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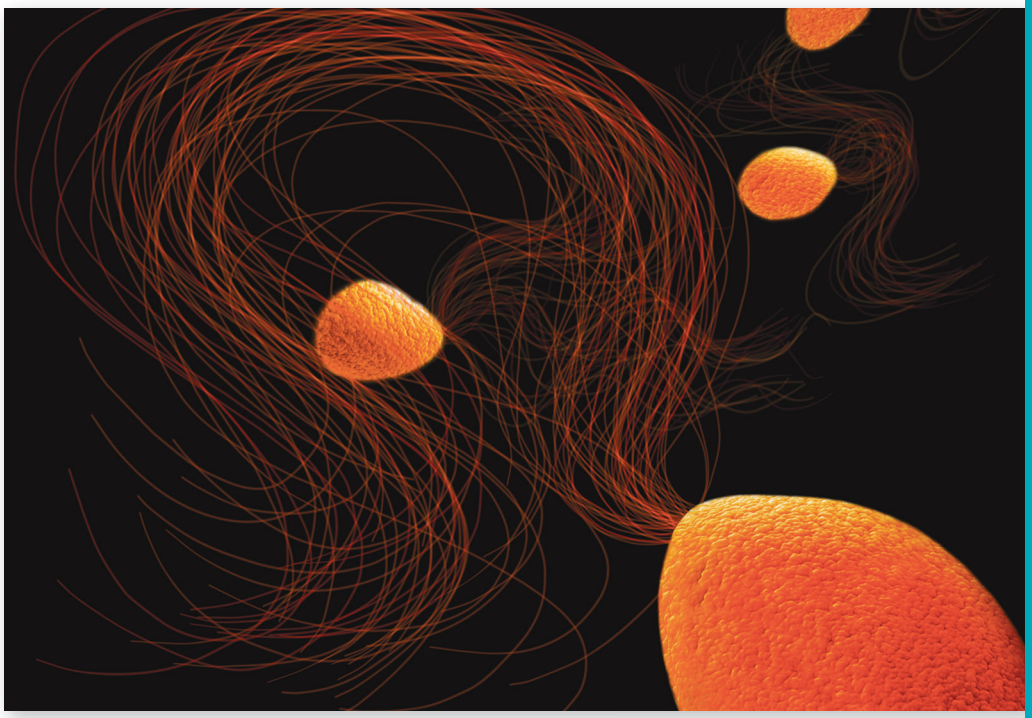
The Ancient Origin of Microbes



Hi there! My name is *Pyrococcus furiosus*. No fears, I am not a furious microbial monster. I am simply an extremophilic, hyperthermophilic archaeon that thrives in extremely hot environments. Maybe now you would prefer me to simply be furious! It isn't that hard to figure me out. I love hot! I mean I really, really love hot. My optimal growth temperature is a mere 100°C (or 212°F). I am also "allergic to oxygen," meaning I need to live in anaerobic environments, such as near hydrothermal vents. In fact, I was first found in waters near Italy, hanging out in a vent. Why should you care about little 'ole me? Well, I am a chemoorganotroph, meaning I break down sulfur to obtain energy. In the process I produce hydrogenases and amylases that are extremely heat-stable and efficient, which makes them valuable for some of your human industrial applications. So, a little kudos to me, please! (Photo from Power and Syred / Science Source)

CHAPTER CONTENTS

Before we begin our exploration of the human microbiome, we must first develop an understanding of microorganisms, also called microbes—those minute creatures, far too small to be seen by the naked eye, that are both the creators and constituents of a breathtaking spectrum of microbiomes found on Earth. As you will learn, microorganisms emerged on our planet shortly after its origin and have spent over 4 billion years adapting to every conceivable environment our planet has to offer, including us! This first chapter provides an overview of the origins and diversification of microbes on Earth, with a special emphasis on what makes microbes so unique among life on our planet.

"If you don't like bacteria, you're on the wrong planet."
—Stewart Brand (Brand, 2014)

1.1 IN THE BEGINNING

If you could peer back in time to the birth of our planet, some 4.5 billion years ago (bya), what might you find? Certainly nothing even remotely resembling the Earth of today. Our young planet had no oceans, although there were plenty of volcanoes spewing out magma, water vapor, and gasses. It had no free oxygen in its atmosphere and no protective **ozone layer**, which is the thin layer of the Earth's atmosphere that absorbs most of the sun's harmful ultraviolet light. It would have been an exceedingly hot place—imagine a surface temperature upwards of 2,000° Celsius (3,632° Fahrenheit). An artist's rendition of early Earth shows a planet that does not appear even remotely hospitable to life (**Figure 1.1**).

Or was it? In fact, some of the earliest signs of life appear in 3.7 bya rock, formed when our planet was just beginning to cool from its volcanic origin (Dodd et al., 2017). Some of this ancient rock has survived the ages and paints a fascinating picture of early life. The dark gray peaks in the cross section of sedimentary rock shown in **Figure 1.2A** have tentatively been identified as fossilized microbial mats, also known as **stromatolites**, which are mounds of layers of lime-secreting bacteria and trapped sediment. Stromatolites were the only biological structures on Earth until about 540 million years ago (mya), and they can still be found in certain lagoons in Australasia (**Figure 1.2B**). In other words, regardless of how inhospitable early Earth might look to us, by 3.7 bya Earth was already teeming with life!

The word **microbe** literally means "small life," from the Greek words *mikros* and *bios*. Microbes are small life forms that are usually too small to be seen without magnification. As we shall learn, they represent the greatest diversity of life on our planet. Although most of us are aware microbes exist, we may be unaware that they appeared very early in Earth's history and have remained the dominant life forms ever since. Exploring present-day **hydrothermal vents** in the seafloor provides valuable clues about how these earliest life forms flourished in the extreme environments of our young planet. Heated, mineral-rich water flows out of these seafloor vents, and it supports untold numbers of **chemolithotrophs**, which are bacteria that harvest energy from the minerals and chemicals that spew from the vents and release compounds that other microorganisms then use for food. Fossils of hydrothermal vents have been discovered in rock as old as 3.8 bya (Cavalazzi et al., 2021).

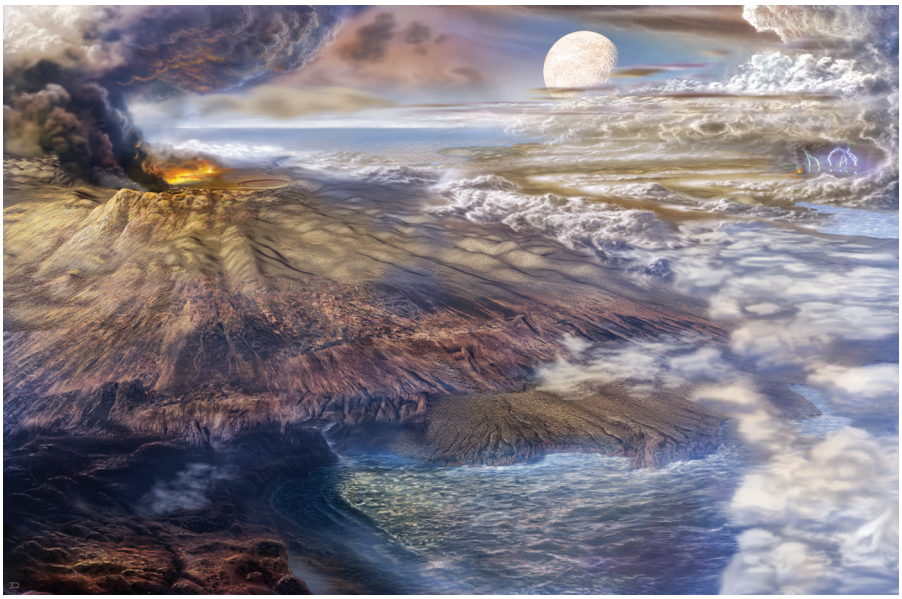


Figure 1.1 Early Earth This artist's rendition provides a glimpse of what early Earth may have looked like. Our planet coalesced just over 4.5 billion years ago from cosmic debris. Transient oceans and lakes existed from the start, although they had been repeatedly vaporized by the massive meteorites that showered our planet back then. The environment of the planet had settled down by about 3.8 million years ago, when the earliest rocks appear in the fossil record in what is now southeast Greenland, and the planet might have looked as this artist portrays it. (Photo © Don Dixon)

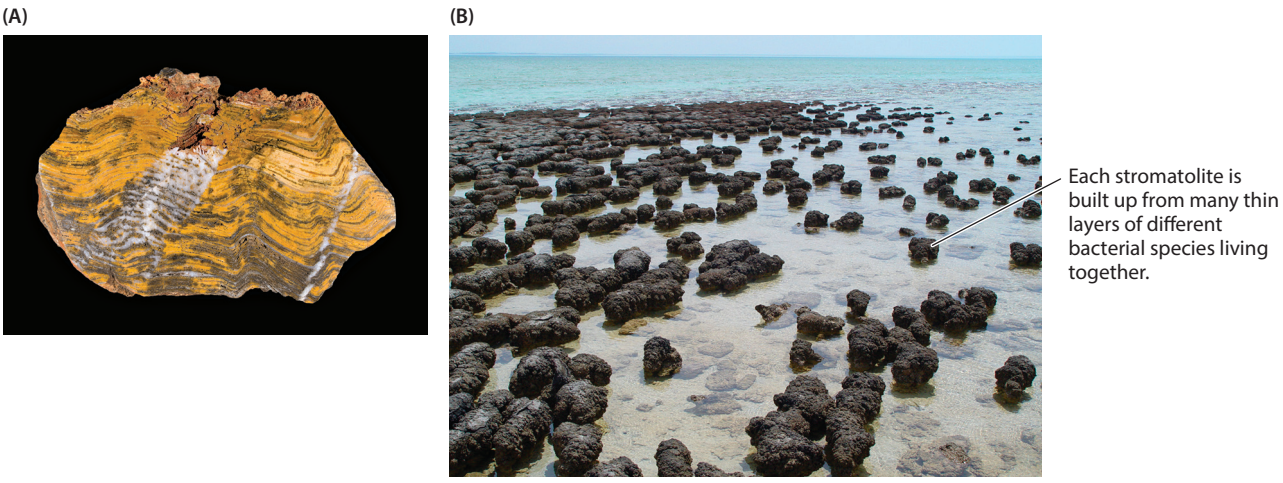


Figure 1.2 **Ancient Microbial Fossils** (A) The earliest fossil evidence of microbial communities. The layering in this rock is very likely due to biological activity. Cyanobacteria form mats of cells that secrete sticky substances that trap sediments in the surrounding water. Over time, these sediments form a mat and then new layers of Cyanobacteria attach. Layers of volcanic ash compacted against these structures, preserving them in the Greenland fossil record for the past ~3.7 billion years. Small fossils like these, buried under billions of years of collected rock, allow us to learn more about life in the distant past. (B) A cluster of living stromatolites from Shark Bay, Australia. There are very few such structures remaining on Earth. (A photo from Muséum de Toulouse, CC BY-SA 4.0, via Wikimedia Commons; B photo from Paul Harrison, CC BY-SA 3.0, via Wikimedia Commons)

One microbial species commonly found in vents, *Methanopyrus kandleri*, uses hydrogen gas as a food source and releases methane as a waste product. This process is known as **methanogenesis**, and it is one of the most ancient forms of energy production. The name of this microbe describes its fondness for extreme environments; *methanopyrus* literally means "methane fire," which is highly appropriate as it can grow in temperatures up to 122°C (252°F), the highest temperature known to be compatible with life. Consider that water boils at 100°C; with this in mind, we can begin to imagine how life emerged on what we had previously considered to be an inhospitable early Earth.

