15

DNA, RNA, and DNA Replication

Learning Objectives

In this chapter, you will learn:

→ Structure of DNA and RNA
→ DNA Replication

Overview

Nucleic acids, DNA and RNA, are the carriers of genetic information in living organisms. This genetic information is transferred from one generation to the next generation through these nucleic acids. While prokaryotes and eukaryotes typically use DNA as the carrier of genetic information between generations, some viruses use RNA as their primary genetic material. Prokaryotes package their DNA into circular chromosomes in a region called the nucleoid. Eukaryotes package their DNA into linear chromosomes in the nucleus. Both prokaryotes and eukaryotes can carry additional genetic information in the form of small, circular pieces of DNA called plasmids, which are referred to as extranuclear DNA.

DNA and RNA have some structural similarities and structural differences, which are important to their respective functions. Both will be discussed within this chapter followed by a review of DNA replication.

Structure of DNA and RNA

DNA and RNA are composed of nucleotides. Each nucleotide has three parts: a nitrogenous base, a five-carbon sugar, and a phosphate group. The nitrogenous bases in DNA are adenine, guanine, cytosine, and thymine. RNA contains the nitrogenous bases adenine, guanine, cytosine, and uracil. These nitrogenous bases are classified into two groups, as shown in Figure 15.1. Adenine and guanine are purines and consist of a two-ringed structure. Cytosine, thymine, and uracil are pyrimidines and have a one-ringed structure.

