

14 | DNA STRUCTURE AND FUNCTION



Figure 14.1 Michael Morton went to jail in 1986 for the murder of his wife. Twenty-five years later, in 2011, he was exonerated of her murder by DNA evidence. (credit: Lauren Gerson)

Chapter Outline

- 14.1: Historical Basis of Modern Understanding**
- 14.2: DNA Structure and Sequencing**
- 14.3: Basics of DNA Replication**
- 14.4: DNA Replication in Prokaryotes**
- 14.5: DNA Replication in Eukaryotes**
- 14.6: DNA Repair**

Introduction

Each person's DNA is unique, and it is possible to detect differences among individuals within a species on the basis of these unique features. DNA analysis has many practical applications, including identifying criminals (forensics), determining paternity, tracing genealogy, identifying pathogens, researching archeological finds, tracing disease outbreaks, and studying human migration patterns. In the medical field, DNA is used in diagnostics, new vaccine development, and cancer therapy. It is often possible to determine predisposition to diseases by sequencing genes.

Sometimes an innocent person is erroneously convicted of a crime and sent to jail. Between 2000 and 2015, evidence from DNA was used to exonerate over 250 innocent people. Twenty of those people were on death row after being convicted of a murder they didn't commit. To learn more about the intense scientific and legal processes used to exonerate those wrongfully convicted, go to The Innocence Project website [here \(http://www.openstaxcollege.org/l/32innocence\)](http://www.openstaxcollege.org/l/32innocence).

14.1 | Historical Basis of Modern Understanding

In this section, you will explore the following questions:

- What is transformation of DNA? How do Griffith's experiments in 1928 relate to our modern understanding of DNA and how it works?
- What are key historic experiments that helped identify DNA as the genetic material?
- What are Chargaff's rules of nitrogenous base pairing?

Connection for AP[®] Courses

Today the three letters “DNA” have become synonymous with crime solving, paternity testing, human identification, and genetic testing. All of these procedures are possible because of the discovery, in the middle of the twentieth century, that DNA is the genetic material. The results of several classic experiments set the stage for an explosion of our knowledge about DNA and how it stores and transmits genetic information. DNA was first isolated from white blood cells by Miescher in the 1860s. Over fifty years later, Griffith's work transforming strains of the bacterium *Streptococcus pneumoniae* provided the first clue that DNA and not protein (as others argued) is the universal molecule of heredity. Griffith's conclusions were later supported by Avery, MacLeod, and McCarty.

Subsequent experiments by Hershey and Chase using the bacteriophage T2 proved decisively that DNA is the genetic material. Shortly thereafter, Chargaff determined the ratios of adenine, thymine, cytosine, and guanine in DNA, suggesting paired relationships (A = T and C = G). He also found that the percentages of A, T, C, and G are different for different species. All of these historically important experiments shaped our current understanding of DNA.

The content presented in this section supports the learning objectives outlined in Big Ideas 3 and 4 of the AP[®] Biology Curriculum Framework. The AP[®] learning objectives merge essential knowledge content with one or more of the seven science practices. These objectives provide a transparent foundation for the AP[®] Biology course, along with inquiry-based laboratory experiences, instructional activities, and AP[®] exam questions.

| | |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Big Idea 3 | Living systems store, retrieve, transmit and respond to information essential to life processes. |
| Enduring Understanding 3.A | Heritable information provides for continuity of life. |
| Essential Knowledge | 3.A.1 DNA, and in some cases RNA, is the primary source of heritable information. |
| Science Practice | 6.5 The student can evaluate alternative scientific explanations. |
| Learning Objective | 3.1 The student is able to construct scientific explanations that use the structures and mechanisms of DNA to support the claim that DNA is the primary source of heritable information. |
| Essential Knowledge | 3.A.1 DNA, and in some cases RNA, is the primary source of heritable information. |
| Science Practice | 4.1 The student can justify the selection of the kind of data needed to answer a particular scientific question. |
| Learning Objective | 3.2 The student is able to justify the selection of data from historical investigations that support the claim that DNA is the source of heritable information. |
| Big Idea 4 | Biological systems interact, and these systems and their interactions possess complex properties. |

| | |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| Enduring Understanding 4.A | Interactions within biological systems lead to complex properties. |
| Essential Knowledge | 4.A.1 The subcomponents of biological molecules and their sequence determine the properties of that molecule. |
| Science Practice | 7.1 The student can connect phenomena and models across spatial and temporal scales. |
| Learning Objective | 4.1 The student is able to explain the connection between the sequence and the subcomponents of a biological polymer and its properties. |

The Science Practice Challenge Questions contain additional test questions for this section that will help you prepare for the AP exam. These questions address the following standards:

[APLO 3.2][APLO 3.28][APLO 1.11][APLO 1.16][APLO 3.1][APLO 4.1]

Modern understandings of DNA have evolved from the discovery of nucleic acid to the development of the double-helix model. In the 1860s, Friedrich Miescher (**Figure 14.2**), a physician by profession, was the first person to isolate phosphate-rich chemicals from white blood cells or leukocytes. He named these chemicals (which would eventually be known as RNA and DNA) nuclein because they were isolated from the nuclei of the cells.



Figure 14.2 Friedrich Miescher (1844–1895) discovered nucleic acids.



To see Miescher conduct an experiment step-by-step, click through [this review \(http://openstaxcollege.org/l/miescher_levene\)](http://openstaxcollege.org/l/miescher_levene) of how he discovered the key role of DNA and proteins in the nucleus.

Why were Phoebus Levene's discoveries important to our current understanding of DNA?

- Phoebus Levene believed that the four nucleotides in DNA are not linked or repeated in the same pattern and that they are held together by phosphodiester bonds.
- He discovered that the nucleotides were held together by phosphodiester bonds, in which two phosphate groups bind two sugars together. This discovery led to our current understanding of DNA.
- He believed that proteins were less likely the vehicles for hereditary information. Later he discovered the four nucleotides in DNA which were linked together and repeated in a wide variety of different ways.
- He believed inaccurately that the four nucleotides in DNA repeated over in the same pattern. Also, he discovered that the nucleotides were held together by phosphodiester bonds in which the phosphate group binds two sugars together.

A half century later, British bacteriologist Frederick Griffith was perhaps the first person to show that hereditary information could be transferred from one cell to another “horizontally,” rather than by descent. In 1928, he reported the first demonstration of bacterial **transformation**, a process in which external DNA is taken up by a cell, thereby changing morphology and physiology. He was working with *Streptococcus pneumoniae*, the bacterium that causes pneumonia. Griffith worked with two strains, rough (R) and smooth (S). The R strain is non-pathogenic (does not cause disease) and is called rough because its outer surface is a cell wall and lacks a capsule; as a result, the cell surface appears uneven under the microscope. The S strain is pathogenic (disease-causing) and has a capsule outside its cell wall. As a result, it has a smooth appearance under the microscope. Griffith injected the live R strain into mice and they survived. In another experiment, when he injected mice with the heat-killed S strain, they also survived. In a third set of experiments, a mixture of live R strain and heat-killed S strain were injected into mice, and—to his surprise—the mice died. Upon isolating the live bacteria from the dead mouse, only the S strain of bacteria was recovered. When this isolated S strain was injected into fresh mice, the mice died. Griffith concluded that something had passed from the heat-killed S strain into the live R strain and transformed it into the pathogenic S strain, and he called this the transforming principle (**Figure 14.3**). These experiments are now famously known as Griffith's transformation experiments.

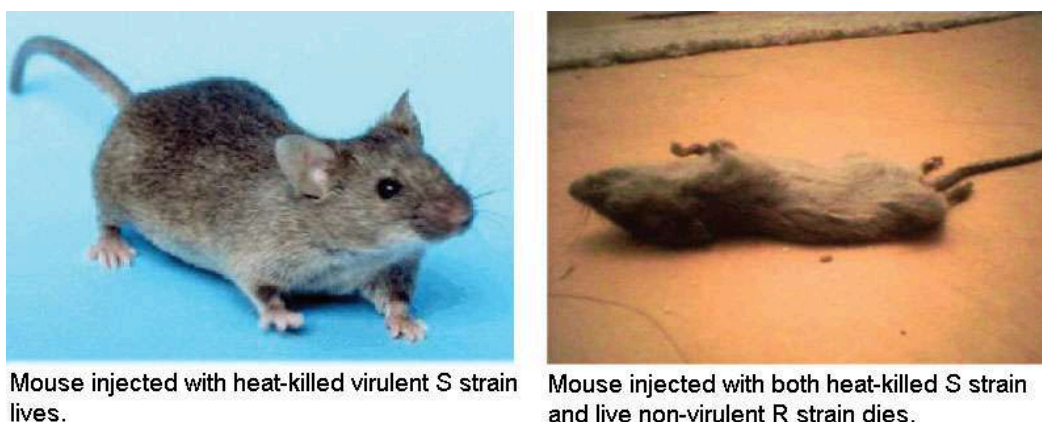


Figure 14.3 Two strains of *S. pneumoniae* were used in Griffith's transformation experiments. The R strain is non-pathogenic. The S strain is pathogenic and causes death. When Griffith injected a mouse with the heat-killed S strain and a live R strain, the mouse died. The S strain was recovered from the dead mouse. Thus, Griffith concluded that something had passed from the heat-killed S strain to the R strain, transforming the R strain into S strain in the process. (credit "living mouse": modification of work by NIH; credit "dead mouse": modification of work by Sarah Marriage)

Scientists Oswald Avery, Colin MacLeod, and Maclyn McCarty (1944) were interested in exploring this transforming principle further. They isolated the S strain from the dead mice and isolated the proteins and nucleic acids, namely RNA and DNA, as these were possible candidates for the molecule of heredity. They conducted a systematic elimination study. They used enzymes that specifically degraded each component and then used each mixture separately to transform the R strain. They found that when DNA was degraded, the resulting mixture was no longer able to transform the bacteria, whereas all of the other combinations were able to transform the bacteria. This led them to conclude that DNA was the transforming principle.

career CONNECTION

Forensic Scientists and DNA Analysis

DNA evidence was used for the first time to solve an immigration case. The story started with a teenage boy returning to London from Ghana to be with his mother. Immigration authorities at the airport were suspicious of him, thinking that he was traveling on a forged passport. After much persuasion, he was allowed to go live with his mother, but the immigration authorities did not drop the case against him. All types of evidence, including photographs, were provided to the authorities, but deportation proceedings were started nevertheless. Around the same time, Dr. Alec Jeffreys of Leicester University in the United Kingdom had invented a technique known as DNA fingerprinting. The immigration authorities approached Dr. Jeffreys for help. He took DNA samples from the mother and three of her children, plus an unrelated mother, and compared the samples with the boy's DNA. Because the biological father was not in the picture, DNA from the three children was compared with the boy's DNA. He found a match in the boy's DNA for both the mother and his three siblings. He concluded that the boy was indeed the mother's son.

Forensic scientists analyze many items, including documents, handwriting, firearms, and biological samples. They analyze the DNA content of hair, semen, saliva, and blood, and compare it with a database of DNA profiles of known criminals. Analysis includes DNA isolation, sequencing, and sequence analysis; most forensic DNA analysis involves polymerase chain reaction (PCR) amplification of short tandem repeat (STR) loci and electrophoresis to determine the length of the PCR-amplified fragment. Only mitochondrial DNA is sequenced for forensics. Forensic scientists are expected to appear at court hearings to present their findings. They are usually employed in crime labs of city and state government agencies. Geneticists experimenting with DNA techniques also work for scientific and research organizations, pharmaceutical industries, and college and university labs. Students wishing to pursue a career as a forensic scientist should have at least a bachelor's degree in chemistry, biology, or physics, and preferably some experience working in a laboratory.

science practices CONNECTION for AP[®] Courses

Activity *DNA Necklace.*

- 1) Using a molecular modeling kit (or an online virtual kit such as jmol), create a model of each of the 4 nucleotides in DNA, based on structural diagrams found in this chapter or elsewhere online.
- 2) Identify where each nucleotide hydrogen-bonds with its complementary base. Add these bonds to secure the two pairs of nucleotides together. How does the hydrogen bonding differ between the two pairs of complementary bases?
- 3) Now look at a structural diagram of a complete DNA molecule. Based on the diagram, connect your two pairs of nucleotides together along your DNA's sugar-phosphate backbone (depending on your model kit, you may have to first disconnect the hydrogen bonds between the complementary bases). Which atoms and molecules did you have to remove and add to create the sugar-phosphate backbone?

Think About It

Explain why radioactive sulfur and phosphorus were used to label T2 bacteriophages in the Hershey-Chase experiments. How did the results of these experiments contribute to the identification of DNA as the genetic material?

Experiments conducted by Martha Chase and Alfred Hershey in 1952 provided confirmatory evidence that DNA was the genetic material and not proteins. Chase and Hershey were studying a bacteriophage, which is a virus that infects bacteria. Viruses typically have a simple structure: a protein coat, called the capsid, and a nucleic acid core that contains the genetic material, either DNA or RNA. The bacteriophage infects the host bacterial cell by attaching to its surface, and then it injects its nucleic acids inside the cell. The phage DNA makes multiple copies of itself using the host machinery, and eventually the host cell bursts, releasing a large number of bacteriophages. Hershey and Chase labeled one batch of phage with radioactive sulfur, ^{35}S , to label the protein coat. Another batch of phage were labeled with radioactive phosphorus, ^{32}P . Because phosphorus is found in DNA, but not protein, the DNA and not the protein would be tagged with radioactive phosphorus.

Each batch of phage was allowed to infect the cells separately. After infection, the phage bacterial suspension was put in a blender, which caused the phage coat to be detached from the host cell. The phage and bacterial suspension was spun down in a centrifuge. The heavier bacterial cells settled down and formed a pellet, whereas the lighter phage particles stayed in the supernatant. In the tube that contained phage labeled with ^{35}S , the supernatant contained the radioactively labeled phage, whereas no radioactivity was detected in the pellet. In the tube that contained the phage labeled with ^{32}P , the radioactivity was detected in the pellet that contained the heavier bacterial cells, and no radioactivity was detected in the supernatant. Hershey and Chase concluded that it was the phage DNA that was injected into the cell and carried information to produce more phage particles, thus providing evidence that DNA was the genetic material and not proteins (**Figure 14.4**).

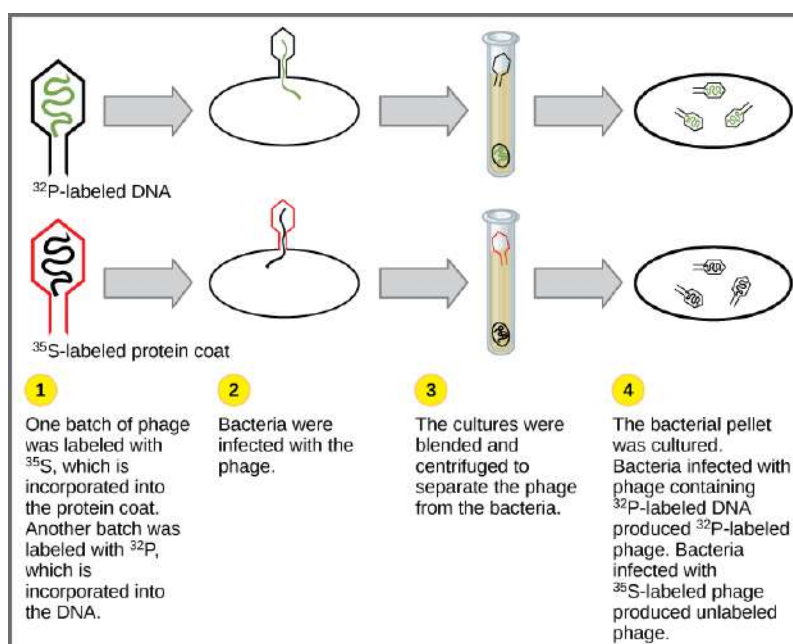


Figure 14.4 In Hershey and Chase's experiments, bacteria were infected with phage radiolabeled with either ^{35}S , which labels protein, or ^{32}P , which labels DNA. Only ^{32}P entered the bacterial cells, indicating that DNA is the genetic material.

Around this same time, Austrian biochemist Erwin Chargaff examined the content of DNA in different species and found that the amounts of adenine, thymine, guanine, and cytosine were not found in equal quantities, and that it varied from species to species, but not between individuals of the same species. He found that the amount of adenine equals the amount of thymine, and the amount of cytosine equals the amount of guanine, or $A = T$ and $G = C$. This is also known as Chargaff's rules. This finding proved immensely useful when Watson and Crick were getting ready to propose their DNA double helix model.

14.2 | DNA Structure and Sequencing

In this section, you will explore the following questions:

- What is the molecular structure of DNA?
- What is the Sanger method of DNA sequencing? What is an application of DNA sequencing?
- What are the similarities and differences between eukaryotic and prokaryotic DNA?

