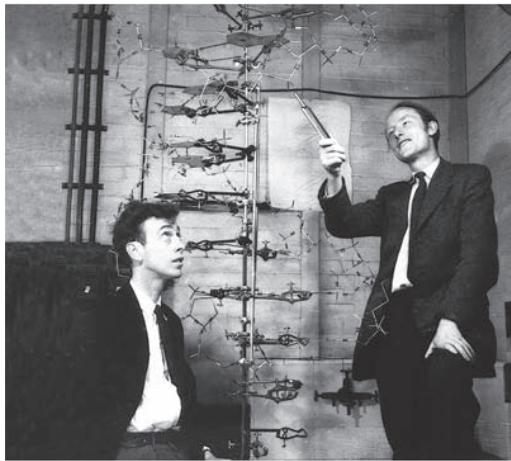


16

The Molecular Basis of Inheritance

KEY CONCEPTS

- 16.1 DNA is the genetic material
- 16.2 Many proteins work together in DNA replication and repair
- 16.3 A chromosome consists of a DNA molecule packed together with proteins



▲ James Watson (left) and Francis Crick with their DNA model.

▲ **Figure 16.1** What is the structure of DNA?

Life's Operating Instructions

In April 1953, James Watson and Francis Crick shook the scientific world by proposing an elegant double-helical model for the structure of deoxyribonucleic acid, or DNA (**Figure 16.1**). The photo at the lower left shows the DNA model they constructed from sheet metal and wire. Over the past 60 years, their model has become an icon of modern biology. Gregor Mendel's heritable factors and Thomas Hunt Morgan's genes on chromosomes are, in fact, composed of DNA. Chemically speaking, your genetic endowment is the DNA you inherited from your parents. DNA, the substance of inheritance, is the most celebrated molecule of our time.

Of all nature's molecules, nucleic acids are unique in their ability to direct their own replication from monomers. Indeed, the resemblance of offspring to their parents has its basis in the accurate replication of DNA and its transmission from one generation to the next. Hereditary information in DNA directs the development of your biochemical, anatomical, physiological, and, to some extent, behavioral traits. In this chapter, you will discover how biologists deduced that DNA is the genetic material and how Watson and Crick worked out its structure. You will also learn how a molecule of DNA is copied during **DNA replication** and how cells repair their DNA. Finally, you will explore how a molecule of DNA is packaged together with proteins in a chromosome.

CONCEPT 16.1

DNA is the genetic material

Today, even schoolchildren have heard of DNA, and scientists routinely manipulate DNA in the laboratory, often to change the heritable traits of cells in their experiments. Early in the 20th century, however, identifying the molecules of inheritance loomed as a major challenge to biologists.

The Search for the Genetic Material: Scientific Inquiry

Once T. H. Morgan's group showed that genes exist as parts of chromosomes (described in Chapter 15), the two chemical components of chromosomes—DNA and protein—emerged as the leading candidates for the genetic material. Until the 1940s, the case for proteins seemed stronger: Biochemists had identified proteins as a class of macromolecules with great heterogeneity and specificity of function, essential requirements for the hereditary material. Moreover, little was known about nucleic acids, whose physical and chemical properties seemed far too uniform to account for the multitude of specific inherited traits exhibited by every organism. This view gradually changed as the role of DNA in heredity was worked out in studies of bacteria and the viruses that infect them, systems far simpler than fruit flies or humans. Let's trace the search for the genetic material as a case study in scientific inquiry.

Evidence That DNA Can Transform Bacteria

In 1928, a British medical officer named Frederick Griffith was trying to develop a vaccine against pneumonia. He was studying *Streptococcus pneumoniae*, a bacterium that causes pneumonia in mammals. Griffith had two strains (varieties) of the bacterium, one pathogenic (disease-causing) and one nonpathogenic (harmless). He was surprised to find that when he killed the pathogenic bacteria with heat and then mixed the cell remains with living bacteria of the nonpathogenic strain, some of the living cells became pathogenic (Figure 16.2). Furthermore, this newly acquired trait of pathogenicity was inherited by all the descendants of the transformed bacteria. Apparently, some chemical component of the dead pathogenic cells caused this heritable change, although the identity of the substance was not known. Griffith called the phenomenon **transformation**, now defined as a change in genotype and phenotype due to the assimilation of external DNA by a cell. Later work by Oswald Avery, Maclyn McCarty, and Colin MacLeod identified the transforming substance as DNA.

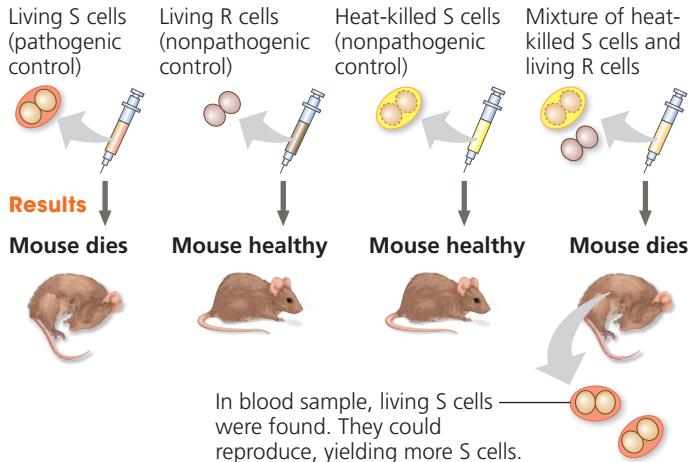
Scientists remained skeptical, however, many still viewing proteins as better candidates for the genetic material. Also, many biologists were not convinced that bacterial genes would be similar in composition and function to those of

▼ Figure 16.2

Inquiry

Can a genetic trait be transferred between different bacterial strains?

Experiment Frederick Griffith studied two strains of the bacterium *Streptococcus pneumoniae*. The S (smooth) strain can cause pneumonia in mice; it is pathogenic because the cells have an outer capsule that protects them from an animal's immune system. Cells of the R (rough) strain lack a capsule and are nonpathogenic. To test for the trait of pathogenicity, Griffith injected mice with the two strains:



Conclusion The living R bacteria had been transformed into pathogenic S bacteria by an unknown, heritable substance from the dead S cells that enabled the R cells to make capsules.

Source: F. Griffith, The significance of pneumococcal types, *Journal of Hygiene* 27:113–159 (1928).

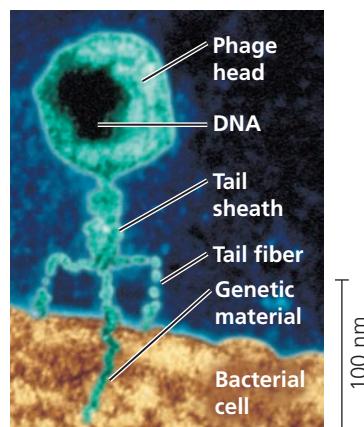
WHAT IF? How did this experiment rule out the possibility that the R cells simply used the dead S cells' capsules to become pathogenic?

more complex organisms. But the major reason for the continued doubt was that so little was known about DNA.

Evidence That Viral DNA Can Program Cells

Additional evidence that DNA was the genetic material came from studies of viruses that infect bacteria (Figure 16.3). These viruses are called **bacteriophages** (meaning “bacteria-eaters”), or **phages** for short. Viruses are much simpler than

► Figure 16.3 A virus infecting a bacterial cell. A phage called T2 attaches to a host cell and injects its genetic material through the plasma membrane, while the head and tail parts remain on the outer bacterial surface (colorized TEM).



cells. A **virus** is little more than DNA (or sometimes RNA) enclosed by a protective coat, which is often simply protein. To produce more viruses, a virus must infect a cell and take over the cell's metabolic machinery.

Phages have been widely used as tools by researchers in molecular genetics. In 1952, Alfred Hershey and Martha Chase performed experiments showing that DNA is the genetic material of a phage known as T2. This is one of many phages that infect *Escherichia coli* (*E. coli*), a bacterium that normally lives in the intestines of mammals and is a model organism for molecular biologists. At that time,

biologists already knew that T2, like many other phages, was composed almost entirely of DNA and protein. They also knew that the T2 phage could quickly turn an *E. coli* cell into a T2-producing factory that released many copies of new phages when the cell ruptured. Somehow, T2 could reprogram its host cell to produce viruses. But which viral component—protein or DNA—was responsible?

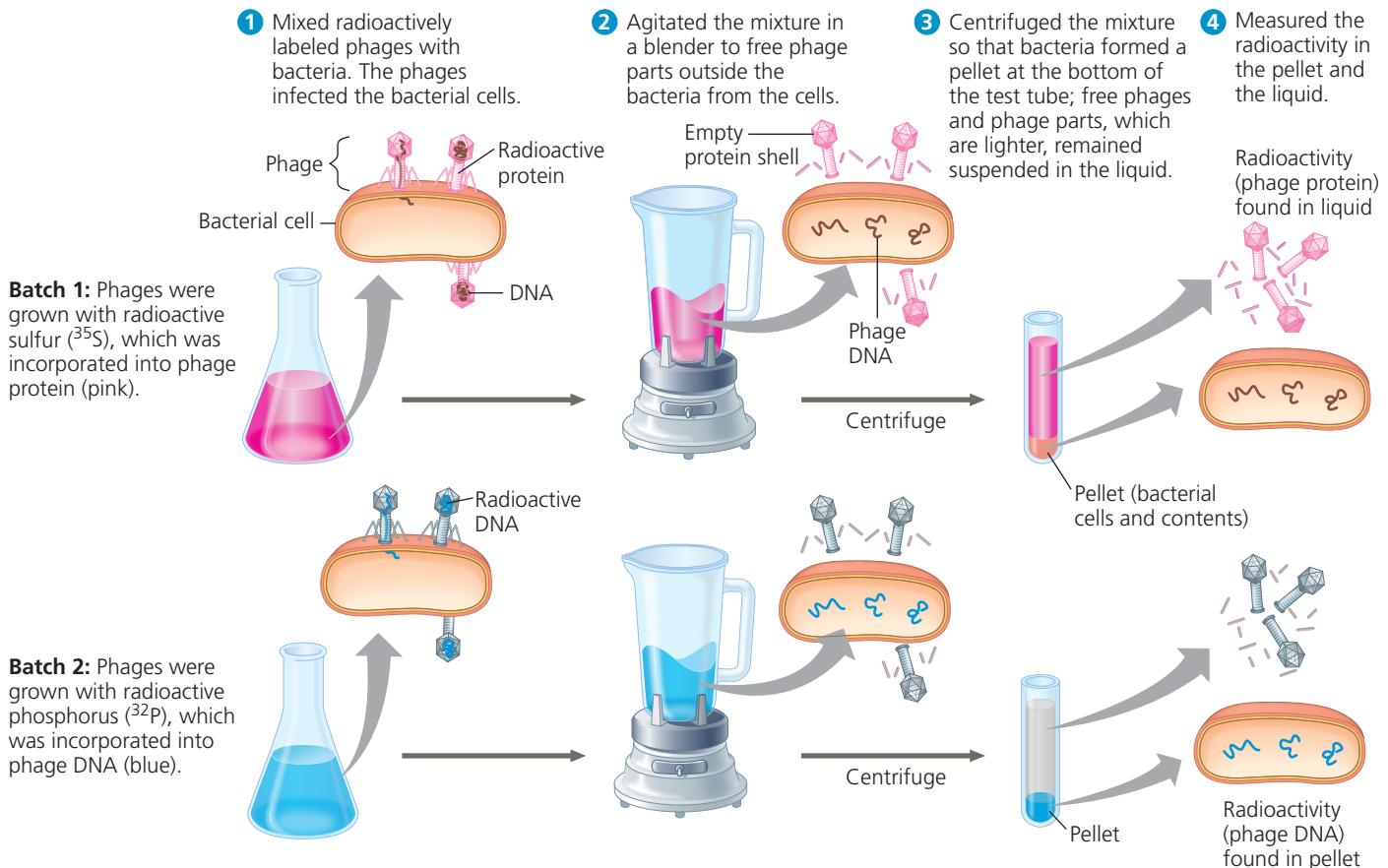
Hershey and Chase answered this question by devising an experiment showing that only one of the two components of T2 actually enters the *E. coli* cell during infection (Figure 16.4). In their experiment, they used a radioactive

▼ Figure 16.4

Inquiry

Is protein or DNA the genetic material of phage T2?

Experiment Alfred Hershey and Martha Chase used radioactive sulfur and phosphorus to trace the fates of protein and DNA, respectively, of T2 phages that infected bacterial cells. They wanted to see which of these molecules entered the cells and could reprogram them to make more phages.



Results When proteins were labeled (batch 1), radioactivity remained outside the cells, but when DNA was labeled (batch 2), radioactivity was found inside the cells. Bacterial cells containing radioactive phage DNA released new phages with some radioactive phosphorus.

Conclusion Phage DNA entered bacterial cells, but phage proteins did not. Hershey and Chase concluded that DNA, not protein, functions as the genetic material of phage T2.

Source: A. D. Hershey and M. Chase, Independent functions of viral protein and nucleic acid in growth of bacteriophage, *Journal of General Physiology* 36:39–56 (1952).

WHAT IF? How would the results have differed if proteins carried the genetic information?

isotope of sulfur to tag protein in one batch of T2 and a radioactive isotope of phosphorus to tag DNA in a second batch. Because protein, but not DNA, contains sulfur, radioactive sulfur atoms were incorporated only into the protein of the phage. In a similar way, the atoms of radioactive phosphorus labeled only the DNA, not the protein, because nearly all the phage's phosphorus is in its DNA. In the experiment, separate samples of nonradioactive *E. coli* cells were infected with the protein-labeled and DNA-labeled batches of T2. The researchers then tested the two samples shortly after the onset of infection to see which type of molecule—protein or DNA—had entered the bacterial cells and would therefore be capable of reprogramming them.