Origin of Life

If we can't rewind the tape of time and return to early Earth, can we ever learn about life's origins? In 1953, a young scientist, Stanley Miller, and his mentor, Harold Urey, showed us the way by answering the question: Could the complex organic molecules necessary for life be created under the conditions of our planet billions of years ago? Miller and Urey designed a glass chamber in which they could create conditions that were believed to mimic those on early Earth (**Figure 1.3**). Starting with simple ingredients, such as heat, which would have been provided by the Earth's molten core; an electrical charge to mimic lightning; water (H_2O); and an early atmosphere made of methane (CH_4), hydrogen (H_2), and ammonia (NH_3) gasses, Miller and Urey showed that complex **organic molecules** could be created from what was a predominately inorganic planet. Organic molecules are primarily made of carbon atoms bonded with hydrogen and other elements and are of biological origin. All living things on Earth are composed of organic molecules. In contrast, inorganic compounds are substances that do not contain both carbon and hydrogen. Hydrogen atoms are contained in many inorganic compounds, such as water (H_2O) and the hydrochloric acid (HCl) produced by your stomach. In contrast, only a handful of inorganic compounds contain carbon atoms. Carbon dioxide (CO_2) is one of the few examples. Miller and Urey showed that with heat, electricity, and simple inorganic ingredients, complex organic molecules, such as amino acids, could be produced. Amino acids are

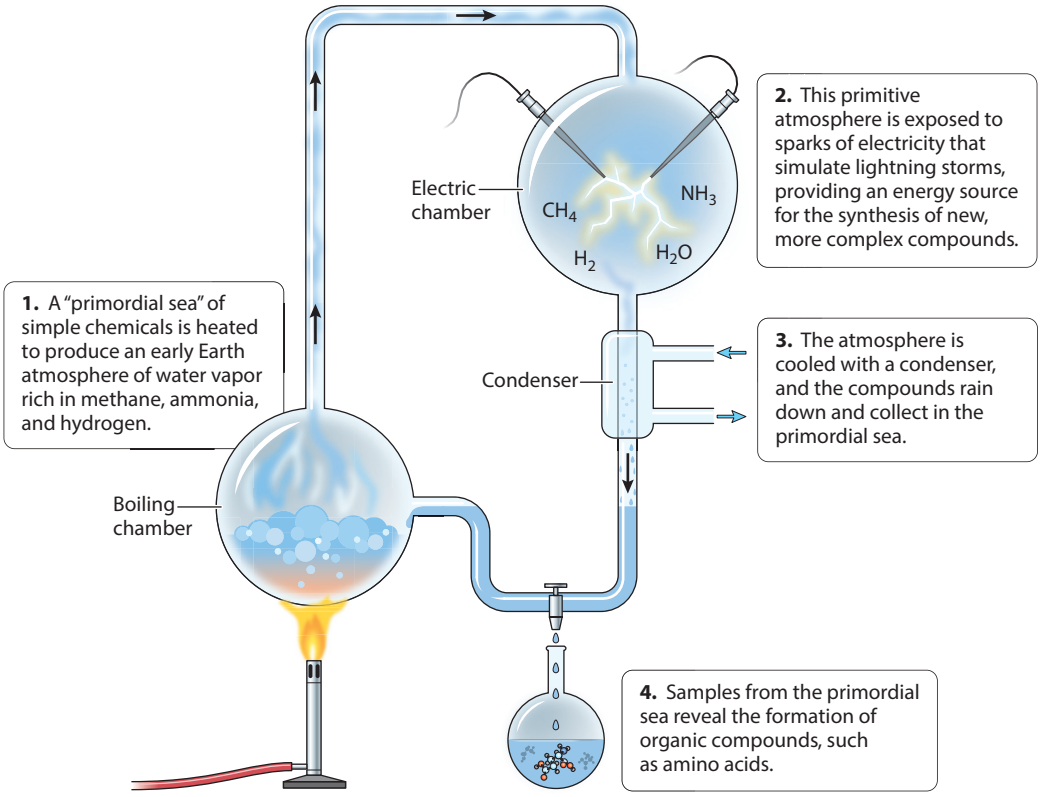


Figure 1.3 **The Miller-Urey Origin of Life Experiment** This experimental apparatus was designed to simulate the origin of organic compounds on early Earth.

the building blocks of **proteins**, the workhorses of cells that carry out many biological functions.

Miller and Urey's findings were extraordinary for several reasons. First, their data suggested that life could have arisen from the simple ingredients present in the "primordial soup" found on early Earth. We now know that many of the essential building blocks of life, such as amino acids and **nucleotides** (the key ingredients of **deoxyribonucleic acid**, or **DNA**), would have rapidly accumulated from simple inorganic constituents. Furthermore, this was the very first experiment in what was to emerge as a rich and exciting field of **abiogenesis**, or the study of the creation of life from nonlife. Their publication helped transform studies of the origin of life into a respectable field of research.

By the 1990s many scientists agreed that at least two functions were required for cellular life to emerge from a nonliving precursor: a means to physically separate the cell's internal functions from the environment (a membrane), and the ability to generate offspring, which involves copying the genetic information and producing daughter cells (replication). **Figure 1.4** provides an overview of how a cell's genetic information, DNA, is transcribed into RNA, which is then used to produce proteins. This theory is known as the **Central Dogma of Molecular Biology**, and there were vigorous debates about which element (DNA, RNA, or protein) came first. One argument emphasized the role of genetics and inheritance (**replication argument**), that is, DNA or RNA was first on the scene. A competing view proposed that creating the cells' structure came first (**membrane argument**), that is, proteins creating the structure of a cell came first. It was at this moment that a particularly innovative thinker entered the field. Jack Szostak, who won a Nobel Prize for his work on **chromosome structure**, which refers to the way DNA is packaged in a cell, decided to retool his lab and to focus on an entirely new research question—the origin of life. Szostak was

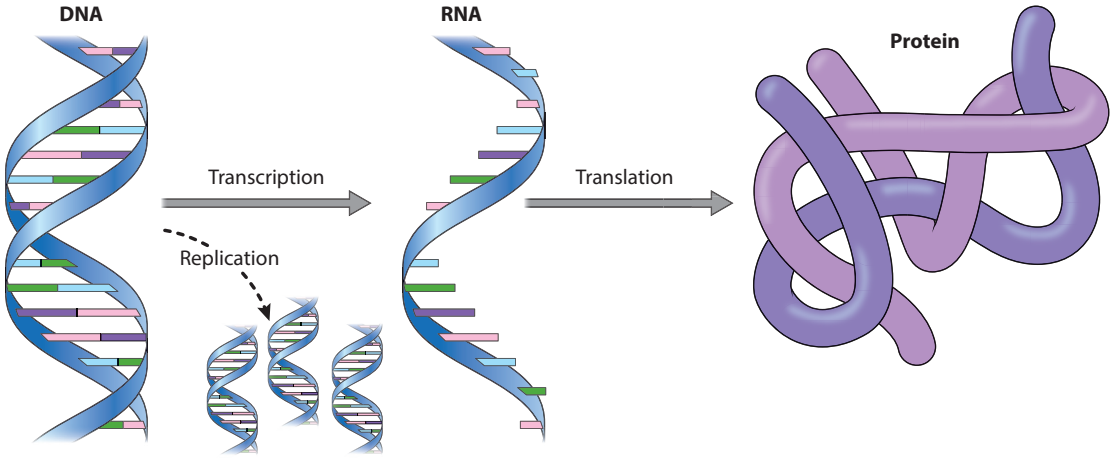


Figure 1.4 **Central Dogma** The Central Dogma of Molecular Biology describes the fundamental biological process by which proteins are built. In most cells, the genetic information is encoded in the DNA, which can be transcribed into a messenger molecule known as RNA. This RNA is then translated into proteins, using complex cellular machinery to "read" the RNA sequence and build the corresponding protein structure. The Central Dogma is essential to our understanding of early Earth because it illustrates the connection between genetic information and cellular processes.

fascinated with the origins debate, and he set off to create a primitive cell, or **protocell**, that would permit him to experimentally explore how the first cell might have evolved (**Box 1.1**).

The Very First Cell

Szostak knew that fatty acids could transition from small spheres (or **micelles**) into multilayered membranes as the pH of the local environment goes from a basic to a more neutral state, so he decided to simply add more membrane-forming molecules (**fatty acids**) to the mix and see what happened (see Box 1.1 and Figure 1.5B). The team added fatty acids, some of which inserted themselves into the cell's membrane. This spontaneous growth process transformed the small spheres into long filamentous vesicles, which could be induced to divide when agitated and then to re-form cells when the agitation stopped. This elegantly simple protocell appears to possess one of the key characteristics of life—a cellular structure that could make copies of itself.

Szostak had proven that the earliest cells could have created a protected environment in which metabolism could take place. Next, he tested whether the RNA fragments located in these protocells were able to replicate, or make copies of themselves, which would permit the identical genetic information to be passed on when the protocell divides. Given that RNA is capable of both replicating itself and performing enzymatic activities, it is often considered the likely ancestor to our own DNA-based mode of inheritance. However, RNA requires high concentrations of magnesium, which can destroy the delicate membranes of the cell. Szostak found conditions that protected the membrane but still provided sufficient magnesium to permit RNA replication. These experiments provide us with a membrane-bound genetic system that is capable of self-replication and growth—two of the hallmarks of life. All done in test tubes in a laboratory!

Since this revolutionary experiment, Szostak and many others continue to dive ever deeper into questions about the origin of life (Mann, 2021). One current focus is on planetary habitability, or the potential for planets to develop and sustain life. A second research area examines the environmental conditions required to produce **biomolecules** (such as carbohydrates, lipids, nucleic acids, and proteins) in concentrations that permit metabolism. A third focus is on determining the ways in which the

precursors to DNA and RNA might have assembled and replicated. These studies are just beginning to answer some fundamental questions about the origin of life (Mann, 2021). Szostak himself notes, "Many challenges remain before we will be close to a full understanding of the origin of life, so the future of research in this field is brighter than ever!" (Szostak, 2017).

BOX 1.1. RESEARCH IN ACTION

Building Life from Scratch—The Quest for a Protocell

Researchers in the Szostak lab made their first protocell out of self-replicating genetic material, in this case a fragment of RNA. **Figure 1.5A** shows the organization of this protocell and reveals the inner compartment created by this structure and the fragments of RNA floating within. This internal environment would have permitted the cell to carry out key functions, such as metabolism, which allows the cell to transform food into energy. However, the first cells would have had none of the machinery needed for their own growth and division. The researchers hypothesized that the coupled growth and division of protocells could be achieved using conditions likely to have been present on Earth over 3 billion years ago.

❖ **Experiment.** A protocell is placed in dilute acid, such that the interior of the protocell is slightly more acidic than the solution. Osmosis will cause water to enter the protocell, resulting in large (~4 mm in diameter) vesicles. Fatty acids are then added to the mixture and modest shear forces are provided (**Figure 1.5B**).

❖ **Results.** The growth of small protocells is achieved by placing them in a solution where liquid permeates the membrane, resulting in the transformation of initially spherical vesicles into long threadlike vesicles that can divide into multiple daughter vesicles.

❖ **Conclusion.** This experiment shows that protocells can be created, enlarged, and replicated in the laboratory, suggesting that similar processes might have occurred under the prebiotic conditions of early Earth.

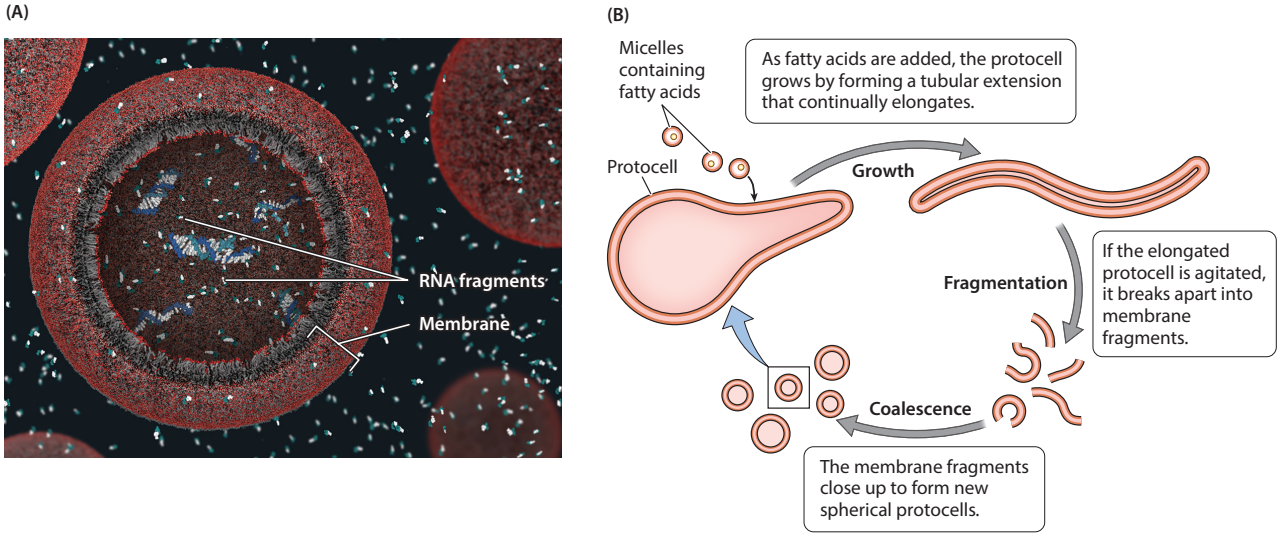


Figure 1.5 The Protocell (A) A computer-generated image of the type of protocell created by the Szostak lab. The protocell is spherical but is shown in cross section here so the inside can be seen. The lipid membrane (red outer circle) provides an internal environment for the protocell to store and replicate its genetic material and undergo metabolic processes to generate energy. Noticeably lacking are more-complex cellular structures that you may already be familiar with, such as a nucleus or mitochondrion. The protocell is surrounded by a "primordial soup" consisting of inorganic and organic molecules. Most of these were small, but some were more complex, such as RNA fragments. (B) The proposed cyclical process of protocell membrane growth and division. The cell incorporates micelles that cause its size to increase until it reaches a point where agitation results in its splitting open. The resulting fragments of the original protocell then reconfigure into new cells. This series of events is a precursor to the modern cell cycle. (A photo courtesy Janet Iwasa, Szostak Laboratory, Harvard Medical School and Massachusetts General Hospital; B after Zhu and Szostak, 2009.)

