10

Genetics of *Bacteria* and *Archaea*

The Ames test uses bacteria to detect mutagens (chemical agents that cause mutations) and is used in the chemical and food industries to ensure that their products are safe for human use.

In this chapter we discuss the traditional principles and techniques of bacterial genetics. Many newer techniques are now routinely used to investigate the genomes of bacteria and other organisms. These newer approaches are discussed in Chapter 11, Genetic Engineering, and Chapter 12, Microbial Genomics. Here we first describe how alterations arise in the genetic material. Then we consider how genes can be transferred from one microorganism to another.

I Mutation

All organisms contain a specific sequence of nucleotide bases in their genome, their genetic blueprint. A **mutation** is a heritable change in the base sequence of that genome. Mutations can lead to changes—some good, some bad, but mostly neutral in effect—in an organism. Genetic alterations can also be brought about by recombination (Section 10.6), the physical exchange of DNA between genetic elements. Recombination creates new combinations of genes even in the absence of mutation. Whereas mutation usually brings about only a very small amount of genetic change in a cell, genetic recombination typically generates much larger changes. Entire genes, sets of genes, or even larger segments of DNA can be transferred between chromosomes or other genetic elements. Taken together, mutation and recombination fuel the evolutionary process.

Unlike most eukaryotes, prokaryotes do not reproduce sexually. However, prokaryotes possess mechanisms of horizontal genetic exchange that allow for both gene transfer and recombination. To detect genetic exchange between two prokaryotes, it is therefore necessary to employ genetic markers whose transfer can be detected. The term "marker" refers to any gene whose presence is monitored during a genetics experiment. If possible, markers are chosen that are relatively easy to detect. Genetically altered strains are used in gene transfer experiments, the alteration(s) being due to one or more mutations in their DNA. These mutations may involve changes in only one or a few base pairs or even the insertion or deletion of entire genes. Before discussing genetic exchange, we will therefore consider the molecular mechanism of mutation and the properties of mutant microorganisms.

10.1 Mutations and Mutants

A mutation is a heritable change in the base sequence of the nucleic acid in the genome of an organism or a virus or any other genetic entity. In all cells, the genome consists of double-stranded DNA. In viruses, by contrast, the genome may consist of single- or double-stranded DNA or RNA. A strain of any cell or virus carrying a change in nucleotide sequence is called a **mutant**. A mutant by definition differs from its parental strain in its **genotype**, the nucleotide sequence of the genome. In addition, the observable properties of the mutant—its **phenotype**—may also be altered relative to its parent. This altered phenotype is called a *mutant phenotype*. It is common to refer to a strain isolated from nature as a **wild-type strain**. The term wild-type may be used to refer to a whole organism or just to the status of a particular gene that is under investigation. Mutant derivatives can be obtained either directly from wild-type strains or from other strains previously derived from the wild type, for example, another mutant.

Genotype versus Phenotype

Depending on the mutation, a mutant strain may or may not differ in phenotype from its parent. By convention in bacterial genetics, the genotype of an organism is designated by three lowercase letters followed by a capital letter (all in italics) indicating a particular gene. For example, the *hisC* gene of *Escherichia coli* encodes a protein called HisC that functions in biosynthesis of the amino acid histidine. Mutations in the *hisC* gene would be designated as *hisC1*, *hisC2*, and so on, the numbers referring to the order of isolation of the mutant strains. Each *hisC* mutation would be different, and each *hisC* mutation might affect the HisC protein in different ways.

The phenotype of an organism is designated by a capital letter followed by two lowercase letters, with either a plus or minus superscript to indicate the presence or absence of that property. For example, a His$^+$ strain of *E. coli* is capable of making its own histidine, whereas a His$^-$ strain is not. The His$^-$ strain would require a histidine supplement for growth. A mutation in the *hisC* gene will lead to a His$^-$ phenotype if it eliminates the function of the HisC protein.

Isolation of Mutants: Screening versus Selection

Virtually any characteristic of an organism can be changed by mutation. However, some mutations are selectable, conferring some type of advantage on organisms possessing them, whereas others are nonselectable, even though they may lead to a very clear change in the phenotype of an organism. A selectable mutation confers a clear advantage on the mutant strain under certain environmental conditions, so the progeny of the mutant cell are able to outgrow and replace the parent. A good example of a selectable mutation is drug resistance: An antibiotic-resistant mutant can grow in the presence of antibiotic concentrations that inhibit or kill the parent (**Figure 10.1*a***) and is thus selected for under these conditions. It is relatively easy to detect and isolate selectable mutants by choosing the appropriate environmental conditions. **Selection** is therefore an extremely powerful genetic tool, allowing the isolation of a single mutant from a population containing millions or even billions of parental organisms.

An example of a nonselectable mutation is color loss in a pigmented organism (Figure 10.1*b*). Nonpigmented cells usually have neither an advantage nor a disadvantage over the pigmented parent cells when grown on agar plates, although pigmented organisms may have a selective advantage in nature. We can detect such mutations only by examining large numbers of colonies and looking for the "different" ones, a process called **screening**.

Isolation of Nutritional Auxotrophs and Penicillin Selection

Although screening is more tedious than selection, methods are available for screening large numbers of colonies for certain types of mutations. For instance, nutritionally defective mutants can be detected by the technique of *replica plating* (**Figure 10.2**). An

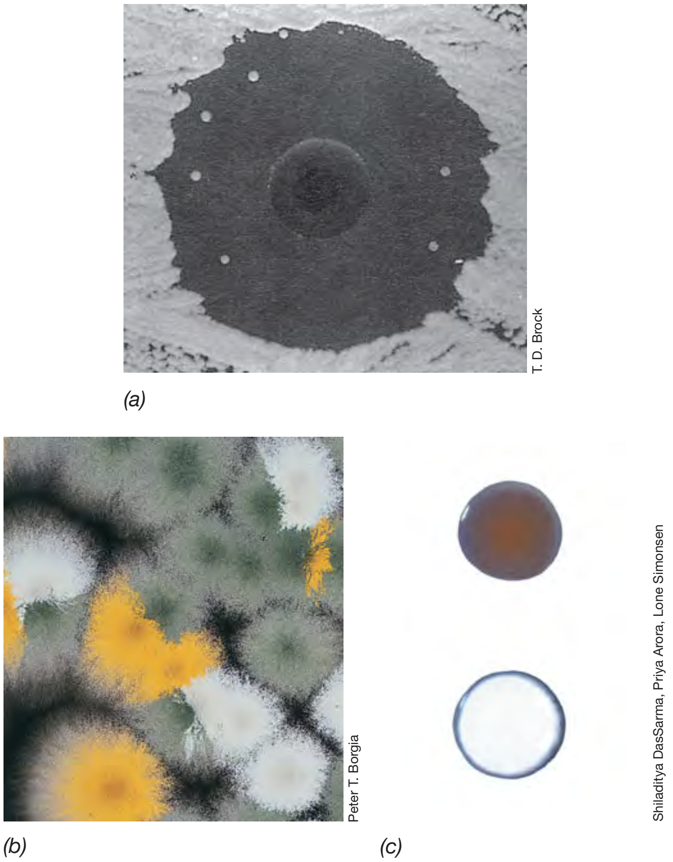

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Figure 10.1 Selectable and nonselectable mutations. *(a)* Development of antibiotic-resistant mutants, a type of easily selectable mutation, within the inhibition zone of an antibiotic assay disc. *(b)* Nonselectable mutations. Spontaneous pigmented and nonpigmented mutants of the fungus *Aspergillus nidulans*. The wild type has a green pigment. The white or colorless mutants make no pigment, whereas the yellow mutants cannot convert the yellow pigment precursor to the normal (green) color. *(c)* Colonies of mutants of a species of *Halobacterium,* a member of the *Archaea*. The wild-type colonies are white. The orangish brown colonies are mutants that lack gas vesicles (Section 3.11). The gas vesicles scatter light and mask the color of the colony.

imprint of colonies from a master plate is made onto an agar plate lacking the nutrient by using sterile velveteen cloth or filter paper. Parental colonies will grow normally, whereas those of the mutant will not. Thus, the inability of a colony to grow on medium lacking the nutrient signals that it is a mutant. The colony on the master plate corresponding to the vacant spot on the replica plate can then be picked, purified, and characterized. A mutant with a nutritional requirement for growth is called an **auxotroph**, and the parent from which it was derived is called a *prototroph*. (A prototroph may or may not be the wild type. An auxotroph may be derived from the wild type or from a mutant derivative of the wild type.) For instance, mutants of *E. coli* with a His^- phenotype are histidine auxotrophs. Although of great utility, replica plating is nevertheless a screening process, and it can be laborious to isolate mutants by screening.

An ingenious method widely used to isolate auxotrophs is penicillin selection. Ordinarily, mutants that require specific nutrients are at a disadvantage in competition with the parent cells and so there is no direct way of isolating them. Moreover, auxotrophic mutants are rare in a mutagenized culture, and it is time consuming to obtain them by replica plating alone. However, penicillin selection can be used to enrich for auxotrophic mutants in a population of mutagenized cells, after which replica plating is much more effective. How does penicillin selection work?

