The oceans of the world are a teeming but invisible forest of microorganisms and viruses. As we discovered in Chapter 1, these microscopic agents are not only found around the world but, as Steven Giovannoni had pointed out for SAR11, being in such numbers implies they must be doing something important.

A substantial portion of the marine microbes represent the phytoplankton (phyto = “plant”; plankto = “wandering”), which are floating communities of prokaryotic cyanobacteria and eukaryotic algae. Besides forming the foundation for the marine food web, the phytoplankton account for 50 percent of the photosynthesis on earth and, in so doing, supply about half the oxygen gas we and other organisms breathe.

While sampling ocean water, scientists from MIT's Woods Hole Oceanographic Institution discovered that many of their samples were full of a marine cyanobacterium, which they eventually named Prochlorococcus. Inhabiting tropical and subtropical oceans, a typical sample often contained more than 200,000 (2 × 10^5) cells in one drop of seawater.

Studies with Prochlorococcus suggest the organism is responsible for more than 50 percent of the photosynthesis among the phytoplankton (FIGURE 3.1). This makes Prochlorococcus the smallest and most abundant marine photosynthetic organism yet discovered.
The success of *Prochlorococcus* is due, in part, to the presence of different ecotypes inhabiting different ocean depths. For example, the high sunlight ecotype occurs in the top 100 meters while the low-light type is found between 100 and 200 meters. This latter ecotype compensates for the decreased light by increasing the amount of cellular chlorophyll that can capture the available light.

In terms of nitrogen sources, the high-light ecotype only uses ammonium ions (NH$_4^+$) (see MicroFocus 2.5). At increasing depth, NH$_4^+$ is less abundant so the low-light ecotype compensates by using a wider variety of nitrogen sources.

These and other attributes of *Prochlorococcus* illustrate how microbes survive through change. They are of global importance to the functioning of the biosphere as well as affecting our lives on Earth.

Once again, we encounter an interdisciplinary group of scientists studying how microorganisms influence our lives and life on this planet. Microbial ecologists study how the phytoplankton communities help in the natural recycling and use of chemical elements such as nitrogen. Evolutionary microbiologists look at these microorganisms to learn more about their taxonomic relationships, while microscopists, biochemists, and geneticists study how *Prochlorococcus* cells compensate for a changing environment of sunlight and nutrients.

This chapter focuses on many of the aspects described above. We examine how prokaryotes maintain a stable internal state and how they can exist in multicellular, complex communities. Throughout the chapter we are concerned with the relationships between prokaryotic and eukaryotic organisms and the many attributes they share. Then, we explore the methods used to name and catalog microorganisms. Finally, we discuss the tools and techniques used to observe microorganisms.

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**Ecotype:**
A subgroup of species having special characteristics to survive in its ecological surroundings.

**Biosphere:**
That part of the earth—including the air, soil, and water—where life occurs.

**FIGURE 3.1 Photosynthesis in the World’s Oceans.** This global satellite image (false color) shows the distribution of photosynthetic organisms on the planet. In the aquatic environments, red colors indicate high levels of chlorophyll and productivity, yellow and green are moderate levels, and blue and purple areas are the “marine deserts.”

*Q: How do the landmasses where photosynthesis is most productive (green) compare in size to photosynthesis in the oceans?*
In Chapter 1 we introduced the concept of prokaryotes and eukaryotes. In the news media or even in scientific magazines and textbooks, bacterial and archaeal cells—the prokaryotes—often are described as “simple organisms” compared to the “complex organisms” representing multicellular plants and animals. This view represents a mistaken perception. Despite their microscopic size, the prokaryotes exhibit every complex feature, or emerging property, common to all living organisms. These include:

- DNA as the hereditary material and complex gene control.
- Complex biochemical patterns of growth and energy conversions.
- Complex responses to stimuli.
- Reproduction.
- Adaptation.
- Complex organization.

Why then have the prokaryotes inherited the misnomer of “simple organisms?” To answer that question, we need to examine the similarities between prokaryotes and eukaryotes. They share many universal processes even though in some cases the structures to carry out these processes may be different. Having done this, we can then better distinguish the significant differences that set prokaryotes apart. In the next chapter, we look more closely at the differences between Bacteria and Archaea.

**Prokaryote/Eukaryote Similarities**

**KEY CONCEPT**

- Prokaryotes undergo biological processes as complex as in eukaryotes.

All organisms continually battle their external environment, where factors such as temperature, sunlight, or toxic chemicals can have serious consequences. Organisms strive to maintain a stable internal state by making appropriate metabolic or structural adjustments. This ability to adjust yet maintain a relatively steady internal state is called homeostasis (*homeo* = “similar”; *stasis* = “state”). Two examples illustrate the concept.

The low-light *Prochlorococcus* ecotype mentioned in the chapter introduction lives at depths of 80 to 200 meters. At these varying depths, transmitted sunlight decreases and any one nitrogen source is less accessible. The ecotype compensates for the light reduction and nitrogen limitation by (1) increasing the amount of cellular chlorophyll to capture light and (2) using a wider variety of available nitrogen sources. These adjustments maintain a relatively stable internal state.

Suppose a patient is given an antibiotic to combat a bacterial infection (FIGURE 3.2). In response, the infecting bacterium may compensate for the change by pumping the drug out of the cell. The adjustment maintains homeostasis in the bacterial cell.

In both these examples, the internal environment is maintained despite a changing environment. Such, often complex, homeostatic controls are as critical to prokaryotes as they are to eukaryotes (MicroFocus 3.1).

**FIGURE 3.2** Homeostasis. A concept map illustrating the ability of a bacterial pathogen to compensate for the presence of an antibiotic. Survival is dependent on its homeostatic abilities.

Q: Redraw the concept map for the low-light response by *Prochlorococcus*. 
Another misunderstanding about prokaryotes is that they represent “unicellular” organisms. And why not? When they are studied, often they appear in the microscope as single cells. The early studies of disease causation done by Koch (see Chapter 1) certainly required pure cultures to associate a specific disease with one specific microbe. However, today it is necessary to abolish the impression that bacteria are self-contained, independent organisms. In nature few species live such a pure and solitary life. In fact, it has been estimated that as many as 99 percent of prokaryotic species live in communal associations called biofilms; that is, in a “multicellular state” where survival requires chemical communication and cooperation between cells.

One example of prokaryotic multicellularity within a species involves a group of soil-dwelling bacteria called the myxobacteria, which exhibit the most complex behavior among the known prokaryotes. The cells represent a social community dependent on cell-to-cell interactions and communication throughout growth and development.

Myxobacterial cells can obtain nutrients by decomposing dead plant or animal matter. More often, when live prey (e.g., bacterial cells, yeasts, or algae) are “sensed” nearby, cell communication within the flat colonies triggers (1) a swarming or so-called “wolf pack” behavior characterized by predatory feeding and (2) an increasing secretion of hydrolytic enzymes by the wolf pack to digest the prey. Like a lone wolf, a single cell could not carry out this behavior.

At the other extreme, under starvation conditions and high cell densities, myxobacterial cells chemically communicate to form a mound of 100,000 cells and cooperate to build a three-dimensional structure called a fruiting body (Figure 3.3). Within this structure, a cell-secreted slime forms the stalk on top of which the remaining viable cells develop into resting structures called myxospores.

Here then is one example where the term unicellular does not apply. MicroFocus 3.2 further examines the concept of prokaryotic multicellularity.

The final misconception to be addressed concerns the reference to prokaryotes as “simple cells” or “simpler organisms” than their eukaryotic counterparts. Historically, when one looks at bacterial cells even with an electron microscope, often there is little to see (Figure 3.4). “Cell structure,” representing the cell’s physical appearance or its components and the “pattern of organization,” referring to the configuration of those structures and their relationships to one another, does give the impression of simpler cells.

But what has been overlooked is the “cellular process,” the activities all cells carry out for the continued survival of the cell (and organism). At this level, the complexity is just as intricate as in any eukaryotic cell. So, in reality, prokaryotes carry out many of the same cellular processes as eukaryotes—only

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**Figure 3.3** A Myxobacterial Fruiting Body. The stalk (lower left) is made of slime secreted by the cells that eventually form the resting spores in the head. (Bar = 10 μm.)

Q: How does the myxobacterial fruiting body represent a multicellular structure?
MICROFOCUS 3.1: Being Skeptical
Are Cyanobacterial Food Supplements Better for What’s Ailing You?

The food supplement business is always looking for new products to boost human health. Take the phytoplankton. The chapter introduction highlighted the importance of these microbial communities to the marine food web. Some individuals therefore have perceived this as indicating these microorganisms must be very nutritious.

Among the items found on today's health food shelves and the Internet are capsules, pills, and powders consisting of dried cyanobacteria, primarily from the fresh-water species *Aphanizomenon flos-aquae* (AFA) and the genus *Spirulina* (see figure). These products are promoted as a treatment or cure for a wide variety of human ailments, including asthma, allergies, chronic viral hepatitis, anxiety, depression, fatigue, hypoglycemia, digestive problems, and attention deficit disorder. The microbial products purported to help individuals lose weight, improve memory and mental ability, boost immune system function, and restore overall cellular balance.

