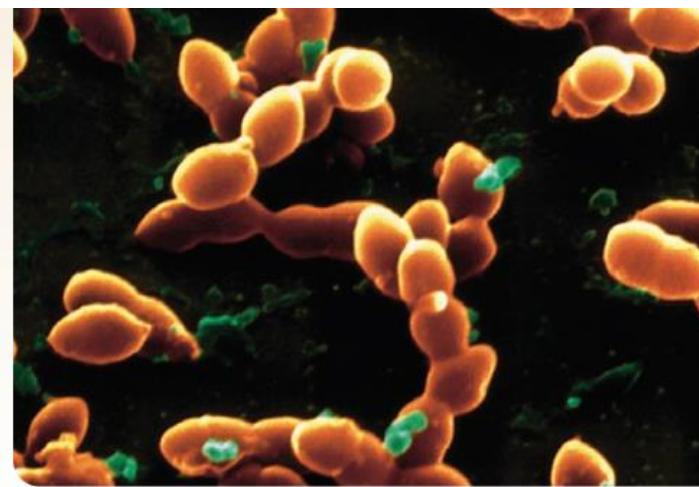


13

Microbial Genetics: Mechanisms of Genetic Variation



The scanning electron micrograph shows *Streptococcus pneumoniae*, the bacterium first used to study transformation and obtain evidence that DNA is the genetic material of organisms.

PREVIEW

- Mutations are stable, heritable alterations in DNA sequence. In prokaryotes, they usually produce phenotypic changes and can occur spontaneously or are induced by chemical mutagens or radiation.
- Microorganisms have several repair mechanisms designed to detect alterations in their genetic material and restore it to its original state. Despite these repair systems, some alterations remain uncorrected and provide material and opportunity for evolutionary change.
- Recombination is the process in which one or more nucleic acid molecules are rearranged or combined to produce new combinations of genes or a new nucleotide sequence.
- Gene transfer is a one-way process in prokaryotes: a piece of genetic material (the exogenote) is donated to the chromosome of a recipient cell (the endogenote) and integrated into it.
- The transfer of genetic material between prokaryotes is called horizontal gene transfer. It takes place in one of three ways: conjugation, transformation, or transduction.
- Transposable elements and plasmids can move genetic material between chromosomes and within chromosomes to cause rapid changes in genomes and drastically alter phenotypes.
- Bacterial chromosomes have been mapped with great precision, using Hfr conjugation in combination with transformational and transductional mapping techniques.
- Recombination of virus genomes occurs when two viruses with homologous chromosomes infect a host cell at the same time.

Chapters 11 and 12 introduce the fundamentals of molecular genetics—the way genetic information is organized, stored, replicated, and expressed. As demonstrated in these chapters, considerable information is embedded in the precise order of nucleotides in DNA. For life to exist with stability,

it is essential that the nucleotide sequence of genes is not disturbed to any great extent. However, sequence changes do occur and can result in altered phenotypes. These changes may be detrimental, but those that are not are important in generating new variability in populations and in contributing to the process of evolution.

In this chapter we focus on processes that contribute to genetic variation in populations of microbes. We begin with an overview of the chemical nature of mutations and the effects of mutations at both the molecular and organismal levels. Because mutants have been put to important uses in the laboratory and in industry, the generation and isolation of mutant organisms are considered. The chapter continues with a discussion of DNA repair mechanisms. Although these repair mechanisms evolved to prevent the occurrence of mutations, as will be seen, some cellular attempts to correct DNA damage actually generate mutations. Finally, we examine microbial recombination and gene transfer in *Bacteria*. These processes have practical implications in terms of antibiotic and drug resistance. In addition, recombination and gene transfer mechanisms observed in *Bacteria* and viruses have been useful for mapping microbial genomes, and these techniques are discussed as well.

13.1 MUTATIONS AND THEIR CHEMICAL BASIS

Mutations [Latin *mutare*, to change] were initially characterized as altered phenotypes, but they are now understood at the molecular level. Several types of mutations exist. Some mutations arise from the alteration of single pairs of nucleotides and from the addition or deletion of one or two nucleotide pairs in the coding regions of a

*Deep in the cavern of the infant's breast
The father's nature lurks, and lives anew.*

—Horace, *Odes*

gene. Such small changes in DNA are sometimes called **microlesions**, and the smallest of these are called **point mutations** because they affect only one base pair in a given location. Larger mutations (**macrolesions**) are also recognized, but 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 occur in the absence of any added agent. (2) **Induced mutations**, on the other hand, are the result of exposure to a **mutagen**, which can be either a physical or a chemical agent. Mutations can be 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.

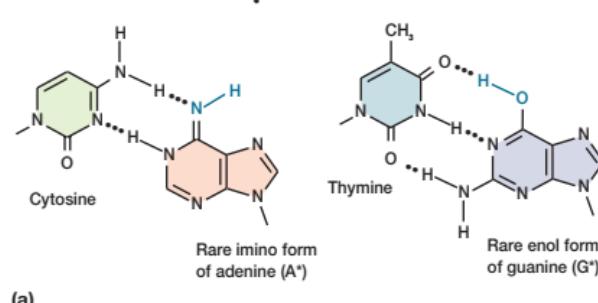
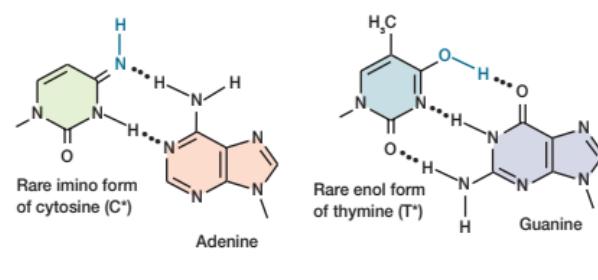
Spontaneous Mutations

Spontaneous mutations arise without exposure to external agents. This class of mutations may result from errors in DNA replication or from the action of mobile genetic elements such as transposons. A few of the more prevalent mechanisms are described here.

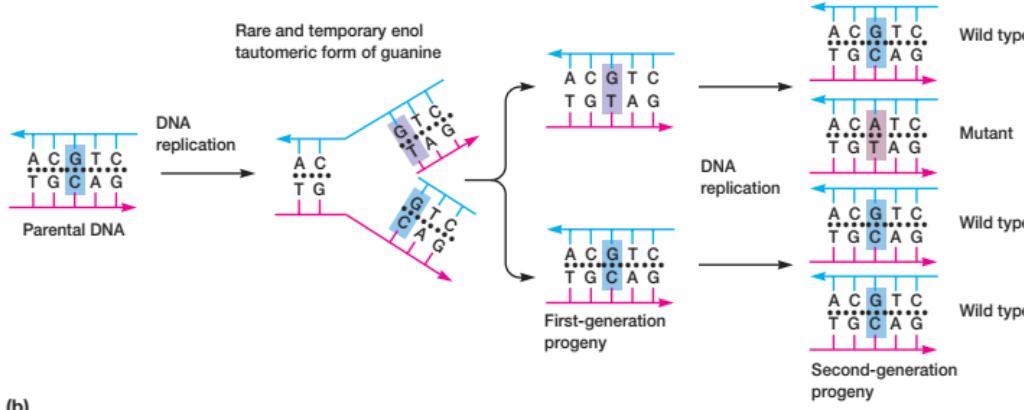
Replication errors can occur when the nitrogenous base of a template nucleotide takes on a rare tautomeric form. Tautomerism is the relationship between two structural isomers that are in chemical equilibrium and readily change into one another. Bases typically exist in the **keto** form. However, they can at times take on either an **imino** or **enol** form (figure 13.1a). These tautomeric shifts change 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 13.1b). Such substitutions are known as

Figure 13.1 Tautomerization and Transition Mutations.

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. (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. The mutation only occurs if the abnormal first-generation GT base pair is missed by repair mechanisms.



(a)



(b)

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 addition and deletion of nucleotides. These mutations generally occur where there is a short stretch of the same nucleotide. In such a location, the pairing of template and new strand can be displaced by the distance of the repeated sequence leading to additions or deletions of bases in the new strand (**figure 13.2**).

Spontaneous mutations can also 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 transition type mutation after the next round of replication. Likewise, pyrimidines can be lost, forming an **apirimidinic site**. Other lesions are caused by reactive forms of oxygen such as oxygen free radicals and peroxy-

ides produced during aerobic metabolism. These may alter DNA bases and cause mutations. For example, guanine can be converted to 8-oxo-7,8-dihydrodeoxyguanine, which often pairs with adenine rather than cytosine during replication.

Finally, spontaneous mutations can result from the insertion of DNA segments into genes. Insertions usually inactivate genes. They are caused by the movement of insertion sequences and transposons. Insertion mutations are very frequent in *Escherichia coli* and many other bacteria. These genetic elements are described in more detail in section 13.5.

Although most geneticists believe that spontaneous mutations occur randomly in the absence of an external agent and are then selected, observations by some microbiologists have led to an alternate and controversial hypothesis. The controversy began when **John Cairns** and his collaborators reported that a mutant *E. coli* strain, unable to use lactose as a carbon and energy source, could regain the ability to do so more rapidly when lactose was added to the culture medium as the only carbon source. Lactose appeared to induce mutations that allow *E. coli* to use the sugar again. One interpretation of these observations is that the mutations are examples of **directed** or **adaptive mutation**—that is, some bacteria seem able to select which mutations occur so that they can better adapt to their surroundings.

Many explanations have been offered to account for this phenomenon without depending on induction of particular mutations. One is the proposal that **hypermutation** can produce such results. Some starving bacteria might rapidly generate multiple mutations through activation of special mutator genes. This would produce many mutant bacterial cells. In such a random process, the rate of production of favorable mutants would increase, with many of these mutants surviving to be counted. There would appear to be directed or adaptive mutation because only mutants with the favorable mutations would survive. There is support for this hypothesis. Mutator genes have been discovered and have been shown to cause hypermutation under nutritional stress. Even if the directed mutation hypothesis is incorrect, it has stimulated much valuable research and led to the discovery of new phenomena.

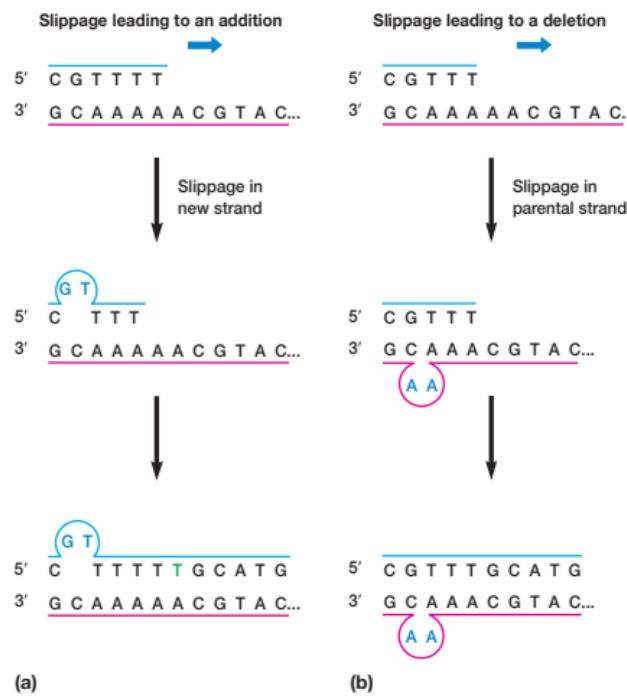


Figure 13.2 Additions and Deletions. A hypothetical mechanism for the generation of additions and deletions during replication. The direction of replication is indicated by the blue arrow. In each case there is strand slippage resulting in the formation of a small loop that is stabilized by the hydrogen 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).

Induced Mutations

Virtually any agent that directly damages DNA, alters its chemistry, or in some way interferes with its functioning will induce mutations. Mutagens can be conveniently classified according to their mode of action. Three common types of chemical mutagens are base analogs, DNA-modifying agents, and intercalating agents. A number of physical agents (e.g., radiation) damage DNA and also are mutagens.

Base analogs are structurally similar to normal nitrogenous bases and can be incorporated into the growing polynucleotide chain during replication (**table 13.1**). 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 analog is 5-bromouracil (5-BU), 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.

Table 13.1 Examples of Mutagens	
Mutagen	Effect(s) on DNA Structure
Chemical	
5-Bromouracil	Base analog
2-Aminopurine	Base analog
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	Causes base deletions, single-strand nicks, cross-linking, and chromosomal breaks

The enol tautomer forms hydrogen bonds like cytosine and directs the incorporation of guanine rather than adenine (**figure 13.3**). The mechanism of action of other base analogs is similar to that of 5-bromouracil.

DNA-modifying agents change a base's structure and therefore alter its base pairing characteristics. Some mutagens in this category are fairly selective; they preferentially react with some bases and produce a specific kind of DNA damage. An example of this type of mutagen is methyl-nitrosoguanidine, an alkylating agent that adds methyl groups to guanine, causing it to mispair with thymine (**figure 13.4**). A subsequent round of replication could then result in a GC-AT transition. Hydroxylamine is another example of a DNA-modifying agent. It hydroxylates the C-4 nitrogen of cytosine, causing it to base pair like thymine. There are many other DNA modifying agents that can cause mispairing.

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, directly damage bases so severely that hydrogen bonding between base pairs is impaired or prevented and the damaged DNA can no longer act as a template for replication. For instance, UV radiation generates cyclobutane type dimers, usually thymine dimers, between adjacent pyrimidines (**figure 13.5**). Other examples are ionizing radiation and carcinogens such as the fungal toxin aflatoxin B1 and other benzo(a)pyrene derivatives.

Retention of proper base pairing is essential in the prevention of mutations. Cells have developed extensive repair mechanisms. Often the damage can be repaired before a mutation is permanently es-

tablished. If a complete DNA replication cycle takes place before the initial lesion is repaired, the mutation frequently becomes stable and inheritable. Repair mechanisms are discussed in section 13.3.

Effects of Mutations

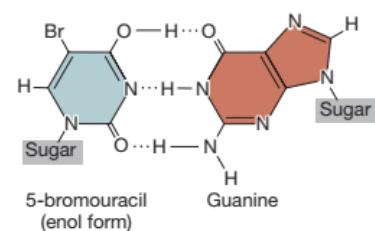
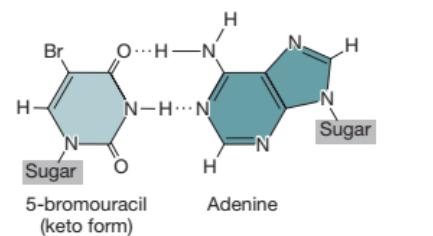
The effects of a mutation can be described at the protein level and in terms of traits or other easily observed phenotypes. In all cases, the impact is readily noticed only if it produces a change in phenotype. In general, the more prevalent form of a gene and its associated phenotype is called the **wild type**. A mutation from wild type to a mutant form is called a **forward mutation** (**table 13.2**). A forward mutation can be reversed by a second mutation that restores the wild-type phenotype. When the second mutation is at the same site as the original mutation, it is called a **reversion mutation**. A true reversion converts the mutant nucleotide sequence back to the wild-type sequence. If the second mutation is 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 will be the focus here.

Mutations in Protein-Coding Genes

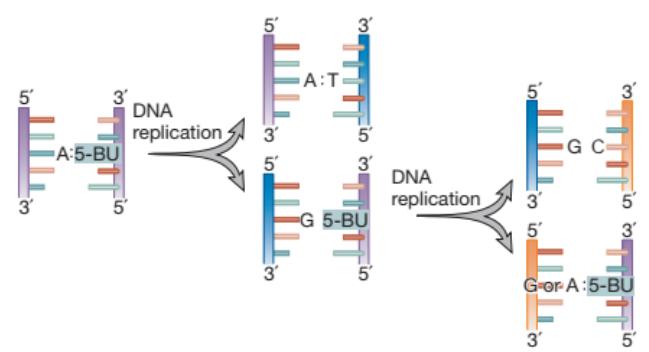
Point mutations in protein-coding genes can affect protein structure in a variety of ways (**table 13.2**). Point mutations are named according to if and how they change the encoded protein. The most common types of point mutations are silent mutations, missense mutations, nonsense mutations, and frameshift mutations. These are described in more detail below.

Silent mutations change the nucleotide sequence of a codon, but do not change the amino acid encoded by that codon. This is possible because of the degeneracy of the genetic code. 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. The mutation can only be detected at the level of the DNA or mRNA. When there is no change in the protein, there is no change in the phenotype of the organism. [The genetic code \(section 11.7\)](#)

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 protein structure, 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



(a) Base pairing of 5-BU with adenine or guanine



(b) How 5-BU causes a mutation in a base pair during DNA replication

Figure 13.3 Mutagenesis by the Base Analog 5-Bromouracil. (a) Base pairing of the normal keto form of 5-BU is shown in the top illustration. The enol form of 5-BU (bottom illustration) base pairs with guanine rather than with adenine as might be expected for a thymine analog. (b) If the keto form of 5-BU is incorporated in place of thymine, its occasional tautomerization to the enol form (BU_e) will produce an AT to GC transition mutation.

acid with another at the protein surface may have little or no effect. Missense mutations actually 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 \(appendix I\)](#)

Nonsense mutations cause the early termination of translation and therefore result in a shortened polypeptide. They are called nonsense mutations because they convert a sense codon to a nonsense or stop codon. Depending on the relative location of the mutation, the phenotype may be more or less severely af-

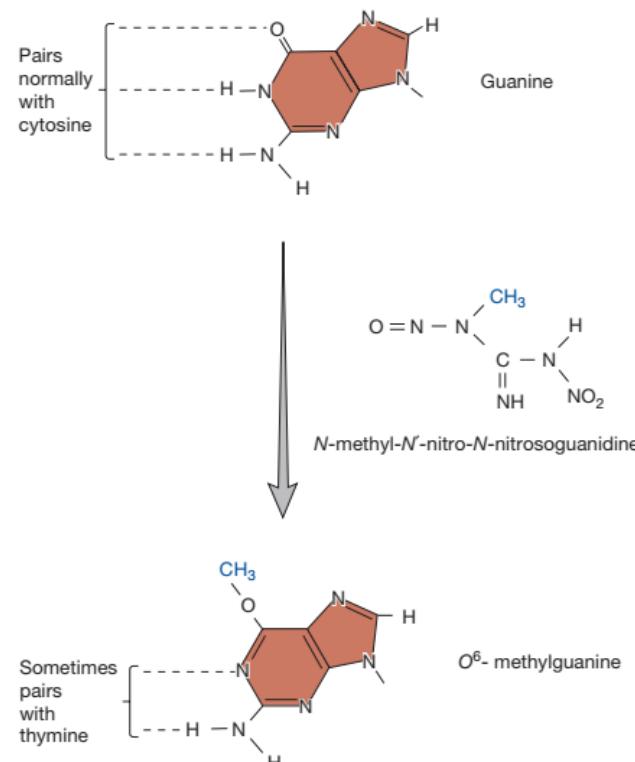


Figure 13.4 Methyl-Nitrosoguanidine Mutagenesis. Mutagenesis by methyl-nitrosoguanidine due to the methylation of guanine.

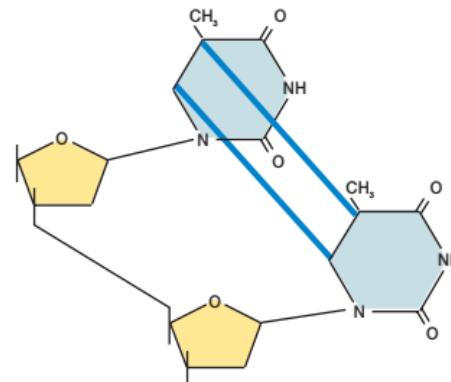


Figure 13.5 Thymine Dimer. Thymine dimers are formed by ultraviolet radiation.

fected. Most proteins retain some function if they are shortened by only one or two amino acids; complete loss of normal function will almost certainly result if the mutation occurs closer to the beginning or middle of the gene.

Table 13.2 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-G-A-A-A-G-G-3'
Silent	Base substitution	Met - Thr - Ser - Pro - Lys - Gly
Missense	Base substitution	5'-A-T-G-A-C-C- A -T-C-C-G-A-A-A-G-G-3'
Nonsense	Base substitution	Met - Thr - Ser - Pro - Lys - Gly
Frameshift	Addition/deletion	5'-A-T-G-A-C-C-T-C-C-G-C-G- T A-A-G-G-3' Met - Thr - Ser - Pro - STOP!
		5'-A-T-G-A-C-C-T-C-C-G-C-G-A-A-A-G-G-3' Met - Thr - Ser - Ala - Glu - Arg
Reverse Mutations		
True reversion	Base substitution	5'-A-T-G-A-C-C-T-C-C- forward → A-T-G- C -C-T-C-C- reverse → A-T-G-A-C-C-T-C-C Met - Thr - Ser - Pro - Ser - Met - Thr - Ser
	Base substitution	5'-A-T-G-A-C-C-T-C-C- forward → A-T-G-A-C-C-T- G -C- reverse → A-T-G-A-C-C-A-G-C Met - Thr - Ser - Cys - Met - Thr - Ser
Equivalent Reversion	Base substitution	5'-A-T-G-A-C-C-T-C-C- forward → A-T-G- C -C-T-C-C- reverse → A-T-G- C -T-C-T-C-C Met - Thr - Ser - Pro - Ser - Met - Leu - Ser (polar amino acid) (nonpolar amino acid) pseudo-wild type
Suppressor Mutations		
Frameshift of opposite sign (intragenic suppressor)	Addition/deletion	5'-A-T-G-A-C-C-T-C-C-G-C-G-A-A-A-G-G-3' Met - Thr - Ser - Ala - Glu - Arg
		Forward mutation Suppressor mutation (deletion) mutation (deletion)
Extragenic suppressor		5'-A-T-G-A-C-C-T-C-C-G-C-G-A-A-A-G-G-3' Met - Thr - Pro - Pro - Lys - Gly
Nonsense suppressor		Gene (e.g., for tyrosine tRNA) undergoes mutational event in its anticodon region that enables it to recognize and align with a mutant nonsense codon (e.g., UAG) to insert an amino acid (tyrosine) and permit completion of translation.
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.

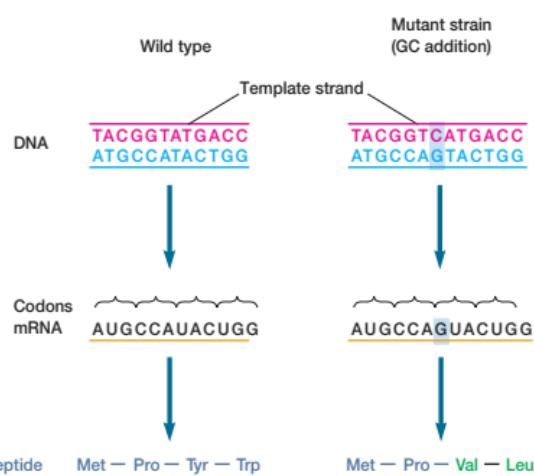


Figure 13.6 Frameshift Mutation. A frameshift mutation resulting from the insertion of a GC base pair. The reading frameshift produces a different peptide after the addition.

