

Introduction to Microbiology

WELCOME TO MICROBIOLOGY: the study of the great variety of living organisms that are too small for us to see without a microscope—the **microbes**, or **microorganisms**. You will learn, as you read this book, that despite their minute size, these organisms form the basis for all life on earth. Their activities produce the soil in which plants grow and the atmospheric gases that plants and animals both use. Their activities regulate the temperature of earth, preventing it from freezing or baking; subterranean gelatinous masses of them may lubricate the movements of tectonic plates, and their chemical activities recycle gases on which all life on land depends.

For nearly three quarters of the history of earth, a period of about 3 billion years, microbes were the only life on earth. To us the planet would have looked barren and uninhabited, except for the colored scum at the edge of ponds and on intertidal rocks, yet the seas and lakes teemed with great masses of life. Even today, after nearly a billion years of abundant plant and animal life, the earth is fundamentally a microbial planet, to which the macro-organisms are recent and relatively unimportant additions. Life on earth is like an iceberg: only a small portion of it is visible (Figure 1.1).

In this chapter, we define our subject and then briefly survey the evolutionary history of life on earth, the major categories of microbial life, and the chemical composition of microbial cells.

1.1 Most microorganisms are microscopic, and they include all life forms other than the plants and animals

Microorganism is a term that is difficult to define precisely. Operationally, it refers to any organism that is too small to be seen by the unaided eye. For most people, that would be about 0.1 to 0.2 mm in diameter. Anything less than this cannot be seen without a microscope of some kind and is in a loose sense a microorganism.

In practice, however, the term microorganism is often used to include some macroscopic forms that belong to a group that is largely microscopic (e.g., the fungi, most of the algae), and it excludes some microscopic forms (e.g., some microscopic animals that despite their small size are multicellular with differentiated tissues and organ systems).

Our recent ability to sketch confidently the outlines of the evolution of life on earth, discussed later here and in Chapter 13, allows us to now define microorganism in evolutionary terms. Broadly speaking, microorganism in this sense would include everything except the animals and the plants.

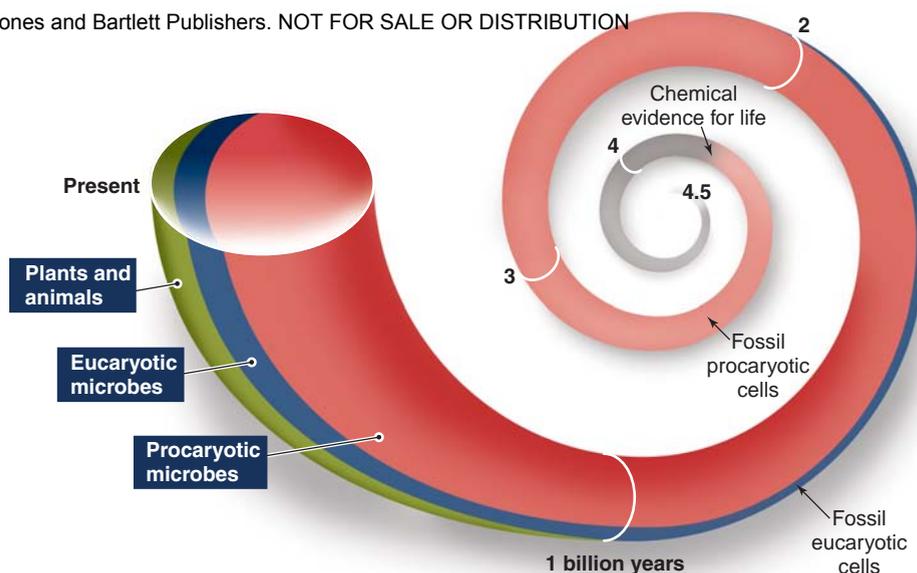


FIGURE 1.1 Time line of life on earth.