Competition Drives Diversification

Szostak's research shows us how an ancestral life form could have emerged on early Earth. However, these primitive processes were inefficient. Each time a new protocell was formed, a new RNA fragment would have been captured in the cell, which would have encoded completely different functions, or none at all. We envision the cycle shown in Figure 1.5B repeating itself billions, if not trillions, of times. Some cells captured RNA that encoded novel functions, and those cells might have survived longer and had a greater likelihood of "reproducing," which at this point means that the protocell divided into two daughter cells that share the same RNA fragment. Imagine a primitive ocean filled with trillions upon trillions of protocells. Those that had features that resulted in the production of more copies would consume more ingredients, which would then not be available for others.

The process just described is known as **natural selection**, and it is one of the most powerful forces affecting life on Earth, through which populations of living organisms adapt to the ever-changing environment. Organisms more adapted to their environment are more likely to survive and pass on the genes that contributed to their success. This process, natural selection, causes species to change and diverge over time. Individuals in a population are naturally variable, meaning that they all differ in some ways. This variation means that some individuals have traits better suited to the present environment than others. Individuals with **adaptive traits**—traits that give them some advantage—are more likely to survive and reproduce. These individuals then pass the adaptive traits on to their offspring. Over time, these advantageous traits become more common in the population. Through this process of natural selection, favorable traits are transmitted through generations, and organisms adapt to their environment.

Overwhelming evidence shows us that all **extant** species (meaning that they are alive today) are related, having descended from a common ancestral protocell. We call this extinct organism the **Last Universal Common Ancestor**, or **LUCA**. LUCA was very likely a single-celled **autotroph**, which means it was able to make its own energy and relied on available inorganic compounds as a food source. It is envisioned that LUCA engaged in **chemolithoautotrophy**, meaning it obtained energy by oxidizing inorganic compounds (like hydrogen or sulfur) and fixing carbon dioxide to produce organic molecules. Its genetic material was almost certainly DNA, and it employed RNA molecules, such as tRNA and mRNA, in translating the information encoded in its DNA into proteins.

Heterotrophs would have emerged next, which are organisms that lack the ability to make their own organic compounds. Instead, heterotrophs obtain their energy by breaking down complex organic molecules, such as carbohydrates, fats, and proteins, which they acquire from other organisms—either by eating plants, animals, or decomposing organic matter. As heterotrophs reproduced and became more numerous, they would have rather quickly consumed the organic compounds being produced by autotrophs, resulting in selection for organisms capable of using alternative foods.

Anaerobic versus Aerobic Respiration

These earliest heterotrophs evolved on a planet with an atmosphere composed of methane, ammonia, and hydrogen cyanide, which derived primarily from the gasses emitted from volcanoes. Free oxygen was present at only trace levels. Therefore, the earliest Earth ecosystems existed in an **anoxic** world, devoid of oxygen, and the microbial communities present were supported by anaerobic respiration. Cellular respiration is the process by which cells break down sugar and turn it into energy, which is then used to perform work at the cellular level. The most primitive form happens in the absence of oxygen and is called anaerobic **respiration** (**Figure 1.6A**). Early anaerobic microbes used chemicals to derive energy for respiration by mediating the oxidation and reduction of inorganic compounds in their environments. For example, methanogens obtain their energy from hydrogen (H_2) and carbon dioxide (CO_2) and release methane (CH_4) as a waste product, hence their name. Similarly, sulfate-reducing

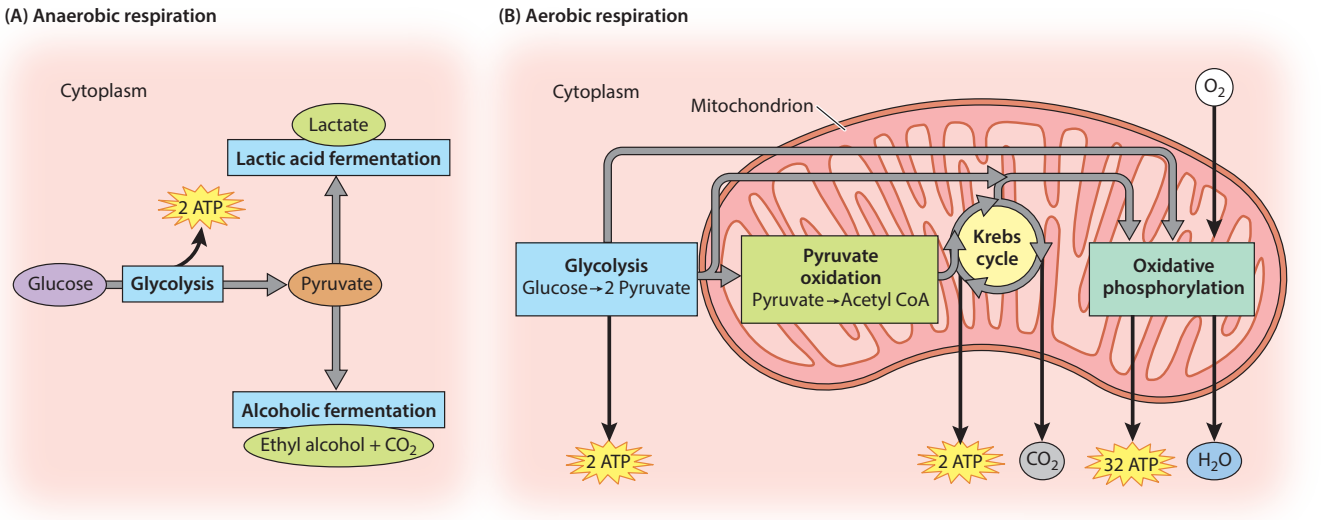


Figure 1.6 **Cellular Respiration** Cellular respiration is the process by which cells release energy by breaking down sugar molecules, such as glucose. (A) Anaerobic respiration, the most primitive form of respiration on Earth, is how cells convert the stored energy of glucose into adenosine triphosphate (ATP) in the absence of free oxygen. It provides energy to the cells very rapidly. (B) Aerobic respiration is the process through which cells break down the glucose molecule to convert its stored biochemical energy into ATP in the presence of oxygen.

microbes feed on sulfate. These **chemoautotrophs**, which use chemicals for energy, would have had an enormous supply of inorganic chemicals to feed on and are still commonly found in environments rich in inorganic compounds, such as near deep-sea hydrothermal vents.

The anaerobic **biosphere** of early Earth, that is, the regions of the planet occupied by living organisms, was less energetically active than our present-day aerobic biosphere—in other words, energy flow from chemicals into and between microbes was slow, roughly 5% of the rate of energy conversion found in our current biosphere. Life forms would have been engaged in intensive competition for the limited energy sources, which would have driven the process of natural selection, resulting in novel approaches to finding and harvesting energy.

Some microbial lineages evolved the ability to use oxygen as an energy source, which we refer to as aerobic respiration (**Figure 1.6B**). This novel form of respiration converts glucose or other organic molecules into energy in the form of **ATP**, or **adenosine triphosphate**, which is essential for various cellular functions. ATP uses the energy stored in its phosphate bonds to power chemical reactions. It is often referred to as the "currency" of the cell. Although anaerobic respiration also produces ATP, aerobic respiration is much more efficient, and it produces ATP much more quickly. This is because oxygen is an excellent electron acceptor for the chemical reactions involved in generating ATP.

Photosynthesis Evolves

One of the truly great metabolic innovations involved the ability to harness the sun's energy, a process called **photosynthesis**. The earliest photosynthetic organisms evolved specialized pigments capable of extracting energy directly from sunlight. These pigments captured the sun's energy and used it to transform carbon dioxide and water into carbohydrates (food) and oxygen (waste product) (**Figure 1.7A**). The first organisms capable of photosynthesis were the ancestors of the modern-day **Cyanobacteria**, a phylum of bacteria also known as blue-green algae. Thanks to photosynthesis, these organisms no longer needed to rely on a limited pool of organic

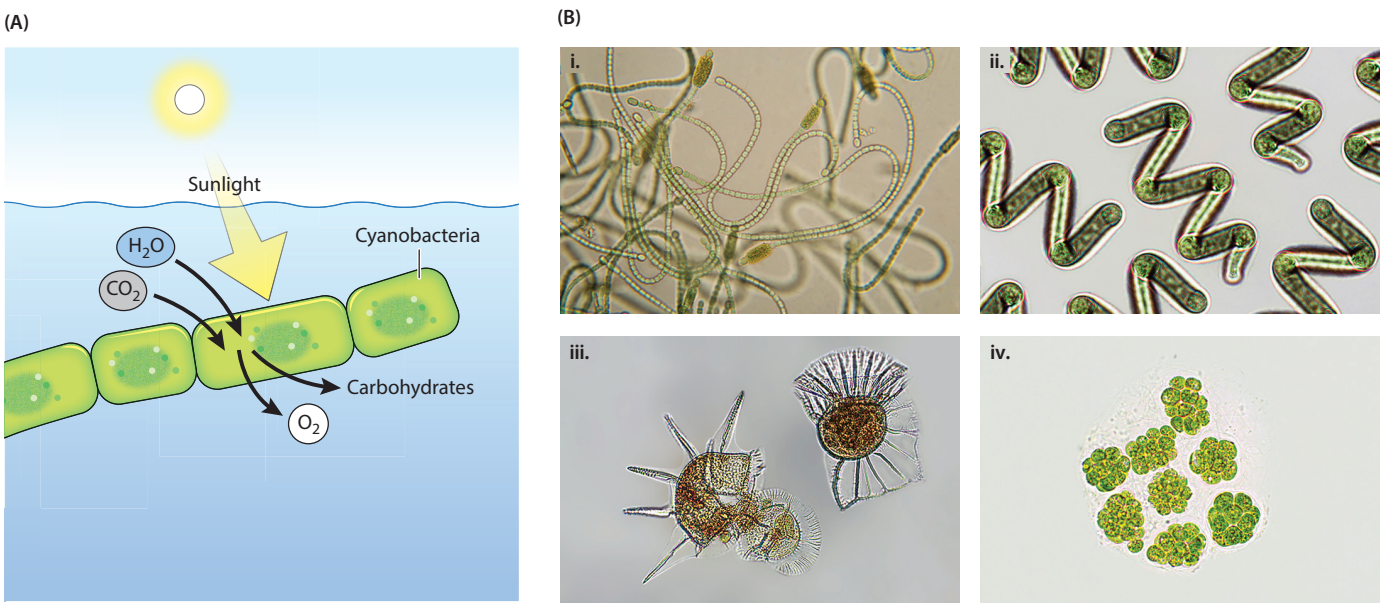


Figure 1.7 **Cyanobacterial Photosynthesis and Diversity** (A) Cyanobacteria use the energy of sunlight to drive photosynthesis, a process where the energy of light is used to synthesize organic compounds from carbon dioxide and water, resulting in oxygen as a waste product. (B) Some of the diverse types of cyanobacteria. Left column: Blue green algae (top), Dinophysis algae (bottom). Right column: Spirulin (top), Pandorina (bottom). (B photos from [i] istock.com /Nnehring; [ii, iii, iv] iStock.com/Elif Bayraktar)

molecules or engage in the far slower process of extracting energy from chemicals and could instead get their energy directly from the sun, which offered them a profound selective advantage. Descendants of these very first photosynthetic cells can be found in almost any water source you examine today. **Figure 1.7B** provides a snapshot of some of the stunning and diverse members of this ancient lineage.

Cyanobacteria played a key role in transforming early Earth's biosphere. Every time a cell broke down a molecule of carbon dioxide, it would release a molecule of oxygen as waste. Imagine trillions upon trillions of cells, each puffing out oxygen over the millennia. At first this free oxygen was captured by minerals, which we see as massive iron oxide (or rust) deposits in the geological record about 2.5 bya (**Figure 1.8**). Once these minerals were saturated with oxygen, the excess began to accumulate in the atmosphere. This period in Earth's history is referred to as the **Great Oxidation Event** (**GOE**), in which the atmosphere was transformed into one rich in oxygen, like Earth's atmosphere today, which is 78% nitrogen (N_2), 21% oxygen (O_2), 0.93% argon (Ar), 0.04% carbon dioxide (CO_2), and trace levels of other chemicals. Figure 1.8 shows the dramatic impact of the GOE on levels of atmospheric oxygen on Earth.

The rising levels of oxygen resulted in one of the first mass **extinction** events on our planet. A mass extinction event is identified when species go extinct faster than new species evolve, defined as about 75% of the world's species being lost in less than 3 million years. Oxygen is toxic to anaerobic bacteria, which do not possess mechanisms to protect their enzymes from oxidants, and thus, most did not survive this period of atmospheric transformation. A lucky few found ways to avoid the oxygen. For example, it is likely that the ancestors of modern-day methanotrophs, microorganisms that produce methane (CH_4) as a by-product of their metabolism, would have continued to flourish in the so-called dead zones in the ocean (areas where the levels of oxygen remain low) and deep in the ocean floor. Our fossil record of that time is limited, and given the microscopic size of the organisms, we are forced to infer features of these ancient life forms.

