Name:_____ Period: _____

Discovering the Structure of DNA

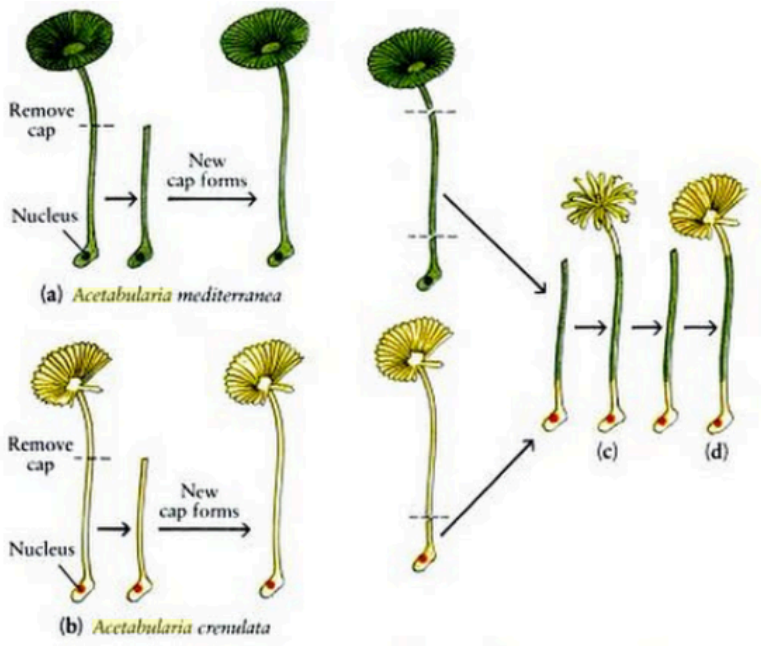
(Note: the inspiration for this activity comes from a book entitled something like "Inductive Biology." I've lost the book, and would love to credit the original author. You can identify the original work by the cut out shapes on the last page…)

Instructions: Working with your lab group, read through the clues in each set below. As you do, take notes on a separate sheet (which will be your lab write-up) summarizing what you've learned (and answering any questions in the text).

Clues Set 1

1. In 1868, Swiss biochemist Friedrich Miescher found that when pepsin (an enzyme known to break apart proteins) was added to chromosomes, atoms of oxygen, carbon, hydrogen, and nitrogen were detected. This made sense, because these were the atoms known to be present in proteins. But he also detected phosphorus atoms. Because phosphorus atoms are never in proteins, he suspected that another type of molecule, in addition to protein, must be present in chromosomes. He named the new molecule ***nuclein***.

2. *Acetabularia* is a single celled marine algae. Despite this organism's single-celled status, it grows large enough to be easily visible with the naked eye. One species, *A. mediterranea*, has a smooth cap, while another species, *A. crenulata*, has a more ragged cap (see diagram below). In all species of *Acetabularia*, the nucleus is located in the bottom of the stalk.



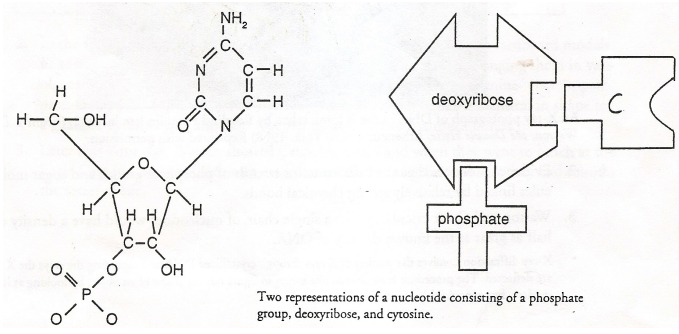
***Acetabulara*, from Curtis, Biology**

The diagrams at the left show the outcome of nuclear transplantation experiments. Note what happens when the nucleus of *A. crenulata* is grafted on to the stalk of *A. mediterranea.* What does this say about the role of the nucleus in determining the traits of the cell?

3. During the 1880s, German biologist Walter Flemming studied the behavior of chromosomes in reproducing cells. His work showed that new individuals begin with the union of sperm and egg cells, which always contain chromosomes and often little else. What does this establish about the role of chromosomes?

