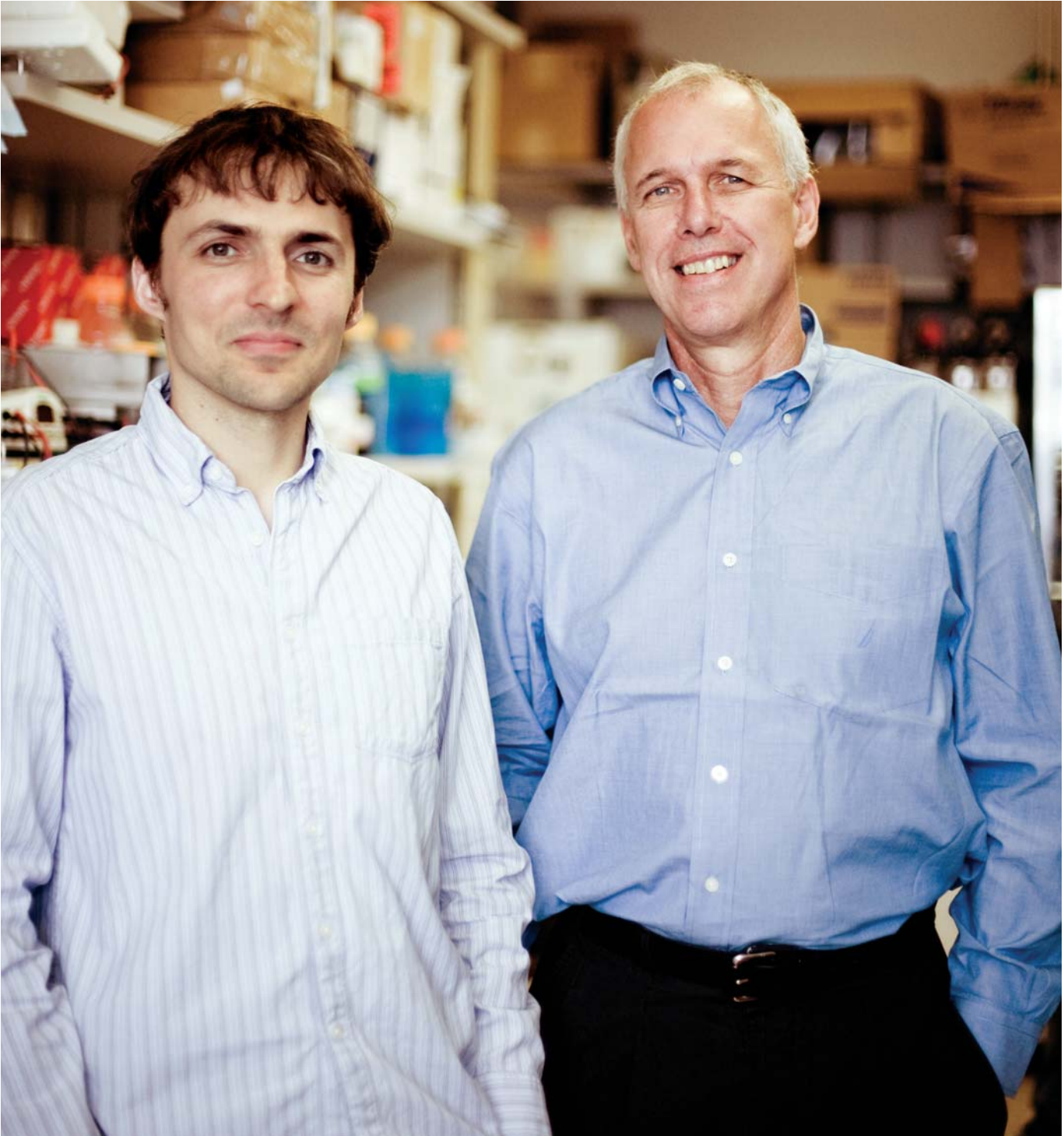


# Seeing Spots

*Relying on old-fashioned techniques allowed these researchers to make a surprising discovery.*



*Skirmantas Kriaucionis and Nathaniel Heintz found a new piece of DNA's genetic code and energized the field of epigenetics research.*

*Elizabeth Weinberg*

THE HISTORY OF SCIENCE GLITTERS WITH ACCIDENTAL DISCOVERIES, FROM Roentgen's x-rays to Fleming's penicillin. And despite what some would consider a creeping loss of flexibility in the modern scientific process, researchers from time to time still stumble across something truly surprising. Just ask HHMI investigator Nathaniel Heintz and his postdoctoral associate Skirmantas Kriaucionis.