Are these claims reasonable? What potentially unique nutrients must these cyanobacterial supplements have to provide relief from all these ailments? The answer—none that we know. The organisms contain large amounts of chlorophyll, but humans cannot carry out photosynthesis so why eat chlorophyll? Cyanobacteria do contain small amounts of protein, beta carotene, and a few vitamins and minerals. However, you would have to eat shovels full of the cyanobacterial powders to get a sufficient level of these nutrients—there are more sensible and cheaper ways to get these same nutrients in easily consumable amounts.

Importantly, having come from natural lakes, the products may be contaminated with toxic substances, such as heavy metals that polluted the lake water. The verdict? Yes, microbes do have many useful and important roles in the human body. However, medical organizations agree there is not enough evidence to support their use for treatment of any medical condition. Whenever a product proclaims such a wide-range of curative powers, be skeptical. As the old adage says, “If it is too good to be true,” it probably is.
MICROFOCUS 3.2: Environmental Microbiology

Multicellular Prokaryotes

As microbiologists continue to study prokaryotic organisms, it is becoming quite obvious that these microbes are social creatures. Whether it is studying myxobacteria, mixed bacterial populations in a biofilm, or other communities of microorganisms, there is more to bacterial life than just existing in a unicellular community.

Quorum Sensing

Several bacterial behaviors are dependent on population density. When population numbers reach a critical level, chemical signals are produced that alter gene activity. Such quorum sensing (QS) can control spore formation as described for the myxobacteria or enhance the ability of a pathogen to cause disease.

Chemical communication can occur between different bacterial species. This QS cross talk allows the cells of one species to detect the presence of other species, which could be a good thing or a bad thing. In biofilms, QS is necessary for the development of the complex structures within the biofilm. However, *Pseudomonas aeruginosa*, a pathogen of the human gut, can sense an immune system response to infection. Through QS, *P. aeruginosa* counters immune activation by strengthening or enhancing its ability to cause disease by disrupting the function of epithelial cells in the gut. These chemical signals would not be produced by solitary, non-communicative cells.

Programmed Cell Death

The multicellular nature of microbial communities also demonstrates cell suicide; that is, genetically programmed cell death (PCD). Such a phenomenon (called apoptosis) was discovered in eukaryotes many years ago, but a death program in bacterial cells is a newly discovered phenomenon that is based on cellular behavioral responses.

One example of PCD is the myxobacteria, where up to 80 percent of the cells die in the process of fruiting body formation, the remaining 20 percent becoming the myxospores. Also, during biofilm development, overcrowding can be prevented by cells dying within the biofilm, which hollows out the structure and makes room for new cell reproduction. “Sensed” by the multicellular aggregate, specific toxic proteins trigger this form of PCD.

Perhaps most interesting (and controversial) is research looking at the death of defective or injured cells resulting from antibiotics. In the face of a chemical attack, cells damaged by the antibiotic undergo PCD so they do not become a burden to the remaining cells. Yet other damaged cells in the community apparently turn off the PCD program and become persistor cells—they survive by growing so slowly, they are not susceptible to the action of antibiotics.

Interestingly, many of the multicellular behaviors observed only occur in wild strains in nature. Evidently, the “good life” in a culture plate has made such cell-cell behaviors unnecessary—the cells have become unicellular “couch bacteria.”

without the need for an elaborate, visible pattern of organization.

Let’s now look at the cell structure relationships in terms of organized patterns and processes.

CONCEPT AND REASONING CHECKS

3.2 Respond to the statement that prokaryotes are simple, unicellular organisms.

Prokaryotes and Eukaryotes: The Similarities in Organizational Patterns

KEY CONCEPT

- There are organizational patterns common to all prokaryotes and eukaryotes.

In the 1830s, Matthias Schleiden and Theodor Schwann developed part of the cell theory by demonstrating all organisms are composed of one or more cells, making the cell the fundamental unit of life. (Note: about 20 years later, Rudolph Virchow added that all cells arise from pre-existing cells.) Although the concept of a microorganism was just in its infancy at the time, the doctrine suggests that there are certain organizational patterns common to prokaryotes and eukaryotes.

Genetic organization. All organisms have a similar genetic organization whereby the hereditary material is communicated or
The organizational pattern for the hereditary material is in the form of one or more chromosomes. Structurally, most prokaryotic cells have a single, circular DNA molecule without an enclosing membrane. Eukaryotic cells, however, have multiple, linear chromosomes enclosed by the membrane envelope of the cell nucleus.

**Compartmentation.** All prokaryotes and eukaryotes have an organizational pattern separating the internal compartments from the surrounding environment but allowing for the exchange of solutes and wastes. The pattern for compartmentation is represented by the cell. All cells are surrounded by a cell membrane (known as the plasma membrane in eukaryotes), where the phospholipids form the impermeable boundary to solutes while proteins regulate the exchange of solutes and wastes across the membrane. We have more to say about membranes in the next chapter.

**Metabolic organization.** The process of metabolism is a consequence of compartmentation. By being enclosed by a membrane, all cells have an internal environment in which chemical reactions occur. This space, called the cytoplasm, represents everything surrounded by the membrane and, in eukaryotic cells, exterior to the cell nucleus. If the cell structures are removed from the cytoplasm, what remains is the cytosol, which consists of water, salts, ions, and organic compounds as described in Chapter 2.

**Protein synthesis.** All organisms must make proteins, which we learned in Chapter 2 are the workhorses of cells and organisms. The structure common to all prokaryotes and eukaryotes is the ribosome, an RNA-protein machine that cranks out proteins based on the
genetic instructions it receives from the DNA (Chapter 7). Although the pattern for protein synthesis is identical, structurally prokaryotic ribosomes are smaller than their counterparts in eukaryotic cells.

### CONCEPT AND REASONING CHECKS

3.3 The cell theory states that the cell is the fundamental unit of life. Summarize those processes all cells have that contribute to this fundamental unit.

#### Prokaryotes and Eukaryotes: The Structural Distinctions

<table>
<thead>
<tr>
<th>KEY CONCEPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Prokaryotes and eukaryotes have distinct subcellular compartments.</td>
</tr>
</tbody>
</table>

Eukaryotic microbes have a variety of structurally discrete, often membrane-enclosed, subcellular compartments called **organelles** (Figure 3.5). Prokaryotic cells also have subcellular compartments—they just are not readily visible or membrane enclosed.

**Protein/lipid transport.** Eukaryotic microbes have a series of membrane-enclosed organelles in the cytosol that compose the cell's **endomembrane system**, which is designed to transport protein and lipid cargo through and out of the cell. This system includes the **endoplasmic reticulum** (ER), which consists of flat membranes to which ribosomes are attached (rough ER) and tube-like membranes without ribosomes (smooth ER). These portions of the ER are involved in protein and lipid synthesis and transport, respectively.

The **Golgi apparatus** is a group of independent stacks of flattened membranes and vesicles where the proteins and lipids coming from the ER are processed, sorted, and packaged for transport. **Lysosomes**, somewhat circular, membrane-enclosed sacs containing digestive (hydrolytic) enzymes, are derived from the Golgi apparatus and are vital to the killing of many pathogens by white blood cells.

Prokaryotes lack an endomembrane system, yet they are capable of manufacturing and modifying proteins and lipids just as their eukaryotic relatives do. However, many bacterial cells contain so-called **microcompartments** surrounded by a protein shell. These microcompartments represent a type of organelle since the shell proteins can control transport similar to membrane-enclosed organelles.

**Energy metabolism.** Cells and organisms carry out one or two types of energy transformations. Through a process called **cellular respiration**, all cells convert chemical energy into cellular energy for cellular work. In eukaryotic microbes, this occurs in the cytosol and in membrane-enclosed organelles called **mitochondria** (sing., mitochondrion). Bacterial and archaeal cells lack mitochondria; they use the cytosol and cell membrane to complete the energy converting process.

A second energy transformation, **photosynthesis**, involves the conversion of light energy into chemical energy. In algal protists, photosynthesis occurs in membrane-bound **chloroplasts**. Some prokaryotes, such as the cyanobacteria we have mentioned, also carry out almost identical energy transformations. Again, the cell membrane or elaborations of the membrane represent the chemical workbench for the process.

**Cell structure and transport.** The eukaryotic **cytoskeleton** is organized into an interconnected system of fibers, threads, and interwoven molecules that give structure to the cell and assist in the transport of materials throughout the cell. The main components of the cytoskeleton are microtubules and actin filaments, each assembled from different protein subunits. Prokaryotes to date have no similar physical cytoskeleton, although proteins related to those that construct microtubules and actin filaments aid in determining the shape in some bacterial cells as we will see in Chapter 4.

