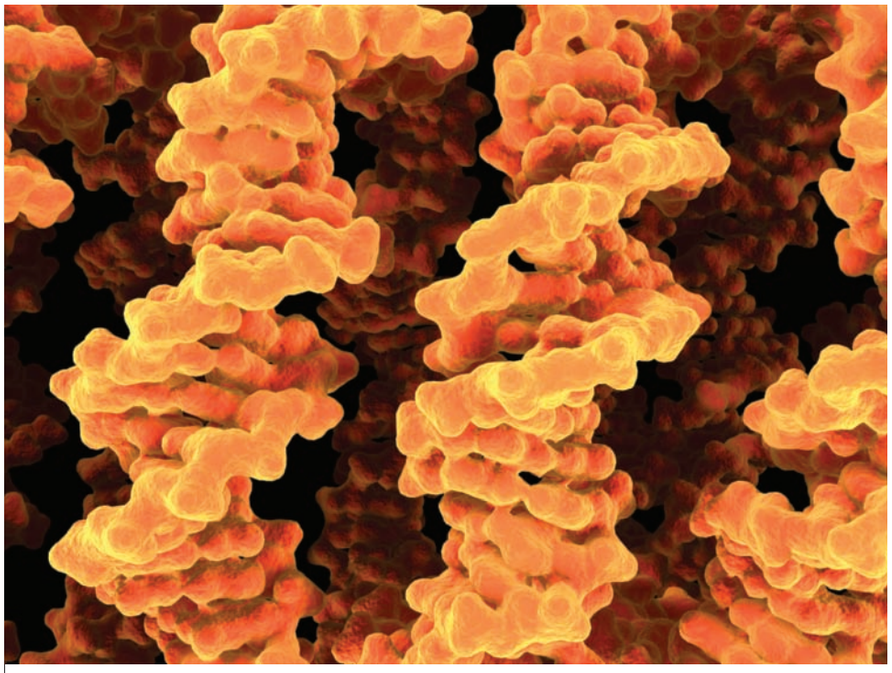


Kenneth Eward/Photo Researchers, Inc.

A digital model of DNA (based on data generated by X-ray crystallography).

Study Plan

14.1 Establishing DNA as the Hereditary Molecule

Experiments began when Griffith found a substance that could genetically transform pneumonia bacteria

Avery and his coworkers identified DNA as the molecule that transforms rough *Streptococcus* to the infective form

Hershey and Chase found the final evidence establishing DNA as the hereditary molecule

14.2 DNA Structure

Watson and Crick brought together information from several sources to work out DNA structure

The new model proposed that two polynucleotide chains wind into a DNA double helix

14.3 DNA Replication

Meselson and Stahl showed that DNA replication is semiconservative

DNA polymerases are the primary enzymes of DNA replication

Helicases unwind DNA to expose template strands for new DNA synthesis

RNA primers provide the starting point for DNA polymerase to begin synthesizing a new DNA chain

One new DNA strand is synthesized continuously; the other, discontinuously

Multiple enzymes coordinate their activities in DNA replication

Telomerases solve a specialized replication problem at the ends of linear DNA molecules

DNA replication begins at replication origins

14.4 Mechanisms That Correct Replication Errors

Proofreading depends on the ability of DNA polymerases to reverse and remove mismatched bases

DNA repair corrects errors that escape proofreading

14.5 DNA Organization in Eukaryotes and Prokaryotes

Histones pack eukaryotic DNA at successive levels of organization

Many nonhistone proteins have key roles in the regulation of gene expression

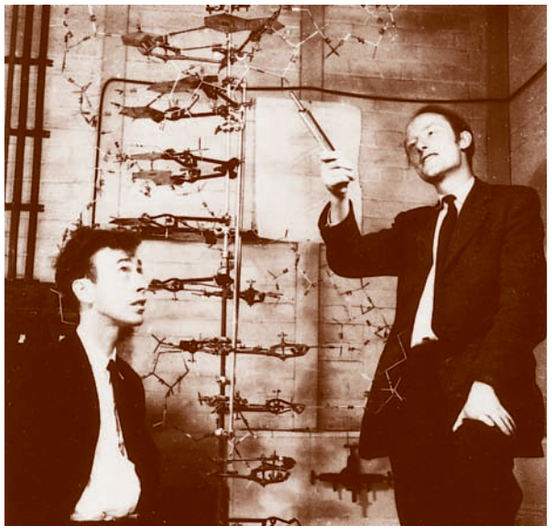
DNA is organized more simply in prokaryotes than in eukaryotes

14 DNA Structure, Replication, and Organization

Why It Matters

One might have wondered, in the spring of 1868, why Johann Friedrich Miescher, a Swiss physician and physiological chemist, was collecting pus cells from discarded bandages. His intentions were purely scientific: Miescher wanted to study the chemical composition of the cell nucleus. He used pus cells because much of the volume of the white blood cells in pus is occupied by the nucleus. From the nuclei of these cells, Miescher extracted large quantities of an acidic substance with a high phosphorus content. He called the unusual substance "nuclein." His discovery is at the root of the development of our molecular understanding of life: nuclein is now known by its modern name, **deoxyribonucleic acid**, or **DNA**, the molecule that is the genetic material of all living organisms.

At the time of Miescher's discovery, scientists knew nothing about the molecular basis of heredity and very little about genetics. Although Mendel had already published the results of his genetic experiments with garden peas, the significance of his findings was not widely known or appreciated. It was not known which chemical substance in cells actually carries the instructions for reproducing parental traits in offspring. Not until 1952, more than 80 years after



A. C. Barrington Brown © 1968 J. D. Watson

Figure 14.1
James D. Watson and Francis H. C. Crick demonstrating their 1953 model for DNA structure, which revolutionized the biological sciences.

Miescher's discovery, did scientists fully recognize that the hereditary molecule was DNA.

After DNA was established as the hereditary molecule, the focus of research changed to the three-dimensional structure of DNA. Among the scientists striving to work out the structure were James D. Watson, a young American postdoctoral student at Cambridge University in England, and the Englishman Francis H. C. Crick, then a graduate student at Cambridge University. Using chemical and physical information about DNA, in particular Rosalind Franklin's analysis of the arrangement of atoms in DNA, the two investigators assembled molecular models from pieces of cardboard and bits of wire. Eventually they constructed a model for DNA that fit all the known data **(Figure 14.1).** Their discovery was of momentous importance in biology. The model enabled scientists to understand key processes in cells for the first time in terms of the structure and interaction of molecules. For example, the model immediately made it possible to understand how genetic information is stored in the structure of DNA and how DNA replicates. Unquestionably, the discovery launched a molecular revolution within biology, making it possible for the first time to relate the genetic traits of living organisms to a universal molecular code present in the DNA of every cell. In addition, Watson and Crick's discovery opened the way for numerous advances in fields such as medicine, forensics, pharmacology, and agriculture, and eventually gave rise to the current rapid growth of the biotechnology industry.

14.1 Establishing DNA as the Hereditary Molecule

In the first half of the twentieth century, many scientists believed that proteins were the most likely candidates for the hereditary molecules because they appeared to offer greater opportunities for information coding than did nucleic acids. That is, proteins contain 20 types of amino acids, whereas nucleic acids have only 4 different nitrogenous bases available for coding. Other scientists believed that nucleic acids were the hereditary molecules. In this section, we describe the experiments showing that DNA, and not protein, is the genetic material.

Experiments Began When Griffith Found a Substance That Could Genetically Transform Pneumonia Bacteria

In 1928, Frederick Griffith, a British medical officer, observed an interesting phenomenon in his experiments with the bacterium *Streptococcus pneumoniae,* which causes a severe form of pneumonia in mammals. Griffith was trying a make a vaccine to prevent pneumonia infections in the epidemics that occurred after World War I. He used two strains of the bacterium in his attempts. The smooth strain—*S*—has a polysaccharide capsule surrounding each cell and forms colonies that appear smooth and glossy when grown on a culture plate. When he injected the *S* strain into mice, it was virulent (highly infective, or pathogenic), causing pneumonia and killing the mice in a day or two (**Figure 14.2,** step 1).The rough strain—*R*—does not have a polysaccharide capsule and forms colonies with a nonshiny, rough appearance. When Griffith injected the *R* strain into mice, it was avirulent (not infective, or nonpathogenic); the mice lived (step 2). Evidently the capsule was responsible for the virulence of the *S* strain.

If Griffith killed the *S* bacteria by heating before injecting them into the mice, the mice remained healthy (step 3). However, quite unexpectedly, Griffith found that if he injected living *R* bacteria along with the heat-killed *S* bacteria, many of the mice died (step 4). Also, he was able to isolate living *S* bacteria with polysaccharide capsules from the infected mice. In some way, living *R* bacteria had acquired the ability to make the polysaccharide capsule from the dead *S* bacteria, and they had changed—transformed—into virulent *S* cells. The transformed bacteria were altered permanently; the smooth, infective trait was stably inherited by the descendants of the transformed bacteria. Griffith called the conversion of *R* bacteria to *S* bacteria *transformation* and called the agent responsible the *transforming principle*. What was the nature of the molecule responsible for the transformation? The most likely candidates were proteins or nucleic acids.

Avery and His Coworkers Identified DNA as the Molecule That Transforms Rough *Streptococcus* to the Infective Form

In the 1940s, Oswald Avery, a physician and medical researcher at the Hospital at Rockefeller Institute for Medical Research, and his coworkers Colin MacLeod

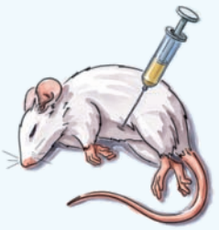
Figure 14.2 Experimental Research

Griffith's Experiment with Infective and Noninfective Strains of Streptococcus pneumoniae

QUESTION: What is the nature of the genetic material?

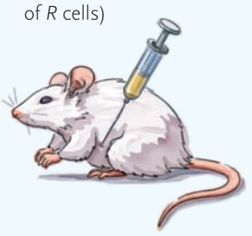
EXPERIMENT: Frederick Griffith studied the conversion of a nonvirulent (noninfective) *R* form of the bacterium *Streptococcus pneumoniae* to a virulent (infective) *S* form. The *S* form has a capsule surrounding the cell, giving colonies of it on a laboratory dish a smooth, shiny appearance. The *R* form has no capsule, so the colonies have a rough, nonshiny appearance. Griffith injected the bacteria into mice and determined how the mice were infected.

1. Mice injected with live, infective *S* cells (control to show effect of *S* cells)



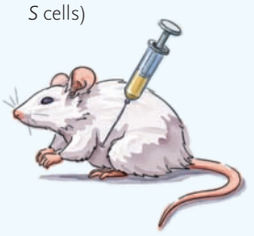
RESULT: Mice die. Live, infective *S* cells in their blood; shows that *S* cells are virulent.

2. Mice injected with live, noninfective *R* cells (control to show effect of *R* cells)



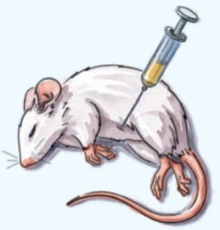
RESULT: Mice live. No live *R* cells in their blood; shows that *R* cells are nonvirulent.

3. Mice injected with heat-killed *S* cells (control to show effect of dead *S* cells)



RESULT: Mice live. No live *S* cells in their blood; shows that live *S* cells are necessary to be virulent to mice.

4. Mice injected with heat-killed *S* cells plus live *R* cells



RESULT: Mice die. Live *S* cells in their blood; shows that living *R* cells can be converted to virulent *S* cells with some factor from dead *S* cells.

CONCLUSION: Griffith concluded that some molecules released when *S* cells were killed could change living *R* cells genetically to the virulent *S* form. He called the molecule the *transforming principle* and the process of genetic change *transformation*.

and Maclyn McCarty performed an experiment designed to identify the chemical nature of the transforming principle that can change *R Streptococcus* bacteria into the *S* infective form. Rather than working with mice, they attempted to reproduce the transformation using bacteria growing in culture tubes. They used heat to kill virulent *S* bacteria and then treated the macromolecules extracted from the cells in turn with enzymes that break down each of the three main candidate molecules for the hereditary material—protein; DNA; or the other nucleic acid, RNA. When they destroyed proteins or RNA, the researchers saw no effect; the extract of *S* bacteria still transformed *R* bacteria into virulent *S* bacteria—the cells had polysaccharide capsules and produced smooth colonies on culture plates. When they destroyed DNA, however, no transformation occurred—no smooth colonies were seen on culture plates.

In 1944, Avery and his colleagues published their discovery that the transforming principle was DNA. At the time, many scientists firmly believed that the genetic material was protein. So, although their findings were clearly revolutionary, Avery and his colleagues presented their conclusions in the paper cautiously, offering several interpretations of their results. Some scientists accepted their results almost immediately. However, those who believed that the genetic material was protein argued that it was possible not all protein was destroyed by their enzyme treatments and, as contaminants in their DNA transformation reaction, these remaining proteins were in fact responsible for the transformation. Further experiments were needed to convince all scientists that DNA is the hereditary molecule.

Hershey and Chase Found the Final Evidence Establishing DNA as the Hereditary Molecule

A final series of experiments conducted in 1952 by bacteriologist Alfred D. Hershey and his laboratory assistant Martha Chase at the Cold Spring Harbor Laboratory removed any remaining doubts that DNA is the hereditary molecule. Hershey and Chase studied the infection of the bacterium *Escherichia coli* by bacteriophage T2. *E. coli* is a bacterium normally found in the intestines of mammals. **Bacteriophages** (or simply **phages**; see Chapter 17) are viruses that infect bacte-

Figure 14.3 Experimental Research

The Hershey and Chase Experiment Demonstrating That DNA Is the Hereditary Molecule

QUESTION: Is DNA or protein the genetic material?

