CHAPTER 1

The Microbial World
As you study the key topics, make sure you review the following elements:

- **Microbiology involves the study of bacteria, archaeons, eukaryal microbes, and viruses.**
  - Table 1.1: Macromolecules in microbial cells
  - Animation: Classification systems
  - Toolbox 1.1: Polymerase chain reaction amplification of rRNA genes
  - Mini-Paper: The three domains of life

- **Studies of microbes have provided insight into the evolution of life and genetics.**
  - Perspective 1.1: Creating life in the laboratory: The Miller-Urey experiment
  - Perspective 1.2: Ribozymes: Evidence for an RNA-based world
  - Animation: Endosymbiosis
  - Figure 1.20: Effects of mutations
  - Figure 1.22: Recombinant DNA techniques

- **The metabolic properties of microbes are related to their habitats.**
  - Figure 1.24: Glycolysis, fermentation, and aerobic respiration
  - Figure 1.25: Role of microbes in the global nitrogen cycle

- **Microbes remain important causes of disease throughout the world.**
  - Microbes in Focus 1.1: Bacillus anthracis
  - Figure 1.30: Infectious disease deaths in the United States during the twentieth century
  - Figure 1.32: Impact of malaria in sub-Saharan Africa

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Anton van Leeuwenhoek was a successful textile merchant in the city of Delft, in the Netherlands, in the late seventeenth century. He used magnifying lenses in his trade to examine cloth, but in 1665, after reading Robert Hooke’s book *Micrographia*, van Leeuwenhoek became fascinated with using microscopes to explore the natural world. Hooke, an Englishman of about the same age as van Leeuwenhoek, had laboriously constructed microscopes that magnified objects roughly 30 times, and used them to examine the fine structure of materials both living and dead. His greatest contribution to biology was the discovery of cells (which he first observed in cork slices) as the units from which living organisms are assembled. Hooke’s writings inspired van Leeuwenhoek, who enjoyed blowing glass and grinding tiny lenses, to fabricate simple but remarkably powerful microscopes. Some of the 400 or so microscopes that van Leeuwenhoek built magnified images almost 300-fold, and could be used to observe objects one-tenth the size that Hooke had seen. If we consider that the best modern light microscopes of today are limited to around 1000-fold magnification, van Leeuwenhoek’s accomplishments are even more astounding!

With his extraordinary lenses, van Leeuwenhoek pushed the frontiers of human knowledge to ever-smaller dimensions. No one had imagined living creatures so small they could not be seen by the human eye, yet van Leeuwenhoek saw them all around us, on us, even inside us. In a letter to the Royal Society of London in 1684, he related that:

> The number of these animals in the scurf of a man’s Teeth, are so many that I believe they exceed the number of Men in a kingdom. For upon the examination of a small parcel of it, no thicker than a Horse-hair, I found too many living Animals therein, that I guess there might have been 1000 in a quantity of matter no bigger than the 1/100 part of a sand.

In another letter, he confided with amazement that:

> Some of these are so exceedingly small that millions of millions might be contained in a single drop of water. I was much surprized at this wonderful spectacle, having never seen any living creature comparable to those for smallness; nor could I indeed imagine that nature had afforded instances of so exceedingly minute animal proportions.

Thus, this modest Dutch merchant revealed a whole new microscopic world to humanity. Van Leeuwenhoek discovered microorganisms.

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**CONNECTIONS** for this chapter:

- Development of antimicrobial and antiviral drugs ([Section 24.2](#))
- Evolution of eukaryal cells through an endosymbiotic process ([Section 3.4](#))
- Oxygenic and anoxygenic photosynthesis ([Section 13.6](#))
- Epidemiology: The study of how infectious diseases spread within populations ([Section 18.3](#))
Introduction

With wonder in his voice, Anton van Leeuwenhoek shared his observations of microbial life with a skeptical public. In the three centuries since van Leeuwenhoek first viewed these “animalcules,” the scientific community and the general public have become much more appreciative of the importance of microbes. We now know that microscopic life on Earth is enormously abundant and diverse, that microbes appeared billions of years before humans, and that the health of the entire biosphere depends on its tiniest inhabitants, the microbes. We also know that microbes interact with each other and with multicellular organisms, including humans, in many ways. Because of our increased understanding of microbes, we now can use them to help us in many agricultural and industrial settings. Because of our increased understanding of microbes, we now better understand how our own bodies work. We also have learned to fear microbes; some of them cause diseases that have resulted in the suffering and death of untold millions of people through the ages.

Throughout this book, we will explore all of these aspects of microbiology. While we will use specific examples to illustrate our points, we will focus on the general principles. We will emphasize the evolutionary relationships between microbes and the evolutionary history of biological processes. We also will learn how the study of microbes relates to various other disciplines, like genetics, chemistry, and environmental science. Finally, we will see that microbiology itself is a dynamic, evolving science. Our knowledge of microbiology is predicated on thoughtful, interesting, exciting experiments. Much more still awaits our discovery. As we will note throughout this book, we do not know all the answers. We probably do not even know all the questions! The field of microbiology is ever changing. Today’s basic research will lead to tomorrow’s revelations.

So, let’s start our exploration of this dynamic field. In this chapter and in the book as a whole, we first will learn about the microbes. Then, we will examine the genetics of microbes. Next, we will look at the metabolism of microorganisms and how microbes interact with their environment. Finally, we will explore the role of microbes in disease. We can frame our initial discussion, then, around these questions:

What is microbiology? (1.1)
What do we know about the evolution of life and the genetics of microbes? (1.2)
How do microbes get energy and interact with the world around them? (1.3)
How are microbes associated with disease? (1.4)

1.1 The microbes

What is microbiology?

Microorganisms are microscopic forms of life—organisms that are too small to see with the unaided eye. They usually consist of a single cell and include bacteria, archaeons, fungi, protozoa, and algae. We will include viruses in many of our discussions as well. Viruses are not living, but they are microscopic; they utilize biological molecules and cellular machinery (borrowed from their host) to replicate, and they can cause infectious diseases like some microorganisms. While viruses are not microorganisms, we can refer to them as microbes, a more general term that includes microorganisms and viruses. Microbiology, then, is the study of microbes.

Our relationship with the microbial world is complex and dynamic. On one hand, harmful bacteria, viruses, fungi, and protozoa kill millions of people each year, and sicken billions. On the other hand, beneficial microbes associated with our bodies help us digest food, and protect us from potentially harmful microbial invaders (Figure 1.1). Some microbes cause crops to fail, while others provide essential nitrogen to plant roots through symbiotic relationships. Some microbes cause food to rot, but others carry out fermentations that produce yogurt, wine, beer, and other foods and beverages (Figure 1.2).

In the past few decades, we have learned so much about the molecular machinery of life through the study of microbes such as the bacterium Escherichia coli that scientists now routinely alter microbial cells to produce high-value, lifesaving medical products (Figure 1.3). Whether helpful or harmful, the microbial world is deeply intertwined with our lives, and with the very fabric of life on Earth. Let’s begin our exploration of microbiology, then, by asking a very fundamental question. What is life?

The basis of life

So, what is life? This question has fascinated humans for millennia—perhaps since our ancestors first developed conscious, introspective thought. As biologists, we will focus on a practical definition of “life” that distinguishes living organisms from non-living objects.

First, living organisms are composed of cells, the smallest units of life as we know them. Second, living organisms are capable of:

- **Metabolism**: a controlled set of chemical reactions that extract energy and nutrients from the environment, and transform them into new biological materials.
- **Growth**: an increase in the mass of biological material.
- **Reproduction**: the production of new copies of the organism.
Figure 1.1. Microbes and humans
A. Some microbes cause horrific infectious diseases, like smallpox. Man with smallpox (left); color enhanced smallpox viruses (right).
B. Other microbes, particularly those that reside in our gut, do not usually cause disease and help us digest the food that we eat (left). Food debris (yellow) and bacteria (purple) in the small intestine (right).

Figure 1.2. Microbes and food
A. Soybean rust, a disease caused by a fungus, causes significant crop losses every year.
B. Nitrogen-fixing bacteria interact with the roots of certain plants, forming nodules. The bacteria provide essential nutrients to plants, thereby aiding in their growth.
C. These rotting tomatoes show growth of fungi.
D. For centuries, microbes have been used by humans to help us produce cheese, yogurt, wine, and beer.

1.1 The Microbes
To accomplish these tasks, organisms contain a biological instruction set to guide their actions. These instructions need to be reproduced as the organism itself reproduces. Other features that living organisms share include:

- Genetic variation, allowing the possibility of **evolution**, or inherited change within a population, through natural selection over the course of multiple generations.
- Response to external stimuli and adaptation to the local environment (within genetic and physiological constraints).
- **Homeostasis**: active regulation of their internal environment to maintain relative constancy.

Does this list represent a complete description of what it means to be alive? Probably not. It’s easy to come up with situations that challenge these criteria. Consider the curious case of bacterial endospores—specialized, metabolically inert cells produced by some bacterial species under highly stressful conditions. After shutting down metabolism, growth, and reproduction, the endospores can remain dormant for long periods of time, even thousands of years, awaiting a favorable environment to germinate. Is an endospore “alive” during this state of suspended animation? These spores have all the components of living cells and, when conditions are appropriate, they will again develop into cells that meet the criteria listed above. As we will see later in this section, viruses—subcellular microbes—represent an even more interesting anomaly to the standard definition of life. So, our definition of life should be applied holistically; an organism may not exhibit all of these traits at all times.

Most microorganisms live and function as single, autonomous cells. A free-living unicellular, or single-celled, organism can carry out all the necessary functions of metabolism, growth, and reproduction without physical connection to any other cells. Multicellular organisms, by contrast, are comprised of many physically connected and genetically identical cells. The constituent cells that contribute to a multicellular organism can have distinct, specialized functions. A complex organism like a human can have hundreds of cell types, organized into tissues and organs. While the distinction between unicellular and multicellular organisms seems obvious, you might rethink this issue later, as we learn more about the microbial world. Some unicellular organisms, for instance, only can survive in close association with cells of another species. Other unicellular microorganisms can communicate, behave socially, form three-dimensional structures containing millions of cells with different functions, and enter into dependent relationships with other cells—behaviors that blur the boundaries between unicellular and multicellular lifestyles. The slime mold *Dictyostelium discoideum*, for instance, exists as a rather typical unicellular organism when food is readily available. During periods of nutrient depletion, however, individual cells aggregate and form a complex structure, with cells differentiating to assume specialized tasks (Figure 1.4). Before we investigate these more unusual arrangements, let’s learn more about the chemical make-up of cells.
Chemical make-up of cells

As we shall see in this section, all cells share some basic features. Notably, all cells are built from macromolecules—large, complex molecules composed of simpler subunits (Table 1.1). Macromolecules, in fact, make up over 90 percent of the dry weight, or weight after the removal of all water, of most cells. In this section, we will explore the four major types of macromolecules found in cells: polypeptides, nucleic acids, lipids, and polysaccharides. For each, we will look briefly at their structure and functions.

