The Microbial World

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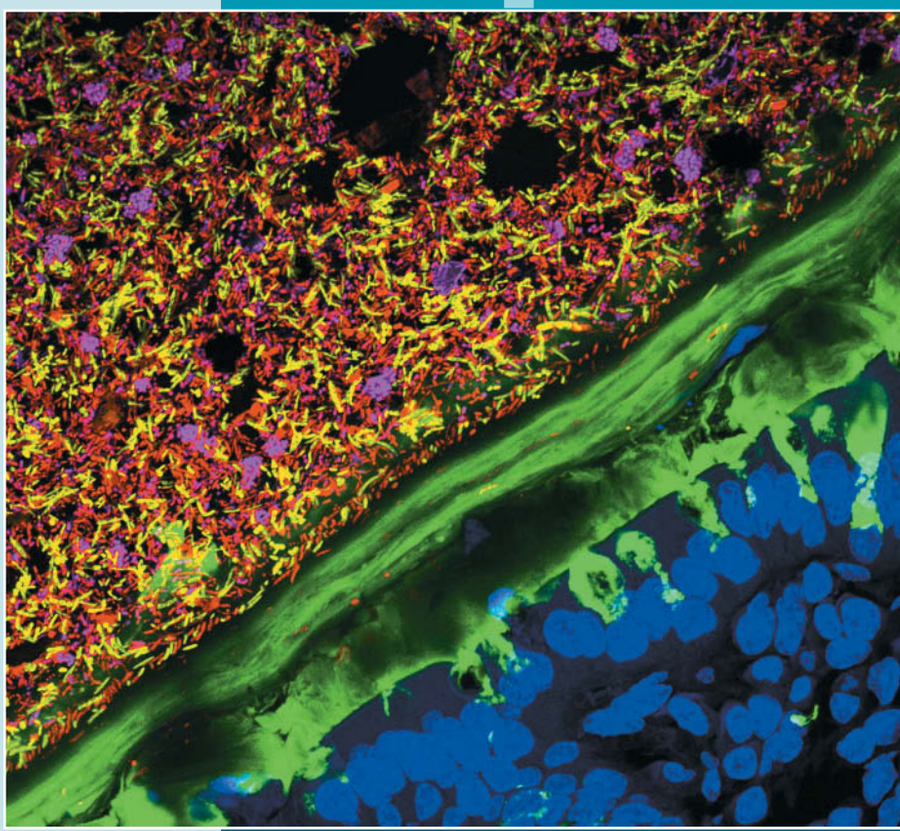
Microorganisms, Our Constant Companions

Microorganisms are everywhere, and though small, their activities have tremendous impacts on everything in our biosphere. As you learn more about microbiology you will realize that many of our day-to-day interactions with the world are influenced, for better or worse, by microbial life. Indeed, hundreds of trillions of bacteria are working within your body now to digest your last meal. The total complement of microbial cells in and on your body—your microbiome—contains thousands of species each adapted to grow best in a particular part of your body. For example, your gut microbiome encodes enzymes that help digest your food and synthesize vitamins critical to your health. The composition of your microbiome changes in response to your diet, your genes, your health, and the medicines you take. Our microbiome is absolutely essential to our health and well-being, yet we are only beginning to understand the diverse ways in which we depend upon our gut microorganisms.

Our knowledge of the microbial world is highly dependent on technological developments. Recent advances in microscopic techniques have made it possible to visualize microbes in intimate association with the lining of the gut (see photo). This image, generated by laser scanning confocal microscopy, reveals a cross section from the colon of a mouse and shows the dense and complex microbial community residing within the gut. Mice, which can be raised in a germ-free condition and then inoculated with a human gut microbiome, are used as a model system to explore microbiome function. Fluorescent stains identify the mucus layer (green) and host cell nuclei (blue) of the gut epithelium. *Bacteria* of the phylum *Firmicutes* stain yellow and those of the family *Bacteroidaceae* stain pink; all other bacteria stain red. Changes in diet alter the thickness of the mucus layer and the potential for microbial interactions with the epithelium. Such interactions can change gut function and possibly lead to inflammation.

In the chapters that follow, the exciting science of microbiology will unfold, and you will see that there is still much to learn about the inner workings of the microbial world.



Source: Earle, K.A., et al. 2015. Quantitative imaging of gut microbiota spatial organization. *Cell Host & Microbe 18:* 478–488.

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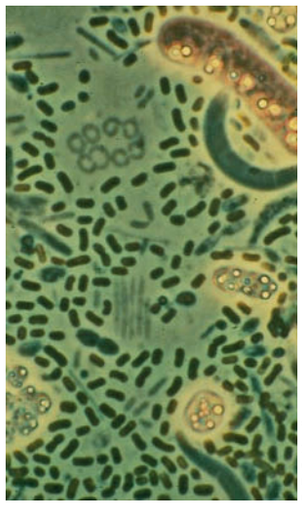
I • Exploring the Microbial World

1.1 Microorganisms, Tiny Titans of the Earth

Microorganisms (also called *microbes*) are life forms too small to be seen by the unaided human eye. These microscopic organisms are diverse in form and function and they inhabit every environment on Earth that supports life. Many microbes are undifferentiated single-celled organisms, but some can form complex structures, and some are even multicellular. Microorganisms typically live in complex **microbial communities** (Figure 1.1), and their activities are regulated by interactions with each other, with their environments, and with other organisms. The science of microbiology is all about microorganisms, who they are, how they work, and what they do.



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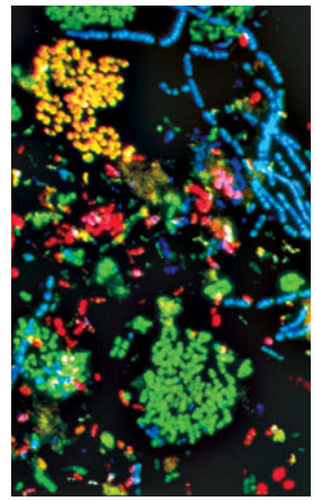

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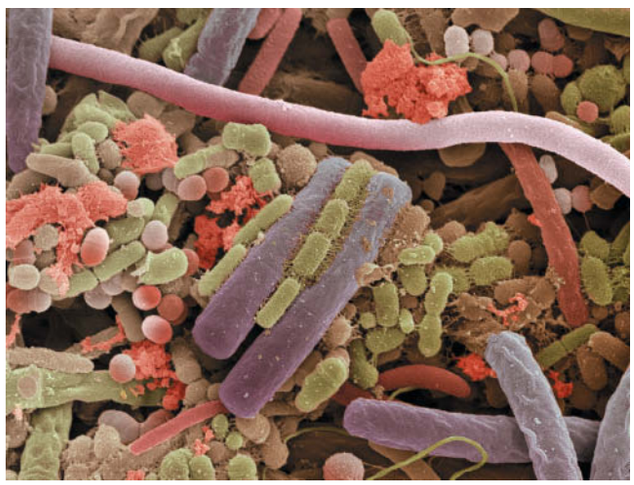


(c)

Figure 1.1 Microbial communities. *(a)* A bacterial community that developed in the depths of a small Michigan lake, showing cells of various phototrophic bacteria. The bacteria were visualized using phase-contrast microscopy. *(b)* A bacterial community in a sewage sludge sample. The sample was stained with a series of dyes, each of which stained a specific bacterial group. From *Journal of Bacteriology 178:* 3496–3500, Fig. 2b. © 1996 American Society for Microbiology. *(c)* Scanning electron micrograph of a microbial community scraped from a human tongue.

Microorganisms were teeming on the land and in the seas for billions of years before the appearance of plants and animals, and their diversity is staggering. Microorganisms represent a major fraction of Earth's biomass, and their activities are essential to sustaining life. Indeed, the very oxygen (O_2) we breathe is the result of microbial activities. Plants and animals are immersed in a world of microbes, and their evolution and survival are heavily influenced by microbial activities, by microbial symbioses, and by pathogens—those microbes that cause disease. Microorganisms are woven into the fabric of human life as well, from infectious diseases, to the food we eat, the water we drink, the fertility of our soils, the health of our animals, and even the fuel we put in automobiles. Microbiology is the study of the dominant form of life on Earth, and the effect that microbes have on our planet and all of the living things that call it home.

Microbiologists have many tools for studying microorganisms. Microbiology was born of the microscope, and microscopy is foundational to microbiology. Microbiologists have developed an array of methods for visualizing microorganisms, and these microscopic techniques are essential to microbiology. The cultivation of microorganisms is also foundational to microbiology. A microbial **culture** is a collection of cells that have been grown in or on a nutrient medium. A **medium** (plural, media) is a liquid or solid nutrient mixture that contains all of the nutrients required for a microorganism to grow. In microbiology, we use the word **growth** to refer to the increase in cell number as a result of cell division. A single microbial cell placed on a solid nutrient medium can grow and divide into millions of cells that form a visible **colony** (Figure 1.2). The formation of visible colonies makes it easier to see and grow microorganisms. Comprehension of the microbial basis of disease and microbial biochemical diversity has relied on the ability to grow microorganisms in the laboratory.

The ability to grow microorganisms rapidly under controlled conditions makes them highly useful for experiments that probe the fundamental processes of life. Most discoveries relating to the molecular and biochemical basis of life have been made using microorganisms. The study of molecules and their interactions is essential to defining the workings of microbial cells, and the tools of molecular biology and biochemistry are foundational to microbiology. Molecular biology has also provided a variety of tools to study microorganisms without need for their cultivation in the laboratory. These molecular tools have greatly expanded our knowledge of microbial ecology and diversity. Finally, the tools of genomics and molecular genetics are also cornerstones of modern microbiology and allow microbiologists to study the genetic basis of life, how genes evolve, and how they regulate the activities of cells.

This chapter begins our journey into the microbial world. Here we will begin to discover what microorganisms are, what they do, and how they can be studied. We will also place microbiology in historical context, as a process of scientific discovery.

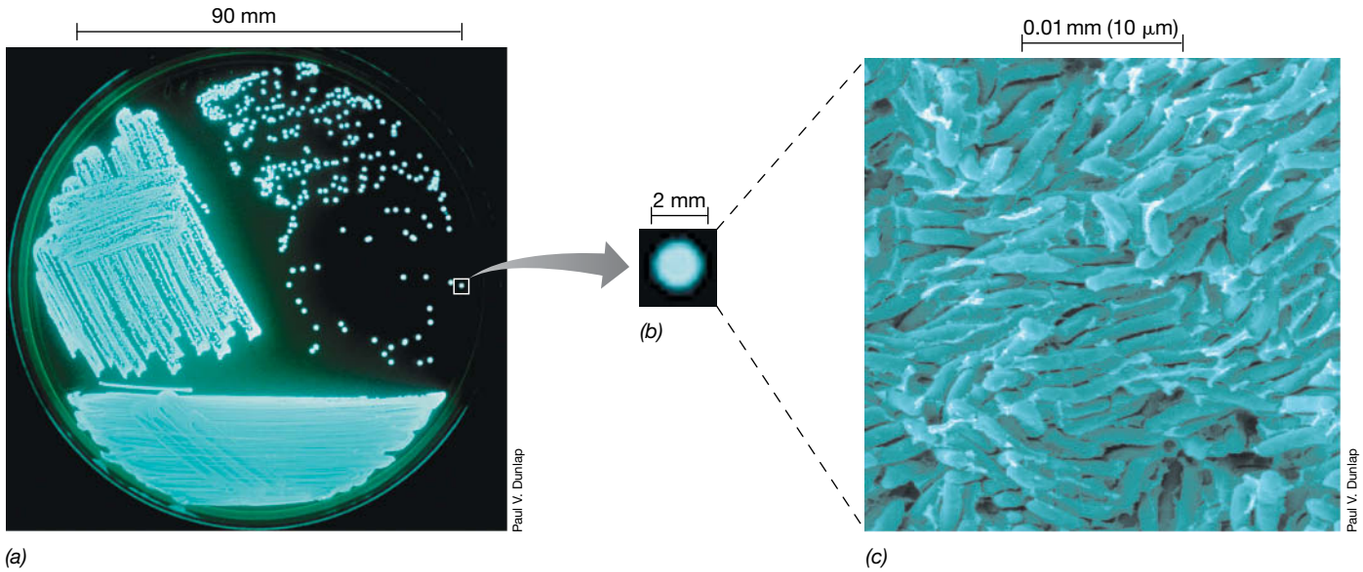


Figure 1.2 Microbial cells. *(a)* Bioluminescent (light-emitting) colonies of the bacterium *Photobacterium* grown in laboratory culture on a Petri plate. *(b)* A single colony can contain more than 10 million (10^7) individual cells. *(c)* Scanning electron micrograph of cells of *Photobacterium*.

MINIQUIZ

- In what ways are microorganisms important to humans?
- Why are microbial cells useful for understanding the basis of life?
- What is a microbial colony and how is one formed?

1.2 Structure and Activities of Microbial Cells

Microbial cells are living compartments that interact with their environment and with other cells in dynamic ways. In Chapter 2 we will examine the structure of cells in detail and relate specific structures to specific functions. Here we present a snapshot of microbial structure and activities. We purposely exclude viruses in most of this discussion because although they resemble cells in many ways, viruses are not cells but instead a special category of microorganism. We consider the structure, diversity, and activities of viruses in Section 1.14 and in Chapters 8 and 10.

Elements of Microbial Structure

All cells have much in common and contain many of the same components (Figure 1.3). All cells have a permeability barrier called the **cytoplasmic membrane** that separates the inside of the cell, the **cytoplasm**, from the outside. The cytoplasm is an aqueous mixture of **macromolecules** (for example proteins, lipids, nucleic acids, and polysaccharides), small organic molecules (mostly the precursors of macromolecules), various inorganic ions, and ribosomes. **Ribosomes** are the structures responsible for protein synthesis and are found in all cells. Some cells have a **cell wall** that lends structural strength to a cell. The cell wall is a relatively permeable structure located outside the cell membrane and is a much stronger layer than the membrane itself. Plant cells and most microorganisms have cell walls, whereas animal cells typically do not.

Examination of cell structure reveals there are two major structural classes of cells, called **prokaryotic** cells and **eukaryotic** cells (Figure 1.3). Eukaryotic cells are found in the phylogenetic domain *Eukarya*. This group includes plants and animals as well as diverse microbial eukaryotes such as algae, protozoa, and fungi. Eukaryotic cells contain an assortment of membrane-enclosed cytoplasmic structures called **organelles** (Figure 1.3*b*). These include, most prominently, the DNA-containing nucleus but also mitochondria and chloroplasts, organelles that specialize in supplying the cell with energy, and various other organelles.

Prokaryotic cells are found in the domains *Bacteria* and *Archaea*. Prokaryotic cells have few internal structures, they lack a nucleus, and they typically lack organelles (Figure 1.3*a*). The prokaryotic cell structure evolved prior to the evolution of the eukaryotic cell (Section 1.3). While *Archaea* and *Bacteria* both contain exclusively prokaryotic cells, these groups have diverged greatly and we will see later that the *Archaea* actually share many molecular and genetic characteristics with cells of *Eukarya*.

Genes, Genomes, Nucleus, and Nucleoid

In addition to a cytoplasmic membrane and ribosomes, all cells also possess a DNA **genome**. The genome is the complement of all genes in a cell. A gene is a segment of DNA that encodes a protein or an RNA molecule. The genome is the living blueprint of an organism; the characteristics, activities, and very survival of a cell are governed by its genome.

The genomes of prokaryotic cells and eukaryotic cells are organized differently. In eukaryotes, DNA is present as several linear molecules within the membrane-enclosed **nucleus**. By contrast, the genomes of *Bacteria* and *Archaea* are typically closed circular chromosomes (though a few prokaryotes have linear

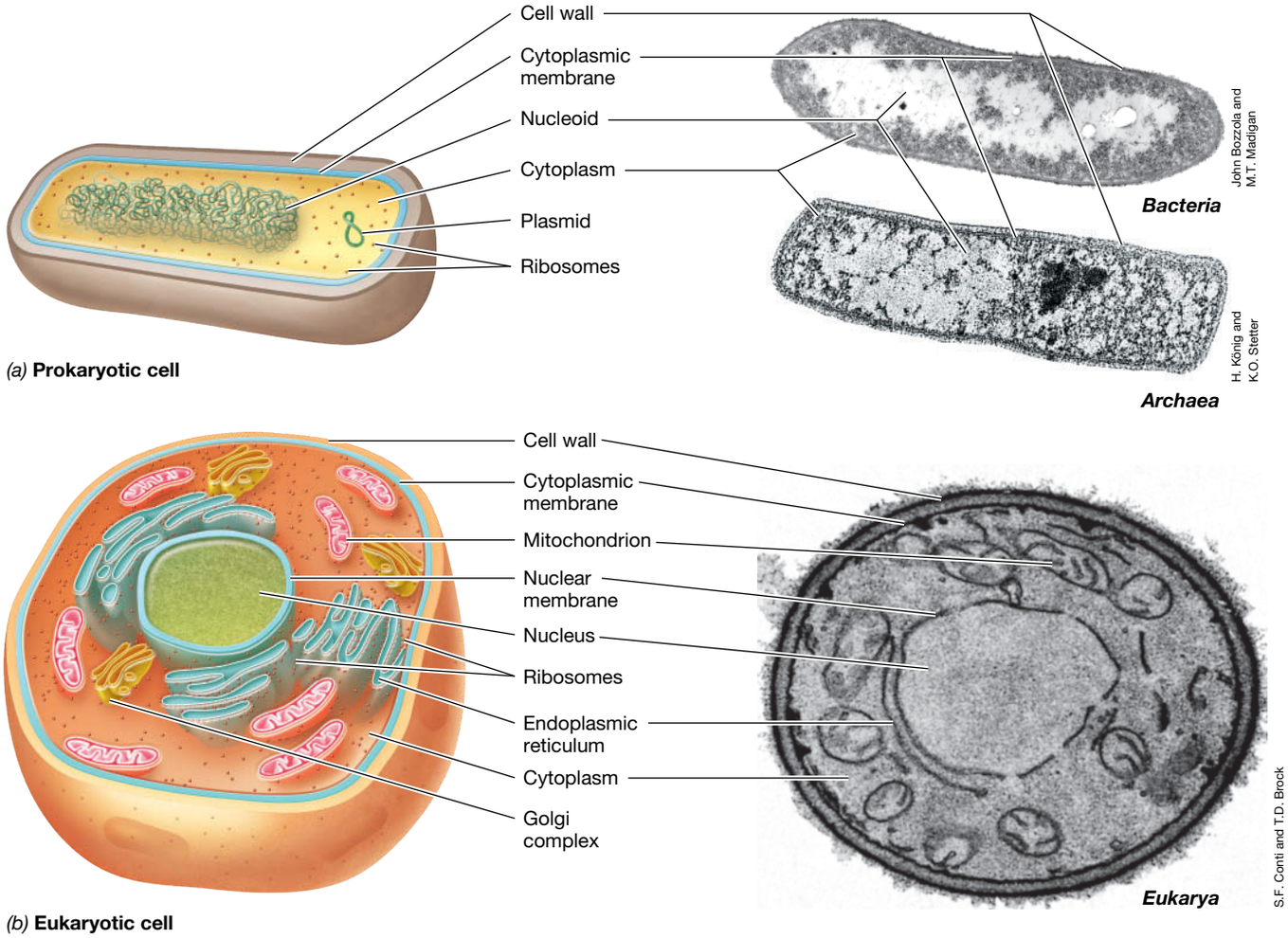


Figure 1.3 Microbial cell structure. *(a)* (Left) Diagram of a prokaryotic cell. (Right) Electron micrograph of *Heliobacterium modesticaldum* (*Bacteria,* cell is about 1 µm in diameter) and *Thermoproteus neutrophilus* (*Archaea,* cell is about 0.5 µm in diameter). *(b)* (Left) Diagram of a eukaryotic cell. (Right) Electron micrograph of a cell of *Saccharomyces cerevisiae* (*Eukarya,* cell is about 8 µm in diameter).

chromosomes). The chromosome aggregates within the prokaryotic cell to form the **nucleoid**, a mass visible in the electron microscope (Figure 1.3*a*). Most prokaryotic cells have only a single chromosome, but many also contain one or more small circles of DNA distinct from that of the chromosome, called **plasmids**. Plasmids typically contain genes that are not essential and confer some special property on the cell (such as a unique metabolism, or antibiotic resistance). The genomes of *Bacteria* and *Archaea* are typically small and compact and most contain between 500 and 10,000 genes encoded by 0.5 to 10 million base pairs. Eukaryotic cells typically have much larger and much less compact genomes than prokaryotic cells. A human cell, for example, contains approximately 3 billion base pairs, which encode about 20,000–25,000 genes.

Activities of Microbial Cells

What activities do microbial cells carry out? We will see that in nature, microbial cells typically live in groups called microbial communities (Figure 1.1). Figure 1.4 considers some of the ongoing cellular activities within the microbial community. All cells show some form of **metabolism** by taking up nutrients from the environment and transforming them into new cell materials and waste products. During these transformations, energy is conserved to support synthesis of new structures. Production of these new structures culminates in the division of the cell to form two cells. Microbial growth results from successive rounds of cell division.

During metabolism and growth, genes are decoded to form proteins that regulate cellular processes. **Enzymes**, those proteins that have catalytic activity, are required to carry out reactions that supply the energy and precursors necessary for the biosynthesis of all cell components. Enzymes and other proteins are synthesized during *gene expression* in the sequential processes of transcription and translation (Figure 1.4). **Transcription** is the process by which the information on DNA is copied into an RNA molecule, and **translation** is the process whereby the information on an RNA molecule is used by a ribosome to synthesize a protein (Chapter 4). Gene expression and enzyme activity in a microbial cell are coordinated and highly regulated to ensure that the cell remains

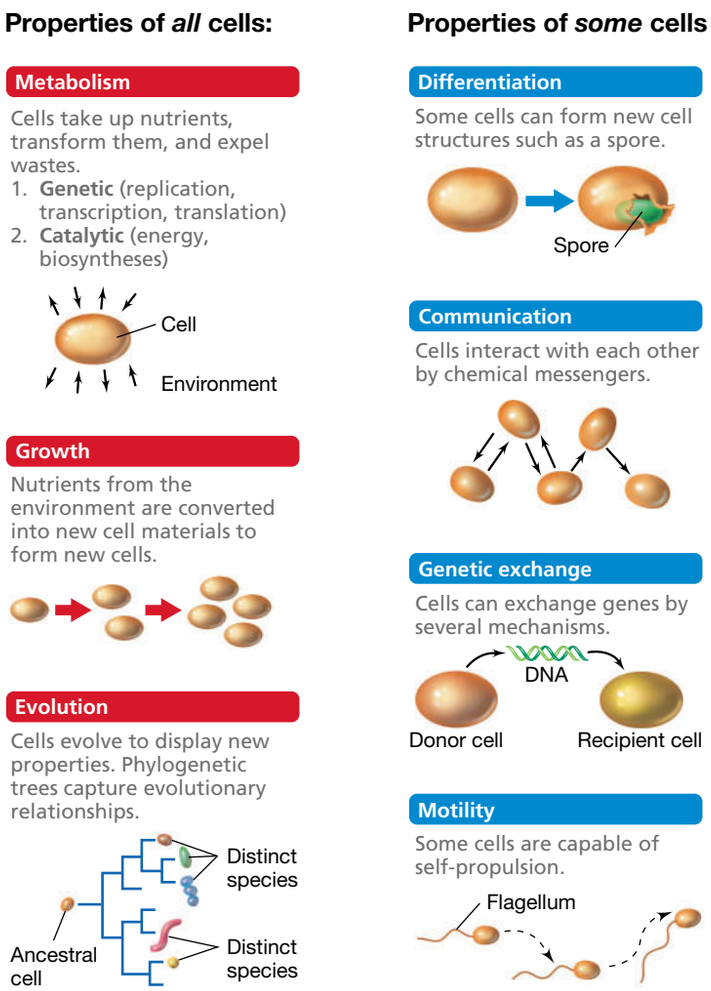


Figure 1.4 The properties of microbial cells. Major activities ongoing in cells in the microbial community are depicted.

optimally tuned to its surroundings. Ultimately, microbial growth requires replication of the genome through the process of **DNA replication**, followed by cell division. All cells carry out the processes of transcription, translation, and DNA replication.

Microorganisms have the ability to sense and respond to changes in their local environment. Many microbial cells are capable of **motility**, typically by self-propulsion (Figure 1.4). Motility allows cells to relocate in response to environmental conditions. Some microbial cells undergo **differentiation**, which may result in the formation of modified cells specialized for growth, dispersal, or survival. Cells respond to chemical signals in their environment, including those produced by other cells of either the same or different species, and these signals often trigger new cellular activities. Microbial cells thus exhibit **intercellular communication**; they are "aware" of their neighbors and can respond accordingly. Many prokaryotic cells can also exchange genes with neighboring cells, either of the same species or of a different species, in the process of **horizontal gene transfer**.

Evolution (Figure 1.4) results when genes in a population of cells change in sequence and frequency over time, leading to descent with modification. The evolution of microorganisms can be very rapid relative to the evolution of plants and animals. For example, the indiscriminate use of antibiotics in human and veterinary medicine has selected for the proliferation of antibiotic resistance in pathogenic bacteria. The rapid pace of microbial evolution can be attributed in part to the ability of microorganisms to grow very quickly and to acquire new genes though the process of horizontal gene transfer.

Not all of the processes depicted in Figure 1.4 occur in all cells. Metabolism, growth, and evolution, however, are universal and will be major areas of emphasis throughout this book.

MINIQUIZ

- What structures are universal to all type of cells?
- What processes are universal to all types of cells?
- What structures can be used to distinguish between prokaryotic cells and eukaryotic cells?

1.3 Microorganisms and the Biosphere

Microbes are the oldest form of life on Earth, and they have evolved to perform critical functions that sustain the biosphere. In this section we will learn how microbes have changed our planet and how they continue to do so.

A Brief History of Life on Earth

Earth is about 4.6 billion years old, and microbial cells first appeared between 3.8 and 4.3 billion years ago (Figure 1.5). During the first 2 billion years of Earth's existence, its atmosphere was anoxic (O_2 was absent), and only nitrogen (N_2), carbon dioxide (CO_2), and a few other gases were present. Only microorganisms capable of anaerobic metabolisms (that is, metabolisms that do not require O_2) could survive under these conditions.

The evolution of phototrophic microorganisms—organisms that harvest energy from sunlight—occurred within 1 billion years of the formation of Earth (Figure 1.5*a*). The first phototrophs were anoxygenic (non-oxygen-producing), such as the purple sulfur bacteria and green sulfur bacteria we know today (Figure 1.6). *Cyanobacteria* (oxygenic phototrophs) (Figure 1.6*f*) evolved nearly a billion years later (Figure 1.5*a*) and began the slow process of oxygenating Earth's atmosphere. These early phototrophs lived in structures called *microbial mats*, which are still found on Earth today (Figure 1.6*a–c*). After the oxygenation of Earth's atmosphere, multicellular life forms eventually evolved, culminating in the plants and animals we know today. But plants and animals have only existed for about half a billion years. The timeline of life on Earth (Figure 1.5*a*) shows that *80% of life's history was exclusively microbial*, and thus in many ways, Earth can be considered a microbial planet.

As evolutionary events unfolded, three major lineages of microbial cells—the *Bacteria*, the *Archaea*, and the *Eukarya* (Figure 1.5*b*)—were distinguished. These three major cell lineages are called **domains**, and all known cellular organisms belong to one of these three domains. All cellular organisms also share certain characteristics and genes. For example, approximately 60 genes are universally present in cells of all domains. Examination of these genes reveals that all three domains have descended from a

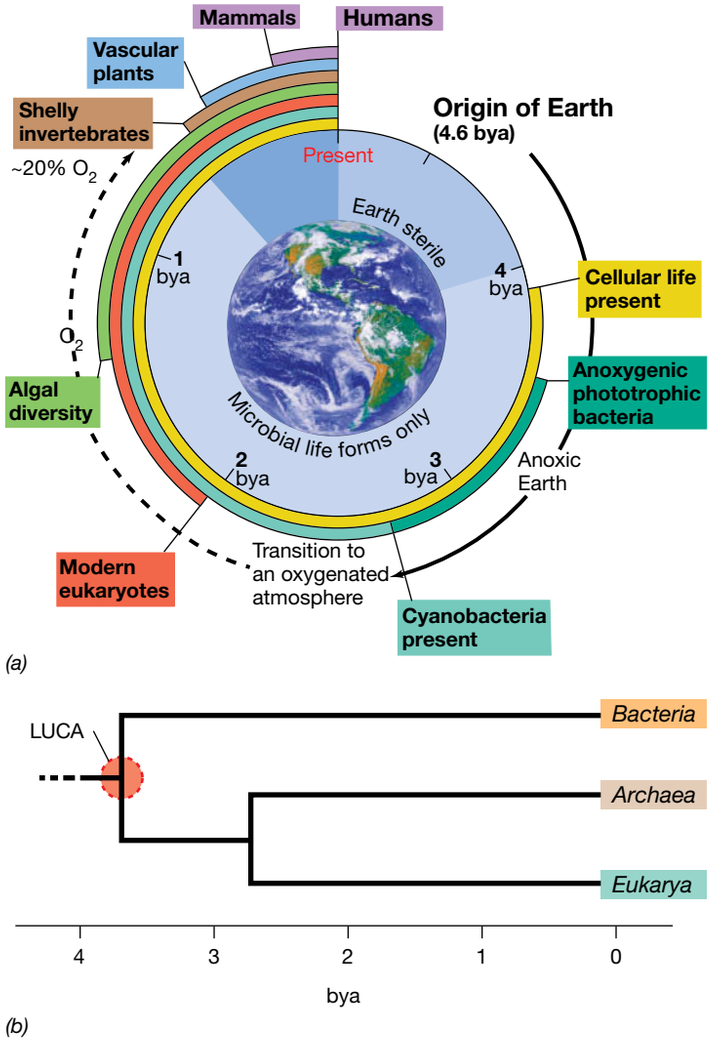


Figure 1.5 A summary of life on Earth through time and origin of the cellular domains. *(a)* At its origin, Earth was sterile. Cellular life was present on Earth by 3.8 billion years ago (bya). Cyanobacteria began the slow oxygenation of Earth about 3 bya, but current levels of O_2 in the atmosphere were not achieved until 500–800 million years ago. *(b)* The three domains of cellular organisms are *Bacteria, Archaea,* and *Eukarya. Archaea* and *Eukarya* diverged long before eukaryotic cells appear in the fossil record. LUCA, last universal common ancestor.

common ancestor, the *last universal common ancestor* (LUCA, Figure 1.5*b*). Over enormous periods of time, microorganisms derived from these three domains have evolved to fill every suitable environment on Earth.

Microbial Abundance and Activity in the Biosphere

Microorganisms are present everywhere on Earth that will support life. They constitute a major fraction of global biomass and are key reservoirs of nutrients essential for life. There are an estimated 2×10^{30} microbial cells on Earth. To put this number in context, the universe in all its vast extent is estimated to contain merely 7×10^{22} stars. The total amount of carbon present in all microbial cells is a significant fraction of Earth's biomass (Figure 1.7). Moreover, the total amount of nitrogen and phosphorus (essential nutrients for life) within microbial cells is nearly four times that in all plant and animal cells combined. Microbes also represent a major fraction of the total DNA in the biosphere (about 31%), and their genetic diversity far exceeds that of plants and animals (see Figure 1.36).

Microbes are even abundant in habitats that are much too harsh for other forms of life, such as volcanic hot springs, glaciers and ice-covered regions, high-salt environments, extremely acidic or alkaline habitats, and deep in the sea or deep in the earth at extremely high pressure. Such microorganisms are called **extremophiles** and their properties define the physiochemical limits to life as we know it (Table 1.1). We will revisit many of these organisms in later chapters and discover the special structural and biochemical properties that allow them to thrive under extreme conditions.

All ecosystems are influenced greatly by microbial activities. The metabolic activities of microorganisms can change the habitats in which they live, both chemically and physically, and these changes can affect other organisms. For example, excess nutrients added to a habitat can cause aerobic (O_2-consuming) microorganisms to grow rapidly and consume O_2, rendering the habitat anoxic (O_2-free). Many human activities release nutrients into the coastal oceans, thereby stimulating excessive microbial growth, which can cause enormous anoxic zones in these waters. These "dead zones" cause massive mortality of fish and shellfish in coastal oceans worldwide, because most aquatic animals require O_2 and die if it is not available. Only by understanding microorganisms and microbiology can we predict and minimize the effects of human activity on the biosphere that sustains us.

Though diverse habitats are influenced strongly by microorganisms, their contributions are often overlooked because of their small sizes. Within the human body, for example, there are between one and ten microbial cells (mainly of *Bacteria*) for every human cell and more than 200 microbial genes for every human gene. These microbes provide nutritional and other benefits that are essential to human health. In later chapters we will return to a consideration of the ways in which microorganisms affect animals, plants, and the entire global ecosystem. This is the science of **microbial ecology**, perhaps the most exciting subdiscipline of microbiology today. We will see that microbes are important to myriad issues of global importance to humans including climate change, agricultural productivity, and even energy policy.

MINIQUIZ

- How old is Earth and when did cells first appear on Earth?
- Name the three domains of life.
- Why were cyanobacteria so important in the evolution of life on Earth?

1.4 The Impact of Microorganisms on Human Society

Microbiologists have made great strides in discovering how microorganisms function, and application of this knowledge has greatly advanced human health and welfare. Besides understanding

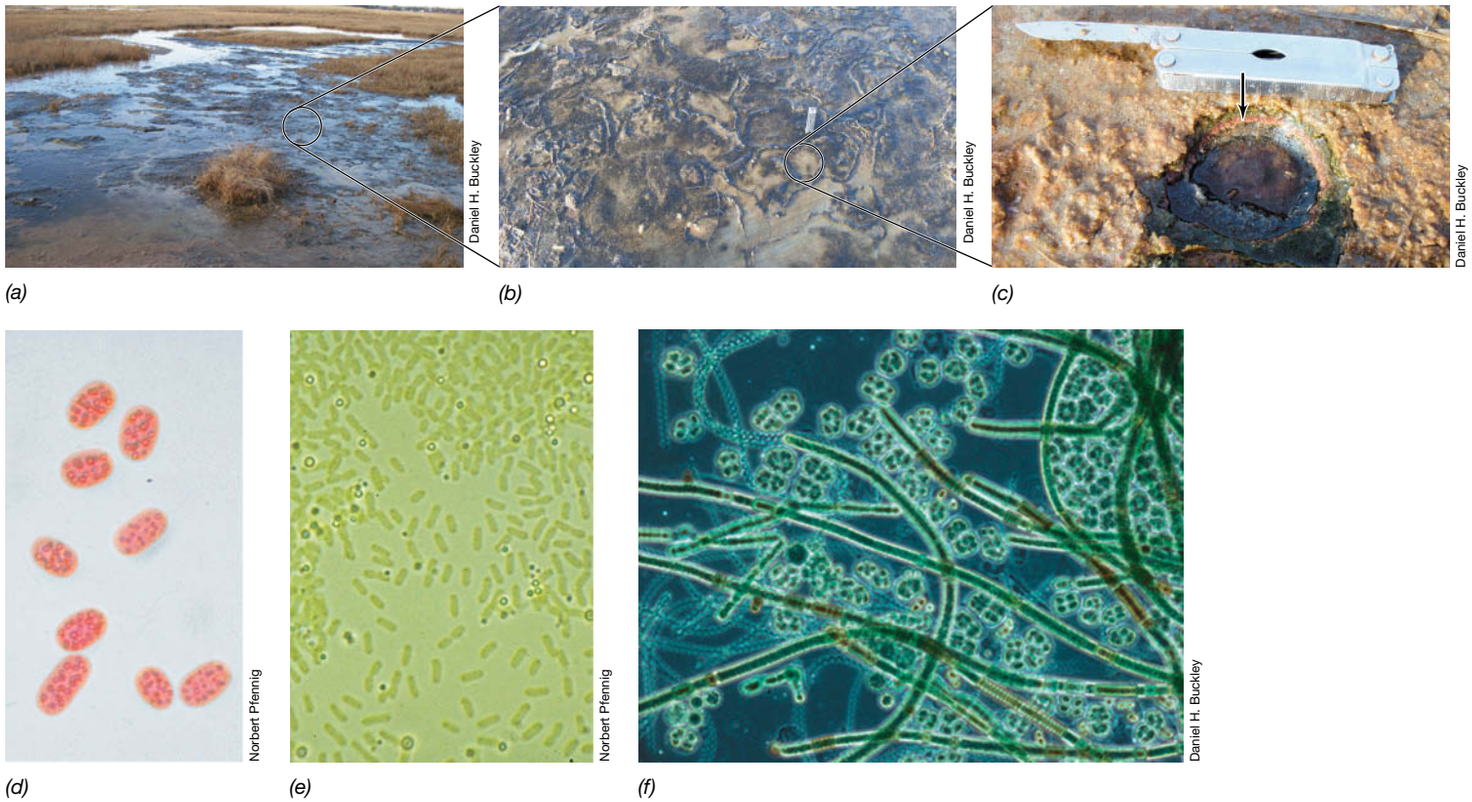


Figure 1.6 Phototrophic microorganisms. The earliest phototrophs lived in microbial mats. *(a)* Photosynthetic microbial mats in the Great Sippewissett Marsh, a salt marsh in Massachusetts, USA. *(b)* Mats develop a cohesive structure that forms at the sediment surface. *(c)* A slice through the mat shows colored layers that form due to the presence of photopigments. Cyanobacteria form the green layer nearest the surface, purple sulfur bacteria form the purple and yellow layers below, and green sulfur bacteria form the bottommost green layer. The scale on the knife is in cm. *(d)* Purple sulfur bacteria, *(e)* green sulfur bacteria, and *(f)* cyanobacteria imaged by bright-field and phase-contrast microscopy. Purple and green sulfur bacteria are anoxygenic phototrophs that appeared on Earth long before oxygenic phototrophs evolved (see Figure 1.5*a*).

microorganisms as agents of disease, microbiology has made great advances in understanding the important roles microorganisms play in food and agriculture, and microbiologists have been able to exploit microbial activities to produce valuable human products, generate energy, and clean up the environment.

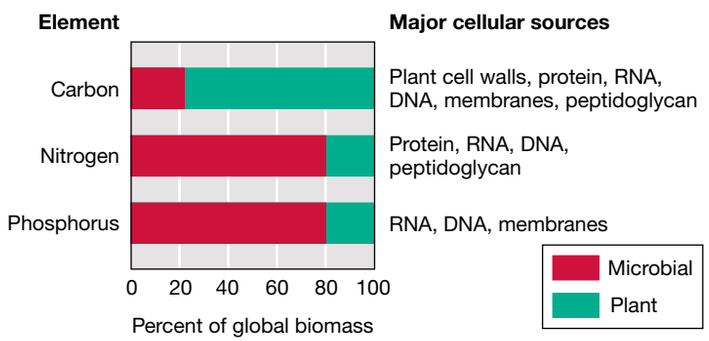


Figure 1.7 Contribution of microbial cells to global biomass. Microorganisms comprise a significant fraction of the carbon (C) and a majority of the nitrogen (N) and phosphorus (P) in the biomass of all organisms on Earth. C, N, and P are the macronutrients required in the greatest quantity by living organisms. Animal biomass is a minor fraction (<0.1%) of total global biomass and is not shown.

Microorganisms as Agents of Disease

The statistics summarized in **Figure 1.8** show how microbiologists and clinical medicine have combined to conquer infectious diseases in the past 100 years. At the beginning of the twentieth century, the major causes of human death were infectious diseases caused by bacterial and viral **pathogens**. In those days children and the aged in particular succumbed in large numbers to microbial diseases. Today, however, infectious diseases are much less deadly, at least in developed countries. Control of infectious disease has come from a combination of advances including our increased understanding of disease processes, improved sanitary and public health practices, active vaccine campaigns, and the widespread use of antimicrobial agents, such as antibiotics. As we will see later in this chapter, the development of microbiology as a science can be traced to pioneering studies of infectious disease.

While pathogens and infectious disease remain a major threat to humanity, and combating these harmful organisms remains a major focus of microbiology, most microorganisms are not harmful to humans. In fact, most microorganisms are beneficial, and in many cases are even essential to human welfare and the functioning of the planet. We turn our attention to these microorganisms and microbial activities now.

TABLE 1.1 Classes and examples of extremophiles[a]

Extreme	Descriptive term	Genus/species	Domain	Habitat	Minimum	Optimum	Maximum
Temperature							
High	Hyperthermophile	*Methanopyrus kandleri*	*Archaea*	Undersea hydrothermal vents	90°C	106°C	122°C[b]
Low	Psychrophile	*Psychromonas ingrahamii*	*Bacteria*	Sea ice	−12°C[c]	5°C	10°C
pH							
Low	Acidophile	*Picrophilus oshimae*	*Archaea*	Acidic hot springs	−0.06	0.7[d]	4
High	Alkaliphile	*Natronobacterium gregoryi*	*Archaea*	Soda lakes	8.5	10[e]	12
Pressure	Barophile (piezophile)	*Moritella yayanosii*	*Bacteria*	Deep ocean sediments	500 atm	700 atm[f]	>1000 atm
Salt (NaCl)	Halophile	*Halobacterium salinarum*	*Archaea*	Salterns	15%	25%	32% (saturation)

[a]The organisms listed are the current "record holders" for growth in laboratory culture at the extreme condition listed.
[b]Anaerobe showing growth at 122°C only under several atmospheres of pressure.
[c]The permafrost bacterium *Planococcus halocryophilus* can grow at −15°C and metabolize at −25°C. However, the organism grows optimally at 25°C and grows up to 37°C and thus is not a true psychrophile.
[d]*P. oshimae* is also a thermophile, growing optimally at 60°C.
[e]*N. gregoryi* is also an extreme halophile, growing optimally at 20% NaCl.
[f]*M. yayanosii* is also a psychrophile, growing optimally near 4°C.

Microorganisms, Agriculture, and Human Nutrition

Agriculture benefits from the cycling of key plant nutrients by microorganisms. For example, legumes are a diverse family of plants that include major crop species such as beans, peas, and lentils, among others. Legumes live in close association with bacteria that form structures called *nodules* on their roots. In the nodules, these bacteria convert atmospheric nitrogen (N_2) into ammonia (NH_3) through the process of *nitrogen fixation*. NH_3 is the major nutrient found in fertilizer and is used as a nitrogen source for plant growth (**Figure 1.9**). In this way bacteria allow legumes to make their own fertilizer, thereby reducing the need for farmers to apply fertilizers produced industrially. Bacteria regulate nutrient cycles, such as the nitrogen cycle and the sulfur cycle (Figure 1.9), transforming and recycling nutrients that form the basis of soil fertility.

Also of major agricultural importance are microorganisms that inhabit the *rumen* of ruminant animals, such as cattle and sheep. The rumen is a microbial ecosystem in which microbial communities digest and ferment the polysaccharide cellulose (Figure 1.9*d*), the major component of plant cell walls. Without these symbiotic microorganisms, ruminants could not thrive on cellulose-rich (but otherwise nutrient-poor) food such as grass and hay. Many domesticated and wild herbivorous mammals—including deer, bison, camels, giraffes, and goats—are also ruminants.

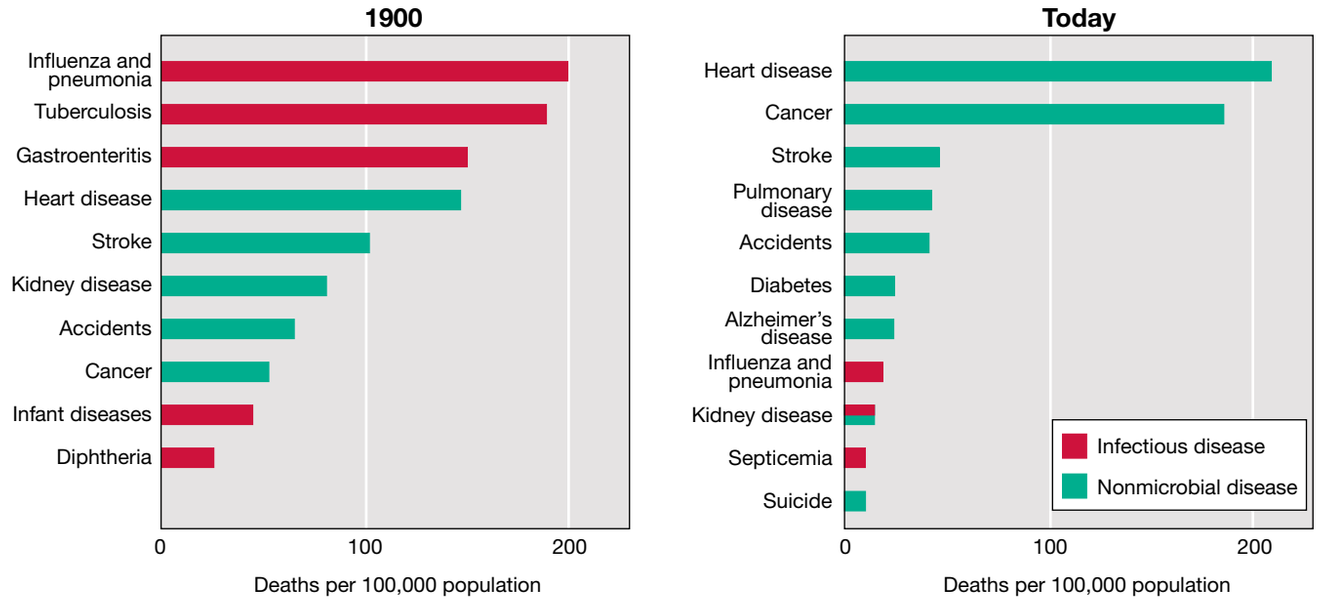


Figure 1.8 Death rates for the leading causes of death in the United States: 1900 and today. Infectious diseases were the leading causes of death in 1900, whereas today they account for relatively few deaths. Kidney diseases can be caused by microbial infections or systemic sources (diabetes, cancers, toxicities, metabolic diseases, etc.). Data are from the United States National Center for Health Statistics and the Centers for Disease Control and Prevention.

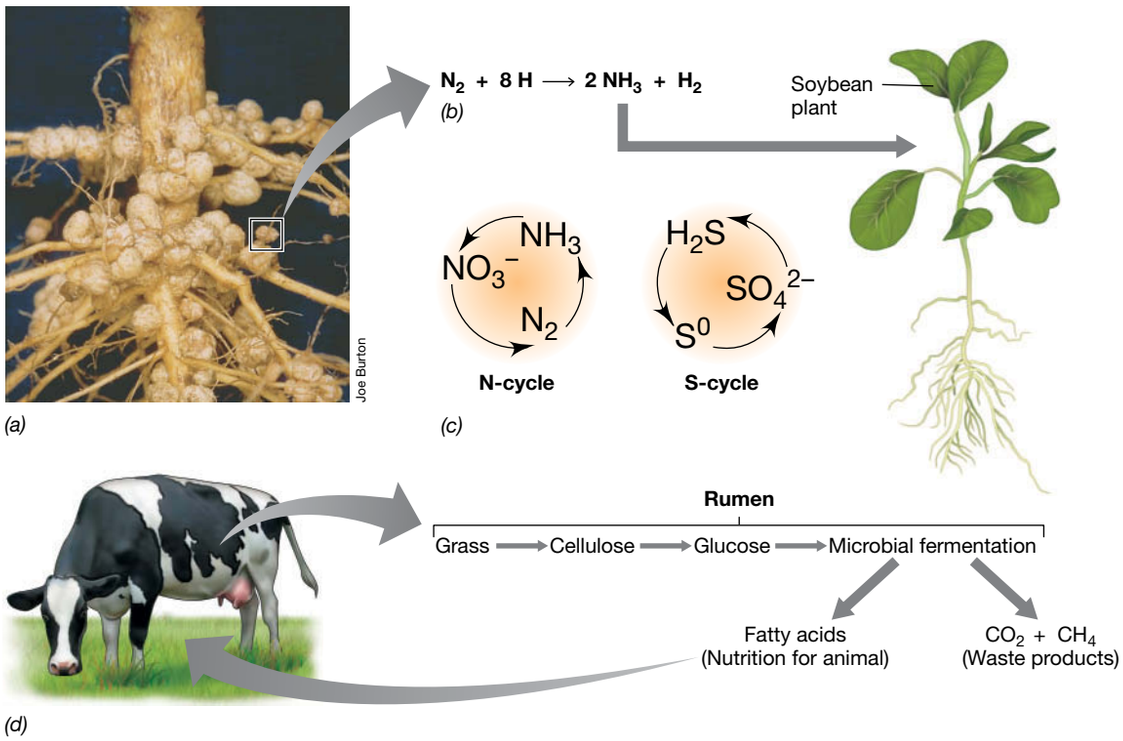


Figure 1.9 Microorganisms in modern agriculture. *(a, b)* Root nodules on this soybean plant contain bacteria that fix molecular nitrogen (N_2) for use by the plant. *(c)* The nitrogen and sulfur cycles, key nutrient cycles in nature. *(d)* Ruminant animals. Microorganisms in the rumen of the cow convert cellulose from grass into fatty acids that can be used by the animal. The other products are not so desirable, as CO_2 and CH_4 are the major gases that cause global warming.

The human gastrointestinal (GI) tract lacks a rumen, but complex carbohydrates (which can represent 10–30% of food energy) are digested by the **gut microbiome**. The colon, or large intestine (Figure 1.10), follows the stomach and small intestine in the digestive tract, and it contains about 10^{11} microbial cells per gram of colonic contents. Microbial cell numbers are low in the very acidic (pH 2) stomach (about 10^4 per gram) but increase to about 10^8 per gram near the end of the small intestine (pH 5) and then reach maximal numbers in the colon (pH 7) (Figure 1.10). The colon contains diverse microbial species that assist in the digestion of complex carbohydrates, and that synthesize vitamins and other nutrients essential to host nutrition. The gut microbiome develops from birth, but it can change over time with the human host. The composition of the gut microbiome has major effects on GI function and human health.

Microorganisms and Food

Microbes are intimately associated with the foods we eat. Microbial growth in food can cause food spoilage and foodborne disease. The manner in which we harvest and store food (e.g., canning, refrigeration, drying, salting, etc.), the ways in which we cook it, and even the spices we use, have all been fundamentally influenced by microbes in order to minimize microbial growth and eliminate harmful organisms. Microbial food safety and prevention of food spoilage is a major focus of the food industry and a major cause of economic loss every year.

While some microbes can cause foodborne disease and food spoilage, not all microorganisms in foods are harmful. Indeed, beneficial microbes have been used for thousands of years to improve food safety and to preserve foods (Figure 1.11). For example, cheeses, yogurt, and buttermilk are all produced by the microbial fermentation of dairy products to produce acids that improve shelf life and prevent the growth of foodborne pathogens. Such microbial fermentations are used to produce a variety of foods including sauerkraut, kimchi, pickles, and certain sausages. Even the production of chocolate and coffee rely on microbial fermentation. Moreover, baked goods and alcoholic beverages rely on the fermentative activities of yeast, which generate carbon dioxide (CO_2) to raise the dough and alcohol as a key component (Figure 1.11), respectively. Fermentation products affect the flavor and taste of foods, and can prevent spoilage as well as the growth of deleterious organisms.

Microorganisms and Industry

Microorganisms play important roles in all manner of human industry. Microbes can grow in almost any habitat containing liquid water, including structures made by humans. For example, microbes often grow on submerged surfaces, forming *biofilms*. Biofilms that grow in pipes and drains can cause fouling and blockages in factory settings and pipelines, in sewers, and even in water distribution systems. In addition, biofilms that grow on ships' hulls can cause marked reductions in speed and efficiency. Biofilms can even grow in tanks that store oil and fuel, leading to spoilage of these products. We will learn that biofilms are also of great importance in medicine, as biofilms that form on implanted medical devices (↻ Figure 5.4*a*) can cause infections that are extremely difficult to treat.

Microorganisms can be harnessed to produce commercially valuable products. In *industrial microbiology*, naturally occurring microorganisms are grown on a massive scale to make large amounts of products at relatively low cost, such as antibiotics, enzymes, and certain chemicals. By contrast, *biotechnology* employs genetically engineered microorganisms to synthesize products of high value, such as insulin or other human proteins, usually on a small scale.

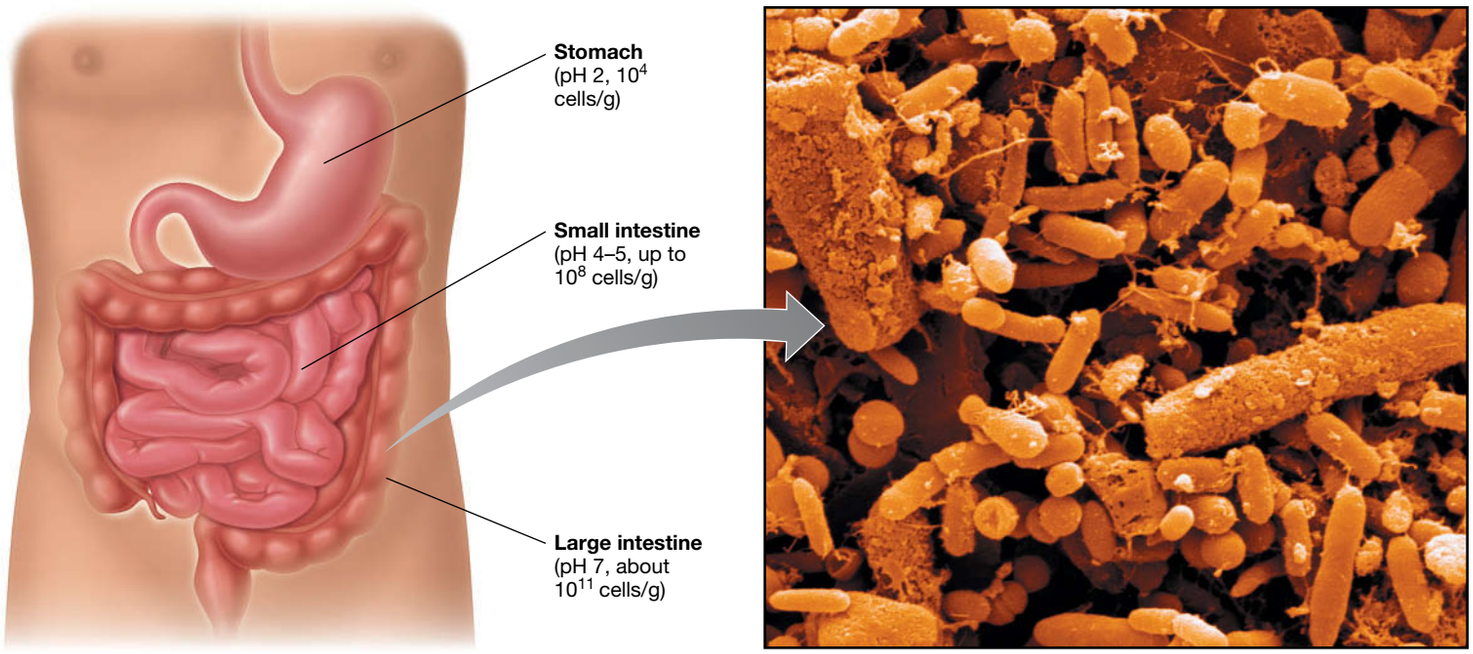


Figure 1.10 The human gastrointestinal tract. *(a)* Diagram of the human GI tract showing the major organs. *(b)* Scanning electron micrograph of microbial cells in the human colon (large intestine). Cell numbers in the colon can reach as high as 10^{11} per gram. As well as high *numbers* of cells, the microbial *diversity* in the colon is also quite high.

Microorganisms can also be used to produce *biofuels*. For example, natural gas (methane, CH_4) is a product of the anaerobic metabolism of a group of *Archaea* called *methanogens*. Ethyl alcohol (ethanol) is a major fuel supplement, which is produced by the microbial fermentation of glucose obtained from carbon-rich feedstocks such as sugarcane, corn, or rapidly growing grasses (**Figure 1.12**). Microorganisms can even convert waste materials, such as domestic refuse, animal wastes, and cellulose, into ethanol and methane.

Microorganisms are also used to clean up wastes. Wastewater treatment is essential to sanitation and human health. Wastewater treatment relies on microbes to treat water contaminated with human waste so that it can be reused or returned safely to the environment. Waterborne disease such as cholera and typhoid can proliferate in the absence of proper wastewater treatment. Microbes can also be used to clean up industrial pollution in a process called *bioremediation*. In bioremediation, microorganisms are used to transform spilled oil, solvents, pesticides, heavy metals, and other environmentally toxic pollutants. Bioremediation accelerates the cleanup process either by adding special microorganisms to a polluted environment or by adding nutrients that stimulate indigenous microorganisms to degrade the pollutants. In either case the goal is to accelerate disappearance of the pollutant.

As these examples show, the influence of microorganisms on humans is great and their activities are essential for the functioning of the planet. Or, as the eminent French chemist and early microbiologist Louis Pasteur so aptly put it: "The role of the infinitely small in nature is infinitely large." Microscopes provide an essential portal though which microbiologists such as Pasteur gazed into the world of microbes. We therefore continue our introduction to the microbial world with an overview of microscopy.

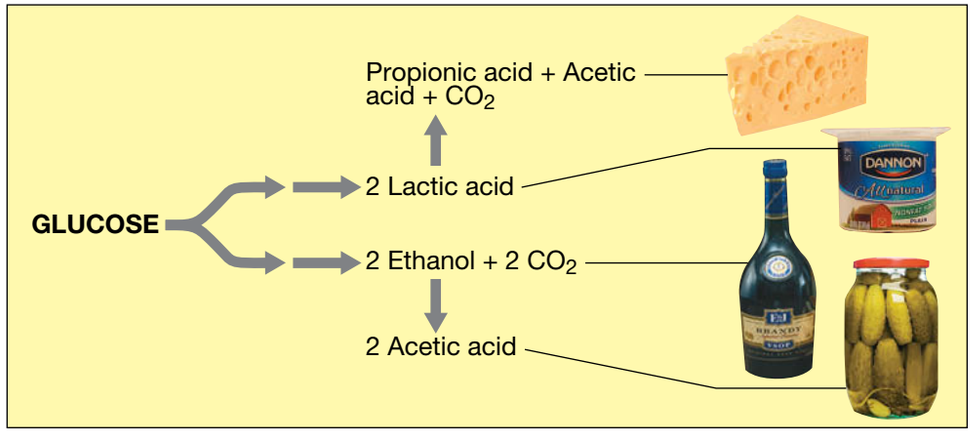


Figure 1.11 Fermented foods. *(a)* Major fermentations in various fermented foods. It is the fermentation product (ethanol, or lactic, propionic, or acetic acids) that both preserves the food and renders in it a characteristic flavor. *(b)* Photo of several fermented foods showing the characteristic fermentation product in each.

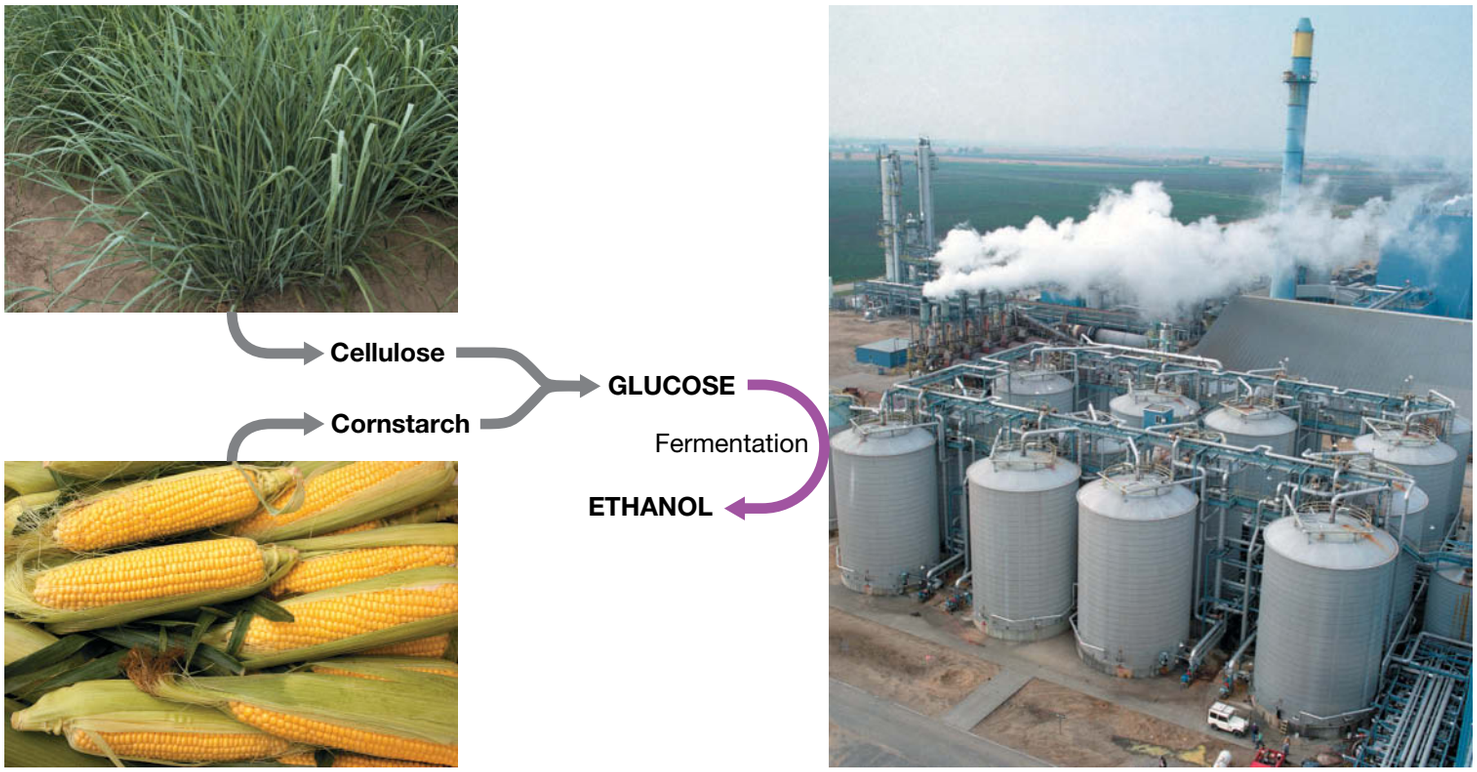


Figure 1.12 Ethanol as a biofuel. *(a)* Major crop plants used as feedstocks for biofuel ethanol production. Top: switchgrass, a source of cellulose. Bottom: corn, a source of cornstarch. Both cellulose and starch are composed of glucose, which is fermented to ethanol by yeast. *(b)* An ethanol plant in the United States. Ethanol produced by fermentation is distilled and then stored in the tanks.

MINIQUIZ

- How do microbes contribute to the nutrition of animals such as humans and cows?
- Describe several ways in which microorganisms are important in the food and agricultural industries.
- What is wastewater treatment and why is it important?

II • Microscopy and the Origins of Microbiology

Historically, the science of microbiology has taken its greatest leaps forward as new tools are developed and old tools improve. The microscope is the microbiologist's oldest and most fundamental tool for studying microorganisms. Indeed, microbiology did not exist before the invention of the microscope. Many forms of microscopy are available and some are extremely powerful. Throughout this text you will see images of microorganisms that were taken through the microscope using a variety of different techniques. So let's take a moment to explore how microscopy can be used to visualize microbial cells, starting at the very beginning with the invention of the microscope.

1.5 Light Microscopy and the Discovery of Microorganisms

Although the existence of creatures too small to be seen with the naked eye had been suspected for centuries, their discovery had to await invention of the microscope. The English mathematician and natural historian Robert Hooke (1635–1703) was an excellent microscopist. In his famous book *Micrographia* (1665), the first book devoted to microscopic observations, Hooke illustrated many microscopic images including the fruiting structures of molds (**Figure 1.13**). This was the first known description of microorganisms.

The first person to see bacteria, the smallest microbial cells, was the Dutch draper and amateur microscopist Antoni van Leeuwenhoek (1632–1723). Van Leeuwenhoek constructed extremely simple microscopes containing a single lens to examine various natural substances for microorganisms (**Figure 1.14**). These microscopes were crude by today's standards, but by careful manipulation and focusing, van Leeuwenhoek was able to see bacteria. He discovered bacteria in 1676 while studying pepper–water infusions, and reported his observations in a series of letters to the prestigious Royal Society of London, which published them in English translation in 1684. Drawings of some of van Leeuwenhoek's "wee animalcules," as he referred to them, are shown in Figure 1.14*b*, and a photo taken through a van Leeuwenhoek microscope is shown in Figure 1.14*c*.

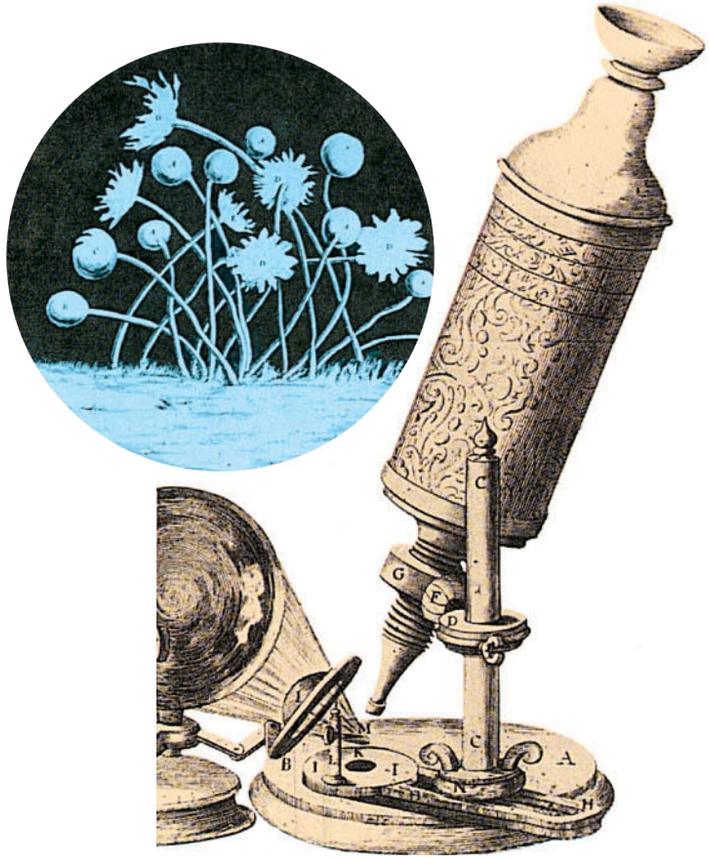


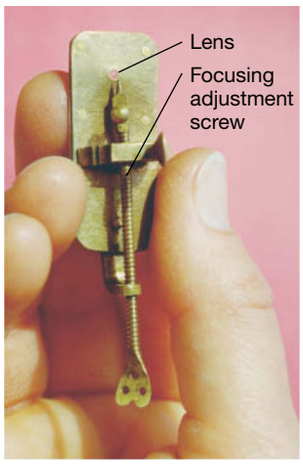
Figure 1.13 Robert Hooke and early microscopy. A drawing of the microscope used by Robert Hooke in 1664. The lens was fitted at the end of an adjustable bellows (G) and light focused on the specimen by a separate lens (1). Inset: Hooke's drawing of a bluish mold he found degrading a leather surface; the round structures contain spores of the mold.

Van Leeuwenhoek's microscope was a *light* microscope, and his design used a simple lens that could magnify an image at least 266 times. In a light microscope the sample is illuminated with visible light. **Magnification** describes the capacity of a microscope to enlarge an image. All microscopes employ lenses that provide magnification. Magnification, however, is not the limiting factor in our ability to see small objects. It is resolution that governs our ability to see the very small. **Resolution** is the ability to distinguish two adjacent objects as distinct and separate. The limit of resolution for a light microscope is about 0.2 µm (µm is the abbreviation for micrometer, 10^{-6} m). What this means is that two objects that are closer together than 0.2 µm cannot be resolved as distinct and separate.

Microscopy has improved considerably since the days of van Leeuwenhoek. Several types of light microscopy are now available, including *bright-field, phase-contrast, differential interference contrast, dark-field*, and *fluorescence*. With the modern compound light microscope, light from a light source is focused on the specimen by the condenser (**Figure 1.15**), and this light passes through the sample and is collected by the lenses. The modern compound light microscope contains two types of lenses, *objective* and *ocular*, that function in combination to magnify the image. Microscopes used in microbiology have ocular lenses that magnify 10–30× and objective lenses that magnify 10–100× (Figure 1.15*b*). The total magnification of a compound light microscope is the *product* of the magnification of its objective and ocular lenses (Figure 1.15*b*). Magnification of 1000× is required to resolve objects 0.2 µm in diameter, which is the limit of resolution for most light microscopes (increasing magnification beyond 1000× provides little improvement in the resolution of a light microscope).

The limit of resolution for a light microscope is a function of the wavelength of light used and the light-gathering ability of the objective lens, a property known as its *numerical aperture*. There is a correlation between the magnification of a lens and its numerical aperture; lenses with higher magnification typically have higher numerical apertures. The diameter of the smallest object resolvable by any lens is equal to 0.5λ/numerical aperture, where λ is the wavelength of light used. With objectives that have a very high numerical aperture (such as the 100× objective), an optical grade oil is placed between the microscope slide and the objective. Lenses on which oil is used are called *oil-immersion* lenses. Immersion oil increases the light-gathering ability of a lens, that is, it increases the amount of light that is collected and viewed by the lens.

In light microscopy, specimens are visualized because of differences in **contrast** that exist between them and their surroundings. In bright-field microscopy, contrast results when cells absorb or scatter light



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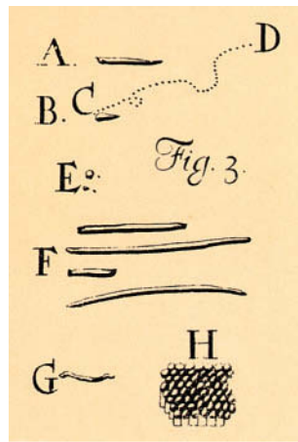
(a)



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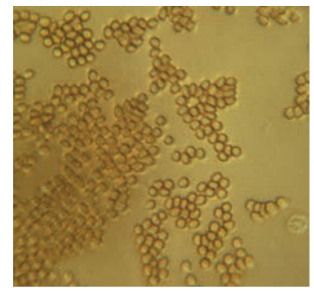


Brian J. Ford

(c)

Figure 1.14 The van Leeuwenhoek microscope. *(a)* A replica of Antoni van Leeuwenhoek's microscope. *(b)* Van Leeuwenhoek's drawings of bacteria, published in 1684. Even from these simple drawings we can recognize several shapes of common bacteria: A, C, F, and G, rods; E, cocci; H, packets of cocci. *(c)* Photomicrograph of a human blood smear taken through a van Leeuwenhoek microscope. Red blood cells are clearly apparent.

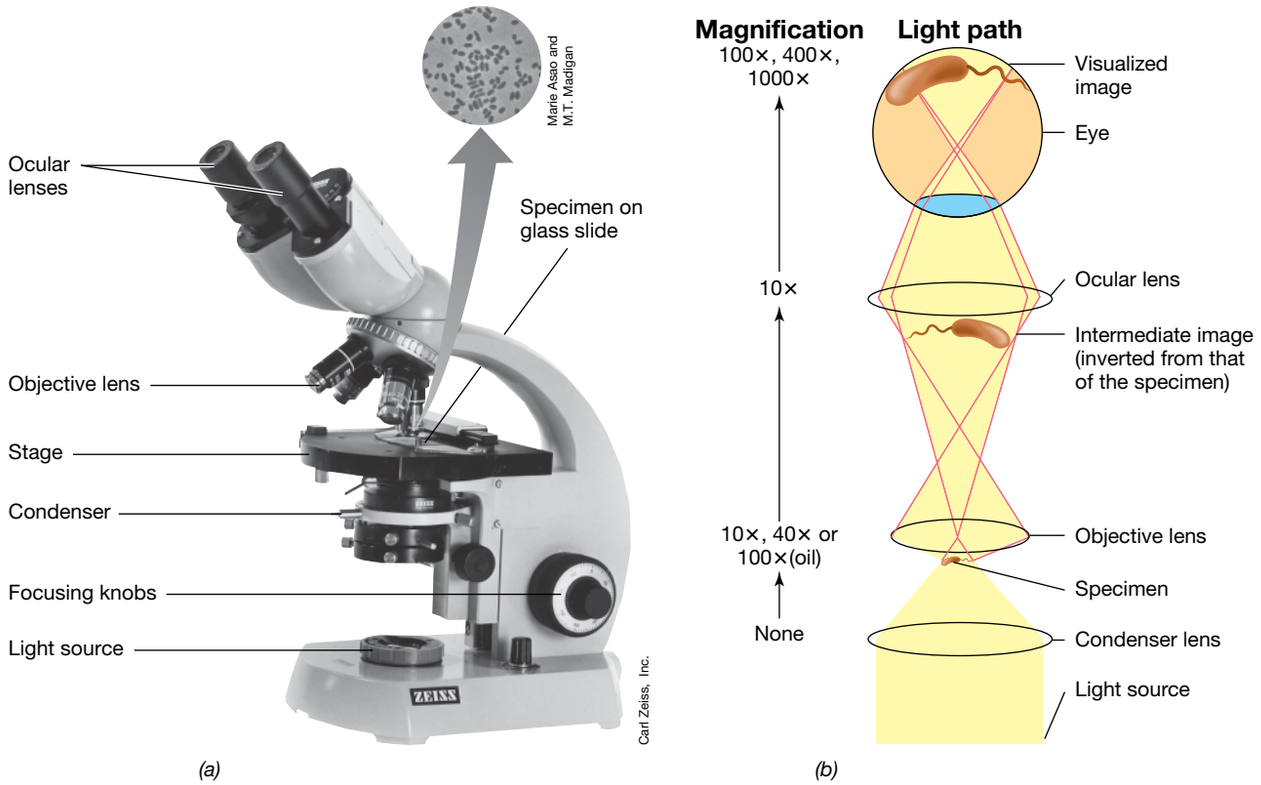


Figure 1.15 Microscopy. *(a)* A compound light microscope (inset photomicrograph of unstained cells taken through a phase-contrast light microscope). *(b)* Path of light through a compound light microscope. Figure 1.19 compares cells visualized by bright field with those visualized by phase contrast.

differently from their surroundings. Bacterial cells typically lack contrast, that is, their optical properties are similar to the surrounding medium, and hence they are difficult to see well with the bright-field microscope. Pigmented microorganisms are an exception because the color of the organism adds contrast, thus improving visualization by bright-field optics (**Figure 1.16**). For cells lacking pigments there are several ways to boost contrast, and we consider these methods in the next section.

MINIQUIZ

- Define the terms magnification and resolution.
- What is the limit of resolution for a bright-field microscope? What defines this limit?

1.6 Improving Contrast in Light Microscopy

Contrast is necessary in light microscopy to distinguish microorganisms from their surroundings. Cells can be stained to improve contrast, and staining is commonly used to visualize bacteria with bright-field microscopy. In addition to staining, other methods of light microscopy have been developed to improve contrast, such as phase contrast, differential interference contrast, dark field, and fluorescence.

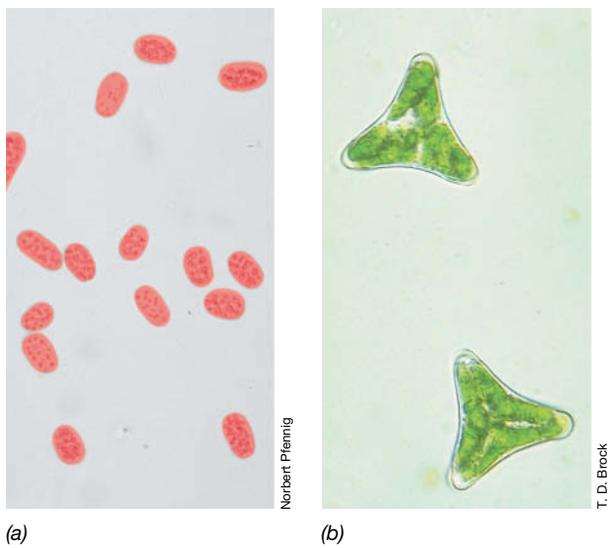


Figure 1.16 Bright-field photomicrographs of pigmented microorganisms. *(a)* Purple phototrophic bacteria (*Bacteria*). The bacterial cells are about 5 µm wide. *(b)* A green alga (eukaryote). The green structures are chloroplasts. The algal cells are about 15 µm wide. Purple bacteria are anoxygenic phototrophs, whereas algae are oxygenic phototrophs. Both groups contain photosynthetic pigments but only oxygenic phototrophs produce O_2 (Section 1.3 and Figure 1.5*a*).

Staining: Increasing Contrast for Bright-Field Microscopy

Dyes can be used to stain cells and increase their contrast so that they can be more easily seen in the bright-field microscope. Each class of dye has an affinity for specific cellular materials. Many dyes used in microbiology are positively charged, and for this reason they are called *basic dyes*. Examples of basic dyes include methylene blue, crystal violet, and safranin. Basic dyes bind strongly to negatively charged cell components, such as nucleic acids and acidic polysaccharides. These dyes also stain the surfaces of cells, because cell surfaces tend to be negatively charged. These properties make basic dyes useful general-purpose stains that nonspecifically stain most bacterial cells.

To perform a simple stain, one begins with dried preparations of cells (Figure 1.17). A clean glass slide containing a dried suspension of cells is flooded for a minute or two with a dilute solution of a basic dye, rinsed several times in water, and blotted dry. Because their cells are so small, it is common to observe dried, stained preparations of bacterial cells with a high-power (oil-immersion) lens.

Differential Stains: The Gram Stain

Stains that render different kinds of cells different colors are called *differential* stains. An important differential-staining procedure used in microbiology is the **Gram stain** (Figure 1.18). On the basis of their reaction in the Gram stain, bacteria can be divided into two major groups: **gram-positive** and **gram-negative**. After Gram staining, gram-positive bacteria appear purple-violet and gram-negative bacteria appear pink (Figure 1.18*b*). The color difference in the Gram stain arises because of differences in the cell wall structure of gram-positive and gram-negative cells (Section 2.4). Staining with a basic dye such as crystal violet renders cells purple in color. Cells are then treated with ethanol, which decolorizes gram-negative cells but not gram-positive cells. Finally, cells are counterstained with a different-colored stain, typically the red stain safranin. As a result, gram-positive and gram-negative cells can be distinguished microscopically by their different colors (Figure 1.18*b*).

Figure 1.17 Staining cells for microscopic observation. Stains improve the contrast between cells and their background. Step 3 center: Same cells as shown in Figure 1.15 inset but stained with a basic dye.

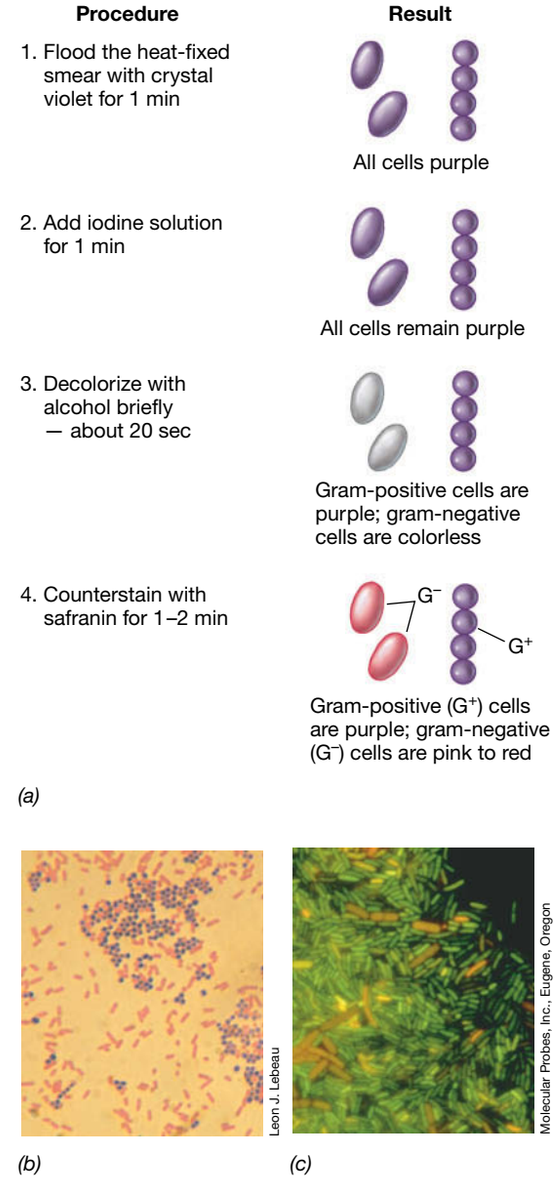


Figure 1.18 The Gram stain. *(a)* Steps in the procedure. *(b)* Microscopic observation of gram-positive (purple) and gram-negative (pink) bacteria. The organisms are *Staphylococcus aureus* and *Escherichia coli*, respectively. *(c)* Cells of *Pseudomonas aeruginosa* (gram-negative, green) and *Bacillus cereus* (gram-positive, orange) stained with a one-step fluorescent staining method. This method allows for differentiating gram-positive from gram-negative cells in a single staining step.

The Gram stain is the most common staining procedure used in microbiology, and it is often performed to begin the characterization of a new bacterium. If a fluorescence microscope is available, the Gram stain can be reduced to a one-step procedure; gram-positive and gram-negative cells fluoresce different colors when treated with a special chemical (Figure 1.18*c*).

Phase-Contrast and Dark-Field Microscopy

Although staining is widely used in light microscopy, staining often kills cells and can distort their features. Two forms of light microscopy improve image contrast of unstained (and thus live) cells. These are phase-contrast microscopy and dark-field microscopy (**Figure 1.19**). The phase-contrast microscope in particular is widely used in teaching and research for the observation of living preparations.

Phase-contrast microscopy is based on the principle that cells differ in refractive index (that is, the ability of a material to alter the speed of light) from their surroundings. Light passing through a cell thus differs in phase from light passing through the surrounding liquid. This subtle difference is amplified by a device in the objective lens of the phase-contrast microscope called the *phase ring*, resulting in a dark image on a light background (Figure 1.19*b*; see also inset to Figure 1.15*a*). The ring consists of a phase plate that amplifies the variation in phase to produce the higher-contrast image.

In the dark-field microscope, light does not pass through the specimen. Instead, light is directed from the sides of the specimen and only light that is scattered when it hits the specimen can reach the lens. Thus, the specimen appears light on a dark background (Figure 1.19*c*). Dark-field microscopy often has better resolution than light microscopy, and some objects can be resolved by dark-field that cannot be resolved by bright-field or even by phase-contrast microscopes. Dark-field microscopy is a particularly good way to observe microbial motility, as bundles of flagella (the structures responsible for swimming motility) are often resolvable with this technique.

Fluorescence Microscopy

The fluorescence microscope visualizes specimens that fluoresce. In fluorescence microscopy, cells are made to fluoresce (to emit light) by illuminating them from above with light of a single color. Filters are used so that only fluorescent light is seen, and thus cells appear to glow in a black background (**Figure 1.20**).

Cells fluoresce either because they contain naturally fluorescent substances such as chlorophyll (autofluorescence, Figure 1.20*b*, *d*) or because they have been stained with a fluorescent dye (Figure 1.20*e*). DAPI (4′,6-*di*a*m*idino-2-*p*heny*l*indole) is a widely used fluorescent dye that stains cells bright blue because it complexes with the cell's DNA (Figure 1.20*e*). DAPI can be used to visualize cells in their natural habitats, such as soil, water, food, or a clinical specimen. Fluorescence microscopy using DAPI is widely used in clinical diagnostic microbiology and also in microbial ecology for enumerating bacteria in a natural environment or in a cell suspension (Figure 1.20*e*).

MINIQUIZ

- What color will a gram-negative cell be after Gram staining by the conventional method?
- What major advantage does phase-contrast microscopy have over staining?
- How can cells be made to fluoresce?

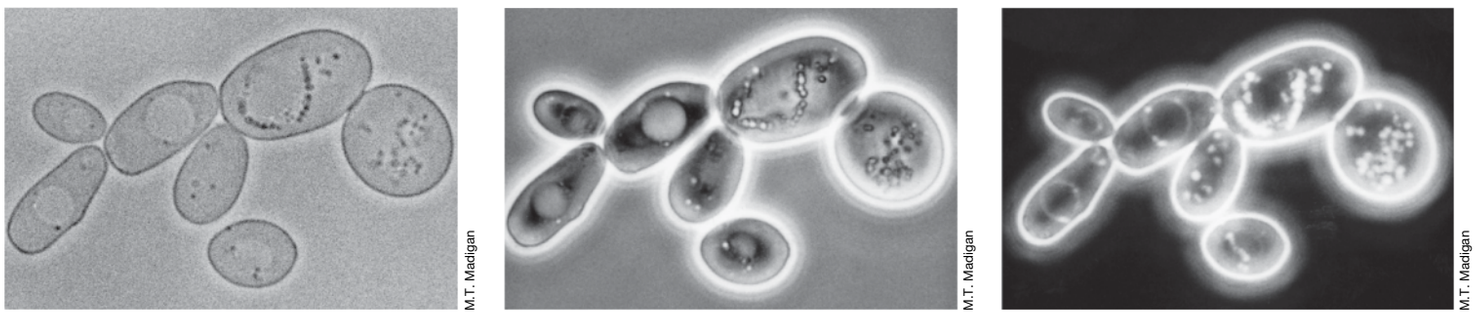
1.7 Imaging Cells in Three Dimensions

Thus far we have only considered forms of microscopy in which the rendered images are two-dimensional. Two methods of light microscopy can render a more three-dimensional image, and in this section we explore these forms of microscopy.

Differential Interference Contrast Microscopy

Differential interference contrast (DIC) microscopy is a form of light microscopy that employs a polarizer in the condenser to produce polarized light (light in a single plane). The polarized light then passes through a prism that generates two distinct beams. These beams pass through the specimen and enter the objective lens, where they are recombined into one. Because the two beams pass through substances that differ in refractive index, the combined beams are not totally in phase but instead interfere with each other. This optical effect provides a three-dimensional perspective, which enhances subtle differences in cell structure.

Using DIC microscopy, cellular structures such as the nucleus of eukaryotic cells (**Figure 1.21**), or endospores, vacuoles, and inclusions of bacterial cells, appear more three-dimensional than in



(a) (b) (c)

Figure 1.19 Cells visualized by different types of light microscopy. The same field of cells of the yeast *Saccharomyces cerevisiae* visualized by *(a)* bright-field microscopy, *(b)* phase-contrast microscopy, and *(c)* dark-field microscopy. Cells average 8–10 µm wide.

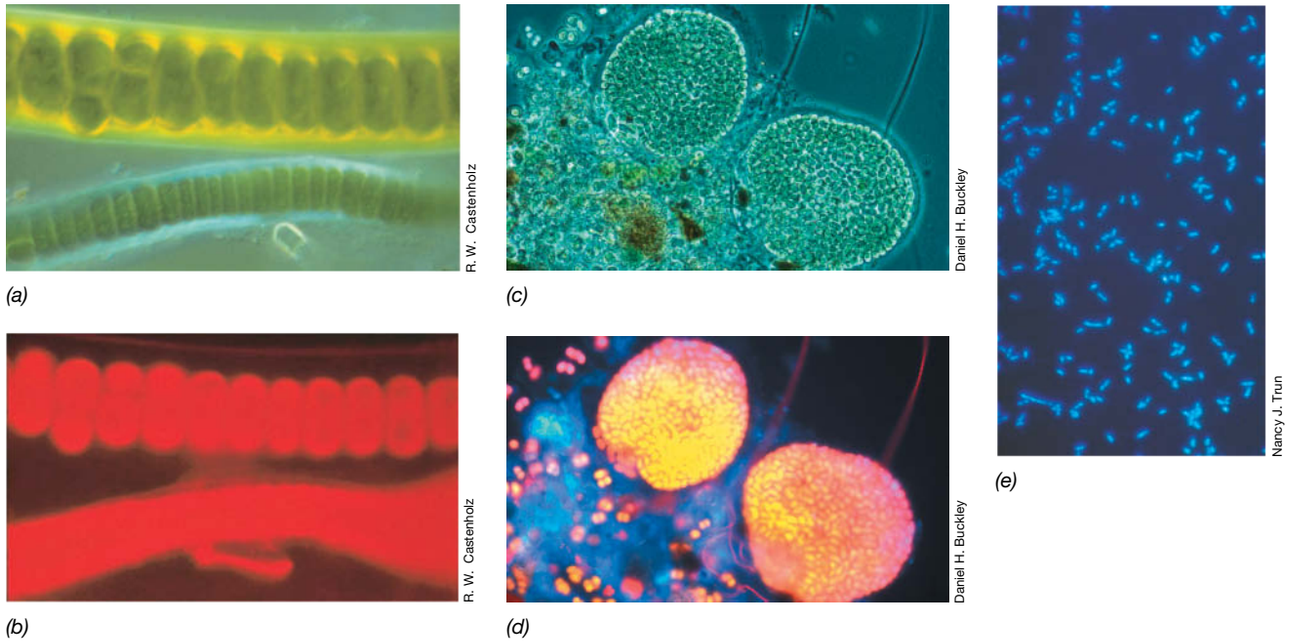


Figure 1.20 Fluorescence microscopy. *(a, b, c, d)* Cyanobacteria. The same cells are observed by phase-contrast microscopy *(a, c)* and by fluorescence microscopy *(b, d)*. The cells fluoresce because they contain chlorophyll *a* and other pigments. The image in *b* was generated using a filter specific for the fluorescence of chlorophyll *a*, while the image in *d* was generated using a permissive filter that shows fluorescence from a range of pigments that occur naturally in cyanobacteria. *(e)* Fluorescence photomicrograph of cells of *Escherichia coli* made fluorescent by staining with the fluorescent dye DAPI, which binds to DNA.

other forms of light microscopy. DIC microscopy is typically used on unstained cells as it can reveal internal cell structures that are nearly invisible by bright-field microscopy without the need for staining (compare Figure 1.19*a* with Figure 1.21).

Confocal Scanning Laser Microscopy

A confocal scanning laser microscope (CSLM) is a computer-controlled microscope that couples a laser to a fluorescence microscope. The laser generates a high-contrast, three-dimensional image and allows the viewer to access several planes of focus in the specimen (**Figure 1.22**). The laser beam is precisely adjusted such that only a particular layer within a specimen is in perfect focus at one time. Cells struck by the laser fluoresce to generate the image as in fluorescence microscopy (Section 1.6).

Cells in CSLM preparations can also be stained with fluorescent dyes to make them more distinct (Figure 1.22*a*). The laser then scans up and down through the layers of the sample, generating an image for each layer. A computer assembles the pictures to compose the many layers into a single high-resolution, three-dimensional image. Thus, for a relatively thick specimen such as a bacterial biofilm (Figure 1.22*a*), not only can cells on the surface of the biofilm be observed, as with conventional light microscopy, but cells in the various layers are also observed by adjusting the plane of focus of the laser beam. CSLM is particularly useful when thick specimens need to be examined.

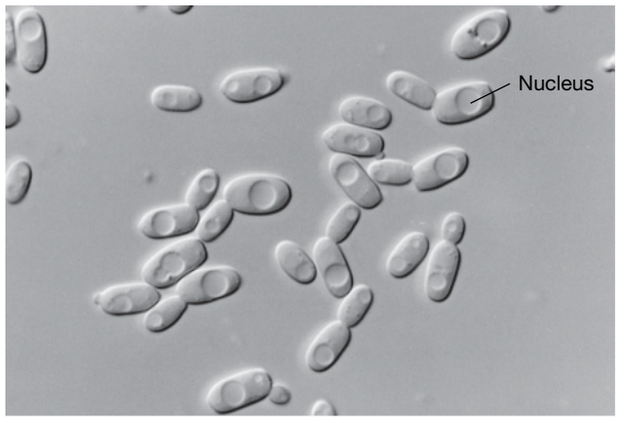


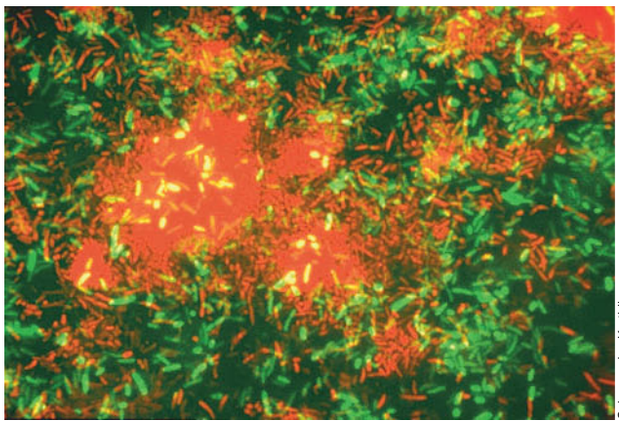
Figure 1.21 Differential interference contrast microscopy. The yeast cells are about 8 μm wide. Note the clearly visible nucleus and compare to the bright-field image of yeast cells in Figure 1.19*a*.

MINIQUIZ

- What structure in eukaryotic cells is more easily seen in DIC than in bright-field microscopy? (*Hint:* Compare Figures 1.19*a* and 1.21).
- Why is CSLM able to view different layers in a thick preparation while bright-field microscopy cannot?

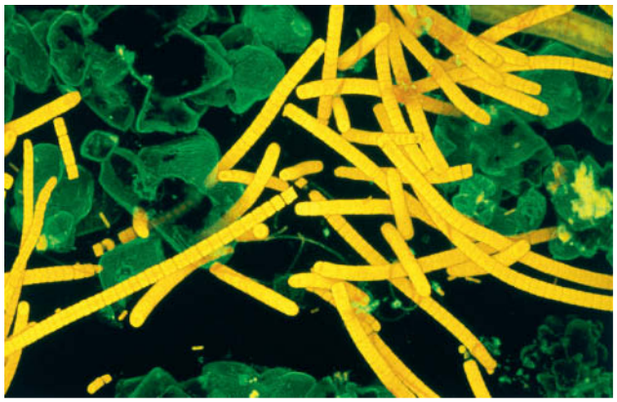
1.8 Probing Cell Structure: Electron Microscopy

Electron microscopes use electrons instead of visible light (photons) to image cells and cell structures. In the electron microscope, electromagnets function as lenses, and the whole system operates in a vacuum (**Figure 1.23**). Electron microscopes are fitted with



Subramanian Karthikeyan

(a)



Gernot Arp and Christian Boeker, Carl Zeiss, Jena

(b)

Figure 1.22 Confocal scanning laser microscopy. *(a)* Confocal image of a microbial biofilm community. The green, rod-shaped cells are *Pseudomonas aeruginosa* experimentally introduced into the biofilm. Cells of different colors are present at different depths in the biofilm. *(b)* Confocal image of a filamentous cyanobacterium growing in a soda lake. Cells are about 5 µm wide.

cameras to allow a photograph, called an *electron micrograph*, to be taken. Two types of electron microscopy are in routine use in microbiology: transmission and scanning.

Transmission Electron Microscopy

The *transmission electron microscope* (TEM) is used to examine cells and cell structure at very high magnification and resolution. The resolving power of a TEM is much greater than that of the light microscope, even allowing one to view structures at the molecular level (**Figure 1.24**). This is because the wavelength of electrons is much shorter than the wavelength of visible light, and, as we have learned, wavelength affects resolution (Section 1.5). For example, whereas the resolving power of a light microscope is about 0.2 *micrometer*, the resolving power of a TEM is about 0.2 *nanometer*, a thousandfold improvement. With such powerful resolution, objects as small as individual protein and nucleic acid molecules can be visualized by transmission electron microscopy (Figure 1.24*b*).

