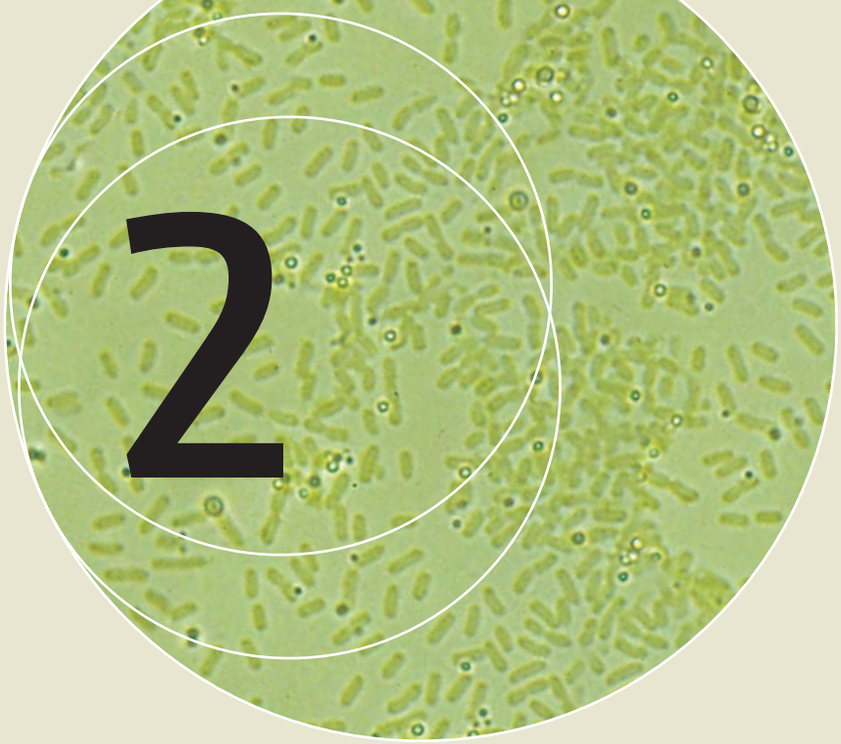


2

A Brief Journey to the Microbial World

Green sulfur bacteria are phototrophic microorganisms that form their own phylogenetic lineage and were some of the first phototrophs to evolve on Earth.

Seeing the Very Small

Historically, the science of microbiology blossomed as the ability to see microorganisms improved; thus, *microbiology* and *microscopy* advanced hand-in-hand. The microscope is the microbiologist's most basic tool, and every student of microbiology needs some background on how microscopes work and how microscopy is done. We therefore begin our brief journey to the microbial world by considering different types of microscopes and the applications of microscopy to imaging microorganisms.

2.1 Some Principles of Light Microscopy

Visualization of microorganisms requires a microscope, either a *light* microscope or an *electron* microscope. In general, light microscopes are used to examine cells at relatively low magnifications, and electron microscopes are used to look at cells and cell structures at very high magnification.

All microscopes employ lenses that magnify (enlarge) the image. Magnification, however, is not the limiting factor in our ability to see small objects. It is instead **resolution**—the ability to distinguish two adjacent objects as distinct and separate—that governs our ability to see the very small. Although magnification can be increased virtually without limit, resolution cannot, because resolution is a function of the physical properties of light.

We begin with the light microscope, for which the limits of resolution are about 0.2 μm (μm is the abbreviation for micrometer, 10^{-6} m). We then proceed to the electron microscope, for which resolution is considerably greater than that of the light microscope.

The Compound Light Microscope

The light microscope uses visible light to illuminate cell structures. Several types of light microscopes are used in microbiology: *bright-field*, *phase-contrast*, *differential interference contrast*, *dark-field*, and *fluorescence*.

With the bright-field microscope, specimens are visualized because of the slight differences in contrast that exist between them and their surrounding medium. Contrast differences arise because cells absorb or scatter light to varying degrees. The compound bright-field microscope is commonly used in laboratory courses in biology and microbiology; the microscopes are called *compound* because they contain two lenses, *objective* and *ocular*, that function in combination to form the image. The light source is focused on the specimen by the condenser (**Figure 2.1**). Bacterial cells are typically difficult to see well with the bright-field microscope because the cells themselves lack significant contrast with their surrounding medium. Pigmented microorganisms are an exception because the color of the organism itself adds contrast, thus improving visualization (**Figure 2.2**). For cells lacking pigments there are ways to boost contrast, and we consider these methods in the next section.

Magnification and Resolution

The total magnification of a compound light microscope is the *product* of the magnification of its objective and ocular lenses

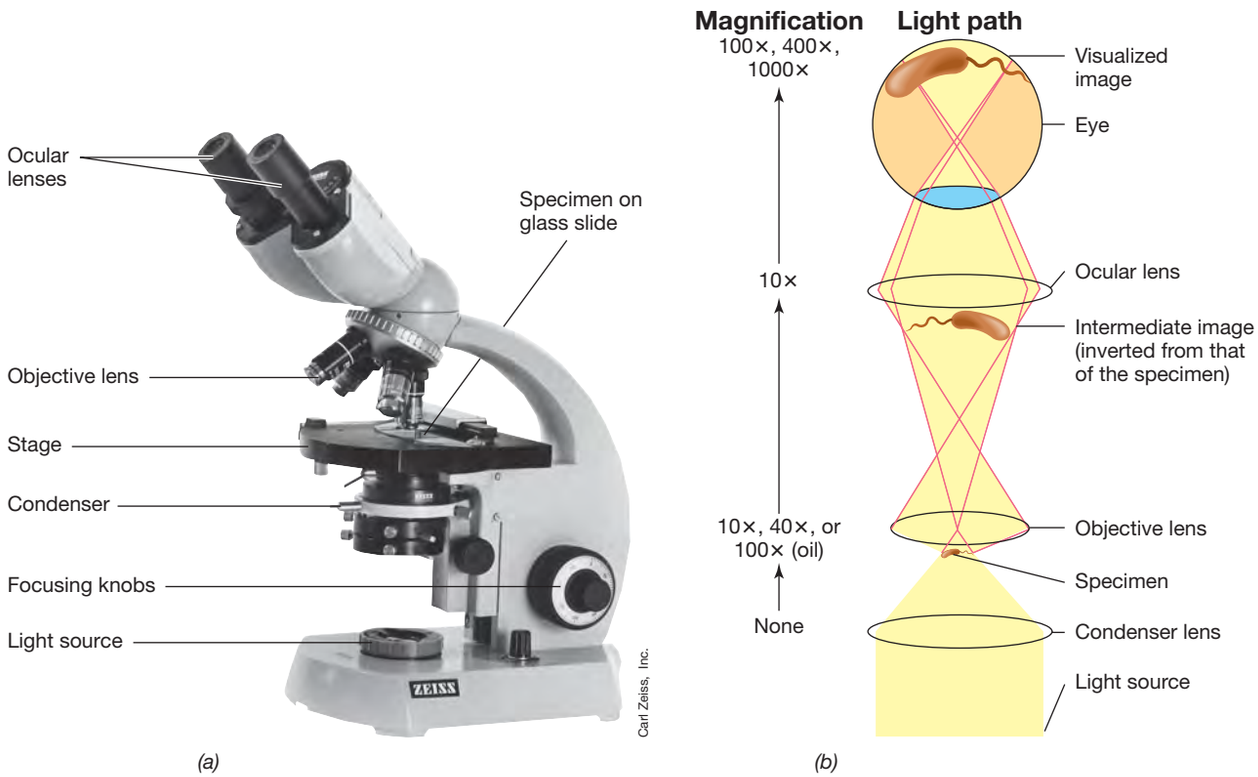
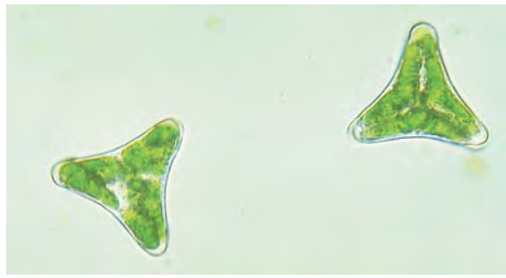


Figure 2.1 Microscopy. *(a)* A compound light microscope. *(b)* Path of light through a compound light microscope. Besides 10×, ocular lenses are available in 15–30× magnifications.



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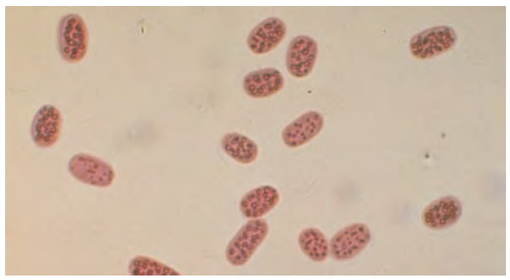


(b)

Figure 2.2 **Bright-field photomicrographs of pigmented microorganisms.** *(a)* A green alga (eukaryote). The green structures are chloroplasts. *(b)* Purple phototrophic bacteria (prokaryote). The algal cell is about 15 μm wide, and the bacterial cells are about 5 μm wide. We contrast prokaryotic and eukaryotic cells in Section 2.5.

(Figure 2.1*b*). Magnifications of about 2000× are the upper limit for light microscopes. At magnifications above this, resolution does not improve. Resolution is a function of the wavelength of light used and a characteristic of the objective lens known as its *numerical aperture*, a measure of light-gathering ability. There is a correlation between the magnification of a lens and its numerical aperture: Lenses with higher magnification typically have higher numerical apertures (the numerical aperture of a lens is stamped on the lens alongside the magnification). 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. Based on this formula, resolution is highest when blue light is used to illuminate a specimen (because blue light is of a shorter wavelength than white or red light) and the objective has a very high numerical aperture. For this reason, many light microscopes come fitted with a blue filter over the condenser lens to improve resolution.

As mentioned, the highest resolution possible in a compound light microscope is about 0.2 μm. What this means is that two objects that are closer together than 0.2 μm cannot be resolved as distinct and separate. Microscopes used in microbiology have ocular lenses that magnify 10–20× and objective lenses of 10–100× (Figure 2.1*b*). At 1000×, objects 0.2 μm in diameter can just be resolved. With the 100× objective, and with certain other objectives of very high numerical aperture, an optical-grade oil is placed between the specimen and the objective. Lenses on which oil is used are called *oil-immersion* lenses. Immersion oil increases the light-gathering ability of a lens by allowing some of the light rays emerging from the specimen at angles (that would otherwise be lost to the objective lens) to be collected and viewed.

MiniQuiz

- Define and compare the terms magnification and resolution.
- What is the useful upper limit of magnification for a bright-field microscope? Why is this so?

2.2 Improving Contrast in Light Microscopy

In microscopy, improving contrast typically improves the final image. Staining is an easy way to improve contrast, but there are many other approaches.

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. Dyes are organic compounds, and 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. Because cell surfaces tend to be negatively charged, these dyes also combine with high affinity to the surfaces of cells, and hence are very useful general-purpose stains.

To perform a simple stain one begins with dried preparations of cells (**Figure 2.3**). 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 bacteria 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 2.4**). On the basis of their reaction to 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 2.4*b*). The color difference in the Gram stain arises because of differences in the cell wall structure of gram-positive and gram-negative cells, a topic we will consider in Chapter 3. After staining with a basic dye, typically crystal violet, treatment with ethanol decolorizes gram-negative but not gram-positive cells. Following counterstaining with a different-colored stain, typically safranin, the two cell types can be distinguished microscopically by their different colors (Figure 2.4*b*).

The Gram stain is one of the most useful staining procedures in microbiology. Typically, one begins the characterization of a new bacterium by determining whether it is gram-positive or

Figure 2.3 **Staining cells for microscopic observation.** Stains improve the contrast between cells and their background.

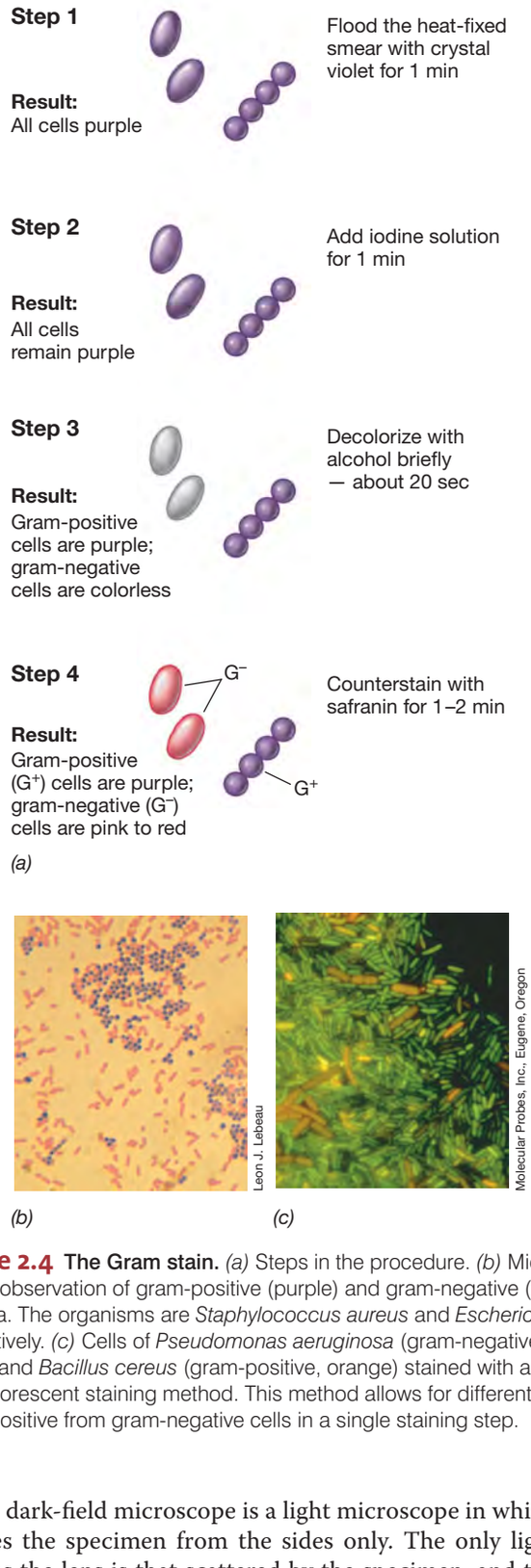


Figure 2.4 **The Gram stain.** *(a)* Steps in the procedure. *(b)* Microscopic observation of gram-positive (purple) and gram-negative (pink) bacteria. The organisms are *Staphylococcus aureus* and *Escherichia coli*, respectively. *(c)* Cells of *Pseudomonas aeruginosa* (gram-negative, green) and *Bacillus 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.

