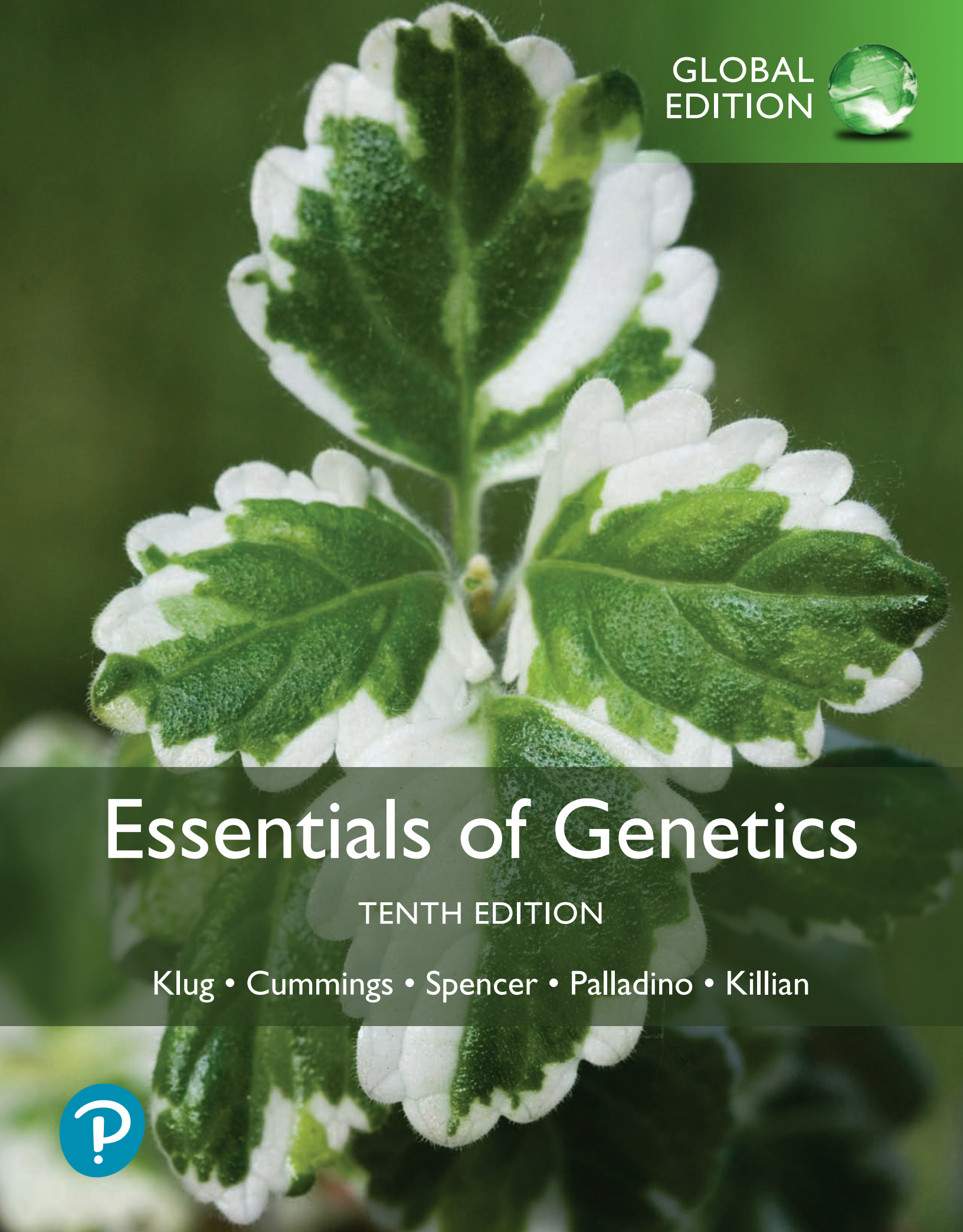
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EVOLVING CONCEPT OF THE GENE

The Evolving Concept of the Gene is a unique feature, integrated into key chapters, which highlights how scientists' understanding of the gene has changed over time. By underscoring how the conceptualization of the gene has evolved, our goal is to help students appreciate the process of discovery that has led to an ever more sophisticated understanding of hereditary information.