Unlike photons, electrons are very poor at penetrating; even a single cell is too thick to penetrate with an electron beam. Consequently, to view the internal structure of a cell, *thin sections* of the

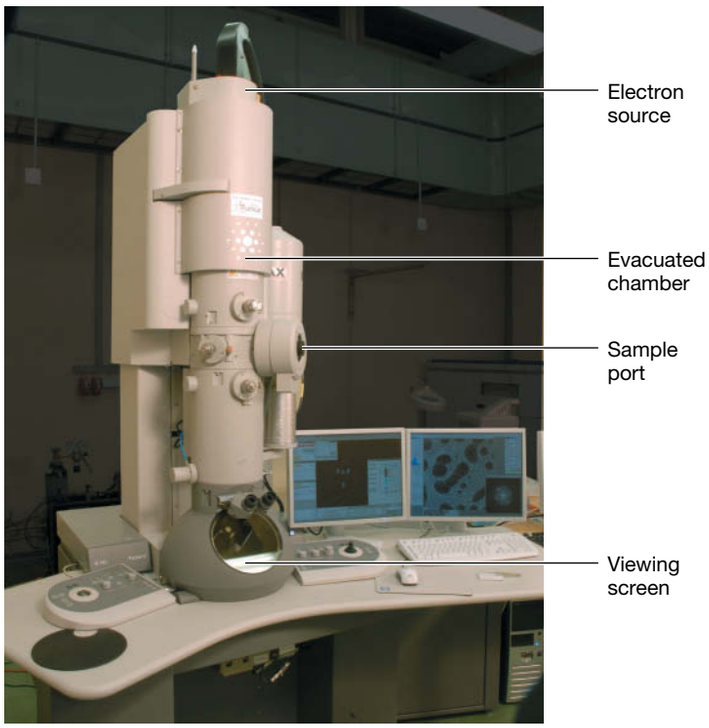


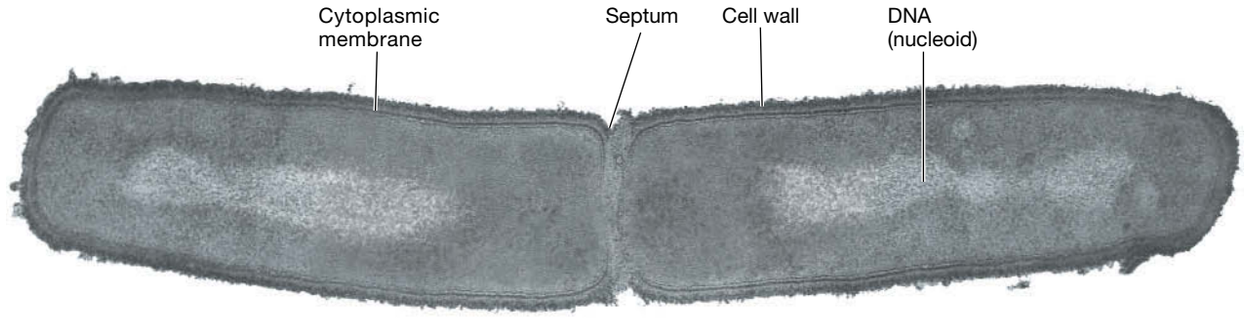
Figure 1.23 The electron microscope. This instrument encompasses both transmission and scanning electron microscope functions.

cell are needed, and the sections must be stabilized and stained with various chemicals to make them visible. A single bacterial cell, for instance, is cut into extremely thin (20–60 nm) slices, which are then examined individually by TEM (Figure 1.24*a*). To obtain sufficient contrast, the sections are treated with stains such as osmic acid, or permanganate, uranium, lanthanum, or lead salts. Because these substances are composed of atoms of high atomic weight, they scatter electrons well and thus improve contrast. If only the *external* features of an organism are to be observed, thin sections are unnecessary. Intact cells or cell components can be observed directly in the TEM by a technique called *negative staining* (Figure 1.24*b*).

Scanning Electron Microscopy

For optimal three-dimensional imaging of cells, a *scanning electron microscope* (SEM) is used. In scanning electron microscopy, the specimen is coated with a thin film of a heavy metal, typically gold. An electron beam then scans back and forth across the specimen. Electrons scattered from the metal coating are collected and projected on a monitor to produce an image (Figure 1.24*c*). In the SEM, even fairly large specimens can be observed, and the depth of field (the portion of the image that remains in sharp focus) is extremely good. A wide range of magnifications can be obtained with the SEM, from as low as 15× up to about 100,000×, but only the *surface* of an object is typically visualized.

Electron micrographs taken by either TEM or SEM are black-and-white images. Although the original image contains the maximum amount of scientific information that is available, color is often added to scanning electron micrographs by manipulating



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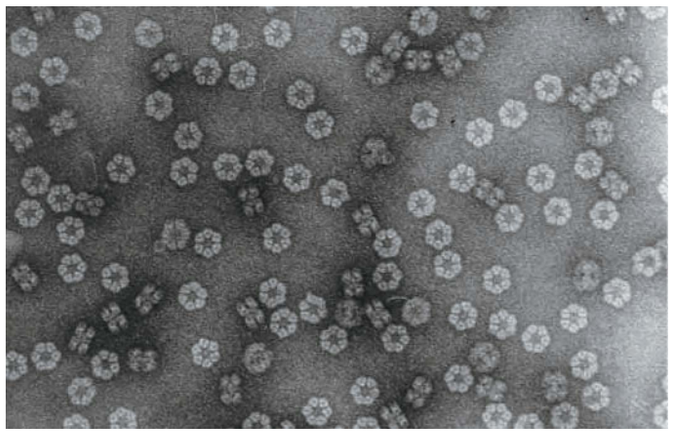

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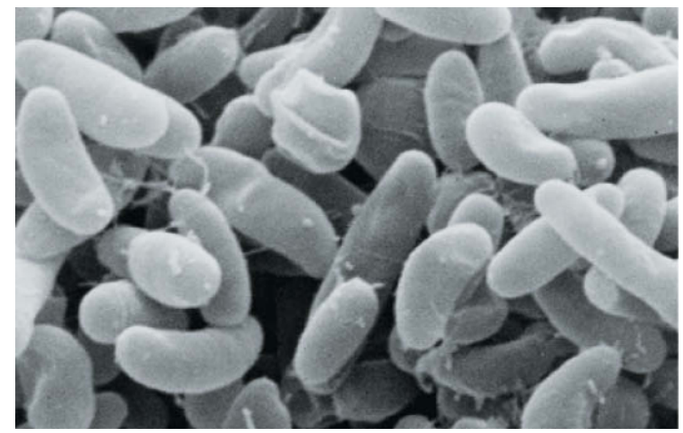


(c)

Figure 1.24 Electron micrographs. *(a)* Micrograph of a thin section of a dividing bacterial cell, taken by transmission electron microscopy (TEM). The cell is about 0.8 μm wide. *(b)* TEM of negatively stained molecules of hemoglobin. Each hexagonal-shaped molecule is about 25 nanometers (nm) in diameter and consists of two doughnut-shaped rings, a total of 15 nm wide. *(c)* Scanning electron micrograph (SEM) of bacterial cells. A single cell is about 0.75 μm wide.

them in a computer. However, such false color does not improve resolution of a micrograph. In this book, false color will be used sparingly in electron micrographs so as to present the micrographs in their original scientific context.

MINIQUIZ

- What is an electron micrograph? Why do electron micrographs have greater resolution than light micrographs?
- What type of electron microscope would be used to view a cluster of cells? What type would be used to observe internal cell structure?

III • Microbial Cultivation Expands the Horizon of Microbiology

Following the discovery of microorganisms driven by microscopic methods, major discoveries in microbiology were fueled by advances in microbial cultivation. Important advances included the development of **aseptic technique**, which is a collection of practices that allow for the preparation and maintenance of **sterile** (that is, without the presence of living organisms) nutrient media and solutions (Figure 5.12). Aseptic technique is essential for the isolation and maintenance of pure cultures of bacteria. **Pure cultures** are those that contain cells from only a single type of microorganism and are of great value for the study of microorganisms. Finally, **enrichment culture techniques**, which allow for the isolation from nature of microbes having particular metabolic characteristics, facilitate the discovery of diverse microorganisms.

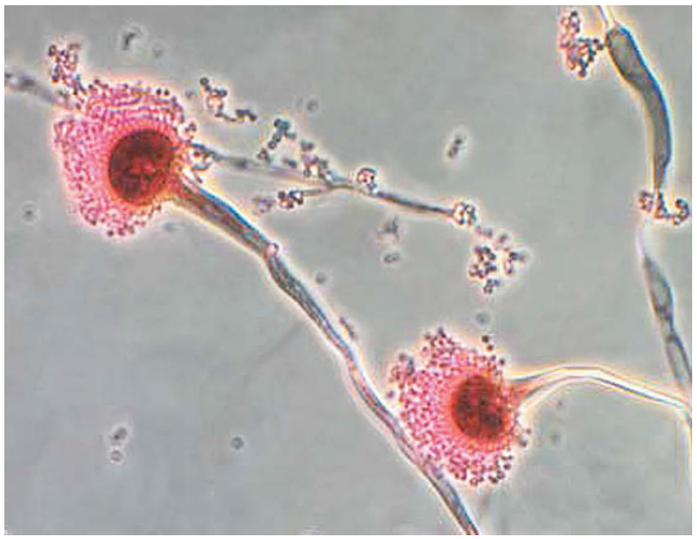
Advances in microbial cultivation are directly responsible for success in fighting infectious disease, the discovery of microbial diversity, and the use of microbes as model systems to discover the fundamental properties of all living cells. Important advances in microbial cultivation occurred in the nineteenth century as microbiologists sought to answer two major questions of that time: (1) Does spontaneous generation occur? (2) What is the nature of infectious disease? Answers to these seminal questions emerged from the work of two giants in the field of microbiology: the French chemist Louis Pasteur and the German physician Robert Koch. We begin with the work of Pasteur.

1.9 Pasteur and Spontaneous Generation

Pasteur was a chemist by training and was one of the first to recognize that many of what were thought to be strictly chemical reactions were actually catalyzed by microorganisms. Pasteur studied the chemistry of crystal formation and he used microscopes to examine crystal structure. His training in chemistry and microscopy prepared him to make a series of foundational discoveries to further the science of microbiology.

The Microbial Basis of Fermentation

Early in his career, Pasteur studied crystals formed during the production of alcohol. Through careful microscopic observation of tartaric acid crystals formed in wine, he observed two types of crystals that had mirror-image structures (**Figure 1.25**). He separated these by hand and observed that each type of crystal bent a beam of polarized light in a different direction. In this way he discovered that chemically identical substances can have *optical isomers*, which have different molecular structures that can influence their properties. Pasteur went on to discover that microorganisms could discriminate between optical isomers. For example, cultures of the mold *Aspergillus* (Figure 1.25*a*) metabolized exclusively D-tartrate but not its optical isomer, L-tartrate (Figure 1.25*b*). The fact that a living organism could discriminate between optical isomers led Pasteur to suspect that many reactions that were thought to be purely chemical in nature were actually catalyzed by specific microorganisms.



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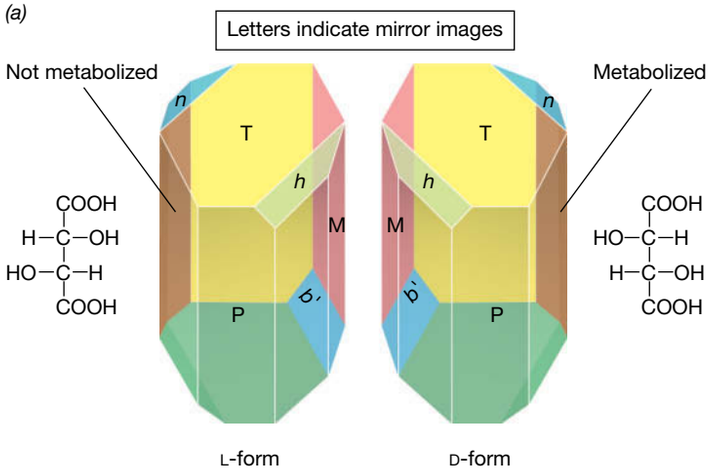
(a)



(b)

Figure 1.25 Louis Pasteur and optical isomers. *(a)* Light micrograph of cells of the mold *Aspergillus*. *(b)* Pasteur's drawings of crystals of tartaric acid. Left-handed L-form crystals bend light to the left, and right-handed D-form crystals bend light to the right. Note that the two crystals are mirror images of one another, a hallmark of optical isomers. Pasteur found that only D-tartrate was metabolized by *Aspergillus*.

While a professor of chemistry, Pasteur encountered a local businessman who produced alcohol industrially from beet juice. The businessman was losing money because many of his vats produced, instead of alcohol, a product that smelled like sour milk, which Pasteur determined to be lactic acid. In the mid-nineteenth century the production of alcohol was thought to be solely a chemical process. Pasteur studied the broth with his microscope, but instead of crystals he observed cells. Pasteur observed that the vats that produced alcohol were full of yeast, but the sour vats were full of rod-shaped bacteria. He hypothesized that these were living organisms whose growth produced either alcohol or lactic acid.

Pasteur needed to grow these organisms to prove his hypothesis. He prepared an extract of yeast cells, deducing that this would contain all of the nutrients that yeast need to grow. He then used a porcelain filter to remove all cells from this yeast extract nutrient medium, rendering it sterile. If he introduced living yeast back into this sterile yeast extract medium he could observe their growth and show the production of alcohol, but if he instead introduced the small rods he then observed lactic acid formation. Heating of these cultures eliminated growth *and* the production of either alcohol or lactic acid. In this way he proved that fermentation is carried out by microorganisms and that different microorganisms perform different fermentation reactions.

During his work on fermentation, Pasteur observed that other organisms would often grow in his yeast extract medium. He deduced that these organisms were being introduced from the air. Pasteur's work on fermentation had prepared him to conduct a series of classic experiments on spontaneous generation, experiments that are forever linked to his name and which helped establish microbiology as a modern science.

Spontaneous Generation

The concept of **spontaneous generation** existed for thousands of years and its basic tenet can be easily grasped. If food or some other perishable material is allowed to stand for some time, it putrefies. When examined microscopically, the putrefied material is teeming with microorganisms. From where do these organisms arise? Prior to Pasteur it was common belief that life arose spontaneously from nonliving materials, that is, by *spontaneous generation*.

Pasteur became a powerful opponent of spontaneous generation. He predicted that microorganisms in putrefying materials were descendants of cells that entered from the air or cells that had been initially present on the decaying materials. Pasteur reasoned that if food were treated in such a way as to destroy all living organisms present—that is, if it were rendered sterile—and if it were kept sterile, it would not putrefy.

Pasteur used heat to kill contaminating microorganisms, and he found that extensive heating of a nutrient solution followed by

sealing kept it from putrefying. Proponents of spontaneous generation criticized these experiments by declaring that "fresh air" was necessary for the phenomenon to occur. In 1864 Pasteur countered this objection simply and brilliantly by constructing a swan-necked flask, now called a *Pasteur flask* (**Figure 1.26**). In such a flask, nutrient solutions could be heated to boiling and sterilized. After the flask cooled, air could reenter, but the bend in the neck prevented particulate matter (including microorganisms) from entering the nutrient solution and initiating putrefaction. Nutrient solutions in such flasks remained sterile indefinitely. Microbial growth was observed only after particulate matter from the neck of the flask was allowed to enter the liquid in the flask (Figure 1.26*c*). This experiment settled the spontaneous generation controversy forever.

Pasteur's work on spontaneous generation demonstrated the importance of sterilization and led to the development of effective sterilization procedures that were eventually standardized and applied widely in microbiology, medicine, and industry. For example, the British physician Joseph Lister (1827–1912) deduced from Pasteur's discoveries that surgical infections were caused by microorganisms. He implemented a range of techniques designed to kill microorganisms and to prevent microbial infection of surgical patients. Lister is credited with the introduction of aseptic techniques for surgeries (1867), and his methods were adopted worldwide; these greatly improved the survival rate of surgical patients. The food industry also benefited from the work of Pasteur, as his principles were quickly adapted for the preservation of milk and many other foods by heat treatment, which we now call *pasteurization*.

Other Accomplishments of Pasteur

Pasteur went on to many other triumphs in microbiology and medicine. Some highlights include his development of vaccines for the diseases anthrax, fowl cholera, and rabies. Pasteur's work on rabies was his most famous success, culminating in July 1885 with the first administration of a rabies vaccine to a human, a young French boy named Joseph Meister who had been bitten by a rabid dog. In those days, a bite from a rabid animal was invariably fatal. News spread quickly of the success of Meister's vaccination, and of one administered shortly thereafter to a young shepherd boy, Jean-Baptiste Jupille (**Figure 1.27*a***). Within a year several thousand people bitten by rabid animals had traveled to Paris to be treated with Pasteur's rabies vaccine.

Pasteur's fame was legendary and led the French government to establish the Pasteur Institute in Paris in 1888 (Figure 1.27*b*). Originally established as a clinical center for the treatment of rabies and other contagious diseases, the Pasteur Institute today is a major biomedical research center focused on antiserum and vaccine research and production. The medical and veterinary breakthroughs of Pasteur not only were highly significant in their own right but helped solidify the concept of the germ theory of disease, whose principles were being developed at about the same time by a second giant of this era, Robert Koch.

MINIQUIZ

- Define the term sterile. What two methods did Pasteur used to make solutions sterile?
- How did Pasteur's experiments using swan-necked flasks defeat the theory of spontaneous generation?
- Besides ending the controversy over spontaneous generation, what other accomplishments do we credit to Pasteur?

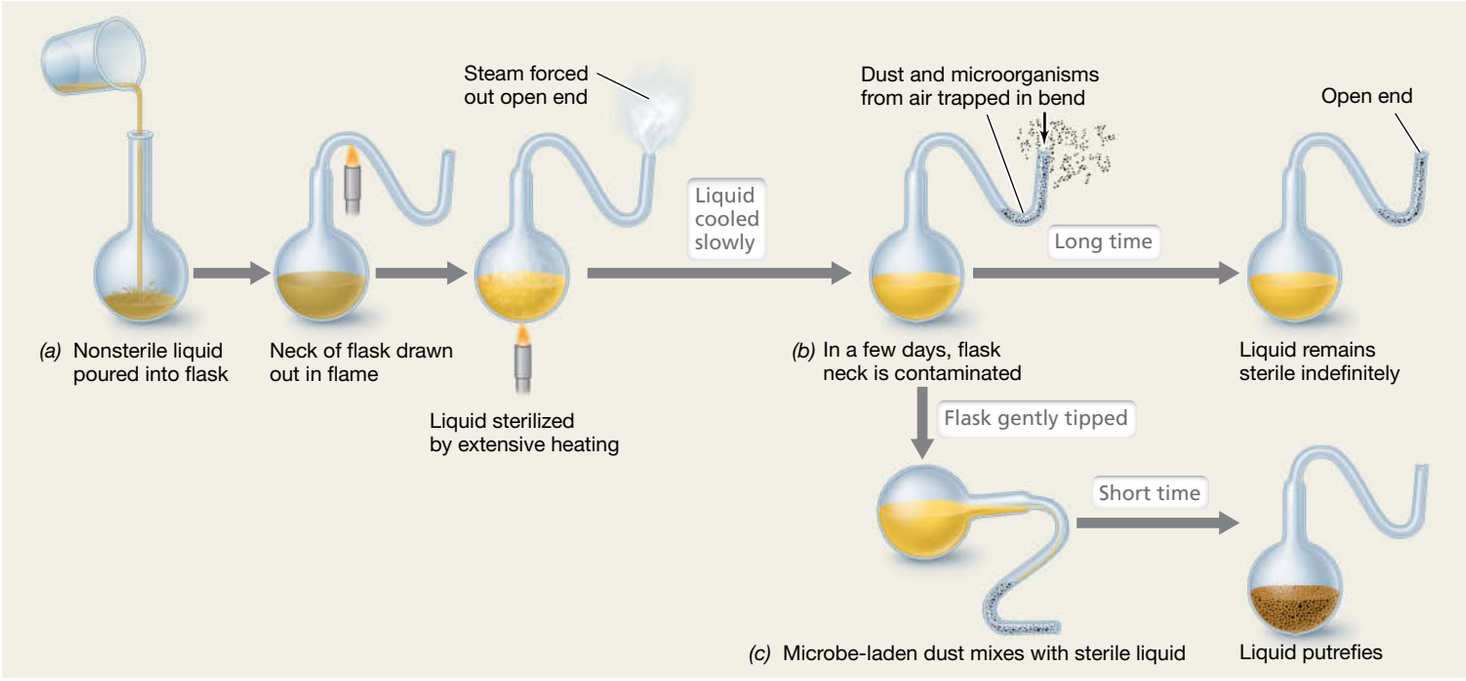
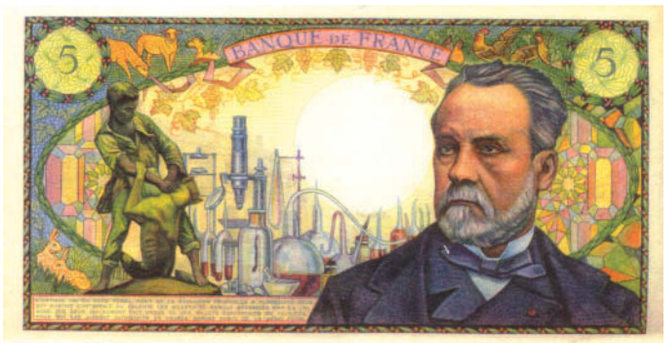


Figure 1.26 The defeat of spontaneous generation: Pasteur's swan-necked flask experiment. In *(c)* the liquid putrefies because microorganisms enter with the dust. The bend in the flask allowed air to enter (a key objection to Pasteur's sealed flasks) but prevented microorganisms from entering.



(a)

M.T. Madigan

(b)

Figure 1.27 Louis Pasteur and some symbols of his contributions to microbiology. *(a)* A French 5-franc note honoring Pasteur. The shepherd boy Jean-Baptiste Jupille is shown killing a rabid dog that had attacked children. Pasteur's rabies vaccine saved Jupille's life. In France, the franc preceded the euro as a currency. *(b)* Part of the Pasteur Institute, Paris, France. Today this structure, built for Pasteur by the French government, houses a museum that displays some of the original swan-necked flasks used in his experiments and a chapel containing Pasteur's crypt.

1.10 Koch, Infectious Diseases, and Pure Cultures

Proof that some microorganisms can cause disease provided the greatest impetus for the development of microbiology as an independent biological science. As early as the sixteenth century it was suspected that some agent of disease could be transmitted from a diseased person to a healthy person. After microorganisms were discovered, a number of individuals proposed that they caused infectious diseases, but skepticism prevailed and definitive proof was lacking. As early as 1847, the Hungarian physician Ignaz Semmelweis promoted sanitary methods including hand washing as a method for preventing infections. His methods are credited with saving many lives, but he could not prove why these methods worked and his advice was met with scorn by most of the medical community. The work of Pasteur and Lister provided strong evidence that microbes were the cause of infectious disease, but it was not until the work of the German physician Robert Koch (1843–1910) (**Figure 1.28**) that the germ theory of infectious disease had direct experimental support.

The Germ Theory of Disease and Koch's Postulates

In his early work Koch studied anthrax, a disease of cattle and occasionally of humans. Anthrax is caused by an endospore-forming bacterium called *Bacillus anthracis*. By careful microscopy and staining, Koch established that the bacteria were always present in the blood of an animal that was succumbing to the disease. However, Koch reasoned that the mere *association* of the bacterium with the disease was not actual proof of *cause and effect*, and he seized the opportunity to study cause and effect experimentally using anthrax and laboratory animals. The results of this study formed the standard by which infectious diseases have been studied ever since.

Koch used mice as experimental animals. Using appropriate controls, Koch demonstrated that when a small drop of blood from a mouse with anthrax was injected into a healthy mouse, the latter quickly developed anthrax. He took blood from this second animal, injected it into another, and again observed the characteristic disease symptoms. However, Koch carried this experiment a critically important step further. He discovered that the anthrax bacteria could be grown in a nutrient medium *outside the host* and

Figure 1.28 Robert Koch. The German physician and microbiologist is credited with founding medical microbiology and formulating his famous postulates.

that even after many transfers in laboratory culture, the bacteria still caused the disease when inoculated into a healthy animal.

On the basis of these experiments and others on the causative agent of tuberculosis, Koch formulated a set of rigorous criteria, now known as **Koch's postulates**, for definitively linking cause and effect in an infectious disease. Koch's postulates, summarized in Figure 1.29, stressed the importance of *laboratory culture* of the putative infectious agent followed by introduction of the suspected agent into virgin animals and recovery of the pathogen from diseased or dead animals. With these postulates as a guide, Koch, his students, and those that followed them discovered the causative agents of most of the important infectious diseases of humans and domestic animals. These discoveries also led to the development of successful treatments for the prevention and cure of many of these diseases, greatly improving the scientific basis of clinical medicine and human health and welfare (Figure 1.8).

Koch, Pure Cultures, and Microbial Taxonomy

The second of Koch's postulates states that the suspected pathogen must be isolated and grown away from other microorganisms in laboratory culture (Figure 1.29); in microbiology we say that such a culture is *pure*. To accomplish this important goal, Koch and his associates developed several simple but ingenious methods of obtaining and growing bacteria in pure culture, and many of these methods are still used today.

Koch started by using natural surfaces such as a potato slice to obtain pure cultures, but he quickly developed more reliable and reproducible growth media employing liquid nutrient solutions solidified with gelatin, and later with agar, an algal polysaccharide with excellent properties for this purpose. Along with his associate Walther Hesse, Koch observed that when a solid surface was incubated in air, masses of microbial cells called colonies developed, each having a characteristic shape and color (Figure 1.30).

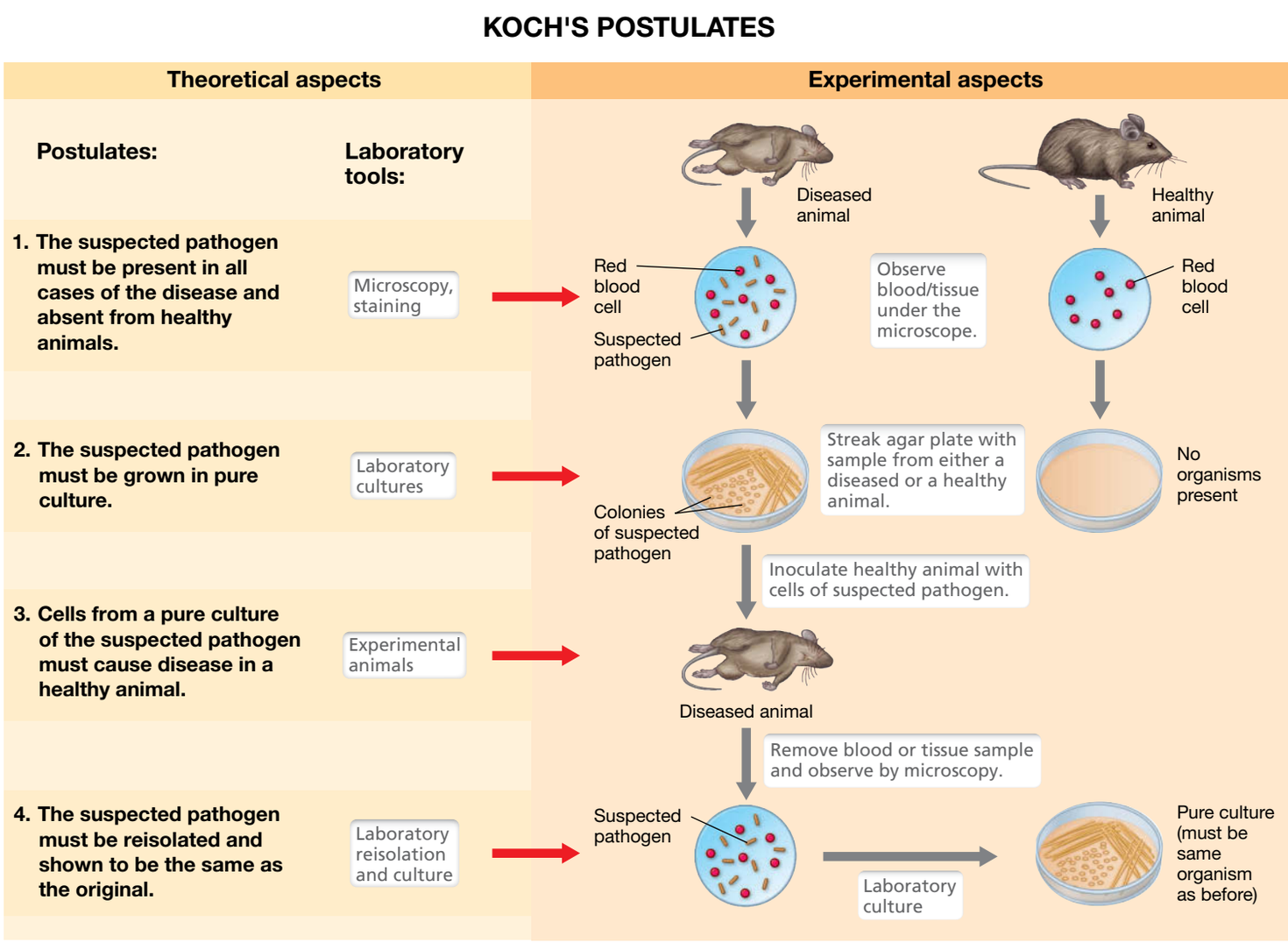


Figure 1.29 Koch's postulates for proving cause and effect in infectious diseases. Note that following isolation of a pure culture of the suspected pathogen, the cultured organism must both initiate the disease and be recovered from the diseased animal. Establishing the correct conditions for growing the pathogen is essential; otherwise it will be missed.

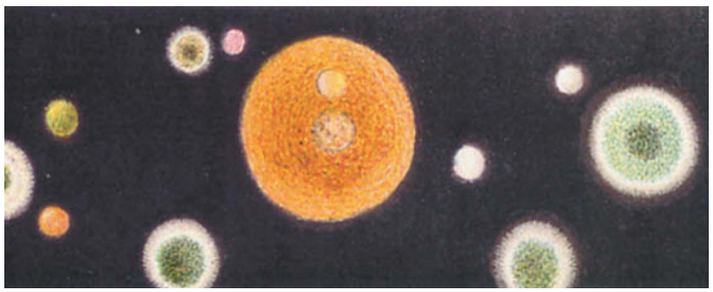


Figure 1.30 A hand-colored photograph taken by Walther Hesse of colonies formed on agar. The colonies include those of molds and bacteria obtained during Hesse's studies of the microbial content of air in Berlin, Germany, in 1882. From Hesse, W. 1884. "Ueber quantitative Bestimmung der in der Luft enthaltenen Mikroorganismen." *Mittheilungen aus dem Kaiserlichen Gesundheitsamte. 2*: 182–207.

He inferred that each colony had arisen from a single bacterial cell that had grown to yield the mass of cells (see also Figure 1.2). Koch reasoned that each colony harbored a pure culture (a population of identical cells), and he quickly realized that solid media provided an easy way to obtain pure cultures. Richard Petri, another associate of Koch, developed the transparent double-sided "Petri dish" in 1887, and this quickly became the standard tool for obtaining pure cultures.

Koch was keenly aware of the implications his pure culture methods had for classifying microorganisms. He observed that colonies that differed in color and size (Figure 1.30) bred true and that cells from different colonies typically differed in size and shape and often in their nutrient requirements as well. Koch realized that these differences were analogous to the criteria taxonomists had established for the classification of larger organisms, such as plant and animal species, and he suggested that the different types of bacteria should be considered as "species, varieties, forms, or other suitable designation." Such insightful thinking was important for the rapid acceptance of microbiology as a new biological science, rooted as biology was in classification during Koch's era.

Koch and Tuberculosis

Koch's crowning scientific accomplishment was his discovery of the causative agent of tuberculosis. At the time Koch began this work (1881), one-seventh of all reported human deaths were caused by tuberculosis (Figure 1.8). There was a strong suspicion that tuberculosis was a contagious disease, but the suspected agent had never been seen, either in diseased tissues or in culture. Following his successful studies of anthrax, Koch set out to demonstrate the cause of tuberculosis, and to this end he brought together all of the methods he had so carefully developed in his previous studies with anthrax: microscopy, staining, pure culture isolation, and an animal model system (Figure 1.29).

The bacterium that causes tuberculosis, *Mycobacterium tuberculosis*, is very difficult to stain because *M. tuberculosis* cells contain large amounts of a waxlike lipid in their cell walls. Nevertheless, Koch devised a staining procedure for *M. tuberculosis* cells in lung tissue samples. Using this method, he observed the blue, rod-shaped cells of *M. tuberculosis* in tubercular tissues but not in healthy tissues (**Figure 1.31**). Obtaining cultures of *M. tuberculosis*

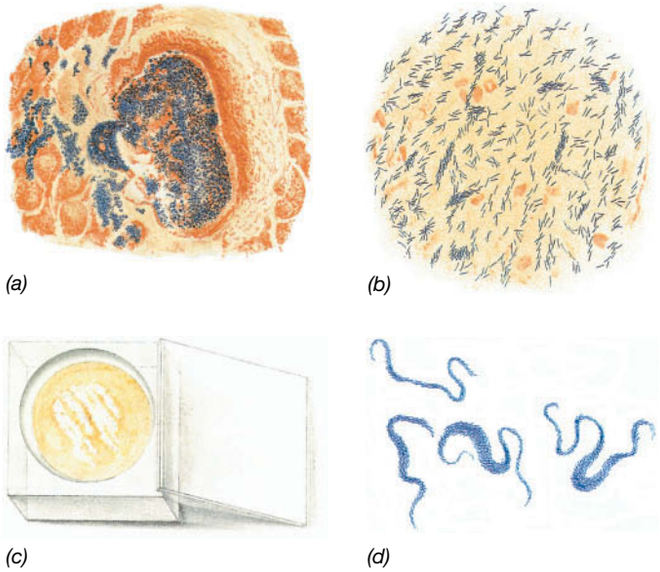


Figure 1.31 Robert Koch's drawings of *Mycobacterium tuberculosis*. *(a)* Section through infected lung tissue showing cells of *M. tuberculosis* (blue). *(b) M. tuberculosis* cells in a sputum sample from a tubercular patient. *(c)* Growth of *M. tuberculosis* on a glass plate of coagulated blood serum stored inside a glass box to prevent contamination. *(d) M. tuberculosis* cells taken from the plate in *c* and observed microscopically; cells appear as long, cordlike forms. Original drawings from Koch, R. 1884. "Die Aetiologie der Tuberkulose." *Mittheilungen aus dem Kaiserlichen Gesundheitsamte 2*: 1–88.

was not easy, but eventually Koch succeeded in growing colonies of this organism on a solidified medium containing blood serum. Under the best of conditions, *M. tuberculosis* grows slowly in culture, but Koch's persistence and patience eventually led to pure cultures of this organism from human and animal sources.

From this point Koch used his postulates (Figure 1.29) to obtain definitive proof that the organism he had isolated was the cause of the disease tuberculosis. Guinea pigs can be readily infected with *M. tuberculosis* and eventually succumb to systemic tuberculosis. Koch showed that tuberculous guinea pigs contained masses of *M. tuberculosis* cells in their lungs and that pure cultures obtained from such animals transmitted the disease to healthy animals. In this way, Koch successfully satisfied all four of his postulates, and the cause of tuberculosis was understood. Koch announced his discovery of the cause of tuberculosis in 1882, and for this accomplishment he was awarded the 1905 Nobel Prize for Physiology or Medicine. Koch had many other triumphs in the growing field of infectious diseases, including the discovery of the causative agent of cholera (the bacterium *Vibrio cholerae*) and the development of methods to diagnose infection with *M. tuberculosis* (the tuberculin skin test).

MINIQUIZ

- How do Koch's postulates ensure that cause and effect of a given disease are clearly differentiated?
- What advantages do solid media offer for the isolation of microorganisms?
- What is a pure culture?

1.11 Discovery of Microbial Diversity

As microbiology entered the twentieth century, its initial focus on basic principles, methods, and medical aspects broadened to include studies of the microbial diversity of soil and water and the metabolic processes that microorganisms carried out in these habitats. Major contributors of this era included the Dutchman Martinus Beijerinck and the Russian Sergei Winogradsky.

Martinus Beijerinck and the Enrichment Culture Technique

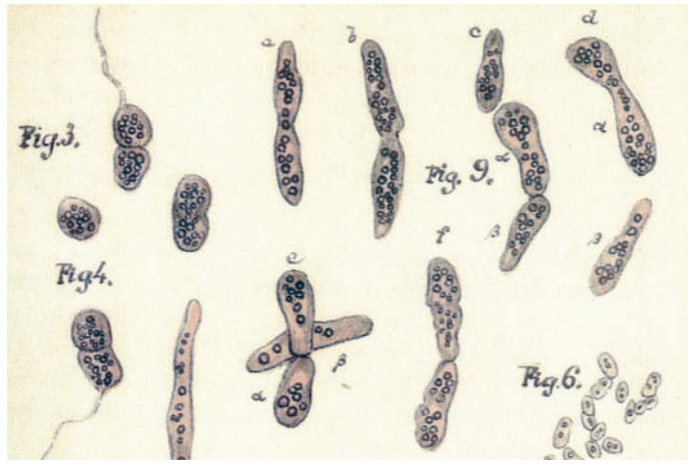
Martinus Beijerinck (1851–1931) was a professor at the Delft Polytechnic School in Holland and was originally trained in botany, so he began his career in microbiology studying plants. Beijerinck's greatest contribution to the field of microbiology was his clear formulation of the *enrichment culture technique*.

The media of Pasteur and Koch were rich in nutrients and while they supported the growth of many organisms, they did not select for specific types of organisms. In enrichment culture, microorganisms are isolated by using highly selective media and incubation conditions that favor a particular metabolic group of organisms. For example, Beijerinck devised a precise chemically defined medium to isolate rhizobia and prove that they are responsible for the formation of the root nodules of legumes (Figure 1.9).

Using the enrichment culture technique, Beijerinck isolated the first pure cultures of many soil and aquatic microorganisms, including sulfate-reducing and sulfur-oxidizing bacteria, lactic acid bacteria, green algae, various anaerobic bacteria, and many others. In addition, in his classic studies of "mosaic disease" of tobacco, Beijerinck used selective filters to show that the infectious agent in this disease (a virus) was smaller than a bacterium and that it somehow became incorporated into cells of the living host plant. In this insightful work, Beijerinck described not only the first virus but also the basic principles of virology, which we expand upon in Chapters 8 and 10.

Sergei Winogradsky, Chemolithotrophy, and Nitrogen Fixation

Like Beijerinck, Sergei Winogradsky (1856–1953) was interested in the bacterial diversity of soils and waters and was highly successful in isolating several notable bacteria from natural samples. Winogradsky was particularly interested in bacteria that cycle nitrogen and sulfur compounds, such as the nitrifying bacteria and the sulfur bacteria (**Figure 1.32**). He studied *Beggiatoa*, which are large bacteria commonly observed in marine sediments. He observed that *Beggiatoa* would not grow on the rich nutrient media used by Koch. He designed specific enrichment media to imitate the environment in which *Beggiatoa* lived. He showed that these bacteria catalyze specific chemical transformations in nature and proposed the important concept of **chemolithotrophy**, the oxidation of *inorganic* compounds to yield energy. Winogradsky further showed that these organisms, which he called *lithotrophs* (meaning, literally, "stone eaters"),



From *Microbiologie du Sol*, used with permission

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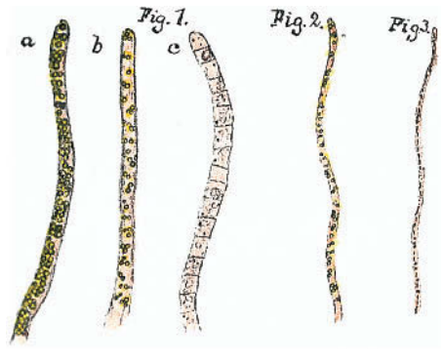


From Winogradsky, S. 1949. *Microbiologie du Sol*. Masson, Paris

(b)

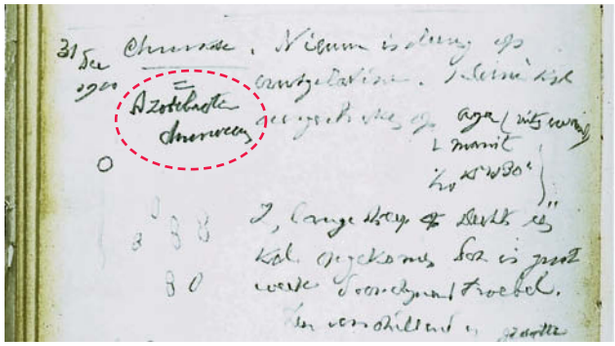
Figure 1.32 Sulfur bacteria. The original drawings were made by Sergei Winogradsky in the late 1880s and then copied and hand-colored by his wife Hèléne. *(a)* Purple sulfur phototrophic bacteria. Figures 3 and 4 show cells of *Chromatium okenii* (compare with photomicrographs of *C. okenii* in Figures 1.1*a* and 1.6*d*, and 1.16*a*). *(b) Beggiatoa,* a sulfur chemolithotroph (compare with Figure 15.27*a*).

are widespread in nature. Winogradsky thus revealed that, like photosynthetic organisms, chemolithotrophic bacteria obtain their carbon from CO_2.

Winogradsky isolated diverse metabolic types of bacteria. Using an enrichment medium that lacked nitrogen, he isolated the anaerobic nitrogen-fixing bacterium *Clostridium pasteurianum*, becoming the first to demonstrate the process of nitrogen fixation. Beijerinck would use a similar technique shortly thereafter to isolate the first aerobic nitrogen-fixing bacterium, *Azotobacter* (**Figure 1.33**). Winogradsky also isolated the first nitrifying bacteria by using an enrichment medium that contained ammonium salts and CO_2.

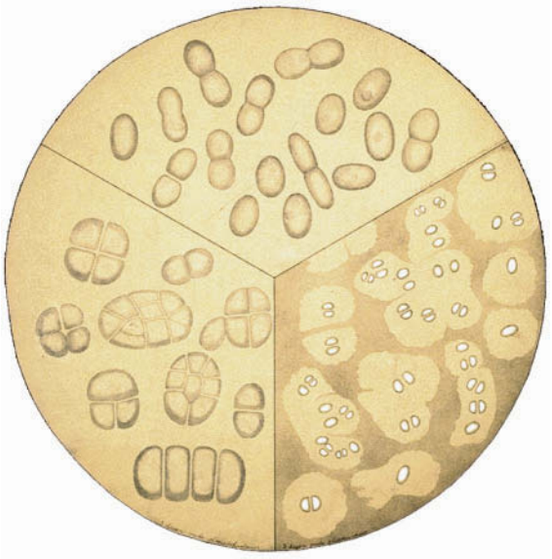
MINIQUIZ

- What is meant by the term "enrichment culture"?
- What is meant by the term "chemolithotrophy"? In what way are chemolithotrophs like plants?



Lesley Robertson and the Kluyver Laboratory Museum, Delft University of Technology

(a)



Lesley Robertson and the Kluyver Laboratory Museum, Delft University of Technology

(b)

Figure 1.33 Martinus Beijerinck and *Azotobacter*. *(a)* A page from the laboratory notebook of M. Beijerinck dated 31 December 1900 describing the aerobic nitrogen-fixing bacterium *Azotobacter chroococcum* (name circled in red). Compare Beijerinck's drawings of pairs of *A. chroococcum* cells with the photomicrograph of cells of *Azotobacter* in Figure 15.32*a*. *(b)* A painting by M. Beijerinck's sister, Henriëtte Beijerinck, showing cells of *A. chroococcum.* Beijerinck used such paintings to illustrate his lectures.

IV • Molecular Biology and the Unity and Diversity of Life

The development of aseptic technique and methods for the enrichment, isolation, and propagation of bacteria at the end of the nineteenth century gave rise to explosive growth in the pace of microbiological discovery. Moreover, microbiologists realized that the ability to grow bacteria rapidly and in controlled laboratory conditions made them excellent model systems in which to explore the fundamental nature of life.

1.12 Molecular Basis of Life

Experiments with bacterial cultures in the twentieth century were critical in describing the foundations of molecular biology, molecular genetics, and biochemistry. Microbiologists came to realize that while microorganisms were incredibly diverse, all cells operated on similar principles.

Unity in Biochemistry

Albert Jan Kluyver (1888–1956) was Beijerinck's successor at what was then called the Delft Institute of Technology. Kluyver recognized that though microbial diversity was tremendous, microorganisms used many of the same biochemical pathways and their metabolic processes faced similar thermodynamic constraints. Kluyver promoted the study of comparative biochemistry to identify the unifying features of all cells. He famously proclaimed, "From elephant to butyric acid bacterium—it is all the same!" This was later reformulated by Jacques Monod (1910–1976) into the expression, "What is true for *E. coli* is also true for the elephant," a statement that proclaimed the importance of working with bacteria to understand the fundamental principles that govern all living things.

The use of microbes as metabolic model systems led to the discovery that certain macromolecules and biochemical reactions are universal, and that to understand their function in one cell is to understand their function in all cells. These discoveries were of central importance to understanding microbial evolution and none were as important as the discovery of DNA as the molecular basis of heredity, a discovery that is less than 80 years old.

Cracking the Code of Life

In the early twentieth century, it was clear that some molecule carried the hereditary information from parent to offspring, but the molecular basis of heredity remained a mystery. Most biologists thought that proteins carried this hereditary information. DNA had been discovered but it was thought to be merely a structural molecule, and too simple in its composition to encode cellular functions. The hunt for the molecular basis of heredity began in earnest with an experiment by Frederick Griffith (1879–1941).

Griffith worked with a virulent strain of *Streptococcus pneumoniae*, a cause of bacterial pneumonia in both humans and mice. This strain, strain S, produced a polysaccharide coat (that is, a capsule, ↩ Section 2.7) that caused cells to form smooth colonies and conferred the ability to kill infected mice (**Figure 1.34*a***). A related strain, strain R, lacked this polysaccharide and produced "rough" colonies that did not cause disease. However, Griffith observed that strain R could be *transformed* to type S, forming smooth colonies and causing disease, when it was mixed with the dead remains of cells of strain S (Figure 1.34*a*). He reasoned that some molecule that contained genetic information must have been transferred from strain R to strain S in this process, and this experiment showed that genetic transfer could be studied in bacteria.

Later, the Avery–MacLeod–McCarty experiment (1944), named for three scientists at the Rockefeller University, would show that this "transforming principle" is DNA. They treated the dead remains of cells of strain S with chemicals and enzymes that destroyed protein and left behind only DNA. They then repeated Griffith's experiment with the pure DNA of strain S and showed that this DNA was sufficient to cause transformation, causing strain R cells to become S-type cells and virulent (Figure 1.34*b*). They also demonstrated that transformation failed if the DNA

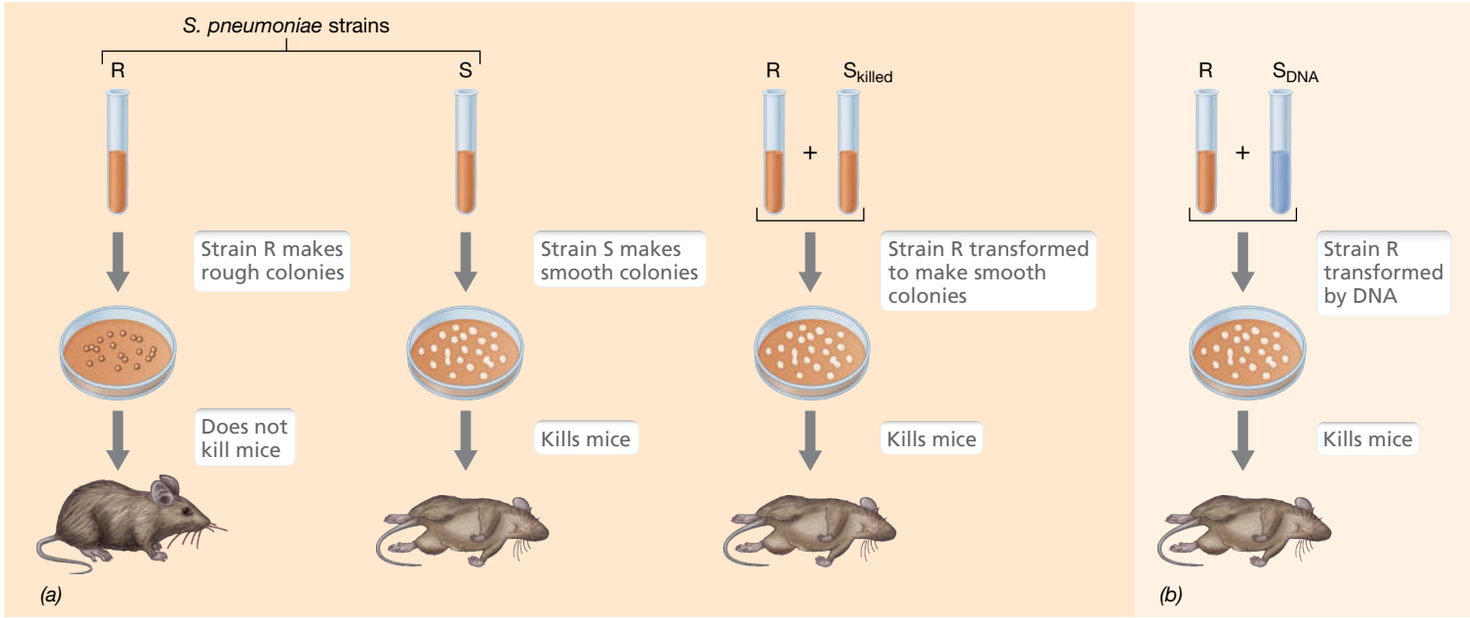


Figure 1.34 Early evidence that DNA is the molecular basis of heredity. *(a)* Griffith's experiment showed that bacteria can transfer genetic information. *Streptococcus pneumoniae* strain R makes rough colonies and does not kill mice, but strain S makes smooth colonies and does kill mice. Heat-killed cells of strain S do not cause disease, but if these killed cells are mixed with cells of strain R, then strain R is "transformed" to the S type and begins to make smooth colonies and kill mice. *(b)* The Avery–MacLeod–McCarty experiment showed that DNA contains genetic information. DNA isolated from strain S can transform strain R to cause disease, though the DNA itself does not cause disease. Degraded DNA lacks the ability to transform strain R.

from strain S was degraded. These experiments proved that DNA is the genetic material of cells.

The discovery that DNA is the basis of heredity was followed by intense effort to understand how this molecule stores genetic information. The structure of DNA was ultimately solved by James D. Watson (1928–) and Francis Crick (1916–2004) using X-ray diffraction images of DNA taken by their colleague Rosalind Franklin (1920–1958). They revealed that DNA is composed of a double helix that contains four nitrogenous bases: guanine, cytosine, adenine, and thymine (↔ Section 4.1). Later research would reveal how the genetic code is read from DNA and translated into a protein alphabet, and these principles are covered in Chapter 4. Once again, however, this research to crack the code of life was enabled by a microbial model system, in this case, the bacterium *Escherichia coli* (commonly called *E. coli*).

Not long after the discovery that genetic information is encoded in the sequence of biological molecules, Emile Zuckerkandl (1922–2013) and Linus Pauling (1901–1994) proposed that molecular sequences could be used to reconstruct evolutionary relationships. They recognized that evolution, as described by Darwin, required variation in offspring and that these variations must be caused by changes in molecular sequences. They predicted that these sequence differences occur randomly in a clocklike fashion over time. This led to the conclusion that the evolutionary history of organisms is inscribed in the sequence of molecules such as DNA. Carl Woese seized upon these insights to pursue the ambitious goal of reconstructing the evolutionary history of all cells.

MINIQUIZ

- Describe the experiments that proved DNA was the transforming principle described by Griffith.
- Why are microbial cells useful tools for basic science?

1.13 Woese and the Tree of Life

Evolutionary relationships between microorganisms remained a mystery until it was discovered that certain molecular sequences maintain a record of evolutionary history. Here we will examine how the sequence of **ribosomal RNA (rRNA)** genes, present in all cells, revolutionized the understanding of microbial evolution and made it possible to construct the first universal tree of life.

Molecular Sequence Data Has Revolutionized Microbial Phylogeny

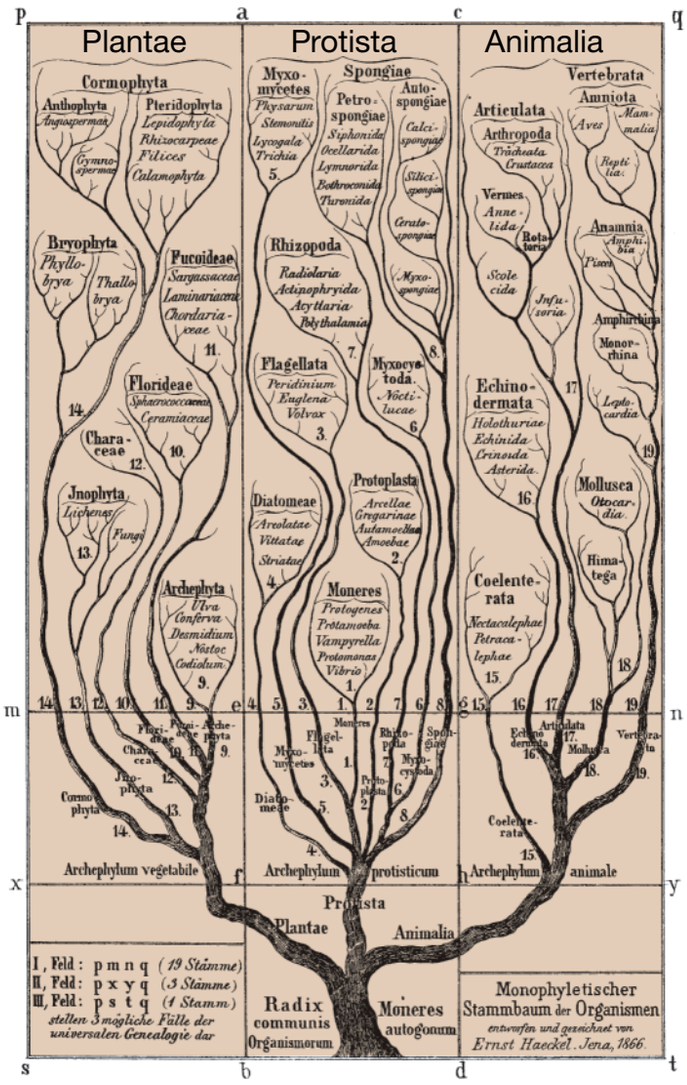
For over a hundred years, following the 1859 publication of Charles Darwin's *On the Origin of Species*, evolutionary history was studied primarily with the tools of paleontology (through examining fossils) and comparative biology (through comparing the traits of living organisms). These approaches led to progress in understanding the evolution of plants and animals, but they were powerless to explain the evolution of microorganisms. The vast majority of microorganisms do not leave behind fossils, and their morphological and physiological traits provide few clues about their evolutionary history. Moreover, microorganisms do not share any morphological traits with plants and animals; thus it

was impossible to create a robust evolutionary framework that included microorganisms.

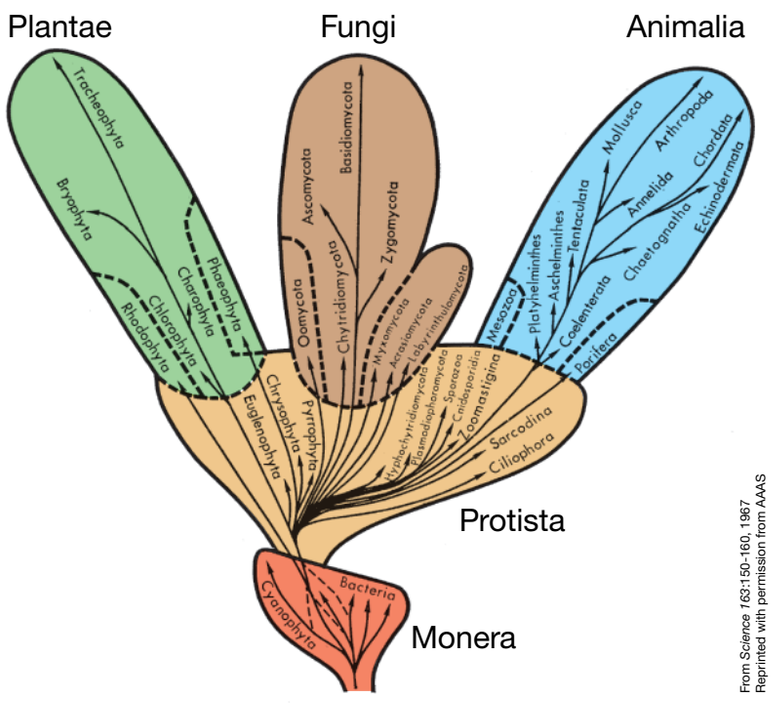
The first attempt to depict the common evolutionary history of all living cells was published by Ernst Haeckel in 1866 (**Figure 1.35*a***). Haeckel correctly suggested that single-cell organisms, which he called *Monera*, were ancestral to other forms of life, but his scheme, which included plants, animals, and protists, did not attempt to resolve evolutionary relationships among microorganisms. The situation was little changed as late as 1967 when Robert Whittaker proposed a five-kingdom classification scheme (Figure 1.35*b*). Whittaker's scheme distinguished the fungi as a distinct lineage, but it was still largely impossible to resolve evolutionary relationships among most microorganisms. Hence, microbial phylogeny had made little progress since Haeckel's day.

Everything changed after the structure of DNA was discovered and it was recognized that evolutionary history is recorded in DNA sequence. Carl Woese (1928–2012), a professor at the University of Illinois (USA), realized in the 1970s that the sequence of ribosomal RNA (rRNA) molecules and the genes that encode them could be used to infer evolutionary relationships between organisms. Ribosomal RNAs are components of ribosomes, the structures that synthesize new proteins in the process of translation (Section 1.2). Woese recognized that genes encoding rRNAs are excellent candidates for phylogenetic analysis because they are (1) universally distributed, (2) functionally constant, (3) highly conserved (that is, slowly changing), and (4) of adequate length to provide a deep view of evolutionary relationships.

Woese compared the sequences of rRNA molecules from many microorganisms. Among the microbes he examined were methanogens. To his astonishment, he found that the rRNA sequences from methanogens were distinct from those of both *Bacteria* and *Eukarya*, the only two domains recognized at that time. He named this new group of prokaryotic cells the *Archaea* (originally *Archaebacteria*) and recognized them as the third domain of life alongside the *Bacteria* and the *Eukarya* (**Figure 1.36*b***). More importantly, Woese demonstrated that the analysis of rRNA gene sequences could be used to reveal evolutionary relationships



(a) **The Haeckel Tree**



(b) **The Whittaker Tree**

From *Science* 163:150-160, 1967
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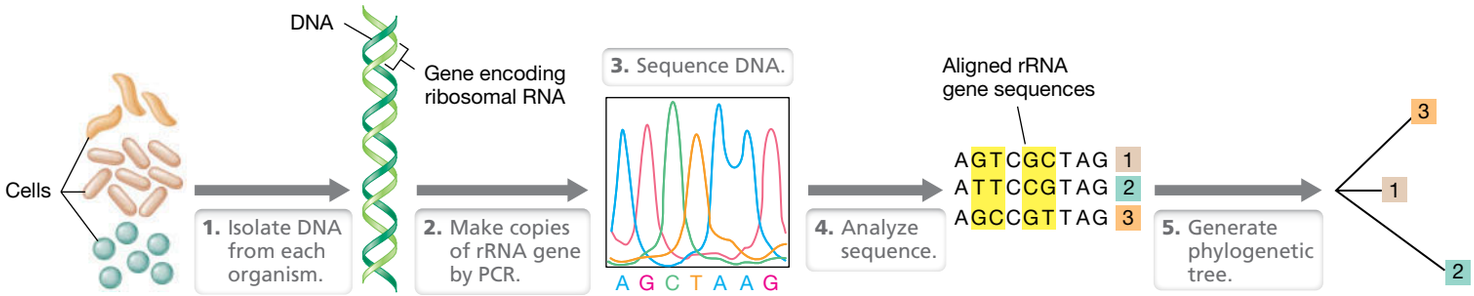
Figure 1.35 Early efforts to depict the universal tree of life. *(a)* Tree of life published in 1866 by Ernst Haeckel in *Generelle Morphologie der Organismen*. *(b)* Tree of life published by Robert H. Whittaker in 1969. The terms "Monera" and "Moneres" are antiquated terms used to refer to prokaryotic cells. Compare these conceptual trees with the tree generated from rRNA gene sequences in Figure 1.36*b*.



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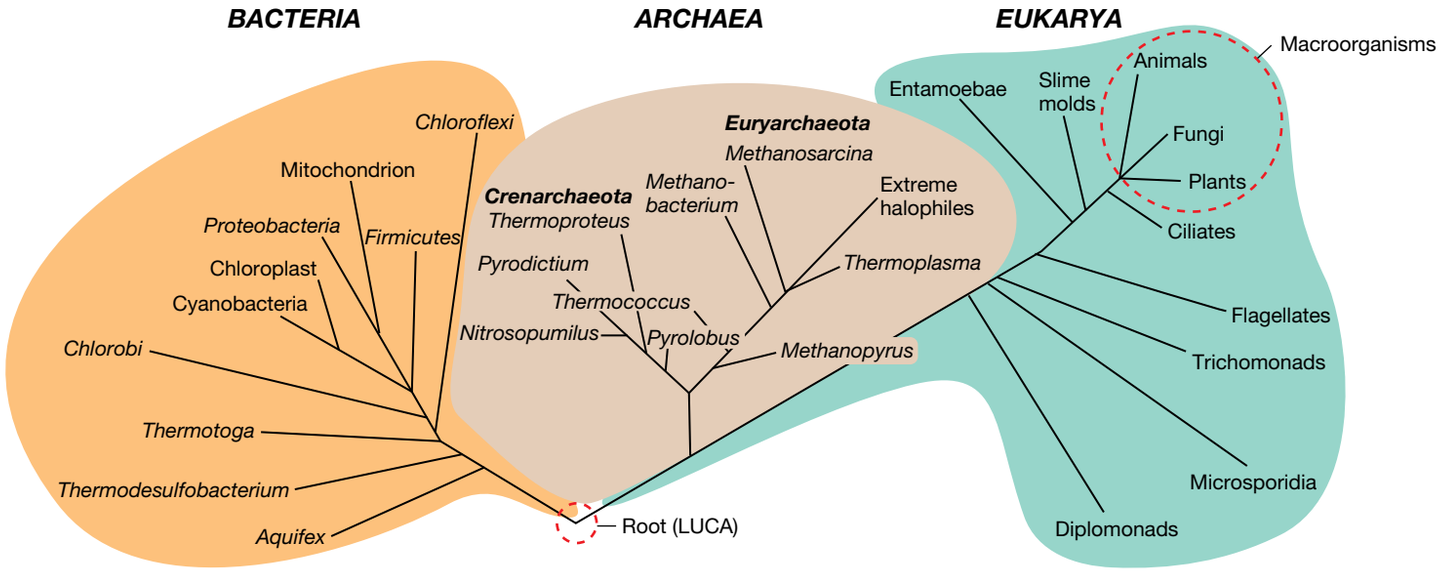


(b)

Figure 1.36 Evolutionary relationships and the phylogenetic tree of life. *(a)* The technology behind ribosomal RNA gene phylogenies. 1. DNA is extracted from cells. 2. Copies of the gene encoding rRNA are made by the polymerase chain reaction (PCR; Section 12.1). 3, 4. The gene is sequenced and the sequence aligned with sequences from other organisms. A computer algorithm makes pairwise comparisons at each base and generates a phylogenetic tree, 5, that depicts evolutionary relationships. In the example shown, the sequence differences are highlighted in yellow and are as follows: organism 1 versus organism 2, three differences; 1 versus 3, two differences; 2 versus 3, four differences. Thus organisms 1 and 3 are closer relatives than are 2 and 3 or 1 and 2. *(b)* The phylogenetic tree of life. The tree shows the three domains of organisms and a few representative groups in each domain.

between *all cells*, providing the first effective tool for the evolutionary classification of microorganisms.

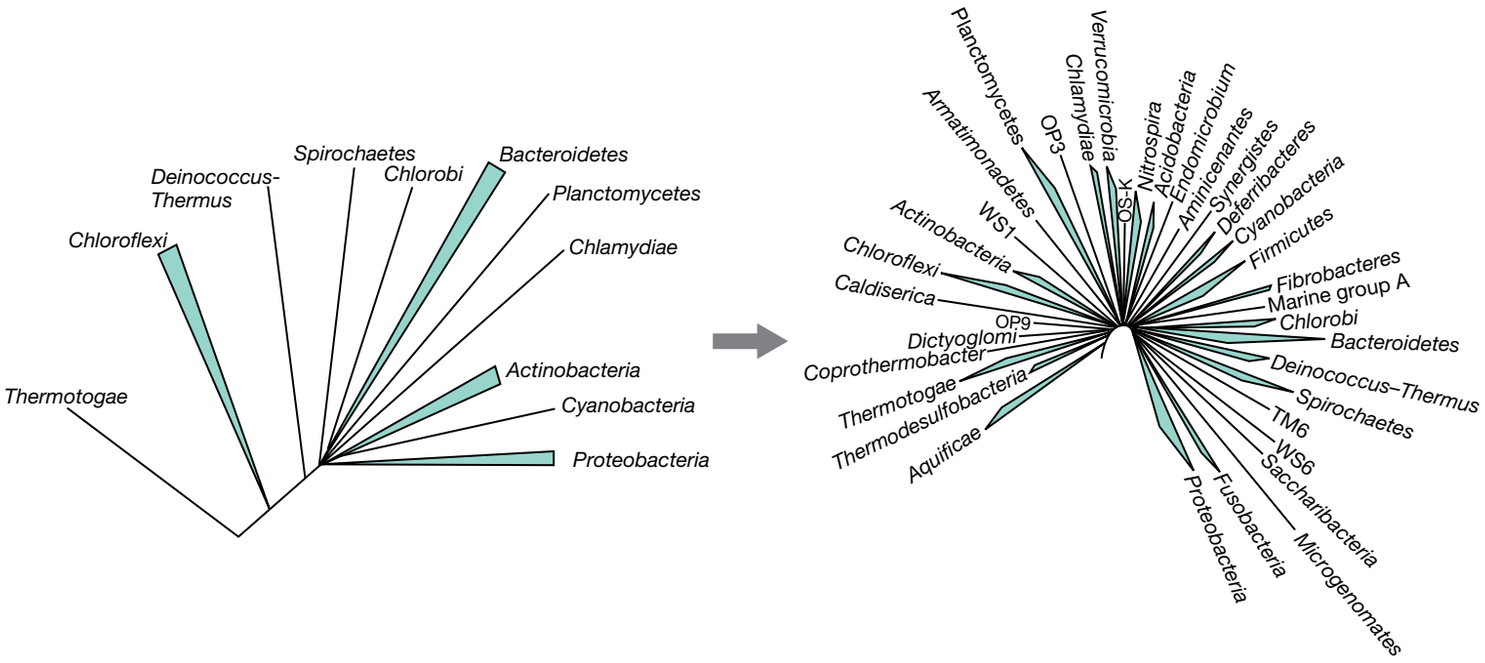
The Tree of Life Based on rRNA Genes

The universal tree of life based on rRNA gene sequences (Figure 1.36*b*) is a genealogy of all life on Earth. It is a true **phylogenetic tree**, a diagram that depicts the evolutionary history—the **phylogeny**—of all cells and clearly reveals the three domains. The root of the universal tree represents a point in time when all extant life on Earth shared a common ancestor, the last universal common ancestor, LUCA (Figures 1.5*b* and 1.36*b*). From the last universal common ancestor of all cells, evolution proceeded along two paths to form the domains *Bacteria* and *Archaea*. At some later time, the domain *Archaea* diverged to distinguish the *Eukarya* from the *Archaea* (Figures 1.5*b* and 1.36*b*). The three domains of cellular life are evolutionarily distinct and yet they share features indicative of their common descent from a universal cellular ancestor.

Revealing the Extent of Microbial Diversity

The tools Woese developed to build the tree of life were first used to determine the evolutionary history of microorganisms in pure culture (Figure 1.36*a*). However, Norman Pace (1942–), a professor at the University of Colorado (USA), realized that Woese's approach could be applied to rRNA molecules isolated *directly from the environment* as a way to probe the diversity of microbial communities without first cultivating their component organisms (Chapter 19).

The cultivation-independent methods of rRNA analysis pioneered by Pace greatly improved our picture of microbial diversity (**Figure 1.37**) and have led to the staggering conclusion that most microorganisms on Earth have yet to be brought into laboratory culture! Furthermore, because the ability of microbiologists to culture the microbial diversity that abounds in nature has lagged behind the ability to detect this diversity, microbiology is now in a position to flesh out the true diversity of microbial life.



(a) ***Before*** **analysis of environmental rRNA genes** (b) ***After*** **analysis of environmental rRNA genes**

Figure 1.37 Analysis of environmental rRNA genes leads to discovery of new phyla of *Bacteria*.
(a) In 1987 Carl Woese described 11 phyla of *Bacteria* from analysis of rRNA genes from cultured species. *(b)* By 1998, analyses of rRNA genes from environmental samples, as described by Norman Pace, had revealed evidence for 36 bacterial phyla. Today there is evidence for more than 80 bacterial phyla.

With an evolutionary framework of the microbial world to guide future research, advances in microbial diversity, both in obtaining cultures and in devising even more powerful methods of assessing diversity, are happening quickly. Besides unveiling the previously hidden concept of three evolutionary domains of life, the contributions of Carl Woese and his associates have given microbiologists the tools they need to understand the scope of microbial diversity at a level similar to biologists' understanding of the diversity of plants and animals.

MINIQUIZ

- What kinds of evidence support the three-domain concept of life?
- What is a phylogenetic tree?
- List three reasons why rRNA genes are suitable for phylogenetic analyses.

1.14 An Introduction to Microbial Life

All cells are unified by the facts that their genetic blueprints are encoded in DNA (Section 1.12) and that evolution is the process by which their blueprints change over time (Section 1.13). We now move on from these fundamental unifying principles to the microbes themselves and take a peek at the diversity of microbial life that evolution has generated.

Microorganisms vary dramatically in size, shape, and structure. And, while much of our focus in this chapter has been on cellular forms of life, not all microbes form cells. In this section we will learn about *Bacteria*, *Archaea*, *Eukarya*, and viruses—the four groups into which all known microorganisms can be classified.

Bacteria

Bacteria have a prokaryotic cell structure (Figure 1.3*a*). Bacteria are often thought of as undifferentiated single cells with a length that ranges from 1 to 10 μm. While bacteria that fit this description are common, the *Bacteria* are actually tremendously diverse in appearance and function. The smallest bacteria are no more than 0.15–0.2 μm in diameter and the largest can be as much as 700 μm long (**Figure 1.38**)! Some bacteria can differentiate to form multiple cell types and others are even multicellular (for example, *Magnetoglobus*, Figure 1.38).

Among the *Bacteria*, 30 major phylogenetic lineages (called phyla) have been described, and some key ones are shown in Figures 1.36 and 1.37. Some of these phyla contain thousands of described species while others contain only a few. More than 90% of bacteria in cultivation belong to one of only four phyla: *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*. The analysis of rRNA gene sequences and even entire genome sequences from environmental samples reveals that at least 80 bacterial phyla likely exist.

Although species in some bacterial phyla are characterized by unique phenotypic traits, most bacterial phyla contain a wide diversity of species and show tremendous physiological diversity. The *Proteobacteria* illustrate this concept well as they include organisms with a diverse array of physiological traits including

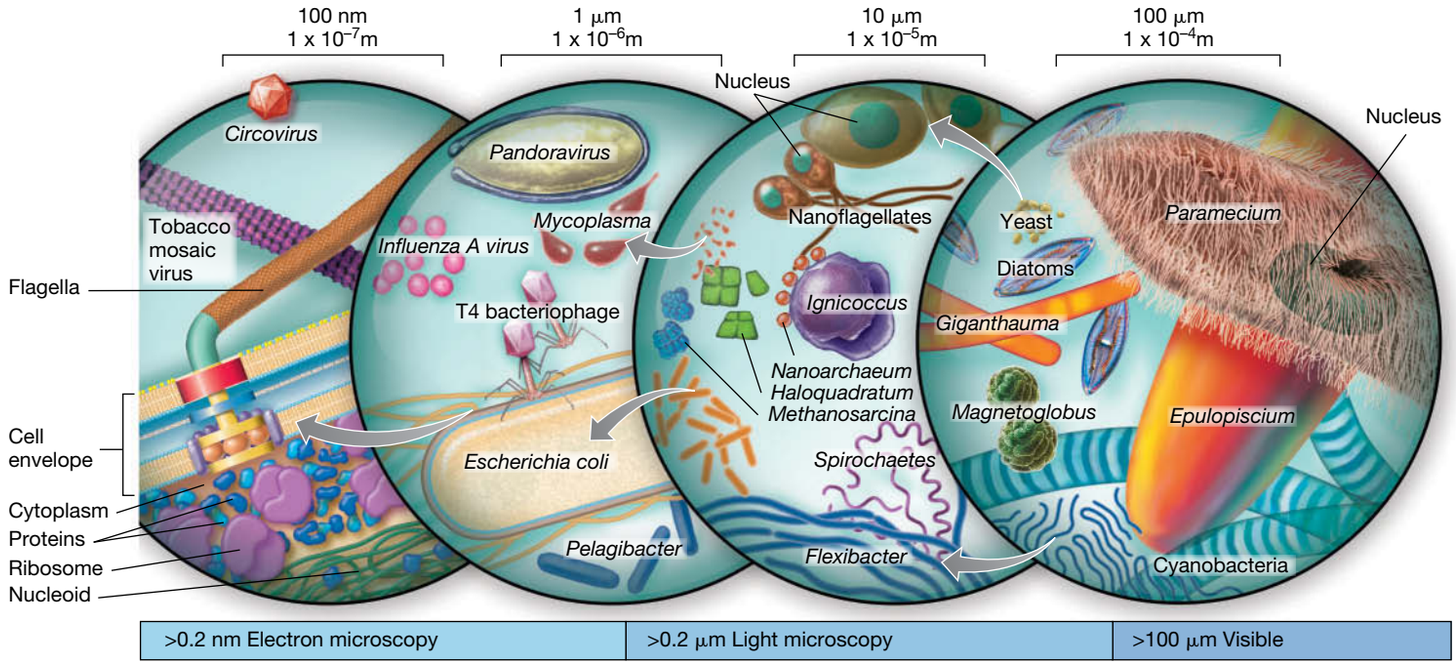


Figure 1.38 Microorganisms vary greatly in size and shape. The smallest known microbe is the circovirus (20 nm) and the largest shown here is the bacterium *Epulopiscium* (700 μm), which represents a 35,000-fold difference in length! Certain protozoa can be even larger than *Epulopiscium* (>2 mm long) and are visible to the unaided eye. Included in the figure are *Eukarya*: *Paramecium* (300 μm × 85 μm), diatoms (*Navicula*, 50 μm × 12 μm), yeast (*Saccharomyces*, 5 μm), and nanoflagellates (*Cafeteria*, 2 μm); *Bacteria*: *Epulopiscium* (700 μm × 80 μm), cyanobacteria (*Oscillatoria*, 10-μm-diameter multicellular filaments), *Magnetoglobus* (multicellular aggregate, 20 μm diameter), *Spirochaetes* (2–10 μm × 0.25 μm), *Flexibacter* (5–100 μm × 0.5 μm filaments), *Escherichia coli* (2 μm × 0.5 μm), *Pelagibacter* (0.4 μm × 0.15 μm), and *Mycoplasma* (0.2 μm); *Archaea*: *Giganthauma* (10-μm-diameter multicellular filament), *Ignicoccus* (6 μm), *Nanoarchaeum* (0.4 μm), *Haloquadratum* (2 μm), *Methanosarcina* (2 μm per cell in packet); and viruses: *Pandoravirus* (1 μm × 0.4 μm), T4 bacteriophage (200 nm × 90 nm), *Influenza A virus* (100 nm), *Tobacco mosaic virus* (300 nm × 20 nm), *Circovirus* (20 nm).

respiration (both with and without oxygen), fermentations of various types, diverse forms of phototrophy, and chemolithotrophic metabolisms using H_2, sulfur or nitrogen compounds, or even metals (as described in Chapters 14 and 15). Species of *Proteobacteria* also possess a wide range of ecological strategies and can be found in all but the hottest and most salty environments on Earth. It is important to remember that while most phyla of plants and animals originated within the last 600 million years (Figure 1.5*a*), bacterial phyla are billions of years old and this time has allowed for extensive experimentation and diversification. The diversity of *Bacteria* is discussed in detail in Chapters 15 and 16.

Archaea

Like *Bacteria*, *Archaea* also have a prokaryotic cell structure (Figure 1.3*a*). While *Archaea* are quite diverse in their physiology, cultured isolates have less morphological diversity than *Bacteria*, and most described *Archaea* exist as undifferentiated cells that are 1 to 10 μm in length. The domain *Archaea* consists of five well-described phyla: *Euryarchaeota, Crenarchaeota, Thaumarchaeota*, *Nanoarchaeota*, and *Korarchaeota*. As for the *Bacteria*, many lineages of *Archaea* are known only from rRNA genes or genome sequences recovered from the environment. Analysis of these environmental DNA sequences indicate more than 12 archaeal phyla likely exist.

Archaea have historically been associated with extreme environments; the first isolates came from hot, salty, or acidic sites. But not all *Archaea* are extremophiles. *Archaea* are indeed common in the most extreme environments that support life, such as those associated with volcanic systems, and species of *Archaea* hold many of the records that define the chemical and physical limits of life (Table 1.1). But in addition to these, *Archaea* are found widely in nature. For example, methanogens are common in wetlands and in the guts of animals (including humans). Methanogenic *Archaea* produce methane and have a major impact on the greenhouse gas composition of our atmosphere. In addition, species of *Thaumarchaeota* inhabit soils and oceans worldwide and are important contributors to the global nitrogen cycle (Chapter 17).

Archaea are also notable in that this domain lacks any known pathogens or parasites of plants or animals. Most described species of *Archaea* fall within the phyla *Crenarchaeota* and *Euryarchaeota* (Figure 1.36*b*) while only a handful of species have been described for the *Nanoarchaeota, Korarchaeota*, and *Thaumarchaeota*. We discuss *Archaea* in detail in Chapter 17.

Eukarya

Plants, animals, and fungi are the most well-characterized groups of *Eukarya*. These groups are relatively young in relation to *Bacteria* and *Archaea*, originating during a burst of evolutionary radiation called the Cambrian explosion, which began about 600 million years ago. The first eukaryotes, however, were unicellular microbes. Microbial eukaryotes, which include diverse algae and protozoa, may have first appeared as early as 2 billion years ago, well before the origin of plants, animals, and fungi (Figure 1.5). The major

lineages of *Eukarya* are traditionally called kingdoms instead of phyla. There are at least six kingdoms of *Eukarya*, and this diverse domain contains microorganisms as well as the plants and animals.

Microbial eukaryotes vary dramatically in size, shape, and physiology (Figure 1.38). Among the smallest are the nanoflagellates, which are microbial predators that can be as small as 2 μm long. In addition, *Ostreococcus*, a genus of green algae that contains species that are 0.8 μm in diameter, is smaller than many bacteria. The largest single-celled organisms are eukaryotes, but they are hardly microbial. Xenophyophores are amoeba-like, single-celled organisms that live exclusively in the deep ocean. Exploration of the Mariana Trench has revealed xenophyophores up to 10 cm in length. In addition, plasmodial slime molds consisting of a single cytoplasmic compartment can be up to 30 cm in diameter. Microbial eukaryotes include diverse phototrophic organisms, microbial predators, symbionts and parasites, along with a range of other physiological types. In Chapter 18 we consider microbial eukaryotes in detail.

Viruses

Viruses are not found on the tree of life. Indeed, it can be argued that they are not truly alive. Viruses are obligate parasites that can only replicate within the cytoplasm of a host cell. Viruses are not cells, and they lack the cytoplasmic membrane, cytoplasm, and ribosomes found in all forms of cellular life. Viruses cannot conserve energy and they do not carry out metabolic processes; instead, they take over the metabolic systems of infected cells and turn them into vessels for producing more viruses. Unlike cells, which all have genomes composed of double-stranded DNA, viruses have genomes composed of DNA *or* RNA that can be either double- or single-stranded. Viral genomes are often quite small, with the smallest having only three genes. The small size of most viral genomes means that no genes are conserved among all viruses, or between all viruses and all cells; hence it may be impossible to ever place viruses into the tree of life or build a universal viral phylogenetic tree that includes all viruses.

Viruses are as diverse as the cells they infect, and viruses are known to infect cells from all three domains of life. Viruses are often classified on the basis of structure, genome composition, and host specificity. Viruses that infect bacteria are called *bacteriophages* (or *phages*, for short). Bacteriophages have been used as model systems to explore many aspects of viral biology. While most viruses are considerably smaller than bacterial cells (Figure 1.38), there are also unusually large viruses such as the *Pandoraviruses,* which can be more than 1 micrometer long and have a genome of as many as 2500 genes, larger than that of many bacteria! We will learn more about viruses in Chapters 8 and 10.

MINIQUIZ

- How are viruses different from *Bacteria*, *Archaea*, and *Eukarya*?
- What four bacterial phyla contain the most well-characterized species?
- What phylum of *Archaea* is common worldwide in soils and in the oceans?

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Chapter Review

I • Exploring the Microbial World

1.1 Microorganisms are single-celled microscopic organisms that are essential for the well-being and functioning of other life forms and the planet. The tools of microscopy, microbial cultivation, molecular biology, and genomics are cornerstones of modern microbiology.

Q What are bacterial colonies and how are they formed?

1.2 Prokaryotic and eukaryotic cells differ in cellular architecture, and an organism's characteristics are defined by its complement of genes—its genome. All cells have a cytoplasmic membrane, a cytoplasm, ribosomes, and a double-stranded DNA genome. All cells carry out activities including metabolism, growth, and evolution.

Q What cellular structures distinguish prokaryotic and eukaryotic cells? What are some differences between a cell wall and a cell membrane? In what types of organisms would you expect to find these structures?

1.3 Diverse microbial populations were widespread on Earth for billions of years before plants and animals appeared. Microbes are abundant in the biosphere and their activities greatly affect the chemical and physical properties of their habitats. *Bacteria, Archaea,* and *Eukarya* are the major phylogenetic lineages (domains) of cells.

Q Why can Earth, in many ways, be considered a microbial planet? Which event in Earth's history eventually lead to the evolution of multicellular life forms?

1.4 Microorganisms can be both beneficial and harmful to humans, although many more microorganisms are beneficial (or even essential) than are harmful. Agriculture, food, energy, and the environment are all affected in major ways by microorganisms.

Q The gut microbiome directly benefits humans by digesting complex carbohydrates and synthesizing vitamins and other nutrients. In what other ways do microorganisms benefit humans?

II • Microscopy and the Origins of Microbiology

1.5 Microscopes are essential for studying microorganisms. Bright-field microscopy, the most common form of microscopy, employs a microscope with a series of lenses to magnify and resolve the image. The limit of resolution for a light microscope is about 0.2 μm.

Q What is the difference between magnification and resolution? Can either increase without the other?

1.6 An inherent limitation of bright-field microscopy is the lack of contrast between cells and their surroundings. This problem can be overcome by the use of stains or by alternative forms of light microscopy, such as phase contrast or dark field.

Q What is the function of staining in light microscopy? What is the advantage of phase-contrast microscopy over bright-field microscopy?

1.7 Differential interference contrast (DIC) microscopy and confocal scanning laser microscopy allow enhanced three-dimensional imaging or imaging through thick specimens.

Q How is confocal scanning laser microscopy different from fluorescence microscopy? In what ways are they similar? How does differential interference contrast microscopy differ from bright-field microscopy?

1.8 Electron microscopes have far greater resolving power than do light microscopes, the limits of resolution being about 0.2 nm. The two major forms of electron microscopy are transmission, used primarily to observe internal cell structure, and scanning, used to examine the surface of specimens.

Q Why does an electron microscope have a higher resolution, or greater resolving power, than a light microscope?

III • Microbial Cultivation Expands the Horizon of Microbiology

1.9 Louis Pasteur devised ingenious experiments proving that living organisms cannot arise spontaneously from nonliving matter. Pasteur introduced many concepts and techniques central to the science of microbiology, including sterilization, and developed a number of key vaccines for humans and other animals.

Q Explain the principle behind the Pasteur flask in studies on spontaneous generation. Why were the results of this experiment inconsistent with the theory of spontaneous generation?

1.10 Robert Koch developed a set of criteria called Koch's postulates for linking cause and effect in infectious diseases. Koch also developed the first reliable and reproducible means for obtaining and maintaining microorganisms in pure culture.

Q What are Koch's postulates and how did they influence the development of microbiology? Why are Koch's postulates still relevant today?

1.11 Martinus Beijerinck and Sergei Winogradsky explored soil and water for microorganisms that carry out important natural processes, such as nutrient cycling and the biodegradation of particular substances. Out of their work came the enrichment culture technique and the concepts of chemolithotrophy and nitrogen fixation.

Q What were the major microbiological interests of Martinus Beijerinck and Sergei Winogradsky? It can be said that both men discovered nitrogen fixation. Explain.

IV • Molecular Biology and the Unity and Diversity of Life

1.12 All cells share certain characteristics, and microorganisms are used as model systems to explore the fundamental processes that define life. The discoveries of DNA as the molecular basis of heredity, and of its structure and function, paved the way for progress in molecular genetics, microbial phylogeny, and genomics.

Q Describe the experiments that proved DNA to be the molecule at the basis of heredity.

1.13 Carl Woese discovered that ribosomal RNA (rRNA) sequences can be used to determine the evolutionary history of microorganisms, and in so doing, he diagrammed the tree of life and discovered the domain *Archaea*. Analysis of rRNA sequences from the environment reveals that microbial diversity is exceptional and that the majority of microorganisms have not yet been cultivated.

Q What insights led to the reconstruction of the tree of life? Which domain, *Archaea* or *Eukarya*, is more closely related to *Bacteria*? What evidence is there to justify your answer?

1.14 The greatest diversity of microorganisms is found in the *Bacteria*, while many extremophiles are found within the *Archaea*. Microbial eukaryotes can vary tremendously in size, with some species being smaller than bacteria. Viruses are acellular and because of this cannot be placed on the tree of life.

Q What features (or lack of features) can be used to distinguish between viruses, *Bacteria*, *Archaea*, and *Eukarya*?

Application Questions

1. Pasteur's experiments on spontaneous generation contributed to the methodology of microbiology, understanding of the origin of life, and techniques for the preservation of food. Explain briefly how Pasteur's experiments affected each of these topics.
2. Describe the lines of proof Robert Koch used to definitively associate the bacterium *Mycobacterium tuberculosis* with the disease tuberculosis. How would his proof have been flawed if any of the tools he developed for studying bacterial diseases had not been available for his study of tuberculosis?
3. Imagine that all microorganisms suddenly disappeared from Earth. From what you have learned in this chapter, why do you think that animals would eventually disappear from Earth? Why would plants disappear? By contrast, if all higher organisms suddenly disappeared, what in Figure 1.5*a* tells you that a similar fate would not befall microorganisms?

Chapter Glossary

Aseptic technique the manipulation of sterile instruments or culture media in such a way as to maintain sterility

Cell wall a rigid layer present outside the cytoplasmic membrane; it confers structural strength on the cell

Chemolithotrophy a form of metabolism in which energy is generated from the oxidation of inorganic compounds

Colony a macroscopically visible population of cells growing on solid medium, arising from a single cell

Contrast the ability to resolve a cell or structure from its surroundings

Culture a collection of microbial cells grown using a nutrient medium

Cytoplasm the fluid portion of a cell, enclosed by the cytoplasmic membrane

Cytoplasmic membrane a semipermeable barrier that separates the cell interior (cytoplasm) from the environment

Differentiation modification of cellular components to form a new structure, such as a spore

Domain one of the three main evolutionary lineages of cells: the *Bacteria*, the *Archaea*, and the *Eukarya*

DNA replication the process by which information from DNA is copied into a new strand of DNA

Enrichment culture technique a method for isolating specific microorganisms from nature using specific culture media and incubation conditions

Enzyme a protein (or in some cases an RNA) catalyst that functions to speed up chemical reactions

Eukaryotic having a membrane-enclosed nucleus and various other membrane-enclosed organelles; cells of *Eukarya*

Evolution a change over time in gene sequence and frequency within a population of organisms, resulting in descent with modification

Extremophiles microorganisms that inhabit environments characterized by extremes of temperature, pH, pressure, or salinity

Genome an organism's full complement of genes

Gram-negative a bacterial cell with a cell wall containing small amounts of peptidoglycan and an outer membrane

Gram-positive a bacterial cell whose cell wall consists chiefly of peptidoglycan; it lacks the outer membrane of gram-negative cells

Gram stain a differential staining procedure that stains cells either purple (gram-positive cells) or pink (gram-negative cells)

Growth in microbiology, an increase in cell number with time

Gut microbiome the microbial communities present in the animal gastrointestinal tract

Horizontal gene transfer the transfer of genes between cells through a process uncoupled from reproduction

Intercellular communication interactions between cells using chemical signals

Koch's postulates a set of criteria for proving that a given microorganism causes a given disease

Macromolecules a polymer of monomeric units, for example proteins, nucleic acids, polysaccharides, and lipids

Magnification the optical enlargement of an image

Medium (plural, media) in microbiology, the liquid or solid nutrient mixture(s) used to grow microorganisms

Metabolism all biochemical reactions in a cell

Microbial community two or more populations of cells that coexist and interact in a habitat

Microbial ecology the study of microorganisms in their natural environments

Microorganism an organism that is too small to be seen by the unaided human eye

Motility the movement of cells by some form of self-propulsion

Nucleoid the aggregated mass of DNA that makes up the chromosome(s) of prokaryotic cells

Nucleus a membrane-enclosed structure in eukaryotic cells that contains the cell's DNA genome

Organelle a bilayer-membrane-enclosed structure such as the mitochondrion, found in eukaryotic cells

Pathogen a disease-causing microorganism

Phylogenetic tree a diagram that depicts the evolutionary history of organisms

Phylogeny the evolutionary history of organisms

Plasmid an extrachromosomal genetic element that is not essential for growth

Prokaryotic lacking a membrane-enclosed nucleus and other organelles; cells of *Bacteria* or *Archaea*

Pure culture a culture containing a single kind of microorganism

Resolution the ability to distinguish two objects as distinct and separate when viewed under the microscope

Ribosomes a structure composed of RNAs and proteins upon which new proteins are made

Ribosomal RNA (rRNA) the types of RNA found in the ribosome

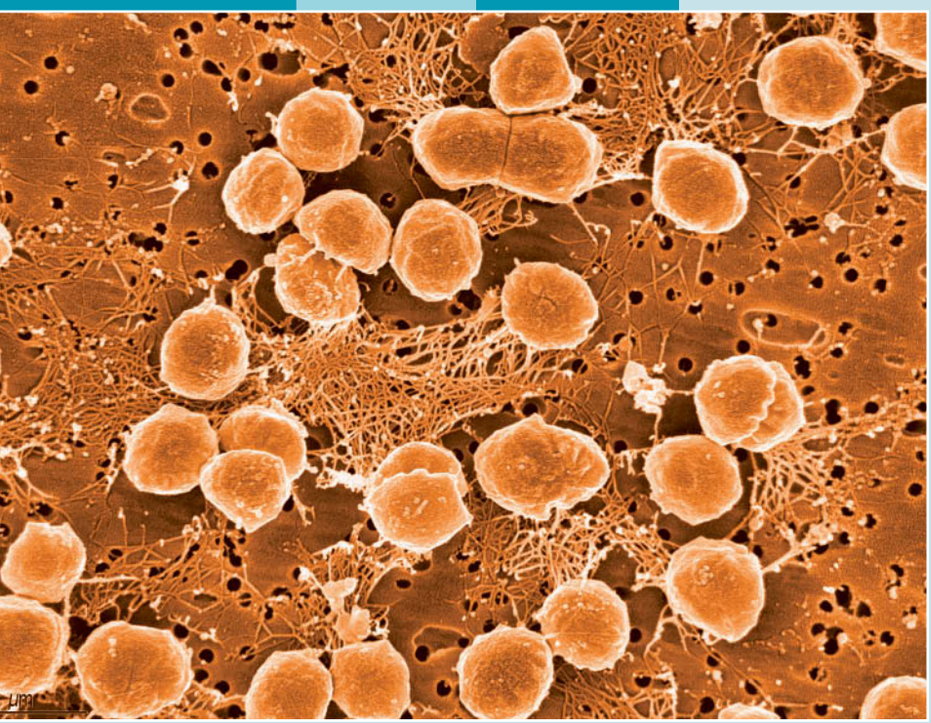
Spontaneous generation the hypothesis that living organisms can originate from nonliving matter

Sterile free of all living organisms (cells) and viruses

Transcription the synthesis of an RNA molecule complementary to one of the two strands of a double-stranded DNA molecule

Translation the synthesis of protein by a ribosome using the genetic information in a messenger RNA as a template

2 Microbial Cell Structure and Function



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microbiologynow

The Archaellum: Motility for the *Archaea*

Motility is important for microbes because it allows cells to explore new habitats and exploit their resources. Motility has been studied for over 50 years in the bacterium *Escherichia coli*, and it is with *E. coli* that scientists first discovered that the bacterial flagellum rotates like a propeller and is powered not by ATP but by the proton motive force. Subsequent studies of other motile *Bacteria* indicated that the structure and function of the flagellum was highly conserved.

When *Archaea* were first discovered, it was clear that some species, such as the archaeon *Methanocaldococcus* (see photo), were also motile and that their flagella also functioned by rotating. So it was only natural to assume that archaeal flagella were structurally related to bacterial flagella. But as scientists began to pick apart the archaeal flagellum, they were in for a big surprise.

Studies showed that archaeal flagella—now called *archaella* to distinguish them from flagella—were thinner than their bacterial counterparts and were composed not of one major protein but of several proteins. For example, whereas the flagellar filament (the actual rotating structure) is composed of a single type of protein called *flagellin*, the archaellar filament is composed of at least three proteins, none of which are structurally related to flagellin. Motor proteins in the flagellum and archaellum are also distinct, as is the overall structure of the motor.

Despite these clear differences, genomic studies of motile *Archaea* surprisingly revealed that the archaellum did have a structural counterpart in *Bacteria*—the type IV pilus. In *Bacteria*, type IV pili do not rotate but facilitate "twitching motility" and are often important in attaching pathogenic (disease-causing) bacteria to their host tissues. However, unlike the flagellum, the activities of type IV pili in *Bacteria* are powered by ATP, and ATP also drives rotation of the archaellum.

The archaellum is thus a "rotating type IV pilus" and is a good example of how evolution can modify a single structure to drive different functions. Such discoveries also demonstrate how even firmly entrenched paradigms (such as the structure and function of the bacterial flagellum) can be turned upside down when scientists begin to probe phylogenetically distinct species.

Source: Albers, S-V., and K.F. Jarrell. 2015. The archaellum: How *Archaea* swim. *Front. Microbiol. 6:* doi: 10.3389/fmicb.2015.00023.

I • Cells of *Bacteria* and *Archaea*

In the opening chapter, we painted a picture of the microbial world using a broad brush. There we considered in a very general way several key aspects of microbiology essential to a modern understanding of the science. In Chapter 2, we move on to begin a more detailed examination of microbial life, with a focus on cell structure and function.

Microscopic examination of microorganisms immediately reveals their shape and size. A variety of cell shapes pervade the microbial world, and although microscopic by their very nature, microbial cells—both prokaryotic and eukaryotic—come in a variety of sizes. Cell shape can be useful for distinguishing different microbial cells and often has ecological significance. Moreover, the very small size of most microbial cells has a profound effect on their ecology and dictates many aspects of their biology. We begin by considering cell shape and then consider cell size.

2.1 Cell Morphology

In microbiology, the term **morphology** means cell shape. Several morphologies are found among *Bacteria* and *Archaea*, and the most common ones are described by terms that are part of the essential lexicon of the microbiologist.

Major Morphologies of Prokaryotic Cells

Common morphologies of prokaryotic cells are shown in Figure 2.1. A cell that is spherical or ovoid in morphology is called a *coccus* (plural, cocci). A cylindrically shaped cell is called a *rod* or a *bacillus*. Some cells form curved or loose spiral shapes and are called *spirilla*. The cells of some *Bacteria* and *Archaea* remain together in groups or clusters after cell division, and the arrangements are often characteristic. For instance, some cocci form long chains (for example, the bacterium *Streptococcus*), others occur in three-dimensional cubes (*Sarcina*), and still others in grapelike clusters (*Staphylococcus*).

Some morphological groups are immediately recognizable by the unusual shapes of their individual cells. Examples include the spirochetes, which are tightly coiled *Bacteria*; bacteria that form extensions of their cells as long tubes or stalks (appendaged forms); and filamentous bacteria, which form long, thin cells or chains of cells (Figure 2.1).

The cell morphologies described here are representative but certainly not exhaustive; many variations of these morphologies are known. For example, there can be fat rods, thin rods, short rods, and long rods, a rod simply being a cell—roughly in the shape of a cylinder—that is longer in one dimension than in the other. As we will see, there are even square bacteria and star-shaped bacteria! Cell morphologies thus form a continuum, with some shapes, such as rods and cocci, being very common, whereas others, such as spiral, budding, and filamentous shapes, are less common.

Morphology and Biology

Although cell morphology is easily determined, it is a poor predictor of other properties of a cell. For example, under the microscope many rod-shaped *Archaea* are indistinguishable from rod-shaped *Bacteria*, yet we know they are of different phylogenetic domains (Section 1.13). With rare exceptions, it is impossible to predict the physiology, ecology, phylogeny, pathogenic (disease-causing) potential, or virtually any other major property of a prokaryotic cell by simply knowing its morphology. Nevertheless, cell morphology is an important characteristic that is always noted when describing a particular species of *Bacteria* or *Archaea*.