1.2 Most microorganisms are unicellular; if multicellular, they lack highly differentiated tissues

The vast majority of microbes are unicellular—that is, the entire organism consists of a single cell. Also fairly common are filaments of cells attached end to end in a row. In some cases, especially in the fungi and the algae (most of which are considered microbes), there are representatives that are multicellular (often macroscopic as well). Even in these forms, however, the cells that make up the organism are not organized into highly differentiated tissues and organs. The differentiation of diverse and very different types of tissues and the organization of these different tissues into organs seem to have been an evolutionary invention of the plants and animals alone (with some rudimentary tissue differentiation in the algae and fungi).

1.3 Microbial life originated shortly after the earth was formed

Cosmologists agree that the earth is about 4.5 billion years old, originating from the coalescence of debris left over after the formation of the sun. It was originally very hot—too hot for liquid water—from the heat of gravitational collapse and from radioactive decay in its core. It was also continually bombarded with large meteorites whose impact released so much energy that nothing living would have been able to survive. It is generally agreed that the earth cooled to habitable temperatures (less than 100°C) about 4.0 billion years ago and that the meteoritic bombardment abated by about 3.8 billion years ago. Coincidentally, this is about the age of the oldest rocks, and these rocks show chemical evidence of microbial life. Shortly thereafter (in geological time), at about 3.5 billion years ago, fossil evidence of microbes exists. Clearly life originated on earth almost immediately after conditions permitted.

The microscope has been as much a tool for geologists as for biologists. Starting in the 19th century, the microscopic organization of mineral grains in rocks was examined by putting very small shards of rock under the microscope and looking through the very thin edges. When the rock is very thin (on the order of 0.1 mm), it is translucent, and light can pass through it, revealing its structure.

It was thus natural that at some point geologists would search shards of rock for fossil microorganisms. The earth's mantle consists of layer on layer of rock, with, unless the rock has been rearranged by tectonic movements, the most recent rock on top and the oldest on the bottom. The top rocks, corresponding to the last quarter of the earth's history, contain macroscopic fossils; the bottom rock, three quarters of the history of the earth, is barren of visible fossils. This discontinuity was recognized even in Darwin's time, and Darwin commented on the apparently sudden appearance of life in the geological era called the Cambrian.

It was an obvious possibility that Precambrian life was microscopic, and thus, geologists were attentive to the possibility of microscopic fossils. They are quite rare, however, and it was not until the 20th century that the first Precambrian microfossils were discovered. In 1918, E. Moore saw fossil cyanobacteria in Precambrian rocks, and similar observations were made by John Gruner in 1923 and Burton Ashley in 1937 (Figure 1B.1). Interest waned, however; the point had been made, and the attention of geologists moved on.

After the Second World War, however, nuclear sciences produced new and precise ways to date rocks (rock dates before this had been little more than educated guesses). This led to the realization that a systematic study of microfossils might reveal when life on earth originated, and there was a resurgence of interest in microbial fossils. These studies have revealed that complex, filamentous organisms similar to modern prokaryotes are present in rocks from nearly 3.6 billion years ago (Figure 1B.2). Older rocks exist—up to about 3.8 billion years old. To date, these have not shown unambiguous fossils.

Modern micropaleontology is a complex science. Rocks to be examined are cleaned many times and shattered into small pieces with a hammer, and then thin sections (typically about 100 μm thick) are cut with a fine saw for microscopy. The rest of the rock sample is pulverized and analyzed chemically for the amount of carbon present and for the ratio of ¹³C to ¹⁴C (which can indicate whether the carbon is of biological origin). Sometimes the rock is assayed for specific compounds, like derivatives of chlorophylls or other complex molecules, that indicate the presence of life.

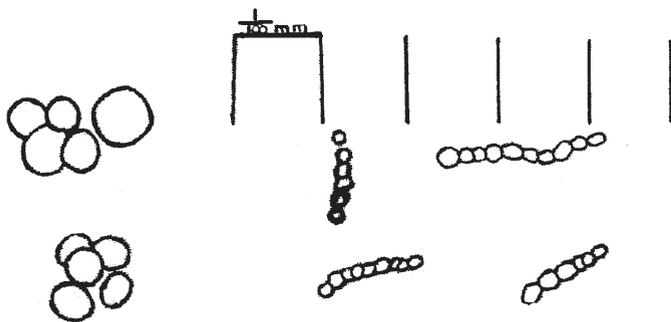


FIGURE 1B.1 Moore's 1918 drawing of microbial fossils.

