Profile of Arthur D. Riggs

A molecular biologist and pioneer in the field of DNA methylation epigenetics, Arthur Riggs was elected to the National Academy of Sciences in 2006. Riggs and his colleagues were the first to produce human insulin in *Escherichia coli*. He is also known for his work on mammalian DNA replication, protein-DNA interactions, and the production of recombinant antibodies.

Riggs has spent most of his career as a researcher, and later the director, at the Beckman Research Institute of the City of Hope National Medical Center, a National Institutes of Health sponsored cancer center in Duarte, California. His current work focuses primarily on mammalian epigenetics.

Riggs' Inaugural Article, published in the January 22, 2009 issue of PNAS (1), investigates genome-wide DNA methylation. Specifically, he and his colleagues examined at high resolution the pattern in DNA of 5-methylcytosine, an epigenetic mark formed by the enzymatic addition of a methyl group to cytosine in double-stranded DNA after replication. Among a number of possible functions, DNA methylation is thought to help pass information with high fidelity from parent cells to daughter cells, providing an information coding system in addition to that of the primary nucleotide sequence.

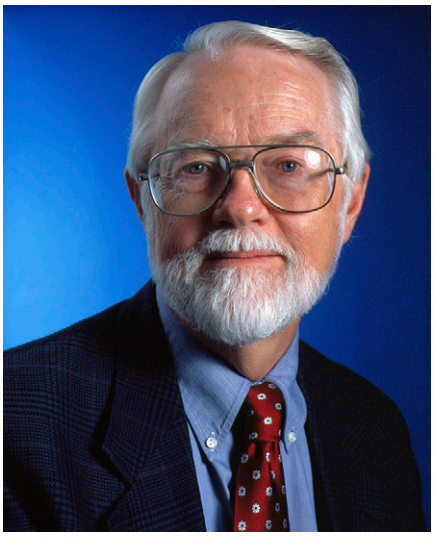
These epigenetic marks are thought to lock genes in an inactive state and help stable maintenance of cell phenotype. In the article, the authors describe the DNA methylation pattern of the genome of a human B cell. Riggs conducted this work in collaboration with Gerd Pfeifer after stepping down from an eight-year stint as director of the Beckman Research Institute.

From Science Fiction to Science

Riggs was born in Modesto, in central California, to parents who lost their farm in the Great Depression and moved to San Bernardino in southern California. His mother, a nurse, encouraged his interest in biology and chemistry and gave him a chemistry set at age 10.

"I thoroughly enjoyed mixing reagents and getting changes in color and carbon dioxide release," he recalls. "That and reading science fiction got me enthusiastic about science in junior high school."

His father built and managed a mobile home trailer park in San Bernardino, designing the electrical and plumbing systems, as well as doing most of the construction work. The elder Riggs also designed, built, and flew small autogyro airplanes as a hobby.



Arthur Riggs.

"Helping my father was a great learning experience," says Riggs. "But I also remember sometimes telling my father that I had homework to do in order to avoid digging ditches. I would then read science fiction rather than do my homework. But I was always a good student and the science classes at San Bernardino High School were quite good."

For college he went to the nearby branch of the University of California, Riverside.

"After I took a class in organic chemistry, I decided to be a chemistry major," Riggs says. "You have to do some mental manipulations when you're thinking about organic compounds and their synthesis. It was fun for me, and I was good at it. Spatial relations are apparently one of my talents. I wound up being the best in the class."

He also took enough biology coursework to get a double major in biology and chemistry, and although he did not complete the thesis requirement, he found himself gravitating toward biochemistry as his graduation neared in 1960.

He applied to and was accepted by the PhD program in biochemistry at the California Institute of Technology (Caltech, Pasadena, CA).

"My plan was to use my chemistry knowledge and talent to make new discoveries about how organisms and biological systems work," he says.