4. During the early 1900s, German chemist Robert Feulgen discovered that all body cells of any particular organism contain precisely the same amount of nuclein but that the amount of protein varies from cell to cell. He also found that egg and sperm cells contain exactly one-half the amount of nuclein present in body cells.

5. Later work determined that the non-protein part of nuclein was *deoxyribonucleic acid* (DNA)—but its structure was still uncertain. It was clear that that the monomer (building block) of DNA was a *nucleotide*. Nucleotides can be broken down into three parts: 1) a five-carbon sugar called *deoxyribose*, 2) an atom of phosphorus surrounded by four atoms of oxygen called a *phosphate group*, and 3) molecules called *nitrogenous bases*, which consist of either a single ring of nitrogen and carbon atoms, or a double ring. Four different kinds of nitrogenous bases were found: adenine, guanine, cytosine, and thymine. In other words, DNA consists of four different kinds of nucleotides, each with one of the four nitrogenous bases. Two representations of a nucleotide containing the nitrogenous base cytosine are shown above and to the right.

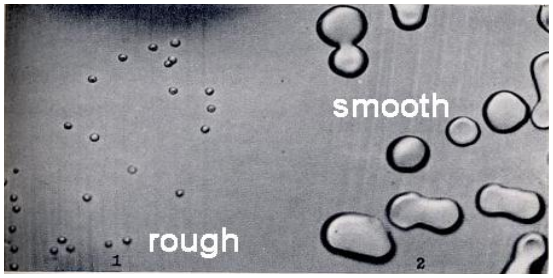


Two representations of a nucleotide consisting of a phosphate group, deoxyribose, and cytosine.

6. During the late 1940s, English scientists discovered that DNA (like sugar) can crystalize when water is removed. This fact suggested that the atoms in DNA must be arranged in a very orderly way, perhaps with many repetitions of a fairly simple pattern. Protein, by contrast, doesn't crystalize.

Clues Set 2

7. During the great influenza of 1918-1919, many of the deaths associated with the flu virus were actually caused by secondary infections involving a type of bacteria called *Pneumococcus*. Infection with this bacteria led to pneumonia, a lung inflammation in which the air sacs fill with pus. This made understanding the biology of *Pneumococcus* an important research topic in the 1920s. One of the researchers whose focus was *Pneumococcus* was the British bacteriologist Frederick Griffith, who was studying *Pneumococcus* in the hopes of developing a vaccine against pneumococcal infection. *Pneumococci* bacteria come in two varieties, or strains. In one strain, the pneumococci are surrounded by a capsule, a layer of polysaccharide just outside of the cell membrane. These are called "smooth." In the second strain, the cells lack a capsule. These are called "rough." You can see the difference between these two strains in the picture of bacterial colonies to your left..



www.bio.miami.edu/dana/pix/S_pneumonia.jpg

These two strains vary in terms of virulence (ability to cause disease), as you can see in the upper part (1 and 2) of the diagram to your right.

In 1928, Griffith carried out a series of experiments on *Pneumococcus*, which are summarized in the diagram above and the text below. .

In diagram 1, smooth strain bacteria were injected into mice. The mice developed pneumonia and died.

