

Ο	Bacteria				•	•	•		•	. A

O Capsule.....B

O Pathogenic Bacteria....C

DNA and	Transformation
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 Dead MouseD
Nonpathogenic BacteriaE
Healthy Mouse......F

Ο	Heat-killed					
	Bacteria	G				
Ο	Bacteria	MixtureH				

<u>Chapter 4-6</u>: DNA and Transformation

Modern biologists know that deoxyribonucleic acid (DNA) is inherited through generations, and that it is the carrier of genetic information. Over the decades, a wealth of evidence has accumulated attesting to this fact, but the relationship between DNA and gene expression was not recognized until the 1940s and 1950s. Prior to that time, scientists were uncertain where the processes of genetics took place, or what cellular components were involved. One of the first to draw conclusions about the role of DNA in heredity was Frederick Griffith, in 1928.

This plate contains diagrams detailing four experiments that were performed by Frederick Griffith in 1928. As a result of these experiments, Griffith managed to transform bacterial cells, converting them

from nonpathogenic (non-disease causing) to pathogenic (disease causing) forms.

Frederick Griffith was a British bacteriologist who worked with the bacteria that cause pneumonia. These bacteria, which are called *Streptococcus pneumoniae*, are composed of a chain of cocci that are spherical. For now we will refer to this organism simply as "bacteria."

Diagram 1 shows one of the experiments performed by Griffith. (You may wish to use bold colors on this diagram.) The **bacteria** (A) are enclosed in **capsules** (B). These bacteria are pathogenic, meaning that they are disease causing. When the **pathogenic bacteria** (C) are placed in a syringe and injected into an animal, the animal gets sick. Note the **dead mouse** (D).

The second diagram shows another experiment performed by Griffith. Here we note that the bacteria (A) are not contained within capsules. These bacteria are harmless, or nonpathogenic. When a syringe is filled with these **nonpathogenic bacteria (E)** and the contents are injected into an animal, the animal lives. Note the **healthy mouse (F)**. As you can see, the bacteria's capsule is the key to its pathogenicity; in its absence the bacteria are harmless.

We now know that the presence of a capsule is the difference between pathogenic bacteria and nonpathogenic bacteria. We now continue with Griffith's experiments as we concentrate on diagram 3. The third experiment performed by Griffith is shown in diagram 3. Here the bacteria were killed by excessive heating. These **heat-killed bacteria (G)** are encapsulated, and a different color should be used for them. When the dead bacteria are injected into the animal, the animal lives. A **healthy mouse (F)** is a result of the third experiment.

We now focus on Griffith's most important experiment. This is where the phenomenon of transformation occurred. Focus on diagram 4 as you read below.

Griffith's key experiment in transformation was performed in the following way. He took live, unencapsulated bacteria (A) and mixed them with heat-killed dead bacteria (G) that were capsulated (B). This **bacterial mixture** (H) of live, unencapsulated bacteria and dead encapsulated bacteria should have been harmless to the mouse; this mixture was unlike the bacteria used in the previous three experiments. However, when a syringeful of the mixture was injected into a mouse, the animal died (D)!

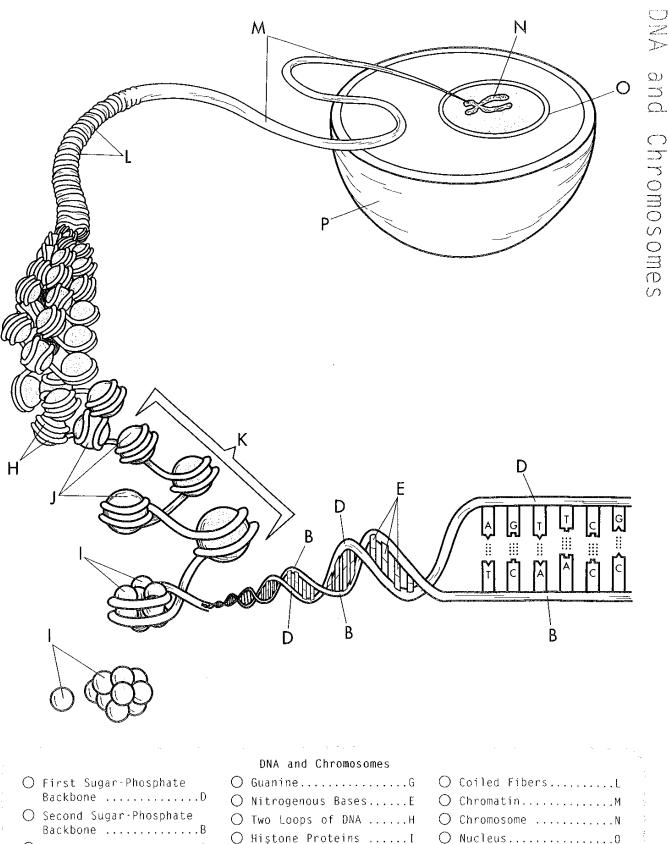
Griffith questioned how a mixture of two kinds of nonpathogenic bacteria could kill the mouse. He obtained tissue from the mouse and examined it under his microscope. His observations were startling; he saw live bacteria (A) that were enclosed in capsules (B)! The mouse had died because it was attacked by live, pathogenic bacteria.

