In-Class Exercise: DNA Replication: A Case Discussion of a Landmark Paper by Meselson and Stahl

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Conceptual Background and Resource Description:

In the concluding statement of Watson and Crick's seminal paper introducing the atomic structure of DNA, they write, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material (Watson, JD and Crick, 1953)." Some consider this one of the biggest understatements in modern biology, but Watson and Crick couldn't confirm the mechanism for copying DNA because of lack of hard evidence. The scientists were racing to publish ahead of their competitors, so they included the statement to be able to introduce the model before proof was available. The base-pairing inherent to the structure of DNA certainly strongly suggested a mechanism for creating an identical copy of the DNA, but it wasn't proven until Meselson and Stahl (1958) carried out their classic study. Not only was this a landmark experiment for the essential process it helped to define, but it was also recognized for its elegant simplicity.

For these reasons, Meselson and Stahl (1958) is an ideal paper to introduce students to the art of reading papers and appreciating beautiful science. This resource is a facilitator's guide to help run a discussion session for senior undergraduate or junior graduate students.

Reference: M Meselson and FW Stahl (1958) The Replication of DNA in *Escherichia Coli.* Proc Natl Acad Sci 44: 671-682.

Resource Justification:

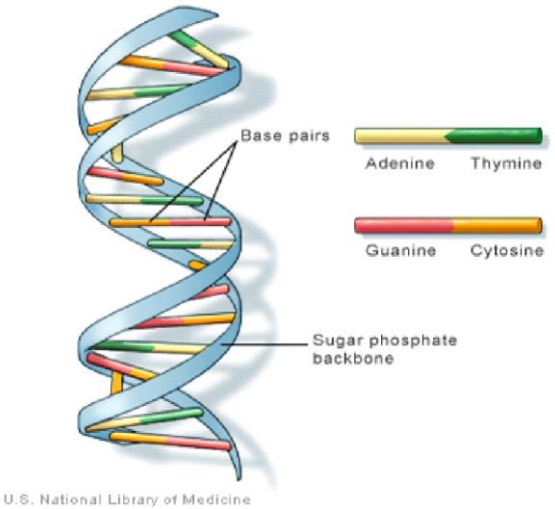
This resource is aligned primarily with the GSA's core category in "the nature of genetic material." This core category encompasses "the mechanisms by which an organism's genome is passed onto the next generation" which begins with the process of DNA replication. In addition to focusing on the actual process of replication, study of this paper also gives students the opportunity to develop core competencies in observational strategies/hypothesis testing/experimental design, evaluation of experimental evidence, and (developing and) interpreting graphs.

Implementation Advice:

Because this session is structured as a small group discussion, I recommend carrying this out in classrooms with 5-7 students. This size allows students to truly participate and engage in the discussion. In larger sections, students can get lost in the crowd, and it can begin to turn into a lecture rather than a discussion. I anticipate discussion of this paper to fill a class of approximately 1 hour in length. No special equipment is needed beyond a whiteboard in the classroom and homework access to the (freely available) paper. Discussion questions are provided below to help the students solve problems and make predictions on their own before linking to the figures in the paper itself. Students should read the paper before coming to class. The questions can be provided to the students in advance or can be introduced during the discussion itself.

Session Outline and Discussion Questions:

1. Just a few years before this paper was published, Watson and Crick (1953) published the structure of DNA. What were the main elements of their model?

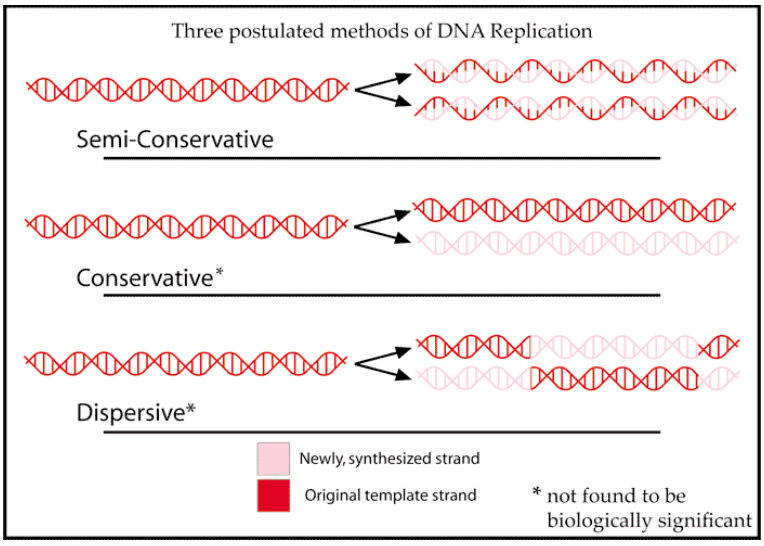


- *Double-stranded*
- *Sugar-phosphate backbone*
- *Anti-parallel*
- *Helical*
- *Purine-Pyrimadine base pairing*
- *Chargaff's rule: AT base pairs and CG base pairs*

Panel 1: Schematic structure of DNA.