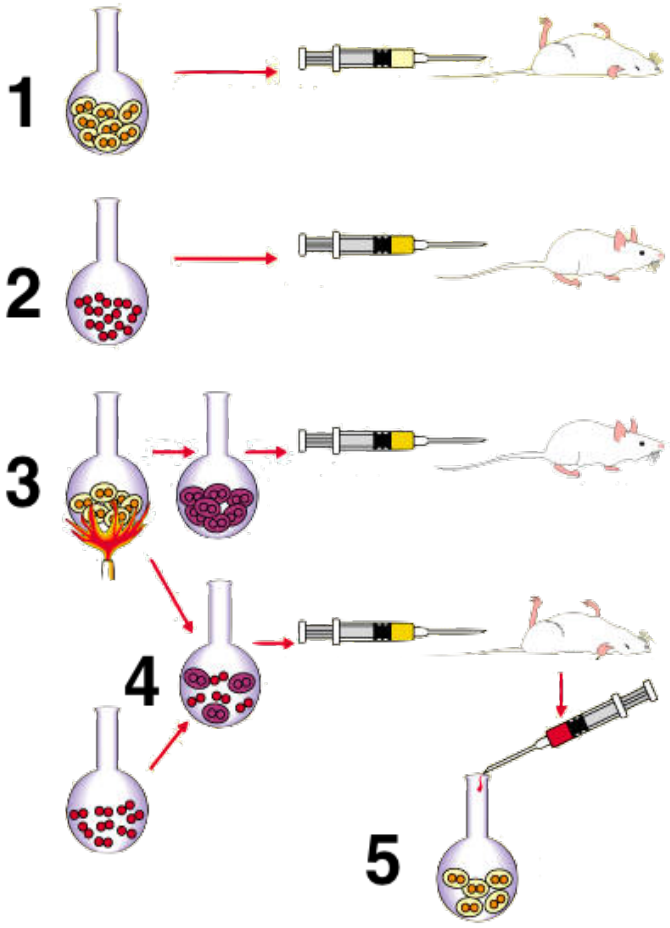
In diagram 2, rough strain bacteria were injected into mice. The mice survived.

In diagram 3, Griffith heated smooth strain bacteria, which killed the cells. When he injected these heat-killed smooth strain bacteria into mice, the mice lived.

Next, Griffith took these heat killed, smooth-strain bacteria and mixed them with living rough strain bacteria (the mixture is shown at 4). He then injected this mixture into mice. The mice died.

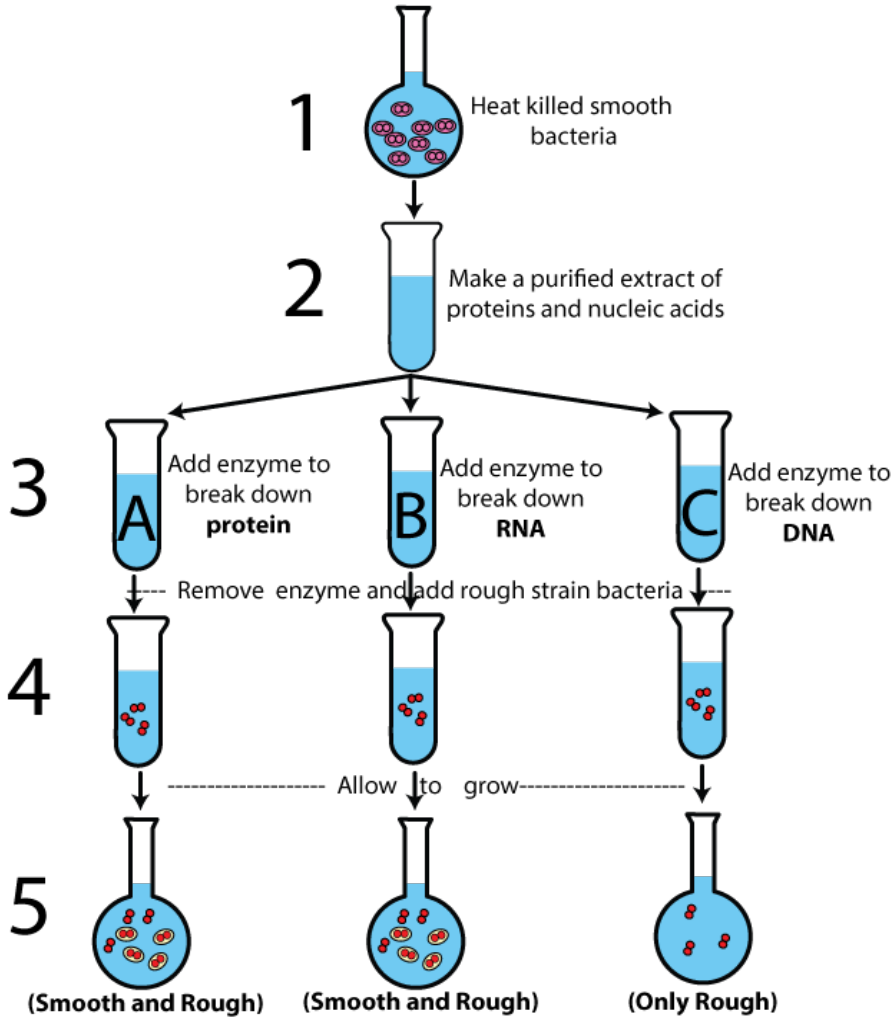
When blood was drawn from these dead mice, Griffith observed living smooth strain bacteria (shown at 5).

