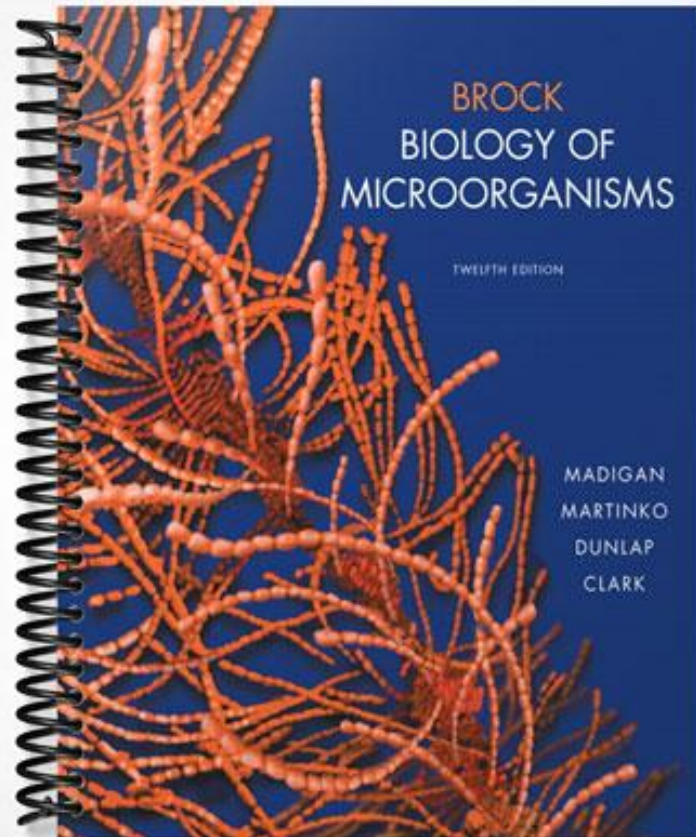


TEST BANK



2: A Brief Journey to the Microbial World

Summary

Chapter 2 is an excellent introductory overview of cell structure, evolution, and microbial diversity, topic areas that are addressed in more detail in future chapters. For courses involving nonmajors, this chapter provides general details on each topic that, if supplemented with material from related chapters later in the text, may be sufficient background for most students. However, it is recommended that Chapter 2 be used to set the stage for more detailed coverage later in the course.

2.1–2.4 | Seeing the Very Small

The variety of microscopic methods available for the observation of the very small prokaryotes must be introduced early, as much of the presentation of structure–function relationships depends upon the excellent photomicrographs that appear throughout the book. Although details of microscopy are more easily introduced in the laboratory portion of the course, the material presented here is pertinent.

- Discuss the basic principles and components of the compound light microscope, including the relationships between resolution and magnification, and numerical aperture (Figure 2.1). Note that although *bright-field microscopy* is fine for visualizing pigmented cells (Figure 2.2), it is not an efficient tool for looking at unstained nonpigmented cells such as bacteria.
- This will lead to a discussion of various methods employed to increase contrast. Discuss the various *simple* dyes used to stain cells, most of which are positively charged, *basic* dyes that are capable of binding to negatively charged cell surfaces (e.g., methylene blue, crystal violet; Figure 2.3). Continue the discussion of *differential stains*, the most useful of which is the Gram stain (Figure 2.4).
- Students should understand that staining not only kills cells, but can often distort their appearance. Discuss *phase-contrast microscopy* (Figure 2.5*b*) and *dark-field microscopy* (Figure 2.5*c*); two tools that allow one to look at living cells without the need for staining.
- *Fluorescence microscopy* is widely used in clinical diagnostic microbiology and environmental microbiology (Figure 2.6). Most students who enter the biotechnology industry or medical profession will work with fluorescent molecules (such as ELISA); the variety and sensitivity of these has increased dramatically over the past decade. This has allowed the development of a wide variety of nonradioactive alternatives to biological assays that are now commonly used in research.
- Students will be interested in the photomicrographs from three-dimensional imaging of cells (Figure 2.7). Depending upon the level of the course, the instructor could discuss the principles of *differential interference contrast microscopy*, *atomic force microscopy*, and *confocal laser microscopy* (Figure 2.8). Next, show and discuss the photomicrographs obtained from *electron microscopy* (Figures 2.9 and 2.10), and the differences between scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Figures 2.11 and 2.12 are excellent photographs showing the difference between prokaryotic and eukaryotic cells with respect to their internal structure and scale.