Frameshift mutations arise from the insertion or deletion of one or two 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 will cause the reading frame to be shifted for all codons downstream. **Figure 13.6** shows the effect of a frameshift mutation on a short section of mRNA and the amino acid sequence it codes for.

Frameshift mutations usually are very deleterious and yield mutant phenotypes resulting from the synthesis of nonfunctional proteins. In addition, frameshift mutations often produce a nonsense or stop codon so that the peptide product is shorter as well as different in sequence. Of course if the frameshift occurred near the end of the gene, or if there were a second frameshift shortly downstream from the first that restored the reading frame, the phenotypic effect might not be as drastic. A second nearby frameshift that restores the proper reading frame is a good example of an intragenic suppressor mutation (table 13.2).

As noted previously, changes in protein structure can lead to changes in protein function, which in turn can alter the phenotype of an organism. The phenotype of a microorganism can be affected in several different 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 in order to be isolated and studied, lethal mutations are recovered only if they are recessive in diploid organisms or are conditional mutations in haploid organisms. **Conditional mutations** are those that are expressed only under certain environmental conditions. For example, a conditional lethal mutation in *E. 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 hypothetical mutant would grow normally at the permissive temperature 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 macromolecule 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 selection 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 \(section 12.2\)](#)

Mutations in tRNA and rRNA Genes

Mutations in rRNA and tRNA 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 will restore normal (or near normal) growth rates. Here a base substitution in the anticodon region of a tRNA allows the insertion of the correct amino acid at a mutant codon (table 13.2).

1. Define or describe the following: mutation, conditional mutation, auxotroph and prototroph, spontaneous and induced mutations, mutagen, transition and transversion mutations, apurinic and apyrimidinic sites, base analog, DNA-modifying agent, intercalating agent, thymine dimer, wild type, forward and reverse mutations, suppressor mutation, point mutation, silent mutation, missense and nonsense mutations, directed or adaptive mutation, and frameshift mutation.
2. List four ways in which spontaneous mutations might arise.
3. How do the mutagens 5-bromouracil, methyl-nitrosoguanidine, proflavin, and UV radiation induce mutations?
4. Give examples of intragenic and extragenic suppressor mutations.

5. Sometimes a point mutation does not change the phenotype. List all the reasons why this is so.
6. Why might a missense mutation at a protein's surface not affect the phenotype of an organism, while the substitution of an internal amino acid does?

13.2 DETECTION AND ISOLATION OF MUTANTS

In order to study microbial mutants, one must be able to detect them readily, even when they are rare, and then efficiently isolate them 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 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, mutations are rare and 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, one must know the normal or wild-type characteristics so as to recognize an altered phenotype. A suitable detection system for the mutant phenotype also is needed. Detection systems in prokaryotes and other haploid organisms are straightforward because any mutation should be seen immediately, even if it is a recessive mutation. Sometimes detection of mutants is direct. For instance, if albino mutants of a normally pigmented bacterium are being studied, detection simply requires visual observation of colony color. Other direct detection systems are more complex. For example, the **replica plating** technique is used to detect 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 13.7**). A lysine auxotroph, for instance, will grow on lysine-supplemented media but not on a medium lacking an adequate supply of lysine because it cannot synthesize this amino acid.

Once a detection method is established, 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 the 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 given it by the mutation, whereas the wild type does not. Selection methods often involve reversion mutations or the development of resistance to an

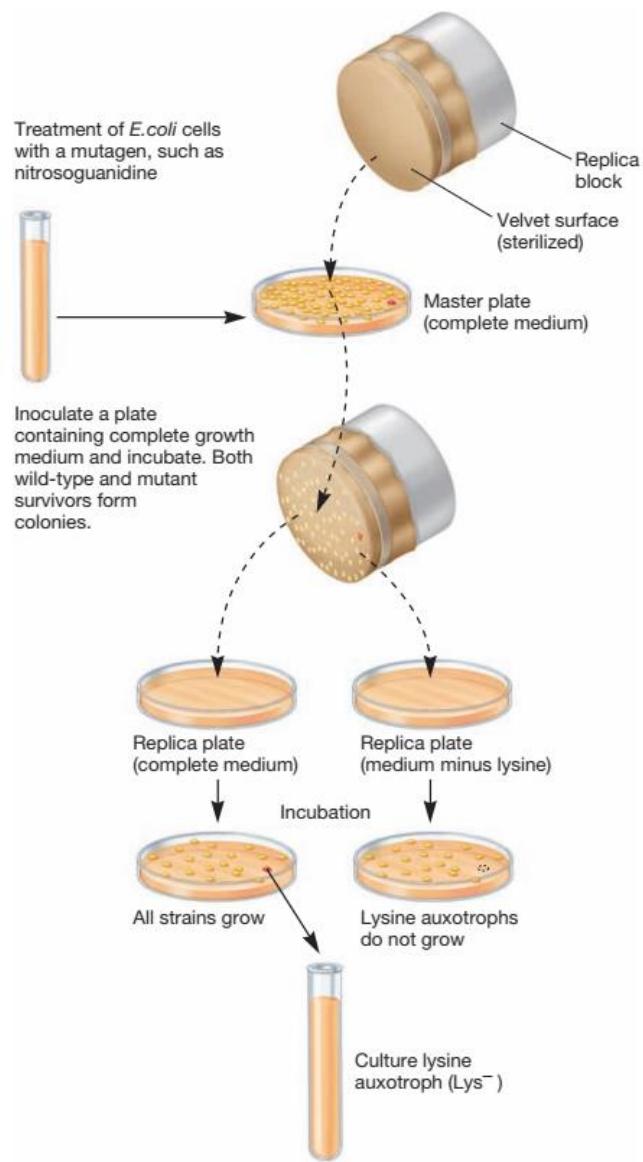


Figure 13.7 Replica Plating. The use of replica plating in isolating a lysine auxotroph. Mutants are generated by treating a culture with a mutagen. The culture containing wild type and auxotrophs is plated on complete medium. After the colonies have developed, a piece of sterile velveteen is pressed 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 determining the location of Lys⁻ colonies growing on the replica with complete medium, the auxotrophs can be isolated and cultured.

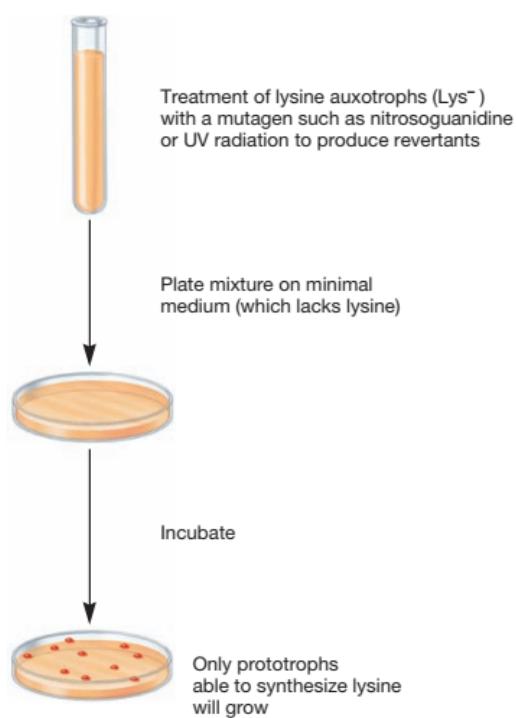


Figure 13.8 **Mutant Selection.** The production and direct selection of auxotroph revertants. In this example, lysine revertants are selected after treatment of a lysine auxotroph culture because the agar contains minimal medium that does not support auxotroph growth.

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 (figure 13.8). 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. This method has proven very useful in determining the relative mutagenicity of many substances.

Methods for selecting mutants resistant to a particular environmental stress follow a similar approach. Often wild-type cells are susceptible to virus attack or antibiotic treatment, so it is possible to grow the bacterium in the presence of the agent and look for surviving organisms. Consider the example of a phage-sensitive wild-type bacterium. When the organism 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. This type of selection can be used for virtually any environmental parameter; resistance to bacteriophages (bacterial viruses), antibiotics, or temperature are most commonly employed.

Substrate utilization mutations also are employed in bacterial selection. 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 detection 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 detection techniques is in the study of carcinogens. The next section briefly describes one of the first and perhaps best known of the carcinogen testing systems.

Carcinogenicity Testing

An increased understanding of the mechanisms of mutation and cancer induction has stimulated efforts to identify environmental carcinogens. The observation that many carcinogenic agents also are mutagenic is the basis for detecting potential carcinogens by testing for mutagenicity while taking advantage of bacterial selection techniques and short generation times. The **Ames test**, developed by **Bruce Ames** in the 1970s, has been widely used to test for carcinogens. The Ames test is a mutational reversion assay employing several special strains of *Salmonella enterica* serovar Typhimurium, each of which has a different mutation in the histidine biosynthesis operon; that is to say, they are histidine auxotrophs. 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 the ability to repair DNA correctly.

In the Ames test these special tester strains of *Salmonella* are plated with the substance being tested and the appearance of visible colonies followed (figure 13.9). 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, as previously discussed, replication is required for the development of a mutation (figure 13.3). 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 in order to estimate the relative mutagenicity of the compound: the more colonies, the greater the mutagenicity.

A mammalian liver extract is also often added to the molten top agar prior to plating. The extract converts potential carcinogens into electrophilic derivatives that readily react with DNA. This process occurs naturally when foreign substances are metabolized in the liver. Because bacteria do not have this activation system, addition of the liver extract promotes the same kind of enzymatic transformations that occur in mammals. Many potential carcinogens, such as aflatoxins, are not actually carcinogenic until they are modified in the liver. The addition of extract shows which

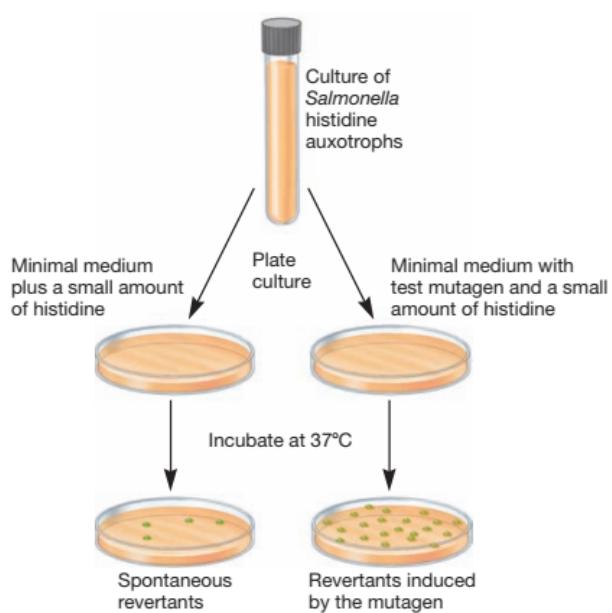


Figure 13.9 The Ames Test for Mutagenicity. See text for details.

compounds have intrinsic mutagenicity and which need activation after uptake. Despite the use of liver extracts, only about half the potential animal carcinogens are detected by the Ames test.

1. Describe how replica plating is used to detect and isolate auxotrophic mutants.
2. Why are mutant selection techniques generally preferable to the direct detection and isolation of mutants?
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.
5. What is the Ames test and how is it carried out? What assumption concerning mutagenicity and carcinogenicity is it based upon?

13.3 DNA REPAIR

Because replication errors and a variety of mutagens can alter the nucleotide sequence, a microorganism must be able to repair changes in the sequence that might be lethal. DNA is repaired by several different mechanisms besides **proofreading** by replication enzymes (DNA polymerases can remove an incorrect nucleotide immediately after its addition to the growing end of the chain). Repair in *E. coli* is best understood and is briefly described in this section. [DNA replication \(section 11.4\)](#)

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 are distinguished by the enzymes used to correct DNA damage. However, 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.

In **nucleotide excision repair**, a repair enzyme called UvrABC endonuclease removes damaged bases and some bases on either side of the lesion. The resulting single-stranded gap, about 12 nucleotides long, is filled by DNA polymerase I, and DNA ligase joins the fragments. [Figure 13.10](#) presents the process in detail. This system can remove thymine dimers (figure 13.5) and repair almost any other injury that produces a detectable distortion in DNA.

Base excision repair employs DNA glycosylases to remove damaged or unnatural bases yielding apurinic or apyrimidinic (AP) sites. Special endonucleases called AP endonucleases recognize the damaged DNA and nick the backbone at the APsite ([figure 13.11](#)). 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**. **Photoreactivation** is the repair of thymine dimers by splitting them apart into separate thymines with the help of visible light in a photochemical reaction catalyzed by the enzyme photolyase ([figure 13.12a](#)). Methyls and some other alkyl groups that have been added to the O⁶ position of guanine can be removed with the help of an enzyme known as alkyltransferase or methylguanine methyltransferase ([figure 13.12b](#)). Thus damage to guanine from mutagens such as methyl-nitrosoguanidine ([figure 13.4](#)) can be repaired directly.

Mismatch Repair

Despite the accuracy of DNA polymerase and continual proofreading, errors still are made during DNA replication. Remaining mismatched bases are usually detected and repaired by the **mismatch repair system** in *E. coli* ([figure 13.13](#)). The mismatch correction 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 with a ligase. In this regard, mismatch repair is similar to excision repair.

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

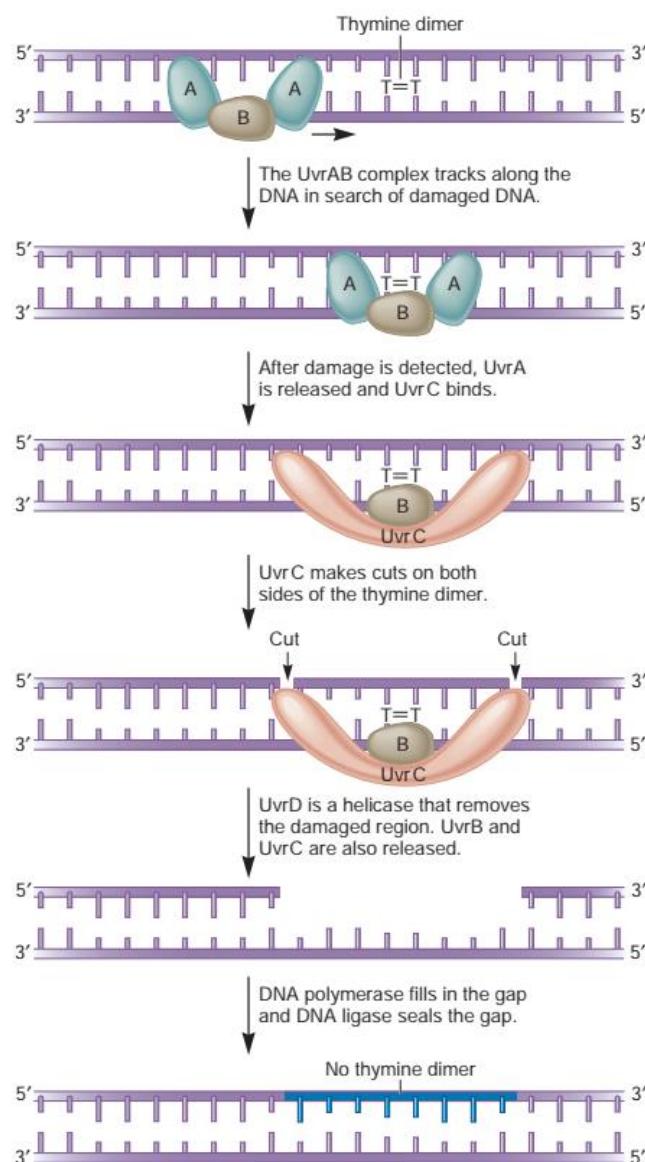


Figure 13.10 Nucleotide Excision Repair in *E. coli*.

(DAM) methylates adenine bases in GATC sequences to form *N*6-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 **hemi-methylated**. The repair system cuts out the mismatch from the unmethylated strand.

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 the **RecA protein** cuts a

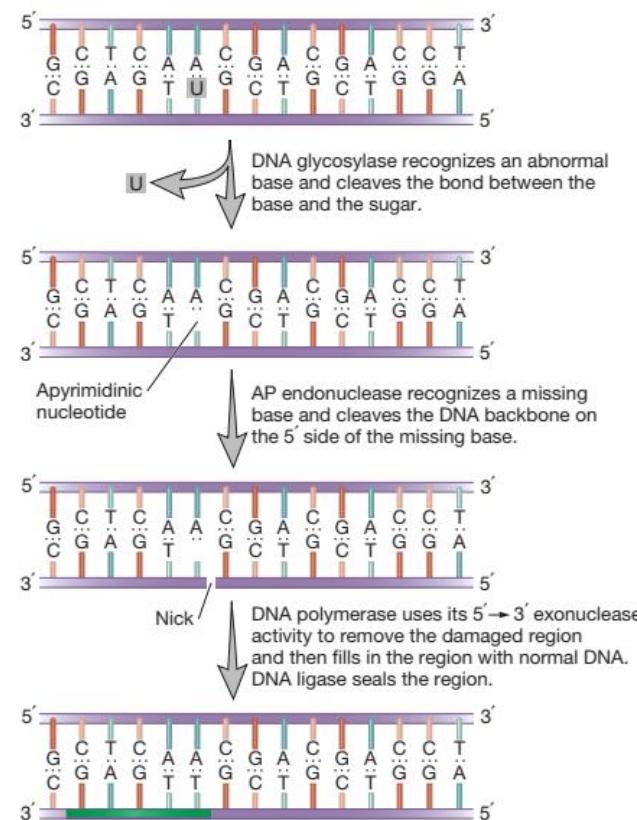
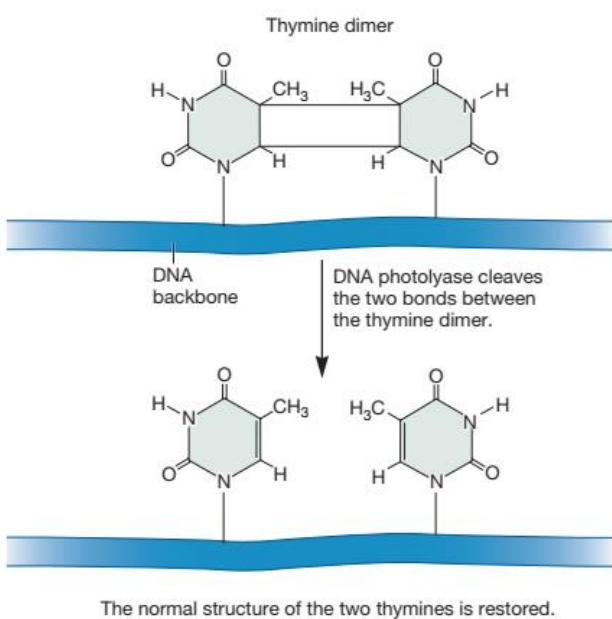


Figure 13.11 Base Excision Repair.

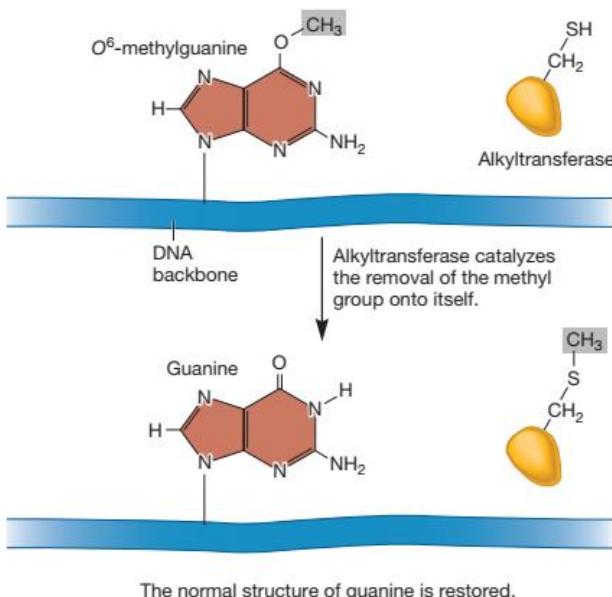
piece of template DNA from a sister molecule and puts it into the gap or uses it to replace a damaged strand (figure 13.14). Although prokaryotes are haploid, another copy of the damaged segment often is available because either it has recently been replicated or the cell is growing rapidly and has more than one copy of its chromosome. Once the template is in place, the remaining damage can be corrected by another repair system.

The 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. The SOS response, like recombinational repair, is dependent on the activity of the RecA protein. 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 a proteolytic function that destroys a repressor protein called LexA. LexA negatively regulates the function of many genes involved in DNA repair and synthesis. Destruction of LexA increases transcription of genes for excision repair and



(a) Direct repair of a thymine dimer



(b) Direct repair of a methylated base

Figure 13.12 Direct Repair. (a) The repair of thymine dimers by photolyase. (b) The repair of methylguanine by the transfer of the methyl group to alkyltransferase.

The MutS protein finds a mismatch. The MutS/MutL complex binds to MutH, which is already bound to a hemimethylated sequence.

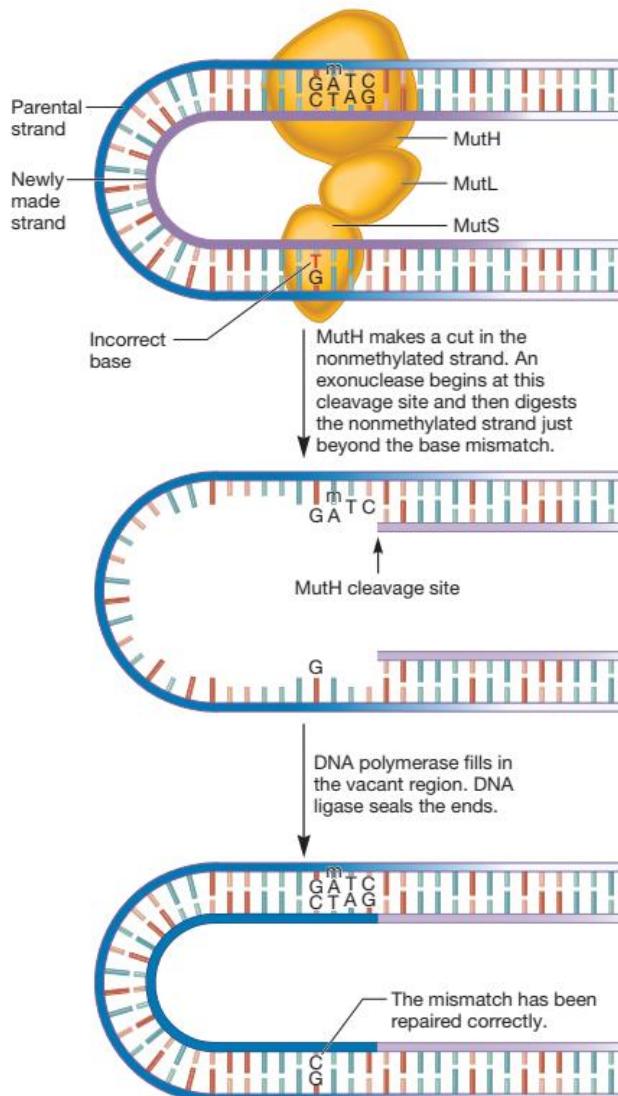


Figure 13.13 Methyl-Directed Mismatch Repair in *E. coli*. MutS slides along the DNA and recognizes base mismatches in the double helix. MutL binds to MutS and acts as a linker between MutS and MutH. The DNA must loop for this interaction to occur. The role of MutH is to identify the methylated strand of DNA, which is the nonmutated parental strand. The methylated adenine is designated with an m.