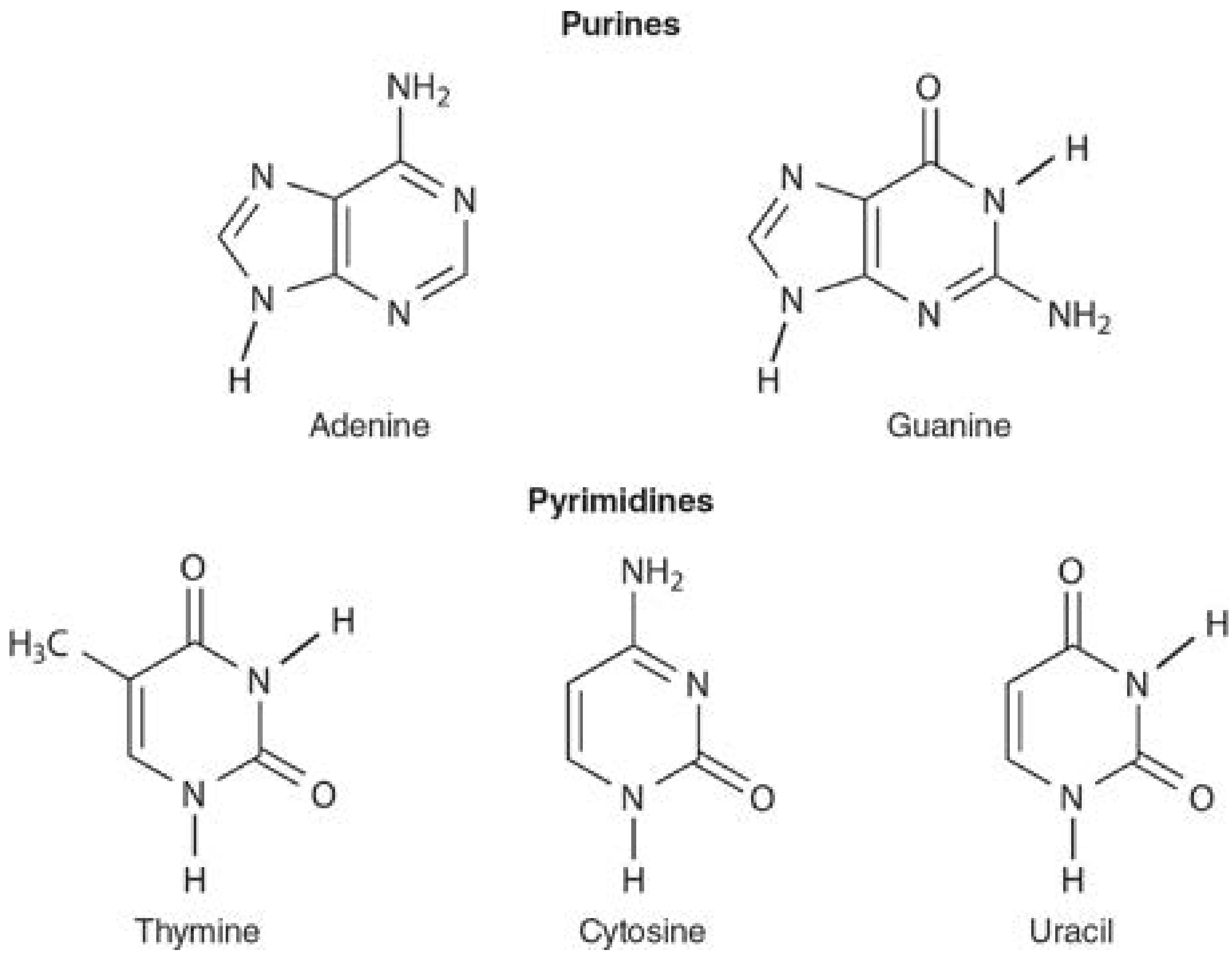


Figure 15.1 Nitrogenous Bases in DNA and RNA

DNA and RNA have consistent base-pairing rules that are seen in all living organisms. In DNA, adenine (A) pairs with thymine (T), and guanine (G) pairs with cytosine (C). RNA base-pairing rules are similar in that guanine pairs with cytosine but different in that adenine (A) pairs with uracil (U). When adenine pairs with another base, either thymine or uracil, it forms two hydrogen bonds. Guanine and cytosine pairs form three hydrogen bonds between them.

The five-carbon sugar also differs slightly in DNA and RNA. DNA contains the five-carbon sugar deoxyribose, while RNA has the five-carbon sugar ribose. An important difference between these sugars is at the 2′ carbon. Deoxyribose has a hydrogen atom attached to the 2′ carbon, while ribose has a hydroxyl (–OH) group attached to the 2′ carbon, as shown in Figure 15.2.

TIP

An easy way to remember which nitrogenous bases are purines and which are pyrimidines are the mnemonic devices "PureAsGold" for Purines are Adenine and Guanine, and "CUtThePy" for Cytosine, Uracil, and Thymine are Pyrimidines.

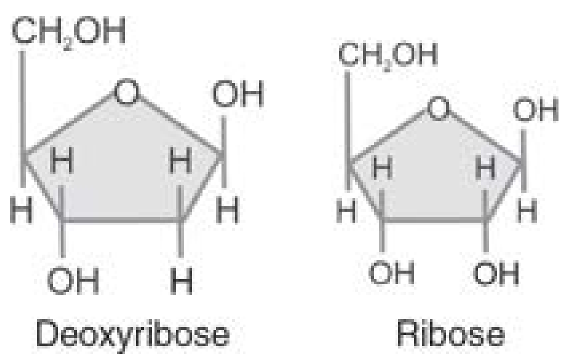


Figure 15.2 Deoxyribose and Ribose

This small difference makes DNA much more stable than RNA. This increased stability of DNA may explain why it is the carrier of genetic information between generations, while RNA usually has more temporary functions. For example, in transcription, mRNA carries the genetic information from the nucleus to the ribosome.

DNA is a double helix in which the two strands are antiparallel. One strand of the DNA is oriented with the 5′ phosphate group at the start of the strand, while the opposite strand has the 3′ hydroxyl (–OH) group at the start of the strand. A purine on one of the strands is always paired with a pyrimidine on the opposite strand. Since purines have a double-ringed structure and pyrimidines have a single-ringed structure, this keeps the width of the double helix consistent.

RNA is typically single-stranded (in mRNAs and microRNAs) but can fold to form three-dimensional structures in rRNAs in the ribosome and in tRNAs.

DNA Replication

The purpose of DNA replication is to ensure the continuity of genetic information between generations. Each of the original two strands in the double helix serves as a template for a new strand, as shown in Figure 15.3. Since each new double helix is composed of one strand from the original piece of DNA and one newly synthesized strand, DNA replication is described as being semiconservative.