2.5 | Elements of Cell and Viral Structure

Students are often confused about what constitutes a cell. Carefully point out the major components common to all cell types, and then those components that differentiate plant, animal, and bacterial cell types (e.g., cell wall, chloroplasts, nucleus vs. nuclear region, etc.). Next discuss differences between eukaryotic and prokaryotic cells, and provide examples (see Figures 2.11–2.13).

- It is difficult for students to mentally visualize the extremely small size of a bacterial cell. Use the analogy from the main text stating that 500 prokaryotic cells placed end to end would fit into the period at the end of a sentence! Eukaryotic cells, on the other hand, are generally much larger. Difference in size is an extremely important concept in understanding the biotic potential of microbes, as is the relationship between surface area and cell volume. This topic is discussed in more detail in Section 4.2, *Cell Size and the Significance of Smallness*.
- Briefly introduce viruses and viral structure (Figure 2.13). Viruses will be discussed in detail in Chapters 10 and 19, and the instructor may want to include some of that material here if those chapters will not be covered in the course.

2.6 | Arrangement of DNA in Microbial Cells

The structural differences in the DNA of prokaryotic and eukaryotic cells, and the rapid advances in the sequencing and analysis of genomes of organisms, are covered in some detail in Chapters 7 and 13. The arrangement of genomes from both cell types is the primary topic of discussion in this section. However, the instructor may want to include some of the material in the next few paragraphs.

The genetic constitution of all cells is referred to as its genome. Analysis and sequencing of genomes, including the human genome, is an exciting and dynamic area of research. This technology is discussed in detail in Chapter 13. Students should become familiar with the concept of a gene as “a segment of DNA that encodes a protein (via messenger RNA), or another RNA molecule (e.g., ribosomal RNA).”

The DNA from all living systems is “localized” in specific regions within the cell. Each cell must “know” where the genetic material is located in time and space to allow for accurate replication and partitioning of daughter DNA molecules prior to cell division. Consequently, the genome of prokaryotes is associated with the cytoplasmic face of the cell membrane in the *nucleoid region* (Figure 2.14), and that of eukaryotes with the inner face of the nuclear membrane.

Prokaryotic genomes are considered haploid (that is, each gene is a single copy), usually consisting of a single, circular chromosome. Most prokaryotes also possess plasmids, which are extrachromosomal DNA molecules not necessary for survival under normal environmental conditions. Point out to students that plasmids often carry genes for properties such as antibiotic resistance, or the ability to degrade unusual or toxic compounds present in their environment, and this confers a selective survival advantage on those cells. Although addressed later in the text, mention the “independent” nature of plasmid replication and its ability to be transferred to other cells by various means. Microbiologists capitalized on the properties of plasmids in the early 1970s and developed recombinant DNA technology that has since spawned what we now call biotechnology.

Eukaryotic genomes are considered diploid (that is, each gene is present in two copies), consisting of linear DNA molecules carefully packaged into structures called chromosomes. Mitosis is the process that results in two identical daughter cells (with the same number and type of chromosomes), whereas meiosis results in daughter cells with half the normal chromosome number (haploid gametes).

