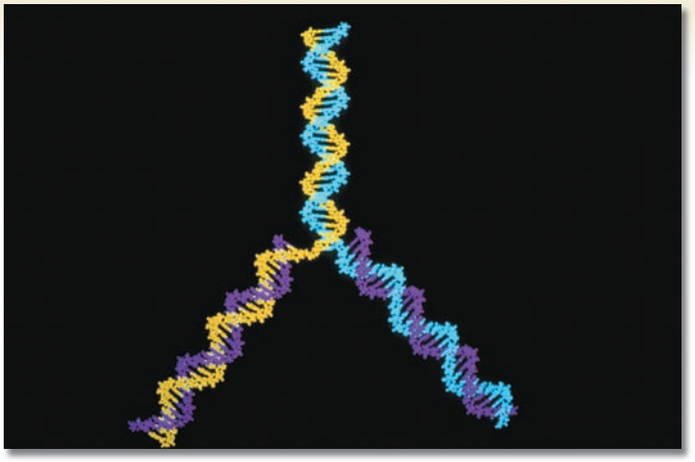
CHAPTER OUTLINE



A model for DNA undergoing replication. *This molecular model shows a DNA replication fork, the site where new DNA strands are made. In this model, the original DNA is yellow and blue. The newly made strands are purple.*

11 DNA REPLICATION

As discussed throughout Chapters 2 to 8, genetic material is transmitted from parent to offspring and from cell to cell. For transmission to occur, the genetic material must be copied. During this process, known as **DNA replication,** the original DNA strands are used as templates for the synthesis of new DNA strands. We will begin Chapter 11 with a consideration of the structural features of the double helix that underlie the replication process. Then we examine how chromosomes are replicated within living cells, addressing the following questions: where does DNA replication begin, how does it proceed, and where does it end? We first consider bacterial DNA replication and examine how DNA replication occurs within living cells, and then we turn our attention to the unique features of the replication of eukaryotic DNA. At the molecular level, it is rather remarkable that the replication of chromosomal DNA occurs very quickly, very accurately, and at the appropriate time in the life of the cell. For this to happen, many cellular proteins play vital roles. In this chapter, we will examine the mechanism of DNA replication and consider the functions of several proteins involved in the process.

11.1 STRUCTURAL OVERVIEW OF DNA REPLICATION

Because they bear directly on the replication process, let's begin by recalling a few important structural features of the double helix from Chapter 9. The double helix is composed of two DNA strands, and the individual building blocks of each strand are nucleotides. The nucleotides contain one of four bases: adenine, thymine, guanine, or cytosine. The double-stranded structure is held together by base stacking and by hydrogen bonding between the bases in opposite strands. A critical feature of the double-helix structure is that adenine hydrogen bonds with thymine, and guanine hydrogen bonds with cytosine. This rule, known as the AT/GC rule, is the basis for the complementarity of the base sequences in double-stranded DNA.

Another feature worth noting is that the strands within a double helix have an antiparallel alignment. This directionality is determined by the orientation of sugar molecules within the sugar-phosphate backbone. If one strand is running in the 5′ to 3′ direction, the complementary strand is running in the 3′ to 5′ direction. The issue of directionality will be important when we consider the function of the enzymes that synthesize new DNA

strands. In this section, we will consider how the structure of the DNA double helix provides the basis for DNA replication.

Existing DNA Strands Act as Templates for the Synthesis of New Strands

As shown in **Figure 11.1a**, DNA replication relies on the complementarity of DNA strands according to the AT/GC rule. During the replication process, the two complementary strands of DNA come apart and serve as **template strands,** or **parental strands,** for the synthesis of two new strands of DNA. After the double helix has separated, individual nucleotides have access to the template strands. Hydrogen bonding between individual nucleotides and the template strands must obey the AT/GC rule. To complete the replication process, a covalent bond is formed between the phosphate of one nucleotide and the sugar of the previous nucleotide. The two newly made strands are referred to as the **daughter strands.** Note that the base sequences are identical in both double-stranded molecules after replication (**Figure 11.1b**). Therefore, DNA is replicated so that both copies retain the same information—the same base sequence—as the original molecule.

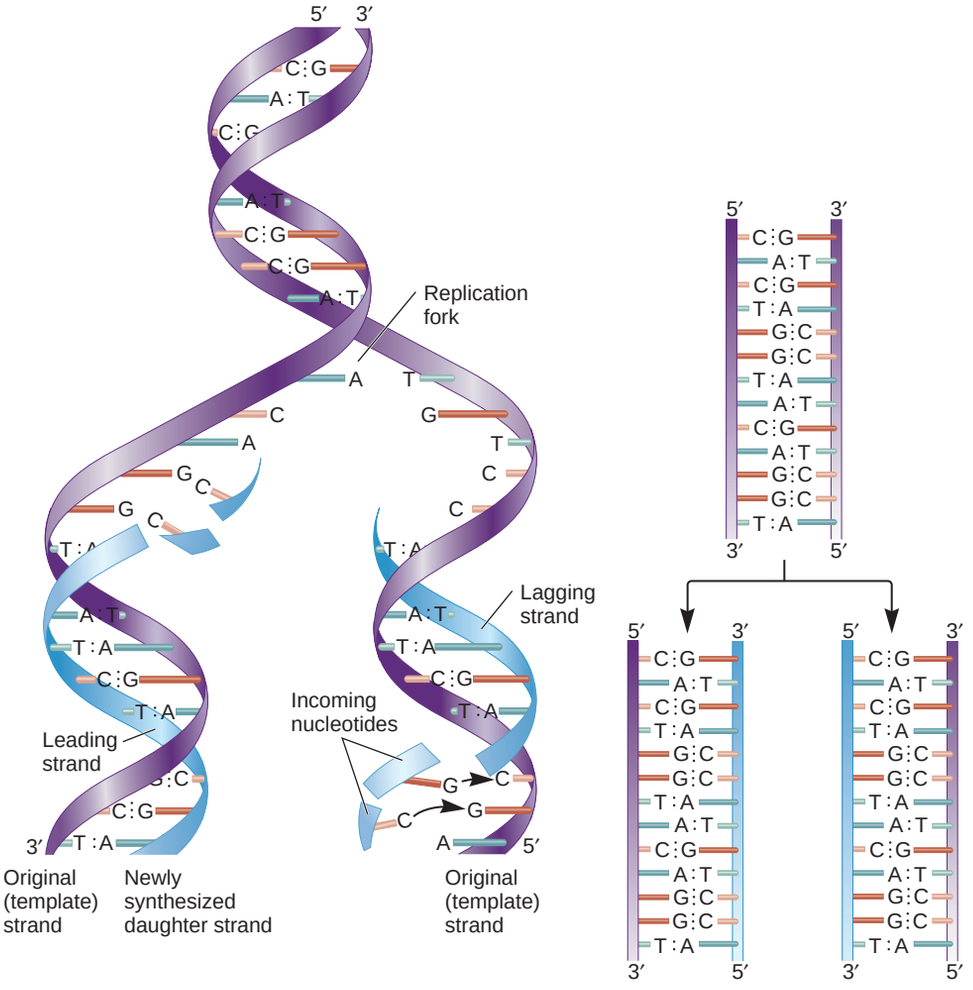


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FIGURE 11.1 The structural basis for DNA replication. **(a)** The mechanism of DNA replication as originally proposed by Watson and Crick. As we will see, the synthesis of one newly made strand (the leading strand) occurs in the direction toward the replication fork, whereas the synthesis of the other newly made strand (the lagging strand) occurs in small segments away from the replication fork. **(b)** DNA replication produces two copies of DNA with the same sequence as the original DNA molecule.

EXPERIMENT 11A

Three Different Models Were Proposed That Described the Net Result of DNA Replication

Scientists in the late 1950s had considered three different mechanisms to explain the net result of DNA replication. These mechanisms are shown in **Figure 11.2.** The first is referred to as a **conservative model.** According to this hypothesis, both strands of parental DNA remain together following DNA replication. In this model, the original arrangement of parental strands is completely conserved, while the two newly made daughter strands also remain together following replication. The second is called a **semiconservative model.** In this mechanism, the double-stranded DNA is half conserved following the replication process. In other words, the newly made double-stranded DNA contains one parental strand and one daughter strand. The third, called the **dispersive model,** proposes that segments of parental DNA and newly made DNA are interspersed in both strands following the replication process. Only the semiconservative model shown in Figure 11.2b is actually correct.

In 1958, Matthew Meselson and Franklin Stahl devised a method to experimentally distinguish newly made daughter strands from the original parental strands. Their technique involved labeling DNA with a heavy isotope of nitrogen. Nitrogen, which is found within the bases of DNA, occurs in both a heavy (^{15}N) and light (^{14}N) form. Prior to their experiment, they grew *Escherichia coli* cells in the presence of ^{15}N for many generations. This produced a population of cells in which all of the DNA was heavy-labeled. At the start of their experiment, shown in **Figure 11.3** (generation 0), they switched the bacteria to a medium that contained only ^{14}N and then collected samples of cells after various time points. Under the growth conditions they employed, 30 minutes is the time required for one doubling, or one generation time. Because the bacteria were doubling in a medium that contained only ^{14}N, all of the newly made DNA strands are labeled with light nitrogen, but the original strands remain in the heavy form.

Meselson and Stahl then analyzed the density of the DNA by centrifugation, using a cesium chloride (CsCl) gradient. (The procedure of gradient centrifugation is described in the Appendix.) If both DNA strands contained ^{14}N, the DNA would have a light density and sediment near the top of the tube. If one strand contained ^{14}N and the other strand contained ^{15}N, the DNA would be half-heavy and have an intermediate density. Finally, if both strands contained ^{15}N, the DNA would be heavy and would sediment closer to the bottom of the centrifuge tube.

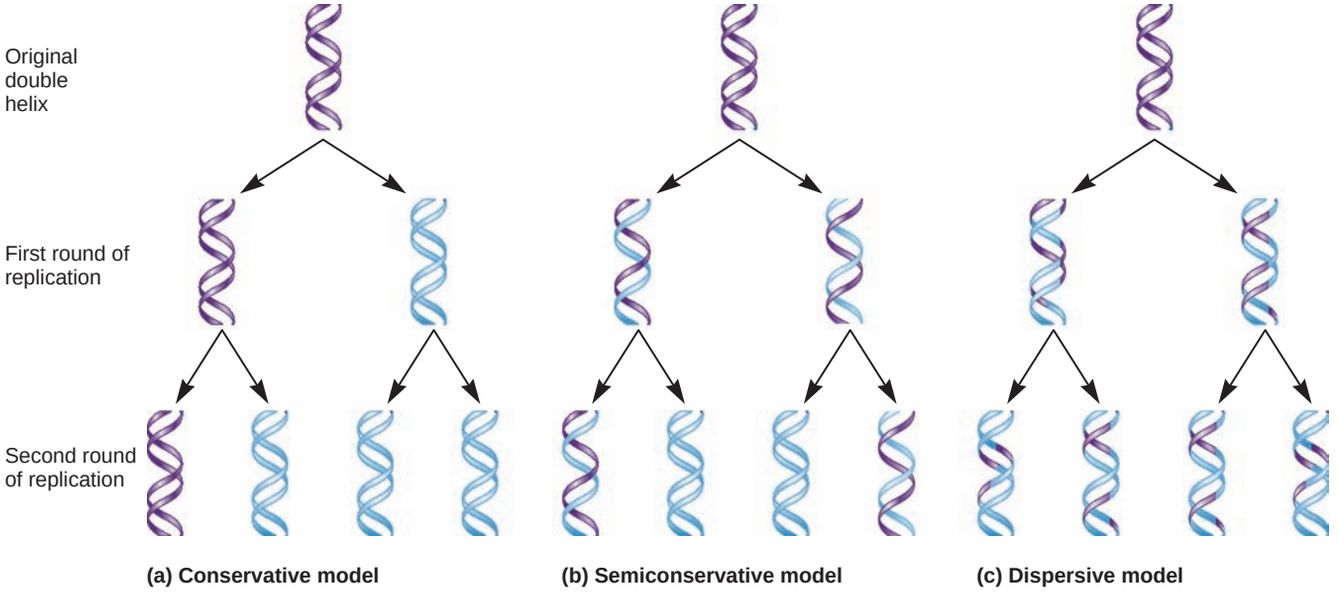


FIGURE 11.2 **Three possible models for DNA replication.** The two original parental DNA strands are shown in purple, and the newly made strands after one and two generations are shown in light blue.

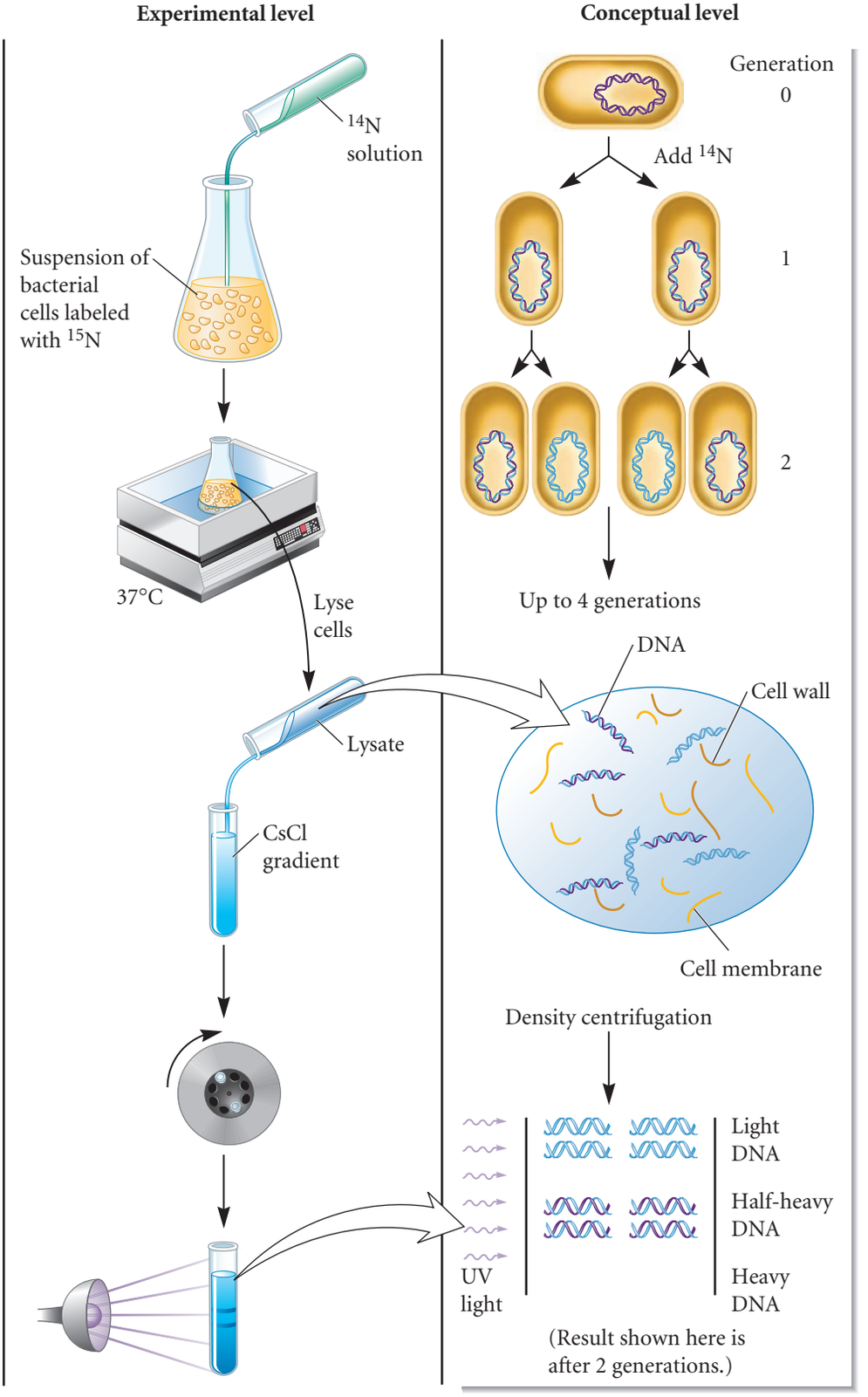
THE HYPOTHESIS

Based on Watson's and Crick's ideas, the hypothesis was that DNA replication is semiconservative. Figure 11.2 also shows two alternative models.

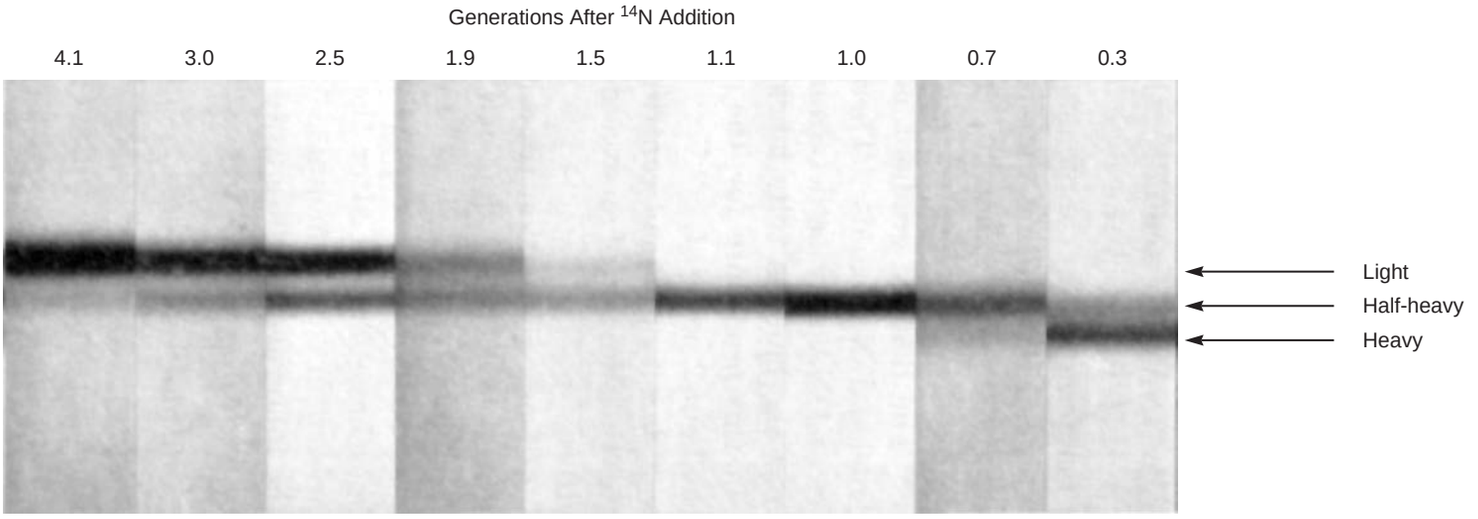
TESTING THE HYPOTHESIS — FIGURE 11.3 Evidence that DNA replication is semiconservative.