Expected To Be Outstanding

At Caltech, Riggs felt that he was not as sophisticated as the students trained at academic powerhouses like Harvard or Stanford.

"They had a better understanding of how science really worked," he says. "But as far as the fundamentals, as far as the chemistry knowledge, my physics and mathematical background, I found that I was actually able to compete very well."

Riggs's graduate school mentor was Herschel (H.K.) Mitchell. "I chose him because I enjoyed playing basketball, and he was the coach of an intramural basketball team," Riggs says.

Mitchell specialized in the developmental genetics of *Drosophila*. Riggs studied *Drosophila* for a few years, but then switched to a project designed to determine the size of a *Mycoplasma* genome.

"Mitchell's policy was to allow his students to do anything they wanted," says Riggs. "Most professors at Caltech allowed a lot of freedom to their students, but we were expected to do outstanding work, and that was the situation with Mitchell."

Just as Riggs began to write his doctoral thesis, he and fellow student Joel Huberman decided to do an experiment that combined their areas of expertise. They wanted to label replicating DNA with pulses of radioactive nucleotides and expose photographic film to the DNA so that they might get a physical picture of what happens during replication. Their supervisors told them not to bother, but the students were undeterred.

"Joel and I didn't exactly sneak in," he adds, "but we did come in at night and did the experiment anyway. Then we had to put it in the freezer for 3 months. I went back to working on my thesis, and Joel went back to his other project. But the results were spectacular."

In fact, once their supervisors saw the results, they gave their full support.

"These experiments led to better understanding of mammalian DNA replication," Riggs says. The investigators learned that chromosomal DNA was composed of many sections that replicated independently and that, at each section's origin, replication forks proceeded in both directions (2).

From the experiment, the students were able to measure the rate of DNA replication. Despite the importance of the findings, Mitchell and Guiseppe Attardi, Huberman's supervisor, insisted on leaving their names off the resulting papers, because both supervisors had initially said not to do the work.

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 671 in issue 3 of volume 106.

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"I'm not sure that advisors would be that generous these days," Riggs observes.

Gene Regulation and Inactivation

After finishing his PhD in 1966, Riggs took a postdoctoral job under Melvin Cohn at the Salk Institute (San Diego, CA).

In collaboration with Susan Bourgeois, Riggs was the first to study protein–DNA interactions by using a purified transcription factor, the *lac* repressor. At the time, only two proteins that bind to DNA and control gene expression had been detected in cell extracts: the *lambda* repressor and the *lac* repressor. His research group was the first to isolate a useful quantity of either (3, 4).

"I was able to obtain milligram amounts of pure *lac* repressor—enabling study of the first transcription factor protein," he says. "That was, in its time, a major accomplishment."

As Riggs began to consider establishing his own lab, he turned toward the regulation of gene transcription in mammalian cells. He was particularly fascinated by X chromosome inactivation, in which one copy of the X chromosome—either the maternal or the paternal—is randomly turned off in each cell in the body of a mammalian female.

That interest in X chromosome inactivation attracted him to the City of Hope's research institute where Susumu Ohno had established a reputation as codiscoverer of X chromosome inactivation and was writing a book on evolution by gene duplication.

"I became aware that there was a possibility of becoming a faculty member in Dr. Ohno's department," Riggs says. "I wanted to work on X chromosome activation, using that as a model system to study gene regulation in mammals. So I decided to come to City of Hope as my first independent job. I have now been at the City of Hope for 40 years."

Somatostatin and Insulin

"Even though X chromosome inactivation was a very interesting puzzle," he says, "it was also difficult to approach experimentally. So for a while I returned to *lac* repressor work and gene regulation in bacteria."

He began collaborating with Caltech's Richard Dickerson and his postdoc, John Rosenberg, and recruited a chemist, Keiichi Itakura, as a faculty member at City of Hope.

