A Master Thief

The archaeon *Haloarcula marismortui* and other haloarchaea have an interesting evolutionary history. They are thought to have evolved from methanogens. Thus they began their existence as strict anaerobes that carried out chemolithotrophy. Today they are found in habitats with plenty of light and lots of organic material. Importantly, they now are metabolically flexible, functioning either as aerobic chemoorganotrophs when oxygen is readily available or as phototrophs when the environment becomes microoxic. How did they make this drastic change?

Evidence suggests that *H. marismortui* and its haloarchaeal cousins successfully morphed into their new way of life by becoming master thieves; they stole genes from other organisms in their environment. In particular, haloarchaea stole many genes from bacteria. *H. marismortui* has done this so well, it has cobbled together several bacterial genes to create a unique anaplerotic pathway called the methylaspartate cycle (*see figure 20.5*). This pathway is especially important when *H. marismortui* is starving. Under starvation conditions, it catabolizes carbon and energy stores such as polyhydroxyalkanoates (PHAs) by a process that yields acetyl-CoA. Acetyl-CoA can then be used not only to make ATP but also to generate many precursor metabolites; thus the need for anaplerotic reactions (*see section 12.5*).

As clever as *H. marismortui* has been, it is not alone in its ability to capture and use genes from other organisms. All bacteria and archaea do so. As more and more bacterial and archaeal genomes have been sequenced and annotated, it has become increasingly clear that taking the genes from other microbes has occurred many times. One outcome of this is to increase the genetic diversity of a population of microbes, thus raising the probability that some members of the population will survive in spite of dramatic environmental changes.

Genetic diversity also is necessary for evolution to occur. Organisms with certain genotypes, and therefore phenotypes, may survive to eventually evolve into new species. Note that the change in phenotype of concern here is not due to changes in gene expression brought about by the types of regulatory processes discussed in chapters 14 and 15. Rather, we are referring to heritable changes in phenotype brought about by changes in the nucleotide sequence of a microbe's genome.

How the genomes of microbes are altered to increase genetic diversity in a population is the topic of this chapter. As you will see, microbes have numerous mechanisms for introducing genetic diversity; our main focus is on how this is accomplished in the absence of sexual reproduction. Some of the mechanisms we discuss can create changes in the genome that are actually detrimental. Thus we also consider processes that help balance the stability of the genome with the capacity to introduce genetic variation.

Readiness Check:

Based on what you have learned previously, you should be able to:

- ✔ Draw a simple diagram that illustrates the three chemical moieties that define a nucleotide (section 13.2)
- ✔ State the base-pairing rules
- ✔ Explain the importance of the reading frame of a protein-coding gene (section 13.6)
- ✔ Describe the major events of meiosis
- ✔ Describe the major types of plasmids (section 3.6)
- ✔ Create a concept map or table that distinguishes the secretion systems observed in Gram-negative bacteria (section 13.8)

16.1 Mutations

After reading this section, you should be able to:

- Distinguish spontaneous from induced mutations, and list the most common ways each arises
- Construct a table, concept map, or picture to summarize how base analogues, DNA-modifying agents, and intercalating agents cause mutations
- Discuss the possible effects of mutations

Perhaps the most obvious way genetic diversity can be created is by **mutations** (Latin *mutare*, to change). Several types of mutations exist. Some arise from the alteration of single pairs of nucleotides and from the addition or deletion of one nucleotide pair in the coding regions of a gene. Such small changes in DNA are called **point mutations** because they affect only one base pair in a given location. Larger mutations are less common. These include large insertions, deletions, inversions, duplications, and translocations of nucleotide sequences.

Mutations occur in one of two ways. (1) **Spontaneous mutations** arise occasionally in all cells and in the absence of any added agent. (2) **Induced mutations** are the result of exposure to a **mutagen,** which can be either a physical or a chemical agent. Mutations are characterized according to either the kind of genotypic change that has occurred or their phenotypic consequences. In this section, the molecular basis of mutations and mutagenesis is first considered. Then the phenotypic effects of mutations are discussed.

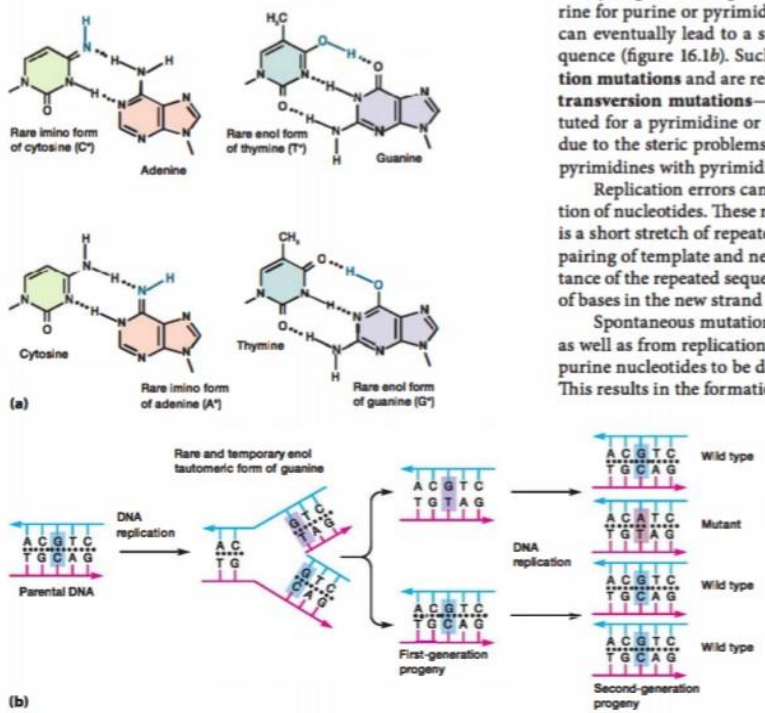

Figure 16.1 Tautomerization and Transition Mutations. Errors in replication due to base tautomerization. (a) Normally AT and GC pairs are formed when keto groups participate in hydrogen bonds. In contrast, enol tautomers produce AC and GT base pairs. The alteration in the base is shown in blue. (b) Mutation as a consequence of tautomerization during DNA replication. The temporary enolization of guanine leads to the formation of an AT base pair in the mutant, and a GC–to–AT transition mutation occurs. The process requires two replication cycles. Mutation only occurs if the abnormal first-generation GT base pair is missed by repair mechanisms. Wild type is the form of the gene before mutation occurred.

Spontaneous Mutations

Spontaneous mutations result from errors in DNA replication, spontaneously occurring lesions in DNA, or the action of mobile genetic elements such as transposons (section 16.5). A few of the more prevalent mechanisms are described here.

