Cell reproduction involves a series of steps that always begin with the processes of interphase. During interphase the cell’s genetic information which is stored in its nucleus in the form of chromatin, composed of both mitotic and interphase chromosomes molecules of protein complexes and DNA strands that are loosely coiled winds tightly to be replicated. It is estimated that the DNA in human cells consists of approximately three billion nucleotides. If a DNA molecule was stretched out it would measure over 20 miles in length and all of it is stored in the microscopic nuclei of human cells. This lesson will help you to understand how such an enormous amount of DNA is coiled and packed in a complicated yet organized manner. During cell reproduction as a cell gets ready to divide the DNA coils even more into tightly compact structures.

Lesson Objectives
• Describe the coiled structure of chromosomes.
• Understand that chromosomes are coiled structures made of DNA and proteins. They form after DNA replicates and are the form in which the genetic material goes through cell division.
• Discover that DNA replication is semi-conservative; half of the parent DNA molecule is conserved in each of the two daughter DNA molecules.
• Outline discoveries that led to knowledge of DNA’s structure and function.
• Examine the processes of DNA replication.

Vocabulary
• centromere
• Chargaff’s rules
• chromatid
• chromatin
• chromosome
• DNA replication
• double helix
• histones
• nucleosomes
• semi-conservative DNA replication
• sister chromatids
• transformation

Introduction
In eukaryotic cells, the nucleus divides before the cell itself divides. The process in which the nucleus divides is called mitosis. Before mitosis occurs, a cell’s DNA is replicated. This is necessary so that each daughter cell will have a complete copy of the genetic material from the parent cell. How is the replicated DNA sorted and separated so that each daughter cell gets a complete set of the genetic material? To understand how this happens, you need to know more chromosomes.
CHROMOSOMES

Chromosomes are coiled structures made of DNA and proteins. Each chromosome is a single DNA molecule. Chromosomes are the form of the genetic material of a cell during cell division. During other phases of the cell cycle, DNA is not coiled into chromosomes. Instead, it exists as a grainy material called chromatin.

In eukaryotic cells during the S phase of interphase, DNA wraps tightly around disc-shaped proteins called histones. The DNA wraps around each histone twice. Histones help chromosomes maintain their shape and aid in the process of tightly packing DNA. When DNA is wrapped around the histones it forms bead-like structures called nucleosomes. Long strings of nucleosomes form thick coils that pack together to make up closed chromatin. During the prophase step of mitosis the chromatin, which is very tightly packed, become chromosomes that are visible under a microscope. These structural changes can be seen in Figure 6.14 below.

![Figure 6.14 DNA’s transition into chromosome form.](image)

Chromosomes are simpler structures in prokaryotic organisms. The DNA of most prokaryotes consists of only one chromosome, which is attached to the inside of the cell membrane. Prokaryotic chromosomes consist of a circular DNA molecule (see Figure 6.15) compactly packed into the cell.

![Figure 6.15 Prokaryotic chromosomes consist of a single circular DNA molecule, while eukaryotic chromosomes are composed of tightly packed chromatin.](image)

**Chromatids and the Centromere**

DNA condenses and coils into the familiar X-shaped form of a chromosome, shown in Figure 6.16, only after it has replicated. This X-shaped form of DNA has already replicated, each chromosome actually consists of two identical copies. The two copies are called sister chromatids. They are attached to one another at a region called the centromere.
**Chromosome Numbers**

Every species of living organism on Earth has its own characteristic number of chromosomes in each of its cells. For example, cats have 32 chromosomes in each of their cells and dogs have 78. Some species even have the same number of chromosomes in each of their cells. Potatoes, plums, and chimpanzees all have 48 chromosomes in each of their cells. Human cells normally have two sets of chromosomes, one set inherited from each parent. There are 23 chromosomes in each set, for a total of 46 chromosomes per cell. Each chromosome in one set is matched by a chromosome of the same type in the other set, so there are actually 23 pairs of chromosomes per cell. Each pair consists of chromosomes of the same size and shape that also contain the same genes. The chromosomes in a pair are known as homologous chromosomes.