Polypeptides, polymers of amino acids, constitute the most abundant class of macromolecules. Polypeptides, also often referred to as proteins, fold into elaborate structures and can execute a vast array of important jobs. Some proteins function as enzymes, macromolecules that catalyze chemical reactions within the cell (Figure 1.5). Other proteins may facilitate the movement of material into or out of the cell. Still other proteins comprise critical structures such as microfilaments, that allow cell movement (Table 1.2).

Nucleic acids, polymers of nucleotides, make up most of the remainder of the macromolecules within a cell. This category includes deoxyribonucleic acid (DNA), a polymer of deoxyribonucleotides, and ribonucleic acid (RNA), a polymer of ribonucleotides. Individual nucleotides, in turn, are composed of a sugar molecule (deoxyribose in DNA, ribose in RNA), a phosphate moiety, and one of four nitrogen-containing bases (abbreviated A, T, C, and

### Table 1.1 Macromolecules in microbial cells

<table>
<thead>
<tr>
<th>Macromolecule</th>
<th>Subunits</th>
<th>Functions</th>
<th>Dry weight of cell (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypeptides</td>
<td>Amino acids</td>
<td>Enzymes catalyze the vast majority of biochemical reactions in the cell. Other proteins are structural components of cells.</td>
<td>50–55</td>
</tr>
<tr>
<td>Nucleic acids</td>
<td>Deoxyribonucleotides</td>
<td>Informational: DNA provides the instructions for assembly and reproduction of the cell.</td>
<td>2–5</td>
</tr>
<tr>
<td></td>
<td>Ribonucleotides</td>
<td>Many functions, most of which are involved in the production of polypeptides. Some serve structural or catalytic functions.</td>
<td>15–20</td>
</tr>
<tr>
<td>Lipids</td>
<td>Diverse structures</td>
<td>Structural: make up cellular membranes that form physical boundary between the inside of cell and surroundings and membranes of internal organelles.</td>
<td>10</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>Sugars</td>
<td>Structural (such as cellulose and chitin) and energy storage (such as glycogen and starch).</td>
<td>6–7</td>
</tr>
</tbody>
</table>

### Table 1.2 Selected functions of polypeptides

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Location</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA polymerase</td>
<td>Cytoplasm of bacteria and archaeons, nucleus of eukarya</td>
<td>Produces RNA molecules from DNA template</td>
</tr>
<tr>
<td>Glycogen phosphorylase</td>
<td>Cytoplasm</td>
<td>Conversion of glycogen into glucose monomers</td>
</tr>
<tr>
<td>K⁺ channel</td>
<td>Plasma membrane</td>
<td>Passive transport of K⁺ across the membrane, from an area of high concentration to an area of low concentration</td>
</tr>
<tr>
<td>Na⁺/K⁺ ATPase</td>
<td>Plasma membrane</td>
<td>Active transport of Na⁺ and K⁺ across the membrane, from areas of low concentration to areas of high concentration</td>
</tr>
<tr>
<td>Flagellin</td>
<td>Bacterial flagellum</td>
<td>Monomers polymerize to form flagellum, which aids in bacterial motility</td>
</tr>
<tr>
<td>FtsZ</td>
<td>Associated with plasma membrane of bacteria</td>
<td>Key component of cell division machinery</td>
</tr>
</tbody>
</table>
G in DNA; A, U, C, and G in RNA). In all cells, DNA constitutes the main informational molecule, containing instructions for the production of RNA molecules. These RNA molecules fulfill numerous functions within the cell, most of which are associated with protein production.

**Lipids**, hydrophobic hydrocarbon molecules, represent another important class of macromolecules. The primary role of lipids in most cells is to form the foundation of the plasma membrane, a barrier surrounding the cell that, quite simply, separates inside from outside. This membrane restricts the movement of materials into and out of the cell, thereby allowing the cell to capture and concentrate nutrients for metabolism and growth, and prevent the products of metabolism from escaping (Figure 1.6).

**Polysaccharides** are polymers of monosaccharides, or sugars. These molecules are comprised entirely of carbon, hydrogen, and oxygen, with the general formula of C\(_m\)(H\(_2\)O)\(_n\). Some polysaccharides serve as energy storage molecules. Starch and glycogen, for instance, both are polymers of the monosaccharide glucose (C\(_6\)H\(_{12}\)O\(_6\)). Other polysaccharides serve as structural molecules. Cellulose, the primary structural component of plant cell walls, also is a polymer of glucose monomers. Chitin, the primary structural component of fungal cell walls, consists of a derivative of glucose: N-acetylglucosamine. Many bacterial and archaenal cells use other polysaccharides for their cell walls.

**The domains of life**

While polypeptides, nucleic acids, lipids, and polysaccharides exist in all living organisms, major groups of organisms also differ in significant ways. Today, we categorize all living organisms, and, by extension, their cells, into three domains: Bacteria, Archaea, and Eukarya. Until the late 1990s, however, biologists divided cells into two types, **prokaryotes** and **eukaryotes** (Figure 1.7). The term “eukaryote” is derived from Greek roots meaning “true kernel,” in contrast to the term “prokaryote,” which translates as “before kernel.” The “kernel” refers to the...
Biologists noted other differences between these cell types. Additional membrane-enclosed organelles exist within eukaryal cells, with each organelle serving a unique and important function. Prokaryotes and eukaryotes also differ strikingly in the organization of their genetic material. Prokaryotes usually contain a single, circular chromosomal DNA molecule. Eukaryotes, in contrast, usually contain multiple, linear DNA molecules. At some point in their life cycle, most eukaryotic organisms have two copies, or a 2n complement, of their genetic material. Most prokaryotes, in contrast, possess a single copy of their genetic material.

Conventional wisdom through the better part of the twentieth century stated that prokaryotes represented a fairly uniform group, until scientists started looking in more detail at the molecular machinery for the synthesis of DNA, RNA, and polypeptides—the most ancient, important, and conserved processes in cells. In the 1970s, microbiologists studying some prokaryotes noted that their molecular machinery resembled that of eukaryotes more than it did other prokaryotes. Leading the way in these studies was Dr. Carl Woese of the University of Illinois. Woese focused his attention on the structure and sequence of one of the RNA molecules that serves as a scaffold for assembly of the ribosome—the small subunit (SSU) ribosomal RNA. This molecule is a critical component of the ribosome in all living organisms and interacts with the messenger RNA during translation (see Section 7.4). His work paved the way for a revolution in thinking about the phylogeny, or evolutionary history, of organisms (Figure 1.8). His studies also led to a major revision in the taxonomy, or the classification, of living organisms. Because of Woese’s work, we now categorize all living organisms into one of three domains: Bacteria, Archaea, or Eukarya (Mini-Paper, p. 14).

Thanks largely to the development of the polymerase chain reaction (PCR), a technique that allows researchers to quickly amplify specific pieces of DNA (Toolbox 1.1, p. 12), we now have a richer, more accurate phylogenetic tree. This tree is consistent with the idea that the archaeal and eukaryal domains shared a common ancestor after they split from the bacterial domain. It probably is impossible, though, to determine when the divergence of these lineages actually occurred. Microorganisms don’t fossilize well, but fossilized stromatolites, mineralized mats built up by layer upon layer of photosynthetic bacteria and other microbes in shallow marine habitats, have been observed in rock formations nearly 3.5 billion years old (see Figure 1.13). If such elaborate microbial communities, including bacteria capable of photosynthesis, existed 3.5 billion years ago, then the split between Bacteria and the Archaea/Eukarya domains probably occurred significantly earlier.

Although all cells share many features, studies have clearly demonstrated that bacteria, archaeans, and eukarya are evolutionarily distinct. Some of their differences are listed in Table 1.3. We will discuss each of these types of cells in much more detail in Chapters 2–4.

**Table 1.3 Selected characteristics of the three domains**

<table>
<thead>
<tr>
<th></th>
<th>Bacteria</th>
<th>Archaea</th>
<th>Eukarya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear membrane</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Membrane-bound organelles</td>
<td>Rare, a few types found in a few species</td>
<td>Rare, a few types found in a few species</td>
<td>Multiple distinct types, found in all species</td>
</tr>
<tr>
<td>Plasma membrane</td>
<td>Similar to Eukarya</td>
<td>Different from Bacteria and Eukarya</td>
<td>Similar to Bacteria</td>
</tr>
<tr>
<td>Cell wall</td>
<td>Found in nearly all species, constructed of peptidoglycan</td>
<td>Found in nearly all species, constructed of various materials</td>
<td>Found in some species, constructed of various materials</td>
</tr>
<tr>
<td>RNA polymerases</td>
<td>Single polymerase</td>
<td>Single polymerase, Eukaryal-like RNA pol II</td>
<td>Three main polymerases (RNA pol I, II, and III)</td>
</tr>
<tr>
<td>Histones</td>
<td>Histone-like proteins</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
**Viruses**

Are viruses alive? They are not cellular, but they certainly replicate and evolve. Viruses, however, require host cells for replication. Outside of a host cell, virus particles are essentially inert. An isolated virus has no metabolism—it takes up no nutrients and extracts no energy from its environment. Viruses also lack most of the basic machinery needed for the synthesis of macromolecules. Viruses do not respond to stimuli, except perhaps when they bind to receptors on a new host cell, and they do not maintain internal homeostasis. When a virus enters a host cell, it does not grow and reproduce in the same sense that cellular organisms do. Virus particles are more or less completely disassembled in the host cell, and new virus particles are only assembled after the genetic material has been replicated and the host cell has synthesized new viral proteins. Cellular organisms have no comparable state of disassembly during their growth and reproduction.

**CONNECTION**  Viruses infect all cellular forms of life. They replicate in various ways, but all depend on using host cell machinery for their replication. This makes them obligate intracellular parasites. We will examine viral replication in Section 8.3.