CHAPTER 3 pg. 66 Based on the pioneering work of Gregor Mendel, the gene was viewed as a heritable unit factor that determines the expression of an observable trait, or phenotype. ■

CHAPTER 4 pg. 82 Based on the work of many geneticists following the rediscovery of Mendel's work in the very early part of the twentieth century, the chromosome theory of inheritance was put forward, which hypothesized that chromosomes are the carriers of genes and that meiosis is the physical basis of Mendel's postulates. In the ensuing 40 years, the concept of a gene evolved to reflect the idea that this hereditary unit can exist in multiple forms, or alleles, each of which can have an impact on the phenotype in different ways, leading to incomplete dominance, codominance, and even lethality. It became clear that the process of mutation was the source of new alleles. ■

CHAPTER 7 pg. 160 Based on the gene-mapping studies in *Drosophila* and many other organisms from the 1920s through the mid-1950s, geneticists regarded genes as hereditary units organized in a specific sequence along chromosomes, between which recombination could occur. Genes were thus viewed as indivisible "beads on a string." ■

CHAPTER 9 pg. 199 Based on the model of DNA put forward by Watson and Crick in 1953, the gene was viewed for the first time in molecular terms as a sequence of nucleotides in a DNA helix that encodes genetic information. ■

CHAPTER 12 pg. 260 The elucidation of the genetic code in the 1960s supported the concept that the gene is composed of a linear series of triplet nucleotides encoding the amino acid sequence of a protein. While this is indeed the case in bacteria and viruses, in 1977, it became apparent that in eukaryotes, the gene is divided into coding sequences, called exons, which are interrupted by noncoding sequences, called introns (intervening sequences), which must be spliced out during production of the mature mRNA. ■

CHAPTER 13 pg. 278 In the 1940s, a time when the molecular nature of the gene had yet to be defined, groundbreaking work of Beadle and Tatum provided the first experimental evidence concerning the product of genes, their "one-gene:one-enzyme" hypothesis. This idea received further support and was later modified to indicate that one gene specifies one polypeptide chain. ■

CHAPTER 15 pg. 319 The groundbreaking work of Jacob, Monod, and Lwoff in the early 1960s, which established the operon model for the regulation of gene expression in bacteria, expanded the concept of the gene to include noncoding regulatory sequences that are present upstream (5′) from the coding region. In bacterial operons, the transcription of several contiguous structural genes whose products are involved in the same biochemical pathway is regulated in a coordinated fashion. ■

CHAPTER 18 pg. 383 Based on the work of the ENCODE project, we now know that DNA sequences that have previously been thought of as "junk DNA," because they do not encode proteins, are nonetheless often transcribed into what we call noncoding RNA (ncRNA). Since the function of some of these RNAs is now being determined, we must consider whether the concept of the gene should be expanded to include DNA sequences that encode ncRNAs. At this writing, there is no consensus, but it is important for you to be aware of these current findings as you develop your final interpretation of a gene. ■

P22 prophages rarely enter the vegetative or lytic phase, reproduce, and are released by the LA-22 cells. Such P22 phages, being much smaller than a bacterium, then cross the filter of the U-tube and subsequently infect and lyse some of the LA-2 cells. In the process of lysis of LA-2, these P22 phages occasionally package a region of the LA-2 chromosome in their heads. If this region contains the *phe*$^+$ and *trp*$^+$ genes and the phages subsequently pass back across the filter and infect LA-22 cells, these newly lysogenized cells will behave as prototrophs. This process of transduction, whereby bacterial recombination is mediated by bacteriophage P22, is diagrammed in **Figure 8.15**.