Penicillin is an antibiotic that kills only growing cells. If penicillin is added to a population of cells growing in a medium lacking the nutrient required by the desired mutant, those cells capable of growth will be killed, whereas any nongrowing mutant cells will survive. After preliminary incubation in the absence of the nutrient in a penicillin-containing medium, the population is washed free of the penicillin and transferred to plates containing the nutrient. The colonies that appear include some wild-type cells that escaped penicillin killing, but also include a relatively increased proportion of the desired mutants. Penicillin selection is thus a kind of negative selection; the selection is not *for* the mutant, but instead *against* the wild type. Penicillin selection is

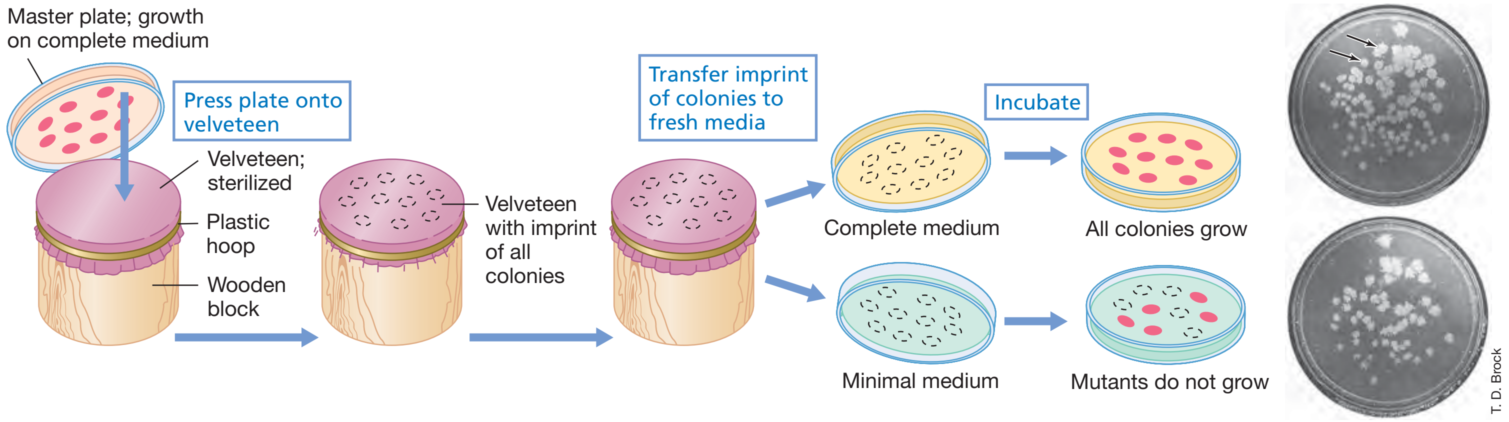

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Figure 10.2 Screening for nutritional auxotrophs. The replica-plating method can be used for the detection of nutritional mutants. Photos: The photograph at the top right shows the master plate. Some of the colonies not appearing on the replica plate are indicated with arrows. The replica plate at bottom right lacked one nutrient (leucine) present in the master plate. Therefore, the colonies indicated with arrows on the master plate are leucine auxotrophs.

Table 10.1 *Kinds of mutants*

Phenotype	*Nature of change*	*Detection of mutant*
Auxotroph	Loss of enzyme in biosynthetic pathway	Inability to grow on medium lacking the nutrient
Temperature-sensitive	Alteration of an essential protein so it is more heat-sensitive	Inability to grow at a high temperature (for example, 40°C) that normally supports growth
Cold-sensitive	Alteration of an essential protein so it is inactivated at low temperature	Inability to grow at a low temperature (for example, 20°C) that normally supports growth
Drug-resistant	Detoxification of drug or alteration of drug target or permeability to drug	Growth on medium containing a normally inhibitory concentration of the drug
Rough colony	Loss or change in lipopolysaccharide layer	Granular, irregular colonies instead of smooth, glistening colonies
Nonencapsulated	Loss or modification of surface capsule	Small, rough colonies instead of larger, smooth colonies
Nonmotile	Loss of flagella or nonfunctional flagella	Compact instead of flat, spreading colonies
Pigmentless	Loss of enzyme in biosynthetic pathway leading to loss of one or more pigments	Presence of different color or lack of color
Sugar fermentation	Loss of enzyme in degradative pathway	Lack of color change on agar containing sugar and a pH indicator
Virus-resistant	Loss of virus receptor	Growth in presence of large amounts of virus

often used as a prelude to replica plating to increase the chances of obtaining auxotrophic mutants.

Examples of common classes of mutants and the means by which they are detected are listed in **Table 10.1**. www.microbiologyplace.com **Online Tutorial 10.1: Replica Plating**

MiniQuiz

- Distinguish between the words “mutation” and “mutant.”
- Distinguish between the words “screening” and “selection.”

10.2 Molecular Basis of Mutation

Mutations can be either spontaneous or induced. **Induced mutations** are those that are due to agents in the environment and include mutations made deliberately by humans. They can result from exposure to natural radiation (cosmic rays, and so on) that alters the structure of bases in the DNA. In addition, a variety of chemicals, including oxygen radicals (⇄ Section 5.18), can chemically modify DNA. For example, oxygen radicals can convert guanine into 8-hydroxyguanine, and this causes mutations. **Spontaneous mutations** are those that occur without external intervention. The bulk of spontaneous mutations result from occasional errors in the pairing of bases during DNA replication.

Mutations that change only one base pair are called **point mutations**. Point mutations are caused by base-pair substitutions in the DNA or by the loss or gain of a single base pair. Most point mutations do not actually cause any phenotypic change, as discussed below. However, as for all mutations, any phenotypic change that does result from a point mutation depends on exactly where the mutation occurs and what the nucleotide change is.

Base-Pair Substitutions

If a point mutation is within the coding region of a gene that encodes a polypeptide, any change in the phenotype of the cell is most likely the result of a change in the amino acid sequence of the polypeptide. The error in the DNA is transcribed into mRNA, and the erroneous mRNA in turn is translated to yield a polypeptide. **Figure 10.3** shows the consequences of various

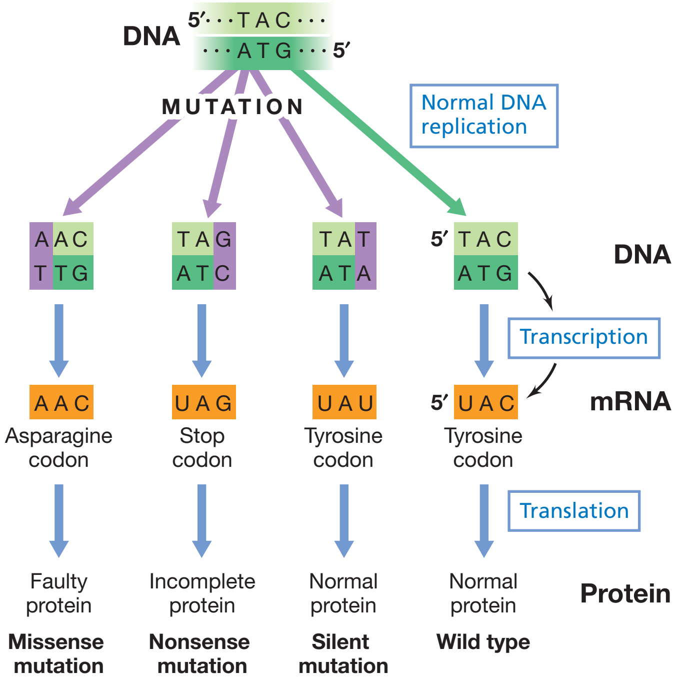

Figure 10.3 Possible effects of base-pair substitution in a gene encoding a protein. Three different protein products are possible from changes in the DNA for a single codon.

base-pair substitutions. (Occasionally, a base change that does not alter the amino acid sequence may nonetheless affect the cellular phenotype by changing the efficiency of translation of an mRNA molecule and thus altering protein levels. This is usually due to changes in secondary structure of the mRNA as a result of altered internal base pairing.)

In interpreting the results of a mutation, we must first recall that the genetic code is degenerate (⇄ Section 6.17 and Table 6.5). Consequently, not all mutations in the base sequence encoding a polypeptide will change the polypeptide. This is illustrated in Figure 10.3, which shows several possible results when the DNA that encodes a single tyrosine codon in a polypeptide is mutated. First, a change in the RNA from UAC to UA*U* would have no apparent effect because UAU is also a tyrosine codon. Although they do not affect the sequence of the encoded polypeptide, such changes in the DNA are indeed still mutations. They are one type of **silent mutation**, that is, a mutation that does not affect the phenotype of the cell. Note that silent mutations in coding regions are almost always in the third base of the codon (arginine and leucine can also have silent mutations in the first position).

Changes in the first or second base of the codon more often lead to significant changes in the polypeptide. For instance, a single base change from *U*AC to *A*AC (Figure 10.3) results in an amino acid change within the polypeptide from tyrosine to asparagine at a specific site. This is referred to as a **missense mutation** because the informational "sense" (precise sequence of amino acids) in the ensuing polypeptide has changed. If the change is at a critical location in the polypeptide chain, the protein could be inactive or have reduced activity. However, not all missense mutations necessarily lead to nonfunctional proteins. The outcome depends on where the substitution lies in the polypeptide chain and on how it affects protein folding and activity. For example, mutations in the active site of an enzyme are more likely to destroy activity than mutations in other regions of the protein.

Another possible outcome of a base-pair substitution is the formation of a nonsense (stop) codon. This results in premature termination of translation, leading to an incomplete polypeptide that would almost certainly not be functional (Figure 10.3). Mutations of this type are called **nonsense mutations** because the change is from a codon for an amino acid (sense codon) to a nonsense codon (⇄ Table 6.5). Unless the nonsense mutation is very near the end of the gene, the incomplete product will be completely inactive.

The terms "transition" and "transversion" are used to describe the type of base substitution in a point mutation. **Transitions** are mutations in which one purine base (A or G) is substituted for another purine, or one pyrimidine base (C or T) is substituted for another pyrimidine. **Transversions** are point mutations in which a purine base is substituted for a pyrimidine base or vice versa.