Starting material: A strain of *E. coli* that has been grown for many generations in the presence of ^{15}N. All of the nitrogen in the DNA is labeled with ^{15}N.

1. Add an excess of ^{14}N-containing compounds to the bacterial cells so all of the newly made DNA will contain ^{14}N.
2. Incubate the cells for various lengths of time. Note: The ^{15}N-labeled DNA is shown in purple and the ^{14}N-labeled DNA is shown in blue.
3. Lyse the cells by the addition of lysozyme and detergent, which disrupt the bacterial cell wall and cell membrane, respectively.
4. Load a sample of the lysate onto a CsCl gradient. (Note: The average density of DNA is around 1.7 g/cm^3, which is well isolated from other cellular macromolecules.)
5. Centrifuge the gradients until the DNA molecules reach their equilibrium densities.
6. DNA within the gradient can be observed under a UV light.



THE DATA



Data from: Meselson, M., and Stahl, F.W. (1958) The Replication of DNA in *Escherichia coli. Proc. Natl. Acad. Sci, USA 44:* 671–682.

INTERPRETING THE DATA

As seen in the data following Figure 11.3, after one round of DNA replication (i.e., one generation), all of the DNA sedimented at a density that was half-heavy. Which of the three models is consistent with this result? Both the semiconservative and dispersive models are consistent. In contrast, the conservative model predicts two separate DNA types: a light type and a heavy type. Because all of the DNA had sedimented as a single band, this model was disproved. According to the semiconservative model, the replicated DNA would contain one original strand (a heavy strand) and a newly made daughter strand (a light strand). Likewise, in a dispersive model, all of the DNA should have been half-heavy after one generation as well. To determine which of these two remaining models is correct, therefore, Meselson and Stahl had to investigate future generations.

After approximately two rounds of DNA replication (i.e., 1.9 generations), a mixture of light DNA and half-heavy DNA was observed. This result was consistent with the semiconservative model of DNA replication, because some DNA molecules should contain all light DNA, and other molecules should be half-heavy (see Figure 11.2b). The dispersive model predicts that after two generations, the heavy nitrogen would be evenly dispersed among four strands, each strand containing 1/4 heavy nitrogen and 3/4 light nitrogen (see Figure 11.2c). However, this result was not obtained. Instead, the results of the Meselson and Stahl experiment provided compelling evidence in favor of only the semiconservative model for DNA replication.

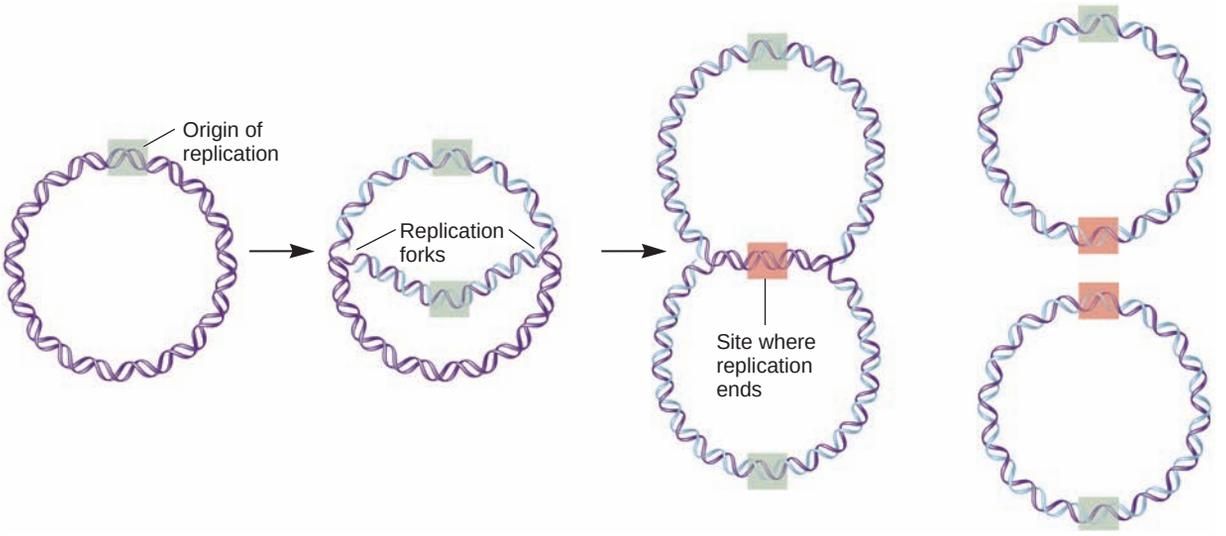
A self-help quiz involving this experiment can be found at www.mhhe.com/brookergenetics4e.

11.2 BACTERIAL DNA REPLICATION

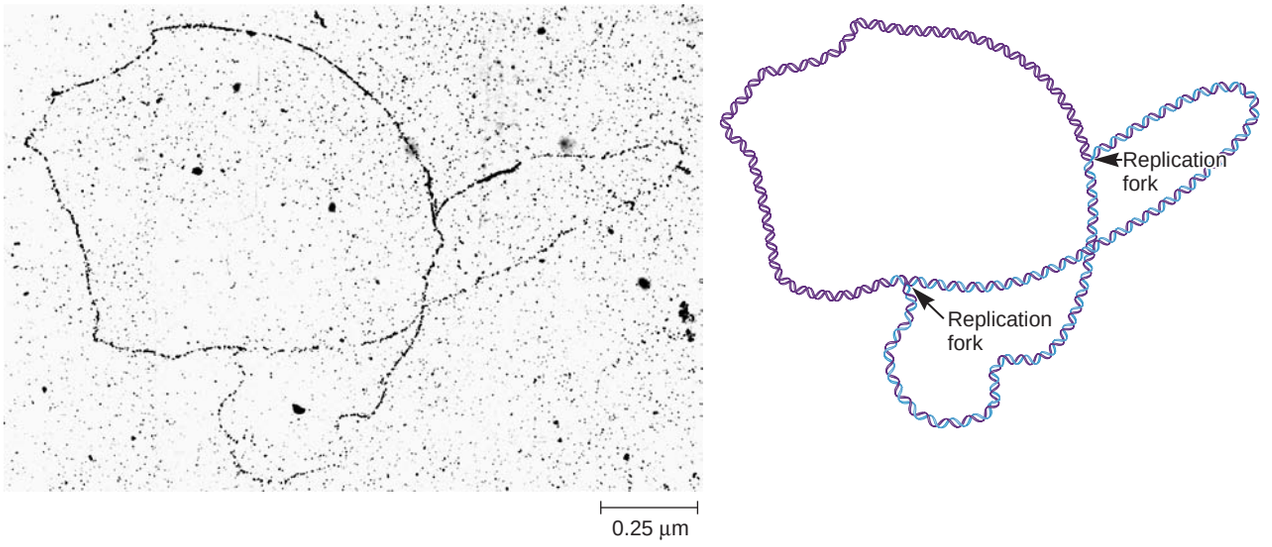
Thus far, we have considered how a complementary, double-stranded structure underlies the ability of DNA to be copied. In addition, the experiments of Meselson and Stahl showed that DNA replication results in two double helices, each one containing an original parental strand and a newly made daughter strand. We now turn our attention to how DNA replication actually occurs within living cells. Much research has focused on the bacterium *E. coli.* The results of these studies have provided the foundation for our current molecular understanding of DNA replication. The replication of the bacterial chromosome is a stepwise process in which many cellular proteins participate. In this section, we will follow this process from beginning to end.

Bacterial Chromosomes Contain a Single Origin of Replication

Figure 11.4 presents an overview of the process of bacterial chromosomal replication. The site on the bacterial chromosome where DNA synthesis begins is known as the **origin of replication.** Bacterial chromosomes have a single origin of replication. The synthesis of new daughter strands is initiated within the origin and proceeds in both directions, or **bidirectionally,** around the bacterial chromosome. This means that two **replication forks** move in opposite directions outward from the origin. A replication fork is the site where the parental DNA strands have separated and new daughter strands are being made. Eventually, these replication forks meet each other on the opposite side of the bacterial chromosome to complete the replication process.



(a) Bacterial chromosome replication



(b) Autoradiograph of an *E. coli* chromosome in the act of replication

FIGURE 11.4 **The process of bacterial chromosome replication.** **(a)** An overview of the process of bacterial chromosome replication. **(b)** A replicating *E. coli* chromosome visualized by autoradiography and transmission electron microscopy (TEM). This chromosome was radiolabeled by growing bacterial cells in media containing radiolabeled thymidine. The diagram at the right shows the locations of the two replication forks. The chromosome is about one-third replicated. New strands are shown in blue.

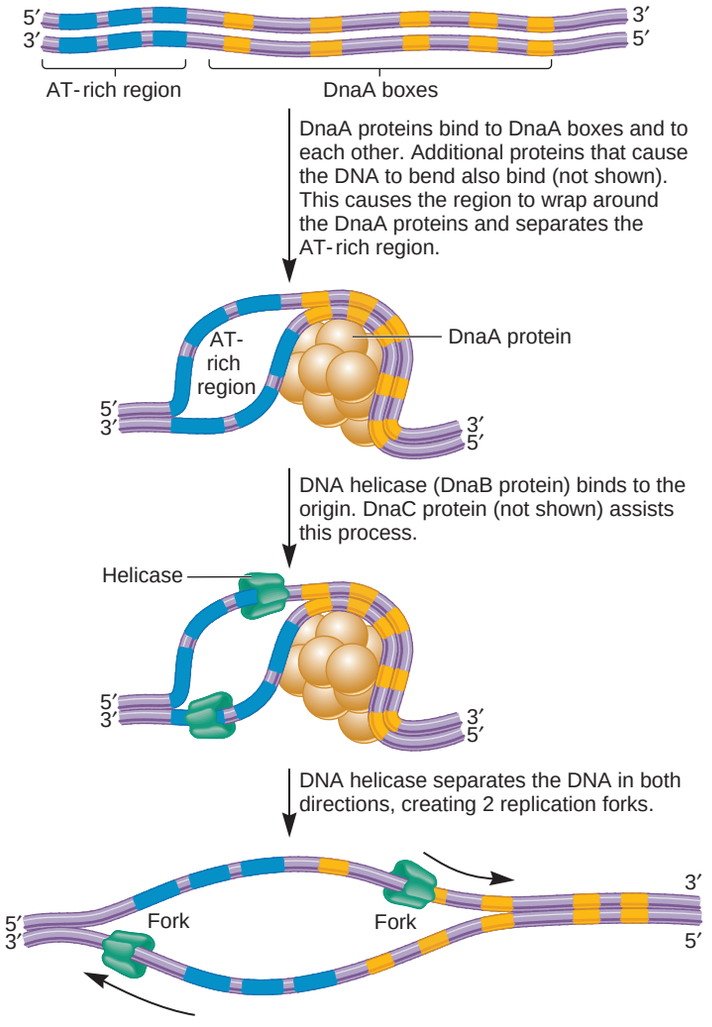
Replication Is Initiated by the Binding of DnaA Protein to the Origin of Replication

Considerable research has focused on the origin of replication in *E. coli*. This origin is named *oriC* for origin of Chromosomal replication (**Figure 11.5**). Three types of DNA sequences are found within *oriC:* an AT-rich region, DnaA box sequences, and GATC methylation sites. The GATC methylation sites will be discussed later in this chapter when we consider the regulation of replication.

DNA replication is initiated by the binding of **DnaA proteins** to sequences within the origin known as **DnaA box sequences.** The DnaA box sequences serve as recognition sites for the binding of the DnaA proteins. When DnaA proteins are in their ATP-bound form, they bind to the five DnaA boxes in *oriC* to initiate DNA replication. DnaA proteins also bind to each other to form a complex (**Figure 11.6**). With the aid of other DNA-binding proteins, such as HU and IHF, this causes the DNA to bend around the complex of DnaA proteins and results in the separation of the AT-rich region. Because only two hydrogen bonds form between AT base pairs, whereas three hydrogen bonds occur between G and C, the DNA strands are more easily separated at an AT-rich region.

Following separation of the AT-rich region, the DnaA proteins, with the help of the DnaC protein, recruit **DNA helicase** proteins to this site. DNA helicase is also known as DnaB protein. When a DNA helicase encounters a double-stranded region,

FIGURE 11.5 The sequence of *oriC* in *E. coli*. The AT-rich region is composed of three tandem repeats that are 13 bp long and highlighted in blue. The five DnaA boxes are highlighted in orange. The GATC methylation sites are underlined.



it breaks the hydrogen bonds between the two strands, thereby generating two single strands. Two DNA helicases begin strand separation within the *oriC* region and continue to separate the DNA strands beyond the origin. These proteins use the energy from ATP hydrolysis to catalyze the separation of the double-stranded parental DNA. In *E. coli,* DNA helicases bind to single-stranded DNA and travel along the DNA in a 5′ to 3′ direction to keep the replication fork moving. As shown in Figure 11.6, the action of DNA helicases promotes the movement of two replication forks outward from *oriC* in opposite directions. This initiates the replication of the bacterial chromosome in both directions, an event termed **bidirectional replication.**

Several Proteins Are Required for DNA Replication at the Replication Fork

Figure 11.7 provides an overview of the molecular events that occur as one of the two replication forks moves around the bacterial chromosome, and **Table 11.1** summarizes the functions of the major proteins involved in *E. coli* DNA replication. Let's begin with strand separation. To act as a template for DNA replication, the strands of a double helix must separate. As mentioned previously, the function of DNA helicase is to break the hydrogen bonds between base pairs and thereby unwind the strands; this action generates positive supercoiling ahead of each replication

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FIGURE 11.6 The events that occur at *oriC* to initiate the DNA replication process. To initiate DNA replication, DnaA proteins bind to the five DnaA boxes, which causes the DNA strands to separate at the AT-rich region. DnaA and DnaC proteins then recruit DNA helicase (DnaB) into this region. Each DNA helicase is composed of six subunits, which form a ring around one DNA strand and migrate in the 5′ to 3′ direction. As shown here, the movement of two DNA helicase proteins serves to separate the DNA strands beyond the *oriC* region.

Functions of key proteins involved with DNA replication

- DNA helicase breaks the hydrogen bonds between the DNA strands.
- Topoisomerase alleviates positive supercoiling.
- Single-strand binding proteins keep the parental strands apart.
- Primase synthesizes an RNA primer.
- DNA polymerase III synthesizes a daughter strand of DNA.
- DNA polymerase I excises the RNA primers and fills in with DNA (not shown).
- DNA ligase covalently links the Okazaki fragments together.

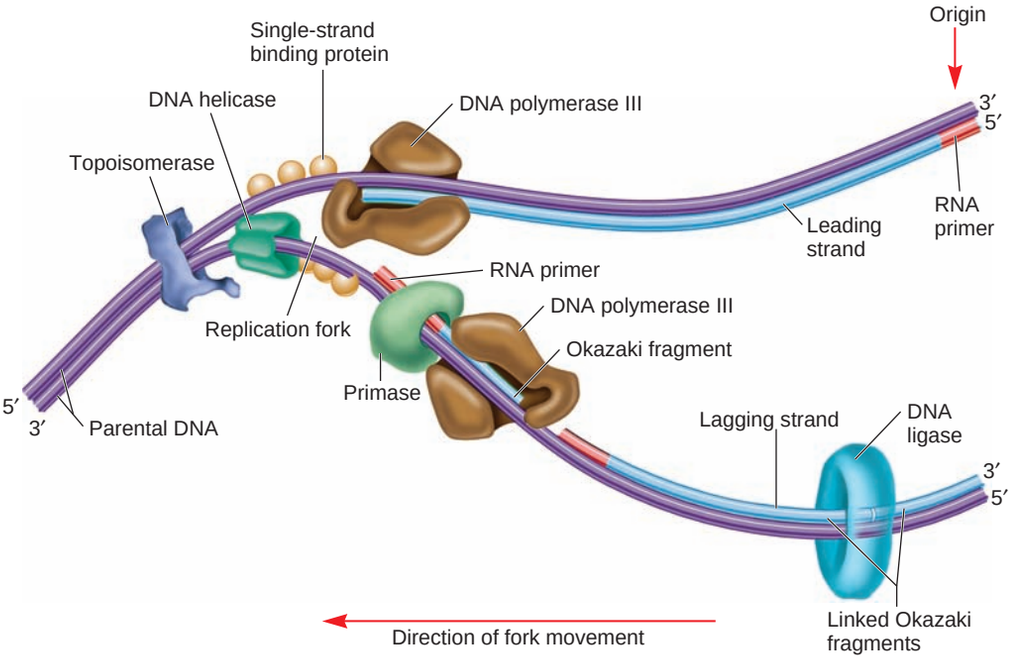

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FIGURE 11.7 The proteins involved with DNA replication.
Note: The drawing of DNA polymerase III depicts the catalytic subunit that synthesizes DNA.

fork. As shown in Figure 11.7, an enzyme known as a **topoisomerase (type II),** also called **DNA gyrase,** travels in front of DNA helicase and alleviates positive supercoiling.

After the two parental DNA strands have been separated and the supercoiling relaxed, they must be kept that way until the complementary daughter strands have been made. What prevents the DNA strands from coming back together? DNA replication requires **single-strand binding proteins** that bind to the strands of parental DNA and prevent them from re-forming a double helix. In this way, the bases within the parental strands are kept in an exposed condition that enables them to hydrogen bond with individual nucleotides.

