13 Synthesis of DNA

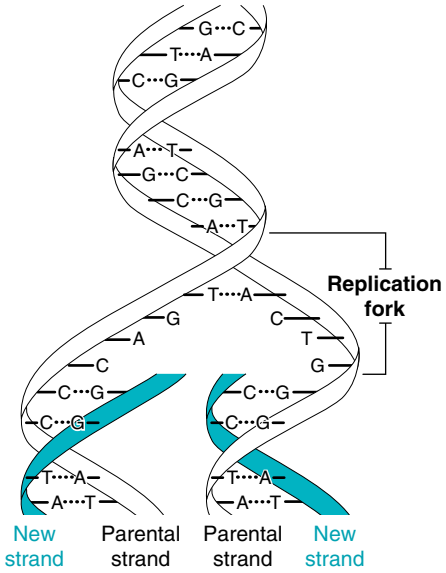


Fig. 13.1. A replicating DNA helix. The parental strands separate at the replication fork. Each parental strand serves as a template for the synthesis of a new strand.

DNA synthesis occurs by the process of ***replication.*** *During replication, each of the two parental strands of DNA serves as a* ***template*** *for the synthesis of a complementary strand. Thus, each DNA molecule generated by the replication process contains one intact parental strand and one newly synthesized strand (Fig. 13.1). In eukaryotes,* ***DNA replication*** *occurs during the* ***S phase*** *of the* ***cell cycle,*** *which is followed by the* G_2 *phase. The cell* ***divides*** *during the next phase* ***(M),*** *and each daughter cell receives an exact copy of the DNA of the parent cell.*

The Replication Fork. *In both prokaryotes and eukaryotes, the site at which replication is occurring at any given moment is called the* ***replication fork.*** *As replication proceeds, the two parental strands separate in front of the fork. Behind the fork, each newly synthesized strand of DNA base-pairs with its complementary parental template strand. A complex of proteins is involved in replication.* ***Helicases*** *and* ***topoisomerases*** *unwind the parental strands, and* ***single-strand binding proteins*** *prevent them from reannealing.*

The major enzyme involved in replication is a ***DNA polymerase*** *that copies each parental template strand in the 3′ to 5′ direction, producing new strands in a 5′ to 3′ direction.* ***Deoxyribonucleoside triphosphates*** *serve as the precursors. One strand of newly synthesized DNA grows continuously, whereas the other strand is synthesized discontinuously in short segments known as* ***Okazaki fragments.*** *These fragments are subsequently joined by* ***DNA ligase.***

Initiation. DNA polymerase cannot initiate *the synthesis of new strands. Therefore, a short* ***primer*** *is produced, which contains ribonucleotides (RNA). DNA polymerase can add deoxyribonucleotides to the 3′ end of this primer. This RNA primer is subsequently removed and replaced by deoxyribonucleotides.*

Telomeres. *The ends of linear chromosomes are called telomeres. The enzyme* ***telomerase,*** *an RNA-dependant DNA polymerase that carries its own RNA template, is required for their replication.*

Errors and Repair. Errors *occurring* ***during replication*** *could lead to deleterious* ***mutations.*** *However, many errors are corrected by enzyme activities associated with the complex at the replication fork. The* ***error rate*** *is thus kept at a very* ***low*** *level.*

Damage *to DNA molecules also causes mutations.* ***Repair mechanisms*** *correct DNA damage, usually by removing and replacing the damaged region. The intact, undamaged strand serves as a template for the DNA polymerase involved in the repair process.*

Recombination. *Although cells have mechanisms to correct replication errors and to repair DNA damage, some genetic change is desirable. It produces new proteins or variations of proteins that may increase the survival rate of the species. Genetic change is produced by unrepaired mutations and by a mechanism known as* ***recombination*** *in which portions of chromosomes are exchanged.*

THE WAITING ROOM

Ivy Sharer is having difficulty complying with her multidrug regimen. She often forgets to take her pills. When she returns for a checkup, she asks whether such a large number of pills are really necessary for treatment of acquired immunodeficiency syndrome (AIDS).

Di Abietes responded to treatment for her diabetes mellitus but subsequently developed a low-grade fever, an increase in urinary urgency and frequency, and burning at the urethral opening with urination (dysuria). A urinalysis showed a large number of white blood cells and many Gram-negative bacilli. A urine culture indicated many colonies of *Escherichia coli*, which is sensitive to several antibiotics, including the quinolone norfloxacin.

Melvin (Mel) Anoma is a 46-year-old man who noted a superficial, brownish-black, 5-mm nodule with irregular borders in the skin on his chest. He was scheduled for outpatient surgery, at which time a wide excision biopsy was performed. (In an excision biopsy, the complete mole is removed and biopsy performed). Examination of the nodule indicated histologic changes characteristic of a malignant melanoma reaching a thickness of only 0.7 mm from the skin surface (Stage I).

Nick O'Tyne is a 62-year-old electrician who has smoked two packs of cigarettes a day for 40 years. He recently noted that his chronic cough had gotten worse. His physician ordered a chest radiograph, which showed a 2-cm nodule in the upper lobe of the right lung. Cytologic study of the sputum by Papanicolaou technique showed cells consistent with the presence of a well-differentiated adenocarcinoma of the lung.

I. DNA SYNTHESIS IN PROKARYOTES

The basic features of the mechanism of DNA replication are illustrated by the processes occurring in the bacterium *E. coli*. This bacillus grows symbiotically in the human colon. It has been extensively studied and serves as a model for the more complex and, consequently, less well-understood processes that occur in eukaryotic cells.

A. Replication Is Bidirectional

Replication of the circular, double-stranded DNA of the chromosome of *E. coli* begins with the binding of approximately 30 molecules of the protein DnaA at a single point of origin, designated *oriC*, where the DNA coils around the DnaA core (Fig. 13.2). With the assistance of other proteins (e.g., a helicase, gyrase, and single-stranded binding protein), the two parental strands separate within this region, and both strands are copied simultaneously. Synthesis begins at the origin and occurs at two replication forks that move away from the origin bidirectionally (in both directions at the same time). Replication ends on the other side of the chromosome at a termination point. One round of synthesis, involving the incorporation of over 4 million nucleotides in each new strand of DNA, is completed in approximately 40 minutes. However, a second round of synthesis can begin at the origin before the first round is finished. These multiple initiations of replication allow bacterial multiplication to occur much more quickly than the time it takes to complete a single round of replication.

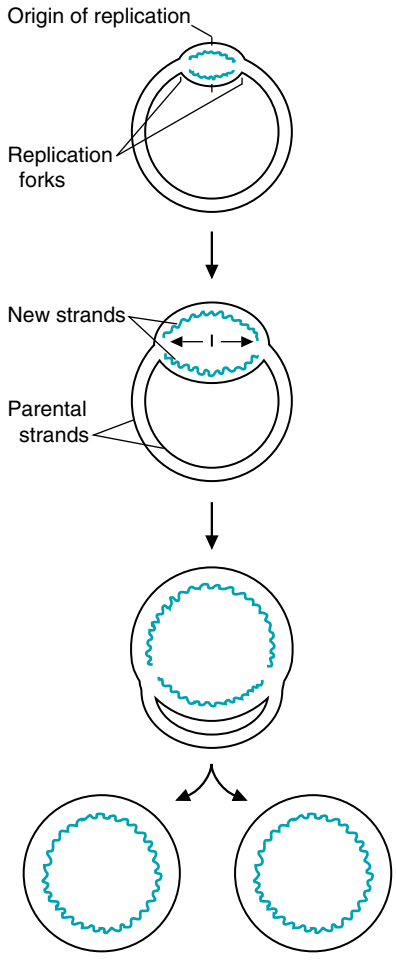


Fig. 13.2. Bidirectional replication of a circular chromosome. Replication begins at the point of origin (*oriC*) and proceeds in both directions at the same time.

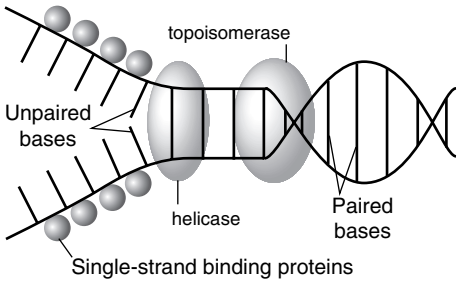
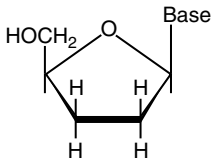


Fig. 13.3. Proteins involved in separating and unwinding parental DNA strands at the replication fork in prokaryotes.

