

DNA: Structure and Replication



Spooling of DNA. [TED M. KINSMAN/
Science Source.]

CHAPTER OUTLINE AND LEARNING OBJECTIVES

7.1 DNA IS THE GENETIC MATERIAL

LO 7.1 Describe evidence demonstrating that DNA is the genetic material.

7.2 DNA STRUCTURE

LO 7.2 Describe evidence used to construct the double helix model of DNA.

LO 7.3 Draw the chemical structure of a DNA double helix.

7.3 DNA REPLICATION IS SEMICONSERVATIVE

LO 7.4 Describe evidence used to support semiconservative DNA replication.

7.4 DNA REPLICATION IN BACTERIA

LO 7.5 Outline the factors and events involved in DNA replication.

7.5 DNA REPLICATION IN EUKARYOTES

LO 7.6 Explain why and how DNA replication differs between bacteria and eukaryotes.

CHAPTER OBJECTIVE

Building upon the finding of early twentieth-century geneticists that heredity involves chromosomes, the broad objective for this chapter is to understand how the structure of DNA, with its base-paired strands, (1) holds the genetic information that determines how organisms are built, and (2) is accurately copied to make it possible for genetic information to be inherited when cells divide and organisms reproduce.

In this chapter, we describe the structure of DNA and the process of DNA replication, which makes an identical copy of DNA every time a cell divides. The story begins in the early 1900s, when results of several experiments led scientists to conclude that DNA, rather than another biological molecule such as carbohydrate, protein, or lipid, is the genetic material. DNA is a simple molecule made up of only four building blocks called **nucleotides**. It was thus necessary to understand how this very simple molecule could be the blueprint for the incredible diversity of organisms on Earth.

A large part of this understanding came from the structure of DNA, which was determined in 1953 by James Watson and Francis Crick through modeling based on the data of others. Their model of the structure of DNA was revolutionary because it defined genes in chemical terms and, in doing so, paved the way for understanding gene action and heredity at a molecular level. A measure of the importance of their discovery is that the double-helical structure of DNA has become a cultural icon that is seen more and more frequently in various forms of art (**Figure 7-1**).

The model of DNA proposed by Watson and Crick was built upon the results of scientists before them. They relied on earlier discoveries of the chemical composition of DNA and the ratios of its nucleotide bases. In addition, pictures of DNA fibers produced by X-ray diffraction revealed to the trained eye that DNA is a helix of precise dimensions. Watson and Crick concluded that DNA is a **double helix** composed of two strands of linked nucleotides that wind around each other.

The proposed structure of DNA immediately suggested that the sequence of nucleotides composing the two DNA strands of the helix could serve as a blueprint for constructing an organism. In addition, the structure hinted at how the blueprint could be copied into all cells in an organism. Because of the rules of base complementarity discovered by Watson and Crick, the sequence of one strand determines the sequence of the other strand. In this way, genetic information in the sequence of DNA can be passed from a mother cell to each daughter cell by having each of the separated strands of DNA serve as a **template** for producing new copies of double-stranded DNA.

In summary, this chapter focuses on the structure of DNA and the molecules and mechanisms that produce

An artistic representation of DNA



FIGURE 7-1 A 15-meter-tall sculpture of DNA is housed at the Prince Felipe Museum of Science in Valencia, Spain. [Peter Blixt/Alamy.]

DNA copies in a process called **DNA replication**. This information is essential for understanding the molecular basis of genes and genetic inheritance. Precisely how DNA is replicated is still an active area of research more than 65 years after the discovery of the double-helix structure. Our current understanding of the mechanism of replication gives a central role to a protein machine called the **replisome**. This complex of proteins coordinates numerous reactions that are necessary for rapid and accurate replication of DNA.

7.1 DNA IS THE GENETIC MATERIAL

LO 7.1 Describe evidence demonstrating that DNA is the genetic material.

Before we see how Watson and Crick solved the structure of DNA, let's review what was known about genes and DNA at the time they began their historic collaboration:

1. Genes—the hereditary “factors” described by Mendel—were known to be associated with specific traits, but their physical nature was not understood. Similarly, mutations were known to alter gene function, but the precise chemical nature of a mutation was not understood.
2. The one-gene–one-enzyme hypothesis (described in Chapter 5) postulated that genes determine the structure of proteins.
3. Genes were known to be carried on chromosomes.
4. Chromosomes were known to consist of DNA and protein.
5. As described next, experiments beginning in the 1920s revealed that DNA is the genetic material.

The discovery of bacterial transformation: the Griffith experiment

In 1928, Frederick Griffith made the puzzling observation that the genotype and phenotype of a live bacterial

strain can be changed, that is, “transformed,” by mixing it with a different, heat-killed bacterial strain. His studies used the bacterium *Streptococcus pneumoniae*, which causes pneumonia in humans and is normally lethal in mice. However, some strains of this bacterial species have evolved to be less virulent (less able to cause disease or death). In experiments summarized in **Figure 7-2**, Griffith used two strains that are distinguishable by the appearance of their colonies when grown in laboratory cultures. One strain was a normal virulent type, deadly to most laboratory animals. The cells of this strain are enclosed in a polysaccharide capsule, giving colonies a smooth appearance; hence, this strain is identified as S. Griffith's other strain was a mutant, nonvirulent type that grows in mice but is not lethal. In this strain, the polysaccharide coat is absent, giving colonies a rough appearance; this strain is called R.

Griffith killed some virulent S cells by boiling them. He then injected the heat-killed cells into mice. The mice survived, showing that the carcasses of the cells do not cause death. However, mice injected with a mixture of heat-killed virulent S cells and live nonvirulent R cells did die. Furthermore, live cells could be recovered from the dead mice; these cells gave smooth colonies and were virulent on subsequent injection. Somehow, the cell debris of the boiled S cells converted some of the live R cells into live S cells. That is, the live R cells were transformed into S cells by picking up some chemical component of the dead S cells. The process, already discussed in Chapter 6, is called *transformation*.

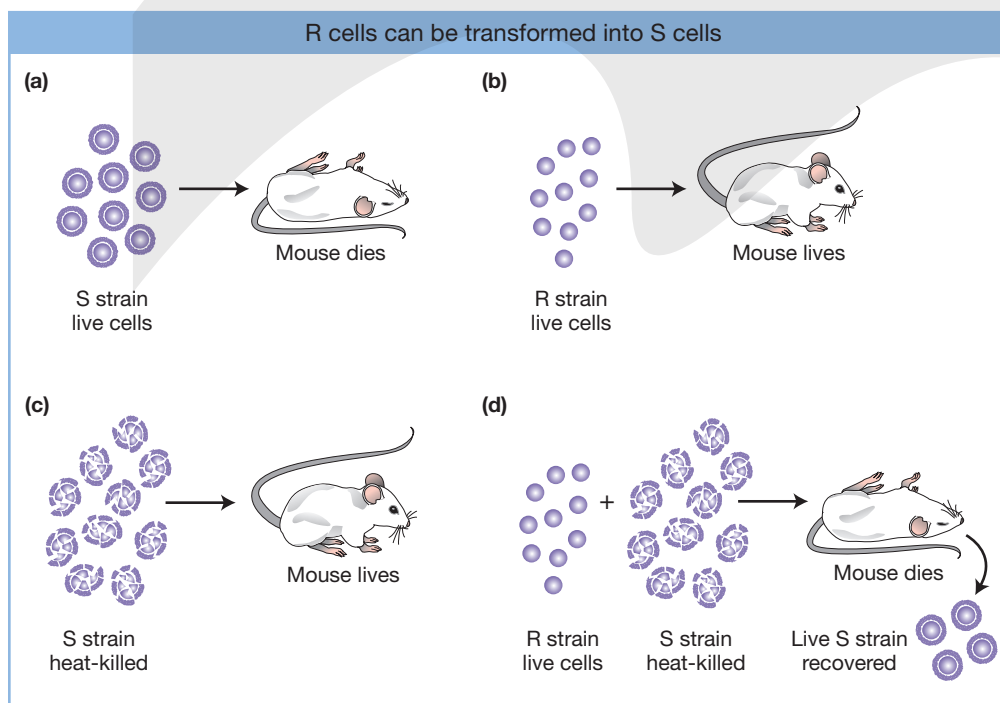


FIGURE 7-2 The presence of heat-killed S cells transforms live R cells into live S cells. (a) Mice die after injection with virulent S cells. (b) Mice survive after injection with R cells. (c) Mice survive after injection with heat-killed S cells. (d) Mice die after injection with a mixture of heat-killed S cells and live R cells. Live S cells were isolated from dead mice, indicating that heat-killed S cells somehow transform nonvirulent R cells into virulent S cells.

Evidence that DNA is the genetic material in bacteria: the Avery, Macleod, and McCarty experiments

The next step was to determine which chemical component of dead S cells caused transformation. This molecule had changed the genotype of the recipient strain and therefore was a candidate for the hereditary material. The problem was solved by experiments conducted in 1944 by Oswald Avery, Colin MacLeod, and Maclyn McCarty (Figure 7-3). Their approach to the problem was to destroy all of the major categories of chemicals in an extract of dead S cells one at a time, and to find out if the extract had lost the ability to transform. Virulent S cells had a smooth polysaccharide coat, whereas nonvirulent R cells did not; hence, polysaccharides were an obvious candidate for the transforming agent. However, when polysaccharides were destroyed, the mixture could still transform. Lipids, RNAs, and proteins were all similarly shown not to be the transforming agent. In contrast, the mixture lost its transforming ability when the donor mixture was treated with the enzyme deoxyribonuclease (DNase), which destroys DNA. These results strongly implicated DNA as the genetic material. It is now known that fragments of the transforming DNA that confer virulence enter the bacterial chromosome and replace their counterparts that confer nonvirulence.

KEY CONCEPT The demonstration that DNA is the transforming agent was the first evidence that genes (the hereditary material) are composed of DNA.

Evidence that DNA is the genetic material in phage: the Hershey–Chase experiment

The experiments conducted by Avery and his colleagues were definitive, but many scientists were reluctant to accept DNA (rather than proteins) as the genetic material. After all, how could such a low-complexity molecule as DNA encode the diversity of all living things? In 1952, Alfred Hershey and Martha Chase provided additional evidence in an experiment that made use of bacteriophage T2 (or phage T2 for short), a virus that infects bacteria. They reasoned that the infecting phage must inject into the bacterium the specific information that directs the production of new viral particles. If they could find out what material the phage was injecting into the bacterial host, they would have determined the genetic material of phages.

The phage is relatively simple in molecular composition. The T2 structure is similar to T4 shown in Figures 6-22 to 6-24. Most of its structure is protein, with DNA contained inside the protein sheath of its “head.” Hershey and Chase used radioisotopes to give DNA and protein distinct labels

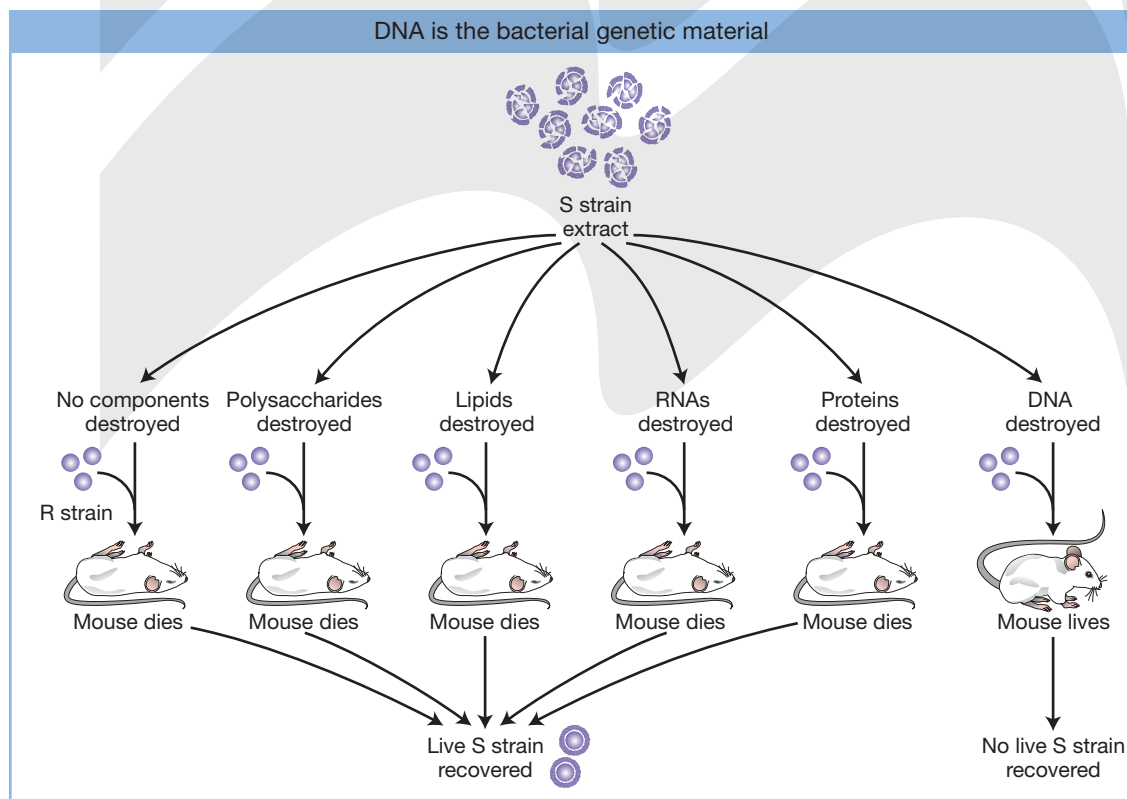


FIGURE 7-3 DNA is the genetic material that transforms nonvirulent R cells into virulent S cells. Mice survive when injected with a mixture of heat-killed S cells with destroyed DNA and live nonvirulent R cells. However, destroying polysaccharides, lipids, RNAs, or proteins does not allow the mice to survive. Thus, DNA, but not polysaccharide, lipid, RNA, or protein, is necessary for transformation.

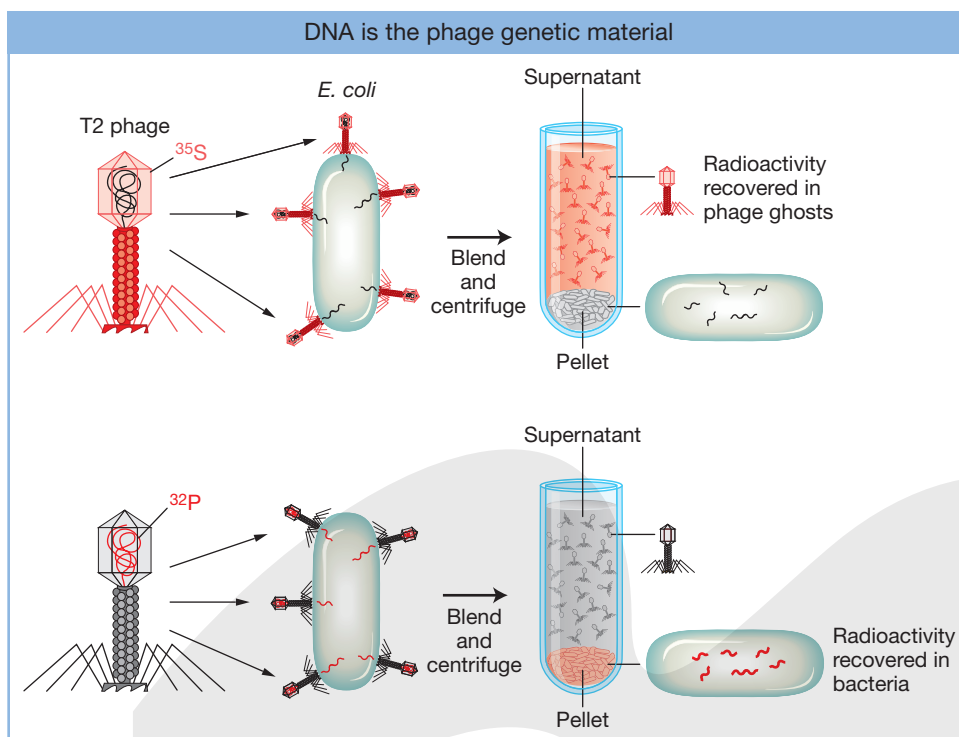


FIGURE 7-4 The Hershey–Chase experiment demonstrates that the genetic material of phages is DNA, not protein. The experiment uses two sets of T2 phage. In one set, the protein coat is labeled with radioactive sulfur (^{35}S), not found in DNA. In the other set, the DNA is labeled with radioactive phosphorus (^{32}P), not found in protein. Only ^{32}P is recovered from *E. coli* and phage progeny, indicating that DNA is the genetic material necessary for production of new phages.

that they could track during infection. Phosphorus is not found in the amino acid building blocks of proteins but is found in DNA; conversely, sulfur is not in the nucleotide building blocks of DNA but is in proteins. Hershey and Chase incorporated a radioisotope of phosphorus (^{32}P) into DNA and that of sulfur (^{35}S) into proteins of separate phage cultures. **Radioisotopes** are unstable (i.e., radioactive) isotopes of an element that emit radiation to transform into a more stable form. Emitted radiation can be measured using instruments such as a scintillation counter or a Geiger counter or by autoradiography (described in Section 7.3).

