6.4 DNA Replication and Repair

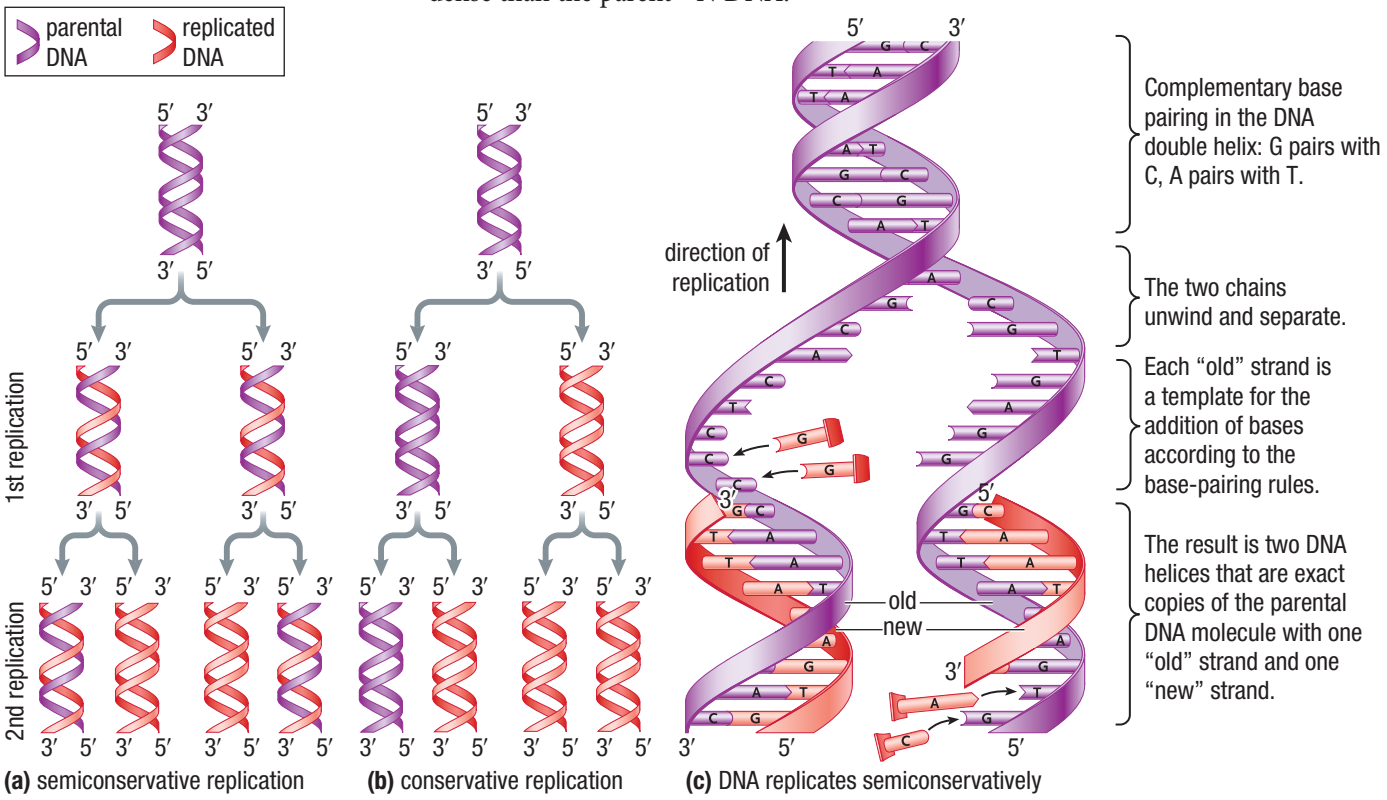
Watson and Crick's double helix model of DNA set the stage for more questions. Scientists knew that DNA replicates itself, but they did not know how. DNA molecules can be millions of nucleotides in length, and replication has to occur very quickly with few (if any) errors. Watson and Crick's new model had to account for this. Scientists now understood the shape, size, and chemical composition of DNA, but they did not know how such a complex and important molecule could replicate itself so well. A great deal of research was needed to understand DNA replication, but Watson and Crick's initial model of the structure of DNA gave scientists a place to start.

DNA Replication Is Semiconservative

semiconservative replication a mechanism of DNA replication in which each of the two strands of parent DNA is incorporated into a new double-stranded DNA molecule

Researchers proposed two potential mechanisms of DNA replication: conservative and semiconservative. **Semiconservative replication** would involve separating the two parent strands and building a new, complementary replacement strand for each. The new molecules would consist of one parent strand and one new strand (**Figure 1(a)**). Alternatively, conservative replication would involve copying the DNA molecule "as is," leaving the two original (parent) strands together (**Figure 1(b)**).

In 1958, Matthew Meselson and Franklin Stahl carried out an experiment that demonstrated that DNA replication is semiconservative (**Figure 1(c)**). Like Hershey and Chase, Meselson and Stahl used isotopes to label the parent DNA strands before replication. The isotope they used was "heavy" nitrogen, ^{15}N. *E. coli* bacteria were grown for 17 generations in a medium that contained ^{15}N, until all the cells had incorporated it completely into their DNA. Meselson and Stahl then transferred the bacteria to a medium that contained only normal, "light" nitrogen, ^{14}N, and allowed one or two rounds of replication (approximately 20 min each). Any new DNA produced should have lighter ^{14}N incorporated into its structure, thus making it less dense than the parent ^{15}N DNA.



