Hybrid Medical Animation/Photo Researchers, Inc.

Escherichia coli, a model research organism for several types of biological studies, including bacterial genetics (computer rendering).

17 Bacterial and Viral Genetics

Study Plan

Why It Matters

In 1885, a Viennese pediatrician, Theodor Escherich, discovered a bacterium that caused severe diarrhea in infants. He named it *Bacterium coli*. Surprisingly, however, researchers discovered that *Bacterium coli* was also present in healthy infants, and was a normal inhabitant of the human intestine. Only certain strains cause human diseases. Further, researchers in the twentieth century found that if they mixed together bacteria of different strains, the organisms produced some progeny with a mixture of traits from more than one strain—evidence that bacteria could have some kind of sexual reproduction.

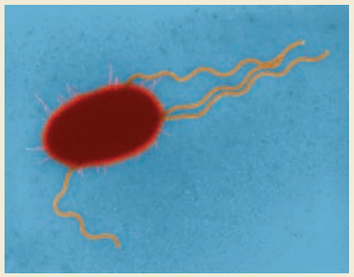
As an organism that is readily available and easy to grow, the bacterium has been of central interest to scientists since its first discovery. Renamed *Escherichia coli* in honor of Escherich, the bacterium brings several distinct advantages to scientific investigation. It can be grown quickly in huge numbers in nutrient solutions that are simple to prepare. And, it is infected with a group of viruses called **bacteriophages** (**phages** for short) that have been just as valuable to scientists as *E. coli* itself because these phages can also be grown by the billions in cultures of the bacterium. The rapid generation times

Focus on Research

Model Research Organisms: *Escherichia coli*

We probably know more about *E. coli* than any other organism. For example, microbiologists have deciphered the complete DNA sequence of the genome of a standard laboratory strain of *E. coli,* including the sequence of the approximately 4400 genes in its genome. The functions of about one third of these genes are still unidentified, however.

E. coli got its start in laboratory research because of the ease with which it can be grown in cultures. Because *E. coli* cells can divide about every 20 minutes under optimal conditions, a clone of 1 billion cells can be grown in a matter of hours, in only 10 mL of culture medium. The same amount of medium can accommodate as many as 10 billion cells before the growth rate begins to slow. *E. coli* strains can be grown in the laboratory with minimal equipment, requiring little more than culture vessels in an incubator held at 37°C.



Dennis Kunkel Microscopy, Inc.

The major advantage of *E. coli* for scientific research in the early days it was used, however, can be summed up in a single word: sex. When Joshua Lederberg and Edward Tatum discovered that *E. coli* can enter into a form of sexual reproduction, they and other scientists realized they could carry out genetic crosses with the bacterium, producing genetic recombinants that could indicate the relative positions of genes on the chromosome. Knowing these relative positions, Lederberg, Tatum, and other scientists were able to generate a genetic map of the *E. coli* chromosome. The map showed that genes with related functions are clustered together, a fact that had significant implications for the regulation of expression of those genes. For example, François Jacob and Jacques Monod's work with the genes for lactose metabolism led to the pioneering operon model (described in Section 16.1). In their work, at The Pasteur Institute in Paris, they used conjugation to map the genes and generated partial diploids to help understand the details of the regulation of transcription of those genes.

The development of *E. coli* as a model organism for studying gene organization and the regulation of gene expression led to the field of molecular genetics. The study of naturally occurring plasmids in *E. coli* and of enzymes that cut DNA at specific sequences eventually resulted in techniques for combining DNA from different sources, such as inserting a gene from an organism into a plasmid. Today *E. coli* is used for creating plasmids that contain inserted genes or other sequences, and for amplifying (cloning) them once they are made.

In essence, the biotechnology industry has its foundation in molecular genetics studies of *E. coli,* and large-scale *E. coli* cultures are widely used as "factories" for production of desired proteins. For example, the human insulin hormone, required for treatment of certain forms of diabetes, can be produced by *E. coli* factories. (Chapter 18 explains more about cloning and other types of DNA manipulation.)

Laboratory strains of *E. coli* are harmless to humans. Similarly, the natural *E. coli* cells in the colon of humans and other mammals are usually harmless. There are pathogenic strains of *E. coli,* though; sometimes they make the news when humans eating food that contains a pathogenic strain develop disease symptoms, such as diarrhea and fever. The genomes of several pathogenic *E. coli* strains have been sequenced, and it is notable that they have more genes than the lab strain, or the strain in the human colon. The extra genes include the genes that make the bacterium pathogenic.

of *E. coli,* its phages, and their numerous offspring make them especially valuable to geneticists. Geneticists have used them to analyze genetic crosses and their outcomes much more quickly than they can with eukaryotes. They can also detect genetic events that occur only once within millions of offspring. The characteristics of these rare events helped scientists to work out the structure, activity, and recombination of genes at the molecular level.

Because *E. coli* can be cultured in completely defined chemical solutions—solutions in which the identity and amount of each chemical is known—it is particularly useful for biochemical investigations. What goes in, what comes out, and what biochemical changes occur inside can be detected and closely followed in normal and mutant bacteria, and in bacteria infected by viruses. These biochemical studies have added immeasurably to the definition of genes and their activities, and have identified many biochemical pathways and the enzymes catalyzing them. (*Focus on Research* tells more about *E. coli*'s advantages as a model laboratory organism.)

After research with bacteria and their viruses showed the way, biologists successfully applied the same techniques to eukaryotes such as *Neurospora* and *Aspergillus,* fungi with short generation times that can also be grown and analyzed biochemically in large numbers. Molecular studies are now easy to carry out with a wide variety of eukaryotes, including yeast, the fruit fly *Drosophila,* and the plant *Arabidopsis*. The results of this research showed that the molecular characteristics of genes discovered in prokaryotes apply to eukaryotes as well.

This chapter outlines the basic findings of molecular genetics in bacteria and their phages. It also describes *transposable elements*—sequences that can move from

place to place in bacterial DNA—and compares the bacterial transposable elements with those of eukaryotes. We begin our discussion with the sex life of bacteria.

17.1 Gene Transfer and Genetic Recombination in Bacteria

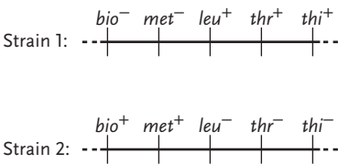
In the first half of the twentieth century, foundational genetic experiments with eukaryotes revealed the processes of genetic recombination during sexual reproduction and led to the construction of genetic maps of chromosomes for a number of organisms (see Chapters 12 and 13). Bacteria became the subject of genetics research in the middle of the twentieth century. A key early question was whether gene transfer and genetic recombination can occur in bacteria even though these organisms do not reproduce sexually by meiosis. For particular bacteria, the answer to the question was yes—genes can be transferred from one bacterium to another by several different mechanisms, and the newly introduced DNA can recombine with DNA already present. Such genetic recombination performs the same function as it does in eukaryotes: it generates genetic variability through the exchange of alleles between homologous regions of DNA molecules from two different individuals.

E. coli is one of the bacteria in which genetic recombination occurs. By the 1940s geneticists knew that *E. coli* and many other bacteria could be grown in a **minimal medium** containing water, an organic carbon source such as glucose, and a selection of inorganic salts including one, such as ammonium chloride, that provides nitrogen. The growth medium can be in liquid form or in the form of a gel made by adding agar to the liquid medium. (Agar is a polysaccharide material, indigestible by most bacteria, that is extracted from algae.)

Since it is not practical to study a single bacterium for most experiments, researchers soon developed techniques for starting bacterial cultures from a single cell, generating cultures with a large number of genetically identical cells. Cultures of this type are called **clones.** To start bacterial clones, the scientist spreads a drop of a bacterial culture over a sterile agar gel in a culture dish. The culture is diluted enough to ensure that cells will be widely separated on the agar surface. Each individual cell divides many times to produce a separate colony that is a clone of the cell. Cells can be removed from a clone and introduced into liquid cultures or spread on agar and grown in essentially any quantity.

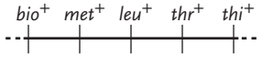
Genetic Recombination Occurs in *E. coli*

In 1946, two scientists at Yale University, Joshua Lederberg and Edward L. Tatum, set out to determine if genetic recombination occurs in bacteria, using *E. coli* as their experimental organism. In essence, they were testing whether bacteria had a sexual reproduction process. As a first step, they induced genetic mutations in *E. coli* bacteria by exposing the cells to mutagens such as X-rays or ultraviolet light. After the exposure, Lederberg and Tatum found that some bacteria had become auxotrophs, mutant strains that could not grow on minimal medium (see Section 15.1). One mutant strain could grow only if the vitamin biotin and the amino acid methionine were added to the culture medium; evidently the genes that encoded the enzymes required to make these substances had mutated in this strain. A second mutant strain did not need biotin or methionine in its growth medium, but could grow only if the amino acids leucine and threonine were added along with the vitamin thiamine. These two genetic strains of *E. coli* were represented in genetic shorthand as:



In this shorthand, *bio* refers to the gene that governs a cell's ability to synthesize biotin from inorganic precursors. The designation bio^+ indicates that the allele is normal; bio^- represents the mutant allele, which produces cells that cannot make biotin for themselves. Similarly, met^+, met^-, leu^+, leu^-, thr^+, thr^-, and thi^+, thi^- are the respective normal and mutant alleles for methionine, leucine, threonine, and thiamine synthesis.

Lederberg and Tatum mixed about 100 million cells of the two mutant strains together and placed them on a minimal medium **(Figure 17.1).** None of the cells were expected to be able to grow on the minimal medium unless some form of recombination between DNA molecules from the two parental types produced the new combination with normal alleles for each of the five genes:



Several hundred colonies grew on the minimal medium, indicating that genetic recombination had actually taken place in the bacteria. Lederberg and Tatum eliminated the possibility that the colonies arose from chance mutations back to normal alleles by placing hundreds of millions of cells of strains 1 or 2 separately on the surface of a minimal medium: no colonies grew.

