30: DNA Structure, Replication, and Repair

Overview

Listen

Nucleic acids are required for the storage and expression of genetic information. There are two chemically distinct types of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid ([RNA], see Chapter 31). DNA, the repository of genetic information (the genome), is present not only in chromosomes in the nucleus of eukaryotic organisms, but also in mitochondria and the chloroplasts of plants. Prokaryotic cells, which lack nuclei, have a single chromosome but may also contain nonchromosomal DNA in the form of plasmids. The genetic information found in DNA is copied and transmitted to daughter cells through DNA replication. Each cell type is specialized, expressing only those genes that are required for it to perform its role in maintaining the organism. The DNA contained in a fertilized egg encodes the information that directs the development of an organism. This development may involve the production of billions of cells. Therefore, DNA must be able not only to replicate precisely each time a cell divides, but also to have the information that it contains be selectively expressed and processed for production of the set of functional RNA and protein products needed for cellular function. Transcription (RNA synthesis) is the first stage in the expression of genetic information (see Chapter 31). Next, the code contained in the nucleotide sequence of messenger RNA molecules is translated (protein synthesis; see Chapter 32), thus completing gene expression. The regulation of gene expression is discussed in Chapter 33.

The flow of information from DNA to RNA to protein is termed the “central dogma” of molecular biology (Fig. 30.1) and is descriptive of all organisms, with the exception of some viruses that have RNA as the repository of their genetic information.

FIGURE 30.1

The “central dogma” of molecular biology.

DNA Structure

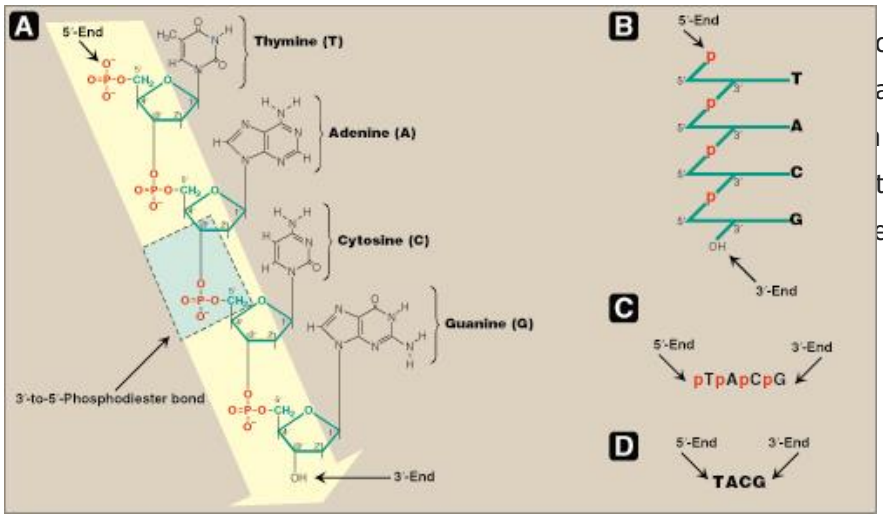
Listen

DNA is a polymer of deoxyribonucleoside monophosphates (dNMP), also called nucleotides, covalently linked by 3′-to-5′ phosphodiester bonds. With the exception of a few viruses that contain single-stranded DNA (ssDNA), DNA exists as a double-stranded molecule (dsDNA), in which the two strands wind around each other, forming a double helix. (Note: The sequence of the linked dNMP is primary structure, whereas the double helix is secondary structure.) In eukaryotic cells, DNA is found associated with various types of proteins (known collectively as nucleoprotein) present in the nucleus, whereas in prokaryotes, the protein–DNA complex is present in a non–membrane-bound region known as the nucleoid.

3′-to-5′ Phosphodiester bonds

Phosphodiester bonds join the 3′-hydroxyl group of the deoxyribose of one nucleotide to the 5′-hydroxyl group of the deoxyribose of an adjacent nucleotide through a phosphoryl group (Fig. 30.2). The resulting long, unbranched chain has polarity, with both a 5′ end (the end with the free phosphate) and a 3′ end (the end with the free hydroxyl) that are not attached to other nucleotides. By convention, the bases located along the resulting deoxyribose-phosphate backbone are always written in sequence from the 5′ end of the chain to the 3′ end. For example, the sequence of the DNA shown in Figure 30.2A is written TACG and is read “thymine, adenine, cytosine, guanine.” Phosphodiester linkages between nucleotides can be hydrolyzed enzymatically by a family of nucleases, deoxyribonucleases for DNA and ribonucleases for RNA, or cleaved hydrolytically by chemicals. (Note: Only RNA is cleaved by alkali.)

FIGURE 30.2



ction. A 3′-to-5′ phosphodiester bond is ackbone is shaded in *yellow* with the in a more stylized form, emphasizing the the nucleotide sequence. **D:** The simplest ence is assumed to be written in the

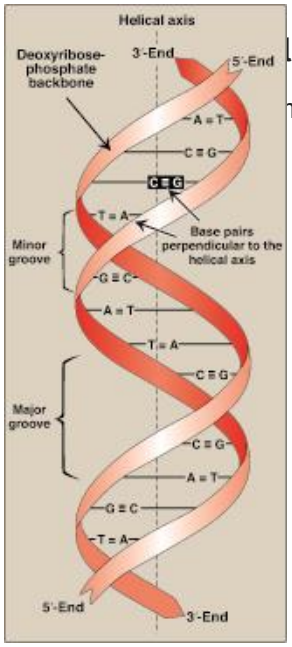
Double helix

In the double helix, the two chains are coiled around a common axis called the helical axis. The chains are paired in an antiparallel manner (i.e., the 5′ end of one strand is paired with the 3′ end of the other strand), as shown in Figure 30.3. In the DNA helix, the hydrophilic deoxyribose-phosphate backbone of each chain is on the outside of the molecule, whereas the hydrophobic bases are stacked inside. The overall structure resembles a twisted ladder. The spatial relationship between the two strands in the helix creates a major (wide) groove and a minor (narrow) groove. These grooves provide access for the binding of regulatory proteins to their specific recognition sequences along the DNA chain. (Note: Certain anticancer drugs, such as dactinomycin [actinomycin D], exert their cytotoxic effect by intercalating into the minor groove of the DNA double helix, thereby interfering with DNA [and RNA] synthesis.)

Base pairing

The bases of one strand of DNA are paired with the bases of the second strand, so that an adenine (A) is always paired with a thymine (T), and a cytosine (C) is always paired with a guanine (G). (Note: The base pairs [bps] are perpendicular to the helical axis [see Fig. 30.3].) Therefore, one polynucleotide chain of the DNA double helix is always the complement of the other. Given the sequence of bases on one chain, the sequence of bases on the complementary chain can be determined (Fig. 30.4). (Note: The specific base-pairing in DNA leads to the Chargaff rule, which states that in any sample of dsDNA, the amount of A equals the amount of T, the amount of G equals the amount of C, and the total amount of purines [A + G] equals the total amount of pyrimidines [T + C].) The base pairs are held together by hydrogen bonds: two between A and T and three between G and C (Fig. 30.5). The base pairs are also stacked along the axis so that the planes of their rings are parallel. The hydrogen bonds of the base pairs, plus the hydrophobic interactions between the stacked bases, stabilize the structure of the double helix.

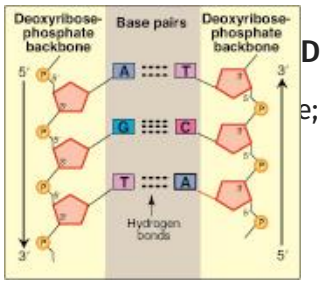
FIGURE 30.3



lustrating some of its major structural features.

ne; C = cytosine; G = guanine.

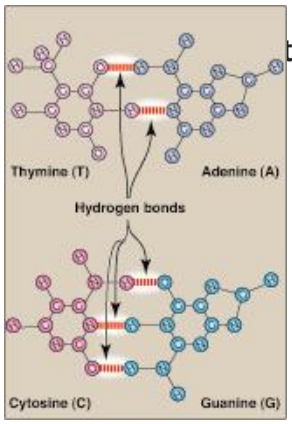
FIGURE 30.4



DNA sequences.

e; C = cytosine; G = guanine.

FIGURE 30.5

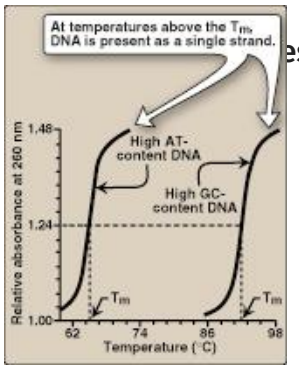


tween complementary bases.

DNA strand separation

The two strands of the double helix separate when hydrogen bonds between the paired bases are disrupted. Disruption can occur in the laboratory if the pH of the DNA solution is altered so that the nucleotide bases ionize, or if the solution is heated. (Note: Covalent phosphodiester bonds are not broken by such treatment.) When DNA is heated, the temperature at which one half of the helical structure is lost and single-stranded regions are formed is defined as the melting temperature (T_m). The loss of helical structure in DNA, called denaturation, can be monitored by measuring its absorbance at 260 nm. (Note: ssDNA has a higher relative absorbance at this wavelength than does dsDNA.) Because there are three hydrogen bonds between G and C but only two between A and T, DNA that contains high concentrations of A and T denatures at a lower temperature than does G- and C-rich DNA (Fig. 30.6). If the DNA solution is cooled or titrated to neutral pH, complementary DNA strands can reform the double helix by the process called renaturation (or, reannealing). (Note: Separation of the two strands over short regions occurs during both DNA and RNA synthesis.)

