PULSE-CHASE PRIMER: THE MESELSON–STAHL EXPERIMENT

This activity can be used in conjunction with the short film *The Double Helix*. After publishing the structure of DNA, Watson and Crick published a second paper that stated their hypothesis of how DNA replicates. They predicted that when a double helix replicates, each of the two daughter molecules of DNA will have one strand from the parental molecule and a newly made strand. This is referred to as semiconservative replication. It can be distinguished from a conservative model of replication in which the two parental strands come back together and the new daughter molecule is made up of two new strands. A third model, dispersive replication, predicts that the two daughter molecules contain a mixture of old and newly made DNA. The experiment that distinguished between these possibilities was the experiment by Matthew Meselson and Franklin Stahl in 1958. This activity uses Meselson and Stahl's classic experiment to provide students with a basic understanding of how a pulse-chase analysis works and how it allowed them to establish that DNA replication follows the semiconservative model.

LEARNING OBJECTIVES

This activity has been designed to engage students in thinking about the following

- Experimental design
- Analysis of experimental results

CURRICULUM CONNECTIONS

Text/Curriculum	Curriculum Topics
NGSS	HS-LS3-1, HS-LS1.A, HS-LS3.A
Common Core*	CCSS.ELA-LITERACY.RST.9-10.4, CCSS.ELA-LITERACY.RST.9-10.5, CCSS.ELA-LITERACY.RST.9-10.6, CCSS.ELA-LITERACY.RST.9-10.7, CCSS.ELA-LITERACY.RST.9-10.8, CCSS.ELA-LITERACY.RST.11-12.1, CCSS.ELA-LITERACY.RST.11-12.2, CCSS.ELA-LITERACY.RST.11-12.3, CCSS.ELA-LITERACY.RST.11-12.4
AP (2012-13 Standards)	3.A.1, 3.A.2, 4.A.1, S.P.1, S.P.5, S.P.6
IB (2009 Standards)	2.5, 3.3, 3.4, 7.1, 7.2

KEY TERMS

DNA, experiment, Meselson, nucleotide, replication, semiconservative, conservative, dispersive, Stahl

TIME REQUIREMENTS

If this activity is done in class, it will require one 45-minute period. This would include 25 minutes to read through the primer and answer the questions, and a 20-minute summary discussion. Additional time will be needed to watch the 16 min. film, *The Double Helix*.

SUGGESTED AUDIENCE

This activity is appropriate for all levels of high school biology (honors, IB, and AP) and undergraduate biology.

PRIOR KNOWLEDGE

Students will benefit from having a basic understanding of DNA structure and DNA replication. Students should know that bacterial cells contain DNA and that when a bacterial cell divides it produces two cells, each containing the same amount of DNA as the original cell.

PROCEDURE

After watching the film, *The Double Helix*, students work through the Pulse-Chase Primer student handout. After completing the handout discuss with students the results of the Meselson and Stahl experiment and what each result reveals about DNA replication.

Results of the Meselson and Stahl experiment:

- The first replication in the ^{14}N medium produced a band of hybrid (^{14}N and ^{15}N) DNA. This result eliminated the conservative model of replication.
- The second replication in the ^{14}N medium produced both light (^{14}N) DNA and hybrid (^{14}N and ^{15}N) DNA. This result eliminated the dispersive model of replication.

TEACHING TIPS

- Students may watch the film *The Double Helix* before doing this activity. The film will provide background about the structure of DNA.
- This activity may be assigned as homework, followed by a class discussion about the results, or it may be completed in class.
- If the activity is done in class, students may work in pairs to discuss the different scenarios.
- If students are not already familiar with a pulse-chase experiment, explain that it is a two-phase technique used to examine cellular processes that take place over a period of time. During the **pulse** phase of the experiment, cells are exposed to a labeled compound. The labeled compound is incorporated into the molecule or pathway being studied. In the **chase** phase, an unlabeled form replaces the labeled compound. The reaction is monitored to see how long it takes the labeled form of the compound to be replaced by the unlabeled form. There are many ways to label a compound for use in a pulse-chase experiment. Radioisotopes or fluorescent labeling using compounds such as green fluorescent protein (GFP) are both popular.

MATERIALS

Each student should have a copy of the Pulse-Chase Primer student handout.

ANSWERS TO QUESTIONS

1. Using Figure 1 as a reference, indicate the location of the band for heavy DNA in Generation Zero in the centrifuge tube represented below.

