CHAPTER

4

Genetics

CHAPTER CONTENTS

INTRODUCTION

The genetic material of a typical bacterium, *Escherichia coli,* consists of a single circular DNA molecule with a molecular weight of about 2×10^9 and is composed of approximately 5×10^6 base pairs. This amount of genetic information can code for about 2000 proteins with an average molecular weight of 50,000. The DNA of the smallest free-living organism, the wall-less bacterium *Mycoplasma,* has a molecular weight of 5×10^8. The DNA of human cells contains about 3×10^9 base pairs and encodes about 100,000 proteins.

Note that bacteria are **haploid;** in other words, they have a single chromosome and therefore a single copy of each gene. Eukaryotic cells (such as human cells) are **diploid,** which means they have a pair of each chromosome and therefore have two copies of each gene. In diploid cells, one copy of a gene (allele) may be expressed as a protein (i.e., be dominant), whereas another allele may not be expressed (i.e., be recessive). In haploid cells, any gene that has mutated—and therefore is not expressed—results in a cell that has lost that trait.

MUTATIONS

A mutation is a change in the base sequence of DNA that usually results in insertion of a different amino acid into a protein and the appearance of an altered phenotype. Mutations result from three types of molecular changes:

(1) The first type is the **base substitution.** This occurs when one base is inserted in place of another. It takes place at the time of DNA replication, either because the DNA polymerase makes an error or because a mutagen alters the hydrogen bonding of the base being used as a template in such a manner that the wrong base is inserted. When the base substitution results in a codon that simply causes a different amino acid to be inserted, the mutation is called a **missense mutation;** when the base substitution generates a termination codon that stops protein synthesis prematurely, the mutation is called a **nonsense mutation.** Nonsense mutations almost always destroy protein function.

(2) The second type of mutation is the **frameshift mutation.** This occurs when one or more base pairs are added or deleted, which shifts the reading frame on the ribosome and results in incorporation of the wrong amino acids "downstream" from the mutation and in the production of an inactive protein.

(3) The third type of mutation occurs when **transposons** or **insertion sequences** are integrated into the DNA. These newly inserted pieces of DNA can cause profound changes in the genes into which they insert and in adjacent genes.

Mutations can be caused by chemicals, radiation, or viruses. Chemicals act in several different ways.

(1) Some, such as nitrous acid and alkylating agents, alter the existing base so that it forms a hydrogen bond preferentially with the wrong base (e.g., adenine would no longer pair with thymine but with cytosine).

(2) Some chemicals, such as 5-bromouracil, are base analogues, since they resemble normal bases. Because the bromine atom has an atomic radius similar to that of a methyl group, 5-bromouracil can be inserted in place of thymine (5-methyluracil). However, 5-bromouracil has less

hydrogen-bonding fidelity than does thymine, and so it binds to guanine with greater frequency. This results in a transition from an A-T base pair to a G-C base pair, thereby producing a mutation. The antiviral drug iododeoxyuridine acts as a base analogue of thymidine.

(3) Some chemicals, such as benzpyrene, which is found in tobacco smoke, bind to the existing DNA bases and cause frameshift mutations. These chemicals, which are frequently carcinogens as well as mutagens, intercalate between the adjacent bases, thereby distorting and offsetting the DNA sequence.

X-rays and ultraviolet light can cause mutations also.

(1) X-rays have high energy and can damage DNA in three ways: (a) by breaking the covalent bonds that hold the ribose phosphate chain together, (b) by producing free radicals that can attack the bases, and (c) by altering the electrons in the bases and thus changing their hydrogen bonding.

(2) Ultraviolet radiation, which has lower energy than X-rays, causes the cross-linking of the adjacent pyrimidine bases to form dimers. This cross-linking (e.g., of adjacent thymines to form a thymine dimer) results in inability of the DNA to replicate properly.

Certain viruses, such as the bacterial virus Mu (mutator bacteriophage), cause a high frequency of mutations when their DNA is inserted into the bacterial chromosome. Since the viral DNA can insert into many different sites, mutations in various genes can occur. These mutations are either frameshift mutations or deletions.