Bacterial Conjugation Brings DNA of Two Cells Together, Allowing Recombination to Occur

Lederberg and Tatum's results led to a major question: How did DNA molecules with different alleles get together to undergo genetic recombination? Recombina-

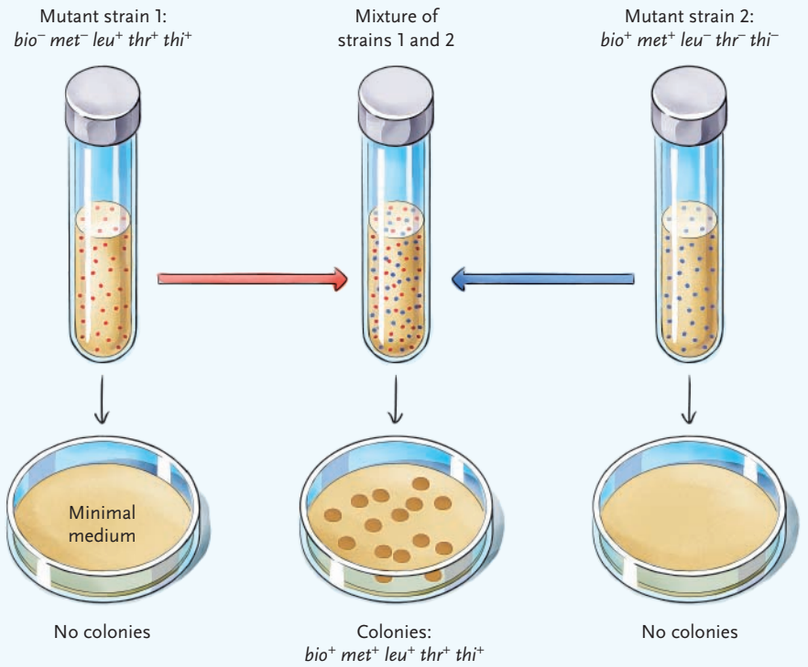
Figure 17.1 Experimental Research

Genetic Recombination in Bacteria

QUESTION: Does genetic recombination occur in bacteria?

EXPERIMENT: Lederberg and Tatum tested whether genetic recombination occurred between two mutant strains of *E. coli*. Mutant strain 1 required biotin and methionine to grow, but not leucine, threonine, or thiamine—its genotype was $bio^-\ met^-\ leu^+\ thr^+\ thi^+$. Mutant strain 2 required leucine, threonine, and thiamine to grow, but not biotin or methionine—its genotype was $bio^+\ met^+\ leu^-\ thr^-\ thi^-$.

Lederberg and Tatum mixed together large numbers of the two mutant strains and plated them on minimal medium, which lacked any of the nutrients the strains needed for growth. As controls, they also plated large numbers of the two mutant strains individually on minimal medium.



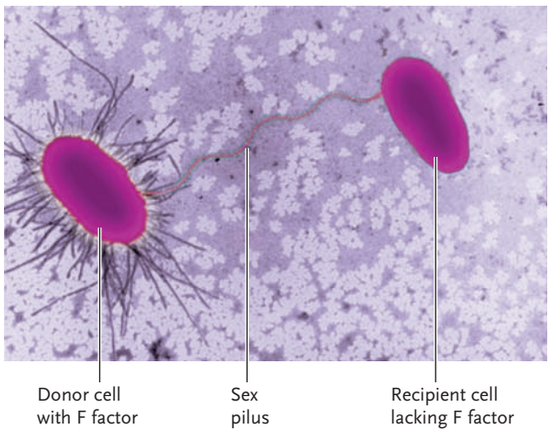
RESULTS: No colonies grew on the control plates, indicating that the mutant alleles in the two strains did not mutate back to normal alleles, which would have produced growth on minimal medium. However, many colonies grew on plates spread with a mixture of mutant strain 1 and mutant strain 2.

CONCLUSION: In order to grow on minimal medium, the bacteria must have the genotype $bio^+\ met^+\ leu^+\ thr^+\ thi^+$. Lederberg and Tatum reasoned that the colonies on the plate must have resulted from genetic recombination between mutant strains 1 and 2.

tion in eukaryotes occurs in diploid cells, by an exchange of segments between the chromatids of homologous chromosome pairs (discussed in Section 13.1). Bacteria typically have a single, circular chromosome—they are haploid organisms (see Section 14.5). At first, bacterial cells were thought to fuse together, producing the prokaryotic equivalent of a diploid zygote. However, it was later established that instead of fusing, bacterial cells *conjugate:* they contact each other, initially becoming connected by a long tubular structure called a *sex pilus* **(Figure 17.2a),** and then forming a cytoplasmic bridge that connects two cells **(Figure 17.2b).** During **conjugation**, a copy of part of the DNA of one cell, the *donor* (the bristly cell in Figure 17.2a), moves through the cytoplasmic bridge into the other cell, the *recipient*. Once donor DNA enters the recipient, it pairs with the homologous region of the recipient cell's DNA, and genetic recombination can occur. Through this unidirectional transfer of DNA, bacterial conjugation thus accomplishes a prokaryotic form of sexual reproduction.

The F Factor and Conjugation. The donor bacterial cell in a pairing initiates conjugation. The ability to conjugate depends on the presence within a donor cell of a

a. Attachment by sex pilus



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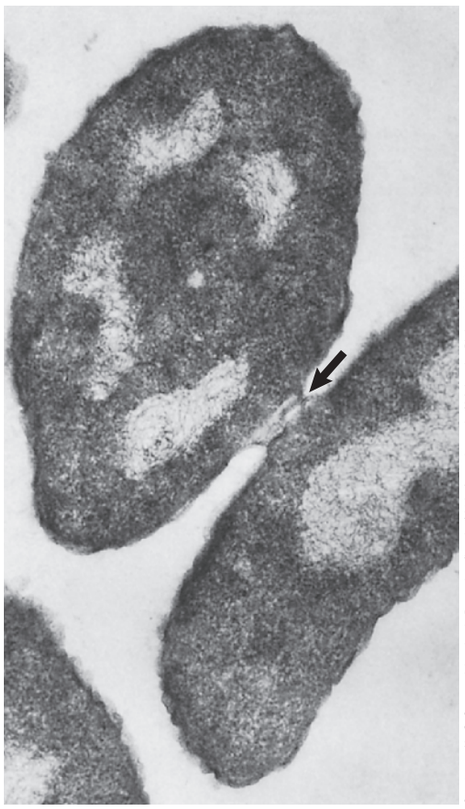
b. Cytoplasmic bridge formed

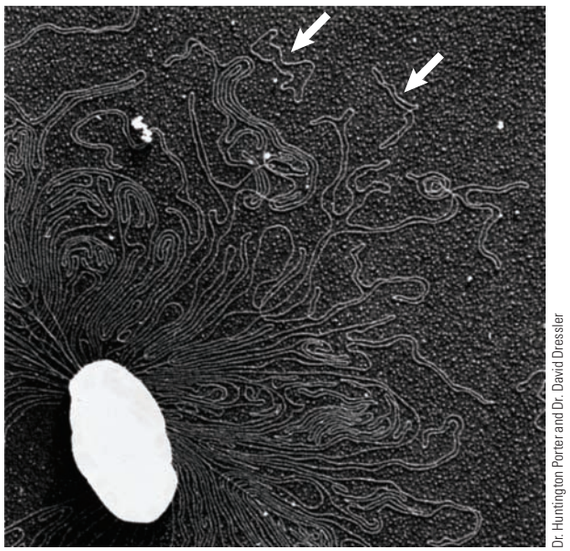


Courtesy of L. G. Caro and Academic Press, Inc. [London] Ltd., from *Journal of Molecular Biology* 16:269, 1966

Figure 17.2
Conjugating *E. coli* cells. **(a)** Initial attachment of two cells by the sex pilus. **(b)** A cytoplasmic bridge (arrow) has formed between the cells, through which DNA moves from one cell to the other.

plasmid called the **F factor** (F = fertility). Plasmids are small circles of DNA that occur in bacteria in addition to the main circular chromosomal DNA molecule **(Figure 17.3).** Plasmids contain several to many genes and a replication origin that permits them to be duplicated and passed on during bacterial division. Donor cells in conjugation are called **F^+ cells** because they contain the F factor. They are able to mate with recipient cells but not with other donor cells. Recipient calls lack the F factor and, hence, are called **F^- cells.**

a. Bacterial DNA released from cell



Dr. Huntington Porter and Dr. David Dressler

b. Plasmid

Prof. Stanley Cohen/SPL/Photo Researchers, Inc.

Figure 17.3
Electron micrographs of DNA released from a disrupted bacterial cell. **(a)** Plasmids (arrows) near the mass of chromosomal DNA. **(b)** A single plasmid at higher magnification (colorized).

The F factor carries about 20 or so genes. Several of the genes encode proteins of the **sex pilus**, also called the **F pilus** (plural, *pili*). The sex pilus is a long, tubular structure on the cell surface that allows an F^+ donor cell to attach to a F^- recipient (see Figure 17.2a). Once attached, the cells form a cytoplasmic bridge and conjugate (**Figure 17.4a,** step 1, and see Figure 17.2b). During conjugation, the F plasmid replicates using a special type of DNA replication. When the two strands of the plasmid DNA separate during replication, one of the strands is transferred from the F^+ cell through the cytoplasmic bridge to the F^- cell (Figure 17.4a, step 2). In the recipient cell, synthesis of the complementary strand to the entering DNA strand occurs (Figure 17.4a, step 3). When the entire F factor strand has entered and its complementary strand has been synthesized, the F factor circularizes into a complete F factor, changing the cell to F^+ (Figure 17.4a, step 4). No chromosomal DNA is transferred between cells in this