Replication errors can occur when the nitrogenous base of a nucleotide shifts to a different form (isomer) called a tautomeric form. Nitrogenous bases typically exist in the keto form (*see figure 13.5*) but are in equilibrium with the rarer imino and enol forms (**figure 16.1*a***). The shift from one form to another changes the hydrogen-bonding characteristics of the bases, allowing purine for purine or pyrimidine for pyrimidine substitutions that can eventually lead to a stable alteration of the nucleotide sequence (figure 16.1*b*). Such substitutions are known as **transition mutations** and are relatively common. On the other hand, **transversion mutations**—mutations where a purine is substituted for a pyrimidine or a pyrimidine for a purine—are rarer due to the steric problems of pairing purines with purines and pyrimidines with pyrimidines.

Replication errors can also result in the insertion and deletion of nucleotides. These mutations generally occur where there is a short stretch of repeated nucleotides. In such a location, the pairing of template and new strand can be displaced by the distance of the repeated sequence, leading to insertions or deletions of bases in the new strand (**figure 16.2**).

Spontaneous mutations can originate from lesions in DNA as well as from replication errors. For example, it is possible for purine nucleotides to be depurinated; that is, to lose their base. This results in the formation of an apurinic site, which does not base pair normally and may cause a mutation after the next round of replication (**figure 16.3**). Likewise, pyrimidines can be lost, forming an apyrimidinic site. Other lesions are caused by reactive forms of oxygen such as oxygen free radicals and peroxides produced during aerobic metabolism. For example, guanine can be converted to 8-oxo-7,8-dihydrodeoxyguanine, which often pairs with adenine, rather than cytosine, during replication.

Induced Mutations

Any agent that damages DNA, alters its chemistry, or in some way interferes with its functioning will probably induce mutations. Mutagens can be conveniently classified according to their mode of

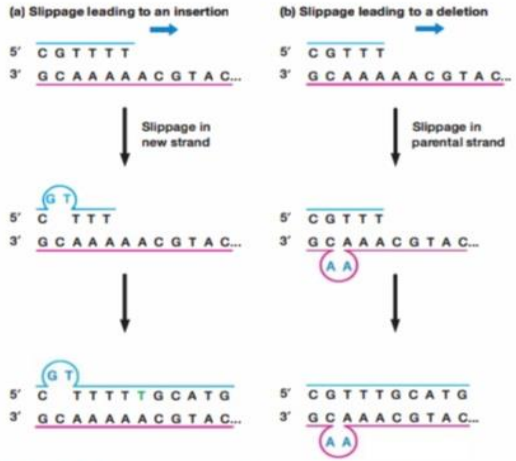

Figure 16.2 Insertions and Deletions. A mechanism for the generation of insertions and deletions during replication. The direction of replication is indicated by the blue arrow. In each case, there is strand slippage resulting in the formation of a small loop that is stabilized by hydrogen bonding in the repetitive sequence, the AT stretch in this example. (a) If the new strand slips, an addition of one T results. (b) Slippage of the parental strand yields a deletion (in this case, a loss of two Ts).

action. Three common types of chemical mutagens are base analogues, DNA-modifying agents, and intercalating agents. A number of physical agents (e.g., radiation) are mutagens that damage DNA.

Base analogues are structurally similar to normal nitrogenous bases and can be incorporated into the growing polynucleotide chain during replication (**table 16.1**). Once in place, these compounds typically exhibit base-pairing properties different from the bases they replace and can eventually cause a stable mutation. A widely used base analogue is 5-bromouracil, an analog of thymine. It undergoes a tautomeric shift from the normal keto form to an enol much more frequently than does a normal base. The enol tautomer forms hydrogen bonds like cytosine, pairing with guanine rather than adenine. The mechanism of action of other base analogues is similar to that of 5-bromouracil.

There are many **DNA-modifying agents**—mutagens that change a base's structure and therefore alter its base-pairing specificity. Some of these mutagens are selective; they preferentially react with certain bases and produce a particular kind of DNA damage. For example, methyl-nitrosoguanidine is an alkylating agent that adds methyl groups to guanine, causing it to mispair with thymine (**figure 16.4**). A subsequent round of replication can then result in a GC-AT transition. Hydroxylamine is another example of a DNA-modifying agent. It hydroxylates the nitrogen attached to the number 4 carbon (C-4) of cytosine (*see figure 13.5*), causing it to base pair like thymine.

Intercalating agents distort DNA to induce single nucleotide pair insertions and deletions. These mutagens are planar and insert themselves (intercalate) between the stacked bases of the helix. This results in a mutation, possibly through the formation of a loop in DNA. Intercalating agents include acridines such as proflavin and acridine orange.

Many mutagens, and indeed many carcinogens, damage bases so severely that hydrogen bonding between base pairs is

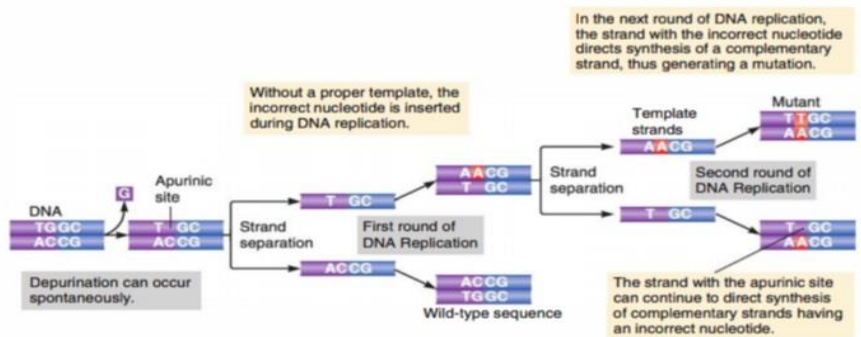

Figure 16.3 Mutation Due to Formation of an Apurinic Site. Apurinic sites can arise spontaneously (depurination). They do not provide a template for DNA replication, and as a result, an incorrect nucleotide can be inserted. A similar process can introduce mutations at sites where apyrimidinic sites arise.