FIGURE 30.6

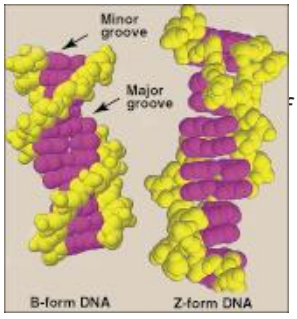


es (T_m) of DNA molecules with different nucleotide compositions.

Structural forms

There are three major structural forms of DNA: the B form (described by Watson and Crick in 1953), the A form, and the Z form. The B form is a right-handed helix with 10 bps per 360-degree turn (or twist) of the helix, and with the planes of the bases perpendicular to the helical axis. Chromosomal DNA is thought to consist primarily of B-DNA (Fig. 30.7 shows a space-filling model of B-DNA). The A form is produced by moderately dehydrating the B form. It is also a right-handed helix, but there are 11 bps per turn, and the planes of the base pairs are tilted 20 degrees away from the perpendicular to the helical axis. The conformation found in DNA–RNA hybrids (see p. 466) or RNA–RNA double-stranded regions is probably very close to the A form. Z-DNA is a left-handed helix that contains 12 bps per turn (see Fig. 30.7). (Note: The deoxyribose-phosphate backbone zigzags, hence, the name Z-DNA.) Stretches of Z-DNA can occur naturally in regions of DNA that have a sequence of alternating purines and pyrimidines (e.g., poly GC). Transitions between the B and Z helical forms of DNA may play a role in regulating gene expression.

FIGURE 30.7



and Z-DNA.

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Linear and circular DNA molecules

Each chromosome in the nucleus of a eukaryote consists of one long, linear molecule of dsDNA, which is bound by a complex mixture of proteins (histone and nonhistone, see p. 473) to form chromatin. Eukaryotes have closed, circular, dsDNA molecules in their mitochondria, as do plant chloroplasts. A prokaryotic organism typically contains a single, circular, dsDNA molecule. Each prokaryotic chromosome is associated with nonhistone proteins that help compact the DNA to form a nucleoid. In addition, most species of bacteria also contain small, circular, extrachromosomal DNA molecules called plasmids. Plasmid DNA carries genetic information and undergoes replication that may or may not be synchronized to chromosomal division. (Note: The use of plasmids as vectors in recombinant DNA technology is described in Chapter 34.)

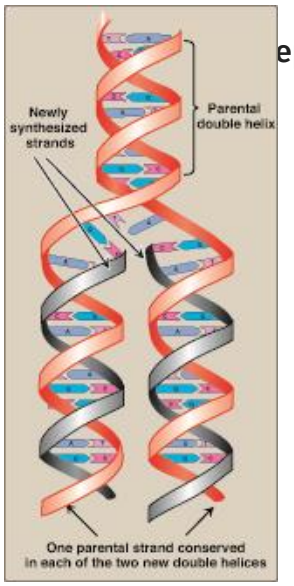
Plasmids may carry genes that convey antibiotic resistance to the host bacterium and may facilitate the transfer of genetic information from one bacterium to another.

Steps in Prokaryotic DNA Replication

Listen

When the two strands of dsDNA are separated, each can serve as a template for the replication (synthesis) of a new complementary strand. This produces two daughter molecules, each of which contains two DNA strands (one old, one new) in an antiparallel orientation (see Fig. 30.3). This process is called semiconservative replication because, although the parental duplex is separated into two halves (and, therefore, is not conserved as an entity), each of the parental strands remains intact in one of the two new duplexes (Fig. 30.8). The enzymes involved in DNA replication are template-directed, magnesium (Mg^{2+})-requiring polymerases that can synthesize the complementary sequence of each strand with extraordinary fidelity. The reactions described in this section were first known from studies of the bacterium *Escherichia coli (E. coli)*, and the description given below refers to the process in prokaryotes. DNA synthesis in higher organisms is more complex but involves the same types of mechanisms. In either case, initiation of DNA replication commits the cell to continue the process until the entire genome has been replicated.

FIGURE 30.8

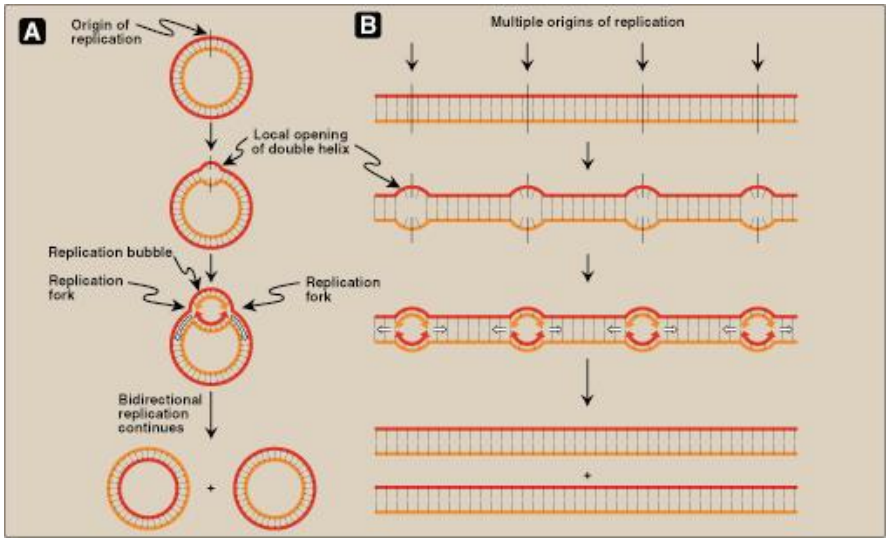


eplication of DNA.

Complementary strand separation

In order for the two complementary strands of the parental dsDNA to be replicated, they must first separate (or "melt") over a small region, because the polymerases use only ssDNA as a template. In prokaryotic organisms, DNA replication begins at a single, unique nucleotide sequence, a site called the origin of replication, or ori (oriC in *E. coli*), as shown in Figure 30.9A. (Note: This sequence is referred to as a consensus sequence, because the order of nucleotides at this site is essentially the same in different bacteria.) The ori includes short, AT-rich segments that facilitate melting. In eukaryotes, replication begins at multiple sites in each chromosome (Fig. 30.9B). Having multiple origins of replication provides a mechanism for rapidly replicating the great length of eukaryotic DNA molecules.

FIGURE 30.9



Replication fork formation

As the two strands unwind and separate, synthesis occurs at two replication forks that move away from the origin in opposite directions (bidirectionally), generating a replication bubble (see Fig. 30.9). (Note: The term "replication fork" derives from the Y-shaped structure in which the tines of the fork represent the separated strands [Fig. 30.10].)

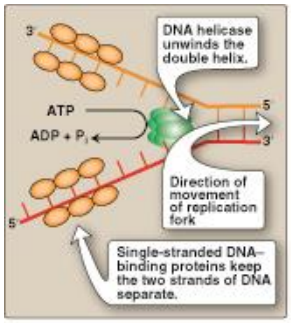
Required proteins

Initiation of DNA replication requires the recognition of the origin (start site) by a group of proteins that form the prepriming complex. These proteins are responsible for melting at the ori, maintaining the separation of the parental strands, and unwinding the double helix ahead of the advancing replication fork. In *E. coli*, these proteins include the following.

DnaA protein

DnaA protein initiates replication by binding to specific nucleotide sequences (DnaA boxes) within oriC. Binding causes an AT-rich region (the DNA unwinding element) in the origin to melt. Melting (strand separation) results in a short, localized region of ssDNA.

FIGURE 30.10



for maintaining the separation of the parental strands and unwinding the

of the advancing replication fork.

sphate; P_i = inorganic phosphate.

DNA helicases

These enzymes bind to ssDNA near the replication fork and then move into the neighboring double-stranded region, forcing the strands apart (in effect, unwinding the double helix). Helicases require energy provided by ATP hydrolysis (see Fig. 30.10). Unwinding at the replication fork causes supercoiling in other regions of the DNA molecule. (Note: DnaB is the principal helicase of replication in *E. coli*. Binding of this hexameric protein to DNA requires DnaC.) Supercoiling is a type of tertiary structure in which the double helix of a chromosome crosses over on itself one or more times to relieve torsional strain in the DNA molecule.

Single-stranded DNA–binding protein

This protein binds to the ssDNA generated by helicases (see Fig. 30.10). Binding is cooperative (i.e., the binding of one molecule of single-stranded binding [SSB] protein makes it easier for additional molecules of SSB protein to bind tightly to the DNA strand). The SSB proteins are not enzymes, but rather serve to shift the equilibrium between dsDNA and ssDNA in the direction of the single-stranded forms. These proteins not only keep the two strands of DNA separated in the area of the replication origin, thus providing the single-stranded template required by polymerases, but also protect the DNA from nucleases that degrade ssDNA.