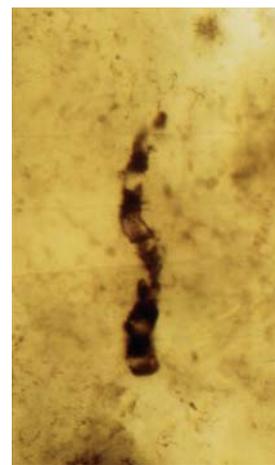


FIGURE 1B.2 Modern micrograph of fossil microbes 3.5 billion years old.

1.4 There are two fundamentally different types of cells: prokaryotic and eucaryotic

Careful examination of microbial cells under the microscope reveals that there are two fundamentally different types: relatively large cells with complex interiors and very small and simple cells. Electron microscopic examination confirms that their internal organization is very different. These two types of cells are termed *eucaryotic* for the large, complex ones and *prokaryotic* for the small simple ones (Figure 1.2). At the ultra-structural level, eucaryotic cells possess complex internal structures composed of membranes that divide the cytoplasm into a number of different compartments. One of these is the nucleus, which contains the DNA-containing chromosomes. The

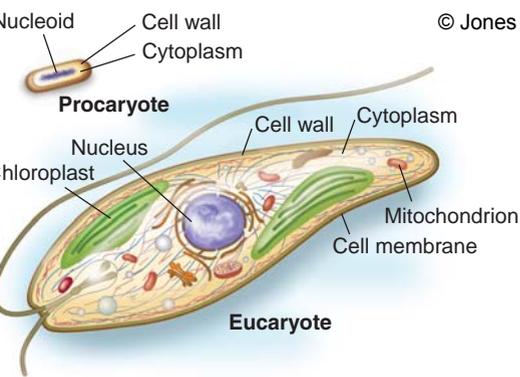


FIGURE 1.2 Prokaryotic and eukaryotic cells.

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membrane around it) instead of a nucleus, and it lacks the internal membrane system. Despite its apparent simplicity at the cellular level, study of the molecular biology of prokaryotic cells makes it clear that they too are highly organized structures; however, much of their organization is at the molecular level and is not visible in the microscope. Chapters 4 and 5 discuss microbial cell structure in more detail.

Although there are many exceptions, it is probably fair to think of the prokaryotes as specialists at rapid growth at the expense of nutrients dissolved in the water that surrounds them. This has entailed the evolution of small, relatively simple cells with highly effective permease systems to take nutrients from a dilute solution, and a highly efficient, tightly regulated metabolism to make the most of them.

Eucaryotic cells, on the other hand, appear to have initially specialized in predation on the smaller, simpler prokaryotes. This move up the trophic scale led to an increase in cell size and complexity and, ultimately, after several billion years, to multicellular organization in the algae, fungi, plants, and animals.

Of course, many exceptions exist to this simple generalization—there are many nonpredatory eucaryotes and many slow-growing prokaryotes. Nevertheless, it is a useful way to think of the broad evolutionary strategies that worked to produce the two cell types. Both strategies have proved successful so that today the planet supports an immense variety of life, both eucaryotic and prokaryotic, microscopic and macroscopic, unicellular and multicellular. These all interact with each other in an unimaginably complex web of physical and chemical interactions at the microscopic, local, and global levels. This web of interactions has proved to be quite stable for eons: for the first 3 billion years as an exclusively microbial biosphere (containing both prokaryotic and eucaryotic microbes) and for the last billion as microscopic and macroscopic organisms commingled. It remains to be seen whether these long-term stabilities can be maintained in the face of the assault of industrial human cultures on the biodiversity and ecological integrity of the planet. We study some of these interactions in Chapters 16–20.

