

Molecular Biology Through Discovery

DNA Replication (Part 2): The Meselson-Stahl Experiment

Meselson MS, Stahl FW (1958) Proc Natl Acad Sci USA 44:671-682

Outline:

- A. Problems raised by the double helix
- B. Meselson and Stahl experiment
- C. Reconciliation of semi-conservative replication with the double helix

A. Problems raised by the double helix

Avery, MacLeod, and McCarty presented the accumulated weight of a decade of experimental work that pointed to DNA as the identity of the genetic material.¹ The article was widely known, but few accepted their conclusions.² Nine years later, Watson and Crick offered their model of the double helix plus a coy hint that their proposed structure explained how the genetic material might be replicated.³ They included essentially no experimental evidence supporting the model, ... yet the world snapped it up as self-evident truth! No scandal here. The key difference was that the double helix had the power to reorganize how one thought about biological information. It isn't always evidence that carries the day. Often explanatory power is even more persuasive.

But the model presented a severe problem to its adherents, one that was immediately identified by Watson's mentor Max Delbrück, first in discussion⁴ and later in print.⁵ Central to the model was the winding of two strands of DNA around themselves. This is certainly a point of esthetic beauty, but Delbrück realized that it would be a real headache to unwind the strands, ... and unwind they must be to execute the mechanism of replication suggested by Watson and Crick (**Fig. 1**).

The problem is that if you take two strands wound about themselves – like a rope -- and pull them apart, either the still wound portion of the rope will start twirling wildly, or it will tangle up into a mess. If the rope is attached to something heavy (e.g. a braid attached to someone's head), then tangle is the only humane choice. It was difficult to see how unwinding a portion of a very long DNA molecule could cause the entire molecule to rotate against the resistance of water, so tangle seemed inevitable. Worse, it soon became apparent that DNA in many organisms was circular (**Fig. 2**),⁶ so rotation was not possible.

SQ1. Suppose you had two shoelaces wound up together, like DNA. How could you separate them? Suppose the shoelaces were many miles long.

Delbrück saw a clever way out of this dilemma, retaining Watson and Crick's structural model but abandoning their notion of how DNA was replicated. The trick (**Fig. 3**) was to replicate the double helix in pieces, cutting and pasting each half-turn of newly synthesized DNA strand to the

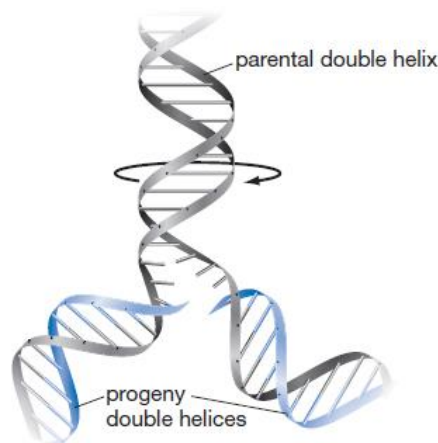


Figure 1. Replication of DNA double helix. (from Ref 9)

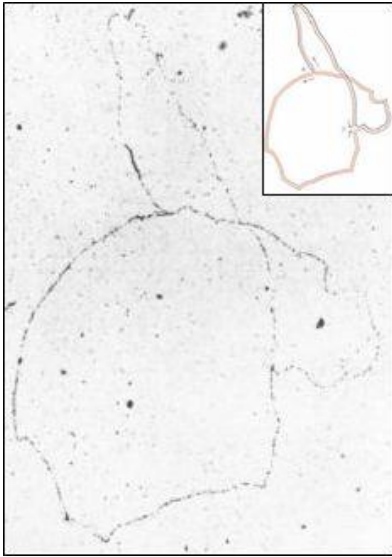


Figure 2. Autoradiograph of replicating DNA from *E. coli*. DNA carefully isolated from *E. coli* grown in [³H]thymine. The dark grains are caused by radioactive decay of the radioactive base. The inset shows an interpretation of the autoradiograph. (image from Ref. 6)