Their serendipitous finding of a DNA constituent called hydroxymethylcytosine—roughly akin to the discovery of a new letter of the AGCT genetic code—has energized the fast-growing field of epigenetics, the study of the all-important mechanisms that maintain genes in an “on” or “off” state within cells.

“Our finding of this nucleotide in animal cells was completely unexpected,” says Heintz, whose lab is based at Rockefeller University in New York City.

It began with a dark spot on a glass chromatography plate—a spot that shouldn't have been there. Under Heintz's guidance, Kriaucionis had been trying to measure levels of a known epigenetic marker, a gene-silencing nucleotide known as methylcytosine (mC), in a sample of DNA from mouse Purkinje cells.

These large neurons, located in the movement-coordinating cerebellum, are difficult to harvest in large quantities, says Heintz: “To get enough DNA to do the analysis was difficult, so we used a very sensitive, old-fashioned methodology for detecting DNA nucleotides.”

The experiment yielded a chromatogram, in which separate spots of clustered material indicate the presence of unique DNA nucleotides within the sample. But near the expected spot for mC Kriaucionis saw another spot in a surprising location, suggesting an unknown nucleotide.

Was it an experimental artifact? “We were suspicious,” Kriaucionis remembers. After searching the literature and repeating

the experiment numerous times with different controls, however, the researchers realized that the mystery spot marked a nucleotide known as hydroxymethylcytosine (hmC). Previously, hmC had been considered a rare DNA modification found only in primitive, bacteria-infecting viruses. Its relative abundance in a mammalian brain cell strongly suggested that it could be a major epigenetic player.

That its presence had been overlooked for so long also suggested that molecular biologists needed to rethink some of their

experimental methods, according to Heintz. The Heintz lab had long emphasized the use of cells taken from animals, but if Kriaucionis had instead relied on immortalized neuronal cell lines, which are more convenient to use but differ subtly from normal neurons, he would have found no hmC. He also determined that the standard sequencing technique for mapping mC in cellular genomes could not distinguish hmC from ordinary mC.

As it turned out, other researchers had been on a similar epigenetic quest. A week after Heintz and Kriaucionis submitted their paper to *Science*, a group led by Harvard Medical School scientist Anjana Rao submitted their own closely related

finding. Hydroxymethylcytosine, they reported, is present in mouse embryonic stem cells and is converted from mC by an enzyme found in humans as well as mice. “Her discovery of an enzyme that actually does this hydroxylation is critically important, for it tells us that there's a dedicated biochemical mechanism for producing this modification,” Heintz says.

The two papers appeared together in *Science* on May 15, 2009, and both labs are now trying to understand hmC's functions in different cell types. Some evidence already suggests that hmC can reverse mC's usual gene-silencing function, although Heintz thinks it could have other, more interesting epigenetic roles. To help clear up the mystery, he and Kriaucionis are first

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NATHANIEL HEINTZ

trying to map hmC's distribution in the DNA of various cell types—a goal complicated by the lack of a high-resolution method that can separate hmC from mC. “It is critical to develop a new chemical strategy to allow us to precisely map the locations of both of these marks in the genome,” says Heintz. The two researchers also have begun collaborating with Rao's group to manipulate hmC levels in different types of mouse neurons to see how it affects their gene expression.

The apparent significance of hmC has drawn keen interest from other researchers, too. Based on queries she's had from other scientists, Rao says “at least fifty other labs are interested in this now.” ■ —JIM SCHNABEL