Why are the cells of a given species the shape they are? Although we know quite a bit about *how* cell shape is controlled, we know relatively little about *why* a particular cell displays the morphology it does. The morphology of a given microbe is undoubtedly the result of the selective forces that have shaped its evolution to

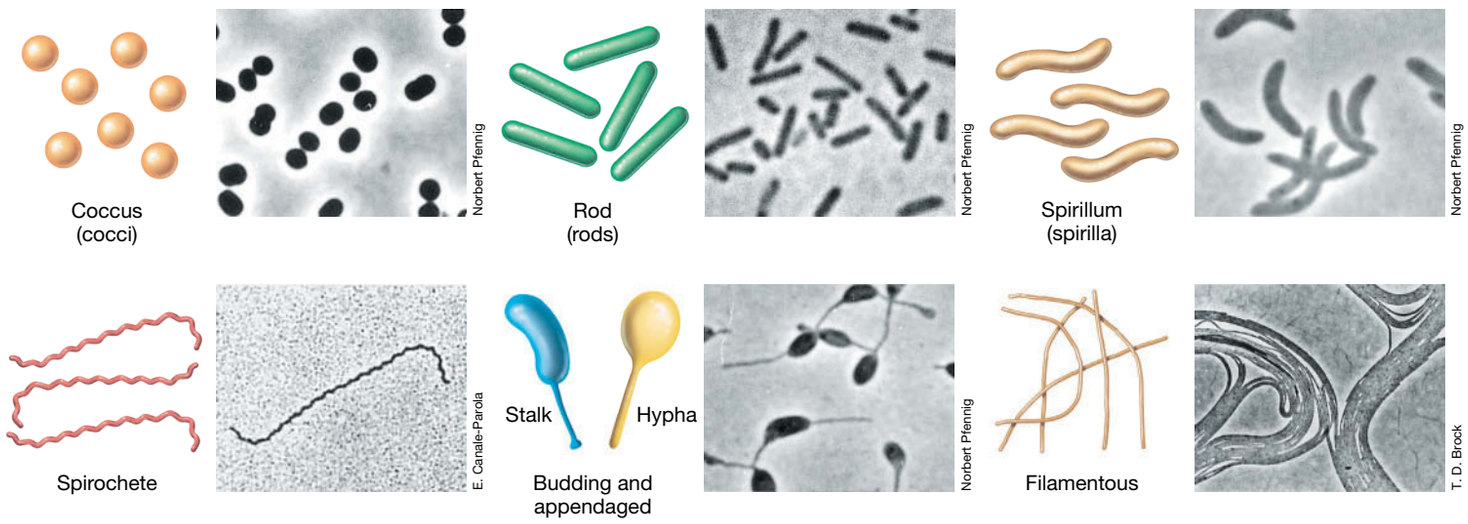


Figure 2.1 Cell morphologies. Beside each drawing is a phase-contrast photomicrograph of cells showing that morphology. Coccus (cell diameter in photomicrograph, 1.5 μm); rod (1 μm); spirillum (1 μm); spirochete (0.25 μm); budding (1.2 μm); filamentous (0.8 μm). All photomicrographs are of species of *Bacteria*. Not all of these morphologies are known among the *Archaea*, but cocci, rods, and spirilla are common.

maximize fitness for competitive success in its habitat. Some examples of these might include evolving an optimal cell shape to maximize nutrient uptake for survival in nutrient-limiting environments (small cells and others with high surface-to-volume ratios, such as appendaged cells), evolving a morphology to exploit swimming motility in viscous environments (helical- or spiral-shaped cells), or evolving a morphology that facilitates gliding motility along a surface (filamentous bacteria) (Figure 2.1).

MINIQUIZ

- How do cocci and rods differ in morphology?
- Using a microscope, could you differentiate a coccus from a spirillum? A pathogen from a nonpathogen?

2.2 The Small World

Cells of *Bacteria* and *Archaea* vary in size from as small as about 0.2 micrometer (μm) in diameter to those more than 700 μm in diameter (**Table 2.1**). The vast majority of rod-shaped species that have been cultured are between 0.5 and 4 μm wide and less than 15 μm long. A few very large *Bacteria* are known, such as *Epulopiscium fishelsoni*, whose cells exceed 600 μm (0.6 millimeter) in length (**Figure 2.2*a***; Figure 1.38). This bacterium, phylogenetically related to the endospore-forming bacterium *Clostridium* and found in the gut of the surgeonfish, contains multiple copies of its genome. The many copies are apparently necessary because the volume of an *Epulopiscium* cell is so large (Table 2.1) that a single copy of its genome would be insufficient to support its transcriptional and translational demands.

Cells of the largest known bacterium, the sulfur-oxidizing chemolithotroph *Thiomargarita* (Figure 2.2*b*), are even larger than those of *Epulopiscium*, about 750 μm in diameter; such cells are just visible to the naked eye. Why these cells are so large is not well understood, although for sulfur bacteria a large cell size may have evolved for storing inclusions of sulfur (used as an energy source). No species of *Archaea* are known that rival *Epulopiscium* or *Thiomargarita* in cell size, but that may simply be because they remain undiscovered.

It is hypothesized that the upper size limit for prokaryotic cells results from the decreasing ability of larger and larger cells to transport nutrients (their surface-to-volume ratio is very small; see the next subsection). Since the metabolic rate of a cell varies inversely with the square of its size, for very large cells, nutrient uptake would eventually limit metabolism to the point that the cell would no longer be competitive with smaller cells.

Very large cells are uncommon in the prokaryotic world. In contrast to *Thiomargarita* or *Epulopiscium* (Figure 2.2), the dimensions of an average rod-shaped bacterium, such as *Escherichia coli*, for example, are about 1–2 μm; these dimensions are typical of cells in the prokaryotic world. By contrast, eukaryotic cells can be as small as 2 to more than 600 μm in diameter, although very small microbial eukaryotes (cells less than about 6 μm in diameter) are uncommon. We explore the world of microbial eukaryotes in Chapter 18.

Surface-to-Volume Ratios, Growth Rates, and Evolution

For a cell, there are advantages to being small. Small cells have more surface area relative to cell volume than do large cells and thus have a higher *surface-to-volume ratio*. To understand this, consider a coccus-shaped cell. The volume of a coccus is a function of the cube of its radius ($V = \frac{4}{3}\pi r^3$), whereas its surface area is a function of the square of the radius ($S = 4\pi r^2$). Therefore, the S/V ratio of a coccus is $3/r$ (**Figure 2.3**). As cell size *increases*, its S/V ratio *decreases*. To illustrate this, consider the S/V ratio for some of the cells of different sizes listed in Table 2.1: *Pelagibacter ubique*, 22; *E. coli*, 4.5; and *E. fishelsoni* (Figure 2.2*a*), 0.05. These are all rods

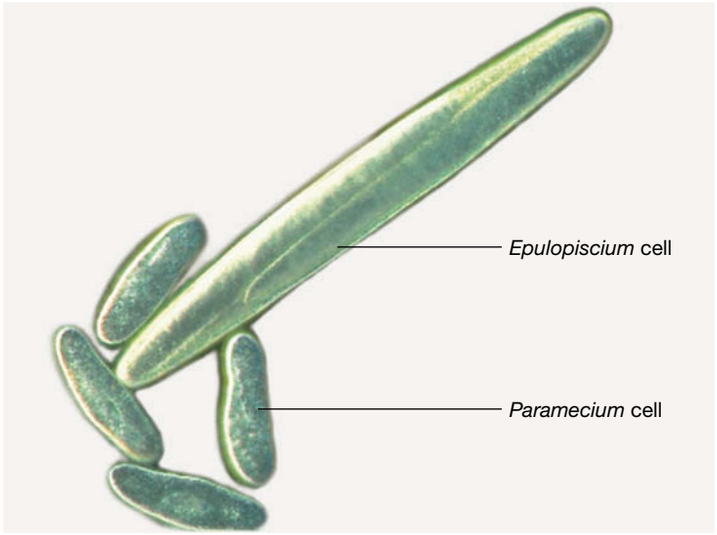
TABLE 2.1 Cell size and volume of some cells of *Bacteria*, from the largest to the smallest

Organism	*Characteristics*	*Morphology*	*Size[a] (μm)³*	*Cell volume (μm)³*	Volumes compared to *E. coli*
Thiomargarita namibiensis	Sulfur chemolithotroph	Cocci in chains	750	200,000,000	100,000,000×
Epulopiscium fishelsoni[a]	Chemoorganotroph	Rods with tapered ends	80 × 600	3,000,000	1,500,000×
Beggiatoa species[a]	Sulfur chemolithotroph	Filaments	50 × 160	1,000,000	500,000×
Achromatium oxaliferum	Sulfur chemolithotroph	Cocci	35 × 95	80,000	40,000×
Lyngbya majuscula	Cyanobacterium	Filaments	8 × 80	40,000	20,000×
Thiovulum majus	Sulfur chemolithotroph	Cocci	18	3,000	1,500×
Staphylothermus marinus[a]	Hyperthermophile	Cocci in irregular clusters	15	1,800	900×
Magnetobacterium bavaricum	Magnetotactic bacterium	Rods	2 × 10	30	15×
Escherichia coli	Chemoorganotroph	Rods	1 × 2	2	1×
Pelagibacter ubique[a]	Marine chemoorganotroph	Rods	0.2 × 0.5	0.014	0.007×
Ultra-small bacteria	Uncultured, from groundwater	Variable	<0.2	0.009	0.0045×
Mycoplasma pneumoniae	Pathogenic bacterium	Pleomorphic[b]	0.2	0.005	0.0025×

[a]Where only one number is given, this is the diameter of spherical cells. The values given are for the largest cell size observed in each species. For example, for *T. namibiensis*, an average cell is only about 200 μm in diameter. But on occasion, giant cells of 750 μm are observed. Likewise, an average cell of *S. marinus* is about 1 μm in diameter. The species of *Beggiatoa* here is unclear and *E. fishelsoni, M. bavaricum*, and *P. ubique* are not formally recognized names in taxonomy.

[b]*Mycoplasma* is a bacterium that lacks a cell wall and can thus take on many shapes (*pleomorphic* means "many shapes").

Source: Data obtained from Schulz, H.N., and B.B. Jørgensen. 2001. *Annu. Rev. Microbiol. 55*: 105–137, and Luef, B., et al. 2015. *Nature Communications.* doi:10.1038/ncomms7372.



Esther R. Angert, Harvard University

(a)

Heide Schulz-Vogt

(b)

Figure 2.2 Two very large *Bacteria*. *(a) Epulopiscium fishelsoni*. The rod-shaped cell is about 600 µm (0.6 mm) long and 75 µm wide and is shown with four cells of the protist *Paramecium* (a microbial eukaryote), each of which is about 150 µm long. *(b) Thiomargarita namibiensis*, a large sulfur chemolithotroph and currently the largest known of all prokaryotic cells. Cell widths vary from 400 to 750 µm.

rather than cocci, but if it is assumed that a rod-shaped cell is a perfect cylinder, the same *S*/*V* principles that hold for cocci also hold for rods; that is, for rods of a given length, cells with a smaller radius have a greater *S*/*V* than do cells with a larger radius.

The *S*/*V* ratio of a cell controls many of its properties, including its growth rate and evolution. Because how fast a cell can grow depends in part on the rate at which it can exchange nutrients and waste products with its environment, the higher *S*/*V* ratio of small cells supports a faster rate of nutrient and waste exchange per unit of cell volume compared with large cells. As a result, free-living smaller cells tend to grow faster than free-living larger cells, and for a given amount of resources (nutrients available to support growth), a larger population of small cells than of large cells can be supported. This in turn can affect a cell's evolution.

Each time a cell divides, its chromosome replicates. As DNA is replicated, occasional errors, called *mutations*, occur. Because mutation rates are roughly the same in all cells, large or small, the more chromosome replications that occur, the greater the total number of mutations in the cell population. Mutations are the "raw material" of evolution; the larger the pool of mutations, the greater the evolutionary possibilities. Thus, because prokaryotic cells are quite small and are also genetically haploid (they typically have only one copy of each gene, allowing mutations to be expressed immediately), prokaryotic cells can grow faster and evolve more rapidly than can larger cells.

Because of their typically larger size, not only is the *S*/*V* ratio of microbial eukaryotes smaller, the diploid character of the eukaryotic cell (a cell has two copies of each gene) allows for a mutation in one gene to be masked by a second, unmutated gene copy. These fundamental differences in size and genetics between prokaryotic and eukaryotic cells help explain why species of *Bacteria* and *Archaea* adapt rapidly to changing environmental conditions and more easily exploit new habitats than do eukaryotic cells. We illustrate this concept in later chapters when we consider the enormous diversity of cells and metabolisms of *Bacteria* and *Archaea* (Chapters 14–17), the rapidity of prokaryotic evolution (Chapter 13), and the ecological ramifications of microbial activities in nature (Chapters 19–23).

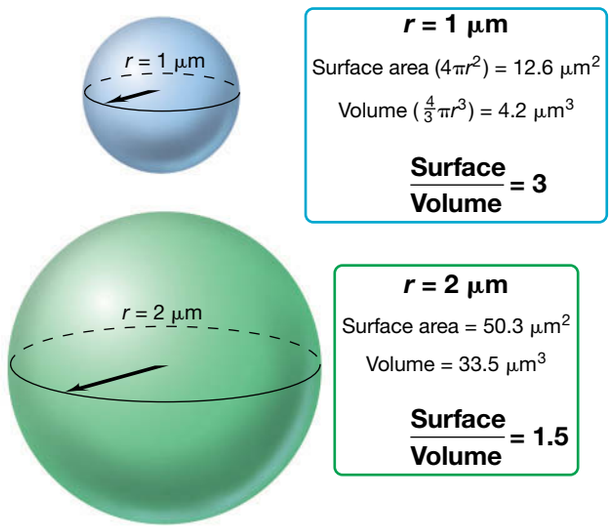


Figure 2.3 Surface area and volume relationships in cells. As a cell increases in size, its *S*/*V* ratio decreases.

Lower Limits to Cell Size

From the foregoing, one would predict that smaller and smaller microbes would have greater and greater selective advantages in nature and that as a consequence, only extremely tiny bacterial cells would exist. However, this is not the case, as there are lower limits to cell size and good reasons why there should be.

TINY CELLS

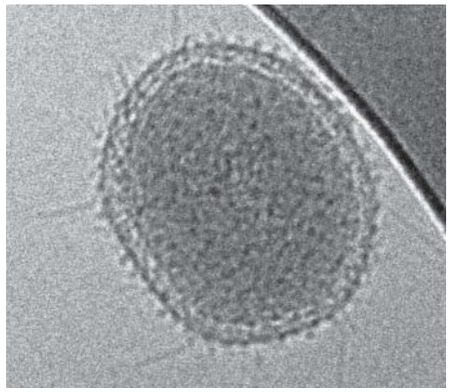
Viruses are very small microbes and range in diameter from as small as 20 nm to almost 750 nm. Although no cells exist that are as small as most viruses, the recent discovery of ultra-small bacterial cells[1, 2] has pushed the lower limits of cell size to what microbiologists feel must be very close to the minimal value.

Microbiologists collected groundwater, which travels through Earth's deep subsurface, from a Colorado (USA) aquifer (**Figure 1**) and ran it through a membrane filter whose pores were only 0.2 µm in diameter. The liquid that passed through the filter was then subjected to microbiological analyses. Surprisingly, since filters with 0.2-µm pores have been used for decades to remove bacterial cells from solutions to generate "sterile solutions," prokaryotic cells were present in the groundwater filtrate. In fact, a diverse array of *Bacteria* were present in the filtrate, revealing that the groundwater was inhabited by a microbial community of tiny cells[1] that microbiologists have come to call "ultramicrobacteria."

Cryo-electron microscopy, in which a specimen is examined at extremely cold temperatures without fixation (chemical treatment that can alter a cell's morphology), showed the groundwater ultramicrobacteria to consist primarily of oval-shaped cells about 0.2 µm in diameter (**Figure 2**). The volume of these cells was calculated to be about 1/100 that of a cell of the bacterium *Escherichia coli* (see Table 2.1) such that nearly 150 of the small cells could fit into one *E. coli* cell! Each of the tiny cells contained about 50 ribosomes, which is also about 1/100 of the number present in a slowly growing (100-min generation time) cell of *E. coli*. The very small size of the

Kenneth H. Willams

Figure 1 Sampling the anoxic groundwater aquifer that parallels the Colorado River near Rifle, Colorado.



Birgit Luef and Jill Banfield

Figure 2 A tiny bacterial cell from anoxic groundwater that passed through a filter with 0.2-µm pores. The cell is not quite 0.2 µm in diameter.

groundwater ultramicrobacteria gives them an enormous surface-to-volume ratio, and it is hypothesized that this advantage benefits them in extracting resources from their nutrient-deficient habitat.

Despite the fact that the tiny groundwater bacteria have yet to be cultured in the laboratory, much is already known about them because their small genomes—less than 1 Mb in size—were obtained and analyzed.[2] From a phylogenetic perspective, the different species detected were distantly related to major phyla of *Bacteria* known from environmental analyses of diverse environments but which have thus far defied laboratory culture. Further analyses showed that genes encoding the enzymes for several core metabolic pathways widely distributed among microorganisms were absent from the genomes of the groundwater ultramicrobacteria. This suggests a metabolically minimalist lifestyle for these tiny cells and a survival strategy of cross-feeding essential nutrients with neighboring species in their microbial community.

Although we do not yet know exactly how small a microbial cell can be, microbiologists are closing in on this number from environmental analyses such as the Colorado groundwater study. From the same samples that yielded ultra-small *Bacteria* in this study, ultra-small *Archaea* were also detected and found to contain small and highly reduced genomes.[2] Apparently, a large diversity of very small prokaryotic cells occurs in nature, and from the continued study of these tiny cells, more precise values for both the lower limits to cell size and the minimal genomic requirements for life should emerge.

[1] Luef, B., et al. 2015. *Nature Communications.* doi:10.1038/ncomms7372.
[2] Castelle, C.J., et al. 2015. *Current Biology. 25:* 1–12.

If one calculates the volume needed to house the essential components of a free-living cell—proteins, nucleic acids, ribosomes, and so on—cells 0.15–0.2 µm in diameter are probably the lower limit, or very close to it. Many small prokaryotic cells are known and several have been grown in the laboratory. Open ocean water, for example, contains 10^5–10^6 prokaryotic cells per milliliter, and these tend to be very small cells, 0.2–0.4 µm in diameter. In addition, populations of bacterial and archaeal cells have been discovered in Earth's deep subsurface that are about 0.2 µm in diameter (see Explore the Microbial World, "Tiny Cells"). Collectively, these cells have been referred to as "ultramicrobacteria" to indicate that they are considerably smaller than typical bacterial cells. We will see later that the cells of many pathogenic bacteria are also very small.

Interestingly, when the genomes of ultramicrobacteria are unraveled, they are typically found to be highly streamlined and to be missing many genes whose products or functions must be supplied to them by other microbial cells or by the host organisms (plants and animals, for example) that harbor them. In these tiny prokaryotic cells, evolutionary success is apparently linked to a minimalist biochemistry and obligate association with one or more other organisms. In some cases, these associations are so

specific and so tightly linked that the tiny cell (and in some cases, the second organism) cannot survive without its respective partner. We consider the genomes—both large and small—of *Bacteria* and *Archaea* in many chapters of this book and focus exclusively on genomes and related aspects of the systems biology of microbial cells in Chapter 9.

MINIQUIZ

- What physical property of cells increases as cells become smaller?
- How can the small size and haploid genome of *Bacteria* and *Archaea* accelerate their evolution?
- What are the approximate limits to how small a cell can be? Why should this be so?

II • The Cell Membrane and Wall

We consider here the structure and function of two of a cell's most essential structures, the *cytoplasmic membrane* and the *cell wall*. The cytoplasmic membrane plays many roles, chief among them as the "gatekeeper" for the entrance and exit of dissolved substances. The cell wall, by contrast, confers structural strength on the cell in order to keep it from bursting due to osmotic pressure.

2.3 The Cytoplasmic Membrane

The **cytoplasmic membrane** surrounds the *cytoplasm*—the mixture of macromolecules and small molecules inside the cell—and separates it from the environment. The cytoplasmic membrane is physically rather weak but it is an ideal structure for its major cellular function: selective permeability. In order for a cell to grow, nutrients must be transported inwards and waste products outwards. Both of these events occur across the cytoplasmic membrane. A variety of proteins located in the cytoplasmic membrane facilitate these reactions, and many other membrane proteins play important roles in energy metabolism.

The Bacterial Cytoplasmic Membrane

The cytoplasmic membrane of all cells is a phospholipid bilayer containing embedded proteins. Phospholipids are composed of both hydrophobic (water-repelling) and hydrophilic (water-attracting) components (**Figure 2.4**). In *Bacteria* and *Eukarya*, the hydrophobic component consists of fatty acids and the hydrophilic component of a glycerol molecule containing phosphate and one of several other functional groups (such as sugars, ethanolamine, or choline) bonded to the phosphate. The fatty acids point inward toward each other to form a hydrophobic region, while the hydrophilic portion remains exposed to either the environment or the cytoplasm (Figure 2.4*b*). That is, the *outer* surface of the cytoplasmic membrane faces the environment while the *inner* surface faces the cytoplasm and interacts with the cytoplasmic milieu. This type of membrane structure is called a *lipid bilayer*, or a *unit membrane* because each phospholipid "leaf" forms half of the unit (see Figure 2.5).

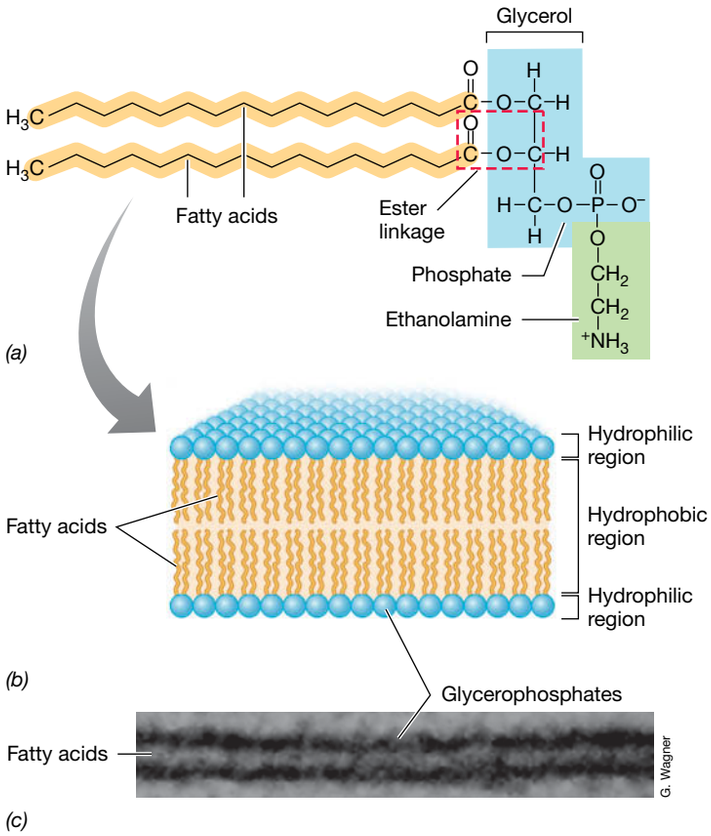


Figure 2.4 Phospholipid bilayer membrane. *(a)* Structure of the phospholipid phosphatidylethanolamine. The side chains are fatty acids and the ester linkage (characteristic of the lipids of *Bacteria* and *Eukarya* but not *Archaea*) is boxed with a red dashed line. *(b)* General architecture of a bilayer membrane; the blue spheres depict glycerol with phosphate and/or other hydrophilic groups. *(c)* Transmission electron micrograph of a membrane. The light inner area is the hydrophobic region of the model membrane shown in *b*.

The cytoplasmic membrane is only 8–10 nanometers wide but can be resolved easily by transmission electron microscopy (Figure 2.4*c*). In addition, although physically weak, the cytoplasmic membranes of some *Bacteria* are strengthened by sterol-like molecules called *hopanoids*. Sterols are rigid and planar molecules that strengthen the membranes of eukaryotic cells (Section 2.14), many of which lack a cell wall.

A variety of proteins are attached to or integrated into the cytoplasmic membrane; membrane proteins typically have hydrophobic domains that span the membrane and hydrophilic domains that contact the environment or the cytoplasm (**Figure 2.5**). Proteins significantly embedded in the membrane are called *integral* membrane proteins. By contrast, *peripheral* membrane proteins are more loosely attached. Some peripheral membrane proteins are lipoproteins, proteins that contain a hydrophobic lipid tail that anchors the protein into the membrane (Figure 2.5). Peripheral membrane proteins typically interact with integral membrane proteins in important cellular processes such as energy metabolism and transport.

Archaeal Membranes

The cytoplasmic membrane of *Archaea* is structurally similar to those of *Bacteria* and *Eukarya*, but the chemistry is somewhat

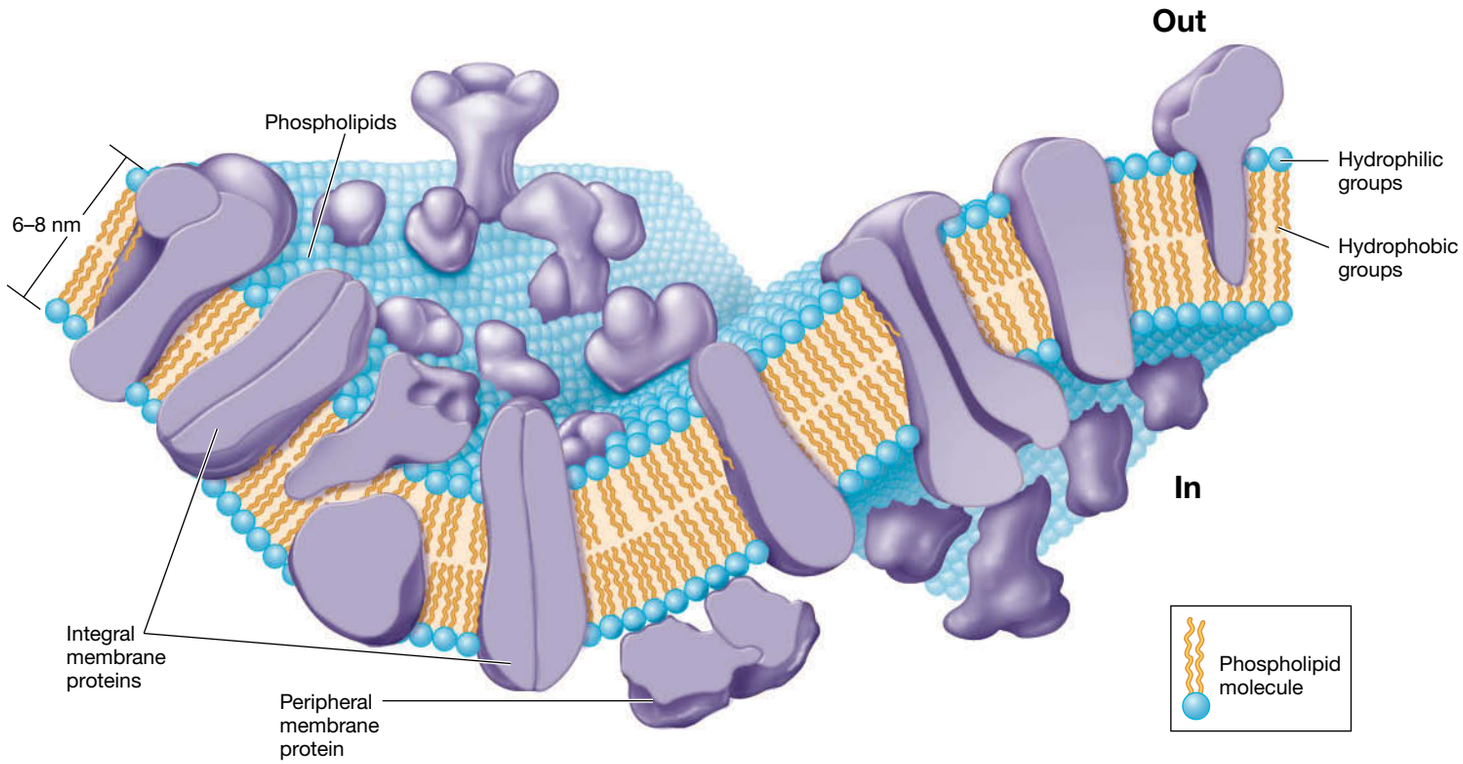


Figure 2.5 Structure of the cytoplasmic membrane. The inner surface (**In**) faces the cytoplasm and the outer surface (**Out**) faces the environment. Phospholipids compose the matrix of the cytoplasmic membrane with proteins embedded (integral) or surface associated (peripheral). The general design of the cytoplasmic membrane is similar in both prokaryotic and eukaryotic cells, although there are chemical differences between different species.

different. In contrast to the lipids of *Bacteria* and *Eukarya* in which *ester* linkages bond *fatty acids* to glycerol (Figure 2.4), the lipids of *Archaea* contain *ether* bonds between glycerol and a hydrophobic side chain that is not a fatty acid (**Figure 2.6**). The hydrophobic region of archaeal membranes is formed from repeating units of the five-carbon hydrocarbon *isoprene*, rather than from fatty acids (compare Figures 2.4 and 2.6).

The cytoplasmic membrane of *Archaea* is constructed from either phosphoglycerol diethers, which have C_{20} side chains (called a *phytanyl* group), or diphosphoglycerol tetraethers (C_{40} side chains, called a *biphytanyl* group) (Figure 2.6). In the tetraether lipid structure, the ends of the inwardly pointing phytanyl groups are covalently linked at their termini to form a *lipid monolayer* (Figure 2.6*e*) instead of a lipid bilayer (Figure 2.6*d*) membrane.

Some archaeal lipids contain rings within the hydrocarbon side chains. For example, *crenarchaeol*, a common membrane lipid in cells of *Crenarchaeota* (a major phylum of *Archaea*) contains four C_5 rings and one C_6 ring (Figure 2.6*c*). These rings affect the chemical properties of the lipids and thus influence membrane function. As in other organisms, the polar head groups in archaeal lipids can be sugars, ethanolamine, or a variety of other molecules. Hopanoids, present in the cytoplasmic membranes of many *Bacteria*, have not been found in the cytoplasmic membranes of *Archaea*.

Despite differences in chemistry between the cytoplasmic membranes of *Archaea* and organisms in the other phylogenetic domains, the fundamental construction of the archaeal cytoplasmic membrane—inner and outer hydrophilic surfaces and a hydrophobic interior—is the same as that of membranes in all cells. Obviously, evolution has selected this fundamental design as the best solution to the major functions of the cytoplasmic membrane, an issue we turn to now.

Cytoplasmic Membrane Function

The cytoplasmic membrane has at least three major functions (**Figure 2.7**). First, it is the cell's permeability barrier, preventing the passive leakage of solutes into or out of the cell. Second, the cytoplasmic membrane anchors several proteins that catalyze a suite of key cell functions. And third, the cytoplasmic membrane of *Bacteria* and *Archaea* plays a major role in energy conservation and consumption.

The cytoplasmic membrane is a barrier to the diffusion of most substances, especially polar or charged molecules. Because the cytoplasmic membrane is so impermeable, most substances that enter or leave the cell must be carried in or out by *transport proteins*. These are not simply ferrying proteins but instead function to *accumulate* solutes against the concentration gradient, a process that diffusion alone cannot do (**Figure 2.8**). Transport, which requires energy, ensures that the cytoplasm has sufficient concentrations of the nutrients it needs to perform biochemical reactions efficiently.

Transport proteins typically display high sensitivity and high specificity. If the concentration of a solute is high enough to saturate the transporter, which often occurs at the very low concentrations of nutrients found in nature, the rate of uptake can be near maximal (Figure 2.8). Some nutrients are transported by a

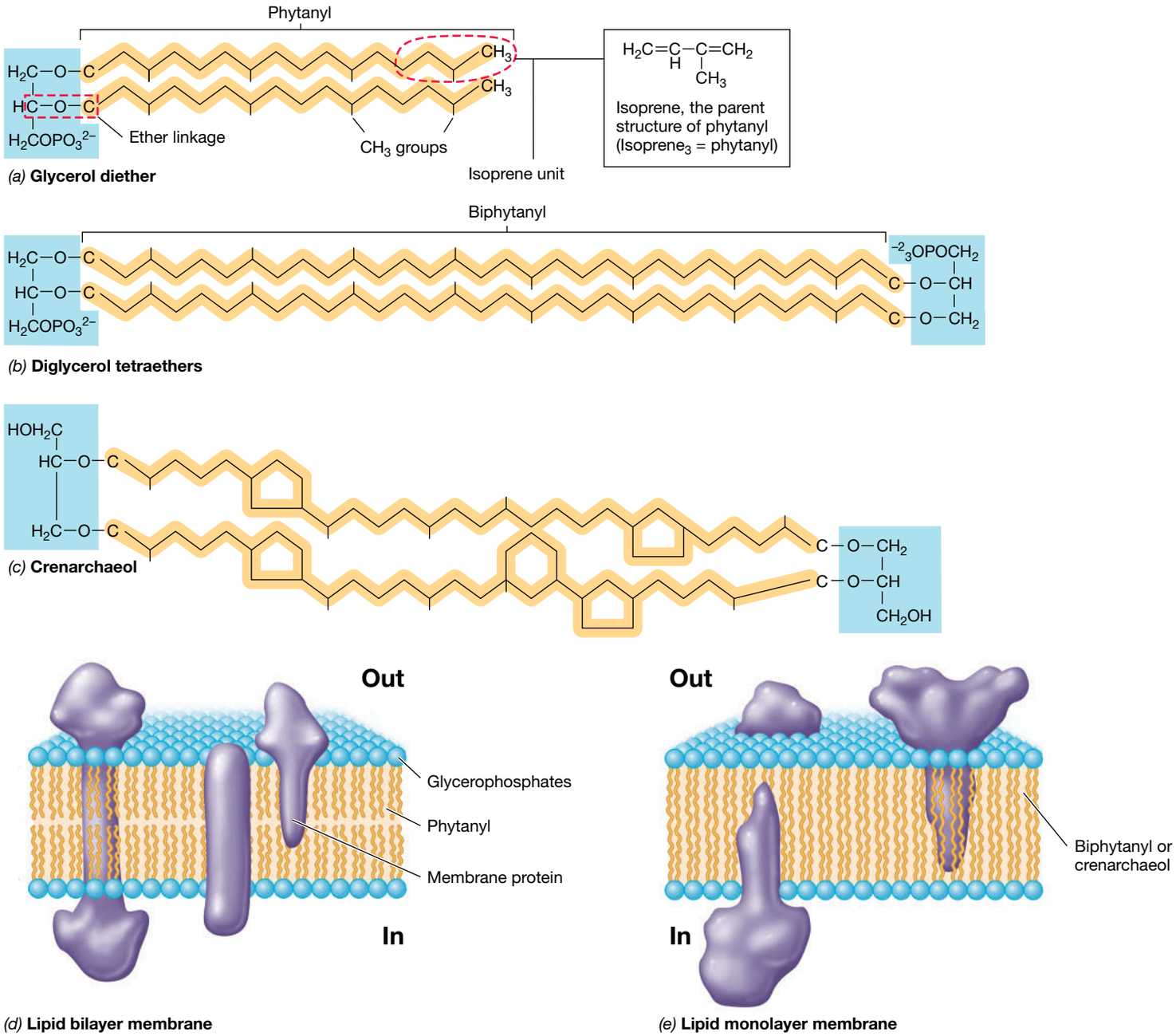


Figure 2.6 Major lipids of *Archaea* and the architecture of archaeal membranes. *(a, b)* Note that the hydrocarbon of the lipid is bonded to the glycerol by an ether linkage (in dashed red box in *a*) in both cases. The hydrocarbon is phytanyl (C_{20}) in *a* and biphytanyl (C_{40}) in *b*; both are multiples of the parent structure, isoprene (in dashed red oval; detailed structure shown in black box). *(c)* A major lipid of *Thaumarchaeota* is crenarchaeol, a lipid containing 5- and 6-carbon rings. *(d, e)* The membrane structure in *Archaea* may form a lipid bilayer or a lipid monolayer (or a mix of both).

low-affinity transporter when present at high external concentration and by a separate, typically higher-affinity, transporter for those present at low concentration (Figure 2.8). Moreover, many transport proteins transport only a single kind of molecule while others carry a related class of molecules, such as different sugars or different amino acids. This economizing reduces the need for separate transport proteins for each different sugar or amino acid. We revisit the important issue of nutrient transport in Section 3.2, where we focus on transport mechanisms.

In addition to its permeability and transport functions, the cytoplasmic membrane of *Bacteria* and *Archaea* is a major site of both energy conservation and consumption. We discuss in Chapter 3 how the cytoplasmic membrane can be energized when protons (H^+) are separated from hydroxyl ions (OH^-) across the membrane

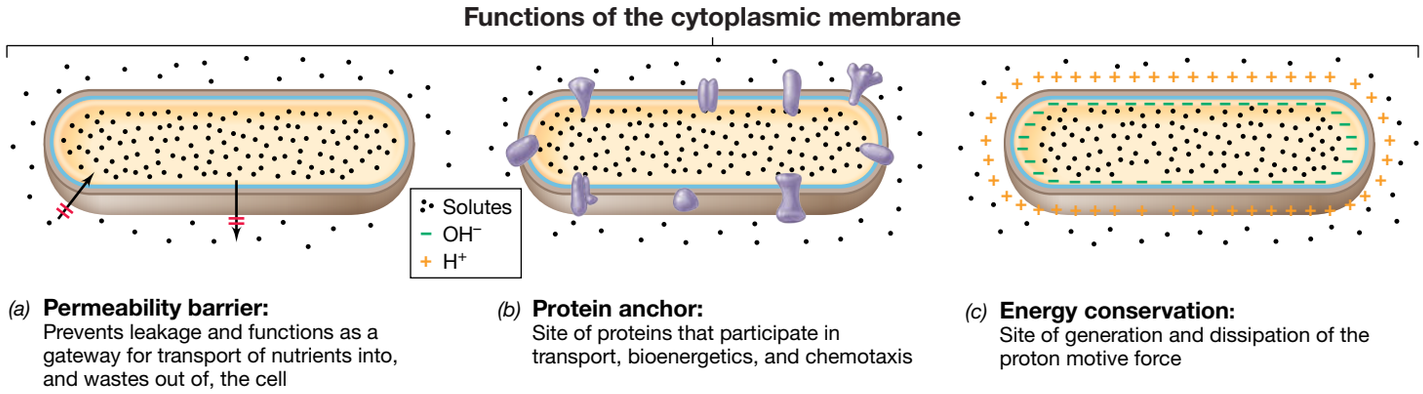


Figure 2.7 The major functions of the cytoplasmic membrane. Although physically weak, the cytoplasmic membrane controls at least three critically important cellular functions: preventing leakage, anchoring proteins, and conserving energy.

surface (Figure 2.7*c*). This charge separation creates an energized state of the membrane called the *proton motive force*, analogous to the potential energy present in a charged battery. Dissipation of the proton motive force can be coupled to several energy-requiring reactions, such as transport, cell locomotion, and the biosynthesis of ATP. In eukaryotic microbial cells, although transport across the cytoplasmic membrane is just as necessary as it is in prokaryotic cells, energy conservation takes place in the membrane systems of the cell's key organelles, the mitochondrion (respiration) and chloroplast (photosynthesis).

MINIQUIZ

- Draw the basic structure of a lipid bilayer and label the hydrophilic and hydrophobic regions. Why is the cytoplasmic membrane a good permeability barrier?
- How are the membrane lipids of *Bacteria* and *Archaea* similar, and how do they differ?
- Describe the major functions of the cytoplasmic membrane.

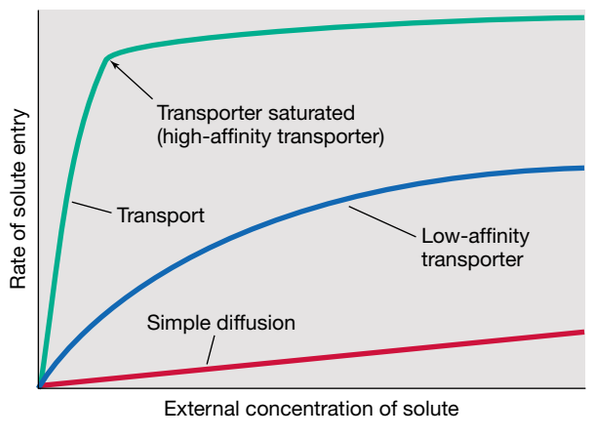


Figure 2.8 The importance of transport in membrane function. In transport, the uptake rate shows saturation at relatively low external concentrations. Both high-affinity and low-affinity transport systems are depicted.

2.4 Bacterial Cell Walls: Peptidoglycan

The cytoplasm of prokaryotic cells maintains a high concentration of dissolved solutes that creates significant osmotic pressure—about 2 atm (203 kPa); this is about the same as the pressure in an automobile tire. To withstand these pressures and prevent bursting—a process called *cell lysis*—most cells of *Bacteria* and *Archaea* have a layer outside the cytoplasmic membrane called the *cell wall*. Besides protecting against osmotic lysis, cell walls also confer shape and rigidity on the cell.

Knowledge of cell wall structure and function is important not only for understanding the biology of microbial cells, but also because certain antibiotics, for example, the penicillins and cephalosporins, target bacterial cell wall synthesis, leaving the cell susceptible to osmotic lysis. Since human cells lack cell walls and are therefore not a target of such antibiotics, these drugs are of obvious benefit for treating bacterial infections.

Cells of *Bacteria* can be divided into two major groups, *gram-positive* and *gram-negative*. The distinction between gram-positive and gram-negative bacteria is based on the Gram stain reaction (Section 1.6), and differences in cell wall structure play a major role in the reaction. The surface of gram-positive and gram-negative cells as viewed in the electron microscope differs markedly, as shown in **Figure 2.9**. The gram-negative cell wall, or *cell envelope* as it is also called, consists of at least two layers, whereas the gram-positive cell wall is typically thicker and consists primarily of a single type of molecule.

Our focus in this section is on a key molecule found in the cell walls of both gram-positive and gram-negative *Bacteria*. In Section 2.5, we describe some additional wall components present in gram-negative *Bacteria*, and in Section 2.6, we describe the cell walls of *Archaea*.

Structure of Peptidoglycan

The walls of cells of *Bacteria* contain a rigid polysaccharide called **peptidoglycan** that confers structural strength on the cell. Peptidoglycan is found in all *Bacteria* that contain a cell wall, but it is not present in the cell walls of *Archaea* or *Eukarya*. Peptidoglycan is composed of alternating repeats of two modified glucose residues

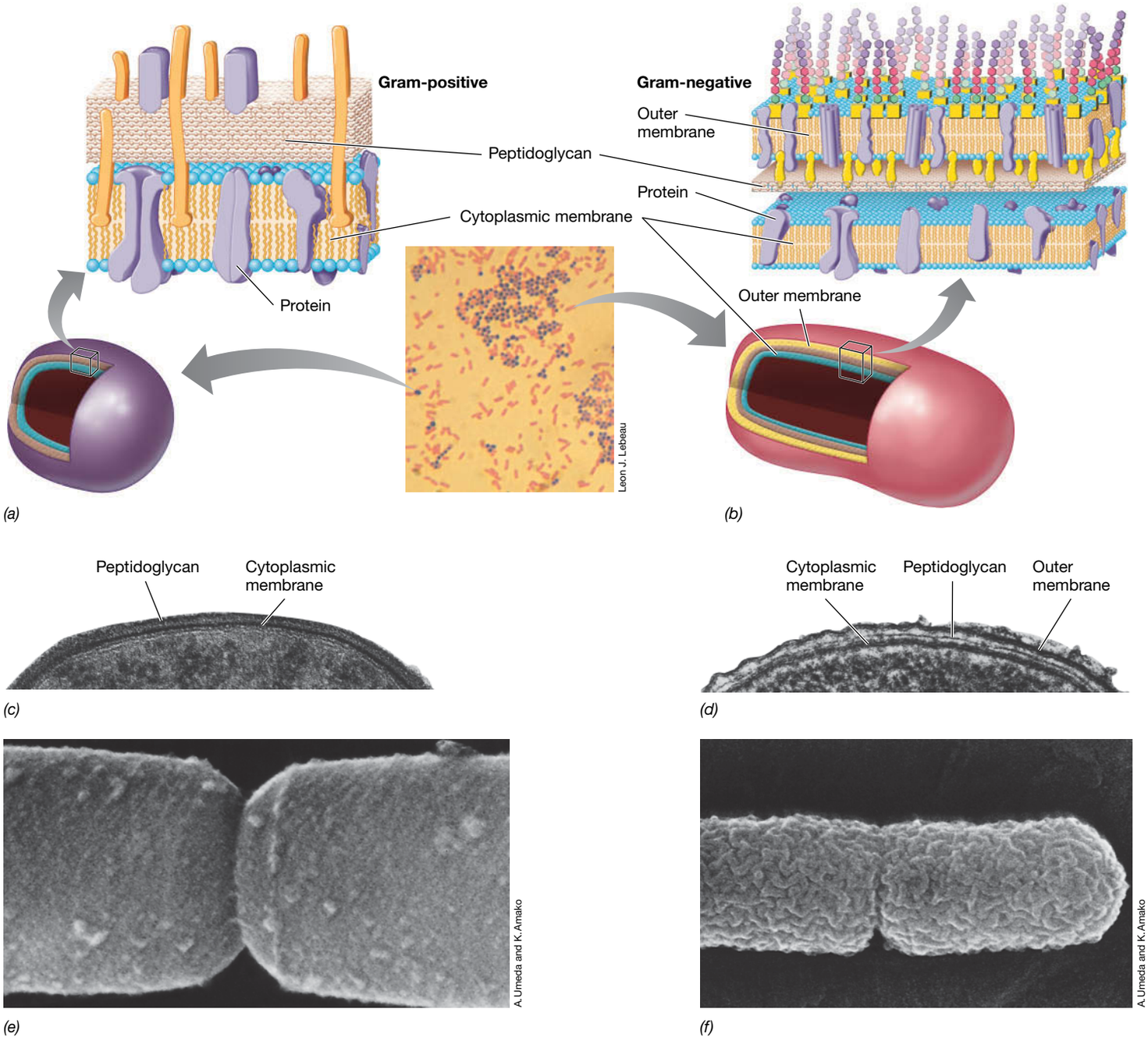


Figure 2.9 **Cell walls of *Bacteria*.** *(a, b)* Schematic diagrams of gram-positive and gram-negative cell walls; the Gram stain procedure was described in Section 1.6 and shown in Figure 1.18. The photo of Gram-stained bacteria in the center shows cells of *Staphylococcus aureus* (purple, gram-positive) and *Escherichia coli* (pink, gram-negative). *(c, d)* Transmission electron micrographs showing the cell wall of a gram-positive bacterium and a gram-negative bacterium, respectively. *(e, f)* Scanning electron micrographs of gram-positive and gram-negative bacteria, respectively. Note differences in surface texture. Each cell is about 1 μm wide.

called *N-acetylglucosamine* and *N-acetylmuramic acid* along with the amino acids L-alanine, D-alanine, D-glutamic acid, and either L-lysine or diaminopimelic acid (DAP). These constituents are connected in an ordered way to form the *glycan tetrapeptide* (**Figure 2.10**), and long chains of this basic unit form peptidoglycan.

Strands of peptidoglycan are biosynthesized adjacent to one another to form a sheet surrounding the cell, and the individual strands are connected by peptide cross-links; this forms a polymer that is strong in both X and Y directions (**Figure 2.11**). In gram-negative bacteria, the cross-link forms from the amino group of DAP of one glycan strand to the carboxyl group of the terminal D-alanine on the adjacent glycan strand (Figure 2.11*a*). In gram-positive bacteria, the cross-link often contains a short peptide "interbridge," the kinds and numbers of amino acids in the

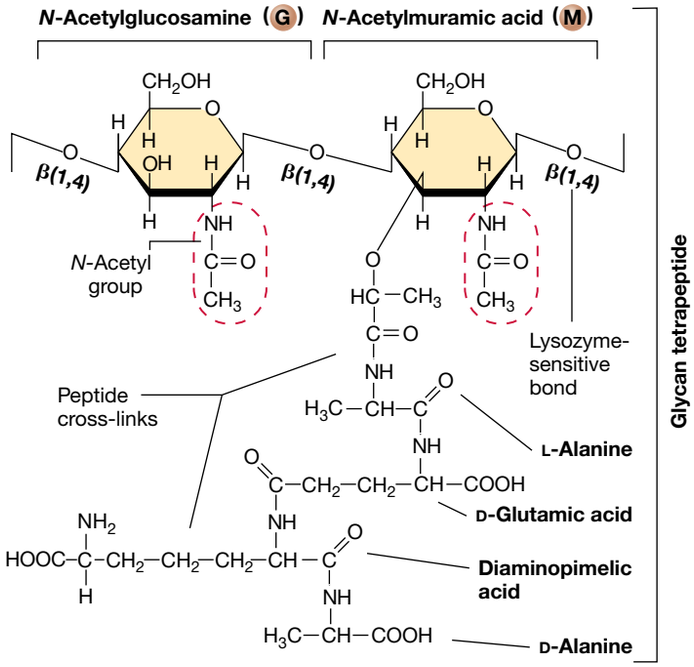


Figure 2.10 Structure of the repeating unit in peptidoglycan, the glycan tetrapeptide. The structure given is that of *Escherichia coli* and most other gram-negative *Bacteria*. In some *Bacteria*, other amino acids are present as cross-linkers.

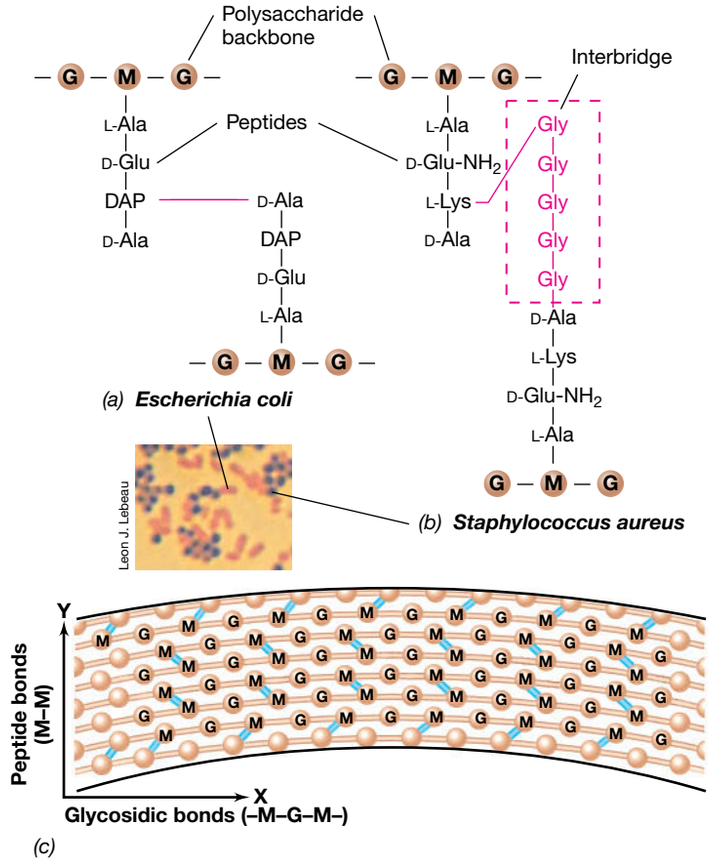


Figure 2.11 Peptidoglycan in *Escherichia coli* and *Staphylococcus aureus*. *(a)* No interbridge is present in *E. coli* peptidoglycan nor in that of other gram-negative *Bacteria*. *(b)* The glycine interbridge in the gram-positive bacterium *S. aureus*. *(c)* Overall structure of peptidoglycan. G, *N*-acetylglucosamine; M, *N*-acetylmuramic acid. Note how glycosidic bonds confer strength on peptidoglycan in the X direction whereas peptide bonds confer strength in the Y direction.

interbridge varying between species. In the gram-positive bacterium *Staphylococcus aureus*, for example, a bacterium whose cell wall chemistry is well understood, the interbridge consists of five glycines (Figure 2.11*b*). The overall structure of peptidoglycan is shown in Figure 2.11*c*.

Peptidoglycan can be destroyed by *lysozyme*, an enzyme that cleaves the glycosidic bond between *N*-acetylglucosamine and *N*-acetylmuramic acid (Figure 2.11). This weakens the peptidoglycan and can cause cell lysis. Lysozyme is present in human secretions including tears, saliva, and other bodily fluids, and functions as a major line of defense against bacterial infection. When we consider peptidoglycan biosynthesis in Chapter 7, we will see that the antibiotic penicillin also destroys peptidoglycan, but in a different way than lysozyme does. Whereas lysozyme destroys preexisting peptidoglycan, penicillin blocks a key step in its biosynthesis; this also weakens the molecule and leads to osmotic lysis.

An unusual feature of peptidoglycan is the presence of two amino acids of the D *stereoisomer*, D-alanine and D-glutamic acid. Teichoic acids, to be described shortly, also contain D-amino acids. By contrast, proteins are always constructed of L-amino acids. All in all, more than 100 chemically distinct peptidoglycans have been described that vary in their peptide cross-links and/or interbridge structures. By contrast, the glycan portion of all peptidoglycans appears to be universal; only alternating repeats of *N*-acetylglucosamine and *N*-acetylmuramic acid in β-1,4 linkage are known (Figures 2.10 and 2.11).

Overview of the Gram-Positive Cell Wall

As much as 90% of the cell wall of a gram-positive bacterium can consist of peptidoglycan. Although some bacteria have only a single layer of peptidoglycan, many gram-positive bacteria form several layers of peptidoglycan stacked one upon another (Figure 2.11*c*). It is thought that peptidoglycan is synthesized by the cell in the form of "cables" about 50 nm wide, with each cable consisting of several glycan strands (**Figure 2.12*a***). As peptidoglycan is synthesized, the cables themselves become cross-linked to form an even stronger cell wall structure.

In addition to peptidoglycan, many gram-positive bacteria produce acidic molecules called **teichoic acids** embedded in their cell wall. Teichoic acids are composed of glycerol phosphate or ribitol phosphate with attached molecules of glucose or D-alanine (or both). Individual alcohol molecules are then connected through their phosphate groups to form long strands, and these are then covalently linked to peptidoglycan (Figure 2.12*b*). Teichoic acids also function to bind divalent metal ions, such as Ca^{2+} and Mg^{2+}, prior to their transport into the cell. Some teichoic acids are covalently bonded to membrane lipids rather than to peptidoglycan, and these are called *lipoteichoic* acids. Figure 2.12*c* summarizes the structure of the cell wall of gram-positive *Bacteria* and shows how teichoic acids and lipoteichoic acids are arranged in the overall wall structure.

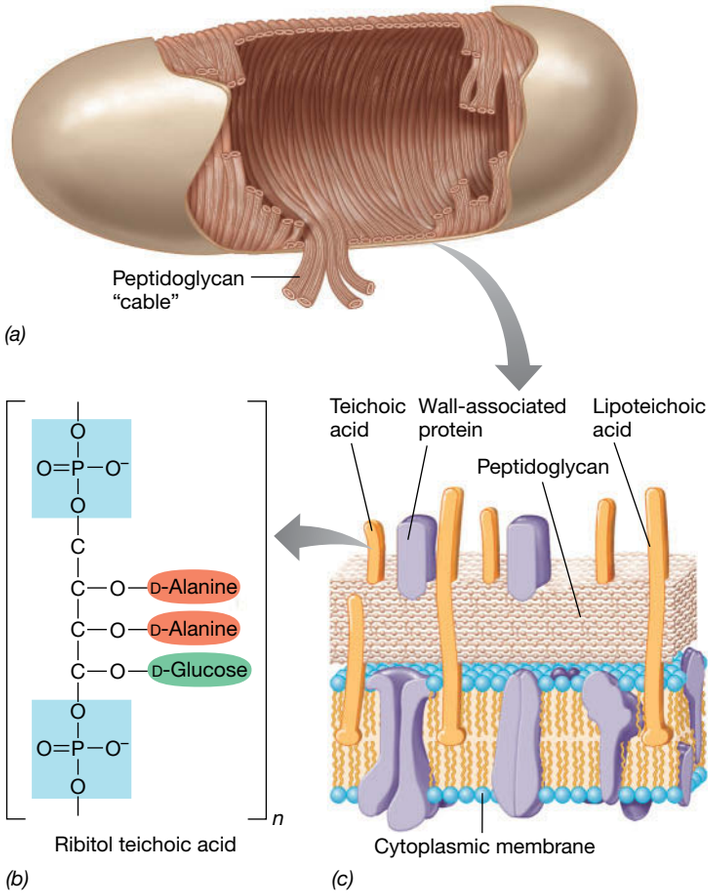


Figure 2.12 Structure of the gram-positive bacterial cell wall. *(a)* Schematic of a gram-positive rod showing the internal architecture of the peptidoglycan "cables." *(b)* Structure of a ribitol teichoic acid. The teichoic acid is a polymer of the repeating ribitol unit shown here. *(c)* Summary diagram of the gram-positive bacterial cell wall.

A very few *Bacteria* and *Archaea* lack cell walls altogether. These include in particular the mycoplasmas, pathogenic *Bacteria* related to gram-positive bacteria that cause a variety of infectious diseases of humans and other animals, and *Thermoplasma* and some of its relatives (*Archaea*). Lacking a cell wall, these cells would be expected to contain unusually tough cytoplasmic membranes, and chemical analyses show that they do. For example, most mycoplasmas contain sterols in their cytoplasmic membranes; these molecules function to add strength and rigidity to the membrane as they do in the cytoplasmic membranes of eukaryotic cells (Section 2.14). *Thermoplasma* membranes contain molecules called *lipoglycans* that serve a similar strengthening function.

MINIQUIZ

- Why do bacterial cells need cell walls? Do all bacteria have cell walls?
- Why is peptidoglycan such a strong molecule?
- What do the enzyme lysozyme and the antibiotic penicillin have in common?

2.5 LPS: The Outer Membrane

In gram-negative bacteria, only a small amount of the total cell wall consists of peptidoglycan, as most of the wall is composed of the **outer membrane**. This layer is effectively a second lipid bilayer, but it is not constructed solely of phospholipid and protein, as is the cytoplasmic membrane (Figures 2.4 and 2.5). Instead, the outer membrane also contains polysaccharide, and the lipid and polysaccharide are linked to form a complex. Hence, the outer membrane is often called the **lipopolysaccharide** layer, or simply **LPS** for short.

The outer membrane confers only modest structural strength on the gram-negative cell (peptidoglycan remains the major strengthening agent), but it acts as an effective barrier against many substances such as lipophilic antibiotics and other harmful agents that might otherwise penetrate the cytoplasmic membrane. Indeed, many antibiotics that are clinically useful against gram-positive bacterial pathogens show little to no activity against gram-negative pathogens because of their outer membrane.

Structure and Activity of LPS

The structure of LPS from several bacteria is known, and there are many variations. As seen in Figure 2.13, the polysaccharide portion of LPS consists of two components, the *core polysaccharide* and the *O-specific polysaccharide*. In *Salmonella* species, where LPS has been well studied, the core polysaccharide consists of ketodeoxyoctonate (KDO), various seven-carbon sugars (heptoses),

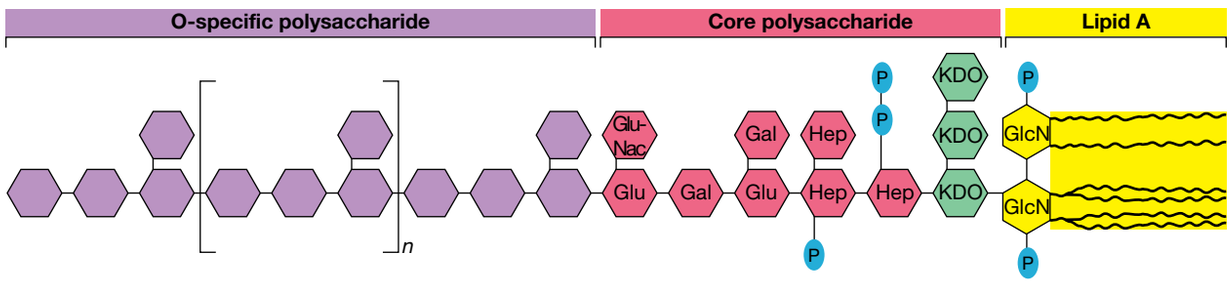


Figure 2.13 Structure of bacterial lipopolysaccharide. The chemistry of lipid A and the polysaccharide components varies among species of gram-negative *Bacteria*, but the major components (lipid A–KDO–core–O-specific) are typically the same. The O-specific polysaccharide is highly variable among species. KDO, ketodeoxyoctonate; Hep, heptose; Glu, glucose; Gal, galactose; GluNac, *N*-acetylglucosamine; GlcN, glucosamine; P, phosphate. Glucosamine and the lipid A fatty acids are linked through the amine groups of GlcN. The lipid A portion of LPS can be toxic to animals and comprises the endotoxin complex (Section 25.7 and Figure 25.18). Compare this figure with Figure 2.14 and follow the LPS components by their color-coding.

glucose, galactose, and *N*-acetylglucosamine. Connected to the core is the O-specific polysaccharide, which typically contains galactose, glucose, rhamnose, and mannose, as well as one or more dideoxyhexoses, such as abequose, colitose, paratose, or tyvelose. These sugars are connected in four- or five-membered sequences, which often are branched. When the sequences repeat, the long O-specific polysaccharide is formed.

The relationship of the LPS layer to the overall gram-negative cell wall is shown in **Figure 2.14**. The lipid portion of the LPS, called *lipid A*, is not a typical glycerol lipid (see Figure 2.4*a*); instead the fatty acids are bonded through the amine groups from a disaccharide composed of glucosamine phosphate. The disaccharide is attached to the core polysaccharide through KDO (Figure 2.13). Fatty acids commonly found in lipid A include caproic (C_6), lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), and stearic (C_{18}) acids. LPS replaces much of the phospholipid in the outer half of the outer membrane, and although the outer membrane is technically a lipid bilayer, its many unique components distinguish it from the cytoplasmic membrane. The outer membrane is anchored to the peptidoglycan layer by the *Braun lipoprotein*, a molecule that spans the gap between the LPS layer and the peptidoglycan layer (in the periplasm, discussed in the next subsection) (Figure 2.14*a*).

An important biological activity of LPS is its toxicity to animals. Common gram-negative pathogens for humans include species of *Salmonella*, *Shigella*, and *Escherichia*, among many others, and some of the gastrointestinal symptoms these pathogens elicit are due to their toxic outer membrane components. Toxicity is specifically linked to the LPS layer, in particular, to lipid A. The term

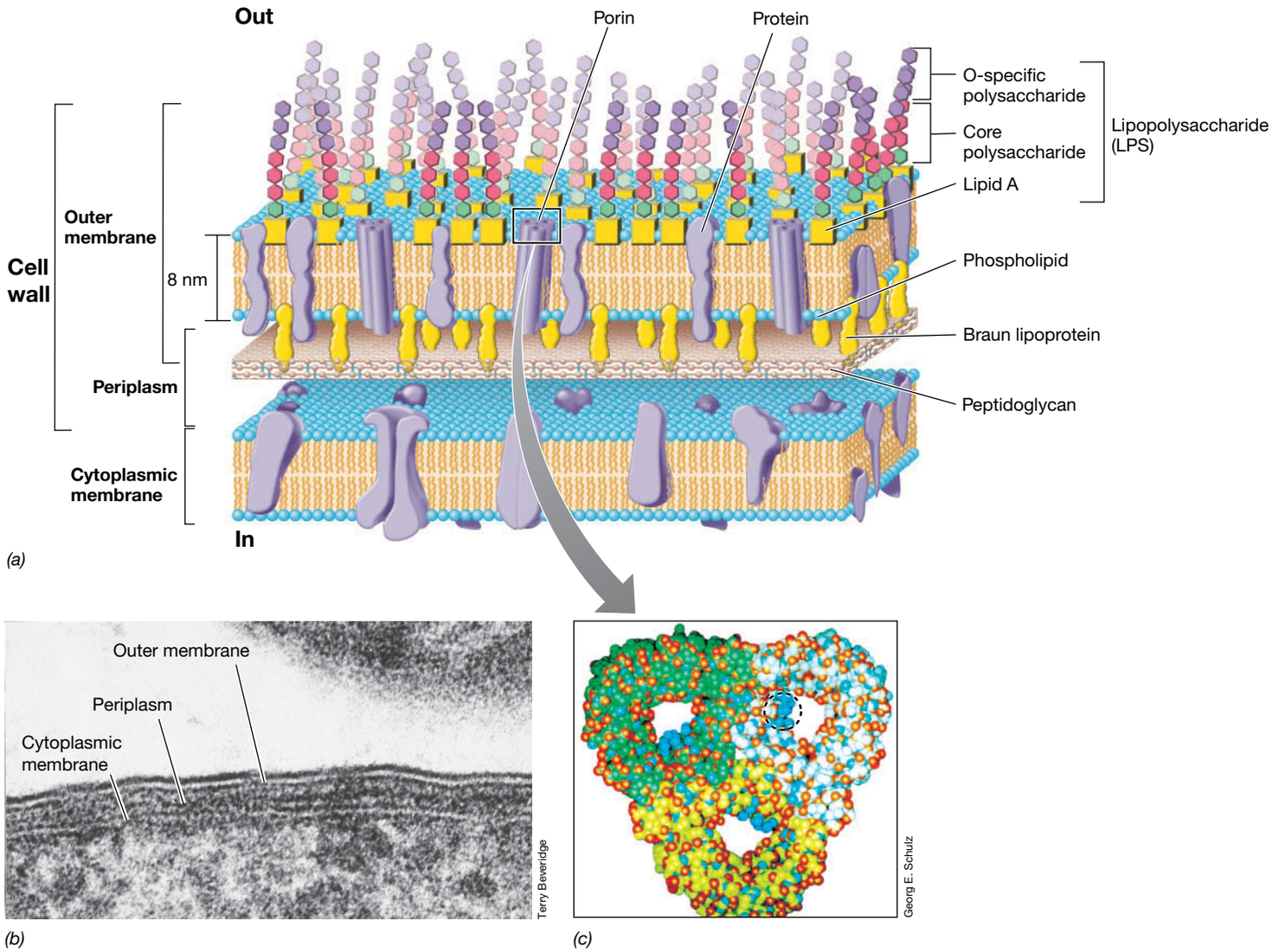


Figure 2.14 The gram-negative bacterial cell wall. *(a)* Arrangement of lipopolysaccharide, lipid A, phospholipid, porins, and Braun lipoprotein in the outer membrane. See Figure 2.13 for details of the structure of LPS. *(b)* Transmission electron micrograph of a cell of *Escherichia coli* showing the cytoplasmic membrane and wall. *(c)* Molecular model of porin proteins. Note the four pores present, one within each of the proteins forming a porin molecule and a smaller central pore (circled) between the porin proteins. The view is perpendicular to the plane of the membrane.

endotoxin refers to this toxic component of LPS. Some endotoxins cause violent symptoms in humans, including gas, diarrhea, and vomiting, and the endotoxins produced by *Salmonella* and enteropathogenic strains of *E. coli* transmitted in contaminated foods are classic examples of this. We discuss major gram-negative enteric pathogens in Chapter 32 and endotoxin in Section 25.7.

The Periplasm and Porins

The outer membrane is impermeable to proteins and other very large molecules. In fact, a major function of the outer membrane is to prevent cellular proteins whose activities must occur outside the cytoplasm from diffusing away from the cell. These extracellular proteins reside in the **periplasm**. This space, located between the outer surface of the cytoplasmic membrane and the inner surface of the outer membrane, spans about 15 nm (Figure 2.14*a*, *b*).

The periplasm may contain several different classes of proteins. These include hydrolytic enzymes, which function in the initial degradation of polymeric substances; binding proteins, which begin the process of transporting substrates (Section 3.2); chemoreceptors, which are proteins that govern the chemotaxis response (Section 2.13); and proteins that construct extracellular structures (such as peptidoglycan and the outer membrane) from precursor molecules secreted through the cytoplasmic membrane. Most periplasmic proteins reach the periplasm by way of a protein-exporting system present in the cytoplasmic membrane (Sections 4.12 and 4.13).

The outer membrane is relatively permeable to small molecules because of proteins called *porins* that function as channels for the entrance and exit of solutes (Figure 2.14*a*, *c*). Several porins are known, including both specific and nonspecific classes. Nonspecific porins form water-filled channels through which virtually any very small hydrophilic substance can pass. By contrast, specific porins contain a binding site for one or a group of structurally related substances. Porins are transmembrane proteins composed of three identical polypeptides. Besides the channel present in each subunit of a porin, the porin subunits aggregate in such a way that a hole about 1 nm in diameter is formed through which very small molecules can travel (Figure 2.14*c*).

The signature molecule of *Bacteria*—peptidoglycan—is absent from the *Archaea*. Nevertheless, cells of *Archaea* face the same osmotic stresses as do cells of *Bacteria* and thus need to counter these stresses with a cell wall. We consider the cell walls of *Archaea* now and see that the cell wall chemistries of these fascinating microbes occasionally hint at those of *Bacteria* but often confer their structural strength in chemically unique ways.

MINIQUIZ

- Describe the major chemical components in the outer membrane of gram-negative bacteria.
- What is the function of porins and where are they located in a gram-negative cell wall?
- What component of the gram-negative cell has endotoxin properties?

2.6 Archaeal Cell Walls

A variety of cell wall structures are found in *Archaea*, including walls containing polysaccharides, proteins, or glycoproteins or some mixture of these macromolecules.

Pseudomurein and Other Polysaccharide Cell Walls

The cell walls of certain methane-producing *Archaea* (methanogens) contain a molecule that is remarkably similar to peptidoglycan, a polysaccharide called *pseudomurein* (the term "murein" is from the Latin word for wall and was an old term for peptidoglycan) (Figure 2.15). The backbone of pseudomurein is formed from alternating repeats of *N*-acetylglucosamine (also present in peptidoglycan) and *N*-acetyltalosaminuronic acid; the latter replaces the *N*-acetylmuramic acid of peptidoglycan (Section 2.4). Pseudomurein also differs from peptidoglycan in that the glycosidic bonds between the sugar derivatives are β-1,3 instead of β-1,4, and the amino acids are all of the L stereoisomer (Figure 2.15).

Because in many respects they are so similar, it is likely that peptidoglycan and pseudomurein are variants of a cell wall polysaccharide originally present in the common ancestor of *Bacteria* and *Archaea*. However, although they are structurally very similar, they differ sufficiently that pseudomurein is immune from destruction by both lysozyme and penicillin, molecules that destroy peptidoglycan (Section 2.4).

Cell walls of some other *Archaea* lack pseudomurein and instead contain other polysaccharides. For example, *Methanosarcina* species have thick polysaccharide walls composed of polymers of glucose, glucuronic acid, galactosamine uronic acid, and acetate. Extremely halophilic (salt-loving) *Archaea* such as *Halococcus*, which are related to *Methanosarcina*, have similar cell walls that contain large amounts of sulfate. The negative charges on the sulfate ion (SO_4^{2-}) bind the abundant Na^+ present in the habitats of *Halococcus*

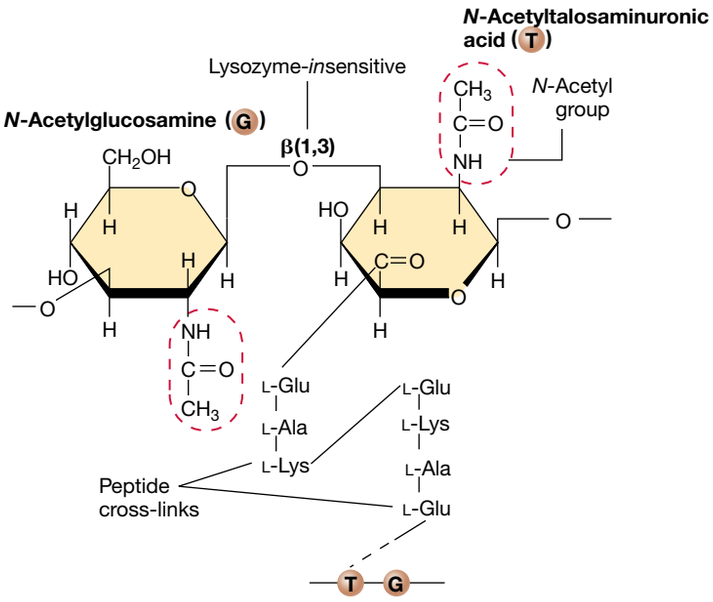


Figure 2.15 Pseudomurein. Structure of pseudomurein, the cell wall polymer of *Methanobacterium* species. Note the similarities and differences between pseudomurein and peptidoglycan (Figures 2.10 and 2.11).

(salt evaporation ponds and saline seas and lakes), and this sulfate–sodium complex helps stabilize the *Halococcus* cell wall.

S-Layers

The most common type of cell wall in *Archaea* is the paracrystalline surface layer, or **S-layer** as it is called. S-layers consist of interlocking molecules of protein or glycoprotein (Figure 2.16). The paracrystalline structure of S-layers can form various symmetries, including hexagonal, tetragonal, or trimeric, depending upon the number and structure of the subunits of which it is composed. S-layers have been found in representatives of all major lineages of *Archaea* and also in some species of *Bacteria* (Figure 2.16).

The cell walls of some *Archaea*, for example the methanogen *Methanocaldococcus jannaschii*, consist only of an S-layer. Thus, S-layers are sufficiently strong to withstand osmotic pressures without any other wall components. However, in many organisms S-layers are present in addition to other cell wall components, usually polysaccharides. When an S-layer accompanies other wall components, the S-layer is always the *outermost* wall layer; that is, the layer that is in direct contact with the environment.

Besides serving as protection from osmotic lysis, S-layers undoubtedly have other functions. For example, as the interface between the cell and its environment, it is likely that the S-layer functions as a selective sieve, allowing the passage of low-molecular-weight solutes while excluding large molecules or structures (such as viruses or lytic enzymes). The S-layer may also function to retain proteins near the cell surface that must function outside the cytoplasmic membrane, much as the outer membrane (Section 2.5) retains periplasmic proteins and prevents their drifting away in gram-negative *Bacteria*.

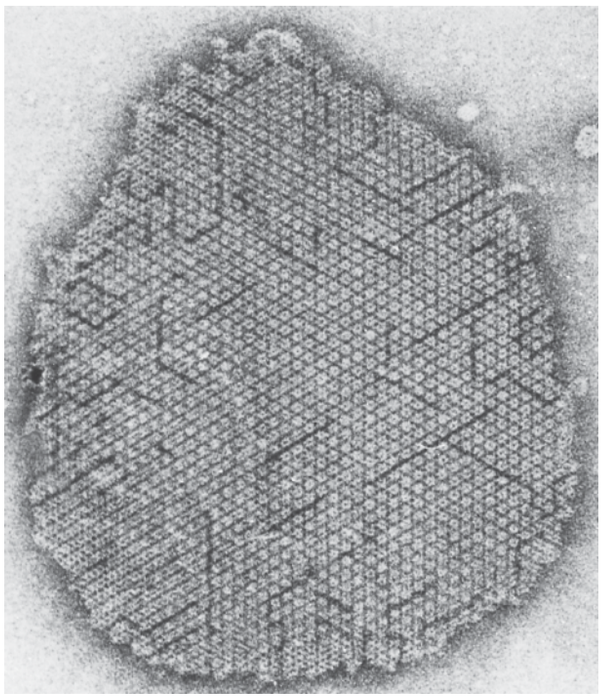


Figure 2.16 The S-layer. Transmission electron micrograph of an S-layer fragment showing its paracrystalline nature. Shown is the S-layer from *Aquaspirillum* (*Bacteria*); this S-layer shows hexagonal symmetry common in S-layers of *Archaea*.

MINIQUIZ

- How does pseudomurein resemble peptidoglycan? How do the two molecules differ?
- What is the structure of an S-layer, and what are its functions?

III • Cell Surface Structures and Inclusions

In addition to the cytoplasmic membrane and cell wall, cells of *Bacteria* and *Archaea* may have other layers or structures in contact with the environment and often contain one or more types of cellular inclusions. We examine some of these here.

2.7 Cell Surface Structures

Many *Bacteria* and *Archaea* secrete sticky or slimy materials on their cell surface that consist of either polysaccharide or protein. However, these are not considered part of the cell wall because they do not confer significant structural strength on the cell. The terms "capsule" and "slime layer" are used to describe these layers.

Capsules and Slime Layers

The terms capsule and slime layer are often used interchangeably, but the two terms do not refer to the same thing. If the layer is organized in a tight matrix that excludes small particles and is tightly attached, it is called a **capsule**. Capsules are readily visible by light microscopy if cells are treated with India ink, which stains the background but not the capsule, and can also be seen in the electron microscope (Figure 2.17*b–d*). By contrast, if the layer is more easily deformed and loosely attached, it will not exclude particles and is more difficult to see microscopically. This form is called a *slime layer* and is easily recognized in colonies of slime-forming species such as the lactic acid bacterium *Leuconostoc* (Figure 2.17*a*).

Outer surface layers have several functions. Surface polysaccharides assist in the attachment of microorganisms to solid surfaces. As we will see later, pathogenic microorganisms that enter the body by specific routes usually do so by first binding specifically to surface components of host tissues; this binding is often facilitated by bacterial cell surface polysaccharides. When the opportunity arises, many bacteria will bind to solid surfaces, often forming a thick layer of cells called a *biofilm*. Extracellular polysaccharides play a key role in the development and maintenance of biofilms as well.

Besides attachment, outer surface layers have other functions. These include acting as virulence factors (molecules that contribute to the pathogenicity of a bacterial pathogen) and preventing dehydration. For example, the causative agent of the diseases anthrax and bacterial pneumonia—*Bacillus anthracis* and *Streptococcus pneumoniae*, respectively—each contain a thick capsule of either protein (*B. anthracis*) or polysaccharide (*S. pneumoniae*). Encapsulated cells of these bacteria avoid destruction by the host's immune system because the immune cells that would otherwise recognize these pathogens as foreign and destroy them are blocked from doing so by the bacterial capsule. In addition to this role in

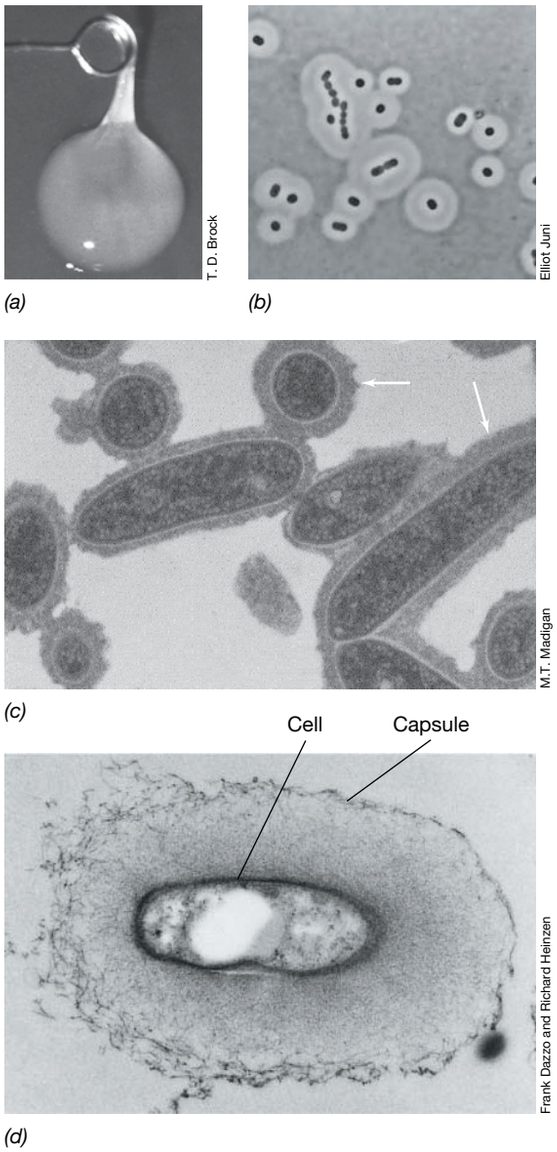


Figure 2.17 Bacterial capsules and slime formation. *(a)* A viscid colony of the bacterium *Leuconostoc mesenteroides* (lifted up by an inoculating loop) contains a thick dextran (glucose polymer) slime layer formed by the cells. *(b)* Capsules of *Acinetobacter* species observed by phase-contrast microscopy after negative staining with India ink. India ink does not penetrate the capsule and so the capsule appears as a light area surrounding the cell, which appears black. *(c)* Transmission electron micrograph of a thin section of cells of *Rhodobacter capsulatus* with capsules (arrows) clearly evident; cells are about 0.9 µm wide. *(d)* Transmission electron micrograph of *Rhizobium trifolii* stained with ruthenium red to reveal the capsule. The cell is about 0.7 µm wide.

disease, outer surface layers of virtually any type bind water and because of this likely protect the cell from desiccation in periods of dryness.

Fimbriae, Pili, and Hami

Fimbriae and pili are thin (2–10 nm in diameter) filamentous structures made of protein that extend from the surface of a cell and can have many functions. *Fimbriae* (Figure 2.18) enable cells to stick to surfaces, including animal tissues in the case of pathogenic bacteria, or to form pellicles (thin sheets of cells on a liquid surface) or biofilms on solid surfaces. **Pili** are similar to fimbriae, but are typically longer and only one or a few pili are present on the surface of a cell. All gram-negative bacteria produce pili of one sort or another, and many gram-positive bacteria also contain these structures. Because pili can be receptors for certain types of viruses, they can be easily seen under the electron microscope when they become coated with virus particles (Figure 2.19).

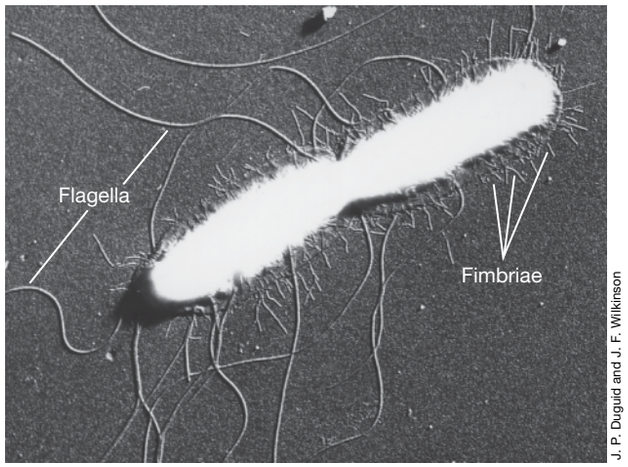


Figure 2.18 Fimbriae. Electron micrograph of a dividing cell of *Salmonella enterica (typhi)*, showing flagella and fimbriae. A single cell is about 0.9 µm wide.

Many classes of pili are known, distinguished by their structure and function. Two very important functions of pili include facilitating genetic exchange between cells in a process called *conjugation* (conjugative or sex pili) and enabling the adhesion of pathogens to specific host tissues that they subsequently invade (type IV and other pili). *Type IV pili* not only facilitate specific adhesion but also support an unusual form of cell movement called *twitching motility* in certain bacterial species. On rod-shaped cells that move by twitching, type IV pili are present only at the poles. Twitching motility is a type of gliding motility, movement along a solid surface (Section 2.12). In twitching motility, extension of pili followed by their retraction drags the cell along a solid surface, and ATP supplies the energy necessary for this movement. The motility of certain species of *Pseudomonas* and *Moraxella* are the best-known examples of twitching motility.

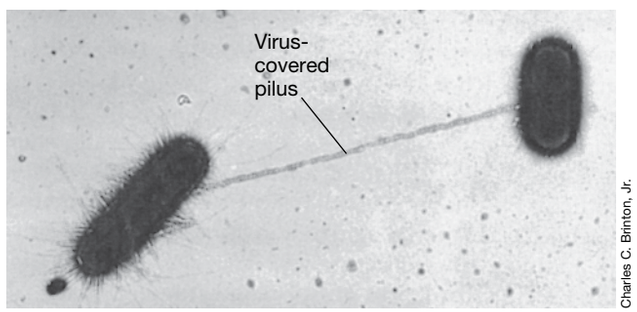


Figure 2.19 Pili. The pilus on an *Escherichia coli* cell that is undergoing conjugation (a form of genetic transfer, Chapter 11) with a second cell is better resolved because viruses have adhered to it. The cells are about 0.8 µm wide.

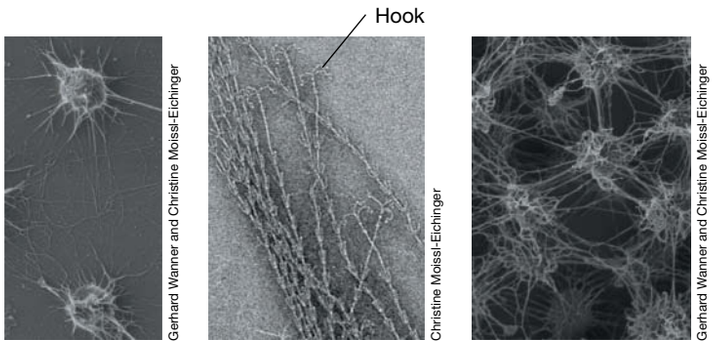


Figure 2.20 Unique attachment structures in the SM1 group of *Archaea*: Hami. *(a)* Cells of SM1 *Archaea* showing the pili-like surface structures called hami. *(b)* Transmission electron micrograph of isolated hami. A hamus "grappling hook" (labeled "Hook" in the micrograph) is about 60 nm in diameter. *(c)* A biofilm of SM1 cells showing the network of hami connecting individual cells.

Type IV pili have also been implicated as key colonization factors for certain human pathogens, including the gram-negative pathogens *Vibrio cholerae* (cholera) and *Neisseria gonorrhoeae* (gonorrhea) and the gram-positive pathogen *Streptococcus pyogenes* (strep throat and scarlet fever). The twitching motility of these organisms assists them in locating specific sites for attachment to initiate the disease process. Type IV pili also mediate genetic transfer by the process of transformation in some bacteria, which, along with conjugation and transduction, are the three known means of horizontal gene transfer in *Bacteria* and *Archaea* (Chapter 11). Type IV pili are also widespread in the *Archaea*, functioning in surface adhesion and cell aggregation events that lead to biofilm formation.

An unusual group of *Archaea*, the SM1 group, forms a unique attachment structure called a *hamus* (plural, hami) that resembles a tiny grappling hook (**Figure 2.20*a, b***). The SM1 group inhabits anoxic groundwater in Earth's deep subsurface, and hami function to affix cells to a surface to form a networked biofilm (Figure 2.20*c*). Hami structurally resemble type IV pili except for their barbed terminus, which functions to attach cells both to surfaces and to each other (Figure 2.20*c*). The biofilms formed by SM1 *Archaea* are likely an ecological strategy that allows these microbes to more efficiently trap the scarce nutrients present in their deep subsurface habitat. Although cells of the SM1 group are not as small as the groundwater ultramicrobacterial cells described earlier (see Explore the Microbial World, "Tiny Cells"), they are less than 1 μm in diameter and live in a similar nutrient-limiting habitat. Thus, their hami probably play an important role in preventing cells from being washed away in groundwater flowage.

MINIQUIZ

- Could a bacterial cell dispense with a cell wall if it had a capsule? Why or why not?
- How do fimbriae differ from pili, both structurally and functionally?
- How can type IV pili facilitate pathogenesis? What are hami?

2.8 Cell Inclusions

Prokaryotic cells often contain inclusions of one sort or another. Inclusions function as energy reserves and/or carbon reservoirs or have special functions. Inclusions can often be seen in cells with the light microscope and are enclosed by a thin membrane that partitions off the inclusion in the cytoplasm. Storing carbon or other substances in an insoluble form is advantageous because it reduces the osmotic stress that the cell would encounter should the same amount be dissolved in the cytoplasm.

Carbon Storage Polymers

One of the most common inclusion bodies in prokaryotic organisms is **poly-β-hydroxybutyric acid (PHB)**, a lipid that is formed from β-hydroxybutyric acid units. The monomers of PHB polymerize by ester linkage and then the polymer aggregates into granules; the granules can be seen by either light or electron microscopy (**Figure 2.21**).

The monomer in the polymer is usually hydroxybutyrate (C_4) but can vary in length from as short as C_3 to as long as C_{18}. Thus,

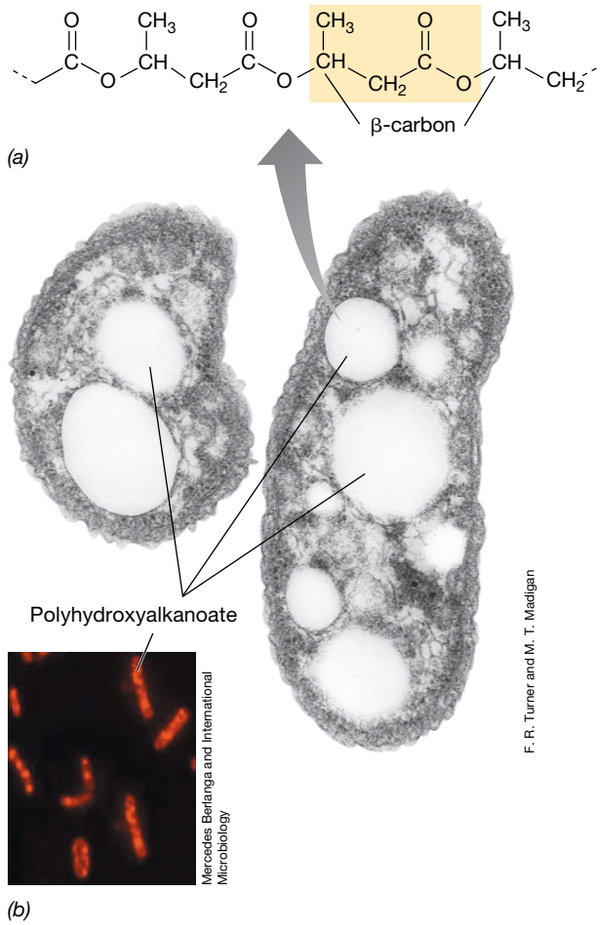


Figure 2.21 Poly-β-hydroxyalkanoates (PHAs). *(a)* Chemistry of poly-β-hydroxybutyrate, a common PHA. A monomeric unit is shown in color. Other PHAs are made by substituting longer-chain hydrocarbons for the $-CH_3$ group on the β-carbon. *(b)* Electron micrograph of a thin section of cells of a bacterium containing granules of PHB. Color photo: Nile red–stained cells of a PHA-containing bacterium.

the more generic term poly-β-hydroxyalkanoate (PHA) is often used to describe this class of carbon- and energy-storage polymers. PHAs are synthesized by cells when there is an excess of carbon and are broken down as carbon or energy sources when conditions warrant. Many *Bacteria* produce PHAs, as do several extremely halophilic species of *Archaea*.

Another storage inclusion is *glycogen*, which is a polymer of glucose; like PHA, glycogen is a reservoir of both carbon and energy and is produced when carbon is in excess. Glycogen resembles starch, the major storage reserve of plants, but differs slightly from starch in the manner in which the glucose units are linked together.

Polyphosphate, Sulfur, and Carbonate Minerals

Many prokaryotic and eukaryotic microbes accumulate inorganic phosphate (PO_4^{3-}) in the form of *polyphosphate* granules (**Figure 2.22*a***). These granules are formed when phosphate is in excess and can be drawn upon as a source of phosphate for nucleic acid and phospholipid biosynthesis when phosphate is limiting. In addition, in some organisms, polyphosphate can be broken down to synthesize the energy-rich compound ATP from ADP.

Many gram-negative *Bacteria* and *Archaea* oxidize reduced sulfur compounds, such as hydrogen sulfide (H_2S); these organisms are the "sulfur bacteria," discovered by the great Russian microbiologist Sergei Winogradsky (Section 1.11). The oxidation of sulfide generates electrons for use in energy metabolism (chemolithotrophy) or CO_2 fixation (autotrophy). In either case, *elemental sulfur* (S^0) from the oxidation of sulfide may accumulate in the cell in microscopically visible granules (Figure 2.22*b*). This sulfur remains as long as the source of reduced sulfur from which it was derived is still present. However, as the reduced sulfur source becomes limiting, the S^0 in the granules is oxidized to sulfate (SO_4^{2-}), and the granules slowly disappear. Interestingly, although sulfur globules appear to reside in the cytoplasm (Figure 2.22*b*), they are actually present in the periplasm (Section 2.5). In these cells the periplasm expands outward to accommodate the growing globules as H_2S is oxidized to S^0 and then contracts inward as S^0 is oxidized to SO_4^{2-} (Section 14.9).

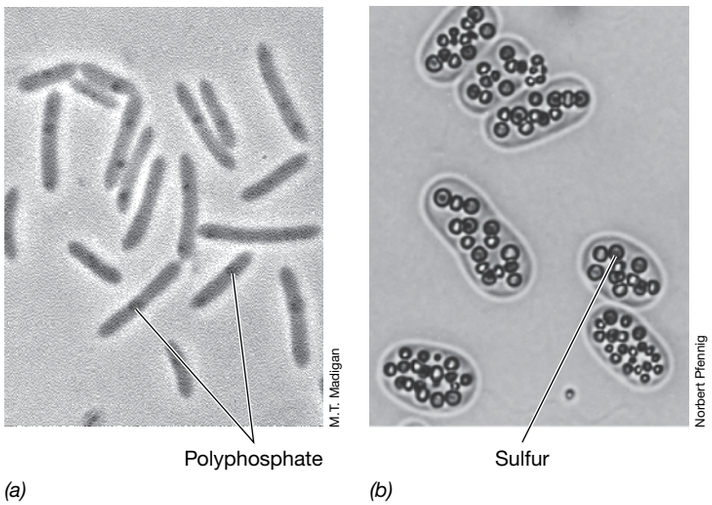


Figure 2.22 Polyphosphate and sulfur storage products. *(a)* Phase-contrast photomicrograph of cells of *Heliobacterium modesticaldum* showing polyphosphate as dark granules; a cell is about 1 µm wide. *(b)* Bright-field photomicrograph of cells of the purple sulfur bacterium *Isochromatium buderi*. The periplasmic inclusions are sulfur globules formed from the oxidation of hydrogen sulfide (H_2S). A cell is about 4 µm wide.

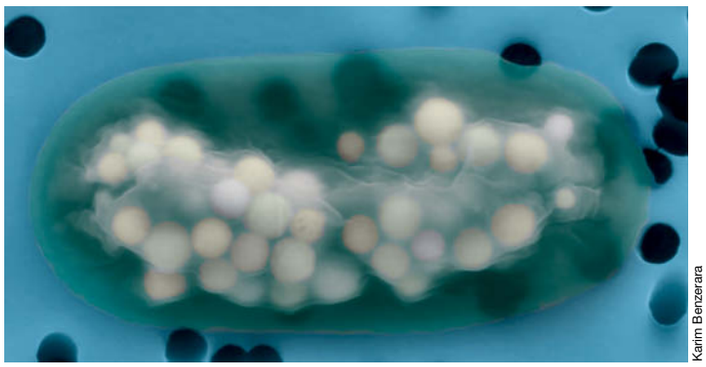


Figure 2.23 Biomineralization by a cyanobacterium. Electron micrograph of a cell of the cyanobacterium *Gloeomargarita* containing granules of the mineral benstonite [$(Ba,Sr)_6(Ca,Mn)_6Mg(CO_3)_{13}$]. A cell is about 2 µm wide.

Filamentous cyanobacteria have long been known to form carbonate minerals on the external surface of their cells. However, some cyanobacteria also form carbonate minerals *inside* the cell, as cell inclusions. For example, the unicellular cyanobacterium *Gloeomargarita* forms intracellular granules of benstonite, a carbonate mineral that contains barium, strontium, and magnesium (**Figure 2.23**). The microbiological process of forming minerals is called *biomineralization*. It is unclear why benstonite is formed by *Gloeomargarita*, although it might function as ballast to maintain cells of this cyanobacterium in their habitat, deep in an alkaline lake. Alternatively (or in addition), the mineral could be a way to sequester carbonate (a source of CO_2) to support autotrophic growth. The biomineralization of several different minerals is catalyzed by various bacteria, but only in the case of *Gloeomargarita* and magnetosomes (to be discussed next) do we see the process yield actual intracellular inclusions.

Magnetic Storage Inclusions: Magnetosomes

Some bacteria can orient themselves within a magnetic field because they contain **magnetosomes**. These structures are biomineralized particles of the magnetic iron oxides magnetite [$Fe(II)Fe(III)_2O_4$] or greigite [$Fe(II)Fe(III)_2S_4$] (**Figure 2.24**). Magnetosomes impart a magnetic dipole on a cell, allowing it to orient itself in a magnetic field. This allows the cell to undergo *magnetotaxis*, the process of migrating along Earth's magnetic field lines. Magnetosomes have been found in several aquatic organisms that grow best at low O_2 concentrations or are anaerobic. It has thus been hypothesized that one function of magnetosomes may be to guide these aquatic cells downward (the direction of Earth's magnetic field) toward the sediments where O_2 is low or absent.

Magnetosome synthesis begins with insertion of magnetosome-specific proteins into the cytoplasmic membrane followed by invagination of the membrane to form a vesicle. The vesicle is then filled with iron—primarily iron in the Fe(II) oxidation state—and biomineralization proceeds through the

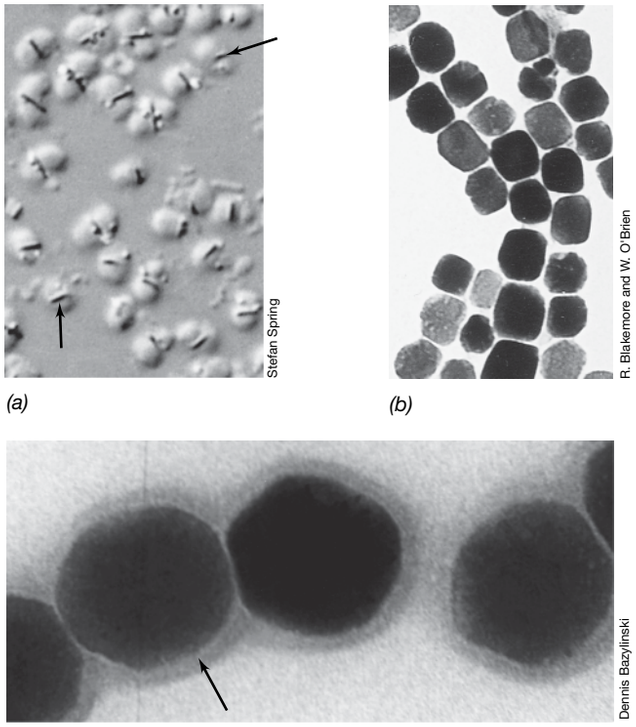


Figure 2.24 Magnetotactic bacteria and magnetosomes. *(a)* Differential interference contrast micrograph of coccoid magnetotactic bacteria; note chains of magnetosomes (arrows). A cell is 2.2 µm wide. *(b)* Magnetosomes isolated from the magnetotactic bacterium *Magnetospirillum magnetotacticum*; each particle is about 50 nm wide. *(c)* Transmission electron micrograph of magnetosomes from an unnamed magnetic coccus. The arrow points to the membrane that surrounds each magnetosome. A single magnetosome is about 90 nm wide.

activities of the magnetosome proteins, which includes an iron oxidase that generates the Fe(III) needed to form the magnetic minerals. The morphology of magnetosomes varies and appears to be species-specific; several morphologies are possible, but square, rectangular, or spike-shaped magnetosomes are most common.