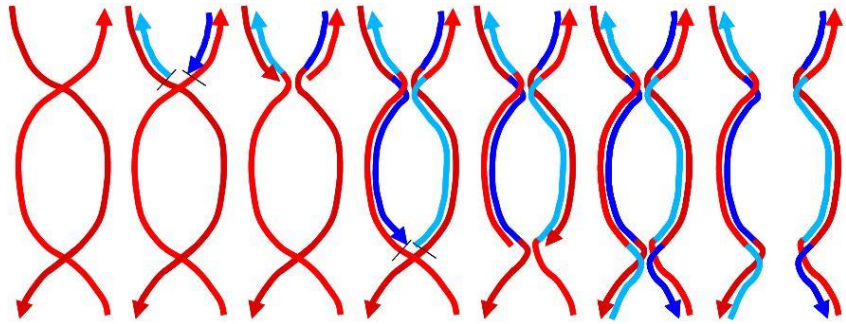


Figure 3. Diagram of dispersive DNA replication as described in Ref. 5. A portion of a DNA molecule is shown replicating, with time proceeding from left to right. Parental DNA is shown at the far left, with two antiparallel strands in red. As synthesis of new strands (in blue) is completed for a half turn of the helix (one loop), the two parental strands are broken and joined to the new strands. The result (far right) is two daughter DNA molecules that are not interconnected and that therefore can separate from each other. Each of the four strands is a mixture of new and old DNA. (Image adapted from Ref. 5)

original strand going in the same direction. By the end of the process, the double helix would be replicated, *and* the two daughter molecules would be separated.⁵ The replication mechanism illustrated in **Fig. 3**, derived from Delbrück's article, is an example of *dispersive* DNA replication, whose distinguishing feature is that the daughter molecules that result from replication are a mixture of DNA derived from parental strands and newly synthesized DNA.

SQ2. Follow Fig. 3 from left to right. What mechanism leads to strands in the right-most frame to alternate in color between red and blue?

So, in the mid 1950's there were two competing views regarding DNA replication: Delbrück's dispersive model (**Fig. 3**) and Watson and Crick's *semiconservative* model (**Fig. 1**), so called because it predicted that each daughter strand carries one strand conserved from the parent molecule. The first explained how replicating DNA avoided getting tangled up, while the second was just much prettier. A third possible was also put forth – *conservative* replication, in which the parent strand remains intact and the daughter strand is composed completely of newly replicated DNA.⁷

SQ3. What are the stakes in determining which (if any) of the three models are correct?

B. Meselson and Stahl experiment⁸

In 1954, Jacques Monod from the Pasteur Institute* visited Cal Tech† and gave a talk regarding his work on how bacteria retool in the face of changing environmental conditions. In the audience was Matt Meselson (**Fig. 4**), a graduate student at Cal Tech in Linus Pauling's group.

* You'll recall from last week that Benzer spent a year at the Pasteur Institute just prior to starting his work to understand the topology of genes.

† You'll recall from last week that Benzer spent two years at Cal Tech working with Max Delbrück before going to the Pasteur Institute.

Monod told his audience about a critical open question – whether proteins induced by environmental conditions (e.g. a change in food source) are newly made or instead modified from existing proteins. Meselson was taking a course from Pauling that had introduced the properties of deuterium bonds as compared to hydrogen bonds, and he had the idea of connecting heavy isotopes to Monod's problem. If the bacterium was not only switched to a new food source but also switched to growth on D₂O (heavy water), then new proteins made in response to the food switch would be heavy (synthesized with D rather than H), while pre-existing proteins would be their normal light density.

Not long after that, Meselson talked with Delbrück and was introduced to the problem of DNA replication. When Meselson met Frank Stahl (**Fig. 4**) at a meeting, he found a soul mate, someone as interested as he in exploring experimental means of determining whether Delbrück's model could be correct. Stahl came to Delbrück's lab as a post-doc in 1955, but any thought of putting to a test the ideas they had discussed had to be shelved until he had finished a project begun as a graduate student and Meselson had finished his own thesis.



