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 procaryotes. 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 Procaryotes

Unlike eucaryotes, procaryotes do not reproduce sexually, nor do they undergo meiosis. This would suggest that genetic variation in populations of procaryotes 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. Procaryotes 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 procaryotes 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 procaryotes to create recombinant organisms, the mechanisms of recombination at the molecular level are remarkably similar.

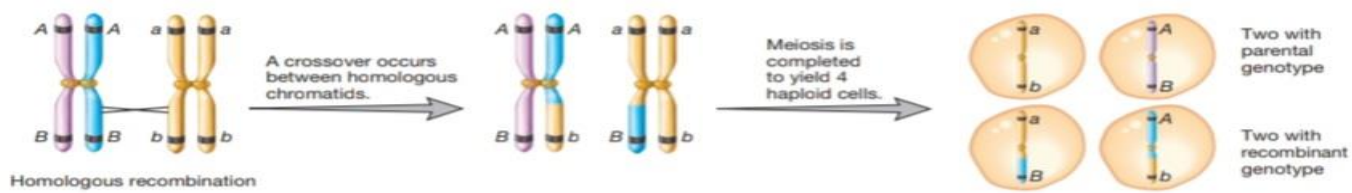


Figure 13.15 Recombination During Meiosis. During meiosis, homologous chromosomes pair and crossing-over can occur. The recombinant genotypes formed are inherited by progeny organisms, where they can result in recombinant phenotypes. Crossing-over involving similar DNA sequences is called homologous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

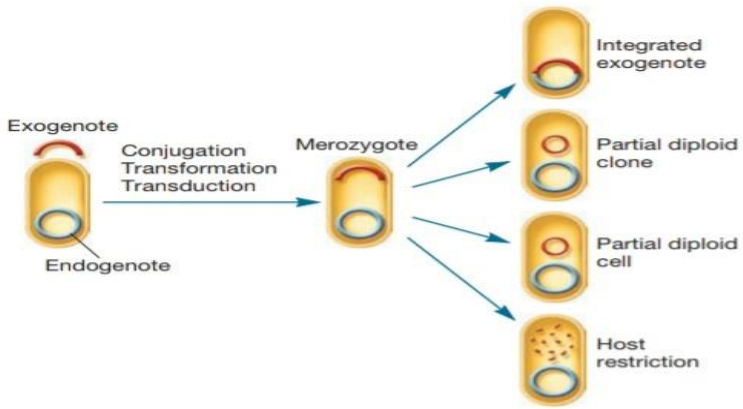


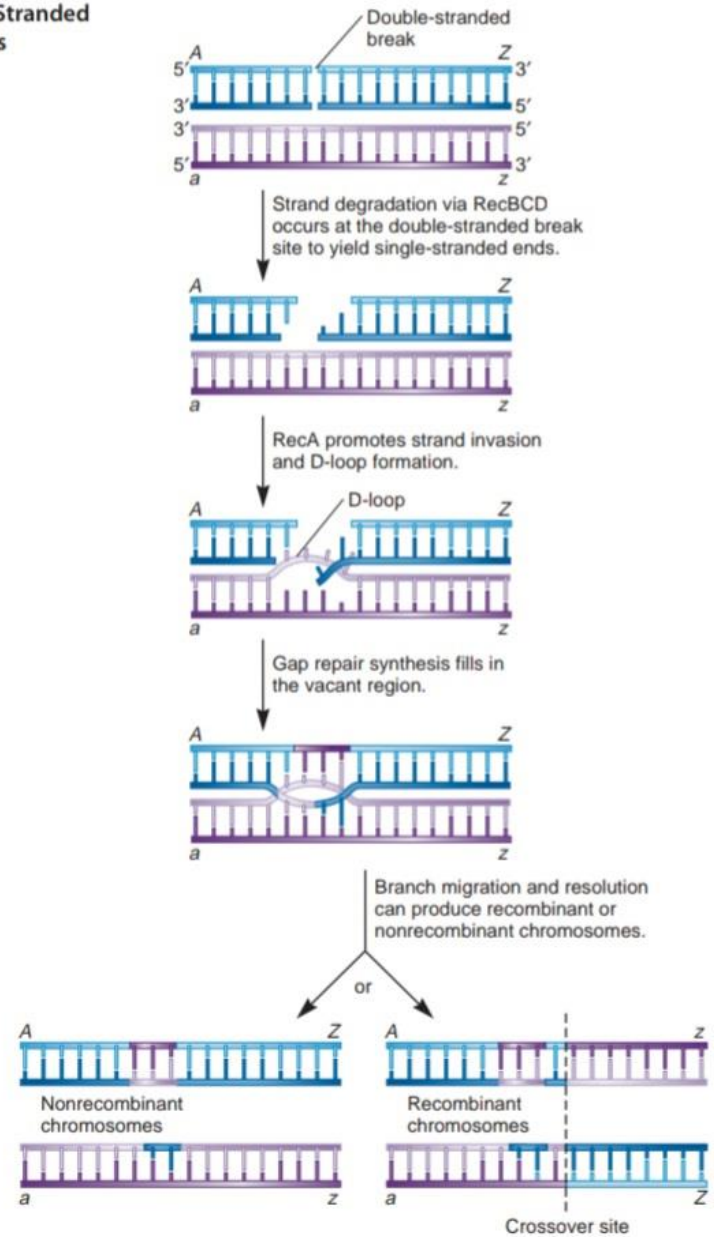
Figure 13.16 The Production and Fate of Merozygotes.
See text for discussion.

1. Define the following terms: recombination, crossing-over, homologous recombination, site-specific recombination, transposition, exogenote, endogenote, horizontal (lateral) gene transfer, merozygote, and host restriction.
2. Distinguish among the three forms of recombination mentioned in this section.
3. 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.



16.6 Bacterial Conjugation

After reading this section, you should be able to:

- Identify the type of plasmids that are important creators of genetic variation
- Describe the features of the F factor that allow it to (1) transfer itself to a new host cell and (2) integrate into a host cell's chromosome
- Outline the events that occur when an F^+ cell encounters an F^- cell
- Distinguish F^+, Hfr, and F′ cells from each other
- Explain how Hfr cells arise
- Outline the events that occur when an Hfr cell encounters an F^- cell

Conjugation, the transfer of DNA by direct cell-to-cell contact, depends on the presence of a **conjugative plasmid**. 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, replicate autonomously, and are stably inherited. Some plasmids are **episomes**, plasmids that can exist either with or without being integrated into host chromosomes.

