Putting It Together

Exploring viral replication pathways has led Carol Carter from the study of measles and reoviruses to the assembly and budding of newly minted HIV.

BY ANNA AZVOLINSKY

Carol Carter entered City College of New York wanting to major in biology and chemistry. But her freshman-year courses left her cold. "The classes were dull and uninspiring, and I was very discouraged," she recalls. Carter went to the professor who served as her freshman advisor and told him how much she hated the intro biology class. "The advisor leaned back in his chair with no change in facial expression and said, 'Wow, you're lucky.' This caught me completely off guard," she says. She remembers the advisor telling her, "You are lucky because you know what you want to do, so nothing is going to discourage you." Carter left the office even more perplexed than when she had entered, but after mulling over the encounter, finally understood the advisor's indirect message: a single experience or data point should not discourage someone with conviction.

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Carter persevered in her study of biology. She was encouraged to apply to graduate school by her undergraduate work-study employer, an ecology professor who became her mentor. "City College was and remains a strong teaching institution, and it prepared me well for graduate school."

As a graduate student at Yale University, Carter became fascinated with viruses. "Animal viruses were the new guys on the scene in the 1960s. Bacteria, phage, fungi, and parasites had held court in microbiology for a long time. It was a new field that attracted many young scientists." To Carter, animal virology was not only very exciting but provided opportunities to impact health and disease. She worked on measles virus and on reovirus, a related but low-virulence model for rotaviruses that cause diarrhea in infants and children, and then switched to studying HIV in the late '80s, a time when very little was known about the virus's biology. Early on, Carter identified virus-encoded targets for AIDS treatment. More recently, she has concentrated on host proteins necessary for HIV particle assembly, and is currently investigating how they work.

Here, Carter talks about pursuing biology despite a frightening experience with a salamander, the importance of inspiring mentors, and how Kaopectate—an over-the-counter medication to treat diarrhea—figured in her postdoctoral work.

CARTER, CULTIVATED

A mind for inventions. In elementary school in Harlem, New York, despite an overwhelming lack of resources in the school system, Carter's teacher noticed her love of reading and managed to provide her with books. "He gave me this tattered brown book called *Inventions*. It described commonly used things people had invented, and I must have read that book a million times. Now it seems like such dry material for kids, but I found it so intriguing."

Unfettered support. "Neither of my parents went beyond the 7th grade, but they strongly believed that education was a good path, so they supported my sisters and me," Carter says. "What I mean by that is they created an environment of support for us. My mother would sit with us while we did homework, and if one of us wanted a cup of milk or tea, she would bring it to us. It was that kind of contribution."

A tale of a tail. Carter landed a work-study position at City College with James Organ, who studied salamander limb regeneration. In her first week in the lab, she was charged with cleaning the animals' cages and got a firsthand introduction to regeneration. "I'm taking the salamanders out and changing the straw in the cages and one of them runs away! So I chase the little guy around the lab and finally grab him by the tail. And, well, it's a salamander, so he drops his tail off and runs away. I start screaming hysterically. Jim comes running into the lab and finds me holding this little wiggling tail. And of course he starts laughing."

Wonderful counselors. Besides having a good sense of humor, Organ was also a great mentor, says Carter. "He knew how to guide students interested in science. He introduced me to other faculty in the biology department." That interaction with professors outside of the classroom was very important for Carter.

Off to Yale. "The expectation in my family was that when I graduated from college I would get a job. But I was encouraged to apply and attend graduate school by Organ," Carter recalls. "My mother was not that open to the idea, but then one of the schools that accepted me was Yale. She was not particularly sophisticated with respect to colleges and universities, but she knew that name and encouraged me to accept."

