

CLASSIC EXPERIMENTS IN MOLECULAR BIOLOGY

The Transforming Principle: Identifying the Molecule of Inheritance

by

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Part I – Molecule of Inheritance

If there's inheritance of traits from parents to children, or from “mother cell” to “daughter cells” in mitosis, then some *thing* must be passed from parent to child. We know today that this *thing* is DNA, in the form of chromosomes. However, someone needed to figure that out!

In the 1930s and 1940s, scientists were very interested in identifying the biochemical nature of the “transforming principle.” The candidate molecules were DNA, RNA, and protein. These molecules were candidates because we knew that nuclei contained chromosomes which are associated with phenotypes (think Morgan’s fruit fly eye color experiments where eye color corresponded to the X- or Y- chromosome content of the fly cells), and isolated nuclei are composed mostly of protein, DNA, and RNA. Most scientists at the time were leaning toward protein being the genetic material because it is the most molecularly diverse of the three.

The investigations into the chemical nature of genetic material were initiated by one very important paper from 1928, written by Fred Griffith at the British Ministry of Health. Griffith was studying the bacterium *Streptococcus pneumoniae*, an important pathogen in the 1920s.

Question

1. Why would studying *S. pneumoniae* be an important topic in the 1920s?

Some forms of *S. pneumoniae* cause disease (pneumonia), others don't. When grown on a laboratory plate, you can make a good guess about the pathogenicity (disease-causing ability) of *S. pneumoniae* because *pathogenic* strains look shiny (smooth) due to a polysaccharide cell coat called a capsule (right side of the picture). The capsule helps the bacteria “hide” from the immune system long enough to cause disease. *Nonpathogenic* strains lack the capsule and appear “rough” on a petri plate (left side of the picture). Generally these colonies are smaller, too.

Griffith used mice as his species for detecting the pathogenicity of the bacteria. Injecting mice with bacteria grown on petri plates either made the mice sick and killed them, or produced no disease.

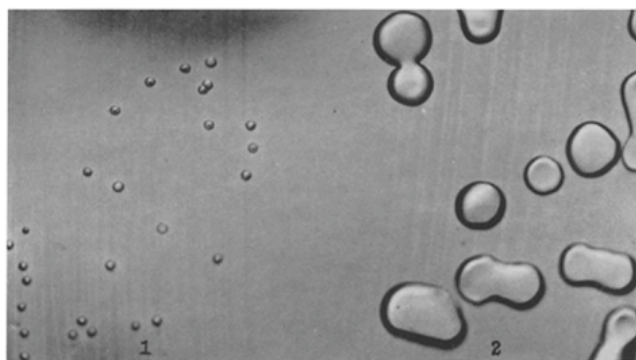


Image credit: Photograph by Joseph B. Haulenbeck, from Avery et al., *Journal of Experimental Medicine* 79 (2): 137–158. ©1944 Rockefeller University Press, used in accordance with the Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported license.

Question

2. Fill out the table below the diagram predicting what you think will happen as a result of each injection (mice live or mice die).

The diagram shows four mice being injected with different bacterial strains. Above each mouse is a syringe and a micrograph of the bacteria:

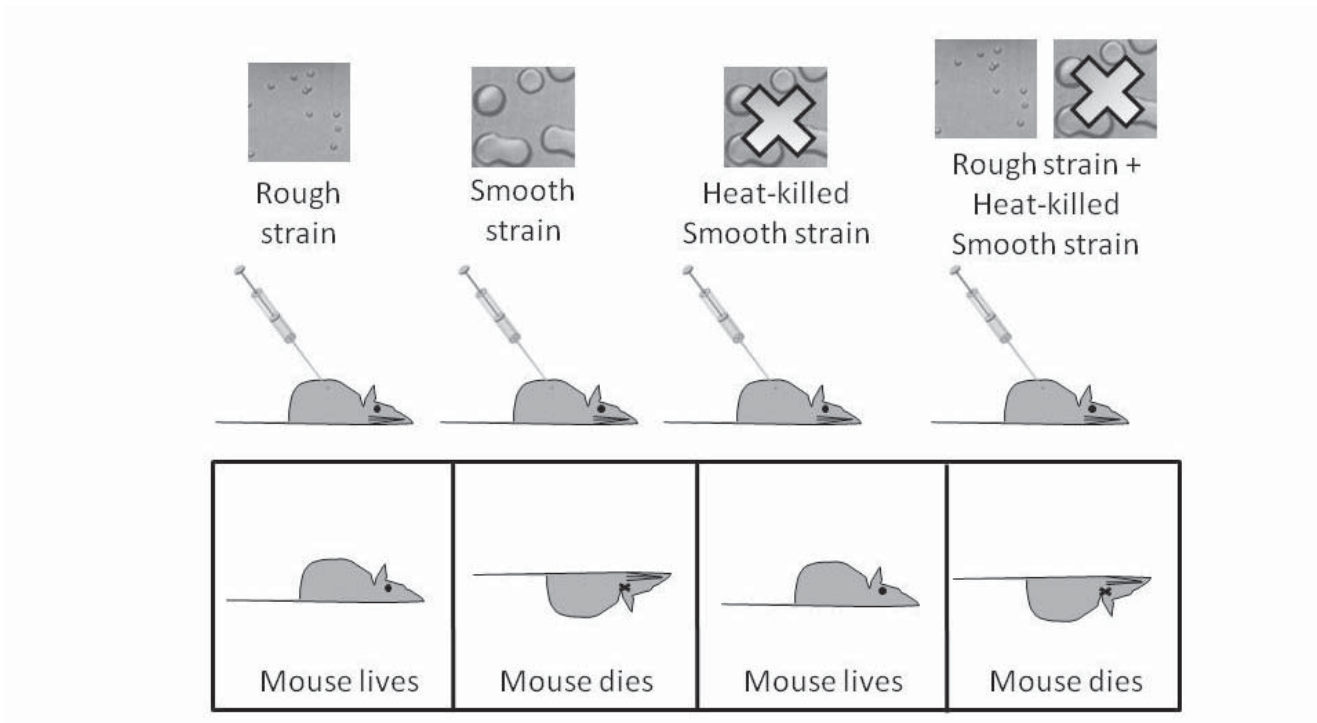
- Mouse 1:** Labeled "Rough strain". The micrograph shows small, irregularly shaped bacteria.
- Mouse 2:** Labeled "Smooth strain". The micrograph shows larger, smooth-surfaced bacteria.
- Mouse 3:** Labeled "Heat-killed Smooth strain". The micrograph shows smooth-surfaced bacteria with a large 'X' over them, indicating they are dead.
- Mouse 4:** Labeled "Rough strain + Heat-killed Smooth strain". The micrograph shows a mixture of rough and smooth bacteria, with a large 'X' over the smooth ones.

Below the mice is a table with four empty cells for predictions:

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Part II – Results

Here are the actual results of the experiment.



Questions

1. Which of the four treatments are controls? Explain your answer.
2. Which of the results is unexpected?
3. What are *two* hypotheses that might explain the unexpected result? In other words, what might be going on in the system?

Part III – Enzymes as Tools

The properties of the rough strain are its *phenotype*. In the experiment you just read about, the *phenotype* of the living rough strain changed from nonpathogenic to pathogenic. Scientists proposed that it changed because the *genotype* changed. The next set of experiments started with the hypothesis that the *genotype change* was due to some extra genetic material being added to the rough bacteria to change their *phenotype* to a smooth phenotype.

The group of scientists who did the next set of experiments consisted of Oswald Avery, Colin MacLeod, and Maclyn McCarty. Their paper was published in 1944. These scientists had three tools to use in their experiments (in addition to the smooth and rough strains, and mice):