2. At the time the paper was written, the authors indicated that "hypotheses for the mechanism of DNA replication differ in the predictions they make concerning the distribution among progeny molecules of atoms derived from parental molecules (Meselson & Stahl, 1958)." What were the main competing models for the mechanism of DNA replication? Which model was favored by Watson and Crick?

The semiconservative model was favored by Watson and Crick. This model also implies a mechanism for fidelity in replication.

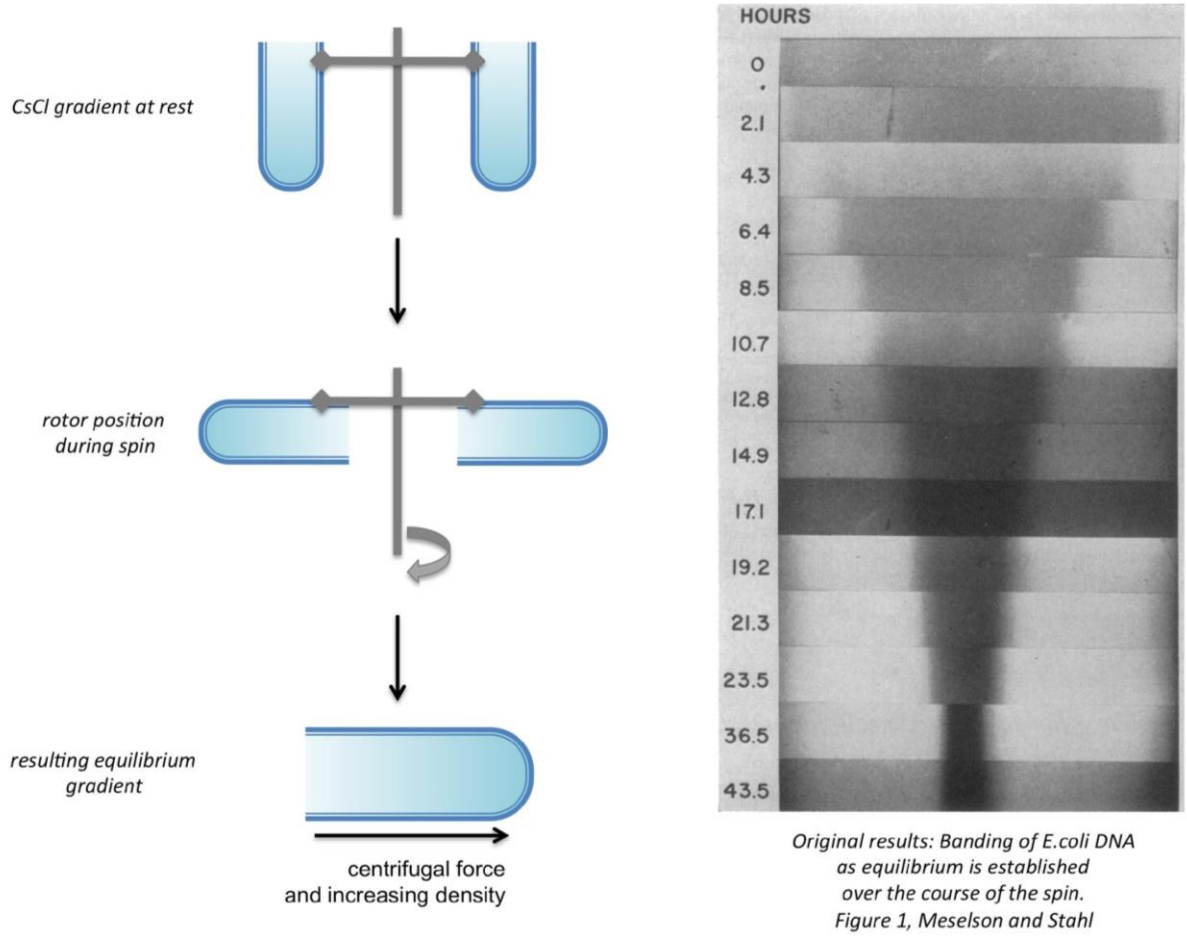


Panel 2: Three competing models of DNA replication.
**Adapted with permission from http://en.wikipedia.org/wiki/File:DNAreplicationModes.png*

3. What technique did Meselson and Stahl use to test whether any of these models appeared to be at work? What were the goals of the control experiments shown in Figures 1 and 2 of Meselson and Stahl?