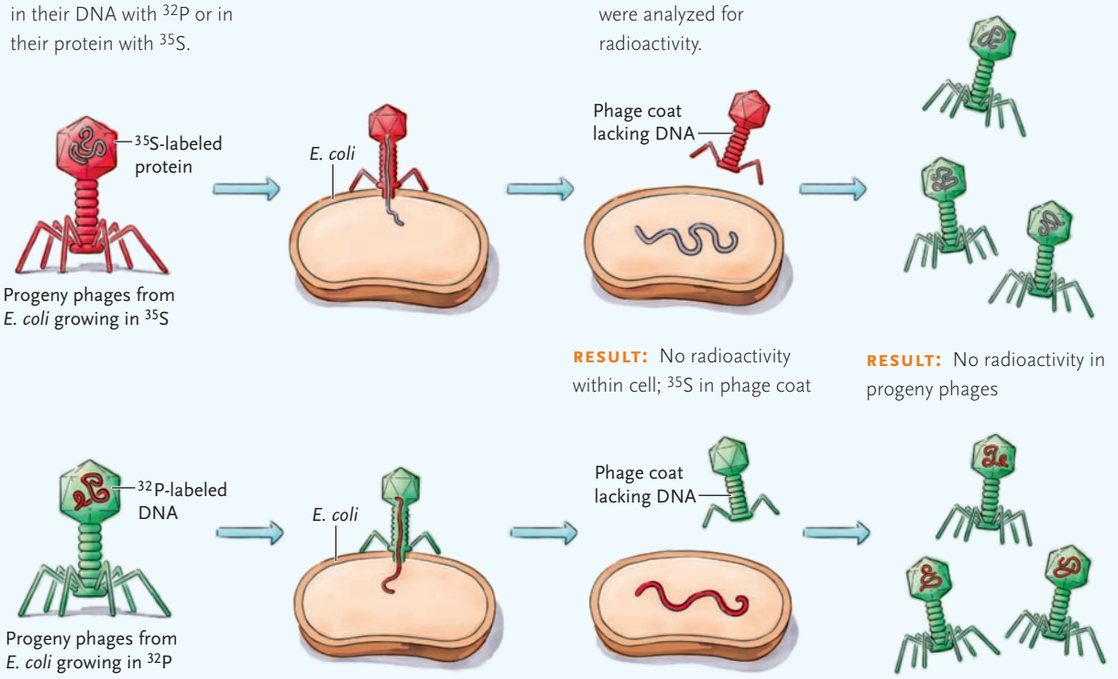
EXPERIMENT: Hershey and Chase performed a definitive experiment to show whether DNA or protein is the genetic material. They used phage T2 for their experiment; it consists only of DNA and protein.

1. They infected *E. coli* growing in the presence of radioactive ^{32}P or ^{35}S with phage T2. The progeny phages were either labeled in their DNA with ^{32}P or in their protein with ^{35}S.

2. Fresh *E. coli* cells were infected with the radioactively labeled phages.

3. After infecting the bacteria, the cells were mixed in a blender to remove the phage coats from the cell surface. The components were analyzed for radioactivity.

4. Progeny phages analyzed for radioactivity.



RESULT: No radioactivity within cell; ^{35}S in phage coat

RESULT: No radioactivity in progeny phages

RESULT: ^{32}P within cell; not in phage coat

RESULT: ^{32}P in progeny phages

CONCLUSION: ^{32}P, the radioisotope used to label DNA, was found within phage-infected cells and in progeny phages, indicating that DNA is the genetic material. ^{35}S, the radioisotope used to label proteins, was found in phage coats after infection, but was not found in the infected cell or in progeny phages, showing that protein is not the genetic material.

ria. A **virus** is an infectious agent that contains either DNA or RNA surrounded by a protein coat. Viruses cannot reproduce except in a host cell. When a virus infects a cell, it can use the cell's resources to produce more virus particles.

The phage life cycle begins when a phage attaches to the surface of a bacterium. For phages such as T2, the infected cell quickly stops producing its own molecules and instead starts making progeny phages. After about 100 to 200 phages are assembled inside the bacterial cell, a viral enzyme breaks down the cell wall, killing the cell and releasing the new phages. The whole life cycle takes approximately 90 minutes.

The T2 phage that Hershey and Chase studied consists of only a core of DNA surrounded by proteins. Therefore, one of these molecules must be the genetic material that enters the bacterial cell and directs the infective cycle within. But which one? Hershey and Chase prepared two batches of phages, one with the protein tagged with a radioactive label and the other with the DNA tagged with a radioactive label. To obtain labeled phages, they added T2 to *E. coli* growing in the presence

of either the radioactive isotope of sulfur (^{35}S) or the radioactive isotope of phosphorus (^{32}P) (**Figure 14.3,** step 1). The progeny phages produced in the ^{35}S medium had labeled proteins and unlabeled DNA because sulfur is a component of proteins but not of DNA. The phages produced in the ^{32}P medium had labeled DNA and unlabeled proteins because phosphorus is a component of DNA but not of proteins.

Hershey and Chase then infected separate cultures of *E. coli* with the two types of labeled phages (step 2). After a short period to allow the genetic material to enter the bacterial cell, they mixed the bacteria in a kitchen blender. They reasoned that only the genetic material was injected into the bacterial cell, leaving the rest of the phage outside. By mixing the cells in a blender, they could shear off the phage parts that did not enter the bacteria and collect them separately for analysis.

When they infected the bacteria with phages that contained labeled protein coats, they found no radioactivity in the bacterial cells but could easily measure it in the material removed by the blender (step 3, top). They also found no radioactivity in the progeny phages (step 4, top). However, if the infecting phages contained radioactive DNA, they found radioactivity inside the infected bacteria but none in the phage coats removed by the blender (step 3, bottom). In addition, radioactivity *was* seen in the progeny phages (step 4, bottom). The results were unequivocal: the genetic material of the phage was DNA, not protein.

When taken together, the experiments of Griffith, Avery and his coworkers, and Hershey and Chase established that DNA, not proteins, carries genetic information. Their research also established the term *transformation,* which is still used in molecular biology. **Transformation** is the conversion of a cell's hereditary type by the uptake of DNA released by the breakdown of another cell, as in the Griffith and Avery experiments. Having identified DNA as the hereditary molecule, scientists turned next to determine its structure.

Study Break

Imagine that ^{35}S labeled *both* protein and DNA, whereas ^{32}P labeled only DNA. How would Hershey and Chase's results have been different?

14.2 DNA Structure

The experiments that established DNA as the hereditary molecule were followed by a highly competitive scientific race to discover the structure of DNA. The race ended in 1953, when Watson and Crick elucidated the structure of DNA, ushering in a new era of molecular biology.

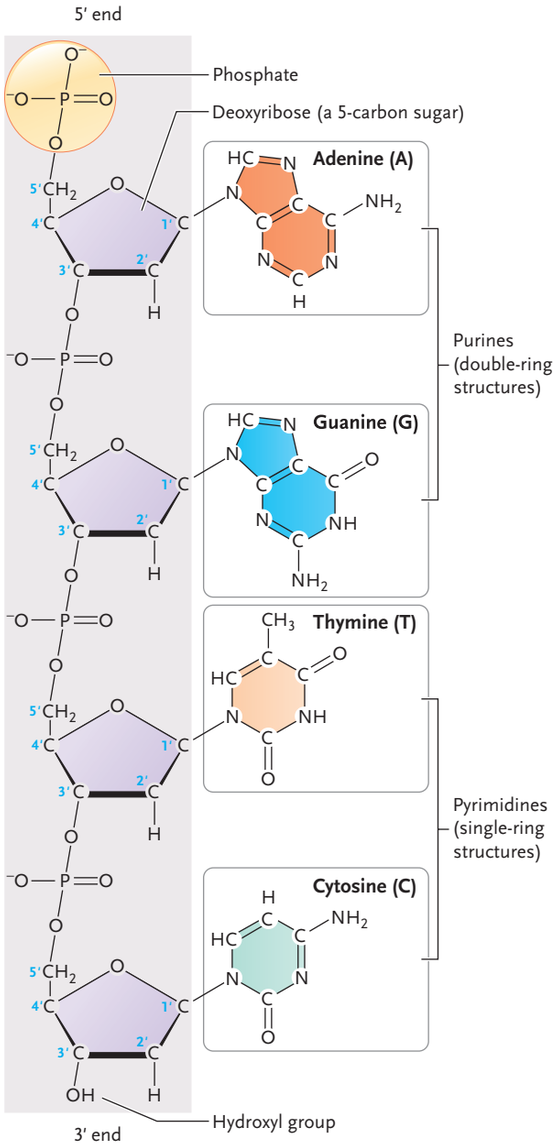


Figure 14.4
The four nucleotide subunits of DNA, linked into a polynucleotide chain. The sugar–phosphate backbone of the chain is highlighted in gray. The connection between adjacent deoxyribose sugars is a phosphodiester bond. The polynucleotide chain has polarity; at one end, the 5′ end, a phosphate group is bound to the 5′ carbon of a deoxyribose sugar, whereas at the other end, the 3′ end, a hydroxyl group is bound to the 3′ carbon of a deoxyribose sugar.

Watson and Crick Brought Together Information from Several Sources to Work Out DNA Structure

Before Watson and Crick began their research, other investigators had established that DNA contains four different nucleotides. Each nucleotide consists of the five-carbon sugar *deoxyribose* (carbon atoms on deoxyribose are numbered with primes from 1′ to 5′), a phosphate group, and one of the four nitrogenous bases—adenine (A), guanine (G), thymine (T), or cytosine (C) **(Figure 14.4).** Two of the bases, **adenine** and

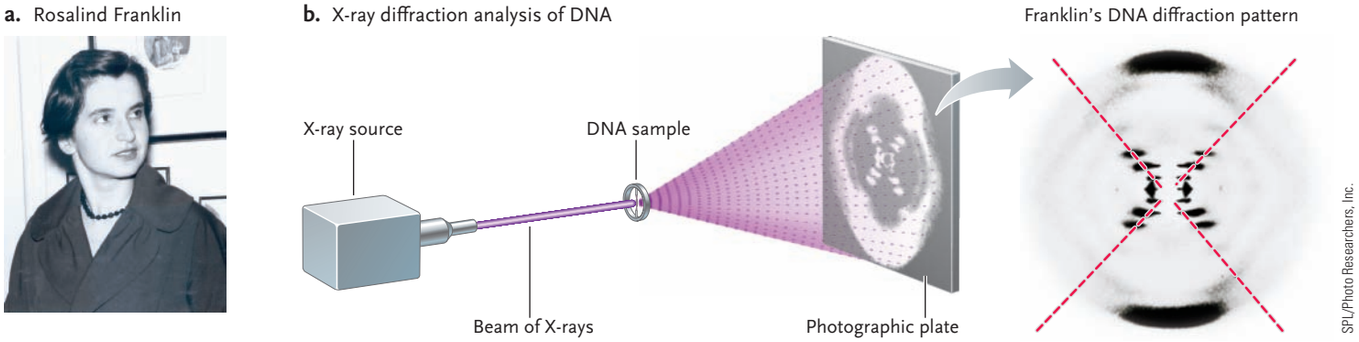


Figure 14.5
X-ray diffraction analysis of DNA. **(a)** Rosalind Franklin. **(b)** The X-ray diffraction method to study DNA and the diffraction pattern Rosalind Franklin obtained. The X-shaped pattern of spots (dashed lines) was correctly interpreted by Franklin to indicate that DNA has a helical structure similar to a spiral staircase.

guanine, are *purines,* nitrogenous bases built from a pair of fused rings of carbon and nitrogen atoms. The other two bases, **thymine** and **cytosine**, are *pyrimidines,* built from a single carbon ring. An organic chemist, Erwin Chargaff, measured the amounts of nitrogenous bases in DNA and discovered that they occur in definite ratios. He observed that the amount of purines equals the amount of pyrimidines, but more specifically, the amount of adenine equals the amount of thymine, and the amount of guanine equals the amount of cytosine; these relationships are known as *Chargaff's rules*.

Researchers had also determined that DNA contains nucleotides joined to form a *polynucleotide chain*. In a polynucleotide chain, the deoxyribose sugars are linked by phosphate groups in an alternating sugar–phosphate–sugar–phosphate pattern, forming a **sugar–phosphate backbone** (highlighted in gray in Figure 14.4). Each phosphate group is a "bridge" between the 3′ carbon of one sugar and the 5′ carbon of the next sugar; the entire linkage, including the bridging phosphate group, is called a *phosphodiester bond*.

The polynucleotide chain of DNA has polarity—directionality. That is, the two ends of the chain are not the same: at one end, a phosphate group is bound to the 5′ carbon of a deoxyribose sugar, whereas at the other end, a hydroxyl group is bonded to the 3′ carbon of a deoxyribose sugar (see Figure 14.4). Consequently, the two ends are called the **5′ end** and **3′ end**, respectively.

Those were the known facts when Watson and Crick began their collaboration in the early 1950s. However, the number of polynucleotide chains in a DNA molecule and the manner in which they fold or twist in DNA were unknown. Watson and Crick themselves did not conduct experiments to study the structure of DNA; instead, they used the research data of others for their analysis, relying heavily on data gathered by physicist Maurice H. F. Wilkins and research associate Rosalind Franklin **(Figure 14.5a),** at King's College, London. These researchers were using X-ray diffraction to study the structure of DNA **(Figure 14.5b).** In **X-ray diffraction**, an X-ray beam is directed at a molecule in the form of a regular solid, ideally in the form of a crystal. Within the crystal, regularly arranged rows and banks of atoms bend and reflect the X-rays into smaller beams that exit the crystal at definite angles determined by the arrangement of atoms in the crystal. If a photographic film is placed behind the crystal, the exiting beams produce a pattern of exposed spots. From that pattern, researchers can deduce the positions of the atoms in the crystal.

Wilkins and Franklin did not have DNA crystals with which to work, but they were able to obtain X-ray diffraction patterns from DNA molecules that had been pulled out into a fiber (see Figure 14.5). The patterns indicated that the DNA molecules within the fiber were cylindrical and about 2 nm in diameter. Separations between the spots showed that major patterns of atoms repeat at intervals of 0.34 and 3.4 nm within the DNA. Franklin interpreted an X-shaped distribution of spots in the diffraction pattern (see dashed lines in Figure 14.5) to mean that DNA has a helical structure.

The New Model Proposed That Two Polynucleotide Chains Wind into a DNA Double Helix

Watson and Crick constructed scale models of the four DNA nucleotides and fitted them together in different ways until they arrived at an arrangement that satisfied both Wilkins' and Franklin's X-ray data and Chargaff's chemical analysis. Watson and Crick's trials led them to a double-stranded model for DNA structure in which two polynucleotide

chains twist around each other in a right-handed way, like a double-spiral staircase **(Figure 14.6).** They were the first to propose the famous double-helix model for DNA.