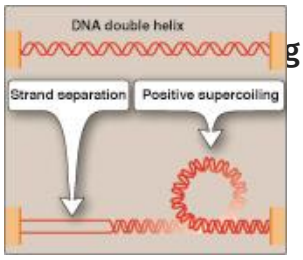
Solving the problem of supercoils

Supercoiling can result from overwinding (positive supercoiling) or underwinding (negative supercoiling) of DNA. As the two strands of the double helix are separated, a problem is encountered, namely, the appearance of positive supercoils in the region of DNA ahead of the replication fork (Fig. 30.11) and negative supercoils in the region behind the fork. The accumulating positive supercoils interfere with further separation of the DNA strands. (Note: Supercoiling can be demonstrated by tightly grasping one end of a helical telephone cord while twisting the other end. If the cord is twisted in the direction of tightening the coils, the cord will wrap around itself in space to form positive supercoils. If the cord is twisted in the direction of loosening the coils, the cord will wrap around itself in the opposite direction to form negative supercoils.) To solve this problem, there is a group of enzymes called DNA topoisomerases, which are responsible for removing supercoils in the helix by transiently cleaving one or both of the DNA strands.

Type I DNA topoisomerases

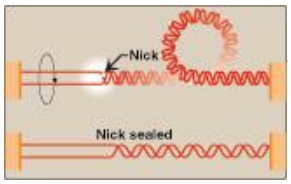
These enzymes reversibly cleave one strand of the double helix and form a covalent bond to the end of the nicked strand. They have both strand-cutting and strand-resealing activities. They do not require ATP, but rather appear to store the energy from the phosphodiester bond they cleave, reusing the energy to reseal the strand (Fig. 30.12). Each time an enzyme creates a transient nick in one DNA strand, it rotates around the intact DNA strand before resealing the nick, thus relieving (relaxing) accumulated supercoils. Type I topoisomerases relax negative supercoils (i.e., those that contain fewer turns of the helix than does relaxed DNA) in *E. coli* and both negative and positive supercoils (i.e., those that contain fewer or more turns of the helix than does relaxed DNA) in many prokaryotic cells (but not *E. coli*) and in eukaryotic cells.

FIGURE 30.11



g resulting from DNA strand separation.

FIGURE 30.12

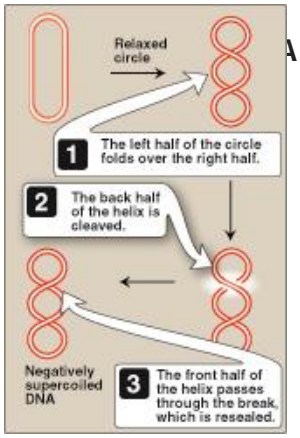


topoisomerases.

Type II DNA topoisomerases

These enzymes bind tightly to the DNA double helix and make transient breaks in both strands. The enzyme then passes a second part of the DNA double helix through the break and, finally, reseals the break (Fig. 30.13). As a result, both negative and positive supercoils can be relieved by this ATP-requiring process. DNA gyrase, a type II topoisomerase found in bacteria and plants, has the unusual property of being able to introduce negative supercoils into circular DNA using energy from the hydrolysis of ATP. This facilitates the replication of DNA because the negative supercoils neutralize the positive supercoils introduced during opening of the double helix. It also aids in the transient strand separation required during transcription (see p. 485).

FIGURE 30.13



A topoisomerase.

Anticancer agents, such as the camptothecins, target human type I topoisomerases, whereas etoposide targets human type II topoisomerases. Bacterial DNA gyrase is a unique target of a group of antimicrobial agents called fluoroquinolones (e.g., ciprofloxacin).

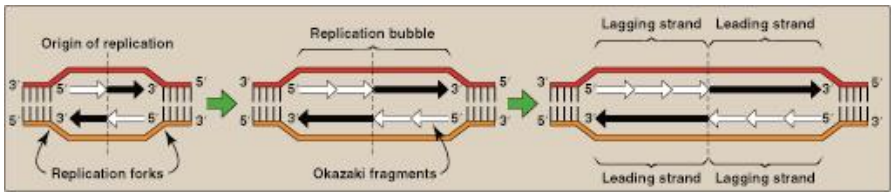
Direction of DNA replication

The DNA polymerases (DNA pols) responsible for copying the DNA templates are only able to read the parental nucleotide sequences in the 3′→5′ direction, and they synthesize the new DNA strands only in the 5′→3′ (antiparallel) direction. Therefore, beginning with one parental double helix, the two newly synthesized stretches of nucleotide chains must grow in opposite directions, one in the 5′→3′ direction toward the replication fork and one in the 5′→3′ direction away from the replication fork (Fig. 30.14). This feat is accomplished by a slightly different mechanism on each strand.

Leading strand

The strand that is being copied in the direction of the advancing replication fork is synthesized continuously and is called the leading strand.

FIGURE 30.14



Lagging strand

The strand that is being copied in the direction away from the replication fork is synthesized discontinuously, with small fragments of DNA being copied near the replication fork. These short stretches of discontinuous DNA, termed Okazaki fragments, are eventually joined (ligated) by ligase to become a single, continuous strand. The new strand of DNA produced by this mechanism is termed the lagging strand.

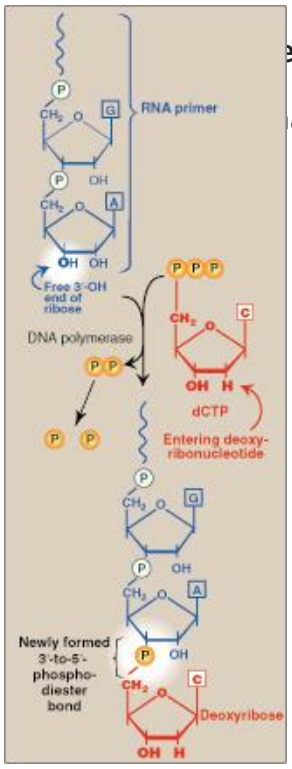
RNA primer

DNA pols cannot initiate synthesis of a complementary strand of DNA on a totally single-stranded template. Rather, they require an RNA primer, which is a short piece of RNA base paired to the DNA template, thereby forming a double-stranded DNA–RNA hybrid. The free hydroxyl group on the 3′ end of the RNA primer serves as the first acceptor of a deoxynucleotide by action of a DNA pol (Fig. 30.15). (Note: Recall that glycogen synthase also requires a primer in the form of a short glycogen molecule [see p. 138].)

Primase

A specific RNA polymerase, called primase (DnaG), synthesizes the short stretches of RNA (~10 nucleotides long) that are complementary and antiparallel to the DNA template. In the resulting hybrid duplex, the uracil (U) in RNA pairs with A in DNA. As shown in Figure 30.16, these short RNA sequences are constantly being synthesized at the replication fork on the lagging strand, but only one RNA sequence at the origin of replication is required on the leading strand. The substrates for this process are 5′-ribonucleoside triphosphates, and pyrophosphate (PP_i) is released as each ribonucleoside monophosphate is added through formation of a 3′-to-5′ phosphodiester bond. (Note: The RNA primer is later removed, as described in F. below.)

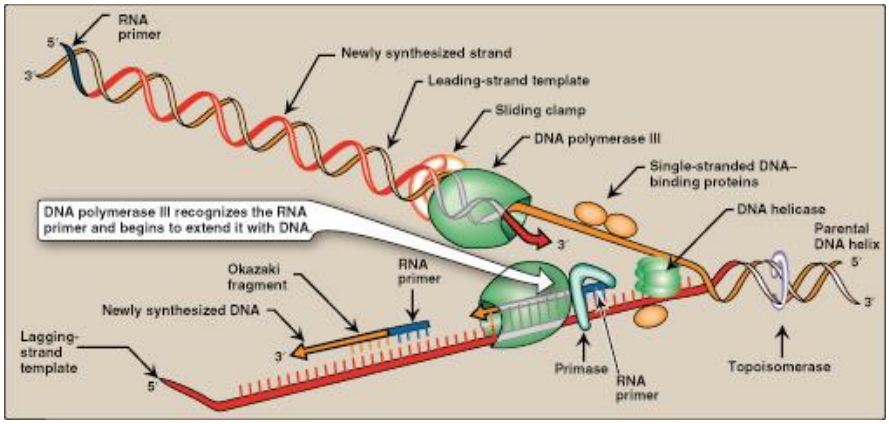
FIGURE 30.15



er to initiate DNA synthesis.

ate; dCTP = deoxycytidine triphosphate.

FIGURE 30.16



Primosome

The addition of primase converts the prepriming complex of proteins required for DNA strand separation (see p. 463) to a primosome. The primosome makes the RNA primer required for leading-strand synthesis and initiates Okazaki fragment formation in discontinuous lagging-strand synthesis. As with DNA synthesis, the direction of synthesis of the primer is 5′→3′.

Chain elongation

Prokaryotic (and eukaryotic) DNA pols elongate a new DNA strand by adding deoxyribonucleotides, one at a time, to the 3′ end of the growing chain (see Fig. 30.16). The sequence of nucleotides that are added is dictated by the base sequence of the parental strand, which serves as a template. Incoming nucleotides, used in the synthesis of the new strand, pair with the bases of the template.

DNA polymerase III

DNA chain elongation is catalyzed by the multisubunit enzyme, DNA pol III. Using the 3′-hydroxyl group of the RNA primer as the acceptor of the first deoxyribonucleotide, DNA pol III begins to add nucleotides along the single-stranded template that specifies the sequence of bases in the newly synthesized chain. DNA pol III is a highly processive enzyme (i.e., it remains bound to the template strand as it moves along and does not diffuse away and then rebind before adding each new nucleotide). The processivity of DNA pol III is the result of the β-subunits of the holoenzyme forming a ring that encircles and moves along the template strand of the DNA, thus serving as a sliding DNA clamp. (Note: Clamp formation is facilitated by a protein complex, the clamp loader, and ATP hydrolysis.) The new (daughter) strand grows in the 5′→3′ direction, antiparallel to the parental strand (see Fig. 30.16). The nucleotide substrates are 5′-deoxyribonucleoside triphosphates. PP_i is released when each new deoxynucleoside monophosphate is added to the free 3′-hydroxyl group of the growing chain through a 3′-to-5′ phosphodiester bond (see Fig. 30.15). Hydrolysis of PP_i to 2 P_i by pyrophosphatase means that a total of two high-energy bonds are used to drive the addition of each deoxynucleotide.