Conditional lethal mutations are of medical interest because they may be useful in vaccines (e.g., influenza vaccine). The word *conditional* indicates that the mutation is expressed only under certain conditions. The most important conditional lethal mutations are the temperature-sensitive ones. Temperature-sensitive organisms can replicate at a relatively low, permissive temperature (e.g., 32°C) but cannot grow at a higher, restrictive temperature (e.g., 37°C). This behavior is due to a mutation that causes an amino acid change in an essential protein, allowing it to function normally at 32°C but not at 37°C because of an altered conformation at the higher temperature. An example of a conditional lethal mutant of medical importance is a strain of influenza virus currently used in an experimental vaccine. This vaccine contains a virus that cannot grow at 37°C and hence cannot infect the lungs and cause pneumonia, but it can grow at 32°C in the nose, where it can replicate and induce immunity.

TRANSFER OF DNA WITHIN BACTERIAL CELLS

Transposons transfer DNA from one site on the bacterial chromosome to another site or to a plasmid. They do so by synthesizing a copy of their DNA and inserting the copy at another site in the bacterial chromosome or the plasmid. The structure and function of transposons are described in Chapter 2, and their role in antimicrobial drug resistance is described in Chapter 11. The transfer of a transposon to a plasmid and the subsequent transfer of the plasmid to another bacterium by conjugation (see later) contributes significantly to the spread of antibiotic resistance.

Transfer of DNA within bacteria also occurs by **programmed rearrangements** (Figure 4–1). These gene rearrangements account for many of the antigenic changes seen in *Neisseria gonorrhoeae* and *Borrelia recurrentis,* the cause of relapsing fever. (They also occur in trypanosomes, which are discussed in Chapter 52.) A programmed rearrangement consists of the movement of a gene from a silent storage site where the gene is not expressed to an active site where transcription and translation occur. There are many silent genes that encode variants of the antigens, and the insertion of a new gene into the active site in a sequential, repeated programmed manner is the source of the consistent antigenic variation. These movements are not induced by an immune response but have the effect of allowing the organism to evade it.

TRANSFER OF DNA BETWEEN BACTERIAL CELLS

The transfer of genetic information from one cell to another can occur by three methods: conjugation, transduction, and transformation (Table 4–1). From a medical viewpoint, the two most important consequences of DNA transfer are (1) **that antibiotic resistance genes are spread from one bacterium to another primarily by conjugation** and (2) **that several important exotoxins are encoded by bacteriophage genes and are transferred by transduction**.

1. Conjugation

Conjugation is the mating of two bacterial cells, during which DNA is transferred from the donor to the recipient cell (Figure 4–2). The mating process is controlled by an **F (fertility) plasmid** (F factor), which carries the genes for the proteins required for conjugation. One of the most important proteins is pilin, which forms the **sex pilus** (conjugation tube). Mating begins when the pilus of the donor male bacterium carrying the F factor (F^+) attaches to a receptor on the surface of the recipient female bacterium, which does not contain an F factor (F^-). The cells are then drawn into direct contact by "reeling in" the pilus. After an enzymatic cleavage of the F factor DNA, one strand is transferred across the conjugal bridge (mating bridge) into the recipient cell. The process is completed by synthesis of the complementary strand to form a double-stranded F factor plasmid in both the donor and recipient cells. The recipient is now an F^+ male cell that is capable of transmitting the plasmid further. Note that in this instance only the F factor, and not the bacterial chromosome, has been transferred.

