Chapter I: The Microbial World

I. Introduction

- **Micro-** prefix: from the Greek *"mikros"*, meaning "small."
- **Bio-** prefix: from the Greek *"bios"*, meaning "life."
- **Logy**: from the Greek *"logia"*, meaning "theory, science."

Microbiology is a subdiscipline of biology that studies microorganisms.

The term microorganism or microbe does not have a precise taxonomic meaning. It refers to tiny organisms that are invisible to the naked eye but visible under an optical microscope or an electron microscope. These include bacteria, archaea, and eukaryotes, distributed across the three domains of life. These organisms are highly diverse in terms of shape, size, and lifestyle. Their small size requires the use of special techniques, such as microscopy, to observe them.

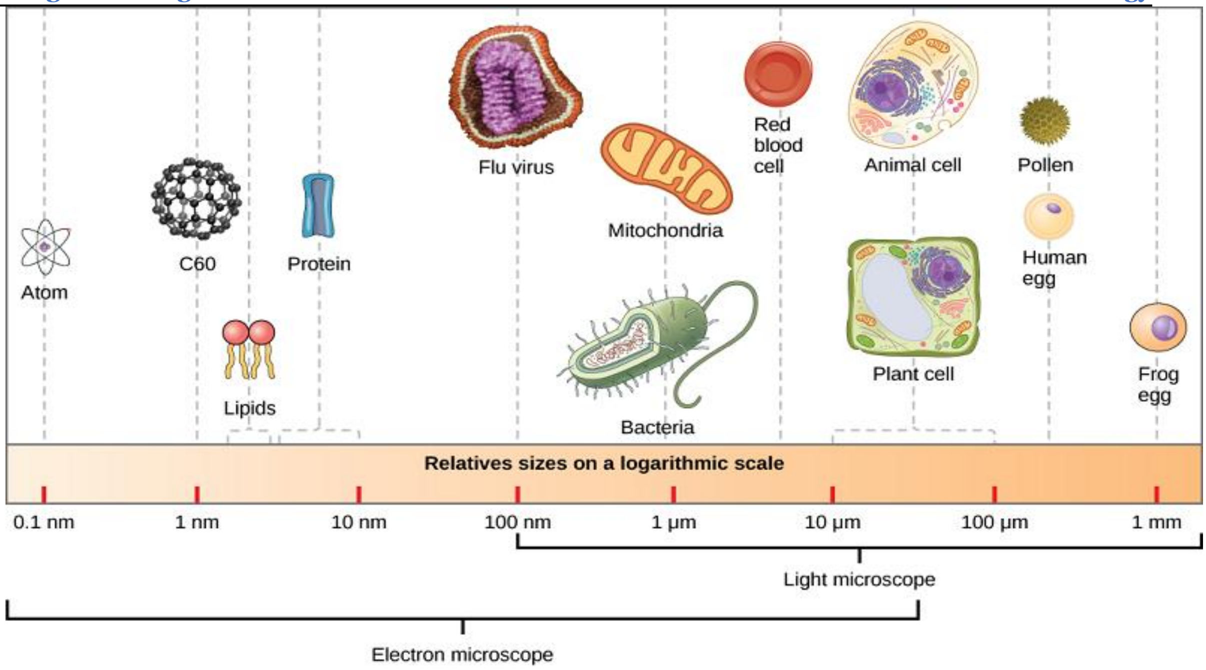
Microbiology is divided into several branches based on the type of microbe studied:

- **Bacteriology**: focuses primarily on bacteria but is often used synonymously with microbiology.
- **Protozoology**: deals with protozoa and is a branch of parasitology that studies protozoan parasites of humans.
- **Mycology**: studies fungi.
- **Phycology or Algology**: studies algae.
- **Virology**: studies viruses.

Microbiology encompasses the study of various aspects of microorganisms, including their structure, function, classification, ecology, reproduction, genetics, and interactions with their environment and other living organisms, including humans.