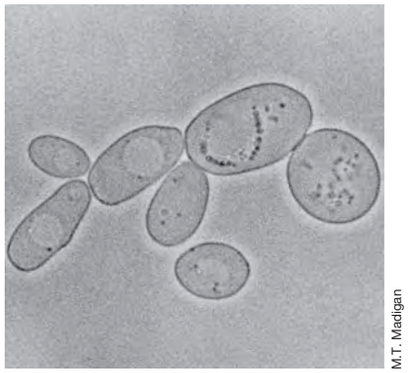
gram-negative. If a fluorescent microscope, discussed below, is available, the Gram stain can be reduced to a one-step procedure in which gram-positive and gram-negative cells fluoresce different colors (Figure 2.4*c*).

Phase-Contrast and Dark-Field Microscopy

Staining, although a widely used procedure in light microscopy, kills cells and can distort their features. Two forms of light microscopy improve image contrast without the use of stain, and thus do not kill cells. These are phase-contrast microscopy and dark-field microscopy (**Figure 2.5**). The phase-contrast microscope in particular is widely used in teaching and research for the observation of wet-mount (living) preparations.

Phase-contrast microscopy is based on the principle that cells differ in refractive index (a factor by which light is slowed as it passes through a material) 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 2.5*b*). The ring consists of a phase plate that amplifies the minute variation in phase. The development of phase-contrast microscopy stimulated other innovations in microscopy, such as fluorescence and confocal microscopy (discussed below), and greatly increased use of the light microscope in microbiology.

The dark-field microscope is a light microscope in which light reaches the specimen from the sides only. The only light that reaches the lens is that scattered by the specimen, and thus the specimen appears light on a dark background (Figure 2.5*c*). Resolution by dark-field microscopy is somewhat better than by light microscopy, and objects can often be resolved by dark-field that cannot be resolved by bright-field or even phase-contrast



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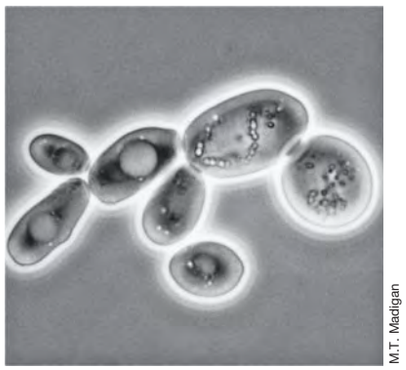


(b)

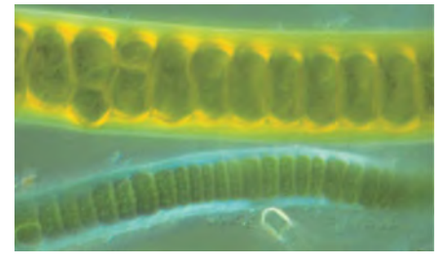
(c)

Figure 2.5 **Cells visualized by different types of light microscopy.** The same field of cells of the baker's yeast *Saccharomyces cerevisiae* visualized by *(a)* bright-field microscopy, *(b)* phase-contrast microscopy, and *(c)* dark-field microscopy. Cells average 8–10 μm wide.

microscopes. Dark-field microscopy is also an excellent way to observe microbial motility, as bundles of flagella (the structures responsible for swimming motility) are often resolvable with this technique (↻ Figure 3.40*a*).

Fluorescence Microscopy

The fluorescence microscope is used to visualize specimens that fluoresce—that is, emit light of one color following absorption of light of another color (**Figure 2.6**). Cells fluoresce either because they contain naturally fluorescent substances such as chlorophyll



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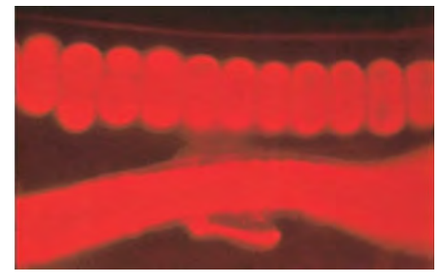

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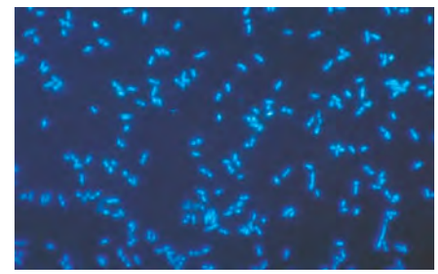


(c)

Figure 2.6 **Fluorescence microscopy.** *(a, b)* Cyanobacteria. The same cells are observed by bright-field microscopy in part a and by fluorescence microscopy in part b. The cells fluoresce red because they contain chlorophyll *a* and other pigments. *(c)* Fluorescence photomicrograph of cells of *Escherichia coli* made fluorescent by staining with the fluorescent dye DAPI.

or other fluorescing components, a phenomenon called *autofluorescence* (Figure 2.6*a*, *b*), or because the cells have been stained with a fluorescent dye (Figure 2.6*c*). DAPI (4′,6-diamidino-2-phenylindole) is a widely used fluorescent dye, staining cells bright blue because it complexes with the cell's DNA (Figure 2.6*c*). DAPI can be used to visualize cells in various habitats, such as soil, water, food, or a clinical specimen. Fluorescence microscopy using DAPI or related stains is therefore widely used in clinical diagnostic microbiology and also in microbial ecology for enumerating bacteria in a natural environment or, as in Figure 2.6*c*, in a cell suspension.

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?

2.3 Imaging Cells in Three Dimensions

Up to now we have considered forms of microscopy in which the images obtained are essentially two-dimensional. How can this limitation be overcome? We will see in the next section that the scanning electron microscope offers one solution to this problem, but certain forms of light microscopy can also improve the three-dimensional perspective of the image.

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 traverse the specimen and enter the objective lens where they are recombined into one. Because the two beams pass through different substances with slightly different refractive indices, the combined beams are not totally in phase but instead create an interference effect. This effect visibly enhances subtle differences in cell structure. Thus, by DIC microscopy, cellular structures such as the nucleus of eukaryotic cells (**Figure 2.7**), or endospores, vacuoles, and granules of bacterial cells, appear more three-dimensional. DIC microscopy is typically used for observing unstained cells because it can reveal internal cell structures that are nearly invisible by the bright-field technique (compare Figure 2.5*a* with Figure 2.7*a*).

Atomic Force Microscopy

Another type of microscope useful for three-dimensional imaging of biological structures is the atomic force microscope (AFM). In atomic force microscopy, a tiny stylus is positioned extremely close to the specimen such that weak repulsive forces are established between the probe on the stylus and atoms on the surface of the specimen. During scanning, the stylus surveys the specimen surface, continually recording any deviations from a flat surface. The pattern that is generated is processed by a series of detectors that feed the digital information into a computer, which then outputs an image (Figure 2.7*b*).

Although the images obtained from an AFM appear similar to those from the scanning electron microscope (compare Figure 2.7*b* with Figure 2.10*c*), the AFM has the advantage that the specimen does not have to be treated with fixatives or coatings. The AFM thus allows living specimens to be viewed, something that is generally not possible with electron microscopes.

Confocal Scanning Laser Microscopy

A confocal scanning laser microscope (CSLM) is a computerized microscope that couples a laser source to a fluorescent microscope. This generates a three-dimensional image and allows the viewer to profile several planes of focus in the specimen (**Figure 2.8**). The laser beam is precisely adjusted such that only a particular layer within a specimen is in perfect focus at one time. By precisely illuminating only a single plane of focus, the CSLM eliminates stray light from other focal planes. Thus, when observing a relatively thick specimen such as a microbial biofilm (Figure 2.8*a*), not only are cells on the surface of the biofilm apparent, as would be the case with conventional light microscopy, but cells in the various layers can also be observed by adjusting the plane of focus of the laser beam. Using CSLM it has been possible to improve on the 0.2-μm resolution of the compound light microscope to a limit of about 0.1 μm.

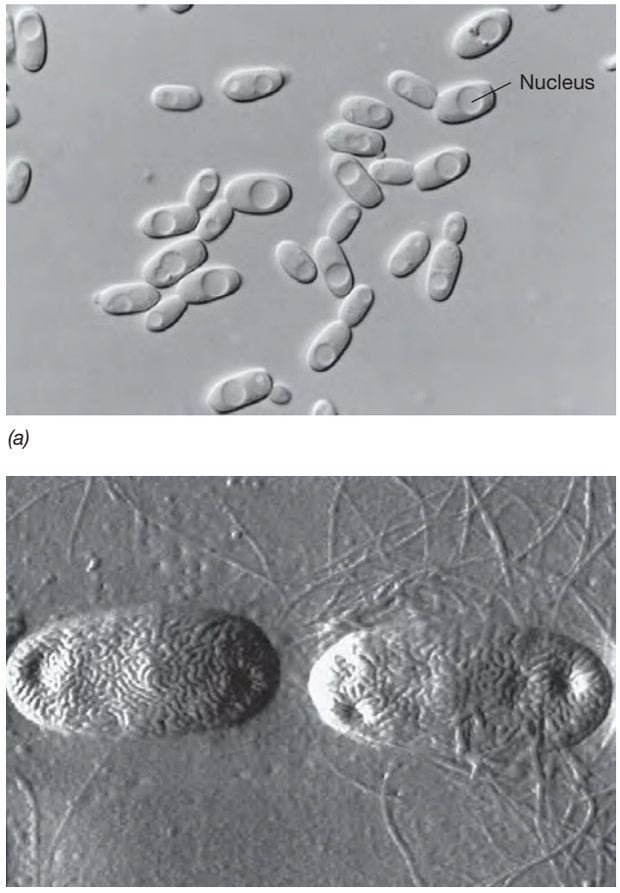
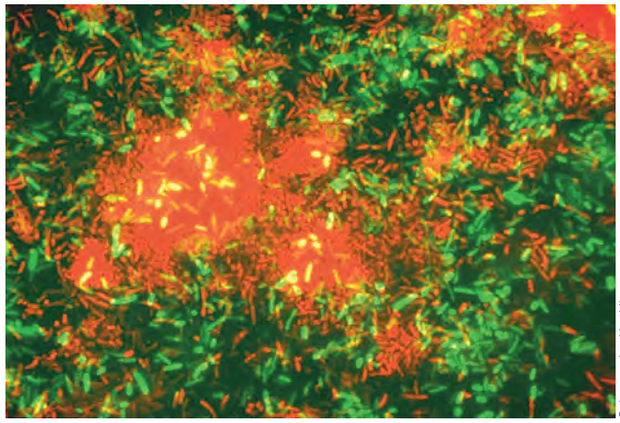


Figure 2.7 Three-dimensional imaging of cells. *(a)* Differential interference contrast and *(b)* atomic force microscopy. The yeast cells in part a are about 8 μm wide. Note the clearly visible nucleus and compare to Figure 2.5a. The bacterial cells in part b are 2.2 μm long and are from a biofilm that developed on the surface of a glass slide immersed for 24 h in a dog's water bowl.

Cells in CSLM preparations are typically stained with fluorescent dyes to make them more distinct (Figure 2.8). Alternatively, false-color images of unstained preparations can be generated such that different layers in the specimen are assigned different colors. The CLSM comes equipped with computer software that assembles digital images for subsequent image processing. Thus, images obtained from different layers can be digitally overlaid to reconstruct a three-dimensional image of the entire specimen (Figure 2.8).

CSLM has found widespread use in microbial ecology, especially for identifying populations of cells in a microbial habitat or for resolving the different components of a structured microbial habitat, such as a biofilm (Figure 2.8*a*). CSLM is particularly useful anywhere thick specimens are assessed for microbial content with depth.



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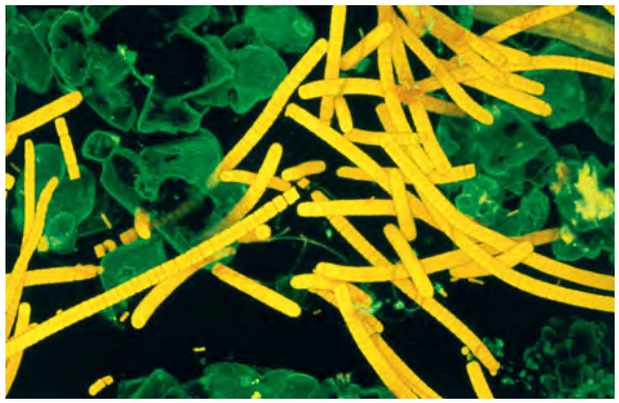


Gernot Arp and Christian Boeker, Carl Zeiss, Jena

(b)

Figure 2.8 Confocal scanning laser microscopy. *(a)* Confocal image of a microbial biofilm community cultivated in the laboratory. The green, rod-shaped cells are *Pseudomonas aeruginosa* experimentally introduced into the biofilm. Other 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.

MiniQuiz

- What structure in eukaryotic cells is more easily seen in DIC than in bright-field microscopy? (*Hint:* Compare Figures 2.5*a* and 2.7*a*).
- How is CSLM able to view different layers in a thick preparation?

2.4 Electron Microscopy

Electron microscopes use electrons instead of visible light (photons) to image cells and cell structures. Electromagnets function as lenses in the electron microscope, and the whole system operates in a vacuum (**Figure 2.9**). Electron microscopes are fitted with cameras to allow a photograph, called an *electron micrograph*, to be taken.

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 enabling one to view structures at the molecular level. This is because the wavelength of electrons is much shorter than the wavelength of visible light, and wavelength affects resolution (Section 2.1). For example, whereas the resolving power of a high-quality light microscope is about 0.2 *micrometer*, the resolving power of a high-quality TEM is about 0.2 *nanometer* (nm, 10^{-9} m). With such powerful resolution, even individual protein and nucleic acid molecules can be visualized in the transmission electron microscope (**Figure 2.10**, and see Figure 2.14*b*).

Unlike visible light, however, electron beams do not penetrate very well; even a single cell is too thick to reveal its internal contents directly by TEM. Consequently, special techniques of thin sectioning are needed to prepare specimens before observing them. A single bacterial cell, for instance, is cut into many, very thin (20–60 nm) slices, which are then examined individually by TEM (Figure 2.10*a*). To obtain sufficient contrast, the preparations 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.