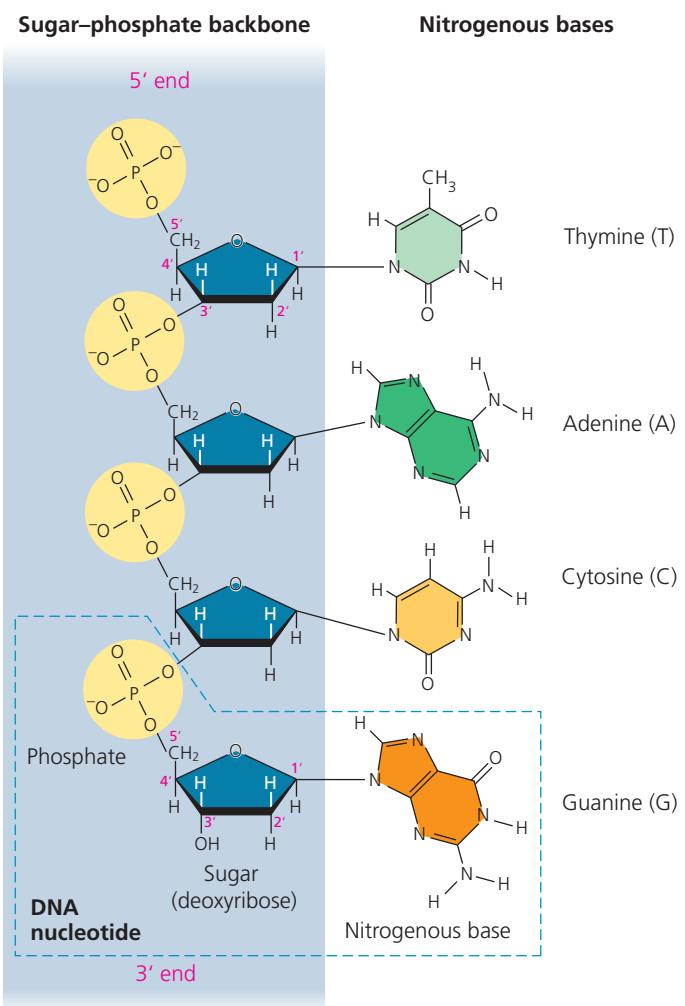
Hershey and Chase found that the phage DNA entered the host cells but the phage protein did not. Moreover, when these bacteria were returned to a culture medium, and the infection ran its course, the *E. coli* released phages that contained some radioactive phosphorus. This result further showed that the DNA inside the cell played an ongoing role during the infection process.

Hershey and Chase concluded that the DNA injected by the phage must be the molecule carrying the genetic information that makes the cells produce new viral DNA and proteins. The Hershey-Chase experiment was a landmark study because it provided powerful evidence that nucleic acids, rather than proteins, are the hereditary material, at least for certain viruses.

Additional Evidence That DNA Is the Genetic Material

Further evidence that DNA is the genetic material came from the laboratory of biochemist Erwin Chargaff. It was already known that DNA is a polymer of nucleotides, each consisting of three components: a nitrogenous (nitrogen-containing) base, a pentose sugar called deoxyribose, and a phosphate group (Figure 16.5). The base can be adenine (A), thymine (T), guanine (G), or cytosine (C). Chargaff analyzed the base composition of DNA from a number of different organisms. In 1950, he reported that the base composition of DNA varies from one species to another. For example, he found that 32.8% of sea urchin DNA nucleotides have the base A, whereas only 30.4% of human DNA nucleotides have the base A and only 24.7% of the DNA nucleotides from the bacterium *E. coli* have the base A. Chargaff's evidence of molecular diversity among species, which most scientists had presumed to be absent from DNA, made DNA a more credible candidate for the genetic material.

Chargaff also noticed a peculiar regularity in the ratios of nucleotide bases. In the DNA of each species he studied, the number of adenines approximately equaled the number



▲ Figure 16.5 The structure of a DNA strand. Each of the four DNA nucleotide monomers consists of a nitrogenous base (T, A, C, or G), the sugar deoxyribose (blue), and a phosphate group (yellow). The phosphate group of one nucleotide is attached to the sugar of the next, forming a “backbone” of alternating phosphates and sugars from which the bases project. The polynucleotide strand has directionality, from the 5' end (with the phosphate group) to the 3' end (with the —OH group of the sugar). 5' and 3' refer to the numbers assigned to the carbons in the sugar ring.

of thymines, and the number of guanines approximately equaled the number of cytosines. In sea urchin DNA, for example, Chargaff's analysis found the four bases in these percentages: A = 32.8% and T = 32.1%; G = 17.7% and C = 17.3%. (The percentages are not exactly the same because of limitations in Chargaff's techniques.)

These two findings became known as *Chargaff's rules*: (1) the base composition v of DNA varies between species, and (2) for each species, the percentages of A and T bases are roughly equal and the percentages of G and C bases are roughly equal. In the **Scientific Skills Exercise**, you can use Chargaff's rules to predict unknown percentages of nucleotide bases. The basis for these rules remained unexplained until the discovery of the double helix.

SCIENTIFIC SKILLS EXERCISE

Working with Data in a Table

Given the Percentage Composition of One Nucleotide in a Genome, Can We Predict the Percentages of the Other Three Nucleotides? Even before the structure of DNA was elucidated, Erwin Chargaff and his coworkers noticed a pattern in the base composition of nucleotides from different organisms: The percentage of adenine (A) bases roughly equaled that of thymine (T) bases, and the percentage of cytosine (C) bases roughly equaled that of guanine (G) bases. Further, the percentage of each pair (A/T or C/G) varied from species to species. We now know that the 1:1 A/T and C/G ratios are due to complementary base pairing between A and T and between C and G in the DNA double helix, and interspecies differences are due to the unique sequences of bases along a DNA strand. In this exercise, you will apply Chargaff's rules to predict the composition of bases in a genome.

How the Experiments Were Done In Chargaff's experiments, DNA was extracted from the given organism, hydrolyzed to break apart the individual nucleotides, and then analyzed chemically. (These experiments provided approximate values for each type of nucleotide. Today, whole-genome sequencing allows base composition analysis to be done more precisely directly from the sequence data.)

Data from the Experiments Tables are useful for organizing sets of data representing a common set of values (here, percentages of A, G, C, and T) for a number of different samples (in this case, from different species). You can apply the patterns that you see in the known data to predict unknown values. In the table at the upper right, complete base distribution data are given for sea urchin DNA and salmon DNA; you will use Chargaff's rules to fill in the rest of the table with predicted values.

Source of DNA	Base Percentage			
	Adenine	Guanine	Cytosine	Thymine
Sea urchin	32.8	17.7	17.3	32.1
Salmon	29.7	20.8	20.4	29.1
Wheat	28.1	21.8	22.7	
<i>E. coli</i>	24.7	26.0		
Human	30.4			30.1
Ox	29.0			

Interpret the Data

- Explain how the sea urchin and salmon data demonstrate both of Chargaff's rules.
- Using Chargaff's rules, fill in the table with your predictions of the missing percentages of bases, starting with the wheat genome and proceeding through *E. coli*, human, and ox. Show how you arrived at your answers.
- If Chargaff's rule—that the amount of A equals the amount of T and the amount of C equals the amount of G—is valid, then hypothetically we could extrapolate this to the combined DNA of all species on Earth (like one huge Earth genome). To see whether the data in the table support this hypothesis, calculate the average percentage for each base in your completed table by averaging the values in each column. Does Chargaff's equivalence rule still hold true?



A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Data from several papers by Chargaff: for example, E. Chargaff et al., Composition of the desoxyribose nucleic acids of four genera of sea-urchin, *Journal of Biological Chemistry* 195:155–160 (1952).

Building a Structural Model of DNA: Scientific Inquiry

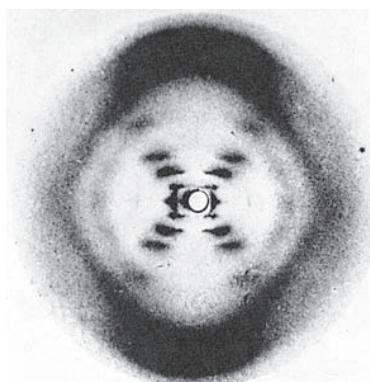
Once most biologists were convinced that DNA was the genetic material, the challenge was to determine how the structure of DNA could account for its role in inheritance. By the early 1950s, the arrangement of covalent bonds in a nucleic acid polymer was well established (see Figure 16.5), and researchers focused on discovering the three-dimensional structure of DNA. Among the scientists working on the problem were Linus Pauling, at the California Institute of Technology, and Maurice Wilkins and Rosalind Franklin, at King's College in London. First to come up with the correct answer, however, were two scientists who were relatively unknown at the time—the American James Watson and the Englishman Francis Crick.

The brief but celebrated partnership that solved the puzzle of DNA structure began soon after Watson journeyed to Cambridge University, where Crick was studying protein structure with a technique called X-ray crystallography (see Figure 5.22). While visiting the laboratory of Maurice Wilkins, Watson saw an X-ray diffraction image of DNA produced by Wilkins's accomplished colleague Rosalind Franklin (Figure 16.6a). Images produced by X-ray crystallography are not actually pictures of molecules. The spots

and smudges in Figure 16.6b were produced by X-rays that were diffracted (deflected) as they passed through aligned fibers of purified DNA. Watson was familiar with the type of X-ray diffraction pattern that helical molecules produce, and an examination of the photo that Wilkins showed him confirmed that DNA was helical in shape. It also augmented earlier data obtained by Franklin and others suggesting the



(a) Rosalind Franklin



(b) Franklin's X-ray diffraction photograph of DNA

▲ Figure 16.6 Rosalind Franklin and her X-ray diffraction photo of DNA. Franklin, a very accomplished X-ray crystallographer, conducted critical experiments resulting in the photo that allowed Watson and Crick to deduce the double-helical structure of DNA.

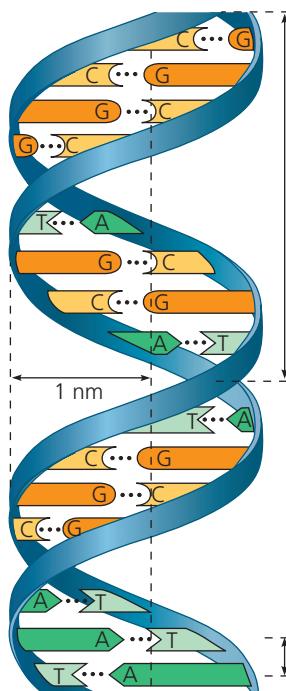
width of the helix and the spacing of the nitrogenous bases along it. The pattern in this photo implied that the helix was made up of two strands, contrary to a three-stranded model that Linus Pauling had proposed a short time earlier. The presence of two strands accounts for the now-familiar term **double helix** (Figure 16.7).

Watson and Crick began building models of a double helix that would conform to the X-ray measurements and what was then known about the chemistry of DNA, including Chargaff's rule of base equivalences. Having also read an unpublished annual report summarizing Franklin's work, they knew she had concluded that the sugar-phosphate backbones were on the outside of the DNA molecule, contrary to their working model. Franklin's arrangement was appealing because it put the relatively hydrophobic nitrogenous bases in the molecule's interior, away from the surrounding aqueous solution, and the negatively charged phosphate groups wouldn't be forced together in the interior. Watson constructed such a model, shown in the lower photo on the first page of this chapter.

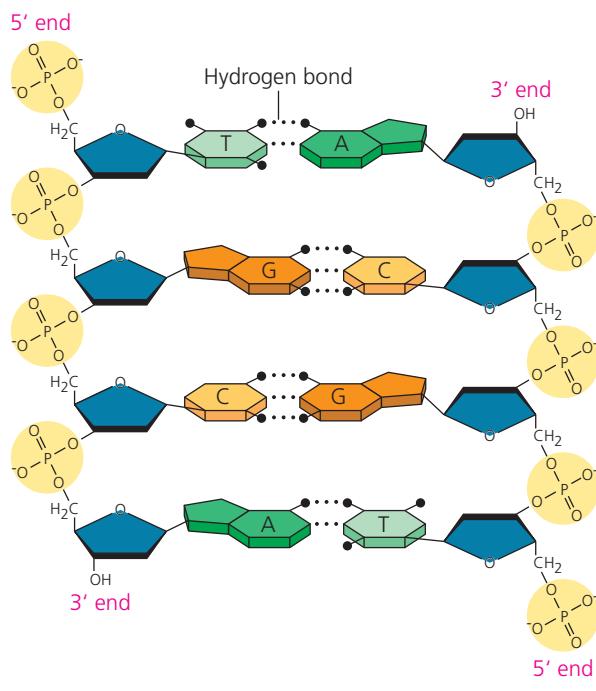
In this model, the two sugar-phosphate backbones are **antiparallel**—that is, their subunits run in opposite directions (see Figure 16.7b). You can imagine the overall

arrangement as a rope ladder with rigid rungs. The side ropes represent the sugar-phosphate backbones, and the rungs represent pairs of nitrogenous bases. Now imagine twisting the ladder to form a helix. Franklin's X-ray data indicated that the helix makes one full turn every 3.4 nm along its length. With the bases stacked just 0.34 nm apart, there are ten layers of base pairs, or rungs of the ladder, in each full turn of the helix.