Microbiology plays an essential role in numerous areas of science and society. It contributes to :

- The prevention and treatment of infectious diseases.
- The production of safe food.
- The development of new medical therapies.
- The discovery of new antibiotics.
- The detection and prevention of epidemics.
- Environnemental protection.
- The sustainable exploitation of natural resources.



II. History

The history of microbiology dates back to antiquity. Although microorganisms are invisible to the naked eye, early observations and theories about these invisible organisms can be traced to ancient Greece, where thinkers like Hippocrates and Aristotle speculated on the existence of "small creatures" responsible for diseases.

- **1665**: **Antony Van Leeuwenhoek** (1632–1723), a Dutch cloth merchant, constructed the first microscope by stacking lenses to examine textiles. With his curious mind, he used this instrument to observe particles from his skin, mouth, and teeth, sketching what he saw. He discovered the microbial world. The term "microbe" did not yet exist, and the terminology of the time referred to these observations as "animalcules," including protozoa, unicellular algae, yeasts, etc.

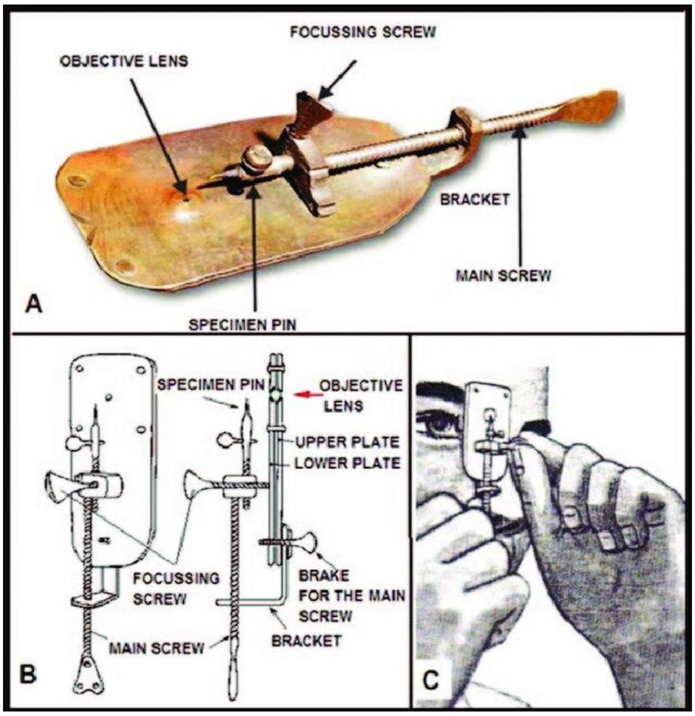


Figure 1: Leeuwenhoek's microscope

- **1729–1799: Lazzaro Spallanzani** found that boiling broth would sterilize it and kill any microorganisms in it. He also found that new microorganisms could settle only in a broth if the broth was exposed to the air.
- **1857–1876**: **Louis Pasteur** expanded upon Spallanzani's findings by exposing boiled broths to the air in vessels that contained a filter to prevent all particles from passing through to the growth medium. He also did this in vessels with no filter at all, with air being admitted via a curved tube that prevented dust particles from coming in contact with the broth. By boiling the broth beforehand, Pasteur ensured that no microorganisms survived within the broths at the beginning of his experiment. Nothing grew in the broths in the course of Pasteur's experiment. This meant that the living organisms that grew in such broths came from outside, as spores on dust, rather than spontaneously generated within the broth.

Louis Pasteur demonstrated the roles of microorganisms in lactic and alcoholic fermentation. He developed pasteurization and sterilization techniques, enabling the creation of pure microbial cultures. These advancements helped disprove the theory of spontaneous generation and supported germ theory instead.

