Bailey & Scott's

DIAGNOSTIC MICROBIOLOGY

fifteenth edition

PATRICIA M. TILLE

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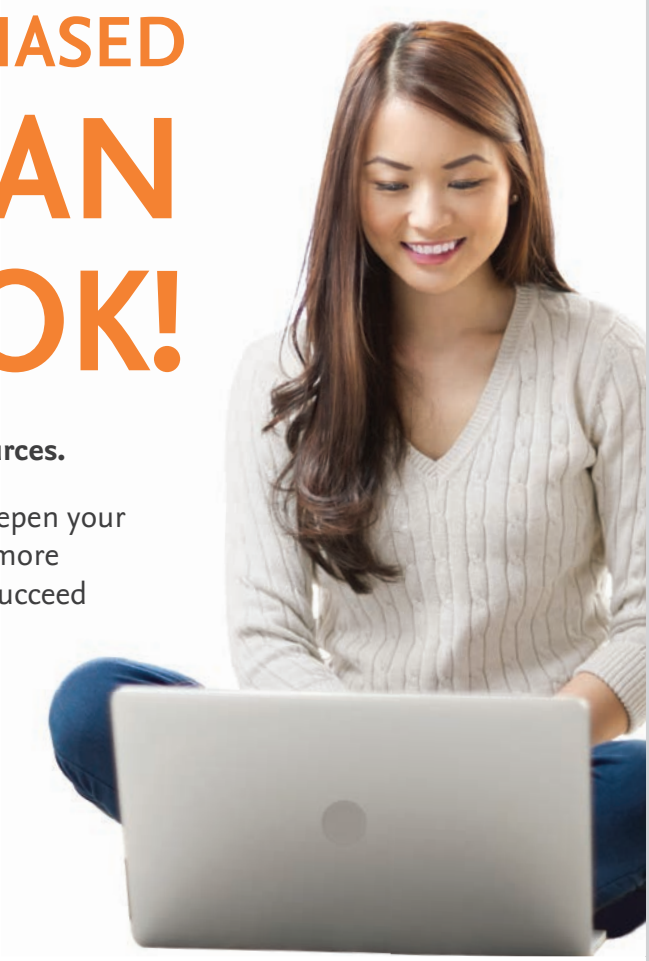

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Bailey & Scott's

DIAGNOSTIC MICROBIOLOGY

Fifteenth Edition

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Life is fleeting, but you must always remember friends, colleagues, and family. I will always thank my husband, David, and our children, Chrissy, Malissa, D.J., and Katie, along with their significant others. I would be remiss if I did not mention the seven little "smiles" that bring daily joy to our lives: Aedan, Milan Jr., Julia, Maja, Jayce, Riley, and Mila!

Lastly, no endeavor such as this would continue to evolve from one edition to the next without the insightful comments and input from numerous professional users and students. Thank you for your dedication, hard work, and humor.

This edition was created during a time of great unrest and challenges in the medical field, with the world facing the COVID-19 pandemic. I myself was struck with the virus and hospitalized for a period. I can say firsthand that the care and compassion of the health care workers was amazing, from physical, occupational, and respiratory therapy; nursing staff; phlebotomists; and laboratory professionals. They all came together to ensure the best care!

This edition is dedicated to all the essential workers who keep the country going and the health care professionals who continue to save lives as we battle this formidable viral adversary! To all the lives lost, may you rest in peace and know that the knowledge developed during the pandemic continues to save more lives every day!

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Preface

This, the fifteenth edition of *Bailey & Scott's Diagnostic Microbiology*, is the third edition that I have had the great pleasure to edit and author with some amazing colleagues. The dynamics of infectious disease trends, along with the technical developments available for diagnosing, treating, and controlling these diseases, continues to present major challenges in the laboratory and medical care. In meeting these challenges, the primary goal for the fifteenth edition is to provide an updated and reliable reference text for practicing clinical microbiologists and technologists, while also presenting this information in a format that supports the educational efforts of all those responsible for preparing others for a career in diagnostic microbiology. The text retains the traditional information needed to develop a solid, basic understanding of diagnostic microbiology while integrating the dynamic expansion of molecular diagnostics and advanced techniques such as matrix-assisted laser desorption time-of-flight mass spectrometry.

We have kept the favorite features and made adjustments in response to important critical input from users of the text. The succinct presentation of each organism group's key laboratory, clinical, epidemiologic, and therapeutic features in tables and figures has been kept and updated. Regarding content, the major changes reflect the changes that the discipline of diagnostic microbiology continues to experience. Also, although the grouping of organisms into sections according to key features (e.g., Gram reaction, catalase or oxidase reaction, growth on MacConkey agar) has remained, changes regarding the genera and species discussed in these sections have been made. These changes, along with changes in organism nomenclature, were made to accurately reflect the changes that have occurred, and continue to occur, in taxonomy. Also, throughout the text, the content has been enhanced with new photographs and artistic drawings. Finally, although some classic methods for bacterial identification and characterization developed over the years (e.g., catalase, oxidase, Gram stain) still play a critical role in today's laboratory, others have given way to commercial identification systems. We realize that in a textbook such as this, a balance is needed for practicing and teaching diagnostic microbiology; our selection of identification methods that received the most detailed attention may not always meet the needs of both groups. However, we have tried to be consistent in selecting those methods that reflect the most current and common practices of today's clinical microbiology laboratories, along with those that present historical information required within an educational program.

Finally, in terms of organization, the fifteenth edition is similar in many aspects to the fourteenth edition, but some changes have been made. Various instructor ancillaries, specifically geared for the fifteenth edition, are available on the Evolve website, including an expanded test bank, updated PowerPoints, a laboratory manual with answers, review questions with answer key, and an electronic image collection. Student resources include a laboratory manual, review questions, online case studies, and online procedures.

We sincerely hope that clinical microbiology practitioners and educators find *Bailey & Scott's Diagnostic Microbiology*, fifteenth edition, to be a worthy and useful tool to support their professional activities.

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I would like to acknowledge the help of my colleagues at Elsevier who guided me through this project: Kristine Feeherty, Health Content Management Specialist, and Betsy McCormac, Content Development Specialist.

Patricia M. Tille

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1 Microbial Taxonomy

OBJECTIVES

1. Define classification, identification, species, genus, type genus, and binomial nomenclature.
2. Properly use binomial nomenclature in the identification of microorganisms, including syntax, capitalization, and punctuation.
3. Identify a microorganism's characteristics as either phenotypic or genotypic.
4. Define polyphasic taxonomy and chemotaxonomic methods and how they are being applied to the classification of microorganisms.
5. Describe how the classification, naming, and identification of organisms play a role in diagnostic microbiology in the clinical setting.

The science of **taxonomy** is a systematic process applied to all living entities, providing a consistent means to classify, name (nomenclature), and identify organisms. This consistency allows biologists worldwide to use a common label for every organism studied within the multitude of biologic disciplines. The common language of taxonomy minimizes confusion about organisms' names, physiology, and biologic relatedness. Taxonomy is important in the **phylogeny** (the evolutionary history of organisms) and scientific study of all living things in virtually every biologic discipline, including microbiology.

As a result of the advances in molecular biology, traditional taxonomy based on genotypic, phenotypic, and phylogenetic or evolutionary relationships currently encompasses a multifaceted analysis of **epigenetic** (variations in gene expression not caused by nucleic acid sequence similarities or differences) and **chemotaxonomic methods**. This method of classification or **polyphasic taxonomy** provides a more detailed but very complex analysis of the current classification system using ribosomal ribonucleic acid (rRNA) sequences, whole genome sequences, epigenetics, and mass spectrometry (MS). The "gold standard" for classification of bacterial species has historically been based on deoxyribonucleic acid (DNA) including DNA hybridization (DDH) patterns and 16S rRNA gene (16S rDNA) sequence homology. With the implementation of next generation sequencing, a more detailed analysis of organism genomes including the average nucleotide identity (ANI), multilocus phylogenetic, and genome-to-genome distance (GGD) analysis permit the resolution of microorganisms from closely related subspecies to specific species. Not all parameters clearly delineate each organism to the species level. In other words, some characteristics may strengthen the organization of the genus, and some may be useful at the species level. Species identification techniques have distinct variations in cutoff values or thresholds for the differentiation of organisms at the genus and species levels. The comparative thresholds indicate the likelihood that two genomes are from the same organism (Table 1.1). When using a single sequence such as the 16S rRNA, the possibility of gene transfer may also affect genotypic classification. Although 16S rRNA sequences are evolutionarily highly conserved, ANI evaluates multiple coding regions across an entire genome, making the genomic analysis more detailed and accurate. Finally, lateral gene transfer among organisms, particularly bacteria, creates difficulty in the classification of organisms according to phenotypic traits or biochemical traits and genotypic criteria such as DNA G + C content, which has historically been the hallmark of diagnostic microbiology. Molecular methods have provided a means for identifying the historical core genomes used in classification and species identification. However, it is important to recognize that phenotypic expression and classification of organisms will continue to be compounded by the variation in genomes as a result of gene transfer among organisms.

In addition to more advanced genomic analysis, **chemotaxonomic methods** are more frequently being applied to the identification and classification of microorganisms. These methods include protein studies, fatty acid analysis, and cell wall composition. MS and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) use the separation and analysis of high-abundance proteins and peptides for the classification and identification of bacterial isolates. Techniques such as rapid evaporative ionization mass spectrometry (REIMS) are able to identify molecules and create images of tissues and microorganisms from laboratory growth medium. This polyphasic analysis beyond genomics provides a mechanism to use the MS data in conjunction with the genomic analysis and phenotypic characteristics to identify and classify organisms, as well as monitor biochemical therapies in complex disease states.

TABLE 1.1 Identification Criteria and Characteristics for Microbial Classification

Criteria	Characteristics
Phenotypic	
Macroscopic morphology	The microbial growth patterns on artificial media as observed when inspected with the unaided eye. Examples include the size, texture, and pigmentation of bacterial colonies.
Microscopic morphology	The size, shape, intracellular inclusions, cellular appendages, and arrangement of cells when observed with the aid of microscopic magnification.
Staining characteristics	The ability of an organism to reproducibly stain a particular color with the application of specific dyes and reagents. Staining is used in conjunction with microscopic morphology for bacterial identification. For example, the Gram stain for bacteria is a critical criterion for differential identification.
Environmental requirements	The ability of an organism to grow at various temperatures, in the presence of oxygen and other gases, at various pH levels, or in the presence of other ions and salts, such as NaCl.
Nutritional requirements	The ability of an organism to use various carbon and nitrogen sources as nutritional substrates when grown under specific environmental conditions.
Resistance profiles	The exhibition of a characteristic inherent resistance to specific antibiotics, heavy metals, or toxins.
Antigenic properties	The profiles of microorganisms established by various serologic and immunologic methods to determine relatedness among various microbial groups.
Subcellular properties	Molecular constituents of the cell that are typical of a particular taxon, or organism group, as established by various analytic methods. Some examples include cell wall components, components of the cell membrane, and enzymatic content of the microbial cell.
Chemotaxonomic properties	The chemical constituents of the cell, such as the structure of teichoic acids, fatty acid analysis, and protein profiles, as determined by analytical methods.
Genotypic	
DNA base composition ratio	DNA comprises four bases (guanine, cytosine, adenine, and thymine). The extent to which the DNA from two organisms is made up of cytosine and guanine (i.e., G + C content) relative to their total base content can be used as an indicator of relatedness or lack thereof. For example, an organism with a G + C content of 50% is not closely related to an organism with a G + C content of 25%.
Nucleic acid (DNA and RNA) base sequence characteristics, including those determined by hybridization assays	The order of bases along a strand of DNA or RNA is known as the **base sequence**. The extent to which sequences are **homologous** (similar) between two microorganisms can be determined directly or indirectly by various molecular methods. The degree of similarity in the sequences may be a measure of the degree of organism relatedness, specifically, the rRNA sequences that remain stable in comparison to the genome as a whole.
Average nucleotide identity (ANI)	This method analyses multiple coding sequences in a microorganism's genome to determine the average nucleotide identity using genome sequencing and computer algorithms. The relatedness of microorganisms is accurate at 95%–96% threshold for organism identification.
Genome-to-Genome Distance (GGD)	This is a computerized calculation that uses inference by in-silico genome comparisons eliminating the limitations and errors associated with wet-lab techniques. Organisms are related with a GGD threshold score of 70% or greater.

DNA, Deoxyribonucleic acid; *RNA,* ribonucleic acid; *rRNA,* ribosomal RNA.

As technology improves, the classification and identification of organisms will undoubtedly continue to evolve along with the changes in the populations of organisms. In diagnostic microbiology, classification, nomenclature, and identification of microorganisms play a central role in providing an accurate, timely diagnosis and monitoring the management of infectious disease. A brief, detailed discussion of the major components of taxonomy is important for a basic understanding of bacterial identification and application to diagnostic microbiology.

Classification

Classification is a method for organizing microorganisms into groups or **taxa** based on similar morphologic, physiologic, and genetic traits. The hierarchical classification system consists of the following taxa:

- Domains (Bacteria, Archaea, and Eukarya)
- Kingdom (contains similar divisions or phyla; most inclusive taxa)
- Phylum (contains similar classes; equivalent to the Division taxa in botany)

- Class (contains similar orders)
- Order (contains similar families)
- Family (contains similar genera)
- Genus (contains similar species)
- Species (specific epithet; lowercase Latin adjective or noun; most exclusive taxa)

Bacteria or **prokaryotes** (prenucleus) are separated into two domains, the Bacteria and the Archaea (ancient bacteria). The Bacteria contain the environmental prokaryotes (blue green or cyanobacteria) and the heterotrophic medically relevant bacteria. The Archaea are environmental isolates that live in extreme habitats such as high salt concentrations, jet fuel, or high temperatures. The third domain, Eukarya, ***eukaryotes*** (true nucleus), also contains medically relevant organisms, including fungi and parasites.

There are several other taxonomic sublevels below the domains, as noted previously; however, the typical application of organism classification in the diagnostic microbiology laboratory primarily uses the taxa beginning at the family designation.

Family

A **family** encompasses a group of organisms that may contain multiple genera and consists of organisms with a common attribute. The name of a family is formed by adding the suffix -aceae to the root name of one of the group's genera, called the **type genus;** for example, the *Streptococcaceae* family type genus is *Streptococcus.* One exception to the rule in microbiology is Enterobacterales; it is named after the "enteric" group of bacteria rather than the type species *Escherichia coli.* Bacterial (prokaryotic)-type species or strains are determined according to guidelines published by the International Committee for the Systematics of Prokaryotes (ICSP) in The International Code of Nomenclature of Prokaryotes (ICNP). This code provides the guidelines for linking nomenclature, classification, and characterization of organisms using the physiologic, biochemical, genetic, and phenotypic traits of organisms. Microorganism type species should be described in detail using diagnostic and comparable methods that are reproducible, and all authentic strains must be available for further analysis.

Genus

Genus (plural, genera), the next taxon, contains different species that have several important features in common. Each species within a genus differs sufficiently to maintain its status as an individual species. Placement of a species within a particular genus is based on various genetic and phenotypic characteristics shared among the species.

Microorganisms do not possess the multitude of physical features exhibited by higher organisms such as plants and animals. For instance, they rarely leave any fossil record, and they exhibit a tremendous capacity to intermix genetic material among seemingly unrelated species and genera. For these reasons, confidently establishing a microorganism's relatedness in higher taxa beyond the genus level is difficult. Although grouping similar genera into common families and similar families into common orders is used for classification of plants and animals, these higher taxa designations (i.e., division, class, and order) are not useful for classifying bacteria.

Species

Species (abbreviated as **sp.**, singular, or **spp.**, plural) is the most basic of the taxonomic groups and can be defined as a collection of bacterial strains that share common physiologic and genetic features and differ notably from other microbial species. Occasionally, taxonomic subgroups within a species, called **subspecies,** are recognized. Furthermore, designations such as **biotype, serotype,** or **genotype** may be given to groups below the subspecies level that share specific but relatively minor characteristics. For example, *Klebsiella pneumoniae* and *Klebsiella oxytoca* are two distinct species within the genus *Klebsiella. Serratia odorifera* biotype 2 and *Treponema pallidum* subsp. *pallidum* are examples of a biotype and a subspecies designation. A biotype is considered the same species with the same genetic makeup but displays differential physiologic characteristics. Subspecies do not display significant enough divergence to be classified as a biotype or a new species. Although these subgroups may have some taxonomic importance, their usefulness in diagnostic microbiology is limited.

Nomenclature

Nomenclature is the naming of microorganisms according to established rules and guidelines set forth in the ICNP. It provides the accepted labels by which organisms are universally recognized. Because genus and species are the groups commonly used by microbiologists, the discussion of rules governing microbial nomenclature is limited to these two taxa. In this **binomial** (two name) system of nomenclature, every organism is assigned a genus and a species of Latin or Greek derivation. Each organism has a scientific "label" consisting of two parts: the genus designation, in which the first letter is always capitalized, and the species designation, in which the first letter is always lowercase. The two components are used simultaneously and are printed in italics or underlined in script. For example, the streptococci include *Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae,* and *Streptococcus bovis,* among others. Alternatively, the name may be abbreviated by using the uppercase form of the first letter of the genus designation followed by a period (.) and the full species name (e.g., *S. pneumoniae, S. pyogenes, S. agalactiae,* and *S. bovis*). Finally, when discussing a single specific organism, the species may be designated using sp., and a group of species within the genus using spp. (e.g., *Staphylococcus* sp. and *Staphylococcus* spp.). Frequently an informal designation (e.g., staphylococci, streptococci, enterococci) may be used to label a particular group of organisms. These designations are not capitalized or italicized.

As more information is gained regarding organism classification and identification, a particular species may be moved to a different genus or assigned a new genus name. The rules and criteria for these changes are beyond the scope of this chapter, but such changes are documented in the *International Journal of Systemic and Evolutionary Microbiology*. Published nomenclature may be found at http://www.bacterio.net for bacteria, http://www.ictvonline.org for viruses, http://www.iapt-taxon.org/nomen/main.php for fungi, and http://www.iczn.org for parasites. It is important to note that the fungi and parasite lists are difficult to maintain and may not reflect the current validity at the time of review. In the diagnostic laboratory, changes in nomenclature are phased in gradually so that physicians and laboratorians have ample opportunity to recognize that a familiar pathogen has been given a new name. This is usually accomplished by using the new genus designation while continuing to provide the previous designation in parentheses; for example, *Stenotrophomonas (Xanthomonas) maltophilia* or *Burkholderia (Pseudomonas) cepacia*.

Identification

Microbial identification is the process by which a microorganism's key features are delineated. Once those features have been established, the profile is compared with those of other previously characterized microorganisms. The organism can then be assigned to the most appropriate taxa and can be given appropriate genus and species names; both are essential aspects of taxonomy in diagnostic microbiology and the management of infectious disease (Box 1.1).

Identification Methods

A wide variety of methods and criteria are used to establish a microorganism's identity. These methods can be separated into either of two general categories: genotypic or phenotypic characteristics. **Genotypic characteristics** relate to an organism's genetic makeup, including the nature of the organism's genes and constituent nucleic acids (see Chapter 2 for more information about microbial genetics). **Phenotypic characteristics** are based on features beyond the genetic level, including both readily observable characteristics and features that may require extensive analytic procedures to be detected. Examples of characteristics used as criteria for bacterial identification and classification are provided in Table 1.1. Modern microbial taxonomy uses a combination of several methods to characterize microorganisms thoroughly to classify and name each organism.

Although the criteria and examples in Table 1.1 are given in the context of microbial identification for classification purposes, the principles and practices of classification parallel the approaches used in diagnostic microbiology for the identification and characterization of microorganisms encountered in the clinical setting. Fortunately, because of the previous efforts and accomplishments of microbial taxonomists, microbiologists do not have to use several burdensome classification and identification schemes to identify infectious agents. Instead, microbiologists use key phenotypic and genotypic features on which to base their identification to provide clinically relevant information in a timely manner (Chapter 12). This should not be taken to mean that the identification of all clinically relevant organisms is easy and straightforward. This is also not meant to imply that microbiologists can identify or recognize only organisms that have already been characterized and named by taxonomists. Indeed, the clinical microbiology laboratory is well recognized as the place where previously unknown or uncharacterized infectious agents are initially encountered, and as such it has an ever-increasing responsibility to be the source of information and reporting for emerging etiologies of infectious disease.

• BOX 1.1 Role of Taxonomy in Diagnostic Microbiology

- Establishes and maintains records of key characteristics of clinically relevant microorganisms
- Facilitates communication among technologists, microbiologists, physicians, and scientists by assigning universal names to clinically relevant microorganisms. This is essential for:
  - Establishing an association of particular diseases or syndromes with specific microorganisms
  - Epidemiology and tracking outbreaks
  - Accumulating knowledge regarding the management and outcome of diseases associated with specific microorganisms
  - Establishing patterns of resistance to antimicrobial agents and recognition of changing microbial resistance patterns
  - Understanding the mechanisms of antimicrobial resistance and detecting new resistance mechanisms exhibited by microorganisms
  - Recognizing new and emerging pathogenic microorganisms
  - Recognizing changes in the types of infections or diseases caused by characteristic microorganisms
  - Revising and updating available technologies for the development of new methods to optimize the detection and identification of infectious agents and the detection of resistance to antiinfective agents (microbial, viral, fungal, and parasitic)
  - Developing new antiinfective therapies (microbial, viral, fungal, and parasitic)

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Chapter Review

1. The most specific and exclusive taxon used in the classification of microorganisms is:
   - **a.** Family
   - **b.** Order
   - **c.** Species
   - **d.** Genus
2. The process consisting of a series of methods designed to provide the microbiologist with relevant and useful clinical information about a microorganism is:
   - **a.** Classification
   - **b.** Identification
   - **c.** Organization
   - **d.** Taxonomy
3. Classification and naming of organisms are useful in diagnostic microbiology for all of the following *except:*
   - **a.** Providing standardized groupings for identification
   - **b.** Standardized groupings are always genotypically similar at 0.98%
   - **c.** Standardized groupings share similar phenotypic traits
   - **d.** The ability of organisms within a standard group may be identified using similar methods
4. Which of the following is *not* a correct use of the binomial nomenclature system? (Select all that apply.)
   - **a.** *Staphylococcus Aureus*
   - **b.** *S. aureus*
   - **c.** *Staphylococcus aureus*
   - **d.** Staphylococcus aureus
5. **Labeling:** *Label each of the following characteristics as either a phenotypic (P) or a genotypic (G) characteristic.*
   _____ Color of growth on artificial media
   _____ The presence of an antibiotic-resistance DNA sequence
   _____ The shape of the bacterial cell
   _____ The arrangement of the bacterial cells on a microscope slide
   _____ The ability of the organism to ferment lactose
6. Mass spectrometry is a technique used to separate and identify the spectrum of proteins and peptides that are expressed by microorganisms. This method is considered a _____method for the characterization and classification of organisms.
   - **a.** phenotypic
   - **b.** chemotaxonomic
   - **c.** genotypic
   - **d.** polyphasic
7. Which of the following methods would be considered chemotaxonmic?
   - **a.** Fatty acid analysis
   - **b.** Protein mass spectrometry
   - **c.** Cell wall composition
   - **d.** All of the answers are correct

2

Bacterial Genetics, Metabolism, and Structure

OBJECTIVES

1. Describe the basic structure and organization of prokaryotic (bacterial) chromosomes, including number, relative size, and cellular location.
2. Outline the basic processes and essential components required for information transfer in replication, transcription, translation, and regulatory mechanisms.
3. Define mutation, recombination, transduction, transformation, and conjugation.
4. Describe how genetic alterations and diversity provide a mechanism for the evolution and survival of microorganisms.
5. Differentiate environmental oxygenation and final electron acceptors (aerobes, facultative anaerobes, and strict anaerobes) in the formation of energy.
6. Compare and contrast the key structural elements, cellular organization, and types of organisms classified as prokaryotic and eukaryotic.
7. State the functions and biologic significance of the following cellular structures: the outer membrane, cell wall, periplasmic space, cytoplasmic membrane, capsule, fimbriae, pili, flagella, nucleoid, and cytoplasm.
8. Differentiate the organization and chemical composition of the cell envelope for a gram-positive and a gram-negative bacterium.

Microbial genetics, metabolism, and structure are the keys to microbial viability and survival. These processes involve numerous pathways that are widely varied, often complicated, and frequently interactive. Essentially, survival requires nutrients and energy to fuel the synthesis of materials necessary to grow, propagate, and carry out other metabolic processes (Fig. 2.1). Although the goal of survival is the same for all organisms, the strategies microorganisms use to accomplish this vary substantially.

Knowledge regarding genetic, metabolic, and structural characteristics of microorganisms provides the basis for understanding almost every aspect of diagnostic microbiology, including:

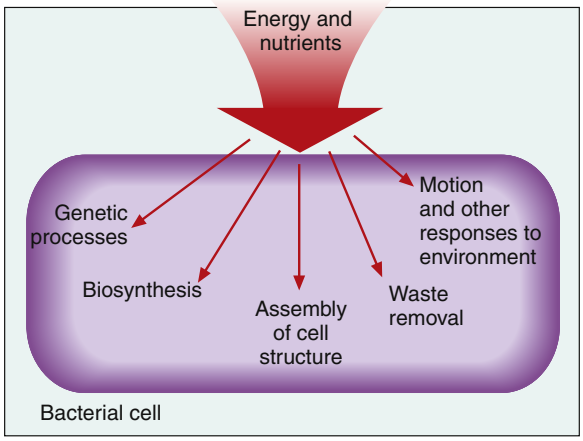
- The mechanisms by which microorganisms cause disease
- The development and implementation of techniques for microbial detection, cultivation, identification, and characterization
- Antimicrobial action and resistance
- The development and implementation of tests for the detection of antimicrobial resistance
- Potential strategies for disease therapy and control of microorganisms

Microorganisms vary significantly in their genomic and metabolic pathways and therefore structure. A detailed consideration of these differences is beyond the scope of this textbook. Therefore, a generalized description of bacterial systems is used as a model to discuss microbial physiology and structure. Information regarding characteristics of fungi, parasites, and viruses can be found in subsequent chapters for each specific taxonomic group.

