

18 Gene Mutations and DNA Repair



People with a mutation in the *tinman* gene, named after Tin Man in *The Wizard of Oz*, often have congenital heart defects. [Mary Evans Picture Library/Alamy.]

A FLY WITHOUT A HEART

The heart of a fruit fly is a simple organ, an open-ended tube that rhythmically contracts, pumping fluid—rather inefficiently—around the body of the fly. Although simple and inelegant, the fruit fly’s heart is nevertheless essential, at least for a fruit fly. Remarkably, a few rare mutant fruit flies never develop a heart and die (not surprisingly) at an early embryonic stage. Geneticist Rolf Bodmer analyzed these mutants in the 1980s and made an important discovery—a gene that specifies the development of a heart. He named the gene *tinman*, after the character in *The Wizard of Oz* who also lacked a heart. Bodmer’s research revealed that *tinman* encodes a transcription factor, which binds to DNA and turns on other genes that are essential for the normal development of a heart. In the mutant flies studied by Bodmer, this gene was lacking, the transcription factor was never produced, and the heart never developed. Findings from subsequent research revealed the existence of a human gene (called *Nkx2.5*) with a sequence similar to that of *tinman*, but the function of the human gene was unknown.

Then, in the 1990s, physicians Jonathan and Christine Seidman began studying people born with abnormal hearts, such as those with a hole in the septum that separates the chambers on the left and right sides of the heart. Such defects result in abnormal blood flow through the heart, causing the heart to work harder than normally and the mixing of oxygenated and deoxygenated blood. Congenital heart defects are not uncommon; they’re found in about 1 of every 125 babies. Some of the defects heal on their own, but others require corrective surgery. Although surgery is often successful in reversing congenital heart problems, many of these patients begin to have irregular heartbeats in their 20s and 30s. The Seidmans and their colleagues found several families in which congenital heart defects and irregular heartbeats were inherited together in an autosomal dominant fashion.

Detailed molecular analysis of one of these families revealed that the gene responsible for the heart problems was located on chromosome 5, at a spot where the human *tinman* gene (*Nkx2.5*) had been previously mapped. All members of this family who inherited the heart defects also inherited a mutation in the *tinman* gene. Subsequent studies with additional patients found that many people with congenital heart defects have a mutation in the *tinman* gene. The human version of this gene, like its counterpart in flies, encodes a transcription factor that controls heart development. Despite tremendous differences in size, anatomy, and physiology, humans and flies use the same gene to make a heart.

The story of *tinman* illustrates the central importance of studying mutations: the analysis of mutants is often a source of key insights into important biological processes. This chapter focuses on gene mutations—how errors arise in genetic instructions and how those errors are studied. We begin with a brief examination of the different types of mutations, including their phenotypic effects, how they can be suppressed, and mutation rates. The next section explores how mutations spontaneously arise in the course of DNA replication and afterward, as well as how chemicals and radiation induce mutations. We then consider the analysis of mutations. Finally, we take a look at DNA repair and some of the diseases that arise when DNA repair is defective.

18.1 Mutations Are Inherited Alterations in the DNA Sequence

DNA is a highly stable molecule that is replicated with amazing accuracy (see Chapters 10 and 12), but changes in DNA structure and errors of replication do occur. A **mutation** is defined as an inherited change in genetic information; the descendants may be cells or organisms.

The Importance of Mutations

Mutations are both the sustainer of life and the cause of great suffering. On the one hand, mutation is the source of all genetic variation, the raw material of evolution. The ability of organisms to adapt to environmental change depends critically on the presence of genetic variation in natural populations, and genetic variation is produced—at least partly—by mutation. On the other hand, many mutations have detrimental effects, and mutation is the source of many diseases and disorders.

Much of the study of genetics focuses on how variants produced by mutation are inherited; genetic crosses are meaningless if all individual members of a species are identically homozygous for the same alleles. Much of Gregor Mendel's success in unraveling the principles of inheritance can be traced to

his use of carefully selected variants of the garden pea; similarly, Thomas Hunt Morgan and his students discovered many basic principles of genetics by analyzing mutant fruit flies.

Mutations are also useful for probing fundamental biological processes. Finding or creating mutations that affect different components of a biological system and studying their effects can often lead to an understanding of the system. This method, referred to as genetic dissection, is analogous to figuring out how an automobile works by breaking different parts of a car and observing the effects; for example, smash the radiator and the engine overheats, revealing that the radiator cools the engine. Using mutations to disrupt function can likewise be a source of insight into biological processes. For example, geneticists have begun to unravel the molecular details of development by studying mutations, such as *tinman*, that interrupt various embryonic stages in *Drosophila* (see Chapter 22). Although this method of breaking “parts” to determine their function might seem like a crude approach to understanding a system, it is actually very powerful and has been used extensively in biochemistry, developmental biology, physiology, and behavioral science (but this method is *not* recommended for learning how your car works).

CONCEPTS

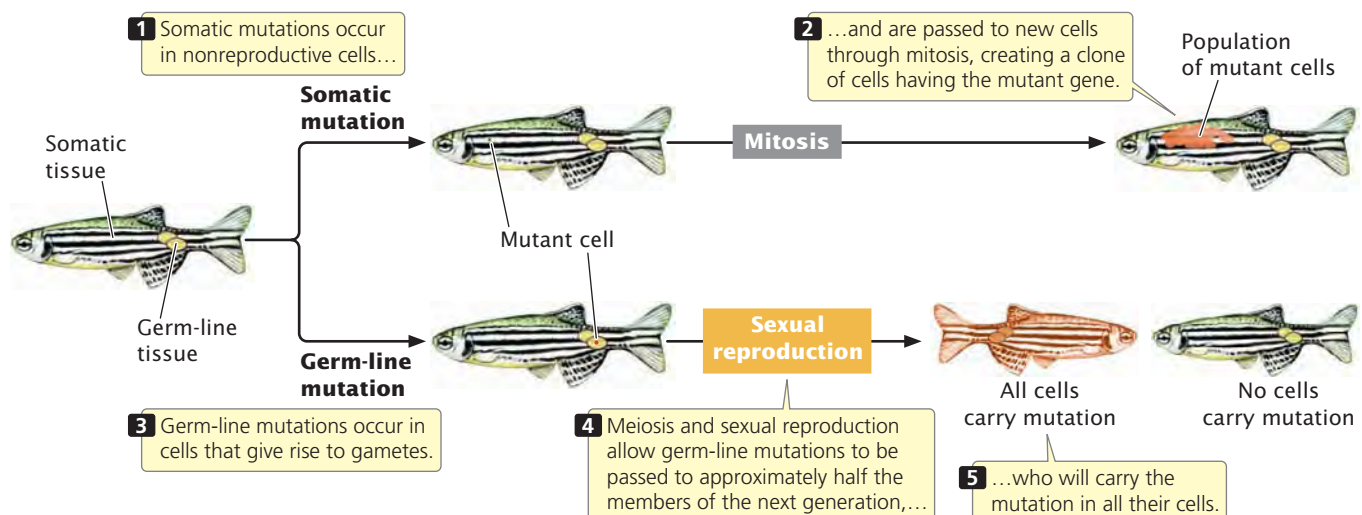
Mutations are heritable changes in DNA. They are essential to the study of genetics and are useful in many other biological fields.

✓ CONCEPT CHECK 1

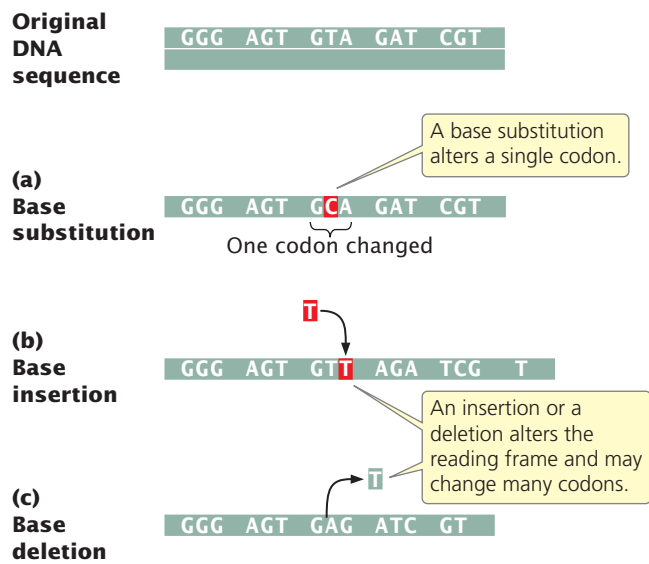
How are mutations used to help in understanding basic biological processes?

Categories of Mutations

In multicellular organisms, we can distinguish between two broad categories of mutations: somatic mutations and germ-line mutations. **Somatic mutations** arise in somatic tissues, which do not produce gametes (Figure 18.1). When



18.1 The two basic classes of mutations are somatic mutations and germ-line mutations.



18.2 Three basic types of gene mutations are base substitutions, insertions, and deletions.

a somatic cell with a mutation divides (mitosis), the mutation is passed on to the daughter cells, leading to a population of genetically identical cells (a clone). The earlier in development that a somatic mutation occurs, the larger the clone of cells within that individual organism that will contain the mutation.

Because of the huge number of cells present in a typical eukaryotic organism, somatic mutations are numerous. For example, there are about 10^{14} cells in the human body. Typically, a mutation arises once in every million cell divisions, and so hundreds of millions of somatic mutations must arise in each person. Many somatic mutations have no obvious effect on the phenotype of the organism, because the function of the mutant cell (even the cell itself) is replaced by that of normal cells. However, cells with a somatic mutation that stimulates cell division can increase in number and spread; this type of mutation can give rise to cells with a selective advantage and is the basis for cancers (see Chapter 23).

Germ-line mutations arise in cells that ultimately produce gametes. A germ-line mutation can be passed to future generations, producing individual organisms that carry the mutation in all their somatic and germ-line cells

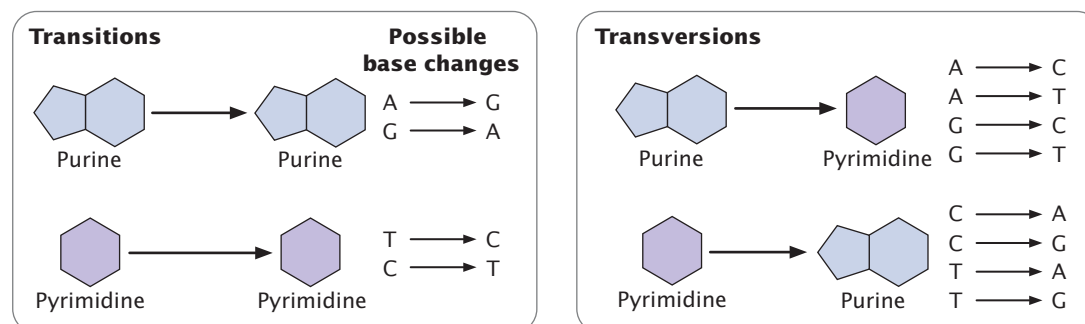
(see Figure 18.1). When we speak of mutations in multicellular organisms, we're usually talking about germ-line mutations.

Historically, mutations have been partitioned into those that affect a single gene, called *gene mutations*, and those that affect the number or structure of chromosomes, called *chromosome mutations*. This distinction arose because chromosome mutations could be observed directly, by looking at chromosomes with a microscope, whereas gene mutations could be detected only by observing their phenotypic effects. Now, DNA sequencing allows direct observation of gene mutations, and chromosome mutations are distinguished from gene mutations somewhat arbitrarily on the basis of the size of the DNA lesion. Nevertheless, it is practical to use the term *chromosome mutation* for a large-scale genetic alteration that affects chromosome structure or the number of chromosomes and to use the term **gene mutation** for a relatively small DNA lesion that affects a single gene. This chapter focuses on gene mutations; chromosome mutations were discussed in Chapter 9.

Types of Gene Mutations

There are a number of ways to classify gene mutations. Some classification schemes are based on the nature of the phenotypic effect, others are based on the causative agent of the mutation, and still others focus on the molecular nature of the defect. Here, we will categorize mutations primarily on the basis of their molecular nature, but we will also encounter some terms that relate the causes and the phenotypic effects of mutations.

Base substitutions The simplest type of gene mutation is a **base substitution**, the alteration of a single nucleotide in the DNA (Figure 18.2a). Base substitutions are of two types. In a **transition**, a purine is replaced by a different purine or, alternatively, a pyrimidine is replaced by a different pyrimidine (Figure 18.3). In a **transversion**, a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine. The number of possible transversions (see Figure 18.3) is twice the number of possible transitions, but transitions arise more frequently. **TRY PROBLEM 18** →



18.3 A transition is the substitution of a purine for a purine or of a pyrimidine for a pyrimidine; a transversion is the substitution of a pyrimidine for a purine or of a purine for a pyrimidine.

Insertions and deletions The second major class of gene mutations contains **insertions** and **deletions**—the addition or the removal, respectively, of one or more nucleotide pairs (collectively called indels, **Figure 18.2b** and **c**). Although base substitutions are often assumed to be the most common type of mutation, molecular analysis has revealed that insertions and deletions are often more frequent. Insertions and deletions within sequences that encode proteins may lead to **frameshift mutations**, changes in the reading frame (see p. 411 in Chapter 15) of the gene. Frameshift mutations usually alter all amino acids encoded by nucleotides following the mutation, and so they generally have drastic effects on the phenotype. Not all insertions and deletions lead to frameshifts, however; insertions and deletions consisting of any multiple of three nucleotides will leave the reading frame intact, although the addition or removal of one or more amino acids may still affect the phenotype. Mutations not affecting the reading frame are called **in-frame insertions** and **deletions**, respectively.

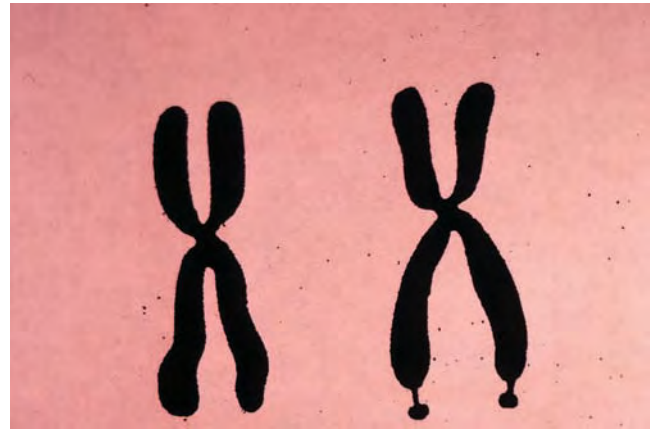
CONCEPTS

Gene mutations consist of changes in a single gene and can be base substitutions (a single pair of nucleotides is altered) or insertions or deletions (nucleotides are added or removed). A base substitution can be a transition (substitution of like bases) or a transversion (substitution of unlike bases). Insertions and deletions often lead to a change in the reading frame of a gene.

✓ CONCEPT CHECK 2

Which of the following changes is a transition base substitution?

- Adenine is replaced by thymine.
- Cytosine is replaced by adenine.
- Guanine is replaced by adenine.
- Three nucleotide pairs are inserted into DNA.