Connection for AP[®] Courses

The currently accepted model of the structure of DNA was proposed in 1953 by Watson and Crick, who made their model after seeing a photograph of DNA that Franklin had taken using X-ray crystallography. The photo showed the molecule's double-helix shape and dimensions. The two strands that make up the double helix are complementary and anti-parallel in nature. That is, one strand runs in the 5' to 3' direction, whereas the complementary strand runs in the 3' to 5' direction. (The significance of directionality will be important when we explore how DNA copies itself.) DNA is a polymer of nucleotides that consists of deoxyribose sugar, a phosphate group, and one of four nitrogenous bases—A, T, C, and G—with a purine always pairing with a pyrimidine (as Chargaff found). The genetic “language” of DNA is found in sequences of the nucleotides. During cell division each daughter cell receives a copy of DNA in a process called replication. In the years since the discovery of the structure of DNA, many technologies, including DNA sequencing, have been developed that enable us to better understand DNA and its role in our genomes.

Information presented and the examples highlighted in the section support concepts outlined in Big Idea 3 of the AP[®] Biology Curriculum Framework. The Learning Objectives listed in the Curriculum Framework provide a transparent foundation for the AP[®] Biology course, an inquiry-based laboratory experience, instructional activities, and AP[®] exam

questions. A Learning Objective merges required content with one or more of the seven science practices.

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| Learning Objective | 3.2 The student is able to justify the selection of data from historical investigations that support the claim that DNA is the source of heritable information. |
| Essential Knowledge | 3.A.1 DNA, and in some cases RNA, is the primary source of heritable information. |
| Science Practice | 6.4 The student can make claims and predictions about natural phenomena based on scientific theories and models. |
| Learning Objective | 3.5 The student can justify the claim that humans can manipulate heritable information by identifying <i>at least two</i> commonly used technologies. |

The Science Practice Challenge Questions contain additional test questions for this section that will help you prepare for the AP exam. These questions address the following standards:

[APLO 3.3][APLO 3.5][APLO 3.13]

The building blocks of DNA are nucleotides. The important components of the nucleotide are a nitrogenous base, deoxyribose (5-carbon sugar), and a phosphate group (**Figure 14.5**). The nucleotide is named depending on the nitrogenous base. The nitrogenous base can be a purine such as adenine (A) and guanine (G), or a pyrimidine such as cytosine (C) and thymine (T).

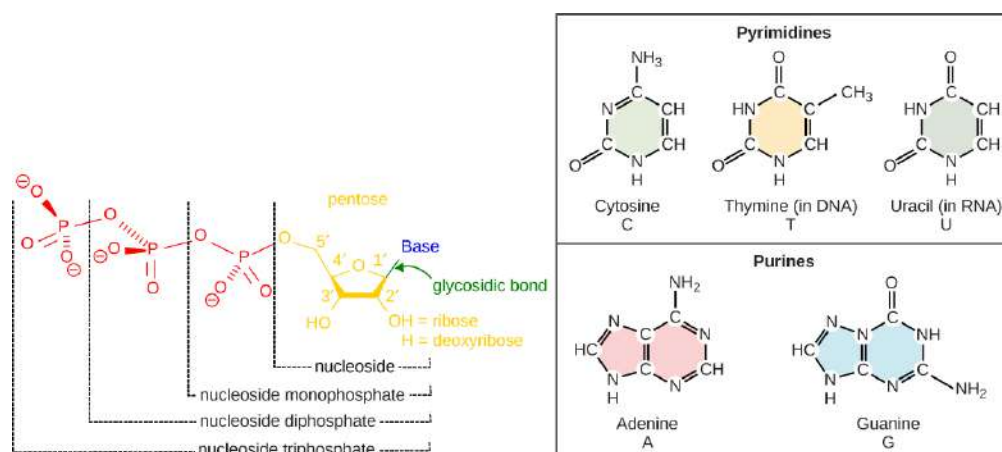


Figure 14.5 Each nucleotide is made up of a sugar, a phosphate group, and a nitrogenous base. The sugar is deoxyribose in DNA and ribose in RNA.

The nucleotides combine with each other by covalent bonds known as phosphodiester bonds or linkages. The purines have a double ring structure with a six-membered ring fused to a five-membered ring. Pyrimidines are smaller in size; they have a single six-membered ring structure. The carbon atoms of the five-carbon sugar are numbered 1', 2', 3', 4', and 5' (1' is read as "one prime"). The phosphate residue is attached to the hydroxyl group of the 5' carbon of one sugar of one nucleotide and the hydroxyl group of the 3' carbon of the sugar of the next nucleotide, thereby forming a 5'-3' phosphodiester bond.

In the 1950s, Francis Crick and James Watson worked together to determine the structure of DNA at the University of Cambridge, England. Other scientists like Linus Pauling and Maurice Wilkins were also actively exploring this field. Pauling had discovered the secondary structure of proteins using X-ray crystallography. In Wilkins' lab, researcher Rosalind Franklin was using X-ray diffraction methods to understand the structure of DNA. Watson and Crick were able to piece together the puzzle of the DNA molecule on the basis of Franklin's data because Crick had also studied X-ray diffraction (**Figure 14.6**). In 1962, James Watson, Francis Crick, and Maurice Wilkins were awarded the Nobel Prize in Medicine. Unfortunately, by then Franklin had died, and Nobel prizes are not awarded posthumously.

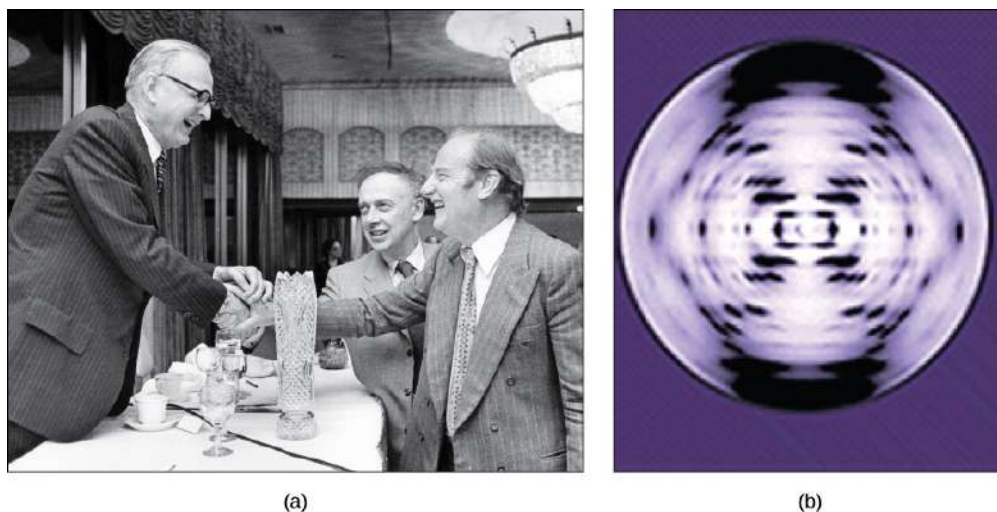


Figure 14.6 The work of pioneering scientists (a) James Watson, Francis Crick, and Maclyn McCarty led to our present day understanding of DNA. Scientist Rosalind Franklin discovered (b) the X-ray diffraction pattern of DNA, which helped to elucidate its double helix structure. (credit a: modification of work by Marjorie McCarty, Public Library of Science)

Watson and Crick proposed that DNA is made up of two strands that are twisted around each other to form a right-handed helix. Base pairing takes place between a purine and pyrimidine; namely, A pairs with T and G pairs with C. Adenine and thymine are complementary base pairs, and cytosine and guanine are also complementary base pairs. The base pairs are stabilized by hydrogen bonds; adenine and thymine form two hydrogen bonds and cytosine and guanine form three hydrogen bonds. The two strands are anti-parallel in nature; that is, the 3' end of one strand faces the 5' end of the other strand. The sugar and phosphate of the nucleotides form the backbone of the structure, whereas the nitrogenous bases are

DNA Sequencing Techniques

Until the 1990s, the sequencing of DNA (reading the sequence of DNA) was a relatively expensive and long process. Using radiolabeled nucleotides also compounded the problem through safety concerns. With currently available technology and automated machines, the process is cheap, safer, and can be completed in a matter of hours. Fred Sanger developed the sequencing method used for the human genome sequencing project, which is widely used today (**Figure 14.8**).



Visit **this site** (http://openstaxcollege.org/l/DNA_sequencing) to watch a video explaining the DNA sequence reading technique that resulted from Sanger's work.

Describe one advantage and a possible limitation to Sanger's method.

- Sanger's method can be used to sequence more than one strand at a time which is less time consuming. Challenges of Sanger's method includes its decreased accuracy to sequence DNA strands.
- Sanger's method is a reliable and accurate way of sequencing DNA strands. However, only one strand at a time can be sequenced at a time. Also, it can look for one base only at a time which can be time consuming.
- Sanger's method is highly inexpensive and less accurate. However, it is not readily adaptable to commercial kits.
- Sanger's method is less time consuming and highly accurate. However, it is more expensive than other methods available for sequencing.

The method is known as the dideoxy chain termination method. The sequencing method is based on the use of chain terminators, the dideoxynucleotides (ddNTPs). The dideoxynucleotides, or ddNTPs, differ from the deoxynucleotides by the lack of a free 3' OH group on the five-carbon sugar. If a ddNTP is added to a growing a DNA strand, the chain is not extended any further because the free 3' OH group needed to add another nucleotide is not available. By using a predetermined ratio of deoxyribonucleotides to dideoxynucleotides, it is possible to generate DNA fragments of different sizes.

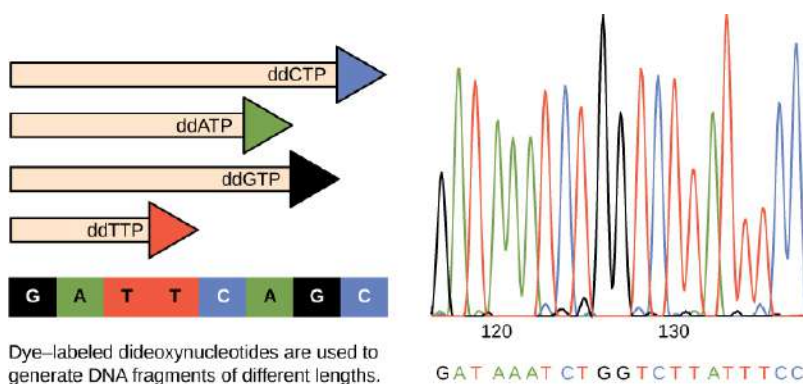


Figure 14.8 In Frederick Sanger's dideoxy chain termination method, dye-labeled dideoxynucleotides are used to generate DNA fragments that terminate at different points. The DNA is separated by capillary electrophoresis on the basis of size, and from the order of fragments formed, the DNA sequence can be read. The DNA sequence readout is shown on an electropherogram that is generated by a laser scanner.

The DNA sample to be sequenced is denatured or separated into two strands by heating it to high temperatures. The DNA is divided into four tubes in which a primer, DNA polymerase, and all four nucleotides (A, T, G, and C) are added. In addition to each of the four tubes, limited quantities of one of the four dideoxynucleotides are added to each tube

respectively. The tubes are labeled as A, T, G, and C according to the ddNTP added. For detection purposes, each of the four dideoxynucleotides carries a different fluorescent label. Chain elongation continues until a fluorescent dideoxy nucleotide is incorporated, after which no further elongation takes place. After the reaction is over, electrophoresis is performed. Even a difference in length of a single base can be detected. The sequence is read from a laser scanner. For his work on DNA sequencing, Sanger received a Nobel Prize in chemistry in 1980.



Sanger's genome sequencing has led to a race to sequence human genomes at a rapid speed and low cost, often referred to as the \$1000 in one day sequence. Learn more by selecting the Sequencing at Speed animation [here](http://openstaxcollege.org/l/DNA_and_genomes) (http://openstaxcollege.org/l/DNA_and_genomes) .

Explain how fast DNA sequencing can change the way doctors treat disease.

- Faster genetic sequencing will help in quick analysis of the genetic makeup of bacteria that can cause diseases in humans for better and more efficient treatments. Also, sequencing of a cancerous cell's DNA can provide better ways to treat or prevent cancer.
- Fast DNA sequencing can help us quickly analyze the genetic information of existing only bacteria (not new strains) only that cause disease in humans, which may lead to more efficient treatments.
- Fast DNA sequencing can help doctors to treat and diagnose diseases which are not rare in populations.
- Faster genetic sequencing can be used to treat and prevent a few types of cancers and thus increase the life expectancy of patients suffering from the diseases.

Gel **electrophoresis** is a technique used to separate DNA fragments of different sizes. Usually the gel is made of a chemical called agarose. Agarose powder is added to a buffer and heated. After cooling, the gel solution is poured into a casting tray. Once the gel has solidified, the DNA is loaded on the gel and electric current is applied. The DNA has a net negative charge and moves from the negative electrode toward the positive electrode. The electric current is applied for sufficient time to let the DNA separate according to size; the smallest fragments will be farthest from the well (where the DNA was loaded), and the heavier molecular weight fragments will be closest to the well. Once the DNA is separated, the gel is stained with a DNA-specific dye for viewing it (**Figure 14.9**).

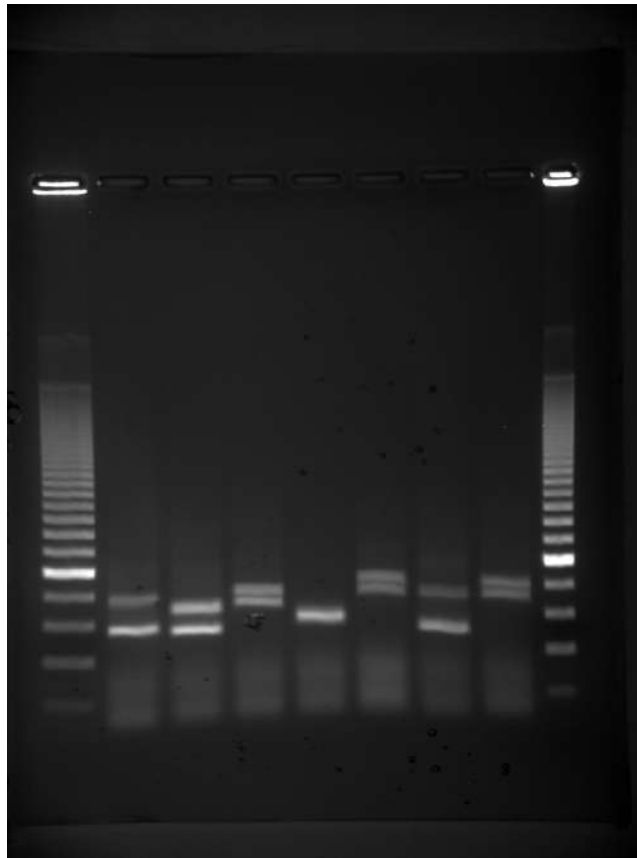


Figure 14.9 DNA can be separated on the basis of size using gel electrophoresis. (credit: James Jacob, Tompkins Cortland Community College)

evolution CONNECTION

Neanderthal Genome: How Are We Related?

The first draft sequence of the Neanderthal genome was recently published by Richard E. Green et al. in 2010.^[1] Neanderthals are the closest ancestors of present-day humans. They were known to have lived in Europe and Western Asia before they disappeared from fossil records approximately 30,000 years ago. Green's team studied almost 40,000-year-old fossil remains that were selected from sites across the world. Extremely sophisticated means of sample preparation and DNA sequencing were employed because of the fragile nature of the bones and heavy microbial contamination. In their study, the scientists were able to sequence some four billion base pairs. The Neanderthal sequence was compared with that of present-day humans from across the world. After comparing the sequences, the researchers found that the Neanderthal genome had 2 to 3 percent greater similarity to people living outside Africa than to people in Africa. While current theories have suggested that all present-day humans can be traced to a small ancestral population in Africa, the data from the Neanderthal genome may contradict this view. Green and his colleagues also discovered DNA segments among people in Europe and Asia that are more similar to Neanderthal sequences than to other contemporary human sequences. Another interesting observation was that Neanderthals are as closely related to people from Papua New Guinea as to those from China or France. This is surprising because Neanderthal fossil remains have been located only in Europe and West Asia. Most likely, genetic exchange took place between Neanderthals and modern humans as modern humans emerged out of Africa, before the divergence of Europeans, East Asians, and Papua New Guineans.

Several genes seem to have undergone changes from Neanderthals during the evolution of present-day humans. These genes are involved in cranial structure, metabolism, skin morphology, and cognitive development. One of the genes that is of particular interest is *RUNX2*, which is different in modern day humans and Neanderthals. This gene is responsible for the prominent frontal bone, bell-shaped rib cage, and dental differences seen in Neanderthals. It is speculated that an evolutionary change in *RUNX2* was important in the origin of modern-day humans, and this affected the cranium and the upper body.

According to the passage, which statement best describes the relationship between humans and Neanderthals?

