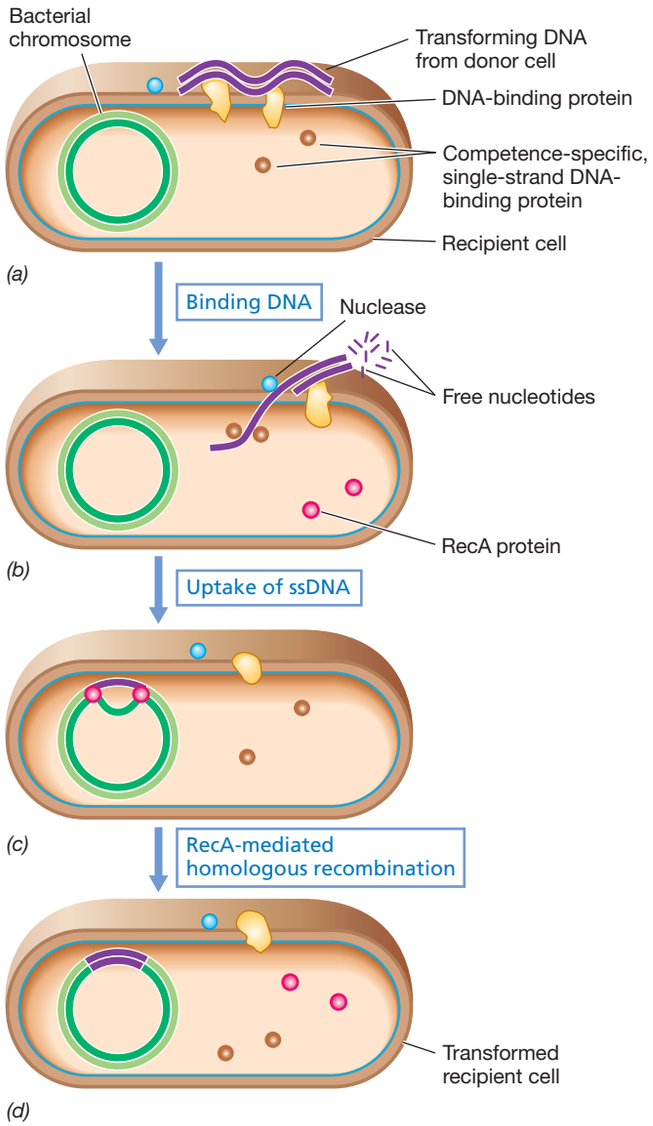

Figure 10.13 Mechanism of transformation in a gram-positive bacterium. *(a)* Binding of double-stranded DNA by a membrane-bound DNA-binding protein. *(b)* Passage of one of the two strands into the cell while nuclease activity degrades the other strand. *(c)* The single strand in the cell is bound by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

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

Transfection

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

MiniQuiz

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

UNIT 4

10.8 Transduction

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

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

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

Generalized Transduction

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

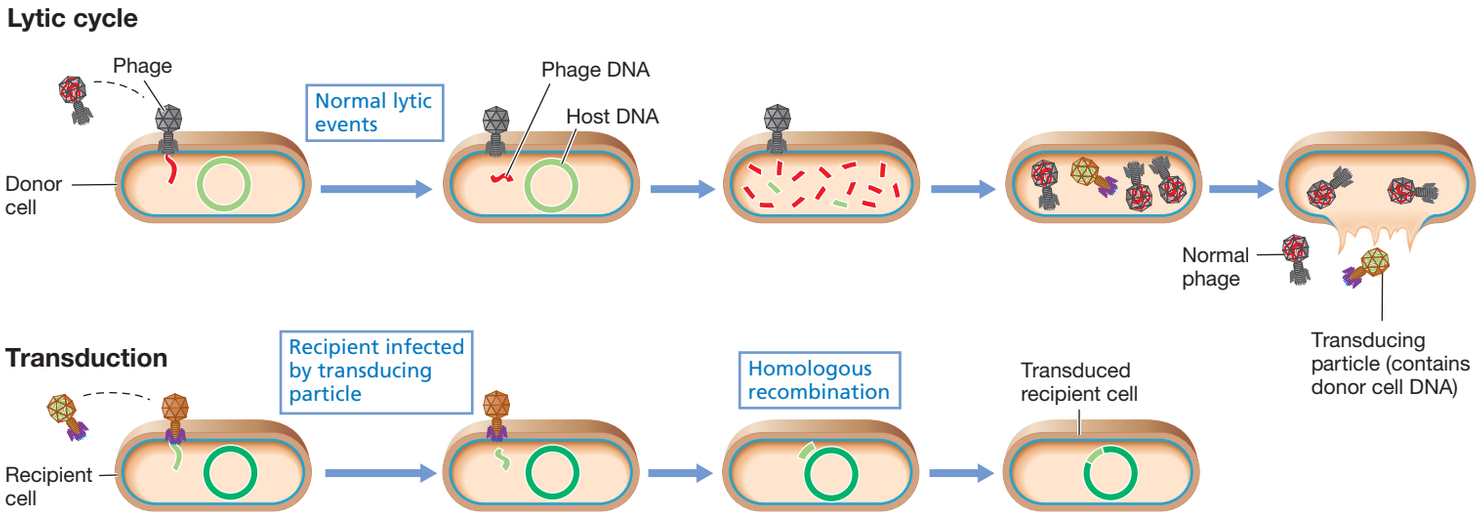

Figure 10.14 **Generalized transduction.** Note that "normal" virions contain phage genes, whereas a transducing particle contains host genes.

transducing particles are formed is given in **Figure 10.14**. When a bacterial cell is infected with a phage, the lytic cycle may occur. However, during lytic infection, the enzymes responsible for packaging viral DNA into the bacteriophage sometimes package host DNA accidentally. The result is called a *transducing particle*. These cannot lead to a viral infection because they contain no viral DNA, and are said to be *defective*. On lysis of the cell, the transducing particles are released along with normal virions that contain the virus genome. Consequently, the lysate contains a mixture of normal virions and transducing particles.

When this lysate is used to infect a population of recipient cells, most of the cells are infected with normal virus. However, a small proportion of the population receives transducing particles that inject the DNA they packaged from the previous host bacterium. Although this DNA cannot replicate, it can recombine with the DNA of the new host. Because only a small proportion of the particles in the lysate are defective, and each of these contains only a small fragment of donor DNA, the probability of a given transducing particle containing a particular gene is quite low. Typically, only about 1 cell in 10^6 to 10^8 is transduced for a given marker.

Phages that form transducing particles can be either temperate or virulent, the main requirements being that they have a DNA-packaging mechanism that accepts host DNA and that DNA packaging occurs before the host genome is completely degraded. Transduction is most likely when the ratio of input phage to recipient bacteria is low so that cells are infected with only a single phage particle; with multiple phage infection, the cell is likely to be killed by the normal virions in the lysate.

Phage Lambda and Specialized Transduction

Generalized transduction allows the transfer of any gene from one bacterium to another, but at a low frequency. In contrast, specialized transduction allows extremely efficient transfer, but is selective and transfers only a small region of the bacterial chromosome. In the first case of specialized transduction to be discovered, the galactose genes were transduced by the temperate phage lambda of *E. coli*.

When lambda lysogenizes a host cell, the phage genome is integrated into the *E. coli* chromosome at a specific site (↻ Section 9.10). This site is next to the cluster of genes that encode the enzymes for galactose utilization. After insertion, viral DNA replication is under control of the bacterial host chromosome. Upon induction, the viral DNA separates from the host DNA by a process that is the reverse of integration (**Figure 10.15**). Usually, the lambda DNA is excised precisely, but occasionally, the phage genome is excised incorrectly. Some of the adjacent bacterial genes to one side of the prophage (for example, the galactose operon) are excised along with phage DNA. At the same time, some phage genes are left behind (Figure 10.15*b*).

One type of altered phage particle, called *lambda dgal* (λ*dgal*; *dgal* means "*d*efective *gal*actose"), is defective because of the lost phage genes. It will not make a mature phage in a subsequent infection. However, a viable lambda virion known as a helper phage can provide those functions missing in the defective particle. When cells are coinfected with λ*dgal* and the helper phage, the culture lysate contains a few λ*dgal* particles mixed in with a large number of normal lambda virions. When a galactose-negative (Gal^-) bacterial culture is infected with such a lysate and Gal^+ transductants selected, many are double lysogens carrying both lambda and λ*dgal*. When such a double lysogen is induced, the lysate contains large numbers of λ*dgal* virions and can transduce at high efficiency, although only for the restricted group of *gal* genes.