Genome size varies among organisms. Original estimates of the number of genes in the human genome ranged from 50,000 to 100,000. More recent analyses of the human genome sequencing data have reduced this number to around 30,000 genes (a still debated issue), which is still about seven times higher than the number of genes known in *E. coli*. The primary products encoded by genes are proteins, and the level of complexity of organisms shows a positive correlation with the number of they proteins possess. A few summary points are listed below:

- Differentiate the terms *nucleus* and *nucleoid* (Figure 2.14) for your students, and the fact that *most* (but not all) prokaryotic cells have a single chromosome. In addition, many prokaryotes possess small circular DNA molecules, called *plasmids*, which often carry genes that confer a selective advantage for the organism (such as antibiotic resistance or an unusual metabolic capability).
- Eukaryotic cells usually contain a lot more DNA than do prokaryotic cells, necessitating the packaging of the DNA into discrete chromosomes to ensure efficient replication and transmission to daughter cells (Figure 2.15; *see also Section 8.5*).
- Using the *E. coli* genome as an example, point out that its 4.68 million base pairs contain about 4,300 genes that code for about 1,900 different proteins. Other bacteria may have three times as many genes or less than one-eighth that number (see Table 13.1). This is in stark contrast to the human genome, which contains more than 1,000 times as much DNA as *E. coli*.

2.7 | The Evolutionary Tree of Life

Now is the time to introduce the three-domain classification of life, two of which contain solely prokaryotic cells (*Archaea* and *Bacteria*), and one whose members all have a eukaryotic cell structure (*Eukarya*). Reinforce the extremely small size of microbial cells, and also the relative difference in sizes of eukaryotic and prokaryotic cells. There are rare exceptions to these size rules for prokaryotes; however, as will be seen in Chapter 4, these large bacteria generally have surface area-to-volume ratios similar to their smaller counterparts. Reinforce the fact discussed in Chapter 1 that, despite their small size, the presence and activities of prokaryotes are essential for the biosphere and all its inhabitants. Scientists are still uncovering novel microbial diversity, including a recently described bacterium that is the smallest bacterial cell described to date. This bacterium, named *Nanoarchaeum equitans*, has a diameter of only 400 nm and appears to be a symbiont of another archaean, *Ignicoccus* (*Nature* vol. 417:63–67). These have

been found growing together in hydrothermal environments at temperatures close to the boiling point of water (see Sections 17.13–17.14).

The science of *phylogeny* attempts to describe evolutionary relationships between living systems in a scientifically meaningful way. Traditional descriptive methodologies for inferring relatedness between organisms have been supplanted by molecular sequencing of key biological macromolecules. Although several macromolecules have served as good evolutionary clocks (e.g., cytochromes, ATPase, and RecA protein), ribosomal RNA has become the molecule of choice. The reasons for using rRNA for phylogenetic studies are detailed in Chapter 14. The instructor may wish to include this information here if Chapter 14 will not be covered in his or her course.

Discuss the rRNA sequencing and phylogeny methodology (Figure 2.16) and the *three-domain* classification scheme resulting from rRNA sequencing data (see Figure 2.17). Several points should be emphasized

- These domains are believed to have diverged from a common ancestor.
- **Eukarya** (including humans) are more closely related to **Archaea** than **Archaea** are to **Bacteria**! Be sure to explain to students that the origin of each branch, and its length, contains important information about the phylogeny of each group of organisms. Branches originating closer to the root contain organisms with a more ancient lineage than ones farther from the root. Also, the total length of the branches separating any two organisms is proportional to the calculated evolutionary distance between them.
- Multicellular organisms evolved from eukaryotic microorganisms, and thus plants and animals can be found near the top of the *Eukarya* domain, indicating a relatively recent evolutionary origin.
- Organelles of eukaryotes (mitochondria and chloroplasts) can be shown to have evolved from ancestors of *Bacteria* that established residence within a primitive eukaryotic cell—a process now referred to as *endosymbiosis* (see Sections 14.4 and 18.4). Point out their location on the universal tree. Students may be interested to know that the bacteria *Agrobacterium*, *Rhizobium*, and the rickettsias are each capable of intracellular existence and are closely related in rRNA sequence to present-day mitochondria (an intracellular symbiont). Similarly, the chloroplast of plants groups closely with the photosynthetic cyanobacteria, thus lending further support for the endosymbiotic theory.