MINIQUIZ

- Under what nutritional conditions would you expect PHAs or glycogen to be produced?
- Why would it be impossible for gram-positive bacteria to store sulfur as gram-negative sulfur-oxidizing chemolithotrophs can?
- How are magnetosomes and the *Gloeomargarita* inclusions similar and how do they differ?

2.9 Gas Vesicles

Some *Bacteria* and *Archaea* are *planktonic*, meaning that they inhabit the water column of lakes and the oceans. Most planktonic organisms move up and down with changes in currents, but some can float because they contain **gas vesicles**, structures that confer buoyancy and allow the cells to position themselves in regions of the water column that best suit their metabolisms.

The most dramatic examples of gas-vesiculate microbes are cyanobacteria that form massive accumulations called *blooms* in lakes or other bodies of water (**Figure 2.25*a***). Cyanobacteria are oxygenic phototrophic bacteria (Section 14.4). Gas-vesiculate cells rise to the surface of the lake and are blown by winds into dense masses. Several other primarily aquatic *Bacteria* and *Archaea* have gas vesicles, but the structures are not found in microbial eukaryotes.

Gas Vesicle Structure

Gas vesicles are conical-shaped structures made of protein; they are hollow yet rigid and of variable length and diameter (Figure 2.25*b*, *c* and see Figure 2.26*a*). Gas vesicles in different species vary in length from about 300 to more than 1000 nm and in width from 45 to 120 nm, but the vesicles of a given species are of constant size. Gas vesicles may number from a few to hundreds per cell and are impermeable to water and solutes but permeable to gases. The presence of gas vesicles in cells can be detected

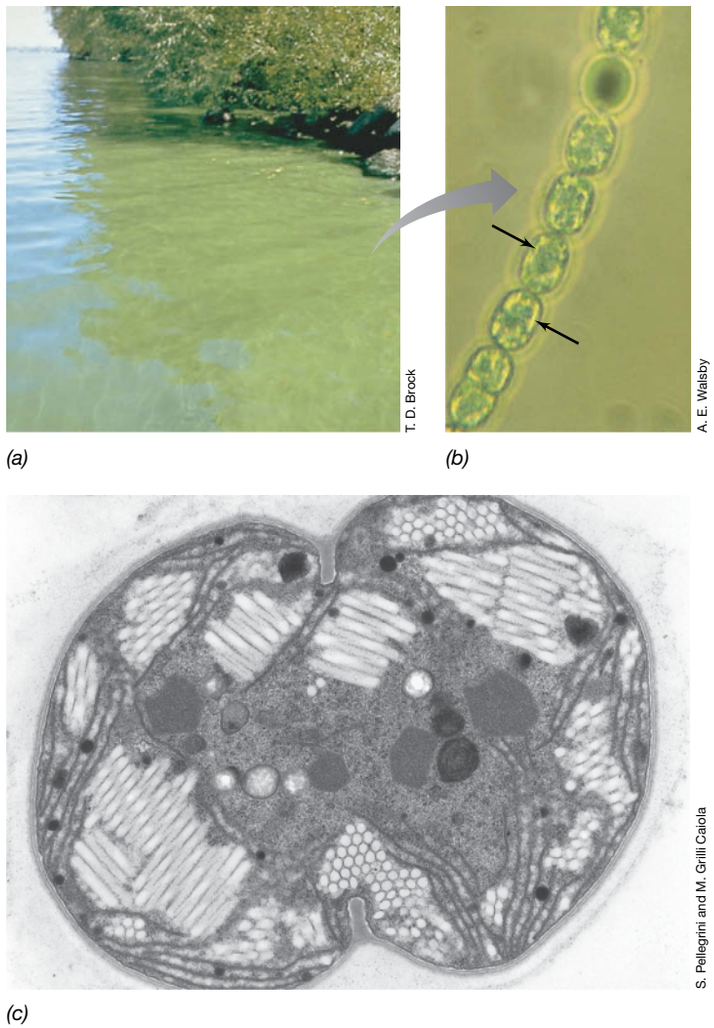


Figure 2.25 Buoyant cyanobacteria and their gas vesicles. *(a)* Flotation of a bloom of gas-vesiculate cyanobacteria in a freshwater lake. *(b)* Phase-contrast photomicrograph of *Anabaena*. Clusters of gas vesicles form phase-bright gas vacuoles (arrows). *(c)* Transmission electron micrograph of *Microcystis*. Gas vesicles are arranged in bundles, here seen in both longitudinal and cross-section. Both cells are about 5 µm wide.

either by light microscopy, where clusters of vesicles, called *gas vacuoles*, appear as irregular bright inclusions (Figure 2.25*b*), or by transmission electron microscopy of cell thin sections (Figure 2.25*c*).

Gas vesicles are composed of two distinct proteins (**Figure 2.26*b***). The major gas vesicle protein, called *GvpA*, forms the watertight vesicle shell and is a small, hydrophobic, and very rigid protein; multiple copies of GvpA align to form the parallel "ribs" of the vesicle. The rigidity is essential for the structure to resist the pressures exerted on it from outside. A minor protein, called *GvpC*, functions to strengthen the shell of the gas vesicle by cross-linking and binding the ribs at an angle to group several GvpA molecules together (Figure 2.26*b*).

The composition and pressure of the gas inside a gas vesicle is that in which the organism is suspended. Because an inflated gas vesicle has a density only one-tenth that of the cell proper, inflated gas vesicles combine to decrease a cell's density and thereby increase its buoyancy. If and when vesicles are collapsed, buoyancy is lost. Phototrophic bacteria (Chapter 14) in particular can benefit from gas vesicles because they allow cells to adjust their vertical position in a water column to sink or rise to regions where conditions (for example, light intensity) are optimal for photosynthesis.

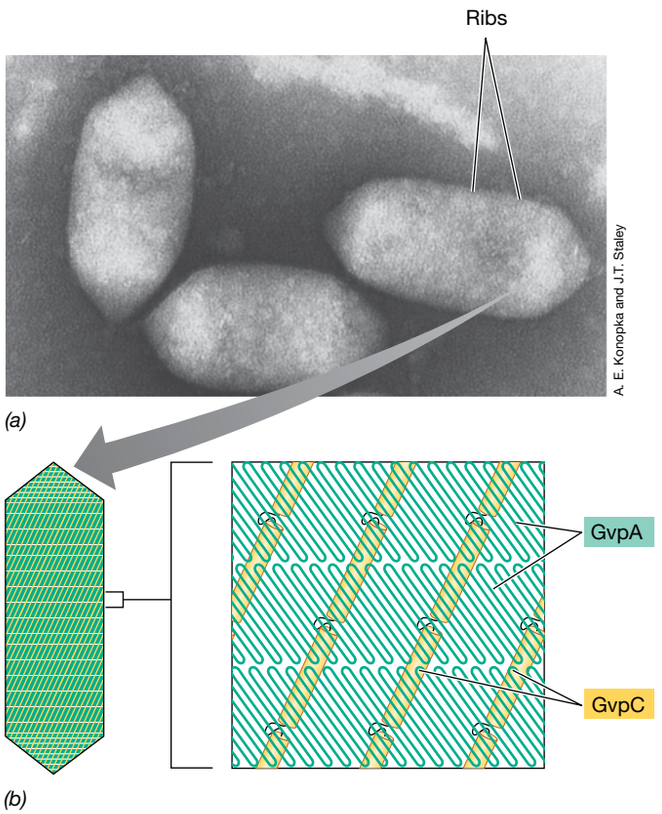


Figure 2.26 Gas vesicle architecture. *(a)* Transmission electron micrograph of gas vesicles purified from the bacterium *Ancylobacter aquaticus* and examined in negatively stained preparations. A single vesicle is about 100 nm in diameter. *(b)* Model of how gas vesicle proteins GvpA and GvpC interact to form a watertight but gas-permeable structure. GvpA, a rigid β-sheet, makes up the rib, and GvpC, an α-helix structure, is the cross-link. (See Figure 4.30*b*, *c* for the structures of a β-sheet and an α-helix.)

MINIQUIZ

- What gas is present in a gas vesicle? Why might a photosynthetic cell benefit from controlling its buoyancy?
- How are the two proteins that make up the gas vesicle, GvpA and GvpC, arranged to form such a water-impermeable structure?

2.10 Endospores

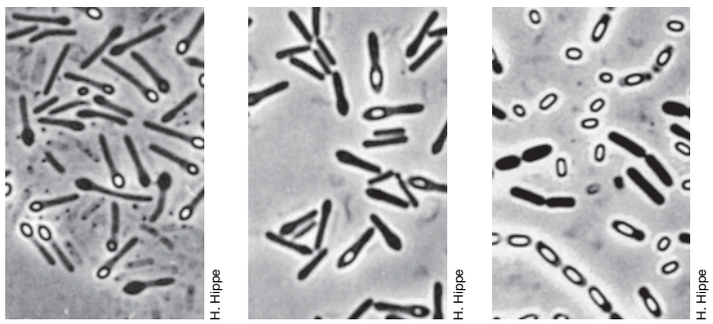
Certain species of *Bacteria* produce structures called **endospores** (**Figure 2.27**) during a process called *endosporulation* (or just *sporulation* for short). Endospores (the prefix *endo-* means "within") are highly differentiated cells that are extremely resistant to heat, harsh chemicals, and radiation. Endospores function as survival structures and enable the organism to endure unfavorable growth conditions, including but not limited to extremes of temperature, drying, or nutrient depletion. Endospores can thus be thought of as the dormant stage of a bacterial life cycle: vegetative cell → endospore → vegetative cell. Endospores are easily dispersed by wind, water, or through the animal gut, and hence endospore-forming bacteria are widely distributed in nature.

The endospore-forming bacteria *Bacillus* and *Clostridium* are common in soil and the best-studied representatives. Some endospore-forming bacteria are serious pathogens of humans and other animals, the endospore stage being an effective way of surviving outside the host or until conditions within the host can support disease. Botulism, tetanus, and several foodborne bacterial infections are caused by species of endospore-forming bacteria.

Endospore Formation and Germination

During endospore formation, a vegetative cell is converted into a nongrowing, heat-resistant, and light-refractive structure (**Figure 2.28**). Cells do not sporulate when they are actively growing but only when growth ceases owing to the exhaustion of an essential nutrient. Thus, cells of *Bacillus*, a typical endospore-forming bacterium, cease vegetative growth and begin sporulation when, for example, a key nutrient such as carbon or nitrogen becomes limiting (Section 7.6).

An endospore can remain dormant for years but can convert back to a vegetative cell rapidly. This process occurs in three



(a) **Terminal endospores** *(b)* **Subterminal endospores** *(c)* **Central endospores**

Figure 2.27 The bacterial endospore. Phase-contrast photomicrographs showing different intracellular locations of endospores in different species of bacteria. Endospores appear bright by phase-contrast microscopy.

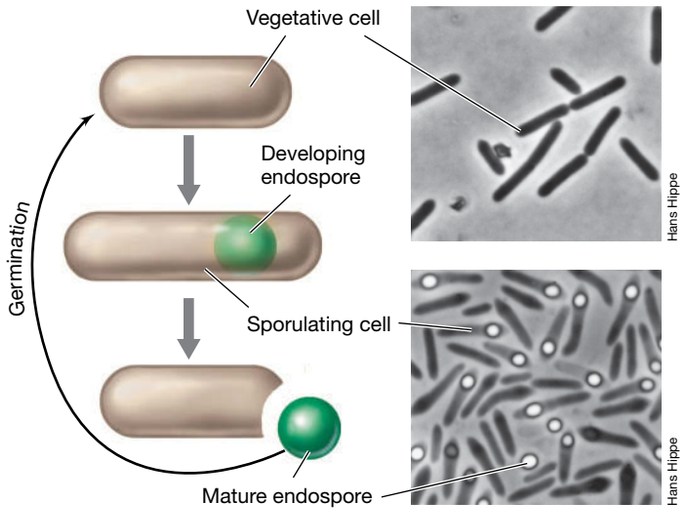


Figure 2.28 The life cycle of an endospore-forming bacterium. The phase-contrast photomicrographs are of cells of *Clostridium pascui*. A cell is about 0.8 µm wide.

steps: *activation, germination*, and *outgrowth* (Figure 2.29). Activation occurs when endospores are heated for several minutes at an elevated but sublethal temperature. Activated endospores are then conditioned to germinate when supplied with certain nutrients, such as certain amino acids. Germination, typically a rapid process (occurring in a matter of minutes), is signaled by the loss of refractility of the endospore (Figure 2.29*b*) and loss of resistance to heat and chemicals. The final stage, outgrowth (Figure 2.29*c, d*), involves visible swelling due to water uptake and synthesis of RNA, proteins, and DNA. The vegetative cell emerges from the broken endospore and begins to grow, remaining in vegetative growth until environmental signals once again trigger sporulation.

Endospore Structure and Features

Endospores are visible by light microscopy as strongly refractile structures (Figures 2.27 and 2.29*a*). Endospores are impermeable to most dyes, so occasionally they are seen as unstained regions within cells that have been stained with basic dyes such as methylene blue. To stain endospores, special stains and procedures must be used. In the classical endospore-staining protocol, the stain malachite green is used and is infused into the spore with steam.

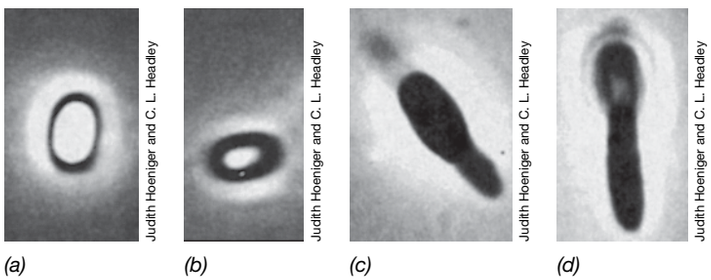


Figure 2.29 Endospore germination in *Bacillus*. Conversion of an endospore into a vegetative cell. The series of phase-contrast photomicrographs shows the sequence of events starting from *(a)* a highly refractile free endospore. *(b)* Activation: Refractility is diminishing. *(c, d)* Outgrowth: The new vegetative cell is emerging.

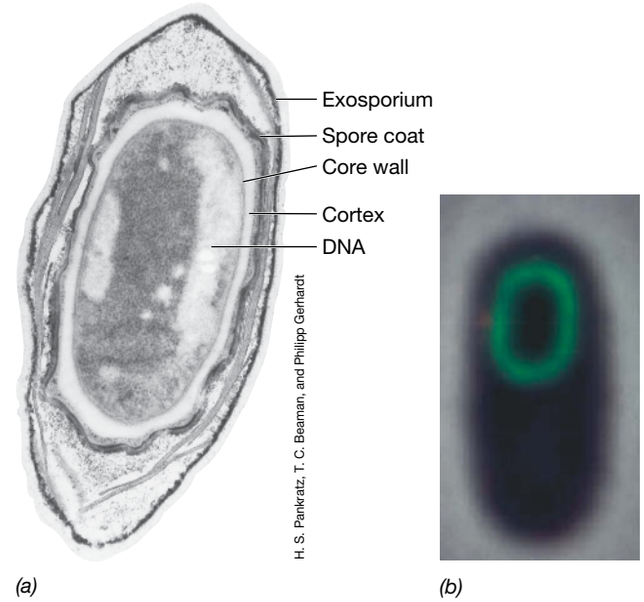


Figure 2.30 Structure of the bacterial endospore. *(a)* Transmission electron micrograph of a thin section through an endospore of *Bacillus megaterium*. *(b)* Fluorescent photomicrograph of a cell of *Bacillus subtilis* undergoing sporulation. The green color is a dye that specifically stains a sporulation protein in the spore coat.

The structure of the endospore as seen with the electron microscope differs distinctly from that of the vegetative cell (Figure 2.30). The endospore contains many layers absent from the vegetative cell. The outermost layer is the *exosporium*, a thin protein covering. Moving inward there are several *spore coats*, composed of layers of spore-specific proteins (Figure 2.30*b*). Below the spore coat is the *cortex*, which consists of loosely cross-linked peptidoglycan, and inside the cortex is the *core*, which contains the core wall, cytoplasmic membrane, cytoplasm, nucleoid, ribosomes, and other cellular essentials. Thus, the endospore differs structurally from the vegetative cell primarily in the kinds of structures found outside the core wall.

One substance found in endospores but not in vegetative cells is **dipicolinic acid** (Figure 2.31*a*), which accumulates in the core. Endospores also contain large amounts of calcium (Ca^{2+}), most of which is complexed with dipicolinic acid (Figure 2.31*b*). The calcium–dipicolinic acid (DPA) complex forms about 10% of the dry weight of the endospore and functions to bind free water within the endospore, helping to dehydrate the developing

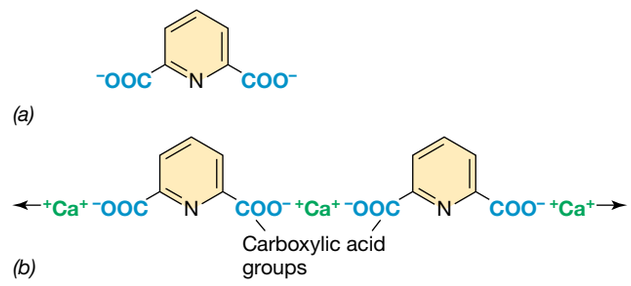


Figure 2.31 Dipicolinic acid (DPA). *(a)* Structure of DPA. *(b)* How Ca^{2+} cross-links DPA molecules to form a complex.

endospore. In addition, the DPA complex inserts between bases in DNA, which helps stabilize DNA against heat denaturation.

The core of the endospore differs significantly from the cytoplasm of the vegetative cell that produced it. The endospore core contains less than one quarter of the water found in the vegetative cell, and thus the consistency of the core cytoplasm is that of a gel. Dehydration of the core greatly increases the heat resistance of macromolecules within the spore. Some bacterial endospores survive heating to temperatures as high as 150°C, although 121°C, the standard for microbiological sterilization (121°C is autoclave temperature, Section 5.15), kills the endospores of most species. Dehydration has also been shown to provide endospores with resistance to toxic chemicals, such as hydrogen peroxide (H_2O_2), and causes enzymes in the core to become inactive. In addition to the low water content of the endospore, the pH of the core is about one unit lower than that of the vegetative cell cytoplasm.

The endospore core contains high levels of *small acid-soluble spore proteins* (SASPs). These proteins are only made during the sporulation process and have at least two functions. SASPs bind tightly to DNA in the core and protect it from potential damage from ultraviolet radiation, desiccation, and dry heat. Ultraviolet resistance is conferred when SASPs alter the molecular structure of DNA from the normal "B" form to the (more compact) "A" form. A-form DNA better resists pyrimidine dimer formation by UV radiation, which can cause mutations (Section 11.4), and resists the denaturing effects of dry heat. In addition, SASPs function as a carbon and energy source for the outgrowth of a new vegetative cell from the endospore during germination.

TABLE 2.2 Differences between endospores and vegetative cells

Characteristic	*Vegetative cell*	*Endospore*
Microscopic appearance	Nonrefractile	Refractile
Calcium content	Low	High
Dipicolinic acid	Absent	Present
Enzymatic activity	High	Low
Respiration rate	High	Low or absent
Macromolecular synthesis	Present	Absent
Heat resistance	Low	High
Radiation resistance	Low	High
Resistance to chemicals	Low	High
Lysozyme	Sensitive	Resistant
Water content	High, 80–90%	Low, 10–25% in core
Small acid-soluble spore proteins	Absent	Present

The Sporulation Cycle

Sporulation is a form of cellular differentiation (Figure 1.4), and many genetically directed changes in the cell occur during the conversion from vegetative growth to sporulation (Table 2.2). The structural changes in sporulating cells of *Bacillus* are shown in Figure 2.32. Sporulation can be divided into several stages. In

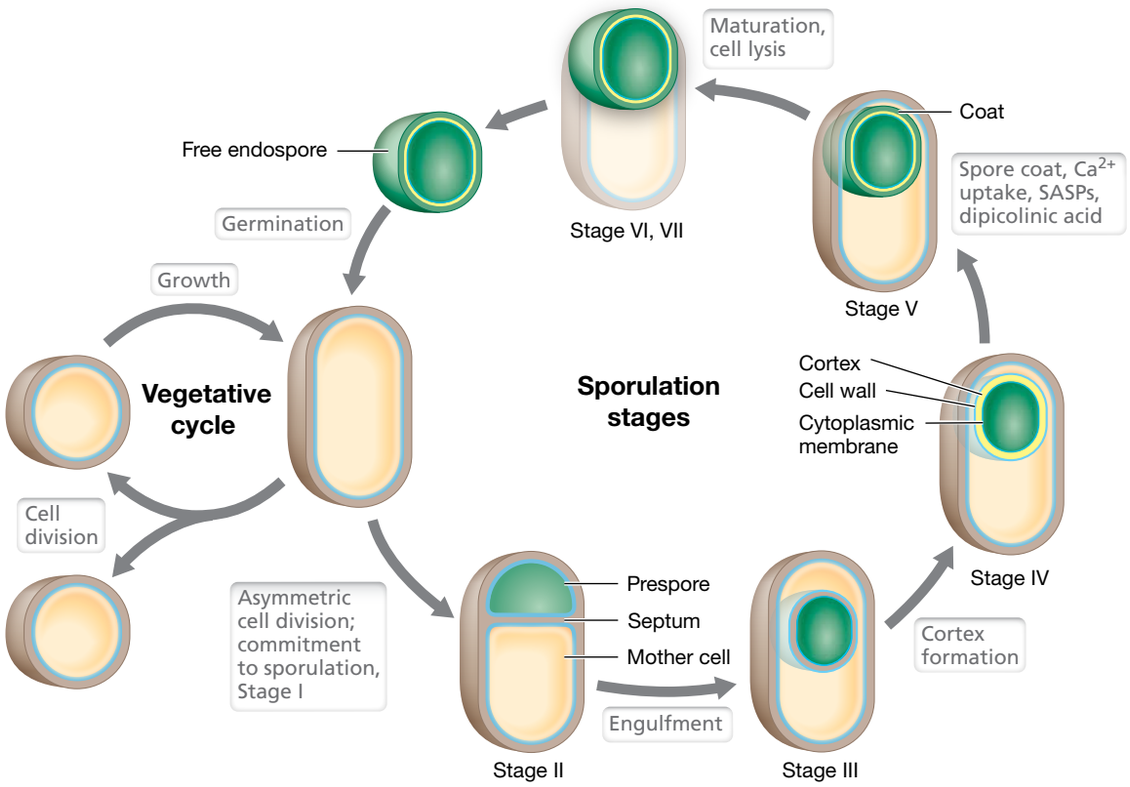


Figure 2.32 Stages in endospore formation. The stages are defined from genetic and microscopic analyses of sporulation in *Bacillus subtilis*, the model organism for studies of sporulation. SASPs, small acid-soluble proteins.

Bacillus subtilis, which has been studied in detail, the conversion of a vegetative cell into an endospore takes about 8 hours and begins with asymmetric cell division (Figure 2.32). Note how key events such as asymmetric cell division, cortex formation, and SASP production take place in a defined sequence and at specific times in the sporulation cycle (Figure 2.32). Genetic studies of mutants of *Bacillus subtilis*, each blocked at one of the stages of endosporulation, indicate that more than 200 spore-specific genes exist.

Endosporulation requires differential protein synthesis. This occurs by the sequential activation of several families of endospore-specific genes and the turning off of many vegetative cell functions. The proteins encoded by sporulation-specific genes catalyze the series of events leading from the moist, metabolizing, vegetative cell to the relatively dry, metabolically inert, but extremely resistant endospore (Table 2.2). In Section 7.6 we examine some of the molecular events that take place during the endosporulation process.

Diversity and Phylogenetic Aspects of Endospore Formation

Nearly 20 genera of *Bacteria* form endospores, although the process has only been studied in detail in a few species. Nevertheless, most of the major events described here, such as the formation of DPA complexes and the production of endospore-specific SASPs, seem universal. From a phylogenetic perspective, the capacity to produce endospores is limited to a particular lineage of the gram-positive bacteria. Despite this, the physiologies of endospore-forming bacteria are highly diverse and include anaerobes, aerobes, phototrophs, and chemolithotrophs. In light of this physiological diversity, the actual triggers for endospore formation may vary with different species and could include signals other than simple nutrient starvation, the major trigger for endospore formation in *Bacillus*. No *Archaea* have been shown to form endospores, suggesting that the capacity to produce endospores evolved sometime after *Bacteria* and *Archaea* diverged about 3.5 billion years ago.

MINIQUIZ

- What is dipicolinic acid and the DPA complex, and where is it found?
- What are SASPs and what is their function?
- What is formed when an endospore germinates?

IV • Cell Locomotion

We finish our survey of prokaryotic structure and function by examining cell locomotion. Many microbial cells can move under their own power. Motility allows cells to reach different parts of their environment, and in nature, a new location may offer additional resources for a cell and spell the difference between life and death.

We examine here the two major types of prokaryotic cell movement, *swimming* and *gliding*. We then consider how motile cells are able to move in a directed fashion toward or away from particular stimuli (phenomena called *taxes*) and present examples of these simple behavioral responses.

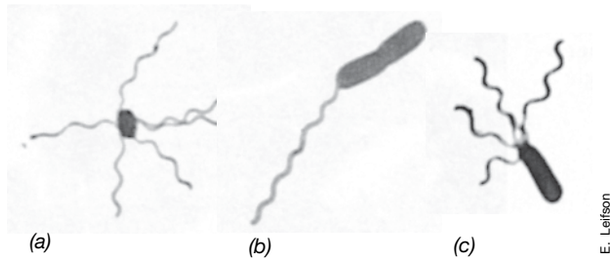


Figure 2.33 Bacterial flagella. Classic light photomicrographs taken by Einar Leifson of bacteria containing different arrangements of flagella. Cells are stained with the Leifson flagella stain. *(a)* Peritrichous. *(b)* Polar. *(c)* Lophotrichous.

2.11 Flagella, Archaella, and Swimming Motility

Many *Bacteria* are motile by swimming due to a structure called the **flagellum** (plural, flagella) (**Figure 2.33**); an analogous structure called the **archaellum** is present in many *Archaea*. Flagella and archaella are tiny rotating machines that function to push or pull the cell through a liquid.

Flagella and Flagellation

Bacterial flagella are long, thin appendages (15–20 nm wide, depending on the species) free at one end and anchored into the

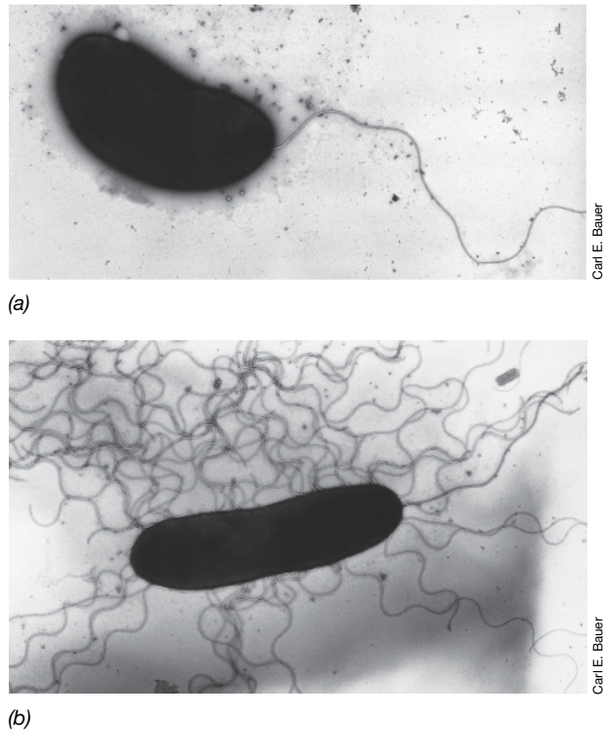


Figure 2.34 Bacterial flagella as observed by negative staining in the transmission electron microscope. *(a)* A single polar flagellum. *(b)* Peritrichous flagella. Both micrographs are of cells of the phototrophic bacterium *Rhodospirillum centenum*, which are about 1.5 µm wide. Cells of *R. centenum* are normally polarly flagellated but under certain growth conditions form peritrichous flagella. See Figure 2.44*b* for a photo of colonies of *R. centenum* cells that move toward an increasing gradient of light (phototaxis).

cell at the other end. Flagella can be stained and observed by light microscopy (Figure 2.33) or electron microscopy (Figure 2.34).

Flagella can be anchored to a cell in different locations. In **polar flagellation**, the flagella are attached at one or both ends of a cell (Figure 2.33*b*). Occasionally a group of flagella (called a *tuft*) may arise at one end of the cell, a type of polar flagellation called *lophotrichous* (Figure 2.33*c*). Tufts of flagella can sometimes be seen in large unstained cells by dark-field or phase-contrast microscopy (Figure 2.35). When a tuft of flagella emerges from both poles of the cell, flagellation is called *amphitrichous*. In **peritrichous flagellation** (Figures 2.33*a* and 2.34*b*), flagella are inserted around the cell surface.

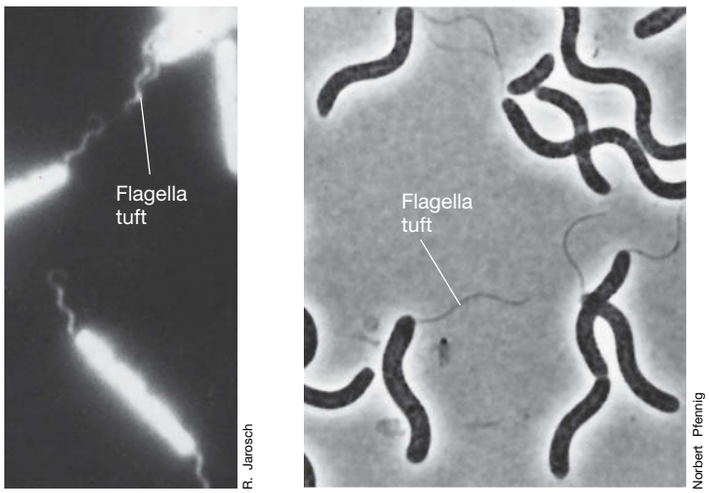
Flagella do not rotate at a constant speed but increase or decrease their rotational speed in relation to the strength of the proton motive force. Flagella can rotate at up to 1000 revolutions per second to support a swimming speed of up to 60 cell-lengths/sec. The fastest known land animal, the cheetah, can move at about 25 body-lengths/sec. Thus, a bacterium swimming at 60 cell-lengths/sec is actually moving over twice as fast—relative to its size—as the fastest animal!

The swimming motions of polarly and lophotrichously flagellated organisms differ from those of peritrichously flagellated organisms, and these can be distinguished microscopically (Figure 2.36). Peritrichously flagellated organisms typically move slowly in a straight line. By contrast, polarly flagellated organisms move more rapidly, often spinning around and seemingly dashing from place to place. The different behavior of flagella on polar and peritrichous organisms, including differences in reversibility of the flagellum, is illustrated in Figure 2.36.

Flagella Structure and Activity

Flagella are not straight structures but are helical. The main part of the flagellum, called the *filament*, is composed of many copies of a protein called *flagellin*. The amino acid sequence of flagellin is highly conserved in *Bacteria*, suggesting that flagellar motility evolved early and has deep roots within this domain. In addition to the filament, a flagellum consists of several other components. A wider region at the base of the filament called the *hook* consists of a single type of protein and connects the filament to the flagellum motor in the base (Figure 2.37).

The flagellum motor is a reversible rotating machine composed of several proteins and is anchored in the cytoplasmic membrane and cell wall. The motor consists of a central rod that passes through a series of rings. In gram-negative bacteria, an outer ring, called the *L ring*, is anchored in the outer membrane (Section 2.5). A second ring, called the *P ring*, is anchored in the peptidoglycan layer. A third set of rings, called the *MS* and *C rings*, are located within the cytoplasmic membrane and the cytoplasm, respectively (Figure 2.37*a*). In gram-positive bacteria, which lack an outer membrane, only the inner pair of rings is present. Surrounding the inner ring and anchored in the cytoplasmic membrane and peptidoglycan are a series of proteins called *Mot proteins*. Another set of proteins, called *Fli proteins* (Figure 2.37*a*), function as the motor switch, reversing the direction of rotation of the flagella in response to intracellular signals.



(a) (b)

Figure 2.35 Bacterial flagella observed in living cells. *(a)* Dark-field photomicrograph of a group of large rod-shaped bacteria with flagellar tufts at each pole (a condition called *amphitrichous flagellation*). A single cell is about 2 µm wide. *(b)* Phase-contrast photomicrograph of cells of the large phototrophic purple bacterium *Rhodospirillum photometricum* with a tuft of lophotrichous flagella that emanate from one of the poles. A cell measures about 4 × 25 µm.

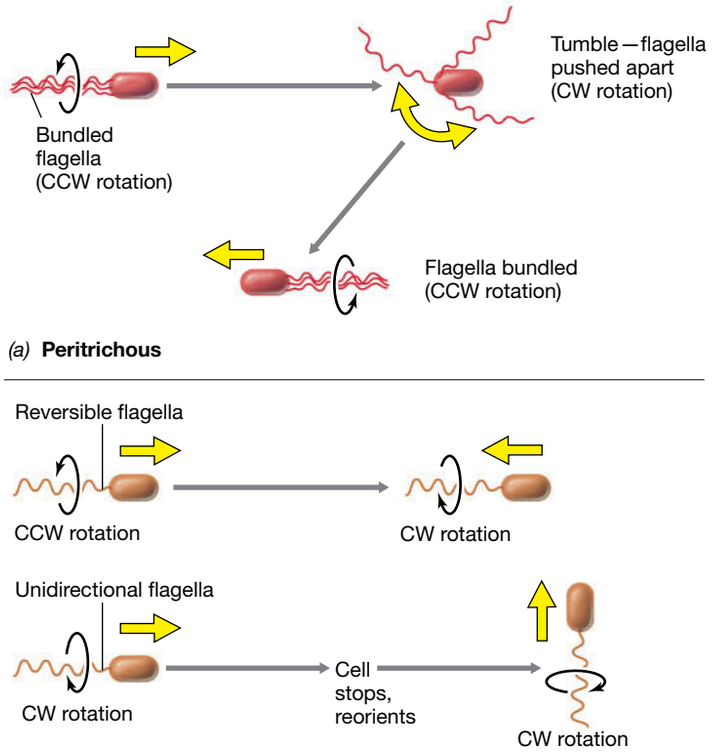
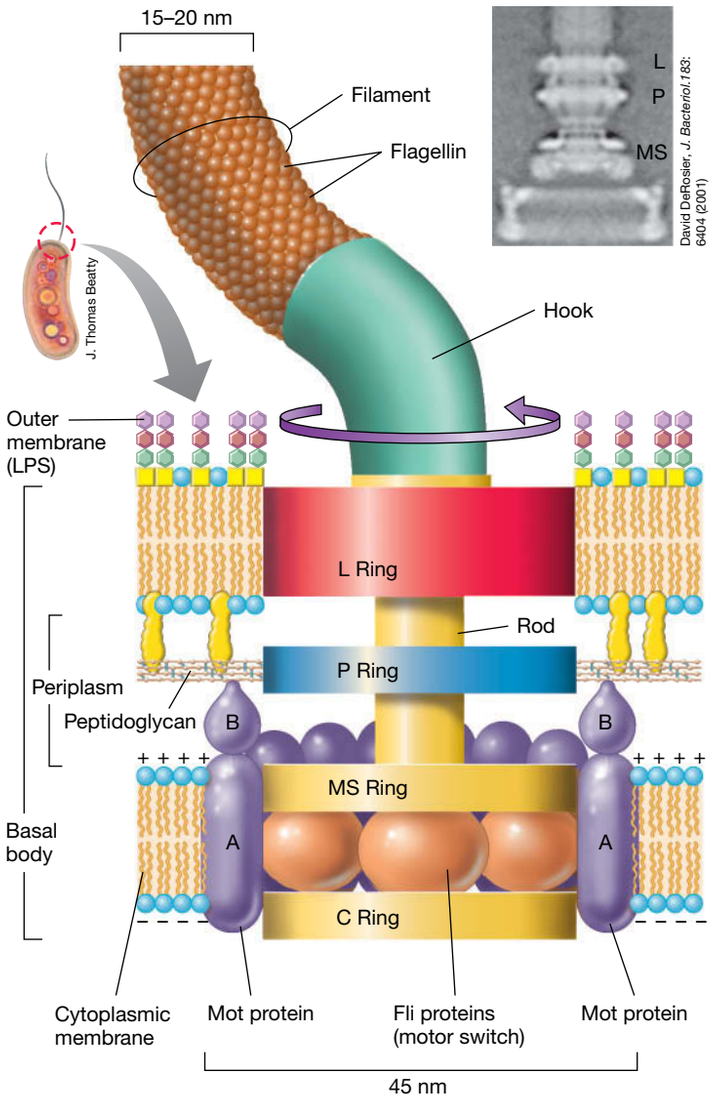


Figure 2.36 Movement in peritrichously and polarly flagellated prokaryotic cells. *(a)* Peritrichous: Forward motion is imparted by all flagella forming into a bundle and rotating counterclockwise (CCW). Clockwise (CW) rotation causes the bundle to break apart and the cell to tumble. A return to counterclockwise rotation leads the cell off in a new direction. *(b)* Polar: Cells change direction by reversing flagellar rotation (thus pulling instead of pushing the cell) or, with unidirectional flagella, by stopping periodically to reorient and then moving forward by clockwise rotation of its flagella. The yellow arrows show the direction the cell is traveling.



The flagellum motor contains two main components: the *rotor* and the *stator*. The rotor consists of the central rod and the L, P, C, and MS rings. Collectively, these structures make up the flagellar **basal body** (Figure 2.37). The stator consists of the Mot proteins that surround the rotor and function to generate torque. Rotation of the flagellum occurs at the expense of the proton motive force (Section 2.3), and it is thought that rotation is imparted to the flagellum by a type of "proton turbine" process. In this model, proton translocation through the Mot complex drives rotation of the flagellum, with about 1200 protons being translocated per each rotation of the flagellum (Figure 2.37*b*). Protons flowing through the Mot proteins exert electrostatic forces on helically arranged charges on the rotor proteins. Alternating attractions between positive and negative charges on the rotor as protons flow though the Mot proteins then cause the entire basal body to rotate. Rotational speed of the flagellum is set by the proton flow rate through the Mot proteins, which is a function of the intensity of the proton motive force.

Flagellar Synthesis

Several genes encode the motility apparatus of *Bacteria*. In *Escherichia* and *Salmonella* species, in which motility studies have been extensive, over 50 genes are linked to motility in one way or another. These genes encode the structural proteins of the flagellum and motor apparatus, of course, but also encode proteins that export the structural proteins through the cytoplasmic membrane to the outside of the cell and proteins that regulate the synthesis of new flagella.

A flagellar filament grows not from its base, as does an animal hair, but from its tip. The MS ring is synthesized first and inserted into the cytoplasmic membrane. Then other anchoring proteins are synthesized along with the hook before the filament forms (**Figure 2.38**). Flagellin molecules synthesized in the cytoplasm pass up through a 3-nm channel inside the filament and add on at the terminus to form the mature flagellum. A protein "cap" is present at the end of the growing flagellum. Cap proteins assist flagellin molecules that have diffused through the filament channel to assemble in the proper fashion at the flagellum terminus (Figure 2.38). Approximately 20,000 flagellin protein molecules are needed to make one filament. The flagellum grows more or less continuously until it reaches its final length. Broken flagella still rotate and can be repaired with new flagellin units passed through the filament channel to replace the lost ones.

Figure 2.37 Structure and function of the flagellum in gram-negative *Bacteria*. *(a)* Structure. The L ring is embedded in the LPS and the P ring in peptidoglycan. The MS ring is embedded in the cytoplasmic membrane and the C ring in the cytoplasm. A narrow channel exists in the rod and filament through which flagellin molecules diffuse to reach the site of flagellar synthesis. The Mot proteins function as the flagellar motor, whereas the Fli proteins function as the motor switch. The flagellar motor rotates the filament to propel the cell through the medium. Inset photos: Top left, a cell of the purple sulfur bacterium *Chromatium* containing a tuft of polar flagella; Top right, transmission electron micrograph of a flagellar basal body from *Salmonella enterica* with the various rings labeled. *(b)* Function. A "proton turbine" model explains rotation of the flagellum. Protons, flowing through the Mot proteins, exert forces on charges present on the C and MS rings, thereby spinning the rotor.

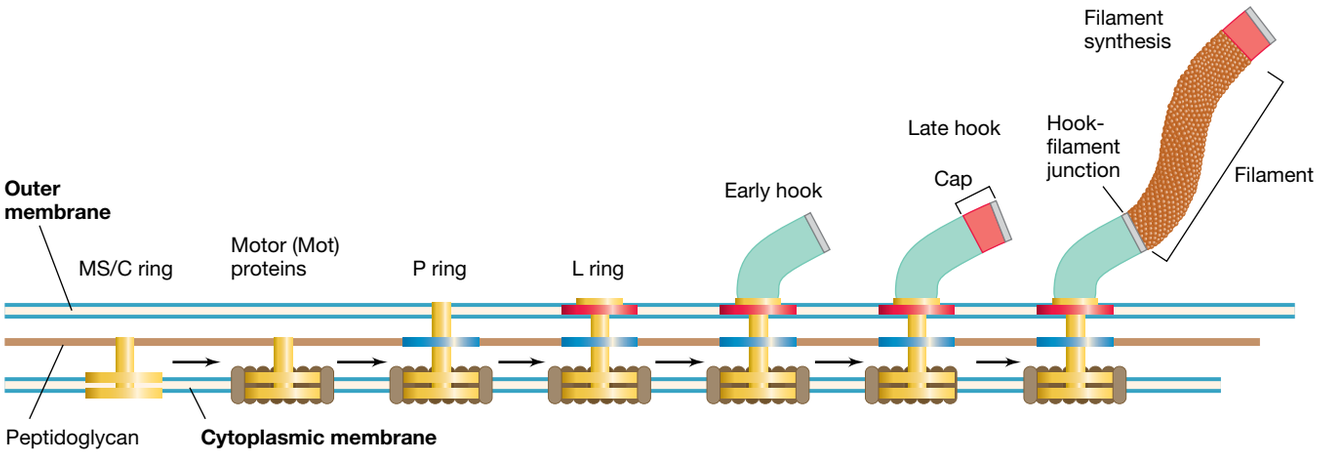


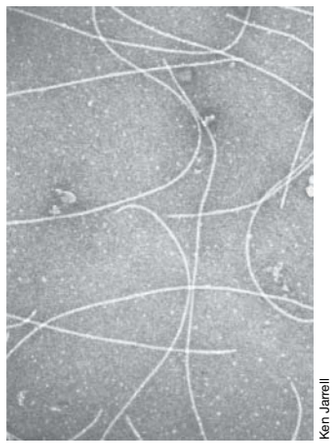
Figure 2.38 Flagella biosynthesis. Synthesis begins with assembly of MS and C rings in the cytoplasmic membrane, followed by the other rings, the hook, and the cap. Flagellin protein flows through the hook to form the filament and is guided into position by cap proteins.

Archaella

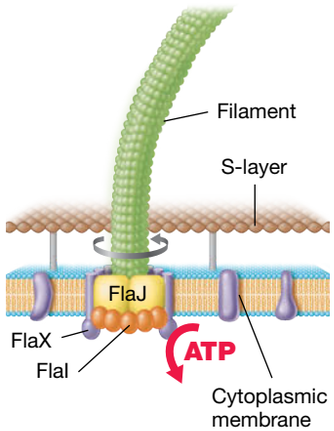
As in *Bacteria*, swimming motility is widespread among species of *Archaea* due to rotation of their flagella analog, the archaellum (see also page 70). These structures are roughly half the diameter of flagella, measuring about 10–13 nm in width (**Figure 2.39*a***), and impart movement to the cell by rotating, as do flagella. However, unlike *Bacteria*, in which a single type of protein makes up the filament, several different filament proteins are known in *Archaea*, and the genes that encode them bear little sequence homology to genes that encode bacterial flagellin. Depending on the archaeal species, 7–12 genes encode the major proteins that make up the archaellum. Archaella have been particularly well studied in the salt-loving archaeon *Halobacterium*, the heat- and acid-loving archaeon *Sulfolobus*, and the methane-producing archaeon *Methanocaldococcus*.

Studies of swimming cells of *Halobacterium* show that they swim at speeds only about one-tenth that of cells of *Escherichia coli*. This could be due to the smaller diameter of the archaellum compared to the flagellum, as this would be expected to reduce the torque of the structure significantly. However, this hypothesis has been questioned since the discovery that some *Archaea* swim incredibly fast. For example, cells of *Methanocaldococcus* (Figure 2.39*c*) swim nearly 50 times faster than cells of *Halobacterium* and 10 times faster than cells of *Escherichia coli* (*Bacteria*). In fact, *Methanocaldococcus* swims at nearly 500 cell lengths per second, which makes it the fastest organism on Earth! Thus, the net torque or rotational speeds of archaella from different species of *Archaea* can obviously vary significantly.

The overall structure of the archaellum bears a strong resemblance to that of type IV pili (Figure 2.39*b*), and it is clear that the archaellum is structurally related to these appendages (Section 2.7).



(a)



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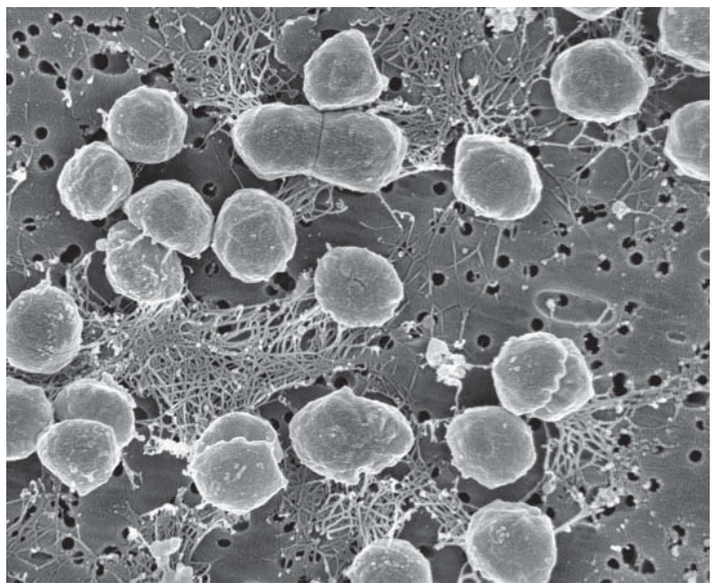


(c)

Figure 2.39 Archaella. *(a)* Transmission electron micrograph of archaella isolated from the methanogen *Methanococcus maripaludis*. A single archaellum is about 12 nm wide. *(b)* Depiction of an archaellum embedded in the archaeal cell wall and cytoplasmic membrane. ATP (rather than the proton motive force, see Figure 2.37*b*) drives archaella rotation. *(c)* Scanning electron micrograph of cells of *Methanocaldococcus jannaschii* containing abundant archaella.

In fact, the archaellum can be considered a rotating type IV pilus capable of both clockwise and counterclockwise rotation. Moreover, in contrast to the flagellum, whose energy requirement is met by dissipation of the proton motive force (Figure 2.37*b*), rotation of the archaellum is driven by the hydrolysis of ATP. Thus, although flagella and archaella are functionally similar—rotating filaments that drive cell propulsion—their flagellar motors are powered in fundamentally different ways. This suggests that swimming motility evolved separately in *Bacteria* and *Archaea* as these domains diverged some 3.5 billion years ago.

MINIQUIZ

- Cells of *Salmonella* are peritrichously flagellated, those of *Pseudomonas* polarly flagellated, and those of *Spirillum* lophotrichously flagellated. Using a sketch, show how each organism would appear in a flagella stain.
- Compare flagella and archaella in terms of their structure, function, and energy source.

2.12 Gliding Motility

Some bacteria are motile but lack flagella. Most of these nonswimming yet still motile cells move by *gliding*. Unlike flagellar motility, in which cells stop and then start off in a different direction, gliding motility is a slower and smoother form of movement and typically occurs along the long axis of the cell.

Diversity of Gliding Motility

Gliding motility is widely distributed among *Bacteria* but has been well studied in only a few groups. The gliding movement itself—up to 10 μm/sec in some gliding bacteria—is considerably slower than propulsion by flagella but still offers the cell a means of moving about its habitat.

Gliding bacteria are typically filamentous or rod-shaped in morphology, and the gliding process requires that the cells be in contact with a solid surface (**Figure 2.40**). The morphology of colonies of a typical gliding bacterium is distinctive because cells glide out and move away from the center of the colony (Figure 2.40*c*). Perhaps the best-known gliding bacteria are the filamentous cyanobacteria (Figure 2.40*a*, *b*), certain gram-negative bacteria such as *Myxococcus* and other myxobacteria, and species of *Cytophaga* and *Flavobacterium* (Figure 2.40*c*, *d*). No gliding *Archaea* are known.

Mechanisms of Gliding Motility

More than one mechanism drives gliding motility. Cyanobacteria glide by secreting a polysaccharide slime from pores onto the outer surface of the cell. The slime contacts both the cell surface and the solid surface against which the cell moves. As the excreted slime adheres to the surface, the cell slides along. The nonphototrophic gliding bacterium *Cytophaga* also glides at the expense of slime excretion, rotating along its long axis as it does.

Cells capable of "twitching motility" also display a form of gliding motility using a mechanism by which repeated extension and retraction of type IV pili (Section 2.7) drag the cell along a surface. The gliding myxobacterium *Myxococcus xanthus* has two forms of gliding motility. One form is driven by type IV pili, whereas the other is distinct from either the type IV pili or the slime extrusion methods. In this form of *M. xanthus* motility, a protein adhesion complex is formed at one pole of the rod-shaped cell and remains at a fixed position on the surface as the cell glides forward. This means that the adhesion complex moves in the direction opposite that of the cell, presumably fueled by some sort of cytoplasmic motility engine.

Neither slime extrusion nor twitching is the mechanism of gliding in other gliding bacteria. In the genus *Flavobacterium* (Figure 2.40*c* and **Figure 2.41**), for example, no slime is excreted and

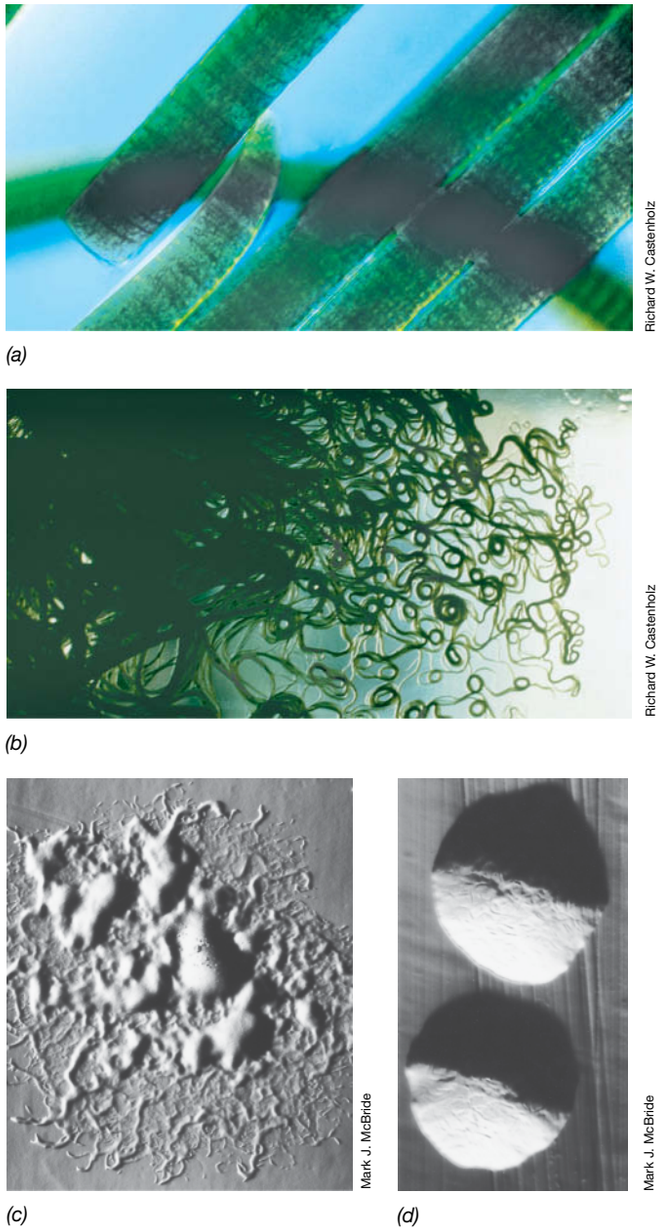


Figure 2.40 Gliding bacteria. *(a, b)* The large filamentous cyanobacterium *Oscillatoria* has cells about 35 μm wide. *(b) Oscillatoria* filaments gliding on an agar surface. *(c)* Masses of the bacterium *Flavobacterium johnsoniae* gliding away from the center of the colony (the colony is about 2.7 mm wide). *(d)* Nongliding mutant strain of *F. johnsoniae* showing typical colony morphology of nongliding bacteria (the colonies are 0.7–1 mm in diameter). See also Figure 2.41.

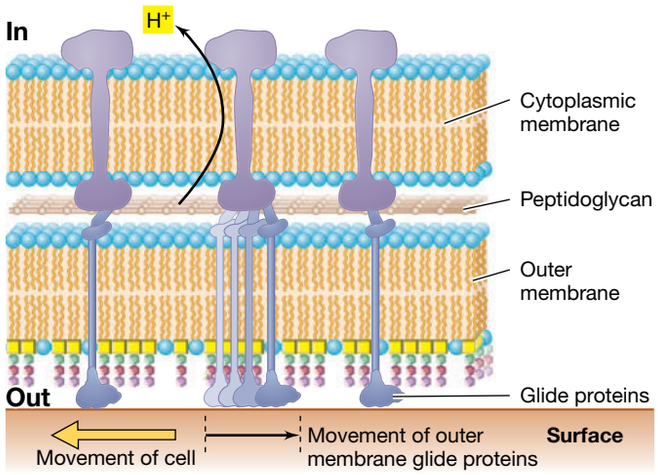


Figure 2.41 Gliding motility in *Flavobacterium johnsoniae*. Tracks (yellow) exist in the peptidoglycan that connect cytoplasmic proteins to outer membrane glide proteins and propel the glide proteins along the solid surface. Note that the glide proteins and the cell proper move in opposite directions.

cells lack type IV pili. Instead of using one of these gliding mechanisms, the movement of proteins on the *Flavobacterium* cell surface supports gliding motility in this organism. Specific motility proteins anchored in the cytoplasmic and outer membranes are thought to propel cells of *Flavobacterium* forward by a ratcheting mechanism (Figure 2.41). Movement of gliding-specific proteins in the cytoplasmic membrane is driven by energy from the proton motive force, and this motion is then transmitted to complementary glide proteins in the outer membrane. Movement of the outer membrane proteins against the solid surface then pulls the cell forward (Figure 2.41).

Like other forms of motility, gliding motility has ecological relevance. Gliding allows a cell to exploit new resources and to interact with other cells. Myxobacteria, such as *Myxococcus xanthus*, have a very social and cooperative lifestyle, and gliding motility may play an important role in the intimate cell-to-cell interactions necessary to complete their life cycle (Section 15.17).

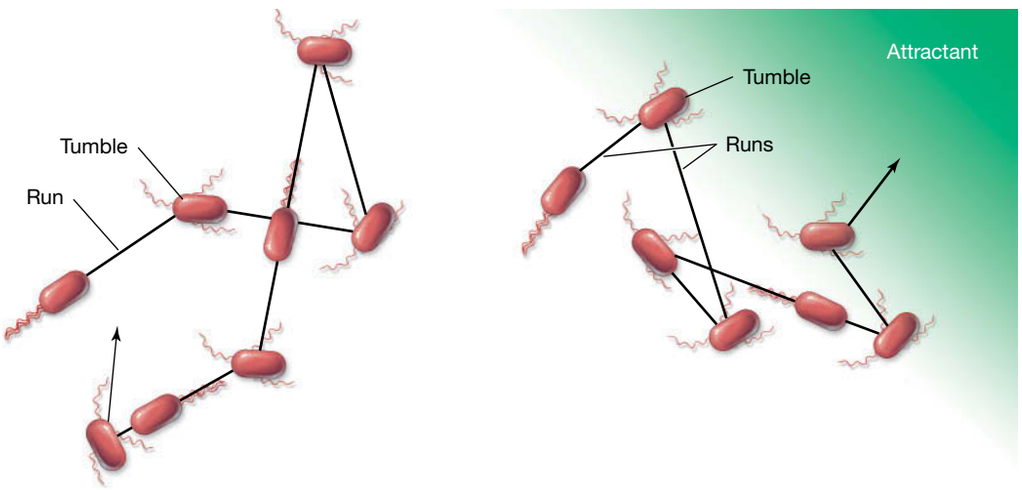
MINIQUIZ

- How does gliding motility differ from swimming motility in both mechanism and requirements?
- Contrast the mechanism of gliding motility in a filamentous cyanobacterium and in *Flavobacterium*.

2.13 Chemotaxis and Other Taxes

Cells of *Bacteria* and *Archaea* often encounter gradients of physical or chemical agents in nature and have evolved means to respond to these gradients by moving either toward or away from the agent. Such a directed movement is called a *taxis* (plural, taxes). **Chemotaxis**, a response to chemicals, and **phototaxis**, a response to light, are two well-studied taxes. The ability of a cell to move toward or away from various stimuli has ecological significance in that the directed movement may enhance a cell's access to resources or allow it to avoid harmful substances that could damage or kill it.

Chemotaxis has been well studied in swimming bacteria, and much is known at the genetic level about how the chemical state of a cell's environment is communicated to the flagellum. Our discussion here will thus deal solely with swimming bacteria. However, some gliding bacteria are also chemotactic, and there are phototactic movements in filamentous cyanobacteria (Figure 2.40*a*, *b*). In addition, many swimming species of *Archaea* are also chemotactic, and several of the proteins that control chemotaxis in *Bacteria* have homologs in these *Archaea*. Here we discuss microbial taxes in a general way. In Section 6.7 we examine the molecular mechanism of chemotaxis and its genetics and regulation in *Escherichia coli* as a general model for the control of taxes in both *Bacteria* and *Archaea*.



(a) **No attractant present: Random movement** *(b)* **Attractant present: Directed movement**

Figure 2.42 Chemotaxis in a peritrichously flagellated bacterium. *(a)* In the absence of a chemical attractant, the cell swims randomly in runs, changing direction during tumbles. *(b)* In the presence of an attractant, runs become biased, and the cell moves up the gradient of the attractant. The attractant gradient is depicted in green, with the highest concentration where the color is most intense.

Chemotaxis in Peritrichously Flagellated *Bacteria*

Much research on chemotaxis has been done with the peritrichously flagellated bacterium *E. coli*. To understand how chemotaxis affects the behavior of *E. coli*, consider the situation in which a cell encounters a gradient of some chemical in its environment (Figure 2.42). In the absence of the gradient, cells move in a random fashion that includes *runs*, in which the cell is swimming forward in a smooth fashion, and *tumbles*, when the cell stops and jiggles about. During forward movement in a run,

the flagellar motor rotates counterclockwise. When flagella rotate clockwise, the bundle of flagella pushes apart, forward motion ceases, and the cells tumble (Figure 2.42).

Following a tumble, the direction of the next run is random. Thus, by means of runs and tumbles, the cell moves about its environment in a random fashion. However, if a gradient of a chemical attractant is present, these random movements become biased. If the organism senses that it is moving toward higher concentrations of the attractant, runs become longer and tumbles are less frequent. The result of this behavioral response is that the organism moves up the concentration gradient of the attractant (Figure 2.42*b*). If the organism senses a repellent, the same mechanism applies, although in this case it is the *decrease* in concentration of the repellent (rather than the *increase* in concentration of an attractant) that promotes runs.

How are chemical gradients sensed? Prokaryotic cells are too small to sense a gradient of a chemical along the length of a single cell. Instead, while moving, cells "monitor" their environment by sampling chemicals periodically and comparing the concentration of a particular chemical with that sensed a few moments before. Bacterial cells thus respond to *temporal* rather than *spatial* differences in the concentration of a chemical as they swim. Sensory information is fed through an elaborate cascade of proteins that eventually affect the direction of rotation of the flagellar motor. The attractants and repellents are sensed by a series of membrane proteins called *chemoreceptors*. These sensory proteins bind the chemicals and begin the process of sensory transduction to the flagellum (Section 6.7). Chemotaxis can thus be considered a type of *sensory response system*, analogous to sensory responses in the nervous system of animals.

Chemotaxis in Polarly Flagellated *Bacteria*

Chemotaxis in polarly flagellated cells is similar but not identical to that in peritrichously flagellated cells such as *E. coli*. Many polarly flagellated bacteria, such as *Pseudomonas* species, can fully reverse the direction of rotation of their flagella. In so doing, they do not tumble but immediately reverse their direction of movement (Figure 2.36*b*). However, in the phototrophic bacterium *Rhodobacter*, cells of which have only a single flagellum that can rotate in just one direction, rotation of the flagellum stops periodically. When the flagellum stops rotating, the cell becomes reoriented by Brownian motion (Figure 2.36*b*). Then as the flagellum begins to rotate again, the cell moves off in a new direction.

Despite this seemingly random activity, cells of *Rhodobacter* are strongly chemotactic to various organic compounds and also to oxygen and light. *Rhodobacter* cannot reverse its flagellar motor and tumble as *E. coli* can, but cells do maintain runs as long as they sense an increasing concentration of attractant. If the cells sense a decreasing concentration of attractant, movement ceases. By such starting and stopping, a cell eventually finds the path of increasing attractant and maintains a run until either its chemoreceptors are saturated or it senses a decrease in the level of attractant.

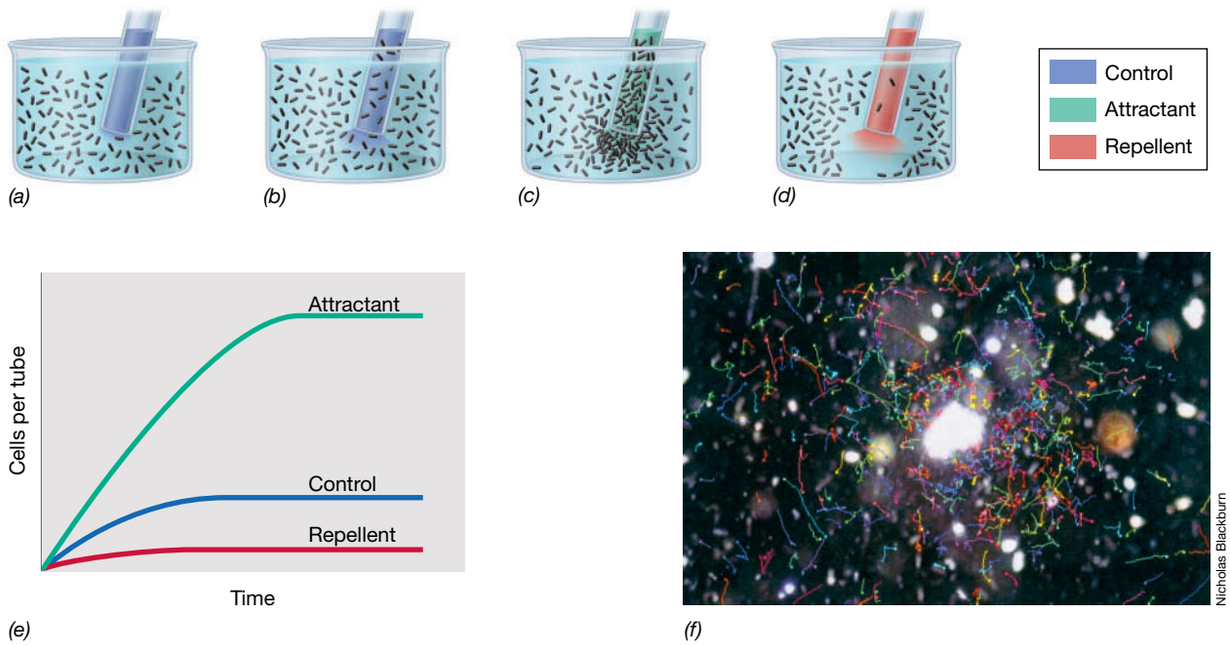


Figure 2.43 Measuring chemotaxis using a capillary tube assay. *(a)* Insertion of the capillary into a bacterial suspension. As the capillary is inserted, a gradient of the chemical begins to form. *(b)* Control capillary contains a salt solution that is neither an attractant nor a repellent. Cell concentration inside the capillary becomes the same as that outside. *(c)* Accumulation of bacteria in a capillary containing an attractant. *(d)* Repulsion of bacteria by a repellent. *(e)* Time course showing cell numbers in capillaries containing various chemicals. *(f)* Tracks of motile bacteria in seawater swarming around an algal cell (large white spot, center) photographed with a tracking video camera system attached to a microscope. The bacterial cells are showing positive aerotaxis by moving toward the oxygen-producing algal cell. The alga is about 60 μm in diameter. The proteins that participate in chemotaxis and the mechanisms by which chemotaxis is regulated, are discussed in detail in Section 6.7.

Measuring Chemotaxis

Bacterial chemotaxis can be demonstrated and quantified by immersing a small glass capillary tube containing an attractant into a suspension of motile bacteria that does not contain the attractant. From the tip of the capillary, a gradient forms into the surrounding medium, with the concentration of chemical gradually decreasing with distance from the tip (**Figure 2.43**). When an attractant is present, chemotactic bacteria will move toward it, forming a swarm around the open tip (Figure 2.43*c*) with many of the bacteria swimming into the capillary itself. Of course, because of random movements some chemotactic bacteria will swim into the capillary even if it contains a solution of the same composition as the medium (control solution, Figure 2.43*b*). However, when an attractant is present, the number of cells within the capillary will be many times higher than external cell numbers. If the capillary is removed after a time period and the cells within it are counted and compared with that of the control, attractants can easily be identified (Figure 2.43*e*).

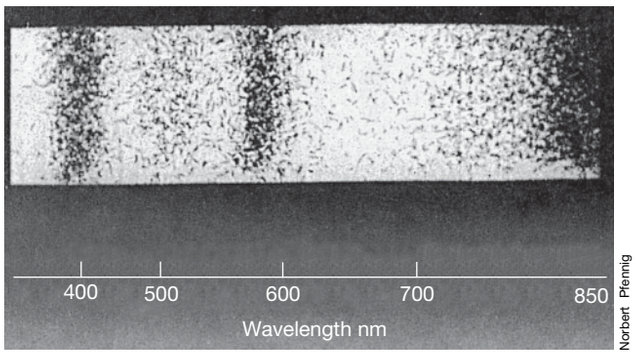
If the inserted capillary contains a repellent, just the opposite occurs; the cells sense an increasing gradient of repellent and the appropriate chemoreceptors affect flagellar rotation to gradually move the cells away from the repellent. In this case, the number of bacteria within the capillary will be fewer than in the control (Figure 2.43*d*). Using this simple capillary method, it is possible to screen chemicals to see if they are attractants or repellents for a given bacterium.

Chemotaxis can also be observed microscopically. Using a video camera that captures the position of bacterial cells with time and shows the motility tracks of each cell, it is possible to see the chemotactic movements of cells (Figure 2.43*f*). This method has been used to study chemotaxis of mixtures of microbes in natural environments. In nature it is thought that the major chemotactic agents for bacteria are nutrients excreted from larger microbial cells or from live or dead macroorganisms. Algae, for example, produce both organic compounds and oxygen (O_2, from photosynthesis) that can trigger chemotactic movements of bacteria toward the algal cell (Figure 2.43*f*).

Phototaxis and Other Taxes

Many phototrophic microorganisms can move toward light, a process called *phototaxis*. Phototaxis allows a phototrophic organism to position itself most efficiently to receive light for photosynthesis. This can be shown if a light spectrum is spread across a microscope slide on which there are motile phototrophic purple bacteria. On such a slide the bacteria accumulate at wavelengths at which their photosynthetic pigments absorb (**Figure 2.44*a***). These pigments include, in particular, bacteriochlorophylls and carotenoids (Chapter 14).

Two different light-mediated taxes are observed in phototrophic bacteria. One, called *scotophobotaxis*, can be observed only microscopically and occurs when a phototrophic bacterium happens to swim outside the illuminated field of view of the microscope into darkness. Entering darkness negatively affects photosynthesis and thus the energy state of the cell and signals the cell to tumble, reverse direction, and once again swim in a run, thus reentering the light. Scotophobotaxis is presumably a mechanism by which phototrophic purple bacteria avoid entering darkened habitats when they are moving about in illuminated ones, and this likely improves their competitive success.



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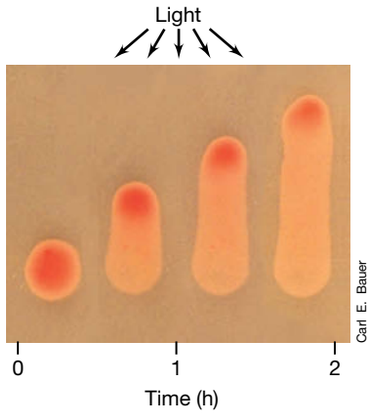


(b)

Figure 2.44 Phototaxis of phototrophic bacteria. *(a)* Scotophobic accumulation of the phototrophic purple bacterium *Thiospirillum jenense* at wavelengths of light at which its pigments absorb. A light spectrum was displayed on a microscope slide containing a dense suspension of the bacteria; after a period of time, the bacteria had accumulated selectively and the photomicrograph was taken. The wavelengths at which the bacteria accumulated are those at which the photosynthetic pigment bacteriochlorophyll *a* absorbs (compare with Figure 14.2*b*). *(b)* Phototaxis of an entire colony of the purple phototrophic bacterium *Rhodospirillum centenum*. These strongly phototactic cells move in unison toward the light source at the top. See Figure 2.34 for electron micrographs of flagellated *R. centenum* cells.

Phototaxis differs from scotophobotaxis in that cells move up a light gradient from lower to higher intensities. Phototaxis is analogous to chemotaxis except that the attractant is light instead of a chemical. In some phototactic organisms, such as the highly motile phototrophic purple bacterium *Rhodospirillum centenum* (Figure 2.34), *entire colonies* of cells show phototaxis and move in unison toward the light (Figure 2.44*b*).

Several components of the regulatory system that govern chemotaxis also control phototaxis. A *photoreceptor*, a protein that functions similarly to a chemoreceptor but senses a gradient of light instead of chemicals, is the initial sensor in the phototaxis response. The photoreceptor then interacts with the same cytoplasmic proteins that control flagellar rotation in chemotaxis, maintaining the cell in a run if it is swimming toward an increasing intensity of light. Section 6.7 describes the activities of these proteins in more detail.

Other bacterial taxes, such as movement toward or away from oxygen (*aerotaxis*, see Figure 2.43*f*) or toward or away from conditions of high ionic strength (*osmotaxis*), are known among various

swimming bacteria. In some gliding cyanobacteria, *hydrotaxis* (movement toward water), has also been observed. Hydrotaxis allows gliding cyanobacteria that inhabit dry environments, such as desert soils, to glide toward a gradient of increasing hydration.

MINIQUIZ

- Define the word chemotaxis. How does chemotaxis differ from aerotaxis?
- What causes a run versus a tumble?
- How can chemotaxis be measured quantitatively?
- How does scotophobotaxis differ from phototaxis?

V • Eukaryotic Microbial Cells

Compared with prokaryotic cells, microbial eukaryotes typically have structurally more complex and much larger cells. We complete our study of microbial cell structure and function with a consideration of structure/function issues in microbial eukaryotes, common models for the study of eukaryotic biology. Microbial eukaryotes include the fungi, the algae, and the protozoa and other protists. We cover the diversity of microbial eukaryotes in Chapter 18.

2.14 The Nucleus and Cell Division

Eukaryotic cells vary in the complement of organelles they contain, but a unit membrane–enclosed nucleus is universal and a hallmark of the eukaryotic cell. Mitochondria are nearly universal among eukaryotic cells, while pigmented chloroplasts are found only in phototrophic cells. Other structures include the Golgi complex, lysosomes, endoplasmic reticula, and microtubules and microfilaments (Figure 2.45). Some microbial eukaryotes have flagella or cilia—structures that confer motility—and a cell wall is present in many, such as the fungi and algae.

Eukaryotic cell membranes contain *sterols*. These molecules, absent from all but a few prokaryotic cells, lend structural strength to the eukaryotic cell, something especially important to those eukaryotes that lack a cell wall, such as the protozoa or animal cells.

The Nucleus

The **nucleus** contains the chromosomes of the eukaryotic cell. DNA within the nucleus is wound around basic (positively charged) proteins called **histones**, which tightly pack the negatively

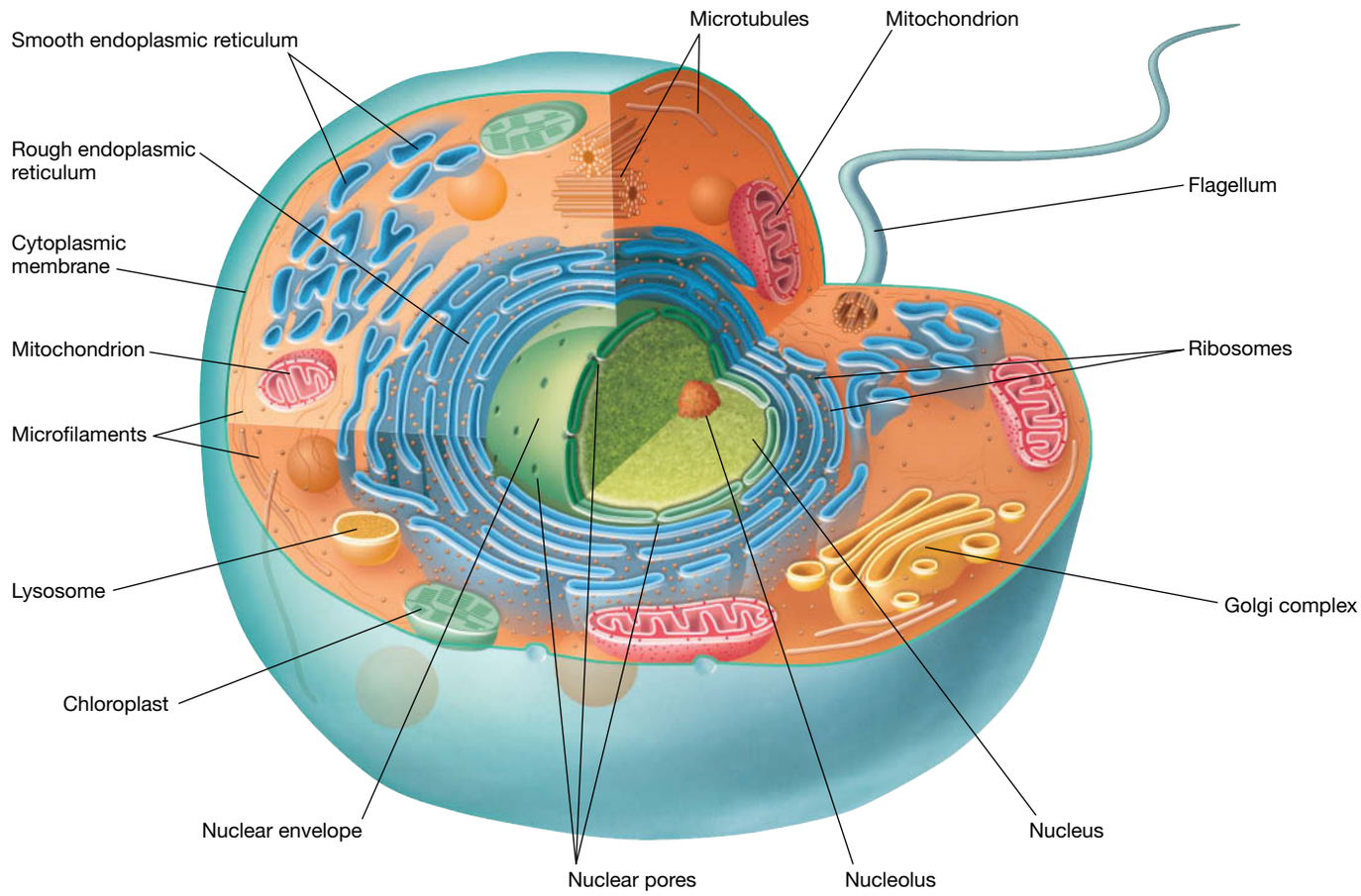


Figure 2.45 Cutaway schematic of a microbial eukaryote. Although all eukaryotic cells contain a nucleus, not all organelles and other structures shown are present in all microbial eukaryotes. Not shown is the cell wall, found in fungi, algae, plants, and a few protists.

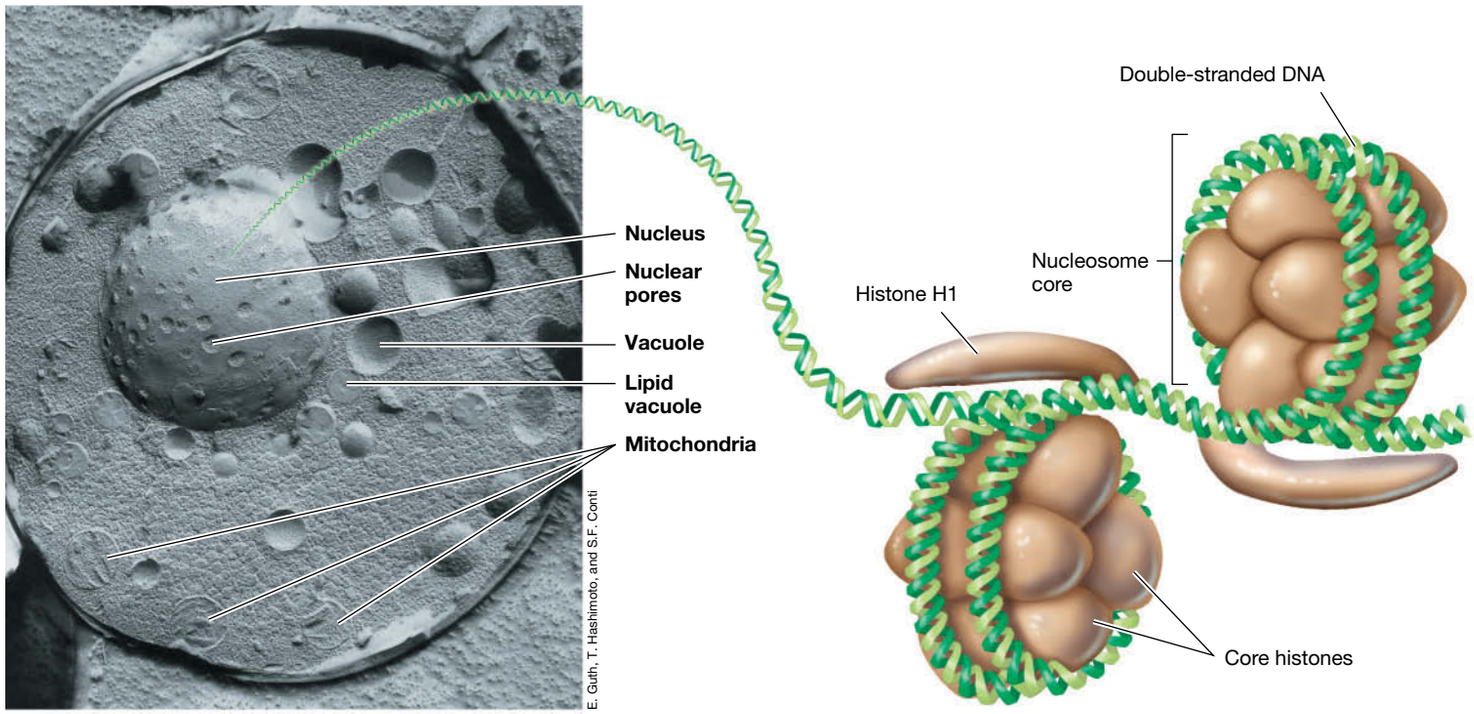


Figure 2.46 The nucleus and DNA packaging in eukaryotes. *(a)* Electron micrograph of a yeast cell prepared in such a way as to reveal a surface view of the nucleus. The cell is about 8 µm wide. *(b)* Packaging of DNA around histone proteins to form a nucleosome. Nucleosomes are arranged along the DNA strand like beads on a string and aggregate to form chromosomes during the process of mitosis (see Figure 2.47).

charged DNA to form nucleosomes (**Figure 2.46**) and from them, chromosomes. The nucleus is enclosed by a pair of membranes, each with its own function, separated by a space. The innermost membrane is a simple sac while the outermost membrane is in many places continuous with the endoplasmic reticulum. The inner and outer nuclear membranes specialize in interactions with the nucleoplasm and the cytoplasm, respectively. The nuclear membranes contain pores (Figures 2.45 and 2.46*a*), formed from holes where the inner and outer membranes are joined. The pores allow transport proteins to import and export other proteins and nucleic acids into and out of the nucleus, a process called *nuclear transport*.

Within the nucleus is found the *nucleolus* (Figure 2.45), the site of ribosomal RNA (rRNA) synthesis. The nucleolus is rich in RNA, and ribosomal proteins synthesized in the cytoplasm are transported into the nucleolus and combine with rRNA to form the small and large subunits of eukaryotic ribosomes. These are then exported to the cytoplasm, where they associate to form the intact ribosome and function in protein synthesis.

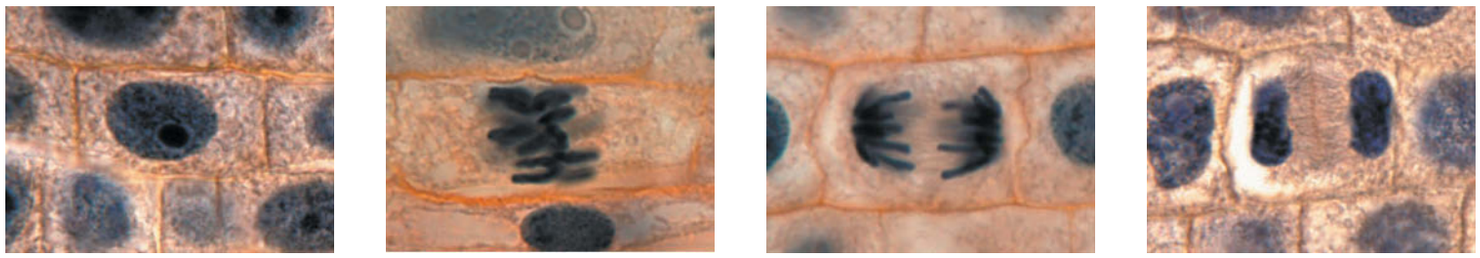
Cell Division

Eukaryotic cells divide by a process in which the chromosomes are replicated, the nucleus is disassembled, the chromosomes are segregated into two sets, and a nucleus is reassembled in each daughter cell (**Figure 2.47**). Whereas prokaryotic cells are genetically haploid, many microbial eukaryotes can exist in either of two genetic states: haploid or diploid. *Diploid* cells have two copies of each chromosome whereas *haploid* cells have only one. For example, the brewer's yeast *Saccharomyces cerevisiae* can exist in the haploid state (16 chromosomes) as well as in the diploid state (32 chromosomes). However, regardless of its genetic state, during cell division the chromosome number is first doubled and later halved to give each daughter cell its correct complement of chromosomes. This is the process of **mitosis**, unique to eukaryotic cells. During mitosis, the chromosomes condense, divide, and are separated into two sets, one for each daughter cell (Figure 2.47).

In contrast to mitosis, **meiosis** converts a diploid cell into several haploid cells. Meiosis consists of two successive cell divisions. In the first meiotic division, homologous chromosomes segregate into separate cells, changing the genetic state from diploid to haploid. The second meiotic division is essentially the same as mitosis, as the two haploid cells divide to form a total of four haploid cells called *gametes*. In higher organisms these are the eggs and sperm; in eukaryotic microorganisms, they are spores or related reproductive structures.

MINIQUIZ

- How is DNA arranged in the chromosomes of eukaryotes?
- What are histones and what do they do?
- What are the major differences between mitosis and meiosis?

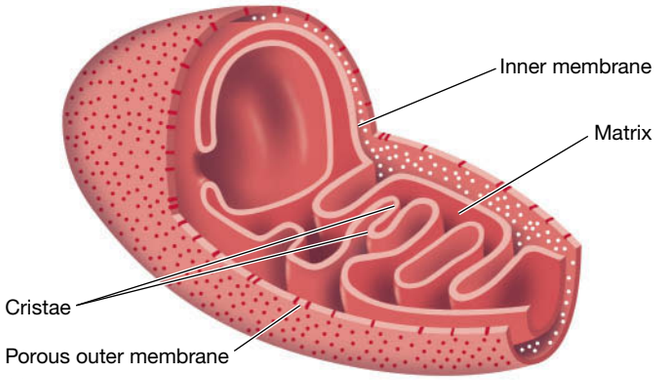


(a) (b) (c) (d)

Figure 2.47 Light micrograph of eukaryotic cells undergoing mitosis. *(a)* Interphase, distinct chromosomes are not apparent. *(b)* Metaphase. Homologous chromosomes are lining up along the cell center. *(c)* Anaphase. Homologous chromosomes are pulling apart. *(d)* Telophase. Chromosomes have separated into the newly forming daughter cells.

2.15 Mitochondria, Hydrogenosomes, and Chloroplasts

Organelles that specialize in energy metabolism in eukaryotes include the mitochondrion or hydrogenosome, and in phototrophic eukaryotes, the chloroplast. These organelles have evolutionary roots within the *Bacteria* and provide ATP to the eukaryotic cell from either the oxidation of organic compounds or from light.



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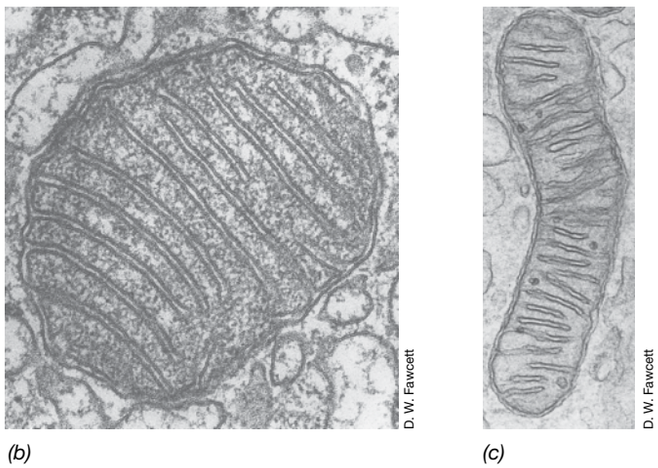


(b) (c)

Figure 2.48 Structure of the mitochondrion. *(a)* Diagram showing the overall structure of the mitochondrion; note the inner and outer membranes. *(b, c)* Transmission electron micrographs of mitochondria from rat tissue showing the variability in morphology; note the cristae.

Mitochondria

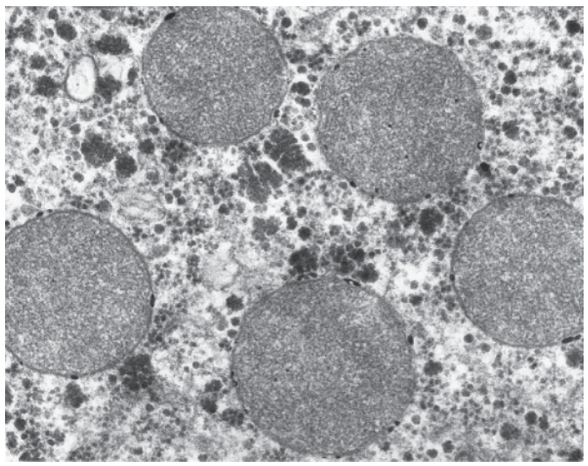
In aerobic eukaryotic cells, respiration occurs in the mitochondrion. **Mitochondria** are of bacterial dimensions and can take on many shapes (Figure 2.48). The number of mitochondria per cell depends somewhat on the cell type and size. A yeast cell may have only a few mitochondria per cell, whereas an animal cell may have over a thousand. The mitochondrion is enclosed by a double membrane system. Like the nuclear membrane, the outermost mitochondrial membrane is relatively permeable and contains pores that allow the passage of small molecules. The innermost membrane is less permeable and its structure more closely resembles that of the cytoplasmic membrane of *Bacteria*.

Mitochondria also contain folded internal membranes called **cristae**. These membranes, formed by invagination of the inner membrane, contain the enzymes needed for respiration and ATP production. Cristae also contain transport proteins that regulate the passage of key molecules such as ATP into and out of the *matrix*, the innermost compartment of the mitochondrion (Figure 2.48*a*). The matrix contains enzymes for the oxidation of organic compounds, in particular, enzymes of the citric acid cycle, the major pathway for the combustion of organic compounds to CO_2 (↻ Section 3.9).

Hydrogenosomes

Some eukaryotic microorganisms are killed by O_2 and, like many *Bacteria* and *Archaea*, live an anaerobic lifestyle. Such cells lack mitochondria and some of them contain structures called **hydrogenosomes** (Figure 2.49). Although similar in size to mitochondria, hydrogenosomes lack citric acid cycle enzymes and also lack cristae. Microbial eukaryotes that contain hydrogenosomes carry out a strictly fermentative metabolism. Examples include the human parasite *Trichomonas* (↻ Sections 18.3 and 33.4) and various protists that inhabit the rumen of ruminant animals (↻ Section 23.13) or anoxic muds and lake sediments.

The major biochemical reaction in the hydrogenosome is the oxidation of pyruvate to H_2, CO_2, and acetate (Figure 2.49*b*). Some anaerobic eukaryotes have H_2-consuming, methane-producing *Archaea* in their cytoplasm. These *methanogens* consume the H_2 and CO_2 produced by the hydrogenosome and combine them to form methane (CH_4). Because hydrogenosomes are anoxic and cannot respire, they cannot oxidize the acetate produced from



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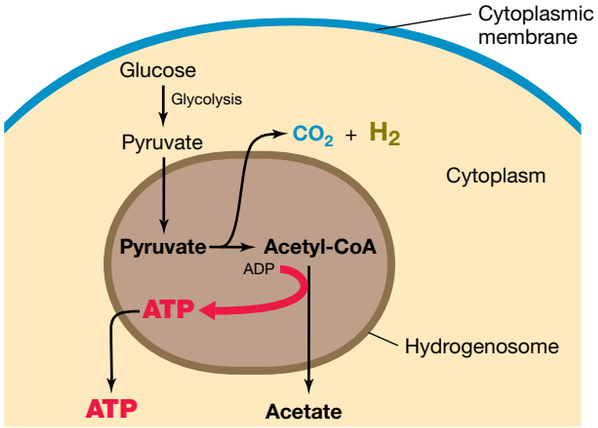


(b)

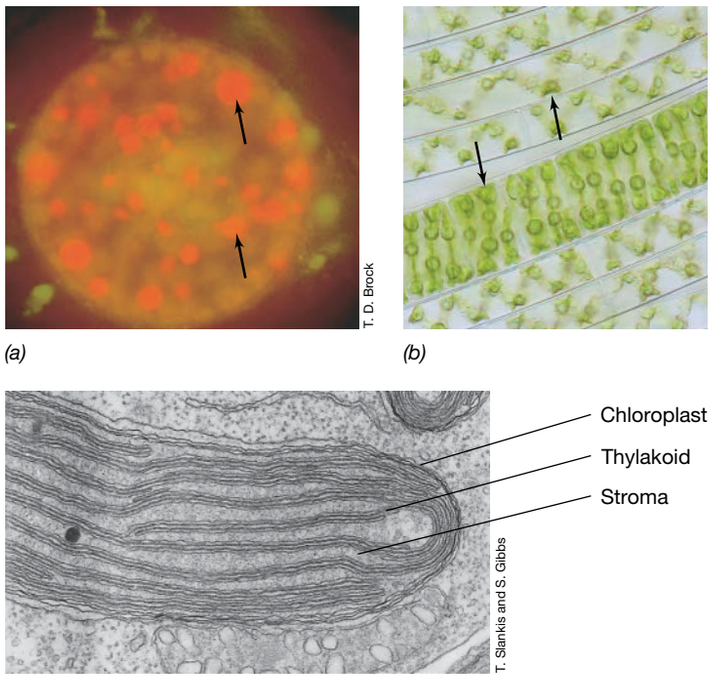
Figure 2.49 The hydrogenosome. *(a)* Electron micrograph of a thin section through a cell of the anaerobic protist *Trichomonas vaginalis* showing five hydrogenosomes in cross section. Compare their internal structure with that of mitochondria in Figure 2.48. *(b)* Biochemistry of the hydrogenosome. Pyruvate is taken up by the hydrogenosome, and H_2, CO_2, acetate, and ATP are produced.

pyruvate oxidation as mitochondria do. Acetate is therefore excreted from the hydrogenosome into the cytoplasm of the host cell (Figure 2.49*b*).

Chloroplasts

Chloroplasts are the chlorophyll-containing organelles of phototrophic microbial eukaryotes such as the algae and function to carry out photosynthesis. Chloroplasts are relatively large and readily visible with the light microscope (**Figure 2.50**), and their number per cell varies among species.

Like mitochondria, chloroplasts have a permeable outer membrane and a much less-permeable inner membrane. The innermost membrane surrounds the **stroma**, analogous to the matrix of the mitochondrion (Figure 2.50*c*). The stroma contains the enzyme *ribulose bisphosphate carboxylase* (RubisCO), the key enzyme of the *Calvin cycle*, the series of biosynthetic reactions by which phototrophs convert CO_2 to organic compounds (↔ Section 14.5). The permeability of the outermost chloroplast membrane allows glucose and ATP produced during



(a) (b) (c)

Figure 2.50 Chloroplasts of a diatom and a green alga cell. *(a)* Fluorescence photomicrograph of a diatom shows chlorophyll fluorescence; arrows, chloroplasts. The cell is about 40 µm wide. *(b)* Phase-contrast photomicrograph of the filamentous green alga *Spirogyra* showing the characteristic spiral-shaped chloroplasts (arrows) of this phototroph. A cell is about 20 µm wide. *(c)* Transmission electron micrograph showing a chloroplast of a diatom; note the thylakoids.

photosynthesis to diffuse into the cell cytoplasm where they are used in biosynthesis.

Chlorophyll and all other components needed for ATP synthesis in chloroplasts are located in a series of flattened membrane discs called **thylakoids** (Figure 2.50*c*). Like the cytoplasmic membrane, the thylakoid membrane is highly impermeable and its major function is to form a proton motive force (Figure 2.7*c*) that results in ATP synthesis.

Organelles and Endosymbiosis

On the basis of their relative autonomy, size, and morphological resemblance to bacteria, it was hypothesized over 100 years ago that mitochondria and chloroplasts were descendants of respiratory and phototrophic bacterial cells, respectively. By associating with nonphototrophic eukaryal hosts, the latter gained a new form of energy metabolism while the symbiotic bacterial cells received a stable and supportive growth environment inside the host. Over time, these originally free-living symbionts became an intimate part of the eukaryotic cell. This idea of symbiotic bacteria as the ancestors of the mitochondrion, hydrogenosome, and chloroplast is called the **endosymbiotic hypothesis** (↔ Sections 13.4 and 18.1) and is now well accepted in biology.

Several lines of evidence support the endosymbiotic hypothesis. These include in particular the fact that mitochondria, hydrogenosomes, and chloroplasts contain their own genomes and

ribosomes. The genomes are arranged in a circular fashion as for bacterial chromosomes (Section 9.3), and the sequence of genes that encode ribosomal RNA (Figure 1.36) in organelles clearly points to their bacterial origin. Thus, the eukaryotic cell is a genetic chimera containing genes from two domains of life: the host cell (*Eukarya*) and the endosymbiont (*Bacteria*).

MINIQUIZ

- What key reactions occur in the mitochondrion and in the chloroplast, and what key product is made in each?
- Compare and contrast pyruvate metabolism in the mitochondrion and the hydrogenosome.
- What is the endosymbiotic hypothesis and what evidence is there to support it?

2.16 Other Eukaryotic Cell Structures

Besides the nucleus and the mitochondrion (or hydrogenosome), and chloroplasts in phototrophic cells, other cytoplasmic structures are present in microbial eukaryotes. These include the endoplasmic reticulum, the Golgi complex, lysosomes, a variety of tubular structures, and structures responsible for motility. However, unlike mitochondria and chloroplasts, these structures lack DNA and are not of endosymbiotic origin. Cell walls are also present in certain microbial eukaryotes and function as they do in prokaryotic cells to provide shape and protect the cell from osmotic lysis. The exact structure of the cell wall varies with the organism, but various polysaccharides and proteins are commonly observed.

