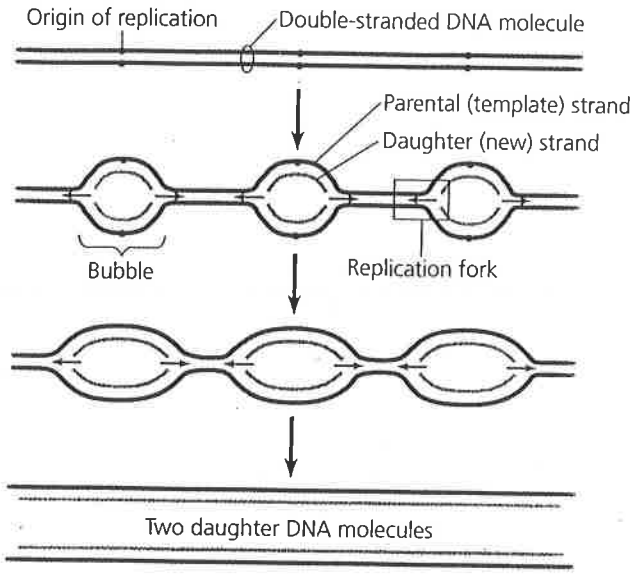
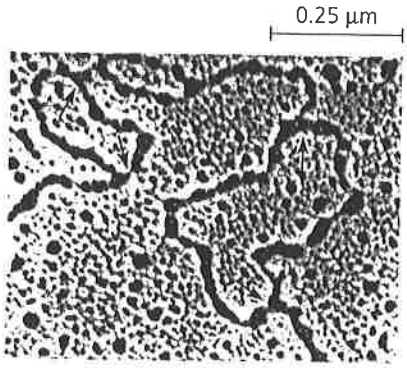
DNA Replication Reading Packet

Getting Started

The replication of a DNA molecule begins at special sites called **origins of replication**, short stretches of DNA having a specific sequence of nucleotides. The *E. coli* chromosome, like many other bacterial chromosomes, is circular and has a single origin. Proteins that initiate DNA replication recognize this sequence and attach to the DNA, separating the two strands and opening up a replication "bubble." Replication of DNA then proceeds in both directions until the entire molecule is copied **(Figure 16.12a)**. In





(b) In each linear chromosome of eukaryotes, DNA replication begins when replication bubbles form at many sites along the giant DNA molecule. The bubbles expand as replication proceeds in both directions. Eventually, the bubbles fuse and synthesis of the daughter strands is complete. The TEM shows three replication bubbles along the DNA of a cultured Chinese hamster cell.

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DRAW IT *In the TEM in (b), add arrows for the third bubble.*

DNA Replication T/F

Directions: Review the assigned DNA reading by indicating whether each DNA statement below is T/F and the textbook page where the answer can be found.

	DNA Replication Statements	After reading T/F	Textbook page
1	Okazaki fragments are joined together by the action of Ligase enzymes		
2	DNA Polymerase enzymes copy in the 5'→ 3' direction on the leading strand and the 3'→ 5" on the lagging strand.		
3	Okazaki fragments are only found on the lagging strands.		
4	DNA Polymerase enzymes in human cells can copy DNA at a rate of 500 nucleotides per second.		
5	RNA primers are only needed on the lagging strands		
6	Human Okazaki fragments are about 10-20 nucleotides long.		
7	Eukaryotic chromosomal DNA is copied relatively quickly because of the fusion of multiple replication bubbles.		
8	DNA Polymerase enzymes cannot build a new DNA strand without a primer containing a free 3' end.		
9	Due to its rapid rate of copying, DNA Polymerase enzymes make about 1 copying error for every 1000 bases copied.		
10	Telomeres are special segments in the middle of a chromosome that do not contain genes but consist of a repeating sequence of the letters CATCAT.		

1. What is a **telomere** and what is its purpose?

2. Explain what **PROBLEM** happens at the end of chromosomes each time the DNA is copied?

contrast to a bacterial chromosome, a eukaryotic chromosome may have hundreds or even a few thousand replication origins. Multiple replication bubbles form and eventually fuse, thus speeding up the copying of the very long DNA molecules **(Figure 16.12b)**. As in bacteria, eukaryotic DNA replication proceeds in both directions from each origin.