What can you conclude from this series of experiments?



8. The result of Griffith's work was identification of an unknown "transforming factor." Something in heat-killed, smooth-strain *Pneumococcus* bacteria could transform non-virulent rough-strain bacteria into the virulent smooth-strain bacteria. But what was the chemical nature of this transforming factor?

It fell upon Oswald Avery, working with his colleagues Colin MacLeod and Maclyn McCarty at the Rockefeller University Hospital in New York City, to figure out what the transforming factor was. Avery's experiments involved taking the transforming factor and digesting it with various enzymes. The diagrams below summarize what they did and what they found.



As is shown in "1," they started with heat-killed, smooth strain bacteria (the virulent variety). Remember that even though the cells were dead, they still contained the "transforming factor."

They broke down the cells, and removed the lipids and carbohydrates, leaving them with a liquid containing the possible contenders for the transforming factor: nucleic acids and protein. This liquid was in test tube 2. Keep in mind that nucleic acids come in two varieties: DNA, and a closely related molecule, RNA.

They subjected the nucleic acid/protein-containing liquid to three enzymes. Enzyme A would digest the protein, creating a protein-free liquid. Enzyme B would digest RNA, and enzyme C would digest DNA. The adding of these enzymes is shown in "3".

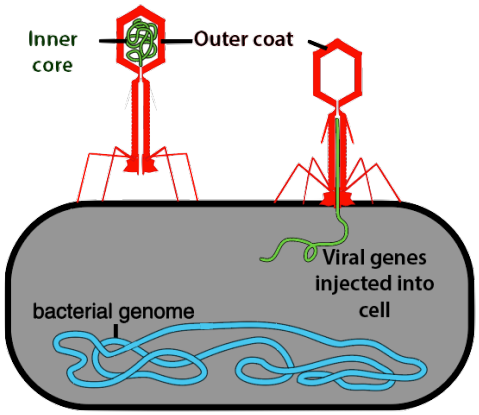
Then they added non-virulent, rough strain bacteria, and allowed the bacteria to grow ("4").

The results are shown in "5." In the flasks where the protein and the RNA had been digested (columns "A" and "B"), they found both rough and smooth strain bacteria. In the flask where DNA had been digested, there were only rough bacteria.

In your notes, describe what Avery and his colleagues had established, and how they had established it.

9. In the early 1950s, Alfred Hershey and Martha Chase, from the Cold Spring Harbor Laboratory in New York, were working with viruses. The particular virus they worked with is called a **phage**: a virus that attacks bacteria. Here's what Hershey and Chase knew about viruses as they were setting up what turned out to be a landmark experiment.

1. Phage viruses infect bacterial cells, turning them into virus factories.
2. Phage viruses are composed mostly of DNA and protein.
3. Phage have a structure that consists of an outer coat, and an inner core. When phage infect bacterial cells, they leave their coat outside the cell and inject something inside which initiates the cycle of viral takeover.