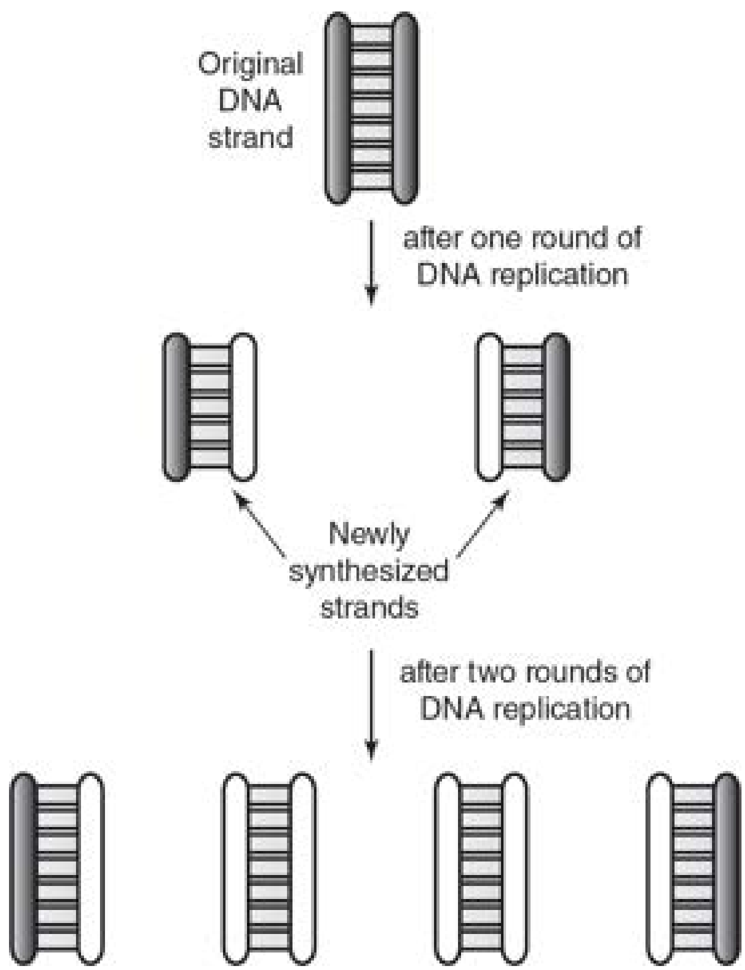


Figure 15.3 DNA Replication Is Semiconservative

To start DNA replication, the enzyme helicase first unwinds the two DNA strands in an area called the origin of replication, or "ori" site. As part of the double helix is unwound, other sections of the double helix become more tightly wound, and this results in supercoiling in those areas. Topoisomerase enzymes make temporary nicks in the sugar-phosphate backbone of DNA to relieve this supercoiling and then reseal these nicks. The enzyme RNA polymerase then synthesizes an RNA primer using a few complementary RNA nucleotides. New DNA nucleotides can then be added to this RNA primer. DNA polymerase is the enzyme that adds new nucleotides to the 3′ hydroxyl group at the end of this RNA primer. DNA polymerase adds new nucleotides in the 5′ to 3′ direction, always connecting the 5′ phosphate on the new nucleotide to the 3′ hydroxyl on the growing nucleotide strand.

TIP

When you think of DNA polymerase adding new nucleotides in the 5′ to 3′ direction, think of adding new cars to a toy train. New cars can only be added to the back end of the train, not to the engine, just like new nucleotides can only be added to the 3′ hydroxyl, not the 5′ phosphate.

As discussed previously, DNA molecules have directionality; the two strands of the DNA double helix are antiparallel (oriented in opposite directions). Because DNA polymerase can only add new nucleotides in the 5′ to 3′ direction, and because the two strands of DNA are antiparallel, DNA must proceed slightly differently on the two strands, as shown in Figure 15.4.

