Straight Shooter

Danny Reinberg chose an unconventional model organism to study gene expression. In the process, he's revealed fascinating things about ants, behavior, and aging.

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THE GOOFY, BESPECTACLED plant is the first thing you see in Danny Reinberg's office at the New York University (NYU) School of Medicine. It's how you know that this serious, accomplished scientist has an irreverent side. "You can see I love plants," says Reinberg, an HHMI investigator, gesturing toward the profuse greenery at his windows overlooking the East River. "But this one didn't make it." Instead of tossing the dead plant, though, his assistant, Michele Giunta, noticed that its bedraggled brown foliage bore an uncanny resemblance to a colleague from another lab. When she put a pair of Groucho glasses on it, the transformation was complete.

"He has a crazy haircut like this, and he wears weird glasses, and has a weird nose," Reinberg explains in his tuneful South American accent (he was born and raised in Chile). "She thought it was a perfect way to remind me constantly of him." It's also a good way to make anyone who walks in to see Reinberg break into a smile.

In his sunny office, Reinberg talks about his journey from a boyhood in Santiago—his father was a German Jew who moved to Chile just before World War II—to his position as a much-lauded biochemist whose curiosity led him to uncover key details of gene transcription, the process by which DNA is copied to RNA as the first step in protein synthesis.

He uses social ants as the model system for his most recent work on the epigenetic aspects of gene transcription. Although ant workers and queens have virtually identical genetic makeups, they express their genes differently. And that expression can change dramatically in response to a change in the environment—a worker can become a queen if the colony loses its queen, or one caste of workers can become a higher caste if the need arises.

Epigenetics is a trendy and complicated concept, so Reinberg has been giving lots of interviews to reporters who want to use his ant model as a way to explain it to their readers. But when we met in his office, he wanted to be sure our conversation went beyond ants to cover the previous 30 years of work that led him to epigenetics research—beginning with his student days in Chile amidst the political upheaval of Salvador Allende's presidency and the subsequent coup.

Supportive and Intense

From a young age, Reinberg set about defying expectations. As the oldest in a family of two boys and two girls, he was expected to take over the family business. But he hated working in his father's stores, where he sold jewelry and furniture during school breaks. Like most young people in his city, he was expected to live at home until he married, but he couldn't wait to get away from a household filled with discord. So at age 19, he moved into his grandfather's small apartment in Viña del Mar, one and a half hours away, and eventually studied science at the Pontificia Universidad Católica de Valparaíso. "My father hoped that if I didn't take over the family business, I'd at least become a doctor or a lawyer," he says. "But that's not what I wanted."

At the university, Reinberg loved all of science: physics, chemistry, and especially biology—the more the better. "In Chile no one worked on weekends," he says. "I was the only crazy person at the microscope on Saturdays and Sundays." He finished with a degree in cell biology in 1976 and started a doctoral program in Santiago in a histology/cell biology lab. When he found the science to be too descriptive, he switched to biochemistry. Through a string of lucky coincidences, he landed at the Albert Einstein College of Medicine, in the Bronx, and met the man who would become his mentor and lifelong friend, biochemist Jerry Hurwitz.

"He was a very ambitious, very smart kid," says Hurwitz, who is now at Memorial Sloan-Kettering Cancer Center. "Very aggressive. Always eager to do more things. Also very upbeat, very positive. And above all, a prodigious worker." Reinberg could be a critical and somewhat demanding co-worker, Hurwitz says, but he was most critical and demanding of himself. He pushed himself and was always looking around for new research topics that would allow him to make a real contribution to the scientific understanding of how genes are replicated, transcribed, and expressed.

With the good fortune of having landed in the lab of the father of protein enzymology, Reinberg set about learning everything he could about how to purify proteins and run assays. As Hurwitz puts it, he was steeped in the brute-force process of purification on a large scale. Reinberg worked hard at it, knowing it would serve him well no matter where he went in protein enzymology. "I knew if I wanted to continue in that field, I had acquired a skill very beneficial to my life as a scientist," he says.

Hurwitz did more than supply Reinberg with crucial lab skills. He also was a role model for how to run a lab, creating the collegial, cooperative, just-competitive-enough atmosphere that Reinberg has tried to copy in his own lab, first at Stony Brook University for a year, then at the University of Medicine and Dentistry of New Jersey (UMDNJ) from 1986 to 2006 (where in 1994 he was named an HHMI investigator), and then at NYU since 2006. "Danny really bends over backward to help out young scientists," says his wife, Lynne Vales, who first met Reinberg when she was doing graduate work in a different biochemistry lab at Albert Einstein. "He gets a kick out of boosting people's careers—the ones who are worthy of it." When asked to describe the atmosphere in his NYU lab, where Vales now works writing and editing grant applications and scientific papers, she says that two words come to mind: "supportive" and "intense."

Hurwitz had a tendency to bark at students and postdocs with some rather salty language, Reinberg recalls, but there was nothing he wouldn't do for them—something Reinberg discovered for himself soon after he earned his PhD in 1982. He went straight to the University of California, Berkeley, to do a postdoctoral fellowship—and lasted four months. California was a bad fit, he says. "It was too laid-

Danny Reinberg, in the ant room, uses two species of social ants to study epigenetics and behavior.