**DISCOVER OF DNA STRUCTURE**

Today, it is commonly known that DNA is the genetic material. For a long time, scientists knew such molecules existed. They were aware that genetic information was contained within organic molecules. However, they didn’t know which type of molecules play this role. In fact, for many decades, scientists thought that proteins were the molecules that carry genetic information. In this section, you will learn how scientists discovered that DNA carries the code of life.

**Griffith Searches for the Genetic Material**

Many scientists contributed to the identification of DNA as the genetic material. In the 1920s, Frederick Griffith made an important discovery. He was studying two different strains of a bacterium, called R (rough) strain and S (smooth) strain. He injected the two strains into mice. The S strain killed (virulent) the mice, but the R strain did not (non-virulent) (see Figure 6.17). Griffith also injected mice with S-strain bacteria that had been killed by heat. As expected, the killed bacteria did not harm the mice. However, when the dead S-strain bacteria were mixed with live R-strain bacteria and injected, the mice died.
Based on his observations, Griffith deduced that something in the killed S-strain was transferred to the previously harmless R-strain, making the R-strain deadly. He called this process transformation, as something was "transforming" the bacteria from one strain into another strain. What was that something? What type of substance could change the characteristics of the organism that received it?

Avery’s Team Makes a Major Contribution
In the early 1940s, a team of scientists led by Oswald Avery tried to answer the question raised by Griffith’s results. They inactivated various substances in the S-strain bacteria. They then killed the S-strain bacteria and mixed the remains with live R-strain bacteria. (Keep in mind, the R-strain bacteria usually did not harm the mice.) When they inactivated proteins, the R-strain was deadly to the injected mice. This ruled out proteins as the genetic material. Why? Even without the S-strain proteins, the R-strain was changed, or transformed, into the deadly strain. However, when the researchers inactivated DNA in the S-strain, the R-strain remained harmless. This led to the conclusion that DNA is the substance that controls the characteristics of organisms. In other words, DNA is the genetic material.

Hershey and Chase Seal the Deal
The conclusion that DNA is the genetic material was not widely accepted at first. It had to be confirmed by other research. In the 1950s, Alfred Hershey and Martha Chase did experiments with viruses and bacteria. Viruses are not cells. They are basically DNA inside a protein coat. To reproduce, a virus must insert its own genetic material into a cell (such as a bacterium). Then it uses the cell’s machinery to make more viruses. The researchers used different radioactive elements to label the DNA and proteins in viruses. This allowed them to identify which molecule the viruses inserted into bacteria. DNA was the molecule they identified. Their experimental procedure can be viewed in Figure 6.18. This confirmed that DNA is the genetic material.

![Figure 6.18](image-url) Hershey and Chase experiments using radioactive markers to trace the genetic material transferred from viruses to bacterium.

Chargaff Writes the Rules
Other important discoveries about DNA were made in the mid-1900s by Erwin Chargaff. He studied DNA from many different species. He was especially interested in the four different nitrogen bases of DNA: adenine (A), guanine (G), cytosine (C), and thymine (T) (see Figure 6.19). Chargaff found that concentrations of the four bases differed from one species to another. However, within each
species, the concentration of adenine was always about the same as the concentration of thymine. The same was true of the concentrations of guanine and cytosine. These observations came to be known as Chargaff’s rules; A pairs with T and G pairs with C. The significance of the rules would not be revealed until the structure of DNA was discovered.

![Nitrogen Bases in DNA](image)

**Figure 6.19** Nitrogen Bases in DNA. The DNA of all species has the same four nitrogen bases.

**The Double Helix**

After DNA was found to be the genetic material, scientists wanted to learn more about it. James Watson and Francis Crick are usually given credit for discovering that DNA has a double helix shape (April, 1953), like a spiral staircase (see **Figure 6.20**). The discovery was based on the prior work of Rosalind Franklin and other scientists, who had used X rays to learn more about DNA’s structure. Franklin and these other scientists have not always been given credit for their contributions, see **Figure 6.21** of Rosalind Franklin’s x-ray diffraction images of the DNA molecule.