**Cell motility.** Many microbial organisms live in watery or damp environments and use the process of cell motility to move from one place to another. Many algae and protozoa have long, thin protein projections called **flagella** (sing., flagellum) that, covered by the plasma membrane, extend from the cell. By beating back and forth, the flagella provide a mechanical force for motility. Many prokaryotic cells also exhibit motility; however, the flagella are structurally different and without a cell membrane covering. The pattern of motility also is different, providing a rotational propeller-like force for motility (Chapter 4).
Some protozoa also have other membrane-enveloped appendages called cilia (sing., cilium) that are shorter and more numerous than flagella. In some motile protozoa, they wave in synchrony and propel the cell forward. No prokaryotes have cilia.

**Water balance.** The aqueous environment in which many microorganisms live presents a situation where the process of diffusion occurs, specifically the movement of water, called osmosis, into the cell. Continuing unabated, the cell would eventually swell and burst (cell lysis) because the cell or plasma membrane does not provide the integrity to prevent lysis.

Many prokaryotic and eukaryotic cells contain a cell wall exterior to the cell or plasma membrane. Although the structure and organization of the wall differs between groups, all cell walls provide support for the cells, give them shape, and help them resist the pressure exerted by the internal water pressure.

A summary of the prokaryote and eukaryote processes and structures is presented in Table 3.1. MicroInquiry 3 examines a scenario for the evolution of the eukaryotic cell.

### Table 3.1 A Comparison of Prokaryotes and Eukaryotes

<table>
<thead>
<tr>
<th>Process</th>
<th>Prokaryotic</th>
<th>Eukaryotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic organization</td>
<td>Circular DNA chromosome</td>
<td>Linear DNA chromosomes</td>
</tr>
<tr>
<td>Compartmentation</td>
<td>Cell membrane</td>
<td>Plasma membrane</td>
</tr>
<tr>
<td>Metabolic organization</td>
<td>Cytoplasm</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>Ribosomes</td>
<td>Ribosomes</td>
</tr>
<tr>
<td>Protein/lipid transport</td>
<td>Cytoplasm</td>
<td>Endomembrane system</td>
</tr>
<tr>
<td>Energy metabolism</td>
<td>Cytoplasm and cell membrane</td>
<td>Mitochondria and chloroplasts</td>
</tr>
<tr>
<td>Cell structure and transport</td>
<td>Proteins in cytoplasm</td>
<td>Protein filaments in cytoplasm</td>
</tr>
<tr>
<td>Cell motility</td>
<td>Prokaryotic flagella</td>
<td>Eukaryotic flagella or cilia</td>
</tr>
<tr>
<td>Water regulation</td>
<td>Cell wall</td>
<td>Cell wall</td>
</tr>
</tbody>
</table>

If you open any catalog, items are separated by types, styles, or functions. For example, in a fashion catalog, watches are separated from shoes and, within the shoes, men’s, women’s, and children’s styles are separated from one another. Even the brands of shoes or their use (e.g., dress, casual, athletic) may be separated.

The human drive to catalog objects extends to the sciences, especially biology where historically the process was not much different from cataloging watches and shoes; it was based on shared characteristics. In this section, we shall explore the principles on which microorganisms are cataloged.

**Classification Attempts to Catalog Organisms**

- **Key Concept**
  - Organisms historically were grouped by shared characteristics.

The science of classification, called taxonomy, involves the systematized arrangement or cataloging of related organisms into logical categories. Taxonomy is essential for understanding the relationships among living organisms, discovering unifying concepts among organisms, and providing a universal "language" for communication among biologists.
Biologists and geologists have speculated for decades about the chemical evolution that led to the origins of the first prokaryotic cells on Earth (see Microfocus 2.1 and 2.6). Whatever the origin, the first ancestral prokaryotes arose about 3.8 billion years ago.

Scientists also have proposed various scenarios to account for the origins of the first eukaryotic cells. A key concern here is figuring out how different membrane compartments arose to evolve into what are found in the eukaryotic cells today. Debate on this long intractable problem continues, so here we present some of the ideas that have fueled such discussions.

At some point around 2 billion years ago, the increasing number of metabolic reactions occurring in some prokaryotes started to interfere with one another. Complexity would necessitate more extensive compartmentation.

The Endomembrane System May Have Evolved through Invagination

Similar to today’s prokaryotic cells, the cell membrane of an ancestral prokaryote may have had specialized regions involved in protein synthesis, lipid synthesis, and nutrient hydrolysis. If the invagination of these regions occurred, the result could have been the internalization of these processes as independent internal membrane systems. For example, the membranes of the endoplasmic reticulum may have originated by multiple invagination events of the plasma membrane (see Figure A1).

Biologists have suggested that the elaboration of the evolving ER created the nuclear envelope. Surrounded and protected by a double membrane, greater genetic complexity could occur as the primitive eukaryotic cell continued to evolve in size and function.

Chloroplasts and Mitochondria Arose from a Symbiotic Union of Engulfed Prokaryotes

Mitochondria and chloroplasts are not part of the extensive endomembrane system. Therefore, these energy-converting organelles probably originated in a different way.

The structure of modern-day chloroplasts and mitochondria is very similar to the description of a bacterial cell. In fact, mitochondria, chloroplasts, and bacteria share a large number of similarities (see Table). In addition, there are bacterial cells alive today that carry out cellular respiration similarly to mitochondria and other bacterial cells that can carry out photosynthesis similarly to chloroplasts.

These similar functional patterns, along with other chemical and molecular similarities, suggested to Lynn Margulis that present-day chloroplasts and mitochondria represent modern representatives of what were once, many eons ago, free-living prokaryotes. Margulis, therefore, proposed the endosymbiotic theory for the origin of mitochondria and chloroplasts. The hypothesis suggests, in part, that mitochondria evolved from a prokaryote that carried out cellular respiration and which was “swallowed” (engulfed) by a primitive eukaryotic cell. The bacterial partner then lived within (endo) the eukaryotic cell in a mutually beneficial association (symbiosis) (see Figure A2).

Likewise, a photosynthetic prokaryote, perhaps a primitive cyanobacterium, was engulfed and evolved into the chloroplasts present in plants and algae today (see Figure A3). The theory also would explain why both organelles have two membranes. One was the cell membrane of the engulfed bacterial cell and the other was the plasma membrane resulting from the engulfment process. By engulfing these prokaryotes and not destroying them, the evolving eukaryotic cell gained energy-conversion abilities, while the symbiotic bacterial cells gained a protected home.

Obviously, laboratory studies can only guess at mechanisms to explain how cells evolved and can only suggest—not prove—what might have happened billions of years ago. The description here is a very simplistic view of how the first eukaryotic cells might have evolved. Short of inventing a time machine, we may never know the exact details for the origin of eukaryotic cells and organelles.

Discussion Point

Determine which endosymbiotic event must have come first: the engulfment of the bacterial progenitor of the chloroplast or the engulfment of the bacterial progenitor of the mitochondrion.
Possible Origins of Eukaryotic Cell Compartments. (A1) Invagination of the cell membrane from an ancient prokaryotic cell may have lead to the development of the cell nucleus as well as to the membranes of the endomembrane system, including the endoplasmic reticulum. (A2) The mitochondrion may have resulted from the uptake and survival of a bacterial cell that carried out cellular respiration. (A3) A similar process, involving a bacterial cell that carried out photosynthesis, could have accounted for the origin of the chloroplast.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mitochondria</th>
<th>Chloroplasts</th>
<th>Prokaryotes</th>
<th>Microbial Eukaryotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average size</td>
<td>1–5 μm</td>
<td>1–5 μm</td>
<td>1–5 μm</td>
<td>10–20 μm</td>
</tr>
<tr>
<td>Nuclear envelope present</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>DNA molecule shape</td>
<td>Circular</td>
<td>Circular</td>
<td>Circular</td>
<td>Linear</td>
</tr>
<tr>
<td>Ribosomes</td>
<td>Yes; bacterial-like</td>
<td>Yes; bacterial-like</td>
<td>Yes</td>
<td>Yes; eukaryotic-like</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>Make some of their proteins</td>
<td>Make some of their proteins</td>
<td>Make all of their proteins</td>
<td>Make all of their proteins</td>
</tr>
<tr>
<td>Reproduction</td>
<td>Binary fission</td>
<td>Binary fission</td>
<td>Binary fission</td>
<td>Mitosis and cytokinesis</td>
</tr>
</tbody>
</table>
The Swedish botanist Carl von Linné, better known to history as Carolus Linnaeus, laid down the foundation of modern taxonomy. Until his time, naturalists referred to organisms using long cumbersome phrases or descriptive terms, which often varied by country. In his Systema Naturae, published in several editions between 1735 and 1759, Linnaeus established a uniform system for naming organisms based on shared characteristics. As a result, he named about 7,700 species of plants and 4,400 species of animals. In reflecting the scant knowledge of microorganisms (animal-cules) at the time and his general disinterest, Linnaeus grouped them separately under the heading of Vermes (vermis = "worm") in the category Chaos (chaos = "an abyss").