The next event in DNA replication involves the synthesis of short strands of RNA (rather than DNA) called **RNA primers.** These strands of RNA are synthesized by the linkage of ribonucleotides via an enzyme known as **primase.** This enzyme synthesizes short strands of RNA, typically 10 to 12 nucleotides in length. These short RNA strands start, or prime, the process of DNA replication. In the **leading strand,** a single primer is made at the origin of replication. In the **lagging strand,** multiple primers are made. As discussed later, the RNA primers are eventually removed.

A type of enzyme known as **DNA polymerase** is responsible for synthesizing the DNA of the leading and lagging strands. This enzyme catalyzes the formation of covalent bonds between adjacent nucleotides and thereby makes the new daughter strands. In *E. coli*, five distinct proteins function as DNA polymerases and are designated polymerase I, II, III, IV, and V. DNA polymerases I and III are involved in normal DNA replication, whereas DNA polymerases II, IV, and V play a role in DNA repair and the replication of damaged DNA.

DNA polymerase III is responsible for most of the DNA replication. It is a large enzyme consisting of 10 different subunits that play various roles in the DNA replication process (**Table 11.2**). The α subunit actually catalyzes the bond formation between adjacent nucleotides, and the remaining nine subunits fulfill other functions. The complex of all 10 subunits

TABLE 11.1
Proteins Involved in *E. coli* DNA Replication

Common Name	Function
DnaA protein	Binds to DnaA boxes within the origin to initiate DNA replication
DnaC protein	Aids DnaA in the recruitment of DNA helicase to the origin
DNA helicase (DnaB)	Separates double-stranded DNA
Topoisomerase	Removes positive supercoiling ahead of the replication fork
Single-strand binding protein	Binds to single-stranded DNA and prevents it from re-forming a double-stranded structure
Primase	Synthesizes short RNA primers
DNA polymerase III	Synthesizes DNA in the leading and lagging strands
DNA polymerase I	Removes RNA primers, fills in gaps with DNA
DNA ligase	Covalently attaches adjacent Okazaki fragments
Tus	Binds to ter sequences and prevents the advancement of the replication fork

together is called DNA polymerase III holoenzyme. By comparison, DNA polymerase I is composed of a single subunit. Its role during DNA replication is to remove the RNA primers and fill in the vacant regions with DNA.

Though the various DNA polymerases in *E. coli* and other bacterial species vary in their subunit composition, several common structural features have emerged. The catalytic subunit of all DNA polymerases has a structure that resembles a human hand. As shown in **Figure 11.8**, the template DNA is threaded through the palm of the hand; the thumb and fingers are wrapped around the DNA. The incoming deoxyribonucleoside triphosphates (dNTPs) enter the catalytic site, bind to the template strand according to the AT/GC rule, and then are covalently attached to the 3′ end of the growing strand. DNA polymerase also contains a 3′ exonuclease site that removes mismatched bases, as described later.

As researchers began to unravel the function of DNA polymerase, two features seemed unusual (**Figure 11.9**). DNA polymerase cannot begin DNA synthesis by linking together the first two individual nucleotides. Rather, this type of enzyme can elongate only a preexisting strand starting with an RNA primer or existing DNA strand (Figure 11.9a). A second unusual feature is the directionality of strand synthesis. DNA polymerase can attach nucleotides only in the 5′ to 3′ direction, not in the 3′ to 5′ direction (Figure 11.9b).

Due to these two unusual features, the synthesis of the leading and lagging strands shows distinctive differences (**Figure 11.10**). The synthesis of RNA primers by primase allows DNA polymerase III to begin the synthesis of complementary daughter strands of DNA. DNA polymerase III catalyzes the attachment of nucleotides to the 3′ end of each primer, in a 5′ to 3′ direction. In the leading strand, one RNA primer is made at the origin, and then DNA polymerase III can attach nucleotides in a 5′ to 3′ direction as it slides toward the opening of the replication fork. The synthesis of the leading strand is therefore continuous.

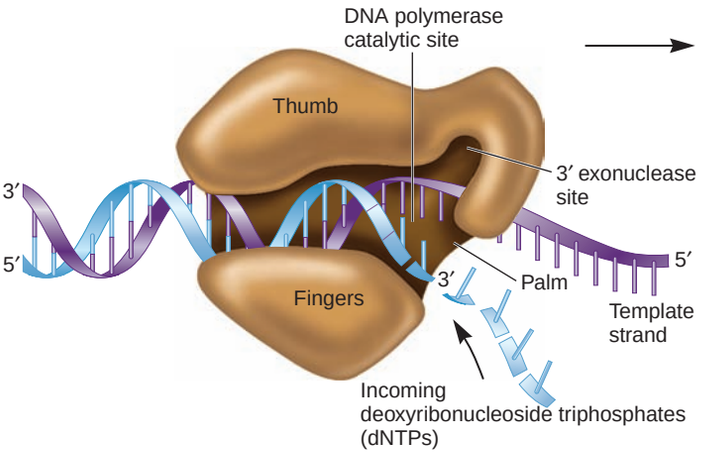
In the lagging strand, the synthesis of DNA also elongates in a 5′ to 3′ manner, but it does so in the direction away from the replication fork. In the lagging strand, RNA primers must repeatedly initiate the synthesis of short segments of DNA; thus, the synthesis has to be discontinuous. The length of these fragments in bacteria is typically 1000 to 2000 nucleotides. In eukaryotes, the fragments are shorter—100 to 200 nucleotides. Each fragment contains a short RNA primer at the 5′ end, which is made by primase. The remainder of the fragment is a strand of DNA made by DNA polymerase III. The DNA fragments made in this manner are known as **Okazaki fragments,** after Reiji and Tuneko Okazaki, who initially discovered them in the late 1960s.

To complete the synthesis of Okazaki fragments within the lagging strand, three additional events must occur: removal of the RNA primers, synthesis of DNA in the area where the primers have been removed, and the covalent attachment of adjacent

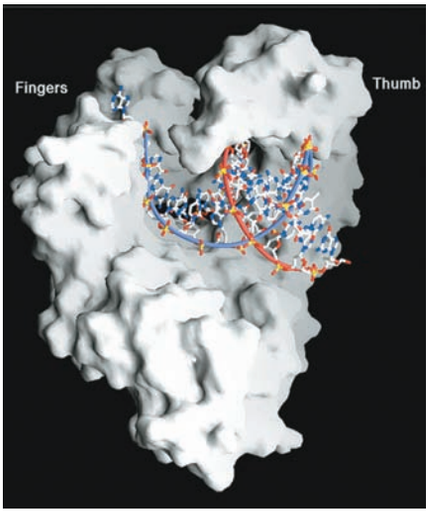
TABLE 11.2

Subunit Composition of DNA Polymerase III Holoenzyme from *E. coli*

Subunit(s)	Function
α	Synthesizes DNA
ε	3′ to 5′ proofreading (removes mismatched nucleotides)
θ	Accessory protein that stimulates the proofreading function
β	Clamp protein, which allows DNA polymerase to slide along the DNA without falling off
τ, γ, δ, δ′, ψ, and χ	Clamp loader complex, involved with helping the clamp protein bind to the DNA



(a) Schematic side view of DNA polymerase III



(b) Molecular model for DNA polymerase bound to DNA

(Reprinted by permission from Macmillan Publishers Ltd. Ying Li, et al. [1998] Crystal structures of open and closed forms of binary and ternary complexes of the large fragment of *Thermus aquaticus* DNA polymerase I: Structural basis for nucleotide incorporation. *Embo J 17*:24, 7514–7525.)

FIGURE 11.8 The action of DNA polymerase. (a) DNA polymerase slides along the template strand as it synthesizes a new strand by connecting deoxyribonucleoside triphosphates (dNTPs) in a 5′ to 3′ direction. The catalytic subunit of DNA polymerase resembles a hand that is wrapped around the template strand. In this regard, the movement of DNA polymerase along the template strand is similar to a hand that is sliding along a rope. **(b)** The molecular structure of DNA polymerase I from the bacterium *Thermus aquaticus*. This model shows a portion of DNA polymerase I that is bound to DNA. This molecular structure depicts a front view of DNA polymerase; part (a) is a schematic side view.

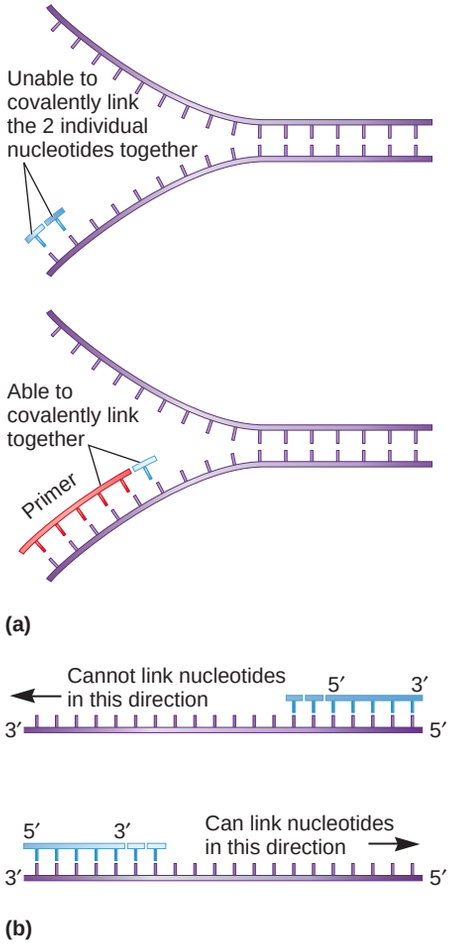


FIGURE 11.9 **Unusual features of DNA polymerase function.** **(a)** DNA polymerase can elongate a strand only from an RNA primer or existing DNA strand. **(b)** DNA polymerase can attach nucleotides only in a 5′ to 3′ direction. Note the template strand is in the opposite, 3′ to 5′, direction.

fragments of DNA (see Figure 11.10 and refer back to Figure 11.7). In *E. coli*, the RNA primers are removed by the action of DNA polymerase I. This enzyme has a 5′ to 3′ exonuclease activity, which means that DNA polymerase I digests away the RNA primers in a 5′ to 3′ direction, leaving a vacant area. DNA polymerase I then synthesizes DNA to fill in this region. It uses the 3′ end of an adjacent Okazaki fragment as a primer. For example, in Figure 11.10, DNA polymerase I would remove the RNA primer from the first Okazaki fragment and then synthesize DNA in the vacant region by attaching nucleotides to the 3′ end of the second Okazaki fragment. After the gap has been completely filled in, a covalent bond is still missing between the last nucleotide added by DNA polymerase I and the adjacent DNA strand that had been previously made by DNA polymerase III. An enzyme known as **DNA ligase** catalyzes a covalent bond between adjacent fragments to complete the replication process in the lagging strand (refer back to Figure 11.7). In *E. coli*, DNA ligase requires NAD^+ to carry out this reaction, whereas the DNA ligases found in archaea and eukaryotes require ATP.

The synthesis of the lagging strand was studied by the Okazakis using radiolabeled nucleotides. They incubated *E. coli* cells

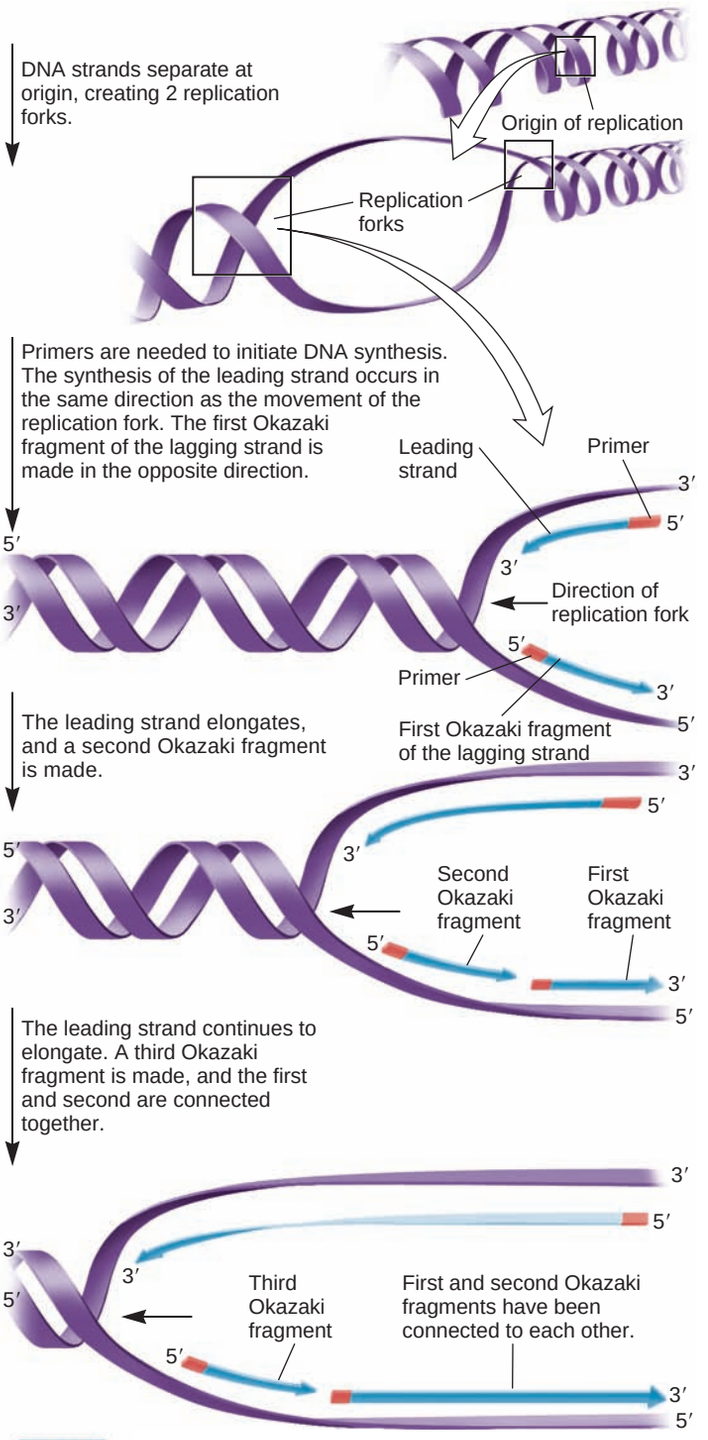


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FIGURE 11.10 **The synthesis of DNA at the replication fork.**

with radiolabeled thymidine for 15 seconds and then added an excess of nonlabeled thymidine. This is termed a **pulse/chase experiment** because the cells were given the radiolabeled compound for a brief period of time—a pulse—followed by an excess amount of unlabeled compound—a chase. They then isolated DNA from samples of cells at timed intervals after the pulse/chase. The DNA was denatured into single-stranded molecules, and the sizes of the radiolabeled DNA strands were determined by

centrifugation. At quick time intervals, such as only a few seconds following the thymidine incubation, the fragments were found to be short, in the range of 1000 to 2000 nucleotides in length. At extended time intervals, the radiolabeled strands became much longer. At these later time points, the adjacent Okazaki fragments would have had enough time to link together.

Now that we understand how the leading and lagging strands are made, **Figure 11.11** shows how new strands are constructed from a single origin of replication. To the left of the origin, the top strand is made continuously, whereas to the right of the origin it is made in Okazaki fragments. By comparison, the synthesis of the bottom strand is just the opposite. To the left of the origin it is made in Okazaki fragments and to the right of the origin the synthesis is continuous.

DNA Polymerase III Is a Processive Enzyme That Uses Deoxyribonucleoside Triphosphates

Let's now turn our attention to other enzymatic features of DNA polymerase. As shown in **Figure 11.12**, DNA polymerases catalyze the covalent attachment between the phosphate in one nucleotide and the sugar in the previous nucleotide. The formation of this covalent (ester) bond requires an input of energy. Prior to bond formation, the nucleotide about to be attached to the growing strand is a dNTP. It contains three phosphate groups attached at the 5′–carbon atom of deoxyribose. The dNTP first enters the catalytic site of DNA polymerase and binds to the template strand according to the AT/GC rule. Next, the 3′–OH group on the previous nucleotide reacts with the phosphate group adjacent to the sugar on the incoming nucleotide. The breakage of a covalent bond between two phosphates in a dNTP is a highly exergonic reaction that provides the energy to form a covalent (ester) bond between the sugar at the 3′ end of the DNA strand and the phosphate of the incoming nucleotide. The formation of this covalent bond causes the newly made strand to grow in the 5′ to 3′ direction. As shown in Figure 11.12, pyrophosphate (PP_i) is released.

As noted in Chapter 9 (Figure 9.10), the term phosphodiester linkage (also called a phosphodiester bond) is used to describe the linkage between a phosphate and two sugar molecules. As its name implies, a phosphodiester linkage involves two ester bonds. In comparison, as a DNA strand grows, a single covalent (ester) bond is formed between adjacent nucleotides (see Figure 11.12). The other ester bond in the phosphodiester linkage—the bond between the 5′-oxygen and phosphorus—is already present in the incoming nucleotide.

DNA polymerase catalyzes the covalent attachment of nucleotides with great speed. In *E. coli*, DNA polymerase III attaches approximately 750 nucleotides per second! DNA polymerase III can catalyze the synthesis of the daughter strands so quickly because it is a **processive enzyme.** This means it does not dissociate from the growing strand after it has catalyzed the covalent joining of two nucleotides. Rather, as depicted in Figure 11.8a, it remains clamped to the DNA template strand and slides along the template as it catalyzes the synthesis of the daughter strand. The β subunit of the holoenzyme, also known as the clamp protein, promotes the association of the holoenzyme with the DNA as it glides along the template strand (refer back to Table 11.2). The β subunit forms a dimer in the shape of a ring; the hole of the ring is large enough to accommodate a double-stranded DNA molecule, and its width is about one turn of DNA. A complex of several subunits functions as a clamp loader that allows the DNA polymerase holoenzyme to initially clamp onto the DNA.