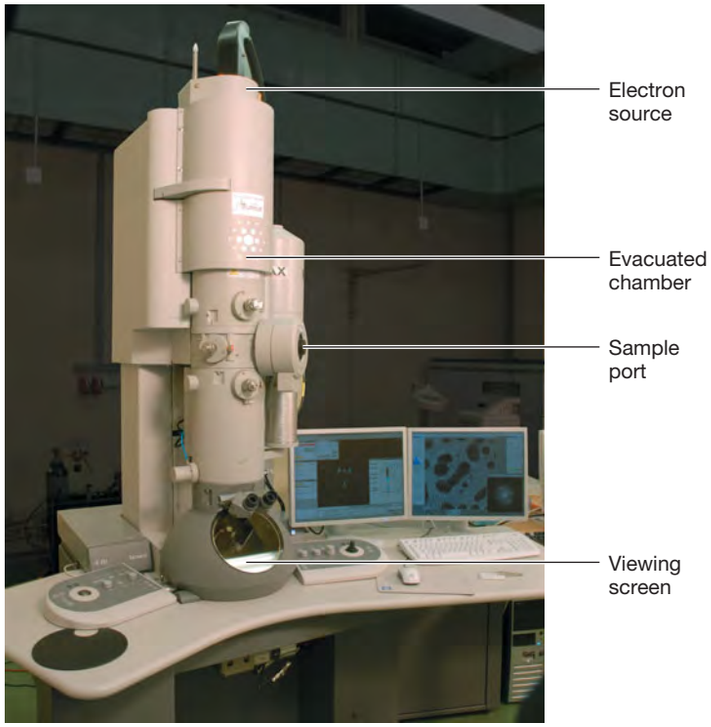
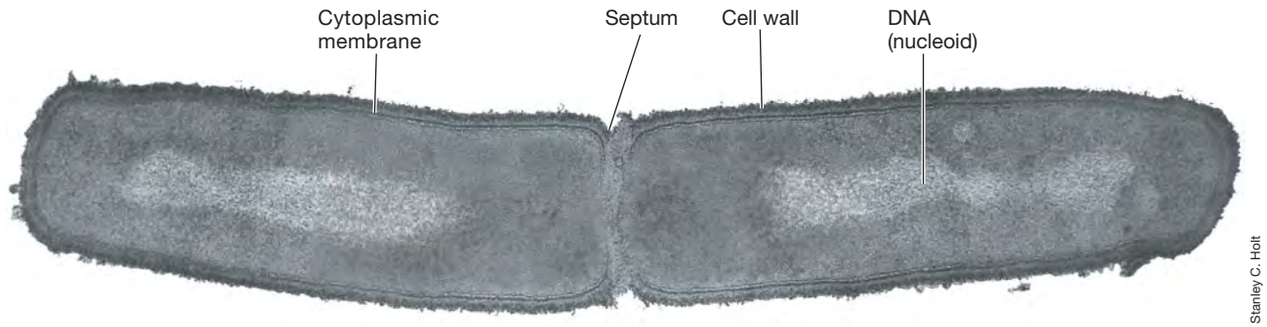


Figure 2.9 The electron microscope. This instrument encompasses both transmission and scanning electron microscope functions.

Scanning Electron Microscopy

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 by TEM with a technique called *negative staining* (Figure 2.10*b*). Alternatively, one can image the specimen using a *scanning electron microscope* (SEM) (Figure 2.9).

In scanning electron microscopy, the specimen is coated with a thin film of a heavy metal, such as gold. An electron beam then



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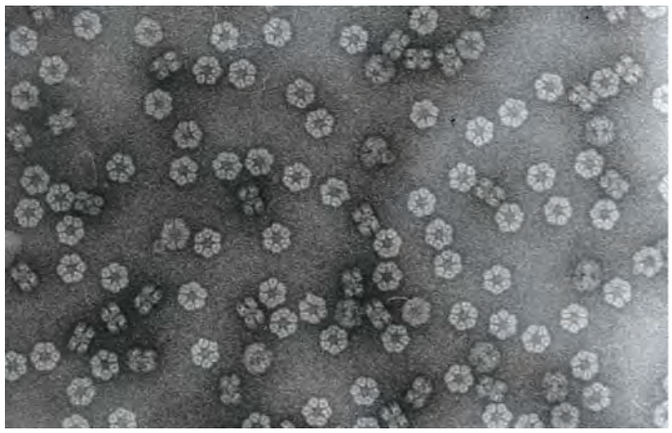

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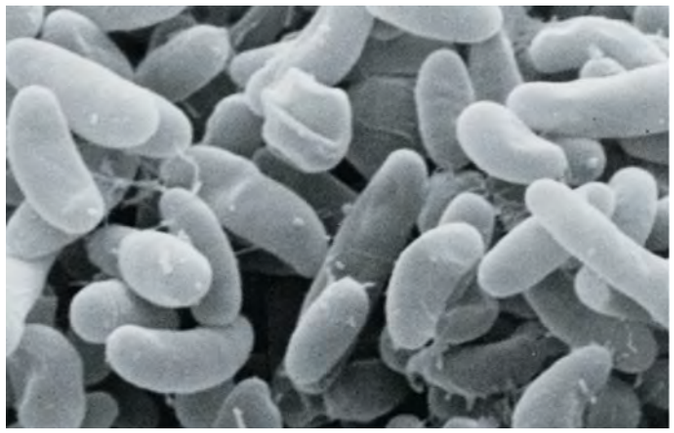


(c)

Figure 2.10 **Electron micrographs.** *(a)* Micrograph of a thin section of a dividing bacterial cell, taken by transmission electron microscopy (TEM). Note the DNA forming the nucleoid. 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 of bacterial cells. A single cell is about 0.75 μm wide.

scans back and forth across the specimen. Electrons scattered from the metal coating are collected and activate a viewing screen to produce an image (Figure 2.10*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. Often times, false color is added to these images to boost their artistic appearance by manipulating the micrographs with a computer. But false color does not improve resolution of the micrograph or the scientific information it yields; resolution is set by the magnification used to take the original micrograph.

MiniQuiz

- What is an electron micrograph? Why do electron micrographs have so much 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?

II Cell Structure and Evolutionary History

We now consider some basic concepts of microbial cell structure that underlie many topics in this book. We first compare the internal architecture of microbial cells and differentiate eukaryotic from prokaryotic cells and cells from viruses. We then explore the evolutionary tree of life to see how the major groups of microorganisms that affect our lives and our planet are related.

2.5 Elements of Microbial Structure

All cells have much in common and contain many of the same components. For example, all cells have a permeability barrier called the **cytoplasmic membrane** that separates the inside of the cell, the **cytoplasm**, from the outside (**Figure 2.11**). The cytoplasm is an aqueous mixture of macromolecules—proteins, lipids, nucleic acids, and polysaccharides—small organic molecules (mainly precursors of macromolecules), various inorganic ions, and **ribosomes**, the cell's protein-synthesizing structures.

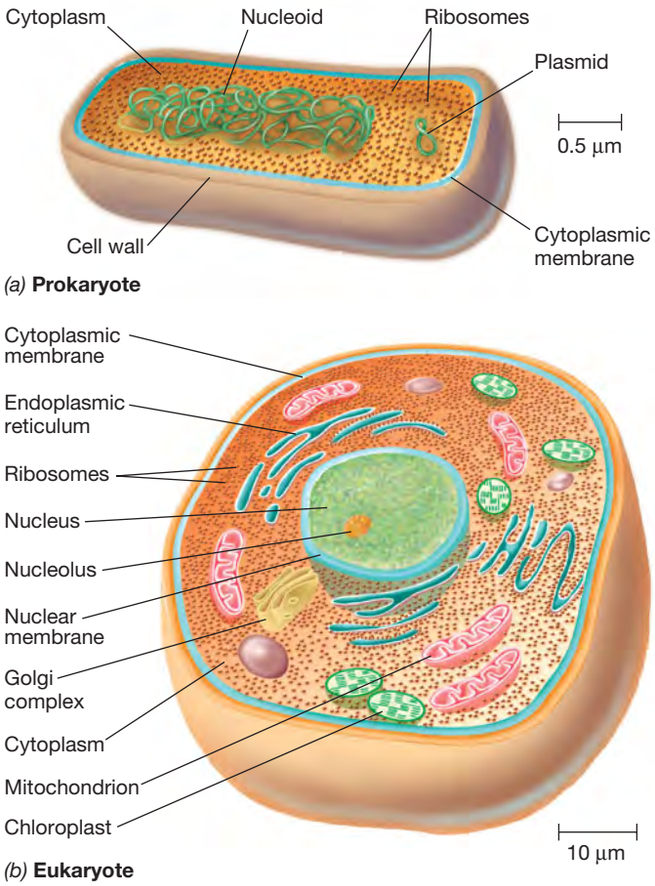


Figure 2.11 **Internal structure of cells.** Note differences in scale and internal structure between the prokaryotic and eukaryotic cells.

Ribosomes interact with cytoplasmic proteins and messenger and transfer RNAs in the key process of protein synthesis (translation).

The **cell wall** lends structural strength to a cell. The cell wall is relatively permeable and located outside the membrane (Figure 2.11*a*); it is a much stronger layer than the membrane itself. Plant cells and most microorganisms have cell walls, whereas animal cells, with rare exceptions, do not.

Prokaryotic and Eukaryotic Cells

Examination of the internal structure of cells reveals two distinct patterns: **prokaryote** and **eukaryote** (**Figure 2.12**). Eukaryotes house their DNA in a membrane-enclosed **nucleus** and are typically much larger and structurally more complex than prokaryotic cells. In eukaryotic cells the key processes of DNA replication, transcription, and translation are partitioned; replication and transcription (RNA synthesis) occur in the nucleus while translation (protein synthesis) occurs in the cytoplasm. Eukaryotic microorganisms include algae and protozoa, collectively called *protists*, and the fungi and slime molds. The cells of plants and animals are also eukaryotic cells. We consider microbial eukaryotes in detail in Chapter 20.

A major property of eukaryotic cells is the presence of membrane-enclosed structures in the cytoplasm called **organelles**. These include, first and foremost, the nucleus, but also mitochondria and chloroplasts (the latter in photosynthetic cells only) (Figures 2.2*a* and 2.12*c*). As mentioned, the nucleus houses the cell's genome and is also the site of RNA synthesis in eukaryotic cells. Mitochondria and chloroplasts are dedicated to energy conservation and carry out respiration and photosynthesis, respectively.

In contrast to eukaryotic cells, prokaryotic cells have a simpler internal structure in which organelles are absent (Figures 2.11*a*

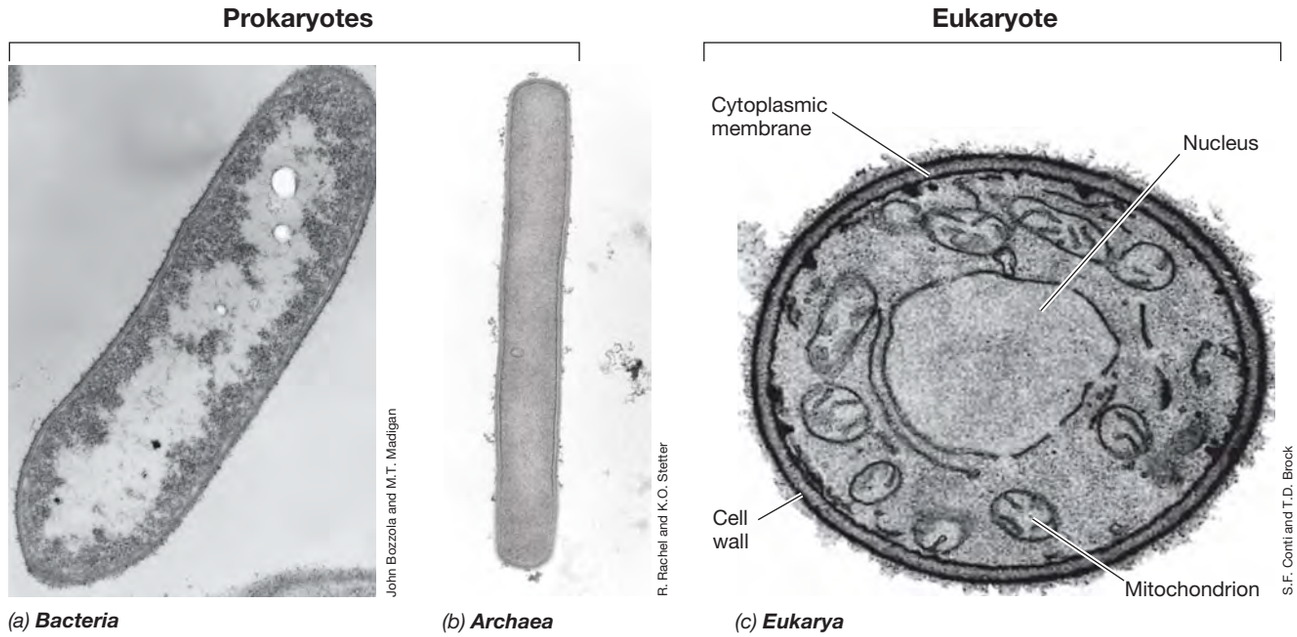


Figure 2.12 **Electron micrographs of sectioned cells from each of the domains of living organisms.** *(a) Heliobacterium modesticaldum*; the cell measures 1 × 3 μm. *(b) Methanopyrus kandleri*; the cell measures 0.5 × 4 μm. Reinhard Rachel and Karl O. Stetter, 1981. *Archives of Microbiology* 128:288–293. © Springer-Verlag GmbH & Co. KG. *(c) Saccharomyces cerevisiae*; the cell measures 8 μm in diameter.

and 2.12*a*, *b*). However, prokaryotes differ from eukaryotes in many other ways as well. For example, prokaryotes can couple transcription directly to translation because their DNA resides in the cytoplasm and is not enclosed within a nucleus as in eukaryotes. Moreover, in contrast to eukaryotes, most prokaryotes employ their cytoplasmic membrane in energy-conservation reactions and have small, compact genomes consisting of circular DNA, as discussed in the next section. In terms of cell size, a typical rod-shaped prokaryote is 1–5 μm long and about 1 μm wide, but considerable variation is possible (↻ Table 3.1). The range of sizes in eukaryotic cells is quite large. Eukaryotic cells are known with diameters as small as 0.8 μm or as large as several hundred micrometers. We revisit the subject of cell size in more detail in Section 3.2.

Despite the many clear-cut *structural* differences between prokaryotes and eukaryotes, it is very important that the word "prokaryote" not be given an *evolutionary* connotation. As was touched on in Chapter 1, the prokaryotic world consists of two evolutionarily distinct groups, the *Bacteria* and the *Archaea*. Moreover, the word "prokaryote" should not be considered synonymous with "primitive," as all cells living today—whether prokaryotes or eukaryotes—are highly evolved and closely adapted to their habitat. In Chapters 6 and 7 we compare and contrast the molecular biology of *Bacteria* and *Archaea*, highlighting their similarities and differences and relating them to molecular processes in eukaryotes.

