Biography of Carol W. Greider

When biochemist and molecular biologist Carol Greider was a first-year graduate student at the University of California, Berkeley, in 1984, she began to study a topic slightly off the beaten path. With her adviser, Elizabeth Blackburn, Greider investigated how a certain single-celled pond organism maintained the tens of thousands of caps on the ends of its minichromosomes—specialized structures known as telomeres that protect against DNA damage. On Christmas Day, 9 months later, Greider spotted signs of a new enzyme, telomerase, that appeared to be responsible for this chromosomal maintenance. The finding helped kick off a field of research that would attract the attention of longevity researchers, cancer biologists, and the biotechnology industry.

Today, so many papers are published with the keyword "telomerase" in the title—about 1,000 each year, at last count—that Greider cannot keep up with them all. Still, she delights in how the field's research questions multiply and expand with each new finding. "I see telomeres as having taken me for a ride," says Greider, who was elected to the National Academy of Sciences in 2003. Her work began in biochemistry but has changed over the years to intersect with the fields of cellular senescence, cancer, DNA recombination, and stem cell failure. In her Inaugural Article (1), published in this issue of PNAS, Greider explores the structure of the RNA subunit of telomerase. "It's fun, because I picked one topic, but the fields keep changing, so I have to keep learning along the way," she says.

Carol W. Greider

Clicking with Biochemistry

Greider grew up in Davis, CA, near the University of California, Davis campus, where her father was a physics professor. While her high school classmates descended on the University of California, Davis, or nearby Berkeley for college, she decided to move down the coast to attend the University of California, Santa Barbara. "I didn't want to do what everyone else was doing," she says. Originally interested in marine biology, she enrolled in the university's College of Creative Studies, whose small size meant its students could enjoy a high faculty-to-student ratio.

Greider's mentor, a dynamic researcher named Bea Sweeney, insisted that she try out research right away, to find out whether hands-on science would suit her as much as textbook learning did. Greider sampled a few laboratories as a freshman, but, when she landed in a biochemistry laboratory the next year, she knew she had found her home. "You can't really know without being in a lab the style of science that it does," she says. "But once you get into an environment that fits your own scientific way of thinking about problems, it just clicks."

Greider stayed in the biochemistry laboratory for two and a half years, enjoying lively conversations with other laboratory members and the mechanistic flavor of thinking that pervaded. Even during her junior year abroad in Germany, she found a biochemistry laboratory in which to work. After this preview of graduate student life and research publishing responsibilities, the career path of an academic scientist still appealed to her. Greider applied to molecular biology graduate programs across California.

Her application package was a bit unusual, Greider says. "I had great research experience, great letters of recommendation, and outstanding grades, but I had poor GREs." Although she did not know it growing up, Greider suffers from dyslexia, which affected her scores on standardized tests. Only two schools—the California Institute of Technology (Pasadena, CA) and the University of California, Berkeley—offered her an interview. When she met with cell biologist Elizabeth Blackburn in Berkeley, things clicked again. "I really liked my conversations with Liz, and there were a number of other people in the department that would be potentially fun to work with, so I went there," says Greider.

First Glimpse of Telomerase

Throughout the late 1970s and early 1980s, Blackburn and other researchers had found that telomeres show unusual behavior and structure. The chromosome caps consist of multiple repeats of a simple motif, which in the pond ciliate *Tetrahymena* was six nucleotides long. The mechanism by which these sequence repeats were added to the ends of telomeres in ciliates and yeast had not yet been identified. Most researchers believed recombination was responsible, but Blackburn favored the explanation of specific repeat addition by an as-yet-unknown enzyme. She decided that *Tetrahymena*, a pond ciliate with a macronucleus and 40,000 telomeres, was a natural place to look. When she started work in Blackburn's laboratory in April 1984, Greider set out to find this hypothetical enzyme in *Tetrahymena*.

It was a tall order for a graduate student, but Greider was clearly up to the task, says Blackburn, now the Morris Herzstein Professor of Biology and Physiology in the Department of Biochemistry and Biophysics at the University of California, San Francisco. "If you were easily intimidated, you wouldn't take on that kind of project," Blackburn says. "We had to be both rigorous and enterprising, and those are exactly the characteristics that Carol has. The combination is a great strength." For her part, Greider worked 12-hour days and supplemented her existing biochemistry knowledge with DNA cloning techniques and other skills needed for the project.