Meselson and Stahl grew the E. coli in media containing N^{15}-ammonium chloride (a heavy isotope of nitrogen which normally exists as N^{14}) as the sole N source for 14 generations. The DNA in the resulting culture was virtually entirely labelled with the heavy N^{15} isotope.

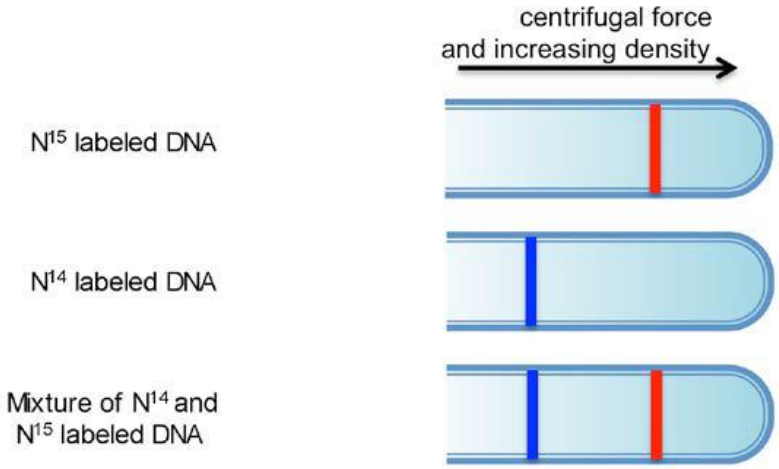
They next verified that this heavy N^{15} DNA could be separated from light (N^{14}) DNA on a CsCl density gradient. The density gradients were created in tubes that were spun using a swinging bucket rotor until the gradients reached equilibrium. The DNA present in the gradients at equilibrium floated at the position in the gradient where the CsCl solution density was equal to the DNA's buoyant density. The establishment of these equilibrium conditions is demonstrated in Figure 1 of Meselson and Stahl, excerpted below. It is worth noting that this technique was invented specifically for the purpose of carrying out these experiments.

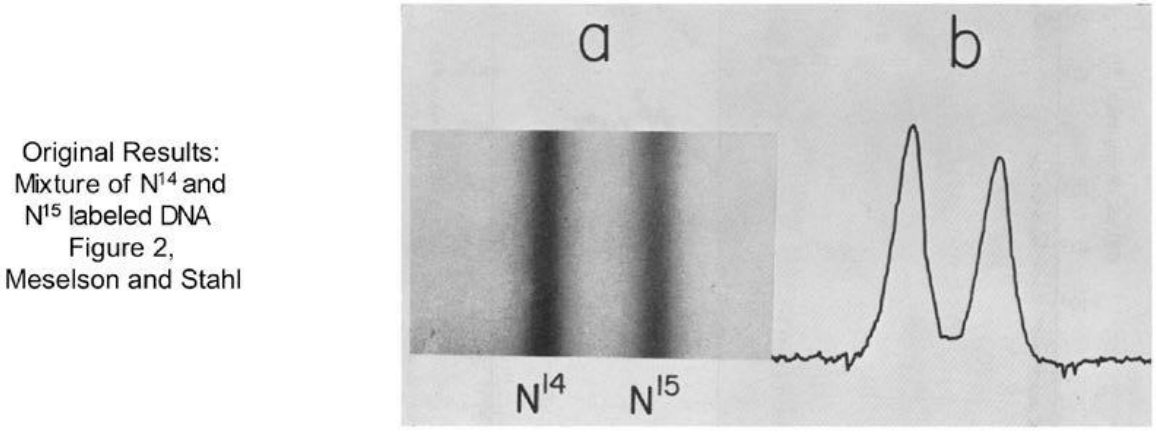


Original results: Banding of E.coli DNA as equilibrium is established over the course of the spin. Figure 1, Meselson and Stahl

Panel 3: Control experiment using CsCl density gradients of bacterial cell lysates to demostrate equilibrium banding of labeled DNA from bacterial cell lysates. The results depicted in the right hand panel correspond to the original results shown in Figure 1 of Meselson and Stahl (1958).