The effects of processivity are really quite remarkable. In the absence of the β subunit, DNA polymerase can synthesize DNA at a rate of approximately only 20 nucleotides per second. On average, it falls off the DNA template after about 10 nucleotides have been linked together. By comparison, when the β subunit is present, as in the holoenzyme, the synthesis rate is approximately 750 nucleotides per second. In the leading strand, DNA polymerase III has been estimated to synthesize a segment of DNA that is over 500,000 nucleotides in length before it inadvertently falls off.

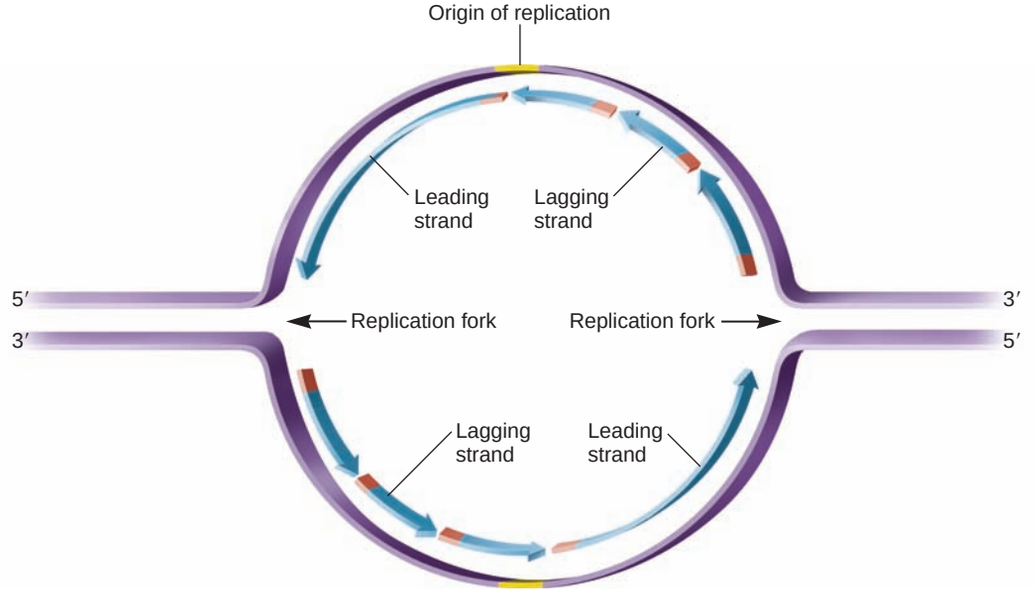


FIGURE 11.11 **The synthesis of leading and lagging strands outward from a single origin of replication.**

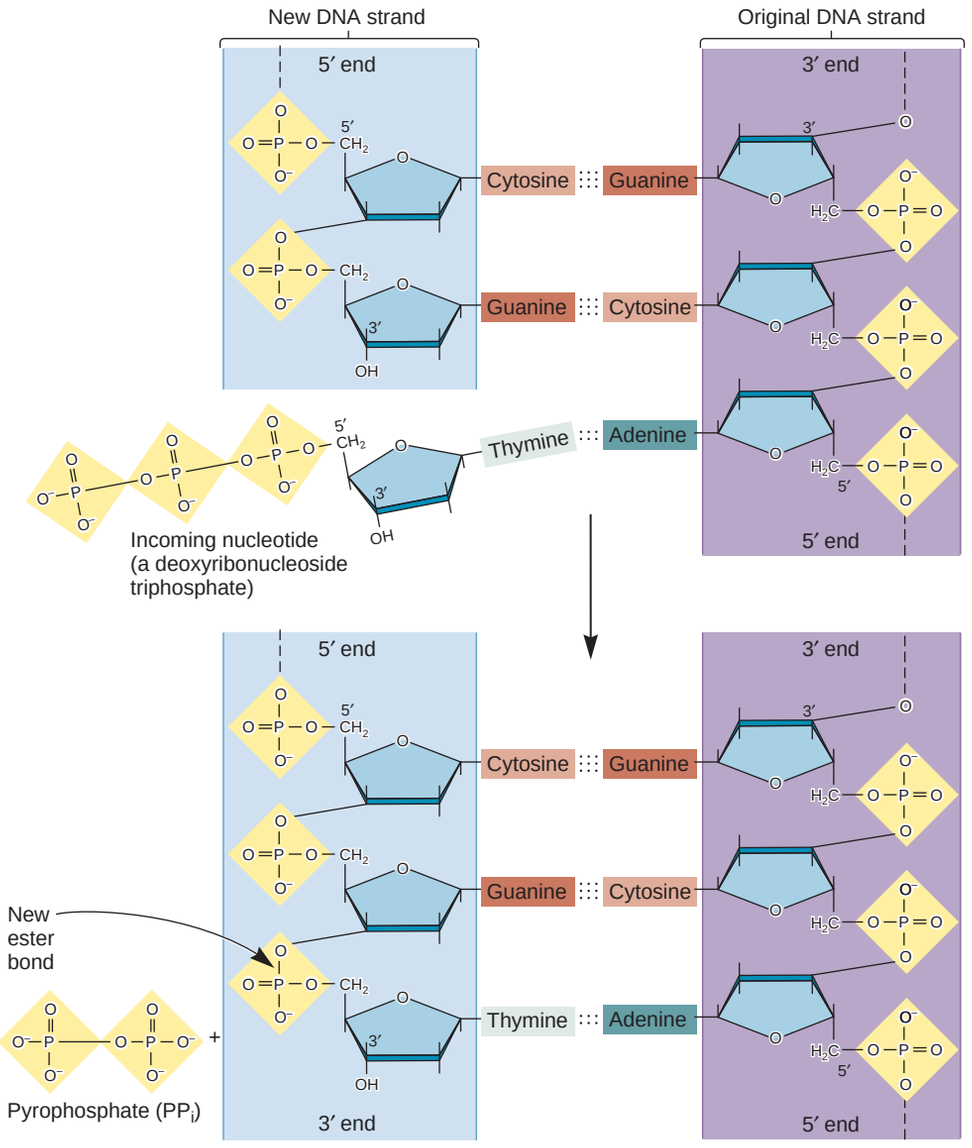


FIGURE 11.12 **The enzymatic action of DNA polymerase.** An incoming deoxyribonucleoside triphosphate (dNTP) is cleaved to form a nucleoside monophosphate and pyrophosphate (PP_i). The energy released from this exergonic reaction allows the nucleoside monophosphate to form a covalent (ester) bond at the 3′ end of the growing strand. This reaction is catalyzed by DNA polymerase. PP_i is released.

Replication Is Terminated When the Replication Forks Meet at the Termination Sequences

On the opposite side of the *E. coli* chromosome from *oriC* is a pair of **termination sequences** called ter sequences. A protein known as the termination utilization substance (Tus) binds to the ter sequences and stops the movement of the replication forks. As shown in **Figure 11.13**, one of the ter sequences designated T1 prevents the advancement of the fork moving left to right, but allows the movement of the other fork (see the inset to Figure 11.13). Alternatively, T2 prevents the advancement of

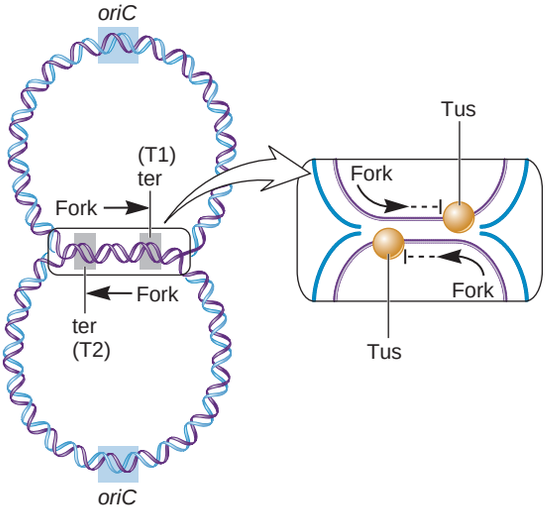


FIGURE 11.13 **The termination of DNA replication.** Two sites in the bacterial chromosome, shown with rectangles, are ter sequences designated T1 and T2. The T1 site prevents the further advancement of the fork moving left to right, and T2 prevents the advancement of the fork moving right to left. As shown in the inset, the binding of Tus prevents the replication forks from proceeding past the ter sequences in a particular direction.

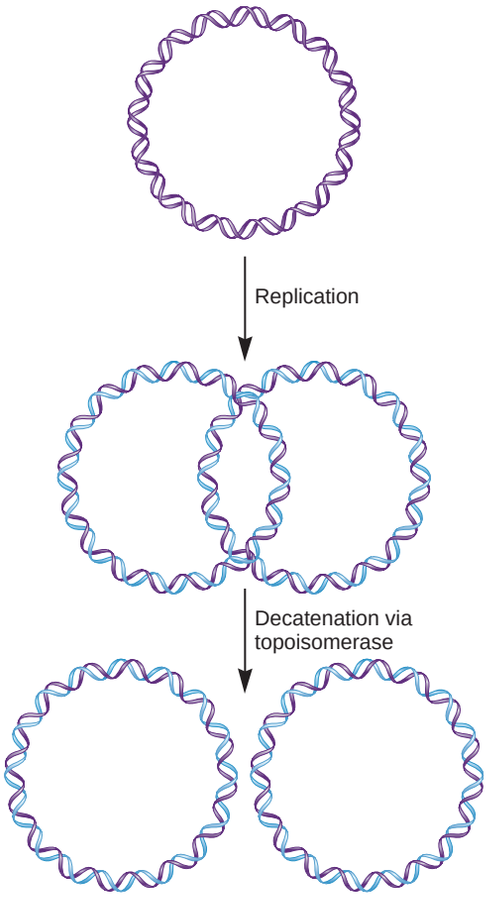


FIGURE 11.14 **Separation of catenanes.** DNA replication can result in two intertwined chromosomes called catenanes. These catenanes can be separated by the action of topoisomerase.

the fork moving right to left, but allows the advancement of the other fork. In any given cell, only one ter sequence is required to stop the advancement of one replication fork, and then the other fork ends its synthesis of DNA when it reaches the halted replication fork. In other words, DNA replication ends when oppositely advancing forks meet, usually at T1 or T2. Finally, DNA ligase covalently links the two daughter strands, creating two circular, double-stranded molecules.

After DNA replication is completed, one last problem may exist. DNA replication often results in two intertwined DNA molecules known as **catenanes** (Figure 11.14). Fortunately, catenanes are only transient structures in DNA replication. In *E. coli*, topoisomerase II introduces a temporary break into the DNA strands and then rejoins them after the strands have become unlocked. This allows the catenanes to be separated into individual circular molecules.

Certain Enzymes of DNA Replication Bind to Each Other to Form a Complex

Figure 11.15 provides a more three-dimensional view of the DNA replication process. DNA helicase and primase are physically bound to each other to form a complex known as a **primosome.** This complex leads the way at the replication fork. The primosome tracks along the DNA, separating the parental strands and synthesizing RNA primers at regular intervals along the lagging strand. By acting within a complex, the actions of DNA helicase and primase can be better coordinated.

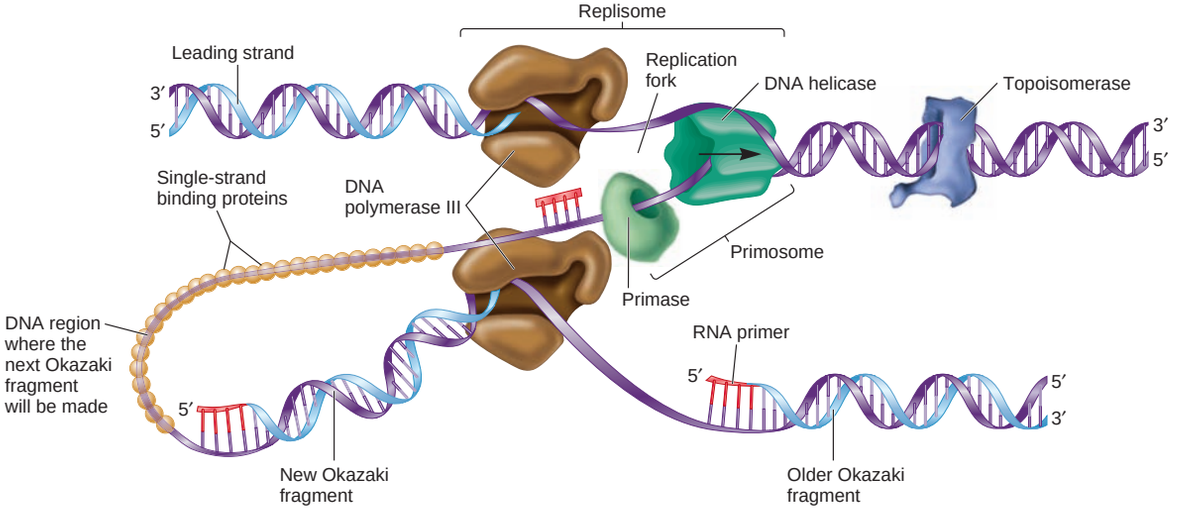


FIGURE 11.15 **A three-dimensional view of DNA replication.** DNA helicase and primase associate together to form a primosome. The primosome associates with two DNA polymerase enzymes to form a replisome.

The primosome is physically associated with two DNA polymerase holoenzymes to form a **replisome.** As shown in Figure 11.15, two DNA polymerase III proteins act in concert to replicate the leading and lagging strands. The term **dimeric DNA polymerase** is used to describe two DNA polymerase holoenzymes that move as a unit toward the replication fork. For this to occur, the lagging strand is looped out with respect to the DNA polymerase that synthesizes the lagging strand. This loop allows the lagging-strand polymerase to make DNA in a 5′ to 3′ direction yet move toward the opening of the replication fork. Interestingly, when this DNA polymerase reaches the end of an Okazaki fragment, it must be released from the template DNA and "hop" to the RNA primer that is closest to the fork. The clamp loader complex (see Table 11.2), which is part of DNA polymerase holoenzyme, then reloads the enzyme at the site where the next RNA primer has been made. Similarly, after primase synthesizes an RNA primer in the 5′ to 3′ direction, it must hop over the primer and synthesize the next primer closer to the replication fork.

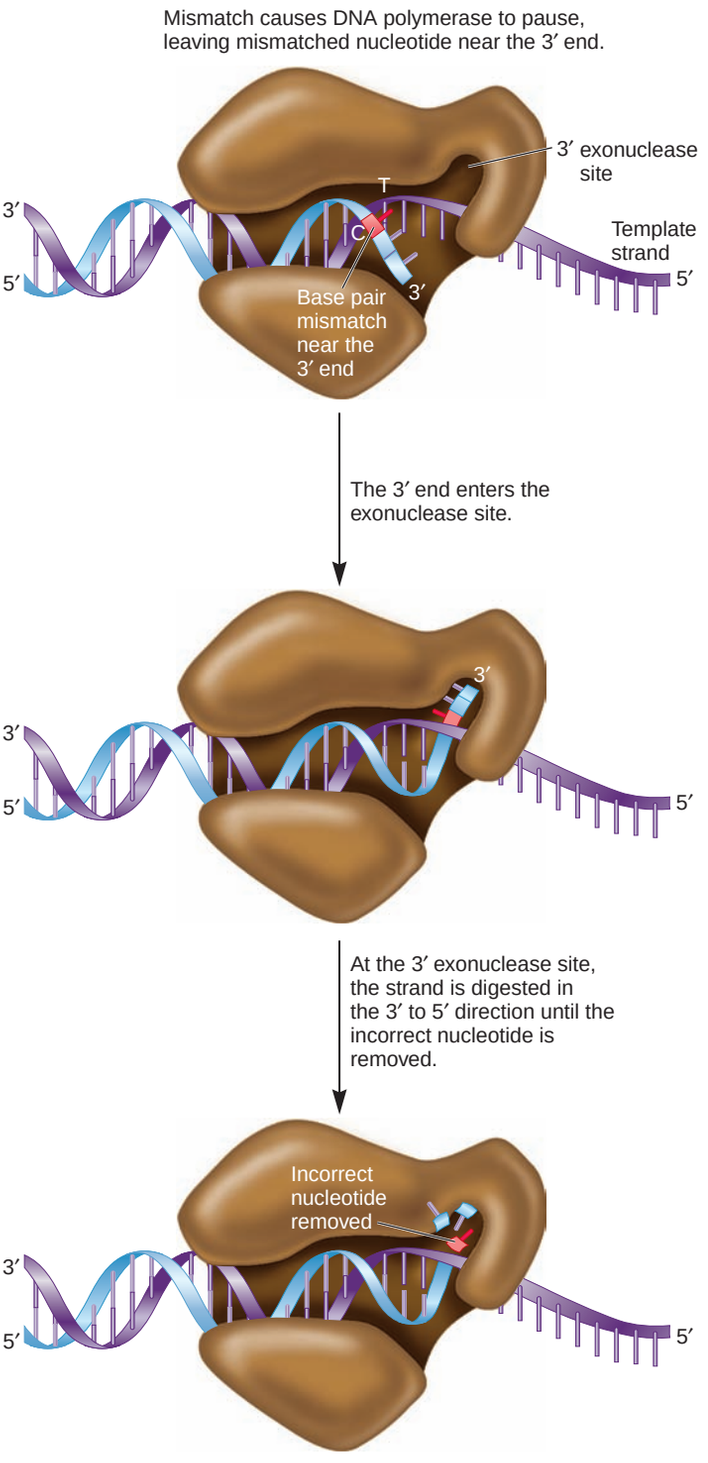
The Fidelity of DNA Replication Is Ensured by Proofreading Mechanisms

With replication occurring so rapidly, one might imagine that mistakes can happen in which the wrong nucleotide is incorporated into the growing daughter strand. Although mistakes can happen during DNA replication, they are extraordinarily rare. In the case of DNA synthesis via DNA polymerase III, only one mistake per 100 million nucleotides is made. Therefore, DNA synthesis occurs with a high degree of accuracy or **fidelity.**

Why is the fidelity so high? First, the hydrogen bonding between G and C or A and T is much more stable than between mismatched pairs. However, this stability accounts for only part of the fidelity, because mismatching due to stability considerations accounts for 1 mistake per 1000 nucleotides.