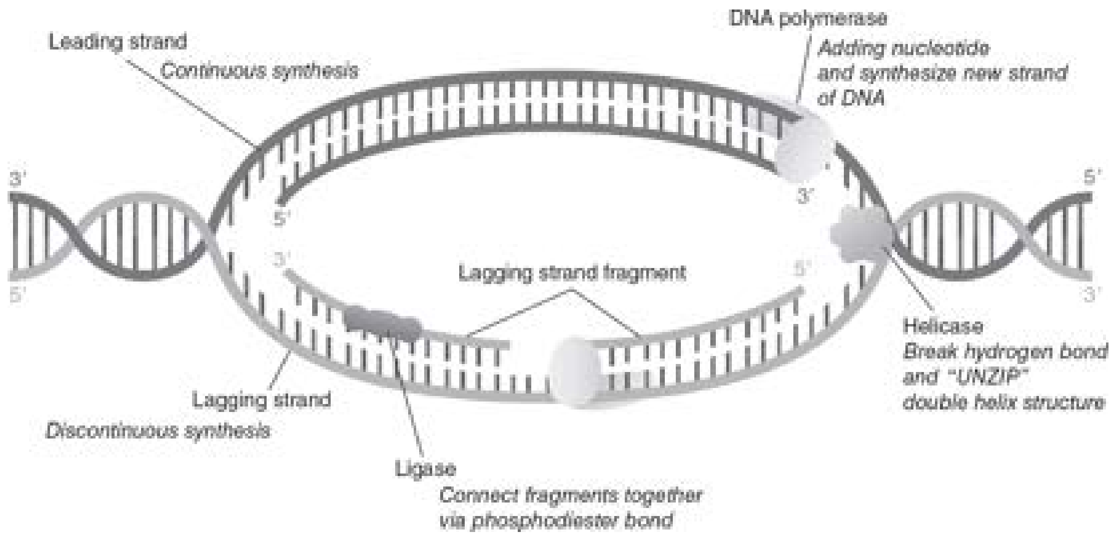


Figure 15.4 DNA Replication

DNA replication can be an intimidating process to learn, and many textbooks have far more detail than you need to know for the AP Biology exam. The most important things to know are why DNA replication is considered semiconservative and the differences between leading and lagging strand replication. Make sure you understand the functions of helicase, topoisomerase, DNA polymerase, RNA polymerase, and ligase in this process.

On one strand of the double helix, DNA polymerase reads the original strand in the 3′ to 5′ direction and can add new nucleotides continuously in the 5′ to 3′ direction. This process is called leading strand replication.

However, the other strand of the double helix is oriented in the 5′ to 3′ direction, which makes the replication process on this strand more complicated. On this strand, DNA polymerase must proceed in the opposite direction in order to read the strand in the 3′ to 5′ direction. Replication on this strand occurs discontinuously, producing short fragments called lagging strand fragments (also known as Okazaki fragments), which are then joined together by the enzyme ligase. This is called lagging strand replication.

Practice Questions

Multiple-Choice

1. The genome of a newly discovered virus has the following nucleotide composition: 22% guanine, 16% cytosine, 34% adenine, and 28% uracil. Based on the nucleotide composition, the genome of this virus is most likely made of which of the following?

   (A) single-stranded DNA
   (B) double-stranded DNA
   (C) single-stranded RNA
   (D) double-stranded RNA

2. Prokaryotic genomes are packaged into _________ chromosomes in the _________.

   (A) circular; nucleoid region
   (B) circular; nucleus
   (C) linear; nucleoid region
   (D) linear; nucleus

3. Energy is required to separate the two strands of the DNA double helix because of the hydrogen bonds between the base pairs. Based on the base pair content, which of the following would require the least amount of energy to separate the strands of DNA?

   (A) 20% G, 20% C, 30% A, 30% T
   (B) 30% G, 30% C, 20% A, 20% T
   (C) 15% A, 15% T, 35% G, 35% C
   (D) 25% G, 25% C, 25% A, 25% T

4. Which of the following correctly describes a structural difference between DNA and RNA?

   (A) DNA has a five-carbon sugar; RNA has a four-carbon sugar.

(B) DNA has thymine; RNA has uracil.
(C) DNA has adenine; RNA has cytosine.
(D) DNA is typically single-stranded; RNA is typically double-stranded.

Questions 5 and 6

N-15, also known as heavy nitrogen, is an isotope of nitrogen that is heavier than the isotope that is typically found in nature, N-14. Conducting chemical reactions in the presence of different isotopes of nitrogen allow a scientist to follow nitrogen atoms in a metabolic pathway. In a classic experiment, Meselson and Stahl allowed parent DNA (containing N-15) to replicate in the presence of N-14.

5. After **one** round of DNA replication, which of the following results would support the statement "DNA replication is semiconservative"?

(A) All DNA molecules have one strand containing N-14 and one strand containing N-15.
(B) 50% of the DNA molecules only contain N-14, and 50% of the DNA molecules only contain N-15.
(C) All DNA molecules only contain N-15.
(D) All DNA molecules only contain N-14.