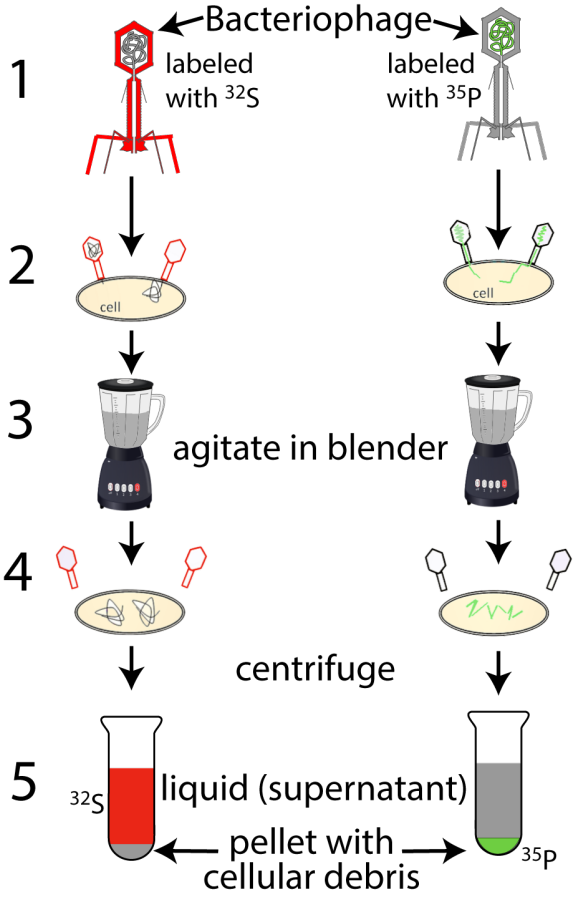


To see if it was the protein or the DNA that was injected inside the cell, Hershey and Chase needed to be able to follow these substances during the viral infection cycle. One way to do this was to radioactively label the protein and DNA. They did this by growing bacteria on two separate food sources. In group one, they used a radioactive isotope of phosphorus, ^{32}P. Phosphorus is present in DNA, but not in protein. In the second group, they used a radioactive isotope of sulfur, ^{35}S. Sulfur is present in protein, but not in DNA. Next, they infected these bacteria with phage. When phage take over bacterial cells, they use them as virus factories to assemble more phage. As a result, whatever the bacteria are made of, the phage will be made of (because even for viruses, "you are what you eat."). Consequently, the phage that would be produced in group one (with radioactive phosphorus) would have radioactive phosphorus built into their DNA. By contrast, the phage produced in group two would have radioactive sulfur built into their protein.

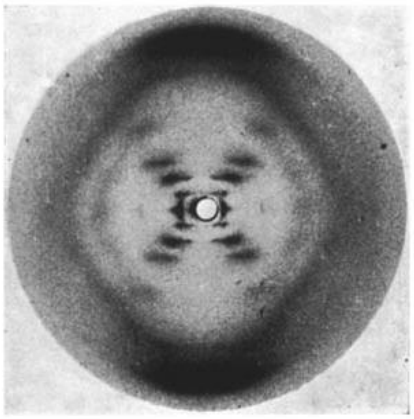
With this labeling system, all that Hershey and Chase had to do was to allow phage to start their infection cycle. If they could isolate infected cells just at the moment after the phage had injected their genes inside the cells that they were attacking, they could then test to see what the phage had injected inside: protein, or DNA. Here's how they did it.

In row 1, at the top of the diagram to the right, you can see the radioactively labeled phage. These phage are allowed to infect cells that have been grown on normal, non-radioactive growth media (shown in row 2). That way, if any radioactivity appeared in the infected cells, Hershey and Chase would know that it was brought in by the radioactive phage.

Now, remember that the goal was to figure out what the genetic material that the phage were injecting into the cells was made of. Hershey and Chase didn't want the phage to go through their entire reproductive cycle, because that would make interpretation of the results impossible (see if you can figure out why after you read what follows). So after just enough time passed for the phage to inject their genes into their bacterial victims, Hershey and Chase placed the mixture of phage and bacteria into a blender (shown in row 3). The blender shook the attacking phage off of the cells they had just attacked (row 4). Then they broke the cells open (using chemicals that dissolve the cells' membranes while not destroying protein or DNA) and placed the mixtures in a centrifuge. A centrifuge spins test tubes around at high speed, and, as it does, it separates whatever is in the test tube by density. The result was a test tube with liquid on top (called the "supernatant") and a pellet of denser, cellular debris on the bottom (see row 5).



Note the results: In the treatment with radioactive phosphorus, ^{35}P was found in the pellet of cellular debris at the bottom of the test tube. And in the treatment with radioactive sulfur, ^{32}S was found only in the supernatant (and not in the debris pellet). How would you interpret this?