Figure 4. Matt Meselson, Frank Stahl, and Martha Chase, 1954. Meselson met Stahl at the 1954 Cold Spring Harbor phage meeting. Chase worked at Cold Spring Harbor lab with Alfred Hershey on phage molecular biology. (image courtesy of Matt Meselson)

SQ4. How might Meselson's idea concerning heavy water and Monod's problem with proteins that may or may not be newly synthesized be applied to the issue of DNA replication?

That brings us to the experiment performed by Meselson and Stahl and published in 1958. You can hear the story behind the experiment in Meselson's own words:

<http://www.youtube.com/watch?v=cvU4kEMvbm4>
(Meselson telling the story behind the experiment)

as well as reading the article, which might be an appropriate action now.

The article

As usual, I suggest that you not read the article like a novel but rather skim it, searching for the **question** the authors addressed by experiment and the **result(s)** reported in the article (not to be confused with the conclusions nor with second-hand reports of other's results)

SQ5. What is the experimentally accessible question taken on by Meselson and Stahl in this article? (Don't say 'What is the mechanism of DNA replication?' That's way too broad for a single experiment.)

SQ6. What results (not conclusions) were reported in this article? Which result is the most pertinent to the question you listed in SQ5?

You've got to admit, this article has perhaps the smallest Results section that you could imagine in a research article! That section points squarely to one figure, the result of one experiment.

SQ7. What experiment led to the results shown in that figure? Outline the steps.

It strikes me as pretty likely that you know very little about the method that underlies the experiment. Since this is an important paper, and there's only one directly pertinent result, based on one experimental method, it follows that if you want to gain from this important paper, you're going to have to do whatever is necessary to understand the underlying method.

SQ8. How could you go about educating yourself on this method?

SQ9. This article was the first (I think) to use this method to answer a biological question about DNA, so Meselson and Stahl say more about the method than you might expect to find in a typical research article. What do they mean by "*The macromolecules of DNA present in this density gradient are driven by the centrifugal field into the region where the solution density is equal to their own buoyant density*"? If you're not sure, break it down. What is meant by the two underlined phrases?

SQ10. If the explanation by the authors doesn't do it for you, find something on the web that does (there's lots) and then try SQ9 again.

SQ11. What is the direction of the centrifugal field in Fig. 2a? Up to down? Down to up? Left to right? Right to left? What are the bands labeled N¹⁴ and N¹⁵? Why are they where they are?

The Results

SQ12. What is meant by "generations" in Fig. 4?

SQ13. The last two lines of Fig. 4 are labeled (under generations) "0 and 1.9 mixed" and "0 and 4.1 mixed". What do these labels signify? Do they look correct?

SQ14. What would you expect from Fig. 4 at 1 generation in each of the three models of DNA replication mentioned above (conservative, semiconservative, dispersive)?

SQ15. Same question as SQ13 but at 2 generations.

Discussion

The **Discussion** section lists three numbered conclusions

SQ16. What in the Results section supports the first assertion (*The nitrogen of a DNA molecule is divided equally...*)?

SQ17. What in the Results section supports the second assertion (*...each daughter molecule has received one parental subunit*)?

SQ18. What in the Results section supports the third assertion (*...The replicative act results in a molecular doubling*)?

SQ19. What are Meselson and Stahl's thoughts concerning the Watson and Crick model and why?

SQ20. What do their results say concerning the disquiet Delbrück had with semiconservative replication and the problem of tangling?

C. Reconciliation of semi-conservative replication with the topological constraints of the double helix

So where do we stand? Suppose we, like many at the time, take Meselson and Stahl's results as supporting semi-conservative replication, as suggested by Watson and Crick. Delbrück thought this was impossible, because replicating by this method would produce a tangled mess. And yet it appears to happen. How is tangling avoided?

Try reading the first chapter of James Wang's book, *Untangling the Double Helix*, the highly pertinent first chapter of which is freely available on-line.⁹

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