- a. Early humans emerged from Africa, then spread out to populate different parts of the globe. An isolated population of these early humans interbred with Neanderthals.
- b. Early humans interbred with Neanderthals, emerged from Africa, then spread out to populate different parts of the globe.
- c. Early humans emerged from Africa, interbred with Neanderthals, then spread out to populate different parts of the globe.
- d. Early humans did not interbreed with Neanderthals, but we have many genetic similarities because we share a common ancestor.

1.

Richard E. Green et al., "A Draft Sequence of the Neandertal Genome," *Science* 328 (2010): 710-22.



Watch **Svante Pääbo's talk** (<http://openstaxcollege.org/l/neanderthal>) explaining the Neanderthal genome research at the 2011 annual TED (Technology, Entertainment, Design) conference.

Which of the statements gives the best explanation for the wider genetic variation in the human population in Africa than the rest of the world?

- a. It has been suggested that all humans most likely descended from Africa. This is supported by the research that genetic variance in Africa was also found in the rest of the world.
- b. The theory that humans descended from Africa was supported by the research that most of the human genomes tested outside of Africa had close ties to the genomes of people in Africa but a genetic variance in Africa was not found in the rest of the world.
- c. Humans have most likely descended from Africa. This research is supported by the fact that all the human genomes tested outside of Africa had close ties to the genomes of people in Africa. Also, there is a genetic variance in Africa that was not found in the rest of the world.
- d. The transition to modern humans occurred within Africa which was sudden. Thus, human genomes tested outside of Africa had close ties to the genomes of people in Africa.

DNA Packaging in Cells

When comparing prokaryotic cells to eukaryotic cells, prokaryotes are much simpler than eukaryotes in many of their features (**Figure 14.10**). Most prokaryotes contain a single, circular chromosome that is found in an area of the cytoplasm called the nucleoid.

visual CONNECTION

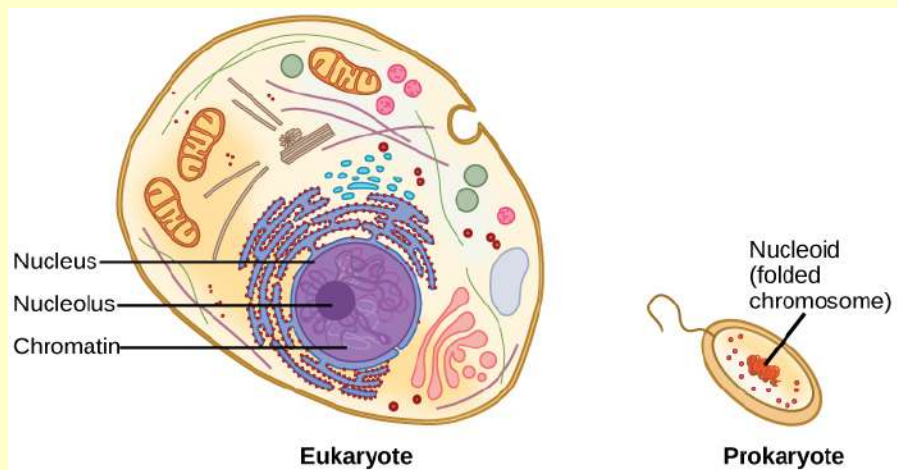


Figure 14.10 A eukaryote contains a well-defined nucleus, whereas in prokaryotes, the chromosome lies in the cytoplasm in an area called the nucleoid.

In eukaryotic cells, DNA and RNA synthesis occur in a separate compartment from protein synthesis. In prokaryotic cells, both processes occur together. What advantages might there be to separating the processes? What advantages might there be to having them occur together?

- Compartmentalization in eukaryotic cells enables the building of more complex proteins and RNA products. In prokaryotes, the advantage is that RNA and protein synthesis occurs much more quickly because it occurs in a single compartment.
- Compartmentalization in prokaryotic cells enables the building of more complex proteins and RNA products. In eukaryotes, the advantage is that RNA and protein synthesis occurs much more quickly because they occur in a single compartment.
- Compartmentalization in eukaryotic cells enables the building of simpler proteins and RNA products. In prokaryotes, the advantage is only simpler proteins and RNA products because complex ones are not needed.
- Compartmentalization in eukaryotic cells enables the building of more complex proteins and RNA products. In prokaryotes, the advantage is that RNA and protein synthesis takes more time because it occurs in a single compartment.

The size of the genome in one of the most well-studied prokaryotes, *E.coli*, is 4.6 million base pairs (approximately 1.1 mm, if cut and stretched out). So how does this fit inside a small bacterial cell? The DNA is twisted by what is known as supercoiling. Supercoiling means that DNA is either under-wound (less than one turn of the helix per 10 base pairs) or over-wound (more than 1 turn per 10 base pairs) from its normal relaxed state. Some proteins are known to be involved in the supercoiling; other proteins and enzymes such as DNA gyrase help in maintaining the supercoiled structure.

Eukaryotes, whose chromosomes each consist of a linear DNA molecule, employ a different type of packing strategy to fit their DNA inside the nucleus (**Figure 14.11**). At the most basic level, DNA is wrapped around proteins known as histones to form structures called nucleosomes. The histones are evolutionarily conserved proteins that are rich in basic amino acids and form an octamer. The DNA (which is negatively charged because of the phosphate groups) is wrapped tightly around the histone core. This nucleosome is linked to the next one with the help of a linker DNA. This is also known as the “beads on a string” structure. This is further compacted into a 30 nm fiber, which is the diameter of the structure. At the metaphase stage, the chromosomes are at their most compact, are approximately 700 nm in width, and are found in association with scaffold proteins.

In interphase, eukaryotic chromosomes have two distinct regions that can be distinguished by staining. The tightly packaged region is known as heterochromatin, and the less dense region is known as euchromatin. Heterochromatin usually contains genes that are not expressed, and is found in the regions of the centromere and telomeres. The euchromatin usually contains genes that are transcribed, with DNA packaged around nucleosomes but not further compacted.

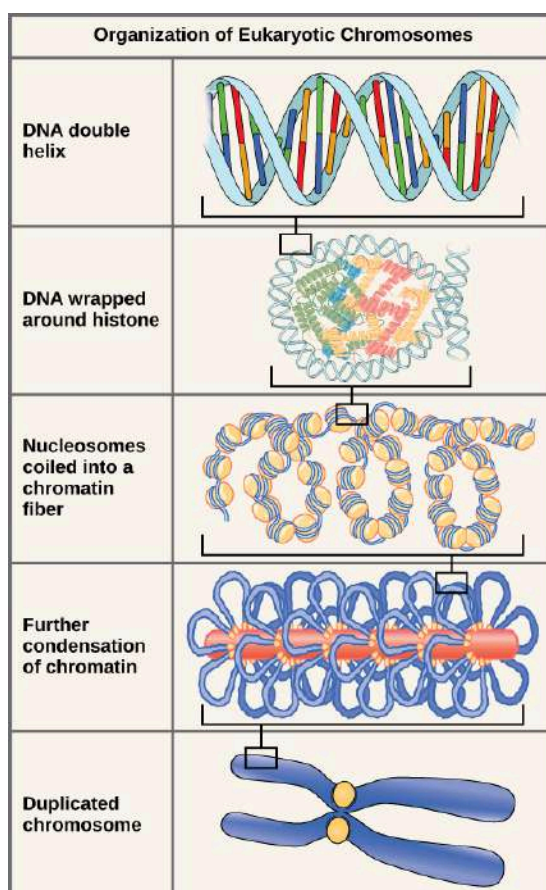


Figure 14.11 These figures illustrate the compaction of the eukaryotic chromosome.

14.3 | Basics of DNA Replication

In this section, you will explore the following questions:

- How does the structure of DNA provide for the process of replication?
- How did the Meselson and Stahl experiments support the semi-conservative nature of replication?

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The Watson and Crick model suggested a way in which DNA could be replicated during cell division. Basically, the two strands unwind and separate where the hydrogen bonds connect the nucleotides. Each parental strand then serves as a template for a new, complementary daughter strand. Replication is said to be semi-conservative because the original information encoded in each parental strand is conserved (kept) in the daughter molecules. Thus, a newly replicated molecule of DNA consists of one “old” strand and one “new” strand. Meselson and Stahl used density differences in nitrogen isotopes to investigate replication, and their experiments supported the semi-conservative model. However, the process of replication is more complex than their model’s simple description.

Information presented and the examples highlighted in the section support concepts outlined in Big Idea 3 and Big Idea 4 of the AP[®] Biology Curriculum Framework. The Learning Objectives listed in the Curriculum Framework provide a transparent foundation for the AP[®] Biology course, an inquiry-based laboratory experience, instructional activities, and AP[®] exam questions. A Learning Objective merges required content with one or more of the seven Science Practices.

| | |
|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Big Idea 3 | Living systems store, retrieve, transmit and respond to information essential to life processes. |
| Enduring Understanding 3.A | Heritable information provides for continuity of life. |
| Essential Knowledge | 3.A.1 DNA, and in some cases RNA, is the primary source of heritable information. |
| Science Practice | 1.2 The student can describe representations and models of natural or man-made phenomena and systems in the domain. |
| Learning Objective | 3.3 The student is able to describe representations and models that illustrate how genetic information is copied for transmission between generations. |

The Science Practice Challenge Questions contain additional test questions for this section that will help you prepare for the AP exam. These questions address the following standards:

[APLO 2.34][APLO 3.3][APLO 4.1]

The elucidation of the structure of the double helix provided a hint as to how DNA divides and makes copies of itself. This model suggests that the two strands of the double helix separate during replication, and each strand serves as a template from which the new complementary strand is copied. What was not clear was how the replication took place. There were three models suggested (**Figure 14.12**): conservative, semi-conservative, and dispersive.

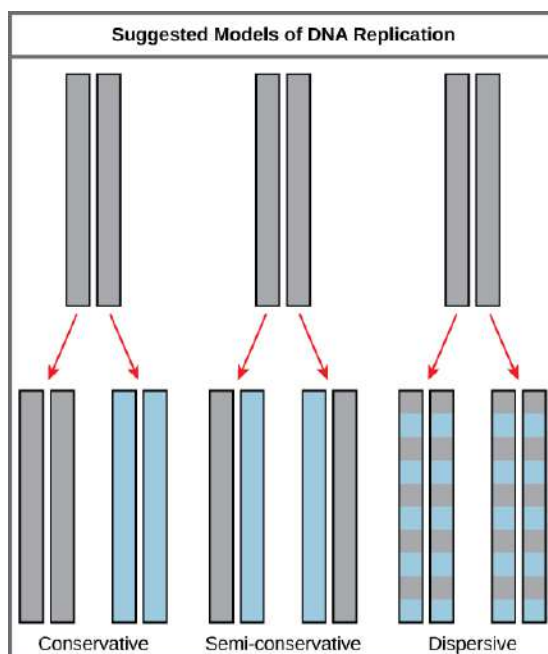


Figure 14.12 The three suggested models of DNA replication. Grey indicates the original DNA strands, and blue indicates newly synthesized DNA.

In conservative replication, the parental DNA remains together, and the newly formed daughter strands are together. The semi-conservative method suggests that each of the two parental DNA strands act as a template for new DNA to be synthesized; after replication, each double-stranded DNA includes one parental or “old” strand and one “new” strand. In the dispersive model, both copies of DNA have double-stranded segments of parental DNA and newly synthesized DNA interspersed.

Meselson and Stahl were interested in understanding how DNA replicates. They grew *E. coli* for several generations in a medium containing a “heavy” isotope of nitrogen (^{15}N) that gets incorporated into nitrogenous bases, and eventually into the DNA (**Figure 14.13**).

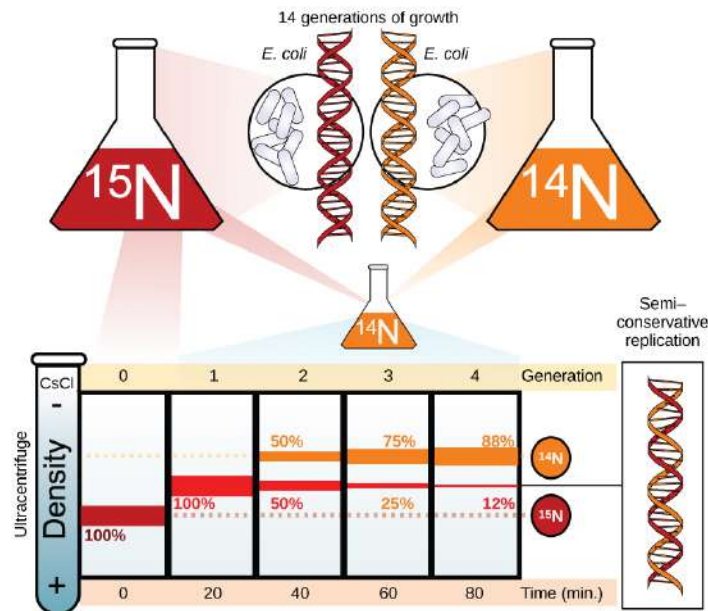


Figure 14.13 Meselson and Stahl experimented with *E. coli* grown first in heavy nitrogen (^{15}N) then in ^{14}N . DNA grown in ^{15}N (red band) is heavier than DNA grown in ^{14}N (orange band), and sediments to a lower level in cesium chloride solution in an ultracentrifuge. When DNA grown in ^{15}N is switched to media containing ^{14}N , after one round of cell division the DNA sediments halfway between the ^{15}N and ^{14}N levels, indicating that it now contains fifty percent ^{14}N . In subsequent cell divisions, an increasing amount of DNA contains ^{14}N only. This data supports the semi-conservative replication model. (credit: modification of work by Mariana Ruiz Villareal)

The *E. coli* culture was then shifted into medium containing ^{14}N and allowed to grow for one generation. The cells were harvested and the DNA was isolated. The DNA was centrifuged at high speeds in an ultracentrifuge. Some cells were allowed to grow for one more life cycle in ^{14}N and spun again. During the density gradient centrifugation, the DNA is loaded into a gradient (typically a salt such as cesium chloride or sucrose) and spun at high speeds of 50,000 to 60,000 rpm. Under these circumstances, the DNA will form a band according to its density in the gradient. DNA grown in ^{15}N will band at a higher density position than that grown in ^{14}N . Meselson and Stahl noted that after one generation of growth in ^{14}N after they had been shifted from ^{15}N , the single band observed was intermediate in position in between DNA of cells grown exclusively in ^{15}N and ^{14}N . This suggested either a semi-conservative or dispersive mode of replication. The DNA harvested from cells grown for two generations in ^{14}N formed two bands: one DNA band was at the intermediate position between ^{15}N and ^{14}N , and the other corresponded to the band of ^{14}N DNA. These results could only be explained if DNA replicates in a semi-conservative manner. Therefore, the other two modes were ruled out.

During DNA replication, each of the two strands that make up the double helix serves as a template from which new strands are copied. The new strand will be complementary to the parental or “old” strand. When two daughter DNA copies are formed, they have the same sequence and are divided equally into the two daughter cells.



Click through **this tutorial** (http://openstaxcollege.org/l/DNA_replicatio2) on DNA replication.

One theory of aging is that the body's ability to fix mistakes in its DNA decreases as we age. How can this affect DNA replication?

- Aging causes accumulation of DNA mutations and DNA damage of only the nuclear DNA and the mistakes will be passed down to new cells causing age related diseases.
- Aging results in ineffective DNA repair mechanism so that the mistakes in the DNA will be passed down to new cells. This could lead to the development of age-related diseases.
- Aging causes DNA polymerase to function abnormally. This is the sole reason which causes defects in DNA replication.
- DNA replication of only fast growing cells is affected by aging.

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Activity

Design (but do not implement) an experiment to test the three models of DNA replication. Summarize the results you would expect if each of the three models of DNA replication were correct. Assume you have access in a laboratory to the following: an experimental organism such as *E. coli*, an unlimited variety of isotopes, test tube and centrifuge, and organic growth media.

14.4 | DNA Replication in Prokaryotes

In this section, you will explore the following questions:

- How is DNA replicated in prokaryotes, and what are the roles of the leading and lagging strands and Okazaki fragments in the process?
- What is the role of DNA polymerase and other enzymes and proteins in supporting replication?

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As was stated previously, DNA replication is more complex than simply unzipping the double helix and making new complementary strands. Replication in prokaryotes starts from a sequence of nucleotides on the chromosome called the origin of replication—the point at which the DNA opens up or unzips. The enzyme helicase opens up the DNA at the point where hydrogen bonds connect the strands, resulting in the formation of a Y-shaped replication fork. Single-strand binding proteins keep the fork open. The enzyme primase synthesizes RNA primers to initiate DNA synthesis by DNA polymerase, which can add nucleotides only in the 5' to 3' direction. DNA polymerase recognizes the 3'-OH end as its landing site; thus, polymerase “reads” the template strand in the 3' to 5' direction and builds the complementary DNA polymer in the 5' to 3' direction. One strand—called the leading strand—is synthesized continuously in the direction of the replication fork (the direction in which helicase is separating the two strands), with polymerase adding new nucleotides one-by-one. However,

replication of the other strand—called the lagging strand—occurs in a direction away from the replication fork, in short stretches of DNA known as Okazaki fragments. (Think of the activities on the lagging strand as analogous to trying to walk on a moving sidewalk that is moving in the opposite direction.) The RNA primers are replaced by DNA nucleotides, and ligase seals the DNA, creating phosphodiester bonds between the 3'-OH of one end and the 5'-phosphate of the other strand. The replicated DNA molecules now consist of one original template strand and one newly synthesized strand.