Two characteristics of DNA polymerase also contribute to the fidelity of DNA replication. First, the active site of DNA polymerase preferentially catalyzes the attachment of nucleotides when the correct bases are located in opposite strands. Helix distortions caused by mispairing usually prevent an incorrect nucleotide from properly occupying the active site of DNA polymerase. By comparison, the correct nucleotide occupies the active site with precision and undergoes induced fit, which is necessary for catalysis. The inability of incorrect nucleotides to undergo induced fit decreases the error rate to a range of 1 in 100,000 to 1 million.

A second way that DNA polymerase decreases the error rate is by the enzymatic removal of mismatched nucleotides. As shown in **Figure 11.16**, DNA polymerase can identify a mismatched nucleotide and remove it from the daughter strand. This occurs by exonuclease cleavage of the bonds between adjacent nucleotides at the 3′ end of the newly made strand. The ability to remove mismatched bases by this mechanism is called the **proofreading function** of DNA polymerase. Proofreading occurs by the removal of nucleotides in the 3′ to 5′ direction at the 3′ exonuclease site. After the mismatched nucleotide is removed, DNA polymerase resumes DNA synthesis in the 5′ to 3′ direction.



ONLINE ANIMATION

FIGURE 11.16 The proofreading function of DNA polymerase. When a base pair mismatch is found, the end of the newly made strand is shifted into the 3′ exonuclease site. The DNA is digested in the 3′ to 5′ direction to release the incorrect nucleotide.

Bacterial DNA Replication Is Coordinated with Cell Division

Bacterial cells can divide into two daughter cells at an amazing rate. Under optimal conditions, certain bacteria such as *E. coli* can divide every 20 to 30 minutes. DNA replication should take place only when a cell is about to divide. If DNA replication occurs too frequently, too many copies of the bacterial chromosome will be found in each cell. Alternatively, if DNA replication does not occur frequently enough, a daughter cell will be left without a chromosome. Therefore, cell division in bacterial cells must be coordinated with DNA replication.

Bacterial cells regulate the DNA replication process by controlling the initiation of replication at *oriC*. This control has been extensively studied in *E. coli*. In this bacterium, several different mechanisms may control DNA replication. In general, the regulation prevents the premature initiation of DNA replication at *oriC*.

After the initiation of DNA replication, DnaA protein hydrolyzes its ATP and therefore switches to an ADP-bound form. DnaA-ADP has a lower affinity for DnaA boxes and does not readily form a complex. This prevents premature initiation. In addition, the initiation of replication is controlled by the amount of the DnaA protein (**Figure 11.17**). To initiate DNA replication, the concentration of the DnaA protein must be high enough so it can bind to all of the DnaA boxes and form a complex. Immediately following DNA replication, the number of DnaA boxes is double, so an insufficient amount of DnaA protein is available to initiate a second round of replication. Also, some of the DnaA protein may be rapidly degraded and some of it may be inactive because it becomes attached to other regions of chromosomal DNA and to the cell membrane during cell division. Because it takes time to accumulate newly made DnaA protein, DNA replication cannot occur until the daughter cells have had time to grow.

Another way to regulate DNA replication involves the GATC methylation sites within *oriC*. These sites can be methylated by an enzyme known as DNA adenine methyltransferase (Dam). The Dam enzyme recognizes the 5′–GATC–3′ sequence, binds there, and attaches a methyl group onto the adenine base, forming methyladenine (**Figure 11.18a**). DNA methylation within

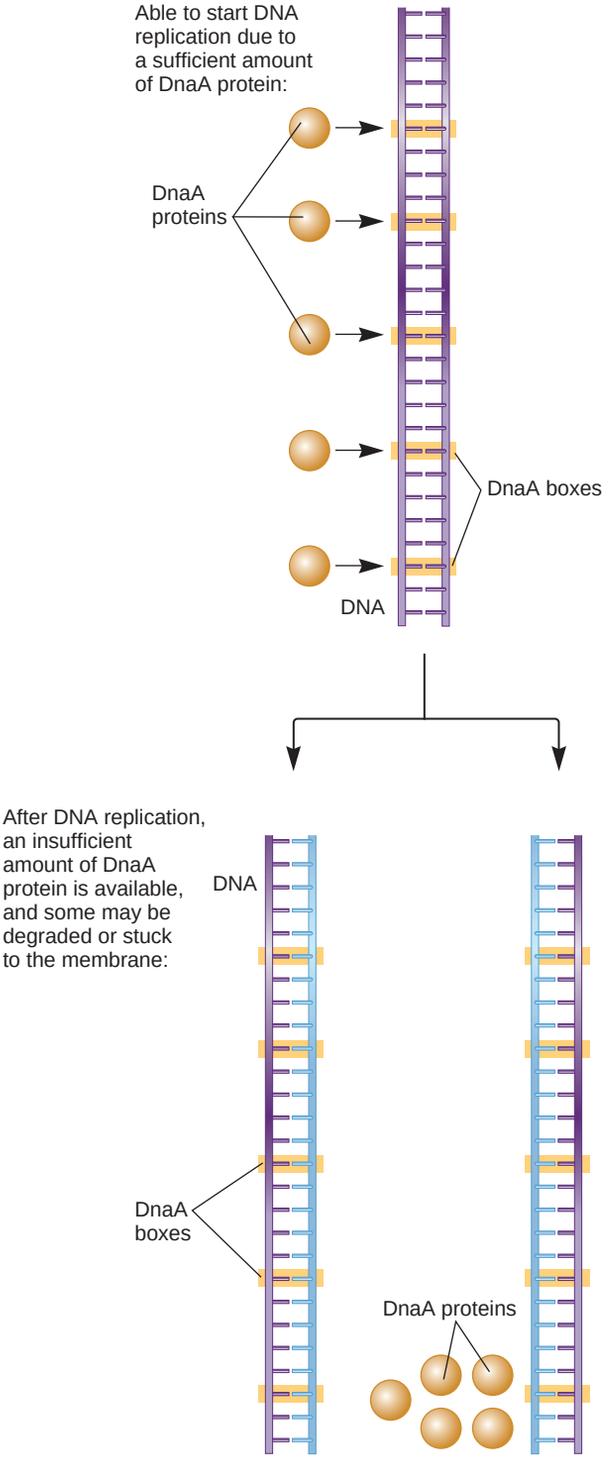
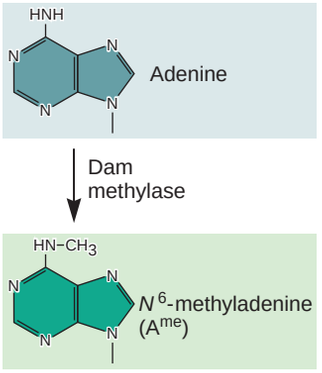
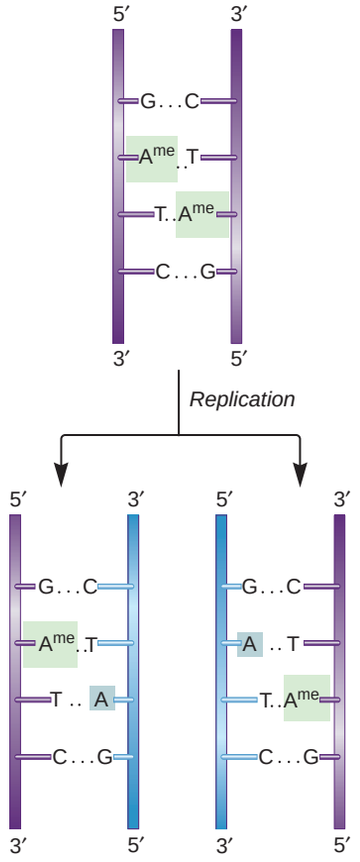


FIGURE 11.17 The amount of DnaA protein provides a way to regulate DNA replication. To begin replication, enough DnaA protein must be present to bind to all of the DnaA boxes. Immediately after DNA replication, insufficient DnaA protein is available to reinitiate a second (premature) round of DNA replication at the two origins of replication. This is because twice as many DnaA boxes are found after DNA replication and because some DnaA proteins may be degraded or stuck to other chromosomal sites and to the cell membrane.



(a) Structure of adenine and methyladenine



(b) Results immediately after DNA replication

oriC helps regulate the replication process. Prior to DNA replication, these sites are methylated in both strands. This full methylation of the 5′–GATC–3′ sites facilitates the initiation of DNA replication at the origin. Following DNA replication, the newly made strands are not methylated, because adenine rather than methyladenine is incorporated into the daughter strands (**Figure 11.18b**). The initiation of DNA replication at the origin does not readily occur until after it has become fully methylated. Because it takes several minutes for Dam to methylate the 5′–GATC–3′ sequences within this region, DNA replication does not occur again too quickly.

FIGURE 11.18 **Methylation of GATC sites in *oriC*.** **(a)** The action of Dam (DNA adenine methyltransferase), which covalently attaches a methyl group to adenine to form methyladenine (A^{me}). **(b)** Prior to DNA replication, the action of Dam causes both adenines within the GATC sites to be methylated. After DNA replication, only the adenines in the original strands are methylated. Several minutes will pass before Dam methylates these unmethylated adenines.

EXPERIMENT 11B

DNA Replication Can Be Studied in Vitro

Much of our understanding of bacterial DNA replication has come from thousands of experiments in which DNA replication has been studied in vitro. This approach was pioneered by Arthur Kornberg in the 1950s, who received the 1959 Nobel Prize in Physiology or Medicine for his efforts.

Figure 11.19 describes Kornberg's approach to monitor DNA replication in vitro. In this experiment, an extract of proteins from *E. coli* was used. Although we do not consider the procedures for purifying replication proteins here, an alternative approach is to purify specific proteins from the extract and study their functions individually. In either case, the proteins are mixed with template DNA and radiolabeled nucleotides. Kornberg correctly hypothesized that dNTPs are the precursors for DNA synthesis. Also, he knew that dNTPs are soluble in an acidic solution, whereas long strands of DNA precipitate out of solution at an acidic pH. This precipitation event provides a method of separating nucleotides—in this case, dNTPs—from strands of DNA. Therefore, after the proteins, template DNA, and nucleotides were incubated for a sufficient time to allow the synthesis of new strands, step 3 of this procedure involved the addition of perchloric acid. This step precipitated strands of DNA, which were then separated from the radiolabeled nucleotides via centrifugation. Newly made strands of DNA, which were radiolabeled, sediment to the pellet, whereas radiolabeled nucleotides that had not been incorporated into new strands remained in the supernatant.

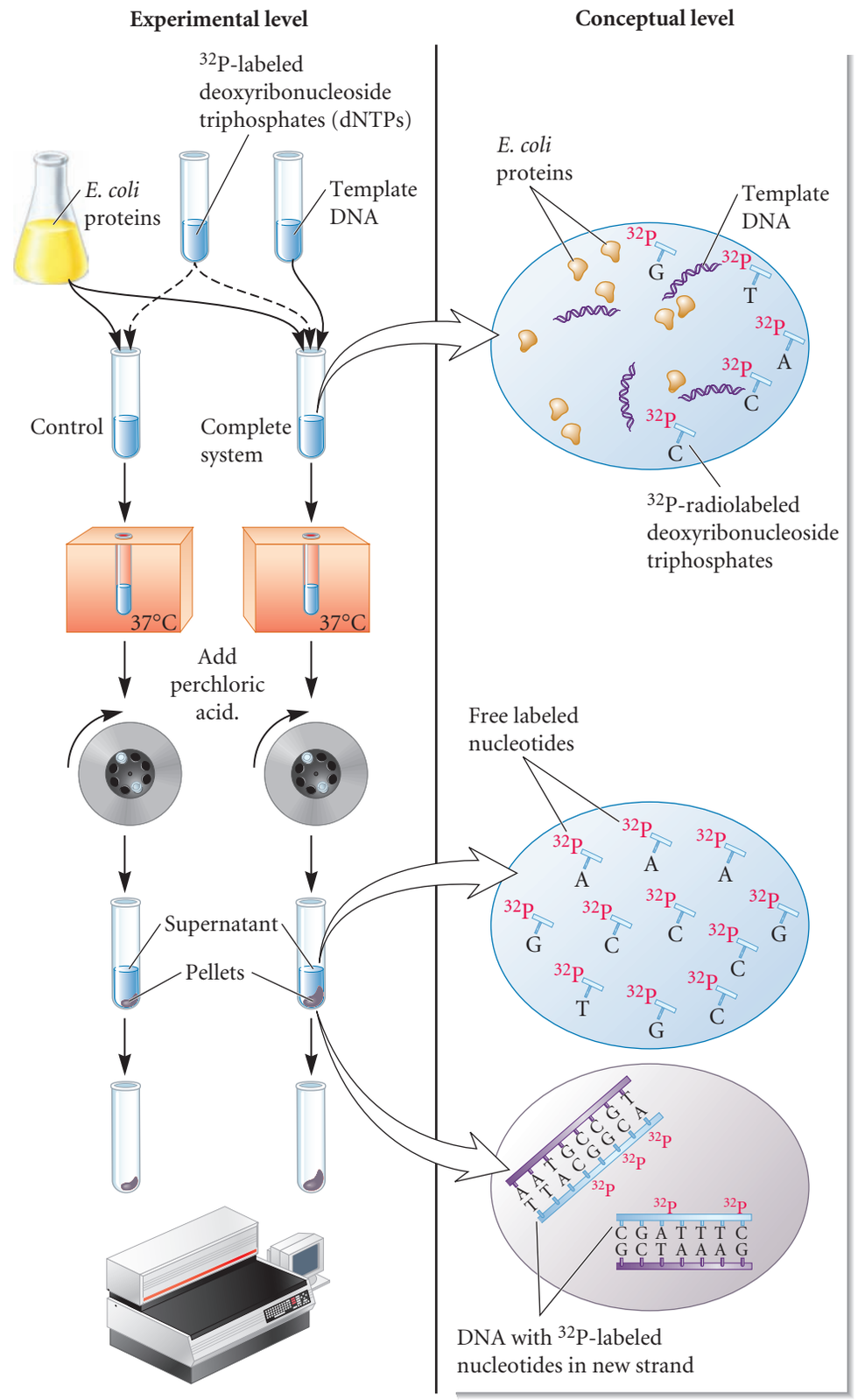
THE HYPOTHESIS

DNA synthesis can occur in vitro if all the necessary components are present.

TESTING THE HYPOTHESIS — FIGURE 11.19 In vitro synthesis of DNA strands.

Starting material: An extract of proteins from *E. coli*.

1. Mix together the extract of *E. coli* proteins, template DNA that is not radiolabeled, and ^{32}P-radiolabeled deoxyribonucleoside triphosphates. This is expected to be a complete system that contains everything necessary for DNA synthesis. As a control, a second sample is made in which the template DNA was omitted from the mixture.
2. Incubate the mixture for 30 minutes at 37°C.
3. Add perchloric acid to precipitate DNA. (It does not precipitate free nucleotides.)
4. Centrifuge the tube.
   Note: The radiolabeled deoxyribonucleoside triphosphates that have not been incorporated into DNA will remain in the supernatant.
5. Collect the pellet, which contains precipitated DNA and proteins. (The control pellet is not expected to contain DNA.)
6. Count the amount of radioactivity in the pellet using a scintillation counter. (See the Appendix.)



THE DATA

Conditions	*Amount of Radiolabeled DNA**
Complete system	3300
Control (template DNA omitted)	0

*Calculated in picomoles of ^{32}P-labeled DNA. Data from: M.J. Bessman, I.R. Lehman, E.S. Simms, and A. Kornberg (1958) Enzymatic synthesis of deoxyribonucleic acid. II. General properties of the reaction. *J Biol Chem 233*:171–177.

INTERPRETING THE DATA

As shown in the data after Figure 11.19, when the *E. coli* proteins were mixed with nonlabeled template DNA and radiolabeled dNTPs, an acid-precipitable, radiolabeled product was formed. This product was newly synthesized DNA strands. As a control, if nonradiolabeled template DNA was omitted from the assay, no radiolabeled DNA was made. This is the expected result, because the template DNA is necessary to make new daughter strands. Taken together, these results indicate that this technique can be used to measure the synthesis of DNA in vitro.

The in vitro approach has provided the foundation for studying the replication process at the molecular level. A common experimental strategy is to purify proteins from cell extracts and to determine their roles in the replication process. In other words, purified proteins, such as those described in Table 11.1, can be mixed with nucleotides, template DNA, and other substances in a test tube to determine if the synthesis of new DNA strands occurs. This approach still continues, particularly as we try to understand the added complexities of eukaryotic DNA replication.

A self-help quiz involving this experiment can be found at www.mhhe.com/brookergenetics4e.

The Isolation of Mutants Has Been Instrumental to Our Understanding of DNA Replication

In the previous experiment, we considered an experimental strategy for studying DNA synthesis in vitro. In his early experiments, Arthur Kornberg used crude extracts containing *E. coli* proteins and monitored their ability to synthesize DNA. In such extracts, the predominant polymerase is DNA polymerase I. Surprisingly, its activity is so high that it is nearly impossible to detect the activities of the other DNA polymerases. For this reason, researchers in the 1950s and 1960s thought that DNA polymerase I was the only enzyme responsible for DNA replication. This conclusion dramatically changed as a result of mutant isolation.

In 1969, Paula DeLucia and John Cairns identified a mutant *E. coli* strain in which DNA polymerase I lacked its 5′ to 3′ polymerase function but retained its 5′ to 3′ exonuclease function, which is needed to remove RNA primers. This mutant was identified by randomly testing thousands of bacterial colonies that had been subjected to mutagens—agents that cause mutations. Because this mutant strain could grow normally, DeLucia and Cairns concluded that the DNA-synthesizing function of DNA polymerase I is not absolutely required for bacteria to replicate their DNA. How is this possible? The researchers speculated that *E. coli* must have other DNA polymerases. Therefore, DeLucia and Cairns set out to find these seemingly elusive enzymes.