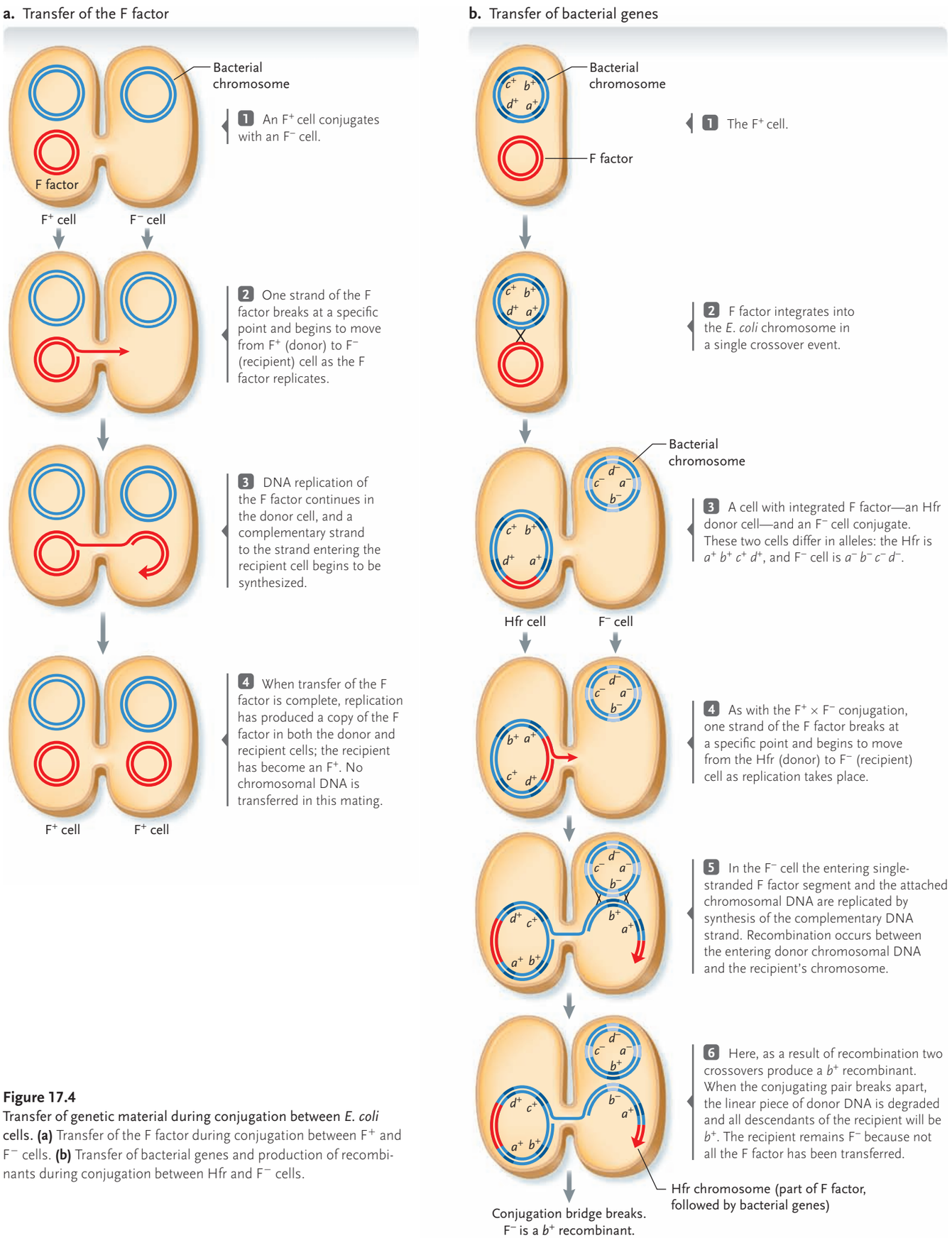


Figure 17.4
Transfer of genetic material during conjugation between *E. coli* cells. **(a)** Transfer of the F factor during conjugation between F^+ and F^- cells. **(b)** Transfer of bacterial genes and production of recombinants during conjugation between Hfr and F^- cells.

process, however, so no genetic recombination results from $F^+ \times F^-$ mating.

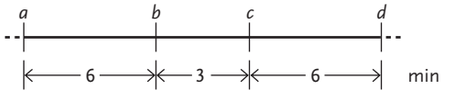
Hfr Cells and Genetic Recombination. How does genetic recombination of bacterial genes occur as a result of conjugation if no chromosomal DNA transfers when an F factor is transferred in a mating? The answer is that in some F^+ cells (**Figure 17.4b,** step 1), the F factor integrates into the bacterial chromosome by crossing-over (Figure 17.4b, step 2), which produces a donor that can transfer genes on the bacterial chromosome to a recipient. These special donor cells are known as **Hfr cells** (Hfr = high frequency recombination). Because the F factor genes are still active when the plasmid is integrated into the bacterial chromosome, an Hfr cell can conjugate with an F^- cell. Figure 17.4b, step 3, shows an Hfr $\times$ F^- mating where the two cell types differ in alleles. DNA replication begins in the middle of the integrated F factor, and a segment of the F factor moves through the conjugation bridge into the recipient (Figure 17.4b, step 4), bringing the chromosomal DNA behind it (Figure 17.4b, step 5). Synthesis of the complementary DNA strand to the entering DNA from the donor occurs in the recipient cell. The conjugation bridge between the mating cells soon breaks, but DNA transfer usually continues for long enough so that at least some genes of the donor cell follow the F segment into the recipient cell. The recipient therefore becomes a **partial diploid** for the donor chromosomal DNA segment that goes through the conjugation bridge.

For our example, the recipient cell in Figure 17.4b, step 5, has become $a^+\ b^+/a^-\ b^-$. The recipient's DNA and the homologous DNA fragment from the donor can pair and recombine. Genetic recombination occurs by a double crossover event exchanging donor gene(s) with recipient gene(s) using essentially the same mechanisms as in eukaryotes (discussed in Section 13.1)—Figure 17.4b, step 6, shows the generation of an b^+ recombinant. In other pairs in the mating population, the a^+ gene could recombine with the homologous recipient gene, or both a^+ and b^+ genes could recombine. The genetic recombinants observed in the Lederberg and Tatum experiment described earlier were produced in this same general way. Since conjugation usually breaks off long before the second part of the F plasmid has been transferred (it would be the last DNA piece transferred), the recipient cell remains F^-.

Recombinants produced during conjugation can be detected only if the alleles of the genes in the DNA transferred from the donor differ from those in the recipient's chromosome. Following recombination, the bacterial DNA replicates and the cell divides normally, producing a cell line with the new combination. Any remnants of the DNA fragment that originally entered the cell are degraded as division proceeds and do not contribute further to genetic recombination or cell heredity.

Mapping Genes by Conjugation. Genetic recombination by conjugation was discovered by two scientists, François Jacob (the same scientist who proposed the operon model for the regulation of gene expression in bacteria; see Section 16.1) and Elie L. Wollman, at the Pasteur Institute in Paris. They began their experiments by mating Hfr and F^- cells that differed in a number of alleles. At regular intervals after conjugation commenced, they removed some of the cells and agitated them in a blender to break apart mating cells. They then cultured the separated cells and analyzed them for recombinants. They found that the longer they allowed cells to conjugate before separation, the greater the number of donor genes that entered the recipient and produced recombinants. From this result, Jacob and Wollman concluded that during conjugation, the Hfr cell slowly injects a copy of its DNA into the F^- cell. Full transfer of an entire DNA molecule to an F^- cell would take about 90 to 100 minutes. In nature, however, the entire DNA molecule is rarely transferred because the cytoplasmic bridge between conjugating cells is fragile and easily broken by random molecular motions before transfer is complete.

The pattern of gene transfer from Hfr to F^- cells was used to map the *E. coli* chromosome. The F factor integrates into one of a few possible fixed positions around the circular *E. coli* DNA. As a result, the genes of the bacterial DNA follow the F factor segment into the recipient cell in a definite order, with the gene immediately behind the F factor segment entering first and the next genes following. In the theoretical example shown in Figure 17.4b, donor genes will enter in the order a^+–b^+–c^+–d^+. By breaking off conjugation at gradually increasing times, investigators allowed longer and longer pieces of DNA to enter the recipient cell, carrying more and more genes from the donor cell (detected by the appearance of recombinants). By noting the order and time at which genes were transferred, investigators were able to map and assign the relative positions of most genes in the *E. coli* chromosome. The resulting genetic map has distances between genes in units of minutes. To this day, the genetic map of *E. coli* shows map distances as minutes, reflecting the mapping of genes by conjugation.



The genetic maps from *E. coli* conjugation experiments indicated that the genes are arranged in a circle, reflecting the circular form of the *E. coli* chromosome. More recently, direct sequencing of the *E. coli* genome has confirmed the results obtained by genetic mapping.

In addition to the F plasmid, bacteria also contain other types of plasmids. Some plasmids contain genes that provide resistance to unfavorable conditions, such as exposure to antibiotics (plasmids providing such resistance genes are called **R plasmids**). The competi-

tive advantage provided by the genes in some plasmids may account for the wide distribution of plasmids of all kinds in prokaryotic cells.

Transformation and Transduction Provide Additional Sources of DNA for Recombination

The discovery of conjugation and genetic recombination in *E. coli* showed that genetic recombination is not restricted to eukaryotes. Further discoveries demonstrated that DNA can transfer from one bacterial cell to another by two additional mechanisms, *transformation* and *transduction*. Like conjugation, these mechanisms transfer DNA in one direction, and create partial diploids in which recombination can occur between alleles in the homologous DNA regions.

Transformation. In **transformation**, bacteria take up pieces of DNA that are released as other cells disintegrate. Fred Griffith, a medical officer in the British Ministry of Health, London, discovered this mechanism in 1928, when he found that a noninfective form of the bacterium *Streptococcus pneumoniae,* unable to cause pneumonia in mice, could be transformed to the infective form if it was exposed to heat-killed cells of an infective strain. The key difference between the strains is a polysaccharide capsule around the infective strain that is absent in the noninfective strain because of a genetic difference between the strains. In 1944, Oswald Avery and his colleagues at New York University found that the substance derived from the killed infective cells, the substance capable of transforming noninfective bacteria to the infective form, was DNA (discussed in Section 14.1).

Subsequently, geneticists established that in the transformation of *Streptococcus,* the linear DNA fragments taken up from disrupted infective cells recombine with the chromosomal DNA of the noninfective cells by double crossovers, much in the same way as genetic recombination takes place in conjugation. The recombination introduces the normal allele for capsule formation into the DNA of the noninfective cells; expression of that normal allele generates a capsule around the cell and its descendants, making them infective.

Only some species of bacteria can take up DNA from the surrounding medium by natural mechanisms. Such bacteria typically have a DNA-binding protein on the outer surface of the cell wall. When DNA from the cell's surroundings binds to the protein, a deoxyribonuclease enzyme breaks the DNA into short pieces that pass through the cell wall and plasma membrane into the cytosol. The entering DNA can then recombine with the recipient cell's chromosome if it contains homologous regions.

E. coli cells do not normally take up DNA from their surroundings. However, they can be induced to take up DNA by *artificial transformation*. One way this is accomplished is to expose *E. coli* cells to calcium ions and the DNA of interest, incubate them on ice, and then give them a quick heat shock. This treatment alters the plasma membrane so that DNA can penetrate and enter. The entering DNA undergoes recombination if it contains regions that are homologous to part of the chromosomal DNA.

Another technique for artificial transformation, called *electroporation,* exposes cells briefly to rapid pulses of an electrical current. The electrical shock alters the plasma membrane so that DNA can enter. The method works well with many of the bacterial species that are unable to take up DNA on their own, and also with many types of eukaryotic cells.

Artificial transformation is often used to insert plasmids containing DNA sequences of interest into *E. coli* cells as a part of cloning techniques. After the cells are transformed, clones of the cells are grown in large numbers to increase the quantity of the inserted DNA to the amounts necessary for sequencing or genetic engineering. (DNA cloning and genetic engineering are discussed further in Chapter 18.)