Students should be made aware of the important and dramatic advances in molecular and sequencing technologies that have allowed science to uncover the incredible diversity and biotechnological and/or medical potential of microorganisms without the need for pure culture methodologies. These topics will be covered in Chapters 14 and 22. Present the major contributions that molecular sequencing and analysis have provided to the field of microbiology (such as microbial ecology and clinical diagnostics).

2.8 | Physiological Diversity of Microorganisms

This section introduces students to microbial diversity within the context of evolution; this topic is covered in detail in Chapters 14–18. Depending upon their chemistry backgrounds, students may have difficulty with the *concept of oxidation and reduction*, which is *key to understanding metabolism and metabolic diversity*. Simply put, oxidation involves the removal of an electron(s) from a substance and reduction involves the addition of an electron(s) to a substance. Thus, these processes are “coupled” during metabolism and the transfer of electrons eventually results in the production of the high-energy compound ATP (either via substrate-level phosphorylation or electron transport and oxidative phosphorylation; see Chapter 5).

The production of ATP can come from the oxidation of organic or inorganic compounds. To impress on students the tremendous diversity of microorganisms, mention that for almost any organic compound present on Earth, there is a microorganism that can utilize it for energy! These oxidations can occur either aerobically (oxygen present) or anaerobically (oxygen absent). The topic of metabolic diversity will be covered in detail in Chapters 5, 6, 20, and 21. The classification categories of microorganisms based upon how they obtain energy are outlined below:

- **Chemoorganotrophs** obtain energy from *oxidizing* (removing electrons from) organic compounds to synthesize ATP (the cell’s energy currency). These organisms represent the majority of microorganisms isolated to date (Figure 2.18).
- **Chemolithotrophs** obtain energy from *oxidizing* inorganic compounds. Inform students that this capability is possessed only by prokaryotes (*Bacteria* and *Archaea*), and because these organisms often use the inorganic waste products produced by chemoorganotrophs (Figure 2.8; H₂, H₂S) they do not compete for metabolic resources within a given environment.

- **Phototrophs** obtain energy by utilizing light to generate ATP. Students will most certainly associate photosynthesis with plants, and will recall that this process releases oxygen as a by-product (*oxygenic photosynthesis*). However, *anoxygenic photosynthesis* predates the former process and does not release oxygen. The evolution of the two photosystems in oxygenic phototrophs can be traced to the acquisition of each photosystem from different groups of anoxygenic phototrophs (see Chapter 17). Stress that the major differences between the two processes lie in their electron donors.
- Organisms are also classified according to their source of cell carbon: **heterotrophs** require organic compounds as a carbon source and **autotrophs** use CO₂ as their source of cell carbon.

The physiological diversity of microorganisms is also demonstrated by the ability of some to occupy habitats defined as “extreme”; these include extremes of temperature, pH, salt, dry, and combinations of these parameters. Table 2.1 shows the current recordholders identified in a given extreme environment (see also Chapters 6, 15, and 17).

2.9 | Bacteria

This section surveys the prokaryotic diversity within the domain *Bacteria*, which contains an enormous variety of physiologies and cell morphologies. Highlight members of the major groups within each domain. A good example of this is the *Proteobacteria*, which contain almost every type of microbial physiology known (Figure 2.19), including phototrophic and chemolithotrophic bacteria (Figure 2.20).

- Figures 2.18–2.20 can be used to take students on a visual tour of the *Proteobacteria*. Point out pathogenic and nonpathogenic representatives, the former arising from an evolutionary history associated with inhabiting animal tissues and cells. Continue your discussion by surveying other groups within this domain: Gram-positive bacteria (Figure 2.21), filamentous cyanobacteria (Figure 2.22), *Plantomyces* (Figure 2.23), Spirochetes (Figure 2.24), and others (Figures 2.25–2.27).