The isolation of mutants was one way that helped researchers identify additional DNA polymerase enzymes, namely DNA polymerase II and III. In addition, mutant isolation played a key role in the identification of other proteins needed to replicate the leading and lagging strands, as well as proteins that recognize the origin of replication and the ter sites. Because DNA replication is vital for cell division, mutations that block DNA replication would be lethal to a growing population of bacterial cells. For this reason, if researchers want to identify loss-of-function mutations in vital genes, they must screen for **conditional mutants.** One type of conditional mutant is a **temperature-sensitive (ts) mutant.** In the case of a vital gene, an organism harboring a ts mutation can survive at the permissive temperature but not at the nonpermissive temperature. For example, a ts mutant might survive and grow at 30°C (the permissive temperature) but fail to grow at 42°C (the nonpermissive temperature). The higher temperature inactivates the function of the protein encoded by the mutant gene.

Figure 11.20 shows a general strategy for the isolation of ts mutants. Researchers expose bacterial cells to a mutagen that increases the likelihood of mutations. The mutagenized cells are plated on growth media and incubated at the permissive temperature. The colonies are then replica plated onto two plates: one incubated at the permissive temperature and one at the nonpermissive temperature. As seen here, this enables researchers to identify ts mutations that are lethal at the nonpermissive temperature.

With regard to the study of DNA replication, researchers analyzed a large number of ts mutants to discover if any of them had a defect in DNA replication. For example, one could expose a ts mutant to radiolabeled thymine (a base that is incorporated into DNA), shift to the nonpermissive temperature, and determine if a mutant strain could make radiolabeled DNA, using procedures that are similar to those described in Figure 11.19. Because *E. coli* has many vital genes not involved with DNA replication, only a small subset of ts mutants would be expected to have mutations in genes that encode proteins that are critical to the replication process. Therefore, researchers had to screen many thousands of ts mutants to identify the few involved in DNA replication. This approach is sometimes called a “brute force” genetic screen.

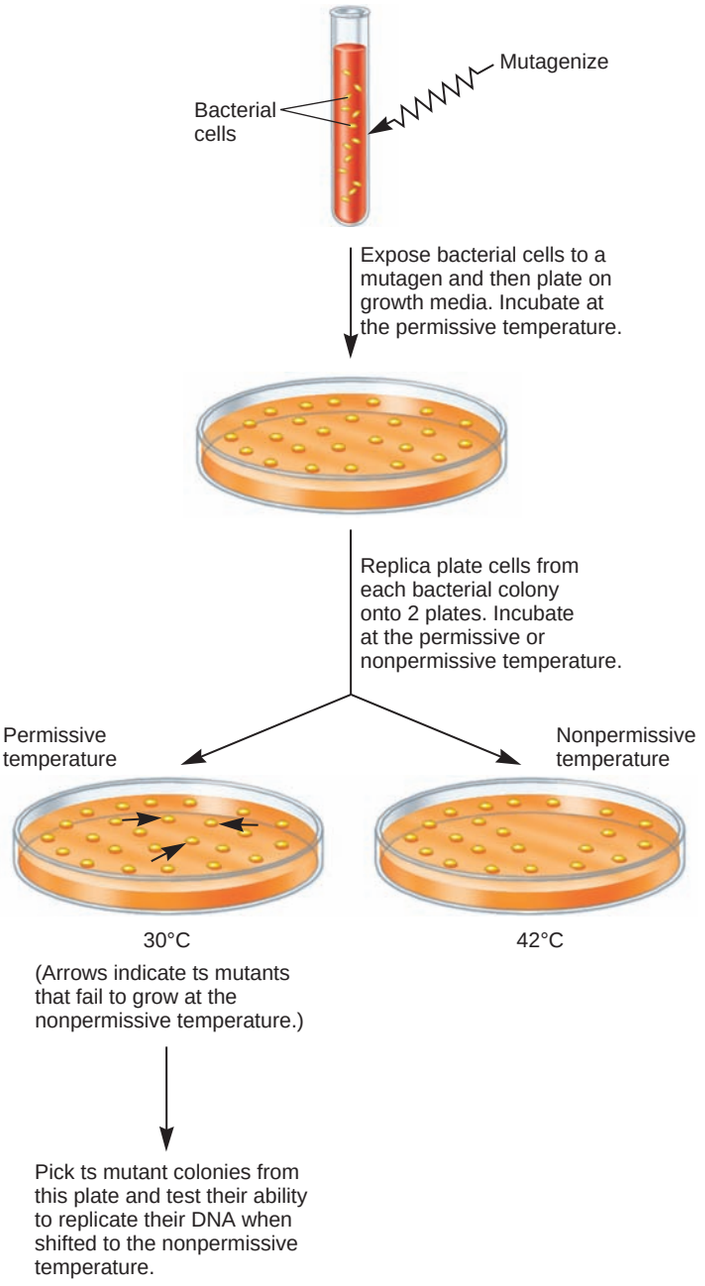


FIGURE 11.20 A strategy to identify ts mutations in vital genes. In this approach, bacteria are mutagenized, which increases the likelihood of mutation, and then grown at the permissive temperature. Colonies are then replica plated and grown at both the permissive and nonpermissive temperatures. (Note: The procedure of replica plating is shown in Chapter 16, Figure 16.7.) Ts mutants fail to grow at the nonpermissive temperature. The appropriate colonies can be picked from the plates, grown at the permissive temperature, and analyzed to see if DNA replication is altered at the nonpermissive temperature.

Table 11.3 summarizes some of the genes that were identified using this type of strategy. The genes were originally designated with the name dna, followed by a capital letter that generally refers to the order in which they were discovered. When shifted to the nonpermissive temperature, certain mutants showed a rapid arrest of DNA synthesis. These so-called rapid-stop mutations inactivated genes that encode enzymes needed for DNA replication. By comparison, other mutants were able to complete their current round of replication but could not start another round. These slow-stop mutants involved genes that encode proteins needed for the initiation of replication at the origin. In later studies, the proteins encoded by these genes were purified, and their functions were studied in vitro. This work contributed greatly to our modern understanding of DNA replication at the molecular level.

TABLE 11.3
Examples of ts Mutants Involved in DNA Replication in *E. coli*

Gene Name	Protein Function
Rapid-Stop Mutants	
dnaE	α subunit of DNA polymerase III, synthesizes DNA
dnaX	τ subunit of DNA polymerase III, promotes the dimerization of two DNA polymerase III proteins together at the replication fork and stimulates DNA helicase
dnaN	β subunit of DNA polymerase III, functions as a clamp protein that makes DNA polymerase a processive enzyme
dnaZ	γ subunit of DNA polymerase III, helps the β subunit bind to the DNA
dnaG	Primase, needed to make RNA primers
dnaB	Helicase, needed to unwind the DNA strands during replication
Slow-Stop Mutants	
dnaA	DnaA protein that recognizes the DnaA boxes at the origin
dnaC	DnaC protein that recruits DNA helicase to the origin

11.3 EUKARYOTIC DNA REPLICATION

Eukaryotic DNA replication is not as well understood as bacterial replication. Much research has been carried out on a variety of experimental organisms, particularly yeast and mammalian cells. Many of these studies have found extensive similarities between the general features of DNA replication in prokaryotes and eukaryotes. For example, DNA helicases, topoisomerases, single-strand binding proteins, primases, DNA polymerases, and DNA ligases—the types of bacterial enzymes described in Table 11.1—have also been identified in eukaryotes. Nevertheless, at the molecular level, eukaryotic DNA replication appears to be substantially more complex. These additional intricacies of eukaryotic DNA replication are related to several features of eukaryotic cells. In particular, eukaryotic cells have larger, linear chromosomes, the chromatin is tightly packed within nucleosomes,

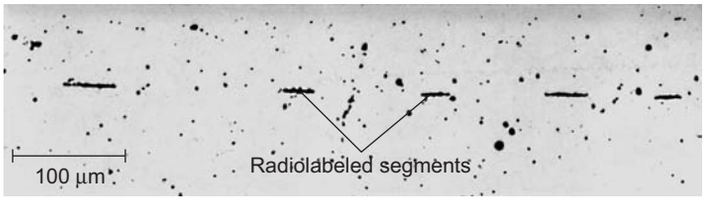


FIGURE 11.21 **Evidence for multiple origins of replication in eukaryotic chromosomes.** In this experiment, cells were given a pulse/chase of ^{3}H-thymidine and unlabeled thymidine. The chromosomes were isolated and subjected to autoradiography. In this micrograph, radiolabeled segments were interspersed among nonlabeled segments, indicating that eukaryotic chromosomes contain multiple origins of replication.

and cell cycle regulation is much more complicated. This section emphasizes some of the unique features of eukaryotic DNA replication.

Initiation Occurs at Multiple Origins of Replication on Linear Eukaryotic Chromosomes

Because eukaryotes have long, linear chromosomes, the chromosomes require multiple origins of replication so the DNA can be replicated in a reasonable length of time. In 1968, Joel Huberman and Arthur Riggs provided evidence for multiple origins of replication by adding a radiolabeled nucleoside (^{3}H-thymidine) to a culture of actively dividing cells, followed by a chase with nonlabeled thymidine. The radiolabeled thymidine was taken up by the cells and incorporated into their newly made DNA strands for a brief period. The chromosomes were then isolated from the cells and subjected to autoradiography. As seen in **Figure 11.21**, radiolabeled segments were interspersed among nonlabeled segments. This result is consistent with the hypothesis that eukaryotic chromosomes contain multiple origins of replication.

As shown schematically in **Figure 11.22**, DNA replication proceeds bidirectionally from many origins of replication during S phase of the cell cycle. The multiple replication forks eventually make contact with each other to complete the replication process.

The molecular features of eukaryotic origins of replication may have some similarities to the origins found in bacteria. At the molecular level, eukaryotic origins of replication have been extensively studied in the yeast *Saccharomyces cerevisiae*. In this organism, several replication origins have been identified and sequenced. They have been named **ARS elements** (for autonomously replicating sequence). ARS elements, which are about 50 bp in length, are necessary to initiate chromosome replication. ARS elements have unique features of their DNA sequences. First, they contain a higher percentage of A and T bases than the rest of the chromosomal DNA. In addition, they contain a copy of the ARS consensus sequence (ACS), ATTTAT(A or G)TTTA, along with additional elements that enhance origin function. This arrangement is similar to bacterial origins.

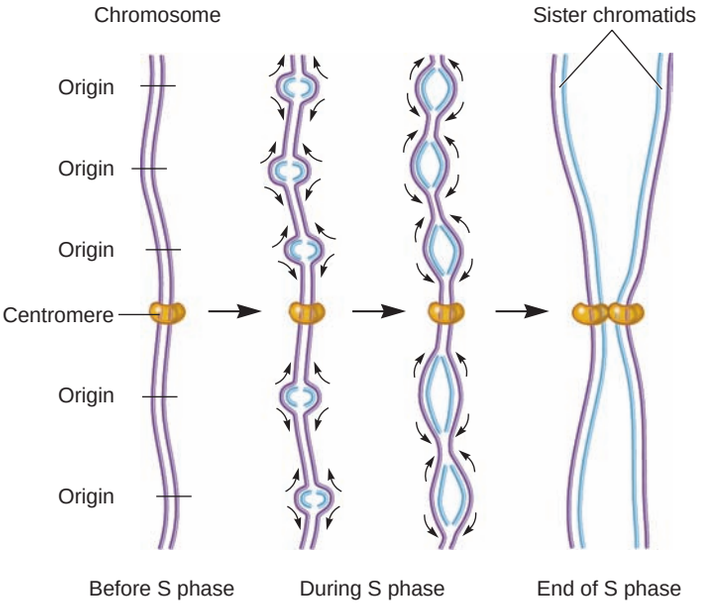


FIGURE 11.22 **The replication of eukaryotic chromosomes.** At the beginning of the S phase of the cell cycle, eukaryotic chromosome replication begins from multiple origins of replication. As S phase continues, the replication forks move bidirectionally to replicate the DNA. By the end of S phase, all of the replication forks have merged. The net result is two sister chromatids attached to each other at the centromere.

In *S. cerevisiae*, origins of replication are determined primarily by their DNA sequences. In animals, the critical features that define origins of replication are not completely understood. In many species, origins are not determined by particular DNA sequences but instead occur at specific sites along a chromosome due to chromatin structure and protein modifications.

DNA replication in eukaryotes begins with the assembly of a **prereplication complex (preRC)** consisting of at least 14 different proteins. Part of the preRC is a group of six proteins called the **origin recognition complex (ORC)** that acts as the initiator of eukaryotic DNA replication. The ORC was originally identified in yeast as a protein complex that binds directly to ARS elements. DNA replication at the origin begins with the binding of ORC, which usually occurs during G_1 phase. Other proteins of the preRC then bind, including a group of proteins called **MCM helicase.**[1] The binding of MCM helicase at the origin completes a process called **DNA replication licensing;** only those origins with MCM helicase can initiate DNA synthesis. During S phase, DNA synthesis begins when preRCs are acted on by at least 22 additional proteins that activate MCM helicase and assemble two divergent replication forks at each replication origin. An

[1] MCM is an acronym for minichromosome maintenance. The genes encoding MCM proteins were originally identified in mutant yeast strains that are defective in the maintenance of minichromosomes in the cell. MCM proteins have since been shown to play a role in DNA replication.

important role of these additional proteins is to carefully regulate the initiation of DNA replication so that it happens at the correct time during the cell cycle and occurs only once during the cell cycle. The precise roles of these proteins are under active research investigation.

Eukaryotes Contain Several Different DNA Polymerases

Eukaryotes have many types of DNA polymerases. For example, mammalian cells have well over a dozen different DNA polymerases (**Table 11.4**). Four of these, designated α (alpha), ε (epsilon), δ (delta), and γ (gamma), have the primary function of replicating DNA. DNA polymerase γ functions in the mitochondria to replicate mitochondrial DNA, whereas α, ε, and δ are involved with DNA replication in the cell nucleus during S phase.

DNA polymerase α is the only eukaryotic polymerase that associates with primase. The functional role of the DNA polymerase α/primase complex is to synthesize a short RNA-DNA primer of approximately 10 RNA nucleotides followed by 20 to 30 DNA nucleotides. This short RNA-DNA strand is then used by DNA polymerase ε or δ for the processive elongation of the leading and lagging strands, respectively. For this to happen, the DNA polymerase α/primase complex dissociates from the replication fork and is exchanged for DNA polymerase ε or δ. This exchange is called a **polymerase switch.** Accumulating evidence suggests that DNA polymerase ε is primarily involved with leading-strand synthesis, whereas DNA polymerase δ is responsible for lagging-strand synthesis.

What are the functions of the other DNA polymerases? Several of them also play an important role in DNA repair, a topic that will be examined in Chapter 16. DNA polymerase β, which has been studied for several decades, is not involved in the replication of normal DNA, but plays an important role in removing incorrect bases from damaged DNA. More recently, several additional DNA polymerases have been identified. While their precise roles have not been elucidated, many of these are in a category called **lesion-replicating polymerases.** When DNA polymerase α, δ, and ε encounter abnormalities in DNA structure, such as abnormal bases or cross-links, they may be unable to replicate over the aberration. When this occurs, lesion-replicating polymerases are attracted to the damaged DNA and have special properties that enable them to synthesize a complementary strand over the abnormal region. Each type of lesion-replicating polymerase may be able to replicate over a different kind of DNA damage.

TABLE 11.4
Eukaryotic DNA Polymerases

Polymerase Types*	Function
α	Initiates DNA replication in conjunction with primase
ε	Replication of the leading strand during S phase
δ	Replication of the lagging strand during S phase
γ	Replication of mitochondrial DNA
η, κ, ι, ξ (lesion-replicating polymerases)	Replication of damaged DNA
α, β, δ, ε, σ, λ, μ, φ, θ, η	DNA repair or other functions†

*The designations are those of mammalian enzymes.
†Many DNA polymerases have dual functions. For example, DNA polymerases α, δ, and ε are involved in the replication of normal DNA and also play a role in DNA repair. In cells of the immune system, certain genes that encode antibodies (i.e., immunoglobulin genes) undergo a phenomenon known as hypermutation. This increases the variation in the kinds of antibodies the cells can make. Certain polymerases in this list, such as η, may play a role in hypermutation of immunoglobulin genes. DNA polymerase σ may play a role in sister chromatid cohesion, a topic discussed in Chapter 10.

Flap Endonuclease Removes RNA Primers During Eukaryotic DNA Replication

Another key difference between bacterial and eukaryotic DNA replication is the way that RNA primers are removed. As discussed earlier in this chapter, bacterial RNA primers are removed by DNA polymerase I. By comparison, a DNA polymerase enzyme does not play this role in eukaryotes. Instead, an enzyme called flap endonuclease is primarily responsible for RNA primer removal.

Flap endonuclease gets its name because it removes small pieces of RNA flaps that are generated by the action of DNA polymerase δ. In the diagram shown in **Figure 11.23**, DNA polymerase δ elongates the left Okazaki fragment until it runs into the RNA primer of the adjacent Okazaki fragment on the right. This causes a portion of the RNA primer to form a short flap, which is removed by the endonuclease function of flap endonuclease. As DNA polymerase δ continues to elongate the DNA, short flaps continue to be generated, which are sequentially removed by flap endonuclease. Eventually, all of the RNA primer is removed, and DNA ligase can seal the DNA fragments together.

Though flap endonuclease is thought to be the primary pathway for RNA primer removal in eukaryotes, it is unable to remove a flap that is too long. In such cases, a long flap is cleaved by the enzyme called Dna2 nuclease/helicase. This enzyme can cut a long flap, thereby generating a short flap. The short flap is then removed via flap endonuclease.

The Ends of Eukaryotic Chromosomes Are Replicated by Telomerase

Linear eukaryotic chromosomes contain **telomeres** at both ends. The term telomere refers to the complex of telomeric sequences within the DNA and the special proteins that are bound to these sequences. Telomeric sequences consist of a moderately repetitive tandem array and a 3′ overhang region that is 12 to 16 nucleotides in length (**Figure 11.24**).