Bacterial Genetics

Genetics, the process of heredity and variation, is the starting point from which all other cellular pathways, functions, and structures originate. The ability of a microorganism to maintain viability, adapt, multiply, and cause disease is determined by the organism's genetic composition. The three major aspects of microbial genetics that require discussion include:

- The structure and organization of genetic material
- Replication and expression of genetic information
- The mechanisms by which genetic information is altered and exchanged among bacteria



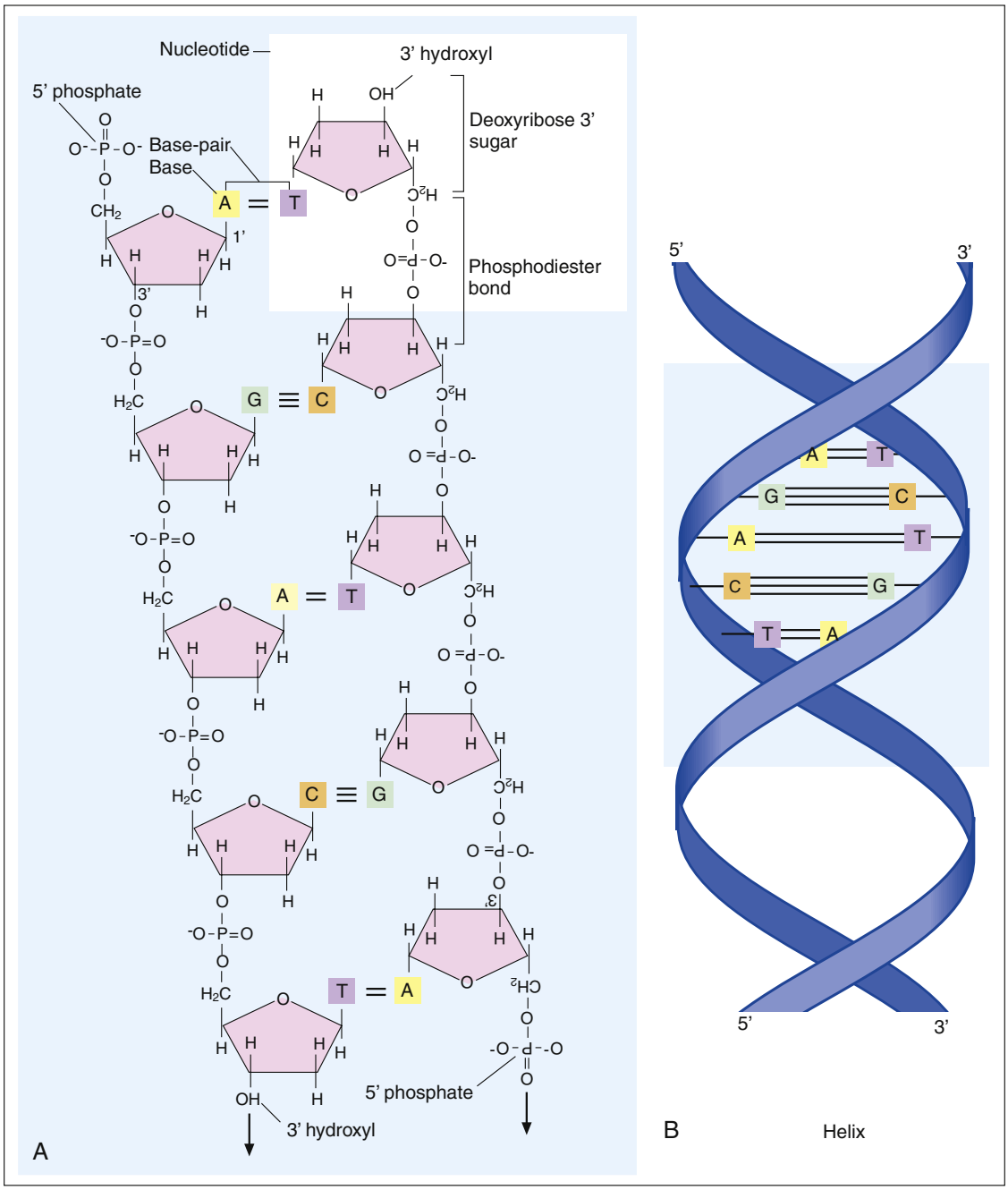
• **Fig. 2.1** General overview of bacterial cellular processes.

Nucleic Acid Structure and Organization

For all living entities, hereditary information resides or is encoded in nucleic acids. The two major classes of nucleic acids are **deoxyribonucleic acid (DNA),** which is the most common macromolecule that encodes genetic information, and **ribonucleic acid (RNA).** In some forms, RNA encodes genetic information for various viruses; in other forms, RNA plays an essential role in several of the genetic processes in prokaryotic and eukaryotic cells, including the regulation and transfer of information. Prokaryotic, or prenuclear, organisms do not have membrane-bound organelles, and the cells' genetic material is therefore not enclosed in a nucleus. Eukaryotic, or "true nucleus," organisms have the genetic material enclosed in a nuclear envelope.

Nucleotide Structure and Sequence

DNA consists of deoxyribose sugars connected by phosphodiester bonds (Fig. 2.2A). The bases that are covalently linked to each deoxyribose sugar are the key to the **genetic code** within the DNA molecule. The four nitrogenous bases



• **Fig. 2.2** (A) Molecular structure of deoxyribonucleic acid (DNA) depicting nucleotide structure, phosphodiester bonds connecting nucleotides, and complementary base pairing (*A,* adenine; *T,* thymine; *G,* guanine; *C,* cytosine) between antiparallel nucleic acid strands. (B) 5′ and 3′ antiparallel polarity and double-helix configuration of DNA.

include two **purines**, adenine (A) and guanine (G), and the two **pyrimidines,** cytosine (C) and thymine (T) (Fig. 2.3). In RNA, uracil replaces thymine. The combined sugar, phosphate, and a base form a single unit referred to as a **nucleotide** (adenosine triphosphate [ATP], guanine triphosphate [GTP], cytosine triphosphate [CTP], and thymine triphosphate [TTP] or uridine triphosphate [UTP]). DNA and RNA are nucleotide polymers (i.e., chains or strands), and the order of bases along a DNA or RNA strand is known as the **base sequence.** This sequence provides the information that codes for the proteins that will be synthesized by microbial cells; that is, the sequence is the genetic code.

Deoxyribonucleic Acid Molecular Structure

The intact DNA molecule is composed of two nucleotide polymers. Each strand has a 5′ (prime) phosphate and a 3′ (prime) hydroxyl terminus (Fig. 2.2A). The two strands run **antiparallel,** with the 5′ of one strand opposed to the 3′ terminal of the other. The strands are also complementary. This adherence to A-T and G-C base pairing results in a double-stranded DNA (dsDNA) molecule (double helix). The two antiparallel single strands of DNA form a "twisted ladder" structure (Fig. 2.2B). In addition, the dedicated base pairs (bp) provide the format for consistent replication and expression of the genetic code. In contrast to DNA, which carries the genetic code, RNA rarely exists as a double-stranded molecule. There are four major types of RNA (**messenger RNA [mRNA], transfer RNA [tRNA],** and **ribosomal RNA [rRNA]**) along with a variety of **noncoding RNA (ncRNA)** molecules such as microRNA (miRNA) that play key roles in posttranscriptional regulation of gene expression.

Genes and the Genetic Code

A DNA sequence that encodes a specific product (RNA or protein) is defined as a **gene.** Thousands of genes in an organism encode messages or blueprints for the production of one or more proteins and RNA products that play essential metabolic roles in the cell. All the genes in an organism comprise the organism's **genome.** The genome of a microorganism includes the chromosomes and the **mobilome** (extrachromosomal mobile genetic elements). The size of a gene and an entire genome are usually expressed in the number of bp present (e.g., kilobases [103 bases], megabases [106 bases]).

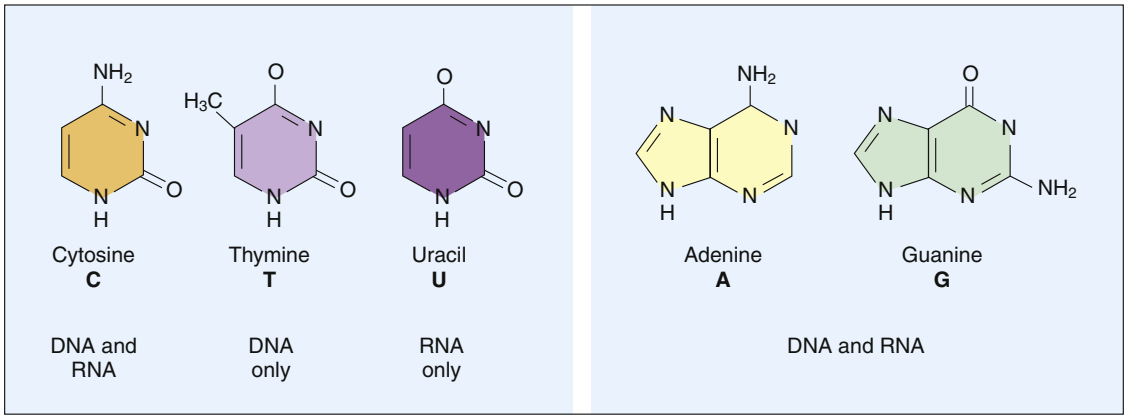
Certain genes are widely distributed among various organisms, whereas others are limited to a particular species. In addition, the base pair sequence for individual genes may be highly conserved (i.e., show limited sequence differences among different organisms) or be widely variable. As discussed in Chapter 8, these similarities and differences in genetic content and sequences are the basis for the development of molecular methods used to detect, identify, and characterize microorganisms.

Chromosomes

The genome is organized into discrete elements known as **chromosomes.** The set of genes within a given chromosome is arranged in a linear fashion, but the number of genes per chromosome is variable. Similarly, although the number of chromosomes per cell is consistent for a given species, this number varies considerably among species. For example, human cells contain 23 pairs (i.e., diploid) of chromosomes, whereas bacteria contain a single, unpaired (i.e., haploid) chromosome.

Bacteria are classified as prokaryotes; therefore the chromosome is not located in a membrane-bound organelle (i.e., **nucleus**). The bacterial chromosome contains the genes essential for viability and exists as a double-stranded, closed, circular macromolecule. The molecule is extensively folded and twisted (i.e., supercoiled) to fit within the confined space of the bacterial cell. The linearized, unsupercoiled chromosome of the bacterium *Escherichia coli* is approximately 130 μm long, but it fits within a cell 1 × 3 μm; this attests to the extreme compact structure of the supercoiled bacterial chromosome. For genes in the compacted chromosome to be expressed and replicated, unwinding or relaxation of the molecule is required.

In contrast to the bacterial chromosome, the chromosomes of parasites and fungi number more than one per cell, are linear, and are housed within a membrane-bound organelle (the nucleus) of the cell. This difference is a major criterion for classifying bacteria as prokaryotes and fungi and parasites as eukaryotes. The genetic makeup of a virus may consist of DNA or RNA contained within a protein coat rather than a cell.



• **Fig. 2.3** Molecular structure of nucleic acid bases. *DNA,* Deoxyribonucleic acid; *RNA,* ribonucleic acid. Pyrimidines: cytosine, thymine, and uracil. Purines: adenine and guanine.

Nonchromosomal Elements (Mobilome)

Although the bacterial chromosome represents the majority of a cell's genome, not all genes are confined to the chromosome. Many genes may also be located on **plasmids** and **transposable elements.** Both of these extrachromosomal elements are able to replicate and encode information for the production of various cellular products. Many of these elements replicate by integration into the host chromosome, whereas others, referred to as **episomes,** are capable of replication independently of the host chromosome. Although considered part of the bacterial genome, they are not as stable as the chromosome and may be lost during cellular replication, often without any detrimental effects on the viability of the cell.

Plasmids exist as double-stranded, closed, circular, autonomously replicating extrachromosomal genetic elements ranging in size from 1 to 2 kilobases up to 1 megabase or more. The number of plasmids per bacterial cell varies extensively, and each plasmid is composed of several genes. Some genes encode products that mediate plasmid replication and transfer between bacterial cells, whereas others encode products that provide a specialized function, such as a determinant of antimicrobial resistance or a unique metabolic process. Unlike most chromosomal genes, plasmid genes do not usually encode for products essential for viability. Plasmids, in whole or in part, may also become incorporated into the chromosome.

Transposable elements are pieces of DNA that move from one genetic element to another, from plasmid to chromosome or vice versa. Unlike plasmids, many are unable to replicate independently and do not exist as separate entities in the bacterial cell. The two types of transposable elements are the **simple transposon** or **insertion sequence (IS)** and the **composite or complex transposon.** Insertion sequences are limited to containing the genes that encode information required for movement from one site in the genome to another. Composite transposons are cassettes (grouping of genes) flanked by insertion sequences. The internal gene embedded in the IS encodes for an accessory function, such as antimicrobial resistance. Plasmids and transposable elements coexist with chromosomes in the cells of many bacterial species. These extrachromosomal elements play a key role in the exchange of genetic material throughout the bacterial microbiosphere, including genetic exchange among clinically relevant bacteria.

DNA Replication

Replication

Bacteria multiply by **binary fission** (a form of cell division), resulting in the production of two daughter cells from one parent cell. As part of this process, the genome must be replicated and each daughter cell receives an identical copy of functional DNA. **Replication** is a complex process mediated by various enzymes, such as DNA polymerase and cofactors; replication must occur quickly and accurately. For descriptive purposes, replication may be considered in four stages (Fig. 2.4):

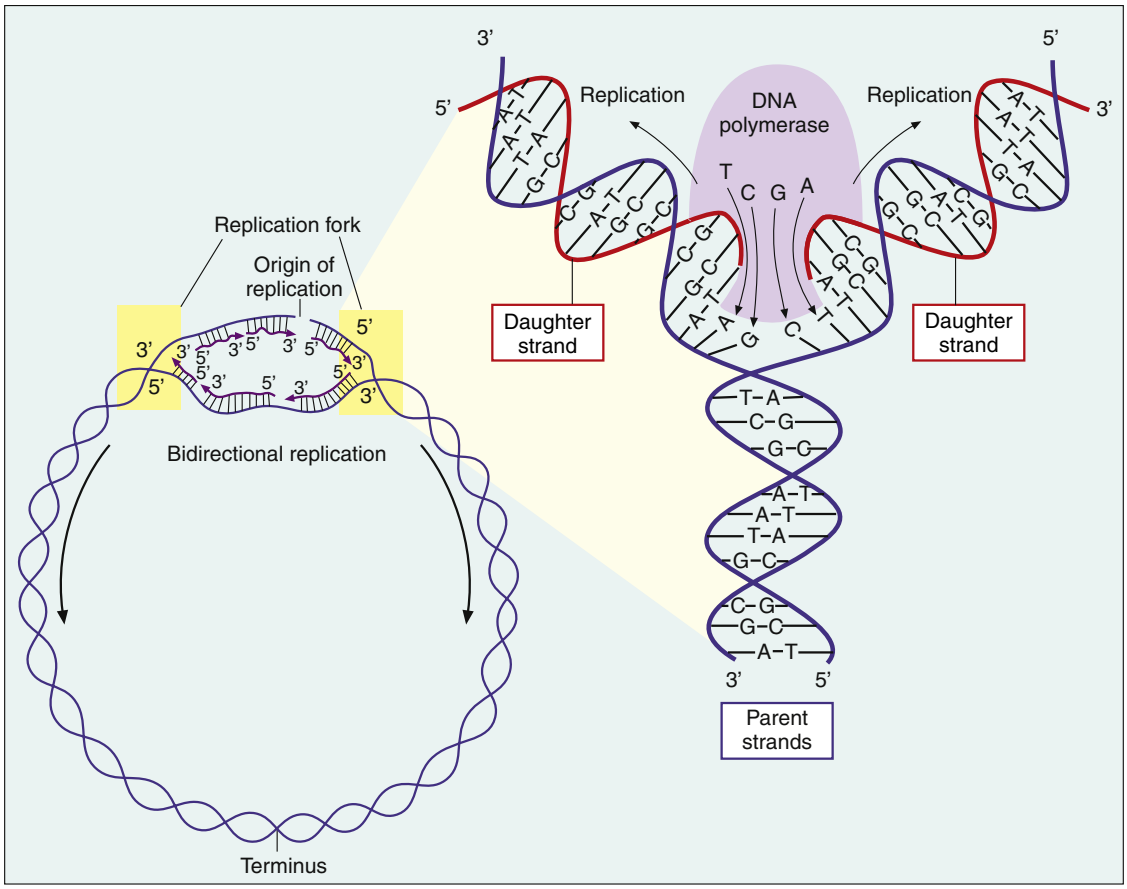
1. Unwinding or relaxation of the chromosome's supercoiled DNA
2. Separation of the complementary strands of the parental DNA. Each strand may serve as a **template** (i.e., pattern) for synthesis of new DNA strands, referred to as **semiconservative replication**
3. Synthesis of the new (i.e., daughter) DNA strands
4. Termination of replication, releasing two identical chromosomes, one for each daughter cell

Relaxation of supercoiled chromosomal DNA is required, which permits the enzymes and cofactors involved in replication access to the DNA molecule at the site where the replication process will originate (i.e., origin of replication). The **origin of replication** (a specific sequence of approximately 300 bp) is recognized by several initiation proteins, followed by the separation of the complementary strands of parental DNA. Each parental strand serves as a template for the synthesis of a new complementary daughter strand. The site of active replication is referred to as the **replication fork;** two bidirectional forks are involved in the replication process. Each replication fork moves through the parent DNA molecule in opposite directions as a bidirectional process. Activity at each replication fork involves different cofactors and enzymes, with **DNA polymerase** playing a central role. Using each parental strand as a template, DNA polymerase adds nucleotide bases to each growing daughter strand in a sequence that is complementary to the base sequence of the template (parent) strand. The complementary bases of each strand are then held together by hydrogen bonding between nucleotides and the hydrophobic nature of the nitrogenous bases. The new nucleotides can be added only to the 3′ hydroxyl end of the growing strand. The synthesis for each daughter strand occurs in the 5′ to 3′ direction.

Termination of replication occurs when the replication forks meet. The result is two complete chromosomes, each containing two complementary strands, one of parental origin and one newly synthesized daughter strand. Although the time required for replication can vary among bacteria, the process generally takes approximately 20 to 40 minutes in rapidly growing bacteria such as *E. coli.* The replication time for a particular bacterial strain can vary depending on environmental conditions, such as the availability of nutrients or the presence of toxic substances (e.g., antimicrobial agents).

Expression of Genetic Information

Gene **expression** is the processing of information encoded in genetic elements (i.e., chromosomes, plasmids, and transposons) that results in the production of biochemically functional molecules, including RNA and proteins. The overall process of gene expression is composed of two steps, **transcription** and **translation.** Gene expression



• **Fig. 2.4** Bacterial deoxyribonucleic acid (DNA) replication with bidirectional movement of two replication forks from the origin of replication. Each parent strand serves as a template for production of a complementary daughter strand and, eventually, two identical chromosomes.

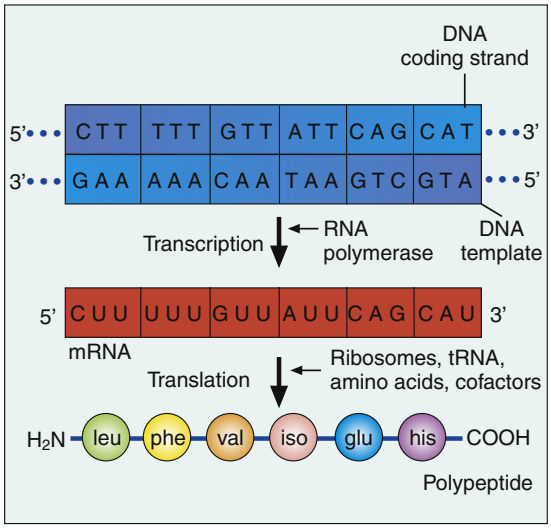
requires various components, including a DNA template representing a single gene or cluster of genes, various enzymes and cofactors, and RNA molecules of specific structure and function.

Transcription

Gene expression begins with transcription. During transcription the DNA base sequence of the gene (i.e., the genetic code) is converted into an mRNA molecule that is complementary to the gene's DNA sequence (Fig. 2.5). Usually only one of the two DNA strands (**sense strand**) encodes for a functional gene product. This same strand is the template for mRNA synthesis.

RNA polymerase is the enzyme central to the transcription process. The enzyme is composed of four protein subunits and a sigma (σ) factor. **Sigma factors** are required for the RNA polymerase to identify the appropriate site on the DNA template where transcription of mRNA is initiated. This initiation site is also known as the **promoter sequence**. The remainder of the enzyme functions to unwind the dsDNA at the promoter sequence and use the DNA strand as a template to sequentially add ribonucleotides (ATP, GTP, UTP, and CTP) to form the growing mRNA strand.

Transcription proceeds in a 5′ to 3′ direction. However, in mRNA, the TTP of DNA is replaced with UTP. TTP



• **Fig. 2.5** Overview of gene expression components: transcription for production of messenger ribonucleic acid (mRNA) and translation for production of a polypeptide (protein). *DNA,* Deoxyribonucleic acid; *RNA,* ribonucleic acid; *tRNA,* transfer RNA.

contains thymine, and UTP contains uracil. Both molecules contain a heterocyclic ring and are classified as pyrimidines. During synthesis and modification of these molecules, a portion of the molecules are dehydroxylated, forming a

2′-deoxynucleotide monophosphate. The dehydroxylated uracil monophosphate (dUMP) is then methylated, forming dehydroxylated thymine monophosphate (dTMP). After phosphorylation, thymine is found only in the final state as deoxythymidine and therefore cannot be incorporated into an RNA molecule. Synthesis of the single-stranded mRNA product ends when specific nucleotide base sequences on the DNA template are encountered. Termination of transcription may be facilitated by a rho (a prokaryotic protein) cofactor or an intrinsic termination sequence. Both of these mechanisms disrupt the mRNA-RNA polymerase template DNA complex.

In bacteria, the mRNA molecules that result from the transcription process are **polycistronic;** that is, they encode for several gene products. Polycistronic mRNA may encode several genes whose products (proteins) are involved in a single or closely related cellular function. When a cluster of genes is under the control of a single promoter sequence, the gene group is referred to as an **operon.**

The transcription process not only produces mRNA but also tRNA, rRNA, and regulatory non coding (ncRNA) molecules. All types of RNA molecules have key roles in protein synthesis. To initiate transcription, accessory factors are needed to localize the RNA polymerase to the promoter upstream of the coding sequence. In bacteria, the σ factor binds to the RNA polymerase and recognizes the gene-specific promotor. In some bacteria a small regulatory RNA (sRNA), 6S RNA, binds the sigma factor to repress transcription in the late stationary phase of bacterial growth. The 6S RNA binds and forms a bulge or loop. The loop serves as an RNA-dependent site for RNA synthesis. The RNA synthesized from the loop is referred to as pRNA. When sufficient pRNA is produced, it causes the 6S RNA to detach from the promotor, permitting transcription to continue.

Transfer RNA (tRNA) binds to the A site in the ribosome and delivers the appropriate amino acid during elongation. However, tRNAs exist in many more diverse forms than once believed. In bacteria, the initiation codon codes for an N-formylmethionine. This modified amino acid is never placed inside the coding sequence of a bacterial protein. In other words, there are two forms of tRNA that are produced in bacteria that are cable of carrying methionine. One is the initiator $tRNA^{Met}$ and the other is the elongation $tRNA^{Met}$. The elongation $tRNA^{Met}$ binds to the A site of the ribosome, whereas the initiation $tRNA^{Met}$ is capable of binding only to the P site within the ribosome. The binding of the elongation-specific tRNA is controlled by transcription elongation factor 1.

Ribosomal RNA, specifically the 16S rRNA, has historically been associated with classification of organisms based on evolutionary relatedness. The 16S rRNA is present in all organisms and is responsible for catalyzing the peptidyl transferase reaction during protein synthesis. A very small portion of the molecule is capable of undergoing genetic changes without deleterious effects to the transcription process, providing a means to monitor the evolutionary development of bacterial species.

In addition to the differences in tRNA specificity, bacteria have developed numerous mechanisms to regulate gene transcription and respond to the environment, including transcriptional and posttranscriptional regulation. Many sensory and regulatory RNA molecules have now been identified that serve as RNA thermosensors and riboswitches. These molecules may undergo structural alterations during temperature changes or serve as antisense RNAs and sRNAs that bind to either nucleic acid–binding proteins modulating their activity or directly to mRNA sequences to suppress and alter gene expression. This reversible regulation is clearly evident in the expression of virulence genes in many known pathogens including *E. coli, Shigella* spp., and *Yersinia* spp. The global changes of RNA expression within the transcriptome of a pathogenic bacteria allows the organism to rapidly adjust to changes in the environment associated with temperature, ionic conditions, oxygen conditions, pH, calcium, iron, and other metals to maintain growth and survival.

Translation

The next phase in gene expression, translation, involves protein synthesis. Through this process the genetic code in mRNA molecules is translated into specific amino acid sequences that are responsible for protein structure and function (Fig. 2.5).

The process of protein translation requires the use of a genetic alphabet or code. The code consists of triplets of nucleotide bases, referred to as **codons;** each codon encodes for a specific amino acid. Because there are 64 different codons for 20 amino acids, an amino acid can be encoded by more than one codon (Table 2.1). Each codon is specific for a single amino acid. The codon sequences in mRNA direct which amino acids are added and in what order. Translation ensures that proteins with proper structure and function are produced. Errors in the process can result in aberrant proteins that are nonfunctional, emphasizing the need for translation to be well controlled and accurate.

To accomplish the task of translation, intricate interactions between mRNA, tRNA, and rRNA are required. Sixty different standard types of tRNA molecules are responsible for transferring different amino acids from intracellular locations to the site of protein synthesis. These molecules, which have a structure that resembles an inverted *t,* contain one **anticodon** (sequence recognition site) for binding to specific codons (3-base sequences) on the mRNA molecule (Fig. 2.6). A second site binds specific amino acids, the building blocks of proteins. Each amino acid is joined to a specific tRNA molecule through the enzymatic activity of aminoacyl-tRNA synthetases. Transfer RNA molecules use the codons of the mRNA molecule as the template for precisely delivering a specific amino acid for polymerization. This process occurs in **ribosomes**, which are compact nucleoproteins, composed of rRNA and proteins. They are central to translation, assisting with the coupling of all required components and controlling the translational process.

TABLE 2.1 The Genetic Code as Expressed by Triplet-Base Sequences of Messenger Ribonucleic Acid[a]

Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid
UUU	Phenylalanine	CUU	Leucine	GUU	Valine	AUU	Isoleucine
UUC	Phenylalanine	CUC	Leucine	GUC	Valine	AUC	Isoleucine
UUG	Leucine	CUG	Leucine	GUG	Valine	AUG (start)[b]	Methionine
UUA	Leucine	CUA	Leucine	GUA	Valine	AUA	Isoleucine
UCU	Serine	CCU	Proline	GCU	Alanine	ACU	Threonine
UCC	Serine	CCC	Proline	GCC	Alanine	ACC	Threonine
UCG	Serine	CCG	Proline	GCG	Alanine	ACG	Threonine
UCA	Serine	CCA	Proline	GCA	Alanine	ACA	Threonine
UGU	Cysteine	CGU	Arginine	GGU	Glycine	AGU	Serine
UGC	Cysteine	CGC	Arginine	GGC	Glycine	AGC	Serine
UGG	Tryptophan	CGG	Arginine	GGG	Glycine	AGG	Arginine
UGA	None (stop signal)	CGA	Arginine	GGA	Glycine	AGA	Arginine
UAU	Tyrosine	CAU	Histidine	GAU	Aspartic	AAU	Asparagine
UAC	Tyrosine	CAC	Histidine	GAC	Aspartic	AAC	Asparagine
UAG	None (stop signal)	CAG	Glutamine	GAG	Glutamic	AAG	Lysine
UAA	None (stop signal)	CAA	Glutamine	GAA	Glutamic	AAA	Lysine

[a]The codons in deoxyribonucleic acid (DNA) are complementary to those given here. Thus U is complementary to the A in DNA, C is complementary to G, G to C, and A to T. The nucleotide on the left is at the 5′ end of the triplet.
[b]AUG codes for N-formylmethionine at the beginning of messenger ribonucleic acid (mRNA) in bacteria.
Modified from Brock TD, Madigan M, Martinko J, et al., eds. *Biology of Microorganisms*. Upper Saddle River, NJ: Prentice Hall; 2009.