Information presented and the examples highlighted in the section support concepts outlined in Big Idea 3 of the AP[®] Biology Curriculum Framework. The Learning Objectives listed in the Curriculum Framework provide a transparent foundation for the AP[®] Biology course, an inquiry-based laboratory experience, instructional activities, and AP[®] exam questions. A Learning Objective merges required content with one or more of the seven Science Practices.

| | |
|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Big Idea 3 | Living systems store, retrieve, transmit and respond to information essential to life processes. |
| Enduring Understanding 3.A | Heritable information provides for continuity of life. |
| Essential Knowledge | 3.A.1 DNA, and in some cases RNA, is the primary source of heritable information. |
| Science Practice | 1.2 The student can describe representations and models of natural or man-made phenomena and systems in the domain. |
| Learning Objective | 3.3 The student is able to describe representations and models that illustrate how genetic information is copied for transmission between generations. |

The Science Practice Challenge Questions contain additional test questions for this section that will help you prepare for the AP exam. These questions address the following standards:

[APLO 4.3][APLO 1.18][APLO 1.21]

DNA replication has been extremely well studied in prokaryotes primarily because of the small size of the genome and the mutants that are available. *E. coli* has 4.6 million base pairs in a single circular chromosome and all of it gets replicated in approximately 42 minutes, starting from a single origin of replication and proceeding around the circle in both directions. This means that approximately 1000 nucleotides are added per second. The process is quite rapid and occurs without many mistakes.

DNA replication employs a large number of proteins and enzymes, each of which plays a critical role during the process. One of the key players is the enzyme DNA polymerase, also known as DNA pol, which adds nucleotides one by one to the growing DNA chain that are complementary to the template strand. The addition of nucleotides requires energy; this energy is obtained from the nucleotides that have three phosphates attached to them, similar to ATP which has three phosphate groups attached. When the bond between the phosphates is broken, the energy released is used to form the phosphodiester bond between the incoming nucleotide and the growing chain. In prokaryotes, three main types of polymerases are known: DNA pol I, DNA pol II, and DNA pol III. It is now known that DNA pol III is the enzyme required for DNA synthesis; DNA pol I and DNA pol II are primarily required for repair.

How does the replication machinery know where to begin? It turns out that there are specific nucleotide sequences called origins of replication where replication begins. In *E. coli*, which has a single origin of replication on its one chromosome (as do most prokaryotes), it is approximately 245 base pairs long and is rich in AT sequences. The origin of replication is recognized by certain proteins that bind to this site. An enzyme called **helicase** unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. ATP hydrolysis is required for this process. As the DNA opens up, Y-shaped structures called **replication forks** are formed. Two replication forks are formed at the origin of replication and these get extended bi-directionally as replication proceeds. **Single-strand binding proteins** coat the single strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix. DNA polymerase is able to add nucleotides only in the 5' to 3' direction (a new DNA strand can be only extended in this direction). It also requires a free 3'-OH group to which it can add nucleotides by forming a phosphodiester bond between the 3'-OH end and the 5' phosphate of the next nucleotide. This essentially means that it cannot add nucleotides if a free 3'-OH group is not available. Then how does it add the first nucleotide? The problem is solved with the help of a primer that provides the free 3'-OH end. Another enzyme, RNA **primase**, synthesizes an RNA primer that is about five to ten nucleotides long and complementary to the DNA. Because this sequence primes the DNA synthesis, it is appropriately called the **primer**. DNA polymerase can now extend this RNA primer, adding nucleotides one by one that are complementary to the template strand (**Figure 14.14**).

visual CONNECTION

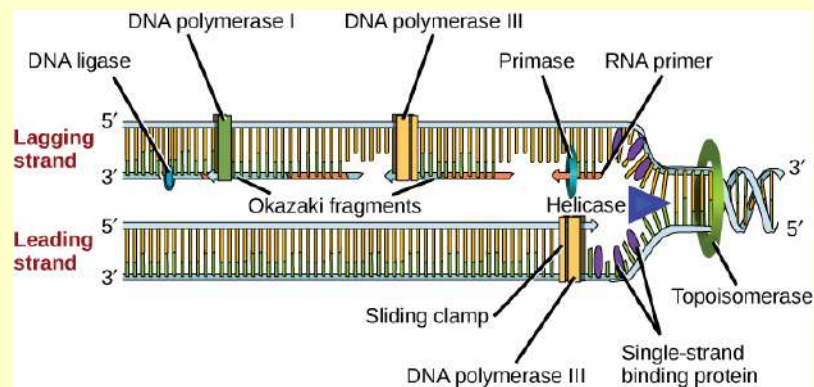


Figure 14.14 A replication fork is formed when helicase separates the DNA strands at the origin of replication. The DNA tends to become more highly coiled ahead of the replication fork. Topoisomerase breaks and reforms DNA's phosphate backbone ahead of the replication fork, thereby relieving the pressure that results from this supercoiling. Single-strand binding proteins bind to the single-stranded DNA to prevent the helix from re-forming. Primase synthesizes an RNA primer. DNA polymerase III uses this primer to synthesize the daughter DNA strand. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches called Okazaki fragments. DNA polymerase I replaces the RNA primer with DNA. DNA ligase seals the gaps between the Okazaki fragments, joining the fragments into a single DNA molecule. (credit: modification of work by Mariana Ruiz Villareal)

You isolate a cell strain in which the joining together of Okazaki fragments is impaired and suspect that a mutation has occurred in an enzyme found at the replication fork. Which enzyme is most likely to be mutated?

- DNA ligase
- DNA polymerase III
- helicase
- topoisomerase

The replication fork moves at the rate of 1000 nucleotides per second. DNA polymerase can only extend in the 5' to 3' direction, which poses a slight problem at the replication fork. As we know, the DNA double helix is anti-parallel; that is, one strand is in the 5' to 3' direction and the other is oriented in the 3' to 5' direction. One strand, which is complementary to the 3' to 5' parental DNA strand, is synthesized continuously towards the replication fork because the polymerase can add nucleotides in this direction. This continuously synthesized strand is known as the **leading strand**. The other strand, complementary to the 5' to 3' parental DNA, is extended away from the replication fork, in small fragments known as **Okazaki fragments**, each requiring a primer to start the synthesis. Okazaki fragments are named after the Japanese scientist who first discovered them. The strand with the Okazaki fragments is known as the **lagging strand**.

The leading strand can be extended by one primer alone, whereas the lagging strand needs a new primer for each of the short Okazaki fragments. The overall direction of the lagging strand will be 3' to 5', and that of the leading strand 5' to 3'. A protein called the **sliding clamp** holds the DNA polymerase in place as it continues to add nucleotides. The sliding clamp is a ring-shaped protein that binds to the DNA and holds the polymerase in place. **Topoisomerase** prevents the over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up; it does so by causing temporary nicks in the DNA helix and then resealing it. As synthesis proceeds, the RNA primers are replaced by DNA. The primers are removed by the exonuclease activity of DNA pol I, and the gaps are filled in by deoxyribonucleotides. The nicks that remain between the newly synthesized DNA (that replaced the RNA primer) and the previously synthesized DNA are sealed by the enzyme **DNA ligase** that catalyzes the formation of phosphodiester linkage between the 3'-OH end of one nucleotide and the 5' phosphate end of the other fragment.

Once the chromosome has been completely replicated, the two DNA copies move into two different cells during cell division. The process of DNA replication can be summarized as follows:

1. DNA unwinds at the origin of replication.

2. Helicase opens up the DNA-forming replication forks; these are extended bidirectionally.
3. Single-strand binding proteins coat the DNA around the replication fork to prevent rewinding of the DNA.
4. Topoisomerase binds at the region ahead of the replication fork to prevent supercoiling.
5. Primase synthesizes RNA primers complementary to the DNA strand.
6. DNA polymerase starts adding nucleotides to the 3'-OH end of the primer.
7. Elongation of both the lagging and the leading strand continues.
8. RNA primers are removed by exonuclease activity.
9. Gaps are filled by DNA pol by adding dNTPs.
10. The gap between the two DNA fragments is sealed by DNA ligase, which helps in the formation of phosphodiester bonds.

Table 14.1 summarizes the enzymes involved in prokaryotic DNA replication and the functions of each.

Prokaryotic DNA Replication: Enzymes and Their Functions

| Enzyme/protein | Specific Function |
|--------------------------------------|---------------------------------------------------------------------------------------------|
| DNA pol I | Exonuclease activity removes RNA primer and replaces with newly synthesized DNA |
| DNA pol II | Repair function |
| DNA pol III | Main enzyme that adds nucleotides in the 5'-3' direction |
| Helicase | Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases |
| Ligase | Seals the gaps between the Okazaki fragments to create one continuous DNA strand |
| Primase | Synthesizes RNA primers needed to start replication |
| Sliding Clamp | Helps to hold the DNA polymerase in place when nucleotides are being added |
| Topoisomerase | Helps relieve the stress on DNA when unwinding by causing breaks and then resealing the DNA |
| Single-strand binding proteins (SSB) | Binds to single-stranded DNA to avoid DNA rewinding back. |

Table 14.1



Review the full process of DNA replication [here \(http://openstaxcollege.org/l/replication_DNA\)](http://openstaxcollege.org/l/replication_DNA).

Explain why errors in DNA replication are rare events in cells.

- Errors in DNA replication are rare events in a cell due to the presence of DNA ligase enzyme which fixes mistakes in the copying process.
- Polymerase I is solely responsible for proofreading and fixing mistakes in the copying process, which explains why so few mistakes are made.
- Polymerase I and II are responsible for proofreading and fixing mistakes in the copying process which explains why errors in DNA replication are rare.
- Errors in DNA replication are rare events in cells due to the action of DNA helicase.

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Activity

Use the model of DNA you constructed in Section 14.2 to demonstrate the process of replication in prokaryotes, showing how the activities differ on the leading and lagging strands. You need to add to your model by including enzymes and other proteins involved in the replication process.

Think About It

You isolate a DNA strand in which the joining together of Okazaki fragments is impaired and suspect that a mutation has occurred in an enzyme found at the replication fork. Which enzyme is most likely mutated?

14.5 | DNA Replication in Eukaryotes

In this section, you will explore the following questions:

- What are the similarities and differences between DNA replication in eukaryotes and prokaryotes?
- What is the role of telomerase in DNA replication?

Connection for AP[®] Courses

Concepts and examples described in this section are not in scope for AP. However, the roles of telomeres and telomerase in aging and cancer are informative and build on your knowledge of DNA replication in prokaryotes.

Eukaryotic genomes are much more complex and larger in size than prokaryotic genomes. The human genome has three billion base pairs per haploid set of chromosomes, and 6 billion base pairs are replicated during the S phase of the cell cycle. There are multiple origins of replication on the eukaryotic chromosome; humans can have up to 100,000 origins of replication. The rate of replication is approximately 100 nucleotides per second, much slower than prokaryotic replication.

In yeast, which is a eukaryote, special sequences known as Autonomously Replicating Sequences (ARS) are found on the chromosomes. These are equivalent to the origin of replication in *E. coli*.

The number of DNA polymerases in eukaryotes is much more than prokaryotes: 14 are known, of which five are known to have major roles during replication and have been well studied. They are known as pol α , pol β , pol γ , pol δ , and pol ϵ .

The essential steps of replication are the same as in prokaryotes. Before replication can start, the DNA has to be made available as template. Eukaryotic DNA is bound to basic proteins known as histones to form structures called nucleosomes. The chromatin (the complex between DNA and proteins) may undergo some chemical modifications, so that the DNA may be able to slide off the proteins or be accessible to the enzymes of the DNA replication machinery. At the origin of replication, a pre-replication complex is made with other initiator proteins. Other proteins are then recruited to start the replication process (**Table 14.2**).

A helicase using the energy from ATP hydrolysis opens up the DNA helix. Replication forks are formed at each replication origin as the DNA unwinds. The opening of the double helix causes over-winding, or supercoiling, in the DNA ahead of the replication fork. These are resolved with the action of topoisomerases. Primers are formed by the enzyme primase, and using the primer, DNA pol can start synthesis. While the leading strand is continuously synthesized by the enzyme pol δ , the lagging strand is synthesized by pol ϵ . A sliding clamp protein known as PCNA (Proliferating Cell Nuclear Antigen) holds the DNA pol in place so that it does not slide off the DNA. RNase H removes the RNA primer, which is then replaced with DNA nucleotides. The Okazaki fragments in the lagging strand are joined together after the replacement of the RNA primers with DNA. The gaps that remain are sealed by DNA ligase, which forms the phosphodiester bond.

Telomere Replication

Unlike prokaryotic chromosomes, eukaryotic chromosomes are linear. As you've learned, the enzyme DNA pol can add nucleotides only in the 5' to 3' direction. In the leading strand, synthesis continues until the end of the chromosome is reached. On the lagging strand, DNA is synthesized in short stretches, each of which is initiated by a separate primer. When the replication fork reaches the end of the linear chromosome, there is no place for a primer to be made for the DNA fragment to be copied at the end of the chromosome. These ends thus remain unpaired, and over time these ends may get progressively shorter as cells continue to divide.

The ends of the linear chromosomes are known as **telomeres**, which have repetitive sequences that code for no particular gene. In a way, these telomeres protect the genes from getting deleted as cells continue to divide. In humans, a six base pair sequence, TTAGGG, is repeated 100 to 1000 times. The discovery of the enzyme telomerase (**Figure 14.16**) helped in the understanding of how chromosome ends are maintained. The **telomerase** enzyme contains a catalytic part and a built-in RNA template. It attaches to the end of the chromosome, and complementary bases to the RNA template are added on the 3' end of the DNA strand. Once the 3' end of the lagging strand template is sufficiently elongated, DNA polymerase can add the nucleotides complementary to the ends of the chromosomes. Thus, the ends of the chromosomes are replicated.

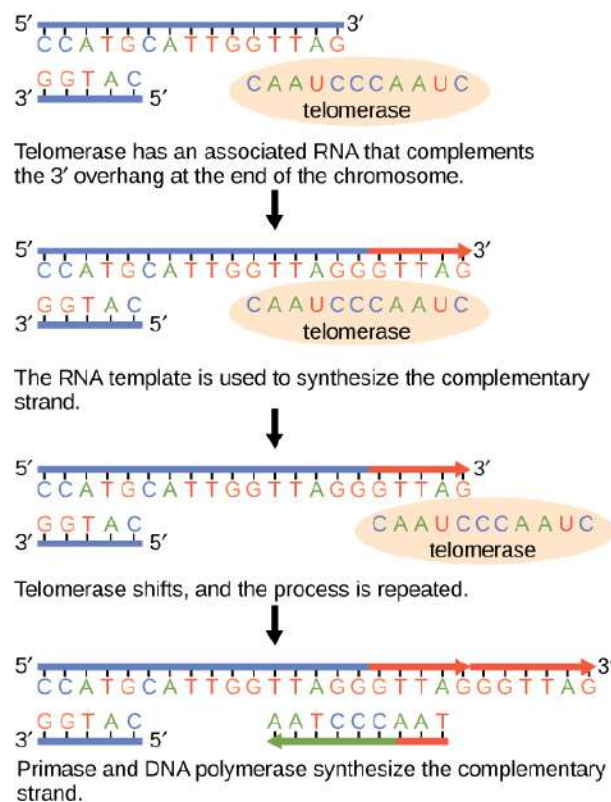


Figure 14.15 The ends of linear chromosomes are maintained by the action of the telomerase enzyme.

Telomerase is typically active in germ cells and adult stem cells. It is not active in adult somatic cells. For her discovery of telomerase and its action, Elizabeth Blackburn (**Figure 14.16**) received the Nobel Prize for Medicine and Physiology in 2009.



Figure 14.16 Elizabeth Blackburn, 2009 Nobel Laureate, is the scientist who discovered how telomerase works. (credit: US Embassy Sweden)

Telomerase and Aging

Cells that undergo cell division continue to have their telomeres shortened because most somatic cells do not make telomerase. This essentially means that telomere shortening is associated with aging. With the advent of modern medicine, preventative health care, and healthier lifestyles, the human life span has increased, and there is an increasing demand for people to look younger and have a better quality of life as they grow older.

In 2010, scientists found that telomerase can reverse some age-related conditions in mice. This may have potential in regenerative medicine.^[2] Telomerase-deficient mice were used in these studies; these mice have tissue atrophy, stem cell depletion, organ system failure, and impaired tissue injury responses. Telomerase reactivation in these mice caused

extension of telomeres, reduced DNA damage, reversed neurodegeneration, and improved the function of the testes, spleen, and intestines. Thus, telomere reactivation may have potential for treating age-related diseases in humans.

