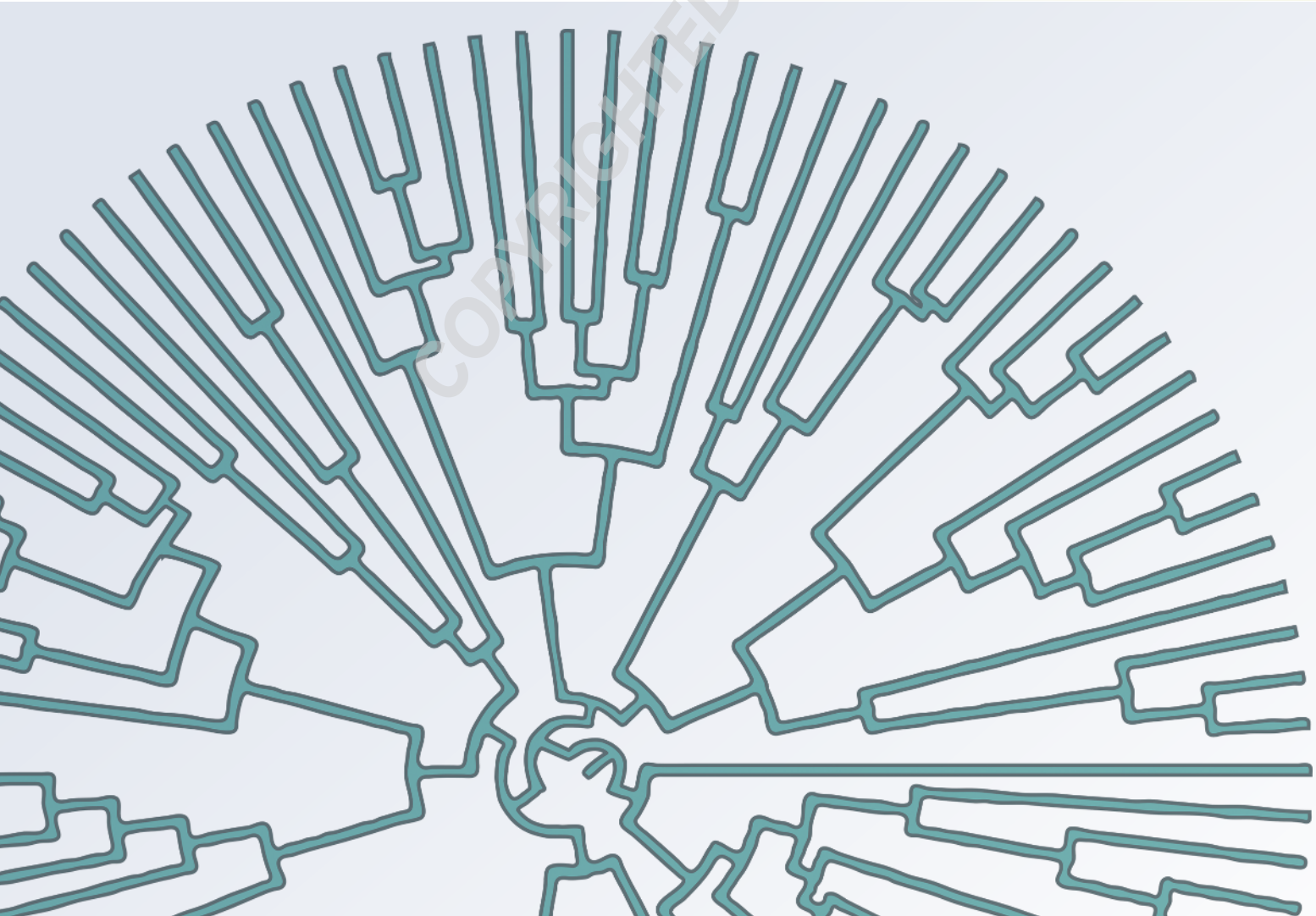


# MICROBIAL PHYLOGENY—
# THE THREE DOMAINS OF LIFE

**After completing the material in this chapter, students should be able to . . .**

1. identify distinguishing characteristics for each of the three domains of the modern molecular-based tree of life
2. discuss the strengths and limitations of using rRNA genes to generate phylogenetic trees
3. interpret a simple phylogenetic tree to determine relatedness
4. explain how endosymbiotic events and horizontal gene transfer can influence phylogenetic relationships
5. describe characteristics of two major phyla of *Archaea*

*Microbial Physiology: Unity and Diversity,* First Edition. Ann M. Stevens, Jayna L. Ditty, Rebecca E. Parales and Susan M. Merkel.

Companion website: www.wiley.com/go/stevens/microbialphysiology

## Introduction

Microbial physiology is, in the most basic terms, the study of the structure and function of microbes. A fundamental understanding of microbial physiology is broad-reaching and has important implications across the field including the subdisciplines of environmental, medical, food, agricultural, and industrial microbiology. This first chapter begins with a background discussion on how microbes are classified, both from a historical and modern perspective, and incorporates the themes of unity and diversity that will be woven throughout this textbook. Molecular components and processes that are common across the microbial world will also be introduced.

## The Three Branches of Life: *Bacteria*, *Archaea*, and *Eukarya*

Historically, microbial cells have been considered prokaryotic or eukaryotic based on their cellular substructure. Eukaryotic cells have organelles, the most obvious of which is the nucleus, but prokaryotes do not. Distinguishing between different prokaryotes is more difficult since most of these organisms look very similar to one another under a microscope. Microbes were initially classified based on their phenotypes with regard to their morphology (e.g., rods, cocci, spirilla, filaments), structures (e.g., flagella, pili), growth characteristics (e.g., aerobes versus anaerobes), and metabolic capacities (e.g., phototrophy, nitrogen fixation). However, since the 1970s it has been possible to classify microbes based on molecular **phylogeny**, which represents the evolutionary relationships between organisms. We now recognize that all organisms belong to three major groups known as **domains**. The prokaryotes consist of the domains *Bacteria* and *Archaea*, which are distinct from the third domain, *Eukarya* (Fig. 1.1). As discussed later in the text, some, but not all, of the phenotypic characteristics described above track with phylogenetic relationships.