"My part of the project was to help prepare large amounts of *E. coli lac* repressor. Itakura's job was to synthesize the *lac* operator, the piece of DNA that the *lac* repressor binds to, and then we were going to mix those together and hopefully get them to crystallize. And if we got crystals, then we would be able to do high-resolution analysis of protein–DNA binding."

The crystallography project led Riggs to work with Herbert Boyer at the University of California, San Francisco. Soon after Riggs began this collaboration, Boyer cofounded Genentech, which funded much of the research they conducted together.

With Herbert Heyneker, a postdoc in Boyer's lab, Riggs and his colleagues cloned the *lac* operator made by Itakura and conducted experiments to determine whether the operator actually worked in live bacteria. It did, which was a landmark result that the researchers published in *Nature* (5).

"These experiments led to better understanding of mammalian DNA replication."

Riggs and colleagues also developed the "linker" method (6), widely adopted thereafter, in which researchers add short links of DNA-containing sites recognized by restriction enzymes to the DNA sequence they wish to clone. The investigators can then easily insert the modified sequence into a bacterial plasmid.

Riggs' team set their targets on developing a way to produce, on a large scale, medically useful reagents.

Insulin became their goal because of its small size and importance as the hormone that regulates blood glucose. However, before tackling insulin, Riggs, Boyer, Itakura, and their coworkers needed to prove their method would work.

For their initial experiment, they chose somatostatin, a 14-unit peptide roughly one-tenth the size of insulin. The researchers did not know the nucleotide sequence of the gene for somatostatin in human DNA, but they did know the amino acid sequence, so they worked backward, using the genetic code to make a gene coding for somatostatin. The gene was thus designed from scratch and designed to work in *E. coli*.

In preliminary experiments, Riggs and his colleagues found that *E. coli* enzymes degraded somatostatin soon after translation, but that if somatostatin was linked to the much larger protein, beta-galactosidase, it could be produced in *E. coli*, isolated as a unit, and then separated from the galactosidase (7).

"It was the first human-designed and man-made gene that functioned in any organism," Riggs says. "It was the first mammalian hormone produced in bacteria, and it jump-started the biotechnology industry."

To produce insulin was then a relatively straightforward task (8).

"We used the same method, simply writing out—and then making—a gene coding for human insulin," Riggs says. "But the insulin was composed of two peptide chains that had to be joined [by disulfide bonds]. Together they were about 10 times larger than the somatostatin."

There was tremendous media interest in the somatostatin and insulin projects (9). Riggs, Boyer, and Itakura were also competing with other groups after the same goal, trying to clone the human gene for insulin.

One competing group was led by Walter Gilbert at Harvard University (Cambridge, MA) and another other was led by William Rutter and Howard Goodman at University of California, San Francisco. Stephen Hall chronicled the competition in a 1987 book, *Invisible Frontiers: The Race to Synthesize a Human Gene* (10).

Despite the media and commercial attention, Riggs continued his research on X chromosome inactivation and continued to work with Genentech.

"I did not actively work in other start-up companies or even as an advisor for other companies," he says. "I had a contract to do work for Genentech until about 1984. After the insulin work, the main work for them was on recombinant antibodies, that is, using recombinant DNA technology to make antibodies."

Epigenetics

Riggs' work with restriction enzymes used for recombinant DNA led him back to his original interest in X inactivation. He had a conceptual breakthrough in 1973 while spending a short time in Boyer's lab learning about the function of restriction enzyme complexes.

"It dawned on me that the properties of an *E. coli* restriction system could be used to explain X chromosome inactivation," he says. "It took me a couple of years to get my idea written down. I eventually got it published—a theoretical paper that correctly predicted a key mechanism for DNA methylation epigenetics."

"The paper was kind of buried, but at least a couple of others noticed it, and we all used the model to guide our experiments," Riggs notes. "And it turns out that the DNA methylation system does work pretty much as I predicted in 1975 (11). Mammalian cells do have an enzyme that adds a methyl group to cytosine very slowly if the DNA site is unmethylated in both strands, but methylation is rapid if the DNA site already has 5-methylcytosine in one strand. This feature allows methylation patterns to be maintained through DNA replication. In 1975, there were only a

handful of papers on epigenetics; now there are thousands, but my 1975 paper is still highly cited."