Although viruses are not cellular, they are still very important biological entities to study (Figure 1.9). Viruses are molecular parasites that probably have been around since...
Microbes as model organisms

Microbes have been used extensively in research. Because they replicate quickly, are cheap to grow, and have relatively simple structures, they have been used extensively to study basic cellular processes like DNA replication, transcription, and translation. Two of the most studied microbial model organisms are A. the bacterium *Escherichia coli* and B. the eukarya *Saccharomyces cerevisiae*, a yeast.

shortly after the first cells evolved. Microbiologists are interested in viruses not only because they cause many important infectious diseases in humans, crop plants, and livestock, but also because they are fascinating biological systems in their own right. Viruses have taught us a great deal about how cellular organisms function. As parasites, viruses must adapt to their host organism. To be taken up by host cells, most viruses have evolved to bind to host cell surface molecules, and often enter cells by hijacking host systems ordinarily used for taking up non-viral molecules. Many viruses rely on the host enzymes for the production of mRNA, and all viruses use host cell ribosomes for the production of proteins. By studying how viruses use the machinery of their host cells, scientists have gained insight into many critical processes in eukaryal, bacterial, and archaeal cells.

**Microbes as research models**

Basic research on the structure and function of microbes has laid a solid foundation for understanding the biology of all cells, including our own. Unicellular microorganisms generally possess the same genetic code and many of the same biochemical pathways as multicellular organisms. Additionally, microbes have many advantages for use in research:

- Many are easily cultivated in the lab; they grow rapidly to high cell density on cheap nutrient sources, using inexpensive equipment.
- They facilitate the production of enzymes, other proteins, and various biomolecules for industrial and medical uses.
- Most have relatively small numbers of genes to analyze. Even the largest bacterial and archaeal genomes are smaller than the smallest eukaryal genomes, and eukaryal microbes have significantly fewer genes than complex multicellular eukarya.
- Many can be genetically manipulated much more easily than complex eukarya.

Popular microbial model systems for research include the intestinal bacterium *Escherichia coli* and the eukaryal yeast *Saccharomyces cerevisiae*, which is also known as “baker’s yeast” or “brewer’s yeast,” because of its long historical use in food and beverage production (Figure 1.10). These model microbes have been subjected to the vast experimental armaments of the fields of biochemistry, genetics, molecular biology, and cell biology. Our current understanding of the complexities of biochemical pathways, DNA replication and cell division, the nature of genes, control of gene expression, and protein synthesis, folding, and function has arisen largely from studies of these microorganisms.

Research on the biology of microbial cells has virtually unlimited practical applications. For example, to understand how some antimicrobial drugs work against their microbial targets, while sparing host cells, we need to understand differences in structure between bacterial and eukaryal cells, or perhaps between fungal and human cells. Paul Ehrlich, a towering figure in the history of medicine and immunology, was among the first to recognize that such differences had medical implications. From his experience in the field of histology, Ehrlich was familiar with dyes that differentially stained bacterial and human cells. Based on this observation, he speculated that molecular “magic bullets” that specifically target microbial invaders were feasible. He had little knowledge of the actual structures present on or in cells of any kind, but this concept that certain drugs may adversely affect specific types of cells, while sparing other types of cells, remains at the heart of our drug development initiatives today.
The method of ribosomal RNA sequencing developed by Carl Woese was extremely laborious. Fortunately, techniques just being developed in the 1970s and 1980s, as Woese and colleagues were initially developing the universal phylogenetic tree, made nucleic acid sequencing much simpler. The most important of these techniques was the polymerase chain reaction (PCR). With this technique, researchers can create millions of copies of a specific piece of DNA.

Polymerase chain reaction

Kary Mullis, then a scientist at Cetus Corporation, a biotechnology company in Emeryville, California, invented PCR in 1983 and was awarded the Nobel Prize in Chemistry in 1993 for this discovery. The technique basically mirrors the process of DNA replication utilized by all cells. Rather than replicating an entire DNA molecule, however, PCR results in the repeated replication, or amplification, of a small, defined segment of a larger DNA molecule. The reaction requires only a few basic reagents:

- DNA containing the sequence to be amplified
- Deoxyribonucleotides (dATP, dCTP, dTTP, and dGTP)
- DNA polymerase
- Oligonucleotide primers

The process begins with the denaturation of double-stranded DNA, making it single-stranded. This step is achieved by heating the DNA to around 95°C for a short period of time. The primers, 15–30 nucleotide-long pieces of single-stranded DNA synthesized in the laboratory, then bind to complementary regions on this newly denatured DNA. The primers are designed such that one primer binds to one strand of the denatured DNA, while the other primer binds to the other strand of the DNA. Additionally, the two primers bind to regions of the DNA flanking the sequence to be amplified (Figure B1.1).

After the primers bind, then the DNA polymerase begins generating new DNA, using the denatured DNA as a template. We will see in Section 7.2 that DNA polymerases generate new DNA by attaching...
Members of Ehrlich’s research group discovered an organic arsenic-containing compound, arsphenamine, which in 1910 became the first effective commercial drug for the treatment of *Treponema pallidum*, the bacterium that causes the sexually transmitted disease syphilis (Figure 1.11). Because it also exhibited toxicity to host cells, arsphenamine, known by its trade name Salvarsan, was abandoned in the 1940s in favor of penicillin, the first widely used antibiotic capable of killing many different kinds of bacteria.

Salvarsan’s historical importance was in establishing that lethal agents specifically targeted at microbial cells are indeed possible. In the century since Salvarsan came on the market, an enormous amount has been learned about the molecular differences between bacterial and eukaryal cells. Hundreds of new antimicrobial and antiviral drugs have been discovered, and hopefully there will be more to come.

**CONNECTION** Basic research into the structure and replication of microbes has led to the development of numerous antimicrobial and antiviral drugs. Many of the currently approved drugs for the treatment of HIV, for instance, interfere with specific viral enzymes needed for the production of new virus particles. We will discuss how a particular class of these drugs—nucleoside analogs—works in Section 24.2.

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**Test Your Understanding**

1. What are the key features of living organisms?
2. Describe the macromolecules found in cells.
3. What are the three domains of living organisms?
4. Explain why microbes are useful model systems in research and provide examples of microbial model systems.
The sequence of this molecule changes very slowly because of the functional constraints on the molecule. Random mutations that occur within the gene encoding the small subunit rRNA often have serious negative consequences, so relatively few changes are passed on to subsequent generations. Nevertheless, there are enough differences in the roughly 1,600 nucleotide sequence to differentiate between species to map patterns of similarity. If one assumes that overall mutation rates are similar between species (which seems to be true with respect to rRNA genes), then one can quantify sequence differences between SSU rRNA genes in multiple species to infer relationships. Ultimately, Woese discovered that the methane-producing microorganisms were no more closely related to other bacteria than they were to the eukaryotes. Let’s examine the scientific work that led to this conclusion.

Experiments

The 1990 Woese et al. paper actually presented no new experimental data. Its importance was in articulating a new view of the phylogeny of life. To understand the genesis of this idea, we should step back and examine the data. The biggest challenge Woese faced in the 1970s in developing ribosomal RNA sequences as a tool for phylogenetic analysis was the difficulty in determining such sequences. Woese and colleagues developed a laborious method to infer the sequence of 16S rRNA molecules, which they described in a 1977 article, “Comparative cataloging of 16S ribosomal ribonucleic acid: A molecular approach to procaryotic systematics,” Journal of Bacteriology, vol. 27, pp. 44–57. First, RNA was extracted from cells. The isolated rRNA then was cut into small chunks using a ribonuclease enzyme that yields short fragments of nucleic acid usually 5–20 bases long. The sequence of each oligonucleotide was determined by further chemical and enzymatic analysis. In its original incarnation, this method did not actually yield a complete rRNA sequence, but rather a catalog of short oligonucleotide sequences present in the rRNA. Catalogs from different species then were compared.

The underlying assumption of molecular sequence comparisons, at least with respect to phylogeny and taxonomy, is that the number of nucleotide differences between two sequences is proportional to the time since the two species diverged from a common ancestor. Species that shared a more recent common ancestor will have fewer differences than species that have been separated for longer periods of time. Exactly how long ago two species separated depends on the rate at which mutations accumulate, which can be very difficult, if not impossible, to know. Fortunately, to determine phylogenetic relationships, we do not need to know exact times of divergence. We are just interested in relative times: if organisms A and B shared a common ancestor after they shared an ancestor with organism C, then A and B would have fewer sequence differences with each other than either would have with C. The Woese method essentially took each 16S rRNA sequence and compared it against all of the other species. A method for quantifying the similarity between sequences was developed to yield
an “association coefficient” between 0 and 1. A perfect match between sequences would result in an association coefficient of 1, whereas no matches would give a score of 0. From these scores, a computer algorithm was used to plot the most likely phylogenetic tree.

Starting with papers in 1977 and continuing through the 1980s, Woese and colleagues built a “universal phylogenetic tree” through comparison of SSU rRNA sequences from diverse organisms, including members of each of the five kingdoms of life, as defined at that time. The great strength of this universal tree is that it compares all organisms using a common standard, a molecule they all possess. Woese noted that this universal tree supports three primary branches of life, not the five kingdoms previously accepted. In the 1990 paper, Woese and his coauthors propose that these very ancient branches—Bacteria, Eukarya (or Eucarya, as it is spelled in the 1990 paper), and Archaea—be referred to as domains (see Figure 1.8).

The domain Archaea is composed of species previously known as archaeabacteria. Though archaeons lack a nucleus, they turned out to be no more similar to bacteria than they are to eukaryotic organisms, or eukarya. In fact, Woese’s tree suggests that Archaea and Eukarya share a more recent common ancestor than either does with Bacteria. As we will see in Chapter 4, archaeons are similar in size and shape to bacteria. Many of the enzymes used by archaeons for DNA replication, transcription, and translation, however, more closely resemble the corresponding enzymes found in eukarya. Perhaps most interestingly, the plasma membrane of archaeons differs chemically from the plasma membranes of bacteria or eukarya. The exact evolutionary history of these organisms is yet to be determined.

In this three-domain phylogenetic tree, Monera and Protista disappear as kingdoms. In fact, if kingdoms are to be defined by equivalent depth of branching—which implies roughly equivalent evolutionary times since divergence—rRNA gene sequence comparisons support many more kingdoms than were previously known, most of which are populated by microorganisms. As we will see in Chapter 4, Woese and coauthors proposed two kingdoms within the Archaeal domain: the Crenarchaeota and the Euryarchaeota. The authors also noted the presence of heat-loving microbes in several other branches of life. It is quite possible, then, that the organism at the root of the tree (the last common ancestor of all life on Earth) was thermophilic, or heat-loving. We will return to this point in Section 1.2.