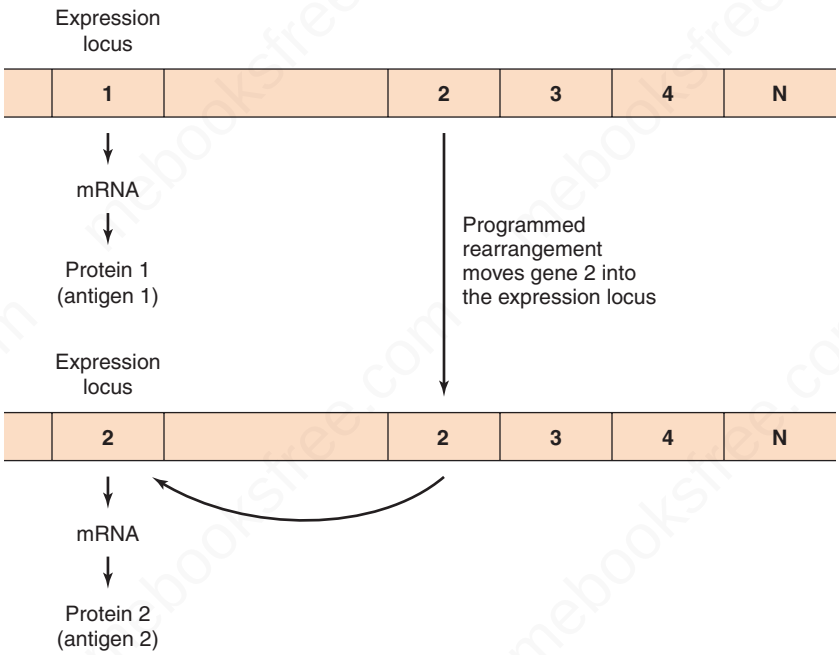

FIGURE 4–1 Programmed rearrangements. In the top part of the figure, the gene for protein 1 is in the expression locus and the mRNA for protein 1 is synthesized. At a later time, a copy of gene 2 is made and inserted into the expression locus. By moving only the copy of the gene, the cell always keeps the original DNA for use in the future. When the DNA of gene 2 is inserted, the DNA of gene 1 is excised and degraded.

Some F^+ cells have their F plasmid integrated into the bacterial DNA and thereby acquire the capability of transferring the chromosome into another cell. These cells are called **Hfr (high-frequency recombination)** cells (Figure 4–3). During this transfer, the single strand of DNA that enters the recipient F^- cell contains a piece of the F factor at the leading end followed by the bacterial chromosome and then by the remainder of the F factor. The time required for complete transfer of the bacterial DNA is approximately 100 minutes. Most matings result in the transfer of only a portion of the donor chromosome because the attachment between the two cells can break. The donor cell genes that are transferred vary since the F plasmid can integrate at several different sites in the bacterial DNA. The bacterial genes adjacent to the leading piece of the F factor are the first and therefore the most frequently transferred. The newly acquired DNA can recombine into the recipient's DNA and become a stable component of its genetic material.

Resistance plasmids (R plasmids) can also be transferred by conjugation. R plasmids can carry one or more genes for a variety of enzymes that can degrade antibiotics and modify membrane transport systems. For example, R plasmids encode the β-lactamases of *Staphylococcus aureus*, *E. coli*, and *Klebsiella pneumoniae*. In addition, they encode the proteins of the transport system that actively export sulfonamides out of the bacterial cell. Note that R plasmids can be transferred not only to cells of the same species, but also to other species and genera (see chapter 11 for more information about R plasmids).

2. Transduction

Transduction is the transfer of cell DNA by means of a bacterial virus (**bacteriophage, phage**) (Figure 4–4). During the growth of the virus within the cell, a piece of bacterial DNA is incorporated into the virus particle and is carried into the recipient cell at the time of infection. Within the recipient cell, the phage DNA can integrate into the cell DNA and the cell can acquire a new trait—a process called **lysogenic conversion** (see the end of Chapter 29). This process can change a nonpathogenic organism into a

TABLE 4–1 Comparison of Conjugation, Transduction, and Transformation

Transfer Procedure	Process	Type of Cells Involved	Nature of DNA Transferred
Conjugation	DNA transferred from one bacterium to another	Prokaryotic	Chromosomal or plasmid
Transduction	DNA transferred by a virus from one cell to another	Prokaryotic	Any gene in generalized transduction; only certain genes in specialized transduction
Transformation	Purified DNA taken up by a cell	Prokaryotic or eukaryotic (e.g., human)	Any DNA