For a lambda virion to be viable, there is a limit to the amount of phage DNA that can be replaced with host DNA. Sufficient phage DNA must be retained to encode the phage protein coat and other phage proteins needed for lysis and lysogeny. However, if a helper phage is used together with a defective phage in a mixed infection, then far fewer phage-specific genes are needed in the defective phage. Only the *att* (attachment) region, the *cos* site (cohesive ends, for packaging), and the replication origin of the lambda genome are absolutely needed for production of a

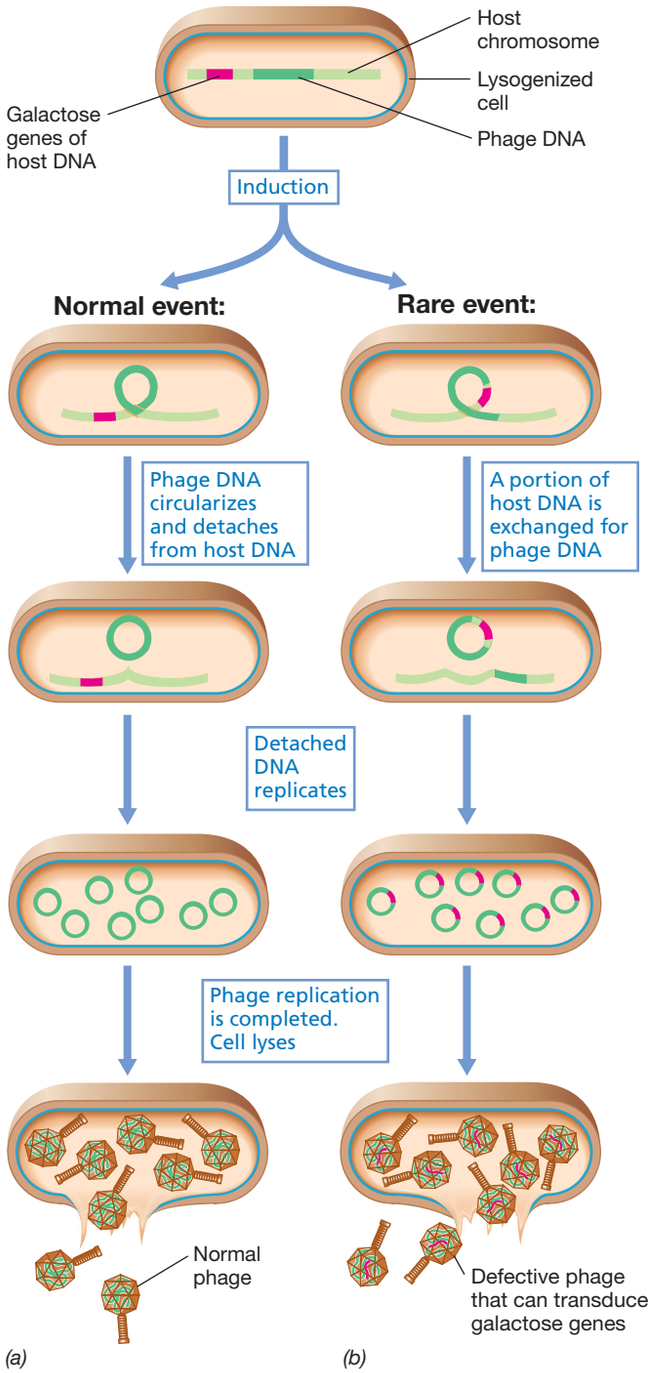

Figure 10.15 **Specialized transduction.** In an *Escherichia coli* cell containing a lambda prophage, *(a)* normal lytic events, and *(b)* the production of particles transducing the galactose genes. Only a short region of the circular host chromosome is shown in the figure.

transducing particle when a helper phage is used. By deleting the normal chromosomal *att* site and forcing lambda to integrate at other locations, specialized transducing phages covering many specific regions of the *E. coli* genome have been isolated. In addition, lambda transducing phages can be constructed by the techniques of genetic engineering to contain genes from any organism (↻ Section 11.9).

Phage Conversion

Alteration of the phenotype of a host cell by lysogenization is called *phage conversion*. When a normal (that is, nondefective) temperate phage lysogenizes a cell and becomes a prophage, the cell becomes immune to further infection by the same type of phage. Such immunity may itself be regarded as a change in phenotype. However, other phenotypic changes unrelated to phage immunity are often observed in lysogenized cells.

Two cases of phage conversion have been especially well studied. One involves a change in structure of a polysaccharide on the cell surface of *Salmonella anatum* on lysogenization with bacteriophage ε^{15}. The second involves the conversion of non-toxin-producing strains of *Corynebacterium diphtheriae* (the bacterium that causes the disease diphtheria) to toxin-producing (pathogenic) strains following lysogeny with phage β (↻ Section 33.3). In both cases, the genes responsible for the changes are an integral part of the phage genome and hence are automatically transferred upon infection by the phage and lysogenization.

Lysogeny probably carries a strong selective value for the host cell because it confers resistance to infection by viruses of the same type. Phage conversion may also be of considerable evolutionary significance because it results in efficient genetic alteration of host cells. Many bacteria isolated from nature are natural lysogens. It seems likely that lysogeny is often essential for survival of the host cells in nature.

MiniQuiz

- What is the major difference between generalized transduction and transformation?
- In specialized transduction, the donor DNA can replicate inside the recipient cell without homologous recombination taking place, but this is not true in generalized transduction. Explain.

10.9 Conjugation: Essential Features

Bacterial **conjugation** (mating) is a mechanism of genetic transfer that involves cell-to-cell contact. Conjugation is a plasmid-encoded mechanism. Conjugative plasmids use this mechanism to transfer copies of themselves to new host cells. Thus the process of conjugation involves a *donor* cell, which contains the conjugative plasmid, and a *recipient* cell, which does not. In addition, genetic elements that cannot transfer themselves can sometimes be mobilized during conjugation. These other genetic elements can be other plasmids or the host chromosome itself. Indeed, conjugation was discovered because the F plasmid of *Escherichia coli* can mobilize the host chromosome (see Figure 10.21). Transfer mechanisms may differ depending on the plasmid involved, but most plasmids in gram-negative *Bacteria* employ a mechanism similar to that used by the F plasmid.

F Plasmid

The F plasmid (F stands for "fertility") is a circular DNA molecule of 99,159 bp. **Figure 10.16** shows a genetic map of the F plasmid. One region of the plasmid contains genes that regulate DNA replication. It also contains a number of transposable elements

UNIT 4

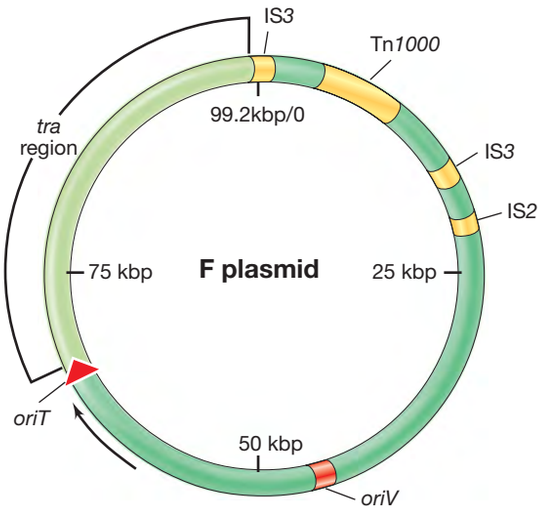

Figure 10.16 **Genetic map of the F (fertility) plasmid of *Escherichia coli*.** The numbers on the interior show the size in kilobase pairs (the exact size is 99,159 bp). The region in dark green at the bottom of the map contains genes primarily responsible for the replication and segregation of the F plasmid. The origin of vegetative replication is *oriV*. The light green *tra* region contains the genes needed for conjugative transfer. The origin of transfer during conjugation is *oriT*. The arrow indicates the direction of transfer (the *tra* region is transferred last). Insertion sequences are shown in yellow. These may recombine with identical elements on the bacterial chromosome, which leads to integration and the formation of different Hfr strains.

(Section 10.13) that allow the plasmid to integrate into the host chromosome. In addition, the F plasmid has a large region of DNA, the *tra* region, containing genes that encode transfer functions. Many genes in the *tra* region are involved in mating pair formation, and most of these have to do with the synthesis of a surface structure, the sex pilus (↻ Section 3.9). Only donor cells produce these pili. Different conjugative plasmids may have slightly different *tra* regions, and the pili may vary somewhat in structure. The F plasmid and its relatives encode F pili.

Pili allow specific pairing to take place between the donor and recipient cells. All conjugation in gram-negative *Bacteria* is thought to depend on cell pairing brought about by pili. The pilus makes specific contact with a receptor on the recipient cell and then is retracted by disassembling its subunits. This pulls the two cells together (**Figure 10.17**). Following this process, donor and recipient cells remain in contact by binding proteins located in the outer membrane of each cell. DNA is then transferred from donor to recipient cell through this conjugation junction.