As shown in **Figure 7-4**, after labeling the phage DNA and proteins, they then infected two *E. coli* cultures with many phage particles per cell: one *E. coli* culture received phage labeled with ^{32}P , and the other received phage labeled with ^{35}S . After allowing sufficient time for infection to take place, they sheared the empty phage carcasses (called *ghosts*) off the bacterial cells in a kitchen blender. They separated the bacterial cells from the phage ghosts in a centrifuge and then measured the radioactivity in the solid pellet of bacteria and the liquid supernatant of phage ghosts. When the ^{32}P -labeled phages were used to infect *E. coli*, the radioactivity ended up inside the bacterial cells, indicating that phage DNA entered the cells. In contrast, when ^{35}S -labeled phages were used, the radioactive material ended up in the phage ghosts, indicating that phage proteins did not enter the bacterial cell. In addition, the progeny of ^{32}P -labeled phages remained labeled, but the progeny of ^{35}S -labeled phages were not labeled. These data once again indicated that DNA is the hereditary material. The phage proteins are mere structural

packaging that is discarded after delivering the viral DNA to the bacterial cell.

7.2 DNA STRUCTURE

LO 7.2 Describe evidence used to construct the double helix model of DNA.

LO 7.3 Draw the chemical structure of a DNA double helix.

Even before the structure of DNA was elucidated, genetic studies indicated that the hereditary material must have three key properties:

1. Because essentially every cell in the body of an organism has the same genetic makeup, accurate replication of the genetic material at every cell division is crucial. Thus, structural features of the genetic material *must allow accurate replication*. The structural features of DNA will be covered in this section of the chapter.
2. Because it must encode the collection of proteins expressed by an organism, structural features of the genetic material *must have informational content*. How information coded in DNA is deciphered to produce proteins is the subject of Chapters 8 and 9.
3. Because hereditary changes, called mutations, provide the raw material for evolutionary selection, genetic material *must be able to change* on rare occasion. Nevertheless, the structure of the genetic material must be stable enough for an organism to rely on its encoded information. The mechanisms of DNA mutations will be covered in Chapter 16.

DNA structure before Watson and Crick

Consider the discovery of the double-helical structure of DNA by Watson and Crick as the solution to a complicated three-dimensional puzzle. To solve this puzzle, Watson and Crick used a process called “model building,” in which they assembled the results of earlier and ongoing experiments (the puzzle pieces) to form the three-dimensional puzzle (the double helix model). To understand how they built the DNA model, we first need to know what pieces of the puzzle were available to them.

The building blocks of DNA The first piece of the puzzle was knowledge of the basic building blocks of DNA. As a chemical, DNA is quite simple. It contains three components: (1) **phosphate**, (2) a sugar called **deoxyribose**, and (3) four nitrogenous **bases**—**adenine**, **guanine**, **cytosine**, and **thymine** (Figure 7-5). The sugar in DNA is called “deoxyribose” because it contains ribose sugars that are missing an oxygen atom. Deoxyribose has a hydrogen atom (H) at the 2'-carbon atom, unlike **ribose** (a component of RNA), which has a hydroxyl (OH) group at that position.

Two of the bases, adenine and guanine, have a double-ring structure characteristic of a class of chemicals called **purines**. The other two bases, cytosine and thymine, have a single-ring structure characteristic of another class of chemicals called **pyrimidines**. Carbon and nitrogen atoms in the rings of the bases are assigned numbers for ease of reference. Carbon atoms in the sugar group also are assigned numbers—in this case, each number is followed by a prime (1', 2', and so forth).

KEY CONCEPT DNA contains four bases—two purines (adenine and guanine) and two pyrimidines (cytosine and thymine).

The chemical subunits of DNA are nucleotides or, more specifically, **deoxynucleotides**, each composed of a phosphate group, a deoxyribose sugar molecule, and one of the four bases (Figure 7-5). It is convenient to refer to each nucleotide by the first letter of the name of its base: A, G, C, or T. The nucleotide with the adenine base is called deoxyadenosine 5'-monophosphate and abbreviated dAMP,

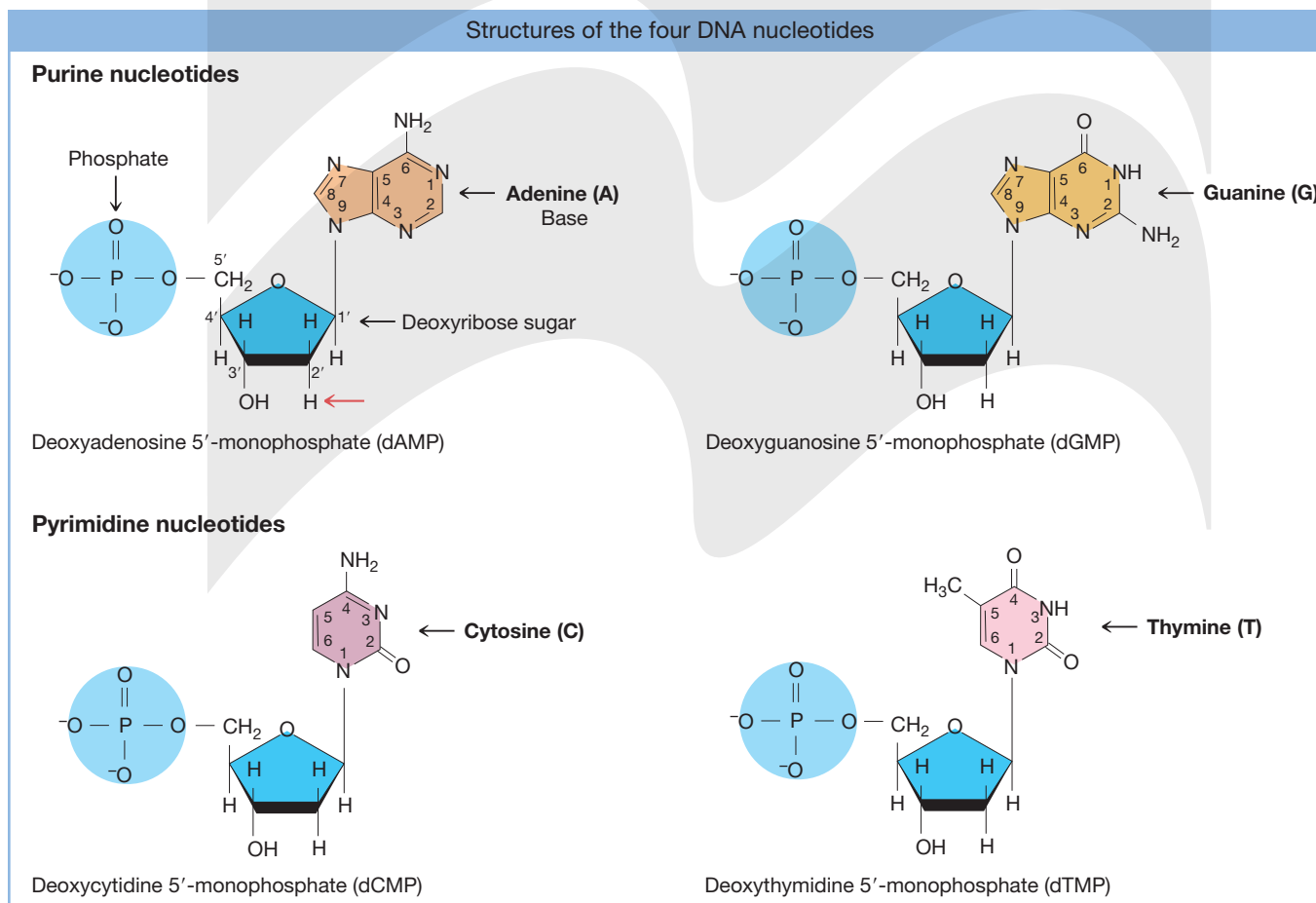


FIGURE 7-5 Nucleotides are the fundamental building blocks of DNA. All nucleotides have a phosphate, a sugar, and a base. The sugar is called deoxyribose because it is a variant of ribose that lacks an oxygen atom, indicated by the red arrow. There are two purine bases (adenine and guanine) and two pyrimidine bases (cytosine and thymine). Note that each of the bases contains nitrogen atoms.

where the 5' refers to the position of the carbon atom in the sugar ring to which the single (mono) phosphate group is attached. The other nucleotides are named using the same convention.

KEY CONCEPT DNA nucleotides are known as deoxynucleotides and are composed of a phosphate, a deoxyribose, and a purine or pyrimidine base.

Chargaff's rules of base composition The second piece of the puzzle used by Watson and Crick came from work done several years earlier by Erwin Chargaff. Studying a large selection of DNAs from different organisms (Table 7-1), Chargaff established certain empirical rules about the amounts of each type of nucleotide found in DNA:

1. The total amount of purine nucleotides (A + G) always equals the total amount of pyrimidine nucleotides (T + C).
2. The amount of A always equals the amount of T, and the amount of G always equals the amount of C; that is, A/T and G/C is close to 1.0, regardless of the source of DNA (Table 7-1).
3. The amount of A + T is not necessarily equal to the amount of G + C, as can be seen in the last column of Table 7-1. The (A + T)/(G + C) ratio varies among different organisms. For example, in sea urchins, the ratio is 1.85, indicating that the sea urchin genome has almost twice as much A + T than G + C; it is said to be AT-rich. In contrast, the *Mycobacterium tuberculosis* genome is GC-rich, with about twice as much G + C than A + T. However, the ratio is virtually the same in different tissues of the same organism (as seen for human tissues in the last three rows of Table 7-1), supporting the idea that all cells of an organism have the same genomic DNA sequence.

KEY CONCEPT DNA contains an equal amount of A and T nucleotides and G and C nucleotides. Organisms vary in the relative amount of A + T versus G + C, but different tissues in the same organism have the same relative amount of A + T versus G + C.

X-ray diffraction analysis of DNA: Rosalind Franklin

The third piece of the puzzle came from the X-ray diffraction pattern of DNA fibers (Figure 7-6a) that was collected by Rosalind Franklin (Figure 7-6b). In this experiment, X rays were fired at DNA fibers that were collected from cells, as shown in the opening photograph of this chapter. The scatter of the X rays from the fibers is detected as spots on photographic film (Figure 7-6a). The angle of scatter represented by each spot on the film gives information about the position of an atom or certain groups of atoms in DNA. Darker spots are where the film was hit multiple times by X rays from repeated parts of DNA such as nucleotide bases. This procedure is not simple to carry out (or to explain), and interpretation of the spot patterns requires complex mathematical treatment that is beyond the scope of this text. The available data suggested that DNA is long and skinny and that it has two similar parts that are parallel to each other and run along the length of the molecule. The X-ray data showed that DNA is helical, like a spiral staircase. Unknown to Franklin, her best X-ray picture (Figure 7-6a) was shown to Watson and Crick, and this crucial piece of the puzzle allowed them to deduce the three-dimensional structure of DNA (Figure 7-7).

KEY CONCEPT The X-ray diffraction pattern of DNA showed that it is a long and skinny, two-stranded helix (that is, a double helix).

TABLE 7-1 Molar Properties of Bases* in DNAs from Various Sources

Organism	Tissue	Adenine	Thymine	Guanine	Cytosine	$\frac{A + T}{G + C}$
<i>E. coli</i> (K12)	—	26.0	23.9	24.9	25.2	1.00
<i>D. pneumoniae</i>	—	29.8	31.6	20.5	18.0	1.59
<i>M. tuberculosis</i>	—	15.1	14.6	34.9	35.4	0.42
Yeast	—	31.3	32.9	18.7	17.1	1.79
Sea urchin	Sperm	32.8	32.1	17.7	18.4	1.85
Herring	Sperm	27.8	27.5	22.2	22.6	1.23
Rat	Bone marrow	28.6	28.4	21.4	21.5	1.33
Human	Thymus	30.9	29.4	19.9	19.8	1.52
Human	Liver	30.3	30.3	19.5	19.9	1.53
Human	Sperm	30.7	31.2	19.3	18.8	1.62

*Defined as moles of nitrogenous constituents per 100 g-atoms phosphate in hydrolysate.

Source: Data from E. Chargaff and J. Davidson, eds., *The Nucleic Acids*. Academic Press, 1955.

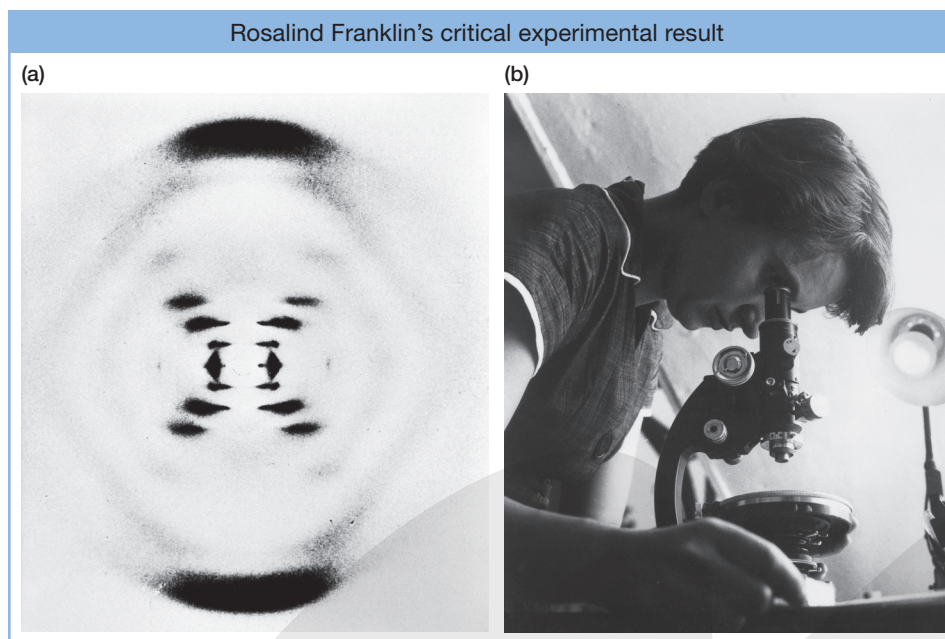


FIGURE 7-6 (a) The X-ray diffraction pattern of DNA determined by (b) Rosalind Franklin. [(a) OMIKRON/Science Source; (b) © World History Archive/Alamy Stock Photo.]

The DNA double helix structure: Watson and Crick

A 1953 paper by Watson and Crick in the journal *Nature* began with two sentences that ushered in a new age of biology: “We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.”¹ The structure of DNA had been a subject of great debate since the experiments of Avery and co-workers in 1944. The general composition of DNA was known, but how the parts fit together was not known. The structure had to fulfill the

main requirements for a hereditary molecule: the ability to store information, the ability to be replicated, and the ability to mutate.

By studying models that they made of the structure, Watson and Crick realized that the observed diameter of the double helix (known from the X-ray data) would be explained if a purine base always pairs (by hydrogen bonding) with a pyrimidine base (Figure 7-8). Such pairing would account for the $(A + G) = (T + C)$ regularity observed by Chargaff, but it would predict four possible pairings: A-T, G-T, A-C, and G-C. However, Chargaff’s data indicate that G pairs only with C, and A pairs only with T. Watson and Crick concluded that each base pair consists of one purine

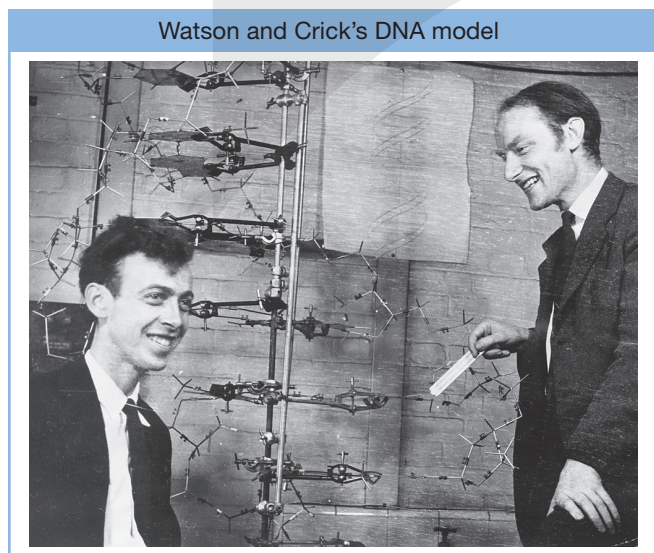


FIGURE 7-7 James Watson (left) and Francis Crick (right) with their three-dimensional DNA model and a two-dimensional drawing of DNA on the wall. [BARRINGTON BROWN/Science Source.]

¹ J. Watson and F. Crick, *Nature* 171:737, 1953.