(a) semiconservative replication **(b)** conservative replication **(c)** DNA replicates semiconservatively

Figure 1 (a) In semiconservative replication, the two parental strands would act as templates for replication and remain separated from each other, incorporated into two new molecules. (b) In conservative replication, the two parental strands would act as templates for replication, but then recombine afterwards. (c) DNA undergoes semiconservative replication.

To determine the density of the DNA, the DNA was isolated and placed in a centrifuge tube that contained a mixture that, when centrifuged, produces a density gradient from most dense at the bottom to least dense at the top. DNA migrates to a level in the tube with a density similar to its own. In this way, heavy parental ^{15}N DNA could be isolated from any new, less dense ^{14}N DNA.

After centrifuging, the original heavy ^{15}N DNA was in a single band (**Figure 2(a)**). After one round of replication, there was a single band of DNA at a density that was consistent with hybrid DNA—DNA containing equal parts of ^{15}N and ^{14}N (**Figure 2(b)**). If replication had been conservative, two bands would have been seen, one containing the parental ^{15}N DNA and one containing only new ^{14}N DNA. The single band of hybrid DNA, lying between these two positions in the gradient, confirmed that DNA replication was indeed semiconservative. Each single strand of parental DNA had been used as a template for a new complementary ^{14}N DNA strand to be built.

The results of centrifuging after two rounds of replication confirmed this conclusion. Two bands of DNA were detected: one that had a density consistent with hybrid DNA and one that corresponded to DNA built only with light ^{14}N nucleotides (**Figure 2(c)**). Each double strand of hybrid DNA had been separated and used to build complementary ^{14}N strands, producing one band of hybrid DNA and one with only ^{14}N. The clarity of the results of Meselson and Stahl's elegant and innovative experiment left little doubt. DNA replication is semiconservative.

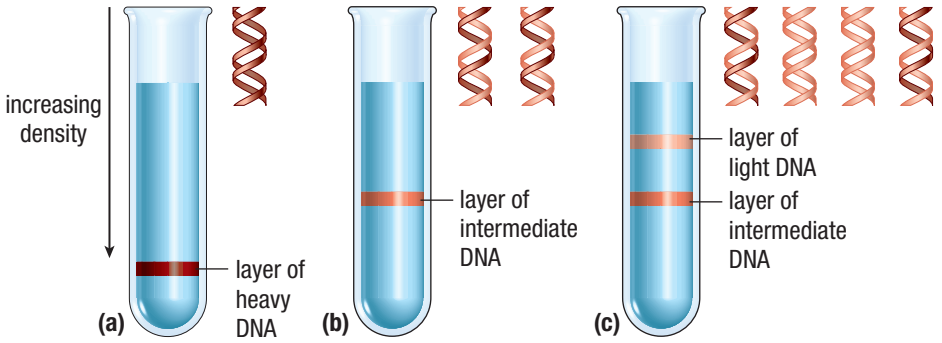


Figure 2 Results of Meselson and Stahl's experiment: (a) position of the original parent heavy ^{15}N DNA, (b) position of hybrid DNA produced after one round of DNA replication, and (c) positions of DNA formed after two rounds of replication

DNA Replication: The Process

Most of our understanding of the process of DNA replication comes from the study of the bacterium *E. coli*. Replication in eukaryotes is similar to replication in prokaryotes in most ways. However, the linear configuration of eukaryotic DNA and its sheer volume in a cell means that the process is more complex. DNA replication has three steps. First, the parental strands of DNA separate. Second, the complementary DNA strands are assembled. Third, the new strands are proofread and repaired.

Step 1: Strand Separation

To begin replication, the DNA strands must be unwound from each other (**Figure 3**, next page). Specific nucleotide sequences on the genome, called **replication origins**, act as starting points. Due to the length of DNA molecules in eukaryotes, there are many replication origins on a DNA strand. An enzyme called **helicase** binds to these origins and begins to unwind the two strands of DNA by breaking the hydrogen bonds between the complementary base pairs. As the two strands separate, they form a Y-shaped structure known as the **replication fork**.

