

الجمهورية الجزائرية الديمقراطية والشعبية
People's Democratic Republic of Algeria

وزارة التعليم العالي والبحث العلمي

Ministry of Higher Education and Scientific Research

المدرسة الوطنية العليا لأساتذة التعليم التكنولوجي بسكيكدة

Higher Normal School of Technological Education of Skikda

قسم العلوم الطبيعية

Department of Natural Sciences



A Brief Textbook for:

- *Natural Sciences Students of Middle & Secondary Education Professors.*
- *Common Core Students in Biology and medicine.*

Cell Biology

Reviewed by:

Berrebah-Djebar Houria,	Prof.	University of Annaba, Algeria
Issaad-Bouguerra Ghazlene,	Associate Professor A	University of Annaba, Algeria
Khafallah Imen,	Associate Professor A.	HSTE of Skikda, Algeria

Prepared by
Dr. Faouzi Dahdouh
Associate Professor in Biological Sciences

2023/2024

Contents

Preface.....	2
Introduction	3
1. Chemical composition of cell.....	5
Elements.....	5
Organic Compounds.....	5
2. Overview of prokaryotic and eukaryotic cells.....	7
Prokaryotic cells	7
Eukaryotic cells.....	8
3. Cellular components.....	11
4. Genetic materials.....	12
DNA Structure	12
Chromosomes	15
Genetic codes	17
Cracking the Code	17
The Flow of Information.....	18
The Code's Impact	18
5. Cell Cycle and Cell Division.....	22
Cell cycle	22
Cell division	23
Regulation of the Cell Cycle	24
6. Methods of Studying Cells	25
Light microscope	25
Electronic microscope.....	27
7. Biological staining	28
8. Centrifugation and ultracentrifugation	29
Conclusion.....	32
References	33

Preface

Welcome to the wondrous world of cell biology!

This textbook will embark on a journey of discovery, delving into the fundamental units of life – cells. From the basic building blocks of macromolecules to the intricate dance of cellular processes, we will explore the fascinating mechanisms that govern life at its most basic level. This knowledge forms the foundation for understanding all living organisms, from the simplest bacteria to the complex human body.

Due to the unavailability of written lectures in Arabic and/or English language provided by the institution Lecturers and Professors, this manuscript serves as the first English-language textbook introduced in my institution (Higher School of Technological Education of Skikda, HSTES) in English language to help the HSTES students to learn and to understand well the cell structure, function, and behavior of cells.

I taught the module of cell biology in the HSTES for nearly four years ago, when I covered the most common module sections, including the lectures of basics in cells, and the experimental tests in cell structure and function.

This textbook is designed for undergraduate students with a basic understanding of biology. It aims to provide a comprehensive yet accessible introduction to cell biology, covering essential topics with clear explanations, engaging illustrations, and relevant examples.

The present textbook includes two major sections, namely Basic cell structure and function, and experimental tools and methods of studying cells.

Introduction

Cell biology, the study of cells as the fundamental units of life, is a cornerstone of biological sciences. Cells are the smallest units capable of performing all the necessary functions of life, including metabolism, growth, and reproduction. This field explores the intricate structures within cells, such as the nucleus, mitochondria, and ribosomes, and their specific roles in maintaining cellular function. The development of advanced microscopy and molecular techniques has allowed scientists to visualize and manipulate cells at unprecedented levels, leading to significant discoveries about cellular processes and their implications for health and disease.

A pivotal concept in cell biology is the distinction between prokaryotic and eukaryotic cells. Prokaryotic cells, found in bacteria and archaea, lack a nucleus and other membrane-bound organelles, presenting a simpler structure compared to eukaryotic cells. Eukaryotic cells, which make up plants, animals, fungi, and protists, contain a nucleus that houses their genetic material and various organelles that compartmentalize different cellular functions. This complexity allows for specialized functions within eukaryotic cells, such as energy production in mitochondria and protein synthesis in the endoplasmic reticulum and ribosomes, facilitating the development of multicellular organisms with diverse tissues and organ systems.

The study of cell biology has profound implications across various scientific and medical fields. Understanding cellular mechanisms enables researchers to develop targeted therapies for diseases, including cancer, where cell division and death are dysregulated. In biotechnology, insights into cellular processes are harnessed to engineer microorganisms for the production of pharmaceuticals, biofuels, and other valuable products. Furthermore, cell biology research is crucial for advancing regenerative medicine, where stem cells are used to repair or replace damaged tissues. As our knowledge of cell biology continues to expand, it holds the promise of innovative solutions to some of the most pressing challenges in health and industry.

1. Definition of cell

Cells are the fundamental units of life. They are the smallest structures capable of carrying out all the functions necessary for an organism to live and reproduce. The study of cells, known as cell biology, is crucial for understanding the complexities of life and has led to significant advancements in various fields of science.

The cell theory, formulated in the 19th century, is a fundamental concept in cell biology. It states that all living organisms are composed of cells, and cells are the basic units of structure and function in living organisms. The cell theory was proposed by Theodor Schwann, a German physiologist, and Matthias Schleiden, a German botanist. Their work built upon the discoveries of other scientists, including Robert Hooke, who first observed cells in 1665 (**Lanza et al., 2005; Ford, 2009**).

Robert Hooke's observations of cork under a microscope led to the discovery of cells. He described the tiny compartments he saw as resembling the small rooms, or "cells," in a monastery. Although Hooke's observations were limited to dead cells, they laid the foundation for further investigations into the structure and function of cells.

Theodor Schwann, a contemporary of Schleiden, extended the cell theory to animal cells. He observed the similarities between plant and animal tissues and concluded that cells are the fundamental units of both plant and animal life. Schwann's contributions to the cell theory solidified its status as one of the fundamental principles of biology (**Pollard and Earnshaw, 2022**).

The cell theory revolutionized our understanding of life and laid the groundwork for the field of cell biology. It provided a framework for studying the structure and functions of cells and opened up new avenues of research. Since then, scientists have made numerous discoveries and advancements in cell biology, uncovering the intricate mechanisms and processes that govern life at the cellular level (**Lanza et al., 2005**).

The study of cell biology has far-reaching implications, from understanding the basics of how cells function to developing treatments for diseases. It encompasses a wide range of topics, including cell metabolism, cell communication, and cell cycle, biochemistry, and cell composition. By studying cells, scientists can gain insights into the inner workings of organisms and develop a deeper understanding of the complexities of life.

1. Chemical composition of cell

The chemical composition of a cell is a crucial aspect of cellular biology. It involves the presence of various elements and compounds that are essential for the cell's structure, function, and overall survival. The cell is primarily composed of organic compounds such as carbohydrates, proteins, lipids, and nucleic acids, which are synthesized from the elements hydrogen, oxygen, nitrogen, and carbon. These compounds play vital roles in the cell, including providing energy, structural support, and storing genetic information. Additionally, inorganic compounds like water and salts are also present in the cell, serving as a medium for biochemical reactions and maintaining osmotic balance (**Figure 1**).

Elements

The cell contains several essential elements, including hydrogen (H), oxygen (O), nitrogen (N), carbon (C), sulfur (S), phosphorus (P), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and chlorine (Cl). These elements are required in varying amounts for the proper functioning of the cell. For example, H, C, O, and N are needed in large quantities for the synthesis of organic compounds, while S, Na, Mg, Ca, Fe, and Cl are required in smaller amounts for specific functions.

Organic Compounds

The organic compounds found in cells can be divided into four main categories: carbohydrates, lipids, proteins, and nucleic acids.

❖ Carbohydrates

Carbohydrates are the primary source of energy for cells and are composed of carbon, hydrogen, and oxygen atoms. They include monosaccharides (e.g., glucose), disaccharides (e.g., sucrose), and polysaccharides (e.g., starch, glycogen, and cellulose).

❖ Lipids

Lipids are a diverse group of compounds that are insoluble in water but soluble in organic solvents. They include fatty acids, phospholipids, and steroids. Lipids serve as energy sources, structural components of cell membranes, and as precursors for various hormones.

❖ Proteins

Proteins are large, complex molecules composed of amino acids linked by peptide bonds. They are involved in various cellular processes, such as structural support, enzymatic catalysis, transport, and signaling.

❖ Nucleic Acids

Nucleic acids, including DNA and RNA, are responsible for storing and transmitting genetic information. They are composed of nucleotides, which consist of a nitrogenous base, a sugar, and a phosphate group.

❖ Inorganic Compounds

The most abundant inorganic compound in the cell is water, which accounts for approximately 70% of a cell's weight. Water serves as a medium for biochemical reactions, a solvent for various molecules, and plays a crucial role in maintaining osmotic balance and cellular structure.

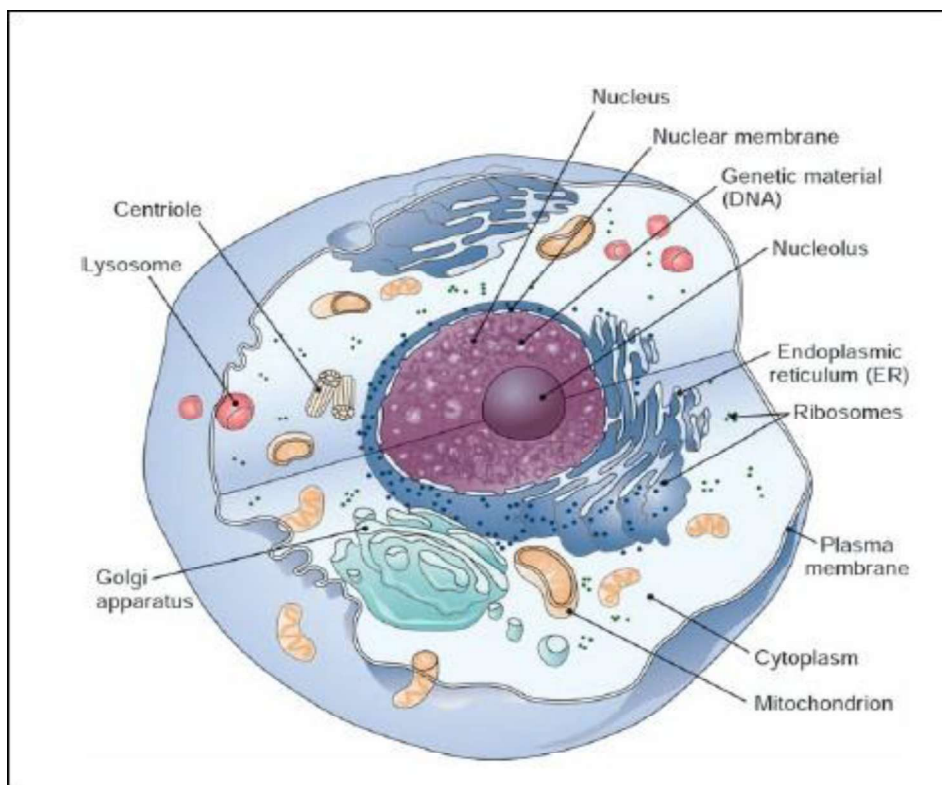


Figure 1. Chemical composition of a typical mammalian cell (Cohen & Hull, 2020).