1.5 Microbes, especially prokaryotic ones, are unbelievably numerous

The sheer number of microbial cells in the world is mind boggling and vastly in excess of the number of macroscopic organisms. This is, of course, a consequence of their small size; very large numbers of them can fit into a very small space, and very small amounts of nutrients can nourish a very large number of them. For instance, *Escherichia coli*, a very well-studied and reasonably typical prokaryote, is a short rod a bit over a micrometer in length and a bit under a micrometer in diameter, weighing about a picogram (10^{-12} g). Although there is much variation in prokaryotic cell size, this is an especially common size and can be taken as typical.

A single individual human being is as much an ecosystem as an individual. It has been estimated, very approximately, that there are about 10^{13} human cells that make up the adult human body. This body is, in turn, inhabited by approximately 10^{14} microbial cells—in the intestine, in the mouth, in the vagina, and on the skin—nearly all of them prokaryotic. A single milliliter of intestinal contents contains more microbial cells than there are humans on earth (Figure 1.3). Other animals are of course similarly populated. Rich soil is also densely inhabited: 1 gram also contains more microbes than there are humans on earth—so too for lake sediments and for the sediments of the continental shelf. Thus, the total number of microbes on earth is beyond comprehension.



FIGURE 1.3 Intestinal microbes.

Most (90% or more) of these organisms are prokaryotes, and most of the eukaryotes, as is usual for larger organisms at a higher trophic level, are vastly outnumbered.

Despite their very small size and mass, the immense numbers of microbes mean that the total microbial biomass (weight of living material) is larger than that of macroscopic life. Indeed, the prokaryotic biomass alone is about half of the planetary biomass. Thus, in nearly all quantitative ways, microbes are the most significant life forms on the planet.

Microbes are not just unbelievably numerous; they are genealogically ancient as well. It has been estimated that the average *E. coli* living in the human digestive tract replicates itself about once every 12 hours—or more than 700 times a year. Thus, by the time a human dies at the age of 75 years, the *E. coli* inside him or her have been through more than 50,000 generations since they first colonized his or her intestinal tract. For comparison, there have probably been only about 5,000 generations of humans since anatomically modern humans appeared about 100,000 years ago.

1.6 The tree of life is almost entirely microbial

Considering that the ancestors of today's microbes were actively multiplying and evolving for 3 billion years before the first animals and plants appeared in the fossil record, it is no wonder that the tree of life is almost entirely microbial. There are about 20 or so major lineages of prokaryotic cells and a comparable number of eukaryotic microbial lineages, all of which are vastly older than the plants and animals. Plants and animals are two closely related twigs of the tree of life (Figure 1.4).

1.7 There are three major lineages of life on earth

To the best of our current ability to reconstruct the early history of life on earth, it appears that all life on earth shares a single common ancestor that lived about 3.8 billion years ago. It was probably prokaryotic in structure, although probably simpler even than the modern prokaryotic cell. It was adapted to a world that was very hot