Transduction. In **transduction**, DNA is transferred to recipient bacterial cells by an infecting phage (see Section 14.1). When new phages assemble in an infected bacterial cell, they sometimes incorporate fragments of the host cell DNA along with or instead of the viral DNA. After the phages are released from the host cell, they may attach to another cell and inject the bacterial DNA (and the viral DNA if it is present) into that cell. The introduction of this DNA, as in conjugation and transformation, makes the recipient cell a partial diploid and allows recombination to take place. Joshua Lederberg and his graduate student, Norton Zinder, then at the University of Wisconsin at Madison, discovered transduction in 1952 in experiments with the bacterium *Salmonella typhimurium* and phage P22. Lederberg received a Nobel Prize in 1958 for his discovery of conjugation and transduction in bacteria.

Replica Plating Allows Genetic Recombinants to Be Identified and Counted

How do researchers identify and count genetic recombinants in conjugation, transformation, or transduction experiments? Joshua Lederberg and Esther Lederberg developed a now widely applied technique called **replica plating** for doing this. In replica plating, a plate of solid growth medium with colonies on it—the master plate—is pressed gently onto sterile velveteen **(Figure 17.5).** This transfers some of each colony to the velveteen in the same pattern as the colonies were on the plate. The velveteen is then pressed gently onto new plates of solid growth medium—the replica plates—thereby transferring some of the cells from each original colony to those plates. The new plates each have been inoculated with a "replica" of the original set of colonies on the starting

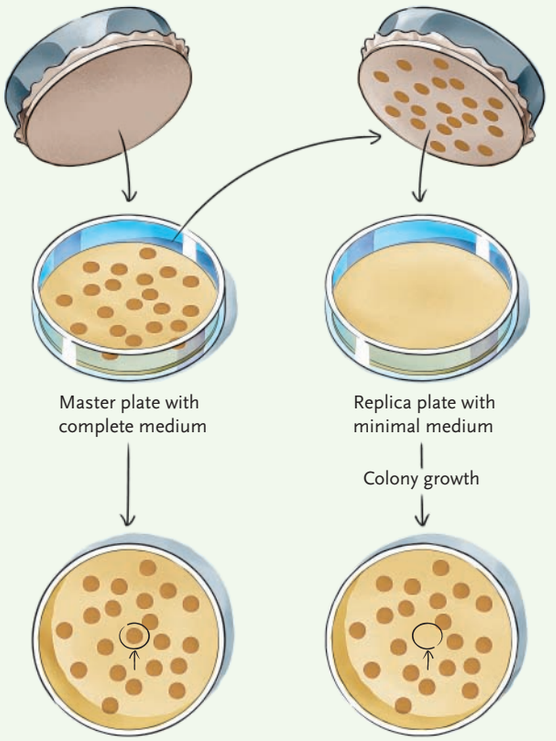
Figure 17.5 Research Method

Replica Plating

PURPOSE: Replica plating is used to identify different strains with respect to their growth requirements in a heterogeneous mixture of strains.

PROTOCOL:

1. Press sterile velveteen gently onto a plate of solid growth medium with colonies on it. Some of each colony transfers to the velveteen in the same pattern as the colonies on the plate. In the example, a mixture of colonies of normal and auxotrophic strains are on a plate of complete medium.
2. Press the velveteen gently onto a new plate—the replica plate—to transfer some of each strain. In the example, the replica plate contains minimal medium. Incubate to allow colonies to grow and compare the pattern of colonies on the replica plate with that on the master plate.



INTERPRETING THE RESULTS: A colony present on the master plate but not on the replica plate indicates that strain requires some growth substance missing from the minimal medium in order to grow. In other words, the strain is an auxotroph. In actual experiments, the compositions of the master plate and replica plate media are chosen to be appropriate for the goals of the experiment.

plate. The new plates are incubated to allow new colonies to grow. Therefore, by replica plating from the original plate to plates containing growth media with different compositions, an investigator can determine the mutations a strain carries. That is, the compositions of the growth media on the new plates are adjusted to promote the growth of colonies with particular characteristics. **Complete medium** is a full complement of nutrient substances, including amino acids and other chemicals that normal strains can make for themselves; in a minimal medium, normal cells will grow but auxotrophic mutants will not, because they are unable to make one or more of the missing substances.

Figure 17.5 shows the identification of auxotrophic mutants of *E. coli* by replica plating colonies that were grown in complete medium onto minimal medium. All strains grow on the complete medium, but auxotrophic mutants will not grow on the minimal medium. Therefore, an investigator compares the colony patterns on the complete medium (original plate) and the minimal medium to determine missing colonies on the minimal medium plate. The corresponding colonies on the original plate are taken and studied further. In an actual experiment, the compositions of the media are appropriate for the goals of the experiment. For example, to identify a met^+ recombinant in a conjugation experiment, the starting plate contains methionine and the colonies are replica plated to a plate lacking methionine. Comparison of the colony patterns on the two plates identifies met^+ recombinants because they grow on the plate lacking methionine, whereas met^- parentals do not.

While the phages mentioned in this section infect only bacteria, viruses infect all living organisms. Many viruses have become important subjects for research into, among other things, the molecular nature of re-

combination and the genetic control of virus infection. Viruses and viral recombination are the subjects of the next section.

Study Break

Describe the properties of F^+, F^-, and Hfr cells of *E. coli*.

17.2 Viruses and Viral Recombination

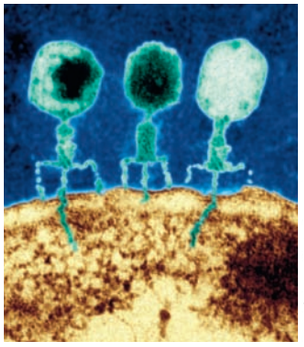
As agents of transduction, the phages that infect bacteria are important tools in research on bacterial genetics. The same viruses are also important for studying *viral* recombination and genetics. Viruses can undergo genetic recombination when the DNA of two viruses, carrying different alleles of one or more genes, infect a single cell. Using phages, researchers study viral genetics with the same molecular and biochemical techniques used to investigate their bacterial hosts.

Viruses in the Free Form Consist of a Nucleic Acid Core Surrounded by a Protein Coat

Viruses in the free form, outside their host cells, consist of a nucleic acid **core** surrounded by a protective protein **coat. Figure 17.6** shows a phage and an animal virus. Some animal viruses have an additional layer—the envelope—derived from the plasma membrane of the host cell. Viruses are carried about passively by random molecular movements and perform none of the metabolic activities of life. However, once they or their nucleic acid genome enters a host cell, viruses typically subvert the host's cellular machinery for the replication of the viral nucleic acid and the synthesis of viral proteins. Viruses of different kinds infect bacterial, plant, and animal cells. (Viruses are described further in Chapter 25.)

The viral nucleic acid molecules—DNA in some viruses, RNA in others—may contain from a few to around a hundred genes. All viruses have genes encoding at least their coat proteins and the enzymes required for nucleic acid replication. Many viruses also have genes encoding recognition proteins that become implanted in the coat surface. These coat proteins recognize and bind to the host cell, promoting entry of the viral particle or its nucleic acid core into that cell.

a. Bacteriophages



Eye of Science/Photo Researchers, Inc.

b. Animal virus

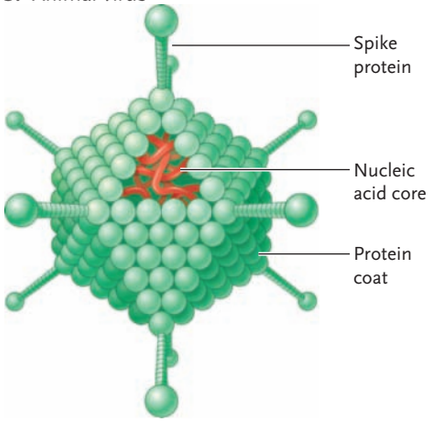


Figure 17.6
Viruses. **(a)** Bacteriophages injecting their DNA into *E. coli*. **(b)** An animal virus. A portion of the protein coat has been cut away to show the nucleic acid core. The spike proteins are recognition proteins that allow the viral particle to bind to the surface of a host cell.

E. coli's Bacteriophages Are Widely Used in Genetic Research

Several bacteriophages that infect *E. coli* are used by scientists studying both bacterial and viral genetics. These include **virulent bacteriophages**, which kill their host cells during each cycle of infection, and **temperate bacteriophages**, which may enter an inactive phase in which the host cell replicates and passes on the bacteriophage DNA for generations before the phage becomes active and kills the host.

***E. coli*'s Virulent Bacteriophages.** Among the virulent bacteriophages infecting *E. coli*, the **T-even bacteriophages** T2, T4, and T6 have been most valuable in genetic studies (shown in Figure 17.6a). The coat of these phages is divided into a *head* and a *tail*. Packed into the head is a single linear molecule of DNA. The tail, assembled from several different proteins, has recognition proteins at its tip that can bind to the surface of the host cell.

As the first step in a cycle of infection, a T-even phage collides randomly with the surface of an *E. coli* cell and the tail attaches to the cell wall (**Figure 17.7**, step 1). The tail then contracts and injects the phage's DNA through the cell wall and plasma membrane and into the cytoplasm (step 2). The coat proteins remain outside. Throughout its life cycle within the bacterial cell, the phage uses host cell machinery to express its genes. One of the proteins produced early in infection is an enzyme that breaks down the bacterial chromosome. Also early in infection, the phage gene for a DNA polymerase that replicates the phage chromosome is expressed, and replication of the phage DNA begins; eventually 100 to 200 new viral DNA molecules are made (step 3). Later in infection, the host cell machinery transcribes the other phage genes and translates their mRNAs, producing the viral coat proteins (step 4). As the head and tail proteins assemble, the replicated viral DNA packs into the heads (step 5).

When viral assembly is complete, a final enzyme encoded in the viral DNA ruptures the cell. The rupture releases viral particles into the surrounding medium; these progeny phages can infect *E. coli* cells they encounter (step 6). This whole series of events from infection of one cell through the release of progeny phages from broken open, or lysed, cells is called the **lytic cycle.**

For some virulent phages (although not T-even phages), fragments of the host DNA may be included in the heads as the viral particles assemble, providing the basis for transduction of bacterial genes during the next cycle of infection. Because genes are randomly incorporated from essentially any DNA fragments, gene transfer by this mechanism is termed **generalized transduction.**

A Scientist's Favorite Temperate *E. coli* Bacteriophage, lambda (λ). The infective cycle of bacteriophage *lambda,* an *E. coli* bacteriophage much used in research, is typical of temperate phages. Phage λ infects *E. coli* in much the same way as the T-even phages do. The phage injects its linear DNA chromosome into the bacterium (**Figure 17.8,** step 1). Once inside, the linear chromosome forms a circle, and then follows one of two paths. Sophisticated molecular switches govern which path is followed at the time of infection.

One path is the lytic cycle, which is like the lytic cycles of virulent phages. The lytic cycle starts with steps 1 and 2 (infection), then goes directly to steps 7 through 9 (production and release of progeny virus), and back to step 1.