2. Overview of prokaryotic and eukaryotic cells

Prokaryotic cells

Prokaryotic cells are the simplest and most ancient forms of life, characterized by their lack of a nucleus and membrane-bound organelles. These unicellular organisms, which include bacteria and archaea, possess a single, circular chromosome located in a region called the nucleoid. Their cellular structure is relatively straightforward, with components such as the plasma membrane, cytoplasm, ribosomes, and sometimes cell walls and flagella. Despite their simplicity, prokaryotic cells are remarkably efficient and versatile, thriving in a wide range of environments, from extreme heat and acidity to high salinity. Their ability to adapt and evolve rapidly through mechanisms such as horizontal gene transfer contributes to their ubiquitous presence and ecological significance on Earth (Madigan et al., 2014; Prescott, 2011; Tortora, Funke, & Case, 2016) (Figure 2).

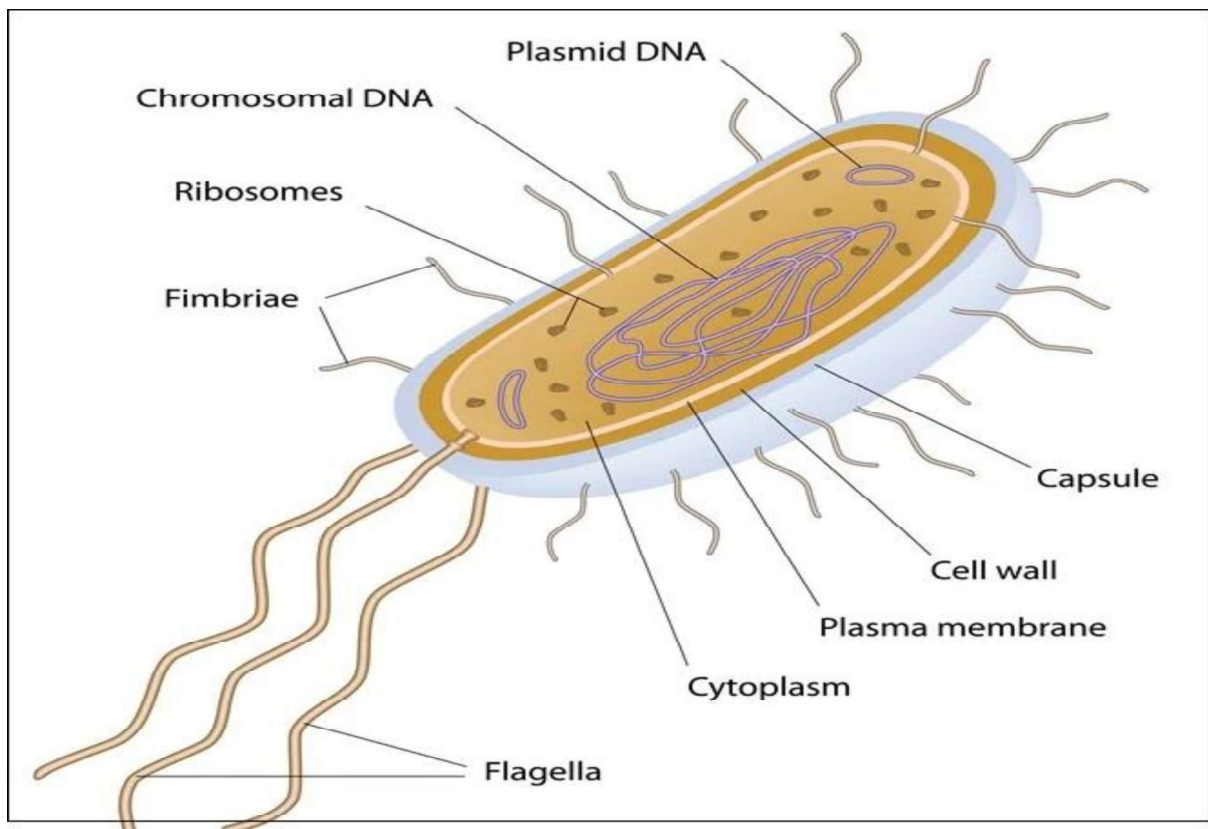


Figure 2. A typical example of prokaryotic cells; case of bacterial cell (Parija, 2023).

Eukaryotic cells

Eukaryotic cells are distinguished by their complex structure and compartmentalization, setting them apart from prokaryotic cells. A defining feature of eukaryotic cells is the presence of a true nucleus, which houses the cell's genetic material within a double membrane, facilitating organized gene expression and regulation. These cells contain an extensive array of membrane-bound organelles that perform specialized functions essential for cellular survival and function. Mitochondria, known as the powerhouses of the cell, generate ATP through cellular respiration, providing energy for various cellular processes. The endoplasmic reticulum (ER) comes in two forms: rough ER, studded with ribosomes, synthesizes proteins, while smooth ER is involved in lipid synthesis and detoxification processes. The Golgi apparatus further modifies sorts, and packages proteins and lipids for delivery to their destinations. Lysosomes contain hydrolytic enzymes crucial for intracellular digestion, recycling cellular components, and apoptosis.

Additionally, the cytoskeleton, composed of microtubules, actin filaments, and intermediate filaments, provides structural support, enables cell movement, and plays a role in intracellular transport and cell division. Plant cells, a subset of eukaryotic cells, possess unique structures such as chloroplasts, which conduct photosynthesis, and a rigid cell wall made of cellulose that maintains cell shape and offers protection. The versatility and adaptability of eukaryotic cells are further highlighted by their ability to form multicellular organisms with complex tissues and organ systems, enabling higher levels of biological organization and functionality. The intricate organization and dynamic nature of eukaryotic cells underscore their evolutionary success and pivotal role in the diversity of life on Earth (**Alberts et al., 2015; Karp, 2013; Cooper, 2018; Pollard & Earnshaw, 2012**) (**Figures 3 and 4**).

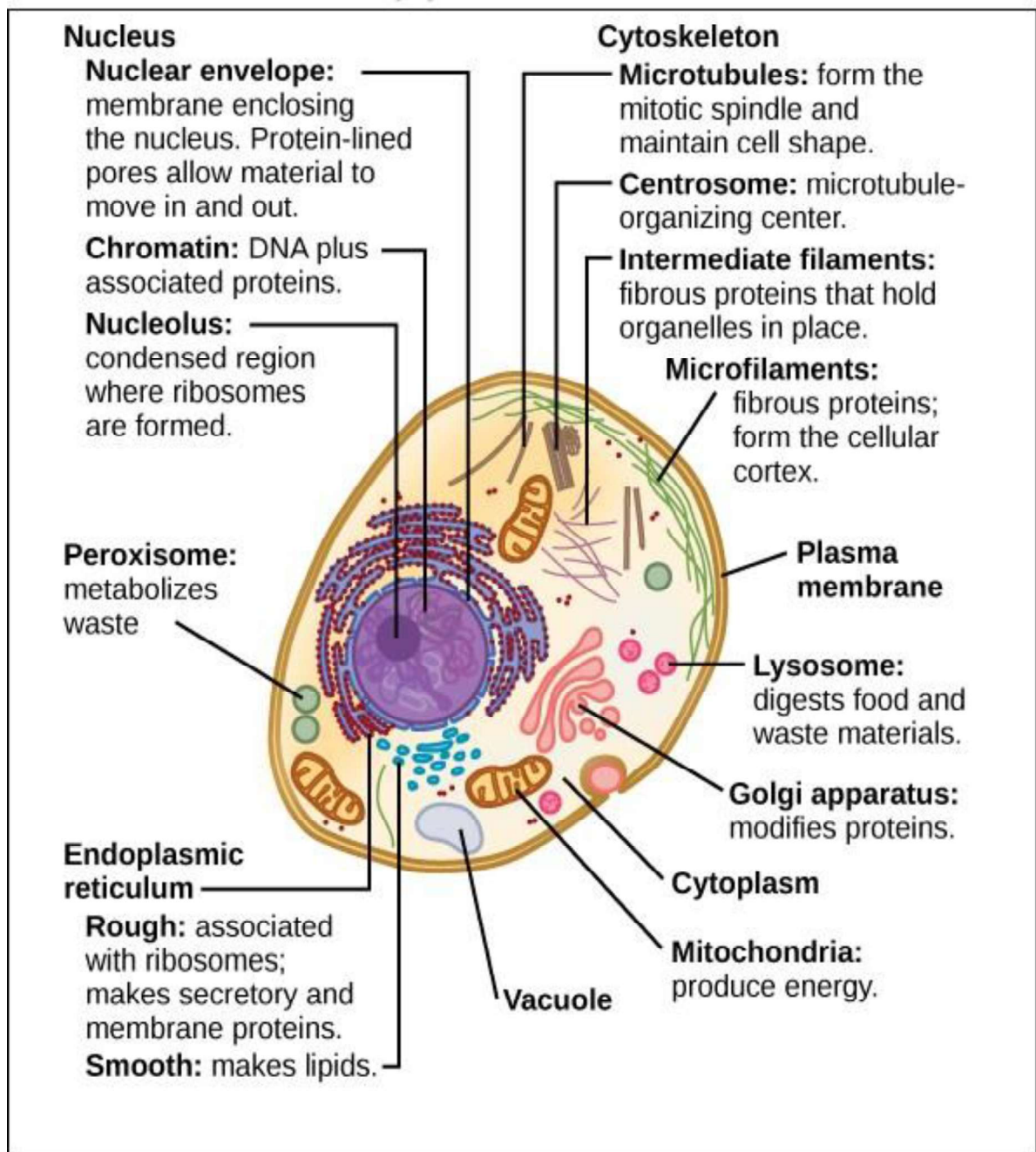


Figure 3. A typical animal cell (Clément, 2007)