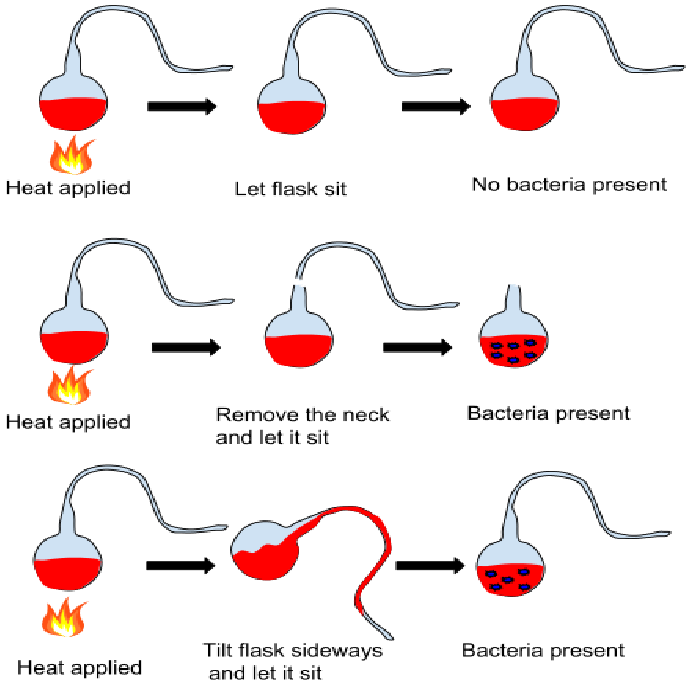


Figure 2: Pasteur's test of spontaneous generation

- **1877–1895**: Louis Pasteur proved that certain diseases are caused by microorganisms. This period saw the first systematic research into the origins of certain diseases and the development of vaccines (vaccination was already known since Edward Jenner's work on smallpox, a viral disease).
- **1873–1882**: **Robert Koch** discovered that the anthrax bacillus forms resistant endospores capable of remaining in the soil. These endospores, were the cause of unexplained "spontaneous" outbreaks of anthrax. Koch revolutionized microbiology by developing techniques for isolating pure cultures, notably the streak plate method, essential for isolating and studying microbes. He also developed techniques to link specific microbes to specific diseases "**Koch's postulates**" for which he was awarded a Nobel Prize in 1905. These postulates stated that an organism is responsible for

a disease if it is present in all sick individuals and absent in healthy ones. These postulates enabled the identification of agents responsible for diseases such as diphtheria, plague, and syphilis. Robert Koch identified the bacillus responsible for tuberculosis (*Mycobacterium tuberculosis*), later known as Koch's bacillus. Koch also discovered tuberculin, an extract of the bacillus, which played a role in diagnosing the disease.

- **1884**: Hans Christian Gram developed a staining technique (Gram stain), still widely used to classify bacteria into two major groups: Gram-positive and Gram-negative bacteria.
- **1929**: Alexander Fleming discovered the antibacterial properties of penicillin, produced by *Penicillium*. This marked the beginning of the antibiotic era, revolutionizing the treatment of bacterial infections.
- **1944**: Albert Schatz and Selman Waksman discovered another antibiotic, streptomycin, which was soon used to treat tuberculosis.
- **1977**: Carl Woese studied ribosomal RNA and discovered a third form of life, Archaea, genetically distinct from bacteria and eukaryotes.
- **1986**: Using an enzyme from the bacterium *Thermus aquaticus*, Kary Mullis invented the Polymerase Chain Reaction (PCR) technology. PCR became a fundamental tool in molecular biology.
- **1995**: Craig Venter and his colleagues at TIGR sequenced the first complete bacterial genome (*Haemophilus influenzae*), ushering microbiology into the era of genomics.

III. The Place of Microorganisms in the Living World

With over 500,000 species, the microbial world is extraordinarily diverse. Even today, it is impossible to provide a single, definitive classification of microorganisms. A review of the major microbial groups through their key anatomical and physiological characteristics helps navigate the successive classifications of microorganisms.

Before the discovery of microorganisms, all living beings were classified into the animal and plant kingdoms:

- **Animal organisms** use organic matter as an energy source, store reserves in the form of fats or glycogen, are mobile, and lack cell walls.
- **Plant organisms**, on the other hand, are photosynthetic, using light as an energy source, synthesize starch as a nutritional reserve, are immobile, and have cell walls.