2.10 | Archaea

This domain contains two phyla, *Euryarchaeota* and *Crenarchaeota*, and most members of this domain are extremophiles (Figures 2.28–2.31). Outline significant metabolic properties of members of these two phyla: halophiles, methanogens, acidophiles, and alkaliphiles. Some *Crenarchaeota* can make a living as either chemoorganotrophs or chemolithotrophs and many grow optimally at extremely high temperatures.

- Summarize the subsection on *Phylogenetic Analysis of Natural Microbial Communities*, as it introduces students to the important new techniques that allow scientists to identify unculturable organisms in an environment. These tools have led to the discovery, cloning, and expression of genes for the production of important commercial and medical products, without having to isolate and culture the organism responsible. In addition, knowledge of the microbial composition of an environment can allow scientists to devise media and growth conditions for isolation of a particular organism based upon its metabolic capabilities (see Sections 13.1 and 22.1).

2.11 | Eukaryotic Microorganisms

The evolutionary history of eukaryotes shows a single long branch from where *Archaea* diverged, leading to the recent emergence of plants, animals, and fungi. It is worth mentioning to students that biology was (and still is, in some schools) taught using the five-kingdom classification of life, with little significance accorded to prokaryotic life. Actually, over 80% of the Earth’s history of life involved prokaryotes only! Thus, multicellular eukaryotes were a relatively recent addition to the planet, and the most ancient members lacked mitochondria and other organelles. Most of the present-day relatives lacking mitochondria (diplomonads, microsporidia, and trichomonads) are pathogens of humans, depending upon an intracellular existence in a host cell.

- Figure 2.32 shows a detailed tree of *Eukarya*. The phylogenetic similarity of plants and animals is put in perspective when compared with the universal tree shown in Figure 2.17. Microbial eukaryotes are discussed in Chapter 18, and representatives are shown in Figures 2.33 and 2.34.

Answers to Review Questions

1. Dyes are used to stain cells and increase their contrast so that they can be more easily seen using the bright-field microscope. Cationic (positively charged) dyes are used because they combine with negatively charged cellular constituents, such as nucleic acids and acidic polysaccharides