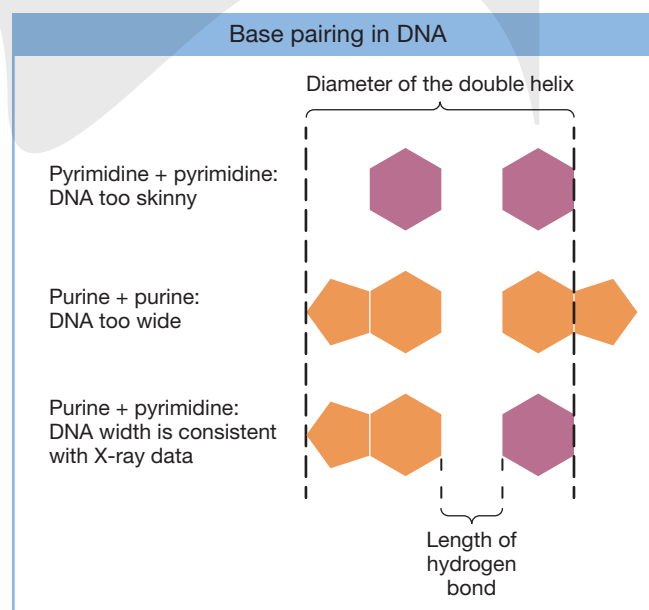


FIGURE 7-8 Pairing of purines with pyrimidines accounts exactly for the diameter of the DNA double helix determined from X-ray data.

base and one pyrimidine base, paired according to the following rule: G pairs with C (G-C), and A pairs with T (A-T). These are called **complementary bases**. The double helix accounted nicely for Franklin's X-ray data as well as Chargaff's base composition data.

KEY CONCEPT The two strands of DNA contain complementary base pairs—G base pairs with C and A base pairs with T.

The three-dimensional structure derived by Watson and Crick is composed of two side-by-side chains ("strands") of nucleotides twisted into the shape of a double helix with 10 base pairs in each complete turn of the helix (Figure 7-9a). DNA is a right-handed helix; in other words, it has the same structure as that of a screw that would be screwed into place using a clockwise turning motion. The two strands are held together by hydrogen bonds between purine and pyrimidine bases of each strand, forming the stairs of a spiral staircase. On the outside of the double helix, the backbone of each

strand is formed by alternating phosphate and deoxyribose sugar units that are connected by phosphodiester linkages (Figure 7-9b). These linkages are used to describe how a nucleotide chain is organized. As already mentioned, the carbon atoms of the sugar groups are numbered 1' through 5'. A phosphodiester linkage connects the 5'-carbon atom of one deoxyribose to the 3'-carbon atom of the adjacent deoxyribose. Thus, each sugar-phosphate backbone is said to have a 5'-to-3' polarity, or direction. Understanding this polarity is essential in understanding how DNA fulfills its roles. In double-stranded DNA, the two backbones are in opposite, or **antiparallel**, orientation; one is oriented 5'-to-3' and the other is oriented 3'-to-5' (Figure 7-9b).

KEY CONCEPT The base-paired strands of DNA are oriented antiparallel to one another—one strand is oriented in the 5'-to-3' direction and the other strand is oriented in the 3'-to-5' direction.

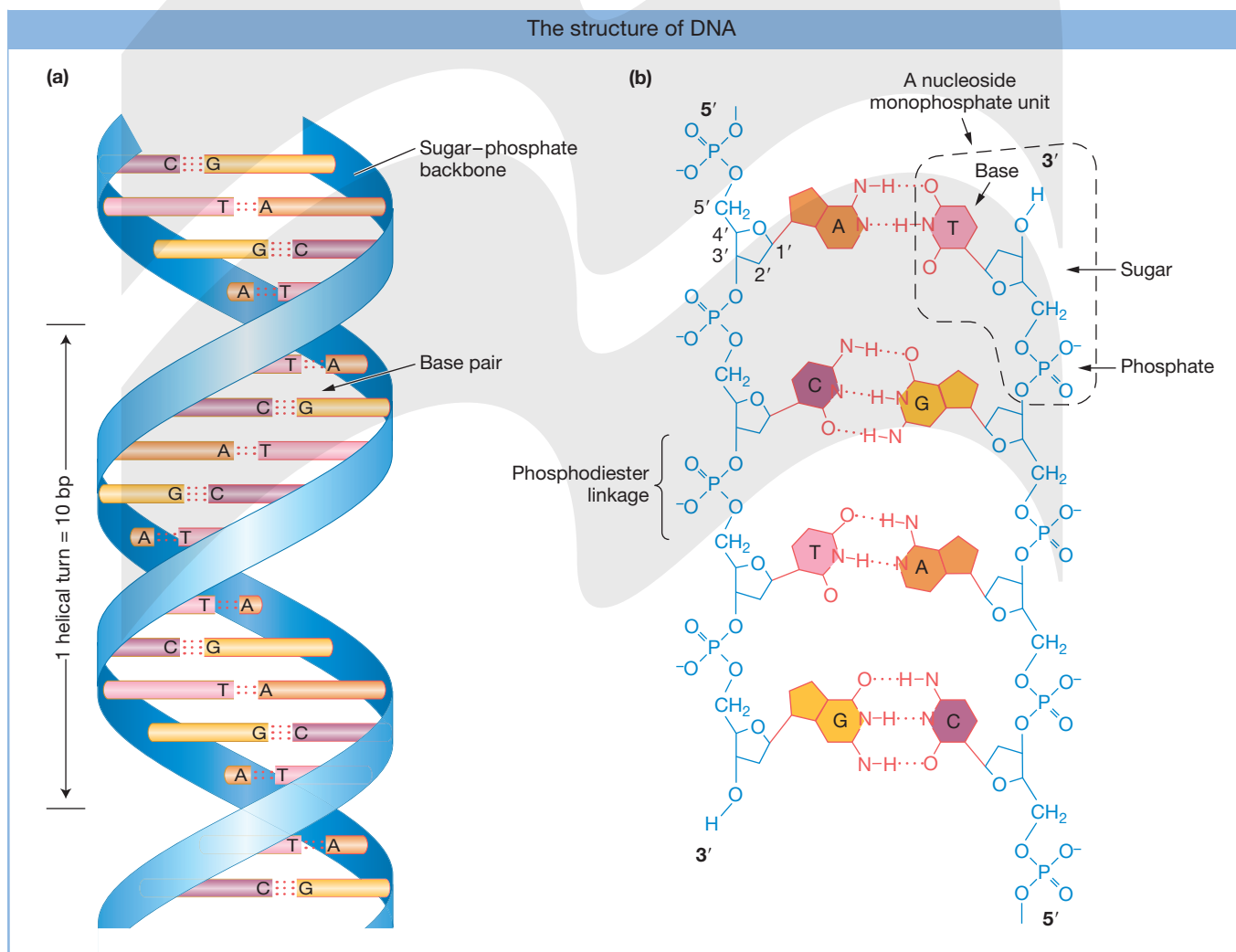


FIGURE 7-9 (a) A simplified model showing the helical structure of DNA. Horizontal sticks represent base pairs; blue ribbons represent the sugar-phosphate backbones of the two antiparallel DNA chains. (b) A chemical diagram of the DNA double helix, unrolled to show the sugar-phosphate backbones (blue) and base-pair rungs (purple,

orange). The backbones run in opposite directions. The strand on the left is oriented 5'-to-3' from top to bottom, and the strand on the right is oriented 3'-to-5' from top to bottom. Each base pair has one purine base, adenine (A) or guanine (G), and one pyrimidine base, thymine (T) or cytosine (C), connected by hydrogen bonds (red dots).

Each base is attached to the 1'-carbon atom of a deoxy-ribose sugar in the backbone of each strand and faces inward toward a base on the other strand. Hydrogen bonds between pairs of purine and pyrimidine bases (indicated by dots in Figure 7-9b) hold the two strands of DNA together. Note that G–C base pairs have three hydrogen bonds, whereas A–T base pairs have only two. We would predict that DNA containing many G–C base pairs would be more stable than DNA containing many A–T base pairs. In fact, this prediction is confirmed. Heat causes the two strands of the DNA double helix to separate (a process called DNA melting or DNA denaturation); DNAs with higher G + C content require higher temperatures to melt because of greater attraction of G–C base pairs.

KEY CONCEPT A–T base pairs have two hydrogen bonds, and G–C base pairs have three.

The two complementary nucleotide strands paired in an antiparallel manner automatically assume a double-helical conformation (Figure 7-10), mainly through the interaction of base pairs. Base pairs, which are flat planar structures, stack on top of one another at the center of the double helix (Figure 7-10a). Stacking adds to the stability of DNA by excluding water molecules from spaces between

the base pairs. A single strand of nucleotides is not helical; the helical shape of DNA depends entirely on pairing and stacking of bases in the antiparallel strands. The most stable form that results from base stacking is a double helix with two distinct sizes of grooves running in a spiral: shallow **major grooves** occur where the sugar–phosphate backbones are far apart, and deep **minor grooves** occur where they are close together. Both types of grooves can be seen in ribbon (Figure 7-10a) and space-filling (Figure 7-10b) models. Proteins that bind DNA interact specifically with either major or minor grooves.

KEY CONCEPT The geometry of base pairs creates shallow, wide major grooves and narrow, deep minor grooves along the DNA helix; features that are recognized for protein binding.

The structure of DNA is considered by some to be the most important biological discovery of the twentieth century. The reason that this discovery is considered so important is that the double helix model, in addition to being consistent with earlier data about DNA structure, fulfilled the three requirements for a hereditary substance:

1. The double-helical structure suggested how the genetic material might determine the structure of proteins. Perhaps the *sequence* of nucleotides in DNA dictates the

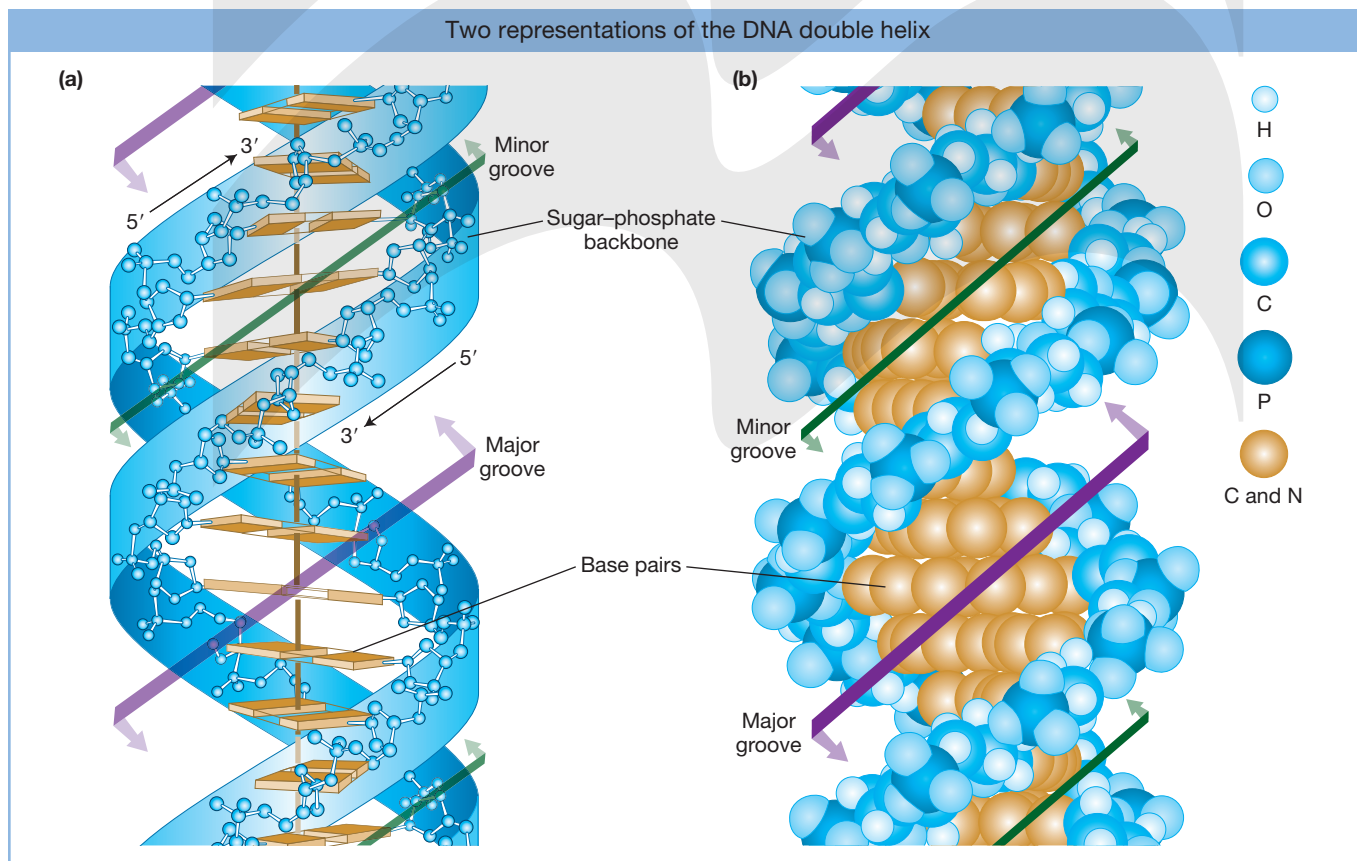


FIGURE 7-10 (a) The ribbon diagram highlights the stacking of base pairs, whereas (b) the space-filling model emphasizes the major and minor grooves. In both models, the sugar–phosphate backbone is blue and the bases are gold.

sequence of amino acids in the protein specified by that gene. In other words, some sort of **genetic code** may write information in DNA as a sequence of nucleotides and then translate it into a different language of amino acid sequence in protein. Just how this is done is the subject of Chapter 9.

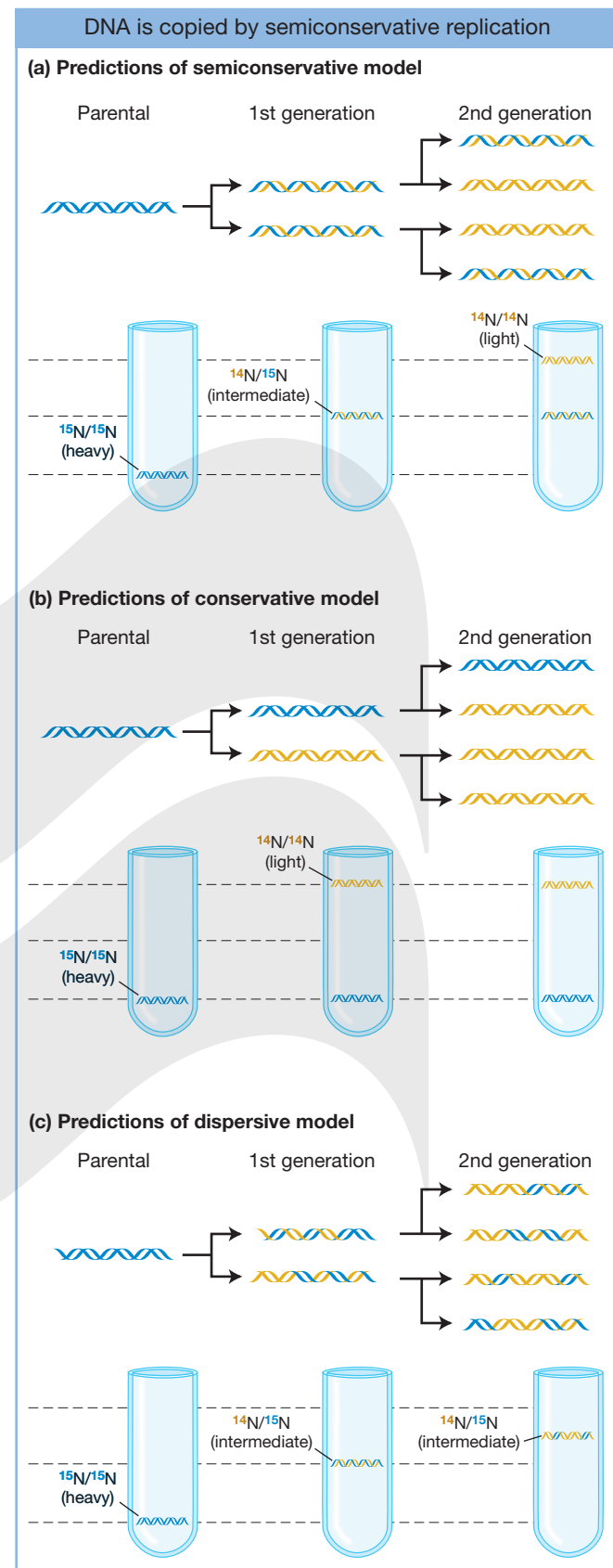
2. As Watson and Crick stated in the concluding words of their 1953 *Nature* paper that reported the double-helical structure of DNA: “It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.”² To geneticists at the time, this statement proposed that DNA is replicated by a semiconservative mechanism, as described in the next section.
3. If the nucleotide sequence of DNA specifies the amino acid sequence, mutations are possible by the substitution of one nucleotide for another at one or more positions. Mutations will be discussed in Chapter 16.

7.3 DNA REPLICATION IS SEMICONSERVATIVE

LO 7.4 Describe evidence used to support semiconservative DNA replication.