6. After **two** rounds of DNA replication, which of the following results would support the statement "DNA replication is semiconservative"?

(A) All DNA molecules have one strand containing N-14 and one strand containing N-15.
(B) 50% of the DNA molecules only contain N-14, and 50% of the DNA molecules only contain N-15.
(C) All DNA molecules only contain N-15.
(D) 50% of the DNA molecules contain only N-14, and 50% of the DNA molecules have one strand containing only N-15 and one strand containing only N-14.

7. Why does DNA replication proceed continuously on one strand of the double helix but discontinuously on the other strand of the double helix?

(A) One stand of the double helix contains thymine, and the other strand contains uracil.
(B) Only G and C nucleotides appear on one strand of the double helix, and only A and T nucleotides appear on the other strand of the double helix.
(C) RNA polymerase can only synthesize RNA primers on one strand of the double helix.
(D) DNA polymerase can only add new nucleotides in the 5′ to 3′ direction.

8. The enzyme __________ unwinds the double helix of DNA, and the enzyme __________ relieves the supercoiling created by this unwinding.

(A) helicase; topoisomerase
(B) DNA polymerase; topoisomerase
(C) DNA polymerase; ligase
(D) helicase; ligase

9. The enzyme __________ adds new nucleotides to both the lagging and leading strand, and the enzyme __________ joins the discontinuous segments synthesized on the lagging strand.

(A) helicase; topoisomerase
(B) DNA polymerase; topoisomerase
(C) DNA polymerase; ligase
(D) helicase; ligase

10. Which of the following are small, circular pieces of extranuclear DNA that can be found in either prokaryotes or eukaryotes?

(A) Okazaki fragments

(B) RNA primers
(C) plasmids
(D) linear chromosomes

Short Free-Response

11. Reverse transcriptase is an enzyme that makes a complementary DNA copy of RNA in retroviruses. This DNA copy can then insert itself into the genome of the host cell. Reverse transcriptase has a higher error rate than DNA polymerase, which results in more mutations in the DNA copy of the RNA. Reverse transcriptase is not typically used by eukaryotic cells for any function.

(a) **Describe** which nucleotides you would expect to find in the genome of a virus that uses reverse transcriptase.
(b) The human immunodeficiency virus (HIV) contains RNA as its genetic material. Reverse transcriptase inhibitors have been shown to be effective in slowing the replication of HIV. **Explain** why reverse transcriptase inhibitors have few side effects in eukaryotic cells.
(c) **Predict** the rate of mutation in a retrovirus compared to that of a DNA virus.
(d) **Justify** your prediction from part (c).

12. An experiment is conducted to study the effect of a ligase inhibitor on DNA replication.

(a) **Describe** the function of ligase in DNA replication.
(b) **Identify** an appropriate control for this experiment.
(c) **Predict** which strand of the DNA would be most affected by a ligase inhibitor.
(d) **Justify** your prediction from part (c).

Long Free-Response

13. Polymerase chain reaction (PCR) uses a heat-stable DNA polymerase and repeated cycles of DNA replication to amplify specific sequences of DNA. Primers specific to the desired DNA sequences are used to direct DNA polymerase to the beginning of the sequence to be amplified. The following table shows the number of copies of the DNA sequence that exist at the end of each cycle.

Number of Cycles of DNA Replication	Number of Copies of Desired DNA Sequence
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	128

(a) **Explain** why a primer is needed to direct DNA polymerase to copy the desired sequence.

(b) **Construct** a graph of the data from the table.

(c) **Analyze** the data, and **state** the mathematical relationship between the number of cycles of PCR and the number of copies of the desired DNA sequence generated.

(d) **Predict** the minimum number of cycles required to generate 1,000 copies of the desired DNA sequence. **Justify** your prediction.

Answer Explanations

Multiple-Choice

1. **(C)** Since the question states that the virus's genome contains uracil, choices (C) and (D) are possibilities since RNA contains uracil and DNA does not; thus, rule out choices (A) and (B). However, since the percentage of guanine does not equal the percentage of cytosine and the percentage of adenine does not equal the percentage of uracil, it cannot be a double-stranded virus, so choice (C) is the best answer.

2. **(A)** Prokaryotes have *circular* chromosomes in the *nucleoid region* of their cells. Choice (B) is incorrect because prokaryotes do not have a nucleus. Choices (C) and (D) are incorrect because prokaryotes do not have linear chromosomes; eukaryotes have linear chromosomes.