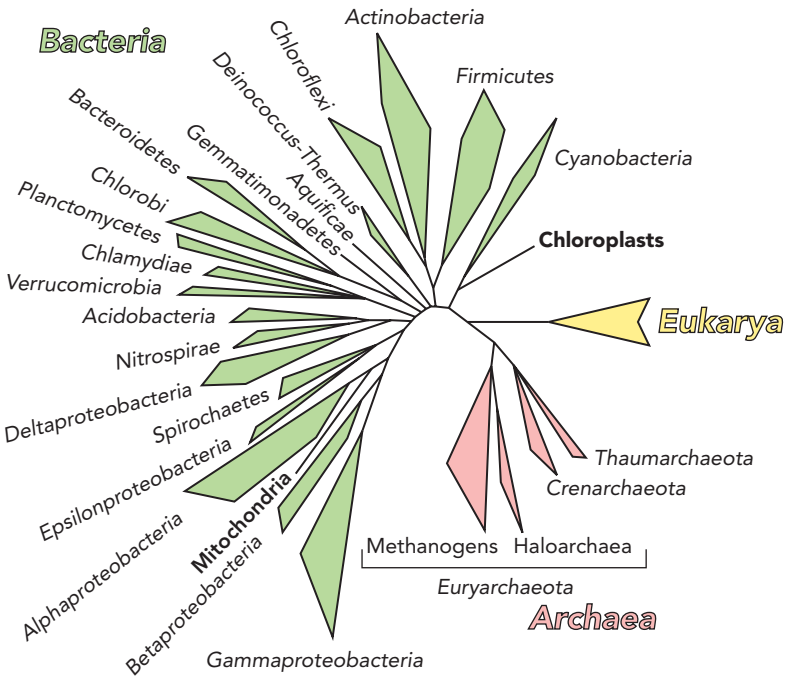



FIGURE 1.1 **Universal phylogenetic tree of life showing the three domains: *Bacteria*, *Archaea*, and *Eukarya*.** This phylogenetic tree is a model based on comparisons of small subunit rRNA (16S and 18S) sequences. Branches indicate some of the major lineages (of organisms in each domain. The major lineages represented here are based upon phyla and classes as categorized by the National Center for Biotechnology Information (NCBI) and terminology common in the literature. It should be noted that phylogenetic organization is frequently revised based on growing knowledge, technological advances, and the identification of new microbial species. As denoted by the line length of each branch, the *Bacteria* (green) and *Archaea* (pink) diverged first and the *Eukarya* (yellow) diverged from the *Archaea*. Note the close relationships of the chloroplast to the *Cyanobacteria* and the mitochondrion to the *Alphaproteobacteria*, which provide evidence for the endosymbiotic theory and the origin of eukaryotic organelles. Similar versions of this tree will be used throughout this book to illustrate the phylogeny of organisms of interest as we discuss their unique physiologies.

**TABLE 1.1 Unique attributes of the three domains of life**

| Attribute | *Bacteria* | *Archaea* | *Eukarya* |
|---|---|---|---|
| Membrane-enclosed nucleus | NO | NO | YES |
| Peptidoglycan cell wall | YES | NO | NO |
| Membrane lipid structure | Ester-linked | Ether-linked | Ester-linked |
| Translation initiation | fMet (formylmethionyl) | Met | Met |
| Ribosome size | 70S (5S, 16S, 23S) | 70S (5S, 16S, 23S) | 80S (5.8S, 18S, 28S) |
| Ribosome is sensitive to | Chloramphenicol, kanamycin, and streptomycin | Diphtheria toxin | Diphtheria toxin |
| RNA polymerase | One core with 4 essential subunits and multiple exchangeable sigma subunits | One with 12 to 14 subunits (like eukaryotic RNA polymerase II) | Three (I, II, and III) |
| Transcription factors required | NO | YES | YES |

In addition to a nucleus, eukaryotic cells contain other membrane-bound organelles such as mitochondria and chloroplasts, which contain their own sets of genetic material. Current evidence indicates that mitochondria and chloroplasts evolved from free-living bacteria that became obligate symbionts within a host eukaryotic cell; this is the basis of the **endosymbiotic theory**. This theory, which is now strongly supported by genetic evidence, posits that a respiring free-living bacterium related to the *Alphaproteobacteria* was engulfed by another cell and evolved to form the mitochondrion. Similarly, a photosynthetic cyanobacterium that was engulfed (by a mitochondria-containing eukaryotic cell) is proposed to be the progenitor of the present-day chloroplast. Mitochondria and chloroplasts, which are roughly the same size as an average bacterium, have remnant genomes and ribosomes. Their ribosomal RNA (rRNA) gene sequences are more similar to those of bacteria than eukaryotic cells. Therefore mitochondria and chloroplasts fall within the *Bacteria* on the **phylogenetic tree** (Fig. 1.1), a diagram that depicts the evolutionary relationships among organisms. Although some *Bacteria* and *Archaea* possess subcellular structures, these are not generally considered to be organelles.