Mechanism of DNA Transfer During Conjugation

DNA synthesis is necessary for DNA transfer by conjugation. This DNA is synthesized not by normal bidirectional replication (↻ Section 6.10), but by **rolling circle replication**, a mechanism also used by some viruses (↻ Section 9.10) and shown in **Figure 10.18**. DNA transfer is triggered by cell-to-cell contact, at which time one strand of the circular plasmid DNA is nicked and is transferred to the recipient. The nicking enzyme required to

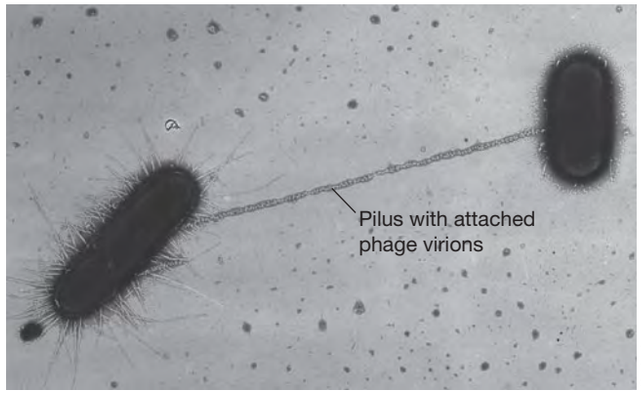

Figure 10.17 **Formation of a mating pair.** Direct contact between two conjugating bacteria is first made via a pilus. The cells are then drawn together to form a mating pair by retraction of the pilus, which is achieved by depolymerization. Certain small phages (F-specific bacteriophages; ↻ Section 21.1) use the sex pilus as a receptor and can be seen here attached to the pilus.

initiate the process, TraI, is encoded by the *tra* operon of the F plasmid. This protein also has helicase activity and thus also unwinds the strand to be transferred. As this transfer occurs, DNA synthesis by the rolling circle mechanism replaces the transferred strand in the donor, while a complementary DNA strand is being made in the recipient. Therefore, at the end of the process, both donor and recipient possess complete plasmids. For transfer of the F plasmid, if an F-containing donor cell, which is designated F^+, mates with a recipient cell lacking the plasmid, designated F^-, the result is two F^+ cells (Figure 10.18).

Transfer of plasmid DNA is efficient and rapid; under favorable conditions virtually every recipient cell that pairs with a donor acquires a plasmid. Transfer of the F plasmid, comprising approximately 100 kbp of DNA, takes about 5 minutes. If the plasmid genes can be expressed in the recipient, the recipient itself becomes a donor and can transfer the plasmid to other recipients. In this fashion, conjugative plasmids can spread rapidly among bacterial populations, behaving much like infectious agents. This is of major ecological significance because a few plasmid-containing cells introduced into a population of recipients can convert the entire population into plasmid-bearing (and thus donating) cells in a short time.

Plasmids can be lost from a cell by curing. This may happen spontaneously in natural populations when there is no selection pressure to maintain the plasmid. For example, plasmids conferring antibiotic resistance can be lost without affecting cell viability if there are no antibiotics in the cells' environment.

MiniQuiz

- In conjugation, how are donor and recipient cells brought into contact with each other?
- Explain how rolling circle DNA replication allows both donor and recipient to end up with a complete copy of plasmids transferred by conjugation.
- Why does F have two different origins of replication?

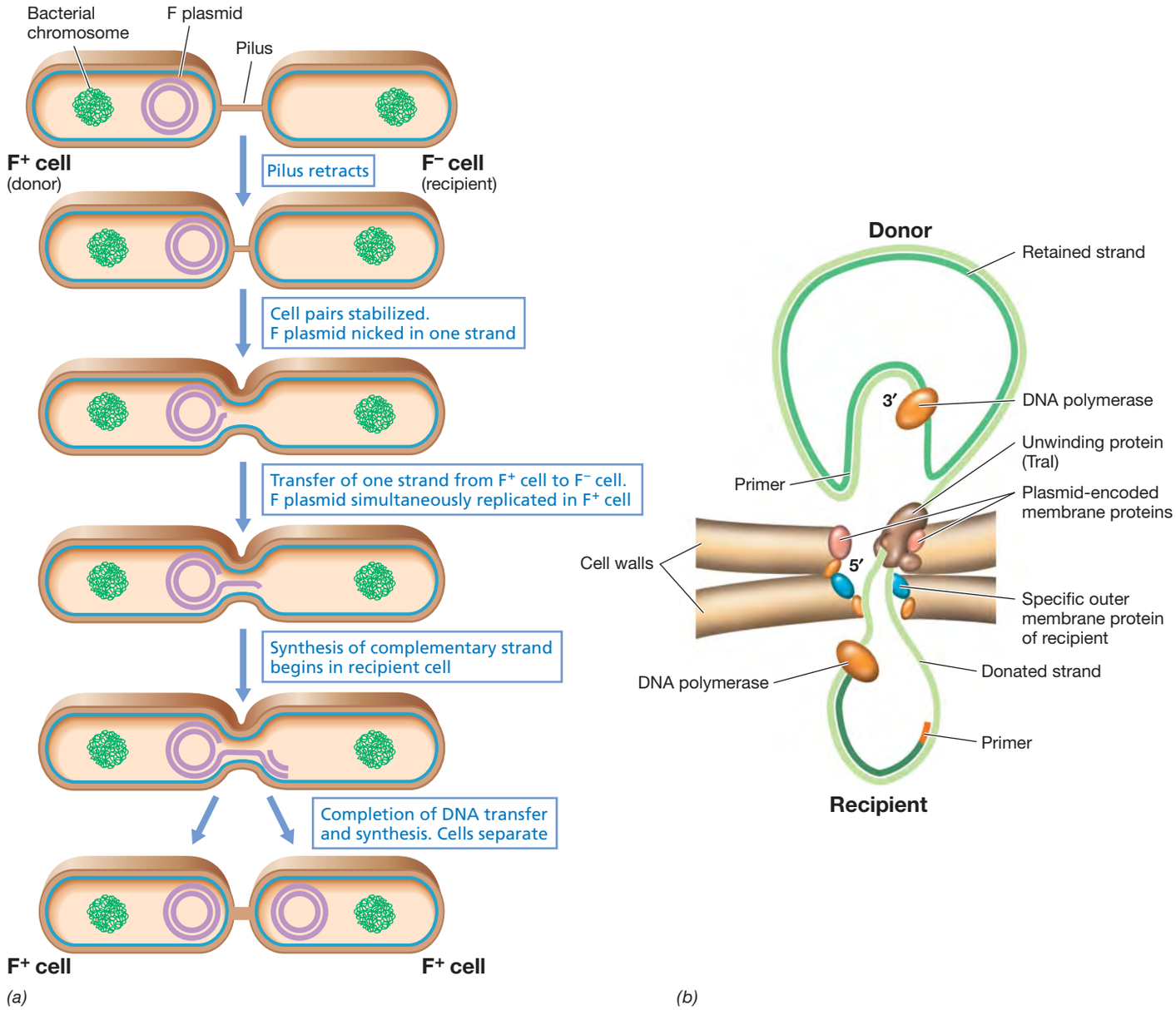

Figure 10.18 **Transfer of plasmid DNA by conjugation.** *(a)* The transfer of the F plasmid converts an F^- recipient cell into an F^+ cell. Note the mechanism of rolling circle replication. *(b)* Details of the replication and transfer process.

10.10 The Formation of Hfr Strains and Chromosome Mobilization

Chromosomal genes can be transferred by plasmid-mediated conjugation. As mentioned above, the F plasmid of *Escherichia coli* can, under certain circumstances, mobilize the chromosome during cell-to-cell contact. The F plasmid is an *episome*, a plasmid that can integrate into the host chromosome. When the F plasmid is integrated, chromosomal genes can be transferred along with the plasmid. Following genetic recombination between donor and recipient DNA, horizontal transfer of chromosomal genes by this mechanism can be very extensive.

Cells possessing a nonintegrated F plasmid are called F^+. Those with an F plasmid integrated into the chromosome are called **Hfr** (for *h*igh *f*requency of *r*ecombination) **cells**. This term refers to the high rates of genetic recombination between genes on the donor and recipient chromosomes. Both F^+ and Hfr cells are donors, but unlike conjugation between an F^+ and an F^-, conjugation between an Hfr donor and an F^- leads to transfer of genes from the host chromosome. This is because the chromosome and plasmid now form a single molecule of DNA. Consequently, when rolling circle replication is initiated by the F plasmid, replication continues on into the chromosome. Thus, the chromosome is also replicated and transferred. Hence, integration of a conjugative plasmid provides a mechanism for *mobilizing* a cell's genome.

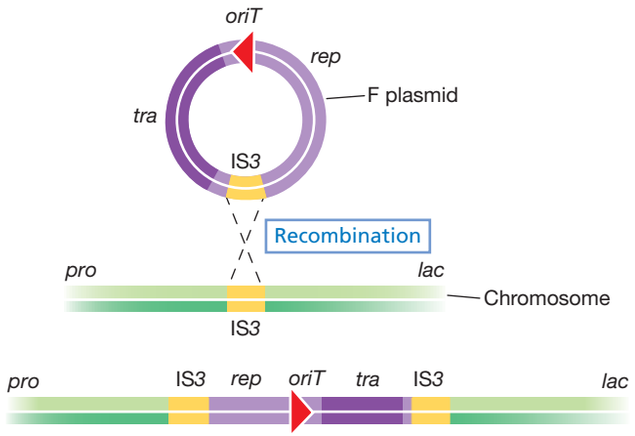

Figure 10.19 **The formation of an Hfr strain.** Integration of the F plasmid into the chromosome may occur at a variety of specific sites where IS elements are located. The example shown here is an IS*3* located between the chromosomal genes *pro* and *lac*. Some of the genes on the F plasmid are shown. The arrow indicates the origin of transfer, *oriT*, with the arrow as the leading end. Thus, in this Hfr *pro* would be the first chromosomal gene to be transferred and *lac* would be among the last.