Translation, diagrammatically shown in Fig. 2.6, involves three steps: **initiation, elongation,** and **termination.** After termination, bacterial proteins often undergo posttranslational modifications as a final step in protein synthesis.

Initiation begins with the association of ribosomal subunits, mRNA, formylmethionine (f-met) tRNA (carrying the initial amino acid of the protein to be synthesized), and various initiation factors (Fig. 2.6A). Assembly of the complex begins at a specific 3- to 9-base sequence (Shine-Dalgarno sequence) on the mRNA approximately 10 bp upstream of the AUG start codon. After the initial complex has been formed, addition of individual amino acids begins.

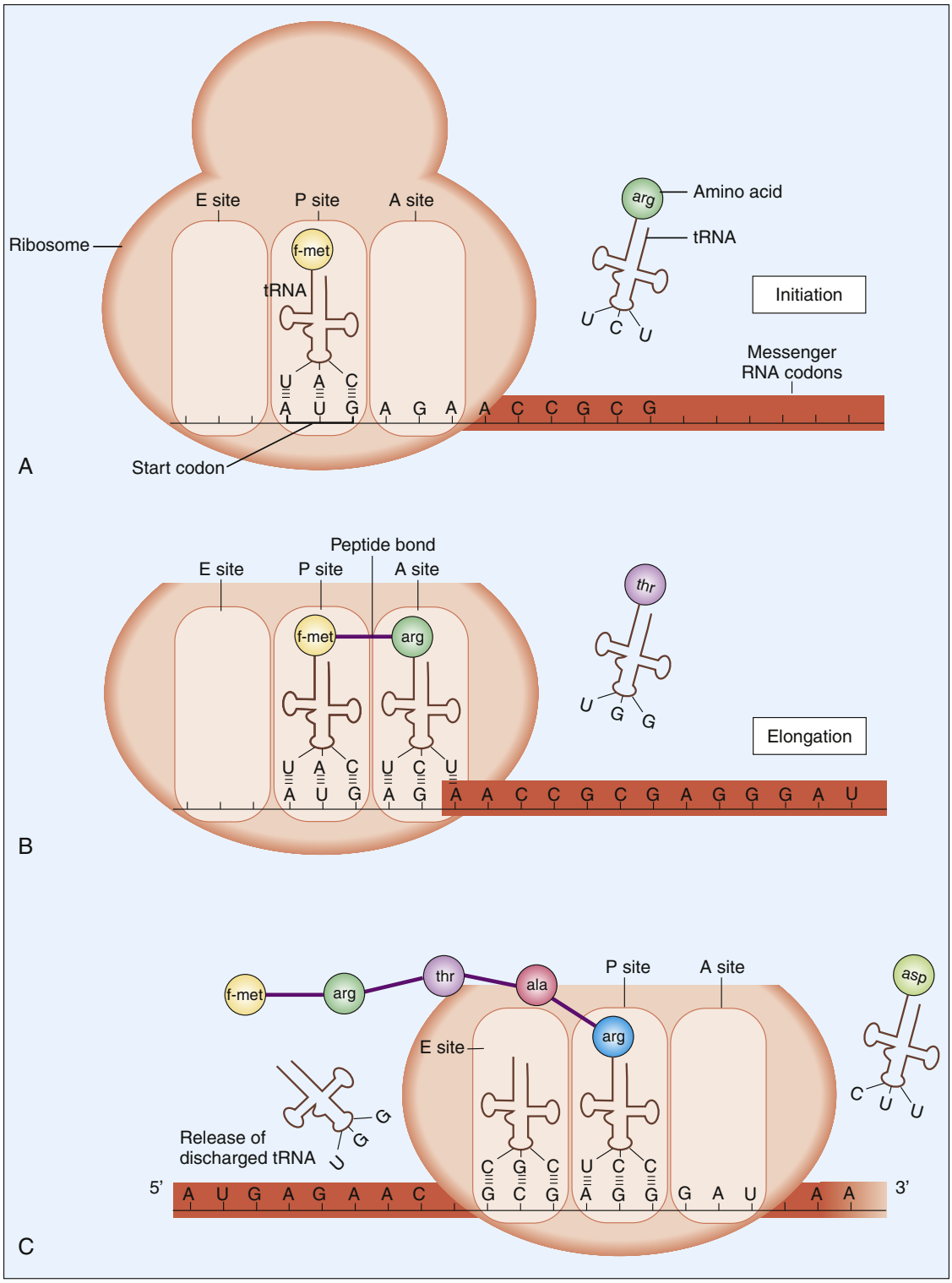
Elongation involves tRNAs and a host of elongation factors that mediate the addition of amino acids in a specific sequence dictated by the codon on the mRNA molecule (Fig. 2.6B and C and Table 2.1). As the mRNA molecule threads through the ribosome in a 5′ to 3′ direction, peptide bonds are formed between adjacent amino acids, still bound by their respective tRNA molecules in the peptide (P) and acceptor (A) sites of the ribosome. During the process, the forming peptide is moved to the P site, and the 5′ tRNA is released from the exit (E) site. This movement vacates the A site, which contains the codon specific for the next amino acid, so that the incoming tRNA–amino acid can join the complex (Fig. 2.6C).

Because multiple proteins encoded on an mRNA strand can be translated at the same time, multiple ribosomes may be simultaneously associated with one mRNA molecule. Such an arrangement is referred to as a **polysome;** its appearance resembles a string of pearls.

Termination, the final step in translation, occurs when the ribosomal A site encounters a stop or non sense codon that does not specify an amino acid (i.e., a "stop signal"; Table 2.1). At this point, the protein synthesis complex disassociates and the ribosomes are available for another round of translation. After termination, most proteins must undergo modification, such as folding or enzymatic trimming, so that protein function, transportation, or incorporation into various cellular structures can be accomplished. This process is referred to as **posttranslational modification.**

Regulation and Control of Gene Expression

The vital role that gene expression and protein synthesis play in the survival of cells dictates that bacteria judiciously



• **Fig. 2.6** Overview of translation in which messenger ribonucleic acid (mRNA) serves as the template for the assembly of amino acids into polypeptides. The three steps include initiation (A), elongation (B and C), and termination (not shown). *tRNA,* transfer RNA.

control these processes. The cell must regulate gene expression and control the activities of gene products so that a physiologic balance is maintained. Regulation and control are also key factors. These are highly complex mechanisms by which single-cell organisms are able to respond and adapt to environmental challenges, regardless of whether the challenges occur naturally or result from medical intervention (e.g., antibiotics).

Regulation occurs at one of three levels of information transfer from the gene expression and protein synthesis pathway: **transcriptional, translational,** or **posttranslational.** The most common is transcriptional regulation. Because

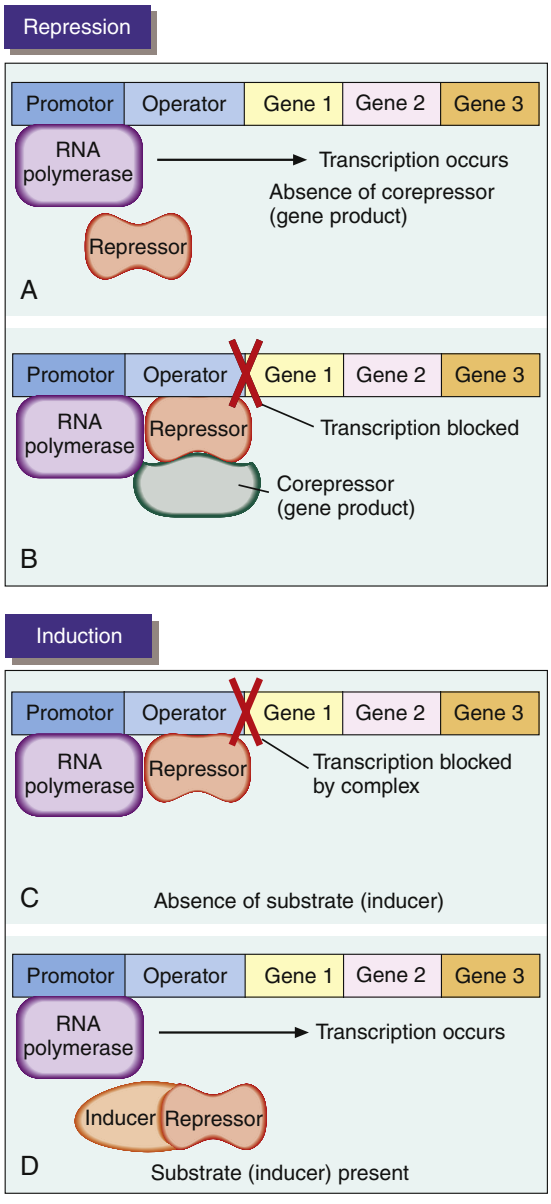
direct interactions with genes and their ability to be transcribed to mRNA are involved, transcriptional regulation is also referred to as **genetic control.** Genes that encode enzymes involved in **anabolic** processes (biosynthesis) and genes that encode enzymes for **catabolic** processes (biodegradation) are examples of genetic control.

In general, genes that encode anabolic enzymes for the synthesis of particular products are **repressed** (i.e., are not transcribed and therefore are not expressed) in the presence of the gene end product. This strategy prevents waste and overproduction of products that are already present in sufficient supply. In this system, the product acts as a corepressor that forms a complex with a repressor molecule. In the absence of corepressor product (i.e., gene product), transcription occurs (Fig. 2.7A). When present in sufficient quantity, the product forms a complex with the repressor. The complex then binds to a specific base region of the gene sequence known as the **operator region** (Fig. 2.7B). This binding blocks RNA polymerase progression from the promoter sequence and inhibits transcription. As the supply of product (corepressor) dwindles, an insufficient amount remains to form a complex with the repressor. The operator region is no longer bound to the repressor molecule. Transcription of the genes for the anabolic enzymes commences and continues until a sufficient supply of end product is again available.

In contrast to repression, genes that encode catabolic enzymes are usually **induced;** that is, transcription occurs when the substrate to be degraded by enzymatic action is present. Production of degradative enzymes in the absence of substrates would be a waste of cellular energy and resources. When the substrate is absent in an inducible system, a repressor binds to the operator sequence of the DNA and blocks transcription of the gene for the degradative enzyme (Fig. 2.7C). In the presence of an inducer, which often is the target substrate for degradation, a complex is formed between the inducer and the repressor that results in the release of the repressor from the operator site, allowing transcription of the genes encoding the specific catabolic enzyme (Fig. 2.7D).

Certain genes are not regulated; that is, they are not under the control of inducers or repressors. These genes are referred to as **constitutive.** Because they usually encode for products that are essential for viability under almost all growth and environmental conditions, these genes are continuously expressed. In addition, not all regulation occurs at the genetic level (i.e., transcriptional regulation). For example, the production of some enzymes may be controlled at the protein synthesis (i.e., translational) level. The activities of other enzymes that have already been synthesized may be regulated at a posttranslational level; that is, certain catabolic or anabolic metabolites may directly interact with enzymes either to increase or to decrease their enzymatic activity.

Among different bacteria and even different genes in the same bacterium, the mechanisms by which inducers and corepressors are involved in gene regulation vary widely. Furthermore, bacterial cells have mechanisms to detect



• **Fig. 2.7** Transcriptional control of gene expression. (A and B) Gene repression. (C and D) Induction. *RNA,* Ribonucleic acid.

environmental changes. These changes can generate signals that interact with the gene expression mechanism, ensuring that appropriate products are made in response to the environmental change. In addition, several complex interactions between different regulatory systems are found within a single cell. Such diversity and interdependence are necessary components of metabolism that allow an organism to respond to environmental changes in a rapid, well-coordinated, and appropriate way.

Genetic Exchange and Diversity

In eukaryotic organisms, genetic diversity is achieved by sexual reproduction, which allows for the mixing of genomes through genetic exchange. Bacteria multiply by simple binary cell division in which two identical daughter

cells result by division of one parent cell. Each daughter cell receives the full genetic complement contained in the original parent cell. This process does not allow for the mixing of genes from other cells and leaves no means of achieving genetic diversity among bacterial progeny. Without genetic diversity and change, the essential ingredients for evolution are lost. However, microorganisms have been on earth for billions of years, and microbiologists have witnessed their ability to change as a result of exposure to chemicals (i.e., antibiotics) and environmental conditions (i.e., temperature or oxygenation). It is evident that these organisms are fully capable of evolving and altering their genetic composition.

Genetic alterations and diversity in bacteria are accomplished by three basic mechanisms: **mutation, genetic recombination,** and **genetic exchange,** with or without recombination. Throughout diagnostic microbiology and infectious diseases, there are numerous examples of the effect these genetic alteration and exchange mechanisms have on clinically relevant bacteria and the management of the infections they cause.

Mutation

Mutation is defined as an alteration in the original nucleotide sequence of a gene or genes within an organism's genome (i.e., a change in the organism's genotype). This alteration may involve a single DNA base in a gene, an entire gene, or several genes. Mutational changes in the sequence may arise spontaneously, perhaps by an error made during DNA replication. Alternatively, mutations may be induced by **mutagens** (i.e., chemical or physical factors) in the environment or by biologic factors, such as the introduction of foreign DNA into the cell. Alterations in the DNA base sequence can result in changes in the base sequence of mRNA during transcription. This, in turn, can affect the types and sequences of amino acids that will be incorporated into the protein during translation.

Depending on the site and extent of the mutation, various outcomes may affect the physiologic functions of the organism. For example, a mutation may be so devastating that it is lethal to the organism; therefore the mutation "dies" along with the organism. In another instance, the mutation may be silent so that no changes are detected in the organism's **phenotype** (i.e., observable properties). Alternatively, the mutation may result in a noticeable alteration in the organism's phenotype, and the change may provide the organism with a survival advantage. This outcome, in Darwinian terms, is the basis for prolonged survival and evolution. Nonlethal mutations are considered stable if they are passed on from one generation to another as an integral part of the cell's genotype (i.e., genetic composition). In addition, genes that have undergone stable mutations may also be transferred to other bacteria by one of the mechanisms of genetic exchange. In other instances, the mutation may be lost as a result of cellular repair mechanisms capable of restoring the original genotype and phenotype, or it may be lost spontaneously during subsequent cycles of DNA replication.

Genetic Recombination

Besides mutations, bacterial genotypes can be altered through **recombination.** In this process, a segment of DNA originating from one bacterial cell (i.e., the donor) enters a second bacterial cell (i.e., the recipient) and is exchanged with a DNA segment of the recipient's genome. This is also referred to as **homologous recombination,** because the pieces of DNA that are exchanged usually have extensive homology or similarities in their nucleotide sequences. Recombination involves a number of binding proteins, with the bacterial recombinase protein (RecA) playing a central role (Fig. 2.8A). RecA is capable of binding single-stranded DNA (ssDNA) to the complementary dsDNA, providing a mechanism for DNA repair and recombination to occur. After recombination, the recipient DNA consists of one original, unchanged strand and a second strand from the donor DNA fragment that has been recombined.

Recombination is a molecular event that occurs frequently in many varieties of bacteria, including most of the clinically relevant species, and it may involve any portion of the organism's genome. However, the recombination event may go unnoticed unless the exchange of DNA results in a distinct alteration in the phenotype. Nonetheless, recombination is a major means by which bacteria may achieve genetic diversity.

Genetic Exchange

An organism's ability to undergo recombination depends on the acquisition of "foreign" DNA from a donor cell. The three mechanisms by which bacteria physically exchange DNA are **transformation, transduction,** and **conjugation.**

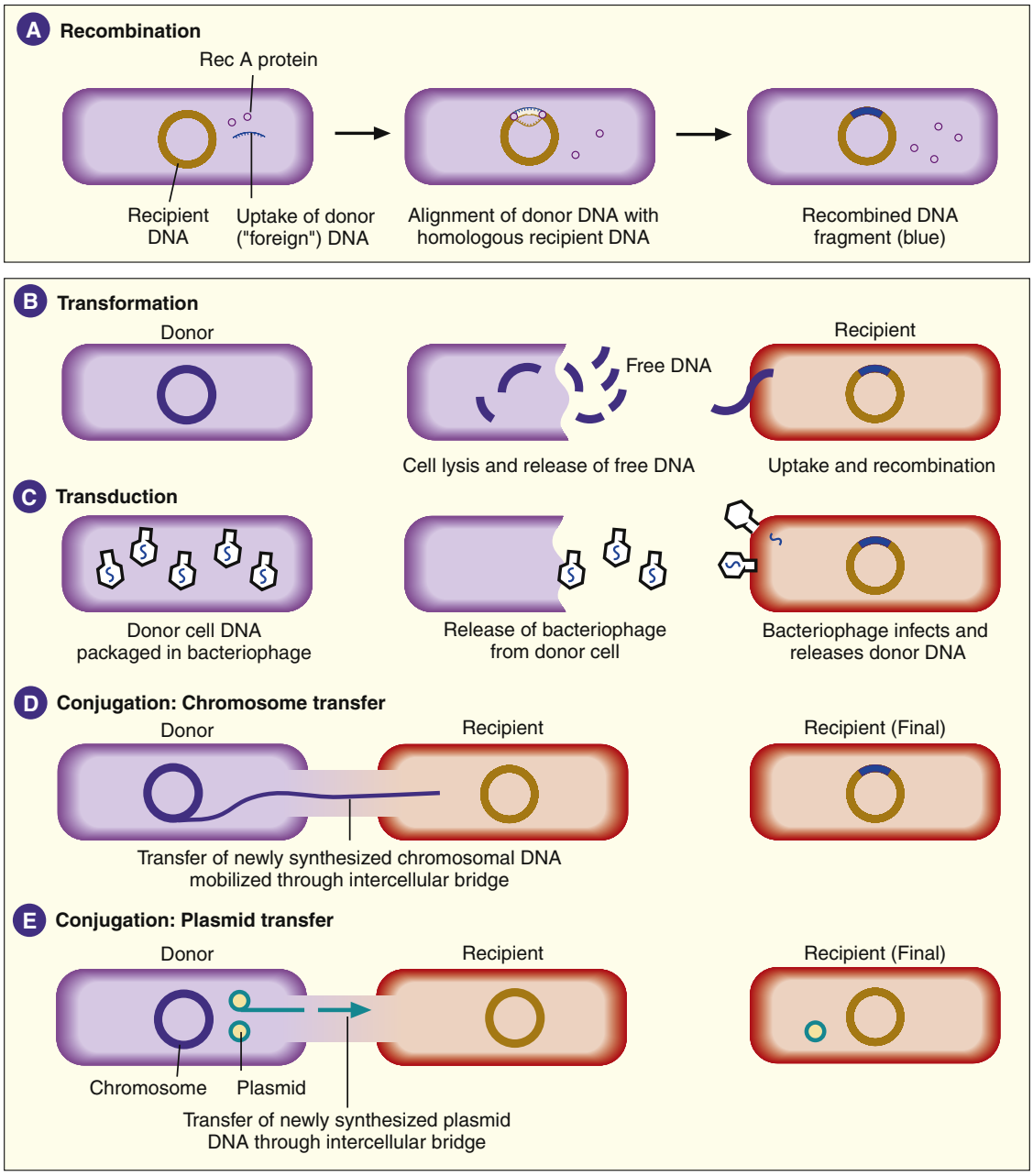
Transformation

Transformation involves recipient cell uptake of naked (free) DNA released into the environment when another bacterial cell (i.e., the donor) dies and undergoes lysis (Fig. 2.8B). This genomic DNA exists as fragments in the environment. Certain bacteria are able to take up naked DNA from their surroundings; that is, they are able to undergo transformation. Such bacteria are said to be **competent.** Among the bacteria that cause human infections, competence is a characteristic commonly associated with members of the genera *Haemophilus, Streptococcus,* and *Neisseria.*

Once the donor DNA, usually as a single strand, gains access to the interior of the recipient cell, recombination with the recipient's homologous DNA can occur. The mixing of DNA between bacteria via transformation and recombination plays a major role in the development of antibiotic resistance and in the dissemination of genes that encode factors essential to an organism's ability to cause disease. In addition, genetic exchange by transformation is not limited to organisms of the same species, thus allowing important characteristics to be disseminated to a greater variety of medically important bacteria.

Transduction

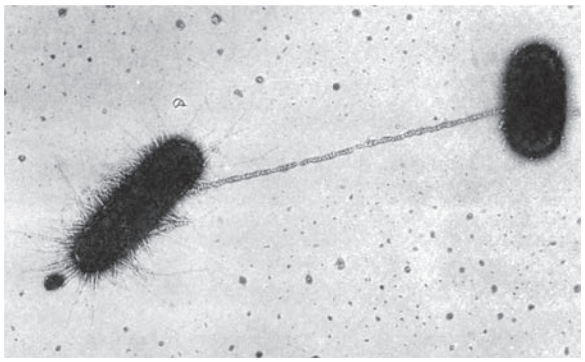
Transduction is a second mechanism by which DNA from two bacteria may come together in one cell, thus allowing for recombination (Fig. 2.8C). This process is mediated



• **Fig. 2.8** (A) Genetic recombination. The mechanisms of genetic exchange between bacteria are transformation (B), transduction (C), and conjugational transfer of chromosomal (D) and plasmid (E) deoxyribonucleic acid *(DNA)*.

through viruses capable of infecting bacteria (i.e., **bacteriophages**). In their "life cycle," these viruses integrate their DNA into the bacterial cell's chromosome, where viral DNA replication and expression occur. When the production of viral products is complete, viral DNA is excised (cut) from the bacterial chromosome and packaged within a protein coat. The excision process is not always accurate, resulting in the removal of genetic material that contains both the bacterial and viral DNA. The newly formed recombinant virion (virus particle), along with the additional multiple virions, is released when the infected bacterial cell lyses.

The bacterial DNA may be randomly incorporated with viral DNA (**generalized transduction**), or it may be incorporated along with specific adjacent viral DNA (**specialized transduction**). In generalized transduction, the viral DNA is inserted randomly into any area of the bacterial genome. However, in specialized transduction, the virus inserts into particular genes in an organism based on sequence specificity and resulting in a higher frequency of genetic material in those regions being transferred through recombination. In either case, when the virus infects another bacterial cell, it releases its DNA, which includes the previously incorporated bacterial donor DNA. The newly infected cell is then the recipient of donor DNA introduced by the bacteriophage, and recombination between DNA from two different cells occurs.



• **Fig. 2.9** Photomicrograph of an *Escherichia coli* sex pilus between a donor and a recipient cell. (From Brock TD, Madigan M, Martinko J, et al, eds. *Biology of Microorganisms.* Upper Saddle River, NJ: Prentice Hall; 2009.)

Conjugation

The third mechanism of DNA exchange between bacterial cells is conjugation. This process involves cell-to-cell contact and requires mobilization of the donor bacterium's chromosome or other mobile genetic element. The nature of intercellular contact is not well characterized in all bacterial species capable of conjugation. However, in *E. coli,* contact is mediated by a sex pilus (Fig. 2.9). The sex pilus originates from the donor and establishes a conjugative bridge that serves as the conduit for DNA transfer from donor to recipient cell. With intercellular contact established, mobilization of the genetic element is undertaken and involves DNA synthesis. One new DNA strand is produced by the donor and is passed to the recipient (Fig. 2.8D). The amount of DNA transferred depends on how long the cells are able to maintain contact, but usually only portions of the donor molecule are transferred. In any case the newly introduced DNA is then available to recombine with the recipient's genome.

In addition to chromosomal DNA, genes encoded in extrachromosomal genetic elements, such as plasmids and transposons, may be transferred by conjugation (Fig. 2.8E). Not all plasmids are capable of conjugative transfer, but for those that are, the donor plasmid usually is replicated so that the donor retains a copy of the plasmid transferred to the recipient. (See the discussion of the F plasmid in the section Cellular Appendages, later in the chapter.) Plasmid DNA may also become incorporated into the host cell's chromosome.

In contrast to plasmids, most transposons do not exist independently in the cell. Except when they are moving from one location to another, many transposons must be incorporated into the chromosome, plasmids, or both. These elements are often referred to as "jumping genes" because of their ability to change location within and even between the genomes of bacterial cells. **Transposition** is the process by which these genetic elements excise from one genomic location and insert into another. Transposons carry genes that have products that help to mediate the transposition process, in addition to genes that encode for other accessory characteristics, such as antimicrobial resistance. Homologous recombination between the genes of plasmids or transposons and the host bacterium's chromosomal DNA may occur.

Plasmids and transposons play a key role in genetic diversity and the dissemination of genetic information among bacteria. Many characteristics that significantly alter the activities of clinically relevant bacteria are encoded and disseminated on these elements. Furthermore, as shown in Fig. 2.10, the variety of strategies that bacteria can use to mix and match genetic elements provides them with a tremendous capacity to genetically adapt to environmental changes, including those imposed by human medical practices. A good example of this is the emergence and widespread dissemination of resistance to antimicrobial agents among clinically important bacteria. Bacteria have used their capacity for disseminating genetic information to establish resistance to many of the commonly prescribed antibiotics. (See Chapter 10 for more information about antimicrobial resistance mechanisms.)

Bacterial Metabolism

Fundamentally, bacterial metabolism involves all the cellular processes required for the organism's survival and replication. Familiarity with bacterial metabolism is essential to understand bacterial interactions with human host cells, the mechanisms bacteria use to cause disease, and the basis of diagnostic microbiology (i.e., the tests and strategies used for laboratory identification of infectious organisms). Because metabolism is an extensive and complicated topic, this section focuses on processes typical of medically relevant bacteria.

For the sake of clarity, metabolism is discussed in terms of four primary, but interdependent, processes: fueling, biosynthesis, polymerization, and assembly (Fig. 2.11).