Each domain of the tree of life has distinguishing characteristics, some of which are summarized in Table 1.1. For example, there are several differences in the components of bacterial, archaeal, and eukaryotic cell envelopes (Chapter 13). In the domain *Bacteria*, cell walls are composed of **peptidoglycan**, a cross-linked polymer of *N*-acetylglucosamine and *N*-acetylmuramic acid, whereas the *Archaea* and *Eukarya* do not utilize this macromolecule. This difference is important in the treatment of human diseases caused by pathogenic bacteria, as antibiotics such as penicillin and other β-lactams target the specific cell wall structure of the *Bacteria* and thus do not harm host eukaryotic cells. A specific type of membrane lipid with ether linkages is present in only *Archaea*, whereas the *Bacteria* and *Eukarya* use ester linkages. The *Eukarya* have larger ribosomes (composed of rRNA and proteins) in comparison to the *Bacteria* and *Archaea*.

## The 16S/18S rRNA Gene as a Basis for Phylogenetic Comparisons

The manner in which living organisms have been classified and compared to one another has changed multiple times over scientific history. Traditionally,

taxonomic classifications were wholly based on structural and physiological traits. Here, we will utilize a modern molecular phylogenetic tree (Fig. 1.1), which is instead based on 16S and 18S rRNA gene sequences. This text focuses on the domains *Bacteria* and *Archaea*.

In the 1970s, Carl Woese**,** a nucleic acid biologist at the University of Illinois at Urbana-Champaign, studied phylogenetic relationships based on 16S rRNA gene sequences. The essential process of translation, whereby cells convert the information in messenger RNA (mRNA) to produce polypeptides, is universally carried out by ribosomes and transfer RNAs (tRNAs) and thus is a highly conserved process across all domains of life. The ribosome is a large complex made of rRNA and protein molecules. The rRNA molecules are classified based on their sedimentation rates when subjected to centrifugation (reported in Svedberg units, "S"). *Bacteria* and *Archaea* contain 70S ribosomes, which are composed of ~50 ribosomal proteins and three types of rRNA: 5S, 16S, and 23S. These single-stranded rRNA molecules are approximately 500, 1600, and 2300 nucleotides in length, respectively. Eukaryotes, in contrast, have larger (80S) ribosomes containing 5.8S, 18S, and 28S rRNAs plus ribosomal proteins.

Back in the 1970s, nucleic acid sequencing was in its infancy and Maxim and Gilbert sequencing was the first method developed. The 16S rRNA molecule, found in *Bacteria* and *Archaea*, was of an appropriate size to yield sufficient genetic information so that it was technically possible to obtain its full sequence, making it useable for phylogenetic comparisons. The 5S rRNA, while easier to sequence, contained less genetic information for comparisons to be made, and the 23S rRNA was at the time more difficult and costlier to sequence. Further reasons for selecting the 16S rRNA, and the corresponding 18S rRNA in eukaryotes, for phylogenetic analysis include the following. First, rRNA molecules are believed to be very ancient and therefore inherited from a common, but unknown, ancestor of all organisms (i.e., **LUCA**, the **last universal common ancestor**). This hypothesis has been supported by the discovery and characterization of small RNA molecules called ribozymes that have the ability to encode genetic information as well as perform enzymatic activities (e.g., self-splicing introns). Thus, it is thought that the evolution of RNA preceded the evolution of DNA and proteins. Second, phylogenetic relationships among organisms can only be inferred using cellular components that are homologous, since they were inherited from a common ancestor. All living cellular organisms contain rRNA, and it performs the same essential function during translation in all organisms. Third, because of the essential role of the ribosome in all cells, mutations in the rRNA gene may be lethal and can only accumulate at a slow rate that allows the molecule to maintain its function. Indeed, within the rRNA molecule, which has a complex secondary structure (Fig. 1.2), there are conserved regions that have been resilient to change over time and variable regions (V1 to V9) with more base changes. Therefore, the 16S and 18S rRNA genes were targeted as the ideal candidates for comparing sequences to determine phylogenetic relatedness.

Based on all of these characteristics, the sequence of the 16S/18S rRNA gene was used to distinguish different microorganisms from one another, and Woese proceeded to perform a comparative analysis of these sequences across a variety of microorganisms (Fig. 1.3). Current methods used to determine 16S

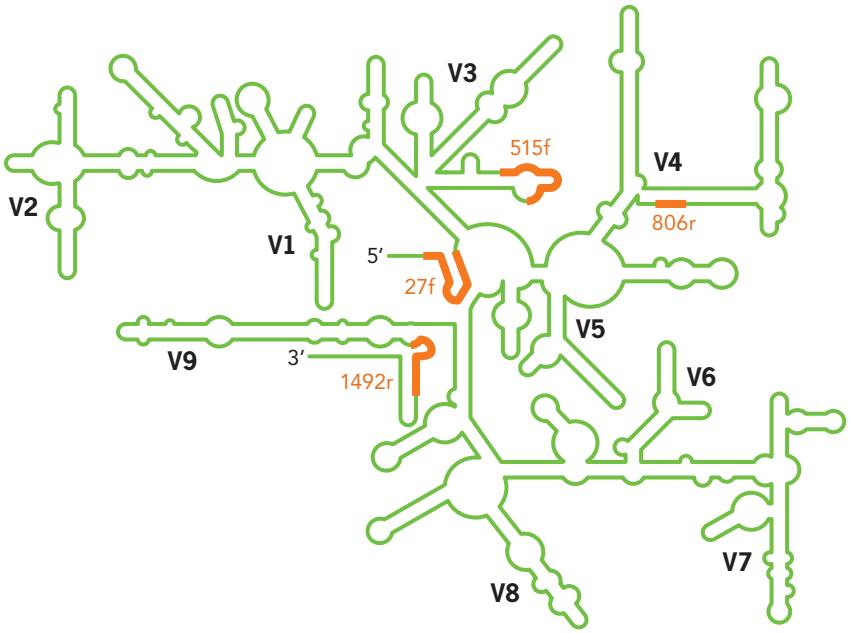



FIGURE 1.2 **Model of the secondary structure of 16S ribosomal RNA.** The conserved structure of 16S rRNA is composed of stems, which are formed by regions of the rRNA with complementary sequences that form base pairs, and loops, which are not base-paired. The molecule has both conserved and variable regions. V1 to V9 indicate the variable regions. Some conserved regions that are used as universal primer sites for PCR amplification are indicated by bold orange lines; numbers indicate nucleotide positions within the 16S rRNA gene, and r and f indicate reverse and forward directions, respectively.