Endoplasmic Reticulum, the Golgi Complex, and Lysosomes

The endoplasmic reticulum (ER) is a network of membranes continuous with the nuclear membrane. Two types of endoplasmic reticulum exist: *rough* ER, which contains attached ribosomes,

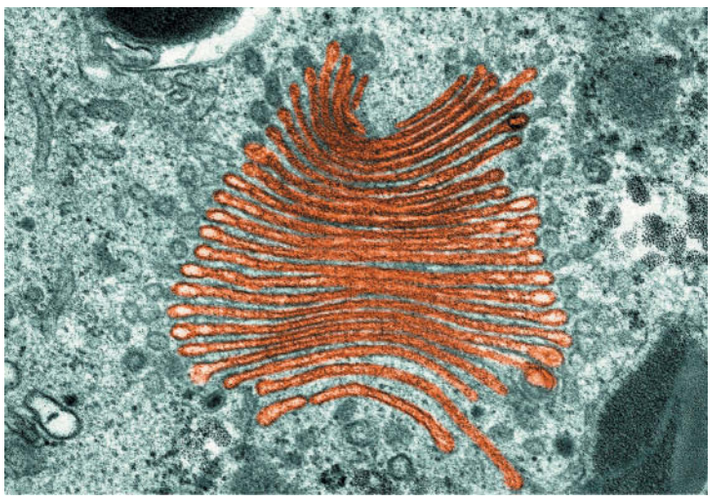
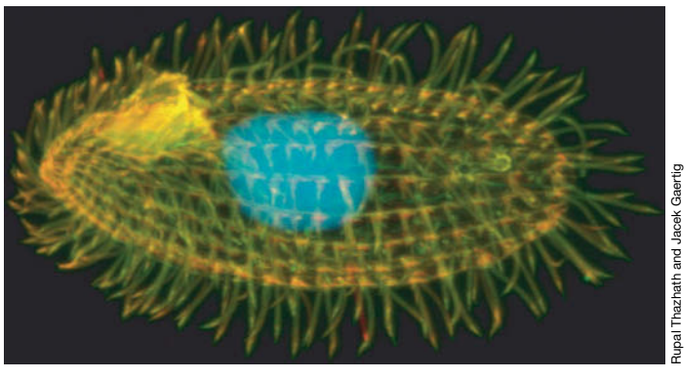


Figure 2.51 The Golgi complex. Transmission electron micrograph of a portion of a eukaryotic cell showing the Golgi complex (colored in gold). Note the multiple folded membranes of the Golgi complex (membrane stacks are 0.5–1.0 µm in diameter).



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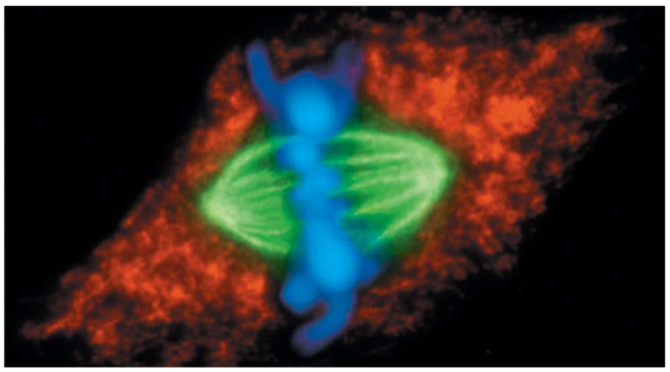

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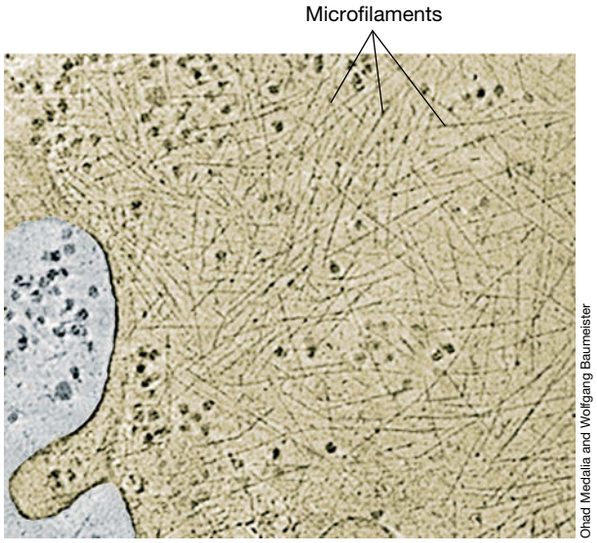

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(c)

Figure 2.52 Tubulin and microfilaments. *(a)* Fluorescence photomicrograph of a cell of *Tetrahymena* with red- and green-labeled antitubulin antibodies (the two combine to give yellow) and with DAPI, which stains DNA (blue, nucleus). A cell is about 10 µm wide. *(b)* An animal cell showing the role of tubulin (green) in separating chromosomes (blue) during metaphase of mitosis (cytoplasmic proteins stain red). *(c)* Electron microscopic image of the cellular slime mold *Dictyostelium discoideum* showing the network of actin microfilaments that along with microtubules functions as the cell cytoskeleton. Microfilaments are about 7 nm in diameter. *D. discoideum* has been used for decades as an experimental model system for eukaryotic cellular development and cell-to-cell cooperation (Figures 18.17 and 18.18).

and *smooth* ER, which does not (Figure 2.45). Smooth ER participates in the synthesis of lipids and in some aspects of carbohydrate metabolism. Rough ER, through the activity of its ribosomes, is a major producer of glycoproteins and also produces new membrane material that is transported throughout the cell to enlarge the various membrane systems before cell division.

The Golgi complex is a stack of membrane-bound sacs called *cisternae* (Figure 2.51) that arise from preexisting Golgi bodies and function in concert with the ER. In the Golgi complex, products of the ER are chemically modified and sorted into those destined for secretion versus those that will function in other membranous structures in the cell. Many of the modifications made in the Golgi complex are glycosylations (addition of sugar residues) that convert the proteins into glycoproteins that can then be targeted to specific locations in the cell.

Lysosomes (Figure 2.45) are membrane-enclosed compartments that contain digestive enzymes that hydrolyze proteins, fats, and polysaccharides. The lysosome fuses with food that enters the cell in vacuoles and then releases digestive enzymes that break down the foods for biosynthesis and energy generation. Lysosomes also function in degrading damaged cellular components and recycling these materials for new biosyntheses. The lysosome thus allows the cell's lytic activities to be partitioned away from the cytoplasm proper. Following the degradation of macromolecules in the lysosome, the resulting nutrients pass from the lysosome into the cytoplasm for use by cytoplasmic enzymes.

Microtubules, Microfilaments, and Intermediate Filaments

Just as buildings are supported by structural reinforcement, the large size of eukaryotic cells and their ability to move requires structural reinforcement. This internal support network consists of *microtubules, microfilaments*, and *intermediate filaments*; together, these structures form the cell **cytoskeleton** (Figure 2.45).

Microtubules are hollow tubes about 25 nm in diameter and are composed of the proteins α-*tubulin* and β-*tubulin*. Microtubules (Figure 2.52*a*) have many functions including maintaining cell shape and cell motility by cilia and flagella, moving chromosomes during mitosis (Figures 2.47 and 2.52*b*), and in movement of organelles within the cell. **Microfilaments** (Figure 2.52*c*) are smaller than microtubules, about 7 nm in diameter, and are polymers of two intertwined strands of the protein *actin*. Microfilaments function in maintaining or changing cell shape, in cell motility by cells that move by amoeboid movement, and during cell division. **Intermediate filaments** are fibrous keratin proteins that form into fibers 8–12 nm in diameter and function in maintaining cell shape and positioning organelles in the cell.

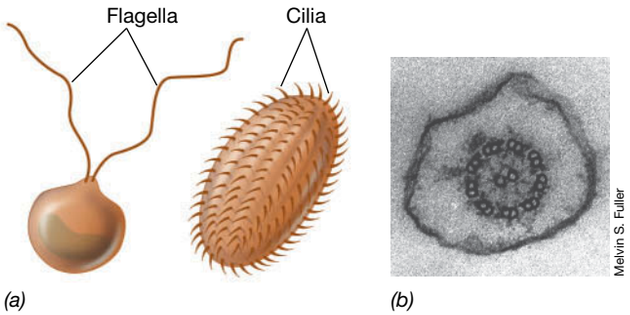


Figure 2.53 Motility organelles in eukaryotic cells: Flagella and cilia. *(a)* Flagella can be present as single or multiple filaments. Cilia are structurally very similar to flagella but much shorter. Eukaryotic flagella move in a whiplike motion. *(b)* Cross section through a flagellum of the fungus *Blastocladiella* showing the outer sheath, the outer nine pairs of microtubules, and the central pair of microtubules.

Flagella and Cilia

Flagella and cilia are present on the surface of many eukaryotic microbes and function as organelles of motility, allowing cells to move by swimming. Motility has survival value, as the ability to move allows motile organisms to move about their habitat and exploit new resources. *Cilia* are essentially short flagella that beat in synchrony to propel the cell—usually quite rapidly—through the medium. *Flagella*, by contrast, are long appendages present singly or in groups that propel the cell along—typically more slowly than by cilia—through a whiplike motion (Figure 2.53*a*). The flagella of eukaryotic cells are structurally quite distinct from bacterial flagella and do not rotate as do the flagella and archaella of *Bacteria* and *Archaea*, respectively (Section 2.11).

In cross section, cilia and flagella appear similar. Each contains a bundle of nine pairs of microtubules surrounding a central pair of microtubules (Figure 2.53*b*). A protein called *dynein* is attached to the microtubules and uses ATP to drive motility. Movement of flagella and cilia is similar. In both cases, movement is the result of the coordinated sliding of microtubules against one another in a direction toward or away from the base of the cell. This movement confers the whiplike motion on the flagellum or cilium that ultimately results in cell propulsion.

MINIQUIZ

- Why are the activities in the lysosome best partitioned away from the cytoplasm proper?
- How is the cell's cytoskeleton held together?
- From a functional standpoint, how does the flagellum of eukaryotic cells differ from that of prokaryotic cells?

Chapter Review

I • Cells of *Bacteria* and *Archaea*

2.1 Prokaryotic cells can have many different shapes; rods, cocci, and spirilla are common cell morphologies. Morphology is a poor predictor of other cell properties and is a genetically directed characteristic that has evolved to best serve the ecology of the cell.

Q What are the major morphologies of prokaryotic cells? Draw cells for each morphology you list.

2.2 Cells of *Bacteria* and *Archaea* are typically smaller than those of eukaryotes, although a few very large bacteria are known. The typical small size of prokaryotic cells affects their physiology, growth rate, ecology, and evolution. The lower limit for the diameter of a coccus-shaped cell is 0.15–0.2 μm.

Q How large can a bacterium be? How small? Why is it that we likely know the lower limit more accurately than the upper limit? What are the dimensions of the rod-shaped bacterium *Escherichia coli*?

II • The Cell Membrane and Wall

2.3 The cytoplasmic membrane is a highly selective permeability barrier constructed of lipids and proteins that form a bilayer, hydrophobic inside and hydrophilic outside. In contrast to *Bacteria* and *Eukarya*, where fatty acids are ester-linked to glycerol, *Archaea* contain ether-linked lipids and some form monolayer instead of bilayer membranes. The major functions of the cytoplasmic membrane are permeability, transport, and energy conservation, and to accumulate nutrients against the concentration gradient, transport systems are necessary.

Q Does the cytoplasmic membrane of prokaryotic cells provide shape and rigid support to the cell? Contrast the typical structure of the cytoplasmic membranes of *Bacteria* and *Archaea*.

2.4 Peptidoglycan is a polysaccharide found only in *Bacteria* that consists of an alternating repeat of *N*-acetylglucosamine and *N*-acetylmuramic acid, the latter cross-linked by tetrapeptides in adjacent strands. The enzyme lysozyme and the antibiotic penicillin both target peptidoglycan, leading to cell lysis.

Q Why is the rigid layer of the bacterial cell wall called peptidoglycan? What are the structural reasons for the rigidity that is conferred on the cell wall by the peptidoglycan structure?

2.5 Gram-negative *Bacteria* have an outer membrane consisting of LPS, protein, and lipoprotein. Porins allow for permeability across the outer membrane. The gap between the outer and cytoplasmic membranes is called the periplasm and contains proteins involved in transport, sensing chemicals, and other important cell functions.

Q Is the outer membrane more selective or less selective than the cytoplasmic membrane? Describe the periplasm and its possible functions.

2.6 Cell walls of *Archaea* are of several types, including pseudomurein, various polysaccharides, and S-layers composed of protein or glycoprotein. As for *Bacteria*, the walls of *Archaea* protect the cell from osmotic lysis.

Q Will the cells of *Archaea* be lysed by the enzyme lysozyme, or the antibiotic penicillin? Why or why not?

III • Cell Surface Structures and Inclusions

2.7 Many prokaryotic cells contain capsules, slime layers, pili, or fimbriae. These structures have several functions, including attachment, genetic exchange, and twitching motility. Hami, present on the surface of certain *Archaea*, function as miniature grappling hooks to attach cells to a surface or to one another.

Q What function(s) do polysaccharide layers outside the cell wall have in prokaryotic cells?

2.8 Prokaryotic cells can contain inclusions of sulfur, polyphosphate, carbon polymers, or various minerals formed by biomineralization. These substances function as nutrient storage materials or, in the case of magnetosomes, in magnetotaxis.

Q What are some of the roles of poly-β-hydroxyalkanoates (PHAs)?

2.9 Gas vesicles are gas-filled structures that confer buoyancy on cells of certain species of *Bacteria* and *Archaea*. Gas vesicles are composed of two different proteins arranged to form a gas-permeable but watertight structure.

Q What is the function of gas vesicles? How are these structures made such that they can remain gas tight?

2.10 The endospore is a highly resistant and differentiated structure produced by certain gram-positive *Bacteria*. Endospores are highly dehydrated and contain calcium dipicolinate and small acid-soluble spore proteins, both of which are absent from vegetative cells. Endospores can remain dormant indefinitely but can germinate quickly when conditions warrant.

Q Is an endospore still the same bacterial cell? What are the different types of endospores as to their location in the cell? Describe the structure of endospores.

IV • Cell Locomotion

2.11 Swimming motility in prokaryotic cells is due to flagella (*Bacteria*) or archaella (*Archaea*). Both structures are composed of several proteins, are anchored in the cell wall and cytoplasmic membrane, and function by rotation. Flagella and archaella differ in structure and how energy is coupled to rotation.

Q Describe the structure and function of a bacterial flagellum. What is the energy source for the flagellum? How do bacterial flagella differ from archaella in size, composition, and power source?

2.12 Bacteria that move by gliding do not employ rotating flagella but instead creep along a solid surface by employing one of several different mechanisms including polysaccharide excretion, twitching, or rotating glide proteins.

Q Contrast the mechanism for motility in *Flavobacterium* with that in *Escherichia coli*.

2.13 Swimming bacteria respond to chemical and physical gradients in their environment by controlling the lengths of runs and frequency of tumbles. Tumbles are controlled by the direction of rotation of the flagellum, which in turn is controlled by a network of sensory and response proteins.

Q In a few sentences, explain how a swimming bacterium is able to sense the direction of an attractant and move toward it. In the experiment described in Figure 2.43, what is the control and why is it essential?

V • Eukaryotic Microbial Cells

2.14 Microbial eukaryotes contain various organelles including the nucleus, mitochondria (or hydrogenosomes), and chloroplasts. The nucleus contains the cell's DNA wrapped around histone proteins. Microbial eukaryotes divide following the process of mitosis and may undergo meiosis as well if a haploid/diploid life cycle occurs.

Q List at least three features of eukaryotic cells that clearly differentiate them from prokaryotic cells. What are histones and what do they do?

2.15 The mitochondrion and hydrogenosome are energy-generating organelles; mitochondria respire, whereas hydrogenosomes ferment. Chloroplasts generate ATP by photosynthesis and also fix CO_2 into cell material. All of these organelles were once free-living *Bacteria* that later established residence inside cells of *Eukarya* (endosymbiosis).

Q How are the mitochondrion and the hydrogenosome similar structurally? How do they differ? How do they differ metabolically? What major physiological processes occur in the chloroplast? What evidence supports the idea that the major organelles of eukaryotes were once *Bacteria*?

2.16 Endoplasmic reticula are membranous structures in eukaryotes that either contain attached ribosomes (rough ER) or not (smooth ER). Flagella and cilia are means of motility and move by a whiplike mechanism instead of by rotation. Lysosomes specialize in degrading large molecules. Microtubules, microfilaments, and intermediate filaments function as internal cell scaffolds to form the cell cytoskeleton.

Q Describe the major functions of the endoplasmic reticulum, Golgi complex, and lysosomes. What makes up the eukaryotic cell cytoskeleton?

Application Questions

1. Calculate the surface-to-volume ratio of a spherical cell 15 μm in diameter and of a cell 2 μm in diameter. What are the consequences of these differences in surface-to-volume ratio for cell function?
2. Assume you are given two cultures, one of a species of gram-negative *Bacteria* and one of a species of *Archaea*. Discuss at least four different ways you could tell which culture was which.
3. Calculate the amount of time it would take a cell of *Escherichia coli* (1×2 μm) swimming at maximum speed (60 cell lengths per second) to travel all the way up a 3-cm-long capillary tube containing a chemical attractant.

Chapter Glossary

Archaellum a long, thin cellular appendage present in many *Archaea* that rotates and is responsible for swimming motility

Basal body the "motor" portion of the bacterial flagellum, embedded in the cytoplasmic membrane and cell wall

Capsule a polysaccharide or protein outermost layer, usually rather slimy, present on some bacteria

Chemotaxis directed movement of an organism toward (positive chemotaxis) or away from (negative chemotaxis) a chemical gradient

Chloroplast the photosynthetic organelle of phototrophic eukaryotes

Cristae the internal membranes of a mitochondrion

Cytoplasmic membrane the permeability barrier of the cell, separating the cytoplasm from the environment

Cytoskeleton the cellular scaffolding typical of eukaryotic cells in which microtubules, microfilaments, and intermediate filaments define the cell's shape

Dipicolinic acid a substance unique to endospores that confers heat resistance on these structures

Endospore a highly heat-resistant, thick-walled, differentiated structure produced by certain gram-positive *Bacteria*

Endosymbiotic hypothesis the idea that mitochondria and chloroplasts originated from *Bacteria*

Flagellum a long, thin cellular appendage that rotates (in *Bacteria*) or has a whiplike motion (in *Eukarya*) and is responsible for swimming motility

Gas vesicles gas-filled cytoplasmic structures bounded by protein and conferring buoyancy on cells

Histones highly basic proteins that compact and wind DNA in the nucleus of eukaryotic cells

Hydrogenosome an organelle of endosymbiotic origin present in certain microbial eukaryotes that oxidizes pyruvate to H_2, CO_2, and acetate, and couples this to ATP synthesis

Intermediate filament a filamentous polymer of fibrous keratin proteins, supercoiled into thicker fibers, that functions in maintaining cell shape and the positioning of certain organelles in the eukaryotic cell

Lipopolysaccharide (LPS) a combination of lipid with polysaccharide and protein that forms the major portion of the outer membrane in gram-negative *Bacteria*

Lysosome an organelle containing digestive enzymes for hydrolysis of proteins, fats, and polysaccharides

Magnetosome a particle of magnetite (Fe_3O_4) enclosed by a nonunit membrane in the cytoplasm of magnetotactic *Bacteria*

Meiosis the nuclear division that halves the diploid number of chromosomes to the haploid

Microfilament a filamentous polymer of the protein actin that helps maintain the shape of a eukaryotic cell

Microtubule a filamentous polymer of the proteins α-tubulin and β-tubulin that functions in eukaryotic cell shape and motility

Mitochondrion the respiratory organelle of eukaryotic organisms

Mitosis nuclear division in eukaryotic cells in which chromosomes are replicated and partitioned into two daughter cells during cell division

Morphology the *shape* of a cell—rod, coccus, spirillum, and so on

Nucleus the organelle that contains the eukaryotic cell's chromosomes

Outer membrane a phospholipid- and polysaccharide-containing unit membrane that lies external to the peptidoglycan layer in cells of gram-negative *Bacteria*

Peptidoglycan a polysaccharide composed of alternating repeats of *N*-acetylglucosamine and *N*-acetylmuramic acid arranged in adjacent layers and cross-linked by short peptides

Periplasm a gel-like region between the outer surface of the cytoplasmic membrane and the inner surface of the lipopolysaccharide layer of gram-negative *Bacteria*

Peritrichous flagellation having flagella located in many places around the surface of the cell

Phototaxis movement of an organism toward light

Pili thin, filamentous structures that extend from the surface of a cell and, depending on type, facilitate cell attachment, genetic exchange, or twitching motility

Polar flagellation having flagella emanating from one or both poles of the cell

Poly-β-hydroxybutyric acid (PHB) a common storage material of prokaryotic cells consisting of a polymer of β-hydroxybutyrate or another β-alkanoic acid or mixtures of β-alkanoic acids

S-layer an outermost cell surface layer composed of protein or glycoprotein present on some *Bacteria* and *Archaea*

Stroma the lumen of the chloroplast, surrounded by the inner membrane

Teichoic acid a phosphorylated polyalcohol found in the cell wall of some gram-positive *Bacteria*

Thylakoid a membrane layer containing the photosynthetic pigments in chloroplasts

Microbial Metabolism

3

microbiologynow

Sugars and Sweets: *Archaea* Do It Their Way

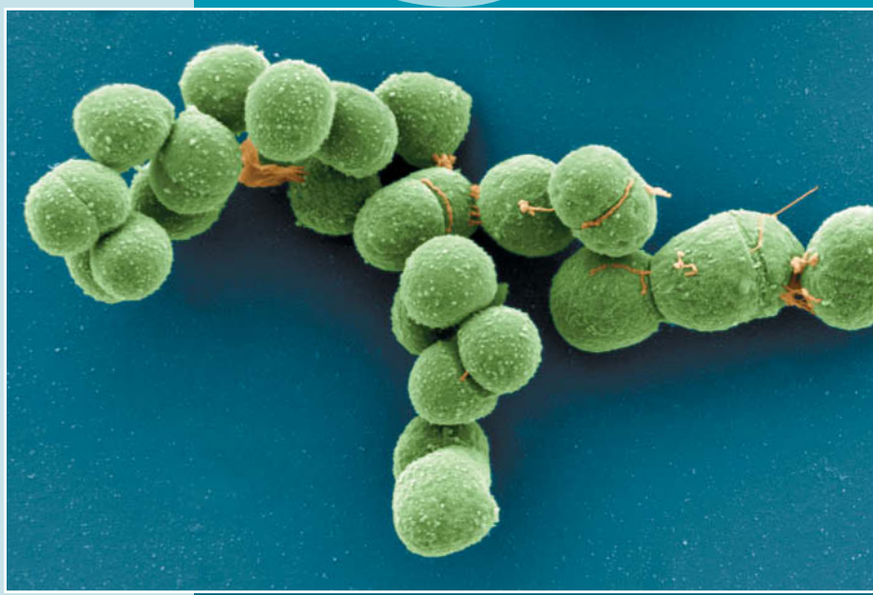
Ever since the 1977 proposal by Carl Woese and George Fox to group life into three domains—*Bacteria, Archaea,* and *Eukarya*—new discoveries with the *Archaea* keep emphasizing their unique biology. Their novel lipids, lack of peptidoglycan, and many eukaryotic-like traits are just some of the fundamental properties that set *Archaea* apart from the other domains of life. However, recent studies have shown that even some "housekeeping biochemistry" in *Archaea* differs from that in the other domains, and a good example is the metabolism of sugars.

Studies of sugar metabolism in *Archaea* have focused on species of the phylum *Crenarchaeota*, most of which grow at extremely high temperatures, and the phylum *Euryarchaeota*, many of which, like the cells of *Halococcus* (see photo), grow at extremely high salt concentrations. Several species in these two phyla can use glucose and some other sugars as carbon and energy sources. But whether sugars are used or not, all *Archaea* must be able to metabolize sugars because they form the backbone of cell structures such as cell walls and macromolecules such as nucleic acids.

Biochemical studies have shown that some of the most fundamental pathways of carbohydrate metabolism—such as glycolysis—are absent in *Archaea* and that instead, modified variants of this mainstream pathway are present. Another important sugar-metabolizing pathway, the pentose phosphate pathway, is absent in some species of *Archaea* but is present in others in a truncated form distinct from the classical pathway of *Bacteria* and *Eukarya*. Several new enzymes have evolved to run these unique archaeal pathways, and their catalytic activities are often regulated in unusual ways.

The discovery that *Archaea* have evolved distinct pathways for such fundamental metabolic transformations is yet another indication that this domain, first established on the basis of gene sequences, is indeed a phylogenetically distinct entity. Now the question arises as to *why* these biochemical pathways in *Archaea* differ so much from those in cells of the two other domains. Stay tuned, as the most exciting part of the story likely lies ahead.

Source: Bräsen, C., D. Esser, B. Rauch, and B. Slebers. 2014. Carbohydrate metabolism in *Archaea*: Current insights into unusual enzymes and pathways and their regulation. *Microbiol. Mol. Biol. Rev. 78*: 89–175.



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IV Biosyntheses 132

I • Microbial Nutrients and Nutrient Uptake

Metabolism is the series of biochemical reactions by which the cell breaks down or biosynthesizes various metabolites. In order to grow, cells must incorporate nutrients from their environment, transform them into precursor molecules, and then use them to construct a new cell. In this chapter we examine some of these processes, with a focus on three areas: (1) defining the basic nutrients of life, (2) exploring seminal metabolic pathways and alternative metabolic lifestyles, (3) and biosynthesizing the building blocks of macromolecules. We will use some of the principles developed here in Chapter 4, where we explore how informational macromolecules—the nucleic acids and proteins—are biosynthesized, and in Chapter 14, where the enormous metabolic diversity of the microbial world unfolds.

3.1 Feeding the Microbe: Cell Nutrition

Because the metabolic capacities of microbes differ, their nutrient requirements also differ. However, all microbes require a core set of nutrients. Some nutrients, called *macronutrients*, are required in large amounts, while others, called *micronutrients*, are required in minute amounts. We begin by dissecting the cell to reveal its chemical composition and then consider the nutrients that all cells require.

Chemical Makeup of a Cell

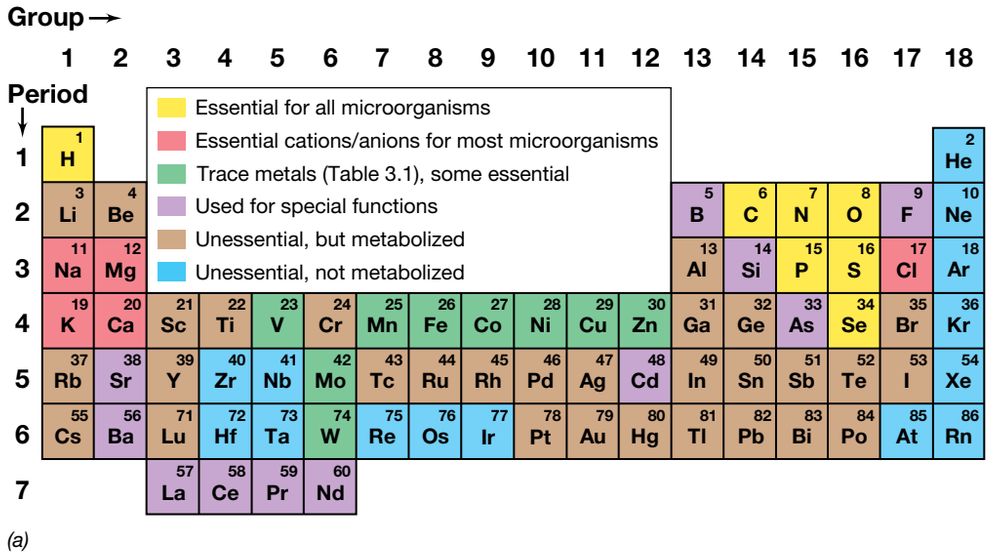
Just a handful of the chemical elements predominate in living systems: hydrogen (H), oxygen (O), carbon (C), nitrogen (N), phosphorus (P), sulfur (S), and selenium (Se). C is needed in the largest amount (50% of a cell's dry weight), O and H are next (combined, 25% of dry weight), and N follows (13%). Although required, P, S, K, Mg, and Se combine for less than 5% of a cell's dry weight. In addition to these, at least 50 other elements either are required by one or more microorganisms or, if not required, are still metabolized in some way (**Figure 3.1**).

About 75% of the wet weight of a microbial cell (a single cell of *Escherichia coli* weighs just 10^{-12} g) is water, and the remainder is primarily macromolecules—proteins, nucleic acids, lipids, and polysaccharides (Figure 3.1*b*). The building blocks of these macromolecules are the amino acids, nucleotides, fatty acids, and sugars, respectively. Proteins dominate the macromolecular composition of a cell, and the diversity of proteins exceeds that of all other macromolecules combined (Figure 3.1*c*). Interestingly, as important as DNA is to a cell (the cell genome), it contributes a very small percentage of a cell's dry weight; RNA is far more abundant (Figure 3.1*c*).

Carbon, Nitrogen, and Other Macronutrients

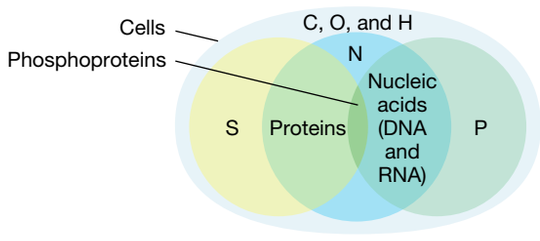
All cells require carbon and nitrogen in large amounts, and most prokaryotic cells require organic compounds as their source of carbon. Cells obtain organic carbon from the breakdown of polymeric substances or from the direct uptake of their monomeric constituents: the amino acids, fatty acids, organic acids, sugars, nitrogen bases, and aromatic and other organic compounds. Some microbes are autotrophs and can synthesize their own organic compounds from carbon dioxide (CO_2). The bulk of nitrogen available in nature is as ammonia (NH_3), nitrate (NO_3^-), or nitrogen gas (N_2). Virtually all microorganisms can use NH_3 as their nitrogen source and many can also use NO_3^-; some microbes can use organic nitrogen sources, such as amino acids; and a few can use N_2 (the nitrogen-fixing bacteria).

In addition to C and N (and O and H from H_2O), many other macronutrients are needed by cells but typically in smaller amounts (Figure 3.1). Phosphorus is required for nucleic acids and phospholipids and is usually incorporated as phosphate (PO_4^{2-}). Sulfur is present



(a)

Elemental composition of informational macromolecules



(b)

Macromolecular composition of a cell

Macromolecule	Percent of dry weight
Protein	55
Lipid	9.1
Polysaccharide	5.0
Lipopolysaccharide	3.4
DNA	3.1
RNA	20.5

(c)

Figure 3.1 Elemental and macromolecular composition of a bacterial cell. *(a)* A microbial periodic table of the elements. With the exception of those shown in row 7, other elements in row 7 and elements in rows beyond row 7 are not known to be metabolized. *(b)* Distribution of the major elements in informational macromolecules. *(c)* Relative abundance of macromolecules in a bacterial cell. Data from Escherichia coli *and* Salmonella typhimurium: *Cellular and Molecular Biology.* ASM, Washington, DC (1996).

in the amino acids cysteine and methionine and also in several vitamins, including thiamine, biotin, and lipoic acid, and is commonly incorporated as sulfate (SO_4^{2-}), sulfide (H_2S), or organic S compounds. Potassium (K) is required for the activity of several enzymes, whereas magnesium (Mg) stabilizes ribosomes, membranes, and nucleic acids and is also required for the activity of many enzymes. Calcium (Ca) and sodium (Na) are essential nutrients for only a few organisms, such as the NaCl requirement of most marine microorganisms.

Micronutrients: Trace Metals and Growth Factors

Microorganisms require several *metals* in very small amounts relative to macronutrients (Figure 3.1); chief among these is iron (Fe), which plays a major role in cellular respiration. Besides iron, many other metals may be required or otherwise metabolized by microorganisms (Figure 3.1*a*). Collectively these metals are called *trace metals* and typically function in the cell as cofactors of certain enzymes (Section 3.5). **Table 3.1** lists the major trace metals and other trace elements of life and examples of enzymes or other molecules in which each is found.

Growth factors differ from trace metals in that they are *organic* (rather than metallic) micronutrients (Table 3.1). Common growth factors include the vitamins, but amino acids, purines, pyrimidines, and several other organic molecules may be growth factors for one or another microorganism. Vitamins are the most frequently required growth factors and a few common ones are shown in Table 3.1. Most vitamins function as enzyme cofactors. Vitamin requirements vary among microorganisms, ranging from none to several. Lactic acid bacteria of the genera *Streptococcus, Lactobacillus*, and *Leuconostoc* inhabit nutrient-rich habitats such as dairy products and the animal gut (Sections 16.6 and 24.2); these bacteria are well known for their vitamin requirements, which are even more extensive than those of humans!

If a cell is to grow and divide, it must take up its macronutrients and micronutrients from the environment. But this process is not as easy as it may seem, primarily because of the impermeability of the cytoplasmic membrane and the fact that the concentration of a given nutrient in the cytoplasm must often be much higher than its concentration in the environment. We examine this situation now and see how cells have overcome these fundamental problems.

MINIQUIZ

- Which four chemical elements make up the bulk of a cell's dry weight?
- Which two classes of macromolecules contain most of a cell's nitrogen?
- Differentiate between trace metals and growth factors. How are these used by the cell?

3.2 Transporting Nutrients into the Cell

In Chapter 2 we learned how the structure of the cytoplasmic membrane is an effective barrier to leakage; solutes leak neither into nor out of a living cell. However, to fuel metabolism and support growth, cells need to import nutrients and export waste products

TABLE 3.1 Micronutrients needed by microorganisms[a]

I. Trace Elements		*II. Growth Factors*	
Element	*Function*	*Growth factor*	*Function*
Boron (B)	Autoinducer for quorum sensing in bacteria; also found in some polyketide antibiotics	PABA (*p*-aminobenzoic acid)	Precursor of folic acid
		Folic acid	One-carbon metabolism; methyl transfers
Cobalt (Co)	Vitamin B_{12}; transcarboxylase (only in propionic acid bacteria)	Biotin	Fatty acid biosynthesis; some CO_2 fixation reactions
Copper (Cu)	In respiration, cytochrome *c* oxidase; in photosynthesis, plastocyanin, some superoxide dismutases	B_{12} (Cobalamin)	One-carbon metabolism; synthesis of deoxyribose
Iron (Fe)[b]	Cytochromes; catalases; peroxidases; iron–sulfur proteins; oxygenases; all nitrogenases	B_1 (Thiamine)	Decarboxylation reactions
		B_6 (Pyridoxal)	Amino acid/keto acid transformations
Manganese (Mn)	Activator of many enzymes; component of certain superoxide dismutases and of the water-splitting enzyme in oxygenic phototrophs (photosystem II)	Nicotinic acid (Niacin)	Precursor of NAD^+
		Riboflavin	Precursor of FMN, FAD
Molybdenum (Mo)	Certain flavin-containing enzymes; some nitrogenases, nitrate reductases, sulfite oxidases, DMSO–TMAO reductases; some formate dehydrogenases	Pantothenic acid	Precursor of coenzyme A
		Lipoic acid	Decarboxylation of pyruvate and α-ketoglutarate
Nickel (Ni)	Most hydrogenases; coenzyme F_{430} of methanogens; carbon monoxide dehydrogenase; urease	Vitamin K	Electron transport
		Coenzymes M and B	Methanogenesis[c]
Selenium (Se)	Formate dehydrogenase; some hydrogenases; the amino acid selenocysteine	F_{420} and F_{430}	Methanogenesis[c]
Tungsten (W)	Some formate dehydrogenases; oxotransferases of hyperthermophiles		
Vanadium (V)	Vanadium nitrogenase; bromoperoxidase		
Zinc (Zn)	Carbonic anhydrase; nucleic acid polymerases; many DNA-binding proteins		

[a]Not all trace elements or growth factors are needed by all organisms, and many growth factors are biosynthesized and not required from the environment.
[b]Iron is typically needed in larger amounts than the other trace metals shown.
[c]The production of methane (CH_4) by methanogens (*Archaea*).

on a more or less continuous basis. To do this, several transport systems reside in the cytoplasmic membrane. We consider the most common of these systems here, with a focus on the well-studied transporters widespread in *Bacteria* and *Archaea*.

Active Transport and Transporters

Active transport is the process by which cells accumulate solutes against the concentration gradient, and three basic mechanisms of active transport are found in prokaryotic cells. **Simple transport** consists only of a transmembrane transport protein, **group translocation** employs a series of proteins in the transport event, and **ABC transport systems** consist of three components: a substrate-binding protein, a transmembrane transporter, and an ATP-hydrolyzing protein. Each of these transport systems is energy-driven, be it from the proton motive force, ATP, or some other energy-rich compound (Figure 3.2).

The transmembrane component of virtually all transport systems is composed of a polypeptide containing 12 domains that weave back and forth through the membrane to form a channel (see Figure 3.3), and it is through this channel that the solute is actually transported into the cell. Transport is linked to a conformational change in this transmembrane protein that occurs when it binds its specific solute. Like a gate swinging open, this conformational change sweeps the solute into the cell.

Simple Transporters and Group Translocation

Simple transport reactions are driven by the energy inherent in the proton motive force (Sections 2.3 and 3.11). The major transport events catalyzed are either *symport* reactions (where a solute and a proton are cotransported in one direction) or *antiport* reactions (where a solute and a proton are transported in opposite directions) (Figure 3.3). A classic example of a simple transporter is the uptake of the disaccharide sugar lactose by way of the *lac permease*, a well-studied symporter in *Escherichia coli*. As each lactose molecule enters the cell, the potential energy in the proton motive force is diminished slightly by the cotransport of a proton (Figure 3.3). The net result is the energy-driven accumulation of lactose in the cytoplasm against the concentration gradient. Many other solutes enter by the activity of their own simple symporters, including phosphate, sulfate, and several different organic compounds.

Group translocation differs from simple transport in two important ways: (1) the transported substance is *chemically modified* during the transport process, and (2) an energy-rich organic compound (rather than the proton motive force) drives the transport event. The best-studied group translocation systems transport the sugars glucose, mannose, and fructose in *E. coli*. During uptake, these compounds are phosphorylated by the *phosphotransferase system*. The phosphotransferase system consists of a family of five proteins that work in concert to transport any given sugar. Before the sugar is transported, the proteins in the phosphotransferase system are themselves alternately phosphorylated and dephosphorylated in a cascading fashion until Enzyme II_c phosphorylates the sugar as it enters the cytoplasm (Figure 3.4). A protein called *HPr*, the enzyme that phosphorylates HPr (Enzyme I), and Enzyme II_a are all cytoplasmic proteins. By contrast, Enzyme II_b is a peripheral membrane protein and Enzyme II_c is the transmembrane component.

In the phosphotransferase system, HPr and Enzyme I are nonspecific components and participate in the uptake of several different sugars. By contrast, distinct Enzyme II proteins exist, one set for each different sugar transported. Energy to drive the phosphotransferase system comes from phosphoenolpyruvate, an energy-rich intermediate in glycolysis (Sections 3.7 and 3.8).

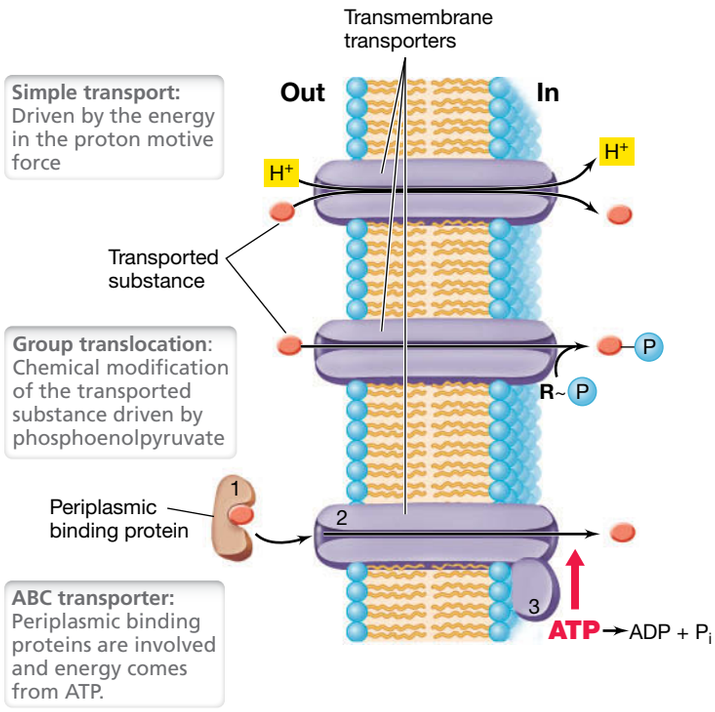


Figure 3.2 The three classes of transport systems. Note how simple transporters and the ABC system transport substances without chemically modifying them, whereas group translocation results in chemical modification (in this case phosphorylation) of the transported substance. The three proteins of the ABC system are labeled 1, 2, and 3.

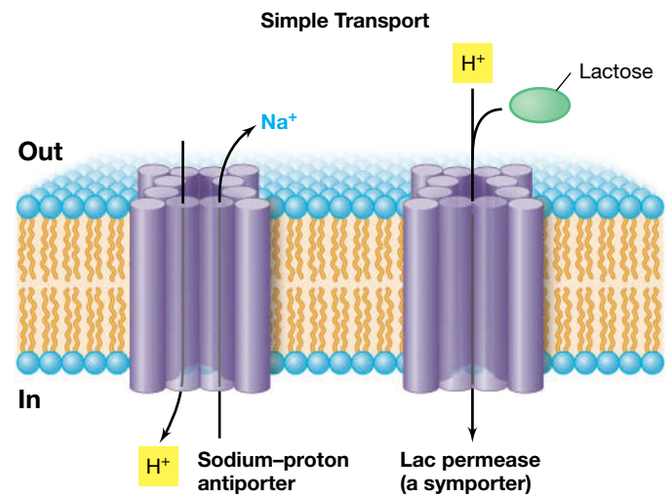


Figure 3.3 Structure of membrane-spanning transporters and symport and antiport events. Transmembrane transporters are composed of a polypeptide that forms 12 α-helices (each shown as a cylinder) that aggregate to form a channel through the membrane. Note how transport is linked to dissipation of the proton motive force.

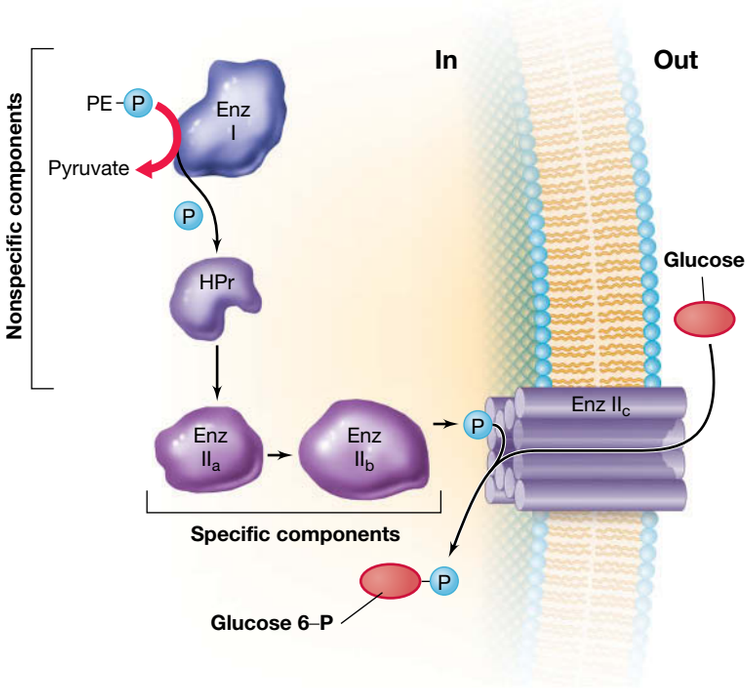


Figure 3.4 Mechanism of the phosphotransferase system of *Escherichia coli*. For glucose transport, the system consists of five proteins: Enzyme (Enz) I, Enzymes II_a, II_b, and II_c, and HPr. A phosphate cascade occurs from phosphoenolpyruvate (PE-P) to Enz II_c and the latter actually transports and phosphorylates the sugar. Proteins HPr and Enz I are nonspecific and participate in the transport of any sugar. By contrast, the three components of Enz II are specific for a particular sugar.

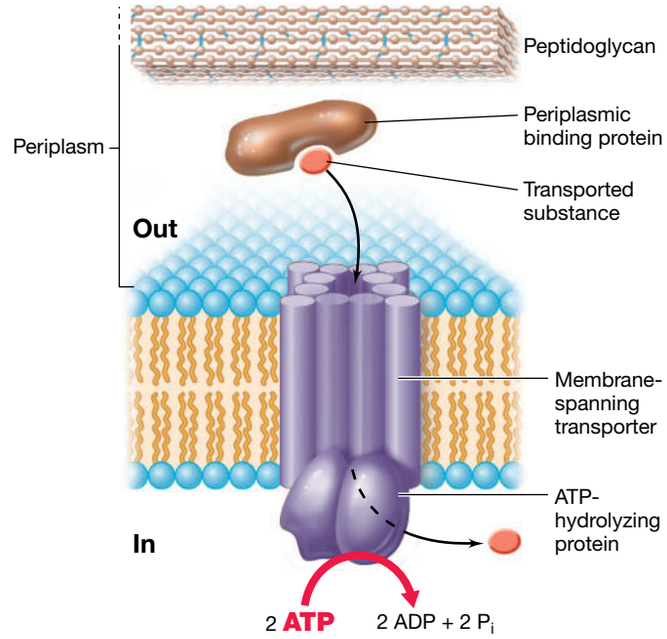


Figure 3.5 Mechanism of an ABC transporter. The periplasmic binding protein has high affinity for substrate, the membrane-spanning proteins form the transport channel, and the cytoplasmic ATP-hydrolyzing proteins supply the energy for the transport event.

Periplasmic Binding Proteins and the ABC System

We learned in Chapter 2 that gram-negative bacteria contain a region called the *periplasm* that lies between the cytoplasmic membrane and the *outer membrane*, part of the gram-negative cell wall (Section 2.5). The periplasm is home to proteins that carry out different functions including transport; the latter is catalyzed by the activity of *periplasmic binding proteins*. Transport systems that employ a periplasmic binding protein along with transmembrane and ATP-hydrolyzing components are called ABC transport systems, the "ABC" standing for *A*TP-*b*inding *c*assette, a structural feature of proteins that bind ATP (**Figure 3.5**). More than 200 different ABC transport systems have been identified in various *Bacteria*, and these catalyze the uptake of a wide variety of organic and inorganic compounds.

A characteristic property of periplasmic binding proteins is their extremely high substrate affinity. These proteins can bind their specific substrate even when it is present at extremely low concentration; for example, less than 1 micromolar (10^{-6} M). Once its specific substrate is bound, the periplasmic binding protein interacts with its respective transmembrane component to transport the substrate into the cell driven by the energy in ATP (Figures 3.2 and 3.5).

Although gram-positive bacteria and most *Archaea* lack a periplasm, they also have ABC transport systems. In gram-positive bacteria, substrate-binding proteins (the functional equivalent of periplasmic binding proteins) are attached to the external surface of the cytoplasmic membrane. Once they bind their substrate, these proteins interact with their transmembrane component to catalyze the ATP-driven uptake of the substrate. ABC systems are also present in a variety of *Archaea* and are primarily employed for the transport of sugars.

MINIQUIZ

- Compare and contrast simple transporters, the phosphotransferase system, and ABC transporters in terms of (1) energy source, (2) chemical alterations of the solute during transport, and (3) number of proteins required.
- Which major characteristic of ABC transport systems makes them ideal for organisms living in nutrient-poor environments?

II • Energetics, Enzymes, and Redox

If a microorganism has all the nutrients it needs and has transported them into its cell, it must next conserve some of the energy released in energy-yielding reactions in order to grow. Here we discuss the different options for energy conservation and use some basic laws of chemistry and physics to guide our understanding of bioenergetics.

3.3 Energy Classes of Microorganisms

Energy-yielding reactions are that part of metabolism called **catabolism**. Here we discuss the various catabolic energy classes of microorganisms, pointing out their similarities and differences.

The terms used to describe the energy classes of microorganisms are important ones and will appear many times in this book.

Chemoorganotrophs, Chemolithotrophs, and Phototrophs

Organisms that conserve energy from chemicals are called *chemotrophs*, and those that use *organic* chemicals are called **chemoorganotrophs** (Figure 3.6). Most microorganisms in laboratory culture are chemoorganotrophs. A wide variety of organic compounds can be catabolized during chemoorganotrophic metabolism, and some of the energy released during their oxidation is conserved in the energy-rich bonds of adenosine triphosphate—ATP, the cell's energy currency—or related energy-rich compounds (see Section 3.7 and Figure 3.13).

Many *Bacteria* and *Archaea* can tap the energy available from the oxidation of *inorganic* compounds, and this form of catabolism is called *chemolithotrophy*. Organisms that carry out chemolithotrophic reactions are called **chemolithotrophs** (Figure 3.6). Several inorganic compounds can be oxidized, for example, gaseous hydrogen (H_2), hydrogen sulfide (H_2S), ammonia (NH_4^+), and ferrous iron (Fe^{2+}). Related groups of chemolithotrophs typically specialize in the oxidation of a group of related inorganic compounds, and thus we have the "sulfur" bacteria, the "iron" bacteria, the "nitrifying" bacteria, and so on.

Phototrophs contain chlorophylls and other pigments that convert light energy into ATP and thus, unlike chemotrophs, do not require chemicals as a source of energy. Two forms of phototrophy are known in *Bacteria*. In one form, called *oxygenic photosynthesis*, oxygen (O_2) is produced. Oxygenic photosynthesis is characteristic of cyanobacteria, a major lineage of *Bacteria,* and is also carried out by algae (eukaryotic microbes). The other form of phototrophy, *anoxygenic photosynthesis*, occurs in at least six phylogenetic lineages of *Bacteria*, including the purple and green bacteria, the heliobacteria, and many others. In anoxygenic photosynthesis, O_2 is not produced. Nevertheless, many strong parallels exist between the mechanisms that underlie anoxygenic and oxygenic photosynthesis, and it is clear that the oxygenic process evolved from the anoxygenic process. We explore these relationships in Chapters 13 and 14.

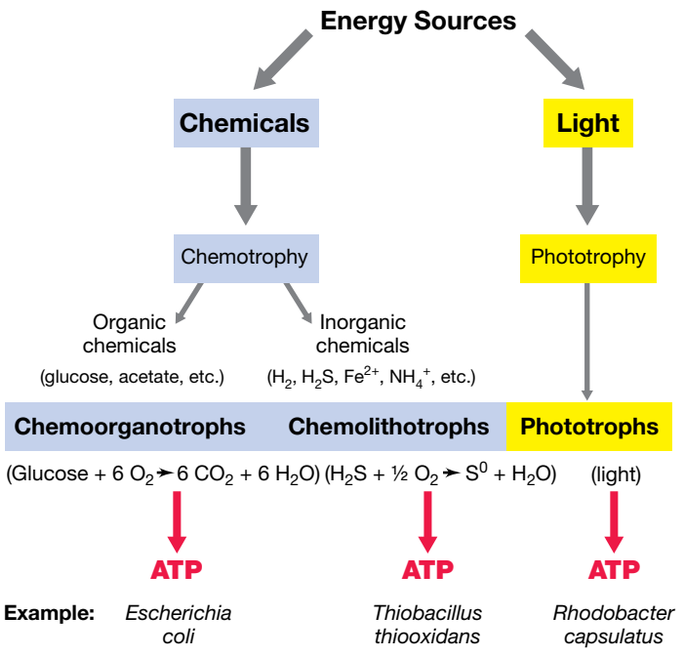


Figure 3.6 Metabolic options for conserving energy by microorganisms. Most organisms employ only one option, but some have two and a few rare species can tap into all three forms of energy conservation.

Heterotrophs and Autotrophs

Regardless of how a microorganism conserves energy, be it from chemicals or from light, all cells require large amounts of carbon in one form or another to make new cell materials (Section 3.1). If an organism is a **heterotroph**, its cell carbon is obtained from one or another organic compound. An **autotroph**, by contrast, uses carbon dioxide (CO_2) as its carbon source. Chemoorganotrophs (Figure 3.6) are by definition also heterotrophs. By contrast, most chemolithotrophs and phototrophs (Figure 3.6) are autotrophs. Autotrophs are also called *primary producers* because they synthesize new organic matter from inorganic carbon (CO_2). Virtually all organic matter on Earth has been synthesized by primary producers, in particular, the phototrophs. The **Calvin cycle** is the major biochemical pathway by which phototrophic organisms incorporate CO_2 into cell material, although we will see in Chapter 14 that many other pathways exist, especially among prokaryotic autotrophs.

MINIQUIZ

- How does a chemoorganotroph differ from a chemolithotroph? A chemotroph from a phototroph?
- How does an autotroph differ from a heterotroph?

3.4 Principles of Bioenergetics

We have just reviewed the options that microbes have in terms of energy conservation: chemoorganotrophy, chemolithotrophy, and phototrophy. But exactly how is the energy available from these processes conserved by the cell? We consider these issues here using chemoorganotrophic metabolism as our model system.

Energy is defined as the ability to do work, and in microbiology, energy transformations are measured in kilojoules (kJ), a unit of heat energy. All chemical reactions in a cell are accompanied by *changes* in energy, energy being either *required* or *released* as a reaction proceeds. To identify which reactions release energy and which require energy, we first need to understand some basic bioenergetic principles.

Basic Bioenergetics

In microbiology we are interested in **free energy** (abbreviated G), which is *the energy available to do work*. Free energy released during a reaction can be conserved by cells in the form of ATP and a handful of other energy-rich substances. The *change* in free energy during a reaction is expressed as $\Delta G^{0\prime}$, where the symbol Δ is read as "change in." The "0" and "prime" in $\Delta G^{0\prime}$ indicate that the

free-energy value is for *standard conditions*: pH 7 (approximate cytoplasmic conditions), 25°C, 1 atmosphere of pressure, and all reactants and products at molar concentrations.

Consider the reaction

$$A + B \rightarrow C + D$$

If the $\Delta G^{0\prime}$ for this reaction is *negative* in arithmetic sign, then the reaction will proceed with the *release* of free energy; such reactions are said to be **exergonic**. However, if $\Delta G^{0\prime}$ is *positive*, the reaction *requires* energy in order to proceed and such reactions are **endergonic**. Thus, exergonic reactions *release* free energy whereas endergonic reactions *require* free energy. With these essentials in hand, how do we calculate $\Delta G^{0\prime}$?

Free Energy of Formation and Calculating Free-Energy Changes ($\Delta G^{0\prime}$)

To calculate the free-energy yield of a reaction, one first needs to know the free energy inherent in the reactants and products of the reaction. This is the *free energy of formation* (G_f^0), the energy released or required during the formation of a given molecule from the elements. Table 3.2 lists the G_f^0 for a few common substances. By convention, the free energy of formation of the *elements* in their elemental and electrically neutral form (for instance, C, H_2, N_2) is zero. However, the free energies of formation of *compounds* are not zero. If the formation of a compound from its elements proceeds exergonically (free energy released), then the G_f^0 of the compound is negative. If the reaction is endergonic (free energy required), then the G_f^0 of the compound is positive.

For most compounds G_f^0 is negative. This reflects the fact that compounds tend to form spontaneously (that is, with a free-energy release) from their elements. However, the positive G_f^0 for nitrous oxide (N_2O) (Table 3.2) indicates that this compound does not form spontaneously. Instead, over time it decomposes spontaneously to yield N_2 and O_2. The compounds listed in Table 3.2 are only a small subset of free energy of formation values available from physical chemistry reference sources.

Using free energies of formation, it is possible to calculate $\Delta G^{0\prime}$ of a reaction. For the reaction A + B → C + D, $\Delta G^{0\prime}$ is calculated by subtracting the sum of the free energies of formation of the reactants (A + B) from that of the products (C + D). Thus

$$\Delta G^{0\prime} = G_f^0[C + D] - G_f^0[A + B]$$

The value obtained for $\Delta G^{0\prime}$ tells us whether the reaction is exergonic (and can be a potential energy source for the cell) or endergonic (and requires an energy input to proceed). The phrase "products minus reactants" is a simple way to recall how to calculate changes in free energy during chemical reactions.

Before free-energy calculations can be made, it is first necessary to balance the reaction. That is, (1) the total number of each kind of atom and ionic charges must be identical on both sides of the reaction, and (2) the oxidation–reduction state must balance such that all of the electrons removed from one substance are transferred to another substance. Once a reaction is balanced, its $\Delta G^{0\prime}$ can be calculated, and from this, the potential of the reaction as a means of energy conservation for a cell can be assessed.

$\Delta G^{0\prime}$ versus ΔG

Although calculations of $\Delta G^{0\prime}$ are often very accurate estimates of actual free-energy changes, in some cases they are not. We will see when we pick up the bioenergetics theme again in Chapter 14 that the actual concentrations of products and reactants in a microbe's natural habitat, which are rarely at the levels used in calculations of $\Delta G^{0\prime}$, can sometimes change the results of bioenergetic calculations

TABLE 3.2 Free energy of formation (G_f^0, kJ/mol) for some common substances[a]

Sugars	*Organic and fatty acids*	*Amino acids and alcohols*	*Gases and inorganic compounds*
Fructose (–951.4)	Acetate (–369.4)	Alanine (–371.5)	O_2, N_2, H_2, S^0, Fe^0 (0)
Glucose (–917.2)	Benzoate (–245.6)	Aspartate (–700.4)	CH_4 (–50.8)
Lactose (–1515.2)	Butyrate (–352.6)	*n*-Butanol (–171.8)	CO_2 (–394.4); CO (–137.4)
Ribose (–369.4)	Caproate (–335.9)	Ethanol (–181.7)	H_2O (–237.2); H^+ (–39.8); OH^- (–198.7)
Sucrose (–757.3)	Citrate (–1168.3)	Glutamate (–699.6)	N_2O (+104.2); NO (+86.6)
	Formate (–351.1)	Glutamine (–529.7)	NO_2^- (–37.2); NO_3^- (–111.3)
	Fumarate (–604.2)	Glycerol (–488.5)	NH_3 (–26.57); NH_4^+ (–79.4)
	Glyoxylate (–468.6)	Mannitol (–942.6)	H_2S (–27.87); HS^- (+12.1)
	Ketoglutarate (–797.5)	Methanol (–175.4)	SO_4^{2-} (–744.6); $S_2O_3^{2-}$ (–513.4)
	Lactate (–517.8)	*n*-Propanol (–175.8)	Fe^{2+} (–78.8); Fe^{3+} (–4.6); FeS (–100.4)
	Malate (–845.1)		
	Propionate (–361.1)		
	Pyruvate (–474.6)		
	Succinate (–690.2)		
	Valerate (–344.3)		

[a]Values for free energy of formation taken from Speight, J. 2005. *Lange's Handbook of Chemistry*, 16th edition, and Thauer, R.K., K. Jungermann, and H. Decker. 1977. Energy conservation in anaerobic chemotrophic bacteria. *Bacteriol. Rev. 41:* 100–180.

in significant ways. In this regard, what is most relevant to a bioenergetic calculation is not $\Delta G^{0\prime}$, but ΔG, the free-energy change that occurs *under the actual conditions* in which the organism is growing. The equation for ΔG takes into account the actual concentrations of reactants and products in the organism's habitat and is expressed as

$$\Delta G = \Delta G^{0\prime} + RT \ln K_{eq}$$

where R and T are physical constants and K_{eq} is the equilibrium constant for the reaction. For the reaction aA + bB → cC + dD, $K_{eq} = [C]^c[D]^d/[A]^a[B]^b$, where A and B are reactants and C and D are products; *a, b, c*, and *d* are the number of molecules of each; and brackets indicate concentrations.

The reason that ΔG rather than $\Delta G^{0\prime}$, may be a more accurate estimate of bioenergetic processes is that the products of a reaction carried out by one microbe are typically consumed by the activities of other microorganisms. In most cases, this does not significantly affect $\Delta G^{0\prime}$. However, in some cases, the consumption of products is so aggressive that it can drive K_{eq} values to less than 1; the logarithm of such a number will be negative in arithmetic sign. Then, when ΔG is calculated, a reaction that might have been endergonic under standard conditions can become exergonic under the actual conditions present in the microbial habitat. We will see examples of this in Chapter 14, especially in reactions in which H_2—a product of syntrophic metabolisms—is consumed by other microbes to levels that approach zero.

So, although $\Delta G^{0\prime}$ and ΔG are not always identical, at this point in our understanding of microbial bioenergetics the expression $\Delta G^{0\prime}$ is all we need to deal with in order to appreciate energy flow in microbial systems. The main point to keep in mind is that only reactions that are exergonic yield energy that can be conserved by the cell, and this will be our focus in the next few sections.

MINIQUIZ

- What is free energy?
- Does glucose formation from the elements release or require energy?
- Using Table 3.2, calculate $\Delta G^{0\prime}$ for the reaction $CH_4 + \frac{1}{2} O_2 \rightarrow CH_3OH$.

3.5 Catalysis and Enzymes

Free-energy calculations reveal only whether energy is released or required in a given reaction; they say nothing about the *rate* of the reaction. If the rate of a reaction is very slow, it may be of no value to a cell. For example, consider the formation of water from O_2 and H_2. The energetics of this reaction are quite favorable: $H_2 + \frac{1}{2} O_2 \rightarrow H_2O$, $\Delta G^{0\prime} = -237$ kJ. However, if O_2 and H_2 were mixed in a sealed bottle, no measurable amount of water would form, even after years. This is because the bonding of O_2 and H_2 to form H_2O requires that these two gases become reactive. This requires that their bonds be broken and requires a small amount of energy. This energy is called **activation energy**.

Activation energy can be viewed as the minimum energy required for a chemical reaction to begin. For an exergonic reaction, the situation is as shown in Figure 3.7. Although the activation energy barrier is virtually insurmountable in the absence of a *catalyst*—a substance that facilitates a reaction but is not consumed by it—in the presence of a proper catalyst, this barrier is reduced, allowing the reaction to proceed.

Enzymes

Catalysts function by lowering the activation energy of a reaction (Figure 3.7), thereby increasing the reaction rate. Catalysts have no effect on the energetics or the equilibrium of a reaction but only affect the *rate* at which a reaction proceeds. Most cellular reactions will not proceed at significant rates without catalysis.

The major catalysts in cells are **enzymes**, which are proteins (or in a few cases, RNAs) that are highly specific for the reactions they catalyze. This specificity is a function of the precise three-dimensional structure of the enzyme. In an enzyme-catalyzed reaction, the enzyme combines with the reactant, called a *substrate*, forming an *enzyme–substrate complex*. Then, as the reaction proceeds, the *product* is released and the enzyme is returned to its original state, ready to catalyze a new round of the reaction (Figure 3.8). The enzyme is generally much larger than the substrate(s), and the portion of the enzyme to which substrate binds is the enzyme's *active site*; the entire enzymatic reaction, from substrate binding to product release, may take only a few milliseconds.

Many enzymes contain small nonprotein molecules that participate in catalysis but are not themselves substrates. These small molecules can be divided into two classes based on the way they associate with the enzyme: *prosthetic groups* and *coenzymes*. Prosthetic groups bind tightly to their enzymes, usually covalently and permanently. The heme group present in cytochromes such as cytochrome *c* (Section 3.10) is an example of a prosthetic group. By contrast, **coenzymes**, with a few exceptions, are loosely and often transiently bound to enzymes; thus, a single coenzyme molecule may associate with a number of different enzymes. Most coenzymes are derivatives of vitamins (Table 3.1).

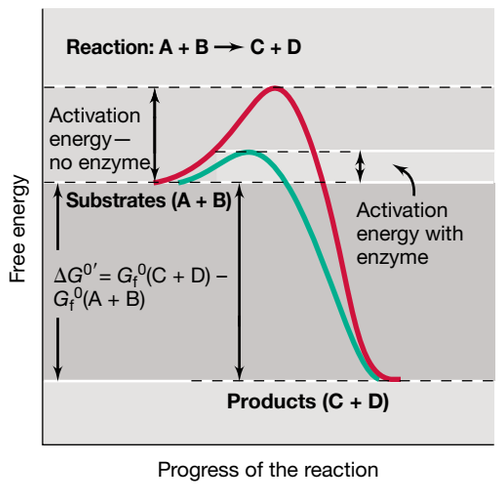


Figure 3.7 Activation energy and catalysis. Even chemical reactions that release energy may not proceed spontaneously if not activated. Once the reactants are activated, the reaction proceeds spontaneously. Catalysts such as enzymes lower the required activation energy.

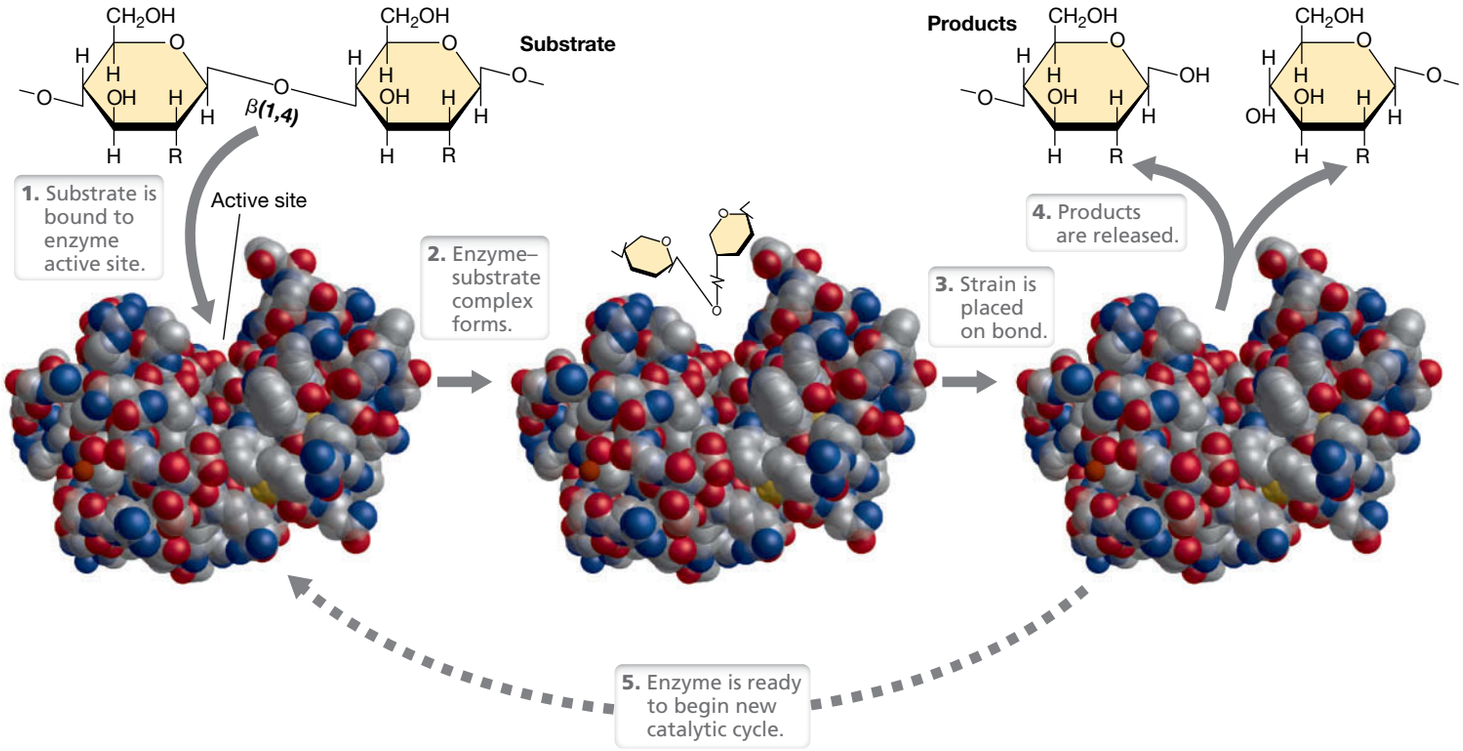


Figure 3.8 The catalytic cycle of an enzyme. The enzyme depicted here, lysozyme, catalyzes the cleavage of the β-1,4-glycosidic bond in the polysaccharide backbone of peptidoglycan. Following substrate binding in the enzyme's active site, strain is placed on the bond, and this favors breakage. Space-filling model of lysozyme courtesy of Richard Feldmann.

Enzyme Catalysis

To catalyze a reaction, an enzyme must bind its substrate and position it properly in its active site. The enzyme–substrate complex (Figure 3.8) aligns reactive groups in the substrate(s) and places strain on specific bonds. This reduces the activation energy required to make the reaction proceed (Figure 3.7). This is shown in Figure 3.8 for the enzyme lysozyme, an enzyme whose substrate is the polysaccharide backbone of peptidoglycan, the bacterial cell wall polymer (↬ Section 2.4).

The reaction depicted in Figure 3.7 is exergonic. By contrast, some enzymes catalyze endergonic reactions where they convert energy-poor substrates into energy-rich products. In these cases not only must an activation energy barrier (Figure 3.7) be overcome, but sufficient free energy must also be put into the reaction in order to raise the energy level of the substrates to that of the products. This is done by *coupling* the energy-requiring reaction to an energy-yielding one, such as the hydrolysis of ATP or dissipation of the proton motive force (Section 3.11), so that the overall reaction proceeds with a free-energy change that is either negative in arithmetic sign or zero.

Theoretically, all enzymes are reversible in their activity. However, enzymes that catalyze highly exergonic or highly endergonic reactions typically function in only one direction. If a particularly exergonic or endergonic reaction needs to be reversed, a different enzyme usually catalyzes the reverse reaction.

MINIQUIZ

- What is the function of a catalyst? What are enzymes made of?
- Where on an enzyme does the substrate bind?
- What is activation energy?

3.6 Electron Donors and Acceptors

Cells conserve energy released from exergonic reactions by coupling the reaction to the biosynthesis of energy-rich compounds, such as ATP. Reactions that release sufficient energy to form ATP require oxidation–reduction biochemistry. An *oxidation* is the removal of an electron (or electrons) from a substance, and a *reduction* is the addition of an electron (or electrons) to a substance. In microbiology, the term *redox* is used as shorthand for oxidation–reduction.

Redox Reactions

Redox reactions occur in pairs. For example, H_2 can release electrons and protons and become oxidized (**Figure 3.9**). However, electrons cannot exist alone in solution; they must be part of atoms or molecules. Thus the oxidation of H_2 is only a *half reaction*, a term that implies the need for a second half reaction to complete the overall reaction. The second half reaction is a reduction, in which another substance is reduced to form the coupled redox reaction (Figure 3.9).

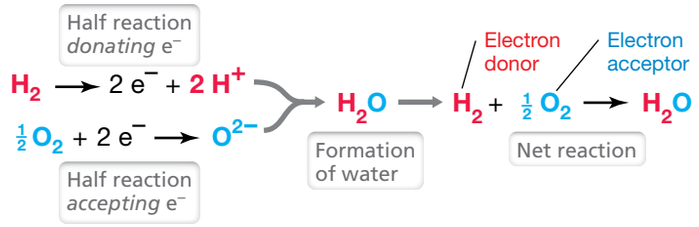


Figure 3.9 Example of an oxidation–reduction reaction. Oxidations are the removal of electrons from a substance, while reductions are the addition of electrons to a substance.

In redox reactions of this type, we refer to the substance *oxidized* (in this case, H_2) as the **electron donor**, and the substance *reduced* (in this case, O_2) as the **electron acceptor** (Figure 3.9). Many different electron donors exist in nature, including a wide variety of organic and inorganic compounds. Many electron acceptors other than O_2 exist as well, including many nitrogen and sulfur compounds, such as NO_3^- and SO_4^{2-}, and many organic compounds (Figure 14.25). As we continue our journey through this book we will see that the concept of electron donors and electron acceptors is very important for understanding microbial physiology, diversity, and ecology, and underlies all aspects of cellular energy metabolism.

Reduction Potentials and Redox Couples

Many substances can be *either* an electron donor *or* an electron acceptor, depending on the substance they couple with in a redox reaction. The constituents on each side of the arrow in half reactions are called a *redox couple*, such as 2 H^+/H_2, or $\frac{1}{2}$ O_2/H_2O in Figure 3.9. By convention, in writing a redox couple, the *oxidized* form of the couple is always placed on the left (before the forward slash) followed by the *reduced* form after the forward slash.

Substances differ in their tendency to donate or accept electrons. This tendency is expressed as their **reduction potential** (E_0', standard conditions), a value measured in volts (V) compared with that of a reference substance, H_2 (see Figure 3.10). Moreover, in biology, reduction potentials are listed for half reactions *written as reductions* at pH 7. In the example of Figure 3.9, the E_0' of the 2 H^+/H_2 couple is −0.42 V and that of the $\frac{1}{2}$ O_2/H_2O couple is +0.82 V. We will learn shortly that these values mean that O_2 is an excellent *electron acceptor* and H_2 is an excellent *electron donor*.

When two redox couples react, the *reduced* substance of the couple whose E_0' is *more negative* donates electrons to the *oxidized* substance of the couple whose E_0' is *more positive*. Thus, in the couple 2 H^+/H_2, H_2 has a greater tendency to donate electrons than does 2 H^+ to accept them, and in the couple $\frac{1}{2}$ O_2/H_2O, H_2O has a poor tendency to donate electrons while O_2 has a strong tendency to accept them. It thus follows that in a redox reaction of H_2 and O_2, *H_2 will be the electron donor* and become oxidized, and *O_2 will be the electron acceptor* and become reduced (Figure 3.9).

Although all half reactions are written as reductions, in an actual reaction between two redox couples, the half reaction with the more negative E_0' proceeds as an oxidation and is therefore written in the opposite direction. For example, in the reaction between H_2 and O_2, H_2 is oxidized and is written in the

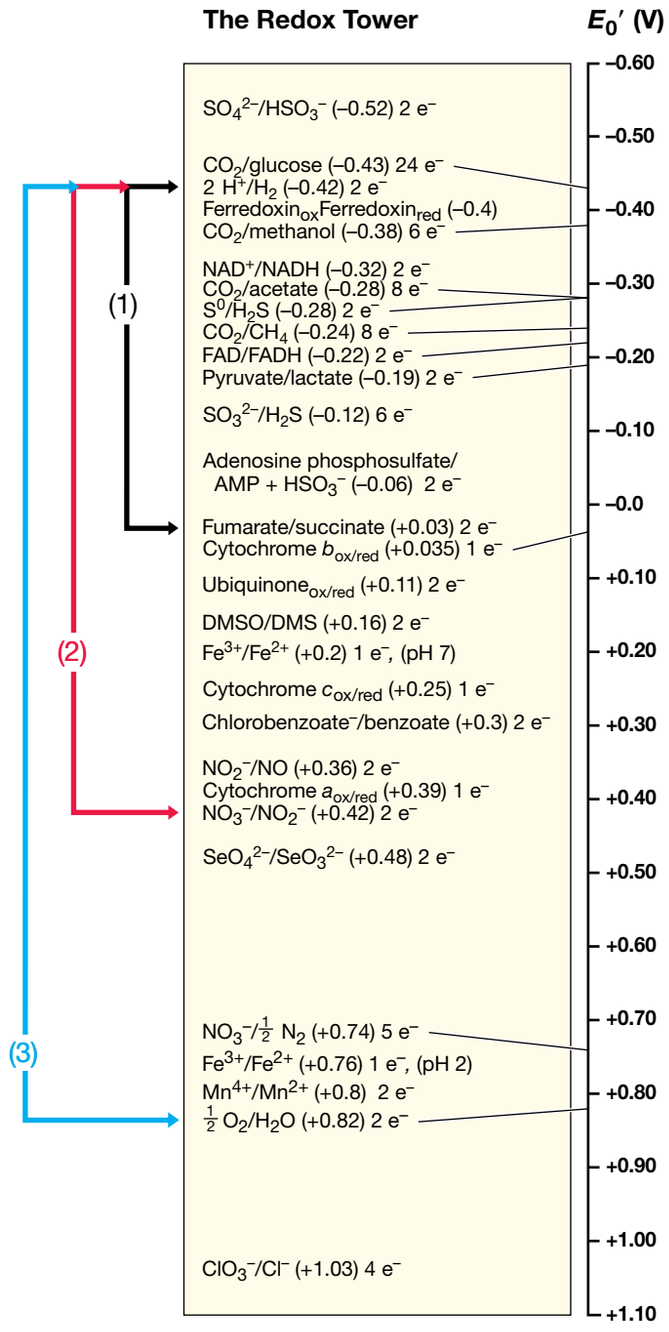


Figure 3.10 The redox tower. Redox couples are arranged from the strongest donors at the top to the strongest acceptors at the bottom. The larger the difference in reduction potential between electron donor and electron acceptor, the more free energy is released. Note the differences in energy yield when H_2 reacts with three different electron acceptors: fumarate (1), nitrate (2), and oxygen (3). The redox tower is placed in the context of anaerobic respiration in Figure 14.25.

TABLE 3.3 Example of free-energy-change calculations using G_f values or electrochemical potentials

For the reaction in which acetate is oxidized completely to CO_2:[a]

$$CH_3COO^- + H^+ + 2\,O_2 \rightarrow 2\,CO_2 + 2\,H_2O$$

1. Calculation from G_f values:
   $\Delta G^{0\prime} = [G_f(\text{products}) - G_f(\text{reactants})]$
   $= [G_f(2\,CO_2 + 2\,H_2O) - G_f\,(CH_3\,COO^- + H^+ + 2\,O_2)]$
   $= -852$ kJ/reaction

2. Calculation from the Nernst equation:[b]
   $\Delta G^{0\prime} = -nF\Delta E_0{}'$
   $= [-8(96.5)(1.1)]$
   $= -849$ kJ/reaction

[a] The reaction is balanced and is an 8-electron oxidation ($n = 8$ in equation 2). G_f^0 values were taken from Table 3.2.
[b] F is the Faraday constant (96.5 kJ/V) and $\Delta E_0{}'$ is calculated from the $E_0{}'$ values in Figure 3.10.

reverse direction from its formal half reaction (Figure 3.9 and see Figure 3.10).

The Redox Tower and Its Relationship to $\Delta G^{0\prime}$

A convenient way of viewing electron transfer reactions is to imagine a vertical tower that represents the entire range of reduction potentials possible for redox couples in nature, from those with the most negative $E_0{}'$ on the top to those with the most positive $E_0{}'$ at the bottom; this is a *redox tower* (**Figure 3.10**). Now imagine electrons from an electron donor near the top of the tower falling and being "caught" by electron acceptors at different levels. The difference in reduction potential between the donor and acceptor redox couples is expressed as $\Delta E_0{}'$.

The further an electron drops before it is caught by an acceptor, the greater is the $\Delta E_0{}'$ between the two redox couples and the greater is the amount of energy released in the net reaction. That is, $\Delta E_0{}'$ is proportional to $\Delta G^{0\prime}$ (Figure 3.10). This relationship is expressed more precisely in the Nernst equation (an expression that equates free energy with electrochemistry), $\Delta G^{0\prime} = -nF\Delta E_0{}'$, where n is the number of electrons transferred and F is the Faraday constant (96.5 kJ/V). We thus see that the $\Delta G^{0\prime}$ of a given redox reaction can be calculated in two ways: from knowledge of the equilibrium constant of the reaction (Section 3.4) or by knowing the $\Delta E_0{}'$ of the two half reactions that make up the full redox reaction. This is illustrated for an example reaction, the oxidation of acetate to CO_2, in **Table 3.3**.

Oxygen (O_2), at the bottom of the redox tower, is the strongest electron acceptor of significance in nature. In the middle of the redox tower, redox couples can be either electron donors or acceptors depending on whom they react with. For instance, the $2\,H^+/H_2$ couple (–0.42 V) can react with the fumarate/succinate couple (+0.03 V), the NO_3^-/NO_2^- couple (+0.42 V), or the $\frac{1}{2}\,O_2/H_2O$ (+0.82 V) couple, with increasing amounts of free energy being released, respectively, because $\Delta G^{0\prime} \propto \Delta E_0{}'$ ($\propto$ means "is proportional to") (Figure 3.10).

Electron Carriers and NAD^+/NADH Cycling

Redox reactions are typically facilitated by coenzymes that associate with the redox enzymes that catalyze the reaction. A very common redox coenzyme is nicotinamide adenine dinucleotide, or

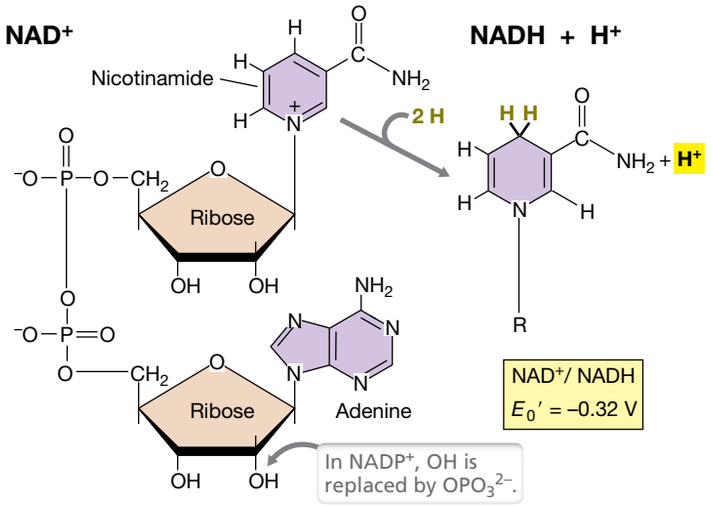


Figure 3.11 The redox coenzyme nicotinamide adenine dinucleotide (NAD^+) and $NADP^+$. NAD^+ undergoes oxidation–reduction as shown and is freely diffusible. "R" is the adenine dinucleotide portion of NAD^+.

NAD^+ for short, whose reduced form is NADH (**Figure 3.11**). NAD^+/NADH is an electron plus proton carrier, transporting 2 e^- and 2 H^+ simultaneously. The reduction potential of the NAD^+/NADH couple is –0.32V, which places it fairly high on the electron tower; that is, NADH is a good electron donor while NAD^+ is a rather weak electron acceptor (Figure 3.10).

Coenzymes such as NAD^+/NADH increase the diversity of redox reactions that are possible in a cell by allowing many different electron donors and acceptors to interact, with the coenzyme being the redox intermediary that services the different enzymes involved (**Figure 3.12**). For example, electrons removed from an electron donor by an enzyme that oxidizes that donor are used to reduce NAD^+ to NADH. The NADH then diffuses away from the enzyme and attaches to a different enzyme that oxidizes NADH back to NAD^+ when it reduces an electron acceptor (Figure 3.12).

Electron shuttling mediated by NAD^+/NADH is common in microbial catabolism. However, in addition to NAD^+/NADH, many other redox coenzymes may participate as electron shuttles. For example, the redox coenzyme nicotinamide adenine dinucleotide phosphate ($NADP^+$) is made from NAD^+ by adding a phosphate molecule. $NADP^+$ (and its reduced form NADPH) participates in anabolic redox reactions (biosynthesis of cellular precursors, Sections 3.14 and 3.15), whereas NAD^+/NADH participates in catabolic redox reactions, many of which we will explore in Sections 3.8–3.12.

MINIQUIZ

- In the reaction $H_2 + \frac{1}{2}\,O_2 \rightarrow H_2O$, what is the electron donor and what is the electron acceptor?
- Why is nitrate (NO_3^-) a better electron acceptor than fumarate?
- Is NADH a better electron donor than H_2? Is NAD^+ a better acceptor than 2 H^+? How does Figure 3.10 tell you this?

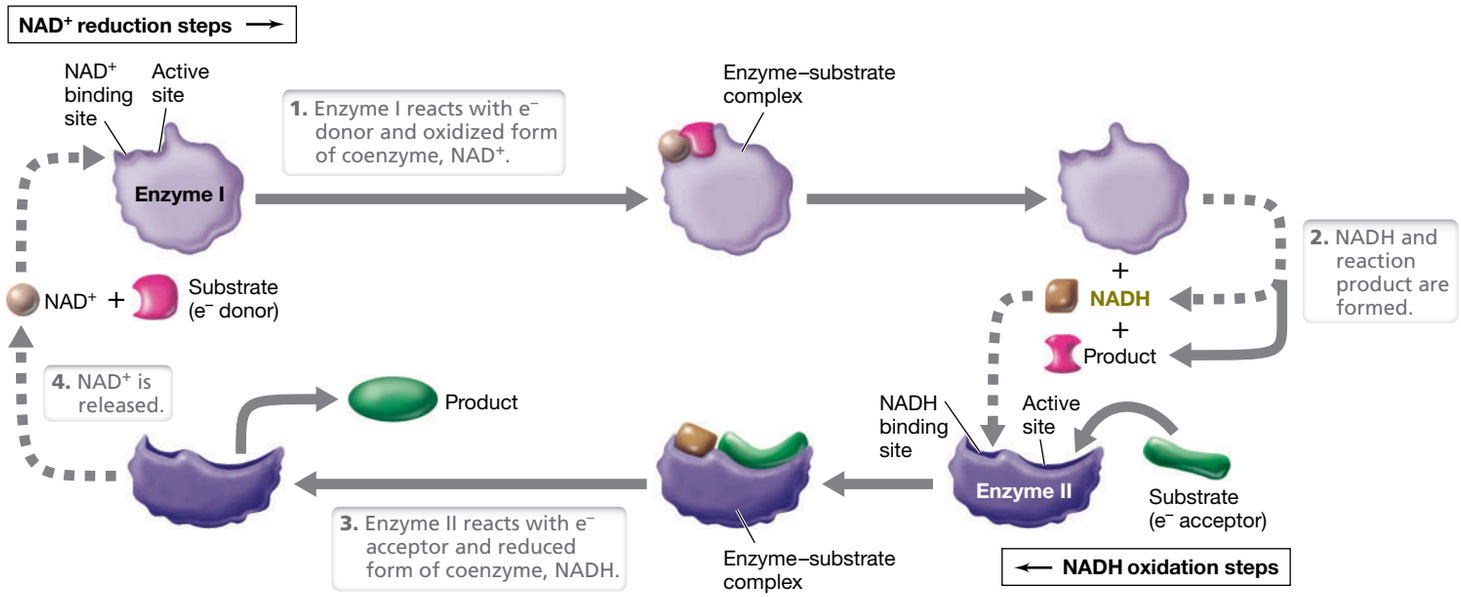


Figure 3.12 NAD⁺/NADH cycling. A schematic example of redox reactions in which two different enzymes are linked by their requirement for either NAD^+ or NADH.

3.7 Energy-Rich Compounds

The energy released from redox reactions fuels energy-requiring cell functions. But the free energy released in the coupled exergonic redox reaction must first be trapped by the cell and conserved. Energy conservation in cells is accomplished through the formation of a set of compounds containing *energy-rich* phosphate or sulfur bonds. The biosynthesis of these compounds functions as the free-energy trap, and their hydrolysis releases this energy to drive endergonic reactions.

Phosphate can be bonded to organic compounds by either *ester* or *anhydride* bonds, as illustrated in **Figure 3.13**. However, not all phosphate bonds are energy-rich. As seen in this figure, the $\Delta G^{0\prime}$ of hydrolysis of the phosphate *ester* bond in glucose 6-phosphate is −13.8 kJ/mol. By contrast, the $\Delta G^{0\prime}$ of hydrolysis of the phosphate *anhydride* bond in phosphoenolpyruvate is −51.6 kJ/mol, almost four times that of glucose 6-phosphate. Although the phosphate in either compound could be hydrolyzed in energy metabolism, cells typically use compounds whose $\Delta G^{0\prime}$ of phosphate hydrolysis exceeds −30 kJ/mol as energy "currencies" in the cell. Thus, phosphoenolpyruvate is energy-rich, whereas glucose 6-phosphate is not (Figure 3.13).

Adenosine Triphosphate

The most important energy-rich phosphate compound in cells is **adenosine triphosphate (ATP)**. ATP consists of the ribonucleoside adenosine to which three phosphate molecules are bonded in series. From the structure of ATP (Figure 3.13), it can be seen that only two of the phosphate bonds (ATP → ADP + P_i and ADP → AMP + P_i) are phosphoanhydrides and thus have free energies of hydrolysis greater than −30 kJ/mol. By contrast, AMP is not energy-rich because its free energy of hydrolysis is only about half that of ADP or ATP (Figure 3.13).

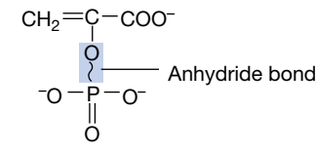
Although the energy released in ATP hydrolysis is −32 kJ/mol, a caveat must be mentioned here to more precisely state the energy requirements for the synthesis of ATP. In an actively growing *Escherichia coli* cell, the ratio of ATP to ADP is maintained at about 7:1, and this increases the actual free-energy requirements for ATP synthesis. Thus, in an actively growing cell, the actual energy expenditure (that is, the ΔG, Section 3.4) for the synthesis of ATP is more like −55 to −60 kJ/mol. Nevertheless, for the purposes of learning and applying the basic principles of bioenergetics, we will assume that reactions conform to "standard conditions" ($\Delta G^{0\prime}$), and thus that the energy required for the biosynthesis of ATP (from ADP + P_i) *or* hydrolysis of ATP (to ADP + P_i) is 32 kJ/mol.

Coenzyme A

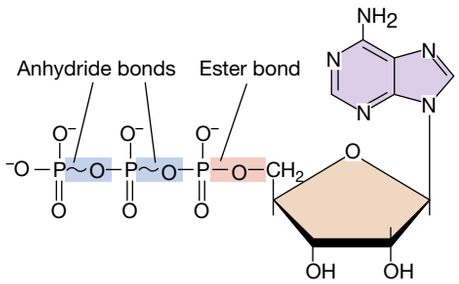
Cells can also use the free energy available in the hydrolysis of a handful of energy-rich compounds other than phosphorylated compounds. These include, in particular, derivatives of *coenzyme A* (for example, acetyl-CoA in Figure 3.13). Coenzyme A derivatives contain energy-rich *thioester* bonds, and hydrolysis of these bonds yields sufficient free energy to be coupled to the synthesis of an energy-rich phosphate bond. For example, in the coupled reaction

$$\text{Acetyl-S-CoA} + H_2O + \text{ADP} + P_i \rightarrow \text{acetate}^- + \text{HS-CoA} + \text{ATP} + H^+$$

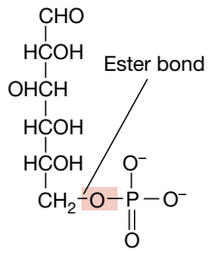
the energy released in the hydrolysis of coenzyme A is conserved in the synthesis of ATP. Coenzyme A derivatives (acetyl-CoA is one of many) are especially important in the energetics of anaerobic microorganisms, in particular those whose energy metabolism depends on fermentation (see Table 3.4). We will return to the importance of coenzyme A derivatives in microbial bioenergetics many times in Chapter 14.



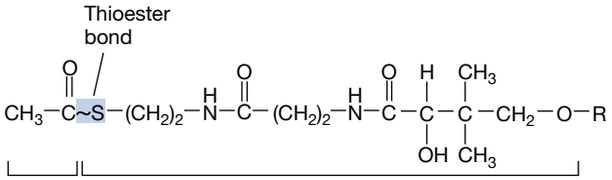
Phosphoenolpyruvate



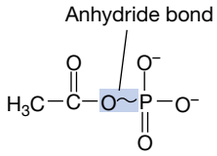
Adenosine triphosphate (ATP)



Glucose 6-phosphate



Acetyl-CoA



Acetyl phosphate

Compound	$G^{0'}$ kJ/mol
$\Delta G^{0'}$ > 30kJ	
Phosphoenolpyruvate	−51.6
1,3-Bisphosphoglycerate	−52.0
Acetyl phosphate	−44.8
ATP	−31.8
ADP	−31.8
Acetyl-CoA	−35.7
$\Delta G^{0'}$ < 30kJ	
AMP	−14.2
Glucose 6-phosphate	−13.8

Figure 3.13 Energy-rich bonds in compounds that conserve energy in microbial metabolism. Notice, by referring to the table, the range in free energy of hydrolysis of the phosphate or sulfur bonds highlighted in the compounds. The "R" group of acetyl-CoA is a 3′-phospho ADP group.

Energy Storage

ATP is a dynamic molecule in the cell; it is continuously broken down to drive anabolic reactions and resynthesized at the expense of catabolic redox reactions. For long-term energy storage, microorganisms typically produce insoluble polymers that can be catabolized later for the production of ATP.

Examples of energy storage polymers include glycogen (polyglucose), poly-β-hydroxybutyrate and other polyhydroxyalkanoates, and elemental sulfur, stored from the oxidation of H_2S by sulfur chemolithotrophs. These polymers are deposited within the cell as granules that can be seen with the light or electron microscope (Section 2.8). In eukaryotic microorganisms, starch (also a polymer of glucose) and simple fats are the major reserve materials. In the absence of an available energy source, a cell can break down these polymers to make new cell material or to supply the very low amount of energy, called *maintenance energy*, needed to maintain cell integrity when a cell is in a nongrowing state. This energy requirement, essentially the power necessary to maintain a bacterium in a living state, is thought to be about 1–100 zeptowatts (one zeptowatt equals 10^{-21} W) per cell, an indication of how little energy is actually necessary to keep a bacterial cell alive.

MINIQUIZ

- How much free energy is released when ATP is converted to ADP + P_i or when AMP is converted to adenosine and P_i?
- What is coenzyme A and why is it important?
- During periods of nutrient abundance, how can cells prepare for periods of nutrient starvation?

III • Catabolism: Fermentation and Respiration

We now explore some major catabolic pathways that result in energy conservation in chemoorganotrophs: fermentation and respiration. **Fermentation** is a form of anaerobic catabolism in which organic compounds both donate electrons and accept electrons, and redox balance is achieved without the need for external electron acceptors. By contrast, **respiration** is a form of aerobic or anaerobic catabolism in which an organic or inorganic electron donor is oxidized with O_2 (in aerobic respiration) or some other compound (in anaerobic respiration) functioning as electron acceptors. We will revisit the concepts of fermentation and respiration in detail in Chapter 14 and so focus here only on the basics.

3.8 Glycolysis and Fermentation

A nearly universal pathway for the catabolism of glucose is the *Embden–Meyerhof–Parnas pathway*, better known as **glycolysis**, a series of reactions in which glucose is oxidized to pyruvate. If glucose is ultimately respired, it is first catabolized through glycolysis before pyruvate is further oxidized to CO_2 in the citric acid cycle (Section 3.9). By contrast, if the glucose is fermented, pyruvate is not oxidized completely to CO_2 but instead is used as an electron acceptor to achieve redox balance in glycolysis.

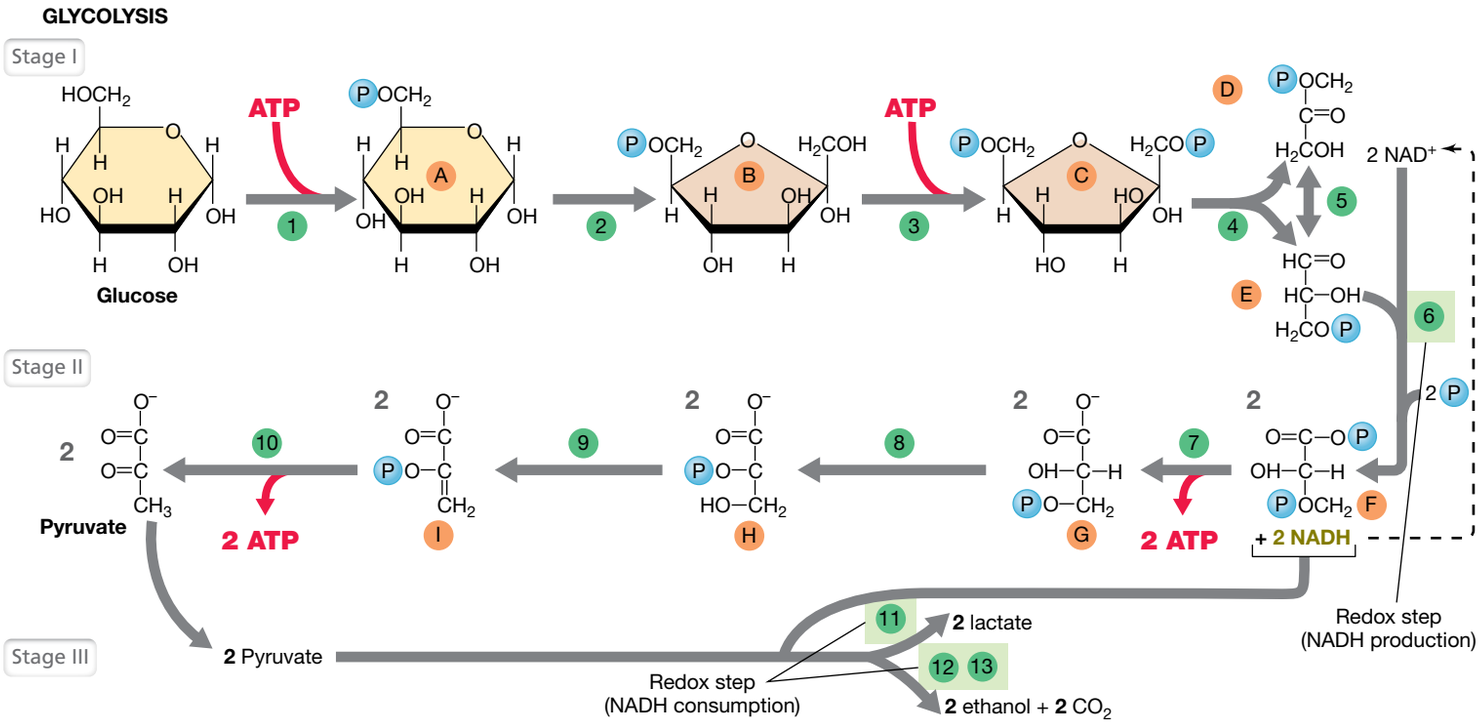
In the series of reactions that are glycolysis, two are redox reactions. During these reactions, free energy is released and is

conserved by the simultaneous production of energy-rich compounds (Figure 3.13). ATP is made from these energy-rich compounds by **substrate-level phosphorylation**, a process whereby the energy-rich phosphate bond on the organic compound is transferred directly to ADP to form ATP.

