7 Microbial Genetics

CHAPTER SUMMARY

The Structure and Replication of Genomes (pp. 197–205)

Genetics is the study of inheritance and inheritable traits. Genes are composed of specific sequences of nucleotides that code for polypeptides or RNA molecules. A genome is the sum of all the genetic material in a cell or virus. Prokaryotic and eukaryotic cells use DNA as their genetic material; some viruses use DNA, and other viruses use RNA.

The Structure of Nucleic Acids

The two strands of DNA are held together by hydrogen bonds between complementary bases of nucleic acids called **base pairs** (BP). In DNA, adenine bonds with thymine, and guanine bonds with cytosine. One end of a DNA strand is called the 5' end because it terminates in a phosphate group attached to a 5' carbon; the opposite end of the strand is called the 3' end because it terminates with a hydroxyl group bound to a 3' carbon of deoxyribose. The two strands are oriented in opposite directions to each other: One strand runs in a 3' to 5' direction, whereas the other runs in a 5' to 3' direction. The lengths of DNA molecules are expressed in base pairs.

The Structure of Prokaryotic Genomes

Prokaryotic genomes consist of one or two **chromosomes**, which are typically circular molecules of DNA associated with protein and RNA molecules, localized in a region of the cytoplasm called the **nucleoid**. Prokaryotic cells may also contain one or more extrachromosomal DNA molecules called **plasmids**, which contain genes that regulate nonessential life functions such as bacterial conjugation; resistance to one or more antimicrobial drugs, heavy metals, or toxins; destruction of competing bacteria; and pathogenicity.

The Structure of Eukaryotic Genomes

In addition to DNA, eukaryotic chromosomes contain proteins called **histones**, arranged as **nucleosomes** (beads of DNA) that clump with other proteins to form chromatin fibers. Eukaryotic cells also contain extrachromosomal DNA in mitochondria, chloroplasts, and plasmids.

DNA Replication

DNA replication is a simple concept: A cell separates the two original strands and uses each as a template for the synthesis of a new complementary strand. The process is *semiconservative* because each daughter DNA molecule is composed of one original strand and one new strand.

In DNA replication, the cell removes histones and other proteins from the DNA molecule. DNA helicase unzips the double helix, breaking hydrogen bonds between complementary base pairs, to form a replication fork. DNA synthesis always moves in the 5' to 3' direction, so the **leading strand** is synthesized toward the replication fork. Synthesis is mediated by enzymes that prime, join, and proofread the pairing of new nucleotides. The **lagging strand** is synthesized in a direction away from the replication fork, and discontinuously in *Okazaki fragments*. It always lags behind the process occurring in the leading strand. DNA ligase seals the gaps between adjacent Okazaki fragments to form a continuous DNA strand.

After bacterial DNA replication, methylation occurs. In **methylation**, a cell adds a methyl group to one or two bases that are part of specific nucleotide sequences. In some cases, genes that are methylated are "turned off" and are not transcribed, whereas in other cases, they are "turned on" and are transcribed. In some bacteria, methylated nucleotide sequences play a role in initiating DNA replication, repairing DNA, or recognizing and protecting against viral DNA.

Eukaryotic DNA replication is similar to that in bacteria with a few exceptions. Eukaryotic cells use four DNA polymerases to replicate DNA. Due to the large size of eukaryotic chromosomes, there are many origins of replication. Okazaki fragments of eukaryotes are smaller than those of bacteria. Finally, plant and animal cells methylate cytosine bases exclusively.

Gene Function (pp. 205–219)

To understand gene function, it is necessary to distinguish between an organism's genotype and phenotype.

The Relationship Between Genotype and Phenotype

The **genotype** of an organism is the actual set of genes in its genome, whereas the **phenotype** is the physical and functional traits expressed by those genes, such as the presence of flagella. Thus, genotype determines phenotype; however, not all genes are active at all times.

The Transfer of Genetic Information

The **central dogma** of genetics states that DNA is transcribed to RNA, which is translated to form polypeptides.