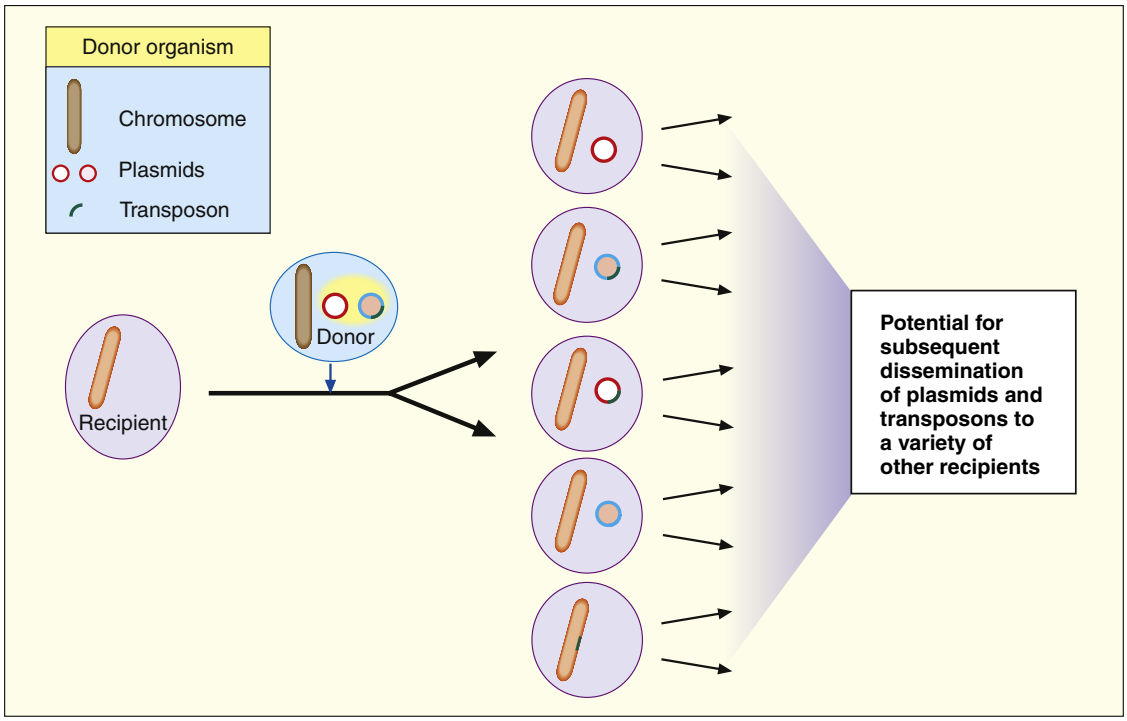
Fueling

Fueling is considered the utilization of metabolic pathways involved in the acquisition of nutrients from the environment, production of precursor metabolites, and energy production.

Acquisition of Nutrients

Bacteria use various strategies for obtaining essential nutrients from the external environment and transporting these substances into the cell's interior. For nutrients to be internalized, they must cross the bacterial cell wall and membrane. These complex structures help to protect the cell from environmental insults, maintain intracellular equilibrium, and transport substances into and out of the cell. Although some key nutrients (e.g., water, oxygen, and carbon dioxide) enter the cell by simple diffusion across the cell membrane, the uptake of other substances is controlled by membrane-selective permeability; still other substances use specific transport mechanisms.

Active transport is among the most common methods used for the uptake of nutrients such as certain sugars, most



• **Fig. 2.10** Pathways for bacterial dissemination of plasmids and transposons, together and independently.

amino acids, organic acids, and many inorganic ions. The mechanism, driven by an energy-dependent pump, involves carrier molecules embedded in the membrane portion of the cell structure. These carriers combine with the nutrients, transport them across the membrane, and release them inside the cell. Group translocation is another transport mechanism that requires energy but differs from active transport in that the nutrients being transported undergo chemical modification. Many sugars, purines, pyrimidines, and fatty acids are transported by this mechanism.

Production of Precursor Metabolites

Once inside the cell, many nutrients serve as the raw materials from which precursor metabolites for subsequent biosynthetic processes are produced. These metabolites, listed in Fig. 2.11, are produced through two central pathways: the Embden-Meyerhof-Parnas (EMP) pathway (glycolysis) and the tricarboxylic acid (TCA) cycle. The two major pathways and their relationship to one another are shown in Fig. 2.12; not shown are the alternative pathways (e.g., the Entner-Doudoroff and the pentose phosphate pathway) that play key roles in redirecting and replenishing the precursors as they are used in subsequent processes. The Entner-Doudoroff pathway catalyzes the degradation of gluconate and glucose. The gluconate is phosphorylated, dehydrated, and converted into pyruvate and glyceraldehyde, leading to ethanol production. Alternatively, the pentose phosphate pathway uses glucose to produce reduced nicotinamide adenine dinucleotide phosphate (NADPH), pentoses, and tetroses for biosynthetic reactions such as nucleoside and amino acid synthesis.

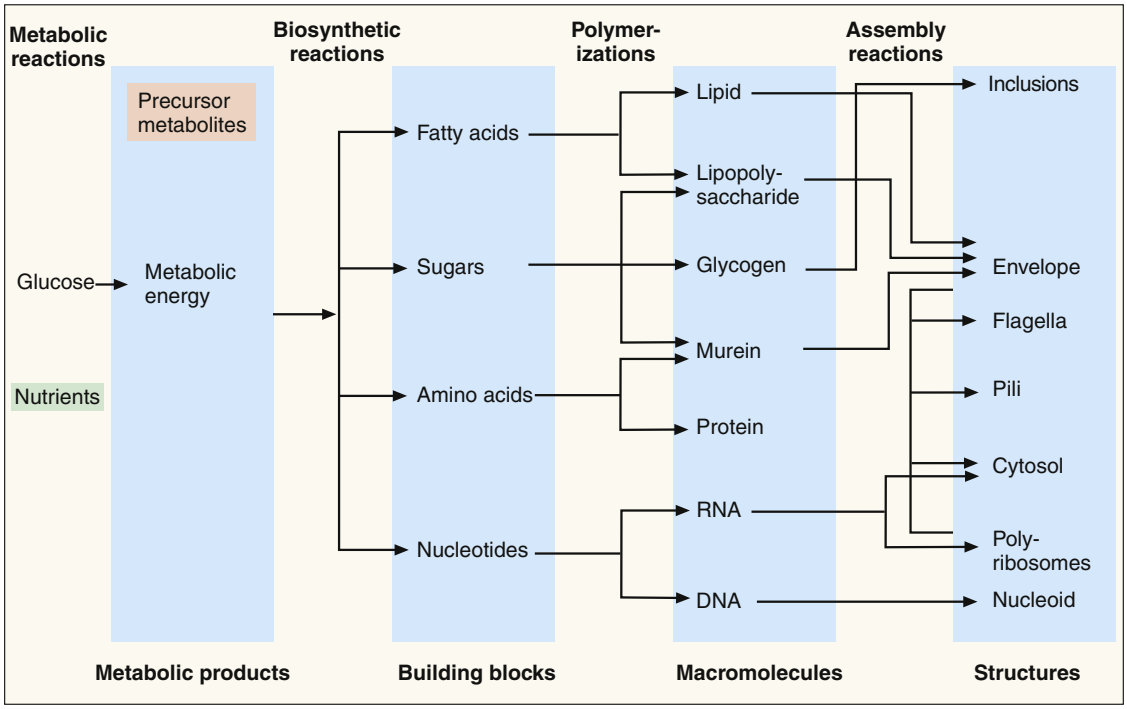
The production efficiency of a bacterial cell resulting from these precursor-producing pathways can vary substantially, depending on the growth conditions and availability of nutrients. This is an important consideration because the accurate identification of medically important bacteria has traditionally depended on methods that measure the presence of products and byproducts of these metabolic pathways.

Energy Production

The third type of fueling pathway is one that produces the energy required for nearly all cellular processes, including nutrient uptake and precursor production. Energy production is accomplished by the breakdown of chemical substrates (i.e., chemical energy) through the degradative process of catabolism coupled with oxidation-reduction reactions. In this process, the energy source molecule (i.e., substrate) is oxidized as it donates electrons to an electron-acceptor molecule, which is then reduced. The transfer of electrons is mediated through carrier molecules, such as nicotinamide-adenine-dinucleotide (NAD^+) and nicotinamide-adenine-dinucleotide-phosphate ($NADP^+$). The energy released by the oxidation-reduction reaction is transferred to phosphate-containing compounds, where high-energy phosphate bonds are formed. ATP is the most common of such molecules. The energy contained in this compound is eventually released by the hydrolysis of ATP under controlled conditions. The release of this chemical energy, coupled with enzymatic activities, specifically catalyzes each biochemical reaction in the cell and drives cellular reactions.

Precursor metabolites

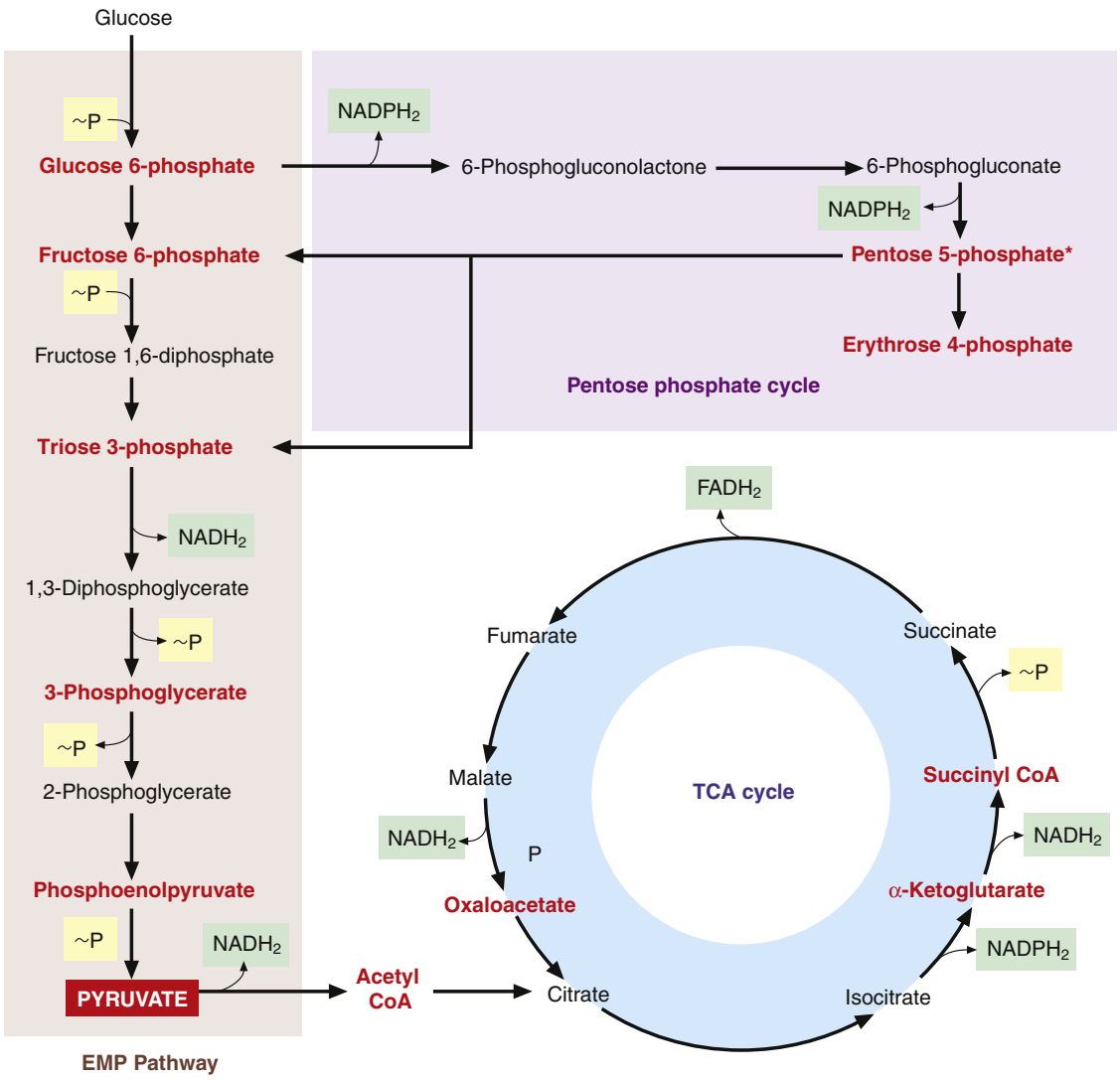
- Glucose 6-phosphate
- Fructose 6-phosphate
- Pentose 5-phosphate
- Erythrose 4-phosphate
- 3-Phosphoglycerate
- Phosphoenolpyruvate
- Pyruvate
- Acetyl CoA
- α-Ketoglutarate
- Succinyl CoA
- Oxaloacetate



Nutrients

- Gases
  - Carbon dioxide (CO_2)
  - Oxygen (O_2)
  - Ammonia (NH_3)
- Organic compounds, including amino acids
- Water (H_2O)
- Nitrate (NO_3^-)
- Phosphate (PO_4^{3-})
- Hydrogen sulfide (H_2S)
- Sulfate (SO_4^{2-})
- Potassium ($K+$)
- Magnesium (Mg^{2+})
- Calcium (Ca^{2+})
- Sodium (Na^+)
- Iron (Fe^{3+})
  - Organic iron complexes

• **Fig. 2.11** Overview of bacterial metabolism, which includes the processes of fueling, biosynthesis, polymerization, and assembly. *CoA,* Coenzyme A; *DNA,* deoxyribonucleic acid; *RNA,* ribonucleic acid. (Modified from Niedhardt FC, Ingraham JL, Schaechter M, eds. *Physiology of the Bacterial Cell: A Molecular Approach.* Sunderland, MA: Sinauer Associates; 1990.)



• **Fig. 2.12** Overview of the central metabolic pathways (Embden-Meyerhof-Parnas [EMP], the tricarboxylic acid [TCA] cycle, and the pentose phosphate shunt). Precursor metabolites (Fig. 2.11) that are produced are highlighted in *red;* production of energy in the form of adenosine triphosphate *(~P)* by substrate-level phosphorylation is highlighted in *yellow;* and reduced carrier molecules for transport of electrons used in oxidative phosphorylation are highlighted in *green*. (Modified from Niedhardt FC, Ingraham JL, Schaechter M, eds. *Physiology of the Bacterial Cell: A Molecular Approach.* Sunderland, MA: Sinauer Associates; 1990.)

The two general mechanisms for ATP production in bacterial cells are **substrate-level phosphorylation** and electron transport, also referred to as **oxidative phosphorylation.** In substrate-level phosphorylation, high-energy phosphate bonds produced by the central pathways are donated to adenosine diphosphate (ADP) to form ATP directly from the substrate as opposed to generation via the electron transport chain (Fig. 2.12). In addition, pyruvate, a primary intermediate in the central pathways, serves as the initial substrate for several other pathways to generate ATP by substrate-level phosphorylation. These other pathways constitute **fermentative metabolism,** which does not require oxygen and produces various end products, including alcohols, acids, carbon dioxide, and hydrogen. The specific fermentative pathways and the end products produced vary with different bacterial species. Detection of these products is an important basis for laboratory identification of bacteria. (See Chapter 7 for more information on the biochemical basis for bacterial identification.)

Oxidative Phosphorylation

Oxidative phosphorylation involves an electron transport system that conducts a series of electron transfers from reduced carrier molecules such as $NADH_2$, $NADPH_2$, and $FADH_2$ (flavin adenine dinucleotide), produced in the central pathways (Fig. 2.12), to a terminal electron acceptor. The energy produced by the series of oxidation-reduction reactions is used to generate ATP from ADP. When oxidative phosphorylation uses oxygen as the terminal electron acceptor, the process is known as **aerobic respiration. Anaerobic respiration** refers to processes that use final electron acceptors other than oxygen.

A knowledge of which mechanisms bacteria use to generate ATP is important for designing laboratory protocols for cultivating and identifying these organisms. For example, some bacteria depend solely on aerobic respiration and are unable to grow in the absence of oxygen (**strictly aerobic bacteria**). Others can use either aerobic respiration or fermentation, depending on the availability of oxygen (**facultative anaerobic bacteria**). For still others, oxygen is absolutely toxic (**strictly anaerobic bacteria**).

Biosynthesis

The fueling reactions essentially bring together all the raw materials needed to initiate and maintain all other cellular processes. The production of precursors and energy is accomplished through catabolic processes and the degradation of substrate molecules. The three remaining pathways for biosynthesis, polymerization, and assembly depend on anabolic metabolism. In **anabolic metabolism,** precursor compounds are joined for the creation of larger molecules (polymers) required for assembly of cellular structures (Fig. 2.11).

Biosynthetic processes use the precursor products in dozens of pathways to produce a variety of building blocks, such as amino acids, fatty acids, sugars, and nucleotides (Fig. 2.11). Many of these pathways are highly complex and interdependent, whereas other pathways are completely independent. In many cases the enzymes that drive the individual pathways are encoded on a single mRNA molecule that has been transcribed from contiguous genes in the bacterial chromosome (i.e., an operon).

As previously mentioned, bacterial genera and species vary extensively in their biosynthetic capabilities. Knowledge of these variations is necessary to determine the optimal conditions for growing organisms under laboratory conditions. For example, some organisms may not be capable of synthesizing an essential amino acid necessary as a building block for proteins. Without the ability to synthesize the amino acid, the bacterium must obtain the building block from the environment. Thus, if the organism is cultivated in the microbiology laboratory, the amino acid must be provided in the artificial culture medium.

Polymerization and Assembly

Various anabolic reactions assemble (polymerize) the building blocks into macromolecules, including lipids, lipopolysaccharides, polysaccharides, proteins, and nucleic acids. This synthesis of macromolecules is driven by energy and enzymatic activity in the cell. Similarly, energy and enzymatic activities also drive the assembly of various macromolecules into the component structures of the bacterial cell. Cellular structures are the product of all the genetic and metabolic processes discussed.

Structure and Function of the Bacterial Cell

Based on key characteristics, all cells are classified into two basic types: prokaryotic and eukaryotic. Although these two cell types share many common features, they have important differences in terms of structure, metabolism, and genetics.

Eukaryotic and Prokaryotic Cells

Among clinically relevant organisms, **bacteria** are single-cell prokaryotic microorganisms. **Fungi** and **parasites** are single-cell or multicellular eukaryotic organisms, as are plants and all higher animals. **Viruses** are dependent on host cells for survival and therefore are not considered cellular organisms but rather infectious agents. **Prions,** which are abnormal infectious proteins, are also not considered living cells.

A notable characteristic of eukaryotic cells, such as parasites and fungi, is the presence of membrane-enclosed organelles that have specific cellular functions. Examples of these organelles and their respective functions include:

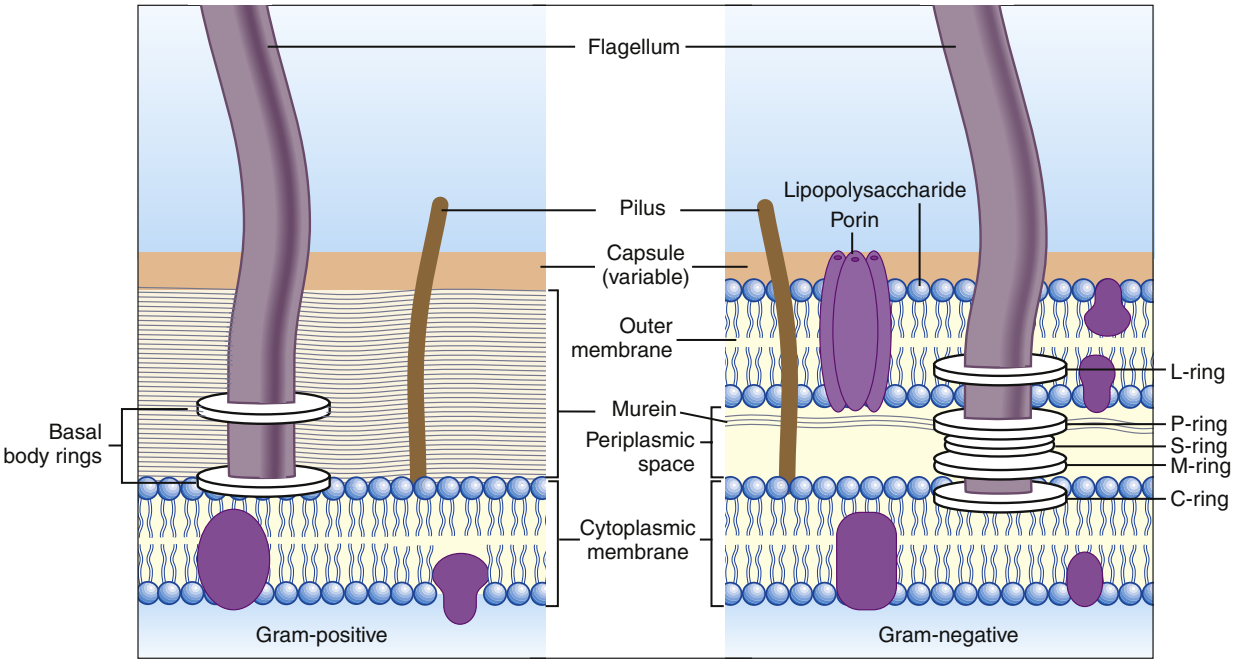
- Endoplasmic reticulum—process and transport proteins
- Golgi body—modification of substances and transport throughout the cell, including internal delivery of molecules, and exocytosis or secretion of other molecules
- Mitochondria—generate energy (ATP)
- Lysosomes—provide an environment for controlled enzymatic degradation of intracellular substances
- Nucleus—provide a membrane enclosure for chromosomes

In addition, eukaryotic cells have an infrastructure, or **cytoskeleton**, which provides support for cellular structure, organization, and movement. The cytoskeleton in eukaryotic cells also plays an essential role in immunology by mediating phagocytosis for the removal of foreign materials from the host, including bacteria, fungi, and viral agents.

Prokaryotic cells, such as bacteria, do not contain organelles. All functions take place in the cytoplasm or cytoplasmic membrane of the cell. Prokaryotic and eukaryotic cell types differ considerably at the macromolecular level, including protein synthesis machinery, chromosomal organization, and gene expression. One notable structure present only in prokaryotic bacterial cells is a **cell wall** composed of **peptidoglycan.** This structure has an immeasurable effect on the practice of diagnostic bacteriology and the management of bacterial diseases.

Bacterial Morphology

Most clinically relevant bacterial species range in size from 0.25 to 1 μm in width and 1 to 3 μm in length, thus requiring microscopy for visualization (see Chapter 6 for more information on microscopy). Just as bacterial species and genera vary in their metabolic processes, their cells also vary in size, morphology, and cell-to-cell arrangements and in the chemical composition and structure of the cell wall. The bacterial cell wall differences provide the basis for the **Gram stain,** a fundamental staining technique used in bacterial identification schemes. This staining procedure separates almost all medically relevant bacteria into two general types: **gram-positive** bacteria, which stain a deep blue or purple, and **gram-negative** bacteria, which stain a pink to red (Fig. 6.3). This simple



• **Fig. 2.13** General structures of the gram-positive and gram-negative bacterial cell envelopes. The outer membrane and periplasmic space are present only in the envelope of gram-negative bacteria. In addition to porins, bacterial membranes contain additional proteins involved in stabilizing the layers of the cellular structure, adherence, or sorting and reacting to chemical signals. The murein layer is substantially more prominent in gram-positive envelopes. (Modified from Niedhardt FC, Ingraham JL, Schaechter M, eds. *Physiology of the Bacterial Cell: A Molecular Approach.* Sunderland, MA: Sinauer Associates; 1990.)

but important color distinction is the result of differences in the constituents of bacterial cell walls that influence the cell's ability to retain differential dyes after treatment with a decolorizing agent.

Common bacterial cellular morphologies include **cocci** (circular), **coccobacilli** (ovoid), and **bacilli** (rod shaped), as well as **fusiform** (pointed end), curved, or spiral shapes. Cellular arrangements are also noteworthy. Cells may characteristically occur singly, in pairs, or grouped as tetrads, clusters, or in chains (see Fig. 6.4 for examples of bacterial staining and morphologies). The determination of the Gram stain reaction and the cell size, morphology, and arrangement are essential aspects of bacterial identification.

Bacterial Cell Components

Bacterial cell components can be divided into those that make up the outer cell structure and its appendages (**cell envelope**) and those associated with the cell's interior. It is important to note that the cellular structures work together to function as a complex and integrated unit.

Cell Envelope

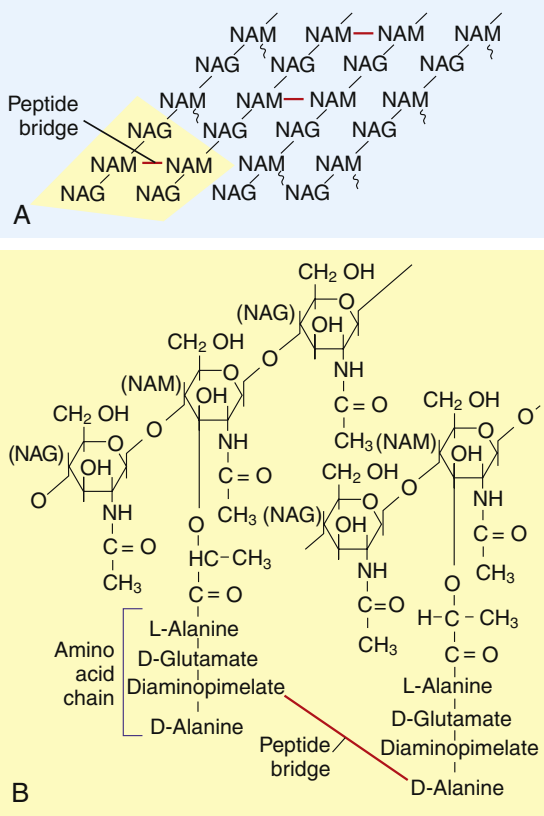
As shown in Fig. 2.13, the outermost structure, the cell envelope, comprises:

- An outer membrane (in gram-negative bacteria only)
- A cell wall composed of the peptidoglycan macromolecule (also known as the murein layer)
- Periplasm (in gram-negative bacteria only)
- The cytoplasmic or cell membrane, which encloses the cytoplasm

Outer Membrane

Outer membranes, which are found only in gram-negative bacteria, function as the cell's initial barrier to the environment. These membranes serve as the primary permeability barriers to hydrophilic and hydrophobic compounds and contain essential enzymes and other proteins located in the periplasmic space. The membrane is a bilayered structure composed of lipopolysaccharide, which gives the surface of gram-negative bacteria a net negative charge. The outer membrane also plays a significant role in the ability of certain bacteria to cause disease.

Scattered throughout the lipopolysaccharide macromolecules are protein structures called **porins.** These water-filled structures control the passage of nutrients and other solutes, including antibiotics, through the outer membrane. The number and types of porins vary with bacterial species. These differences can substantially influence the extent to which various substances pass through the outer membranes of different bacteria. In addition to porins, other proteins (murein lipoproteins) facilitate the attachment of the outer membrane to the next internal layer in the cell envelope, the cell wall, and may serve as adhesions for attachment to a host cell or as transporters.