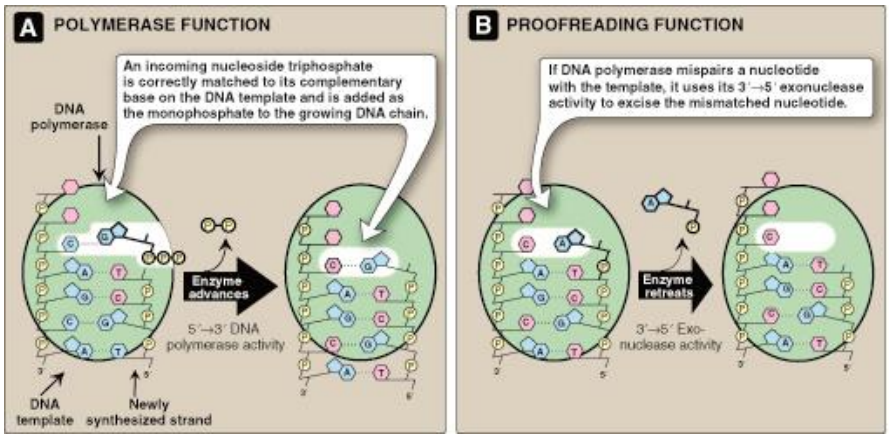
> The production of PP_i with subsequent hydrolysis to 2 P_i is a common theme in biochemistry. Removal of the PP_i drives the reaction that generates PP_i in the forward direction, making it essentially irreversible.

All four substrates (deoxyadenosine triphosphate [dATP], deoxythymidine triphosphate [dTTP], deoxycytidine triphosphate [dCTP], and deoxyguanosine triphosphate [dGTP]) must be present for DNA elongation to occur. DNA synthesis stalls when the concentration of the nucleotide falls below the K_m for the polymerase binding to the nucleotide.)

Proofreading newly synthesized DNA

It is highly important for the survival of an organism that the nucleotide sequence of DNA be replicated with as few errors as possible. Misreading of the template sequence could result in deleterious, perhaps lethal, mutations. To ensure replication fidelity, DNA pol III has a proofreading activity (3′→5′ exonuclease, Fig. 30.17) in addition to its 5′→3′ polymerase activity. As each nucleotide is added to the chain, DNA pol III checks to make certain the base of the newly added nucleotide is, in fact, the complement of the base on the template strand. If it is not, the 3′→5′ exonuclease activity removes the erroneously added nucleotide in the direction opposite to polymerization. (Note: Because the exonuclease function of DNA pol III requires an improperly base-paired 3′-hydroxy terminus, it does not degrade correctly paired nucleotide sequences.) For example, if the template base is C and the enzyme inserts an A instead of a G into the new chain, the 3′→5′ exonuclease activity hydrolytically removes the misplaced nucleotide. The 5′→3′ polymerase activity then repeats the nucleotide addition step and inserts the correct nucleotide containing G (see Fig. 30.17). (Note: The 5′→3′ polymerase and 3′→5′ exonuclease domains are located on different subunits of DNA pol III.)

FIGURE 30.17



fread the newly synthesized DNA

Sickle cell anemia is caused by a single nucleotide change, an error of inserting a T in the place of an A, in the β-globin gene. This mutation results in an incorrect amino acid (a valine in the place of a glutamate) in the β-globin protein that alters the function of the protein in the red blood cell.

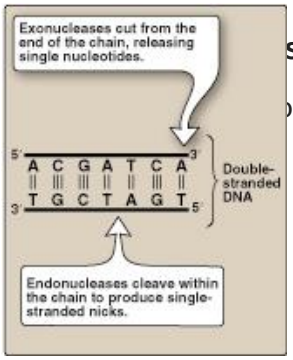
RNA primer excision and replacement by DNA

DNA pol III continues to synthesize DNA on the lagging strand until it nears the 5′ end of an RNA primer. When this occurs, the RNA is excised and the gap between Okazaki fragments is filled by DNA pol I.

5′→3′ Exonuclease activity

In addition to having the 5′→3′ polymerase activity that synthesizes DNA and the 3′→5′ exonuclease activity that proofreads the newly synthesized DNA like DNA pol III, monomeric DNA pol I also has a 5′→3′ exonuclease activity that is able to hydrolytically remove the RNA primer. (Note: Exonucleases remove nucleotides from the end of the DNA chain, rather than cleaving the chain internally as do endonucleases [Fig. 30.18].) First, DNA pol I locates the space (nick) between the 3′ end of the DNA newly synthesized by DNA pol III and the 5′ end of the adjacent RNA primer. Next, DNA pol I hydrolytically removes the RNA nucleotides ahead of itself, moving in the 5′→3′ direction (5′→3′ exonuclease activity). As it removes ribonucleotides, DNA pol I replaces them with deoxyribonucleotides, synthesizing DNA in the 5′→3′ direction (5′→3′ polymerase activity). As it synthesizes the DNA, it also proofreads using its 3′→5′ exonuclease activity to remove errors. This removal/synthesis/proofreading continues until the RNA primer is totally degraded, and the gap is filled with DNA (Fig. 30.19). (Note: DNA pol I uses its 5′→3′ polymerase activity to fill in gaps generated during most types of DNA repair [see p. 476].)

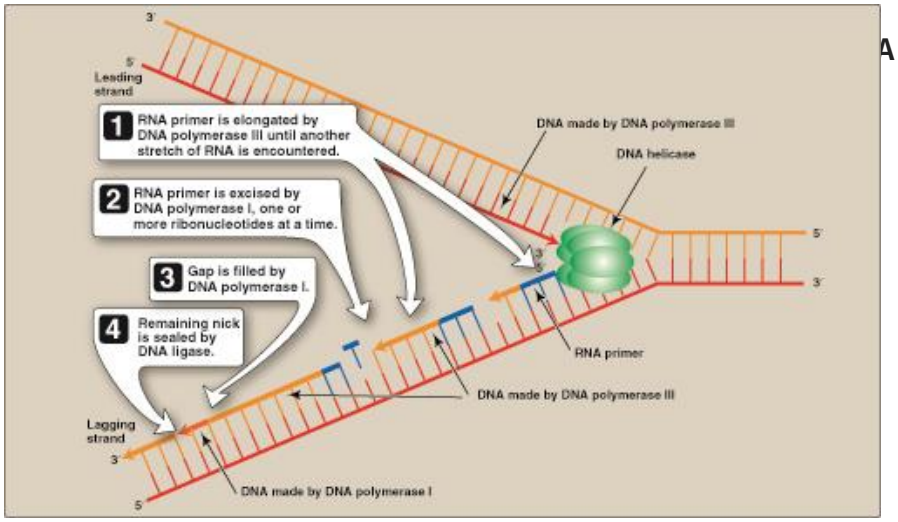
FIGURE 30.18



s exonuclease activity.

onucleases (see p. 532) cleave both strands.)

FIGURE 30.19



A polymerase I.

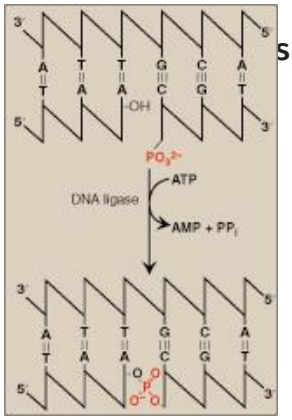
Comparison of 5′→3′ and 3′→5′ exonuclease activities

The 5′→3′ exonuclease activity of DNA pol I allows the polymerase, moving 5′→3′, to hydrolytically remove one or more nucleotides at a time from the 5′ end of the ~10-nucleotide–long RNA primer. In contrast, the 3′→5′ exonuclease activity of DNA pol I and pol III allows these polymerases, moving 3′→5′, to hydrolytically remove one misplaced nucleotide at a time from the 3′ end of a growing DNA strand, increasing the fidelity of replication such that newly replicated DNA has no more than one error per 10^7 nucleotides.

DNA ligase

DNA pol can only catalyze phosphodiester bond formation between a DNA strand and a mononucleotide and cannot join two sections of a DNA strand. The final phosphodiester linkage between the 5′-phosphate group on the DNA synthesized by DNA pol III and the 3′-hydroxyl group on the DNA made by DNA pol I is catalyzed by DNA ligase (Fig. 30.20). The joining (ligation) of these two stretches of DNA requires energy, which in most organisms is provided by the cleavage of ATP to adenosine monophosphate + PP_i.

FIGURE 30.20



sphodiester bond by DNA ligase.

Termination

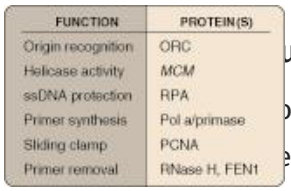
Replication termination in *E. coli* is mediated by sequence-specific binding of the protein, terminus utilization substance (Tus) to replication termination (Ter) sites on the DNA, stopping the movement of the replication fork.