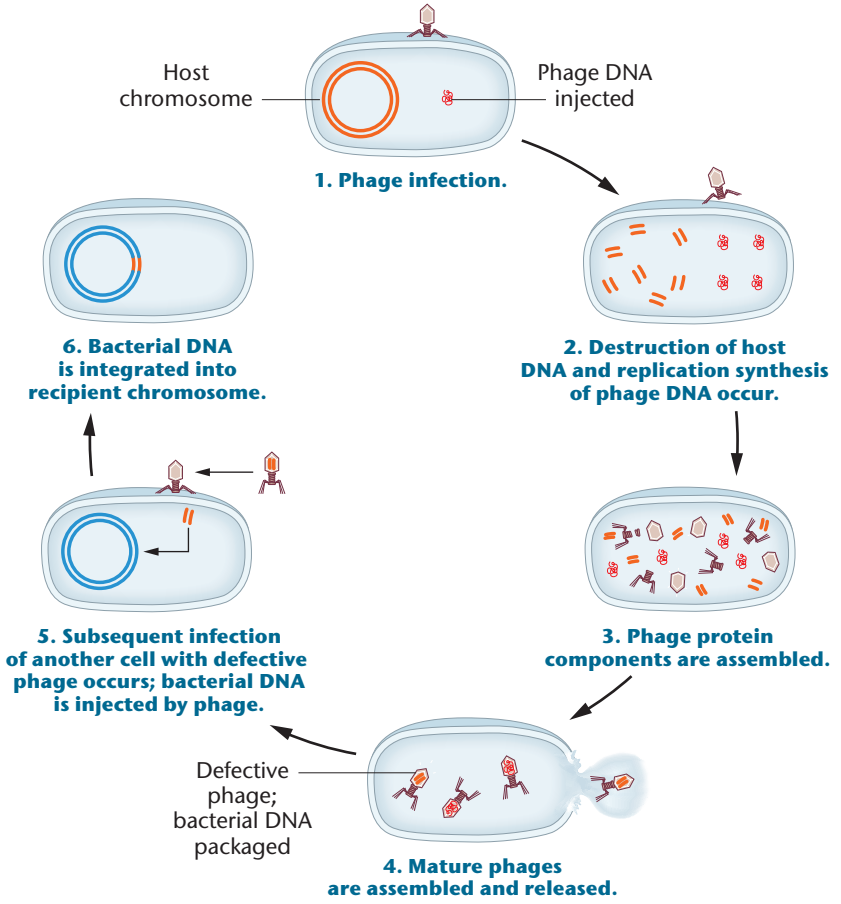


FIGURE 8.15 The process of transduction, where bacteriophages mediate bacterial recombination.

Transduction and Mapping

Like transformation, transduction was used in linkage and mapping studies of the bacterial chromosome. The fragment of bacterial DNA involved in a transduction event is large enough to include numerous genes. As a result, two genes that closely align (are linked) on the bacterial chromosome can be simultaneously transduced, a process called **cotransduction.** Two genes that are not close enough to one another along the chromosome to be included on a single DNA fragment require two independent events to be transduced into a single cell. Since this occurs with a much lower probability than cotransduction, linkage can be determined.

By concentrating on two or three linked genes, transduction studies can also determine the precise order of these genes. The closer linked genes are to each other, the greater the frequency of cotransduction. Mapping studies involving three closely aligned genes can thus be executed, and the analysis of such an experiment is predicated on the same rationale underlying other mapping techniques.

ESSENTIAL POINT

Transduction is virus-mediated bacterial DNA transfer and can be used to map phage genes. ■

GENETICS, ETHICS, AND SOCIETY

Multidrug-Resistant Bacteria: Fighting with Phage

The worldwide spread of *multidrug-resistant (MDR)* pathogenic bacteria has become an urgent threat to human and animal health. More than two million people in the United States become infected with antibiotic-resistant bacteria each year, and more than 23,000 of them will die from their infections. In 2015, approximately 480,000 cases of MDR tuberculosis occurred worldwide and another 100,000 cases were resistant to at least one antibiotic. In the United States, cases of drug-resistant enterobacteriaceae infections increased three-fold between 2001 and 2012. In 2016, a woman in Nevada died of a *Klebsiella pneumoniae* infection caused by a strain that was resistant to 26 different antibiotics, including colistin, which is considered the "last resort" antibiotic.

One factor leading to the spread of MDR bacteria is the selective pressure brought about by repeated exposure to antibiotics. Worldwide, livestock consume

as much as 80 percent of all antibiotics, used as feed supplements. The routine use of antibiotics in livestock feed and the overuse of human antibiotic prescriptions are thought to be the most significant contributors to the spread of MDR bacteria.

A second factor leading to the new "post-antibiotic era" is the reduction in antibiotic drug development by pharmaceutical companies. Economic issues are significant. Drug companies spend hundreds of millions of dollars to develop and test a new drug. However, they receive less profit from antibiotics than from more expensive drugs such as chemotherapies or diabetes drugs.