The Administrator

Starting in 1979, Riggs became increasingly involved in administration at City of Hope. First, he was appointed associate chairman of its division of biology and then became chairman, a position he held for most of the next 20 years. In the early 1990s, he helped create a standalone PhD program at City of Hope, unaffiliated with any university, for which he served as dean from 1994 to 1998. In 1999, he accepted the position of director of the Beckman Research Institute, the City of Hope's laboratory research arm.

"Administration can be and probably should be a full-time job," he says. "But I prefer research, so by 2007 I felt it was time to step down and get back to research."

Since his return to laboratory work, Riggs is, once again, focused on DNA methylation epigenetics.

"It's a cellular memory mechanism dependent on the formation of 5-methylcytosine in DNA," he says. "Enzymes decorate DNA with methyl groups in a way that can be read as a record of a cell's developmental history, and this information is passed on to daughter cells."

In his Inaugural Article (1), Riggs and colleagues described a human B cell's "methylome," which refers to the DNA methylation pattern of the entire genome. They chose B cells for analysis because the cells provided a relatively uniform population. Many of the features they observed are likely the same for all cells, whereas some will be specific to cell type.

"One of the more interesting things we learned was that methylation is correlated with chromosome bands seen by classical staining methods" he says. "We also made the surprising observation that methylation within genes increases, rather than decreases, with transcription rate." This was opposite to expectation; methylation is generally thought to prevent transcription of DNA.

"Overall," he adds, "we obtained almost too much information, and it's going to take years to figure out the full significance. But it was a milestone to have a complete profile of DNA methylation throughout the entire genome at 100 base pair resolution."

And it is not just any genome: Riggs donated the blood used to isolate the B cells. "It could have been anyone's DNA, but as a pioneer in DNA methylation epigenetics, there is something special to me about it being my methylome," Riggs says.

Kaspar D. Mossman, *Science Writer*

1. Rauch TA, Wu X, Zhong X, Riggs AD, Pfeifer GP (2009) A human B cell methylome at 100 base pair resolution. *Proc Natl Acad Sci USA* 106:671–678.
2. Huberman JA, Riggs AD (1968) On the mechanisms of DNA replication in mammalian chromosomes. *J Mol Biol* 32:327–341.
3. Riggs AD, Bourgeois S (1968) On the assay, isolation, and characterization of the lac repressor. *J Mol Biol* 34: 36l–364.
4. Riggs AD, Bourgeois S, Newby RF, Cohn M (1968) DNA binding of the lac repressor. *J Mol Biol* 34:365–368.
5. Heyneker HL, et al. (1976) Synthetic *lac* operator DNA is functional in vivo. *Nature* 263:748–752.
6. Scheller RH, Dickerson RE, Boyer HW, Riggs AD, Itakura K (1977) Chemical synthesis of restriction enzyme recognition sites useful for cloning. *Science* 196: 177–180.
7. Itakura K, et al. (1977) Expression in Escherichia coli of a chemically synthesized gene for the hormone somatostatin. *Science* 198:1056–1062.
8. Goeddel DV, et al. (1979) Expression in Escherichia coli of chemically synthesized genes for human insulin. *Proc Natl Acad Sci USA* 76:106–110.
9. McElheny V (December 2, 1977) Coast concern plans bacterial use for brain hormone and insulin. *New York Times,* Section D, p1.
10. Hall S (1987) *Invisible Frontiers: The Race to Synthesize a Human Gene* (A Morgan Entrekin Book/Atlantic Monthly Press, New York).
11. Riggs AD (1975) X inactivation, differentiation, and DNA methylation. *Cytogenet Cell Genet* 14:9–25.