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

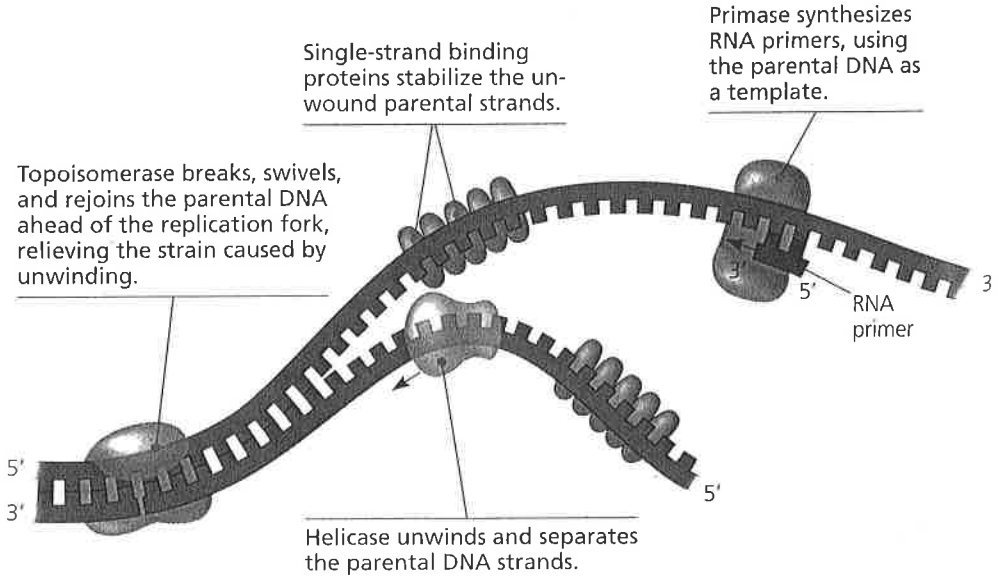
The unwound sections of parental DNA strands are now available to serve as templates for the synthesis of new complementary DNA strands. However, the enzymes that synthesize DNA cannot *initiate* the synthesis of a polynucleotide; they can only add nucleotides to the end of an already existing chain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

Synthesizing a New DNA Strand

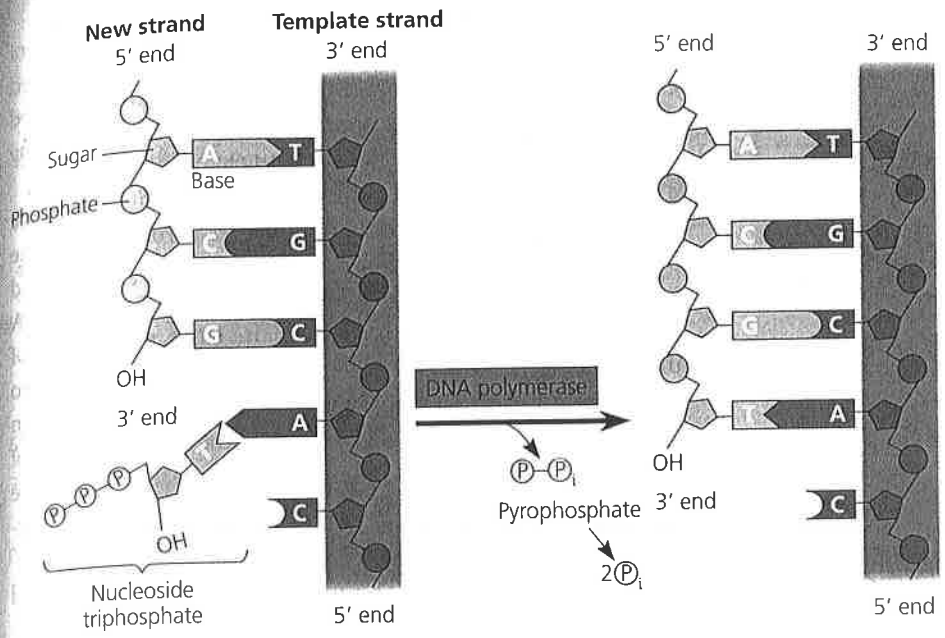
Enzymes called **DNA polymerases** catalyze the synthesis of new DNA by adding nucleotides to a preexisting chain. In *E. coli*, there are several different DNA polymerases, but two appear to play the major roles in DNA replication: DNA polymerase III and DNA polymerase I. The situation in eukaryotes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