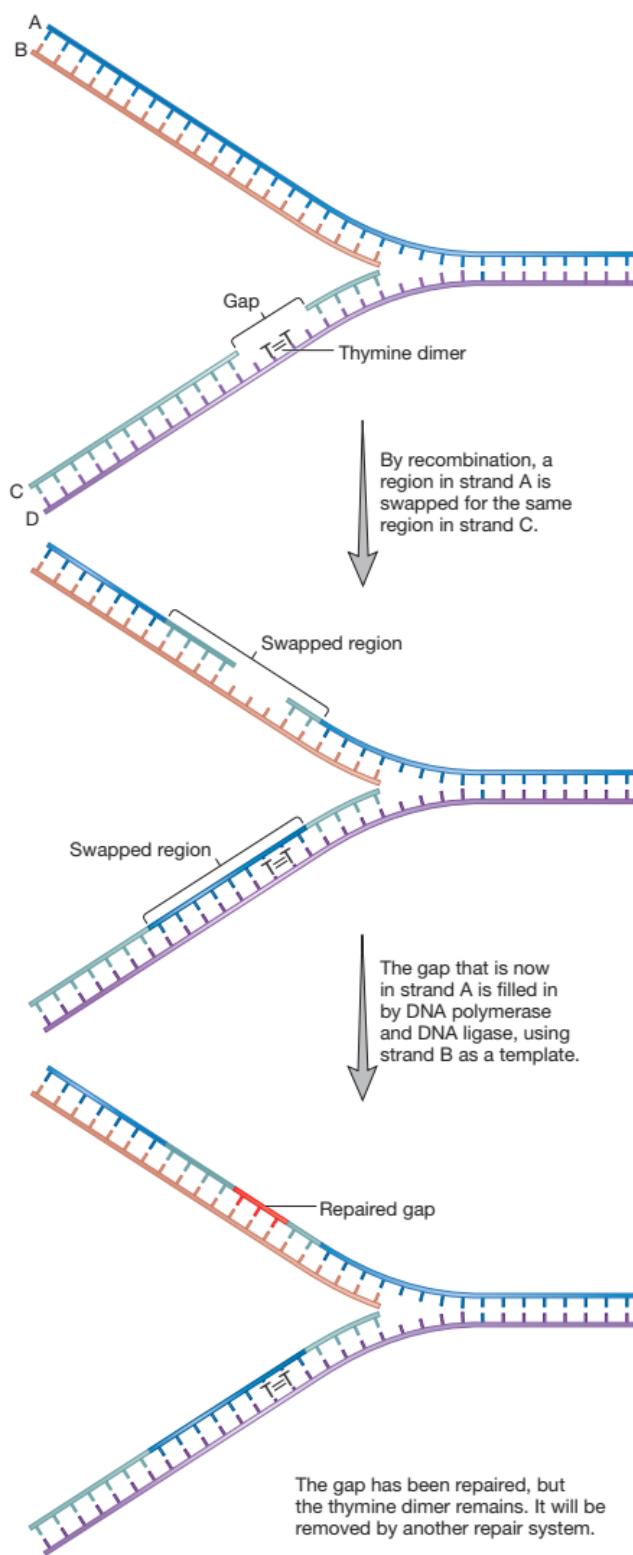


Figure 13.14 Recombinational Repair.

recombinational repair, in particular. The first genes to be transcribed are those that encode the Uvr proteins needed for nucleotide excision repair (figure 13.10). Then genes involved in recombinational repair are further upregulated. 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 about 40 minutes, a process called **translesion DNA synthesis** is triggered. In this process, DNA polymerases IV (also known as DinB) and V (UmuCD) 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 SOS response 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 a response 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.

1. Define the following: proofreading, excision repair, photoreactivation, methylguanine methyltransferase, mismatch repair, direct repair, DNA methylation, recombinational repair, RecA protein, SOS response, and LexA repressor.
2. Describe in general terms the mechanisms of the following repair processes: excision repair, recombinational repair, direct repair, and SOS response.
3. 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.

13.4 CREATING GENETIC VARIABILITY

As discussed previously, the consequences of mutations can range from no effect to being lethal, depending not only on the nature of the mutation but also on the environment in which the organism lives. Thus all mutations are subject to selective pressure, and this determines if a mutation will survive in a population. Each mutant form that survives is called an **allele**, an alternate form of the gene. Mutant alleles, as well as the wild-type allele, can be combined with other genes, leading to an increase in the genetic variability within a population. Each genotype in a population can be selected for or selected against. Organisms with genotypes, and therefore phenotypes, that are best suited to the environment survive and are able to pass on their genes. Shifts in environmental pressures can lead to changes in the population and ultimately result in the evolution of new species. The mechanisms by which new combinations of genes are generated are the topic of this section. All involve **recombination**, the process in which one or more nucleic acid molecules are rearranged or combined to produce a new nucleotide sequence. This is normally accompanied by a phenotypic change. Geneticists refer to organisms produced following a recombination event as recombinant organisms or simply **recombinants**.

Recombination in Eucaryotes

The processes that create genetic variability in eucaryotes differ from those in prokaryotes. Recombinant genotypes can arise from the integration of viruses into the host chromosomes and movement of mobile genetic elements. However, the most important recombination events occur during the sexual cycle, including meiosis, of those eucaryotes capable of sexual reproduction. During meiosis, **crossing-over** between homologous chromosomes—chromosomes containing identical sequences of genes (figure 13.15)—generates new combinations of alleles. This is followed by segregation of chromosomes into gametes and then by zygote formation, which further increases genetic variability. This transfer of genes from parents to progeny is sometimes called **vertical gene transfer**.

Horizontal Gene Transfer in Prokaryotes

Unlike eucaryotes, prokaryotes do not reproduce sexually, nor do they undergo meiosis. This would suggest that genetic variation in populations of prokaryotes would be relatively limited, only occurring with the advent of a new mutation or by the integration of viruses and mobile genetic elements into the chromosome. However, this is not the case. Prokaryotes have evolved three different mechanisms for creating recombinants. These mechanisms are referred to collectively as **horizontal (or lateral) gene transfer (HGT)**. HGT is distinctive from vertical gene transfer because genes from one independent, mature organism are transferred to another, often creating a stable recombinant having characteristics of both the donor and the recipient.

It was once thought that HGT occurred primarily between members of the same species. However, it is increasingly clear that HGT has been important in the evolution of many species, and that it is still commonplace in many environments. Furthermore, there are clear examples of DNA from one species being transferred to distantly related species. The importance of HGT cannot be overstated. Its recognition as an evolutionary force has caused evolutionary biologists to reconsider the universal tree of life first proposed by **Carl Woese** in the 1970s. It is felt by some that phylogenetic relationships are better represented by a web or network of relationships rather than a tree (see figure 19.15).

HGT is still shaping genomes. For instance, it has been demonstrated that prokaryotes sharing an ecological niche can exchange genes and this alters the nature of the microbial community in a habitat. Another important example is the evolution and spread of antibiotic-resistance genes among pathogenic bacteria. [Microbial evolution \(section 19.1\)](#)

During HGT, a piece of donor DNA, the **exogenote**, must enter and become a stable part of the recipient cell. This can be accomplished in two ways, depending on the nature of the exogenote. If the exogenote is a DNA fragment that is incapable of replicating itself and is susceptible to degradation by nucleases present in the recipient (e.g., a small, linear piece of the donor's chromosome), then the exogenote must integrate into the recipient cell's chromosome (**endogenote**), replacing a portion of the recipient cell's genetic material. As this occurs, the recipient becomes temporarily diploid for a portion of its genome and is called a **merozygote** (figure 13.16). However, if the exogenote is capable of self-replication and is resistant to attack by the recipient cell's nucleases (e.g., a plasmid), then it need not integrate into the recipient cell's chromosome. Instead, it is maintained independent of the endogenote.

Horizontal gene transfer can take place in three ways: direct transfer between two bacteria temporarily in physical contact (conjugation), transfer of a naked DNA fragment (transformation), and transport of bacterial DNA by bacterial viruses (transduction). Whatever the mode of transfer, the exogenote has only four possible fates in the recipient (figure 13.16). First, when the exogenote has a sequence homologous to that of the endogenote, integration may occur; that is, it may pair with the recipient DNA and be incorporated to yield a recombinant genome. Second, the foreign DNA sometimes persists outside the endogenote and replicates to produce a clone of partially diploid cells. Third, the exogenote may survive, but not replicate, so that only one cell is a partial diploid. Finally, host cell nucleases may degrade the exogenote, a process called **host restriction**.

Recombination at the Molecular Level

Although different processes are used in eucaryotes and prokaryotes to create recombinant organisms, the mechanisms of recombination at the molecular level are remarkably similar.

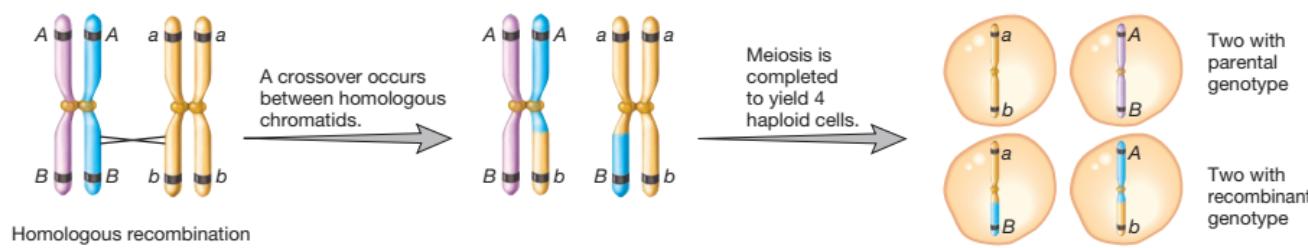


Figure 13.15 Recombination During Meiosis. During meiosis, homologous chromosomes pair and crossing-over can occur. The recombinant genotypes formed are inherited by progeny organisms, where they can result in recombinant phenotypes. Crossing-over involving similar DNA sequences is called homologous recombination.

Three types of recombination are observed: homologous recombination, site-specific recombination, and transposition. **Homologous recombination**, the most common form of recombination, usually involves a reciprocal exchange between a pair of DNA molecules with the same nucleotide sequence. It can occur anywhere on the chromosome, and it results from DNA strand breakage and reunion leading to crossing-over. Homologous recombination is carried out by the products of the *rec* genes, including the RecA protein, which is also important for DNA repair (table 13.3). The most widely accepted model of homologous recombination is the **double-strand break model** (figure 13.17). It proposes that duplex DNA with a double-stranded break is processed to create DNA with single-stranded ends. RecA promotes the insertion of one single-stranded end into an intact, homologous piece of DNA. This is called **strand invasion**. As can be seen in figure 13.17, strand invasion results in the formation of two gaps in the two parent DNA molecules. The gaps are filled, yielding a structure with **heteroduplex DNA**; that is, it contains strands derived from both parent mol-

ecules. The two parental DNA molecules are now linked together by two structures called **Holliday junctions**. These structures move along the DNA molecule during **branch migration** until they are finally cut and the two DNA molecules are separated. Depending on how this occurs, the resulting DNA molecules will be either recombinant or nonrecombinant. In some cases, a nonreciprocal form of homologous recombination occurs (figure 13.18). In **nonreciprocal homologous recombination**, a piece of genetic material is inserted into the chromosome through the incorporation of a single strand to form a stretch of heteroduplex DNA. The second type of recombination, **site-specific recombination**, is particularly important in the integration of virus genomes into host chromosomes. In site-specific recombination, the genetic material bears only a small region of homology with the chromosome it joins. The enzymes responsible for this event are often specific for sequences within the particular virus and its host. The third kind of recombination is **transposition**, which also does not depend on sequence homology. It can occur at many sites in the genome and will be discussed in more detail in section 13.5.

Until about 1945, the primary focus in genetic analysis was on the recombination of genes in plants and animals. The early work on recombination in higher eucaryotes led to the foundation of classical genetics, but it was the development of bacterial and phage genetics between about 1945 and 1965 that really stimulated a rapid advance in our understanding of molecular genetics. Therefore recombination in the *Bacteria* and viruses is the major focus of the following discussion of recombination. We begin with a consideration of transposons and plasmids—genetic elements that can be involved in recombination events—and then turn to mechanisms of horizontal gene transfer in *Bacteria*.

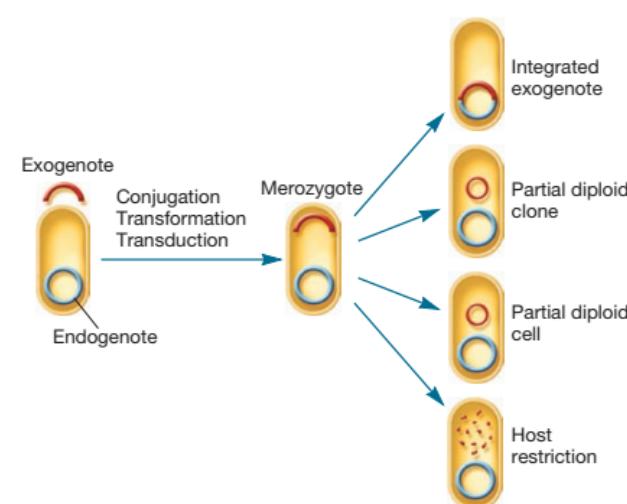


Figure 13.16 The Production and Fate of Merozygotes.

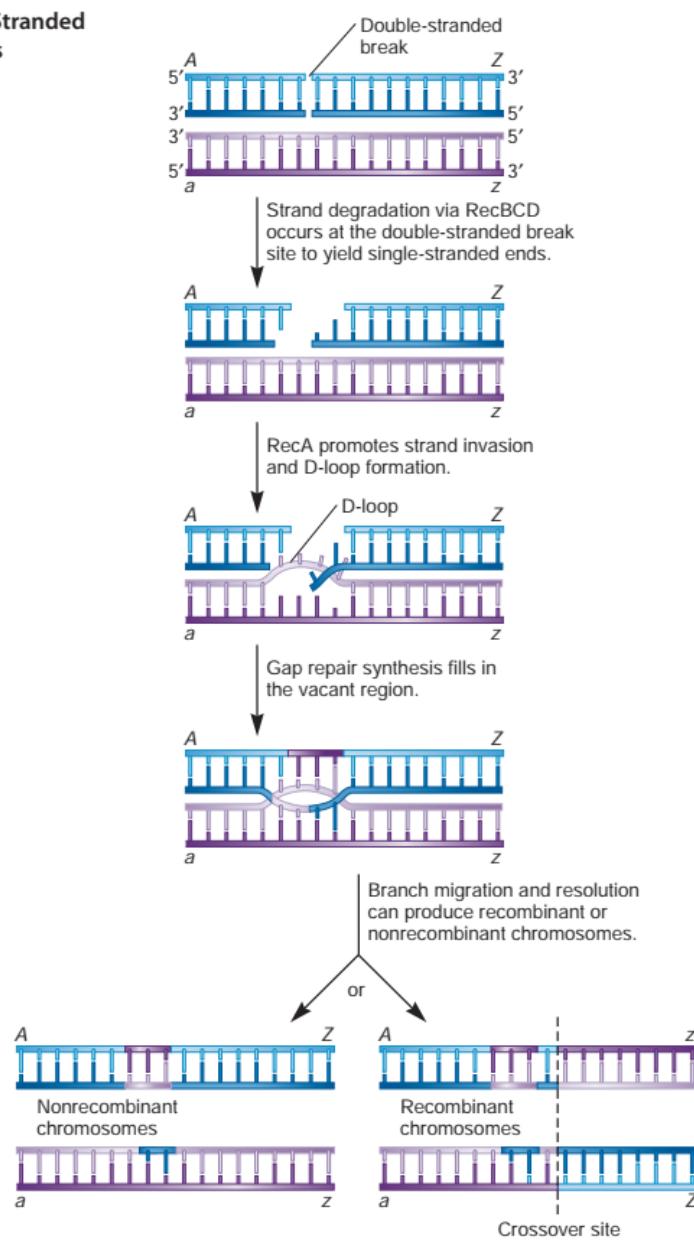
See text for discussion.

- Define the following terms: recombination, crossing-over, homologous recombination, site-specific recombination, transposition, exogenote, endogenote, horizontal (lateral) gene transfer, merozygote, and host restriction.
- Distinguish among the three forms of recombination mentioned in this section.
- What four fates can DNA have after entering a bacterium?

Table 13.3 *E. coli* Homologous Recombination Proteins

Protein	Description
Rec BCD	Recognizes double-stranded breaks and then generates single-stranded regions at the break site that are involved in strand invasion
Single-strand binding protein	Prevents excessive strand degradation by RecBCD
RecA	Promotes strand invasion and displacement of complementary strand to generate D loop
RecG	Helps form Holliday junctions and promotes branch migration
RuvABC	Endonuclease that binds Holliday junctions, promotes branch migration, and cuts strands in the Holliday junction in order to separate chromosomes

Figure 13.17 The Double-Stranded Break Model of Homologous Recombination.



13.5 TRANSPOSABLE ELEMENTS

The chromosomes of prokaryotes, viruses, and eukaryotic cells contain pieces of DNA that can move and integrate into different sites in the chromosomes. Such movement is called transposition, and it plays important roles in the generation of new gene combinations. DNA segments that carry the genes required for transposition are **transposable elements** or **transposons**, sometimes called “jumping genes.” Unlike other processes that reorganize DNA, transposition does not require extensive areas of homology

between the transposon and its destination site. Transposons were first discovered in the 1940s by Barbara McClintock during her studies on maize genetics (a discovery for which she was awarded the Nobel prize in 1983). They have been most intensely studied in *Bacteria*.

The simplest transposable elements are **insertion sequences** or IS elements (figure 13.19a). An IS element is a short sequence of DNA (around 750 to 1,600 base pairs [bp] in length) containing only the genes for those enzymes required for its transposition and bounded at both ends by identical or very similar sequences

of nucleotides in reversed orientation known as **inverted repeats**. Inverted repeats are usually about 15 to 25 base pairs long and vary among IS elements so that each type of IS has its own characteristic inverted repeats. Between the inverted repeats is a gene that codes for an enzyme called **transposase**. This enzyme is required for transposition and accurately recognizes the ends of the IS. Each type of element is named by giving it the prefix IS followed by a number. In *E. coli* several copies of different IS elements have been observed; some of their properties are given in **table 13.4**.

Transposable elements also can contain genes other than those required for transposition (for example, antibiotic resistance or toxin genes). These elements often are called **composite transposons**. Composite transposons often consist of a central region containing the extra genes, flanked on both sides by IS elements that are identical or very similar in sequence (figure 13.19b). Many composite transposons are simpler in organization. They are bounded by

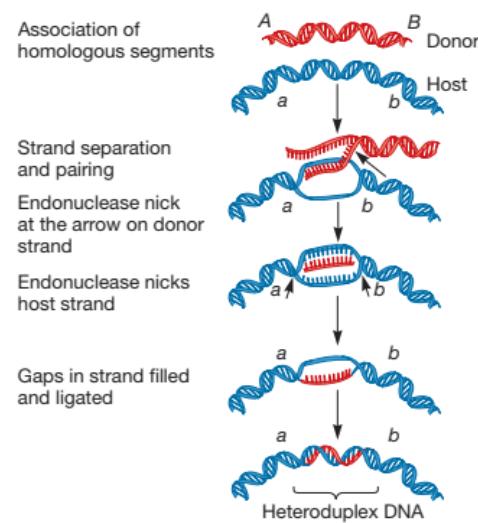


Figure 13.18 Nonreciprocal Homologous Recombination. The Fox model for nonreciprocal homologous recombination. This mechanism has been proposed for the recombination occurring during transformation in some bacteria.

short inverted repeats, and the coding region contains both transposition genes and the extra genes. It is believed that composite transposons are formed when two IS elements associate with a central segment containing one or more genes. This association could arise if an IS element replicates and moves only a gene or two down the chromosome. Composite transposon names begin with the prefix Tn. Some properties of selected composites are given in **table 13.5**.

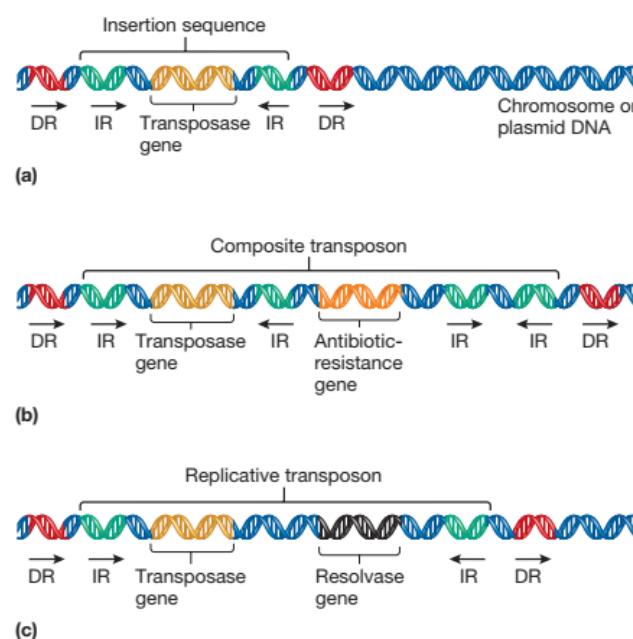


Figure 13.19 Transposable Elements. All transposable elements contain common elements. These include inverted repeats (IRs) at the ends of the element and a transposase gene. (a) Insertion sequences consist only of IRs on either side of the transposase gene. (b) Composite transposons and (c) replicative transposons contain additional genes. Insertion sequences and composite transposons move by simple (cut-and-paste) transposition. Replicative transposons move by replicative transposition. DRs, direct repeats in host DNA, flank a transposable element.