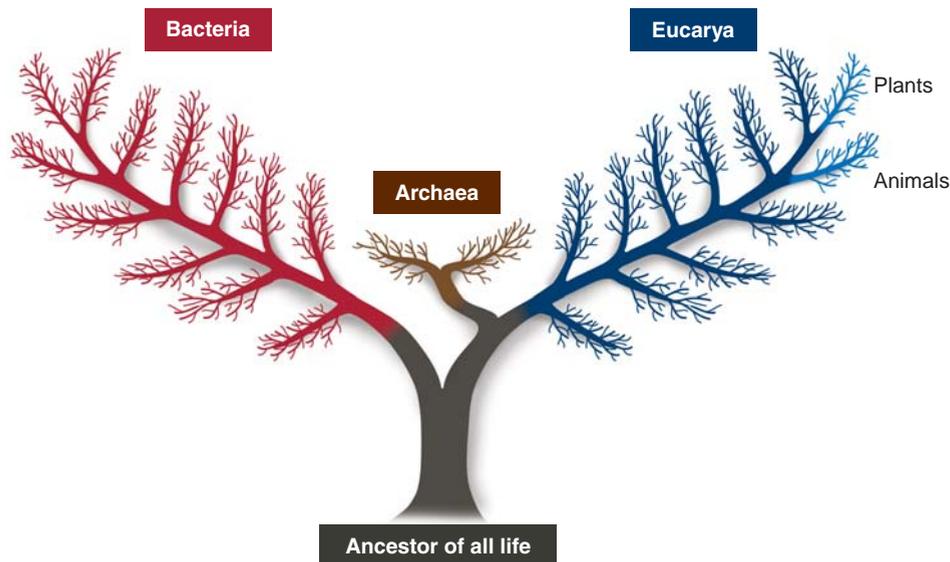


FIGURE 1.4 Phylogenetic tree of life on earth.

organism split originally into two major lineages. One of them, called the **bacteria**, produced about a score of known major sublineages, all of them procaryotic.

The other initial branch split again shortly thereafter to produce two lineages, one procaryotic and one that developed onto the eucaryotic branch. These lineages are the **archaea** (procaryotic) and the **eucarya** (eucaryotic). Within the eucaryotic lineage there are another 20 or so known sublineages, plants and animals being two of the most recent newcomers.

Of course when plants and animals first evolved, their bodies provided a new set of habitats for microbes to colonize, with the result that now each individual macroscopic organism is a complex ecosystem inhabited by a large number and wide diversity of microbes. Each of these microbes is specifically adapted to inhabit its host and to take its nutrients from the host. Nutrients may be provided by secretions, such as sebaceous secretions of skin or mucous on mucous membranes or in the case of intestinal bacteria by ingested food that feeds the microbes as well as the host.

Many of the microbes that live in us and on us are benign, even helpful. Others, however, damage their hosts and cause disease. Thus, another reason that microbes are of great interest to humans is the great damage that a few of them cause us—directly by causing human disease or indirectly by causing diseases of our crops or domestic animals.

1.8 Procaryotes inhabit an immense range of habitats

Microbes, particularly procaryotes, inhabit a wide range of habitats, including some that have qualities that would suggest they are completely uninhabitable—for instance, boiling hot springs or highly alkaline or acidic ponds. In general, if a place contains liquid water, a source of energy (light or a reduced organic or inorganic compound), and some dissolved minerals, some procaryotic organism has probably evolved to inhabit it. Procaryotic habitats range in temperature from -10°C to nearly 120°C (as long as the water is liquid due to dissolved solutes or hydrostatic pressure) and in pH from less than 1 to more than 10. Procaryotes are found miles under the surface of the earth growing on inorganic minerals or on hydrocarbons, and they are found in all surface soils and waters that are not permanently frozen. This enormous range probably defines the limits of life based on the kind of chemistry that characterizes terrestrial life.

1.9 Procaryotes are essential to all life on earth

Clearly microbes are sufficient to maintain a sustainable global ecosystem—after all, for several billion years, there were only microbes on earth, and the planet did just fine. The emergence of multicellular life introduced new complexity to the planetary ecology and introduced new habitats for microbes (the tissues of the new multicellular organisms). Multicellular life, however, has not become essential to the biosphere. Even plants, which we usually think of as essential for their primary productivity, are not in fact essential. In their absence, as in the first few billion years on earth, photosynthetic microbes, especially the cyanobacteria, could substitute. Indeed, if all plants and animals, or even if all eucaryotes, were eliminated from the earth, life would continue indefinitely. There would, of course, be centuries of severe ecological disruption, but eventually, a new and sustainable steady state would be established.

The reason that procaryotes are essential to all life on earth is that some of them catalyze transformations of chemical compounds that are essential to the sustain-

1.10 The dry weight of microbial cells consists mainly of macromolecules and lipids

Like all cells, microbes consist mostly of water. *E. coli*, for instance, is about 70% water; only 30% of its weight consists of other chemical compounds. This 30% is called the **dry weight**, and it, in turn, consists principally of a variety of different macromolecules and of lipid (Figure 1.5). **Macromolecules** are very large molecules, typically with molecular weights above 10,000 daltons. They have a polymeric structure, being composed of many similar monomers covalently linked together. Lipids are much smaller, with molecular weights typically under 1,000 daltons; they are not polymeric in structure, and they are hydrophobic.