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

Each nucleotide added to a growing DNA strand comes from a nucleoside triphosphate, which is a nucleoside (a sugar and a base) with three phosphate groups. You have already encountered such a molecule—ATP (adenosine triphosphate; see Figure 8.8). The only difference between the ATP of energy metabolism and dATP, the nucleoside triphosphate that supplies an adenine nucleotide to DNA, is the sugar component, which is deoxyribose in the building block of DNA, but ribose in ATP. Like ATP, the nucleoside triphosphates used for DNA synthesis are chemically reactive, partly because their triphosphate tails have an unstable cluster of negative charge. As each monomer joins the growing end of a DNA strand, two phosphate groups are lost as a molecule of pyrophosphate Ⓟ—Ⓟ_i. Subsequent hydrolysis of the pyrophosphate to two molecules of inorganic phosphate Ⓟ_i is a coupled exergonic reaction that helps drive the polymerization reaction **(Figure 16.14)**.



▶ Figure 16.13 Some of the proteins involved in the initiation of DNA replication. The same proteins function at both replication forks in a replication bubble. For simplicity, only one fork is shown.



◀ Figure 16.14 Incorporation of a nucleotide into a DNA strand. DNA polymerase catalyzes the addition of a nucleoside triphosphate to the 3′ end of a growing DNA strand, with the release of two phosphates.

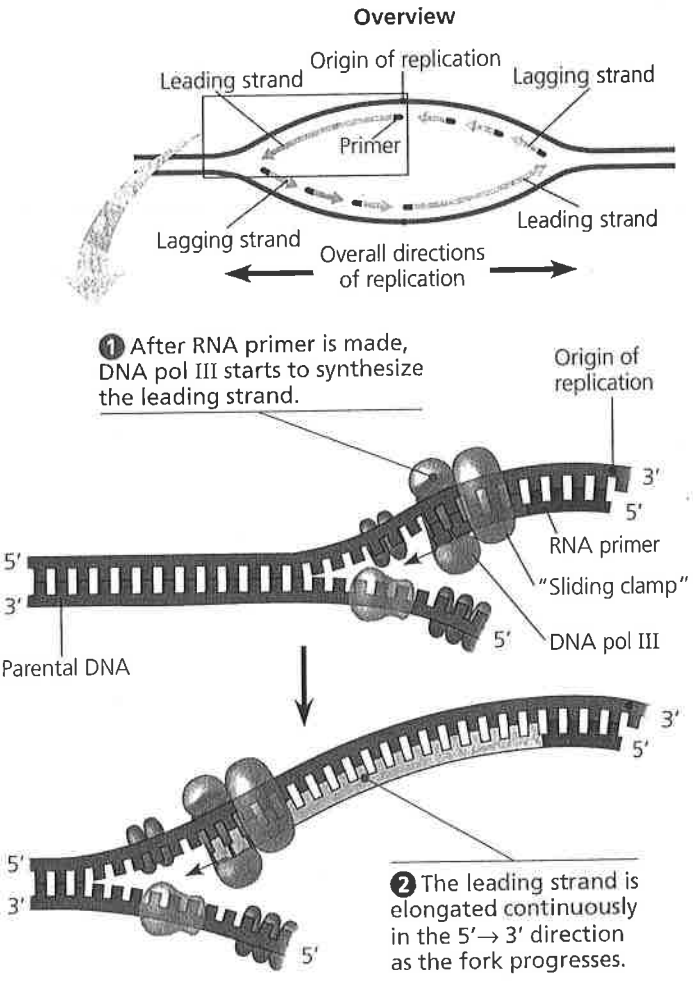
? *Use this diagram to explain what we mean when we say that each DNA strand has directionality.*

Antiparallel Elongation

As we have noted previously, the two ends of a DNA strand are different, giving each strand directionality, like a one-way street (see Figure 16.5). In addition, the two strands of DNA in a double helix are antiparallel, meaning that they are oriented in opposite directions to each other, like a divided highway (see Figure 16.14). Clearly, the two new strands formed during DNA replication must also be antiparallel to their template strands.