Cancer is characterized by uncontrolled cell division of abnormal cells. The cells accumulate mutations, proliferate uncontrollably, and can migrate to different parts of the body through a process called metastasis. Scientists have observed that cancerous cells have considerably shortened telomeres and that telomerase is active in these cells. Interestingly, only after the telomeres were shortened in the cancer cells did the telomerase become active. If the action of telomerase in these cells can be inhibited by drugs during cancer therapy, then the cancerous cells could potentially be stopped from further division.

Difference between Prokaryotic and Eukaryotic Replication

| Property | Prokaryotes | Eukaryotes |
|-----------------------|--------------------|-------------------------------|
| Origin of replication | Single | Multiple |
| Rate of replication | 1000 nucleotides/s | 50 to 100 nucleotides/s |
| DNA polymerase types | 5 | 14 |
| Telomerase | Not present | Present |
| RNA primer removal | DNA pol I | RNase H |
| Strand elongation | DNA pol III | Pol δ , pol ϵ |
| Sliding clamp | Sliding clamp | PCNA |

Table 14.2

14.6 | DNA Repair

In this section, you will explore the following questions:

- What are different types of mutations in DNA and the significance of mutations?
- What are examples of mechanisms that repair mutations in DNA?

Connection for AP[®] Courses

DNA polymerase is an efficient enzyme but it can make mistakes while adding nucleotides during replication. It edits the DNA by proofreading every newly added base. An incorrect base is removed and replaced by the correct base. If a base remains mismatched, special repair enzymes can often recognize the wrongly incorporated base, excise it from the DNA, and replace it with the correct base. Most mistakes are corrected, but if they are not they may result in a mutation, which is defined as a permanent change in a DNA sequence. A mutation can be passed to daughter cells through DNA replication and cell division. There are several types of DNA mutations, including substitution, deletion, insertion, and translocation. Mutations in repair genes may lead to serious consequences, such as cancer. Mutations can be induced by environmental factors, such as UV radiation, or they can occur spontaneously. (We will explore the effects of mutation in more detail in a later chapter. Remember that mutations are not always detrimental. They can be beneficial, too. Changes in DNA increase genetic variation—the foundation of evolution by natural selection.)

Information presented and the examples highlighted in the section support concepts outlined in Big Idea 3 of the AP[®] Biology Curriculum Framework. The Learning Objectives listed in the Curriculum Framework provide a transparent foundation for the AP[®] Biology course, an inquiry-based laboratory experience, instructional activities, and AP[®] exam questions. A Learning Objective merges required content with one or more of the seven Science Practices.

Big Idea 3 Living systems store, retrieve, transmit, and respond to information essential to life processes.

Enduring Understanding 3.C The processing of genetic information is imperfect and is a source of genetic variation.

2. Jaskelioff et al., “Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice,” *Nature* 469 (2011): 102-7.

| | |
|----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Essential Knowledge | 3.C.1 Changes in genotype can result in changes in phenotype. |
| Science Practice | 6.4 The student can make claims and predictions about natural phenomena based on scientific theories and models. |
| Science Practice | 7.2 The student can connect concepts in and across domain(s) to generalize or extrapolate in and/or across enduring understandings and/or big ideas. |
| Learning Objective | 3.24 The student is able to predict how a change in genotype, when expressed as a phenotype, provides a variation that can be subject to natural selection. |

| | |
|----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Essential Knowledge | 3.C.1 Changes in genotype can result in changes in phenotype. |
| Science Practice | 1.1 The student can create representations and models of natural or man-made phenomena and systems in the domain. |
| Learning Objective | 3.25 The student can create a visual representation to illustrate how changes in a DNA nucleotide sequence can result in a change in the polypeptide produced. |

| | |
|----------------------------|------------------------------------------------------------------------------------------------------------------------------------|
| Essential Knowledge | 3.C.2 Biological systems have multiple processes that increase genetic variation. |
| Science Practice | 6.2 The student can construct explanations of phenomena based on evidence produced through scientific practices. |
| Learning Objective | 3.28 The student is able to construct an explanation of the multiple processes that increase variation within a population. |

DNA replication is a highly accurate process, but mistakes can occasionally occur, such as a DNA polymerase inserting a wrong base. Uncorrected mistakes may sometimes lead to serious consequences, such as cancer. Repair mechanisms correct the mistakes. In rare cases, mistakes are not corrected, leading to mutations; in other cases, repair enzymes are themselves mutated or defective.

Most of the mistakes during DNA replication are promptly corrected by DNA polymerase by proofreading the base that has been just added (**Figure 14.17**). In **proofreading**, the DNA pol reads the newly added base before adding the next one, so a correction can be made. The polymerase checks whether the newly added base has paired correctly with the base in the template strand. If it is the right base, the next nucleotide is added. If an incorrect base has been added, the enzyme makes a cut at the phosphodiester bond and releases the wrong nucleotide. This is performed by the exonuclease action of DNA pol III. Once the incorrect nucleotide has been removed, a new one will be added again.

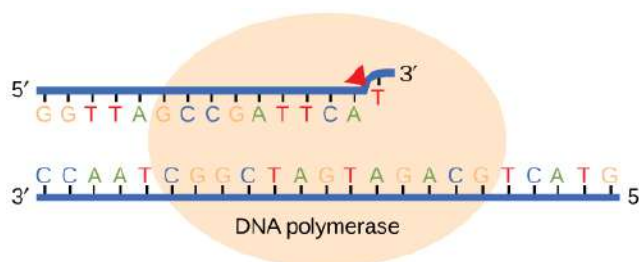


Figure 14.17 Proofreading by DNA polymerase corrects errors during replication.

Some errors are not corrected during replication, but are instead corrected after replication is completed; this type of repair is known as **mismatch repair** (**Figure 14.18**). The enzymes recognize the incorrectly added nucleotide and excise it; this is then replaced by the correct base. If this remains uncorrected, it may lead to more permanent damage. How do mismatch repair enzymes recognize which of the two bases is the incorrect one? In *E. coli*, after replication, the nitrogenous base

adenine acquires a methyl group; the parental DNA strand will have methyl groups, whereas the newly synthesized strand lacks them. Thus, DNA polymerase is able to remove the wrongly incorporated bases from the newly synthesized, non-methylated strand. In eukaryotes, the mechanism is not very well understood, but it is believed to involve recognition of unsealed nicks in the new strand, as well as a short-term continuing association of some of the replication proteins with the new daughter strand after replication has completed.

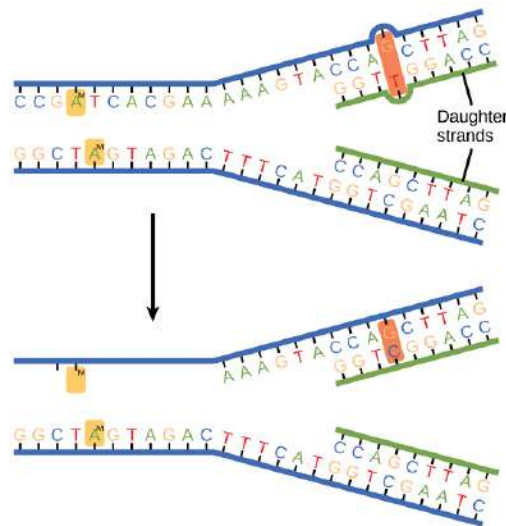


Figure 14.18 In mismatch repair, the incorrectly added base is detected after replication. The mismatch repair proteins detect this base and remove it from the newly synthesized strand by nuclease action. The gap is now filled with the correctly paired base.

In another type of repair mechanism, **nucleotide excision repair**, enzymes replace incorrect bases by making a cut on both the 3' and 5' ends of the incorrect base (**Figure 14.19**). The segment of DNA is removed and replaced with the correctly paired nucleotides by the action of DNA pol. Once the bases are filled in, the remaining gap is sealed with a phosphodiester linkage catalyzed by DNA ligase. This repair mechanism is often employed when UV exposure causes the formation of pyrimidine dimers.

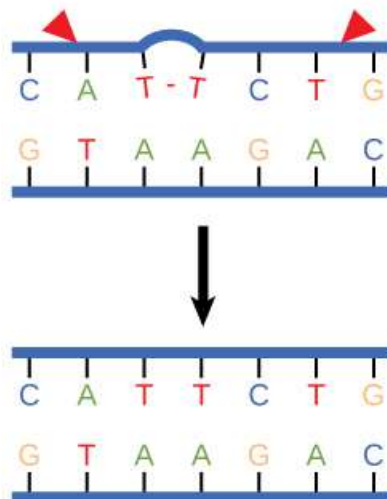


Figure 14.19 Nucleotide excision repairs thymine dimers. When exposed to UV, thymines lying adjacent to each other can form thymine dimers. In normal cells, they are excised and replaced.

A well-studied example of mistakes not being corrected is seen in people suffering from xeroderma pigmentosa (**Figure 14.20**). Affected individuals have skin that is highly sensitive to UV rays from the sun. When individuals are exposed to UV, pyrimidine dimers, especially those of thymine, are formed; people with xeroderma pigmentosa are not able to repair the damage. These are not repaired because of a defect in the nucleotide excision repair enzymes, whereas in normal individuals, the thymine dimers are excised and the defect is corrected. The thymine dimers distort the structure of the DNA double helix, and this may cause problems during DNA replication.



Figure 14.20 Xeroderma pigmentosa is a condition in which thymine dimerization from exposure to UV is not repaired. Exposure to sunlight results in skin lesions. (credit: James Halpern et al.)

Errors during DNA replication are not the only reason why mutations arise in DNA. **Mutations**, variations in the nucleotide sequence of a genome, can also occur because of damage to DNA. Such mutations may be of two types: induced or spontaneous. **Induced mutations** are those that result from an exposure to chemicals, UV rays, x-rays, or some other environmental agent. **Spontaneous mutations** occur without any exposure to any environmental agent; they are a result of natural reactions taking place within the body.

Mutations may have a wide range of effects. Some mutations are not expressed; these are known as **silent mutations**. **Point mutations** are those mutations that affect a single base pair. The most common nucleotide mutations are substitutions, in which one base is replaced by another. These can be of two types, either transitions or transversions. **Transition substitution** refers to a purine or pyrimidine being replaced by a base of the same kind; for example, a purine such as adenine may be replaced by the purine guanine. **Transversion substitution** refers to a purine being replaced by a pyrimidine, or vice versa; for example, cytosine, a pyrimidine, is replaced by adenine, a purine. Mutations can also be the result of the addition of a base, known as an insertion, or the removal of a base, also known as deletion. Sometimes a piece of DNA from one chromosome may get translocated to another chromosome or to another region of the same chromosome; this is also known as translocation. These mutation types are shown in **Figure 14.22**.

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Sometimes a nucleotide is overlooked by the DNA repair system for no known reason. This malignant melanoma is the result of DNA not undergoing repair after too much UV exposure.



Figure 14.21

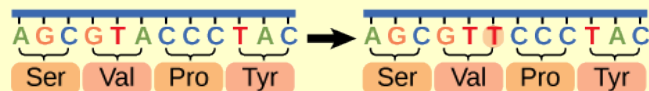
Which statement about the above malignant melanoma is most likely true?

- a. it was the result of a spontaneous mutation
- b. it was caused by thymine dimer formation
- c. it was caused by a transition substitution
- d. it was caused by a transversion substitution

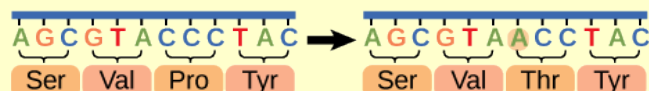
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Point Mutations

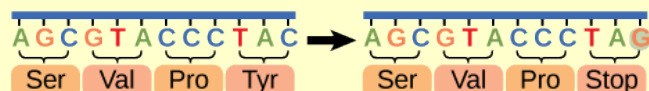
Silent: has no effect on the protein sequence



Missense: results in an amino acid substitution



Nonsense: substitutes a stop codon for an amino acid



Frameshift Mutations

Insertions or deletions of nucleotides may result in a shift in the reading frame or insertion of a stop codon.

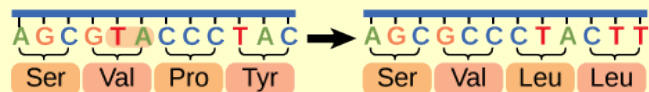


Figure 14.22 Mutations can lead to changes in the protein sequence encoded by the DNA.

A frameshift mutation that results in the insertion of three nucleotides is often less deleterious than a mutation that results in the insertion of one nucleotide. Why?

- Addition of three nucleotides does not shift the reading frame.
- Addition of three nucleotides shifts the reading frame.
- Addition of three nucleotides incorporates two amino acids.
- Addition of three nucleotides removes two amino acids.

Mutations in repair genes have been known to cause cancer. Many mutated repair genes have been implicated in certain forms of pancreatic cancer, colon cancer, and colorectal cancer. Mutations can affect either somatic cells or germ cells. If many mutations accumulate in a somatic cell, they may lead to problems such as the uncontrolled cell division observed in cancer. If a mutation takes place in germ cells, the mutation will be passed on to the next generation, as in the case of hemophilia and xeroderma pigmentosa.

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Think About It

Infertility can sometimes be explained by chromosome translocations. Explain how chromosome translocations can cause infertility. Are there times when a chromosome translocation might *not* result in infertility?

KEY TERMS

electrophoresis technique used to separate DNA fragments according to size

helicase during replication, this enzyme helps to open up the DNA helix by breaking the hydrogen bonds

induced mutation mutation that results from exposure to chemicals or environmental agents

lagging strand during replication, the strand that is replicated in short fragments and away from the replication fork

leading strand strand that is synthesized continuously in the 5'-3' direction which is synthesized in the direction of the replication fork

ligase enzyme that catalyzes the formation of a phosphodiester linkage between the 3' OH and 5' phosphate ends of the DNA

mismatch repair type of repair mechanism in which mismatched bases are removed after replication

mutation variation in the nucleotide sequence of a genome

nucleotide excision repair type of DNA repair mechanism in which the wrong base, along with a few nucleotides upstream or downstream, are removed

Okazaki fragment DNA fragment that is synthesized in short stretches on the lagging strand

point mutation mutation that affects a single base

primase enzyme that synthesizes the RNA primer; the primer is needed for DNA pol to start synthesis of a new DNA strand

primer short stretch of nucleotides that is required to initiate replication; in the case of replication, the primer has RNA nucleotides

proofreading function of DNA pol in which it reads the newly added base before adding the next one

replication fork Y-shaped structure formed during initiation of replication

silent mutation mutation that is not expressed

single-strand binding protein during replication, protein that binds to the single-stranded DNA; this helps in keeping the two strands of DNA apart so that they may serve as templates

sliding clamp ring-shaped protein that holds the DNA pol on the DNA strand

spontaneous mutation mutation that takes place in the cells as a result of chemical reactions taking place naturally without exposure to any external agent

telomerase enzyme that contains a catalytic part and an inbuilt RNA template; it functions to maintain telomeres at chromosome ends

telomere DNA at the end of linear chromosomes

topoisomerase enzyme that causes underwinding or overwinding of DNA when DNA replication is taking place

transformation process in which external DNA is taken up by a cell

transition substitution when a purine is replaced with a purine or a pyrimidine is replaced with another pyrimidine

transversion substitution when a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine

CHAPTER SUMMARY

14.1 Historical Basis of Modern Understanding

DNA was first isolated from white blood cells by Friedrich Miescher, who called it nuclein because it was isolated from nuclei. Frederick Griffith's experiments with strains of *Streptococcus pneumoniae* provided the first hint that DNA may be the transforming principle. Avery, MacLeod, and McCarty proved that DNA is required for the transformation of bacteria. Later experiments by Hershey and Chase using bacteriophage T2 proved that DNA is the genetic material. Chargaff found that the ratio of A = T and C = G, and that the percentage content of A, T, G, and C is different for different species.

14.2 DNA Structure and Sequencing

The currently accepted model of the double-helix structure of DNA was proposed by Watson and Crick. Some of the salient features are that the two strands that make up the double helix are complementary and anti-parallel in nature. Deoxyribose sugars and phosphates form the backbone of the structure, and the nitrogenous bases are stacked inside. The diameter of the double helix, 2 nm, is uniform throughout. A purine always pairs with a pyrimidine; A pairs with T, and G pairs with C. One turn of the helix has ten base pairs. During cell division, each daughter cell receives a copy of the DNA by a process known as DNA replication. Prokaryotes are much simpler than eukaryotes in many of their features. Most prokaryotes contain a single, circular chromosome. In general, eukaryotic chromosomes contain a linear DNA molecule packaged into nucleosomes, and have two distinct regions that can be distinguished by staining, reflecting different states of packaging and compaction.

14.3 Basics of DNA Replication

The model for DNA replication suggests that the two strands of the double helix separate during replication, and each strand serves as a template from which the new complementary strand is copied. In conservative replication, the parental DNA is conserved, and the daughter DNA is newly synthesized. The semi-conservative method suggests that each of the two parental DNA strands acts as template for new DNA to be synthesized; after replication, each double-stranded DNA includes one parental or “old” strand and one “new” strand. The dispersive mode suggested that the two copies of the DNA would have segments of parental DNA and newly synthesized DNA.