The Events in Transcription

The transfer of genetic information begins with **transcription** of the genetic code from DNA to RNA, in which **RNA polymerase** links RNA nucleotides that are complementary to genetic sequences in DNA. Transcription begins at a region of DNA called a **promoter** (recognized by RNA polymerase) and ends with a sequence called a **terminator**. Other proteins may assist in termination, or it may depend solely on the nucleotide sequence of the transcribed RNA.

Cells transcribe four types of RNA from DNA:

- RNA primer molecules for DNA polymerase to use during DNA replication.
- **Messenger RNA (mRNA)** molecules, which carry genetic information from chromosomes to ribosomes.
- **Ribosomal RNA (rRNA)** molecules, which combine with ribosomal polypeptides to form ribosomes, the organelles that synthesize polypeptides.
- **Transfer RNA (tRNA)** molecules, which deliver amino acids to the ribosomes.

Eukaryotic transcription differs from bacterial transcription in several ways. Eukaryotic cells transcribe RNA in the nucleus, while prokaryotic transcription occurs in the cytosol. Eukaryotes have three types of nuclear RNA polymerase and multiple transcription factors. Eukaryotic cells process mRNA before translation. RNA processing involves capping, polyadenylation, and splicing.

Translation

In **translation**, the sequence of genetic information carried by mRNA is used by ribosomes to construct polypeptides with specific amino acid sequences. To understand how four DNA nucleotides can specify the 20 different amino acids commonly found in proteins requires an understanding of the genetic code. Scientists define the genetic code as the complete set of triplets of mRNA nucleotides called **codons** that code for specific amino acids. These bind to complementary **anticodons** on tRNA. The code is redundant; that is, more than one codon is associated with all the amino acids except methionine and tryptophan.

The smaller subunit of a ribosome is shaped to accommodate three codons at one time. Each ribosome also has three binding sites that are named for their function:

- The **A site** accommodates a tRNA delivering an amino acid.
- The **P site** holds a tRNA and the growing polypeptide.
- Discharged tRNAs exit from the **E site**.

Prokaryotic translation proceeds in three stages: In *initiation*, an initiation complex is formed. During *elongation*, tRNAs sequentially deliver amino acids as directed by the codons of mRNA. Ribosomal RNA in the large ribosomal subunit catalyzes a peptide bond between the amino acid at the A site and the growing polypeptide at the P site. The third stage, *termination*, does not involve tRNA; instead, proteins called *release factors* halt elongation. The ribosome then dissociates into its subunits.

Regulation of Genetic Expression

About 75% of genes are expressed at all times; other genes are regulated so that the polypeptides they encode are synthesized only when a cell has need of them. Cells may stop synthesis by stopping transcription or by stopping translation directly.

Some regulation of genetic expression is at the level of translation. A riboswitch is a molecule of mRNA that folds in such a way as to block ribosomes and translation of the polypeptide they encode when that polypeptide is not needed. Translation can also be controlled by short interference RNA (siRNA). siRNA is an RNA molecule complementary to a portion of mRNA, tRNA, or a gene. siRNA binds to its target and renders it inactive.

An **operon** consists of a promoter, an adjacent regulatory element called an **operator**, and a series of genes all either repressed or induced by one regulatory gene located elsewhere. **Inducible operons** such as the *lac operon* are not usually transcribed and must be activated by inducers. **Repressible operons** such as the *trp operon* are transcribed continually until deactivated by repressors.

Mutations of Genes (pp. 220–227)

A **mutation** is a change in the nucleotide sequence of a genome.

Types of Mutations

Mutations range from large changes in an organism's genome, such as the loss or gain of an entire chromosome, to the most common type of mutation, **point mutations**,

in which just one or a few nucleotide base pairs are affected. Point mutations include the following:

- **Substitutions**, in which a single nucleotide is substituted for another, possibly leaving the amino acid sequence unaffected because of the redundancy of the genetic code.
- **Frameshift mutations**, including **insertions** and **deletions** of nucleotides, in which nucleotide triplets subsequent to an insertion or deletion are displaced, creating new sequences of codons that result in vastly altered polypeptide sequences.