In **semiconservative replication** hypothesized by Watson and Crick, the double helix is unwound and each DNA strand acts as a template to direct assembly of complementary bases following the A–T and G–C base-pairing rules to create two double helices that are identical to the original. This mode of replication is called semiconservative because each of the new helices conserves one of the original strands (that is, the **parental molecule**) and the other strand (that is, the **daughter molecule**) is new (Figure 7-11a). However, two other modes of replication were also hypothesized. In **conservative replication**, the parent DNA double helix is conserved, and a daughter double helix is produced consisting of two newly synthesized strands (Figure 7-11b). In **dispersive replication**, two new DNA double helices are produced, with each strand containing segments of *both* parental DNA and newly synthesized daughter DNA (Figure 7-11c).

FIGURE 7-11 Three mechanisms were hypothesized for how DNA is replicated: (a) semiconservative, (b) conservative, and (c) dispersive. The Meselson–Stahl experiment demonstrates that DNA is copied by semiconservative replication. DNA centrifuged in a cesium chloride (CsCl) gradient will form bands according to its density. (a) In accord with semiconservative replication, when cells grown in heavy ^{15}N are transferred to light ^{14}N medium, the first generation produces a single DNA band of intermediate density and the second generation produces two bands: one intermediate and one light. (b and c) In contrast, the data do not match results predicted for conservative and dispersive replication.



² J. Watson and F. Crick, *Nature* 171:737, 1953.

Evidence that DNA replication is semiconservative: the Meselson–Stahl experiment

In 1958, Matthew Meselson and Franklin Stahl set out to discover whether DNA replication was semiconservative, conservative, or dispersive. Their idea was to allow parental DNA containing nucleotides of one density to replicate using nucleotides of a different density. They realized that after two rounds of DNA replication, the three proposed replication mechanisms could be distinguished by differences in the density of the newly replicated DNA (Figure 7-11, left).

To carry out their experiment, Meselson and Stahl grew *E. coli* cells in liquid medium containing the heavy isotope of nitrogen (^{15}N) rather than the normal light (^{14}N) form. The ^{15}N isotope was used by the cells to synthesize nitrogenous bases, which then were incorporated into newly synthesized DNA strands. After many cell divisions in ^{15}N , the DNA was almost completely labeled with the heavy isotope. Cells were then removed from the ^{15}N medium and placed into ^{14}N medium; after one and two cell divisions, DNA was isolated from each sample and analyzed.

Meselson and Stahl were able to distinguish DNA of different densities using a separation procedure called *cesium chloride gradient centrifugation*. If cesium chloride (CsCl) is spun in a centrifuge at a tremendously high speed (50,000 revolutions per minute, or rpm) for many hours, the cesium and chloride ions are pushed by centrifugal force toward the bottom of the tube. Ultimately, a gradient of ions is established in the tube, with the highest ion concentration, or density, at the bottom and the lowest density at the top. When DNA is centrifuged along with cesium chloride, it forms a band in the gradient at a position identical to its density (Figure 7-11, right). DNA of different densities will form bands at different places in the gradient. Cells initially grown in the heavy isotope ^{15}N showed DNA of high density. This DNA is shown in blue in the left-most tube of Figure 7-11. After growing these cells in the light isotope ^{14}N for one generation, they found that the DNA was of intermediate density, shown in half blue (^{15}N) and half gold (^{14}N) in the middle tube of Figure 7-11. This banding pattern supported the semiconservative (Figure 7-11a) and dispersive (Figure 7-11c) models and disproved the conservative (Figure 7-11b) model. Meselson and Stahl continued the experiment through a second *E. coli* generation so that they could distinguish semiconservative from dispersive replication. After two generations, they observed two bands of intermediate and low density, supporting the semiconservative model (right-most tube of Figure 7-11a) and disproving the dispersive model (right-most tube of Figure 7-11c).

KEY CONCEPT DNA is replicated semiconservatively by unwinding the two strands of the double helix and building a new complementary strand on each of the separated strands of the original double helix.

Evidence for a replication fork: the Cairns experiment

The next problem was to determine where replication initiates on the chromosome. The possibilities were that replication could initiate at one site or many sites, and that the sites could be random or defined. In 1963, John Cairns addressed this problem by allowing replicating DNA in bacterial cells to incorporate tritiated thymidine ($[^3\text{H}]\text{thymidine}$)—a **nucleoside** (a base linked to a sugar) labeled with a radioactive hydrogen isotope called tritium. In the cells, the nucleoside was converted to a nucleotide by phosphorylation and incorporated into newly replicated DNA. After varying the number of replication cycles in the presence of tritiated thymidine, Cairns carefully isolated the DNA and covered it with photographic emulsion for several weeks. This procedure, called autoradiography, allowed Cairns to develop a picture of the location of ^3H in the DNA. As ^3H decays, it emits a beta particle (an energetic electron). A photograph of black spots results from a chemical reaction that occurs wherever a beta particle strikes the emulsion. Hence, each tritiated thymidine incorporated into the DNA appears as a black spot on the photograph.

Since DNA is replicated semiconservatively, after one round of DNA replication, each newly synthesized daughter chromosome should contain one radioactive (“hot”) strand (with ^3H) that is detected in the autoradiograph, and another nonradioactive (“cold”) strand that is not detected. Indeed, after one replication cycle in $[^3\text{H}]\text{thymidine}$, a ring of black spots appeared in the autoradiograph. Cairns interpreted this ring as a newly formed radioactive strand in a circular daughter DNA molecule, as shown in **Figure 7-12a**. It is thus apparent that the bacterial chromosome is circular—a fact that also emerged from genetic analysis described earlier (see Chapter 6). Furthermore, Cairns found that chromosomes captured in the middle of a second replication cycle formed a structure that resembled the Greek letter theta (θ), with a thin circle of dots consisting of a single radioactive strand and a thick curve of dots cutting through the interior of the circle of DNA consisting of two radioactive strands (Figure 7-12b). Thus, this type of replication is often called theta replication. The ends of the thick curve of dots defined two sites of ongoing DNA replication and are referred to as **replication forks**. Cairns saw all sizes of theta autoradiographic patterns, suggesting that replication begins at one place and the replication forks progressively move around the ring. Other experiments showed that DNA replication initiates at a single, specific DNA sequence and spreads bidirectionally (that is, in opposite directions) from this site, and both DNA strands are simultaneously replicated.

KEY CONCEPT The Cairns experiment provided additional evidence for semiconservative replication and also demonstrated that replication in bacteria begins at one site in the genome and spreads bidirectionally by means of two replication forks.

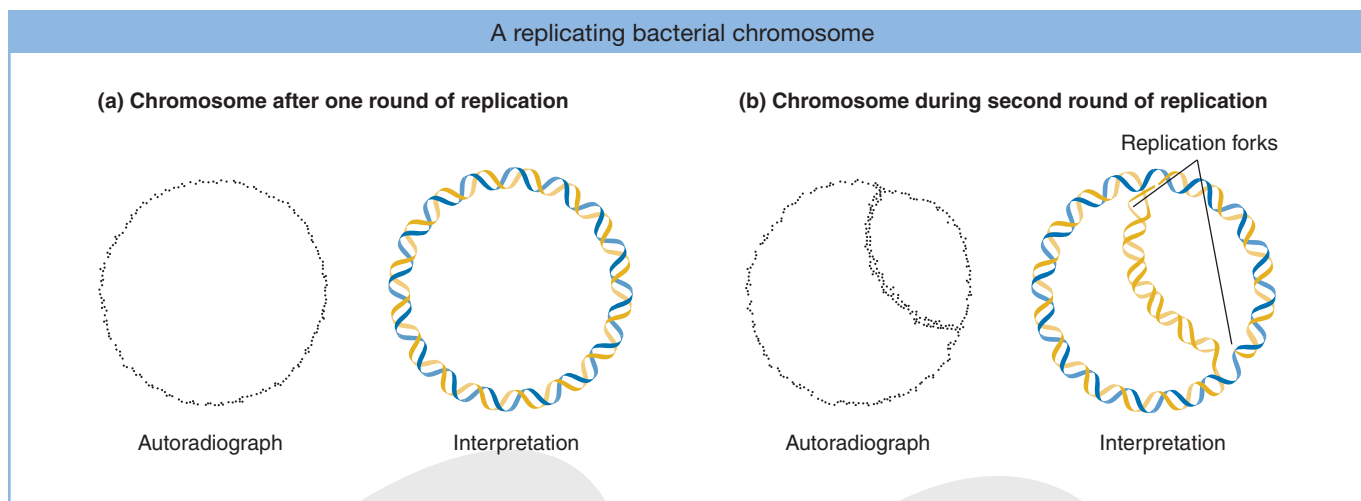


FIGURE 7-12 A replicating bacterial chromosome has two replication forks. (a) *Left:* An autoradiograph of a bacterial chromosome after one generation of replication in tritiated thymidine. *Right:* An interpretation of the autoradiograph. The gold helix represents the tritiated strand. In agreement with the semiconservative model of replication, one of the two strands should be radioactive. (b) *Left:* An autoradiograph of a bacterial chromosome in the second generation of replication in tritiated thymidine. *Right:* An interpretation of the autoradiograph. Replication forks occur at the two sites of ongoing, bidirectional DNA replication. Once again, in accord with the semiconservative model of replication, the newly replicated double helix that crosses the circle consists of two radioactive strands (if the parental strand was the radioactive one).

7.4 DNA REPLICATION IN BACTERIA

LO 7.5 Outline the factors and events involved in DNA replication.

In this section, we walk through the steps of DNA replication in bacteria, emphasizing the activities of enzymes in the replisome, the multi-protein molecular machine that carries out DNA replication. Similar steps occur in eukaryotes, and they are carried out by analogous enzymes ([Table 7-2](#)).

Unwinding the DNA double helix

When the double helix was proposed in 1953, a major objection was that replication of such a structure would require unwinding the double helix and breaking hydrogen bonds that hold the strands together. How could DNA be unwound so rapidly and, even if it could, wouldn't that overwind the DNA behind the fork and make it hopelessly tangled? The problem can be envisioned by thinking of two strands of a rope that are separated at one end while the other end is held stationary ([Figure 7-13a](#)). We now know that the replisome contains proteins that open the helix and

TABLE 7-2 Analogous DNA Replication Factors in Bacteria and Eukaryotes

Function	Bacteria (<i>E. coli</i>)	Eukaryotes (humans)
Recognizes origins	DnaA	ORC (origin recognition complex)
Unwinds double-stranded DNA	DnaB helicase	MCM2-7 (minichromosome maintenance 2-7) helicase
Assists helicase binding	DnaC	Cdc6 and ORC
Stabilizes single-stranded DNA	SSB	RPA (replication factor A)
Removes twists and supercoils	Gyrase	Topoisomerases
Synthesizes RNA primers	Primase	DNA pol α -primase complex
Elongates DNA	DNA pol III	DNA pol ϵ (leading strand) and δ (lagging strand)
Sliding clamp	β -clamp	PCNA (proliferating cell nuclear antigen)
Clamp loader	τ complex	RFC (replication factor C)
Removes RNA primers	DNA pol I	FEN1
Replaces RNA primers with DNA	DNA pol I	DNA pol ϵ
Ligates Okazaki fragments	DNA ligase	DNA ligase I

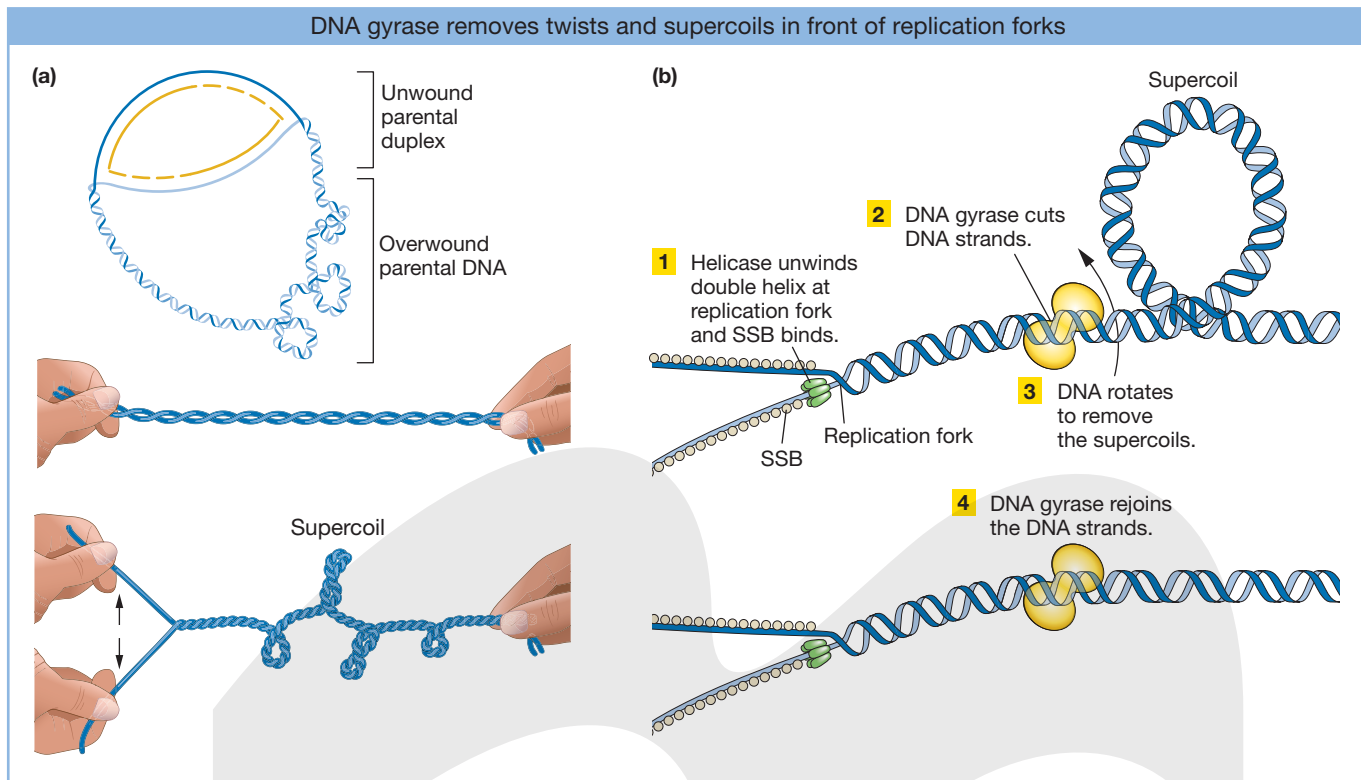


FIGURE 7-13 (a) Twisted and supercoiled regions accumulate ahead of the replication fork as parental DNA strands separate for replication, analogous to what happens when a rope is separated into individual strands. (b) Helicase encircles one DNA strand at each replication fork and uses the energy of ATP hydrolysis to break hydrogen bonds between bases, unzipping the two strands and causing twisting and supercoiling. SSB molecules bind the single-stranded DNA to prevent their reannealing. A topoisomerase called DNA gyrase counteracts twisting and supercoiling by cutting the DNA strands, allowing them to rotate, and then rejoining the strands.

prevent overwinding; they are **helicases** and **topoisomerases**, respectively.

Helicases are enzymes that disrupt hydrogen bonds that hold the two strands of the double helix together. The DNA replication helicase is a ring-shaped homohexamer of DnaB proteins (that is, a complex of six copies of DnaB) that encircles single-stranded DNA at the replication forks. From this position, helicases use energy from ATP hydrolysis to rapidly unzip the double helix ahead of DNA synthesis (Figure 7-13b, step 1). Unwound DNA is stabilized by **single-strand DNA-binding (SSB) proteins**, which bind to single-stranded DNA and prevent the duplex from re-forming.

Unwinding of DNA by helicases causes extra twisting to occur ahead of replication forks, and supercoils form to release the strain of the extra twisting (Figure 7-13b, steps 1). Twists and supercoils must be removed (commonly called *relaxed*) to allow replication to continue. This is done by enzymes termed topoisomerases, of which an example is **DNA gyrase** (Figure 7-13b, steps 2 and 3). Topoisomerases relax supercoiled DNA by breaking either a single DNA strand or both strands, which allows DNA to rotate into a relaxed molecule. Topoisomerases finish their job by religating the strands of the now-relaxed DNA (Figure 7-13b, step 4).

KEY CONCEPT Helicases, topoisomerases, and single-strand-binding proteins generate and maintain single-stranded DNA that is used as a template for DNA replication.

