University Abderrahmane MIRA-BEJAIA
Natural and Life Sciences Faculty
Department of Biological and Environmental Sciences
Applied Zoology and Animal Ecophysiology Laboratory

جامعة بجاية
Tasdawit n Bgayet
Université de Béjaïa

abdelmadjid.chelli@univ-bejaia.dz
https://orcid.org/0000-0001-9133-2761

Essential Cell Biology

Teaching Manuel

Presented by Dr CHELLI A. Madjid

FOREWORD

This course in Cytology has been specifically designed for students in Biomedical Sciences, while remaining a valuable resource for learners in other areas of Biological Sciences. It aims to provide a clear, structured, and accessible foundation in cell biology, enabling students to build a solid understanding of the fundamental principles that govern cellular structure and function.

Inspired by the organization of Essentials of Cell Biology, this course is divided into twelve units that guide students through key concepts of modern cytology. These units cover the basics of cell theory, the principal methods used to study cells, and the structural organization of both prokaryotic and eukaryotic cells. Special attention is given to the nature of viruses and their unique biological characteristics.

Furthermore, the course explores the ultrastructure of the cell membrane and delves into the major cellular organelles, including the nucleus, mitochondria, ribosomes, endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes, and the cytoskeleton, highlighting their architectures and essential functions.

By integrating fundamental knowledge with detailed cellular mechanisms, this course aims to support biomedical students in developing the scientific background required for their future academic and professional endeavors, while remaining a useful reference for all students in the life sciences.

Recommandations :

- There's no point in trying to learn a course before you've understood it.
- Learning by heart is generally an unnecessary and inefficient task.
- There are many different learning methods. Each student must find (invent) the one that suits them best.

« We make science out of facts like a house out of stones, but an accumulation of facts is no more a science than a pile of stones is a house. »

Henri Poincaré

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CHAPTER I : INTRODUCTION TO CELL BIOLOGY

Lesson Objectives

By the end of this lesson, students will be able to:

- *Identify the scientists that first observed cells.*
- *Outline the importance of microscopes in the discovery of cells.*
- *Summarize what the cell theory proposes.*
- *Identify the limitations on cell size.*

Introduction

Cell biology also known as cytology. It deals with cells and its organelles. Cell, in biology, the basic membrane-bound unit that contains the fundamental molecules of life and of which all living things are composed. A single cell is often a complete organism, such as a bacterium or yeast. The cell is the structural and functional unit of all living organisms and is sometimes called the "building block of life." All living things are made from one or more cells. A cell is the simplest unit of life and they are responsible for keeping an organism alive and functioning. Almost every different type of cell contains genetic material, a membrane and cytoplasm. The most basic categorization of Earth's organisms is determined by different types of cells. All cells can be divided into one of two classifications : prokaryotic cells and eukaryotic cells. Prokaryotic cells are found in bacteria and archaea. Eukaryotic cells are found in organisms from the domain Eukaryota which includes animals, plants, fungi and protists. Cell metabolism is the process by which individual cells process nutrient molecules. Metabolism has two distinct divisions : catabolism, in which the cell breaks down complex molecules to produce energy and reducing power, and anabolism, in which the cell uses energy and reducing power to construct complex molecules and perform other biological functions. Cells were discovered by Robert Hooke in 1665, who named them for their resemblance to cells inhabited by Christian monks in a monastery. Cell theory, first developed in 1839 by Matthias Jakob Schleiden and Theodor Schwann, states that all organisms are composed of one or more cells, that cells are the fundamental unit of structure and function in all living organisms, and that all cells come from pre-existing cells. Cells emerged on Earth at least 3.5 billions years ago. The study of cells is called cell biology or cellular biology.

Cell biology is therefore an important discipline that has made it possible to observe and study cells for decades. It has become particularly important for differentiating and determining different cell types and cellular processes, as well as for understanding the various diseases and disorders associated with cell malfunction.

In biomedical research, cell biology is used to find out more about how cells normally work, and how disturbances in this normal function can result in disease

1. Definitions

Biology : is the science of life. Its name is derived from the Greek words "bios" (life) and "logos" (study). Biologists study the structure, function, growth, origin, evolution and distribution of living organisms.

Cell biology : (also called cytology, from the Greek kytos, "vessel") is a branch of biology that studies the structure and function of the cell, which is the basic unit of life. Cell biology is concerned with the physiological properties, metabolic processes, signalling pathways, life cycle, chemical composition and interactions of the cell with their environment. Research in cell biology is closely related to genetics, biochemistry, molecular biology, immunology and cytochemistry.

2. Discovery of cell and Cell Theory

The discovery of cells followed from the invention of the microscope by Robert Hooke, and its refinement by Anton Leewenhoek. Robert Hooke was the first to discover and name the cell in 1665. He remarked that it looked strangely similar to cellular or small rooms which monks inhabited, thus depriving the name. However, what Hooke truly saw under the microscope were the dead cell walls of plant cells (cork). Anton Van Leeuwenhoek was the first to examine live cells in his study of algae a few years later, in 1674. Leeuwenhoek studied the tiny organisms under the microscope and named them ‘animalcules’, which included protozoa and other unicellular organisms, like bacteria. Antony van Leeuwenhoek is regarded as the ‘Father of Microbiology’. He is known for the discovery of bacteria. All of this came before the cell hypothesis, which argues that cells make up all living things and that cells are the functional and structural unit of organisms. In 1838, plant scientist Matthias Schleiden and animal scientist Theodor Schwann observed live cells in plant and animal tissue, respectively, and came to this conclusion. Rudolf Virchow added to the cell hypothesis 19 years later, statAnton Van Leeuwenhoek was the first to examine live cells in his study of algae a few years later, in 1674. ing that all cells arise through the division of pre-existing cells.

• *English scientist* *looked at a thin slice of cork (oak cork) through a compoundmicroscope* *observed tiny, hollow, room-like structures* *called these structures 'cells' because they reminded him of the rooms that monks lived in* *only saw the outer walls (cell walls) because cork cells are not living*	 ***Robert Hooke (1665)***
• *Dutch fabric merchant and amateur scientist* *looked at blood, rainwater, scrapings from teeth through a simple microscope of one lens* *observed living cells; called some 'animalcules'* *some of the small 'animalcules' are now called bacteria.*	 ***A.V. Leeuwenhoek (1674)***
• *German botanist* *viewed plant parts under a microscope* *discovered that plant parts are made of cells.*	 ***Matthias Schleiden (1838)***
• *German zoologist* *viewed animal parts under a microscope* *discovered that animal parts are made of cells*	 ***Theodor Schwann (1839)***
• *German physician* *stated that all living cells come only from other living cells.*	 ***Rudolph Virchow (1855)***

Cell Theory is one of the basic principles of biology, Credit for the formulation of this theory is givento German scientists Theodor Schwann, Matthias Schleiden, and Rudolph Virchow. The cell theory formulates the following three principles :

- ❖ All living organisms are composed of one or more cells.
- ❖ The cell is the most basic unit of life.
- ❖ All cells arise only from pre-existing cells.

4. Fundamentals of Cell

The cell is the fundamental unit of life. All living organisms on planet Earth are composed unicellular (single cell) or multicellular (many cells). A cell may be defined as a unit of protoplasm bounded by a plasma or cell membrane and possessing a nucleus. Protoplasm is the life giving substance and includes the cytoplasm and the nucleus. The cytoplasm has in it organelles, such as Ribosomes, Mitochondria, Golgi Bodies, Plastids, Lysosomes and Endoplasmic Reticulum. Plant cells have in their cytoplasm large vacuoles containing non-living inclusions like crystals, pigments, etc. The bacteria have neither organelles nor a well formed nucleus. But the basic components and functions of the cell are common to all cells. There are many different types of cells, but all cells have a few things in common. These are :

• plasma membrane
• cytoplasm
• ribosomes for protein synthesis
• DNA (genetic information)

In addition, these cells all have common abilities, such as getting and using food energy, responding to the external environment, and reproducing. A cell's shape determines its function.

Basically, the cells form tissues and multiple tissues make up an organ, different organs create an organ system, such as digestive system, respiratory system, circulatory system, nervous system, etc., to perform specific functions in the human body and any other living organism. Therefore, Cells → Tissue → Organ → System → Organism/Human Body

5. Cell shape and size

Cells are commonly measured in units of micrometers (1 μm = 10^{-6} meter) and nanometers (1 nm = 10^{-9} meter).

There are many cells in an individual, which performs several functions throughout the life. Cells differ greatly in size, shape and activities. Cells range in its size from a millimeter to microns and generally varies in their shapes. Few cells are flat, oval, rod, curved, spherical, concave, rectangular (Fig.1). Most of the cells are microscopic in size and can only be seen under the microscope. Some cells are fairly long and large. For example, Mycoplasmas, the smallest cells, are only 0.3 µm in length while bacteria could be 3 to 5 µm. The largest isolated single cell is the egg of an ostrich. Among multicellular organisms, human red blood cells are about 7.0 µm in diameter. Nerve cells are some of the longest cells. Cells also vary greatly in their shape. They may be disc-like, polygonal, columnar, cuboid, thread like, or even irregular. The shape of the cell may vary with the function they perform.

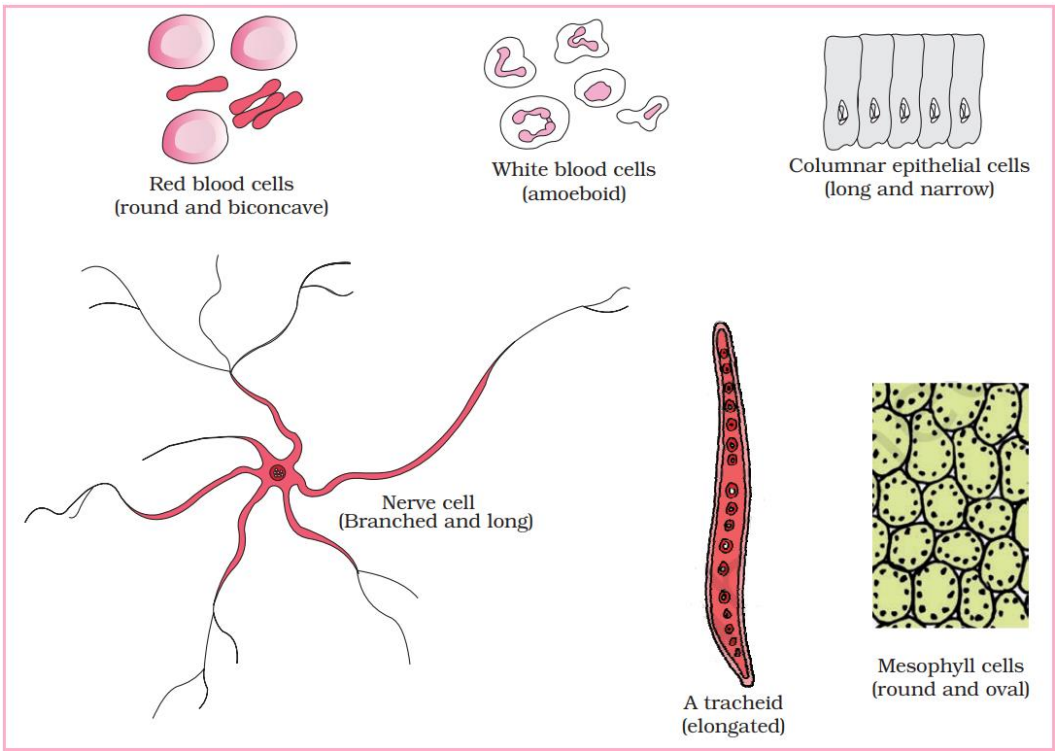


Figure1. Diagram showing different shapes of the cells

Scale of Measurements

1 centimeter (cm) = 10 millimeters (mm) = 10^{-2} meters (m)
1 mm = 1000 micrometers (µm) = 10^{-3} m
1 µm = 1000 nanometers (nm) = 10^{-6} m
1nm = 10^{-3} µm