Effects of Mutations

Some base-pair substitutions produce **silent mutations**: the substitution does not change the amino acid sequence because of the redundancy of the genetic code. A change in a nucleotide sequence resulting in a codon that specifies a different amino acid is called a **missense mutation**; what gets transcribed and translated makes sense, but not the right sense. In a **nonsense mutation**, a base-pair substitution changes an amino acid codon into a stop codon. Nearly all nonsense mutations result in nonfunctional proteins. Frameshift mutations (insertions or deletions) typically result in drastic missense and nonsense mutations.

Mutagens

Mutations can be spontaneous, or result from recombination. Physical or chemical agents called **mutagens**, which include radiation and several types of DNA-altering chemicals, induce mutations. Radiation in the form of X-rays and gamma rays can cause mutations. Additionally, nonionizing radiation in the form of ultraviolet light causes adjacent pyrimidine bases to bond to one another to form **pyrimidine dimers**. The presence of dimers prevents hydrogen bonding with the nucleotides in the complementary strand, distorts the sugar-phosphate backbone, and prevents proper replication and transcription.

Chemical mutagens include **nucleotide analogs**, compounds that are structurally similar to normal nucleotides but, when incorporated into DNA, cause mutations. Some *nucleotide-altering chemicals* alter the structure of nucleotides, causing base-pair substitution mutations. Aflatoxins are nucleotide-altering chemicals that result in missense mutations and cancer. Still other mutagenic chemicals insert or delete nucleotide base pairs, resulting in frameshift mutations.

Frequency of Mutation

About one of every ten million genes contain an error. Mutagens typically increase the mutation rate by a factor of 10–1000 times.

DNA Repair

Cells have numerous methods of repairing damaged DNA. In **light repair**, cells use DNA photolyase to break the bonds between adjoining pyrimidine nucleotides. In **dark repair**, enzymes repair pyrimidine dimers by cutting damaged DNA from the molecule, creating a gap that is repaired by DNA polymerase I and DNA ligase. In **base-excision repair**, an enzyme system excises the erroneous base and DNA polymerase I fills in the gap. In **mismatch repair**, enzymes scan newly synthesized unmethylated DNA looking for mismatched bases, which they remove and replace. Once a new DNA strand is methylated, mismatch repair enzymes cannot correct any errors that remain. When damage is so extensive that these mechanisms are

overwhelmed, bacterial cells resort to an **SOS response** involving the production of a novel DNA polymerase capable of copying less-than-perfect DNA.

Identifying Mutants, Mutagens, and Carcinogens

If a cell does not repair a mutation, it and its descendents are called **mutants**. In contrast, cells normally found in nature are called **wild type** cells. Researchers have developed methods to recognize mutants amidst their wild type neighbors. These include:

- **Positive selection**, which involves selecting a mutant by eliminating wild type phenotypes.
- **Negative selection** (also called **indirect selection**), a process in which a researcher attempts to culture *auxotrophs*.
- The **Ames test**, which is used to identify potential **carcinogens** (cancer-causing agents).

Genetic Recombination and Transfer (pp. 227–234)

Genetic recombination refers to the exchange of nucleotide sequences between two DNA molecules, often mediated by segments that are composed of identical or nearly identical nucleotide sequences called *homologous sequences*. DNA molecules that contain new arrangements of nucleotide sequences are called **recombinants**. Scientists first observed recombinants in eukaryotes during *crossing over*, a process in which portions of homologous chromosomes are recombined during the formation of gametes (sex cells). (See Chapter 12.)

Horizontal Gene Transfer Among Prokaryotes

Vertical gene transfer is the transmission of genes from parents to offspring. In **horizontal gene transfer**, DNA from a **donor cell** is transmitted to a **recipient cell**. A **recombinant cell** results from genetic recombination between donated and recipient DNA.