Frameshifts and Other Insertions or Deletions

Because the genetic code is read from one end of the nucleic acid in consecutive blocks of three bases (that is, as codons), any deletion or insertion of a single base pair results in a shift in the reading frame. These frameshift mutations often have serious consequences. Single base insertions or deletions change the primary sequence of the encoded polypeptide, typically in a major way (**Figure 10.4**). Such microinsertions or microdeletions can result from replication errors. Insertion or deletion of two base pairs also causes a frameshift; however, insertion or deletion of three base pairs adds or removes a whole codon. This results in addition or deletion of a single amino acid in the polypeptide sequence. Although this may well be deleterious to protein function, it is usually not as bad as a frameshift, which scrambles the entire polypeptide sequence after the mutation point.

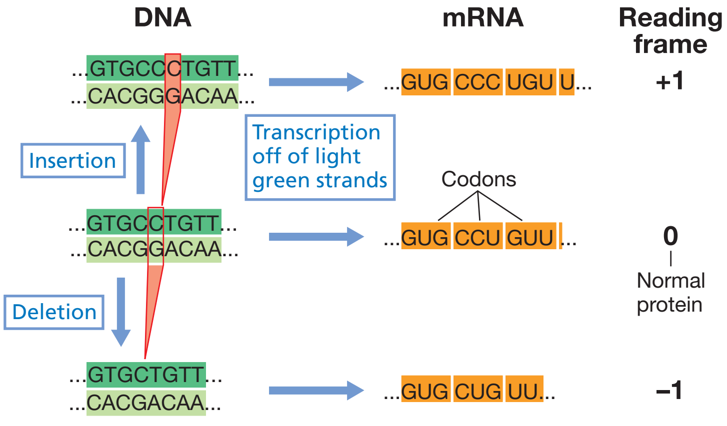

Figure 10.4 Shifts in the reading frame of mRNA caused by insertions or deletions. The reading frame in mRNA is established by the ribosome, which begins at the 5′ end (toward the left in the figure) and proceeds by units of three bases (codons). The normal reading frame is referred to as the 0 frame, that missing a base the −1 frame, and that with an extra base the +1 frame.

Insertions or deletions can also result in the gain or loss of hundreds or even thousands of base pairs. Such changes inevitably result in complete loss of gene function. Some deletions are so large that they may include several genes. If any of the deleted genes are essential, the mutation will be lethal. Such deletions cannot be restored through further mutations, but only through genetic recombination. Indeed, one way in which large deletions are distinguished from point mutations is that the latter are reversible through further mutations, whereas the former are not. Larger insertions and deletions may arise as a result of errors during genetic recombination. In addition, many insertion mutations are due to the insertion of specific identifiable DNA sequences 700–1400 base pairs (bp) in length called *insertion sequences*, a type of transposable element (Section 10.13). The effect of transposable elements on the evolution of bacterial genomes is discussed further in Section 12.12.

Other types of large-scale mutations are rearrangements brought about by errors in recombination. These include translocations, in which a large section of chromosomal DNA is moved to a new location (and in eukaryotes often to a different chromosome), and inversions, in which the orientation of a particular segment of DNA is reversed relative to the surrounding DNA. www.microbiologyplace.com **Online Tutorial 10.2: The Molecular Basis for Mutations**

Site-Directed Mutagenesis and Transposons

The mutations that we have considered thus far have been random, that is, not directed at any particular gene. However, recombinant DNA technology and the use of synthetic DNA make it possible to induce specific mutations in specific genes. The approach of generating mutations at specific sites is called *site-directed mutagenesis* and is discussed further in Chapter 11. Mutations can also be deliberately introduced by transposon mutagenesis (Section 10.13). If a transposable element inserts within a gene, loss of gene function generally results. Because transposable elements can insert into the chromosome at various locations, transposons are widely used to generate mutations.

Back Mutations or Reversions

Point mutations are typically reversible, a process known as **reversion**. A revertant is a strain in which the original phenotype that was changed in the mutant is restored. Revertants can be of two types. In *same-site revertants*, the mutation that restores activity is at the same site as the original mutation. If the back mutation is not only at the same site but also restores the original sequence, it is called a *true revertant.*

In second-site revertants, the mutation is at a different site in the DNA. Second-site mutations can restore a wild-type phenotype if they function as suppressor mutations—mutations that compensate for the effect of the original mutation. Several classes of suppressor mutations are known. These include (1) a mutation somewhere else in the same gene that restores enzyme function, such as a second frameshift mutation near the first that restores the original reading frame; (2) a mutation in another gene that restores the function of the original mutated gene; and (3) a mutation in another gene that results in the production of an enzyme that can replace the mutated one.

An interesting class of suppressor mutations are those due to alterations in tRNA. Nonsense mutations can be suppressed by changing the anticodon sequence of a tRNA molecule so that it now recognizes a stop codon. Such an altered tRNA is known as a suppressor tRNA and will insert the amino acid it carries at the stop codon that it now reads. Suppressor tRNA mutations would be lethal unless a cell has more than one tRNA for a particular codon. One tRNA may then be mutated into a suppressor, and the other performs the original function. Most cells have multiple tRNAs and so suppressor mutations are reasonably common, at least in microorganisms. Sometimes the amino acid inserted by the suppressor tRNA is identical to the original amino acid and the protein is fully restored. In other cases, a different amino acid is inserted and a partially active protein may be produced.

Unlike point mutations, large-scale deletions do not revert. By contrast, large-scale insertions can revert as the result of a subsequent deletion that removes the insertion. Typically, frameshift mutations of any magnitude are difficult to restore to the wild type, and mutants carrying frameshift mutations are therefore genetically quite stable. For this reason, geneticists often use them in genetic crosses to avoid accidental reversion of mutant strains during the course of a genetic study.

MiniQuiz

- What does it mean to say that point mutations can spontaneously revert?
- Do missense mutations occur in genes encoding tRNA? Why or why not?

10.3 Mutation Rates

The rates at which different kinds of mutations occur vary widely. Some types of mutations occur so rarely that they are almost impossible to detect, whereas others occur so frequently that they present difficulties for an experimenter trying to maintain a genetically stable stock culture. Furthermore, all organisms possess a variety of systems for DNA repair. Consequently, the observed mutation rate depends not only on the frequency of DNA alterations but also on the efficiency of DNA repair.

Spontaneous Mutation Frequencies

For most microorganisms, errors in DNA replication occur at a frequency of 10^{-6} to 10^{-7} per kilobase pair during a single round of replication. A typical gene has about 1000 base pairs. Therefore, the frequency of a mutation *in a given gene* is also in the range of 10^{-6} to 10^{-7} per generation. For instance, in a bacterial culture having 10^8 cells/ml, there are likely to be a number of different mutants for any given gene in each milliliter of culture. Eukaryotes with very large genomes tend to have replication error rates about 10-fold lower than typical bacteria, whereas DNA viruses, especially those with very small genomes, may have error rates 100-fold to 1000-fold higher than those of cellular organisms. RNA viruses have even higher error rates.

Single base errors during DNA replication are more likely to lead to missense mutations than to nonsense mutations because most single base substitutions yield codons that encode other amino acids (🔗 Table 6.5). The next most frequent type of codon change caused by a single base change leads to a silent mutation. This is because for the most part alternate codons for a given amino acid differ from each other by a single base change in the "silent" third position. A given codon can be changed to any of 27 other codons by a single base substitution, and on average, about two of these will be silent mutations, about one a nonsense mutation, and the rest will be missense mutations. There are also some DNA sequences, typically areas containing short repeats, that are hot spots for mutations because the error frequency of DNA polymerase is relatively high there. The error rate at hot spots is affected by the base sequence in the vicinity.

Unless a mutation can be selected for, its experimental detection is difficult, and much of the skill of the microbial geneticist involves increasing the efficiency of mutation detection. As we see in the next section, it is possible to greatly increase the mutation rate by mutagenic treatments. In addition, the mutation rate may change in certain situations, such as under high-stress conditions.

Mutations in RNA Genomes

Whereas all cells have DNA as their genetic material, some viruses have RNA genomes (🔗 Section 9.1). These genomes can also undergo mutation. Interestingly, the mutation rate in RNA genomes is about 1000-fold higher than in DNA genomes. Why should this be so?

Some RNA polymerases have proofreading activities like those of DNA polymerases (Section 6.10), thus limiting the total number of polymerase errors. However, although there are several repair systems for DNA that can correct changes before they become fixed in the genome as mutations (Section 10.4), comparable RNA repair mechanisms do not exist. This leads to heightened mutation rates for RNA genomes. This high mutation rate in RNA viruses has dramatic consequences. For example, the RNA genomes of viruses that cause disease can mutate very rapidly, presenting a constantly changing and evolving population of viruses. Such changes are one of many challenges to human medicine posed by the AIDS virus, HIV, which is an RNA virus with a notorious ability to undergo genetic changes that affect its virulence (Section 21.11).

MiniQuiz

- Which class of mutation, missense or nonsense, is more common, and why?
- Why are RNA viruses genetically highly variable?

10.4 Mutagenesis

The spontaneous rate of mutation is very low, but there are a variety of chemical, physical, and biological agents that can increase the mutation rate and are therefore said to induce mutations. These agents are called **mutagens**. We discuss some of the major categories of mutagens and their activities here.

