

Meselson and Stahl: The art of DNA replication

In 2003, the scientific community celebrated the 50th anniversary of James Watson and Francis Crick's landmark 1953 paper on the structure of DNA (1). The double helix, whose form has become the icon of biological research, was not an instant hit however. The model did not gain wide acceptance until the publication of another paper 5 years later.

Matthew Meselson and Franklin Stahl's experiments on the replication of DNA, published in PNAS in 1958 (2), helped cement the concept of the double helix. Meselson, a graduate student, and Stahl, a postdoctoral researcher, both at the California Institute of Technology (Pasadena), gave validity to a model that many scientists saw as speculation: how two intertwined and tangled strands of a helix could physically code for the material of inheritance. The Perspective by Philip Hanawalt of Stanford University (Stanford, CA), in this issue of PNAS (3), reviews the scientific evolution of this crowning achievement and outlines its subsequent impact on four decades of DNA replication, recombination, and repair research. The two men behind the laborious steps in discovering the semiconservative replication of DNA credit much of their success to timing, hard work, and serendipity.

A Partnership Begins

During his third year of graduate school at the University of Rochester (Rochester, NY), one of Stahl's advisors suggested that he take a physiology course and sent him to the Marine Biological Laboratory in Woods Hole, MA. "I partied my way through that course," Stahl confesses. "During the partying, I met Meselson," who was also temporarily at Woods Hole, working as a teaching assistant. During that summer of 1954, the double helix model had been well received but was only truly accepted by an enthusiastic minority of scientists. "Matt had the idea that one ought to be able to use density labels to test Watson's hypothesis," said Stahl. Although at Woods Hole, Meselson was a graduate student with Linus Pauling at the time at Caltech. There, Meselson had heard Jacques Monod speak about the nature of chemical bonds and enzyme synthesis, which gave Meselson a new technique idea for working with β -galactosidase in bacterial protein synthesis and measuring changes in protein density.

To explore the project, Pauling, whose work centered on x-ray crystallography, sent Meselson to another Caltech professor, Max Delbrück, to learn about the

biological aspects of the necessary experiments. Meselson credits Delbrück with giving him the information that would change the nature of the project. As he thrust the Watson and Crick papers toward the young scientist, "He said, 'Read these and don't come back until you have,'" Meselson recalls. Up to that point, Meselson admits that he had not been aware of Watson and Crick's work or their DNA structure model.

Stahl planned to go to Caltech for his postdoctoral work, and at Woods Hole he and Meselson decided to collaborate on the density label project in their spare time. "Caltech is a cozy community. It's ruled by ideas, not by walls," says Stahl. When he arrived at Caltech, Stahl began a bacteriophage project that did not end well after he inadvertently switched the labels on some culture plates. "In the midst of this gloom and doom, Matt came in," Stahl says. Meselson had finished his main research project and was ready to tackle Watson and Crick's hypothesis. Thus, Stahl changed his focus from bacteriophages to DNA replication.

Not as Simple as It Seems

Meselson and Stahl faced a tangled problem. The Watson and Crick double helix seemed to suggest that the two strands dissociated, each giving rise to a new, complementary strand. The two daughter molecules would thus contain one strand each from the parent molecule, in a semiconservative replication fashion. If replication were conservative, however, the intertwined strands would be replicated as a whole. This would produce one daughter molecule with all original information and one with all new information. The third model, termed dispersive replication, considered that each strand of the daughter molecule could consist of DNA that had been shuffled around so each strand was a hybrid of old and new.

According to Meselson, "There were 2 years of things that didn't work" followed by a year of successful experiments. Jan Drake, then a postdoctoral researcher at Woods Hole, reflected on the years he shared a rented house with Meselson and Stahl and recalls that they all worked the same hard hours kept by many graduate students and postdoctoral researchers today. They would often discuss their work over dinner before returning to the laboratory in the evening. Despite the long hours, results were not immediately forthcoming. Yet perseverance prevailed, and Meselson

and Stahl finally designed a successful experiment that would help distinguish new daughter strands from the parent strand.

Hanawalt's Perspective (3) outlines the intricacies of the differential nitrogen (^{14}N and ^{15}N) labeling and subsequent separation of the DNA. The experiments demonstrated that Watson and Crick's model of the double helix could replicate itself in a concerted, semiconservative fashion, and the results were published in PNAS after being communicated by Delbrück.

The Legacy of Elegant Peaks

Now, more than 45 years later, the paper is still held aloft for its clarity. Looking back, though, Meselson says the paper has "one thing I wish weren't there." At the time, published research from an established scientist, Paul Doty (4), seemed to show that salmon sperm DNA did not come apart when heated. Meselson and Stahl's research could then have two implications: either Doty was incorrect or *Escherichia coli* DNA actually had four strands. Hence, Meselson and Stahl were cautious with their wording and used the term "subunit" instead of "strand." "We were little graduate students," Meselson says. He and Stahl were wary of contradicting an established scientist. "I wish we had had the courage. You should believe in your convictions," says Meselson. Doty's conclusions were later found to be incorrect because the instruments used were not sensitive enough to detect the DNA molecular weight changes.

Stahl credits the beauty and success of their paper to two things. First, the "delightfully clean data" were serendipitous. The clean data peaks they observed resulted from the DNA fragmenting during handling; unfragmented DNA would not have separated as nicely. Stahl likens pipetting DNA to "throwing spaghetti over Niagara Falls." The stress of the pipetting caused tremendous shearing of the DNA, although they did not realize this at the time, nor did they realize how critical this would be to obtaining clean peaks. In addition, Meselson was a "stickler for clarity," said Stahl. "Every single word in that paper was discussed several times before being allowed to keep its position in the sentence."

Such clean data and clear writing, in addition to the significance of the paper for the field of molecular biology, have

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placed Meselson and Stahl's experiment on the pages of many a syllabus. At the Massachusetts Institute of Technology (Cambridge, MA), Professor of Biology Tania Baker says the experiment is part of a course required of all molecular biology graduate students. "It is a very nice test of a model of replication," she says. "Conceptually, it's a very important technique."

Today, the "little graduate students" stay in touch. Stahl is a professor at the University of Oregon (Eugene, OR), and Meselson is a professor at Harvard University (Cambridge, MA). As definitive as the 1958 paper may appear in its elegance and simplicity, its greater legacy is the subsequent research it has fostered. Cold Spring Harbor Laboratory (Cold Spring Harbor, NY) hosts a

meeting for scientists in the field of DNA replication every other year. President and CEO Bruce Stillman acknowledges that it is not a large field—the attendees can fit into a single auditorium—but states that it is a very active one. Stillman says, "Forty-five years after Meselson and Stahl, we've still got work to do."

Tinsley H. Davis, *Science Writer*

1. Watson, J. D. & Crick, F. H. C. (1953) *Nature* **171**, 964–967.
2. Meselson, M. & Stahl, F. W. (1958) *Proc. Natl.*

Acad. Sci. USA **44**, 671–682.

3. Hanawalt, P. C. (2004) *Proc. Natl. Acad. Sci. USA* **101**, 17889–17894.

4. Rice, S. A. & Doty, P. (1957) *J. Am. Chem. Soc.* **79**, 3937–3947.