Transformation, transduction, and bacterial conjugation are types of horizontal gene transfer:

- In **transformation**, a **competent** recipient cell takes up DNA from the environment. Competency is found naturally and can be created artificially in some cells.
- In **transduction**, a virus such as a bacteriophage carries DNA from a donor cell to a recipient cell. Donor DNA is accidentally incorporated in **transducing phages**.
- In **conjugation**, a bacterium containing an **F fertility plasmid** (**factor**) forms a **conjugation pilus** that attaches to an F^- recipient bacterium. Plasmid genes are transferred to the recipient, which becomes F^+ as a result. **Hfr** (**high frequency of recombination**) **cells** result when an F plasmid integrates into a prokaryotic chromosome. Hfr cells form conjugation pili and transfer cellular genes more frequently than normal F^+ cells.

Transposons and Transposition

Transposons are DNA segments that code for the enzyme transposase and contain palindromic sequences known as **inverted repeats** (**IR**) at each end. (A *palindrome* is a word, phrase, or sentence that has the same sequence of letters when read backward or forward.) Transposons move among locations in chromosomes in

eukaryotes and prokaryotes. The simplest transposons, known as **insertion sequences** (**IS**), consist only of inverted repeats and transposase. **Complex transposons** contain other genes as well.

KEY THEMES

So far we have focused on the structural elements of microbial cells and the processes of metabolism and growth. We have yet to address the concept, however, of from whence these structures and functions arise. Evolution ultimately works on the phenotype, but it is the genotype, the sum total of our genes, that ultimately produces the phenotypes we see. While studying this chapter on genetics, focus on the following key points:

- *Genomes are large, even among microbes, but not all of what they code for is expressed:* Only a small fraction of the entire genome is actually translated into protein. Expression is highly regulated, a fact essential to survival.
- *Mutation is the foundation of evolutionary adaptation:* Without the process of mutation, genes would not change, and ultimately there would be no variation among organisms.
- *Many microbes exchange genes horizontally:* Horizontal gene transfer allows for the rapid spread of genetic elements among "adult" populations. This can be enormously beneficial to the microbes, but devastating for us.

QUESTIONS FOR FURTHER REVIEW

Answers to these questions can be found in the answer section at the back of this study guide. Refer to the answers only after you have attempted to solve the questions on your own.

Multiple Choice

1. DNA genomes are found in:
   a. All organisms and all viruses
   b. All organisms, but only some viruses
   c. Eukaryotic cells only
   d. All eukaryotic cells, but only some prokaryotic cells and some viruses
2. Plasmid DNA:
   a. Is the same as genomic DNA
   b. Is DNA in addition to genomic DNA
   c. Generally codes for extra functions
   d. Both b and c
3. Which of the following cells does not use histones to compact DNA?
   a. Archaea
   b. Bacteria
   c. Eukaryotic cells
   d. All use histones to compact DNA
4. Plasmids cannot be found among representatives of which of the following microbes?
   a. Algae
   b. Archaea
   c. Bacteria
   d. Fungi

5. Leading strand synthesis is initiated by:
   a. A DNA primer
   b. An RNA primer
   c. An amino acid primer
   d. A protein primer

6. Which of the following molecules is needed in lagging strand synthesis but not in leading strand synthesis?
   a. Primer
   b. Polymerase
   c. Ligase
   d. Helicase

7. Which of the following is not a role of methylation?
   a. Initiating DNA replication
   b. Regulating gene expression
   c. Identifying host DNA from viral DNA
   d. All are functions of methylation

8. Of the types of RNA listed below, which is/are not translated into protein?
   a. mRNA
   b. rRNA
   c. tRNA
   d. Both b and c

9. Transcription in prokaryotes requires which of the following molecules that is not used in eukaryotic transcription?
   a. RNA polymerase
   b. Sigma factor
   c. Transcription factors
   d. All are needed in eukaryotic transcription

10. Which of the following statements is true concerning DNA polymerases and RNA polymerases?
    a. Both require the aid of a helicase
    b. RNA polymerase is faster than DNA polymerase
    c. RNA polymerase uses ribonucleotides while DNA polymerase uses dexyribonucleotides
    d. Both have proofreading capabilities