The tandem array that occurs within the telomere has been studied in a wide variety of eukaryotic organisms. A common feature is that the telomeric sequence contains several guanine

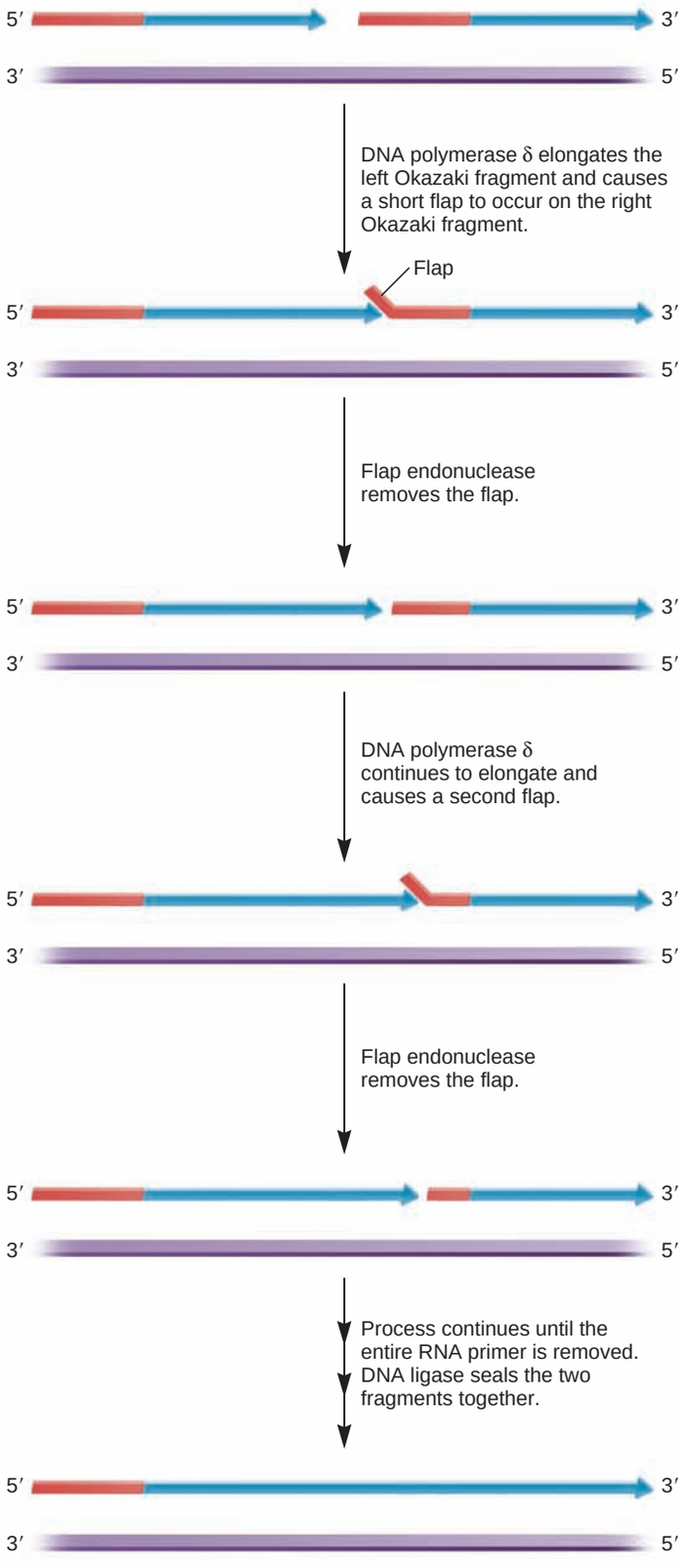
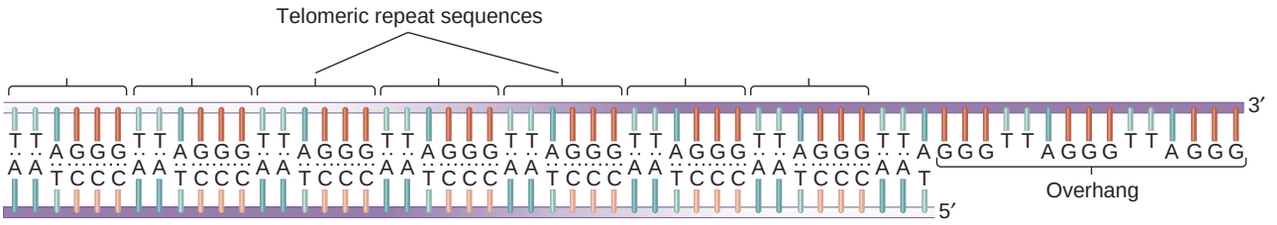


FIGURE 11.23 **Removal of an RNA primer by flap endonuclease.**

nucleotides and often many thymine nucleotides (**Table 11.5**). Depending on the species and the cell type, this sequence can be tandemly repeated up to several hundred times in the telomere region.

One reason why telomeric repeat sequences are needed is because DNA polymerase is unable to replicate the 3′ ends of DNA strands. Why is DNA polymerase unable to replicate this region? The answer lies in the two unusual enzymatic features of this enzyme. As discussed previously, DNA polymerase synthesizes DNA only in a 5′ to 3′ direction, and it cannot link together the first two individual nucleotides; it can elongate only preexisting strands. These two features of DNA polymerase function pose a problem at the 3′ ends of linear chromosomes. As shown in **Figure 11.25**, the 3′ end of a DNA strand cannot be replicated by DNA polymerase because a primer cannot be made upstream from this point. Therefore, if this problem were not solved, the chromosome would become progressively shorter with each round of DNA replication.

To prevent the loss of genetic information due to chromosome shortening, additional DNA sequences are attached to the ends of telomeres. In 1984, Carol Greider and Elizabeth Blackburn discovered an enzyme called **telomerase** that prevents chromosome shortening. It recognizes the sequences at the ends of eukaryotic chromosomes and synthesizes additional repeats of telomeric sequences. They received the 2009 Nobel Prize in physiology or medicine for their discovery. **Figure 11.26** shows the interesting mechanism by which telomerase works. The telomerase enzyme contains both protein subunits and RNA. The RNA part of telomerase contains a sequence complementary to the DNA sequence found in the telomeric repeat. This allows telomerase to bind to the 3′ overhang region of the telomere. Following binding, the RNA sequence beyond the binding site functions as a template allowing the synthesis of a six-nucleotide sequence at the end of the DNA strand. This is called polymerization, because it is analogous to the function of DNA polymerase. It is catalyzed by two identical protein subunits called **telomerase reverse transcriptase (TERT).** TERT's name indicates that it uses an RNA template to synthesize DNA. Following polymerization, the telomerase can then move—a process called translocation—to the new end of this DNA strand and attach another six nucleotides to the end. This binding-polymerization-translocation cycle occurs many times in a row, thereby greatly lengthening the 3′ end of the DNA strand in the telomeric region. The complementary strand is then synthesized by primase, DNA polymerase, and DNA ligase, as described earlier in this chapter.



ONLINE ANIMATION

FIGURE 11.24 General structure of telomeric sequences. The telomere DNA consists of a tandemly repeated sequence and a 12- to 16-nucleotide overhang.

TABLE **11.5**

Telomeric Repeat Sequences Within Selected Organisms

Group	Examples	Telomeric Repeat Sequence
Mammals	Humans	TTAGGG
Slime molds	*Physarum, Didymium*	TTAGGG
	Dictyostelium	$AG_{(1–8)}$
Filamentous fungi	*Neurospora*	TTAGGG
Budding yeast	*Saccharomyces cerevisiae*	$TG_{(1–3)}$
Ciliates	*Tetrahymena*	TTGGGG
	Paramecium	TTGGG(T/G)
	Euplotes	TTTTGGGG
Higher plants	*Arabidopsis*	TTTAGGG

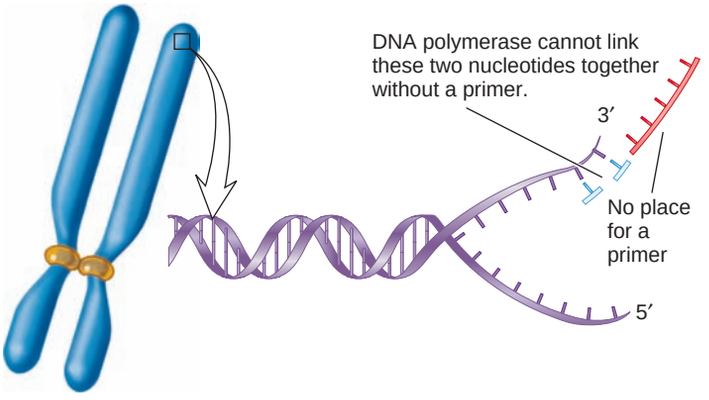
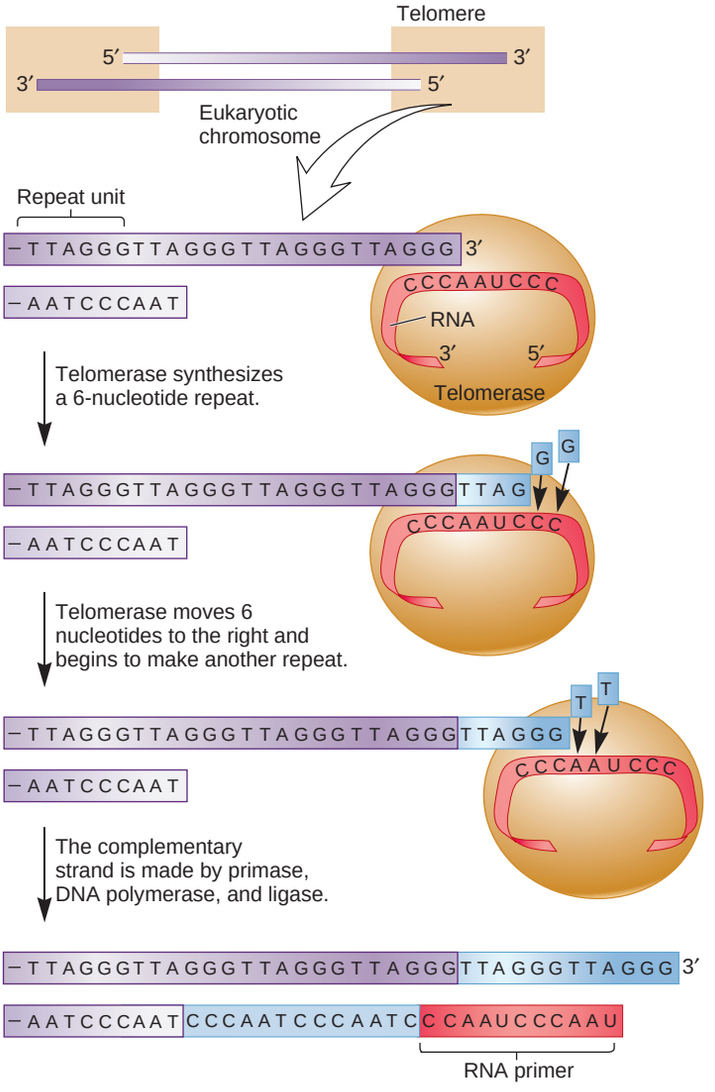


FIGURE 11.25 The replication problem at the ends of linear chromosomes. DNA polymerase cannot synthesize a DNA strand that is complementary to the 3′ end because a primer cannot be made upstream from this site.



ONLINE ANIMATION

FIGURE 11.26 The enzymatic action of telomerase. A short, three-nucleotide segment of RNA within telomerase causes it to bind to the 3′ overhang. The adjacent part of the RNA is used as a template to make a short, six-nucleotide repeat of DNA. After the repeat is made, telomerase moves six nucleotides to the right and then synthesizes another repeat. This process is repeated many times to lengthen the top strand shown in this figure. The bottom strand is made by DNA polymerase, using an RNA primer at the end of the chromosome that is complementary to the telomeric repeat sequence in the top strand. DNA polymerase fills in the region, which is sealed by ligase.

KEY TERMS

Page 270. DNA replication
Page 271. template strands, parental strands, daughter strands
Page 272. conservative model, semiconservative model, dispersive model
Page 274. origin of replication, bidirectionally, replication forks
Page 275. DnaA proteins, DnaA box sequences, DNA helicase
Page 276. bidirectional replication
Page 277. topoisomerase (type II), DNA gyrase, single-strand binding proteins, RNA primers, primase, leading strand, lagging strand, DNA polymerase
Page 278. Okazaki fragments
Page 279. DNA ligase, pulse/chase experiment
Page 280. processive enzyme
Page 281. termination sequences
Page 282. catenanes, primosome
Page 283. replisome, dimeric DNA polymerase, fidelity, proofreading function
Page 287. conditional mutants, temperature-sensitive (ts) mutant
Page 289. ARS elements, prereplication complex (preRC), origin recognition complex (ORC), MCM helicase, DNA replication licensing
Page 290. polymerase switch, lesion-replicating polymerases, flap endonuclease, telomeres
Page 291. telomerase, telomerase reverse transcriptase (TERT)

CHAPTER SUMMARY

- DNA replication is the process in which existing DNA strands are used to make new DNA strands.

11.1 Structural Overview of DNA Replication

- DNA replication occurs when the strands of DNA unwind and each strand is used as a template to make a new strand according to the AT/GC rule. The resulting DNA molecules have the same base sequence as the original DNA (see Figure 11.1).
- By labeling DNA with heavy and light isotopes of nitrogen and using centrifugation, Meselson and Stahl showed that DNA replication is semiconservative (see Figures 11.2, 11.3).

11.2 Bacterial DNA Replication

- Bacterial DNA replication begins at a single origin of replication and proceeds bidirectionally around the circular chromosome (see Figure 11.4).
- In *E. coli*, DNA replication is initiated when DnaA proteins bind to five DnaA boxes at the origin of replication and cause the AT-rich region to unwind. DNA helicases then promote the movement of two forks (see Figures 11.5, 11.6).
- At each replication fork, DNA helicase unwinds the DNA and topisomerase alleviates positive supercoiling. Single-strand binding proteins coat the DNA to prevent the strands from coming back together. Primase synthesizes RNA primers and DNA polymerase synthesizes complementary strands of DNA. DNA ligase seals the gaps between Okazaki fragments (see Figure 11.7, Table 11.1).
- DNA polymerase III in *E. coli* is an enzyme with several subunits that wraps around the DNA like a hand (see Figure 11.8, Table 11.2).
- DNA polymerase enzymes need a primer to synthesize DNA and make new DNA strands in a 5′ to 3′ direction (see Figure 11.9).
- During DNA synthesis, the leading strand is made continuously in the direction of the replication fork, whereas the lagging strand is made as Okazaki fragments in the direction away from the fork (see Figures 11.10, 11.11).
- DNA polymerase III is a processive enzyme that uses deoxynucleoside triphosphates to make new DNA strands (see Figure 11.12).
- In *E. coli*, DNA replication is terminated at ter sequences (see Figure 11.13).
- Following DNA replication, interlocked catenanes sometimes need to be unlocked via topoisomerase II (see Figure 11.14).
- The primosome is a complex between helicase and primase. The replisome is a complex between the primosome and dimeric DNA polymerase (see Figure 11.15).
- The high fidelity of DNA replication is a result of (1) the stability of hydrogen bonding between the correct bases, (2) the phenomenon of induced fit, and (3) the proofreading ability of DNA polymerase (see Figure 11.16).
- Bacterial DNA replication is regulated by the amount of DnaA protein and by the methylation of GATC sites in *oriC* (see Figures 11.17, 11.18).
- Kornberg devised a method to measure DNA replication in vitro (see Figure 11.19).
- The isolation and characterization of temperature-sensitive mutants was a useful strategy for identifying proteins involved with DNA replication (see Figure 11.20, Table 11.3).

11.3 Eukaryotic DNA Replication

- Eukaryotic chromosomes contain multiple origins of replication. Part of the prereplication complex is formed from a group of six proteins called the origin recognition complex. The binding of MCM helicase completes a process called DNA replication licensing (see Figures 11.21, 11.22).
- Eukaryotes have several different DNA polymerases with specialized roles. Different types of DNA polymerases switch with each other during the process of DNA replication (see Table 11.4).
- Flap endonuclease is an enzyme that removes RNA primers from Okazaki fragments (see Figure 11.23).

- The ends of eukaryotic chromosomes contain telomeres, which are composed of short repeat sequences and proteins (see Figure 11.24, Table 11.5).
- DNA polymerase is unable to replicate the very end of a eukaryotic chromosome (see Figure 11.25).
- Telomerase uses a short RNA molecule as a template to add repeat sequences onto telomeres (see Figure 11.26).

PROBLEM SETS & INSIGHTS

Solved Problems

S1. Describe three ways to account for the high fidelity of DNA replication. Discuss the quantitative contributions of each of the three ways.

Answer:

First: AT and GC pairs are preferred in the double-helix structure. This provides fidelity to around 1 mistake per 1000.

Second: Induced fit by DNA polymerase prevents covalent bond formation unless the proper nucleotides are in place. This increases fidelity another 100- to 1000-fold, to about 1 error in 100,000 to 1 million.

Third: Exonuclease proofreading increases fidelity another 100- to 1000-fold, to about 1 error per 100 million nucleotides added.

S2. What do you think would happen if the ter sequences were deleted from the bacterial DNA?

Answer: Instead of meeting at the ter sequences, the two replication forks would meet somewhere else. This would depend on how fast they were moving. For example, if one fork was advancing faster than the other, they would meet closer to where the slower-moving fork started. In fact, researchers have actually conducted this experiment. Interestingly, *E. coli* without the ter sequences seemed to survive just fine.

S3. Summarize the steps that occur in the process of chromosomal DNA replication in *E. coli*.

Answer:

Step 1. DnaA proteins bind to the origin of replication, resulting in the separation of the AT-rich region.

Step 2. DNA helicase breaks the hydrogen bonds between the DNA strands, topoisomerases alleviate positive supercoiling, and single-strand binding proteins hold the parental strands apart.

Step 3. Primase synthesizes one RNA primer in the leading strand and many RNA primers in the lagging strand. DNA polymerase III then synthesizes the daughter strands of DNA. In the lagging strand, many short segments of DNA (Okazaki fragments) are made. DNA polymerase I removes the RNA primers and fills in with DNA, and DNA ligase covalently links the Okazaki fragments together.

Step 4. The processes described in steps 2 and 3 continue until the two replication forks reach the ter sequences on the other side of the circular bacterial chromosome.