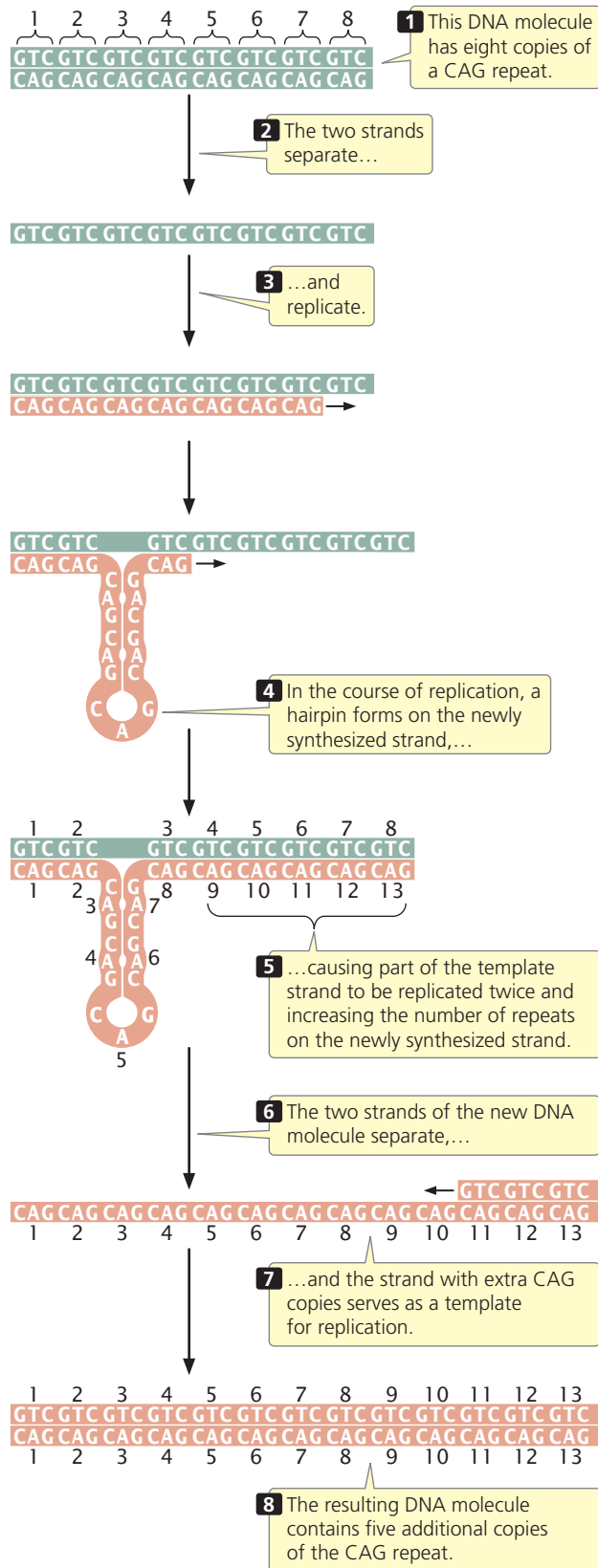
18.4 The fragile-X chromosome is associated with a characteristic constriction (fragile site) on the long arm. [Visuals Unlimited.]

Expanding nucleotide repeats Mutations in which the number of copies of a set of nucleotides increases in number are called **expanding nucleotide repeats**. This type of mutation was first observed in 1991 in a gene called *FMR-1*, which causes fragile-X syndrome, the most common hereditary cause of mental retardation. The disorder is so named because, in specially treated cells from persons having the condition, the tip of each long arm of the X chromosome is attached by only a slender thread (**Figure 18.4**). The normal *FMR-1* allele (not containing the mutation) has 60 or fewer copies of CGG but, in persons with fragile-X syndrome, the allele may harbor hundreds or even thousands of copies.

Expanding nucleotide repeats have been found in almost 30 human diseases, several of which are listed in **Table 18.1**. Most of these diseases are caused by the expansion of a set of three nucleotides (called a trinucleotide), most often CNG, where N can be any nucleotide. However,

Table 18.1 Examples of genetic diseases caused by expanding nucleotide repeats

Disease	Repeated Sequence	Number of Copies of Repeat	
		Normal Range	Disease Range
Spinal and bulbar muscular atrophy	CAG	11–33	40–62
Fragile-X syndrome	CGG	6–54	50–1500
Jacobsen syndrome	CGG	11	100–1000
Spinocerebellar ataxia (several types)	CAG	4–44	21–130
Autosomal dominant cerebellar ataxia	CAG	7–19	37–220
Myotonic dystrophy	CTG	5–37	44–3000
Huntington disease	CAG	9–37	37–121
Friedreich ataxia	GAA	6–29	200–900
Dentatorubral-pallidoluysian atrophy	CAG	7–25	49–75
Myoclonus epilepsy of the Unverricht–Lundborg type	CCCCGCCCGCG	2–3	12–13



18.5 A model of how the number of copies of a nucleotide repeat may increase in replication.



www.whfreeman.com/pierce4e Try Animation 18.1 to see how copies of a nucleotide repeat can increase.

some diseases are caused by repeats of four, five, and even twelve nucleotides. The number of copies of the nucleotide repeat often correlates with the severity or age of onset of the disease. The number of copies of the repeat also corresponds to the instability of nucleotide repeats: when more repeats are present, the probability of expansion to even more repeats increases. This association between the number of copies of nucleotide repeats, the severity of the disease, and the probability of expansion leads to a phenomenon known as anticipation (see pp. 122–123 in Chapter 5), in which diseases caused by nucleotide-repeat expansions become more severe in each generation. Less commonly, the number of nucleotide repeats may decrease within a family. Expanding nucleotide repeats have also been observed in some microbes and plants.

Increases in the number of nucleotide repeats can produce disease symptoms in different ways. In several of the diseases (e.g., Huntington disease), the nucleotide expands within the coding part of a gene, producing a toxic protein that has extra glutamine residues (the amino acid encoded by CAG). In other diseases, the repeat is outside the coding region of a gene and affects its expression. In fragile-X syndrome, additional copies of the nucleotide repeat cause the DNA to become methylated, which turns off the transcription of an essential gene.

The mechanism that leads to the expansion of nucleotide repeats is not completely understood. Evidence suggests that expansion occurs in the course of DNA replication and appears to be related to the formation of hairpins and other special DNA structures that form in single-stranded DNA consisting of nucleotide repeats. Such structures may interfere with normal replication by causing strand slippage, misalignment of the sequences, or stalling of replication. One model of how repeat hairpins might result in repeat expansion is shown in **Figure 18.5**.

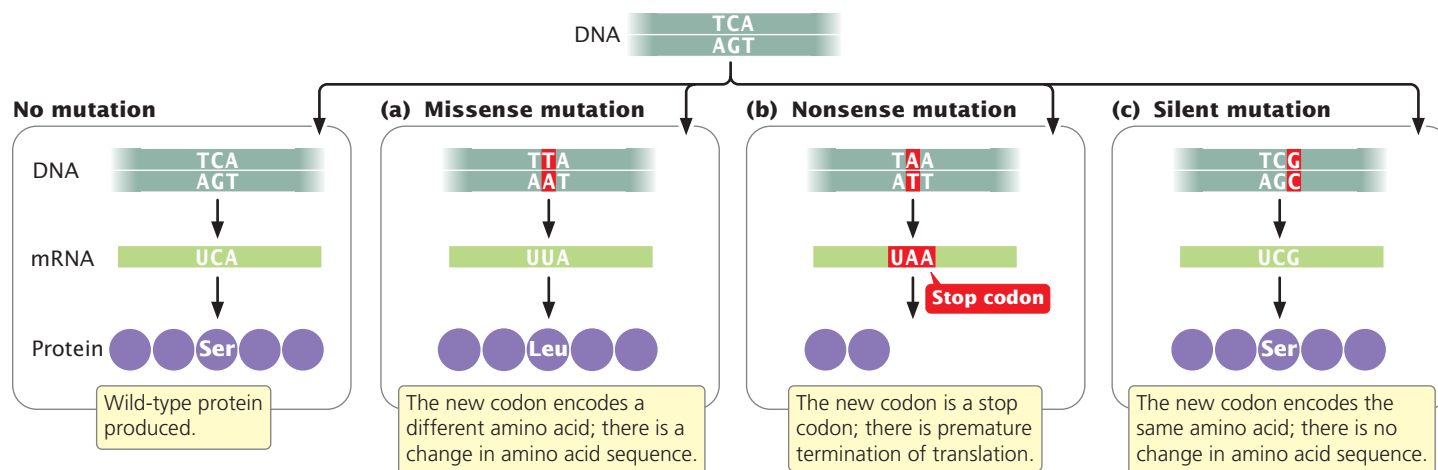
CONCEPTS

Expanding nucleotide repeats are regions of DNA that consist of repeated copies of sets of nucleotides. Increased numbers of nucleotide repeats are associated with several genetic diseases.

Phenotypic Effects of Mutations

Another way that mutations are classified is on the basis of their phenotypic effects. At the most general level, we can distinguish a mutation on the basis of its phenotype compared with the wild-type phenotype. A mutation that alters the wild-type phenotype is called a **forward mutation**, whereas a **reverse mutation** (a *reversion*) changes a mutant phenotype back into the wild type.

Geneticists use other terms to describe the effects of mutations on protein structure. A base substitution that results in a different amino acid in the protein is referred to as a **missense mutation** (**Figure 18.6a**). A **nonsense mutation**



18.6 Base substitutions can cause (a) missense, (b) nonsense, and (c) silent mutations.

changes a sense codon (one that specifies an amino acid) into a nonsense codon (one that terminates translation), as shown in **Figure 18.6b**. If a nonsense mutation occurs early in the mRNA sequence, the protein will be greatly shortened and will usually be nonfunctional.

Because of the redundancy of the genetic code, some different codons specify the same amino acid. A **silent mutation** changes a codon to a synonymous codon that specifies the same amino acid (**Figure 18.6c**), altering the DNA sequence without changing the amino acid sequence of the protein. Not all silent mutations, however, are truly silent: some do have phenotypic effects. For example, silent mutations may have phenotypic effects when different tRNAs (called isoaccepting tRNAs, see Chapter 15) are used for different synonymous codons. Because some isoaccepting tRNAs are more abundant than others, which synonymous codon is used may affect the rate of protein synthesis. The rate of protein synthesis can influence the phenotype by affecting the amount of protein present in the cell and, in a few cases, the folding of the protein. Other silent mutations may alter sequences near the exon–intron junctions that affect splicing (see Chapter 14). Still other silent mutations may influence the folding of the mRNA, affecting its stability.

A **neutral mutation** is a missense mutation that alters the amino acid sequence of the protein but does not change its function. Neutral mutations occur when one amino acid is replaced by another that is chemically similar or when the affected amino acid has little influence on protein function. For example, neutral mutations occur in the genes that encode hemoglobin; although these mutations alter the amino acid sequence of hemoglobin, they do not affect its ability to transport oxygen.

Loss-of-function mutations cause the complete or partial absence of normal protein function. A loss-of-function mutation so alters the structure of the protein that the protein no longer works correctly or the mutation can occur in regulatory regions that affect the transcription, translation,

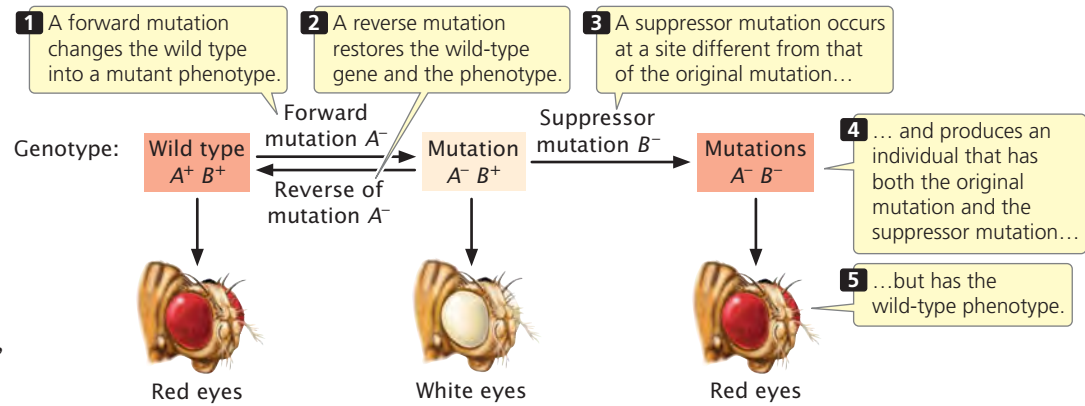
or splicing of the protein. Loss-of-function mutations are frequently recessive, and an individual diploid organism must be homozygous for a loss-of-function mutation before the effects of the loss of the functional protein can be exhibited. The mutations that cause cystic fibrosis are loss-of-function mutations: these mutations produce a nonfunctional form of the cystic fibrosis transmembrane conductance regulator protein, which normally regulates the movement of chloride ions into and out of the cell (see Chapter 5).

In contrast, a **gain-of-function mutation** produces an entirely new trait or it causes a trait to appear in an inappropriate tissue or at an inappropriate time in development. For example, a mutation in a gene that encodes a receptor for a growth factor might cause the mutated receptor to stimulate growth all the time, even in the absence of the growth factor. Gain-of-function mutations are frequently dominant in their expression. Still other types of mutations are **conditional mutations**, which are expressed only under certain conditions. For example, some conditional mutations affect the phenotype only at elevated temperatures. Others are **lethal mutations**, causing premature death (Chapter 5).

TRY PROBLEM 21 →

Suppressor Mutations

A **suppressor mutation** is a genetic change that hides or suppresses the effect of another mutation. This type of mutation is distinct from a reverse mutation, in which the mutated site changes back into the original wild-type sequence (**Figure 18.7**). A suppressor mutation occurs at a site that is distinct from the site of the original mutation; thus, an individual with a suppressor mutation is a double mutant, possessing both the original mutation and the suppressor mutation but exhibiting the phenotype of an unmutated wild type. Like other mutations, suppressors arise randomly. Geneticists distinguish between two classes of suppressor mutations: intragenic and intergenic.



18.7 Relation of forward, reverse, and suppressor mutations.

Intragenic suppressor mutation An **intragenic suppressor mutation** is in the same gene as that containing the mutation being suppressed and may work in several ways. The suppressor may change a second nucleotide in the same codon altered by the original mutation, producing a codon that specifies the same amino acid as that specified by the original, unmutated codon (**Figure 18.8**). Intragenic suppressors may also work by suppressing a frameshift mutation. If the original mutation is a one-base deletion, then the addition of a single base elsewhere in the gene will restore the former reading frame. Consider the following nucleotide sequence in DNA and the amino acids that it encodes:

DNA	AAA	TCA	CTT	GGC	GTA	CAA
Amino acids	Phe	Ser	Glu	Pro	His	Val

Suppose a one-base deletion occurs in the first nucleotide of the second codon. This deletion shifts the reading frame by one nucleotide and alters all the amino acids that follow the mutation.

One-nucleotide deletion

AAA	C CAC	TTG	GCG	TAC	AA
Phe	Val	Asn	Arg	Met	

If a single nucleotide is added to the third codon (the suppressor mutation), the reading frame is restored, although two of the amino acids differ from those specified by the original sequence.