Perhaps the best-studied conjugative plasmid is **F factor**. It plays a major role in conjugation in *E. coli*, and it was the first conjugative plasmid to be described (**figure 16.16**). The F factor is about 100,000 bases long and bears genes responsible for cell attachment and plasmid transfer between specific *E. coli* cells. Most

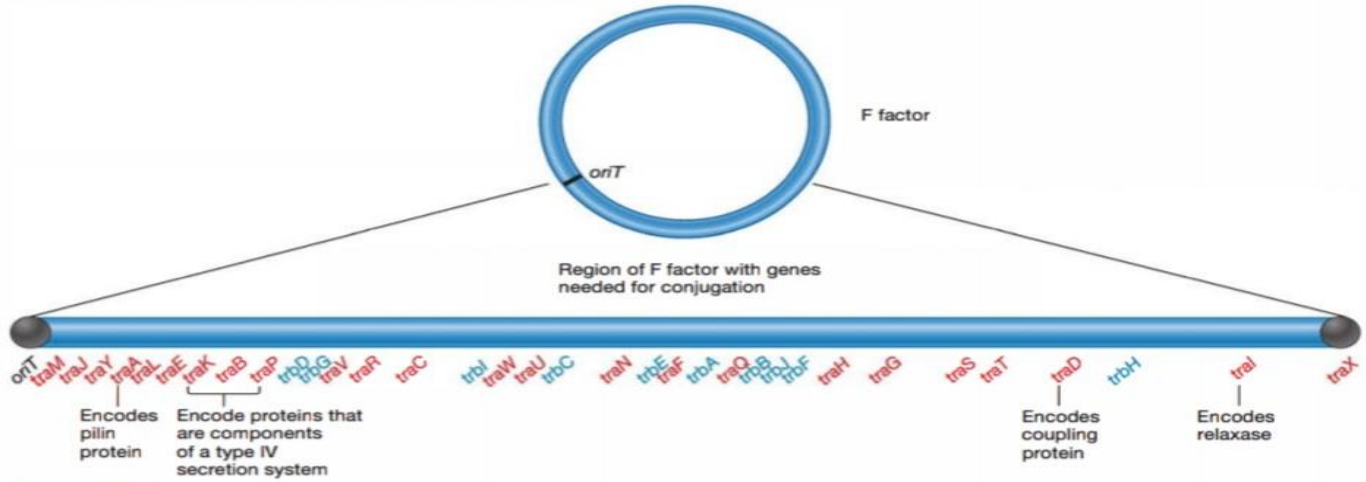


Figure 16.16 The F plasmid. Transfer (*tra*) genes are shown in red, and some of their functions are indicated. The plasmid also contains three insertion sequences and a transposon. The site for initiation of rolling-circle replication and gene transfer during conjugation is *oriT*.

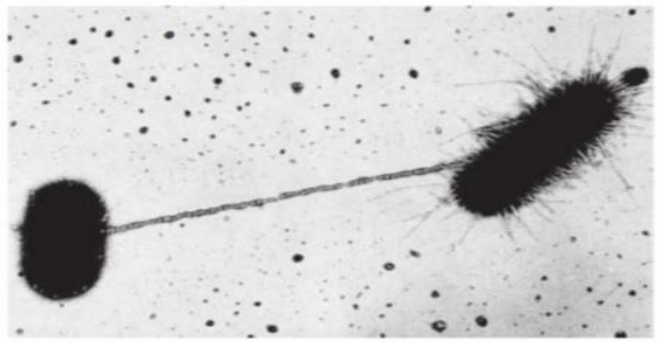


Figure 16.17 Bacterial Conjugation. An electron micrograph of two *E. coli* cells in an early stage of conjugation. The F^+ cell to the right is covered with fimbriae, and a sex pilus connects the two cells.

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 16.17**). Other gene products aid DNA transfer. In addition, the F factor has several IS elements that assist plasmid integration into the host cell's chromosome. Thus the F factor is an episome that can exist outside the bacterial chromosome or be integrated into it (**figure 16.18**).

The initial evidence for bacterial conjugation came from an elegant experiment performed in 1946 by Joshua Lederberg (1925–2008) and Edward Tatum (1909–1975). 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 a reversion or suppressor mutation, they used double and triple auxotrophs on the assumption that two or three simultaneous reversion or suppressor mutations would be extremely rare. When recombinant prototrophic colonies appeared on the minimal medium after incubation, they concluded that 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 several years later by Bernard Davis (1919–1994), who constructed a U-tube consisting of two pieces of curved glass tubing fused at the base to form a U shape with a glass filter between the halves. The filter allowed passage of media but not bacteria. The U-tube was filled with a growth medium and each side inoculated with a different auxotrophic strain of *E. coli*. During incubation, the medium was pumped back and forth through the filter to ensure medium exchange between the halves. When the bacteria were later plated on minimal medium, Davis discovered that if the two auxotrophic strains were separated from each other by the filter, gene transfer did not take place. Therefore direct contact was required for the recombination that Lederberg and Tatum had observed.

Bacterial Conjugation

$F^+ \times F^-$ Mating

In 1952 William Hayes (1913–1994) demonstrated that the gene transfer observed by Lederberg and Tatum was unidirectional. That is, there were definite donor (F^+, or fertile) and recipient (F^-, or nonfertile) strains, and gene transfer was nonreciprocal. He also

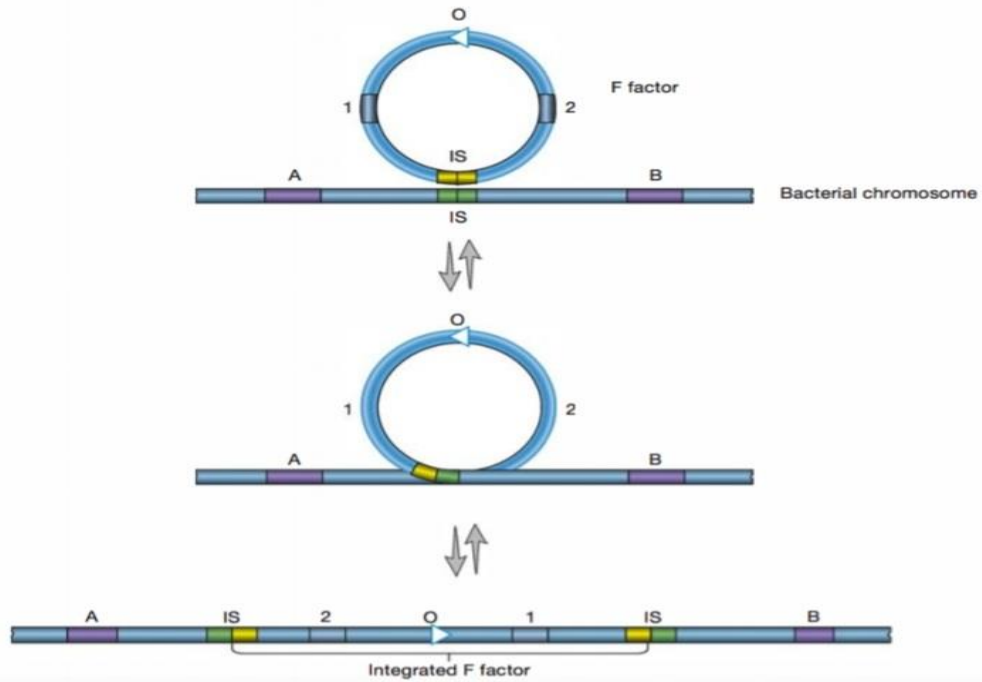


Figure 16.18 F Plasmid Integration. The reversible integration of an F plasmid or factor into a host bacterial chromosome. The process begins with association between plasmid and bacterial insertion sequences. The O arrowhead (white) indicates the site at which oriented transfer of chromosome to the recipient cell begins. A, B, 1, and 2 represent genetic markers.

MICRO INQUIRY *What does the term episome mean?*

found that in $F^+ \times F^-$ mating, the progeny were only rarely changed with regard to auxotrophy (i.e., chromosomal genes usually were not transferred). However, F^- strains frequently became F^+.