Clues Set 3 (1952)

10. At this point, it was clear that genes were made of DNA. So what structure could DNA have that could allow it to store and transmit genetic information? A key clue related to DNA's structure was provided by the work of Rosalind Franklin. Franklin, working with her colleague Maurice Wilkins and her graduate student Raymond Gosling at Cambridge University in England was trying to decipher the structure of DNA by crystallizing it, and then photographing the crystals using X-rays. The X-rays would create a diffraction pattern that could be used to infer the arrangement of the atoms in the DNA crystal.
https://upload.wikimedia.org/wikipedia/en/b/b2/Photo_51_x-ray_diffraction_image.jpg

Some of the thinking involved in interpreting these images is demonstrated below by Stephen Carr:
(https://www.mun.ca/biology/scarr/Franklins_crystallograph.html)

"...The X-ray crystallograph (at left, also known as "*Photo 51*") shows an exceptionally clear diffraction pattern of a crystallized DNA molecule. The X-pattern in the middle is characteristic of a helical molecule with regular repeats." The image also allowed one to infer the distance between the monomers making up the DNA polymer (3.4 Angstroms, or 0.34 nanometers), and that the width of the DNA stayed constant throughout its entire length."

Historians of science have speculated that Franklin and Wilkins, with a bit more time, might have deduced the structure of DNA. But the prize was won by two other researchers who were also working in Cambridge: American physicist James Watson and British biologist Francis Crick. Their method involved synthesizing data, building models, and using their rival's data (they didn't get along with Franklin, who also had a difficult relationship with her colleague Wilkins. Watson and Crick convinced Wilkins to let them take a look at one of Franklin's best photographs of DNA). Watson and Crick also had access to other clues (which others had access to as well):

Chargaff's Data: Relative % of Bases in DNA

Organism	%A	%G	%C	%T
Maize	26.8	22.8	23.2	27.2
Octopus	33.2	17.6	17.6	31.6
Chicken	28.0	22.0	21.6	28.4
Rat	28.6	21.4	20.5	28.4
Human	29.3	20.7	20.0	30.0
Grasshopper	29.3	20.5	20.7	29.3
Sea Urchin	32.8	17.7	17.3	32.1
Wheat	27.3	22.7	22.8	27.1
Yeast	31.3	18.7	17.1	32.9
E. coli	24.7	26.0	25.7	23.6

11. In the 1940s, Erwin Chargaff, a Swiss biochemist, analyzed the DNA of many species and organized the data in the table that's shown to your left. This data gives an enormous clue to DNA's structure…Figure it out and write it down in your lab report.

12. Additional evidence indicated that the DNA helix contains strands of phosphate groups and sugar molecules linked by covalent chemical bonds.

13. Watson and Crick calculated that a single chain of nucleotides would have a density only half as great as the known density of DNA.

14. In the spring of 1953, Watson arrived at the lab early one day, cut out cardboard models of nucleotides containing adenine, thymine, guanine, and cytosine molecules, and began arranging them in various combinations and patterns on his desk. He discovered that an adenine-thymine pair presumably held together by relatively weak hydrogen bonds is identical in shape to a guanine-cytosine pair also held together by hydrogen bonds.

DRAW A CONCLUSION:

Later that same day, Watson showed Crick his result, and when they went to lunch at the Eagle Cafe in London, Crick told everyone within hearing distance that they had found the secret of life. Now it's your turn to discover the same secret:

YOUR FINAL JOB: After you've drawn inferences from all the clues above, get a kit of cardboard cutouts, and do the following:

1) Create a model of DNA that contains at least 8 nucleotides
2) Make a drawing of this model in your notes. In your drawing, indicate which bonds are covalent bonds, and which bonds are hydrogen bonds.
3) Create a bullet point list where you explain how the model you've created is supported by various pieces of evidence that you've gleaned from this activity.
4) Propose a method by which DNA could be replicated (something that Watson and Crick did in their 1953 paper describing the structure of DNA).

Cut out the shapes below for DNA model construction. Each lab group will need about three copies of this sheet.