Di Abietes' urinary tract infection was treated with norfloxacin, a fluorinated member of the quinolone family. This group of drugs inhibits bacterial DNA gyrase, a topoisomerase that unwinds the closed circular bacterial DNA helix ahead of the replication fork, and thus inhibits bacterial DNA synthesis. Because eukaryotic cells have linear DNA and do not contain DNA gyrase, they are not affected by quinolones.

One of the drugs used to treat **Ivy Sharer** was didanosine (ddI). It is a dideoxynucleoside, an example of which is shown below.



A dideoxynucleoside

The dideoxynucleosides do not have a hydroxyl group on either the 2′- or 3′-carbon. They can be converted to dideoxynucleoside triphosphates in cells and, like ZDV, terminate chain growth when incorporated into DNA. In the case of the dideoxynucleosides, chain termination results from the absence of a hydroxyl group on the 3′ carbon. The HIV virus mutates very rapidly (mostly because reverse transcriptase lacks 3′ to 5′ exonuclease activity, the proofreading activity) and frequently develops resistance to one or more of these drugs. Therefore, it is recommended that AIDS patients take a number of drugs, including more than one reverse transcriptase inhibitor.

B. Replication Is Semiconservative

Each daughter chromosome contains one of the parental DNA strands and one newly synthesized, complementary strand. Therefore, replication is said to be semiconservative; i.e., the parental strands are conserved but are no longer together. Each one is paired with a newly synthesized strand (see Figs. 13.1 and 13.2).

C. Unwinding of Parental Strands

Replication requires separation of the parental DNA strands and unwinding of the helix ahead of the replication fork. Helicases (DnaB) separate the DNA strands and unwind the parental duplex. Single-strand binding proteins prevent the strands from reassociating and protect them from enzymes that cleave single-stranded DNA (Fig. 13.3). Topoisomerases, enzymes that can break phosphodiester bonds and rejoin them, relieve the supercoiling of the parental duplex caused by unwinding. DNA gyrase is a major topoisomerase in bacterial cells.

D. Action of DNA Polymerase

Enzymes that catalyze the synthesis of DNA are known as DNA polymerases. *E. coli* has three DNA polymerases, Pol I, Pol II, and Pol III. Pol III is the major replicative enzyme (Table 13.1). All DNA polymerases that have been studied copy a DNA template strand in its 3′ to 5′ direction, producing a new strand in the 5′ to 3′ direction (Fig. 13.4). Deoxyribonucleoside triphosphates (dATP, dGTP, dCTP, and dTTP) serve as substrates for the addition of nucleotides to the growing chain.

The incoming nucleotide forms a base pair with its complementary nucleotide on the template strand. Then an ester bond is formed between the first 5′-phosphate of the incoming nucleotide and the free 3′-hydroxyl group at the end of the growing chain. Pyrophosphate is released. The release of pyrophosphate and its subsequent cleavage by a pyrophosphatase provide the energy that drives the polymerization process.

DNA polymerases that catalyze the synthesis of new strands during replication exhibit a feature called processivity. They remain bound to the parental template strand while continuing to "process" down the chain, rather than dissociating and reassociating as each nucleotide is added. Consequently, synthesis is much more rapid than it would be with an enzyme that was not processive.

E. Elimination of Base-Pairing Errors

In *E. coli*, the replicative enzyme Pol III also performs a proofreading or editing function. This enzyme has 3′–5′-exonuclease activity in addition to its polymerase activity (see Table 13.1). If the nucleotide at the end of the growing chain is incorrectly base-paired with the template strand, Pol III removes this nucleotide before continuing to lengthen the growing chain. This proofreading activity eliminates

Table 13.1. Functions of Bacterial DNA Polymerases

Polymerases	Functions[a]	Exonuclease Activity[b]
Pol I	Filling of gap after removal of RNA primer DNA repair Removal of RNA primer in conjunction with RNAse H	5′ to 3′ and 3′ to 5′
Pol II	DNA repair	3′ to 5′
Pol III	Replication – synthesis of DNA	3′ to 5′

[a] Synthesis of new DNA strands always occurs 5′ to 3′
[b] Exonucleases remove nucleotides from DNA strands and act at the 5′ end (cleaving 5′ to 3′) or at the 3′ end (cleaving 3′ to 5′).

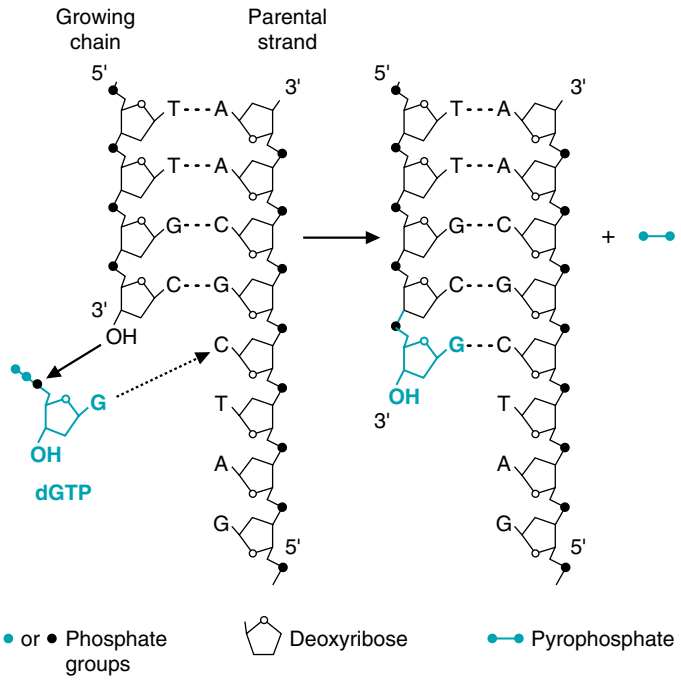


Fig. 13.4. Action of DNA polymerase. Deoxyribonucleoside triphosphates serve as precursors (substrates) used by DNA polymerase to lengthen the DNA chain. DNA polymerase copies the DNA template strand in the 3′ to 5′ direction. The new strand grows 5′ to 3′.

most base-pairing errors as they occur. Only about one base pair in a million is mismatched in the final DNA product; the error rate is about 10^{-6}. If this proofreading activity is experimentally removed from the enzyme, the error rate increases to about 10^{-3}.

After replication, other mechanisms replace mismatched bases that escaped proofreading so that the fidelity of DNA replication is very high. The two processes of proofreading and postreplication mismatch repair result in an overall error rate of about 10^{-10}, that is, less than one mismatched base pair in 10 billion.

F. Function of RNA Primers

DNA polymerase cannot initiate the synthesis of new strands; it requires the presence of a free 3′-OH group to function. Therefore, a primer is required to supply the free 3′-OH group. This primer is an RNA oligonucleotide. It is synthesized in a 5′ to 3′ direction by an RNA polymerase (primase) that copies the DNA template strand. DNA polymerase initially adds a deoxyribonucleotide to the 3′-hydroxyl group of the primer and then continues adding deoxyribonucleotides to the 3′-end of the growing strand (Fig. 13.5).

G. DNA Synthesis at the Replication Fork

Both parental strands are copied at the same time in the direction of the replication fork, an observation difficult to reconcile with the known activity of DNA polymerase, which can produce chains only in a 5′ to 3′ direction. Because the parental strands run in opposite directions relative to each other, synthesis should occur in a 5′ to 3′ direction **toward** the fork on one template strand and in a 5′ to 3′ direction **away** from the fork on the other template strand.

Okazaki resolved this dilemma by showing that synthesis on one strand, called the leading strand, is continuous in the 5′ to 3′ direction toward the fork. The other

Nucleases cleave phosphodiester bonds. Endonucleases cleave bonds within polynucleotide chains. Exonucleases cleave bonds at the ends of polynucleotide chains, removing one nucleotide at a time. 5′-Exonucleases start at the 5′-end of the chain and cleave 5′ to 3′. 3′-Exonucleases start at the 3′-end and cleave 3′ to 5′.