These results are now understood and readily explained in the following way. An 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 16.19**). Once contact is made, the pilus retracts, bringing the cells into close physical contact. The F^+ cell 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 16.20**). The F factor then replicates by rolling-circle replication. *Conjugation: Transfer of the F Plasmid*

During **rolling-circle replication,** one strand of the circular DNA is nicked, and the free 3′-hydroxyl end is extended by replication enzymes (**figure 16.21**). The 3′ end is lengthened while the growing point rolls around the circular template and the 5′ end of the strand is displaced to form an ever-lengthening tail, much like the peel of an apple is displaced by a knife as an apple is pared. The single-stranded tail may be converted to the double-stranded form by complementary strand synthesis. We are concerned here with rolling-circle replication of a plasmid. However, rolling-circle replication is also observed during the replication of some viral genomes (e.g., phage lambda). *Rolling-Circle Replication*

During conjugation, rolling-circle replication is initiated by a complex of proteins called the relaxosome, which are encoded by the F factor (figure 16.16). The relaxosome 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. During plasmid transfer, the entering strand is copied to produce double-stranded DNA. When this is completed, the F^- recipient cell becomes F^+.

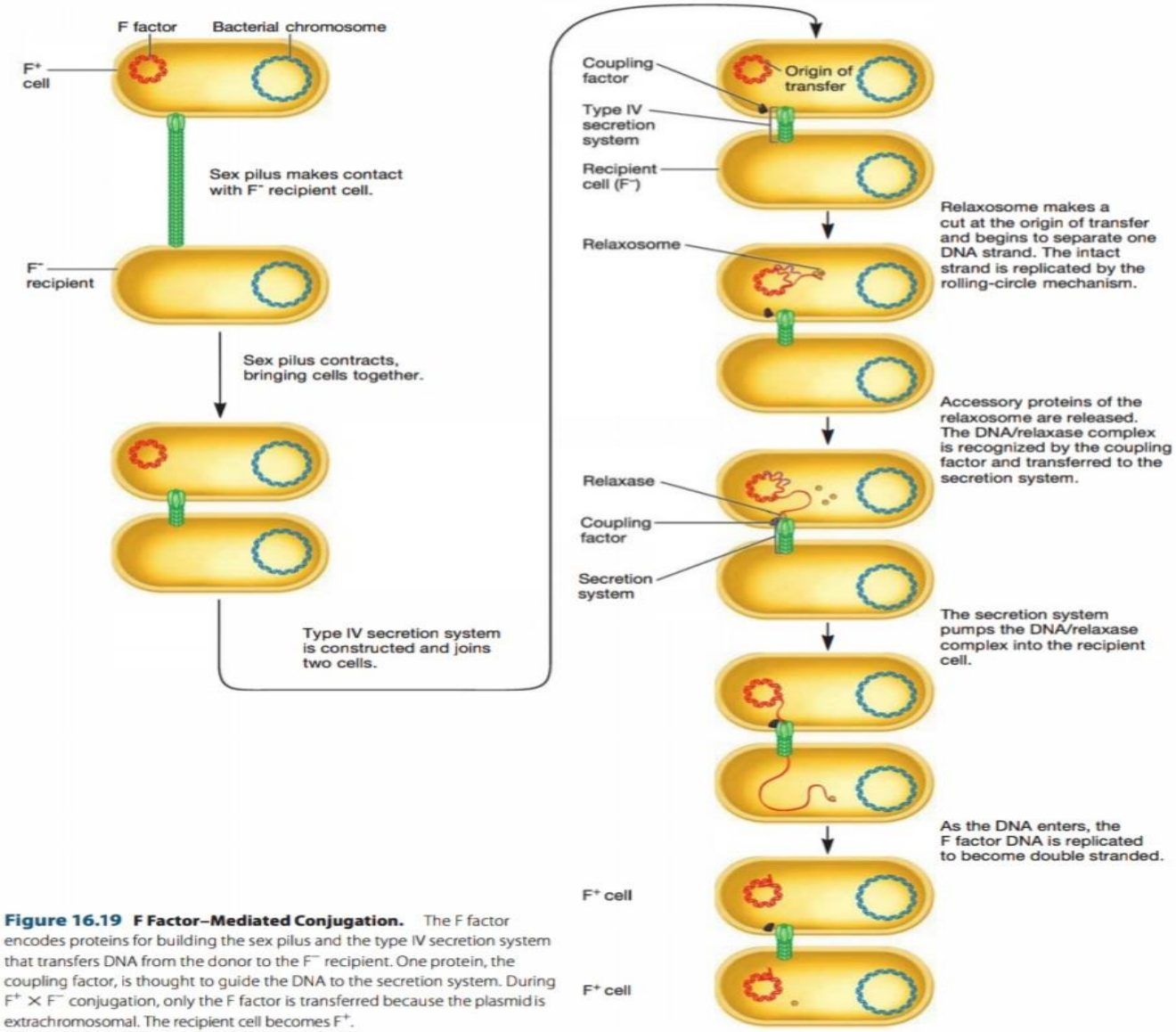


Figure 16.19 F Factor–Mediated Conjugation. The F factor encodes proteins for building the sex pilus and the type IV secretion system that transfers DNA from the donor to the F^- recipient. One protein, the coupling factor, is thought to guide the DNA to the secretion system. During $F^+ \times F^-$ conjugation, only the F factor is transferred because the plasmid is extrachromosomal. The recipient cell becomes F^+.

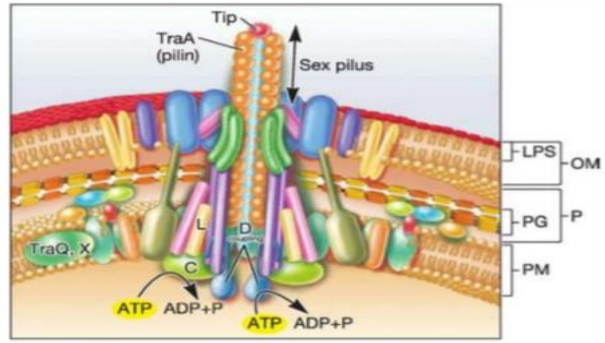


Figure 16.20 The Type IV Secretion System Encoded by F Factor. The F factor–encoded type IV secretion system is composed of numerous Tra proteins, including TraA proteins, which form the sex pilus, and TraD, which is the coupling factor. Some Tra proteins are located in the plasma membrane (PM); others extend into the periplasm (P) and pass through the peptidoglycan layer (PG) into the outer membrane (OM) and its lipopolysaccharide (LPS) layer.

Hfr Conjugation

By definition, an F^+ cell has the F factor free from the chromosome, so in an $F^+ \times F^-$ mating, chromosomal DNA is not transferred. However, within this population, a few cells have the F plasmid integrated into their chromosomes. This explains why 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 *h*igh *f*requency of recombinants produced by this mating, it is referred to as **Hfr conjugation** and the donor is called an **Hfr strain.**

Hfr strains contain the F factor integrated into their chromosome, rather than free in the cytoplasm (figure 16.22*a*). 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 just itself, the F factor also directs the transfer of the host chromosome. DNA transfer begins when the integrated F factor is nicked at *oriT*. As it is replicated, the F factor begins to move into the recipient (figure 16.22*b*). Initially only part of the F factor is transferred, followed by the donor's chromosome. If the cells remain connected, the entire chromosome with the rest of the integrated F factor will be transferred; this takes about 100 minutes to accomplish. However, the connection between the cells usually breaks before this process is finished. Thus a complete F factor is rarely transferred, and the recipient remains F^-.