There are two major elements to Linnaeus' classification system: the use of binomial nomenclature and a hierarchical organization of species.

Nomenclature Gives Scientific Names to Organisms

KEY CONCEPT

- The binomial system identifies each organism by a universally-accepted scientific name.

In his Systema Naturae, Linnaeus popularized a two word (binomial) scheme of nomenclature, the two words usually derived from Latin or Greek stems. Each organism's name consists of the genus to which the organism belongs and a specific epithet, a descriptor that further describes the genus name. Together these two words make up the species name. For example, the common bacterium Escherichia coli resides in the gut of all humans (Homo sapiens) (MicroFocus 3.3).

Notice in these examples that when a species name is written, only the first letter of the genus name is capitalized, while the specific epithet is not. In addition, both words are printed in italics or underlined. After the first

MICROFOCUS 3.3: Tools

Naming Names

As you read this book, you have and will come across many scientific names for microbes, where a species name is a combination of the genus and specific epithet. Not only are many of these names tongue twisting to pronounce (they are all listed with their pronunciation inside the front and back covers), but how in the world did the organisms get those names? Here are a few examples.

Genera Named after Individuals

Escherichia coli: named after Theodore Escherich who isolated the bacterial cells from infant feces in 1885. Being in feces, it commonly is found in the colon.

Neisseria gonorrhoeae: named after Albert Neisser who discovered the bacterial organism in 1879. As the specific epithet points out, the disease it causes is gonorrhea.

Genera Named for a Microbe's Shape

Vibrio cholerae: vibrio means “comma-shaped,” which describes the shape of the bacterial cells that cause cholera.

Staphylococcus epidermidis: staphylo means “cluster” and coccus means “spheres.” So, these bacterial cells form clusters of spheres that are found on the skin surface (epidermis).

Genera Named after an Attribute of the Microbe

Saccharomyces cerevisiae: in 1837, Theodor Schwann observed yeast cells and called them Saccharomyces (saccharo = “sugar,” myce = “fungus”) because the yeast converted grape juice (sugar) into alcohol; cerevisiae (from cervisia = “beer”) refers to the use of yeast since ancient times to make beer.

Myxococcus xanthus: myxo means “slime,” so these are slime-producing spheres that grow as yellow (xantho = “yellow”) colonies on agar.

Thiomargarita namibiensis: see MicroFocus 3.5.
time a species name has been spelled out, biologists usually abbreviate the genus name using only its initial genus letter or some accepted substitution, together with the full specific epithet; that is, *E. coli* or *H. sapiens*. A cautionary note: often in magazines and newspapers, proper nomenclature is not followed, so our gut bacterium would be written as *Escherichia coli*.

### Concept and Reasoning Checks

3.5 Which one of the following is a correctly written scientific name for the bacterium that causes anthrax? (a) *bacillus Anthracis*; (b) *Bacillus Anthracis*; or (c) *Bacillus anthracis*.

### Classification Uses a Hierarchical System

**Key Concept**

- Species can be organized into higher, more inclusive groups.

Linnaeus’ cataloging of plants and animals used shared and common characteristics. Such similar organisms that could interbreed were related as a species, which formed the least inclusive level of the hierarchical system. Part of Linnaeus’ innovation was the grouping of species into higher taxa that also were based on shared, but more inclusive, similarities.

Today several similar species are grouped together into a genus (pl. genera). A collection of similar genera makes up a family and families with similar characteristics make up an order. Different orders may be placed together in a class and classes are assembled together into a phylum (pl. phyla) or division (in bacteriology and botany). All phyla or divisions would be placed together in a kingdom and/or domain, the most inclusive level of classification. [Table 3.2](#)

<table>
<thead>
<tr>
<th>Taxa (sing. Taxon):</th>
<th>Subdivisions used to classify organisms.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biotype:</strong></td>
<td>A population or group of individuals having the same genetic constitution (genotype).</td>
</tr>
</tbody>
</table>

### Concept and Reasoning Checks

3.6 How would you describe an order in the taxonomic classification?

### Kingdoms and Domains: Trying to Make Sense of Taxonomic Relationships

**Key Concept**

- Historically, most organisms were assigned to one of several kingdoms.

As more microorganisms were described after Linnaeus’ time, some were considered plants (bacterial, algal, and fungal organisms) while the protozoa were categorized as animals.

In 1866, the German naturalist Ernst H. Haeckel disturbed the tidiness of Linnaeus’ plant and animal kingdoms. How could mushrooms be plants when they do not carry out photosynthesis? Haeckel therefore coined the term “protist” for a microorganism, and

| TABLE 3.2 Taxonomic Classification of Humans, Brewer’s Yeast, and a Common Bacterium |
|-------------------------------------|-------------------------------------|-------------------------------------|
| **Domain**                          | **Eukarya**                        | **Eukarya**                        |
| **Kingdom**                         | **Animalia**                      | **Fungi**                          |
| **Phylum (Division)**               | **Chordata**                      | **Ascomycota**                     |
| **Class**                           | **Mammalia**                      | **Saccharomycotina**               |
| **Order**                           | **Primates**                      | **Saccharomycetales**              |
| **Family**                          | **Hominidae**                     | **Saccharomycetaceae**             |
| **Genus**                           | **Homo**                          | **Saccharomyces**                  |
| **Species**                         | **H. sapiens**                    | **S. cerevisiae**                 |
| **Escherichia coli**                |                                     | **E. coli**                        |
he placed all bacterial, protozoal, algal, and fungal species in this third kingdom. Although not everyone liked his system, it did provoke discussions about the nature of microorganisms.

During the twentieth century, advances in cell biology and interest in evolutionary biology led scientists to question the two- or three-kingdom classification schemes. In 1969, Robert H. Whittaker of Cornell University proposed a system that quickly gained wide acceptance in the scientific community. Further expanded in succeeding years by Lynn Margulis of the University of Massachusetts, the five kingdom system of organisms was established (FIGURE 3.6).

In the five-kingdom system, bacterial organisms were so different from other organisms they must be in their own kingdom. Protista were limited to the protozoa and the unicellular algae. Fungi included non-green, non-photosynthetic eukaryotic organisms that, along with other characteristics, had cell walls that were chemically different from those in bacterial, algal, and plant cells.

### Concept and Reasoning Checks

3.7 Could an organism be assigned to a kingdom based on one characteristic? Two characteristics? How many characteristics?

#### The Three-Domain System Places the Prokaryotes in Separate Lineages

**Key Concept**

- The three domain system uses nucleotide sequence analysis to catalog organisms.

Often it is difficult to make sense of taxonomic relationships because new information that is more detailed keeps being discovered about organisms. This then motivates taxonomists to figure out how the new information fits into the known classification schemes—or how the schemes need to be modified to fit the new information. This is no clearer than the most recent taxonomic revolution that, as the opening quote states, “It’s as if [he] lifted a whole submerged continent out of the ocean.”

“He” is Carl Woese, who along with his coworkers at the University of Illinois proposed a new classification scheme with a new most inclusive taxon, the domain. The new scheme primarily came from work that compared the nucleotide base sequences of the RNA in ribosomes, those protein manufacturing machines needed by all cells. Woese’s results in the late 1970s were especially relevant when comparing those sequences from a group of bacterial organisms formerly called the archaebacteria (archae = “ancient”). Many of these bacterial forms are known for their ability to live under extremely harsh environments. Woese discovered that the nucleotide sequences in these archaebacteria were different from those in other bacterial species and in eukaryotes. After finding other differences, including cell wall composition, membrane lipids, and sensitivity to certain antibiotics, the evidence pointed to there being three taxonomic lines to the tree of life.

In Woese’s three domain system, one domain includes the former archaebacteria and is called the domain Archaea (FIGURE 3.7). The second encompasses all the remaining true bacteria and is called the domain Bacteria. The third domain, the Eukarya, includes the
four remaining kingdoms (i.e., Protista, Plantae, Fungi, and Animalia).

Scientists initially were reluctant to accept the three-domain system of classification, and many deemed it a threat to the tenet that all living things are either prokaryotes or eukaryotes. Then, in 1996, Craig Venter and his coworkers deciphered the DNA base sequence of the archaean *Methanococcus jannaschii* and showed that almost two thirds of its genes are different from those of the *Bacteria*. They also found that proteins replicating the DNA and involved in RNA synthesis have no counterpart in the *Bacteria*. It appears that the three-domain system now is on firm ground.

**Concept and Reasoning Checks**

3.8 What is the “whole submerged continent” that Woese lifted out of the ocean and why is the term “lifted” used?

**Distinguishing between Prokaryotes**

**Key Concept**

- Taxonomy now includes many criteria to distinguish one prokaryote from another.

We shall consider the taxonomy of most groups of microorganisms in their respective chapters. Prokaryotic taxonomy (referring to the *Bacteria* and *Archaea*), however, merits special attention because these organisms occupy an important position in microbiology and have had a complex system of taxonomy. David Hendricks Bergey devised one of the first systems of classification for the prokaryotes in 1923. His book, *Bergey’s Manual of Determinative Bacteriology*, was updated and greatly expanded in the decades that followed. Now in its 9th edition, *Bergey’s Manual* is available for use as a reference guide to identifying unknown bacteria that previously have been isolated in pure culture.