14.4 DNA Replication in Prokaryotes

Replication in prokaryotes starts from a sequence found on the chromosome called the origin of replication—the point at which the DNA opens up. Helicase opens up the DNA double helix, resulting in the formation of the replication fork. Single-strand binding proteins bind to the single-stranded DNA near the replication fork to keep the fork open. Primase synthesizes an RNA primer to initiate synthesis by DNA polymerase, which can add nucleotides only in the 5' to 3' direction. One strand is synthesized continuously in the direction of the replication fork; this is called the leading strand. The other strand is synthesized in a direction away from the replication fork, in short stretches of DNA known as Okazaki fragments. This strand is known as the lagging strand. Once replication is completed, the RNA primers are replaced by DNA nucleotides and the DNA is sealed with DNA ligase, which creates phosphodiester bonds between the 3'-OH of one end and the 5' phosphate of the other strand.

14.5 DNA Replication in Eukaryotes

Replication in eukaryotes starts at multiple origins of replication. The mechanism is quite similar to prokaryotes. A primer is required to initiate synthesis, which is then extended by DNA polymerase as it adds nucleotides one by one to the growing chain. The leading strand is synthesized continuously, whereas the lagging strand is synthesized in short stretches called Okazaki fragments. The RNA primers are replaced with DNA nucleotides; the DNA remains one continuous strand by linking the DNA fragments with DNA ligase. The ends of the chromosomes pose a problem as polymerase is unable to extend them without a primer. Telomerase, an enzyme with an inbuilt RNA template, extends the ends by copying the RNA template and extending one end of the chromosome. DNA polymerase can then extend the DNA using the primer. In this way, the ends of the chromosomes are protected.

14.6 DNA Repair

DNA polymerase can make mistakes while adding nucleotides. It edits the DNA by proofreading every newly added base. Incorrect bases are removed and replaced by the correct base, and then a new base is added. Most mistakes are corrected during replication, although when this does not happen, the mismatch repair mechanism is employed. Mismatch repair enzymes recognize the wrongly incorporated base and excise it from the DNA, replacing it with the correct base. In yet

another type of repair, nucleotide excision repair, the incorrect base is removed along with a few bases on the 5' and 3' end, and these are replaced by copying the template with the help of DNA polymerase. The ends of the newly synthesized fragment are attached to the rest of the DNA using DNA ligase, which creates a phosphodiester bond.

Most mistakes are corrected, and if they are not, they may result in a mutation defined as a permanent change in the DNA sequence. Mutations can be of many types, such as substitution, deletion, insertion, and translocation. Mutations can be induced or may occur spontaneously.

REVIEW QUESTIONS

1. Who was the first person to isolate the material that came to be known as nucleic acids?

- a. Frederick Griffith
- b. Friedrich Miescher
- c. James Watson
- d. Oswald Avery

2. What is bacterial transformation?

- a. The transformation of a bacterium occurs during replication.
- b. It is the transformation of a bacterium into a pathogenic form.
- c. Transformation of bacteria involves changes in its chromosome.
- d. Transformation is a process in which external DNA is taken up by a cell, thereby changing morphology and physiology.

3. What type of nucleic acid material is analyzed the most frequently in forensics cases?

- a. cytoplasmic rRNA
- b. mitochondrial DNA
- c. nuclear chromosomal DNA
- d. nuclear mRNA

4. The experiments by Hershey and Chase helped confirm that DNA was the hereditary material on the basis of the finding of what?

- a. Radioactive phages were found in the pellet.
- b. Radioactive cells were found in the supernatant.
- c. Radioactive sulfur was found inside the cell.
- d. Radioactive phosphorus was found in the cell.

5. If DNA of a particular species was analyzed and it was found that it contains 27% A, what would be the percentage of C?

- a. 23%
- b. 27%
- c. 30%
- d. 54%

6. If the sequence of the 5' to 3' strand is AATGCTAC, then the complementary sequence has the following

sequence:

- a. 3'-AATGCTAC-5'
- b. 3'-CATCGTAA-5'
- c. 3'-TTACGATG-5'
- d. 3'-GTAGCATT-5'

7. The DNA double helix does not have which of the following?

- a. antiparallel configuration
- b. complementary base pairing
- c. major and minor grooves
- d. uracil

8. What is a purine?

- a. a double ring structure with a six-membered ring fused to a five-membered ring
- b. a single six-membered ring
- c. a six-membered ring
- d. three phosphates covalently bonded by phosphodiester bonds

9. What is the name of the method developed by Fred Sanger to sequence DNA?

- a. Dideoxy Chain Termination method
- b. Double Helix Determination
- c. Polymerase Chain Reaction
- d. Polymer Gel Electrophoresis

10. What happens when a dideoxynucleotide is added to a developing DNA strand?

- a. The chain extends to the end of the DNA strand.
- b. The DNA strand is duplicated.
- c. The chain is not extended any further.
- d. The last codon is repeated.

11. In eukaryotes, what is DNA wrapped around?

- a. histones
- b. polymerase
- c. single-stranded binding proteins
- d. sliding clamp

12. Which enzyme is only found in prokaryotic organisms?

- a. DNA gyrase
 - b. helicase
 - c. ligase
 - d. telomerase
- 13.** Uracil is found where?
- a. chromosomal DNA
 - b. helicase
 - c. mitochondrial DNA
 - d. mRNA
- 14.** What prevents the further development of a DNA strand in Sanger sequencing?
- a. the addition of DNA reductase
 - b. the addition of dideoxynucleotides
 - c. the elimination of DNA polymerase
 - d. the addition of uracil
- 15.** Which of the following is not one of the proteins involved during the formation of the replication fork?
- a. helicase
 - b. ligase
 - c. origin of replication
 - d. single-strand binding proteins
- 16.** In which direction does DNA replication take place?
- a. 5' to 3'
 - b. 3' to 5'
 - c. 5'
 - d. 3'
- 17.** Meselson and Stahl's experiments proved that DNA replicates by which mode?
- a. conservative
 - b. converse
 - c. dispersive
 - d. semi-conservative
- 18.** Which set of results was found in the Meselson and Stahl's experiments?
- a. The original chromosome was kept intact and a duplicate was made.
 - b. The original chromosome was split and half went to each duplicate.
 - c. The original chromosome was mixed with new material and each duplicate strand contained both old and new.
 - d. The original chromosome was used as a template for two new chromosomes and discarded.
- 19.** Which enzyme initiates the splitting of the double DNA strand during replication?
- a. DNA gyrase
 - b. helicase
 - c. ligase
 - d. telomerase
- 20.** Which enzyme is most directly responsible for the main process of producing a new DNA strand?
- a. DNA pol I
 - b. DNA pol II
 - c. DNA pol III
 - d. DNA pol I, DNA pol II, and DNA pol III
- 21.** Which portion of a chromosome contains Okazaki fragments?
- a. helicase
 - b. lagging strand
 - c. leading strand
 - d. primer
- 22.** Which of the following does the enzyme primase synthesize?
- a. DNA primer
 - b. Okazaki fragments
 - c. phosphodiester linkage
 - d. RNA primer
- 23.** The ends of the linear chromosomes are maintained by what?
- a. DNA polymerase
 - b. helicase
 - c. primase
 - d. telomerase
- 24.** What is the difference in the rate of replication of nucleotides between prokaryotes and eukaryotes?
- a. Eukaryotes are 50 times slower.
 - b. Eukaryotes are 20 times faster.
 - c. Prokaryotes are 100 times slower.
 - d. Prokaryotes are 10 times faster.
- 25.** What are Autonomously Replicating Sequences (ARS)?

- a. areas of prokaryotic chromosomes that initiate copying
 - b. portions of prokaryotic chromosomes that can be transferred from one organism to another
 - c. areas of eukaryotic chromosomes that are equivalent to the origin of replication in *E. coli*
 - d. portions of eukaryotic chromosomes that replicate independent of the parent chromosome
- 26.** What type of body cell does not exhibit telomerase activity?
- a. adult stem cells
 - b. embryonic cells
 - c. germ cells
 - d. liver cells
- 27.** During proofreading, which of the following enzymes reads the DNA?
- a. DNA polymerase
 - b. helicase
 - c. topoisomerase
 - d. primase
- 28.** If a prokaryotic cell is replicating nucleotides at a rate of 100 per second, how fast would a eukaryotic cell be replicating nucleotides?
- a. 1000 per second
 - b. 100 per second
 - c. 10 per second
 - d. 1 per second
- 29.** Which type of point mutation would have no effect on gene expression?
- a. frameshift
 - b. missense
 - c. nonsense
 - d. silent
- 30.** Which type of point mutation would result in the substitution of a stop codon for an amino acid?
- a. frameshift
 - b. missense
 - c. nonsense
 - d. silent
- 31.** You have developed skin cancer and you are pregnant. You are worried that your child will be born with the cancer you have while carrying the baby. Should you be worried?
- a. Yes, the cancer can spread to the baby.
 - b. No, the mutations causing the cancer are in somatic cells, not reproductive germ cells.
 - c. Yes, the mutations can be passed on to the child through the placenta.
 - d. No, UV light only affects adult, somatic cells.
- 32.** What is the initial mechanism for repairing nucleotide errors in DNA?
- a. DNA polymerase proofreading
 - b. mismatch repair
 - c. nucleotide excision repair
 - d. thymine dimers
- 33.** Nucleotide excision repair is often employed when UV exposure causes the formation of what?
- a. phosphodiester bonds
 - b. purine conjugates
 - c. pyrimidine dimers
 - d. tetrad disassembly

CRITICAL THINKING QUESTIONS

- 34.** Explain Griffith's transformation experiments. What did he conclude from them?

- a. Two strains of *S. pneumoniae* were used for the experiment. Griffith injected a mouse with heat-inactivated S strain (pathogenic) and R strain (non-pathogenic). The mouse died and S strain was recovered from the dead mouse. He concluded that external DNA is taken up by a cell that changed morphology and physiology.
 - b. Two strains of *Vibrio cholerae* were used for the experiment. Griffith injected a mouse with heat-inactivated S strain (pathogenic) and R strain (non-pathogenic). The mouse died and S strain was recovered from the dead mouse. He concluded that external DNA is taken up by a cell that changed morphology and physiology.
 - c. Two strains of *S. pneumoniae* were used for the experiment. Griffith injected a mouse with heat-inactivated S strain (pathogenic) and R strain (non-pathogenic). The mouse died and R strain was recovered from the dead mouse. He concluded that external DNA is taken up by a cell that changed morphology and physiology.
 - d. Two strains of *S. pneumoniae* were used for the experiment. Griffith injected a mouse with heat-inactivated S strain (pathogenic) and R strain (non-pathogenic). The mouse died and S strain was recovered from the dead mouse. He concluded that mutation occurred in the DNA of the cell that changed morphology and physiology.
- 35.** Explain why radioactive sulfur and phosphorous were used to label bacteriophages in the Hershey and Chase experiments.
- a. Protein was labeled with radioactive sulfur and DNA was labeled with radioactive phosphorous. Phosphorous is found in DNA, so it will be tagged by radioactive phosphorous.
 - b. Protein was labeled with radioactive phosphorous and DNA was labeled with radioactive sulfur. Phosphorous is found in DNA, so it will be tagged by radioactive phosphorous.
 - c. Protein was labeled with radioactive sulfur and DNA was labeled with radioactive phosphorous. Phosphorous is found in DNA, so DNA will be tagged by radioactive sulfur.
 - d. Protein was labeled with radioactive sulfur and DNA was labeled with radioactive phosphorous. Phosphorous is found in DNA, so DNA will be tagged by radioactive sulfur.
- 36.** How can Chargaff's rules be used to identify different species?
- a. The amount of adenine, thymine, guanine, and cytosine varies from species to species and are not found in equal quantities. They do not vary between individuals of the same species and can be used to identify different species.
 - b. The amount of adenine, thymine, guanine, and cytosine varies from species to species and is found in equal quantities. They do not vary between individuals of the same species and can be used to identify different species.
 - c. The amount of adenine and thymine is equal to guanine and cytosine and is found in equal quantities. They do not vary between individuals of the same species and can be used to identify different species.
 - d. The amount of adenine, thymine, guanine, and cytosine varies from species to species and they are not found in equal quantities. They vary between individuals of the same species and can be used to identify different species.
- 37.** In the Avery, Macleod, and McCarty experiments, what conclusion would the scientists have drawn if the use of proteases prevented the transformation of R strain bacteria?
- 38.** Describe the structure and complementary base pairing of DNA.

- a. DNA is made up of two strands that are twisted around each other to form a helix. Adenine pairs up with thymine and cytosine pairs with guanine. The two strands are anti-parallel in nature; that is, the 3' end of one strand faces the 5' end of other strand. Sugar, phosphate and nitrogenous bases contribute to the DNA structure.
 - b. DNA is made up of two strands that are twisted around each other to form a helix. Adenine pairs up with cytosine and thymine pairs with guanine. The two strands are anti-parallel in nature; that is, the 3' end of one strand faces the 5' end of other strand. Sugar, phosphate and nitrogenous bases contribute to the DNA structure.
 - c. DNA is made up of two strands that are twisted around each other to form a helix. Adenine pairs up with thymine and cytosine pairs with guanine. The two strands are parallel in nature; that is, the 3' end of one strand faces the 3' end of other strand. Sugar, phosphate and nitrogenous bases contribute to the DNA structure.
 - d. DNA is made up of two strands that are twisted around each other to form a helix. Adenine pairs up with thymine and cytosine pairs with guanine. The two strands are anti-parallel in nature; that is, the 3' end of one strand faces the 5' end of other strand. Only sugar contributes to the DNA structure.
- 39.** Provide a brief summary of the Sanger sequencing method.
- a. Frederick Sanger's sequencing is a chain termination method that is used to generate DNA fragments that terminate at different points using dye-labeled dideoxynucleotides. DNA is separated by electrophoresis on the basis of size. The DNA sequence can be read out on an electropherogram generated by a laser scanner.
 - b. Frederick Sanger's sequencing is a chain elongation method that is used to generate DNA fragments that elongate at different points using dye-labeled dideoxynucleotides. DNA is separated by electrophoresis on the basis of size. The DNA sequence can be read out on an electropherogram generated by a laser scanner.
 - c. Frederick Sanger's sequencing is a chain termination method that is used to generate DNA fragments that terminate at different points using dye-labeled dideoxynucleotides. DNA is joined together by electrophoresis on the basis of size. The DNA sequence can be read out on an electropherogram generated by a laser scanner.
 - d. Frederick Sanger's sequencing is a chain termination method that is used to generate DNA fragments that terminate at different points using dye-labeled dideoxynucleotides. DNA is separated by electrophoresis on the basis of size. The DNA sequence can be read out on an electropherogram generated by a magnetic scanner.
- 40.** Compare and contrast the similarities and differences between eukaryotic and prokaryotic DNA.

- a. Eukaryotes have a single, circular chromosome, while prokaryotes have multiple, linear chromosomes. Prokaryotes pack their chromosomes by super coiling, managed by DNA gyrase. Eukaryote chromosomes are wrapped around histone proteins that create heterochromatin and euchromatin, which is not present in prokaryotes.
 - b. Prokaryotes have a single, circular chromosome, while eukaryotes have multiple, linear chromosomes. Prokaryotes pack their chromosomes by super coiling, managed by DNA gyrase. Eukaryote chromosomes are wrapped around histone proteins that could form heterochromatin, which is not present in prokaryotes.
 - c. Prokaryotes have a single, circular chromosome, while eukaryotes have multiple, linear chromosomes. Eukaryotes pack their chromosomes by super coiling, managed by DNA gyrase. Prokaryotes chromosomes are wrapped around histone proteins that could form heterochromatin, which is not present in eukaryotes.
 - d. Prokaryotes have a single, circular chromosome, while eukaryotes have multiple, linear chromosomes. Prokaryotes pack their chromosomes by super coiling, managed by DNA gyrase. Eukaryote chromosomes are wrapped around histone proteins that could form heterochromatin, which is present in prokaryotes.
- 41.** DNA replication is bidirectional and discontinuous; explain your understanding of those concepts.
- a. DNA polymerase reads the template strand in the 3' to 5' direction and adds nucleotides only in the 5' to 3' direction. The leading strand is synthesized in the direction of the replication fork. Replication on the lagging strand occurs in the direction away from the replication fork in short stretches of DNA called Okazaki fragments.
 - b. DNA polymerase reads the template strand in the 5' to 3' direction and adds nucleotides only in the 5' to 3' direction. The leading strand is synthesized in the direction of the replication fork. Replication on the lagging strand occurs in the direction away from the replication fork in short stretches of DNA called Okazaki fragments.
 - c. DNA polymerase reads the template strand in the 3' to 5' direction and adds nucleotides only in the 5' to 3' direction. The leading strand is synthesized in the direction away from the replication fork. Replication on the lagging strand occurs in the direction of the replication fork in short stretches of DNA called Okazaki fragments.
 - d. DNA polymerase reads the template strand in the 5' to 3' direction and adds nucleotides only in the 3' to 5' direction. The leading strand is synthesized in the direction of the replication fork. Replication on the lagging strand occurs in the direction away from the replication fork in long stretches of DNA called Okazaki fragments.
- 42.** Discuss how the scientific community learned that DNA replication takes place in a semiconservative fashion.