Impact
For the first time, biologists could create a natural taxonomic system in which all organisms are compared by the same criteria. The realization that the prokaryotes could not be united as a phylogenetic group raised many questions regarding the validity of the five kingdom system. Not surprisingly, there was initial resistance. Many scientists challenged the validity of this approach and the computational methods on which it was based. Since Woese first conducted his experiments, there have been methodological improvements, most significantly the ability to amplify entire rRNA genes using the polymerase chain reaction (PCR; see Toolbox 1.1), followed by rapid and straightforward DNA sequencing. The basic concept of using rRNA gene sequence comparisons to derive phylogenetic relationships is now accepted as an essential method of phylogenetic analysis.

Sequence-based phylogenies have had more impact on microbiology than any other branch of science. Since this paper was published in 1990, databases containing ribosomal RNA gene sequences have grown explosively. The universal phylogenetic tree is now much richer and more complex, but the three-domain organization remains unchallenged. Using PCR, microbiologists can characterize organisms using rRNA gene sequences even if the microorganisms cannot be grown in culture. Since the majority of microbes apparently will not grow in laboratory culture (see Section 6.3), this approach is enormously important for understanding the true diversity of life on Earth, and its evolutionary history. Proposals for new bacterial and archaeal kingdoms, based on rRNA gene analysis of uncultured organisms, have appeared regularly since 1990. Classification of eukaryal microorganisms also is affected by rRNA-based phylogenetics.

Ribosomal RNA sequences, however, do not tell the entire evolutionary story of an organism. In the last decade, the DNA sequences of hundreds of entire genomes have been determined, most of them from microorganisms. It is clear that microbes are rampant sharers of genes, which we will discuss more in Chapters 9, 10, and 21. While it is likely that rRNA genes are rarely shared and do accurately reflect the evolutionary history of the “core” genome, large fractions of genetic material in many organisms may have distinct histories—a finding that Carl Woese could scarcely have imagined when he began his revolutionary efforts to clarify microbial taxonomy.

Questions for Discussion

1. What features make SSU rRNA gene sequences ideal for phylogenetic studies?
2. What drawbacks do you see with the use of rRNA for these studies?
3. If we discover forms of life on another planet, would studies of rRNA gene sequences be useful for categorizing these life forms?
1.2 Microbial genetics

What do we know about the evolution of life and the genetics of microbes?

While groups of microbes may be different from each other, they all share common information processes. Indeed, one of the most remarkable aspects of life as we know it is the constancy of these information processes. In all cells, the main informational molecule is double-stranded DNA. In all cells, a specific type of RNA, messenger RNA, or mRNA, serves as the conduit between the information in DNA and the actual production of proteins. In all cells, the code used to convert the information present in DNA to RNA to protein is the same. This conserved genetic code probably represents the most compelling evidence for evolution. All living organisms share a common informational pathway, suggesting that all living organisms share a common ancestor.

Because all living organisms, and the genetic processes of these organisms, are evolutionarily related, we will begin our exploration of microbial genetics by examining the origins of life. We then will look at how genetic processes occur in microbes and how microbiologists study these processes. We will end this section with a brief overview of how researchers today use these processes to learn more about living organisms.

The evolution of life on Earth

Earth is home to a huge variety of microbes. To understand how this incredible diversity evolved, we need to consider the history of Earth and the origins of life itself. The geochemical changes that have occurred on Earth in the past 4 billion years—in the oceans, on land, and in the atmosphere—have been dramatic. These changes have profoundly affected, and were profoundly affected by, microorganisms. The vast majority of the living organisms we see today, at least without microscopes, are large, multicellular eukarya that arose within the last few hundred million years, the last 10 percent of Earth’s history. But most of the major evolutionary events that moved life toward today’s world occurred in the distant past, when microbes alone ruled the planet. To get some perspective on this point, let’s take a brief walk through the history of life.

Prebiotic Evolution

When Earth formed approximately 4.5 billion years ago (abbreviated ybp, for years before present), it was a hot, sterile place. Oceans of liquid water formed around 4 billion ybp, once the crust and atmosphere had cooled sufficiently for liquid water to condense (Figure 1.12). These oceans may have been partially or completely converted to steam on multiple occasions by the energy of asteroid impacts, which were far more common in the early solar system. Depending on when the first life forms evolved, such impacts could have resulted in mass extinctions, coupled with selection for life forms that could live in this extreme environment. By 3.8 billion ybp, life clearly had gained a permanent foothold. The first microorganisms appeared as life transformed from a semi-organized set of chemicals and
reactions to a true cellular form. By 3.5 billion ybp, microbial cells were abundant on Earth, as is evident from fossilized stromatolites containing cyanobacteria-like structures (Figure 1.13). Cyanobacteria, we should note, are photosynthetic bacteria. The evolution of these organisms, and their oxygen-releasing photosynthetic capabilities, led to the eventual oxygenating of Earth’s atmosphere. Given that multicellular algae and marine invertebrates are not evident in the fossil record until 0.5 billion ybp, it appears that microbial life ruled Earth for over 3 billion years. Only during the last 500 million years has Earth seen the rise of plants and animals! Our planet has changed drastically since its violent birth, but with the exception of dramatic events like asteroid impacts and volcanic eruptions, changes have occurred gradually. Microbes had plenty of time to evolve an incredible array of talents, allowing them to exploit every possible niche. Given the eons that have gone by, we can only imagine the diversity of microbial life that has existed since Earth’s origins; we still do not fully comprehend the richness of microbial life on present-day Earth.

When life first appeared, Earth was a harsh place. The average temperature was quite hot, probably over 50°C. The composition of the atmosphere is not known for sure, but researchers hypothesize that it had a high concentration of CO₂, perhaps up to 30 percent. Other atmospheric gases may have included nitrogen (N₂) and hydrogen (H₂). Whether gases such as ammonia (NH₃), methane (CH₄), cyanide (HCN), and hydrogen sulfide (H₂S) were present in substantial concentrations is not known with certainty. It is clear, though, that there was little or no molecular oxygen (O₂). The oceans probably were fairly acidic due to the high concentration of dissolved CO₂. By comparison, today’s atmosphere consists of about 0.03 percent CO₂ and 21 percent O₂, with a moderate average temperature of 13°C. What changed the O₂ and CO₂ concentrations so dramatically since life began? Microbial activities over the past 4 billion years are part of the answer.

The First Microbial Life

It is generally assumed that life forms present on early Earth have not survived unaltered to modern times. Conditions on our planet have changed radically over the past four billion years, and it is reasonable to assume that evolutionary innovations incorporated into living systems during that time out-competed and displaced primitive cells long ago. Nevertheless, the biochemical origins of life are of great interest. As we look outward for life elsewhere in our solar system and beyond, simple living systems—microorganisms—are far more likely to be discovered than are advanced civilizations in flying saucers. The better we understand the evolution of life on Earth, the better idea we have of what to look for elsewhere.

Many hypotheses have addressed the origin of life. The Miller–Urey experiment envisioned an early Earth where organic molecules accumulated in the oceans, creating a rich “prebiotic soup” from which organized cellular life eventually emerged (Perspective 1.1). Perhaps the organic molecules would have required a surface on which to accumulate, rather than simply floating in the open ocean. Günter Wächtershäuser
**Perspective 1.1**
CREATING LIFE IN THE LABORATORY:
THE MILLER–UREY EXPERIMENT

We cannot go back in time to the prebiotic world to watch how life actually evolved. We can imagine scenarios, formulate hypotheses, and test them in the laboratory to gain insight into potential pathways to life. In 1953, Stanley Miller described the first laboratory investigation intended to simulate prebiotic Earth. Miller, then a graduate student at the University of Chicago, designed a reactor with his mentor, Harold Urey, to test for abiotic production of biologically relevant molecules. The Miller–Urey experiment revolutionized the thinking of many scientists, and a fair number of non-scientists as well, about the origin of life on Earth.

The Miller–Urey experiment started with a water-filled flask, heated by a burner. The atmosphere in the apparatus was intended to simulate that of primitive Earth, and consisted of a mixture of ammonia (NH₃), methane (CH₄), and hydrogen (H₂). The water was heated to boiling, at which time water vapor mixed with the various gases in the atmosphere and circulated through the apparatus. A separate chamber discharged electrical sparks through the atmosphere, after which the tubing was cooled so that water vapor would condense and drip back into the original flask (Figure B1.3).

After a few days of continuous operation, Miller observed that the water in the flask was changing color. Within a week, the solution in the flask had turned a deep red, and had become turbid. Miller examined the solution after a week and found that the solution contained organic molecules, or molecules containing carbon-hydrogen bonds. Most notably, the simple amino acids glycine and alanine were readily detectable, along with aspartic acid. These amino acids are among the 20 primary amino acids used by life on Earth for protein synthesis, and presumably were abundant as life evolved. Miller’s flask provided the first evidence that such molecules could be synthesized from inorganic precursors under conditions believed to mimic primordial Earth, where energy inputs could have included electrical discharge from lightning strikes as well as heat from both the sun and Earth’s crust.

The Miller–Urey experiment stimulated other scientists to design experiments to simulate hypothetical atmospheric, oceanic, and geological conditions on prebiotic Earth. Experiments done in hydrogen-rich reducing atmospheres similar to Miller’s showed that many amino acids, nitrogenous bases, and other organic compounds could be produced abiotically. Questions about these experiments arose, however, as the consensus of scientists shifted in the 1970s to favor an atmosphere on early Earth that was much richer in CO₂ and N₂. In a Miller-type apparatus, these atmospheric conditions yield few or no organic molecules such as amino acids. Models of early Earth conditions continued to evolve. By 2005, scenarios with much higher levels of H₂ in early atmospheres, coexisting with CO₂, were tested. Jeffrey Bada, a professor at Scripps Institution of Oceanography and a former graduate student of Miller’s, further altered the composition and atmosphere in a Miller-type reactor and was able to reproduce Miller’s yield of organic material.

Controversies continue as we do not know the exact chemical and physical conditions on early Earth. Predicting the pathway by which life arose depends greatly on what conditions are assumed to have existed. To further complicate the situation, some evidence suggests that substantial amounts of organic molecules could have been delivered to Earth by comets and asteroids crashing into the planet during the tumultuous early days of the solar system. Hydrocarbons and complex nitrogen-containing organic molecules, including amino acids, have been detected on comets in space, and in meteorites on Earth. The magnitude of the contribution of these impacts remains unclear.
has theorized that life evolved on iron-containing surfaces, such as iron pyrite (FeS₂), an insoluble, positively charged surface with affinity for organic compounds. Metabolic processes that occur in modern cells could have evolved from reactions among surface-bound organic compounds. Many modern proteins, such as cytochromes and hemes, bind iron and other metallic atoms; many enzymes, including DNA polymerases, require bound metals for activity. These interactions with metals may reflect a very ancient relationship.