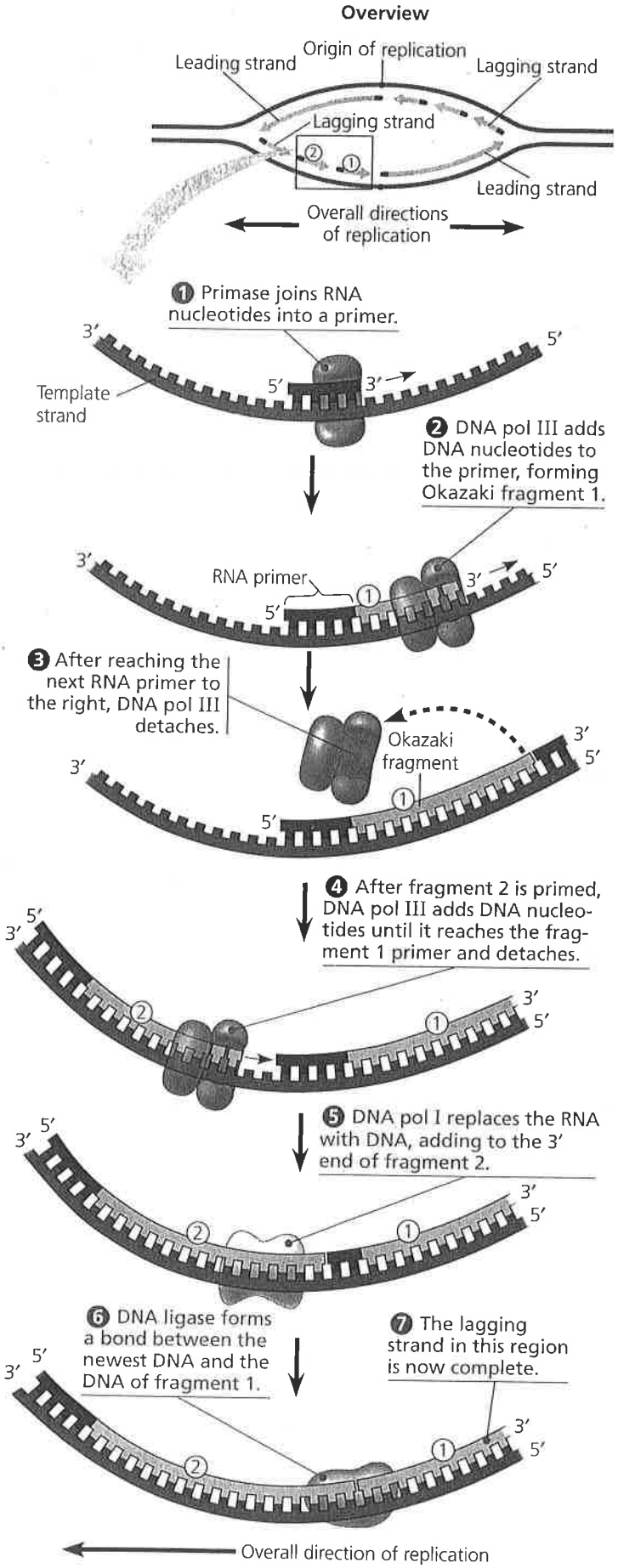
How does the antiparallel arrangement of the double helix affect replication? Because of their structure, DNA polymerases can add nucleotides only to the free 3′ end of a primer or growing DNA strand, never to the 5′ end (see Figure 16.14). Thus, a new DNA strand can elongate only in the 5′→3′ direction.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

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



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

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



▲ **Figure 16.16 Synthesis of the lagging strand.**

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

Figure 16.16 illustrates the steps in the synthesis of the lagging strand. Whereas only one primer is required on the leading strand, each Okazaki fragment on the lagging strand must be primed separately. Another DNA polymerase, DNA polymerase I (DNA pol I), replaces the RNA nucleotides of the primers with DNA versions, adding them one by one onto the 3′ end of the adjacent Okazaki fragment (fragment 2 in Figure 16.16). But DNA pol I cannot join the final nucleotide of this replacement DNA segment to the first DNA nucleotide of the Okazaki fragment whose primer was just replaced (fragment 1 in Figure 16.16). Another enzyme, **DNA ligase**, accomplishes this task, joining the sugar-phosphate backbones of all the Okazaki fragments into a continuous DNA strand.