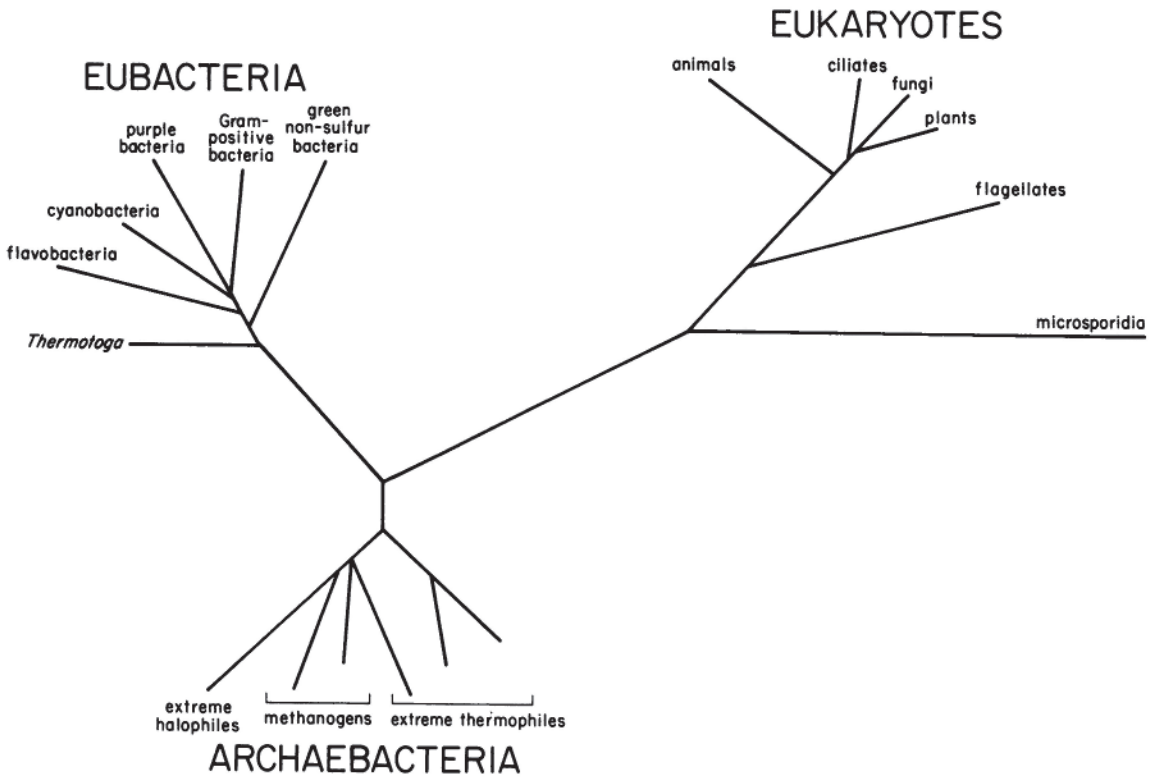



FIGURE 1.3 **An unrooted Woese phylogenetic tree.** Using rRNA sequence comparisons, Carl Woese was the first person to identify the domain *Archaea*, which he originally named the *Archaebacteria*. This is one of his early phylogenetic trees with the *Archaebacteria* broken into three groups based on physiological traits (i.e., extreme halophiles, methanogens, and extreme thermophiles). Compare this to the more recent tree shown in Fig. 1.1. Reprinted from Woese CR. 1987. *Microbiol Rev* 51(2):221–271.

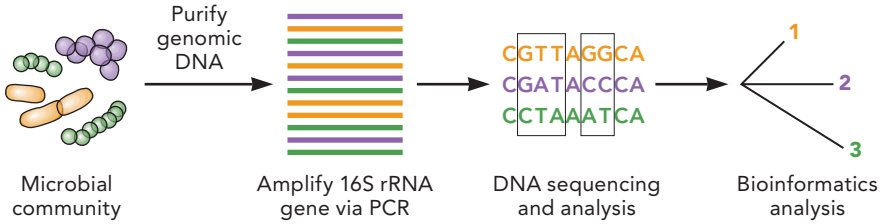



FIGURE 1.4 **Process of generating a phylogenetic tree.** Genomic DNA is first purified from a microbial community (shown) or a pure culture. The 16S rRNA genes are amplified by PCR using universal oligonucleotide primers that bind to conserved sequences in the gene. The amplified DNA fragments are then sequenced and compared to one another using bioinformatics to generate a phylogenetic tree. The length of the line between organisms represents their phylogenetic distance from one another. This method allows for the sequencing, and potential identification, of microorganisms that cannot be grown in culture.

rRNA gene sequences (Fig. 1.4) involve first isolating the genomic DNA from the organism of interest (or from an environmental sample) and then using the **polymerase chain reaction (PCR)** to amplify specific regions of the 16S or 18S rRNA gene. Typically, this involves the use of universal primers (that anneal to conserved regions in any rRNA gene) to amplify across a variable region or across the whole rRNA gene. DNA sequencing is performed and then the obtained sequences are compared using different bioinformatics methods to generate a phylogenetic tree.