Eukaryotic DNA Replication

Listen

The process of eukaryotic DNA replication closely follows that of prokaryotic DNA synthesis. Some differences, such as the multiple origins of replication in eukaryotic cells versus single origins of replication in prokaryotes, have already been noted. Eukaryotic origin recognition proteins, ssDNA-binding proteins, and ATP-dependent DNA helicases have been identified, and their functions are analogous to those of the prokaryotic proteins previously discussed. In contrast, RNA primers are removed by RNase H and flap endonuclease 1 (FEN1) rather than by a DNA pol (Fig. 30.21).

FIGURE 30.21



FUNCTION	PROTEIN(S)
Origin recognition	ORC
Helicase activity	MCM
ssDNA protection	RPA
Primer synthesis	Pol a/primase
Sliding clamp	PCNA
Primer removal	RNase H, FEN1

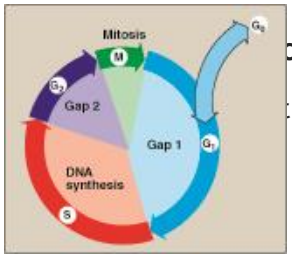
unction in eukaryotic replication.

on complex; MCM = minichromosome maintenance (complex); RPA = replication protein A; ell nuclear antigen; FEN = flap endonuclease.

Eukaryotic cell cycle

The events surrounding eukaryotic DNA replication and cell division (mitosis) are coordinated to produce the cell cycle (Fig. 30.22). The period preceding replication is called the Gap 1 phase (G_1). DNA replication occurs during the synthesis (S) phase. Following DNA synthesis, there is another phase (G_2, or Gap 2) before mitosis (M). Cells that have stopped dividing, such as mature T lymphocytes, are said to have gone out of the cell cycle into the G_0 phase. Such quiescent cells can be stimulated to reenter the G_1 phase to resume division. (Note: The cell cycle is controlled at a series of checkpoints that prevent entry into the next phase of the cycle until the preceding phase has been completed. Two key classes of proteins that control the progress of a cell through the cell cycle are the cyclins and cyclin-dependent kinases [Cdks].)

FIGURE 30.22



cycle.

he cell cycle and enter a reversible quiescent state called G_0.)

Eukaryotic DNA polymerases

At least five high-fidelity eukaryotic DNA pols have been identified and categorized on the basis of molecular weight, cellular location, sensitivity to inhibitors, and the templates or substrates on which they act. They are designated by Greek letters rather than by Roman numerals (Fig. 30.23).

Pol α

Pol α is a multisubunit enzyme. One subunit has primase activity, which initiates strand synthesis on the leading strand and at the beginning of each Okazaki fragment on the lagging strand. The primase subunit synthesizes a short RNA primer that is extended by the 5′→3′ polymerase activity of pol α, generating a short piece of DNA that is later extended by a more processive DNA pol such as pol ε or pol δ. (Note: Pol α is also referred to as pol α/primase.)

FIGURE 30.23

POLY-MERASE	FUNCTION	PROOF-READING*
Pol α (alpha)	• Contains primase • Initiates DNA synthesis	–
Pol β (beta)	• Repair	–
Pol δ (delta)	• Elongates Okazaki fragments of the lagging strand	+
Pol ε (epsilon)	• Elongates the leading strand	+
Pol γ (gamma)	• Replicates mitochondrial DNA	+

…otic DNA polymerases (pol).

…denotes 3′→5′ exonuclease activity.)

Pol ε and pol δ

Pol ε is recruited to complete DNA synthesis on the leading strand, whereas pol δ elongates the Okazaki fragments of the lagging strand, each using 3′→5′ exonuclease activity to proofread the newly synthesized DNA. (Note: DNA pol ε associates with proliferating cell nuclear antigen [PCNA], a protein that serves as a sliding DNA clamp in much the same way the β subunits of DNA pol III do in *E. coli*, thus ensuring high processivity.)

Pol β and pol γ

Pol β is involved in gap filling in DNA repair. Pol γ replicates mitochondrial DNA.

Telomeres

Telomeres are complexes of DNA, associated with proteins (collectively known as shelterin) located at the ends of linear chromosomes. They maintain the structural integrity of the chromosome, preventing attack by nucleases, and allow repair systems to distinguish a true end from a break in dsDNA. In humans, telomeric DNA consists of several thousand tandem repeats of a noncoding hexameric sequence, AGGGTT, base paired to the repeating AACCCT sequence. The strand with AGGGTT repeats (the “G-rich strand”) is longer than its complementary strand with AACCCT repeats (the “C-rich strand”), leaving ssDNA a few hundred nucleotides in length at the 3′ end. The single-stranded region is thought to fold back on itself, forming a loop structure that is stabilized by protein.

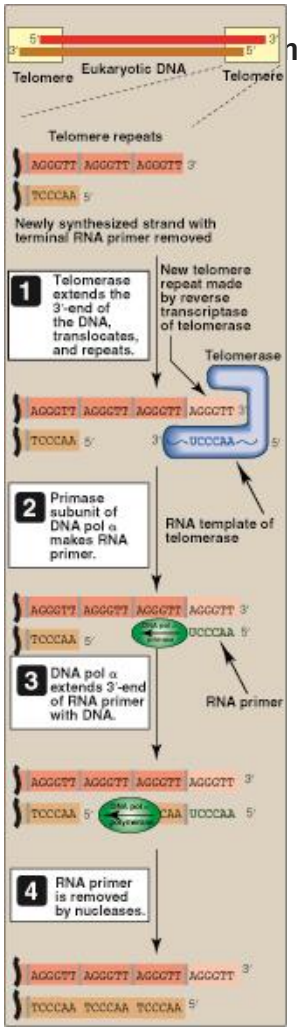
Telomere shortening

Eukaryotic cells face a special problem in replicating the ends of their linear DNA molecules. Following removal of the RNA primer from the extreme 5′ end of the lagging strand, there is no way to fill in the remaining gap with DNA. Consequently, in most normal human somatic cells, telomeres shorten with each successive cell division. Once telomeres are shortened beyond some critical length, the cell is no longer able to divide and is said to be senescent. In germ cells and stem cells, as well as in cancer cells, telomeres do not shorten and the cells do not senesce. This is a result of the activity of the ribonucleoprotein telomerase, which maintains telomeric length in these cells.

Telomerase

This complex contains a protein, TERT that acts as a reverse transcriptase and a short piece of RNA, TERC that acts as a template. The C-rich RNA template base pairs with the G-rich, single-stranded 3′ end of telomeric DNA (Fig. 30.24). The reverse transcriptase uses the RNA template to synthesize DNA in the usual 5′→3′ direction, extending the already longer 3′ end. Telomerase then translocates to the newly synthesized end, and the process is repeated. Once the G-rich strand has been lengthened, primase activity of DNA pol α can use it as a template to synthesize an RNA primer. The primer is extended by DNA pol α and then removed by nucleases.

FIGURE 30.24



n of telomerase, a ribonucleoprotein.

Telomeres may be viewed as mitotic clocks in that their length in most cells is inversely related to the number of times the cells have divided. The study of telomeres provides insight into the biology of normal aging, diseases of premature aging (the progerias), and cancer.

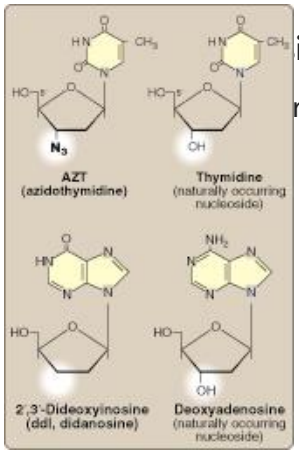
Reverse transcriptases

As seen with telomerase, reverse transcriptases are RNA-directed DNA pols. A reverse transcriptase is involved in the replication of retroviruses, such as human immunodeficiency virus (HIV). These viruses carry their genome in the form of ssRNA molecules. Following infection of a host cell, the viral enzyme reverse transcriptase uses the viral RNA as a template for the 5′→3′ synthesis of viral DNA, which then becomes integrated into host chromosomes. Reverse transcriptase activity is also seen with transposons, DNA elements that can move about the genome (see p. 527). In eukaryotes, most transposons are transcribed to RNA, the RNA is used as a template for DNA synthesis by a reverse transcriptase encoded by the transposon, and the DNA is randomly inserted into the genome. (Note: Transposons that involve an RNA intermediate are called retrotransposons or retroposons.)

DNA replication inhibition by nucleoside analogs

DNA chain growth can be blocked by the incorporation of certain nucleoside analogs that have been modified on the sugar portion (Fig. 30.25). For example, removal of the hydroxyl group from the 3′ carbon of the deoxyribose ring as in 2′,3′ dideoxyinosine ([ddI] also known as didanosine), or conversion of the deoxyribose to another sugar, such as arabinose, prevents further chain elongation. By blocking DNA synthesis, these compounds slow the division of rapidly proliferating cancer cells and the replication of viruses. Cytosine arabinoside (cytarabine, or araC) has been used in anticancer chemotherapy, whereas adenine arabinoside (vidarabine, or araA) is an antiviral agent. Substitution on the sugar moiety, as seen in azidothymidine (AZT), also called zidovudine (ZDV), also terminates DNA chain elongation. (Note: These drugs are generally supplied as nucleosides, which are then converted to nucleotides by cellular kinases.)

FIGURE 30.25



ide analogs that lack a 3′-hydroxyl group.

rted to its active form [dideoxy ATP].)

