Beyond the NEXT RIDGE

BY STEVE OLSON

Nobel laureate Craig Mello's role in the discovery of RNA interference is consistent with a lifetime of impatience to see what's next.

AS A TEENAGER GROWING UP IN THE SUBURBS OF WASHINGTON, D.C., Craig Mello and his family used to go on long hikes in the nearby Blue Ridge Mountains. All the Mellos were good athletes and accustomed to difficult hikes. Yet Craig walked so quickly that the rest of his family struggled to keep up. "I just want to see what's past that next ridge," he would tell them. "You go and look, and then come back and tell us," his mother would say.

PHOTOGRAPHS BY ERIKA LARSEN

Mello, an HHMI investigator at the University of Massachusetts Medical School, has spent his life searching for answers. And the discoveries he's brought back from his explorations have helped change how biologists think about the functioning of cells, with profound scientific and medical implications. On October 2, 2006, Mello's work was accorded the ultimate honor when he and his colleague Andrew Fire (of the Stanford University School of Medicine) learned they were to be awarded the Nobel Prize in Physiology or Medicine for their discovery of the remarkable biological effect called RNA interference.

Mello Memories

LAST NOVEMBER, MELLO AND HIS PARENTS and siblings gathered on a rainy Sunday afternoon at the parents' farm in the Blue Ridge foothills. The Mellos are an unusually close family, but they hadn't all been together since the Nobel announcement. Amidst hugs and congratulations, the family discussed who would be going to Stockholm in December for the Nobel ceremonies and who would be staying home to take care of the farm. "It's the busiest time of the year for us," said the family patriarch, James Mello.

Jim, an open and cheerful man who sets the tone for the family's conversations, was a paleontologist at the Smithsonian Institution in Washington, D.C., until the last of his four children graduated from college. He and his wife, Sally, then built a log house on property they had bought near Culpeper, Virginia, and began raising cut-your-own Christmas trees and organic vegetables that they sell in the local farmers' market. Over the next two decades, all their children except Craig moved to their own farms within a quarter mile of their parents' house. The oldest, Jeanne Day, is a librarian and artist who sells her hooked rugs in the area. The second-oldest, Frank, is an engineer and metalworker whose wrought-iron sconces decorate his parents' home. The youngest, Roger, is principal of the local high school, renowned for both his discipline and the poetry he quotes to his students.

For two hours that Sunday afternoon, as clouds scudded across the ridgeline opposite the Mellos' hilltop house, the family talked about school, sports, science, and the Nobel Prize. In many ways, the Mellos were a typical suburban family while the kids were growing up. Jeanne rode horses and the boys joined their high school wrestling and football teams. Craig and his siblings spent hours wandering through their neighborhood catching snakes, toads, and salamanders. (Craig once brought some of the toads to his high school biology class to dissect.)

Once Craig built a crossbow in the family workshop and convinced his younger brother to lie with him in the backyard while they shot arrows into the air. "The game was to shoot an arrow as straight up as you could," said Craig, an experimentalist even then. "It was fun to watch it go up, and then it would turn around and come down. But no matter how hard you tried, you could never make it come straight back down. So I told [Roger] we were totally safe, just stay next to me. And sure enough nobody got hurt."

"We figured Craig was either going to win a Nobel Prize or a Darwin Award," said Roger.

The Mellos also exhibited a fierce devotion to education, achievement, and clear thinking. Jim and Sally both attended Brown University in the 1950s, and Sally—a painter in the time she could spare from raising four children—oversaw an intellectually rich household. After she prepared dinner, the family would sit down together and devote their mealtime conversation to the topic of the day.

Jim:* I worked in the museum, and I would bring home stuff about what was going on in science. We would talk about it at the dinner table. Craig was the one who would always challenge me. Do you remember those days, Craig?

Craig: My favorite line was, "That's the most ridiculous thing I've ever heard."

Jim: I remember when the first tube worms were discovered. We actually got specimens—these big six-foot-tall, encased worms—at the museum. I was always interested in this sort of thing, and maybe some of the enthusiasm that I had for it carried over.