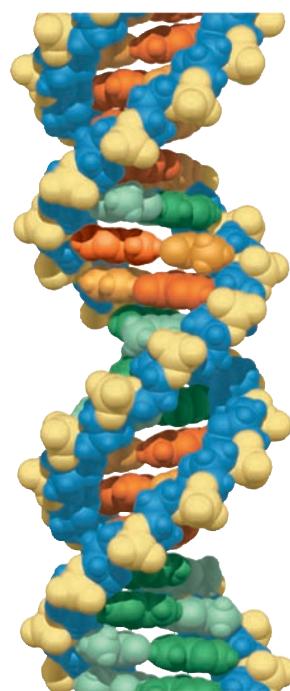
The nitrogenous bases of the double helix are paired in specific combinations: adenine (A) with thymine (T), and guanine (G) with cytosine (C). It was mainly by trial and error that Watson and Crick arrived at this key feature of DNA. At first, Watson imagined that the bases paired like with like—for example, A with A and C with C. But this model did not fit the X-ray data, which suggested that the double helix had a uniform diameter. Why is this requirement inconsistent with like-with-like pairing of bases? Adenine and guanine are purines, nitrogenous bases with two organic rings, while cytosine and thymine are nitrogenous bases called pyrimidines, which have a single ring. Thus, purines (A and G) are about twice as wide as pyrimidines (C and T). A purine-purine pair is too wide and a pyrimidine-pyrimidine pair too narrow to account for the 2-nm



(a) Key features of DNA structure. The “ribbons” in this diagram represent the sugar-phosphate backbones of the two DNA strands. The helix is “right-handed,” curving up to the right. The two strands are held together by hydrogen bonds (dotted lines) between the nitrogenous bases, which are paired in the interior of the double helix.



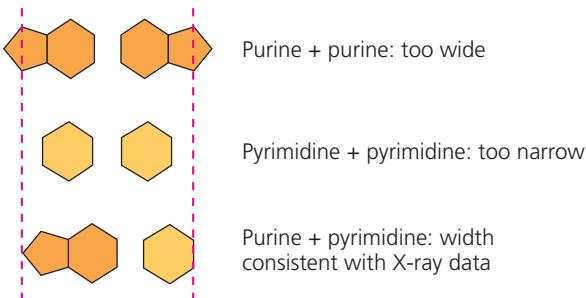
(b) Partial chemical structure. For clarity, the two DNA strands are shown untwisted in this partial chemical structure. Strong covalent bonds link the units of each strand, while weaker hydrogen bonds between the bases hold one strand to the other. Notice that the strands are antiparallel, meaning that they are oriented in opposite directions, like the lanes of a divided highway.



(c) Space-filling model. The tight stacking of the base pairs is clear in this computer-generated, space-filling model. Van der Waals interactions between the stacked pairs play a major role in holding the molecule together.

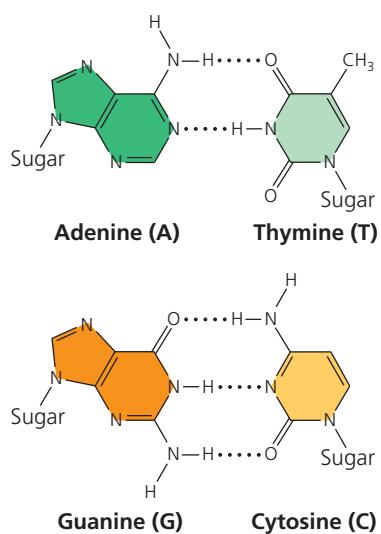
▲ **Figure 16.7** The structure of the double helix.

diameter of the double helix. Always pairing a purine with a pyrimidine, however, results in a uniform diameter:



Watson and Crick reasoned that there must be additional specificity of pairing dictated by the structure of the bases. Each base has chemical side groups that can form hydrogen bonds with its appropriate partner: Adenine can form two hydrogen bonds with thymine and only thymine; guanine forms three hydrogen bonds with cytosine and only cytosine. In shorthand, A pairs with T, and G pairs with C (**Figure 16.8**).

The Watson-Crick model took into account Chargaff's ratios and ultimately explained them. Wherever one strand of a DNA molecule has an A, the partner strand has a T. Similarly, a G in one strand is always paired with a C in the complementary strand. Therefore, in the DNA of any organism, the amount of adenine equals the amount of thymine, and the amount of guanine equals the amount of cytosine. (Modern DNA sequencing techniques have confirmed that the amounts are exactly equal.) Although the base-pairing rules dictate the combinations of nitrogenous bases that form the “rungs” of the double helix, they do not restrict the sequence of nucleotides *along* each DNA strand. The linear sequence of the four bases can be varied in countless ways, and each gene has a unique base sequence.



▲ Figure 16.8 Base pairing in DNA. The pairs of nitrogenous bases in a DNA double helix are held together by hydrogen bonds, shown here as black dotted lines.

In April 1953, Watson and Crick surprised the scientific world with a succinct, one-page paper that reported their molecular model for DNA: the double helix, which has since become the symbol of molecular biology. Watson and Crick, along with Maurice Wilkins, were awarded the Nobel Prize in 1962 for this work. (Sadly, Rosalind Franklin had died at the age of 38 in 1958 and was thus ineligible for the prize.) The beauty of the double helix model was that the structure of DNA suggested the basic mechanism of its replication.

CONCEPT CHECK 16.1

- Given a polynucleotide sequence such as GAATTC, can you tell which is the 5' end? If not, what further information do you need to identify the ends? (See Figure 16.5.)
- WHAT IF?** Griffith did not expect transformation to occur in his experiment. What results was he expecting? Explain.

For suggested answers, see Appendix A.

CONCEPT 16.2

Many proteins work together in DNA replication and repair

The relationship between structure and function is manifest in the double helix. The idea that there is specific pairing of nitrogenous bases in DNA was the flash of inspiration that led Watson and Crick to the double helix. At the same time, they saw the functional significance of the base-pairing rules. They ended their classic paper with this wry statement: “It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.”* In this section, you will learn about the basic principle of DNA replication, as well as some important details of the process.

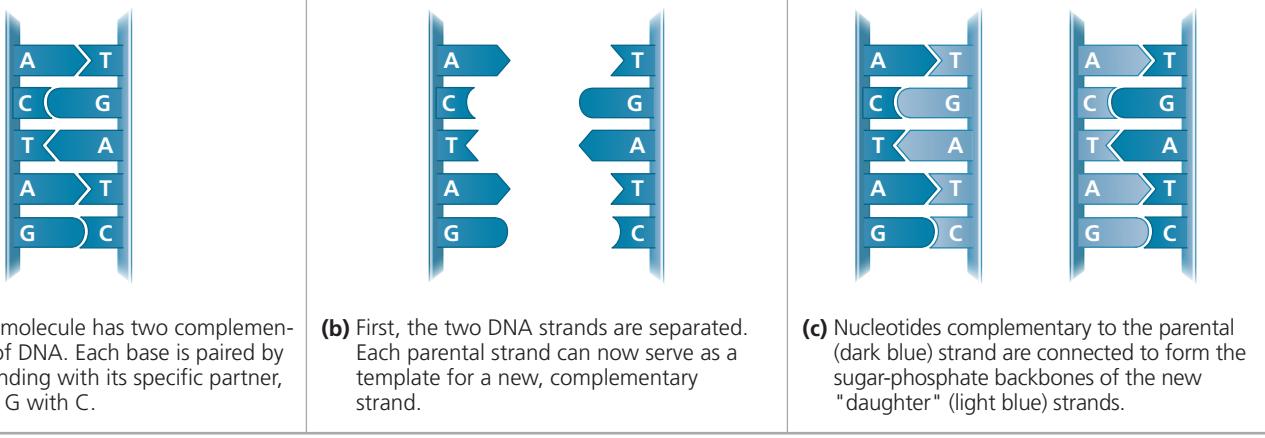
The Basic Principle: Base Pairing to a Template Strand

In a second paper, Watson and Crick stated their hypothesis for how DNA replicates:

Now our model for deoxyribonucleic acid is, in effect, a pair of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds are broken, and the two chains unwind and separate. Each chain then acts as a template for the formation on to itself of a new companion chain, so that eventually we shall have two pairs of chains, where we only had one before. Moreover, the sequence of the pairs of bases will have been duplicated exactly.†

*J. D. Watson and F. H. C. Crick, Molecular structure of nucleic acids: a structure for deoxyribose nucleic acids, *Nature* 171:737–738 (1953).

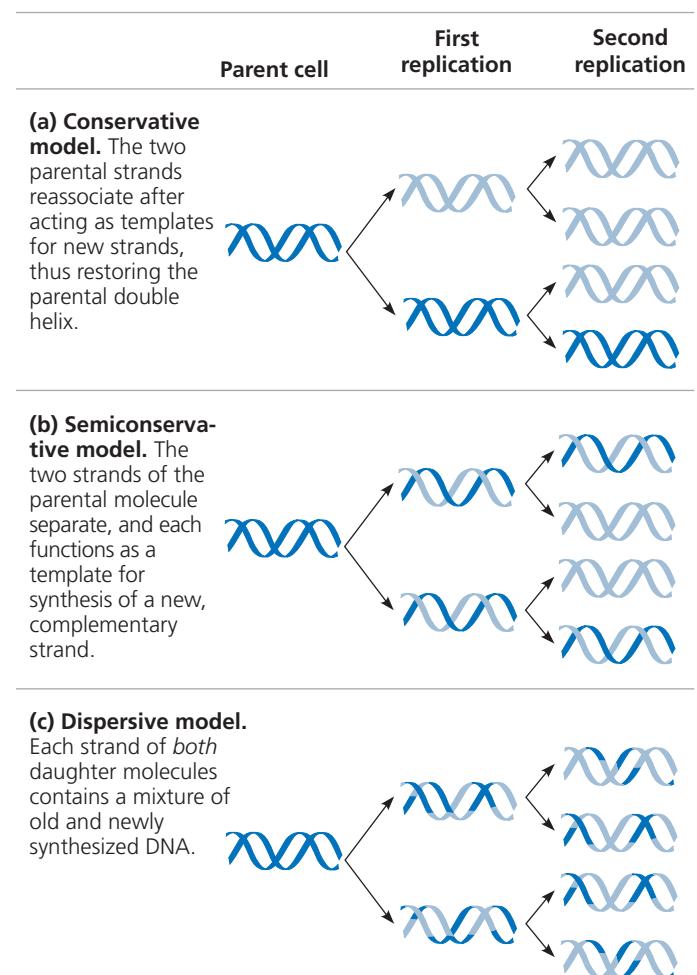
†J. D. Watson and F. H. C. Crick, Genetical implications of the structure of deoxyribonucleic acid, *Nature* 171:964–967 (1953).



▲ **Figure 16.9** A model for DNA replication: the basic concept. In this simplified illustration, a short segment of DNA has been untwisted. Simple shapes symbolize the four kinds of bases. Dark blue represents DNA strands present in the parental molecule; light blue represents newly synthesized DNA.

Figure 16.9 illustrates Watson and Crick's basic idea. To make it easier to follow, we show only a short section of double helix in untwisted form. Notice that if you cover one of the two DNA strands of Figure 16.9a, you can still determine its linear sequence of nucleotides by referring to the uncovered strand and applying the base-pairing rules. The two strands are complementary; each stores the information necessary to reconstruct the other. When a cell copies a DNA molecule, each strand serves as a template for ordering nucleotides into a new, complementary strand. Nucleotides line up along the template strand according to the base-pairing rules and are linked to form the new strands. Where there was one double-stranded DNA molecule at the beginning of the process, there are soon two, each an exact replica of the "parental" molecule. The copying mechanism is analogous to using a photographic negative to make a positive image, which can in turn be used to make another negative, and so on.

This model of DNA replication remained untested for several years following publication of the DNA structure. The requisite experiments were simple in concept but difficult to perform. Watson and Crick's model predicts that when a double helix replicates, each of the two daughter molecules will have one old strand, from the parental molecule, and one newly made strand. This **semiconservative model** can be distinguished from a conservative model of replication, in which the two parental strands somehow come back together after the process (that is, the parental molecule is conserved). In yet a third model, called the dispersive model, all four strands of DNA following replication have a mixture of old and new DNA. These three models are shown in **Figure 16.10**. Although mechanisms for conservative or dispersive DNA replication are not easy to devise, these models remained possibilities until they could be



▲ **Figure 16.10** Three alternative models of DNA replication. Each short segment of double helix symbolizes the DNA within a cell. Beginning with a parent cell, we follow the DNA for two more generations of cells—two rounds of DNA replication. Parental DNA is dark blue; newly made DNA is light blue.

ruled out. After two years of preliminary work at the California Institute of Technology in the late 1950s, Matthew Meselson and Franklin Stahl devised a clever experiment that distinguished between the three models, described in **Figure 16.11**. The results of their experiment supported the semiconservative model of DNA replication, as predicted by Watson and Crick, and is widely acknowledged among biologists to be a classic example of elegant experimental design.

The basic principle of DNA replication is conceptually simple. However, the actual process involves some complicated biochemical gymnastics, as we will now see.

DNA Replication: A Closer Look

The bacterium *E. coli* has a single chromosome of about 4.6 million nucleotide pairs. In a favorable environment, an *E. coli* cell can copy all this DNA and divide to form two genetically identical daughter cells in less than an hour. Each of your cells has 46 DNA molecules in its nucleus, one long double-helical molecule per chromosome. In all, that represents about 6 billion nucleotide pairs, or over a thousand times more DNA than is found in a bacterial cell. If we were to print the one-letter symbols for these bases (A, G, C, and T) the size of the type you are now reading, the 6 billion nucleotide pairs of information in a diploid human cell would fill about 1,400 biology textbooks. Yet it takes one of your cells just a few hours to copy all of this DNA. This replication of an enormous amount of genetic information is achieved with very few errors—only about one per 10 billion nucleotides. The copying of DNA is remarkable in its speed and accuracy.

More than a dozen enzymes and other proteins participate in DNA replication. Much more is known about how this “replication machine” works in bacteria (such as *E. coli*) than in eukaryotes, and we will describe the basic steps of the process for *E. coli*, except where otherwise noted. What scientists have learned about eukaryotic DNA replication suggests, however, that most of the process is fundamentally similar for prokaryotes and eukaryotes.