Several alternative approaches are in development and early clinical trials. One very unique approach is the use of therapeutic bacteriophages (phages). Phages have been used to treat bacterial infections since the early 1900s, especially in Europe, but were abandoned in the mid-twentieth century after the introduction of antibiotics. Researchers are returning to phage, using modern molecular tools to modify phage and phage-derived products for use as antibacterial drugs. No phage or phage products are yet approved for human therapies in the United States or Europe; however, several phage preparations, targeted at pathogens such as *Listeria*, are approved for topical use on fresh and prepared foods, and at least one phage therapy is in clinical trials.

Although scientific and regulatory challenges must still be overcome, we may be on the verge of the Age of the Phage.

Your Turn

Take time, individually or in groups, to consider the following questions. Investigate the references dealing with the technical and ethical challenges of combating drug-resistant bacteria.

1. How do phage therapies work, and what are the main advantages and disadvantages of using phage to treat bacterial infections?

These topics are discussed in Potera, C. (2013). Phage renaissance: New hope against antibiotic resistance (https://ehp.niehs.nih.gov/121-a48) *and* Cooper, C. J., et al. (2016). Adapting drug approval pathways for bacteriophage-based therapeutics. https://doi.org/10.3389/fmicb.2016.01209

2. Two significant reasons for the spread of MDR bacteria are the overuse of agricultural antibiotics and the reluctance of pharmaceutical companies to develop new antibiotics. Discuss the ethical concerns surrounded these two situations. For example, how do we balance our need for both abundant food and infection control? Also, how can we resolve the ethical disconnect between private-sector profits and the public good?

These topics are discussed in Littmann, J., and Viens, A. M. (2015). The ethical significance of antimicrobial resistance. *Public Health Ethics* 8:209–224.

CASE STUDY To test or not to test

A 4-month-old infant had been running a moderate fever for 36 hours, and a nervous mother made a call to her pediatrician. Examination and tests revealed no outward signs of infection or cause of the fever. The anxious mother wanted a prescription for antibiotics, but the pediatrician recommended watching the infant for two days before making a decision. He explained that decades of rampant use of antibiotics in medicine and agriculture has caused a global surge in antibiotic-resistant bacteria, drastically reducing the effectiveness of antibiotic therapy for infections. He pointed out that bacteria can exchange antibiotic resistance traits and that many pathogenic strains are now resistant to several antibiotics. The mother was not placated by these explanations and insisted that her baby receive antibiotics immediately. This situation raises several issues.

1. Was the pediatrician correct in stating that bacteria can exchange antibiotic resistance genes? If so, how is this possible?
2. If the infant was given antibiotics, how might this have contributed to the production of resistant bacteria?
3. If you were an anxious parent of the patient, would it change your mind if you learned that a woman died in 2016 from a bacterial infection that was resistant to all 26 antibiotics available in the United States?
4. How should the pediatrician balance his ethical responsibility to provide effective treatment to the present patient with his ethical responsibility to future patients who may need antibiotics for effective treatment?

See Garau, J. (2006). Impact of antibiotic restrictions: The ethical perspective. *Clin. Microbiol. Infect.* 12 (Supplement 5):16–24. See also the Genetics, Ethics, and Society essay above.

INSIGHTS AND SOLUTIONS

1. Time mapping is performed in a cross involving the genes *his, leu, mal,* and *xyl*. The recipient cells are auxotrophic for all four genes. After 25 minutes, mating is interrupted, with the results in recipient cells shown below. Diagram the positions of these genes relative to the origin (*O*) of the F factor and to one another.

   (a) 90% are xyl^+

   (b) 80% are mal^+

   (c) 20% are his^+

   (d) None are leu^+

Solution: The *xyl* gene is transferred most frequently, so it is closest to *O* (very close). The *mal* gene is next and reasonably close to *xyl,* followed by the more distant *his* gene. The *leu* gene is far beyond these three, since no recovered recombinants include it. The diagram shows these relative locations along a piece of the circular chromosome.

(continued)

Insights and Solutions continued

2. In four Hfr strains of bacteria, all derived from an original F^+ culture grown over several months, a group of hypothetical genes is studied and shown to transfer in the orders shown in the following table.

   (a) Assuming *b* is the first gene along the chromosome, determine the sequence of all genes shown. (b) One strain creates an apparent dilemma. Which one is it? Explain why the dilemma is only apparent, not real.