• **Fig. 2.14** Peptidoglycan sheet (A) and subunit (B) structure. Multiple peptidoglycan layers compose the murein structure, and different layers are extensively cross-linked by peptide bridges. Note that amino acid chains are only derived from NAM. *NAG,* N-acetylglucosamine; *NAM,* N-acetylmuramic acid. (Modified from Saylers AA, Whitt DD. *Bacterial Pathogenesis: A Molecular Approach.* Washington, DC: American Society for Microbiology Press; 2010.)

Cell Wall (Murein Layer)

The cell wall, also referred to as the peptidoglycan, or **murein layer,** is an essential structure found in nearly all clinically relevant bacteria. This structure gives the bacterial cell shape and strength to withstand changes in environmental osmotic pressures that would otherwise result in cell lysis. The murein layer protects against mechanical disruption of the cell and offers some barrier to the passage of larger substances. Because this structure is essential for the survival of bacteria, its synthesis and structure are often the primary target for the development and design of several antimicrobial agents.

The structure of the cell wall is unique and is composed of disaccharide-pentapeptide subunits. The disaccharides N-acetylglucosamine and N-acetylmuramic acid are the alternating sugar components (moieties) with the amino acid chain linked to N-acetylmuramic acid molecules (Fig. 2.14). Polymers of these subunits cross-link to one another by means of peptide bridges to form peptidoglycan sheets. In turn, layers of these sheets are cross-linked with one another, forming a multilayered, cross-linked structure of considerable strength. Referred to as the **murein sacculus,** or sack, this peptidoglycan structure surrounds the entire cell.

A notable difference between the cell walls of gram-positive and gram-negative bacteria is the substantially thicker peptidoglycan layer in gram-positive bacteria (Fig. 2.13). In addition, the cell wall of gram-positive bacteria contains **teichoic acids** (i.e., glycerol or ribitol phosphate polymers combined with various sugars, amino acids, and amino sugars). Some teichoic acids are linked to N-acetylmuramic acid, and others (e.g., lipoteichoic acids) are linked to the next underlying layer, the **cellular** or **cytoplasmic membrane.** Other bacteria (e.g., *Mycobacteria*) have waxy substances within the murein layer, such as mycolic acids. Mycolic acids make the cells more refractory to toxic substances, including acids. Bacteria with mycolic acid in the cell wall require unique staining procedures and growth media in the diagnostic laboratory.

Periplasmic Space

The **periplasmic space** typically is found only in gram-negative bacteria (whether it is present in gram-positive organisms is a subject of debate). The periplasmic space is bounded by the internal surface of the outer membrane and the external surface of the cellular membrane. This area, which contains the murein layer, consists of gel-like substances that assist in the capture of nutrients from the environment. This space also contains several enzymes involved in the degradation of macromolecules and detoxification of environmental solutes, including antibiotics that enter through the outer membrane.

Cytoplasmic (Inner) Membrane

The **cytoplasmic** (inner) **membrane** is present in both gram-positive and gram-negative bacteria and is the deepest layer of the cell envelope. The cytoplasmic membrane is heavily laced with various proteins, including a number of enzymes vital to cellular metabolism. The cell membrane serves as an additional osmotic barrier and is functionally similar to the membranes of several eukaryotic cellular organelles (e.g., mitochondria, Golgi complexes, lysosomes). The cytoplasmic membrane functions include:

- Transport of solutes into and out of the cell
- Housing of enzymes involved in outer membrane synthesis, cell wall synthesis, and the assembly and secretion of extracytoplasmic and extracellular substances
- Generation of chemical energy (i.e., ATP)
- Cell motility
- Mediation of chromosomal segregation during replication
- Housing of molecular sensors that monitor chemical and physical changes in the environment

Cellular Appendages

In addition to the components of the cell envelope, cellular appendages (i.e., capsules, fimbriae, and flagella) are associated with or proximal to this portion of the cell. The presence of these appendages, which can play a role in the mediation of infection and in laboratory identification, varies among bacterial species and even among strains within the same species.

The **capsule** is immediately exterior to the murein layer of gram-positive bacteria and the outer membrane of gram-negative bacteria. The capsule is composed of high-molecular-weight polysaccharides, the production of which may depend on the environment and growth conditions surrounding the bacterial cell. The capsule does not function as an effective permeability barrier or add strength to the cell envelope, but it does protect bacteria from attack by components of the human immune system. The capsule also facilitates and maintains bacterial colonization of biologic (e.g., teeth) and inanimate (e.g., prosthetic heart valves) surfaces through the formation of "slime layers" or biofilms. Both **slime layers** and **biofilms** imply the presence of an extracellular polymer matrix that varies in composition and structure in different organisms. A biofilm may consist of a monomicrobic or polymicrobic group of bacteria housed in a complex biochemical matrix. This extracellular matrix stabilizes the cell to protect the organism from hydrodynamic forces in the host and plays a protective role against biocides and agents of the host's immune system. (See Chapter 3 for further discussion of microbial biofilms.)

Fimbriae, or **pili,** are hairlike, proteinaceous structures that extend from the cell membrane into the external environment; some may be up to 2 μm long. Fimbriae may serve as adhesins that help bacteria attach to animal host cell surfaces, often as the first step in establishing infection. In addition, a pilus may be referred to as a **sex pilus;** this structure, which is well characterized in the gram-negative bacillus *E. coli,* serves as the conduit for the passage of DNA from the donor to the recipient during conjugation. The sex pilus is present only in cells that produce a protein referred to as the **F factor.** F-positive cells initiate mating or conjugation only with F-negative cells, thereby limiting the conjugative process to cells capable of transporting genetic material through the hollow sex pilus.

Flagella are complex structures, mostly composed of the protein flagellin, that are intricately embedded in the cell envelope. These structures are responsible for bacterial motility. Although not all bacteria are motile, motility plays an important role in survival and the ability of bacteria to cause disease. Depending on the bacterial species, a single flagellum may be located at one end of the cell **(monotrichous flagella),** a group of flagella may be located at one end of the cell **(lophotrichous flagella),** a single flagellum may reside at both ends of the cell **(amphitrichous flagella),** or the entire cell surface may be covered with flagella **(peritrichous flagella).** The flagellum acts as a rotary motor containing a complex set of rings that act as bushings to control cellular movement. Gram-negative flagella are equipped with a basal body structure that contains five rings, the L-ring that is embedded in the lipid bilayer, the P-ring in the periplasmic space, a smaller S-ring (stator ring) attached to the M-ring or motor ring, and the C-ring, which anchors the entire complex to the cell. Because gram-positive organisms have a much more stable complex cellular structure because of the thick layer of peptidoglycan, the flagella contain only two basal body rings: One is embedded in the peptidoglycan layer, which is very stable, and the second is embedded in the cell membrane.

Cell Interior

Those structures and substances that are bound internally by the cytoplasmic membrane compose the cell interior and include the cytosol, polysomes, inclusions, nucleoid, plasmids, and endospores.

The **cytosol,** where nearly all other functions not conducted by the cell membrane occur, contains thousands of enzymes and is the site of protein synthesis. The cytosol has a granular appearance caused by the presence of many polysomes (mRNA complexed with several ribosomes during translation and protein synthesis) and inclusions (i.e., storage reserve granules). The number and nature of the inclusions vary depending on the bacterial species and the nutritional state of the organism's environment. Two common types of granules include glycogen, a storage form of glucose, and polyphosphate granules, a storage form for inorganic phosphates. These granules may be microscopically visible in bacteria stained with specific dyes.

Unlike eukaryotic chromosomes, the bacterial chromosome is not enclosed within a membrane-bound nucleus. Instead the bacterial chromosome exists as a **nucleoid** in which the highly coiled DNA is intermixed with RNA, polyamines, and various proteins that lend structural support. At times, depending on the stage of cell division, more than one chromosome may be present per bacterial cell. Plasmids are the other genetic elements that exist independently in the cytosol, and their numbers may vary from none to several hundred per bacterial cell.

The final bacterial structure to be considered is the **endospore.** Under adverse physical and chemical conditions or when nutrients are scarce, some bacterial genera (*Bacillus* and *Clostridium* spp.) are able to form spores (i.e., sporulate). Sporulation involves substantial metabolic and structural changes in the bacterial cell. Essentially, the cell transforms from an actively metabolic and growing state to a dormant state, with a decrease in cytosol and a concomitant increase in the thickness and strength of the cell envelope. The endospore remains in a dormant state until favorable conditions for growth are again encountered. This survival tactic is demonstrated by a number of clinically relevant bacteria and complicates thorough sterilization of materials and food for human use.

Visit the Evolve site for a complete list of procedures, review questions, and case studies.

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Chapter Review

1. The periplasmic space is required for:
   - **a.** Nutrient collection in both gram-positive and gram-negative bacteria
   - **b.** Collection and enzymatic degradation of nutrients in gram-negative bacteria
   - **c.** Nutrient detoxification and enzymatic degradation in all bacteria
   - **d.** None of the above
2. Prokaryotic chromosomes:
   - **a.** Are double-stranded RNA molecules
   - **b.** Are single-copy, double-stranded DNA molecules
   - **c.** Are linear double-stranded DNA molecules
   - **d.** Are unable to replicate independently of plasmids
3. Bacterial cells genetically evolve by:
   - **a.** Recombination with plasmids, transposons, and other bacterial chromosomes
   - **b.** Mutation and recombination
   - **c.** Use of the mechanisms of transduction, transformation, and conjugation
   - **d.** All of the above
4. Transcription is the:
   - **a.** Copying of DNA to RNA
   - **b.** Changing of DNA to RNA
   - **c.** Production of a complementary DNA
   - **d.** Completion of a protein sequence
5. A eukaryotic cell:
   - **a.** Is smaller and less complex than a prokaryotic cell
   - **b.** Is able to grow only in aerobic conditions
   - **c.** Contains membrane-bound organelles
   - **d.** Is unable to grow outside of another cell
6. **Matching:** *Match each term with the correct description.*

_____ capsule	a. involved in transcriptional regulation
_____ replication	b. able to grow in the presence or absence of oxygen
_____ repressor	c. maintains selective permeability and cell shape
_____ tRNA	d. provides a mechanism to evade the human immune system
_____ facultative anaerobe	e. the process of making a new DNA molecule
_____ gram-negative	f. involved in protein translation
_____ gram-positive	g. mobile genetic elements
_____ aerobic	h. contains a thick layer of peptidoglycan
_____ cell envelope	i. final electron acceptor is oxygen
_____ mobilome	j. has an outer and inner membrane
_____ genome	k. all genetic elements within a cell

7. Which chemical or physical property is essential for the conservation of genetic information?
   - **a.** Complementation between base pairs
   - **b.** Double-stranded
   - **c.** Antiparallel structure
   - **d.** All are equally important
8. Expression of a biochemical molecule in an organism requires:
   - **a.** Replication only
   - **b.** Transcription only
   - **c.** Transcription and translation of a protein
   - **d.** All of the above
9. **True or False**
   - _____ All bacteria are considered competent.
   - _____ Conjugation requires cell-to-cell contact.
   - _____ Oxidative phosphorylation occurs across the cell membrane in bacteria.
10. **Short Answer**
    Provide an explanation for why bacteria are capable of rapidly responding to changes in their environment based on molecular and cellular structure. Bacteria are prokaryotes; the genetic material is not contained within a nucleus, allowing replication transcription and translation to occur simultaneously.

3 Host-Microorganism Interactions

OBJECTIVES

1. List the various reservoirs (environments) that facilitate host-microorganism interactions.
2. Define direct versus indirect transmission, and provide examples of each.
3. Define and differentiate the interactions between the host and microorganism, including colonization, infection, microbiota, microbiome, pathogens, opportunistic pathogens, and nosocomial (health care–acquired or –associated) and community-acquired infection.
4. List and describe the components involved in specific versus nonspecific immune defenses, including inflammation, phagocytosis, antibody production, and cellular responses.
5. Identify elements involved in the two arms of the immune system: humoral and cell-mediated immunity.
6. Provide specific examples of disease prevention strategies, including preventing transmission, controlling reservoirs, and minimizing risk of exposure.
7. Differentiate between bacterial endotoxins and exotoxins, and provide examples of each.
8. Given a patient history of an infectious process, identify and differentiate a sign versus a symptom.
9. Define and differentiate between an acute infectious process and one that is chronic and/or latent.
10. Describe the three major steps in the formation of a microbial biofilm, and list the advantages of biofilm formation to the microorganism and the disadvantages to the infected host.

Interactions between humans and microorganisms are exceedingly complex and far from being completely understood. The interactions between these two living entities plays an important role in the practice of diagnostic microbiology and in the management of infectious disease. Understanding these interactions is necessary for establishing methods to isolate specific microorganisms from patient specimens and for developing effective treatment strategies. This chapter provides the framework for understanding the various aspects of host-microorganism interactions. Box 3.1 lists a variety of terms and definitions associated with host-microorganism interactions.

Host-microorganism interactions should be viewed as bidirectional in nature. Humans use the abilities and natural products of microorganisms in various settings, including the food and fermentation industry, as biologic insecticides for agriculture; to genetically engineer a multitude of products; and even for biodegrading industrial waste. However, microbial populations share the common goal of survival with humans, using their relationship with humans for food, shelter, and dissemination, and they have been successful at achieving those goals. Which participant in the relationship is the user and which is the used is a fine and intricate balance of nature. This is especially true when considering the microorganisms most closely associated with humans and human disease.

In 2008, the National Institutes of Health initiated a project referred to as the *Human Microbiome Project* (http://commonfund.nih.gov/hmp/index). The human **microbiome** consists of microorganisms that are present in and on the human body at any given time without causing harm. Phase I (2008–2012) of the microbiome project focused on four major goals:

(1) identify and characterize a core human microbiome in healthy individuals, both male and female;

(2) determine whether changes in the human microbiome correlate with health and disease;

(3) develop new technology and bioinformatic tools to manage the project data; and

(4) address the ethical, legal, and social implications associated with the microbiome project.

Interestingly, the study has elucidated that the microbiome complex ecosystem varies significantly across the body and between individuals. Analysis of the human microbiome has demonstrated that it is clearly an emergent property. One hundred and thirteen females were examined for the presence of microorganisms at 18 body sites and 129 males at 15 body sites (excluding vaginal collections). The data demonstrated that dependent on the body site, both low diversity of microorganisms and a high diversity of microorganisms correlates with the development of disease. Phase I also examined the relationships between the microbiome and characteristics of the host, including age, body mass index, and available medical history. The second phase of the project, the *Integrative Human Microbiome Project*, has begun to analyze data from phase I and apply it to host interactions in healthy and disease states. Established in 2014, Phase II focuses on three major areas: (1) the vaginal

• BOX 3.1 Definitions of Selected Epidemiologic Terms

- **Carrier:** A person who harbors the etiologic agent but shows no apparent signs or symptoms of infection or disease
- **Common source:** A single source or reservoir from which an etiologic agent responsible for an epidemic or outbreak originates
- **Community-associated infection:** Infection acquired in an activity or group that is not in a health care setting or environment.
- **Disease incidence:** The number of new diseases or infected persons in a population
- **Disease prevalence:** The percentage of diseased persons in a given population at a particular time
- **Endemic:** A disease constantly present at some rate of occurrence in a particular location
- **Epidemic:** A larger-than-normal number of diseased or infected individuals in a particular location
- **Etiologic agent:** A microorganism responsible for causing infection or infectious disease
- **Health care–associated infection:** Infections acquired as a result of a short- or long-term admission into a health care facility
- **Iatrogenic:** Infection acquired as a result of a medical procedure.
- **Microbiome**: An individual's microbiologic environment, present in or on the human host
- **Mode of transmission:** The means by which etiologic agents are brought in contact with the human host (e.g., infected blood, contaminated water, insect bite)
- **Morbidity:** The state of disease and its associated effects on the host
- **Morbidity rate:** The incidence of a particular disease state
- **Mortality:** Death resulting from disease
- **Mortality rate:** The incidence in which a disease results in death
- **Nosocomial infection:** Infection for which the etiologic agent was acquired in a hospital or long-term health care center or facility
- **Outbreak:** A larger than normal number of diseased or infected individuals that occurs over a relatively short period
- **Pandemic:** An epidemic that spans the world
- **Reservoir:** The origin of the etiologic agent or location from which it disseminates (e.g., water, food, insects, animals, other humans)
- **Strain typing:** Laboratory-based characterization of etiologic agents designed to establish their relatedness to one another during a particular outbreak or epidemic
- **Surveillance:** Any type of epidemiologic investigation that involves data collection for characterizing circumstances surrounding the incidence or prevalence of a particular disease or infection
- **Vector:** A living entity (animal, insect, or plant) that transmits the etiologic agent
- **Vehicle:** A nonliving entity that is contaminated with the etiologic agent and as such is the mode of transmission for that agent

microbiome associated with pregnancy and preterm birth; (2) gastrointestinal microbiome and the development of inflammatory bowel disease; and (3) microbiome and the development of type 2 diabetes. Undoubtedly, this research will continue to evolve and potentially provide insight into the characterization, risk, and prevention of disease. The relationship between host and microorganism is ultimately associated with the variation and balance of the normal human microbiome and the appearance of a potentially infectious agent.

The complex relationships between the human host and medically relevant microorganisms are demonstrated in the sequential steps associated with microbe-host interactions and the subsequent development of infection and disease. The stages of interaction include (1) the physical encounter between the host and microorganism; (2) colonization or survival of the microorganism on an internal (gastrointestinal, respiratory, or genitourinary tract) or external (skin) surface of the host; (3) microbial entry, invasion, and dissemination to deeper tissues and organs of the human body; and (4) resolution or outcome.

The Encounter Between Host and Microorganism

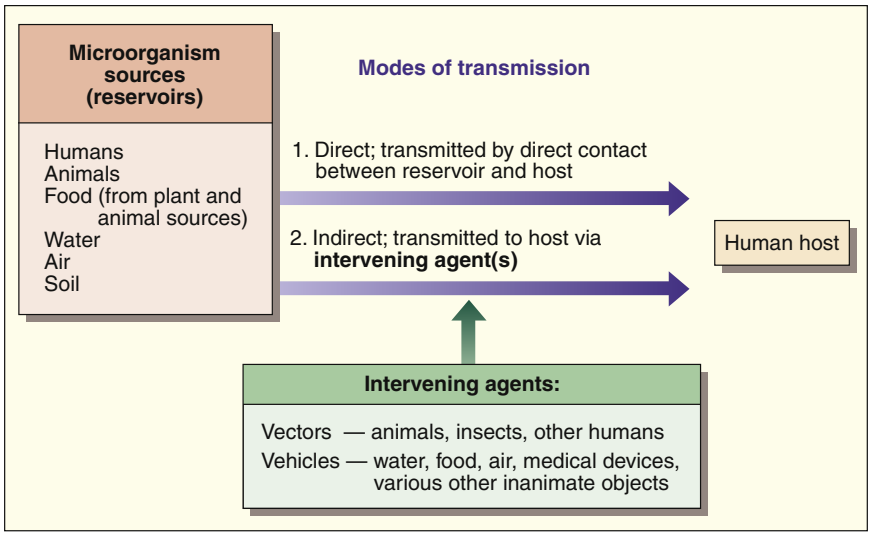
The Human Host's Perspective

Because microorganisms are ubiquitous in nature, human encounters are inevitable, but the means of encounter vary widely. Which microbial population and the mechanism of exposure are often direct consequences of a person's activity or behaviors. Certain activities carry different risks for an encounter. There is a wide spectrum of activities and situations over which a person may or may not have absolute control. For example, acquiring salmonellosis because one fails to cook the holiday turkey thoroughly is avoidable, whereas contracting tuberculosis living in conditions of extreme poverty and overcrowding may be unavoidable. The role that human activities play in the encounter between humans and microorganisms cannot be overstated. Most of the crises associated with infectious disease are preventable or can be greatly reduced with changes in human behavior and living conditions.

Microbial Reservoirs and Transmission

Humans encounter microorganisms when they enter or are exposed to the same environment in which the microbial agents live or when the infectious agents are brought to the human host by indirect means. The environment or place of origin of the infecting agent is the **reservoir.** As shown in Fig. 3.1, microbial reservoirs include humans, animals, water, food, air, and soil. The human host may acquire microbial agents by various means or **modes of transmission.** The mode of transmission is direct when the host directly contacts the microbial reservoir and is indirect when the host encounters the microorganism by an intervening agent of transmission.

The agents of transmission that bring the microorganism from the reservoir to the host may be a living entity, such as



• **Fig. 3.1** Summary of microbial reservoirs and modes of transmission to humans.

an insect, in which case they are called **vectors,** or they may be a nonliving entity, referred to as a **vehicle** or **fomite.** In addition, some microorganisms may have a single mode of transmission, whereas others may spread by various methods. From a diagnostic microbiology perspective, knowledge about an infectious agent's mode of transmission is often important for determining optimal specimens for isolation of the organism and for implementing precautions that minimize the risk of laboratory or health care–associated infections (HAIs) (see Chapters 4, 78, and 79 for more information regarding laboratory safety, infection control, and sentinel laboratory responses, respectively).

Human and Microbe Interactions

Humans play a substantial role as microbial reservoirs. In fact, the passage of a neonate from the sterile environment of the mother's womb through the birth canal, which is heavily colonized with various microbial agents, is a primary example of one human directly (i.e., **direct transmission**) acquiring a microorganism from another human serving as the reservoir. This is the mechanism that newborns first encounter microbial agents. Other examples in which humans serve as the microbial reservoir include the acquisition of streptococcal pharyngitis through touching; hepatitis through blood transfusions; gonorrhea, syphilis, and acquired immunodeficiency syndrome (AIDS) through sexual contact; and tuberculosis and the common cold through aerosolized droplets associated with coughing or sneezing. **Indirect transmission** can occur when microorganisms from one individual contaminate a vehicle of transmission, such as water (e.g., cholera), that is then ingested by another person. In the medical setting, indirect transmission of microorganisms from one human host to another by means of a medical procedure (i.e., **iatrogenic**) and contaminated medical devices helps to disseminate infections in hospitals. Hospital-acquired, health care–associated, or long-term care–associated infections are considered **nosocomial infections. Health care–associated infections (HAIs)** include exposure in a variety of settings and not confined to in-patient care in a health care institution. These exposures occur during field containment or transportation of infectious agents as well as in daily contact with infected patients in clinics. In addition, HAIs are not limited to health care professionals and patients, but also include visitors, support staff, and students.

In addition, humans are routinely exposed to infectious agents through participation in activities and events throughout their daily lives. These activities include direct and indirect transmission of infectious agents in community settings. These infections are considered **community-associated (CA) infections.**

Animals as Microbial Reservoirs

Infectious agents from animal reservoirs are transmissible directly to humans through an animal bite (e.g., rabies) or indirectly through the bite of insect vectors that feed on both animals and humans (e.g., Lyme disease and Rocky Mountain spotted fever). Animals may also transmit infectious agents by acquiring them from or depositing them in water and food supplies. For example, beavers heavily colonized with parasites can cause infection of the human gastrointestinal tract. These parasites may be encountered and subsequently acquired when stream water is contaminated by the beaver and is used by a vacationing camper. Alternatively, animals used for human food carry numerous bacteria (e.g., *Salmonella* and *Campylobacter*) that, if not destroyed through appropriate cooking during preparation, can cause severe gastrointestinal illness.

Many other infectious diseases can be encountered through direct or indirect animal contact, and information regarding a patient's exposure to animals is often a key component in the diagnosis of these infections. Some microorganisms primarily infect animal populations and on occasion accidentally encounter and infect humans. When

a human infection results from such an encounter, it is a **zoonotic infection.** More specifically, if the human infection is a result of regular interaction with animals for food production, the infection is **livestock-associated.**

Insects as Vectors

The most common role of insects (arthropods) in the transmission of infectious disease is as vectors rather than as reservoirs. A variety of arthropods can transmit viral, parasitic, and bacterial disease from animals to humans, whereas others transmit microorganisms between human hosts without an intermediate animal reservoir. Malaria, a deadly disease, is a prime example of an infectious disease maintained in the human population by the feeding and survival of an insect vector, the mosquito. Still other arthropods may themselves be agents of disease. These include organisms such as lice and scabies spread directly between humans and cause skin irritations but do not penetrate the body. Because they are able to survive on the skin of the host without gaining access to internal tissues, they are **ectoparasites** (Chapter 46). In addition, nonfungal infections may result when microbial agents in the environment, such as endospores, are introduced mechanically through the bite of a vector, scratch, or other penetrating wound.

The Environment as a Microbial Reservoir

The soil and natural environmental debris are reservoirs for countless types of microorganisms. It is not surprising that these also serve as reservoirs for microorganisms that can cause infection in humans. Many of the fungal agents (see Part V: Mycology) are acquired by inhalation of soil and dust particles containing microorganisms (e.g., San Joaquin Valley fever). Other, nonfungal infections (e.g., tetanus endospores) may result when microbial agents in the environment are introduced into the human body by a penetrating wound.

The Microorganism's Perspective

Clearly, numerous activities can result in human encounters with microorganisms. Because humans are engaged in all of life's complex activities, the tendency is to perceive the microorganism as having a passive role in the encounter process. However, this assumption is a gross oversimplification.

Microorganisms are driven by survival, and the environment of the reservoirs they occupy must allow their metabolic and genetic needs to be fulfilled. Reservoirs can be inhabited by hundreds or thousands of different microorganisms. Yet human encounters with the reservoirs, either directly or indirectly, do not result in all microorganisms establishing an association with the human host. Although some microorganisms have evolved strategies that do not require a human host to ensure survival, others have included humans to a lesser or greater extent as part of their survival tactics. These organisms often have mechanisms that enhance their chances for a human encounter.

Depending on factors associated with both the human host and the microorganism involved, the encounter may have a beneficial, disastrous, or inconsequential effect on each of the participants.

Microorganism Colonization of Host Surfaces

The Host's Perspective

Once a microbe is in contact with a human host, the outcome of the encounter depends on what happens during each step of the interaction, beginning with **colonization.** The human host's role in microbial colonization, defined as the persistent survival of microorganisms on a surface of the human body, is dictated by the defenses that protect vital internal tissues and organs against microbial invasion. The first defenses are the external and internal body surfaces that are in direct contact with the external environment and are the anatomic regions where the microorganisms will initially encounter the human host. These surfaces include:

- Skin (including the conjunctival epithelium covering the eye)
- Mucous membranes lining the mouth or oral cavity, the respiratory tract, the gastrointestinal tract, and the genitourinary tract

Because body surfaces are always present and provide protection against all microorganisms, skin and mucous membranes are constant and **nonspecific defense mechanisms.** As is discussed later in this text, other protective mechanisms are produced in response to the presence of microbial agents (inducible defenses), and some are directed specifically at particular microorganisms (**specific defense mechanisms**).