The discovery of new microscopic life forms made it increasingly difficult to classify them within the animal or plant kingdoms. Among these :

- Algae and fungi were more closely related to plants.
- Protozoa, being mobile and non-photosynthetic, were considered animals.
- The placement of bacteria remained unresolved.

To avoid arbitrarily placing unicellular organisms into either kingdom, the German zoologist Haeckel proposed in 1866 a third kingdom, the **Protists (*"Protista"*)**, derived from the Greek "***protestos***", a superlative of ***protos***, meaning "**first**." This kingdom grouped algae, protozoa, and bacteria. In his 1886 version, fungi were also included. Protists are primarily characterized by a rudimentary biological organization.

In 1937, due to the invention of the electron microscope, Edward Chatton distinguished two types of cells:

- **Eukaryotic cells**: with a nucleus surrounded by a membrane and containing cellular organelles.
- **Prokaryotic cells**: with a nucleus lacking a membrane and a very simple organization.

This led to the subdivision of protists into two groups:

- **Lower protists (“*Protista inferior*”)**, encompassing prokaryotes (bacteria).
- **Higher protists (“*Protista superior*”)**, comprising eukaryotic organisms such as protozoa, fungi, and unicellular algae.

In 1938, H.F. Herbert F. Copeland created a classification system with four kingdoms (based on Haeckel’s classification system): ***Monera***, ***Protista***, ***Plantae***, and ***Animalia***. He notably created a new kingdom, ***Monera***, which includes bacteria and cyanobacteria, which had previously been classified under the kingdom **Protista**.

The development of molecular biology techniques allowed the characterization of genes encoding ribosomal RNA (rRNA). By comparing numerous 16S rRNA sequences from various living organisms, researchers divided all living organisms into three domains and six kingdoms:

- The ***Eucarya* domain** includes eukaryotic organisms, which can be unicellular or multicellular. It encompasses the following kingdoms :
  - ***Fungi***
  - ***Plantae*** (plants)
  - ***Animalia*** (animals)
  - Unicellular eukaryotic organisms (protists “***Protista***”), including:
    - ✓ **Unicellular algae**, whose cells resemble plant cells.
    - ✓ **Protozoa**, whose cells resemble animal cells.
    - ✓ **Yeasts**, whose cells resemble fungal cells.

Prokaryotic living beings are divided into two very different domains:

- The ***Bacteria* domain**, including unicellular organisms known as bacteria.
- The ***Archaea* domain**, previously considered bacteria and initially grouped under the term “*archaebacteria*.” This prefix, introduced by Carl Woese in 1977, reflects the initial assumption that they were ancient life forms, a notion that has since been challenged.