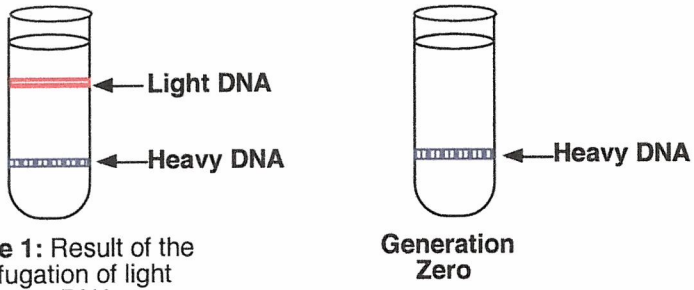
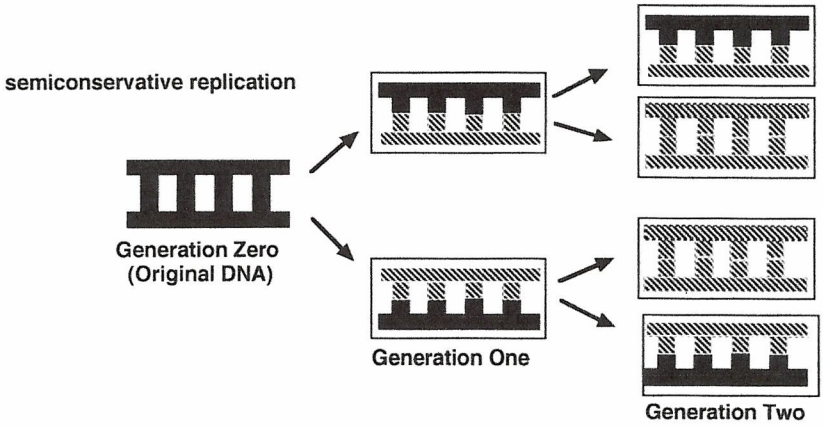


Figure 1: Result of the centrifugation of light and heavy DNA

2. If DNA replication is semiconservative, explain how the results of the centrifugation of the DNA from Generation One would be different than the results obtained from Generation Zero. Assume that each bacterium divided exactly once. Use the key provided below to illustrate the arrangement of light and heavy isotopes of nitrogen in the DNA molecules formed in Generation One and in Generation Two.



Explanation: **The original strand contains only heavy ^{15}N. If replication is semiconservative, in Generation One, each of the two DNA molecules will consist of one strand of the original DNA and one strand of complementary DNA made using ^{14}N available in the media. The original DNA serves as a template for replication. The DNA separates between the nitrogen bases. E. coli synthesizes new nucleotides. These nucleotides are what bond to the original strands that have separated.**

In Generation Two, when the DNA molecules separate during replication, two of the new DNA molecules receive an original heavy strand of DNA. The other two DNA molecules consist entirely of DNA strands synthesized using ^{14}N available in the media.

3. Using the tube on the left as the standard, sketch where the bands of DNA would collect in the tubes for Generations Zero, One, and Two if DNA replication is semiconservative.

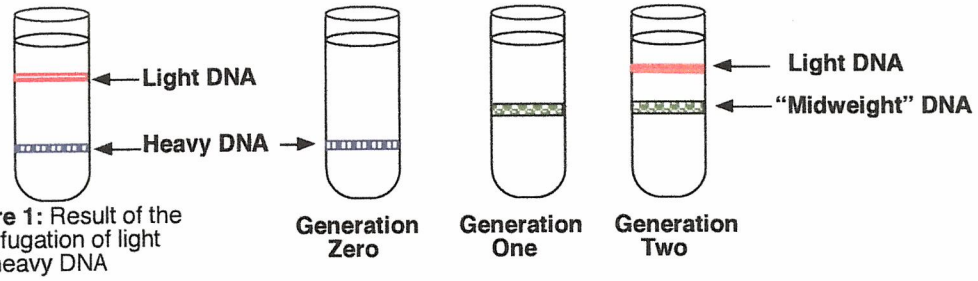
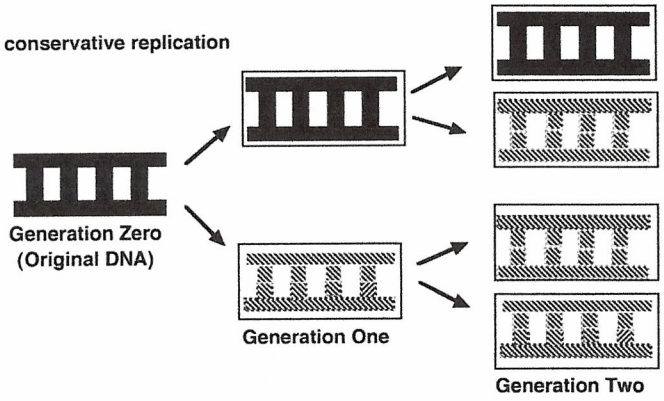


Figure 1: Result of the centrifugation of light and heavy DNA

4. Using the key provided in question 2, illustrate the location of light and heavy isotopes of nitrogen in the strands of DNA in Generations Zero, One, and Two if DNA replication is conservative.