This seemingly simple separation presents two challenges. First, if you have ever tried to separate the fibres in a rope or string by pulling them apart, you know that the tension

replication origin a specific sequence of DNA that acts as a starting point for replication

helicase a replication enzyme that separates and unwinds the DNA strands

replication fork the point of separation of the two parent DNA strands during replication

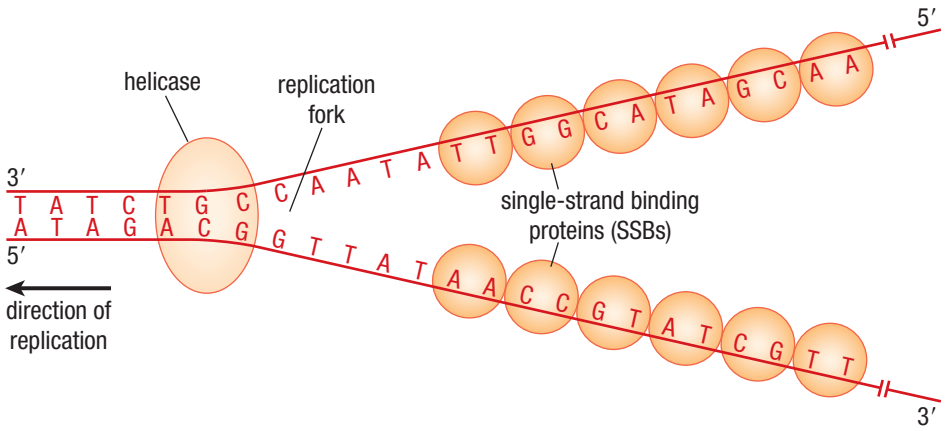


Figure 3 The double-stranded DNA is unwound by helicase. Single-strand proteins bind to the exposed bases to prevent them from annealing (reattaching).

topoisomerases a class of enzymes that relieve tension caused by the unwinding of parent DNA; they cleave one or two of the DNA strands, allow the strand(s) to untwist, and then rejoin the strand(s)

single-strand binding protein (SSB) a replication enzyme that prevents parent DNA strands from annealing to each other once they have been separated by helicase

replication bubble the separating of DNA in both directions during replication

created can lead to twisting and tangles. A class of enzymes called **topoisomerases** relieves this tension in DNA strands by cutting one or two of the strands near the replication fork, allowing the strands to untangle, and then rejoining the cut strand(s). Second, after the two strands are separated, they have a tendency to rejoin, or anneal, because they are complementary. **Single-strand binding proteins (SSBs)** prevent annealing by attaching to the DNA strands to stabilize them and keep them separated (Figure 3). These proteins are removed during the next phase of replication but are used again when needed.

Helicase complexes can separate DNA strands in both directions from a replication origin. Soon after separation, new complementary nucleotides are added to the naked strands. As the two replication forks proceed in opposite directions, the space between them that is filled with newly replicated DNA is called a **replication bubble** (**Figure 4**). Due to the great length of eukaryotic DNA, there are many replication bubbles along a DNA strand. Each bubble expands along the length of the molecule until it meets and merges with another bubble, eventually producing two separate daughter strands.

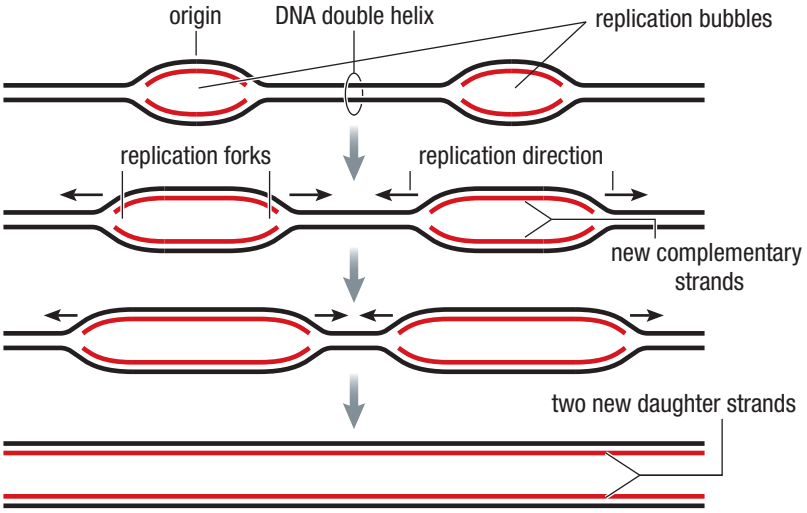


Figure 4 Replication bubbles form, grow, and merge.

If it were not for multiple replication origins, each producing a replication bubble, replicating the genome of a typical human would take an impractical amount of time. In eukaryotes, new DNA is made at the rate of about 50 base pairs per second at each replication fork. At this rate, if the replication process depended on only one replication origin, the entire genome of a human would be reproduced in about a month. Multiple replication origins decrease the time to about 1 h.

Step 2: Building Complementary Strands

During replication, new nucleotides are joined by a group of enzymes called DNA polymerases. Although both prokaryotes and eukaryotes have DNA polymerases, the following description will refer to the polymerases that occur in prokaryotes. Eukaryotes have several more specialized DNA polymerases, and the system is quite complex.

Prokaryotic DNA polymerases add nucleotides to the 3′ end of a new developing strand while moving along and "reading" the template strand in its 3′ to 5′ direction (**Figure 5(a)**). This is the important feature of DNA polymerases: they can only add nucleotides to the 3′ end of an existing DNA (or RNA) strand. Therefore, the new strand is always assembled in the 5′ to 3′ direction. You will learn more about this later.