As we try to envision the origins of life, the formation of chains of ribonucleotides (RNA) and amino acids (polypeptides) clearly represents a necessary event. These polymers, as we noted, contain information and carry out cellular functions. Some researchers have suggested that information was initially stored in RNA molecules, rather than DNA. We now know that certain RNA molecules, ribozymes, can catalyze chemical reactions (Perspective 1.2) Early in the history of life, then, this macro molecule may have had dual functions.

We noted earlier that all cells contain a plasma membrane. As we think about the origins of life, let’s ask another question. How did the first membranes form? We know that hydrocarbons coupled to charged groups, such as phosphates, form polar lipids, which can spontaneously organize into micelles and even bilayer membranes that close back upon themselves to form a sealed...
compartment (Figure 1.14). Perhaps such primitive membranes initially formed around fragments of minerals, such as FeS₂, that also happened to serve as surfaces for the aggregation of organic molecules, as discussed above. If a membrane encapsulated an informational molecule and a catalytic molecule, like a ribozyme, then something like a cell would have been formed.

The Appearance of Eukarya

How did complex cells arise? Most biologists now agree that mitochondria and chloroplasts, two of the most distinctive organelles in eukaryal cells (Figure 1.15), are derived from bacterial cells, through a process known as endosymbiosis. These organelles have their own circular genomes, though these genomes are far smaller than the genomes of contemporary bacteria. The genes present in the chloroplast and mitochondrial genomes include a gene encoding 16S rRNA, the SSU rRNA molecule present in bacterial ribosomes. Sequence analysis of the mitochondrial and chloroplast 16S rRNA gene indicates that mitochondria and chloroplasts are related to specific groups of bacteria.

Lynn Margulis, a biologist at the University of Massachusetts–Amherst, has long championed this idea to explain the origins of mitochondria and chloroplasts. Indeed, substantial molecular evidence indicates that these organelles became part of eukaryal cells through endosymbiotic relationships that became permanent. Mitochondria probably were added first to a developing eukaryal cell; they are present in most, but not all, modern eukarya. We can imagine that the endosymbiont provided the host with extra ATP. In return, the host provided the endosymbiont with nutrients and a safe place to live. The extra ATP ultimately allowed for an increase in cell size, and for multicellular arrangements.

Chloroplasts probably were added later, leading to the evolution of algae and plants. A bacterium capable of photosynthesis, the ability to harvest the energy present in sunlight to produce organic molecules, would provide a host with a new energy source and allow the host to expand into new niches. During the evolution of eukarya, multiple endosymbiotic events leading to chloroplasts may have occurred. We now know that some differences exist between the chloroplasts present in different algal and plant groups. Regardless, photosynthesis brought solar power to eukaryal cells. Algae and plants, as a result, have been phenomenally successful, and now account for a major fraction of the biomass on land (plants) and sea (algae). Once these mitochondria and chloroplasts became permanently established in eukaryal hosts, their own genomes degenerated until only a few essential genes were left.

Connection

We will look more closely at the origins of eukaryal cells in Section 3.4 and explore the evidence supporting the endosymbiotic theory. In Chapter 17, we’ll see that endosymbiotic relationships between microbes and host cells are actually very common in nature. In most cases, the host cannot survive without the essential functions supplied by the endosymbiont.

In the earliest cells, RNA may have been the major informational and catalytic molecule. Eventually, double-stranded DNA supplanted RNA as the major informational molecule and proteins arose as the major catalytic molecules, giving rise to the first living organism. Carl Woese refers to this first living organism as a progenote, a cell hypothesized to store information in genes not yet linked together on chromosomes (Figure 1.16).
By 500 million years ago, multicellular eukarya had begun to dominate the macroscopic landscape of life on Earth. Microbes had set the stage for them. As we will see in the next section, the evolution of oxygenic photosynthesis in cyanobacteria led to the abundance of molecular oxygen we see in the atmosphere today. Highly efficient aerobic respiration became possible. Aerobically respiring bacteria then entered into the symbioses that led to mitochondria in eukaryal cells. Without mitochondria to efficiently power cellular metabolism, large multicellular eukaryal organisms would never have appeared.

**DNA and RNA: The genetic molecules**

Today, in all organisms, genes are linked together in long molecules of double-stranded DNA. DNA is an elegant, if enormously long, molecule whose structure is beautifully suited for information storage and replication. Because each strand of a double-stranded DNA molecule is complementary to the other strand, one can easily imagine a mechanism of faithfully replicating the molecule. If the two strands denature, or come apart, then each strand contains the information necessary to re-form the other strand (Figure 1.17). When a cell divides, identical copies of DNA can be produced so that each progeny cell will contain the exact same informational molecule.

When a progenote replicated itself, each of the progeny may have had a different subset of the parental genes, and thus a number of variations could have arisen in one generation. Genetic variation and mutation were probably frequent, given that these primitive cells would have lacked the sophisticated genetic repair mechanisms seen in modern cells. Though they might not have looked much like modern life, progenotes would have been just as subject to Darwinian evolution. Darwinian evolution, we should note, depends on (1) genetic variation in a population, (2) the environment exerting selective pressure(s), and (3) differential reproductive success among genetic variants as a result of that selective pressure. Primitive cells that not only survived, but reproduced more efficiently and passed gene sets to their progeny, would have spread most rapidly. Assuming that genetic variation was common in progenotes, the rate of evolutionary change could have been quite high.
As we noted earlier, the functional versatility of RNA has contributed catalytically to peptide bond formation. Ribosomes are the protein synthesis factories of the cell. During translation (polypeptide synthesis), transfer RNAs (tRNAs) deliver amino acids to the ribosome. The ribosome itself is composed of proteins and ribosomal RNA (rRNA) molecules, which provide a structural framework. One of the rRNAs contributes catalytically to peptide bond formation. As we noted earlier, the functional versatility of RNA has suggested to scientists that it could have played a central role in the origin of living systems.

The companion nucleic acid, RNA, also plays a critical role in the information flow in cells (Figure 1.18). Messenger RNA (mRNA) is synthesized from a DNA template during the process of transcription, and delivers instructions to the ribosome for the production of a specific chain of amino acids. Ribosomes are the protein synthesis factories of the cell. During translation (polypeptide synthesis), transfer RNAs (tRNAs) deliver amino acids to the ribosome. The ribosome itself is composed of proteins and ribosomal RNA (rRNA) molecules, which provide a structural framework. One of the rRNAs contributes catalytically to peptide bond formation. As we noted earlier, the functional versatility of RNA has suggested to scientists that it could have played a central role in the origin of living systems.

The ability to faithfully replicate the genome and convert the information contained within the genome into functional proteins only represents part of the story. As we noted earlier, living organisms must contain some genetic variation in order for evolution to occur. The ultimate source of this variation is mutation, a heritable change in the genome. As we will first see in Section 7.5, mutations can result from errors made during replication or from various physical or chemical insults to the DNA. Regardless of their source, these changes in the genotype, or genetic composition of an organism, can be passed on when the genome replicates and the cell divides (Figure 1.19). More importantly, these mutations may alter the genetic information in such a way that the proteins produced by the cell differ (Figure 1.20). These changes in the proteins, in turn, can alter the phenotype, or observable characteristics of the cell.

While bacteria do not undergo sexual reproduction by the formation and uniting of gametes, they do exchange genes between cells, a process referred to as horizontal gene transfer. As we will see in Section 9.5, genetic material can be transferred between bacteria in several ways. Moreover, more and more evidence suggests that genetic material can be transferred not only between different bacterial types, but also between organisms in different domains. This exchange of genetic material muddies somewhat our ability to construct a perfect phylogenetic tree of all living organisms (Figure 1.21).

In recent years, improved DNA sequencing techniques have allowed researchers to determine the exact DNA sequences of entire genomes of organisms. Rather than just studying specific genes, we now can study entire genomes, a field of inquiry known as genomics. Along with these improved sequencing techniques, we also now have improved computing power. The computer tools available today allow us to analyze and compare genomes, a burgeoning field referred to as bioinformatics. With these new tools at our disposal, we are learning more about the evolutionary history of life, the diversity of organisms, and the functioning of our cells. The flood of microbial genome sequences in recent years has clearly shown that horizontal gene transfer is ubiquitous in microorganisms. In reality, most microbial genomes are composites of DNA fragments with distinct evolutionary histories. Microbial genomes, then, are quite plastic. It’s probably more accurate to say that all members of a microbial type share a common pool of genes.

Genomic analysis

While most living organisms share a common genetic code, significant differences in some of the details of the code and various genetic processes exist between members of the domains Eukarya, Archaea, and Bacteria. We will examine translation more in Section 7.4 and explore some of these domain and organism-specific differences.

CONNECTION

CONNECTION

Genomics has revealed the profound impacts of gene transfer in regard to pathogen evolution. In Section 21.3, we’ll explore several examples of how transfer events have led to the development of a pathogen.
Finally, this recombinant DNA molecule can be added back to bacteria. As the bacteria replicate, the recombinant plasmid also replicates. More importantly, foreign genes inserted into the plasmid will be expressed (Figure 1.22).

Biotechnology and industrial microbiology

Scientists studying genetics and molecular biology revolutionized all of biology in the late 1970s with the development of recombinant DNA molecules—DNA sequences linked together to form a single molecule that never existed previously in the natural world. This revolutionary technology has allowed genes from humans and many other organisms to be inserted into bacteria and other microbes. This technique, in turn, has allowed microbes to be used as low-cost manufacturing plants for the production of valuable proteins such as human insulin and human growth hormone. The incredible range of applications for recombinant DNA methods could not have been realized without the availability of well-studied microorganisms such as E. coli.

The process of creating recombinant DNA is surprisingly simple. From bacteria, researchers first can isolate plasmids, small circular DNA molecules that replicate independently of the chromosome. Using restriction endonucleases, bacterial enzymes that cleave DNA at specific locations, researchers can insert foreign DNA into a plasmid, creating a new molecule.
Following a related process, researchers at Genentech, a biotechnology company based in San Francisco, announced in 1978 that they had engineered E. coli to produce human insulin. This achievement marked the first time that a medical product was produced via recombinant DNA technology. Today, a vast majority of insulin used by people with diabetes is produced in an analogous fashion.

**CONNECTION** As we will see in Section 12.2, researchers now can use techniques like site-directed mutagenesis to intentionally modify natural gene products. In Section 12.4, we will investigate how biotechnology may help to alleviate our dependence on fossil fuels.