Table 16.1 Examples of Mutagens	
Mutagen	**Effect(s) on DNA Structure**
Chemical	
5-Bromouracil	Base analogue
2-Aminopurine	Base analogue
Ethyl methanesulfonate	Alkylating agent
Hydroxylamine	Hydroxylates cytosine
Nitrogen mustard	Alkylating agent
Nitrous oxide	Deaminates bases
Proflavin	Intercalating agent
Acridine orange	Intercalating agent
Physical	
UV light	Promotes pyrimidine dimer formation
X rays	Cause base deletions, single-strand nicks, cross-linking, and chromosomal breaks

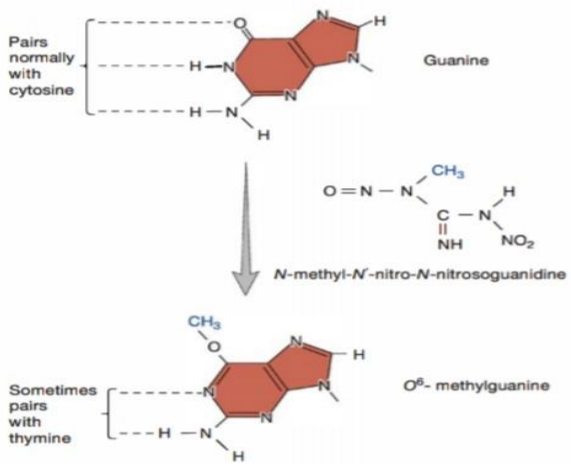

Figure 16.4 Methyl-Nitrosoguanidine Mutagenesis. Methylnitrosoguanidine methylates guanine.

impaired or prevented and the damaged DNA can no longer act as a template for replication. For instance, ultraviolet (UV) radiation generates cyclobutane dimers, usually thymine dimers, between adjacent pyrimidines (figure 16.5). Other examples are ionizing radiation and carcinogens such as the fungal toxin aflatoxin B1 and other benzo(a)pyrene derivatives.

Effects of Mutations

The effects of a mutation can be described at the protein level and in terms of observed phenotypes. In all cases, the impact is readily noticed only if it produces a change in phenotype. In general, the more prevalent form of a gene and its associated phenotype is called the **wild type.** A mutation from wild type to a mutant form is a **forward mutation** (table 16.2). The effect of a forward mutation can be reversed by a second mutation that restores the wild-type phenotype. The second mutation can occur at the same site as the original mutation or at another site. When the second mutation is at the same site as the original mutation (e.g., the same base pair in a codon), it is called a **reversion mutation.** Some reversion mutations re-establish the original wild-type sequence. Others create a new codon that codes for the same amino acid. Reversions

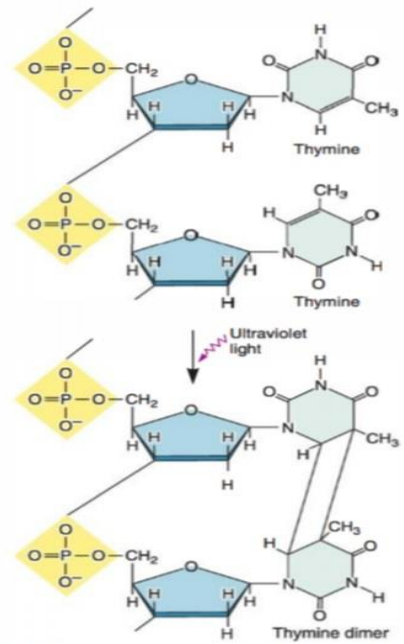

Figure 16.5 Thymine Dimers Are Formed by Ultraviolet Radiation.

Table 16.2 Types of Point Mutations

Type of Mutation	Change in DNA	Example
Forward Mutations		
None	None	5′-A-T-G-A-C-C-T-C-C-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Ser - Pro - Lys - Gly
Silent	Base substitution	5′-A-T-G-A-C-A-T-C-C-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Ser - Pro - Lys - Gly
Missense	Base substitution	5′-A-T-G-A-C-C-T-G-C-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Cys - Pro - Lys - Gly
Nonsense		5′-A-T-G-A-C-C-T-C-C-C-C-G-T-A-A-G-G-G-3′ Met - Thr - Ser - Pro - STOP!
Frameshift	Insertion/deletion	5′-A-T-G-A-C-C-T-C-C-G-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Ser - Ala - Glu - Arg
Reverse Mutations		
	Base substitution	5′-A-T-G-A-C-C-T-C-C $\xrightarrow{\text{forward}}$ A-T-G-C-C-C-T-C-C $\xrightarrow{\text{reverse}}$ A-T-G-A-C-C-T-C-C Met - Thr - Ser Met - Pro - Ser Met - Thr - Ser
	Base substitution	5′-A-T-G-A-C-C-T-C-C $\xrightarrow{\text{forward}}$ A-T-G-A-C-C-T-G-C $\xrightarrow{\text{reverse}}$ A-T-G-A-C-C-A-G-C Met - Thr - Ser Met - Thr - Cys Met - Thr - Ser
	Base substitution	5′-A-T-G-A-C-C-T-C-C $\xrightarrow{\text{forward}}$ A-T-G-C-C-C-T-C-C $\xrightarrow{\text{reverse}}$ A-T-G-C-T-C-T-C-C Met - Thr - Ser Met - Pro - Ser Met - Leu - Ser (polar amino acid) (nonpolar amino acid) (polar amino acid) pseudo-wild type
Suppressor Mutations		
Frameshift of opposite sign (intragenic suppressor)	Insertion/deletion	5′-A-T-G-A-C-C-T-C-C-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Ser - Pro - Lys - Gly ↓ Forward mutation 5′-A-T-G-A-C-C-T-C-C-G-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Ser - Ala - Glu - Arg ↓ Suppressor mutation (deletion) 5′-A-T-G-A-C-C-C-C-G-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Pro - Pro - Lys - Gly
Extragenic suppressor Nonsense suppressor		Gene (e.g., for tyrosine tRNA) undergoes a mutation in its anticodon region that enables it to recognize and align with a nonsense mutation (e.g., UAG). Thus an amino acid (tyrosine) is inserted at the mutant stop codon and translation continues.
Physiological suppressor		A defect in one chemical pathway is circumvented by another mutation; for example, one that opens up another chemical pathway to the same product or one that permits more efficient uptake of a compound produced in small quantities because of the original mutation.

can also restore the wild-type phenotype by creating a codon that specifies an amino acid that is similar to the amino acid found at that location in the wild-type protein (e.g., both amino acids are nonpolar). If the wild-type phenotype is restored by a second mutation at a different site than the original mutation, it is called a **suppressor mutation.** Suppressor mutations may be within the same gene (intragenic suppressor mutation) or in a different gene (extragenic suppressor mutation). Because point mutations are the most common types of mutations, their effects are the focus here.