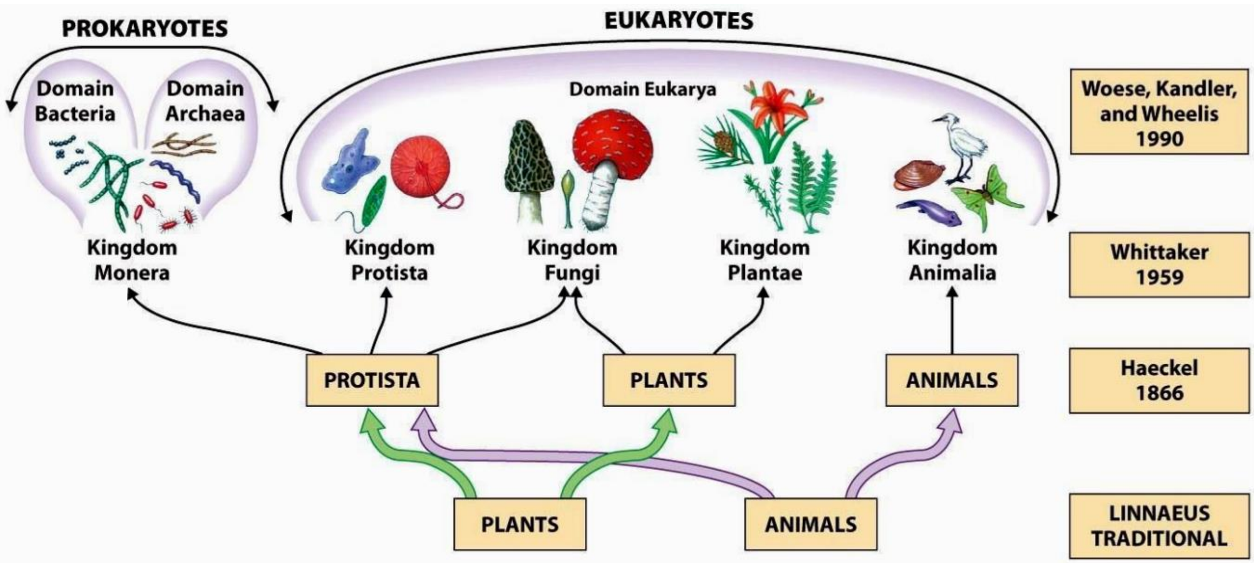


Figure 3: Classification of the microbial world

Thus, the following are classified among microorganisms:

✓ **Microalgae**

Microalgae are **eukaryotic** microorganisms capable of **photosynthesis**. These are **autotrophic** organisms possessing **chloroplasts** in their cells. Additionally, they are distinguished from other categories of eukaryotes by the presence of a rigid cell wall impregnated with cellulose and pectin. Microalgae can be unicellular or consist of several cells that associate to form filaments, colonies, or sheets.

Most often, algae reproduce through binary fission (asexual reproduction). The size of unicellular species is generally between 5 and 10 µm.

Only about fifty microalgae species have been studied so far, categorized into several groups. The most common include:

- **Diatoms**: *Skeletonema, Thalassiosira, Phaeodactylum, Chaetoceros* (90% of marine plankton).
- **Flagellates**: *Isochrysis, Monochrysis, Dunaliella*.
- **Chlorophyceae**: *Chlorella, Scenedesmus*.
- **Chrysophyceae**.

And **Cyanophyceae** (cyanobacteria or blue-green algae): These are prokaryotes closely related to bacteria, characterized by their possession of chlorophyll and their ability to produce carbon-based matter via photosynthesis.

Example of cyanophyceae: *Anabaena sphaerica*.

✓ **Protozoa**

Protozoa are unicellular eukaryotes of variable forms and sizes but generally microscopic. The smallest measure around 1 µm. They are characterized by **motility**, which is a general rule for organisms in this group, the absence of a cellulose cell wall, and the inability to perform photosynthesis. Protozoa feed on organic matter extracted from the environment, making them heterotrophs. Most live freely in natural environments, especially in aquatic habitats, while other species are parasitic in human and animal tissues.

Protozoa reproduce most often through binary fission (asexual reproduction). Sexual reproduction occurs occasionally.

✓ **Fungi (Mycophyta)**

Fungi are nonmotile eukaryotes with a classic cell nucleus and rigid chitin-impregnated walls. They contain no photosynthetic pigments and are carbon heterotrophic, that is, they utilize various organic nutrient substrates (in contrast to carbon autotrophic plants). Of more than 50 000 fungal species, only about 300 are known to be human pathogens. For reproduction, fungi use various asexual and sexual processes.

Two categories of fungi are distinguished:

- Molds.
- Yeasts.

✓ ***Bacteria***

Bacteria are unicellular microbes with a simple structure, lacking a nucleus or membrane-bound organelles. They possess a cell wall made of peptidoglycan (found only in bacteria) and have 70S ribosomes. These organisms are ubiquitous, present in soil, water, food, and the human body.

✓ ***Archaea***

Also called archaeobacteria, *Archaea* is a relatively new domain, since these organisms used to be grouped with the bacteria. There are some obvious similarities, since they are mostly unicellular, cells lack a nucleus or any other organelle, they have 70S ribosomes, and all Archaea are microbes. But they have completely different cell walls that can vary markedly in composition (but notably lack peptidoglycan and might have pseudomurien instead). In addition, their rRNA sequences have shown that they are not closely related to Bacteria at all.