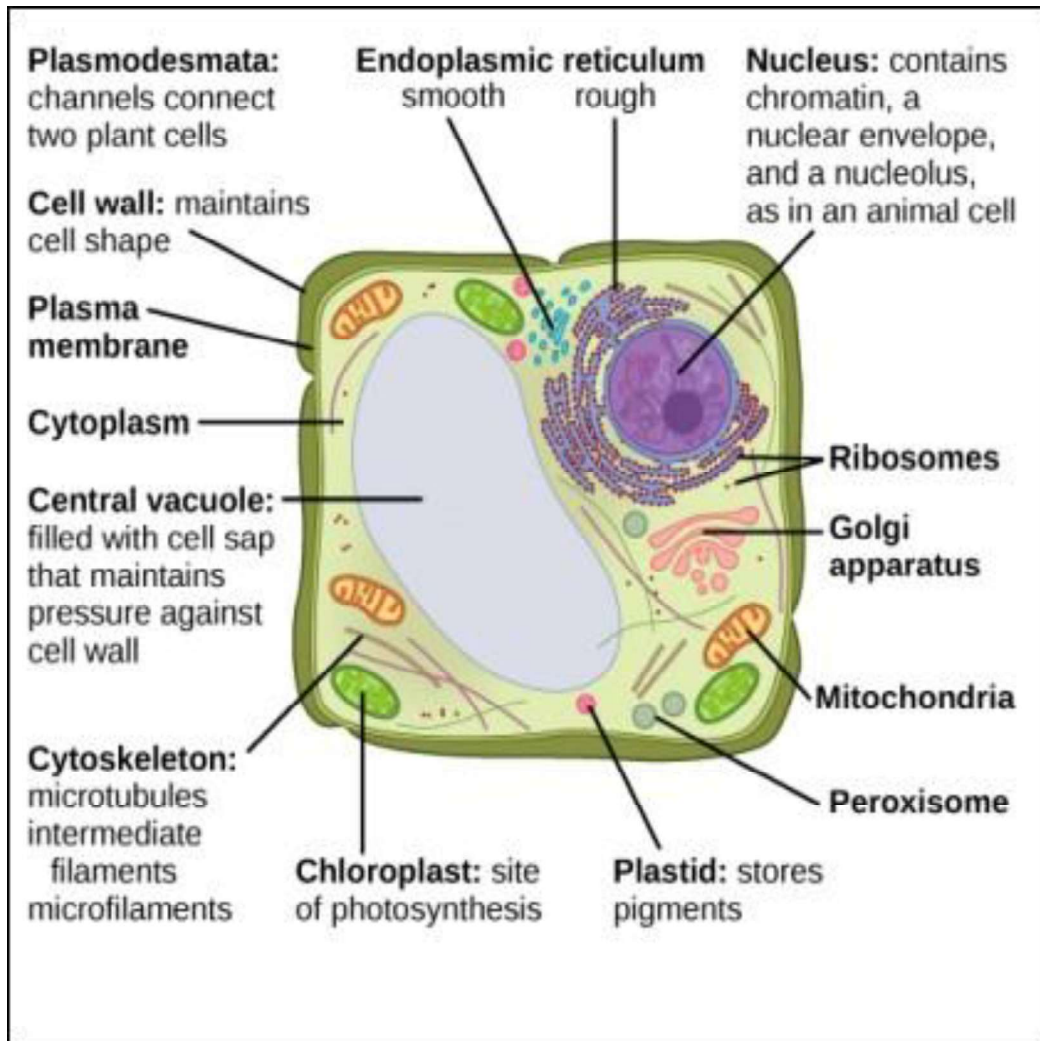


Figure 4. A typical plant cell (Clément, 2007)

3. Cellular components

The cells are mainly composed of plasma membrane, nucleus, ribosomes, endoplasmic reticulum, Golgi apparatus, mitochondria, lysosomes and cytoskeleton (Figures 3 and 4).

- ✚ **Plasma Membrane:** Composed of a lipid bilayer embedded with proteins, the plasma membrane regulates the passage of molecules and ions in and out of the cell, while also providing structural support (Alberts et al., 2015).
- ✚ **Nucleus:** Enclosed within a double membrane-bound structure called the nuclear envelope, the nucleus houses the genetic material, DNA, controlling gene expression and cellular activities (Alberts et al., 2015).
- ✚ **Ribosomes:** These small structures, composed of RNA and protein, serve as the site of protein synthesis, translating genetic information into functional proteins (Alberts et al., 2015).
- ✚ **Endoplasmic Reticulum (ER):** A network of membranes, the ER includes rough ER with ribosomes involved in protein synthesis and smooth ER involved in lipid metabolism and detoxification (Lodish et al., 2016).
- ✚ **Golgi Apparatus:** Functioning in protein modification, sorting, and packaging for secretion or delivery to other cellular locations, the Golgi apparatus plays a crucial role in intracellular transport (Cooper, 2018).
- ✚ **Mitochondria:** Known as the powerhouse of the cell, mitochondria produce ATP through aerobic respiration, providing energy for cellular activities (Alberts et al., 2015).
- ✚ **Lysosomes:** These membrane-bound vesicles contain digestive enzymes for intracellular degradation and recycling, contributing to cellular waste management (Madigan et al., 2014).
- ✚ **Cytoskeleton:** Comprising microtubules, actin filaments, and intermediate filaments, the cytoskeleton maintains cell shape, facilitates cell movement, and assists in intracellular transport (Alberts et al., 2015).

4. Genetic materials

DNA Structure

Human genes are built up by the deoxyribonucleic acid (DNA), known as the key molecule containing the overall genetic information of the cell life and structure. As a result, DNA is able to pass on this information during the cell division. Herein, we would describe the structure and properties of DNA molecules, the way in which our DNA is packaged into chromosomes, and how the information stored within DNA is retrieved via the genetic code. Deoxyribonucleic acid is a long polymer of deoxyribonucleotides composed mainly of deoxyribose, having no OH group on its 2 carbon, two purine bases, namely adenine (A) and guanine (G), and two pyrimidine bases: cytosine (C) and thymine (T). The combined base and sugar is known as a nucleoside to distinguish it from the phosphorylated form, which is called a nucleotide (**Figure 5**) [1]. We note thus, the following possible nucleotides: 2'-deoxyguanosine-5'-triphosphate (dGTP), 2'-deoxycytidine-5'-triphosphate (dCTP), and 2'-deoxythymidine-5'-triphosphate (dTTP). Moreover, eukaryotic cells are made up of a very large DNA molecule, since prokaryotic cells, i. g. the DNA molecules of bacterium *Escherichia coli* is included into a single circular molecule comprising 9 million nucleotides. Humans have 46 DNA molecules in each cell, each forming one chromosome. We inherit 23 chromosomes from each parent. Each set of 23 chromosomes encodes a complete copy of our genome and is made up of 6×10^9 nucleotides (or 3×10^9 base pairs). Furthermore, the iconic double helix structure of DNA resembles a twisted ladder. The two sugar-phosphate backbones form the rigid sides of the ladder, with the phosphate groups of one sugar molecule linked to the sugar molecule of the opposite strand. These linkages, called phosphodiester bonds, are strong covalent bonds formed between the phosphate group's phosphorus atom and an oxygen atom on the sugar molecule's 3' (three prime) carbon on one strand and the 5' (five prime) carbon on the sugar of the other strand (**Travers& Muskhelishvili, 2015; Carrol et al., 2015**). This specific 3'-5' phosphodiester bond orientation gives directionality to the DNA strands, which is crucial for DNA replication. The rungs of the ladder are formed by complementary pairs of nitrogenous bases (adenine with thymine and cytosine with guanine) that connect the two sugar-phosphate backbones

via hydrogen bonds. This elegant design allows DNA to be both stable due to the strong phosphodiester bonds and easily copied during cell division thanks to the specific base pairing held by hydrogen bonds (**Figure 6**) (Travers& Muskhelishvili, 2015; Carrol et al., 2015).