Chemical Mutagens

An overview of some of the major chemical mutagens and their modes of action is given in **Table 10.2**. Several classes of chemical mutagens exist. The *nucleotide base analogs* are molecules that resemble the purine and pyrimidine bases of DNA in structure yet display faulty pairing properties (**Figure 10.5**). If a base analog is incorporated into DNA in place of the natural base, the DNA may replicate normally most of the time. However, DNA replication errors occur at higher frequencies at these sites due to

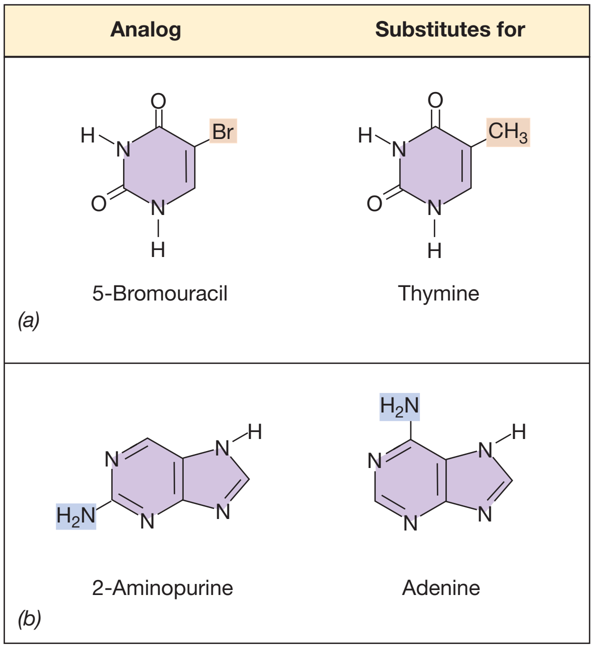

Figure 10.5 Nucleotide base analogs. Structure of two common nucleotide base analogs used to induce mutations and the normal nucleic acid bases for which they substitute. *(a)* 5-Bromouracil can base-pair with guanine, causing AT to GC substitutions. *(b)* 2-Aminopurine can base-pair with cytosine, causing AT to GC substitutions.

Table 10.2 *Chemical and physical mutagens and their modes of action*

Agent	*Action*	*Result*
Base analogs		
5-Bromouracil	Incorporated like T; occasional faulty pairing with G	AT → GC and occasionally GC → AT
2-Aminopurine	Incorporated like A; faulty pairing with C	AT → GC and occasionally GC → AT
Chemicals reacting with DNA		
Nitrous acid (HNO_2)	Deaminates A and C	AT → GC and GC → AT
Hydroxylamine (NH_2OH)	Reacts with C	GC → AT
Alkylating agents		
Monofunctional (for example, ethyl methanesulfonate)	Puts methyl on G; faulty pairing with T	GC → AT
Bifunctional (for example, mitomycin, nitrogen mustards, nitrosoguanidine)	Cross-links DNA strands; faulty region excised by DNase	Both point mutations and deletions
Intercalating dyes		
Acridines, ethidium bromide	Inserts between two base pairs	Microinsertions and microdeletions
Radiation		
Ultraviolet	Pyrimidine dimer formation	Repair may lead to error or deletion
Ionizing radiation (for example, X-rays)	Free-radical attack on DNA, breaking chain	Repair may lead to error or deletion

incorrect base pairing. The result is the incorporation of a wrong base into the new strand of DNA and thus introduction of a mutation. During subsequent segregation of this strand in cell division, the mutation is revealed.

Other chemical mutagens induce *chemical modifications* in one base or another, resulting in faulty base pairing or related changes (Table 10.2). For example, alkylating agents (chemicals that react with amino, carboxyl, and hydroxyl groups by substituting them with alkyl groups) such as nitrosoguanidine, are powerful mutagens and generally induce mutations at higher frequency than base analogs. Unlike base analogs, which have an effect only when incorporated during DNA replication, alkylating agents can introduce changes even in nonreplicating DNA. Both base analogs and alkylating agents tend to induce base-pair substitutions (Section 10.2).

Another group of chemical mutagens, the acridines, are planar molecules that function as *intercalating agents*. These mutagens become inserted between two DNA base pairs and push them apart. During replication, this abnormal conformation can lead to single base insertions or deletions in acridine-containing DNA. Thus, acridines typically induce frameshift mutations (Section 10.2). Ethidium bromide, which is often used to detect DNA in electrophoresis, is also an intercalating agent and therefore a mutagen.

Radiation

Several forms of radiation are highly mutagenic. We can divide mutagenic electromagnetic radiation into two main categories, nonionizing and ionizing (**Figure 10.6**). Although both kinds of radiation are used to generate mutations, nonionizing radiation such as ultraviolet (UV) radiation has the widest use.

The purine and pyrimidine bases of nucleic acids absorb UV radiation strongly, and the absorption maximum for DNA and RNA is at 260 nm (Figure 6.7). Killing of cells by UV radiation is due primarily to its effect on DNA. Although several effects are known, one well-established effect is the production of pyrimidine dimers, in which two adjacent pyrimidine bases (cytosine or thymine) on the same strand of DNA become covalently bonded to one another. This results either in impeding DNA polymerase or in a greatly increased probability of DNA polymerase misreading the sequence at this point.

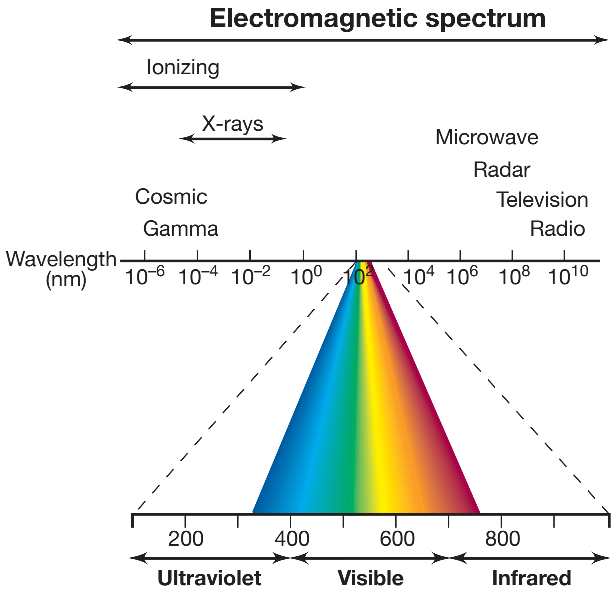

Figure 10.6 Wavelengths of radiation. Ultraviolet radiation consists of wavelengths just shorter than visible light. For any electromagnetic radiation, the shorter the wavelength, the higher the energy. DNA absorbs strongly at 260 nm.

The UV radiation source most commonly used for mutagenesis is the germicidal lamp, which emits UV radiation in the 260-nm region. A dose of UV radiation is used that kills about 50–90% of the cell population, and mutants are then selected or screened for among the survivors. If much higher doses of radiation are used, the number of surviving cells is too low. If lower doses are used, damage to DNA is insufficient to generate enough mutations. When used at the correct dose, UV radiation is a very convenient tool for isolating mutants and avoids the need to handle toxic chemicals.

Ionizing Radiation

Ionizing radiation is a more powerful form of radiation than UV radiation and includes short-wavelength rays such as X-rays, cosmic rays, and gamma rays (Figure 10.6). These rays cause water and other substances to ionize, and mutagenic effects result indirectly from this ionization. Among the potent chemical species formed by ionizing radiation are chemical free radicals, the most important being the hydroxyl radical, OH• (Section 5.18).

Free radicals react with and damage macromolecules in the cell, including DNA. This causes double-stranded and single-stranded breaks that may lead to rearrangements or large deletions. At low doses of ionizing radiation only a few "hits" on DNA occur, but at higher doses, multiple hits cause fragmentation of DNA that sometimes cannot be repaired and thus leads to the death of the cell. In contrast to UV radiation, ionizing radiation penetrates readily through glass and other materials. Therefore, ionizing radiation is used frequently to induce mutations in animals and plants because its penetrating power makes it possible to reach the gamete-producing cells of these organisms. However, because ionizing radiation is more dangerous and is less readily available than UV radiation, it finds less use in microbial genetics.

DNA Repair Systems

By definition, a mutation is a *heritable* change in the genetic material. Therefore, if damaged DNA can be corrected before the cell divides, no mutation will occur. Most cells have a variety of different DNA repair processes to correct mistakes or repair damage. Most of these DNA repair systems are virtually error-free. However, some are error-prone and the repair process itself introduces the mutation. DNA repair processes may be grouped into three categories: direct reversal, repair of single-strand damage, and repair of double-strand damage.

Direct reversal applies to bases that have been chemically altered but whose identity is still recognizable. No base pairing (that is, no template strand) is needed. For example, some alkylated bases are repaired by direct chemical removal of the

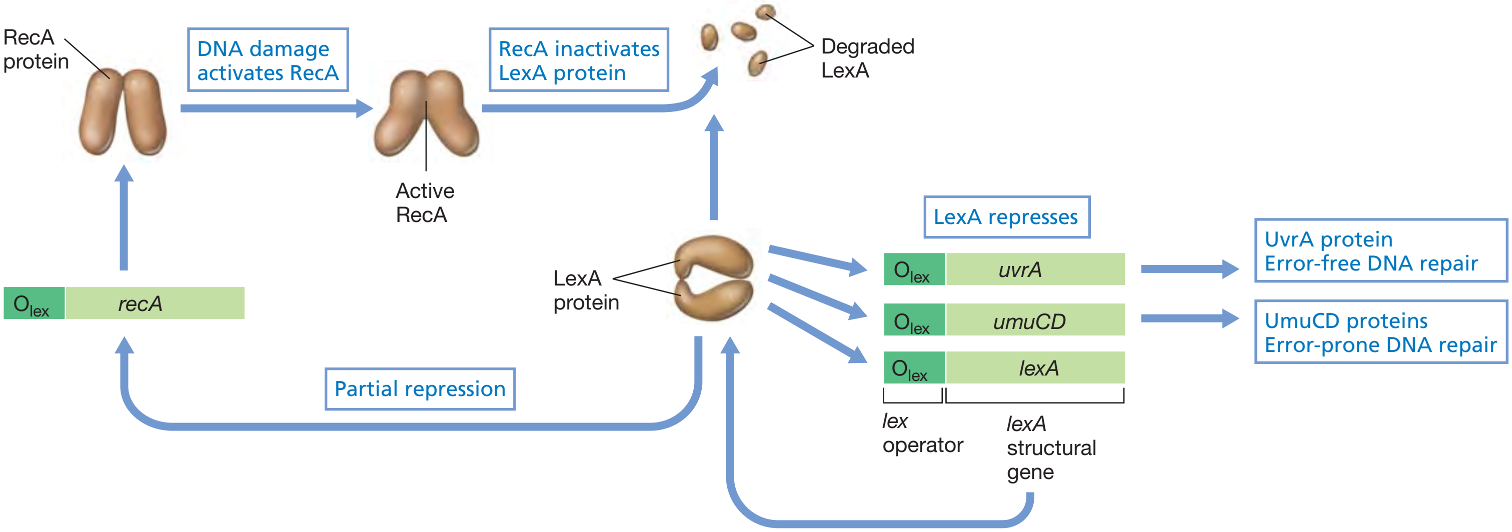

Figure 10.7 Mechanism of the SOS response. DNA damage activates RecA protein, which in turn activates the protease activity of LexA. The LexA protein then cleaves itself. LexA protein normally represses the activities of the *recA* gene and the DNA repair genes *uvrA* and *umuCD* (the UmuCD proteins are part of DNA polymerase V). However, repression is not complete. Some RecA protein is produced even in the presence of LexA protein. With LexA inactivated, these genes become highly active.