Nine months after she began the project, and after much trial and error finding the right substrate and assay, Greider identified the first signs of her enzyme. On Christmas Day in 1984, she developed one of her gels and saw a ladder of the characteristic *Tetrahymena* 6-base telomeric repeats—exactly the pattern that would be expected from a telomere-synthesizing enzyme. But she and Blackburn did not celebrate right

This is a Biography of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 8080.

away. Greider says, "When you find something that is really exciting that you think may be something new, the first things that go through your mind should be, 'What else could it be? How could I be being fooled?'"

Many potential sources of artifacts existed, and Greider and Blackburn wanted to rule out as many as possible. In June 1985 came the persuasive experiment, which showed that yeast telomeres functioned in *Tetrahymena* and indicated that they were seeing a new enzyme activity. This time, Greider went home and celebrated. Publishing in December 1985, Greider and Blackburn originally called the newly discovered activity "*Tetrahymena* telomere terminal transferase" because it seemed to add telomere repeats in a manner similar to terminal transferase (2). Deciding that this name was "a mouthful," Greider says, they later shortened it to "telomerase."

Freedom in the Academic Laboratory

For the next two and a half years, Greider purified and further characterized telomerase. In the laboratory, she and Blackburn enjoyed hashing out the problems, Greider says, and they would often end up arguing opposite sides of a debate until each had convinced the other of her side. "Carol is a marvelous person to discuss science with," Blackburn says. "She's very lively and interactive."

On the advice of her thesis committee, Greider sought a postdoctoral fellowship at Cold Spring Harbor Laboratory in Long Island, NY, in 1987. The laboratory gave her the option to bypass the usual process of working in one researcher's laboratory and instead to work completely independently in her own laboratory as a Cold Spring Harbor Fellow. "That was a really good position for Carol, given her interest and her high abilities," Blackburn says. When she moved to Cold Spring Harbor, Greider continued work to clone the gene that encoded the RNA component of telomerase. She published the results of this work in 1989 (3).

Greider soon found a graduate student who wanted to work in her laboratory, and she tried to squeeze the student into her limited funding. Cold Spring Harbor then told Greider that if she could obtain a grant from the National Institutes of Health (NIH; Bethesda, MD), she could have a graduate student as well as be a full staff member. Funds were tight, Greider says, but she managed to obtain the NIH funding. Lea Harrington joined Greider's laboratory as her first graduate student, and in 1990 Greider was promoted to Assistant Investigator.

During this time, Greider began work with Calvin Harley at McMaster University (Hamilton, Ontario, Canada). The collaboration combined Harley's interest in cellular senescence and Greider's interest in telomeres. Together, in 1990, they provided early evidence that telomere length was related to cellular aging (4). Greider's laboratory also collaborated with Harley's to investigate telomere shortening in cancer cells. They found that the gene for telomerase is activated in cancer cells, which allows these cells to bypass cellular senescence and continue growing as immortalized cells (5). Both of these results posed great implications for understanding aging and treating cancer.

"We wanted to ask genetic questions about what happens to cells when they lose telomerase."

Greider first concentrated her efforts on cancer because the relationship between telomeres and cancer cells made telomerase a potential target for cancer chemotherapy. "Being a molecular biology and genetics lab, we wanted to ask genetic questions about what happens to cells when they lose telomerase," Greider says. Instead of inhibiting the enzyme, Greider and her group developed a mouse model and knocked out the gene for telomerase to mimic a telomerase inhibitor. "That's when we set out to clone the mouse and human RNA component, so that we could do those kinds of studies," she says.