Scientists in Reinberg's lab are learning to stimulate epigenetic changes that convert one kind of worker ant to another.

back for me." Even nearby San Francisco wasn't urban enough; the bars closed at 11, and the city was always foggy.

Then one probably foggy Saturday morning in late 1982, Hurwitz telephoned out of the blue. "Come back, son," he said. "We'll take care of you."

The following year, Reinberg took a postdoc position in the lab of Robert Roeder at Manhattan's Rockefeller University, where he set about trying to characterize the complex process of gene transcription. (Vales also eventually went to Rockefeller to do a postdoc at a different lab. She and Reinberg got reacquainted there and married in 1986.) "Many labs were working on gene transcription, trying to identify factors that allow the enzyme that makes RNA to be recruited to specific genes," Reinberg says. His goal was to be the first to make the fullest identification of the proteins involved and how they function.

Transcription in a Test Tube

At the time, not much was known about the biochemistry of gene transcription in eukaryotes, though scientists knew it was set in motion by RNA polymerase II, an enzyme that travels along DNA to produce RNA along with other protein fractions. In Roeder's lab, Reinberg used the human HeLa cell line to help identify factors that are required for transcription, which he and Roeder published in 1987 in an extraordinary series of three papers in a single issue of *The Journal of Biological Chemistry*. In the first paper, they described two transcription factors, TFIIE and TFIIB, that initiate the process at all promoter sites, the stretches of DNA adjoining genes that spark their transcription. In the second paper they described the actions of two more transcription factors, TFIIA and TFIID. In the third paper they described another factor, TFIIS, involved in the

Reinberg's group can make carpenter ant foragers and fighters switch roles.

elongation of the RNA chain being transcribed.

By the time these papers appeared, Reinberg had established his own laboratory at Stony Brook. Years later, Reinberg's group discovered other factors required for transcription, TFIIF and TFIIH, and finally reconstituted transcription in a test tube using DNA strands–so-called naked DNA. They went on to reconstitute transcription working with DNA in the form of chromatin, as occurs naturally in the nucleus of a cell.

His lab was a spirited place to work. "Danny speaks his mind," says Gary LeRoy, who was a grad student in the UMDNJ lab from 1995 to 2000 and recently re-joined Reinberg at NYU as a research scientist. His directness earned him the nickname "Chili Pepper," but LeRoy says he liked Reinberg's approach–especially because he so obviously cared about his students.

Stories of the lighthearted moments in the lab tend to be a bit nerdy. Like the time in the late 1990s when LeRoy was doing "bucket biochemistry" (formally known as biochemical fractionation) to try to isolate an enzyme. LeRoy was in the cold room and Reinberg was standing outside, looking in through the window, waving. LeRoy would look up and see Reinberg waving, go back to his work, look up again and see Reinberg still waving. Finally he came out of the cold room to ask what was going on. "I'm waving goodbye to your protein if you think you're going to put it on that gigantic column," Reinberg said. The story makes LeRoy laugh in the retelling, but it's clearly a joke only a biochemist could love.

Other stories reveal something more serious about the kind of scientist and mentor Reinberg is. LeRoy talks about the time he asked Reinberg why he had accepted him as a grad student. "Because you had ideas," Reinberg told him. "They were very immature ideas which you will never do in my laboratory, but at least you had ideas."

During his early UMDNJ years, Reinberg continued to live in Manhattan, enjoying the city's lively social life. He and Vales moved to New Jersey in 1990, but it wasn't until 1999, when they walked into a 120-year-old farmhouse in the New Jersey town of Warren and instantly fell in love with it, that Reinberg fully embraced suburbia and became an avid gardener. When he moved his lab to NYU in 2006, he stayed in New Jersey–the house and its garden were too beautiful to leave behind–and once again became a commuter.

During this time, Reinberg kept working on chromatin, the complex of nucleic acids and proteins that condenses into chromosomes during cell division. He helped to refine the understanding of how gene transcription is enabled and disabled through modifications to histones, the proteins that are the main component of chromatin. These studies led him to epigenetics. He was wondering how he could design an epigenetic study of social behavior at the organism level when the answer arrived in an unusual way–on a hot bus stuck in traffic in Mexico.

Social Creatures

It was 2004, and Reinberg had flown into Mexico City for a scientific conference that was an hour from the airport. Because of anti-government protests on the streets, the bus ride to the meeting site stretched to two hours, then three, then four. Luckily he was sitting with his friend Shelley Berger, director of the epigenetics program at the University of Pennsylvania. As the bus crawled through the snarled traffic, they chatted about the question he'd been mulling: what model systems would work best to study epigenetics? He rejected yeast, the organism Berger worked with. He rejected worms. "I told her that I wanted to work on something that is social," he says, but that bees were too much of a hassle; you need to suit up to handle them, and you get stung anyway. That led Berger to describe some fascinating behavior she'd observed among leaf-cutter ants during a family trip to Costa Rica. "That's the system we want to work on," Reinberg remembers thinking. "Ants!"