3. **(A)** Since it has the lowest G-C content and since each G-C pair has three hydrogen bonds between them (instead of the two hydrogen bonds that are between each A-T pair), choice (A) would require the least amount of energy to break those hydrogen bonds and separate the DNA strands. Choices (B), (C), and (D) are all incorrect because they have higher G-C contents than choice (A).

4. **(B)** Only DNA contains thymine, and only RNA contains uracil. Choice (A) is incorrect because RNA has a five-carbon sugar (ribose), not a four-carbon sugar. Both DNA and RNA contain adenine and cytosine, so choice (C) is incorrect. Choice (D) is incorrect because DNA is typically double-stranded and RNA is typically single-stranded.

5. **(A)** After one round of DNA replication, every molecule of DNA would contain one parent strand (containing N-15) and one newly synthesized strand (containing N-14). Choice (B) is incorrect because it describes the expected result from conservative DNA replication, not semiconservative replication. Choices (C) and (D) are both incorrect because after one round of DNA replication, no DNA molecules would contain solely N-15 or N-14.

6. **(D)** As shown in Figure 15.3, after two rounds of DNA replication, 50% of the DNA molecules would contain strands with only N-14. The other 50% of the DNA molecules would contain one strand with only N-15 and one strand with only N-14. Choice (A) describes the result of only one round of DNA replication if DNA replication was semiconservative, not the result of two rounds of DNA replication. So (A) is incorrect. Conservative DNA replication would produce the result described in choice (B), so (B) is incorrect. Choice (C) is incorrect because it describes the result if no replication was happening at all.

7. **(D)** Because the two strands of DNA are antiparallel and because DNA polymerase can only add new nucleotides in the 5′ to 3′ direction, replication must occur differently on the two strands of DNA. On the strand of the double helix that is oriented in the 3′ to 5′ direction, DNA polymerase can perform replication continuously, adding a new antiparallel strand one nucleotide at a time in the 5′ to 3′ direction. On the opposite strand of the double helix that is oriented in the 5′ to 3′ direction, DNA polymerase must work in the opposite direction, performing replication discontinuously. Choices (A), (B), and (C) are all incorrect statements.

8. **(A)** *Helicase* unwinds the double helix of DNA, and *topoisomerase* relieves the stress caused by the supercoiling (created when helicase unwinds the DNA). Choices (B) and (C) are incorrect because DNA polymerase adds new nucleotides; it does not have an unwinding function. Choices (C) and (D) are incorrect because ligase joins together short segments of DNA created on the lagging strand by discontinuous replication.

9. **(C)** *DNA polymerase* adds new nucleotides to the growing DNA strand, and *ligase* joins the discontinuous segments of DNA on the lagging strand together. Choice (A) is incorrect because helicase unwinds the DNA double helix and topoisomerase relieves the supercoiling created by this unwinding. Since topoisomerase relieves supercoiling, choice (B) is also incorrect. Choice (D) is incorrect because helicase is

responsible for unwinding the DNA double helix, not adding new nucleotides.

10. **(C)** Plasmids are small, circular pieces of DNA outside of the nucleus that can be found in prokaryotes and eukaryotes. Okazaki fragments are the short segments of DNA created by discontinuous replication on the lagging strand of DNA, so choice (A) is incorrect. Choice (B) is incorrect because RNA primers are used to give DNA polymerase a place to start adding nucleotides to on the growing DNA strand. Linear chromosomes are found in eukaryotes, not prokaryotes, and they are in the nucleus (they are not extranuclear). Thus, choice (D) is also incorrect.

Short Free-Response

11. (a) A virus that uses reverse transcriptase would have RNA as its genetic material, so its genome would contain the nucleotides adenine, cytosine, guanine, and uracil. (Thymine is not found in RNA.)

    (b) Eukaryotic cells contain DNA as their genetic material and do not need to use reverse transcriptase to make a DNA copy of their genetic material. Therefore, eukaryotic cells do not contain reverse transcriptase. A reverse transcriptase inhibitor would have few, if any, side effects on eukaryotic cells.

    (c) Retroviruses would be expected to have a higher mutation rate than that of DNA viruses.