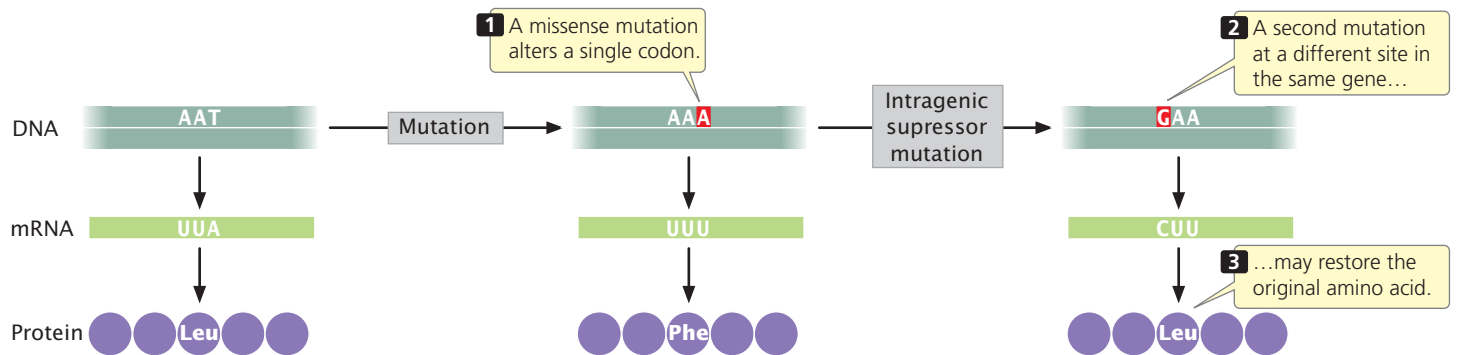
One-nucleotide insertion

AAA	CAC	TTT	GGC	GTA	CAA
Phe	Val	Lys	Pro	His	Val

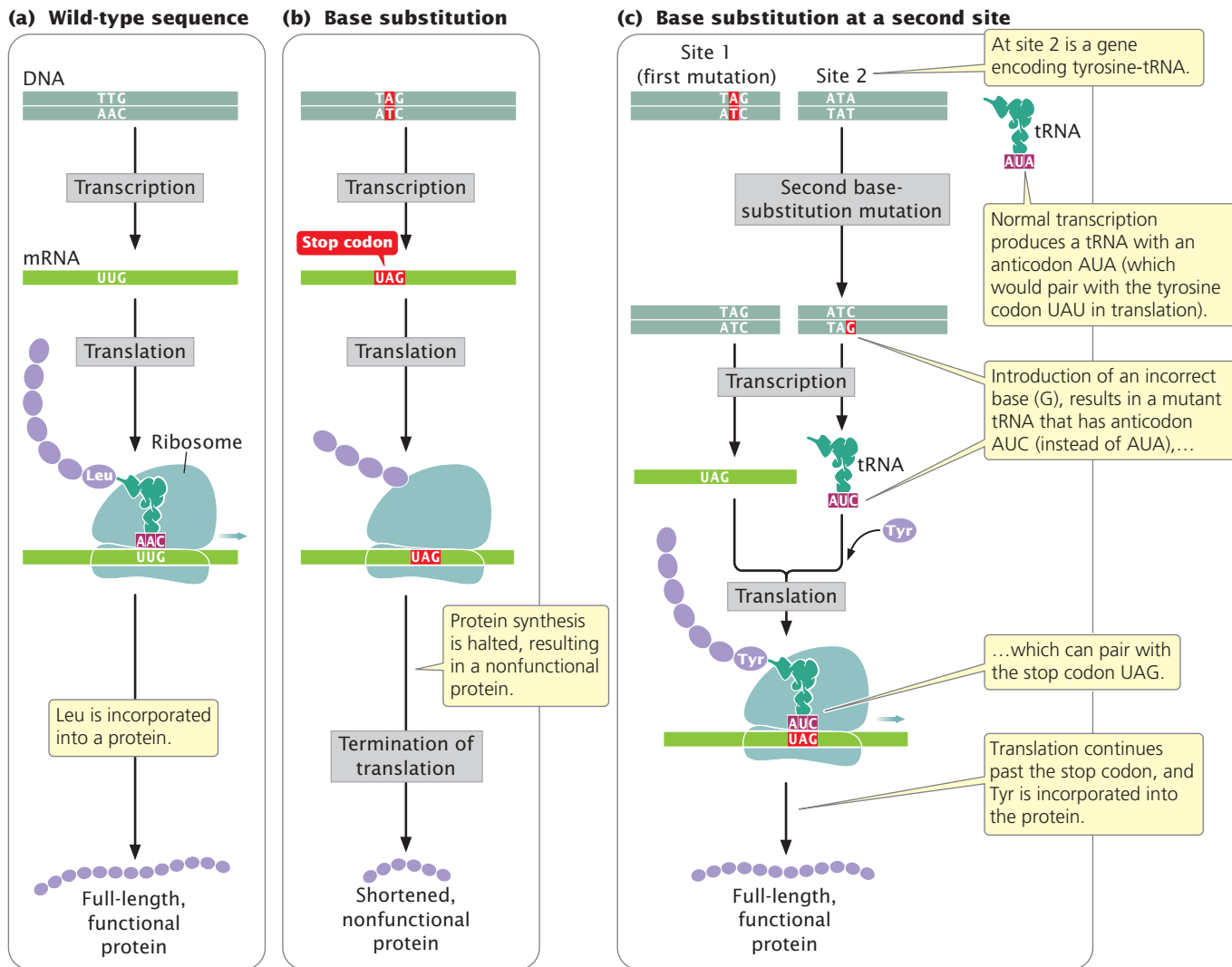
Similarly, a mutation due to an insertion may be suppressed by a subsequent deletion in the same gene.

A third way in which an intragenic suppressor may work is by making compensatory changes in the protein. A first missense mutation may alter the folding of a polypeptide chain by changing the way in which amino acids in the protein interact with one another. A second missense mutation at a different site (the suppressor) may recreate the original folding pattern by restoring interactions between the amino acids.

Intergenic suppressors mutations An **intergenic suppressor mutation**, in contrast, occurs in a gene other than the one bearing the original mutation. These suppressors sometimes work by changing the way that the mRNA is translated. In the example illustrated in **Figure 18.9a**, the



18.8 An intragenic suppressor mutation occurs in the gene containing the mutation being suppressed.



18.9 An intergenic suppressor mutation occurs in a gene other than the one bearing the original mutation.

(a) The wild-type sequence produces a full-length, functional protein. (b) A base substitution at a site in the same gene produces a premature stop codon, resulting in a shortened, nonfunctional protein. (c) A base substitution at a site in another gene, which in this case encodes tRNA, alters the anticodon of tRNA^{Tyr}; tRNA^{Tyr} can pair with the stop codon produced by the original mutation, allowing tyrosine to be incorporated into the protein and translation to continue.

original DNA sequence is AAC (UUG in the mRNA) and specifies leucine. This sequence mutates to ATC (UAG in mRNA), a termination codon (**Figure 18.9b**). The ATC nonsense mutation could be suppressed by a second mutation in a different gene that encodes a tRNA; this second mutation would result in a codon capable of pairing with the UAG termination codon (**Figure 18.9c**). For example, the gene that encodes the tRNA for tyrosine (tRNA^{Tyr}), which has the anticodon AUA, might be mutated to have the anticodon AUC, which will then pair with the UAG stop codon. Instead of translation terminating at the UAG codon, tyrosine would be inserted into the protein and a full-length protein would be produced, although tyrosine would now substitute for leucine. The effect of this change would depend on the role of this amino acid in the overall structure of the protein,

but the effect of the suppressor mutation is likely to be less detrimental than the effect of the nonsense mutation, which would halt translation prematurely.

Because cells in many organisms have multiple copies of tRNA genes, other unmutated copies of tRNA^{Tyr} would remain available to recognize tyrosine codons in the transcripts of the mutant gene in question and other genes being expressed concurrently. We might expect that the tRNAs that have undergone the suppressor mutation described would also suppress the normal termination codons at the ends of other coding sequences, resulting in the production of longer-than-normal proteins, but this event does not usually take place.

Intergenic suppressors can also work through genic interactions (see p. 106 in Chapter 5). Polypeptide chains that are produced by two genes may interact to produce a

Table 18.2 Characteristics of different types of mutations

Type of Mutation	Definition
Base substitution	Changes the base of a single DNA nucleotide
Transition	Base substitution in which a purine replaces a purine or a pyrimidine replaces a pyrimidine
Transversion	Base substitution in which a purine replaces a pyrimidine or a pyrimidine replaces a purine
Insertion	Addition of one or more nucleotides
Deletion	Deletion of one or more nucleotides
Frameshift mutation	Insertion or deletion that alters the reading frame of a gene
In-frame deletion or insertion	Deletion or insertion of a multiple of three nucleotides that does not alter the reading frame
Expanding nucleotide repeats	Repeated sequence of a set of nucleotides in which the number of copies of the sequence increases
Forward mutation	Changes the wild-type phenotype to a mutant phenotype
Reverse mutation	Changes a mutant phenotype back to the wild-type phenotype
Missense mutation	Changes a sense codon into a different sense codon, resulting in the incorporation of a different amino acid in the protein
Nonsense mutation	Changes a sense codon into a nonsense codon, causing premature termination of translation
Silent mutation	Changes a sense codon into a synonymous codon, leaving unchanged the amino acid sequence of the protein
Neutral mutation	Changes the amino acid sequence of a protein without altering its ability to function
Loss-of-function mutation	Causes a complete or partial loss of function
Gain-of-function mutation	Causes the appearance of a new trait or function or causes the appearance of a trait in inappropriate tissue or at an inappropriate time
Lethal mutation	Causes premature death
Suppressor mutation	Suppresses the effect of an earlier mutation at a different site
Intragenic suppressor mutation	Suppresses the effect of an earlier mutation within the same gene
Intergenic suppressor mutation	Suppresses the effect of an earlier mutation in another gene

functional protein. A mutation in one gene may alter the encoded polypeptide such that the interaction between the two polypeptides is destroyed, in which case a functional protein is not produced. A suppressor mutation in the second gene may produce a compensatory change in its polypeptide, therefore restoring the original interaction. Characteristics of some of the different types of mutations are summarized in **Table 18.2**. **TRY PROBLEM 22** →

CONCEPTS

A suppressor mutation overrides the effect of an earlier mutation at a different site. An intragenic suppressor mutation occurs within the *same* gene as that containing the original mutation, whereas an intergenic suppressor mutation occurs in a *different* gene.

✓ CONCEPT CHECK 3

How is a suppressor mutation different from a reverse mutation?

Worked Problem

A gene encodes a protein with the following amino acid sequence:

Met-Arg-Cys-Ile-Lys-Arg

A mutation of a single nucleotide alters the amino acid sequence to:

Met-Asp-Ala-Tyr-Lys-Gly-Glu-Ala-Pro-Val

A second single-nucleotide mutation occurs in the same gene and suppresses the effects of the first mutation (an intragenic suppressor). With the original mutation and the intragenic suppressor present, the protein has the following amino acid sequence:

Met-Asp-Gly-Ile-Lys-Arg

What is the nature and location of the first mutation and of the intragenic suppressor mutation?

• Solution

The first mutation alters the reading frame, because all amino acids after Met are changed. Insertions and deletions affect the reading frame; the original mutation consists of a single-nucleotide insertion or deletion in the second codon. The intragenic suppressor restores the reading frame; the intragenic suppressor also is most likely a single-nucleotide insertion or deletion. If the first mutation is an insertion, the suppressor must be a deletion; if the first mutation is a deletion, then the suppressor must be an insertion. Notice that the protein produced by the suppressor still differs from the original protein at the second and third amino acids, but the second amino acid produced by the suppressor is the same as that in the protein produced by the original mutation. Thus, the suppressor mutation must have occurred in the third codon, because the suppressor does not alter the second amino acid.

➔ For more practice with analyzing mutations, try working Problem 23 at the end of the chapter.

Mutation Rates

The frequency with which a wild-type allele at a locus changes into a mutant allele is referred to as the **mutation rate** and is generally expressed as the number of mutations per biological unit, which may be mutations per cell division, per gamete, or per round of replication. For example, achondroplasia is a type of hereditary dwarfism in humans that results from a dominant mutation. On average, about four achondroplasia mutations arise in every 100,000 gametes, and so the mutation rate is $4/100,000$, or 0.00004 mutations per gamete. The mutation rate provides information about how often a mutation arises.

Factors affecting mutation rates Calculations of mutation rates are affected by three factors. First, they depend on the frequency with which a change takes place in DNA. A change in the DNA can arise from spontaneous molecular changes in DNA or it can be induced by chemical, biological, or physical agents in the environment.

The second factor influencing the mutation rate is the probability that, when a change takes place, it will be repaired. Most cells possess a number of mechanisms for repairing altered DNA, and so most alterations are corrected before they are replicated. If these repair systems are effective, mutation rates will be low; if they are faulty, mutation rates will be elevated. Some mutations increase the overall rate of mutation at other genes; these mutations usually occur in genes that encode components of the replication machinery or DNA-repair enzymes.

The third factor is the probability that a mutation will be recognized and recorded. When DNA is sequenced, all

mutations are potentially detectable. In practice, however, mutations are usually detected by their phenotypic effects. Some mutations may appear to arise at a higher rate simply because they are easier to detect.

Variation in mutation rates Mutation rates vary among genes and species (Table 18.3), but we can draw several general conclusions about mutation rates. First, spontaneous mutation rates are low for all organisms studied. Typical mutation rates for bacterial genes range from about 1 to 100 mutations per 10 billion cells (from 1×10^{-8} to 1×10^{-10}). The mutation rates for most eukaryotic genes are a bit higher, from about 1 to 10 mutations per million gametes (from 1×10^{-5} to 1×10^{-6}). These higher values in eukaryotes may be due to the fact that the rates are calculated per gamete, and several cell divisions are required to produce a gamete, whereas mutation rates in prokaryotic cells are calculated *per cell division*.

The differences in mutation rates among species may be due to differing abilities to repair mutations, unequal exposures to mutagens, or biological differences in rates of spontaneously arising mutations. Even within a single species, spontaneous rates of mutation vary among genes. The reason for this variation is not entirely understood, but some regions of DNA are known to be more susceptible to mutation than others.

Several recent studies have measured mutation rates directly by sequencing genes of organisms before and after a number of generations. These new studies suggest that mutation rates are often higher than those previously measured on the basis of changes in phenotype. In one study, geneticists sequenced randomly chosen stretches of DNA in the nematode worm *Caenorhabditis elegans* and found about 2.1 mutations per genome per generation, which was 10 times as high as previous estimates based on phenotypic changes. The researchers found that about half of the mutations were insertions and deletions.

Adaptive mutation As will be discussed in Chapters 24 through 26, evolutionary change that brings about adaptation to new environments depends critically on the presence of genetic variation. New genetic variants arise primarily through mutation. For many years, genetic variation was assumed to arise randomly and at rates that are independent of the need for adaptation. However, some evidence now suggests that stressful environments—where adaptation may be necessary to survive—can induce more mutations in bacteria, a process that has been termed **adaptive mutation**. The idea of adaptive mutation has been intensely debated; critics counter that most mutations are expected to be deleterious, and so increased mutagenesis would likely be harmful most of the time.