The five-volume *Bergey’s Manual of Systematic Bacteriology* is intended as a classification guide and the official listing for all recognized *Bacteria* and *Archaea*. Since the first edition in 1984, bacterial taxonomy has gone through tremendous changes. For example, in Volume 1 of the second edition (2001), more than 2,200 new species and 390 new genera have been added.

There are several traditional and more modern criteria that microbiologists can use to identify (and classify) prokaryotes. Let’s briefly review these methods.

**Physical characteristics.** These include staining reactions to help determine the organism’s shape (morphology), and the size and
arrangement of cells. Other characteristics can include oxygen, pH, and growth temperature requirements. Spore-forming ability and motility are additional determinants. Unfortunately, there are many prokaryotes that have the same physical characteristics, so other distinguishing features are needed.

**Biochemical tests.** As microbiologists better understood bacterial physiology, they discovered there were certain metabolic properties that were present only in certain groups. Today, a large number of biochemical tests exist and often a specific test can be used to eliminate certain groups of prokaryotes from the identification process. Among the more common tests are: fermentation of carbohydrates, the utilization of a specific substrate, and the production of specific products or waste products. But, as with the physical characteristics, often several biochemical tests are needed to differentiate between species.

These identification tests are important clinically, as they can be part of the arsenal available to the clinical lab that is trying to identify a pathogen. Many of these tests can be rapidly identified using modern procedures and automated systems. Further, various forms of dichotomous keys, but one very useful construction is a flow chart where a series of positive or negative test procedures are listed down the page. Based on the dichotomous nature of the test (always a positive or negative result), the flow chart immediately leads to the next test result. The result is the identification of a specific organism. A simplified example is shown in *MicroFocus 3.4.*

**Molecular taxonomy** is based on the universal presence of ribosomes in all living organisms. In particular, it is the RNAs in the ribosome, called ribosomal RNA (rRNA), which are of most interest and the primary basis of Woese's construction of the three-domain system. Many scientists today believe the rRNA molecule is the ultimate “molecular chronometer” that allows for precise bacterial classification in all taxonomic classes. Other techniques, including the polymerase chain reaction and nucleic acid hybridization, will be mentioned in later chapters.

The vast number of tests and analyses available for bacterial and archaeal cells can make it difficult to know which are relevant taxonomically for classification purposes or medically for pathogen identification purposes. One widely used technique in many disciplines is the **dichotomous key.** There are various forms of dichotomous keys, but one very useful construction is a flow chart where a series of positive or negative test procedures are listed down the page. Based on the dichotomous nature of the test (always a positive or negative result), the flow chart immediately leads to the next test result. The result is the identification of a specific organism. A simplified example is shown in *MicroFocus 3.4.*

**CONCEPT AND REASONING CHECKS**

3.9 Why are so many tests often needed to identify a specific prokaryotic species?
A medical version of a taxonomic key (in the form of a dichotomous flow chart) can be used to identify very similar bacterial species based on physical and biochemical characteristics.

In this simplified scenario, an unknown bacterium has been cultured and several tests run. The test results are shown in the box at the bottom. Using the test results and the flow chart, identify the bacterial species that has been cultured.

**Microbiology Test Results**
- Gram stain: gram-negative rods
- Biochemical tests:
  - Citrate test: negative
  - Lactose fermentation: positive
  - Indole test: positive
  - Methyl red test: positive

**Dichotomous Key Flow Chart**

1. **Unknown Bacterium**
   - Gram staining:
     - Positive
     - Negative
   - Ability to ferment lactose:
     - Positive
     - Negative
   - Indole production:
     - Positive
     - Negative
   - Use of citrate as sole carbon source:
     - Positive
     - Negative
   - Methyl red reaction:
     - Positive
     - Negative

**Species Identification**
- Citrobacter intermedius
- Escherichia coli
- Citrobacter freundii
- Enterobacter aerogenes
CHAPTER 3 Concepts and Tools for Studying Microorganisms

The ability to see small objects all started with the microscopes used by Robert Hooke and Anton van Leeuwenhoek. By now, you should be aware that microorganisms usually are very small. Before we examine the instruments used to “see” these tiny creatures, we need to be familiar with the units of measurement.

**Most Microbial Agents Are In the Micrometer Size Range**

**KEY CONCEPT**
- Metric system units are the standard for measurement.

One physical characteristic used to study microorganisms and viruses is their size. Because they are so small, a convenient system of measurement is used that is the scientific standard around the world. The measurement system is the metric system, where the standard unit of length is the meter and is a little longer than a yard (see Appendix A). To measure microorganisms, we need to use units that are a fraction of a meter. In microbiology, the common unit for measuring length is the **micrometer** (μm), which is equivalent to a millionth (10^-6) of a meter. To appreciate how small a micrometer is, consider this: Comparing a micrometer to an inch is like comparing a housefly to New York City’s Empire State Building, 1,472 feet high.

Microbial agents range in size from the relatively large, almost visible protozoa (100 μm) down to the incredibly tiny viruses (0.02 μm) (MicroFocus 3.5). Most bacterial and archaeal cells are about 1 μm to 5 μm in length, although notable exceptions have been discovered recently. Because most viruses are a fraction of one micrometer, their size is expressed in nanometers. A **nanometer** (nm) is equivalent to a billionth (10^-9) of a meter; that is, 1/1,000 of a μm. Using nanometers, the size of the poliovirus, among the smaller viruses, measures 20 nm (0.02 μm) in diameter.

**CONCEPT AND REASONING CHECKS**

3.10 If a bacterial cell is 0.75 μm in length, what is its length in nanometers?

**Light Microscopy Is Used to Observe Most Microorganisms**

**KEY CONCEPT**
- Light microscopy uses visible light to magnify and resolve specimens.

The basic microscope system used in the microbiology laboratory is the **light microscope**, in which visible light passes directly through the lenses and specimen (FIGURE 3.10A). Such an optical configuration is called **bright-field microscopy**. Visible light is projected through a condenser lens, which focuses the light into a sharp cone (FIGURE 3.10B). The light then passes through the opening in the stage. When hitting the glass slide, the light is reflected or refracted as it passes through the specimen. Next, light passing through the specimen enters the objective lens to form a magnified intermediate image inverted from that of the specimen. This intermediate image becomes the object magnified by the ocular lens (eyepiece) and seen by the observer. Because this microscope has several lenses, it also is called a **compound microscope**.

A light microscope usually has at least three objective lenses: the low-power, high-power, and oil-immersion lenses. In general, these lenses magnify an object 10, 40, and 100 times, respectively. (Magnification is represented by the multiplication sign, ×.) The ocular lens then magnifies the intermediate image produced by the objective lens by 10×. Therefore, the **total magnification** achieved is 100×, 400×, and 1,000×, respectively.

For an object to be seen distinctly, the lens system must have good **resolving power**; that is, it must transmit light without variation and allow closely spaced objects to be clearly distinguished. For example, a car seen in the distance at night may appear to have a single headlight because at that distance the unaided eye lacks resolving power. However, using binoculars, the two headlights can be seen clearly as the resolving power of the eye increases.

When switching from the low-power (10×) or high-power (40×) lens to the oil-
immersion lens (100x), one quickly finds that the image has become fuzzy. The object lacks resolution, and the resolving power of the lens system appears to be poor. The poor resolution results from the refraction of light.

Both low-power and high-power objectives are wide enough to capture sufficient light for viewing. The oil-immersion objective, on the other hand, is so narrow that most light bends away and would miss the objective lens. The index of refraction (or refractive index) is a measure of the light-bending ability of a medium. Immersion oil has an index of refraction of 1.5, which is almost identical to the index of refraction of glass. Therefore, by immersing the 100x lens in oil, the light does not bend away from the lens as it passes from the glass slide and the specimen.

The oil thus provides a homogeneous pathway for light from the slide to the objective, and
the resolution of the object increases. With the oil-immersion lens, the highest resolution possible with the light microscope is attained, which is near 0.2 μm (200 nm) (MicroFocus 3.6).

**Concept and Reasoning Checks**

3.11 What are the two most important properties of the light microscope?

**Staining Techniques Provide Contrast**

**Key Concept**

- Specimens stained with a dye are contrasted against the microscope field.

Microbiologists commonly stain bacterial cells before viewing them because the cytoplasm of bacterial cells lacks color, making it hard to see the cells on a bright background. Several staining techniques have been developed to provide contrast for bright-field microscopy.

To perform the simple stain technique, bacterial cells in a droplet of water or broth are smeared on a glass slide and the slide air-dried. Next, the slide is passed briefly through a flame in a process called heat fixation, which bonds the cells to the slide, kills any organisms still alive, and increases stain absorption. Now the slide is flooded with a basic (cationic) dye such as methylene blue (Figure 3.11A). Because cationic dyes have a positive charge, the dye is attracted to the cytoplasm and cell wall, which primarily have negative charges. By contrasting the blue cells against the bright background, the staining procedure allows the observer to measure cell size and determine cell shape. It also can provide information about how cells are arranged with respect to one another (Chapter 4).