In the **double-helix model** the two sugar–phosphate backbones are separated from each other by a regular distance. The bases extend into and fill this central space. A purine and a pyrimidine, if paired together, are exactly wide enough to fill the space between the backbone chains in the double helix. However, a purine–purine base pair is too wide to fit the space exactly, and a pyrimidine–pyrimidine pair is too narrow. From Chargaff's data, Watson and Crick proposed that the purine–pyrimidine base pairs in DNA are A-T and G-C pairs. That is, wherever an A occurs in one strand, a T must be opposite it in the other strand; wherever a G occurs in one strand, a C must be opposite it. This feature of DNA is called **complementary base pairing,** and one strand is said to be *complementary* to the other. The base pairs, which fit together like pieces of a jigsaw puzzle, are stabilized by hydrogen bonds—two between A and T and three between G and C (see Figures 14.6 and 3.30; hydrogen bonds are discussed in Section 2.3). The hydrogen bonds between the paired bases, repeated along the double helix, hold the two strands together in the helix.

The base pairs lie in flat planes almost perpendicular to the long axis of the DNA molecule. In this state, each base pair occupies a length of 0.34 nm along the long axis of the double helix (see Figure 14.6). This spacing accounts for the repeating 0.34-nm pattern noted in the X-ray diffraction patterns. The larger 3.4-nm repeat pattern was interpreted to mean that each full turn of the double helix takes up 3.4 nm along the length of the molecule and therefore 10 base pairs are packed into a full turn.

Watson and Crick also realized that the two strands of a double helix fit together in a stable chemical way only if they are **antiparallel,** that is, only if they run in opposite directions (see Figure 14.6, arrows). In other words, the *3′ end* of one strand is opposite the *5′ end* of the other strand. This antiparallel arrangement is highly significant for the process of replication, which is discussed in the next section.

As hereditary material, DNA must faithfully store and transmit genetic information for the entire life cycle of an organism. Watson and Crick recognized that this information is coded into the DNA by the particular sequence of the four nucleotides. Although only four different kinds of nucleotides exist, combining them in groups allows an essentially infinite number of different sequences to be "written," just as the 26 letters of the alphabet can be combined in groups to write a virtually unlimited number of words. Chapter 15 shows how taking the four nucleotides in groups of three forms enough words to spell out the structure of any conceivable protein.

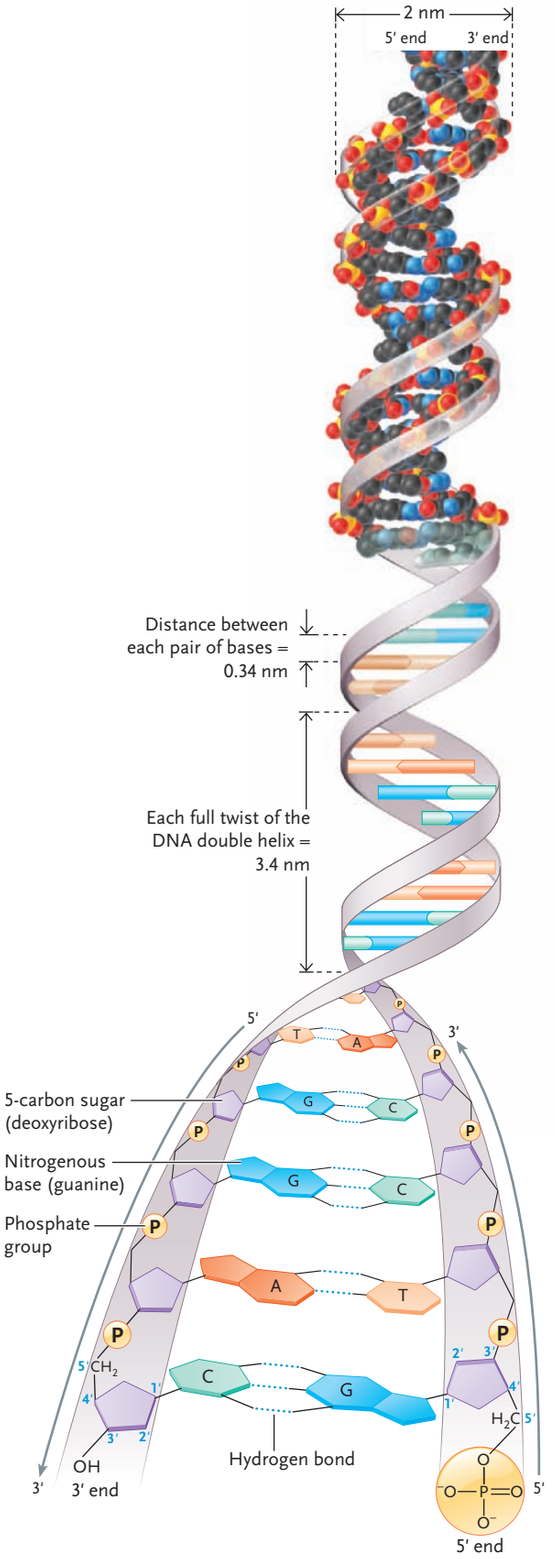


Figure 14.6
DNA double helix. Arrows and labeling of the ends show that the two polynucleotide chains of the double helix are antiparallel—that is, they have opposite polarity in that they run in opposite directions. In the space-filling model at the top, the spaces occupied by atoms are indicated by spheres. There are 10 base pairs per turn of the helix; only 8 base pairs are visible because the other 2 are obscured where the backbones pass over each other.

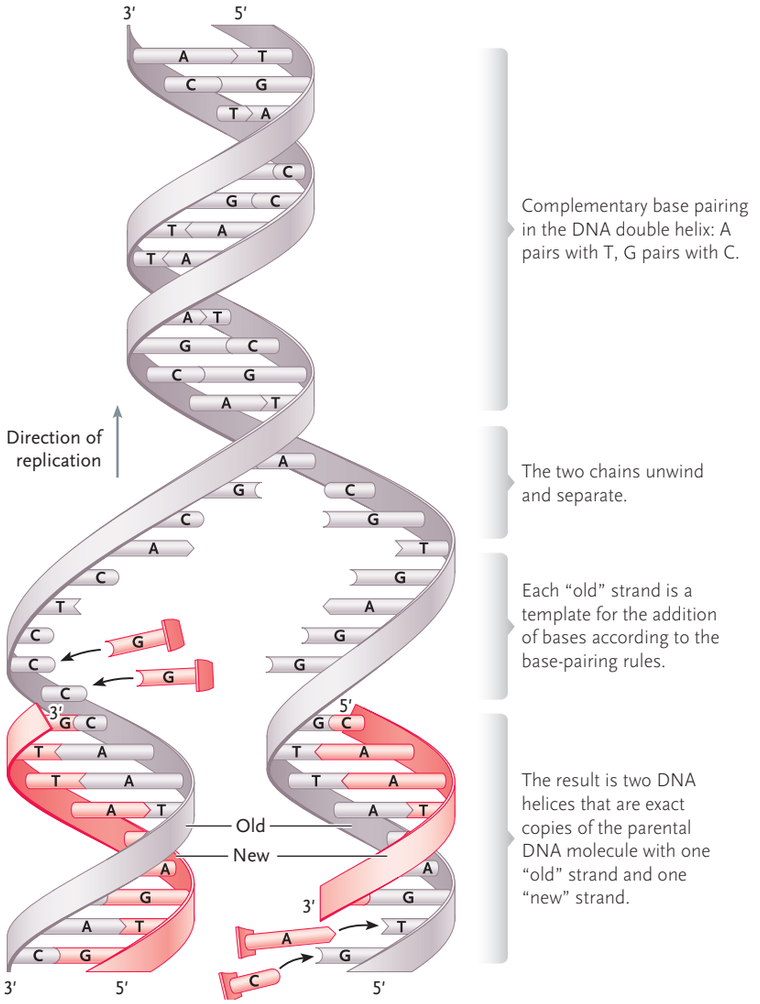


Figure 14.7
Watson and Crick's model for DNA replication. The original DNA molecule is shown in gray. A new polynucleotide chain (red) is assembled on each original chain as the two chains unwind. The template and complementary copy chains remain wound together when replication is complete, producing molecules that are half old and half new. The model is known as the semiconservative model for DNA replication.

Watson and Crick announced their model for DNA structure in a brief but monumental paper published in the journal *Nature* in 1953. Watson and Crick shared a Nobel Prize with Wilkins in 1962 for their discovery of the molecular structure of DNA. Rosalind Franklin might have been a candidate for a Nobel Prize had she not died of cancer at age 38 in 1958. (The Nobel Prize is given only to living investigators.) Unquestionably, Watson and Crick's discovery of DNA structure opened the way to molecular studies of genetics and heredity, leading to our modern understanding of gene structure and action at the molecular level.

Study Break

1. Which bases in DNA are purines? Which are pyrimidines?
2. What bonds form between complementary base pairs? Between a base and the deoxyribose sugar?
3. Which features of the DNA molecule did Watson and Crick describe?
4. The percentage of A in a double-stranded DNA molecule is 20. What is the percentage of C in that DNA molecule?

14.3 DNA Replication

Once they had discovered the structure of DNA, Watson and Crick realized immediately that complementary base pairing between the two strands could explain how DNA replicates **(Figure 14.7).** They imagined that, for replication, the hydrogen bonds between the two strands break, and the two strands unwind and separate. Each strand then acts as a template for the synthesis of its partner strand. When replication is complete, there are two double helices, each of which has one strand derived from the parental DNA molecule base paired with a newly synthesized strand. Most important, each of the two new double helices has the identical base-pair sequence as the parental DNA molecule.

The model of replication Watson and Crick proposed is termed **semiconservative replication (Figure 14.8a).** Other scientists proposed two other models for replication. In the *conservative replication model,* the two strands of the original molecule serve as templates for the two strands of a new DNA molecule, then rewind into an all "old" molecule **(Figure 14.8b).** After the two complementary copies separate from their templates, they wind together into an all "new" molecule. In the *dispersive replication model,* neither parental strand is conserved and both chains of each replicated molecule contain old and new segments **(Figure 14.8c).**

Meselson and Stahl Showed That DNA Replication Is Semiconservative

A definitive experiment published in 1958 by Matthew Meselson and Franklin Stahl of the California Institute of Technology demonstrated that DNA replication is semiconservative **(Figure 14.9).** In their experiment, Meselson and Stahl had to be able to distinguish parental DNA strands from newly synthesized DNA. To do this they used a nonradioactive "heavy" nitrogen isotope to tag the parental DNA strands. The heavy isotope, ^{15}N, has one more neutron in its nucleus than the

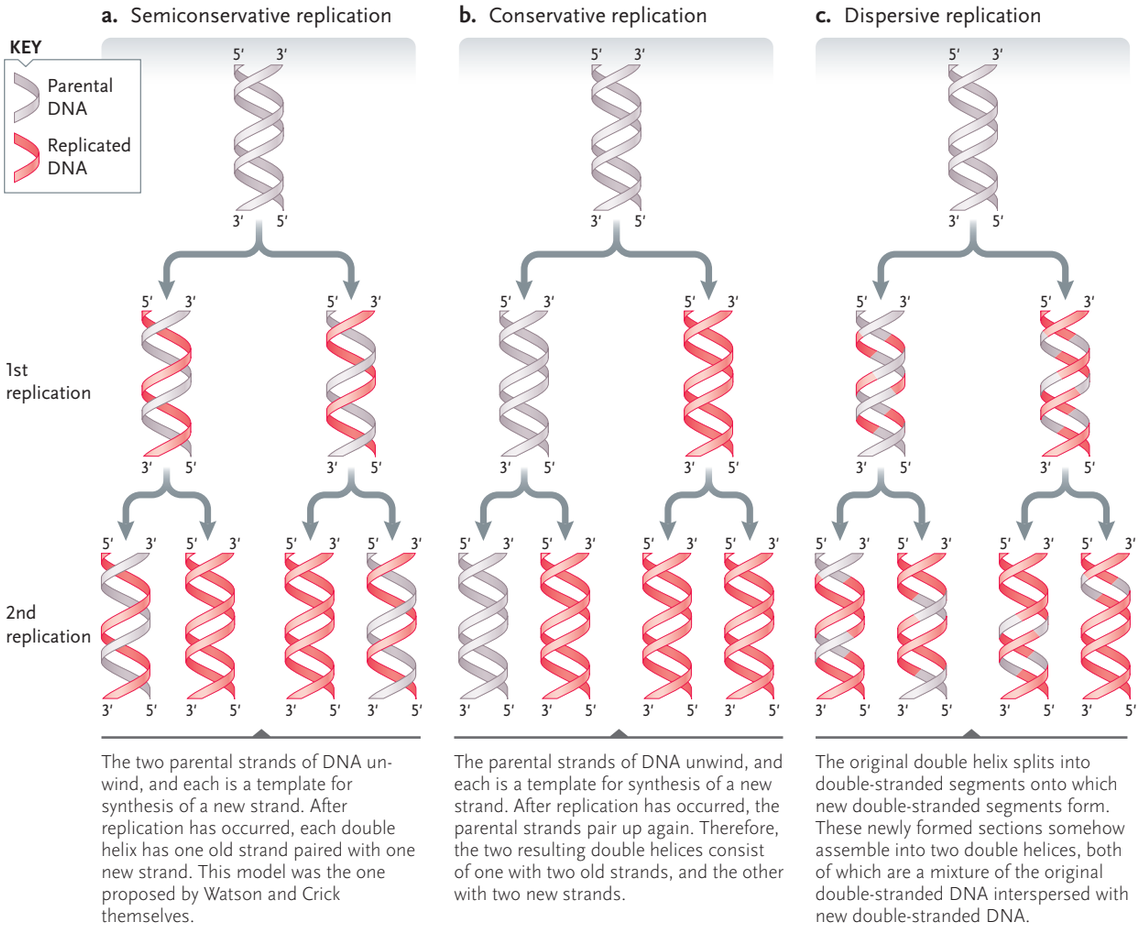


Figure 14.8
Semiconservative **(a),** conservative **(b),** and dispersive **(c)** models for DNA replication.

normal ^{14}N isotope. Molecules containing ^{15}N are measurably heavier (denser) than molecules of the same type containing ^{14}N.

As the first step in their experiment, Meselson and Stahl grew the bacterium *E. coli* in a culture medium containing the heavy ^{15}N isotope (Figure 14.9, step 1). The heavy isotope incorporated into the nitrogenous bases of DNA, resulting in all the DNA being labeled with ^{15}N. Then they transferred the bacteria to a culture medium containing the light ^{14}N isotope (step 2). All new DNA synthesized after the transfer contained the light isotope. Just before the transfer to the medium with the ^{14}N isotope, and after each round of replication following the transfer, they took a sample of the cells and extracted the DNA (step 3).