Nurturing lab culture. At Yale in 1968, Carter joined the laboratory of virologist and epidemiologist Francis L. Black, who

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CAROL CARTER

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Greatest Hits

- Discovered that measles virus has a nuclear phase of replication
- Contributed to studies that established the subacute sclerosing panencephalitis agent as a variant of measles virus
- Demonstrated that oligo(A) in reovirions is not necessary for replication
- Among the first to demonstrate that self-assembly of the capsid protein of HIV could be executed in vitro using recombinant protein
- Contributed to defining the specificity of the HIV protease to better target the enzyme with small-molecule inhibitors
- Among the first to identify Tsg101 as a cellular protein that interacts with the HIV Gag precursor protein, pinpointed the Gag binding sequence, and showed that Tsg101 facilitates Gag release by a mechanism distinct from that used by related viruses
- Established that HIV recruits calcium signaling machinery to stabilize viral assembly platforms at the cell membrane

studied how measles virus spreads in human populations and how it replicates in cell culture. "He was also a fun advisor and really took his role as mentor seriously, taking me to meetings and introducing me to his colleagues." A collaborator of his in the department, epidemiologist Ann Schluederberg, was another valued counselor and an "inspiring and supportive person with whom I could discuss my experimental results and ideas. Ann was never discouraging. If she didn't like my idea, she'd say, 'Why don't you think about that and let's pick it up next week,' which I learned was a cue that maybe the idea was not so great!"

Years later, Carter recalls that Shirley Kenny, the first woman president of Stony Brook University in New York, encouraged incoming undergraduates to find "homes," by which she meant laboratories, where they could build relationships and get to know the people doing research. "I think that notion of finding a laboratory that becomes your own community is key, and that is the experience I had at both City College and Yale."

Virus gone haywire. In the early 1970s, when Carter was working towards a PhD, measles was the second most common cause of childhood mortality worldwide, responsible for about a million deaths every year. It was thought that measles only replicated in the cytoplasm, but Carter's thesis work showed that the virus's RNA genome was also present in the host cell's nucleus, providing evidence that measles has a nuclear phase of replication. She also found that measles strains recovered from patients who had a persistent infection in the central nervous system (CNS) were distinct from those strains responsible for acute measles infections. This CNS infection, called subacute sclerosing panencephalitis (SSPE), is a potentially deadly inflammation of the brain occurring in about one in every 10,000 measles cases. Others later discovered that the virus is able to infect the CNS when mutations result in a defective viral protein.

CARTER CARVES HER PATH

A serendipitous meeting. Carter met Aaron Shatkin, a virologist at the Roche Institute of Molecular Biology who later discovered the 5' cap on reovirus messenger RNA (mRNA) molecules, on the way to a 1972 Gordon conference on animal viruses in Tilton, New Hampshire. "The airline lost my luggage, so I had no toothbrush or comb. Aaron saw me looking distressed and offered to drive me to the nearest town to get what I needed. He was very kind to me at the meeting and at the end, offered me a postdoctoral fellowship in his lab." Carter's work on reovirus

showed that, unlike in other viruses, reovirus-encoded oligo(A)s are not essential for infectivity.

A sense of humor. Shatkin's laboratory was having trouble growing high-enough titers of reovirus. Carter suggested a trick she had used in graduate school to get measles virus to grow well in tissue culture—Kaopectate. "I suggested this to Aaron and it turned out to work beautifully with reovirus. A few years later, Aaron was giving a seminar and told the audience, 'Let me tell you about a really shitty experiment my postdoc did,' and then proceeded to describe the Kaopectate experiment," Carter recalls, laughing.

A new opportunity. In 1975, Carter joined the faculty of the Department of Genetics and Microbiology at Stony Brook University as an assistant professor, and she has been there ever since. "The chair of the department who recruited me, Joseph Kates (the discoverer of polyA on mRNA), was the youngest department chair at Stony Brook. What attracted me to Stony Brook was how unconventional Joe was. He had very nontraditional ideas, and he attracted faculty who were interactive, collaborative, and diverse."