Mutations in Protein-Coding Genes

Point mutations in protein-coding genes can affect protein structure in a variety of ways. Point mutations are named according to if and how they change the encoded protein. The most common types of point mutations are silent mutations, missense mutations, nonsense mutations, and frameshift mutations. Examples of each are shown in table 16.2.

Silent mutations change the nucleotide sequence of a codon but do not change the amino acid encoded by that codon. This is possible because the genetic code exhibits degeneracy. Therefore when there is more than one codon for a given amino acid, a single base substitution may result in the formation of a new codon for the same amino acid. For example, if the codon CGU were changed to CGC, it would still code for arginine, even though a mutation had occurred. When there is no change in the protein, there is no change in the phenotype of the organism. ◀◀ *The genetic code (section 13.6)*

Missense mutations involve a single base substitution that changes a codon for one amino acid into a codon for another. For example, the codon GAG, which specifies glutamic acid, could be changed to GUG, which codes for valine. The effects of missense mutations vary. They alter the primary structure of a protein, but the effect of this change may range from complete loss of activity to no change at all. This is because the effect of missense mutations on protein function depends on the type and location of the amino acid substitution. For instance, replacement of a nonpolar amino acid in the protein's interior with a polar amino acid can drastically alter the protein's three-dimensional structure and therefore its function. Similarly the replacement of a critical amino acid at the active site of an enzyme often destroys its activity. However, the replacement of one polar amino acid with another at the protein surface may have little or no effect. Such mutations are called **neutral mutations.** Missense mutations play a very important role in providing new variability to drive evolution because they often are not lethal and therefore remain in the gene pool. ▶▶ *Proteins (appendix I)*
Mutation by Base Substitution

Nonsense mutations convert a sense codon (i.e., one that codes for an amino acid) to a nonsense codon (i.e., a stop codon: one that does not code for an amino acid). This causes the early termination of translation and therefore results in a shortened polypeptide. Depending on the location of the mutation in the gene, the phenotype may be more or less severely affected. Most proteins retain some function if they are shortened by only one or two amino acids; complete loss of normal function usually results if the mutation occurs closer to the beginning or middle of the gene.

Frameshift mutations arise from the insertion or deletion of base pairs within the coding region of the gene. Since the code consists of a precise sequence of triplet codons, the addition or deletion of fewer than three base pairs causes the reading frame to be shifted for all subsequent codons downstream. Frameshift mutations usually are very deleterious and yield mutant phenotypes resulting from the synthesis of nonfunctional proteins. In addition, frameshift mutations often produce a stop codon so that the peptide product is shorter as well as different in sequence. Of course, if the frameshift occurs near the end of the gene or if there is a second frameshift shortly downstream from the first that restores the reading frame, the phenotypic effect might not be as drastic. A second, nearby frameshift that restores the proper reading frame is an example of an intragenic suppressor mutation (table 16.2). *Addition and Deletion Mutations*

Changes in protein structure can alter the phenotype of an organism in many ways. Morphological mutations change the microorganism's colonial or cellular morphology. Lethal mutations, when expressed, result in the death of the microorganism. Because a microbe must be able to grow to be isolated and studied, lethal mutations are recovered only if they are conditional mutations. **Conditional mutations** are those that are expressed only under certain environmental conditions. For example, a conditional lethal mutation in *Escherichia coli* might not be expressed under permissive conditions such as low temperature but would be expressed under restrictive conditions such as high temperature. Thus the mutant would grow normally at cooler temperatures but would die at high temperatures.

Biochemical mutations are those causing a change in the biochemistry of the cell. Since these mutations often inactivate a biosynthetic pathway, they frequently eliminate the capacity of the mutant to make an essential molecule such as an amino acid or nucleotide. A strain bearing such a mutation has a conditional phenotype: it is unable to grow on medium lacking that molecule but grows when the molecule is provided. Such mutants are called **auxotrophs,** and they are said to be auxotrophic for the molecule they cannot synthesize. If the wild-type strain from which the mutant arose is a chemoorganotroph able to grow on a minimal medium containing only salts (to supply needed elements such as nitrogen and phosphorus) and a carbon source, it is called a **prototroph.** Another type of biochemical mutant is the resistance mutant. These mutants have acquired resistance to some pathogen, chemical, or antibiotic. Auxotrophic and resistance mutants are quite important in microbial genetics due to the ease of their detection and their relative abundance.

Mutations in Regulatory Sequences

Some of the most interesting and informative mutations studied by microbial geneticists are those that occur in the regulatory sequences responsible for controlling gene expression. Constitutive lactose operon mutants in *E. coli* are excellent examples. Many of these mutations map in the operator site and produce altered operator sequences that are not recognized by the repressor protein. Therefore the operon is continuously transcribed, and β-galactosidase is always synthesized. Mutations in promoters also have been identified. If the mutation renders the promoter sequence nonfunctional, the mutant will be unable to synthesize the product, even though the coding region of the structural gene is completely normal. Without a fully functional promoter, RNA polymerase rarely transcribes a gene as well as wild type. ◀◀ *Regulation of transcription initiation (section 14.2)*

Mutations in tRNA and rRNA Genes

Mutations in tRNA and rRNA alter the phenotype of an organism through disruption of protein synthesis. In fact, these mutants often are initially identified because of their slow growth. On the other hand, a suppressor mutation involving tRNA restores normal (or near normal) growth rates. In these mutations, a base substitution in the anticodon region of a tRNA allows the insertion of the correct amino acid at a mutant codon (table 16.2).

Retrieve, Infer, Apply

1. List three ways in which spontaneous mutations might arise.
2. Compare and contrast the means by which the mutagens 5-bromouracil, methyl-nitrosoguanidine, proflavin, and UV radiation induce mutations.
3. Give examples of intragenic and extragenic suppressor mutations.
4. Sometimes a point mutation does not change the phenotype. List all the reasons why this is so.
5. Why might a missense mutation at a protein's surface not affect the phenotype of an organism, whereas the substitution of an internal amino acid does?

16.2 Detection and Isolation of Mutants

After reading this section, you should be able to:

- Differentiate mutant detection from mutant selection
- Design an experiment to isolate mutant bacteria that are threonine auxotrophs
- Propose an experiment to isolate revertants of a threonine auxotroph and predict the types of mutations that might lead to the revertant phenotype
- Explain how the Ames test is used to screen for potential carcinogens and evaluate its effectiveness

Mutations often arise spontaneously and provide genetic diversity, which enhances survival during changing environmental conditions; thus mutations are of value to microbes. Mutations are also of practical importance to microbial geneticists. Mutant strains have been used to reveal mechanisms of complex processes such as DNA replication, endospore formation, and regulation of transcription. They are also useful as selective markers in recombinant DNA procedures. ▶▶| *Recombinant DNA technology (chapter 17)*

To study microbial mutants, they must be readily detected, even when they are rare, and then efficiently isolated from wild-type organisms and other mutants that are not of interest. Microbial geneticists typically increase the likelihood of obtaining mutants by using mutagens to increase the rate of mutation. The rate can increase from the usual one mutant per 10^7 to 10^{11} cells to about one per 10^3 to 10^6 cells. Even at this rate, carefully devised means for detecting or selecting a desired mutation must be used. This section describes some techniques used in mutant detection, selection, and isolation.