**Excerpts have been edited for the* Bulletin. *To hear more of the Mello family conversations, go to www.hhmi.org/bulletin/feb2007.*

Clockwise:
FRANK MELLO, CRAIG MELLO, ROGER MELLO
JEANNE DAY, JIM MELLO, SALLY MELLO

Jeanne: I remember the blue-tailed skink conversation. Dad had just given us a nice talk about lizards, and he said, "You could always tell a blue-tailed skink because it always has a blue tail." And Craig said, "But that's not necessarily true, because you just told us that sometimes they lose their tails."

Craig: One time, when I was really into astronomy, I said Sirius was a southern star, and you couldn't see it from North America because it was in the southern hemisphere. Jeanne pointed out that she had seen it, so I must be wrong, because she had never been to the southern hemisphere. That was such a crushing argument.

Those conversations were great for our self-esteem. You [Jim] would listen to what we had to say and never put us down. If you disagreed with what we said, you would argue with us, and you didn't get upset if I said, "That's ridiculous."

Jim: No, I was delighted.

Understanding Genes

MELLO BECAME FASCINATED WITH one science after another as he was growing up—paleontology, evolution, geology, astronomy—but he traces his interest in molecular biology to June 12, 1978. A few days before graduating from high school, he read an article in the *Washington Post* titled "Bacterium Is Used in Producing Insulin." It described one of the first applications of genetic engineering: the insertion of a human gene into bacteria to produce insulin for diabetics. "I was absolutely amazed to learn that bacteria could read the human genetic code," Mello says. "I realized that I would love to be able to understand how genes function by taking them out and putting them back into an organism."

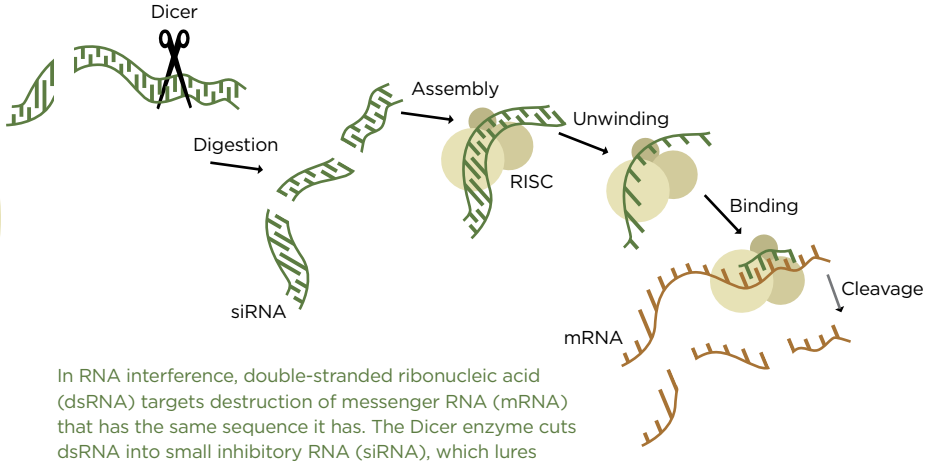
Like their parents, Mello and his older brother and sister had gone to Brown University. (Roger, the only exception in the family, attended the University of Virginia on a wrestling scholarship after becoming state champion in high school.) After Brown, Mello went to graduate school at the University of Colorado at Boulder, where he began the research that would lead to his Nobel Prize. Working in the laboratory of David Hirsh, he focused on techniques to introduce genes into a widely used model organism, the tiny worm *Caenorhabditis elegans*. Around that time, Andrew Fire, who was doing graduate and postdoctoral work at the Massachusetts Institute of Technology and the Medical Research Council Laboratory of Molecular Biology in England, developed a way to microinject DNA directly into the nucleus of a worm egg. But the technique was so difficult that Mello developed an alternative microinjection strategy, and in the process the two got to know each other and began collaborating. "He was very gracious," says Mello. "The worm community is a very sharing community."