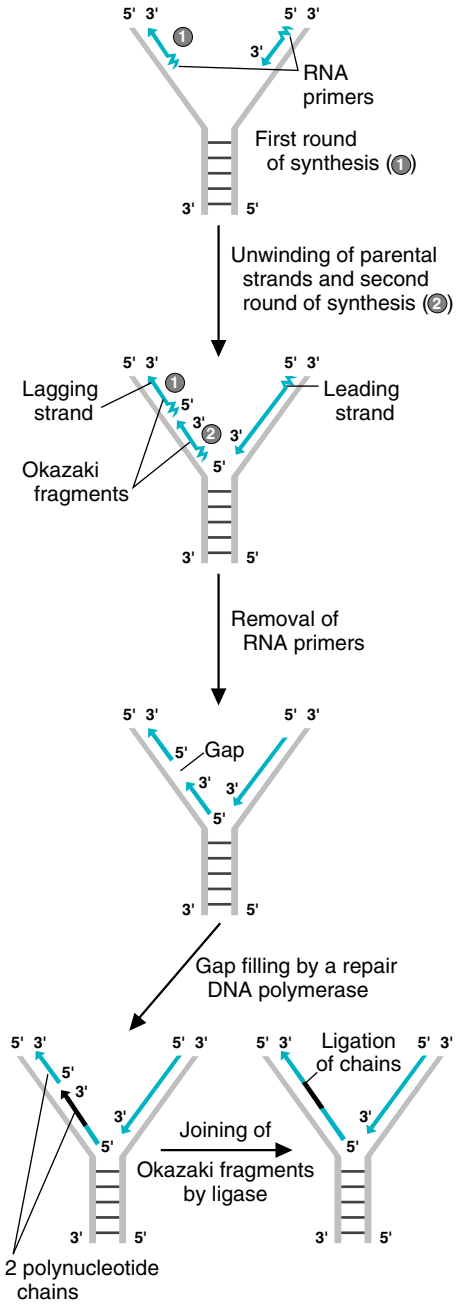


Fig. 13.5. Synthesis of DNA at the replication fork. (See Fig. 13.6 for the ligation reaction.)

RNase H is a ribonuclease that specifically degrades RNA from an RNA-DNA hybrid. The HIV virus (see Chapter 12) converts an RNA genome to a double-stranded DNA copy using the enzyme reverse transcriptase. An intermediate in the conversion of the single-stranded RNA genome to double-stranded DNA is an RNA-DNA hybrid. To remove the RNA so a double-stranded DNA molecule can be made, reverse transcriptase contains RNase H activity. Scientists are currently attempting to target the RNase H activity of reverse transcriptase with inhibitors as a means of controlling spread of the virus.

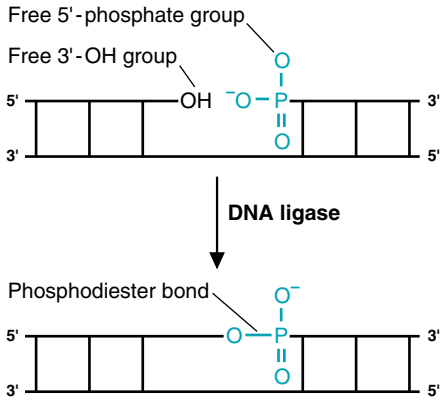


Fig. 13.6. Action of DNA ligase. Two polynucleotide chains, one with a free 3′-OH group and one with a free 5′-phosphate group, are joined by DNA ligase, which forms a phosphodiester bond.

strand, called the lagging strand, is synthesized discontinuously in short fragments (see Fig. 13.5). These fragments, named for Okazaki, are produced in a 5′ to 3′ direction (away from the fork), but then joined together so that, overall, synthesis proceeds toward the replication fork.

H. Function of DNA Ligase

As replication progresses, the RNA primers are removed from Okazaki fragments, probably by the combined action of DNA polymerase I (Pol I, using its 5′ → 3′ exonuclease activity) and RNase H. Pol I fills in the gaps produced by removal of the primers. Because DNA polymerases cannot join two polynucleotide chains together, an additional enzyme, DNA ligase, is required to perform this function. The 3′-hydroxyl group at the end of one fragment is ligated to the phosphate group at the 5′-end of the next fragment (Fig. 13.6).

II. DNA SYNTHESIS IN EUKARYOTES

The process of replication in eukaryotes is similar to that in prokaryotes. Differences in the processes are related mainly to the vastly larger amount of DNA in eukaryotic cells (over 1,000 times the amount in *E. coli*) and the association of eukaryotic DNA with histones in nucleosomes. Enzymes with DNA polymerase, primase, ligase, helicase, and topoisomerase activity are all present in eukaryotes, although these enzymes differ in some respects from those of prokaryotes.

A. Eukaryotic Cell Cycle

The cell cycle of eukaryotes consists of four phases (Fig. 13.7). The first three phases (G_1, S, and G_2) constitute interphase. Cells spend most of their time in these three phases, carrying out their normal metabolic activities. The fourth phase is mitosis, the process of cell division. This phase is very brief.

The first phase of the cell cycle, G_1 (the first "gap" phase), is the most variable in length. Late in G_1, the cells prepare to duplicate their chromosomes (e.g., by producing nucleotide precursors). In the second or S phase, DNA replicates. Nucleosomes disassemble as the replication forks advance. Throughout S phase, the synthesis of histones and other proteins associated with DNA is markedly increased.

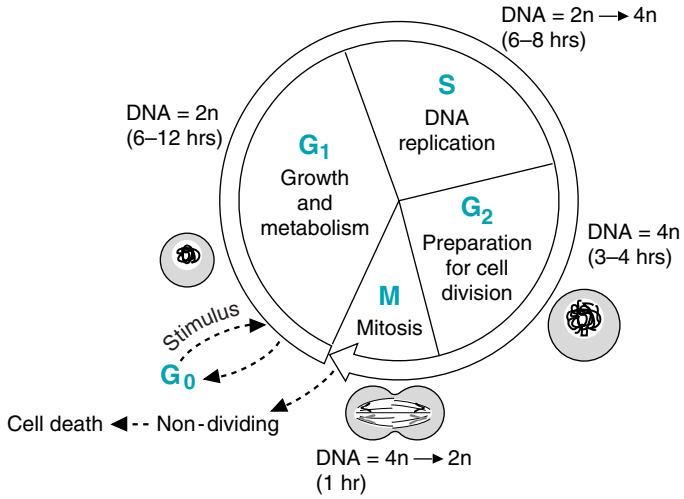


Fig. 13.7. Eukaryotic cell cycle. The times given for the length of each phase are for cells growing in culture.

The amount of DNA and histones both double, and chromosomes are duplicated. Histones complex with DNA, and nucleosomes are formed very rapidly behind the advancing replication forks.

During the third phase of the cell cycle, G_2 (the second "gap" phase), the cells prepare to divide and synthesize tubulin for construction of the microtubules of the spindle apparatus. Finally, division occurs in the brief mitotic or M phase.

After mitosis, some cells reenter G_1, repeatedly going through the phases of the cell cycle and dividing. Other cells leave the cycle after mitosis, never to divide again, or they enter an extended G_1 phase (sometimes called G_0), in which they remain for long periods. On the appropriate signal, cells in G_0 are stimulated to reenter the cycle and divide.

B. Points of Origin for Replication

In contrast to bacterial chromosomes (see section I.A. of this chapter), eukaryotic chromosomes have multiple points of origin at which replication begins. "Bubbles" appear at these points on the chromosomes. At each end of a bubble, a replication fork forms; thus, each bubble has two forks. DNA synthesis occurs at each of these forks, as illustrated in Figure 13.8. As the bubbles enlarge, they eventually merge, and replication is completed. Because eukaryotic chromosomes contain multiple points of origin of replication (and, thus, multiple replicons-units of replication), duplication of such large chromosomes can occur within a few hours.

C. Eukaryotic DNA polymerases

At least nine DNA polymerases exist in eukaryotic cells (α, β, γ, δ, ϵ, ζ, κ, η, and ι) (Table 13.2). Polymerase δ (pol δ) is the major replicative enzyme. Pol α and pol ϵ are also involved in relication. Polymerases β and ϵ, as well as pol α, appear to be involved in DNA repair. Pol γ is located in mitochondria and replicates the DNA of this organelle. Polymerases ζ, κ, η and ι, which lack $3' \rightarrow 5'$ exonuclease activity, are used when DNA is damaged.