Table 13.4 The Properties of Selected Insertion Sequences

Insertion Sequence	Length (bp)	Inverted Repeat (Length in bp)	Target Site (Length in bp)	Number of Copies on <i>E. coli</i> Chromosome
IS1	768	23	9 or 8	6–10
IS2	1,327	41	5	4–13(1) ^a
IS3	1,400	38	3–4	5–6(2)
IS4	1,428	18	11 or 12	1–2
IS5	1,195	16	4	10–11

^aThe value in parentheses indicates the number of IS elements on the F factor plasmid.

Table 13.5 The Properties of Selected Composite Transposons				
Transposon	Length (bp)	Terminal Repeat Length	Terminal Module	Genetic Markers
Tn3	4,957	38		Ampicillin resistance
Tn501	8,200	38		Mercury resistance
Tn951	16,500	Unknown		Lactose utilization
Tn5	5,700		IS50	Kanamycin resistance
Tn9	2,500		IS1	Chloramphenicol resistance
Tn10	9,300		IS10	Tetracycline resistance
Tn903	3,100		IS903	Kanamycin resistance
Tn1681	2,061		IS1	Heat-stable enterotoxin
Tn2901	11,000		IS1	Arginine biosynthesis

The process of transposition in prokaryotes can occur by two basic mechanisms. **Simple transposition**, also called **cut-and-paste transposition**, involves transposase-catalyzed excision of the transposon, followed by cleavage of a new target site and ligation of the transposon into this site (figure 13.20). Target sites are specific sequences about five to nine base pairs long. When a transposon inserts at a target site, the target sequence is duplicated so that short, direct-sequence repeats flank the transposon's terminal inverted repeats.

The second transposition mechanism is **replicative transposition**. In this mechanism, the original transposon remains at the parental site on the chromosome and a replicate is inserted at the target DNA site (figure 13.21). The transposition of the Tn3 transposon is a well-studied example of replicative transposition. In the first stage, DNA containing Tn3 fuses with the target DNA to form a cointegrate molecule (figure 13.21, step 1). This process requires the *tnpA* gene (figure 13.22). Note that the cointegrate has two copies of the Tn3 transposon. In the second stage the cointegrate is resolved to yield two DNA molecules, each with a copy of the transposon (figure 13.21, step 3). Resolution involves a crossover and is catalyzed by a resolvase enzyme coded for by the *tnpR* gene (figure 13.22).

Transposable elements produce a variety of important effects. They can insert within a gene to cause a mutation or stimulate DNA rearrangement, leading to deletions of genetic material. Because some transposons carry stop codons or termination sequences, when transposed into genes they may block translation or transcription, respectively. Likewise, other transposons carry promoters and can activate genes near the point of insertion. Thus transposons can turn genes on or off. Transposons also are located in plasmids and participate in such processes as plasmid fusion, insertion of plasmids into chromosomes, and plasmid evolution.

The role of transposons in plasmid evolution is of particular note. Plasmids can contain several different transposon-target sites. Therefore, transposons frequently move between plasmids. Of concern is the fact that many transposons contain antibiotic-

resistance genes. Thus as they move from one plasmid to another, resistance genes are introduced into the target plasmid, creating a resistance (R) plasmid. Multiple drug-resistance plasmids can arise from the accumulation of transposons in a plasmid (figure 13.22). Many R plasmids are able to move from one cell to another during conjugation, which spreads the resistance genes throughout a population. Finally, because transposons also move between plasmids and chromosomes, drug resistance genes can exchange between these two molecules, resulting in the further spread of antibiotic resistance.

Some transposons bear transfer genes and can move between bacteria through the process of conjugation, as discussed in section 13.7. A well-studied example of a **conjugative transposon** is Tn916 from *Enterococcus faecalis*. Although Tn916 cannot replicate autonomously, it can transfer itself from *E. faecalis* to a variety of recipients and integrate into their chromosomes. Because it carries a gene for tetracycline resistance, this conjugative transposon also spreads drug resistance.

13.6 BACTERIAL PLASMIDS

Conjugation, the transfer of DNA between bacteria involving direct contact, depends on the presence of an "extra" piece of DNA known as a plasmid. Plasmids play many important roles in the lives of prokaryotes. They also have proved invaluable to microbiologists and molecular geneticists in constructing and transferring new genetic combinations and in cloning genes as described in chapter 14.

Recall from chapter 3 that **plasmids** are small double-stranded DNA molecules that can exist independently of host chromosomes. They have their own replication origins and autonomously replicate and are stably inherited. Some plasmids are **episomes**, plasmids that can exist either with or without being integrated into host chromosomes. Although there are a variety of plasmid types, our concern here is with **conjugative plasmids**. These plasmids can transfer copies of themselves to other bacteria during the process of conjugation, which is discussed in section 13.7.

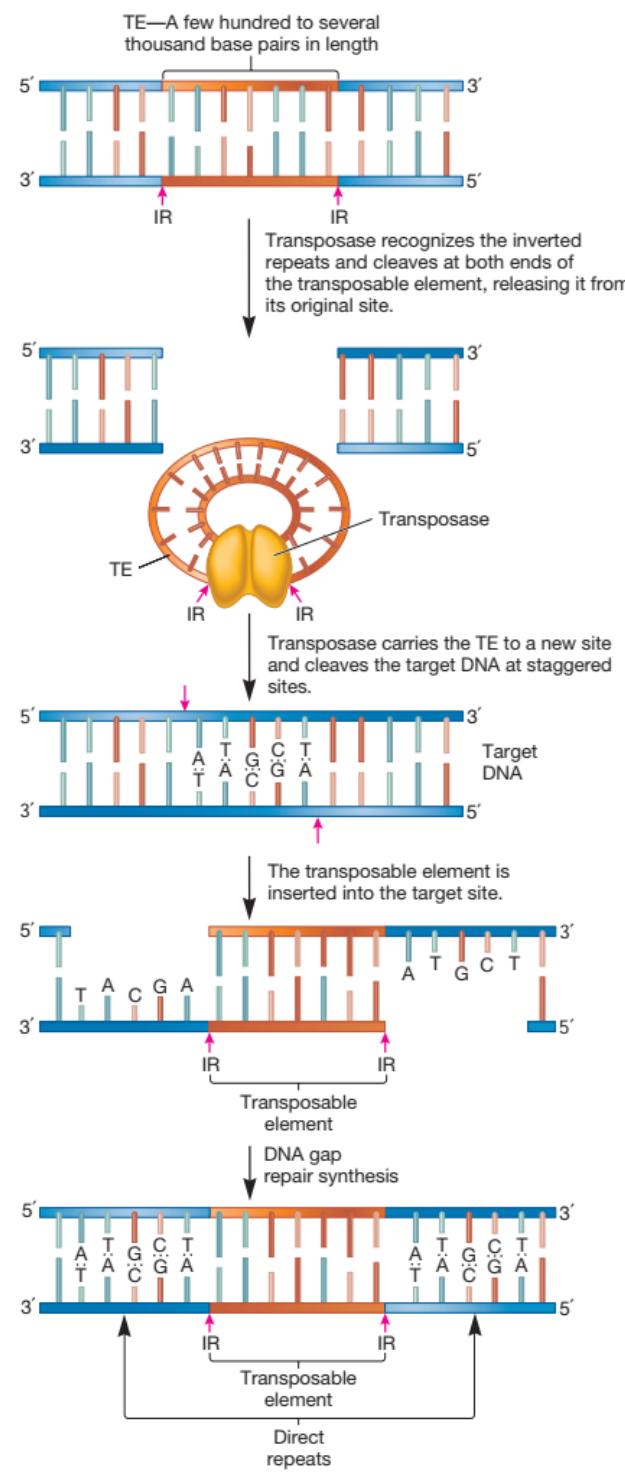


Figure 13.20 Simple Transposition. TE, transposable element; IR, inverted repeat.

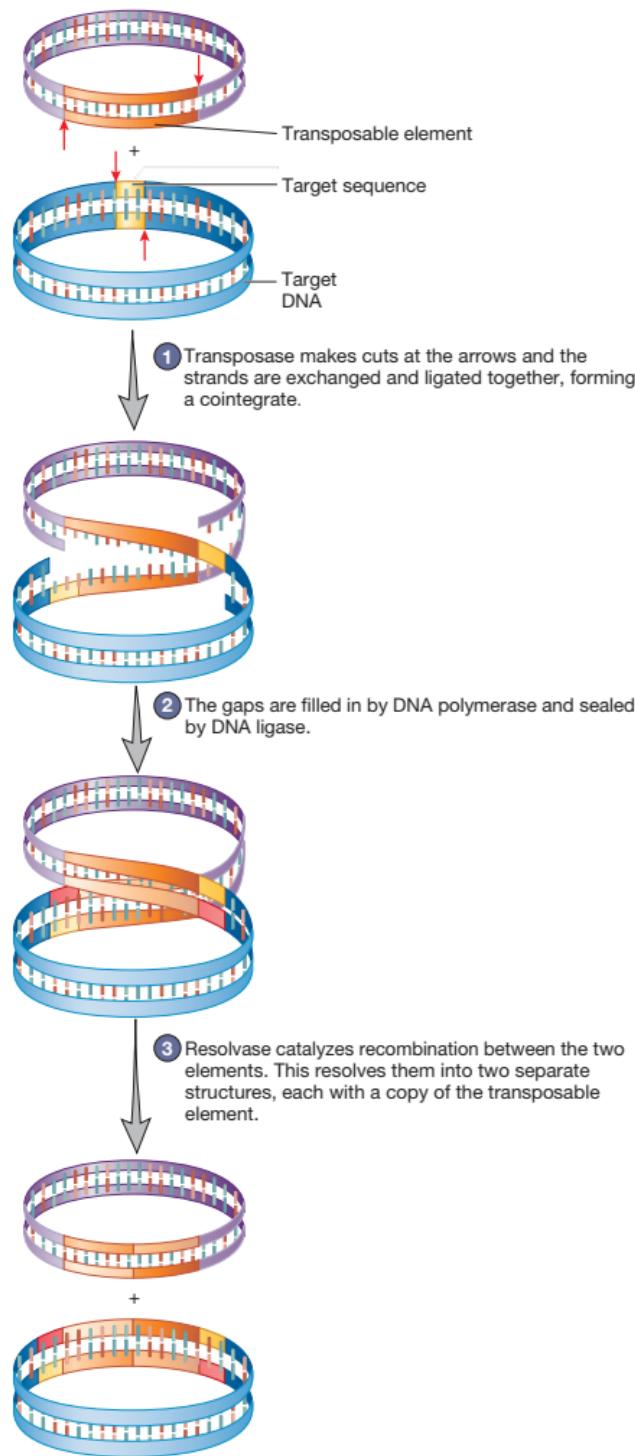
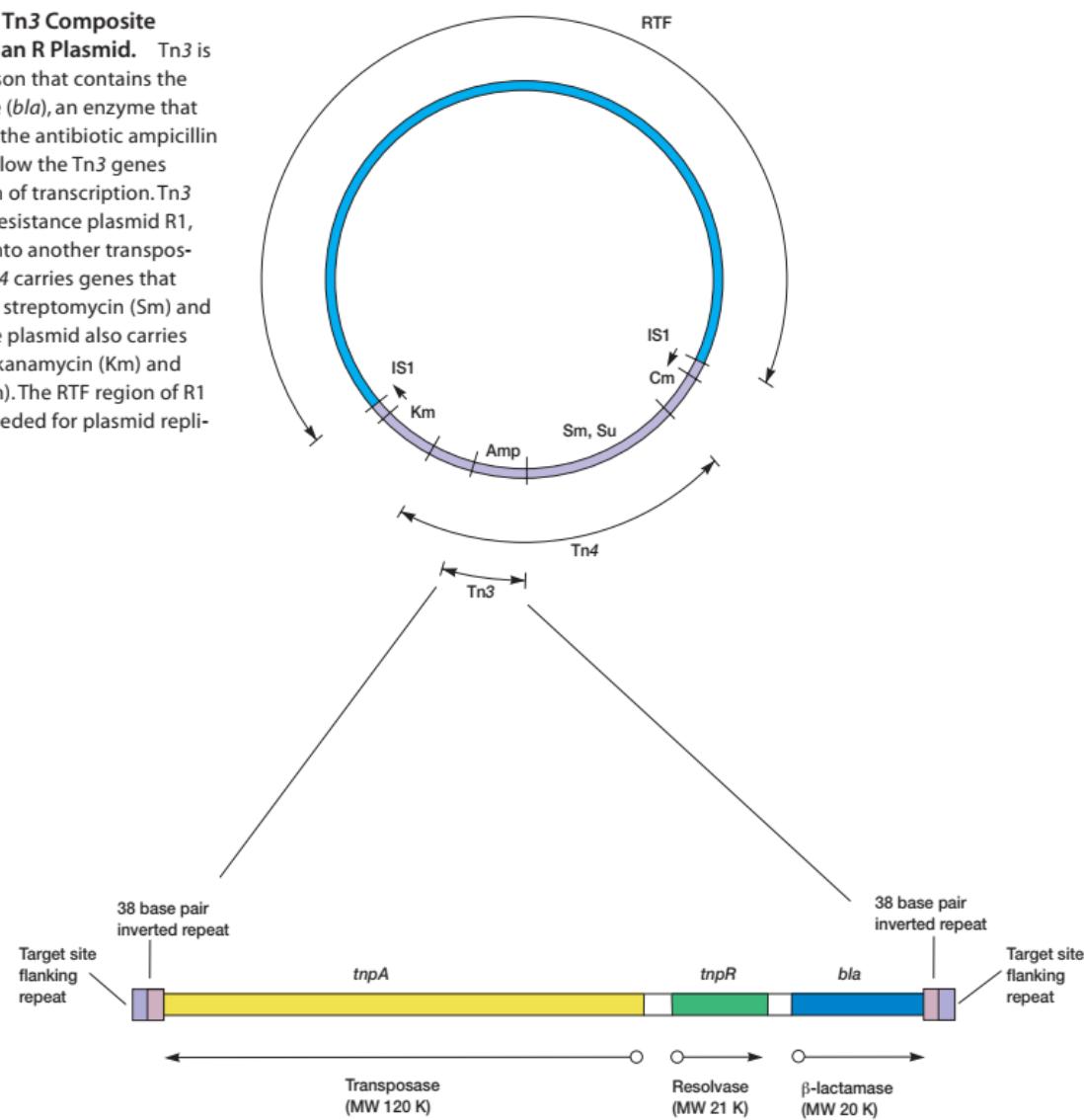


Figure 13.21 Replicative Transposition.

Figure 13.22 The Tn3 Composite Transposon within an R Plasmid. Tn3 is a replicative transposon that contains the gene for β -lactamase (*bla*), an enzyme that confers resistance to the antibiotic ampicillin (Amp). The arrows below the Tn3 genes indicate the direction of transcription. Tn3 can be found in the resistance plasmid R1, where it is inserted into another transposable element, Tn4. Tn4 carries genes that provide resistance to streptomycin (Sm) and sulfonamide (Su). The plasmid also carries resistance genes for kanamycin (Km) and chloramphenicol (Cm). The RTF region of R1 codes for proteins needed for plasmid replication and transfer.



Perhaps the best-studied conjugative plasmid is **F factor**. It plays a major role in conjugation in *E. coli* and was the first conjugative plasmid to be described (figure 13.23). The F factor is about 100 kilobases long and bears genes responsible for cell attachment and plasmid transfer between specific bacterial strains during conjugation. Most of the information required for plasmid transfer is located in the **tra operon**, which contains at least 28 genes. Many of these direct the formation of sex pili that attach the F⁺ cell (the donor cell containing an F plasmid) to an F⁻ cell (figure 13.24). Other gene products aid DNA transfer. In addition, the F factor has several segments called insertion sequences that assist plasmid integration into the host cell chromosome. Thus the F factor is an episome that can exist outside the bacterial chromosome or can be integrated into it (figure 13.25).

1. Define the following: episome, conjugative plasmid, transposition, transposase, and conjugative transposon.
2. Compare and contrast plasmids and transposable elements. Compare and contrast insertion sequences, composite transposons, and replicative transposons.
3. How might one demonstrate the presence of a plasmid in a host cell?
4. What is simple (cut-and-paste) transposition? What is replicative transposition? How do the two mechanisms of transposition differ? What happens to the target site during transposition?
5. What effect would you expect the existence of transposable elements and plasmids to have on the rate of microbial evolution? Give your reasoning.
6. How do multiple-drug-resistant plasmids often arise?

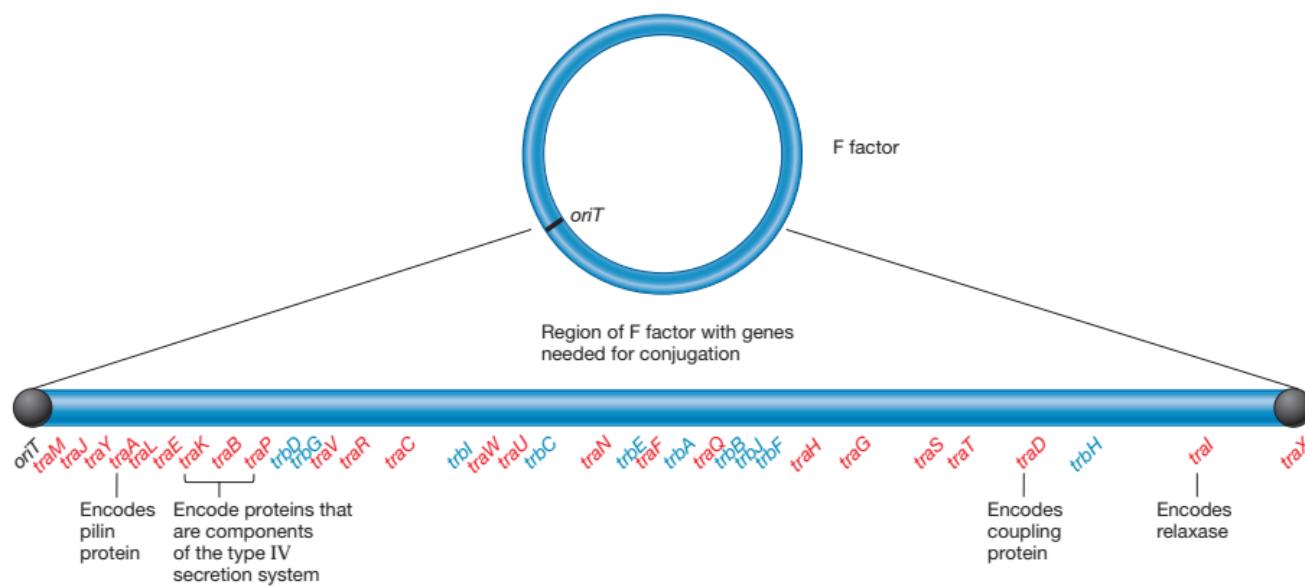


Figure 13.23 The F plasmid. Genes that play a role in conjugation are shown, and some of their functions are indicated. The plasmid also contains three insertion sequences and a transposon. The site for initiation of rolling-circle replication and gene transfer during conjugation is *oriT*.

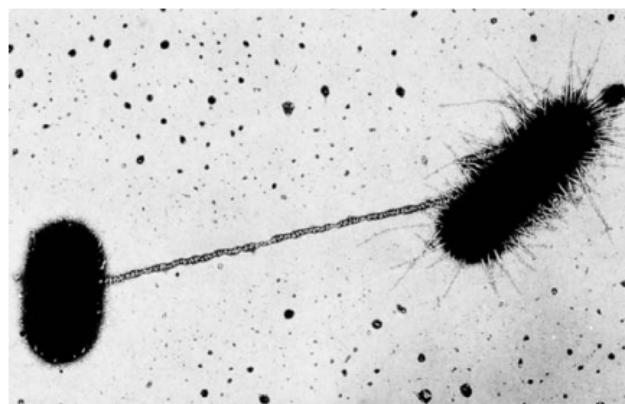


Figure 13.24 Bacterial Conjugation. An electron micrograph of two *E. coli* cells in an early stage of conjugation. The *F*⁺ cell to the right is covered with small pili or fimbriae, and a sex pilus connects the two cells.

13.7 BACTERIAL CONJUGATION

The initial evidence for bacterial **conjugation**, the transfer of DNA by direct cell to cell contact, came from an elegant experiment performed by **Joshua Lederberg** and **Edward Tatum** in 1946. They mixed two auxotrophic strains, incubated the culture for

several hours in nutrient medium, and then plated it on minimal medium. To reduce the chance that their results were due to simple reversion, they used double and triple auxotrophs on the assumption that two or three simultaneous reversions would be extremely rare. For example, one strain required biotin (Bio^-), phenylalanine (Phe^-), and cysteine (Cys^-) for growth, and another needed threonine (Thr^-), leucine (Leu^-), and thiamine (Thi^-). Recombinant prototrophic colonies appeared on the minimal medium after incubation (figure 13.26). Thus the chromosomes of the two auxotrophs were able to associate and undergo recombination.

Lederberg and Tatum did not directly prove that physical contact of the cells was necessary for gene transfer. This evidence was provided by **Bernard Davis** (1950), who constructed a U tube consisting of two pieces of curved glass tubing fused at the base to form a U shape with a fritted glass filter between the halves. The filter allowed the passage of media but not bacteria. The U tube was filled with nutrient medium and each side inoculated with a different auxotrophic strain of *E. coli* (figure 13.27). During incubation, the medium was pumped back and forth through the filter to ensure medium exchange between the halves. After a 4 hour incubation, the bacteria were plated on minimal medium. Davis discovered that when the two auxotrophic strains were separated from each other by the fine filter, gene transfer could not take place. Therefore direct contact was required for the recombination that Lederberg and Tatum had observed. F factor-mediated conjugation is one of the best-studied conjugation systems. It is the focus of this section.