6. The Classification of Living Things

The binomial system of nomenclature was devised by Carolus Linnaeus in 1735 as a method for identifying organisms This initial system grouped all organisms into two kingdoms – vegetable (plants) and animal. The binomial system of nomenclature is still used by scientists today, however several revisions have occurred over the years (Tab.1):

• In 1866, Ernst Haeckel proposed the addition of a third kingdom – Protista

• In 1925, Edouard Chatton recognised the distinction between prokaryotes and eukaryotes and proposed a two empire system

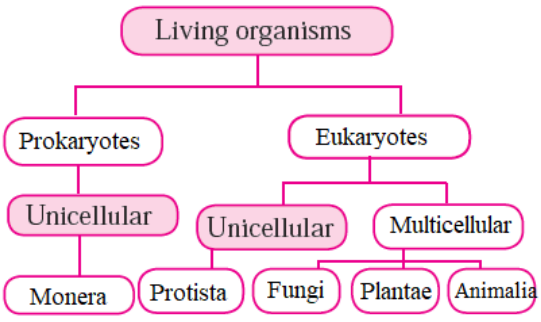
• In 1938, Herbert Copeland incorporated prokaryotic cells into a fourth kingdom – Monera

• In 1969, Robert Whittaker proposed a five kingdom system, which included a kingdom for Fungi

• In 1977, Carl Woese identified differences in prokaryotes and in 1990 proposed the currently recognised three domain system : Archeobacteria - Eubacteria - Eukaryota

At present, the biological classification includes:

- Kingdom Monera
- Kingdom Protista
- Kingdom Fungi
- Kingdom Plantae
- Kingdom Animalia

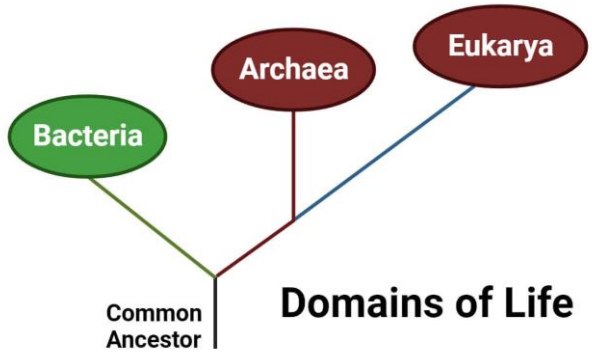


On the basis of the presence or absence of a well-developed nucleus and gene structure,carl woese (1990) classifies the living things into three major domains. These domains are as following :

Table 1. Classification of Living Things

1735 Linnaeus	1866 Haeckel	1938 Copeland	1969 Whittaker	1990 Woese
2 Kingdoms	**3 Kingdoms**	**4 Kingdoms**	**5 Kingdoms**	**3 Domains**
Plant	Protist	Monera	Monera	Eubacteria
				Archaea
		Protist	Protist	Eukaryote
	Plant	Plant	Fungi	
			Plant	
Animal	Animal	Animal	Animal	

- Archeobacteria : thermophilic or heat-loving bacteria living in hight-temperature vents.
- Eubacteria : single-celled organisms without a well-developed nucleus ;
- Eukaryota : all other organisms with a well-developed nucleus in their cell /cells



7. Classification System

The first person to formalize the classification of living things was Carl von Linnae, also known as Linnaeus (the Latin form). Linnaeus' method of classification was based on similarities and differences; a logical place to start. He began by dividing all the organisms he knew of into separate Kingdoms based on the greatest physical similarities and differences. Subdivisions within Kingdoms were called Phyla. Phyla are subdivided into Classes. Classes are subdivided into Orders. Orders are subdivided into Families. Families are subdivided into Genera. Finally,

the Genera are subdivided into species (Tab.2). Each species is defined as a group of organisms with the potential to interbreed. Members of different species cannot, by definition, mate and successfully produce fertile offspring. All names are written in Latin, it never changes and is used only by scientists. The scientific name of an organism is written as Genus species. By convention, the genus and species names are either italicized or underlined. Genus name starts with an Uppercase letter while species name starts with a lowercase letter. Here are a few examples:

Table 2. Linnean system of classification sorts living things into smaller and smaller categories based on similarities and differences.

Common Name	**Human**	**Chimpanzee**	**Lynx**	**Honeybee**	**Nightcrawler**
Kingdom	Animalia	Animalia	Animalia	Animalia	Animalia
Phylum	Chordata	Chordata	Chordata	Arthropoda	Annelida
Class	Mammalia	Mammalia	Mammalia	Insecta	Clitellata
Order	Primata	Primata	Carnivora	Hymenoptera	Haplotaxida
Family	Hominidae	Hominidae	Felidae	Apidae	Lumbricidae
Genus	*Homo*	*Pan*	*Lynx*	*Apis*	*Lumbricus*
species	*sapiens*	*troglodytes*	*lynx*	*mellifera*	*terrestris*

The lesson in a nutshell

The most important concept of cellular biology is the cell theory which states three important principles that all organisms are composed of one or more cells, the cell is the basic unit of life in all living things and all cells are produced by the division of pre-existing cells. Cell biology is concerned with the form and function of a cell, from the most basic traits shared by all cells to the unique, highly complex tasks exclusive to specialised cells. Cells are the smallest common denominator of life. Some cells are organisms unto themselves; others are part of multicellular organisms. Cells can be placed in two major categories as a result of ancient evolutionary events: prokaryotes, with their cytoplasmic genomes, and eukaryotes, with their nuclear-encased genomes and other membrane-bound organelles. Though they are small, cells have evolved into a vast variety of shapes and sizes. Together they form tissues that themselves form organs, and eventually entire organisms.

Living organisms described up to now is included in 05 kingdoms : Animalia, Plantae, Fungi, Protista, Monera, or in 03 domains, namely : Eukaryota, Eubacteria, Archeobacteria.

1) Cell Theory (Core Concept)

Three principles

1. *All living organisms are made of **one or more cells**.*
2. *The **cell is the basic unit** of structure and function.*
3. *All cells arise from **pre-existing cells**.*

***Key idea:** Cells are the smallest units of life capable of metabolism, response, and reproduction.*

2) Discovery of Cells (Important Scientists)

- ***Robert Hooke (1665):** first observed and named "cells" (cork).*
- ***A. van Leeuwenhoek (1674):** first observed **living cells** (microorganisms).*
- ***Schleiden & Schwann (1838–1839):** plants and animals are made of cells.*
- ***Rudolf Virchow (1855):** cells come from pre-existing cells.*

3) Basic Features Common to All Cells

All cells contain:

- ***Plasma membrane***
- ***Cytoplasm***
- ***DNA (genetic material)***
- ***Ribosomes** (protein synthesis)*

Cells perform:

- *Metabolism (catabolism + anabolism)*
- *Growth and reproduction*
- *Response to environment*

4) Classification of Living Organisms

Five Kingdoms

- *Monera*
- *Protista*
- *Fungi*
- *Plantae*
- *Animalia*

Three Domains

- ***Bacteria (Eubacteria)***
- ***Archaea***
- ***Eukarya***

CHAPTER II : PROKARYOTIC AND EUKARYOTIC CELLS : STRUCTURE AND FONCTION

Lesson Objectives

By the end of this lesson, students will be able to:

- *Know the various components of a cell*
- *Compare prokaryotic and eukaryotic cells.*
- *Compare and contrast plant and animal cells*
- *Provide an overview of the origin and evolution of the eukaryotic cell*

Introduction

The cell is the basic functional and structural unit of life. Cell plays a vital role in all biological activities and include membrane-bound organelles, which perform several individual functions to keep the cell alive and active.

All living organisms (bacteria, blue green algae, plants and animals) have cellular organization and may contain one or many cells. The organisms with only one cell in their body are called unicellular organisms (bacteria, blue green algae, some algae, Protozoa, etc.). The organisms having many cells in their body are called multicellular organisms (fungi, most plants and animals). Any living organism may contain only one type of cell either (***Prokaryotic cells***) ; (***Eukaryotic cells***). This classification is based on their complexcity.

Superficially at least, cells exhibit a staggering diversity. Some lead a solitary existence ; others live in communities; some have defined, geometric shapes; others have flexible boundaries; some swim, some crawl, and some are sedentary; many are green (some are even red, blue, or purple); others have no obvious coloration. Given these differences, it is perhaps surprising that there are only two types of cell. Bacterial cells are said to be **Prokaryotic** (Greek for "before nucleus") because they have very little visible internal organization so that, for instance, the genetic material is free within the cell. They are also small, the vast majority being 1-2 μm in length. The cells of all other organisms, from protists to mammals to fungi to plants, are **Eukaryotic** (Greek for "with a nucleus"). These are generally larger (5-100µm, although some eukaryotic cells are large enough to be seen with the naked eye; and structurally more complex. Eukaryotic cells contain a variety of specialized structures known collectively as organelles, surrounded by a viscous substance called cytosol. The largest organelle, the nucleus, contains the genetic information stored in the molecule deoxyribonucleic acid (DNA). The structure and function of organelles will be described in detail in subsequent chapters.

I. Structure of Prokaryotic and Eukaryotic cells

Based on whether they have a nucleus, the cytologists recognize two basic types of cells as Eukaryotic cell and Prokaryotic cell (Refer to Fig.6). Their differences have been tabulated below (Refer to Tab.5). Organisms which do not possess a well formed nucleus are prokaryotes, such as the bacteria. All others possess a welldefined nucleus, covered by a nuclear membrane and are termed as eukaryotes. In most of the cases, prokaryotes are single cells whereas eukaryotes are either single cells or part of multicellular tissues system. Besides this, both types of cells have several structural and metabolic differences as given in (Tab.5).

A. Structure of Prokaryotic Cells

Prokaryotes are generally unicellular microorganisms, except for a majority of Cyanobacteria (multicellular microorganisms). They are subdivided into two domains, the ***(Eubacteria)*** and the ***(Archaea).*** The latter differ from Eubacteria by certain characteristics. Both domains have the characteristic to reproduce by scissiparity (absence of mitosis and meiosis). A prokaryotic cell is much simpler and smaller than eukaryotic cells. It lacks membrane bound organelles including nucleus. The structure and components of a typical prokaryotic cell is shown in (Fig.6.c). The description of different structural feature of prokaryotic cells is as follows.

- ***Essential elements***

The essential elements are common to all prokaryotic cells: the bacterial wall, the plasmamembrane, the nuclear apparatus (nucleoid) and the cytoplasm rich in ribosomes and polyribosomes

- **Bacterial wall**

It's an external, rigid and resistant structure that determines shape and provides protection. In eubacteria, it generally consists of sugar polymers (essentiallyN-acetyl muranic acid and N-acetyl glucosamine) linked by peptide bridges : peptidoglycans (PG). The Gram staining technique, based on the chemical composition of the cell wall, has revealed the existence of 2 types of bacteria (Fig.3) :

Gram-positive (+) bacteria : with a thick wall consisting mainly of PG (20 to 80nm) and few lipids (lipoteichoic acid bound to PG and plasma membrane lipids).

Gram-negative (-) bacteria : have a wall consisting of a thin layer of PG (1 to 3nm) associated with an abundant lipoprotein. This lipoprotein is also linked to an additional outer membrane, rich in lipopolysaccharides (LPS). The space between the 2 membranes, in which the PG bathe, is an aqueous gel called periplasm.