D. The Eukaryotic Replication Complex

Many proteins bind at or near the replication fork and participate in the process of duplicating DNA (Fig. 13.9)(Table 13.3). Polymerase δ (pol δ) is the major replica-

Table 13.2. Functions of Eukaryotic DNA Polymerases

Polymerase	Functions[a]	Exonuclease Activity
Pol α	Replication (in a complex with primase and aids in starting the primer) DNA repair	None
Pol β	DNA repair exclusively	None
Pol γ	DNA replication in mitochondria	3′ to 5′
Pol δ	Replication (processive DNA synthesis on leading and lagging strands) DNA repair	3′ to 5′
Pol ϵ	Replication (in some tissues takes the place of Pol d) DNA repair	3′ to 5′
Pol κ	DNA repair (bypass polymerase)[b]	None
Pol η	DNA repair (bypass polymerase)	None
Pol ζ	DNA repair (bypass polymerase)	None
Pol ι	DNA repair (bypass polymerase)	None

[a] Synthesis of new DNA strands always occurs 5′ to 3′.
[b] Bypass polymerase are able to "bypass" areas of DNA damage and continue DNA replication. Some enzymes are error-free and insert the correct bases; other enzymes are error prone and insert random bases.

HeLa cells, derived from a human cervical carcinoma, are rapidly dividing cells that can be grown in culture flasks. Their cell cycle is approximately 20 hours. Only 1 hour of this time is spent in mitosis.

Cells within the body that divide less frequently, such as liver cells, spend days, weeks, or months in interphase before going through a brief mitotic phase.

Although Prometheus was chained to a rock as punishment for his theft of fire from the gods, and a vulture pecked at his liver each day, he survived. Can you guess why?

In the human body, many cells cycle frequently, e.g., hair follicles, skin cells, and cells of the duodenal crypts. Other cells, such as the precursors of red blood cells, divide a number of times, then lose their nuclei and leave the cell cycle to form mature red blood cells. These cells transport oxygen and carbon dioxide between the lungs and other tissues for about 120 days, then die. Other cells are normally quiescent (in G_0). However, they can be stimulated to divide. In many instances, the stimuli are growth factors or hormones (e.g., mammary aveolar cells and uterine cells). In the case of liver cells, the stimulus is produced by death of some of the cells.

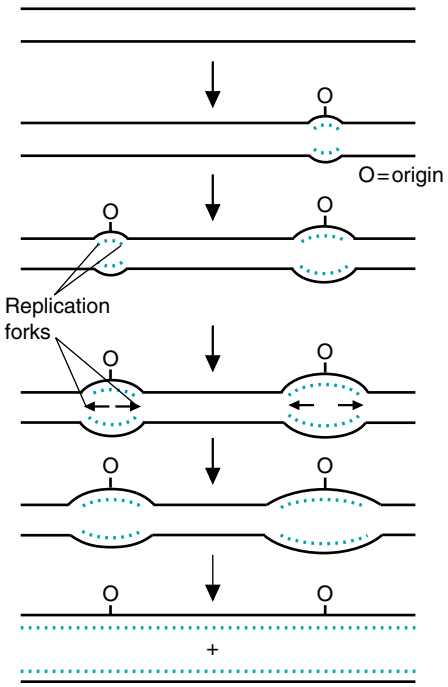


Fig. 13.8. Replication of a eukaryotic chromosome. Synthesis is bidirectional from each point of origin (O) and semiconservative, each daughter DNA helix contains one intact parental strand (solid line) and one newly synthesized strand (dashed line).

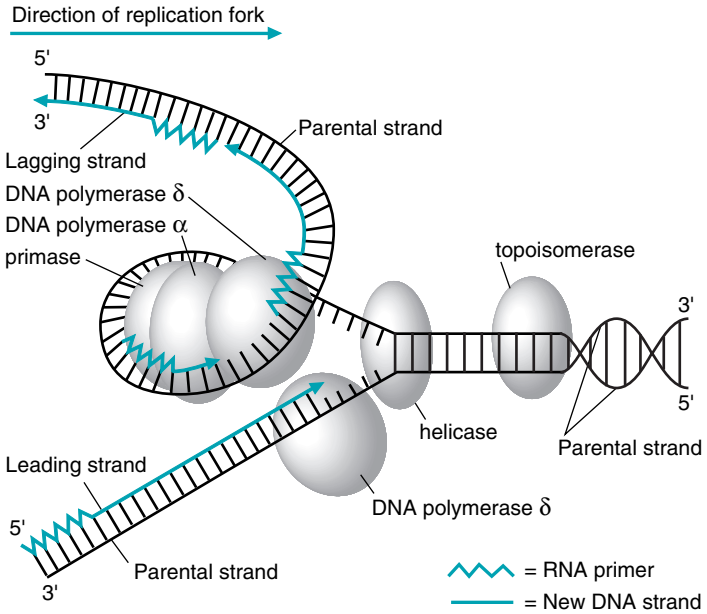


Fig. 13.9. Replication complex in eukaryotes. The lagging strand is shown looped around the replication complex. Single-strand binding proteins (not shown) are bound to the unpaired, single-stranded DNA. Other proteins also participate in this complex (see text).

Okazaki fragments are much smaller in eukaryotes than in prokaryotes (about 200 nucleotides vs. 1,000 to 2,000). Because the size of eukaryotic Okazaki fragments are equivalent to the size of the DNA found in nucleosomes, it seems likely that one nucleosome at a time may release its DNA for replication.

A: Liver cells are in G_0. Up to 90% of the human liver can be removed. The remaining liver cells are stimulated to re-enter the cell cycle and divide, regenerating a mass equivalent to the original mass of the liver within a few weeks. The myth of Prometheus indicates that the capacity of the liver to regenerate was recognized even in ancient times.

tive enzyme. However, before it acts, a primase associated with polymerase α (pol δ) produces an RNA primer (approximately 10 nucleotides in length). Then pol α adds about 20 deoxyribonucleotides to this RNA and dissociates from the template, because of the low processivity of pol α. On the leading strand, pol δ adds deoxyribonucleotides to this RNA-DNA primer, continuously producing this strand. Pol δ is a highly processive enzyme.

The lagging strand is produced from a series of Okazaki fragments (see Fig. 13.5). Synthesis of each Okazaki fragment is initiated by pol α and its associated primase, as described above. After pol α dissociates, pol δ adds deoxyribonucleotides to the primer, producing an Okazaki fragment. Pol δ stops synthesizing one fragment when it reaches the start of the previously synthesized Okazaki fragment (see Fig. 13.5). The primer of the previously synthesized Okazaki fragment is

Table 13.3 Major proteins involved in replication

DNA polymerases	- add nucleotides to a strand growing 5′ → 3′, copying a DNA template 3′ → 5′
Primase	- synthesizes RNA primers
Helicases	- Separate parental DNA strands, i.e., unwind the double helix
Single-strand binding proteins	- Prevent single strands of DNA from reassociating
Topoisomerases	- Relieve torsional strain on parental duplex caused by unwinding
Enzymes that remove primers	- RNase H – hydrolyzes RNA of DNA-RNA hybrids - Flap endonucleases 1 (FEN1) – recognizes "flap" (Unannealed portion of RNA) near 5′-end of primer and cleaves downstream in DNA region of primer
DNA ligase	- Joins, by forming a phosphodiester bond, two adjacent DNA strands that are bound to the same template

removed by flap endonuclease 1 (FEN1) and Rnase H. The gap left by the primer is filled by a DNA polymerase that uses the parental DNA strand as its template and the newly synthesized Okazaki fragment as its primer. DNA ligase subsequently joins the Okazaki fragments together (see Fig. 13.6).

Obviously, eukaryotic replication requires many proteins. The complexity of the fork and the fact that it is not completely understood limits the detail shown in Figure 13.9. One protein not shown is proliferating cell nuclear antigen (PCNA), which is involved in organizing and orchestrating the replication process.

Additional activities that occur during replication include proofreading and DNA repair. Pol δ, which is part of the replication complex, has the 3′ → 5′-exonuclease activity required for proofreading. Enzymes that catalyze repair of mismatched bases are also present (see section III.B.3 of this chapter). Consequently, eukaryotic replication occurs with high fidelity; approximately one mispairing occurs for every 10^9 to 10^{12} nucleotides incorporated into growing DNA chains.