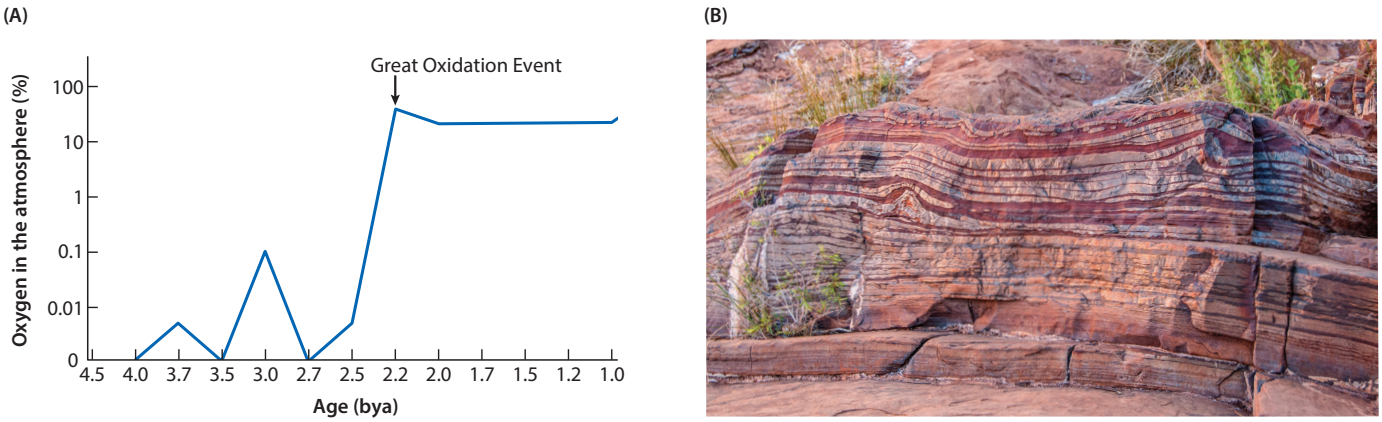


Figure 1.8 **The Great Oxidation Event** (A) Timeline of atmospheric oxygen levels on early Earth. Note the dramatic increase, labeled Great Oxidation Event, that corresponds with the saturation of minerals with oxygen, resulting in iron oxide sediments. (B) The red inserts of sedimentary rock are rust deposits providing evidence of the Great Oxidation Event. Rust is the common name for the chemicals that result when iron reacts with oxygen and water. Sedimentary rock is built by layering different rocks and soils, where the oldest layers are at the bottom. (A after R.A. White III 2020; oxygen data were provided by Dr. Sean Crowe [University of British Columbia] with permission, D.E. Canfield 2005, C. Dupraz and P. T. Visscher 2005, T. W. Lyons et al. 2014, and S.A. Crowe et al., 2013; B photo from Graeme Churchard from Bristol, UK, CC BY 2.0, via Wikimedia Commons)

Endosymbiosis and the Origin of Eukaryotes

With the rise in atmospheric oxygen and the advent of aerobic respiration, large, complex multicellular organisms first appear in the fossil record. Multicellularity has several obvious advantages over single-celled life forms. One of the earliest selective pressures for it may have been related to the fact that a group of cells presents a great challenge for a predator. As cells group together, their survival rate increases. Further, multicellular organisms can have longer lifespans—the organism survives even when individual cells die. Finally, multicellularity also permits increasing complexity by allowing differentiation of cell types, or tissue specificity (Pentz et al., 2020). These changes paved the way for evolution of circulatory and respiratory systems and intestines that break down food sources and extract nutrients from them.

For the first half of the history of life on Earth, single-celled **prokaryotes**, whose genetic information is found floating in the cell's cytoplasm, were the sole inhabitants. However, sometime around 2 bya, a new type of cellular life form arose: the **eukaryotes**, whose DNA is enclosed in a protective membrane called the **nucleus**. Figure 1.9 provides a comparison of a simple prokaryote and a more complex eukaryotic cell.

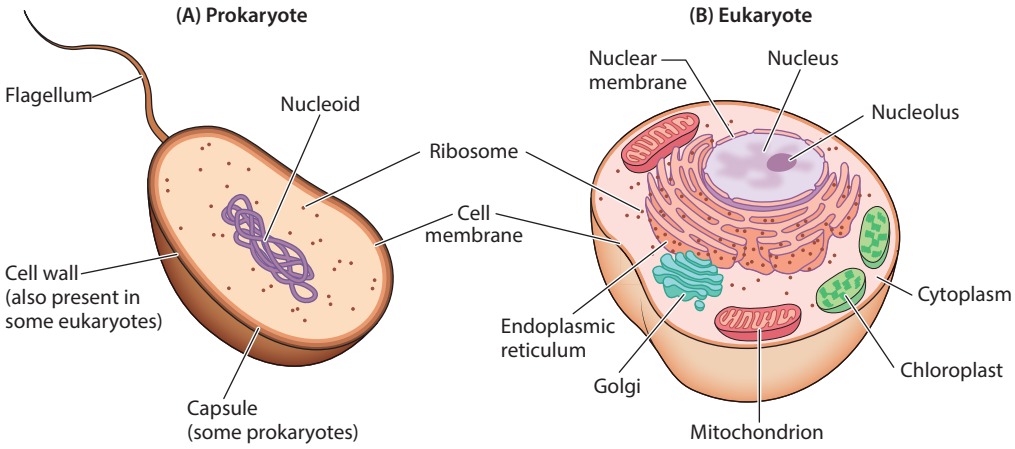


Figure 1.9 **Prokaryote versus Eukaryote Cellular Complexity** This diagram illustrates the similarities and differences between prokaryotic and eukaryotic cells. Both contain genetic material, a cell membrane, and ribosomes. Eukaryotic cells also contain membrane-bound organelles, such as the nucleus, mitochondria, and Golgi body, whereas prokaryotic cells do not.

In 1967 Lynn Margulis, a microbiologist and evolutionary biologist at the University of Massachusetts, proposed that the eukaryotic cell was the result of a chance fusion between two prokaryotes. An ancestral prokaryotic host cell engulfed, but didn't digest, a second prokaryotic cell, one capable of aerobic metabolism (**Figure 1.10A** shows this with a eukaryotic cell). The engulfed cell, or **endosymbiont**, provided its host with the ability to use oxygen to release energy stored in nutrients. In turn, the host cell protected the endosymbiont from predators. Over time, a **symbiotic** relationship, which refers to a close, long-term interaction between two different species, where at least one of the species benefits from the relationship, developed between the two organisms to the point that neither could survive on its own. This endosymbiotic event is immortalized in eukaryotic cells by the presence of the **mitochondrion**, which is the descendent of that ancient, engulfed aerobic symbiont and now serves as the energy factory in nearly all eukaryotic cells today.

Margulis's idea was largely ridiculed, and some 15 journals rejected her research findings before they were published (Sagan, 1967). She spent much of her career defending the hypothesis until enough experimental evidence was garnered to support its recognition as a valid theory. In fact, it is now clear that a series of symbiotic events (**serial endosymbiosis**) occurred. One endosymbiosis resulted in eukaryotic cells possessing a mitochondrion, which became the cell's energy factory (**Figure 1.10B**). Plant cells went even further, with chloroplasts resulting from a fusion of a heterotrophic bacterium with a photosynthetic cyanobacterium (**Figure 1.10C**). Chloroplasts are the membrane-bound organelles in plants and algae where photosynthesis takes place. Margulis was an extraordinary scientist, one who remained steadfast in her then-revolutionary belief that eukaryotic origins could be found. When questioned about the controversy surrounding her proposal of endosymbiosis, she replied, "I don't consider my ideas controversial. I consider them right" (Teresi, 2011).

With the advent of the eukaryotic cell, the diversification of life took on a whole new dimension. A tidal wave of biological diversification occurred about 540 mya. This period, known as the **Cambrian Explosion**, was literally that, an explosion of macroscopic life forms that appear all at once in the fossil record during the geological period known as the Cambrian. What was previously a planet dominated by microscopic prokaryotes is now rich with complex macroscopic, multicellular life

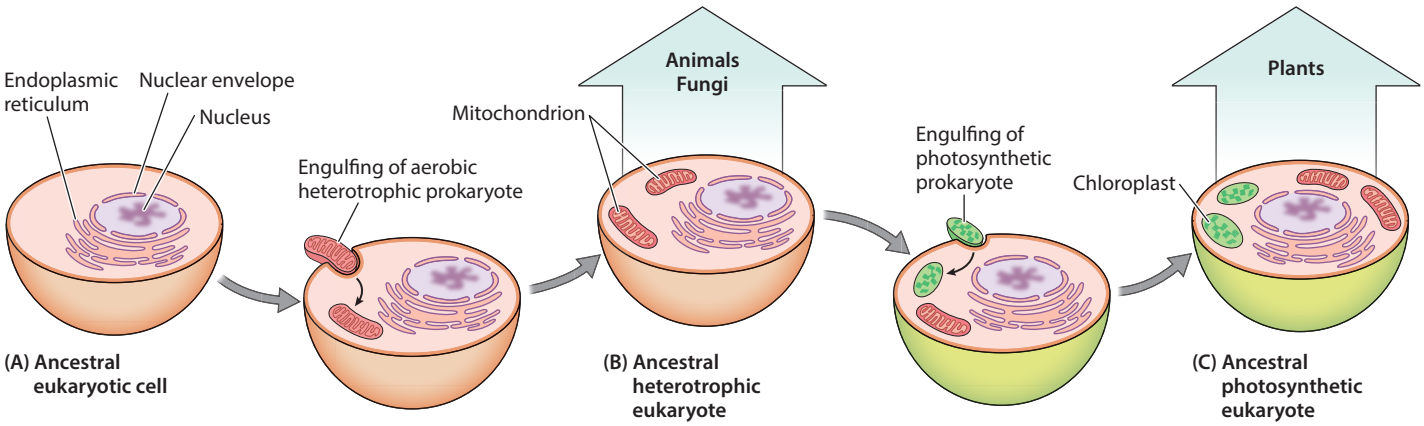


Figure 1.10 **The First Endosymbiotic Events** Imagine an ancestral eukaryotic cell (A), similar to a present-day amoeba. It engaged in phagocytosis, gaining energy from ingested organic matter, such as prokaryotic cells. The endosymbiotic theory posits that in several instances, the ingested cells survived and developed a symbiotic relationship with the host. Mitochondria (in B) and chloroplasts (in C) were the result of this process and were capable of aerobic respiration or photosynthesis, respectively.

forms that fuel successive waves of ecological and environmental transformations on Earth.

1.2 THE GREAT TREE OF LIFE

Now that our planet is teeming with microscopic and macroscopic life, we need a system to name all this diversity. In 1735, Carolus Linnaeus proposed a hierarchical scheme of classification that started with the most inclusive groupings, **kingdoms**, and descended into smaller and smaller subgroups, ultimately ending with a **species** name. Linnaeus would assign each species a unique two-word Latin name, or **binomial**, such as *Homo sapiens*, the binomial for humans. It consists of the species designation (*sapiens* or "wise man") preceded by the **genus** (*Homo*). Genera were grouped into **families**, families into superfamilies, and so on until the level of kingdom was reached. **Figure 1.11** shows a portion of the hierarchical levels of the Linnaean classification system and provides an example of how the human species is classified. Beyond the level of order, humans are members of the class Mammalia, the phylum Chordata, and the kingdom Animalia.

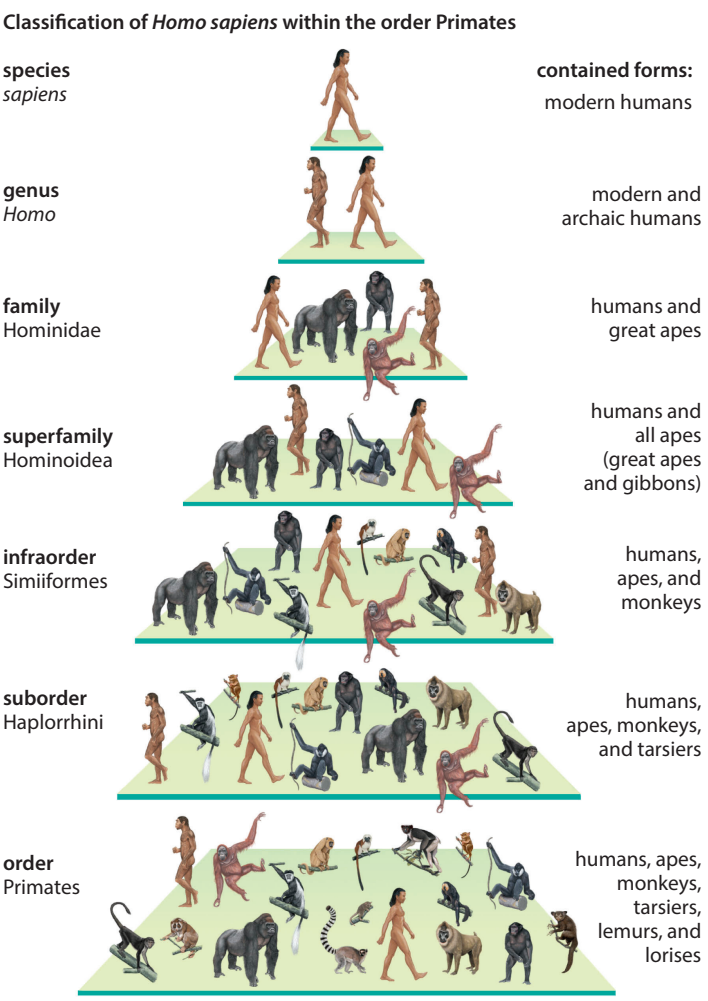


Figure 1.11 Hierarchical Classification This image shows a portion of the Linnaean classification of humans, or *Homo sapiens*. The broadest level of Linnaeus's classification system is kingdom. The kingdom Animalia includes all animals, including humans. The groups become more specific as classification continues. Humans are in the genus *Homo*, which contains modern humans as well as now-extinct humans, such as Neanderthals. A species' name consists of its genus name followed by its species name, which is specific to it, so humans are given the name *Homo sapiens*. (Photo from Universal Images Group North America LLC/Alamy Stock Photo)

Although Linnaeus sought to classify organisms based upon similarities, his methods often resulted in clusters that reflected evolutionary relationships, which we can represent in a phylogenetic tree. A **phylogenetic tree** (also **phylogeny** or **evolutionary tree**) is a branching diagram showing the evolutionary relationships among organisms based upon similarities and differences in their physical or genetic characteristics. In 1859, when Charles Darwin published his thesis on the origin of species, he introduced the concept of a great **tree of life** (**ToL**) connecting all living and extinct life forms to a common ancestor (Darwin, 1859). He envisioned an ever-growing tree whose root is our common ancestor (LUCA), with the branches representing distinct lineages terminating in foliage, which represent the species. **Figure 1.12A** shows Darwin's illustration of his tree of life. He went so far as to describe the fallen limbs and leaves as those extinct lineages that we know only from the fossil record: "Buds give rise by growth to fresh buds, and these, if vigorous, branch out and overtop on all sides many a feebler branch, so by generation I believe it has been with the great Tree of Life, which fills with its dead and broken branches the crust of the earth, and covers the surface with its ever branching and beautiful ramifications" (Darwin, 1859).