DNA polymerase builds the new strand of DNA using **nucleoside triphosphates (Figure 5(b))**. These molecules are very similar to the nucleotides in the finished DNA. They contain a deoxyribose sugar bonded to one of the four nitrogenous bases (adenine, thymine, cytosine, or guanine), as well as a group of three phosphate molecules. Figure 5 shows how the nucleoside triphosphates are joined to the growing DNA strand.

nucleoside triphosphate a building block and energy source for replicating DNA

For a new strand to be built, energy is needed to power the reactions carried out by the DNA polymerase (**Figure 5(c)**). This energy is provided by the phosphate groups attached to the nucleoside molecule. Energy is released by the hydrolysis reaction that cleaves two phosphates from the nucleoside triphosphate and by the formation of the phosphodiester bond between the one remaining phosphate and the hydroxyl group on the 3′ carbon at the 3′ end of the growing DNA strand. The two released phosphates are hydrolysed to two inorganic phosphates (P_i). The energy released is also used to drive DNA synthesis.

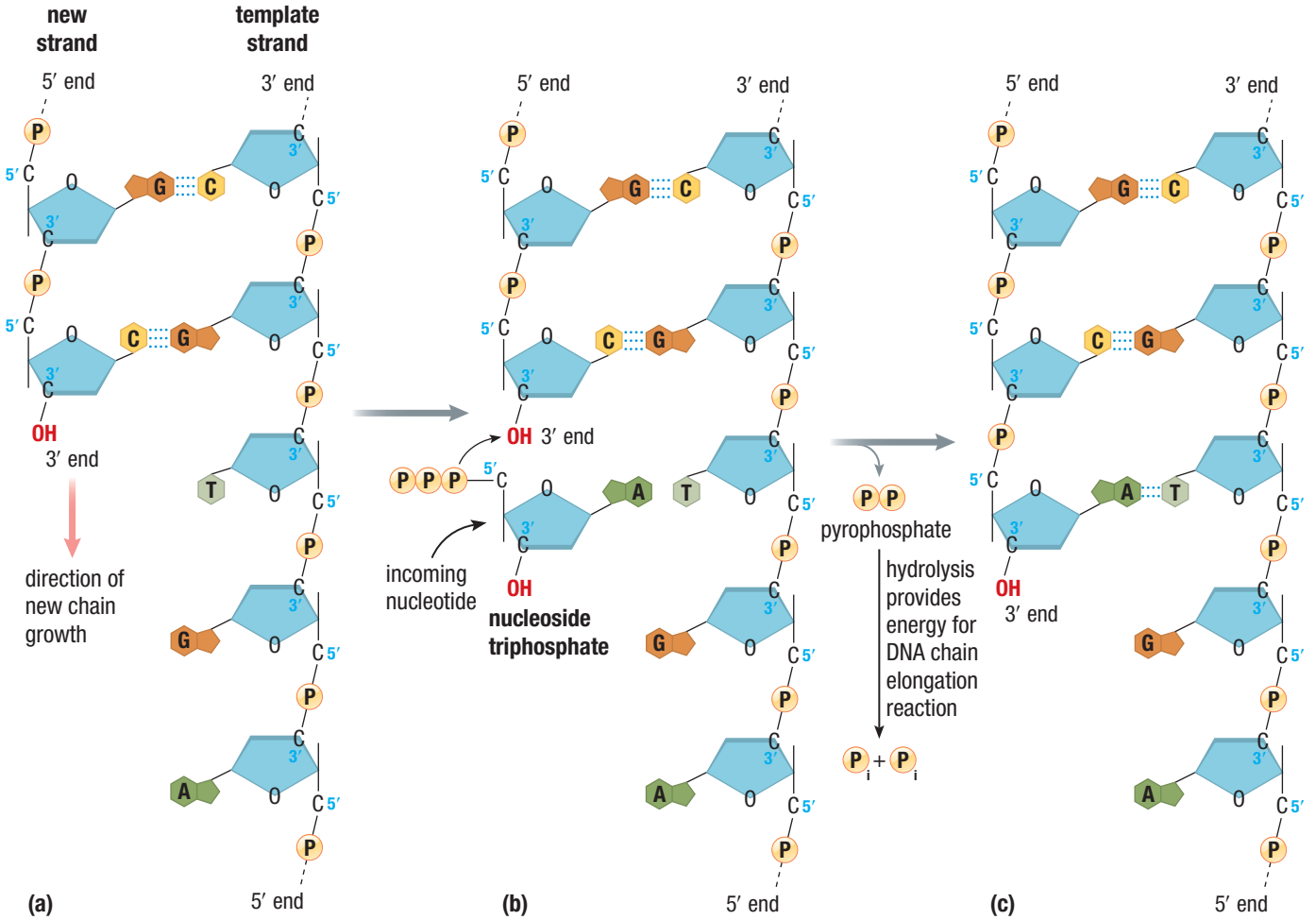


Figure 5 New nucleotides are added to the 3′ end of the new strand. (a) The template strand is read as two nucleotides are assembled on the new strand. (b) A nucleoside triphosphate with an A base forms a complementary base pair with the next nucleotide of the template strand. (c) A phosphodiester bond forms, linking the newly added nucleotide to the end of the new strand.