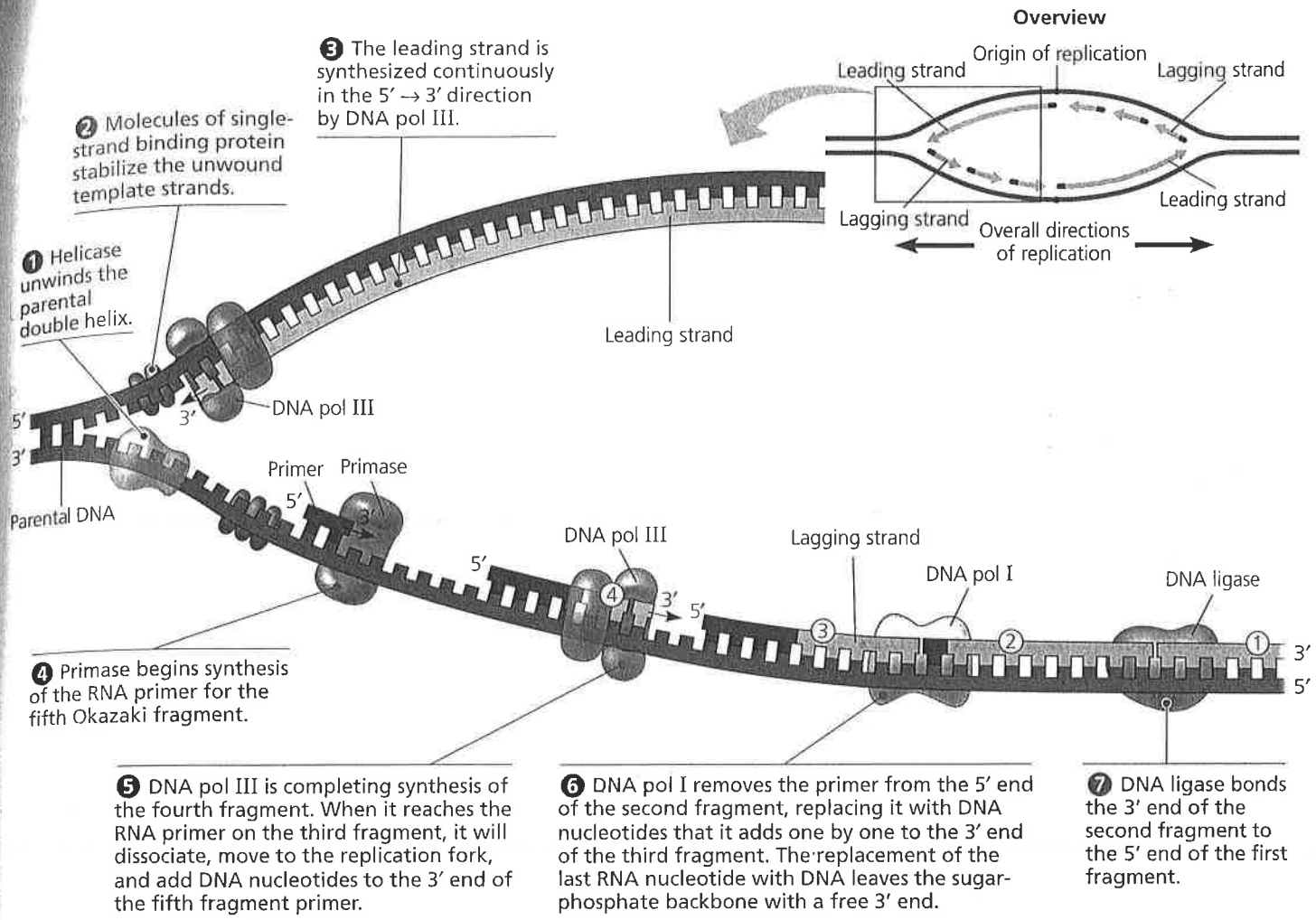
Figure 16.17 and **Table 16.1**, on the next page, summarize DNA replication. Study them carefully before proceeding.