alkyl group. Another direct repair system is photoreactivation, which cleaves pyrimidine dimers generated by UV radiation. The enzyme photolyase absorbs blue light and uses the energy to drive the cleavage reaction.

Several systems exist that repair single-strand DNA damage. In these cases, the damaged DNA is removed from only one strand. Then the opposite (undamaged) strand is used as a template for replacing the missing nucleotides. In base excision repair, a single damaged base is removed and replaced. In nucleotide excision repair and mismatch repair, a short stretch of single-stranded DNA containing the damage is removed and replaced. Double-strand damage, including both cross-strand links and double-stranded breaks, is especially dangerous. These lesions are repaired by recombinational mechanisms and may require error-prone repair.

Mutations That Arise from DNA Repair: The SOS System

Some types of DNA damage, especially large-scale damage from highly mutagenic chemicals or large doses of radiation, may interfere with replication if such lesions are not removed before replication occurs. Lesions on the template DNA may lead to stalling of DNA replication, which is a lethal event. Stalled replication, as well as certain types of major DNA damage, activate the *SOS repair system*. The SOS system initiates a number of DNA repair processes, some of which are error-free. However, the SOS system also allows DNA repair to occur without a template, that is, without base pairing; as expected, this results in many errors and hence many mutations. This permits cell survival under conditions that are otherwise lethal.

In *Escherichia coli* the SOS repair system regulates the transcription of approximately 40 genes located throughout the chromosome that are involved in DNA damage tolerance and DNA repair. In DNA damage tolerance, DNA lesions remain in the DNA, but are bypassed by specialized DNA polymerases that can move past DNA damage—a process known as translesion synthesis. Even if no template is available to allow insertion of the correct bases, it is less dangerous to fill the gap than let it remain. Consequently, translesion synthesis generates many errors. In *E. coli*, in which the process of mutagenesis has been studied in great detail, the two error-prone repair polymerases are DNA polymerase V, an enzyme encoded by the *umuCD* genes (Figure 10.7), and DNA polymerase IV, encoded by *dinB*. Both are induced as part of the SOS repair system.

The SOS system is a **regulon**, that is, a set of genes that are coordinately regulated although they are transcribed separately. The SOS system is regulated by two proteins, LexA and RecA. LexA is a repressor that normally prevents expression of the SOS regulon. The RecA protein, which normally functions in genetic recombination (Section 10.6), is activated by the presence of DNA damage, in particular by the single-stranded DNA that results when replication stalls (Figure 10.7). The activated form of RecA stimulates LexA to inactivate itself by self-cleavage. This leads to derepression of the SOS system and results in the coordinate expression of a number of proteins that take part in DNA repair. Because some of the DNA repair mechanisms of the SOS system are inherently error-prone, many mutations arise. Once the DNA damage has been repaired, the SOS regulon is repressed and further mutagenesis ceases.

Changes in Mutation Rate

High fidelity (low error frequency) in DNA replication is essential if organisms are to remain genetically stable. On the other hand, perfect fidelity is counterproductive because it would prevent evolution. Therefore, a mutation rate has evolved in cells that is very low, yet detectable. This allows organisms to balance the need for genetic stability with that for evolutionary improvement.

The fact that organisms as phylogenetically distant as *Archaea* and *E. coli* have about the same mutation rate might suggest that evolutionary pressure has selected organisms with the lowest possible mutation rates. However, this is not so. The mutation rate in an organism is subject to change. For example, mutants of some organisms that are hyperaccurate in DNA replication and repair have been selected in the laboratory. However, in these strains, the improved proofreading and repair mechanisms have a significant metabolic cost; thus, hyperaccurate mutants might well be at a disadvantage in the natural environment. On the other hand, some organisms seem to benefit from enhanced DNA repair systems that enable them to occupy particular niches in nature. A good example is the bacterium *Deinococcus radiodurans* (⇄ Section 18.17). This organism is 20 times more resistant to UV radiation and 200 times more resistant to ionizing radiation than is *E. coli*. This resistance, dependent in part upon redundant DNA repair systems and on a mechanism for exporting damaged nucleotides, allows the organism to survive in environments in which other organisms cannot, such as near concentrated sources of radiation or on the surfaces of dust particles exposed to intense sunlight.

In contrast to hyperaccuracy, some organisms actually benefit from an increased mutation rate. DNA repair systems are themselves genetically encoded and thus subject to mutation. For example, the protein subunit of DNA polymerase III involved in proofreading (⇄ Section 6.10) is encoded by the gene *dnaQ*. Certain mutations in *dnaQ* lead to mutants that are still viable but have an increased rate of mutation. These are known as hypermutable or **mutator strains**. Mutations leading to a mutator phenotype are known in several other DNA repair systems as well. The mutator phenotype is apparently selected for in complex and changing environments because strains of bacteria with mutator phenotypes appear to be more abundant under these conditions. Presumably, whatever disadvantage an increased mutation rate may have in such environments is offset by the ability to generate greater numbers of useful mutations. These mutations ultimately increase evolutionary fitness of the population and make the organism more successful in its ecological niche.

As indicated earlier, a mutator phenotype may be induced in wild-type strains by stressful situations. For instance, the SOS repair system includes error-prone repair. Therefore, when the SOS repair system is activated, the mutation rate increases. In some cases this is merely an inevitable by-product of DNA repair, but in other cases, the increased mutation rate may itself be of selective value to the organism for survival purposes.

MiniQuiz

- How do mutagens work?
- Why might a mutator phenotype be successful in an environment experiencing rapid changes?
- What is meant by "error prone" DNA repair?

10.5 Mutagenesis and Carcinogenesis: The Ames Test

The Ames test makes practical use of bacterial mutations to detect potentially hazardous chemicals in the environment. Because selectable mutants can be detected in large populations of bacteria with very high sensitivity, bacteria can be used to screen chemicals for potential mutagenicity. This is relevant because many mutagenic chemicals are also carcinogenic, capable of causing cancer in humans or other animals.

The variety of chemicals, both natural and artificial, that humans encounter through agricultural and industrial exposure is enormous. There is good evidence that some human cancers have environmental causes, most likely from various chemicals, making the detection of chemical carcinogens an important matter. Not every mutagen is also a carcinogen. The correlation, however, is high, and knowing that a compound is mutagenic to bacteria is a warning of possible danger. Bacterial tests for carcinogen screening were developed primarily by Bruce Ames and colleagues at the University of California in Berkeley and consequently, the mutagenicity test for carcinogens is known as the *Ames test* (**Figure 10.8**).

The standard way to test chemicals for mutagenesis is to look for an increase in the rate of back mutation (reversion) in auxotrophic strains of bacteria in the presence of the suspected mutagen. It is important that the auxotrophic strain carry a point mutation so that the reversion rate is measurable. Cells of such an auxotroph do not grow on a medium lacking the required nutrient (for example, an amino acid), and even very large populations of cells can be spread on the plate without formation of visible colonies. However, if back mutants (revertants) are present, those cells form colonies. Thus, if 10^8 cells are spread on the surface of a single plate, even as few as 10–20 revertants can be detected by the 10–20 colonies they form (Figure 10.8, left photo). However, if the reversion rate is increased by the presence of a chemical mutagen, the number of revertant colonies is

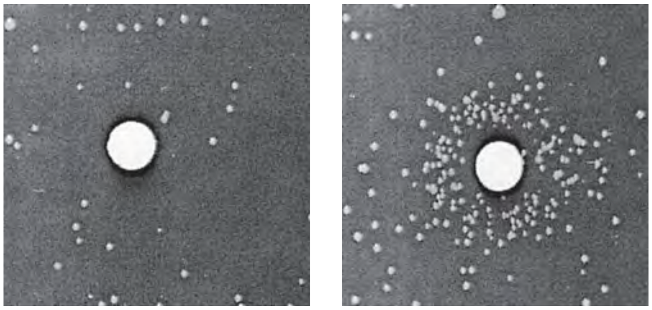
T. D. Brock

Figure 10.8 The Ames test for assessing the mutagenicity of a chemical. Two plates were inoculated with a culture of a histidine-requiring mutant of *Salmonella enterica*. The medium does not contain histidine, so only cells that revert back to wild type can grow. Spontaneous revertants appear on both plates, but the chemical on the filter-paper disc in the test plate (right) has caused an increase in the mutation rate, as shown by the large number of colonies surrounding the disc. Revertants are not seen very close to the test disc because the concentration of mutagen is lethally high there. The plate on the left was the negative control; its filter-paper disc had only water added.

even greater. Histidine auxotrophs of *Salmonella enterica* (Figure 10.8) and tryptophan auxotrophs of *Escherichia coli* have been the major tools of the Ames test.