Skin and Skin Structures

Skin serves as a physical and chemical barrier to microorganisms; its protective characteristics are summarized in Table 3.1 and Fig. 3.2. The acellular, outermost layer of the skin, along with the tightly packed cellular layers underneath, provides an impenetrable physical barrier to all microorganisms, unless damaged. In addition, these layers continuously shed, thus dislodging bacteria that have attached to the outer layers. The skin is also a dry and cool environment, which is incompatible with the growth requirements of many microorganisms that thrive in a warm, moist environment.

The follicles and glands of the skin produce various natural antibacterial substances, including sebum and sweat. However, many microorganisms can survive the conditions of the skin. These bacteria, or the skin microbiome, are **skin colonizers,** and they often produce substances that may be toxic and inhibit the growth of more harmful microbial agents. The skin human microbiome differs among healthy individuals more than any other body site. Beneath the outer layers of skin are various host cells that protect against organisms that breach the surface barriers. These cells, collectively known as **skin-associated lymphoid tissue,**

TABLE 3.1 Protective Characteristics of the Skin and Skin Structures

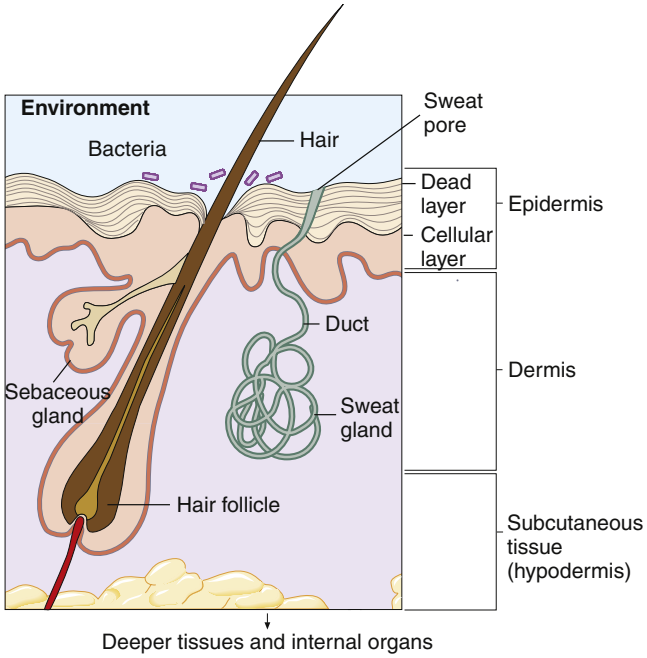
Skin Structure	Protective Activity
Outer (dermal) layers	• Act as a physical barrier to microbial penetration • Remove attached bacteria through sloughing of the outer layers • Provide dry, acidic, and cool conditions that limit bacterial growth
Hair follicles, sweat glands, sebaceous glands	• Produce acids, alcohols, and toxic lipids that limit bacterial growth
Eyes/conjunctival epithelium	• Flushing action of tears: removes microorganisms • Lysozyme in tears: destroys the bacterial cell wall • Mechanical blinking of the eyelid: removes microorganisms
Skin-associated lymphoid tissue	• Mediates specific and nonspecific protection mechanisms against microorganisms that penetrate the outer tissue layers

TABLE 3.2 Protective Characteristics of Mucous Membranes

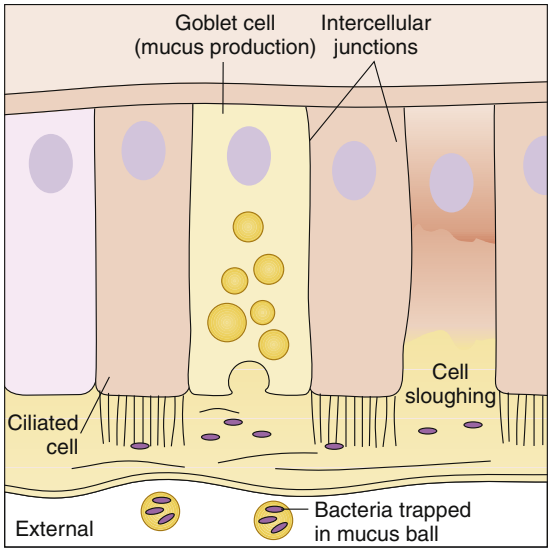
Mucous Membrane	Protective Activity
Mucosal cells	• Rapid sloughing for bacterial removal • Tight intercellular junctions: prevent bacterial penetration

Goblet cells	• Mucus production: protective lubrication of cells; bacterial trapping; contains specific antibodies with specific activity against bacteria
	• Provision of antibacterial substances to mucosal surface:
	  • Lysozyme (degrades bacterial cell wall)
	  • Lactoferrin (competes for bacterial iron supply)
	  • Lactoperoxidase (production of substances toxic to bacteria)

Mucosa-associated lymphoid tissue	• Mediates specific responses against bacteria that penetrate the outer layer



• **Fig. 3.2** Skin and skin structures.



• **Fig. 3.3** General features of mucous membranes, highlighting the protective features such as ciliated cells, mucus production, tight intercellular junctions, and cell sloughing.

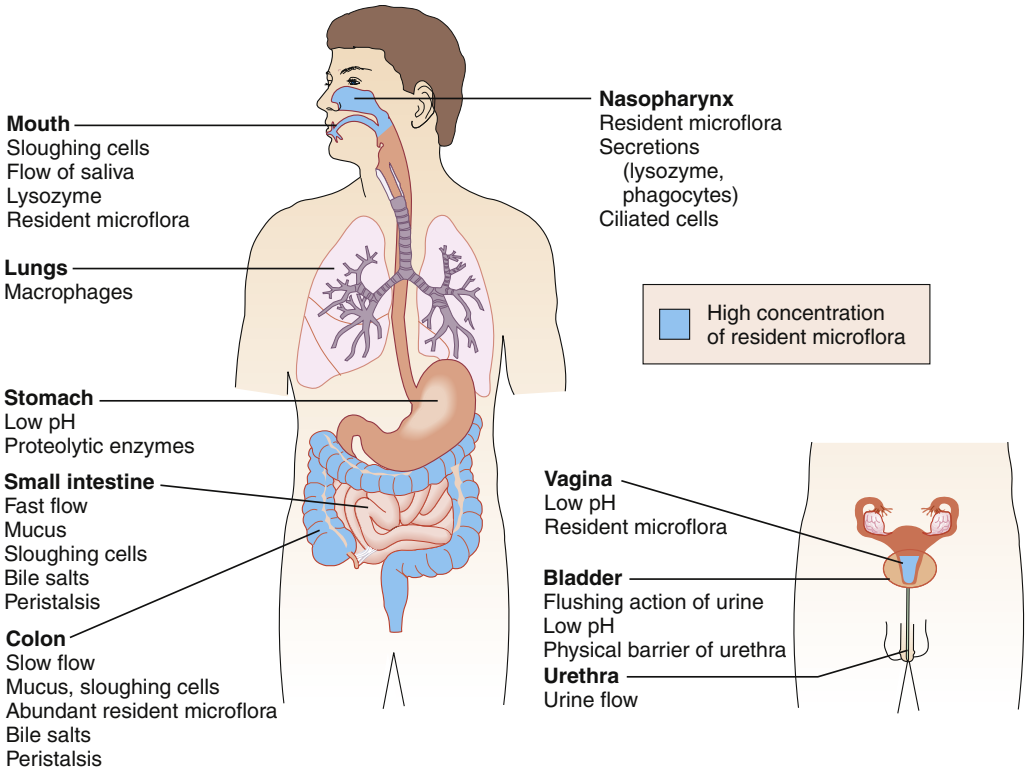
mediate specific and nonspecific responses directed at controlling microbial invaders.

Mucous Membranes

Because cells that line the respiratory tract, gastrointestinal tract, and genitourinary tract are involved in numerous functions besides protection, they are not covered with a hardened, acellular layer. However, the cells that compose these membranes still exhibit various protective characteristics (Table 3.2 and Fig. 3.3).

General Protective Characteristics

Mucus is a major protective component of the membranes. This substance serves to trap bacteria before they can reach the outer surface of the cells, lubricates the cells to prevent damage that promotes microbial invasion, and contains specific chemical (i.e., antibodies) and nonspecific antibacterial substances. In addition to the chemical properties and physical movement of the mucus and trapped microorganisms mediated by ciliary action, rapid cellular shedding and tight intercellular connections provide effective barriers to infection. As is the case with the skin, specific cell clusters,



• **Fig. 3.4** Protective characteristics associated with the mucosal linings of different internal body surfaces.

known as **mucosa-associated lymphoid tissue,** exist below the outer cell layer and mediate specific protective mechanisms against microbial invasion.

Specific Protective Characteristics

Besides the general protective properties of mucosal cells, the mucosal linings throughout the body have characteristics specific to each anatomic site (Fig. 3.4).

The mouth, or **oral cavity,** is protected by the flow of saliva that physically carries microorganisms away from cell surfaces and contains antibacterial substances, such as antibodies (immunoglobulin A [IgA]) and **lysozyme** that participate in the destruction of bacterial cells. The mouth is heavily colonized with protective microorganisms that produce substances that hinder successful invasion by harmful organisms.

In the gastrointestinal tract, the low pH and proteolytic (protein-digesting) enzymes of the stomach prevent the growth of many microorganisms. In the small intestine, bile salts provide protection that disrupts bacterial membranes, and by peristaltic movement and the fast flow of intestinal contents, which hinder microbial attachment to mucosal cells. Although the large intestine also contains bile salts, the movement of bowel contents is slower, permitting a higher concentration of microbial agents the opportunity to attach to the mucosal cells and inhabit the gastrointestinal tract. As in the oral cavity, the high concentration of normal microbial inhabitants in the large bowel also contributes significantly to protection.

In the upper respiratory tract, nasal hairs keep out large airborne particles that may contain microorganisms. The cough-sneeze reflex significantly contributes to the removal of potentially infective agents. The cells lining the trachea contain **cilia** (hairlike cellular projections) that move microorganisms trapped in mucus upward and away from the delicate cells of the lungs (Fig. 3.3) by the **mucociliary escalator.** These barriers are so effective that only inhalation of particles smaller than 2 to 3 μm have a chance of reaching the lungs.

In the female urogenital tract, the vaginal lining and the cervix are protected by colonization with normal microbial inhabitants and a low pH. A thick mucus plug in the cervical opening is a substantial barrier that keeps microorganisms from ascending and invading the delicate tissues of the uterus, uterine tubes, and ovaries. The anterior urethra of males and females is colonized with microorganisms, and a stricture at the urethral opening provides a physical barrier that, combined with a low urine pH and the flushing action of urination, protects against bacterial invasion of the bladder, ureters, and kidneys.

The Microorganism's Perspective

As previously discussed, microorganisms that inhabit many surfaces of the human body (Fig. 3.4) are referred to as **colonizers, normal flora, normal microbiota,** and collectively as the **human microbiome.** Some are **transient colonizers** because they are able to survive, but do not multiply, on

• BOX 3.2 Microbial Factors That Contribute to Colonization of Host Surfaces

Survival Against Environmental Conditions

- Localization in moist areas
- Protection in ingested or inhaled debris
- Expression of specific metabolic characteristics (e.g., salt tolerance)

Achieving Attachment and Adherence to Host Cell Surfaces

- Pili
- Adherence proteins
- Biofilms
- Various protein adhesins

Other Factors

- Motility
- Production of substances that compete with the host for acquisition of essential nutrients (e.g., siderophores to capture iron)
- Ability to coexist with other colonizing microorganisms

the surface and frequently shed with the host cells. Others, called **resident microbiota,** not only survive but also thrive and multiply; their presence is more persistent.

The body's microbiota varies considerably with anatomic location. For example, environmental conditions, such as temperature and oxygen availability, differ considerably between the nasal cavity and the small bowel. Only microorganisms with the metabolic capability to survive under the physiologic conditions of the anatomic location are inhabitants of those particular body surfaces.

Knowledge of the microbiota of the human body is extremely important in diagnostic microbiology, especially for determining the clinical significance of microorganisms isolated from patient specimens. Organisms considered normal microbiota are often in clinical specimens. This may be a result of contamination of normally sterile specimens during the collection process or because the colonizing organism is actually involved in the infection. Microorganisms considered as normal colonizers of the human body and the anatomic locations they colonize are addressed in Part VII.

Microbial Colonization

Colonization may be the last step in the establishment of a long lasting, mutually beneficial (i.e., commensal) or harmless relationship between a colonizer and the human host. Alternatively, colonization may be the first step in the process for the development of infection and disease. Whether colonization results in a harmless or damaging infection depends on the characteristics of the host and the microorganism. In either case, successful initial colonization depends on the microorganism's ability to survive the conditions first encountered on the host surface (Box 3.2).

To avoid the dryness of the skin, organisms often seek moist areas of the body, including hair follicles, sebaceous (oil or **sebum**) and sweat glands, skin folds, underarms, the genitals or anus, the face, the scalp, and areas around the mouth. Microbial penetration of mucosal surfaces is mediated when an organism embedded in food particles survives oral and gastrointestinal conditions or is contained within airborne particles to aid survival in the respiratory tract. Microorganisms also exhibit metabolic capabilities that assist in their survival. For example, the ability of staphylococci to thrive in relatively high salt concentrations enhances their survival in and among the sweat glands of the skin.

Besides surviving the host's physical and chemical conditions, colonization also requires that microorganisms **attach** and **adhere** to host surfaces (Box 3.2). Attachment can be particularly challenging in places such as the mouth and bowel, in which the surfaces are frequently flushed with passing fluids. Pili, the rodlike projections of bacterial envelopes; various molecules (e.g., adherence proteins and adhesins); and biochemical complexes (e.g., biofilm) work together to enhance attachment of microorganisms to the host cell surface. Biofilm is discussed in more detail later in this chapter. (For more information concerning the structure and functions of pili, see Chapter 2.)

In addition, microbial motility with flagella allows organisms to move around and actively seek optimum conditions. Finally, because no single microbial species is a lone colonizer, successful colonization also requires that a microorganism be able to coexist with other microorganisms.

Microorganism Entry, Invasion, and Dissemination

The Host's Perspective

In most instances, to establish infection, microorganisms must **penetrate** or circumvent the host's physical barriers (i.e., skin or mucosal surfaces); overcoming these defensive barriers depends on both host and microbial factors. When these barriers are broken, numerous other host defensive strategies activate.

Disruption of Surface Barriers

Any situation that disrupts the physical barrier of the skin and mucosa, alters the environmental conditions (e.g., loss of stomach acidity or dryness of the skin), changes the functioning of surface cells, or alters the normal microbiota facilitates the penetration of microorganisms past the barriers and into deeper host tissues. Disruptive factors may vary from accidental or intentional (medical) trauma resulting in surface destruction to the use of antibiotics that remove normal, protective, colonizing microorganisms (Box 3.3). A number of these factors are a result of a medical intervention or procedure.

Responses to Microbial Invasion of Deeper Tissue

Once an organism circumvents surface barriers, the host responds to a microbial presence in the underlying tissue in various ways. Some of these responses are nonspecific, because they occur regardless of the type of invading

organism; other responses are more specific and involve the host's immune system. Both nonspecific and specific host responses are critical if the host is to survive. Without them, microorganisms would multiply and invade vital tissues and organs, resulting in severe damage to the host.

• BOX 3.3 Factors That Contribute to the Disruption of the Skin and Mucosal Surface

Trauma

- Penetrating wounds
- Abrasions
- Burns (chemical and fire)
- Surgical wounds
- Needle sticks

Inhalation

- Noxious or toxic gases
- Particulate matter
- Smoking

Implantation of Medical Devices

Other Diseases

- Malignancies
- Diabetes
- Previous or simultaneous infections
- Alcoholism and other chemical dependencies

Childbirth

Overuse of Antibiotics

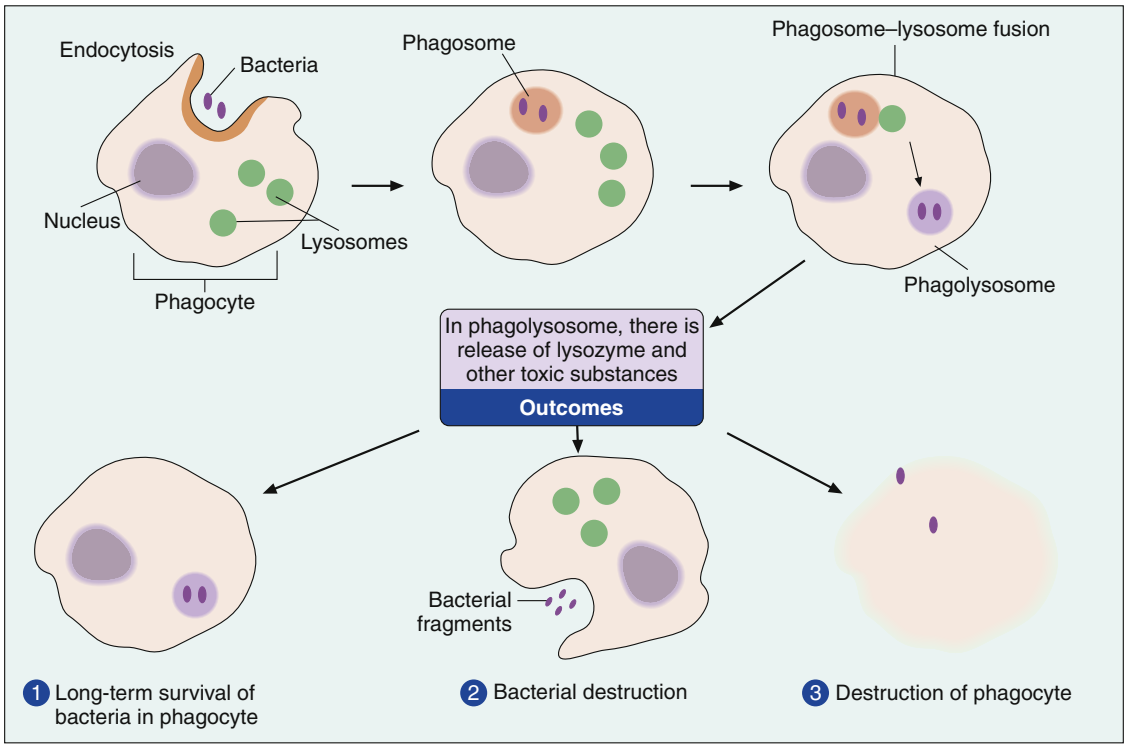
Nonspecific Responses

Some nonspecific responses are biochemical; others are cellular. Biochemical factors remove essential nutrients, such as iron, from tissues so that it is unavailable for use by invading microorganisms. Cellular responses are central to tissue and organ defenses, and the primary cells responsible are **phagocytes.**

Phagocytes

Phagocytes are cells that ingest and destroy bacteria and other foreign particles. The types of phagocytes are polymorphonuclear leukocytes, also known as **neutrophils (PMNs), monocytes** (circulating mononuclear white blood cells) or **macrophages (mononuclear white blood cells found in tissue),** and **dendritic cells.** Phagocytes ingest bacteria by a process known as **phagocytosis** and engulf them in a membrane-lined structure called a **phagosome** (Fig. 3.5). The phagosome fuses with a second structure, the **lysosome.** When the lysosome, which contains toxic chemicals and destructive enzymes, combines with the phagosome, the bacteria that are trapped within the **phagolysosome** are neutralized and destroyed. This destructive process occurs inside membrane-lined structures to prevent the noxious substances contained within the phagolysosome from destroying the phagocyte itself. This is evident during the course of rampant infections when thousands of phagocytes exhibit "sloppy" ingestion of the microorganisms and toxic substances spill from the cells, damaging the surrounding host tissue.

The two major phagocytes, PMNs and mononuclear cells, differ in viability and anatomic distribution. PMNs develop in the bone marrow and spend their short lives (usually a day



• **Fig. 3.5** Overview of phagocyte activity and possible outcomes of phagocyte-bacterial interactions.

TABLE 3.3 Components of Inflammation

Component	Functions
Phagocytes (polymorphonuclear neutrophils [PMNs], dendritic cells, and monocytes)	• Ingest and destroy microorganisms
Complement system (coordinated group of serum proteins)	• Attracts phagocytes to the site of infection (chemotaxis) • Helps phagocytes to recognize and bind to bacteria (opsonization) • Directly kills gram-negative bacteria (membrane attack complex)
Coagulation system (wide variety of proteins and other biologically active compounds)	• Attracts phagocytes to the site of infection • Increases blood and fluid flow to the site of infection • Walls off the site of infection, physically inhibiting the spread of microorganisms
Cytokines (proteins secreted by macrophages and other cells)	• Multiple effects that enhance the activities of many different cells essential to nonspecific and specific defensive responses

or less) circulating in blood and tissues. Widely dispersed in the body, PMNs usually are the first cells on the scene of bacterial invasion. Mononuclear cells (**monocytes**) also develop in the bone marrow. When deposited in tissue or at a site of infection, monocytes transform into mature macrophages. In the absence of infection, macrophages usually reside in specific organs, such as the spleen, lymph nodes, liver, or lungs, where they live for days to several weeks, awaiting encounters with invading bacteria. In addition to the ingestion and destruction of bacteria, macrophages play an important role in mediating immune system defenses (see Specific Responses—The Immune System later in this chapter).

In addition to the inhibition of microbial proliferation by phagocytes and biochemical substances such as lysozyme, microorganisms are "washed" from tissues during the flow of lymph fluid. The fluid carries infectious agents through the lymphatic system, where they are deposited in tissues and organs (e.g., lymph nodes and the spleen) heavily populated with phagocytes. This process functions as an efficient filtration system.

Inflammation

Because microbes may survive the initial encounters with phagocytes (Fig. 3.5), the inflammatory response plays an extremely important role as a primary mechanism against microbial survival and proliferation in tissues and organs. Inflammation has both cellular and biochemical components that interact in various complex ways (Table 3.3).

The **complement system** is composed of a coordinated group of proteins activated by the immune system because of the presence of invading microorganisms. On activation of this system, a cascade of biochemical events occurs that attracts (**chemotaxis**) and enhances the activities of phagocytes. Because PMNs and macrophages are widely dispersed throughout the body, signals attract and concentrate these cells at the point of invasion, and serum complement proteins provide many of these signals. **Cytokines** are chemical substances, or proteins secreted by a cell, that have effects on the activities of other cells. Cytokines draw more phagocytes toward the infection and activate the maturation of monocytes to macrophages.

Additional protective functions of the complement system is enhanced by hemostasis, which works to increase blood flow to the area of infection and can effectively wall off the infection through the production of clots and barriers composed of cellular debris.

The manifestations of **inflammation** are readily evident and are familiar to most adults; they include the following:

- Swelling—caused by an increased flow of fluid and cells to the affected body site
- Redness—results from vasodilation of blood vessels and increased blood flow at the infection site
- Heat—results from increased cellular metabolism and energy production in the affected area
- Pain—caused by tissue damage and pressure on nerve endings from an increased flow of fluid and cells

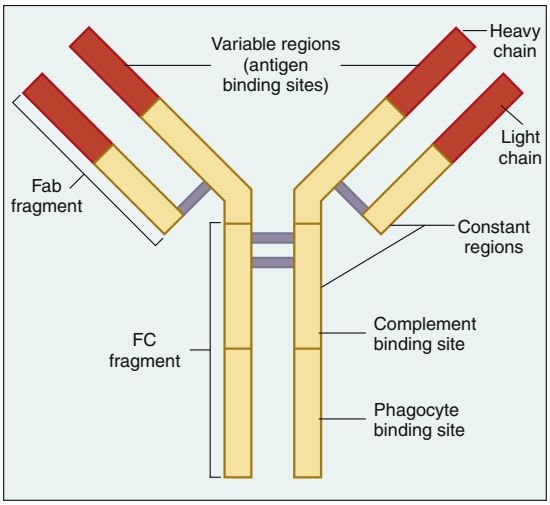
On a microscopic level, the presence of phagocytes at the infection site is an important observation in diagnostic microbiology. Microorganisms associated with these host cells are frequently identified as the cause of a particular infection.

Specific Responses—The Immune System

The immune system provides the human host with the ability to mount a specific protective response to the presence of the invading microorganism. In addition to this specificity, the immune system has a "memory." When a microorganism is encountered a second or third time, an immune-mediated defensive response is immediately available. However, nonspecific (i.e., phagocytes, inflammation) and specific (i.e., the immune system) host defensive systems are interdependent in their efforts to limit the spread of infection.

Components of the Immune System

The central molecule of the immune response is the antibody. **Antibodies,** also referred to as **immunoglobulins,** are specific glycoproteins produced by **plasma cells** (activated B cells) in response to the presence of a molecule recognized as foreign to the host (referred to as an **antigen**). In the case of infectious diseases, antigens are chemicals or toxins secreted by the invading microorganism or components of the organism's structure and are usually composed of proteins or polysaccharides. Antibodies circulate in the plasma or liquid portion of the host's blood and are present in secretions such as saliva. These molecules have two active areas: the antigen-binding site (**Fab region**) and the phagocyte and complement binding sites (**Fc region**) (Fig. 3.6).



• **Fig. 3.6** General structure of the immunoglobulin G (IgG)-class antibody molecule.

Five major classes or **isotypes** of antibody exist: IgG, IgA, IgM, IgD, and IgE. Each class has distinctive molecular configurations. **IgM** is the largest and first antibody produced when an invading microorganism is encountered in the host; production of the most abundant antibody, **IgG,** follows. IgG consists of four subclasses, IgG1 to IgG4, that have variations in their constant regions resulting in different effector functions related to phagocytosis, complement activation, and antibody-dependent cell-mediated cytotoxicity. **IgA** is secreted in various body fluids (e.g., saliva and tears) and primarily protects body surfaces lined with mucous membranes. IgA also includes subclasses: IgA1 is predominantly located in the blood stream, and IgA2, which is more resistant to proteolytic cleavage, is located predominantly in secretions. Increased **IgE** is associated with parasitic infections and allergies. **IgD** is attached to the surface of specific immune system cells and is involved in the regulation of antibody production. As is discussed in Chapter 9, the ability to measure specific antibody production is a valuable tool for the laboratory diagnosis of infectious diseases.

Regarding the cellular components of the immune response, there are three major types of cells: **B lymphocytes (B cells), T lymphocytes (T cells),** and **natural killer cells (NK cells)** (Box 3.4). B lymphocytes originate from stem cells and develop into B cells in the bone marrow before being widely distributed to lymphoid tissues throughout the body. These cells primarily function as antibody producers (plasma cells). T lymphocytes also originate from bone marrow stem cells, but they mature in the thymus and either directly destroy infected cells (cytotoxic T cells, TC or CTLs) or work with B cells (**helper T cells, TH**) to regulate antibody production. **Regulatory T cells (Tregs)** suppress autoimmune responses by other T lymphocytes and mediate immune tolerance. NK cells are a subset of T cells. There are different types of NK cells, with the most prevalent referred to as **invariant natural killer T (NKT) cells.** NKT cells develop in the thymus from the same precursor cells as other T lymphocytes. NKT cells have a limited repertoire of T-cell receptors that respond to synthetic, bacterial, and fungal glycolipids. NKT cells are activated by the release of cytokines during viral infections. Each of the three cell types is strategically located in lymphoid tissue throughout the body to maximize the chances of encountering invading microorganisms that the lymphatic system drains from the site of infection.