- a. Meselson and Stahl experimented with *E. coli*. DNA grown in ^{15}N was heavier than DNA grown in ^{14}N . When DNA in ^{15}N was switched to ^{14}N media, DNA sedimented halfway between the ^{15}N and ^{14}N levels after one round of cell division, indicating fifty percent presence of ^{14}N . This supports the semi-conservative replication model.
 - b. Meselson and Stahl experimented with *S. pneumonia*. DNA grown in ^{15}N was heavier than DNA grown in ^{14}N . When DNA in ^{15}N was switched to ^{14}N media, DNA sedimented halfway between the ^{15}N and ^{14}N levels after one round of cell division, indicating fifty percent presence of ^{14}N . This supports the semi-conservative replication model.
 - c. Meselson and Stahl experimented with *E. coli*. DNA grown in ^{14}N was heavier than DNA grown in ^{15}N . When DNA in ^{15}N was switched to ^{14}N media, DNA sedimented halfway between the ^{15}N and ^{14}N levels after one round of cell division, indicating fifty percent presence of ^{14}N . This supports the semi-conservative replication model.
 - d. Meselson and Stahl experimented with *S. pneumonia*. DNA grown in ^{15}N was heavier than DNA grown in ^{14}N . When DNA in ^{15}N was switched to ^{14}N media, DNA sedimented halfway between the ^{15}N and ^{14}N levels after one round of cell division, indicating complete presence of ^{14}N . This supports the semi-conservative replication model.
- 43.** Explain why half of DNA is replicated in a discontinuous fashion.
- a. Replication of the lagging strand occurs in the direction away from the replication fork in short stretches of DNA, since access to the DNA is always from the 5' end. This results in pieces of DNA being replicated in a discontinuous fashion.
 - b. Replication of the leading strand occurs in the direction away from the replication fork in short stretches of DNA, since access to the DNA is always from the 5' end. This results in pieces of DNA being replicated in a discontinuous fashion.
 - c. Replication of the lagging strand occurs in the direction of the replication fork in short stretches of DNA, since access to the DNA is always from the 5' end. This results in pieces of DNA being replicated in a discontinuous fashion.
 - d. Replication of the lagging strand occurs in the direction away from the replication fork in short stretches of DNA, since access to the DNA is always from the 3' end. This results in pieces of DNA being replicated in a discontinuous fashion.
- 44.** Explain the events taking place at the replication fork. If the gene for helicase is mutated, what part of replication will be affected?

- a. Helicase separates the DNA strands at the origin of replication. Topoisomerase breaks and reforms DNA's phosphate backbone ahead of the replication fork, thereby relieving the pressure. Single-stranded binding proteins prevent reforming of DNA. Primase synthesizes RNA primer which is used by DNA polymerase to form a daughter strand. If helicase is mutated, the DNA strands will not be separated at the beginning of replication.
 - b. Helicase joins the DNA strands together at the origin of replication. Topoisomerase breaks and reforms DNA's phosphate backbone after the replication fork, thereby relieving the pressure. Single-stranded binding proteins prevent reforming of DNA. Primase synthesizes RNA primer which is used by DNA polymerase to form a daughter strand. If helicase is mutated, the DNA strands will not be joined together at the beginning of replication.
 - c. Helicase separates the DNA strands at the origin of replication. Topoisomerase breaks and reforms DNA's sugar backbone ahead of the replication fork, thereby increasing the pressure. Single-stranded binding proteins prevent reforming of DNA. Primase synthesizes DNA primer which is used by DNA polymerase to form a daughter strand. If helicase is mutated, the DNA strands will be separated at the beginning of replication.
 - d. Helicase separates the DNA strands at the origin of replication. Topoisomerase breaks and reforms DNA's sugar backbone ahead of the replication fork, thereby relieving the pressure. Single-stranded binding proteins prevent reforming of DNA. Primase synthesizes DNA primer which is used by RNA polymerase to form a parent strand. If helicase is mutated, the DNA strands will be separated at the beginning of replication.
- 45.** What are Okazaki fragments and how they are formed?
- a. Okazaki fragments are short stretches of DNA on the lagging strand, which is synthesized in the direction away from the replication fork.
 - b. Okazaki fragments are long stretches of DNA on the lagging strand, which is synthesized in the direction of the replication fork.
 - c. Okazaki fragments are long stretches of DNA on the leading strand, which is synthesized in the direction away from the replication fork.
 - d. Okazaki fragments are short stretches of DNA on the leading strand, which is synthesized in the direction of the replication fork.
- 46.** Compare and contrast the roles of DNA polymerase I and DNA ligase in DNA replication.
- a. DNA polymerase I removes the RNA primers from the developing copy of DNA. DNA ligase seals the ends of the new segment, especially the Okazaki fragments.
 - b. DNA polymerase I adds the RNA primers to the already developing copy of DNA. DNA ligase separates the ends of the new segment, especially the Okazaki fragments.
 - c. DNA polymerase I seals the ends of the new segment, especially the Okazaki fragments. DNA ligase removes the RNA primers from the developing copy of DNA.
 - d. DNA polymerase I removes the enzyme primase from the developing copy of DNA. DNA ligase seals the ends of the old segment, especially the Okazaki fragments.
- 47.** If the rate of replication in a particular prokaryote is 900 nucleotides per second, how long would it take to make two copies of a 1.2 million base pair genome?
- a. 22.2 minutes
 - b. 44.4 minutes
 - c. 45.4 minutes
 - d. 54.4 minutes
- 48.** How do the linear chromosomes in eukaryotes ensure that their ends are replicated completely?
- a. The ends of the linear chromosomes are maintained by the activity of the telomerase enzyme.
 - b. The ends of the linear chromosomes are maintained by the formation of a replication fork.
 - c. The ends of the linear chromosomes are maintained by the continuous joining of Okazaki fragments.
 - d. The ends of the linear chromosomes are maintained by the action of the polymerase enzyme.
- 49.** Compare and contrast prokaryotic and eukaryotic DNA replication.

- a. A prokaryotic organism's rate of replication is ten times faster than that of eukaryotes. Prokaryotes have a single origin of replication and use five types of polymerases, while eukaryotes have multiple sites of origin and use fourteen polymerases. Telomerase is absent in prokaryotes. DNA pol I is the primer remover in prokaryotes, while in eukaryotes it is RNase H. DNA pol III performs strand elongation in prokaryotes and pol δ and pol ϵ do the same in eukaryotes.
- b. A prokaryotic organism's rate of replication is ten times slower than that of eukaryotes. Prokaryotes have a single origin of replication and use five types of polymerases, while eukaryotes have multiple sites of origin and use fourteen polymerases. Telomerase is absent in eukaryotes. DNA pol I is the primer remover in prokaryotes, while in eukaryotes it is RNase H. DNA pol III performs strand elongation in prokaryotes and pol δ and pol ϵ do the same in eukaryotes.
- c. A prokaryotic organism's rate of replication is ten times faster than that of eukaryotes. Prokaryotes have five origins of replication and use a single type of polymerase, while eukaryotes have a single site of origin and use fourteen polymerases. Telomerase is absent in prokaryotes. DNA pol I is the primer remover in prokaryotes, while in eukaryotes it is RNase H. DNA pol III performs strand elongation in prokaryotes and pol δ and pol ϵ do the same in eukaryotes.
- d. A prokaryotic organism's rate of replication is ten times slower than that of eukaryotes. Prokaryotes have a single origin of replication and use five types of polymerases, while eukaryotes have multiple sites of origin and use fourteen polymerases. Telomerase is absent in prokaryotes. DNA pol I is the primer remover in eukaryotes, while in prokaryotes it is RNase H. DNA pol III performs strand elongation in prokaryotes and pol δ and pol ϵ do the same in eukaryotes.

50. What would be the consequence of a mutation in a mismatch repair enzyme? How will this affect the function of a gene?

- a. Mismatch repair corrects the errors after the replication is completed by excising the incorrectly added nucleotide and adding the correct base. Any mutation in a mismatch repair enzyme would lead to more permanent damage.
- b. Mismatch repair corrects the errors during the replication by excising the incorrectly added nucleotide and adding the correct base. Any mutation in the mismatch repair enzyme would lead to more permanent damage.
- c. Mismatch repair corrects the errors after the replication is completed by excising the added nucleotides and adding more bases. Any mutation in the mismatch repair enzyme would lead to more permanent damage.
- d. Mismatch repair corrects the errors after the replication is completed by excising the incorrectly added nucleotide and adding the correct base. Any mutation in the mismatch repair enzyme would lead to more temporary damage.

51. A mutation has occurred in the DNA and in the mRNA for a gene. Discuss which would have a more significant effect on gene expression. Why?

- a. Both will result in the production of defective proteins. The DNA mutation, if not corrected, is permanent, while the mRNA mutation will only affect proteins made from that mRNA strand. Production of defective protein ceases when the mRNA strand deteriorates.
- b. Both will result in the production of defective proteins. The DNA mutation, if not corrected, is permanent, while the mRNA mutation will not affect proteins made from that mRNA strand. Production of defective protein continues when the mRNA strand deteriorates.
- c. Only DNA will result in the production of defective proteins. The DNA mutation, if not corrected, is permanent. Production of defective protein ceases when the DNA strand deteriorates.
- d. Only mRNA will result in the production of defective proteins. The mRNA mutation will only affect proteins made from that mRNA strand. Production of defective protein ceases when the mRNA strand deteriorates.

52. Discuss the effects of point mutations on a DNA strand.

- a. Mutations can cause a single change in an amino acid. A nonsense mutation can stop the replication or reading of that strand. Insertion or deletion mutations can cause a frame shift. This can result in non-functional proteins.
- b. Mutations can cause a single change in amino acid. A missense mutation can stop the replication or reading of that strand. Insertion or deletion mutations can cause a frame shift. This can result in non-functional proteins.
- c. Mutations can cause a single change in amino acid. A nonsense mutation can stop the replication or reading of that strand. Substitution mutations can cause a frame shift. This can result in non-functional proteins.
- d. Mutations can cause a single change in amino acid. A nonsense mutation can stop the replication or reading of that strand. Insertion or deletion mutations can cause a frame shift. This can result in functional proteins.

53. Discuss the significance of mutations in tRNA and rRNA.

- a. Mutations in tRNA and rRNA would lead to the production of defective proteins or no protein production.
- b. Mutations in tRNA and rRNA would lead to changes in the semi-conservative mode of replication of DNA.
- c. Mutations in tRNA and rRNA would lead to production of a DNA strand with a mutated single strand and normal other strand.
- d. Mutations in tRNA and rRNA would lead to skin cancer in patients of xeroderma pigmentosa.

TEST PREP FOR AP® COURSES

54. What would Chase and Hershey have concluded if the supernatant contained radioactive labeled-phosphorus?

- a. DNA was the primary source of heritable information.
- b. RNA was the primary source of heritable information.
- c. Protein was the primary source of heritable information.
- d. Phages were the primary source of heritable information.

55. Which piece of evidence supports that the material Miescher discovered was DNA?

- a. The precipitate contained sulfur.
- b. The precipitate contained oxygen.
- c. The precipitate contained phosphorus.
- d. The precipitate contained protein.

56. Explain how forensic scientists are able to use DNA analysis to identify individuals.

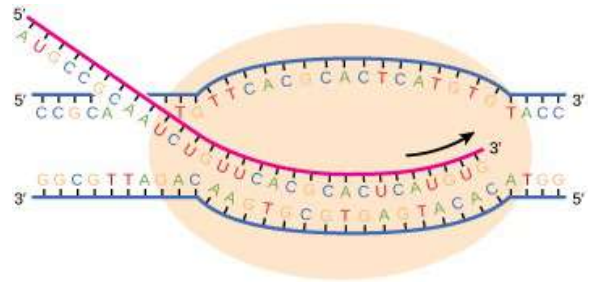
- a. Comparison of DNA from a known source or individual with analysis of the sequence of an unknown sample of DNA allows scientists to find out if both of them are similar or not.
- b. DNA from the unknown sample is sequenced and analyzed. The result of the analysis is then matched with any random population. The matching individual then helps in forensics.
- c. Comparison of DNA from a known source or individual with analysis of the sequence of bases in strands of an unknown sample of RNA allows scientists to find out if both of them are similar or not.
- d. Comparison of DNA from a known source or individual with analysis of the sugars and phosphates in strands of an unknown sample of DNA allows scientists to find out if both of them are similar or not.

57. Discuss the contributions of Francis Crick, James Watson, and Rosalind Franklin to the discovery of the structure of DNA.

- a. Rosalind Franklin used X-ray diffraction methods to demonstrate the helical nature of DNA, while Watson and Crick formulated the double stranded structural model of DNA.
 - b. Rosalind Franklin, Watson and Crick first employed the technique of X-ray diffraction to understand the storage of DNA. Since it did not work out, Watson and Crick then ran experiments to ascertain the DNA structure.
 - c. Rosalind Franklin, Watson and Crick used X-ray diffraction methods to demonstrate the helical nature of DNA, while Rosalind Franklin formulated the double stranded structural model of DNA.
 - d. Watson and Crick used X-ray diffraction methods to demonstrate the helical nature of DNA, while Rosalind Franklin formulated the double stranded structural model of DNA.
- 58.** What do RNA and DNA have in common?
- a. Both contain four different nucleotides.
 - b. Both are usually double-stranded molecules.
 - c. Both contain adenine and uracil.
 - d. Both contain ribose.
- 59.** Which of the following would be a good application of plasmid transformation?
- a. to make copies of DNA
 - b. to isolate a change in a single nucleotide
 - c. to separate DNA fragments
 - d. to sequence DNA
- 60.** Explain how the components of DNA fit together.
- a. DNA is composed of nucleotides, consisting of a 5 carbon sugar, a phosphate, and a nitrogenous base. DNA is a double helical structure in which complementary base pairing occurs. Adenine pairs with thymine and guanine pairs with cytosine. Adenine and thymine form two hydrogen bonds and cytosine and guanine form three hydrogen bonds. The two individual strands of DNA are held together by covalent bonds between the phosphate of one nucleotide and sugar of the next. The two strands run antiparallel to each other.
 - b. DNA is composed of nucleotides, consisting of a 5 carbon sugar, a phosphate, and a nitrogenous base. DNA is a double helical structure in which complementary base pairing occurs. Adenine pairs with cytosine and guanine pairs with thymine. Adenine and cytosine form two hydrogen bonds and guanine and thymine form three hydrogen bonds. The two individual strands of DNA are held together by covalent bonds between the phosphate of one nucleotide and sugar of the next. The two strands run antiparallel to each other.
 - c. DNA is composed of nucleotides, consisting of a 5 carbon sugar, a phosphate, and a nitrogenous base. DNA is a double helical structure in which complementary base pairing occurs. Adenine pairs with cytosine and guanine pairs with thymine. Adenine and cytosine form three hydrogen bonds and guanine and thymine form two hydrogen bonds. The two individual strands of DNA are held together by covalent bonds between the phosphate of one nucleotide and sugar of the next. The two strands run antiparallel to each other.
 - d. DNA is composed of nucleotides, consisting of a 5 carbon sugar, a phosphate, and a nitrogenous base. DNA is a double helical structure in which complementary base pairing occurs. Adenine pairs with cytosine and guanine pairs with thymine. Adenine and cytosine form three hydrogen bonds and guanine and thymine form two hydrogen bonds. The two individual strands of DNA are held together by covalent bonds between the phosphate of one nucleotide and sugar of the next. The two strands run parallel to each other.
- 61.** Describe the Sanger DNA sequencing method used for the human genome sequencing project.

- a. A DNA sample is denatured by heating and then put into four tubes. A primer, DNA polymerase and all four nucleotides are added. Limited quantities of one of the four dideoxynucleotides (ddNTPs) are added to each tube respectively. Each one of them carries a specific fluorescent label. Chain elongation continues until a fluorescent ddNTP is added to the growing chain, after which chain termination occurs. Gel electrophoresis is performed and the length of each base is detected by laser scanners with wavelengths specific to the four different ddNTPs's.
- b. A DNA sample is denatured by heating and then put into four tubes. A primer, RNA polymerase and all four nucleotides are added. Limited quantities of one of the four dideoxynucleotides (ddNTPs) are added to each tube respectively. Each one of them carries a specific fluorescent label. Chain elongation continues until a fluorescent ddNTP is added to the growing chain, after which chain termination occurs. Gel electrophoresis is performed and the length of each base is detected by laser scanners with wavelengths specific to the four different ddNTPs's.
- c. A DNA sample is denatured by heating and then put into four tubes. A primer, DNA polymerase and all four nucleotides are added. Limited quantities of one of the four dideoxynucleotides (ddNTPs) are added to each tube respectively. Each one of them carries a specific fluorescent label. Chain elongation continues until a fluorescent ddNTP is removed from the growing chain, after which chain termination occurs. Gel electrophoresis is performed and the length of each base is detected by laser scanners with wavelengths specific to the four different ddNTPs's.
- d. A DNA sample is denatured by heating and then put into four tubes. A primer, DNA polymerase and all four nucleotides are added. Limited quantities of one of the four deoxynucleotides (dNTPs) are added to each tube respectively. Each one of them carries a specific fluorescent label. Chain elongation continues until a fluorescent dNTP is added the growing chain, after which chain termination occurs. Gel electrophoresis is performed and the length of each base is detected by laser scanners with wavelengths specific to the four different dNTPs's.