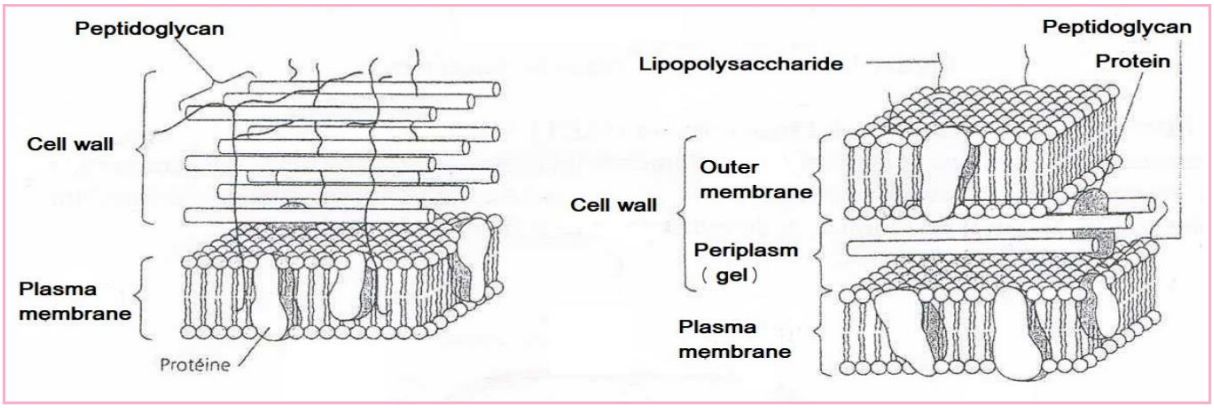


Figure 3. Schematic representation of the walls of Gram (+) and Gram (-) eubacteria.

Most prokaryotes are bounded by a cell wall with the exceptions of Mycoplasma (bacteria) and Thermoplasma (archaea).

The walls of archaea do not contain PG, and have a different organization and chemical composition from those of eubacteria.

- **Plasma membrane**

In TEM, the plasma membrane of prokaryotes is similar to that of eukaryotic cells; it is trilamellar, asymmetrical, 7.5 nm thick and has a fluid mosaic molecular architecture. However, its chemical composition is different, containing 70% proteins, 30% lipids (without cholesterol) and rare carbohydrates. The plasma membrane performs several functions, some of which are specific to the cell (respiration and biosynthesis), while others are common to those of eukaryotic cells (exchanges with the external environment, without membrane deformation).

In archaea, lipids are branched and made up of longer chains, and are also linked together by ether bonds. Whereas in eubacteria, as in eukaryotic cells, lipids are unbranched, consisting of shorter chains linked by ester bonds.

- **Cytoplasm**

It is an aqueous gel with a cytoskeleton-like, whose proteins are homologous to those of Eukaryotic cells, it contains ribosomes and polyribosomes characterizedby a sedimentation

coefficient of 70S. It is the site of all metabolic activities, but also of transcription and translation, which take place simultaneously, as is characteristic of prokaryotes.The tRNA that initiates translation is f-methionine in eubacteria and methionine in archaea as well as in eukaryotes.

- **Nucleoid or nuclear apparatus**

Also known as a chromosome, it is diffused in the cytoplasm and is made up of a single double-stranded DNA molecule, circular, supercoiled and forming several loops due to the action of enzymes and t o its association with histone-like proteins, similar to the histones of eukaryotic cells.

- ***Optional elements***

Optional elements are specific to certain prokaryotic species and absent in others: plasmids, capsule, flagella, pilus, chromatophore and gas vacuole.

- **Plasmid**

It's a very small molecule of extranuclear, double-stranded, circular DNA, located in the cytoplasm. Their number varies from 1 to several depending on the species. Plasmids carry genes that are useful but not essential to the normal growth and division of prokaryotic cells. They duplicate independently of the nucleoid; some have the ability to transfer from one bacterium to another, in which case they are called fertility plasmids (F) if they carry fertility genes, or resistance plasmids (R) if they carry antibiotic resistance genes.

- **Capsule**

This is the outermost structure. Layers of polysaccharide or protein-aqueous material, called a thin layer if it is diffuse and easily destroyed, or a capsule if well organized. They ensure the protection and/or adhesion of prokaryotes to surfaces.

- **Flagellum**

A rigid, cylindrical appendage extending outside the plasma membrane and cell wall, of variable length up to 20µm. Its organization, chemical composition and operating mechanism are very different from those of the eukaryotic flagellum. It is made up of a specific protein: flagellin. Variable in number and position, it enables mobile bacteria to move rapidly.

- **Pilus Pili (in the plural)**

Are extensions of the plasma membrane. There are two types, somatic pili and sexual pili. The first ones serve for the adhesion of bacteria to different surfaces and the latter are of genetic material (e.g., copy of the plasmid) from one bacterium to another.

- **Chromatophores**

These are membrane systems or thylakoids diffused throughout the cytoplasm, rich in specific pigments. They are found only in photosynthetic eubacteria (ex : Cyanobacterium).

- **Mesosome**

Mesosome is a convoluted membranous structure formed in a prokaryotic cell by the invagination of the plasma membrane. It performs the function of mitochondria in bacteria, and also help in carrying out the respiration in the bacteria.

B. Structure of Eucaryote Cells

1. *Animal cells*

An animal cell is a form of eukaryotic cell that makes up many tissues in animals. (Fig.6.a) depicts a typical animal cell. The animal cell is distinct from other eukaryotes, most notably plant cells, as they lack cell walls and chloroplasts, and they have smaller vacuoles. Due to the lack of a rigid cell wall, animal cells can adopt a variety of shapes, and a phagocytic cell can even engulf other structures. There are many different cell types. For instance, there are pproximately 210 distinct cell types in the adult human body.

Cell organelles in animal cell:

- **Cytosol**

Cytosol is the liquid part filled inside the cell and it contains water, salt, macromolecules (Protein, Lipid, RNA). It has an array of microtubule fiber running throughout the cytosol to give vesicular structure to its destination.

- **Cell membrane**

Plasma membrane is the thin layer of protein and fat that surrounds the cell, but is insidethe cell wall. The cell membrane is semipermeable, allowing selective substances to pass into the cell and blocking others.

- **Nucleus**

They are spherical body containing many organelles, including the nucleolus. The nucleus controls many of the functions of the cell (by controlling protein synthesis) and contains DNA (in chromosomes). The nucleus is surrounded by the nuclear membrane and possesses the nucleolus which is an organelle within the nucleus - it is where ribosomal RNA is produced.

- **Golgi apparatus**

It is a flattened, layered, sac-like organelle involved in packaging proteins and carbohydrates into membrane-bound vesicles for export from the cell.

- **Ribosome and Endoplasmic reticulum**

Ribosomes are small organelles composed of RNA-rich cytoplasmic granules that are sites of protein synthesis and Endoplasmic reticulum are the sites of protein maturation and they can be divided into the following types :

Rough endoplasmic reticulum: These are a vast system of interconnected, membranous, infolded and convoluted sacks that are located in the cell's cytoplasm (the ER is continuous with the outer nuclear membrane). Rough ER is covered with ribosomes that give it a rough appearance. Rough ER transport materials through the cell and produces proteins in sacks called cisternae (which are sent to the Golgi body, or inserted into the cell membrane). ***Smooth endoplasmic reticulum:*** These are a vast system of interconnected, membranous, infolded and convoluted tubes that are located in the cell's cytoplasm (the ER is continuous with the outer nuclear membrane). The space within the ER is called the ER lumen. Smooth ER transport materials through the cell. It contains enzymes and produces and digests lipids (fats) and membrane proteins; smooth ER buds off from rough ER, moving the newly-made proteins and lipids to the Golgi body and membranes.

- **Mitochondria**

These are spherical to rod-shaped organelles with a double membrane. The inner membrane is infolded many times, forming a series of projections (called cristae). The mitochondrion converts the energy stored in glucose into ATP (adenosine triphosphate) for the cell.

- **Lysosome**

Lysosomes are cellular organelles that contain the hydrolase enzymes which breaks down waste materials and cellular debris. They can be described as the stomach of the cell. They are found in animal cells, while in yeast and plants the same roles are performed by lytic vacuoles.Lysosomes digest excess or worn-out organelles, food particles, and engulf viruses or bacteria. The membrane around a lysosome allows the digestive enzymes to work at the 4.5 pH they require. Lysosomes fuse with vacuoles and dispense their enzymes into the vacuoles, digesting their contents. They are created by the addition of hydrolytic enzymes to early endosomes from the Golgi apparatus.

- **Centrosome**

They are small body located near the nucleus and has a dense center and radiating tubules. The centrosomes are the destination where microtubules are made. During mitosis, the centrosome divides and the two parts move to opposite sides of the dividing cell. Unlike the centrosomes in animal cells, plant cell centrosomes do not have centrioles.

- **Peroxisome**

Peroxisomes are organelles that contain oxidative enzymes, such as D-amino acid oxidase, ureate oxidase, and catalase. They may resemble a lysosome, however, they are not formed in the Golgi complex. Peroxisomes are distinguished by a crystalline structure inside a sac which also contains amorphous gray material. They are self replicating, like the mitochondria. Components accumulate at a given site and they can be assembled into a peroxisome. Peroxisomes function to rid the body of toxic substances like hydrogen peroxide, or other metabolites. They are a major site of oxygen utilization and are numerous in the liver where toxic byproducts accumulate.

- **Vacuoles and vesicles**

Vacuoles are single-membrane organelles that are essentially part of the outside that is located within the cell. The single membrane is known in plant cells as a tonoplast. Many organisms will use vacuoles as storage areas. Vesicles are much smaller than vacuoles and function in transporting materials both within and to the outside of the cell.

2. *Plant cells*

Plant cells are eukaryotic cells that differ in several key aspects from the cells of other eukaryotic organisms. In contrast to animal cells, plant cells are enclosed within a rigid cell wall that gives shape to the cell and structural rigidity to the organism. Plant cells frequently contain one or more vacuoles that can occupy up to 75% of the cell volume. Cells of photosynthetic plant tissues contain a special organelle, the chloroplast, that houses the light-harvesting and carbohydrate-generating systems of photosynthesis. Plant cells lack centrosomes (Fig.6.b) although these are found in many algae. Their distinctive features include the following organelles :

- **Vacuole**

It is present at the centre and is water-filled volume enclosed by a membrane known as the tonoplast. The function is to maintain the cell's turgor, pressure by controlling movement of molecules between the cytosol and sap, stores useful material and digests waste proteins and organelles.

- **Cell Wall**

It is the extracellular structure surrounding plasma membrane. The cell wall is composed of cellulose, hemicellulose, pectin and in many cases lignin, is secreted by the protoplast on the outside of the cell membrane. This contrasts with the cell walls of fungi (which are made of chitin), and of bacteria, which are made of peptidoglycan. An important function of the cell wall is that it controls turgity. The cell wall is divided into the primary cell wall and the secondary cell wall. The Primary cell wall: extremely elastic and the secondary cell wall forms around primary cell wall after growth are complete.

- **Plasmodesmata**

Pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous.

- **Plastids**

The plastids are chloroplasts, which contain chlorophyll and the biochemical systems for light harvesting and photosynthesis. The other plastids are amyloplasts specialized for starch storage, elaioplasts specialized for fat storage, and chromoplasts specialized for synthesis and storage of pigments. As in mitochondria, which have a genome encoding 37

genes, plastids have their own genomes of about 100-120 unique genes and, it is presumed, arose as prokaryotic endosymbionts living in the cells of an early eukaryotic ancestor of the land plants and algae (Refe to Fig.6).