Archaea can be categorized into:

- **Methanogens** (e.g., *Methanobrevibacter smithii*), which have specific metabolic pathways, such as methanogenesis.
- **Halophiles** (e.g., *Halobacterium, Haloarcula, Haloquadrata*), which thrive in environments saturated with salt, such as salt lakes or salt marshes.
- **Thermoacidophiles** (e.g., *Sulfolobus solfataricus*), which live in hot, acidic terrestrial springs.

Classification of Living Things

DOMAIN	Bacteria	Archaea	Eukarya			
KINGDOM	Eubacteria	Archaebacteria	Protista	Fungi	Plantae	Animalia
CELL TYPE	Prokaryote	Prokaryote	Eukaryote	Eukaryote	Eukaryote	Eukaryote
CELL STRUCTURES	Cell walls with peptidoglycan	Cell walls without peptidoglycan	Cell walls of cellulose in some; some have chloroplasts	Cell walls of chitin	Cell walls of cellulose; chloroplasts	No cell walls or chloroplasts
NUMBER OF CELLS	Unicellular	Unicellular	Most unicellular; some colonial; some multicellular	Most multicellular; some unicellular	Multicellular	Multicellular
MODE OF NUTRITION	Autotroph or heterotroph	Autotroph or heterotroph	Autotroph or heterotroph	Heterotroph	Autotroph	Heterotroph
EXAMPLES	*Streptococcus, Escherichia coli*	Methanogens, halophiles	*Amoeba, Paramecium,* slime molds, giant kelp	Mushrooms, yeasts	Mosses, ferns, flowering plants	Sponges, worms, insects, fishes, mammals

Viruses

All these microorganisms share the following common characteristics:

- They all exhibit a **cellular** type of organization.
- They are capable of carrying out their own metabolic activities.

These criteria do not apply to viruses, which are distinguished by their non-cellular organization and their inability to produce the energy necessary for performing vital activities.

Viruses are not part of the Three Domain Classification, since they lack ribosomes and therefore lack rRNA sequences for comparison. They are classified separately, they are essentially large molecules of nucleic acid (DNA or RNA), either single or fragmented into multiple chromosomes, combined with proteins forming a shell around the nucleic acid called a capsid. They can be considered a separate world, at the boundary between life and inanimate matter.

Viruses are typically described as "obligate intracellular parasites," a reference to their strict requirement for a host cell in order to replicate or increase in number. These acellular entities are often agents of disease, a result of their cell invasion.

V. General characteristics of prokaryotic cells

Prokaryotes (*Prokaryota* or *Prokarya*), from the Greek pro (*before*) and karyon (*nucleus*), exhibit the following characteristics, as identified through advances in electron microscopy, which distinguish them from eukaryotic cells:

1. **Absence of a nucleus:**

Unlike eukaryotic cells, prokaryotic cells lack a nucleus enclosed by a membrane. Their DNA, in the form of a single, naked chromosome, floats freely in the cytoplasm.

2. **Reduced size:**

Prokaryotic cells are generally smaller than eukaryotic cells.

3. **Limited organelles:**

Intracellular organelles such as mitochondria, the endoplasmic reticulum, and the Golgi apparatus are absent in prokaryotic cells. However, they may contain less complex structures, such as ribosomes, plasmids (small circular DNA molecules), and vacuoles.

4. **Cell wall:**

Most prokaryotic cells have a rigid cell wall composed of peptidoglycan (in bacteria). This wall provides mechanical strength and protection.