## 1.3 Microbial metabolism and ecology

**How do microbes get energy and interact with the world around them?**

So far we have seen that microbes share many structural and functional features, but also exhibit a great deal of diversity. We also have seen, at least briefly, how life may have evolved and how genetic information is transmitted and interpreted within microbes. As we continue our introduction to microbiology, let’s ask two more important questions. First, how do microorganisms get the energy needed to support biosynthesis and growth? Second, how do microorganisms interact with their environment? As we will see, these two questions are intricately linked.

Bacteria and archaeons have greater metabolic diversity and inhabit more diverse environments than eukarya, particularly multicellular eukarya. Environments inhabited by bacteria and archaeons range from deep sea thermal vents with temperatures of greater than 110°C, to Antarctic ice sheets, to deserts that rarely if ever see a drop of rain, to porous rocks a kilometer or more beneath Earth’s surface. Microbes thrive in acidic hot springs with a pH of 1 and alkaline lakes with a pH of greater than 11. They live in distilled water taps, and saturated brine solutions in salt evaporating ponds. Their ability to live in these varied habitats reflects their ability to acquire energy from these environments. Their metabolic capabilities, in other words, dictate the habitats in which they can live.

### Photosynthesis, respiration, and the appearance of atmospheric oxygen

All microorganisms, indeed, all living organisms, must acquire energy and produce macromolecules. Most macroscopic eukarya exhibit relatively limited types of metabolism. Microbes, as we will see briefly in this section and more fully in Chapter 13, exhibit more diverse types of metabolism. This metabolic diversity allows microbes to inhabit a wide range of habitats. Because different microbes can utilize various nutrients, they can exist in environments that may be uninhabitable by other organisms.

In a very basic sense, all living organisms need organic molecules. **Heterotrophs**, or “other” feeders, ingest organic molecules. These pre-formed molecules can be used for the biosynthesis of other macromolecules or as an energy source. **Autotrophs**, or “self” feeders, can produce their own organic molecules from an inorganic carbon source. We typically think of plants when we think of autotrophs. These photosynthetic organisms harvest the energy present in sunlight to convert the carbon in CO₂ into organic molecules.

In cyanobacteria, the presumed descendants of the organisms that gave rise to chloroplasts, membrane-bound pigment molecules absorb the energy present in sunlight. This absorbed energy results in the transfer of an electron from the pigment molecule to a series of membrane-bound proteins. Ultimately, the movement of this electron powers the formation of ATP, the energy currency of the cell (Figure 1.23). The cells can use this ATP to incorporate inorganic carbon from CO₂ into an organic molecule. To complete the process, the pigment molecule must regain an electron to replace the one that was lost. In cyanobacteria and plants, the pigment molecule regains this electron by removing electrons from water, resulting in the liberation of O₂:

\[
2\text{H}_2\text{O} \rightarrow 4\text{H}^+ + 4\text{e}^- + \text{O}_2
\]

This liberation of oxygen via photosynthesis ultimately led to the increased atmospheric O₂ concentration that we discussed previously.

**CONNECTION** Not all photosynthetic organisms produce O₂ as a by-product. Some photosynthetic organisms gain electrons from hydrogen sulfide (H₂S), instead of water. For these organisms, elemental sulfur (S), not O₂, is liberated. This anoxygenic photosynthesis will be discussed in more detail in Section 13.6.

Regardless of how organisms acquire organic molecules, all living organisms also need a mechanism of oxidizing those molecules to generate ATP. One of the simplest means of acquiring...
energy from organic molecules is glycolysis, the reaction in which glucose is converted to pyruvate, with the subsequent generation of two ATP molecules:

\[
\text{Glucose} + 2 \text{ADP} + 2 \text{P} \rightarrow 2 \text{pyruvate} + 2 \text{ATP} + 2 \text{NAD} + 2 \text{H}^+.
\]

In some cases, glycolysis is coupled with fermentation, a process in which the NAD produced by glycolysis is converted back to NAD\(^+\) and the pyruvate molecules are converted to a waste product, such as ethanol or lactate. While this system of obtaining energy from glucose is fairly simple, it is not particularly efficient; much of the potential energy present in the original glucose molecule is not converted to ATP (Figure 1.24).

A more effective means of obtaining ATP from glucose involves respiration, a set of metabolic processes whereby the glucose is more completely utilized, resulting in the production of larger quantities of ATP. Plants, animals, and many bacteria undergo aerobic respiration, a form of respiration that involves the addition of electrons to O\(_2\), resulting in the formation of H\(_2\)O. Some bacteria perform respiration in which a terminal electron acceptor other than oxygen is used. This type of respiration generally results in the formation of less ATP than respiration involving oxygen.

The presence of O\(_2\) in the atmosphere had profound consequences for life on Earth. Respiration utilizing oxygen allows organisms to harvest a significant amount of energy from organic molecules. During this process, though, oxygen is converted to a series of toxic by-products. These by-products can damage cells through their ability to oxidize other molecules and their ability to generate even more potent toxins through interactions with light. Until oxygenic photosynthesis evolved, all life, by necessity, was anaerobic. When O\(_2\) began accumulating in the atmosphere, organisms either had to develop strategies for defending themselves against these dangerous by-products, or retreat to niches where O\(_2\) remained less abundant or absent.

This remains true today. Anaerobic microbes are still with us, living in specialized habitats free from molecular oxygen.

**Connection** During aerobic respiration, O\(_2\) can be converted to hydrogen peroxide (H\(_2\)O\(_2\)). This reactive molecule can damage cells. As a result, most organisms that can survive in the presence of O\(_2\) produce catalase, an enzyme that converts H\(_2\)O\(_2\) to H\(_2\)O and O\(_2\). We will learn more about toxic oxygen species in Section 6.3.

The increase of O\(_2\) in the atmosphere also created an ozone layer in the stratosphere, approximately 10–25 miles above Earth’s surface. Ozone (O\(_3\)) is generated naturally from O\(_2\) and strongly absorbs short wavelength ultraviolet (UV) light. UV light is dangerous to cells because it causes damaging chemical reactions in DNA. Because UV light does not penetrate water very well, aquatic life is fairly well protected from its harmful effects. Terrestrial organisms, however, are not so lucky. The accumulation of ozone in the atmosphere reduced the amount of UV radiation reaching Earth’s surface. The rise of O\(_2\) in the atmosphere, therefore, indirectly facilitated the colonization of land by microorganisms.

1.3 Microbial Metabolism and Ecology 25
Microorganisms and biogeochemical cycling

Microbes have been intimately involved in modulating conditions within the biosphere, those regions of Earth that can support life. Not only did microbes, through photosynthesis, create the oxygen-rich atmosphere on which most life on Earth relies, but they are also involved in biogeochemical cycling, the transitioning of various chemicals between organic and inorganic forms. The amount of carbon contained within living bacteria on Earth, for instance, is estimated to be nearly as great as the amount of carbon in all the multicellular organisms combined. Photosynthetic cyanobacteria convert CO$_2$ from the atmosphere into organic molecules. Microbial metabolism ultimately converts much of this organic carbon back into CO$_2$.

As we saw in Section 1.1, nucleic acids and polypeptides, two important categories of cellular macromolecules, contain nitrogen. Only certain types of bacteria can convert nitrogen gas (N$_2$) from the atmosphere into forms that can be readily used by other organisms to form these molecules. This nitrogen fixation is accomplished both by free-living bacteria, and by bacteria living in symbiotic associations with plants. Bacteria also convert nitrogen present in organic material back to N$_2$ gas, through both denitrification and ammonia oxidation. Denitrification is primarily a terrestrial process that is of special concern to farmers, as it decreases the fertility of agricultural soils. Ammonia oxidation is largely a marine phenomenon, which limits ocean productivity.

Microbial interactions

Before we leave this section, we should note that microbes do not exist in isolation. As we will investigate more thoroughly in Chapter 15, microbes exist in diverse communities of organisms that interact with each other and the environment. As we will see in Chapter 3, vast numbers of eukaryal microbes exist within the Rio Tinto, a river in Spain with a pH of approximately 2. As we will see in Chapter 4, communities of organisms live in the waters surrounding deep sea thermal vents, dependent, in large part, on archaeons that thrive in the superheated water near these thermal vents. As we will see in Chapter 17, many microbes live with, in, or on various plants, invertebrates, and vertebrates.

Microbes, of course, also live in and on humans. Van Leeuwenhoek, remember, described microorganisms living on a person’s teeth over 300 years ago. In fact, we can view the human body as a complex ecosystem. Microbes live throughout our bodies, often in a symbiotic relationship with us. Microbes within our body produce vitamins for us and help us digest our food. Some microbes, as we will discuss in Chapter 23, even help us control the replication of other, unwanted microbes, thereby aiding in the prevention of disease.

1.3 Fact Check

1. Differentiate between the terms heterotroph and autotroph.
2. Describe the roles of glycolysis, fermentation, and respiration in energy production in different environments.
3. Explain how bacteria are involved in nitrogen cycling.
Today, we know that microbes can cause diseases. Throughout most of human history, however, people thought that diseases had various causes from angry gods to bad air. These views prevailed mostly because people could not see the bacteria associated with bacterial infections. Even after the development of the microscope, people still did not understand that microbes could be transmitted from person to person. Rather, many assumed that microbes arose from inanimate materials, a process known as spontaneous generation. This belief negated a need to even consider the transmission or prevention of microbial diseases. If microbes arose spontaneously, then there was no reason to investigate how a person became infected.

While many scientists worked to disprove the widely held belief in spontaneous generation, Louis Pasteur’s experiments in the mid-1800s provided the most compelling evidence refuting this idea. As with so many classic experiments, his experiment was both simple and elegant. Pasteur added nutrient broths to swan-necked flasks and then boiled the broths to kill any contaminating microorganisms. He then observed the broths for signs of microbial growth (Figure 1.26).

With this approach, Pasteur reasoned, outside air could enter the flask. Bacteria present in the outside air, though, would become trapped in the neck of the flask, never coming in contact with the sterile broth. No bacteria, he hypothesized, would grow. If the flask were tilted such that the broth traveled to the neck of the flask, however, then bacteria trapped there would gain access to the nutrient broth and growth would occur. Microbial life in the broth, in other words, would only result from microbial life present in the neck of the flask. It would not arise spontaneously.

**How are microbes associated with disease?**

**Microbes in Focus 1.1**

**BACILLUS ANTHRACIS**

**Habitat:** Infects various mammals, including cattle, sheep, and horses. Also can infect humans. Spores can be found in soil.