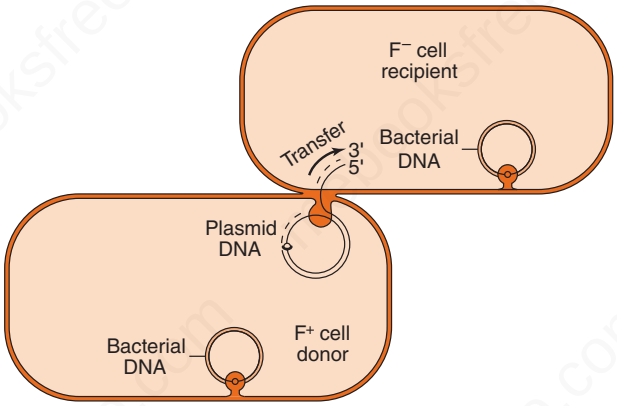

FIGURE 4–2 Conjugation. An F plasmid is being transferred from an F^+ donor bacterium to an F^- recipient. The transfer is at the contact site made by the sex pilus. The new plasmid in the recipient bacterium is composed of one parental strand (solid line) and one newly synthesized strand (dashed line). The previously existing plasmid in the donor bacterium now consists of one parental strand (solid line) and one newly synthesized strand (dashed line). Both plasmids are drawn with only a short region of newly synthesized DNA (dashed lines), but at the end of DNA synthesis, both the donor and the recipient contain a complete copy of the plasmid DNA. (Adelberg EA, Doudoroff MJ, Fowlks RL, et al. *The Microbial World (Stainer).* 3rd edition, © 1970. Reprinted by permission of Pearson Education Inc, New York, New York.)

pathogenic one. Diphtheria toxin, botulinum toxin, cholera toxin, and erythrogenic toxin (*Streptococcus pyogenes*) are encoded by bacteriophages and can be transferred by transduction.

There are two types of transduction: generalized and specialized. The **generalized** type occurs when the virus carries a segment from any part of the bacterial chromosome. This occurs because the cell DNA is fragmented after phage infection and pieces of cell DNA the same size as the viral DNA are incorporated into the virus particle at a frequency of about 1 in every 1000 virus particles. The **specialized** type occurs when the bacterial virus DNA that has integrated into the cell DNA is excised and carries with it an adjacent part of the cell DNA. Since most lysogenic (temperate) phages integrate at specific sites in the bacterial DNA, the adjacent cellular genes that are transduced are usually specific to that virus.

3. Transformation

Transformation is the transfer of DNA itself from one cell to another. This occurs by either of the two following methods. First, in nature, dying bacteria may release their DNA, which may be taken up by recipient cells. Certain bacteria, such as *Neisseria* and *Haemophilus*, synthesize receptors on