Hfr Strain	Order of Transfer					
1	*e*	*r*	*i*	*u*	*m*	*b*
2	*u*	*m*	*b*	*a*	*c*	*t*
3	*c*	*t*	*e*	*r*	*i*	*u*
4	*r*	*e*	*t*	*c*	*a*	*b*

Solution:

(a) The sequence is found by overlapping the genes in each strain.

Strain 2	*u*	*m*	***b***	***a***	***c***	*t*				
Strain 3				*c*	***t***	***e***	***r***	*i*	*u*	
Strain 1						*e*	*r*	***i***	***u***	***m***	*b*

Starting with *b* in strain 2, the gene sequence is *bacterium.*

(b) Strain 4 creates a dilemma, which is resolved when we realize that the F factor is integrated in the opposite orientation. Thus, the genes enter in the opposite sequence, starting with gene *r*.

$$retcab \longrightarrow$$

Problems and Discussion Questions

Mastering Genetics Visit for instructor-assigned tutorials and problems.

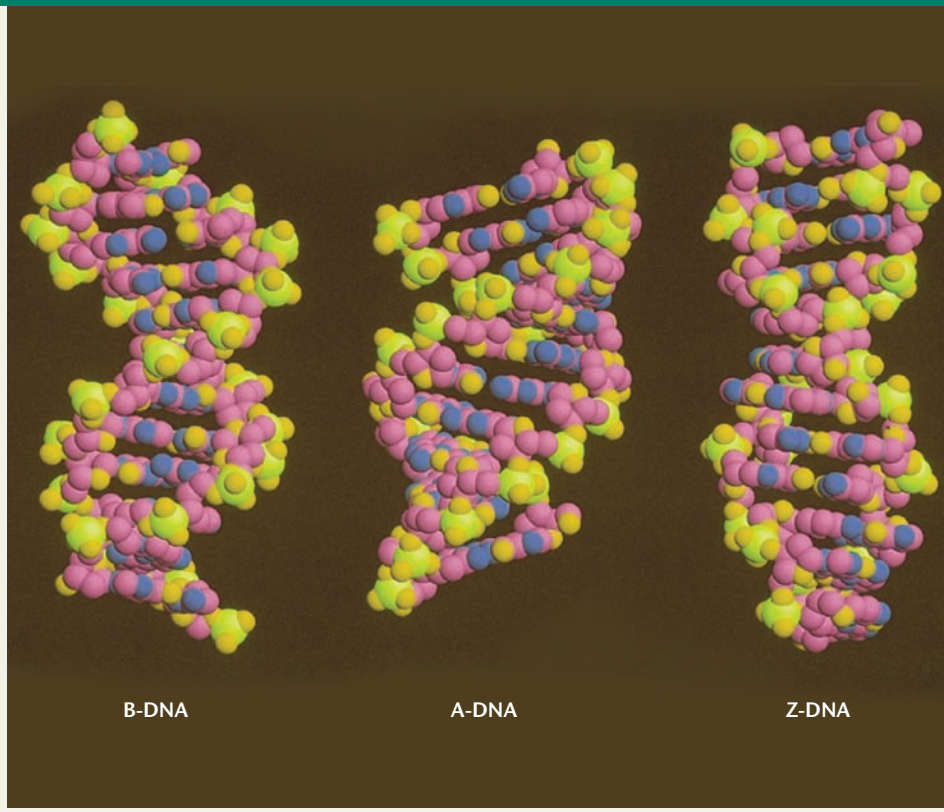
1. **HOW DO WE KNOW?** In this chapter, we have focused on genetic systems present in bacteria and the viruses that use bacteria as hosts (bacteriophages). In particular, we discussed mechanisms by which bacteria and their phages undergo genetic recombination, the basis of chromosome mapping. Based on your knowledge of these topics, answer several fundamental questions:
   (a) How do we know that bacteria undergo genetic recombination, allowing the transfer of genes from one organism to another?
   (b) How do we know that conjugation leading to genetic recombination between bacteria involves cell contact, which precedes the transfer of genes from one bacterium to another?
   (c) How do we know that during transduction bacterial cell-to-cell contact is not essential?