The second and more common path is the **lysogenic cycle.** This cycle begins when the circular lambda chromosome integrates into the host cell's DNA by crossing-over (Figure 17.8, step 3). The DNA of a temperate phage typically inserts at one or possibly a few specific sites in the bacterial chromosome through the action of a phage-encoded enzyme that recognizes certain sequences in the host DNA. In the case of lambda, there is one integration site in the *E. coli* chromosome. Once integrated, the lambda genes are mostly inactive and, therefore, no phage components are made. As a consequence, the phage does not affect its host cell and its descendants. The viral genome is known as a **prophage** while it is inserted in the host cell DNA. In the integrated state, the viral DNA is replicated and passed on in division along with the host cell DNA (steps 4 and 5).

In response to certain environmental signals, such as UV irradiation, the lambda phage in an infected cell becomes active and enters the lytic cycle. Genes that were inactive in the prophage are now transcribed. Among the first viral proteins synthesized in response to the environmental signal are enzymes that excise the lambda chromosome from the host chromosome (step 6). Excision occurs by a crossing-over process that reverses the integration step. The result is a circular

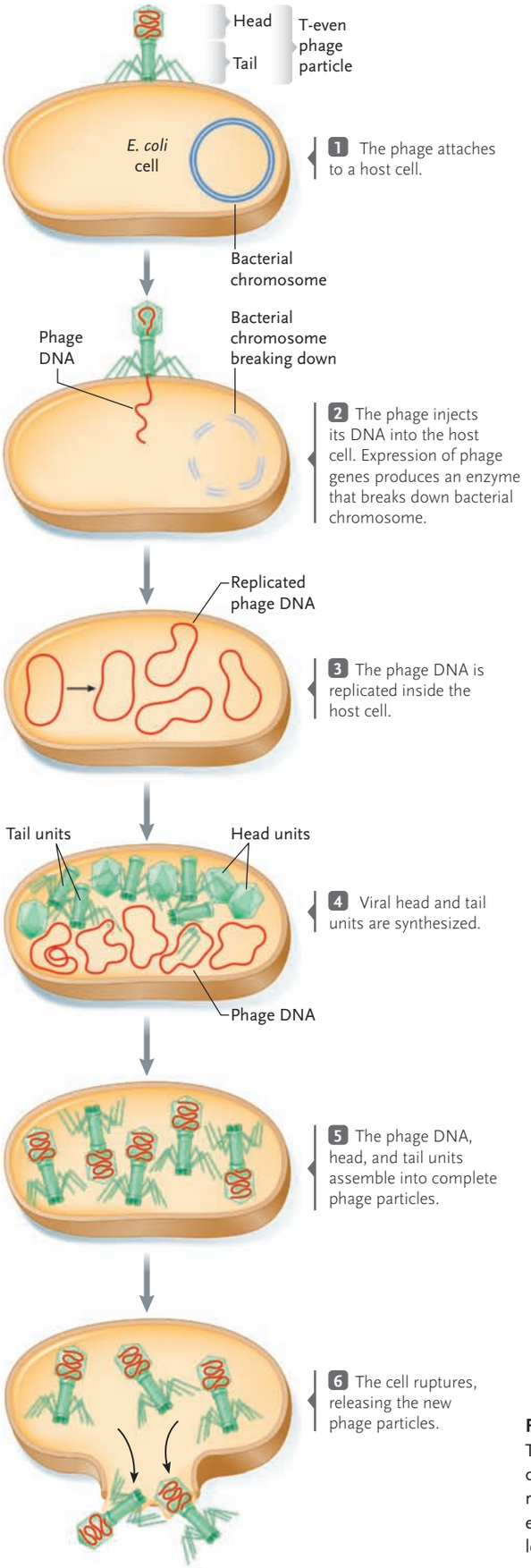


Figure 17.7
The infective cycle of a T-even bacteriophage, an example of a virulent phage.

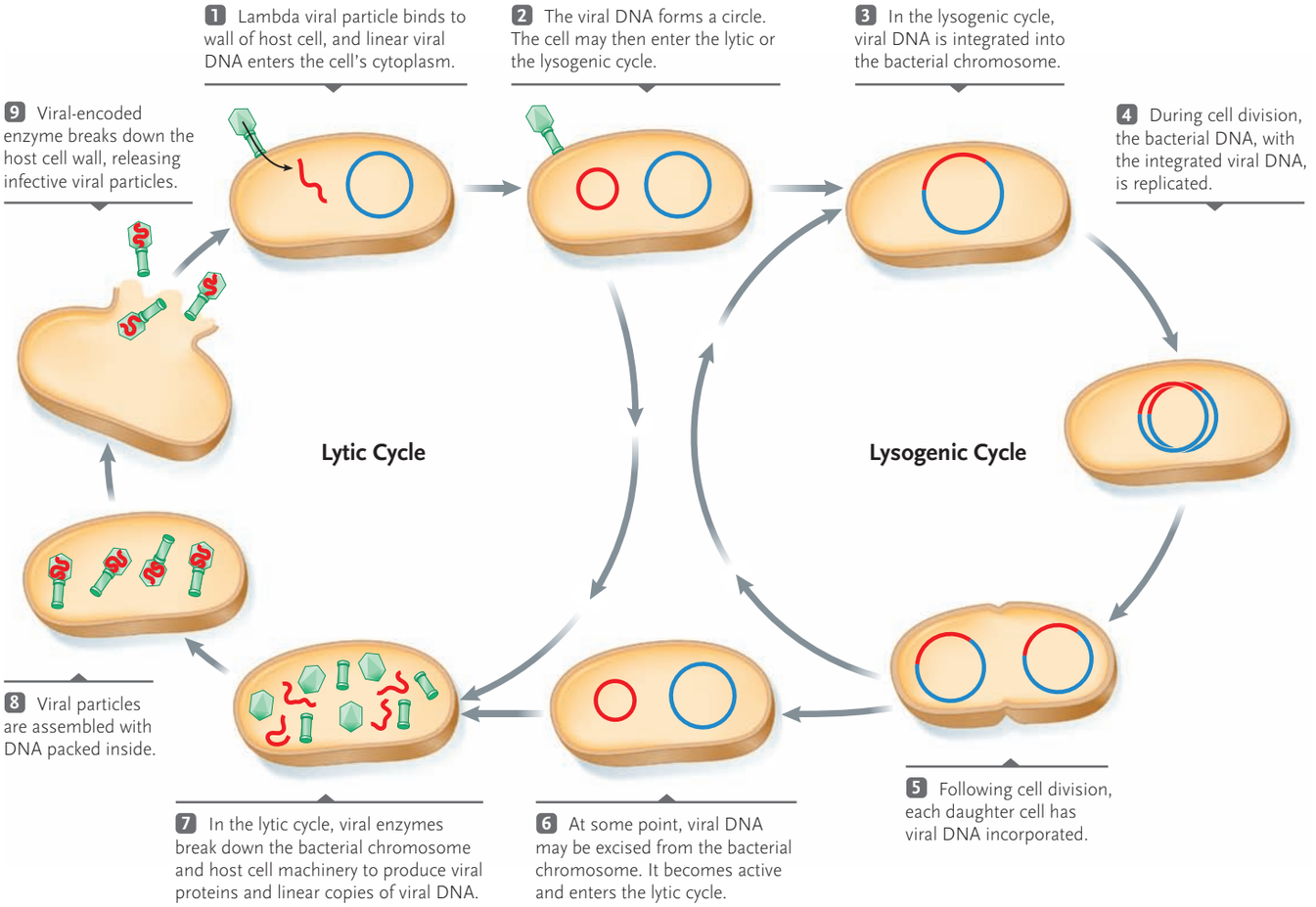


Figure 17.8
The infective cycle of lambda, an example of a temperate phage, which can go through the lytic cycle or the lysogenic cycle.

lambda chromosome. Replication of that chromosome produces many copies of linear lambda chromosomes. Expression of genes on those chromosomes generates coat proteins, which assemble with the chromosomes to produce the viral particles (steps 7 and 8). This active stage culminates in rupture of the host cell with the release of infective viral particles (step 9), and the beginning of a new cycle (step 1).

At times, the excision of the lambda chromosome from the *E. coli* DNA is not precise, resulting in the inclusion of one or more host cell genes. These genes are replicated with the viral DNA and packed into the coats, and may be carried to a new host cell in the next cycle of infection. Clearly only genes that are adjacent to the integration site(s) of a temperate phage can be cut out with the viral DNA, included in phage particles during the lytic stage, and undergo transduction. Accordingly, this mechanism of gene transfer is termed **specialized transduction.**

We have seen several examples of how bacteria and viruses generate genetic variability through exchange of genes between organisms. We now turn to a source of genetic variability that involves gene transfer within the genome of a single organism.

Study Break

What is the difference between a virulent phage and a temperate phage?

17.3 Transposable Elements

All organisms contain particular segments of DNA that can move from one place to another within a cell's genome. The movable sequences are called *transposable genetic elements,* or more simply, **transposable elements (TEs)**. They have also been called "jumping genes."

The movement of TEs, called *transposition,* involves a type of genetic recombination process. However, the location in the DNA where the TE moves to—the *target site*—is not homologous with the TE. In this respect, transposition differs from the genetic recombination process in bacteria conjugation, transformation, and transduction and in eukaryote meiosis, which involves crossing-over between homologous DNA molecules.

Transposition of a TE occurs at a low frequency. Depending on the TE, transposition occurs in one of two ways: (1) a cut-and-paste process, in which the TE leaves its original location and transposes to a new location **(Figure 17.9a);** or (2) a copy-and-paste process, in which a copy of a TE transposes to a new location, leaving the original TE behind **(Figure 17.9b).** For most TEs, transposition starts with contact between the TE and the target site. This also means that TEs do not exist free of the DNA in which they are integrated; hence, their popular name of "jumping genes" is actually inaccurate.

TEs are important because of the genetic changes they cause. For example, they produce mutations by transposing into genes and knocking out their functions, and they increase or decrease gene expression by transposing into regulatory sequences of genes. As such, TEs are an important source of genetic variability.

Insertion Sequence Elements and Transposons Are the Two Major Types of Bacterial Transposable Elements

Bacterial TEs were discovered in the 1960s. They have been shown to move from site to site within the bacterial chromosome, between the bacterial chromosome and plasmids, and between plasmids. The frequency of transposition is low but constant for a given TE. Some bacterial TEs insert randomly, at any point in the DNA, while others recognize certain sequences as "hot spots" for insertion and insert preferentially at these locations.