Eukaryotic DNA Organization

Listen

A typical (diploid) human somatic cell contains 46 chromosomes, whose total DNA is ~2 m long! It is difficult to imagine how such a large amount of genetic material can be effectively packaged into a volume the size of a cell nucleus so that it can be efficiently replicated and its genetic information expressed. To do so requires the interaction of DNA with a large number of proteins, each of which performs a specific function in the ordered packaging of these long molecules of DNA. Eukaryotic DNA is associated with tightly bound basic proteins, called histones. These serve to order the DNA into fundamental structural units, called nucleosomes, which resemble beads on a string. Nucleosomes are further arranged into increasingly more complex structures that organize and condense the long DNA molecules into chromosomes that can be segregated during cell division. (Note: The complex of DNA and protein found inside the nuclei of eukaryotic cells is called chromatin.)

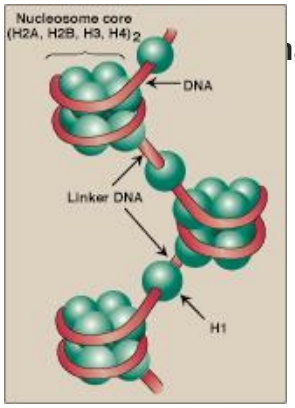
Histones and nucleosome formation

There are five classes of histones, designated H1, H2A, H2B, H3, and H4. These small, evolutionally conserved proteins are positively charged at physiologic pH as a result of their high content of lysine and arginine. Because of their positive charge, they form ionic bonds with negatively charged DNA. Histones, along with ions such as Mg^{2+}, help neutralize the negatively charged DNA phosphate groups.

Nucleosomes

Two molecules each of H2A, H2B, H3, and H4 form the octameric core of the individual nucleosome "beads." Around this structural core, a segment of dsDNA is wound nearly twice (Fig. 30.26). Winding eliminates a helical turn, causing negative supercoiling. (Note: The N-terminal ends of these histones can be acetylated, methylated, or phosphorylated. These reversible covalent modifications influence how tightly the histones bind to the DNA, thereby affecting the expression of specific genes. Histone modification is an example of epigenetics, or heritable changes in gene expression caused without alteration of the nucleotide sequence.) Neighboring nucleosomes are joined by linker DNA ~50 bp long. H1 is not found in the nucleosome core, but instead binds to the linker DNA chain between the nucleosome beads. H1 is the most tissue specific and species specific of the histones. It facilitates the packing of nucleosomes into more compact structures.

FIGURE 30.26

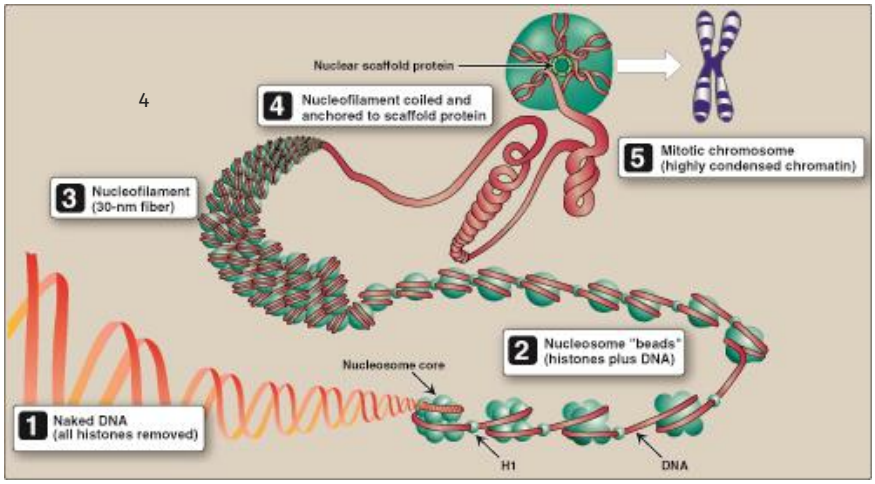


an DNA, illustrating the structure of nucleosomes.

Higher levels of organization

Nucleosomes can be packed more tightly (stacked) to form a nucleofilament. This structure assumes the shape of a coil, often referred to as a 30-nm fiber. The fiber is organized into loops that are anchored by a nuclear scaffold containing several proteins. Additional levels of organization lead to the final chromosomal structure (Fig. 30.27).

FIGURE 30.27



Nucleosome fate during DNA replication

Parental nucleosomes are disassembled to allow access to DNA during replication. Once DNA is synthesized, nucleosomes form rapidly. Their histone proteins come both from *de novo* synthesis and from the transfer of parental histones.

DNA Repair

Listen

Despite the elaborate proofreading system employed during DNA synthesis, errors (including incorrect base-pairing or insertion of one to a few extra nucleotides) can occur. In addition, DNA is constantly being subjected to environmental insults that cause the alteration or removal of nucleotide bases. The damaging agents can be either chemicals (e.g., nitrous acid, which can deaminate bases) or radiation (e.g., nonionizing ultraviolet [UV] radiation from sunlight, which can fuse two pyrimidines adjacent to each other in the DNA, and high-energy ionizing radiation, which can cause double-strand breaks). Bases are also altered or lost spontaneously from mammalian DNA at a rate of many thousands per cell per day. If the damage is not repaired, a permanent change (mutation) is introduced that can result in any of a number of deleterious effects, including loss of control over the proliferation of the mutated cell, leading to cancer. Luckily, cells are remarkably efficient at repairing damage done to their DNA, especially when the damage affects only one or two bases at a location on the same strand of the DNA duplex. Most of the repair systems (which are called excision repair systems) involve recognition of the damage (lesion) on the DNA, removal, or excision of the damage, filling the gap left by excision using the undamaged, complementary strand as a template for DNA synthesis, and ligation to restore the continuity of the repaired strand. These excision repair systems remove one to tens of nucleotides. (Note: Repair synthesis of DNA can occur outside of the S phase.) Damage may also affect both strands of the DNA at location (e.g., double-strand breaks). These forms of damage are repaired by different repair systems than those removing damage to one strand.

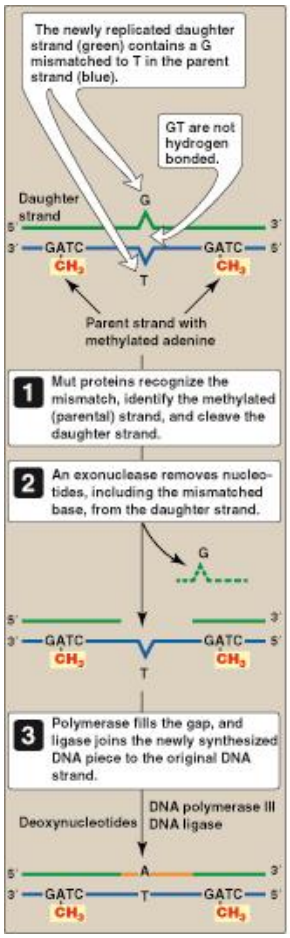
Mismatch repair

Sometimes replication errors escape the proofreading activity during DNA synthesis, causing a mismatch of one to several bases. In *E. coli*, mismatch repair (MMR) is mediated by a group of proteins known as the Mut proteins (Fig. 30.28). Homologous proteins are present in humans. (Note: MMR occurs within minutes of replication and reduces the error rate of replication from 1 in 10^7 to 1 in 10^9 nucleotides.)

Mismatched strand identification

When a mismatch occurs, the Mut proteins that identify the mispaired nucleotide(s) must be able to discriminate between the correct strand and the strand with the mismatch. In prokaryotes, discrimination is based on the degree of methylation. GATC sequences, which are found once every thousand nucleotides, are methylated on the A residue by DNA adenine methylase (DAM). This methylation is not done immediately after synthesis, so the DNA is hemimethylated (i.e., the parental strand is methylated, but the daughter strand is not). The methylated parental strand is assumed to be correct, and it is the daughter strand that gets repaired. (Note: The exact mechanism by which the daughter strand is identified in eukaryotes is not yet known, but likely involves recognition of nicks in the newly synthesized strand.)

FIGURE 30.28



match repair in *Escherichia coli.*

cognizes the mismatch and recruits Mut L. The complex activates Mut H, which cleaves ghter] strand.)

Repair procedure

When the strand containing the mismatch is identified, an endonuclease nicks the strand, and the mismatched nucleotide(s) is/are removed by an exonuclease. Additional nucleotides at the 5′ and 3′ ends of the mismatch are also removed. The gap left by removal of the nucleotides is filled, using the sister strand as a template, by a DNA pol, typically DNA pol III. The 3′ hydroxyl of the newly synthesized DNA is joined to the 5′ phosphate of the remaining stretch of the original DNA strand by DNA ligase.

Defects in the proteins involved in MMR in humans are associated with Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC). Mutations in MSH2 and MLH1 (two human homologs of bacterial Mut proteins) account for 90% of patients with Lynch syndrome.

Although HNPCC confers an increased risk for developing colon cancer (as well as other cancers), only about 5% of all colon cancer is the result of mutations in MMR.

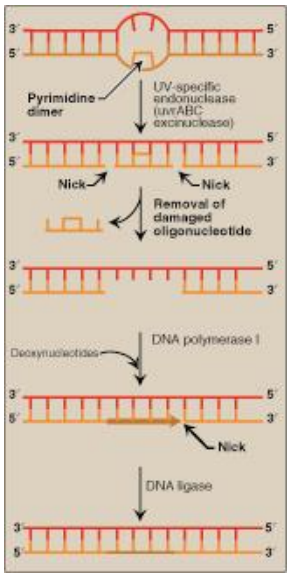
Nucleotide excision repair

Exposure of a cell to UV radiation can result in the covalent joining of two adjacent pyrimidines (usually Ts), producing a dimer. These intrastrand cross-links prevent DNA pol from replicating the DNA strand beyond the site of dimer formation. T dimers are excised in bacteria by UvrABC proteins in a process known as nucleotide excision repair (NER), as illustrated in Figure 30.29. The NER pathway is also present in humans (see 2. below). NER has two DNA damage recognition mechanisms, global genomic repair that finds damage throughout chromosomes and transcription-coupled repair that identifies DNA lesions encountered by RNA polymerases.