Research findings have shown that mutation rates in bacteria collected from the wild do increase in stressful environments, such as those in which nutrients are limited. There

Table 18.3 Mutation rates of different genes in different organisms

Organism	Mutation	Rate	Unit
Bacteriophage T2	Lysis inhibition	1×10^{-8}	Per replication
	Host range	3×10^{-9}	
<i>Escherichia coli</i>	Lactose fermentation	2×10^{-7}	Per cell division
	Histidine requirement	2×10^{-8}	
<i>Neurospora crassa</i>	Inositol requirement	8×10^{-8}	Per asexual spore
	Adenine requirement	4×10^{-8}	
Corn	Kernel color	2.2×10^{-6}	Per gamete
<i>Drosophila</i>	Eye color	4×10^{-5}	Per gamete
	Allozymes	5.14×10^{-6}	
Mouse	Albino coat color	4.5×10^{-5}	Per gamete
	Dilution coat color	3×10^{-5}	
Human	Huntington disease	1×10^{-6}	Per gamete
	Achondroplasia	1×10^{-5}	
	Neurofibromatosis (Michigan)	1×10^{-4}	
	Hemophilia A (Finland)	3.2×10^{-5}	
	Duchenne muscular dystrophy (Wisconsin)	9.2×10^{-5}	

is considerable disagreement about whether (1) the increased mutagenesis is a genetic strategy selected to increase the probability of evolving beneficial traits in the stressful environment or (2) mutagenesis is merely an accidental consequence of error-prone DNA-repair mechanisms that are more active in stressful environments (see Section 18.4 on DNA repair).

CONCEPTS

Mutation rate is the frequency with which a specific mutation arises. Rates of mutations are generally low and are affected by environmental and genetic factors.

✓ CONCEPT CHECK 4

What three factors affect mutation rates?

18.2 Mutations Are Potentially Caused by a Number of Different Natural and Unnatural Factors

Mutations result from both internal and external factors. Those that occur under normal conditions are termed **spontaneous mutations**, whereas those that result from changes caused by environmental chemicals or radiation are **induced mutations**.

Spontaneous Replication Errors

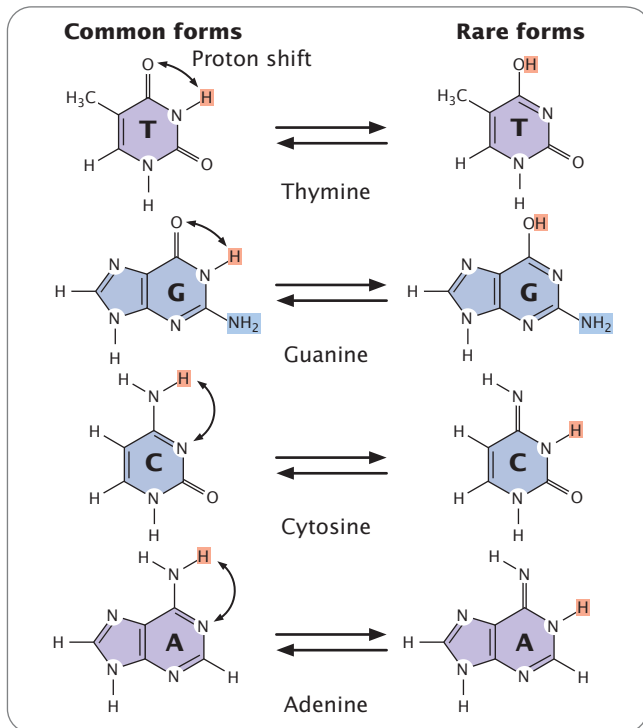
Replication is amazingly accurate: less than one error in a billion nucleotides arises in the course of DNA synthesis (see Chapter 12). However, spontaneous replication errors do occasionally occur.

Tautomeric shifts The primary cause of spontaneous replication errors was formerly thought to be tautomeric shifts, in which the positions of protons in the DNA bases change. Purine and pyrimidine bases exist in different chemical forms called tautomers (**Figure 18.10a**). The two tautomeric forms of each base are in dynamic equilibrium, although one form is more common than the other. The standard Watson-and-Crick base pairings—adenine with thymine, and cytosine with guanine—are between the common forms of the bases, but, if the bases are in their rare tautomeric forms, other base pairings are possible (**Figure 18.10b**).

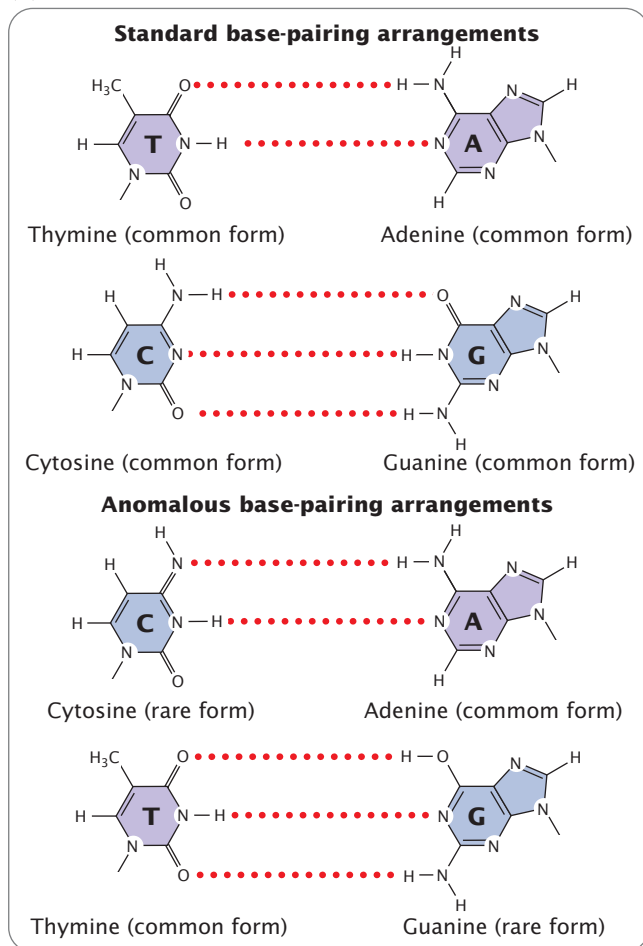
Watson and Crick proposed that tautomeric shifts might produce mutations, and, for many years, their proposal was the accepted model for spontaneous replication errors. However, there has never been convincing evidence that the rare tautomers are the cause of spontaneous mutations. Furthermore, research now shows little evidence of tautomers in DNA.

Mispairing due to other structures Mispairing can also occur through wobble, in which normal, protonated,

(a)



(b)

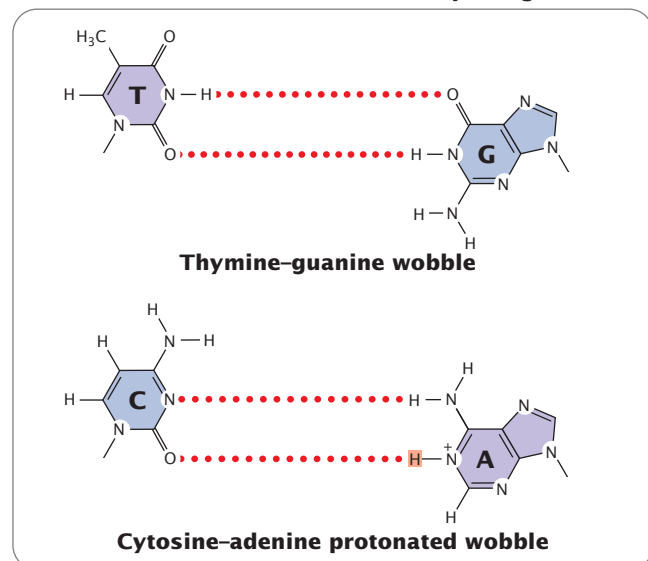
**18.10 Purine and pyrimidine bases exist in different forms called tautomers.**

(a) A tautomeric shift takes place when a proton changes its position, resulting in a rare tautomeric form. (b) Standard and anomalous base-pairing arrangements that arise if bases are in the rare tautomeric forms.

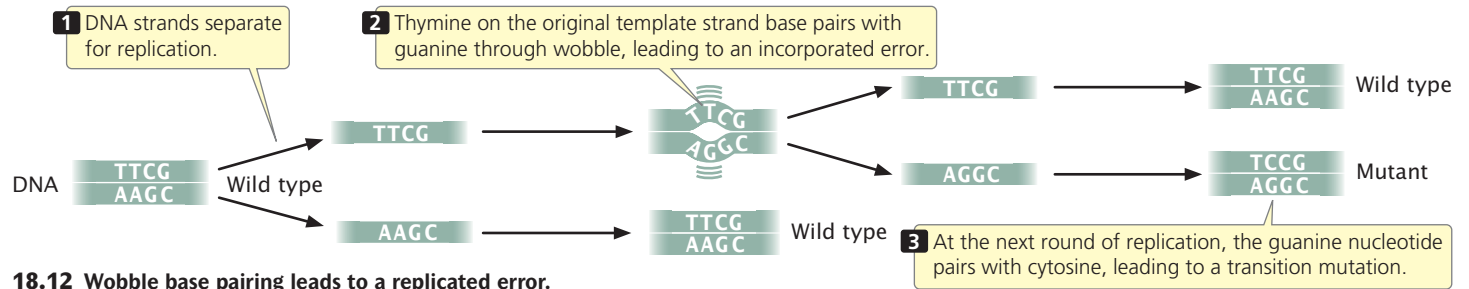
and other forms of the bases are able to pair because of flexibility in the DNA helical structure (**Figure 18.11**). These structures have been detected in DNA molecules and are now thought to be responsible for many of the mispairings in replication.

Incorporation errors and replication errors When a mismatched base has been incorporated into a newly synthesized nucleotide chain, an **incorporated error** is said to have occurred. Suppose that, in replication, thymine (which normally pairs with adenine) mispairs with guanine through wobble (**Figure 18.12**). In the next round of replication, the two mismatched bases separate, and each serves as template for the synthesis of a new nucleotide strand. This time, thymine pairs with adenine, producing another copy of the original DNA sequence. On the other strand, however, the incorrectly incorporated guanine serves as the template and pairs with cytosine, producing a new DNA molecule that has an error—a C·G pair in place of the original T·A pair (a T·A → C·G base substitution). The original incorporated error leads to a **replication error**, which creates a permanent mutation, because all the base pairings are correct and there is no mechanism for repair systems to detect the error.

Causes of deletions and insertions Mutations due to small insertions and deletions also arise spontaneously in

Non-Watson-and-Crick base pairing

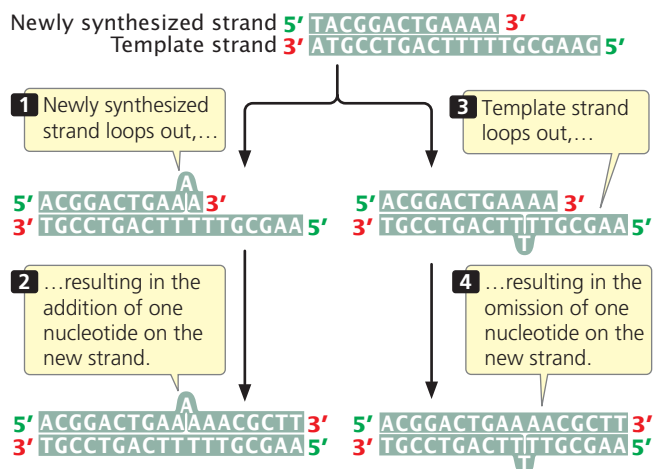
18.11 Nonstandard base pairings can occur as a result of the flexibility in DNA structure. Thymine and guanine can pair through wobble between normal bases. Cytosine and adenine can pair through wobble when adenine is protonated (has an extra hydrogen atom).



18.12 Wobble base pairing leads to a replicated error.

replication and crossing over. **Strand slippage** can occur when one nucleotide strand forms a small loop (**Figure 18.13**). If the looped-out nucleotides are on the newly synthesized strand, an insertion results. At the next round of replication, the insertion will be replicated and both strands will contain the insertion. If the looped-out nucleotides are on the template strand, then the newly replicated strand has a deletion, and this deletion will be perpetuated in subsequent rounds of replication.

Another process that produces insertions and deletions is unequal crossing over. In normal crossing over, the homologous sequences of the two DNA molecules align, and crossing over produces no net change in the number of nucleotides in either molecule. Misaligned pairing can cause **unequal crossing over**, which results in one DNA molecule with an insertion and the other with a deletion (**Figure 18.14**). Some DNA sequences are more likely than others to undergo strand slippage or unequal crossing over. Stretches of repeated sequences, such as nucleotide repeats or homopolymeric repeats (more than five repeats of the same base in a row), are prone to strand slippage. Duplicated or repetitive sequences may misalign during pairing, leading to unequal crossing over. Both strand slippage and unequal crossing over produce duplicated copies of sequences, which in turn promote further strand slippage and unequal cross-



18.13 Insertions and deletions may result from strand slippage.

ing over. This chain of events may explain the phenomenon of anticipation often observed for expanding nucleotide repeats.

CONCEPTS

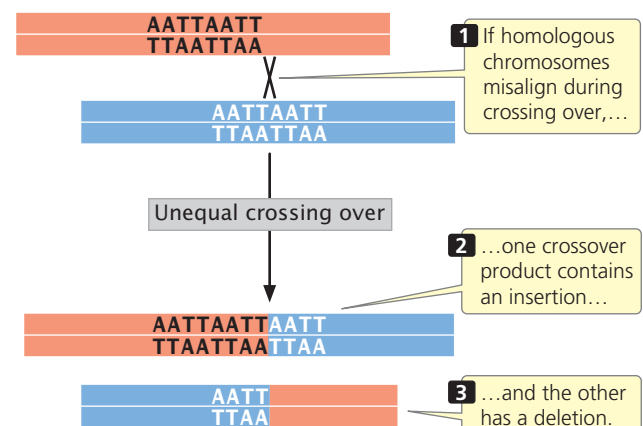
Spontaneous replication errors arise from altered base structures and from wobble base pairing. Small insertions and deletions can occur through strand slippage in replication and through unequal crossing over.