Viruses

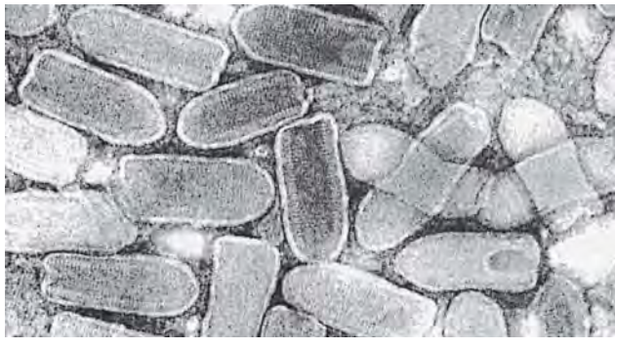
Viruses are a major class of microorganisms, but they are not cells (**Figure 2.13**). Viruses are much smaller than cells and lack many of the attributes of cells (↻ Figure 1.3). Viruses vary in size, with the smallest known viruses being only about 10 nm in diameter.

Instead of being a dynamic open system, a virus particle is static and stable, unable to change or replace its parts by itself. Only when a virus infects a cell does it acquire the key attribute of a living system—replication. Unlike cells, viruses have no metabolic capabilities of their own. Although they contain their own genomes, viruses lack ribosomes. So to synthesize proteins, viruses depend on the biosynthetic machinery of the cells they have infected. Moreover, unlike cells, viral particles contain only a single form of nucleic acid, either DNA or RNA (this means, of course, that some viruses have RNA genomes).

Viruses are known to infect all types of cells, including microbial cells. Many viruses cause disease in the organisms they infect. However, viral infection can have many other effects on cells, including genetic alterations that can actually improve the capabilities of the cell. We discuss the field of virology and viral diversity in detail in Chapters 9 and 21, respectively.

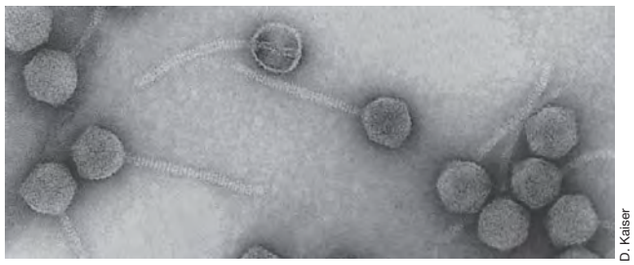
MiniQuiz

- What important functions do the following play in a cell: cytoplasmic membrane, ribosomes, cell wall?
- By looking inside a cell how could you tell if it was a prokaryote or a eukaryote?
- How are viruses like cells, and in which major ways do they differ?



Erskine Caldwell

(a)



D. Kaiser

(b)

Figure 2.13 **Viruses.** *(a)* Particles of rhabdovirus (a virus that infects plants and animals). A single virus particle, called a *virion*, is about 65 nm (0.065 μm) wide. *(b)* Bacterial virus (bacteriophage) lambda. The head of each lambda virion is also about 65 nm wide. Viruses are composed of protein and nucleic acid and do not have structures such as walls or a cytoplasmic membrane.

2.6 Arrangement of DNA in Microbial Cells

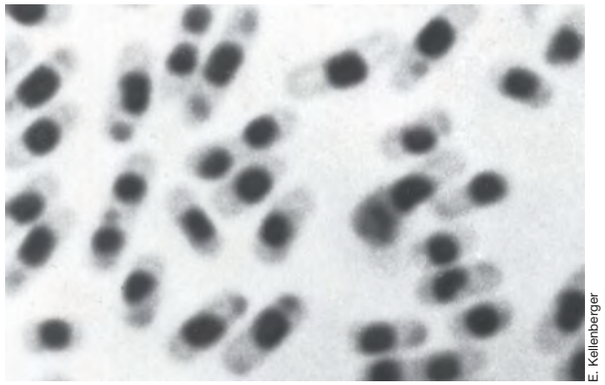
The life processes of any cell are governed by its complement of genes, its *genome*. A gene is a segment of DNA (or RNA in RNA viruses) that encodes a protein or an RNA molecule. Here we consider how genomes are organized in prokaryotic and eukaryotic cells and consider the number of genes and proteins present in a model prokaryotic cell.

Nucleus versus Nucleoid

The genomes of prokaryotic and eukaryotic cells are organized differently. In most prokaryotic cells, DNA is present in a circular molecule called the *chromosome*; a few prokaryotes have a linear instead of a circular chromosome. The chromosome aggregates within the cell to form a mass called the **nucleoid**, visible in the electron microscope (**Figure 2.14**; see also Figure 2.10*a*).

Most prokaryotes have only a single chromosome. Because of this, they typically contain only a single copy of each gene and are therefore genetically *haploid*. Many prokaryotes also contain one or more small circles of DNA distinct from that of the chromosome, called **plasmids**. Plasmids typically contain genes that confer a special property (such as a unique metabolism) on a cell, rather than essential genes. This is in contrast to genes on the chromosome, most of which are needed for basic survival.

In eukaryotes, DNA is arranged in linear molecules within the membrane-enclosed nucleus; the DNA molecules are packaged



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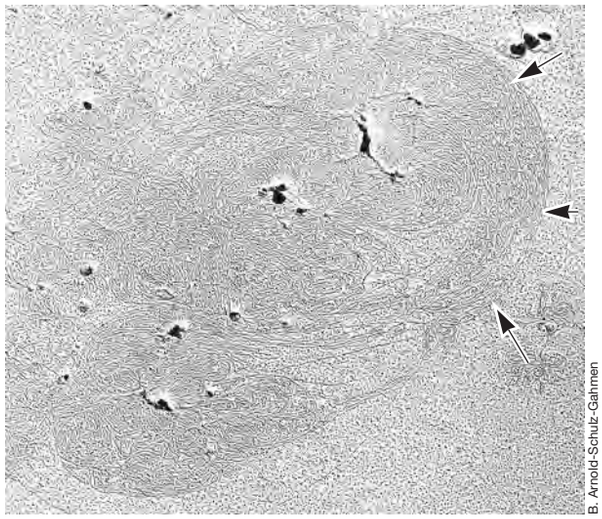


(b)

Figure 2.14 **The nucleoid.** *(a)* Photomicrograph of cells of *Escherichia coli* treated in such a way as to make the nucleoid visible. A single cell is about 3 μm and a nucleoid about 1 μm long. *(b)* Transmission electron micrograph of an isolated nucleoid released from a cell of *E. coli*. The cell was gently lysed to allow the highly compacted nucleoid to emerge intact. Arrows point to the edge of DNA strands.

with proteins and organized to form **chromosomes**. Chromosome number varies by organism. For example, a diploid cell of the baker's yeast *Saccharomyces cerevisiae* contains 32 chromosomes arranged in 16 pairs while human cells contain 46 chromosomes (23 pairs). Chromosomes in eukaryotes contain proteins that assist in folding and packing the DNA and other proteins that are required for transcription. A key genetic difference between prokaryotes and eukaryotes is that eukaryotes typically contain two copies of each gene and are thus genetically *diploid*. During cell division in eukaryotic cells the nucleus divides (following a doubling of chromosome number) in the process called *mitosis* (**Figure 2.15**). Two identical daughter cells result, with each daughter cell receiving a full complement of genes. The diploid genome of eukaryotic cells is halved in the process of *meiosis* to form haploid gametes for sexual reproduction. Fusion of two gametes during zygote formation restores the cell to the diploid state.

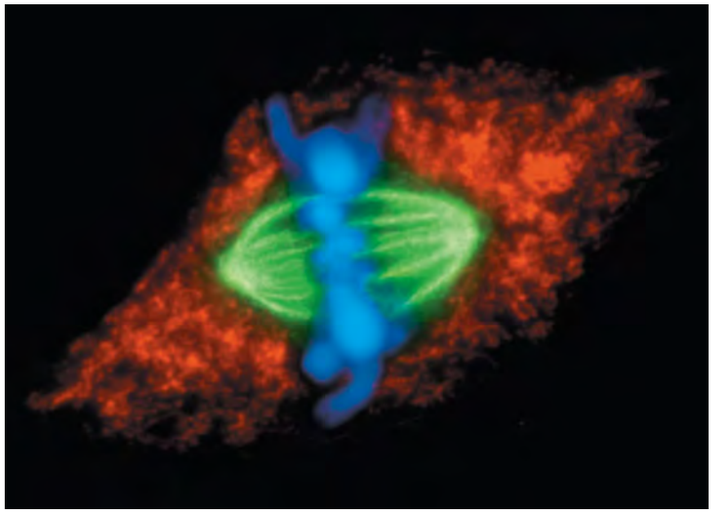


Figure 2.15 **Mitosis in stained kangaroo rat cells.** The cell was photographed while in the metaphase stage of mitotic division; only eukaryotic cells undergo mitosis. The green color stains a protein called tubulin, important in pulling chromosomes apart. The blue color is from a DNA-binding dye and shows the chromosomes.

Genes, Genomes, and Proteins

How many genes and proteins does a cell have? The genome of *Escherichia coli,* a model bacterium, is a single circular chromosome of 4,639,221 base pairs of DNA. Because the *E. coli* genome has been completely sequenced, we also know that it contains 4288 genes. The genomes of a few prokaryotes have three times this many genes, while the genomes of others contain fewer than one-twentieth as many. Eukaryotic cells typically have much larger genomes than prokaryotes. A human cell, for example, contains over 1000 times as much DNA as a cell of *E. coli* and about seven times as many genes.

Depending somewhat on growth conditions, a cell of *E. coli* contains about 1900 different kinds of proteins and about 2.4 million individual protein molecules. However, some proteins in *E. coli* are very abundant, others are only moderately abundant, and some are present in only one or a very few copies per cell. Thus, *E. coli* has mechanisms for regulating its genes so that not all genes are *expressed* (transcribed and translated) at the same time or to the same extent. Gene regulation is important to all cells, and we focus on the major mechanisms of gene regulation in Chapter 8.

MiniQuiz

- Differentiate between the nucleus and the nucleoid.
- What does it mean to say that a bacterial cell is haploid?
- Why does it make sense that a human cell would have more genes than a bacterial cell?

2.7 The Evolutionary Tree of Life

Evolution is the process of descent with modification that generates new varieties and eventually new species of organisms. Evolution occurs in any self-replicating system in which variation is

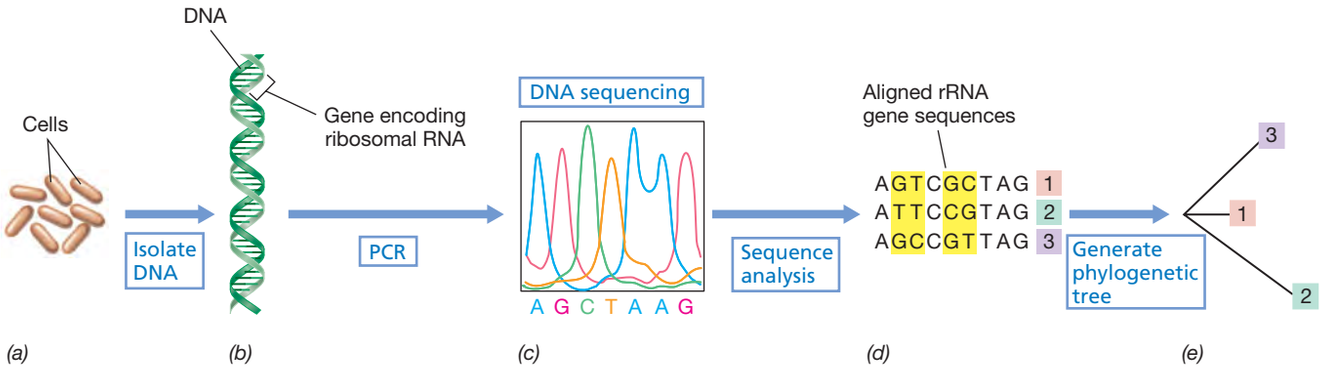


Figure 2.16 Ribosomal RNA (rRNA) gene sequencing and phylogeny. *(a)* DNA is extracted from cells. *(b)* Many identical copies of a gene encoding rRNA are made by the polymerase chain reaction (↻ Section 6.11). *(c, d)* The gene is sequenced and the sequence aligned with rRNA sequences from other organisms. A computer algorithm makes pairwise comparisons at each base and generates a phylogenetic tree *(e)* that depicts evolutionary divergence. 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.

the result of mutation and selection is based on differential fitness. Thus, over time, both cells and viruses evolve.

Determining Evolutionary Relationships

The evolutionary relationships between organisms are the subject of **phylogeny**. Phylogenetic relationships between cells can be deduced by comparing the genetic information (nucleotide or amino acid sequences) that exists in their nucleic acids or proteins. For reasons that will be presented later, macromolecules that form the ribosome, in particular *ribosomal RNAs* (*rRNA*), are excellent tools for discerning evolutionary relationships. Because all cells contain ribosomes (and thus rRNA), this molecule can and has been used to construct a phylogenetic tree of all cells, including microorganisms (see Figure 2.17). Carl Woese, an American microbiologist, pioneered the use of comparative rRNA sequence analysis as a measure of microbial phylogeny and, in so doing, revolutionized our understanding of cellular evolution. Viral phylogenies have also been determined, but because these microorganisms lack ribosomes, other molecules have been used for evolutionary metrics.

The steps in generating an RNA-based phylogenetic tree are outlined in **Figure 2.16**. In brief, genes encoding rRNA from two or more organisms are sequenced and the sequences aligned and scored, base-by-base, for sequence differences and identities using a computer; the greater the sequence variation between any two organisms, the greater their evolutionary divergence. Then, using a treeing algorithm, this divergence is depicted in the form of a phylogenetic tree.

The Three Domains of Life

From comparative rRNA sequencing, three phylogenetically distinct cellular lineages have been revealed. The lineages, called **domains**, are the ***Bacteria*** and the ***Archaea*** (both consisting of prokaryotic cells) and the ***Eukarya*** (eukaryotes) (**Figure 2.17**). The domains are thought to have diverged from a common ancestral organism (LUCA in Figure 2.17) early in the history of life on Earth.