The two major types of bacterial TEs are *insertion sequences* (*IS*) and *transposons* **(Figure 17.10). Insertion sequences** are the simplest TEs. They are relatively small and contain only genes for their transposition, notably the gene for **transposase,** an enzyme that catalyzes some of the reactions inserting or removing

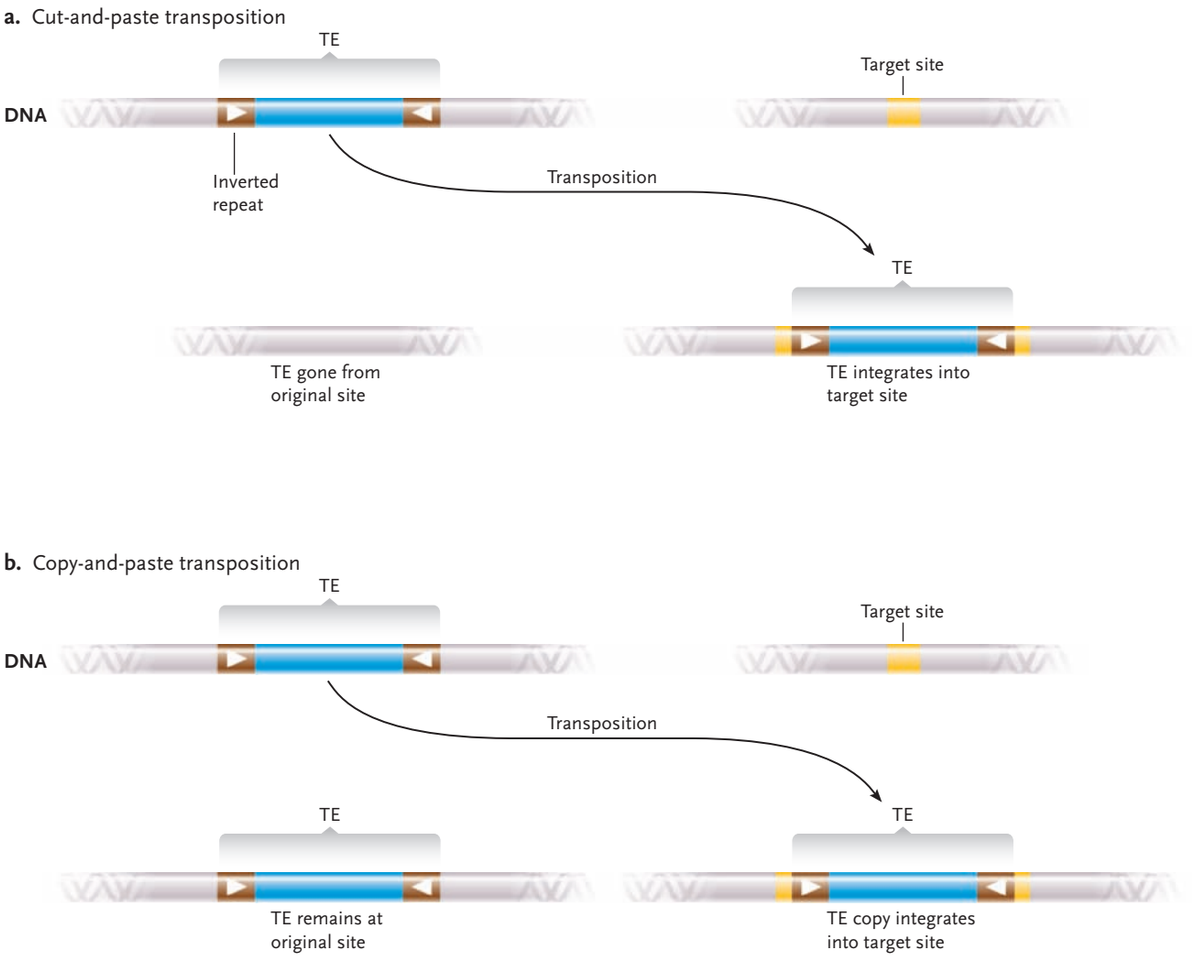


Figure 17.9
Two transposition processes for transposable elements. **(a)** Cut-and-paste transposition, in which the TE leaves one location in the DNA and moves to a new location. **(b)** Copy-and-paste transposition, in which a copy of the TE moves to a new location, leaving the original TE behind.

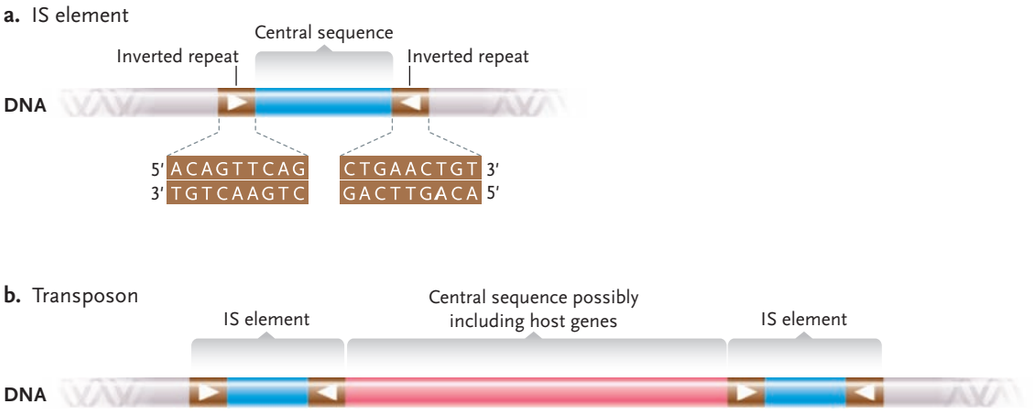


Figure 17.10
Types of bacterial transposable elements. **(a)** Insertion sequence. **(b)** Transposon.

the TE from the DNA (see Figure 17.10a). At the two ends of an IS is a short *inverted repeat* sequence—the same DNA sequence running in opposite directions (shown by directional arrows in the figure). The inverted repeat sequences enable the transposase enzyme to identify the ends of the TE when it catalyzes transposition.

The second type of bacterial TE, called a **transposon**, has an inverted repeat sequence at each end enclosing a central region with one or more genes. In a number of bacterial transposons, the inverted repeat sequences are insertion sequences, which provide the transposase for movement of the element (see Figure 17.10b). Bacterial transposons without IS ends have short inverted repeat end sequences, and a transposase gene is within the central region. Additional gene(s) in the central region typically are for antibiotic resistance; they originated from the main bacterial DNA circle or from plasmids. These non-IS genes included in transposons are carried along as the TEs move from place to place within and between species.

Many antibiotics, such as penicillin, erythromycin, tetracycline, ampicillin, and streptomycin, that were once successful in curing bacterial infections have lost much of their effectiveness because of resistance genes carried in transposons. Movements of the transposons, particularly to plasmids that have been transferred by conjugation within and between bacterial species, greatly increase the spread of genes providing antibiotic resistance. Resistance genes have made many bacterial diseases difficult or impossible to treat with standard antibiotics. (Chapter 25 discusses bacterial resistance further.)

Figure 17.11
Barbara McClintock and corn kernels showing different color patterns due to the movement of transposable elements. As TEs move into or out of genes controlling pigment production in developing kernels, the ability of cells and their descendants to produce the dark pigment is destroyed or restored. The result is random patterns of pigmented and colorless (yellow) segments in individual kernels.

Transposable Elements Were First Discovered in Eukaryotes

TEs were first discovered in a eukaryote, maize (corn), in the 1940s by Barbara McClintock, a geneticist working with corn at the Cold Spring Harbor Laboratory in New York. McClintock noted that some mutations affecting kernel and leaf color appeared and disappeared rapidly under certain conditions. Mapping the alleles by linkage studies produced a surprising result—the map positions changed frequently, indicating that the alleles could move from place to place in the corn chromosomes. Some of the movements were so frequent that changes in their effects could be noticed at different times in a single developing kernel **(Figure 17.11).**

When McClintock first reported her results, her findings were regarded as an isolated curiosity, possibly applying only to corn. This was because the then-prevailing opinion among geneticists was that genes are fixed in the chromosomes and do not move to other locations. Her conclusions were widely accepted only after TEs were detected and characterized in bacteria in the 1960s. By the 1970s, further examples of TEs were discovered in other eukaryotes, including yeast and mammals. McClintock was awarded a Nobel Prize in 1983 for her pioneering work, after these discoveries

confirmed her early findings that TEs are probably universally distributed among both prokaryotes and eukaryotes.

Eukaryotic Transposable Elements Are Classified as Transposons or Retrotransposons

Eukaryotic TEs fall into two major classes, *transposons* and *retrotransposons,* distinguished by the way the TE sequence moves from place to place in the DNA. However, eukaryotes have no TEs resembling insertion sequences. Researchers detect both classes of eukaryotic TEs through DNA sequencing or through their effects on genes at or near their sites of insertion.

Eukaryotic transposons are similar to bacterial transposons in their general structure and in the way they transpose. A gene for transposase is in the central region of the transposon, and most have inverted repeat sequences at their ends. Depending on the transposon, transposition is by the cut-and-paste or copy-and-paste mechanism (see Figure 17.9).

Members of the other class of eukaryotic TEs, the **retrotransposons,** transpose by a copy-and-paste mechanism but, unlike the other TEs we have discussed, their transposition occurs via an intermediate RNA copy of the TE **(Figure 17.12).** First, the retrotransposon, which is a DNA element integrated into the chromosomal DNA, is transcribed into a complementary RNA copy. Next, an enzyme called **reverse transcriptase,** which is encoded by one of the genes of the retrotransposon, uses the RNA as a template to make a DNA copy of the retrotransposon. The DNA copy is then inserted into the DNA at a new location, leaving the original in place. Some retrotransposons are bounded by sequences that are directly repeated rather than in inverted form; others have no repeated sequences at their ends.

Cellular genes may become incorporated into the central region of either a transposon or a retrotransposon and travel with it as it moves to a new location. The trapped genes may become continuously active through the effects of regulatory sequences in the TE. The trapped genes may also become abnormally active if moved in a TE to the vicinity of an enhancer or promoter of an intensely transcribed cellular gene. Certain forms of cancer have been linked to the TE-instigated abnormal activation of genes that regulate cell division.

Once TEs are inserted in the chromosomes, they become more or less permanent residents, duplicated and passed on during cell division along with the rest of the DNA. TEs inserted into the DNA of reproductive cells that produce gametes may be inherited, thereby becoming a permanent part of the genetic material of a species. *Insights from the Molecular Revolution* tells about a transposon that has become established in *Drosophila melanogaster* in recent times.