E. Replication of the Ends of Chromosomes

Eukaryotic chromosomes are linear, and the ends of the chromosomes are called telomeres. As DNA replication approaches the end of the chromosome, a problem develops in the lagging strand (Fig. 13.10). Either primase cannot lay down a primer at the very end of the chromosome, or, after DNA replication is complete, the RNA at the end of the chromosome is degraded. Consequently, the newly synthesized strand is shorter at the 5′ end, and there is a 3′-overhang in the DNA strand being replicated. If the chromosome became shorter with each successive replication, genes would be lost. How is this problem solved?

The 3′ overhang is lengthened by the addition of telomeres so that primase can bind and synthesize the complementary strand. Telomeres consist of a repeating sequence of bases (TTAGGG for humans), which may be repeated thousands of times. The enzyme telomerase contains both proteins and RNA and acts as an RNA-dependent DNA polymerase (just like reverse transcriptase). The RNA within telomerase contains the complementary copy of the repeating sequence in the telomeres and can base pair with the existing 3′-overhang (Fig. 13.11). The polymerase activity of telomerase then uses the existing 3′- hydroxyl group of the overhang as a primer, and its own RNA as a template, and synthesizes new DNA that lengthens the 3′- end of the DNA strand. The telomerase moves down the DNA toward the new 3′- end and repeats the process a number of times. When the 3′-overhang is sufficiently long, primase binds, and synthesis of the complementary strand is completed. The 3′ overhang can also form a complicated structure with telomere binding proteins to protect the ends of the chromosomes from damage and nuclease attack once they have been lengthened

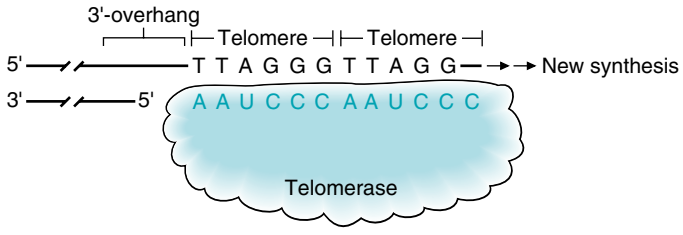


Fig. 13.11. Telomerase action. The RNA present in telomerase base-pairs with the overhanging 3′-end of telomeres and extends it by acting both as a template and a reverse transcriptase. After copying a small number of repeats, the complex moves down to the 3′-end of the overhang and repeats the process.

Proliferating cell nuclear antigen (PCNA) is used clinically as a diagnostic marker for proliferating cells.

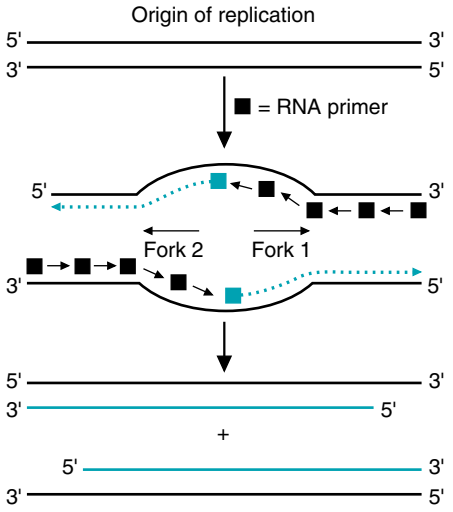


Fig. 13.10. The "end-replication" problem in linear chromosomes. After replication, the telomeres have 3′-overhangs. When these molecules are replicated, chromosome shortening will result. The figure depicts a linear chromosome with one origin of replication. At the origin, two replication forks are generated, each moving in the opposite direction, labeled as Fork I and Fork II. As Fork I moves to the right, the bottom strand is read in the 3′ to 5′ direction, which means it is the template for the leading strand. The newly synthesized DNA complementary to the upper strand at Fork 1 will be the lagging strand. Now consider Fork 2. As this replication fork moves to the left, the upper strand is read in the 3′ to 5′ direction, so the newly synthesized DNA complementary to this strand will be the leading strand. For this fork, the newly synthesized DNA complementary to the bottom strand will be the lagging strand.

An inability to replicate telomeres has been linked to cell aging and death. Many somatic cells do not express telomerase; when placed in culture they survive a fixed number of population doublings, enter senescence, and then die. Analysis has shown significant telomere shortening in those cells. In contrast, stem cells do express telomerase and appear to have an infinite lifetime in culture. Research is underway to understand the role of telomeres in cell aging, growth, and cancer.

Nick O'Tyne has been smoking for 40 years in spite of the warnings on cigarette packs that this habit can be dangerous and even deadly. The burning of tobacco, and, for that matter, the burning of any organic material, produces many different carcinogens, such as benzo[*a*]pyrene. These carcinogens coat the airways and lungs. They can cross cell membranes and interact with DNA, causing damage to bases that interferes with normal base pairing. If these DNA lesions cannot be repaired or if they are not repaired rapidly enough, a permanent mutation can be produced when the cells replicate. Some mutations are silent, whereas other mutations can lead to abnormal cell growth, and cancer results.

Melanomas develop from exposure of the skin to the ultraviolet rays of the sun. The ultraviolet radiation causes pyrimidine dimers to form in DNA. Mutations may result that produce melanomas, appearing as dark brown growths on the skin.

Fortunately, **Mel Anoma**'s malignant skin lesion was discovered at an early stage. Because there was no evidence of cancer in the margins of the resected mass, full recovery was expected. However, lifelong surveillance for return of the melanoma was planned.

Pyrimidine dimers, most commonly thymine dimers, can be repaired by photoreactivating enzymes that cleave the bonds between the bases by using energy from visible light. In this process, nucleotides are not removed from the damaged DNA. This repair process is used by bacteria and might serve as a very minor repair mechanism in human cells.

III. DNA REPAIR

A. Actions of Mutagens

Despite proofreading and mismatch repair during replication, some mismatched bases do persist. Additional problems may arise from DNA damaged by mutagens, chemicals produced in cells, inhaled, or absorbed from the environment that cause mutations. Mutagens that cause normal cells to become cancer cells are known as carcinogens. Unfortunately, mismatching of bases and DNA damage produce thousands of potentially mutagenic lesions in each cell every day. Without repair mechanisms, we could not survive these assaults on our genes.

DNA damage can be caused by radiation and by chemicals (Fig. 13.12). These agents can directly affect the DNA or they can act indirectly. For example, x-rays, a type of ionizing radiation, act indirectly to damage DNA by exciting water in the cell and generating the hydroxyl radical, which reacts with DNA, thereby altering the structure of the bases or cleaving the DNA strands.

While exposure to x-rays is infrequent, it is more difficult to avoid exposure to cigarette smoke and virtually impossible to avoid exposure to sunlight. Cigarette smoke contains carcinogens such as the aromatic polycyclic hydrocarbon benzo[*a*]pyrene (see Fig. 13.12). When this compound is oxidized by cellular enzymes, which normally act to make foreign compounds more water soluble and easy to excrete, it becomes capable of forming bulky adducts with guanine residues in DNA. Ultraviolet rays from the sun, which also produce distortions in the DNA helix, excite adjacent pyrimidine bases on DNA strands, causing them to form covalent dimers (Fig. 13.13).

B. Repair Mechanisms

The mechanisms used for the repair of DNA have many similarities (Fig. 13.14). First, a distortion in the DNA helix is recognized, and the region containing the distortion is removed. The gap in the damaged strand is replaced by the action of a DNA polymerase that uses the intact, undamaged strand as a template. Finally, a ligase seals the nick in the strand that has undergone repair.

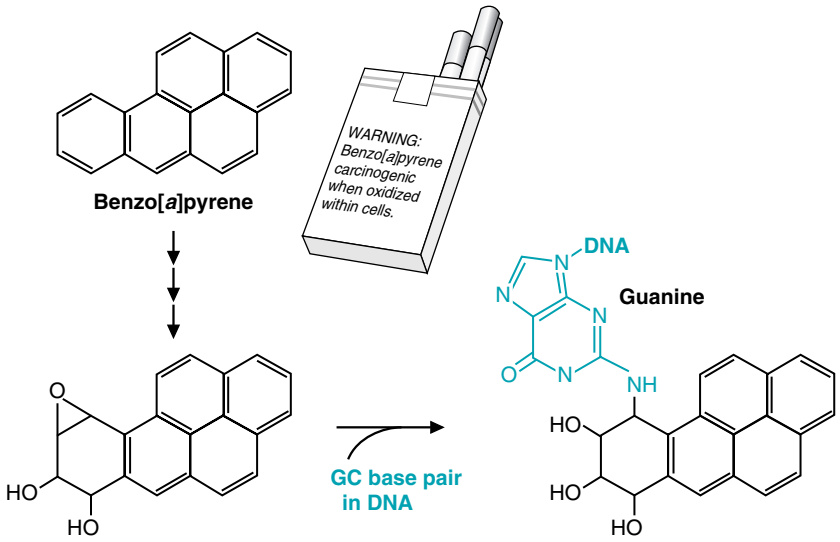


Fig. 13.12. Oxidation of benzo[*a*]pyrene and covalent binding to DNA. Benzo[*a*]pyrene is not carcinogenic until it is oxidized within cells. Then it can covalently bind to guanine residues in DNA, interrupting hydrogen bonding in G-C base pairs and producing distortions of the helix.