Meselson and Stahl then mixed the DNA samples with cesium chloride (CsCl) and centrifuged the mixture at very high speed (step 4). During the centrifugation, the CsCl forms a density gradient and DNA molecules move to a position in the gradient where their density matches that of the CsCl. Therefore, DNA of different densities is separated into bands, with the densest DNA settling closer to the bottom of the tube. Figure 14.9 "Result" shows the outcome of these experiments, and "Conclusions" shows why the results were compatible with only the semiconservative replication model.

DNA Polymerases Are the Primary Enzymes of DNA Replication

During replication, complementary nucleotide chains are assembled from individual nucleotides by enzymes known as **DNA polymerases.** More than one kind of DNA polymerase is required for DNA replication in both eukaryotes and prokaryotes. *Nucleoside triphosphates* are substrates for the polymerization reaction catalyzed by DNA polymerases **(Figure 14.10).** A nucleoside triphosphate is a nitrogenous base linked to a sugar, which is linked, in turn, to a chain of three phosphate groups. You have encountered a nucleoside triphosphate before, namely the ATP produced in cellular respiration (see Chapter 8). The nucleoside triphosphates used in DNA replication differ from ATP by having the sugar deoxyribose rather than the sugar ribose. Because four different bases are found in DNA—adenine (A), guanine (G), cytosine (C), and

Figure 14.9 Experimental Research

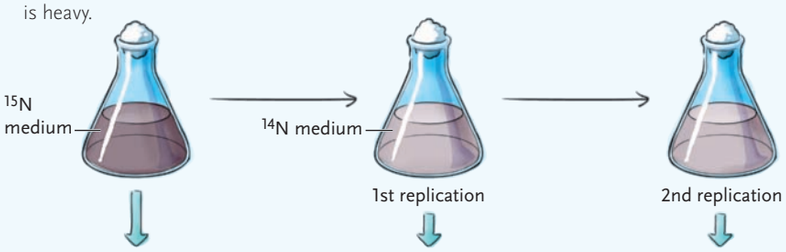
The Meselson and Stahl Experiment Demonstrating the Semiconservative Model to Be Correct

QUESTION: Does DNA replicate semiconservatively?

EXPERIMENT: Matthew Meselson and Franklin Stahl proved that the semiconservative model of DNA replication is correct and that the conservative and dispersive models are incorrect.

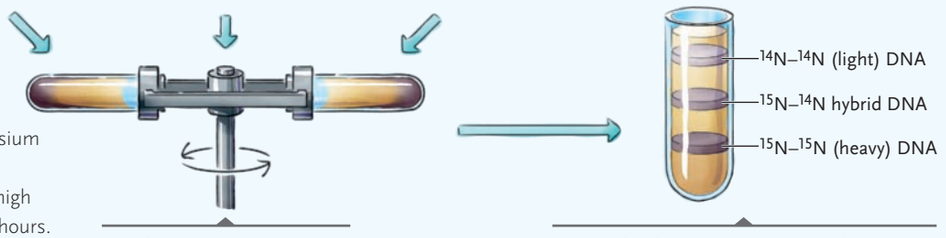
1. Bacteria grown in ^{15}N (heavy) medium. All DNA is heavy.

2. Bacteria transferred to ^{14}N (light) medium and allowed to grow and divide for several generations. All new DNA is light.



3. DNA extracted from bacteria cultured in ^{15}N medium and after each generation in ^{14}N medium.

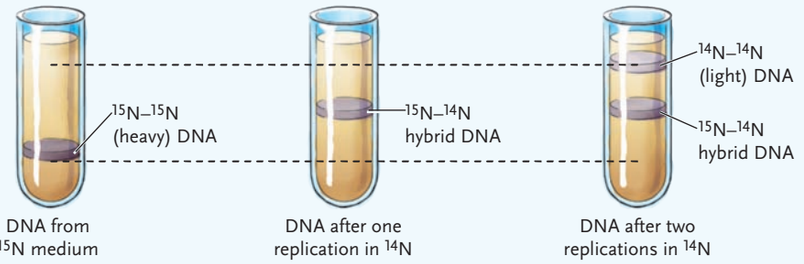
4. DNA mixed with cesium chloride (CsCl) and centrifuged at very high speed for about 48 hours.



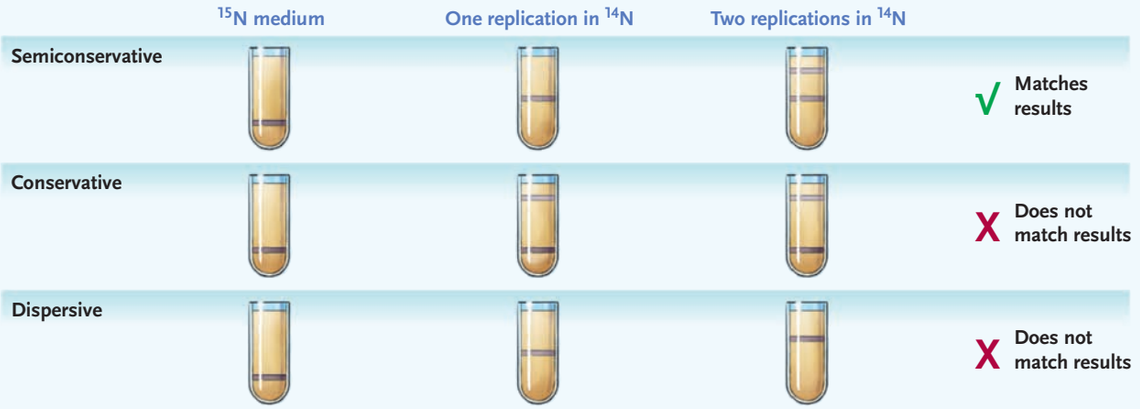
CsCl forms a density gradient during centrifugation, with the highest density at the bottom of the tube.

DNA molecules move to positions where their density equals that of the CsCl solution and form bands. Shown are the positions of differently labeled DNA molecules. Experimentally the bonds are detected by absorbance of UV light.

RESULT: Meselson and Stahl obtained the following results:



CONCLUSIONS: The predicted DNA banding patterns for the three DNA replication models were:



The results support the semiconservative model.

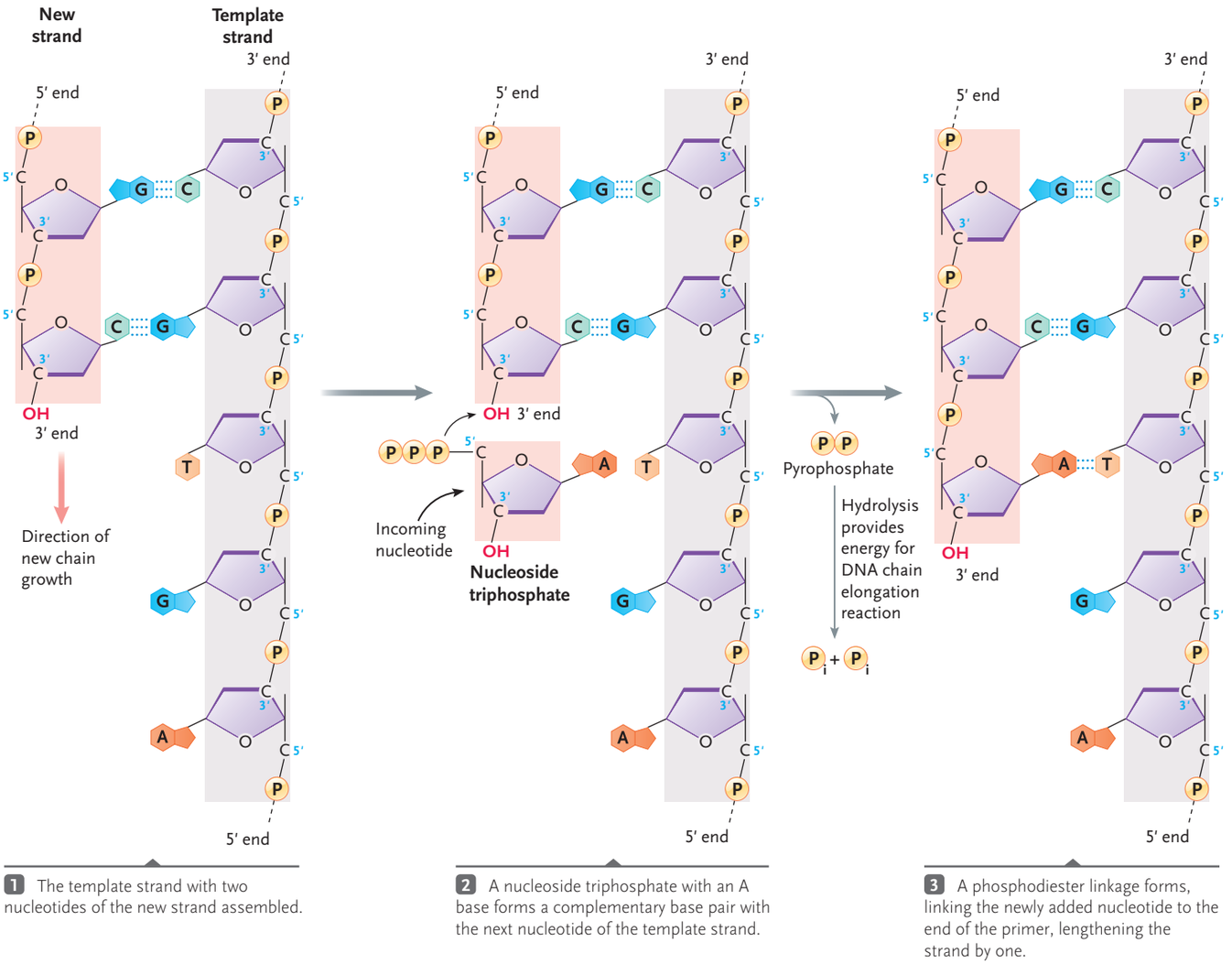


Figure 14.10 Reactions assembling a complementary chain in the 5′→3′ direction on a template DNA strand, showing the phosphodiester linkage created when the DNA polymerase enzyme adds each nucleotide to the chain.

thymine (T)—four different nucleoside triphosphates are used for DNA replication. By analogy with the ATP naming, the nucleoside triphosphates for DNA replication are given the short names dATP, dGTP, dCTP, and dTTP, where the "d" stands for "deoxyribose."

Figure 14.10 presents a section of a DNA polynucleotide chain being replicated to show how DNA polymerase catalyzes the assembly of a new DNA strand that is complementary to the template strand. To understand Figure 14.10, remember that the carbons in the deoxyriboses of nucleotides are numbered with primes. Each DNA strand has two distinct ends: the 5′ end has an exposed phosphate group attached to the 5′ carbon of the sugar, and the 3′ end has an exposed hydroxyl group attached to the 3′ carbon of the sugar. As we learned earlier, because of the antiparallel nature of the DNA double helix, the 5′ end of one strand is opposite the 3′ end of the other.

Part of a template strand with two nucleotides of a new strand hydrogen bonded to it by complementary base pairing is shown in step 1 of Figure 14.10. One of the characteristics of DNA polymerase is that it can add a nucleotide *only to the 3′ end of an existing nucleotide chain*. The next template nucleotide has a T base. This means the DNA polymerase will bind a nucleoside triphosphate with an A base (dATP) from the surrounding solution (step 2). The enzyme then catalyzes the formation of the phosphodiester bond involving the 3′−OH group at the end of the existing chain and the innermost of the three phosphate groups of the dATP, releasing the other two phosphates as a pyrophosphate molecule (step 3). Hydrolysis of the bond between the two phosphates provides the energy for the formation of the new bond.

The DNA polymerase then moves to the next base on the DNA template, shown as guanine in step 3, binds a dCTP, and, using the reaction just described, catalyzes the formation of a phosphodiester bond, inserting the C nucleotide to the growing new strand. The process then continues, adding complementary nucleotides one by one to the growing DNA strand.

As a new DNA strand is assembled, a 3′−OH group is always exposed at its "newest" end; the "old-

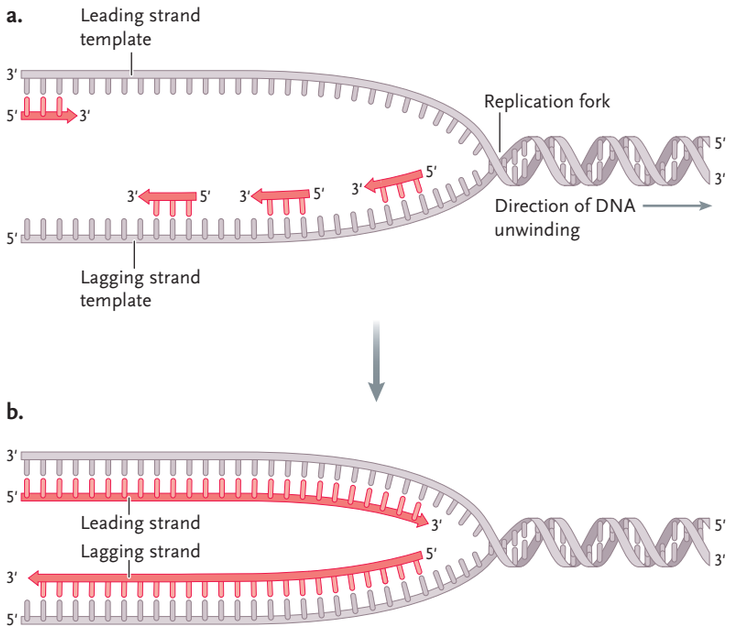


Figure 14.11
How antiparallel template strands are replicated at a fork. The template strand presented to DNA polymerase in the "wrong" 5′→3′ direction—the strand on the bottom in **(a)**—is copied in short lengths that run opposite to the direction of fork movement. The short lengths are then linked into a continuous chain **(b).** The overall effect is synthesis of both strands in the direction of fork movement.

est" end of the new chain has an exposed 5′ triphosphate. DNA polymerases are therefore said to assemble nucleotide chains in the 5′→3′ direction. Because of the antiparallel nature of DNA, the template strand is "read" in the 3′→5′ direction for this new synthesis.