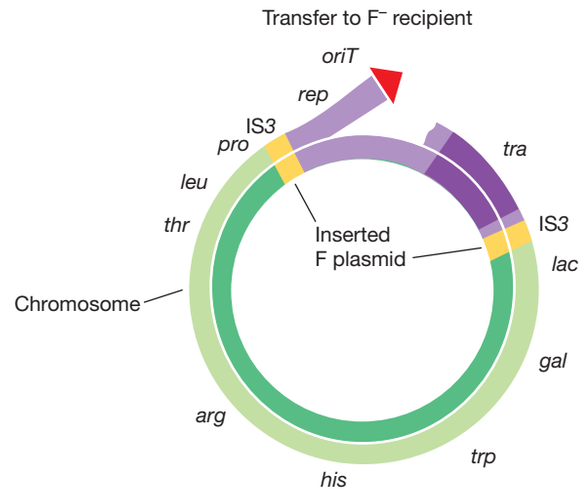

Figure 10.20 **Transfer of chromosomal genes by an Hfr strain.** The Hfr chromosome breaks at the origin of transfer within the integrated F plasmid. The transfer of DNA to the recipient begins at this point. DNA replicates during transfer as for a free F plasmid (Figure 10.18). This figure is not to scale; the inserted F plasmid is actually less than 3% of the size of the *Escherichia coli* chromosome.

Overall, the presence of the F plasmid results in three distinct alterations in the properties of a cell: (1) the ability to synthesize the F pilus, (2) the mobilization of DNA for transfer to another cell, and (3) the alteration of surface receptors so the cell can no longer act as a recipient in conjugation and is unable to take up a second copy of the F plasmid or genetically related plasmids.

Integration of F and Chromosome Mobilization

The F plasmid and the chromosome of *E. coli* both carry several copies of mobile elements called *insertion sequences* (IS; Section 10.13). These provide regions of sequence homology between chromosomal and F plasmid DNA. Consequently, homologous recombination between an IS on the F plasmid and a corresponding IS on the chromosome results in integration of the F plasmid into the host chromosome, as shown in **Figure 10.19**. Once integrated, the plasmid no longer replicates independently, but the *tra* operon still functions normally and the strain synthesizes pili. When a recipient is encountered, conjugation is triggered just as in an F^+ cell, and DNA transfer is initiated at the *oriT* (origin of transfer) site. However, because the plasmid is now part of the chromosome, after part of the plasmid DNA is transferred, chromosomal genes begin to be transferred (**Figure 10.20**). As in the case of conjugation with just the F plasmid itself (Figure 10.18), chromosomal DNA transfer also involves replication.

Because the DNA strand typically breaks during transfer, only part of the donor chromosome is transferred. Consequently, the recipient does not become Hfr (or F^+) because only part of the integrated F plasmid is transferred (**Figure 10.21**). However, after transfer, the Hfr strain remains Hfr because it retains a copy of the integrated F plasmid. Because a partial chromosome cannot replicate, for incoming donor DNA to survive, it must recombine with the recipient chromosome. Following recombination, the recipient cell may express a new phenotype due to incorporation of donor genes. Although Hfr strains transmit chromosomal

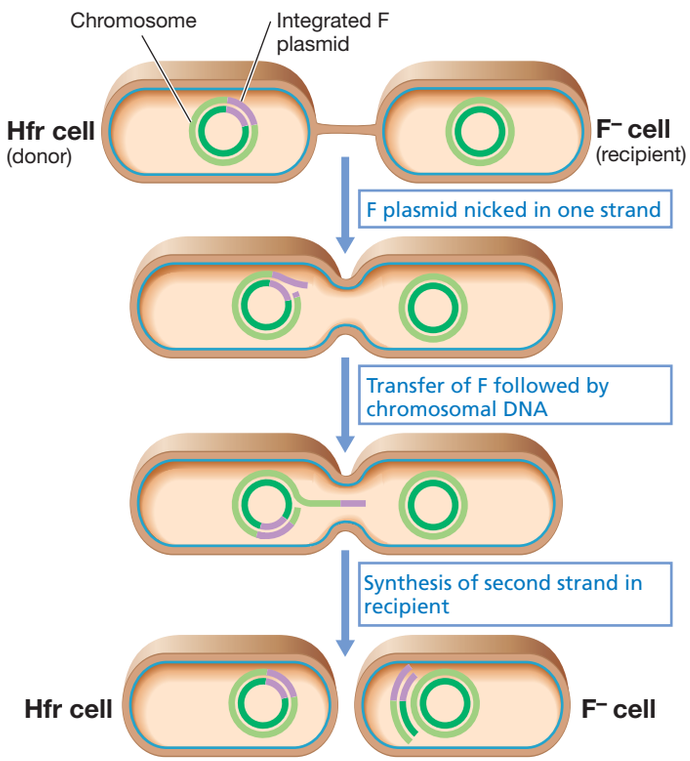

Figure 10.21 **Transfer of chromosomal DNA by conjugation.** Transfer of the integrated F plasmid from an Hfr strain results in the cotransfer of chromosomal DNA because this is linked to the plasmid. The steps in transfer are similar to those in Figure 10.18*a*. However, the recipient remains F^- and receives a linear fragment of donor chromosome attached to part of the F plasmid. For donor DNA to survive, it must be recombined into the recipient chromosome after transfer (not shown).

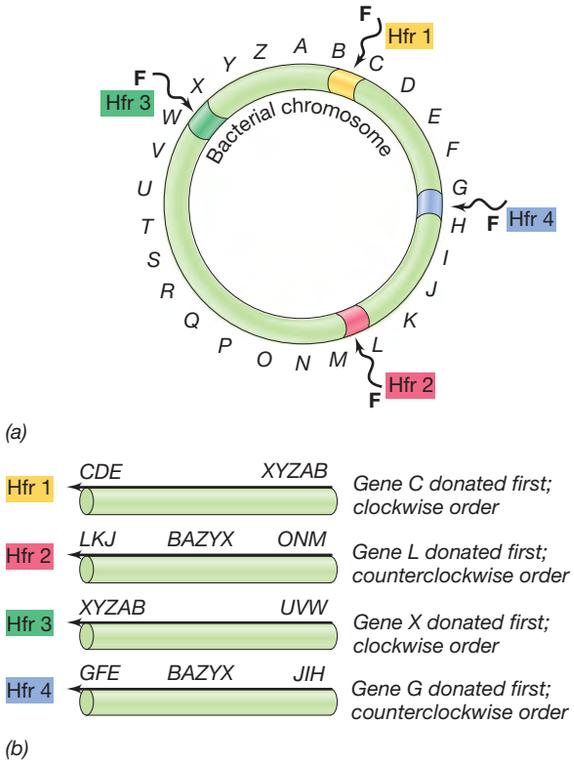

Figure 10.22 **Formation of different Hfr strains.** Different Hfr strains donate genes in different orders and from different origins. *(a)* F plasmids can be inserted into various insertion sequences on the bacterial chromosome, forming different Hfr strains. *(b)* Order of gene transfer for different Hfr strains.

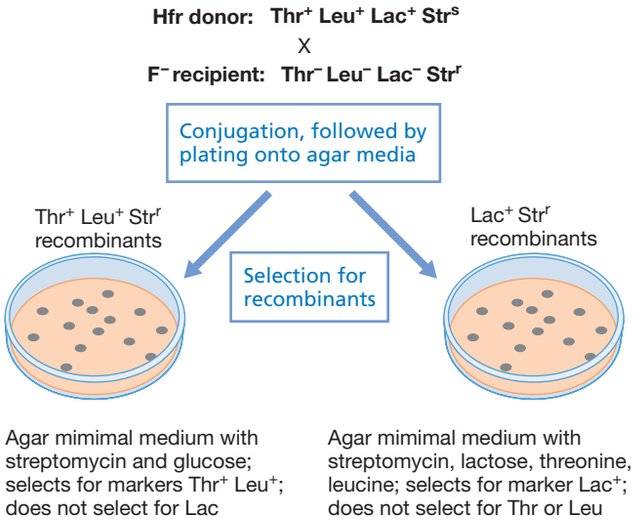

Figure 10.23 **Example experiment for the detection of conjugation.** Thr, threonine; Leu, leucine; Lac, lactose; Str, streptomycin. Note that each medium selects for specific classes of recombinants. The controls for the experiment are made by plating samples of the donor and the recipient before they are mixed. Neither should be able to grow on the selective media used.

UNIT 4

genes at high frequency, they generally do not convert F^- cells to F^+ or Hfr because the entire F plasmid is rarely transferred. Instead, an Hfr × F^- cross yields the original Hfr and an F^- cell that now has a new genotype. As in transformation and transduction, genetic recombination between Hfr genes and F^- genes involves homologous recombination in the recipient cell.

Because several distinct insertion sequences are present on the chromosome, a number of distinct Hfr strains are possible. A given Hfr strain always donates genes in the same order, beginning at the same position. However, Hfr strains that differ in the chromosomal integration site of the F plasmid transfer genes in different orders (**Figure 10.22**). At some insertion sites, the F plasmid is integrated with its origin pointing in one direction, whereas at other sites the origin points in the opposite direction. The orientation of the F plasmid determines which chromosomal genes enter the recipient first (Figure 10.22). By using various Hfr strains in mating experiments, it was possible to determine the arrangement and orientation of most of the genes in the *E. coli* chromosome long before it was sequenced.