Explanation: **If replication is conservative, the original molecule serves as the template. During the chase, the only nitrogen available for the synthesis of new DNA molecules is light. Therefore, all of the newly synthesized molecules will be light DNA. The original DNA molecule will remain intact and composed entirely of heavy DNA. Analysis will show an increasing amount of light DNA from one generation to the next.**

5. In the tubes below, illustrate the banding patterns Meselson and Stahl would have observed if the results of their experiment supported the conservative model of DNA replication.

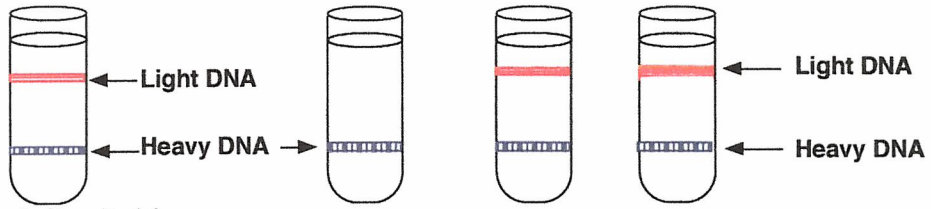
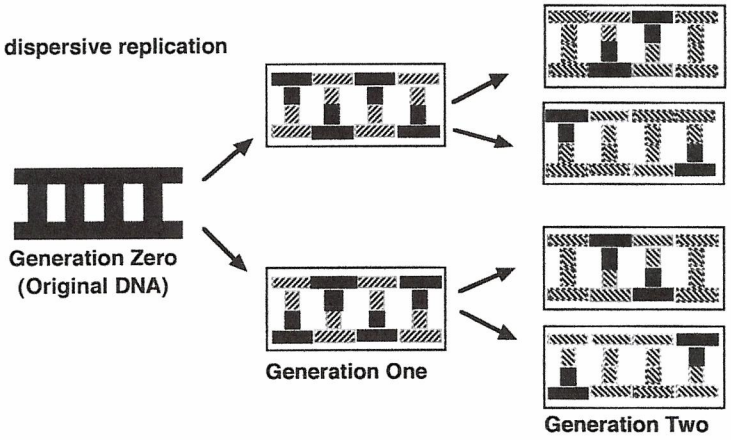


Figure 1: Result of the centrifugation of light and heavy DNA

6. Using the key provided in question 2, illustrate the location of light and heavy isotopes of nitrogen in the strands of DNA in Generations Zero, One, and Two if DNA replication is dispersive.



Explanation: **Answers will vary. The distribution of heavy and light nucleotides in the newly synthesized DNA will be random. However, the number of heavy DNA nucleotides cannot increase from one generation to the next. The number of light DNA nucleotides will increase.**

7. In the tubes below, illustrate the banding patterns Meselson and Stahl would have observed if the results of their experiment supported the dispersive model of DNA replication.

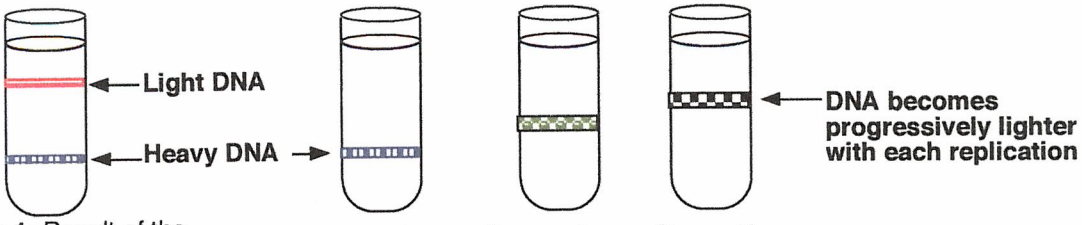


Figure 1: Result of the centrifugation of light and heavy DNA

8. Which model of replication did the actual results of the Meselson-Stahl experiment support? Explain your answer.

The results of the Meselson-Stahl experiment supported a semiconservative model of DNA replication. The first replication in the ^{14}N medium produced a band of hybrid (^{14}N and ^{15}N) DNA. This result eliminated the conservative model of replication. The second replication in the ^{14}N medium produced both light (^{14}N) DNA and hybrid (^{14}N and ^{15}N) DNA. This result eliminated the dispersive model of replication.

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