✓ CONCEPT CHECK 5

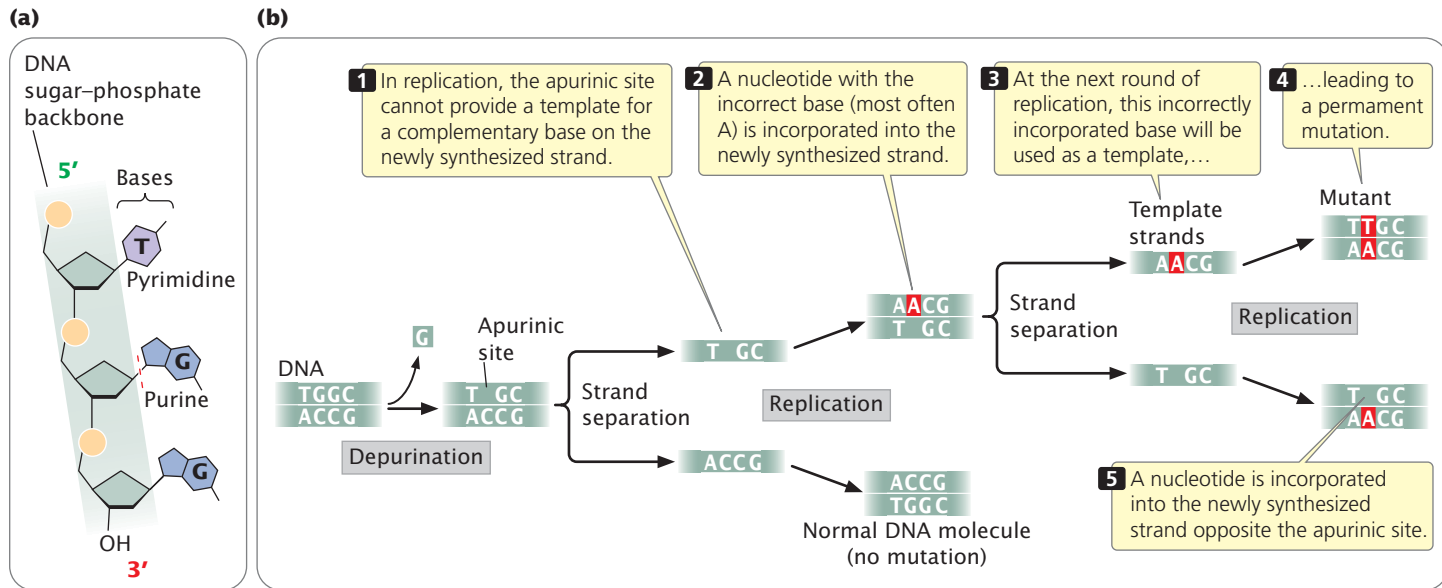
How does an incorporated error differ from a replicated error?

Spontaneous Chemical Changes

In addition to spontaneous mutations that arise in replication, mutations also result from spontaneous chemical changes in DNA. One such change is **depurination**, the loss of a purine base from a nucleotide. Depurination results when the covalent bond connecting the purine to the 1'-carbon atom of the deoxyribose sugar breaks (**Figure 18.15a**), producing an apurinic site, a nucleotide that lacks its purine base. An apurinic site cannot act as a template for a complementary base in replication. In the absence of base-pairing constraints, an



18.14 Unequal crossing over produces insertions and deletions.



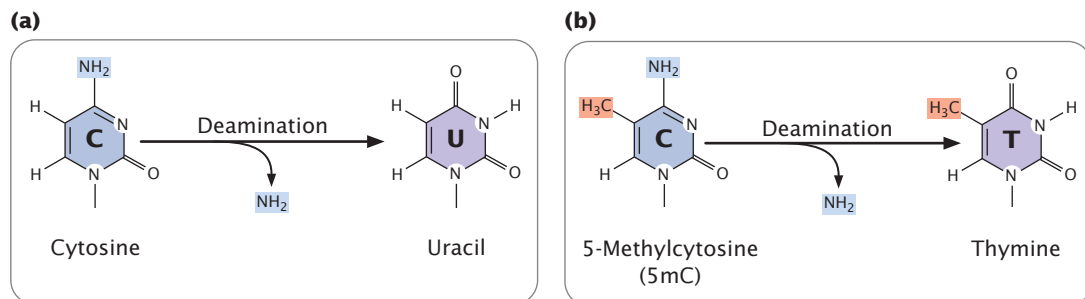
18.15 Depurination (the loss of a purine base from a nucleotide) produces an apurinic site.

incorrect nucleotide (most often adenine) is incorporated into the newly synthesized DNA strand opposite the apurinic site (**Figure 18.15b**), frequently leading to an incorporated error. The incorporated error is then transformed into a replication error at the next round of replication. Depurination is a common cause of spontaneous mutation; a mammalian cell in culture loses approximately 10,000 purines every day.

Another spontaneously occurring chemical change that takes place in DNA is **deamination**, the loss of an amino group (NH_2) from a base. Deamination can be spontaneous or be induced by mutagenic chemicals.

Deamination can alter the pairing properties of a base: the deamination of cytosine, for example, produces uracil (**Figure 18.16a**), which pairs with adenine in replication. After another round of replication, the adenine will pair with thymine, creating a T·A pair in place of the original C·G pair ($\text{C}\cdot\text{G}\rightarrow\text{U}\cdot\text{A}\rightarrow\text{T}\cdot\text{A}$); this chemical change is a transition mutation. This type of mutation is usually repaired by enzymes that remove uracil whenever it is found in DNA. The ability to recognize the product of cytosine deamination may explain why thymine, not uracil, is found in DNA.

18.16 Deamination alters DNA bases.



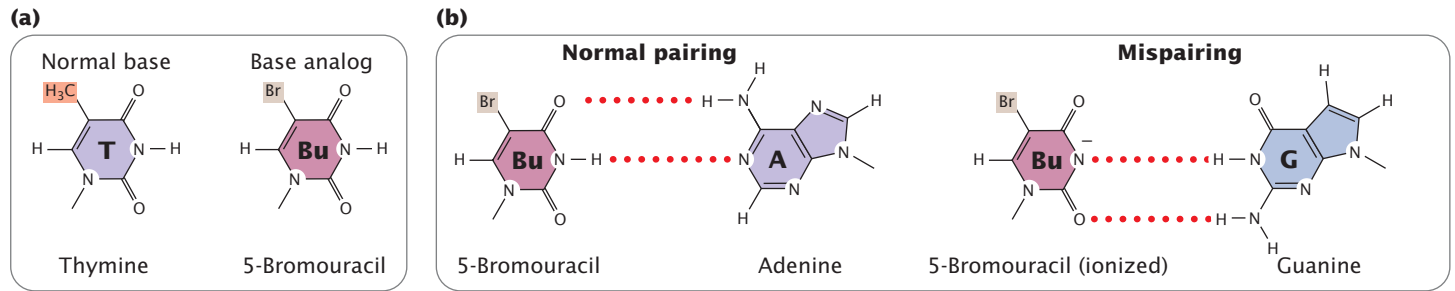
In mammals, including humans, some cytosine bases in DNA are naturally methylated and exist in the form of 5-methylcytosine (5mC; see p. 285 in Chapter 10 and Figure 10.18). When deaminated, 5mC becomes thymine (**Figure 18.16b**). Because thymine pairs with adenine in replication, the deamination of 5-methylcytosine changes an original C·G pair to T·A ($\text{C}\cdot\text{G}\rightarrow\text{5mC}\cdot\text{G}\rightarrow\text{T}\cdot\text{G}\rightarrow\text{T}\cdot\text{A}$). Consequently, C·G \rightarrow T·A transitions are frequent in mammalian cells, and 5mC sites are mutation hotspots in humans. **TRY PROBLEM 27** \rightarrow

CONCEPTS

Some mutations arise from spontaneous alterations to DNA structure, such as depurination and deamination, which may alter the pairing properties of the bases and cause errors in subsequent rounds of replication.

Chemically Induced Mutations

Although many mutations arise spontaneously, a number of environmental agents are capable of damaging DNA, including certain chemicals and radiation. Any environmental agent



18.17 5-Bromouracil (a base analog) resembles thymine, except that it has a bromine atom in place of a methyl group on the 5-carbon atom. Because of the similarity in their structures, 5-bromouracil may be incorporated into DNA in place of thymine. Like thymine, 5-bromouracil normally pairs with adenine but, when ionized, it may pair with guanine through wobble.

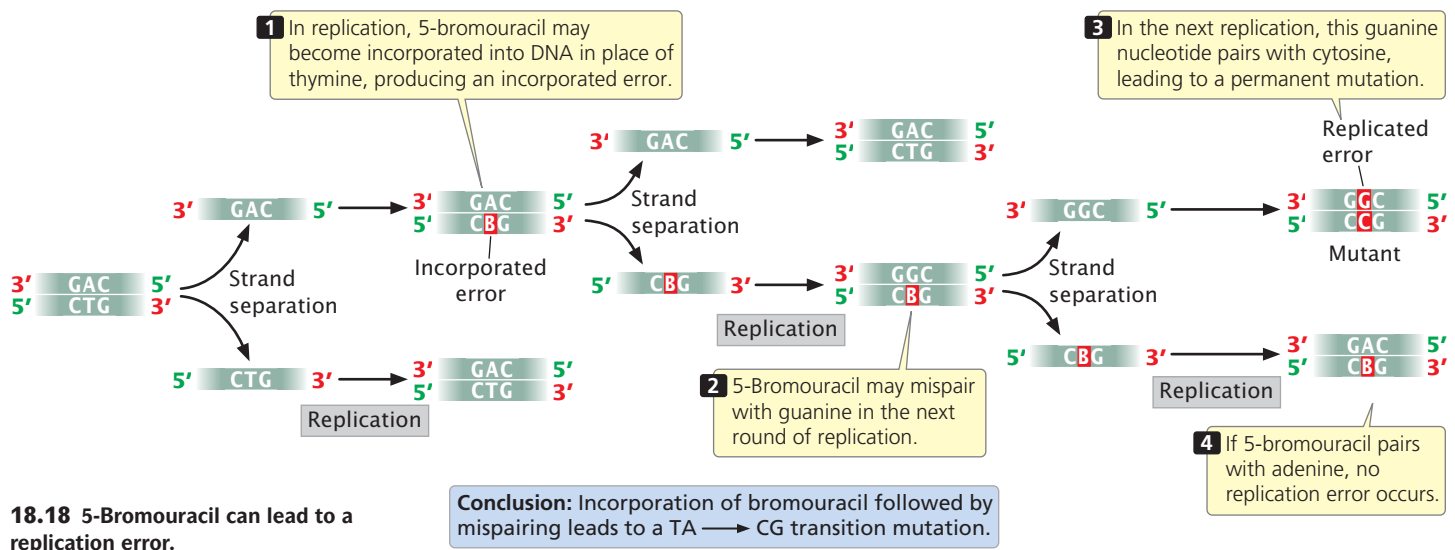
that significantly increases the rate of mutation above the spontaneous rate is called a **mutagen**.

The first discovery of a chemical mutagen was made by Charlotte Auerbach, who started her career in Berlin researching the development of mutants in *Drosophila*. Faced with increasing anti-Semitism in Nazi Germany, Auerbach emigrated to Britain. There she continued her research on *Drosophila* and collaborated with pharmacologist John Robson on the mutagenic effects of mustard gas, which had been used as a chemical weapon in World War I. The experimental conditions were crude. They heated liquid mustard gas over a Bunsen burner on the roof of the pharmacology building, and the flies were exposed to the gas in a large chamber. After developing serious burns on her hands from the gas, Auerbach let others carry out the exposures, and she analyzed the flies. Auerbach and Robson showed that mustard gas is indeed a powerful mutagen, reducing the viability of gametes and increasing the numbers of mutations seen in the offspring of exposed flies. Because the research was part of the secret war effort, publication of their findings was delayed until 1947.

Base analogs One class of chemical mutagens consists of **base analogs**, chemicals with structures similar to that of any

of the four standard bases of DNA. DNA polymerases cannot distinguish these analogs from the standard bases; so, if base analogs are present during replication, they may be incorporated into newly synthesized DNA molecules. For example, 5-bromouracil (5BU) is an analog of thymine; it has the same structure as that of thymine except that it has a bromine (Br) atom on the 5-carbon atom instead of a methyl group (**Figure 18.17a**). Normally, 5-bromouracil pairs with adenine just as thymine does, but it occasionally mispairs with guanine (**Figure 18.17b**), leading to a transition ($T \cdot A \rightarrow 5BU \cdot A \rightarrow 5BU \cdot G \rightarrow C \cdot G$), as shown in **Figure 18.18**. Through mispairing, 5-bromouracil can also be incorporated into a newly synthesized DNA strand opposite guanine. In the next round of replication 5-bromouracil pairs with adenine, leading to another transition ($G \cdot C \rightarrow G \cdot 5BU \rightarrow A \cdot 5BU \rightarrow A \cdot T$).

Another mutagenic chemical is 2-aminopurine (2AP), which is a base analog of adenine. Normally, 2-aminopurine base pairs with thymine, but it may mispair with cytosine, causing a transition mutation ($T \cdot A \rightarrow T \cdot 2AP \rightarrow C \cdot 2AP \rightarrow C \cdot G$). Alternatively, 2-aminopurine may be incorporated through mispairing into the newly synthesized DNA opposite cytosine and then later pair with thymine, leading to a $C \cdot G \rightarrow C \cdot 2AP \rightarrow T \cdot 2AP \rightarrow T \cdot A$ transition.



18.19 Chemicals may alter DNA bases.

	Original base	Mutagen	Modified base	Pairing partner	Type of mutation
(a)	 Guanine	EMS Alkylation	 <i>O</i> ⁶ -Ethylguanine	 Thymine	CG → TA
(b)	 Cytosine	Nitrous acid (HNO ₂) Deamination	 Uracil	 Adenine	CG → TA
(c)	 Cytosine	Hydroxylamine (NH ₂ OH) Hydroxylation	 Hydroxylamino-cytosine	 Adenine	CG → TA

Thus, both 5-bromouracil and 2-aminopurine can produce transition mutations. In the laboratory, mutations caused by base analogs can be reversed by treatment with the same analog or by treatment with a different analog.

Alkylating agents Alkylating agents are chemicals that donate alkyl groups, such as methyl (CH₃) and ethyl (CH₃–CH₂) groups, to nucleotide bases. For example, ethylmethylsulfonate (EMS) adds an ethyl group to guanine, producing *O*⁶-ethylguanine, which pairs with thymine (**Figure 18.19a**). Thus, EMS produces C·G→T·A transitions. EMS is also capable of adding an ethyl group to thymine, producing 4-ethylthymine, which then pairs with guanine, leading to a T·A→C·G transition. Because EMS produces both C·G→T·A and T·A→C·G transitions, mutations produced by EMS can be reversed by additional treatment with EMS. Mustard gas is another alkylating agent.

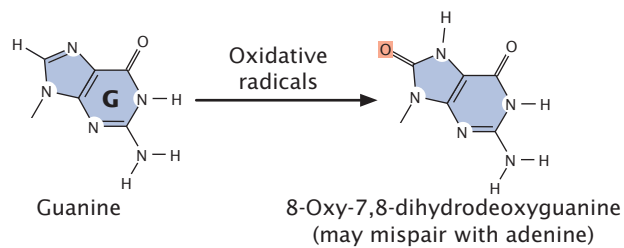
Deamination In addition to its spontaneous occurrence (see **Figure 18.16**), deamination can be induced by some chemicals. For instance, nitrous acid deaminates cytosine, creating uracil, which in the next round of replication pairs with adenine (**Figure 18.19b**), producing a C·G→T·A transition mutation. Nitrous acid changes adenine into hypoxanthine, which pairs with cytosine, leading to a T·A→C·G transition. Nitrous acid also deaminates guanine, producing xanthine, which pairs with cytosine just as guanine does; however, xanthine can also pair with thymine, leading to a C·G→T·A transition. Nitrous acid

produces exclusively transition mutations and, because both C·G→T·A and T·A→C·G transitions are produced, these mutations can be reversed with nitrous acid.