Macromolecules constitute more than 85% of the dry weight; about 10% is lipid. Thus, all of the small molecules of the cell combined constitute less than 5% of the dry weight. These include all of the various monomeric building blocks from which macromolecules are assembled (amino acids, nucleotides, sugars), all of the various intermediates in biochemical pathways, a number of enzyme cofactors, and a variety of inorganic ions.

1.11 Among the macromolecules, proteins are the largest and most diverse category

Among the macromolecules, there are several major subcategories: proteins, nucleic acids, polysaccharides, and heteropolymers. The last is a category of macromolecule in which two different types of monomers are covalently combined. In the case of bacteria such as *E. coli*, the principal heteropolymer combines many short peptides and a polysaccharide core to form a structure called **murein**, found in the cell wall.

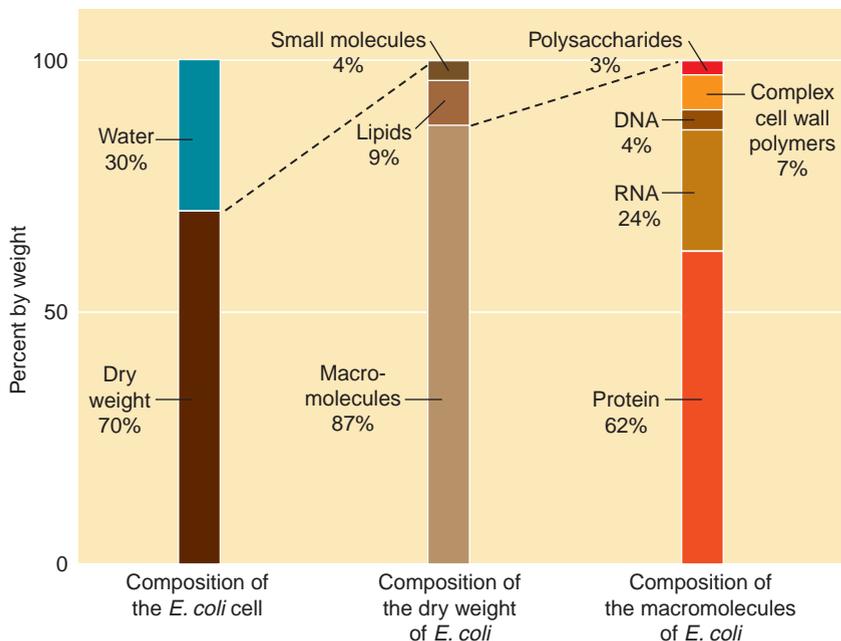


FIGURE 1.5 Cell composition.

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The major class of macromolecule in the *E. coli* cell wall combines polysaccharide with lipid to form **lipopolysaccharide**.

The major class of macromolecule, at least from a quantitative point of view, is protein. Proteins are polymers of amino acids, with molecular weights that vary from somewhat below 10,000 daltons to well over 100,000 daltons. They are not only the most abundant, but also the most diverse of the macromolecules; even such a small cell as *E. coli* has over 2 million protein molecules, of more than 1,000 different types.

Proteins are so diverse because of the many roles they play. Almost every one of the more than 1,000 biochemical reactions of cellular metabolism is catalyzed by a separate enzyme. In addition, there are many permeases in the cell membrane to transport nutrients in and wastes out. There are structural proteins that form organelles within the cell or on its surface, and there are receptor proteins in the membrane that communicate information about the external environment, etc.