RNA primase a replication enzyme that produces RNA primers

RNA primer a replication molecule that acts as a starting point for replication

DNA polymerase III a prokaryotic replication enzyme that builds new DNA strands from nucleotides

When a replication fork first opens, there is nothing for DNA polymerase to "add to." Recall that DNA polymerase can only assemble a strand by adding nucleotides to the 3′ end of an existing strand. To overcome this problem, **RNA primase** enzymes begin the replication process by building a small complementary RNA segment on the strand at the beginning of the replication fork (**Figure 6(a)**, next page). These short, 10 to 60 ribonucleotide pieces of RNA are called **RNA primers**. As the replication fork continues to open and separate the parent strands, one strand is oriented in the 3′ to 5′ direction while the other is oriented in the opposite 5′ to 3′ direction. Once an RNA primer is in place, the enzyme **DNA polymerase III** begins adding DNA nucleotides to the RNA primer. Since DNA polymerase III only builds the new strand in the 5′ to 3′ direction, the two new strands begin to be assembled in opposite directions (**Figure 6(b)**, next page). The result is that, as the replication fork continues to open, the DNA polymerase III that is building a strand toward the fork is able to keep moving forward continuously. There is no need, nor opportunity, for RNA primase to add any additional primers to the strand. This strand is called the **leading strand**.

leading strand the DNA strand that is copied in the direction toward the replication fork

lagging strand the DNA strand that is copied in the direction away from the replication fork

Okazaki fragment the piece of new DNA on the lagging strand

On the opposite strand, however, DNA polymerase III is moving away from the opening replication fork. This strand is the **lagging strand**. When enough fork has opened, RNA primase attaches another primer to the parent strand, allowing another DNA polymerase III to begin from the new starting point. The pattern created on the second strand is a series of RNA primers and short DNA fragments called **Okazaki fragments**. Okazaki fragments are thought to be between 1000 and 2000 bases long in prokaryotes and much shorter (between 100 and 200 nucleotides long) in eukaryotes. Because the second strand is not made in one continual process, it is called the lagging strand (**Figure 6(c)**, next page).

DNA polymerase I a prokaryotic replication enzyme that fills in gaps in the lagging strand between Okazaki fragments; also proofreads the final strands

DNA ligase an enzyme that catalyzes the formation of a phosphodiester bond between two DNA strands, as well as between Okazaki fragments

As each fragment extends in the 5′ to 3′ direction, it eventually runs into the RNA primer attached to the Okazaki fragment ahead of it. Another polymerase, **DNA polymerase I**, removes the RNA nucleotides one at a time and replaces them with DNA nucleotides. Once the primer is replaced, the last nucleotide is linked to the Okazaki fragment in front of it by the formation of a phosphodiester bond. An enzyme called **DNA ligase** catalyzes this reaction (**Figure 6(d)**, next page). Eventually, all the RNA primers are removed and replaced by DNA (**Figure 6(e)**, next page).

Step 3: Dealing with Errors during DNA Replication

The DNA polymerase enzymes that carry out replication play another important role. As they assemble the new DNA strands, they proofread and correct errors. These errors are usually base-pair mismatches. For example, if a thymine is added across from a cytosine, they cannot form hydrogen bonds and the strand is unstable. DNA polymerase III cannot move forward if base pairs are mismatched (**Figure 7(a)**, page 288). It usually backs up, replaces the incorrect base with the correct one, and then continues on.

After a strand has been replicated, rare mismatching errors may still be present—usually an average of only one error for every million base pairs. However, even this low number of errors could have serious implications for an organism. Special DNA repair complexes read the strands for errors that might have been missed by DNA polymerase III. These repair complexes are composed of proteins and enzymes, including DNA polymerase I and **DNA polymerase II**. DNA polymerase II is a slow enzyme and often works to repair damage to DNA that occurs between replication events. Since mismatched base pairs cannot bond correctly, they distort the shape of the DNA. The DNA repair complexes move along the strand, locating these distortions. Once a distortion is found, a repair complex determines which of the two helices contains the incorrect base. The repair complex can even recognize the original (correct) strand! An enzyme removes a portion of the strand around the mismatch. The resultant gap is filled in by a DNA polymerase and completed with DNA ligase (**Figure 7(b)**, page 288).

DNA polymerase II a prokaryotic replication enzyme that repairs damage to DNA, including damage that occurs between replication events

Similar repair mechanisms help to correct damage caused by chemicals and radiation, including ultraviolet light. Without these repair mechanisms, DNA would accumulate many errors. The errors could lead to the loss or change of function, or even cancer.