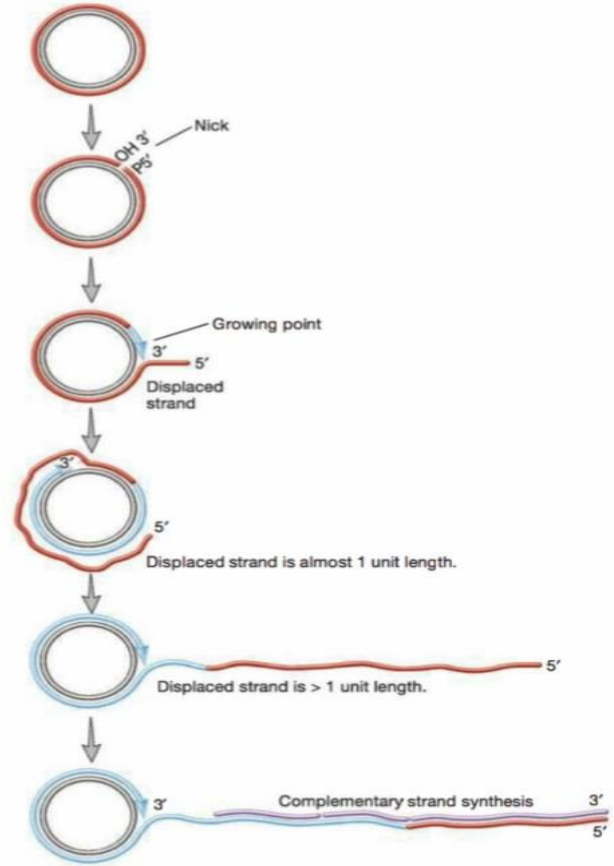


Figure 16.21 Rolling-Circle Replication. A single-stranded tail, often composed of more than one genome copy, is generated and can be converted to the double-stranded form by synthesis of a complementary strand. The "free end" of the rolling-circle strand is probably bound to the primosome. OH 3′ is the 3′-hydroxyl and P 5′ is the 5′-phosphate created when the DNA strand is nicked.

When an Hfr strain participates in conjugation, bacterial genes are transferred to the recipient in either a clockwise or a counterclockwise direction around a 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.

Transfer of Chromosomal DNA

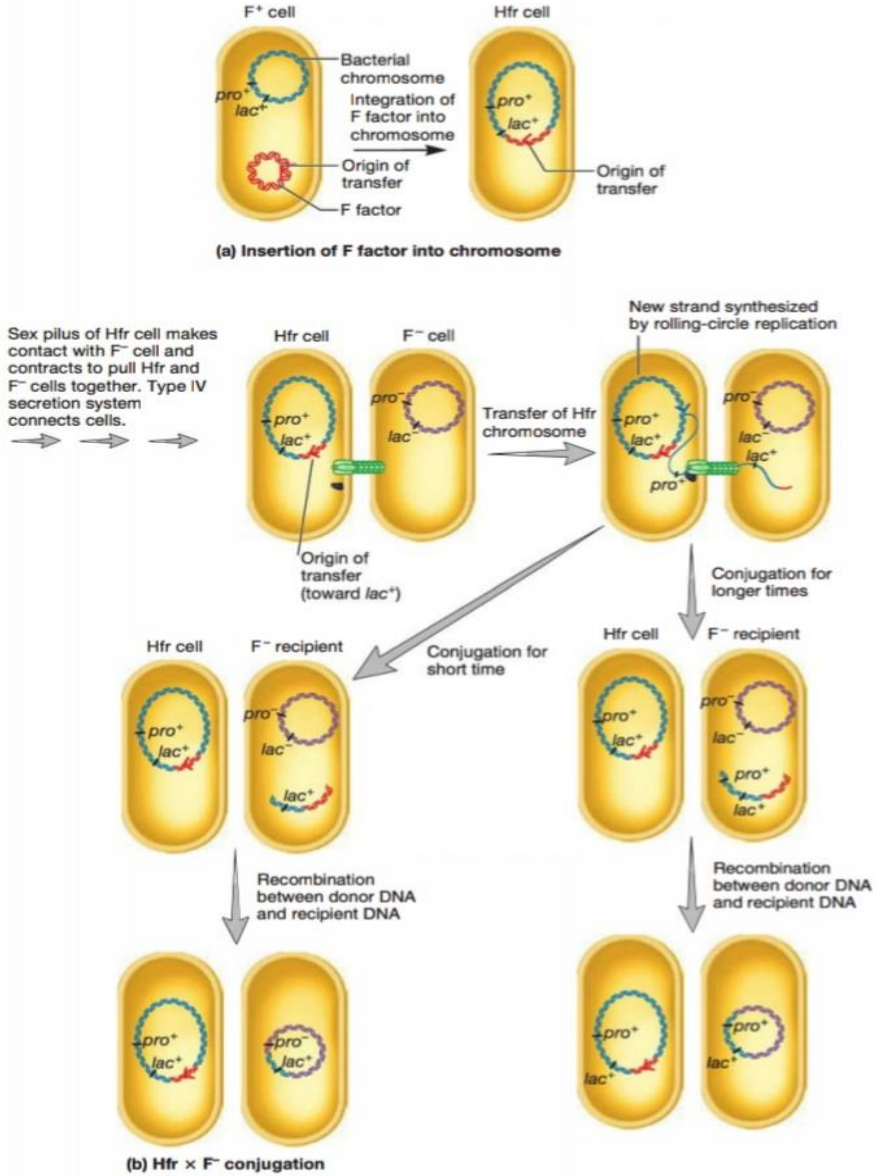


Figure 16.22 **Creation of Hfr Strains and Hfr × F⁻ Conjugation.** (a) Integration of the F factor into the donor cell's chromosome creates an Hfr cell. (b) During Hfr × F⁻ conjugation, some plasmid genes and some chromosomal genes are transferred to the recipient. Note that only a portion of the F factor 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.

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 excision an error occurs and a portion of the chromosome is excised, becoming part of the F plasmid.

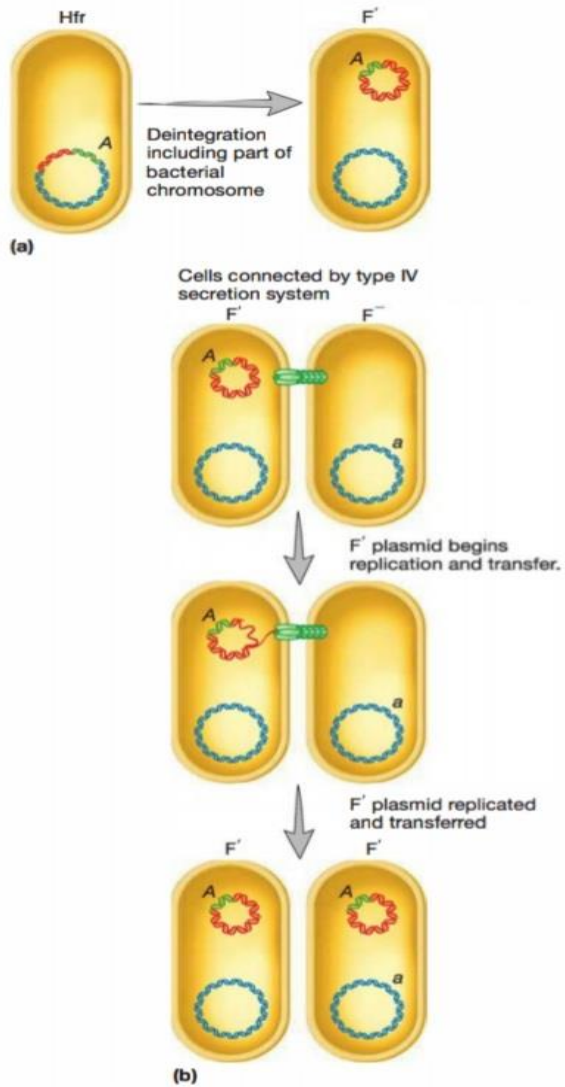


Figure 16.23 F′ Conjugation. (a) Due to an error in excision, the *A* gene of an Hfr cell is picked up by the F factor. (b) During conjugation, the *A* gene is transferred to a recipient, which becomes diploid for that gene (i.e., Aa).