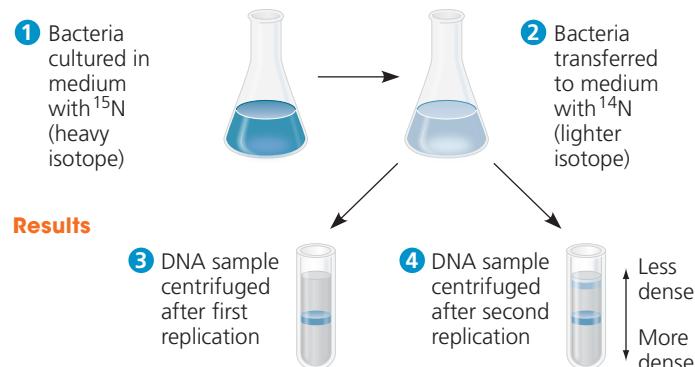
Getting Started

The replication of a chromosome begins at particular sites called **origins of replication**, short stretches of DNA having a specific sequence of nucleotides. The *E. coli* chromosome, like many other bacterial chromosomes, is circular and has a single origin. Proteins that initiate DNA replication recognize this sequence and attach to the DNA, separating the two strands and opening up a replication “bubble.” Replication of DNA then proceeds in both directions until the entire molecule is copied (**Figure 16.12a**). In contrast to a bacterial chromosome, a eukaryotic chromosome may have hundreds or even a few thousand replication origins. Multiple replication bubbles form and eventually fuse, thus speeding up the copying of the very long DNA molecules

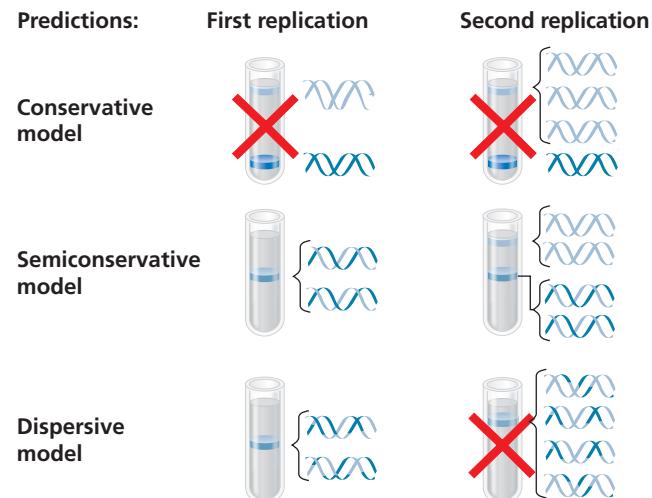
▼ **Figure 16.11** Inquiry

Does DNA replication follow the conservative, semiconservative, or dispersive model?

Experiment Matthew Meselson and Franklin Stahl cultured *E. coli* for several generations in a medium containing nucleotide precursors labeled with a heavy isotope of nitrogen, ^{15}N . They then transferred the bacteria to a medium with only ^{14}N , a lighter isotope. A sample was taken after the first DNA replication; another sample was taken after the second replication. They extracted DNA from the bacteria in the samples and then centrifuged each DNA sample to separate DNA of different densities.



Conclusion Meselson and Stahl compared their results to those predicted by each of the three models in Figure 16.10, as shown below. The first replication in the ^{14}N medium produced a band of hybrid (^{15}N - ^{14}N) DNA. This result eliminated the conservative model. The second replication produced both light and hybrid DNA, a result that refuted the dispersive model and supported the semiconservative model. They therefore concluded that DNA replication is semiconservative.

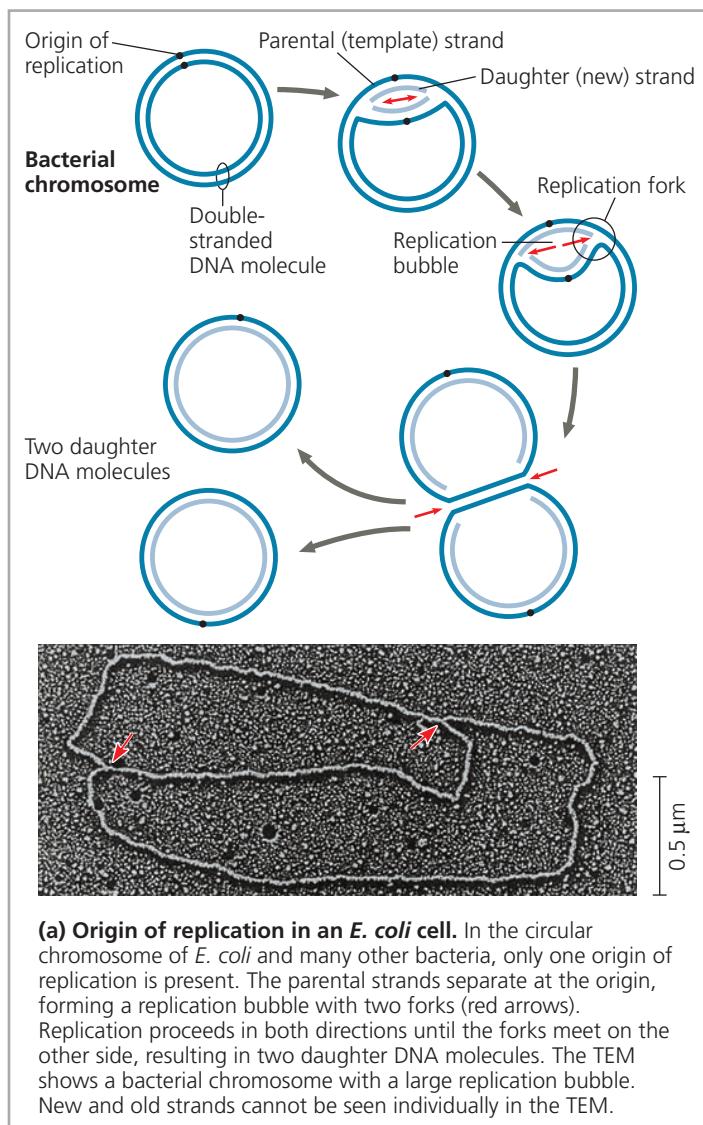


Source: M. Meselson and F. W. Stahl, The replication of DNA in *Escherichia coli*, *Proceedings of the National Academy of Sciences USA* 44:671–682 (1958).

Inquiry in Action Read and analyze the original paper in *Inquiry in Action: Interpreting Scientific Papers*.

A related Experimental Inquiry Tutorial can be assigned in MasteringBiology.

WHAT IF? If Meselson and Stahl had first grown the cells in ^{14}N -containing medium and then moved them into ^{15}N -containing medium before taking samples, what would have been the result?



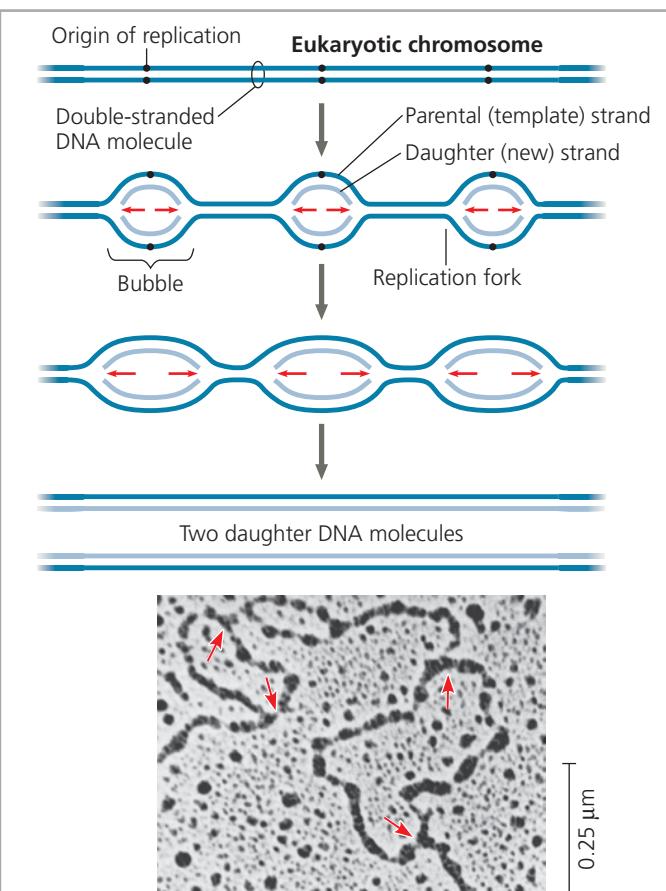
▲ Figure 16.12 Origins of replication in *E. coli* and eukaryotes.

The red arrows indicate the movement of the replication forks and thus the overall directions of DNA replication within each bubble.

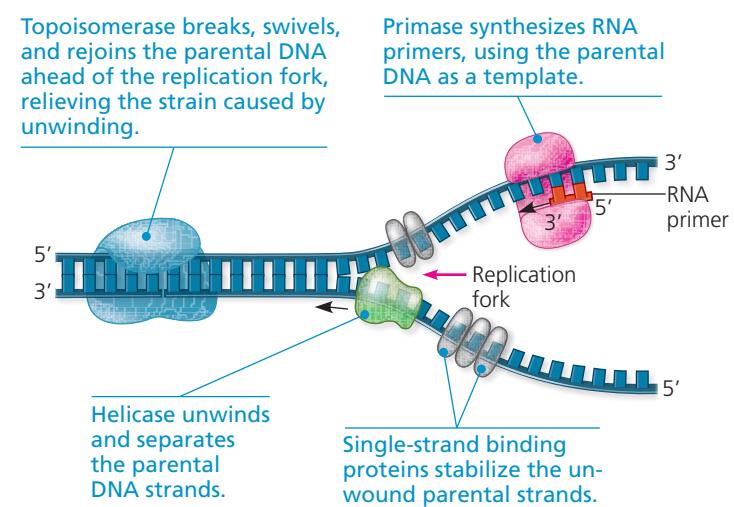
(Figure 16.12b). As in bacteria, eukaryotic DNA replication proceeds in both directions from each origin.

At each end of a replication bubble is a **replication fork**, a Y-shaped region where the parental strands of DNA are being unwound. Several kinds of proteins participate in the unwinding (Figure 16.13). **Helicases** are enzymes that untwist the double helix at the replication forks, separating the two parental strands and making them available as template strands. After the parental strands separate, **single-strand binding proteins** bind to the unpaired DNA strands, keeping them from re-pairing. The untwisting of the double helix causes tighter twisting and strain ahead of the replication fork. **Topoisomerase** helps relieve this strain by breaking, swiveling, and rejoining DNA strands.

The unwound sections of parental DNA strands are now available to serve as templates for the synthesis of new



DRAW IT In the TEM, add arrows in the forks of the third bubble.



▲ Figure 16.13 Some of the proteins involved in the initiation of DNA replication. The same proteins function at both replication forks in a replication bubble. For simplicity, only the left-hand fork is shown, and the DNA bases are drawn much larger in relation to the proteins than they are in reality.

complementary DNA strands. However, the enzymes that synthesize DNA cannot *initiate* the synthesis of a polynucleotide; they can only add DNA nucleotides to the end of an already existing chain that is base-paired with the template strand. The initial nucleotide chain that is produced during DNA synthesis is actually a short stretch of RNA, not DNA. This RNA chain is called a **primer** and is synthesized by the enzyme **primase** (see Figure 16.13). Primase starts a complementary RNA chain from a single RNA nucleotide, adding more RNA nucleotides one at a time, using the parental DNA strand as a template. The completed primer, generally 5–10 nucleotides long, is thus base-paired to the template strand. The new DNA strand will start from the 3' end of the RNA primer.

Synthesizing a New DNA Strand

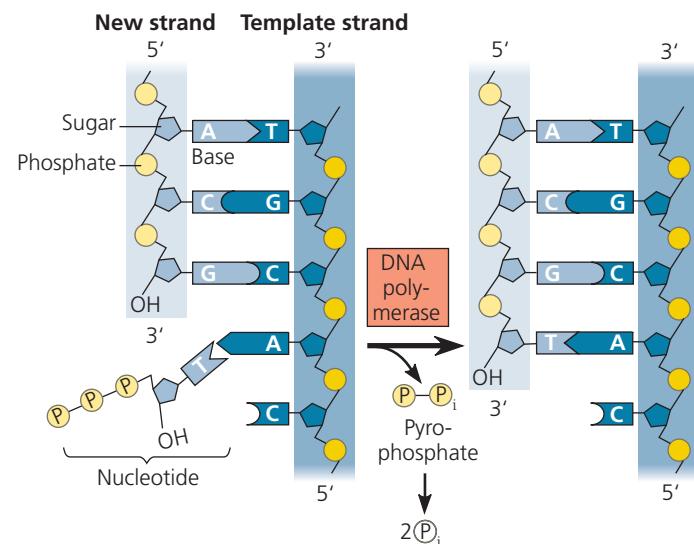
Enzymes called **DNA polymerases** catalyze the synthesis of new DNA by adding nucleotides to a preexisting chain. In *E. coli*, there are several different DNA polymerases, but two appear to play the major roles in DNA replication: DNA polymerase III and DNA polymerase I. The situation in eukaryotes is more complicated, with at least 11 different DNA polymerases discovered so far; however, the general principles are the same.

Most DNA polymerases require a primer and a DNA template strand, along which complementary DNA nucleotides are lined up. In *E. coli*, DNA polymerase III (abbreviated DNA pol III) adds a DNA nucleotide to the RNA primer and then continues adding DNA nucleotides, complementary to the parental DNA template strand, to the growing end of the new DNA strand. The rate of elongation is about 500 nucleotides per second in bacteria and 50 per second in human cells.