A fresh start. NIH funding for reovirus research began to dwindle in the 1980s because illness caused by reovirus was not a major problem in the U.S. At the same time, "HIV emerged in a very dramatic way in this country," says Carter. Her transition to studying the virus that causes AIDS arose from a collaboration with Eckard Wimmer, who worked on poliovirus across the hall. Carter and Wimmer recognized certain similar strategies used by poliovirus and HIV: to make infectious particles, both form a precursor polyprotein that must be cleaved to carry out critical structural and enzymatic functions. They applied for and received a grant to use poliovirus as a model to begin to ask questions about HIV.

HIV drug targets. In the late 1980s, little was known about the function of HIV-encoded proteins, although it had been shown that the virus manufactures a protease related to one that causes hypertension. This enabled Carter to collaborate with several pharmaceutical companies seeking to identify AIDS therapies and to develop HIV-specific diagnostics, efforts that began to bear fruit in the early '90s. Carter's grad students Kathy Partin and Gabriele Zybarth and Wimmer's postdoc Hans-Georg Kräusslich helped define the specificity of the HIV protease to better target the enzyme with small-molecule inhibitors. Carter's laboratory also studied the virus's capsid protein, p24, as a potential drug target. Lorna Ehrlich, a research associate in the lab, demonstrated self-assembly of the capsid protein in vitro using recombinant protein. "There are still no FDA-approved drugs against the capsid, but some are now in development," Carter says.

Hijacking cellular machinery. In 2001, Carter's grad student Beth Agresta identified several cellular proteins, including cyclophillin A and Tsg101, the product of *tumor susceptibility gene 101*, that interact with HIV's Gag—the major viral precursor polyprotein necessary and sufficient for viral assembly. Along with postdoc Fadila Bouamr and undergrad Traci LaGrassa, grad student Lynn VerPlank identified the sequence in Gag used to recruit Tsg101. "Tsg101 is important for sending proteins the cell no longer wants to the cell's garbage pail to be degraded," explains Carter. "But HIV uses Tsg101 to help it escape from the plasma membrane." Grad student Jay Goff showed that blocking Tsg101 prevents HIV particles from budding. Later, in 2005 and 2008, Gisselle Medina from the lab provided evidence that Tsg101 directs particle release through a pathway that is distinct from those used by related retroviruses. Recently, Carter's laboratory found that Tsg101 enables HIV to recruit calcium signaling machinery to help stabilize the viral assembly platforms at the cell's plasma membrane. "We think that this ability to use calcium signaling is the means by which the virus is able to maintain the Tsg101 ESCRT machinery at the budding site, and this may help us in trying to target Tsg101 effectively," Carter says.

CARTER COMMUNICATES

HIV drugs. "Drugs targeting the HIV protease changed the complexion of AIDS treatment in the early 1990s when the major therapy was AZT, which targeted the virus's reverse transcriptase," says Carter. "AZT was a really tough drug for many people to tolerate. Once the FDA approved these newer inhibitors and they were combined with anti–reverse transcriptase drugs, one really began to see an impact on the disease."

Fast pace. "The field is moving fast, but still, at this stage, every drug targeting a viral-encoded gene product requires physician monitoring, because resistant mutants emerge," Carter says. "On the one hand, we are very fortunate that the arsenal is strong enough to use these drugs for both treatment and prevention. But drug development is still critical until we have a drug the virus can't evade, or a vaccine."

Re-education. "The number of new infections each year in the U.S. has not declined. In the beginning years of the epidemic, there were many young people with many, many sexual partners," Carter says. "But as the evidence accrued that this was a risk factor in HIV transmission, there was an impressive sobering that shrank this high-risk population. Now, we are trending back, because people forget what constitutes risk behavior and the disease is not in the public eye. Continuous re-education is needed to inform and to remind."

Beyond HIV. "Tsg101 is critical for budding, not only of HIV but other viruses as well." Coming full circle, Carter's laboratory, in collaboration with Jon Leis, has identified small molecules that target Tsg101 and other budding factors. "If we can get some of these compounds to work, we may be able to target other diseases as well, including Ebola," she says. ■