2. **CONCEPT QUESTION** Review the Chapter Concepts list on p. 168. A number of these center around the findings that genetic recombination occurs in bacteria. Write a short summary that describes ways in which recombination may occur in bacteria. ■
3. Distinguish between vertical and horizontal gene transfer.
4. With respect to F^+ and F^- bacterial matings,
   (a) How was it established that physical contact was necessary?
   (b) How was it established that chromosome transfer was unidirectional?
   (c) What is the genetic basis of a bacterium being F^+?
5. What is the F factor and why does its behavior differ in F^+ and Hfr bacterial strains?
6. Describe the basis for chromosome mapping in the Hfr $\times$ F^- crosses.
7. What is the consequence of the excision of the F factor from the chromosome in an Hfr strain?
8. Describe the origin of F′ bacteria and merozygotes.
9. Describe the main characteristics of a plasmid.
10. The bacteriophage genome consists primarily of genes encoding proteins that make up the head, collar and tail, and tail fibers. When these genes are transcribed following phage infection, how are these proteins synthesized, since the phage genome lacks genes essential to ribosome structure?
11. Describe the structure of the T4 phage.
12. In the plaque assay, what is the precise origin of a single plaque?
13. Explain the basis of the plaque assay. How is it used?
14. A plaque assay is performed beginning with 1.0 mL of a solution containing bacteriophages. This solution is serially diluted three times by taking 0.1 mL and adding it to 9.9 mL of liquid medium. 0.1mL of the final dilution is plated and yields 17 plaques. What is the initial density of bacteriophages in the original 1.0 mL?
15. Why are the lytic and the lysogenic cycles both replicative phage strategies?
16. Define the term *prophage*.
17. Explain the observations that led Zinder and Lederberg to conclude that the prototrophs recovered in their transduction experiments were not the result of Hfr-mediated conjugation.
18. Describe the execution of and rationale behind linkage and mapping studies of bacterial genes during transduction experiments.
19. If a single bacteriophage infects one *E. coli* cell present in a culture of bacteria and, upon lysis, yields 200 viable viruses, how many phages will exist in a single plaque if three more lytic cycles occur?
20. A phage-infected bacterial culture was subjected to a series of dilutions, and a plaque assay was performed in each case, with the following results. What conclusion can be drawn in the case of each dilution?

	Dilution Factor	Assay Results
(a)	10^4	All bacteria lysed
(b)	10^6	14 plaques
(c)	10^8	0 plaques

9

DNA Structure and Analysis



Computer-generated space-filling models of alternative forms of DNA.

CHAPTER CONCEPTS

- With the exception of some viruses, DNA serves as the genetic material in all living organisms on Earth.
- According to the Watson–Crick model, DNA exists in the form of the right-handed double helix.
- The strands of the double helix are antiparallel and held together by hydrogen bonding between complementary nitrogenous bases.
- The structure of DNA provides the basis for storing and expressing genetic information.
- RNA has many similarities to DNA but exists mostly as a single-stranded molecule.
- In some viruses, RNA serves as the genetic material.
- Many techniques have been developed that facilitate the analysis of nucleic acids, most based on detection of the complementarity of nitrogenous bases.

Up to this point in the text, we have described chromosomes as structures containing genes that control phenotypic traits that are transmitted through gametes to future offspring. Logically, genes must contain some sort of information that, when passed to a new generation, influences the form and characteristics of each individual. We refer to that information as the **genetic material.** Logic also suggests that this same information in some way directs the many complex processes that lead to an organism's adult form.

Until 1944, it was not clear what chemical component of the chromosome makes up genes and constitutes the genetic material. Because chromosomes were known to have both a nucleic acid and a protein component, both were candidates. In 1944, however, direct experimental evidence emerged showing that the nucleic acid DNA serves as the informational basis for heredity.

Once the importance of DNA in genetic processes was realized, work intensified with the hope of discerning not only the structural basis of this molecule but also the relationship of its structure to its function. Between 1944 and 1953, many scientists sought information that might answer the most significant and intriguing question in the history of biology: How does DNA serve as the genetic basis for the living process? Researchers believed the answer depended strongly on the chemical structure of the DNA molecule, given the complex but orderly functions ascribed to it.

These efforts were rewarded in 1953 when James Watson and Francis Crick set forth their hypothesis for the double-helical nature of DNA. The

assumption that the molecule's functions would be clarified more easily once its general structure was determined proved to be correct. In this chapter, we initially review the evidence that DNA is the genetic material and then discuss the elucidation of its structure. We conclude the chapter with a discussion of several analytical techniques useful during the study of nucleic acids, DNA and RNA.