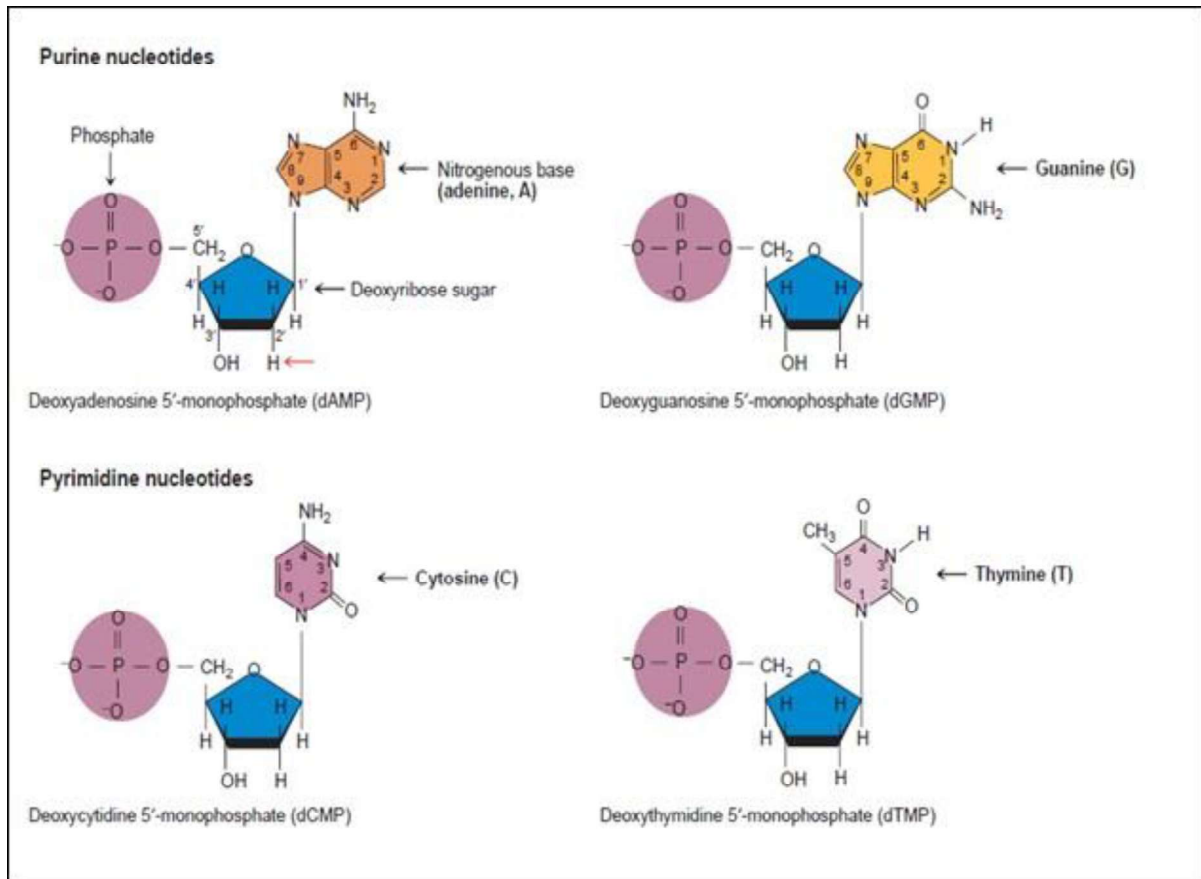


Figure 5. The purine and pyrimidine nucleotides of DNA molecule (Carrol et al., 2015).

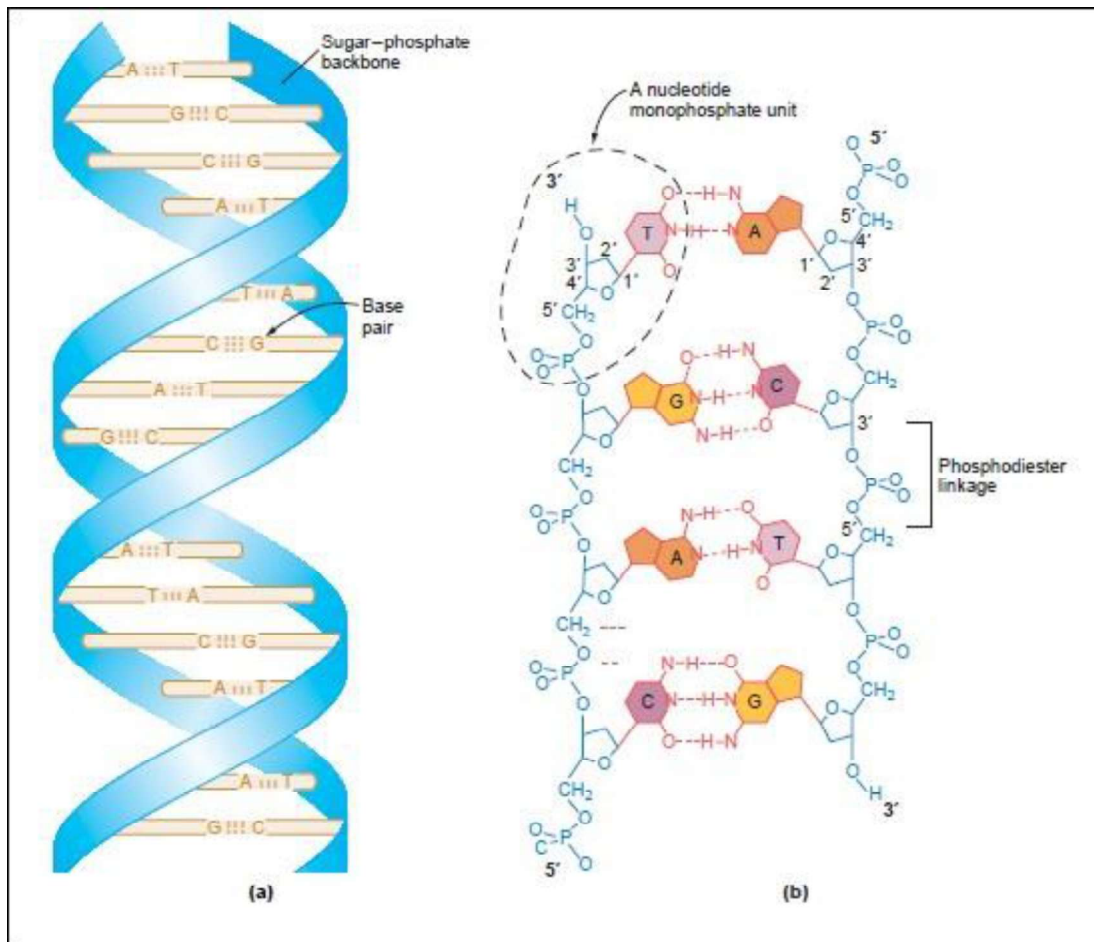


Figure 6. The structure of DNA. (a) A simplified model showing the helical structure of DNA. The sticks represent base pairs, and the ribbons represent the sugar-phosphate backbones of the two antiparallel chains. (b) An accurate chemical diagram of the DNA double helix, unrolled to show the sugar-phosphate backbones (blue) and base-pair rungs (red). The backbones run in opposite directions; the 5' and 3' ends are named for the orientation of the 5' and 3' carbon atoms of the sugar rings. Each base pair has one purine base, adenine (A) or guanine (G), and one pyrimidine base, thymine (T) or cytosine (C), connected by hydrogen bonds (Carrol et al., 2015).

Chromosomes

The DNA molecule in the nucleus of each cell is packaged into a complex structure called chromosomes, in each, DNA is tightly coiled many times around histone proteins, stabilizing its structure. They appear as rod shaped dark stained bodies during the metaphase stage of mitosis when cells are stained with a suitable basic dye and viewed under a light microscope (**Figure 7**). Chromosomes, the carriers of our genetic information in eukaryotic cells, are complex structures built around DNA. Each chromosome consists of a single, very long DNA molecule tightly coiled and packaged with protein molecules called histones. These histones are positively charged due to their abundance in lysine and arginine amino acids. This positive charge allows them to bind strongly to the negatively charged phosphate groups in the DNA backbone. While some non-histone proteins are present, histones are the main players in DNA packaging. Interestingly, these non-histone proteins are often transcription factors that regulate which parts of the DNA get turned into RNA. Most of the time, chromosomes exist in an elongated, decondensed state and are invisible under a microscope. However, during the S phase of cell division, they replicate their DNA entirely. As mitosis, cell division, approaches, these replicated chromosomes condense dramatically, becoming short and easily observable under a microscope after staining. These condensed, duplicated chromosomes are called sister chromatids because they are identical copies of the original chromosome, held together at a specialized region called the centromere (**Figure 8**).

The centromere itself is a fascinating structure composed of repetitive DNA sequences. These repetitive sequences are thought to play a role in chromosome movement during cell division. The attachment point for spindle fibers, which pull sister chromatids apart during cell division (anaphase), is called the kinetochore. Kinetochores are intricate structures made up of a staggering 80 different proteins. Finally, the two arms extending from the centromere have designated names: the shorter arm is the p arm, and the longer arm is the q arm. In summary, chromosomes are dynamic structures that transition between decondensed states for gene expression and condensed states for cell division. Histones play a key role in DNA packaging, while specialized regions like the centromere and kinetochore ensure accurate chromosome segregation during cell division (**Carrol et al., 2015**).