**Evolutionary distance** is a very simple method for comparing sequences, whereby the number of different nucleotides found between two sequences is used as the primary criterion for comparing two organisms. That is, the more different the 16S rRNA gene sequences are, the more distantly related the organisms. **Maximum parsimony** (an algorithm that assumes the smallest number of changes in all probability reflects reality) is an alternative approach that considers not only the number of differences between sequences, but also the precise position of the change in the sequence and the nature of the substitution (i.e., purine for purine, pyrimidine for pyrimidine, or purine for pyrimidine). Some methods also consider the secondary structure of the rRNA as well as the primary sequence when making comparisons. Additional computer algorithms are used to convert the resulting sequence alignments into a phylogenetic tree. The sum of the linear distance on a phylogenetic tree is typically proportional to the evolutionary distance between two organisms. The greater the length of the line, the more distantly related the two organisms are. However, because we cannot know how many times a particular base change has occurred at any position over time, it is likely that these methods underestimate evolutionary distances. It is important to note that any phylogenetic tree is a snapshot of the relationship between the organisms being analyzed at one given point in time.

While the use of 16S/18S rRNA genes has revolutionized how we understand evolutionary relationships between microorganisms, the interpretation of phylogenetic relationships can also be complicated by endosymbiotic events (i.e., mitochondria and chloroplasts). More commonly, **horizontal gene transfer** events (also known as lateral gene transfer), in which DNA is transferred between unrelated cells rather than by hereditary or vertical transmission from parent to offspring, can also complicate the interpretation of evolutionary relationships. DNA can be horizontally transferred to bacterial and archaeal cells through conjugation of plasmids or transduction by phage, and in some cases

through natural transformation, which involves the uptake of DNA from the environment. These types of genetic exchange are taken into consideration in the work of Ford Doolittle and others that represent relationships in a "Web of Life" (Fig. 1.5). One common characteristic of a horizontally transferred gene is a significant deviation in its G+C content relative to that of the whole genome of an organism. Thus, it is important to base phylogenetic analyses on genes or proteins that are unlikely to have been acquired by horizontal transfer. It is generally assumed that "housekeeping genes" that encode essential functions for all cells (e.g., transcription and translation) are acquired by vertical transmission. Currently, in addition to 16S rRNA gene sequence comparisons, researchers often use comparisons of the deduced amino acid sequences of various housekeeping genes (e.g., *rpoB* or *gyrB*) to determine phylogenetic relationships. In some cases, concatenated (linked) amino acid sequences of multiple housekeeping proteins or even whole genome nucleotide sequences are used to analyze phylogeny.

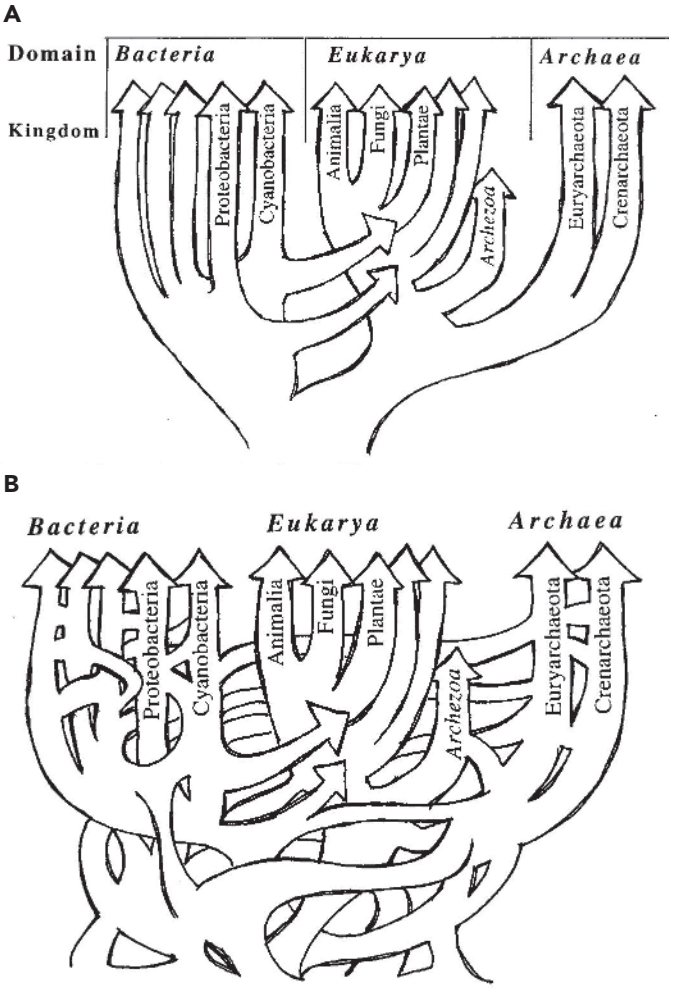



FIGURE 1.5 **A Doolittle phylogenetic tree illustrating the impact of horizontal gene transfer.** (A) An illustration of the role of horizontal gene transfer in the evolution of mitochondria and chloroplasts from free-living bacteria through endosymbiosis. (B) The impact of horizontal gene transfer through mechanisms like transformation, transduction, and conjugation is shown through this reticulated (web-like) phylogenetic tree. Reprinted from Doolittle WF. 1999. *Science* 284:2124–2128, with permission.

