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.

KEY CONCEPTS AND LEARNING OBJECTIVES

- DNA replication is semiconservative.

After participating in this activity, students will be able to:

- Explain that DNA replication is semiconservative.
- Discuss how experiments can be designed to distinguish between various models.
- Analyze 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 (2016)	2.6, 2.7, 7.1

KEY TERMS

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

SUGGESTED AUDIENCE

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

TIME REQUIREMENTS

If this activity is done in class, it will require one 45-minute period for AP students or undergraduate biology. This would include 25 minutes to read through the activity and answer the questions, as well as a 20-minute summary discussion. Additional time will be needed to watch the 16 minute film, *The Double Helix*. Honors or regular biology students will probably need additional time.

.

PRIOR KNOWLEDGE

Students will benefit from having a basic understanding of DNA structure and DNA replication, including that the DNA molecule is double stranded and will untwist and unzip during 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. Students should also understand what it means to culture bacteria, what a centrifuge does, and what isotopes are.

MATERIALS

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

PROCEDURE

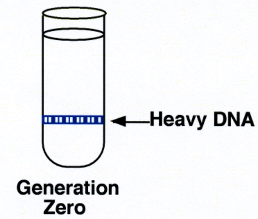
1. Have students watch *The Double Helix*. The film will provide background about the structure of DNA.
2. Have students work through the Pulse-Chase Primer student handout. This activity may be assigned as homework or it may be completed in class. If the activity is done in class, students may work in pairs to discuss the different scenarios.
3. After completing the handout, discuss with students the results of the Meselson and Stahl experiment and what each result reveals about DNA replication.

TEACHING TIPS

- 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.
- Consider how much support your students will need with the activity. AP level students should be able to work through the activity on their own or in small groups, but 9^{th} or 10^{th} grade Biology students might not grasp the concepts as intuitively and might need regular check-ins from the teacher
- Student Misconception: Some students have difficulty understanding that one strand of ^{15}N DNA and a complementary strand of ^{14}N DNA will result in a "midweight" DNA (not all heavy, not all light).
- Optional Manipulatives: Some students could benefit from being able to physically manipulate the nucleotides to model replication. Cut out each of the nucleotides at the end of this document if you wish students to be able to build small models.
- Advanced students could follow up this activity by reading Meselson and Stahl's 1958 paper.
- Animations of DNA replication are available on the BioInteractive website.

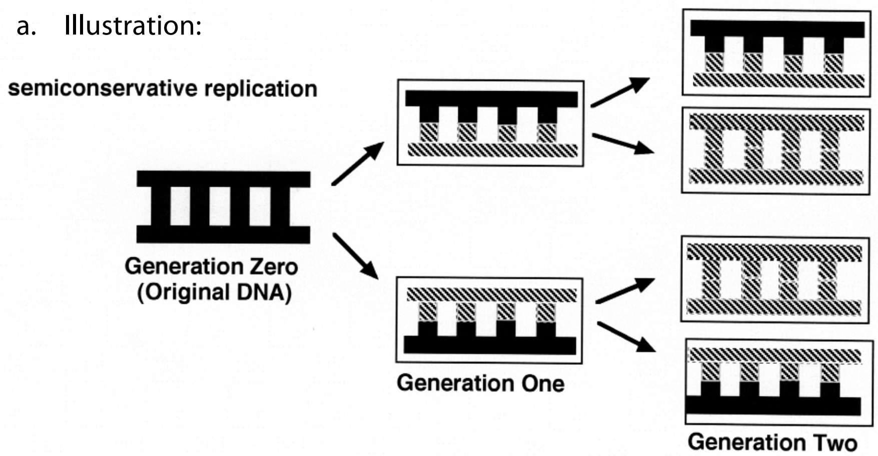
ANSWERS TO QUESTIONS

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



2. If DNA replication is **semiconservative**, use the key provided to illustrate the arrangement of light and heavy isotopes of nitrogen in the DNA molecules formed in Generation One and in Generation Two. Assume that each bacterium divided exactly once per generation. Use the same color as earlier for heavy (^{15}N) and choose a new color for light (^{14}N).