5. **Flagella:**

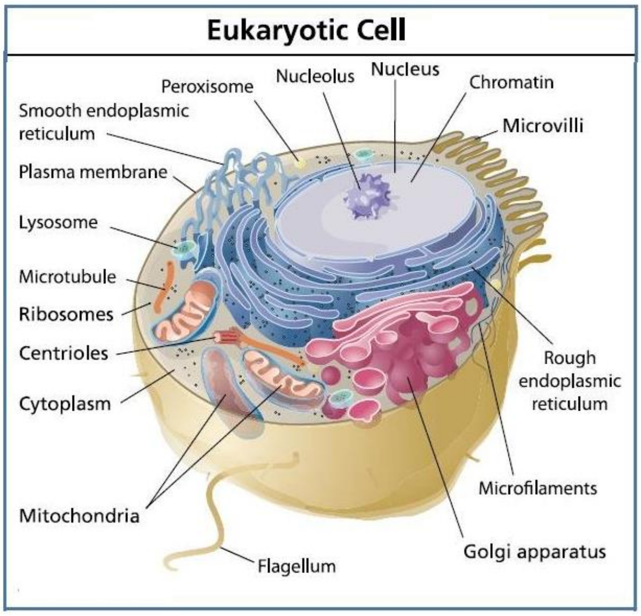
Some prokaryotic cells are equipped with flagella, filamentous structures used for locomotion.

6. **Asexual reproduction:**

Prokaryotic cells primarily reproduce through asexual cell division, specifically binary fission.

7. **Metabolic diversity:**

Prokaryotic cells exhibit significant metabolic diversity. Some are **autotrophic**, capable of producing their own food from inorganic materials, while others are **heterotrophic**, relying on organic sources for nutrition.



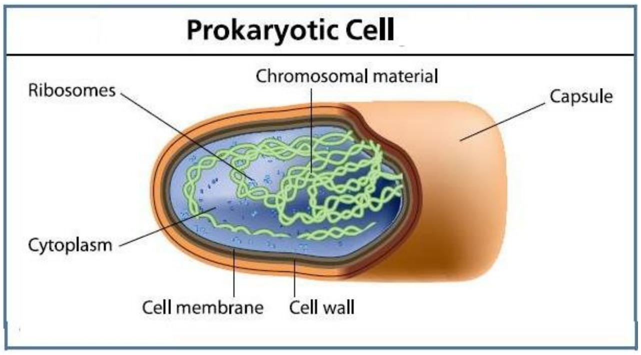


Figure 4: Difference between Eukaryotic and Prokaryotic cells

Properties	Prokaryote	Eukaryote
Phylogenetic groups	Bacteria, Archaea	Eukarya: Algae, fungi, protozoa, plants, animals
Nuclear Structure and Function:		
Nuclear membrane	Absent	Present
Nucleolus	Absent	Present
DNA	Single molecule generally covalently closed and circular, not complexed with histones(other DNA in plasmids)	Linear, present in several chromosomes, usually complexed with histones
Division	No mitosis	Mitosis, mitotic apparatus with microtubular spindle
Sexual reproduction	Fragmentary Process, unidirectional; no meiosis; usually only portions of genetic complement reassorted	Regular process; meiosis; reassortment of whole chromosome complement
Introns in genes	Rare	Common
Cytoplasmic Structure and Organization:		
Cytoplasmic membrane	Usually lacks sterols; hopanoids may be present	Sterols usually present; hopanoids absent
Internal membranes	Relatively simple; limited to specific groups	Complex; endoplasmic reticulum; golgi apparatus
Ribosome	70S in size	80S, excepts for ribosomes of mitochondria and chloroplasts, which are 70S
Membranous organelles	Absent	Several present
Photosynthetic pigments	In internal membranes of chromosomes; chloroplasts absent	In chloroplasts
Respiratory system	Part of cytoplasmic membrane; mitochondria absent	In mitochondria
Cell walls	Present (In most); Composed of peptidoglycan (Bacteria); other polysaccharides, protein, glycoprotein	Present in plants, algae, fungi, usually polysaccharide; absent in animals, most protozoa
Endospores	Present (In some), very heat-resistant	Absent
Gas vesicles	Present (in some)	Absent
Forms of motility:		
Flagellar movement	Flagella composed of single type of protein arranged in a fiber; flagella rotate	Flagella or cilia; composed of microtubules; do not rotate
Nonflagellar movement	Gliding motility; gas vesicle-mediated	Cytoplasmic streaming and ameboid movement; gliding motility
Size	Generally small, usually < 2µm in diameter	Usually larger, 2 to > 100µm in diameter