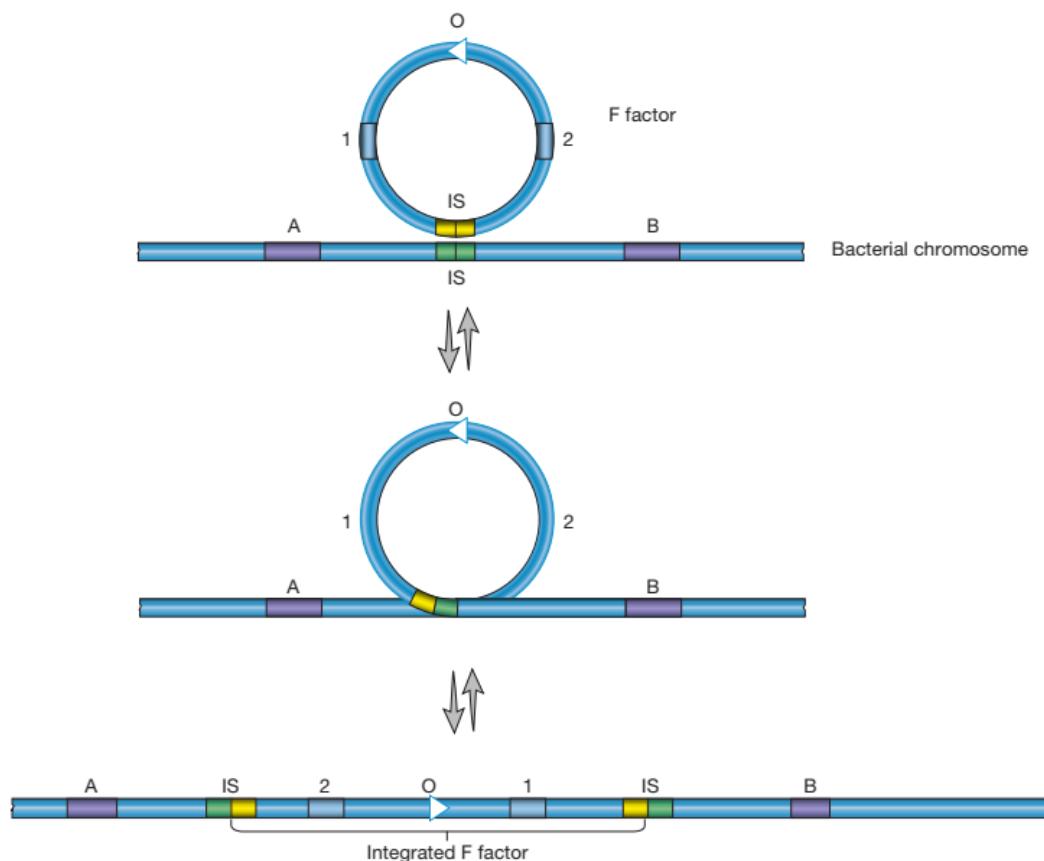


Figure 13.25 F Plasmid Integration. The reversible integration of an F plasmid or factor into a host bacterial chromosome. The process begins with association between plasmid and bacterial insertion sequences. The O arrowhead (white) indicates the site at which oriented transfer of chromosome to the recipient cell begins. A, B, 1, and 2 represent genetic markers.

F⁺ × F⁻ Mating

In 1952 William Hayes demonstrated that the gene transfer observed by Lederberg and Tatum was polar. That is, there were definite donor (F⁺, or fertile) and recipient (F⁻, or nonfertile) strains, and gene transfer was nonreciprocal. He also found that in F⁺ × F⁻ mating the progeny were only rarely changed with regard to auxotrophy (that is, chromosomal genes were not often transferred), but F⁻ strains frequently became F⁺.

These results are readily explained in terms of the F factor previously described (figure 13.23). The F⁺ strain contains an extrachromosomal F factor carrying the genes for sex pilus formation and plasmid transfer. The **sex pilus** is used to establish contact between the F⁺ and F⁻ cells (figure 13.28a). Once contact is made, the pilus retracts, bringing the cells into close physical contact. The F⁺ cell then prepares for DNA transfer by assembling a **type IV secretion apparatus**, using many of the same genes used for sex pilus biogenesis; the sex pilus is embedded in the secretion structure (figure 13.29). The F factor then replicates by a rolling-circle mechanism (see figure 11.12). Replication is initiated by a complex of proteins called the **relaxosome**, which

nicks one strand of the F factor at a site called *oriT* (for origin of transfer). Relaxase, an enzyme associated with the relaxosome, remains attached to the 5' end of the nicked strand. As F factor is replicated, the displaced strand and the attached relaxase enzyme move through the type IV secretion system to the recipient cell. Because the pilus is embedded in the secretion apparatus, it has been suggested that the DNA moves through a lumen in the pilus. However, studies of a related conjugation system, that of the plant pathogen *Agrobacterium tumefaciens*, provide strong evidence that the DNA does not move through the sex pilus. However, it should be noted that although the F factor system and the *Agrobacterium* system are related, there is one important difference between the two. The F factor system is used to transfer DNA from one bacterium to another, whereas the *Agrobacterium* system moves DNA from the bacterium into its plant host. Whatever the route of transfer, as the plasmid is transferred, the entering strand is copied to produce double-stranded DNA. The recombination frequency is low because chromosomal genes are rarely transferred with the independent F factor. **Microorganism associations with vascular plants: *Agrobacterium*** (section 29.5)

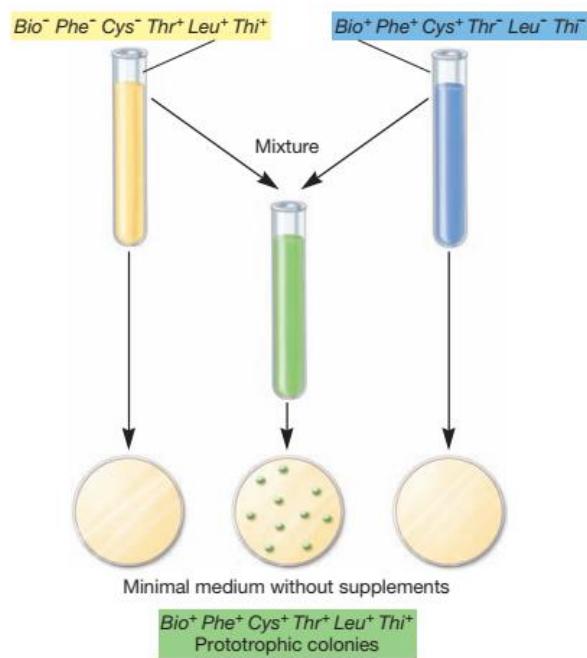


Figure 13.26 Evidence for Bacterial Conjugation.
Lederberg and Tatum's demonstration of genetic recombination using triple auxotrophs. See text for details.

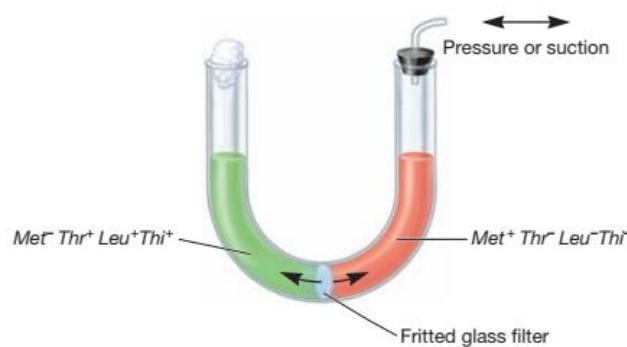


Figure 13.27 The U-tube Experiment. The U-tube experiment used to show that genetic recombination by conjugation requires direct physical contact between bacteria. See text for details.

Hfr Conjugation

Not long after the discovery of $F^+ \times F^-$ mating, a second type of F factor-mediated conjugation was discovered. In this type of conjugation, the donor transfers chromosomal genes with great efficiency, but does not change the recipient bacteria into F^+ cells. Because of the high frequency of recombinants produced by this mating, it is referred to as **Hfr conjugation** and the donor is called an **Hfr strain**. Although initially the mechanism of Hfr

conjugation was not known, eventually it was determined that Hfr strains contain the F factor integrated into their chromosome, rather than free in the cytoplasm (figure 13.28b). When integrated, the F plasmid's *tra* operon is still functional; the plasmid can direct the synthesis of pili, carry out rolling-circle replication, and transfer genetic material to an F^- recipient cell. However, rather than transferring itself, the F factor directs the transfer of host chromosome. DNA transfer begins when the integrated F factor is nicked at its site of transfer origin. As it is replicated, the chromosome moves to the recipient. Transfer of the entire chromosome with the integrated F factor requires about 100 minutes in *E. coli*, and the connection between the cells usually breaks before this process is finished. Thus a complete F factor usually is not transferred, and the recipient remains F^- .

As mentioned earlier, when an Hfr strain participates in conjugation, bacterial genes are frequently transferred to the recipient. Gene transfer can be in either a clockwise or counterclockwise direction around the circular chromosome, depending on the orientation of the integrated F factor. After the replicated donor chromosome enters the recipient cell, it may be degraded or incorporated into the F^- genome by recombination.

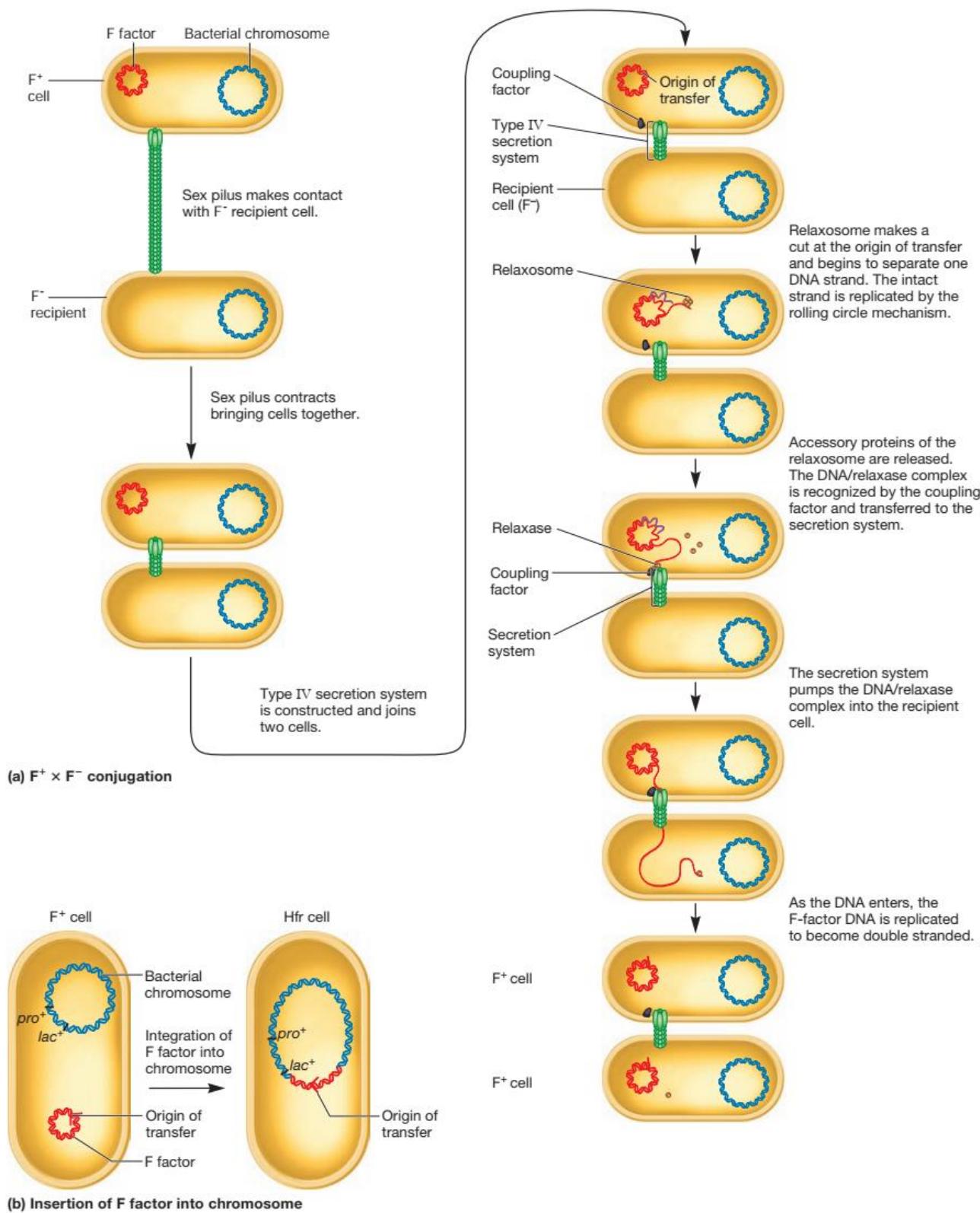
F' Conjugation

Because the F plasmid is an episome, it can leave the bacterial chromosome and resume status as an autonomous F factor. Sometimes during this process the plasmid makes an error in excision and picks up a portion of the chromosome. Because it is now genetically distinct from the original F factor, it is called an **F' plasmid** (figure 13.30a). It is not unusual to observe the inclusion of one or more chromosomal genes in excised F plasmids. A cell containing an F' plasmid retains all of its genes, although some of them are on the plasmid. It mates only with an F^- recipient. $F' \times F^-$ conjugation is similar to $F^+ \times F^-$ mating. Once again, the plasmid is transferred as it is copied by rolling-circle replication. However, bacterial genes on the chromosome usually are not transferred (figure 13.30b). Bacterial genes acquired during excision of the F' plasmid are transferred with it and need not be incorporated into the recipient chromosome to be expressed. The recipient becomes F' and is a partially diploid merozygote because the same bacterial genes present on the F' plasmid are also found on the recipient's chromosome. In this way specific bacterial genes may spread rapidly throughout a bacterial population.

F' conjugation is very important to the microbial geneticist. A partial diploid's behavior shows whether the allele carried by an F' plasmid is dominant or recessive to the chromosomal gene. The formation of F' plasmids also is useful in mapping the chromosome because if two genes are picked up by an F factor they must be neighbors.

Other Examples of Bacterial Conjugation

Although most research on plasmids and conjugation has been done using *E. coli* and other gram-negative bacteria, self-transmissible



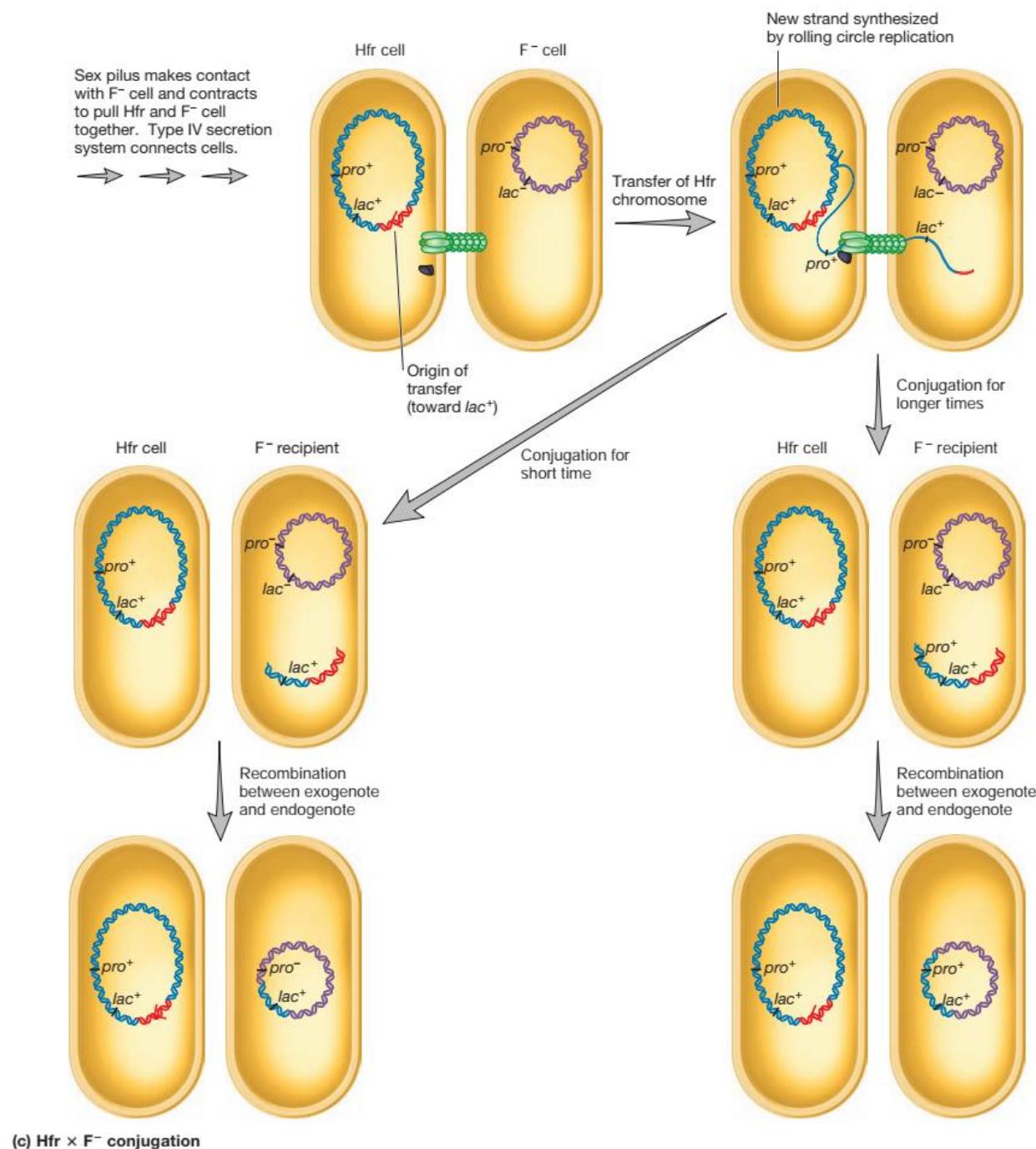
(c) Hfr \times F $^{-}$ conjugation

Figure 13.28 F Factor-Mediated Conjugation. The F factor encodes proteins for building the sex pilus and proteins needed to construct the type IV secretion system that will transfer DNA from the donor to the F $^{-}$ recipient. One protein, the coupling factor, is thought to guide the DNA to the secretion system. (a) During F $^{+}$ \times F $^{-}$ conjugation, only the F factor is transferred because the plasmid is extrachromosomal. The recipient cell becomes F $^{+}$. (b) Integration of the F factor into the chromosome creates an Hfr cell. (c) During Hfr \times F $^{-}$ conjugation, some plasmid genes and some chromosomal genes are transferred to the recipient. Note that only a portion of the F factor moves into the recipient. Because the entire plasmid is not transferred, the recipient remains F $^{-}$. In addition, the incoming DNA must recombine into the recipient's chromosome if it is to be stably maintained.

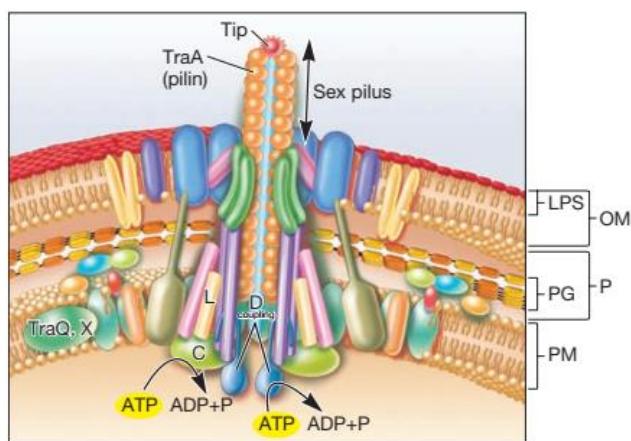


Figure 13.29 The Type IV Secretion System Encoded by F Factor. The F factor-encoded type IV secretion system is composed of numerous Tra proteins, including TraA proteins, which form the sex pilus, and TraD, which is the coupling factor. Some Tra proteins are located in the plasma membrane (PM), others extend into the periplasm (P) and pass through the peptidoglycan layer (PG) into the outer membrane (OM) and its lipopolysaccharide (LPS) layer.

plasmids are present in gram-positive bacterial genera such as *Bacillus*, *Streptococcus*, *Enterococcus*, *Staphylococcus*, and *Streptomyces*. Much less is known about these systems. It appears that fewer transfer genes are involved, possibly because a sex pilus may not be required for plasmid transfer. For example, *Enterococcus faecalis* recipient cells release short peptide chemical signals that activate transfer genes in donor cells containing the proper plasmid. Donor and recipient cells directly adhere to one another through special plasmid-encoded proteins released by the activated donor cell. Plasmid transfer then occurs.

1. What is bacterial conjugation and how was it discovered?
2. Distinguish between F^+ , Hfr, and F^- strains of *E. coli* with respect to their physical nature and role in conjugation.
3. Describe in some detail how $F^+ \times F^-$ and Hfr conjugation processes proceed, and distinguish between the two in terms of mechanism and the final results.
4. What is F' conjugation and why is it so useful to the microbial geneticist? How does the F' plasmid differ from a regular F plasmid?

13.8 DNA TRANSFORMATION

The second way DNA can move between bacteria is through transformation, discovered by **Fred Griffith** in 1928. **Transformation** is the uptake by a cell of a naked DNA molecule or fragment from the medium and the incorporation of this molecule into the recipient chromosome in a heritable form. In natural

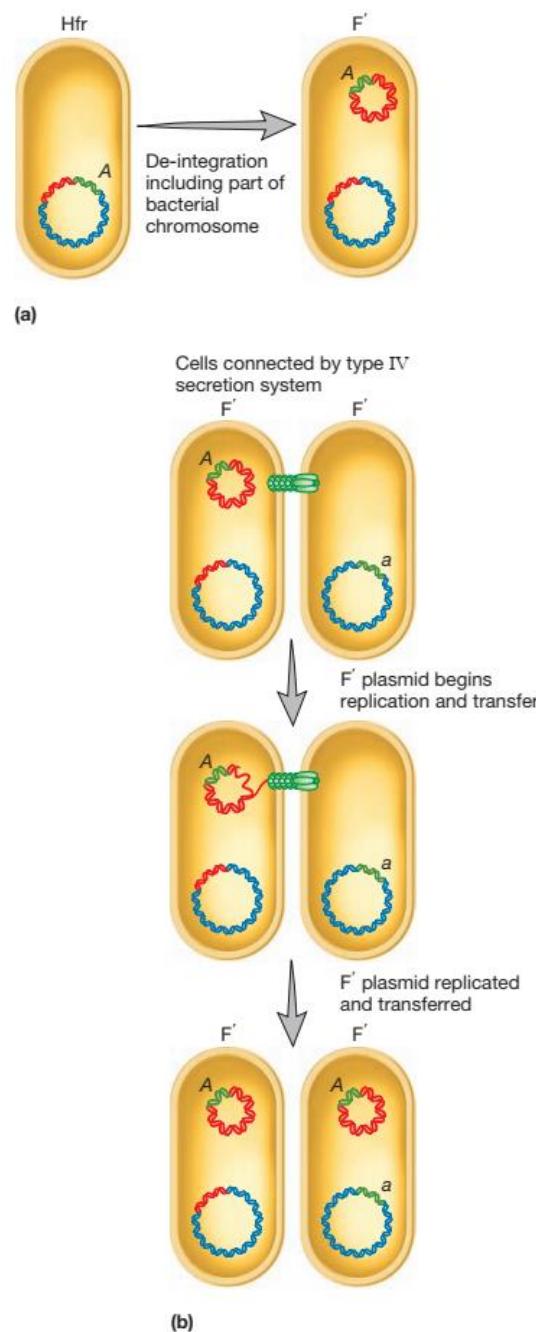


Figure 13.30 F' Conjugation. (a) Due to an error in excision, the A gene of an Hfr cell is picked up by the F factor. (b) The A gene is then transferred to a recipient during conjugation. See text for explanation.