Griffith concluded that the live unencapsulated bacteria (A) were transformed into live, encapsulated bacteria through interaction with the dead encapsulated bacteria. Griffith hypothesized that the substance responsible for this transformation of genetic material was a protein, and called it the "transforming principle".

Griffith's work showed that bacteria could be transformed, but the method of the transformation was still unknown. Many years would pass before Oswald Avery and his group would identify the transforming substance as deoxyribonucleic acid (DNA). This occurred in 1944.

4-6: DNA & Transformation

- a. Who was the first to draw conclusions about DNA & heredity & when did he do it?
- b. With what type of bacteria did he work?
- c. Harmful bacteria were enclosed in:
- d. What does pathogenic mean?
- e. Injecting a mouse with pathogenic bacteria yielded what result?
- f. Injecting a mouse with nonpathogenic bacteria yielded what result?
- g. Injecting a mouse with heat-killed pathogenic bacteria yielded what result?
- h. Injecting a mouse with heat-killed pathogenic & live nonpathogenic bacteria yielded what result?
- i. What did Griffith see under the microscope?
- j. What did Griffith hypothesize that the transforming agent was?
- k. Who later identified the transforming agent as DNA & when did he do it?



○ Condensed HistonesJ

O Nucleosomes.....K

○ Eukaryotic Cell.....P

- O Adenine......A
- O Thymine.....T
- O CytosineC

Chapter 4-5: DNA and Chromosomes

It is important to understand how DNA is packaged into genes and chromosomes since it will help you understand the cycles of condensation and unraveling that occur during mitosis and cell division. It is also important to understand how DNA is arranged into chromosomes because the spatial arrangement of DNA influences gene expression.

In addition, understanding the way in which DNA is coiled into chromosomes will help you understand how more than two meters (about six feet) of DNA fits into forty-six chromosomes in a nucleus that's less than five micrometers in diameter. This plate explores the current model for chromosome organization in eukaryotic cells and shows how DNA is organized with protein in chromosomes.

Starting at the bottom of the plate, notice the molecule of DNA. The double-stranded molecule will become progressively more coiled and eventually forms the chromosome. We will describe that coiling as we proceed in the plate.

Electron microscopic studies and biochemical research have helped biologists understand how DNA associates with protein to form chromosomes. This packaging enables DNA to direct protein synthesis and replicate, but also prevents it from tangling in the process of mitosis.

Again note the double-stranded molecule of DNA. The first sugar-phosphate backbone (D) is at the top, and the second sugar-phosphate backbone (B) is at the bottom. These sugar-phosphate backbones should be colored different colors and traced in the DNA molecule until they are no longer visible in the diagram.

Associated with the sugar-phosphate backbones are the four nitrogenous bases of DNA. They are **adenine** (A), thymine (T), cytosine (C), and guanine (G). Four bold colors should be used to distinguish these **nitrogenous bases** (E). As you can see, they are initially distinct, but as the DNA molecule condenses, they are more difficult to pinpoint and color, so you might want to trace them.

We have reviewed the structure of the DNA molecule, and we now move on to show its association with histone proteins, the result of which is the nucleosome. Continue your reading below as you color.