Table 3. Cell organelles and their functions in the cell

Cell Organelle	Function
Nucleus	Contains genetic material and instructions for cell
Cell membrane	Exterior barrier with channels and pores built from protein molecules to allow certain substances to enter or leave the cell
Cytoskeleton	Provides support in the cell and is made of interlinking protein filaments
Cytosol	Cell fluid that contains organelles and cytoskeleton and allows intracellular movement
Vacuoles	A cell's storage spaces for storing cellular material waste and water
Centrosome	Zone where microtubules are produced during mitosis
Mitochondria	Provides energy to the cell
Ribosome	Site of protein synthesis
Golgi Apparatus	Processes and packages proteins in a cell
Endoplasmic reticulum	Sites of protein maturation
Lysosomes	Breaks down and recycles wastes and pathogens for use in the making of new cellular parts
Peroxisome	Rid the body of toxic substances like hydrogen peroxide, or other metabolites.
Chloroplasts	Plant cell organelles that convert sunlight in stable forms ofenergy
Cell Wall	provide structural strength and support

II. Comparison of cell types

A. Comparison between plant and animal cells

Table 4. Difference between plant and animal cells

S.No	*Animal cell*	*Plant cell*
1	Animal cells are generally small in size	Plant cells are larger than animal cells.
2	Cell wall is absent.	The plasma membrane of plant cells is surrounded by a rigid cell wall of cellulose.
3	Except the protozoan Euglena no animal cell possesses plastids.	Plastids are present.
4	Vacuoles in animal cells are many and small.	Most mature plant cells have a large central sap vacuole
5	Animal cells have a single highly complex Golgi	Plant cells have many simpler units of and prominent Golgi apparatus. apparatus, called dictyosomes.
6	Animal cells have centrosome and centrioles.	Plant cells lack centrosome and centrioles.

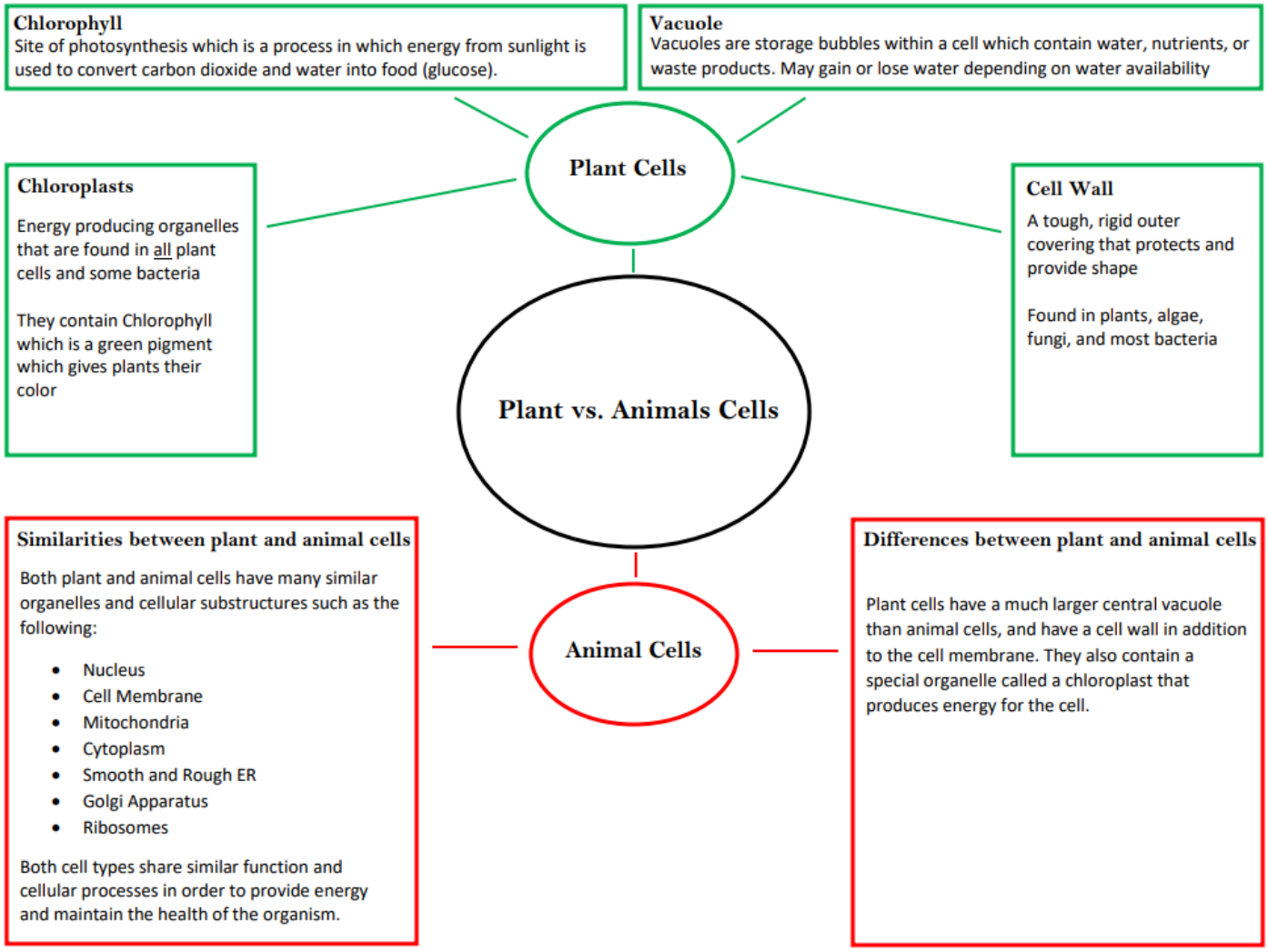


Figure 4. Similarities and differences between plant and animal cells

B. Comparison between Eukaryotic and Prokaryotic Cell

Table 5. Difference between Eukaryotic and Prokaryotic Cell

Characteristic	***Prokaryote (Bacteria and Archaea)***	***Eucaryote (Plant and Animal cell)***
Type of Cell	Always unicellular	Unicellular and multicellular
First appeared	3.5×10^9 years ago	1.5×10^9 years ago
Size	Smal, in µm rang Usually 1–2 µm	Variable size, upto 40 µm in diametre. Usually 5–100 µm
Genetic materiel	Nucleus Absent. Circular DNA present in cytosol as free materiel	Nucleus present, bounded by nuclear envelope, no direct connection with cytosol. Multiple molecules (chromosomes), linear, associated with protein
Genes	No intron	Presence of intron
Replication	Single origine of replication	Multiple origine of replication
Transcription and translation	Occurs together	Transcription in nucleus, translation in cytosol
Ribosome	Smaller size 70S	Larger size 80S ; smaller size (70S) in organelles
Cell division	Through binary fission	Through mitosis
Sexual reproduction	No meiosis; transfer of DNA fragments only (conjugation)	Involves Meiosis
Organelles	No membrane bound organelle	Membrane bound organelles with well defined fonction
Plasma membrane	No carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors present
Cytoskeleton	Absent	Microtubules, microfilaments, intermediate filaments
Cell walls	Very complex cell wall	Except fungi and plant, Eukaryotic cells are devoid of a think cell wall

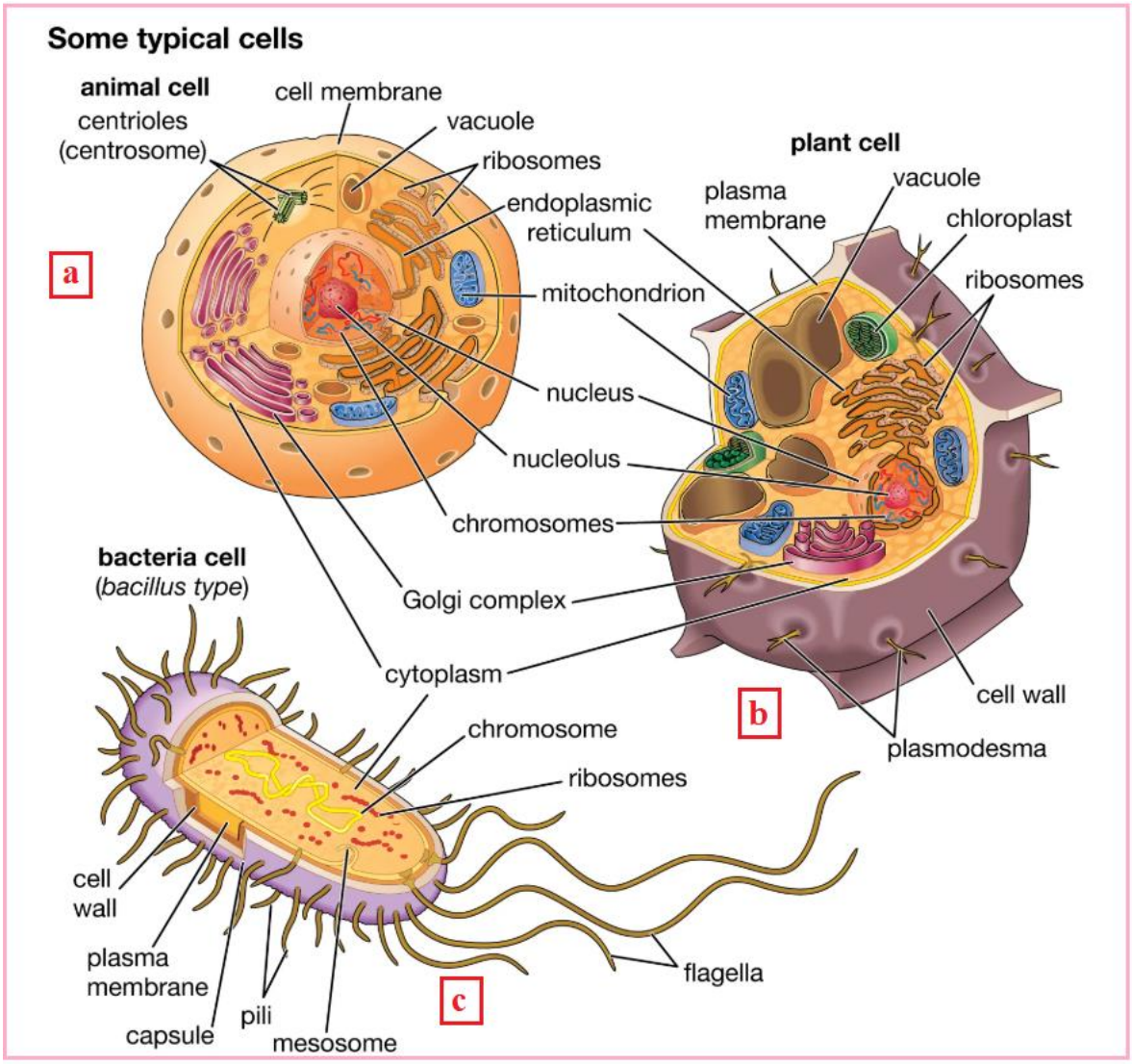


Figure 6. Structure of different types of cell (a : Animal cell, b :Plant cell, c : Bacteria cell)

III. The Evolution of Eukaryotic Cells

Eukaryotes are organisms made of one or more eukaryotic cells. The earliest eukaryotes, like the first prokaryotes, were single-celled organisms. They arose about 1 billion years later than the earliest prokaryotes. Later, multicellular eukaryotes arose. Every type of multicellular organism that exists is made up of eukaryotic cells. There is compelling evidence that mitochondria and chloroplasts were once primitive bacterial cells. This evidence is described in the endosymbiotic theory. How did this theory get its name? Symbiosis occurs when two different species benefit from living and working together. When one organism actually lives inside the other it's called endosymbiosis. The endosymbiotic theory describes how a large host cell and ingested bacteria could easily become dependent on one another for survival, resulting in a permanent relationship. Over millions of years of evolution, mitochondria and chloroplasts have become more specialized and today they cannot live outside the cell.