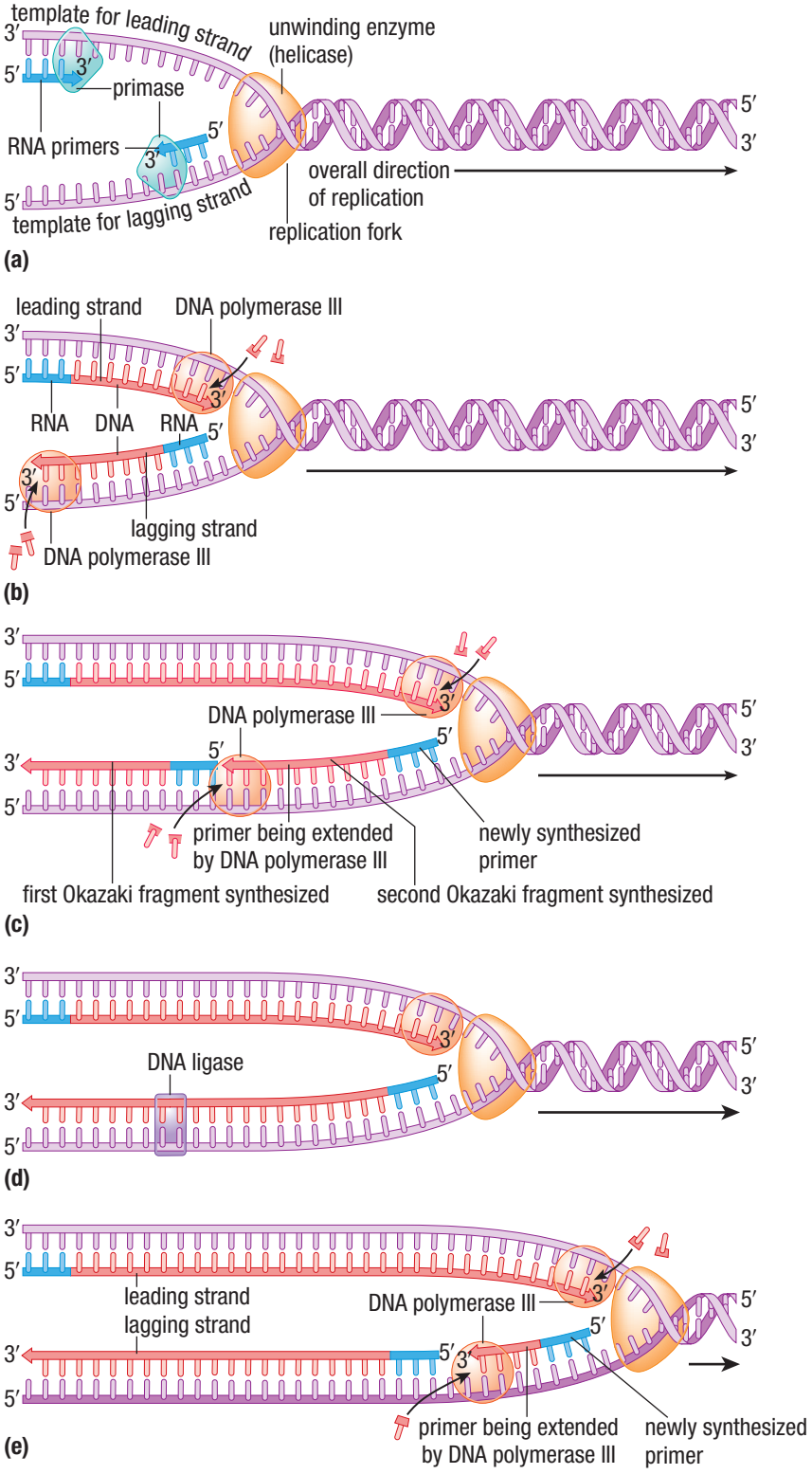


Figure 6 (a) As the replication fork opens, RNA primase attaches RNA primers to both the leading and lagging strands. These primers are oriented in opposite directions. (b) A DNA polymerase III enzyme attaches to the 3′ end of each primer and begins assembling the new DNA strands in opposite (5′ to 3′) directions. (c) As the replication fork continues to open, the new leading strand continues to be assembled in one continuous process. On the lagging strand, a new RNA primer is attached and DNA polymerase III begins assembling a new DNA fragment. (d) The resulting DNA fragments are then joined by DNA ligase. (e) The entire process results in the formation of two new daughter DNA molecules: one consisting of a parent strand and the new leading strand, and the other formed by the other parent strand and the new lagging strand.

