Profile of James C. Carrington

James Carrington attributes the trajectory of his scientific career to "a lot of Thomas Hardy moments," that is, completely random events.

For example, Carrington chose to pursue plant science after asking a high school classmate what he planned to study the following year at university. "Environmental science and plant science," his classmate replied.

Carrington, who enjoyed science and biology, in particular, thought the idea sounded intriguing and followed the same path. Had his peer chosen to study Shakespeare, Carrington admits, he might have ended up studying the Bard's work in Stratford. Fortunately, Carrington enjoyed plant science and "eventually found a niche," as he says in his characteristic understated manner.

Today, Carrington is a professor of botany and plant pathology at Oregon State University (Corvallis, OR) where he also directs the Center for Genome Research and Biocomputing. By focusing on plant viruses early in his career, Carrington unknowingly primed himself for the field of RNA silencing and has played a major role in unraveling some of the mechanisms by which small snippets of RNA orchestrate plant growth and behavior. Carrington has received many awards for his work, including the Presidential Young Investigator Award from the National Science Foundation and the Ruth Allen Award from the American Society for Phytopathology. In 2008, he was elected to the National Academy of Sciences.

A Talented Dishwasher

Carrington, born in 1960 in Redondo Beach, CA, was not the most likely candidate for a scientific career. He confesses to hating all of his college lab courses, which he describes as "canned laboratory exercises... just like building a kit from instructions."

However, during his sophomore year at the University of California, Riverside (Riverside, CA), his circumstances changed, and he needed a job. He landed one in the laboratory of plant physiologist John Einset; it was his first entry into a real laboratory. After washing dishes for 6 months Carrington was bored. Einset and Bill Dawson, a plant virologist, recognized Carrington's potential and hired him as an undergraduate research assistant. During his time as an undergraduate researcher, Carrington glimpsed the investigative nature of bona fide laboratory research. "It was more like a puzzle," says Carrington who, after he got hooked, began spending all his free time in the laboratory.

James C. Carrington.

A pivotal event that secured Carrington's resolve to pursue research in graduate school occurred during Thanksgiving vacation his senior year. Dawson had asked Carrington to characterize what Dawson thought was a new virus infecting cowpeas in central California. Carrington was working alone in the laboratory the day after Thanksgiving when Dawson dropped by. Dawson was impressed by Carrington's work ethic, diligence, and data. He remarked that if Carrington stayed in the business, he was going to be successful. The comment boosted Carrington's confidence and made him believe he had something to contribute.

The pathogen Carrington isolated from the cowpeas turned out to be a common one, but the experience piqued his interest in plant viruses nonetheless.

Following His Hunches

Carrington's interest in plant virology led him to the University of California, Berkeley (Berkeley, CA), where he began his graduate work with virologist Jack Morris. "Morris was the perfect advisor for me," says Carrington, "because he was very hands off and let people in his lab follow their own interests and hunches."

Shortly after beginning work with Morris in 1983, Carrington took a hands-on biochemistry methods course called Grad Lab, taught by Robert Tjian, who later developed the field of eukaryotic transcription. The course covered new cloning, sequencing, and recombinant DNA techniques. Carrington found the course empowering, and Morris was happy to let him use the methods he had learned to analyze several small plant viruses.

In retrospect, Carrington was doing viral genomics. His graduate work revealed the genome organization and structure of some of the smallest known viruses. The viruses Carrington analyzed may have been tiny, but they were incredibly informative and revealed some general principles of viral genomes that were composed of RNA. In particular, his thesis focused on genomes of two small RNA viruses called the carnation mottle and turnip crinkle viruses. After sequencing their genomes, he studied how the genes were expressed and what some of them did (1).

At the time, Carrington had no inkling of the many roles RNA played in gene regulation. His work with RNA merely reflected the fact that most plant viruses have RNA genomes.

When Carrington completed his PhD in 1986, he was still largely fixated on mechanisms of viral gene expression but recognized that he needed to expand his expertise. He went to North Carolina State University (Raleigh, NC) to do postdoctoral work with William Dougherty, who studied plant viruses that used a different strategy of gene expression. Carrington's goal was to identify proteases encoded by viruses and study their specificity. "That turned out to be very productive and had interesting and long-lasting, unforeseen consequences," he says.

While working with Dougherty, Carrington discovered the NIa protease, which became known commonly as the tobacco etch virus (TEV) protease. This proteolytic enzyme has an unusually specific requirement for cleavage: a seven amino acid recognition motif. This finding led to molecular biologists using the protease in the popular tandem affinity purification (TAP) tag system, in which a fusion protein—made of the protein of interest and the recognition motif—is released from an affinity column after being cleaved with TEV protease. This useful and unplanned biotech application facilitated the production of large quantities of highly pure protein, Carrington says. "That was probably the most long-lasting, important thing that I did as a postdoc," he adds.

An Imported Texan

In 1988, Carrington accepted his first faculty position at Texas A&M University (College Station, TX). He was drawn to the biology department largely because it was in the midst of a hiring spree and was bringing on board many young faculty. "These weren't just good scientists. They were young, interesting, fun people, many of whom remain good friends to this day."

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 20055 in issue 51 of volume 105.