After more graduate work at Harvard University and a postdoctoral fellowship at Seattle's Fred Hutchinson Cancer Research Center, Mello set up his own laboratory at the University of Massachusetts Medical School in 1994. The same year, a graduate student at Cornell University named Su Guo made a puzzling discovery. She was injecting molecules known as antisense RNA into *C. elegans* to block the action of a particular gene. (Antisense RNA molecules are designed to bind with single-stranded RNA molecules that have an exactly opposite genetic sequence.) But when Guo injected "sense" (or same-sequence) RNA—which should not bind with messenger RNA—into the cell to establish a control, the action of the gene also was blocked. "That was a surprise," says Mello. "But everyone assumed that there was antisense contaminating the sense RNA preparation."

Mello's lab worked primarily on the developmental biology of *C. elegans*, but he was so intrigued by Guo's observation that he launched a separate project to explore it. One day, after injecting antisense RNA into *C. elegans*, he got distracted and did not check the worms until after they had reproduced. Amazingly, he found that their progeny also exhibited gene silencing, even though there was no obvious way for the effect to travel from one generation to the next. "That to me was stunning, unbelievable," he says.

Not long afterward, another fortunate accident occurred. Sam Driver, a new graduate student in Mello's lab, was trying to inject RNA into particular cells and kept missing. Yet even when he hit cells that weren't anywhere near his target, the gene silencing spread from one cell to another, though there was not nearly enough RNA for the effect to be so widespread. "We had no clue what was going on," Mello recalls.

Fire, who was then at the Carnegie Institution of Washington's Department of Embryology in Baltimore, had been conducting similar experiments, and he and Mello often compared results over the phone and at meetings. One day Fire made an off-the-wall suggestion. Maybe the preparations of single-stranded RNA also contained small amounts of double-stranded RNA, and the



Nucleus

In RNA interference, double-stranded ribonucleic acid (dsRNA) targets destruction of messenger RNA (mRNA) that has the same sequence it has. The Dicer enzyme cuts dsRNA into small inhibitory RNA (siRNA), which lures mRNA into the RISC (RNA-induced silencing complex) protein complex where it is destroyed.

Illustration adapted from HHMI BioInteractive

double-stranded RNA was causing the effect. The idea seemed improbable. Double-stranded RNA should be relatively inert in a cell, whereas single-stranded RNA would bind to complementary strands. But Mello was beginning to wonder if the single-stranded RNA was working alone in *C. elegans*. The only way for the effect to spread from cell to cell or from parent to offspring was if it was being amplified by some sort of cellular machinery.

Fire's idea cracked open the case. When he isolated double-stranded RNA from the preparations of single-stranded DNA, he found that the double-stranded RNA had a much stronger silencing effect than either the antisense or the sense single-stranded RNA.

Mello, Fire, and their colleagues published their results in *Nature* in 1998, calling the effect RNA interference (or RNAi) after polling other worm researchers for an appropriate name. Their paper triggered an avalanche of new findings. Mello, Fire, and other researchers soon uncovered a cellular mechanism that explains not only the original findings but other unexplained occurrences of gene silencing in organisms such as petunias.

Furthermore, the mechanism they discovered turned out to be part of a much larger system that regulates the expression of many genes in multicellular organisms, including humans. When double-stranded RNA is introduced into a cell, a protein known as Dicer cleaves it into double-stranded fragments 20 to 25 base pairs long. The fragments are then incorporated into a molecular structure known as RISC (RNA-induced silencing complex), which degrades messenger RNAs that have the same nucleotide sequence as the RNA fragments, blocking the action of the gene that produced the messenger RNA.

As soon as it was discovered, RNAi was put to work as a powerful research tool. Researchers use it to silence genes one by one to determine the effects of each gene on a cell's growth and function. The same technique can block the action of genes that have gone awry in disease. Today, RNAi is being tested for its ability to treat respiratory infections, macular degeneration, hepatitis, cancer, and other illnesses.