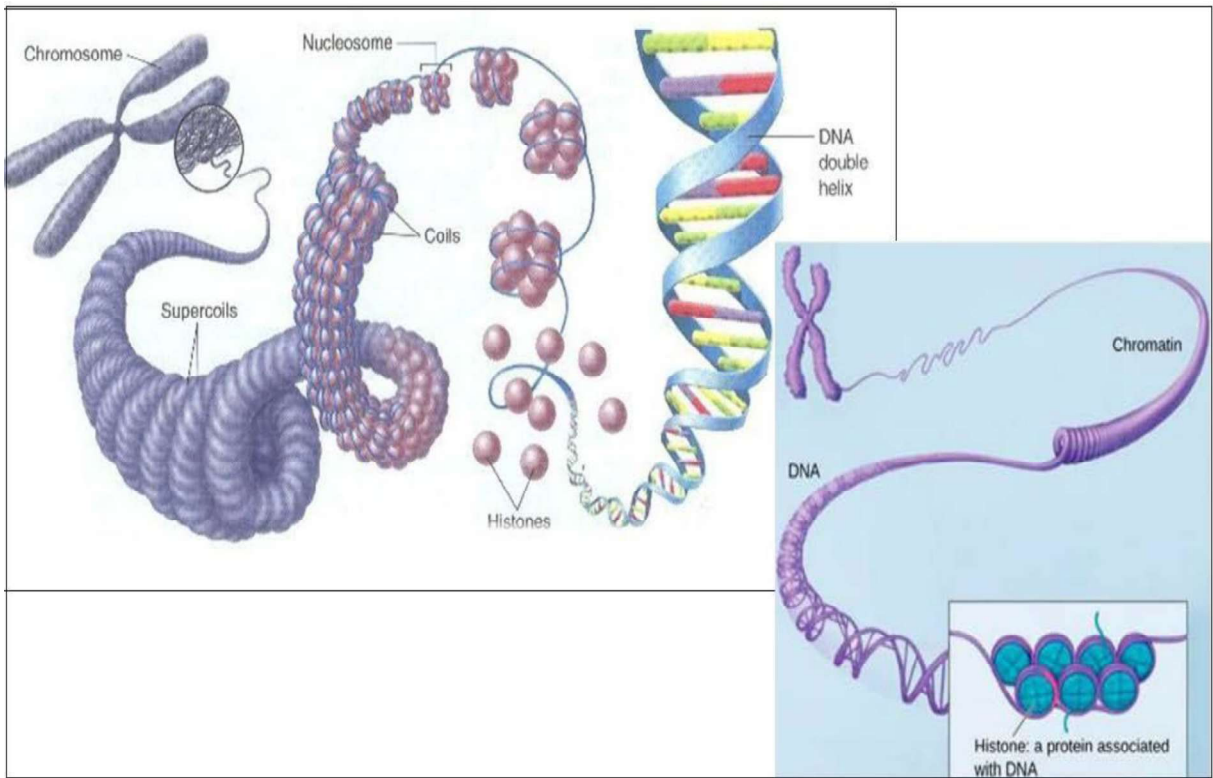


Figure 7. Structure of chromosomes

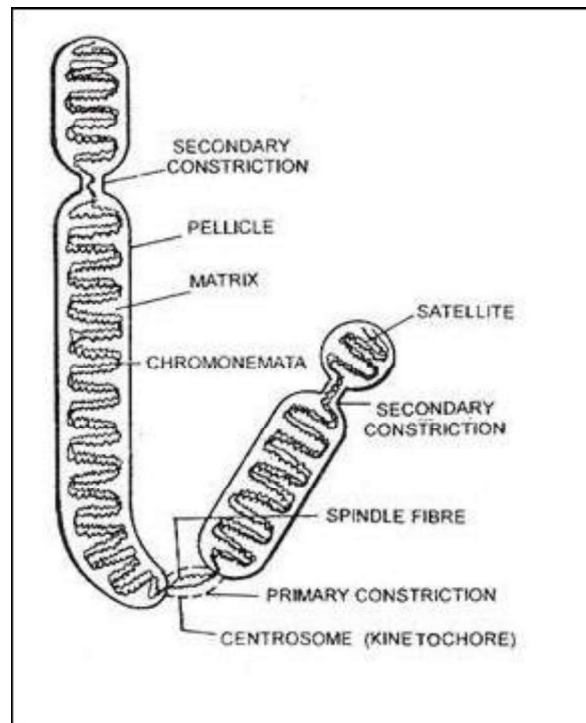


Figure 8. Structure of chromosome at anaphase stage of mitosis (Carrol et al., 2015).

Genetic codes

The genetic code is the cornerstone of life, the hidden language that translates the blueprint within our DNA into the functional building blocks of our cells – proteins. This intricate set of rules dictates how the sequence of nucleotides in DNA, the building blocks of DNA itself, is converted into the sequence of amino acids that make up proteins. Proteins, in turn, are the workhorses of the cell, carrying out a multitude of functions essential for life. The genetic codes are strongly related to the following molecule players:

- **DNA:** Deoxyribonucleic acid, the molecule that stores our genetic information. DNA is a double helix composed of two strands, each strand built from a sequence of nucleotides. There are four types of nucleotides in DNA, distinguished by their nitrogenous bases: adenine (A), guanine (G), cytosine (C), and thymine (T) (**Figure 6**).
- **RNA:** Ribonucleic acid, a single-stranded molecule that plays a crucial role in protein synthesis. There are also four types of nucleotides in RNA, but uracil (U) replaces thymine (T) in RNA. It exists in three major RNA molecules involved in protein synthesis, namely transfer RNA (tRNA) (**Figure 9a, b**), ribosomal RNA (rRNA) (**Figure 10**), and messenger RNA (mRNA) (**Figure 11**).
- **Codons:** These are the key elements of the genetic code. Codons are three-nucleotide sequences within DNA or RNA. Each codon acts as a specific instruction, dictating the incorporation of a particular amino acid into a growing protein chain.
- **Amino Acids:** The building blocks of proteins. There are 20 different amino acids, each with a unique side chain that gives it specific chemical properties. The order and combination of amino acids determine the unique structure and function of a protein.

Cracking the Code: The genetic code is a triplet code, meaning that every three nucleotides (codon) specifies an amino acid. The order of these nucleotides is crucial, as even a single change can alter the encoded message and potentially lead to a malfunctioning protein. For instance, the codon AUG

codes for the amino acid methionine, which typically initiates protein synthesis (Figure 12).

The Flow of Information:

The process of translating the genetic code into proteins is known as protein synthesis. It primarily occurs in the ribosomes, cellular factories dedicated to protein construction. Messenger RNA (mRNA) carries the genetic information from DNA in the nucleus to the ribosomes in the cytoplasm. mRNA codons are "read" by transfer RNA (tRNA) molecules, each of which carries a specific amino acid and an anticodon that can bind to a complementary codon on the mRNA. The ribosome orchestrates the linking of amino acids based on the mRNA sequence, forming the growing protein chain.

The Code's Impact:

Understanding the genetic code has revolutionized our understanding of biology and medicine. It allows scientists to study how mutations in genes can lead to genetic diseases, develop diagnostic tools, and even engineer new proteins with desired functionalities. The genetic code is a testament to the elegance and efficiency of life's processes, a language that continues to inspire awe and hold the key to future advancements in healthcare and biotechnology.

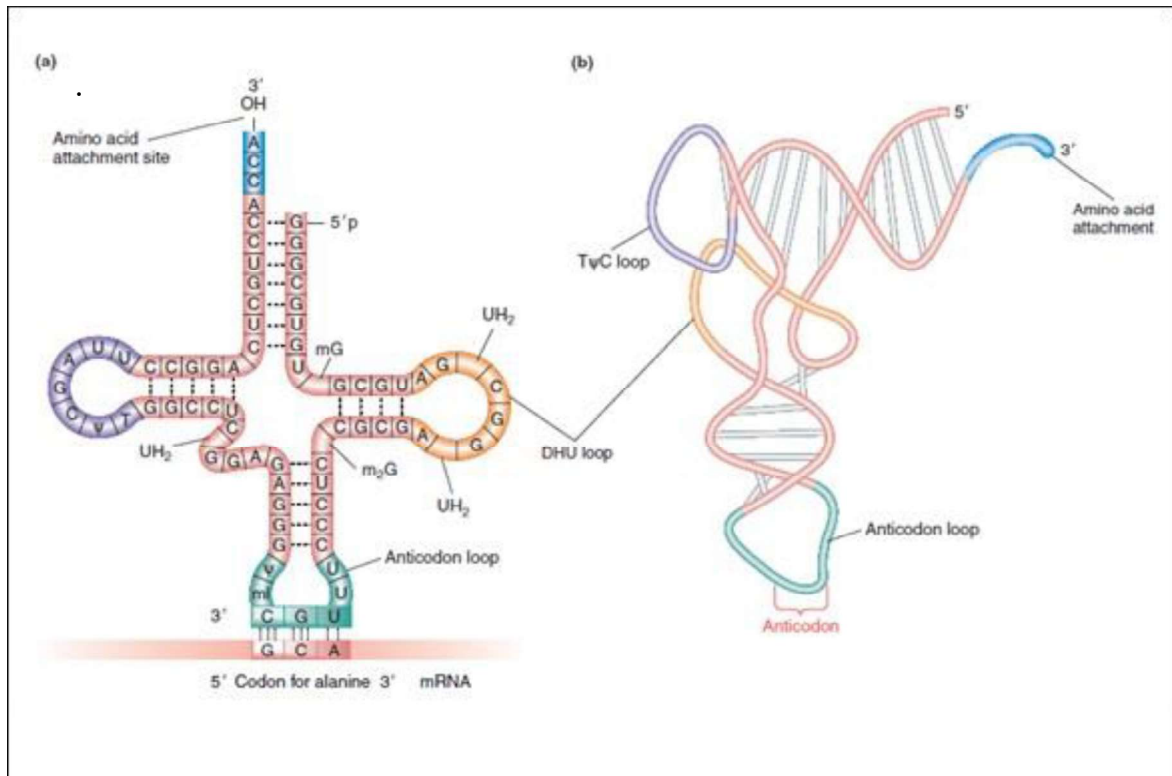


Figure 9. The structure of transfer RNA. (a) The structure of yeast alanine tRNA, showing the anticodon of the tRNA binding to its complementary codon in mRNA. (b) Diagram of the actual three-dimensional structure of yeast phenylalanine tRNA (Carrol et al., 2015).

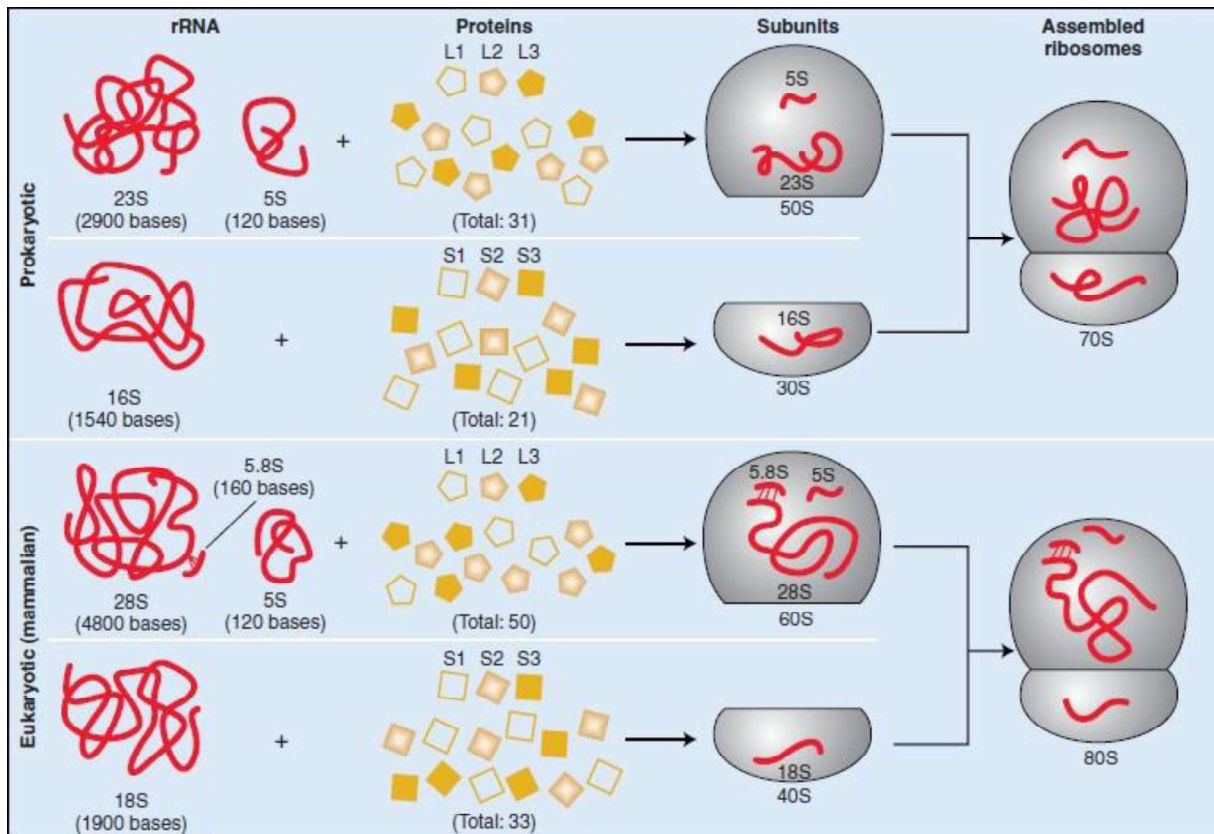


Figure 10. A ribosome contains a large and a small subunit. Each subunit contains both rRNA of varying lengths and a set of proteins. There are two principal rRNA molecules in all ribosomes (shown in the column on the left). Prokaryotic ribosomes also contain one 120-base-long rRNA that sediments at 5S, whereas eukaryotic ribosomes have two small rRNAs: a 5S RNA molecule similar to the prokaryotic 5S, and a 5.8S molecule 160 bases long. The proteins of the large subunit are named L1, L2, and so forth, and those of the small subunit S1, S2, and so forth (Carrol et al., 2015).