1. NUCLEOTIDE EXCISION REPAIR

Nucleotide excision repair involves local distortions of the DNA helix, such as mismatched bases or bulky adducts (e.g., oxidized benzo[*a*]pyrene) (Fig. 13.15, see also Fig. 13.14). Endonucleases cleave the abnormal chain and remove the distorted region. The gap is then filled by a DNA polymerase that adds deoxyribonucleotides, one at a time, to the 3′-end of the cleaved DNA, using the intact, complementary DNA strand as a template. The newly synthesized segment is joined to the 5′-end of the remainder of the original DNA strand by a DNA ligase.

2. BASE EXCISION REPAIR

DNA glycosylases recognize small distortions in DNA involving lesions caused by damage to a single base (e.g., the conversion of cytosine to uracil). A glycosylase cleaves the *N*-glycosidic bond that joins the damaged base to deoxyribose (see Fig. 13.15). The sugar–phosphate backbone of the DNA now lacks a base at this site (known as an apurinic or apyrimidinic site, or an AP site). Then an AP endonuclease cleaves the sugar–phosphate strand at this site. Subsequently, the same types of enzymes involved in other types of repair mechanisms restore this region to normal.

3. MISMATCH REPAIR

Mismatched bases (bases that do not form normal Watson-Crick base pairs) are recognized by enzymes of the mismatch repair system. Because neither of the bases in a mismatch is damaged, these repair enzymes must be able to determine which base of the mispair to correct.

The mismatch repair enzyme complex acts during replication when an incorrect, but normal base (i.e., A, G, C, or T) is incorporated into the growing chain (Fig. 13.16). In bacteria, parental DNA strands contain methyl groups on adenine bases in specific sequences. During replication, the newly synthesized strands are not immediately methylated. Before methylation occurs, the proteins involved in mismatch repair can distinguish parental from newly synthesized strands. A region of the new, unmethylated strand, containing the mismatched base, is removed and replaced.

Human enzymes also can distinguish parental from newly synthesized strands and repair mismatches. However, the mechanisms have not yet been as clearly defined as those in bacteria.

Pyrimidine dimers occur frequently in the skin. Usually repair mechanisms correct this damage, and cancer rarely occurs. However, in individuals with xeroderma pigmentosum, cancers are extremely common. These individuals have defects in their DNA repair systems. The first defect to be identified was a deficiency of the endonuclease involved in removal of pyrimidine dimers from DNA. Because of the inability to repair DNA, the frequency of mutation increases. A cancer develops once proto-oncogenes or tumor suppressor genes mutate. By scrupulously avoiding light, these individuals can reduce the number of skin cancers that develop.

Spontaneous deamination occurs frequently in human DNA and converts cytosine bases to uracil. This base is not normally found in DNA and is potentially harmful because U pairs with A, forming U-A base pairs instead of the normal C-G pairs. To prevent this change from occurring, a uracil *N*-glycosylase removes uracil, and it is replaced by a cytosine via base excision repair.

Hereditary nonpolyposis colorectal cancer (a human cancer that does not arise from intestinal polyps) is caused by mutations in genes for proteins involved in mismatch repair (hMSH1, hMSH2, hPMS1, or hPMS2). The inability to repair mismatches increases the mutation frequency, resulting in cancers from mutations in growth regulatory genes.

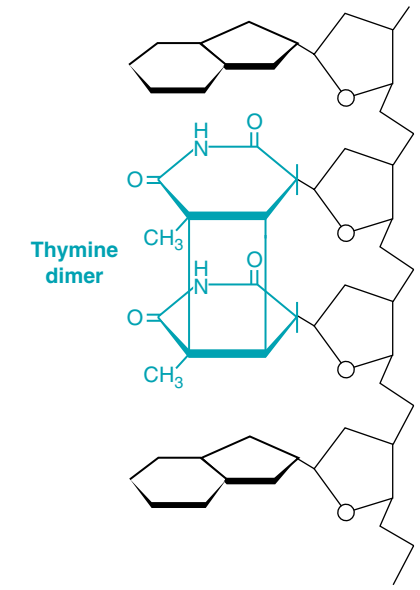


Fig. 13.13. A thymine dimer in a DNA strand. Ultraviolet light can cause two adjacent pyrimidines to form a covalent dimer.

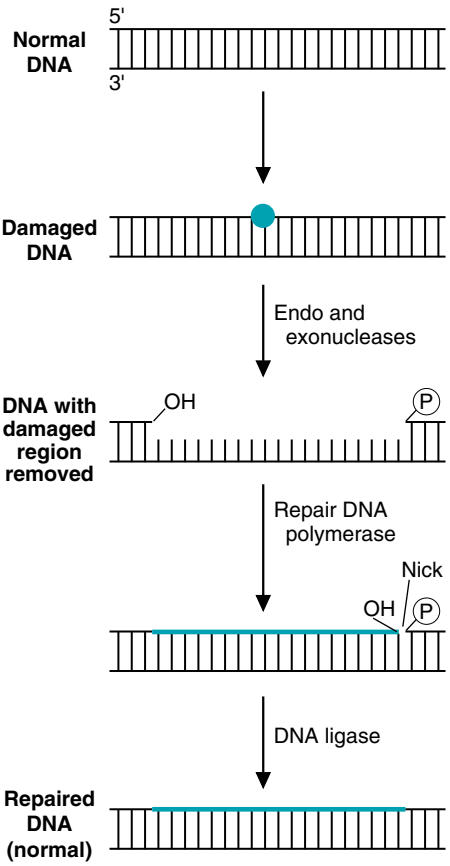


Fig. 13.14. Common steps in DNA repair mechanisms.

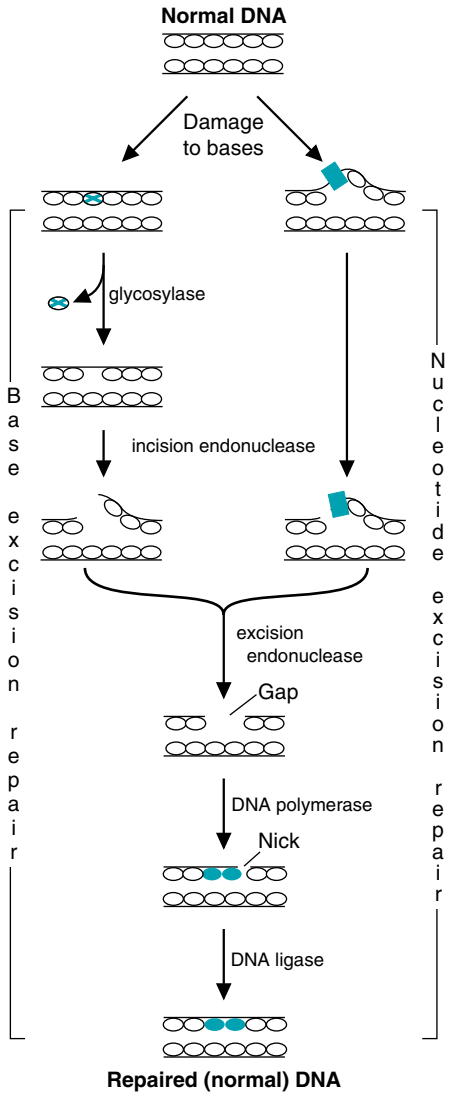


Fig. 13.15. Types of damage and various repair mechanisms. In base excision repair, the glycosylase cleaves the glycosidic bond between the altered base (shown with an X) and ribose. In nucleotide excision repair, the entire nucleotide is removed at once. The gap formed by the incision (cut) and excision (removal) endonucleases is usually several nucleotides wider than the one shown.