![DNA Double Helix](image)

**Figure 6.20** The DNA molecule has a double helix shape. This is the same basic shape as a spiral staircase. Do you see the resemblance? Which parts of the DNA molecule are like the steps of the spiral staircase?

![X-ray diffraction image](image)

**Figure 6.21** X-ray diffraction image of DNA taken by Rosalind Franklin.
The double helix shape of DNA, together with Chargaff’s rules, led to a better understanding of DNA. DNA, as a nucleic acid, is made from nucleotide monomers, and the DNA double helix consists of two polynucleotide chains. Each nucleotide consists of a sugar (deoxyribose), a phosphate group, and a nitrogen-containing base (A, C, G, or T).

Scientists concluded that bonds (hydrogen bonds) between complementary bases hold together the two polynucleotide chains of DNA. Adenine always bonds with its complementary base, thymine. Cytosine always bonds with its complementary base, guanine. If you look at the nitrogen bases in Figure 6.19, you will see why. Adenine and guanine have a two-ring structure. Cytosine and thymine have just one ring. If adenine were to bind with guanine and cytosine with thymine, the distance between the two DNA chains would be variable. However, when a one-ring molecule binds with a two-ring molecule, the distance between the two chains is kept constant. This maintains the uniform shape of the DNA double helix. These base pairs (A-T or G-C) stick into the middle of the double helix, forming, in essence, the steps of the spiral staircase.

In the Figure 6.22 you can see what James Watson’s and Francis Crick’s model of DNA constructed from cardboard and wire looked like.

![Figure 6.22 Watson and Crick and their cardboard and wire DNA molecule model (1953).](image)

**DNA REPLICATION**

Knowledge of DNA’s structure helped scientists understand how DNA replicates. DNA replication is the process in which DNA is copied. It occurs during the synthesis (S) phase of the eukaryotic cell cycle. DNA replication begins when an enzyme breaks the bonds between complementary bases in DNA (see Figure 6.23). This exposes the bases inside the molecule so they can be “read” by another enzyme and used to build two new DNA strands with complementary bases. The two daughter molecules that result each contain one strand from the parent molecule and one new strand that is complementary to it. As a result, the two daughter molecules are both identical to the parent molecule.

The process of DNA replication is actually much more complex than this simple summary. You can see a detailed animation of the process at this link:

http://www.youtube.com/watch?v=-mtLXpgjHL0&NR=1http://www.youtube.com/watch?v=-mtLXpgjHL0&#.
DNA Replication is Semi-Conservative

DNA replication of one helix of DNA results in two identical helices. If the original DNA helix is called the "parental" DNA, the two resulting helices can be called "daughter" helices. Each of these two daughter helices is a nearly exact copy of the parental helix (it is not 100% the same due to mutations). DNA creates "daughters" by using the parental strands of DNA as a template or guide. Each newly synthesized strand of DNA (daughter strand) is made by the addition of a nucleotide that is complementary to the parent strand of DNA. In this way, DNA replication is semi-conservative, meaning that one parent strand is always passed on to the daughter helix of DNA, Figure 6.24.

Figure 6.23  DNA Replication. DNA replication is a semi-conservative process. Half of the parent DNA molecule is conserved in each of the two daughter DNA molecules.

Figure 6.24  The semi-conservative nature of DNA replication.
Replication Forks and Origins of Replication

The first step in DNA replication is the separation of the two DNA strands that make up the helix that is to be copied. DNA Helicase untwists the helix at locations called replication origins. The replication origin forms a Y shape, and is called a replication fork, Figure 6.25. The replication fork moves down the DNA strand, usually from an internal location to the strand's end. The result is that every replication fork has a twin replication fork, moving in the opposite direction from that same internal location to the strand's opposite end. Single-stranded binding proteins (SSB) work with helicase to keep the parental DNA helix unwound. It works by coating the unwound strands with rigid subunits of SSB that keep the strands from snapping back together in a helix. The SSB subunits coat the single-strands of DNA in a way as not to cover the bases, allowing the DNA to remain available for base-pairing with the newly synthesized daughter strands.