## The Modern Molecular Phylogenetic Tree of Life

As discussed in the previous section, our modern molecular phylogenetic tree has three domains. How did Woese discover the *Archaea*? It was actually by coincidence. As the story goes, Woese asked his colleagues at the University of Illinois for samples of the various organisms that they studied in their laboratories. Ralph Wolfe was studying methanogens, which back in the 1970s were thought to be bacteria. When Woese sequenced the 16S rRNA gene of a methanogen, he discovered that the sequence was quite different from the 16S rRNA gene of comparison bacteria. Wolfe recalled this moment: "He told me methanogens weren't bacteria, and I said of course they're bacteria. They look like bacteria under the microscope." (Peterson D. 2015. The microbial man. *MCB* 9:17). When published, Woese's findings were met with great skepticism. Woese's phylogenetic tree clearly shows three domains with the *Archaea* being more closely related to the *Eukarya* than they are to the *Bacteria*. Over time Woese's theory has been validated and he was eventually awarded the Crafoord Prize in Biological Sciences, given by the Royal Swedish Academy of Sciences for accomplishments in fields not covered by the Nobel Prize.

### THE *ARCHAEA*

The *Archaea* are currently divided into multiple phyla, the best studied of which are the *Crenarchaeota* and *Euryarchaeota*. Many of the microbes within the archaeal domain exhibit unique metabolic abilities. For example, the strictly anaerobic archaeal methanogens (*Euryarchaeota*) are the only microbes on the planet capable of reducing carbon dioxide fully to methane due to their repertoire of specialized coenzymes (Chapter 11). They are found in the sediments of lakes, ponds, and swamps; in sewage; and in the intestinal tracts of animals and humans. Some *Archaea* are able to thrive in extreme environments (**extremophiles**). Other members of the *Euryarchaeota* are extreme halophiles that live in environments such as salt lakes and solar evaporation ponds with high salt concentrations (e.g., 3 to 5 M; Chapter 10). While thermophiles, which grow optimally at temperatures >45°C, can be found in both the bacterial and archaeal domains, the extreme hyperthermophiles that grow at temperatures >95°C are generally *Archaea,* many of which are members of the *Crenarchaeota*. Similarly, examples of both bacterial and archaeal acidophiles and alkaliphiles that inhabit low- and high-pH environments, respectively, have been identified. Therefore, *Bacteria* and *Archaea* cannot be differentiated phylogenetically based on their ability to grow in extreme environments.

### THE *BACTERIA*

Looking at a modern molecular tree of life (Fig. 1.1), it becomes readily apparent that microorganisms are the dominant and most diverse organisms on the planet. The visible world, including plants, animals, and some fungi, occupies just the tip of one branch of the tree (Fig. 1.3). This text will focus predominantly on the physiology of the *Bacteria* but will also touch upon the *Archaea*. The well-studied Gram-negative *Escherichia coli* (member of the *Gammaproteobacteria*) will be used as a model to discuss many basic physiological processes,

and the well-studied Gram-positive organism *Bacillus subtilis* (member of the *Firmicutes*) will serve a similar purpose for that distinctive branch of the tree. Although almost all *Bacteria* have cell walls composed of peptidoglycan, there are two distinct types of bacterial envelope structures that can be differentiated by a specific type of cell staining known as the Gram stain (Fig. 1.6). Gram-positive bacteria have a very thick layer of peptidoglycan surrounding their cytoplasmic membrane that is associated with positively charged molecules (e.g., **teichoic acid** and lipoteichoic acid), whereas Gram-negative bacteria have a thin single layer of peptidoglycan surrounded by an outer membrane containing **lipopolysaccharide (LPS)**. The space between the two membranes