9.1 The Genetic Material Must Exhibit Four Characteristics

For a molecule to serve as the genetic material, it must possess four major characteristics: **replication, storage of information, expression of information,** and **variation by mutation.** Replication of the genetic material is one facet of the cell cycle, a fundamental property of all living organisms. Once the genetic material of cells replicates and is doubled in amount, it must then be partitioned equally into daughter cells. During the formation of gametes, the genetic material is also replicated but is partitioned so that each cell gets only one-half of the original amount of genetic material—the process of meiosis. Although the products of mitosis and meiosis differ, both of these processes are part of the more general phenomenon of cellular reproduction.

Storage of information requires the molecule to act as a repository of genetic information that may or may not be expressed by the cell in which it resides. It is clear that while most cells contain a complete copy of the organism's genome, at any point in time they express only a part of this genetic potential. For example, in bacteria many genes "turn on" in response to specific environmental conditions and "turn off" when conditions change. In vertebrates, skin cells may display active melanin genes but never activate their hemoglobin genes; in contrast, digestive cells activate many genes specific to their function but do not activate their melanin genes.

Expression of the stored genetic information is the basis of the process of **information flow** within the cell (**Figure 9.1**). The initial event is the **transcription** of DNA, in which three main types of RNA molecules are synthesized: messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). Of these, mRNAs are translated into proteins. Each mRNA is the product of a specific gene and directs the synthesis of a different protein. In **translation,** the chemical information in mRNA directs the construction of a chain of amino acids, called a polypeptide, which then folds into a protein. Collectively, these processes form the **central dogma of molecular genetics:** "DNA makes RNA, which makes proteins."

The genetic material is also the source of variation among organisms through the process of mutation. If a mutation—a change in the chemical composition of DNA—occurs, the alteration may be reflected during transcription and translation, affecting the specific protein. If such a mutation is present in gametes, it may be passed to future generations and, with time, become distributed throughout the population. Genetic variation, which also includes alterations of chromosome number and rearrangements within and between chromosomes, provides the raw material for the process of evolution.

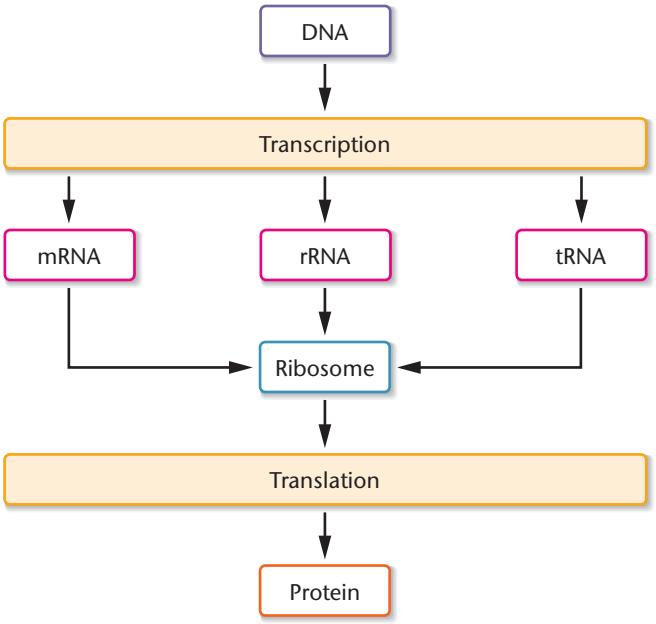


FIGURE 9.1 Simplified view of information flow (the central dogma) involving DNA, RNA, and proteins within cells.

9.2 Until 1944, Observations Favored Protein as the Genetic Material

The idea that genetic material is physically transmitted from parent to offspring has been accepted for as long as the concept of inheritance has existed. Beginning in the late nineteenth century, research into the structure of biomolecules progressed considerably, setting the stage for describing the genetic material in chemical terms. Although both proteins and nucleic acid were major candidates for the role of the genetic material, until the 1940s many geneticists favored proteins. This is not surprising because a diversity of proteins was known to be abundant in cells, and much more was known about protein chemistry.