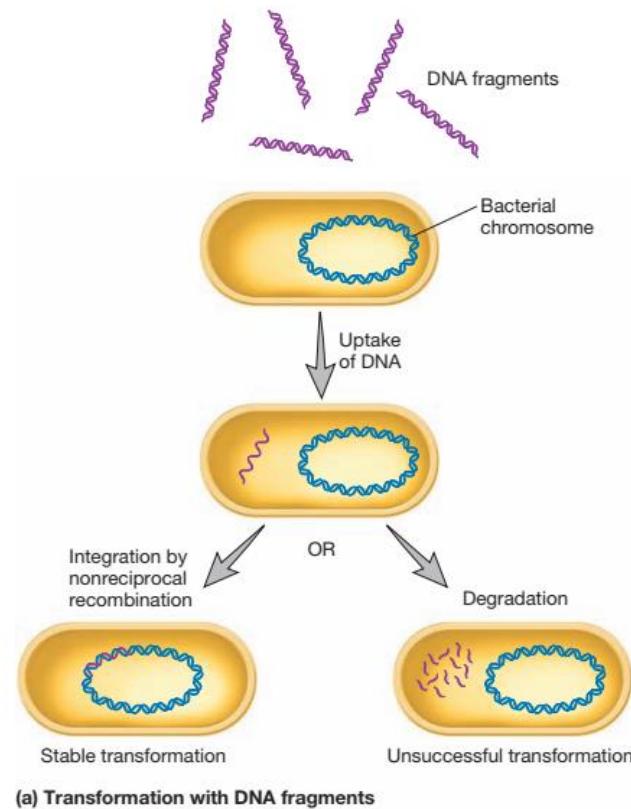
transformation the DNA comes from a donor bacterium. The process is random, and any portion of a genome may be transferred between bacteria. [DNA as genetic material \(section 11.1\)](#)

When bacteria lyse, they release considerable amounts of DNA into the surrounding environment. These fragments may be relatively large and contain several genes. If a fragment contacts a **competent cell**, a cell that is able to take up DNA and be transformed, the DNA can be bound to the cell and taken inside (figure 13.31a). The transformation frequency of very competent cells is around 10^{-3} for most genera when an excess of DNA is used. That is, about one cell in every thousand will take up and integrate the gene. Competency is a complex phenomenon and is dependent on several conditions. Bacteria need to be in a certain stage of growth; for example, *Streptococcus pneumoniae* becomes competent during the exponential phase when the population reaches about 10^7 to 10^8 cells per ml. When a population becomes competent, bacteria such as *S. pneumoniae* secrete a small protein called the competence factor that stimulates the production of 8 to 10 new proteins required for transformation. Natural transformation has been discovered so far only in certain genera including *Streptococcus*, *Bacillus*, *Thermoactinomyces*, *Haemophilus*, *Neisseria*, *Moraxella*, *Acinetobacter*, *Azotobacter*, *Helicobacter*, and *Pseudomonas*. Gene transfer by this process occurs in soil and aquatic ecosystems and may be an important route of genetic exchange in biofilm and other microbial communities.

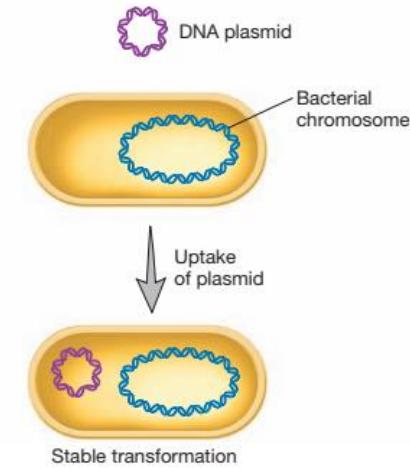
The mechanism of transformation has been intensively studied in *S. pneumoniae* (figure 13.32). A competent cell binds a double-stranded DNA fragment if the fragment is moderately large; the process is random, and donor fragments compete with each other. The DNA then is cleaved by endonucleases to double-stranded fragments about 5 to 15 kilobases in size. DNA uptake requires energy expenditure. One strand is hydrolyzed by an envelope-associated exonuclease during uptake; the other strand associates with small proteins and moves through the plasma membrane. The single-stranded fragment can then align with a homologous region of the genome and be integrated, probably by a mechanism similar to that depicted in figure 13.18.

Transformation in *Haemophilus influenzae*, a gram-negative bacterium, differs from that in *S. pneumoniae* in several respects. *H. influenzae* does not produce a competence factor to stimulate the development of competence, and it takes up DNA from only closely related species (*S. pneumoniae* is less particular about the source of its DNA). Double-stranded DNA, complexed with proteins, is taken in by membrane vesicles. The specificity of *H. influenzae* transformation is due to a special 11 base pair sequence (5'AACTGCG-GTCA3') that is repeated over 1,400 times in *H. influenzae* DNA. DNA must have this sequence to be bound by a competent cell.

The protein complexes that take up free DNA must be able to move it through gram-negative and gram-positive walls, which may be both thick and complex. As expected, the machinery is quite large and complicated and appears related to protein secretion systems. Figure 13.33a shows a schematic diagram of the complex used by the gram-negative bacterium *Neisseria gonorrhoeae*. PilQ aids in the movement across the outer membrane, and the pilin complex PilE moves the DNA through the periplasm and peptidoglycan. ComE is a DNA binding protein; N is the nuclease that degrades one strand before the DNA enters the cytoplasm through the transmembrane channel formed by



(a) Transformation with DNA fragments



(b) Transformation with a plasmid

Figure 13.31 Bacterial Transformation. Transformation with (a) DNA fragments and (b) plasmids. Transformation with a plasmid often is induced artificially in the laboratory. The transforming DNA is in purple and integration is at a homologous region of the genome.

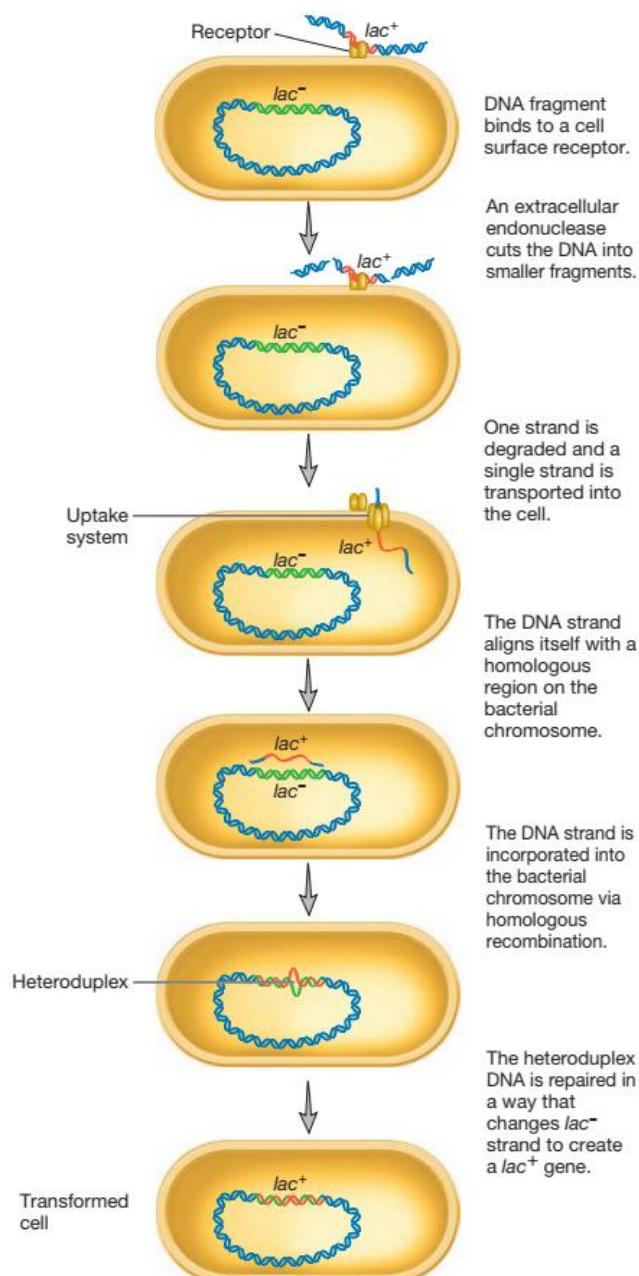


Figure 13.32 Bacterial Transformation as Seen in *S. pneumoniae*.

ComA. The machinery in the gram-positive bacterium *Bacillus subtilis* is depicted in figure 13.33b. It is localized to the poles of the cell, and as can be seen, many of the components are similar to those of *N. gonorrhoeae*: the pilin complex (ComGC), DNA binding protein (ComEA), nuclease (N), and channel protein (ComEC). ComFA is a DNA translocase that moves the DNA into the cyto-

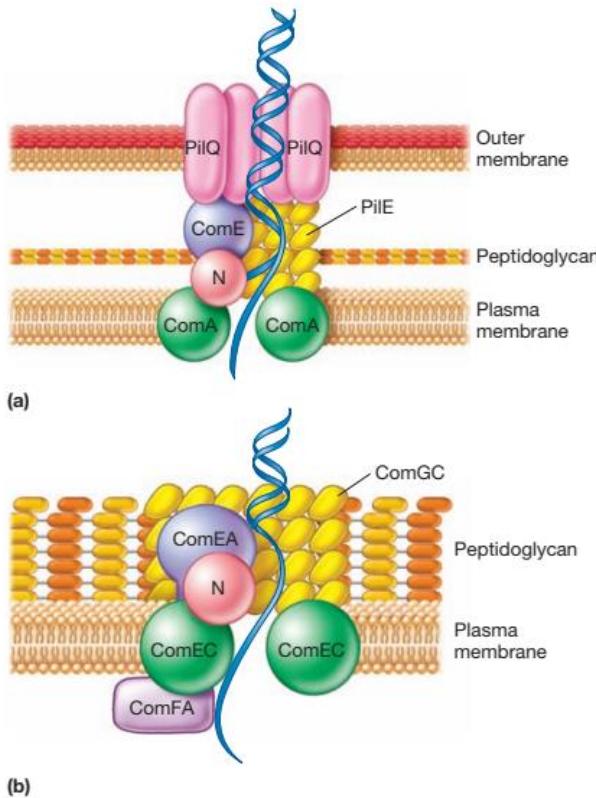


Figure 13.33 DNA Uptake Systems. (a) DNA uptake machinery in *N. gonorrhoeae*. (b) Uptake machinery in *B. subtilis*. See text for details.

plasm. A gram-negative equivalent of ComFA has not been identified yet in *N. gonorrhoeae*.

Microbial geneticists exploit transformation to move DNA (usually recombinant DNA) into cells. However, as already noted, many species, including *E. coli*, are not naturally transformation competent. Fortunately, these bacteria can be made artificially competent by certain treatments. Two common techniques are electrical shock and exposure to calcium chloride. Both approaches render the cell membrane more permeable to DNA and both have been used to make artificially competent *E. coli* cells. To increase the transformation frequency with *E. coli*, strains that lack one or more nucleases are used. These strains are especially important when transforming the cells with linear DNA, which is vulnerable to attack by nucleases. It is easier to transform bacteria with plasmid DNA since plasmids are not as easily degraded as linear fragments and can replicate within the host (figure 13.31b).

1. Define transformation and competence.
2. Describe how transformation occurs in *S. pneumoniae*. How does the process differ in *H. influenzae*?
3. Discuss two ways in which artificial transformation can be used to place functional genes within bacterial cells.

13.9 TRANSDUCTION

The third mode of bacterial gene transfer is **transduction**. It is a frequent mode of horizontal gene transfer in nature and is mediated by viruses. The morphology and life cycle of bacterial viruses or bacteriophages is not discussed in detail until chapter 17. Nevertheless, it is necessary to briefly describe the life cycle here as background for a consideration of their role in gene transfer.

Viruses are structurally simple, often composed of just a nucleic acid genome protected by a protein coat called the capsid. They are unable to replicate autonomously. Instead, they infect and take control of a host cell, forcing the host to make many copies of the virus. Viruses that infect bacteria are called bacteriophages, or phages for short. Some phages are replicated by their bacterial host immediately after entry. After the number of replicated phages reaches a certain number, they cause the host to lyse, so they can be released and infect new host cells (figure 13.34). These phages are called **virulent bacteriophages** and the process is called the **lytic cycle**. Other bacteriophages do not immediately kill their host. Many of these viruses enter the host bacterium and, instead of replicating, insert their genomes into the bacterial chromosome. Once inserted, the viral genome is called a **prophage**. The host bacterium is unharmed by this, and the phage genome is passively replicated as the host cell's genome is replicated. These bacteriophages are called **temperate bacteriophages** and the relationship between these viruses and their host is called **lysogeny** (figure 13.34). Bacteria that have been lysogenized are called **lysogens**. Temperate phages can remain inactive in their hosts for many generations. However, they can be induced to switch to a lytic cycle of growth under certain conditions, including UV ir-

diation. When this occurs, the prophage is excised from the bacterial genome and the lytic cycle proceeds.

Transduction is the transfer of bacterial genes by viruses. Bacterial genes are incorporated into a phage capsid because of errors made during the virus life cycle. The virus containing these genes then injects them into another bacterium, completing the transfer. There are two different kinds of transduction: generalized and specialized.

Generalized Transduction

Generalized transduction occurs during the lytic cycle of virulent and some temperate phages and can transfer any part of the bacterial genome (figure 13.35). During the assembly stage, when the viral chromosomes are packaged into protein capsids, random fragments of the partially degraded bacterial chromosome also may be packaged by mistake. Because the capsid can contain only a limited quantity of DNA, the viral DNA is left behind. The quantity of bacterial DNA carried depends primarily on the size of the capsid. The P22 phage of *Salmonella enterica* serovar Typhimurium usually carries about 1% of the bacterial genome; the P1 phage of *E. coli* and a variety of gram-negative bacteria carries about 2.0 to 2.5% of the genome. The resulting virus particle often injects the DNA into another bacterial cell but cannot initiate a lytic cycle. This phage is known as a **generalized transducing particle** or phage and is simply a carrier of genetic information from the original bacterium to another cell. As in transformation, once the DNA has been injected, it must be incorporated into the recipient cell's chromosome to preserve the transferred genes. The DNA remains double stranded during

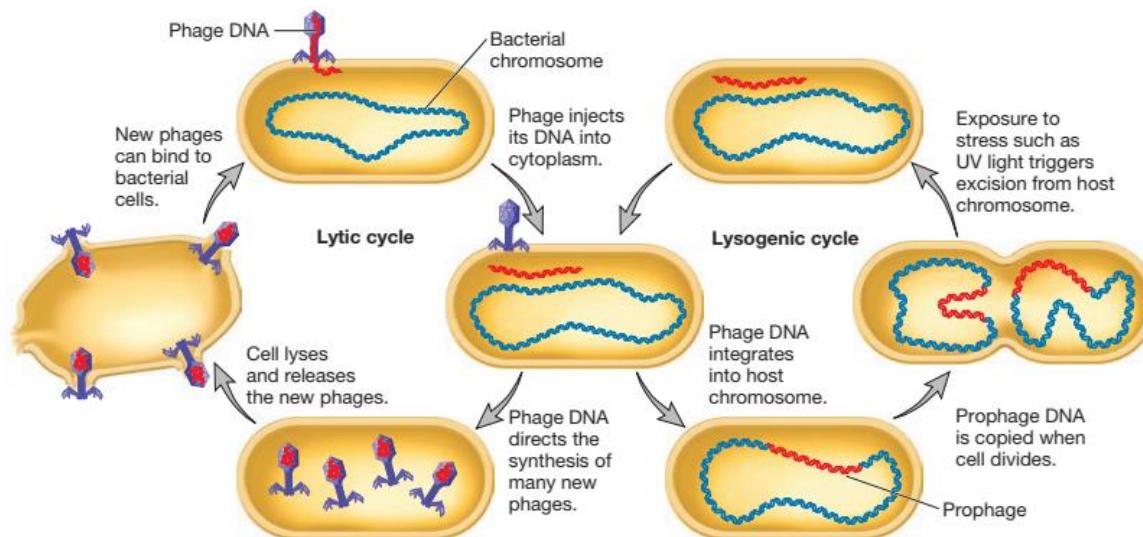


Figure 13.34 Lytic and Lysogenic Cycles of Temperate Phages. Virulent phages undergo only the lytic cycle. Temperate phages have two phases to their life cycles. The lysogenic cycle allows the genome of the virus to be replicated passively as the host cell's genome is replicated. Certain environmental factors such as UV light can cause a switch from the lysogenic cycle to the lytic cycle. In the lytic cycle, new virus particles are made and released when the host cell lyses. Virulent phages are limited to just the lytic cycle.

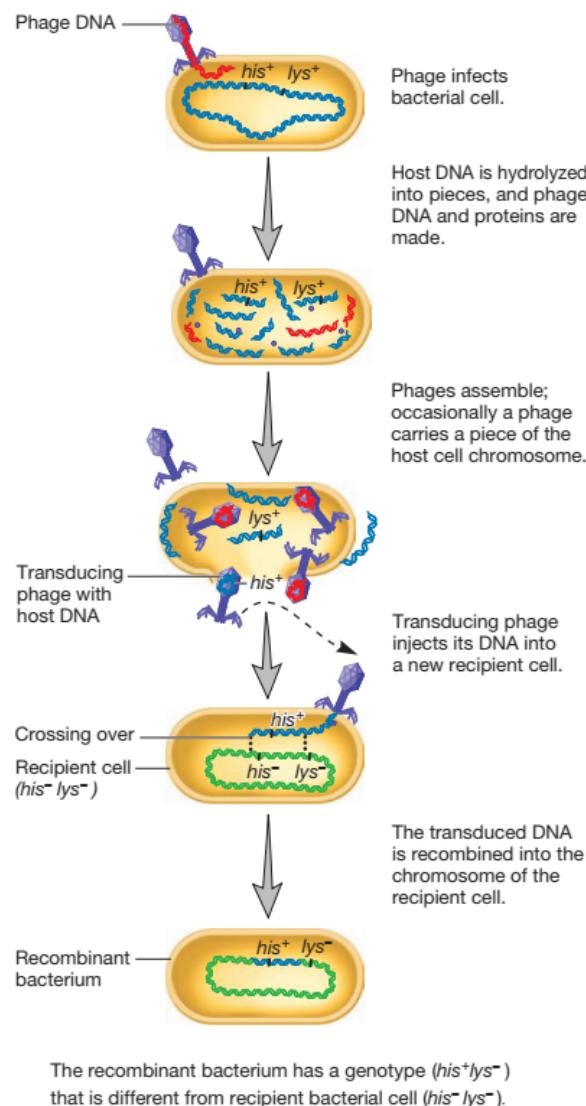


Figure 13.35 Generalized Transduction in Bacteria.

transfer, and both strands are integrated into the endogenote's genome. About 70 to 90% of the transferred DNA is not integrated but often is able to survive temporarily and be expressed. **Abortive transductants** are bacteria that contain this nonintegrated, transduced DNA and are partial diploids.

Generalized transduction was discovered in 1951 by **Joshua Lederberg** and **Norton Zinder** during an attempt to show that conjugation, discovered several years earlier in *E. coli*, could occur in other bacterial species. Lederberg and Zinder were repeating the earlier experiments with *S. enterica* serovar Typhimurium. They found that incubation of a mixture of two multiply auxotrophic strains yielded prototrophs at the level of about one in 10^5 . This seemed like good evidence for bacterial recombination,

and indeed it was, but their initial conclusion that the transfer resulted from conjugation was not borne out. When these investigators performed the U-tube experiment (figure 13.27) with *Salmonella*, they still recovered prototrophs. The filter in the U tube had pores that were small enough to block the movement of bacteria between the two sides but allowed phage P22 to pass. Lederberg and Zinder had intended to confirm that conjugation was present in another bacterial species but instead discovered a completely new mechanism of bacterial gene transfer. This seemingly routine piece of research led to surprising and important results. A scientist must always keep an open mind about results and be prepared for the unexpected.

Specialized Transduction

In **specialized transduction**, the transducing particle carries only specific portions of the bacterial genome. Specialized transduction is made possible by an error in the lysogenic life cycle of phages that insert their genomes into a specific site in the host chromosome. When a prophage is induced to leave the host chromosome, excision is sometimes carried out improperly. The resulting phage genome contains portions of the bacterial chromosome (about 5 to 10% of the bacterial DNA) next to the integration site, much like the situation with F' plasmids (figure 13.36). A transducing phage genome usually is defective and lacks some part of its attachment site. The transducing particle will inject bacterial genes into another bacterium, even though the defective phage cannot reproduce without assistance. The bacterial genes may become stably incorporated under the proper circumstances.

The best-studied example of specialized transduction is carried out by the *E. coli* phage lambda. The lambda genome inserts into the host chromosome at specific locations known as attachment or *att* sites (figure 13.37, see also figures 17.19 and 17.22). The phage *att* sites and bacterial *att* sites are similar and can complex with each other. The *att* site for lambda is next to the *gal* and *bio* genes on the *E. coli* chromosome; consequently, specialized transducing lambda phages most often carry these bacterial genes. The lysate, or product of cell lysis, resulting from the induction of lysogenized *E. coli* contains normal phage and a few defective transducing particles. These particles are called either lambda *dgal* because they carry the galactose utilization genes or lambda *dbio* because they carry the *bio* from the other side of the *att* site (figure 13.37). Because these lysates contain only a few transducing particles, they often are called **low-frequency transduction lysates (LFT lysates)**. Whereas the normal phage has a complete *att* site, defective transducing particles have a nonfunctional hybrid integration site that is part bacterial and part phage in origin. Integration of the defective phage chromosome does not readily take place. Transducing phages also may have lost some genes essential for reproduction. Stable transductants can arise only if there is a double cross-over event on each side of the *gal* site (figure 13.37). **Temperate bacteriophages and lysogeny** (section 17.5)

Defective lambda phages carrying the *gal* or *bio* genes can integrate if there is a normal lambda phage in the same cell. We will continue our discussion of this with a phage carrying the *gal* gene.