1. Proteases
2. DNase
3. RNase

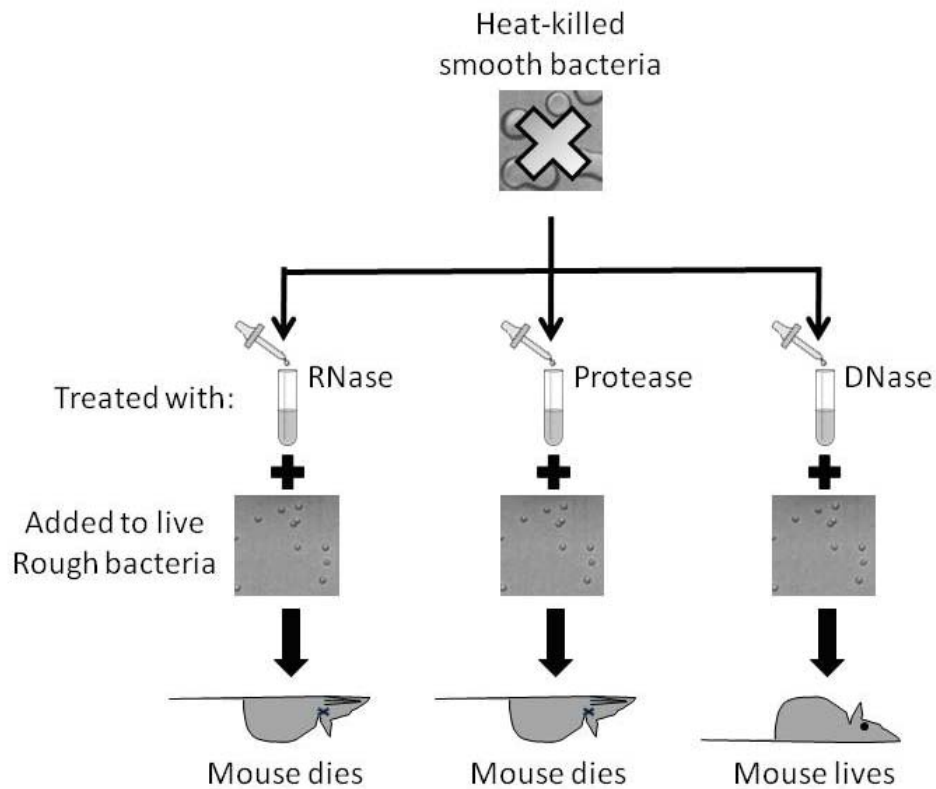
All three of these tools are enzymes, as you can guess because their names end in “ase.”

Questions

1. Given the names of these three enzymes, what reaction do you expect each one to catalyze? Write your answers in the space to the right of the name above.
2. How could you treat dead smooth bacteria with these enzymes to determine whether DNA, RNA, or protein is the genetic material?

Part IV –More Results

The scientists treated heat-killed smooth bacteria with either RNase, Protease, or DNase, then combined that reaction with living rough bacteria and injected mice with the mixture. The results are shown in the diagram.



Question

1. What conclusion can be drawn from these data?

Take-Home Assignment

Address the following using a well-constructed essay paragraph. If you use diagrams, you must have text to thoroughly explain the diagrams rather than just drawing a picture.

Describe the experiments by Griffith, Avery, McCarty, and MacLeod that determined the role for DNA as the genetic material (include the logic behind the experiment).



References

Avery, O.T., CM. MacLeod, and M. McCarty (1944). Studies on the chemical nature of the substance inducing transformation of pneumococcal types: Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. *Journal of Experimental Medicine* 79 (2): 137–158.

Griffith, F. (1928). The significance of pneumococcal types. *Journal of Hygiene* 27 (2): 113–59.



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Meselson and Stahl Experiment: The Nature of DNA Replication

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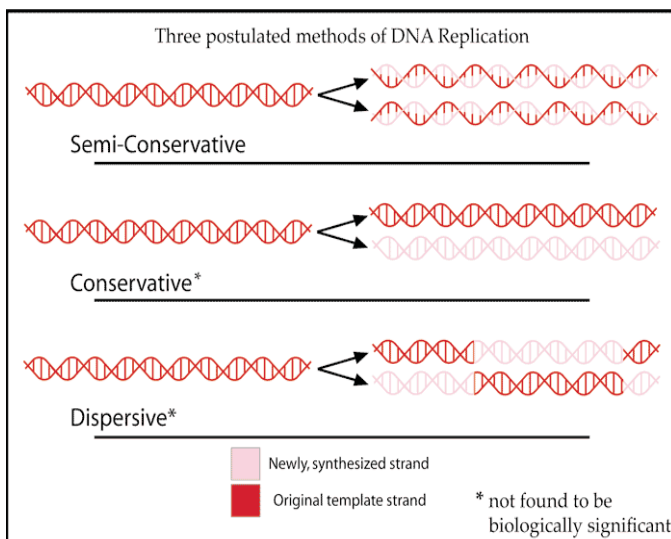
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Part I – Three Possibilities