1.12 The cytoplasm is a dense suspension of ribosomes

Although proteins are very diverse, with over 1,000 different kinds found in even simple procaryotic cells such as *E. coli*, fewer than 100 proteins make up most of the protein mass of the cell. These are the ribosomal proteins. Ribosomes are organelles composed of protein and RNA; their role is to synthesize protein (in collaboration with mRNA and charged tRNAs). In most procaryotes, there are three molecules of RNA and about 80 protein molecules per ribosome. The number of ribosomes per cell varies; the *E. coli* cell that we are considering here contains about 18,000. Thus, over 1.4 million molecules of ribosomal protein are present in an average *E. coli*, or about three-fourths of all the protein of the cell, and the cytoplasm consists largely of a very concentrated suspension of ribosomes (Figure 1.6).

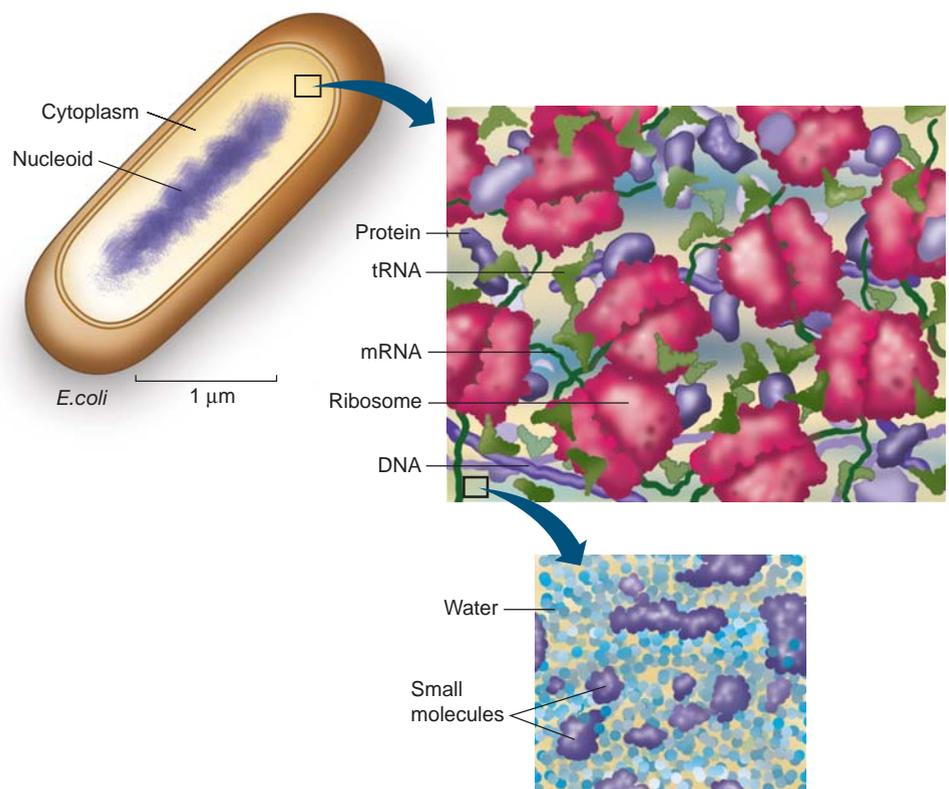


FIGURE 1.6 The procaryotic cytoplasm.

Why are there so many ribosomes in a cell? Protein synthesis is the most significant task during multiplication, and protein is the most abundant molecule in the cell (62% of 87%), the most significant task during multiplication is to make protein. Furthermore, protein synthesis is slower than that of other macromolecules. Only two replisomes (the complex of enzymes that replicates DNA) can duplicate the *E. coli* chromosome in 40 minutes; a few thousand enzyme molecules can double the mass of RNA, polysaccharide, and heteropolymers in 40 minutes or so. To double the mass of protein in a comparable time, however, takes 10,000 or more ribosomes working at their maximum rate.

1.13 The small molecules of the cell are in constant flux

The small molecules of the cell, despite their minor contribution to the dry weight, are abundant and diverse. How can they be abundant if they are only 5% of the dry weight? Because they are so much smaller than the macromolecules, a very small mass of them consists of a very large number of molecules. If we assume an average molecular weight of about 100, then an average *E. coli* cell would contain approximately 70 million small molecules. Because there are so many different kinds of small molecules in the cell, however, the number of any particular molecule is much smaller—typically 1,000 to 100,000.