Mutant Detection

When collecting mutants of a particular organism, the wild-type characteristics must be known so that an altered phenotype can be recognized. A suitable detection system for the mutant phenotype also is needed. The use of detection systems is called screening. Screening for mutant phenotypes in haploid organisms is straightforward because the effects of most mutations can be seen immediately. Some screening procedures require only examination of colony morphology. For instance, if albino mutants of a normally pigmented bacterium are being studied, detection simply requires visual observation of colony color. Other screening methods are more complex. For example, the **replica plating** technique is used to screen for auxotrophic mutants. It distinguishes between mutants and the wild-type strain based on their ability to grow in the absence of a particular biosynthetic end product (**figure 16.6**). A lysine auxotroph, for instance, grows on lysine-supplemented media but not on a medium lacking an adequate supply of lysine because it cannot synthesize this amino acid.

Once a screening method is devised, mutants are collected. However, mutant collection can present practical problems. Consider a search for the albino mutants mentioned previously. If the mutation rate were around one in a million, on average a million or more organisms would have to be tested to find one albino mutant. This probably would require several thousand plates. The task of isolating auxotrophic mutants in this way would be even more taxing with the added labor of replica plating. Thus, if possible, it is more efficient to use a selection system employing some environmental factor to separate mutants from wild-type microorganisms. Examples of selection systems are described next.

Mutant Selection

An effective selection technique uses incubation conditions under which the mutant grows because of properties conferred by the mutation, whereas the wild type does not. Selection methods often involve reversion or suppressor mutations or the development of resistance to an environmental stress. For example, if the intent is to isolate revertants from a lysine auxotroph (Lys^-), the approach is quite easy. A large population of lysine auxotrophs is plated on minimal medium lacking lysine, incubated, and examined for colony formation. Only cells that have mutated to restore the ability to manufacture lysine will grow on minimal medium. Several million cells can be plated on a single Petri dish, but only the rare revertant cells will grow. Thus many cells can be tested for mutations by scanning a few Petri dishes for growth.

Methods for selecting mutants resistant to a particular environmental stress follow a similar approach. Often wild-type cells are susceptible to virus attack, antibiotic treatment, or specific temperatures, so it is possible to grow the microbe in the presence of the stress and look for surviving organisms. Consider the example of a phage-sensitive wild-type bacterium. When it is cultured in medium lacking the virus and then plated on selective medium containing viruses, any colonies that form are resistant to virus attack and very likely are mutants in this regard.

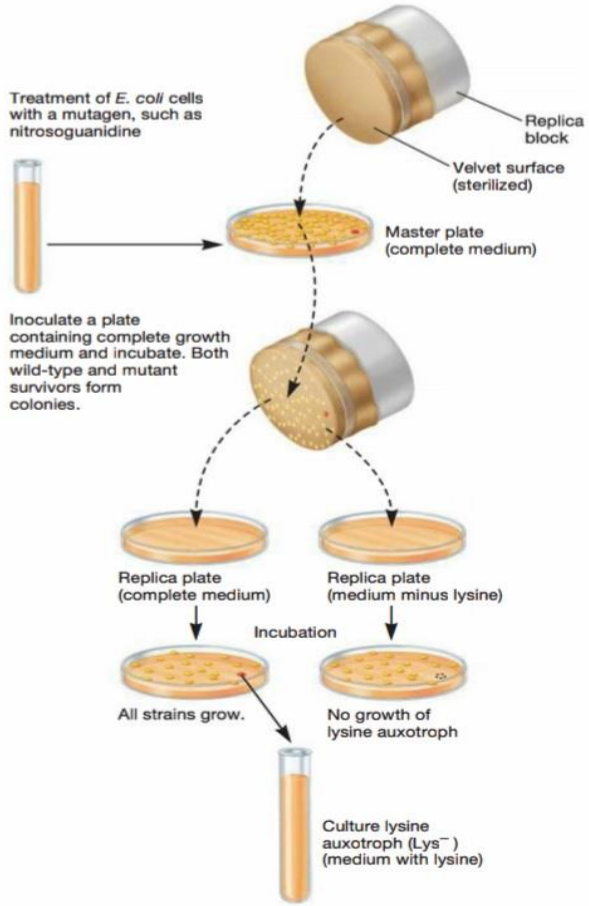

Figure 16.6 Replica Plating. The use of replica plating to isolate a lysine auxotroph. After growth of a mutagenized culture on a complete medium, a piece of sterile velvet is pressed on the plate surface to pick up bacteria from each colony. Then the velvet is pressed to the surface of other plates, and organisms are transferred to the same position as on the master plate. After the location of Lys^- colonies growing on the replica with complete medium is determined, the auxotrophs can be isolated and cultured.

MICRO INQUIRY *How would you screen for a tryptophan auxotroph? How would you select for a mutant that is resistant to the antibiotic ampicillin but sensitive to tetracycline (assume the parental stain is resistant to both antibiotics)?*

Substrate utilization mutations also can be selected. Many bacteria use only a few primary carbon sources. With such bacteria, it is possible to select mutants by plating a culture on medium containing an alternate carbon source. Any colonies that appear can use the substrate and are probably mutants.

Mutant screening and selection methods are used for purposes other than understanding more about the nature of genes or the biochemistry of a particular microorganism. One very important role of mutant selection and screening techniques is in the study of carcinogens. Next we briefly describe one of the first and perhaps best known of the carcinogen testing systems.

Mutagens and Carcinogens

An increased understanding of the mechanisms of mutation and their role in cancer has stimulated efforts to identify environmental carcinogens—agents that cause cancer. The observation that many carcinogens also are mutagens was the basis for development of the Ames test by Bruce Ames in the 1970s. This test determines if a substance increases the rate of mutation; that is, if it is a mutagen. If the substance is a mutagen, then it is also likely to be carcinogenic if an animal is exposed to it at sufficient levels. Note that the test does not directly test for carcinogenicity. This is because it uses a bacterium as the test organism. Carcinogenicity can only be directly demonstrated with animals. Such testing is extremely expensive and takes much longer to complete than does the Ames test. Thus the Ames test serves as an inexpensive screening procedure to identify chemicals that may be carcinogenic and thus deserve further testing.