How did you spend New Year’s Day this year? In 1958, Matthew Meselson and Frank Stahl celebrated the first day of the new year by having breakfast with college friends in Chicago and passing a photograph around the table, not of a girlfriend or a new baby, but of the data behind what is sometimes called the “most elegant experiment in molecular biology”—the experiment that first demonstrated how DNA replication occurs (Judson 1996, p612). Others followed (and won Nobel prizes too) by giving us details about the enzymes involved, but Meselson and Stahl’s experiment is so important and well designed that it has become necessary knowledge for one to be called an educated biologist.

Here are three different possibilities for DNA replication. Only one really happens, but until Meselson & Stahl conducted their experiment, each of these was plausible.

To the right of each diagram below, write two or three sentences describing how the starting DNA molecule differs from the molecule after replication. Do this for each of the three postulated methods of replication.



Credit: Original uploader was Adenosine at en.wikipedia; used in accordance with CC-BY-SA-2.5 license. <http://commons.wikimedia.org/wiki/File:DNAreplicationModes.png>

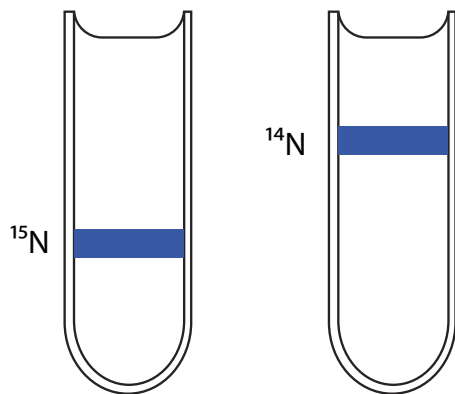
Part II – Nitrogen

Meselson and Stahl had tools like Avery, McCarty, and McLeod: ^{14}N and ^{15}N .

Questions

1. Why is a nitrogen label a good tool for studying DNA?
2. What other molecules in a cell have nitrogen in them?
3. What's the difference between ^{14}N and ^{15}N at the atomic level?
4. What's the term for two atoms of the same element with different molecular masses?
5. Give an example of another element that has atoms of more than one molecular mass.

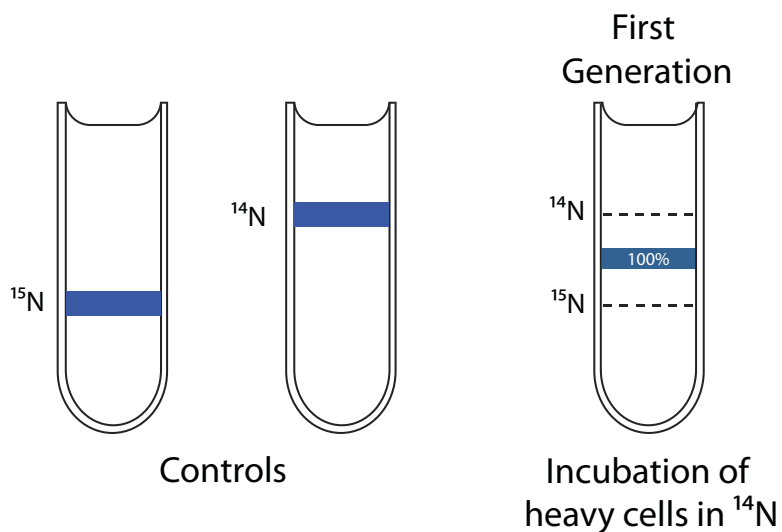
Bacteria in the laboratory can grow on plates or in broth cultures composed of precisely defined mixtures of chemicals. Meselson and Stahl grew bacteria in cultures containing only ^{15}N nitrogen for many generations so that the DNA was almost entirely composed of ^{15}N -containing nucleotides. These chromosomes are more dense than ^{14}N chromosomes, and by spinning chromosomes at very high speeds in a density gradient tube, Meselson & Stahl could tell the difference between the two kinds of chromosomes. Heavy chromosomes sank farther to the bottom of the tube, where the liquid was more dense. Lighter chromosomes floated in the less dense liquid toward the top of the tube. This can be represented by the diagram below. The blue lines represent the location of chromosomes in the tubes after centrifugation.