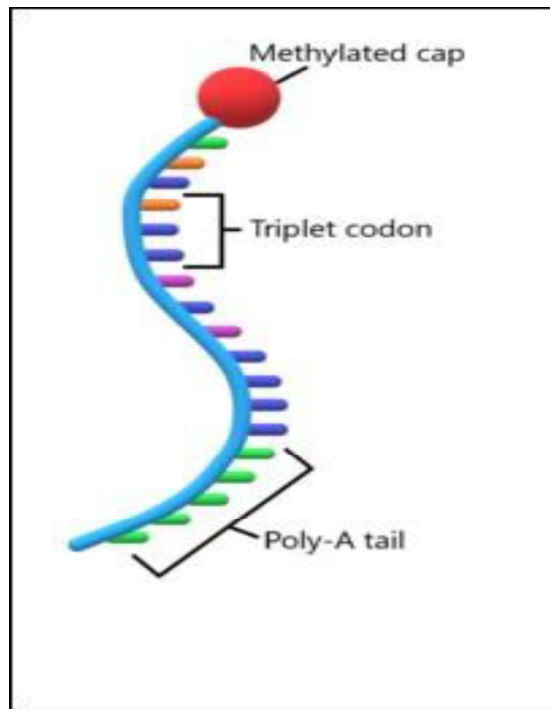


Figure 11. A mature messenger RNA of Eukaryotic cells, poly adenine tail at the end 3', and methylated cap at the end 5' (Sonenberg, 1988).

		Second letter				
		U	C	A	G	
First letter	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA } Stop UAG } Stop	UGU } Cys UGC } UGA } Stop UGG } Trp	U C A G
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G
	A	AUU } AUC } Ile AUA } AUG } Met	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G
						Third letter

Figure 12. The genetic code.

5. Cell Cycle and Cell Division

The cell cycle is a series of stages that cells go through to grow and divide. It is composed of four main phases: G1 (Gap 1), S (Synthesis), G2 (Gap 2), and M (Mitosis). Cells spend most of their time in interphase, which includes G1, S, and G2 phases, where they grow, replicate their DNA, and prepare for division.

Cell cycle

The cell cycle includes the interphase, including following 4 phases (**Mir and Jan, 2023; Lopez et al., 2022**):

- **G1 Phase (Gap 1):** During the G1 phase, the cell grows and synthesizes proteins and organelles. It is a period of intense biochemical activity where the cell increases in size and prepares the necessary machinery for DNA replication. Cells can also enter a resting state known as G0 if they are not ready to proceed to the next phase .
- **S Phase (Synthesis):** In the S phase, DNA replication occurs, resulting in the duplication of the cell's genetic material. Each chromosome now consists of two sister chromatids held together by a centromere. This ensures that each daughter cell will receive an identical set of chromosomes.
- **G2 Phase (Gap 2):** The G2 phase is a second period of growth and preparation for mitosis. The cell continues to grow and produces proteins and organelles needed for cell division. It also undergoes critical checks to ensure that DNA replication has been completed successfully and that the cell is ready to enter mitosis (**Mir and Jan, 2023; Lopez et al., 2022**):

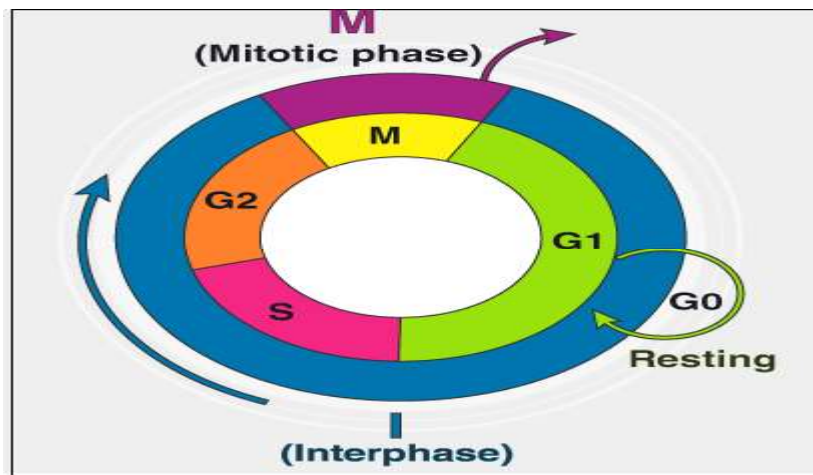


Figure 13. Cell cycle (Miller, 2020)

Cell division

- **M Phase (Mitosis):** Mitosis is the process where the cell's nucleus divides, followed by the division of the cytoplasm (cytokinesis). Mitosis itself is divided into several stages: prophase, metaphase, anaphase, and telophase. During these stages, the replicated chromosomes are aligned, separated, and distributed into two new nuclei
- **Prophase:** During prophase, chromatin condenses into visible chromosomes, the nuclear envelope breaks down, and the mitotic spindle begins to form. The spindle fibers attach to the centromeres of the chromosomes, which start to move towards the metaphase plate.
- **Metaphase:** In metaphase, the chromosomes align at the cell's equatorial plane, known as the metaphase plate. This alignment ensures that each daughter cell will receive one copy of each chromosome during cell division.
- **Anaphase:** During anaphase, the sister chromatids are pulled apart by the spindle fibers and move toward opposite poles of the cell. This separation ensures that each new cell will have an identical set of chromosomes.
- **Telophase and Cytokinesis:** In telophase, the separated chromatids reach the opposite poles of the cell, and new nuclear membranes form around each set of chromosomes. The chromosomes begin to de-condense back into chromatin.

Cytokinesis, the division of the cytoplasm, occurs concurrently or soon after telophase, resulting in two distinct daughter cells (Mir and Jan, 2023; Lopez et al., 2022) (Figure 14).

Regulation of the Cell Cycle:

The cell cycle is tightly regulated by a series of checkpoints and proteins, such as cyclins and cyclin-dependent kinases (CDKs). These checkpoints ensure that each stage of the cell cycle is completed accurately before the cell progresses to the next stage. Errors in regulation can lead to uncontrolled cell division and are often associated with cancer (Lopez et al., 2022).

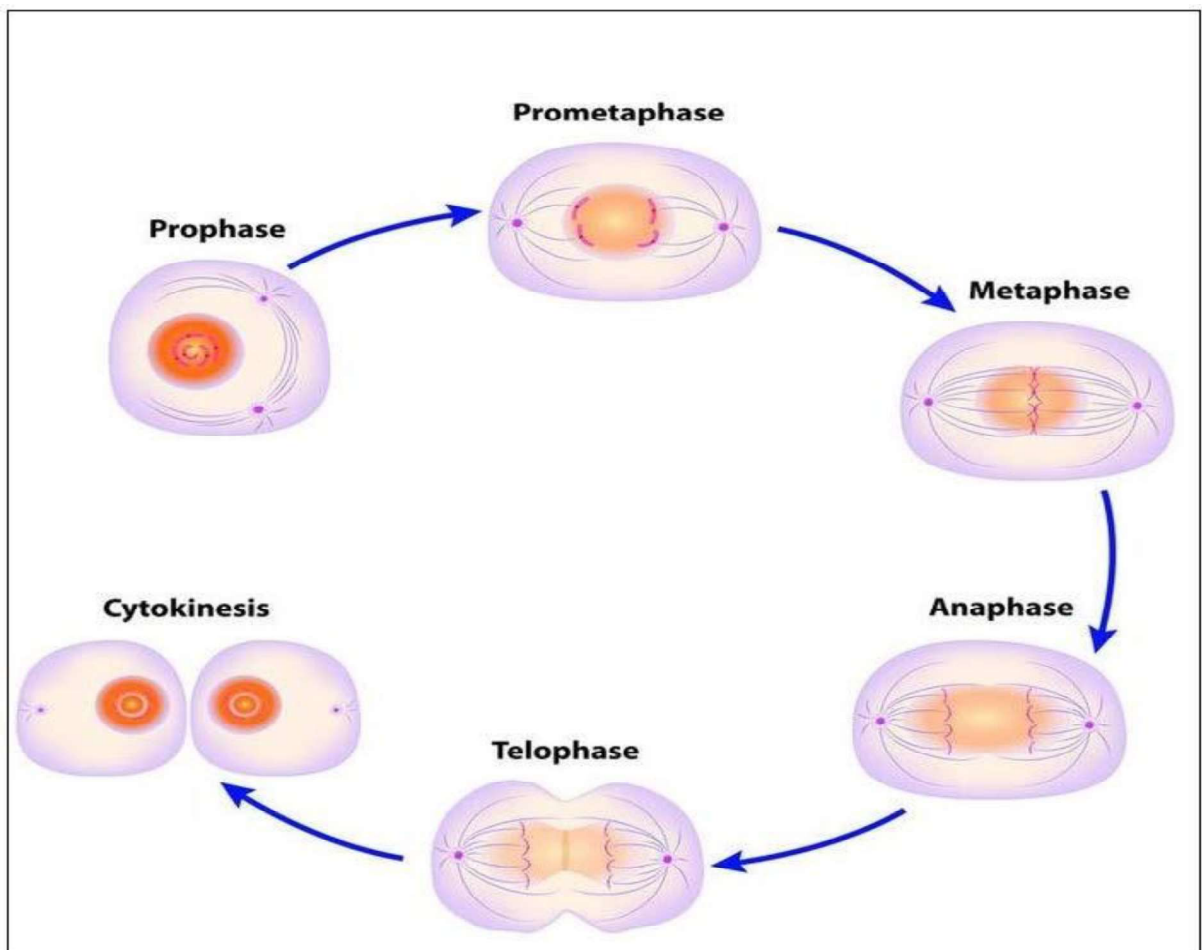


Figure 14. Cell division (mitosis) (Miller, 2020)

6. Methods of Studying Cells

Light microscope

Light microscopy is a technique that uses visible light and a system of lenses to generate magnified images of small objects. It is a core technique in many areas of science and technology, including life sciences, biology, materials sciences, nanotechnology, industrial inspection, and forensics.