4-5: DNA & Chromosomes

a. How long is DNA? How big is the nucleus?

b. What are the proteins with which chromosomes are associated?

c. In clusters of how many do these proteins occur?

d. How many loops of DNA surround each histone?

e. The product of the histone & looped DNA is called a:

f. What further packages the nucleosomes?

g. These coiled fibers are collectively known as:

h. These condense even further during prophase & metaphase producing the traditional:

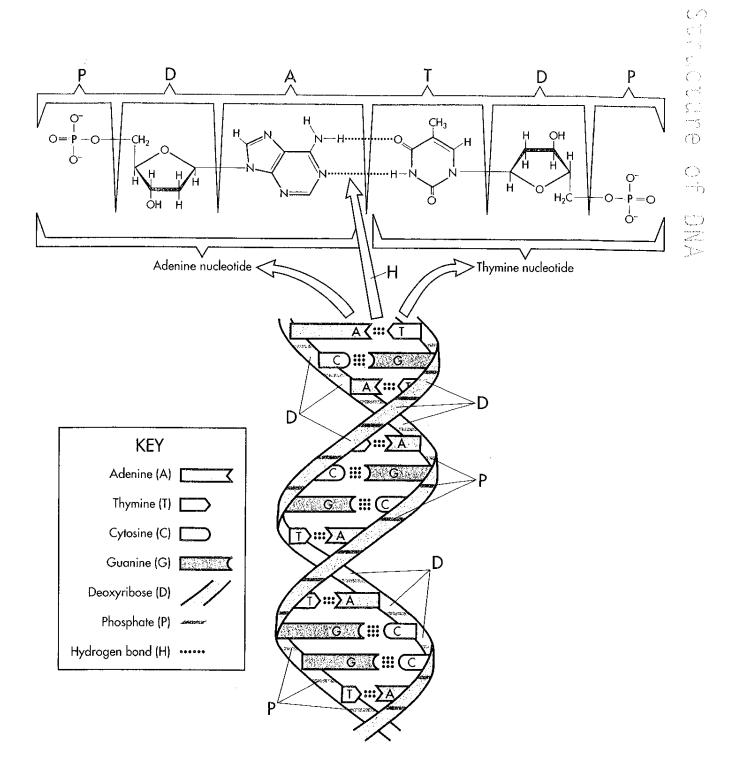
In eukaryotic cells, chromosomes are associated with proteins known as histones. **Histone proteins (I)** occur in clusters of eight molecules, as the plate shows. These histones are small, basic proteins that facilitate DNA packaging. The eight histone proteins are shown distinctly at first, then for simplicity we show them collectively as **condensed histones (J)**.

Note in the diagram that **two loops of DNA (H)** surround each histone. The product of this looping is a unit called a nucleosome. Several **nucleosomes (K)** are outlined by a bracket, which should be colored in a dark color. The nucleosome is the fundamental packing unit for DNA. You should try to color several of the condensed histones and their double loops of DNA.

Having studied the fundamental packing unit of DNA in the chromosome, we now examine how the nucleosomes combine with one another.

A particular type of protein locks the nucleosomes together so that DNA cannot unwind from its histone core; the nucleosomes remain strung together like beads on a necklace. The winding of DNA around the histones shortens its length considerably, but the strand must be further shortened if the chromosome is to fit in the cell's nucleus. This is accomplished when the nucleosomes are further packed into thick **coiled fibers (L)**. A light color is recommended to avoid obscuring these coils.

The coiling of nucleosomes into coiled fibers produces thicker fibers that contain even more compact DNA. These fibers are collectively known as **chromatin** (M). During interphase and early prophase, the DNA of a cell exists as these ultramicroscopic fibers, but during late prophase and metaphase, the chromatin condenses even further. This final compacting produces the traditional **chromosome** (N). The diagram shows two chromosomes joined at the centromere just before separation, during anaphase. You can see the **nucleus** (O), which indicates that it is a **eukaryotic cell** (P). (Few of the details of the cell are shown here since we are concentrating on the DNA and the chromosome.) In bacterial cells, DNA exists without protein wrapping. 4.1 Structure of DNA



. 6		and a second	and a second
15 7 8		Structure of DNA	
1	○ Phosphate GroupP	○ AdenineA	O CytosineC
7 2 2	○ DeoxyriboseD	○ ThymineT	⊖ GuanineG
s.,		⊖ Hydrogen BondH	
		an a	

<u>Chapter 4-1:</u> Structure of DNA

Two types of nucleic acids exist: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the genetic material of organisms, while RNA is used during the construction of proteins. This plate will examine the structure of DNA. RNA's structure is studied in a succeeding plate in this chapter.