Two additional elements have been introduced in the Ames test to make it much more powerful. The first of these is to use test strains that almost exclusively use error-prone pathways to repair DNA damage; normal repair mechanisms are thus thwarted (Section 10.4). The second important element in the Ames test is the addition of liver enzyme preparations to convert the chemicals to be tested into their active mutagenic (and potentially carcinogenic) forms. It has been well established that many carcinogens are not directly carcinogenic or mutagenic themselves, but undergo modifications in the human body that convert them into active substances. These changes take place primarily in the liver, where enzymes called mixed-function oxygenases, whose normal function is detoxification, generate activated forms of the compounds that are highly reactive (and thus mutagenic) toward DNA.

In the Ames test, a preparation of enzymes from rat liver is first used to activate the test compound. The activated complex is then soaked into a filter-paper disc, which is placed in the center of a plate on which the proper bacterial strain has been overlaid. After overnight incubation, the mutagenicity of the compound can be detected by looking for a halo of back mutations in the area around the paper disc (Figure 10.8). It is necessary to carry out this test with several different concentrations of the compound and with appropriate positive (known mutagens) and negative (no mutagen) controls, because compounds vary in their mutagenic activity and may be lethal at higher levels. A wide variety of chemicals have been subjected to the Ames test, and it has become one of the most useful screens for determining the potential carcinogenicity of a compound.

MiniQuiz

- Why does the Ames test measure the rate of back mutation rather than the rate of forward mutation?
- Of what significance is the detection of mutagens to the prevention of cancer?

II Gene Transfer

For genetic analyses, the microbial geneticist must cross strains of an organism that have different genotypes (and phenotypes) and look for recombinants. Three mechanisms of genetic exchange are known in prokaryotes: (1) *transformation*, in which free DNA released from one cell is taken up by another (Section 10.7); (2) *transduction*, in which DNA transfer is mediated by a virus (Section 10.8); and (3) *conjugation*, in which DNA transfer involves cell-to-cell contact and a conjugative plasmid in the donor cell (Sections 10.9 and 10.10). These processes are contrasted in **Figure 10.9**.

Before discussing the mechanisms of transfer, we must consider the fate of the transferred DNA. Whether it is transferred by transformation, transduction, or conjugation, the incoming DNA faces three possible fates: (1) It may be degraded by restriction enzymes; (2) it may replicate by itself (but only if it possesses its own origin of replication such as a plasmid or phage genome); or (3) it may recombine with the host chromosome.

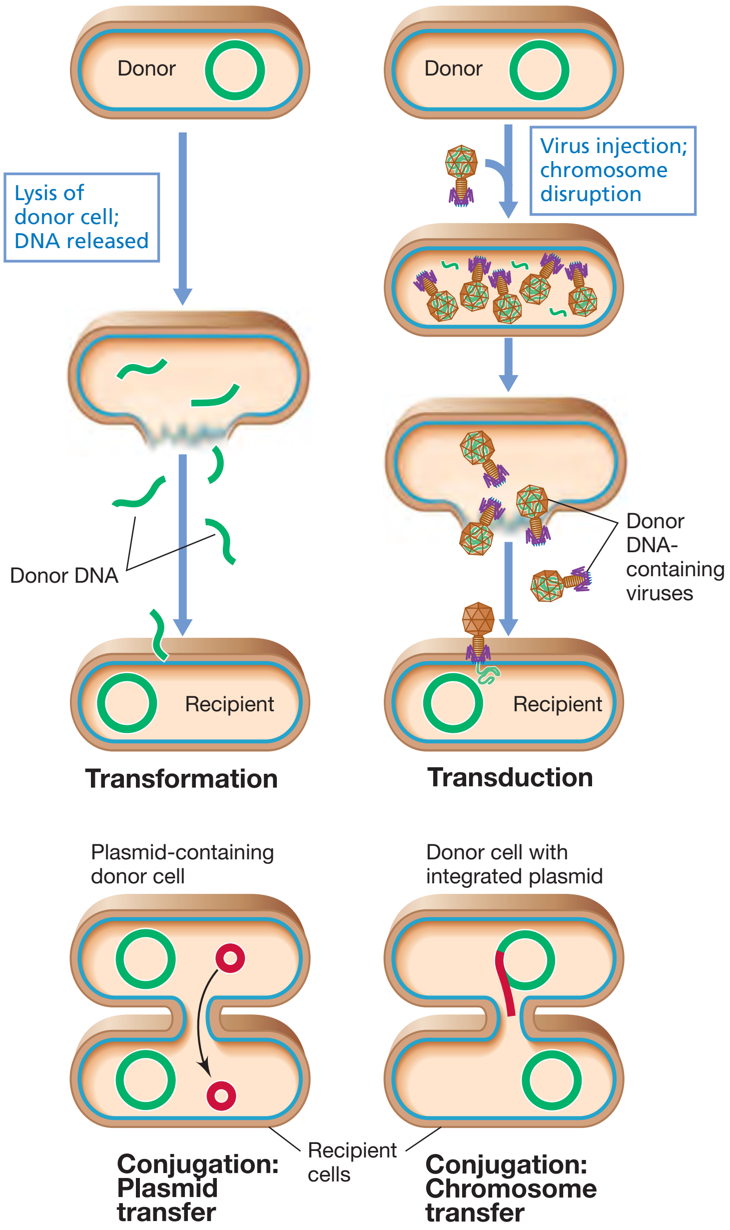

Figure 10.9 Processes by which DNA is transferred from donor to recipient bacterial cell. Just the initial steps in transfer are shown.

10.6 Genetic Recombination

Recombination is the physical exchange of DNA between genetic elements. In this section we focus on *homologous* recombination, a process that results in genetic exchange between homologous DNA sequences from two different sources. Homologous DNA sequences are those that have nearly the same sequence; therefore, bases can pair over an extended length of the two DNA molecules. This type of recombination is involved in the process referred to as "crossing over" in classical genetics.

Molecular Events in Homologous Recombination

The RecA protein, previously mentioned in regard to the SOS repair system (Section 10.4), is the key to homologous recombination. RecA is essential in nearly every homologous recombination pathway. RecA-like proteins have been identified in all bacteria examined, as well as in the *Archaea* and most *Eukarya*.

A molecular mechanism for homologous recombination between two DNA molecules is shown in **Figure 10.10**. An enzyme that cuts DNA in the middle of a strand, known as an endonuclease, begins the process by nicking one strand of the first DNA molecule. This nicked strand is separated from the other strand by proteins with helicase activity (🔗 Section 6.9).

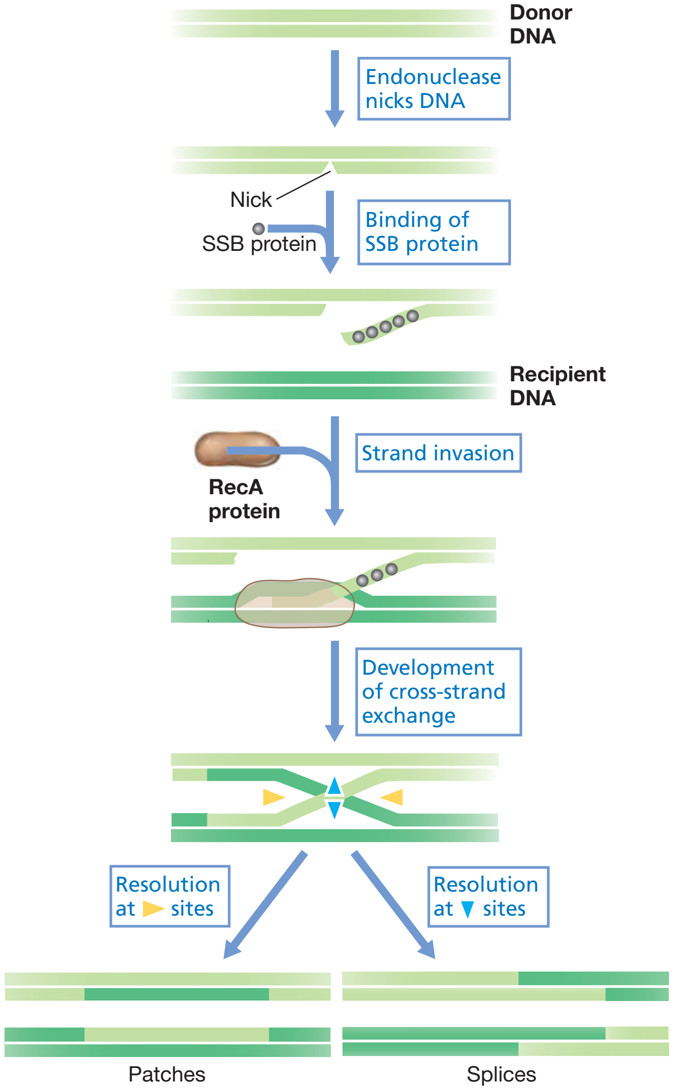

Figure 10.10 A simplified version of homologous recombination. Homologous DNA molecules pair and exchange DNA segments. The mechanism involves breakage and reunion of paired segments. Two of the proteins involved, single-strand binding (SSB) protein and the RecA protein, are shown. The other proteins involved are not shown. The diagram is not to scale: Pairing may occur over hundreds or thousands of bases. Resolution occurs by cutting and rejoining the cross-linked DNA molecules. Note that there are two possible outcomes, patches or splices, depending on where strands are cut during the resolution process.