The Three Stages of Glycolysis

Glycolysis can be divided into three stages, each consisting of one or more enzymatic reactions. Stage I consists of "preparatory" reactions; these are not redox reactions and do not release energy but instead form a key intermediate of the pathway. In Stage II, redox reactions occur, energy is conserved, and two molecules of pyruvate are formed. In Stage III, redox balance is achieved and fermentation products are formed (Figure 3.14).

To begin glycolysis, glucose is phosphorylated to form glucose 6-phosphate. The latter is then isomerized to fructose 6-phosphate, and a second phosphorylation leads to the production of fructose 1,6-bisphosphate. These steps consume, rather than produce, ATP. The enzyme aldolase then splits fructose 1,6-bisphosphate into two 3-carbon molecules, *glyceraldehyde 3-phosphate* and its isomer, *dihydroxyacetone phosphate*, which is converted into glyceraldehyde 3-phosphate. To this point, all of the reactions, including the consumption of ATP, have proceeded without any redox changes (Figure 3.14).



GLYCOLYTIC INTERMEDIATES AND ENZYMES

Intermediates

- A Glucose 6-P
- B Fructose 6-P
- C Fructose 1,6-P
- D Dihydroxyacetone-P
- E Glyceraldehyde-3-P
- F 1,3-Bisphosphoglycerate
- G 3-P-Glycerate
- H 2-P-Glycerate
- I Phosphoenolpyruvate

Enzymes

1. Hexokinase
2. Isomerase
3. Phosphofructokinase
4. Aldolase
5. Triosephosphate isomerase
6. Glyceraldehyde-3-P dehydrogenase
7. Phosphoglycerokinase
8. Phosphoglyceromutase
9. Enolase
10. Pyruvate kinase
11. Lactate dehydrogenase
12. Pyruvate decarboxylase
13. Alcohol dehydrogenase

ENERGETICS

Organism	Fermentation	Energy conserved (kJ/mol)	Efficiency (ATP/kJ released)
Yeast	Glucose → 2 ethanol + 2 CO_2	−239	27%
Lactic acid bacteria	Glucose → 2 lactate	−196	33%

Figure 3.14 Embden–Meyerhof–Parnas pathway (glycolysis). (Top) The sequence of reactions in the catabolism of glucose to pyruvate and then on to fermentation products. Pyruvate is the end product of glycolysis, and fermentation products are made from it. (Bottom) Intermediates, enzymes, and contrasting fermentation balances of yeast and lactic acid bacteria.

The first redox reaction of glycolysis occurs when glyceraldehyde 3-phosphate is oxidized to 1,3-bisphosphoglyceric acid. In this reaction (which occurs twice, once for each of the two glyceraldehyde 3-phosphates), the enzyme glyceraldehyde-3-phosphate dehydrogenase reduces NAD^+ to NADH. Simultaneously, each glyceraldehyde 3-phosphate molecule is phosphorylated by the addition of a molecule of inorganic phosphate. This reaction, in which *inorganic* phosphate is converted to *organic* form, sets the stage for energy conservation, since 1,3-bisphosphoglyceric acid is an energy-rich compound (Figure 3.13). ATP is then synthesized by substrate-level phosphorylation when: (1) each molecule of 1,3-bisphosphoglyceric acid is converted to 3-phosphoglyceric acid, and (2) each molecule of phosphoenolpyruvate is converted to pyruvate (Figure 3.14). During the first two stages of glycolysis, *two* ATP molecules are consumed and *four* ATP molecules are synthesized. Thus, *the net energy yield in glycolysis is two molecules of ATP per molecule of glucose fermented*.

The final stage of glycolysis achieves redox balance. Recall that during the formation of the two 1,3-bisphosphoglyceric acid molecules, two NAD^+ were reduced to NADH (Figure 3.14). This NADH needs to be oxidized (returned to NAD^+) for the next round of glycolysis, and this occurs when pyruvate is reduced by an NADH-containing enzyme to fermentation products; during this reaction, NAD^+ is regenerated (Figure 3.14). In fermentation by yeast, pyruvate is reduced to ethanol (ethyl alcohol) and carbon dioxide (CO_2). By contrast, lactic acid bacteria reduce pyruvate to lactate. Pyruvate can be reduced to many other fermentation products (see next subsection), but the rationale for product formation is the same in each case: NADH must be reoxidized to NAD^+ to keep glycolysis in redox balance (Figure 3.14).

Fermentative Diversity

Not all compounds are inherently fermentable, but sugars—such as glucose and other hexoses as well as most disaccharides and other relatively small sugars—are preeminently fermentable. Since glucose is needed for glycolysis, sugars other than glucose must first be converted to glucose by isomerase enzymes. Polysaccharides such as cellulose and starch are also fermentable by bacteria that produce enzymes that attack these large molecules and produce sugars from them; if the latter are not glucose, they must first be converted to glucose before they enter glycolysis.

Different types of fermentations are classified by either the substrate fermented or the products formed, and Table 3.4 summarizes some major fermentations of glucose on the basis of the products formed. All of the organisms listed in Table 3.4 (except for the bacterium *Zymomonas*) use the glycolytic pathway to catabolize glucose, the major difference in the fermentations being in the products formed from pyruvate to achieve redox balance.

In addition to the two (net) ATP produced in glycolysis (Figure 3.14), some of the fermentations listed in Table 3.4 allow for additional ATP synthesis by substrate-level phosphorylation. This is possible if the fermentation product is a fatty acid because the fatty acid is formed from its coenzyme-A precursor. Recall that CoA derivatives of fatty acids, such as acetyl-CoA, are energy-rich (Section 3.7 and Figure 3.13). Thus, when *Clostridium butyricum* forms butyric acid, the final step is

$$\text{Butyryl-CoA} + \text{ADP} + P_i \rightarrow \text{butyric acid} + \text{ATP} + \text{CoA}$$

The formation of a coenzyme-A derivative during a fermentation increases the yield of ATP, although the yield increase falls far shy of what is possible by glucose respiration (Section 3.9).

Many organic compounds other than sugars can be fermented and may or may not require glycolytic reactions. For instance, some endospore-forming species of *Clostridium* ferment amino acids while others ferment purines and pyrimidines, the products of nucleic acid degradation. Some fermentative anaerobes even ferment aromatic compounds. In most of these cases, the formation of CoA fatty acid derivatives in the fermentative pathway is key to energy conservation.

Some fermentations are catalyzed by only a small group (or even a single species) of anaerobic bacteria. For example, the coupled fermentation of ethanol and acetate is apparently carried out in nature only by the bacterium *Clostridium kluyveri* (Table 3.4).

TABLE 3.4 Common fermentations and some of the organisms carrying them out

Type	*Reaction (substrate → products)*	*Organisms*
Alcoholic	Hexose[a] → 2 ethanol + 2 CO_2	Yeast, *Zymomonas*
Homolactic	Hexose → 2 $lactate^-$ + 2 H^+	*Streptococcus*, some *Lactobacillus*
Heterolactic	Hexose → $lactate^-$ + ethanol + CO_2 + H^+	*Leuconostoc*, some *Lactobacillus*
Propionic acid	3 $Lactate^-$ → 2 $propionate^-$ + $acetate^-$ + CO_2 + H_2O	*Propionibacterium*, *Clostridium propionicum*
Mixed acid[b,c]	Hexose → ethanol + 2,3-butanediol + $succinate^{2-}$ + $lactate^-$ + $acetate^-$ + $formate^-$ + H_2 + CO_2	Enteric bacteria including *Escherichia, Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter*
Butyric acid[c]	Hexose → $butyrate^-$ + 2 H_2 + 2 CO_2 + H^+	*Clostridium butyricum*
Butanol[c]	2 Hexose → butanol + acetone + 5 CO_2 + 4 H_2	*Clostridium acetobutylicum*
Caproate/Butyrate	6 Ethanol + 3 $acetate^-$ → 3 $butyrate^-$ + $caproate^-$ + 2 H_2 + 4 H_2O + H^+	*Clostridium kluyveri*
Acetogenic	Fructose → 3 $acetate^-$ + 3 H^+	*Clostridium aceticum*

[a]Glucose is the starting substrate for glycolysis. However, many other C_6 sugars (hexoses) can be fermented following their conversion to glucose. Except for *Zymomonas*, all organisms catabolize glucose by the glycolytic pathway.
[b]Not all organisms produce all products. In particular, butanediol production is limited to only certain enteric bacteria. The reaction is not balanced.
[c]Other products include some acetate and a small amount of ethanol (butanol fermentation only).

Organisms such as *C. kluyveri* are metabolic specialists, having evolved the capacity to ferment organic compounds not readily catabolized anaerobically by other bacteria. Nevertheless, these fermenters are ecologically important because of their role in the degradation of dead plants, animals, and microorganisms in anoxic environments in nature. We explore these unusual fermentations in more detail in Chapter 14.

Human Benefits of Fermentation and the Fermentation–Respiration Switch

As we have seen, during glycolysis, glucose is consumed, ATP is made, and fermentation products are generated (Figure 3.14). For the organism the crucial product is ATP, fermentation products being merely waste products that must be discarded. However, fermentation products are not waste products to humans. Instead, they are the foundation of the baking and fermented beverage industries (**Figure 3.15**) and are key ingredients in many other fermented foods, such as the lactic and other acids in fermented dairy products (yogurt, sour cream, buttermilk, and the like), cheeses, pickles, and certain sausages and fish products. In the baking and alcohol industries, the metabolic capacities of the key catalyst, the baker's and brewer's yeast *Saccharomyces cerevisiae*, take center stage. However, *S. cerevisiae* can carry out two modes of glucose catabolism, *fermentation*, as we have discussed, and *respiration*, which we consider in the next section.

Cells that can both ferment and respire metabolize in whichever way most benefits them energetically. In this regard, the energy available from a molecule of glucose is much greater if it is respired to CO_2 than if it is fermented. This is because in contrast to CO_2, organic fermentation products such as ethanol or lactic acid discarded by the fermenter still contain a significant amount of free energy. Thus, when O_2 is available, yeast cells respire glucose rather than ferment it. But the key here is O_2 availability; only when conditions are anoxic do yeasts carry out fermentation. This fact has practical significance. Since the brewer and baker need the *products* of yeast fermentation (Figure 3.15) rather than the cells themselves, care must be taken to ensure that the yeast is forced into a fermentative lifestyle. If air is available, respiration will occur, and we see why this is the case over the course of the next three sections.

Barton Spear

Figure 3.15 **Common food and beverage products resulting from the alcoholic fermentation of *Saccharomyces cerevisiae*.**

MINIQUIZ

- Which reactions in glycolysis are redox steps?
- What is the role of NAD^+/NADH in glycolysis?
- Why are fermentation products made during glycolysis? What major fermentation product is present in fermented milk products?

3.9 Respiration: Citric Acid and Glyoxylate Cycles

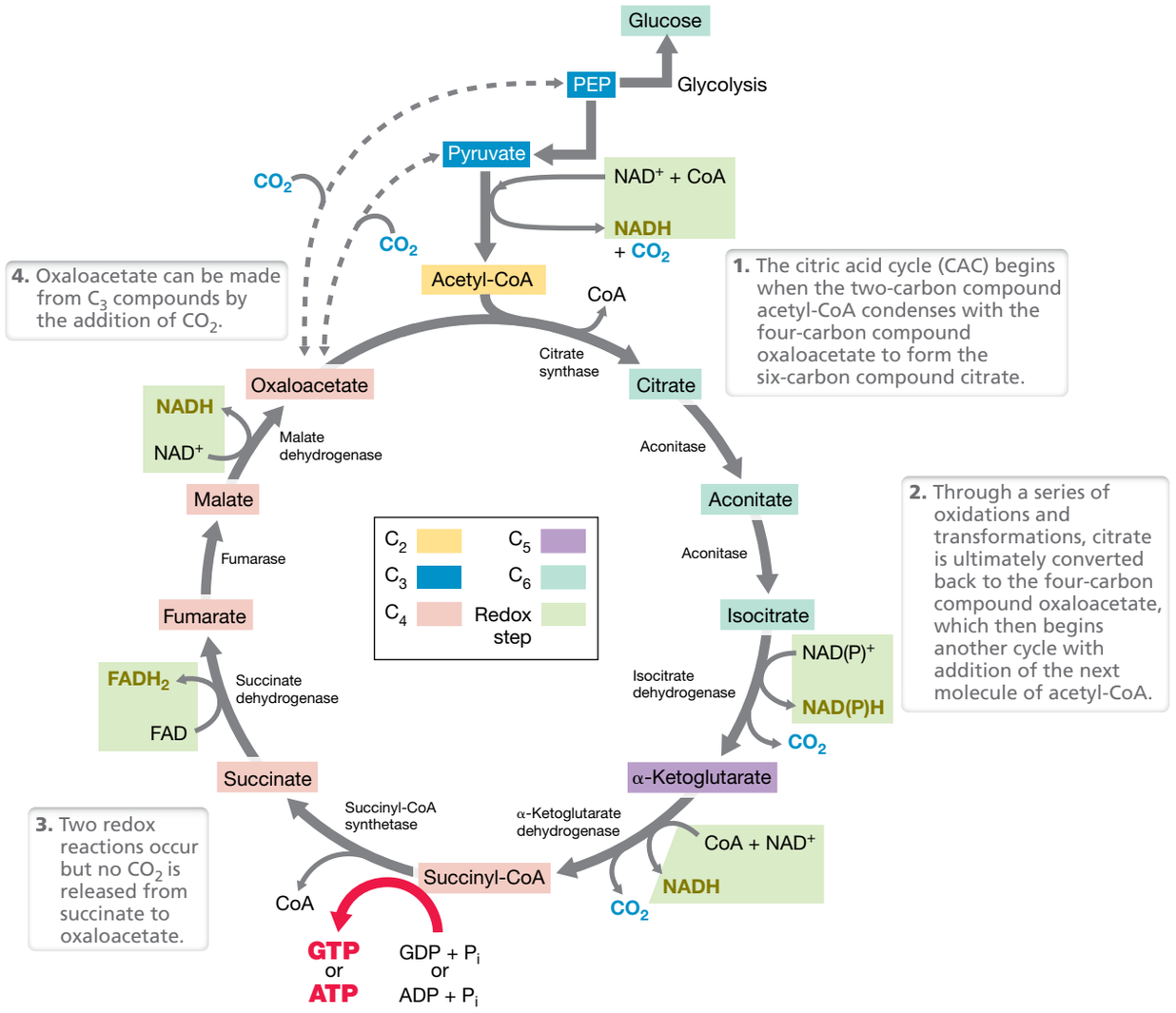
As an alternative to fermentation, glucose can be respired. During respiration, glucose is first catabolized through glycolysis. But instead of reducing pyruvate to fermentation products and discarding them (Figure 3.14), in respiration, *pyruvate is fully oxidized to CO_2* through activities of the citric acid and glyoxylate cycles, major pathways for respiring organic compounds.

Respiration of Glucose

The pathway by which pyruvate is oxidized to CO_2 is called the **citric acid cycle** (CAC) (**Figure 3.16**). In the CAC, pyruvate is first decarboxylated, leading to the production of CO_2, NADH, and the energy-rich substance *acetyl-CoA* (Figure 3.13). The acetyl group of acetyl-CoA then combines with the four-carbon CAC intermediate oxaloacetate, forming the six-carbon compound citric acid, for which the CAC is named. A sequence of reactions follows, and two additional CO_2 molecules, three more NADH, and one $FADH_2$ are formed per pyruvate oxidized. Ultimately, oxaloacetate is regenerated as the next acetyl acceptor, thus completing the cycle (Figure 3.16).

For each 2 pyruvate oxidized through the citric acid cycle (2 pyruvate are produced from one glucose, Figure 3.14), 6 molecules of CO_2, 8 NADH, and 2 $FADH_2$ are produced (Figure 3.16). $FADH_2$ is a redox prosthetic group that can exist in either an oxidized (FAD) or a reduced ($FADH_2$) form (Section 3.10). And, just as for NADH (Figure 3.12), $FADH_2$ must be reoxidized for the CAC to continue. Both NADH and $FADH_2$ are oxidized in redox reactions that occur in the electron transport chain, a series of reactions that both consumes electrons (through the reduction of O_2) and produces ATP (through the proton motive force) (Sections 3.10 and 3.11).

The combined activities of the CAC and electron transport chain result in the complete oxidation of glucose to CO_2 along with a much greater yield of energy than is possible in fermentation. Whereas only *2 ATP* are produced per glucose fermented in alcoholic or lactic acid fermentations (Figure 3.14), a total of *38 ATP* can be made by aerobically respiring the same glucose molecule to



ENERGETICS BALANCE SHEET FOR AEROBIC RESPIRATION

(1) **Glycolysis:** Glucose + 2 NAD^+ → 2 Pyruvate + **2 ATP** + **2 NADH**

- (a) Substrate-level phosphorylation: 2 ADP + P_i → **2 ATP**
- (b) Oxidative phosphorylation: **2 NADH** → **6 ATP**

8 ATP

2 Pyruvate → to CAC; **2 NADH** → to Complex I

(2) **CAC:** 2 Pyruvate + 8 NAD^+ + 2 GDP + 2 FAD → **6 CO_2** + **8 NADH** + **2 $FADH_2$** + **2 GTP** (ATP)

- (a) Substrate-level phosphorylation: 2 GDP + P_i → **2 GTP** (ADP) (ATP)
- (b) Oxidative phosphorylation: **8 NADH** → **24 ATP**; **2 $FADH_2$** → **4 ATP**

30 ATP

8 NADH → to Complex I; **2 $FADH_2$** → to Complex II (See Figure 3.22)

(3) **Glycolysis plus CAC:** Glucose → **6 CO_2** + 6 H_2O → **38 ATP**

Figure 3.16 The citric acid cycle. (Top) The citric acid cycle begins when the two-carbon compound acetyl-CoA condenses with the four-carbon compound oxaloacetate to form the six-carbon compound citrate. Through a series of oxidations and transformations, citrate is converted to two CO_2 and the acetyl acceptor molecule, oxaloacetate. (Bottom) The overall balance sheet of fuel (NADH and $FADH_2$) for the electron transport chain and CO_2 generated in the citric acid cycle. NADH and $FADH_2$ feed into electron transport chain Complexes I and II, respectively (Figure 3.22). The reactions between PEP and oxaloacetate and between pyruvate and oxaloacetate are typically reversible. These reactions connect the citric acid cycle with glycolysis and are particularly important when citric acid cycle intermediates are drawn off to be used as carbon sources.

CO_2. This is because in aerobic respiration, NADH oxidation yields 3 ATP and $FADH_2$ oxidation yields 2 ATP (Figure 3.16, bottom, and see NADH and $FADH_2$ oxidations in Figure 3.22).

Biosynthesis and the Citric Acid Cycle

Besides its role in combusting pyruvate to CO_2, the citric acid cycle plays another important role in the cell. The cycle is composed of several key organic compounds, small amounts of which are drawn off during growth to produce new cell material. Particularly important in this regard are α-ketoglutarate and oxaloacetate, which are precursors of several amino acids (Section 3.14), and succinyl-CoA, needed to form cytochromes, chlorophyll, and related molecules. Any shortage of oxaloacetate is corrected by the addition of CO_2 (carboxylation) to pyruvate or phosphoenolpyruvate (Figure 3.16).

Oxaloacetate is also an important intermediate because it can be converted to phosphoenolpyruvate (a precursor of glucose) if necessary (Section 3.13). In addition, acetate is important because it provides the raw material for fatty acid biosynthesis (Section 3.15). The CAC thus plays two major roles in the cell: *glucose respiration coupled to energy conservation* and *the biosynthesis of key metabolites*. The same can be said about the glycolytic pathway, as certain intermediates from this pathway are also drawn off for biosynthetic needs as well (Section 3.13) and then replenished from glucose in the next round of glycolysis.

The Glyoxylate Cycle

Citrate, malate, fumarate, and succinate are common natural products, and as for glucose, organisms that use these C_4 or C_6 compounds as electron donors in energy metabolism employ the citric acid cycle for their catabolism. By contrast, two-carbon compounds such as acetate cannot be oxidized by the citric acid cycle alone. This is because the citric acid cycle can continue only if oxaloacetate is regenerated at each turn of the cycle. If oxaloacetate is drawn off to biosynthesize glucose and amino acid precursors, as it must be when cells are growing on nonglucose substrates, the cycle would starve for what it needs to continue functioning (Figure 3.16). Thus, when acetate is used as an electron donor, a variation on the citric acid cycle called the **glyoxylate cycle** (so named because the C_2 compound *glyoxylate* is a key intermediate) becomes active and functions to replenish oxaloacetate used for biosyntheses (**Figure 3.17**).

The glyoxylate cycle is composed of several citric acid cycle enzymatic reactions plus two additional enzymes: *isocitrate lyase*, which cleaves isocitrate into succinate and glyoxylate, and *malate synthase*, which converts glyoxylate and acetyl-CoA to malate (Figure 3.17). The succinate formed can be used for biosynthesis while the glyoxylate combines with acetyl-CoA (C_2) to yield malate (C_4). From malate, the acceptor molecule oxaloacetate is produced and can enter a new round of acetyl-CoA oxidation in the citric acid cycle (Figure 3.17).

Three-carbon compounds such as pyruvate or compounds that are converted to pyruvate (for example, lactate or carbohydrates) also cannot be catabolized through the citric acid cycle alone, but here the glyoxylate cycle is unnecessary. This is because any shortage of C_4 CAC intermediates is compensated by synthesizing

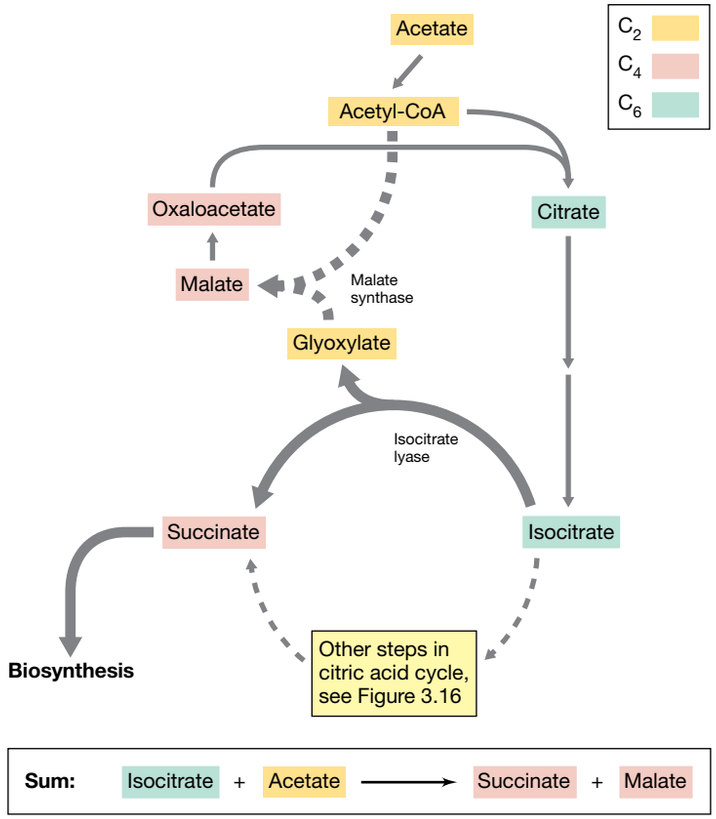


Figure 3.17 The glyoxylate cycle. These reactions occur in conjunction with the citric acid cycle when cells grow on two-carbon electron donors, such as acetate. The glyoxylate cycle regenerates oxaloacetate (from malate) to maintain an acceptor for the citric acid cycle.

oxaloacetate from pyruvate or phosphoenolpyruvate (Figure 3.16). This occurs by carboxylation reactions catalyzed by the enzymes *pyruvate carboxylase* or *phosphoenolpyruvate carboxylase*, respectively.

MINIQUIZ

- How many molecules of CO_2, NADH, and $FADH_2$ are released per pyruvate oxidized in the citric acid cycle?
- What two major roles do the citric acid cycle and glycolysis have in common?
- Why is the glyoxylate cycle necessary for growth on acetate but not on succinate?

3.10 Respiration: Electron Carriers

We have just seen how the citric acid cycle generates CO_2 and the reduced forms of two redox coenzymes, NADH and $FADH_2$ (Figure 3.16). The oxidation of these coenzymes back to NAD^+ and FAD, respectively, is linked to energy conservation through the electron transport chain. In the next section we consider electron transport itself. In this section, we lay the groundwork by considering redox aspects of electron transport and the molecules that participate.

NADH Dehydrogenases and Flavoproteins

Electron transport reactions occur in membranes; in prokaryotic cells this means the cytoplasmic membrane or any internal membranes derived from the cytoplasmic membrane. Several types of oxidation–reduction enzymes participate in electron transport. These include *NADH dehydrogenases, flavoproteins, iron–sulfur proteins*, and *cytochromes*. Also participating are small nonprotein electron carriers called *quinones*. The carriers are arranged in the membrane in order of *increasingly more positive* reduction potential, with NADH dehydrogenase first and the cytochromes last (see Figure 3.22).

NADH dehydrogenases contain an active site that binds NADH. The 2 e^- + 2 H^+ from NADH are transferred by the dehydrogenase to a flavoprotein, the next carrier in the chain. This generates NAD^+ that is then released from the dehydrogenase and free to react with an enzyme that requires it as coenzyme (Figure 3.12). Flavoproteins contain a derivative of the vitamin riboflavin (**Figure 3.18**). The flavin portion, which is bound to its protein as a prosthetic group (Section 3.5), is reduced as it accepts 2 e^-+ 2 H^+ and oxidized when 2 e^- are passed on to the next carrier in the chain (note that flavoproteins *accept* 2 e^-+ 2 H^+ but *donate* only electrons; the fate of the two protons will be considered later). Two types of flavins are commonly found in cells, flavin mononucleotide (FMN, Figure 3.18) and flavin adenine dinucleotide (FAD). The vitamin riboflavin is a source of flavin and is a required growth factor for some organisms (Table 3.1).

Cytochromes, Other Iron Proteins, and Quinones

The cytochromes are proteins that contain heme prosthetic groups (**Figure 3.19**). Cytochromes undergo oxidation and reduction through loss or gain of an electron by the iron atom that exists as either Fe^{2+} or Fe^{3+}. Several classes of cytochromes are known, differing widely in their reduction potentials (Figure 3.10). Different classes of cytochromes are designated by letters, such as cytochrome *a*, cytochrome *b*, cytochrome *c*, and so on, depending upon the type of heme they contain. Occasionally, cytochromes form into complexes with other cytochromes or with iron–sulfur proteins. An important example is the cytochrome bc_1 complex, which contains two different *b*-type cytochromes and one *c*-type cytochrome. The cytochrome bc_1 complex plays an important role in energy metabolism, as we will see in the next section.

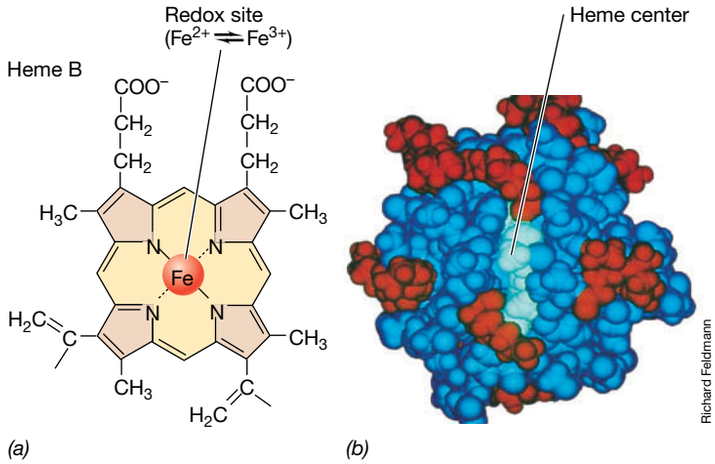


Figure 3.19 Cytochrome and its structure. *(a)* Structure of heme, the iron-containing portion of cytochromes. Cytochromes carry electrons only, and the redox site is the iron atom, which can alternate between the Fe^{2+} and Fe^{3+} oxidation states. *(b)* Space-filling model of cytochrome *c*; heme (light blue) is covalently linked via disulfide bridges to cysteine residues in the protein (dark blue). Cytochromes are tetrapyrroles, composed of four pyrrole rings.

In addition to the cytochromes, in which iron is bound to heme, one or more proteins with nonheme iron are also components of electron transport chains. These proteins contain prosthetic groups made up of clusters of iron and sulfur atoms, with Fe_2S_2 and Fe_4S_4 clusters being the most common (**Figure 3.20**). For example, bacterial *ferredoxin*, a nonheme iron–sulfur protein of low reduction potential (about –0.4V) (Figure 3.10), contains an Fe_4S_4 cluster. The reduction potentials of iron–sulfur proteins vary from –0.2 to about –0.45V, depending on the iron–sulfur cluster present and how the cluster is embedded in the protein. Thus, different iron–sulfur proteins can function at different locations in the electron transport chain. Like cytochromes, iron–sulfur proteins carry electrons only.

Quinones (**Figure 3.21**) are small hydrophobic redox molecules that lack a protein component. Because they are small and hydrophobic, quinones can move about within the membrane. Like the flavins (Figure 3.18), quinones accept 2 e^- + 2 H^+ but transfer only 2 e^- to the next carrier in the chain. Quinones typically function to link iron–sulfur proteins (Figure 3.20) and the initial cytochrome (Figure 3.19) in the electron transport chain. Several types of quinones are known, but ubiquinone (also called coenzyme Q) and menaquinone are the most common quinones and are widely distributed in species of *Bacteria* and *Archaea*.

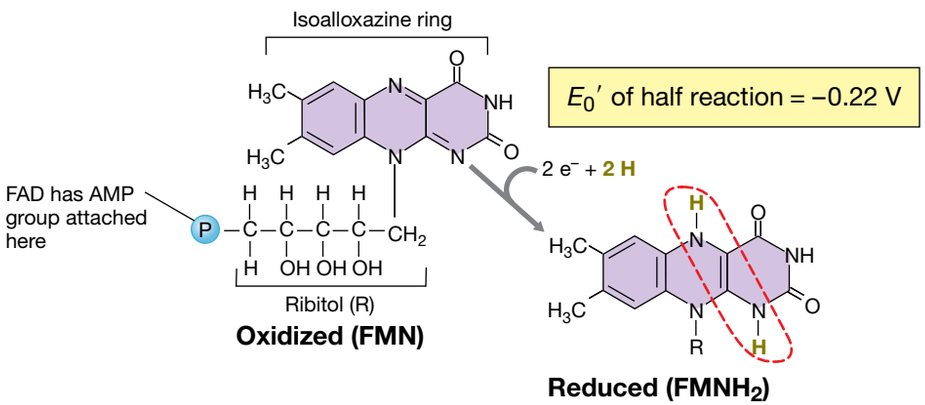


Figure 3.18 Flavin mononucleotide (FMN), a hydrogen atom carrier. The site of oxidation–reduction (dashed red circle) is the same in FMN and the related coenzyme flavin adenine dinucleotide (FAD, not shown). FAD contains an adenosine monophosphate (AMP) group bonded through the phosphate group on FMN.

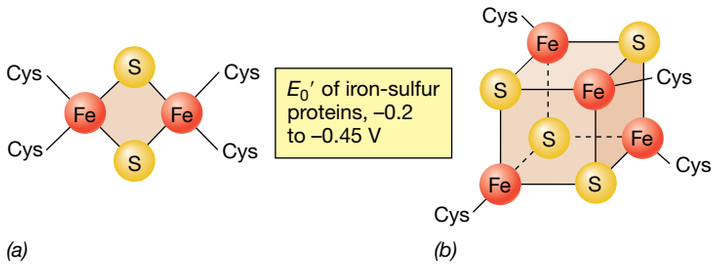


Figure 3.20 Arrangement of the iron–sulfur centers of nonheme iron–sulfur proteins. *(a)* Fe_2S_2 center. *(b)* Fe_4S_4 center. The cysteines (Cys) link the protein to its Fe/S cluster.

MINIQUIZ

- In what major way do quinones differ from other electron carriers in the membrane?
- Which electron carriers described in this section accept $2\ e^- + 2\ H^+$? Which accept electrons only?

3.11 Electron Transport and the Proton Motive Force

Energy conservation in respiration is linked to an energized state of the membrane (Figure 2.7), and this energized state is established by reactions of the electron transport chain.

Electron Transport

Understanding how electron transport is linked to ATP synthesis requires an appreciation for how the electron transport chain is organized in the cytoplasmic membrane. The electron transport carriers we just discussed (Figures 3.11 and 3.18–3.21) are oriented in the membrane in such a way that when redox reactions occur, protons are separated from electrons across the membrane. Two electrons plus two protons enter the electron transport chain when NADH is oxidized to NAD^+ (through activity of the enzyme NADH dehydrogenase) to begin the process of electron transport. Components of the electron transport chain are arranged in the membrane in order of their *increasingly positive* reduction potential (Figure 3.10), with the final carrier in the chain donating the electrons plus protons to a terminal electron acceptor such as O_2.

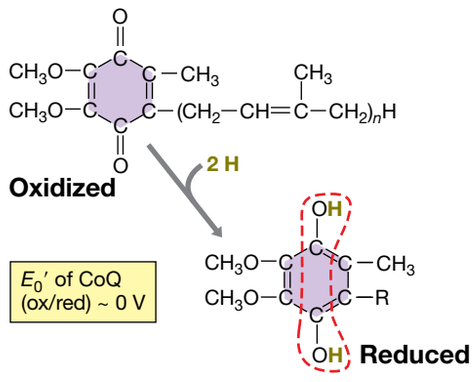


Figure 3.21 Structure of oxidized and reduced forms of ubiquinone (coenzyme Q, or CoQ). The five-carbon unit in the side chain (an isoprenoid) occurs in multiples, typically 6–10. Oxidized ubiquinone requires $2e^-$ and $2H^+$ to become fully reduced (dashed red circles).

During electron transport, H^+ ions are extruded to the *outer surface* of the membrane. These protons originate from two sources: (1) NADH and (2) the dissociation of H_2O into H^+ and OH^- in the cytoplasm. The extrusion of H^+ to the environment results in the accumulation of OH^- on the inside of the cytoplasmic membrane. However, despite their small size, neither H^+ nor OH^- can diffuse through the membrane because they are charged and highly polar.

As a result of the separation of H^+ and OH^-, the inner and outer surfaces of the membrane differ in charge, pH, and electrochemical potential. This latter is called the **proton motive force (pmf)** and energizes the membrane, much like a battery (Figure 2.7). Some of the potential energy in the pmf is then conserved when its charged state is dissipated to drive the biosynthesis of ATP. In addition, the energy of the pmf can also be tapped to support other forms of work in the cell, such as nutrient transport, flagellar rotation, and other energy-requiring reactions.

Figure 3.22 depicts the electron transport chain of the bacterium *Paracoccus*, one of many different electron transport schemes known. Three features are characteristic of this and indeed all electron transport chains: (1) the carriers are arranged in order of increasingly more positive E_0', (2) there is some alternation of electron-only and electron-plus-proton carriers in the chain, and (3) the net result is reduction of a terminal electron acceptor (such as O_2) and generation of a proton motive force.

Generation of the Proton Motive Force: Complexes I and II

We now examine the electron transport process in more detail. The proton motive force forms from the activities of flavins, quinones, the cytochrome bc_1 complex, and the terminal protein, cytochrome oxidase. Following the oxidation of NADH + H^+ to form $FMNH_2$, 4 H^+ are released to the outer surface of the membrane when $FMNH_2$ donates 2 e^- to nonheme iron (Fe/S) proteins that form *Complex I* (Figure 3.22). The term *complex* refers to the fact that several proteins are present that function as a unit (in *Escherichia coli*, Complex I contains 14 distinct proteins). Complex I is also called *NADH: quinone oxidoreductase* because the overall reaction is one in which NADH is oxidized and quinone is reduced. Two protons are taken up from the cytoplasm by ubiquinone when it is reduced by the Fe/S protein in Complex I (Figure 3.22).

Complex II bypasses Complex I and feeds electrons from $FADH_2$ directly to quinones. Complex II is also called the *succinate dehydrogenase complex* because succinate (a product of the citric acid cycle, Section 3.9) as well as fatty acids donate electrons (through $FADH_2$) when they are oxidized. However, because Complex II bypasses Complex I (where electrons enter from NADH at a more negative reduction potential), four fewer protons are pumped per 2 e^- that enter from $FADH_2$ at Complex II than those that enter at Complex I (Figure 3.22); this reduces the ATP yield by one per two electrons consumed.

Complexes III and IV: bc_1 and *a*-Type Cytochromes

Reduced ubiquinone (ubiquinol, QH_2) passes electrons one at a time to the cytochrome bc_1 complex (*Complex III*, Figure 3.22).

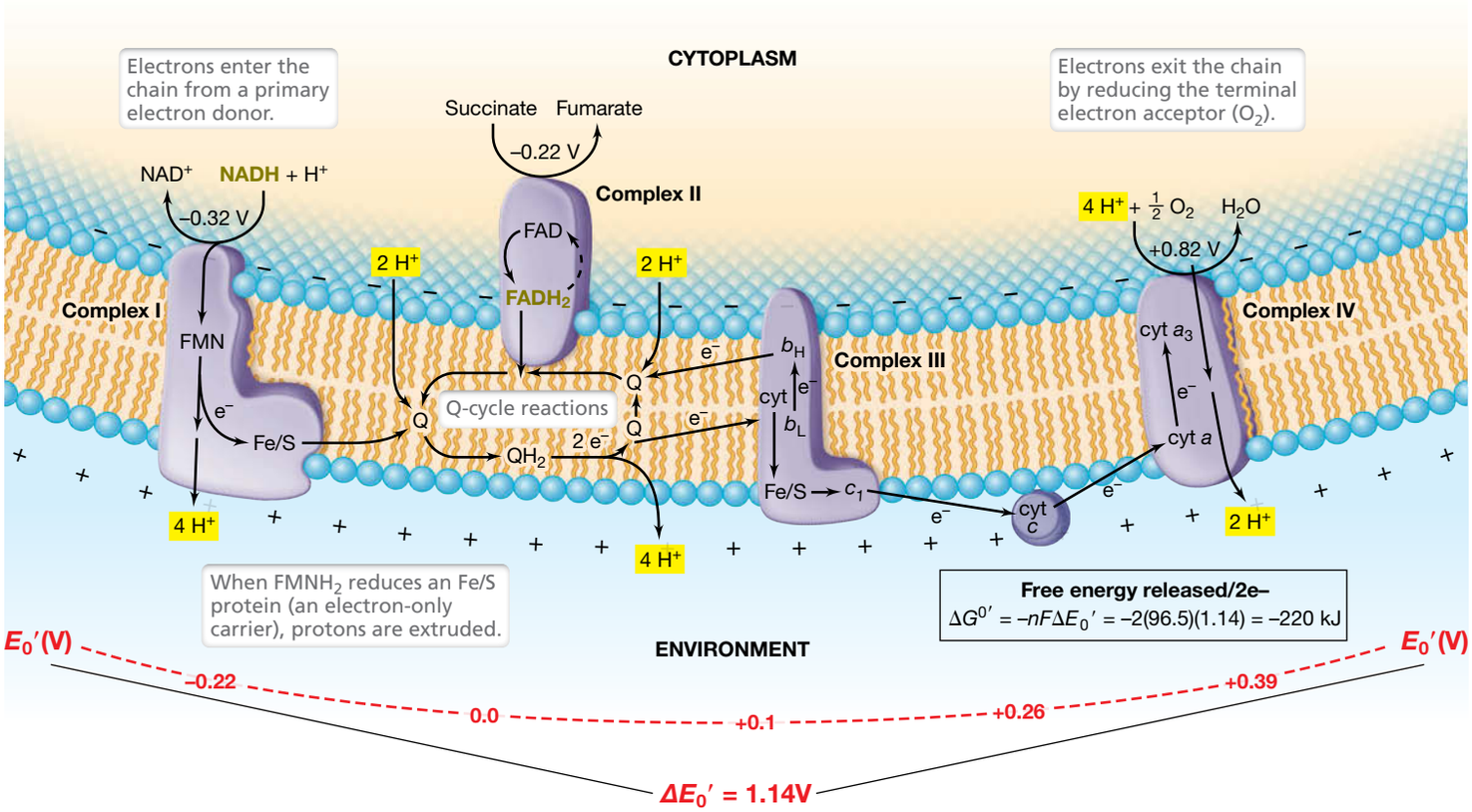


Figure 3.22 Generation of the proton motive force during aerobic respiration. The orientation of electron carriers in the cytoplasmic membrane of *Paracoccus denitrificans*. The $^+$ and $^-$ charges at the inner and outer membrane surfaces represent H^+ and OH^-, respectively. Abbreviations: FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; Q, quinone; Fe/S, iron–sulfur protein; cyt *a*, *b*, *c*, cytochromes (b_L and b_H, low- and high-potential *b*-type cytochromes, respectively). At the quinone site, electrons are recycled from Q to bc_1 from reactions of the "Q cycle." Electrons from QH_2 can be split in the bc_1 complex between the Fe/S protein and the *b*-type cytochromes. Electrons that travel through the cytochromes reduce Q (in two, one-electron steps) back to QH_2, thus increasing the number of protons pumped at the Q-bc_1 site. Electrons that travel to the Fe/S protein proceed to reduce cytochrome c_1, and from there cytochrome *c*. Complex II, the succinate dehydrogenase complex, bypasses Complex I and feeds electrons directly into the quinone pool at a more positive E_0' than NADH (see the redox tower in Figure 3.10).

Complex III consists of several proteins that contain two *b*-type hemes of different E_0' (b_L and b_H), one *c*-type heme (c_1), and one iron–sulfur cluster. The bc_1 complex is present in the electron transport chain of virtually every organism that can respire and also plays a role in photosynthetic electron flow in phototrophic organisms (Sections 14.3 and 14.4).

The major function of the cytochrome bc_1 complex is to transfer e^- from quinones to cytochrome *c*, which is located in the periplasm. Cytochrome *c* functions as a periplasmic shuttle to transfer e^- to the high-redox-potential cytochromes *a* and a_3 (*Complex IV*, Figure 3.22). Complex IV functions as the terminal oxidase and reduces O_2 to H_2O in the final step of the electron transport chain. Complex IV also pumps protons to the outer surface of the membrane, thereby increasing the strength of the proton motive force (Figure 3.22).

Besides transferring e^- to cytochrome *c*, the cytochrome bc_1 complex also interacts with quinones in such a way that on average, two additional H^+ are pumped at the Q-bc_1 site. This happens in a series of electron exchanges between cytochrome bc_1 and Q, called the *Q cycle*. Because quinone and the *b*-type cytochrome in the bc_1 complex have roughly the same E_0' (near 0 V, Figure 3.10), quinone molecules can alternately become oxidized and reduced using electrons returned to the quinone pool from the bc_1 complex. This mechanism allows on average a total of 4 H^+ (instead of 2 H^+) to be pumped to the outer surface of the membrane at the Q-bc_1 site for every two electrons that enter the chain in Complex I (Figure 3.22). This strengthens the proton motive force, and as we will see now, it is the proton motive force that fuels ATP synthesis.

ATP Synthase

How does the proton motive force generated from electron transport actually drive ATP synthesis? Interestingly, a strong parallel exists between the mechanism of ATP synthesis and the mechanism behind the motor that drives rotation of the bacterial flagellum (Section 2.11). In analogy to how dissipation of the pmf applies torque that rotates the bacterial flagellum, the pmf also creates torque in the large membrane protein complex that synthesizes ATP. This complex is called **ATP synthase (ATPase)**. The activity of ATPase is driven by the pmf, and the formation of ATP from respiratory electron flow is called **oxidative phosphorylation** (contrast this with substrate-level phosphorylation in fermentation, Section 3.8).

ATPases consist of two components, a multiprotein complex called F_1 that sticks into the cytoplasm and actually catalyzes ATP

synthesis, and a membrane-integrated multiprotein complex called F_o that carries out proton-translocation across the membrane (**Figure 3.23**). The structure of ATPase proteins is highly conserved throughout all the domains of life, indicating that this mechanism of energy conservation was an early evolutionary invention.

ATPase catalyzes a reversible reaction between ATP and ADP + P_i, and F_1 and F_o are actually two rotary motors. The movement of H^+ through F_o into the cytoplasm is coupled to the rotation of its *c* proteins. This generates a torque that is transmitted to F_1 via the coupled rotation of the γε subunits (Figure 3.23). The rotation causes conformational changes in the β subunits of F_1 that allows them to bind ADP + P_i. ATP is synthesized when the β subunits return to their original conformation; the free energy captured in the rotated β subunits is released and coupled to ATP synthesis.

Quantitative measures of the number of H^+ consumed by ATPase per ATP produced yield a number between 3 and 4. Hence, per two electrons that enter the electron transport chain, about 3 ATP (whose biosynthesis requires 96 kJ of free energy under standard conditions) are produced from the roughly 220 kJ of energy released as the electrons are transported to O_2 (Figure 3.22). This yields a respiratory efficiency of about 44%, which compares with an efficiency of about 27% for yeast fermenting glucose to ethanol plus CO_2 (Figure 3.14).

ATPases are reversible motors. The hydrolysis of ATP supplies the torque necessary for γε to rotate in the opposite direction from that of ATP synthesis and pump H^+ from the cytoplasm through F_o to the environment (Figure 3.23). The net result in this case is *generation of* instead of *dissipation of* the proton motive force. Reversibility of the ATPase explains why strictly fermentative bacteria that lack electron transport chains and are unable to carry out oxidative phosphorylation still contain ATPases. Many important reactions in the cell, such as flagellar rotation and some forms of transport, are coupled to energy from the pmf rather than directly from ATP. Thus, the ATPase of organisms incapable of respiration functions unidirectionally in the cell to generate a pmf from ATP formed from substrate-level phosphorylation (Section 3.8) in fermentation.

MINIQUIZ

- How do electron transport reactions generate the proton motive force?
- How much energy is released per NADH oxidized through the electron transport chain of *Paracoccus* shown in Figure 3.22? At which sites in the chain is the proton motive force being established?
- What structure in the cell links the proton motive force to ATP synthesis? How does it function?

3.12 Options for Energy Conservation

Thus far our discussion of catabolism has been restricted to microbes that use organic electron donors—the chemoorganotrophs. We now briefly consider catabolic diversity and some of the alternatives to fermentation and aerobic respiration. These include *anaerobic respiration, chemolithotrophy,* and *phototrophy* (**Figure 3.24**). We return to this theme of catabolic diversity and explore its many facets in Chapter 14.

Anaerobic Respiration

Under anoxic conditions, electron acceptors other than O_2 support respiration in a wide variety of *Bacteria* and *Archaea* in the process called **anaerobic respiration**. Some of the electron acceptors used in anaerobic respiration include nitrate (NO_3^-, reduced to nitrite, NO_2^-, by *Escherichia coli* or to N_2 by most *Pseudomonas* species), ferric iron (Fe^{3+}, reduced to Fe^{2+} by *Geobacter* and many other species), sulfate (SO_4^{2-}, reduced to hydrogen sulfide, H_2S, by *Desulfovibrio* and other sulfate-reducing species), carbon dioxide (CO_2, reduced to

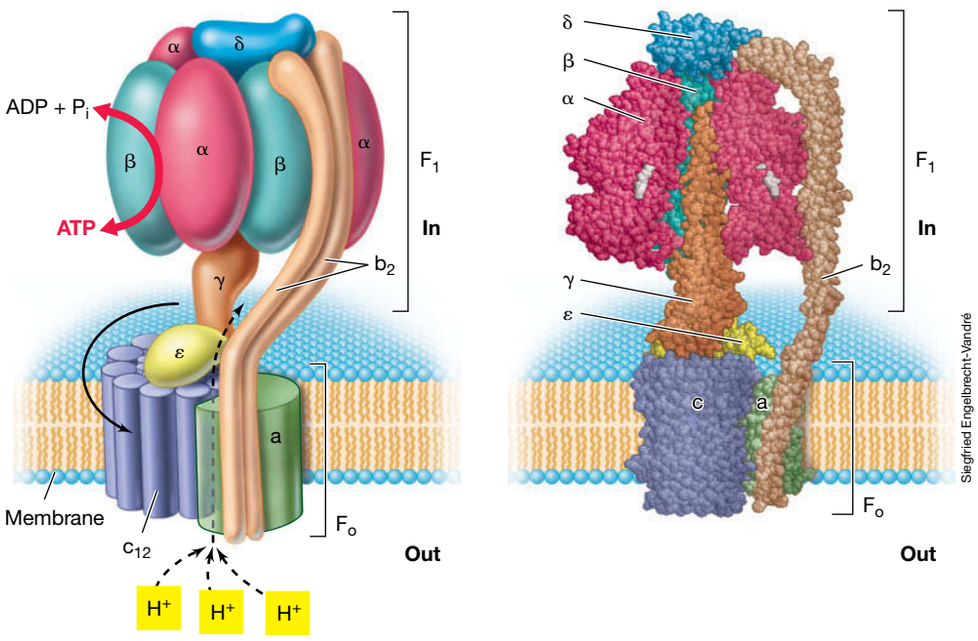


Figure 3.23 Structure and function of the reversible ATP synthase (ATPase) in *Escherichia coli*. *(a)* Schematic. F_1 consists of five different polypeptides forming an $\alpha_3\beta_3\gamma\varepsilon\delta$ complex, the stator. F_1 is the catalytic complex responsible for the interconversion of ADP + P_i and ATP. F_o, the rotor, is integrated in the membrane and consists of three polypeptides in an ab_2c_{12} complex. As protons enter, the dissipation of the proton motive force drives ATP synthesis (3 H^+/ATP). *(b)* Space-filling model. The color-coding corresponds to the art in part *a*. Since proton translocation from outside the cell to inside the cell leads to ATP synthesis by ATPase, it follows that proton translocation from inside to outside in the electron transport chain (Figure 3.22) represents work done on the system and a source of potential energy. All cells, including cells of the *Archaea*, contain ATPases. Also, some bacterial and archaeal ATPases are linked to a sodium (Na^+) rather than a proton (H^+) gradient.

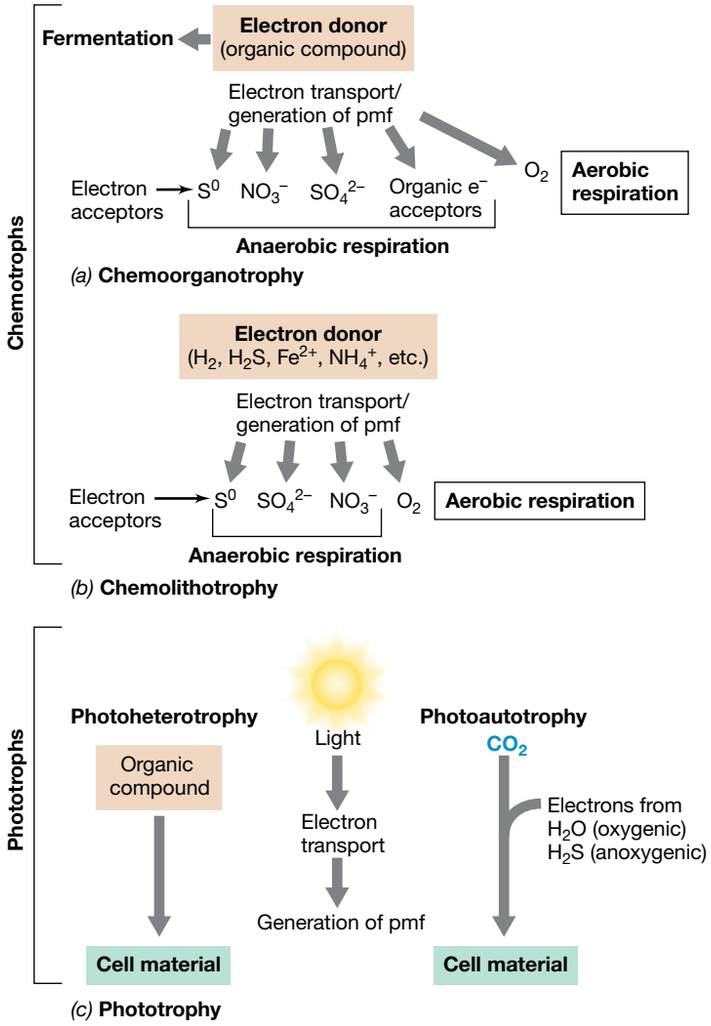


Figure 3.24 Catabolic diversity. *(a)* Chemoorganotrophs. *(b)* Chemolithotrophs. *(c)* Phototrophs. Note the importance of the proton motive force driven by electron transport in energy conservation in both forms of respiration and in photosynthesis.

methane, CH_4, by methanogens or to acetate by acetogens), and even certain organic compounds, such as the citric acid cycle intermediate fumarate (which is reduced to succinate).

Because none of these alternative electron acceptor couples has an E_0' as positive as that of the O_2/H_2O couple (Figure 3.10), less energy is conserved when they are reduced compared with the reduction of O_2 to H_2O (recall that $\Delta G^{0\prime}$ is proportional to $\Delta E^{0\prime}$ as was shown in Figure 3.10 and Table 3.3 and discussed in Section 3.6). O_2 is often limiting or even totally absent in many microbial habitats, and so anaerobic respirations can be very important means of energy conservation. As in aerobic respiration (Figure 3.22), anaerobic respirations require electron transport, generate a proton motive force, and employ ATPase to make ATP (Chapter 14).

Chemolithotrophy and Phototrophy

Organisms able to use *inorganic* chemicals as electron donors are called *chemolithotrophs*. Examples of common inorganic electron donors include H_2S, H_2, Fe^{2+}, and NH_4^+. Many of these compounds are the waste products of chemoorganotrophic organisms, and for this reason, it is common for chemoorganotrophs and chemolithotrophs to coexist in nature.

Chemolithotrophic metabolisms are typically aerobic and begin with the oxidation of the inorganic electron donor and the electrons entering an electron transport chain. Electron flow generates a proton motive force, as we have already seen for the oxidation of organic electron donors by chemoorganotrophs (Figure 3.22). So, respiration by these two groups of organisms is simply a variation on a common theme of oxidative phosphorylation. However, chemoorganotrophs and chemolithotrophs differ significantly in their source of cell carbon. Chemoorganotrophs are heterotrophs and thus use organic compounds (glucose, acetate, and the like) as carbon sources. By contrast, chemolithotrophs use carbon dioxide (CO_2) as a carbon source and are therefore autotrophs (Section 3.3). We discuss autotrophic biosynthetic pathways in Chapter 14.

In the process of photosynthesis, carried out by *phototrophs*, light energy is used instead of a chemical to drive electron flow and generate a proton motive force. During these events, ATPase generates ATP by **photophosphorylation**, the light-driven analog of oxidative phosphorylation (Section 3.11). Most phototrophs assimilate CO_2 as their carbon source and are therefore *photoautotrophs*. However, some phototrophs use organic compounds as carbon sources with light as the energy source; these are the *photoheterotrophs* (Figure 3.24). Despite major differences regarding O_2, oxygenic and anoxygenic phototrophs (Section 3.3) show great parallels in the mechanism by which light drives ATP synthesis, a result of the fact that oxygenic photosynthesis evolved from the simpler anoxygenic system some 3 billion years ago (Chapter 13).

The PMF and Catabolic Diversity

With the exception of fermentation, in which substrate-level phosphorylation occurs (Section 3.8), all other mechanisms of microbial energy conservation are linked to the proton motive force (or as we will see in Chapter 14, a few organisms employ a gradient of sodium ions, Na^+, instead of protons). Whether electrons come from the oxidation of organic or inorganic chemicals or are mediated by light-driven processes, in both respiration and photosynthesis, energy conservation is the result of electron transport reactions and the formation of a pmf. The pmf is then tapped by ATPase to form ATP (Figure 3.23).

Said another way, respiration and anaerobic respiration can be viewed as variations on a theme of *different electron acceptors*, whereas chemoorganotrophy and chemolithotrophy are variations on a theme of *different electron donors*. Phototrophy, as we will see in Chapter 14, is a special case in terms of electron input and output, but the process still draws strong parallels with respiration. Electron transport and the pmf link all of these energy-yielding mechanisms, bringing these seemingly quite different forms of energy conservation into a common focus.

MINIQUIZ

- In terms of their electron donors, how do chemoorganotrophs differ from chemolithotrophs?
- What is the carbon source for autotrophic organisms?
- Why can it be said that the proton motive force is a unifying theme in most of bacterial metabolism?

IV • Biosyntheses

We conclude Chapter 3 with an overview of how the building blocks of the four classes of cellular macromolecules—sugars (polysaccharides), amino acids (proteins), nucleotides (nucleic acids), and fatty acids (lipids)—are biosynthesized and also look at how polysaccharides and lipids are biosynthesized in a general way. The biosynthesis of *informational* macromolecules—proteins and nucleic acids—is the theme of Chapter 4. Collectively, these biosyntheses are that aspect of metabolism we call **anabolism**.

3.13 Sugars and Polysaccharides

Polysaccharides are key components of microbial cell walls, and cells often store carbon and energy reserves in the form of the polysaccharides glycogen or starch (Chapter 2). How are such large molecules made?

Polysaccharide Biosyntheses and Gluconeogenesis

Polysaccharides are synthesized from *activated* forms of glucose, either uridine diphosphoglucose (*U*DPG; Figure 3.25*a*) or adenosine diphosphoglucose (*A*DPG). UDPG is the precursor of several glucose derivatives used in the biosynthesis of important structural polysaccharides, such as *N*-acetylglucosamine and *N*-acetylmuramic acid in peptidoglycan or the lipopolysaccharide component of the gram-negative outer membrane (↔ Sections 2.4 and 2.5). Polysaccharides are biosynthesized by adding activated glucose to a preexisting polymer fragment. For example, glycogen is synthesized as ADPG + glycogen → ADP glycogen-glucose.

When a cell is growing on a hexose such as glucose, obtaining glucose for polysaccharide synthesis is obviously not a problem. But when the cell is growing on other carbon compounds, glucose must be biosynthesized. This process, called *gluconeogenesis*, uses phosphoenolpyruvate, one of the intermediates of glycolysis, as a starting material and travels backwards through the glycolytic pathway (Figure 3.14) to form glucose. Phosphoenolpyruvate can be synthesized from oxaloacetate, a citric acid cycle intermediate (Figure 3.16). An overview of gluconeogenesis is shown in Figure 3.25*b*.

Pentose Metabolism and the Pentose Phosphate Pathway

Pentoses (5-carbon sugars) are formed by the removal of one carbon atom from a hexose, typically as CO_2. The pentoses needed for nucleic acid synthesis, ribose (in RNA) and deoxyribose (in DNA), are formed as shown in Figure 3.25*c*. The enzyme ribonucleotide reductase converts ribose into deoxyribose by reduction of the hydroxyl (–OH) group on the 2′ carbon of the 5-carbon pentose ring. This reaction occurs after, not before, synthesis of nucleotides. Thus, *ribo*nucleotides are biosynthesized, and some of them are later reduced to *deoxy*ribonucleotides for use as precursors of DNA.

The major pathway for pentose production is the **pentose phosphate pathway** (Figure 3.26). In this pathway, glucose, a hexose, is oxidized to CO_2, NADPH, and the key intermediate, *ribulose 5-phosphate*; from the latter, several different pentose derivatives can be formed. When pentoses are used as electron donors for energy conservation, they feed directly into the pentose phosphate pathway, typically becoming phosphorylated to form ribose phosphate or a related compound before being further catabolized (Figure 3.26*b*).

Besides its importance in pentose metabolism, the pentose phosphate pathway is also responsible for producing many other important sugars in the cell, including those containing 4 to 7 carbons. These sugars can eventually be converted to hexoses for either catabolic or biosynthetic purposes (Figures 3.25 and 3.26). A final important role of the pentose phosphate pathway is that it generates NADPH, a coenzyme used in many biosyntheses and in particular as a reductant for the production of deoxyribonucleotides

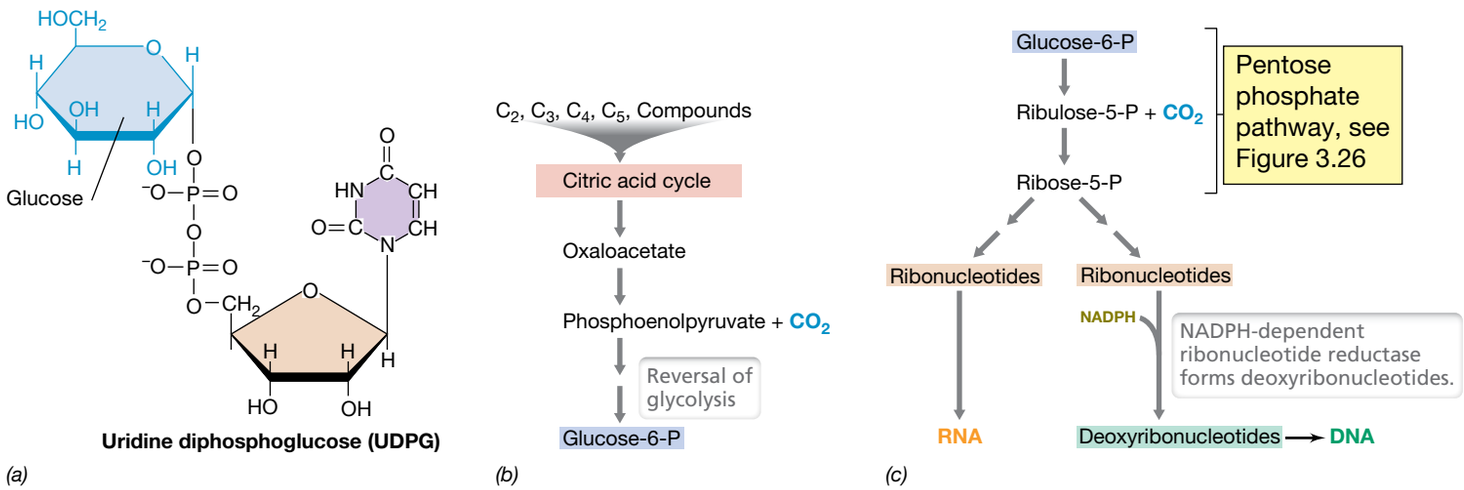


Figure 3.25 Sugar metabolism. *(a)* Polysaccharides are synthesized from activated forms of hexoses such as UDPG. *(b)* Gluconeogenesis. When glucose is needed, it can be biosynthesized from other carbon compounds, generally by the reversal of steps in glycolysis. *(c)* Pentoses for nucleic acid synthesis are formed by decarboxylation of hexoses such as glucose 6-phosphate. Note how the precursors of DNA are produced from the precursors of RNA by the enzyme ribonucleotide reductase.

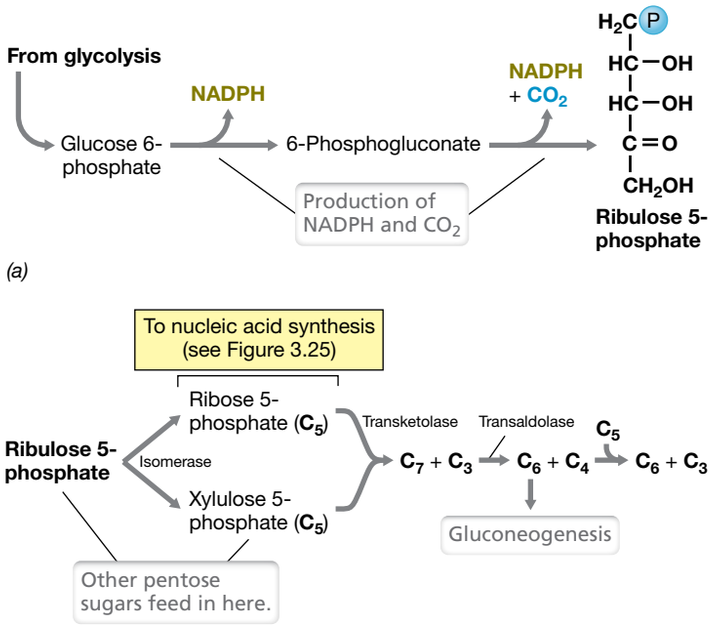


Figure 3.26 Pentose phosphate pathway. This pathway generates pentoses from other sugars for biosynthesis and also functions to catabolize pentose sugars. *(a)* Production of the key intermediate, ribulose 5-phosphate. *(b)* Other reactions in the pentose phosphate pathway.

(Figure 3.25*c*) and in the biosynthesis of fatty acids (see Figure 3.30). Although most cells have an exchange mechanism for converting NADH into NADPH, the pentose phosphate pathway is the major means by which NADPH is synthesized directly.

MINIQUIZ

- What form of activated glucose is used in the biosynthesis of glycogen by bacteria?
- What is gluconeogenesis?
- What functions does the pentose phosphate pathway play in the cell?

3.14 Amino Acids and Nucleotides

The monomers in proteins and nucleic acids are the amino acids and nucleotides, respectively. Their biosyntheses are typically multistep biochemical pathways that we need not consider in detail here. Instead, we identify the carbon skeletons needed for the biosynthesis of amino acids and nucleotides, look at their origins in pathways we have already considered, and summarize the mechanisms by which they are made.

Monomers of Proteins: Amino Acids

Organisms that cannot obtain some or all of their amino acids preformed from the environment must synthesize them from glucose or other carbon sources. Amino acids are grouped into structurally related *families* that share several biosynthetic steps. The carbon skeletons for amino acids come almost exclusively from intermediates of glycolysis (Figure 3.14) or the citric acid cycle (Figure 3.16) (**Figure 3.27**).

The amino group ($-NH_2$) of amino acids is typically derived from some inorganic nitrogen source, such as ammonia (NH_3). Ammonia is most often incorporated during biosynthesis of the amino acids glutamate or glutamine by the enzymes *glutamate dehydrogenase* and *glutamine synthetase*, respectively (**Figure 3.28**). When NH_3 is present at high levels, glutamate dehydrogenase or other amino acid dehydrogenases are used. However, when NH_3 is present at low levels, glutamine synthetase, with its energy-consuming reaction mechanism (Figure 3.28*b*) and correspondingly high affinity for its substrates, is employed. The enzymes glutamate dehydrogenase and glutamine synthetase are present in most *Bacteria* and *Archaea*.

Once ammonia is incorporated into glutamate or glutamine, the amino group of these amino acids can be transferred to form other nitrogenous compounds. For example, glutamate can donate its amino group to oxaloacetate in a transaminase reaction, producing α-ketoglutarate and aspartate (Figure 3.28*c*). Alternatively, glutamine can react with α-ketoglutarate to form two molecules of glutamate in an aminotransferase reaction (Figure 3.28*d*). The end result of these types of reactions is the shuttling of ammonia into various carbon skeletons from which further biosynthetic reactions occur to form all 22 of the

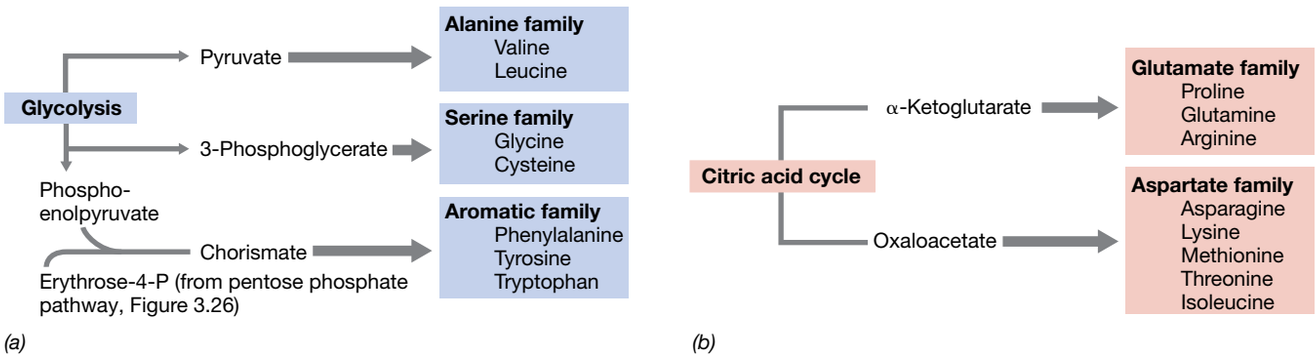


Figure 3.27 Amino acid families. Glycolysis *(a)* and the citric acid cycle *(b)* provide the carbon skeletons for most amino acids. Synthesis of the various amino acids in a family may require many steps starting with the parent amino acid (shown in bold as the name of the family).

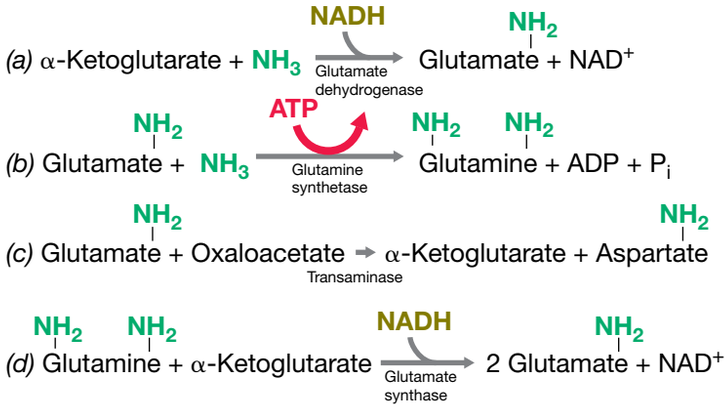


Figure 3.28 Ammonia incorporation in bacteria. Ammonia (NH_3) and the amino groups of all amino acids are shown in green. Two major pathways for NH_3 assimilation in bacteria are those catalyzed by the enzymes *(a)* glutamate dehydrogenase and *(b)* glutamine synthetase. *(c)* Transaminase reactions transfer an amino group from an amino acid to an organic acid. *(d)* The enzyme glutamate synthase forms two glutamates from one glutamine and one α-ketoglutarate.

amino acids needed to make proteins (Figure 4.28) and other nitrogen-containing biomolecules.

Monomers of Nucleic Acids: Nucleotides

The biochemistry behind purine and pyrimidine biosynthesis is quite complex and so only an outline of their biosyntheses is necessary here. Purines are constructed literally atom by atom from several carbon and nitrogen sources, including even CO_2 (Figure 3.29). The purine nucleotide skeleton, inosinic acid (Figure 3.29*b*), is the precursor of the purine nucleotides *adenine* and *guanine*. Once these are synthesized (in their triphosphate forms) and attached to ribose, they are ready to be incorporated into DNA (following ribonucleotide reductase activity, Figure 3.25*c*) or RNA.

Like the purine ring, the pyrimidine ring is also constructed from several sources (Figure 3.29*c*). From the pyrimidine nucleotide skeleton uridylate (Figure 3.29*d*), all of the pyrimidines—*thymine, cytosine,* and *uracil*—are derived. Structures of all of the purines and pyrimidines are shown in Chapter 4 (Figure 4.1*c*).

MINIQUIZ

- What is an amino acid family?
- List the steps required for the cell to incorporate NH_3 into amino acids.
- Which nitrogen bases are purines and which are pyrimidines?

3.15 Fatty Acids and Lipids

Lipids are major components of the cytoplasmic membrane and of the outer membrane of gram-negative bacteria; lipids can also be carbon and energy reserves. Fatty acids are the backbone of microbial lipids. However, recall that fatty acids, per se, are found only in *Bacteria* and *Eukarya*. *Archaea* do not contain fatty acids in their lipids but instead have hydrophobic isoprenoid side chains that play a similar structural role. All of these concepts were presented in Chapter 2. Our focus here is on the biosynthesis of fatty acids in *Bacteria*.

Fatty Acid Biosynthesis

Fatty acids are biosynthesized two carbon atoms at a time by the activity of a protein called *acyl carrier protein* (ACP). ACP holds the growing fatty acid as it is being constructed and releases it once it has reached its final length (Figure 3.30). Although fatty acids are constructed *two* carbons at a time, each C_2 unit originates from the *three*-carbon compound *malonate*, which is attached to the ACP to form malonyl-ACP. As each malonyl residue is donated, one molecule of CO_2 is released (Figure 3.30).

The fatty acid composition of cellular lipids varies from species to species and can also vary in a given organism with differences in growth temperature. Growth at low temperatures promotes the biosynthesis of shorter-chain and unsaturated fatty acids, whereas growth at higher temperatures promotes the biosynthesis of longer-chain and more saturated fatty acids. The most common fatty acids in lipids of *Bacteria* are those with chain lengths of C_{12}–C_{20}.

In addition to saturated, even-carbon-number fatty acids, fatty acids can also be unsaturated, branched, or have an odd number of carbon atoms. Unsaturated fatty acids contain one or more double bonds in the long hydrophobic portion of the molecule. The number and position of these double bonds is often

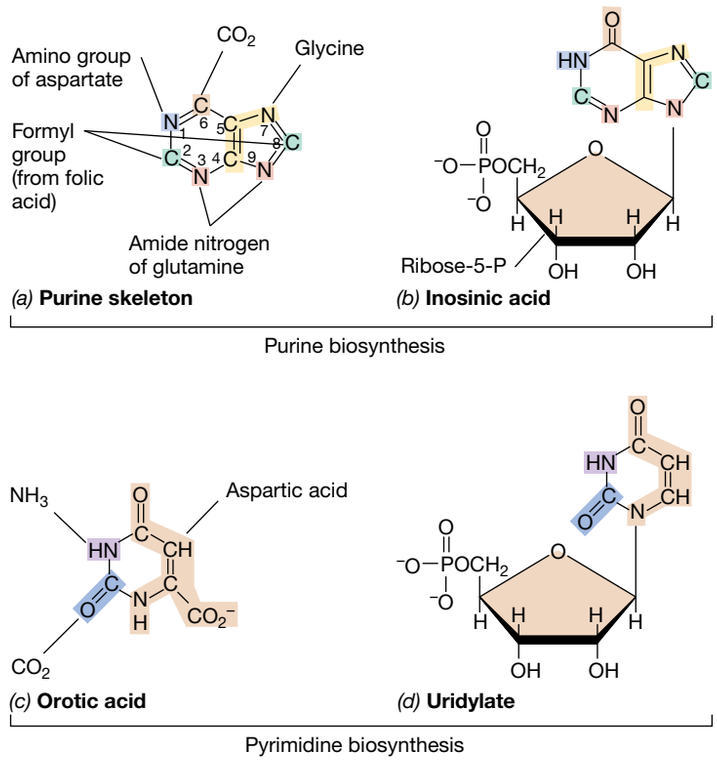


Figure 3.29 Biosynthesis of purines and pyrimidines. *(a)* Components of the purine skeleton, labeled with their sources. *(b)* Inosinic acid, the precursor of all purine nucleotides. *(c)* Components of the pyrimidine skeleton, orotic acid, labeled with their sources. *(d)* Uridylate, the precursor of all pyrimidine nucleotides. Uridylate is formed from orotate following a decarboxylation and the addition of ribose 5-phosphate.

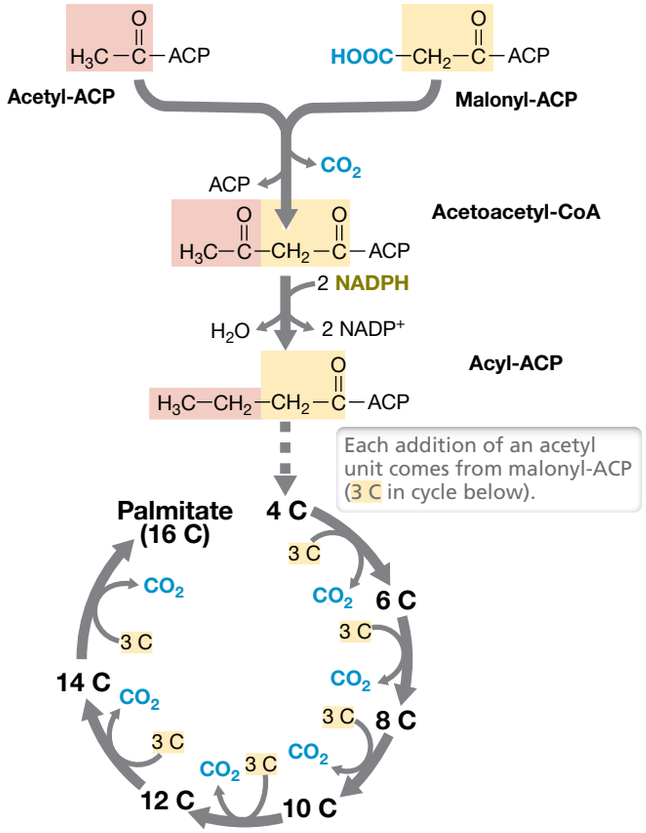


Figure 3.30 The biosynthesis of the C_{16} fatty acid palmitate. The condensation of acetyl-ACP and malonyl-ACP forms acetoacetyl-CoA. Each successive addition of an acetyl unit comes from malonyl-ACP.

species-specific or group-specific, and the double bonds are formed by desaturation of a saturated fatty acid. Branched-chain fatty acids are biosynthesized using a branched-chain initiating molecule, and odd-carbon-number fatty acids (for example, C_{13}, C_{15}, C_{17}) are biosynthesized using an initiating molecule that contains a propionyl (C_3) group instead of acetyl.

Lipid Biosynthesis

In the assembly of lipids in cells of *Bacteria* and *Eukarya*, fatty acids are first added to a molecule of glycerol. For simple triglycerides (fats), all three glycerol carbons are esterified with fatty acids. To form complex lipids, one of the carbon atoms in glycerol is embellished with a molecule of phosphate, ethanolamine, carbohydrate, or some other polar substance (Figure 2.4*a*). In *Archaea*, although membrane lipids are constructed from isoprene to form the phytanyl (C_{15}) or biphytanyl (C_{30}) side chains, the glycerol backbone of archaeal membrane lipids contains a polar group (sugar, phosphate, sulfate, or polar organic compound) as for the lipids of *Bacteria* and *Eukarya*. Polar groups are important in lipids for forming the canonical membrane architecture: a hydrophobic interior with hydrophilic inner and outer surfaces (Section 2.3 and Figures 2.4 and 2.5).

MINIQUIZ

- Explain how fatty acids are constructed two carbon atoms at a time while the immediate donor of these carbons is a three-carbon compound.
- What differences exist in lipids from the three domains of life?

Chapter Review

I • Microbial Nutrients and Nutrient Uptake

3.1 Cells are primarily composed of the elements H, O, C, N, P, and S. Nutrients required by a cell in large amounts are called macronutrients while those required in very small amounts, such as trace elements or growth factors, are micronutrients. Proteins are the most abundant class of macromolecules in the cell.

Q What is the major difference between macronutrients and micronutrients? List a few macronutrients and micronutrients.

3.2 The active transport of nutrients into the cell is an energy-requiring process driven by ATP (or some other energy-rich compound) or by the proton motive force. At least three classes of transport systems are known: simple, group translocation, and ABC systems. Each functions to accumulate solutes against the concentration gradient.

Q Cells of *Escherichia coli* transport lactose via lac permease, glucose via the phosphotransferase system, and maltose via an ABC-type transporter. For each of these sugars describe: (1) the components of the transport system and (2) the source of energy that drives the transport event.

II • Energetics, Enzymes, and Redox

3.3 All microorganisms conserve energy from either the oxidation of chemicals or from light. Chemoorganotrophs use organic chemicals as their electron donors, while chemolithotrophs use inorganic chemicals. Phototrophic organisms convert light energy into chemical energy (ATP) and include both oxygenic and anoxygenic species.

Q When a microorganism is growing on glucose as both an energy and a carbon source, to which energy and carbon utilization classes does it belong? When a

microorganism can synthesize its own organic compounds from carbon dioxide when growing on H_2, to which energy and carbon utilization classes does it belong?

3.4 Chemical reactions in the cell are accompanied by changes in energy, expressed in kilojoules. Reactions either release or consume free energy. $\Delta G^{0\prime}$ is a measure of the energy released or consumed in a reaction under standard conditions and reveals which reactions can be used by an organism to conserve energy.

Q Calculate $\Delta G^{0\prime}$ for the following reaction: glucose + 6 $O_2 \rightarrow$ 6 CO_2 + 6 H_2O. Is this reaction exergonic or endergonic? Distinguish between $\Delta G^{0\prime}$, ΔG, and G_f^0.

3.5 Enzymes are protein catalysts that increase the rate of biochemical reactions by activating the substrates that bind to their active site. Enzymes are highly specific in the reactions they catalyze, and this specificity resides in the three-dimensional structures of the polypeptide(s) that make up the protein(s).

Q What makes enzymes highly specific?

3.6 Oxidation–reduction reactions require electron donors and electron acceptors. The tendency of a compound to accept or release electrons is expressed by its reduction potential (E_0'). Redox reactions in a cell often employ redox coenzymes such as NAD^+/NADH as electron shuttles.

Q The following is a series of coupled electron donors and electron acceptors (written as donor:acceptor). Using the data in Figure 3.10, order this series from most energy yielding to least energy yielding: H_2:Fe^{3+}, NO:Mn^{4+}, H_2S:O_2, methanol: NO_3^- (producing NO_2^-), H_2:O_2, Fe^{2+}:O_2, NO_2^-:Fe^{3+} (producing NO_3^-), and H_2S:NO_3^-.

3.7 The energy released in redox reactions is conserved in compounds that contain energy-rich phosphate or sulfur bonds. The most common of these compounds is ATP, the prime energy carrier in the cell. Longer-term storage of energy is linked to the formation of polymers, which can be consumed to yield ATP.

Q Why is acetyl phosphate considered an energy-rich compound while glucose 6-phosphate is not?

III • Catabolism: Fermentation and Respiration

3.8 The glycolytic pathway is used to break down glucose to pyruvate and is a widespread mechanism for energy conservation by fermentative anaerobes that employ substrate-level phosphorylation. The pathway releases a small amount of ATP (2–3/glucose) and large amounts of fermentation products. Besides glucose, the fermentation of other sugars, amino acids, nucleotides and polymeric compounds is possible.

Q How is ATP made in fermentation and in respiration? Where in glycolysis is NADH produced, where is it consumed, and why are fermentation products such as ethanol and lactic acid made?

3.9 Respiration offers an energy yield much greater than that of fermentation. The citric acid cycle generates CO_2 and electrons for the electron transport chain and is also a source of biosynthetic intermediates. The glyoxylate cycle is necessary for the catabolism of two-carbon electron donors, such as acetate.

Q How much more ATP is possible from aerobically respiring glucose instead of fermenting it to lactate? Why is this so? Does the citric acid cycle only have a catabolic function?

3.10 Electron transport chains are composed of membrane-associated redox proteins that are arranged in order of their increasing E_0' values. The electron transport chain functions in a concerted fashion to carry electrons from the primary electron donor to the terminal electron acceptor, which is O_2 in aerobic respiration.

Q List some of the key electron carriers found in electron transport chains.

3.11 During electron transport, protons are extruded to the outside of the membrane to form the proton motive force. Key electron carriers include flavins, quinones, the cytochrome bc_1 complex, and other cytochromes. The cell uses the proton motive force to make ATP through the activity of ATPase.

Q How is the proton motive force generated during electron transport? How do cells generate ATP from a proton motive force?

3.12 When conditions are anoxic, several electron acceptors can substitute for O_2 in anaerobic respiration. Chemolithotrophs use inorganic compounds as electron donors, whereas phototrophs use light energy. The proton motive force underlies energy conservation in all forms of respiration and photosynthesis.

Q What is the major difference between aerobic respiration and anaerobic respiration? Which metabolic option yields more energy, and why? Give an example of a chemolithotrophic electron donor.

IV • Biosyntheses

3.13 Polysaccharides are important structural components of cells and are biosynthesized from activated forms of their monomers. Gluconeogenesis is the production of glucose from nonsugar precursors.

Q What is the importance of the enzyme ribonucleotide reductase in the metabolism of sugars? What is the difference between "free" and "activated" glucose?

3.14 Amino acids are formed from carbon skeletons to which ammonia is added from glutamate, glutamine, or a few other amino acids. Nucleotides are biosynthesized using carbon skeletons from several different sources.

Q Name two common enzymes that function to incorporate NH_3 into the cell. How do their reaction mechanisms differ?

3.15 Fatty acids are synthesized from the three-carbon precursor malonyl-ACP, and fully formed fatty acids are attached to glycerol to form lipids. Only the lipids of *Bacteria* and *Eukarya* contain fatty acids.

Q Describe the process by which a fatty acid such as palmitate (a C_{16} straight-chain saturated fatty acid) is synthesized in a cell.

Application Questions

1. Using the data of Figure 3.10, predict the sequence of electron carriers in the membrane of an organism growing aerobically that has the following electron carriers: ubiquinone, cytochrome aa_3, cytochrome *b*, NADH, cytochrome *c*, FAD.
2. Explain the following observation in light of the redox tower: Cells of *Escherichia coli* fermenting glucose grow faster when NO_3^- is supplied to the culture (NO_2^- is produced) and then grow even faster (and stop producing NO_2^-) when the culture is highly aerated.

Chapter Glossary

ABC (ATP-binding cassette) transport system a membrane transport system consisting of three proteins, one of which hydrolyzes ATP; the system transports specific nutrients into the cell

Activation energy the energy required to bring the substrate of an enzyme to the reactive state

Adenosine triphosphate (ATP) a nucleotide that is the primary form in which chemical energy is conserved and utilized in cells

Anabolic reactions (Anabolism) the sum total of all biosynthetic reactions in the cell

Anaerobic respiration a form of respiration in which oxygen is absent and alternative electron acceptors are reduced

ATPase (ATP synthase) a multiprotein enzyme complex embedded in the cytoplasmic membrane that catalyzes the synthesis of ATP coupled to dissipation of the proton motive force

Autotroph an organism capable of biosynthesizing all cell material from CO_2 as the sole carbon source

Calvin cycle the series of biosynthetic reactions by which most phototrophs and many chemolithotrophs convert CO_2 into organic compounds

Catabolic reactions (catabolism) biochemical reactions leading to energy conservation (usually as ATP) by the cell

Chemolithotroph an organism that can grow with inorganic compounds as electron donors in energy metabolism

Chemoorganotroph an organism that obtains its energy from the oxidation of organic compounds

Citric acid cycle a cyclical series of reactions resulting in the conversion of acetate to two molecules of CO_2

Coenzyme a small and loosely bound nonprotein molecule that participates in a reaction as part of an enzyme

Electron acceptor a substance that can accept electrons from an electron donor, becoming reduced in the process

Electron donor a substance that can donate electrons to an electron acceptor, becoming oxidized in the process

Endergonic a reaction that requires free energy

Enzyme a protein that can speed up (catalyze) a specific chemical reaction (a few RNAs are also enzymes)

Exergonic a reaction that releases free energy

Fermentation anaerobic catabolism in which an organic compound is both an electron donor and an electron acceptor and ATP is produced by substrate-level phosphorylation

Free energy (*G*) energy available to do work; $G^{0\prime}$ is free energy under standard conditions

Glycolysis a biochemical pathway in which glucose is oxidized to pyruvate, which is either used in respiration or fermented; yields ATP and, in fermentation, various fermentation products (also called the Embden–Meyerhof–Parnas pathway)

Glyoxylate cycle a modification of the citric acid cycle in which isocitrate is cleaved to form succinate and glyoxylate during growth on two-carbon electron donors such as acetate

Group translocation an energy-dependent transport system in which the substance transported is chemically modified during the process of being transported by a series of proteins

Heterotroph an organism that uses organic compounds as a carbon source

Oxidative phosphorylation the production of ATP from a proton motive force formed by electron transport of electrons from organic or inorganic electron donors

Pentose phosphate pathway a series of reactions in which pentoses are catabolized to generate precursors for nucleotide biosynthesis or to synthesize glucose

Photophosphorylation the production of ATP from a proton motive force formed from light-driven electron transport

Phototrophs organisms that use light as their source of energy

Proton motive force (pmf) a source of energy resulting from the separation of protons from hydroxyl ions across the cytoplasmic membrane, generating a membrane electrochemical potential

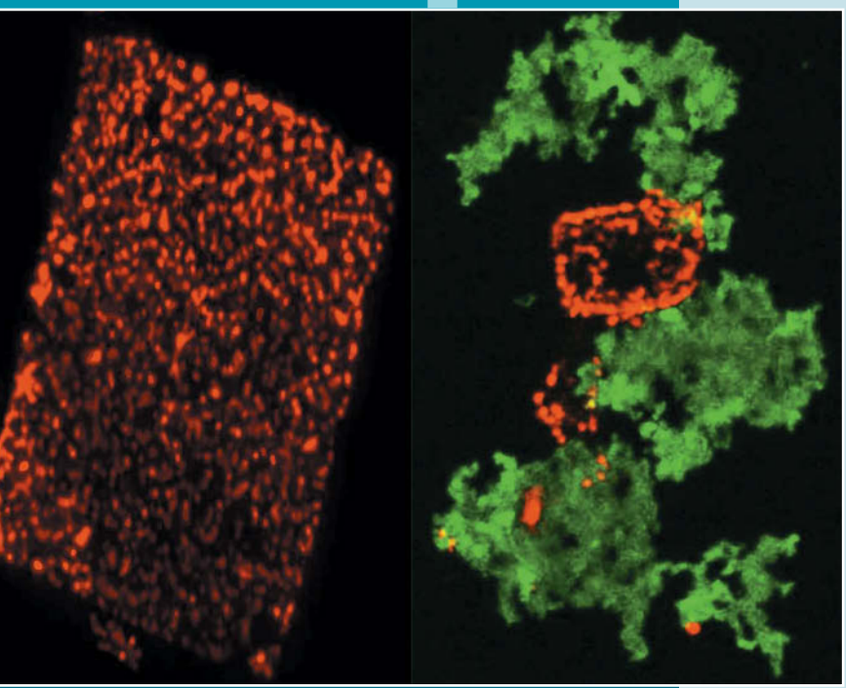
Reduction potential (E_0') the inherent tendency, measured in volts under standard conditions, of a compound to donate or to accept electrons

Respiration the process in which a compound is oxidized with O_2 (or an O_2 substitute) as the terminal electron acceptor, usually accompanied by ATP production by oxidative phosphorylation

Simple transport system a transporter that consists of only a membrane-spanning protein and is typically driven by energy from the proton motive force

Substrate-level phosphorylation the production of ATP by the direct transfer of an energy-rich phosphate molecule from a phosphorylated organic compound to ADP

4 Molecular Information Flow and Protein Processing



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microbiologynow

Synthesis of Jumbo Proteins: Secretion of Halomucin

The genetic blueprint of individual cells is responsible for the distinctive attributes and survival mechanisms observed in all life forms. Because the flow of essential biological information—from fairly inert DNA to the synthesis of proteins—requires considerable resources, the discovery of extremely long polypeptides in *Archaea* and *Bacteria* is surprising. While the average protein size in all three domains of life is <500 amino acids, the square-celled archaeon *Haloquadratum walsbyi* is predicted to encode a 9159-amino-acid protein called *halomucin*. As its name suggests, halomucin shares a structural similarity with animal mucin, which protects the eyes and bronchial epithelium from dehydration and chemical stress. Halomucin is also predicted to contain a signal sequence that suggests it is secreted outside of the cell. As you will see in this chapter, considerable energy is required not only to synthesize a protein, but also to get it to its final destination. Does *H. walsbyi* really produce this jumbo protein, and if so, why would it expend so many resources to do so?

H. walsbyi grows under extremely salty conditions and possesses a unique square but thin morphology ($5 \times 5 \times 0.1$ μm). This shape gives cells of *H. walsbyi* the greatest ratio of surface area to volume of all known microbes. Despite the gigantic nature of halomucin, is it possible that the predicted protein is synthesized and then, using its signal sequence, secreted outside of the cell to protect the cell from desiccation?

Scientists have recently answered this question by using fluorescent labeling techniques. The left photo illustrates the unusual cell morphology of *H. walsbyi* by using a red fluorescent stain targeting the abundant carbon storage polymers (poly-β-hydroxyalkanoates) produced by this organism. The right photo shows the same red cell with green-labeled secreted halomucin surrounding the cell. While translation and secretion of this jumbo protein are undoubtedly energetically draining on the cell, the production of halomucin is likely critical for retaining sufficient moisture near the cell surface to allow *H. walsbyi* to survive at the limits of water activity.

Source: Zenke, R., S. von Gronau, H. Bolhuis, M. Gruska, F. Pfeiffer, and D. Oesterhelt. 2015. Fluorescence microscopy visualization of halomucin, a secreted 927 kDa protein surrounding *Haloquadratum walsbyi* cells. *Front. Microbiol. 6:* 249.

Cells can be viewed as both chemical machines and coding devices. As chemical machines, cells transform nutrients into new cell material (Chapter 3). As coding devices, they store, process, and use genetic information. This chapter highlights the coding aspects of a microbe's activities, events that are foundational to the very being of an organism and the entire science of microbiology. Our focus will be on processes as they occur in *Bacteria*, particularly in *Escherichia coli*—the model organism for molecular biology—but we will also consider these processes in *Archaea*, and briefly in eukaryotic cells.

I • Molecular Biology and Genetic Elements

We begin by considering some basic principles of molecular biology—a refresher course, if you will. In Part I we examine the structure of DNA and the basic processes in genetic information flow and then move on to consider the various types of genetic elements. Part I lays the groundwork for a detailed consideration of DNA replication in prokaryotic cells (Part II) and the mechanisms behind RNA and protein synthesis (Parts III and IV).

4.1 DNA and Genetic Information Flow

An overview of the major macromolecules and processes of molecular biology is shown in Figure 4.1*a*. The functional unit of genetic information is the **gene**, and genes make up parts of chromosomes or other large molecules known collectively as **genetic elements**; the total complement of genetic elements is the **genome**. Genetic information is embedded in the sequence of nucleotides in the nucleic acids **DNA** and **RNA**. DNA carries the cell's genetic blueprint while RNA, produced in transcription, carries a copy of this blueprint. One form of RNA called messenger RNA is converted by translation into defined amino acid sequences in proteins. Collectively, nucleic acids and proteins are called **informational macromolecules** (Figure 4.1*a*).

The monomers of nucleic acids are called **nucleotides** and so DNA and RNA are **polynucleotides**. A nucleotide has three components: a pentose sugar (either ribose in RNA or deoxyribose in DNA), a nitrogenous base, and a molecule of phosphate, PO_4^{3-} (Figure 4.1*b*). A **nucleoside** has a pentose sugar and a nitrogenous base but does not include a phosphate group. The nitrogenous bases in nucleic acids are either **pyrimidines** or **purines**. The purines guanine and adenine and the pyrimidine cytosine are present in both DNA and RNA, whereas the pyrimidines thymine and uracil are only present (with minor exceptions) in DNA and RNA, respectively (Figure 4.1*c*).