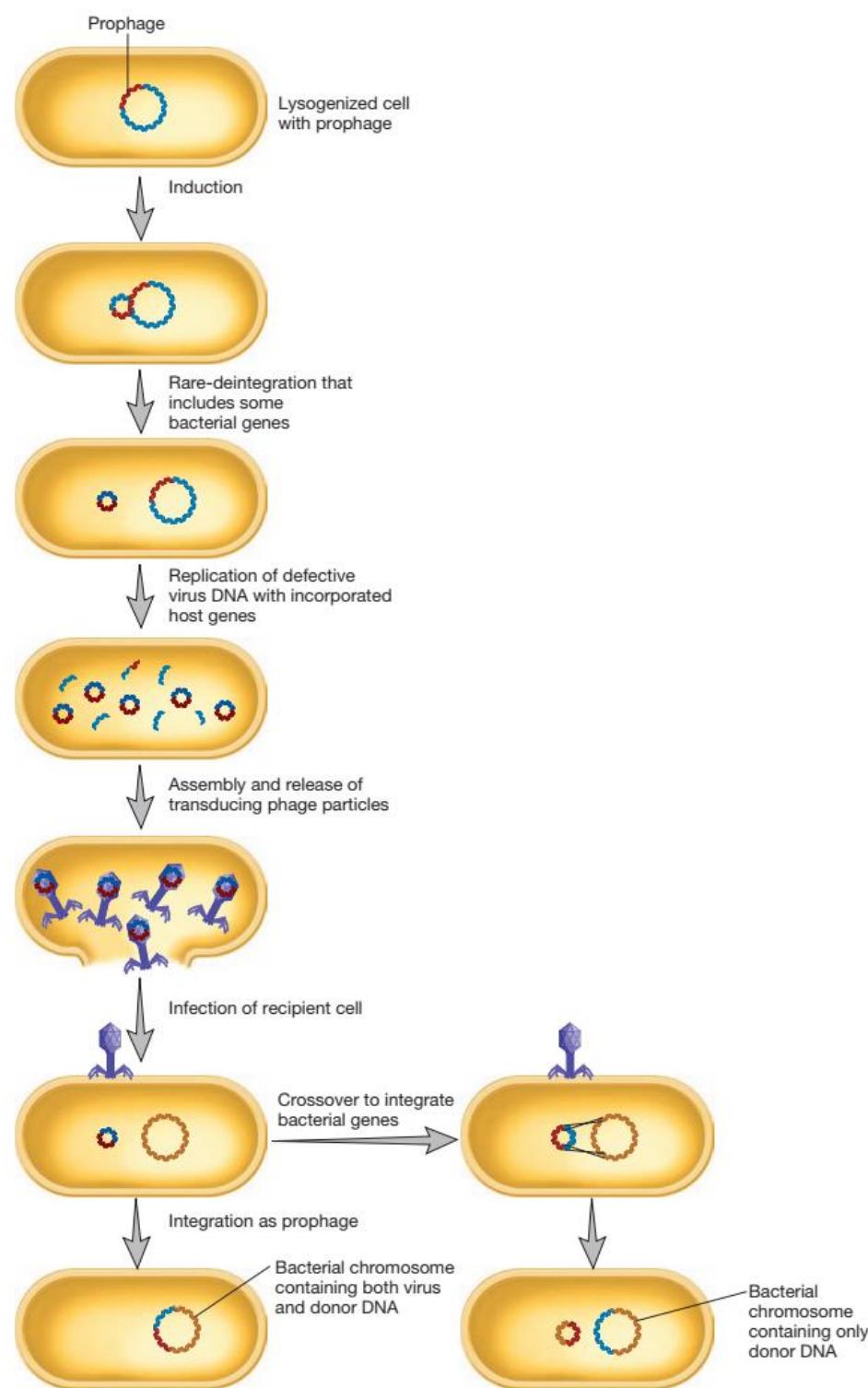


Figure 13.36 Specialized Transduction by a Temperate Bacteriophage. Recombination can produce two types of transductants.

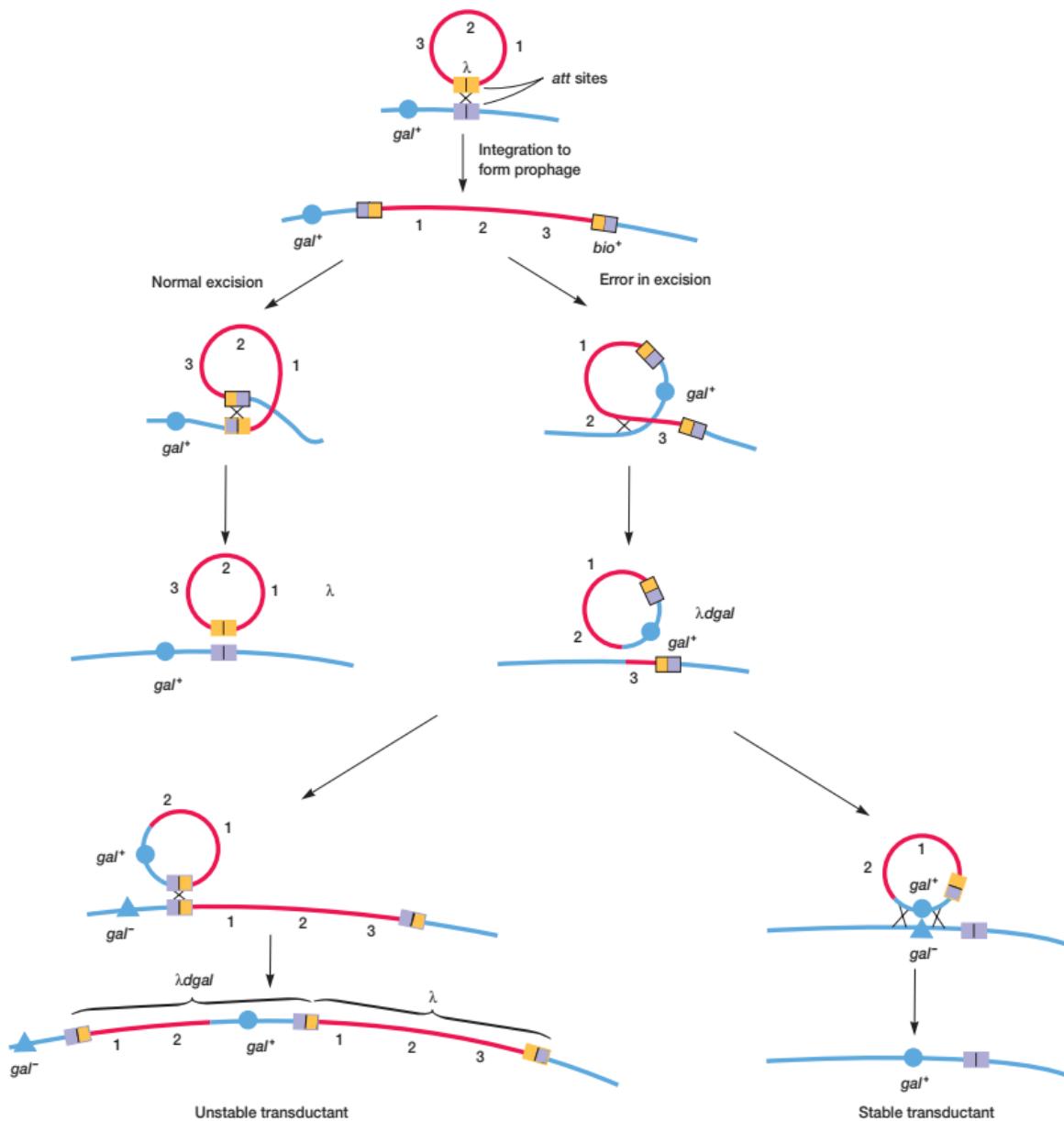


Figure 13.37 The Mechanism of Transduction for Phage Lambda and *E. coli*. Integrated lambda phage lies between the *gal* and *bio* genes. When it excises normally (top left), the new phage is complete and contains no bacterial genes. Rarely excision occurs asymmetrically (top right), and either the *gal* or *bio* genes are picked up and some phage genes are lost (only aberrant excision involving the *gal* genes is shown). The result is a defective lambda phage that carries bacterial genes and can transfer them to a new recipient.

The normal phage integrates, yielding two bacterial/phage hybrid *att* sites where the defective lambda *dgal* phage can insert (figure 13.37). It also supplies the genes missing in the defective phage. The normal phage in this instance is termed the **helper phage** because it aids integration and reproduction of the defective phage.

These transductants are unstable because the prophages can be induced to excise by agents such as UV radiation. Excision, however, produces a lysate containing a fairly equal mixture of defective lambda *dgal* phage and normal helper phage. Because it is very effective in transduction, the lysate is called a **high-frequency trans-**

duction lysate (HFT lysate). Reinfestation of bacteria with this mixture will result in the generation of considerably more transductants. LFT lysates and those produced by generalized transduction have one transducing particle in 10^5 or 10^6 phages; HFT lysates contain transducing particles with a frequency of about 0.1 to 0.5.

1. Briefly describe the lytic and lysogenic viral reproductive cycles. Define lysogeny, lysogen, temperate phage, prophage, and transduction.
2. Describe generalized transduction, how it occurs, and the way in which it was discovered. What is an abortive transductant?
3. What is specialized transduction and how does it come about? Distinguish between LFT and HFT lysates and describe how they are formed.
4. How might one tell whether horizontal gene transfer was mediated by generalized or specialized transduction?
5. Why doesn't a cell lyse after successful transduction with a temperate phage?
6. Describe how conjugation, transformation, and transduction are similar. How are they different?

13.10 MAPPING THE GENOME

Before the advent of genome sequencing, microbial geneticists only had one general approach for elucidating the organization of genes in a bacterial chromosome—to carry out **linkage analysis**. Such analyses yield a genetic map showing the position of genes relative to each other. Genetic mapping using linkage analysis is a very complex task. This section surveys approaches to mapping the bacterial genome, using *E. coli* as an example. All three modes of gene transfer and recombination have been used in mapping.

Hfr conjugation is frequently used to map the relative location of bacterial genes. This technique rests on the observation that during conjugation, the chromosome moves from donor to recipient at a constant rate. In an **interrupted mating experiment** the conjugation bridge is broken and Hfr \times F $^-$ mating is stopped at various intervals after the start of conjugation by mixing the culture vigorously in a blender (figure 13.38a). The order and timing of gene transfer can be determined because they are a direct reflection of the order of genes on the bacterial chromosome (figure 13.38b). For example, extrapolation of the curves in figure 13.38b back to the x-axis gives the time at which each gene just began to enter the recipient. The result is a circular chromosome map with distances expressed in terms of the minutes elapsed until a gene is transferred. This technique can fairly precisely locate genes 3 minutes or more apart. The heights of the plateaus in figure 13.38b are lower for genes that are more distant from the F factor (the origin of transfer) because there is an ever-greater chance that the conjugation bridge will spontaneously break before these genes are transferred. Because of the relatively large size of the *E. coli* genome, it is not possible to generate a map from one Hfr strain. Therefore several Hfr strains with the F plasmid integrated at different locations must be used and their maps superimposed on one another. The overall map is adjusted to 100 minutes, although complete transfer may require somewhat more than 100 minutes. In a sense, minutes are an indication of map distance and not strictly a measure of time. Zero time is set at the threonine (*thr*) locus.

Gene linkage, or the proximity of two genes on a chromosome, can be determined from transformation by measuring the frequency with which two or more genes simultaneously transform a recipient cell. Consider the case for **cotransformation** by two genes. In theory, a bacterium could simultaneously receive two genes, each carried on a separate DNA fragment. However, it is much more likely that genes residing on the same fragment will be simultaneously transferred. If two genes are closely linked on the chromosome, then they should be able to cotransform. The closer the genes are together, the more often they will be carried on the same fragment and the higher will be the frequency of cotransformation. If genes are spaced a great distance apart, they will be carried on separate DNA fragments and the frequency of double transformants will equal the product of the individual transformation frequencies.

Generalized transduction can be used to obtain linkage information in much the same way as transformation. Linkages usually are expressed as **cotransduction** frequencies, using the argument that the closer two genes are to each other, the more likely they both will reside on the DNA fragment incorporated into a single phage capsid. The *E. coli* phage P1 is often used in such mapping because it can randomly transduce up to 1 to 2% of the genome (figure 13.39).

Specialized transduction is used to find which phage attachment site is close to a specific gene. The relative locations of specific phage *att* sites are known from conjugational mapping, and the genes linked to each *att* site can be determined by means of specialized transduction. These data allow precise placement of genes on the chromosome.

A simplified genetic map of *E. coli*K12 is given in figure 13.40. Because conjugation data are not high resolution and cannot be used to position genes that are very close together, the map was developed using several mapping techniques. Interrupted mating data were combined with those from cotransduction and cotransformation studies. Data from recombination studies also were used. New genetic markers in the *E. coli* genome were located within a relatively small region of the genome (10 to 15 minutes long) using a series of Hfr strains with F factor integration sites scattered throughout the genome. Once the genetic marker was located with respect to several genes in the same region, its position relative to nearby neighbors was more accurately determined using transformation and transduction studies. Such analyses are no longer performed in *E. coli* and other microbes for which a genome sequence has been published.

Using these techniques, researchers mapped about 2,200 genes of *E. coli*K12 and compared this with the actual nucleotide sequence of the genome (i.e., a physical map of the genome). Genome sequencing has revealed about 4,300 possible genes. Thus genetic analysis defined over half of the potential genes. The genetic map approximates the physical map, but they do not correspond perfectly. This is because the genetic map is derived from genetic linkage frequencies that do not correlate exactly with the number of nucleotides that separate two genes. Roughly speaking, one minute of the *E. coli* genetic map corresponds to 40 kilobases of DNA sequence.

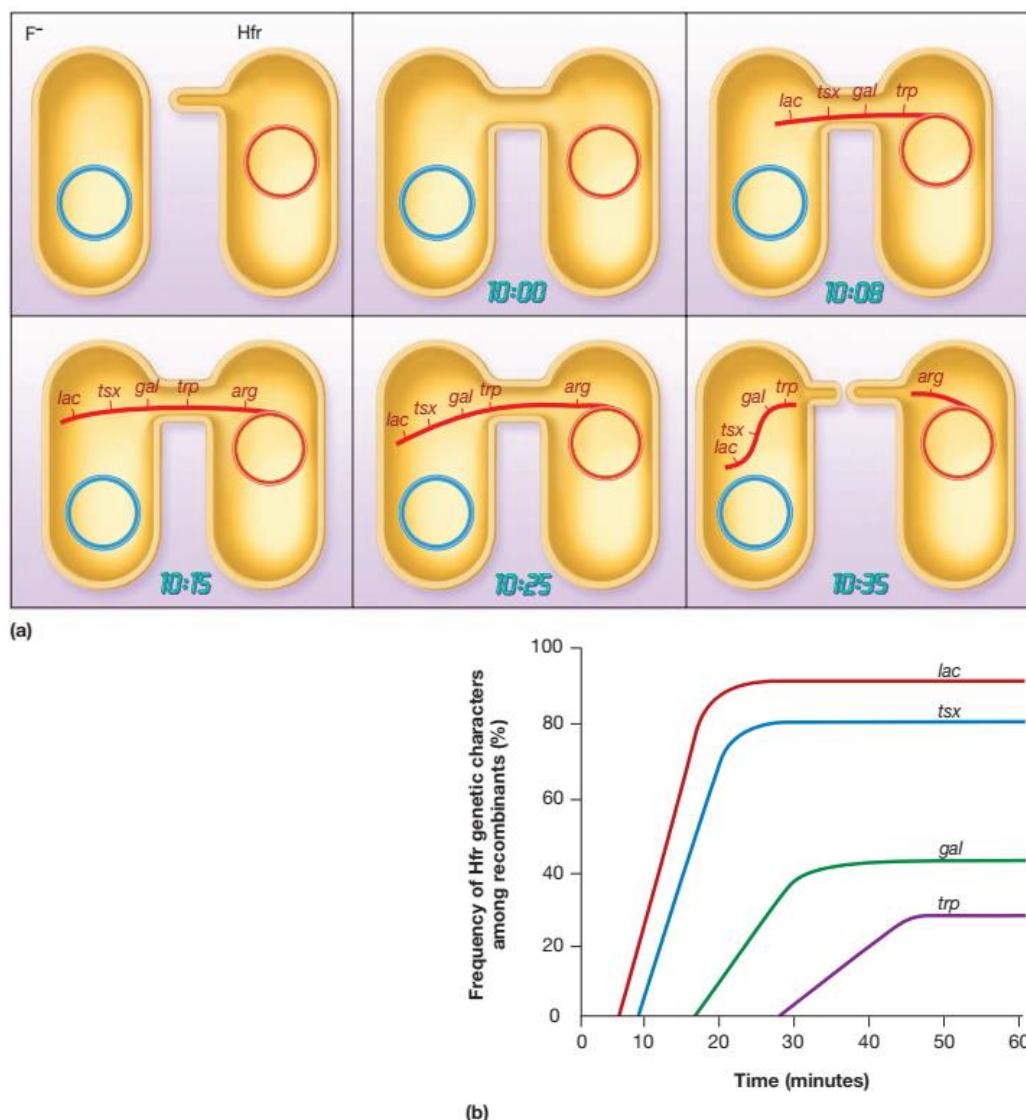


Figure 13.38 An Interrupted Mating Experiment. An interrupted mating experiment using Hfr × F⁻ conjugation. (a) The linear transfer of genes is stopped by breaking the conjugation bridge to study the sequence of gene entry into the recipient cell. (b) An example of the results obtained by an interrupted mating experiment. The gene order is lac-tsx-gal-trp.

13.11 RECOMBINATION AND GENOME MAPPING IN VIRUSES

Bacteriophage genomes also undergo recombination, although the process is different from that in bacteria. Because phages reproduce within cells and cannot recombine directly, crossing-over must occur inside a host cell. In principle, a virus recombination experiment is easy to carry out. If bacteria are mixed with enough phages so that on average at least two viruses will infect each cell, genetic recombination should be observed.

Phage progeny in the resulting lysate can be checked for alternate combinations of the initial parental genotypes.

Alfred Hershey initially demonstrated recombination in the phage T2, using two strains with differing phenotypes. Two of the parental strains in Hershey's crosses were *h*⁺ *r*⁺ and *hr* (figure 13.41). The gene *h* influences host range; when gene *h* changes, T2 infects different strains of *E. coli*. The *r* gene of phage T2 affects plaque morphology. Plaques are visible manifestations of the phage lytic cycle, when the host is cultured on a solid growth medium (figure 13.42a). Phages with the *r*⁺ gene

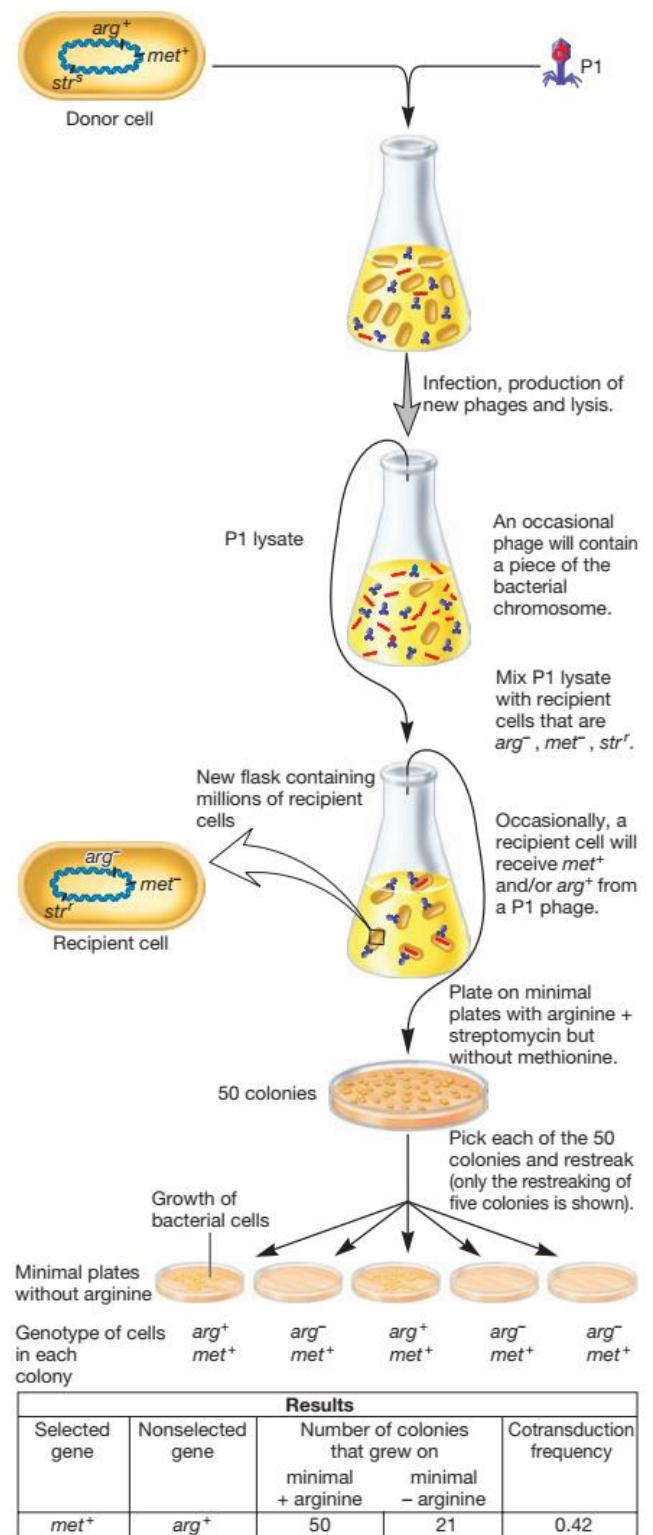


Figure 13.39 A Cotransduction Experiment. In this experiment, the donor is able to synthesize the amino acids arginine and methionine (arg^+ and met^+) but is killed by the antibiotic streptomycin (str^s). The recipient is unable to synthesize arginine and methionine, but is resistant to streptomycin (str^r). The phage lysate made by infecting the donor bacterium is mixed with the recipient bacterium. The mixture is then plated onto a medium containing streptomycin but lacking methionine. Therefore, the only cells able to grow are those recipient cells that have received the functional methionine gene from the donor. The colonies that grow are then tested to see if they also received the gene for arginine biosynthesis from the donor. This is determined by plating the cells on a minimal medium lacking arginine. Only those cells that can synthesize arginine grow.

Figure 13.40 *E. coli* Genetic Map. A circular genetic map of *E. coli* K12 with the location of selected genes. The inner circle shows the origin and direction of transfer of several Hfr strains. The map is divided into 100 minutes, the time required to transfer the chromosome from an Hfr cell to F⁻ at 37°C.

