking, credit in science goes to the leader of the lab, as do any prizes that follow. "That's just how the system works, and I accept my role in this system," says Martin Jinek, another Doudna lab alumnus. "But yeah, it's something you can't help but think about."

ILLUSTRATION BY YASAVA

Sometimes people may take note of the first author, but not in a meaningful way, says Rachel Haurwitz, a former Doudna graduate student and now president of Caribou Biosciences in Berkeley, California. "They'll say 'the 2012 Jinek paper' but most people have no idea who Martin Jinek is," she says.

Jinek was co-first author on a seminal paper[3] showing that the enzyme Cas9 can be programmed to target specific sequences of DNA using only a short strand of RNA — and he found that his life became defined by CRISPR. When he entered the job market, he couldn't even discuss the work in interviews because the patent had not been filed. Even so, he got an attractive offer from the University of Zurich in Switzerland, and has since built a lab there that focuses on the basic biology of CRISPR more than its applications.

As interest in CRISPR–Cas9 gene editing grew, his schedule became packed: he now travels to talks two to three times a month. Although he appreciates the professional boost that the CRISPR frenzy has given him, he also struggles to find a balance between running his lab and other obligations.

Haurwitz has faced obstacles, too. She spent her PhD characterizing the CRISPR-based microbial immune system and the structure of a CRISPR-associated enzyme called Cys4 (ref. 4). In 2011, she co-founded Caribou along with Doudna and others to commercialize research tools based on CRISPR. The early days were tough, but Caribou has since formed partnerships with major industry players, and the company announced in May that its latest round of fundraising had brought in US$30 million. Yet as the firm has grown, some investors have pushed to replace Haurwitz with a more seasoned leader. Doudna has quashed the idea. "There's no reason to replace her," says Doudna. "She keeps showing that she has the talent to be successful."

RIDING THE WAVE

For many early career scientists, working in such a hot field has clear advantages. As a postdoc, bioengineer Prashant Mali helped to launch the CRISPR project in Church's lab. He was a co-first author on the lab's 2013 paper[5] demonstrating that CRISPR–Cas9 could be used to edit the genome in human induced pluripotent stem cells.

The discovery sent CRISPR excitement to fever pitch — a wave of enthusiasm that Mali rode into the job market later that year. "I definitely got a lot of endorsements," he says. (There is, however, no mention of him in 'Heroes of CRISPR' — a sore point with Church.) Eventually settling at the University of California, San Diego, Mali continues to study stem-cell development and develop CRISPR-based tools. He accepts the intensity of the field as a small price to pay. His lab is just 18 months old — too young to have been scooped yet, he says — but competition is inevitable. "There will obviously be a lot of overlap of good ideas."

CRISPR threw open doors for Luhan Yang, the other first author on the 2013 *Science* paper from Church's lab. Soon after the paper was published, the lab was contacted by several researchers who study organ transplantation. They wanted to know whether genome editing could now be used to engineer pig organs so that they would be less likely to provoke an immune response in humans. Yang seized the idea with gusto, says Church.

> ***"The idea that scientific discovery involves just one, two or three people is so nineteenth-century."***

The pig genome is home to retroviral DNA, and concern that those retroviruses might become reactivated in a human host led many researchers to flee the field in the late 1990s. Yang reasoned that the retroviral sequences are so similar to one another that a single CRISPR–Cas9 experiment might knock out many of them at once. She and her three co-first authors now hold the world record[6] for the largest number of sequences targeted in a single CRISPR–Cas9 experiment: 62. And Yang is raising money to launch a company with Church called eGenesis, to further the work. "George always gave me the opportunity to establish my leadership," she says.

Across the Charles River from the Church lab, graduate student Le Cong worked side-by-side, late into the night with his mentor, bioengineer Feng Zhang at the Broad Institute, to develop CRISPR gene editing in mammalian cells. Zhang was himself a young investigator just launching a lab when Cong joined; Cong remembers opening the box containing the lab's first centrifuge and sitting with Zhang at a computer googling 'DNA-binding protein' to look for new ways to edit genomes. The two became a tightly knit team.

When they embarked on the CRISPR project, it seemed like a long shot as Cong screened enzymes and reaction conditions, trying to find those that would work in human cells.

But Cong was willing to take the risk. He and Zhang had previously pioneered the use of a different gene-editing system, called TALENs, in mammalian cells, and he reasoned that this early success would be enough to allow him to graduate if the CRISPR project failed. He never had to test that hypothesis: in 2013, Cong and his fellow graduate student Fei Ann Ran co-first authored a *Science* paper[7] showing that the system works in mammalian cells — the paper was published simultaneously with that of Mali, Church and their team.

At that point, Cong was advised that he could skip the postdoc and go straight to a faculty position. But he worried that doing so would limit him: he could be pigeonholed as 'the CRISPR guy'. "I felt uncomfortable about that," he says. "I was not only looking to develop technology." Instead, Cong opted for another postdoc; he is now embarking on a faculty job search, and plans to use his lab to study allergies and autoimmune disorders.

Cong says that he feels no resentment at being largely excluded from the CRISPR media frenzy and attention centred on Zhang. "I do think I've been recognized," he says; Zhang has been generous in giving him credit within the scientific community, and has encouraged Cong to give talks in his stead.

And Cong, like others interviewed for this story, is himself insistent about giving credit to others in the field. He sprinkles in references to work done in other labs, including some of the earliest microbiology work characterizing the CRISPR system. Wiedenheft says that's characteristic of the CRISPR community. "It's competitive, but it's friendly."

Outside that community, however, the accolades continue to be heaped on senior investigators. "We need to invent ways to expand the medals podium," says Lander. "The idea that scientific discovery involves just one, two or three people is so nineteenth-century."

There are many more unsung heroes of CRISPR than this article could do justice to. One often overlooked group is headed by Virginijus Siksnys at Vilnius University in Lithuania — where Giedrius Gasiunas began his PhD in 2007. He plugged away for years, tackling the biochemistry of CRISPR–Cas9 and, like Jinek, eventually came to the conclusion that the Cas9 enzyme could be programmed to cut isolated DNA at specific sites.

In 2012, the lab sent a paper to *Cell*, where it was rejected without review. Gasiunas then submitted the paper to the *Proceedings of the National Academy of Sciences* and waited. A few months later, while his paper was still under review, the now-legendary Jinek paper appeared in *Science*. The two papers had key differences, but reached similar conclusions. Gasiunas had been scooped[8].

Seeing other scientists collect awards for CRISPR gene editing sometimes irks Gasiunas, now a postdoc in Siksnys's lab. But the experience has not entirely soured him on the subject. Although he has since been scooped again, he finds it no longer stings as much as it did. "It's a risky field," he says. "But I think if you want to achieve something great, you need to take risks." ■

Heidi Ledford *is a senior reporter for* Nature *based in London.*

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