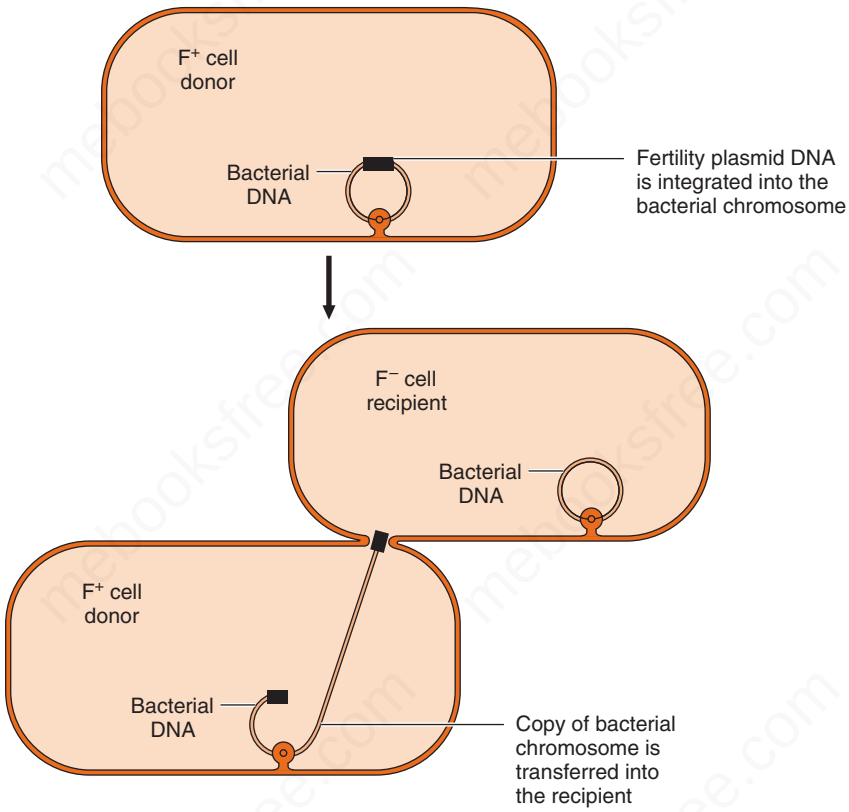

FIGURE 4–3 High-frequency recombination. **Top:** A fertility (F) plasmid has integrated into the bacterial chromosome. **Bottom:** The F plasmid mediates the transfer of the bacterial chromosome of the donor into the recipient bacteria.

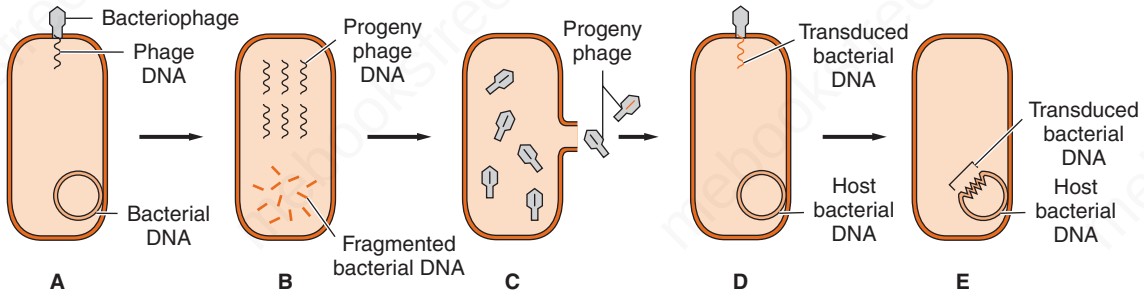

FIGURE 4–4 **Transduction. A:** A bacteriophage infects a bacterium, and phage DNA enters the cell. **B:** The phage DNA replicates, and the bacterial DNA fragments. **C:** The progeny phages assemble and are released; most contain phage DNA, and a few contain bacterial DNA. **D:** Another bacterium is infected by a phage-containing bacterial DNA. **E:** The transduced bacterial DNA integrates into host DNA, and the host acquires a new trait. This host bacterium survives because no viral DNA is transduced; therefore, no viral replication can occur. (Another type of transduction mechanism is depicted in Figure 29–8.)

the cell surface that play a role in the uptake of DNA from the environment. The role of DNA uptake in bacterial pathogenesis is unclear.

Second, in the laboratory, an investigator may extract DNA from one type of bacteria and introduce it into genetically different bacteria. When purified DNA is injected into the nucleus of a eukaryotic cell, the process is called **transfection.** Transfection is frequently used in genetic engineering procedures.

The experimental use of transformation has revealed important information about DNA. In 1944, it was shown that DNA extracted from encapsulated smooth pneumococci could transform nonencapsulated rough pneumococci into encapsulated smooth organisms. This demonstration that the transforming principle was DNA marked the first evidence that DNA was the genetic material.

RECOMBINATION

Once the DNA is transferred from the donor to the recipient cell by one of the three processes just described, it can integrate into the host cell chromosome by recombination. There are two types of recombination:

(1) **Homologous recombination,** in which two pieces of DNA that have extensive homologous regions pair up and exchange pieces by the processes of breakage and reunion.

(2) **Nonhomologous recombination,** in which little, if any, homology is necessary.