In some recombination pathways specialized enzymes, such as the RecBCD enzyme of *Escherichia coli*, combine the endonuclease and helicase activities. Single-strand binding protein (🔗 Section 6.9) then binds to the resulting single-stranded segment. Next, the RecA protein binds to the single-stranded region. This results in a complex that promotes base pairing with the complementary sequence in the second DNA molecule. This in turn displaces the other strand of the second DNA molecule (Figure 10.10) and is therefore called *strand invasion*. The base pairing of one strand from each of the two DNA molecules over long stretches generates recombination intermediates containing long **heteroduplex** regions, where each strand has originated from a different chromosome. These structures are called *Holliday junctions* (after Robin Holliday, who proposed this model in 1964) and can migrate along the DNA; this migration is energized by a complex of several other proteins. Finally, the linked molecules are separated or "resolved" by resolvases that cut and rejoin the second (previously unbroken) strands of both original DNA molecules. In *E. coli*, the RecG and RuvC proteins both function as resolvases, and their activity generates two recombined DNA molecules. Depending on the orientation of the Holliday junction during resolution, two types of products, referred to as "patches" or "splices," are formed that differ in the conformation of the heteroduplex regions remaining after resolution (Figure 10.10).

Effect of Homologous Recombination on Genotype

For homologous recombination to generate new genotypes, the two homologous sequences must be related but genetically distinct. This is obviously the case in a diploid eukaryotic cell, which has two sets of chromosomes, one from each parent. In prokaryotes, genetically distinct but homologous DNA molecules are brought together in different ways, but the process of genetic recombination is equivalent. Genetic recombination in prokaryotes occurs after fragments of homologous DNA from a donor chromosome are transferred to a recipient cell by transformation, transduction, or conjugation. It is only after the transfer event, when the DNA fragment from the donor is in the recipient cell, that homologous recombination occurs. In prokaryotes, only part of a chromosome is transferred; therefore, if recombination does not occur, the DNA fragment will be lost because it cannot replicate independently. Thus, in prokaryotes, transfer is just the first step in generating recombinant organisms.

Detection of Recombination

To detect physical exchange of DNA segments, the cells resulting from recombination must be phenotypically different from both parents. Genetic crosses in bacteria usually depend on using recipient strains that lack some selectable character that the recombinants will gain. For instance, the recipient may be unable to grow on a particular medium, and genetic recombinants are selected that can. Various kinds of selectable markers, such as drug resistance and nutritional requirements, were discussed in Section 10.1.

The exceedingly great sensitivity of the selection process allows even a few recombinant cells to be detected in a large population of nonrecombinant cells (**Figure 10.11**). The only requirement for effective detection of recombination is that the back

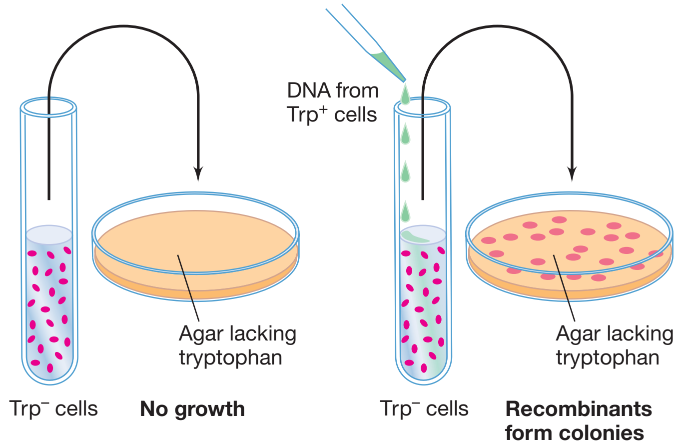

Figure 10.11 Using a selective medium to detect rare genetic recombinants. On the selective medium only the rare recombinants form colonies even though a very large population of bacteria was plated. Procedures such as this, which offer high resolution for genetic analyses, can ordinarily be used only with microorganisms. The type of genetic exchange being illustrated is transformation.

mutation rate for the selected characteristic should be low, because revertants will also form colonies. This problem can often be overcome by using double mutants—strains that carry two different mutations—in genetic crosses because it is very unlikely that two back mutations will occur in the same cell. Alternatively, frameshift mutants can be used, because their reversion rates are typically extremely low.

Much of the skill of the bacterial geneticist lies in the choice of proper mutants and selective media for efficient detection of genetic recombination. Because selection is so powerful and because crosses can be made using billions of individual cells, recombinational analysis following gene transfer is an important tool for the microbial geneticist.

MiniQuiz

- Which protein, found in all prokaryotes, facilitates the pairing required for homologous recombination?
- In eukaryotes, recombination involves entire chromosomes, but this is not true in prokaryotes. Explain.

10.7 Transformation

Transformation is a genetic transfer process by which free DNA is incorporated into a recipient cell and brings about genetic change. Several prokaryotes are naturally transformable, including certain species of both gram-negative and gram-positive *Bacteria* and also some species of *Archaea* (Section 10.12). Because the DNA of prokaryotes is present in the cell as a large single molecule, when the cell is gently lysed, the DNA pours out. Because of their extreme length (1700 μm in *Bacillus subtilis,* for example), bacterial chromosomes break easily. Even after gentle extraction, the *B. subtilis* chromosome of 4.2 megabase pairs (Mbp) is converted to fragments of about 10 kbp each. Because the DNA that corresponds to an average gene is about 1000 nucleotides, each of the fragments of *B. subtilis* DNA therefore contains about ten genes. This is a typical transformable size. A single cell usually incorporates only one or a few DNA fragments, so only a small proportion of the genes of one cell can be transferred to another by a single transformation event.

Transformation in the History of Molecular Biology

The discovery of transformation was one of the key events in biology, as it led to experiments demonstrating that DNA was the genetic material. This discovery became a cornerstone of molecular biology and modern genetics.

The British scientist Frederick Griffith obtained the first evidence of bacterial transformation in the late 1920s. Griffith was working with *Streptococcus pneumoniae* (pneumococcus), a bacterium that owes its ability to invade the body in part to the presence of a polysaccharide capsule (Section 3.9). Mutants can be isolated that lack this capsule and thus cannot cause disease. Such mutants are called *R strains* because their colonies appear rough on agar, in contrast to the smooth appearance of encapsulated strains, called *S strains.* A mouse infected with only a few cells of an S strain succumbs in a day or two to a massive pneumococcus infection. By contrast, even large numbers of R cells do not cause death when injected. Griffith showed that if heat-killed S cells were injected along with living R cells, the mouse developed a fatal infection and the bacteria isolated from the dead mouse were of the S type (**Figure 10.12**). Because the S cells isolated in such an experiment always had the capsule type of the heat-killed S cells, Griffith concluded that the R cells had been transformed into a new type. This process set the stage for the discovery of DNA.

Oswald T. Avery and his associates at the Rockefeller Institute in New York provided the molecular explanation for the transformation of pneumococcus in a series of studies during the 1930s and 1940s. Avery and his coworkers showed that transformation could be carried out in the test tube instead of the mouse and that a cell-free extract of heat-killed cells could induce transformation. In a series of painstaking biochemical experiments, the active fraction was purified from cell-free extracts and was shown to be DNA. The transforming activity of purified DNA preparations was very high, and only a very small amount of material was necessary. Subsequently, others showed that transformation in pneumococcus affected not only the capsule but also other genetic characteristics such as antibiotic resistance and sugar fermentation.

In 1953, James Watson and Francis Crick published their model of the structure of DNA, providing a theoretical framework for how DNA could serve as genetic material. Thus, three types of studies, the bacteriological ones of Griffith, the biochemical ones of Avery, and the structural ones of Watson and Crick, solidified the concept of DNA as the genetic material. In subsequent years, this work led to the whole field of molecular biology and molecular genetics.

Competence in Transformation

Even within transformable genera, only certain strains or species are transformable. A cell that is able to take up DNA and be

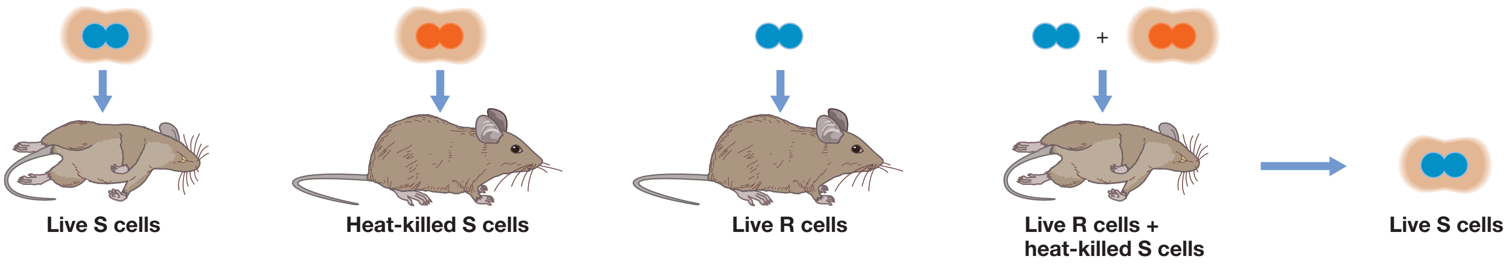

Figure 10.12 Griffith's experiments with pneumococcus. Live smooth (S) cells have a capsule and kill mice because immune cells cannot kill the encapsulated bacteria; the cells proliferate in the lung and cause a fatal pneumonia. Rough (R) cells have no capsule and are not pathogenic. But a combination of live R and dead S cells kill mice, and live S cells can be isolated from the animals. DNA carrying genes for capsule production is released from dead S cells and taken up by R cells, thus transforming them into S cells.

transformed is said to be *competent*, and this capacity is genetically determined.