Assembling the replisome: replication initiation

Assembly of the replisome is an orderly process that begins at a precise site on the chromosome called the **origin of replication**, or simply origin. *E. coli* replication begins from a single origin (a locus called *oriC*) and then proceeds in both directions (with moving forks at both ends, as shown in Figure 7-12b) until the forks merge. *OriC* is 245 base pairs long and contains five copies of 9-base-pair sequences called DnaA boxes and an adjacent DNA unwinding element that is AT-rich (Figure 7-14). The first step in replisome assembly is binding of a protein called DnaA to the DnaA boxes, which helps other copies of DnaA bind at the origin in a process called oligomerization. Subsequent binding of DnaA to the AT-rich region promotes unwinding to form a single-stranded DNA bubble. Recall that A–T base pairs are held together with only two hydrogen bonds, whereas G–C base pairs are held

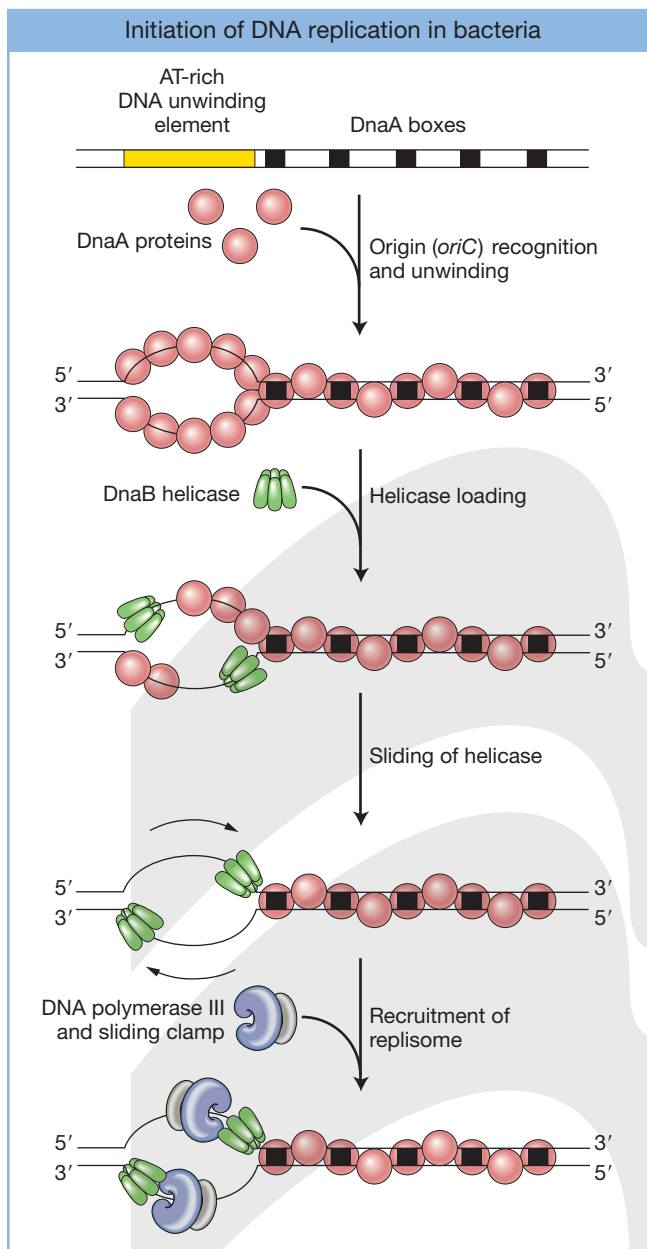


FIGURE 7-14 DNA synthesis is initiated at the origin of replication (*oriC*) in bacteria. DnaA proteins (pink) first bind DnaA boxes and then oligomerize throughout the origin. At the AT-rich DNA unwinding element, DnaA separates the two strands of the double helix and recruits the DnaB helicase (green) and other replisome components (blue) to the two replication forks.

together with three. Thus, it is easier to separate (melt) the double helix at stretches of DNA that are enriched in A–T base pairs.

After unwinding begins, additional DnaA proteins bind to the newly unwound single-stranded regions. With DnaA coating the origin, two DnaB helicases now bind and slide in a 5′-to-3′ direction to begin unzipping the helix at the replication forks. Although DnaA is necessary for the assembly of the replisome, it is not part of the replication machinery. Rather, its job is solely to bring the replisome to the correct place in the circular chromosome

for the initiation of replication. As replication progresses through the origin, the replisome displaces DnaA from the DNA.

KEY CONCEPT Where and when replication takes place are carefully controlled by ordered assembly of the replisome at a precise site called the origin.

DNA polymerases catalyze DNA chain elongation

Although scientists suspected that enzymes play a role in synthesizing DNA, that possibility was not verified until 1959, when Arthur Kornberg isolated a DNA polymerase from *E. coli* and demonstrated its enzymatic activity in vitro. This enzyme adds deoxyribonucleotides to the 3′ end of a growing nucleotide chain, using for its template a single strand of DNA that has been exposed by localized unwinding of the double helix (Figure 7-15). The substrates for DNA polymerases are the triphosphate forms of the deoxyribonucleotides, dATP, dGTP, dCTP, and dTTP (dNTP is used to refer to any of four deoxynucleoside triphosphates). Addition of each base to the growing polymer is accompanied by removal of two of the three phosphates in the form of pyrophosphate (PP_i). Energy produced by cleaving this bond and the subsequent hydrolysis of pyrophosphate to two inorganic phosphate molecules help drive the process of building a DNA polymer.

Five DNA polymerases are now known in *E. coli*. The enzyme that Kornberg purified is called **DNA polymerase I** or **DNA pol I**. This enzyme has three activities, which appear to be located in different parts of the molecule: (1) a polymerase activity that catalyzes DNA chain growth in the 5′-to-3′ direction, (2) a 3′-to-5′ exonuclease activity that removes mismatched nucleotides, and (3) a 5′-to-3′ exonuclease activity that degrades single strands of DNA or RNA. We will return to the significance of the two exonuclease activities later in this chapter.

Although DNA pol I has a role in DNA replication (see the next section), some scientists suspected that it was not responsible for the majority of DNA synthesis because it was too slow (~20 nucleotides/second; at this rate it would take ~30 hours to replicate the *E. coli* genome) and too abundant (~400 molecules/cell, which is more than needed for the two replication forks), and it was not processive (it dissociated from DNA after incorporating only 20 to 50 nucleotides). In 1969, John Cairns and Paula DeLucia settled this matter when they demonstrated that an *E. coli* strain harboring a mutation in the DNA pol I gene that had less than 1% of DNA pol I activity was still able to grow normally and replicate its DNA. They concluded that another DNA polymerase catalyzes DNA synthesis at the replication fork. This enzyme was later shown to be **DNA polymerase III** (DNA pol III).

KEY CONCEPT DNA polymerases synthesize DNA in the 5′-to-3′ direction using single-stranded DNA as a template.

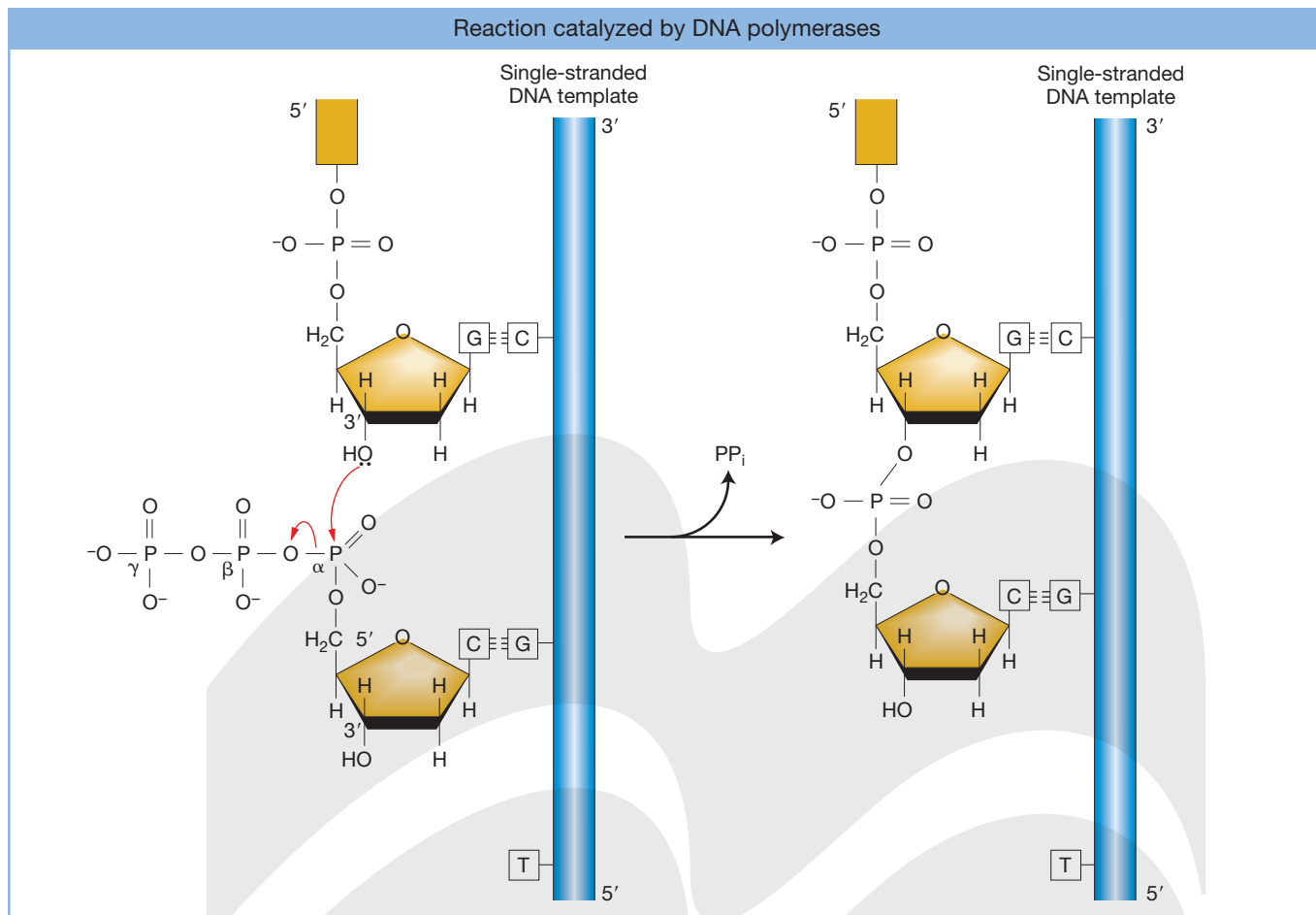


FIGURE 7-15 DNA polymerases catalyze the chain-elongation reaction. A dNTP, in this case dCTP, base pairs to the single-stranded DNA template, the free 3'-OH group at the end of the growing DNA chain is activated to attack the alpha (α) phosphate of the dNTP, resulting in attachment of dNMP to the 3' end and release of linked β and γ phosphates (PP_i). Thus, the DNA chain is elongated in the 5'-to-3' direction by DNA polymerases.

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The nucleotide polymerization process

DNA replication is semidiscontinuous

Another problem in DNA replication arises because DNA polymerases can extend a chain but cannot start a chain. Therefore, synthesis must be initiated by a **primer**, a short chain of nucleotides that forms a segment of duplex nucleic acid (Figure 7-16). Primers are synthesized by a set of proteins called a **primosome**, of which a central component is an RNA polymerase called **primase** (Figure 7-17). Primase copies the template DNA in the 5'-to-3' direction, producing a short RNA of about 11 nucleotides. DNA pol III then takes over and continues to copy the template DNA, extending off the 3' end of the RNA primer.

Because DNA polymerases synthesize DNA only in the 5'-to-3' direction, only one of the two DNA template strands can serve as a template for replication in the same direction as movement of each replication fork (see Figure 7-16). For this strand, called the **leading strand**, synthesis takes place in a smooth continuous manner. Synthesis

on the other template is also in the 5'-to-3' direction, but because it is in the direction opposite to that of replication fork movement it must be in short segments. Reinitiation of synthesis occurs for each segment as the growing fork exposes new DNA template. The 1000–2000 nucleotide stretches of newly synthesized DNA are called **Okazaki fragments**, in honor of their discoverer Reiji Okazaki. As with synthesis of the leading strand, each Okazaki fragment is primed at the 5' end by an RNA primer synthesized by primase. Thus, for this strand, called the **lagging strand**, synthesis takes place in a discontinuous manner. Because DNA replication is continuous for the leading strand and discontinuous for the lagging strand, the overall process is described as **semidiscontinuous**.

KEY CONCEPT DNA replication is described as semidiscontinuous because one DNA template strand is synthesized continuously and the other strand is synthesized as a series of discontinuous fragments.

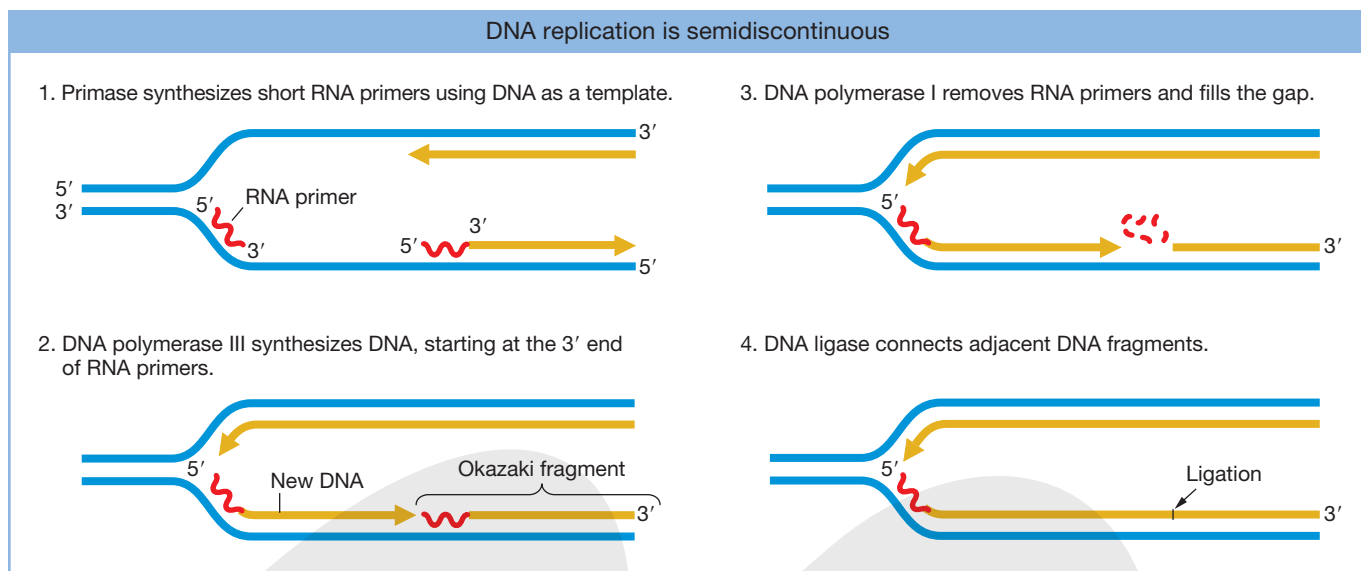


FIGURE 7-16 DNA replication takes place at the replication fork, where the double helix is unwound and the two strands are separated. DNA replication proceeds continuously in the direction of the unwinding replication fork for the leading strand. In contrast, DNA is synthesized in short segments in the direction away from the replication fork for the lagging strand. DNA polymerase requires a primer, a short chain of nucleotides, to be in place to begin synthesis. Additional details are provided in the text.

A different DNA polymerase, DNA pol I, removes the RNA primers with its 5'-to-3' exonuclease activity and fills in the gaps with its 5'-to-3' polymerase activity (Figure 7-17). As mentioned earlier, DNA pol I is the enzyme originally purified by Kornberg. Another enzyme, **DNA ligase**, joins the 3' end of the gap-filling DNA to the 5' end of the downstream Okazaki fragment. In general, DNA ligases join broken pieces of DNA by catalyzing formation of a phosphodiester bond between a 5' phosphate of one fragment and a 3' OH group of an adjacent fragment.

KEY CONCEPT DNA synthesis by DNA polymerase III requires an RNA primer, synthesized by the primase enzyme, an RNA polymerase.

DNA replication is accurate and rapid

A hallmark of DNA replication is its accuracy, also called fidelity: overall, less than one error occurs every 10^{10} nucleotides. Part of the reason for the accuracy of DNA replication is that both DNA pol I and DNA pol III possess a 3'-to-5' exonuclease activity, which serves a “proofreading” function by excising incorrectly inserted mismatched bases (Figure 7-18). Once the mismatched base is removed, the polymerase has another chance to add the correct complementary base.

As you would expect, mutant strains lacking a functional 3'-to-5' exonuclease activity have a higher rate of mutation. In addition, because primase lacks a proofreading function,

the RNA primer is more likely than DNA to contain errors. The need to maintain the high fidelity of replication is one reason why RNA primers at the ends of Okazaki fragments must be removed and replaced with DNA. Only after the RNA primer is gone does DNA pol I catalyze DNA synthesis to replace the primer. Mismatches that escape proofreading are corrected by DNA repair mechanisms that will be covered in detail in Chapter 15.

KEY CONCEPT DNA polymerases I and III have proofreading activity but primase does not.