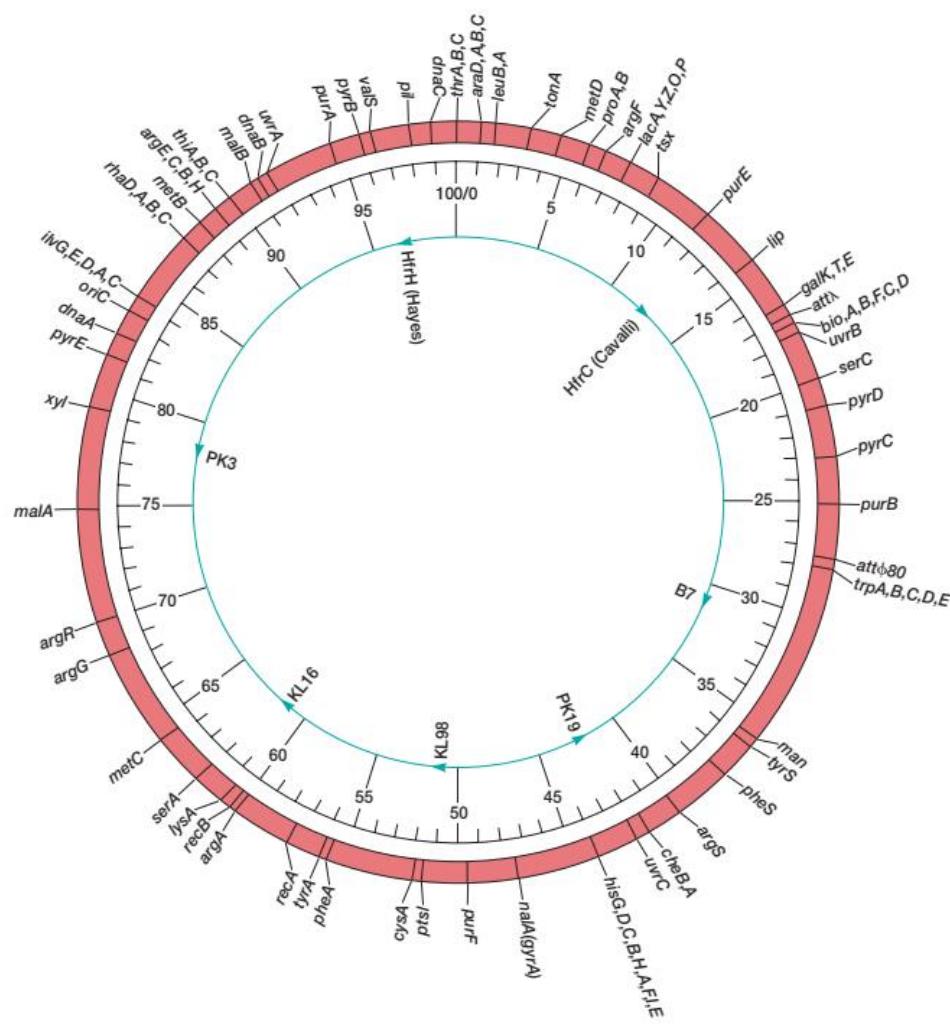
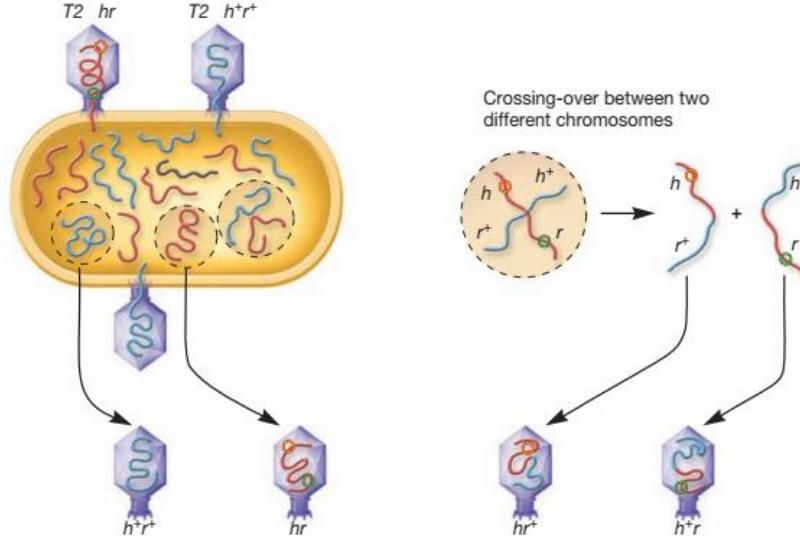


Figure 13.41 Genetic Recombination in Bacteriophages. A summary of a genetic recombination experiment with the *hr* and *h⁺r⁺* strains of the T2 phage. The *hr* chromosome is red; the *h⁺r⁺* chromosome is blue.



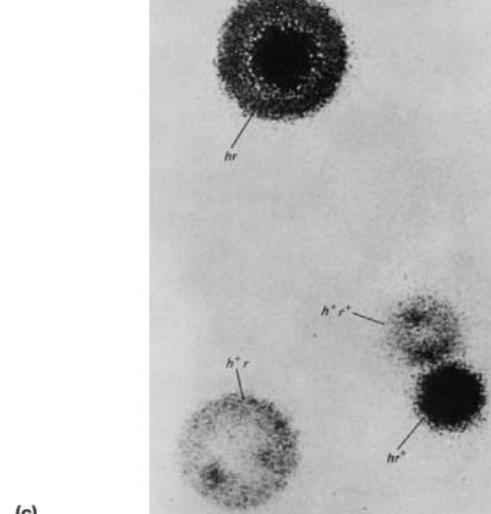
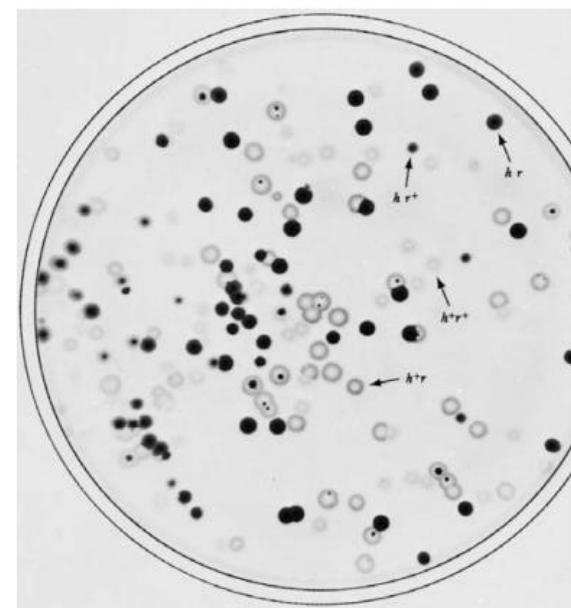
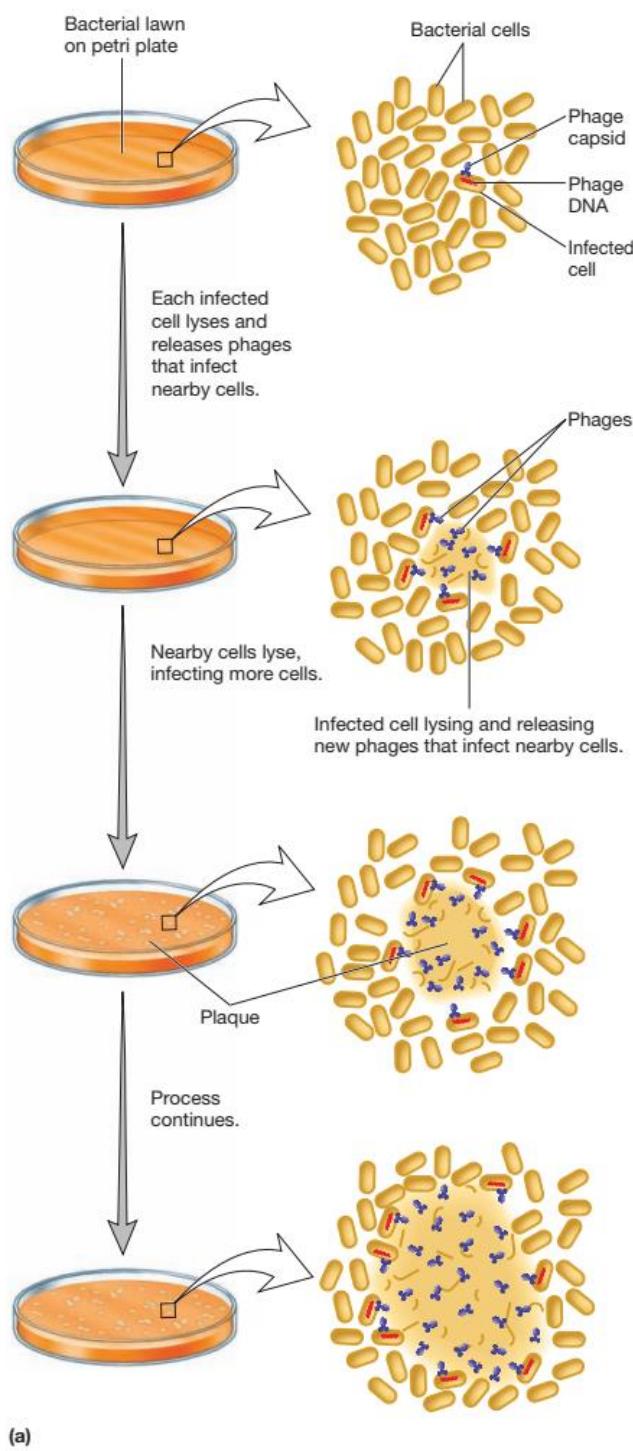


Figure 13.42 The Formation of Phage Plaques. (a) When phages and host bacterial cells are mixed at an appropriate ratio, only a portion of the cells will be initially infected. When this mixture is plated, the infected cells will be separated from each other. The infected cells eventually lyse, releasing progeny phages. They infect nearby cells, which eventually lyse, releasing more phages. This continues and ultimately gives rise to a clear area within a lawn of bacteria. The clear area is a plaque. (b) The types of plaques produced by a recombination experiment between T2 *hr* and T2 *h^rr⁺* on a lawn of *E. coli* cells. (c) A close-up of the four plaque types.

have wild type plaque morphology, whereas T2 with the *r* genotype has a rapid lysis phenotype and produces larger than normal plaques with sharp edges (figures 13.42b and 13.42c). In one experiment Hershey infected *E. coli* with large quantities of the *h⁺r⁺* and *hr* T2 strains (figure 13.41). He then plated out the lysates with a mixture of two different host strains and was able to detect significant numbers of *h⁺r* and *hr⁺* recombinants, as well as parental type plaques. As long as there are detectable phenotypes and methods for carrying out the crosses, it is possible to map phage genes in this way.

Phage genomes are so small that often it is convenient to map them without determining recombination frequencies. Some techniques actually generate physical maps, which often are most useful in genetic engineering. Several of these methods require manipulation of the DNA with subsequent examination in the electron microscope. For example, heteroduplex mapping involves direct comparison of wild-type and mutant viral chromosomes. The two chromosomes are denatured, mixed, and allowed to anneal due to base pairing. When annealed, the homologous regions of the different DNA molecules form a regular double helix. In locations where the bases do not pair due to the presence of a mutation such as a deletion or insertion, bubbles are visible in the electron microscope.

Several other direct techniques are used to generate physical maps of viral genomes or parts of them. Certain enzymes called restriction endonucleases can be used to cut viral DNA at specific sites. The fragments of DNA can be separated from each other based on size by gel electrophoresis—a process in which molecules move in an electrical field (see figures 14.3 and 14.11). By comparing genomes of different virus strains, deletions, insertions, and other mutations can be located. Phage genomes also can be directly sequenced to locate particular mutations and analyze the changes that have taken place.

1. Describe how the bacterial genome can be mapped using Hfr conjugation, transformation, generalized transduction, and specialized transduction. Include both a description of each technique and any assumptions underlying its use.
2. Why is it necessary to use several different techniques in genome mapping? How is this done in practice?
3. Describe how you would precisely locate the *recA* gene and show that it was between 58 and 58.5 minutes on the *E. coli* chromosome.
4. How does recombination in viruses differ from that in bacteria? How did Hershey first demonstrate virus recombination?
5. Describe heteroduplex mapping.

Summary

13.1 Mutations and Their Chemical Basis

- a. A mutation is a stable, heritable change in the nucleotide sequence of the genetic material.
- b. Spontaneous mutations can arise from replication errors (transition, transversion, and addition and deletion of nucleotides), from DNA lesions (apurinic sites, apyrimidinic sites, oxidation of DNA), and from insertions (figures 13.1 and 13.2).
- c. Induced mutations are caused by mutagens. Mutations may result from the incorporation of base analogs, specific mispairing due to alterations of a base caused by DNA-modifying agents, the presence of intercalating agents, and severe damage to the DNA caused by exposure to radiation.
- d. Mutations are usually recognized when they cause a change from the more prevalent wild-type phenotype. A mutant phenotype can be restored to wild type by either reversions or suppressor mutations (table 13.2).
- e. There are four important types of point mutations: silent mutations, missense mutations, nonsense mutations, and frameshift mutations (table 13.2).
- f. Mutations can affect phenotype in numerous ways. Some major types of mutations categorized based on their effects on phenotype are morphological, lethal, conditional, biochemical, and resistance mutations.

13.2 Detection and Isolation of Mutants

- a. A sensitive and specific detection method is needed for detecting and isolating mutants. An example is replica plating for the detection of auxotrophs (figure 13.7).
- b. One of the most effective mutant isolation techniques is to select for a specific mutation by adjusting environmental conditions so that the mutant will grow while the wild type does not.
- c. Because many carcinogens are also mutagenic, one can test for mutagenicity with the Ames test and use the results as an indirect indication of carcinogenicity (figure 13.9).

13.3 DNA Repair

- a. Cells have multiple mechanisms for correcting mispaired and damaged DNA.
- b. Excision repair systems remove damaged portions from a single strand of DNA (e.g., thymine dimers), and use the other strand as a template for filling in the gap (figures 13.10 and 13.11).
- c. Direct repair systems correct damaged DNA without removing damaged regions. For instance, during photoreactivation thymine dimers are repaired by splitting the two thymines apart. This is catalyzed in the presence of light by the enzyme photolyase (figure 13.12).
- d. Mismatch repair is similar to excision repair, except that it replaces mismatched base pairs (figure 13.13).
- e. Recombinational repair removes damaged DNA by recombination of the damaged DNA with a normal DNA strand elsewhere in the cell (figure 13.14).
- f. When DNA damage is severe, DNA replication is halted. This triggers the SOS response. During the SOS response, genes of the repair systems are transcribed at a higher rate. In addition, special DNA polymerases are produced. These are able to replicate damaged DNA. However, they do so without a proper template and therefore create mutations.

13.4 Creating Genetic Variability

- a. In recombination, genetic material from two different DNA molecules is combined to form a new hybrid molecule.
- b. In eukaryotes capable of sexual reproduction, crossing-over during meiosis is important in creating genetic variation (figure 13.15).
- c. Horizontal gene transfer is an important mechanism for creating genetic diversity in prokaryotes. It is a one-way process in which the exogenote is transferred from the donor to a recipient and integrated into the endogenote (figure 13.16).
- d. There are three types of recombination: homologous recombination, site-specific recombination, and transposition.

13.5 Transposable Elements

- a. Transposons or transposable elements are DNA segments that move about the genome in a process known as transposition.
- b. There are three types of transposable elements: insertion sequences, composite transposons, and replicative transposons (**figure 13.19**).
- c. Simple (cut-and-paste) transposition and replicative transposition are two distinct mechanisms of transposition (**figures 13.20** and **13.21**).
- d. Transposable elements can cause mutations, turn genes on and off, aid F plasmid insertion, and carry antibiotic resistance genes.

13.6 Bacterial Plasmids

- a. Plasmids are small, autonomously replicating DNA molecules that can exist independent of the host chromosome.
- b. Episomes are plasmids that can be reversibly integrated with the host chromosome.
- c. The F factor is one type of conjugative plasmid; that is, it is able to transfer itself from one bacterium to another (**figure 13.23**).

13.7 Bacterial Conjugation

- a. Conjugation is the transfer of genes between bacteria that depends upon direct cell-cell contact. F factor conjugation is mediated by a sex pilus and a type IV secretion system.
- b. In $F^+ \times F^-$ mating the F factor remains independent of the chromosome and a copy is transferred to the F^- recipient; donor genes are not usually transferred (**figure 13.28a**).
- c. Hfr strains transfer bacterial genes to recipients because the F factor is integrated into the host chromosome. A complete copy of the F factor is not often transferred (**figure 13.28b, c**).
- d. When the F factor leaves an Hfr chromosome, it occasionally picks up some bacterial genes to become an F' plasmid, which readily transfers these genes to other bacteria (**figure 13.30**).

13.8 DNA Transformation

- a. Transformation is the uptake of naked DNA by a competent cell and its incorporation into the genome (**figure 13.31** and **13.32**).

13.9 Transduction

- a. Bacterial viruses or bacteriophages can reproduce and destroy the host cell (lytic cycle) or become a latent prophage that remains within the host (lysogenic cycle) (**figure 13.34**).
- b. Transduction is the transfer of bacterial genes by viruses.
- c. In generalized transduction any host DNA fragment can be packaged in a virus capsid and transferred to a recipient (**figure 13.35**).
- d. Certain temperate phages carry out specialized transduction by incorporating bacterial genes during prophage induction and then donating those genes to another bacterium (**figure 13.37**).

13.10 Mapping the Genome

- a. The bacterial genome can be mapped by following the order of gene transfer during Hfr conjugation (**figure 13.38**); transformational and transductional mapping techniques also may be used (**figure 13.39**).

13.11 Recombination and Genome Mapping in Viruses

- a. When two viruses simultaneously enter a bacterial cell, their chromosomes can undergo recombination (**figure 13.41**).
- b. Virus genomes are mapped by recombination (genetic mapping). Physical maps can be created by heteroduplex mapping and other techniques.

Key Terms

abortive transductants	346	exogenote	330	lysogen	345	reversion mutation	320
adaptive (directed) mutation	319	F factor	336	lysogeny	345	sex pilus	338
allele	329	F' plasmid	339	lytic cycle	345	silent mutation	320
Ames test	325	forward mutation	320	merozygote	330	simple (cut-and-paste)	
apurinic site	319	frameshift mutation	323	mismatch repair system	326	transposition	334
apyrimidinic site	319	generalized transducing particle	345	missense mutation	320	site-specific recombination	331
auxotroph	323	generalized transduction	345	mutagen	318	SOS response	327
base analog	319	helper phage	348	mutation	317	specialized transduction	346
base excision repair	326	heteroduplex DNA	331	nonreciprocal homologous		spontaneous mutations	318
competent cell	343	Hfr conjugation	339	recombination	331	suppressor mutation	320
composite transposon	333	Hfr strain	339	nonsense mutation	321	temperate bacteriophage	345
conditional mutation	323	high-frequency transduction lysates	(HFT lysates) 348	nucleotide excision repair	326	transduction	345
conjugation	337	homologous recombination	331	photoreactivation	326	transformation	342
conjugative plasmid	334	horizontal (lateral) gene transfer	(HGT) 330	plasmid	334	transition mutation	319
conjugative transposon	334	host restriction	330	point mutation	318	translesion DNA synthesis	329
crossing-over	330	induced mutations	318	proofreading	326	transposable element	332
directed (adaptive) mutation	319	insertion sequence	332	prophage	345	transposase	333
direct repair	326	intercalating agent	320	prototroph	323	transposition	331
DNA methylation	326	interrupted mating experiment	349	RecA protein	327	transposon	332
DNA-modifying agent	320	lateral (horizontal) gene transfer	330	recombinants	329	transversion mutation	319
double-strand break model	331	low-frequency transduction lysate	(LFT lysates) 346	recombination	329	virulent bacteriophage	345
endogenote	330			recombinational repair	327	wild type	320
episome	334			replica plating	324		
excision repair	326			replicative transposition	334		

Critical Thinking Questions

- Mutations are often considered harmful. Give an example of a mutation that would be beneficial to a microorganism. What gene would bear the mutation? How would the mutation alter the gene's role in the cell, and what conditions would select for this mutant allele?
- Mistakes made during transcription affect the cell, but are not considered "mutations." Why not?
- Given what you know about the differences between bacterial and eucaryotic cells, give two reasons why the Ames test detects only about half of potential carcinogens, even when liver extracts are used.
- Diagram a double crossover event and a single crossover event. Which is more infrequent and why? Suggest experiments in which you would use one or the other event and what types of genetic markers you would employ. What kind of recognition features and catalytic capabilities would the recombination machinery need to possess?
- Suppose that transduction took place when a U-tube experiment was conducted. How would you confirm that something like a virus was passed through the filter and transduced the recipient?
- Suppose that you carried out a U-tube experiment with two auxotrophs and discovered that recombination was not blocked by the filter but was stopped by treatment with deoxyribonuclease. What gene transfer process is responsible? Why would it be best to use double or triple auxotrophs in this experiment?
- What would be the evolutionary advantage of having a period of natural "competence" in a bacterial life cycle? What would be possible disadvantages?

Learn More

- Barkay, T., and Smets, B. F. 2005. Horizontal gene flow in microbial communities. *ASM News* 71(9):412–19.
- Brock, T. D. 1990. *The emergence of bacterial genetics*. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press.
- Brooker, R. J. 2005. *Genetics: Analysis and principles*, 2d ed. Boston: McGraw-Hill.
- Foster, P. L. 2004. Adaptive mutation in *Escherichia coli*. *J. Bacteriol.* 186(15): 4846–52.
- Gogarten, J. P., and Townsend, J. P. 2005. Horizontal gene transfer, genome innovation and evolution. *Nature Rev. Microbiol.* 3:679–87.
- Grohmann, E.; Muth, G.; and Espinosa, M. 2003. Conjugative plasmid transfer in gram-positive bacteria. *Microbiol. Molec. Biol. Rev.* 67(2):277–301.
- Hahn, J.; Maier, B.; Hajjema, B. J.; Sheetz, M.; and Dubnau, D. 2005. Transformation proteins and DNA uptake localize to the cell poles in *Bacillus subtilis*. *Cell* 122:59–71.
- Lawley, T. D.; Klimke, W. A.; Gubbins, M. J.; and Frost, L. S. 2003. F factor conjugation is a true type IV secretion system. *FEMS Microbiol. Lett.* 224:1–15.
- Roth, J. R., and Andersson, D. I. 2004. Adaptive mutation: How growth under selection stimulates lac⁺ reversion by increasing target copy number. *J. Bacteriol.* 186(15):4855–60.
- Schröder, G., and Lanka, E. 2005. The mating pair formation system of conjugative plasmids—A versatile secretion machinery for transfer of proteins and DNA. *Plasmid* 54:1–25.
- Sutton, M. D.; Smith, B. T.; Godoy, V. G.; and Walker, G. C. 2000. The SOS response: Recent insights into *umuDC*-dependent mutagenesis and DNA damage tolerance. *Annu. Rev. Genet.* 34:479–97.

Please visit the Prescott website at www.mhhe.com/prescott7
for additional references.