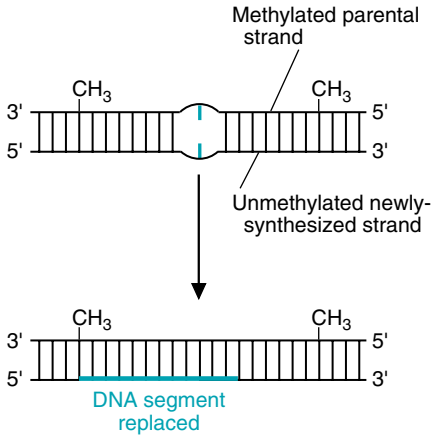


Fig. 13.16. Mismatch repair. Normal, undamaged but mismatched bases bind proteins of the mismatch repair system. In bacteria, these proteins recognize the older, parental strand because it is methylated and replace a segment of newly synthesized (and unmethylated) DNA containing the mismatched base. The mechanism for distinguishing between parental and newly synthesized strands in humans is not as well understood.

4. TRANSCRIPTION-COUPLED REPAIR

Genes that are actively transcribed to produce mRNA are preferentially repaired. The RNA polymerase that is transcribing a gene (see Chapter 14 for a description of the process) stalls when it encounters a damaged region of the DNA template. Excision repair proteins are attracted to this site and repair the damaged region. Subsequently, RNA polymerase can resume transcription.

IV. GENETIC REARRANGEMENTS

The exchange of segments between DNA molecules occurs quite frequently and is responsible for genetic alterations that can have beneficial or devastating consequences for the affected individuals and, in some instances, for their offspring. The DNA segments that are exchanged may be homologous (that is, of very similar sequence) or they may be totally unrelated. The size of these segments can range from a few nucleotides to tens of thousands and can include many different genes or portions of genes. Many of the enzymes involved in these exchanges are the same as or similar to those used for replication and repair and include endonucleases, exonucleases, unwinding enzymes, topoisomerases, DNA polymerases, and ligases.

One type of genetic rearrangement that has been observed for many years is "crossing-over" between homologous chromosomes during meiosis. Another type occurs in stem cells as they differentiate into lymphocytes. Segments of the genes of stem cells are rearranged so that the mature cell is capable of producing only a single type of antibody (see Chapter 16). Other types of genetic exchanges involve transposable elements (transposons) that can move from one site in the genome to another or produce copies that can be inserted into new sites. Translocations occur when chromosomes break and portions randomly become joined to other chromosomes, producing gross changes that can be observed under the light microscope. Genetic exchanges can even occur between species, for example, when foreign DNA is inserted into the human genome as a result of viral infection.

A. General or Homologous Recombination

Various models, supported by experimental evidence, have been proposed for the mechanism of recombination between homologous DNA sequences. Although

these mechanisms are complex, a simplified scheme for one type of recombination is presented in Figure 13.17.

Initially, two homologous chromosomes or segments of double-helical (duplex) DNA that have very similar, but not necessarily identical, sequences become aligned (see Fig. 13.17). One strand of one duplex is nicked by an enzyme and invades the other DNA duplex, base-pairing with a region of complementary sequence. The match between the sequences does not have to be perfect, but a significant number of bases must pair so that the strand displaced from its partner can form a displacement (D) loop. This D loop is nicked, and the displaced strand now base-pairs with the former partner of the invading strand. Ligation occurs, and a Holliday structure is generated (see Fig. 13.17). The branch point of the Holliday structure can migrate and may move many thousands of nucleotides from its original position. The Holliday structure, named for the scientist who discovered it, is finally cleaved and then religated, forming two chromosomes that have exchanged segments. In addition to enzymes similar to those used in DNA replication, enzymes for strand invasion, branch migration, and cleavage of the Holliday structure are required.

B. Translocations

Breaks in chromosomes, caused by agents such as x-rays or chemical carcinogens, can result in gross chromosomal rearrangements (Fig. 13.18). If the free ends of the DNA at the break point reseal with the free ends of a different broken chromosome, a translocation is produced. These exchanges of large portions of chromosomes can have deleterious effects and are frequently observed in cancer cells.

C. Transposable Elements

Movable (or transposable) genetic elements, "jumping genes," were first observed by Barbara McClintock in the 1940s. Her work, initially greeted with skepticism, was ultimately accepted, and she was awarded the Nobel Prize in 1983.

Transposons are segments of DNA that can move from their original position in the genome to a new location (Fig. 13.19). They are found in all organisms. Transposons contain the gene for an enzyme called a transposase, which is involved in cleaving the transposon from the genome and moving it from one location to another.

Retroposons are similar to transposons except that they involve an RNA intermediate. Reverse transcriptase (see below) makes a single-stranded DNA copy of the RNA. A double-stranded DNA is then produced that is inserted into the genome at a new location.

V. REVERSE TRANSCRIPTASE

Reverse transcriptase is an enzyme that uses a single-stranded RNA template and makes a DNA copy (Fig. 13.20). The RNA template can be transcribed from DNA by RNA polymerase or obtained from another source, such as an RNA virus. The DNA copy of the RNA produced by reverse transcriptase is known as complementary DNA (because it is complementary to the RNA template), or cDNA. Retroviruses (RNA viruses) contain a reverse transcriptase, which copies the viral RNA genome. A double-stranded cDNA is produced, which can become integrated into the human genome (see Fig. 12.23). After integration, the viral genes may be inactive, or they may be transcribed, sometimes causing diseases such as AIDS or cancer (see Chapter 18).

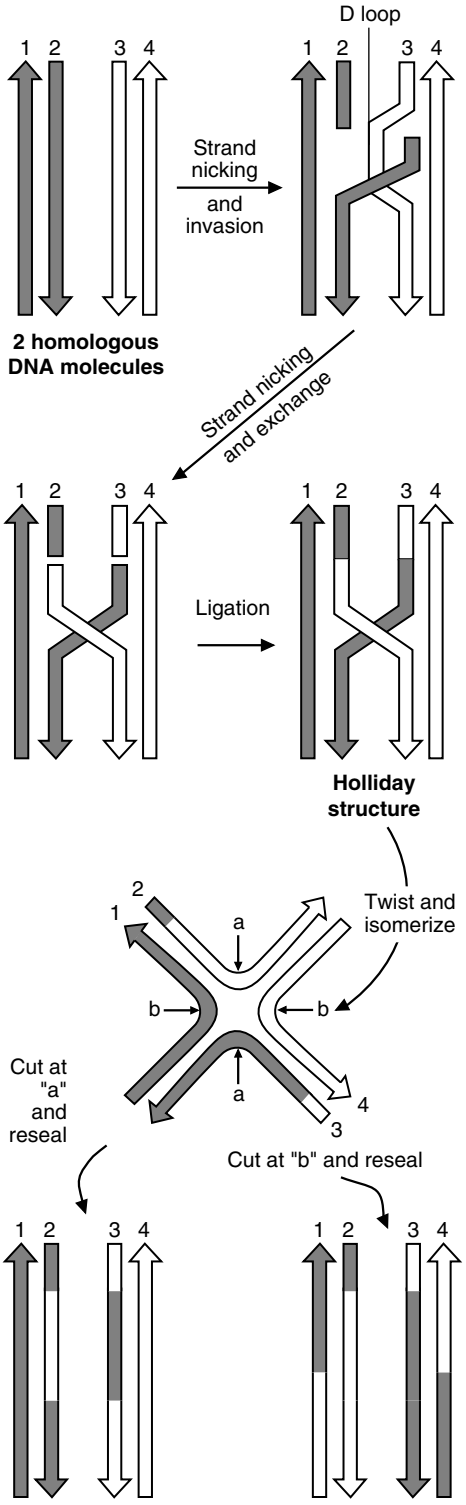


Fig. 13.17. Key steps in recombination.

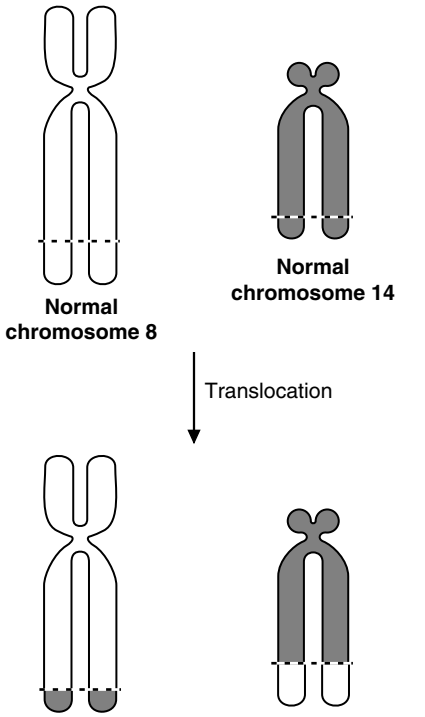


Fig. 13.18. A chromosomal translocation. A portion of the long arm of chromosome 8 is exchanged for a portion of the long arm of chromosome 14. This chromosomal translocation occurs in Burkitt's lymphoma.