The Ames test is a mutational reversion assay employing several "tester" strains of *Salmonella enterica* serovar Typhimurium. Each tester strain has a different mutation in the histidine biosynthesis operon and therefore is a histidine auxotroph. The bacteria also have mutational alterations of their cell walls that make them more permeable to test substances. To further increase assay sensitivity, the strains are defective in their ability to repair DNA.

In the Ames test, tester strains of *S.* Typhimurium are plated with the substance being tested and the number of visible colonies that form are determined (**figure 16.7**). To ensure that DNA replication can take place in the presence of the potential mutagen, the bacteria and test substance are mixed in dilute molten top agar to which a trace of histidine has been added. This molten mix is then poured on top of minimal agar plates and incubated for 2 to 3 days at 37°C. All of the histidine auxotrophs grow for the first few hours in the presence of the test compound until the histidine is depleted. This is necessary because replication is required for the development of a mutation (figure 16.1). However, this initial growth does not produce a visible colony. Once the histidine supply is exhausted, only revertants that have mutationally regained the ability to synthesize histidine continue to grow and produce visible colonies. These colonies need only be counted and compared to controls to estimate the relative mutagenicity of the compound: the more colonies, the greater the mutagenicity.

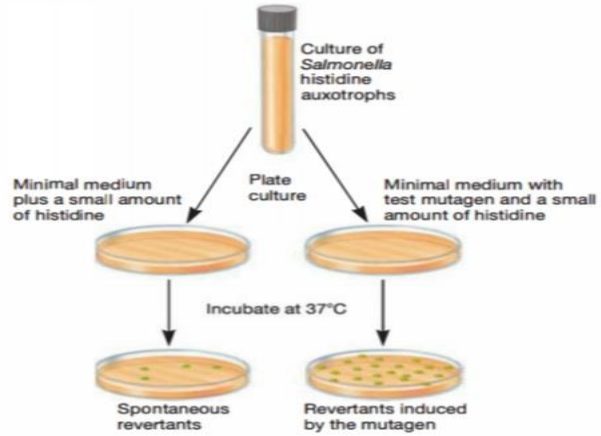

Figure 16.7 The Ames Test for Mutagenicity.

Some chemicals tested may not be mutagenic unless they are transformed into another, more active form. In animals, such transformations occur in the liver. Indeed, many known carcinogens (e.g., aflatoxins; *see figure 41.6*) are not actually carcinogenic until they are modified in the liver by enzymes that function to destroy toxins and other materials that may be circulating in the blood. However, in some cases, these enzymes transform chemicals into more dangerous forms. For this reason, a mammalian liver extract is often added to the molten top agar prior to plating the bacterial cells used in the Ames test. The extract converts potential mutagens into derivatives that readily react with DNA, mimicking the enzymatic transformations that occur in mammals. Carrying out the Ames test with and without the addition of the extract shows which compounds have intrinsic mutagenicity and which need activation after uptake. Despite the use of liver extracts, only about half of all potential carcinogens are detected by the Ames test.

Retrieve, Infer, Apply

1. Describe how replica plating is used to detect and isolate auxotrophic mutants.
2. Why are mutant selection techniques generally preferable to screening methods?
3. Briefly discuss how reversion mutations, resistance to an environmental factor, and the ability to use a particular nutrient can be employed in mutant selection.
4. Describe how you would isolate a mutant that required histidine for growth and was resistant to penicillin. The wild type is a prototroph.
5. What is the Ames test and how is it carried out? What assumption concerning mutagenicity and carcinogenicity is it based upon?

16.3 DNA Repair

After reading this section, you should be able to:

- Compare and contrast excision repair, direct repair, mismatch repair, and recombinational repair
- Propose a scenario that would elicit the SOS response and describe the response to those conditions

If there is a microbial equivalent of an extreme sport, *Deinococcus radiodurans*'s ability to repair its genome after it has been blasted apart by a high dose of radiation might be a contender. Surprisingly, this ability is primarily related to the resistance of *D. radiodurans* proteins and the structure of its genome, rather than to its DNA repair mechanisms. Its radiation-resistant proteins are able to begin repairing the genome quickly. Repair is aided by the genome consisting of two chromosomes, each having numerous areas of homology. This allows the DNA fragments to anneal to each other, facilitating the piecing together of the shattered genome. Other than this, *D. radiodurans* uses pretty much the same DNA repair mechanisms as other organisms. Obviously mutations can have disastrous effects. Therefore it is imperative that a microorganism be able to repair changes. Microbes have numerous repair mechanisms. Repair in *E. coli* is best understood and is briefly described in this section. ▶▶| Deinococcus-Thermus *(section 21.2)*

Proofreading: The First Line of Defense

As we discuss in chapter 13, replicative DNA polymerases sometimes insert the incorrect nucleotide during DNA replication. However, these DNA polymerases have the ability to evaluate the hydrogen bonds formed between the newly added nucleotide and the template nucleotide, and correct any errors immediately; that is, before the next nucleotide is added. This ability is called **proofreading.** When a DNA polymerase detects that a mistake has been made, it backs up, removing the incorrect nucleotide with its 3′ to 5′ exonuclease activity (*see figure 13.16*). It then restarts DNA replication, this time inserting the correct nucleotide. Proofreading is very efficient, but it does not always correct errors in replication. Furthermore, it is not useful for correcting induced mutations. *E. coli* uses other repair mechanisms to help ensure the stability of its genome. |◀◀ *DNA replication in bacteria (section 13.3)*

Mismatch Repair

When proofreading by replicative DNA polymerases fails, mismatched bases are usually detected and repaired by the **mismatch repair** system (**figure 16.8**). In *E. coli* the enzyme MutS scans the newly replicated DNA for mismatched pairs. Another enzyme, MutH, removes a stretch of newly synthesized DNA around the mismatch. A DNA polymerase then replaces the excised nucleotides, and the resulting nick is sealed by DNA ligase.