Another hallmark of DNA replication is speed. It takes *E. coli* about 40 minutes to replicate its chromosome. Therefore, its genome of about 5 million base pairs must be copied at a rate of about 2000 nucleotides per second. From the experiment of Cairns, we know that *E. coli* uses only two replication forks to copy its entire genome. Thus, each fork must be able to move at a rate of about 1000 nucleotides per second. What is remarkable about the entire process of DNA replication is that it does not sacrifice speed for accuracy. How can it maintain both speed and accuracy, given the complexity of the reactions at the replication fork? The answer is that DNA polymerase is part of a large complex that coordinates the activities at the replication fork. This complex, the replisome, is an example of a “molecular machine.” You will encounter other examples in later chapters. The discovery that most major functions

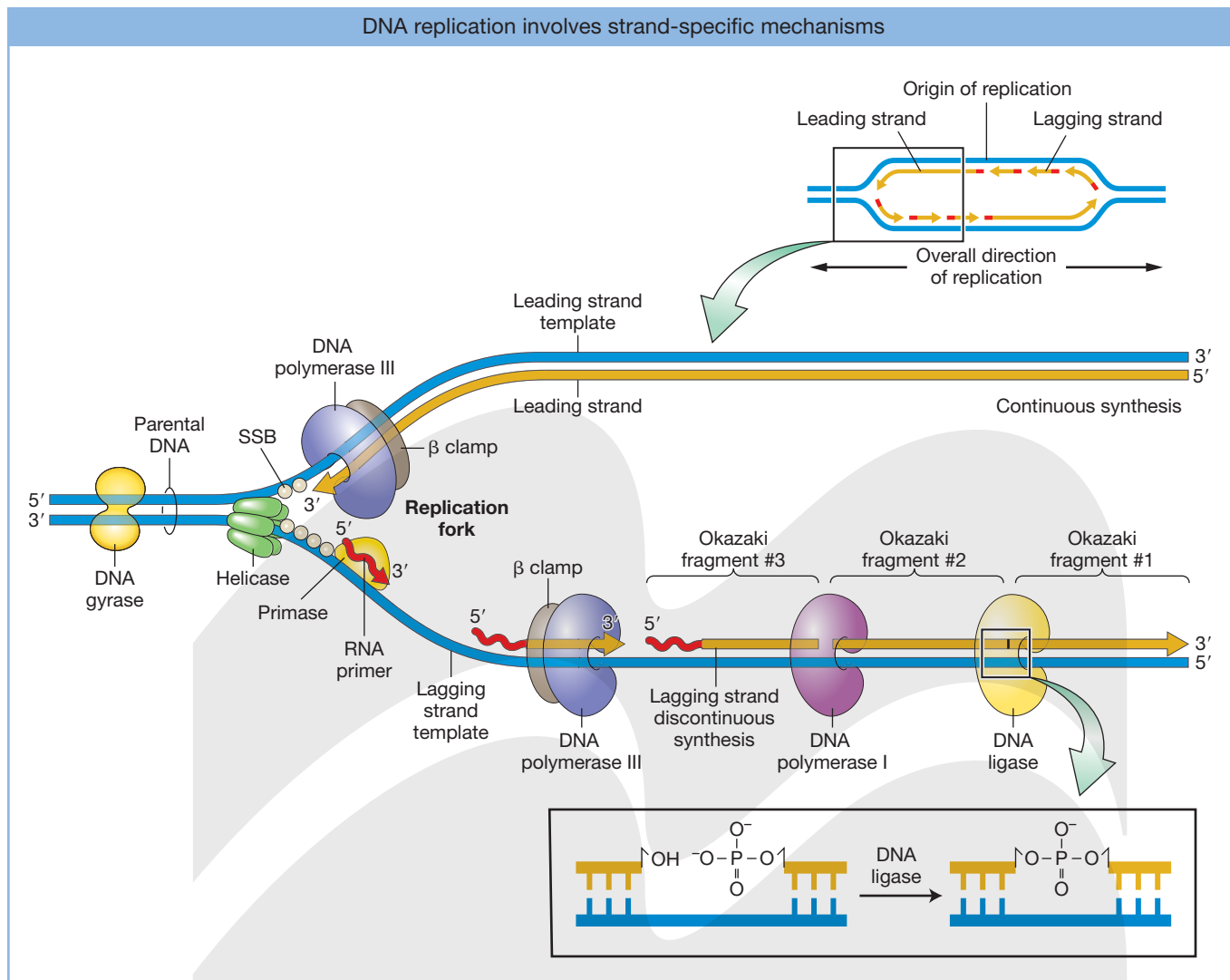


FIGURE 7-17 DNA replication occurs in both directions. The same protein factors are required for synthesis of both the leading and lagging strands, but synthesis occurs in a single continuous stretch for the leading strand and in short stretches for the lagging strand. The β clamp is required for processive DNA synthesis. Helicase separates the strands of parental DNA and gyrase (a topoisomerase) removes the twists and supercoils in DNA. Single-strand DNA-binding proteins (SSBs) prevent the separated DNA strands from reannealing. Primase makes short RNA primers for continuous synthesis by DNA pol III of the leading strand as well as discontinuous synthesis of the lagging strand as Okazaki fragments. DNA pol I removes RNA primers and fills in the resulting gaps. Lastly, DNA ligase joins the DNA fragments together.

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Leading and lagging strand synthesis

of cells—replication, transcription, and translation, for example—are carried out by large multisubunit complexes has changed the way that we think about cells. To begin to understand why, let's look at the replisome more closely.

Some of the interacting components of the replisome in *E. coli* are shown in **Figure 7-19**. At the replication fork, the catalytic core of DNA pol III is part of a much larger complex, called the **DNA pol III holoenzyme**, which consists of two catalytic cores and several accessory proteins. One of the catalytic cores handles synthesis of the leading strand while the other handles the lagging strand. The lagging strand is shown looping around so that the replisome can coordinate the synthesis of both strands and move in the direction of the replication fork. Some of the accessory

proteins (not visible in **Figure 7-19**) form a connection that bridges the two catalytic cores, thus coordinating synthesis of the leading and lagging strands.

KEY CONCEPT A molecular machine called the replisome carries out DNA synthesis. It includes two DNA polymerase units to handle synthesis on each strand, and it coordinates the activity of accessory proteins required for unwinding the double helix, stabilizing the single strands and processing RNA primers.

Attachment of DNA pol III to the DNA template is maintained by other accessory proteins, the **β clamp** (also known as the **sliding clamp**), which encircles the DNA like a

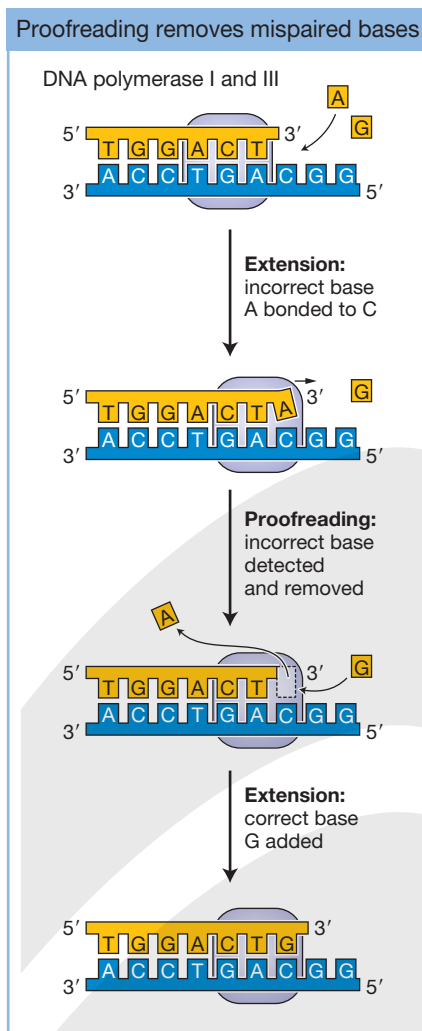


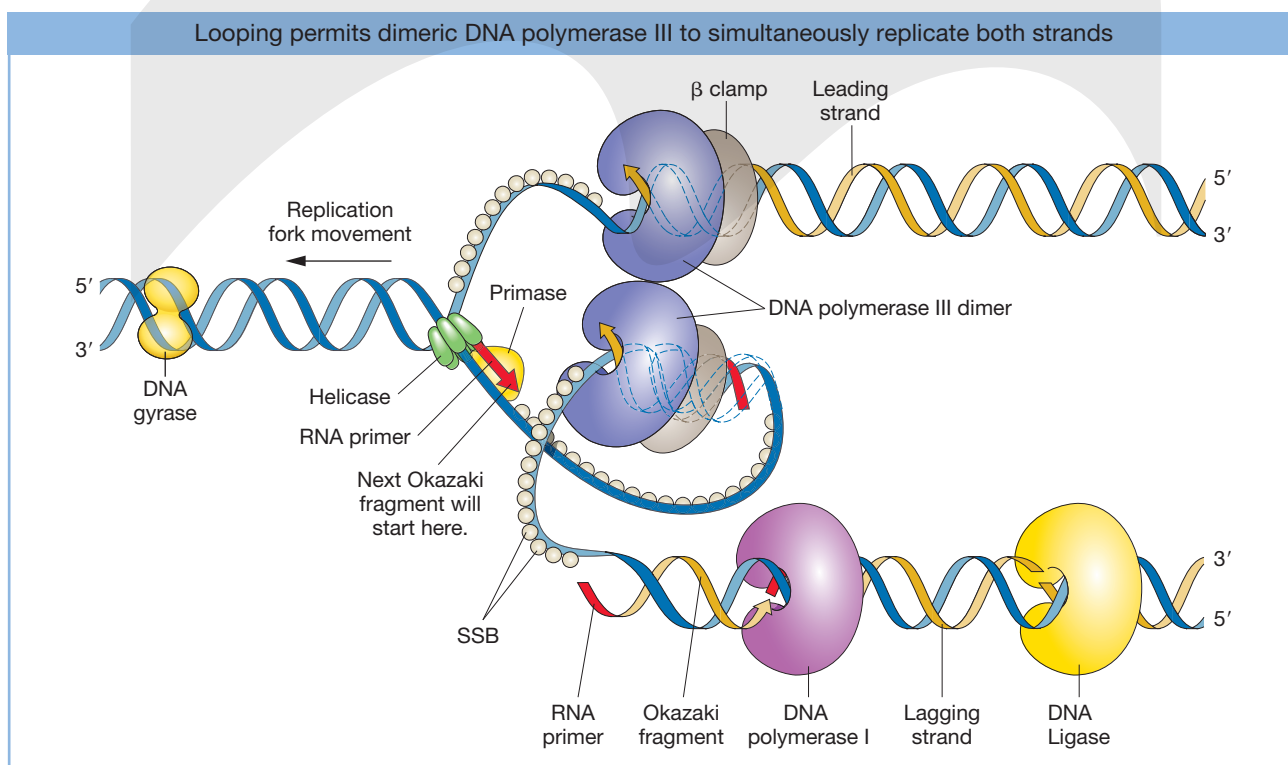
FIGURE 7-18 DNA polymerases I and III use their 3'-to-5' exonuclease activity to remove the A-C mismatch.

donut, and the clamp loader (also called τ complex), which assembles β clamps onto DNA. The β clamp transforms DNA pol III from an enzyme that can add only 10 nucleotides before falling off the template (termed a **distributive enzyme**) into an enzyme that stays at the moving fork and adds tens of thousands of nucleotides (termed a **processive enzyme**). In sum, through the action of accessory proteins, synthesis of both the leading and lagging strands is rapid and highly coordinated.

Note that primase, the enzyme that synthesizes the RNA primer, is not touching the clamp protein. Therefore, primase acts as a distributive enzyme—it adds only a few ribonucleotides before dissociating from the template. This mode of action makes sense because primers only need to be long enough to form a suitable duplex starting point for DNA pol III.

KEY CONCEPT The β clamp converts DNA polymerase III from a distributive to a processive enzyme.

FIGURE 7-19 A dimer of DNA pol III enzymes coordinates replication of the leading and lagging DNA strands. Looping of the template for the lagging strand orients it for synthesis by DNA pol III in the 5'-to-3' direction. DNA pol III releases the lagging strand template after synthesizing 1000–2000 nucleotides, a new loop is formed, and primase synthesizes an RNA primer to initiate another Okazaki fragment.



7.5 DNA REPLICATION IN EUKARYOTES

LO 7.6 Explain why and how DNA replication differs between bacteria and eukaryotes.

DNA replication in both bacteria and eukaryotes uses a semiconservative mechanism and employs leading and lagging strand synthesis. For this reason, it should not come as a surprise that components of the bacterial and eukaryotic replisomes are very similar (see Table 7-2). However, because eukaryotic genomes are larger and have linear, not circular, chromosomes, there are additional mechanistic complexities and associated factors. Bacteria such as *E. coli* usually complete replication in about 40 minutes, but in eukaryotes, the amount of time to complete replication can vary from a few minutes to many hours depending on many features, including the size of the genome, the number of origins, and the type of cell. Eukaryotes also have to solve the problem of coordinating the replication of more than one chromosome.

Eukaryotic origins of replication

To understand eukaryotic replication origins, we will first turn our attention to the simple eukaryote yeast (*Saccharomyces cerevisiae*). Many eukaryotic proteins having roles at replication origins were first identified in yeast because of the ease of genetic analysis (see the yeast Model Organism box in Chapter 12). Origins of replication in yeast are referred to as autonomously replicating sequences (ARSs) and are very much like *oriC* in *E. coli*. ARSs are about 100 to 200 base pairs long and contain several conserved DNA sequence elements, including an AT-rich element that melts when an initiator protein binds to adjacent elements. Unlike bacterial chromosomes, each eukaryotic chromosome has many replication origins to quickly

replicate the much larger eukaryotic genomes. Approximately 400 replication origins are dispersed throughout the 16 chromosomes of yeast, and humans have 40,000 to 80,000 origins among the 23 chromosomes. Thus, in eukaryotes, replication proceeds in both directions from multiple points of origin (Figure 7-20). Double helices that are produced at each origin elongate and eventually join one another. When replication of the two strands is complete, two identical daughter molecules of DNA result.

KEY CONCEPT Replication proceeds in both directions from hundreds or thousands of origins on linear eukaryotic chromosomes.

DNA replication and the yeast cell cycle

DNA synthesis takes place only in S (synthesis) phase of the eukaryotic cell cycle (Figure 7-21). How is the onset of DNA synthesis limited to this single phase? In yeast, the method of control is to link replisome assembly to the cell cycle. Figure 7-22 shows the process. In yeast, three proteins are required to begin assembly of the replisome. The **origin recognition complex (ORC)** first binds to sequences in yeast origins, much as DnaA protein does in *E. coli*. ORC then acts as a landing pad to recruit Cdc6 to origins early in gap 1 (G1) phase of the cell cycle. Together, ORC and Cdc6 then load a complex of Cdt1 and helicase. A second helicase-Cdt1 complex is recruited through association with the already assembled helicase-Cdt1 complex. Once the helicases are on the DNA in early S phase, Cdc6 and Cdt1 are released and DNA polymerases are loaded onto the DNA. Replication is linked to the cell cycle through the availability of Cdc6 and Cdt1. In yeast, these proteins are synthesized during late mitosis (M) and G1 and are destroyed by proteolysis at the beginning of S phase. In this way, the replisome can only be assembled before S phase.

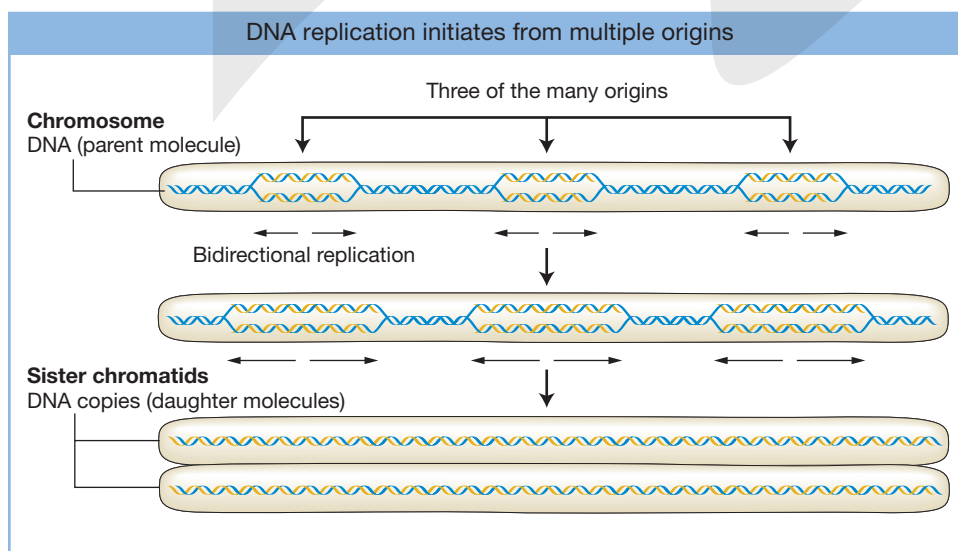


FIGURE 7-20 DNA replication proceeds in both directions from an origin of replication. Three origins of replication are shown in this example.