The DNA Replication Complex

It is traditional—and convenient—to represent DNA polymerase molecules as locomotives moving along a DNA "railroad track," but such a model is inaccurate in two important ways. First, the various proteins that participate in DNA replication actually form a single large complex, a "DNA replication machine." Many protein-protein interactions facilitate the efficiency of this complex. For example, by interacting with other proteins at the fork, primase apparently acts as a molecular brake, slowing progress of the replication fork and coordinating the rate of replication on the leading and lagging strands. Second, the DNA replication complex does not move along the DNA; rather, the DNA moves through the complex during the replication process. In eukaryotic cells, multiple copies of the complex, perhaps grouped into "factories," may be anchored to the nuclear matrix, a framework of fibers extending through the interior of the nucleus. Recent studies support a model in which two DNA polymerase molecules, one on each template strand, "reel in" the parental DNA and extrude newly made daughter DNA molecules. Additional evidence suggests that the lagging strand is looped back through the complex, so that when a DNA polymerase completes synthesis of an Okazaki fragment and dissociates, it doesn't have far to travel to reach the primer for the next fragment, near the replication fork. This looping of the lagging strand enables more Okazaki fragments to be synthesized in less time.

Proofreading and Repairing DNA

We cannot attribute the accuracy of DNA replication solely to the specificity of base pairing. Although errors in the completed DNA molecule amount to only one in 10 billion nucleotides, initial pairing errors between incoming nucleotides and those in



▲ Figure 16.17 A summary of bacterial DNA replication. The detailed diagram shows one replication fork, but as indicated in the overview (upper right), replication usually occurs simultaneously at two forks, one at either end of a replication bubble. Viewing each daughter strand in its entirety in the overview, you can see that half of it is made continuously as the leading strand, while the other half (on the other side of the origin) is synthesized in fragments as the lagging strand.

the template strand are 100,000 times more common—an error rate of one in 100,000 nucleotides. During DNA replication, DNA polymerases proofread each nucleotide against its template as soon as it is added to the growing strand. Upon finding an incorrectly paired nucleotide, the polymerase removes the nucleotide and then resumes synthesis. (This action is similar to fixing a word processing error by using the "delete" key and then entering the correct letter.)

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

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

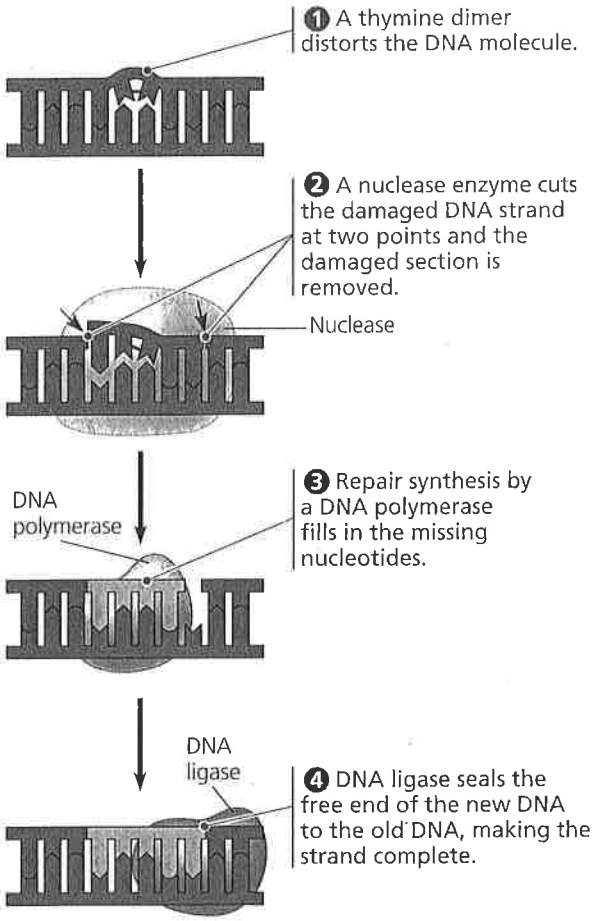
Table 16.1 Bacterial DNA Replication Proteins and Their Functions