DNA was first studied in 1868 by a Swiss chemist, Friedrich Miescher. He isolated cell nuclei and derived an acid substance containing DNA that he called **nuclein.** As investigations progressed, however, DNA, which was shown

to be present in chromosomes, seemed to lack the chemical diversity necessary to store extensive genetic information. This conclusion was based largely on Phoebus A. Levene's observations in 1910 that DNA contained approximately equal amounts of four similar molecules called *nucleotides.* Levene postulated incorrectly that identical groups of these four components were repeated over and over, which was the basis of his **tetranucleotide hypothesis** for DNA structure. Attention was thus directed away from DNA, favoring proteins. However, in the 1940s, Erwin Chargaff showed that Levene's proposal was incorrect when he demonstrated that most organisms do not contain precisely equal proportions of the four nucleotides. We shall see later that the structure of DNA accounts for Chargaff's observations.

ESSENTIAL POINT

Although both proteins and nucleic acids were initially considered as possible candidates, proteins were initially favored to serve as the genetic material. ■

9.3 Evidence Favoring DNA as the Genetic Material Was First Obtained during the Study of Bacteria and Bacteriophages

Oswald Avery, Colin MacLeod, and Maclyn McCarty's 1944 publication on the chemical nature of a "transforming principle" in bacteria was the initial event that led to the acceptance of DNA as the genetic material. Their work, along with subsequent findings of other research teams, constituted the first direct experimental proof that DNA, and not protein, is the biomolecule responsible for heredity. It marked the beginning of the era of molecular genetics, a period of discovery in biology that made biotechnology feasible and has moved us closer to understanding the basis of life. The impact of the initial findings on future research and thinking paralleled that of the publication of Darwin's theory of evolution and the subsequent rediscovery of Mendel's postulates of transmission genetics. Together, these events constitute the three great revolutions in biology.

Transformation Studies

The research that provided the foundation for Avery, MacLeod, and McCarty's work was initiated in 1927 by Frederick Griffith, a medical officer in the British Ministry of Health. He experimented with several different strains of the bacterium *Diplococcus pneumoniae.** Some were *virulent strains*, which cause pneumonia in certain vertebrates (notably humans and mice), while others were *avirulent strains*, which do not cause illness.

The difference in virulence depends on the existence of a polysaccharide capsule; virulent strains have this capsule, whereas avirulent strains do not. The nonencapsulated bacteria are readily engulfed and destroyed by phagocytic cells in the animal's circulatory system. Virulent bacteria, which possess the polysaccharide coat, are not easily engulfed; they multiply and cause pneumonia.

The presence or absence of the capsule causes a visible difference between colonies of virulent and avirulent strains. Encapsulated bacteria form *smooth colonies* (*S*) with a shiny surface when grown on an agar culture plate; nonencapsulated strains produce *rough colonies* (*R*). Thus, virulent and avirulent strains are easily distinguished by standard microbiological culture techniques.

Each strain of *Diplococcus* may be one of dozens of different types called *serotypes.* The specificity of the serotype is due to the detailed chemical structure of the polysaccharide constituent of the thick, slimy capsule. Serotypes are identified by immunological techniques and are usually designated by Roman numerals. Griffith used the avirulent type II*R* and the virulent type III*S* in his critical experiments. **Table 9.1** summarizes the characteristics of these strains.

Griffith knew from the work of others that only living virulent cells produced pneumonia in mice. If heat-killed virulent bacteria were injected into mice, no pneumonia resulted, just as living avirulent bacteria failed to produce the disease. Griffith's critical experiment involved injecting mice with living II*R* (avirulent) cells combined with heat-killed III*S* (virulent) cells. Since neither cell type caused death in mice when injected alone, Griffith expected that the double injection would not kill the mice. But, after five days, all of the mice that had received both types of cells were dead. Paradoxically, analysis of their blood revealed large numbers of living type III*S* bacteria.

As far as could be determined, these III*S* bacteria were identical to the III*S* strain from which the heat-killed cell preparation had been made. Control mice, injected only with living avirulent II*R* bacteria, did not develop pneumonia and remained healthy. This ruled out the possibility that the avirulent II*R* cells simply changed (or mutated) to virulent III*S* cells in the absence of the heat-killed III*S* bacteria. Instead, some type of interaction had taken place between living II*R* and heat-killed III*S* cells.

TABLE 9.1 Strains of *Diplococcus pneumoniae* Used by Frederick Griffith in His Original Transformation Experiments

Serotype	Colony Morphology	Capsule	Virulence
II*R*	Rough	Absent	Avirulent
III*S*	Smooth	Present	Virulent

*This organism is now named *Streptococcus pneumoniae*.