Each nucleotide to be added to a growing DNA strand consists of a sugar attached to a base and to three phosphate groups. You have already encountered such a molecule—ATP (adenosine triphosphate; see Figure 8.9). The only difference between the ATP of energy metabolism and dATP, the adenine nucleotide used to make DNA, is the sugar component, which is deoxyribose in the building block of DNA but ribose in ATP. Like ATP, the nucleotides used for DNA synthesis are chemically reactive, partly because their triphosphate tails have an unstable cluster of negative charge. As each monomer joins the growing end of a DNA strand, two phosphate groups are lost as a molecule of pyrophosphate ($\text{P}_\text{i} - \text{P}_\text{i}$). Subsequent hydrolysis of the pyrophosphate to two molecules of inorganic phosphate (P_i) is a coupled exergonic reaction that helps drive the polymerization reaction (Figure 16.14).

Antiparallel Elongation

As we have noted previously, the two ends of a DNA strand are different, giving each strand directionality, like a one-way street (see Figure 16.5). In addition, the two strands of



▲ Figure 16.14 Incorporation of a nucleotide into a DNA strand. DNA polymerase catalyzes the addition of a nucleotide to the 3' end of a growing DNA strand, with the release of two phosphates.

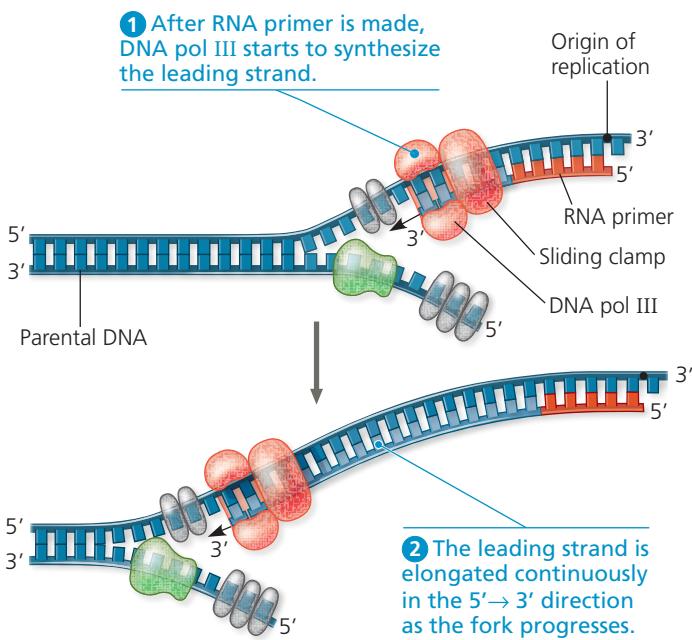
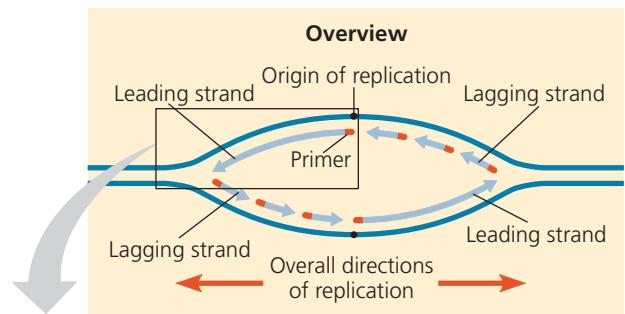
? Use this diagram to explain what we mean when we say that each DNA strand has directionality.

DNA in a double helix are antiparallel, meaning that they are oriented in opposite directions to each other, like the lanes of a divided highway (see Figure 16.14). Therefore, the two new strands formed during DNA replication must also be antiparallel to their template strands.

How does the antiparallel arrangement of the double helix affect replication? Because of their structure, DNA polymerases can add nucleotides only to the free 3' end of a primer or growing DNA strand, never to the 5' end (see Figure 16.14). Thus, a new DNA strand can elongate only in the 5' → 3' direction. With this in mind, let's examine one of the two replication forks in a bubble (Figure 16.15). Along one template strand, DNA polymerase III can synthesize a complementary strand continuously by elongating the new DNA in the mandatory 5' → 3' direction. DNA pol III remains in the replication fork on that template strand and continuously adds nucleotides to the new complementary strand as the fork progresses. The DNA strand made by this mechanism is called the **leading strand**. Only one primer is required for DNA pol III to synthesize the entire leading strand (see Figure 16.15).

To elongate the other new strand of DNA in the mandatory 5' → 3' direction, DNA pol III must work along the other template strand in the direction *away from* the replication fork. The DNA strand elongating in this direction is called the **lagging strand**.* In contrast to the leading strand,

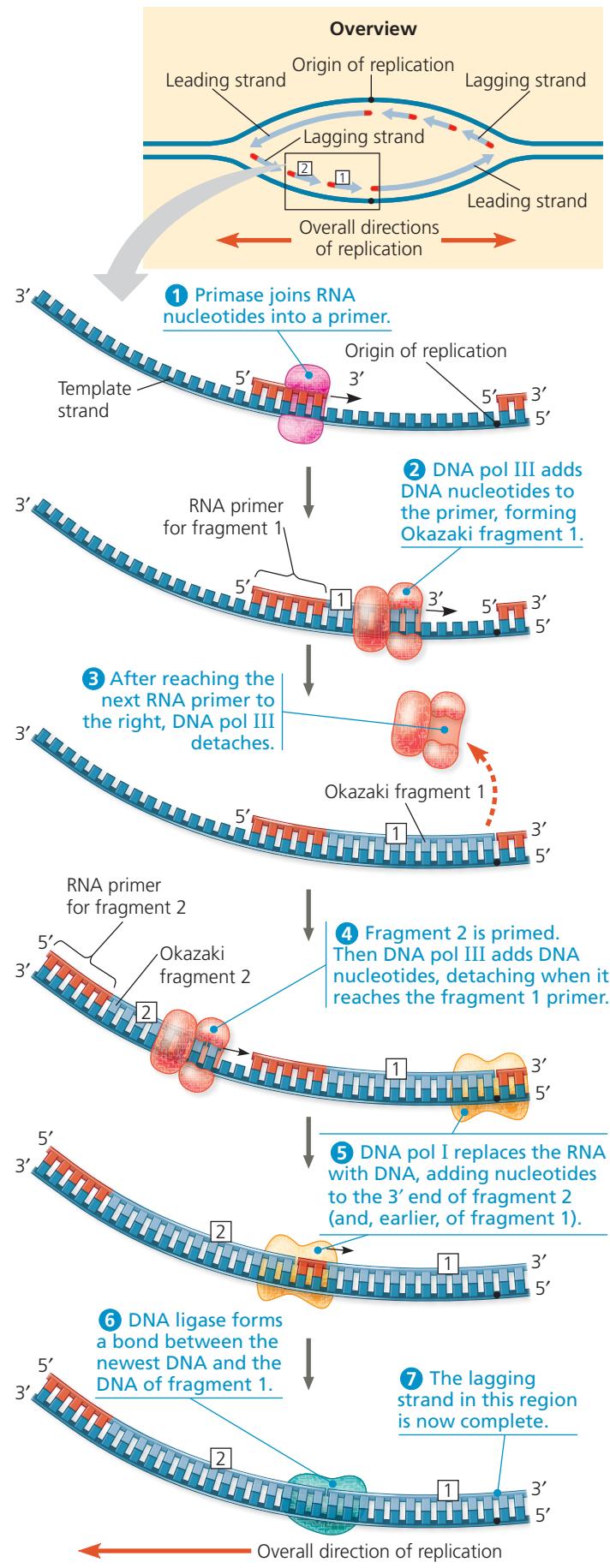
*Synthesis of the leading strand and synthesis of the lagging strand occur concurrently and at the same rate. The lagging strand is so named because its synthesis is delayed slightly relative to synthesis of the leading strand; each new fragment of the lagging strand cannot be started until enough template has been exposed at the replication fork.



▲ Figure 16.15 Synthesis of the leading strand during DNA replication. This diagram focuses on the left replication fork shown in the overview box. DNA polymerase III (DNA pol III), shaped like a cuffed hand, is shown closely associated with a protein called the “sliding clamp” that encircles the newly synthesized double helix like a doughnut. The sliding clamp moves DNA pol III along the DNA template strand.

which elongates continuously, the lagging strand is synthesized discontinuously, as a series of segments. These segments of the lagging strand are called **Okazaki fragments**, after the Japanese scientist who discovered them. The fragments are about 1,000–2,000 nucleotides long in *E. coli* and 100–200 nucleotides long in eukaryotes.

Figure 16.16 illustrates the steps in the synthesis of the lagging strand at one fork. Whereas only one primer is required on the leading strand, each Okazaki fragment on the lagging strand must be primed separately (steps **4** and **4**). After DNA pol III forms an Okazaki fragment (steps **2**–**4**), another DNA polymerase, DNA polymerase I (DNA pol I), replaces the RNA nucleotides of the adjacent primer with DNA nucleotides (step **5**). But DNA pol I cannot join the final nucleotide of this replacement DNA segment to the first DNA nucleotide of the adjacent Okazaki fragment. Another enzyme, **DNA ligase**, accomplishes this task, joining the sugar-phosphate backbones of all the Okazaki fragments into a continuous DNA strand (step **6**).



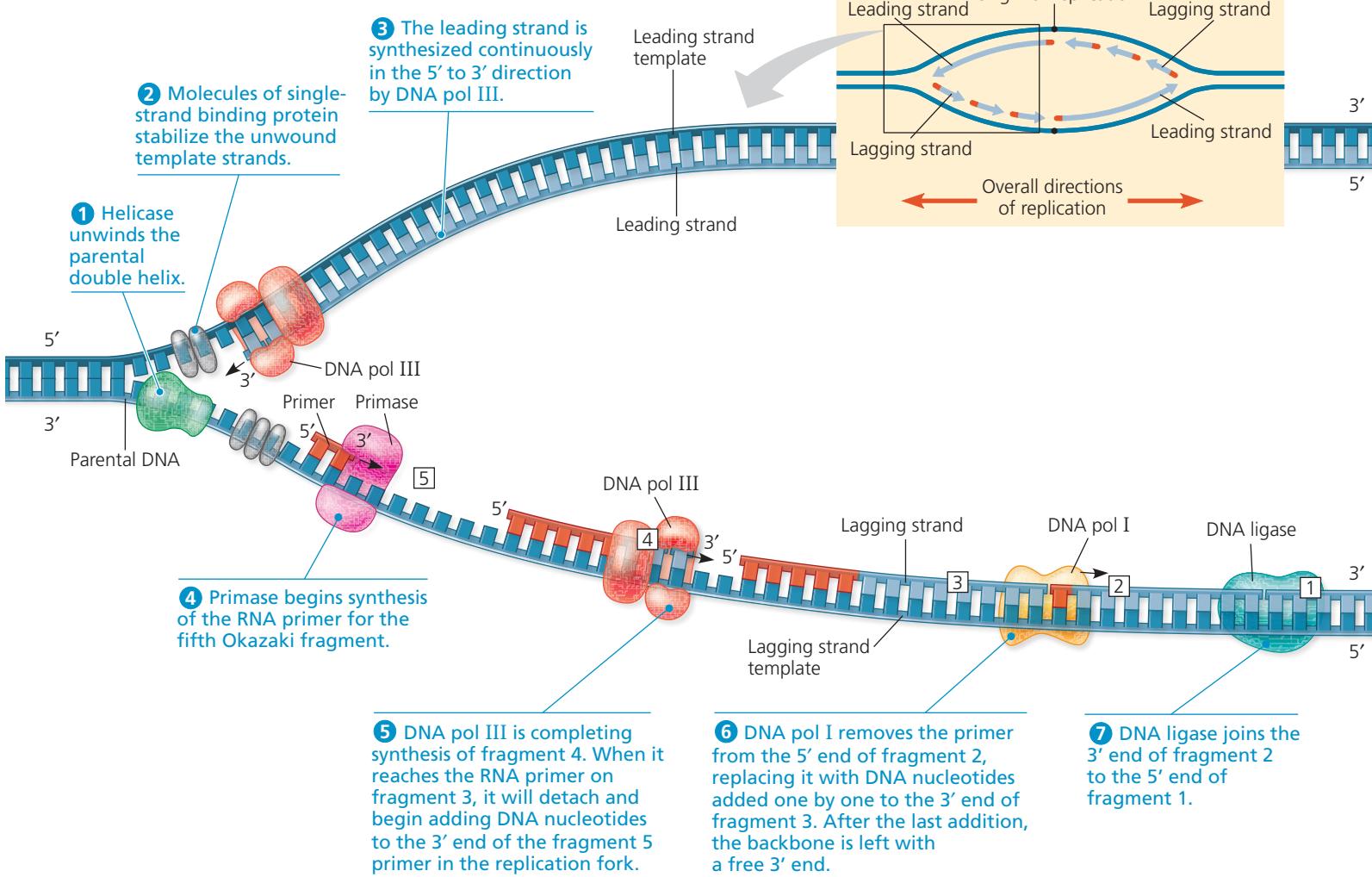
▲ Figure 16.16 Synthesis of the lagging strand.

Figure 16.17 and **Table 16.1** summarize DNA replication. Please study them carefully before proceeding.

The DNA Replication Complex

It is traditional—and convenient—to represent DNA polymerase molecules as locomotives moving along a DNA railroad track, but such a model is inaccurate in two important ways. First, the various proteins that participate in DNA replication actually form a single large complex, a “DNA replication machine.” Many protein-protein interactions facilitate the efficiency of this complex. For example, by interacting with other proteins at the fork, primase apparently acts as a molecular brake, slowing progress of the replication fork and coordinating the placement of primers and

the rates of replication on the leading and lagging strands. Second, the DNA replication complex may not move along the DNA; rather, the DNA may move through the complex during the replication process. In eukaryotic cells, multiple copies of the complex, perhaps grouped into “factories,” may be anchored to the nuclear matrix, a framework of fibers extending through the interior of the nucleus. Experimental evidence supports a model in which two DNA polymerase molecules, one on each template strand, “reel in” the parental DNA and extrude newly made daughter DNA molecules. In this so-called trombone model, the lagging strand is also looped back through the complex (**Figure 16.18**).