- ***The endosymbiotic theory***

The endosymbiotic theory is usually used to explain the origin of eukaryotic cells. Mitochondria and chloroplasts have striking similarities to bacteria cells. They have their own DNA, which is separate from the DNA found in the nucleus of the cell. And both organelles use their DNA to produce many proteins and enzymes required for their function. A double membrane surrounds both mitochondria and chloroplasts, further evidence that each was ingested by a primitive host (Fig.7). The two organelles also reproduce like bacteria, replicating their

own DNA and directing their own division.

- The endosymbiotic theory states that some of the organelles in today's eukaryotic cells were once prokaryotic microbes.
- In this theory, the first eukaryotic cell was probably an amoeba-like cell that got nutrients by phagocytosis and contained a nucleus that formed when a piece of the cytoplasmic membrane pinched off around the chromosomes.
- Some of these amoeba-like organisms ingested prokaryotic cells that then survived within the organism and developed a symbiotic relationship.
- Mitochondria formed when bacteria capable of aerobic respiration were ingested; chloroplasts formed when photosynthetic bacteria were ingested. They eventually lost their cell wall and much of their DNA because they were not of benefit within the host cell.
- The endosymbiotic theory describes how a large host cell and ingested bacteria could easily become dependent on one another for survival, resulting in a permanent relationship.
- Over millions of years of evolution, mitochondria and chloroplasts have become more specialized, and today they cannot live outside the cell.

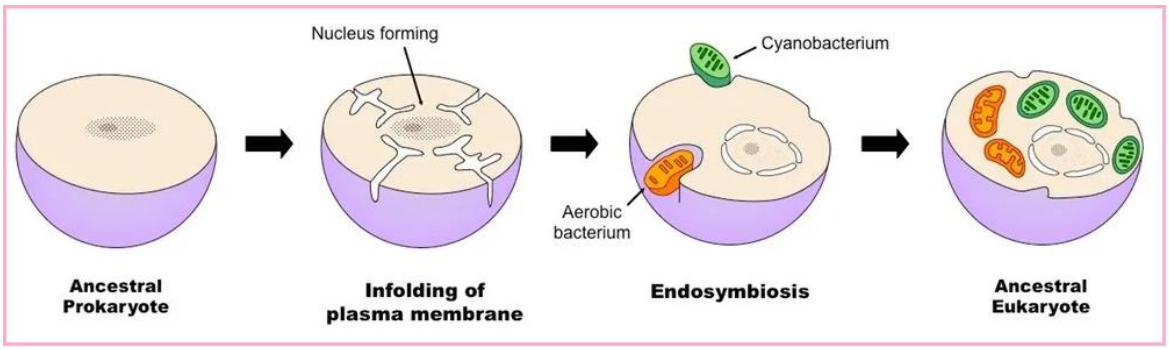


Figure 7. Schematic explaining the endosymbiosis theorem

The lesson in a nutshell

1) The Cell (Basic Concept)

- *The **cell** is the smallest structural and functional unit of life.*
- *Organisms can be:*
  - ***Unicellular** (bacteria, protozoa)*
  - ***Multicellular** (plants, animals, fungi)*
- *Two fundamental cell types :*
  - ***Prokaryotic***
  - ***Eukaryotic***

2) Prokaryotic Cells (Bacteria & Archaea)

General features

- *Size: **1–2 µm***
- *Usually **unicellular***
- ***No true nucleus** (DNA in nucleoid)*
- ***No membrane-bound organelles***
- *Ribosomes: **70S***
- *Division: **binary fission***
- *Transcription and translation occur **simultaneously***

Main structures

- *Cell wall (peptidoglycan in bacteria)*
- *Plasma membrane*
- *Cytoplasm*
- *Nucleoid (circular DNA)*

Optional structures

- *Plasmids (extra DNA)*
- *Capsule (protection/adhesion)*
- *Flagella (motility)*
- *Pili (attachment/DNA transfer)*

3) Eukaryotic Cells

General features

- *Size: **5–100 µm***
- *Uni- or multicellular*
- ***True nucleus** with nuclear envelope*
- ***Membrane-bound organelles***
- *Ribosomes: **80S***
- *Division by **mitosis** (and meiosis for sexual reproduction)*

4) Plant-specific structures

- *Chloroplasts (photosynthesis)*
- *Cell wall (support, protection)*
- *Plasmodesmata (cell communication)*

5) Quick Memory Tips

Prokaryote = No nucleus, No organelles
Eukaryote = Nucleus + Organelles
Plants = Cell wall + Chloroplast + Large vacuole
Animals = No wall, No chloroplast

CHAPTER I : VIRUSES

Lesson Objectives

By the end of this lesson, students will be able to:

- Note that viruses are obligatory intracellular parasites
- Understand the common structural components of viruses
- Know the stages of the viral life cycle

Introduction

Viruses are microscopic agents that exist worldwide and are present in humans, animals, plants, and other living organisms in which they can cause devastating diseases.

Viruses were discovered at the end of the nineteenth century following several observations. In 1892, the Russian botanist Dmitri Ivanovsky noticed that suspensions of plant tissues afflicted with mosaic tobacco disease were still infectious after passage through ceramic filters that retained bacteria. He thought that his filter had most probably leaked and that the causative agent was a bacterium. In 1898, Martinus Beijerinck made a similar observation and thought that the infectious agent, the “virus,” was the liquid. The same year Friedrich Loeffler and Paul Frosch, while studying the cause of the foot-and-mouth disease, determined that the causative agent was also constituted by “filterable” particles that were nonetheless retained by filters of finer grains than those used for bacteria (Murphy 2011). Viruses had been identified as extremely small infectious particles. After one century of research, notably thanks to the development of molecular biology and electron microscopy, our knowledge about viruses, their nature, their diversity, their infectious cycles, and their role in biology has enormously increased. Viruses are strict molecular parasites which depend on a cell to develop their reproductive cycle. Viral infectious particles, or virions, are composed of a nucleic acid (DNA or RNA) surrounded by a protein shell (the capsid) and sometimes by an additional lipid envelope.

1. Definition of viruses

is infectious particle containing one type of nucleic acid and surrounded by protein coat. The viral particle has ability to replicate only in living host cell, and cause disease.

2. General features of viruses

- The Latin word virus means "poisonous", and this is just what seemed to the first virologists.

- Virus depends upon the living host to reproduce and multiply. They cannot replicate when they are outside the living host. They are obligate intracellular parasites
- They are also known to be acellular because they do not contain cellular components such as cell organelles and ribosomes but it also contains certain enzyme.
- No built-in metabolic machinery: Viruses have no metabolic enzymes and cannot generate their own energy.
- No ribosomes, viruses cannot synthesize their own proteins. For this they utilize host cell ribosomes during replication.
- Only one type of nucleic acid: Viruses contain either DNA or RNA (never both) as their genetic material. The nucleic acid can be single-stranded or double stranded.
- The genome is enclosed in a protein coat known as a capsid. (genome + capsid + other components in many cases= virion).
- small size: cannot be viewed with a light microscope. They are 100 times smaller than bacteria and are known to be a microscopic parasite whose size ranges from 5 to 300 nanometers.

3. Organisation of the virion

The term virion designates the fully assembled, extracellular form of a virus, in which the viral genome and associated proteins are packaged into a nucleocapsid, itself composed of the nucleic acid and the surrounding protein capsid, and, in enveloped viruses, further enclosed within a host-derived lipid membrane bearing viral glycoproteins. This modular organisation is conserved across the virosphere and allows a limited set of viral structural proteins to build particles that are both mechanically robust and capable of highly specific recognition and entry into target cells.

Viruses exhibit tremendous diversity in size, shape, and composition, but some common structural elements are:

- **Genetic Material:** This can be single-stranded DNA, double-stranded DNA, single-stranded RNA, or double-stranded RNA.
- **Capsid:** This is a protective layer of protein surrounding the genome. It consists of multiple copies of capsomere subunits tightly arranged to form a shell.
- **Envelope:** Some groups of viruses have a membrane envelope made of lipids, proteins, and glycoproteins. Envelopes are acquired by budding from host cell membranes.

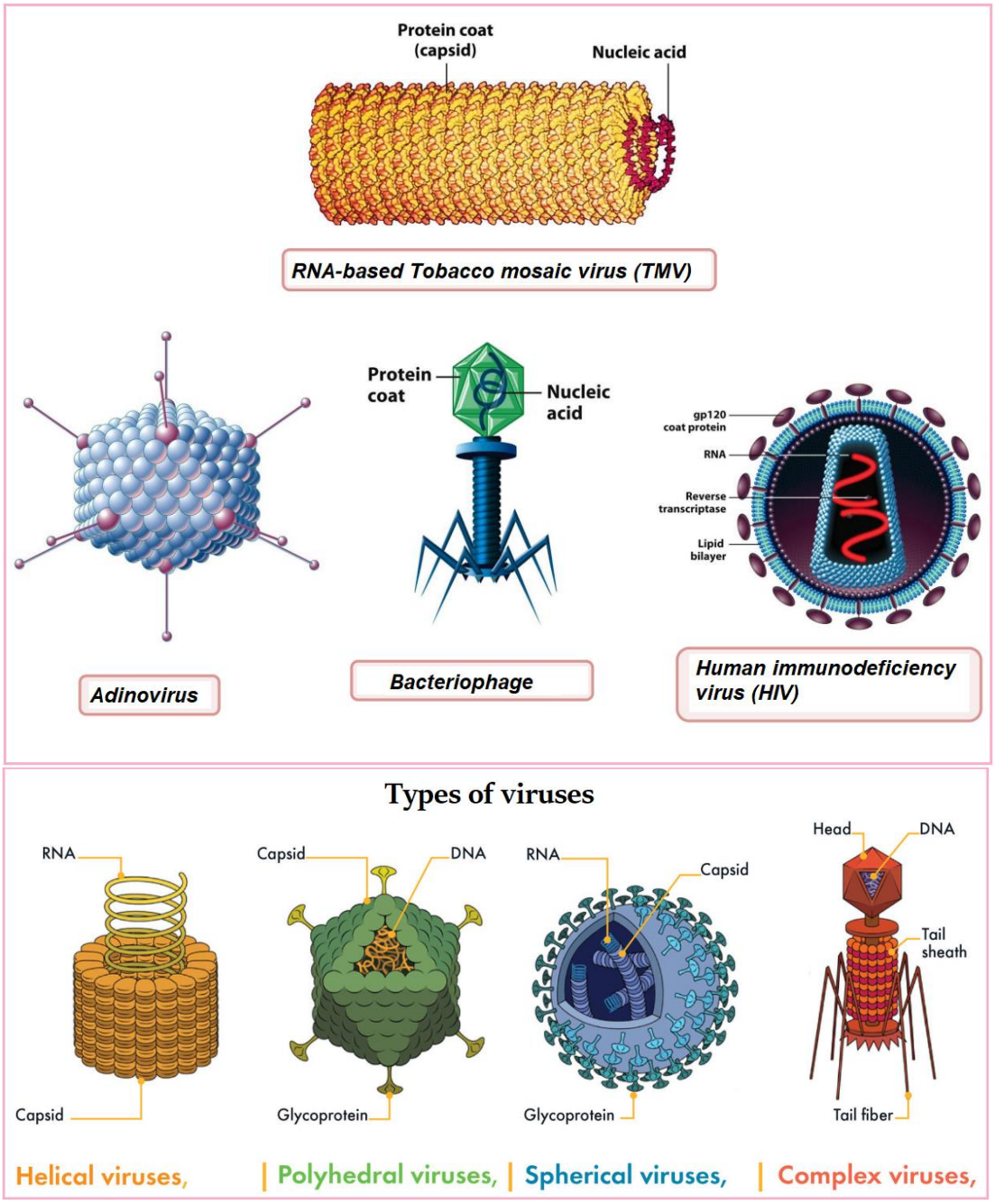


Figure 8. Different Forms and Structures of Viruses

4. Classification of viruses.

Viruses are classified on the basis of their nucleic acid's characteristics, capsid symmetry, the presence or absence of an envelope, their host, and other properties.