• BOX 3.4 Cells of the Immune System

B Lymphocytes (B Cells)

- Location: Lymphoid tissues (lymph nodes, spleen, gut-associated lymphoid tissue, tonsils)
- Function: Antibody-producing cells
- Subtypes:
  - B lymphocytes: Cells waiting to be stimulated by an antigen
  - Plasma cells: Activated B lymphocytes that secrete antibody in response to an antigen
  - B memory cells: Long-living cells preprogramed to an antigen for subsequent exposure

T Lymphocytes (T Cells)

- Location: Circulate and reside in lymphoid tissues (lymph nodes, spleen, gut-associated lymphoid tissue, tonsils)
- Functions: Multiple (see different subtypes)
- Subtypes:
  - Helper T cells (TH): Interact with B cells to facilitate antibody production
  - Cytotoxic T cells (TC): Recognize and destroy host cells that have been invaded by microorganisms
  - Suppressor T cells (TS): Mediate regulatory responses within the immune system
  - T memory cells: Long-living cells preprogramed to an antigen for subsequent exposure

Natural Killer Cells (NK Cells)

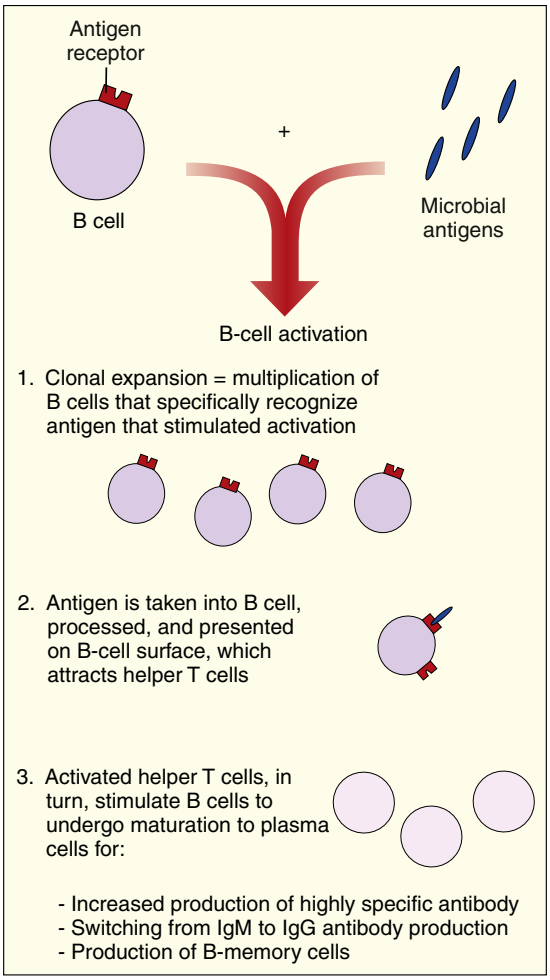
- Function: Similar to that of cytotoxic T cells but do not require the presence of an antigen to stimulate function

Two Branches of the Immune System

The immune system provides immunity through two main branches:

- **Antibody-mediated immunity,** or **humoral immunity**
- **Cell-mediated immunity,** or **cellular immunity**

Antibody-mediated immunity involves the activities of B cells and the production of antibodies. When a B cell encounters a microbial antigen, the cell is activated and initiates a series of events. These events are mediated by helper T cells and the release of cytokines. Cytokines mediate **clonal expansion** and the number of B cells capable of recognizing the antigen increases. Cytokines also activate the maturation of B cells into plasma cells that produce antibodies specific for the antigen. The process results in the production of B memory cells (Fig. 3.7). **B memory cells** remain quiescent in the body until a second (anamnestic) or subsequent exposure to the original antigen occurs. With secondary exposure, the B memory cells are preprogrammed



• **Fig. 3.7** Overview of B-cell activation, which is central to antibody-mediated immunity.

to produce specific antibodies immediately upon encountering the original antigen.

Antibodies protect the host in a number of ways:

- Helping phagocytes to ingest and kill microorganisms through a coating mechanism, referred to as **opsonization**
- Neutralizing microbial toxins that are detrimental to host cells and tissues
- Promoting bacterial clumping (**agglutination**) that facilitates clearing from the infection site
- Inhibiting bacterial motility
- **Viral neutralization;** blocking the virus from entering the host cell
- Combining with microorganisms to activate the complement system and inflammatory response

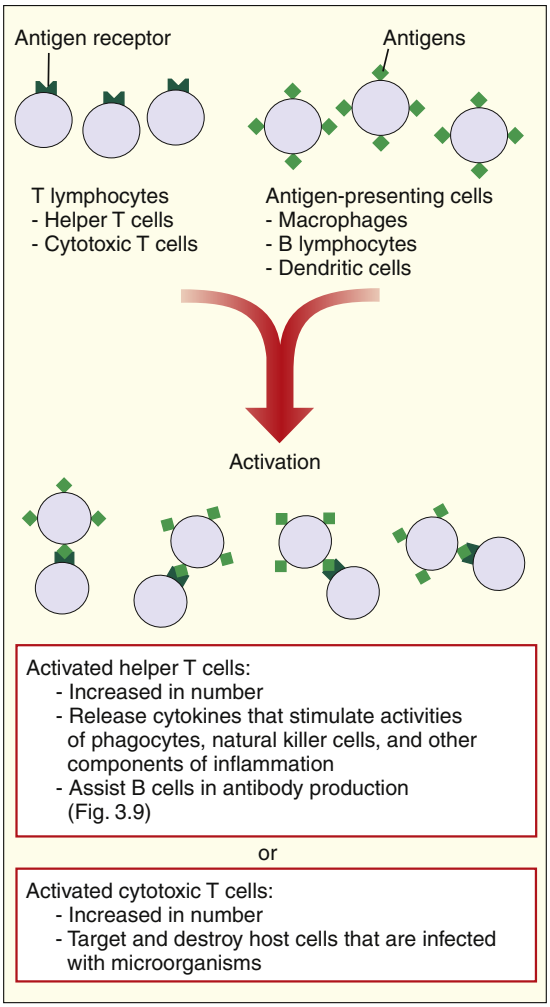
Because a population of activated specific B cells is a developmental process that results from exposure to microbial antigens, antibody production is delayed when the host is first exposed to an infectious agent. This delay in the **primary antibody response** underscores the importance of nonspecific response defenses, such as inflammation, that work to hold the invading organisms in check while antibody production begins. This also emphasizes the importance of B memory cell production. By virtue of this memory, any subsequent exposure or **secondary antibody response** to the same microorganism results in rapid production of protective antibodies avoiding the delays characteristic of the primary exposure.

Some antigens, such as bacterial capsules and outer membranes, activate B cells to produce antibodies without the intervention of helper T cells. However, this activation does not result in the production of B memory cells, and subsequent exposure to the same bacterial antigens does not result in a rapid host memory response.

The primary cells involved in cell-mediated immunity are T lymphocytes (cytotoxic T cells) that recognize and destroy human host cells infected with microorganisms. This function is extremely important for the destruction and elimination of infecting microorganisms. Cytotoxic T cells activated during the primary immune response also form a subset of memory T cells that are able to respond quickly to a subsequent infection from a previously encountered pathogen. Some pathogens (e.g., viruses, tuberculosis, some parasites, and fungi) are able to survive in host cells, protected from antibody interaction. Antibody-mediated immunity targets microorganisms outside human cells, whereas cell-mediated immunity targets microorganisms inside human cells. However, in many instances, these two branches of the immune system overlap and work together.

Like B cells, T cells must become activated in order to be effective. T-cell activation occurs through interactions with other cells that process microbial antigens and present them on their surface (e.g., macrophages, dendritic cells, and B cells). The responses of activated T cells are very different and depend on the subtype of T cell (Fig. 3.8). Activated helper T cells work with B cells for antibody production (Fig. 3.7) and facilitate inflammation by releasing cytokines. Cytotoxic T cells directly interact with and destroy host cells containing microorganisms or other infectious agents, such as viruses. The activated T cell subset, helper or cytotoxic cells, are controlled by an extremely complex series of biochemical pathways and genetic diversity within the **major histocompatibility complex (MHC).** MHC molecules are present on cells and form a complex with the antigen to present them to the T cells. The two primary classes of major histocompatibility molecules are **MHC I** and **MHC II.** MHC I molecules are located on every nucleated cell in the body and are predominantly responsible for the recognition of **endogenous proteins** expressed from within the cell. MHC II molecules are located on specialized cell types, including macrophages, dendritic cells, and B cells, for the presentation of extracellular molecules or **exogenous proteins.**

In summary, the host presents a spectrum of challenges to invading microorganisms, from physical barriers, including the skin and mucous membranes, to the interactive cellular and biochemical components of inflammation and the immune system. All these systems work together to minimize microbial invasion and prevent damage to vital tissues and organs resulting from the presence of infectious agents.



• **Fig. 3.8** Overview of T-cell activation, which is central to cell-mediated immunity.

The Microorganism's Perspective

Given the complexities of the human host's defense systems, it is no wonder that microbial strategies designed to survive these systems are equally complex.

Colonization and Infection

Many surfaces on the human body are colonized with a wide variety of microorganisms or microbiota without apparent detriment. In contrast, an **infection** involves the growth and multiplication of microorganisms that result in damage to the host. The extent and severity of the damage depend on many factors, including the microorganism's ability to cause disease, the site of the infection, and the general health of the individual infected. **Disease** results when the infection produces notable changes in human physiology associated with damage or loss of function to one or more of the body's organ systems.

Pathogens and Virulence

Microorganisms that cause infections or disease are considered **pathogens,** and the characteristics that enable them to cause disease are referred to as **virulence factors.** Most virulence factors protect the organism against host attack or mediate damaging effects on host cells. The terms **pathogenicity** and **virulence** reflect the degree to which a microorganism is capable of causing disease. Pathogenicity specifically refers to the organism's ability to cause disease, whereas virulence refers to the measure or degree of pathogenicity of an organism. An organism of high pathogenicity is very likely to cause disease, whereas an organism of low pathogenicity is much less likely to cause infection. When disease does occur, highly virulent organisms often severely damage the human host. The degree of severity decreases with diminishing virulence of the microorganism.

Because host factors play a role in the development of infectious diseases, the distinction between a **pathogenic** and **nonpathogenic** organism and colonizer is not always clear. For example, many organisms that colonize the skin usually do not cause disease (i.e., exhibit low pathogenicity) under normal circumstances. However, when damage to the skin occurs (Box 3.3) or when the skin is disrupted in some other way, these organisms can gain access to deeper tissues and establish an infection.

Organisms that cause infection when one or more of the host's defense mechanisms are disrupted or malfunction are known as **opportunistic pathogens,** and the infections they cause are referred to as **opportunistic infections.** On the other hand, several pathogens known to cause serious infections can be part of an individual's microbiome (i.e., **carriers**) and never cause disease. However, the same organism can cause life-threatening infection when transmitted to other individuals. The reasons for these inconsistencies are not fully understood, but such widely different results undoubtedly involve complex interactions between microorganism and human. Recognizing and separating a pathogenic from a nonpathogenic organism present one of the greatest challenges in interpreting diagnostic microbiology laboratory results.

Microbial Virulence Factors

Virulence factors provide microorganisms with the capacity to avoid host defenses and damage host cells, tissues, and organs in a number of ways. Some virulence factors are specific for certain pathogenic genera, species, or strains of a microorganism, and substantial differences exist in the way bacteria, viruses, parasites, and fungi cause disease. Knowledge of a microorganism's capacity to cause specific types of infections plays a major role in the development of diagnostic microbiology procedures used for isolating and identifying microorganisms. (See Part VII for more information regarding diagnosis by organ system.)

Attachment

Whether humans encounter microorganisms in the air, through ingestion, or by direct contact, the first step of infection and disease development, a process referred to as **pathogenesis,** is microbial **attachment** to a surface

(exceptions being instances in which the organisms are directly introduced by trauma or other means into deeper tissues).

Many of the microbial factors that facilitate attachment of pathogens are the same as those used by nonpathogenic colonizers (Box 3.2). Most pathogenic organisms are not part of the normal human microbiota, and attachment to the host requires that they outcompete the microbiota for a place on the body's surface. Medical interventions, such as the overuse of antimicrobial agents, result in the destruction of the normal microbiota, creating a competitive advantage for the invading pathogenic organism.

Invasion

Once surface attachment has been secured, microbial **invasion** into subsurface tissues and organs (i.e., infection) is accomplished by disruption of the skin and mucosal surfaces by several mechanisms (Box 3.3) or by the direct action of an organism's virulence factors. Some microorganisms produce factors that force **mucosal surface phagocytes (M cells)** to ingest them and then release them unharmed into the tissue below the surface. Other organisms, such as staphylococci and streptococci, are not so subtle. These organisms produce an array of enzymes (e.g., hyaluronidases, nucleases, collagenases) that hydrolyze host proteins and nucleic acids, destroying host cells and tissues. This destruction allows the pathogen to "burrow" through minor openings in the outer surface of the skin and into deeper tissues. Once a pathogen has penetrated the body, it uses a variety of strategies to survive attack by the host's inflammatory and immune responses. Alternatively, some pathogens cause disease at the site of attachment without further penetration. For example, in diseases such as diphtheria and whooping cough, the bacteria produce toxic substances that destroy surrounding tissues. The organisms generally do not penetrate the mucosal surface they inhabit.

Survival Against Inflammation

If a pathogen is to survive, the action of the phagocytes and the complement components of inflammation must be avoided or controlled (Box 3.5). Some organisms, such as *Streptococcus pneumoniae,* a common cause of bacterial pneumonia and meningitis, avoid phagocytosis by producing a large capsule that inhibits the phagocytic process. Other pathogens may not be able to avoid phagocytosis but are not effectively destroyed once internalized and are able to survive within phagocytes. This is the case for *Mycobacterium tuberculosis,* the bacterium that causes tuberculosis. Still other pathogens use toxins and enzymes to attack and destroy phagocytes before the phagocytes attack and destroy them.

The defenses offered by the complement system depend on a series of biochemical reactions triggered by specific microorganism molecular structures. Therefore microbial avoidance of complement activation requires that the infecting agent either mask its activating molecules (e.g., via production of a capsule that covers bacterial surface antigens) or produce substances (e.g., enzymes) that disrupt critical biochemical components of the complement pathway.

• BOX 3.5 Microbial Strategies for Surviving Inflammation

Avoid Killing by Phagocytes (Polymorphonuclear Leukocytes)

- Producing a capsule, thereby inhibiting phagocytes' ability to ingest them
- Antigenic variation, changing surface antigens to limit the number of cells recognized by the immune system

Avoid Phagocyte-Mediated Killing

- Inhibiting phagosome-lysosome fusion
- Being resistant to destructive agents (e.g., lysozyme) released by lysosomes
- Actively and rapidly multiplying within a phagocyte
- Releasing toxins and enzymes that damage or kill phagocytes

Avoid Effects of the Complement System

- Using a capsule to hide surface molecules that would otherwise activate the complement system, including the formation of a complex protein polysaccharide matrix (biofilm)
- Producing substances that inhibit the processes involved in complement activation
- Producing substances that destroy specific complement proteins

Any single microorganism may possess numerous virulence factors, and several may be expressed simultaneously. For example, while trying to avoid phagocytosis, an organism may also secrete other enzymes and toxins that destroy and penetrate tissue and produce other factors designed to interfere with the immune response. Microorganisms may also use host systems to their own advantage. For example, the lymphatic and circulatory systems used to carry monocytes and lymphocytes to the site of infection may serve to disperse the organism throughout the body.

Survival Against the Immune System

Microbial strategies to avoid the defenses of the immune system are outlined in Box 3.6. Again, a pathogen can use more than one strategy to avoid immune-mediated defenses, and microbial survival does not necessarily require devastation of the immune system. The pathogen may merely need to "buy" time to reach a safe area in the body or to be transferred to the next susceptible host. In addition, microorganisms can avoid much of the immune response if they do not penetrate the surface layers of the body. This strategy is the hallmark of diseases caused by microbial toxins.

Microbial Toxins

Toxins are biochemically active substances released by microorganisms that have a particular effect on host cells. Microorganisms use toxins to establish infections and multiply within the host. Alternatively, a pathogen may be restricted to

• BOX 3.6 Strategies That Microbial Pathogens Use to Survive the Immune Response

- Rapid invasion and multiplication resulting in damage to the host before the immune response can be fully activated, or organism's virulence is so great that the immune response is insufficient
- Invasion and destruction of cells involved in the immune response
- Survival in host cells and avoiding detection by the immune system
- Masking the organism's antigens with a capsule or biofilm so that an immune response is not activated
- Altering the expression and presentation of antigens so that the immune system is constantly fighting a primary encounter (i.e., the memory of the immune system is neutralized)
- Production of enzymes (proteases) that directly destroy or inactivate antibodies

• BOX 3.7 Summary of Bacterial Toxins

Endotoxins

- General toxin common to almost all gram-negative bacteria
- Composed of the lipopolysaccharide portion of cell envelope
- Released when a gram-negative bacterial cell is destroyed
  - Effects on host include:
    - Disruption of clotting, causing clots to form throughout the body (i.e., disseminated intravascular coagulation [DIC])
    - Fever
    - Activation of complement and immune systems
    - Circulatory changes that lead to hypotension, shock, and death

Exotoxins

- Most commonly associated with gram-positive bacteria
- Produced and released by living bacteria; do not require bacterial death for release
- Specific toxins target specific host cells; the type of toxin varies with the bacterial species
- Some kill host cells and help spread bacteria in tissues (e.g., enzymes that destroy key biochemical tissue components or specifically destroy host cell membranes)
- Some destroy or interfere with specific intracellular activities (e.g., interruption of protein synthesis, interruption of internal cell signals, or interruption of the neuromuscular system)

a particular body site from which toxins are released to cause systemic damage throughout the body. Toxins also can cause human disease in the absence of the pathogens that produced them. This common mechanism of food poisoning involves ingestion of preformed bacterial toxins (present in the food at the time of ingestion) and is referred to as **intoxication,** a notable example of which is botulism.

Endotoxin and **exotoxin** are the two general types of bacterial toxins (Box 3.7). Endotoxin is a component of the cellular structure of gram-negative bacteria and can have devastating effects on the body's metabolism, the most serious being endotoxic shock, which often results in death. The effects of exotoxins produced by gram-positive bacteria tend to be more limited and specific than the effects of gram-negative endotoxin. The activities of exotoxins range from enzymes produced by many staphylococci and streptococci that augment bacterial invasion by damaging host tissues and cells to highly specific activities (e.g., diphtheria toxin inhibits protein synthesis, and cholera toxin interferes with host cell signals). Examples of other highly active and specific toxins are those that cause botulism and tetanus by interfering with neuromuscular functions.

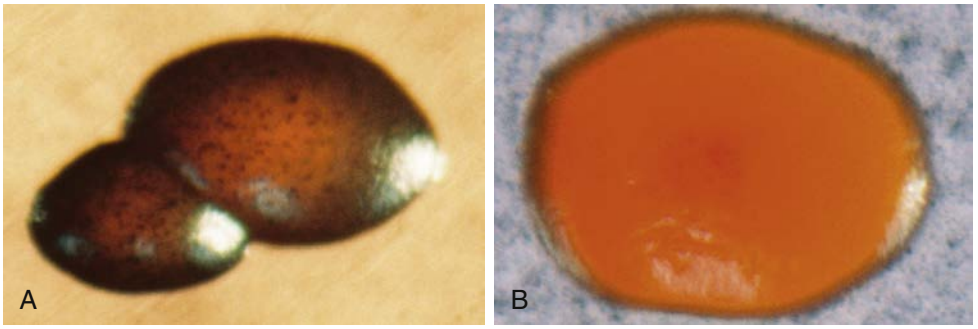
Genetics of Virulence: Pathogenicity Islands

Many virulence factors are encoded in genomic regions of pathogens known as **pathogenicity islands (PAIs).** These mobile genetic elements contribute to the change and spread of virulence factors among bacterial populations of a variety of species. These genetic elements are believed to have evolved from lysogenic bacteriophages and plasmids and are spread by horizontal gene transfer (see Chapter 2 for information about bacterial genetics). PAIs typically comprise one or more virulence-associated genes and "mobility" genes (i.e., integrases and transposases) that mediate movement between various genetic elements (e.g., plasmids and chromosomes) and among different bacterial strains. In essence, PAIs facilitate the dissemination of virulence capabilities among bacteria in a manner similar to the mechanism diagramed in Fig. 2.10; this also facilitates dissemination of antimicrobial resistance genes (Chapter 10). PAIs are widely disseminated among medically important bacteria. For example, PAIs have been identified as playing a role in virulence for each of the following organisms:

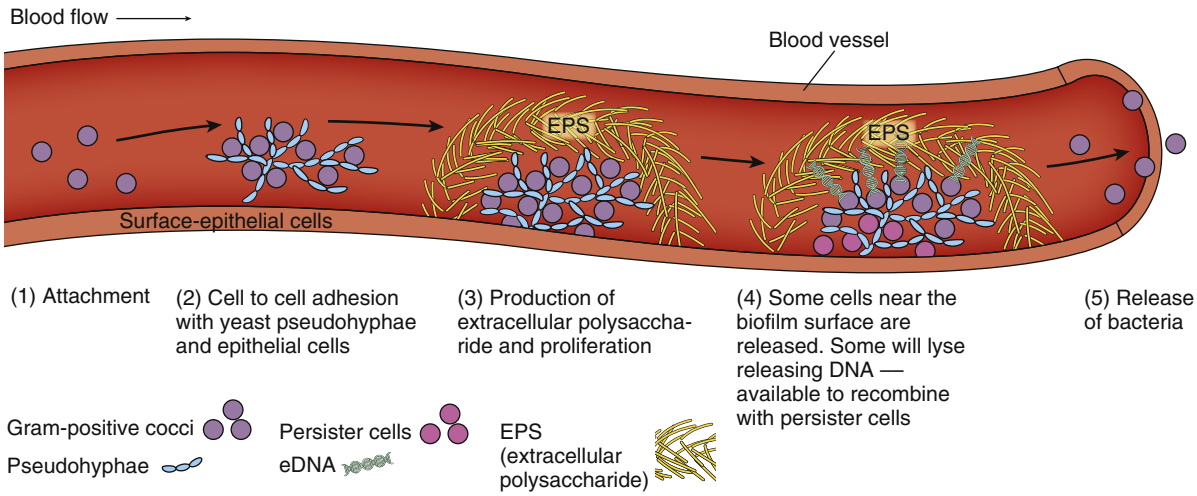
Helicobacter pylori
Pseudomonas aeruginosa
Shigella spp.
Yersinia spp.
Vibrio cholerae
Salmonella spp.
Escherichia coli (enteropathogenic, enterohemorrhagic or serotoxigenic, verotoxigenic, uropathogenic, enterotoxigenic, enteroinvasive, enteroaggregative, meningitis-sepsis associated; Chapter 19)
Neisseria spp.
Bacteroides fragilis
Listeria monocytogenes
Staphylococcus aureus
Streptococcus spp.
Enterococcus faecalis
Clostridioides difficile

Biofilm Formation

Microorganisms typically exist as a group or community of organisms capable of adhering to each other or to other surfaces. A variety of bacterial pathogens, along with other microorganisms, are capable of forming biofilms, such as *S. aureus, P. aeruginosa, Aggregatibacter* spp., *Salmonella* spp.,



• **Fig. 3.9** (A) Biofilm forming isolate of *Staphylococcus aureus* cultivated on Congo red agar. Biofilm production results in the formation of a black precipitate. (B) Non–biofilm-producing strain of *S. aureus* cultivated on Congo red agar.



• **Fig. 3.10** Overview of biofilm formation, maturation, and dissemination of infection.

Citrobacter koseri, and *Candida albicans.* A biofilm is an accumulation of microorganisms embedded in a complex matrix composed of proteins, polysaccharides, extracellular DNA (eDNA), and other molecules. Pathogenic microorganisms use the formation of biofilm to adhere to implants and prosthetic devices. For example, health care–related infections with *Staphylococcus* spp. (Fig. 3.9) associated with implants have become more prevalent. Biofilm-forming strains have a much more complex antibiotic resistance profile, indicating failure of the antibiotic to penetrate the polysaccharide layer. In addition, some of the cells in a **sessile** or stationary biofilm may experience nutrient deprivation and therefore exist in a slow-growing or starved state (i.e., **persister cells**), displaying reduced susceptibility to antimicrobial agents. These organisms also have demonstrated a differential gene expression compared with their **planktonic** or free-floating counterparts. The biofilm-forming communities are able to adapt and respond to changes in their environment, similar to a multicellular organism.

Biofilms may form from the accumulation of a single microorganism (**monomicrobic aggregation**) or from the accumulation of numerous species (**polymicrobic aggregation**). The initial stage in biofilm formation begins with the synthesis of an extracellular polymer matrix accompanied by aggregation and recognition. This process is facilitated by the formation of polysaccharides, proteins, and eDNA. The formation of the biofilm protects the organism from desiccation, forms a barrier against toxic compounds, and prevents the loss of protective organic and inorganic molecules. Once the initial biofilm has developed, a process of maturation of the biofilm occurs, which takes approximately 4 to 6 hours, depending on the growth rate of the microorganism. This includes the complex formation of a three-dimensional architecture, including pores and channels within the polymer matrix. During biofilm accumulation, the cells reach a critical mass that result in the alteration in metabolism and gene expression in the persister cells. This is accomplished through a mechanism of signaling between cells or organisms through chemical signals or inducer molecules, such as acyl homoserine lactone (AHL) in gram-negative bacteria or oligopeptides in gram-positive bacteria. These signals are capable of interspecies and intraspecies communication. In addition, the formation of a complex polymicrobial biofilm provides favorable conditions for the exchange of genetic information and horizontal gene transfer. Fig. 3.10 provides an overview of biofilm formation, maturation, and seeding that results in further dissemination and infection.

Microbial biofilm formation is important to many disciplines, including environmental science, industry, and public health. Biofilm formation affects the efficient treatment of wastewater; it is essential for the effective production of beer, which requires aggregation of yeast cells; and it affects bioremediation for toxic substances such as oil. It has been reported that approximately 65% of hospital-associated infections are associated with biofilm formation. Box 3.8 provides an overview of pathogenic organisms associated with biofilm formation in human infections.

Outcome and Prevention of Infectious Diseases

Outcome of Infectious Diseases

Given the complexities of host defenses and microbial virulence, it is not surprising that the factors determining outcome between these two living entities are also complicated. Outcome depends on the state of the host's health, the virulence of the pathogen, and whether the host can clear the pathogen before infection and disease cause irreparable harm or death (Fig. 3.11).