Long-standing TEs are subject to mutation along with other sequences in the DNA. Such mutations may accumulate in a TE, gradually altering it into a nonmobile, residual sequence in the DNA. The DNA of many

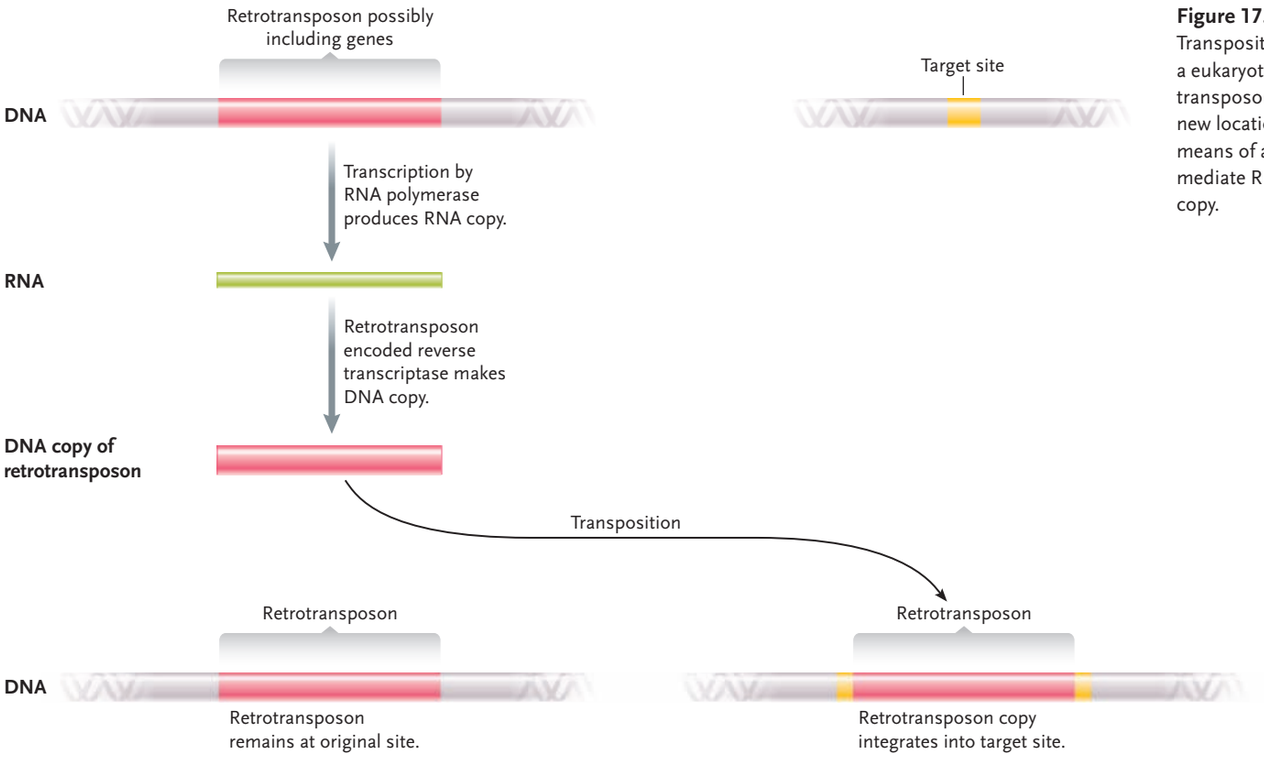


Figure 17.12
Transposition of a eukaryotic retrotransposon to a new location by means of an intermediate RNA copy.

Insights from the Molecular Revolution

Genes That Jump a Mite Too Far

The fruit fly *Drosophila melanogaster* is cultured in genetic laboratories all over the world. Many of the flies in laboratory cultures are home to a transposon known as a *P element*. Strains of flies that have this transposon in their DNA are known as *P strains;* strains of flies without the element are *M strains.*

A curious feature is the absence of P elements in *D. melanogaster* laboratory cultures that have been maintained in total isolation since 1950. The worldwide distribution of P elements among wild *D. melanogaster* populations is also uneven, as if the elements have not had time to become established in wild fruit flies in all parts of the world. These characteristics make it likely that P elements invaded wild *D. melanogaster* populations recently, probably within the last 30 to 50 years.

Margaret G. Kidwell of the University of Arizona and Marilyn A. Houck, now at Texas Tech University, hypothesized that the P element first appeared in *D. melanogaster* by "jumping" from another *Drosophila* species, *D. willistoni,* in which the element is universally distributed. Even though the two species do not interbreed, the P elements in *D. willistoni* and *D. melanogaster* are almost identical. The alternative hypothesis—that P elements were inherited from a common ancestor and were present in both species when *D. melanogaster* and *D. willistoni* separated millions of years ago—predicts that the elements would likely have mutated into significantly different forms in the two species, and clearly this is not the case.

How might P elements have moved from *D. willistoni* to *D. melanogaster*? Kidwell and Houck point to a mite, *Protolaelaps regalis,* as the possible vehicle. (Mites are small relatives of spiders.) The mite feeds on fruit flies and their eggs. Houck noticed that the mouth parts of the mite resemble the microscopic needles used by investigators to transfer DNA from cell to cell. This resemblance gave her the idea that the mites, which feed on both *D. willistoni* and *D. melanogaster,* transferred DNA containing the P elements from one species to the other. After having fed on a *D. willistoni* fly, a mite with DNA containing P elements on its mouth parts may have fed on a *D. melanogaster* egg, at the same time injecting the P element DNA into the egg. If the egg survived, and the P element DNA became integrated into the egg's DNA, an adult produced from the egg once it is fertilized would be a P-strain *D. melanogaster.*

In support of their idea, the investigators have found mites with P element DNA on their mouth parts and in their digestive tracts after feeding on P-strain flies. If the DNA transfer actually occurred in this way, the interspecies transfer also opens the possibility that evolution may occur through genes introduced by this mechanism of natural genetic engineering.

eukaryotes, including humans, contains many nonfunctional TEs likely created in this way.

Retroviruses Are Similar to Retrotransposons

RNA to DNA reverse transcription is associated with certain viruses as well as with retrotransposons. A **retrovirus** is a virus with an RNA genome that replicates via a DNA intermediate. When a retrovirus infects a host cell, a reverse transcriptase carried in the viral particle is released and copies the single-stranded RNA genome into a double-stranded DNA copy. The viral DNA is then inserted into the host DNA, where it is replicated and passed to progeny cells during cell division. Similar to the prophage of temperate bacteriophages, the inserted viral DNA is known as a provirus **(Figure 17.13).**

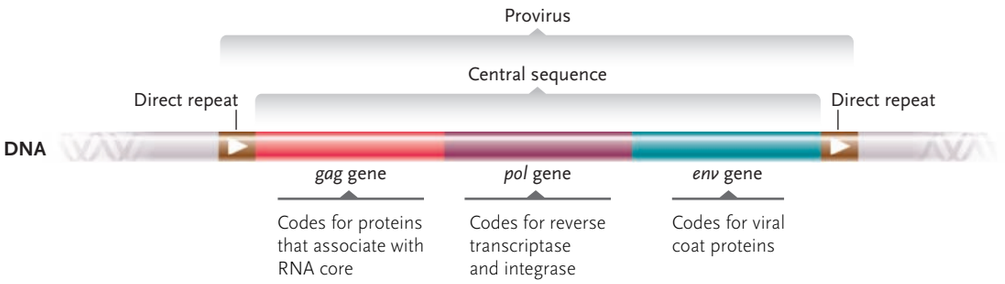


Figure 17.13
A mammalian retrovirus in the provirus form in which it is inserted into chromosomal DNA. The direct repeats at either end contain sequences capable of acting as enhancer, promoter, and termination signals for transcription. The central sequence contains genes coding for proteins, concentrated in the *gag, pol,* and *env* regions. The provirus of HIV, the virus that causes AIDS, takes this form.

Retroviruses are found in a wide range of organisms, with most so far identified in vertebrates. You, as well as most other humans and mammals, probably contain from one to as many as 100 or more retroviruses in your genome as proviruses. Many of these retroviruses do not ever produce viral particles. However, they may sometimes cause genetic disturbances of various kinds, including alterations of gene activity or DNA rearrangements such as deletions and translocations, some of which may be harmful to the host. The AIDS retrovirus, called *HIV* (for *H*uman *I*mmunodeficiency *V*irus), does produce viral particles. When HIV infects a human, its primary effect is to interfere with white blood cells of the immune system (HIV and AIDS are discussed further in Chapter 43).

Some retroviruses, such as the *avian sarcoma virus,* have been linked to cancer (in this case in chickens). Many of these cancer-causing retroviruses have picked up a host gene that triggers entry of cells into uncontrolled DNA replication and cell division. When included in a retrovirus, the gene comes under the influence of the highly active retroviral promoter, which makes the gene continually active and leads to the uncontrolled cell division characteristic of cancer. In other words, the host gene has become an oncogene, a gene that promotes the development of cancer by stimulating cell division (see Section 16.4). Usually, the host gene replaces one or more retrovirus genes, making the virus unable to produce viral particles.

Retroviruses also activate genes related to cell division by moving them to the vicinity of an active host cell promoter or enhancer, or by delivering an enhancer or active promoter to the vicinity of a host cell gene. In either case, the result may be uncontrolled cell division. Harmless retroviruses are being developed as a means to introduce genes into mammalian and other animal cells for genetic engineering (discussed further in Chapter 18).

The close similarities between retrotransposons and retroviruses have led to the proposal that retroviruses may have evolved from retrotransposons. This might have occurred through the chance enclosure of the RNA intermediates of retrotransposons by the coat proteins of infecting viruses, giving the retrotransposons the ability to escape from an original host and move to new individuals. The reverse process, the evolution of retrotransposons from retroviruses, is thought to be less likely because eukaryotic cells contain several additional types of retrotransposons that are not related to retroviruses except that they also move through formation of an RNA intermediate. For example, one of these non-retroviral retroposons, called *Alu,* is

Unanswered Questions

How is movement of transposable elements regulated?

You learned in this chapter that transposable elements (TEs) are mobile genetic elements in genomes. TEs are found in most living organisms—unlike viruses, they are permanent residents. Research with a variety of TEs and organisms has shown that there is a delicate balance between the transposons and the host genome. By studying the types, numbers, and genetic locations of TEs in genomes, researchers can learn about the evolution of the mechanisms the elements use to move and about the regulation of that movement.

A TE may cause a mutation if it moves into the coding region of a gene or if it inactivates its promoter. However, in normal cells, TE movement around the genome is actively restricted, and therefore, mutational damage is low. Researchers are finding that this negative regulation of TE movement can involve factors and mechanisms provided by the host cell and/or by gene regulatory limitations specific to the transposon. Through continued research, scientists hope to get a much better understanding of the molecular interactions between host cell and TE that bring about this relatively peaceful relationship, and of how these interactions developed during evolution.