Hydroxylamine Hydroxylamine is a very specific base-modifying mutagen that adds a hydroxyl group to cytosine, converting it into hydroxylaminocytosine (**Figure 18.19c**). This conversion increases the frequency of a rare tautomer that pairs with adenine instead of guanine and leads to C·G→T·A transitions. Because hydroxylamine acts only on cytosine, it will not generate T·A→C·G transitions; thus, hydroxylamine will not reverse the mutations that it produces. **TRY PROBLEM 26** →

Oxidative reactions Reactive forms of oxygen (including superoxide radicals, hydrogen peroxide, and hydroxyl radicals) are produced in the course of normal aerobic metabolism, as well as by radiation, ozone, peroxides, and certain drugs. These reactive forms of oxygen damage DNA and induce mutations by bringing about chemical changes in DNA. For example, oxidation converts guanine into 8-oxy-7,8-dihydrodeoxyguanine (**Figure 18.20**), which frequently mispairs with adenine instead of cytosine, causing a G·C→T·A transversion mutation.

Intercalating agents Proflavin, acridine orange, ethidium bromide, and dioxin are **intercalating agents** (**Figure 18.21a**), which produce mutations by sandwiching



18.20 Oxidative radicals convert guanine into 8-oxy-7,8-dihydrodeoxyguanine.

themselves (intercalating) between adjacent bases in DNA, distorting the three-dimensional structure of the helix and causing single-nucleotide insertions and deletions in replication (**Figure 18.21b**). These insertions and deletions frequently produce frameshift mutations, and so the mutagenic effects of intercalating agents are often severe. Because intercalating agents generate both additions and deletions, they can reverse the effects of their own mutations.

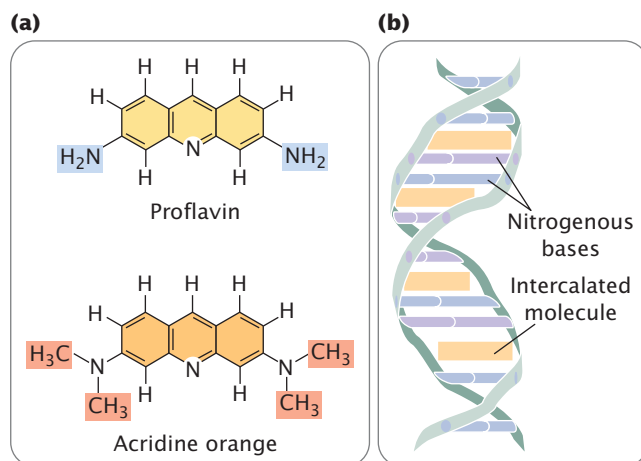
CONCEPTS

Chemicals can produce mutations by a number of mechanisms. Base analogs are inserted into DNA and frequently pair with the wrong base. Alkylating agents, deaminating chemicals, hydroxylamine, and oxidative radicals change the structure of DNA bases, thereby altering their pairing properties. Intercalating agents wedge between the bases and cause single-base insertions and deletions in replication.

✓ CONCEPT CHECK 6

Base analogs are mutagenic because of which characteristic?

- They produce changes in DNA polymerase that cause it to malfunction.
- They distort the structure of DNA.
- They are similar in structure to the normal bases.
- They chemically modify the normal bases.



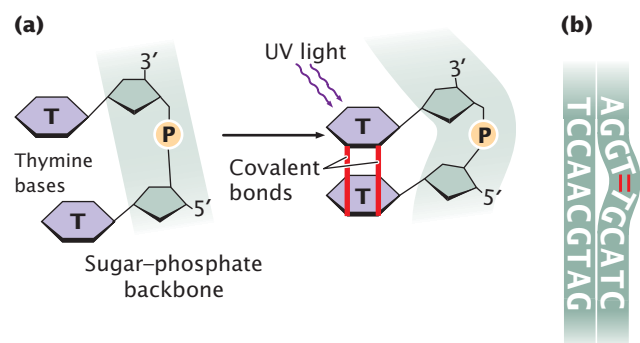
18.21 Intercalating agents. Proflavin and acridine orange (a) insert themselves between adjacent bases in DNA, distorting the three-dimensional structure of the helix (b).

Radiation

In 1927, Hermann Muller demonstrated that mutations in fruit flies could be induced by X-rays. The results of subsequent studies showed that X-rays greatly increase mutation rates in all organisms. Because of their high energies, X-rays, gamma rays, and cosmic rays are all capable of penetrating tissues and damaging DNA. These forms of radiation, called ionizing radiation, dislodge electrons from the atoms that they encounter, changing stable molecules into free radicals and reactive ions, which then alter the structures of bases and break phosphodiester bonds in DNA. Ionizing radiation also frequently results in double-strand breaks in DNA. Attempts to repair these breaks can produce chromosome mutations (discussed in Chapter 9).

Ultraviolet (UV) light has less energy than that of ionizing radiation and does not eject electrons but is nevertheless highly mutagenic. Purine and pyrimidine bases readily absorb UV light, resulting in the formation of chemical bonds between adjacent pyrimidine molecules on the same strand of DNA and in the creation of **pyrimidine dimers** (**Figure 18.22a**). Pyrimidine dimers consisting of two thymine bases (called thymine dimers) are most frequent, but cytosine dimers and thymine–cytosine dimers also can form. Dimers distort the configuration of DNA (**Figure 18.22b**) and often block replication. Most pyrimidine dimers are immediately repaired by mechanisms discussed later in this chapter, but some escape repair and inhibit replication and transcription.

When pyrimidine dimers block replication, cell division is inhibited and the cell usually dies; for this reason, UV light kills bacteria and is an effective sterilizing agent. For a mutation—a hereditary error in the genetic instructions—to occur, the replication block must be overcome. Bacteria can sometimes circumvent replication blocks produced by pyrimidine dimers and other types of DNA damage by means of the **SOS system**. This system allows replication blocks to be overcome but, in the process, makes numerous mistakes and greatly increases the rate of mutation. Indeed, the very reason that replication can proceed in the presence of a block is that the enzymes in the SOS system do not strictly adhere to the base-pairing rules. The trade-off is that



18.22 Pyrimidine dimers result from ultraviolet light. (a) Formation of thymine dimer. (b) Distorted DNA.

replication may continue and the cell survives, but only by sacrificing the normal accuracy of DNA synthesis.

The SOS system is complex, including the products of at least 25 genes. A protein called RecA binds to the damaged DNA at the blocked replication fork and becomes activated. This activation promotes the binding of a protein called LexA, which is a repressor of the SOS system. The activated RecA complex induces LexA to undergo self-cleavage, destroying its repressive activity. This inactivation enables other SOS genes to be expressed, and the products of these genes allow replication of the damaged DNA to proceed. The SOS system allows bases to be inserted into a new DNA strand in the absence of bases on the template strand, but these insertions result in numerous errors in the base sequence.

CONCEPTS

Ionizing radiation such as X-rays and gamma rays damage DNA by dislodging electrons from atoms; these electrons then break phosphodiester bonds and alter the structure of bases. Ultraviolet light causes mutations primarily by producing pyrimidine dimers that disrupt replication and transcription. The SOS system enables bacteria to overcome replication blocks but introduces mistakes in replication.

18.3 Mutations Are the Focus of Intense Study by Geneticists

Because mutations often have detrimental effects, they are often studied by geneticists. These studies have included the development of tests to determine the mutagenic properties of chemical compounds and the investigation of human populations tragically exposed to high levels of radiation.

Detecting Mutations with the Ames Test

People in industrial societies are surrounded by a multitude of artificially produced chemicals: more than 50,000 different chemicals are in commercial and industrial use today, and from 500 to 1000 new chemicals are introduced each year. Some of these chemicals are potential carcinogens and may cause harm to humans. One method for testing the cancer-causing potential of chemicals is to administer them to laboratory animals (rats or mice) and compare the incidence of cancer in the treated animals with that of control animals. These tests are unfortunately time consuming and expensive. Furthermore, the ability of a substance to cause cancer in rodents is not always indicative of its effect on humans. After all, we aren't rats!

In 1974, Bruce Ames developed a simple test for evaluating the potential of chemicals to cause cancer. The **Ames test** is based on the principle that both cancer and mutations result from damage to DNA, and the results of experiments have demonstrated that 90% of known carcinogens are also

mutagens. Ames proposed that mutagenesis in bacteria could serve as an indicator of carcinogenesis in humans.

The Ames test uses different auxotrophic strains of the bacterium *Salmonella typhimurium* that have defects in the lipopolysaccharide coat, which normally protects the bacteria from chemicals in the environment. Furthermore, the DNA-repair system in these strains has been inactivated, enhancing their susceptibility to mutagens.

The most recent version of the test (called Ames II) uses several auxotrophic strains that detect different types of base-pair substitutions. Other strains detect different types of frameshift mutations. Each strain carries a *his⁻* mutation, which renders it unable to synthesize the amino acid histidine, and the bacteria are plated onto medium that lacks histidine (**Figure 18.23**). Only bacteria that have undergone a reverse mutation of the histidine gene (*his⁻ → his⁺*) are able to synthesize histidine and grow on the medium. Different dilutions of a chemical to be tested are added to plates inoculated with the bacteria, and the number of mutant bacterial colonies that appear on each plate is compared with the number that appear on control plates with no chemical (i.e., that arose through spontaneous mutation). Any chemical that significantly increases the number of colonies appearing on a treated plate is mutagenic and is probably also carcinogenic.

Some compounds are not active carcinogens but can be converted into cancer-causing compounds in the body. To make the Ames test sensitive for such *potential* carcinogens, a compound to be tested is first incubated in mammalian liver extract that contains metabolic enzymes.

The Ames test has been applied to thousands of chemicals and commercial products. An early demonstration of its usefulness was the discovery, in 1975, that many hair dyes sold in the United States contained compounds that were mutagenic to bacteria. These compounds were then removed from most hair dyes.

CONCEPTS

The Ames test uses *his⁻* strains of bacteria to test chemicals for their ability to produce *his⁻ → his⁺* mutations. Because mutagenic activity and carcinogenic potential are closely correlated, the Ames test is widely used to screen chemicals for their cancer-causing potential.

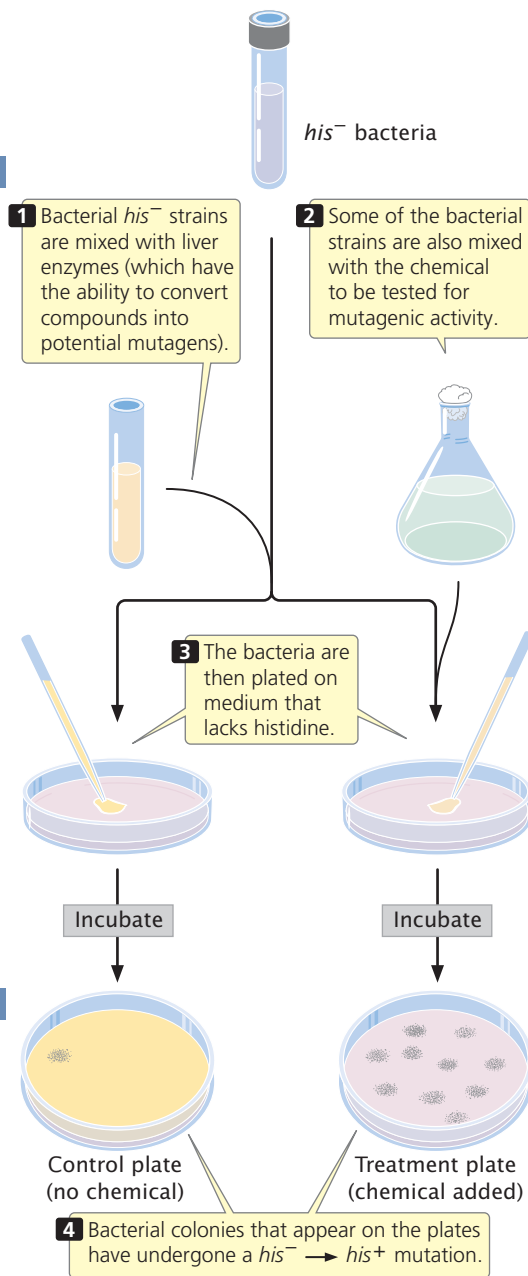
Radiation Exposure in Humans

People are routinely exposed to low levels of radiation from cosmic, medical, and environmental sources, but there have also been tragic events that produced exposures of much higher degree.

On August 6, 1945, a high-flying American airplane dropped a single atomic bomb on the city of Hiroshima, Japan. The explosion devastated an area of the city measuring 4.5 square miles, killed from 90,000 to 140,000 people,

Experiment

Question: How can chemicals be quickly screened for their ability to cause cancer?

Methods**Results**

Conclusion: Any chemical that significantly increases the number of colonies appearing on the treatment plate is mutagenic and therefore probably also carcinogenic.

18.23 The Ames test is used to identify chemical mutagens.

and injured almost as many (Figure 18.24). Three days later, the United States dropped an atomic bomb on the city of Nagasaki, this time destroying an area measuring 1.5 square miles and killing between 60,000 and 80,000 people. Huge amounts of radiation were released during these explosions, and many people were exposed.

After the war, a joint Japanese–U.S. effort was made to study the biological effects of radiation exposure on the survivors of the atomic blasts and their children. Somatic mutations were examined by studying radiation sickness and cancer among the survivors; germ-line mutations were assessed by looking at birth defects, chromosome abnormalities, and gene mutations in children born to people that had been exposed to radiation.

Geneticist James Neel and his colleagues examined almost 19,000 children of people who were within 2000 meters (1.2 miles) of the center of the atomic blast at Hiroshima or Nagasaki, along with a similar number of children whose parents did not receive radiation exposure. Radiation doses were estimated for a child's parents on the basis of careful assessment of the parents' location, posture, and position at the time of the blast. A blood sample was collected from each child, and gel electrophoresis was used to investigate amino acid substitutions in 28 proteins. When rare variants were detected, blood samples from the child's parents also were analyzed to establish whether the variant was inherited or a new mutation.

Of a total of 289,868 genes examined by Neel and his colleagues, only one mutation was found in the children of exposed parents; no mutations were found in the control group. From these findings, a mutation rate of 3.4×10^{-6} was estimated for the children whose parents were exposed to the blast, which is within the range of spontaneous mutation rates observed for other eukaryotes. Neel and his colleagues also examined the frequency of chromosome mutations, sex ratios of children born to exposed parents, and frequencies of chromosome aneuploidy. There was no evidence in any of these assays for increased mutations among the children of the people who were exposed to radiation from the atomic explosions, suggesting that germ-line mutations were not elevated.

Animal studies clearly show that radiation causes germ-line mutations; so why was there no apparent increase in germ-line mutations among the inhabitants of Hiroshima and Nagasaki? The exposed parents did exhibit an increased incidence of leukemia and other types of cancers; so somatic mutations were clearly induced. The answer to the question is not known, but the lack of germ-line mutations may be due to the fact that those persons who received the largest radiation doses died soon after the blasts.

The Techa River in southern Russia is another place where people have been tragically exposed to high levels of radiation. The Mayak nuclear facility produced plutonium for nuclear warheads in the early days of the Cold War. Between 1949 and 1956, this plant dumped some 76 million cubic meters of radioactive sludge into the Techa River. People downstream used the river for drinking water and crop irrigation; some received radiation doses 1700 times the annual amount considered safe by today's standards. Radiation in the area was further elevated by a series of nuclear accidents at the Mayak plant; the worst was an explosion of a radioactive liquid storage tank in 1957, which



18.24 Hiroshima was destroyed by an atomic bomb on August 6, 1945. The atomic explosion produced many somatic mutations among the survivors. [Stanley Troutman/AP.]

showered radiation over a 27,000-square-kilometer (10,425-square-mile) area.