Because most of these small molecules are intermediates in biochemical pathways or the monomeric precursors of macromolecules, they are continually removed and transformed into something else (into another intermediate in the case of biochemical intermediates or into macromolecules in the case of monomers). Thus, the collection of molecules of any given particular type, which we call a “pool,” is in constant flux. Molecules are continually being removed by being transformed into something else, and these are continually replaced by conversion of molecules from a precursor pool. The rates can be very high; in *E. coli* multiplying at a rapid rate (one generation every 30 to 40 minutes), these small molecule pools turn over entirely (i.e., the number of molecules removed and replaced is equal to the total number in the pool) in a matter of a minute or less. Thus, what we see as a relatively stable chemical composition is in fact a highly dynamic equilibrium.

As an example of the highly dynamic pools, let us calculate an approximate average turnover time for the amino acid pools in *E. coli*. The average *E. coli* cell contains 2 million protein molecules, averaging about 300 amino acids in length. Thus, there are 600 million amino acids polymerized into protein in a single *E. coli* cell. For that cell to double in size and then divide into two *E. coli* cells, it has to double the number of proteins to 4 million before dividing, requiring a further 600 million amino acids to be polymerized. Because there are 20 different amino acids, an average of 30 million molecules of each is polymerized. If we assume an average pool size for amino acids of 100,000 molecules (amino acid pools tend to be larger than most), then the pools turn over 300 times over the course of one cell division. If we take 40 minutes as the time that it takes for this (*E. coli* can multiply faster or slower than this, but this is a common time in the laboratory), then each pool is completely replaced every 10 seconds.

Thus, we see that the procaryotic cell is a highly complex, concentrated mixture of macromolecules and ribosomes in constant frenetic chemical activity. Eucaryotic microbes are even more complex, with their multiple subcellular compartments and intracellular motility and cytoskeletal systems. All cells are highly dynamic entities. Although we necessarily draw them as static, remember that every picture or drawing of a cell is an instantaneous snapshot that freezes what is, in the living cell, a seething mass of chemical activity.

Microbes are the most abundant organisms on earth by many orders of magnitude, and their biomass is equal to that of all multicellular organisms combined. They are critical to the sustainability of life on earth. They inhabit a wide range of habitats that are lethal for multicellular organisms: temperatures over 100°C, or pH of over 10 or under 2. They are the foundation of all life on earth, and their activities have changed the planet in fundamental ways. They can easily be regarded as the most important life forms on earth.

Study questions

1. Briefly summarize the argument that procaryotes are the most important life form on the planet; or, if you wish, argue that other organisms are more important. If you choose the latter, be sure that you consider and rebut the case for the procaryotes.
2. Summarize the basic features of the procaryotic cell and how it differs from the eucaryotic cell.
3. Draw a time line showing the history of life on earth, showing the approximate times of emergence of procaryotic cells, eucaryotic cells, multicellular plants and animals, and humans. Draw to scale.
4. Assume that the average *E. coli* cell has a volume of $1 \mu\text{m}^3$ and contains 8×10^{-15} grams of small organic molecules (the thousand or so building blocks, precursor metabolites, and pathway intermediates). Assume that the average molecular weight of these compounds is 200 g/mole. What is the approximate total concentration of these molecules?
5. Assume that the average *E. coli* cell has a volume of $1 \mu\text{m}^3$ and contains 2×10^6 molecules of protein. What is the approximate total concentration of these molecules?
6. Assume that the average *E. coli* cell has a volume of $1 \mu\text{m}^3$ and contains ATP at a concentration of 10 mM. How many ATP molecules are there per cell?
7. Assume that the average *E. coli* cell has a volume of $1 \mu\text{m}^3$ and its cytoplasm is approximately pH 7. How many protons would you expect to find per cell?
8. Use your answer for question 6 to calculate how fast the ATP pool turns over. Assume that it takes 40 millimoles of ATP to make one gram (dry weight) of cells and that each cell contains 3×10^{-13} grams of dry weight.