The control experiment (seen in Figure 2 of Meselson and Stahl, included as an excerpt in Panel 4 below) involved running a mixture of E. coli lysates from bacteria grown in either N^{14} or N^{15} media on the CsCl gradients described above, and the results of this control experiment proved that this technique could separate the differentially-labelled DNA .





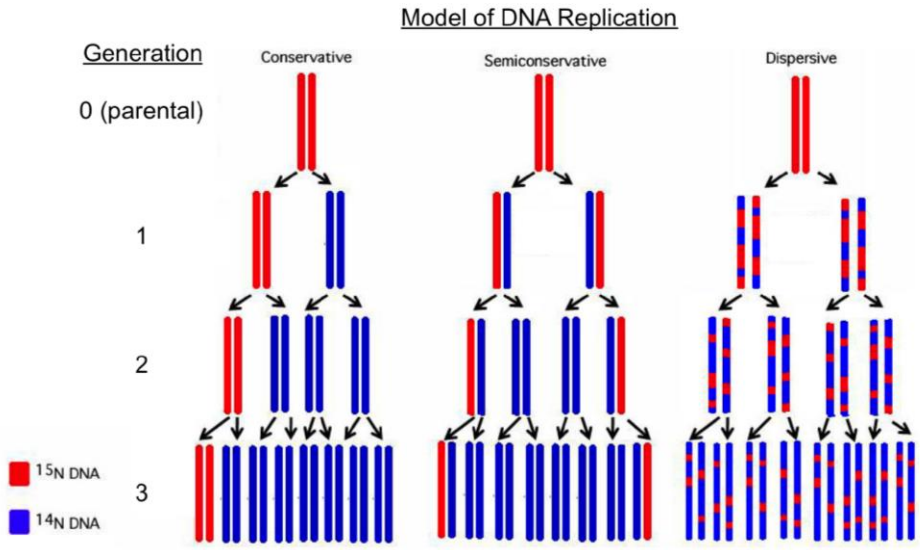
Panel 4: Control experiment using CsCl density gradients of bacterial cell lysates to show positioning of labeled DNA bands. The results depicted in the third panel correspond to the original results shown in Figure 2 of Meselson and Stahl (1958).

Because this method could be used to efficiently separate the labeled DNA species, the next step was to set up a pulse-chase experiment whereby the E.coli were initially labeled in N^{15} containing media. After 14 generations, the cultures were shifted to N^{14} containing media. Next, timepoints were taken to run on the density gradients and visualize the DNA species present in subsequent generations.

4. Why did the authors measure the number of cells growing in the bacterial cell populations over time, as graphed in Figure 3 of Meselson and Stahl?

The authors expected one round of DNA replication to occur during each cell division cycle, so they wanted to accurately measure the doubling time of the bacterial cultures in two given growth conditions (experiment #1 and #2). This way, they would be able to relate time points from subsequent experiments to generation number by dividing by the doubling time.

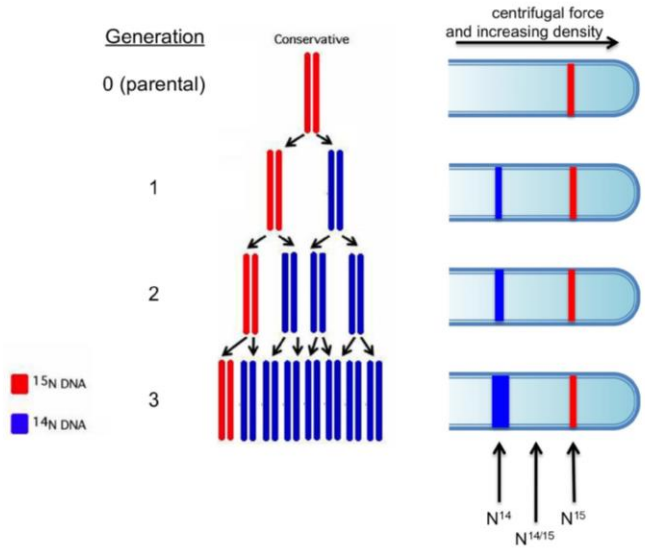
5. Predict the experimental results if each of the models of DNA replication were true. Begin by sketching out the predicted daughter DNA species for each model of replication in the first three generations following the shift to N^{14}-containing media.