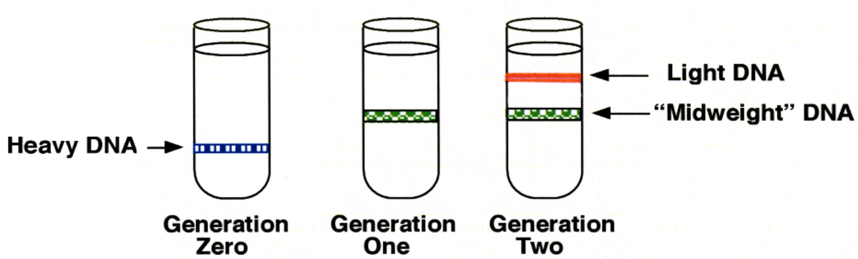
   a. Illustration:



> **Teaching note:**
> This is a good point at which to check in with students to see if they understand that one strand of ^{15}N DNA and a complementary strand of ^{14}N DNA will result in a "midweight" DNA (not all heavy, not all light).

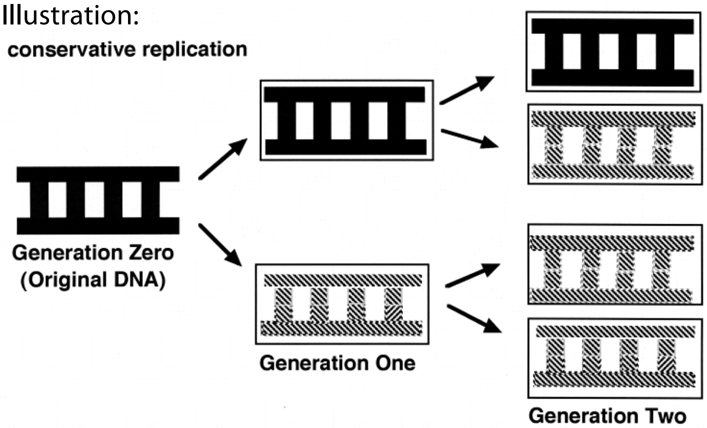
   b. Explanation: **The original strand contains only heavy ^{15}N. If replication is semiconservative, in Generation One, each of the two DNA molecules will consist of one strand of the original DNA and one strand of complementary DNA made using ^{14}N available in the media. The original DNA serves as 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.**

   c. Using **Figure 1** 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.



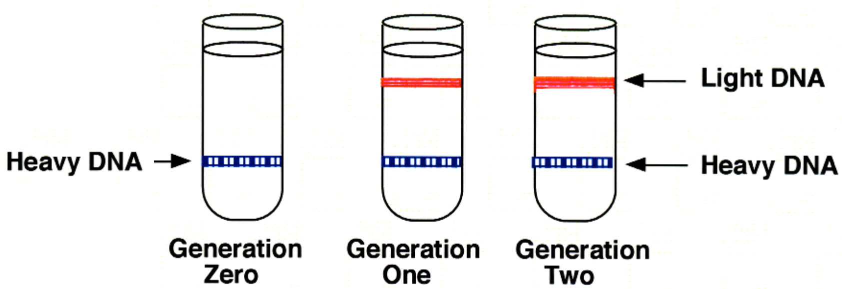
3. Using the key provided in question 2, illustrate the location of light and heavy isotopes of nitrogen in the strands of DNA in Generations Zero, One, and Two if DNA replication is **conservative**.

   a. Illustration:



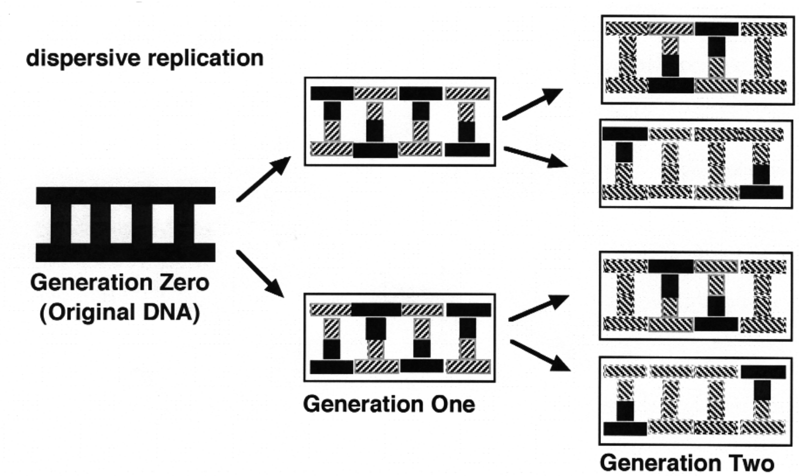
   b. Explanation: **If replication is conservative, the original molecule serves as the template. During the chase, the only nitrogen available for the synthesis of new DNA molecules is light. Therefore, all of the newly synthesized molecules will be light DNA. The original DNA molecule will remain intact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

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



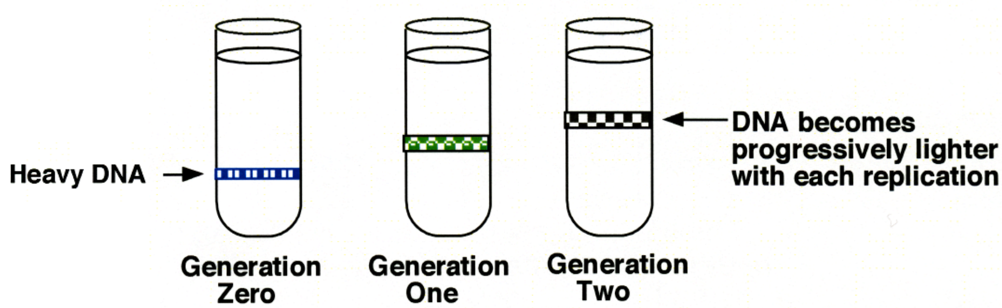
4. Using the key provided in question 2, illustrate the location of light and heavy isotopes of nitrogen in the strands of DNA in Generations Zero, One, and Two if DNA replication is **dispersive**.

   a. Illustration



   b. 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.**

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



5. 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.

AUTHORS

Written by Mary Colvard, Cobleskill-Richmondville High School (retired); Satoshi Amagai, PhD, Jennifer Bricken, Dennis Liu, PhD, HHMI

Edited by Laura Bonetta, PhD, HHMI and Susan Dodge, consultant; copyedited by Linda Felaco

FIELD TESTERS

Charles Bender, Guilderland Central School, Guilderland, NY; Pat Black, Niskayuna High School, Niskayuna, NY; Stephanie Branley, Ravena-Coeymans-Selkirk High Schoo, Ravena, NY; Annie Chien, Schenectady City School District, Schenectady, NY; Deb Cole, Shaker High School, Latham, NY; Donna Graves, Bethlehem Central School District, Delmar, NY; Kathy Hoffman, Shaker High School, Latham, NY; Kelly McHale-Sullivan, Voorheesville, NY; Becky Raymond, Deposit Central School, Deposit, NY; Kelly Ryan, Shaker High School, Latham, NY; Barry Greenwald, Beth Kunze, Cheryl Coronado, Jennifer Kaltenbch, Judith Pinto, Kristen Kohli, David Knuffke, Theresa Kutza, Laura Julien, Luisa McHugh, Ellen Perry, Beth Pethel, Stacey Strandberg, Valerie May

OPTIONAL MANIPULATIVES