▲ Figure 16.17 A summary of bacterial DNA replication. The detailed diagram shows the left-hand replication fork of the replication bubble shown in the overview (upper right). Viewing each daughter strand in its entirety in the overview, you can see that half of it is made continuously as the leading strand, while the other half (on the other side of the origin) is synthesized in fragments as the lagging strand.

DRAW IT Draw a similar diagram showing the right-hand fork of this bubble, numbering the Okazaki fragments appropriately. Label all 5' and 3' ends.

Table 16.1 Bacterial DNA Replication Proteins and Their Functions

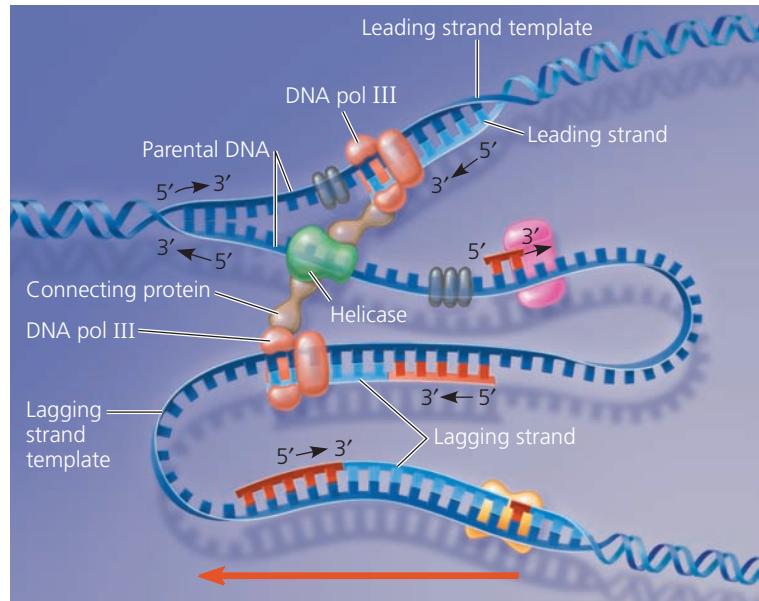
Protein	Function
Helicase	Unwinds parental double helix at replication forks
Single-strand binding protein	Binds to and stabilizes single-stranded DNA until it is used as a template
Topoisomerase	Relieves overwinding strain ahead of replication forks by breaking, swiveling, and rejoining DNA strands
Primase	Synthesizes an RNA primer at 5' end of leading strand and at 5' end of each Okazaki fragment of lagging strand
DNA pol III	Using parental DNA as a template, synthesizes new DNA strand by adding nucleotides to an RNA primer or a pre-existing DNA strand
DNA pol I	Removes RNA nucleotides of primer from 5' end and replaces them with DNA nucleotides
DNA ligase	Joins Okazaki fragments of lagging strand; on leading strand, joins 3' end of DNA that replaces primer to rest of leading strand DNA

Proofreading and Repairing DNA

We cannot attribute the accuracy of DNA replication solely to the specificity of base pairing. Initial pairing errors between incoming nucleotides and those in the template strand occur at a rate of one in 10^5 nucleotides. However, errors in the completed DNA molecule amount to only one in 10^{10} (10 billion) nucleotides, an error rate that is 100,000 times lower. This is because during DNA replication, DNA polymerases proofread each nucleotide against its template as soon as it is covalently bonded to the growing strand. Upon finding an incorrectly paired nucleotide, the polymerase removes the nucleotide and then resumes synthesis. (This action is similar to fixing a word processing error by deleting the wrong letter and then entering the correct letter.)

Mismatched nucleotides sometimes evade proofreading by a DNA polymerase. In **mismatch repair**, other enzymes remove and replace incorrectly paired nucleotides that have resulted from replication errors. Researchers spotlighted the importance of such repair enzymes when they found that a hereditary defect in one of them is associated with a form of colon cancer. Apparently, this defect allows cancer-causing errors to accumulate in the DNA faster than normal.

Incorrectly paired or altered nucleotides can also arise after replication. In fact, maintenance of the genetic



▲ Figure 16.18 A current model of the DNA replication complex. Two DNA polymerase III molecules work together in a complex with helicase and other proteins. One DNA polymerase acts on each template strand. The lagging strand template DNA loops through the complex, resembling the slide of a trombone. (This is often called the **trombone model**.)

DRAW IT Draw a line tracing the lagging strand template along the entire stretch of DNA shown here.

ANIMATION

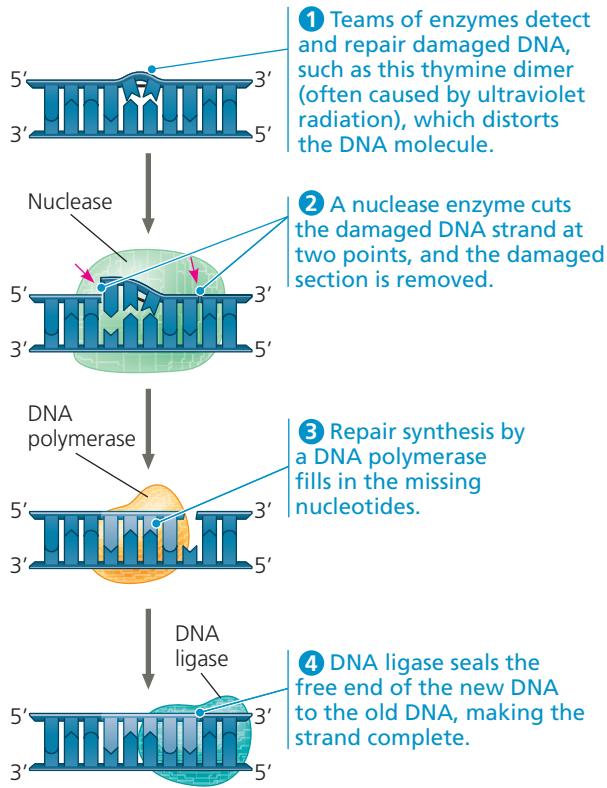


BioFlix

Visit the Study Area in **MasteringBiology** for the BioFlix® 3-D Animation on DNA Replication. BioFlix Tutorials can also be assigned in MasteringBiology.

information encoded in DNA requires frequent repair of various kinds of damage to existing DNA. DNA molecules are constantly subjected to potentially harmful chemical and physical agents, such as X-rays, as we'll discuss in Chapter 17. In addition, DNA bases often undergo spontaneous chemical changes under normal cellular conditions. However, these changes in DNA are usually corrected before they become permanent changes—*mutations*—perpetuated through successive replications. Each cell continuously monitors and repairs its genetic material. Because repair of damaged DNA is so important to the survival of an organism, it is no surprise that many different DNA repair enzymes have evolved. Almost 100 are known in *E. coli*, and about 130 have been identified so far in humans.

Most cellular systems for repairing incorrectly paired nucleotides, whether they are due to DNA damage or to replication errors, use a mechanism that takes advantage of the base-paired structure of DNA. In many cases, a segment of the strand containing the damage is cut out (excised) by a DNA-cutting enzyme—a **nuclease**—and the resulting gap is then filled in with nucleotides, using the undamaged strand as a template. The enzymes involved in filling the gap are



▲ Figure 16.19 Nucleotide excision repair of DNA damage.

a DNA polymerase and DNA ligase. One such DNA repair system is called **nucleotide excision repair** (Figure 16.19).

An important function of the DNA repair enzymes in our skin cells is to repair genetic damage caused by the ultraviolet rays of sunlight. One type of damage, shown in Figure 16.19, is the covalent linking of thymine bases that are adjacent on a DNA strand. Such *thymine dimers* cause the DNA to buckle and interfere with DNA replication. The importance of repairing this kind of damage is underscored by a disorder called xeroderma pigmentosum, which in most cases is caused by an inherited defect in a nucleotide excision repair enzyme. Individuals with this disorder are hypersensitive to sunlight; mutations in their skin cells caused by ultraviolet light are left uncorrected, resulting in skin cancer.

Evolutionary Significance of Altered DNA Nucleotides

EVOLUTION Faithful replication of the genome and repair of DNA damage are important for the functioning of the organism and for passing on a complete, accurate genome to the next generation. The error rate after proofreading and repair is extremely low, but rare mistakes do slip through. Once a mismatched nucleotide pair is replicated, the sequence change is permanent in the daughter molecule that has the incorrect nucleotide as well as in any subsequent copies. As we mentioned earlier, a permanent change in the DNA sequence is called a mutation.

Mutations can change the phenotype of an organism (as you'll learn in Chapter 17). And if they occur in germ cells, which give rise to gametes, mutations can be passed on from generation to generation. The vast majority of such changes either have no effect or are harmful, but a very small percentage can be beneficial. In either case, mutations are the original source of the variation on which natural selection operates during evolution and are ultimately responsible for the appearance of new species. (You'll learn more about this process in Unit Four.) The balance between complete fidelity of DNA replication or repair and a low mutation rate has, over long periods of time, allowed the evolution of the rich diversity of species we see on Earth today.

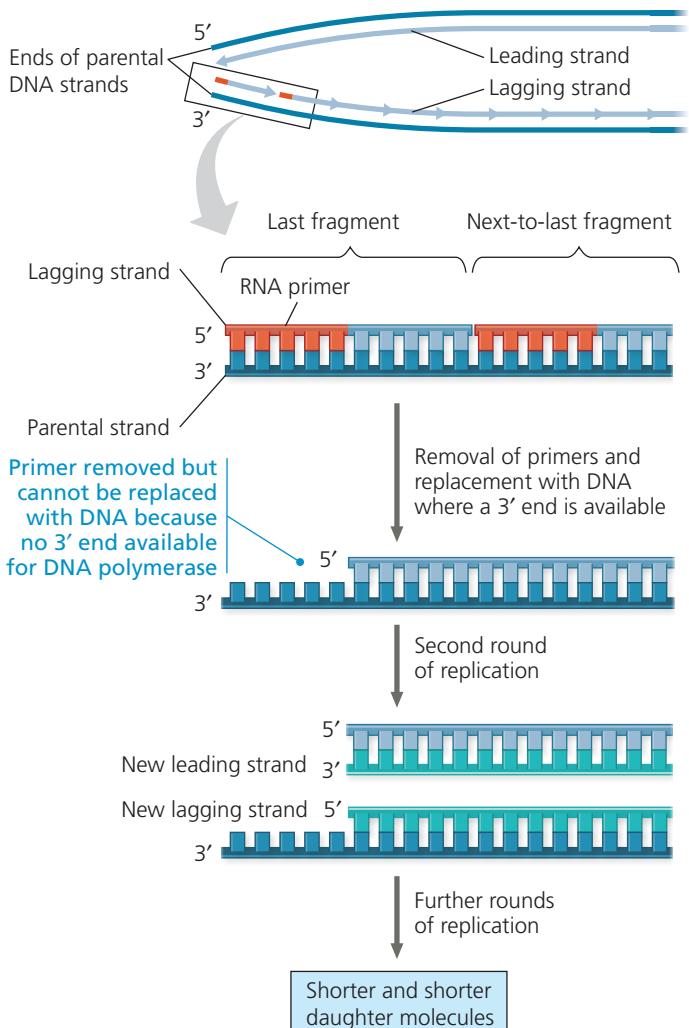
Replicating the Ends of DNA Molecules

For linear DNA, such as the DNA of eukaryotic chromosomes, the usual replication machinery cannot complete the 5' ends of daughter DNA strands. This is a consequence of the fact that a DNA polymerase can add nucleotides only to the 3' end of a preexisting polynucleotide. Even if an Okazaki fragment can be started with an RNA primer bound to the very end of the template strand, once that primer is removed, it cannot be replaced with DNA because there is no 3' end available for nucleotide addition (Figure 16.20). As a result, repeated rounds of replication produce shorter and shorter DNA molecules with uneven ("staggered") ends.

Most prokaryotes have a circular chromosome, with no ends, so the shortening of DNA does not occur. But what protects the genes of linear eukaryotic chromosomes from being eroded away during successive rounds of DNA replication? Eukaryotic chromosomal DNA molecules have special nucleotide sequences called **telomeres** at their ends (Figure 16.21). Telomeres do not contain genes; instead, the DNA typically consists of multiple repetitions of one short nucleotide sequence. In each human telomere, for example, the six-nucleotide sequence TTAGGG is repeated between 100 and 1,000 times. Telomeres have two protective functions.

First, specific proteins associated with telomeric DNA prevent the staggered ends of the daughter molecule from activating the cell's systems for monitoring DNA damage. (Staggered ends of a DNA molecule, which often result from double-strand breaks, can trigger signal transduction pathways leading to cell cycle arrest or cell death.) Second, telomeric DNA acts as a kind of buffer zone that provides some protection against the organism's genes shortening, somewhat like how the plastic-wrapped ends of a shoelace slow its unraveling. However, telomeres do not prevent the erosion of genes near the ends of chromosomes; they merely postpone it.