Reinberg went home and did what he usually does when he gets excited about something: he bought books on the subject and read voraciously. What makes ants ideal for epigenetics research, he found, is that in any one colony the ants are genetically identical, yet they have different behaviors, lifespans, and brain size. How does the social environment during development affect how those genes are expressed? And does a change in the environment change gene expression even in adulthood, after an ant's fate seems to have been sealed?

Berger agreed that ant societies would be an excellent model for studying how epigenetic changes are linked to changes in behavior, reproduction, aging, and neurobiology. Fortunately, just as Berger and Reinberg were talking about working together, HHMI launched the HHMI Collaborative Innovation Awards (HCIA).

In 2008, Reinberg and Berger received a large HCIA award and could begin sequencing the genomes of two species of social ants: *Camponotus floridanus* (a carpenter ant from Florida) and *Harpegnathos saltator* (a.k.a. Jerdon's jumping ant). They also were able to bring in another collaborator, Jürgen Liebig, of Arizona State University, an evolutionary biologist interested in how insect societies maintain the division of labor.

The timing of the HCIA grant program was propitious, allowing Reinberg's lab to launch the new project and benefit from the different mindsets of his collaborators. As Berger describes it, Liebig has a big-picture view of ant society typical of evolutionary biologists, and she and Reinberg are more interested in the details, as biochemists usually are. "We really challenge each other," she says, especially Reinberg, who loves to ask provocative questions.

After they sequenced the ant genomes, the real work could begin: studying epigenetic changes in response to changes in the environment. Reinberg set up an ant room off the lab's main corridor—essentially, a series of shelves holding big Tupperware containers, each home to a colony. Any time a lab worker puts on gloves and reaches in with tweezers to retrieve an ant, the colony gets worked up, ants scurrying up the plastic walls like convicts trying to make a jail break.

When that gloved hand reaches into a *Harpegnathos* colony to remove the queen, it provokes an especially dramatic change. Queens have a social role and lifespan quite different from other females in a colony; they spend their lives laying eggs and can live for as long as 12 years. The female worker ants, on the other hand, are rendered sterile in the presence of the queen and live on average just one year. (The males in the colony—haploids, with only half the full genome complement—are not much more than sperm with legs; they live only as long as it takes them to fertilize the queen's eggs, and then they die.) The brains of queens and workers are different, too: once the queen establishes the colony, she doesn't need to see or engage in any activity other than egg-laying, so her brain shrinks accordingly, while worker brains are more fully developed.

In the *Harpegnathos* colonies in Reinberg's ant room, removing the queen creates a change in the other ants: the once-cooperative workers start fighting to see who will become the new queen. (In the *Camponotus* colonies, no such power struggle occurs; remove the queen and the whole colony dies.) Eventually a few workers become dominant, and they develop the physical traits of the queen—that is, they become capable of reproducing. They're called gamergates, or, as Reinberg calls them, pseudoqueens.

Reinberg's team has identified several intriguing biochemical changes in pseudoqueens, including increased gene expression in at least two genes associated with longevity in mammals, one for the enzyme telomerase, the other for the enzyme sirtuin-1. Preliminary findings suggest that the pseudoqueens live longer than a typical worker, too (though not quite as long as a true queen, probably just three to four years). Could activation of telomerase or sirtuin-1 help explain the several-fold increase in lifespan in queens?

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—SHELLEY BERGER

As for the *Camponotus*, even though removing the queen is too drastic, the HCIA team has found a way to induce other interesting changes. *Camponotus* has two worker castes, major and minor, that differ in size (the majors are bigger) and behaviors (the majors are fighters, the minors are foragers). Scientists in Reinberg's lab are learning to stimulate epigenetic changes that convert one kind of worker to another. They can induce minors to become majors in the *Camponotus*, just as they are learning to create pseudoqueens in the *Harpegnathos*. "We can propagate *Harpegnathos* in the lab," Reinberg says, "which means we can start injecting the embryos or larvae with different things that may affect gene expression." He wants to keep the details vague for now, saying only that the genes are "important for the transition from workers to gamergates, aging, etc."

Berger has enjoyed working with Reinberg. "Danny is very rigorous, dispassionate, and deeply questioning," she says. "He loves the science. He loves big questions. The attraction of this project is what fantastic questions there are to ask."

As rigorous and demanding as he is as a co-worker, Berger is quick to add, Reinberg is also "a great guy, lots of fun at meetings, someone who knows everybody and wants to interact with everybody." Which is why she plans to join a big group of Reinberg's colleagues and friends for a blow-out 60th birthday party in January. "I wouldn't miss it for the world," she says. "I'm trying to think of a couple of good stories to tell."

The birthday celebration will begin with an all-day symposium presented in Reinberg's honor, after which a shuttle will take attendees to a floating restaurant in the East River for the festivities: a jazz band and cocktails, followed by dinner and roasts until midnight. Lab manager Heike Pelka is coordinating the event, with 120 guests, some coming from as far away as China. She doesn't know exactly what to expect, but she says one speaker will be delivering a talk called "Keeping Danny Activated." From the looks of it, what keeps him activated is science—the chance to do it, share it, talk about it, and then do it some more. ■