62.



What process is illustrated in the figure?

- a. transcription
- b. mutation
- c. excision
- d. translation

63. Describe how the model of DNA replication illustrates the function of topoisomerase.

- a. Topoisomerase relieves the pressure that results from supercoiling by breaking and reforming DNA's phosphate backbone ahead of the replication fork.
- b. Topoisomerase increases the pressure to increase supercoiling by breaking and reforming DNA's phosphate backbone ahead of the replication fork.
- c. Topoisomerase relieves the pressure that results from supercoiling by breaking and reforming DNA's nucleotide base pairs ahead of the replication fork.
- d. Topoisomerase relieves the pressure that results from separation of DNA strands by breaking and reforming DNA's phosphate backbone ahead of the replication fork.

64. Flamingos have genotypes for white feathers yet often appear with pink feathers within the same population. What is most likely affecting the phenotype of some flamingos, causing their feathers to turn pink in an isolated population?

- a. weather variations
- b. dietary changes
- c. DNA mutations
- d. translation failure

65. What can be the result of DNA failing to undergo repair after too much UV exposure?

- a. second degree burns
- b. a malignant melanoma
- c. a breakdown of deep layers of the skin
- d. a sun burn

66. Identify the type of change that can occur in the DNA of a chromosome that is termed a chromosomal mutation.

- a. substitution
- b. translocation
- c. missense
- d. transversion

67. Explain why patients with Xeroderma Pigmentosa are more prone to cancer than the rest of the population.

- a. Xeroderma Pigmentosa patients cannot employ the nucleotide excision repair mechanism. When these patients are exposed to UV light, thymine dimers are formed and they are not able to repair this defect. These dimers distort the structure of DNA and cause them to have a high risk of contracting skin cancer.
- b. Xeroderma Pigmentosa patients can employ the nucleotide excision repair mechanism. When these patients are exposed to UV light, the thymine dimers are formed and they are able to repair this defect. These dimers do not distort the structure of DNA and they have moderate risk of contracting skin cancer.
- c. Xeroderma Pigmentosa patients cannot employ the nucleotide excision repair mechanism. When these patients are exposed to UV light, the adjacent adenine forms dimers and they are not able to repair this defect. These dimers distort the structure of DNA and they have high risk of contracting skin cancer.
- d. Xeroderma Pigmentosa patients cannot employ the nucleotide excision repair mechanism. When these patients are exposed to UV light, the adjacent thymine cannot form thymine dimers and they are not able to repair this defect. The non-formation of dimers distorts the structure of DNA and they have high risk of contracting skin cancer.

68. You are looking at two fragments of DNA. Both have the sequence CATTCTG on one strand and GTAAGAC on the other. One of the fragments is exposed to UV light, the other is not. What will happen to the fragments and how might these mutations be repaired?

- a. The fragment exposed to UV light contains thymine dimers. Thymines lying adjacent to each other can form thymine dimers when exposed to UV light. They can be repaired by nucleotide excision.
- b. The fragment exposed to UV light contains adenine dimers. Adenines lying adjacent to each other can form dimers when exposed to UV light. They can be repaired by nucleotide excision.
- c. The fragment exposed to UV light contains thymine dimers. Thymines lying parallel to each other can form thymine dimers when exposed to UV light. They can be repaired by nucleotide excision.
- d. The fragment exposed to UV light contains thymine dimers. Thymines lying adjacent to each other can form thymine dimers when exposed to UV light. They can be synthesized by nucleotide excision.

69. Discuss how mutations can increase variation within a population.

- a. Substitution mutations may cause a different amino acid to be placed at a specific location, causing small changes in the protein. Frameshift mutations usually cause multiple amino acid changes, increasing chances that a new protein will form, leading to radically different characteristics in the offspring.
- b. Substitution mutations may cause multiple amino acid changes, increasing chances that a new protein will form, leading to radically different characteristics in the offspring. Frameshift mutations may cause a different amino acid to be placed at a specific location, causing small changes in a protein.
- c. Substitution mutations may cause a different amino acid to be placed at a specific location, resulting in major changes to the protein and leading to radically different characteristics in the offspring. Frameshift mutations cause multiple amino acid differences in a protein, leading to small changes in the protein.
- d. Substitution mutations result in a different amino acid being placed at a specific position in a protein, causing small changes. Silent mutations could result in new characteristics possessed by an offspring when a stop codon is substituted for an amino acid.

SCIENCE PRACTICE CHALLENGE QUESTIONS

70. The proof that DNA, not protein, is the carrier of genetic information involved a number of historical experiments, including transformation or horizontal gene

transfer (HGT), which is the uptake and expression of extracellular DNA.

A. As described in Figure 14.3, transformation or HGT

was first reported by Griffith in 1928 in an experiment in which the following occurred:

1. heat-treated, pathogenic bacteria recovered their pathogenicity when incubated with nonpathogenic bacteria
2. plasmids were transferred to nonpathogenic bacteria from pathogenic bacteria through conjugation
3. nonpathogenic bacteria acquired pathogenicity when incubated in a broth containing heat-treated, pathogenic bacteria
4. polysaccharide cell capsules from pathogenic bacteria were transferred to nonpathogenic bacteria

B. Griffith's experiment, however, left undetermined the identity of the cellular component that encoded genetic information. The identity of DNA as the carrier of genetic information was resolved through the experiments by Martha Chase and Alfred Hershey because they observed the following:

1. injections with a serum containing chemically isolated polysaccharides and nonpathogenic bacteria were not lethal
2. pathogenic bacterial DNA that was radioactively labeled using a phosphorus isotope was not present in mice that died
3. bacteriophages from a bacterial culture grown in a nutrient-containing medium and radioactively labeled using a sulfur isotope transferred the label to bacteria incubated in an unlabeled nutrient-containing medium
4. bacteriophages from a bacterial culture grown in a nutrient-containing medium and radioactively labeled using a sulfur isotope did not transfer the label to bacteria incubated in an unlabeled nutrient-containing medium

C. Transformation and transduction increase variation within populations of bacteria and archaeobacteria by the following:

- a. transferring DNA among different species
- b. transferring free DNA across the cell membrane without energy expenditure
- c. transferring DNA between different strains of the same species of bacteria
- d. phagocytosis of bacteriophages

The evolution of antibiotic resistance via HGT poses a challenge to medical technology. On the other hand, transformation is often assayed by incorporating an antibiotic-resistance gene in the plasmid to be transferred into the host organism. In natural environments, bacterial and archaeobacterial cells become competent (able to transport DNA through the cytoplasmic membrane) in response to stress such as UV radiation, high population

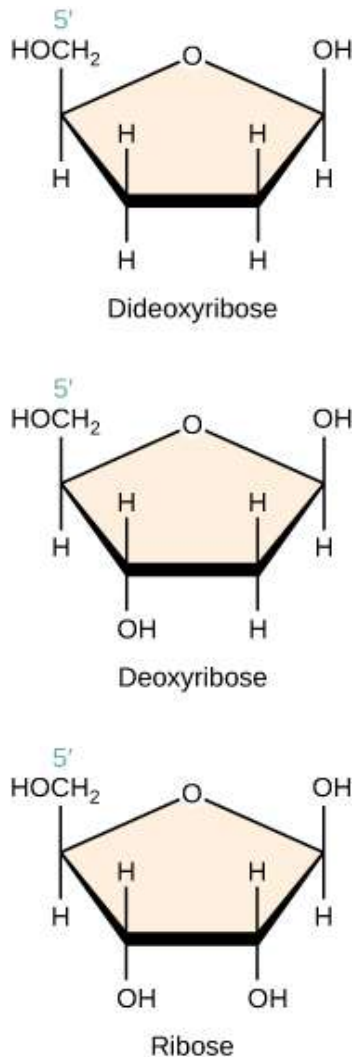
density, or heat shock. Such conditions are often difficult to model in the laboratory, where competence can be induced by high concentrations of divalent cations, Ca^{+2} or Mg^{+2} , or electrical shock. In either setting, extracellular DNA can be transported into the cell, and (to a good approximation) uptake is proportional to the concentration of extracellular DNA.

D. **Identify** a factor that might affect transformation or HGT. Then, **design a plan** to evaluate the dependence of transformational efficiency (defined as the number of transformations per gram of extracellular DNA) of plasmids that transfer antibiotic resistance to a particular strain of *Escherichia coli* that is not resistant on that factor.

71. Prior to the work of Hershey and Chase, scientists thought that inheritance involved “nucleoproteins.” The amount of information to be transmitted between generations did not seem consistent with the chemical simplicity of the few nucleotides found in polymers of deoxyribonucleic acids in comparison to the diversity of protein polymers. Briefly **explain**:

- the relationship between the structure of polymeric DNA and the information stored
- the relationship between the interactions between base pairs on complementary strands of the double helix and Chargaff's observation on the relative abundance of nucleotides in DNA
- the meaning of the statement from the *Nature* publication on the structure of DNA by Watson and Crick: “It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.”

72. In 1977, Fred Sanger developed a method to determine the order of nucleotides in a strand of DNA. Sanger won a Nobel Prize for his work, and his method of sequencing based on dideoxy chain termination (Figure 14.8) has been foundational to the rapid development of more modern, rapid, and cheap methods of sequencing. The challenge of the \$1,000 in one-day sequencing of the human genome was achieved in 2016 by next-generation sequencing (NGS), a “catch-all” term describing several sequencing methods.

**Figure 14.23**

A. Using the diagrams shown above for reference, **explain** the effect of the addition of dideoxynucleotides on chain growth of the DNA strand that is copied during sequencing in terms of the structures of dideoxyribose and deoxyribose.

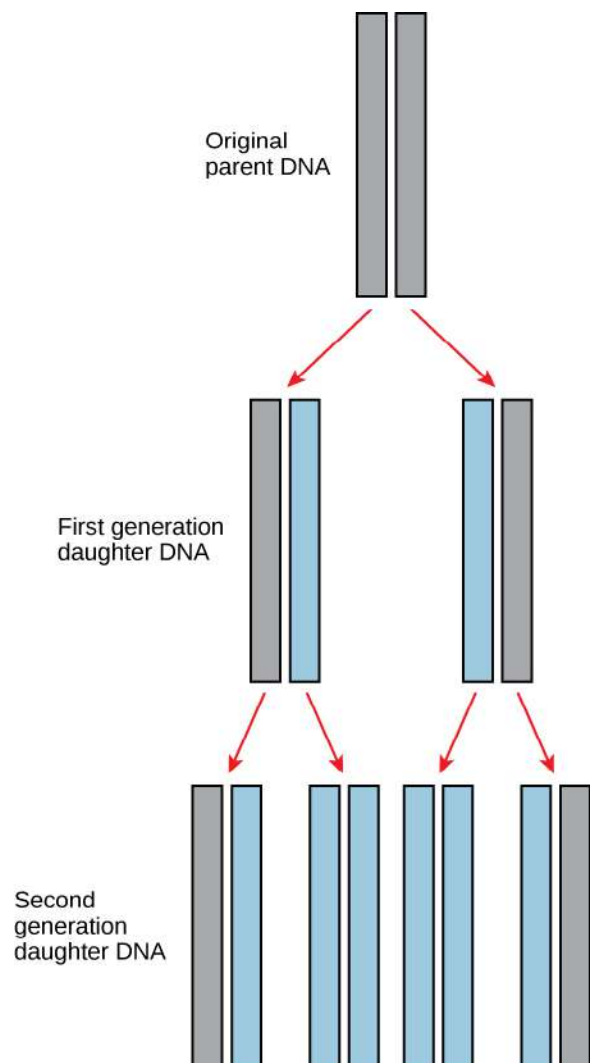
B. Suppose that a single strand to be sequenced is 5'CGAGTACG3'. In the presence of each of the four deoxynucleotides and the dideoxynucleotide ddCTP, **describe** the strands that would be formed from this template. Include in your description an annotation indicating the 3' and 5' ends of the fragments resulting from the procedure.

C. Next-generation sequencing makes termination technology very rapid and relatively inexpensive. All babies born in the U.S. are currently screened by state-mandated tests for several genetic conditions. The number of conditions tested ranges from 29 (GA and KS) to 59 (IL and MS). It is proposed that whole-genome sequencing should be mandatory for all newborns. The Genetic Information Nondiscrimination Act (2008) prevents health

insurers from denying coverage or increasing costs of premiums based on genetic information. It also prohibits employers from making use of these data for hiring, firing, or promotion. The act passed in the House with a vote of 420 to 3, although it was lobbied against by organizations representing business (human resources, health insurance, and manufacturers), including the U.S. Chamber of Commerce. The act does not cover life, long-term care, or disability insurance. **Pose** three **questions** that are relevant to the use of whole-genome data.

73. Our understanding of the mechanisms of DNA replication is important to research on cancer and aging. Additionally, the molecular basis of Mendelian genetics was established.

A. The mechanism of DNA replication was investigated by Meselson and Stahl. The diagram below from their 1958 paper summarizes their findings. **Describe** how this representation illustrates the manner in which DNA is copied for transmission between generations.

**Figure 14.24**

B. During the synthesis of new strands of DNA from the

parent strands, DNA polymerase can only add nucleotides at the terminal 3' of a growing strand. Using the diagram below, **describe** the similarities and differences between the DNA replication of both strands.

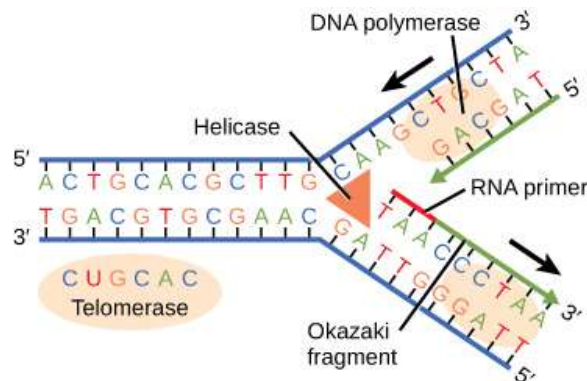


Figure 14.25

C. Shown at the left end of the upper parent strand is the six-base repeat sequence TTAGGG. In humans, this is the repeated, telomeric sequence that is attached to the telomere. The RNA primer in humans spans 10 base pairs, unlike in the drawing where it spans only three. In somatic cells, an enzyme called telomerase no longer functions.

Explain the function of telomerase in the development of stem cells and cancer cells, and the inhibition of telomerase in programmed cell death or apoptosis.

74. The mitochondria of eukaryote cells contain their own circular DNA (mtDNA), consistent with their origin according to the theory of endosymbiosis. The mitochondrial genome is highly conserved in Eukarya. In humans, the 50 to 100 mitochondria in each of the cells in most tissues have 5 to 10 copies of the genome. Each has 37 genes that primarily encode proteins of the electron transport chain. Point mutations in which a single

nucleotide is incorrectly placed is not repaired because the error-checking provided by DNA polymerase is not present in the mitochondria. The mutation rate for mtDNA is approximately 100 times higher than the mutation rate for nuclear DNA. The simultaneous existence of multiple alleles in each cell is likely, a condition called heteroplasmy. In mammals, sperm mitochondria are destroyed prior to fertilization.

A. **Explain** how point mutations in mtDNA can result in a loss of function in critical cellular components such as cytochrome c yet not be lethal to the cell.

B. Oocyte mitochondria are randomly segregated during meiosis, resulting in variation in the frequency of mtDNA mutations in offspring relative to the parent. **Explain** how a loss of function does not accumulate, lowering the metabolic performance from generation to generation.

As described in the Evolution Connection in this chapter of the text, a fossil fingertip found in a Siberian cave revealed an evolutionary link between Neanderthals and Denisovans. Fossils from 28 individuals were located in the “pit of bones,” Sima de los Huesos, in Spain, thousands of miles from the Siberian cave. In 2013, mtDNA from a femur of one of these individuals was compared with mtDNA of Denisovans, Neanderthals, and modern humans. It was found that the Sima fossil shared many more alleles with Denisovans than with either Neanderthals or modern humans. In 2016, the same group of scientists who sequenced the mtDNA from the femur of one of the Sima fossils partially sequenced the DNA from that fossil, showing a clear connection to Neanderthals.

C. **Analyze** these data to draw alternative conclusions regarding the relatedness of the three fossils and support each with evidence.

D. **Design** a plan to differentiate or resolve these alternative conclusions.