2. A differential interference contrast microscope polarizes light, generating two distinct beams. When these beams recombine, they are not totally in phase and therefore subtle differences within the cell are intensified. This is not possible with a bright-field microscope. Phase-contrast microscopy employs the principle that different substances refract light differently. Therefore, this microscope can be used to observe living specimens (wet mount preparations).
3. The major advantage the electron microscope has over the light microscope is magnification. A scanning electron microscope would be used to view the three-dimensional features of the cell.
4. The cell membrane provides a barrier separating the inside from the outside of the cell, and allows the selective transport of nutrients to the inside and waste materials to the outside. The lipid bilayer structure should not permit the free diffusion of charged particles even as small as ions.
5. Organisms belonging to the domains *Archaea* and *Bacteria* have a prokaryotic structure. Prokaryotic cell structure correlates with evolutionary relationships in some respects, but the evolutionary histories of *Bacteria* and *Archaea* are dramatically different.
6. An *E. coli* cell is about 2 μm long. A person who is 6 feet tall ($\sim 1,828,800 \mu\text{m}$) would be almost one million times longer (actually $\sim 914,400$ times longer)! The prokaryotic cell structure is linked to phylogenetic status, as it defines the two domains of prokaryotes: *Bacteria* and *Archaea*.
7. Viruses resemble cells in being able to reproduce (although dependent upon a suitable host) and contain their own (albeit limited) genetic information. They are very different from cells in that they are not dynamic open systems (taking in nutrients and expelling wastes); they have no metabolic capabilities of their own; and they cannot synthesize their own proteins.
8. *Genome* refers to the total complement of genes possessed by an organism. In prokaryotic cells, DNA is present in an aggregated mass of a single double-stranded circular DNA molecule called the *nucleoid*, and the cell is considered haploid. The DNA in eukaryotes is organized into chromosomes (the number of which varies between organisms) and is present as linear DNA molecules.
9. *E. coli* contains about 4,300 genes, whereas a human cell has about seven times as many genes.
10. The endosymbiotic theory suggests that the eukaryotic organelles, mitochondria and chloroplasts, were once free-living cells that established a cooperative arrangement with cells in *Eukarya* eons ago, perhaps for protection or metabolic cooperation of some kind. There is considerable molecular support for this theory based upon organellar genome arrangement, ribosome structure, and 16S rRNA analysis (discussed later).
11. Molecular evidence does indeed suggest that species of *Archaea* are more closely related to eukaryotes than are species of *Bacteria*. These data indicate that evolution from the “universal ancestor” originally went in two directions, *Bacteria* versus the “other,” with the latter diverging into *Archaea* and *Eukarya*. The genomes of eukaryotes contain genomes from cells of two of the domains. Other major evidence can be found in Chapters 14–18.
12. Chemoorganotrophs obtain their energy from organic compounds, whereas chemolithotrophs utilize inorganic compounds for energy generation. Chemoorganotrophs are obviously heterotrophs and many chemolithotrophs (not all) are autotrophs.
13. *Proteobacteria* belong to the domain *Bacteria*, and contain the largest number of prokaryotes and the most diverse metabolic activities within any phylum (see Section 2.9).
14. *Pyrolobus* is the current “recordholder” for optimum temperature for growth (113°C).
15. *Pyrolobus* is an extreme thermophile; *Halobacterium* is an extreme halophile that can synthesize ATP via a light-sensitive pigment system; and *Thermoplasma* is a cell wall-less thermoacidophile.
16. “Env-marine” refers to species defined as being of “environmental, marine” lineage.
17. *Giardia* lacks mitochondria and thus appears to be a modern descendant of primitive eukaryotic cells that either did not undergo endosymbiosis or lost their endosymbionts.

Answers to Application Questions

1. The diameter of the smallest object resolvable by any lens in a light microscope is equal to $0.5\lambda/\text{numerical aperture (NA)}$. Using a 600-nm wavelength and an NA of 1.32, this would be 227 nm, or $\sim 0.23 \mu\text{m}$. The resolution could be improved using this same lens by using a shorter wavelength in the blue light range.

2. Plasmids are extrachromosomal elements that carry genetic information not essential for the organism under normal conditions, and thus they are not subjected to the regulatory partitioning mechanism by the cellular machinery prior to cell division, as the chromosome is.
3. The study of the evolution of macroorganisms long preceded that of microorganisms and was an easier task in that it was based upon descriptive techniques from very similar and narrow lineages from the fossil record. The lack of morphological and even metabolic differences between evolutionarily related microorganisms made these types of criteria useless for making comparisons. The evolutionary story of bacteria had to await the development of molecular tools, especially the rRNA analysis technique pioneered by Carl Woese. The reconstruction of the evolutionary lineage of horses would have been an easier task than that of prokaryotes, given the latter's very recent and brief evolutionary history.
4. Keeping the branches on the tree the same and switching organisms 2 and 3 would make the tree incorrect, as it would imply that organism 2 is more closely related to organism 1, which is not the case.
5. The use of in situ rRNA phylogenetic probes and microbial community analysis techniques have shown scientists that laboratory techniques for isolating microbial community representatives show only an extremely small percentage of organisms that are actually present. Ribosomal RNA signatures thus give important information for assaying the microbial ecology of a given ecosystem.
6. The best argument against describing extremophiles as organisms that are just "hanging on" in a given habitat is the fact that they are represented in virtually every branch of *Bacteria* and *Archaea*. Student answers will vary.
7. Without the evolution of cyanobacteria and their oxygenic phototrophic metabolism (as in present-day plants), there would have been no selective pressure for the evolution of prokaryotic respiratory metabolism, and thus no evolution of "higher organisms" such as plants and animals.