The ToL envisioned by Darwin was transformed over the next hundred years as more and more organisms were discovered, described, and added. **Figure 1.12B** provides a version of the ToL popular in the mid-20th century, called the five-kingdom ToL, with animals, plants, fungi, protists, and monera identified as the five categories, or kingdoms, of life. Animals and plants are obvious; however, you may be less familiar with the other kingdoms. **Fungi** refers to spore-

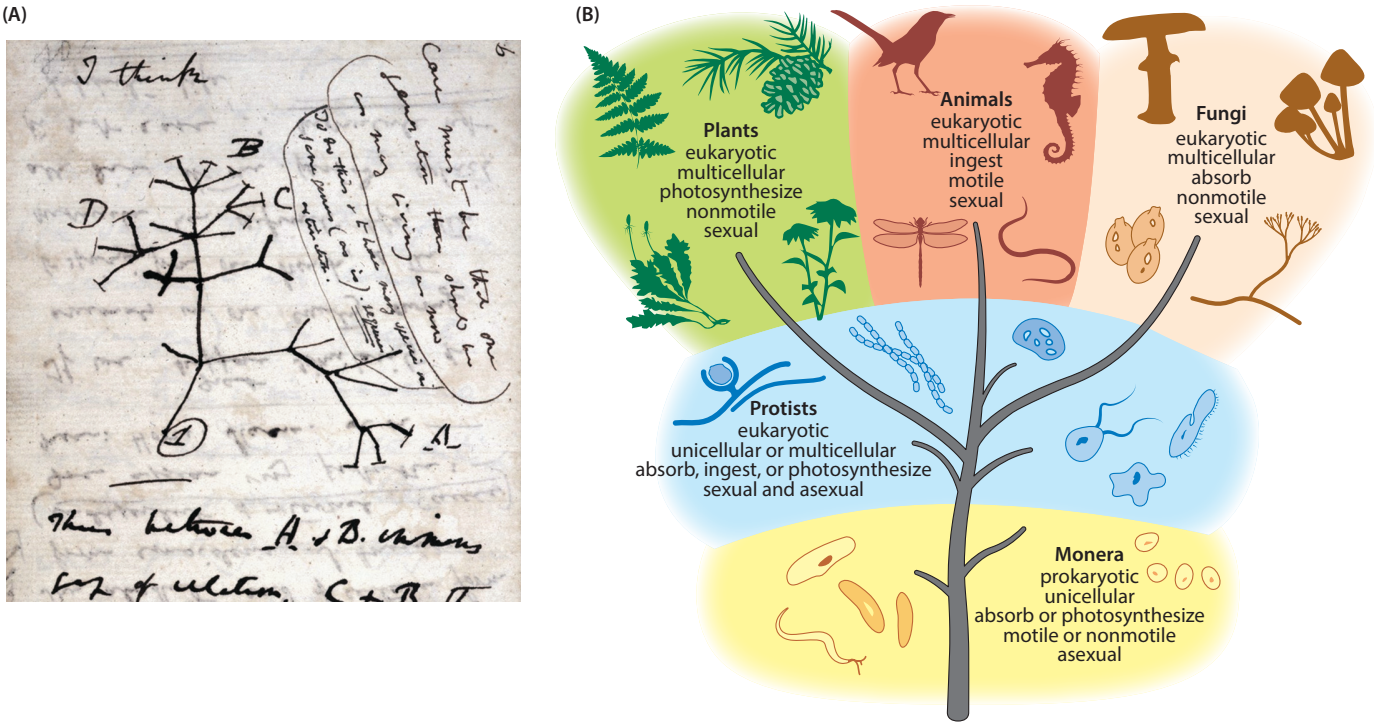


Figure 1.12 **Darwin's Tree of Life** (A) Darwin's illustration of the tree of life, which was first drawn in one of his notebooks in 1837. The base of the tree, which is labeled by the number 1, represents the cenancestor (or LUCA), and the ends of the branches represent species. A version of this illustration was included in his landmark book on evolution, On the Origin of Species, which was published 12 years after Darwin's original tree drawing. (B) The five-kingdom ToL was widely used until molecular technology became advanced enough to permit us a window into the incredible diversity of Protista and Monera, which we now recognize as the prokaryotes. Monera, which includes bacteria and other prokaryotes in this ToL, is at the base. Monera was seen as a more primitive group, from which more-advanced multicellular life evolved. The uppermost branches of the tree represent plants, animals, and fungi. Scientists could easily observe these large, multicellular life forms, so their diversity was better understood and took up most of the branches of the tree. (A illustration reproduced by kind permission of the Syndics of Cambridge University Library.)

producing organisms that feed on organic matter, including molds, yeast, mushrooms, and toadstools. **Protists** are single-celled eukaryotic organisms, such as protozoa or simple algae. **Monera** is the kingdom into which prokaryotes, such as bacteria, are placed. This view of life's diversity focuses your attention first and foremost on the macroscopic organisms and suggests that protists and monera are somewhat more primitive and less diverse. In truth, scientists at that time couldn't make sense of the evolutionary relationships among monera, simply because they didn't have **phenotypes**, or observable characteristics, to compare.

A Molecular Tree of Life

In 1977, Carl Woese tackled this formerly intractable problem, inferring the evolutionary relationships among the monera, or prokaryotes (Woese & Fox, 1977). Lacking visible physical traits with which to classify microorganisms, Woese turned to molecules. He chose the ribosome, which is a complex of RNA and associated proteins that functions to synthesize proteins, and which is one of the most ancient and well-conserved biochemical structures shared by all life. This means that any two species' ribosomes are similar, even if the species are not otherwise closely related. The ribosome is essentially a mini factory that translates genetic information into proteins

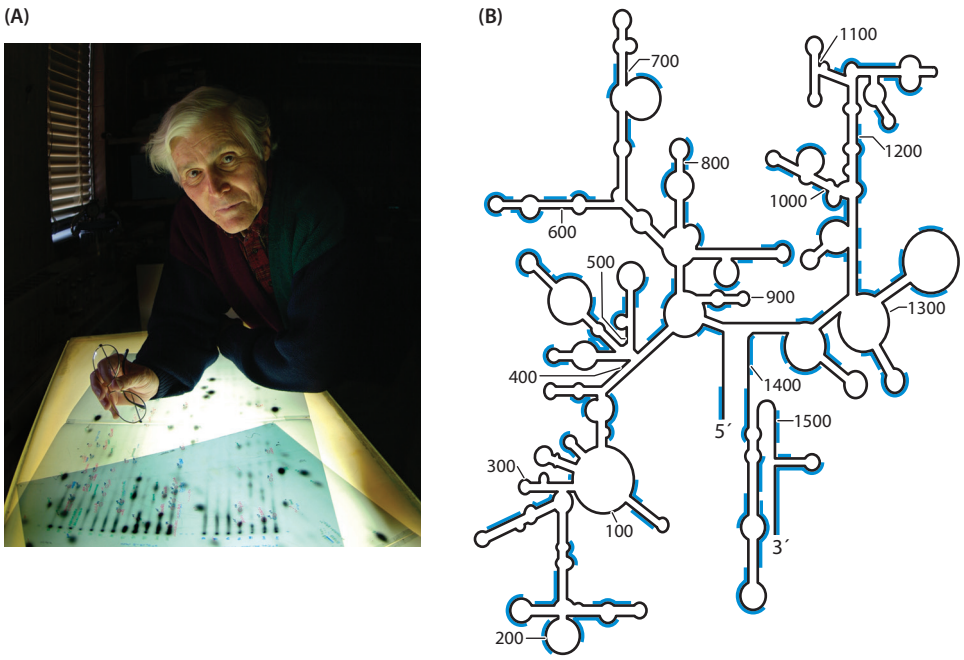


Figure 1.13 **Carl Woese and a Molecular-Based Tree of Life** (A) Photograph of Carl Woese peering at a radiograph that shows the ribosomal fragments of a microorganism's 16S rRNA separated based upon electrical charge. (B) Two-dimensional structure of the 16S rRNA molecule with regions indicated in blue that are cleaved during the RNA digestion procedure employed by Woese. (A photo courtesy of Jason Lindsey, University of Illinois Urbana-Champaign)

(**Figure 1.13**). All life forms use this same fundamental process for making proteins, so they all share at least some portions of the ribosomal RNA–protein complex.

Woese proposed that by comparing portions of the ribosomal complex among all life forms, it would be possible to group organisms in the same manner that they had been grouped previously using physical traits, or phenotypes. Within the ribosomal complex are subunits made of RNA and protein. The RNA molecules within those subunits are named based upon their weight in Svedberg units, such as 16S and 18S. All organisms, even those from across the three domains of life, have ribosomal subunits. Woese used information obtained by cleaving the RNA sequences of these ribosomal subunits and comparing the resulting fragments to estimate how closely related two organisms are. Pairs of taxa that are more similar in their ribosomal fragments are inferred to be more closely related. The number of differences between the ribosomal RNA fragments then serves as a measure of the amount of evolutionary time that separates a pair of taxa. These evolutionary distances can be used to create a phylogeny.

The Three Domains of Life

Woese first focused on a subunit of the ribosome (the 16S subunit) that is present in all bacteria. He produced fragments of the 16S ribosomal RNA (rRNA) for a diverse sample of what he thought of as bacteria and immediately noticed something striking. There was one cluster of fragments that was quite different from all the others. The organisms represented by that cluster were methanogens, prokaryotic cells that produce methane as a waste product. Woese quickly realized the significance of this finding: methanogens were not bacteria, but something completely different. He then employed an additional subunit of the ribosome (the 18S subunit), which is related to the 16S subunit but is found in eukaryotes, so that he could include eukaryotes in his clustering process. Although methanogens looked superficially like bacteria, their ribosomes reveal a very different ancestry. To his surprise the ribosomal fragments of methanogens were more like those found in eukaryotes than in bacteria. Woese named this new lineage **Archaea**, which is a Latin term meaning "primitive."