In 1991, while at Texas A&M, Carrington received the prestigious Presidential Young Investigator Award from the National Science Foundation. "The really good thing about that award was that it is not tied to a specific project," recalls Carrington. "That certainly let me go in new directions."

In the early 1990s, Carrington's research began to shift away from pure virology. He had been focusing on how viruses interacted with their host cells. But, by the mid-1990s, he realized that many of the questions he was asking could not be easily addressed using tobacco plants, which he had used as a host for virus infection. Buoyed by the enthusiasm of postdoctoral researcher Steve Whitham and graduate students Stephen Chisholm and Sunita Mahajan, Carrington's lab began using *Arabidopsis* as a model host for viruses. This model organism catalyzed a shift to studying the genetics of virus–host interactions and has since remained a constant focus in their lab.

RNA Silencing: A New Field

After 9 years in Texas, Carrington joined the Institute for Biological Chemistry at Washington State University (Pullman, WA), in part because the institute offered unique scientific opportunities and it allowed him to live closer to his children. He then published a paper that changed the course of his career, and he realized that the future of his research lay in another field that would become known as RNA silencing or RNA interference.

The field of RNA silencing emerged in the late 1980s and early 1990s when plant biologists discovered that newly introduced transgenes were frequently inactive. Researchers began to recognize that this transgene silencing was caused by an effect at the RNA level in some cases and at the transcriptional or DNA methylation level in other cases—but the molecular basis for the phenomenon was unclear.

Carrington's movement into RNA silencing in the mid-1990s was driven by his discovery that HC-Pro, a viral protease he had identified, was required for TEV to trigger systemic infection in plants. Loss of HC-Pro function resulted in TEV mutants that were unable to spread through plants. These results, coupled with the work of Vicki Vance at the University of South Carolina, led Carrington to hypothesize that HC-Pro suppressed a natural defense response. Working with graduate student and later postdoc Kristin Kasschau, he tested the effects of HC-Pro on RNA silencing. The resulting paper, published in *Cell*, showed that HC-Pro protein made by TEV blocked posttranscriptional silencing of genes in plants. Combined with other work by Vance and David Baulcombe at the Sainsbury Laboratory in the United Kingdom, this work provided powerful evidence that a natural function for RNA silencing in plants was antiviral defense. The virus, in a counterstrategy, used HC-Pro to block the plant's defense, making it more susceptible to infection (2).

"That's why the paper was so important," says Carrington."It assigned a clear biological role, at least in plants, to RNA silencing."

Carrington encourages undergraduates to come into the laboratory.

By 2001, the field had exploded. New classes of small RNAs were being linked to regulating endogenous processes in both single and multicellular organisms. New components had been discovered, and biochemical pathways were defined. Hundreds of microRNAs were identified in flies, worms, and mammals. Additionally, research showed that it was possible to experimentally trigger RNAi in mammalian cell cultures and perform knock-downs in human cells.

Oregon Bound

Carrington's discovery of virus-encoded suppressors of RNAi underscored his transition into the field. After a move to Oregon State in 2001, he began working with postdoctoral researcher Cesar Llave to identify different biological roles for the RNA silencing pathways in plants.

Carrington initially asked Llave to focus on the siRNAs that were made in plants when double-stranded RNA was introduced. If long double-stranded RNA enters cells, it gets diced to make small RNAs, which silence expression of any mRNA with a complementary sequence. Llave figured out how to clone and then sequence small RNAs. "That was kind of tricky," Carrington says.

This work enabled Llave and Carrington to identify a diverse set of endogenous small RNAs in *Arabidopsis*, some of which looked like microRNAs. In two landmark papers, the researchers revealed that, in plants, small RNAs came in a couple of different flavors: endogenous micro-RNAs and endogenous siRNAs. Carrington and Llave proposed that the molecules suppressed gene expression from endogenous genes (3). In a follow-up paper published in *Science*, the researchers showed that at least some plant microRNA functioned by guiding the irreversible cleavage of targets (4).

Today, one of Carrington's primary interests is how evolution has embedded this basic regulatory pathway in so many different contexts: development, antiviral response, and the control of chromatin structure.

One of the major questions that occupies his time, and is the focus of his Inaugural Article, is why the RNA silencing mechanism is specific only to certain transcripts. He and others have deciphered the roles of RNA-dependent RNA polymerases in amplifying siRNAs from some, but not all, targets. Why is the silencing response amplified in some cases but not in others?

In his Inaugural Article (5), Carrington's team, led by Taiowa Montgomery, reveals special features of miR173 that attract RNA-dependent RNA polymerase, transforming single-stranded RNA into the double-stranded form, which can then be chopped up by endoribonuclease Dicer and eventually, used for silencing.

Although his Inaugural Article focuses on a mechanism, Carrington says that other areas of his research are oriented to the genome: looking at genome-wide patterns of small RNAs or genome-wide patterns of how small RNAs interact with their targets. He is also intrigued by how these silencing systems are influenced by the environment.

At Oregon State, Carrington encourages undergraduates to come into the laboratory. "We grow a lot of plants and we wash a lot of dirty dishes. . .we hire lots of undergraduates to do those things, but at the same time, we're always looking for people who have an interest in research." From his own experience, Carrington knows that a good undergraduate student can be as productive as a graduate student. "Not all of them are, but occasionally, you get one who is just phenomenal."

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