The basic working principle of light microscopy involves using lenses to refract and focus light, which then interacts with the sample and forms a magnified image. The sample is placed on a stage and illuminated from below. The objective lens collects the light that has passed through or been reflected by the sample and forms an enlarged image. This image is further magnified by the eyepiece lens and can be observed directly or captured by a camera.

There are several types of light microscopy techniques, each with its own advantages and applications (Lacey, 1999; Thom, 2016):

- ✓ **Bright field microscopy:** The most basic technique, producing a dark image against a bright background. Widely used to view plant and animal cell organelles and some parasites after staining.
- ✓ **Dark field microscopy:** Reveals small structures and surface roughness, allowing imaging of unstained samples.
- ✓ **Phase contrast microscopy:** Enables imaging of transparent samples by enhancing contrast.
- ✓ **Fluorescence microscopy:** Allows individual fluorophores and particular areas of interest in a sample to be singled out, overcoming the resolution limit.
- ✓ **Confocal microscopy:** Provides low background signal and the ability to create 3D images by optical sectioning (Lacey, 1999; Thom, 2016).

The choice of technique depends on the specific sample and the information needed. Light microscopy has a resolution limit due to the wavelength of visible light, typically around 200 nm. Techniques like electron microscopy can achieve much higher resolutions but require more complex sample preparation and instrumentation (Lacey, 1999; Thom, 2016) (Figure 15).

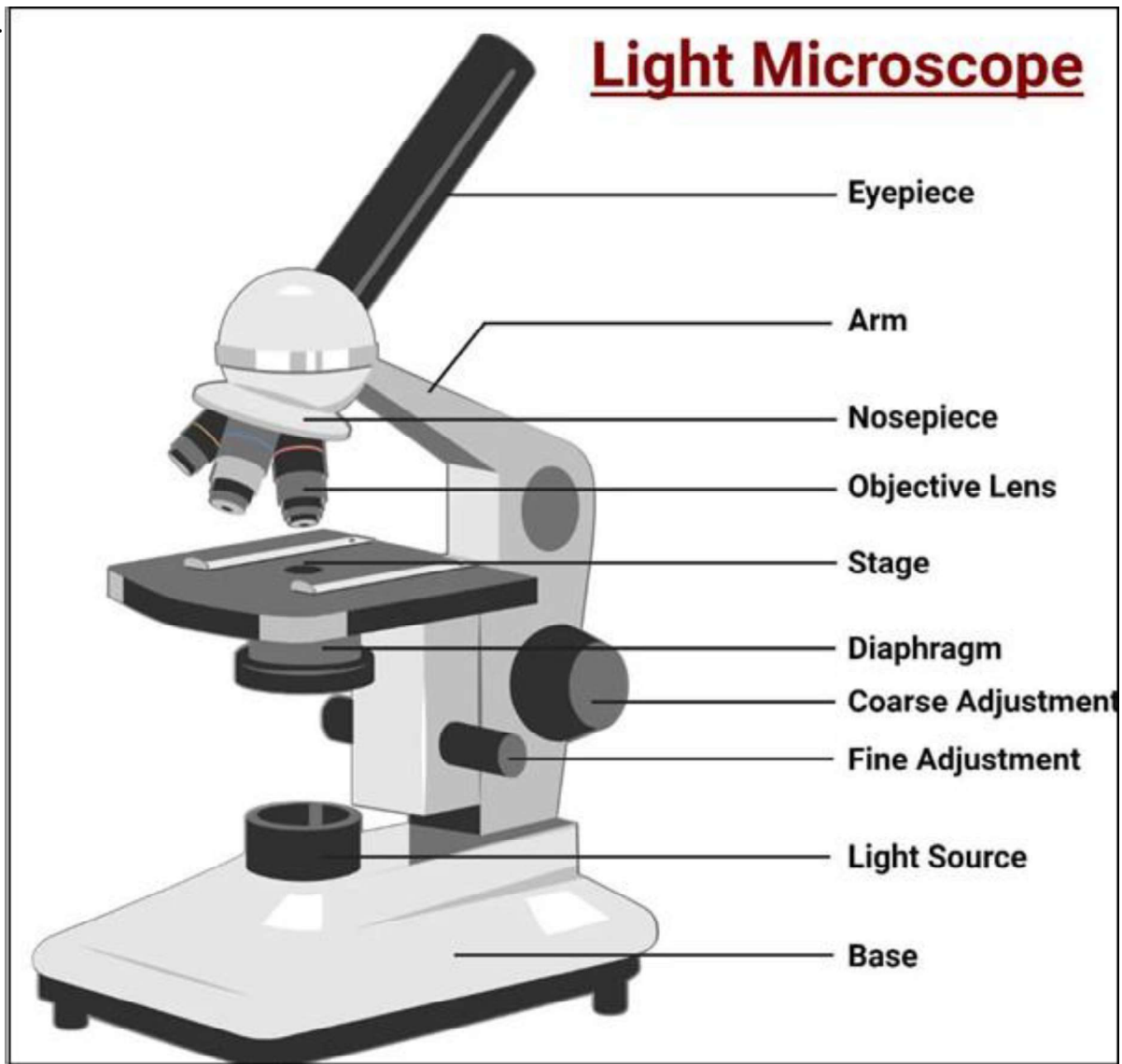


Figure 15. Light microscope (Thom, 2016)

Electronic microscope

An electronic microscope, also known as an electron microscope, is a powerful tool that uses a beam of electrons instead of visible light to illuminate samples, allowing for extremely high-resolution imaging. Electron microscopes offer much higher resolution compared to light microscopes, typically around 0.1 nm compared to 200 nm for light microscopes. There are several types of electron microscopes, including Transmission Electron Microscopy (TEM), Scanning Transmission Electron Microscopy (STEM), Scanning Electron Microscopy (SEM), and others, each with specific applications and advantages (**Figure 16**) (**Seiler, 1983**)

- ✓ **Transmission Electron Microscope (TEM):** Utilizes a high voltage electron beam to illuminate thin samples, allowing for high-resolution imaging and analysis of internal structures at the atomic level.
- ✓ **Scanning Transmission Electron Microscope (STEM):** Similar to TEM but with a scanned electron probe, enabling high-resolution imaging and analytical techniques.
- ✓ **Scanning Electron Microscope (SEM):** Produces images by scanning the surface of a sample with a focused beam of electrons, providing detailed information about surface topography and composition.

Electron microscopes are essential in various fields, including materials science, biology, nanotechnology, and industrial research, due to their ability to visualize structures at the nanoscale with exceptional detail and resolution.

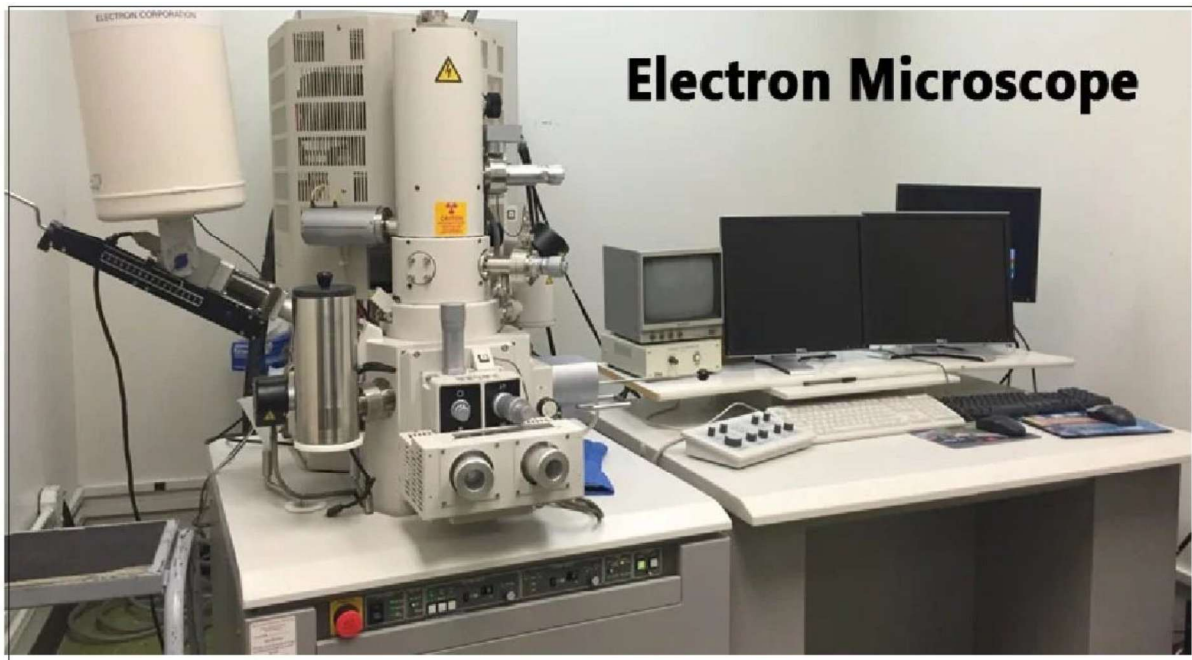


Figure 16. Electron microscope (Seiler, 1983)

7. Biological staining

Biological staining techniques are methods used to enhance the visibility of biological samples under a microscope. These techniques involve the use of chemical dyes or other substances that bind to specific structures or molecules within the sample, allowing for better visualization and analysis. Here are some common biological staining techniques (Horobin, 2002; PENNEY et al., 2002).