Based upon these results, Woese created a new ToL, which required a higher level of organization than the five kingdoms. He identified and named three groups within a higher level of biological relationships: Eukarya (animals, plants, fungi, and

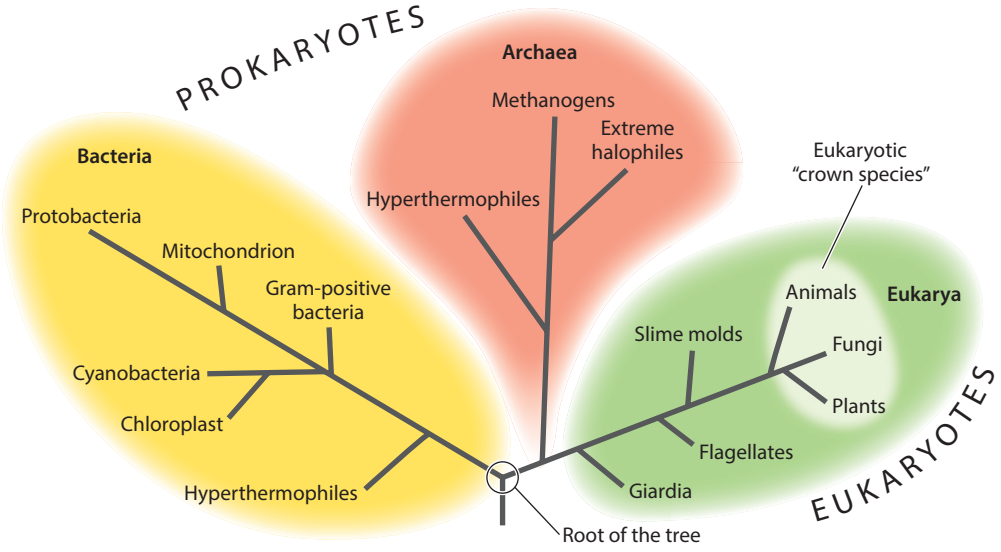


Figure 1.14 The Ribosomal RNA–Based Tree of Life This phylogenetic tree was developed using data from rRNA sequences. While eukaryotes made up most of the five-kingdom-view-based tree, they are only a small portion of the modern tree of life. Monera was found to include two distinct domains: Bacteria and Archaea. Although archaeans are microorganisms like bacteria, they are actually more closely related to eukaryotes, like us, than they are to bacteria! (After M. T. Madigan and M. Martinko, 2006.)

protists), Bacteria, and Archaea (**Figure 1.14**). The discovery of Archaea stimulated both enormous interest and intense skepticism at first. However, as more lineages of Archaea were identified, it became clear that it did, indeed, represent a novel and ancient branch on the ToL. **Table 1.1** summarizes some of the similarities and differences observed between members of the three domains. The prokaryotes, which encompass members of the domains Archaea and Bacteria, share certain characteristics, such as size and a lack of intracellular organelles, while the eukaryotes appear to be chimeras, sharing key characteristics with both archaeans and bacteria. If we think back to the endosymbiotic theory, these patterns of similarities and differences begin to make sense. Eukaryotes, which were created through a series of endosymbiotic events, may very well have been derived from an ancestral archaean host that harbored a bacterial endosymbiont.

Woese's breakthrough was momentous for several reasons. First, by focusing on the ribosome, he had identified a way to compare all cellular life. Second, Woese revealed our ignorance of one of the three main branches of life, the Archaea. Further, he showed us that microbes occupy a dominant place in Earth's biodiversity. If we compare the five-kingdom and three-domain views of biodiversity, we see a fundamental shift from a view of life in which the eukaryotic **crown species** (plants, animals, and fungi) dominate, to one in which these eukaryotes are in the minority (see Figures 1.12B and 1.14). Woese himself described how unsettling this new view of life's diversity truly was: "Imagine walking out in the countryside and not being able to tell a snake from a cow from a mouse from a blade of grass, that's been the level of our ignorance" (Blakeslee, 1996).

Table 1.1 Comparison of Domains

	EUKARYA	BACTERIA	ARCHAEA
Cell type	Eukaryotic	Prokaryotic	Prokaryotic
Chromosomes	Linear	Circular	Circular
Membrane-bound organelles	Yes	No	No
Nuclear envelope	Yes	No	No
RNA polymerase	Many	One	Many
Cell wall composition	Not always present Plants—cellulose Fungi—chitin	Peptidoglycan	Lacks peptidoglycan
Cell membrane composition	Ester linked lipid with proteins (straight chain)	Ester linked lipids with D-glycerol (straight chain)	Ester linked lipids with L-glycerol (branched chain)

The Tiniest Microbes

There is one group of microbes that were not included in Woese's molecular tree of life, the viruses. Viruses are microscopic organisms that require a living cell, or host, to multiply. They are ubiquitous and may even be the most abundant biological entities on our planet. Viruses are simple in structure, with a genetic material (DNA or RNA) and a protein coat (**Figure 1.15A**). Some sport an additional outer layer, the envelope, which may have spikes that help the virus latch onto and enter a host cell. If the cellular conditions are right, the viruses then multiply within their host, often killing the host cell in the process.

Each type of virus has its own **host range**, which refers to the breadth of hosts it can infect. Some have a narrow host range; for example, *Variola virus*, which causes smallpox, can only infect humans. Other viruses have broad host ranges; for example, SARS-CoV-2, the causative agent of COVID-19, may infect hundreds of different hosts, including humans and other primates, bats, pangolins, ferrets, and camels.

Viruses are generally not given species names, so they don't fit neatly into the Linnaean classification system. In fact, many scientists don't consider them to be alive! They lack some of the basic features we think of when we attempt to define life, such as being cellular, maintaining homeostasis (or a stable internal state), growing, and making or acquiring energy. They do, however, replicate—using the host's replication machinery—and they adapt to their environment. Whether they are alive or not, viruses are one of the most abundant and diverse forms of microorganisms on Earth. They are categorized according to various characteristics they possess, includ-

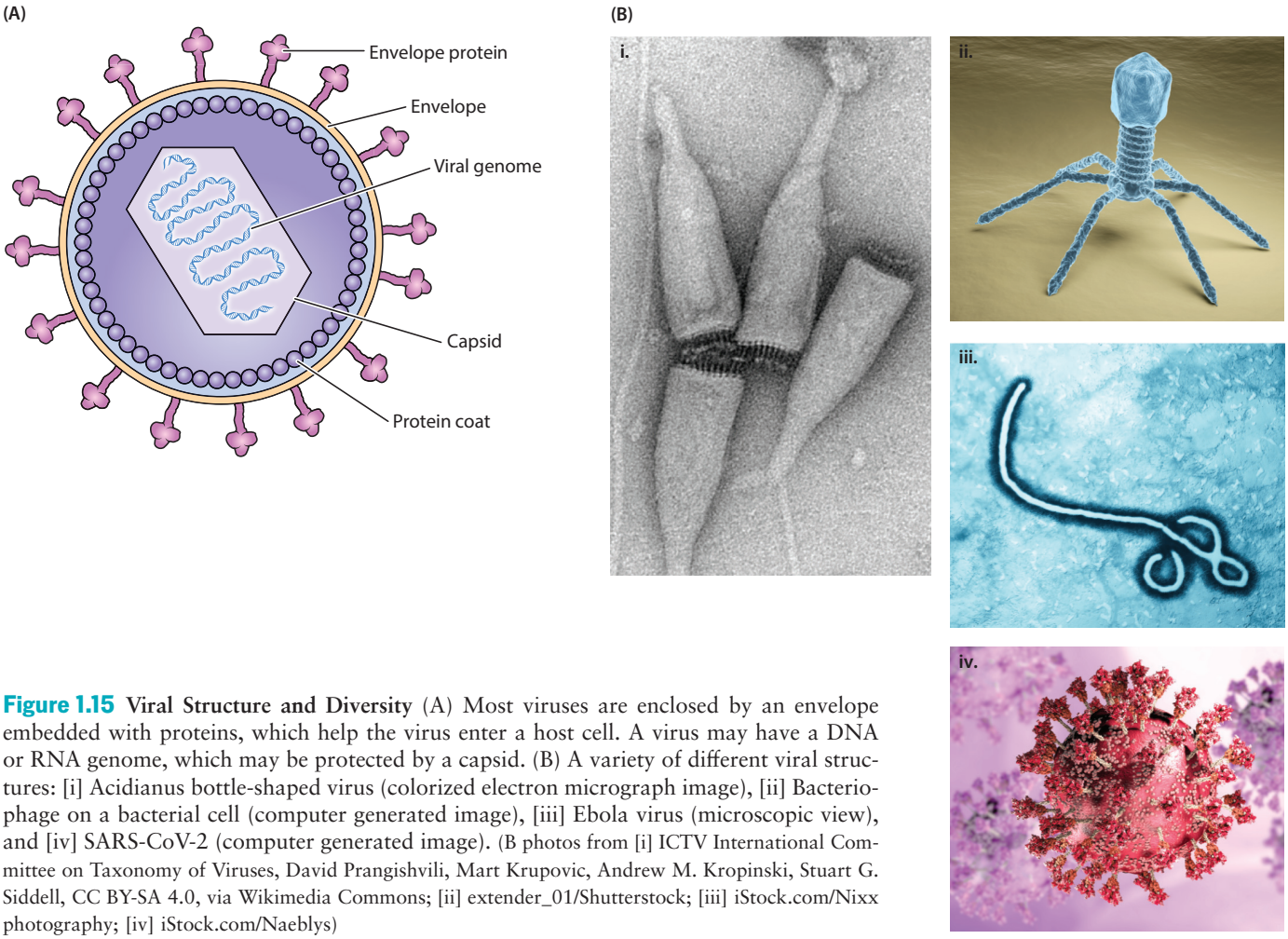


Figure 1.15 **Viral Structure and Diversity** (A) Most viruses are enclosed by an envelope embedded with proteins, which help the virus enter a host cell. A virus may have a DNA or RNA genome, which may be protected by a capsid. (B) A variety of different viral structures: [i] Acidianus bottle-shaped virus (colorized electron micrograph image), [ii] Bacteriophage on a bacterial cell (computer generated image), [iii] Ebola virus (microscopic view), and [iv] SARS-CoV-2 (computer generated image). (B photos from [i] ICTV International Committee on Taxonomy of Viruses, David Prangishvili, Mart Krupovic, Andrew M. Kropinski, Stuart G. Siddell, CC BY-SA 4.0, via Wikimedia Commons; [ii] extender_01/Shutterstock; [iii] iStock.com/Nixx photography; [iv] iStock.com/Naeblys)

ing their shape and size, the type of genetic material they possess (DNA or RNA), and whether they have an envelope layer. **Figure 1.15B** illustrates the major types of viruses.

It is challenging to identify the origin of viruses, as they don't leave fossils. In addition, some viruses can insert their genetic material into their hosts' genomes, which makes it difficult to untangle viral from host evolutionary histories. Since viruses do not share homologous genes or proteins with members of the three domains (Bacteria, Archaea, and Eukarya), we are not able to place them onto one or more branches of the ToL, leaving their relationships with other life forms in question.

1.3 MAKING THE INVISIBLE VISIBLE

With Woese's transformation of the ToL, microbes took center stage in our understanding of the diversity of life for the first time. In fact, according to Woese, microbes are the core of life on Earth: "If you wiped all multicellular life-forms off the face of the earth, microbial life might shift a tiny bit, if microbial life were to disappear, that would be it—instant death for the planet" (Blakeslee, 1996).

Before the 16S rRNA ToL revolution, we hadn't appreciated the immense diversity of microbes on our planet. In large part this was due to their seemingly simple morphology, which resulted in our tendency to group these simple life forms together. In the five-kingdom view of life, we see the microbial lineages clustered in two pools at the base of the tree (see Figure 1.12B). These pools represent the protists and monera (Bacteria and Archaea) with virtually no branches to represent what we now know is an incredible diversity of microscopic life.

We have known that microbes exist for over 400 years, ever since Robert Hooke invented the first microscope and explored the detailed structure of all sorts of biological entities, such as sponges, seaweed, and wood. Of particular interest here are his observations of mold. He describes its appearance on numerous decaying substances and notes that these creatures "will not be unworthy of our more serious speculation and examination" (Hooke, 1665). In short, Hooke was describing a microorganism's appearance for the first time.

The First Sightings of Bacteria

Inspired by Hooke, Antonie van Leeuwenhoek developed an even more powerful microscope and explored numerous samples from his own body, such as stool. In 1677, he reported to the British Royal Society that he had discovered over 1,000 "**animalcules**," or little animals, that differed from one location in the body to another (**Figure 1.16**) (van Leeuwenhoek, 1677). When he examined scrapings from his teeth, van Leuwenhoek noted, "I then most always saw, with great wonder, that in the said matter there were many very little living animalcules, very prettily a-moving. The biggest sort . . . had a very strong and swift motion and shot through the water (or spittle) like a pike does through the water. The second sort . . . oft-times spun round like a top . . . and these were far more in number" (van Leeuwenhoek, 1677). These were the very first observations of living bacteria ever recorded, and they inspired the development of an entirely new field of study, **microbiology**, or the branch of science that deals with microorganisms. Van Leuwenhoek is considered the father of microbiology, and from the

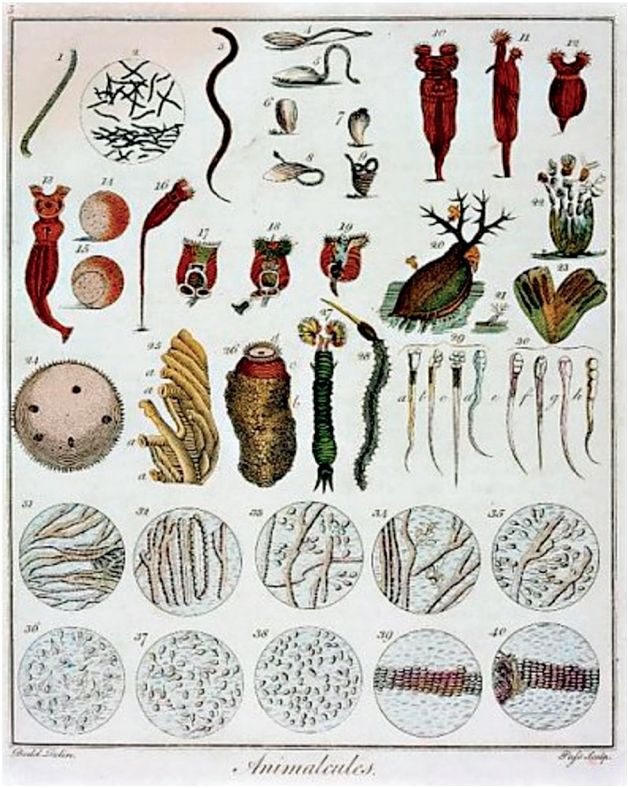


Figure 1.16 Animalcules Antonie van Leeuwenhoek was the first person to record observations of the microbiome. He obtained microbiome samples from various body parts and viewed them under a microscope. The "animalcules," or microorganisms, he saw are illustrated in this figure. (Photo from The Picture Art Collection/Alamy Stock Photo)

late 1600s to present day, scientists have been exploring the rich diversity of microbes on Earth.