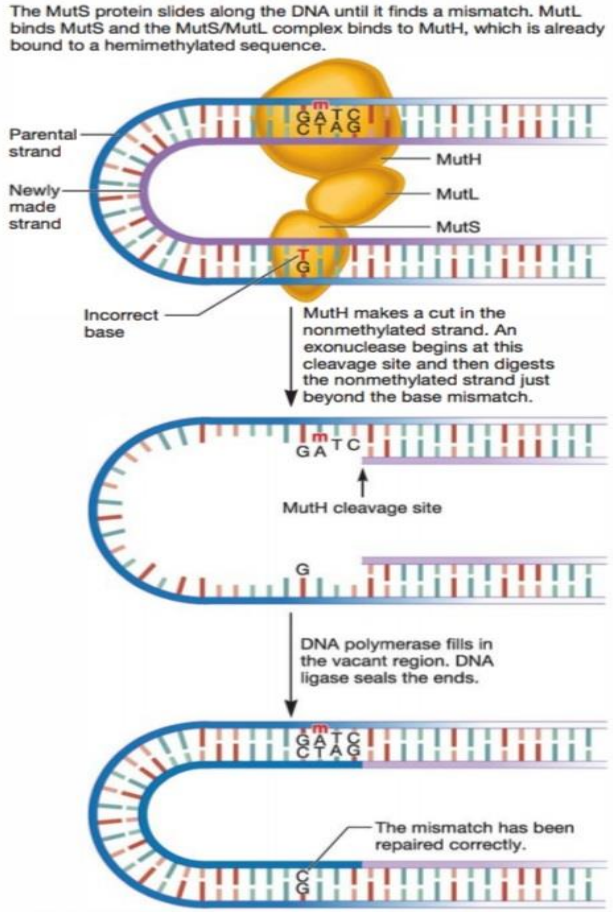

Figure 16.8 Methyl-Directed Mismatch Repair in *E. coli*. The role of MutH is to identify the methylated strand of DNA, which is the nonmutated parental strand. The methylated adenine is designated by an m.

MICRO INQUIRY *How is mismatch repair similar to DNA polymerase proofreading? How is it different?*

Successful mismatch repair depends on the ability of enzymes to distinguish between old and newly replicated DNA strands. This distinction is possible because newly replicated DNA strands lack methyl groups on their bases, whereas older DNA has methyl groups on the bases of both strands. DNA methylation is catalyzed by DNA methyltransferases and results in three different products: N6-methyladenine, 5-methylcytosine, and N4-methylcytosine. After strand synthesis, the *E. coli* DNA adenine methyltransferase (DAM) methylates adenine bases in GATC sequences to form N6-methyladenine. For a short time after the replication fork has passed, the new strand lacks methyl groups while the template strand is methylated. In other words, the DNA is temporarily hemimethylated. The repair system cuts out the mismatch from the unmethylated strand.

Excision Repair

Excision repair corrects damage that causes distortions in the double helix. Two types of excision repair systems have been described: nucleotide excision repair and base excision repair. They both use the same approach to repair: Remove the damaged portion of a DNA strand and use the intact complementary strand as the template for synthesis of new DNA. They are distinguished by the enzymes used to correct DNA damage.

In **nucleotide excision repair,** an *E. coli* enzyme called UvrABC endonuclease removes damaged nucleotides and a few nucleotides on either side of the lesion. The resulting single-stranded gap is filled by DNA polymerase I, and DNA ligase joins the fragments (**figure 16.9**). This system can remove thymine dimers (figure 16.5) and repair almost any other injury that produces a detectable distortion in DNA.

Base excision repair employs enzymes called DNA glycosylases. These enzymes remove damaged or unnatural bases yielding apurinic or apyrimidinic (AP) sites. Enzymes called AP endonucleases recognize the damaged DNA and nick the backbone at the AP site (**figure 16.10**). DNA polymerase I removes the damaged region, using its 5′ to 3′ exonuclease activity. It then fills in the gap, and DNA ligase joins the DNA fragments.

Direct Repair

Thymine dimers and alkylated bases often are corrected by **direct repair.** For instance, **photoreactivation** repairs thymine dimers (figure 16.5) by splitting them apart with the help of visible light. This photochemical reaction is catalyzed by the enzyme photolyase. Methyl and some other alkyl groups that have been added to guanine can be removed with the help of an enzyme known as alkyltransferase or methylguanine methyltransferase. Thus damage to guanine from mutagens such as methyl-nitrosoguanidine (figure 16.4) can be repaired directly.
Direct Repair

Recombinational Repair

Recombinational repair corrects damaged DNA in which both bases of a pair are missing or damaged, or where there is a gap opposite a lesion. In this type of repair, a protein called **RecA** cuts a piece of template DNA from a sister molecule and puts it into the gap or uses it to replace a damaged strand

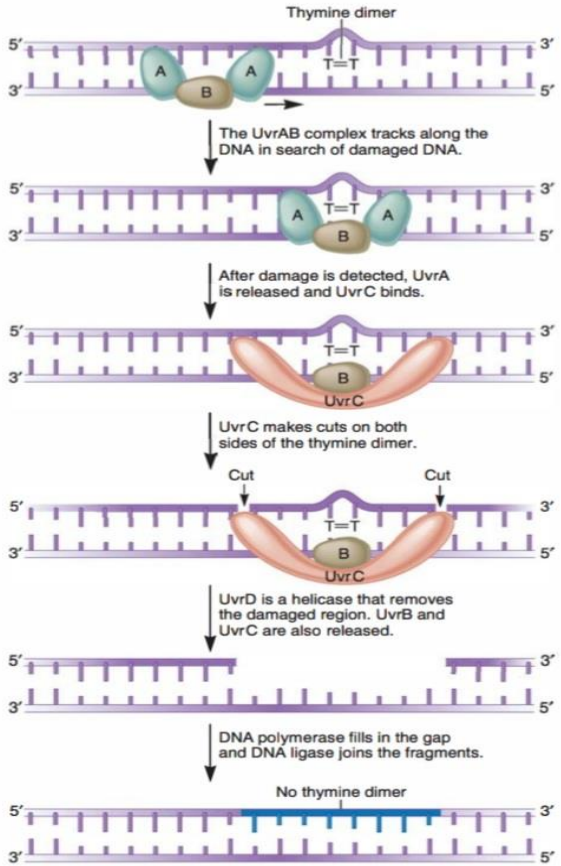

Figure 16.9 Nucleotide Excision Repair in *E. coli*.

MICRO INQUIRY *How is damaged DNA recognized by the UvrAB complex?*

(figure 16.11). Although bacterial cells are haploid, another copy of the damaged segment often is available because either it has recently been replicated or the cell is growing rapidly and has more than one copy of its chromosome. Once the template is in place, the remaining damage can be corrected by another repair system.