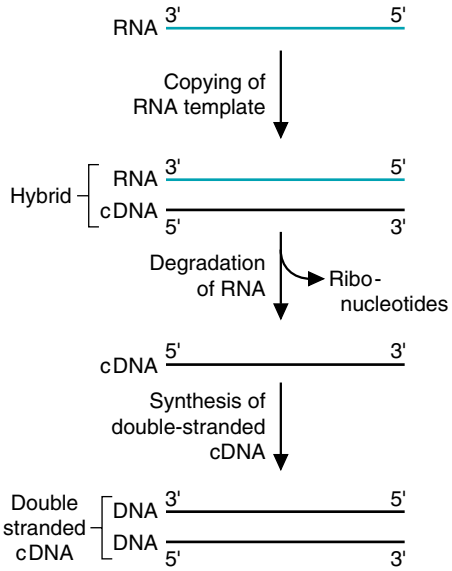


Fig. 13.20. Action of reverse transcriptase. This enzyme catalyzes the production of a DNA copy from an RNA template. The RNA of a DNA-RNA hybrid is degraded, and the single DNA strand is used as a template to make double-stranded DNA. This figure is a simplified version of a more complex process.

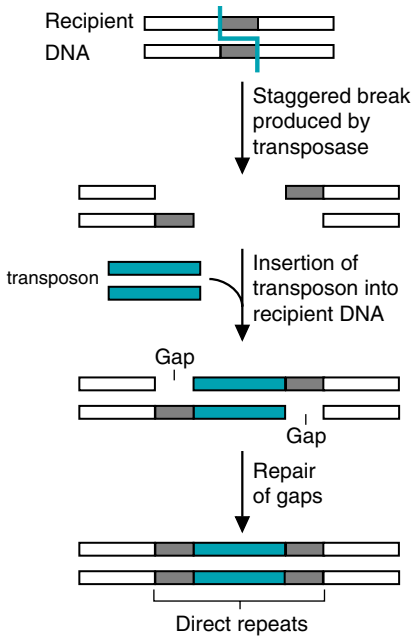


Fig. 13.19. Transposons. The steps involved in transposition are shown. Direct repeats are regions of DNA that have the same base sequence in the 5′ → 3′ direction.

CLINICAL COMMENTS

Ivy Sharer. Ivy Sharer contracted AIDS when she used needles contaminated with HIV to inject drugs intravenously. Intravenous drug abusers account for 15 to 20% of new AIDS cases in the United States. HIV mutates rapidly, and, therefore, current treatment involves a combination of drugs that affect different aspects of its life cycle. This multidrug therapy lowers the viral titer (the number of viral particles found in a given volume of blood), sometimes to undetectable levels. However, if treatment is not followed carefully (i.e., if the patient is not "compliant"), the titer increases rapidly. Therefore, Ivy's physician emphasized that she must carefully follow her drug regimen.

Di Abietes. Di Abietes' poorly controlled diabetes mellitus predisposed her to a urinary tract infection because glucose in the urine serves as a "culture medium" for bacterial growth. The kidney glomerulotubular unit reabsorbs filtered glucose so that normally, the urine is glucose-free. However, when serum blood glucose levels exceed 175 to 185 mg/dL (the tubular threshold for glucose), the capacity for reabsorption is exceeded. In Ms. Abietes' case, blood glucose levels frequently exceed this threshold.

Mel Anoma. The average person has 20 moles on the body surface; however, only seven people of every 100,000 develop a malignant melanoma. The incidence of malignant melanoma, however, is rising rapidly. Because 35 to 40% of patients with malignant melanoma die as a result of this cancer, the physician's decision to perform a biopsy on a pigmented mole with an irregular border and variation of color probably saved **Mel Anoma's** life.

Nick O'Tyne. Lung cancer currently accounts for one fifth of all cancers in men and one tenth in women. The overall 5-year survival rate is still less than 15%. For those who smoke two or more packs of cigarettes daily, as does

Nick O'Tyne, the death rate is 265 per 100,000 population. Thankfully, cigarette smoking has declined in the United States. Whereas 50% of men and 32% of women smoked in 1965, these figures have currently fallen to 26% and 24%, respectively.

BIOCHEMICAL COMMENTS

Chemical carcinogens and tumor promoters. Once it was realized that a number of chemicals react with DNA, leading to mutations, and that mutations may lead to tumor formation, toxicologists searched for chemicals with the ability to cause skin tumors in rats. To test a chemical, it was applied to an area of shaved skin on the back of a rat to see whether a tumor would develop. The chemicals fell into two groups: Group I, the initiators, caused mutations in DNA and group II, the promoters, greatly enhanced the probability that cells that had been previously initiated would develop a tumor. Compounds falling into group II activate a protein kinase (protein kinase C), which is usually only transiently activated when normal cells are stimulated to grow. Tumor promoters, however, are long-lived, and lead to an enhanced activation of protein kinase C.

Is there an easy way to determine whether a compound is an initiator? Bruce Ames developed a rapid and simple test to determine whether chemicals are mutagens. The basic test uses bacteria that have a mutation in a gene necessary for histidine biosynthesis and require histidine for growth. The bacteria are treated with the test chemicals and the number that can grow in the absence of histidine measured. Bacteria that no longer require histidine for growth must have acquired a second, chemically induced mutation that opposed the inactivating, original mutation. In this fashion, the ability of chemicals to alter DNA can be determined. Because many chemicals (such as benz[o]pyrene) do not become carcinogenic in humans until they are metabolized by the liver, the Ames test also involves incubating the chemical with a liver extract, and then testing the metabolized extract for DNA-modifying activity.

Suggested References

Hanawalt P. Transcription-coupled repair and human disease. Science 1994;266:1957–1958.
Kunkel T. DNA replication fidelity. J Biol Chem 1992;267:18251–18254.
Modrich P. Mismatch repair, genetic stability, and cancer. Science 1994;266:1959–1960.
Sancar A. Mechanisms of DNA excision repair. Science 1994;266:1954–1956.
Waga S, Stillman B. The DNA replication fork in eukaryotic cells. Annu Rev Biochem 1998;67:721-751.

REVIEW QUESTIONS—CHAPTER 13

1. Reverse transcriptase, an RNA-dependant DNA polymerase, differs from DNA polymerase δ by which of the following?

   (A) Synthesizes DNA in the 5′ to 3′ direction
   (B) Contains 3′ to 5′ exonuclease activity
   (C) Follows Watson-Crick base pair rules
   (D) Synthesizes DNA in the 3′ to 5′ direction
   (E) Can insert inosine into a growing DNA chain

2. If a 1,000-kilobase fragment of DNA has 10 evenly spaced and symmetric replication origins and DNA polymerase moves at 1 kilobase per second, how many seconds will it take to produce two daughter molecules (ignore potential problems at the ends of this linear piece of DNA)? Assume that the 10 origins are evenly spaced from each other, but not from the ends of the chromosome.

   (A) 20
   (B) 30
   (C) 40
   (D) 50
   (E) 100

3. Primase is not required during DNA repair processes because of which of the following?

   (A) All of the primase is associated with replication origins.
   (B) RNA would be highly mutagenic at a repair site.
   (C) DNA polymerase I does not require a primer.
   (D) DNA polymerase III does not require a primer.
   (E) DNA polyemerase I or III can use any 3′ -OH for elongation.

4. Which of the following enzymes is required to actively enhance the separation of DNA strands during replication?

   (A) Helicase
   (B) 3′ to 5′ exonuclease
   (C) DNA ligase
   (D) Primase
   (E) AP endonuclease

5. The key mechanistic failure in patients with xeroderma pigmentosum involves which of the following?

   (A) Mutation in the primase gene
   (B) Inability to excise a section of the UV-damaged DNA
   (C) Mutation of one of the mismatch repair components
   (D) Inability to synthesize DNA across the damaged region
   (E) Loss of proofreading capacity