**MICROFOCUS 3.5: Environmental Microbiology**

**Biological Oxymorons**

An oxymoron is a pair of words that seem to refer to opposites, such as jumbo shrimp, holy war, old news, and sweet sorrow. One of the characteristics we used for microorganisms is that most are invisible to the naked eye; you need a microscope to see them. Always true? So how about the oxymoron: macroscopic microorganism?

In 1993, researchers at Indiana University discovered near an Australian reef macroscopic bacterial cells in the gut of surgeonfish. Each cell was so large that a microscope was not needed to see it. The spectacular giant, measuring over 0.6 mm in length (that’s 600 μm compared to 2 μm for *Escherichia coli*) even dwarfs the protozoan *Paramecium*.

While on an expedition off the coast of Namibia (western coast of southern Africa), Heide Schultz and teammates from the Max Planck Institute for Marine Microbiology in Bremen, Germany, found another bacterial monster in sediment samples from the sea floor (see figure). These bacterial cells were spherical being about 0.1 to 0.3 mm in diameter—but some as large as 0.75 mm—about the diameter of the period in this sentence. Their volume is about 3 million times greater than that of *E. coli*. The cells, shining white with enclosed sulfur granules, were held together in chains by a mucus sheath looking like a string of pearls. Thus, the bacterial species was named *Thiomargarita namibiensis* (meaning “sulfur pearl of Namibia”).

How does a prokaryotic cell survive in so large a size? The trick is to keep the cytoplasm as a thin layer plastered against the edge of the cell so materials do not need to travel (diffuse) far to get into or out of the cell. The rest of the cell is a giant “bubble,” called a vacuole, in which nitrate and sulfur are stored as potential energy sources. Thus, the actual cytoplasmic layer is microscopic and as close to the surface as possible.

Yes, the vast majority of microorganisms are microscopic, but exceptions have been found in some exotic places.
3.3 Microscopy

The Light Microscope. (A) The light microscope is used in many instructional and clinical laboratories. Note the important features of the microscope that contribute to the visualization of the object. (B) Image formation in the light microscope requires light to pass through the objective lens, forming an intermediate image. This image serves as an object for the ocular lens, which magnifies the image and forms the final image the eye perceives. (C) When using the oil immersion lens (100×), oil must be placed between and continuous with the slide and objective lens.

Q: Why must oil be used with the 100× oil-immersion lens?
The negative stain technique works in the opposite manner (FIGURE 3.11B). Bacterial cells are mixed on a slide with an acidic (anionic) dye such as nigrosin (a black stain) or India ink (a black drawing ink). The mixture then is pushed across the face of the slide and allowed to air-dry. Because the anionic dye carries a negative charge, it is repelled from the cell wall and cytoplasm. The stain does not enter the cells and the observer sees clear or white cells on a black or gray background. Because this technique avoids chemical reactions and heat fixation, the cells appear less shriveled and less distorted than in a simple stain. They are closer to their natural condition.

The Gram stain technique is an example of a differential staining procedure; that is, it allows the observer to differentiate (separate) bacterial cells visually into two groups based on staining differences. The Gram stain technique is named for Christian Gram, the Danish physician who first perfected the technique in 1884.

The first two steps of the technique are straightforward (FIGURE 3.12A). Air-dried, heat-fixed smears are (1) stained with crystal violet, rinsed, and then (2) a special Gram's iodine solution is added. All bacterial cells would appear blue-purple if the procedure was stopped and the sample viewed with the light
Next, the smear is rinsed with a decolorizer, such as 95 percent alcohol or an alcohol-acetone mixture. Observed at this point, certain bacterial cells may lose their color and become transparent. These are the gram-negative bacterial cells. Others retain the crystal violet and represent the gram-positive bacterial cells. The last step uses safranin, a red cationic dye, to counterstain the gram-negative organisms; that is, give them a orange-red color. So, at the technique's conclusion, gram-positive cells are blue-purple while gram-negative cells are orange-red (Figure 3.12B). Similar to the simple staining, gram staining also allows the observer to determine size, shape, and arrangement of cells.

Knowing whether a bacterial cell is gram-positive or gram-negative is important for microbiologists and clinical technicians who use the results from the Gram stain technique to classify it in Bergey’s Manual or aid in the identification of an unknown bacterial species (Textbook Case 3).

Gram-positive and gram-negative bacterial cells also differ in their susceptibility to chemical substances such as antibiotics (gram-positive cells are more susceptible to penicillin, gram-negative cells to tetracycline). Also, gram-negative cells have more complex cell walls, as
described in Chapter 4, and gram-positive and gram-negative bacterial species can produce different types of toxins. One other differential staining procedure, the acid-fast technique, deserves mention. This technique is used to identify members of the genus Mycobacterium, one species of which causes tuberculosis. These bacterial organisms are normally difficult to stain with the Gram stain because the cells have very waxy walls. However, the cell will stain red when treated with carbol-fuchsin (red dye) and heat (or a lipid solubilizer) (Figure 3.13). The cells then retain their color when washed with a dilute acid-alcohol solution. Other stained genera lose the red color easily during the acid-alcohol wash. The Mycobacterium species, therefore, is called acid resistant or acid-fast. Because they stain red and break sharply when they reproduce, Mycobacterium species often are referred to as “red snappers.”

CONCEPT AND REASONING CHECKS

3.12 What would happen if a student omitted the alcohol wash step when doing the Gram stain procedure?

---

**Textbook CASE 3**

**Bacterial Meningitis and a Misleading Gram Stain**

1. A woman comes to the hospital emergency room complaining of severe headache, nausea, vomiting, and pain in her legs. On examination, cerebral spinal fluid (CFS) was observed leaking from a previous central nervous system (CNS) surgical site.

2. The patient indicates that 6 weeks and 8 weeks ago she had undergone CNS surgery after complaining of migraine headaches and sinusitis. Both surgeries involved a spinal tap. Analysis of cultures prepared from the CFS indicated no bacterial growth.

3. The patient was taken to surgery where a large amount of CFS was removed from underneath the old incision site. The pinkish, hazy fluid indicated bacterial meningitis, so among the laboratory tests ordered was a Gram stain.

4. The patient was placed on antibiotic therapy, consisting of vancomycin and cefotaxime.

5. Laboratory findings from the gram-stained CFS smear showed a few gram-positive, spherical bacterial cells that often appeared in pairs. The results suggested a Streptococcus pneumoniae infection.

6. However, upon reexamination of the smear, a few gram-negative spheres were observed.

7. When transferred to a blood agar plate, growth occurred and a prepared smear showed many gram-negative spheres (see figure). Further research indicated that several genera of gram-negative bacteria, including Acinetobacter, can appear gram-positive due to under-decolorization.

8. Although complicated by the under-decolorization outcome, the final diagnosis was bacterial meningitis due to Acinetobacter baumanii.

**Questions**

A. From the gram-stained CSF smear, what color were the bacterial spheres?

B. After reexamination of the CFS smear, assess the reliability of the gram-stained smear.

C. What reagent is used for the decolorization step in the Gram stain?


For additional information see http://www.cdc.gov/ncidod/hip/aresist/acin_general.htm.
3.3 Microscopy

Light Microscopy Has Other Optical Configurations

**KEY CONCEPT**

- Different optical configurations provide detailed views of cells.

Bright-field microscopy provides little contrast (FIGURE 3.14A). However, a light microscope can be outfitted with other optical systems to improve contrast of prokaryotic and eukaryotic cells without staining. Three systems commonly employed are mentioned here.

**Phase-contrast microscopy** uses a special condenser and objective lenses. This condenser lens on the light microscope splits a light beam and throws the light rays slightly out of phase. The separated beams of light then pass through and around the specimen, and small differences in the refractive index within the specimen show up as different degrees of brightness and contrast. With phase-contrast microscopy, microbiologists can see organisms alive and unstained (FIGURE 3.14B). The structure of yeasts, molds, and protozoa is studied with this optical configuration.

**Dark-field microscopy** also uses a special condenser lens mounted under the stage. The condenser scatters the light and causes it to hit the specimen from the side. Only light bouncing off the specimen and into the objective lens makes the specimen visible, as the surrounding area appears dark because it lacks background light (FIGURE 3.14C). Dark-field microscopy provides good resolution and often illuminates parts of a specimen not seen with bright-field optics. Dark-field microscopy also is the preferred way to study motility of live cells.

Dark-field microscopy helps in the diagnosis of diseases caused by organisms near the limit of resolution of the light microscope. For example, syphilis, caused by the spiral bacterium *Treponema pallidum*, has a diameter of...
only about 0.15 μm. Therefore, this bacterial species may be observed in scrapings taken from a lesion of a person who has the disease and observed with dark-field microscopy.