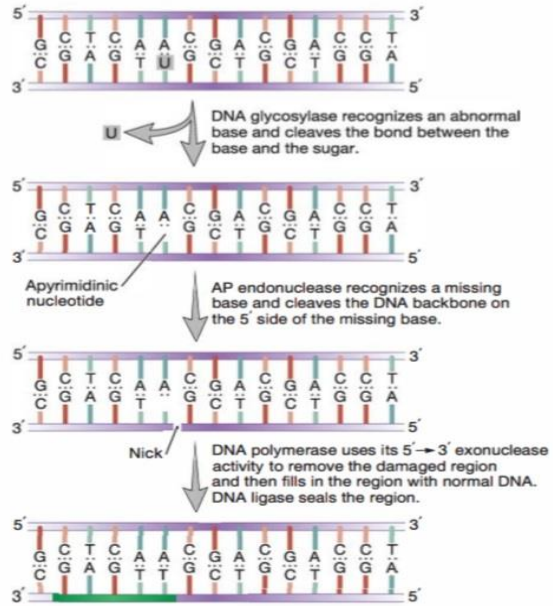

Figure 16.10 Base Excision Repair.

SOS Response

Despite having multiple repair systems, sometimes the damage to an organism's DNA is so great that the normal repair mechanisms just described cannot repair all the damage. As a result, DNA synthesis stops completely. In such situations, a global control network called the **SOS response** is activated. In this response, over 40 genes are activated when a transcriptional repressor called LexA is destroyed. LexA negatively controls these genes, and once it is destroyed, they are transcribed and the SOS response ensues.

The SOS response, like recombinational repair, depends on the activity of RecA. RecA binds to single- or double-stranded DNA breaks and gaps generated by cessation of DNA synthesis. RecA binding initiates recombinational repair. Simultaneously RecA takes on coprotease function. It interacts with LexA, causing LexA to destroy itself (autoproteolysis). Destruction of LexA increases transcription of genes for excision repair and recombinational repair, in particular.

The first genes transcribed in the SOS response are those that encode the Uvr proteins needed for nucleotide excision repair (figure 16.9). Then expression of genes involved in recombinational repair is further increased. To give the cell time to repair its DNA, the protein SfiA is produced; SfiA blocks cell division. Finally, if the DNA has not been fully repaired after

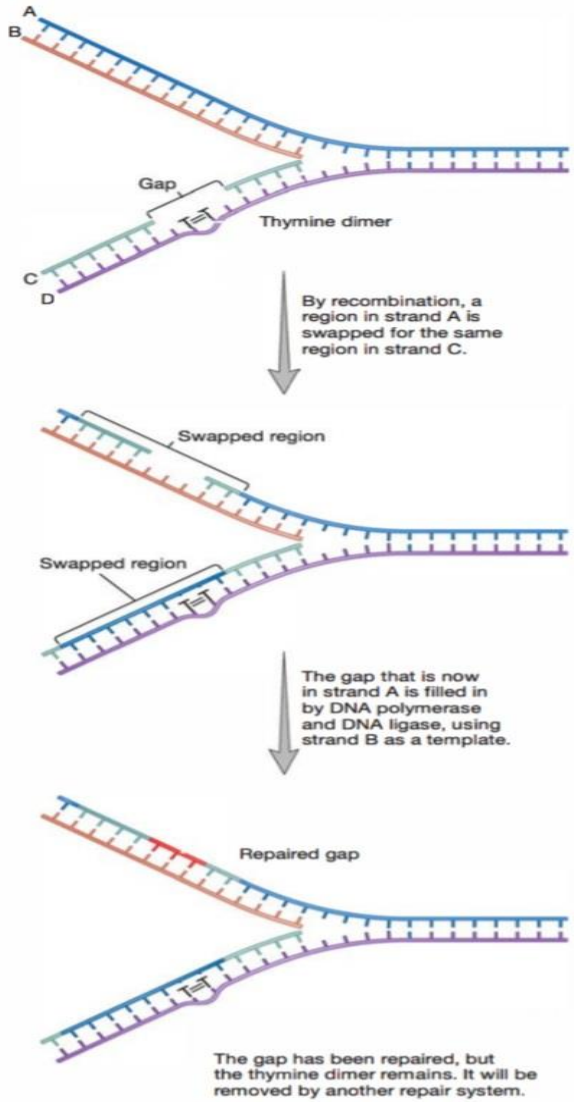

Figure 16.11 **Recombinational Repair.**

about 40 minutes, a process called **translesion DNA synthesis** is triggered. In this process, DNA polymerase IV (also known as DinB; Din is short for *d*amage *in*ducible) and DNA polymerase V (UmuCD; Umu is short for *U*V *mu*tagenesis) synthesize DNA across gaps and other lesions (e.g., thymine dimers) that had stopped DNA polymerase III. However, because an intact template does not exist, these DNA polymerases often insert incorrect bases. Furthermore, they lack proofreading activity. Therefore even though DNA synthesis continues, it is highly error prone and results in the generation of numerous mutations.

The SOS response is so named because it is made in a life-or-death situation. The response increases the likelihood that some cells will survive by allowing DNA synthesis to continue. For the cell, the risk of dying because of failure to replicate DNA is greater than the risk posed by the mutations generated by this error-prone process.

Retrieve, Infer, Apply

1. Compare and contrast the two types of excision repair.
2. What role does DNA methylation play in mismatch repair?
3. When *E. coli* cells are growing rapidly, they may contain up to four copies of their chromosomes. Why is this important if *E. coli* needs to carry out recombinational repair?
4. Explain how the following DNA alterations and replication errors would be corrected (there may be more than one way): base addition errors by DNA polymerase III during replication, thymine dimers, AP sites, methylated guanines, and gaps produced during replication.

16.4 Creating Additional Genetic Variability

After reading this section, you should be able to:

- Describe in general terms how recombinant eukaryotic organisms arise
- Distinguish vertical gene transfer from horizontal gene transfer
- Summarize the four possible outcomes of horizontal gene transfer
- Compare and contrast homologous recombination and site-specific recombination

In section 16.1, we discuss the consequences of mutations in terms of their effect on a protein and the phenotype of the organisms bearing the mutation. However, the effect also depends on the environment in which the organism lives. In other words, those mutations that don't immediately kill the organism are subject to selective pressure. Selective pressure determines if a mutation will persist in a population to become an alternate form of the gene. An alternate form of a gene is termed an **allele.** The existence of alleles for each gene in a genome means each organism in a population can have a distinctive (and probably a unique) set of alleles making up its genome; that is, its genotype. Each genotype in a population can be selected for or selected against. Organisms with genotypes, and therefore phenotypes, that are best suited to the