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DNA replication: replication of a chromosome

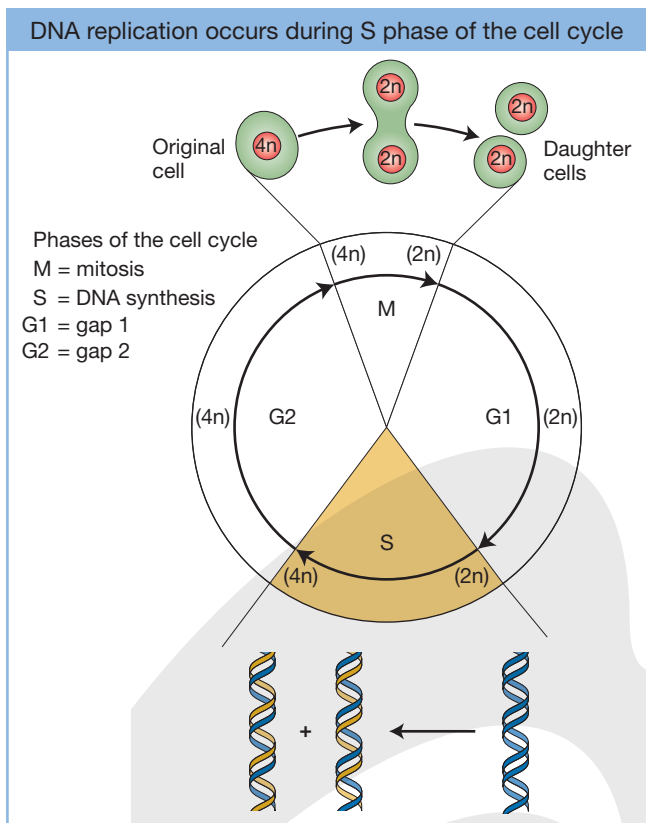


FIGURE 7-21 The cell cycle is composed of G1, S, G2, and M phases. DNA replication occurs during S phase, and chromosome segregation and cell division occur during M phase. Numbers in parentheses indicate the ploidy (that is, the number of sets of chromosomes) at each phase of the cell cycle in a diploid organism.

Once replication has begun, new replisomes cannot form at origins, because Cdc6 and Cdt1 are no longer available.

KEY CONCEPT DNA replication in eukaryotes requires Cdc6 and Cdt1, proteins that are only available during late mitosis (M) and G1 phase, ensuring that the genome is only replicated once per cell cycle.

Replication origins in higher eukaryotes

As already stated, most of the approximately 400 origins of replication in yeast are composed of similar DNA sequence motifs (100–200 base pairs in length) that are recognized by ORC subunits. Although all characterized eukaryotes have similar ORC proteins, origins of replication in higher eukaryotes such as humans are much longer, possibly as long as tens of thousands or hundreds of thousands of base pairs. Significantly, they have limited sequence similarity. Thus, although yeast ORC recognizes specific DNA sequences in yeast chromosomes, what the related ORCs of higher eukaryotes recognize is not clear at this time, but the feature recognized is probably not a specific DNA sequence. In practical terms, this uncertainty means

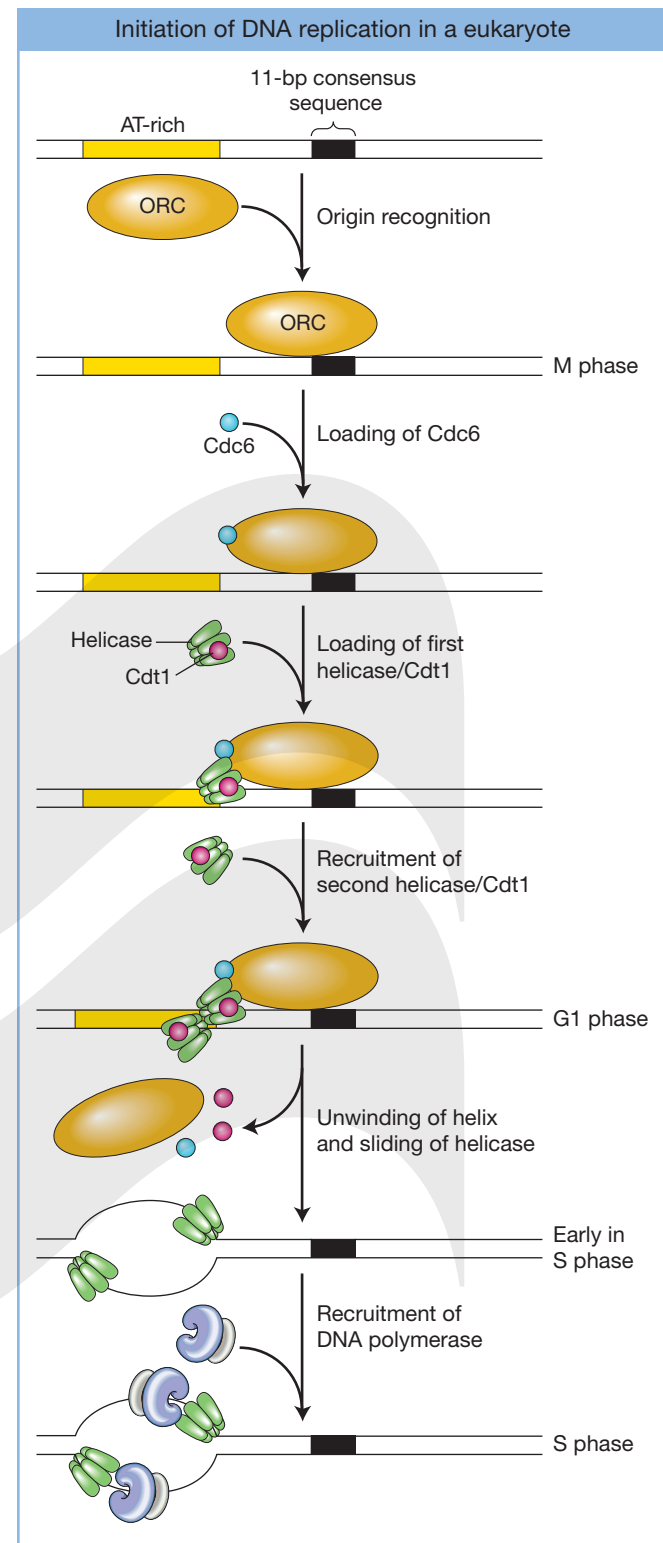


FIGURE 7-22 This eukaryotic example from yeast shows initiation of DNA synthesis at an origin of replication. Similar to initiation in bacteria (see Figure 7-14), sequences at the origin of replication are bound by a protein complex, in this case the origin recognition complex (ORC), which recruits helicases to separate the two strands of the double helix. The cell cycle-regulated factors Cdc6 and Cdt1 are essential for recruitment of the helicases, thereby linking DNA replication to the cell cycle.

that it is much harder to isolate origins from humans and other higher eukaryotes, because scientists cannot use an isolated DNA sequence of one human origin, for example, to perform a computer search of the entire human genome sequence to find other origins.

If the ORCs of higher eukaryotes do not interact with a specific sequence scattered throughout the chromosomes, then how do they find the origins of replication? These ORCs are thought to interact indirectly with origins by associating with other protein complexes that are bound to chromosomes. Such a recognition mechanism may have evolved so that higher eukaryotes can regulate the timing of DNA replication during S phase. Gene-rich regions of the chromosome (the euchromatin) have been known for some time to replicate early in S phase, whereas gene-poor regions, including the densely packed heterochromatin, replicate late in S phase (see Chapter 12 for more about euchromatin and heterochromatin).

KEY CONCEPT Yeast origins of replication, like origin in bacteria, contain a conserved DNA sequence that is recognized by ORC and other proteins needed to assemble the replisome. In contrast, origins of higher eukaryotes have been difficult to isolate and study because they are long and complex and do not contain a conserved DNA sequence.

Telomeres and telomerase: replication termination

Replication of the linear DNA in a eukaryotic chromosome proceeds in both directions from numerous replication origins, as shown in Figure 7-20. This process replicates most of the chromosomal DNA, but there is an inherent problem in replicating the two ends of linear DNA, the regions called **telomeres**. Continuous synthesis for the leading strand can proceed right up to the very tip of the template. However, lagging strand synthesis requires primers ahead of the process; so, when the last primer is removed, sequences are missing at the end of the strand (Figure 7-23, terminal gap). At each subsequent replication cycle, the telomere would continue to shorten, losing essential coding information.

Cells have evolved a specialized system to prevent this loss. The solution has two parts. First, the ends of chromosomes have a simple sequence that is repeated many times. Thus, every time a chromosome is replicated and shortened, only these repeated sequences, which do not contain protein-coding information, are lost. Second, an enzyme called **telomerase** adds these repeated sequences back to the chromosome ends.

KEY CONCEPT Telomeres stabilize chromosomes by preventing loss of genomic information after each round of DNA replication.

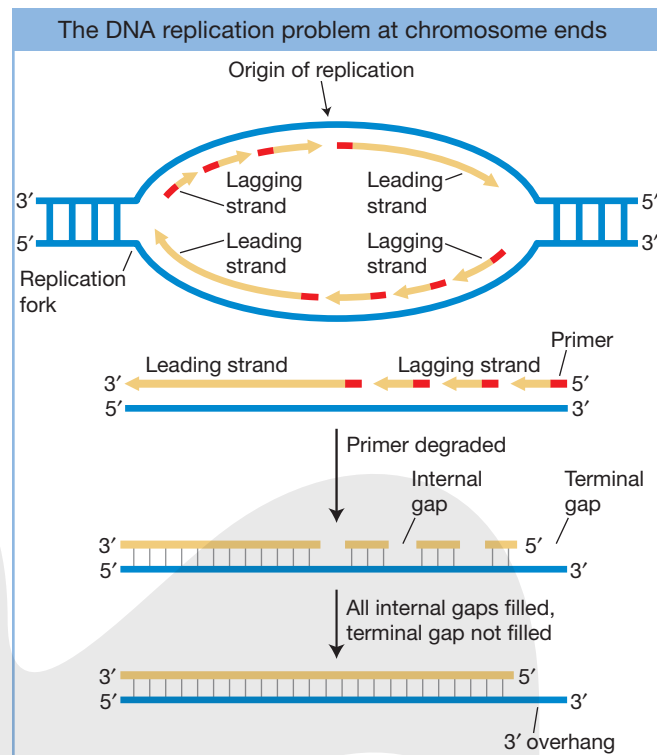


FIGURE 7-23 *Top:* The replication of each Okazaki fragment for the lagging strand begins with a primer. *Bottom:* The fate of the bottom strand in the transcription bubble. When the primer for the last Okazaki fragment of the lagging strand is removed, there is no way to fill the gap by conventional replication. A shortened chromosome would result when the chromosome containing the gap is replicated.

The discovery that the ends of chromosomes are made up of sequences repeated in tandem was made in 1978 by Elizabeth Blackburn and Joe Gall, who were studying DNA in the unusual macronucleus of the single-celled ciliate *Tetrahymena*. Like other ciliates, *Tetrahymena* has a conventional micronucleus and an unusual macronucleus in which the chromosomes are fragmented into thousands of gene-size pieces with new ends added to each piece. With so many chromosome ends, *Tetrahymena* has about 40,000 telomeres and, as such, was the perfect choice to determine telomere composition. Blackburn and Gall were able to isolate fragments containing the genes for ribosomal RNA (fragments called rDNA; see Chapter 9 for more on ribosomes) by using CsCl gradient centrifugation, the technique developed by Meselson and Stahl to study newly replicated *E. coli* DNA. The ends of rDNA fragments contained tandem arrays of the sequence TTGGGG (that is, TTGGGGTTGGGGTTGGGG...). We now know that virtually all eukaryotes have short tandem repeats at their chromosome ends; however, the sequence is not exactly the same. Human chromosomes, for example, end in about 10 to 15 kb of tandem repeats of the sequence TTAGGG.

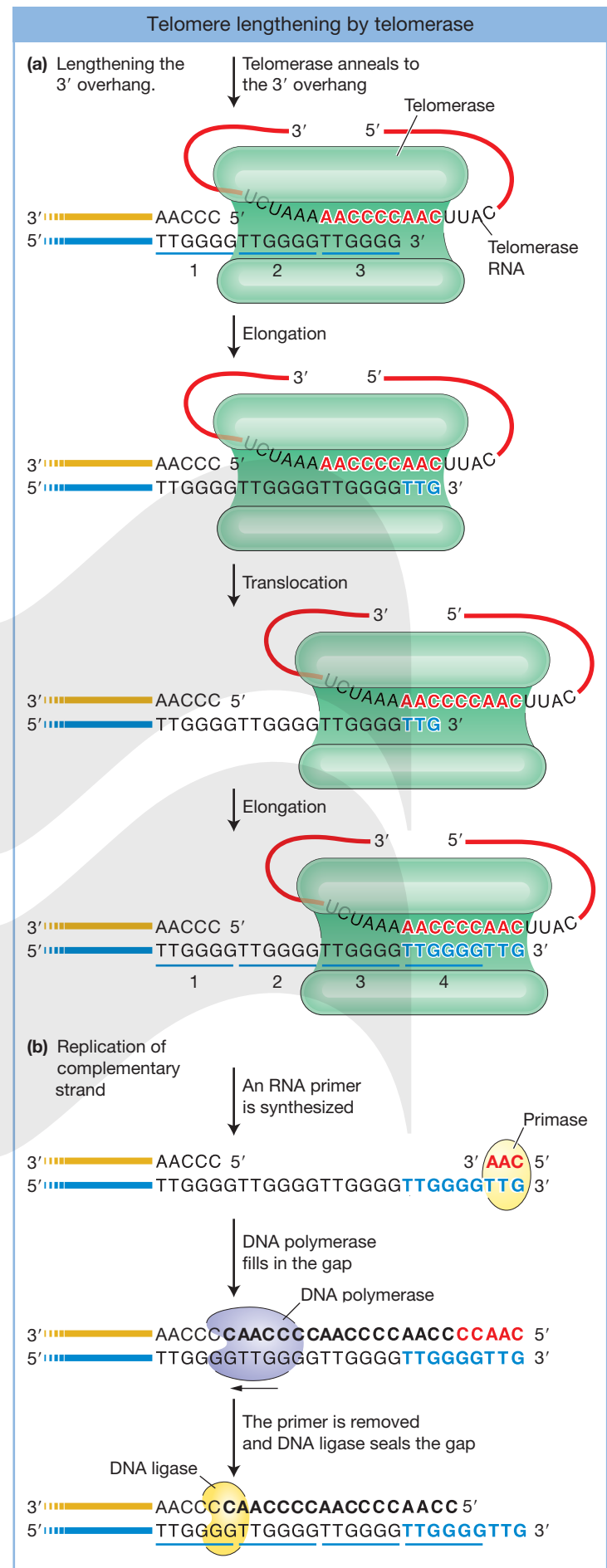
The question of how these repeats are added to chromosome ends after each round of replication was addressed by

FIGURE 7-24 (a) Telomerase carries a small RNA (red letters) that acts as a template for addition of a complementary DNA sequence, which is added to the 3' overhang (blue letters). To add another repeat, telomerase translocates to the end of the repeat that it just added. (b) The extended 3' overhang then serves as template for conventional DNA replication.

Elizabeth Blackburn and Carol Greider. They hypothesized that an enzyme catalyzed the process. Working again with extracts from the *Tetrahymena* macronucleus, they identified the telomerase enzyme, which adds the short repeats to the 3' ends of DNA. Telomerase is an RNA-protein complex, also called a ribonucleoprotein (RNP) complex. The protein component of the telomerase complex is a special type of DNA polymerase known as **reverse transcriptase** that uses RNA as a template to synthesize DNA. The RNA component of the telomerase complex varies in length from 159 nucleotides in *Tetrahymena* to 450 nucleotides in humans and about 1300 nucleotides in the yeast *Saccharomyces cerevisiae*. In all vertebrates, including humans, a region in the telomerase RNA contains the sequence 3'-AAUCCC-5' that serves as the template for synthesis of the 5'-TTAGGG-3' repeat unit by the mechanism shown in **Figure 7-24**. Briefly, the telomerase RNA first anneals to the 3'-end DNA overhang, which is then extended with the use of the telomerase's two components: the RNA and the reverse transcriptase protein. After addition of a repeat to the 3' end, the telomerase RNA moves along the DNA so that the 3' end can be further extended by its polymerase activity. The 3' end continues to be extended by repeated movement of the telomerase RNA. Primase and DNA polymerases then use the very long 3' overhang as a template to fill in the end of the other DNA strand. Working with Blackburn, a third researcher, Jack Szostak, went on to show that telomeres also exist in the less unusual eukaryote yeast. For contributing to the discovery of how telomerase protects chromosomes from shortening, Blackburn, Greider, and Szostak were awarded the 2009 Nobel Prize in Medicine or Physiology.

KEY CONCEPT Telomeres are specialized structures at the ends of linear chromosomes that contain tandem repeats of a short DNA sequence that is added to the 3' end by the enzyme telomerase.