Because this erroneously excised plasmid is larger and genotypically distinct from the original F factor, it is called an **F′ plasmid** (**figure 16.23*a***). 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, and $F' \times F^-$ conjugation is similar to an $F^+ \times F^-$ mating. Once again, the plasmid is transferred as it is copied by rolling-circle replication. Bacterial genes on the chromosome are not transferred (figure 16.23*b*), but bacterial genes on the F′ plasmid are transferred. These genes need not be incorporated into the recipient chromosome to be expressed. The recipient becomes F′ and is partially diploid 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.

Other Examples of Bacterial Conjugation

Although most research on plasmids and conjugation has been done using *E. coli* and other Gram-negative bacteria, conjugative plasmids are present in Gram-positive bacteria belonging to 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 plasmid-encoded proteins released by the activated donor cell. Plasmid transfer then occurs. *Transfer of a Plasmid*

Retrieve, Infer, Apply

1. What is bacterial conjugation and how was it discovered?
2. For F^+, Hfr, and F^- strains of *E. coli*, indicate which acts as a donor during conjugation, which acts as a recipient, and which transfers chromosomal DNA.
3. Describe how $F^+ \times F^-$ and Hfr conjugation processes proceed, and distinguish between the two in terms of mechanism and the final results.
4. Compare and contrast $F^+ \times F^-$ and $F' \times F^-$ conjugation.

16.7 Bacterial Transformation

After reading this section, you should be able to:

- Describe the factors that contribute to a bacterium being naturally transformation competent
- Predict the outcomes of transformation using a DNA fragment versus using a plasmid
- Design an experiment to transform bacteria that are not naturally competent with a plasmid that carries genes encoding ampicillin resistance and the protein that generates green fluorescence

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 DNA, either a plasmid or a fragment of linear DNA, from the surroundings and maintenance of the DNA in the recipient in a heritable form. In natural transformation, the DNA comes from a donor bacterium. The process is random, and any portion of the donor's genome may be transferred.

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 16.24*a***). 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 depends 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 milliliter. When a population becomes competent, bacteria such as *S. pneumoniae* secrete a small protein called competence factor that stimulates the production of eight to 10 new proteins required for transformation. Natural transformation has been discovered in some archaea and in members of several bacterial phyla (e.g., *Deinococcus-Thermus, Cyanobacteria, Chlorobi, Proteobacteria,* and *Firmicutes*). Natural transformation occurs in soil and aquatic ecosystems and in vivo during infection; it also is an important route of HGT in biofilm and other microbial communities.

The mechanism of transformation has been intensively studied in *S. pneumoniae* (**figure 16.25**). 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 5,000 to 15,000 base pairs 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 into the chromosome.

Transformation in *Haemophilus influenzae,* a Gram-negative bacterium, differs from that in *S. pneumoniae* in several respects. *H. influenzae* does not produce a protein factor to stimulate the development of competence, and it takes up DNA from only closely related species (*S. pneumoniae* and most other naturally competent bacteria are less particular about the source of the DNA). Double-stranded DNA, complexed with proteins, is taken in by membrane vesicles. The specificity of *H. influenzae* transformation is due to an 11 base pair sequence that is repeated over 1,400 times in the *H. influenzae* genome. 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 the bacterial cell wall. As expected,

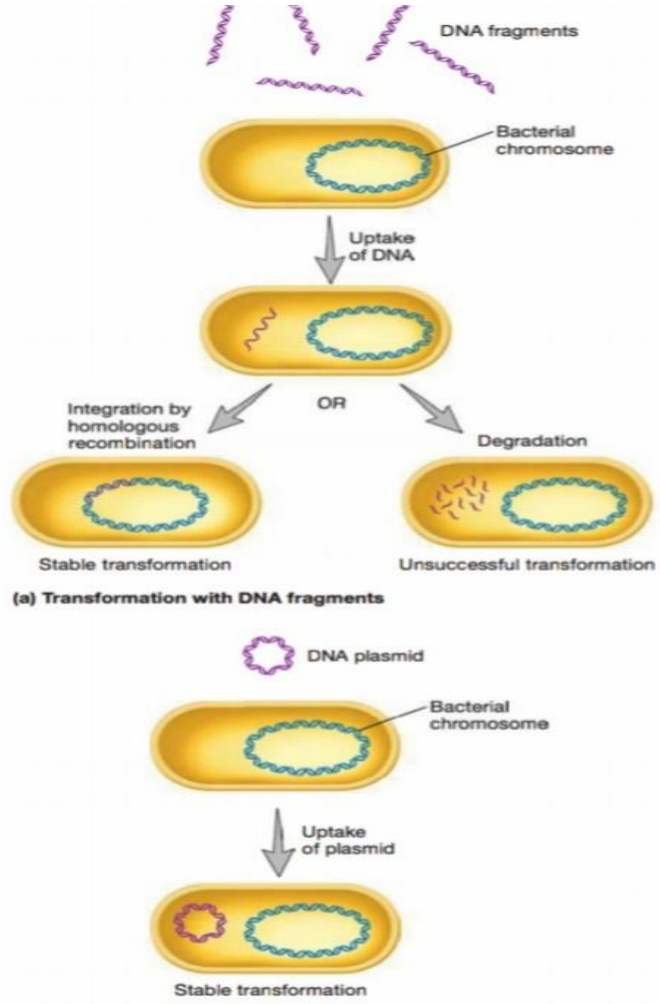


Figure 16.24 Bacterial Transformation. Transformation with (a) DNA fragments and (b) plasmids. Transformation with a plasmid often is induced artificially in the laboratory. The transforming DNA is in purple, and integration is at a homologous region of the genome.

the machinery is quite large and complicated. **Figure 16.26*a*** illustrates the complex used by the Gram-negative bacterium *Neisseria gonorrhoeae*. The protein 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