Although Soviet authorities suppressed information about the radiation problems along the Techa until the 1990s, Russian physicians led by Mira Kossenko quietly began studying cancer and other radiation-related illnesses among the inhabitants in the 1960s. They found that the overall incidence of cancer was elevated among people who lived on the banks of the Techa River.

Most data on radiation exposure in humans are from the intensive study of the survivors of the atomic bombing of Hiroshima and Nagasaki. However, the inhabitants of Hiroshima and Nagasaki were exposed in one intense burst of radiation, and these data may not be appropriate for understanding the effects of long-term low-dose radiation. Today, U.S. and Russian scientists are studying the people of the Techa River region in an attempt to better understand the effects of chronic radiation exposure on human populations.

18.4 A Number of Pathways Repair Changes in DNA

The integrity of DNA is under constant assault from radiation, chemical mutagens, and spontaneously arising changes. In spite of this onslaught of damaging agents, the rate of mutation remains remarkably low, thanks to the efficiency with which DNA is repaired.

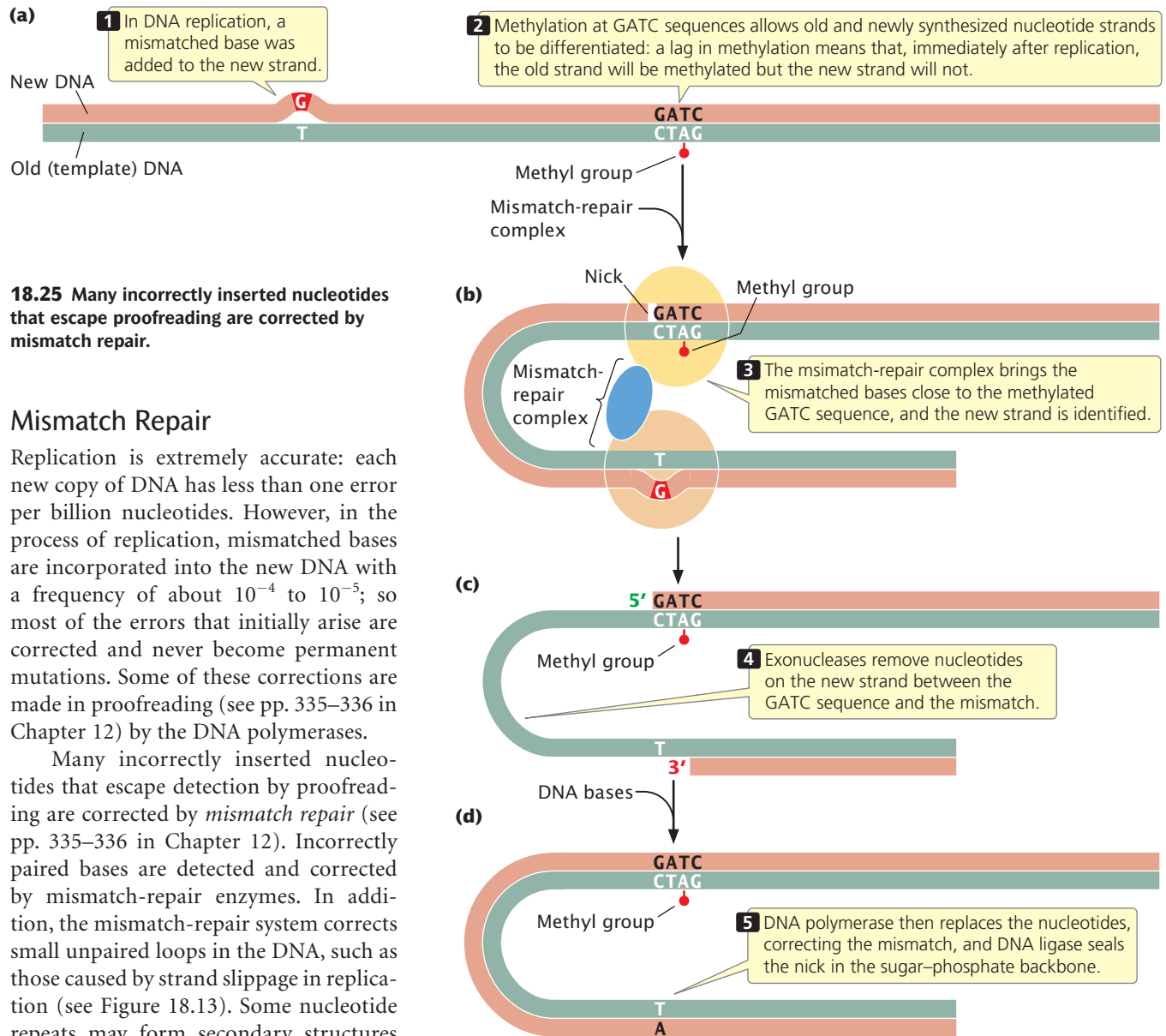
There are a number of complex pathways for repairing DNA, but several general statements can be made about DNA repair. First, most DNA-repair mechanisms require two nucleotide strands of DNA because most replace whole nucleotides, and a template strand is needed to specify the base sequence.

A second general feature of DNA repair is redundancy, meaning that many types of DNA damage can be corrected by more than one pathway of repair. This redundancy testifies to the extreme importance of DNA repair to the survival of the cell: it ensures that almost all mistakes are corrected. If a mistake escapes one repair system, it's likely to be repaired by another system.

We will consider several general mechanisms of DNA repair: mismatch repair, direct repair, base-excision repair, nucleotide-excision repair, and repair of double-strand breaks (Table 18.4).

Table 18.4 Summary of common DNA repair mechanisms

Repair System	Type of Damage Repaired
Mismatch	Replication errors, including mispaired bases and strand slippage
Direct	Pyrimidine dimers; other specific types of alterations
Base excision	Abnormal bases, modified bases, and pyrimidine dimers
Nucleotide excision	DNA damage that distorts the double helix, including abnormal bases, modified bases, and pyrimidine dimers
Homologous recombination	Double-strand breaks
Nonhomologous end joining	Double-strand breaks



18.25 Many incorrectly inserted nucleotides that escape proofreading are corrected by mismatch repair.

Mismatch Repair

Replication is extremely accurate: each new copy of DNA has less than one error per billion nucleotides. However, in the process of replication, mismatched bases are incorporated into the new DNA with a frequency of about 10^{-4} to 10^{-5} ; so most of the errors that initially arise are corrected and never become permanent mutations. Some of these corrections are made in proofreading (see pp. 335–336 in Chapter 12) by the DNA polymerases.

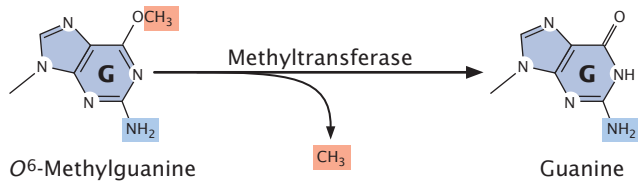
Many incorrectly inserted nucleotides that escape detection by proofreading are corrected by *mismatch repair* (see pp. 335–336 in Chapter 12). Incorrectly paired bases are detected and corrected by mismatch-repair enzymes. In addition, the mismatch-repair system corrects small unpaired loops in the DNA, such as those caused by strand slippage in replication (see Figure 18.13). Some nucleotide repeats may form secondary structures on the unpaired strand (see Figure 18.5), allowing them to escape detection by the mismatch-repair system.

After the incorporation error has been recognized, mismatch-repair enzymes cut out a section of the newly synthesized strand and fill the gap with new nucleotides by using the original DNA strand as a template. For this strategy to work, mismatch repair must have some way of distinguishing between the old and the new strands of the DNA so that the incorporation error, but not part of the original strand, is removed.

The proteins that carry out mismatch repair in *E. coli* differentiate between old and new strands by the presence of methyl groups on special sequences of the old strand. After replication, adenine nucleotides in the sequence GATC are methylated. The process of methylation is delayed and so, immediately after replication, the old strand is methylated and the new strand is not (Figure 18.25a). The mismatch-

repair complex brings an unmethylated GATC sequence in close proximity to the mismatched bases. It nicks the unmethylated strand at the GATC site (Figure 18.25b), and degrades the strand between the nick and the mismatched bases (Figure 18.25c). DNA polymerase and DNA ligase fill in the gap on the unmethylated strand with correctly paired nucleotides (Figure 18.25d).

Mismatch repair in eukaryotic cells is similar to that in *E. coli*, but how the old and new strands are recognized in eukaryotic cells is not known. In some eukaryotes, such as yeast and fruit flies, there is no detectable methylation of DNA, and yet mismatch repair still takes place. Humans who possess mutations in mismatch-repair genes often exhibit elevated somatic mutations and are frequently susceptible to colon cancer.



18.26 Direct repair changes nucleotides back into their original structures.

CONCEPTS

Mismatched bases and other DNA lesions are corrected by mismatch repair. Enzymes cut out a section of the newly synthesized strand of DNA and replace it with new nucleotides.

✓ CONCEPT CHECK 7

Mismatch repair in *E. coli* distinguishes between old and new strands of DNA on the basis of

- differences in base composition of the two strands.
- modification of histone proteins.
- base analogs on the new strand.
- methyl groups on the old strand.

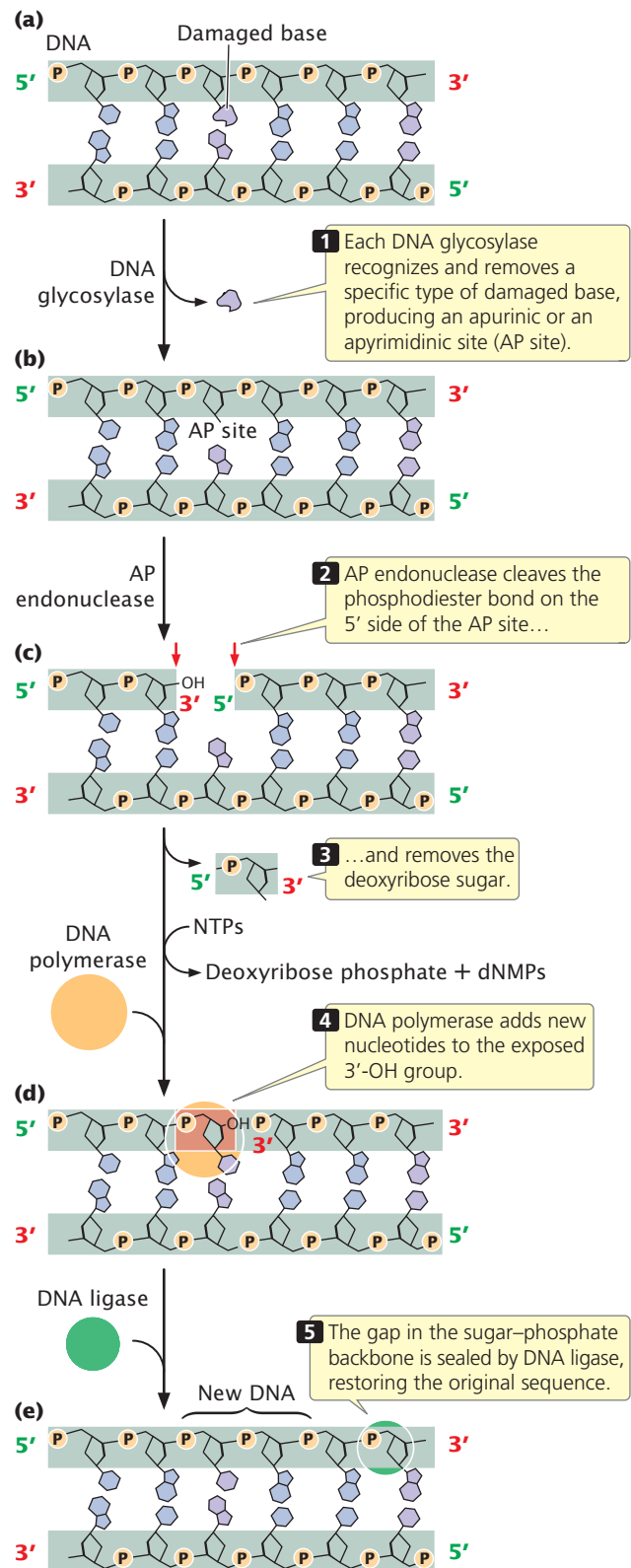
Direct Repair

Direct repair does not replace altered nucleotides but, instead, changes them back into their original (correct) structures. One of the most well understood direct-repair mechanisms is the photoreactivation of UV-induced pyrimidine dimers. *E. coli* and some eukaryotic cells possess an enzyme called photolyase, which uses energy captured from light to break the covalent bonds that link the pyrimidines in a dimer.

Direct repair also corrects *O*⁶-methylguanine, an alkylation product of guanine that pairs with adenine, producing G·C→T·A transversions. An enzyme called *O*⁶-methylguanine-DNA methyltransferase removes the methyl group from *O*⁶-methylguanine, restoring the base to guanine (Figure 18.26).

Base-Excision Repair

In **base-excision repair**, a modified base is first excised and then the entire nucleotide is replaced. The excision of modified bases is catalyzed by a set of enzymes called DNA glycosylases, each of which recognizes and removes a specific type of modified base by cleaving the bond that links that base to the 1'-carbon atom of deoxyribose sugar (Figure 18.27a). Uracil glycosylase, for example, recognizes and removes uracil produced by the deamination of cytosine. Other glycosylases recognize hypoxanthine, 3-methyladenine, 7-methylguanine, and other modified bases.



18.27 Base-excision repair excises modified bases and then replaces one or more nucleotides.

After the base has been removed, an enzyme called AP (apurinic or apyrimidinic) endonuclease cuts the phosphodiester bond, and other enzymes remove the deoxyribose sugar (Figure 18.27b). DNA polymerase then adds one or more new nucleotides to the exposed 3'-OH group (Figure 18.27c), replacing a section of nucleotides on the damaged strand. The nick in the phosphodiester backbone is sealed by DNA ligase (Figure 18.27d), and the original intact sequence is restored (Figure 18.27e).

Bacteria use DNA polymerase I to replace excised nucleotides, but eukaryotes use DNA polymerase β , which has no proofreading ability and tends to make mistakes. On average, DNA polymerase β makes one mistake per 4000 nucleotides inserted. About 20,000 to 40,000 base modifications per day are repaired by base excision, and so DNA polymerase β may introduce as many as 10 mutations per day into the human genome. How are these errors corrected? Recent research results show that some AP endonucleases have the ability to proofread. When DNA polymerase β inserts a nucleotide with the wrong base into the DNA, DNA ligase cannot seal the nick in the sugar-phosphate backbone, because the 3'-OH and 5'-P groups of adjacent nucleotides are not in the correct orientation for ligase to connect them. In this case, AP endonuclease 1 detects the mispairing and uses its 3'→5' exonuclease activity to excise the incorrectly paired base. DNA polymerase β then uses its polymerase activity to fill in the missing nucleotide. In this way, the fidelity of base-excision repair is maintained.

CONCEPTS

Direct-repair mechanisms change altered nucleotides back into their correct structures. In base-excision repair, glycosylase enzymes recognize and remove specific types of modified bases. The entire nucleotide is then removed and a section of the polynucleotide strand is replaced.