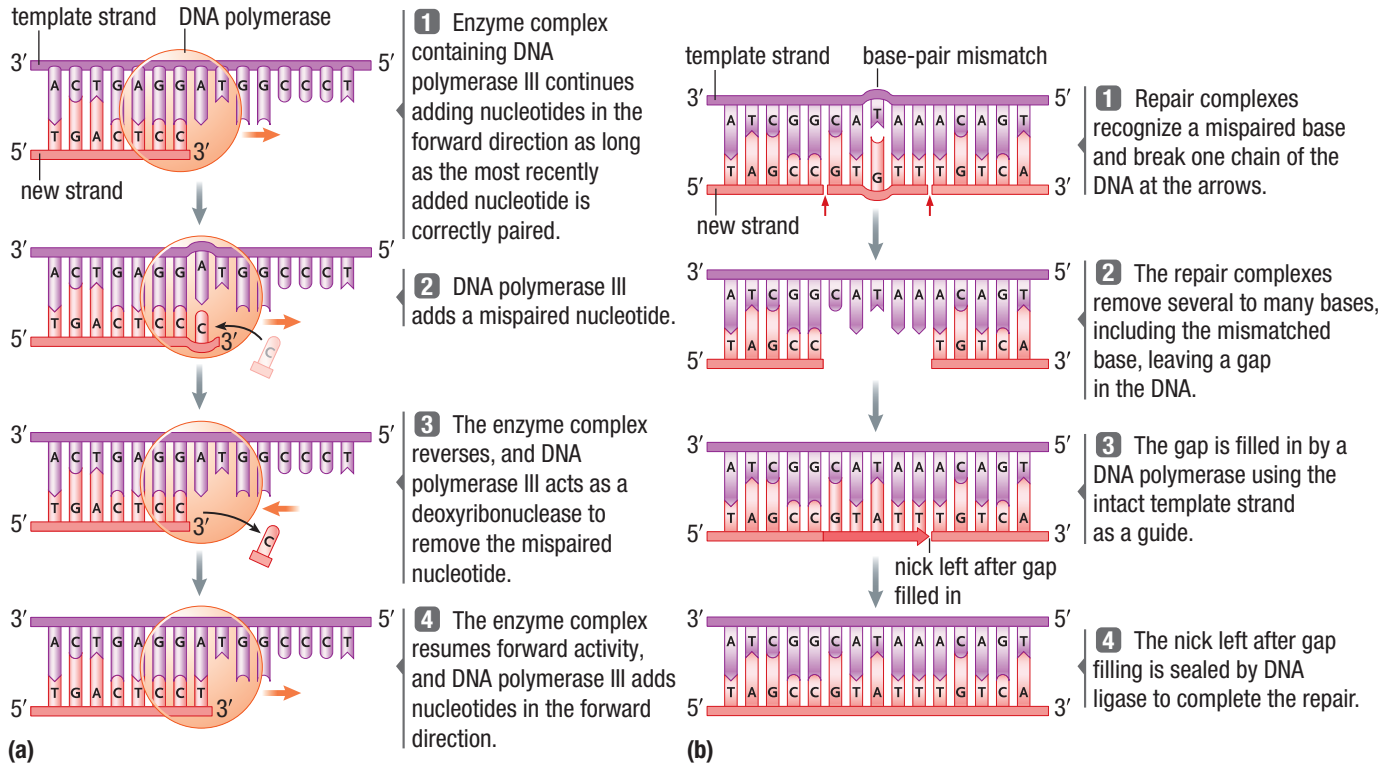


Figure 7 Repair mechanisms fix damage and base-pair mismatches. (a) DNA polymerase enzymes proofread the new DNA strands. (b) Mismatched bases in replicated DNA are repaired.

As you have seen, the process of DNA replication requires many enzymes and other molecules. **Table 1** summarizes their functions. WEB LINK

Table 1 Enzymes and Other Molecules Involved in DNA Replication

Enzyme/Molecule	Function
helicase	unwinds DNA helix
single-strand binding protein (SSB)	stops the two separated parent strands from annealing
topoisomerases	cleave and then reattach one or two of the DNA strands to relieve tension created by the unwinding process
RNA primase	places RNA primers on template strands
RNA primers	act as starting strands for DNA polymerase
DNA polymerases	several closely related enzymes that assemble nucleotides into new DNA strands; remove RNA primer nucleotides and replace them with DNA nucleotides; proofread and repair replication errors and other damage to DNA molecules
DNA ligase	forms the phosphodiester bond that joins the ends of DNA that make up the Okazaki fragments

DNA Replication in Prokaryotes and Eukaryotes

As mentioned at the beginning of this section, most of our knowledge of DNA replication has come from the study of bacteria. Research into DNA replication in eukaryotes has confirmed that the process is essentially the same. However, due to differences in genomic content and structure, there are some differences in the process in eukaryotes. For example, the genomes of prokaryotes are much smaller than the genomes of eukaryotes and are circular in structure. Usually only one replication origin and only one replication bubble occur. Eukaryotic genomes are very long and linear, necessitating thousands of origins and as many replication bubbles. The ends of linear DNA strands also present a replication dilemma, which you will learn about in Sections 6.5 and 6.6.

Another difference occurs in the enzymes that are involved in DNA replication and repair. The polymerases identified in this section are found in bacteria. The DNA polymerases that are found in eukaryotes are more varied, but they function in the same way.

As new research reveals the intricacies of DNA replication in prokaryotes and eukaryotes, new possibilities become increasingly apparent. Understanding DNA replication could have significant implications in the control of growth, aging, cloning, tissue regeneration, cancer, and biotechnology in general. Scientists did not even know what DNA looked like 60 years ago. Now, every question that is answered brings new questions to the forefront.

Investigation 6.4.1

Role-Playing DNA Replication (p. 301)
In this activity, you will demonstrate your knowledge and understanding of DNA replication by designing a role-play with some of your classmates.

Mini Investigation

Constructing a Model of DNA Replication

Skills: Performing, Analyzing, Evaluating, Communicating

SKILLS HANDBOOK A2.1

The structure of DNA ensures that replication occurs with a minimal number of errors. In this investigation, you will model DNA replication, and then compare your model to what you know about the process. For simplicity, you will ignore the need for RNA primers during the process.

Equipment and Materials: ruler; scissors; coloured paper (white, red, blue, brown, green, and black); transparent tape

1. Work in a group. Divide the task of cutting out pieces of paper among the group members (**Table 2**). Write the capital letter for the name of each part of the DNA model on the correct piece of paper.