Culturing the Invisible

Ever since the invention of the microscope, microbiologists have developed a rich toolbox with which to further explore microscopic life forms. The most common approach is to **culture** the cells, which allows them to grow and divide until there are enough for us to see. The basic procedure is straightforward. Say you want to see some of the microorganisms present in a nearby pond. You start with a sample of pond water and spread a drop of it on a rich growth medium. Each cell lands on a unique spot on the growth medium. If its requirements for growth are present, it grows and divides in this spot, and its daughter cells then replicate and eventually form a visible "colony" of hundreds of thousands of identical cells (**Figure 1.17**). In our pond water sample, we might find 50 or more different types of microbes growing on the food source we provide.

By altering the nutrients offered in growth medium to meet different species' growth requirements, scientists have identified several thousand prokaryotic and protist species. However, that seemingly impressive number pales in comparison with the number that actually inhabit the pond water. If we were to apply Woese's molecular methods of comparing all the 16S rDNA present in our pond water sample, we might find several thousand microbial species. This discrepancy between what we can grow in artificial media and what microscopic life is present in a sample is known as the **great plate count anomaly**, and it hindered progress in microbiology for decades. We simply didn't know what (or how much) we didn't know! For example, it is common knowledge that urine is sterile, unless you have a urinary tract infection. And yet, if you take a sample of supposedly sterile urine from a bladder and sequence the 16S rDNA present, you will find a wealth of different microbes have made urine, or the bladder, their home. For every novel environment we sample, we identify an ever-greater breadth and depth of microbial diversity.

Extremophiles, Life on the Edge

With the advent of molecular tools for identifying microbes, microbiologists engaged in an expansive hunt for novel microorganisms. We now know that microbes exist in

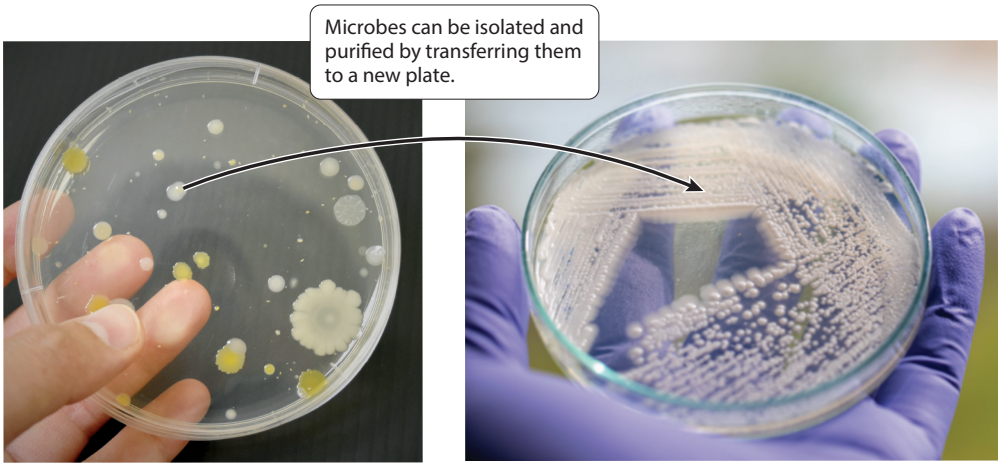


Figure 1.17 **Bacterial Culture** To isolate a genetically identical group of bacteria, a sample can be spread across a nutrient-filled petri dish to isolate individual cells, which can grow and divide to form visible colonies (A). Every member of a colony is a descendant of the first individual cell that landed on that spot on the petri dish. To obtain a pure culture of each individual cell from the original sample, cells from a colony are transferred to a fresh petri dish and grown in isolation (B). (Photos from [left] iStock.com/aorphoto; [right] iStock.com/Sinhyu)

some of Earth's most extreme environments. Some thrive in ice or salt, in the most acidic or basic conditions, living in organic solvents, consuming heavy metals and even toxic waste. Such **extremophiles** have been found in every imaginable, and even the most unimaginable, conditions on Earth. In every extreme environment investigated, a variety of organisms have been shown to not only tolerate the conditions there, but often require them to survive. **Table 1.2** shows just a sliver of the extreme environments where extremophiles have been identified so far.

Table 1.2 Types of Extreme Environments

Hot springs
Deep sea hydrothermal vents
Salt lakes
Polar regions
Volcanic areas
Acidic mine drainage
Deserts
Environments with high radiation levels

The term *extremophile* means "lover of the extreme," and the Archaea domain is where most extremophiles are found. In fact, when archaeans were unveiled to the world, they were thought of as extremophile weirdos. We now know that archaeans can readily adapt to extreme conditions, which may be due, in part, to the composition of their cell membrane. All cells have a plasma membrane made of a phospholipid bilayer, which evolved from the lipid-based protocell membrane we discussed earlier. The archaeans employ ether bonds in that bilayer, while bacteria and eukaryotes use ester bonds. This distinction is important because ether bonds are more resistant to chemical activity, which permits archaeal cells to survive in more extreme environments.

Some archaeans are among the most extremely thermophilic (heat tolerant), acidophilic (acid tolerant), alkaliphilic (base tolerant), and halophilic (salt tolerant) microorganisms known. **Figure 1.18** shows the location where extremophiles were first discovered, in the hot springs of Yellowstone National Park. The genus *Picrophilus*, a member of Archaea, includes the most acidophilic organisms known, which can grow at a pH of 0.06, which is more acidic than hydrochloric acid. Despite their heat-loving reputation, archaeans are also found in very cold places, like Arctic seawater. Aside from our fascination with how extremophiles adapt to their extreme environments, this relatively unknown domain of life is particularly important to humans, due to its position on the ToL. Eukaryotes share a more recent common ancestor with Archaea than they do with Bacteria. Archaeans are our sister lineage, and there is so much more we must learn from them about them, and thus our own place in the biosphere.

Figure 1.18 Extremophiles at Yellowstone National Park Extremophiles were first discovered in Yellowstone National Park's hot springs, where the water regularly reaches 189°F. The thermophiles that live in the hot springs give the pool its ring of colors. To survive at such high temperatures, these bacteria have evolved very stable membranes and proteins. One of these proteins, Taq polymerase, is now used in an important technique for creating copies of DNA, known as the polymerase chain reaction, or PCR. Taq is able to maintain its structure and function even at the high temperatures required for PCR. We can thank extremophiles for our ability to perform PCR for COVID-19 testing, gene sequencing, forensic testing, and more! (Photo from Framalicious/Shutterstock)

1.4 THE MICROBES WITHIN US

We now understand that microbes have a long and rich evolutionary history on Earth, one that is essentially as old as the planet itself. They continuously adapt to novel environments, invent new methods of energy capture, and in the process, have transformed our planet. Given this central role of microbes in the biosphere, it may be less surprising to learn that microbes have also adapted to living in and on us. We refer to these invisible residents as members of our **microbiome** (from the Greek terms *micro* meaning "small" and *bios* meaning "life"). The formal definition of a microbiome refers to a characteristic microbial community occupying a defined habitat that has certain properties. We can find microbiomes essentially everywhere we look—in our gut, in the soil surrounding the roots of a plant, in clouds, and even in the plume from a hydrothermal vent.

A Universe of Microbes within Us

The term *microbiome* refers to both the microorganisms present and the functions they provide, while the term **microbiota** refers simply to which species are present. For example, our

gut microbiome is home to approximately 300 to 500 species of microbes, collectively called the gut microbiota. These members together with the functions they provide, such as digesting some of the food we ingest, are called our gut microbiome. Each microbiome is integrated into its host or ecosystem and is crucial for the proper functioning and health of the organism(s) in that niche.

Our goal in this textbook is to explore what microbes are present in humans, what functions they encode in their genomes, and how those functions impact us, their human hosts, in both healthy and diseased states. This knowledge may force us to redefine what it means to be human. Rather than consider ourselves as distinct biological entities, separate from all other life forms, we must now acknowledge that humans, indeed all multicellular organisms, are composed of numerous complex ecosystems each consisting of a mixture of their own and microbial cells. This new entity, the human with all its microbiomes, is referred to as the **holobiont**, a term derived from the Greek *hólos* or "whole" and *biont* for "unit of life." The term was coined by Lynn Margulis in the 1990s as she was exploring the endosymbiotic origin of eukaryotes. Her intent was to provide a term that would acknowledge the key role of symbiotic relationships in the evolution and diversification of multicellular eukaryotic organisms, such as when an ancestral prokaryotic cell gave rise to mitochondria or chloroplasts. However, the term is equally appropriate to refer to a human body with its invisible microbial symbionts that, as you will learn, provide the key to our health while at the same time serving as the harbingers of certain diseases.

Each of us consists of about 30 trillion human cells, which carry our genetic blueprint and the machinery required to translate that information into what becomes the visible "us." These cells form collections of tissues and organs, which play critical roles in keeping our bodies functioning. For example, skin serves as our frontline defense against invading pathogens, while the heart provides the force required to ensure all of our cells receive the oxygen-rich blood they require. For several thousand years physicians and scientists have explored our cells, tissues, and organs in their quest to understand what makes us uniquely human, what keeps us healthy, and what can go wrong in our bodies to cause disease and death.

We have long known that bacteria and viruses could invade our bodies and cause illness; however, they were considered temporary intruders that our bodies, or the medications we took, would fight to eliminate. In just the past 20 years we have gained an entirely new perspective on the important role microorganisms play in keeping our bodies healthy, leading some to argue that the microbiome should be considered the 11th critical organ, equal in importance to our brain! Let's explore this new organ and learn a bit about its role in keeping us healthy.

How Much of You Is Human?

It's estimated that we have about 35 trillion microbes in and on our bodies—about 5 trillion more than the number of human cells! This count excludes viruses, whose numbers may dwarf the human and microbial cell counts combined. Those numbers translate into a weight of just over 1 kg (2.5 pounds), with a volume of about 1.5 liters (6 cups) of cells. That's nearly half a gallon of microbes per human!

Our body hosts numerous, distinct microbiomes (**Figure 1.19**). We have an oral microbiome in our mouth, one that covers our skin, another in our urinary tract, one in our gut, and even one deep in our lungs. There are far more fine-tuned distinctions we could make. For example, the microbes that inhabit the surface of our tongue are distinct from those that live under our gums, which are different from those that live attached to our teeth, and so on.

These distinct microbial communities also vary greatly in their cell densities. Blood is a virtual microbial desert, while the large intestine contains one of the densest microbial communities on Earth (Bojanova & Bordenstein, 2016)! While the precise number of microbes may differ, each microbiome is highly diverse, with over

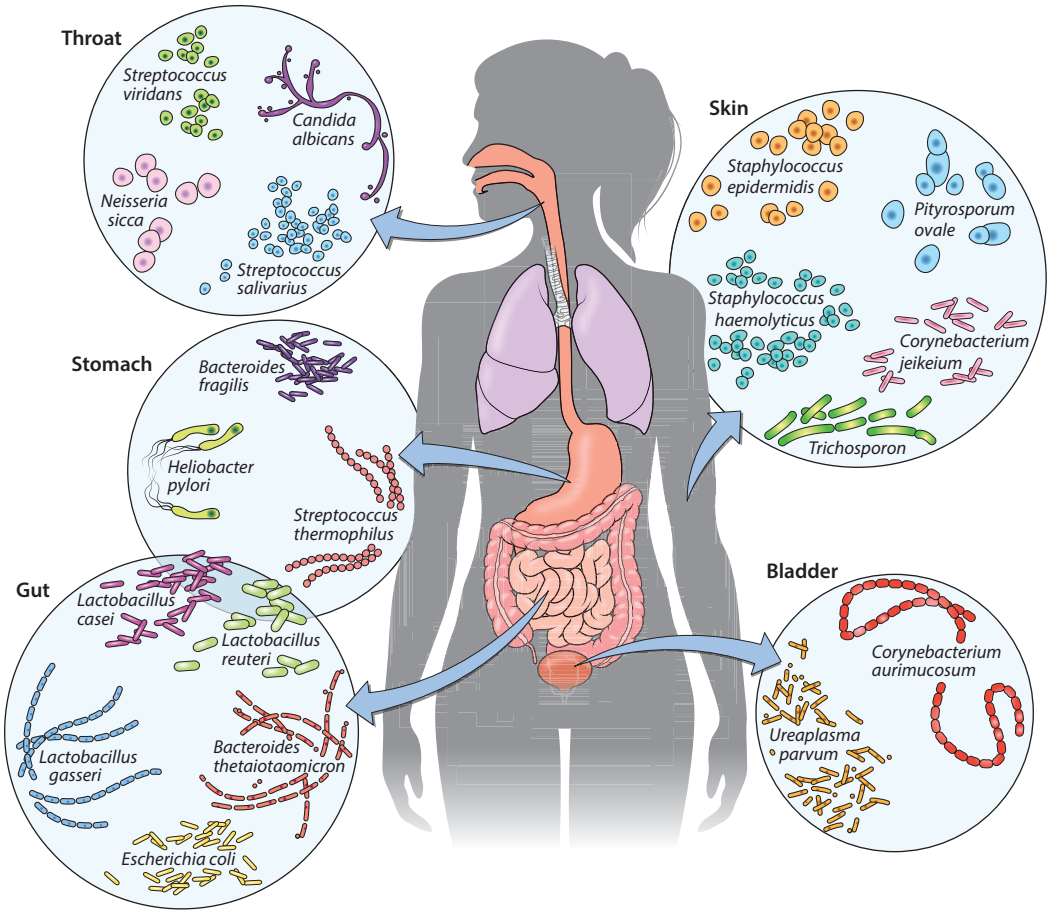


Figure 1.19 **So Many Human Microbiomes** The human microbiome includes many different microbial communities, each with its own unique composition of species and role in maintaining our health. (After V. D. Appanna, 2018.)