**Fluorescence microscopy** is a major asset to clinical and research laboratories. The technique has been applied to the identification of many microorganisms and is a mainstay of modern microbial ecology and especially clinical microbiology. With fluorescence microscopy, objects emit a specific color (wavelength) of light after absorbing a shorter wavelength radiation.

Microorganisms are coated with a fluorescent dye, such as fluorescein, and then illuminated with ultraviolet (UV) light. The energy in UV light excites electrons in fluorescein, and they move to higher energy levels. However, the electrons quickly drop back to their original energy levels and give off the excess energy as visible light. The coated microorganisms thus appear to fluoresce; in the case of fluorescein, they glow a greenish yellow. Other dyes produce other colors (FIGURE 3.15).

![Fluorescence Microscopy](image)

**Fluorescence Microscopy.** Fluorescence microscopy of sporulating cells of *Bacillus subtilis*. DNA has been stained with a dye that fluoresces red and a sporulating protein with fluorescein (green). RNA synthesis activity is indicated by a dye that fluoresces blue. (Bar = 15 μm.)

Q: What advantage is gained by using fluorescence optics over the other light microscope optical configurations?

An important application of fluorescence microscopy is the **fluorescent antibody technique** used to identify an unknown organism. In one variation of this procedure, fluorescein is chemically attached to antibodies, the protein molecules produced by the body’s immune system. These “tagged” antibodies are mixed with a sample of the unknown organism. If the antibodies are specific for that organism, they will bind to it and coat the cells with the dye. When subjected to UV light, the organisms will fluoresce. If the organisms fail to fluoresce, the antibodies were not specific to that organism and a different tagged antibody is tried.

**CONCEPT AND REASONING CHECKS**

3.13 What optical systems can improve specimen contrast over bright-field microscopy?

**Electron Microscopy Provides Detailed Images of Cells, Cell Parts, and Viruses**

**KEY CONCEPT**

- Electron microscopy uses a beam of electrons to magnify and resolve specimens.

The **electron microscope** grew out of an engineering design made in 1933 by the German physicist Ernst Ruska (winner of the 1986 Nobel Prize in Physics). Ruska showed that electrons will flow in a sealed tube if a vacuum is maintained to prevent electron scattering. Magnets, rather than glass lenses, pinpoint the flow onto an object, where the electrons are absorbed, deflected, or transmitted depending on the density of structures within the object (FIGURE 3.16). When projected onto a screen underneath, the electrons form a final image that outlines the structures. As mentioned in Chapter 1, the early days of electron microscopy produced **electron micrographs** that showed bacterial cells indeed were cellular but their structure was different from eukaryotic cells. This led to the development of the prokaryotic and eukaryotic groups of organisms.

The power of electron microscopy is the extraordinarily short wavelength of the beam of electrons. Measured at 0.005 nm (compared to 550 nm for visible light), the short wavelength dramatically increases the resolving power of the system and makes possible the visualization of viruses and detailed cellular structures, often called the **ultrastructure** of cells. The practical limit of resolution of biological samples with
the electron microscope is about 2 nm, which is 100× better than the resolving power of the light microscope. The drawback of the electron microscope is that the method needed to prepare a specimen kills the cells or organisms.

Two types of electron microscopes are currently in use. The transmission electron microscope (TEM) is used to view and record detailed structures within cells. Ultrathin sections of the prepared specimen must be cut because the electron beam can penetrate matter only a very short distance. After embedding the specimen in a suitable plastic mounting medium or freezing it, scientists cut the specimen into sections with a diamond knife. In this manner, a single bacterial cell can be sliced, like a loaf of bread, into hundreds of thin sections.

Several of the sections are placed on a small grid and stained with heavy metals such as lead and osmium to provide contrast. The microscopist then inserts the grid into the vacuum chamber of the microscope and focuses a 100,000-volt electron beam on one portion of a section at a time. An image forms on the screen below or can be recorded on film. The electron micrograph may be enlarged with enough resolution to achieve a final magnification of over 20 million ×.

The scanning electron microscope (SEM) was developed in the late 1960s to enable researchers to see the surfaces of objects in the natural state and without sectioning. The specimen is placed in the vacuum chamber and covered with a thin coat of gold. The electron beam then scans across the specimen and knocks loose showers of electrons that are captured by a detector. An image builds line by line, as in a television receiver. Electrons that strike a sloping surface yield fewer electrons, thereby producing a darker contrasting spot and a sense of three dimensions. The resolving power of the conventional SEM is about 7 nm and magnifications with the SEM are limited to about 50,000×. However, the instrument provides vivid and undistorted views of an organism’s surface details.

The electron microscope has added immeasurably to our understanding of the structure and function of microorganisms by letting us penetrate their innermost secrets. In the chapters ahead, we will encounter many of the...
structures displayed by electron microscopy, and we will better appreciate microbial physiology as it is defined by microbial structures.

The various types of light and electron microscopy are compared in Table 3.3. 

### Table 3.3: Comparison of Various Types of Microscopy

<table>
<thead>
<tr>
<th>Type of Microscopy</th>
<th>Special Feature</th>
<th>Appearance of Object</th>
<th>Magnification Range</th>
<th>Objects Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Light</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bright-field</td>
<td>Visible light illuminates object</td>
<td>Stained microorganisms on clear background</td>
<td>100× – 1,000×</td>
<td>Arrangement, shape, and size of killed microorganisms (except viruses)</td>
</tr>
<tr>
<td>Phase-contrast</td>
<td>Special condenser throws light rays “out of phase”</td>
<td>Unstained microorganisms with contrasted structures</td>
<td>100× – 1,000×</td>
<td>Internal structures of live, unstained eukaryotic microorganisms</td>
</tr>
<tr>
<td>Dark-field</td>
<td>Special condenser scatters light</td>
<td>Unstained microorganisms on dark background</td>
<td>100× – 1,000×</td>
<td>Live, unstained microorganisms (e.g., spirochetes)</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>UV light illuminates fluorescent-coated objects</td>
<td>Fluorescing microorganisms on dark background</td>
<td>100× – 1,000×</td>
<td>Outline of microorganisms coated with fluorescent-tagged antibodies</td>
</tr>
<tr>
<td><strong>Electron</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmission</td>
<td>Short-wavelength electron beam penetrates sections</td>
<td>Alternating light and dark areas contrasting internal cell structures</td>
<td>100× – 200,000×</td>
<td>Ultrathin slices of microorganisms and internal components of eukaryotic and prokaryotic cells</td>
</tr>
<tr>
<td>Scanning</td>
<td>Short-wavelength electron beam knocks loose electron showers</td>
<td>Microbial surfaces</td>
<td>10× – 50,000×</td>
<td>Surfaces and textures of microorganisms and cell components</td>
</tr>
</tbody>
</table>

### Concept and Reasoning Checks

3.14 What type of electron microscope would be used to examine (a) the surface structures on a Paramecium cell and (b) the organelles in an algal cell?
Learning Objectives

After understanding the textbook reading, you should be capable of writing a paragraph that includes the appropriate terms and pertinent information to answer the objective.

1. Identify the six emerging properties common to prokaryotes and eukaryotes.
2. Assess the importance of homeostasis to cell (organismal) survival.
3. Contrast prokaryotes as unicellular and multicellular organisms.
4. Apply the concepts of cell structure, the pattern of organization, and cellular process to prokaryotes and eukaryotes.
5. Describe the four organizational patterns common to all organisms.
6. Identify the structural distinctions between prokaryotic and eukaryotic cells.
7. Explain how prokaryotic cells carry out cellular processes common to eukaryotic cells without needing membrane-bound organelles.
8. Distinguish between the processes of invagination and endosymbiosis for the origin of eukaryotic cells.
9. Evaluate Linnaeus’ contributions to modern taxonomy.
10. Write scientific names of organisms using the binomial system.

11. Identify the taxa used to classify organisms from least to most inclusive taxa.
12. Discuss the assigning of organisms according to the five kingdom system of classification.
13. Explain the assigning of organisms to the three domain system of classification.
14. Contrast the procedures used to identify and classify bacterial and archaean species.
15. Use a dichotomous key.
16. Measure sizes of microbial agents using metric system units.
17. Assess the importance of magnification and resolving power to microscopy.
18. Compare and contrast the procedures used to stain bacterial cells.
19. Summarize the Gram stain procedure.
20. Identify the optical configurations that provide contrast with light microscopy.
21. Compare the uses of the transmission and scanning electron microscopes.
### SELF-TEST

Answer each of the following questions by selecting the one answer that best fits the question or statement. Answers to even-numbered questions can be found in Appendix C.

1. ______ describes the ability of organisms to maintain a stable internal state.
   - A. Metabolism
   - B. Homeostasis
   - C. Biosphere
   - D. Ectype
   - E. None of the above (A–D) is correct.