The phylogenetic tree of life reveals two very important evolutionary facts: (1) As previously stated, all prokaryotes are *not* phylogenetically closely related, and (2) *Archaea* are actually more closely related to *Eukarya* than to *Bacteria* (Figure 2.17). Thus, from the last universal common ancestor (LUCA) of all life forms on Earth, evolutionary diversification diverged to yield the ancestors of the *Bacteria* and of a second main lineage (↻ Figure 1.6). The latter once again diverged to yield the ancestors of the *Archaea*, a lineage that retained a prokaryotic cell structure, and the *Eukarya*, which did not. The universal tree of life shows that LUCA resides very early within the *Bacteria* domain (Figure 2.17).

Eukarya

Because the cells of animals and plants are all eukaryotic, it follows that eukaryotic microorganisms were the ancestors of multicellular organisms. The tree of life clearly bears this out. As expected, microbial eukaryotes branch off early on the eukaryotic lineage, while plants and animals branch near the crown of the tree (Figure 2.17). However, molecular sequencing and several other lines of evidence have shown that eukaryotic cells contain genes from cells of two domains. In addition to the genome in the chromosomes of the nucleus, mitochondria and chloroplasts of eukaryotes contain their own genomes (this DNA is arranged in a circular fashion, as in most prokaryotes), and ribosomes. Using molecular phylogenetic analyses (Figure 2.16), these organelles have been shown to be highly derived ancestors of specific lineages of *Bacteria* (Figure 2.17 and Section 2.9). Mitochondria and chloroplasts are therefore descendants of what are thought to have been free-living bacterial cells that developed an intimate intracellular association with cells of the *Eukarya* domain eons ago. The theory of how this stable arrangement of cells led to the modern eukaryotic cell with organelles has been called **endosymbiosis** (*endo* means "inside") and is discussed in Chapters 16 and 20.

Contributions of Molecular Sequencing to Microbiology

Molecular phylogeny has not only revealed the evolutionary connections between all cells—prokaryotes and eukaryotes—it has

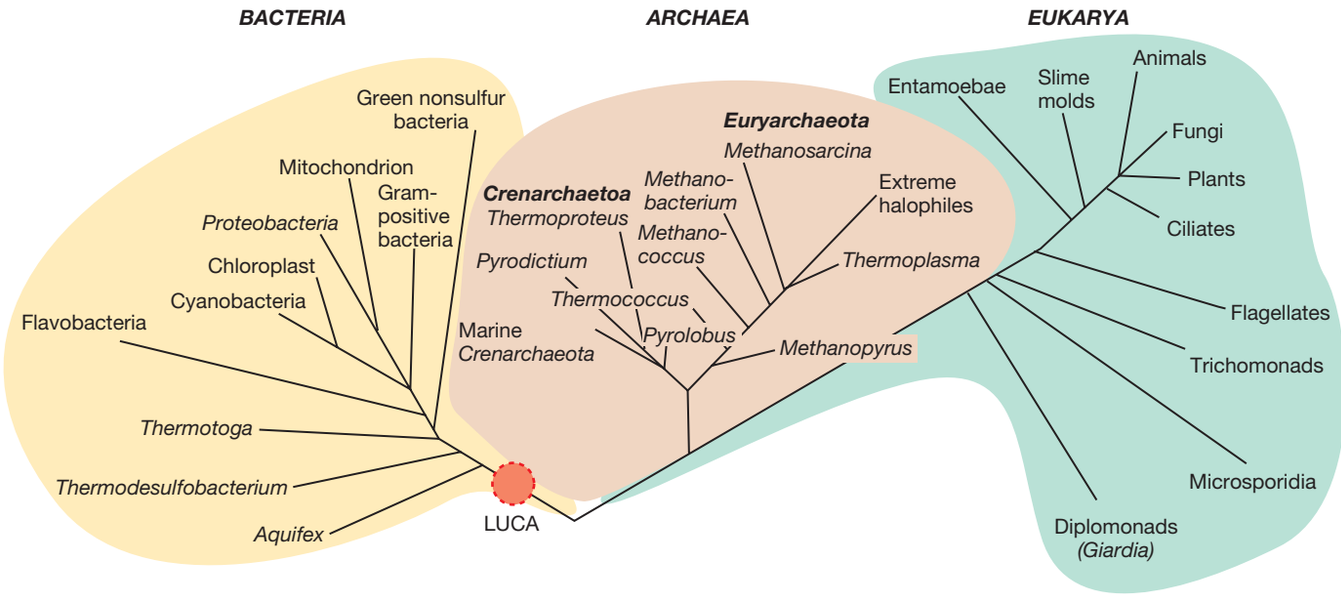


Figure 2.17 The phylogenetic tree of life as defined by comparative rRNA gene sequencing. The tree shows the three domains of organisms and a few representative groups in each domain. All *Bacteria* and *Archaea* and most *Eukarya* are microscopic organisms; only plants, animals, and fungi contain macroorganisms. Phylogenetic trees of each domain can be found in Figures 2.19, 2.28, and 2.32. LUCA, last universal common ancestor.

formed the first evolutionary framework for the prokaryotes, something that the science of microbiology had been without since its inception. In addition, molecular phylogeny has spawned exciting new research tools that have affected many subdisciplines of microbiology, in particular, microbial systematics and ecology, and clinical diagnostics. In these areas molecular phylogenetic methods have begun to shape our concept of a bacterial species and given microbial ecologists and clinical microbiologists the capacity to identify organisms without actually culturing them. This has greatly improved our picture of microbial diversity and has led to the staggering conclusion that most of the microbial diversity that exists on Earth has yet to be brought into laboratory culture.

MiniQuiz

- How can species of *Bacteria* and *Archaea* be distinguished by molecular criteria?
- What is endosymbiosis, and in what way did it benefit eukaryotic cells?

III Microbial Diversity

The diversity of microorganisms we see today is the result of nearly 4 billion years of evolution. Microbial diversity can be seen in many ways besides phylogeny, including cell size and morphology (shape), physiology, motility, mechanism of cell division, pathogenicity, developmental biology, adaptation to environmental extremes, and so on. In the following sections we paint a picture of microbial diversity with a broad brush. We then return to reconsider the topic in more detail in Chapters 16–21.

Our discussion of *microbial* diversity begins with a brief consideration of *metabolic* diversity. The two topics are closely linked. Through eons, microorganisms, especially the prokaryotes, have come to exploit every means of "making a living" consistent with the laws of chemistry and physics. This enormous metabolic versatility has allowed prokaryotes to thrive in every potential habitat on Earth suitable for life.

2.8 Metabolic Diversity

All cells require an energy source and a metabolic strategy for conserving energy from it to drive energy-consuming life processes. As far as is known, energy can be tapped from three sources in nature: organic chemicals, inorganic chemicals, and light (**Figure 2.18**).

Chemoorganotrophs

Organisms that conserve energy from chemicals are called *chemotrophs*, and those that use *organic* chemicals are called **chemoorganotrophs** (Figure 2.18). Thousands of different organic chemicals can be used by one or another microorganism. Indeed, all natural and even most synthetic organic compounds can be metabolized. Energy is conserved from the *oxidation* of the compound and is stored in the cell in the energy-rich bonds of the compound adenosine triphosphate (ATP).

Some microorganisms can obtain energy from an organic compound only in the presence of oxygen; these organisms are called *aerobes*. Others can obtain energy only in the absence of oxygen (*anaerobes*). Still others can break down organic compounds in either the presence or absence of oxygen. Most microorganisms that have been brought into laboratory culture are chemoorganotrophs.

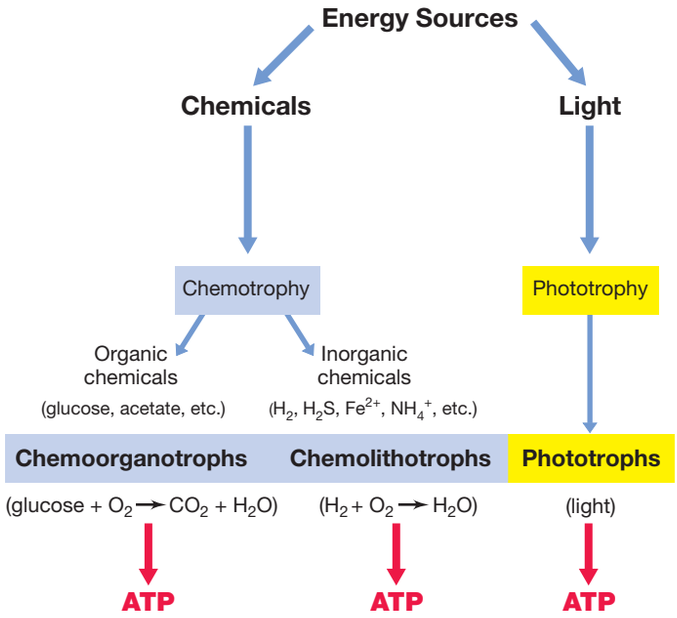


Figure 2.18 Metabolic options for conserving energy. The organic and inorganic chemicals listed here are just a few of the chemicals used by one organism or another. Chemotrophic organisms oxidize organic or inorganic chemicals, which yields ATP. Phototrophic organisms use solar energy to form ATP.

Chemolithotrophs

Many prokaryotes can tap the energy available from the oxidation of *inorganic* compounds. This form of metabolism is called *chemolithotrophy* and was discovered by the Russian microbiologist Winogradsky (↻ Section 1.9). Organisms that carry out chemolithotrophic reactions are called **chemolithotrophs** (Figure 2.18). Chemolithotrophy occurs only in prokaryotes and is widely distributed among species of *Bacteria* and *Archaea*. Several inorganic compounds can be oxidized; for example, H_2, H_2S (hydrogen sulfide), NH_3 (ammonia), and Fe^{2+} (ferrous iron). Typically, a related group of chemolithotrophs specializes in the oxidation of a related group of inorganic compounds, and thus we have the "sulfur" bacteria, the "iron" bacteria, and so on.

The capacity to conserve energy from the oxidation of inorganic chemicals is a good metabolic strategy because competition from chemoorganotrophs, organisms that require organic energy sources, is not an issue. In addition, many of the inorganic compounds oxidized by chemolithotrophs, for example H_2 and H_2S, are actually the waste products of chemoorganotrophs. Thus, chemolithotrophs have evolved strategies for exploiting resources that chemoorganotrophs are unable to use, so it is common for species of these two physiological groups to live in close association with one another.

Phototrophs

Phototrophic microorganisms contain pigments that allow them to convert light energy into chemical energy, and thus their cells appear colored (Figure 2.2). Unlike chemotrophic organisms, then, **phototrophs** do not require chemicals as a source of energy. This is a significant metabolic advantage because competition with chemotrophic organisms for energy sources is not an issue and sunlight is available in many microbial habitats on Earth.

Two major forms of phototrophy are known in prokaryotes. In one form, called *oxygenic* photosynthesis, oxygen (O_2) is produced. Among microorganisms, oxygenic photosynthesis is characteristic of cyanobacteria and algae. The other form, *anoxygenic* photosynthesis, occurs in the purple and green bacteria and the heliobacteria, and does not yield O_2. However, both oxygenic and anoxygenic phototrophs have great similarities in their mechanism of ATP synthesis, a result of the fact that oxygenic photosynthesis evolved from the simpler anoxygenic form, and we return to this topic in Chapter 13.

Heterotrophs and Autotrophs

All cells require carbon in large amounts and can be considered either **heterotrophs**, which require organic compounds as their carbon source, or **autotrophs**, which use carbon dioxide (CO_2) as their carbon source. Chemoorganotrophs are by definition heterotrophs. By contrast, most chemolithotrophs and phototrophs are autotrophs. Autotrophs are sometimes called *primary producers* because they synthesize new organic matter from CO_2 for both their own benefit and that of chemoorganotrophs. The latter either feed directly on the cells of primary producers or live off products they excrete. Virtually all organic matter on Earth has been synthesized by primary producers, in particular, the phototrophs.

Habitats and Extreme Environments

Microorganisms are present everywhere on Earth that will support life. These include habitats we are all familiar with—soil, water, animals, and plants—as well as virtually any structures made by humans. Indeed, sterility (the absence of life forms) in a natural sample is extremely rare.

Some microbial habitats are ones in which humans could not survive, being too hot or too cold, too acidic or too caustic, or too salty. Although such environments would pose challenges to any life forms, they are often teeming with microorganisms. Organisms inhabiting such extreme environments are called **extremophiles**, a remarkable group of microorganisms that collectively define the physiochemical limits to life (**Table 2.1**).

Extremophiles abound in such harsh environments as volcanic hot springs; on or in the ice covering lakes, glaciers, or the polar seas; in extremely salty bodies of water; in soils and waters having a pH as low as 0 or as high as 12; and in the deep sea, where hydrostatic pressure can exceed 1000 times atmospheric. Interestingly, these prokaryotes do not just *tolerate* their particular environmental extreme, they actually *require* it in order to grow. That is why they are called extremophiles (the suffix *-phile* means "loving"). Table 2.1 summarizes the current "record holders" among extremophiles and lists the terms used to describe each class and the types of habitats in which they reside. We will revisit many of these organisms in later chapters and examine the special properties that allow for their growth in extreme environments.

Table 2.1 *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	**5°C**	10°C
pH							
Low	Acidophile	*Picrophilus oshimae*	*Archaea*	Acidic hot springs	−0.06	**0.7**[c]	4
High	Alkaliphile	*Natronobacterium gregoryi*	*Archaea*	Soda lakes	8.5	**10**[d]	12
Pressure	Barophile (Piezophile)	*Moritella yayanosii*[e]	*Bacteria*	Deep ocean sediments	500 atm	**700 atm**	>1000 atm
Salt (NaCl)	Halophile	*Halobacterium salinarum*	*Archaea*	Salterns	15%	**25%**	32% (saturation)

[a]The organisms listed are the current "record holders" for growth at a particular extreme condition.
[b]Anaerobe showing growth at 122°C only under several atmospheres of pressure.
[c]*P. oshimae* is also a thermophile, growing optimally at 60°C.
[d]*N. gregoryi* is also an extreme halophile, growing optimally at 20% NaCl.
[e]*M. yayanosii* is also a psychrophile, growing optimally near 4°C.

MiniQuiz

- In terms of energy generation, how does a chemoorganotroph differ from a chemolithotroph?
- In terms of carbon acquisition, how does an autotroph differ from a heterotroph?
- What are extremophiles?

2.9 *Bacteria*

As we have seen, prokaryotes have diverged into two phylogenetically distinct domains, the *Archaea* and the *Bacteria* (Figure 2.17). We begin with the *Bacteria*, because most of the best-known prokaryotes reside in this domain.