The time from exposure to an infectious agent and the development of a disease or infection depends on host and microbial factors. Infectious processes that develop quickly are referred to as **acute infections,** and those that develop and progress slowly, sometimes over a period of years, are known as **chronic infections.** Some pathogens, particularly certain viruses, can be clinically silent inside the body without any noticeable effect on the host before suddenly causing a severe and acute infection. During the silent phase, the infection is said to be **latent.** Again, depending on host and microbial factors, acute, chronic, or latent infections can result in any of the outcomes detailed in Fig. 3.11.

Medical intervention can help the host to fight the infection but usually is not instituted until after the host is aware that an infectious process is underway. The clues that an infection is occurring are known as the signs and symptoms of disease and result from host responses (e.g., inflammatory and immune responses) to the action of microbial virulence factors (Box 3.9). **Signs** are measurable indications or physical observations, such as an increase in body temperature (fever) or the development of a rash or swelling. **Symptoms** are indictors as described by the patient, such as headache, aches, fatigue, and nausea. The signs and symptoms reflect the stages of infection. In turn, the stages of infection generally reflect the stages in host-microorganism interactions (Fig. 3.12).

Whether medical procedures contribute to controlling or clearing an infection depends on key factors, including:

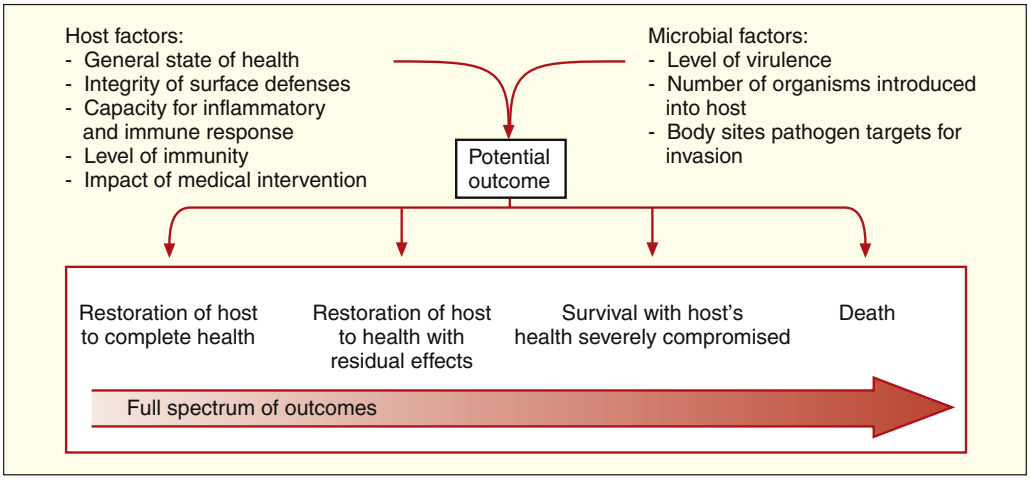
- The severity of the infection, which is determined by the host and microbial interactions already discussed
- Accuracy in diagnosing the pathogen or pathogens causing the infection

• BOX 3.8 Biofilms and Human Infections

These pathogenic organisms have been associated with biofilm formation in human infections.

- *Acinetobacter* spp.
- *Aeromonas* spp.
- *Candida albicans*
- *Citrobacter* spp.
- Coagulase-negative staphylococci
- *Cronobacter* spp.
- *Enterobacter* spp.
- *Enterococcus* spp.
- *Escherichia coli*
- *Klebsiella* spp.
- *Listeria monocytogenes*
- *Proteus* spp.
- *Pseudomonas aeruginosa*
- *Serratia* spp.
- *Staphylococcus aureus*
- *Streptococcus* spp.
- *Listeria monocytogenes*

Not intended to be an all-inclusive list.



• Fig. 3.11 Possible outcomes of infections and infectious diseases.

- Whether the patient receives appropriate treatment for the infection (which depends on accurate diagnosis)

Prevention of Infectious Diseases

The treatment of an infection is often difficult and not always successful. Because much of the damage may already have been done before appropriate medical intervention is provided, the microorganisms gain too much of a "head start." Another strategy for combating infectious diseases is to stop infections before they start (i.e., disease prevention). As discussed at the beginning of this chapter, the first step in any host-microorganism relationship is the encounter and exposure to the infectious agent. Therefore, strategies to prevent disease involve interrupting or minimizing the risk of infection when exposures occur. As outlined in Box 3.10, interruption of encounters may be accomplished by preventing transmission of the infecting agents and by controlling or destroying reservoirs of human pathogens. Interestingly, most of these measures do not really involve medical practices but rather social practices and policies.

• BOX 3.9 Signs and Symptoms of Infection and Infectious Diseases

- General or localized aches and pains
- Headache
- Fever
- Fatigue
- Swollen lymph nodes
- Rashes
- Redness and swelling
- Cough and sneezes
- Congestion of nasal and sinus passages
- Sore throat
- Nausea and vomiting
- Diarrhea

Immunization

Medical strategies exist for minimizing the risk of disease development when exposure to infectious agents occurs. One of the most effective methods is **vaccination,** also referred to as **immunization.** This practice

• BOX 3.10 Strategies for Preventing Infectious Diseases

Preventing Transmission

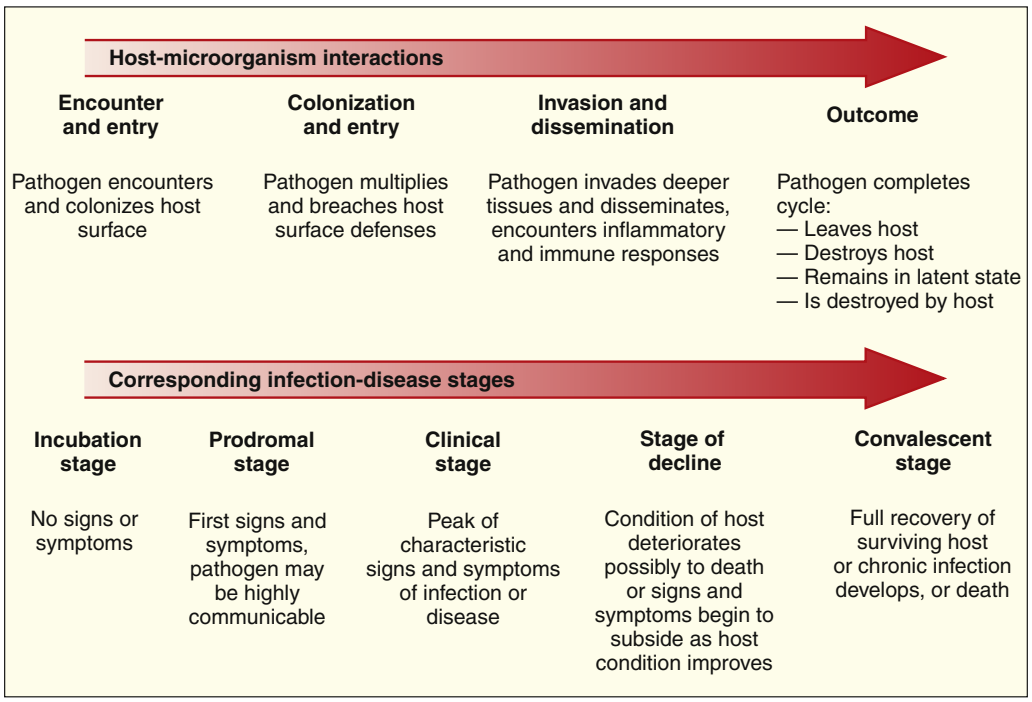
- Avoid direct contact with infected persons or take protective measures when direct contact will occur (e.g., wear gloves, wear condoms).
- Block the spread of airborne microorganisms by wearing masks or isolating persons with infections transmitted by air.
- Use sterile medical techniques.

Controlling Microbial Reservoirs

- Sanitation and disinfection
- Sewage treatment
- Food preservation
- Water treatment
- Control of pests and insect vector populations

Minimizing Risk Before or Shortly After Exposure

- Immunization or vaccination
- Cleansing and use of antiseptics
- Prophylactic use of antimicrobial agents



• **Fig. 3.12** Host-microorganism interactions and stages of infection or disease.

takes advantage of the specificity and memory of the immune system. The two basic approaches to immunization are **active immunization** and **passive immunization.** With active immunization, modified antigens from pathogenic microorganisms are introduced into the body and cause an immune response. If or when the host encounters the pathogen in nature, the memory of the immune system ensures minimal delay in the immune response, thus affording strong protection. With passive immunization, antibodies against a particular pathogen that have been produced in one host are transferred to a second host, where they provide temporary protection. The passage of maternal antibodies to the newborn is a key example of natural passive immunization. Active immunity is generally longer lasting, because the immunized host's own immune response has been activated. However, for complex reasons, naturally acquired active immunity has had limited success for relatively few infectious diseases, necessitating the development of vaccines. Successful immunization has proven effective against many infectious diseases, including diphtheria, whooping cough (pertussis), tetanus, influenza, polio, smallpox, measles, hepatitis, and certain *S. pneumoniae* and *Haemophilus influenzae* infections.

Prophylactic antimicrobial therapy, the administration of antibiotics when the risk of developing an infection is high, is another common medical intervention for preventing infection.

Epidemiology

To prevent infectious diseases, information is required regarding the sources of pathogens, the mode of transmission to and among humans, human risk factors for encountering the pathogen and developing infection, and factors that contribute to good and bad outcomes resulting from the exposure. **Epidemiology** is the science that characterizes these aspects of infectious diseases and monitors the effect diseases have on public health. Fully characterizing the circumstances associated with the acquisition and dissemination of infectious diseases gives researchers a better chance of preventing and eliminating the diseases. In addition, many epidemiologic strategies developed for use in public health systems also apply in long-term care facilities (e.g., nursing homes, hospitals, assisted-living centers) for the control of HAI infections (i.e., nosocomial infections; for more information on infection control, see Chapter 79).

The field of epidemiology is broad and complex. Diagnostic microbiology laboratory personnel and epidemiologists often work closely to investigate problems. Familiarity with certain epidemiologic terms and concepts is important (Box 3.1).

Because the central focus of epidemiology is on tracking and characterizing infections and infectious diseases, this field depends heavily on diagnostic microbiology. Epidemiologic investigations cannot proceed unless researchers first know the **etiologic** or **causative agents.** Therefore the procedures and protocols used in diagnostic microbiology to detect, isolate, and characterize human pathogens are essential for patient care and also play a central role in epidemiologic studies focused on disease prevention and the general improvement of public health. In fact, microbiologists who work in clinical laboratories are often the first to recognize patterns that suggest potential outbreaks or epidemics.

Visit the Evolve site for a complete list of procedures, review questions, and case studies.

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CASE STUDY 3.1

An 8-year-old boy presents to the emergency department (ED) with right upper abdominal pain associated with vomiting, headache, and fever. The boy had been seen in the ED approximately 1.5 months previously for a sore throat, cough, and headache. After the first visit to the ED, the patient was treated with amoxicillin. The boy was born in northern Africa in a refugee camp. He and his family had emigrated from Africa approximately 8 months ago. Generally, the boy appears to be in good health. His immunizations are current, and he has no allergies. He currently resides with his parents and three siblings, who all appear to be in good health. His mother speaks very little English.

The attending physician orders an abdominal computed tomography (CT) scan and identifies a mass in the left hepatic lobe. There appears to be no evidence of gastrointestinal bleeding. The attending physician orders a complete work-up on the patient, including a complete blood count, microbiology tests, chemistry, coagulation, and a hepatitis panel. The laboratory results indicate some type of infection and inflammatory condition. The patient has an elevated white blood cell (WBC) count that correlates with his erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level. The ESR and the CRP level are clear indicators of an inflammatory process.

Questions

1. Identify and differentiate the patient's signs and symptoms.
2. Explain whether this patient likely has an acute or a chronic infection.

Chapter Review

1. An infection acquired from working with an animal reservoir is:
   - **a.** Acquired from a vehicle
   - **b.** Transmitted by a vector
   - **c.** A zoonotic infection
   - **d.** An example of indirect transmission
2. Which of the following is considered an indirect mode of transmission?
   - **a.** A cut with a dirty knife
   - **b.** Ingesting contaminated potato salad
   - **c.** Inhaling a droplet containing a bacterium
   - **d.** Drinking water from a contaminated source
3. Nonspecific immunity includes all of the following *except:*
   - **a.** Inflammation
   - **b.** Phagocytosis by neutrophils
   - **c.** B-cell activation to produce antibodies
   - **d.** Resident normal microbiota
4. Humoral immunity:
   - **a.** Is activated for all infectious agents
   - **b.** Is specific for any organism
   - **c.** Is specifically targeted to an antigen
   - **d.** Provides a broad immune response to any microorganism
5. Bacterial endotoxins are:
   - **a.** All the same
   - **b.** Part of the gram-negative cell wall
   - **c.** Capable of causing a systemic shock response
   - **d.** All of the above
6. A sign is different from a symptom in all of the following ways *except:*
   - **a.** It provides measurable data.
   - **b.** It is believed to be associated with the etiology of the disease.
   - **c.** It is clearly visible.
   - **d.** It includes the temperature, respiratory rate, and pulse.
7. A short-lived infection that manifests with a short incubation period and serious illness is considered to be:
   - **a.** Persistent
   - **b.** Chronic
   - **c.** Latent
   - **d.** Acute
8. A microorganism that colonizes the skin but is capable of causing infection under the appropriate conditions is referred to as:
   - **a.** A pathogenic organism
   - **b.** An opportunistic pathogen
   - **c.** Normal microbiota
   - **d.** A nosocomial pathogen
9. All of the following are involved in humoral immunity *except*:
   - **a.** Cytotoxic T cells
   - **b.** Complement proteins
   - **c.** Plasma cells
   - **d.** Glycoproteins
10. Microorganisms that live in or on the human body without causing damage include:
    - **a.** Colonizers
    - **b.** Normal flora
    - **c.** Microbiota
    - **d.** Human microbiome
    - **e.** All of the above
11. Biofilm formation within a host results in:
    - **a.** Easy clearance of bacterial pathogens
    - **b.** Availability of organic nutrients to the host
    - **c.** Inability of the immune system to remove the pathogen
    - **d.** Starvation of the microorganisms

12. Matching: *Match each term with the correct description.*

_____ vector
_____ nosocomial
_____ fomite
_____ colonization
_____ monocytes
_____ complement
_____ virulence factor
_____ exotoxin
_____ immunization

a. injection of antigens or antibodies to provide immunity
b. inanimate source of infection
c. limited and specific effect
d. long-term health care–associated infection
e. characteristic of a disease-causing organism
f. serum proteins activated in the immune system
g. circulate in the blood before activation
h. insect that carries an infectious agent
i. association between normal microbiota and host

13. Short Answer

Compare and contrast the components of the specific and nonspecific immune defenses, including the occurrence and process of inflammation, phagocytic cells, antibody production, cellular response, and natural physical or chemical properties of the human body.

4 Laboratory Safety

OBJECTIVES

1. Define and differentiate sterilization, disinfection, decontamination, and antiseptic.
2. List the factors that influence the effectiveness of disinfectants in the microbiology laboratory.
3. Describe the methods used for the disposal of hazardous waste, including physical and chemical methods, and the material and/or organisms effectively eliminated by each method.
4. Define a chemical hygiene plan and describe the purpose of the methods and items that are elements of the plan, including proper labeling of hazardous materials, training programs, and safety data sheets (SDS).
5. Name the four types of fire extinguishers and the specific flammables that each is effective in controlling.
6. Describe the process of Standard Precautions in the microbiology laboratory, including handling of infectious materials, personal hygiene, use of personal protective equipment (PPE), handling sharp objects, and hand-washing procedures.
7. Define Biosafety Levels 1 through 4, including the precautions required for each and type of facility; identify a representative organism for each.
8. Outline the basic guidelines for packing and shipping infectious substances.
9. Describe the management and response required during a biologic or chemical exposure incident in the laboratory.

Microbiology laboratory safety practices were first published in 1913. They included admonitions such as the necessity to (1) wear gloves, (2) wash hands after working with infectious materials, (3) disinfect all instruments immediately after use, (4) use water to moisten specimen labels rather than the tongue, (5) disinfect all contaminated waste before discarding, and (6) report to appropriate personnel all accidents or exposures to infectious agents.

These guidelines are still incorporated into safety programs in the diagnostic microbiology laboratory. Safety programs have been expanded to include the proper handling of biologic hazards encountered in processing patient specimens and handling infectious microorganisms that include standard precautions and transmission-based precautions, engineering and work place controls and risk assessment; fire and electrical safety; the safe handling, storage, and disposal of chemicals and radioactive substances; and techniques for safely lifting or moving heavy objects. In areas of the country prone to natural disasters (e.g., earthquakes, hurricanes, snowstorms), safety programs include disaster preparedness plans that outline the steps to take in an emergency. Although all microbiologists are responsible for their own health and safety, the institution and supervising personnel are required to provide safety training to familiarize microbiologists with known hazards in the workplace and to prevent exposure. Infection control is also a vital part of laboratory safety and is discussed in detail in Chapter 78.

Laboratory safety is considered an integral part of overall laboratory services, and federal law in the United States mandates preemployment safety training, followed by quarterly safety in-services. Safety training regulations are enforced by the United States Department of Labor Occupational Safety and Health Administration (OSHA). Regulations and requirements may vary based on the type of laboratory and updated regulations. It is recommended that the laboratory review these requirements as outlined by OSHA (www.osha.gov).

Microbiologists should be knowledgeable, properly trained, and equipped with the proper protective materials, engineering, and working controls while performing duties in the laboratory. Investigation of the causes of accidents indicates that unnecessary exposures to infectious agents occur when individuals become sloppy in performing their duties or when they deviate from standardized safety precautions.

TABLE 4.1 Classification Scheme of Items Requiring Sterilization or Disinfection

Classification	Description	Items	Methods
Critical items	Pose a high risk of infection if contaminated with infectious agents.	Surgical instruments Cardiac and urinary catheters Implants Ultrasound probes used in sterile body cavities	Purchased as sterilized Heat-sensitive objects: ethylene oxide, hydrogen peroxide gas plasma Chemical: glutaraldehyde, stabilized hydrogen peroxide with or without peracetic acid in specific concentrations
Semi-critical items	Generally items that are exposed to the mucous membranes or nonintact skin. These items should be free of all infectious agents including vegetative bacteria, fungi, and viruses.	Respiratory therapy and anesthesia equipment, endoscopes, laryngoscope blades, esophageal manometry probes, cystoscopes, anorectal manometry catheters, and diaphragm fitting rings.	Glutaraldehyde, hydrogen peroxide, ortho-phthaladehyde, hydrogen peroxide with peracetic acid
Non-critical items	Items that contact intact skin but not mucous membranes.	Noncritical patient care items such as bedpans, blood pressure cuffs, computers, crutches etc. Noncritical environmental surfaces: bed rails, bedside tables, patient furniture, and floors.	

Adapted from Centers for Disease and Control Guideline for Disinfection and Sterilization in Healthcare Facilities; https://www.cdc.gov/infectioncontrol/pdf/guidelines/disinfection-guidelines.pdf

Sterilization, Disinfection, and Decontamination

The Guideline for Disinfection and Sterilization in Healthcare Facilities provides evidence-based recommendations for all cleaning, disinfection, and sterilization of medical devices and the healthcare environment (Table 4.1). Equipment and services associated with patient care and the healthcare environment are all subject to regulations and recommendations for the level of sterilization and/or disinfection based on the risk of infection to the patient. These items as well as considerations for disinfection in the ambulatory care and home care environment are included in Chapter 78.

Sterilization is a process that kills all forms of microbial life, including bacterial endospores. **Disinfection** is a process that destroys pathogenic organisms, but not necessarily all microorganisms, endospores, or prions. However, some disinfectants will kill endospores with prolonged exposure times (3 to 12 hours). These disinfectants are **chemical sterilants. Decontamination** is the removal of pathogenic microorganisms so items are safe to handle or dispose of. Many factors limit the success or degree of sterilization, disinfection, or decontamination in a health care setting, such as organic load (organisms and other contaminating materials such as blood or body fluids), the type of organisms present, the concentration and exposure time to the germicide, the physical and chemical nature of the object or surface (hinges, cracks, rough or smooth surfaces), temperature, pH, humidity, and presence of a biofilm. These processes may be accomplished by a variety of physical or chemical methods.

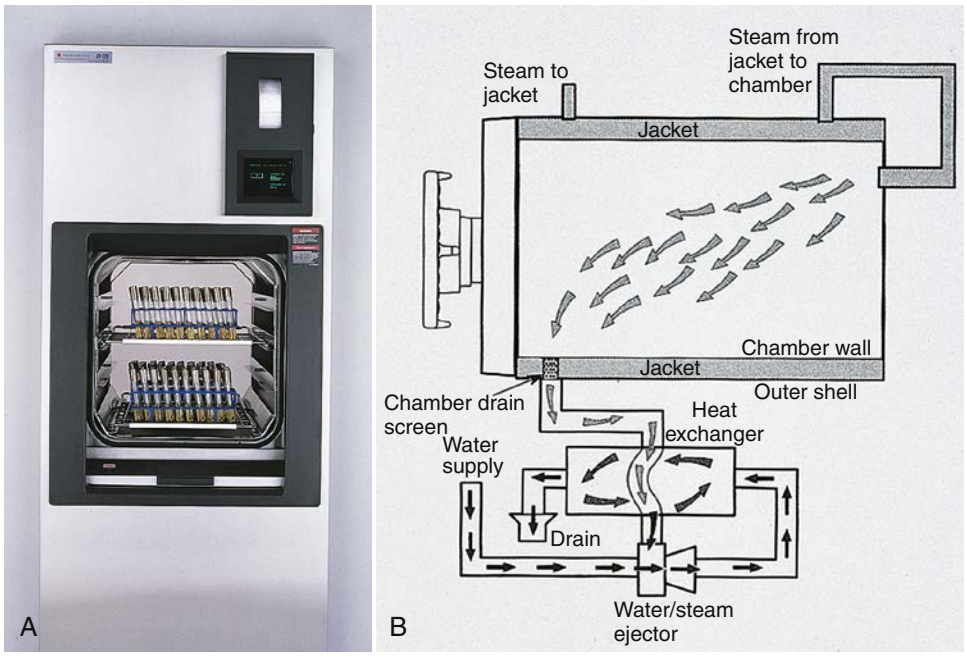
Methods of Sterilization

The physical methods of sterilization include:

- Incineration
- Moist heat
- Dry heat
- Filtration
- Ionizing (gamma) radiation
- Chemicals (ethylene oxide [EtO] gas, hydrogen peroxide gas plasma, vaporized hydrogen peroxide, and other liquid chemicals)

Incineration is a method of treating infectious waste. Hazardous material is literally burned to ashes at temperatures of 870°C to 980°C. Incineration is the safest method to ensure that no infective materials remain in samples or containers when disposed. Prions (infective proteins) are not eliminated using conventional methods. Therefore incineration is recommended. Toxic air emissions and the presence of heavy metals in ash have limited the use of incineration in the United States.

Moist heat (steam under pressure) is used to sterilize biohazardous trash and heat-stable objects; an **autoclave** is used for this purpose. An autoclave is essentially a large pressure cooker. Moist heat in the form of saturated steam under one atmosphere (15 pounds per square inch [psi]) of pressure causes the irreversible denaturation of enzymes and structural proteins. The most commonly used steam



• **Fig. 4.1** Gravity displacement type of autoclave. (A) Typical Eagle Century Series sterilizer for laboratory applications. (B) Typical Eagle 3000 sterilizer piping diagram. The arrows show the entry of steam into the chamber and the displacement of air. (Courtesy AMSCO International, a subsidiary of STERIS Corp., Mentor, Ohio.)

sterilizer in the microbiology laboratory is the gravity displacement autoclave (Fig. 4.1). Steam enters at the top of the sterilizing chamber; because steam is lighter than air, it displaces the air in the chamber and forces it out the bottom through the drain vent. The two common sterilization temperatures are 121°C and 132°C. Biologic waste that includes broth or solid media is usually autoclaved for 30 minutes at 121°C in a displacement sterilizer or 4 minutes at 132°C in a prevacuum sterilizer. Infectious medical waste containing body fluids or blood, on the other hand, is often sterilized at 132°C for 30 to 60 minutes to allow penetration of the steam throughout the waste and the displacement of air trapped inside the autoclave bag. Prions require a much more extensive sterilization process. Several options are recommended for the removal of prions from surgical instruments or other laboratory materials contaminated with high-risk tissue such as brain, spinal cord, and eye tissue. There are four methods for sterilization: (1) autoclave at 134°C for 18 minutes in a prevacuum sterilizer; (2) autoclave at 132°C for 1 hour in a gravity displacement sterilizer; (3) immerse in 1 N sodium hydroxide for 1 hour, remove and rinse with water, then autoclave at 121°C in a gravity displacement or 134°C in a prevacuum sterilizer for 1 hour; or (4) immerse in 1 N sodium hydroxide for 1 hour and heat in a gravity displacement at 121°C for 30 minutes, then clean and subject to routine equipment sterilization. Moist heat is the fastest and simplest physical method of sterilization.

Dry heat requires longer exposure times (1.5 to 3 hours) and higher temperatures than moist heat (160° to 180°C). Dry heat ovens are used to sterilize items such as glassware, oil, petrolatum, or powders. **Filtration** is the method of choice for antibiotic solutions, toxic chemicals, radioisotopes, vaccines, and carbohydrates, which are all heat sensitive. Filtration of liquids is accomplished by pulling the solution through a cellulose acetate or cellulose nitrate membrane with a vacuum. Filtration of air is accomplished using high-efficiency particulate air (HEPA) filters designed to remove organisms larger than 0.3 μm from isolation rooms, operating rooms, and biologic safety cabinets (BSCs). Although considered a method of sterilization, filtration simply removes microorganisms and particles larger than the pore size; smaller particles will not be removed using this method. The ionizing radiation used in microwaves and radiograph machines is composed of short-wavelength and high-energy gamma rays. Ionizing radiation is used for sterilizing disposables such as plastic syringes, catheters, or gloves before use. The most common chemical sterilant is EtO, which is used in gaseous form for sterilizing heat-sensitive objects. The main disadvantages of EtO use are the lengthy cycle times and the potential health hazards it produces. Vapor-phase hydrogen peroxide (an oxidizing agent) has been used to sterilize HEPA filters in BSCs, metals, and nonmetal devices such as medical instruments (e.g., scissors). There are no toxic byproducts produced using vapor-phase hydrogen peroxide. Hydrogen peroxide gas plasma is another method that uses hydrogen peroxide and generates plasma by exciting the gas in an enclosed chamber under deep vacuum with the use of radiofrequency or microwave energy.