Recognition and excision of UV-induced dimers

A UV-specific endonuclease (called uvrABC excinuclease) recognizes the bulky dimer and cleaves the damaged strand on both the 5′ side and 3′ side of the lesion. A short oligonucleotide containing the dimer is excised, leaving a gap in the DNA strand. This gap is filled in using DNA pol I and DNA ligase. The human NER pathway uses additional proteins to remove pyrimidine dimers that form in skin cells and to repair DNA damage that is created by chemical exposure, such as G adducts caused by benzo[a]pyrene from cigarette smoke. NER occurs throughout the cell cycle.

FIGURE 30.29

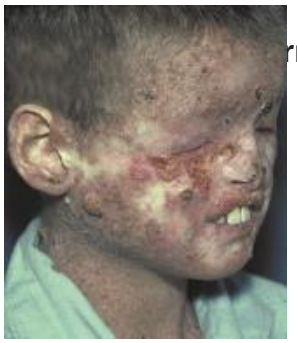


repair of pyrimidine dimers in *Escherichia coli* DNA.

UV radiation and cancer

In the rare, human genetic disease xeroderma pigmentosum (XP), an individual's skin cells cannot repair pyrimidine dimers caused by sunlight, resulting in extensive accumulation of mutations and, consequently, early and numerous skin cancers (Fig. 30.30). XP can be caused by defects in seven genes that code for the XP proteins required for NER of UV damage.

FIGURE 30.30



rma pigmentosum.

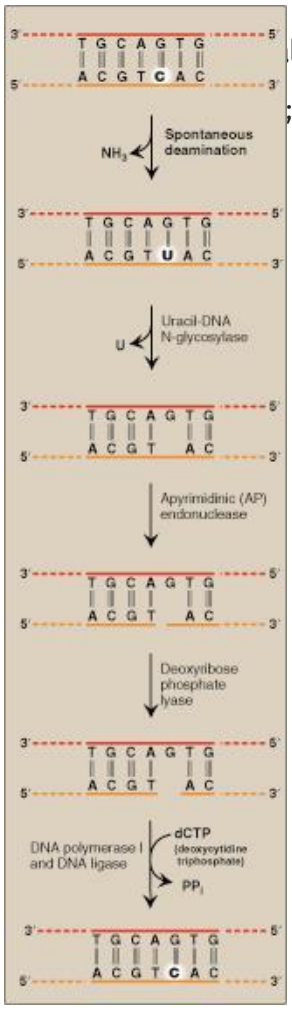
Base excision repair

DNA bases can be altered, either spontaneously, as is the case with C, which slowly undergoes deamination (the loss of its amino group) to form U, or by the action of deaminating or alkylating compounds. For example, nitrous acid, which is formed by the cell from precursors such as the nitrates, deaminates C, A (to hypoxanthine), and G (to xanthine). Dimethyl sulfate can alkylate (methylate) A. Bases can also be lost spontaneously by hydrolysis from the deoxyribose sugar backbone. For example, ~10,000 purine bases are lost this way per cell per day. Lesions involving base alterations or loss can be corrected by base excision repair ([BER], Fig. 30.31).

Abnormal base removal

In BER, abnormal bases, such as U, which can occur in DNA by either deamination of C or improper use of dUTP instead of dTTP during DNA synthesis, are recognized by specific DNA glycosylases that hydrolytically cleave them from the deoxyribose-phosphate backbone of the strand. This leaves an apyrimidinic site, or apurinic if a purine was removed, both referred to as AP sites.

FIGURE 30.31



lterations by base excision repair.

; NH_3 = ammonia; PP_i = pyrophosphate.

AP site recognition and repair

Specific AP endonucleases recognize that a base is missing and initiate the process of excision and gap filling by making an endonucleolytic cut just to the 5′ side of the AP site. A deoxyribose phosphate lyase removes the single, base-free, sugar phosphate residue. DNA pol I and DNA ligase complete the repair process.

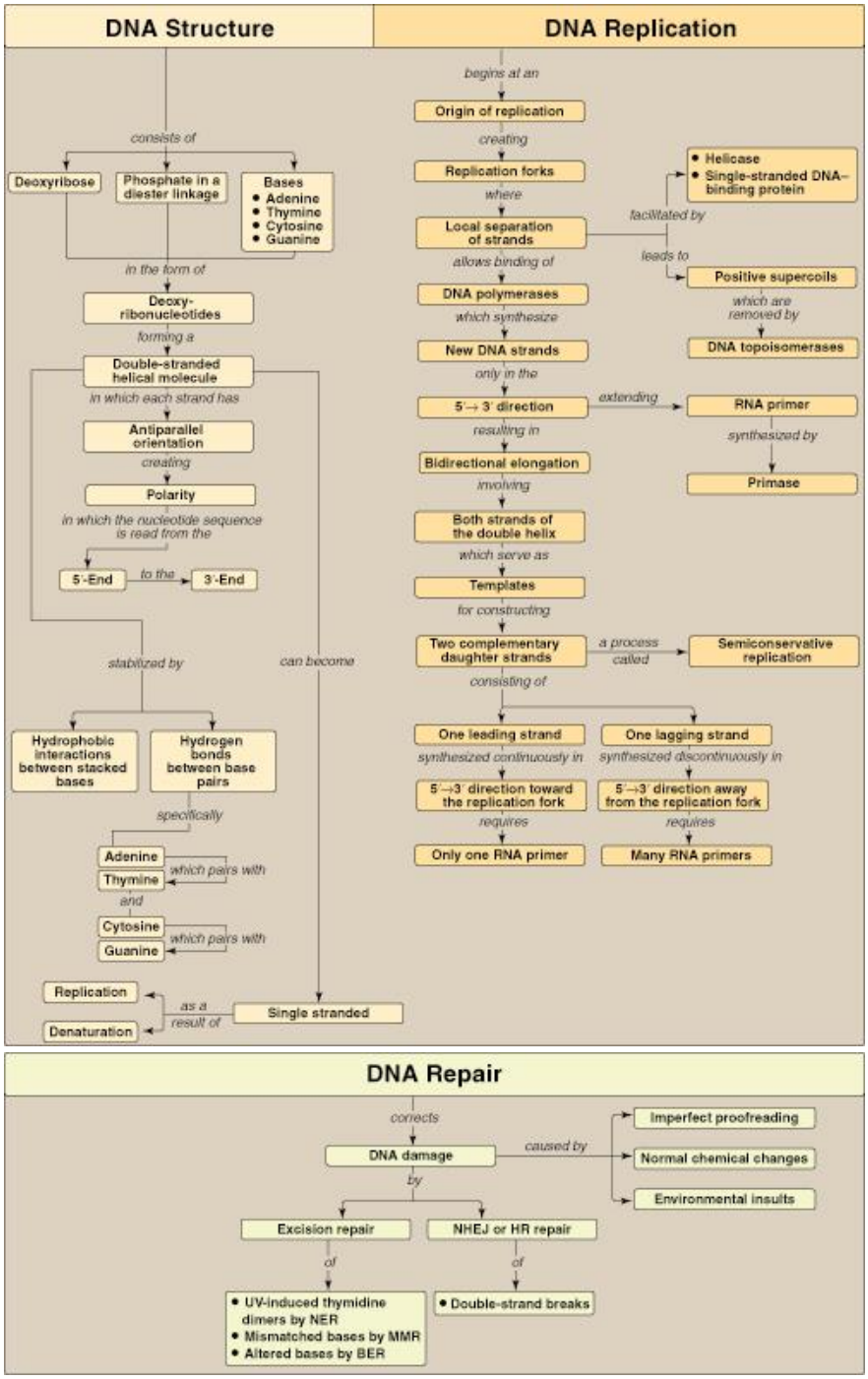
Double-strand break repair

Ionizing radiation, chemotherapeutic agents such as doxorubicin, and oxidative free radicals (see p. 163) can cause double-strand breaks in DNA that can be lethal to the cell. (Note: Such breaks also occur naturally during genetic recombination.) dsDNA breaks cannot be corrected by the previously described strategy of excising the damage on one strand and using the undamaged strand as a template for replacing the missing nucleotide(s). Instead, they are repaired by one of two systems. The first is nonhomologous end joining (NHEJ), in which a group of proteins mediates the recognition, processing, and ligation of the ends of two DNA fragments. However, some DNA is lost in the process. Consequently, NHEJ is error prone and mutagenic. Defects in NHEJ are associated with a predisposition to cancer and immunodeficiency syndromes. The second repair system, homologous recombination (HR), uses the enzymes that normally perform genetic recombination between homologous chromosomes during meiosis. This system is much less error prone (“error free”) than NHEJ because any DNA that was lost is replaced using homologous DNA as a template. HR occurs in late S and G_2 of the cell cycle, whereas NHEJ can occur anytime. (Note: Mutations to the proteins BRCA1 or BRCA2 [breast cancer 1 or 2], which are involved in HR, increase the risk for developing breast and ovarian cancer.)