    (d) One reason why a retrovirus would be expected to have a higher mutation rate than that of a DNA virus is because retroviruses use reverse transcriptase to copy their genome. Reverse transcriptase is less accurate and generates more mutations than DNA polymerase, which would lead to a higher mutation rate in retroviruses.

12. (a) The function of ligase in DNA replication is to join the fragments created during replication of the lagging strand in DNA.

(b) An appropriate control would be to conduct replication of the same DNA sequence without the presence of the ligase inhibitor.

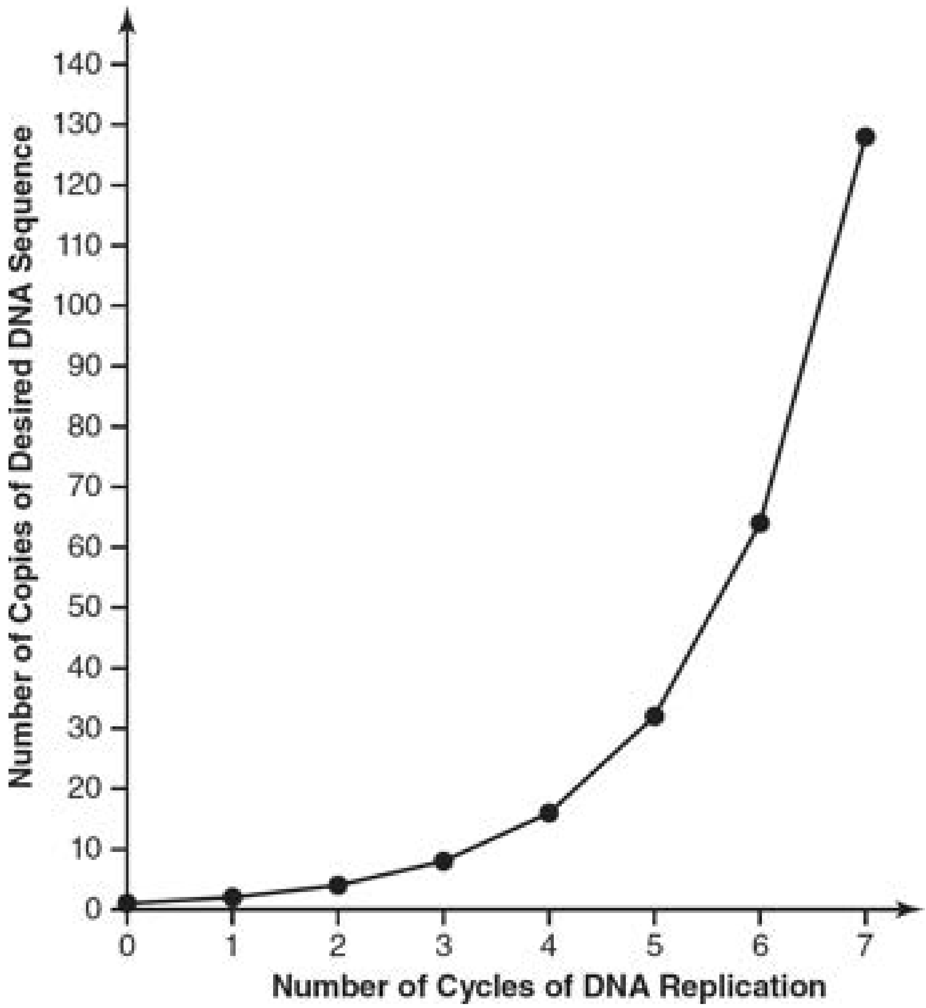
(c) The lagging strand of DNA would be most affected by a ligase inhibitor.

(d) Replication of the lagging strand of DNA would produce multiple short fragments that would need to be joined by ligase. An inhibitor of ligase would prevent this from happening.

Long Free-Response

13. (a) DNA polymerase needs a 3′ hydroxyl group to which it can add new nucleotides, so a primer is necessary to allow DNA polymerase to add the first new nucleotide.

(b)



(c) Each cycle of PCR doubles the number of copies of the desired DNA sequence. In other words, this is an exponential growth relationship.

(d) Since the number of copies of the desired DNA sequence doubles with each cycle, a minimum of 10 cycles would be required to produce at least 1,000 copies. After 8 cycles, 256 copies would be present. After 9 cycles, 512 copies would be present, and after 10 cycles, 1,024 copies would be present.