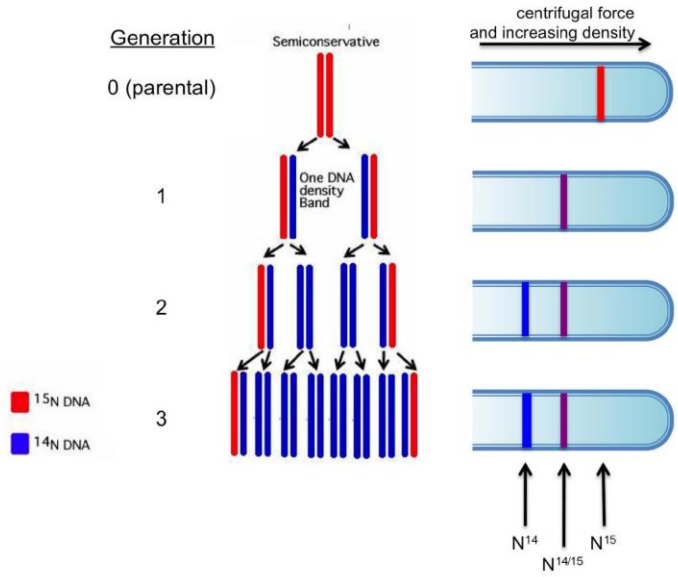
Panel 5: Three models of DNA replication over three generations.
**Adapted from http://www.visionlearning.com/en/library/Inside-Science/58/Meselson-and-Stahl/187*

6. Given these predicted daughter DNA species, what would the banding patterns in the CsCl gradients look like for each model?

If the conservative model of DNA replication held true, the parental strand would remain intact over multiple generations, so the band at the N^{15} position would persist, although it would become a decreasing fraction of the overall population of DNA molecules.

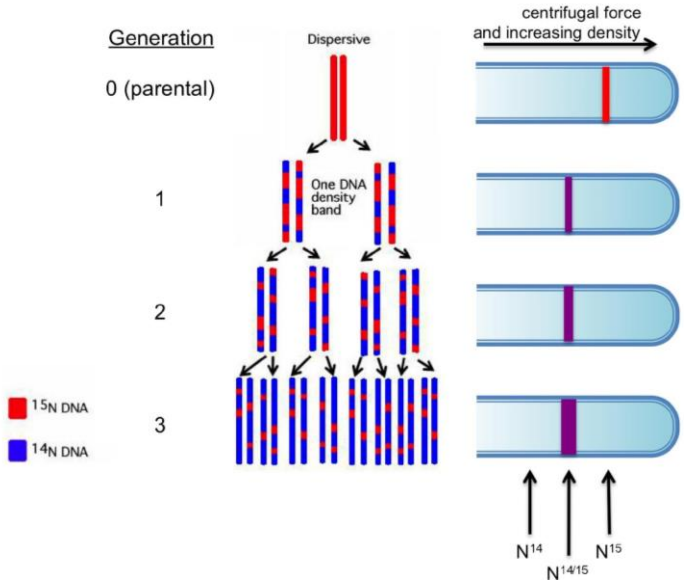


Panel 6: Conservative DNA replication over three generations, non-denaturing conditions.
If the semiconservative model of DNA replication was true, the original parental strands would be split over two daughter strands, and the original N^{15} species would be lost during the first generation.



Panel 7: Semiconservative DNA replication over three generations, non-denaturing conditions.