11. In prokaryotes, which codon serves the dual purpose of being a start signal for translation and coding for *N*-formylmethionine?
    a. UAG
    b. UGA
    c. AUG
    d. GUA

12. Which of the following is true regarding eukaryotic mRNA?
    a. It always codes for only a single polypeptide
    b. Translation of the mRNA can begin before transcription is complete
    c. mRNA is transcribed in the cytosol
    d. None of the above are true about eukaryotic mRNA

13. Anticodons are found as part of:
    a. mRNA structure
    b. tRNA structure
    c. rRNA structure
    d. Ribosomal structure

14. Which stage of translation does not require energy?
    a. Initiation
    b. Elongation
    c. Termination
    d. All steps require energy

15. Which of the following is not a part of the initiation complex in prokaryotic translation?
    a. mRNA
    b. $tRNA^{MET}$
    c. Small ribosomal subunit
    d. All are part of the initiation complex

16. Termination of translation requires:
    a. tRNA
    b. Protein release factors
    c. Recognition of a stop codon
    d. Both b and c

17. Which of the following is a true statement concerning the *lac* operon in *E. coli*?
    a. The *lac* operon is an inducible operon that is generally inactive
    b. Lactose is an inducer of the operon
    c. The *lac* operon allows for the conservation of energy by making catabolic enzymes for lactose only when lactose is present
    d. All of the above are true statements

18. This type of mutation almost always results in a completely nonfunctional protein:
    a. Silent mutation
    b. Missense mutation
    c. Frameshift mutation
    d. Nonsense mutation

19. Pyrimidine dimers are caused by:
    a. Ionizing radiation
    b. Ultraviolet light
    c. Nucleotide analogs
    d. Nucleotide-altering chemicals

20. Horizontal gene transfer among microbes:
    a. Occurs at a high frequency in microbial populations
    b. Involves donor and recipient cells within the same generation
    c. Does not include the method of transformation
    d. Does not occur at all

21. Which of the following is a true statement regarding transduction?
    a. Transduction involves replicating viruses that move nonviral DNA between cells
    b. Transduction only occurs among prokaryotic cells
    c. Transduction involves both generalized and specialized processes
    d. Both a and c

22. Donor cells remain alive in which method of horizontal gene transfer?
    a. Transformation
    b. Transduction
    c. Conjugation
    d. Donor cells die in all three processes

23. Which process below is most likely to create frameshift mutations in the DNA of "recipient" cells?
    a. Transformation
    b. Transduction
    c. Conjugation
    d. Transposition

24. Hfr conjugation:
    a. Creates F^+ cells out of the recipient
    b. Requires competent recipient cells
    c. Involves transfer of part of the donor chromosome
    d. Requires transposase

25. Which of the molecules or entities listed below could not only carry an antibiotic resistance gene, but also move the gene into a recipient cell with a high degree of success?
    a. Complex transposon
    b. Insertion sequence
    c. Hfr cell
    d. Transducing phage

Fill in the Blanks

1. In RNA, _____ replaces _____ to bond with adenine.
2. Eukaryotic genomes, as compared to prokaryotic genomes, are usually _____ (linear/circular). Additionally, eukaryotic genomes generally have _____ (more/fewer) chromosomes than prokaryotic genomes.
3. Monomers and energy needed to anabolically synthesize new strands of DNA are provided by the same molecule, specifically _____.
4. During semiconservative replication, the _____ strand is synthesized continuously while the _____ strand is synthesized in short pieces.
5. The process of _____ produces RNA templates from DNA while _____ produces protein from RNA templates.
6. Transcription can terminate in one of two ways: _____ or _____.
7. In mRNA, nucleotides called _____ code for specific amino acids.
8. The process of transciption produces three types of RNA: _____, _____, and _____. These RNA molecules are then used in the process of _____.
9. Transfer RNA is _____ (specific/nonspecific) for the amino acids carried. Individual tRNA molecules can recognize _____ (one/more than one) codon.