Use of Hfr Strains in Genetic Crosses

As for any system of bacterial gene transfer, the experimenter selects recombinants from conjugation. However, unlike transformation and transduction, both donor and recipient cells are viable during conjugation. It is thus necessary to choose selection conditions in which the desired recombinants can grow, but where neither of the parental strains can grow. Typically, a recipient is used that is resistant to an antibiotic, but is auxotrophic for some nutrient, and a donor is used that is sensitive to the antibiotic, but is prototrophic for the same nutrient. Thus, on minimal medium containing the antibiotic, only recombinant cells will grow following the mating.

For instance, in the experiment shown in **Figure 10.23**, an Hfr donor that is sensitive to streptomycin (Str^s) and is wild type for synthesis of the amino acids threonine and leucine (Thr^+ and Leu^+) and for utilization of lactose (Lac^+), is mated with a recipient cell that cannot make these amino acids or use lactose, but that is resistant to streptomycin (Str^r). The selective minimal medium contains streptomycin so that only recombinant cells can grow. The composition of each selective medium is varied depending on which genotypic characteristics are desired in the recombinant, as shown in Figure 10.23. The frequency of gene transfer is measured by counting the colonies grown on the selective medium.

The order of genes on the donor chromosome can also be determined by following the kinetics of transfer of individual markers. For example, in the process called *interrupted mating*, conjugating cells are separated by agitation in a mixer or blender. If mixtures of Hfr and F^- cells are agitated at various times after mixing and the genetic recombinants scored, it is found that the longer the time between pairing and agitation, the greater the number of genes from the Hfr that are found in the recombinant. As shown in **Figure 10.24**, genes located closer to the origin of transfer enter the recipient first and are present in a higher percentage of the recombinants than genes that are transferred later. In addition to showing that gene transfer from donor to recipient occurs sequentially, experiments of this kind allow the order of the genes on the bacterial chromosome to be determined.

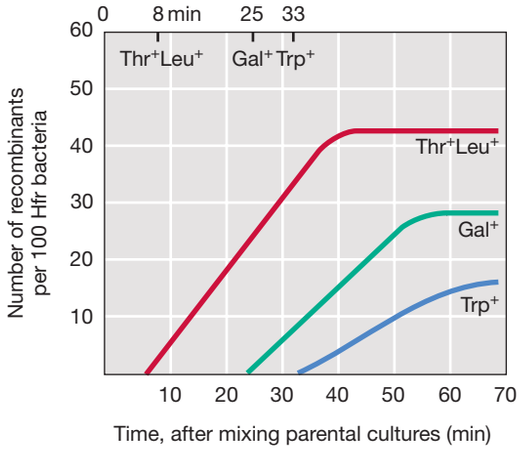

Figure 10.24 Time of gene entry in a mating culture. The rate of appearance of recombinants containing different genes after mating Hfr and F^- bacteria is shown. The location of the genes along the Hfr chromosome is shown at the upper left. Genes closest to the origin (0 min) are the first to be transferred. The experiment is done by mixing Hfr and F^- cells under conditions in which most Hfr cells find recipients. At various times, samples of the mixture are shaken violently to separate mating pairs and plated onto selective medium on which only recombinants can form colonies.

Transfer of Chromosomal Genes to the F Plasmid

Occasionally, integrated F plasmids may be excised from the chromosome. During excision, chromosomal genes may sometimes be incorporated into the liberated F plasmid. This can happen because both the F plasmid and the chromosome contain multiple identical insertion sequences where recombination can occur (Figure 10.20). F plasmids containing chromosomal genes are called *F′ plasmids*. When F′ plasmids promote conjugation, they transfer the chromosomal genes they carry at high frequency to the recipients. F′-mediated transfer resembles specialized transduction (Section 10.8) in that only a restricted group of chromosomal genes is transferred by any given F′ plasmid. Transferring a known F′ into a recipient allows one to establish diploids (two copies of each gene) for a limited region of the chromosome. Such partial diploids are important for complementation tests, as we will see in the next section.

Other Conjugation Systems

Although we have discussed conjugation almost exclusively as it occurs in *E. coli*, conjugative plasmids have been found in many other gram-negative *Bacteria*. Conjugative plasmids of the incompatibility group IncP can be maintained in virtually all gram-negative *Bacteria* and even transferred between different genera. Conjugative plasmids are also known in gram-positive *Bacteria* (for example, in *Streptococcus* and *Staphylococcus*). A process of genetic transfer similar to conjugation in *Bacteria* also occurs in some *Archaea* (Section 10.12).

MiniQuiz

- In conjugation involving the F plasmid of *Escherichia coli*, how is the host chromosome mobilized?
- Why does an Hfr × F^- mating not yield two Hfr cells?
- At which sites in the chromosome can the F plasmid integrate?

10.11 Complementation

In all three methods of bacterial gene transfer, only a portion of the donor chromosome enters the recipient cell. Therefore, unless recombination takes place with the recipient chromosome, the donor DNA will be lost because it cannot replicate independently in the recipient. Nonetheless, it is possible to stably maintain a state of partial diploidy for use in bacterial genetic analysis, and we consider this now.

Merodiploids and Complementation

A bacterial strain that carries two copies of any particular chromosomal segment is known as a partial diploid or *merodiploid*. In general, one copy is present on the chromosome itself and the second copy on another genetic element, such as a plasmid or a bacteriophage. Because it is possible to create specialized transducing phages or specific plasmids using recombinant DNA techniques (Chapter 11), it is possible to put any portion of the bacterial chromosome onto a phage or plasmid.

Consequently, if the chromosomal copy of a gene is defective due to a mutation, it is possible to supply a functional (wild-type) copy of the gene on a plasmid or phage. For example, if one of the genes for tryptophan biosynthesis has been inactivated, this will give a Trp^- phenotype. That is, the mutant strain will be a tryptophan auxotroph and will require the amino acid tryptophan for growth. However, if a copy of the wild-type gene is introduced into the same cell on a plasmid or viral genome, this gene will encode the necessary protein and restore the wild-type phenotype. This process is called *complementation* because the wild-type gene is said to complement the mutation, in this case converting the Trp^- cell into Trp^+.

Complementation Tests and the Cistron

When two mutant strains are genetically crossed (whether by conjugation, transduction, or transformation), homologous recombination can yield wild-type recombinants unless both mutations affect exactly the same base pairs. For example, if two different Trp^- *Escherichia coli* mutants are crossed and Trp^+ recombinants are obtained, it is obvious that the mutations in the two strains were not in the same base pairs. However, this kind of experiment cannot determine whether two mutations are in two different genes that both affect tryptophan synthesis or in different regions of the same gene. This can be determined by a complementation test.

To perform a complementation test, two copies of the region of DNA under investigation must be present and carried on two different molecules of DNA. One copy is normally present on the chromosome and the other is carried on a second DNA molecule, typically a plasmid. For example, if we are analyzing mutants in tryptophan biosynthesis, then two copies of the whole tryptophan operon must be present.

Suppose that we wish to know if two Trp^- strains have a mutation in the same gene. To do this we must arrange for one mutation to be present on the chromosome and the other on a plasmid. The mutations are then referred to as being in *trans* with respect to one another. If the two mutations are in the *same* gene, the recombinant cell will have two defective copies of the same gene and will

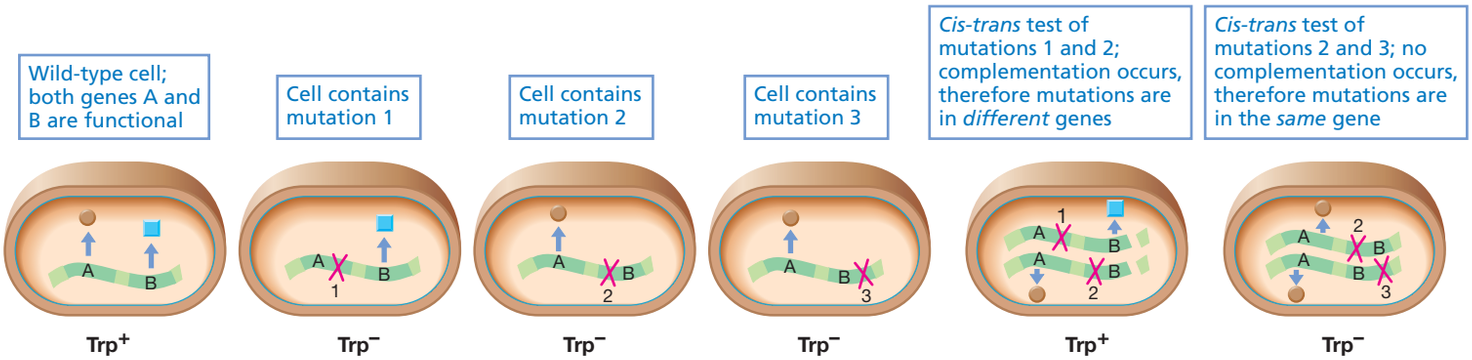

Figure 10.25 Complementation analysis. In this example, the proteins encoded by both genes A and B are required to synthesize tryptophan. Mutations 1, 2, and 3 each lead to the same phenotype, a requirement for tryptophan (Trp^-). Complementation analysis indicates that mutations 2 and 3 are in the same gene but mutation 1 is in a separate gene.

display the negative phenotype. Conversely, if the two mutations are in *different* genes, the recipient cell will have one unmutated copy of each gene (one on the chromosome and the other on the plasmid) and be able to synthesize tryptophan. The possible combinations are shown diagrammatically in **Figure 10.25**. If one DNA molecule carries both mutations (that is, the mutations are in *cis*), a second DNA molecule can serve as a complement if it is wild type for both genes. Having the mutations in *cis* serves as a positive control in a complementation experiment. This type of complementation test is therefore called a *cis-trans* test.