**Description:** Gram-positive rod-shaped bacterium, measuring approximately 1 μm in width and 3 μm in length.

**Key Features:** When nutrients are lacking, *B. anthracis* can form endospores, metabolically inert structures that are largely resistant to harsh environmental conditions. The endospores can begin replicating again when conditions improve, even after years in the endospore state. While naturally occurring human infections with *B. anthracis* are rare, this bacterium still remains actively studied by researchers, mainly because of its potential use as a bioweapon. We will explore this topic more in Chapter 6.

**The identification of infectious agents**

About 200 years after van Leeuwenhoek’s first observations of microbes, and just 15 years after Pasteur showed that microorganisms do not arise by spontaneous generation, Robert Koch provided the first clear proof that a bacterium, *Bacillus anthracis* (Microbes in Focus 1.1), was the cause of a specific disease, anthrax, in livestock.
Robert Koch was a German physician and scientist, and a true pioneer in the field of microbiology. Aside from his anthrax work (Figure 1.27), he was responsible not only for developing important laboratory techniques—for example, the use of media solidified with agar for the isolation of bacteria in the laboratory—but also for developing clear criteria linking particular microorganisms to specific diseases. We will explore the details of these criteria, referred to as Koch’s postulates, in Section 18.4.

Koch used these criteria to establish the cause of other important diseases, including tuberculosis, which is caused by the bacterium *Mycobacterium tuberculosis*. The identification of other infectious microbes quickly followed. The late 1800s, in fact, were a golden age of sorts, in which medical science finally began to achieve real insight into many infectious diseases. Many disease agents, though, continued to be elusive, and even Koch realized his criteria had limitations. For example, he knew that many microorganisms suspected to cause disease, such as the agent of cholera, *Vibrio cholerae*, could be isolated from both sick and healthy people, invalidating his first postulate.

**CONNECTION** As our understanding of infectious diseases, and our experimental techniques, have changed, so too have our interpretations of Koch’s postulates. Molecular correlates of his basic postulates are explored in Section 18.4.

The effects of infectious diseases
As van Leeuwenhoek observed, many microbes exist in and on our bodies. Most of the time, these microbial partners are just that—partners. When the relationship between microbes and host becomes unbalanced, then disease can arise. Microbial diseases, as we all know, have profoundly affected human life. As we will see in the final section of this book, our understanding of how microbes cause disease—and how we can prevent these diseases or alleviate their effects—is constantly changing.

We all feel the effects of microbial infections through the course of our lives. A runny nose and a cough often are signs of “a cold,” which may be due to a rhinovirus infection of the upper respiratory tract. The onset of winter portends the flu...
season, when hundreds of millions of people each year suffer from fevers, headaches, coughs, and muscle aches caused by the influenza virus. The average person in the United States experiences several bouts of diarrhea per year, which could have any number of different causes, including viruses, bacteria, or protozoa, all of which can be acquired through food or drink. If you are studying this book in the United States, Canada, or Europe, it’s unlikely (but not unheard of) that you will have to deal with the constant threat of infections with serious, or even life-threatening consequences. If you live in one of the many regions of the world where affordable effective public health measures are lacking, infectious diseases may have a more prominent role in your life. AIDS, malaria, tuberculosis, and other diseases of microbial origin take millions of lives per year in these regions; we are far from a solution to this enormous problem.

History is full of examples of the powerful impact of infectious diseases. Modern medicine has greatly improved our ability to deal with these diseases. We now understand how infectious diseases spread, and the widespread availability of vaccines and antimicrobial drugs facilitates prevention and treatment. But we have not eliminated the threat of new infectious diseases. The human immunodeficiency virus (HIV), which was only discovered in the 1980s, has caused over 40 million deaths since then, and there is still no vaccine to prevent HIV infection. The influenza virus has caused devastating pandemics, or worldwide outbreaks, in centuries past, and now there is great concern that the avian influenza serotype H5N1 or the recently identified H1N1 strain will mutate to become more deadly, with potentially catastrophic consequences.

**CONNECTION** As we saw during the summer and fall of 2009, the H1N1 strain of influenza virus quickly spread throughout the world. What factors led to the rapid dissemination of this microbe? We will discuss epidemiology, or the study of how diseases spread in populations, in Section 18.3.

An exceptionally devastating pandemic occurred when plague, popularly referred to as “The Black Death,” killed a third or more of the population of Europe, Asia, and North Africa in a 60-year span from 1340 to 1400 (Figure 1.28). Plague...
is caused by the bacterium *Yersinia pestis*, which infects rodents and humans, and is transmitted between them by fleas (Figure 1.29). This bacterium is commonly present in rodents such as mice, rats, and squirrels in many parts of the world. The plague pandemic of the 1300s is thought to have originated in central Asia or China, and probably moved westward with trading caravans and/or Mongol armies. Historical records indicate that plague was absolutely devastating. Up to 60 million people, perhaps half of its population, may have died from plague in China in the 1300s. In Europe, 25–50 million people perished, largely between 1347 and 1351. Several million more people were killed by plague in the Middle East and northern Africa.

To put the global death toll due to the plague pandemic of the 1300s into perspective, imagine a modern pandemic that resulted in the death of over 1 billion people within a few years! Even the modern AIDS and influenza pandemics pale by comparison. In the Middle Ages, people did not understand the underlying cause of this horrifying disease. Supernatural explanations of disease were universally accepted; diseases were punishments from God or the results of curses, witchcraft, or “bad air.” The true cause of plague, in fact, was not discovered until 1893, when Alexandre Yersin isolated the bacterium *Yersinia pestis* during a plague outbreak in Hong Kong. Today, plague is rare and easily treatable with antibiotics.

The massive social disruptions caused by epidemics such as plague in the Middle Ages are rivaled only by the effects of war, famine, and natural disasters. From the perspective of public health, we should note, these events are not unrelated. Until very recently, most people who died during wartime, both military and civilian, were victims of microbes. Before the advent of antibiotics during World War II, battlefield wounds were highly likely to become infected, the end result of which was often death. Troops in the field for prolonged periods often were affected by disease. Cholera, typhus, and dysentery were common, due to the poor sanitation conditions. Diseases spread by carriers such as lice and fleas, which thrive in crowded conditions, also are a great risk to soldiers and displaced civilians (refugees) during wartime. Malnutrition resulting from war or famine (or both) blunts the effectiveness of the immune system, leaving soldiers and civilians more susceptible to disease.

**Control of infectious diseases**

As researchers learned more about the causes of infectious diseases in the late 1800s and early 1900s, the medical treatment of these diseases gained a scientific basis. Even before Koch linked specific microbes to specific diseases, the British physician Joseph Lister had discovered the value of cleanliness and disinfection measures in reducing mortality from post-surgical and post-childbirth infections. Doctors’ offices and hospitals strove for the universal application of such disinfection measures to decrease the incidence of disease. While these methods of preventing infections were effective, methods for treating infectious diseases remained inadequate.

While Salvarsan was the first scientifically developed antimicrobial to be commercially marketed, it was not until penicillin
Recall that bacteria are masters at sharing genes through horizontal gene transfer. This facilitates the passing of genes for antimicrobial resistance. History has shown that the more effective an antimicrobial drug is, the more it is prescribed, and the faster resistance spreads, rendering the drug ineffective. See Section 24.3 for more on the acquisition of drug resistance.

Vaccines also have had an enormous impact in reducing the sickness and death associated with infectious diseases. Vaccination involves exposing a person to an inactivated or weakened version of a microbe, or even just a part of the microbe, to create immunity to a disease. The past century has seen vaccines developed for many deadly diseases, including polio, diphtheria, rabies, and many others. Historically, vaccination began roughly 2,000 years ago in China and India as a defense against smallpox. Smallpox, a viral disease, was common in Europe and Asia, in some areas being responsible for 20 percent of all deaths. It was fatal to 1 of every 4 people infected. Those who survived carried characteristic smallpox scars for the rest of their lives, but they also carried a lifelong protection from the disease.

Vaccination against smallpox was occasionally practiced in eighteenth century Europe, but it was the famous experiment of English physician Edward Jenner in 1796 that popularized the procedure. Jenner used material from a cowpox infection of a milkmaid to inoculate a boy, who was later shown to be immune to smallpox (Figure 1.31). We now know

Figure 1.30. Infectious disease deaths in the United States during the twentieth century. Deaths associated with infectious diseases decreased dramatically in the United States over the past century. The spike in deaths due to infectious diseases around 1920 represents the increased deaths caused by the 1918 influenza pandemic.

and sulfa drugs came into use in the 1940s that antimicrobial drugs had a major impact on the treatment of infectious diseases. Indeed, people in developed countries saw a dramatic decrease in deaths due to infectious disease during the twentieth century, because of improved sanitation and the development of antimicrobial drugs and vaccines (Figure 1.30). A new problem, however, now faces us. Increasingly, infections result from antibiotic-resistant bacteria. Our existing antibiotics are not effective against these microorganisms, and our ability to treat some infectious diseases is declining.

Figure 1.31. Edward Jenner and the development of vaccination
While the Chinese practiced forms of vaccination against smallpox for hundreds of years, vaccination was not practiced in Europe until the end of the eighteenth century. In 1796, Edward Jenner showed that he could inoculate a boy with material obtained from the pox marks on a woman infected with cowpox and provide the boy with immunity to smallpox. Global vaccinations have eliminated smallpox. A. 1798 illustration from Jenner of cowpox lesions on a milkmaid’s hand. B. Painting of Jenner vaccinating a person against smallpox. C. Vaccinia virus, the causative agent of smallpox.
As a result, we now are faced with what Dr. Paul Farmer refers to as the “great epi (epidemiology) divide.” People in developing countries and people in developed countries who are simultaneously affected by at least two of these diseases, lowering their life expectancy even further.

People in developing regions of the world, particularly in sub-Saharan Africa, continue to suffer from infectious diseases, particularly AIDS, tuberculosis, and malaria, which combined take nearly 5 million lives per year (Figure 1.32). Vaccines are not yet available that can prevent HIV or malaria infection, and the current tuberculosis vaccine is far from ideal. Drugs to treat these three diseases are available, but they are expensive, which means that many people do not have adequate access to these therapies. Additionally, it is not uncommon to find people in developing countries who are simultaneously affected by at least two of these diseases, lowering their life expectancy even further.

Other major historical factors that have reduced death from infectious diseases include improvements in personal hygiene, public sanitation, and food and water safety. Indoor plumbing, water treatment measures, and large-scale sewage disposal systems have led to a decrease in water-borne infectious diseases. Pasteurization, the process in which milk is heated briefly to kill most microorganisms, freezers, and refrigerators all have contributed to the increased safety of food. Many of us take these advances for granted. Unfortunately, these simple measures are not universally available. As a result, we now are faced with what Dr. Paul Farmer refers to as the “great epi (epidemiology) divide.” People in developing countries and people in developed countries without access to adequate health care suffer a disproportionate infectious disease burden.