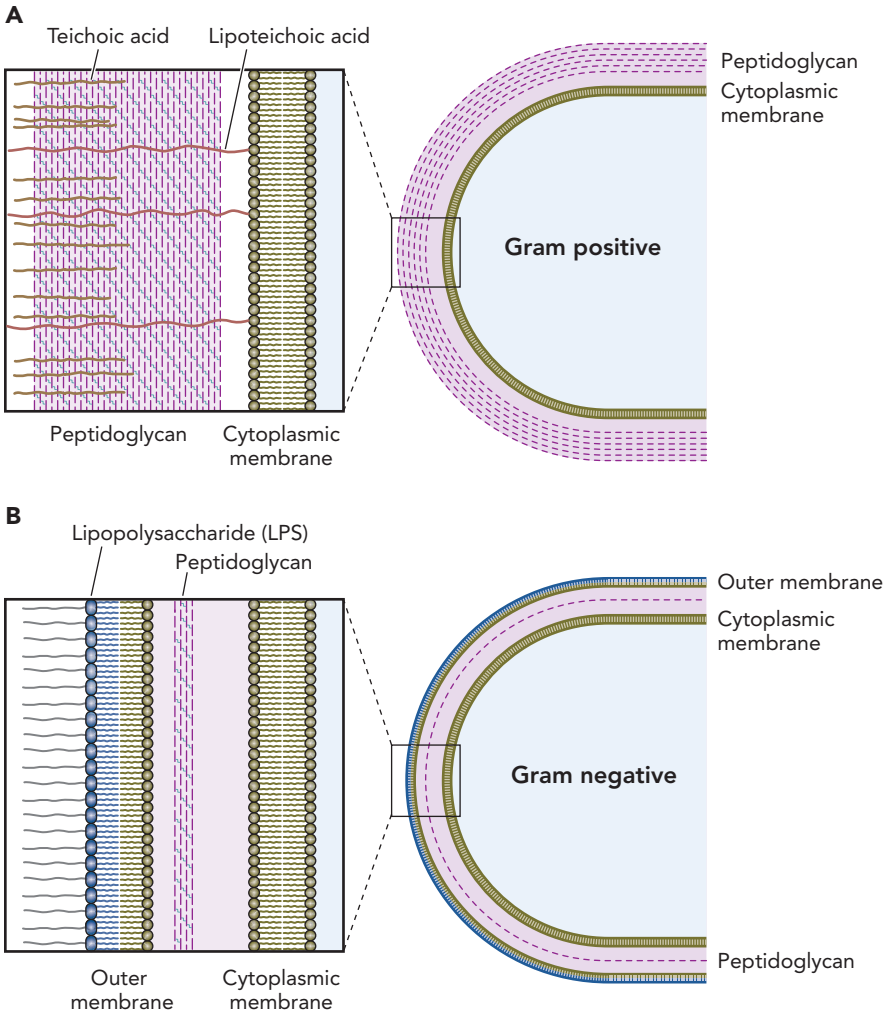



FIGURE 1.6 **Cell envelopes of Gram-positive and Gram-negative bacteria.** (A) Gram-positive bacteria have a cytoplasmic membrane and a thick peptidoglycan cell wall that is associated with other charged constituents (e.g., teichoic acid and lipoteichoic acid), whereas (B) Gram-negative bacteria have both a cytoplasmic (inner) membrane and an outer membrane that flank a thin layer of peptidoglycan. Unlike the inner membrane, which is a phospholipid bilayer, the outer membrane is asymmetrical, with the inner leaflet composed of phospholipids and the outer leaflet composed of lipopolysaccharide. Note that all inner and outer membranes also have loosely associated (peripheral) and embedded (integral) proteins (not shown).

is called the **periplasm**. This difference in cell structure has consequences for the physiology and permeability of cells that will become apparent in later chapters (Chapter 13). It is also notable that all Gram-positive bacteria (*Firmicutes* and *Actinobacteria*) are clustered together on the phylogenetic tree (Fig. 1.1) suggesting that this type of cell envelope evolved once in the last common ancestor of this group.

Although it might appear that we have a great deal of information about microbes, in actuality it is estimated that <5% of the bacteria present on Earth can currently be grown in pure cultures in the laboratory. This is likely due to the great interconnectivity that exists between microorganisms with regard to the generation and utilization of products or substrates that move between different types of cells. Many phylogenetic trees include microorganisms that have been detected in environmental samples using rRNA gene amplification and sequencing but have never been grown in the laboratory (Fig. 1.4).

## Phylogenetics and Earth History

The phylogenetic tree of life can be mapped to Earth's geological history, correlating the evolutionary relationships of the domains with the geological time scale (Fig. 1.7). Based on cells and organic molecules preserved in rocks, cellular life is believed to have evolved between 3.5 billion and 4 billion years ago. However, the first original progenitor cell is unknown, as it no longer exists. Most phylogenetic trees are rooted at a point called the last universal common ancestor (LUCA). Given that the atmosphere on Earth was anaerobic and the surface temperatures were very hot, this organism likely was an anaerobic thermophile. Today, thermophiles can be found distributed in both the *Bacteria* and *Archaea* and most grow optimally in the range of 55 to 100°C in environments such as

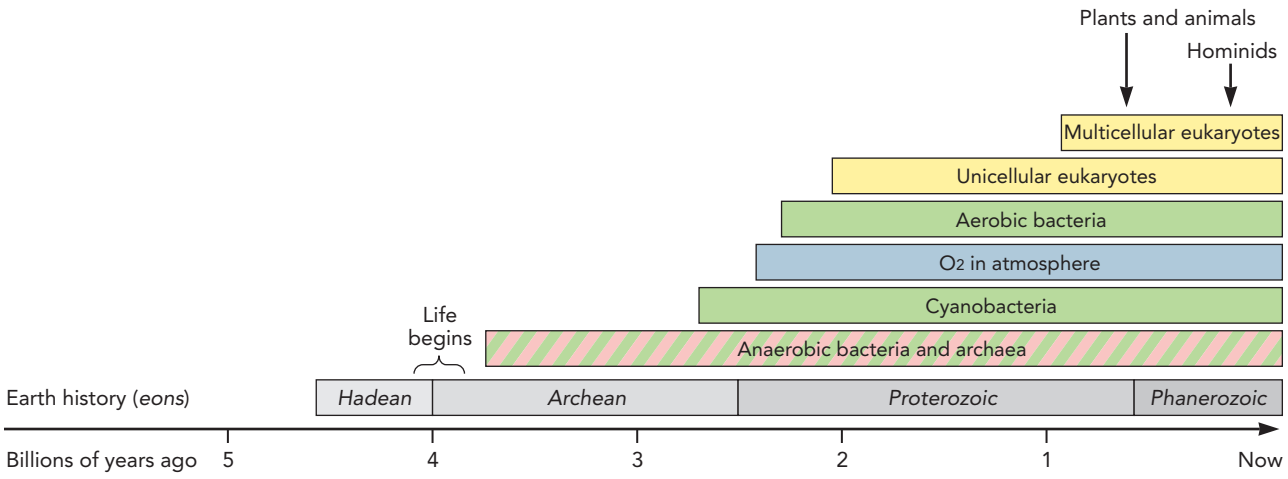



FIGURE 1.7 **Timeline of the evolution of life on Earth.** The origin of life is estimated to have occurred approximately 4 billion years ago when the planet was still hot and anoxic. *Bacteria* (green) and *Archaea* (pink) diverged early in evolution when atmospheric oxygen was completely absent. The evolution of cyanobacteria, which release oxygen as a by-product of oxygenic photosynthesis, led to the gradual oxygenation of the atmosphere. The presence of oxygen led to the evolution of aerobic forms of metabolism in *Bacteria* and *Archaea*. Early unicellular eukaryotes (yellow) with mitochondria evolved approximately 2 billion years ago, and multicellular eukaryotes began evolving less than 1 billion years ago.

hot springs, geysers, and hydrothermal vents. Some archaeal thermophiles are even capable of growth at temperatures well over 100°C. The evolution of oxygenic photosynthesis in the cyanobacteria (~3 billion years ago) resulted in the gradual accumulation of oxygen in the atmosphere, which led to the evolution of life forms capable of respiring oxygen and eventually to the first eukaryotic life (~1.5 billion to 2 billion years ago). The resulting environmental changes paved the way for the great diversification of microbial life on Earth as microbes adapted and evolved to fill all possible biological niches. As will be seen in the "Diversity" section of this book, microbial metabolism is absolutely essential to drive the Earth's carbon, nitrogen, and sulfur cycles.