Chapter Summary

- DNA is composed of two polymers (chains) of dNMP (nucleotides). Each chain has **polarity**, with both a 5′ end (free phosphate) and a 3′ end (free hydroxyl). The nucleotide sequences of the chains are read from the 5′ end to the 3′ end (Fig. 30.32).

FIGURE 30.32



- DNA exists as a dsDNA, in which the two chains are paired in an **antiparallel** manner and form a **double helix**. Through hydrogen bonding, **A** pairs with **T**, and **C** pairs with **G**.
- Each DNA strand serves as a **template** for constructing a **complementary** daughter strand (**semiconservative replication**). DNA replication begins at an **origin of replication** where the two strands unwind and separate and synthesis occurs bidirectionally at two **replication forks** that move away from the origin. As **helicase**

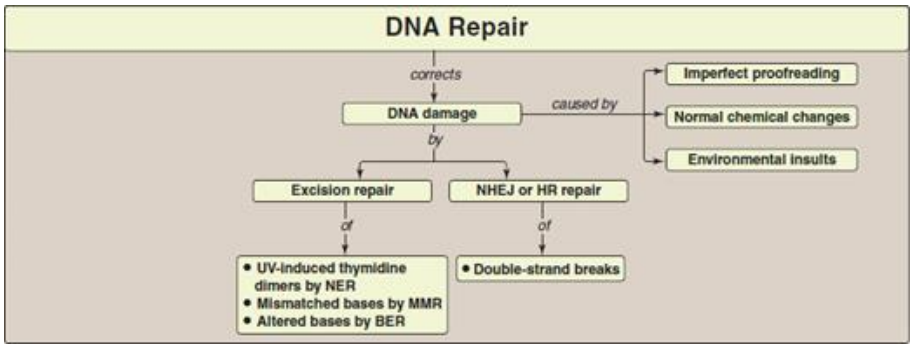
separates the two strands, positive **supercoils** are produced in the region of DNA ahead of the replication fork and negative supercoils behind the fork. **DNA topoisomerases types I** and **II** remove supercoils.

- **DNA pols** synthesize new DNA strands only in the **5′→3′** direction and require a short RNA **primer** created by **primase**. One of the new strands must grow in the 5′→3′ direction toward the replication fork (**leading strand**) while the other grows in the 5′→3′ direction away from the replication fork (**lagging strand**). Leading-strand synthesis is continuous from one RNA primer, whereas the lagging strand needs many primers (**discontinuous** synthesis involving **Okazaki fragments**).
- In *E. coli,* DNA chain elongation is catalyzed by **DNA pol III**, using **5′-deoxyribonucleoside triphosphates** as substrates. The enzyme **proofreads** the newly synthesized DNA, removing terminal mismatched nucleotides with its **3′→5′ exonuclease** activity. RNA primers are removed by **DNA pol I**, using its **5′→3′ exonuclease** activity. This enzyme fills the gaps with DNA, proofreading as it synthesizes. The final phosphodiester linkage is catalyzed by **DNA ligase**.
- There are at least five high-fidelity **eukaryotic DNA pols**. **Pol α** is a multisubunit enzyme, one subunit of which is a **primase**. Pol α 5′→3′ polymerase activity adds a short piece of DNA to the RNA primer. **Pol ε** completes DNA synthesis on the leading strand, whereas **pol δ** elongates each lagging strand fragment. **Pol β** is involved with DNA repair, and **pol γ** replicates mitochondrial DNA. Pols ε, δ, and γ use 3′→5′ exonuclease activity to proofread.
- **Nucleoside analogs** containing modified sugars can be used to block DNA chain growth. They are useful in anticancer and antiviral chemotherapy.
- **Telomeres** are stretches of **highly repetitive DNA** that are bound by protein and protect the **ends** of linear chromosomes. As most cells divide and age, these sequences are shortened, contributing to senescence. In cells that do not senesce (e.g., germline and cancer cells), the ribonucleoprotein **telomerase** employs its protein component **reverse transcriptase** to extend the telomeres, using its **RNA** component as a **template**.
- Pairs of the positively charged histone proteins (H2A, H2B, H3, and H4) form an octameric structural core around which DNA is wrapped, creating a **nucleosome**. The DNA connecting the nucleosomes, called **linker DNA**, is bound to H1. Nucleosomes are packed more tightly to form a nucleofilament. Additional levels of organization create a chromosome.
- Three types of DNA repair correct most DNA damage in chromosomes: NER, BER, and MMR. Each removes specific types of DNA damage (Fig. 30.33). NER removes **pyrimidine dimers** caused by UV radiation, BER replaces abnormal bases and AP sites, and MMR corrects mispaired bases caused by DNA pol errors. Defects in the **XP proteins** needed for NER in humans result in **XP**. Defective MMR in humans, mainly caused by mutations in the MSH2 and MLH1 genes, is associated with **HNPCC**.

FIGURE 30.33

Key concept map for DNA repair.

NHEJ = nonhomologous end joining; HR = homologous recombination; UV = ultraviolet; NER = nucleotide excision repair; MMR = mismatch repair; BER = base excision repair.



- Double-strand breaks in DNA are repaired by **NHEJ** (error prone) and template-requiring **HR** ("error free").

Study Questions

Listen

Choose the ONE best answer.

30.1. A 10-year-old female is brought by her parents to the dermatologist. She has many freckles on her face, neck, arms, and hands, and the parents report that she is unusually sensitive to sunlight. Two basal cell carcinomas are identified on her face. Based on the clinical picture, which of the following processes is most likely to be defective in this patient?

A. Repair of double-strand breaks by error-prone homologous recombination

B. Removal of mismatched bases from the 3′ end of Okazaki fragments by a methyl-directed process

C. Removal of pyrimidine dimers from DNA by nucleotide excision repair

D. Removal of uracil from DNA by base excision repair

E. Removal of incorrectly paired nucleotides by Pol ε 3′→5′ exonuclease activity

Correct answer = C. The sensitivity to sunlight, extensive freckling on parts of the body exposed to the sun, and presence of skin cancer at a young age indicate that the patient most likely suffers from xeroderma pigmentosum (XP). These patients are deficient in any one of several XP proteins required for nucleotide excision repair of pyrimidine dimers in ultraviolet radiation–damaged DNA. Double-strand breaks are repaired by nonhomologous end joining (error prone) or homologous recombination ("error free"). Methylation is not used for strand discrimination in eukaryotic mismatch repair. Uracil is removed from DNA molecules by a specific glycosylase in base excision repair, but a defect in this process does not cause XP.

30.2. Telomeres are complexes of DNA and protein that protect the ends of linear chromosomes. In most normal human somatic cells, telomeres shorten with each division. In stem cells and cancer cells, however, telomeric length is maintained. In the synthesis of telomeres:

A. telomerase, a ribonucleoprotein, provides both the RNA and the protein needed for synthesis.

B. the RNA of telomerase serves as a primer.

C. the RNA of telomerase is a ribozyme.

D. the protein of telomerase is a DNA-directed DNA polymerase.

E. the shorter C-rich strand gets extended.

F. the direction of synthesis is 3′→5′.

Correct answer = A. Telomerase is a ribonucleoprotein particle required for telomere maintenance. Telomerase contains an RNA that serves as the template, not the primer, for the synthesis of telomeric DNA by the reverse transcriptase of telomerase. Telomeric RNA has no catalytic activity. As a reverse transcriptase, telomerase synthesizes DNA using its RNA template and so is an RNA-directed DNA polymerase. The direction of synthesis, as with all DNA synthesis, is 5′→3′, and it is the 3′ end of the already longer G-rich strand that gets extended.

30.3. While studying the structure of a small gene that was sequenced during the Human Genome Project, an investigator notices that one strand of the DNA molecule contains 20 A, 25 G, 30 C, and 22 T. How many of each base is found in the complete double-stranded molecule?

A. A = 40, G = 50, C = 60, T = 44

B. A = 42, G = 55, C = 55, T = 42

C. A = 44, G = 60, C = 50, T = 40

D. A = 45, G = 45, C = 52, T = 52

E. A = 50, G = 47, C = 50, T = 47

Correct answer = B. The two DNA strands are complementary to each other, with A base paired with T and G base paired with C. So, for example, the 20 A on the first strand would be paired with 20 T on the second strand, the 25 G on the first strand would be paired with 25 C on the second strand, and so forth. When these are all added together, the correct numbers of each base are indicated in choice B. Notice that, in the correct answer, A = T and G = C.

30.4. List the order in which the following enzymes participate in leading strand synthesis during prokaryotic replication.

A. Ligase

B. Polymerase I (3′→5′ exonuclease activity)

C. Polymerase I (5′→3′ exonuclease activity)

D. Polymerase I (5′→3′ polymerase activity)

E. Polymerase III

F. Primase

Correct answer: F, E, C, D, B, A. Primase makes the RNA primer; polymerase (pol) III extends the primer with DNA (and proofreads); pol I removes the primer with its 5′→3′ exonuclease activity, fills in the gap with its 5′→3′ polymerase activity, and removes errors with its 3′→5′ exonuclease activity; and ligase makes the 5′-to-3′ phosphodiester bond that links the DNA made by pols I and III.

30.5. Dideoxynucleotides lack a 3′-hydroxyl group. Why would incorporation of a dideoxynucleotide into DNA stop replication?

The lack of the 3′-OH group prevents formation of the 3′ hydroxyl-to-5′-phosphate bond that links one nucleotide to the next in DNA.