In addition to preventing the erosion of genetic material after each round of replication, telomeres preserve chromosomal integrity by associating with proteins such as WRN, TRF1, and TRF2, to form a protective structure called a **telomeric loop (t-loop)** (**Figure 7-25**). These structures sequester the 3' single-stranded overhang, which can be as much as 100 nucleotides long. Without t-loops, the ends of chromosomes would be mistaken for double-strand breaks by the cell and dealt with accordingly. As you will see in Chapter 15, double-strand breaks are potentially very dangerous because they can result in chromosomal instability that can lead to cancer and a variety of



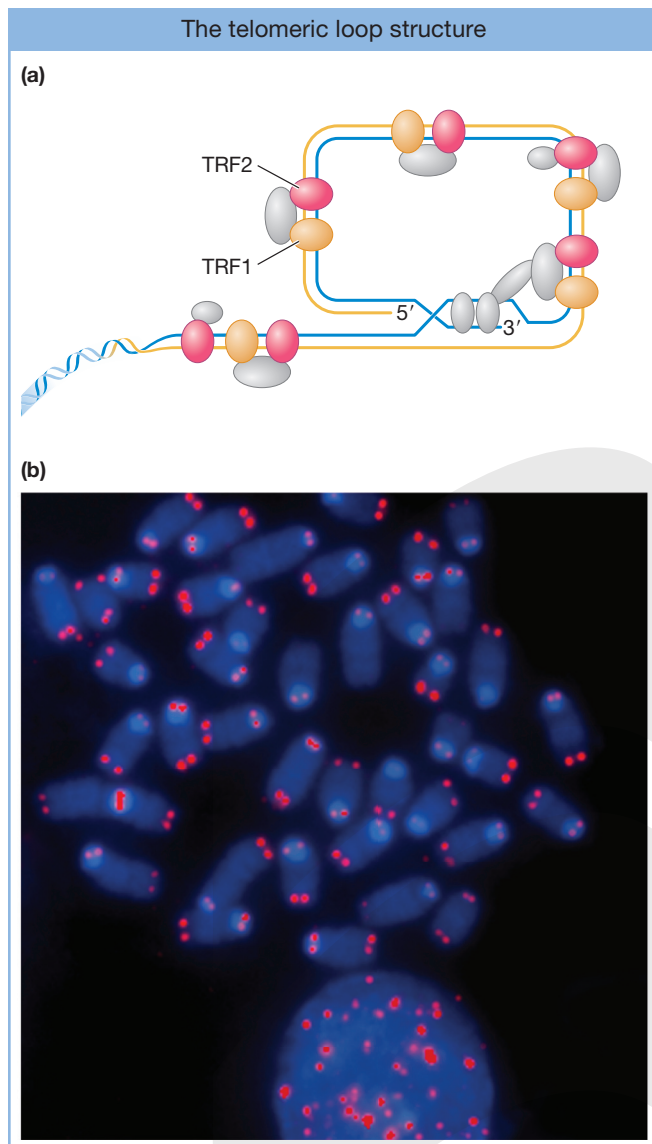


FIGURE 7-25 A telomeric loop (t-loop) structure protects the ends of chromosomes. (a) The 3' overhang is “hidden” when it displaces a DNA strand in a region where telomeric repeats are double-stranded. The proteins TRF1 and TRF2 bind to telomeric repeats, while other proteins, including WRN, bind to TRF1 and TRF2, thus forming the protective structure. (b) Visualized by immunofluorescence microscopy in pink are telomeres at the ends of chromosomes, shown in blue. [SCIENCE SOURCE/Science Source.]

phenotypes associated with aging. For this reason, when a double-strand break is detected, cells respond in a variety of ways, depending, in part, on the cell type and the extent of the damage. For example, a double-strand break can be fused to another break, or the cell can limit damage to the organism by stopping further cell division (called senescence) or by initiating a cell-death pathway (called apoptosis).

KEY CONCEPT Telomeres stabilize chromosomes by associating with proteins to form a structure that “hides” chromosome ends from the cell’s DNA repair machinery.

Werner syndrome causes premature aging

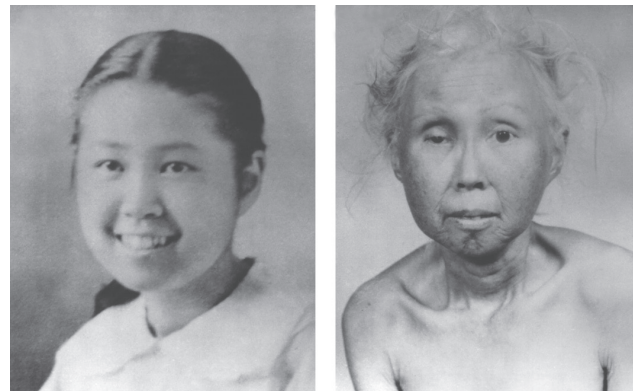


FIGURE 7-26 A woman with Werner syndrome at ages 15 (left) and 48 (right). [International Registry of Werner Syndrome, www.wernersyndrome.org.]

Surprisingly, although most germ cells have ample telomerase, somatic cells produce very little or no telomerase. For this reason, chromosomes of proliferating somatic cells get progressively shorter with each cell division until the cell stops all divisions and enters a senescence phase. This observation led many investigators to suspect that there was a link between telomere shortening and aging. Geneticists studying human diseases that lead to a premature-aging phenotype have uncovered evidence that supports such a connection. People with Werner syndrome experience early onset of many age-related events, including wrinkling of skin, cataracts, osteoporosis, graying of hair, and cardiovascular disease (**Figure 7-26**). Genetic and biochemical studies have found that afflicted people have shorter telomeres than those of normal people owing to a mutation in a gene called WRN (conferring Werner syndrome), which encodes a helicase that associates with proteins that comprise the telomeric loop (TRF2, **Figure 7-25**). This mutation is hypothesized to disrupt the normal telomere, resulting in chromosomal instability and premature aging. Patients with another premature-aging syndrome called dyskeratosis congenita also have shorter telomeres than those of healthy people of the same age, and they harbor mutations in genes required for telomerase activity.

Geneticists are also very interested in connections between telomeres and cancer. Unlike normal somatic cells, about 80% of cancer cells have telomerase activity. The ability to maintain functional telomeres may be one reason why cancer cells, but not normal cells, can grow in cell culture for decades and are considered to be immortal. As such, many pharmaceutical companies are seeking to capitalize on this difference between cancerous and normal cells by developing drugs that selectively target cancer cells by inhibiting telomerase activity.

KEY CONCEPT Telomeres and telomerase are associated with aging and cancer.

SUMMARY

Experimental work on the molecular nature of hereditary material has demonstrated conclusively that DNA (not protein, lipids, or carbohydrates) is the genetic material. Using data obtained by others, Watson and Crick deduced a double-helical model with two DNA strands, wound around each other, running in antiparallel fashion. Binding of the two strands together is based on the fit of adenine (A) to thymine (T) and guanine (G) to cytosine (C). The former pair is held by two hydrogen bonds; the latter, by three.

The Watson–Crick model shows how DNA can be replicated in an orderly fashion—a prime requirement for genetic material. Replication is accomplished semiconservatively in both bacteria and eukaryotes. One double helix is replicated to form two identical helices, each with their nucleotides in the identical linear order; each of the two new double helices is composed of one old and one newly polymerized strand of DNA.

The DNA double helix is unwound at a replication fork, and the two single strands serve as templates for polymerization of free nucleotides. Nucleotides are polymerized by the enzyme DNA polymerase, which adds new nucleotides only to the 3' end of a growing DNA chain. Because addition is only at 3' ends, polymerization on one template is continuous, producing the leading strand; and on the other, it is discontinuous in short stretches (Okazaki fragments), producing the lagging strand. Synthesis of the leading strand and of every Okazaki fragment is primed by a short

RNA primer (synthesized by primase) that provides a 3' end for deoxyribonucleotide addition.

The multiple events that have to occur accurately and rapidly at the replication fork are carried out by a biological machine called the replisome. This protein complex includes two DNA polymerase units, one to produce the leading strand and the other to produce the lagging strand. In this way, the more complex synthesis and joining of Okazaki fragments into a continuous strand can be temporally coordinated with the less complex synthesis of the leading strand. Where and when replication takes place is carefully controlled by the ordered assembly of the replisome at certain sites on chromosomes called origins. Eukaryotic genomes can have tens of thousands of origins. Assembly of replisomes at origins takes place only at a specific time in the cell cycle.

The ends of linear chromosomes (telomeres) in eukaryotes present a problem for the replication system because there is always a short stretch on one strand that cannot be primed. The enzyme telomerase adds numerous short, repetitive sequences to maintain the length of telomeres. Telomerase carries a small RNA that acts as the template for synthesis of telomeric repeats. These noncoding telomeric repeats associate with proteins to form a telomeric loop that protects against DNA damage. Telomeres shorten with age because telomerase is not produced in somatic cells. Individuals who have defective telomeres experience premature aging.

KEY TERMS

adenine (p. 244)
antiparallel (p. 247)
bases (p. 244)
 β -clamp (sliding clamp) (p. 256)
cell cycle (p. 258)
complementary bases (p. 247)
conservative replication (p. 249)
cytosine (p. 244)
daughter molecule (p. 249)
deoxynucleotide (p. 244)
deoxyribose (p. 244)
dispersive replication (p. 249)
distributive enzyme (p. 257)
DNA gyrase (p. 252)
DNA ligase (p. 255)
DNA polymerase I (DNA pol I) (p. 253)
DNA polymerase III (DNA pol III) (p. 253)

DNA pol III holoenzyme (p. 256)
DNA replication (p. 240)
double helix (p. 240)
genetic code (p. 249)
guanine (p. 244)
helicase (p. 252)
lagging strand (p. 254)
leading strand (p. 254)
major groove (p. 248)
minor groove (p. 248)
nucleoside (p. 250)
nucleotide (p. 240)
Okazaki fragment (p. 254)
origin of replication (origin) (p. 252)
Origin recognition complex (ORC) (p. 258)
parental molecule (p. 249)
phosphate (p. 244)
primase (p. 254)





primer (p. 254)
primosome (p. 254)
processive enzyme (p. 257)
purine (p. 244)
pyrimidine (p. 244)
radioisotope (p. 243)
replication fork (p. 250)
replisome (p. 240)
reverse transcriptase (p. 261)
ribose (p. 244)
semiconservative replication (p. 249)
semidiscontinuous (p. 254)
single-strand DNA-binding (SSB) protein (p. 252)
telomerase (p. 260)
telomere (p. 260)
telomeric loop (t-loop) (p. 261)
template (p. 240)
thymine (p. 244)
topoisomerase (p. 252)

PROBLEMS


Visit SaplingPlus for supplemental content. Problems with the  icon are available for review/grading. Problems with the  icon have an Unpacking the Problem exercise.



WORKING WITH THE FIGURES

(The first 27 questions require inspection of text figures.)

- In Table 7-1, complete the table for a genome that is 20 percent adenine. 
- In Figure 7-1, what features of the sculpture of DNA are correct or incorrect?
- In Figure 7-2, speculate as to why Griffith did not conduct the experiment the other way around, that is, with heat-killed R cells and live S cells.
- In Figure 7-3, what types of enzymes could Avery, Macleod, and McCarty have used to destroy proteins and RNAs?
- In Figure 7-4, what part of the DNA structure is labeled by ^{32}P ?
- In Figure 7-5, draw 7-methylguanine and 5-methylcytosine. A methyl group is CH_3 .
- In Figure 7-6, what information did Rosalind Franklin's X-ray diffraction data provide that was key to determining the structure of DNA?
- In Figure 7-7, why do you think that Watson and Crick built a three-dimensional model of DNA rather than only a two-dimensional model?
- In Figure 7-8, would the diameter of DNA change if the pyrimidine was on the left and the purine on the right? Justify your answer.
- In Figure 7-9a, why are there two rows of dots between A–T base pairs but three rows between G–C base pairs?
- In Figure 7-10a, is a purine or a pyrimidine on the left in the bottom base pair?
- In Figure 7-11, draw cesium chloride gradients for a Meselson–Stahl experiment in which cells are first grown in ^{14}N and then in ^{15}N for two generations.
- In Figure 7-12, draw an autoradiograph for a chromosome during the second round of replication in which the DNA that crosses the circle has one blue parental strand. 
- In Figure 7-13a, what would happen in the rope demonstration if you cut one of the two strands in the supercoiled region.
- In Figure 7-14, in the second to last diagram, why do the arrows show the two helicase molecules moving in opposite directions?
- In Figure 7-15, draw the chemical reaction that occurs to add the next nucleotide in the DNA chain.
- In Figure 7-16, draw an analogous diagram for the other replication fork.
- In Figure 7-17, which factors are involved in lagging strand synthesis but not leading strand synthesis?
- In Figure 7-18, draw the phosphodiester linkage between T and the misincorporated A in the strand being synthesized, and place an arrow at the bond that is broken by the 3'-to-5' exonuclease activity of DNA polymerase.
- In Figure 7-19, why is the DNA looped for one strand but not for the other strand when they are both serving as templates for DNA synthesis by the DNA polymerase III dimer?
- In Figure 7-20, could the spacing of origins affect the amount of time that it takes to replicate a chromosome?
- In Figure 7-21, how much DNA would a cell contain if it went through two cell cycles that did not include an M phase? 
- In Figure 7-22, why does replication not initiate at origins in G₂ phase?
- In Figure 7-23, analogous to the last diagram in the figure, draw the top DNA strand in the bubble after the primers are degraded and the gaps are filled. Based on this drawing, is telomerase required for the replication of both ends of chromosomes?
- In Figure 7-24a, the telomerase RNA template contains one and a half copies of the repeat sequence. Circle the full copy and put a box around the half copy. 
- In Figure 7-25b, fluorescent antibodies to what protein may have been used to detect the telomeres?
- In Figure 7-26, if Figure 7-25b represents chromosomes from this individual at a young age, how might the image in Figure 7-25b differ at the older age?

BASIC PROBLEMS

- Does the Hershey–Chase experiment definitively demonstrate that DNA is the genetic material, or just that it is consistent with being the genetic material? Justify your answer.
- Does the Avery, MacLeod, and McCarty experiment definitively demonstrate that DNA is the genetic material or just that it is consistent with being the genetic material? Justify your answer.
- Draw a cesium chloride gradient for the third generation of DNA produced by semiconservative replication in the Meselson–Stahl experiment. 

31. Write the sequence of the telomerase RNA that serves as a template for the telomere repeat sequence 5'-TTAGGG-3'. 
32. Why might Werner syndrome increase the chances of getting cancer?
33. Draw 2',3' dideoxyadenosine and predict what would happen if this nucleotide was incorporated into the growing DNA chain during replication.
34. Explain how DNA fulfills the three main requirements for a hereditary molecule: (1) the ability to store information, (2) the ability to be replicated, and (3) the ability to mutate.
35. Match the protein with its function. 

A. DNA polymerase	creates RNA primers
B. Helicase	links short DNA chains
C. Ligase	helps hold polymerase on DNA
D. Primase	separates DNA strands
E. Gyrase	prevents reannealing of DNA
F. Sliding clamp	extends DNA strand
G. SSB	removes supercoils in DNA
36. Why is telomerase not required for replication of the bacterial genome?
37. Explain what is meant by the terms *conservative* and *semiconservative replication*.
38. Describe two pieces of evidence indicating that DNA polymerase I is not the chromosomal replicase.
39. What is meant by a *primer*, and why are primers necessary for DNA replication?
40. A molecule of composition



5'-AAAAAAAAAAAAA-3'

3'-TTTTTTTTTTTTTT-5'

is replicated in a solution containing unlabeled (not radioactive) dGTP, dCTP, and dTTP plus dATP with all its phosphorus atoms in the form of the radioactive isotope ^{32}P . Will both daughter molecules be radioactive? Explain. Then repeat the question for the molecule

5'-ATATATATATATAT-3'

3'-TATATATATATATA-5'


41. Why is DNA synthesis continuous on one strand and discontinuous on the opposite strand?
42. Explain why cutting one strand of supercoiled DNA removes the supercoiling.
43. Describe how the enzymatic activities of DNA polymerases I and III are similar and different. 
44. If the GC content of a DNA is 48 percent, what are the percentages of the four bases (A, T, G, and C) in this molecule? 

45. Would the Meselson–Stahl experiment have worked if diploid eukaryotic cells had been used instead?
46. Consider the following segment of DNA, which is part of a much longer molecule constituting a chromosome:

5' ... ATTCGTACGATCGACTGACTGACAGTC ... 3'

3' ... TAAGCATGCTAGCTGACTGACTGTCAG ... 5'


If the DNA polymerase starts replicating this segment from the right,

- a. which will be the template for the leading strand?
 - b. draw the molecule when the DNA polymerase is halfway along this segment.
 - c. draw the two complete daughter molecules.
47. The DNA polymerases are positioned over the following DNA segment (which is part of a much larger molecule) and moving from right to left. If we assume that an Okazaki fragment is made from this segment, what will be the fragment's sequence? Label its 5' and 3' ends. 

5' ... CCTAAGACTAACTACTTACTGGGATC ... 3'

3' ... GGAATTCTGATTGATGAATGACCCTAG ... 5'

CHALLENGING PROBLEMS

48. If you extract the DNA of the coliphage ϕX174 , you will find that its composition is 25 percent A, 33 percent T, 24 percent G, and 18 percent C. Does this composition make sense in regard to Chargaff's rules? How would you interpret this result? How might such a phage replicate its DNA? 
49. Given what you know about the structure and function of telomerase, provide a plausible model to explain how a species could exist with a combination of two different repeats (for example, TTAGGG and TTGTGG) on each of their telomeres.
50. Why is it unlikely that continuous replication of both DNA strands occurs but is yet to be discovered?

GENETICS AND SOCIETY

In this chapter, you learned that the shortening of chromosome telomeres due to diminished telomerase activity is associated with aging. This raises the possibility that gene therapy aimed at overexpression of telomerase will increase longevity. Do you think that it is ethical to use this approach to increase the longevity of normal, healthy people? Does your answer change if you consider that there are nongenetic means such as calorie restriction that may increase longevity, or that gene therapy is being pursued to treat numerous diseases?