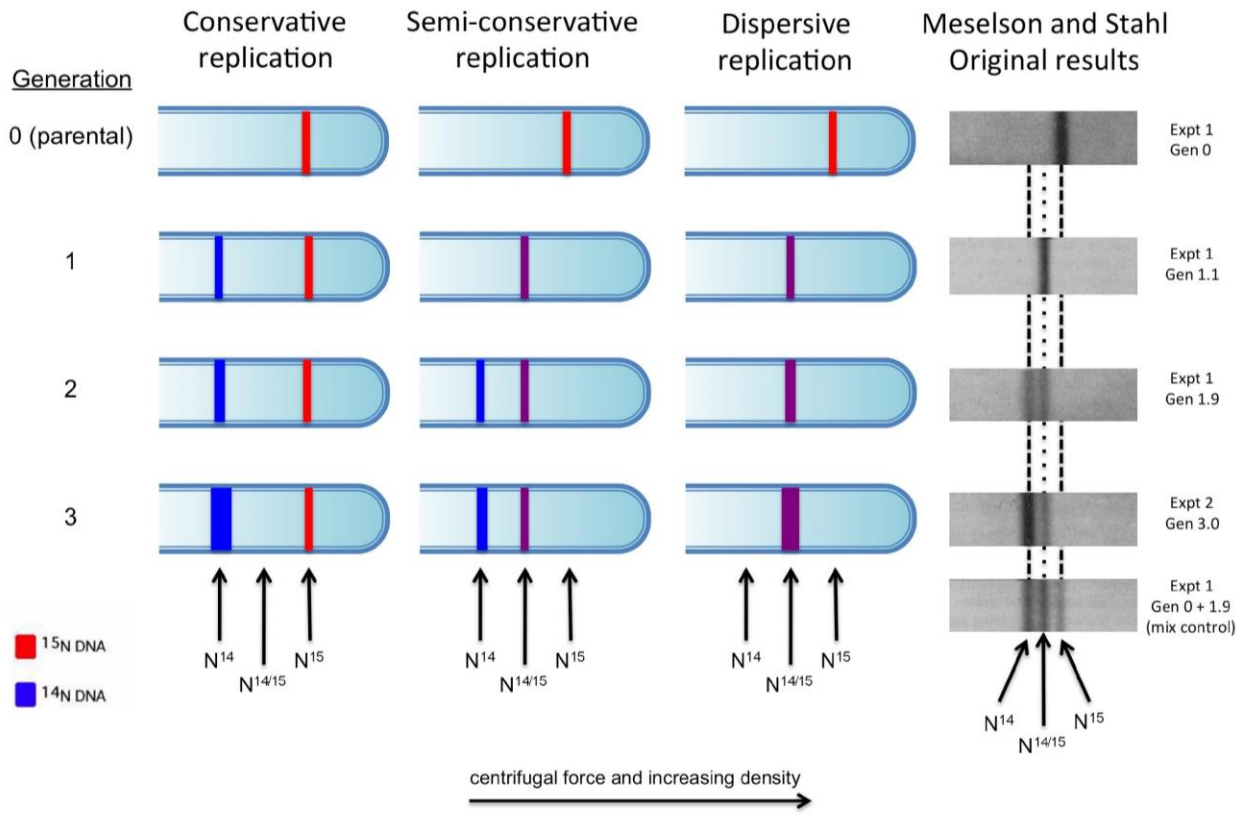
If the dispersive model of DNA replication held true, the parental DNA would be divided over the daughter molecules during every replication cycle, so both the N^{15} population would be lost, and the pure N^{14} population would not appear.



Panel 8: Dispersive DNA replication over three generations, non-denaturing conditions.

7. Now that the predicted results have been delineated, ask the students to compare the results. How many generations of DNA replication must be examined to reach a definitive conclusion? Do Meselson and Stahl's results point to a particular model of DNA replication?

One of the most beautiful aspects of this body of work is that the design of the experiment clearly differentiates between all three major models of DNA replication. During the first generation, the N^{15} species is retained in the conservative model, and it is lost in the semi-conservative and dispersive models. To further distinguish between the semi-conservative model and the dispersive model, one must compare the results for at least a second generation. In the second generation of a semi-conservative model, the N^{14} species will appear whereas in the dispersive model, the intermediate $N^{14/15}$ species will persist.

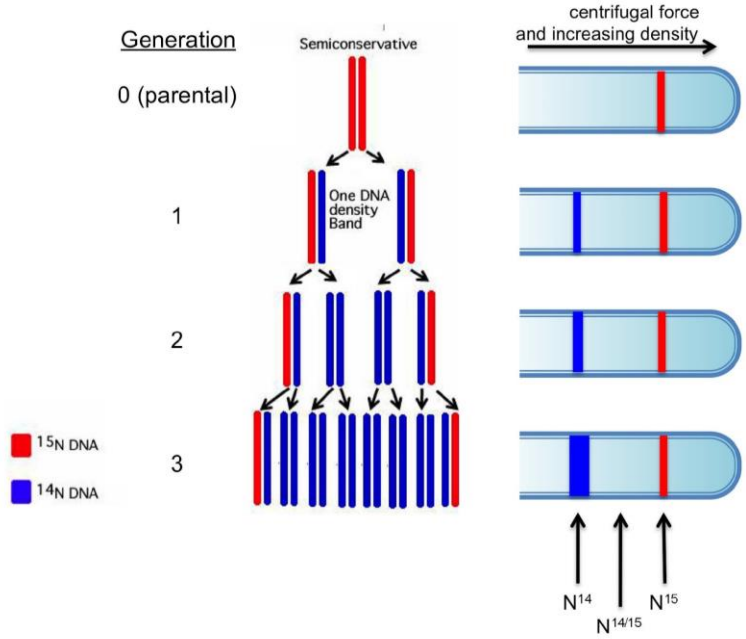


Panel 9: Comparison of predicted results for three models of DNA replication with Meselson and Stahl's experimental results in Figure 4 of their paper.