A gene as defined by the *cis-trans* test is called a **cistron** and is equivalent to defining a structural gene as a segment of DNA that encodes a single polypeptide chain. If two mutations occur in genes encoding different enzymes, or even different protein subunits of the same enzyme, complementation of the two mutations is possible, and the mutations are therefore not in the same cistron (Figure 10.25). It is important to note that complementation does not rely on recombination; the two genes in question remain on separate genetic elements.

Although genetic crosses to test complementation are still done in bacterial genetics, it is often easier to sequence the gene in question to identify the nature and location of any mutations. This is especially true if the sequence of the wild-type gene is already known. The word "cistron" is now rarely used in microbial genetics except when describing whether an mRNA has the genetic information from one gene (*monocistronic* mRNA) or from more than one gene (*polycistronic* mRNA) (Section 6.15).

MiniQuiz

- What is a merodiploid?
- Complementation tests have been referred to as *cis-trans* tests. Explain.

10.12 Gene Transfer in *Archaea*

Although *Archaea* contain a single circular chromosome like most *Bacteria* (**Figure 10.26**) and the genomes of several species of *Archaea* have been entirely sequenced, the development of gene transfer systems lags far behind that for *Bacteria*. Practical problems include the need to grow many *Archaea* under extreme conditions. Thus, the temperatures necessary to culture some hyperthermophiles will melt agar, and alternative materials are required to form solid media and obtain colonies.

Another problem is that most antibiotics do not affect *Archaea*. For example, penicillins do not affect *Archaea* because their cell walls lack peptidoglycan. The choice of selectable markers for genetic crosses is therefore often limited. However, novobiocin (a DNA gyrase inhibitor) and mevinolin (an inhibitor of isoprenoid biosynthesis) are used to inhibit extreme halophiles, and puromycin and neomycin (both protein synthesis inhibitors) inhibit methanogens.

No single species of *Archaea* has become a model organism for archaeal genetics, although more genetic work has probably been done on select species of extreme halophiles (*Halobacterium*, *Haloferax*, Section 19.2) than on any other *Archaea*. Instead, individual mechanisms for gene transfer have been found scattered among a range of *Archaea*. Examples of transformation, transduction, and conjugation are known. In addition, several plasmids have been isolated from *Archaea* and some have been used to construct cloning vectors, allowing genetic analysis through cloning and sequencing rather than traditional genetic crosses. Transposon

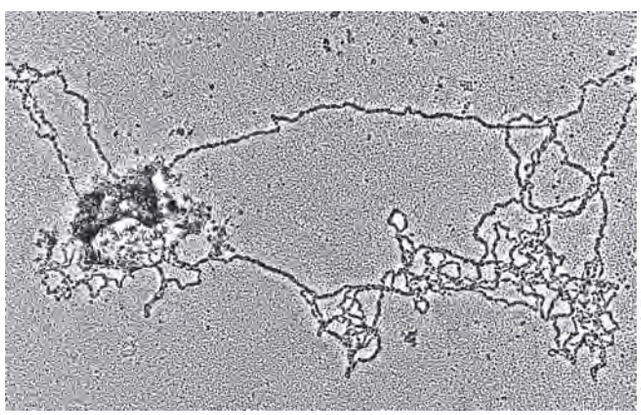

Figure 10.26 An archaeal chromosome, as shown in the electron microscope. The circular chromosome is from the hyperthermophile *Sulfolobus*, a member of the *Archaea*.

mutagenesis has been well developed in certain methanogen species including *Methanococcus* and *Methanosarcina*, and other tools such as shuttle vectors and other *in vitro* methods of genetic analysis have been developed for study of the highly unusual biochemistry of the methanogens (↻ Section 19.3).

Transformation works reasonably well in several *Archaea*. Transformation procedures vary in detail from organism to organism. One approach involves removal of divalent metal ions, which in turn results in the disassembly of the glycoprotein cell wall layer surrounding many archaeal cells and hence allows access by transforming DNA. However, *Archaea* with rigid cell walls have proven difficult to transform, although electroporation sometimes works. One exception is in *Methanosarcina* species, organisms with a thick cell wall, for which high-efficiency transformation systems have been developed that employ DNA-loaded lipid preparations (liposomes) to deliver DNA into the cell.

Although viruses that infect *Archaea* are plentiful, transduction is extremely rare. Only one archaeal virus, which infects the thermophilic methanogen *Methanothermobacter thermautotrophicus*, has been shown to transduce the genes of its host. Unfortunately the low burst size (about six phages liberated per cell) makes using this system for gene transfer impractical.

Two types of conjugation have been detected in *Archaea*. Some strains of *Sulfolobus solfataricus* (↻ Section 19.9) contain plasmids that promote conjugation between two cells in a manner similar to that seen in *Bacteria*. In this process, cell pairing occurs before plasmid transfer, and DNA transfer is unidirectional. However, most of the genes encoding these functions seem to have little similarity to those in gram-negative *Bacteria*. The exception is a gene similar to *traG* from the F plasmid, whose protein product is involved in stabilizing mating pairs. It thus seems likely that the actual mechanism of conjugation in *Archaea* is quite different from that in *Bacteria*.

Some halobacteria, in contrast, perform a novel form of conjugation. No fertility plasmids are involved, and DNA transfer is bidirectional. Cytoplasmic bridges form between the mating cells and appear to be used for DNA transfer. Neither type of conjugation has been developed to the point of being used for routine gene transfer or genetic analysis. However, these genetic resources will likely be useful for developing facile genetic systems in these organisms.

MiniQuiz

- Why is it usually more difficult to select recombinants with *Archaea* than with *Bacteria*?
- Why do penicillins not kill members of the *Archaea*?

10.13 Mobile DNA: Transposable Elements

As we have seen, molecules of DNA may move from one cell to another, but to a geneticist, "mobile DNA" has a specialized meaning. Mobile DNA refers to discrete segments of DNA that move as units from one location to another *within* other DNA molecules. Although the DNA of certain viruses can be inserted into and excised from the genome of the host cell, most mobile DNA consists of **transposable elements**. These are stretches of DNA that can move from one site to another. However, transposable elements are always found inserted into another DNA molecule such as a plasmid, a chromosome, or a viral genome. Transposable elements do not possess their own origin of replication. Instead, they are replicated when the host DNA molecule into which they are inserted is replicated.

Transposable elements move by a process called *transposition* that is important both in evolution and in genetic analysis. The frequency of transposition is extremely variable, and ranges from 1 in 1000 to 1 in 10,000,000 per transposable element per cell generation, depending on both the transposable element and the organism. Transposition was originally observed in corn (maize) in the 1940s by Barbara McClintock before the DNA double helix was even discovered! She later received the Nobel Prize for this discovery. The molecular details of transposition were revealed using *Bacteria* due to the powerful genetic analyses possible in these organisms. Transposable elements are widespread in nature and can be found in the genomes of all three domains of life as well as in many viruses and plasmids.

Transposons and Insertion Sequences

The two major types of transposable elements in *Bacteria* are *insertion sequences* (IS) and *transposons*. Both elements have two important features in common: They carry genes encoding transposase, the enzyme necessary for transposition, and they have short inverted terminal repeats at their ends that are also needed for transposition. Note that the ends of transposable elements are not free but are continuous with the host DNA molecule into which the transposable element has inserted. **Figure 10.27** shows genetic maps of the insertion element IS*2* and of the transposon Tn*5*.