BEEN THERE, DONE THAT

BY RICHARD SALTUS

Along with receiving the greatest scientific honor that exists, 2006 Nobel Prize winner Craig Mello will enter a new world of expectations and competition for his time at the lab bench, say his colleagues who have lived the dream. Asked what words of advice they would give (or have given) Mello, four Nobelists had this to say:

"I would advise Craig to view the Nobel Prize as a test of one's ability to remain highly focused on his research program."
ROD MACKINNON (2003), *an HHMI investigator at The Rockefeller University*

"Within a few days I realized I would have a new responsibility: communicating the importance of basic science to the public. This creates an organizational challenge when one's primary interest is in doing research."
LINDA BUCK (2004), *an HHMI investigator at the Fred Hutchinson Cancer Research Center*

"I told Craig that one of the most special experiences he will have in his life is about to occur—the week in Sweden celebrating the Nobel Prize. Sweden has a wonderful style for this celebration and is a gracious host. He should take the time to enjoy the experience. I would also advise him to reserve time for his family and research over the next few years as the demands for his attention are going to greatly increase."
PHIL SHARP (1993), *MIT and past member, HHMI Medical Advisory Board*

ROBERT HORVITZ (2002), *an HHMI investigator at MIT, agreed with the others and said to Mello,* "Preserve your time for those things you really care about, or would really enjoy," *adding as a final postscript:* "Ask the Nobel Foundation to award the Prize money after January 1, so you don't have to pay taxes on it in 2006. The United States is the only country in the world that requires Nobel Prize recipients to pay income tax on the award."

Don't Hang Up

THE INSULIN GENE, WHICH FIRST ATTRACTED Mello to biology, has continued to influence his life. Four years ago, his daughter Victoria, who is now six, was diagnosed with type 1 diabetes. Mello's wife Edit, whom he met at a dance the same year the *Nature* paper was published ("it was a good year," Mello says), is a nurse, and the two of them meticulously monitor Victoria's blood sugar level as a part of a daily regimen of insulin injections. "Our pediatrician recommended that we check her every night in the middle of the night," Mello says. "Usually we check her about 1 a.m., and if all is well we can sleep for the rest of the night. But if at 1:00 her blood sugar is high, we have to give her insulin, and then we have to check her later to make sure that everything is okay."

When the call from the Nobel committee came on October 2, Mello was actually in Victoria's room checking her blood sugar. Edit didn't know that the prize was being announced that night and hung

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up on the first call. "She thought it was a crank call, because who calls at that time, anyway?" But Mello's father had mentioned to him the previous day that the prize was being announced that night.

Craig: [As the phone rang again] I said to her, in a calm voice, "Yeah, but they're announcing the Nobel Prize tonight, so I'd better pick it up."

Jim: He called me after he got the news. At first I was elated, and then I said, "Craig, are you sure this isn't a joke." And [Craig] said, "I'm watching the Nobel screen right now; and, yup, there comes Andy's and my name at the top of their Website."

Craig: It was just amazing.

Mello plans to use his Nobel Prize to make the case that great opportunities await those who are bold enough to pursue them. When a phenomenon like RNA interference is discovered, those in charge of research funding face a critical decision, he says. "I like to compare it to discovering a new continent. You could keep sending out a few more discoverers. Or you could say, look, we have something really valuable here that could allow us to make a lot of headway in medical science in the next 10 years. We may develop new drugs. We may cure neurodegenerative diseases. We may make lots of progress on infectious diseases. We're maybe going to figure out how to prevent obesity and diabetes. It's all at our fingertips. But we have to aim to steadily increase the expenditures we've been making so that we can reap the rewards."

In the process, Mello says, "We're going to create good jobs in this country and to help people all over the world. It's a win-win situation. [And] for the Bush Administration, what I would say is that this was discovered under your watch. It's your opportunity to make history."

Mello has just one regret, he says. After the commercial success of the iPod, "I wish I had patented that little 'i.'" ■