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

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

Most cellular systems for repairing incorrectly paired nucleotides, whether they are due to DNA damage or to replication errors, use a mechanism that takes advantage of the base-paired structure of DNA. Often, a segment of the strand containing the damage is cut out (excised) by a DNA-cutting enzyme—a **nuclease**—and the resulting gap is then filled in with nucleotides, using the undamaged strand as a template. The enzymes involved in filling the gap are a DNA polymerase and DNA ligase. One such DNA repair system is called **nucleotide excision repair (Figure 16.18)**.

An important function of the DNA repair enzymes in our skin cells is to repair genetic damage caused by the ultraviolet rays of sunlight. One type of damage, shown in Figure 16.18, is the covalent linking of thymine bases that are adjacent on a DNA strand. Such *thymine dimers* cause the DNA to buckle and interfere with DNA replication. The importance of repairing this kind of damage is underscored by the disorder xeroderma pigmentosum, which in most cases is caused by an inherited defect in a nucleotide excision repair enzyme. Individuals with this disorder are hypersensitive to sunlight; mutations in their skin cells caused by ultraviolet light are left uncorrected and cause skin cancer.

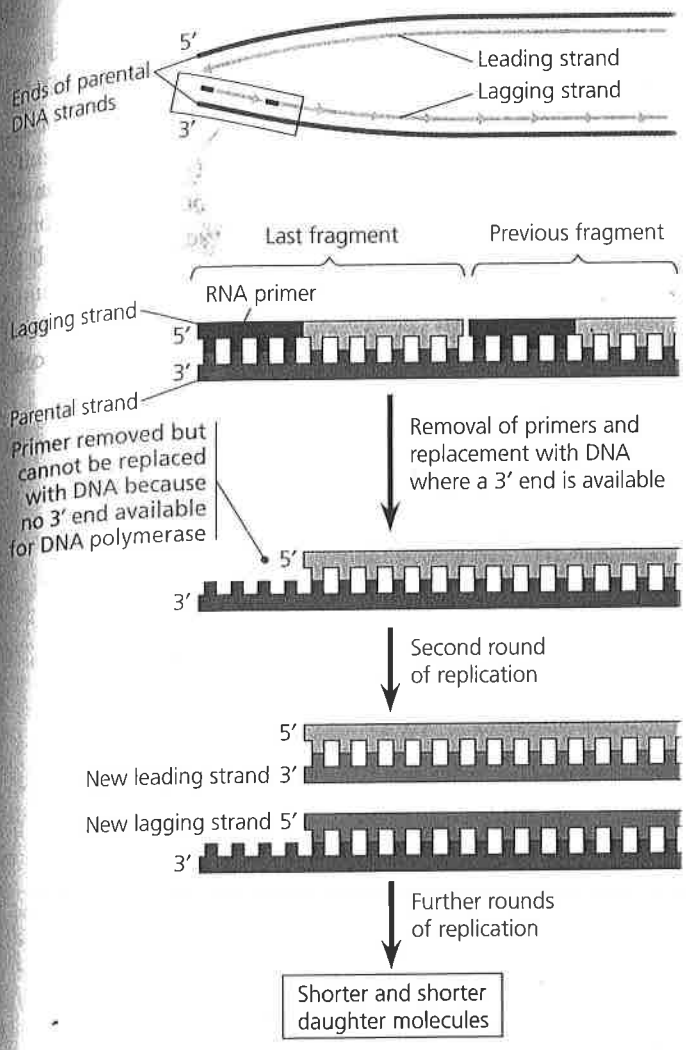


▲ **Figure 16.18 Nucleotide excision repair of DNA damage.** A team of enzymes detects and repairs damaged DNA. This figure shows DNA containing a thymine dimer, a type of damage often caused by ultraviolet radiation. A nuclease enzyme cuts out the damaged region of DNA, and a DNA polymerase (in bacteria, DNA pol I) replaces it with nucleotides complementary to the undamaged strand. DNA ligase completes the process by closing the remaining break in the sugar-phosphate backbone.