Step 5. Topoisomerases unravel the intertwined chromosomes, if necessary.

S4. If a strain of *E. coli* overproduced the Dam enzyme, how would that affect the DNA replication process? Would you expect such a strain to have more or fewer chromosomes per cell compared with a normal strain of *E. coli*? Explain why.

Answer: If a strain overproduced the Dam enzyme, DNA would replicate more rapidly. The GATC methylation sites in the origin of replication have to be fully methylated for DNA replication to occur. Immediately after DNA replication, a delay occurs before the next round of DNA replication because the two copies of newly replicated DNA are hemimethylated. A strain that overproduces Dam would rapidly convert the hemimethylated DNA into fully methylated DNA and more quickly allow the next round of DNA replication to occur. For this reason, the overproducing strain might have more copies of the *E. coli* chromosome because it would not have a long delay in DNA replication.

Conceptual Questions

C1. What key structural features of the DNA molecule underlie its ability to be faithfully replicated?

C2. With regard to DNA replication, define the term bidirectional replication.

C3. Which of the following statements is not true? Explain why.

A. A DNA strand can serve as a template strand on many occasions.

B. Following semiconservative DNA replication, one strand is a newly made daughter strand and the other strand is a parental strand.

C. A DNA double helix may contain two strands of DNA that were made at the same time.

D. A DNA double helix obeys the AT/GC rule.

E. A DNA double helix could contain one strand that is 10 generations older than its complementary strand.

C4. The compound known as nitrous acid is a reactive chemical that replaces amino groups ($-NH_2$) with keto groups (=O). When nitrous acid reacts with the bases in DNA, it can change cytosine to uracil and change adenine to hypoxanthine. A DNA double helix has the following sequence:

TTGGATGCTGG
AACCTACGACC

A. What would be the sequence of this double helix immediately after reaction with nitrous acid? Let the letter H represent hypoxanthine and U represent uracil.

B. Let's suppose this DNA was reacted with nitrous acid. The nitrous acid was then removed, and the DNA was replicated for two generations. What would be the sequences of the DNA products after the DNA had replicated twice? Your answer should contain the sequences of four double helices. Note: During DNA replication, uracil hydrogen bonds with adenine, and hypoxanthine hydrogen bonds with cytosine.

C5. One way that bacterial cells regulate DNA replication is by GATC methylation sites within the origin of replication. Would this mechanism work if the DNA was conservatively (rather than semiconservatively) replicated?

C6. The chromosome of *E. coli* contains 4.6 million bp. How long will it take to replicate its DNA? Assuming DNA polymerase III is the primary enzyme involved and this enzyme can actively proofread during DNA synthesis, how many base pair mistakes will be made in one round of DNA replication in a bacterial population containing 1000 bacteria?

C7. Here are two strands of DNA.

————————————————DNA polymerase–>

————————————————————————————————

The one on the bottom is a template strand, and the one on the top is being synthesized by DNA polymerase in the direction shown by the arrow. Label the 5′ and 3′ ends of the top and bottom strands.

C8. A DNA strand has the following sequence:

5′–GATCCCGATCCGCATACATTTACCAGATCACCACC–3′

In which direction would DNA polymerase slide along this strand (from left to right or from right to left)? If this strand was used as a template by DNA polymerase, what would be the sequence of the newly made strand? Indicate the 5′ and 3′ ends of the newly made strand.

C9. List and briefly describe the three types of sequences within bacterial origins of replication that are functionally important.

C10. As shown in Figure 11.5, five DnaA boxes are found within the origin of replication in *E. coli*. Take a look at these five sequences carefully.

A. Are the sequences of the five DnaA boxes very similar to each other? (Hint: Remember that DNA is double-stranded; think about these sequences in the forward and reverse direction.)

B. What is the most common sequence for the DnaA box? In other words, what is the most common base in the first position, second position, and so on until the ninth position? The most common sequence is called the consensus sequence.

C. The *E. coli* chromosome is about 4.6 million bp long. Based on random chance, is it likely that the consensus sequence for a DnaA box occurs elsewhere in the *E. coli* chromosome? If so, why aren't there multiple origins of replication in *E. coli*?

C11. Obtain two strings of different colors (e.g., black and white) that are the same length. A length of 20 inches is sufficient. Tie a knot at one end of the black string, and tie a knot at one end of the white string. Each knot designates the 5′ end of your strings. Make a double helix with your two strings. Now tape one end of the double helix to a table so that the tape is covering the knot on the black string.

A. Pretend your hand is DNA helicase and use your hand to unravel the double helix, beginning at the end that is not taped to the table. Should your hand be sliding along the white string or the black string?

B. As in Figure 11.15, imagine that your two hands together form a dimeric replicative DNA polymerase. Unravel your two strings halfway to create a replication fork. Grasp the black string with your left hand and the white string with your right hand. Your thumbs should point toward the 5′ end of each string. You need to loop one of the strings so that one of the DNA polymerases can synthesize the lagging strand. With such a loop, the dimeric replicative DNA polymerase can move toward the replication fork and synthesize both DNA strands in the 5′ to 3′ direction. In other words, with such a loop, your two hands can touch each other with both of your thumbs pointing toward the fork. Should the black string be looped, or should the white string be looped?

C12. Sometimes DNA polymerase makes a mistake, and the wrong nucleotide is added to the growing DNA strand. With regard to pyrimidines and purines, two general types of mistakes are possible. The addition of an incorrect pyrimidine instead of the correct pyrimidine (e.g., adding cytosine where thymine should be added) is called a transition. If a pyrimidine is incorrectly added to the growing strand instead of purine (e.g., adding cytosine where an adenine should be added), this type of mistake is called a transversion. If a transition or transversion is not detected by DNA polymerase, a mutation is created that permanently changes the DNA sequence. Though both types of mutations are rare, transition mutations are more frequent than transversion mutations. Based on your understanding of DNA replication and DNA polymerase, offer three explanations why transition mutations are more common.

C13. A short genetic sequence, which may be recognized by DNA primase, is repeated many times throughout the *E. coli* chromosome. Researchers have hypothesized that DNA primase may recognize this sequence as a site to begin the synthesis of an RNA primer for DNA replication. The *E. coli* chromosome is roughly 4.6 million bp in length. How many copies of the DNA primase recognition sequence would be necessary to replicate the entire *E. coli* chromosome?

C14. Single-strand binding proteins keep the two parental strands of DNA separated from each other until DNA polymerase has an opportunity to replicate the strands. Suggest how single-strand binding proteins keep the strands separated and yet do not impede the ability of DNA polymerase to replicate the strands.

C15. The ability of DNA polymerase to digest a DNA strand from one end is called its exonuclease activity. Exonuclease activity is used to digest RNA primers and also to proofread a newly made DNA strand. Note: DNA polymerase I does not change direction while it is removing an RNA primer and synthesizing new DNA. It does change direction during proofreading.

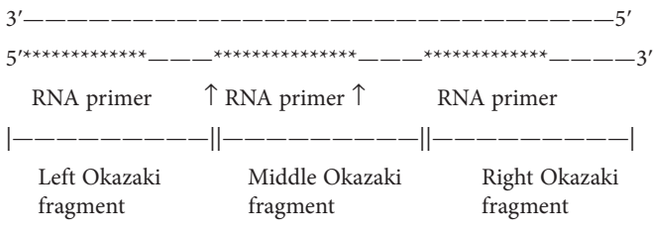
A. In which direction, 5′ to 3′ or 3′ to 5′, is the exonuclease activity occurring during the removal of RNA primers and during the proofreading and removal of mistakes following DNA replication?

B. Figure 11.16 shows a drawing of the 3′ exonuclease site. Do you think this site would be used by DNA polymerase I to remove RNA primers? Why or why not?

C16. In the following drawing, the top strand is the template DNA, and the bottom strand shows the lagging strand prior to the action of

DNA polymerase I. The lagging strand contains three Okazaki fragments. The RNA primers have not yet been removed.

The top strand is the template DNA



A. Which Okazaki fragment was made first, the one on the left or the one on the right?

B. Which RNA primer would be the first one to be removed by DNA polymerase I, the primer on the left or the primer on the right? For this primer to be removed by DNA polymerase I and for the gap to be filled in, is it necessary for the Okazaki fragment in the middle to have already been synthesized? Explain why.

C. Let's consider how DNA ligase connects the left Okazaki fragment with the middle Okazaki fragment. After DNA polymerase I removes the middle RNA primer and fills in the gap with DNA, where does DNA ligase function? See the arrows on either side of the middle RNA primer. Is ligase needed at the left arrow, at the right arrow, or both?

D. When connecting two Okazaki fragments, DNA ligase needs to use NAD^+ or ATP as a source of energy to catalyze this reaction. Explain why DNA ligase needs another source of energy to connect two nucleotides, but DNA polymerase needs nothing more than the incoming nucleotide and the existing DNA strand. Note: You may want to refer to Figure 11.12 to answer this question.

C17. What is DNA methylation? Why is DNA in a hemimethylated condition immediately after DNA replication? What are the functional consequences of methylation in the regulation of DNA replication?

C18. Describe the three important functions of the DnaA protein.

C19. If a strain of bacteria was making too much DnaA protein, how would you expect this to affect its ability to regulate DNA replication? With regard to the number of chromosomes per cell, how might this strain differ from a normal bacterial strain?

C20. Draw a picture that illustrates how DNA helicase works.

C21. What is an Okazaki fragment? In which strand of DNA are Okazaki fragments found? Based on the properties of DNA polymerase, why is it necessary to make these fragments?

C22. Discuss the similarities and differences in the synthesis of DNA in the lagging and leading strands. What is the advantage of a primosome and a replisome as opposed to having all replication enzymes functioning independently of each other?

C23. Explain the proofreading function of DNA polymerase.

C24. What is a processive enzyme? Explain why this is an important feature of DNA polymerase.

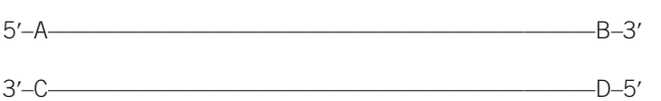
C25. Why is it important for living organisms to regulate DNA replication?

C26. What enzymatic features of DNA polymerase prevent it from replicating one of the DNA strands at the ends of linear chromosomes? Compared with DNA polymerase, how is telomerase different in its ability to synthesize a DNA strand? What does telomerase use as its template for the synthesis of a DNA strand? How does the use of this template result in a telomere sequence that is tandemly repetitive?

C27. As shown in Figure 11.26, telomerase attaches additional DNA, six nucleotides at a time, to the ends of eukaryotic chromosomes. However, it works in only one DNA strand. Describe how the opposite strand is replicated.

C28. If a eukaryotic chromosome has 25 origins of replication, how many replication forks does it have at the beginning of DNA replication?

C29. A diagram of a linear chromosome is shown here. The end of each strand is labeled with an A, B, C, or D. Which ends could not be replicated by DNA polymerase? Why not?



C30. As discussed in Chapter 10, some viruses contain RNA as their genetic material. Certain RNA viruses can exist as a provirus in which the viral genetic material has been inserted into the chromosomal DNA of the host cell. For this to happen, the viral RNA must be copied into a strand of DNA. An enzyme called reverse transcriptase, encoded by the viral genome, copies the viral RNA into a complementary strand of DNA. The strand of DNA is then used as a template to make a double-stranded DNA molecule. This double-stranded DNA molecule is then inserted into the chromosomal DNA, where it may exist as a provirus for a long period of time.

A. How is the function of reverse transcriptase similar to the function of telomerase?

B. Unlike DNA polymerase, reverse transcriptase does not have a proofreading function. How might this affect the proliferation of the virus?

C31. Telomeres contain a 3′ overhang region, as shown in Figure 11.24. Does telomerase require a 3′ overhang to replicate the telomere region? Explain.

Experimental Questions

E1. Answer the following questions that pertain to the experiment of Figure 11.3.

A. What would be the expected results if the Meselson and Stahl experiment were carried out for four or five generations?

B. What would be the expected results of the Meselson and Stahl experiment after three generations if the mechanism of DNA replication was dispersive?

C. As shown in the data, explain why three different bands (i.e., light, half-heavy, and heavy) can be observed in the CsCl gradient.

E2. An absentminded researcher follows the steps of Figure 11.3, and when the gradient is viewed under UV light, the researcher does not see any bands at all. Which of the following mistakes could account for this observation? Explain how.

A. The researcher forgot to add ^{14}N-containing compounds.

B. The researcher forgot to add lysozyme.

C. The researcher forgot to turn on the UV lamp.

E3. Figure 11.4b shows an autoradiograph of a replicating bacterial chromosome. If you analyzed many replicating chromosomes, what types of information could you learn about the mechanism of DNA replication?

E4. The experiment of Figure 11.19 described a method for determining the amount of DNA made during replication. Let's suppose that you can purify all of the proteins required for DNA replication. You then want to "reconstitute" DNA synthesis by mixing together all of the purified components necessary to synthesize a complementary strand of DNA. If you started with single-stranded DNA as a template, what additional proteins and molecules would you have to add for DNA synthesis to occur? What additional proteins would be necessary if you started with a double-stranded DNA molecule?

E5. Using the reconstitution strategy described in experimental question E4, what components would you have to add to measure the ability of telomerase to synthesize DNA? Be specific about the type of template DNA that you would add to your mixture.

E6. As described in Figure 11.19, perchloric acid precipitates strands of DNA, but it does not precipitate free nucleotides. (Note: The term free nucleotide means nucleotides that are not connected covalently to other nucleotides.) Explain why this is a critical step in the experimental procedure. If a researcher used a different reagent that precipitated DNA strands and free nucleotides instead of using perchloric acid (which precipitates only DNA strands), how would that affect the results?

E7. Would the experiment of Figure 11.19 work if the ^{32}P-labeled nucleotides were deoxyribonucleoside monophosphates instead of dNTPs? Explain why or why not.

E8. To synthesize DNA in vitro, single-stranded DNA can be used as a template. As described in Figure 11.19, you also need to add DNA polymerase, dNTPs, and a primer in order to synthesize a complementary strand of DNA. The primer can be a short sequence of DNA or RNA. The primer must be complementary to the template DNA. Let's suppose a single-stranded DNA molecule is 46 nucleotides long and has the following sequence:

GCCCCGGTACCCCGTAATATACGGGACTAGGCCGGAGGTCCGGGCG

This template DNA is mixed with a primer with the sequence 5′–CGCCCGGACC–3′, DNA polymerase, and dNTPs. In this case, a double-stranded DNA molecule is made. However, if the researcher substitutes a primer with the sequence 5′–CCAG-GCCCGC–3′, a double-stranded DNA molecule is not made.

A. Which is the 5′ end of the DNA molecule shown, the left end or the right end?

B. If you added a primer that was 10 nucleotides long and complementary to the left end of the single-stranded DNA, what would be the sequence of the primer? You should designate the 5′ and 3′ ends of the primer. Could this primer be used to replicate the single-stranded DNA?

E9. The technique of dideoxy sequencing of DNA is described in Chapter 18. The technique relies on the use of dideoxyribonucleotides (shown in Figures 18.18 and 18.19). A dideoxyribonucleotide has a hydrogen atom attached to the 3′-carbon atom instead of an −OH group. When a dideoxyribonucleotide is incorporated into a newly made strand, the strand cannot grow any longer. Explain why.

E10. Another technique described in Chapter 18 is the polymerase chain reaction (PCR) (see Figure 18.6). This method is based on our understanding of DNA replication. In this method, a small amount of double-stranded template DNA is mixed with a high concentration of primers. Nucleotides and DNA polymerase are also added. The template DNA strands are separated by heat treatment, and when the temperature is lowered, the primers can bind to the single-stranded DNA, and then DNA polymerase replicates the DNA. This increases the amount of DNA made from the primers. This cycle of steps (i.e., heat treatment, lower temperature, allow DNA replication to occur) is repeated again and again and again. Because the cycles are repeated many times, this method is called a chain reaction. It is called a polymerase chain reaction because DNA polymerase is the enzyme needed to increase the amount of DNA with each cycle. In a PCR experiment, the template DNA is placed in a tube, and the primers, nucleotides, and DNA polymerase are added to the tube. The tube is then placed in a machine called a thermocycler, which raises and lowers the temperature. During one cycle, the temperature is raised (e.g., to 95°C) for a brief period and then lowered (e.g., to 60°C) to allow the primers to bind. The sample is then incubated at a slightly higher temperature for a few minutes to allow DNA replication to proceed. In a typical PCR experiment, the tube may be left in the thermocycler for 25 to 30 cycles. The total time for a PCR experiment is a few hours.

A. Why is DNA helicase not needed in a PCR experiment?

B. How is the sequence of each primer important in a PCR experiment? Do the two primers recognize the same strand or opposite strands?

C. The DNA polymerase used in PCR experiments is a DNA polymerase isolated from thermophilic bacteria. Why is this kind of polymerase used?

D. If a tube initially contained 10 copies of double-stranded DNA, how many copies of double-stranded DNA (in the region flanked by the two primers) would be obtained after 27 cycles?

Questions for Student Discussion/Collaboration

1. The complementarity of double-stranded DNA is the underlying reason that DNA can be faithfully copied. Propose alternative chemical structures that could be faithfully copied.
2. The technique described in Figure 11.19 makes it possible to measure DNA synthesis in vitro. Let's suppose you have purified the following proteins: DNA polymerase, DNA helicase, ligase, primase, single-strand binding protein, and topoisomerase. You also have the following reagents available:

   A. Radiolabeled nucleotides (labeled with ^{32}P, a radioisotope of phosphorus)

   B. Nonlabeled double-stranded DNA

   C. Nonlabeled single-stranded DNA

   D. An RNA primer that binds to one end of the nonlabeled single-stranded DNA

   With these reagents, how could you show that DNA helicase is necessary for strand separation and primase is necessary for the synthesis of an RNA primer? Note: In this question, think about conditions where DNA helicase or primase would be necessary to allow DNA replication and other conditions where they would be unnecessary.
3. DNA replication is fast, virtually error-free, and coordinated with cell division. Discuss which of these three features you think is the most important.

Note: All answers appear at the website for this textbook; the answers to even-numbered questions are in the back of the textbook.