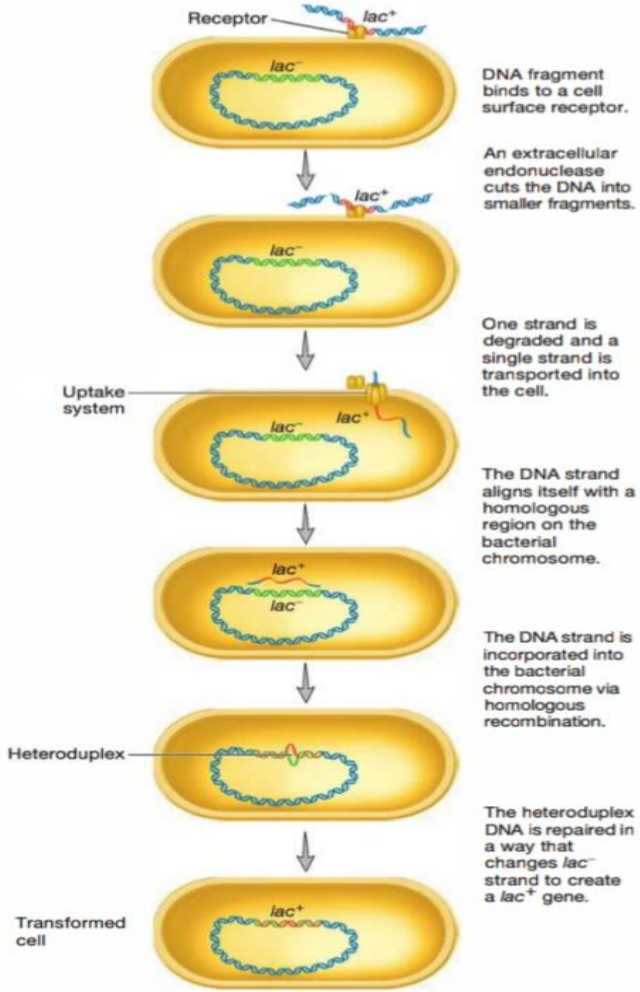


Figure 16.25 Bacterial Transformation as Seen in *S. pneumoniae.*

MICRO INQUIRY *According to this model, what would happen if DNA that lacked homology to the* S. pneumoniae *chromosome were taken into the cell?*

through the transmembrane channel formed by ComA. The machinery in the Gram-positive bacterium *Bacillus subtilis* is depicted in figure 16.26*b*. It is localized to the poles of the cell, and 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 cytoplasm. A Gram-negative equivalent of ComFA has not been identified yet in *N. gonorrhoeae.*

Bacterial Transformation

Microbial geneticists exploit transformation to move DNA (usually recombinant DNA) into cells. Because many species, including *E. coli,* are not naturally transformation competent, these bacteria must be made artificially competent by certain treatments. Two common techniques are electrical shock and exposure to calcium chloride. Both approaches render the cell membrane temporarily more permeable to DNA, and both are used to transform *E. coli* cells that have been made artificially competent. 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 can replicate within the host and are not as easily degraded as are linear fragments (figure 16.24*b*).

Introducing recombinant DNA into host cells (section 17.5)

Retrieve, Infer, Apply

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.

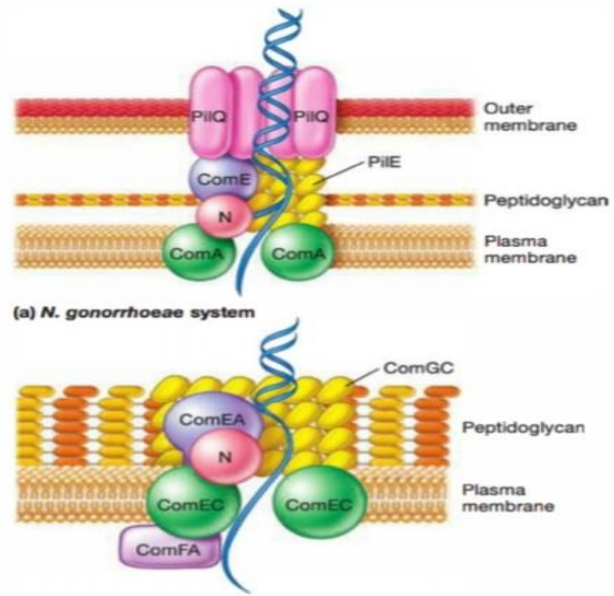


Figure 16.26 DNA Uptake Systems.

16.8 Transduction

After reading this section, you should be able to:

- Differentiate generalized transduction from specialized transduction
- Correlate a phage's life cycle to its capacity to mediate generalized or specialized transduction
- Draw a figure, create a concept map, or construct a table that distinguishes conjugation, transformation, and transduction

The third mode of bacterial gene transfer is **transduction**, which is mediated by viruses. It is a frequent mode of horizontal gene transfer in nature. Indeed evidence suggests that the number of genes moved by marine viruses from one host cell to another is huge (perhaps 10^{24} per year). Furthermore, viruses in marine environments and hot springs move genes between organisms in all three domains of life. ▶▶| *Aquatic viruses (section 30.2)*

Recall from chapter 6 that virus particles are structurally simple, often composed of just a nucleic acid genome protected by a protein coat called the capsid. Viruses are unable to multiply 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. **Virulent bacteriophages** multiply in their bacterial host immediately after entry. After the progeny phage particles reach a certain number, they cause the host to lyse, so they can be released and infect new host cells (**figure 16.27**). Thus this process is called the **lytic cycle. Temperate bacteriophages,** on the other hand, do not immediately kill their host. Many temperate phages enter the host bacterium and insert their genomes into the bacterial chromosome. The inserted 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. The relationship between these viruses and their host is called **lysogeny,** and 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 under certain conditions, including UV irradiation. When this occurs, the prophage is excised from the bacterial genome and the lytic cycle proceeds. |◀◀ *Types of viral infections (section 6.4)*

Transduction is the transfer of bacterial or archaeal genes by virus particles. It is important to understand that host genes are packaged in the virus particle because of errors made during the virus's life cycle. The virion containing these genes then transfers them to a recipient cell. Two kinds of bacterial transduction have been described: generalized and specialized.

Generalized Transduction

Generalized transduction occurs during the lytic cycle of virulent and some temperate phages. Any part of the bacterial genome

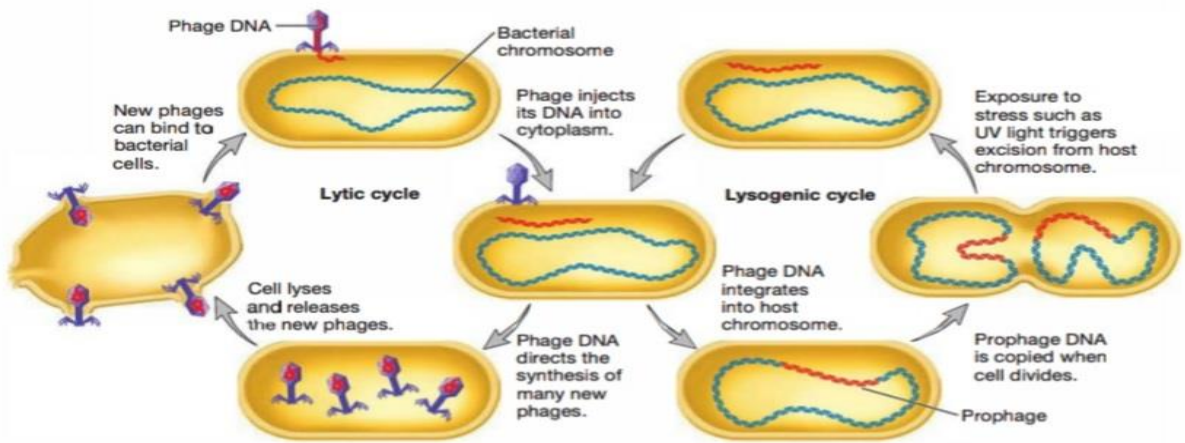
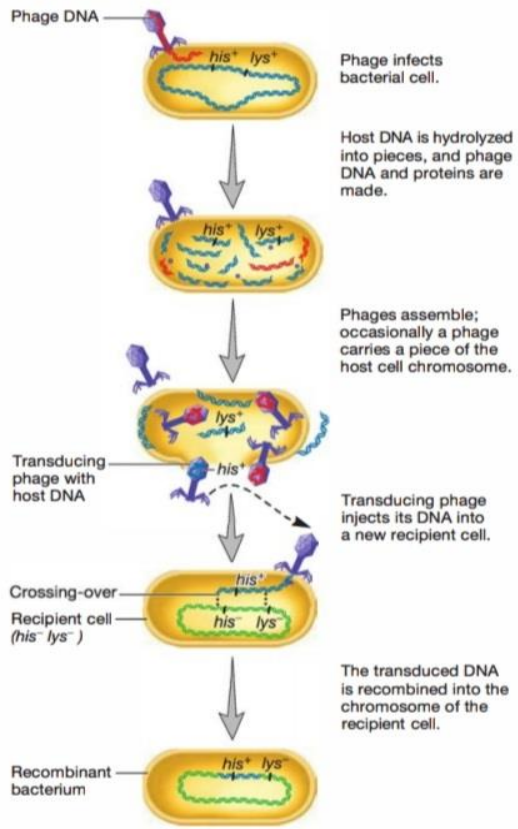