Is there an adaptive function for a retrotransposon?

Amazingly, over 70% of the barley genome consists of retrotransposons. You would expect, therefore, that any changes in the number of these retrotransposons would have a significant impact on genome size. An interesting research project has shown a nearly threefold variation in the copy number of one barley retrotransposon, BARE-1, over a 300-meter span of a particular canyon in Israel. The highest copy numbers were found in plants in drier areas higher in the canyon. The researchers hypothesize that this might be because a stress-response regulatory sequence in the BARE-1 promoter activates the retrotransposon to cause transposition of the element in these plants. The movement leaves the original element in place, so the effect is to increase the number of BARE-1 elements. The scientists propose that the increase in retrotransposon number may have adaptive value for the plants growing under dry conditions where their growth rates are relatively slow. Possibly the increase in retrotransposon number, and its effect on overall genome size, produces larger cell sizes, which would offset the slower growth rates. The model is an interesting one, and is being pursued through further research with this system, and with others involving expanding retrotransposon numbers.

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clearly derived evolutionarily from an RNA molecule that occurs widely in eukaryotes as part of the signal-recognition particle (the signal-recognition particle, described in Section 15.4, is an RNA-protein complex that helps attach ribosomes to the endoplasmic reticulum during protein synthesis). In total, retrotransposons and retroviruses of all types occupy some 40% of the human genome.

The genetic elements discussed in this chapter, particularly plasmids and retroviruses, often act as natural genetic engineers by moving genes between species. The next chapter describes how human genetic engineers manipulate and clone DNA and how they analyze genomes at the DNA level.

Study Break

Among eukaryotic transposable elements, how do transposons, retrotransposons, and retroviruses differ?

Review

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17.1 Gene Transfer and Genetic Recombination in Bacteria

- The rapid generation times and numerous offspring of bacteria and viruses make it possible to trace genetic crosses and their outcomes much more quickly than in eukaryotes. These characteristics make it possible to detect rare genetic events. The results of these crosses show that recombination may occur within the boundaries of a gene as well as between genes.
- Recombination occurs in both bacteria and eukaryotes by exchange of segments between homologous DNA molecules. In bacteria, the DNA of the bacterial chromosome may recombine with DNA brought into the cell from outside.
- Three primary mechanisms bring DNA into bacterial cells from the outside: conjugation, transformation, and transduction.
- In conjugation, which is the basis of bacterial sexual reproduction, two bacterial cells form a cytoplasmic bridge, and part or all of the DNA of one cell moves into the other through the bridge. The donated DNA can then recombine with homologous sequences of the recipient cell's DNA (Figures 17.1, 17.2, and 17.4).
- *E. coli* bacteria that are able to act as DNA donors in conjugation have an F plasmid, making them F^+; recipients have no F plasmid and are F^-. In Hfr strains of *E. coli,* the F plasmid is within the main chromosome. As a result, genes of the main chromosome are often transferred into F^- cells along with a portion of the F-plasmid DNA. Researchers have mapped genes on the *E. coli* chromosome by noting the order in which they are transferred from Hfr to F^- cells during conjugation (Figure 17.4).
- In transformation, intact cells absorb pieces of DNA released from cells that have disintegrated. The entering DNA fragments can recombine with the recipient cell's DNA.
- In transduction, DNA is transferred from one cell to another by an infecting virus.

Practice: Distinguishing between the three major processes: conjugation, transformation, and transduction

17.2 Viruses and Viral Recombination

- When in the free form, viruses consist of a nucleic acid core, either DNA or RNA, surrounded by a protein coat (Figure 17.6).
- The cycle of viral infection begins when the nucleic acid molecule of a virus is introduced into a host cell and replicated. Viral coat proteins are made and assembled with the DNA into new viral particles (Figure 17.7).
- Virulent phages kill the host cell by releasing an enzyme that ruptures the plasma membrane and cell wall and releases the new viral particles (Figure 17.7).
- Temperate phages do not always kill their host cell. They may enter the lytic cycle, in which the viral DNA becomes active, exits the host DNA, and begins replication, or a lysogenic cycle. In the lysogenic cycle, the phage's DNA is integrated into the host cell's DNA and may remain for many generations. At some point, the virus may enter the lytic cycle and begin replication. After production of viral coats, the DNA is assembled into new viral particles, which are released as the cell ruptures (Figure 17.8).
- During a cycle of viral infection with particular phages, one or more fragments of host cell DNA may be incorporated into viral particles. As an infected cell breaks down, it releases the viral particles containing host cell DNA. These particles, which form the basis of bacterial transduction, may infect a second cell and introduce the bacterial DNA segment into the new host, where it may recombine with the host DNA.

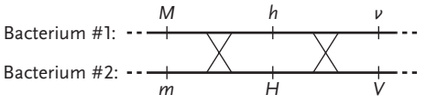
17.3 Transposable Elements

- Both prokaryotes and eukaryotes contain TEs (transposable elements)—DNA sequences that can move from place to place in the DNA. The TEs may move from one location in the DNA to another, or generate duplicated copies that insert in new locations while leaving the "parent" copy in its original location (Figure 17.9).
- Genes of the host cell DNA may become incorporated into a TE and may be carried with it to a new location. There, the genes may become abnormally active when placed near sequences that control the activity of genes within the TE, or near the control elements of active host genes.
- Eukaryotic TEs occur as transposons, which release from one location in the DNA and insert at a different site, or as retrotransposons, which move by making an RNA copy, which is then replicated into a DNA copy that is inserted at a new location. The "parent" copy remains at the original location. Like retrotransposons, retroviruses integrate into chromosomal DNA by making a DNA copy of their RNA genome. Retroviruses may have evolved from retrotransposons (Figures 17.11–17.13).
- TE-instigated abnormal activation of genes regulating cell division has been linked to the development of some forms of cancer in humans and other complex animals.

Questions

Self-Test Questions

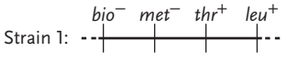
1. When studying the differences in the genes of bacteria, researchers:
   a. do not grow bacteria on minimal medium as the medium lacks needed nutrients.
   b. use a bacterial clone, which is a group of cells from different bacteria of varying genetic makeup.
   c. use bacteria diploid for their full genome because they can grow on minimal medium.
   d. can study only one genetic trait in a single recombinant event.
   e. can measure the passage of genes between cells during conjugation, transduction, and transformation.
2. If crossovers occurred between two bacterial genomes as shown in the figure, the result would be:

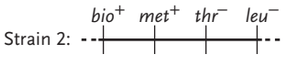


   a. *MHv* and *mhV*.
   b. *MHV* and *mhv*.
   c. *Mhv* and *mHv*.
   d. *MHV* and *mhV*.
   e. *mhv* and *MhV*.
3. In conjugation, when a bacterial F factor is transferred:
   a. the donor cell becomes F^-.
   b. the recipient cell becomes F^+.
   c. the recipient cell becomes F^-.
   d. the donor cell turns into a recipient cell.
   e. viral DNA integrates into the recipient DNA.
4. Which of the following is *not* correct for bacterial conjugation?
   a. Both Hfr and F^+ bacteria have the ability to code for a sex pilus.
   b. After an F^- cell has conjugated with an F^+, its plasmid holds the F^+ factor.
   c. The recipient cell usually becomes Hfr following conjugation.
   d. In an Hfr $\times$ F^- mating, DNA of the main chromosome moves to a recipient cell.
   e. Genes on the F factor encode proteins of the sex pilus.
5. Which of the following is *not* correct for bacterial transformation?
   a. Artificial transformation is used in cloning procedures.
   b. Avery was able to transform live noninfective bacteria with DNA from dead infective bacteria.
   c. The cell wall and plasma membrane must be penetrated for transformation to proceed.
   d. A virus is required for the process.
   e. Electroporation is a form of artificial transformation used to introduce DNA into cells.
6. Transduction:
   a. may allow recombination of newly introduced DNA with host cell DNA.
   b. is the movement of DNA from one bacterial cell to another by means of a plasmid.
   c. can cause the DNA of the donor to change but not the DNA of the recipient.
   d. is the movement of viral DNA but not bacterial DNA into a recipient bacterium.
   e. requires a physical contact between two bacterium.
7. Viruses:
   a. have a protein core.
   b. have a nucleic acid coat.
   c. that infect bacteria are called bacteriophages.
   d. were probably the first forms of life on Earth.
   e. if temperate, kill host cells.
8. A virus in its lysogenic cycle:
   a. is lysing the host cell.
   b. is transducing a bacterial cell.
   c. is assembling viral particles for cell rupture.
   d. is damaging the host cell.
   e. has its genome integrated in host DNA.
9. Which of the following is *not* correct about transposable elements?
   a. They can be recognized by their ends of inverted transposable elements.
   b. They have an internal portion that can be transcribed.
   c. They encode a transposase enzyme.
   d. They have no harmful effects on cell function.
   e. They move by a cut-and-paste or copy-and-paste mechanism.
10. Which is *not* correct about retroviruses?
    a. They are RNA viruses.
    b. They are believed to be the source of retrotransposons.
    c. They encode an enzyme for their insertion into host cell DNA.
    d. They encode single-stranded viral DNA from viral RNA.
    e. They encode a reverse transcriptase enzyme for RNA to DNA synthesis.

Questions for Discussion

1. You set up an experiment like the one carried out by Lederberg and Tatum, mixing millions of *E. coli* of two strains with the following genetic constitutions:





   Among the bacteria obtained after mixing, you find some cells that do not require threonine, leucine, or biotin to grow, but still need methionine. How might you explain this result?
2. As a control for their experiments with bacterial recombination, Lederberg and Tatum placed cells of either "parental" strain 1 or 2 on the surface of a minimal medium. If you set up this control and a few scattered colonies showed up, what might you propose as an explanation? How could you test your explanation?
3. Experimental systems have been developed in which transposable elements can be induced to move under the control of a researcher. Following the induced transposition of a yeast TE element, two mutants were identified with altered activities of enzyme X. One of the mutants lacked enzyme activity completely, while the other had five times as much enzyme activity as normal cells did. Both mutants were found to have the TE inserted into the gene for enzyme X. Propose hypotheses for how the two different mutant phenotypes were produced.

Experimental Analysis

You have a culture of Hfr *E. coli* cells that cannot make biotin for themselves. To this culture you add some wild-type *E. coli* cells that have been heat killed, and then subject the culture to electroporation. After the addition, you find some cells that can grow on minimal medium. How could you establish whether the wild-type bio^+ allele was inserted in a plasmid or the chromosomal DNA of the Hfr cells?

Evolution Link

Are viruses evolutionarily derived from complex organisms that can reproduce themselves, or are they remnants of precellular "life"? Argue your case.