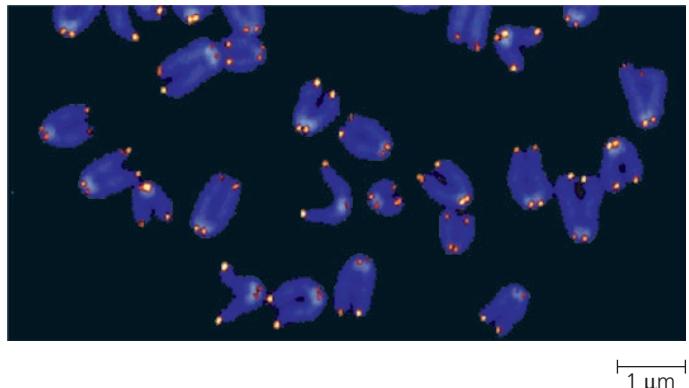
As shown in Figure 16.20, telomeres become shorter during every round of replication. Thus, as expected, telomeric DNA tends to be shorter in dividing somatic cells of older



▲ Figure 16.20 Shortening of the ends of linear DNA molecules. Here we follow the end of one strand of a DNA molecule through two rounds of replication. After the first round, the new lagging strand is shorter than its template. After a second round, both the leading and lagging strands have become shorter than the original parental DNA. Although not shown here, the other ends of these DNA molecules also become shorter.

individuals and in cultured cells that have divided many times. It has been proposed that shortening of telomeres is somehow connected to the aging process of certain tissues and even to aging of the organism as a whole.

But what about cells whose genome must persist virtually unchanged from an organism to its offspring over many generations? If the chromosomes of germ cells became shorter in every cell cycle, essential genes would eventually be missing from the gametes they produce. However, this does not occur: An enzyme called *telomerase* catalyzes the lengthening of telomeres in eukaryotic germ cells, thus restoring their original length and compensating for the shortening that occurs during DNA replication. Telomerase is not active in most human somatic cells, but its activity varies from tissue to tissue. The activity of telomerase in germ cells results in telomeres of maximum length in the zygote.



▲ Figure 16.21 Telomeres. Eukaryotes have repetitive, noncoding sequences called telomeres at the ends of their DNA. Telomeres are stained orange in these mouse chromosomes (LM).

Normal shortening of telomeres may protect organisms from cancer by limiting the number of divisions that somatic cells can undergo. Cells from large tumors often have unusually short telomeres, as we would expect for cells that have undergone many cell divisions. Further shortening would presumably lead to self-destruction of the tumor cells. Telomerase activity is abnormally high in cancerous somatic cells, suggesting that its ability to stabilize telomere length may allow these cancer cells to persist. Many cancer cells do seem capable of unlimited cell division, as do immortal strains of cultured cells (see Chapter 12). For several years, researchers have studied inhibition of telomerase as a possible cancer therapy. Thus far, while studies that inhibited telomerase in mice with tumors have led to the death of cancer cells, eventually the cells have restored the length of their telomeres by an alternative pathway. This is an area of ongoing research that may eventually yield useful cancer treatments.

Thus far in this chapter, you have learned about the structure and replication of a DNA molecule. In the next section, we'll take a step back and examine how DNA is packaged into chromosomes, the structures that carry the genetic information.

CONCEPT CHECK 16.2

1. What role does complementary base pairing play in the replication of DNA?
2. Identify two major functions of DNA pol III in DNA replication.
3. **MAKE CONNECTIONS** What is the relationship between DNA replication and the S phase of the cell cycle? See Figure 12.6.
4. **WHAT IF?** If the DNA pol I in a given cell were non-functional, how would that affect the synthesis of a *leading strand*? In the overview box in Figure 16.17, point out where DNA pol I would normally function on the top leading strand.

For suggested answers, see Appendix A.

CONCEPT 16.3

A chromosome consists of a DNA molecule packed together with proteins

The main component of the genome in most bacteria is one double-stranded, circular DNA molecule that is associated with a small amount of protein. Although we refer to this structure as the bacterial chromosome, it is very different from a eukaryotic chromosome, which consists of one linear

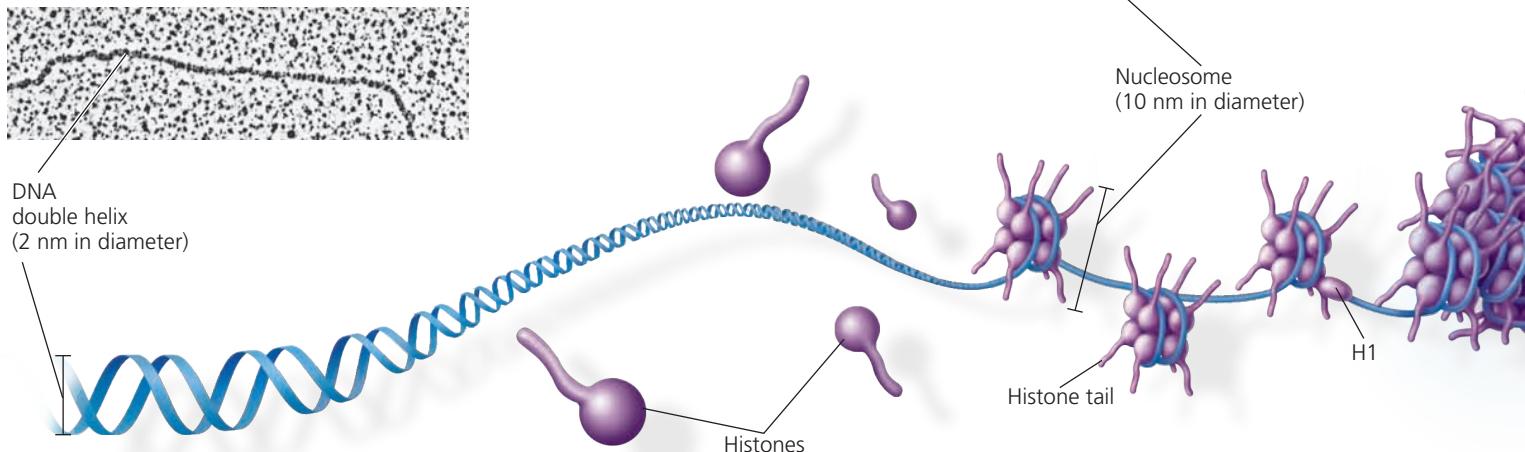
DNA molecule associated with a large amount of protein. In *E. coli*, the chromosomal DNA consists of about 4.6 million nucleotide pairs, representing about 4,400 genes. This is 100 times more DNA than is found in a typical virus, but only about one-thousandth as much DNA as in a human somatic cell. Still, that is a tremendous amount of DNA to be packaged in such a small container.

Stretched out, the DNA of an *E. coli* cell would measure about a millimeter in length, which is 500 times longer than the cell. Within a bacterium, however, certain proteins

▼ Figure 16.22

Exploring Chromatin Packing in a Eukaryotic Chromosome

This series of diagrams and transmission electron micrographs depicts a current model for the progressive levels of DNA coiling and folding. The illustration zooms out from a single molecule of DNA to a metaphase chromosome, which is large enough to be seen with a light microscope.



DNA, the double helix

Shown here is a ribbon model of DNA, with each ribbon representing one of the sugar-phosphate backbones. Recall that the phosphate groups along the backbone contribute a negative charge along the outside of each strand. The TEM shows a molecule of naked (protein-free) DNA; the double helix alone is 2 nm across.

Histones

Proteins called **histones** are responsible for the first level of DNA packing in chromatin. Although each histone is small—containing only about 100 amino acids—the total mass of histone in chromatin roughly equals the mass of DNA. More than a fifth of a histone's amino acids are positively charged (lysine or arginine) and therefore bind tightly to the negatively charged DNA.

Four types of histones are most common in chromatin: H2A, H2B, H3, and H4. The histones are very similar among eukaryotes; for example, all but two of the amino acids in cow H4 are identical to those in pea H4. The apparent conservation of histone genes during evolution probably reflects the important role of histones in organizing DNA within cells.

These four types of histones are critical to the next level of DNA packing. (A fifth type of histone, called H1, is involved in a further stage of packing.)

Nucleosomes, or “beads on a string” (10-nm fiber)

In electron micrographs, unfolded chromatin is 10 nm in diameter (the *10-nm fiber*). Such chromatin resembles beads on a string (see the TEM). Each “bead” is a **nucleosome**, the basic unit of DNA packing; the “string” between beads is called *linker DNA*.

A nucleosome consists of DNA wound twice around a protein core of eight histones, two each of the main histone types (H2A, H2B, H3, and H4). The amino end (N-terminus) of each histone (the *histone tail*) extends outward from the nucleosome.

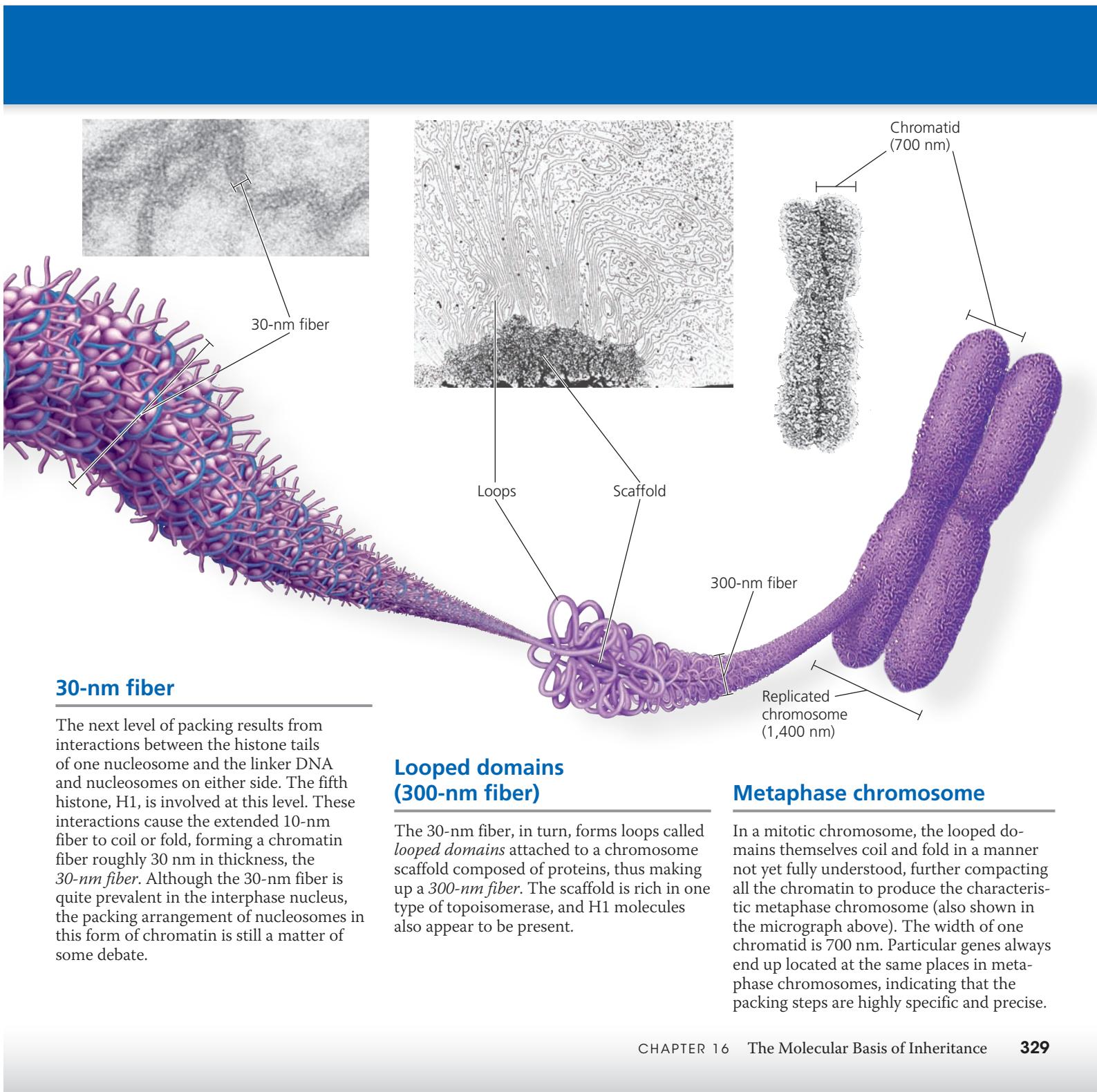
In the cell cycle, the histones leave the DNA only briefly during DNA replication. Generally, they do the same during transcription, another process that requires access to the DNA by the cell's molecular machinery. Nucleosomes, and in particular their histone tails, are involved in the regulation of gene expression.

cause the chromosome to coil and “supercoil,” densely packing it so that it fills only part of the cell. Unlike the nucleus of a eukaryotic cell, this dense region of DNA in a bacterium, called the nucleoid, is not bounded by membrane (see Figure 6.5).

Each eukaryotic chromosome contains a single linear DNA double helix that, in humans, averages about 1.5×10^8 nucleotide pairs. This is an enormous amount of DNA relative to a chromosome’s condensed length. If completely stretched out, such a DNA molecule would be about 4 cm

long, thousands of times the diameter of a cell nucleus—and that’s not even considering the DNA of the other 45 human chromosomes!

In the cell, eukaryotic DNA is precisely combined with a large amount of protein. Together, this complex of DNA and protein, called **chromatin**, fits into the nucleus through an elaborate, multilevel system of packing. Our current view of the successive levels of DNA packing in a chromosome is outlined in **Figure 16.22**. Study this figure carefully before reading further.



30-nm fiber

The next level of packing results from interactions between the histone tails of one nucleosome and the linker DNA and nucleosomes on either side. The fifth histone, H1, is involved at this level. These interactions cause the extended 10-nm fiber to coil or fold, forming a chromatin fiber roughly 30 nm in thickness, the **30-nm fiber**. Although the 30-nm fiber is quite prevalent in the interphase nucleus, the packing arrangement of nucleosomes in this form of chromatin is still a matter of some debate.

Looped domains (300-nm fiber)

The 30-nm fiber, in turn, forms loops called *looped domains* attached to a chromosome scaffold composed of proteins, thus making up a **300-nm fiber**. The scaffold is rich in one type of topoisomerase, and H1 molecules also appear to be present.