300 distinct bacterial species identified in the human gut microbiome alone (Almeida et al., 2021).

Even more compelling than their sheer numbers is the fact that the genetic information our microbiomes encode far exceeds our own. The human genome encodes 20,000 genes, while our microbiomes provide an additional 45 million, each encoding functions with the potential to impact us, their host. For example, if not for genes carried by certain species of bacteria, we would not be able to digest most of the fiber we consume.

1.5 OUR MICROBIOMES, OUR HEALTH

The rapidly growing field of microbiome science is revealing the complex roles these fellow travelers serve in human health. There is now overwhelming evidence that most functions of our body, such as growth, development, and metabolism, depend on our microbiome. Our immune system is trained first by our mother's microbiome during pregnancy and then by our own microbiome, particularly during the first few years of life. Dysfunctions in the gut microbiome are associated with several autoimmune diseases such as arthritis, fibromyalgia, and multiple sclerosis. Our gut microbiome also plays a role in several intestinal conditions, such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), while obesity is often associated with an imbalance in the members of our gut microbiome.

Microbiomes and Human Nutrition

Another example of the key role our microbiome serves is in nutrition. Sugars and starches are two classes of carbohydrates synthesized by all organisms. The plants we eat contain thousands of different carbohydrates, which are broken down to their simplest components to provide us with energy. The human genome has fewer than 20 **enzymes** involved in digesting carbohydrates. Enzymes are proteins that act as biological catalysts by accelerating chemical reactions. Those carbohydrates we can't digest end up in the large intestine, where our microbiome takes over. The microbes in our gut encode thousands of carbohydrate-digesting enzymes in their genomes, which they employ to break down, or ferment, carbohydrates that are not digestible by humans, for energy.

Microbial Metabolites, Key to Human Health

One outcome of the microbiome's digestive efforts is their waste, some of which is essential for human health. These waste molecules, also known as **by-products**, serve key roles in our nutrition and metabolism. For example, our bodies require vitamins, which are organic compounds that are essential for maintaining various body systems, including the immune and nervous systems. You might have learned that the vitamins our bodies need can only be obtained from the food we eat. In fact, our microbes can produce several key vitamins for us. Many vitamins are **metabolites**, or intermediaries, produced during the fermentation of fibrous foods by the microbes living in our gut. Bacteria in the microbiome also produce **short-chain fatty acids** (**SCFAs**), which are fatty acids with fewer than six carbon atoms. They are primarily produced through the fermentation of dietary fibers by gut bacteria in the colon. SCFAs are an essential energy source for our intestinal cells. It is an elegant symbiosis: our gut provides an energy-rich environment that supports an incredible diversity of microbial life, while that life, in turn, provides us with some of the key ingredients required to ensure our health.

Reflections on Your Microbiome

Let's think about our microbiomes from a slightly different perspective. As you walk from one lecture hall to another, passing people who may look very different from you—in height, weight, skin, or eye color—consider this fact: your genome differs by about 0.1% from any other human genome. Regardless of how different you look, you are nearly identical in terms of your DNA content. Now, look again at those passing by, and imagine that you can see the members of their microbiomes as easily as you see their facial features. Each person's microbiome differs by as much as 90% in terms of the species present, not to mention the genetic repertoires those species possess.

All these facts are causing us to reconsider how we think of ourselves as uniquely "us." Traditional explanations for what makes an individual unique focus on our brain or the contents of our genome. However, as you will learn, our microbial residents communicate directly with our brain, and they provide far more gene functions than does our own genome. We are realizing that humans are not discrete entities of human cells and genes; rather, each of us is a consortium of thousands of organisms that result in a functioning, hopefully healthy, human. Indeed, it takes a microbial village to be a human!

Take a moment to reflect on what this new understanding of our microbial partnerships means to you. Does it scare you (or gross you out) to imagine the astronomical numbers of microbes in and on your body? Do you get excited about the genetic potential we carry inside us? Or do your thoughts turn to the role these microbes have played in our evolutionary history? Perhaps you wonder if you can take advantage of them to improve your health. Simply said, we are not alone, and it can feel empowering to understand that you have a fair bit of help in keeping your body healthy.

CHECK YOUR UNDERSTANDING

1. Approximately when do we think life emerged on this planet?
   a. 4 billion years ago
   b. 1 million years ago
   c. 0.5 million years ago
   d. 1,000 years ago

2. The Miller-Urey experiment was designed to test whether
   a. early Earth's conditions could be mimicked.
   b. organic molecules could be created under early Earth conditions.
   c. inorganic molecules could create life.
   d. life could be created in a glass chamber.

3. Which represents the Central Dogma of Molecular Biology?
   a. RNA → DNA → protein
   b. Membrane → DNA → protein
   c. DNA → RNA → protein
   d. DNA → membrane → protein

4. The advanced protocell created by Szostak's lab was essentially a
   a. membrane-bound cell containing DNA.
   b. fragment of RNA that could replicate itself.
   c. cellular structure that could make copies of itself.
   d. cellular structure that was unable to replicate itself.

5. Natural selection occurs when
   a. an individual organism gains new, advantageous traits during its lifespan.
   b. individuals with advantageous traits are better able to survive and reproduce, and those traits become more common in the population over time.
   c. random events result in organisms better able to survive and reproduce.
   d. a population of organisms survives to reproduce.

6. Hydrothermal vents provide a rich nutrient source that some of the earliest life forms likely took advantage of.
   a. True
   b. False

7. What are deep-sea hydrothermal vents?
   a. Magma transmitted from the Earth's core
   b. The ocean's equivalent of geysers
   c. Very hot plumes of air at the bottom of the ocean
   d. Underwater volcanoes

8. The two competing arguments about the origin of life are the replication argument and the cell division argument.
   a. True
   b. False

9. The protocell membrane was created with
   a. DNA.
   b. RNA.
   c. fatty acids.
   d. proteins.

10. What's the difference between autotrophs and heterotrophs?
    a. An autotroph makes its own food.
    b. A heterotroph makes its own food.
    c. A heterotroph uses the sun's energy to fuel itself.
    d. An autotroph uses the sun's energy to fuel itself.

11. How did the Great Oxidation Event affect life?
    a. Anaerobic life largely went extinct.
    b. Aerobic life largely went extinct.
    c. It created the rust deposits found in some sedimentary rocks.
    d. It enabled anaerobic life to flourish.

12. Identify 2 characteristics of eukaryotes not found in prokaryotes.
    a. Cell membranes, flagella
    b. Nuclei, mitochondria
    c. Nuclei, flagella
    d. Golgi bodies, cell membranes

13. Lynn Margulis proposed that eukaryotic cells came from a chance fusion of 2 protists.
    a. True
    b. False

14. How did cyanobacteria transform Earth's atmosphere?
    a. By producing methane
    b. By consuming all the existing oxygen
    c. By producing oxygen
    d. By consuming all the existing carbon dioxide

15. LUCA was the very first organism.
    a. True
    b. False

16. What technology allowed the microbiome to be viewed for the first time?
    a. Telescope
    b. Microscope
    c. Electron microscope
    d. 16S ribosomal sequence

17. What genus are humans members of?
    a. Eukarya
    b. Sapiens
    c. Mammalia
    d. *Homo*

18. Which kingdom were prokaryotes a part of in the 5-kingdom view of life?
    a. Monera
    b. Fungi
    c. Protists
    d. Bacteria

19. What did Carl Woese use to infer relationships between prokaryotes?
    a. Whole genome sequencing
    b. Phenotypic observations
    c. Metabolic pathways
    d. 16S rRNA

20. What are the 3 domains of life?
    a. Eukarya, Prokarya, and Monera
    b. Eukarya, Bacteria, and Archaea
    c. Fungi, Protista, and Bacteria
    d. Eukarya, Bacteria, and Protista

21. What is the cause of the great plate anomaly?
    a. Some bacteria have RNA genomes.
    b. Many bacteria cannot be cultured using available techniques.
    c. It is difficult to find bacteria in the environment.
    d. It is impossible to isolate a single species from a sample.

22. Extremophiles are microbes that survive in intense conditions, such as very high or low temperatures.
    a. True
    b. False

23. Which human microbiome is less dense than the others?
    a. Gut microbiome
    b. Oral microbiome
    c. Blood microbiome
    d. Skin microbiome

24. A human and their microbiome have about the same number of enzymes involved in digesting carbohydrates.
    a. True
    b. False

25. Vitamins, short-chain fatty acids, and other metabolites are produced when certain microbes digest which compounds in food?
    a. Simple sugars
    b. Fatty acids
    c. Lipids
    d. Fibers

Answers: 1A, 2B, 3C, 4C, 5B, 6A, 7B, 8B, 9C, 10A, 11A, 12B 13B, 14C, 15B, 16B, 17D, 18A, 19D, 20B, 21B, 22A, 23C, 24B, 25D

DIVING DEEPER

1. Why were deep-sea hydrothermal vents advantageous locations for early life?
2. How did Miller and Urey show that the organic molecules necessary for life could form from inorganic material?
3. What were the two competing views about the origin of life, and what did Jack Szostak's protocell reveal?
4. What's the difference between autotrophs and heterotrophs?
5. How did the Great Oxidation Event affect life?
6. Can you explain three differences and three similarities between prokaryotes and eukaryotes?
7. According to Lynn Margulis's endosymbiotic theory, how did eukaryotic cells acquire mitochondria and chloroplasts?
8. Identify three differences between the five-kingdoms and three-domains views of life's diversity.
9. Why is the ribosome a good tool to use for inferring the tree of life?
10. Why was Woese's use of 16S rDNA sequencing revolutionary?
11. What technology allowed the microbiome to be viewed for the first time?
12. Why are bacterial culture techniques limited, and what technology solves this problem?
13. Can you give examples of the environments that extremophiles are able to live in?
14. What is a virus's host range?
15. Why can't viruses be placed on the tree of life, and how are they different from Bacteria, Archaea, and Eukarya?
16. What's the difference between the microbiome and microbiota?

17. Lynn Margulis introduced the term holobiont to explain what?
18. Can you list five microbiomes found in/on humans?
19. How does the human microbiome vary by body part?
20. Why is the microbiome necessary for carbohydrate digestion?
21. What are the two main metabolites bacteria produce as waste, and why are they important for human health?

DISCUSSING AND REFLECTING

1. Lynn Margulis's serial endosymbiosis theory was a harbinger of the discovery of the microbiome. Explain what is meant by that statement.
2. Woese's impact on our understanding of biodiversity has been enormous. Describe the key features of biodiversity that we were ignorant about before Woese's research revealed the three-domain tree of life.
3. What can extremophiles tell us about the origin of life on Earth and the possibility of life existing on other planets?
4. Reflection. Carl Woese said, "If you wiped all multicellular life-forms off the face of the earth, microbial life might shift a tiny bit, if microbial life were to disappear, that would be it—instant death for the planet" (Blakeslee, 1996). How do you feel now that you know the importance of microbes, and how does this affect your view of life on this planet?

RECOMMENDED READINGS

Popular Science Reviews

O'Donnell, E. (2019, June 7). How Life Began: Jack Szostak's Pursuit of the Biggest Questions on Earth. *Harvard Magazine*, 40–79.

Quammen, D. (2018, August 13). The Scientist Who Scrambled Darwin's Tree of Life. *New York Times*, 34.

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Sagan, D. (2012). *Lynn Margulis: The Life and Legacy of a Scientific Rebel*. Chelsea Green.

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Gray, M. W. (2017). Lynn Margulis and the Endosymbiont Hypothesis: 50 Years Later. *Molecular Biology of the Cell*, 28(10), 1285–1287. https://doi.org/10.1091/mbc.e16-07-0509

Nasir, A., Romero-Severson, E., & Claverie, J.-M. (2020). Investigating the Concept and Origin of Viruses. *Trends in Microbiology*, 28(12), 959–967. https://doi.org/10.1016/j.tim.2020.08.003

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