Controls

Part III – Results

After growing the bacteria in ^{15}N , producing bacteria with “heavy” chromosomes, they shifted the bacteria to growth conditions where only ^{14}N was present—making all the newly synthesized DNA from this less dense form of nitrogen. Here are the results of their density gradients after exactly one generation of bacterial growth:

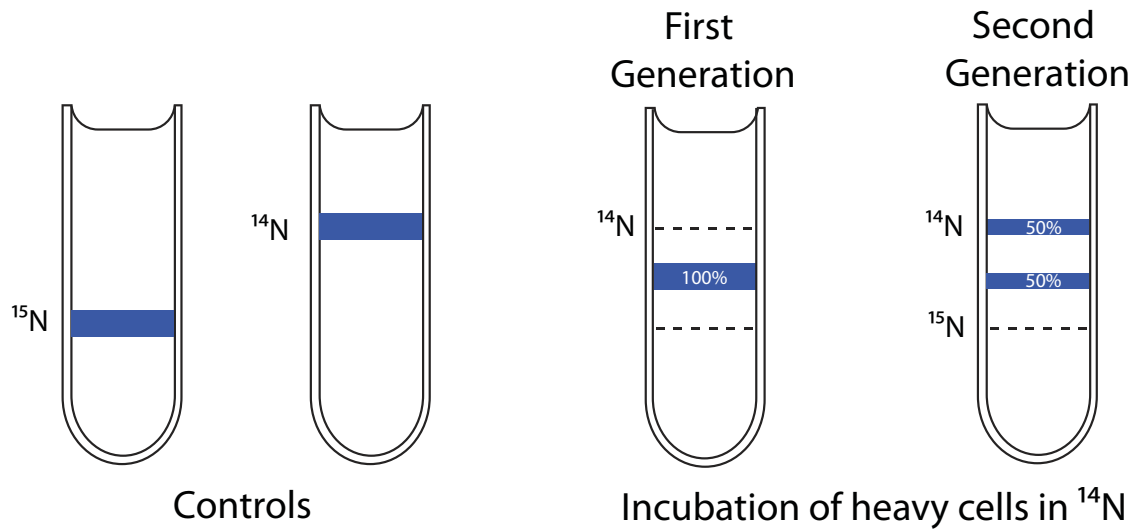


Questions

1. Which of the three models for DNA replication are ruled out by this experiment?
2. What would the data look like if the model you ruled out was what was really happening? Include a diagram of a tube as part of your answer.
3. What could they do to tell which of the two remaining models is actually happening, using the tools that have already been described?

Part IV – Second Generation

The diagram below shows the results from the experiment of the 2nd generation of cells grown in ¹⁴N.



Questions

1. Which model has now been ruled out by these results?
2. What would the data look like if this model was actually happening? Include a diagram of a test tube as part of your answer.

Take-Home Assignment

Address the following question using a well-constructed essay paragraph. If you use diagrams, you must have text to thoroughly explain the diagrams rather than just drawing a picture.

Describe in detail the chemical structure of DNA, how DNA is replicated, and the experiment by Meselson and Stahl that determined the method of DNA replication.



References

Judson, H.F. (1996). *The Eighth Day of Creation: Makers of the Revolution in Biology*, Expanded Edition. New York: Cold Spring Harbor Laboratory Press.

Meselson, M., and F.W. Stahl (1958). The replication of DNA in *Escherichia coli*. *PNAS* 44:671-682.



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