Replicating the Ends of DNA Molecules

In spite of the impressive capabilities of DNA polymerases, there is a small portion of the cell's DNA that DNA polymerases can neither replicate nor repair. For linear DNA, such as the DNA of eukaryotic chromosomes, the fact that a DNA polymerase can add nucleotides only to the 3′ end of a preexisting polynucleotide leads to an apparent problem. The usual replication machinery provides no way to complete the 5′ ends of daughter DNA strands. Even if an Okazaki fragment can be started with an RNA primer bound to the very end of the template strand, once that primer is removed, it cannot be replaced with DNA because there is no 3′ end available for nucleotide addition **(Figure 16.19)**. As a result, repeated rounds of replication produce shorter and shorter DNA molecules with uneven ("staggered") ends.

The shortening of DNA does not occur in most prokaryotes because their DNA is circular and therefore has no ends. But what protects the genes of eukaryotes from being eroded away during successive rounds of DNA replication? It turns out that eukaryotic chromosomal DNA molecules have special nucleotide sequences called **telomeres** at their ends **(Figure 16.20)**. Telomeres do not contain genes; instead, the DNA typically consists of multiple repetitions of one short nucleotide sequence. In each human telomere, for example, the six-nucleotide sequence TTAGGG is repeated between 100 and 1,000 times. Telomeric DNA protects the organism's genes. In addition, specific proteins associated with telomeric DNA prevent the staggered ends of the daughter molecule from activating the cell's systems for monitoring DNA damage. (Staggered ends of a DNA molecule, which often result from double-strand breaks, can trig-



▲ **Figure 16.19 Shortening of the ends of linear DNA molecules.** Here we follow the end of one strand of a DNA molecule through two rounds of replication. After the first round, the new lagging strand is shorter than its template. After a second round, both the leading and lagging strands have become shorter than the original parental DNA. Although not shown here, the other ends of these DNA molecules also become shorter.

ger signal transduction pathways leading to cell cycle arrest or cell death.)

Telomeres do not prevent the shortening of DNA molecules due to successive rounds of replication; they just postpone the erosion of genes near the ends of DNA molecules. As shown in Figure 16.19, telomeres become shorter during every round of replication. As we would expect, telomeric DNA does tend to be shorter in dividing somatic cells of older individuals and in cultured cells that have divided many times. It has been proposed that shortening of telomeres is somehow connected to the aging process of certain tissues and even to aging of the organism as a whole.

But what about the cells whose genomes must persist unchanged from an organism to its offspring over many generations? If the chromosomes of germ cells (which give rise to gametes) became shorter in every cell cycle, essential genes

▲ **Figure 16.20 Telomeres.** Eukaryotes have repetitive, noncoding sequences called telomeres at the ends of their DNA. Telomeres are stained orange in these mouse chromosomes (LM).

would eventually be missing from the gametes they produce. However, this does not occur: An enzyme called **telomerase** catalyzes the lengthening of telomeres in eukaryotic germ cells, thus restoring their original length and compensating for the shortening that occurs during DNA replication. Telomerase is not active in most human somatic cells, but its activity in germ cells results in telomeres of maximum length in the zygote.

Normal shortening of telomeres may protect organisms from cancer by limiting the number of divisions that somatic cells can undergo. Cells from large tumors often have unusually short telomeres, as one would expect for cells that have undergone many cell divisions. Further shortening would presumably lead to self-destruction of the tumor cells. Intriguingly, researchers have found telomerase activity in cancerous somatic cells, suggesting that its ability to stabilize telomere length may allow these cancer cells to persist. Many cancer cells do seem capable of unlimited cell division, as do immortal strains of cultured cells (see Chapter 12). If telomerase is indeed an important factor in many cancers, it may provide a useful target for both cancer diagnosis and chemotherapy.

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

CONCEPT CHECK 16.2

1. What role does complementary base pairing play in the replication of DNA?
2. Identify two major functions of DNA pol III in DNA replication.
3. **WHAT IF?** If the DNA pol I in a given cell were nonfunctional, how would that affect the synthesis of a *leading* strand? In the overview box in Figure 16.17, point out where DNA pol I would normally function on the top leading strand.

For suggested answers, see Appendix A.