❖ **Classification of virus on the basis of host range :**

a. Bacteriophage:

- Phage are virus infecting bacteria. Eg, λ phage, T2, T4, φ174, MV-11

b. Plant virus:

- Those virus that infects plants. Eg. TMV, cauliflower mosaic virus

c. Animal virus:

- Those virus that infects animals. Eg. Polio virus, Retro virus, Herpes virus, Adeno virus

d. Insect virus:

- Virus that infects insects. Eg. Baculovirus, Sacbrood virus, Entomopox virus, Granulosis virus

❖ **Classification of virus on the basis of mode of transmission:**

a. Virus transmitted through respiratory route:

- Eg, Swine flu, Rhino virus

b. Virus transmitted through faeco-oral route:

- Eg. Hepatitis A virus, Polio virus, Rota virus

c. Virus transmitted through sexual contacts:

- Eg. Retro virus

d. Virus transmitted through blood transfusion:

- Eg. Hepatitis B virus, HIV

e. Zoonotic virus: virus transmitted through biting of infected animals;

- Eg. Rabies virus, Alpha virus, Flavi virus

❖ **Classification of virus on the basis of capside symmetry**

***a. Cubical virus*:** they are also known as icosahedral symmetry virus

- eg. Reo virus, Picorna virus

b. Spiral virus: they are also known as helical symmetry virus

- eg. Paramyxovirus, orthomyxovirus

c. Radial symmetry virus:

- eg.Bacteriophage

d. Complex symmetry virus:

- eg. Pox virus

❖ **Classification of virus on the basis of nucleic acid**

a. *DNA virus:* viral genome is DNA

- Double stranded DNA virus: eg. Adenovirus, Herpesvirus
- Single stranded DNA virus: eg. Parvovirus, φ174 virus

b. *RNA virus:* genome is RNA

- Double stranded RNA virus: eg. Reo virus
- Single stranded RNA virus: these are further classified into two groups
  - Positive sense RNA (+RNA): Polio virus, Hepatitis A
  - Negative sense RNA (-RNA): Rabies virus, Influenza virus

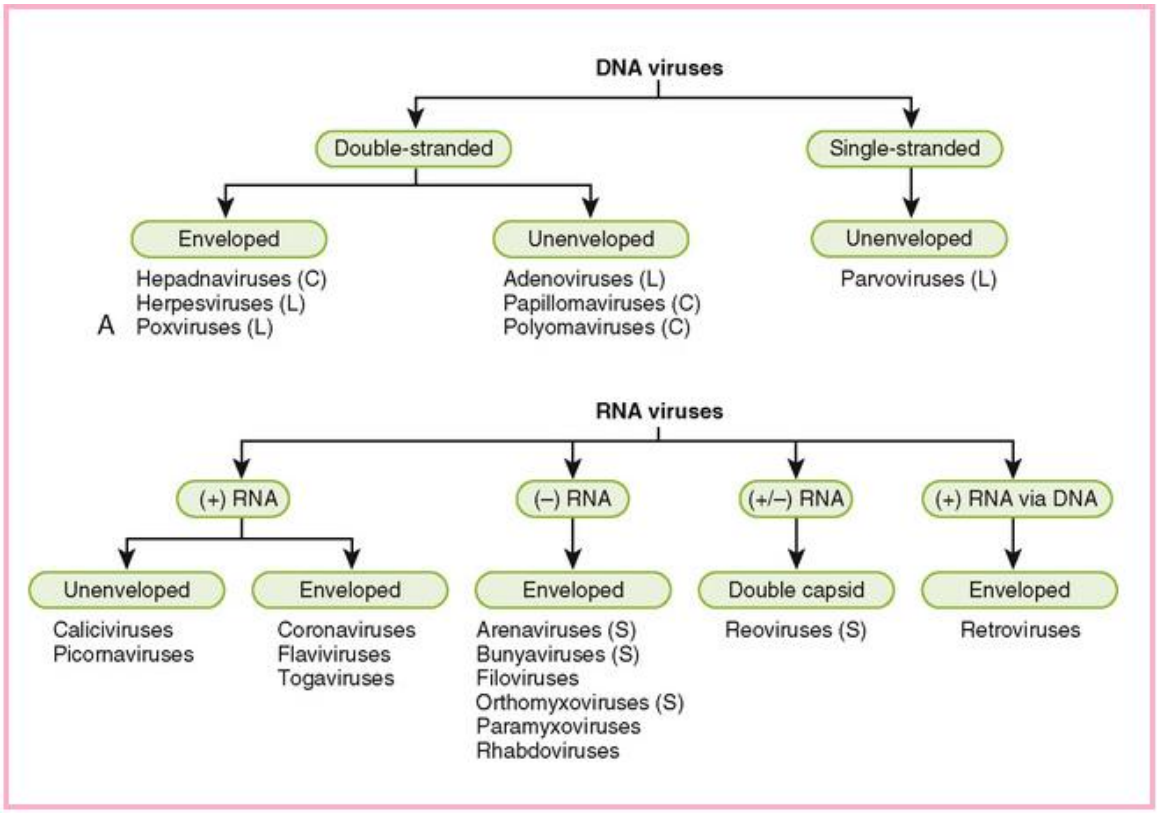


Figure 9. Classification of viruses according to the nature and structure of the nucleic acid

5. Viral replication

The replication cycle of a virus consists of a series of ordered stages attachment, penetration, uncoating, replication of the genome, synthesis of viral proteins, assembly of new nucleocapsids and release of progeny virions that collectively transform a single infectious

particle into tens to thousands of progeny, depending on the virus and host cell. These events are tightly coupled to the host cell cycle and metabolic state ; for example, DNA tumour viruses often require host cells in S phase to ensure the availability of nucleotide pools and replication enzymes, thereby perturbing normal cell-cycle regulation and sometimes contributing to oncogenesis.

a. Lytic cycle

In the lytic cycle, exemplified by bacteriophage T4 and many acute animal viruses, infection culminates in rapid production of progeny virions and destruction of the host cell, either by osmotic lysis or by virus-induced cell death. Following adsorption to specific receptors, the viral genome is injected or released into the cytoplasm, early genes are expressed to subvert cellular machinery, viral nucleic acid is replicated, and late structural proteins assemble into nucleocapsids; finally, progeny virions are released, often in a burst of roughly 100-300 particles per infected bacterium after a latent period of 20-60 minutes in many phage systems. In cytology, lytic infections manifest as cytopathic effects such as cell rounding, fusion into multinucleated syncytia, inclusion bodies, or frank necrosis, which can be appreciated in cell culture or tissue sections and guide virological diagnosis.

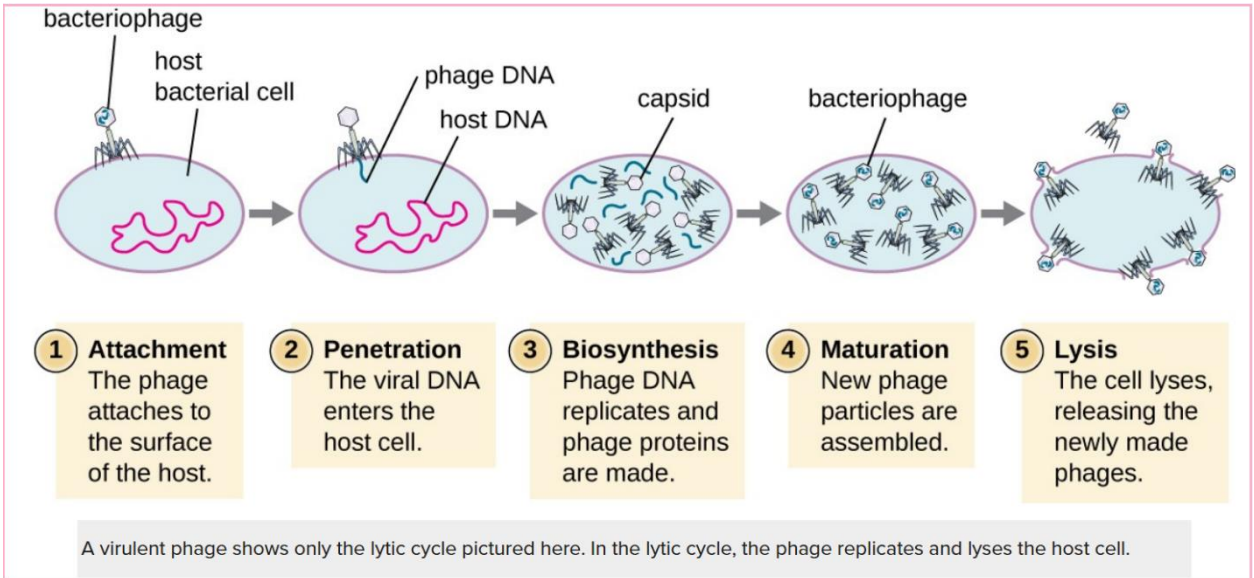


Figure 10. Multiplication of a bacteriophage through the lytic cycle

b. Lysogenic (temperate) cycle

In contrast, the lysogenic or temperate cycle, classically described for bacteriophage λ, is characterised by integration of the viral genome into the host chromosome or its persistence as a stable plasmid-like episome, forming a prophage that is replicated passively with the host

DNA and transmitted to daughter cells without immediate lysis. Environmental stresses such as UV irradiation or DNA-damaging agents can trigger induction, whereby prophage genes encoding repressors are inactivated, the lytic programme is launched, and the infected cell eventually lyses, releasing progeny phages, a switch that has become a paradigm for gene regulatory circuits controlling developmental decisions. Analogous relationships exist in eukaryotes; for example, retroviruses integrate as proviruses in the host genome, and certain DNA viruses can establish latent infections in specific cell types, so that cytological examination may reveal cells harbouring viral genomes or latent proteins without overt cytopathic changes, which has major implications for chronic infection, oncogenesis and reactivation disease.