The key molecular events of DNA replication described in this section are:

1. The two strands of the DNA molecule unwind for replication to occur.
2. Nucleotides are added only to an existing chain.
3. The overall direction of new synthesis is in the 5′→3′ direction, which is a direction antiparallel to that of the template strand.
4. Nucleotides enter into a newly synthesized chain according to the A-T and G-C complementary base-pairing rules.

The following sections describe how enzymes and other proteins conduct these molecular events.

Helicases Unwind DNA to Expose Template Strands for New DNA Synthesis

For replication to be semiconservative, the two strands of the parental DNA molecule must unwind and separate to expose template strands for new DNA synthesis during the replication process. The unwinding produces a Y-shaped structure called a **replication fork**, which consists of the two unwound template strands transitioning to double-helical DNA. An enzyme, **DNA helicase**, catalyzes the unwinding, which exposes both strands for the next steps in replication. The helicase uses the energy of ATP hydrolysis to unwind the DNA helix. The exposed single-stranded segments of DNA become coated with **single-stranded binding proteins**, which stabilize the DNA for the replication process. These proteins are displaced as the replication enzymes make the new polynucleotide chain on the template strands.

Let us consider a possible consequence of the unwinding of DNA by helicases. If the DNA is circular, as is the case for the genomes of most bacteria, unwinding the DNA will eventually cause the still-wound DNA ahead of the unwinding to become knotted. You can visualize this by making a small circular double helix with a pair of shoelaces. Now pick a place and pull apart the laces. You will see that the more you pull, the more the laces become overtwisted and strained on the other side of the circle. In the cell, the overtwisting and strain of DNA ahead of the replication fork during replication is avoided by the action of enzymes known as **topoisomerases**, which remove the overtwisting as it forms.

RNA Primers Provide the Starting Point for DNA Polymerase to Begin Synthesizing a New DNA Chain

DNA polymerases can add nucleotides only to the 3′ end of an existing strand. How, then, can a new strand begin, since there is no existing strand in place? The answer lies in a short nucleotide chain called a **primer**, made of RNA instead of DNA. The primer, assembled by the enzyme **primase**, is laid down as the first series of nucleotides in a new DNA strand. RNA primers are removed and replaced with DNA later in replication.

One New DNA Strand Is Synthesized Continuously; the Other, Discontinuously

DNA polymerases assemble a new DNA strand on a template strand in the 5′→3′ direction. Because the two strands of a DNA molecule are antiparallel, only one of the template strands runs in a direction that allows DNA polymerase to make a 5′→3′ complementary copy in the direction of unwinding. That is, on this template strand (top strand in **Figure 14.11**), the new DNA strand is synthesized continuously in the direction of unwinding of the double helix. However, the other template strand (bottom strand in Figure 14.11) runs in the opposite direction; this means DNA polymerase has to copy it in the direction opposite to the unwinding.

How is the new DNA strand made in the opposite direction to the unwinding? The polymerases make this strand in short lengths that are actually synthesized in the direction opposite to that of DNA unwinding (see Figure 14.11). The short lengths produced by this **discontinuous replication** are then covalently linked into a continuous polynucleotide chain. The short lengths are called *Okazaki fragments,* in honor of Reiji Okazaki, the Japanese scientist who first detected them. The new DNA strand assembled in the direction of DNA unwinding is called the **leading strand** of DNA replication; the strand assembled discontinuously in the opposite direction is called the **lagging strand.** The template strand for the leading strand is the *leading strand template,* and the template strand for the lagging strand is the *lagging strand template*.

Multiple Enzymes Coordinate Their Activities in DNA Replication

Helicase, primase, and DNA polymerase coordinate their activities with additional enzymes to replicate DNA. In the first step of the process, a helicase unwinds the template DNA to produce a replication fork (**Figure 14.12,** step 1). Just behind the site of unwinding, primases lay down short RNA primers about 10 nucleotides in length. The primers are assembled in the 5′→3′ direction on both template chains—in the direction of unwinding on one chain and in the opposite direction on the other.

DNA polymerase then adds DNA nucleotides to the RNA primers (step 2). Helicase continues to unwind the DNA. Leading strand synthesis continues in the direction of unwinding, whereas on the lagging strand template, primase creates a new RNA primer and DNA polymerase adds DNA nucleotides to the new primer (step 3). When this second fragment reaches the primer of the first fragment, the DNA polymerase leaves and a different type of DNA polymerase binds. This polymerase removes the RNA primer on the first fragment, replacing the RNA nucleotides with DNA nucleotides (step 4). At this point, the two newly synthesized fragments are not covalently joined—they have a "nick" between them (see step 4). Another enzyme, **DNA ligase** (*ligare* = to tie), closes the nick, joining the two fragments into one larger fragment (step 5). The replication process continues in the same way until the entire DNA molecule is copied (step 6). **Table 14.1** summarizes the activities of the major enzymes replicating DNA.

The entire replication mechanism, including the activities of the helicase, primase, DNA polymerases, DNA ligase, and other proteins involved in the process, advances at a rate of about 500 to 1000 nucleotides per second in prokaryotes and at a rate of about 50 to 100 per second in eukaryotes. The entire process is so rapid that the RNA primers and gaps left by discontinuous synthesis persist for only seconds or fractions of a second. Consequently, the replication enzymes operate only at the replication fork. A short distance behind the fork, the new DNA chains are fully continuous and wound with their template strands into complete DNA double helices. Each helix consists of one "old" and one "new" polynucleotide chain.

Researchers identified the enzymes that replicate DNA through experiments with a variety of prokaryotes and eukaryotes and with viruses that infect both types of cells. Experiments with the bacterium *E. coli* have provided the most complete information about DNA replication, particularly in the laboratory of Arthur Kornberg at Stanford University. Kornberg received a Nobel Prize in 1959 for his discovery of the mechanism for DNA synthesis.

Telomerases Solve a Specialized Replication Problem at the Ends of Linear DNA Molecules

The priming mechanism outlined in Figure 14.12 leaves one major problem unsolved for linear chromosomes, such as those in eukaryotes. Think about replication that occurs at one end of a chromosome (**Figure 14.13,** step 1). To begin the new strand, an RNA primer is laid down opposite the end of the template strand and then a DNA polymerase adds new DNA nucleotides from the end of this primer (step 2). Once replication is under way, this first primer is removed, leaving a gap at the beginning (5′) end of the new strand (as in step 3). In a similar way, a gap is produced at the 5′ end of the new strand made starting at the other end of the chromosome. Therefore, when these new, now shortened DNA strands are used as a template for the next round of DNA replication, the new chromosome will be shorter. Indeed, when most somatic cells go through the cell cycle, the chromosomes shorten with each division. Deletion of genes by such shortening would have serious, eventually lethal, consequences for the cell.

In most chromosomes, however, the genes are protected by a buffer of noncoding DNA. That is, at the ends of each eukaryotic chromosome are telomeres (*telo* = end; *mere* = segment). **Telomeres** are short sequences repeated hundreds to thousands of times, which do not code for proteins. In humans, the repeated sequence, the *telomere repeat,* is 5′-TTAGGG-3′ on the leading template strand. With each replication, a fraction of the telomere repeats is lost but the genes are unaffected. The buffering fails only when the entire telomere is lost.

The enzyme **telomerase** can stop the shortening by adding telomere repeats to the chromosome ends.

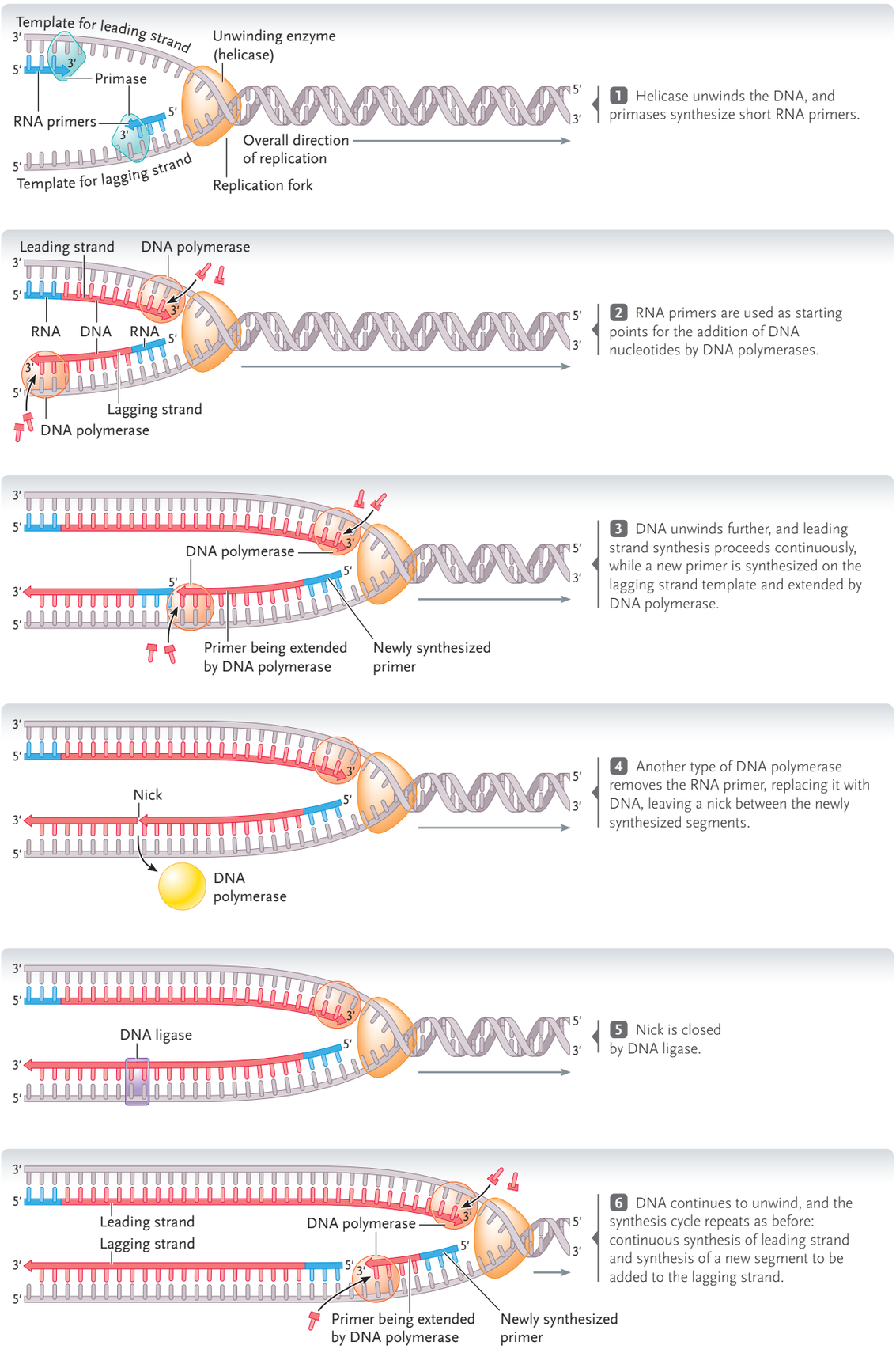


Figure 14.12
Steps in DNA replication, including the activities of the helicase, primase, DNA polymerases, and DNA ligase taking part in the process. Primer synthesis, removal, gap filling, and nick sealing occur primarily in the lagging strand. The drawings simplify the process. In reality, the enzymes assemble at the fork, replicating both strands from that position as the template strands fold and pass through the assembly.

Table 14.1 **Major Enzymes of DNA Replication**

Enzyme	Activity
Helicase	Unwinds DNA helix
Single-stranded binding proteins	Stabilize DNA in single-chain form
Primase	Assembles RNA primers
DNA polymerases	Assemble DNA chains on primers; replace primers while simultaneously replacing primer nucleotides with DNA nucleotides
DNA ligase	Seals nicks left after RNA primers replaced with DNA
Topoisomerases	Relieve overtwisting and strain of DNA ahead of replication fork (in circular DNA)

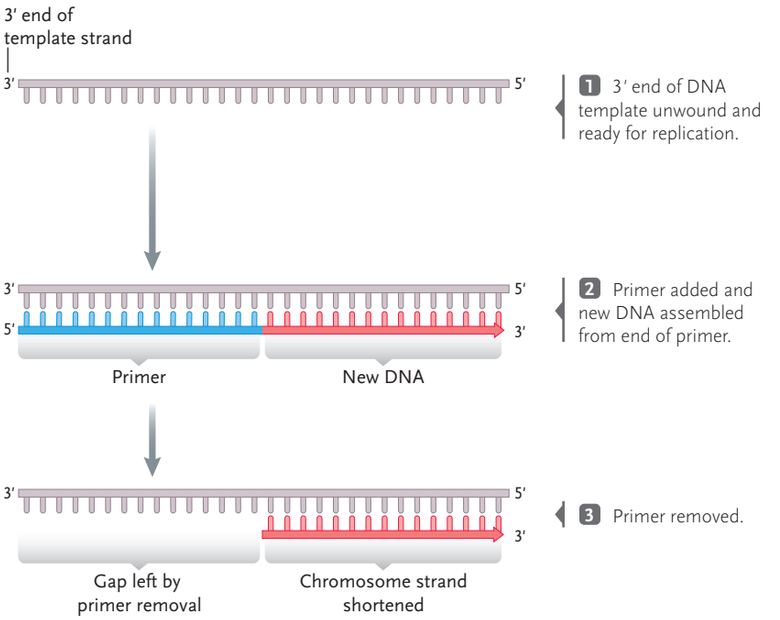


Figure 14.13
How a gap is left by primer removal at the 5′ end of a replicating linear DNA molecule.

Discovered in 1985 by Elizabeth H. Blackburn and her graduate student Carol W. Greider at the University of California, Berkeley, telomerase adds additional telomere repeats to the end of the *template strand* before DNA replication begins (**Figure 14.14,** step 1; compare with Figure 14.13, step 1). After the addition, the primer of the leading strand is laid down, using the newly added telomere repeats as the template. A DNA polymerase then extends the new DNA strand as usual (Figure 14.14, step 2). The primer is removed, which still leaves an unfilled gap at the beginning of the leading chain (step 3). However, the chromosome has not shortened because it now has the extra telomere repeats added by the telomerase. The telomerase enzyme, which appears to be present in all eukaryotes, is an unusual enzyme that consists of protein subunits complexed with RNA; the RNA part is the template for making the extra telomere repeats.