Properties of the Double Helix

The nucleic acid backbone is a polymer of alternating sugar and phosphate molecules, and nucleotides are linked by phosphate between the 3′-carbon of one sugar and the 5′-carbon of the next sugar, the **phosphodiester bond** (Figure 4.1*b*, Figure 4.2). The *sequence* of nucleotides in a DNA or RNA molecule is its

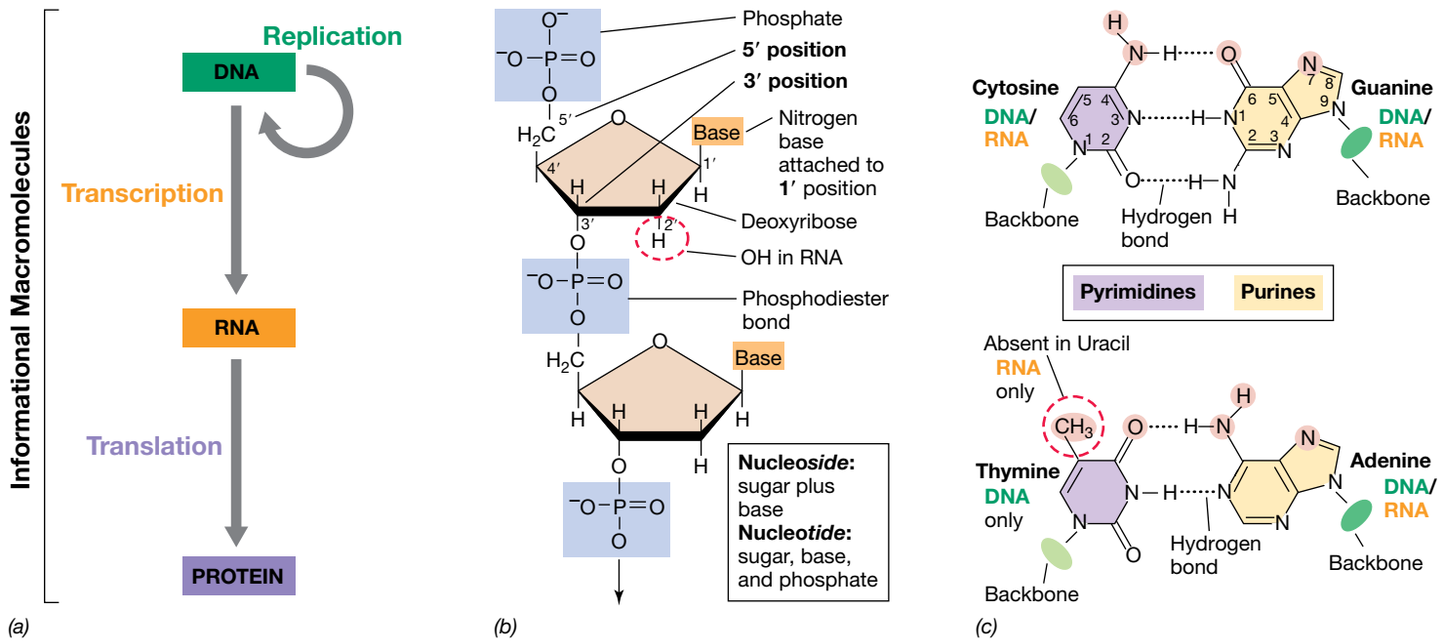

Figure 4.1 Genetic information flow and the components of the nucleic acids. *(a)* An overview of the types of informational macromolecules. *(b)* Part of a DNA chain. The numbers on the sugar of the nucleotide contain a prime (′) to differentiate them from the numbering on the rings of the nitrogen bases. In DNA, a hydrogen is present on the 2′-carbon of the pentose sugar. In RNA, an OH group occupies this position. The nucleotides are linked by a phosphodiester bond. *(c)* The nitrogen bases of DNA and RNA and the specific pairing between cytosine (C) and guanine (G) and between thymine (T) and adenine (A) via hydrogen bonds. Uracil (U) instead of thymine is present in RNA. Note the numbering system of the rings in that a pyrimidine base bonds through N-1 to the sugar–phosphate backbone and that a purine base bonds through N-9. Atoms that are found in the major groove of the double helix (see Figure 4.3) and that interact with proteins are highlighted in pink.

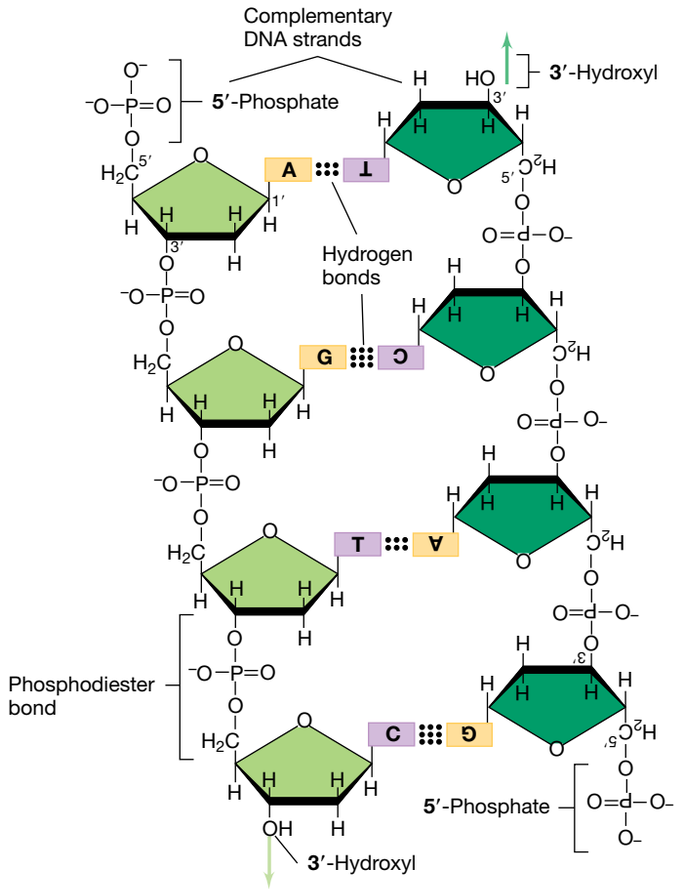

Figure 4.2 DNA structure. Complementary and antiparallel nature of DNA. Note that one chain ends in a 5′-phosphate group, whereas the other ends in a 3′-hydroxyl. The purple bases represent the pyrimidines cytosine (C) and thymine (T), and the yellow bases represent the purines adenine (A) and guanine (G).

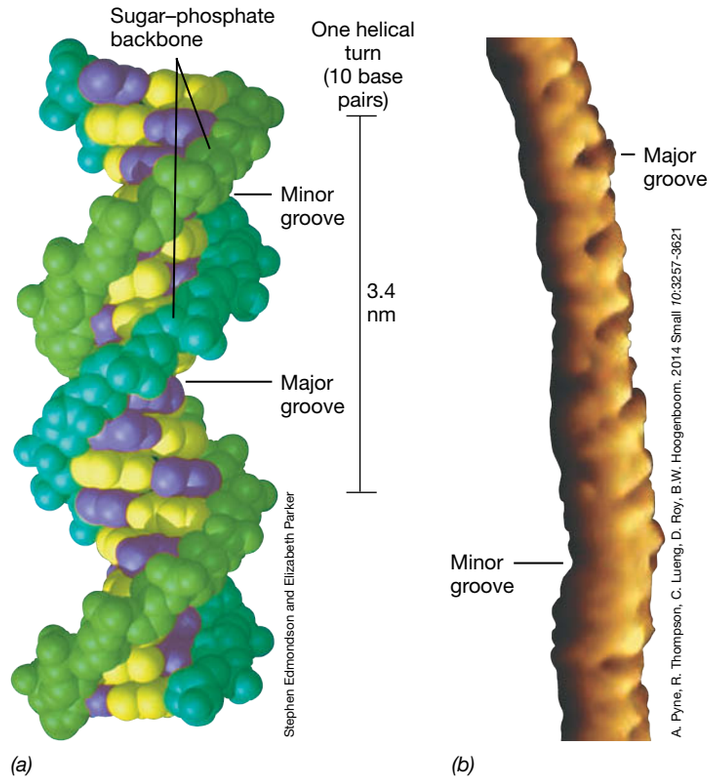

Figure 4.3 Arrangement of the DNA double helix. *(a)* A computer model of a short segment of DNA showing one of the sugar–phosphate backbones in blue and the other in green. The pyrimidine bases are shown in purple and the purines in yellow. Note the locations of the major and minor grooves. One helical turn contains 10 base pairs. *(b)* Atomic force microscopy showing the biomolecular structure of a small piece of DNA. Note the locations of the major and minor grooves.

primary structure and encodes the genetic information. In cells, DNA is *double-stranded*, the strands being held together by hydrogen bonds between the bases in the two strands (Figure 4.1*c*). Specific base pairing, A with T and G with C, ensures that the two strands of DNA are **complementary** in base sequence, and this complementarity is essential for the faithful replication of the molecule. The two strands of the DNA molecule are also arranged in an **antiparallel** fashion; one strand runs 5′ to 3′ (top to bottom), whereas its complement runs 5′ to 3′ (bottom to top) (Figure 4.2).

The complementary and antiparallel strands of DNA are wrapped around each other to form a double helix (Figure 4.3). The helix naturally forms two distinct grooves, the *major groove* and the *minor groove*. Most proteins that interact specifically with DNA bind in the major groove, where space is abundant. Because the double helix is a regular structure, some atoms of each base are always exposed in the major groove (and some in the minor groove). These key regions of the DNA that are important in interactions with proteins can be seen in Figure 4.3, and the atoms of the major groove that interact with proteins are indicated in Figure 4.1*c*.

Size, Shape, and Supercoiling of DNA

The size of a DNA molecule is expressed as its total number of nucleotide base pairs. Thus, a double-stranded DNA molecule consisting of 1000 bases is one kilobase pair (kbp) of DNA. The bacterium *Escherichia coli* has about 4640 kbp (4.64 megabase pairs, Mbp) of DNA in its genome. If this molecule were extended linearly it would be several hundred times longer than the cell itself. To accommodate their genome, cells of *Bacteria* and *Archaea* must compact the DNA, and this is done by the process of *supercoiling* (Figure 4.4).

Supercoils are inserted or removed in DNA by enzymes called *topoisomerases*. The activity of supercoiling puts the DNA molecule under torsion (Figure 4.5), and DNA can be supercoiled in either a positive or a negative manner. Negative supercoiling results when the DNA is twisted about its axis in the opposite sense from the right-handed double helix and is the form found in most cells. In the *E. coli* chromosome, more than 100 supercoiled domains exist, each stabilized by specific proteins bound to the DNA. Inserting supercoils into DNA requires energy from ATP, whereas releasing supercoils does not. In *Bacteria* and most *Archaea*, the topoisomerase **DNA gyrase** inserts negative supercoils into DNA by making double-strand breaks (Figure 4.5*b*). We will see in Chapters 5 and 17 that some *Archaea* live at very high

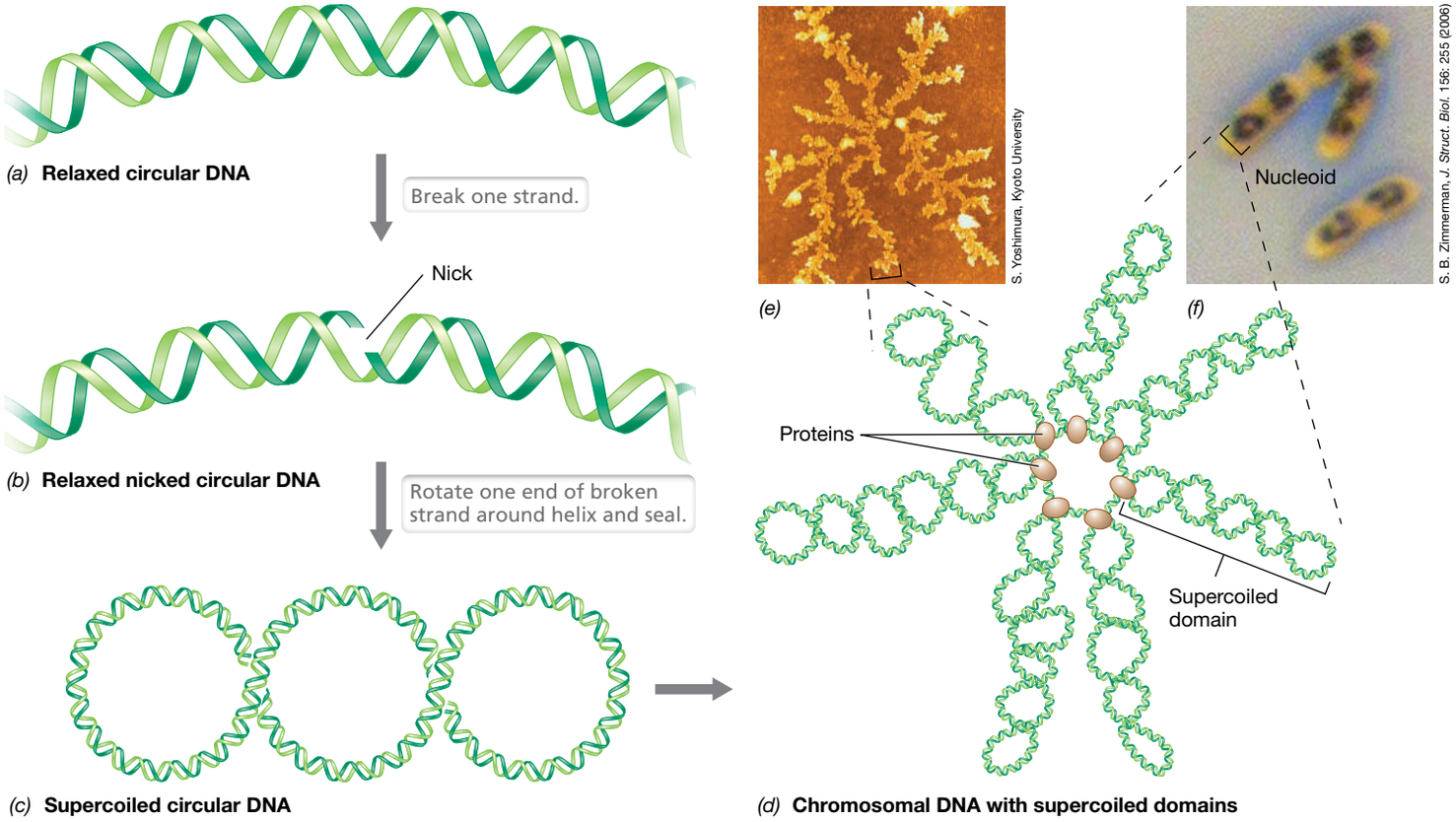

Figure 4.4 Supercoiled DNA. *(a–c)* Relaxed, nicked, and supercoiled circular DNA. A nick is a break in a phosphodiester bond of one strand. *(d)* In fact, the double-stranded DNA in the bacterial chromosome is arranged not in one supercoil but in several supercoiled domains, as shown here. *(e)* Atomic force microscopy of the *Escherichia coli* nucleoid. *(f)* Simultaneous phase-contrast and fluorescence image of *E. coli* illustrating the location of the nucleoid within growing cells. Cells were treated with a fluorescent dye specific for DNA and the color was inverted to show the nucleoids as black.

temperatures—above the boiling point in some cases. These species have chromosomes that are *positively* (instead of negatively) supercoiled, and this genomic feature helps to maintain DNA structure (that is, it prevents the two strands from melting apart) at such high temperatures (Section 17.12). Supercoiling is not a feature of eukaryotes since their genomic DNA is linear rather than circular. However, eukaryotic DNA must still be compacted, and this occurs when the DNA is highly wound around histone proteins.

Genes and the Steps in Biological Information Flow

Genetic information flow is a fundamental process in all cells and is the *central dogma of molecular biology* (Figure 4.1*a* and Figure 4.6). When genes are *expressed*, the genetic information encoded in DNA is transferred to ribonucleic acid (RNA). While several classes of RNA exist in cells, three main classes of RNA participate in protein synthesis. **Messenger RNAs (mRNAs)** are single-stranded molecules that carry the genetic information from DNA to the ribosome. **Transfer RNAs (tRNAs)** help convert the genetic information in the nucleotide sequences of RNA into a defined sequence of amino acids in proteins. **Ribosomal RNAs (rRNAs)** are important catalytic and structural components of the ribosome. The molecular processes of genetic information flow can be divided into three stages (Figure 4.6):

1. **Replication.** During replication, the DNA double helix is duplicated. Replication is catalyzed by the enzyme *DNA polymerase*.
2. **Transcription.** The transfer of genetic information from DNA to RNA is called transcription. Transcription is catalyzed by the enzyme *RNA polymerase*.
3. **Translation.** The formation of a polypeptide using the genetic information transferred to mRNA by DNA is a process that occurs on the ribosome.

Many different RNA molecules can be transcribed from a relatively short region of the long DNA molecule. In eukaryotes, each gene is transcribed to yield a single mRNA, whereas a single mRNA molecule may encode several different proteins in *Bacteria* and *Archaea*. However, a linear correspondence exists between the base sequence of a gene and the amino acid sequence of a polypeptide, and as we will see, each group of *three bases* on an mRNA molecule encodes a single amino acid (Section 4.9).

Eukaryotes differ from *Bacteria* and *Archaea* in that the first two steps of the central dogma, replication and transcription (Figures 4.1 and 4.6), occur in the nucleus. Because ribosomes are not present in

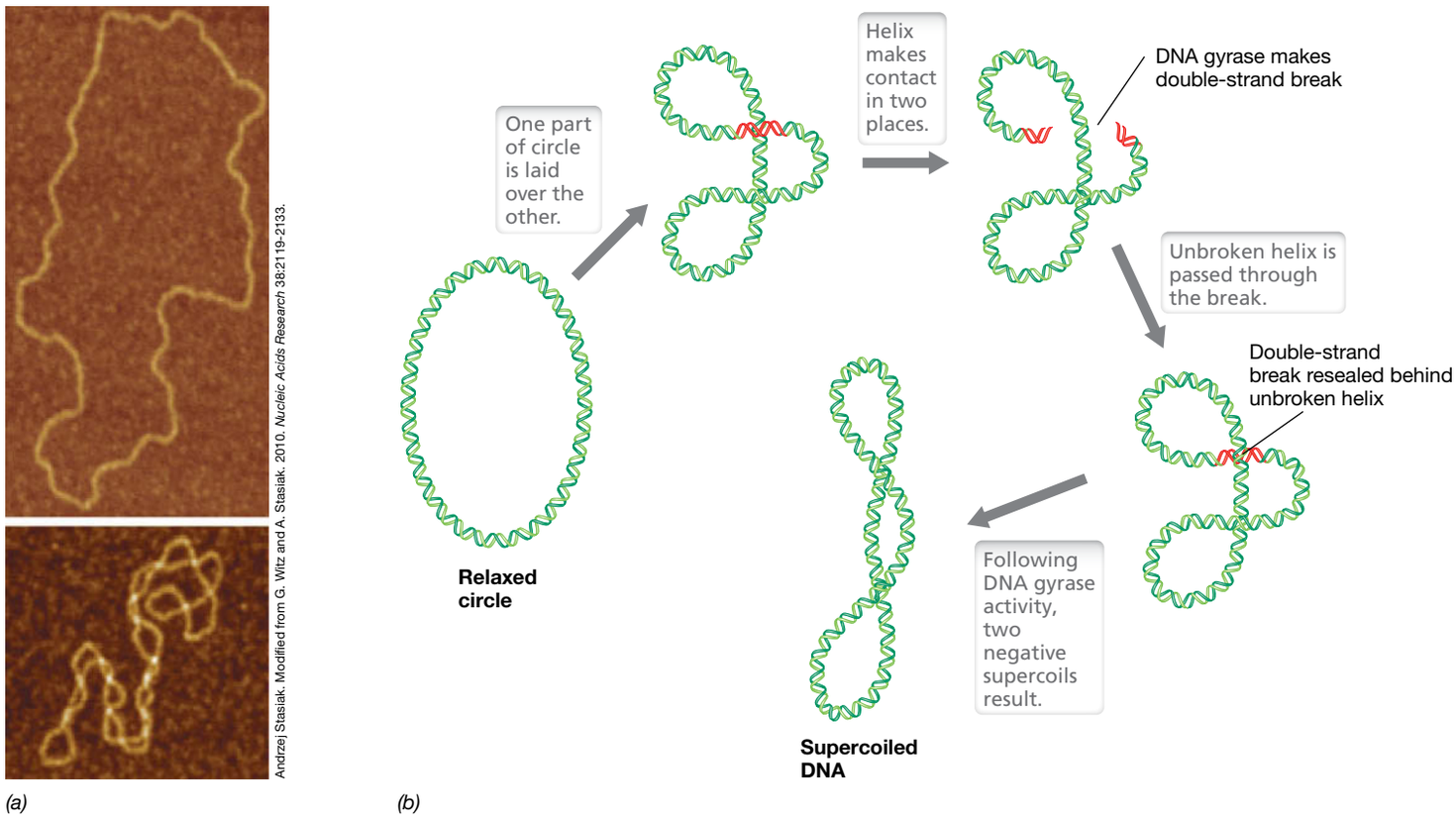

Figure 4.5 DNA gyrase. *(a)* Atomic force microscopy visualization of torsionally relaxed (top) and negatively supercoiled (bottom) plasmid DNA. *(b)* Schematic showing the introduction of negative supercoiling into circular DNA by the activity of DNA gyrase (topoisomerase II), which makes double-strand breaks.

the nucleus, mRNAs as well as other RNAs must be transported outside of the nucleus for translation. By contrast, in prokaryotic cells, mRNAs do not have to be exported from an organelle to be translated. Because of this fundamental difference, transcription and translation in *Bacteria* and *Archaea* can occur simultaneously in a process known as *coupled transcription and translation* (Figure 4.7). During this process, a ribosome initiates translation of an mRNA before RNA polymerase has finished synthesizing it. This allows rapidly growing cells to produce proteins at a maximal rate and also allows the cell to rapidly adapt to changes in growth conditions by quickly expressing the new protein sets required.

While the central dogma of molecular biology (Figures 4.1*a* and 4.6) is invariant in *cells*, we will see later that some *viruses* (which are not cells, Section 1.14) violate the dogma in many interesting ways (Chapters 8 and 10). But for now we move on to consider the different genetic elements present in prokaryotic cells.

MINIQUIZ

- What is a genome and what is it composed of? What is the central dogma of molecular biology?
- Define the terms complementary and antiparallel as they pertain to DNA.
- Why is supercoiling essential to a bacterial cell? What enzyme facilitates this process?

4.2 Genetic Elements: Chromosomes and Plasmids

Structures containing genetic material (DNA in cells but RNA in some viruses) are called *genetic elements*, and the main genetic element in prokaryotic cells is the **chromosome**. However, other genetic elements play important roles in microbes and these include *virus genomes, plasmids, organellar genomes*, and *transposable elements* (Table 4.1). Most *Bacteria* and *Archaea* contain a single circular chromosome containing all (or most) of the organism's genes. Although a single chromosome is the rule in prokaryotic cells, there are exceptions, as a few contain two or even three chromosomes. Eukaryotic genomes, by contrast, are composed of two or more chromosomes containing linear DNA. The genomes of viruses consist of *either* DNA or RNA and can be single- or double-stranded and either linear or circular.

Plasmids are circular or linear double-stranded DNA molecules that replicate separately from the chromosome and are typically much smaller than chromosomes. **Transposable elements** are sequences of DNA that are inserted into other DNA molecules but can move from one site on the DNA molecule to another, either within the same molecule or on a different DNA molecule. Chromosomes, plasmids, virus genomes, and any other type of DNA molecule may host a transposable element. Transposable elements are found in both prokaryotic and eukaryotic cells and play important roles in genetic variation (Section 11.11).

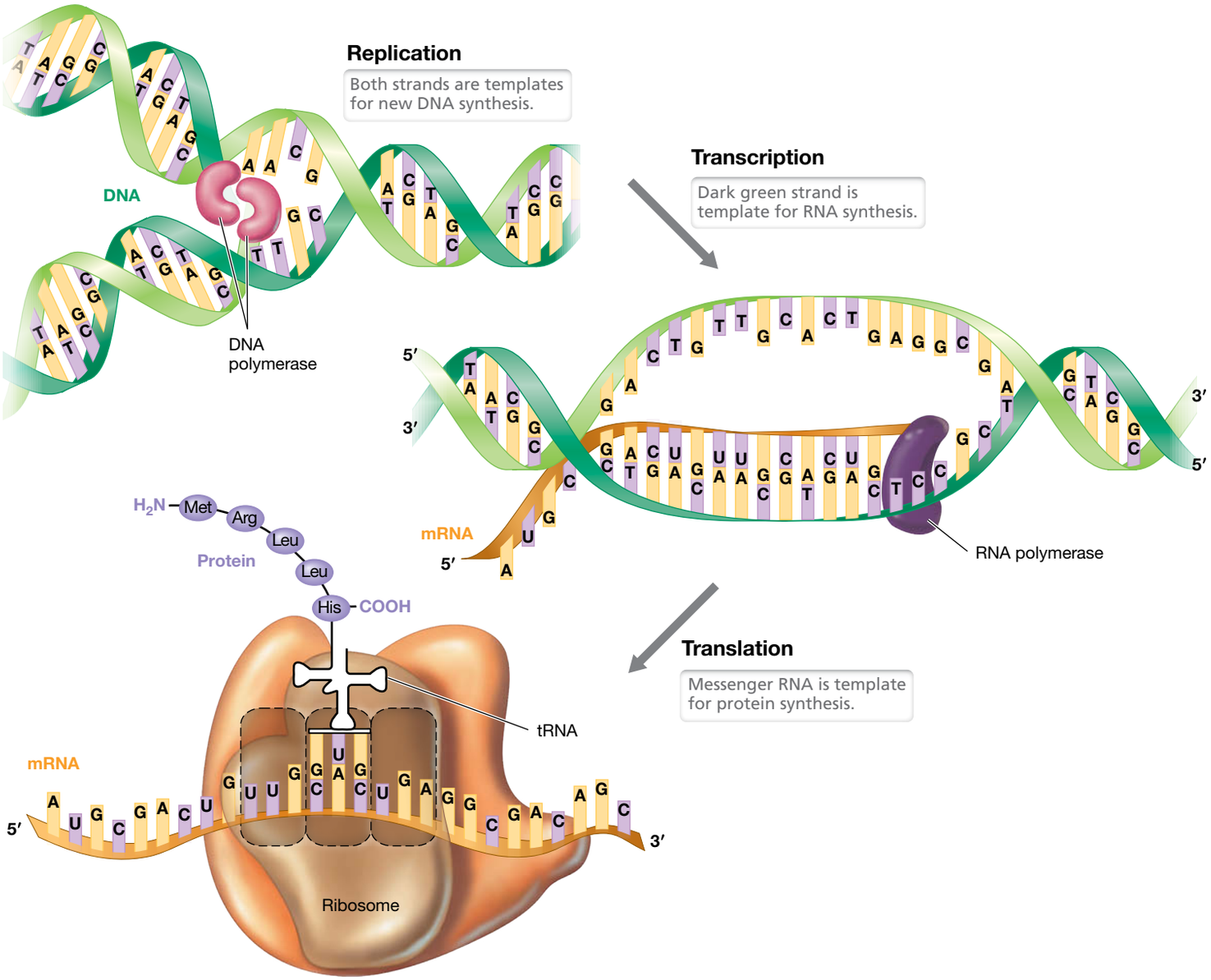

Figure 4.6 Synthesis of the three types of informational macromolecules. Note that for any particular gene only one of the two strands of the DNA double helix is transcribed.

Chromosomal Gene Arrangements and the Operon

Thousands of genomes from species of *Bacteria* and *Archaea* have been completely sequenced, thus revealing the number and location (the genetic map) of the genes they possess. The genetic map of the 4,639,675-bp chromosome of a widely studied strain of *Escherichia coli* is presented in **Figure 4.8**, with only a few of the organism's several thousand genes depicted. Analysis of the *E. coli* genome has revealed 4288 possible protein-encoding genes that account for 88% of the *E. coli* genome. Approximately 1% of the genome encodes tRNAs and rRNAs, and the remaining genes consist of regulatory sequences that may or may not be transcribed (but are not translated) or have other functions. The compact genomes of *Bacteria* and *Archaea* stand in contrast to the genomes of eukaryotes, which typically contain much more DNA than is needed to encode all the proteins required for cell function. This "extra" DNA in eukaryotes is present as intervening DNA between coding sequences (the intervening sequences are removed before translation) or as repetitive sequences, some of which are repeated hundreds or thousands of times (Chapter 9).

Genetic mapping of the genes encoding the enzymes that function in steps of the same biochemical pathway in *E. coli* has shown that these genes are sometimes clustered. On the genetic map in Figure 4.8, a few such clusters are shown (for example, the *gal, trp*, and *his* clusters); each of these groups is called an **operon**. An operon is transcribed to form a single mRNA that encodes several different proteins and is regulated as a unit. In contrast to these, the genes for many other biochemical pathways in *E. coli* are not clustered. For example, genes for maltose degradation (*mal* genes,

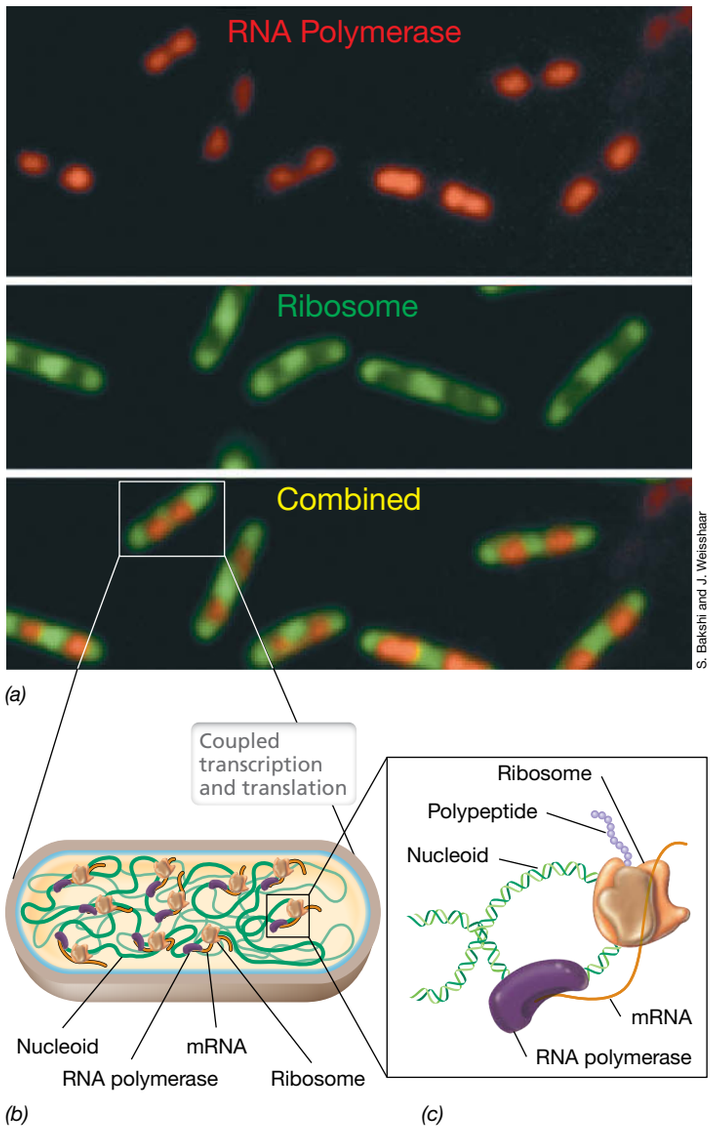

Figure 4.7 Coupled transcription and translation in prokaryotic cells. *(a)* Fluorescence microscopy and protein tagging of actively growing *Escherichia coli* cells illustrating the position of RNA polymerases and ribosomes performing transcription and translation, respectively. The combined photo (bottom image) shows that transcription and translation are occurring concurrently in the cell. *(b)* Location of the nucleoid, RNA polymerases, mRNA, and ribosomes in the cell during coupled transcription and translation. *(c)* Blowup illustration of a ribosome actively translating an mRNA as it is being synthesized by RNA polymerase.

Figure 4.8) are scattered throughout the chromosome. In fact, analysis of the *E. coli* chromosome has shown that over 70% of the predicted or known transcriptional units are of only a single gene while only 6% of operons have four or more genes. Thus operons, as efficient an arrangement of genes as they may be, appear to be the exception rather than the rule.

Plasmids

Many *Bacteria* and *Archaea* contain plasmids in addition to their chromosome(s). Most plasmids are nonessential since with rare exception they do not contain genes required for growth under all conditions. Thousands of different plasmids are known, and over 300 different plasmids have been isolated from strains of *E. coli* alone. Virtually all plasmids consist of double-stranded DNA and exist in the cytoplasm as free DNA. Most plasmids are circular, but many are linear and vary in size from approximately 1 kbp to more than 1 Mbp.

Typical plasmids are less than 5% of the size of the chromosome (**Figure 4.9**), and some bacteria contain several different plasmids. Moreover, different plasmids may be present in different *copy number*. For example, some plasmids may be present in only one or a few copies per cell, whereas others may be present in over 100 copies. Enzymes that replicate chromosomal DNA also replicate plasmids. Some of the genes encoded on a plasmid function to direct the initiation of plasmid replication and to partition replicated plasmids between daughter cells.

Although by definition plasmids do not encode functions essential to the host, plasmids may carry genes that profoundly influence host cell physiology; for example, plasmid genes may encode enzymes for some special metabolism that ensures survival under certain conditions. Among the most widespread and well-studied groups of plasmids are the resistance plasmids, called *R plasmids*, which confer resistance to antibiotics or other growth inhibitors. The resistance genes encode proteins that either inactivate the antibiotic or protect the cell in some other way, and several antibiotic resistance genes can be encoded on a single R plasmid. Plasmid R100 (**Figure 4.10**), for example, encodes resistance to sulfonamides, streptomycin, spectinomycin, fusidic acid, chloramphenicol, and tetracycline, as well as the toxic heavy metal mercury. Pathogenic bacteria resistant to antibiotics are of considerable medical significance, and their increasing incidence is correlated with the increasing use of antibiotics for treating infectious diseases in humans and animals (Chapter 28).

Pathogenic bacteria express a variety of plasmid-encoded *virulence factors* that assist them in establishing infections. For example,

TABLE 4.1 Kinds of genetic elements

Organism	*Element*	*Type of nucleic acid*	*Description*
Virus	Virus genome	Single- or double-stranded DNA or RNA	Relatively short, circular or linear
Bacteria/Archaea	Chromosome	Double-stranded DNA	Extremely long, usually circular
Eukaryote	Chromosome	Double-stranded DNA	Extremely long, linear
Mitochondrion or chloroplast	Organellar genome	Double-stranded DNA	Medium length, usually circular
All organisms	Plasmid[a]	Double-stranded DNA	Relatively short circular or linear, extrachromosomal
All organisms	Transposable element	Double-stranded DNA	Always found inserted into another DNA molecule

[a]Plasmids are uncommon in eukaryotes.

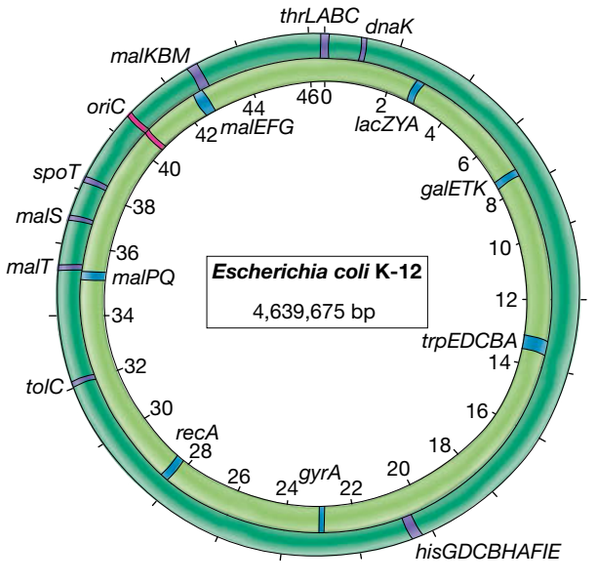

Figure 4.8 The chromosome of *Escherichia coli* strain K-12. Map distances are given in 100 kilobases of DNA. The chromosome contains 4,639,675 base pairs and 4288 open reading frames (genes). Depending on the DNA strand, the locations of a few genes and operons are indicated. Replication (Figure 4.6 and see Figures 4.16 and 4.17) proceeds in both directions from the origin of DNA replication, *oriC*, indicated in red.

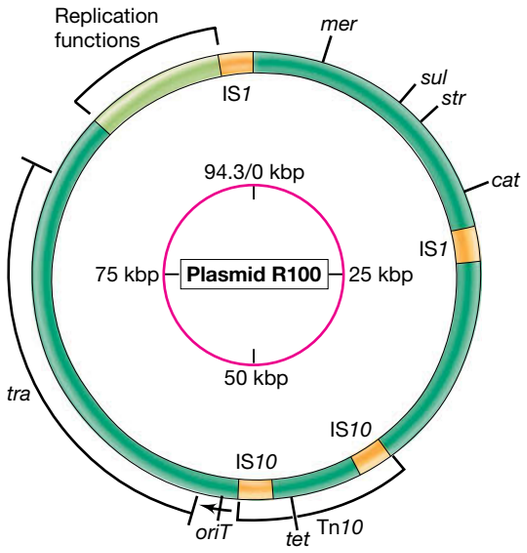

Figure 4.10 Genetic map of the resistance plasmid R100. The inner circle shows the size in kilobase pairs. The outer circle shows the location of major antibiotic resistance genes and other key functions: *mer*, mercuric ion resistance; *sul*, sulfonamide resistance; *str*, streptomycin resistance; *cat*, chloramphenicol resistance; *tet*, tetracycline resistance; *oriT*, origin of conjugative transfer; *tra*, transfer functions. The locations of insertion sequences (IS) and the transposon *Tn10* are also shown. Genes for plasmid replication are found in the region from 88 to 92 kbp.

the ability of a pathogen to attach to and colonize specific host tissues and to produce toxins, enzymes, and other invasive molecules that damage the host are sometimes plasmid encoded. Some bacteria also produce proteins called **bacteriocins** that inhibit or kill closely related species of bacteria (or even different strains of the same species of bacteria), and the genes encoding these bacteriocins and other proteins that protect the producing organism are typically found on plasmids.

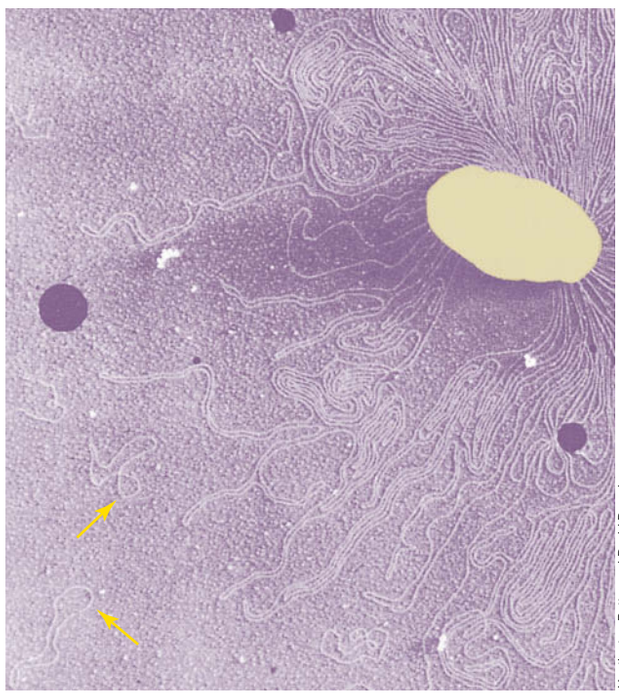

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Figure 4.9 The bacterial chromosome and bacterial plasmids, as seen in the electron microscope. The plasmids (arrows) are the circular structures and are much smaller than the main chromosomal DNA. The cell (large, tan structure) was broken gently so the DNA would remain intact.

In a few cases plasmids encode properties that are fundamental to the ecology of the bacterium. For example, the ability of the soil bacterium *Rhizobium* to form nitrogen-fixing nodules on the roots of plants (Section 23.3) requires certain functions encoded by plasmids. Other plasmids confer special metabolic properties. For example, the ability to degrade hydrocarbons or toxic pollutants, such as polychlorinated biphenyls (PCBs) and herbicides or other pesticides, is often plasmid encoded. In addition, plasmids play a crucial role in the horizontal gene transfer process called conjugation that we consider in detail later (Chapter 11).

MINIQUIZ

- Approximately how large is the *Escherichia coli* genome in base pairs? How many genes does it contain?
- What are viruses and plasmids?
- What properties does an R plasmid confer on its host cell?

II • Copying the Genetic Blueprint: DNA Replication

DNA replication is necessary for cells to divide, whether to reproduce new organisms, as in unicellular microorganisms, or to produce new cells as part of a multicellular organism. To successfully transmit genetic information from a mother cell to an

identical daughter cell, DNA replication must be extremely accurate. We review the basic principles of DNA replication here as a prelude to focusing on the process as it occurs in prokaryotic cells.

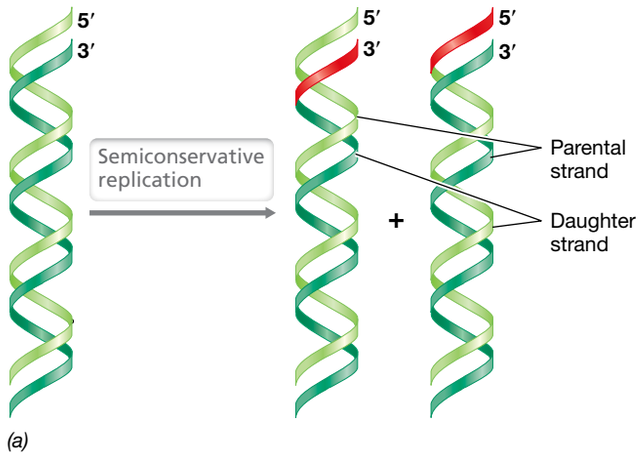
4.3 Templates, Enzymes, and the Replication Fork

DNA exists in cells as a double helix of two complementary strands (Figures 4.2 and 4.6), and if the helix is opened up, a new strand can be synthesized as the complement of each parental strand. As shown in **Figure 4.11*a***, replication is thus a **semiconservative** process, meaning that the two resulting double helices consist of one new strand and one parental strand. The DNA strand that is used to make a complementary daughter strand is called the *template strand*, and in DNA replication, each parental strand is a template for one newly synthesized strand (Figure 4.11*a*). The precursor of each new nucleotide in the DNA strand is a deoxynucleoside 5′-triphosphate. During insertion of this molecule, the two terminal phosphates are removed and the remaining phosphate is bonded to a deoxyribose of the growing chain (Figure 4.11*b*). This addition of the incoming nucleotide requires the presence of a free hydroxyl group, which is available only at the 3′ end of the molecule. This leads to the important principle that DNA replication always proceeds *from the 5′ end to the 3′ end*, the 5′-phosphate of the incoming nucleotide being attached to the 3′-hydroxyl of the previously added nucleotide (Figure 4.11*b*).

Replication Enzymes

Enzymes that catalyze the polymerization of deoxynucleotides are called **DNA polymerases** (abbreviated DNA Pol), and there are five different enzymes in *Escherichia coli*, DNA Pol I–V. DNA Pol III is the primary enzyme for replicating chromosomal DNA, although DNA Pol I also plays a lesser role. The other DNA polymerases function to repair damaged DNA (Section 11.4). DNA Pol enzymes are just some of the many enzymes that are required for DNA replication (**Table 4.2**).

All DNA polymerases synthesize DNA in the 5′→3′ direction, but none of them can initiate a new chain de novo; they can only add a nucleotide onto a preexisting 3′-OH group. Thus, in order to start a new DNA chain, a **primer**, a nucleic acid molecule to which DNA polymerase can attach the first nucleotide, is required, and this primer is a short stretch of *RNA* rather than DNA (**Figure 4.12**). When the DNA helix is first opened, the enzyme **primase** makes this RNA primer, synthesizing a short stretch (11–12 nucleotides) of RNA complementary in base pairing to the template strand DNA. At the growing end of this RNA primer is a 3′-OH group to which DNA polymerase adds the first deoxyribonucleotide. Continued extension of the molecule thus occurs as DNA rather than RNA (Figure 4.12), and the primer is eventually removed and replaced with DNA (described shortly).



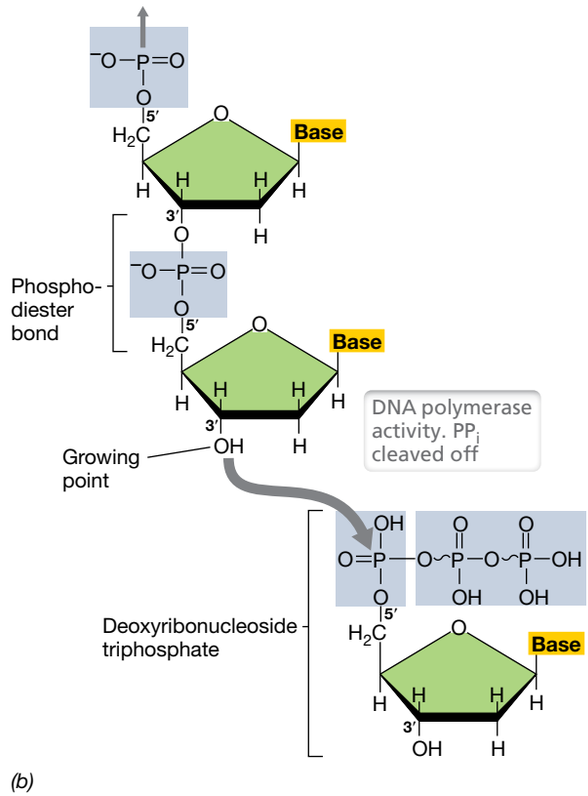


Figure 4.11 Overview of DNA replication. *(a)* DNA replication is a semiconservative process in all cells. Note that the new double helices each contain one new daughter strand (shown topped in red) and one parental strand. *(b)* Extension of a DNA chain by adding a deoxyribonucleoside triphosphate at the 3′ end. Growth proceeds from the 5′-phosphate to the 3′-hydroxyl end. DNA polymerase catalyzes the reaction. The four precursors are deoxythymidine triphosphate (dTTP), deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), and deoxycytidine triphosphate (dCTP). Upon nucleotide insertion, the two terminal phosphates of the triphosphate are split off as pyrophosphate (PP_i). Thus, two energy-rich phosphate bonds are consumed when adding each nucleotide.

TABLE 4.2 Major enzymes that participate in DNA replication in *Bacteria*

Enzyme	*Encoding genes*	*Function*
DNA gyrase	*gyrAB*	Replaces supercoils ahead of replisome
Origin-binding protein	*dnaA*	Binds origin of replication to open double helix
Helicase loader	*dnaC*	Loads helicase at origin
Helicase	*dnaB*	Unwinds double helix at replication fork
Single-strand binding protein	*ssb*	Prevents single strands from annealing
Primase	*dnaG*	Primes new strands of DNA
DNA polymerase III		Main polymerizing enzyme
Sliding clamp	*dnaN*	Holds Pol III on DNA
Clamp loader	*holA–E*	Loads Pol III onto sliding clamp
Dimerization subunit (Tau)	*dnaX*	Holds together the two core enzymes for the leading and lagging strands
Polymerase subunit	*dnaE*	Strand elongation
Proofreading subunit	*dnaQ*	Proofreading
DNA polymerase I	*polA*	Excises RNA primer and fills in gaps
DNA ligase	*ligA, ligB*	Seals nicks in DNA
Tus protein	*tus*	Binds terminus and blocks progress of the replication fork
Topoisomerase IV	*parCE*	Unlinking of interlocked circles

Initiation of DNA Synthesis

Before replication can begin, the double helix must be unwound to expose the template strands, the so-called **replication fork**. The enzyme **DNA helicase** unwinds the double helix (using energy from ATP) and exposes a short single-stranded region (Figure 4.13). Helicase moves along the DNA and separates the strands just in advance of the replication fork. The single-stranded region is immediately covered with copies of single-strand binding protein to stabilize the single-stranded DNA and prevent the double helix from re-forming. DNA synthesis begins at a single site on the chromosome, the origin of replication (*oriC*), where the protein DnaA (Table 4.2) binds and opens up the double helix. Next to assemble is helicase (DnaB), which is helped onto the DNA by a loader protein (DnaC) (Figure 4.13*b*). Two helicases are loaded, one onto each strand, facing in opposite directions. Finally, two primase and two DNA polymerase enzymes are loaded onto the DNA behind the helicases and initiation of DNA replication begins. As replication proceeds, the replication fork appears to move along the DNA (Figure 4.13*a*).

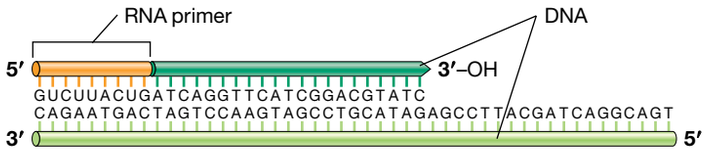


Figure 4.12 The RNA primer. Structure of the RNA–DNA hybrid formed during initiation of DNA synthesis. Orange depicts the RNA primer.

Leading and Lagging Strands and the Replication Process

Figure 4.14 depicts DNA replication at the replication fork. Recall that replication always proceeds from 5′ to 3′ (5′→3′), always adding a new nucleotide to the 3′-OH of the growing chain. On the strand growing from the 5′-PO_4^{2-} to the 3′-OH, called the **leading strand**, DNA synthesis occurs *continuously* because there is always a free 3′-OH at the replication fork to which a new nucleotide can be added; the leading strand must therefore be primed only once. By contrast, on the opposite strand, called the **lagging strand**, DNA synthesis occurs *discontinuously* because there is no 3′-OH at the replication fork to which a new nucleotide can attach; on this strand, primase must synthesize multiple RNA primers in order to provide free 3′-OH groups for DNA Pol III (Figure 4.14). As a result, the lagging strand forms from several short DNA fragments that are combined later to yield a continuous strand of DNA.

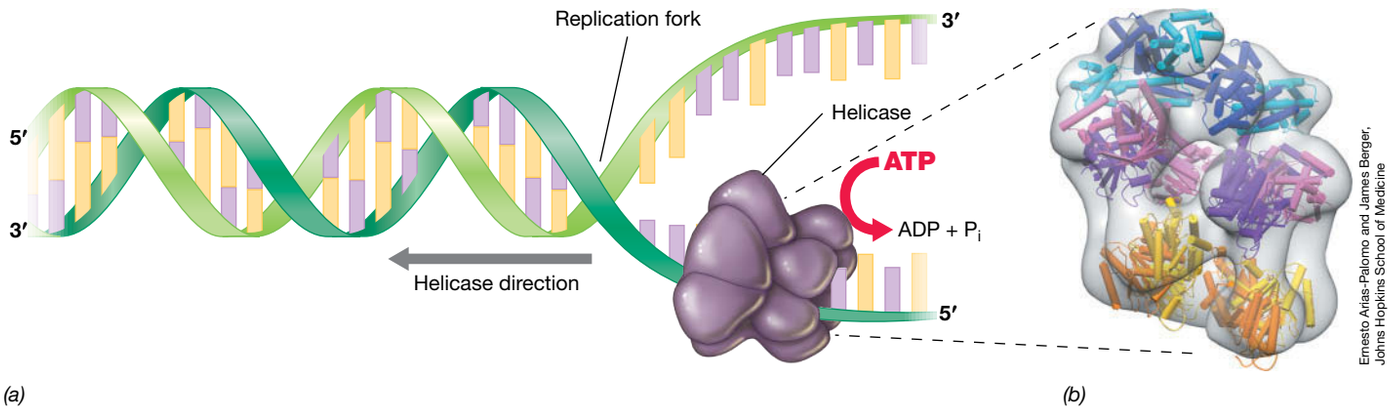


Figure 4.13 DNA helicase unwinding a double helix. *(a)* In this figure, the helicase is denaturing or pulling the two antiparallel strands of DNA apart beginning from the right and moving to the left. *(b)* A three-dimensional model of the helicase (DnaB) along with its loader protein ((DnaC, yellow and orange) based on cryo-electron microscopy.

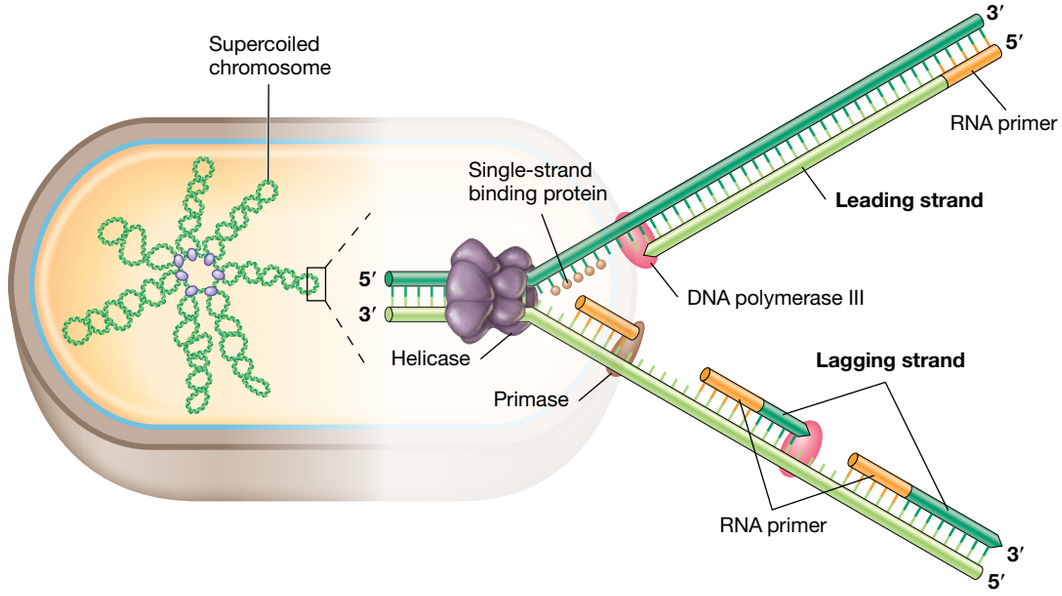


Figure 4.14 Events at the DNA replication fork on the nucleoid. Note the polarity and antiparallel nature of the DNA strands. Helicase unwinds the DNA while primase adds the RNA primer. For the steps in introducing and removing supercoils from DNA, see Figure 4.5. Further events in DNA synthesis including sealing replicated fragments are shown in Figure 4.15.

After synthesizing the RNA primer, primase is replaced by DNA Pol III. This enzyme complex (Table 4.2) is held on the DNA by a "sliding clamp," which encircles and slides along the single template strands of DNA. Consequently, the replication fork contains two polymerase core enzymes and two sliding clamps, one set for each strand. After assembly on the lagging strand, the elongation activity of DNA Pol III adds deoxyribonucleotides sequentially until it reaches previously synthesized DNA (**Figure 4.15**); at this point, activity of DNA Pol III stops.

To complete DNA synthesis, DNA Pol I catalyzes two different reactions. Besides synthesizing DNA, Pol I has a 5′→3′ exonuclease activity that removes the RNA primer (Figure 4.15). When the primer has been excised and replaced with DNA, Pol I is released. The *very last* phosphodiester bond in replicating DNA is made by **DNA ligase**. This enzyme seals nicks in DNAs that have an adjacent 5′-PO_4^{2-} and 3′-OH (something that DNA Pol I and Pol III are unable to do), and along with DNA Pol I, it also participates in DNA repair. DNA ligase is also important for sealing genetically manipulated DNA during molecular cloning (↪ Section 12.2).

We now put DNA synthesis in the context of *Archaea* and *Bacteria* to see how replication events occur around the covalently closed and circular chromosomes typical of these organisms.

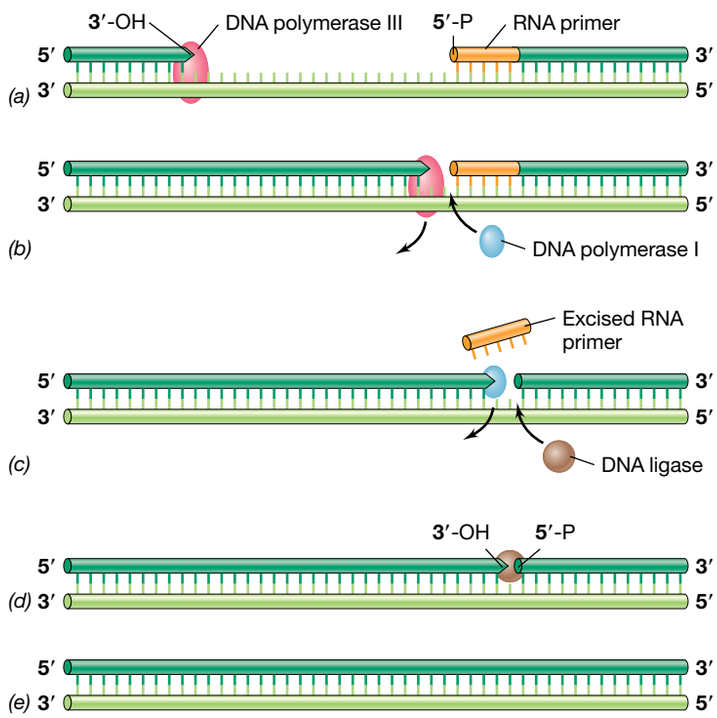


Figure 4.15 Sealing two fragments on the lagging strand. Unlike the leading strand, where synthesis occurs in a continuous fashion, on the lagging strand, DNA fragments need to be sealed to form the intact DNA strand. *(a)* DNA polymerase III is synthesizing DNA in the 5′→3′ direction toward the RNA primer of a previously synthesized fragment on the lagging strand. *(b)* On reaching the fragment, DNA polymerase III leaves and is replaced by DNA polymerase I. *(c)* DNA polymerase I continues synthesizing DNA while removing the RNA primer from the previous fragment, and DNA ligase replaces DNA polymerase I after the primer has been removed. *(d)* DNA ligase seals the two fragments together. *(e)* The final product, complementary and antiparallel double-stranded DNA.

MINIQUIZ

- What is the difference between a template strand and a daughter strand of DNA?
- To which end (5′ or 3′) of a newly synthesized strand of DNA does DNA polymerase add a nucleotide?
- In DNA replication, what is the primer composed of and why are there leading and lagging strands?
- What are the functions of DNA Pol I and III and DNA helicase and DNA ligase?

4.4 Bidirectional Replication, the Replisome, and Proofreading

The circular nature of the bacterial and archaeal chromosome accelerates the genomic replication process. In *Escherichia coli*, and probably in all cells with circular chromosomes, DNA replication occurs *bidirectionally* from the origin of replication. There are thus *two* replication forks on each chromosome, each moving in opposite directions. In circular DNA, bidirectional replication leads to the formation in the replicating molecules of characteristic shapes (so-called "theta structures") as synthesis proceeds in both a leading and a lagging fashion on each template strand (Figure 4.16). In an actively growing cell of *E. coli*, DNA Pol III adds nucleotides at the rate of about 1000 per second; hence, replication of the entire chromosome takes about 40 min.

The Replisome

Figure 4.14 shows the enzymes that participate in replication, and from such a schematic it may appear that the enzymes are working independently. However, this is not the case. Instead, replication proteins aggregate to form a large replication complex called the **replisome** (Figure 4.17). The lagging strand of DNA actually loops out to allow the replisome to move smoothly along both strands, the complex literally pulling the DNA template through it as replication proceeds. In addition to the replisome, helicase and primase form their own subcomplex within the replisome called the *primosome*. This close association facilitates the sequential activities of these two enzymes during the replication process (Figure 4.17). Table 4.2 summarizes the functions of proteins essential for DNA replication in *Bacteria*.

Eventually the work of the replisome is finished, and this is signaled when the replication forks collide at a site located on the opposite side of the chromosome from the origin called the *terminus of replication*. In the terminus region are several DNA sequences called *Ter* sites that are recognized by a protein called Tus, whose function is to block progress of the replication forks. When replication of the circular chromosome is complete, the two circular molecules are linked together, much like the links of a chain. After replication, the DNA is partitioned so that each daughter cell receives a copy of the chromosome; DNA partitioning is facilitated by FtsZ, a protein that orchestrates several key events in the cell division process (Chapter 7).

Fidelity of DNA Replication: Proofreading

DNA replication occurs with a remarkably low error rate. Nevertheless, when errors do occur, a mechanism exists to detect and correct them. Errors in DNA replication introduce *mutations*, changes in DNA sequence. Mutation rates in cells are extremely low, between 10^{-8} and 10^{-11} errors per base pair inserted. This accuracy can be achieved because DNA polymerases effectively get two chances to incorporate the correct base at any given site. The first chance comes when DNA Pol III inserts bases according to the base-pairing rules (Figure 4.1*c*). The second chance comes when a process called *proofreading* takes place (Figure 4.18).

During replication, if an incorrect base has been inserted, a mismatch in base pairing (Figure 4.1*c*) occurs. Both DNA Pol I and Pol III possess a 3′→5′ exonuclease activity that can remove such mismatched nucleotides. The polymerase detects the error because incorrect base pairing causes a slight distortion in the topology of the double helix. After the removal of a mismatched nucleotide, the polymerase then gets a second chance to insert the correct nucleotide (Figure 4.18). With the extremely low error rate of DNA polymerases, the chance of inserting the wrong base at the same

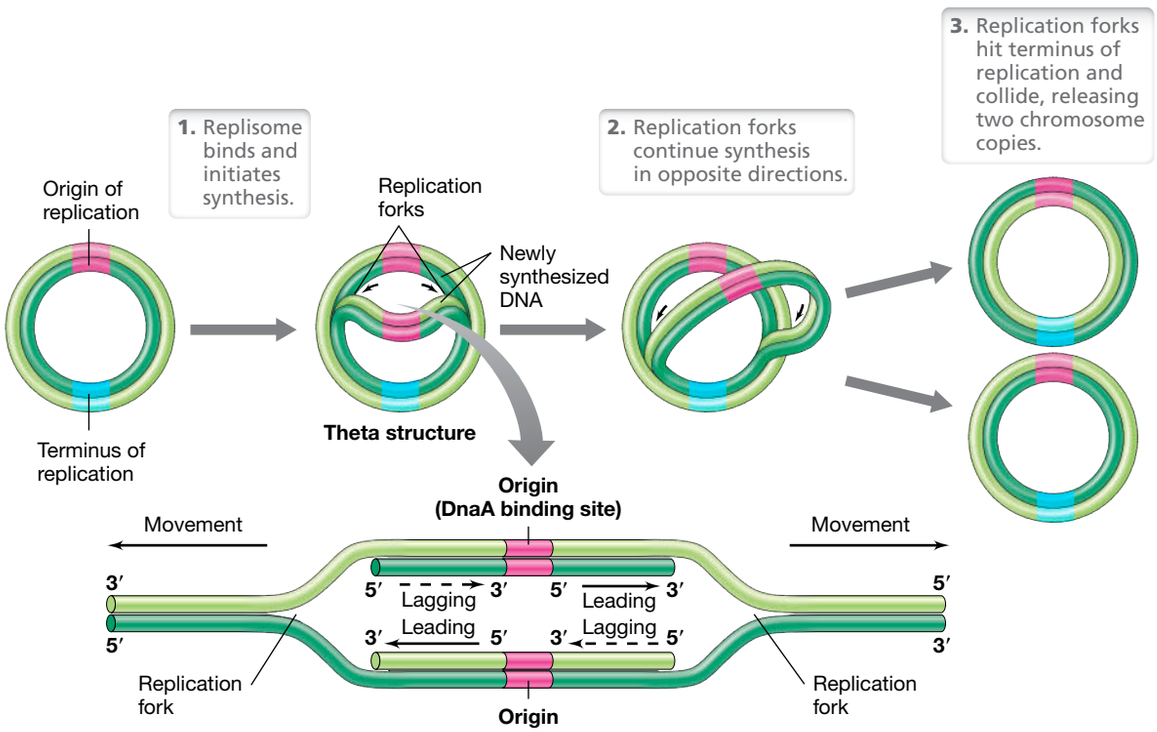


Figure 4.16 Replication of circular DNA: the theta structure. In circular DNA, bidirectional replication from an origin forms an intermediate structure resembling the Greek letter theta (θ). Blowup shows dual replication forks in the circular chromosome. In *Escherichia coli*, the origin of replication is recognized by the DnaA protein and the terminus of replication is recognized by the Tus protein. Note that DNA synthesis is occurring in both a leading and a lagging manner on each of the new daughter strands until the replication forks hit the terminus. Compare this figure with the illustration of the replisome in Figure 4.17.

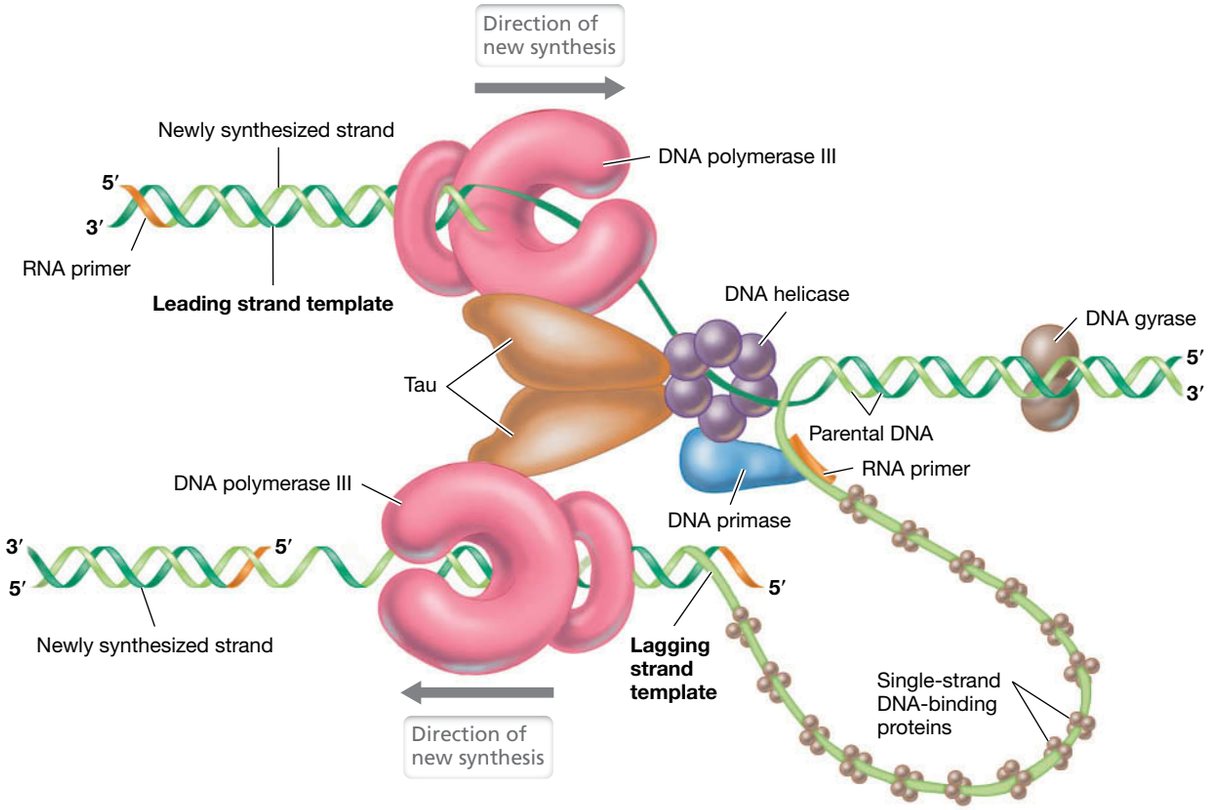


Figure 4.17 The replisome. The replisome consists of two copies of DNA polymerase III and DNA gyrase, plus helicase and primase (together forming the primosome), and many copies of single-strand DNA-binding protein. The Tau subunits hold the two DNA polymerase assemblies and helicase together. Just upstream of the rest of the replisome, DNA gyrase removes supercoils in the DNA to be replicated. Note that the two polymerases are replicating the two individual strands of DNA in opposite directions. Consequently, the lagging-strand template loops around so that the whole replisome moves in the same direction along the chromosome.

site twice is vanishingly small. Exonuclease proofreading occurs in *Bacteria* and *Archaea*, eukaryotes, and viral DNA replication systems.

We now move on from replicating genes to consider *gene expression* as a prelude to examining synthesis of the proteins encoded by the transcribed genes.

MINIQUIZ

- What is the replisome and what are its components?
- How are the activities of the replisome stopped?
- How are errors in DNA replication kept extremely low?

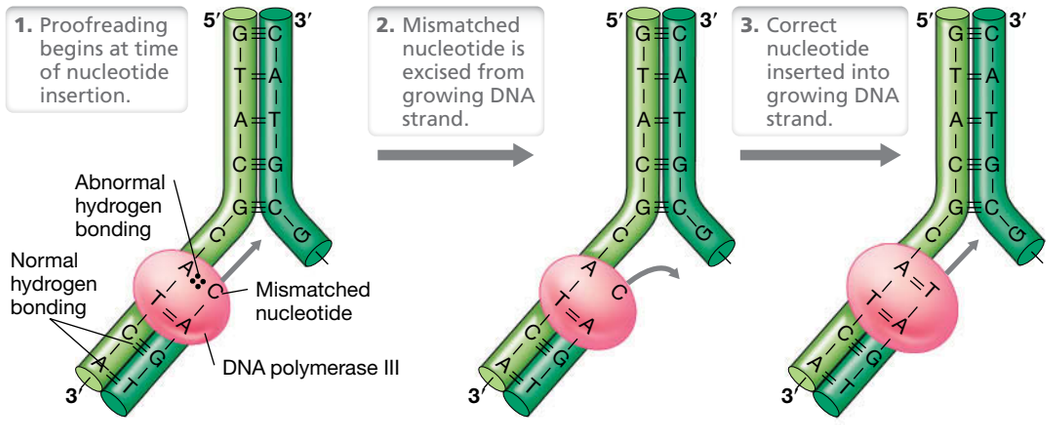


Figure 4.18 Proofreading by the 3′→5′ exonuclease activity of DNA polymerase III. A mismatch in base pairing at the terminal base pair causes the polymerase to pause briefly. This signals the proofreading activity to cut out the mismatched nucleotide, after which the correct base is inserted by the polymerase activity.

III • RNA Synthesis: Transcription

Transcription—RNA synthesis off of a DNA template—yields three main forms of RNA: *messenger* (mRNA), *transfer* (tRNA), and *ribosomal* (rRNA) (Section 4.1). Several other minor classes of RNA exist but most of these function in regulation (Chapter 6). RNA is both a genetic and a functional molecule. At the genetic level, mRNA encodes genetic information from the genome and carries it to the ribosome. In contrast, rRNAs play both a structural and a functional role in ribosomes, while tRNAs function as the carriers of amino acids to the ribosome for protein synthesis.

There are two key differences in the chemistry of RNA and DNA: (1) RNA contains ribose instead of deoxyribose; and (2) RNA contains uracil instead of thymine. The change from deoxyribose to ribose dramatically affects the chemistry of a nucleic acid, and enzymes that act on DNA typically have no effect on RNA, and vice versa. However, the change from thymine to uracil does not affect base pairing, as these two bases pair with adenine equally well.

With the exception of a few viruses that contain double-stranded RNA genomes (Chapter 10), RNA is a single-stranded molecule. However, the primary structure (sequence of nucleotides) of some RNAs allows them to fold and exploit complementary base pairing. The term **secondary structure** refers to this folding, and the functional role an RNA plays in the cell may depend critically on its secondary structure. For example, messenger RNAs, which are typically unfolded, exist in *Bacteria* (and *Archaea*) for only a few minutes before enzymes called *ribonucleases* degrade them. By contrast, rRNAs and tRNAs (referred to as *stable RNAs*) are long-lived because their secondary structures prevent ribonuclease attack. The rapid turnover of mRNAs in *Bacteria* and *Archaea* allows them to quickly adapt to changing environmental conditions and halt the translation of mRNAs whose products are no longer needed.

We begin with transcription in *Bacteria* and contrast this in the following section with transcriptional events in *Archaea* and *Eukarya*.

4.5 Transcription in *Bacteria*

Transcription is catalyzed by the enzyme **RNA polymerase**. Like DNA polymerase, RNA polymerase forms phosphodiester bonds but between the *ribo*nucleotides rather than *deoxyribo*nucleotides (Figure 4.1*b*). Polymerization is driven by energy released from the hydrolysis of two energy-rich phosphate bonds of the incoming ribonucleoside triphosphates. The mechanism of RNA synthesis is thus quite similar to that of DNA synthesis (Figure 4.11*b*): During elongation of an RNA chain, ribonucleoside triphosphates are added to the 3′-OH of the ribose of the preceding nucleotide. Thus chain growth is 5′→3′ just as in DNA synthesis, and the newly synthesized strand of RNA runs antiparallel to the DNA template strand it was transcribed from. A summary of bacterial transcription is illustrated in Figure 4.19.

RNA polymerase uses DNA as a template, but for any given gene, only one of the two strands is transcribed. Unlike DNA polymerase, RNA polymerase can initiate new RNA on its own; no priming is necessary as it is for DNA synthesis (Figure 4.12). Transcription

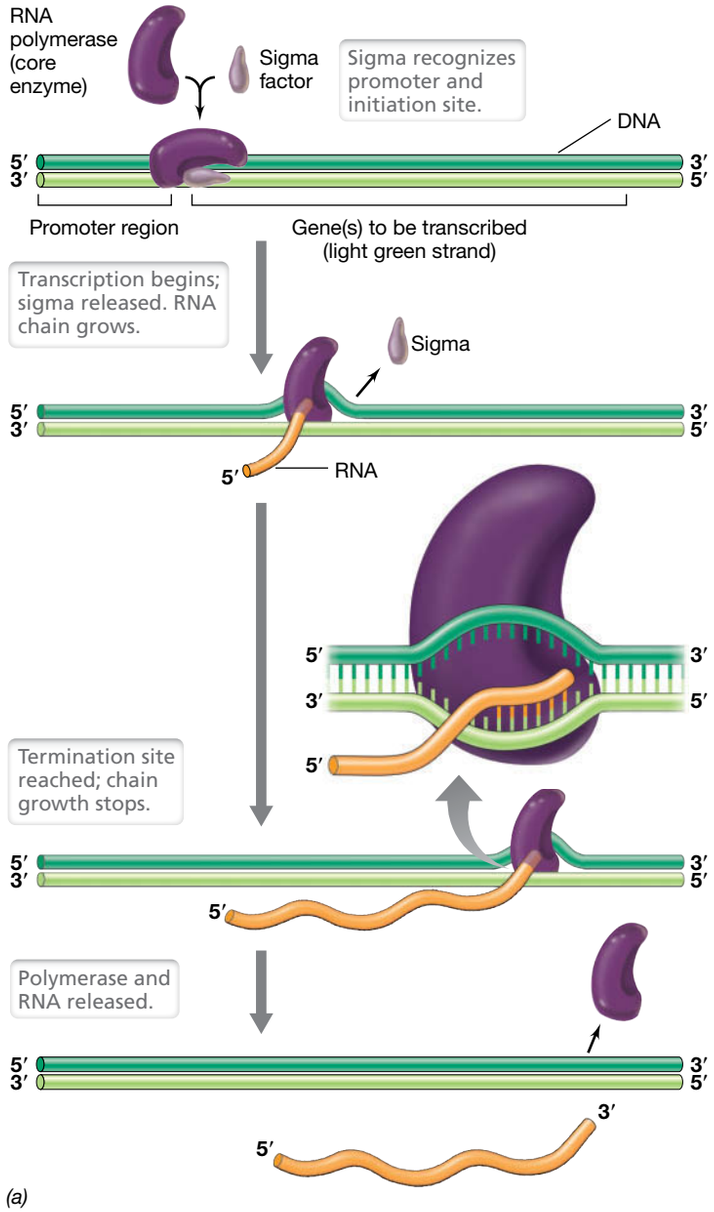


Figure 4.19 Transcription. *(a)* Steps in RNA synthesis. The initiation site (promoter) and termination site are specific nucleotide sequences on the DNA. RNA polymerase moves down the DNA chain, temporarily opening the double helix and transcribing one of the DNA strands. *(b)* Electron micrograph illustrates transcription along a gene on the *Escherichia coli* chromosome. Transcription is proceeding from left to right, with the shorter transcripts on the left becoming longer as transcription proceeds.

continues until specific sequences called *transcription terminators* are reached, but unlike DNA replication, which copies the entire genome, transcription occurs on much smaller units of DNA, often as little as a single gene. This system allows the cell to transcribe different genes at different frequencies, depending on the needs of the cell for different proteins. Said a different way, gene expression is a *highly regulated* process. Transcriptional regulation can occur in prokaryotic cells in many different ways, but the different mechanisms have a common outcome: Cell resources are conserved and cell fitness enhanced (Chapter 6).

RNA Polymerases and the Promoter Sequence

The RNA polymerase from *Bacteria*, which has the simplest structure and about which most is known, consists of five different subunits, designated β, β′, α, ω (omega), and σ (sigma), with α present in two copies (**Figure 4.20**). The subunits form an enzyme complex called the *RNA polymerase holoenzyme*. Sigma is not as tightly bound as the other subunits and easily dissociates to yield the *RNA polymerase core enzyme*, $\alpha_2\beta\beta'\omega$. The core enzyme alone synthesizes RNA, and sigma functions only to recognize the appropriate site on the DNA for transcription to begin (sigma dissociates from the holoenzyme once a short sequence of RNA has been formed) (Figure 4.19).

To begin transcription, RNA polymerase must first recognize initiation sites on the DNA; these sites are called **promoters**. In *Bacteria*, promoters are recognized by sigma, and once RNA polymerase has bound to a promoter, transcription can proceed (Figure 4.19). In this process, the DNA helix at the promoter site is opened up by RNA polymerase, and as the polymerase moves, it unwinds the DNA in short segments to expose template DNA. Because some genes reside on one strand of DNA while other genes reside on the other strand of DNA, promoters are present on both strands; as a result, transcription occurs in *opposite directions* on the two different strands of DNA.

Sigma Factors, Consensus Sequences, and Transcriptional Termination

Promoters are specific DNA sequences; **Figure 4.21** shows the sequence of several promoters from *Escherichia coli*. All of these sequences are recognized by the same *E. coli* sigma factor called σ^{70} (the superscript 70 indicates the size of this protein, 70 kilodaltons). Although these sequences are not identical, sigma recognizes two highly conserved regions within the promoter. These conserved sequences are upstream of (prior to) the transcription start site. One is 10 bases upstream, the −10 region, or *Pribnow box*. Although promoter sequences differ slightly, comparison of many −10 regions gives a consensus sequence of TATAAT. The second conserved region is about 35 bases upstream of the start site and its consensus sequence is TTGACA (Figure 4.21). In *E. coli*, promoters that conform most closely to the consensus sequence are more effective in binding RNA polymerase. Such promoters are called *strong promoters* and are very useful in genetic engineering (Chapter 12).

While most genes in *E. coli* require σ^{70} for transcription, several alternative sigma factors exist that recognize different consensus sequences (**Table 4.3**). Each alternative sigma factor is specific for a group of genes required under special circumstances, and thus the presence or absence of a specific sigma factor is a mechanism for regulating gene expression. That is, by changing the rate of either synthesis or degradation of a particular sigma factor, the cell can control the transcription of entire gene families.

Units of Transcription and Polycistronic mRNA

Genetic information is organized into *transcriptional units*, segments of DNA that are transcribed into a single RNA molecule bounded by their initiation and termination sites. Some transcriptional units contain RNA transcribed from a single gene, whereas

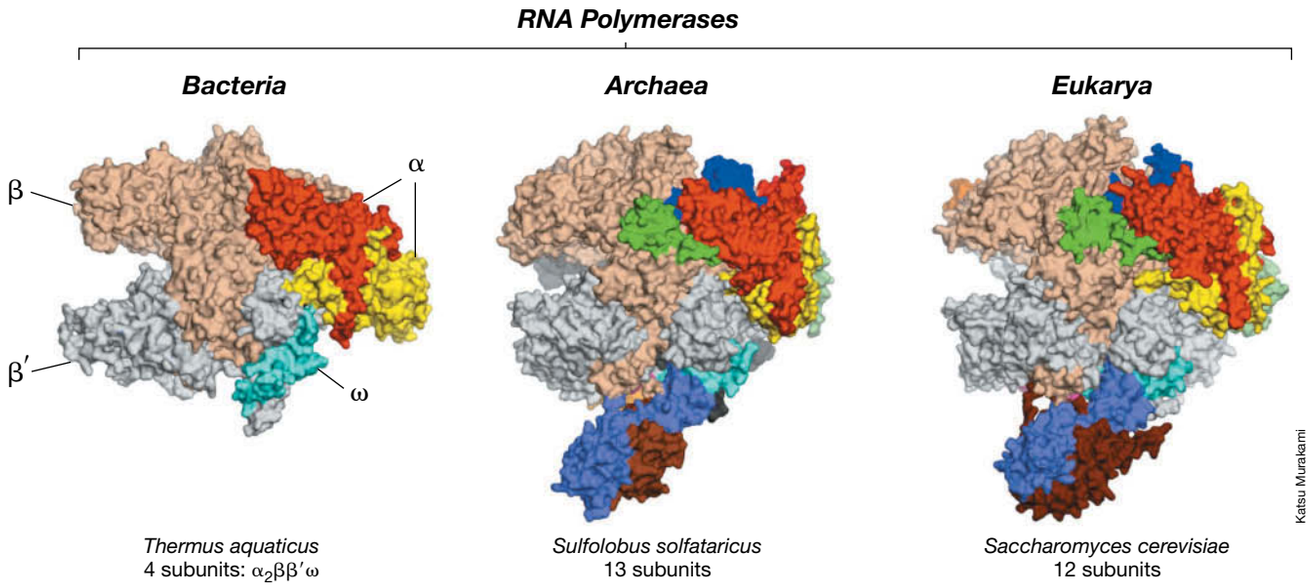


Figure 4.20 RNA polymerase from the three domains. Surface representation of multi-subunit cellular RNA polymerase structures from *Bacteria* (left, *Thermus aquaticus* core enzyme), *Archaea* (center, *Sulfolobus solfataricus*), and *Eukarya* (right, *Saccharomyces cerevisiae* RNA Pol II). Orthologous subunits are depicted by the same color. A unique subunit in the *S. solfataricus* RNA polymerase is not shown in this view.

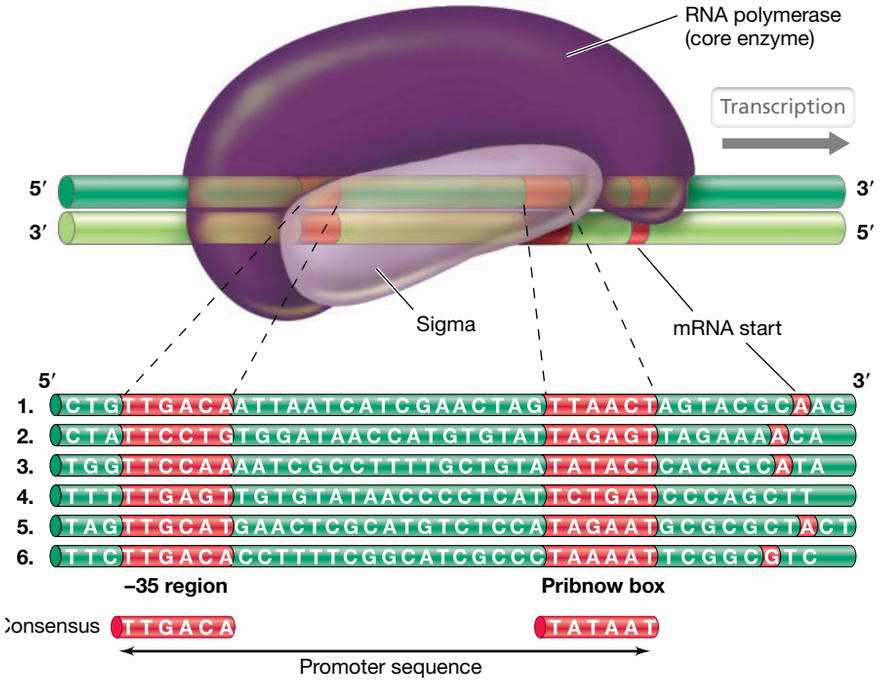


Figure 4.21 The interaction of RNA polymerase with a bacterial promoter. Shown below the RNA polymerase and DNA are six different promoter sequences identified in *Escherichia coli*. The contacts of the RNA polymerase with the −35 region and the Pribnow box (−10 sequence) are shown. Transcription begins at a unique base just downstream from the Pribnow box. Below the actual sequences at the −35 and Pribnow box regions are consensus sequences derived from comparing many promoters. Note that although sigma recognizes the promoter sequences on the 5′⟶3′ (dark green) strand of DNA, the RNA polymerase core enzyme will actually transcribe the light green strand (that runs 3′⟶5′) because core enzyme synthesizes only in a 5′⟶3′ direction.

others are formed from two or more genes (*cotranscribed genes*). Most genes encode proteins, but others encode nontranslated RNAs, such as ribosomal or transfer RNAs. For example, prokaryotic cells produce three size classes of rRNA: 16S, 23S, and 5S (the S refers to *Svedberg units*, a measure of particle size), and their genes are cotranscribed to form a single transcriptional unit that also includes a tRNA (Figure 4.22). This transcriptional unit is subsequently "processed" by proteins that cut them to form the individual rRNAs or tRNAs.

As we have previously considered, genes that encode several enzymes of a particular metabolic pathway in prokaryotic cells, for example the biosynthesis of a particular amino acid, are often clustered together in an *operon* (Section 4.2). Assembling genes for the same biochemical pathway or genes needed under the same conditions into an operon allows their expression to be coordinated. During transcription, RNA polymerase proceeds through the operon and transcribes the entire set of genes into a single mRNA called a *polycistronic mRNA* (Figure 4.23). Polycistronic mRNAs contain multiple *open reading frames*, portions of the mRNA that actually encode amino acids (Section 4.9). When this mRNA is translated, several polypeptides are synthesized sequentially by the same ribosome.

Termination of Transcription

In a growing bacterial cell, only those genes that need to be expressed are usually transcribed; therefore, it is critical that transcription end at the correct position. **Termination** of transcription is governed by specific base sequences on the DNA. In *Bacteria* a common termination signal is a GC-rich sequence containing an *inverted repeat* with a central nonrepeating segment. When such a DNA sequence is transcribed, the RNA forms a stem–loop structure by intra-strand base

TABLE 4.3 Sigma factors in *Escherichia coli*

Name[a]	Upstream recognition sequence[b]	Function
σ^{70} RpoD	TTGACA	For most genes, major sigma factor for normal growth
σ^{54} RpoN	TTGGCACA	Nitrogen assimilation
σ^{38} RpoS	CCGGCG	Stationary phase, plus oxidative and osmotic stress
σ^{32} RpoH	TNTCNCCTTGAA	Heat shock response
σ^{28} FliA	TAAA	For genes involved in flagella synthesis
σ^{24} RpoE	GAACTT	Response to misfolded proteins in periplasm
σ^{19} FecI	AAGGAAAAT	For certain genes in iron transport

[a]Superscript number indicates size of protein in kilodaltons. Many factors also have other names, for example, σ^{70} is also called σ^{D}.

[b]N = any nucleotide.

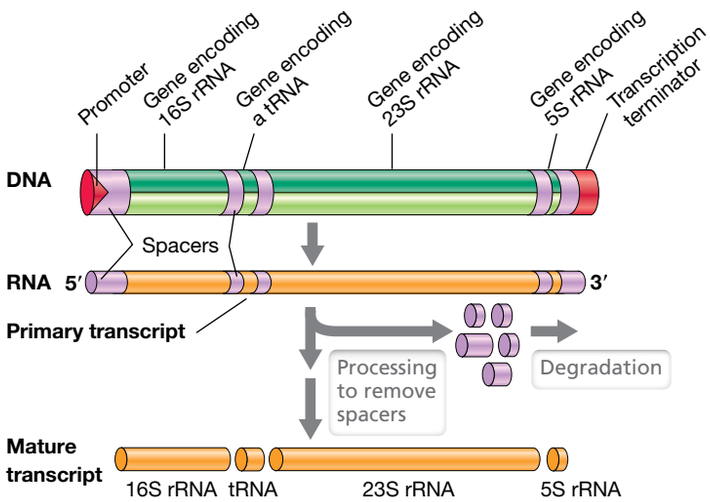


Figure 4.22 A ribosomal rRNA transcription unit from *Bacteria* and its subsequent processing. In *Bacteria*, all rRNA transcription units have the genes in the order 16S rRNA, 23S rRNA, and 5S rRNA (shown approximately to scale). Note that in this particular transcription unit the spacer between the 16S and 23S rRNA genes contains a tRNA gene. In other transcription units this region may contain more than one tRNA gene. Often one or more tRNA genes also follow the 5S rRNA gene and are cotranscribed. *Escherichia coli* contains seven rRNA transcription units.

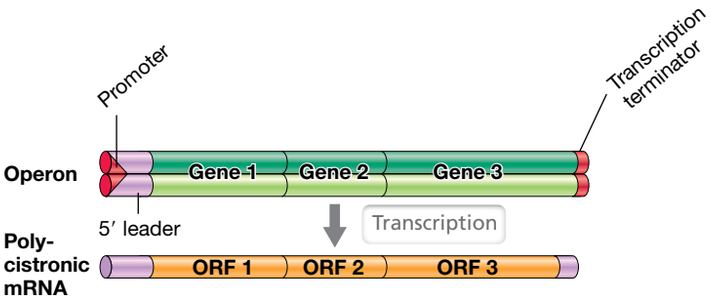


Figure 4.23 Operon and polycistronic mRNA structure. Note that a single promoter controls the three genes within the operon and that the polycistronic mRNA molecule contains an open-reading frame (ORF) corresponding to each gene.

pairing (Figure 4.24). Stem–loops followed by a run of adenines in the DNA template (which yield a run of uridines in the mRNA) are strong transcription terminators because a stretch of U:A base pairs are formed that hold the RNA and DNA together. However, this structure is very weak since U:A base pairs have only two hydrogen bonds rather than the three that form in T:A pairs (Figure 4.1*c*). Thus, RNA polymerase pauses at the stem–loop, and the DNA and RNA dissociate at the run of uridines, terminating transcription.

A second mechanism for stopping transcription is catalyzed by the terminator protein Rho. Rho does not bind to RNA polymerase or to the DNA, but binds tightly to RNA and moves down the chain toward the RNA polymerase–DNA complex. Once RNA polymerase has paused at a Rho-dependent termination site (a specific sequence on the DNA template), Rho causes both the RNA and RNA polymerase to be released from the DNA, thus terminating transcription.

Now that we have grasped the essentials of transcription in *Bacteria*, we turn our attention to this crucial cell process in *Archaea* and *Eukarya*, where the phylogenetic connection between these two domains (Section 1.13) will be apparent in their mechanisms of transcription.

MINIQUIZ

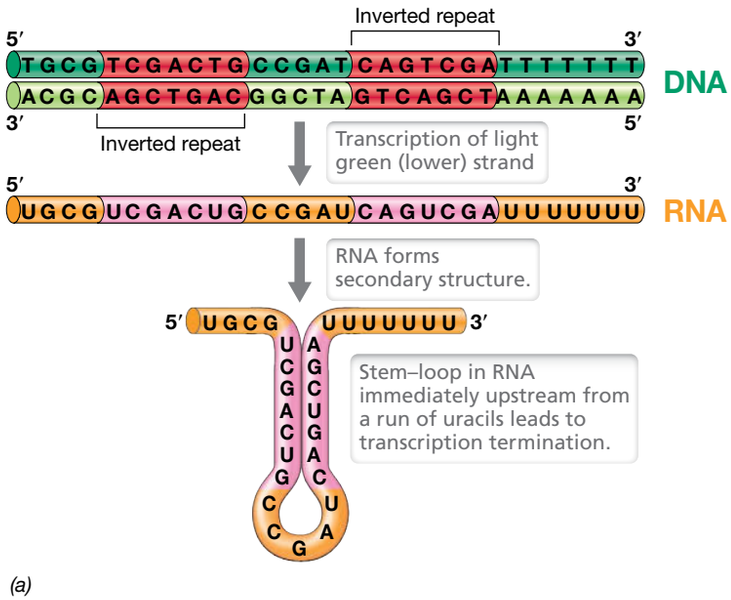
- What enzyme catalyzes transcription? What is a promoter and what protein recognizes promoters in *Bacteria*?
- What is the role of messenger RNA (mRNA)? What are the other two classes of RNA?
- How does polycistronic mRNA allow for gene families to be controlled as a group?
- What type of structures lead to transcription termination?

4.6 Transcription in *Archaea* and *Eukarya*

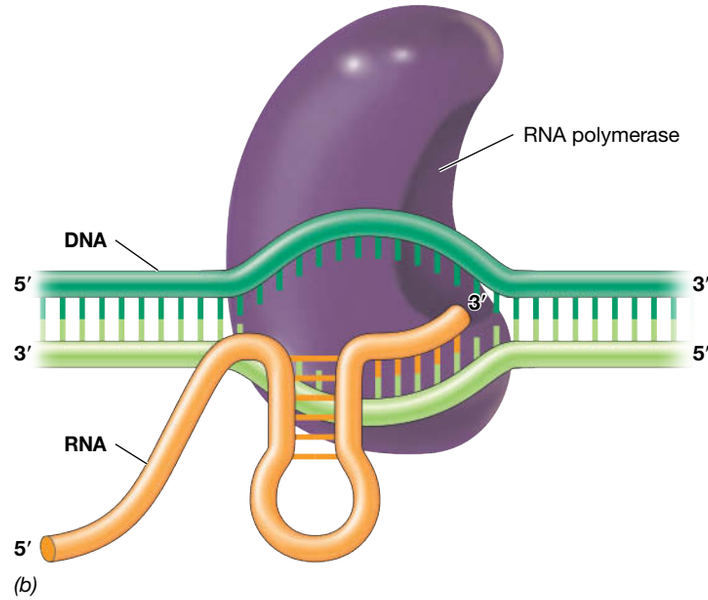
Here we discuss key elements of transcription in *Archaea* and *Eukarya* that differ from those of *Bacteria*. Although in both *Archaea* and *Eukarya* the overall flow of genetic information is the same as in *Bacteria*, some details differ, and in eukaryotic cells the presence of the nucleus complicates the routing of genetic information. Many of the details of transcription (and translation) in *Archaea* resemble those in *Eukarya* more closely than *Bacteria*. However, *Archaea* also share some transcriptional similarities with *Bacteria*, such as operons. We begin our discussion at center stage with a consideration of RNA polymerase.

Archaeal and Eukaryotic RNA Polymerases, Promoters, and Terminators

Archaeal and eukaryotic RNA polymerases are similar and more complex than those of *Bacteria*. *Archaea* contain only a single RNA polymerase while eukaryotes have three. The archaeal polymerase most closely resembles eukaryotic RNA polymerase II and is



(a)



(b)

Figure 4.24 Inverted repeats and transcription termination. *(a)* Inverted repeats in transcribed DNA form a stem–loop structure in the RNA that terminates transcription when followed by a run of uracils. *(b)* Schematic indicating the formation of the terminator stem–loop in the RNA within the RNA polymerase.

composed of 11–13 subunits, depending on the species (eukaryotic RNA polymerase II has 12 or more subunits). These contrast with the comparatively simple four-subunit RNA polymerase core enzyme of *Bacteria* (Figure 4.20).

We learned the importance of the promoter and its recognition sequences to the overall process of transcription in Section 4.5. The most important recognition sequence in archaeal and eukaryotic promoters is the 6- to 8-base-pair "TATA" box, located 18–27 nucleotides upstream of the transcriptional start site (Figure 4.25). The TATA box is recognized by the *TATA-binding protein* (TBP), one of the many *transcription factors* present in *Archaea* and eukaryotes. Upstream of the TATA box is the *B recognition element* (BRE) sequence that is recognized by archaeal transcription factor B (TFB). In addition, a specific initiator element sequence is located at the start of transcription. Once TBP has bound to the TATA box and TFB has bound to the BRE, then archaeal RNA polymerase can bind and initiate transcription. This process is similar in eukaryotes except that several additional transcription factors are required.

Less is known about transcription termination in *Archaea* than in *Bacteria*, although some archaeal genes have inverted repeats followed by an AT-rich sequence similar to those present in many bacterial transcription terminators (Section 4.5). One other type of suspected transcription terminator lacks inverted repeats but contains repeated runs of thymines. In eukaryotes, the termination process differs depending on the RNA polymerase and often requires a specific termination factor protein. No Rho-like proteins (Section 4.5) have been found in either *Archaea* or *Eukarya*.

RNA Processing in Eukaryotes and Intervening Sequences in *Archaea*

In contrast to *Bacteria*, *Eukarya* contain many genes that are split into two or more coding regions separated by noncoding regions. The coding sequences are called **exons**, while **introns** are the intervening noncoding regions. The transcripts from these genes thus require alterations—known as **RNA processing**—to form *mature RNAs* suitable for translation. The term **primary transcript** refers to the RNA molecule that is originally transcribed before the introns are removed to form the mature mRNA containing only exons. The process by which introns are removed and exons are joined is called *splicing* (Figure 4.26).

RNA splicing occurs in the nucleus by the activity of a macromolecular complex containing both RNA and protein called the **spliceosome**. The proteins of the spliceosome excise the intron(s) from the primary transcript and link the flanking exons to form a contiguous protein-encoding mature mRNA (Figure 4.26). While intervening sequences in genes encoding proteins are extremely rare in *Archaea*, several archaeal tRNA- and rRNA-encoding genes contain introns that must be removed after transcription to generate the mature tRNA or rRNA. In analogy to the introns of eukaryotes, these intervening sequences are called "archaeal introns"; however, their processing is catalyzed by a special ribonuclease rather than by the spliceosome complex.