![Replication Fork Diagram](image)

**Figure 6.25 Replication Fork**

As you can see in, when the two parent strands of DNA are separated to begin replication, one strand is oriented in the 5’ to 3’ direction while the other strand is oriented in the 3’ to 5’ direction, Figure 6.26. DNA replication, however, is inflexible: the enzyme that carries out the replication, DNA polymerase, only functions in the 5’ to 3’ direction. This characteristic of DNA polymerase means that the daughter strands synthesize through different methods, one adding nucleotides one by one in the direction of the replication fork, the other able to add nucleotides only in chunks. The first strand, which replicates nucleotides one by one is called the leading strand; the other strand, which replicates in chunks, is called the lagging strand.

**The Leading and Lagging Strands**

**The Leading Strand**

Since DNA replication moves along the parent strand in the 5' to 3' direction, replication can occur very easily on the leading strand. As seen in, the nucleotides are added in the 5’ to 3’ direction. Triggered by RNA primase, which adds the first nucleotide to the nascent chain, the DNA polymerase simply sits near the replication fork, moving as the fork does, adding nucleotides one after the other, preserving the proper anti-parallel orientation. This sort of replication, since it involves one nucleotide being placed right after another in a series, is called continuous.

**The Lagging Strand**

Whereas the DNA polymerase on the leading strand can simply follow the replication fork, because DNA polymerase must move in the 5’ to 3’ direction, on the lagging strand the enzyme must move away from the fork. But if the enzyme moves away from the fork, and the fork is uncovering new DNA that needs to be replicated, then how can the lagging strand be replicated at all? The problem posed by this question is answered through an ingenious method. The lagging strand replicates in small segments, called Okazaki fragments. These fragments are stretches of 100 to 200 nucleotides in humans (1000 to 2000 in bacteria) that are synthesized in the 5’ to 3’ direction away from the replication fork. Yet while each individual segment is replicated away from the replication fork, each subsequent Okazaki fragment is replicated more closely to the receding replication fork than the fragment before. These fragments are then stitched together by DNA ligase, creating a continuous strand. This type of replication is called discontinuous.
As you can see in the figure above, the first synthesized Okazaki fragment on the lagging strand is the furthest away from the replication fork, which is itself receding to the right. Each subsequent Okazaki fragment starts at the replication fork and continues until it meets the previous fragment. The two fragments are then stitched together by DNA ligase, Figure 6.27.

In figure above, we can also see how replication on the lagging strand remains slightly behind that on the leading strand. Because synthesis on the lagging strand takes place in a "backstitching" mechanism, its replication is slightly delayed in relation to synthesis on the leading strand. The lagging strand must wait for a patch of the parent helix to open up a short distance in front of the newly synthesized strand before it can begin its synthesis back to the end of the daughter strand. This "lag" time does not occur in the leading strand because it synthesizes the new strand by following right behind as the helix unwinds at the replication fork.

Another complication to replication on the lagging strand is the initiation of replication. Whereas the RNA primer on the leading strand only has to trigger the initiation of the strand once, on the lagging strand each individual Okazaki fragment must be triggered. On the lagging strand, then, an enzyme called primase that moves with the replication fork synthesizes numerous RNA primers, each of which triggers the growth of an Okazaki fragment. The RNA primers are eventually removed leaving gaps that are filled by the replication machinery.

Lesson Summary
- Chromosomes are coiled structures made of DNA and proteins.
- Chromosomes form after DNA replicates; prior to replication, DNA exists as chromatin.
- Human cells normally have 46 chromosomes, made up of two sets of chromosomes, one set inherited from each parent.
- The work of several researchers led to the discovery that DNA is the genetic material.
- Along the way, Griffith discovered the process of transformation.
- Chargaff’s rules state that the amount of A is similar to the amount of T, and the amount of G is similar to the amount of C.
- Watson and Crick discovered that DNA has a double helix shape, consisting of two polynucleotide chains held together by bonds between complementary bases.
- DNA replication is semi-conservative: half of the parent DNA molecule is conserved in each of the two daughter DNA molecules.
References/ Multimedia Resources

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