Insertion sequences are the simplest type of transposable element. They are short DNA segments, about 1000 nucleotides

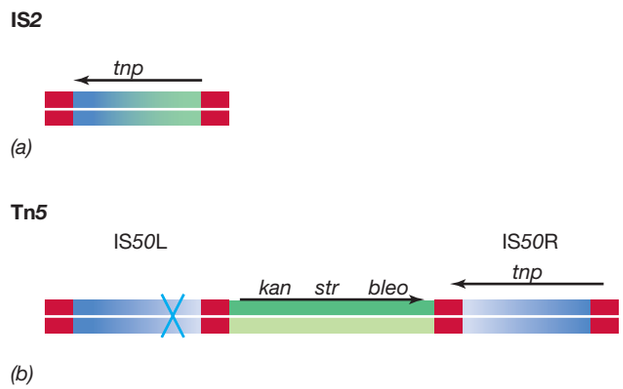

Figure 10.27 **Maps of the transposable elements IS*2* and Tn*5*.** Inverted repeats are shown in red. The arrows above the maps show the direction of transcription of any genes on the elements. The gene encoding the transposase is *tnp*. *(a)* IS*2* is an insertion sequence of 1327 bp with inverted repeats of 41 bp at its ends. *(b)* Tn*5* is a composite transposon of 5.7 kbp containing the insertion sequences IS*50*L and IS*50*R at its left and right ends, respectively. IS*50*L is not capable of independent transposition because there is a nonsense mutation, marked by a blue cross, in its transposase gene. Otherwise, the two IS*50* elements are almost identical. The genes *kan, str*, and *bleo* confer resistance to the antibiotics kanamycin (and neomycin), streptomycin, and bleomycin. Tn*5* is commonly used to generate mutants in *Escherichia coli* and other gram-negative bacteria.

long, and typically contain inverted repeats of 10–50 bp. Each different IS has a specific number of base pairs in its terminal repeats. The only gene they possess is for the transposase. Several hundred distinct IS elements have been characterized. IS elements are found in the chromosomes and plasmids of both *Bacteria* and *Archaea*, as well as in certain bacteriophages. Individual strains of the same bacterial species vary in the number and location of the IS elements they harbor. For instance, one strain of *Escherichia coli* has five copies of IS*2* and five copies of IS*3*. Many plasmids, such as the F plasmid, also carry IS elements. Indeed, integration of the F plasmid into the *E. coli* chromosome is due to homologous recombination between identical IS elements on the F plasmid and the chromosome (Section 10.10).

Transposons are larger than IS elements, but have the same two essential components: inverted repeats at both ends and a gene that encodes transposase. The transposase recognizes the inverted repeats and moves the segment of DNA flanked by them from one site to another. Consequently, any DNA that lies between the two inverted repeats is moved and is, in effect, part of the transposon. Genes included inside transposons vary widely. Some of these genes, such as antibiotic resistance genes, confer important new properties on the organism harboring the transposon. Because antibiotic resistance is both important and easy to detect, most highly investigated transposons have antibiotic resistance genes as selectable markers. Examples include transposon Tn*5*, which carries kanamycin resistance (Figure 10.27) and Tn*10*, with tetracycline resistance.

Because any genes lying between the inverted repeats become part of a transposon, it is possible to get hybrid transposons that display complex behavior. For example, conjugative transposons contain *tra* genes and can move between bacterial species by conjugation as well as transpose from place to place within a single bacterial genome. Even more complex is bacteriophage Mu, which is both a virus and a transposon (↻ Section 21.4). In this case a complete virus genome is contained within a transposon. Other composite genetic elements consist of a segment of DNA lying between two identical IS elements. This whole structure can move as a unit and is called a *composite transposon*. The behavior of composite transposons indicates that novel transposons likely arise periodically in cells that contain IS elements located close to one another.

Mechanisms of Transposition

Both the inverted repeats found at the ends of transposable elements and transposase are essential for transposition. The transposase recognizes, cuts, and ligates the DNA during transposition. When a transposable element is inserted into target DNA, a short sequence in the target DNA at the site of integration is duplicated during the insertion process (**Figure 10.28**). The duplication arises because single-stranded DNA breaks are made by the transposase. The transposable element is then attached to the single-stranded ends that have been generated. Finally, enzymes of the host cell repair the single-strand portions, which results in the duplication.

Two mechanisms of transposition are known: *conservative* and *replicative* (**Figure 10.29**). In conservative transposition, as occurs with the transposon Tn*5*, the transposon is excised from one location and is reinserted at a second location. The copy number of a conservative transposon therefore remains at one. By contrast, during replicative transposition, a new copy is produced and is inserted at the second location. Thus, after a replicative transposition event, one copy of the transposon remains at the original site, and there is a second copy at the new site.

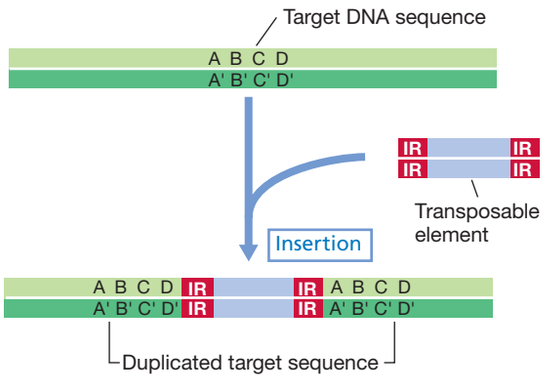

Figure 10.28 Transposition. Insertion of a transposable element generates a duplication of the target sequence. Note the presence of inverted repeats (IR) at the ends of the transposable element.

Transposition is a type of recombination called *site-specific* recombination, because specific DNA sequences (the inverted repeats and target sequence) are recognized by a protein (the transposase). This contrasts with *homologous* recombination (Section 10.6) in which homologous DNA sequences recognize each other by base pairing.

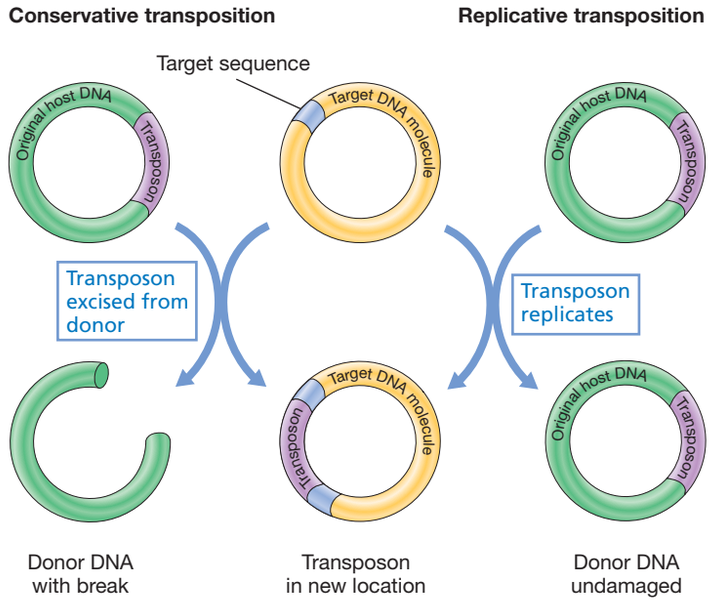

Figure 10.29 Two mechanisms of transposition. Donor DNA (carrying the transposon) is shown in green, and recipient DNA carrying the target sequence is shown in yellow. In both conservative and replicative transposition the transposase inserts the transposon (purple) into the target site (blue) on the recipient DNA. During this process, the target site is duplicated. In conservative transposition, the donor DNA is left with a double-stranded break at the previous location of the transposon. In contrast, after replicative transposition, both donor and recipient DNA possess a copy of the transposon.

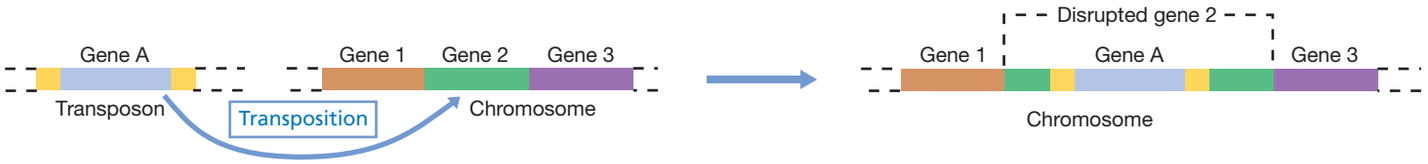

Figure 10.30 **Transposon mutagenesis.** The transposon moves into the middle of gene 2. Gene 2 is now disrupted by the transposon and is inactivated. Gene A in the transposon is expressed in both locations.

Mutagenesis with Transposons

When a transposon inserts itself within a gene, a mutation occurs in that particular gene (**Figure 10.30**). Mutations due to transposon insertion do occur naturally. However, deliberate use of transposons is a convenient way to create bacterial mutants in the laboratory. Typically, transposons carrying antibiotic resistance genes are used. The transposon is introduced into the target cell on a phage or plasmid that cannot replicate in that particular host. Consequently, antibiotic-resistant colonies will mostly be due to insertion of the transposon into the bacterial genome.

Because bacterial genomes contain relatively little noncoding DNA, most transposon insertions will occur in genes that encode proteins. If inserted into a gene encoding an essential protein, the mutation may be lethal under certain growth conditions and be suitable for genetic selection. For example, if transposon insertions are selected on rich medium on which all auxotrophs can grow, they can subsequently be screened on minimal medium supplemented with various nutrients to determine if a nutrient is required. Further analyses can then be performed to reveal which gene the transposon has disrupted. Auxotrophic mutations due to transposon insertions are very useful in bacterial genetics. Normally, auxotrophic recombinants cannot be isolated by positive selection. However, the presence of a transposon with an antibiotic resistance marker allows for positive selection.

Two transposons widely used for mutagenesis of *Escherichia coli* and related bacteria are Tn*5* (Figure 10.27), which confers neomycin and kanamycin resistance, and Tn*10*, which confers tetracycline resistance. Many *Bacteria*, a few *Archaea*, and the yeast *Saccharomyces cerevisiae* have all been mutagenized using transposon mutagenesis. More recently, transposons have even been used to isolate mutations in animals, including mice.

MiniQuiz

- Which features do insertion sequences and transposons have in common?
- What is the significance of the terminal inverted repeats of transposons?