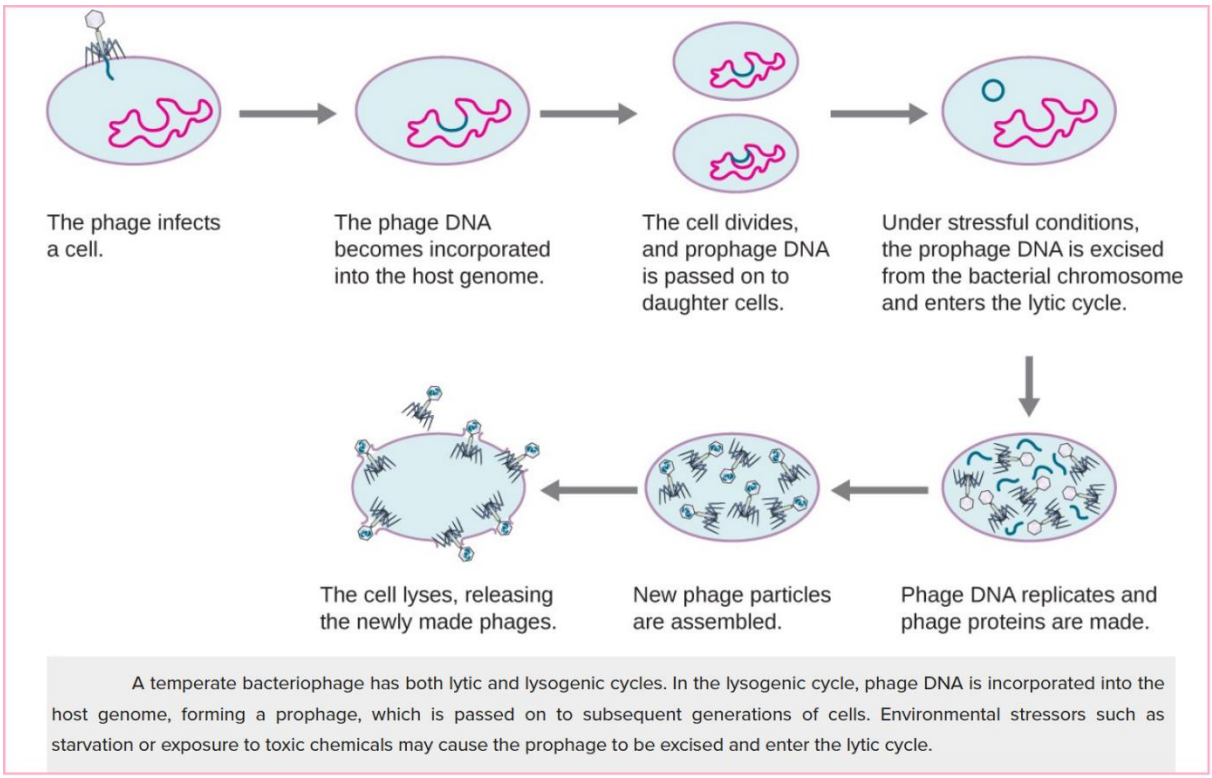


Figure 11. Multiplication of a bacteriophage λ through the lysogenic cycle

The lesson in a nutshell

1) What is a Virus?

- ***Acellular infectious particle***
- *Contains **one type of nucleic acid**: DNA or RNA (never both)*
- *Surrounded by a **protein coat (capsid)***
- *Sometimes enclosed by a **lipid envelope***
- *Replicates **only inside a living host cell***
- ***Obligate intracellular parasite***

Key facts

- *No ribosomes*
- *No metabolism or energy production*
- *Cannot reproduce outside host*
- *Size: **5–300 nm** (much smaller than bacteria*

2) Virus Classification

- *Based on Host*
- *Based on Genome*
- *Based on Capsid Symmetry*

3) Viral Life Cycle (General Steps)

- ***Attachment** – virus binds host receptor*
- ***Penetration** – entry into cell*
- ***Uncoating** – release of genome*
- ***Replication** – viral genome copied*
- ***Protein synthesis** – using host ribosomes*
- ***Assembly** – new virions formed*
- ***Release** – new viruses exit cell*

4) Transmission Routes (Examples)

- *Respiratory: influenza*
- *Fecal–oral: poliovirus*
- *Blood/sexual: HIV, Hepatitis B*
- *Zoonotic (animal bite): rabies*

5) Quick Memory Box

- ***Virus = DNA/RNA + capsid ± envelope***
- ***No metabolism, no ribosomes***
- ***Replicates only inside host***
- ***Two cycles: Lytic (kill) / Lysogenic (latent)***

CHAPTER IV : TECHNIQUES IN CELL BIOLOGY

Lesson Objectives

In this chapter, students will be able to:

- Identify and describe the parts of a light microscope,
- Explain the distinguishing features and typical applications of different types of microscopes,
- Understand and carry out the series of steps required to prepare samples for observation under light microscopy and electron microscopy.

Introduction

Cell biology aims to understand the structure and function of cells, the fundamental units of life. To achieve this, scientists use a range of techniques that allow the visualization and analysis of cellular organization. Histological techniques for light microscopy are essential for preparing, sectioning, and staining cells and tissues, making it possible to observe their internal structures and overall morphology. In addition, several methods are specifically designed to study cell shape and surface features, such as negative contrast (negative staining), metal shadowing, and cryofracture (freeze-fracture), which provide detailed information about surface topography and membrane organization.

Alongside imaging approaches, cell fractionation methods such as centrifugation techniques play a key role in modern cell biology. These techniques separate cellular components according to their size and density, allowing the isolation of organelles and macromolecules for further analysis. Together, these methodological approaches provide powerful tools to explore cellular architecture and function, forming the foundation for advances in biological research, biotechnology, and medicine.

1. Microscopy

Microscopy refers to the set of techniques that use optical or electron beams and lens systems to produce magnified images of structures too small to be resolved by the unaided eye, and constitutes the foundation of modern cytology and histology. The human eye has a limit of resolution of about 0.2 mm, whereas a high-quality light microscope, using visible light (wavelength ~400-700 nm) and objectives of high numerical aperture, can reach a theoretical resolution of about 0.2 µm, thereby improving resolving power by roughly three orders of magnitude. Transmission electron microscopes use accelerated electrons with subnanometre

wavelengths and can achieve resolutions on the order of 0.2-0.5 nm, making it possible to visualise membranes, ribosomes and macromolecular complexes.

These differences in resolution and contrast determine which subcellular features are accessible to each modality: light microscopy allows routine examination of whole cells and tissues in paraffin or frozen sections, while electron microscopy reveals ultrastructural details such as the trilaminar appearance of membranes, the internal organisation of mitochondria and the fine architecture of junctional complexes. In cytology and diagnostic pathology, the two levels of magnification are complementary: light microscopy pres rapid, cost-effective screening, whereas electron microscopy is reserved for selected questions that require ultrastructural confirmation, especially in renal, muscle and ciliary disorders.

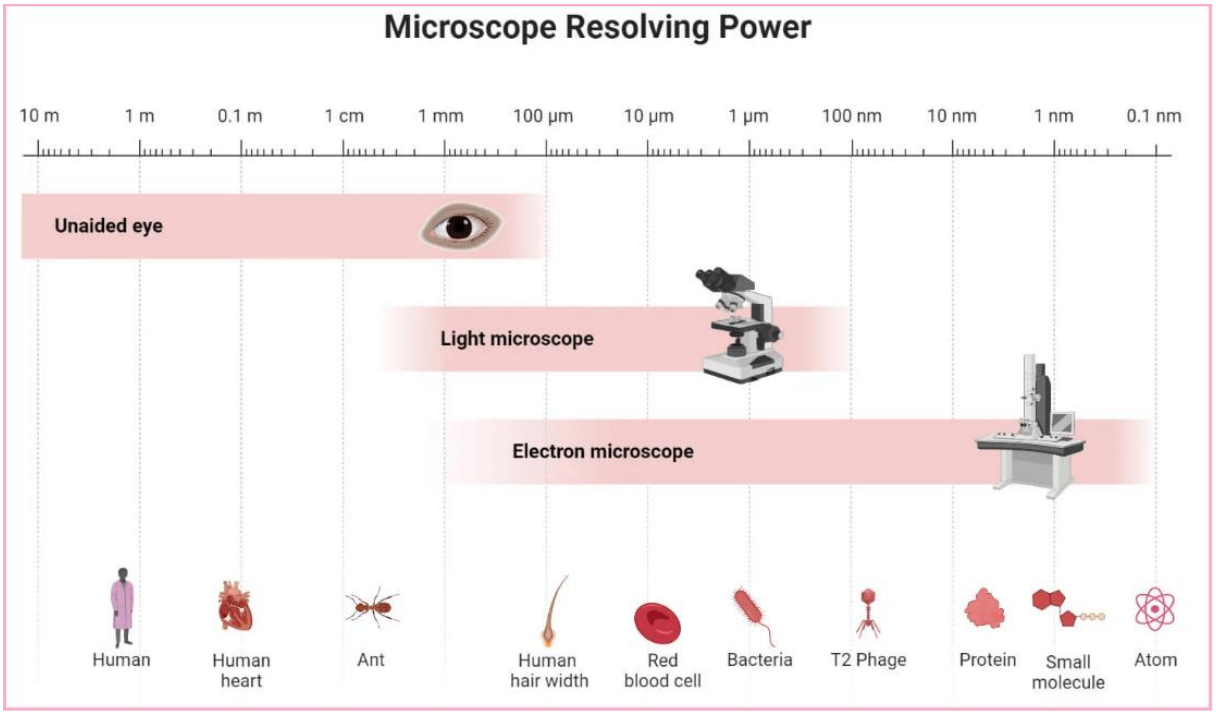


Figure 12. Diagram showing the resolution and resolving power of the human eye, optical microscope, and electron microscope.

a. Light microscope

The modern light microscope (LM) uses visible light transmitted through or reflected by a specimen and refracted by a series of glass lenses (objective and ocular) to produce a magnified image, typically up to 1000-1500× total magnification with oil-immersion objectives. Its resolving power is limited by the wavelength of light and the numerical aperture of the objective and condenser, with the best instruments reaching a practical limit of about 0.2 µm ; objects

smaller than this, such as ribosomes (~25 nm), can be visualised only as unresolved basophilic material in routine histology.

In routine bright-field microscopy, tissue sections are stained with dyes such as hematoxylin and eosin to convert subtle chemical differences into visible contrasts, and the observer scans the slide systematically at low power (×4-×10 objectives) before examining cytological details at higher magnification (×40, then ×100 oil immersion). Alternative contrast-enhancing techniques, such as phase-contrast and differential interference contrast, exploit differences in refractive index to visualise living, unstained cells, while fluorescence microscopy uses fluorochromes coupled to antibodies or nucleic acid probes to detect specific molecules; however, in the context of basic cytology teaching, the reference technique remains bright-field examination of fixed, stained preparations.