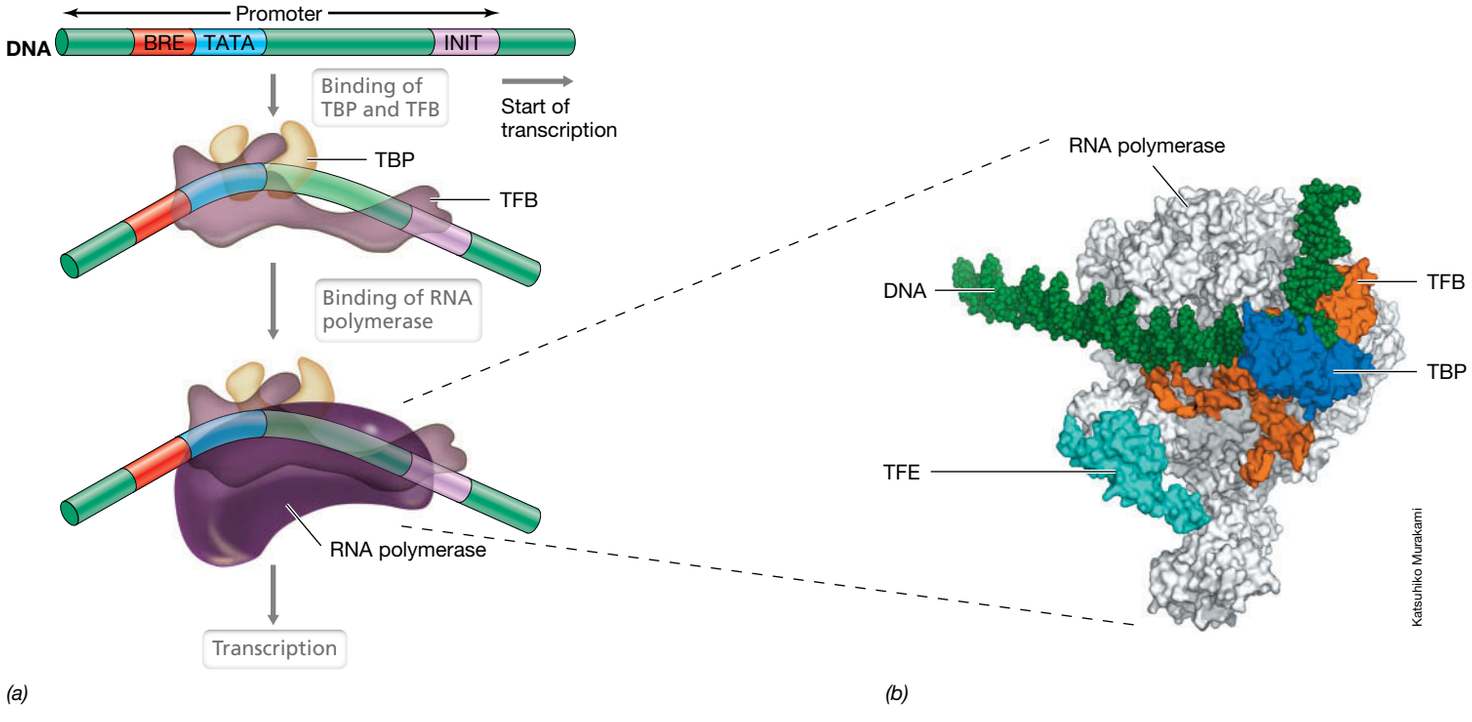


Figure 4.25 Promoter architecture and transcription in *Archaea*. *(a)* Three promoter elements are critical for promoter recognition in *Archaea*: the initiator element (INIT), the TATA box, and the B recognition element (BRE). The TATA-binding protein (TBP) binds the TATA box; transcription factor B (TFB) binds to both BRE and INIT. Once both TBP and TFB are in place, RNA polymerase binds. *(b)* Surface representation of the archaeal pre-initiation complex (with TBP and TFB) and including transcription factor E (TFE). TFE is an optional transcription factor frequently associated with the archaeal pre-initiation complex.

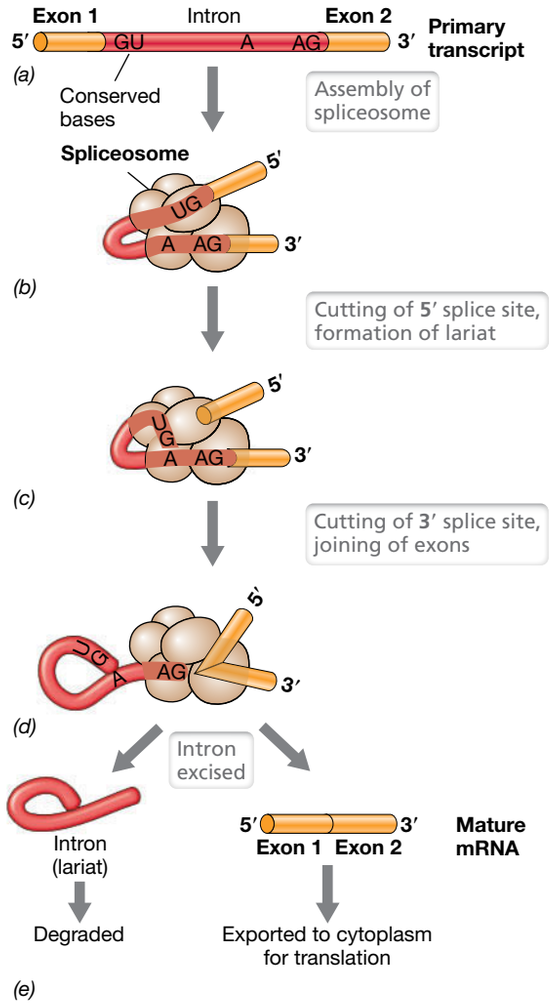


Figure 4.26 Activity of the spliceosome. Removal of an intron from the primary transcript of a protein-coding gene in a eukaryote. *(a)* A primary transcript containing a single intron. The sequence GU is conserved at the 5′ splice site, and AG is conserved at the 3′ splice site. There is also an interior A that serves as a branch point. *(b)* Several small ribonucleoprotein particles (shown in light tan) assemble on the RNA to form a spliceosome. Each of these particles contains distinct small RNA molecules that take part in the splicing mechanism. *(c)* The 5′ splice site has been cut with the simultaneous formation of a branch point. *(d)* The 3′ splice site has been cut and the two exons have been joined. Note that overall, two phosphodiester bonds were broken, but two others were formed. *(e)* The final products are the joined exons (the mRNA) and the released intron.

Two other steps in the processing of mRNA in *Eukarya* are unique to this domain, and both steps take place in the nucleus prior to splicing (Figure 4.27). The first step, called *capping*, occurs before transcription is complete. Capping is the addition of a methylated guanine nucleotide at the 5′-phosphate end of the mRNA (Figure 4.27). The cap is added in reverse orientation relative to the rest of the mRNA molecule and is needed to initiate translation. The second step, *polyadenylation*, consists of trimming the 3′ end of the primary transcript and adding 100–200 adenine residues, called the *poly(A) tail* (Figure 4.27). The poly(A) tail stabilizes mRNA against nuclease attack and must be removed before the mRNA can be degraded.

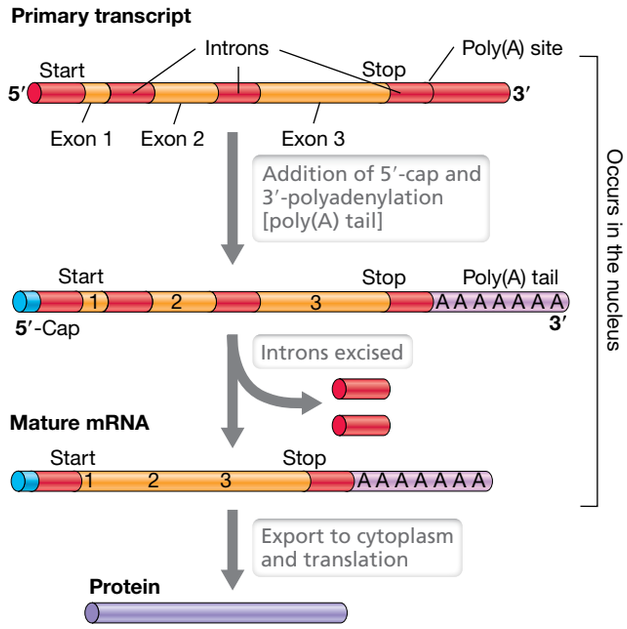


Figure 4.27 Processing of the primary transcript into mature mRNA in eukaryotes. The processing steps include adding a cap at the 5′ end, removing the introns, and clipping the 3′ end of the transcript while adding a poly(A) tail. All these steps are carried out in the nucleus. The location of the start and stop codons to be used during translation are indicated.

We now move on to the culmination of genetic information flow: protein synthesis. Here we will see many events common to all cells with a few exceptions that once again link *Archaea* with *Eukarya*.

MINIQUIZ

- What three major components make up an archaeal promoter?
- What specific eukaryotic enzyme does the archaeal RNA polymerase resemble?
- What steps take place in the processing of eukaryotic RNA?

IV • Protein Synthesis: Translation

Once transcription has occurred, the mRNAs are translated into protein. Translation requires many proteins and RNAs (in addition to mRNA) and a key cellular structure, the ribosome. How these interact to produce a cell's array of proteins is what we consider now, and we begin with a refresher section on the basic properties of proteins.

4.7 Amino Acids, Polypeptides, and Proteins

Proteins play major roles in cell function. Two major classes of proteins are *catalytic proteins* and *structural proteins*. *Enzymes* are the catalysts for chemical reactions that occur in cells (Section 3.5).

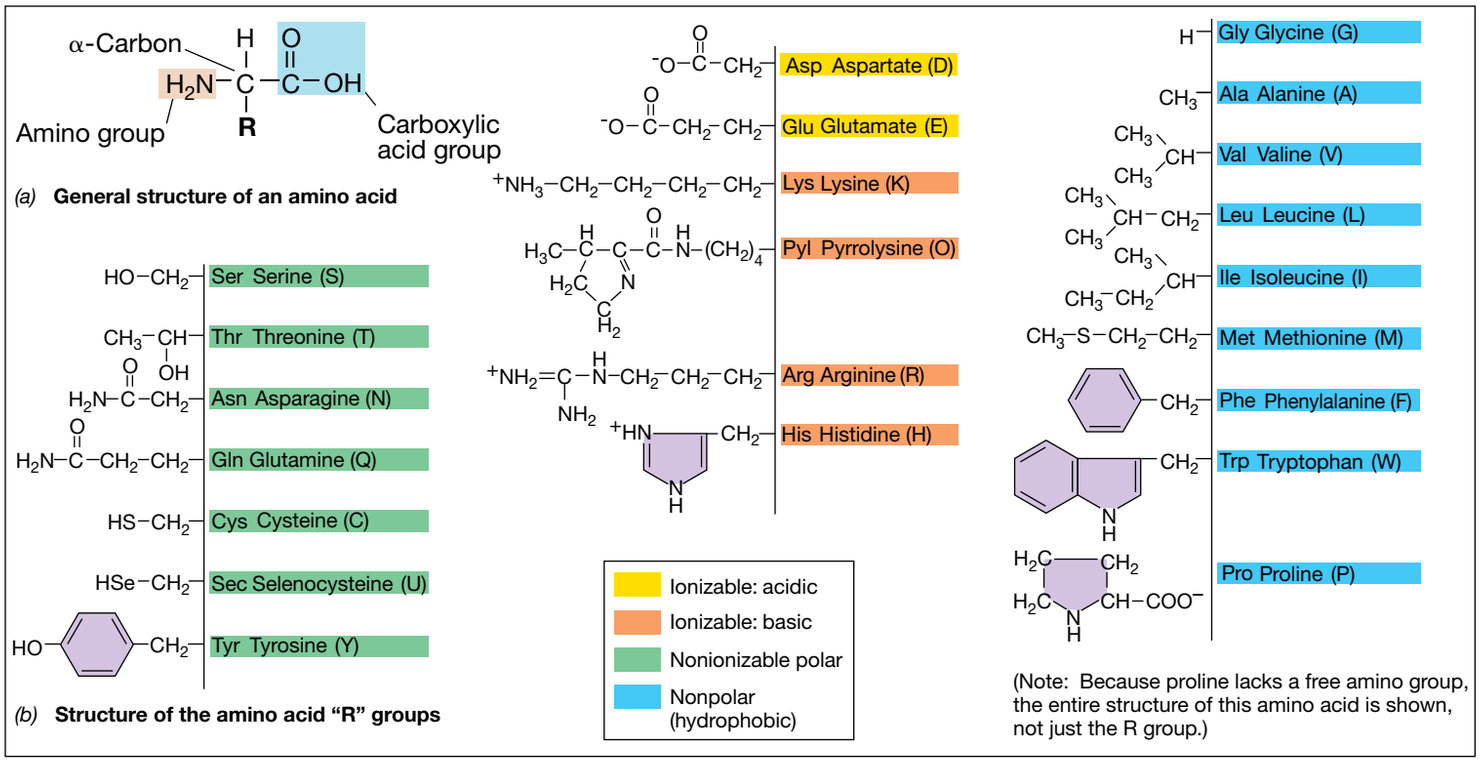


Figure 4.28 Structure of the 22 genetically encoded amino acids. *(a)* General structure. *(b)* R group structure. The three-letter codes for the amino acids are to the left of the names, and the one-letter codes are in parentheses to the right of the names. Pyrrolysine has thus far been found only in certain methanogenic *Archaea* (Section 17.2).

Structural proteins are parts of the major structures of the cell: membranes, walls, ribosomes, and so on. Regulatory proteins control most cell processes by a variety of mechanisms, including binding to DNA and affecting transcription (Chapter 6). However, all proteins show certain basic features in common.

Composition

Proteins are polymers of **amino acids**, organic compounds that contain both an amino group ($-NH_2$) and a carboxylic acid group (–COOH) attached to the α-carbon (**Figure 4.28*a***). Bonds between the carboxyl carbon of one amino acid and the amino nitrogen of a second (formed through the elimination of water) are known as **peptide bonds** (**Figure 4.29**). Two amino acids bonded by peptide linkage constitute a *dipeptide*; three amino acids, a *tripeptide*; and so on. When many amino acids are linked, they form a **polypeptide**. A protein consists of one or more polypeptides. The number of amino acids differs greatly from one protein to another, from as few as 15 to as many as 10,000 (see page 138).

Each amino acid has a unique side chain (abbreviated R), which governs the chemical properties of the amino acid. Side chains vary considerably, from as simple as a hydrogen atom in the amino acid glycine to aromatic rings in phenylalanine, tyrosine, and tryptophan (Figure 4.28*b*). Amino acids with chemically related side chains often show similar chemical properties and are thus grouped into related amino acid "families" (Figure 4.28*b*). For example, the side chain may contain a carboxylic acid group, as in aspartic acid or glutamic acid, rendering the amino acid *acidic*.

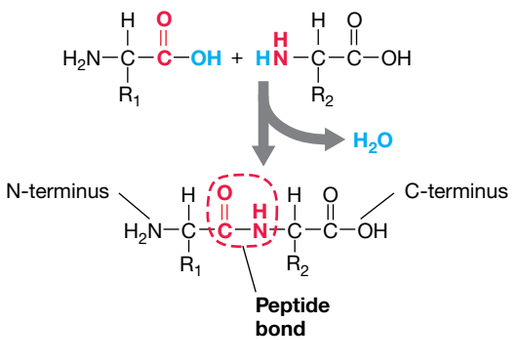


Figure 4.29 Peptide bond formation. R_1 and R_2 refer to the side chains of the amino acids. Note that following peptide bond formation, a free OH group is present at the C-terminus for formation of the next peptide bond.

Others contain additional amino groups, making them positively charged and *basic*. Several amino acids contain hydrophobic side chains and are grouped together as *nonpolar* amino acids. Cysteine contains a sulfhydryl group (–SH). Using their sulfhydryl groups, two cysteines can form a disulfide linkage (R–S–S–R) that connects two polypeptide chains.

Protein Diversity and Structures

The diversity of chemically distinct amino acids makes possible an enormous number of structurally unique proteins that can have

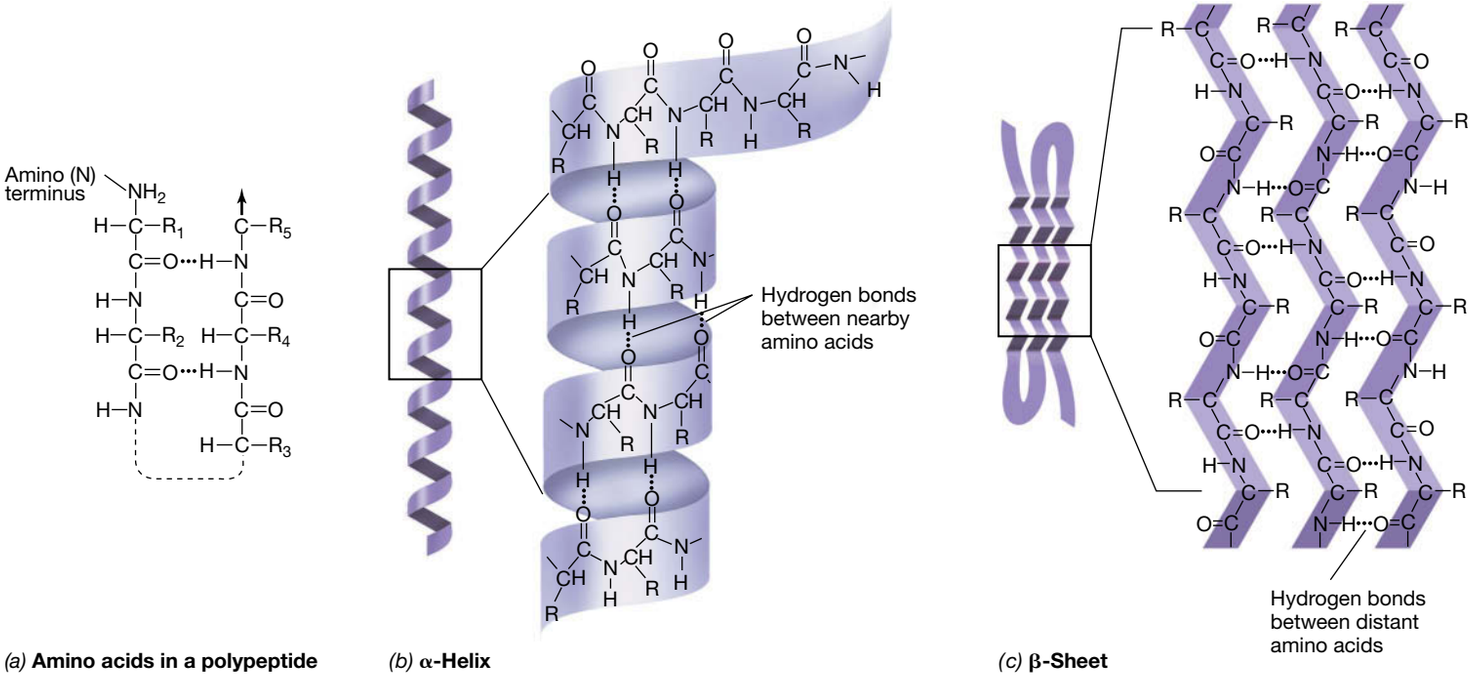


Figure 4.30 Secondary structure of polypeptides. *(a)* Hydrogen bonding in protein secondary structure. R represents the side chain of the amino acid. *(b)* α-Helix secondary structure. *(c)* β-Sheet secondary structure. Note that the hydrogen bonding is between atoms in the peptide bonds and does not involve the R groups.

widely different biochemical properties. If one assumes that an average polypeptide contains 300 amino acids, then 22^{300} different polypeptide sequences are theoretically possible. No cell has anywhere near this many different proteins. A cell of *Escherichia coli* contains around 2000 different kinds of proteins, with the exact number being highly dependent on the resources (nutrients) and growth conditions employed.

The linear sequence of amino acids in a polypeptide is its *primary structure*. This ultimately determines the folding pattern of the polypeptide, which in turn determines its biological activity. Even as little as a single amino acid change in the primary structure of a protein can affect its activity. Once formed, a polypeptide proceeds to fold to form a more stable structure. Hydrogen bonding between the oxygen and nitrogen atoms of two peptide bonds generates the *secondary structure*, either as an *α-helix* (imagine a polypeptide wound around a cylinder) or as a *β-sheet* (a repeated "back and forth" type of folding) (**Figure 4.30**). A single polypeptide can contain regions, called *domains*, of α-helix and regions of β-sheet secondary structure. The type of folding and its location in the molecule are determined by the primary structure and the available opportunities for hydrogen bonding.

Interactions between the R groups of the amino acids in a polypeptide generate higher-order structures. A protein's **tertiary structure** depends largely on hydrophobic interactions, with lesser contributions from hydrogen bonds, ionic bonds, and disulfide bonds, and generates the overall three-dimensional shape of the polypeptide (**Figure 4.31**). Many proteins consist of two or more polypeptides and thus show **quaternary structure**. In such proteins, the quaternary structure describes the number and secondary structure of polypeptides (referred to as *subunits*) in the molecule. Quaternary structures may be stabilized by various interactions and also by disulfide bonds; if cysteines located in two different polypeptides are joined, the disulfide bond links the two subunits.

When proteins are exposed to extremes of heat or pH or to certain chemicals that affect their folding, they may undergo **denaturation**. This results in the loss of a protein's secondary, tertiary, and quaternary structure along with its biological properties. However, because peptide bonds are usually not broken, the denatured polypeptide retains its primary structure. Depending on the severity of the denaturing conditions, the polypeptide may

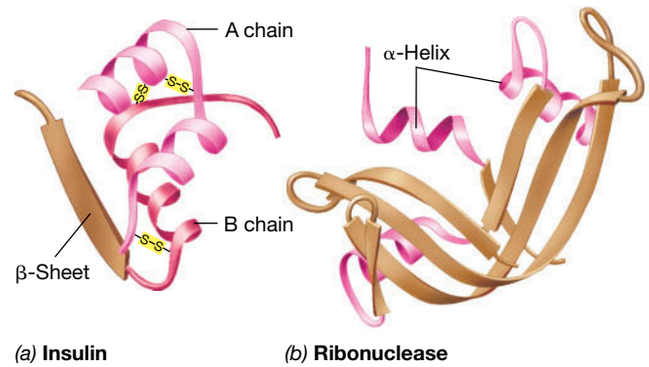


Figure 4.31 Tertiary structure of polypeptides. *(a)* Insulin, a protein containing two polypeptide chains; note how the B chain contains both α-helix and β-sheet secondary structure and how disulfide linkages (S–S) help in dictating folding patterns (tertiary structure). *(b)* Ribonuclease, a large protein with several regions of α-helix and β-sheet secondary structure.

properly refold after the denaturant is removed. However, if refolding is not correct, the protein is effectively "dead" and is degraded by proteases to release its amino acids for new protein synthesis.

MINIQUIZ

- Draw the structure of a dipeptide containing the amino acids alanine and tyrosine and outline the peptide bond.
- Differentiate between the different classes of protein structure.
- What is denaturation and why is the process harmful to a cell?

4.8 Transfer RNA

With a primer on proteins behind us, we now consider protein synthesis. But to do so, we must first understand the role of transfer RNA (tRNA). Transfer RNAs function to carry amino acids to the translation machinery. To ensure that they carry the correct amino acid, each tRNA contains a specific three-nucleotide sequence called the **anticodon**, the group of three bases that recognizes a codon (a three-base code for an amino acid) on the mRNA (Section 4.10). The correct amino acid (called the *cognate* amino acid) is linked to a specific tRNA by an enzyme called an **aminoacyl-tRNA synthetase**. For each amino acid, a separate aminoacyl-tRNA synthetase exists that specifically binds both the cognate amino acid and the tRNA that contains the corresponding anticodon, thus ensuring that each tRNA receives its correct amino acid.

General Structure of Transfer RNA

There are about 60 different tRNAs in prokaryotic cells and 100–110 in human cells. Transfer RNAs are short (73–93 nucleotides), single-stranded molecules that contain extensive secondary structure. Certain base sequences and secondary structures are invariant for tRNAs, whereas other parts are variable. Transfer RNAs also contain some purine and pyrimidine bases that are modified from the bases found in other classes of RNA, and these modifications occur after transcription. These unusual bases include pseudouridine (ψ), inosine, dihydrouridine (D), ribothymidine, methyl guanosine, dimethyl guanosine, and methyl inosine. The mature and active tRNA also contains extensive double-stranded regions formed by internal base pairing when the single-stranded molecule folds back on itself (Figure 4.32).

A tRNA is often depicted in the shape of a cloverleaf (Figure 4.32*a*). Some regions of tRNA secondary structure are named after the modified bases found there (for example, the TψC and D loops) or after their functions (for example, the anticodon loop and acceptor stem). The three-dimensional model of a tRNA shown in Figure 4.32*b* is a more realistic view of the molecule and shows how bases that appear widely separated in the cloverleaf model may actually be much closer together when viewed in 3D. This close proximity allows some of the bases in one loop to pair with bases in another loop (Figure 4.32*b*).

At the 3′ end (the acceptor end) of all tRNAs are three unpaired nucleotides. The sequence of these three nucleotides is always *c*ytosine-*c*ytosine-*a*denine (CCA), and they are absolutely essential for function. However, in most organisms the 3′ CCA is not encoded in the tRNA gene on the chromosome, but instead, each nucleotide is added sequentially by a protein called *CCA-adding enzyme*, using CTP and ATP as substrates. The cognate amino acid is then covalently attached to the terminal adenosine of the CCA end of its corresponding tRNA. From this location, the amino acid

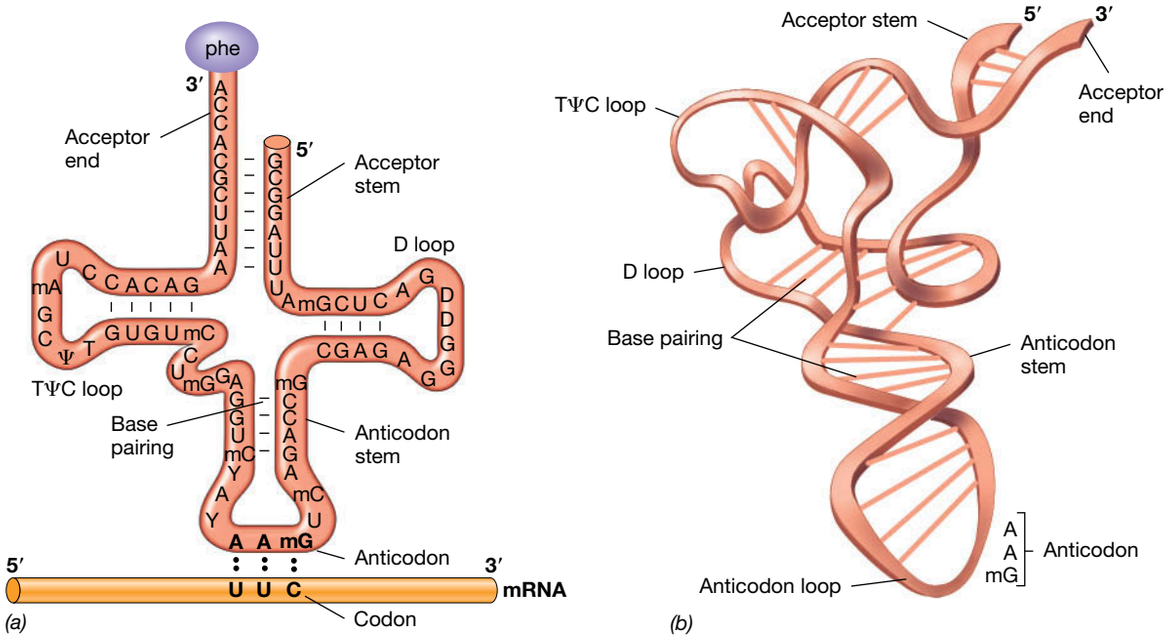


Figure 4.32 Structure of a transfer RNA. *(a)* The conventional cloverleaf structural drawing of yeast phenylalanine tRNA. The amino acid is attached to the ribose of the terminal A at the acceptor end. A, adenine; C, cytosine; U, uracil; G, guanine; T, thymine; ψ, pseudouracil; D, dihydrouracil; m, methyl; Y, a modified purine. *(b)* In fact, the tRNA molecule folds so that the D loop and TψC loops are close together and associate by hydrophobic interactions.

is incorporated into the growing polypeptide chain on the ribosome by a mechanism to be described in Section 4.9.

Recognition, Activation, and Charging of tRNAs

Recognition of the correct tRNA by an aminoacyl-tRNA synthetase is obviously crucial to the fidelity of translation and requires that specific contacts be made between regions of the tRNA and the synthetase (**Figure 4.33**). As might be expected because of its unique sequence, the anticodon of the tRNA is important in recognition by the synthetase. However, other contact sites between the tRNA and the synthetase are also important, including parts of the acceptor stem and D loop of the tRNA (Figure 4.32*a*).

The specific reaction between amino acid and tRNA catalyzed by the aminoacyl-tRNA synthetase begins with *activation* of the amino acid by reaction with ATP:

$$\text{Amino acid} + \text{ATP} \leftrightarrow \text{aminoacyl—AMP} + \text{P—P}$$

The aminoacyl-AMP intermediate formed remains bound to the tRNA synthetase until collision with the appropriate tRNA molecule. Then, as shown in Figure 4.33*a*, the activated amino acid is attached to the CCA stem of its tRNA to form a *charged tRNA*:

$$\text{Aminoacyl—AMP} + \text{tRNA} \leftrightarrow \text{Aaminoacyl—tRNA} + \text{AMP}$$

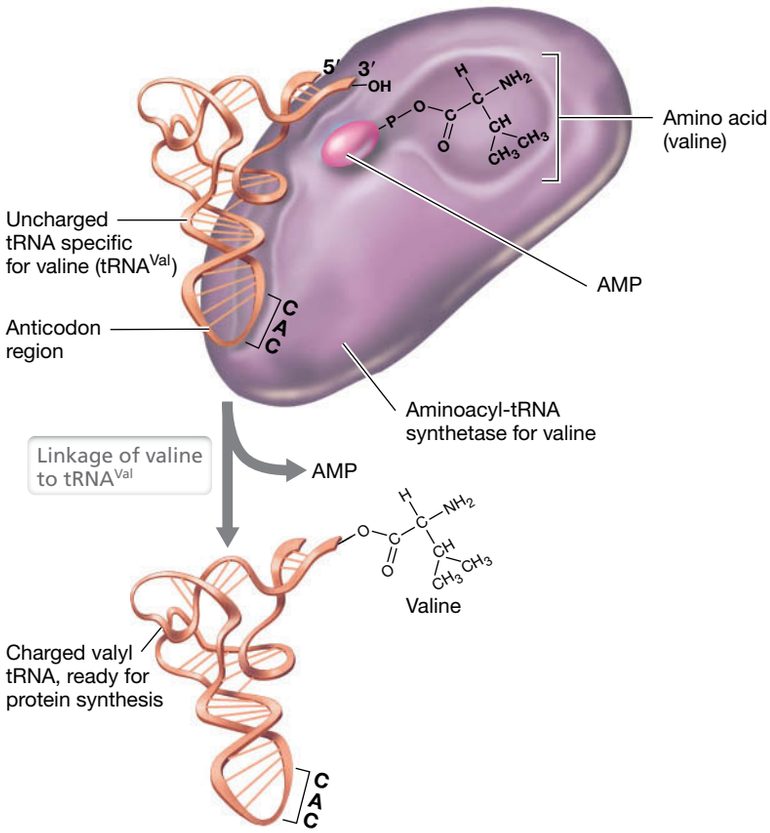
The pyrophosphate (PP_i) formed in the first reaction is split into two molecules of inorganic phosphate. Because ATP is used and AMP is formed in these reactions, a total of *two* energy-rich phosphate bonds are expended to charge a tRNA with its cognate amino acid. After activation and charging, the aminoacyl-tRNA leaves the synthetase. In the next step, it will be bound by a ribosome where actual polypeptide synthesis occurs.

MINIQUIZ

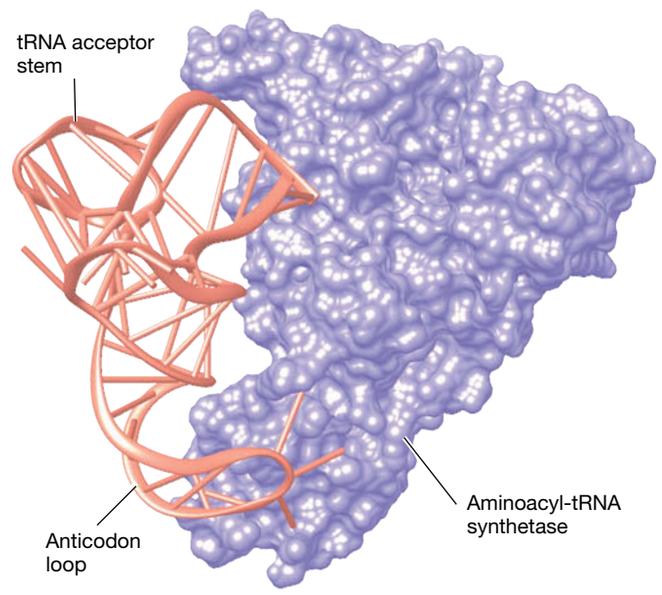
- What is the function of the anticodon of a tRNA?
- What is the function of the acceptor stem of a tRNA?
- What steps are required to form a charged tRNA?

4.9 Translation and the Genetic Code

The heart of genetic information transfer is the correspondence between the nucleic acid template and the amino acid sequence of a polypeptide. This correspondence is rooted in the **genetic code**.



(a)



(b)

Modified from G. Caetano-Anollés and A. Nasir. 2012. *Frontiers in Genetics* 3:00172.

Figure 4.33 Aminoacyl-tRNA synthetase. *(a)* Mode of activity of an aminoacyl-tRNA synthetase. Recognition of the correct tRNA by a particular synthetase involves contacts between specific nucleic acid sequences in the D loop and acceptor stem of the tRNA and specific amino acids of the synthetase. In this diagram, valyl-tRNA synthetase (specific for the amino acid valine) is shown catalyzing the final step of the reaction, where the valine in valyl-AMP is transferred to tRNA. *(b)* A computer model showing the interaction of the prolyl-tRNA synthetase from *Thermus thermophilus* with its tRNA.

TABLE 4.4 The genetic code as expressed by triplet base sequences of mRNA

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

[a]AUG encodes *N*-formylmethionine at the beginning of polypeptide chains of *Bacteria*.

An mRNA triplet of three bases, called a **codon**, encodes each specific amino acid (the codons themselves are encoded by the organism's genome). The 64 possible codons (four bases taken three at a time = 4^3) are shown in Table 4.4. Note that in addition to the codons for amino acids, there are also codons for starting and stopping translation. Here we focus on translation in *Bacteria*.

Properties of the Genetic Code

There are 22 naturally occurring amino acids and because there are 64 codons, several amino acids can be encoded by more than one codon. A code such as this that lacks one-to-one correspondence between "word" (that is, the amino acid) and code (codon) is called a *degenerate code*. A codon is recognized by specific base pairing with a complementary sequence on the *anticodon*, located on a tRNA (Section 4.8). If this base pairing were always the standard pairing of A with U and G with C, then at least one specific tRNA would be needed to recognize each codon. In some cases, this is true. For instance, there are six different tRNAs in *Escherichia coli* for the amino acid leucine, one for each codon (Table 4.4). By contrast, some tRNAs can recognize more than one codon. For example, although there are two lysine codons in *E. coli*, there is only one lysyl tRNA, whose anticodon can base-pair with *either* AAA or AAG. In these cases, the anticodon forms standard base pairs at only the first two positions of the codon and tolerates irregular base pairing at the third position. This phenomenon is called **wobble** and is illustrated in Figure 4.34.

If an amino acid is encoded by multiple codons, the codons are typically closely related in base sequence, usually differing at only their third position (Table 4.4) to allow for wobble (Figure 4.34). Interestingly, not all multiple codons for a given amino acid are used at the same frequency, leading to a **codon bias** that varies from organism to organism. Codon bias is correlated with a corresponding bias in the concentration of different tRNA molecules. That is, a tRNA whose anticodon corresponds to a rarely used codon is typically produced at low levels.

Start and Stop Codons and Reading Frames

Messenger RNA is translated beginning with its **start codon** (AUG, Table 4.4), which encodes a chemically modified methionine in *Bacteria* called *N-formylmethionine* (although AUG at the *beginning* of a coding region encodes *N*-formylmethionine, AUG *within* the coding region encodes methionine). By contrast, *Archaea* and *Eukarya* insert an unmodified methionine as the first amino acid in a polypeptide.

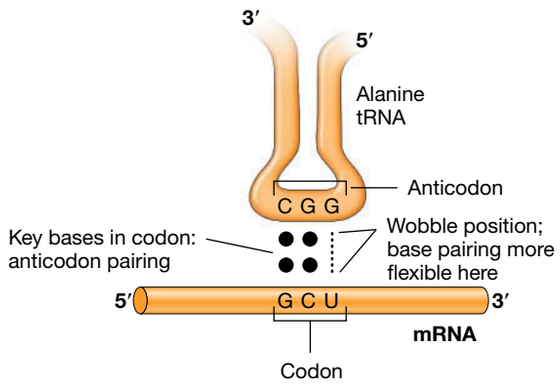


Figure 4.34 The wobble concept. Base pairing is more flexible for the third base of the codon than for the first two. Only a portion of the tRNA is shown here.

With a triplet code it is critical for translation to begin at the correct nucleotide. If it does not, the whole reading frame of the mRNA will be shifted and thus an entirely different (and likely inactive) protein will be made. Alternatively, if the shift introduces a stop codon (Table 4.4) into the reading frame, the polypeptide will terminate prematurely. The reading frame that when translated yields the polypeptide encoded by the gene is called the *0* (zero) *frame*; the other possible reading frames (−1 and +1) do not encode the same amino acid sequence (**Figure 4.35**). Reading frame fidelity is governed by interactions between mRNA and rRNA within the ribosome. In *Bacteria*, ribosomal RNA recognizes a specific AUG on the mRNA as a start codon with the aid of an upstream sequence in the mRNA called the *ribosome-binding site* (RBS) (also called the *Shine–Dalgarno sequence* after its discoverers). This upstream alignment requirement explains why some mRNAs from *Bacteria* can use other start codons, such as GUG. However, because of the RBS, even these unusual start codons direct the incorporation of *N*-formylmethionine as the initiator amino acid (see Figure 4.36).

The codons UAA, UAG, and UGA (Table 4.4) are **stop codons**, and they signal the termination of translation of a protein-coding sequence on the mRNA. Stop codons are also called **nonsense codons**, because they interrupt the "sense" of the growing polypeptide when they terminate translation. In a few rare cases in *Bacteria* and *Archaea*, nonsense codons encode the unusual amino acids selenocysteine and pyrrolysine (Figure 4.28). When this occurs, specific tRNAs are employed whose anticodons read these stop codons. What controls this unusual occurrence is a recognition sequence just downstream of the now-coding stop codon that signals the incorporation of tRNAs containing selenocysteine or pyrrolysine rather than stopping translation. A few other microbes use conventional stop codons to encode amino acids, but in these cases the organisms have simply dispensed with using these particular stop codons as translational stop sites.

If an mRNA can be translated, it is because it contains an **open reading frame** (ORF): a start codon followed by a number of codons and then a stop codon in the same reading frame as the start codon. Using computational methods, a DNA base sequence can be scanned to search for open reading frames. In addition to looking for start and stop codons, computer analyses may include a search for promoters and ribosome-binding sequences as well to confirm the ORF as protein encoding. The search for ORFs is central to the field of genomics (Chapter 9), for if an unknown piece of DNA has been sequenced, the presence of an ORF implies that it can encode protein.

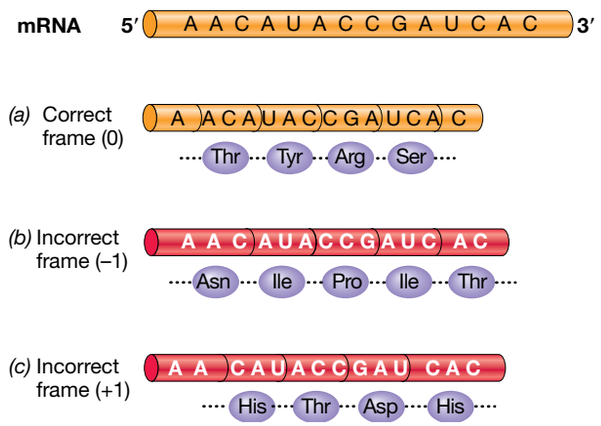


Figure 4.35 Possible reading frames in an mRNA. An interior sequence of an mRNA is shown. *(a)* The amino acids that would be encoded if the ribosome is in the correct reading frame (designated the "0" frame). *(b)* The amino acids that would be encoded by this region of the mRNA if the ribosome were in the −1 reading frame. *(c)* The amino acids that would be encoded if the ribosome were in the +1 reading frame.

MINIQUIZ

- What are start codons and stop codons? Why is it important for the ribosome to read "in frame"?
- What is codon bias?
- If you were given a nucleotide sequence, how would you find ORFs?

4.10 The Mechanism of Protein Synthesis

Protein synthesis is a dynamic process and can be broken down into three major steps: *initiation*, *elongation*, and *termination*. In addition to mRNA, tRNA, and ribosomes, translation requires a number of initiation, elongation, and termination proteins and the energy-rich compound guanosine triphosphate (GTP) to provide the energy for the process.

Ribosomes and the Initiation of Translation

Ribosomes are the sites of protein synthesis. A cell may have many thousands of ribosomes, the number increasing at higher growth rates. Each ribosome consists of two subunits. *Bacteria* and *Archaea* have 30S and 50S ribosomal subunits that yield intact 70S ribosomes. Each ribosomal subunit contains specific ribosomal RNAs and ribosomal proteins. The 30S subunit contains 16S rRNA and 21 proteins, and the 50S subunit contains 5S and 23S rRNA and 31 proteins. Thus, in *Escherichia coli*, there are 52 distinct ribosomal proteins, most present at one copy per ribosome. The ribosome is a highly dynamic structure, and its subunits alternately associate and dissociate during the translational process and also interact with many other proteins. In addition, several cytoplasmic proteins called *translation factors* are essential for translation and interact with the ribosome at various stages of the translational process.

In *Bacteria*, initiation of protein synthesis begins with a free 30S ribosomal subunit (**Figure 4.36**). From this, an *initiation complex* forms consisting of the 30S subunit, mRNA, formylmethionine tRNA (the initiator tRNA in *Bacteria*; after polypeptide completion, the formyl group is removed), and the initiation factors IF1, IF2, and IF3. GTP is also required for this step. Next, a 50S ribosomal subunit is added to the initiation complex to form the active 70S ribosome. Just preceding the start codon on the mRNA is a sequence of three to nine nucleotides that compose the ribosome-binding site (RBS in Figure 4.36). This site is toward the 5′ end of the mRNA and is complementary to base sequences in the 3′ end of the 16S rRNA, which is part of the ribosome. Base pairing between these two RNAs holds the ribosome–mRNA

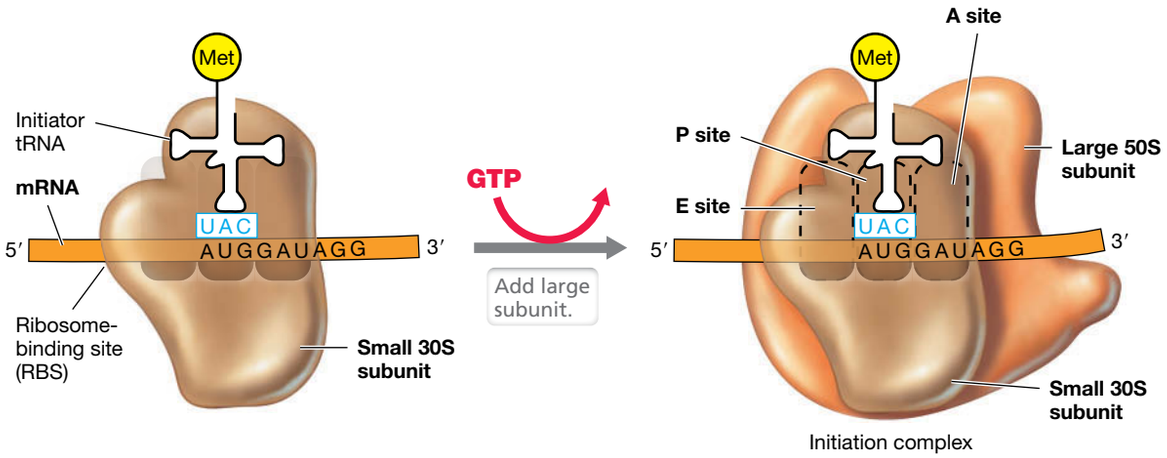


Figure 4.36 The ribosome and initiation of protein synthesis. The mRNA and initiator tRNA, carrying *N*-formylmethionine ("Met"), bind first to the small subunit of the ribosome. Initiation factors (not shown) use energy from GTP to promote the addition of the large ribosomal subunit. The initiator tRNA starts out in the P site (labeled in the structure on the right-hand side of the arrow).

complex securely together in the correct reading frame. Polycistronic mRNA (Section 4.5) contains several RBS sequences, one upstream of each coding sequence. This allows bacterial ribosomes to translate several genes on the same mRNA because the ribosome can locate each initiation site within a message by binding to its RBS.

Elongation, Translocation, and Termination

During translation, the mRNA threads through the ribosome bound to the 30S subunit. The ribosome contains other sites where the tRNAs interact. Two of these sites are located primarily on the 50S subunit and are termed the *A* (*acceptor*) *site* and the *P* (*peptide*) *site* (**Figure 4.37**). The A site is where the incoming charged tRNA first attaches, and the loading of a tRNA into the A site is assisted by the elongation factor EF-Tu. The P site is where the growing polypeptide chain is attached to the prior tRNA. During peptide bond formation, the growing polypeptide chain moves to the tRNA at the A site as a new peptide bond is formed. In addition to EF-Tu, elongation factor EF-Ts, as well as more GTP, is required (Figure 4.37).

Following elongation, the tRNA holding the polypeptide is translocated from the A site to the P site, thus opening the A site for a new charged tRNA; this requires elongation factor EF-G and one molecule of GTP for each translocation event (Figure 4.37). In each translocation, the ribosome advances three nucleotides (one codon) along the mRNA, exposing a new codon at the A site. Translocation pushes the now amino acid–free tRNA to a third site, called the E (exit) site, and it is from here that the tRNA is released from the ribosome. The precision of the translocation step is critical to the accuracy of protein synthesis. That is, the ribosome must move *exactly* one codon at each step or the fidelity of translation will be compromised.

Several ribosomes can translate a single mRNA molecule simultaneously, forming a complex called a *polysome* (**Figure 4.38**). Polysomes increase both the speed and efficiency of translation because each ribosome in the polysome makes a complete polypeptide. Note in Figure 4.38 how ribosomes in the polysome that are closest to the 5′ end (the beginning) of the mRNA molecule have short polypeptides attached to them because only a few codons have been read, while ribosomes closest to the 3′ end of the mRNA have nearly finished polypeptides.

Translation terminates when the ribosome reaches a stop codon (Table 4.4) because no tRNA binds to a stop codon. Instead, proteins called *release factors* (RFs) recognize the stop codon and cleave the attached polypeptide from the final tRNA, releasing the finished product. Following this, the ribosomal subunits dissociate, and the 30S and 50S subunits are then free to form new initiation complexes (Figure 4.36) and repeat the process.

Role of Ribosomal RNA in Protein Synthesis

Ribosomal RNA plays major roles in all stages of translation, from initiation to termination. By contrast, the primary role of the proteins in the ribosome is to form a scaffold to position key sequences in the ribosomal RNAs. In *Bacteria*, 16S rRNA facilitates initiation by base pairing with the ribosome-binding site on the mRNA, and, along with ribosomal proteins, holds the mRNA in position on either side of the A and P sites. Ribosomal RNA also plays a role in ribosome subunit association, as well as in positioning tRNAs on the ribosome (Figures 4.36 and 4.37). Although charged tRNAs recognize the correct codon by codon–anticodon base pairing (Figure 4.34), they are also bound to the ribosome by interactions between the anticodon stem–loop of the tRNA and specific sequences in the 16S rRNA. Moreover, the acceptor end of the tRNA (Figures 4.36 and 4.37) base-pairs with sequences in 23S rRNA.

In addition to roles in mRNA alignment and translocation along the transcript, rRNA also catalyzes the actual formation of peptide bonds. The peptidyl transferase reaction occurs on the 50S subunit of the ribosome and is catalyzed solely by 23S rRNA. This rRNA also plays a role in translocation and interacts with the elongation factors. Thus, in addition to its role as the structural backbone of the ribosome, ribosomal RNA plays a major catalytic role in the translation process.

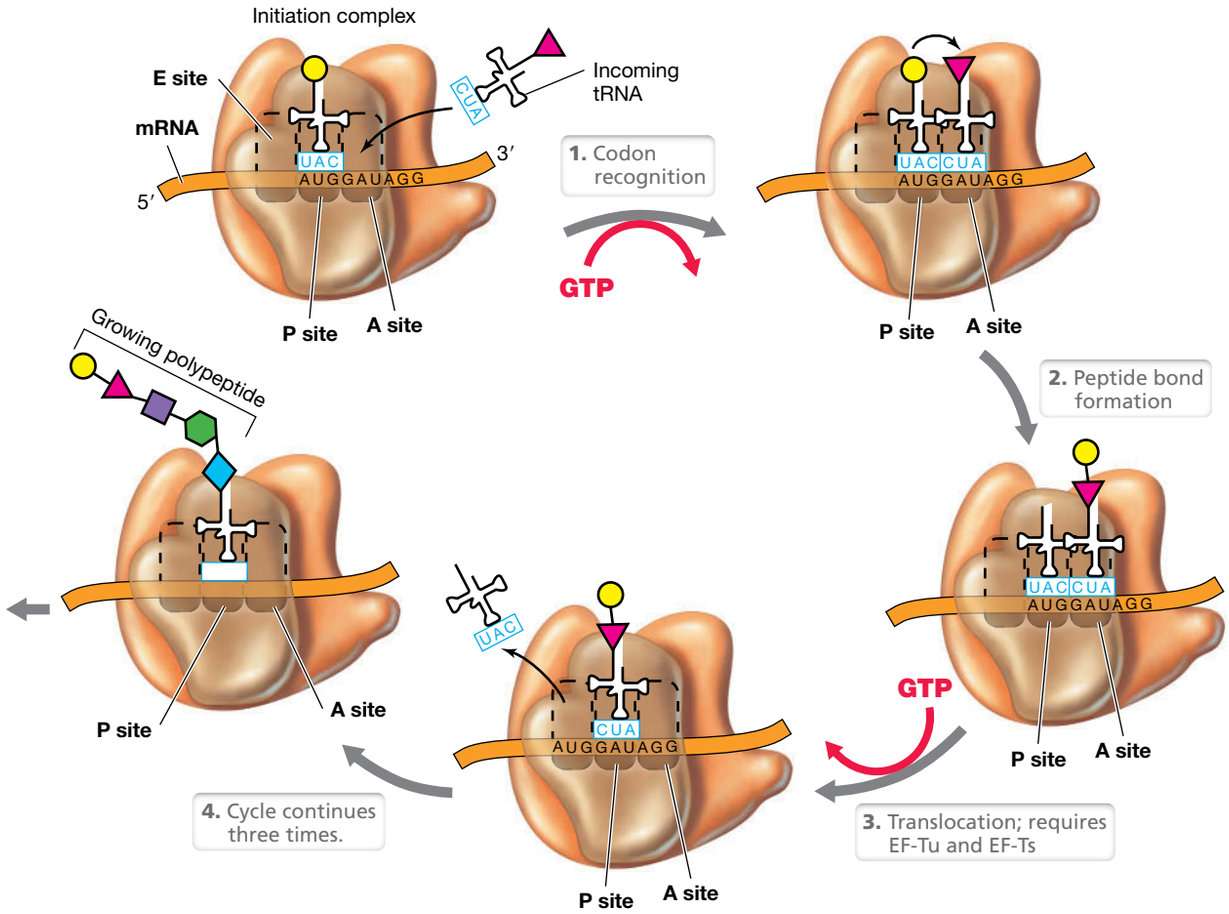


Figure 4.37 Elongation cycle of translation. 1. Elongation factors (not shown) use GTP to install the incoming tRNA into the A site. 2. Peptide bond formation is then catalyzed by the 23S rRNA. 3. Translocation of the ribosome along the mRNA from one codon to the next requires hydrolysis of another GTP and results in movement of the tRNA with the growing peptide to the P site. The outgoing tRNA is released from the E site. 4. The next charged tRNA binds to the A site and the cycle repeats. The genetic code, expressed as codons of mRNA, is shown in Table 4.4.

Freeing Trapped Ribosomes

A defective mRNA that lacks a stop codon cannot be properly translated. Such a defect may arise, for example, from a mutation that removed the stop codon, from defective synthesis of the mRNA, or when partial degradation of an mRNA occurs before it is translated. If a ribosome reaches the end of an mRNA molecule and there is no stop codon, release factor cannot bind and the ribosome cannot be released from the mRNA; the ribosome is effectively "trapped."

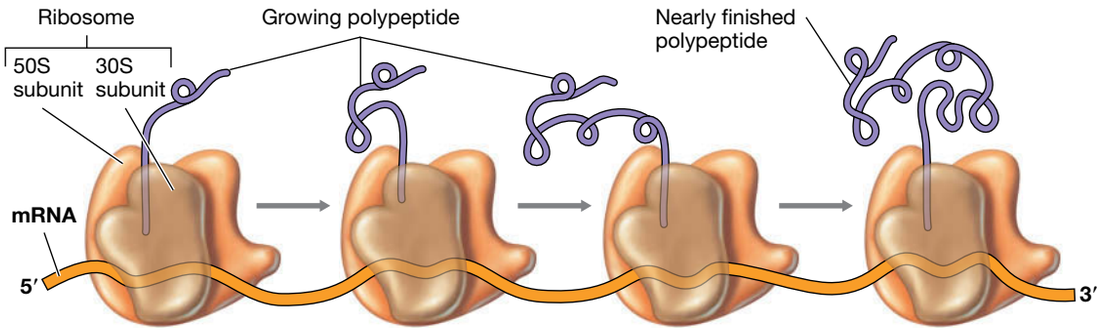


Figure 4.38 Polysomes. Translation by several ribosomes on a single messenger RNA forms the polysome. Note how the ribosomes nearest the 5′ end of the message are at an earlier stage in the translation process than ribosomes nearer the 3′ end, and thus only a relatively short portion of the final polypeptide has been made.

To deal with this problem, bacterial cells produce a small RNA molecule called *tmRNA* that frees stalled ribosomes (**Figure 4.39**). The "tm" in its name refers to the fact that tmRNA mimics both tRNA, in that it carries an amino acid (alanine), and mRNA, in that it contains a short stretch of RNA that can be translated. When tmRNA collides with a stalled ribosome, it binds alongside the defective mRNA. Protein synthesis can

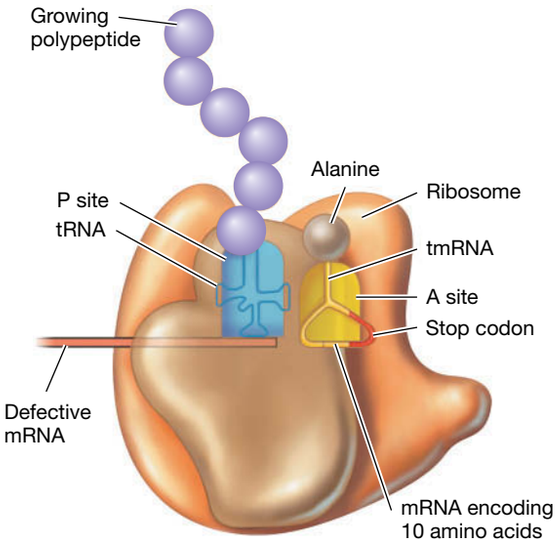


Figure 4.39 Freeing of a stalled ribosome by tmRNA. A defective mRNA lacking a stop codon stalls a ribosome that has a partly synthesized polypeptide attached to a tRNA (blue) in the P site. Binding of tmRNA (yellow) in the A site allows translation to continue up to the stop codon provided by the tmRNA.

then proceed, first by adding the alanine on the tmRNA and then by translating the short tmRNA message. The tmRNA contains a stop codon that allows release factor to bind and disassemble the ribosome. The protein made as a result of this rescue operation is defective and is subsequently degraded. This is accomplished by a short sequence of amino acids encoded by tmRNA and added to the end of the defective protein; the sequence is a signal for a specific protease to degrade the protein. Thus, through the activity of tmRNA, stalled ribosomes are not inactivated but instead are freed up to participate in protein synthesis once again.

MINIQUIZ

- What are the components of a ribosome? What functional roles does rRNA play in protein synthesis?
- How is a completed polypeptide chain released from the ribosome?
- How does tmRNA free stalled ribosomes?

V • Protein Processing, Secretion, and Targeting

While translation is the last step in genetic information flow (Figures 4.1*a* and 4.6), some proteins require subsequent *processing* before they become functional. This processing may include assistance in folding or, for some enzymes, the incorporation of *cofactors* or other nonprotein groups. To perform their assigned activities, some proteins must also be targeted to membranes, the periplasm, or even through the membranes of other cells. These processing and targeting activities often require accessory proteins to assist with folding and transport as well as intrinsic signals within the translated protein itself. In the final part of this chapter, we consider the process of assisted protein folding and mechanisms of protein secretion and targeting.

4.11 Assisted Protein Folding and Chaperones

In Section 4.7 we saw how proteins show several levels of structure including primary, secondary, tertiary, and, in multi-subunit proteins, quaternary structure. Many proteins fold spontaneously into their active form, even while they are being synthesized (Figure 4.38). However, some do not, and these require assistance to achieve an active functional state.

Major Chaperones of *Bacteria*

Bacteria produce a series of proteins called **chaperones** that catalyze a variety of macromolecular folding events. These events include folding proteins that do not fold spontaneously, refolding partially denatured proteins, assembling multiprotein complexes, preventing the improper aggregation of proteins, untangling RNAs, and incorporating cofactors into enzymes. Chaperones are found in all domains of life, and their sequences are highly conserved among all organisms.

Four key chaperones in *Escherichia coli* are the proteins DnaK, DnaJ, GroEL, and GroES. DnaK and DnaJ are ATP-dependent enzymes that bind to newly formed polypeptides and prevent them from folding too quickly, which would increase the risk of improper folding (**Figure 4.40*a***). If the DnaKJ complex is unable to fold the protein properly, it may transfer the partially folded protein to the two multi-subunit proteins GroEL and GroES. The protein first enters GroEL, a large, barrel-shaped protein that uses the energy of ATP hydrolysis to fold the protein properly. GroES assists in this (Figure 4.40*a*). It is estimated that about 100 of the several thousand proteins in a cell of *E. coli* need help in folding from the GroEL–GroES complex, and of these, approximately a dozen are essential for survival of the bacterium.

Other Functions of Chaperones

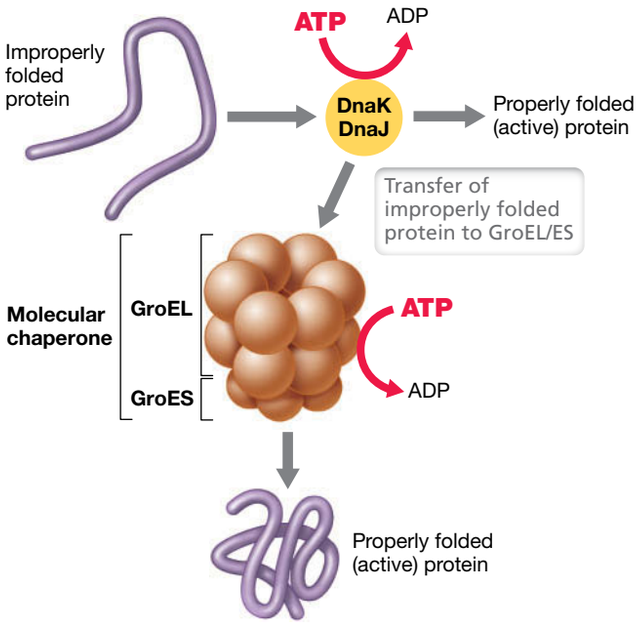
Chaperones can also refold proteins that have partially denatured in the cell. A protein may denature for many reasons, but often it is because the organism has temporarily experienced high temperatures. Chaperones are thus one type of *heat shock protein*, and their synthesis is greatly accelerated when a cell is stressed by excessive heat (Section 6.10). The heat shock response is an attempt by the cell to refold its partially denatured proteins for reuse before proteases recognize them as improperly folded and destroy them. In contrast to heat shock proteins, *cold shock proteins* are produced when the cell experiences a sudden shift to very cold temperatures. Cold (but nonfreezing) conditions do not affect most proteins but can affect RNAs. CspA, a major cold shock protein of *E. coli*, is an RNA chaperone instead of a protein chaperone and prevents the spontaneous formation of secondary structures in mRNA that could compromise their ability to be translated. Many other cold shock proteins are known in *E. coli* including ones that refold cold-sensitive proteins rather than acting on mRNAs.

Some chaperones help assemble cofactor-containing enzymes such as those that catalyze redox or electron transport reactions (Section 3.10). For example, several multi-subunit molybdoenyzmes such as

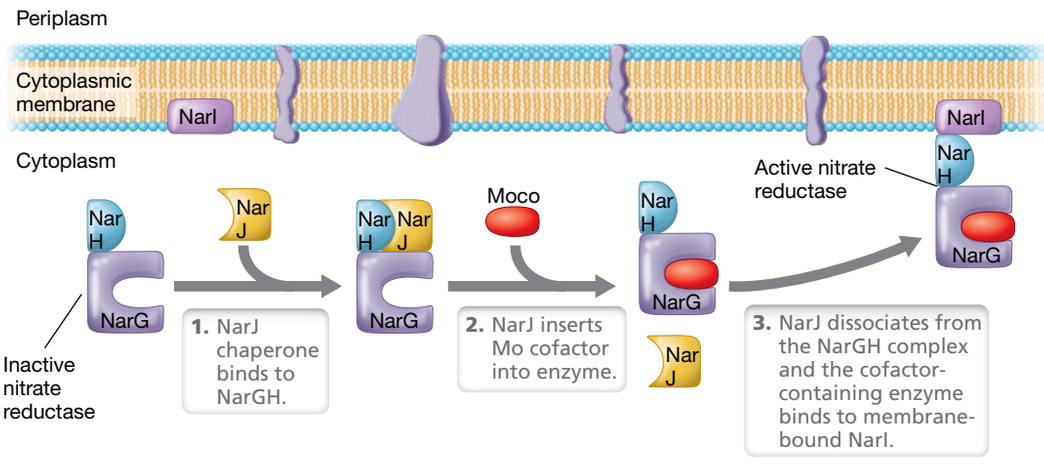
the *E. coli* membrane-bound nitrate reductase require that a molybdenum cofactor (Moco) be inserted during folding, and cofactor insertion is assisted by enzyme-specific chaperone proteins (Figure 4.40*b*). Nitrate reductase is a key enzyme in anaerobic respiration (Sections 3.12 and 14.13) and is composed of three subunits: NarGHI. For the enzyme to be functional, the cytoplasmic chaperone protein NarJ must first insert the Moco group into NarG. Once this cofactor has been inserted, the NarG and NarH subunits associate with the membrane-bound NarI subunit to form the active nitrate reductase enzyme complex (Figure 4.40*b*).

MINIQUIZ

- What are molecular chaperones and why are they necessary?
- What macromolecules are protected by heat shock proteins?
- How do chaperones assist the *Escherichia coli* cell to make nitrate reductase?



(a)



(b)

Figure 4.40 The activity of chaperone proteins. *(a)* An improperly folded protein can be refolded by either the DnaKJ complex or by the GroEL–GroES complex. In both cases, energy for refolding comes from ATP. *(b)* Formation of an active nitrate reductase enzyme by chaperone-facilitated incorporation of a cofactor. The chaperone protein NarJ carries the molybdenum cofactor (Moco) to the inactive cytoplasmic NarGH complex. After incorporation of Moco, NarJ dissociates and the complex binds to NarI in the membrane becoming an active enzyme.

4.12 Protein Secretion: The Sec and Tat Systems

While many proteins exist in the cell's cytoplasm, some must be transported outside the cytoplasmic membrane into the periplasm (of gram-negative cells) or inserted into the cytoplasmic or outer membranes to facilitate nutrient transport or bioenergetic events. Other proteins such as toxins and extracellular enzymes (exoenzymes) must be secreted from the cell entirely to be active in the environment or to invade another cell. These secreted proteins must get from their site of synthesis on ribosomes into or through the cytoplasmic membrane in order to be functional, and the cell has evolved several systems to get this job done.

The Signal Sequence

Proteins called *translocases* transport specific proteins into or through the membranes of *Bacteria* and *Archaea*. For example, the Sec translocase system both exports unfolded proteins and inserts integral membrane proteins into the cytoplasmic membrane. By contrast, the Tat translocase system transports previously folded proteins through the cytoplasmic membrane. Both the general secretion (Sec) and Tat systems are universal in *Bacteria* and *Archaea*.

Most proteins that must be transported into or through bacterial or archaeal cytoplasmic membranes are synthesized with an amino acid sequence of 15–20 residues—called the **signal sequence**—at the beginning (N-terminus, Figure 4.29) of the protein molecule. Signal sequences are variable, but they typically contain a few positively charged amino acids at the beginning, a central region of hydrophobic residues, and then a more polar region at their end. The signal sequence is so named because it "signals" to a translocase system that this particular protein is to be exported and also helps prevent the protein from completely folding, a process that could interfere with its secretion. Because the signal sequence is the first part of the protein to be synthesized, the early steps in export may actually begin before the protein is completely synthesized (Figure 4.41).

Sec and Tat Translocases

In the Sec system, unfolded proteins to be exported from the cytoplasm are recognized by either the *SecA protein* or the *signal recognition particle* (*SRP*) (Figure 4.41). Typically, SecA binds proteins that are to be exported into the periplasm, whereas the SRP binds proteins that are to be inserted into the membrane (but not released on the other side). SRPs are found in all cells, but in *Bacteria*, they consist of a single protein and a small, noncoding RNA molecule (4.5S RNA). Both SecA and the SRP deliver proteins to the membrane secretion complex, and after crossing the membrane (Sec-mediated) or inserting into the membrane (SRP-mediated), which is fueled by ATP hydrolysis (Sec) or the proton motive force (Tat), the signal sequence is removed by a protease and the proteins fold to their active form.

Some proteins must be folded before they are translocated because they contain cofactors that must be inserted as the protein folds; for example, iron–sulfur proteins, cytochromes, and other respiratory enzymes (Section 3.10). Such proteins are processed by the Tat translocase system (for example, nitrate reductase shown in Figure 4.40*b*). Tat stands for "*t*win-*a*rginine *t*ranslocase" because the transported proteins contain a short signal sequence that has a pair of arginine residues. This signal sequence is recognized by TatBC, which carries the folded protein to TatA—the membrane transporter. Following transport, the signal sequence is removed by a protease.

MINIQUIZ

- What is the signal sequence and what does it signal?
- What is the signal recognition particle composed of?
- How do the translocases Sec and Tat differ in the molecules they secrete?

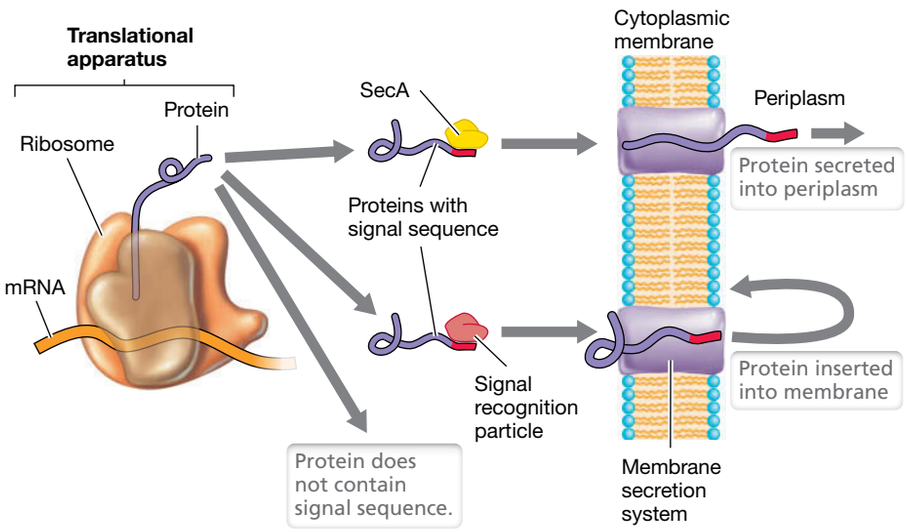


Figure 4.41 Export of proteins via the SecA secretory system. The signal sequence is recognized either by SecA or by the signal recognition particle, which carries the protein to the membrane secretion system. The signal recognition particle binds proteins that are inserted into the membrane, whereas SecA binds proteins that are secreted across the cytoplasmic membrane.

4.13 Protein Secretion: Gram-Negative Systems

In order to insert proteins or other small molecules known as *effectors* into the outer membrane of gram-negative *Bacteria*, or to secrete them outside of the cell, special secretion systems known as types I through VI are used. Gram-positive bacteria and *Archaea* possess secretion systems similar to some of these, but in these organisms, the machinery only has to transport the proteins or effector molecules across the cytoplasmic membrane.

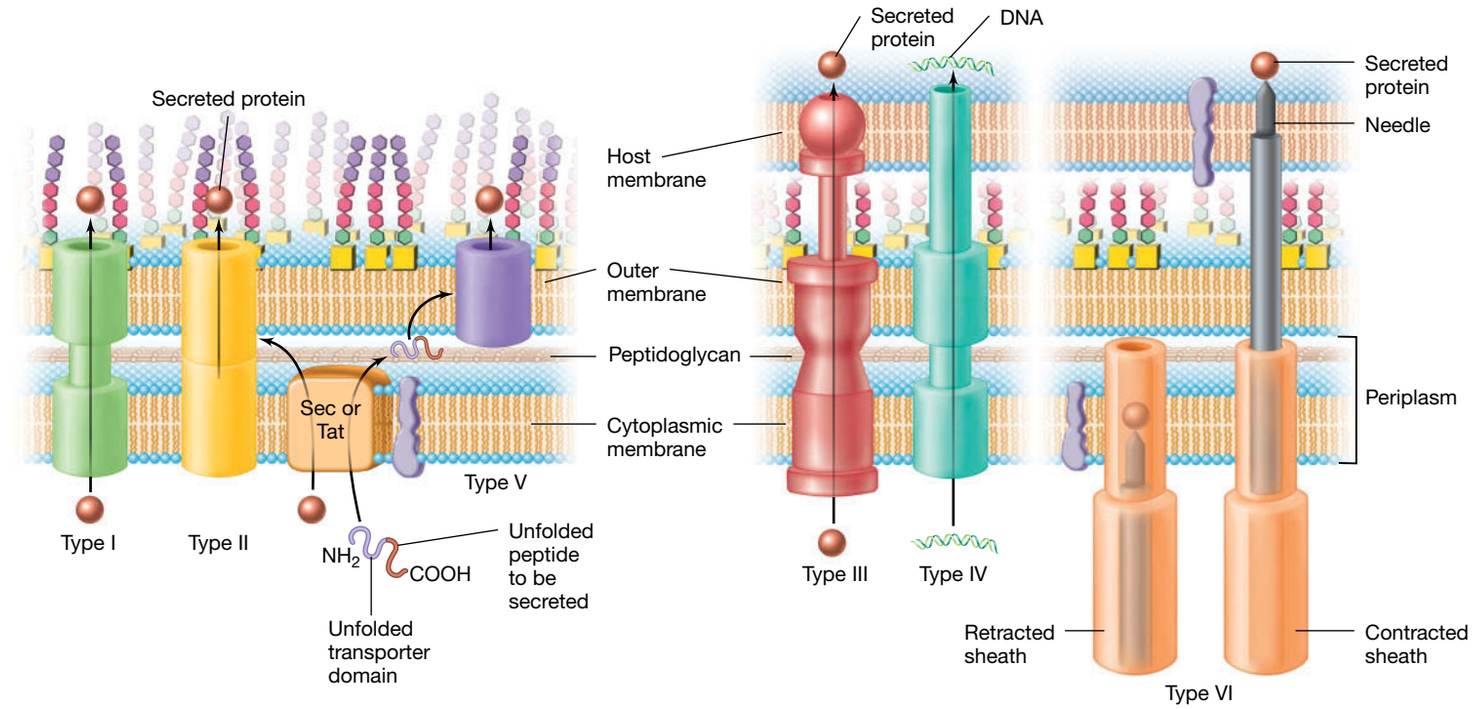
Types I–VI secretion systems facilitate several cellular activities including symbiotic interactions, biofilm formation, extracellular enzyme secretion, transfer of DNA, release of antibiotics, and delivery of proteins into host cells. Thus, molecules secreted by these systems allow bacteria to interact with the environment and other organisms. Each of these systems specifically recognizes its substrate based on amino acid residues and is composed of a large complex of proteins that forms a translocase channel spanning one or more membranes through which the secreted molecule travels (**Figure 4.42**).

Types II and V Secretion Systems

Secretion systems can be grouped into *one-step* and *two-step* classes. Type II and type V systems are two-step translocases because they depend on either the Sec or Tat system (Section 4.12) to transport the secreted protein or a portion of the translocase channel from the cytoplasm through the inner membrane (Figure 4.42*a*). Because types II and V are dependent on the Sec or Tat systems, they generally do not require ATP or the proton motive force to secrete proteins across the outer membrane of the cell.

Type II systems are found in a wide variety of gram-negative pathogenic and nonpathogenic bacteria, and they transport proteins from the periplasm to the extracellular environment. The translocase machinery of type II systems includes a secretion pore in the outer membrane that is anchored to the cytoplasmic membrane by proteins that span the periplasm (Figure 4.42*a*). While the type II system does possess cytoplasmic membrane and periplasmic components, proteins to be secreted do not translocate through these and are instead delivered to the secretion pore in the outer membrane by either the Sec or Tat system. A key to the specificity of the type II system is that the secretion pore only opens to proteins specific to the type II system. Examples of proteins secreted by a type II system include the toxin produced by *Vibrio cholerae* (the causative agent of cholera) and a glucanase exoenzyme produced by *Klebsiella pneumoniae* to degrade large extracellular starch molecules.

Type V systems are the structurally simplest of the secretion systems (Figure 4.42*a*) and are also called *autotransporters* in that the protein to be secreted is fused to a transmembrane protein



(a) **Types I, II, and V** *(b)* **Types III, IV, and VI**

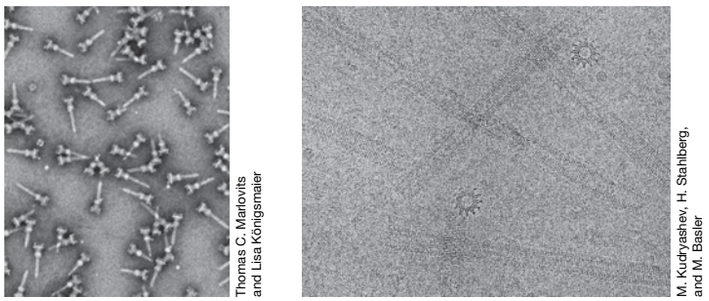
Figure 4.42 Secretion systems in gram-negative bacteria. *(a)* Types I, II, and V secrete proteins outside of the bacterial cell. Type I systems secrete proteins in a single step. Types II and V first require the Sec or Tat system to transport the protein to be secreted across the inner membrane. Note that during type V secretion, the Sec system first transports the unfolded transporter domain linked to the unfolded secretion protein through the inner membrane. The translocase then folds in the outer membrane and delivers the folded secretion protein through the outer membrane. *(b)* Types III, IV, and VI secrete molecules outside of the bacterial cell and into a host cell. Type III systems have been termed the injectisome, while type IV systems are similar to a pilus and also secrete DNA into a host cell. Type VI systems contain a sheath or needle in the cytoplasm that contracts to deliver a protein into a host cell.

domain essential to the protein's transport across the outer membrane. After this unfolded multidomain protein is itself transported through the cytoplasmic membrane by the Sec system, a transmembrane domain called the transporter forms a secretion pore in the outer membrane that allows the remainder of the protein (the passenger domain) to pass through and ultimately be secreted outside of the cell (Figure 4.42*a*). Thus proteins to be moved outside of the cell by a type V system are initially transported in the unfolded state, and both the transporter and passenger domains require chaperone proteins (Section 4.11) for proper folding. The folding of the passenger domain (instead of ATP hydrolysis) drives type V secretion. Examples of proteins secreted by the type V system are adhesion proteins used by *E. coli* and *Haemophilus influenzae* (a causative agent of pneumonia) to attach to host cells.

Types I, III, IV, and VI Secretion Systems

The second class of translocases moves proteins through the outer membrane in a single step. Types I, III, IV, and VI are one-step systems because they form channels through both the cytoplasmic and outer membranes and do not require Sec or Tat. *Type I systems* are characterized by three protein components: (1) a cytoplasmic membrane transporter coupled to (2) an outer membrane pore by (3) a membrane fusion protein. The cytoplasmic membrane transporter binds specifically to the protein to be secreted and requires ATP to initiate transport to the outside of the cell (Figure 4.42*a*). While the cytoplasmic membrane transporter is specific to its substrate, a wide range of polypeptide sizes can be secreted. Type I systems secrete small molecules such as a toxic protein (bacteriocin) produced by *E. coli* to kill competing bacterial cells and can also secrete large proteins such as those necessary for biofilm formation on plant surfaces by the soil bacterium *Pseudomonas fluorescens*.

Type III systems are commonly used by pathogenic bacteria not only to secrete toxic proteins outside of the cell but to inject these molecules directly into eukaryotic host cells (Figure 4.42*b*). The entire type III structure is highly complex and composed of over 100 proteins that facilitate substrate recognition, coordinated assembly of the machinery for translocations, and the transport process itself. ATP hydrolysis provides the energy for the secretion and injection. The entire structure, 30–70 nm in length, has been termed the "injectisome" for its similarity to a syringe in both structure and function (**Figure 4.43*a***). Proteins injected into host cells by the type III system often aid in pathogen infection and host invasion. Type III–injected proteins include certain effector molecules of *Chlamydia* (the cause of trachoma and a sexually transmitted disease) and *Salmonella* (a foodborne and waterborne pathogen). However, type III secretion systems are not limited to



(a) (b)

Figure 4.43 Electron micrographs of secretion machinery. *(a)* Purified type III injectisomes from *Salmonella enterica* (*typhimurium*). *(b)* Purified type VI contractile sheaths from *Vibrio cholerae*.

pathogens. Nitrogen-fixing bacteria deliver molecules critical for establishing a symbiotic relationship with plant roots (root nodules) through type III secretion machinery (Section 23.3).

Type IV systems (Figure 4.42*b*) are the most ubiquitous and are present in many *Bacteria* and *Archaea*. This system is also used to deliver secreted proteins or other molecules *into* other cells. While this system can be used to transport toxins into host cells, its primary role is to transfer DNA to other cells though the process of conjugation (Section 11.8), one mechanism that prokaryotic cells have to exchange genes. The type IV translocase is similar to a pilus that extends through the outer membrane into another cell during conjugation. Once the tip of the pilus makes contact with a receptor on the host cell, the DNA is recognized by an inner membrane protein of the donor and then transferred using ATP-facilitated transport (Section 11.8). Not only can DNA be transferred from one bacterial cell to another using a type IV system, DNA from the plant pathogenic bacterium *Agrobacterium* can be transferred to host plant cells through the system encoded on the Ti plasmid (crown gall disease, Section 23.5).

Type VI systems are widely distributed in gram-negative bacteria, and like type IV systems they are capable of delivering a diversity of proteins directly into the cytoplasm of other cells using a one-step, ATP-requiring process similar to type III and IV systems. However, unlike the injectisome or pilus-like structure of the type III and IV translocase systems, the type VI translocase is cytoplasmic and forms a needle-like protein with a pore-forming protein that contracts all the way through the donor cell's two membranes and directly into a host cell once a substrate molecule is recognized (Figures 4.42*b* and 4.43*b*). The overall delivery process is similar to the mechanism that the tailed bacteriophage T4 uses to deliver DNA into an *E. coli* cell through tail contraction (Section 8.5). Type VI systems are used by bacteria as weapons to compete with other bacterial cells or to attack eukaryotic cells. In *Pseudomonas aeruginosa*, a type VI system is used to inject a toxin into competing bacterial cells. Similarly, *Vibrio cholerae* uses its type VI system (Figure 4.43*b*) to inject enzymes into competing bacteria; the enzymes degrade the cell wall and membrane. *V. cholerae* also uses the system to deliver toxins to human intestinal cells during a cholera infection.

It should be obvious by now that protein secretion is a crucial aspect of the biology of *Bacteria* and *Archaea* and that these cells have evolved diverse structures and unique mechanisms to deal with protein secretion. However, these elaborate systems are of interest to more than just basic science. Since many of these systems are essential virulence factors for the pathogenic bacteria that produce them, they are currently targets for vaccine development. The rationale here is quite simple: If the activity of these systems could be blocked by the immune system, the pathogen would be unable to establish an infection and cause disease.

We now move on from our focus on the central dogma of biology, protein processing, and protein secretion to tackle the concepts of Chapter 5, where we will examine another process of great importance to a cell's well-being: growth. Without increasing cell numbers, a microbial population will eventually wither away. And as we have just witnessed with the secretion process, aspects of both biochemical unity and diversity underlie the process of microbial growth.

MINIQUIZ

- Compared with gram-positive bacteria, why is it important for gram-negative bacteria to have additional secretion pathways?
- How do the types I–VI secretion processes differ from Sec and Tat secretion?
- Why is the injectisome so named?

Chapter Review

I • Molecular Biology and Genetic Elements

4.1 The informational content of a nucleic acid is determined by the sequence of nitrogen bases along the polynucleotide chain. Both RNA and DNA are informational macromolecules, as are the proteins they encode. DNA forms a double-stranded helix whose strands are complementary and antiparallel. DNA is supercoiled before it is packaged into cells. The three key processes of macromolecular synthesis are: (1) DNA replication; (2) transcription (RNA synthesis); and (3) translation (protein synthesis).

Q Describe the central dogma of molecular biology. With regards to DNA, what is supercoiling and what is meant by the terms antiparallel and complementary?

4.2 In addition to the chromosome, other genetic elements can exist in cells. Plasmids are DNA molecules that exist separately from the chromosome and may confer a selective growth advantage under certain conditions. Viruses contain an RNA or DNA genome, and transposable elements exist as a part of other genetic elements.

Q How are chromosomes and plasmids similar, and how do they differ? What are R plasmids and why are they of medical concern?

II • Copying the Genetic Blueprint: DNA Replication

4.3 Both strands of the DNA helix are templates for the synthesis of new strands (semiconservative replication). The new strands are elongated by addition of deoxyribonucleotides to the 3′ end. DNA polymerases require a primer made of RNA by the enzyme primase, and synthesis begins at an origin of replication. The double helix is unwound by helicase and is stabilized by single-strand binding proteins. Extension of the DNA occurs continuously on the leading strand but discontinuously on the lagging strand, resulting in fragments that must be joined together.

Q What is meant by the term semiconservative replication? What are the functions of DNA Pol I and III, helicase, and primase? How does a leading strand differ from a lagging strand?

4.4 Starting from a single origin on a circular chromosome, two replication forks synthesize DNA simultaneously in both directions until the forks meet at the terminus region. The proteins at the replication fork form a large complex known as the replisome. Most errors in base pairing that occur during replication are corrected by the proofreading functions of DNA polymerases.

Q What is the replisome and what does it contain? Why can replication occur faster on circular DNA than on linear DNA? What is proofreading and why is it important?

III • RNA Synthesis: Transcription

4.5 In *Bacteria*, promoters are recognized by the sigma subunit of RNA polymerase. Alternative sigma factors allow joint regulation of large families of genes in response to growth conditions. Transcription by RNA polymerase continues until specific sites called transcription terminators are reached. These terminators function at the level of RNA. In *Bacteria* and *Archaea* a single mRNA molecule may encode more than one polypeptide. A cluster of genes that are transcribed together from a single promoter is called an operon.

Q What do promoters do in *Bacteria*? How is a transcript in *Bacteria* terminated?

4.6 The transcription apparatus and the promoter architecture of *Archaea* and *Eukarya* have many features in common, although the components are usually relatively more simple in *Archaea*. In contrast, the processing of eukaryotic primary transcripts is unique and has three distinct steps: splicing, capping, and adding a poly(A) tail.

Q How does the archaeal RNA polymerase differ from that in *Bacteria*? How does the initiation of transcription in the two domains differ? Why do eukaryotic mRNAs have to be "processed" whereas most prokaryotic RNAs do not?

IV • Protein Synthesis: Translation

4.7 Polypeptide chains contain many amino acids linked via peptide bonds. Twenty-two different amino acids are genetically encoded. The primary structure of a protein is its amino acid sequence, but the higher-order structure (folding) of the polypeptide determines its cellular function.

Q Describe the two types of secondary structure a polypeptide can attain. Which proteins can achieve quaternary structure? Which protein structure(s) are altered by denaturation?

4.8 One or more tRNAs exist for each amino acid incorporated into polypeptides by the ribosome. Enzymes called aminoacyl-tRNA synthetases attach amino acids to their cognate tRNAs.

Q Why are transfer RNAs important in translation? Do genes for tRNAs have promoters, and are tRNAs translated? What are aminoacyl-tRNA synthetases, what are their substrates, and what do they do?

4.9 The genetic code is expressed as RNA, and a single amino acid may be encoded by several different but related codons. In addition to the stop (nonsense) codons, there is also a specific start codon that signals the initiation of translation.

Q Why is the genetic code a degenerate code? What is wobble and how does it accommodate fidelity in the genetic code?

4.10 Translation occurs on the ribosome and requires mRNA and aminoacyl-tRNAs. The ribosome has three sites: acceptor, peptide, and exit (A, P, and E). During each step of translation, the ribosome advances one codon along the mRNA, and the tRNA in the acceptor site moves to the peptide site. Protein synthesis terminates when a stop codon, which does not have a corresponding tRNA, is reached.

Q Where on the ribosome do tRNAs bind, and what is the energy source that supports translocation?

V • Protein Processing, Secretion, and Targeting

4.11 Polypeptides do not remain linear in structure following translation but require proper folding and additional processing for functional activity. Proteins called

chaperones help fold some proteins that are unable to fold spontaneously and also assist in the incorporation of cofactors and refolding partially denatured proteins.

Q What is the primary function of chaperones? How do cells sometimes reuse partially denatured proteins?

4.12 Many proteins need to be transported into or through the cytoplasmic membrane. These proteins contain a signal sequence that is recognized by the cellular translocase systems of Sec and Tat.

Q What are the functions of the Sec and Tat systems and why are they necessary? How do these systems know which proteins to act upon?

4.13 A large diversity of secretion systems are employed by gram-negative bacteria to secrete proteins into or through their outer membrane and even into other cells, and types I–VI secretion systems function in this regard. Types II and V are two-step translocases and rely on the activity of Sec or Tat, whereas types I, III, IV, and VI are one-step translocases.

Q What are the major differences between one-step and two-step translocases? Which are used to secrete proteins into other cells?

Application Questions

1. The genome of the bacterium *Neisseria gonorrhoeae* consists of one double-stranded DNA molecule that contains 2220 kilobase pairs. If 85% of this DNA molecule is made up of the open reading frames of genes encoding proteins, and the average protein is 300 amino acids long, how many protein-encoding genes does *Neisseria* have? What kind of genetic information is present in the other 15% of the DNA?
2. Compare and contrast the activity of DNA and RNA polymerases. What is the function of each? What are the substrates of each? What is the main difference in the behavior of the two polymerases?
3. What would be the result (in terms of protein synthesis) if RNA polymerase initiated transcription one base upstream of its normal starting point? Why? By inspecting Table 4.4, discuss how the genetic code has evolved to help minimize the impact of mutations.

Chapter Glossary

Amino acid one of the 22 different monomers that make up proteins; chemically, a two-carbon carboxylic acid containing an amino group and a characteristic substituent on the alpha carbon

Aminoacyl-tRNA synthetase an enzyme that catalyzes attachment of an amino acid to its cognate tRNA

Anticodon a sequence of three bases in a tRNA molecule that base-pairs with a codon during protein synthesis

Antiparallel in reference to double-stranded DNA, the two strands run in opposite directions (one runs 5′→3′ and the complementary strand 3′→5′)

Bacteriocin a toxic protein secreted by bacteria that inhibits or kills other, related bacteria

Chaperone a protein that helps other proteins fold or refold from a partly denatured state

Chromosome a genetic element, usually circular in prokaryotes, carrying genes essential to cellular function

Codon a sequence of three bases in mRNA that encodes an amino acid

Codon bias nonrandom usage of multiple codons encoding the same amino acid

Complementary nucleic acid sequences that can base-pair with each other

Denaturation loss of the correct folding of a protein, leading (usually) to protein aggregation and loss of biological activity

DNA (deoxyribonucleic acid) a polymer of deoxyribonucleotides linked by phosphodiester bonds that carries genetic information

DNA gyrase an enzyme found in most prokaryotes that introduces negative supercoils in DNA

DNA helicase an enzyme that uses ATP to unwind the double helix of DNA

DNA ligase an enzyme that seals nicks in the backbone of DNA

DNA polymerase an enzyme that synthesizes a new strand of DNA in the 5′→3′ direction using an antiparallel DNA strand as a template

Exons the coding DNA sequences in a split gene (contrast with intron)

Gene a segment of DNA specifying a protein (via mRNA), a tRNA, an rRNA, or any other noncoding RNA

Genetic code the correspondence between nucleic acid sequence and amino acid sequence of proteins

Genetic element a structure that carries genetic information, such as a chromosome, a plasmid, or a virus genome

Genome the total complement of genes contained in a cell or virus

Informational macromolecule any large polymeric molecule that carries genetic information, including DNA, RNA, and protein

Introns the intervening noncoding DNA sequences in a split gene (contrast with exons)

Lagging strand the new strand of DNA that is synthesized in short pieces and then joined together later

Leading strand the new strand of DNA that is synthesized continuously during DNA replication

Messenger RNA (mRNA) an RNA molecule that contains the genetic information to encode one or more polypeptides

Nonsense codon another name for a stop codon

Nucleoside a nitrogenous base (adenine, guanine, cytosine, thymine, or uracil) plus a sugar (either ribose or deoxyribose) but lacking phosphate

Nucleotide a monomer of a nucleic acid containing a nitrogenous base (adenine, guanine, cytosine, thymine, or uracil), one or more molecules of phosphate, and a sugar, either ribose (in RNA) or deoxyribose (in DNA)

Open reading frame (ORF) a sequence of DNA or RNA that could be translated to give a polypeptide

Operon a cluster of genes that are cotranscribed as a single messenger RNA

Peptide bond a type of covalent bond linking amino acids in a polypeptide

Phosphodiester bond a type of covalent bond linking nucleotides together in a polynucleotide

Plasmid an extrachromosomal genetic element that is usually not essential to the cell

Polynucleotide a polymer of nucleotides bonded to one another by covalent bonds called phosphodiester bonds

Polypeptide a polymer of amino acids bonded to one another by peptide bonds

Primary structure the precise sequence of monomers in a macromolecule such as a polypeptide or a nucleic acid

Primary transcript an unprocessed RNA molecule that is the direct product of transcription

Primase the enzyme that synthesizes the RNA primer used in DNA replication

Primer an oligonucleotide to which DNA polymerase attaches the first deoxyribonucleotide during DNA synthesis

Promoter a site on DNA to which RNA polymerase binds to commence transcription

Protein a polypeptide or group of polypeptides forming a molecule of specific biological function

Purine one of the nitrogenous bases of nucleic acids that contain two fused rings; adenine and guanine

Pyrimidine one of the nitrogenous bases of nucleic acids that contain a single ring; cytosine, thymine, and uracil

Quaternary structure in proteins, the number and types of individual polypeptides in the final protein molecule

Replication synthesis of DNA using DNA as a template

Replication fork the site on the chromosome where DNA replication occurs and where the enzymes replicating the DNA are bound to untwisted, single-stranded DNA

Replisome a DNA replication complex that consists of two copies of DNA polymerase III, DNA gyrase, helicase, primase, and copies of single-strand binding protein

Ribosomal RNA (rRNA) types of RNA found in the ribosome; some participate actively in protein synthesis

Ribosome a cytoplasmic particle composed of ribosomal RNA and protein, whose function is to synthesize proteins

RNA (ribonucleic acid) a polymer of ribonucleotides linked by phosphodiester bonds that plays many roles in cells, in particular, during protein synthesis

RNA polymerase an enzyme that synthesizes RNA in the 5′→3′ direction using a complementary and antiparallel DNA strand as a template

RNA processing the conversion of a primary transcript RNA to its mature form

Secondary structure the initial pattern of folding of a polypeptide or a polynucleotide, usually dictated by opportunities for hydrogen bonding

Semiconservative replication DNA synthesis yielding two new double helices, each consisting of one parental and one progeny strand

Signal sequence a special N-terminal sequence of approximately 20 amino acids that signals that a protein should be incorporated into or exported across the cytoplasmic membrane

Spliceosome a complex of ribonucleoproteins that catalyze the removal of introns from primary RNA transcripts

Start codon a special codon, usually AUG, that signals the start of a protein

Stop codon a codon that signals the end of a protein

Termination stopping the elongation of an RNA molecule at a specific site

Tertiary structure the final folded structure of a polypeptide that has previously attained secondary structure

Transcription the synthesis of RNA using a DNA template

Transfer RNA (tRNA) a small RNA molecule used in translation that possesses an anticodon at one end and has the corresponding amino acid attached to the other end

Translation the synthesis of protein using the genetic information in RNA as a template

Transposable element a piece of DNA able to move (transpose) from one site to another on host DNA molecules

Wobble a less rigid form of base pairing allowed only in codon–anticodon pairing

Microbial Growth and Its Control

5

microbiologynow

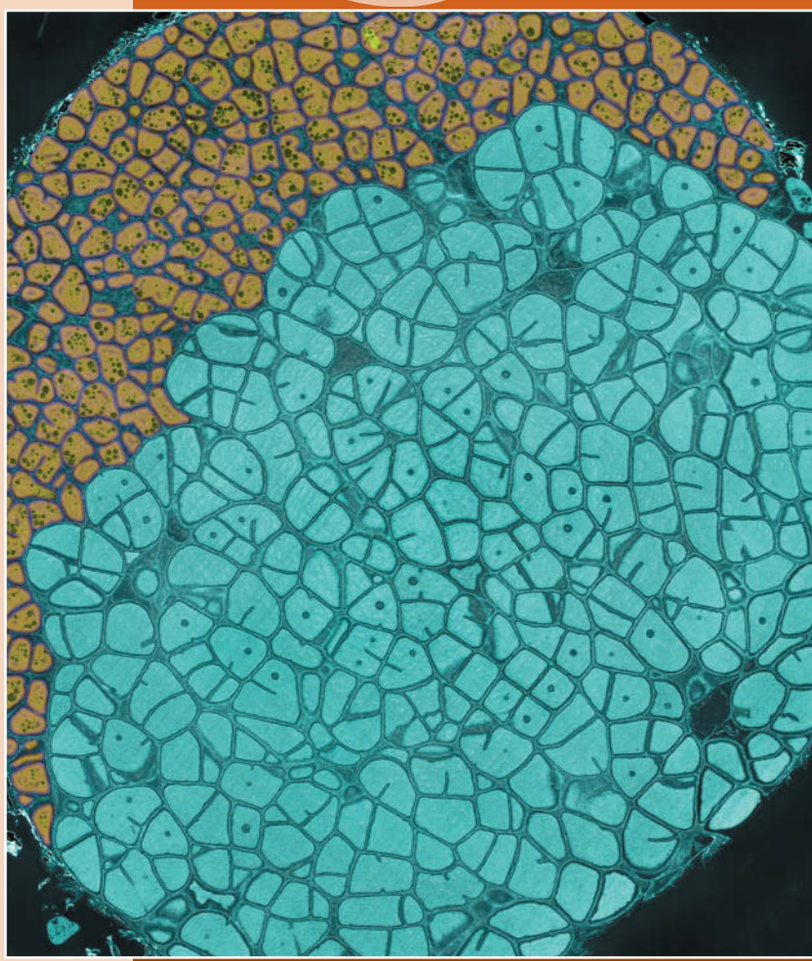
Picking Apart a Microbial Consortium

In nature, certain metabolic processes are carried out by microbes that team up to get the job done, a cozy arrangement called a consortium. Such is the case with the oxidation of methane (CH_4) linked to the reduction of sulfate (SO_4^{2-}) in anoxic marine sediments. The overall reaction ($CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$) is exergonic and the small amount of energy released is shared between two distinct microbes. The methane oxidizer in the consortium is a species of *Archaea* nicknamed ANME (for *an*aerobic *me*thanotroph, blue in photo), and its sulfate-reducing partner is a species of *Bacteria* (brown in photo). The consortium is thought to play a key role in the carbon cycle as a major methane sink, and thus a detailed picture of how it works is important to our understanding of the global carbon economy, climate change, and marine biogeochemistry.

Researchers have tried for years to separate the consortium into its components but always found that methane oxidation required both organisms. However, some researchers hypothesized that it might be possible to replace the sulfate reducer with an artificial electron acceptor and that this might unlock the consortium and allow the methanotroph to grow in pure culture. Using an electron acceptor called AQDS, the scientists discovered that they could turn off sulfate reduction in the consortium while maintaining CH_4 oxidation. During this process, the methanotroph used electrons from CH_4 to reduce AQDS rather than passing them on to its sulfate-reducing partner. Several other electron acceptors known to support anaerobic respiration also sustained methane oxidation, giving hope that ANME may eventually be obtained in pure culture.

The ability to grow a microbe in pure culture is the "gold standard" for the study of its physiology, biochemistry, regulation, and several other aspects of its biology. In the case of the ANME–sulfate reducer consortium, several physiologies were active at once, and resolving these many reactions proved to be a major scientific challenge. However, if further work shows that ANME can be removed from the consortium and grown in pure culture, detailed aspects of its biology can be studied that were not possible when the organism was tightly coupled to its partner in the consortium (photo).

Source: Scheller, S., H. Yu, G.L. Chadwick, S.E. McGlynn, and V.J. Orphan. 2016. Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction. *Science 351:* 703–706.



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