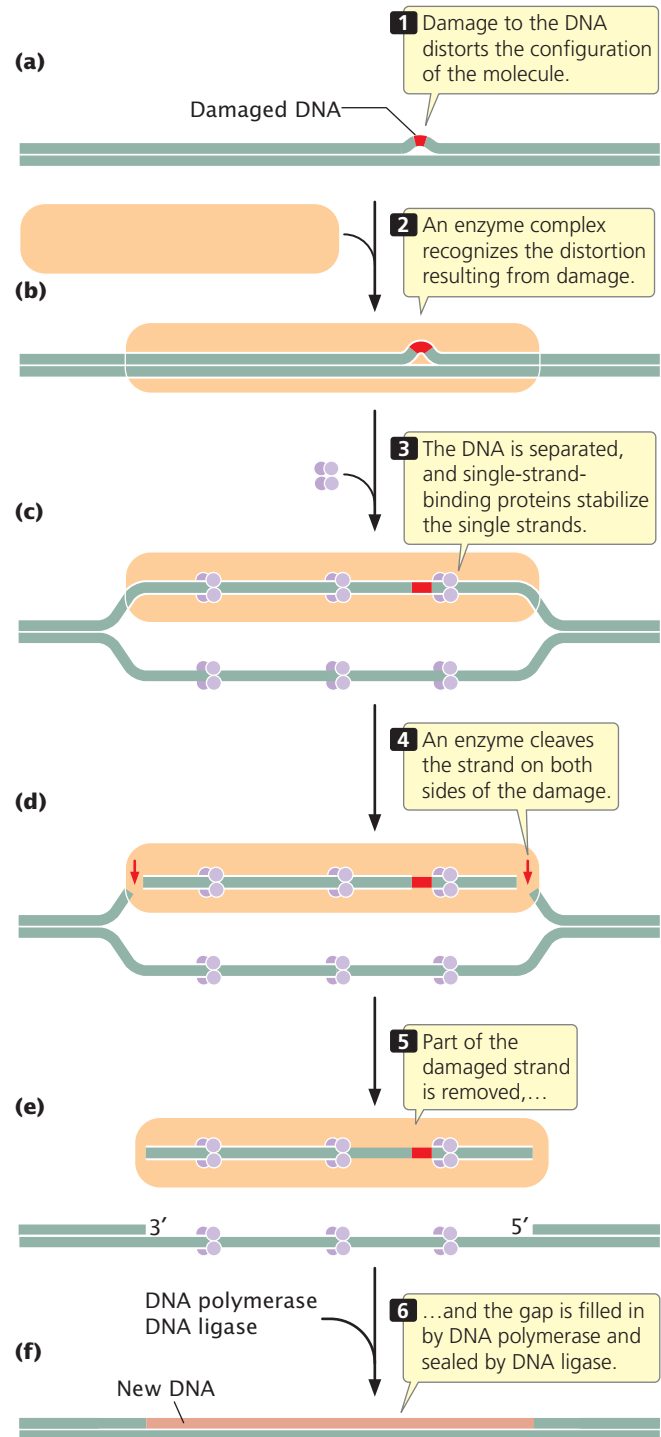
✓ CONCEPT CHECK 8

How do direct-repair mechanisms differ from mismatch repair and base-excision repair?

Nucleotide-Excision Repair

Another repair pathway is **nucleotide-excision repair**, which removes bulky DNA lesions (such as pyrimidine dimers) that distort the double helix. Nucleotide-excision repair is quite versatile and can repair many different types of DNA damage. It is found in cells of all organisms from bacteria to humans and is among the most important of all repair mechanisms.

The process of nucleotide excision is complex; in humans, a large number of genes take part. First, a complex of enzymes scans DNA, looking for distortions of its



18.28 Nucleotide-excision repair removes bulky DNA lesions that distort the double helix.

three-dimensional configuration (Figure 18.28a and b). When a distortion is detected, additional enzymes separate the two nucleotide strands at the damaged region, and single-strand-binding proteins stabilize the separated strands (Figure 18.28c). Next, the sugar-phosphate backbone of

the damaged strand is cleaved on both sides of the damage (**Figure 18.28d**). Part of the damaged strand is peeled away by helicase enzymes (**Figure 18.28e**), and the gap is filled in by DNA polymerase and sealed by DNA ligase (**Figure 18.28f**).

CONCEPTS

Nucleotide-excision repair removes and replaces many types of damaged DNA that distort the DNA structure. The two strands of DNA are separated, a section of the DNA containing the distortion is removed, DNA polymerase fills in the gap, and DNA ligase seals the filled-in gap.

CONNECTING CONCEPTS

The Basic Pathway of DNA Repair

We have now examined several different mechanisms of DNA repair. What do these methods have in common? How are they different? Most methods of DNA repair depend on the presence of two strands, because nucleotides in the damaged area are removed and replaced. Nucleotides are replaced in mismatch repair, base-excision repair, and nucleotide-excision repair but are not replaced by direct-repair mechanisms.

Repair mechanisms that include nucleotide removal utilize a common four-step pathway:

1. **Detection:** The damaged section of the DNA is recognized.
2. **Excision:** DNA-repair endonucleases nick the phosphodiester backbone on one or both sides of the DNA damage and one or more nucleotides are removed.
3. **Polymerization:** DNA polymerase adds nucleotides to the newly exposed 3'-OH group by using the other strand as a template and replacing damaged (and frequently some undamaged) nucleotides.
4. **Ligation:** DNA ligase seals the nicks in the sugar-phosphate backbone.

The primary differences in the mechanisms of mismatch, base-excision, and nucleotide-excision repair are in the details of detection and excision. In base-excision and mismatch repair, a single nick is made in the sugar-phosphate backbone on one side of the damage; in nucleotide-excision repair, nicks are made on both sides of the DNA lesion. In base-excision repair, DNA polymerase displaces the old nucleotides as it adds new nucleotides to the 3' end of the nick; in mismatch repair, the old nucleotides are degraded; and, in nucleotide-excision repair, nucleotides are displaced by helicase enzymes. All three mechanisms use DNA polymerase and ligase to fill in the gap produced by the excision and removal of damaged nucleotides.

Repair of Double-Strand Breaks

A common type of DNA damage is a double-strand break, in which both strands of the DNA helix are broken. Double-strand breaks are caused by ionizing radiation, oxidative free

radicals, and other DNA-damaging agents. These types of breaks are particularly detrimental to the cell because they stall DNA replication and may lead to chromosome rearrangements, such as deletions, duplications, inversions, and translocations. There are two major pathways for repairing double-strand breaks: homologous recombination and non-homologous end joining.

Homologous recombination Homologous recombination repairs a broken DNA molecule by using the identical or nearly identical genetic information contained in another DNA molecule, usually a sister chromatid. DNA repair through homologous recombination uses the same mechanism employed in the process of homologous recombination that is responsible for crossing over (see Chapter 12). Homologous recombination begins with the removal of some nucleotides at the broken ends, followed by strand invasion, displacement, and replication (see Figure 12.21). Many of the same enzymes that carry out crossing over are utilized in the repair of double-strand breaks by homologous recombination. Two enzymes that play a role in homologous recombination are BRCA1 and BRCA2. The genes for these proteins are frequently mutated in breast cancer cells.

Nonhomologous end joining Nonhomologous end joining repairs double-strand breaks without using a homologous template. This pathway is often used when the cell is in G₁ and a sister chromatid is not available for repair through homologous recombination. Nonhomologous end joining uses proteins that recognize the broken ends of DNA, bind to the ends, and then joins them together. Non-homologous end joining is more error prone than homologous recombination and often leads to deletions, insertions, and translocations.

Translesion DNA Polymerases

As discussed in Chapter 12, the high-fidelity DNA polymerases that normally carry out replication operate at high speed and, like a high-speed train, require a smooth track—an undistorted template. Some mutations, such as pyrimidine dimers, produce distortions in the three-dimensional structure of the DNA helix, blocking replication by the high-speed polymerases. When distortions of the template are encountered, specialized translesion DNA polymerases take over replication and bypass the lesion.

The translesion polymerases are able to bypass bulky lesions but, in the process, often make errors. Thus, the translesion polymerases allow replication to proceed past bulky lesion but at the cost of introducing mutations into the sequence. Some of these mutations are corrected by DNA-repair systems, but others escape detection.

An example of a translesion DNA polymerase is polymerase η (eta), which bypasses pyrimidine dimers in eukaryotes.

Polymerase η inserts AA opposite a pyrimidine dimer. This strategy seems to be reasonable because about two-thirds of pyrimidine dimers are thymine dimers. However, the insertion of AA opposite a CT dimer results in a C·G→A·T transversion. Polymerase η therefore tends to introduce mutations into the DNA sequence.

CONCEPTS

Two major pathways exist for the repair of double-strand breaks in DNA: homologous recombination and nonhomologous end joining. Special translesion DNA polymerases allow replication to proceed past bulky distortions in the DNA but often introduce errors as they bypass the distorted region.

Genetic Diseases and Faulty DNA Repair

Several human diseases are connected to defects in DNA repair. These diseases are often associated with high incidences of specific cancers, because defects in DNA repair lead to increased rates of mutation. This concept is discussed further in Chapter 23.

Among the best studied of the human DNA-repair diseases is xeroderma pigmentosum (**Figure 18.29**), a rare autosomal recessive condition that includes abnormal skin pigmentation and acute sensitivity to sunlight. Persons who have this disease also have a strong predisposition to skin cancer, with an incidence ranging from 1000 to 2000 times that found in unaffected people.



18.29 Xeroderma pigmentosum results from defects in DNA repair. The disease is characterized by frecklelike spots on the skin (shown here) and a predisposition to skin cancer. [Ken Greer/Visuals Unlimited.]

Sunlight includes a strong UV component; so exposure to sunlight produces pyrimidine dimers in the DNA of skin cells. Although human cells lack photolyase (the enzyme that repairs pyrimidine dimers in bacteria), most pyrimidine dimers in humans can be corrected by nucleotide-excision repair (see **Figure 18.28**). However, the cells of most people with xeroderma pigmentosum are defective in nucleotide-excision repair, and many of their pyrimidine dimers go uncorrected and may lead to cancer.

Xeroderma pigmentosum can result from defects in several different genes. Some persons with xeroderma pigmentosum have mutations in a gene encoding the protein that recognizes and binds to damaged DNA; others have mutations in a gene encoding helicase. Still others have defects in the genes that play a role in cutting the damaged strand on the 5' or 3' sides of the pyrimidine dimer. Some persons have a slightly different form of the disease (xeroderma pigmentosum variant) owing to mutations in the gene encoding polymerase η , a translesion DNA polymerase that bypasses pyrimidine dimers.

Two other genetic diseases due to defects in nucleotide-excision repair are Cockayne syndrome and trichothiodystrophy (also known as brittle-hair syndrome). Persons who have either of these diseases do not have an increased risk of cancer but do exhibit multiple developmental and neurological problems. Both diseases result from mutations in some of the same genes that cause xeroderma pigmentosum. Several of the genes taking part in nucleotide-excision repair produce proteins that also play a role in recombination and the initiation of transcription. These other functions may account for the developmental symptoms seen in Cockayne syndrome and trichothiodystrophy.

Another genetic disease caused by faulty DNA repair is an inherited form of colon cancer called hereditary non-polyposis colon cancer (HNPCC). This cancer is one of the most common hereditary cancers, accounting for about 15% of colon cancers. Research findings indicate that HNPCC arises from mutations in the proteins that carry out mismatch repair (see **Figure 18.25**). Some additional genetic diseases associated with defective DNA repair are summarized in **Table 18.5**. **TRY PROBLEM 32** →

CONCEPTS

Defects in DNA repair are the underlying cause of several genetic diseases. Many of these diseases are characterized by a predisposition to cancer.

✓ CONCEPT CHECK 9

Why are defects in DNA repair often associated with increases in cancer?

Table 18.5 Genetic diseases associated with defects in DNA-repair systems

Disease	Symptoms	Genetic Defect
Xeroderma pigmentosum	Frecklelike spots on skin, sensitivity to sunlight, predisposition to skin cancer	Defects in nucleotide-excision repair
Cockayne syndrome	Dwarfism, sensitivity to sunlight, premature aging, deafness, mental retardation	Defects in nucleotide-excision repair
Trichothiodystrophy	Brittle hair, skin abnormalities, short stature, immature sexual development, characteristic facial features	Defects in nucleotide-excision repair
Hereditary nonpolyposis colon cancer	Predisposition to colon cancer	Defects in mismatch repair
Fanconi anemia	Increased skin pigmentation, abnormalities of skeleton, heart, and kidneys, predisposition to leukemia	Possibly defects in the repair of interstrand cross-links
Li-Fraumeni syndrome	Predisposition to cancer in many different tissues	Defects in DNA damage response
Werner syndrome	Premature aging, predisposition to cancer	Defect in homologous recombination

CONCEPTS SUMMARY

- Mutations are heritable changes in genetic information. Somatic mutations occur in somatic cells; germ-line mutations occur in cells that give rise to gametes.
- The simplest type of mutation is a base substitution, a change in a single base pair of DNA. Transitions are base substitutions in which purines are replaced by purines or pyrimidines are replaced by pyrimidines. Transversions are base substitutions in which a purine replaces a pyrimidine or a pyrimidine replaces a purine.
- Insertions are the addition of nucleotides, and deletions are the removal of nucleotides; these mutations often change the reading frame of the gene.
- Expanding nucleotide repeats are mutations in which the number of copies of a set of nucleotides increases with the passage of time; they are responsible for several human genetic diseases.
- A missense mutation alters the coding sequence so that one amino acid substitutes for another. A nonsense mutation changes a codon that specifies an amino acid into a termination codon. A silent mutation produces a synonymous codon that specifies the same amino acid as the original sequence, whereas a neutral mutation alters the amino acid sequence but does not change the functioning of the protein. A suppressor mutation reverses the effect of a previous mutation at a different site and may be intragenic (within the same gene as the original mutation) or intergenic (within a different gene).
- Mutation rate is the frequency with which a particular mutation arises in a population. Mutation rates are influenced by both genetic and environmental factors.
- Some mutations occur spontaneously. These mutations include the mispairing of bases in replication and spontaneous depurination and deamination.
- Insertions and deletions can arise from strand slippage in replication or from unequal crossing over.
- Base analogs can become incorporated into DNA in the course of replication and pair with the wrong base in subsequent replication events. Alkylating agents and hydroxylamine modify the chemical structure of bases and lead to mutations. Intercalating agents insert into the DNA molecule and cause single-nucleotide additions and deletions. Oxidative reactions alter the chemical structures of bases.
- Ionizing radiation is mutagenic, altering base structures and breaking phosphodiester bonds. Ultraviolet light produces pyrimidine dimers, which block replication. Bacteria use the SOS response to overcome replication blocks produced by pyrimidine dimers and other lesions in DNA.
- The Ames tests uses bacteria to assess the mutagenic potential of chemical substances.
- Most damage to DNA is corrected by DNA-repair mechanisms. These mechanisms include mismatch repair, direct repair, base-excision repair, nucleotide-excision repair, and other repair pathways. Most require two strands of DNA and exhibit some overlap in the types of damage repaired.
- Double-strand breaks are repaired by homologous recombination and nonhomologous end joining. Special translesion DNA polymerases allow replication to proceed past bulky distortions in the DNA.
- Defects in DNA repair are the underlying cause of several genetic diseases.