- ✓ **Hematoxylin and Eosin (H&E) Staining:** A standard staining technique used to differentiate between different types of tissue in histology. Hematoxylin stains the nucleus blue, while eosin stains the cytoplasm pink.
- ✓ **Giemsa Staining:** Used to stain blood smears and detect parasites like malaria. It stains the nucleus blue and the cytoplasm pink.
- ✓ **Fluorescence Staining:** Uses fluorescent dyes to bind to specific molecules or structures within the sample. This allows for the visualization of specific biological processes or structures under a fluorescence microscope.
- ✓ **Immunofluorescence Staining:** A technique that uses antibodies to bind to specific proteins or antigens within the sample, followed by the application of fluorescent dyes to visualize these bindings.

-
- ✓ **In Situ Hybridization (ISH) Staining:** A method that uses nucleic acid probes to detect specific RNA or DNA sequences within the sample. This is often used to study gene expression or detect viral infections.
 - ✓ **Acid Fuchsin Staining:** Used to stain bacteria and other microorganisms. It stains the cell walls red or pink.
 - ✓ **Gram Staining:** A technique used to differentiate between Gram-positive and Gram-negative bacteria. Gram-positive bacteria retain the stain, while Gram-negative bacteria do not.
 - ✓ **Methylene Blue Staining:** Used to stain bacteria and other microorganisms. It stains the cell walls blue.

8. Centrifugation and ultracentrifugation

Centrifugation and ultracentrifugation are two closely related techniques used to separate particles in a liquid medium based on differences in size, shape, density, and viscosity. The main difference between the two is the speed at which they operate.

Centrifugation uses a lower speed, typically up to 15,000 rpm, to separate coarse precipitates or whole cells. It is a widely used technique in biochemical research and applications, such as isolating cells, subcellular fractions, and supramolecular complexes. Bench centrifuges can develop centrifugal fields of 3,000-7,000 g and accommodate various containers and plates (**Figure 17**).

Ultracentrifugation, on the other hand, uses much higher speeds, up to 150,000 rpm, to separate very fine particles dispersed in a liquid medium. It can achieve centrifugal forces of up to 1,000,000 g. Ultracentrifugation is used for both analytical and preparative purposes:

- ✓ Analytical ultracentrifugation studies the molecular interactions and properties of purified macromolecules or isolated supramolecular assemblies.
- ✓ Preparative ultracentrifugation isolates and purifies particles using techniques like density gradient centrifugation.
- ✓ Ultracentrifuges operate in a vacuum to avoid overheating of samples and require proper rotor selection and maintenance for safe and effective use.

Both centrifugation and ultracentrifugation are essential techniques in various fields, including biochemistry, molecular biology, and polymer science. They enable the separation and analysis of complex biological samples, contributing to our understanding of cellular processes and the development of new applications in medicine and research (**Figure 18**).



Figure 17. Centrifugation apparatus



Figure 18. Ultracentrifugation apparatus

9. Autoradiography technique

Autoradiography is a bioanalytical technique used to visualize the distribution of radioactive substances within biological samples. It involves placing a radioactive sample in direct contact with a photographic emulsion, which is then exposed to the radiation. The radiation causes the silver halide crystals in the emulsion to reduce, resulting in a dark color band that can be viewed under a microscope or other imaging tools. Autoradiography has various applications in biology, including:

- ✓ **Localization of Radioactive Substances:** It helps determine the localization of radioactive substances within tissues or cells, which can be useful in understanding metabolic pathways, receptor distribution, and other biological processes.
- ✓ **Cell Cycle Analysis:** Radioactive precursors of DNA or RNA can be introduced into living cells to study the timing of different phases of the cell cycle.
- ✓ **Hormonal Production and Metabolic Pathways:** Autoradiography can be used to study hormonal production and other metabolic pathways by introducing radioactive precursors into the metabolic pathway.
- ✓ **Receptor Autoradiography:** This technique involves using radiolabeled ligands to determine the tissue distribution of receptors, which is useful in understanding the binding of drugs to receptors.
- ✓ **In Situ Hybridization:** Autoradiography can be used to locate specific RNA transcripts in tissue sections by using radiolabeled, complementary oligonucleotides or ribonucleic acids.

Autoradiography is a powerful tool in various biological and medical applications, enabling researchers to visualize and analyze the distribution of radioactive substances within biological samples.

Conclusion

This journey through cell biology has hopefully ignited your curiosity about the fundamental building blocks of life. We have explored the intricate structures within cells, from the membranous envelope that guards their integrity to the bustling machinery that orchestrates their functions. We have unraveled the secrets of the genetic code, the blueprint that dictates cellular form and function, and witnessed the awe-inspiring processes of cell division that ensure life's continuity.

However, the world of the cell is far from static. We stand at the threshold of exciting discoveries – the potential for cell-based therapies, the manipulation of cellular processes to combat diseases, and the ongoing quest to understand the very origins of life itself. The knowledge you have gained in this book provides a springboard for further exploration, equipping you to delve deeper into these fascinating frontiers.

Remember, cells are not just the building blocks of life; they are the very essence of it. They are the dynamic units that carry out the processes that define living organisms – growth, reproduction, adaptation, and the ability to respond to stimuli. As you continue your scientific journey, may you carry this newfound appreciation for the microscopic marvels that underpin the grand narrative of life?

References

- Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2015). *Molecular Biology of the Cell*. New York: Garland Science. *Taylor and Francis Group*, 4, 973-975.
- Carroll, S. B., Doebley, J., Griffiths, A. J., & Wessler, S. R. (2015). *Introduction to genetic analysis*. WH Freeman.
- Clément, P. (2007). Introducing the cell concept with both animal and plant cells: A historical and didactic approach. *Science & Education*, 16, 423-440.
- Cohen, B. J., & Hull, K. L. (2020). *Memmler's the human body in health and disease*. Jones & Bartlett Learning.
- Cooper, G. M., & Hausman, R. E. (2018). *The Cell: A Molecular Approach*, Eight Edition.
- Ford, B J. On intelligence in cells: the case for whole cell biology. *Interdisciplinary Science Reviews*, 2009, vol. 34, no 4, p. 350-365.
- Horobin, R. W. (2002). Biological staining: mechanisms and theory. *Biotechnic & histochemistry*, 77(1), 3-13.
- Joklik, W. K., & Pierce, M. M. (2014). *Virus-Access Science from McGraw-Hill Education* [http://www. Access science. com/content/virus/733500](http://www.Accessscience.com/content/virus/733500).
- Karp, G. (2009). *Cell and molecular biology: concepts and experiments*. John Wiley & Sons.
- Lacey, A. J. (Ed.). (1999). *Light microscopy in biology: a practical approach* (Vol. 195). OUP Oxford.
- Lanza, R., Gearhart, J., Hogan, B., Melton, D., Pedersen, R., Thomas, E. D., & Thomson, J. A. (Eds.). (2005). *Essentials of stem cell biology*. Elsevier.
- Lopez, E. N. B., Marañón, C. M. B., Origenes, R. W., Pizaña, A. D., Salmo, J. M., & Picardal, J. P. (2022). Content analysis of cell division concepts in Senior High School Biology textbooks. *JPBI (Jurnal Pendidikan Biologi Indonesia)*, 8(2), 159-177.
- Madigan, M. T., Martinko, J. M., Bender, K. S., Buckley, D. H., & Stahl, D. A. (2014). *Brock biology of microorganisms*. 14th Edn Pearson Education.
- Madigan, M. T., Martinko, J. M., Bender, K. S., Buckley, D. H., & Stahl, D. A. (2014). *Brock biology of microorganisms*. 14th Edn Pearson Education.

Miller, C. (2020). 4.12 Cell Cycle and Cell Division. *Human Biology-Excerpts for BBIO 053*.

Mir, M. A., & Jan, A. (2023). Introduction to Cell Cycle and Its Regulators. In *Therapeutic potential of Cell Cycle Kinases in Breast Cancer* (pp. 53-82). Singapore: Springer Nature Singapore.

Parija, S. C. (2023). Microscopy. In *Textbook of Microbiology and Immunology* (pp. 21-25). Singapore: Springer Nature Singapore.

PENNEY, D. P., POWERS, J. M., FRANK, M., *et al.* Analysis and testing of biological stains--the Biological Stain Commission Procedures. *Biotechnic & histochemistry*, 2002, vol. 77, no 5-6, p. 237-275.

Pollard, T. D., Earnshaw, W. C., Lippincott-Schwartz, J., & Johnson, G. (2022). *Cell Biology E-Book: Cell Biology E-Book*. Elsevier Health Sciences.

Pollard, T. D., Earnshaw, W. C., Lippincott-Schwartz, J., & Johnson, G. (2022). *Cell Biology E-Book: Cell Biology E-Book*. Elsevier Health Sciences.

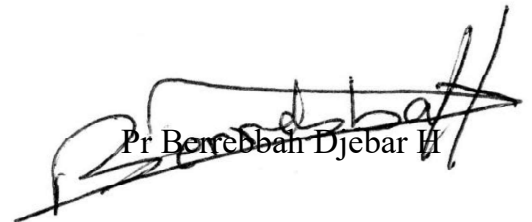
Seiler, H. (1983). Secondary electron emission in the scanning electron microscope. *Journal of Applied Physics*, 54(11), R1-R18.

Thorn, K. (2016). A quick guide to light microscopy in cell biology. *Molecular biology of the cell*, 27(2), 219-222.

Tortora, G. J., Funke, B. R., & Case, C. L. (2016). *Microbiology-An Introduction 12th Edition*. K. Churchman, ed.

Travers, A., & Muskhelishvili, G. (2015). DNA structure and function. *The FEBS journal*, 282(12), 2279-2295.

Sonenberg, N. (1988). Cap-binding proteins of eukaryotic messenger RNA: functions in initiation and control of translation. *Progress in nucleic acid research and molecular biology*, 35, 173-207.



Pr. Benrebhan Djebar