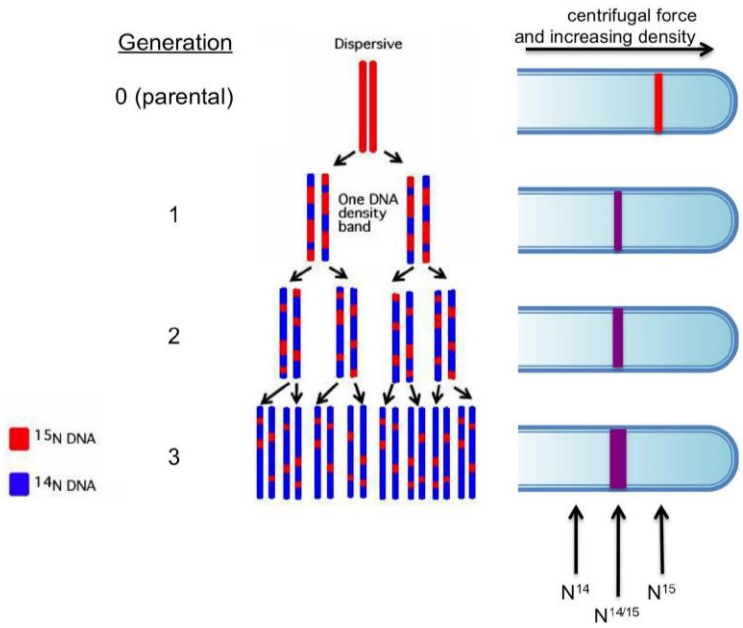
Examining Figure 4 of Meselson and Stahl (1958), one can see that generation 0 begins with the pure N^{15} species, generation 1.0-1.1 has a band at the intermediate $N^{14/15}$ position, and at generation 1.9, we can observe the emergence of a band at the N^{14} position. Representative original data from these timepoints is displayed alongside the predicted experimental results for each model of DNA replication in Panel 9 above. These results are in line with the predictions of the semiconservative model of DNA replication!

8. Although the experiments suggest a particular model of DNA replication, Meselson and Stahl decide to carry out heat denaturation experiments with the DNA isolated from the intermediate position of the CsCl gradients. What is the rationale behind this experiment, and what results would you predict for each model in question?

This experiment was done to further distinguish between the semiconservative and dispersive models of DNA replication. They wanted to be certain that the intermediate density DNA species resulted from the base pairing of one heavy polynucleotide chain and one light polynucleotide chain, as predicted by the semiconservative model, rather than a double helix composed of a mixed assortment of parental and daughter DNA, as in the dispersive model. In order to accomplish this, they heat denatured DNA obtained after one generation of growth time (predicted to run at the intermediate position of the original CsCl gradient) and ran this denatured sample on a CsCl gradient. There are no intermediate DNAs created in the conservative model, but in the semiconservative model, the intermediate species should be able to dissociate into both a light and a heavy chain (Panel 10, below). In contrast, the dispersive model predicts that even after heat denaturation, the dissociated hybrid species will continue to run at the intermediate position (Panel 11).