Different genetic loci govern these two types, and so it is presumed that different enzymes are involved. Although it is known that a variety of endonucleases and ligases are involved, the precise sequence of events is unknown.

PEARLS

- Bacteria have only one copy of their genome DNA (i.e., they are **haploid**). In contrast, eukaryotic cells have two copies of their genome DNA (i.e., they are **diploid**). Bacterial DNA is circular; human nuclear DNA is linear.
- The transfer of DNA within bacterial cells occurs by two processes: movement of transposons and programmed rearrangements. **Transposons** are small pieces of DNA that move readily from one site on the bacterial chromosome to another or from the bacterial chromosome to a plasmid. Medically, transposons are important because they commonly **carry antibiotic resistance genes**. The transfer of transposons on plasmids to other bacteria by conjugation contributes significantly to antibiotic resistance.
- **Programmed rearrangements** are the movement of genes from inactive (storage) sites into active sites, where they are expressed as new proteins. Medically, this is important because bacteria can acquire new proteins (antigens) on their surface and evade the immune system. Two important organisms in which this occurs are *N. gonorrhoeae*, the cause of gonorrhea, and *Trypanosoma brucei,* a protozoan that causes African sleeping sickness.
- The transfer of DNA between bacterial cells occurs mainly by two processes: conjugation and transduction. **Conjugation** is the process by which DNA, either plasmid or chromosomal, is transferred directly from one bacterium to another. For conjugation to occur, the donor bacterium must have a "fertility" plasmid (F plasmid) that encodes the proteins that mediate this process, the most important of which are the proteins that form the **sex pilus**. The DNA transferred by conjugation to the recipient bacterium is a new copy that allows the donor to keep a copy of the DNA. Plasmids carrying antibiotic resistance genes are commonly transferred by conjugation.
- **Transduction** is the process by which DNA, either plasmid or chromosomal, is transferred from one bacterium to another by a **virus**. The transferred DNA integrates into the chromosomal DNA of the recipient, and new proteins, such as exotoxins, are made—a process called **lysogenic conversion.**
- **Transformation** is the process by which DNA itself, either DNA released from dying cells or DNA purified in the laboratory, enters a recipient bacterium. Medically, this process appears to be less important than conjugation and transduction.

SELF-ASSESSMENT QUESTIONS

1. The emergence of antibiotic-resistant bacteria, especially in enteric gram-negative rods, is a medically important phenomenon. This most commonly occurs by a process that involves a sex pilus and the subsequent transfer of plasmids carrying one or more transposons. Which one of the following is the name that best describes this process?

(A) Conjugation
(B) Transduction
(C) Transformation
(D) Translocation
(E) Transposition

2. Several important pathogenic bacteria have the ability to translocate pieces of their DNA in a process called *programmed rearrangements*. Which one of the following is the most important known consequence of this ability?

(A) The number of plasmids increases significantly, which greatly enhances antibiotic resistance.
(B) The amount of endotoxin increases significantly, which greatly enhances the ability to cause septic shock.
(C) The surface antigens of the bacteria vary significantly, which greatly enhances the ability to avoid opsonization by antibody.
(D) The ability of the bacterium to be lysogenized is significantly increased, which greatly enhances the ability to produce increased amounts of exotoxins.
(E) The ability of the bacterium to survive intracellularly is greatly increased.

3. Which statement is the most accurate regarding transposons?

(A) They encode enzymes that degrade the ends of the bacterial chromosome.
(B) They are short sequences of DNA that often encode enzymes that mediate antibiotic resistance.
(C) They are short sequences of RNA that silence specific regulatory genes.
(D) They are a family of transfer RNAs that enhance mutations at "hot spots" in the bacterial genome.

4. *Corynebacterium diphtheriae* causes the disease diphtheria by producing diphtheria toxin. The gene encoding the toxin is integrated into bacterial genome during lysogenic conversion. The toxin gene was acquired by which process?

(A) Conjugation
(B) Transduction
(C) Transformation
(D) Translocation
(E) Transposition

ANSWERS

(1) **(A)**
(2) **(C)**
(3) **(B)**
(4) **(B)**

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 709. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 751.