When Harley moved his laboratory from McMaster University to be part of Geron, a new biotechnology company in Menlo Park, CA, their relationship began to change. At first, Greider continued her collaboration with Harley and was a founding member of the advisory board for Geron. Together, their laboratories cloned the gene for human telomerase RNA, and, later, Greider's laboratory cloned the mouse gene (6, 7). Yet Greider began to see the business and academic research paradigms clash. Geron, like most biotechnology companies, had an active public relations division. Small biotech companies particularly need to have their names publicized, Greider says, and Geron was successful at this. "But that really conflicts with what I saw as a very straightforward, honest science principle: Don't overstate things," she says. Just as Greider was cautious not to prematurely celebrate her original *Tetrahymena* telomerase finding, she also wanted to take this careful approach with advances at Geron. "The rhetoric in an academic area and in a business are very different. So I went off the advisory board."

Juggling Students and Post-Docs

Over time, Greider became increasingly interested in the physiological effects of telomerase in mammals and how the biochemistry of mammalian telomerase differs from that of microorganisms. Her laboratory created a knockout mouse allowing them to address such questions (8). "I decided to focus on the mouse and separate myself from [Geron] to some degree, so I could do the kinds of really academic studies I wanted to do," she says.

In 1997, Greider and her husband, Nathaniel, a science historian, moved to Maryland to take on positions at neighboring universities. Her husband worked at The George Washington University in Washington, DC, while Greider accepted an associate professor position at The Johns Hopkins University School of Medicine in Baltimore, MD. It was a good move, she says, because she wanted to work with more graduate students. Cold Spring Harbor is "post-doc heavy," she explains. "With all post-docs, there's a lot of pressure in terms of them getting a job and having their own territory. Graduate students are a little more open to trying different things. There's a different speed at which they learn." Students now fill more than half her laboratory at Johns Hopkins. "I select for people that like to be very independent," she says. "It's more like having a day-to-day collaboration with smart people, and I find that fun."

One group within Greider's laboratory has continued her original biochemistry research, working to establish the structural and functional regions within the telomerase RNA (9, 10). Another group is exploring genetic questions with yeast. "It turns out that the biology of something as fundamental as telomeres is so conserved in a variety of organisms, that what you find out in yeast also turns out to be true in mammalian cells," Greider explains. Her laboratory has performed experiments studying the role of telomeres in genomic stability, DNA damage, and cell death (11–13).

In her Inaugural Article, Greider and former postdoctoral student Jiunn-Liang Chen explore the structure of the RNA subunit of human telomerase (1). "My roots are in the biochemistry of telomer-

ase," she says. "Since I started in that area, I've spread out into more cell and organismal biology, cancer, and aging. I thought it appropriate that the Inaugural Article goes back to the biochemistry that was the heart of how I started." Their work looks specifically at the pseudoknot structure of the RNA component, which has been conserved in all vertebrates and is essential for telomerase activity. Some researchers have proposed that the pseudoknot essentially switches between two different conformations. In their article, Greider and Chen give strong evidence against this idea, and their results support a static pseudoknot structure.

New Links to Old Interests

Greider's latest line of research takes advantage of these structural studies for a clinical application to dyskeratosis congenita, a rare, inherited disorder related to stem cell failure. Recently, various forms of the disease have been linked to mutations in proteins that bind to telomerase RNA or in telomerase RNA itself. Persons with dyskeratosis congenita cannot maintain the telomerase in their bone marrow and eventually die of bone marrow failure. "Suddenly, the studies that we had done, very careful studies on the structural function of the RNA, were pertinent to this disease," Greider says. "It's another example of curiosity-driven research ending up having a direct medical implication."

Greider is intrigued to find stem cells linked with telomeres and is currently developing mouse models to understand how telomerase may play a role in stem cell failure. This might lead to a clearer link between organismal aging and telomeres, she says. "I'm not a believer that aging is going to be one thing, that there's going to be one gene that controls all of aging. I think there are going to be multiple different failures, and that the loss of stem cells can play a role in a number of them."

This area is also a chance to explore another line of research, something Greider always enjoys. "It's completely new, and I'm intrigued by new things. Any time you do a series of experiments, there are going to be three or four new questions that come up when you think you've answered one." She plans to keep following her research instincts wherever they take her. "You might as well set up your lab and the kinds of experiments you do to give yourself the most freedom," she says. "It's going to be hard work whether you think it's fun or not, so you might as well have fun while you're doing the hard work."

Regina Nuzzo, *Science Writer*

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