In this chapter, we have barely scratched the surface of microbiology. We have been introduced to the microbes. We have learned about the evolution of life and the genetics of microbes. We have begun to explore how microorganisms acquire energy. Finally, we have seen how microbes can cause diseases. In later chapters, we will further explore these topics. We will see how important microbes are in our world. As we investigate these topics, we will see that extensive experimental evidence supports our current understanding of the microbial world. We also will see that many questions and ambiguities still remain.

Throughout this book, we will see that microbiology is a dynamic science. Microbiologists continue to make new discoveries using an expanding selection of laboratory tools. They also continue to refine, modify, and, occasionally, reject existing hypotheses. As we saw in the Mini-Paper, inquisitive minds, continuous experimentation, and the development of new tools can combine to result in completely new ways of seeing the world around us. Such dramatic advances have occurred throughout the history of microbiology and certainly will continue to occur (Table 1.4).

Figure 1.32. Impact of malaria in sub-Saharan Africa Worldwide, deaths associated with malaria, an infectious disease caused by the protozoan Plasmodium falciparum, have decreased dramatically. This decrease, however, has not been evident in sub-Saharan Africa, where malaria remains a major problem. Today, infectious diseases disproportionately affect people in developing countries and people without clean water or access to adequate health care.

### Table 1.4 Selected advances in microbiology

<table>
<thead>
<tr>
<th>Year</th>
<th>Scientist</th>
<th>Advance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late 1600s</td>
<td>Anton van Leeuwenhoek</td>
<td>Uses microscope to see microorganisms</td>
</tr>
<tr>
<td>1860s</td>
<td>Louis Pasteur</td>
<td>Disproves idea of spontaneous generation</td>
</tr>
<tr>
<td>1876</td>
<td>Joseph Lister</td>
<td>Practices infection control</td>
</tr>
<tr>
<td>1876</td>
<td>Robert Koch</td>
<td>Identifies Bacillus anthracis as cause of anthrax</td>
</tr>
<tr>
<td>1928</td>
<td>Alexander Fleming</td>
<td>Discovers penicillin</td>
</tr>
<tr>
<td>1950s</td>
<td>Jonas Salk and Albert Sabin</td>
<td>Develop poliovirus vaccines</td>
</tr>
<tr>
<td>1966</td>
<td>Lynn Margulis</td>
<td>Proposes endosymbiotic theory</td>
</tr>
<tr>
<td>1983</td>
<td>Kary Mullis</td>
<td>Invents PCR</td>
</tr>
<tr>
<td>1990</td>
<td>Carl Woese</td>
<td>Proposes three-domain classification of living organisms</td>
</tr>
<tr>
<td>1995</td>
<td>Craig Venter</td>
<td>Publishes first complete bacterial genome sequence</td>
</tr>
</tbody>
</table>

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During our exploration, we will make connections between topics. We will see how intimately related microbes are to each other, to their environment, and to us. We will see how the genetics, physiology, and habitat of microbes are intertwined. We also will see how, as the evolutionary biologist Theodosius Dobzhansky wrote in 1973, “Nothing in biology makes sense except in the light of evolution.” Most importantly, we will see that the microbial world presents as many surprises to us today as it did to van Leeuwenhoek over 300 years ago.

The Rest of the Story

Van Leeuwenhoek noted that the number of microbes on a person’s teeth may “exceed the number of Men in a kingdom.” It most certainly does. Today, many researchers estimate that the human body consists of $10^{13}$ human cells. On us and in us, though, there may be $10^{14}$ microbial cells. As Dr. David Relman from Stanford University noted, “we are 10 parts microbe, and one part human.” The study of these microbes has moved well beyond the use of the microscope. In this chapter, we reviewed how the microbes present today have evolved to occupy a plethora of environments and niches using a vast array of metabolic processes, even within the human body. Growth conditions for many of these microbes are well understood, and they have been isolated and grown in the laboratory. However, a large subset still remains a mystery because their specific growth microenvironments have not been determined, or cannot be reproduced experimentally.

In 2007, the Human Microbiome Project was launched with an aim to identify, analyze, and catalog the hundreds of microbial species residing in or on the human body (the human microbiota). One strategy being employed by project researchers involves amplifying and studying small subunit ribosomal RNA (SSU rRNA) genes, as described in Section 1.1. Examination of rRNA gene sequences will reveal the diversity of microbes in the human body and provide evidence of the presence of unclassified, rare, and new microbial species that have anonymously inhabited our bodies. This “non-cultivation-based” approach will ultimately facilitate the genetic sequencing and more complete understanding of the microbes that have a major influence on human health and disease.

Image in Action

Van Leeuwenhoek is examining a sample of pond water using the microscope he built by hand. Today, microbiologists also can analyze DNA sequences (represented in the upper left) to study environmental samples.

1. What types of microbes (and from what domains of life) did Anton van Leeuwenhoek most likely observe? What microbes was van Leeuwenhoek unable to observe with his simple microscope? Explain.
2. Imagine that you were given access to the same drop of pond water observed by van Leeuwenhoek. How could you use PCR to identify or characterize some of the microbes he observed or even those he didn’t observe?

Summary

Section 1.1: What is microbiology? Microbiology is the study of microorganisms, including bacteria, archaeons, and eukaryal microbes, and viruses. Collectively, we can refer to all of them as microbes.

- All living organisms share certain features, including metabolism, growth, reproduction, genetic variation resulting in evolution, response to outside stimuli, and internal homeostasis.
- A cell is the simplest structure capable of carrying out all the processes of life.
- All cells contain various macromolecules, including polypeptides, nucleic acids, lipids, and polysaccharides. Many polypeptides function as enzymes.
- Historically, all living organisms were classified as eukaryotes or prokaryotes, depending on whether they did, or did not, have a nucleus. Today, the taxonomy of living organisms consists of three domains, Bacteria, Archaea, and Eukarya.
- This classification scheme reflects the phylogeny of all living organisms.

- Our ability to classify microorganisms has been aided greatly by the polymerase chain reaction (PCR).
- Viruses are sub-cellular and can be classified as microbes.
  Because of their relatively simple structures, microbes have been useful research models.

Section 1.2: What do we know about the evolution of life and the genetics of microbes?

All living organisms possess remarkably similar informational molecules and processes for converting this genetic information into functional molecules. This conservation of genetic processes provides compelling evidence that all living organisms are evolutionarily related.

- Life probably evolved on Earth around 3.8 billion years ago.
  Simple organic molecules, possibly associated with iron-containing surfaces, became enclosed within a lipid membrane.
- The identification of ribozymes lends support to the idea that the precursors of life, the so-called progenote, may have used RNA as the major informational molecule.
Section 1.3: How do microbes get energy and interact with the world around them?

All living organisms must obtain organic molecules. Heterotrophs ingest them. Autotrophs produce their own organic molecules.

Most autotrophs generate organic molecules through photosynthesis.

Organic molecules generally are converted into ATP through the processes of glycolysis, fermentation, and respiration.

Microbial metabolism has affected the biosphere, and microbes are intimately involved in the biogeochemical cycling of many chemicals, including nitrogen, phosphorus, and sulfur, in the biosphere.

Microbes interact with each other and other organisms in many complex ways.

Section 1.4: How are microbes associated with disease?

The work of a number of microbiologists, including Louis Pasteur, led to the development and acceptance of the germ theory of disease.

Koch’s postulates, developed in the 1800s, provide a means of demonstrating that a particular microorganism causes a particular disease.

Infectious diseases have had, and continue to have, a profound impact on humans.

Today, we have an assortment of antibiotics, antivirals, and vaccines that treat or prevent many infectious diseases. The development of these therapies has depended, in large part, on our understanding of the structure of these microbes and their replication strategies. Other techniques, like pasteurization, also have led to a decrease in the incidence of certain infectious diseases.

Unfortunately, infectious diseases remain horrific threats to people throughout the world.

Application Questions

1. A researcher is studying a newly discovered microbe and must first determine whether this microbe should be considered alive. What characteristics will she examine to make this determination?

2. Given what we learned about proteins in Section 1.1, what might be the effects of an antimicrobial drug that halts protein synthesis in cells?

3. Imagine a researcher is examining several cell samples in the laboratory and needs to categorize each sample as either bacterial or eukaryal. What features should the researcher examine and why?

4. Inspired by the Miller–Urey experiment described in Perspective 1.1, a researcher continues to explore the origins of life on Earth. The first task is to design a simulation of prebiotic Earth. What conditions should be considered when creating this simulation?

5. According to the endosymbiotic theory, mitochondria and chloroplasts are derived from bacterial cells. What evidence exists to support this theory?

6. If a species of microbe were discovered that did not appear to mutate, scientists probably would hypothesize that its lack of mutation would be detrimental to its evolution. Explain why the scientists would make this prediction.

7. In Section 1.3, oxygenic photosynthesis and aerobic respiration were described. Based on our discussion of these two processes, explain why oxygenic photosynthesis must have evolved on Earth before aerobic respiration.

8. Oxygen is not always a “good thing.” In fact, O₂ can be considered dangerous. Explain why oxygen can be harmful and what microbes must do to deal with the dangers of an oxygenated world.

9. Section 1.4 discusses the great epi (epidemiology) divide between developing and developed countries. List several policy and funding changes that could be implemented in developing countries to decrease the number of infectious disease cases. Explain each of these recommendations.

10. A research group has received funding to study the diversity and evolutionary relatedness of microbes in a marine ecosystem. The head of the laboratory has decided to sequence and analyze the SSU rRNA genes of the microbes in this ecosystem.

    a. What are SSU rRNA genes?

    b. Why are these gene sequences ideal for studying the evolutionary relationships of the microbes found there?

    c. How is this process conducted?

    d. After sequencing and analyzing three gene samples, the researchers conclude that two of the samples are from closely related microbes, while the third sample is very distantly related. Describe the sequencing results that would lead to this conclusion.

11. In Section 1.1, we discussed Paul Ehrlich’s foundational research in which he noted differences between bacterial and human cells. Those observed differences became the basis for the development of antimicrobial drugs that “spare” eukaryal human cells. Based on these observed differences between bacterial and eukaryal cells:

    a. Identify an aspect of bacteria that would NOT be a good target for a drug because it would not “spare” the host. Explain why this bacterial component is not a good choice for an antimicrobial drug.

    b. Identify an aspect of bacteria that might be worth further exploration as a potential target for an antimicrobial drug that would not harm human cells. Explain.
Suggested Reading