Proteobacteria

The domain *Bacteria* contains an enormous variety of prokaryotes. All known disease-causing (pathogenic) prokaryotes are *Bacteria*, as are thousands of nonpathogenic species. A large variety of morphologies and physiologies are also observed in this domain. The ***Proteobacteria*** make up the largest phylum of *Bacteria* (**Figure 2.19**). Many chemoorganotrophic bacteria are *Proteobacteria,* including *Escherichia coli*, the model organism of microbial physiology, biochemistry, and molecular biology. Several phototrophic and chemolithotrophic species are also *Proteobacteria* (**Figure 2.20**). Many of these use H_2S in their metabolism, producing elemental sulfur (S^0) that is stored either inside or outside the cell (Figure 2.20). Sulfur is an oxidation product of H_2S and is further oxidized to sulfate (SO_4^{2-}). Sulfide and sulfur are oxidized to fuel important metabolic functions such as CO_2 fixation (autotrophy) or energy conservation (Figure 2.18).

Several other common prokaryotes of soil and water, and species that live in or on plants and animals in both harmless and disease-causing ways, are *Proteobacteria.* These include species of *Pseudomonas,* many of which can degrade complex or toxic natural and synthetic organic compounds, and *Azotobacter,* a bacterium that fixes nitrogen (utilizes gaseous nitrogen as a nitrogen source, Figure 1.9). A number of key pathogens are *Proteobacteria*, including *Salmonella* (gastrointestinal diseases), *Rickettsia* (typhus and Rocky Mountain spotted fever), *Neisseria* (gonorrhea), and many others. And finally, the key respiratory organelle of eukaryotes, the mitochondrion, has evolutionary roots within the *Proteobacteria* (Figure 2.17).

Gram-Positive Bacteria

As we learned in Section 2.2, bacteria can be distinguished by the Gram-staining procedure, a technique that stains cells either gram-positive or gram-negative. The gram-positive phylum of *Bacteria* (Figure 2.19) contains many organisms that are united by their common phylogeny and cell wall structure. Here we find the endospore-forming *Bacillus* (discovered by Ferdinand Cohn,

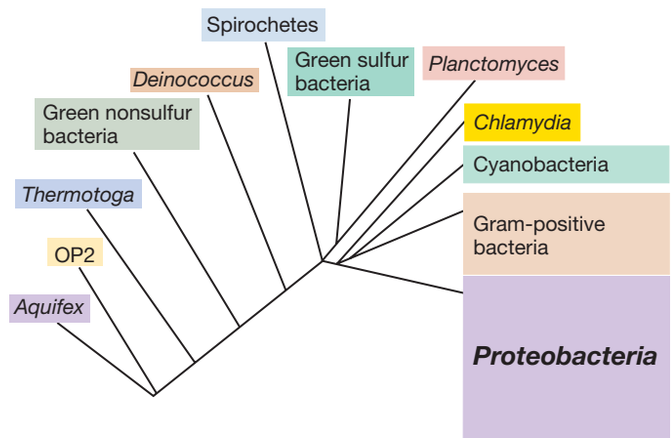
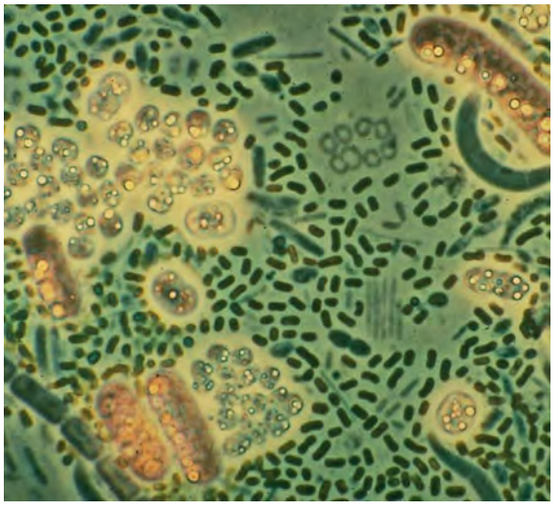


Figure 2.19 **Phylogenetic tree of some representative *Bacteria*.** The *Proteobacteria* are by far the largest phylum of *Bacteria* known. The lineage on the tree labeled OP2 does not represent a cultured organism but instead is an rRNA gene isolated from an organism in a natural sample. In this example, the closest known relative of OP2 would be *Aquifex*. Many thousands of other environmental sequences are known, and they branch all over the tree. Environmental sequences are also called *phylotypes*, and the technology for deriving them is considered in Section 22.4.



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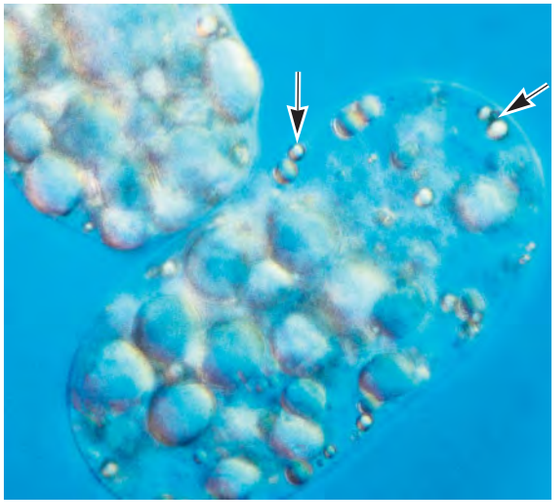

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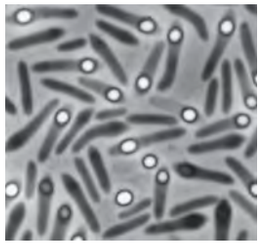
Figure 2.20 **Phototrophic and chemolithotrophic *Proteobacteria.*** *(a)* The phototrophic purple sulfur bacterium *Chromatium* (the large, red-orange, rod-shaped cells in this photomicrograph of a natural microbial community). A cell is about 10 μm wide. *(b)* The large chemolithotrophic sulfur-oxidizing bacterium *Achromatium*. A cell is about 20 μm wide. Globules of elemental sulfur can be seen in the cells (arrows). Both of these organisms oxidize hydrogen sulfide (H_2S).

Section 1.6) (**Figure 2.21**) and *Clostridium* and related spore-forming bacteria, such as the antibiotic-producing *Streptomyces*. Also included here are the lactic acid bacteria, common inhabitants of decaying plant material and dairy products that include organisms such as *Streptococcus* (Figure 2.21*b*) and *Lactobacillus*. Other interesting bacteria that fall within the gram-positive bacteria are the mycoplasmas. These bacteria lack a cell wall and have very small genomes, and many of them are pathogenic. *Mycoplasma* is a major genus of pathogenic bacteria in this medically important group. Cells of some *Archaea*, such as *Thermoplasma* (see Figure 2.31) and *Ferroplasma*, also lack cell walls.



(a)

(b)

Figure 2.21 **Gram-positive bacteria.** *(a)* The rod-shaped endospore-forming bacterium *Bacillus*. Note the presence of endospores (bright refractile structures) inside the cells. Endospores are extremely resistant to heat, chemicals, and radiation. Cells are about 1.6 μm in diameter. *(b) Streptococcus*, a spherical cell that forms cell chains. Streptococci are widespread in dairy products, and some are potent pathogens. Cells are about 0.8 μm in diameter.

Cyanobacteria

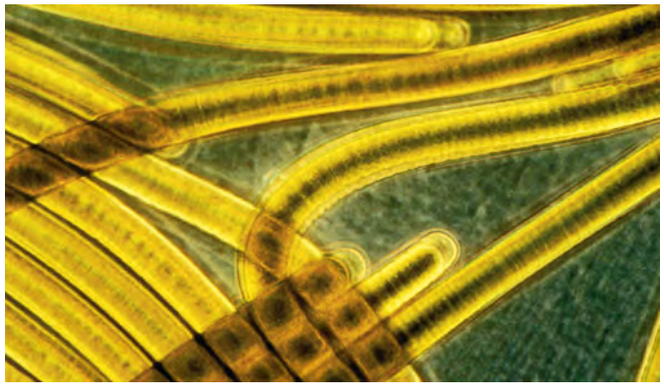
The **cyanobacteria** are phylogenetic relatives of gram-positive bacteria (Figure 2.19) and are oxygenic phototrophs. The photosynthetic organelle of eukaryotic phototrophs, the chloroplast (Figure 2.2*a*), is related to the cyanobacteria (Figure 2.17). Cyanobacteria were key players in the evolution of life, as they were the first oxygenic phototrophs to evolve on Earth. The production of O_2 on an originally anoxic Earth paved the way for the evolution of cells that could respire using oxygen. The development of higher organisms, such as the plants and animals, followed billions of years later when Earth had a more oxygen-rich environment (Figure 1.6). Cells of some cyanobacteria join to form filaments (**Figure 2.22**). Many other morphological forms of cyanobacteria are known, including unicellular, colonial, and heterocystous. Species in the latter group contain special structures called *heterocysts* that carry out nitrogen fixation.

Other Major Phyla of *Bacteria*

Several phyla of *Bacteria* contain species with unique morphologies and almost all of these stain gram-negatively. These lineages include the aquatic planctomycetes, characterized by cells with a distinct stalk that allows the organisms to attach to a solid substratum (**Figure 2.23**), and the helically shaped spirochetes (**Figure 2.24**). Several diseases, most notably syphilis and Lyme disease, are caused by spirochetes.

Two other major phyla of *Bacteria* are phototrophic: the green sulfur bacteria and the green nonsulfur bacteria (*Chloroflexus* group) (**Figure 2.25**). Species in both of these lineages contain similar photosynthetic pigments and are also autotrophs. *Chloroflexus* is a filamentous phototroph that inhabits hot springs and associates with cyanobacteria to form microbial mats, which are laminated microbial communities containing both phototrophs and chemotrophs. *Chloroflexus* is also noteworthy because its ancient relatives may have been the first phototrophic bacteria on Earth.

Other major phyla of *Bacteria* include the *Chlamydiae* and *Deinococcus-Thermus* groups (Figure 2.19). The phylum *Chlamydiae* harbors respiratory and sexually transmitted pathogens of humans. Chlamydia are intracellular parasites, cells



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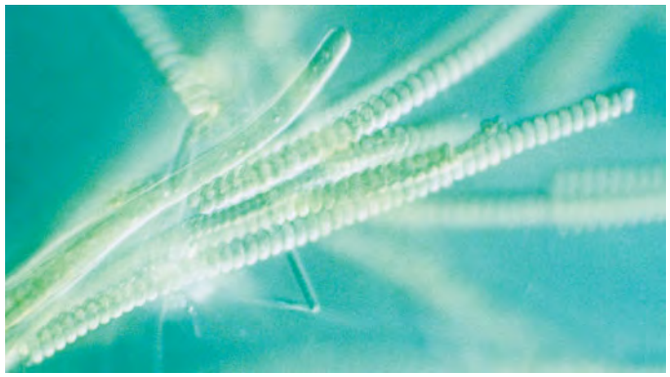

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Figure 2.22 **Filamentous cyanobacteria.** *(a) Oscillatoria*, *(b) Spirulina*. Cells of both organisms are about 10 μm wide. Cyanobacteria are oxygenic phototrophs.

that live *inside* the cells of higher organisms, in this case, human cells. Several other pathogenic bacteria (for example, *Rickettsia,* described previously, and the gram-positive *Mycobacterium tuberculosis,* the cause of tuberculosis) are also intracellular pathogens. By living inside their host's cells, these pathogens avoid destruction by the host's immune response.

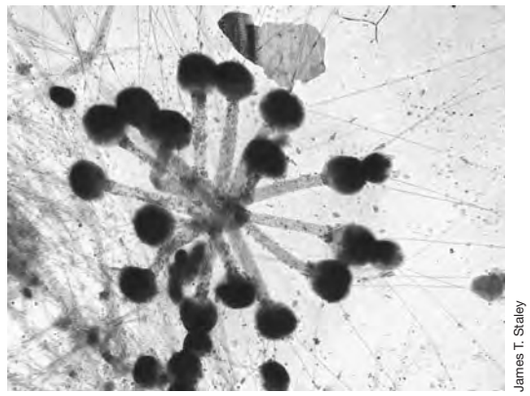


Figure 2.23 **The morphologically unusual stalked bacterium *Planctomyces.*** Shown are several cells attached by their stalks to form a rosette. Cells are about 1.4 μm wide.

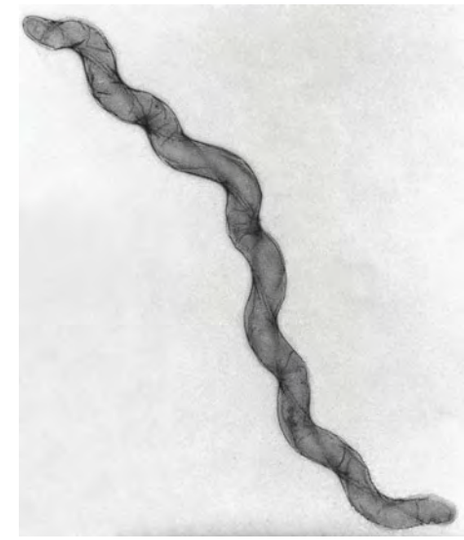
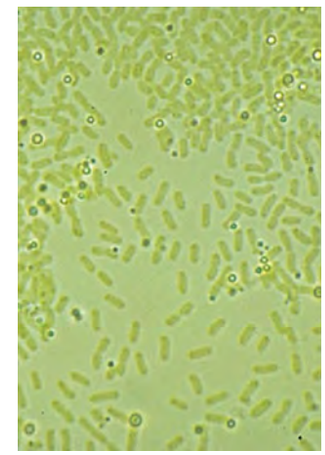


Figure 2.24 **Spirochetes.** Scanning electron micrograph of a cell of *Spirochaeta zuelzerae*. The cell is about 0.3 μm wide and tightly coiled.

The phylum *Deinococcus-Thermus* contains species with unusual cell walls and an innate resistance to high levels of radiation; *Deinococcus radiodurans* (**Figure 2.26**) is a major species in this group. This organism can survive doses of radiation many times greater than that sufficient to kill humans and can actually reassemble its chromosome after it has been shattered by intense radiation. We learn more about this amazing organism in Section 18.17.