Figure 16.27 Lytic and Lysogenic Cycles of Temperate Phages. Virulent phages undergo only the lytic cycle. Temperate phages have two phases to their life cycles. The lysogenic cycle allows the genome of the virus to be replicated passively as the host cell's genome is replicated. Certain environmental factors such as UV light can cause a switch from the lysogenic cycle to the lytic cycle. In the lytic cycle, new virions are made and released when the host cell lyses.

MICRO INQUIRY *What is the term used to describe a lysogenic phage genome when it is integrated into the host genome?*

can be transferred (figure 16.28). During the assembly stage, when the viral chromosomes are packaged into capsids, random fragments of the partially degraded bacterial chromosome may mistakenly be packaged. 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 *S.* Typhimurium can carry about 1% of the bacterial genome; the P1 phage of *E. coli* and a variety of Gram-negative bacteria can package about 2.0 to 2.5% of the genome. The resulting virion often injects the DNA into another bacterial cell but cannot initiate a lytic cycle. This phage particle is known as a generalized transducing particle and is simply a carrier of genetic information from the original bacterium to another cell. As in transformation, once the DNA fragment has been injected, it must be incorporated into the recipient cell's chromosome to preserve the transferred genes. The DNA remains double stranded during transfer, and both strands are integrated into the recipient's chromosome. About 70 to 90% of the transferred DNA is not integrated but often is able to remain intact temporarily and be expressed. Abortive transductants are bacteria that contain this nonintegrated, transduced DNA and are partial diploids.



The recombinant bacterium has a genotype (*his*$^+$*lys*$^-$) that is different from recipient bacterial cell (*his*$^-$ *lys*$^-$).

Figure 16.28 Generalized Transduction in Bacteria.

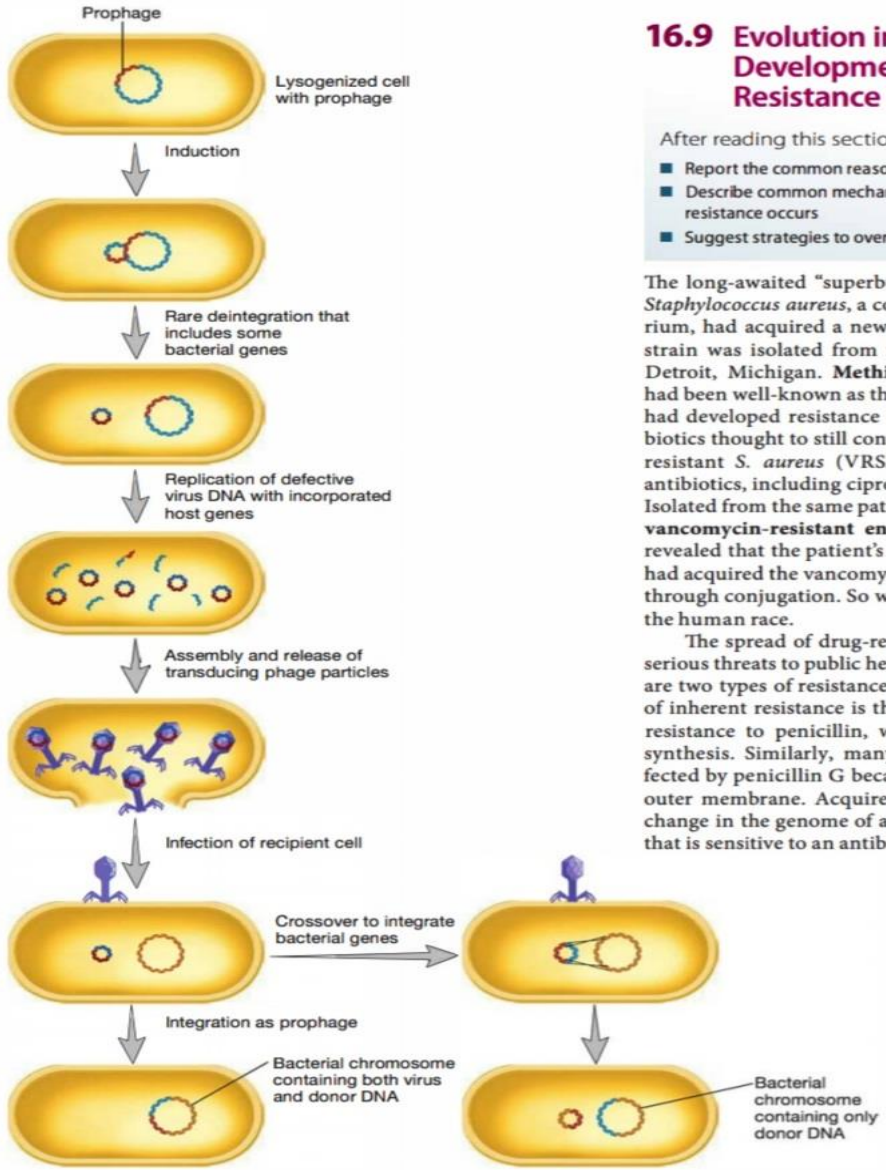
Specialized Transduction

In **specialized transduction,** only specific portions of the bacterial genome are carried by transducing particles. 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 16.29). However, the transducing particle is defective because it lacks some viral genes and cannot reproduce without assistance. In spite of this, it will inject the remaining viral genome and any bacterial genes it carries into another bacterium. 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 16.30). 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 when lambda excises incorrectly to generate a specialized transducing particle, these bacterial genes are most often present. The product of cell lysis (lysate) resulting from the induction of lysogenized *E. coli* contains normal phage and a few defective transducing particles. These particles are called lambda *dgal* if they carry the galactose utilization genes or lambda *dbio* if they carry the *bio* from the other side of the *att* site (figure 16.30). ▶▶| *Bacteriophage lambda (section 27.2)* ❁ *Specialized Transduction*

Retrieve, Infer, Apply

1. Describe generalized transduction and how it occurs. What is an abortive transductant?
2. What is specialized transduction and how does it come about?
3. How might one tell whether horizontal gene transfer was mediated by generalized or specialized transduction?
4. Why doesn't a cell lyse after successful transduction with a temperate phage?
5. Describe how conjugation, transformation, and transduction are similar. How are they different?