2. Which one of the following phrases would not apply to prokaryotes?
   - A. Programmed cell death
   - B. Multicellular communities
   - C. Cell-cell communication
   - D. Cell cooperation
   - E. Neutlated cells

3. Proteins are made by the
   - A. mitochondria
   - B. lysosomes
   - C. Golgi apparatus
   - D. ribosomes
   - E. cytoskeleton

4. Cell walls are necessary for
   - A. nutrient transport regulation.
   - B. DNA compartmentation.
   - C. protein transport.
   - D. energy metabolism.
   - E. water balance.

5. Which one of the following is not found in prokaryotic cells?
   - A. Cell membrane
   - B. Ribosomes
   - C. DNA
   - D. Mitochondria
   - E. Cytoplasm

6. The two functions of the endoplasmic reticulum are
   - A. protein and lipid transport.
   - B. cell respiration and photosynthesis.
   - C. osmotic regulation and genetic control.
   - D. cell respiration and protein synthesis.
   - E. sorting and packaging of proteins.

7. Mitochondria differ from chloroplasts in that only mitochondria
   - A. carry out photosynthesis.
   - B. are membrane-bound.
   - C. are found in the Eukarya.
   - D. convert chemical energy to cellular energy.
   - E. transform sunlight into chemical energy.

8. Who is considered to be the father of modern taxonomy?
   - A. Woese
   - B. Whittaker
   - C. Aristotle
   - D. Haeckel
   - E. Linnaeus

9. Which one of the following is the correct genus name for the bacterial organism that causes syphilis?
   - A. treponema
   - B. pallidum
   - C. Treponema
   - D. pallidum
   - E. T. pallidum

10. Several classes of organisms would be classified into one
    - A. family
    - B. genus
    - C. species
    - D. order
    - E. phylum (division).

11. The domain Eukarya includes all the following except
    - A. fungi.
    - B. protozoa.
    - C. archaeal cells.
    - D. algae.
    - E. animals.

12. ______ was first used to catalog organisms into one of three domains.
    - A. Photosynthesis
    - B. Ribosomes
    - C. Ribosomal RNA
    - D. Nuclear DNA
    - E. Mitochondrial DNA

13. Resolving power is the ability of a microscope to
    - A. estimate cell size.
    - B. magnify an image.
    - C. see two close objects as separate.
    - D. keep objects in focus.
    - E. Both B and D are correct.

14. Calculation of total magnification involves which microscope or lenses?
    - A. Ocular and condenser
    - B. Objective only
    - C. Ocular only
    - D. Objective and ocular
    - E. Objective and condenser

15. Before bacterial cells are simple stained and observed with the light microscope, they must be
    - A. smeared on a slide.
    - B. heat fixed.
    - C. killed.
    - D. air dried.
    - E. All the above (A–D) are correct.

16. The counterstain used in the Gram stain procedure is
    - A. iodine.
    - B. alcohol.
    - C. carbol-fuchsin.
    - D. safranin.
    - E. malachite green.

17. The practical limit of resolution with the transmission electron microscope is ______.
    - A. 10 μm
    - B. 1 μm
    - C. 50 nm
    - D. 10 nm
    - E. 2 nm

18. For transmission electron microscopy, contrast is provided by
    - A. heavy metals.
    - B. a coat of gold.
    - C. crystal violet.
    - D. plastic.
    - E. copper ions.

19. If you wanted to study the surface of a bacterial cell, you would use
    - A. a transmission electron microscope.
    - B. a light microscope with phase-contrast optics.
    - C. a scanning electron microscope.
    - D. a light microscope with dark-field optics.
    - E. a light microscope with bright-field optics.

20. If you wanted to study bacterial motility you would most likely use
    - A. a transmission electron microscope.
    - B. a light microscope with phase-contrast optics.
    - C. a scanning electron microscope.
    - D. a light microscope with dark-field optics.
    - E. a light microscope with bright-field optics.
Questions for Thought and Discussion

Answers to even-numbered questions can be found in Appendix C.

1. A local newspaper once contained an article about “the famous bacteria E. coli.” How many things can you find wrong in this phrase? Rewrite the phrase correctly.

2. Microorganisms have been described as the most chemically diverse, the most adaptable, and the most ubiquitous organisms on Earth. Although your knowledge of microorganisms still may be limited at this point, try to add to this list of “mosts.”

3. Prokaryotes lack the cytoplasmic organelles commonly found in the eukaryotes. Provide a reason for this structural difference.

4. A new bacteriology laboratory is opening in your community. What is one of the first books that the laboratory director will want to purchase? Why is it important to have this book?

5. In 1987, in a respected science journal, an author wrote, “Linnaeus gave each life form two Latin names, the first denoting its genus and the second its species.” A few lines later, the author wrote, “Man was given his own genus and species Homo sapiens.” What is conceptually and technically wrong with both statements?

6. A student of general biology observes a microbiology student using immersion oil and asks why the oil is used. “To increase the magnification of the microscope” is the reply. Do you agree? Why?

7. Every state has an official animal, flower, or tree, but only Oregon has a bacterial species named in its honor: Methanohalophilus oregonese. The specific epithet oregonese is obvious, but can you decipher the meaning of the genus name?

Applications

Answers to even-numbered questions can be found in Appendix C.

1. A student is performing the Gram stain technique on a mixed culture of gram-positive and gram-negative bacterial cells. In reaching for the counterstain in step 4, he inadvertently takes the methylene blue bottle and proceeds with the technique. What will be the colors of gram-positive and gram-negative bacteria at the conclusion of the technique?

2. Would the best resolution with a light microscope be obtained using red light ($\lambda = 680$ nm), green light ($\lambda = 520$ nm), or blue light ($\lambda = 500$ nm)? Explain your answer.

3. The electron micrograph below shows several bacterial cells. The micrograph has been magnified 12,000×. At this magnification, the cells are about 25 mm in length. Calculate the actual length of the bacterial cells in micrometers ($\mu$m).
Match the statement on the left to the term on the right by placing the letter of the term in the available space. Appendix C contains the correct answers to the even-numbered statements.

<table>
<thead>
<tr>
<th>Statement</th>
<th>Term</th>
</tr>
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<tbody>
<tr>
<td>1. System of nomenclature used for microorganisms and other living things.</td>
<td>A. Archaea</td>
</tr>
<tr>
<td>2. Unit of measurement used for viruses and equal to a billionth of a meter.</td>
<td>B. Bergey</td>
</tr>
<tr>
<td>3. Major group of organisms whose cells have no nucleus or organelles in the cytoplasm.</td>
<td>C. Binomial</td>
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<td>4. Bacterial organisms capable of photosynthesis.</td>
<td>D. Boldface</td>
</tr>
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<td>5. Devised the three domain system of classification in which two domains contain prokaryotes.</td>
<td>E. Chloroplast</td>
</tr>
<tr>
<td>6. Type of microscope that uses a special condenser to split the light beam.</td>
<td>F. Cyanobacteria</td>
</tr>
<tr>
<td>7. Type of electron microscope for which cell sectioning is not required.</td>
<td>G. Dark-field</td>
</tr>
<tr>
<td>8. The ability of an organism to maintain a stable internal state.</td>
<td>H. Eukarya</td>
</tr>
<tr>
<td>9. These structures carry out protein synthesis in all cells.</td>
<td>I. Family</td>
</tr>
<tr>
<td>10. The organelle, absent in prokaryotes, which carries out the conversion of chemical energy to cellular energy in eukaryotes.</td>
<td>J. Fluorescence</td>
</tr>
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<td>11. Domain in which many prokaryotes are classified.</td>
<td>K. Fungi</td>
</tr>
<tr>
<td>12. Staining technique that differentiates bacterial cells into two groups.</td>
<td>L. Gram</td>
</tr>
<tr>
<td>13. Author of an early system of classification for bacterial organisms.</td>
<td>M. Haeckel</td>
</tr>
<tr>
<td>14. Category into which two or more genera are grouped.</td>
<td>N. Homeostasis</td>
</tr>
<tr>
<td>15. Coined the name protista for microorganisms.</td>
<td>O. Italics</td>
</tr>
<tr>
<td>16. The staining technique employing a single cationic dye.</td>
<td>P. Micrometer</td>
</tr>
<tr>
<td>17. The convention for writing the binomial name of microorganisms.</td>
<td>Q. Mitochondrion</td>
</tr>
<tr>
<td>18. Unit of measurement for bacterial cells and equal to a millionth of a meter.</td>
<td>R. Nanometer</td>
</tr>
<tr>
<td>19. Type of microscopy using UV light to excite a dye-coated specimen.</td>
<td>S. Negative</td>
</tr>
<tr>
<td>20. Staining technique in which the background is colored and the cells are clear.</td>
<td>T. Prokaryotes</td>
</tr>
</tbody>
</table>

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The site features learning, an on-line review area that provides quizzes and other tools to help you study for your class. You can also follow useful links for in-depth information, read more MicroFocus stories, or just find out the latest microbiology news.
Identify the cell structures indicated in drawings (A) and (B) below. Answers to the even-numbered structures can be found in Appendix C.