Finally, several phyla branch off early in the phylogenetic tree of *Bacteria* (Figure 2.19). Although phylogenetically distinct, these groups are unified by their ability to grow at very high temperatures (*hyperthermophily*, Table 2.1). Organisms



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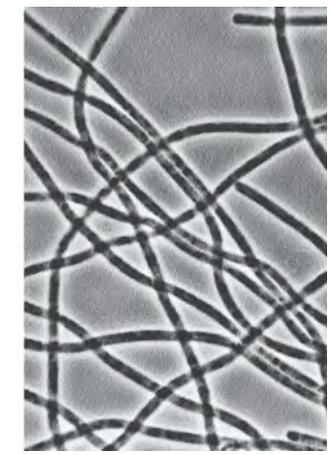
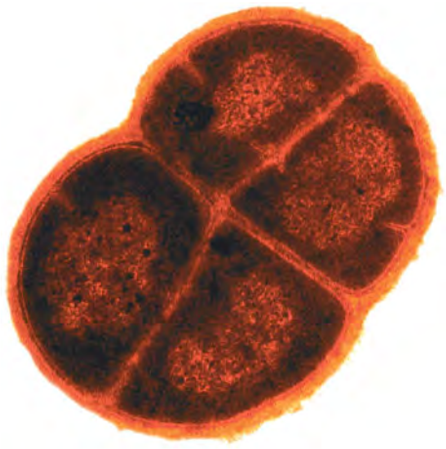


(b)

Figure 2.25 **Phototrophic green bacteria.** *(a) Chlorobium* (green sulfur bacteria). A single cell is about 0.8 μm wide. *(b) Chloroflexus* (green nonsulfur bacteria). A filament is about 1.3 μm wide. Despite sharing many features such as pigments and photosynthetic membrane structures, these two genera are phylogenetically distinct (Figure 2.19).



Michael J. Daly

Figure 2.26 **The highly radiation-resistant bacterium *Deinococcus radiodurans.*** Cells of *D. radiodurans* divide in two planes to yield clusters of cells. A single cell is about 2.5 μm wide.

such as *Aquifex* (**Figure 2.27**) and *Thermotoga* grow in hot springs that are near the boiling point. The early branching of these phyla on the phylogenetic tree (Figure 2.19) is consistent with the widely accepted hypothesis that the early Earth was much hotter than it is today. Assuming that early life forms were hyperthermophiles, it is not surprising that their closest living relatives today would also be hyperthermophiles. Interestingly, the phylogenetic trees of both *Bacteria* and *Archaea* are in agreement here; hyperthermophiles such as *Aquifex, Methanopyrus,* and *Pyrolobus* lie near the root of their respective phylogenetic trees.

MiniQuiz

- What is the largest phylum of *Bacteria*?
- In which phylum of *Bacteria* does the Gram stain reaction predict phylogeny?
- Why can it be said that the cyanobacteria prepared Earth for the evolution of higher life forms?
- What is physiologically unique about *Deinococcus*?

R. Rachel and K. O. Stetter

Figure 2.27 **The hyperthermophile *Aquifex.*** This hot spring organism uses H_2 as its energy source and can grow in temperatures up to 95°C. Transmission electron micrograph using a technique called freeze-etching, where a frozen replica of the cell is made and then visualized. The cell is about 0.5 μm wide.

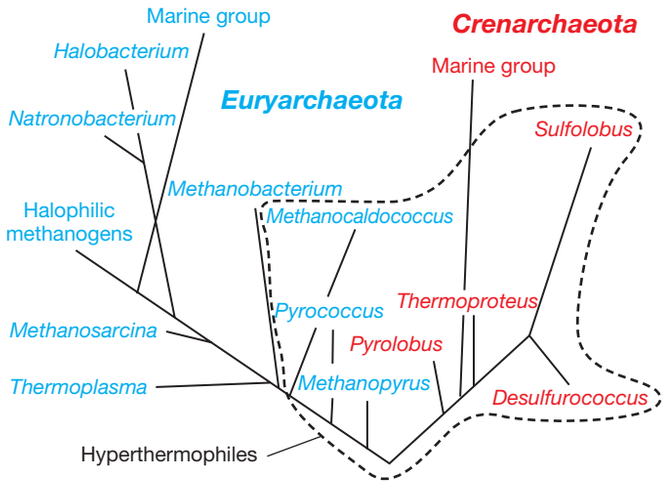
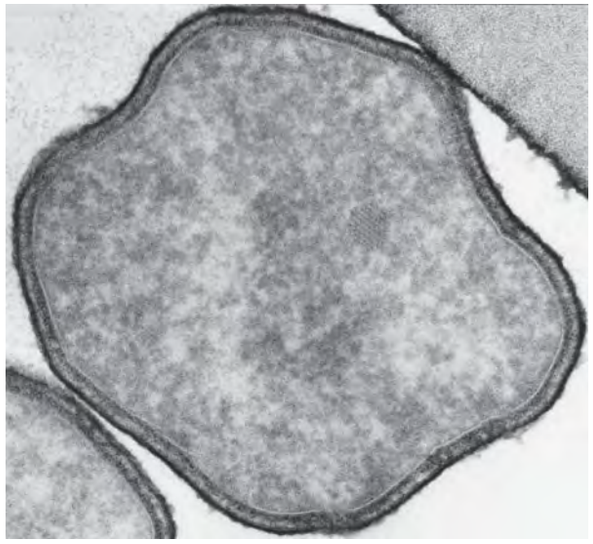


Figure 2.28 **Phylogenetic tree of some representative *Archaea.*** The organisms circled are hyperthermophiles, which grow at very high temperatures. The two major phyla are the *Crenarchaeota* and the *Euryarchaeota*. The "marine group" sequences are environmental rRNA sequences from marine *Archaea*, most of which have not been cultured.

2.10 *Archaea*

Two phyla exist in the domain *Archaea,* the *Euryarchaeota* and the *Crenarchaeota* (**Figure 2.28**). Each of these forms a major branch on the archaeal tree. Most cultured *Archaea* are extremophiles, with species capable of growth at the highest temperatures, salinities, and extremes of pH known for any microorganism. The organism *Pyrolobus* (**Figure 2.29**), for example, is a hyperthermophile capable of growth at up to 113°C, and the methanogen *Methanopyrus* can grow up to 122°C (Table 2.1).

Although all *Archaea* are chemotrophic, *Halobacterium* can use light to make ATP but in a way quite distinct from that of phototrophic organisms (see later discussion). Some *Archaea* use

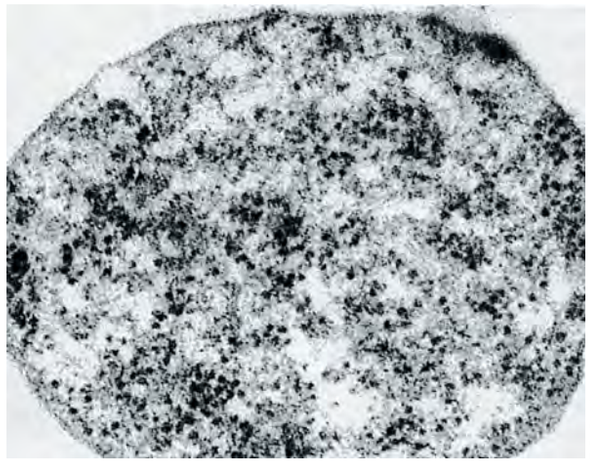


R. Rachel and K. O. Stetter

Figure 2.29 ***Pyrolobus.*** This hyperthermophile grows optimally above the boiling point of water. The cell is 1.4 μm wide.

William D. Grant

Figure 2.30 Extremely halophilic *Archaea*. A vial of brine with precipitated salt crystals contains cells of the extreme halophile, *Halobacterium*. The organism contains red and purple pigments that absorb light and lead to ATP production. Cells of *Halobacterium* can also live within salt crystals themselves (↻ Microbial Sidebar, Chapter 3, "Can an Endospore Live Forever?").



T. D. Brock

Figure 2.31 Extremely acidophilic *Archaea*. The organism *Thermoplasma* lacks a cell wall. The cell measures 1 μm wide.

organic compounds in their energy metabolism, while many others are chemolithotrophs, with hydrogen gas (H_2) being a widely used inorganic substance. Chemolithotrophic metabolisms are particularly widespread among hyperthermophilic *Archaea*.

Euryarchaeota

The *Euryarchaeota* branch on the tree of *Archaea* (Figure 2.28) contains four groups of organisms, the methanogens, the extreme halophiles, the thermoacidophiles, and some hyperthermophiles. Some of these require O_2 whereas others are actually killed by it, and some grow at the upper or lower extremes of pH (Table 2.1). For example, methanogens such as *Methanobacterium* are strict anaerobes and cannot tolerate even very low levels of O_2. The metabolism of methanogens is unique in that energy is conserved during the production of methane (natural gas). Methanogens are important organisms in the anaerobic degradation of organic matter in nature, and most of the natural gas found on Earth is a result of their metabolism.

The extreme halophiles are relatives of the methanogens (Figure 2.28), but are physiologically distinct from them. Unlike methanogens, which are killed by oxygen, most extreme halophiles require oxygen, and all are unified by their requirement for very large amounts of salt (NaCl) for metabolism and reproduction. It is for this reason that these organisms are called *halophiles* (salt lovers). In fact, organisms like *Halobacterium* are so salt loving that they can actually grow on and within salt crystals (**Figure 2.30**).

As we have seen, many prokaryotes are phototrophic and can generate adenosine triphosphate (ATP) using light energy (Section 2.8). Although *Halobacterium* species do not produce chlorophyll, they do synthesize a light-activated pigment that can trigger ATP synthesis (↻ Section 19.2). Extremely halophilic *Archaea* inhabit salt lakes, salterns (salt evaporation ponds), and other very salty environments. Some extreme halophiles, such as *Natronobacterium,* inhabit soda lakes, environments characterized by high levels of salt and high pH. Such organisms are *alkaliphilic* and grow at the highest pH of all known organisms (Table 2.1).

The third group of *Euryarchaeota* are the thermoacidophiles, organisms that grow best at high temperatures plus acidic pH. These include *Thermoplasma* (**Figure 2.31**), an organism that like *Mycoplasma* (Section 2.9) lacks a cell wall. *Thermoplasma* grows best at 60–70°C and pH 2. The thermoacidophiles also include *Picrophilus,* the most acidophilic (acid-loving) of all known prokaryotes (Table 2.1).

The final group of *Euryarchaeota* consists of hyperthermophilic species, organisms whose growth temperature optimum lies above 80°C. These organisms show a variety of physiologies including methanogenesis (*Methanopyrus*), sulfate reduction (*Archaeoglobus*), iron oxidation (*Ferroglobus*) and sulfur reduction (*Pyrococcus*). Most of these organisms obtain their cell carbon from CO_2 and are thus autotrophs.

Crenarchaeota

The vast majority of cultured *Crenarchaeota* are hyperthermophiles (Figure 2.29). These organisms are either chemolithotrophs or chemoorganotrophs and grow in hot environments such as hot springs and hydrothermal vents (ocean floor hot springs). For the most part cultured *Crenarchaeota* are anaerobes (because of the high temperature, their habitats are typically anoxic), and many of them use H_2 present in their habitats as an energy source.

Some *Crenarchaeota* inhabit environments that contrast dramatically with thermal environments. For example, many of the prokaryotes suspended in the open oceans are *Crenarchaeota,* in an environment that is fully oxic and cold (~3°C). Some marine *Crenarchaeota* are chemolithotrophs that use ammonia (NH_3) as their energy source, but we know little about the metabolic activities of most marine *Archaea*. *Crenarchaeota* have also been detected in soil and freshwaters and are thus widely distributed in nature.

MiniQuiz

- What are the major phyla of *Archaea*?
- What is unusual about the genus *Halobacterium*? What group of *Archaea* is responsible for producing natural gas?

2.11 Phylogenetic Analyses of Natural Microbial Communities

Although thus far we have cultured only a small fraction of the *Archaea* and *Bacteria* that exist in nature, we still know a lot about their diversity, which is extensive. This is because it is possible to do phylogenetic analyses on rRNA genes obtained from cells in a natural sample without first having to culture the organisms that contained them. If a sample of soil or water contains rRNA, it is because organisms that made that rRNA are present in the sample. Thus, if we isolate all of the different rRNA genes from a natural sample, a relatively easy task, we can use the techniques described in Figure 2.16 to place them on the phylogenetic tree. Conceptually, this is equivalent to isolating pure cultures of every organism in the sample (a task that is currently not possible) and then extracting and analyzing their rRNA genes. These powerful techniques of molecular microbial community analysis bypass the culturing step—often the bottleneck in microbial diversity studies—and instead focus on the rRNA genes themselves.

From studies carried out using molecular community analysis it has become clear that microbial diversity far exceeds that which laboratory cultures have revealed. For example, a sampling of virtually any habitat will show that the vast majority of microorganisms present there have never been obtained in laboratory cultures. The phylogeny of these uncultured organisms, known as they are only from environmental rRNA gene sequences (phylotypes), is depicted in phylogenetic trees as lineages identified by letters or numbers (Figure 2.19, and lumped together in Figure 2.28 as "marine groups") instead of actual genus and species names.

In addition to sending the clear message that the breadth of microbial diversity is staggering, molecular microbial community analyses have stimulated innovative new culturing techniques to grow the "uncultured majority" of prokaryotes that we know exist. Moreover, full genomic analyses of uncultured *Archaea* and *Bacteria* (environmental genomics, Section 22.7) are also possible. Using environmental genomics to display the full complement of genes in uncultured organisms often reveals important secrets about their metabolic capacities that point to ways to bring them into laboratory culture.

MiniQuiz

- How can we know the microbial diversity of a natural habitat without first isolating and growing the organisms it contains?

2.12 Microbial *Eukarya*

Eukaryotic microorganisms are related by cell structure and phylogenetic history. The phylogeny of *Eukarya* based on ribosomal RNA sequencing (**Figure 2.32**) shows plants and animals to be farthest out on the branches of the tree; such late-branching groups are said to be the "most derived." By contrast, some of the earlier-branching *Eukarya* are structurally simple eukaryotes, lacking mitochondria and some other organelles. We will see in Chapter 20 that it has proven difficult to accurately track the phylogeny of eukaryotes using ribosomal RNA sequencing alone, so other techniques have been used to supplement the general picture we present here.