Big Ideas

10.1
Mutation is a heritable change in DNA sequence and may lead to a change in phenotype. Selectable mutations are those that give the mutant a growth advantage under certain environmental conditions and are especially useful in genetic research. If selection is not possible, mutants must be identified by screening.

10.2
Mutations, which can be either spontaneous or induced, arise because of changes in the base sequence of the nucleic acid of an organism's genome. A point mutation, which is due to a change in a single base pair, can lead to a single amino acid change in a polypeptide or to no change at all, depending on the particular codon. In a nonsense mutation, the codon becomes a stop codon and an incomplete polypeptide is made. Deletions and insertions cause more dramatic changes in the DNA, including frameshift mutations that often result in complete loss of gene function.

10.3
Different types of mutations occur at different frequencies. For a typical bacterium, mutation rates of 10^{-6} to 10^{-7} per kilobase pair are generally seen. Although RNA and DNA polymerases make errors at about the same rate, RNA genomes typically accumulate mutations at much higher frequencies than DNA genomes.

10.4
Mutagens are chemical, physical, or biological agents that increase the mutation rate. Mutagens can alter DNA in many different ways. However, alterations in DNA are not mutations unless they are inherited. Some DNA damage can lead to cell death if not repaired, and both error-prone and high-fidelity DNA repair systems exist.

10.5
The Ames test employs a sensitive bacterial assay system to identify chemical mutagens.

10.6
Homologous recombination occurs when closely related DNA sequences from two distinct genetic elements are combined together in a single element. Recombination is an important evolutionary process, and cells have specific mechanisms for ensuring that recombination takes place.

10.7
Certain prokaryotes exhibit competence, a state in which cells are able to take up free DNA released by other bacteria. Incorporation of donor DNA into a recipient cell requires the activity of single-strand binding protein, RecA protein, and several other enzymes. Only competent cells are transformable.

10.8
Transduction is the transfer of host genes from one bacterium to another by a bacterial virus. In generalized transduction, defective virus particles randomly incorporate fragments of the cell's chromosomal DNA, but the transducing efficiency is low. In specialized transduction, the DNA of a temperate virus excises incorrectly and takes adjacent host genes along with it; the transducing efficiency here may be very high.

10.9
Conjugation is a mechanism of DNA transfer in prokaryotes that requires cell-to-cell contact. Conjugation is controlled by genes carried by certain plasmids (such as the F plasmid) and involves transfer of the plasmid from a donor cell to a recipient cell. Plasmid DNA transfer involves replication via the rolling circle mechanism.

10.10
The donor cell chromosome can be mobilized for transfer to a recipient cell. This requires an F plasmid to integrate into the chromosome to form the Hfr phenotype. Transfer of the host chromosome is rarely complete but can be used to map the order of the genes on the chromosome. F′ plasmids are previously integrated F plasmids that have excised and captured some chromosomal genes.

10.11
A defective copy of a gene may be complemented by the presence of a second, unmutated copy of that gene. The construction of merodiploids carrying two copies of a specific gene or genes allows for complementation tests to determine if two mutations are in the same or different genes. This is necessary when mutations in different genes in the same pathway yield the same phenotype. Recombination does not occur in complementation tests.

10.12
Archaea lag behind *Bacteria* in the development of systems for gene transfer. Many antibiotics are ineffective against *Archaea*, making it difficult to select recombinants effectively. The unusual growth conditions needed by many *Archaea* also make genetic experimentation difficult. Nevertheless, the genetic transfer systems of *Bacteria*—transformation, transduction, and conjugation—are all known in *Archaea*.

10.13
Transposons and insertion sequences are genetic elements that can move from one location on a host DNA molecule to another by transposition, a type of site-specific recombination. Transposition can be either replicative or conservative. Transposons often carry genes encoding antibiotic resistance and can be used as biological mutagens.

Review of Key Terms

Auxotroph an organism that has developed a nutritional requirement, often as a result of mutation

Cistron a gene as defined by the *cis-trans* test; a segment of DNA (or RNA) that encodes a single polypeptide chain

Conjugation the transfer of genes from one prokaryotic cell to another by a mechanism involving cell-to-cell contact

Genotype the complete genetic makeup of an organism; the complete description of a cell's genetic information

Heteroduplex a DNA double helix composed of single strands from two different DNA molecules

Hfr cell a cell with the F plasmid integrated into the chromosome

Induced mutation a mutation caused by external agents such as mutagenic chemicals or radiation

Insertion sequence (IS) the simplest type of transposable element, which carries only genes involved in transposition

Missense mutation a mutation in which a single codon is altered so that one amino acid in a protein is replaced with a different amino acid

Mutagen an agent that causes mutation

Mutant an organism whose genome carries a mutation

Mutation a heritable change in the base sequence of the genome of an organism

Mutator strain a mutant strain in which the rate of mutation is increased

Nonsense mutation a mutation in which the codon for an amino acid is changed to a stop codon

Phenotype the observable characteristics of an organism

Plasmid an extrachromosomal genetic element that has no extracellular form

Point mutation a mutation that involves a single base pair

Recombination the process by which DNA molecules from two separate sources exchange sections or are brought together into a single DNA molecule

Regulon a set of genes or operons that are transcribed separately but are coordinately controlled by the same regulatory protein

Reversion an alteration in DNA that reverses the effects of a prior mutation

Rolling circle replication a mechanism of replicating double-stranded circular DNA that starts by nicking and unrolling one strand and using the other (still circular) strand as a template for DNA synthesis

Screening a procedure that permits the identification of organisms by phenotype or genotype, but does not inhibit or enhance the growth of particular phenotypes or genotypes

Selection placing organisms under conditions that favor or inhibit the growth of those with a particular phenotype or genotype
Silent mutation a change in DNA sequence that has no effect on the phenotype
Spontaneous mutation a mutation that occurs "naturally" without the help of mutagenic chemicals or radiation
Transduction the transfer of host cell genes from one cell to another by a virus
Transformation the transfer of bacterial genes involving free DNA (but see alternative usage in Chapter 9)
Transition a mutation in which a pyrimidine base is replaced by another pyrimidine or a purine is replaced by another purine
Transposable element a genetic element able to move (transpose) from one site to another on host DNA molecules
Transposon a type of transposable element that carries genes in addition to those involved in transposition
Transversion a mutation in which a pyrimidine base is replaced by a purine or vice versa
Wild-type strain a bacterial strain isolated from nature or one used as a parent in a genetics investigation

Review Questions

1. Write a one-sentence definition of the term "genotype." Do the same for "phenotype." Does the phenotype of an organism automatically change when a change in genotype occurs? Why or why not? Can phenotype change without a change in genotype? In both cases, give examples to support your answer (Section 10.1).
2. Explain why an *Escherichia coli* strain that is His^- is an auxotroph and one that is Lac^- is not. (*Hint:* Think about what *E. coli* does with histidine and lactose.) (Section 10.1)
3. What are silent mutations? From your knowledge of the genetic code, why do you think most silent mutations affect the third position in a codon (Section 10.2)?
4. Microinsertions that occur in promoters are not frameshift mutations. Define the terms microinsertion, frameshift, and mutation. Explain why this statement is true (Section 10.2).
5. Explain how it is possible for a frameshift mutation early in a gene to be corrected by another frameshift mutation farther along the gene (Section 10.2).
6. What is the average rate of mutation in a cell? Can this rate change (Section 10.3)?
7. Give an example of one biological, one chemical, and one physical mutagen and describe the mechanism by which each causes a mutation (Section 10.4).
8. How can the Ames test, an assay using bacteria, have any relevance to human cancer (Section 10.5)?
9. How does homologous recombination differ from site-specific recombination (Section 10.6)?
10. Explain why in generalized transduction one always refers to a transducing particle but in specialized transduction one refers to a transducing virus (or transducing phage) (Section 10.8).
11. What is a sex pilus and which cell type, F^- or F^+, would produce this structure (Section 10.9)?
12. What does an F^+ cell need to do before it can transfer chromosomal genes (Section 10.10)?
13. What does it mean to complement a mutation "in *trans*" (Section 10.11)?
14. Explain why performing genetic selections is difficult when studying *Archaea*. Give examples of some selective agents that work well with *Archaea* (Section 10.12).
15. What are the major differences between insertion sequences and transposons (Section 10.13)?
16. The most useful transposons for isolating a variety of bacterial mutants are transposons containing antibiotic resistance genes. Why are such transposons so useful for this purpose (Section 10.13)?

Application Questions

1. A constitutive mutant is a strain that continuously makes a protein that is inducible in the wild type. Describe two ways in which a change in a DNA molecule could lead to the emergence of a constitutive mutant. How could these two types of constitutive mutants be distinguished genetically?
2. Although a large number of mutagenic chemicals are known, none is known that induces mutations in only a single gene (gene-specific mutagenesis). From what you know about mutagens, explain why it is unlikely that a gene-specific chemical mutagen will be found. How then is site-specific mutagenesis accomplished?
3. Why is it difficult in a single experiment to transfer a large number of genes to a recipient cell using transformation or transduction?
4. Transposable elements cause mutations when inserted within a gene. These elements disrupt the continuity of a gene. Introns also disrupt the continuity of a gene, yet the gene is still functional. Explain why the presence of an intron in a gene does not inactivate that gene but insertion of a transposable element does.

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