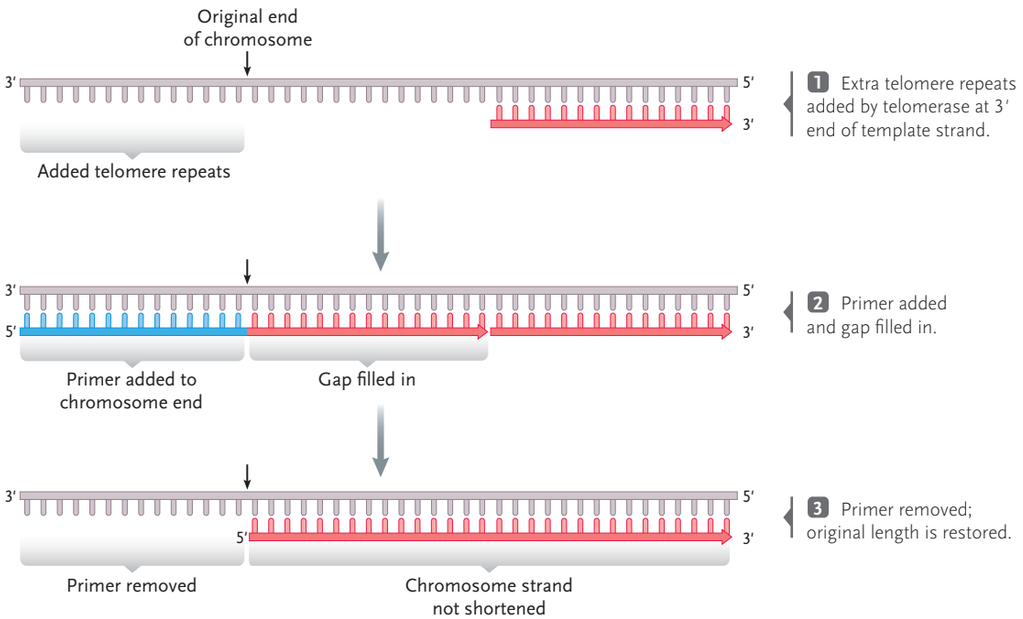


Figure 14.14
How telomere repeats added to eukaryotic chromosomes prevent chromosome shortening.

Telomerase is active in some cells but not in others. In particular, telomerase is active in sperm and eggs, which is necessary to maintain chromosome length from generation to generation. It is also active in the rapidly dividing cells of the early embryo. However, telomerase becomes inactive after a number of divisions, meaning subsequent telomeres shorten as the cells continue to divide. As a result, a cell is capable of only a certain number of mitotic divisions before it stops dividing and dies. Could telomere shortening, then, contribute to the aging process in multicellular animals? Telomere shortening has indeed been linked to the aging process, but it is unknown whether it contributes to or is a result of aging.

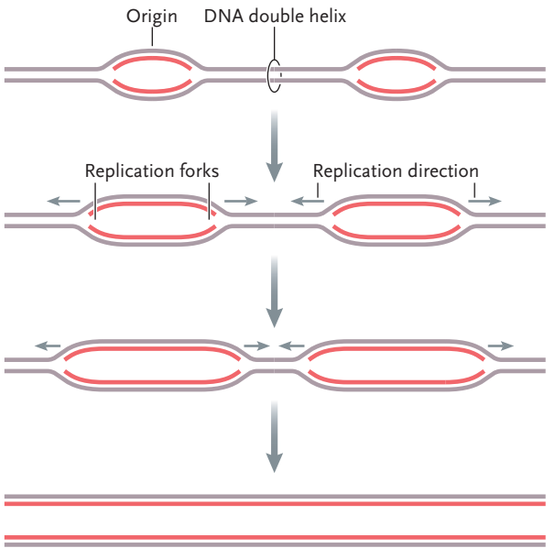


Figure 14.15
Replication from multiple origins in the chromosomes of eukaryotes.

Some observations have made it difficult to draw firm conclusions on this issue. For example, humans, a long-lived species, have telomeres that are much shorter than mice, which live just a few years. Clearly telomeres alone do not determine the life span of an organism.

An unexpected link between telomerases and cancer was found when investigators discovered that more than 90% of cancer cells have fully active telomerase enzymes, regardless of the type of body cell from which they are derived. Evidently, as body cells develop into cancer cells, their telomerases are reactivated, preserving chromosome length during the rapid divisions characteristic of cancer. A positive side of this discovery is that it may lead to an effective cancer treatment, if a means can be found to switch off the telomerases in tumor cells. The chromosomes in the rapidly dividing cancer cells would then eventually shorten to the length at which they break down, leading to cell death and elimination of the tumor.

DNA Replication Begins at Replication Origins

Replication begins at sites called **replication origins.** Hundreds of replication origins may be present in the long chromosomes of eukaryotes. The origins are recognized by proteins that bind to the DNA and stimulate helicases to start the unwinding, followed by primer synthesis and DNA replication. In most cases, replication proceeds from both sides of a replication origin, producing two replication forks that move in opposite directions **(Figure 14.15).** (This means that the leading strands and lagging strands are reversed on the two sides.) The forks eventually meet along the chromosomes to produce fully replicated DNA molecules.

Normally, a replication origin is activated only once during the S phase of a eukaryotic cell cycle, so no portion of the DNA is replicated more than once. *Insights from the Molecular Revolution* describes abnormal extra DNA replication that underlies a common cause of mental retardation in humans.

Study Break

1. What is the importance of complementary base pairing to DNA replication?
2. Why is a primer needed for DNA replication? How is the primer made?
3. Two DNA polymerases are used in DNA replication. What are their roles?
4. Why are telomeres important?

14.4 Mechanisms That Correct Replication Errors

DNA polymerases make very few errors as they assemble new nucleotide chains. Most of the mistakes that do occur, called **base-pair mismatches**, are corrected, either by a proofreading mechanism carried out during replication by the DNA polymerases themselves or by a DNA repair mechanism that corrects mismatched base pairs after replication is complete.

Proofreading Depends on the Ability of DNA Polymerases to Reverse and Remove Mismatched Bases

The **proofreading mechanism,** first proposed in 1972 by Arthur Kornberg and Douglas L. Brutlag of Stanford University, depends on the ability of DNA polymerases to back up and remove mispaired nucleotides from a DNA strand. Only when the most recently added base is correctly paired with its complementary base on the template strand can the DNA polymerases continue to add nucleotides to a growing chain. The correct pairs allow the fully stabilizing hydrogen bonds to form (**Figure 14.16,** step 1). If a newly added nucleotide is mismatched (step 2), the DNA polymerase reverses, using a built-in deoxyribonuclease to remove the newly added incorrect nucleotide (step 3). The enzyme resumes working forward, now inserting the correct nucleotide (step 4).

Several experiments have confirmed that the major DNA polymerases of replication can actually proofread their work. For example, when the pri-

mary DNA polymerase that replicates DNA in bacteria is intact, with its reverse activity working, its overall error rate is astonishingly low—only about 1 mispair survives in the DNA for every 1 million nucleotides assembled in the test tube. If the proofreading activity of the enzyme is experimentally inhibited, the error rate increases to about 1 mistake for every 1000 to 10,000 nucleotides assembled. Experiments with eukaryotes have yielded similar results.

DNA Repair Corrects Errors That Escape Proofreading

Any base-pair mismatches that remain after proofreading face still another round of correction by **DNA repair mechanisms.** These **mismatch repair** mechanisms increase the accuracy of DNA replication well beyond the one-in-a million errors that persist after proofreading. As noted earlier, the "correct" A-T and G-C base pairs fit together like pieces of a jigsaw puzzle, and their dimensions separate the sugar–phosphate backbone chains by a constant distance. Mispaired bases are too large or small to maintain the correct separation, and they cannot form the hydrogen bonds characteristic of the normal base pairs. As a result, base mismatches distort the structure of the DNA helix. These distortions provide recognition sites for the enzymes catalyzing mismatch repair.

The repair enzymes move along newly replicated DNA molecules, "scanning" the DNA for distortions in the newly synthesized nucleotide chain. If the enzymes encounter a distortion, they remove a portion of the new chain, including the mismatched nucleotides (**Figure 14.17,** step 1). The gap left by the removal (step 2) is then filled by a DNA polymerase, using the template strand as a guide (step 3). The repair is completed by a DNA ligase, which seals the nucleotide chain into a continuous DNA molecule (step 4).

The same repair mechanisms also detect and correct alterations in DNA caused by the damaging effects of chemicals and radiation, including the ultraviolet light in sunlight. Some idea of the importance of the repair mechanisms comes from the unfortunate plight of individuals with *xeroderma pigmentosum,* a hereditary disorder in which the repair mechanism is faulty. Because of the effects of unrepaired alterations in their DNA, skin cancer can develop quickly in these individuals if they are exposed to sunlight.

Very few replication errors remain in DNA after proofreading and DNA repair. The errors that persist, although extremely rare, are a primary source of **mutations**, differences in DNA sequence that appear and remain in the replicated copies. When a mutation occurs in a gene, it can alter the property of the pro-

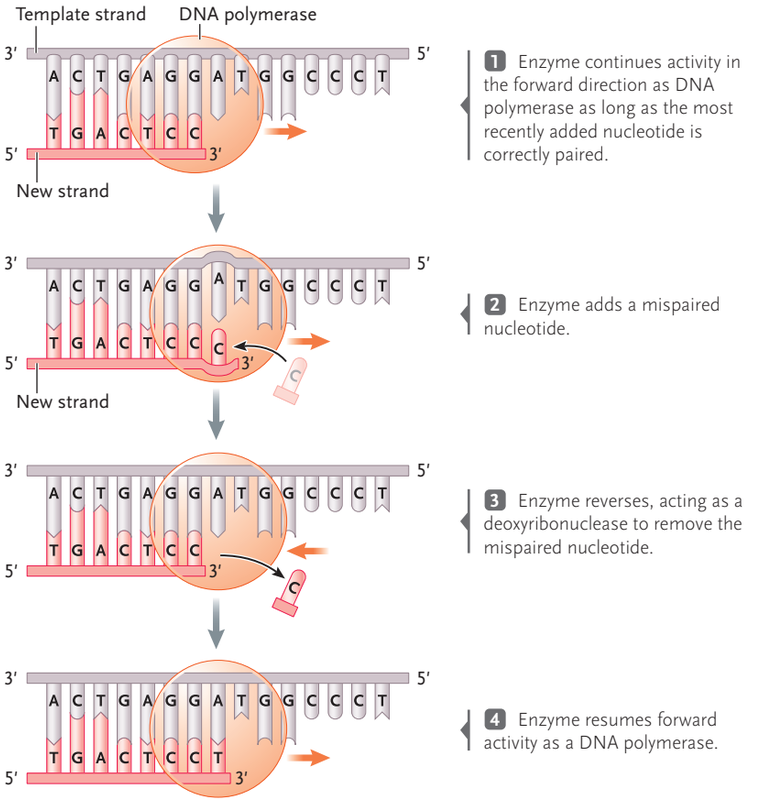


Figure 14.16
Proofreading by a DNA polymerase.

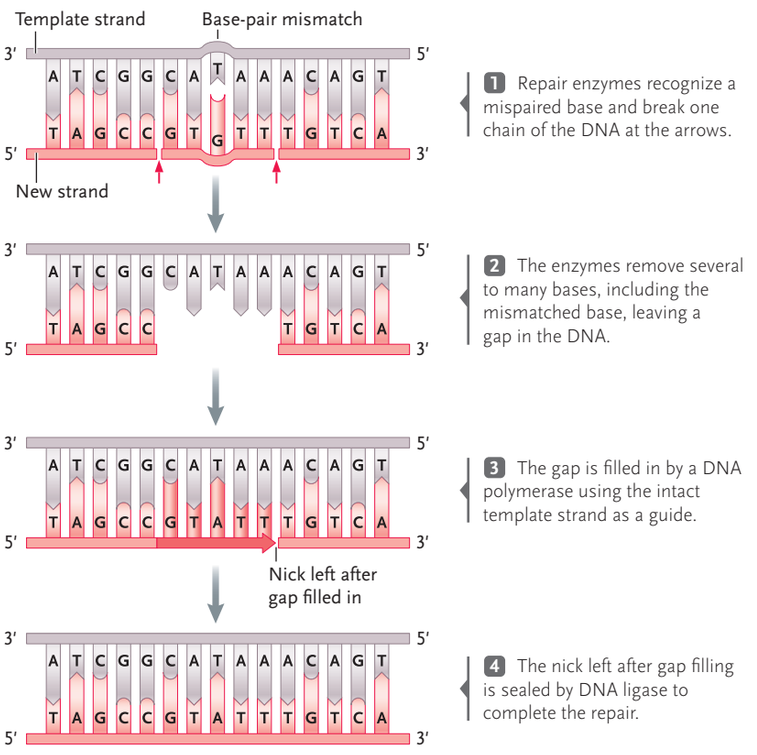


Figure 14.17
Repair of mismatched bases in replicated DNA.

Insights from the Molecular Revolution

A Fragile Connection between DNA Replication and Mental Retardation

One of the most common sources of inherited mental retardation in humans results from breaks that occur in a narrow, constricted region near one end of the X chromosome (see figure). Because the region breaks easily when cultured cells divide, the associated disabilities are called the *fragile X syndrome*. In addition to mental retardation, affected individuals may have an unusually long face and protruding ears; affected males may have oversized testes.

The disorder affects males more frequently than females, as is typical with X-linked traits—about 1 in 1500 male and 1 in 2500 female births are affected by the fragile X syndrome. However, the inheritance pattern of the syndrome also has some unusual characteristics. The disease is passed from a grandfather through his daughter to his grandchild. The grandfather and daughter have apparently normal X chromosomes, although the daughters sometimes have symptoms; however, abnormal X chromosomes and symptoms appear with high frequency in the grandchildren.