Table 2 Coloured Pieces of Paper

Part of DNA model	Colour	Size (cm x cm)	Number needed
sugar	white	2 x 2	72
phosphate	red	1 x 2	72
adenine	blue	1 x 2	16
thymine	brown	1 x 1	16
guanine	green	1 x 2	20
cytosine	black	1 x 1	20

2. Construct a nucleotide of thymine by taping together a sugar, a phosphate group, and a thymine, using the correct configuration as shown in Figure 4, page 276 (Section 6.2).
3. Assemble other nucleotides using the other three bases.
4. Assemble one strand of DNA with a sequence of 5′-TCCGTAGAACTGTGATTA-3′. Label the 5′ and 3′ ends.
5. Assemble the complementary strand of DNA. Be sure that it is antiparallel to the first side. Label the 5′ and 3′ ends.
6. Label the back of each strand as "original parent strand." Do not tape the strands together.
7. Lay the two strands so that the base pairs line up according to base pairing rules. Gently slide them apart at one end. The other end should still be touching. This action models the formation of the replication fork.
8. DNA polymerase reads an original strand from 3′ to 5′ and adds complementary nucleotides in the 5′ to 3′ direction. Simulate this by adding complementary nucleotides one at a time. Follow the base-pairing rules to make sure that you add the correct nucleotide. You should be adding nucleotides and building the new strand toward the replication fork. This represents the leading strand.
9. At any point on the other original strand, begin adding nucleotides. Be sure to add them in the correct direction (5′ to 3′), away from your replication fork. The section you have built represents an Okazaki fragment. As you continue to open the replication fork, make another Okazaki fragment toward the first. When this fragment meets the end of the first fragment, join the two fragments. The strand you are building represents the lagging strand.
10. Once the original strands have been totally separated, and complementary nucleotides have been added to each strand, you should have two "ladders." Each ladder should have one old strand and one new strand.
11. To model a DNA molecule, tape each template strand to its newly synthesized complementary strand.

A. What type of bonding does the tape in Step 11 represent? T/I

B. Why is DNA said to replicate semiconservatively? Explain by referring to your model. K/U C

C. Your two new strands were not replicated in the same direction. Why? K/U T/I

D. Go online and watch a few DNA replication simulations and animations. From your knowledge of DNA replication, comment on the accuracy of each simulation or animation. Which would you recommend to your classmates? T/I

WEB LINK

6.4 Review

Summary

- DNA is copied by semiconservative replication. Each strand of the original DNA is incorporated into one of the new copies.
- When DNA strands separate, topoisomerases help relieve tension caused by unwinding, and single-strand binding proteins (SSBs) prevent strands from rejoining.
- Replication occurs in the general direction toward the replication fork, and always in a 5′ to 3′ direction on the newly synthesized strand. This results in a leading (continuously replicated) strand and a lagging strand. The lagging strand consists of Okazaki fragments.
- All replication begins with the formation of RNA primers on the parent DNA strands. DNA polymerases begin the synthesis of new DNA strands on the 3′ ends of the RNA primers. The primers are later replaced with DNA by DNA polymerase I.
- Base-pair mismatching can occur during replication. DNA polymerase and other repair enzymes proofread the strands for errors and correct mismatched bases.
- DNA replication in prokaryotes and eukaryotes differs.

Questions

1. (a) Without looking at the text, try to list and summarize the three steps that are involved in replication.
   (b) Check what you wrote. What did you miss? Did you miss anything that is crucial to the replication process? K/U
2. Place the following enzymes in the order in which they are used in DNA replication: topoisomerase, DNA ligase, DNA polymerase III, RNA primase, helicase, DNA polymerase I. K/U
3. What would happen if each of the following were not available for DNA replication? How would this affect the process? K/U T/I
   (a) topoisomerase
   (b) DNA ligase
   (c) single-strand binding protein
4. Explain why the process of DNA replication is slower on the lagging strand than on the leading strand. K/U T/I
5. Is DNA an example of a polymer? Explain. K/U
6. Why can both template strands not be replicated continuously? K/U
7. Helicase separates and unwinds the DNA strands from each other. Why is an enzyme not required to bring the strands back together (re-anneal them)? T/I
8. Upon examining an organism, you discover that it produces DNA polymerase III but does not produce DNA polymerase I. Describe two possible scenarios that could be occurring in this organism during DNA replication. Include the assumptions that you must make. K/U T/I
9. During your examination of an alien life form, you notice the DNA replication proceeds in both directions. What questions about the DNA replication would you investigate? Why? K/U T/I A
10. When Watson and Crick first proposed their double helix model of the DNA molecule, they suggested a semiconservative mode of DNA replication. Explain why their model justifies the semiconservative mode of reproduction. K/U T/I
11. Without DNA repair, an organism is likely to suffer from significant problems. Xeroderma pigmentosum is a serious human disorder that is caused by mutations in the gene for DNA repair. Do online research to determine the symptoms that are associated with this disorder. What type of DNA damage are individuals with this disorder most susceptible to? T/I

WEB LINK