## Learning Outcomes: After completing the material in this chapter, students should be able to . . .

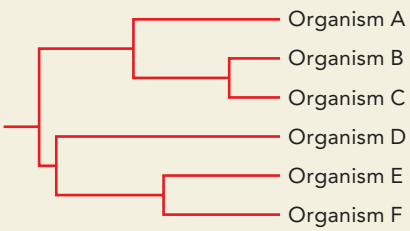
1. identify distinguishing characteristics for each of the three domains of the modern molecular-based tree of life
2. discuss the strengths and limitations of using rRNA genes to generate phylogenetic trees
3. interpret a simple phylogenetic tree to determine relatedness
4. explain how endosymbiotic events and horizontal gene transfer can influence phylogenetic relationships
5. describe characteristics of two major phyla of *Archaea*

## Check Your Understanding

1. Define this terminology: phylogeny, domains, endosymbiotic theory, phylogenetic tree, peptidoglycan, LUCA, polymerase chain reaction (PCR), evolutionary distance, maximum parsimony, horizontal gene transfer, extremophiles, periplasm, teichoic acid, lipopolysaccharide (LPS)
2. Who originated the idea of a three-domain system for the phylogeny of life on Earth? What is the basis of the three-domain system? (LO2)
3. While we now know that *Archaea* and *Bacteria* are very different, before the advent of rRNA gene sequencing, *Archaea* were considered to be in the same kingdom as *Bacteria*. List two traits that caused scientists to group them together and two traits that support the hypothesis that they are very distantly related. (LO2)
4. List and describe five reasons why the 16S/18S rRNA gene is used as the basis for molecular phylogenetic comparisons. (LO2)
5. A segment of the 16S rRNA gene sequence of *Escherichia coli* is shown below. Based on the concept of evolutionary distance, which organism is *most* closely related to *E. coli*? How did you come to this conclusion? (LO3)

   *E. coli*: T G G C A C G G T A G C
   Organism A: T G A C A G C G T A A C
   Organism B: T C G A C G C G T A G C
   Organism C: A G T G A C T G T A T A

6. This phylogenetic tree was created based on 16S rRNA gene sequences. Which two organisms do you think are most closely related? On what did you base your conclusion? (LO3)




7. The purpose of completing the following information table is to help you compare and contrast important characteristics relative to the classification of *Bacteria*, *Eukarya*, and *Archaea* and the importance of two groups of *Bacteria*, the Gram-positive and Gram-negative bacteria. For each characteristic structure, describe the function it serves for the cell and

indicate if it is present (P) or absent (A) in the *Eukarya* and/or *Archaea*, and if it is present (P) or absent (A) in the Gram-positive and/or Gram-negative bacteria. (LO1)

| **Characteristic (Does the cell have it?)** | **Definition (What is it?)** | ***Eukarya*** | ***Archaea*** | ***Bacteria*** | |
|---|---|---|---|---|---|
| | | | | **Gram +** | **Gram –** |
| Peptidoglycan | | | | | |
| Teichoic acid | | | | | |
| Lipopolysaccharide | | | | | |
| 16S rRNA | | | | | |
| 18S rRNA | | | | | |
| Ester-linked lipids | | | | | |
| Ether-linked lipids | | | | | |

8. Extremophiles can be found in both the *Archaea* and *Bacteria* domains. List three general categories of extremophiles and give one characteristic of each. (LO5)
9. While the use of rRNA gene sequencing has revolutionized the way in which we approach establishing evolutionary relationships among organisms, endosymbiotic events and horizontal gene transfer complicate our interpretation of these relationships. Explain how this is true. (LO4)

## Dig Deeper

10. You have isolated a new bacterial species from a volcanic hot spring. Results of the many laboratory experiments you conducted are outlined below. (LO1)

| **Test** | **Result** | |
|---|---|---|
| Growth | | |
| Temperature | Range: 55 to 77°C | Optimal: 70°C |
| pH | Range: 5 to 8 | Optimal: 7.5 |
| Salt | Range: 0 to 1.5% NaCl | Optimal: 0.1% |
| Structures | | |
| Lipids | Fatty acid esters | |
| Cell wall | Penicillin sensitive | |

    a) Would you classify this organism as a thermophile, acidophile, or halophile?
    b) Based on the data provided, would you classify this new species as belonging to the *Bacteria*, *Archaea*, or *Eukarya*? Defend your answer by explaining your rationale for all the results provided.

11. In some cases, 16S rRNA gene sequences may not be sufficiently variable to ensure differentiation between closely related species of bacteria. An alternative approach to create a phylogenetic tree is to use the deduced amino acid sequence of a protein-encoding gene. For each entry listed below, state whether the trait would be useful to generate phylogenetic trees. Explain your reasoning. (LO2)
    Suppose the gene:
    - can undergo horizontal gene transfer.
    - coded for a nonessential protein.
    - had hypervariable regions or constant regions between species.

12. The three phylogenetic trees shown below were constructed using 16S rRNA gene sequences, the amino acid sequences of GyrB (the subunit B protein of DNA gyrase, required for DNA replication), and the amino acid sequences of NifH (a protein involved in nitrogen fixation) as labeled. Which gene was likely transferred via horizontal gene transfer and which via vertical transfer? How do you know? (LO4)