16.9 Evolution in Action: The Development of Antibiotic Resistance in Bacteria

After reading this section, you should be able to:

- Report the common reasons for increasing drug resistance
- Describe common mechanisms by which antimicrobial drug resistance occurs
- Suggest strategies to overcome drug resistance

The long-awaited "superbug" arrived in the summer of 2002. *Staphylococcus aureus*, a common but sometimes deadly bacterium, had acquired a new antibiotic-resistance gene. The new strain was isolated from foot ulcers on a diabetic patient in Detroit, Michigan. **Methicillin-resistant *S. aureus* (MRSA)** had been well-known as the bane of hospitals. This newer strain had developed resistance to vancomycin, one of the few antibiotics thought to still control *S. aureus*. This new vancomycin-resistant *S. aureus* (VRSA) strain also resisted most other antibiotics, including ciprofloxacin, methicillin, and penicillin. Isolated from the same patient was another dread of hospitals—**vancomycin-resistant enterococci (VRE)**. Genetic analyses revealed that the patient's own vancomycin-sensitive *S. aureus* had acquired the vancomycin-resistance gene, *vanA*, from VRE through conjugation. So was born a new threat to the health of the human race.

The spread of drug-resistant pathogens is one of the most serious threats to public health in the twenty-first century. There are two types of resistance: inherent and acquired. An example of inherent resistance is that of the cell wall-less mycoplasma's resistance to penicillin, which interferes with peptidoglycan synthesis. Similarly, many Gram-negative bacteria are unaffected by penicillin G because it cannot penetrate the bacterial outer membrane. Acquired resistance occurs when there is a change in the genome of a bacterium that converts it from one that is sensitive to an antibiotic to one that is now resistant. This

Figure 16.29 Specialized Transduction by a Temperate Bacteriophage. Recombination can produce two types of transductants.

MICRO INQUIRY *Compare the number of transducing particles that arise during generalized (figure 16.28) and specialized transduction. Why is there such a big difference?*

section describes the ways in which bacteria acquire drug resistance and how resistance spreads within a bacterial population. ⏮ *Synthesis of peptidoglycan (section 12.4); Typical Gram-negative cell walls (section 3.4);* ⏭ *Class* Mollicutes *(Phylum* Tenericutes*; section 21.3)*

Mechanisms of Drug Resistance

Bacteria have evolved several resistance mechanisms (**figure 16.31**). One is to modify the target of the antibiotic by mutating a gene that functions in the synthesis of the target. This is possible because each chemotherapeutic agent acts on a specific target enzyme or cellular structure. For instance, resistance to vancomycin arises when bacteria change the terminal D-alanine in the pentapeptide of peptidoglycan into either D-lactate or D-serine (*see figure 12.10*). The affinity of ribosomes for erythromycin and chloramphenicol also can be decreased by a change in the 23S rRNA to which they bind. Antimetabolite action may be resisted through alteration of susceptible enzymes. For example, in sulfonamide-resistant bacteria, the enzyme that uses *p*-aminobenzoic acid during folic acid synthesis often has a much lower affinity for sulfonamides. ⏮ *Antibacterial drugs (section 9.4)*

A second resistance strategy is **drug inactivation.** The best-known example is the hydrolysis of the β-lactam ring of penicillins by penicillinase and other β-lactamase enzymes. Drugs also are inactivated by the addition of chemical groups. For example, chloramphenicol contains two hydroxyl groups that can be modified by the addition of acetyl-CoA. Aminoglycosides (*see figure 9.8a*) can be modified and inactivated in several ways. For instance, acetyltransferases catalyze the acetylation of amino groups. Some aminoglycoside-modifying enzymes catalyze the addition of phosphates (by phosphotransferases) or adenyl groups (by adenyl transferases) to hydroxyl groups present on the aminoglycoside. Because they involve gaining a new function, this resistance strategy is most often accomplished by the acquisition of a gene (or genes) by HGT.

Some bacteria pump the drug out of the cell after it has entered, using translocases, often called **efflux pumps**, that expel drugs. Efflux pumps are relatively nonspecific and pump many different drugs; therefore, they often confer multidrug-resistance. Many efflux pumps are drug/proton antiporters; that is, protons enter the cell as the drug leaves. Such systems are present in *E. coli, Pseudomonas aeruginosa*, and *S. aureus*, to name a few.

Finally, resistant bacteria may either use an **alternate pathway** to bypass the sequence inhibited by the agent or increase the production of the target metabolite. For example, some bacteria are resistant to sulfonamides simply because they use preformed folic acid from their surroundings, rather than synthesize it themselves. Other strains increase their rate of folic acid production and thus counteract sulfonamide inhibition. Again, these changes are most often mediated by HGT.

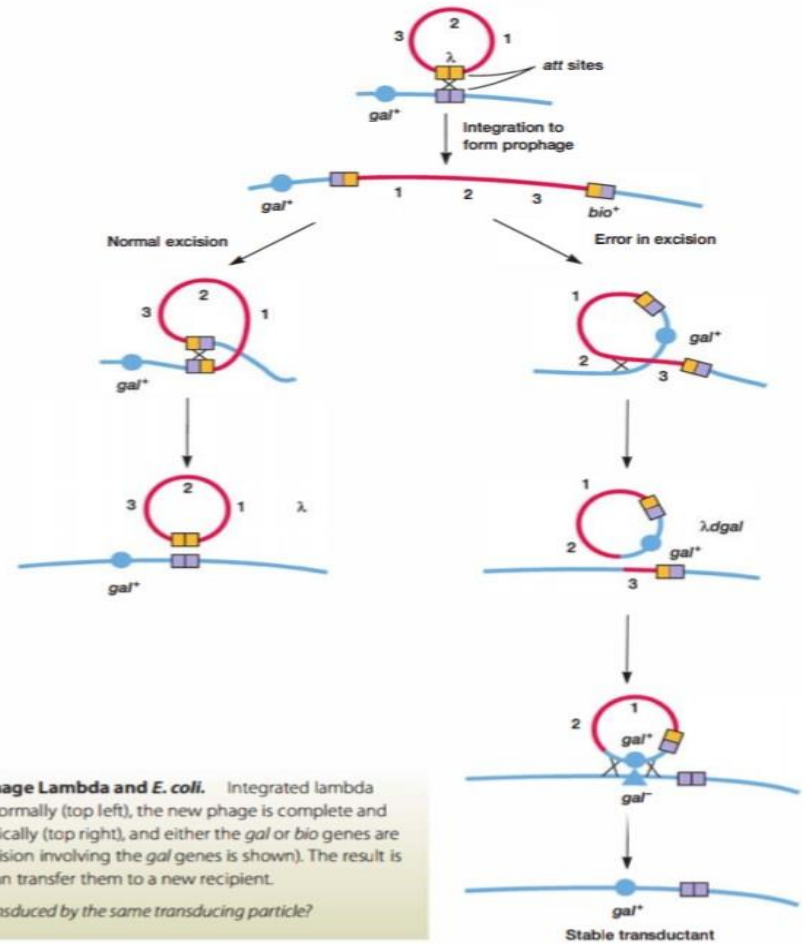


Figure 16.30 The Mechanism of Transduction for Phage Lambda and *E. coli*. Integrated lambda phage lies between the *gal* and *bio* genes. When it excises normally (top left), the new phage is complete and contains no bacterial genes. Excision rarely occurs asymmetrically (top right), and either the *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.

MICRO INQUIRY *Why can't the* gal *and* bio *genes be transduced by the same transducing particle?*