Metaphase chromosome

In a mitotic chromosome, the looped domains themselves coil and fold in a manner not yet fully understood, further compacting all the chromatin to produce the characteristic metaphase chromosome (also shown in the micrograph above). The width of one chromatid is 700 nm. Particular genes always end up located at the same places in metaphase chromosomes, indicating that the packing steps are highly specific and precise.

Chromatin undergoes striking changes in its degree of packing during the course of the cell cycle (see Figure 12.7). In interphase cells stained for light microscopy, the chromatin usually appears as a diffuse mass within the nucleus, suggesting that the chromatin is highly extended. As a cell prepares for mitosis, its chromatin coils and folds up (condenses), eventually forming a characteristic number of short, thick metaphase chromosomes that are distinguishable from each other with the light microscope (**Figure 16.23a**).

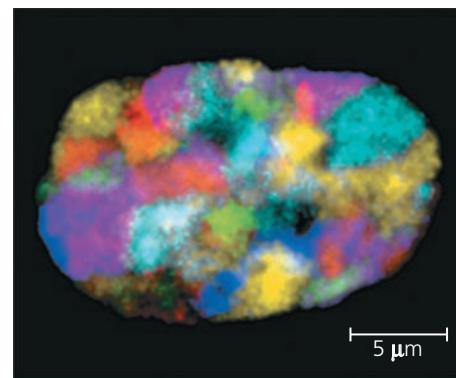
Though interphase chromatin is generally much less condensed than the chromatin of mitotic chromosomes, it shows several of the same levels of higher-order packing. Some of the chromatin comprising a chromosome seems to be present as a 10-nm fiber, but much is compacted into a 30-nm fiber, which in some regions is further folded into looped domains. Early on, biologists assumed that interphase chromatin was a tangled mass in the nucleus, like a bowl of spaghetti, but this is far from the case. Although an interphase chromosome lacks an obvious scaffold, its looped domains appear to be attached to the nuclear lamina, on the inside of the nuclear envelope, and perhaps also to fibers of the nuclear matrix. These attachments may help organize regions of chromatin where genes are active. The chromatin of each chromosome occupies a specific restricted area within the interphase nucleus, and the chromatin fibers of different chromosomes do not appear to be entangled (**Figure 16.23b**).

Even during interphase, the centromeres and telomeres of chromosomes, as well as other chromosomal regions in some cells, exist in a highly condensed state similar to that seen in a metaphase chromosome. This type of interphase chromatin, visible as irregular clumps with a light microscope, is called **heterochromatin**, to distinguish it from the less compacted, more dispersed **euchromatin** ("true chromatin"). Because of its compaction, heterochromatic DNA is largely inaccessible to the machinery in the cell responsible for transcribing the genetic information coded in the DNA, a crucial early step in gene expression. In contrast, the looser packing of euchromatin makes its DNA accessible to this machinery, so the genes present in euchromatin can be transcribed. The chromosome is a dynamic structure that is condensed, loosened, modified, and remodeled as necessary for various cell processes, including mitosis, meiosis, and gene activity. Chemical modifications of histones affect the state of chromatin condensation and also have multiple effects on gene activity, as you'll see in Chapter 18.

In this chapter, you have learned how DNA molecules are arranged in chromosomes and how DNA replication provides the copies of genes that parents pass to offspring. However, it is not enough that genes be copied and transmitted; the information they carry must be used by the cell. In other words, genes must also be expressed. In the next chapter, we will examine how the cell expresses the genetic information encoded in DNA.



(a) These metaphase chromosomes have been "painted" so that the two homologs of a pair are the same color. Above is a spread of treated chromosomes; on the right, they have been organized into a karyotype.



(b) The ability to visually distinguish among chromosomes makes it possible to see how the chromosomes are arranged in the interphase nucleus. Each chromosome appears to occupy a specific territory during interphase. In general, the two homologs of a pair are not located together.

▲ Figure 16.23 "Painting" chromosomes. Researchers can treat ("paint") human chromosomes with molecular tags that cause each chromosome pair to appear a different color.

MAKE CONNECTIONS If you arrested a human cell in metaphase I of meiosis and applied this technique, what would you observe? How would this differ from what you would see in metaphase of mitosis? Review Figure 13.8 and Figure 12.7.

CONCEPT CHECK 16.3

1. Describe the structure of a nucleosome, the basic unit of DNA packing in eukaryotic cells.
2. What two properties, one structural and one functional, distinguish heterochromatin from euchromatin?
3. **MAKE CONNECTIONS** Interphase chromosomes appear to be attached to the nuclear lamina and perhaps also the nuclear matrix. Describe these two structures. See Figure 6.9 and the associated text.

For suggested answers, see Appendix A.

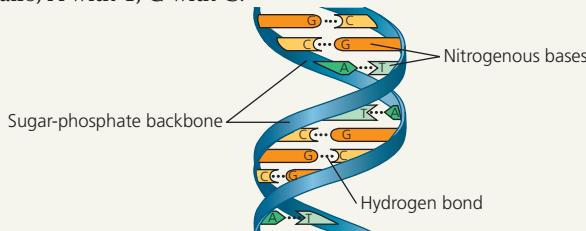
16 Chapter Review

SUMMARY OF KEY CONCEPTS

CONCEPT 16.1

DNA is the genetic material (pp. 313–318)

- Experiments with bacteria and with **phages** provided the first strong evidence that the genetic material is DNA.
- Watson and Crick deduced that DNA is a **double helix** and built a structural model. Two **antiparallel** sugar-phosphate chains wind around the outside of the molecule; the nitrogenous bases project into the interior, where they hydrogen-bond in specific pairs, A with T, G with C.

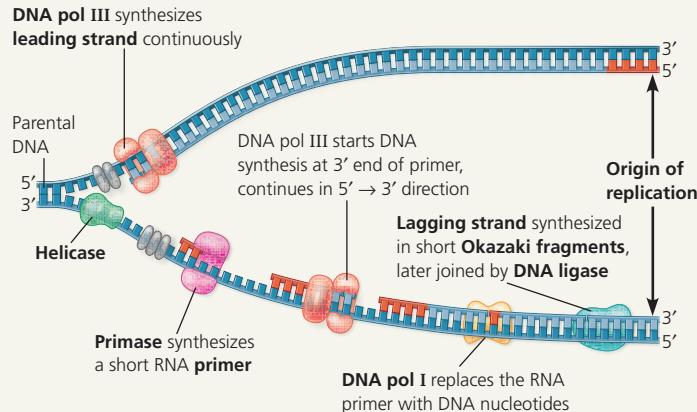


What does it mean when we say that the two DNA strands in the double helix are antiparallel? What would an end of the double helix look like if the strands were parallel?

CONCEPT 16.2

Many proteins work together in DNA replication and repair (pp. 318–327)

- The Meselson-Stahl experiment showed that **DNA replication** is **semiconservative**: The parental molecule unwinds, and each strand then serves as a template for the synthesis of a new strand according to base-pairing rules.
- DNA replication at one **replication fork** is summarized here:



- DNA polymerases proofread new DNA, replacing incorrect nucleotides. In **mismatch repair**, enzymes correct errors that persist. **Nucleotide excision repair** is a process by which **nucleases** cut out and other enzymes replace damaged stretches of DNA.
- The ends of eukaryotic chromosomal DNA get shorter with each round of replication. The presence of **telomeres**, repetitive sequences at the ends of linear DNA molecules, postpones the erosion of genes. Telomerase catalyzes the lengthening of telomeres in germ cells.

Compare DNA replication on the leading and lagging strands, including both similarities and differences.

CONCEPT 16.3

A chromosome consists of a DNA molecule packed together with proteins (pp. 328–330)

- The chromosome of most bacterial species is a circular DNA molecule with some associated proteins, making up the nucleoid. The **chromatin** making up a eukaryotic chromosome is composed of DNA, **histones**, and other proteins. The histones bind to each other and to the DNA to form **nucleosomes**, the most basic units of DNA packing. Histone tails extend outward from each bead-like nucleosome core. Additional coiling and folding lead ultimately to the highly condensed chromatin of the metaphase chromosome. Chromosomes occupy restricted areas in the interphase nucleus. In interphase cells, most chromatin is less compacted (**euchromatin**), but some remains highly condensed (**heterochromatin**). Euchromatin, but not heterochromatin, is generally accessible for transcription of genes.

Describe the levels of chromatin packing you'd expect to see in an interphase nucleus.

TEST YOUR UNDERSTANDING

LEVEL 1: KNOWLEDGE/COMPREHENSION

- In his work with pneumonia-causing bacteria and mice, Griffith found that
 - the protein coat from pathogenic cells was able to transform nonpathogenic cells.
 - heat-killed pathogenic cells caused pneumonia.
 - some substance from pathogenic cells was transferred to nonpathogenic cells, making them pathogenic.
 - the polysaccharide coat of bacteria caused pneumonia.
- What is the basis for the difference in how the leading and lagging strands of DNA molecules are synthesized?
 - The origins of replication occur only at the 5' end.
 - Helicases and single-strand binding proteins work at the 5' end.
 - DNA polymerase can join new nucleotides only to the 3' end of a pre-existing strand.
 - DNA ligase works only in the 3' → 5' direction.
- In analyzing the number of different bases in a DNA sample, which result would be consistent with the base-pairing rules?
 - A = G
 - A + G = C + T
 - A + T = G + C
 - A = C
- The elongation of the leading strand during DNA synthesis
 - progresses away from the replication fork.
 - occurs in the 3' → 5' direction.
 - does not require a template strand.
 - depends on the action of DNA polymerase.
- In a nucleosome, the DNA is wrapped around
 - histones.
 - ribosomes.
 - polymerase molecules.
 - a thymine dimer.

LEVEL 2: APPLICATION/ANALYSIS

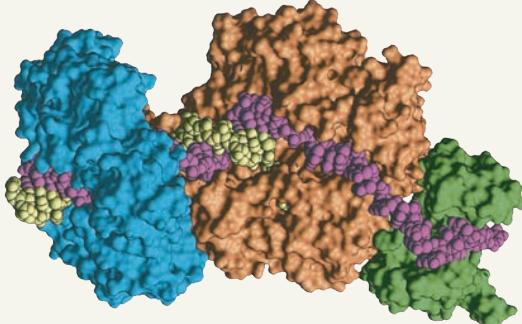
6. *E. coli* cells grown on ^{15}N medium are transferred to ^{14}N medium and allowed to grow for two more generations (two rounds of DNA replication). DNA extracted from these cells is centrifuged. What density distribution of DNA would you expect in this experiment?
- one high-density and one low-density band
 - one intermediate-density band
 - one high-density and one intermediate-density band
 - one low-density and one intermediate-density band
7. A biochemist isolates, purifies, and combines in a test tube a variety of molecules needed for DNA replication. When she adds some DNA to the mixture, replication occurs, but each DNA molecule consists of a normal strand paired with numerous segments of DNA a few hundred nucleotides long. What has she probably left out of the mixture?
- DNA polymerase
 - DNA ligase
 - Okazaki fragments
 - primase
8. The spontaneous loss of amino groups from adenine in DNA results in hypoxanthine, an uncommon base, opposite thymine. What combination of proteins could repair such damage?
- nuclease, DNA polymerase, DNA ligase
 - telomerase, primase, DNA polymerase
 - telomerase, helicase, single-strand binding protein
 - DNA ligase, replication fork proteins, adenyl cyclase
9. **MAKE CONNECTIONS** Although the proteins that cause the *E. coli* chromosome to coil are not histones, what property would you expect them to share with histones, given their ability to bind to DNA (see Figure 5.14)?

LEVEL 3: SYNTHESIS/EVALUATION

10. EVOLUTION CONNECTION

Some bacteria may be able to respond to environmental stress by increasing the rate at which mutations occur during cell division. How might this be accomplished? Might there be an evolutionary advantage of this ability? Explain.

11. SCIENTIFIC INQUIRY

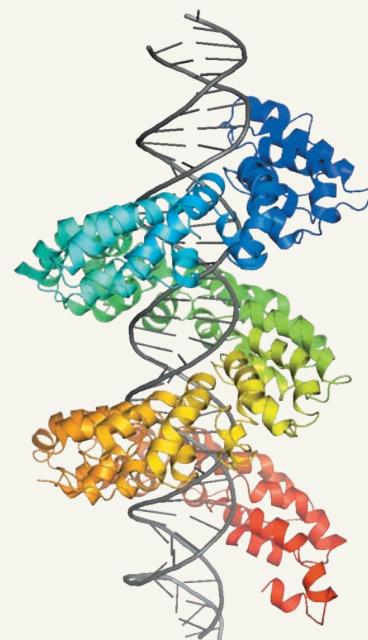


DRAW IT Model building can be an important part of the scientific process. The illustration shown above is a computer-generated model of a DNA replication complex. The parental and newly synthesized DNA strands are color-coded differently, as are each of the following three proteins: DNA pol III, the sliding clamp, and single-strand binding protein. Use what you've learned in this chapter to clarify this model by labeling each DNA strand and each protein and indicating the overall direction of DNA replication.

12. WRITE ABOUT A THEME: INFORMATION

The continuity of life is based on heritable information in the form of DNA, and structure and function are correlated at all levels of biological organization. In a short essay (100–150 words), describe how the structure of DNA is correlated with its role as the molecular basis of inheritance.

13. SYNTHESIZE YOUR KNOWLEDGE



This image shows DNA interacting with a computer-generated model of a TAL protein, one of a family of proteins found only in a species of the bacterium *Xanthomonas*. The bacterium uses proteins like this one to find particular gene sequences in cells of the organisms it infects, such as tomatoes, rice, and citrus fruits. Researchers are excited about working with this family of proteins. Their goal is to generate modified versions that can home in on specific gene sequences. Such proteins could then be used in an approach called gene therapy to “fix” mutated genes in individuals with genetic diseases. Given what you know about DNA structure and considering the image above, discuss how the TAL protein’s structure suggests that it functions.

For selected answers, see Appendix A.

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