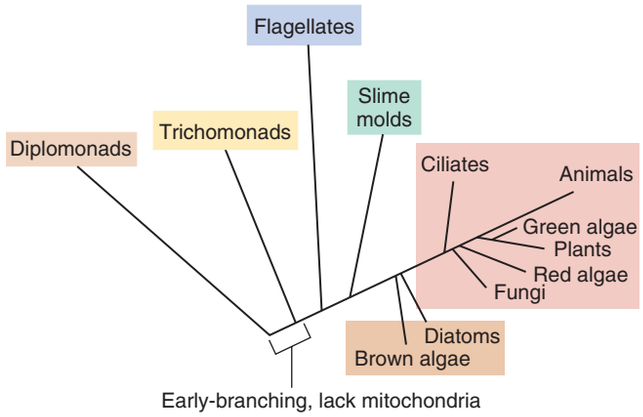


Figure 2.32 **Phylogenetic tree of some representative *Eukarya*.** This tree is based only on comparisons of genes encoding ribosomal RNA. Some early-branching species of *Eukarya* lack organelles other than the nucleus. Note that plants and animals branch near the apex of the tree. Not all known lineages of *Eukarya* are depicted.

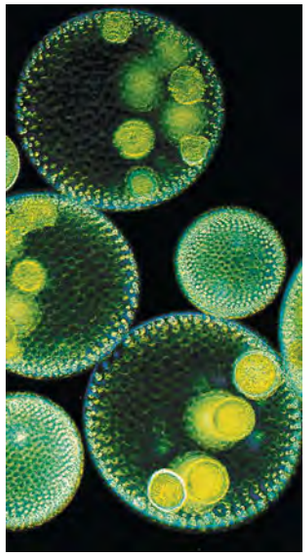
Eukaryotic Microbial Diversity

The major groups are **protists** (algae and protozoa), fungi, and slime molds. Some protists, such as the algae (**Figure 2.33*a***), are phototrophic. Algae contain chloroplasts and can live in environments containing only a few minerals (for example, K, P, Mg, N, S), water, CO_2, and light. Algae inhabit both soil and aquatic habitats and are major primary producers in nature. Fungi (Figure 2.33*b*) lack photosynthetic pigments and are either unicellular (yeasts) or filamentous (molds). Fungi are major agents of decomposition in nature and recycle much of the organic matter produced in soils and other ecosystems.

Cells of algae and fungi have cell walls, whereas the protozoa (Figure 2.33*c*) and slime molds do not. Protozoans are typically motile, and different species are widespread in nature in aquatic habitats or as pathogens of humans and other animals. Examples of protozoa are found throughout the phylogenetic tree of *Eukarya*. Some, like the flagellates, are fairly early-branching species, whereas others, like the ciliates such as *Paramecium* (Figure 2.33*c*), appear later on the phylogenetic tree (Figure 2.32).

The slime molds resemble protozoa in that they are motile and lack cell walls. However, slime molds differ from protozoa in both their phylogeny and by the fact that their cells undergo a complex life cycle. During the slime mold life cycle, motile cells aggregate to form a multicellular structure called a *fruiting body* from which spores are produced that yield new motile cells. Slime molds are the earliest branching organisms on the tree of *Eukarya* to show the cellular cooperation needed to form multicellular structures.

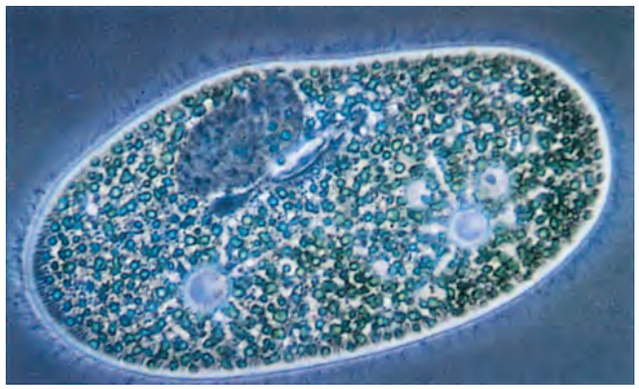
Lichens are leaflike structures often found growing on the surfaces of rocks and trees (**Figure 2.34**). Lichens are an example of a microbial mutualism, a partnership in which two organisms live together for mutual benefit. Lichens consist of a fungus and a phototrophic partner organism, either an alga (a eukaryote) or a



(a)

Barry Katz, Mycosearch

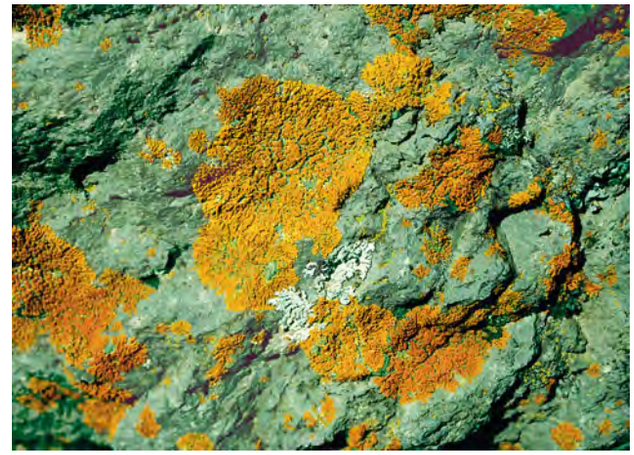
(b)



Sydney Tamm

(c)

Figure 2.33 **Microbial *Eukarya.*** *(a)* Algae; dark-field photomicrograph of the colonial green alga *Volvox*. Each spherical cell contains several chloroplasts, the photosynthetic organelle of phototrophic eukaryotes. *(b)* Fungi; interference-contrast photomicrograph of spores of a typical mold. Each spore can give rise to a new filamentous fungus. *(c)* Protozoa; phase-contrast photomicrograph of the ciliated protozoan *Paramecium*. Cilia function like oars in a boat, conferring motility on the cell.



M. T. Madigan

(a)

M. T. Madigan

(b)

Figure 2.34 **Lichens.** *(a)* An orange-pigmented lichen growing on a rock, and *(b)* a yellow-pigmented lichen growing on a dead tree stump, Yellowstone National Park, USA. The color of the lichen comes from the pigmented (algal) component. Besides chlorophyll(s), lichen algae contain carotenoid pigments, which can be yellow, orange, brown, red, green, or purple.

cyanobacterium (a prokaryote). The phototrophic component is the primary producer while the fungus provides an anchor for the entire structure, protection from the elements, and a means of absorbing nutrients. Lichens have thus evolved a successful strategy of mutualistic interaction between two quite different microorganisms.

Postscript

Our tour of microbial diversity here is only an overview. The story expands in Chapters 16–21. In addition, the viruses were excluded because they are not cells. Nevertheless, viruses show enormous genetic diversity, and cells in all domains of life have viral parasites. So we devote some of Chapter 9 and all of Chapter 21 to this important topic.

We proceed now from our brief tour of microbial diversity to study some of the key remaining principles of microbiology: cell structure and function (Chapter 3), metabolism (Chapter 4), growth (Chapter 5), molecular biology (Chapters 6–8), and genetics and genomics (Chapters 9–12). Once we have mastered these important basics, we will be better prepared to revisit microbial diversity and many other aspects of microbiology in a more thorough way.

MiniQuiz

- List at least two ways algae differ from cyanobacteria.
- List at least two ways algae differ from protozoa.
- How do each of the components of a lichen benefit each other?

Big Ideas

2.1
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.

2.2
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.

2.3
Differential interference contrast microscopy and confocal scanning laser microscopy allow enhanced three-dimensional imaging or imaging through thick specimens. The atomic force microscope gives a very detailed three-dimensional image of live preparations.

2.4
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.

2.5
All microbial cells share certain basic structures, such as their cytoplasmic membrane and ribosomes; most bacterial cells have a cell wall. Two structural patterns of cells are recognized: the prokaryote and the eukaryote. Viruses are not cells and depend on cells for their replication.

2.6
Genes govern the properties of cells, and a cell's complement of genes is called its genome. DNA is arranged in cells as chromosomes. Most prokaryotic species have a single circular chromosome; eukaryotic species have multiple chromosomes containing DNA arranged in linear fashion.

2.7
Comparative rRNA gene sequencing has defined three domains of life: *Bacteria*, *Archaea*, and *Eukarya*. Molecular sequence comparisons have shown that the organelles of *Eukarya* were originally *Bacteria* and have spawned new tools for microbial ecology and clinical microbiology.

2.8
All cells need sources of carbon and energy for growth. Chemoorganotrophs, chemolithotrophs, and phototrophs use organic chemicals, inorganic chemicals, or light, respectively, as their source of energy. Autotrophs use CO_2 as their carbon source, while heterotrophs use organic compounds. Extremophiles thrive under environmental conditions of high pressure or salt, or extremes of temperature or pH.

2.9
Several phyla of *Bacteria* are known, and an enormous diversity of cell morphologies and physiologies are represented. *Proteobacteria* are the largest group of *Bacteria* and contain many well-known bacteria, including *Escherichia coli*. Other major phyla include gram-positive bacteria, cyanobacteria, spirochetes, and green bacteria.

2.10
Two major phyla of *Archaea* are known, the *Euryarchaeota* and the *Crenarchaeota*, and most cultured representatives are extremophiles.

2.11
Retrieval and analysis of rRNA genes (phylotypes) from cells in natural samples have shown that many phylogenetically distinct *Bacteria* and *Archaea* exist in nature but remain to be cultured.

2.12
Microbial eukaryotes are a diverse group that includes algae and protozoa (protists), fungi, and slime molds. Some algae and fungi have developed mutualistic associations called lichens.

Review of Key Terms

Archaea one of two known domains of prokaryotes; compare with *Bacteria*
Autotroph an organism able to grow with carbon dioxide (CO_2) as its sole carbon source
Bacteria one of two known domains of prokaryotes; compare with *Archaea*
Cell wall a rigid layer present outside the cytoplasmic membrane; confers structural strength to the cell and protection from osmotic lysis
Chemolithotroph an organism that obtains its energy from the oxidation of inorganic compounds
Chemoorganotroph an organism that obtains its energy from the oxidation of organic compounds
Chromosome a genetic element containing genes essential to cell function
Cyanobacteria prokaryotic oxygenic phototrophs
Cytoplasm the aqueous internal portion of a cell, bounded by the cytoplasmic membrane
Cytoplasmic membrane the cell's permeability barrier to the environment; encloses the cytoplasm
Domain the highest level of biological classification
Endosymbiosis the theory that mitochondria and chloroplasts originated from *Bacteria*
Eukarya the domain of life that includes all eukaryotic cells
Eukaryote a cell having a membrane-enclosed nucleus and usually other membrane-enclosed organelles
Evolution change in a line of descent over time leading to new species or varieties within a species
Extremophile an organism that grows optimally under one or more environmental extremes
Gram stain a differential staining technique in which bacterial cells stain either pink

(gram-negative) or purple (gram-positive) depending upon their structural makeup

Heterotroph an organism that requires organic carbon as its carbon source

Nucleoid the aggregated mass of DNA that constitutes the chromosome of cells of *Bacteria* and *Archaea*

Nucleus a membrane-enclosed structure that contains the chromosomes in eukaryotic cells

Organelle a membrane-enclosed structure, such as a mitochondrion or chloroplast, present in the cytoplasm of eukaryotic cells

Phototroph an organism that obtains its energy from light

Phylogeny the evolutionary relationships between organisms

Plasmid an extrachromosomal genetic element nonessential for growth

Prokaryote a cell that lacks a membrane-enclosed nucleus and other organelles

Proteobacteria a large phylum of *Bacteria* that includes many of the common gram-negative bacteria, such as *Escherichia coli*

Protists algae and protozoa

Resolution in microbiology, the ability to distinguish two objects as distinct and separate under the microscope

Ribosome a cytoplasmic particle that functions in protein synthesis

Virus a genetic element that contains either a DNA or an RNA genome, has an extracellular form (the virion), and depends on a host cell for replication

Review Questions

1. What is the function of staining in light microscopy? Why are cationic dyes used for general staining purposes (Sections 2.1 and 2.2)?
2. What is the advantage of a differential interference contrast microscope over a bright-field microscope? A phase-contrast microscope over a bright-field microscope (Sections 2.2 and 2.3)?
3. What is the major advantage of electron microscopes over light microscopes? What type of electron microscope would be used to view the three-dimensional features of a cell (Section 2.4)?
4. Which domains of life have a prokaryotic cell structure? Is prokaryotic cell structure a predictor of phylogenetic status (Section 2.5)?
5. How long is a cell of the bacterium *Escherichia coli*? How much larger are you than this single cell (Section 2.5)?
6. How do viruses resemble cells? How do they differ from cells (Section 2.5)?
7. What is meant by the word genome? How does the chromosome of prokaryotes differ from that of eukaryotes (Section 2.6)?
8. How many genes does an organism such as *Escherichia coli* have? How does this compare with the number of genes in one of your cells (Section 2.6)?
9. What is meant by the word endosymbiosis (Section 2.7)?
10. How would you explain the fact that many proteins of *Archaea* resemble their counterparts in eukaryotes more closely than those of *Bacteria* (Section 2.7)?
11. From the standpoint of energy metabolism, how do chemoorganotrophs differ from chemolithotrophs? What carbon sources do members of each group use? Are they heterotrophs or autotrophs (Section 2.8)?
12. What domain contains the phylum *Proteobacteria*? What is notable about the *Proteobacteria* (Section 2.9)?
13. What is unusual about the organism *Pyrolobus* (Sections 2.8 and 2.10)?
14. What similarities and differences exist between the following three organisms: *Pyrolobus, Halobacterium,* and *Thermoplasma* (Section 2.10)?
15. How have rRNA sequencing studies improved our understanding of microbial diversity (Section 2.11)?
16. What are the major similarities and differences between protists, fungi, and the slime molds (Section 2.12)?

Application Questions

1. Calculate the size of the smallest resolvable object if 600-nm light is used to observe a specimen with a 100× oil-immersion lens having a numerical aperture of 1.32. How could resolution be improved using this same lens?
2. Explain why a bacterium containing a plasmid can typically be "cured" of the plasmid (that is, the plasmid can be permanently removed) with no ill effects, whereas removal of the chromosome would be lethal.
3. It has been said that knowledge of the evolution of macroorganisms greatly preceded that of microorganisms. Why do you think that reconstruction of the evolutionary lineage of horses, for example, might have been an easier task than doing the same for any group of prokaryotes?
4. Examine the phylogenetic tree shown in Figure 2.16. Using the sequence data shown, describe why the tree would be incorrect if its branches remained the same but the positions of organisms 2 and 3 on the tree were switched.
5. Explain why even though microbiologists have cultured a great diversity of microorganisms, they know that an even greater diversity exists, despite having never seen or grown them in the laboratory.
6. What data from this chapter could you use to convince your friend that extremophiles are not just organisms that were "hanging on" in their respective habitats?
7. Defend this statement: If cyanobacteria had never evolved, life on Earth would have remained strictly microbial.