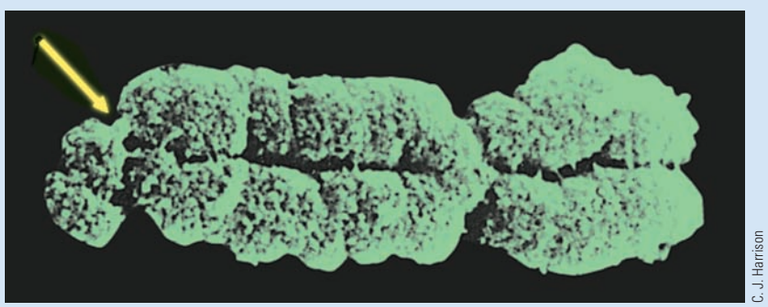
Geneticists were baffled by this unusual pattern of inheritance until a partial explanation was supplied by findings in the laboratories of Grant R. Sutherland of Adelaide Children's Hospital in Australia and others. The investigators examined DNA from individuals with fragile X syndrome using "probes"—short, artificially synthesized DNA sequences that are complementary to, and can pair with, DNA sequences that are of interest. They found that probes containing C and G nucleotides in high proportions paired most strongly with DNA in the fragile X region. Sequencing of DNA that paired with those probes showed that the region contains many repeats of the three-nucleotide sequence CCG.

Interestingly, the number of CCG repeats varies in the different groups: from 6 to 50 copies in people without the syndrome, 50 to 200 copies in people with mild or no symptoms who transmit the syndrome, and about 230 to 1000 copies in seriously affected people. Somehow the number of CCG repeats increases, which initiates the serious disease symptoms.

The increase in copies occurs by overreplication of the CCG sequence, which begins to occur when the number of copies exceeds about 50. As more CCG copies are added, the region becomes increasingly unstable and the tendency for overreplication also increases. This feature of the process explains why symptoms of the disease often become worse in successive generations.

Scientists still do not understand what causes the overreplication or how it causes fragile X syndrome. However, the increase in CCG copies appears to turn off a nearby gene called *FMR-1*, which is necessary for normal mental development. The increase also inhibits other genes located elsewhere that have the same effect. One hypothesis takes note of the fact that methyl groups are added to cytosines as part of the controls that turn off large blocks of genes in mammals. According to this idea, the extra cytosines of the added CCG groups provide many additional methylation sites, leading to inactivation of the genes near the fragile X region.

The probe that pairs with CCG groups can be used to estimate the number of copies of the sequence in people without the syndrome who may have increased numbers of the sequence in the fragile X region. If the number is elevated above 50 repeats, these individuals can be counseled about the possibility that they could transmit the disease to their offspring several generations down the line.



C. J. Harrison

The constricted region *(arrow)* in the human X chromosome associated with fragile X syndrome. The chromosome is double because it has been duplicated in preparation for cell division.

tein encoded by the gene, which, in turn, may alter how the organism functions. Hence, mutations are highly important to the evolutionary process because they are the ultimate source of the variability in offspring acted on by natural selection.

We now turn from DNA replication and error correction to the arrangements of DNA in eukaryotic and prokaryotic cells. These arrangements organize superstructures that fit the long DNA molecules into the microscopic dimensions of cells and also contribute to the regulation of DNA activity.

Study Break

Why is a proofreading mechanism important for DNA replication, and what are the mechanisms that correct errors?

14.5 DNA Organization in Eukaryotes and Prokaryotes

Enzymatic proteins are the essential catalysts of every step in DNA replication. In addition, numerous proteins of other types organize the DNA in both eukaryotes and prokaryotes and control its function.

In eukaryotes, two major types of proteins, the histone and nonhistone proteins, are associated with DNA structure and regulation in the nucleus. These proteins are known collectively as the **chromosomal proteins** of eukaryotes. The complex of DNA and its associated proteins, termed **chromatin**, is the structural building block of a chromosome.

By comparison, the single DNA molecule of a prokaryotic cell is more simply organized and has fewer associated proteins. However, prokaryotic DNA is still associated with two classes of proteins with functions similar to those of the eukaryotic histones and nonhistones: one class that organizes the DNA structurally and one that regulates gene activity. We begin this section with the major DNA-associated proteins of eukaryotes.

Histones Pack Eukaryotic DNA at Successive Levels of Organization

The **histones** are a class of small, positively charged (basic) proteins that are complexed with DNA in the chromosomes of eukaryotes. (Most other cellular proteins are larger and are neutral or negatively charged.) The histones link to DNA by an attraction between their positive charges and the negatively charged phosphate groups of the DNA.

Five types of histones exist in most eukaryotic cells: H1, H2A, H2B, H3, and H4. The amino acid sequences of these proteins are highly similar among eukaryotes, suggesting that they perform the same functions in all eukaryotic organisms.

One function of histones is to pack DNA molecules into the narrow confines of the cell nucleus. For example, each human cell nucleus contains 2 meters of DNA. Combination with the histones compacts this length so much that it fits into nuclei that are only about 10 μm in diameter. Another function is the regulation of DNA activity.

Histones and DNA Packing. The histones pack DNA at several levels of chromatin structure. In the most fundamental structure, called a **nucleosome**, two molecules each of H2A, H2B, H3, and H4 combine to form a beadlike, eight-protein **nucleosome core particle** around which DNA winds for almost two turns **(Figure 14.18).** A short segment of DNA, the **linker,** extends between one nucleosome and the next. Under the electron microscope, this structure looks like beads on a string. The diameter of the beads (the nucleosomes) gives this structure its name—the **10-nm chromatin fiber** (see Figure 14.18).

Each nucleosome and linker includes about 200 base pairs of DNA. Nucleosomes compact DNA by a factor of about 7; that is, a length of DNA becomes about 7 times shorter when it is wrapped into nucleosomes.

Histones and Chromatin Fibers. The fifth histone, H1, brings about the next level of chromatin packing. One H1 molecule binds both to the nucleosome at

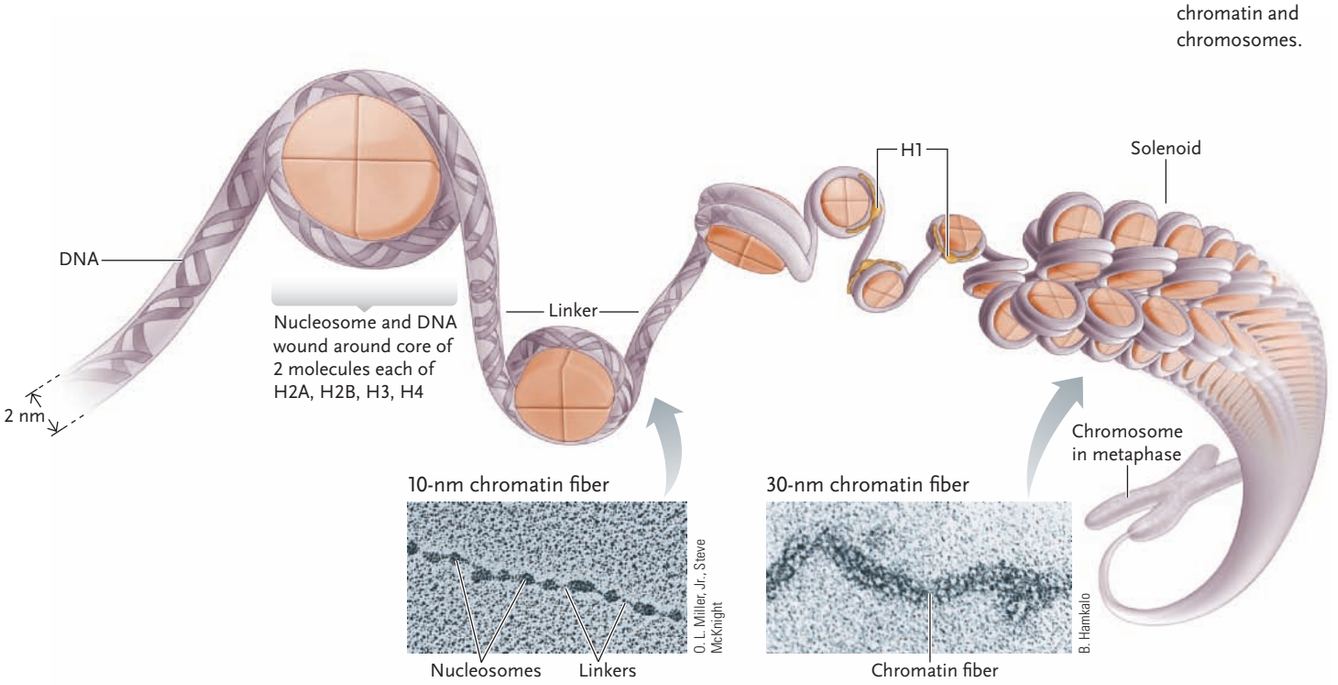


Figure 14.18
Levels of organization in eukaryotic chromatin and chromosomes.

the point where the DNA enters and leaves the core particle and to the linker DNA. This binding causes the nucleosomes to package into a coiled structure 30 nm in diameter, called the **30-nm chromatin fiber** or **solenoid**, with about six nucleosomes per turn (see Figure 14.18).

The arrangement of DNA in nucleosomes and solenoids compacts the DNA and probably also protects it from chemical and mechanical damage. In the test tube, DNA wound into nucleosomes and chromatin fibers is much more resistant to attack by deoxyribonuclease (a DNA-digesting enzyme) than when it is not bound to histone proteins. Therefore, DNA must unwind almost entirely from solenoids and nucleosomes when it becomes active. When genes become active, however, their DNA becomes almost as susceptible to attack as naked DNA in the test tube.

Packing at Still Higher Levels: Euchromatin and Heterochromatin. In interphase nuclei, chromatin fibers are loosely packed in some regions and densely packed in others. The loosely packed regions are known as **euchromatin** (*eu* = true, regular, or typical), and the densely packed regions are called **heterochromatin** (*hetero* = different). Chromatin fibers also fold and pack into the thick, rodlike chromosomes visible during mitosis and meiosis. Some experiments indicate that links formed between H1 histone molecules contribute to the packing of chromatin fibers, both into heterochromatin and into the chromosomes visible during nuclear division (see discussion in Section 10.2 as well as the more detailed discussion in Section 15.2). However, the exact mechanism for the more complex folding and packing is not known.

Several experiments indicate that heterochromatin represents large blocks of genes that have been turned off and placed in a compact storage form. For example, recall the process of X-chromosome inactivation in mammalian females (see Section 13.2). As one of the two X chromosomes becomes inactive in cells early in development, it packs down into a block of heterochromatin called the *Barr body*, which is large enough to see under the light microscope. These findings support the idea that, in addition to organizing nuclear DNA, histones play a role in regulating gene activity.

Many Nonhistone Proteins Have Key Roles in the Regulation of Gene Expression

Nonhistone proteins are loosely defined as all the proteins associated with DNA that are not histones. Nonhistones vary widely in structure; most are negatively charged or neutral, but some are positively charged. They range in size from polypeptides smaller than histones to some of the largest cellular proteins.

Many nonhistone proteins help control the expression of individual genes. (The regulation of gene expression is the subject of Chapter 16.) For example, expression of a gene requires that the enzymes and proteins for that process be able to access the gene in the chromatin. If a gene is packed into heterochromatin, it is unavailable for activation. If the gene is in the more-extended euchromatin, it is more accessible. Many nonhistone proteins affect gene accessibility by modifying histones to change how the histones associate with DNA in chromatin, either loosening or tightening the association. Other nonhistone proteins are regulatory proteins that activate or repress the expression of a gene. Yet others are components of the enzyme–protein complexes that are needed for the expression of any gene.

DNA Is Organized More Simply in Prokaryotes Than in Eukaryotes

Several features of DNA organization in prokaryotes differ fundamentally from eukaryotic DNA. In contrast to the linear DNA in eukaryotes, the primary DNA molecule of most prokaryotic cells is circular, with only one copy per cell. In parallel with eukaryotic terminology, the DNA molecule is called a **bacterial chromosome.** The chromosome of the best-known bacterium, *E. coli,* includes about 1460 μm of DNA, which is equivalent to 4.6 million base pairs. There are exceptions: some bacteria have two or more different chromosomes in the cell, and some bacterial chromosomes are linear.

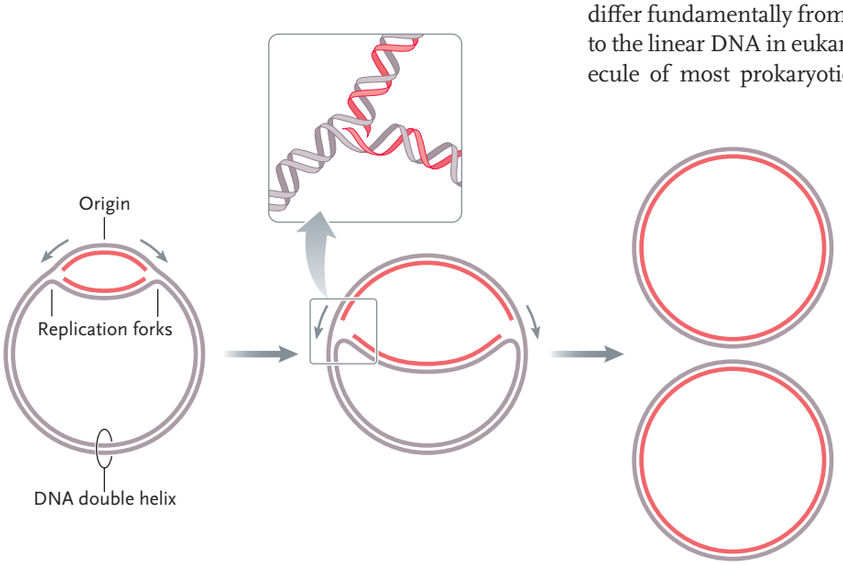


Figure 14.19
Replication from a single origin in the DNA circle of prokaryotes.

Replication begins from a single origin in the DNA circle, forming two forks that travel around the circle in opposite directions. Eventually, the forks meet at the opposite side from the origin to complete replication **(Figure 14.19).**

Inside prokaryotic cells, the DNA circle is packed and folded into an irregularly shaped mass called the **nucleoid** (shown in Figure 5.7). The DNA of the nucleoid is suspended directly in the cytoplasm with no surrounding membrane.

Many prokaryotic cells also contain other DNA molecules, called **plasmids**, in addition to the main chromosome of the nucleoid. Most plasmids are circular, although some are linear. Plasmids have replication origins and are duplicated and distributed to daughter cells together with the bacterial chromosome during cell division.

Although bacterial DNA is not organized into nucleosomes, there are positively charged proteins that combine with bacterial DNA. Some of these proteins help organize the DNA into loops, thereby providing some compaction of the molecule. Bacterial DNA also combines with many types of genetic regulatory proteins that have functions similar to those of the nonhistone proteins of eukaryotes (see Chapter 16).