This plate illustrates the components of a molecule of DNA. Letters have been correlated with the names of some of the components; most textbooks use these letter abbreviations. Light colors such as grays and yellows should be used for the first part of the plate.

DNA exists in the chromosome of the living eukaryotic cell, and in the cytoplasm of prokaryotic cells. DNA is composed of repeating units known as nucleotides. Each nucleotide has three components: a molecule of the carbohydrate deoxyribose, a phosphate group, and a nitrogenous base. At the upper portion of the plate, two nucleotides are shown. At the left is a nucleotide composed of a **phosphate group** (**P**), a **deoxyribose molecule** (**D**), and a nitrogenous base called **adenine** (**A**). The three components should be lightly shaded to avoid obscuring their individual atoms.

The deoxyribose molecule contains a five-carbon carbohydrate ring bound to the phosphate group at its -CH₂ group. On its opposite side, the deoxyribose molecule is bonded to the adenine molecule. The adenine contains five nitrogen atoms, which is why it is called a nitrogenous base.

A second nucleotide is shown at the right. It consists of a nitrogenous base called **thymine (T)**, bonded to a deoxyribose molecule (D) which is inverted here. The deoxyribose is in turn bonded to a phosphate group (P). As before, light shading should be used to denote the three portions of the nucleotide.

Adenine and thymine nucleotides are held to one another by two **hydrogen bonds (H)**, one of which is indicated by an arrow, which should be colored boldly. Hydrogen bonds are weak chemical bonds formed between hydrogen and nearby electronegative atoms. In DNA, two hydrogen bonds exist between A and T, and three exist between G and C. We will now examine how the nucleotides bind to one another to form DNA. Continue your coloring as you read, and use the same colors in the DNA molecule that you used for the nucleotides.

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The four nitrogenous bases that make up DNA are thymine, adenine, **cytosine (C)**, and **guanine (G)**. Let's take a look at the DNA double helix.

Begin at the top of the molecule, and note that the first nucleotide contains adenine (A), and that it is attached to deoxyribose (D). The deoxyribose is connected to a phosphate group (P) which in turn is connected to another deoxyribose molecule. The latter is connected to a cytosine (C) molecule, as well as another phosphate group (P). A deoxyribose molecule (D) follows, which is connected to an adenine (A). This pattern continues with alternating deoxyribose molecules and phosphate groups as the ribbon-like strand continues and curves. Each deoxyribose molecule is connected to one of the four nitrogenous bases.

Now move to the right side of the molecule and follow the ribbon, beginning at the upper right. As you follow it, note that it contains deoxyribose molecules that alternate with phosphate groups, and that again, connected to each deoxyribose molecule is one of the four nitrogenous bases. The second strand of DNA is very similar to the first strand.

We will complete the plate by noting how the two strands of DNA unite to form the double-stranded DNA molecule. If you have not yet completed your coloring of all the parts of the two strands, do so at this point. Then read below.

In the complete DNA molecule, two single strands oppose one another in a ladder-like arrangement, in which the nitrogenous bases line up opposite one another according to the principle of complementary base pairing. Adenine always lines up opposite thymine, and cytosine always lines up opposite guanine. As we mentioned earlier, hydrogen bonds then hold the bases together. The nitrogenous bases thus form rungs of a ladder.

4-1: Structure of DNA

- a. What two types of nucleic acids exist?
- b. What is the function of DNA? Of RNA?
- c. Where is DNA located in eukaryotic cells? In prokaryotic cells?
- d. What are the three components of a nucleotide?
- e. How are nucleotides (such as adenine & thymine) held together?
- f. How many hydrogen bonds exist between A&T? G&C?
- g. What are the four nitrogenous bases that make up DNA?
- h. On the sides of the DNA molecule, deoxyribose alternates with what group?
- i. What also is connected to deoxyribose, forming the middle of the molecule?
- j. With what base does adenine always pair? Cytosine?