10. In translation, the _____ site receives incoming amino acids, the _____ site is involved in elongation, and the _____ site releases spent tRNAs.
11. Operons consist of the following elements: _____, _____, and sometimes a(n) _____.
12. Two types of operons exist, _____ and _____.
13. Catabolic pathways are often associated with _____ (inducible/repressible) operons.
14. The erroneous incorporation of uracil instead of thymine into replicating DNA would be repaired by _____.
15. Negative selection is used to isolate _____, microbes that have different nutritional requirements from those of wild-type microbes.
16. Genetic recombination involves the exchange of _____, two genetic elements of nearly identical nucleotide sequence.
17. Cells must be competent in order for the process of _____ to occur naturally between certain species of bacteria.
18. Conjugation requires that donor cells possess an _____ that codes for a _____ that connects the donor to the recipient.

Short-Answer Questions for Thought and Review

1. Describe three ways in which plasmids can increase the pathogenic nature of bacteria.

2. Explain what this statement means: ". . . the information of a genotype is not always expressed as a phenotype."

3. Summarize the differences between prokaryotic and eukarytoic mRNA.

4. Explain why anticodon wobbling is useful to the cell in terms of protection against mistakes *and* the conservation of anabolic energy that would otherwise have to be spent manufacturing tRNAs.

5. Table 7.3 compares the genetic processes that occur in the cell. For each process, indicate where it occurs in prokaryotes and where it occurs in eukaryotes.

6. You are a researcher who has been given the task of determining the relative mutagenic potential of 3 compounds: X, Y, and Z. You set up a set of experiments similar to what is shown in Figure 7.27 on page 226. Without any mutagens, you see 5 colonies that are antibiotic resistant. Exposure of cells to X produces 6 colonies, Y produces 28, and Z produces 15. Determine the rate of mutation for each compound, and list the compounds in order of increasing mutagenic potential.

7. In the Ames Test, the assumption is made that if something is mutagenic in *Salmonella*, it is likely to be mutagenic in humans also and thus should be investigated further for the ability to cause cancer in animals. What is the genetic basis for this assumption?

Critical Thinking

1. Semiconservative replication of DNA results in the production of new daughter molecules that contain one new strand paired with one original parent strand. Some RNA viruses have only a single genomic strand, not complementary strands as in dsDNA. Explain, based on this structural difference alone, why RNA viruses have a higher rate of mutation than any dsDNA genome (viral or otherwise).

2. Below is a sequence of prokaryotic DNA. Transcribe the DNA into mRNA and then into protein using Figure 7.11 which shows the genetic code.

   5' — TACAAAGAGTAGGGAGGCAGCATCGGCCAT— 3'

3. You are given the prokaryotic DNA sequence below. When you translate it into mRNA you will see that there is no start codon and thus the DNA codes for "nothing." Write out the RNA sequence and then show one substitution, one insertion, and one deletion that would result in a sequence with a start codon that could be translated. Once you have done this, translate each sequence into protein.

   5' — TACGCGTCTATCACG — 3'

Concept Building Questions

1. In previous chapters, we have learned about microbial structure, function, and metabolism. In this chapter we learned about microbial genetics. Ultimately, a cell is the product of its genes, but it is the phenotype, not the genotype, that is subject to evolutionary processes. Explain why it is the appearance, function, and metabolism of a microbe, rather than its actual genes, that determines if a microbe can survive in the environment.
2. In Chapter 3, we learned about prokaryotic structure, and in particular about transporters that move molecules across the cell membrane. Assume symporter A brings galactose into the cell along a sodium gradient. Let us assume that the symporter is encoded in an operon along with enzymes needed to catabolize galactose. Propose a mechanism that would lead to the shut down of the operator and the removal or inactivation of the symporter upon loss of galactose from the environment.
3. In Chapter 2, we learned about chemical bonding and protein structure. Globular proteins are generally hydrophobic on the interior and hydrophilic on the surface. Indicate how a missense mutation that results in a hydrophobic amino acid being placed on the surface of the protein could change the physical structure of the protein and how it interacts with other proteins and molecules in the surrounding environment.