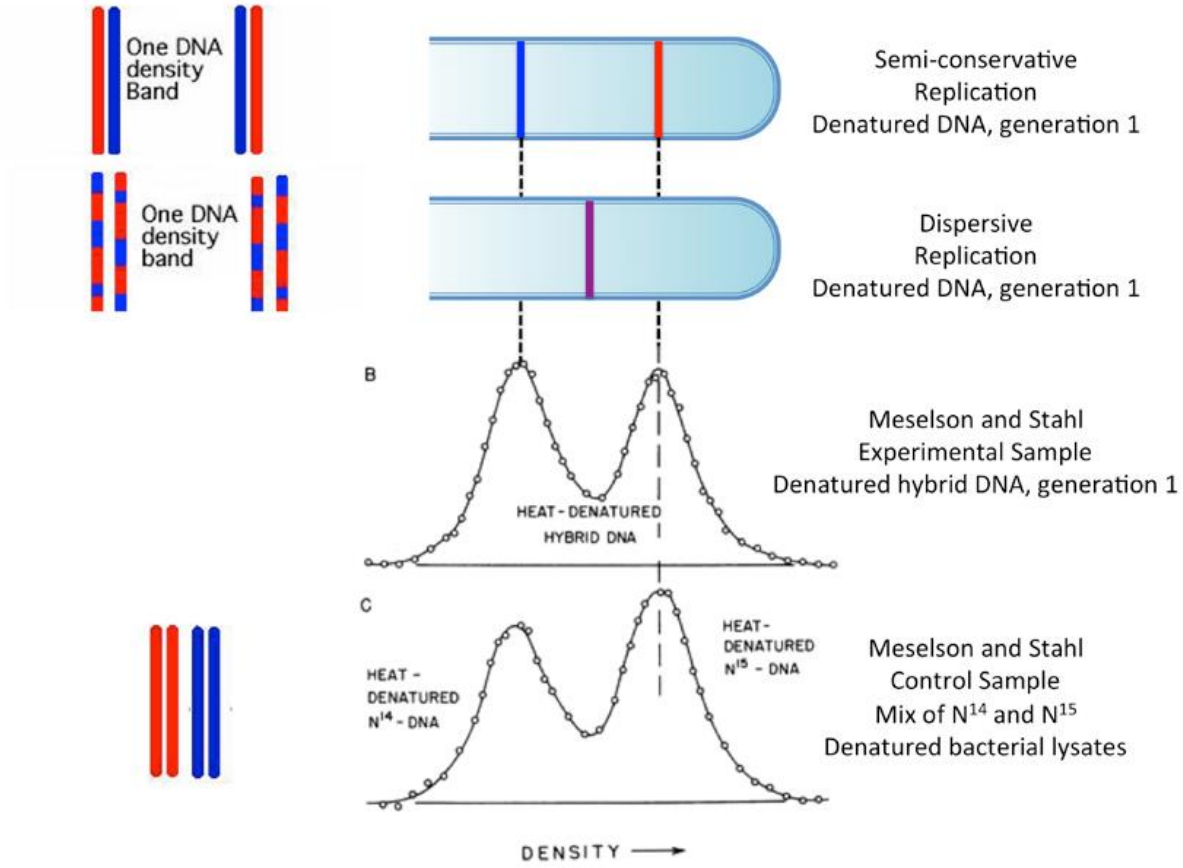


*Panel 10: Semiconservative DNA replication over three generations, **denaturing** conditions.*



*Panel 11: Dispersive DNA replication over three generations, **denaturing** conditions.*

9. When Meselson and Stahl ran their gradients on denatured samples, what did the results ultimately demonstrate?



Panel 12: Comparison of predicted results for semi-conservative and dispersive models of DNA replication with Meselson and Stahl's experimental results in Figure 9 of their paper.
In Figure 9 of their paper, Meselson and Stahl (1958) demonstrated that the intermediate species found after one generation of growth can be heat denatured into individual N^{14} and N^{15} peaks. Furthermore, these peaks run at the same density as the peaks corresponding to heat-denatured control samples composed of a mix of N^{14} and N^{15}-labelled DNA. Therefore, this offers further confirmation of the semiconservative model.

For more advanced students, one may also wish to ask students how they might do a similar set of experiments using modern techniques, and answers may involve fluorescent labeling of products of DNA replication to be analyzed by chromatin fiber assays, for example, but truly, these elegant methods from 1958 remain direct and informative. There are few experimental strategies that would withstand the test of time as well as these experiments have.

Conclusions:

Meselson and Stahl's landmark paper is an excellent study for primary reading in the genetics classroom. It tests fundamentally important hypotheses about essential biological processes. It also relies on one primary method, so students new to paper reading and research study design can focus on the logic of the scientific method rather than get bogged down with numerous varieties of experimental details. Furthermore, there are many opportunities to engage the students in the exercise of creating a hypothesis, using it to predict experimental results, subsequently analyze those results, and use the conclusions to refine their hypotheses.

References:

Meselson, M., & Stahl, F. W. (1958). The Replication of DNA in Escherichia Coli. *Proceedings of the National Academy of Sciences of the United States of America*, *44*, 671–682.

Watson, JD and Crick, F. (1953). A Structure for Deoxyribose Nucleic Acid. *Nature*, *171*(4356), 737–738.