Unanswered Questions

In this chapter, we learned about the structure and replication of DNA, the key role of telomeres in maintaining the ends of chromosomes, and the packaging of DNA into chromosomes in eukaryotes. Research is ongoing in all of these areas, both to understand basic cellular processes and in applied areas such as understanding and treating human diseases and infections.

Does DNA polymerase affect cellular aging?

As cells grow and divide, they eventually become senescent and then die. Understanding cellular aging is critical to understanding the aging process of an organism and, therefore, to developing treatments for age-related diseases.

Senescing cells eventually fail to initiate DNA synthesis and are therefore unable to transition from the G_1 to the S phase of the cell cycle (see Chapter 10). This transition requires the expression of a particular set of genes, some of which encode proteins involved in DNA replication. Loss of regulation of expression of these genes is hypothesized to contribute to cellular aging. In particular, research has shown that the activity and function of a DNA polymerase involved in the initiation of Okazaki fragments on the lagging strand template may be a crucial determinant of aging. Research is ongoing to test this hypothesis, focusing on correlating changes in the activity of the enzyme with cellular aging and the interactions of the enzyme with other replication proteins.

Can DNA replication be an effective target for antiherpes drugs?

Certain antiviral drugs target DNA replication enzymes involved in viral reproduction inside cells. For example, acyclovir, first developed in the 1970s, is the standard drug for treating various herpes simplex viruses (HSV). The genome of HSV is DNA, and a virus-encoded DNA polymerase replicates the viral DNA. Not only can HSV produce sores on the mouth and genitals, it can also kill people who have compromised immune systems, including newborns, elderly individuals, and AIDS patients. Acyclovir is a chemical analog of a DNA nucleoside; that is, it resembles a natural DNA nucleoside (deoxyribose sugar + base) in structure. Acyclovir is taken into cells. If the cell is infected with HSV, a virus-encoded enzyme efficiently converts the acyclovir to acyclovir monophosphate, which cellular enzymes then convert to acyclovir triphosphate. The triphosphate derivative is used by the HSV-encoded DNA polymerase during viral DNA replication, but incorporation of the analog blocks further DNA synthesis. In short, acyclovir blocks viral DNA replication by the viral DNA polymerase. Fortunately, acyclovir does not inhibit cellular DNA polymerase at the concentrations that are effective against the viral enzyme. Furthermore, the efficient conversion of acyclovir to acyclovir triphosphate depends on the presence of a viral enzyme, which means that acyclovir has little effect on cells not infected with HSV.

Other viral enzymes are the target of a recently developed new class of antiherpes drugs. Drugs in this class target the HSV-encoded helicase and primase enzymes used for viral DNA replication; these two proteins work as a complex at the replication fork. The new drugs have been shown to be highly effective in blocking HSV replication in tissue culture cells, as well as reducing the death rate of virus-infected mice. Moreover, they are more potent than acyclovir. Another benefit of these drugs is that they are effective when given 65 hours after mice were infected, whereas acyclovir must be given within a few hours after infection. Further research is needed to determine whether the drugs will be effective in humans, and at what safe dose. However, research in this area requires significant amounts of money, but pharmaceutical companies appear reluctant to invest in such projects because of the large profits that can be made with acyclovir.

More broadly, the results of the experiments indicate that targeting aspects of replication of viral genomes can be an effective mode of treatment.

What is the role of telomerase in cell division?

The University of California, San Francisco, research group of Elizabeth Blackburn, one of the discoverers of telomerase, is investigating the function of telomeres and telomerase in yeast and human cancer cells. Blackburn has demonstrated that some mutants of the RNA molecule in telomerase cause telomere shortening and cellular aging (senescence) in the protozoan *Tetrahymena*. In addition, she found that chemical inhibitors of telomerase cause human telomeres to shorten. Other mutations of the telomere length regulation system cause telomeres to become too long, triggering degradation of the telomeres and potentially causing the cells to stop mitosis at anaphase. Continuing research is directed at determining the precise roles of telomeres in the cell division process. The research findings are also being used as a platform for developing an anticancer strategy, using cells from human breast, prostate, and bladder cancers.

Peter J. Russell

With this description of prokaryotic DNA organization, our survey of DNA structure and its replication and organization is complete. The next chapter revisits the same structures and discusses how they function in the expression of information encoded in the DNA.

Study Break

1. What is the structure of the nucleosome?
2. What is the role of histone H1 in eukaryotic chromosome structure?

Review

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14.1 Establishing DNA as the Hereditary Molecule

- Griffith found that a substance derived from killed infective pneumonia bacteria could transform noninfective living pneumonia bacteria to the infective type (Figure 14.2).
- Avery and his coworkers showed that DNA, and not protein or RNA, was the molecule responsible for transforming pneumonia bacteria into the infective form.
- Hershey and Chase showed that the DNA of a phage, not the protein, enters bacterial cells to direct the life cycle of the virus. Taken together, the experiments of Griffith, Avery and his coworkers, and Hershey and Chase established that DNA is the hereditary molecule (Figure 14.3).

Animation: Griffith's experiment

Animation: The Hershey and Chase experiments

14.2 DNA Structure

- Watson and Crick discovered that a DNA molecule consists of two polynucleotide chains twisted around each other into a right-handed double helix. Each nucleotide of the chains consists of deoxyribose, a phosphate group, and either adenine, thymine, guanine, or cytosine. The deoxyribose sugars are linked by phosphate groups to form an alternating sugar–phosphate backbone. The two strands are held together by adenine–thymine and guanine–cytosine base pairs. Each full turn of the double helix involves 10 base pairs (Figures 14.4 and 14.6).
- The two strands of the DNA double helix are antiparallel.

Animation: The nucleotides of DNA

Animation: The DNA double helix

Practice: Constructing DNA

14.3 DNA Replication

- DNA is duplicated by semiconservative replication, in which the two strands of a parental DNA molecule unwind and each serves as a template for the synthesis of a complementary copy (Figures 14.7–14.9).
- DNA replication is catalyzed by several enzymes. Helicase unwinds the DNA; primase synthesizes an RNA primer used as a starting point for nucleotide assembly by DNA polymerases. DNA polymerases assemble nucleotides into a chain one at a time, in a sequence complementary to the sequence of bases in the template strand. After a DNA polymerase removes the primers and fills in the resulting gaps, DNA ligase closes the remaining single-chain nicks (Figures 14.10 and 14.12).
- As the DNA helix unwinds, only one template strand runs in a direction allowing the new DNA strand to be made continuously in the direction of unwinding. The other template strand is copied in short lengths that run in the direction opposite to unwinding. The short lengths produced by this discontinuous replication are then linked into a continuous strand (Figures 14.11 and 14.12).
- The ends of eukaryotic chromosomes consist of telomeres, short sequences repeated hundreds to thousands of times. These repeats provide a buffer against chromosome shortening during replication. Although most somatic cells show this chromosome shortening, some cell types do not because they have a telomerase enzyme that adds telomere repeats to the chromosome ends (Figures 14.13 and 14.14).
- DNA synthesis begins at sites that act as replication origins and proceeds from the origins as two replication forks moving in opposite directions (Figure 14.15).

Animation: Overview of DNA replication and base pairing

Animation: DNA replication in detail

14.4 Mechanisms That Correct Replication Errors

- In proofreading, the DNA polymerase reverses and removes the most recently added base if it is mispaired as a result of a replication error. The enzyme then resumes DNA synthesis in the forward direction (Figure 14.16).
- In DNA mismatch repair, enzymes recognize distorted regions caused by mispaired base pairs and remove a section of DNA that includes the mispaired base from the newly synthesized nucleotide chain. A DNA polymerase then resynthesizes the section correctly, using the original template chain as a guide (Figure 14.17).

14.5 DNA Organization in Eukaryotes and Prokaryotes

- Eukaryotic chromosomes consist of DNA complexed with histone and nonhistone proteins.
- In eukaryotic chromosomes, DNA is wrapped around a nucleosome consisting of two molecules each of histones H2A, H2B, H3, and H4. Linker DNA connects adjacent nucleosomes. The binding of histone H1 causes the nucleosomes to package into a coiled structure called a solenoid (Figure 14.18).
- Chromatin is distributed between euchromatin, a loosely packed region in which genes are active in RNA transcription, and heterochromatin, densely packed masses in which the genes are inactive. Chromatin also folds and packs to form thick, rodlike chromosomes during nuclear division.
- Nonhistone proteins help control the expression of individual genes.

- The bacterial chromosome is a closed, circular molecule of DNA; it is packed into the nucleoid region of the cell. Replication begins from a single origin and proceeds in both directions. Many bacteria also contain plasmids, which replicate independently of the host chromosome (Figure 14.19).
- Bacterial DNA is organized into loops through interaction with proteins. Other proteins similar to eukaryotic nonhistones regulate gene activity in prokaryotes.

Animation: Chromosome structural organization

Questions

Self-Test Questions

1. Working on the Amazon River, a biologist isolated DNA from two unknown organisms, P and Q. He discovered that the adenine content of P was 15% and the cytosine content of Q was 42%. This means that:
   a. the amount of guanine in P is 15%.
   b. the amount of guanine and cytosine combined in P is 70%.
   c. the amount of adenine in Q is 42%.
   d. the amount of thymine in Q is 21%.
   e. it takes more energy to unwind the DNA of P than the DNA of Q.
2. The Hershey and Chase experiment showed that viral:
   a. ^{35}S entered bacterial cells.
   b. ^{32}P remained outside of bacterial cells.
   c. protein entered bacterial cells.
   d. DNA entered bacterial cells.
   e. DNA mutated in bacterial cells.
3. Pyrimidines include:
   a. cytosine and thymine.
   b. adenine, cytosine, and guanine.
   c. adenine and thymine.
   d. cytosine and guanine.
   e. adenine and guanine.
4. Which of the following statements about DNA replication is *false*?
   a. Synthesis of the new DNA strand is from 3′ to 5′.
   b. Synthesis of the new DNA strand is from 5′ to 3′.
   c. DNA unwinds, primase adds RNA primer, and DNA polymerases synthesize the new strand and remove the RNA primer.
   d. Many initiation points exist in each eukaryotic chromosome.
   e. Okazaki fragments are synthesized in the opposite direction from the direction in which the replication fork moves.
5. Which of the following statements about DNA is *false*?
   a. Phosphate is linked to the 5′ and 3′ carbons of adjacent deoxyribose molecules.
   b. DNA is bidirectional in its synthesis.
   c. Each side of the helix is antiparallel to the other.
   d. The binding of adenine to thymine is through three hydrogen bonds.
   e. Avery identified DNA as the transforming factor in crosses between smooth and rough bacteria.
6. In the Meselson and Stahl experiment, the DNA in the parental generation was all $^{15}N^{15}N$, and after one round of replication, the DNA was all $^{15}N^{14}N$. What DNAs were seen after three rounds of replication, and in what ratio were they found?
   a. one $^{15}N^{14}N$: one ^{14}N:^{14}N
   b. one $^{15}N^{14}N$: two ^{14}N:^{14}N
   c. one $^{15}N^{14}N$: three ^{14}N:^{14}N
   d. one $^{15}N^{14}N$: four ^{14}N:^{14}N
   e. one $^{15}N^{14}N$: seven ^{14}N:^{14}N
7. During replication, DNA is synthesized in a 5′→3′ direction. This implies that:
   a. the template is read in a 5′→3′ direction.
   b. successive nucleotides are added to the 3′–OH end of the newly forming chain.
   c. because both strands are replicated nearly simultaneously, replication must be continuous on both.
   d. ligase unwinds DNA in a 5′→3′ direction.
   e. primase acts on the 3′ end of the replicating strand.
8. Telomerase:
   a. is active in cancer cells.
   b. is more active in adult than embryonic cells.
   c. complexes with the ribosome to form telomeres.
   d. acts on unique genes called telomeres.
   e. shortens the ends of chromosomes.
9. Mismatch repair is the ability:
   a. to seal Okazaki fragments with ligase into a continual DNA strand.
   b. of primase to remove the RNA primer and replace it with the correct DNA.
   c. of some enzymes to sense the insertion of an incorrect nucleotide, remove it, and use a DNA polymerase to insert the correct one.
   d. to correct mispaired chromosomes in prophase I of meiosis.
   e. to remove worn-out DNA by telomerase and replace it with newly synthesized nucleotides.
10. Prokaryotic DNA:
    a. is surrounded by densely packed histones.
    b. has many sites for the initiation of DNA replication.
    c. has both strands synthesized in the same direction.
    d. is packaged as euchromatin and heterochromatin.
    e. is packaged as a large circular chromosome.

Questions for Discussion

1. Chargaff's data suggested that adenine pairs with thymine and guanine pairs with cytosine. What other data available to Watson and Crick suggested that adenine–guanine and cytosine–thymine pairs normally do not form?
2. Eukaryotic chromosomes can be labeled by exposing cells to radioactive thymidine during the S phase of interphase. If cells are exposed to radioactive thymidine during the S phase, would you expect both or only one of the sister chromatids of a duplicated chromosome to be labeled at metaphase of the following mitosis (see Section 10.2)?
3. If the cells in question 2 finish division and then enter another round of DNA replication in a medium that has been washed free of radioactive label, would you expect both or only one of the sister chromatids of a duplicated chromosome to be labeled at metaphase of the following mitosis?
4. During replication, an error uncorrected by proofreading or mismatch repair produces a DNA molecule with a base mismatch at the indicated position:

```
AATTCCGACTCCTATGG
TTAAGGTTGAGGATACC
      ↑
```

The mismatch results in a mutation. This DNA molecule is received by one of the two daughter cells produced by mitosis. In the next round of replication and division, the mutation appears in only one of the two daughter cells. Develop a hypothesis to explain this observation.

5. Strains of bacteria that are resistant to an antibiotic sometimes appear spontaneously among other bacteria of the same type that are killed by the antibiotic. In view of the information in this chapter about DNA replication, what might account for the appearance of this resistance?

Experimental Analysis

Design an experiment using radioactive isotopes to show that the process of bacterial transformation involves DNA and not protein.

Evolution Link

The amino acid sequences of the DNA polymerases found in bacteria show little similarity to those of the DNA polymerases found in eukaryotes and in archaeans. By contrast, the amino acid sequences of the DNA polymerases of eukaryotes and archaeans show a high degree of similarity. Interpret these observations from an evolutionary point of view.