Competence in most naturally transformable bacteria is regulated, and special proteins play a role in the uptake and processing of DNA. These competence-specific proteins include a membrane-associated DNA-binding protein, a cell wall autolysin, and various nucleases. One pathway of natural competence in *B. subtilis*—an easily transformed species—is regulated by quorum sensing (a regulatory system that responds to cell density; ⇄ Section 8.9). Cells produce and excrete a small peptide during growth, and the accumulation of this peptide to high concentrations induces the cells to become competent. In *Bacillus*, roughly 20% of the cells in a culture become competent and stay that way for several hours. However, in *Streptococcus*, 100% of the cells can become competent, but only for a brief period during the growth cycle.

High-efficiency, natural transformation is rare among *Bacteria*. For example, *Acinetobacter*, *Bacillus*, *Streptococcus*, *Haemophilus*, *Neisseria*, and *Thermus* are naturally competent and easy to transform. By contrast, many *Bacteria* are poorly transformed, if at all, under natural conditions. *Escherichia coli* and many other gram-negative bacteria fall into this category. However, if cells of *E. coli* are treated with high concentrations of calcium ions and then chilled for several minutes, they become adequately competent. Cells of *E. coli* treated in this manner take up double-stranded DNA, and therefore transformation of this organism by plasmid DNA is relatively efficient. This is important because getting DNA into *E. coli*—the workhorse of genetic engineering—is critical for biotechnology, as we will see in Chapter 15.

Electroporation is a physical technique that is used to get DNA into organisms that are difficult to transform, especially those with thick cell walls. In electroporation, cells are mixed with DNA and then exposed to brief high-voltage electrical pulses. This makes the cell envelope permeable and allows entry of the DNA. Electroporation is a quick process and works for most types of cells, including *E. coli*, most other *Bacteria*, some members of the *Archaea*, and even yeast and certain plant cells.

Uptake of DNA in Transformation

During natural transformation, competent bacteria reversibly bind DNA. Soon, however, the binding becomes irreversible. Competent cells bind much more DNA than do noncompetent cells—as much as 1000 times more. As noted earlier, the sizes of the transforming fragments are much smaller than that of the whole genome, and the fragments are further degraded during the uptake process. In *S. pneumoniae* each cell can bind only about ten molecules of double-stranded DNA of 10–15 kbp each. However, as these fragments are taken up, they are converted into single-stranded pieces of about 8 kb, with the complementary strand being degraded. The DNA fragments in the mixture compete with each other for uptake, and if excess DNA that does not contain the genetic marker under observation is added, the number of transformants decreases.

In preparations of transforming DNA, typically only about 1 out of 100–300 DNA fragments contains the genetic marker being studied. Thus, at high concentrations of DNA, the competition between DNA molecules results in saturation of the system, so even under the best of conditions it is impossible to transform all the cells in a population for a given marker. The maximum frequency of transformation that has so far been obtained is about 20% of the population; the usual values are between 0.1% and 1.0%. But when recipient population sizes are very high, even this low frequency is easy to detect. The minimum concentration of DNA yielding detectable transformants is about 0.01 ng/ml, which is so low that it is chemically undetectable.

Interestingly, transformation in *Haemophilus influenzae* requires the DNA fragment to have a particular 11-bp sequence for irreversible binding and uptake to occur. This sequence is found at an unexpectedly high frequency in the *Haemophilus* genome. Evidence such as this, and the fact that certain bacteria become competent in their natural environment, suggests that transformation is not a laboratory artifact but plays an important role in horizontal gene transfer in nature. By promoting new combinations of genes, naturally transformable bacteria increase the diversity and fitness of the microbial community as a whole.

Integration of Transforming DNA

Transforming DNA is bound at the cell surface by a DNA-binding protein. Next, either the entire double-stranded fragment is taken up, or a nuclease degrades one strand and the remaining strand is taken up, depending on the organism (**Figure 10.13**). After uptake, the DNA is bound by a competence-specific protein. This protects the DNA from nuclease attack until it reaches

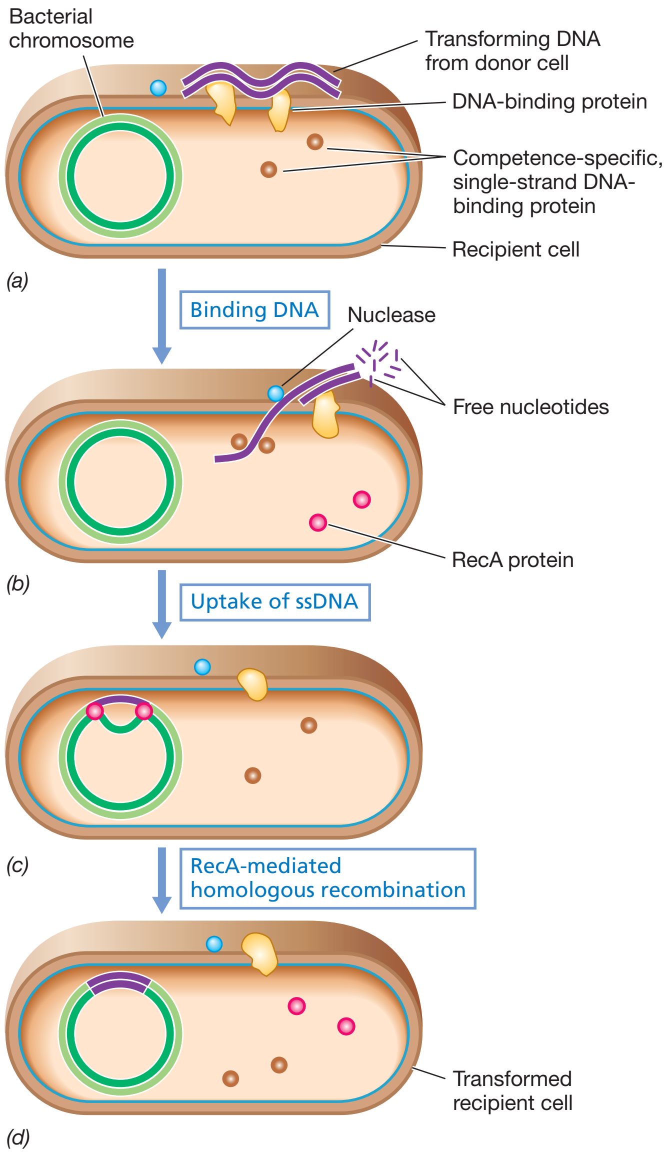

Figure 10.13 Mechanism of transformation in a gram-positive bacterium. *(a)* Binding of double-stranded DNA by a membrane-bound DNA-binding protein. *(b)* Passage of one of the two strands into the cell while nuclease activity degrades the other strand. *(c)* The single strand in the cell is bound by specific proteins, and recombination with homologous regions of the bacterial chromosome is mediated by RecA protein. *(d)* Transformed cell.

the chromosome, where the RecA protein takes over. The DNA is integrated into the genome of the recipient by recombination (Figures 10.13 and 10.10). If single-stranded DNA is integrated, a heteroduplex DNA is formed. During the next round of chromosomal replication, one parental and one recombinant DNA molecule are generated. On segregation at cell division, the recombinant molecule is present in the transformed cell, which is now genetically altered compared to its parent. The preceding applies only to small pieces of linear DNA. Many naturally transformable *Bacteria* are transformed only poorly by plasmid DNA because the plasmid must remain double-stranded and circular in order to replicate.

Transfection

Bacteria can be transformed with DNA extracted from a bacterial virus rather than from another bacterium. This process is called *transfection*. If the DNA is from a lytic bacteriophage, transfection leads to virus production and can be measured by the standard phage plaque assay (⇄ Section 9.4). Transfection is useful for studying the mechanisms of transformation and recombination because the small size of phage genomes allows the isolation of a nearly homogeneous population of DNA molecules. By contrast, in conventional transformation the transforming DNA is typically a random assortment of chromosomal DNA fragments of various lengths, and this tends to complicate experiments designed to study the mechanism of transformation.

MiniQuiz

- The donor bacterial cell in a transformation is probably dead. Explain.
- Even in naturally transformable cells, competence is usually inducible. What does this mean?

10.8 Transduction

In **transduction**, a bacterial virus (bacteriophage) transfers DNA from one cell to another. Viruses can transfer host genes in two ways. In the first, called *generalized transduction*, DNA derived from virtually any portion of the host genome is packaged inside the mature virion in place of the virus genome. In the second, called *specialized transduction*, DNA from a specific region of the host chromosome is integrated directly into the virus genome—usually replacing some of the virus genes. This occurs only with certain temperate viruses (⇄ Section 9.10). The transducing bacteriophage in both generalized and specialized transduction is usually noninfectious because bacterial genes have replaced all or some necessary viral genes.

In generalized transduction, the donor genes cannot replicate independently and are not part of a viral genome. Unless the donor genes recombine with the recipient bacterial chromosome, they will be lost. In specialized transduction, homologous recombination may also occur. However, since the donor bacterial DNA is actually a part of a temperate phage genome, it may be integrated into the host chromosome during lysogeny (⇄ Section 9.10).

Transduction occurs in a variety of *Bacteria*, including the genera *Desulfovibrio*, *Escherichia*, *Pseudomonas*, *Rhodococcus*, *Rhodobacter*, *Salmonella*, *Staphylococcus*, and *Xanthobacter*, as well as *Methanothermobacter thermautotrophicus*, a species of *Archaea*. Not all phages can transduce, and not all bacteria are transducible, but the phenomenon is sufficiently widespread that it likely plays an important role in gene transfer in nature.

Generalized Transduction

In generalized transduction, virtually any gene on the donor chromosome can be transferred to the recipient. Generalized transduction was first discovered and extensively studied in the bacterium *Salmonella enterica* with phage P22 and has also been studied with phage P1 in *Escherichia coli*. An example of how