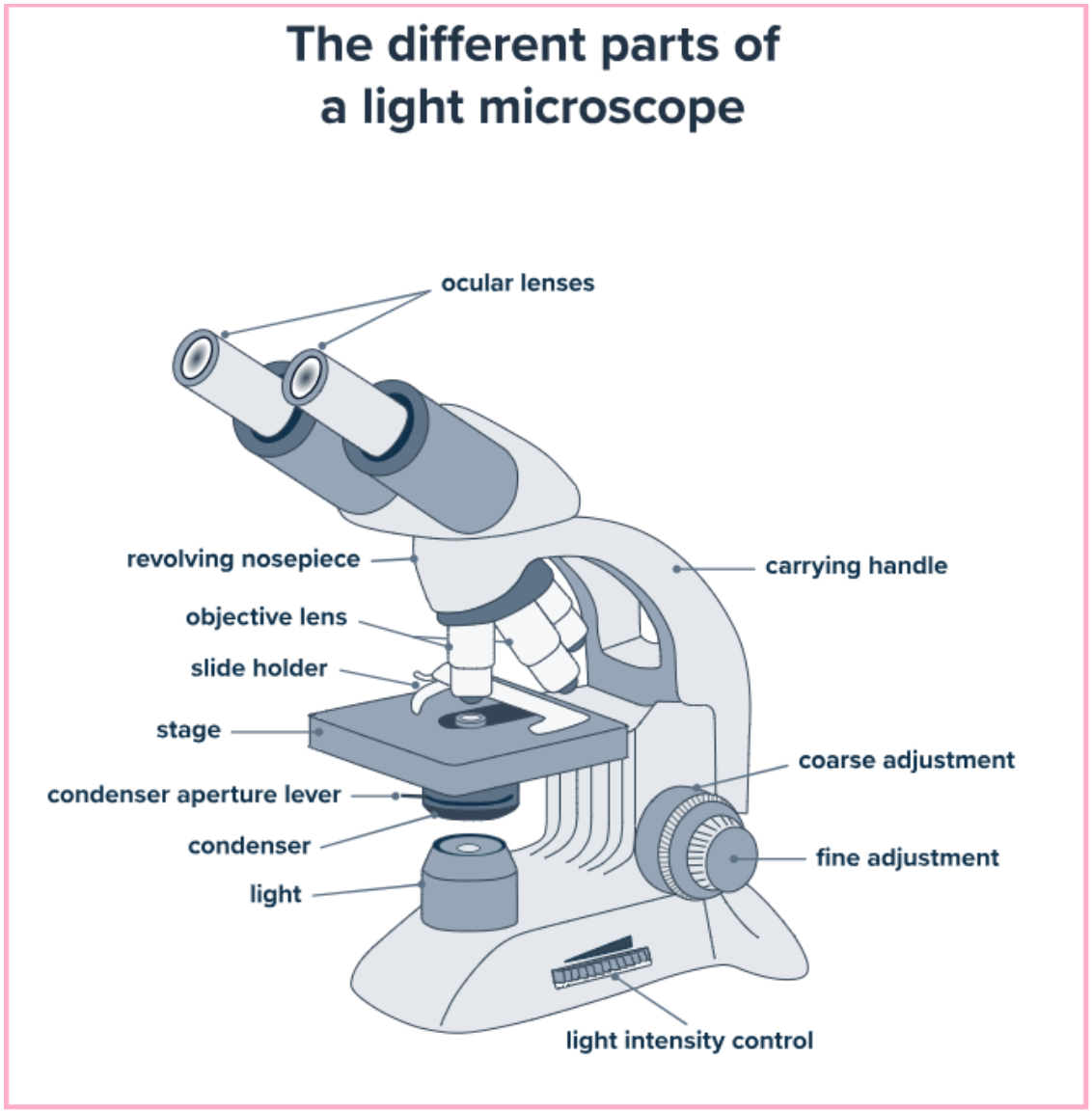


Figure 13. Différent part of a light microscope

b. Electron microscope

The electron microscope replaces photons with electrons accelerated under high voltage (typically 60-200 kV) and focuses them with electromagnetic lenses under high vacuum, allowing a resolving power in the range of 0.2-1 nm, nearly a thousand-fold better than that of the light microscope. In transmission electron microscopy (TEM), a finely focused electron beam passes through ultrathin sections of resin-embedded specimens (usually 60-90 nm thick), and differences in electron scattering by heavy-metal stained structures are translated into image contrast on a phosphorescent screen or digital camera.

In scanning electron microscopy (SEM), a focused electron beam scans the surface of a specimen that has been dehydrated and coated with a thin metal film, and secondary electrons emitted from the surface are collected to generate a high-resolution, pseudo-three-dimensional view of topography at magnifications up to ~10^4-10^5×. SEM is particularly suited to study cell surfaces, microvilli, cilia and extracellular matrices, whereas TEM remains the method of choice for internal ultrastructure ; both require more complex specimen preparation than light microscopy but pre essential information in research and selected diagnostic settings.

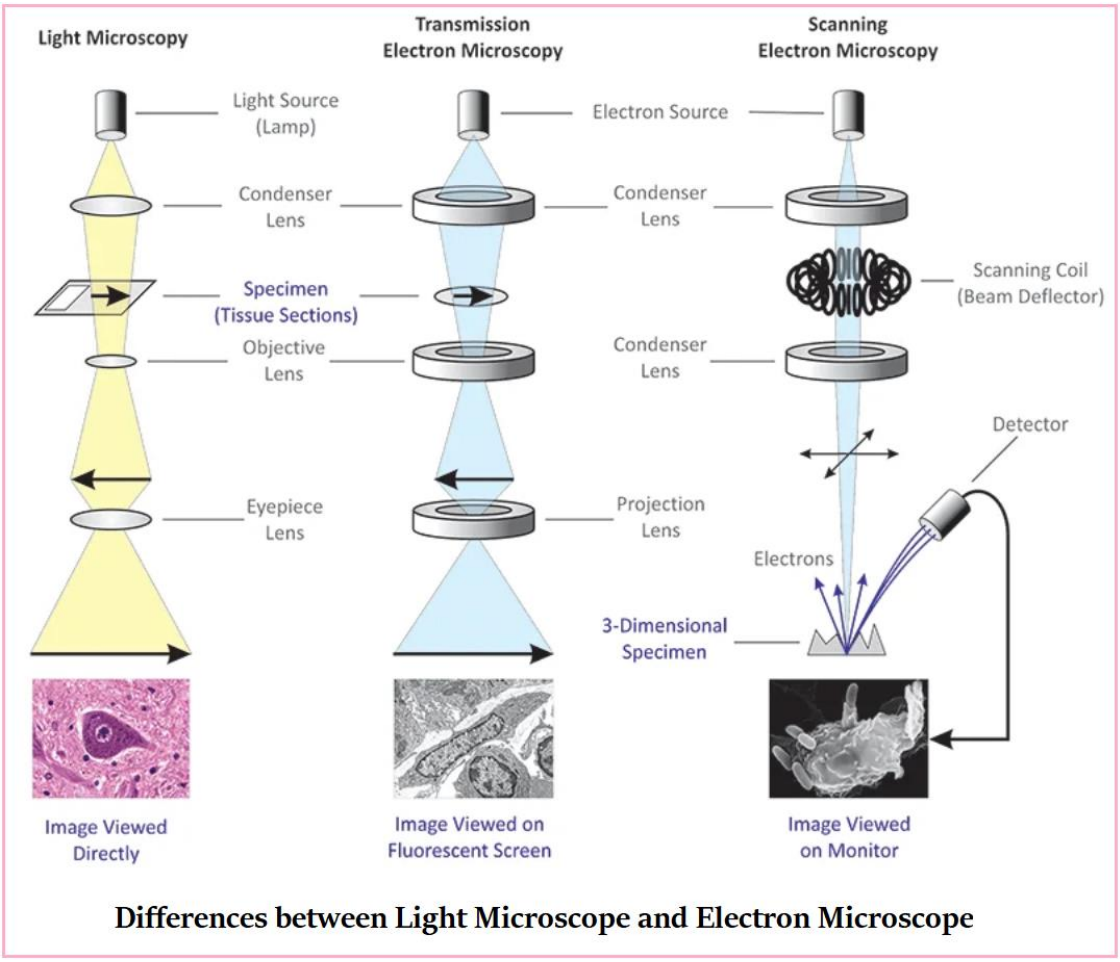


Figure14. Différences between Light Microscope and Electron Microscope

Table 6. Light Microscope vs Electron Microscope

Character	Light Microscope	Electron Microscope
Types/ Variants	Dark-field microscope Phase-contrast microscope Fluorescent microscope Confocal microscope	Transmission electron microscope (TEM) Scanning electron microscope (SEM)
Application	It is used for the study of detailed gross internal structure.	It is used in the study of the external surface, the ultrastructure of cells, and very small organisms.
Principle	The image is formed by the absorption of light waves.	The image is formed by scattering or transmission of electrons.
Lenses used	Lenses are made of glass.	Lenses are made of electromagnets.
Specimen type	Fixed or unfixed, stained or unstained, living or non-living.	Fixed, stained, and non-living.
Specimen observed	Both live and dead specimens can be observed.	Only dead specimens are possible to be observed.
Living processes	Visualization of living processes such as microscopic pond life in action and even cell division is possible.	Living processes cannot be viewed.
Thickness of specimen	5 micrometer or thicker	Ultra-thin, 0.1 micrometers or below
Dehydration of Specimen	Specimens need not be dehydrated before viewing.	Only dehydrated specimens are used.
Coating of specimen	Stained by colored dyes for proper visualization.	Coated with heavy metals to reflect electrons.
Mounting of specimen	Mounted on the glass slide.	Mounted on the metallic grid (mostly copper).
Magnification power	Low magnification of up to 1,500x.	High magnification of up to 1,000,000x.
Resolving power	Low resolving power, usually below 0.30µm.	The high resolving power of up to 0.001µm, about 250 times higher than the light microscope.
Viewing of the image formed	Light microscope images can be viewed directly. Images are viewed by the eyes through the eyepiece.	Images are viewed on a photographic plate or zinc sulfate fluorescent screen.
Advantages	Easy to use Cheap True color but sometimes require staining Live specimens	High resolution Provide detailed images of surface structures and interior structures High magnification 3D images
Disadvantages	Low resolution due to shorter wavelength of light (0.2nm) Low magnification The specimen used is thin.	Expensive Requires extensive training Sample must be dead Black and white/false-color image

2. Techniques Used in Light and Electron Microscopy

2.1 Histological techniques for light microscopy

In routine histology, tissues obtained by biopsy, surgical excision, or autopsy must be processed into thin, stable sections suitable for light microscopic examination, following a sequence of fixation, processing, embedding, sectioning, staining, and mounting. The standard fixative in diagnostic pathology is 10% neutral buffered formalin, corresponding to approximately 4% formaldehyde in phosphate buffer, which cross-links proteins and preserves tissue architecture; specimens are immersed immediately after removal in a fixative volume at least ten times the tissue volume, with slices no thicker than 3-4 mm, and fixation proceeds for roughly 12-48 h at room temperature depending on tissue size.

After fixation, trimmed specimens are placed in labelled perforated cassettes and subjected to tissue processing, which consists of dehydration in ascending alcohol series (for example 70%, 80%, 95% and two changes of 100% ethanol), clearing in an intermediate solvent such as xylene or xylene substitutes, and infiltration with molten paraffin wax at about 56-60 °C; automated processors standardise times and temperatures, but the underlying principle is the gradual replacement of water by a medium compatible with paraffin. Proper control of processing prevents artefacts such as over-shrinkage, vacuolisation or incomplete infiltration, all of which degrade the quality of histological and cytological interpretation.

Following processing, tissues are embedded in paraffin blocks that pre mechanical support for thin sectioning ; blocks are oriented so that diagnostically important surfaces face the cutting plane, rapidly cooled on a cold plate and stored until sectioning. A rotary microtome is then used to cut ribbons of paraffin sections typically 3-5 μm thick for general histology, with thinner sections (1-2 μm) reserved for special applications; the ribbons are floated on a warm water bath to flatten wrinkles, picked up onto glass slides coated for adhesion and dried at 37-60 °C to ensure proper attachment and removal of residual.

For routine staining, sections are first deparaffinised in xylene, rehydrated through descending alcohols to water, and then subjected to hematoxylin eosin (H&E) staining, which remains the cornerstone of diagnostic histology and cytology. Hematoxylin, usually oxidised and combined with a mordant such as aluminium to form a lake, stains basophilic structures rich in nucleic acids (nuclei, ribosomes) a blue-violet hue after differentiation and “blueing” in alkaline solution, whereas eosin, an acidic dye, stains cytoplasmic proteins and extracellular matrix components pink to red; after staining, sections are dehydrated, cleared and mounted with a synthetic resin under a coverslip.

In addition to paraffin sections, frozen section techniques allow rapid intraoperative diagnosis by snap-freezing tissue (for example in isopentane cooled in liquid nitrogen), cutting 5-10 µm sections in a cryostat at -20 to -25 °C, and staining with a rapid H&E protocol within minutes; although frozen sections display somewhat inferior morphology compared with paraffin sections, they preserve lipids and enzyme activities and are indispensable for evaluating surgical margins and certain metabolic diseases.

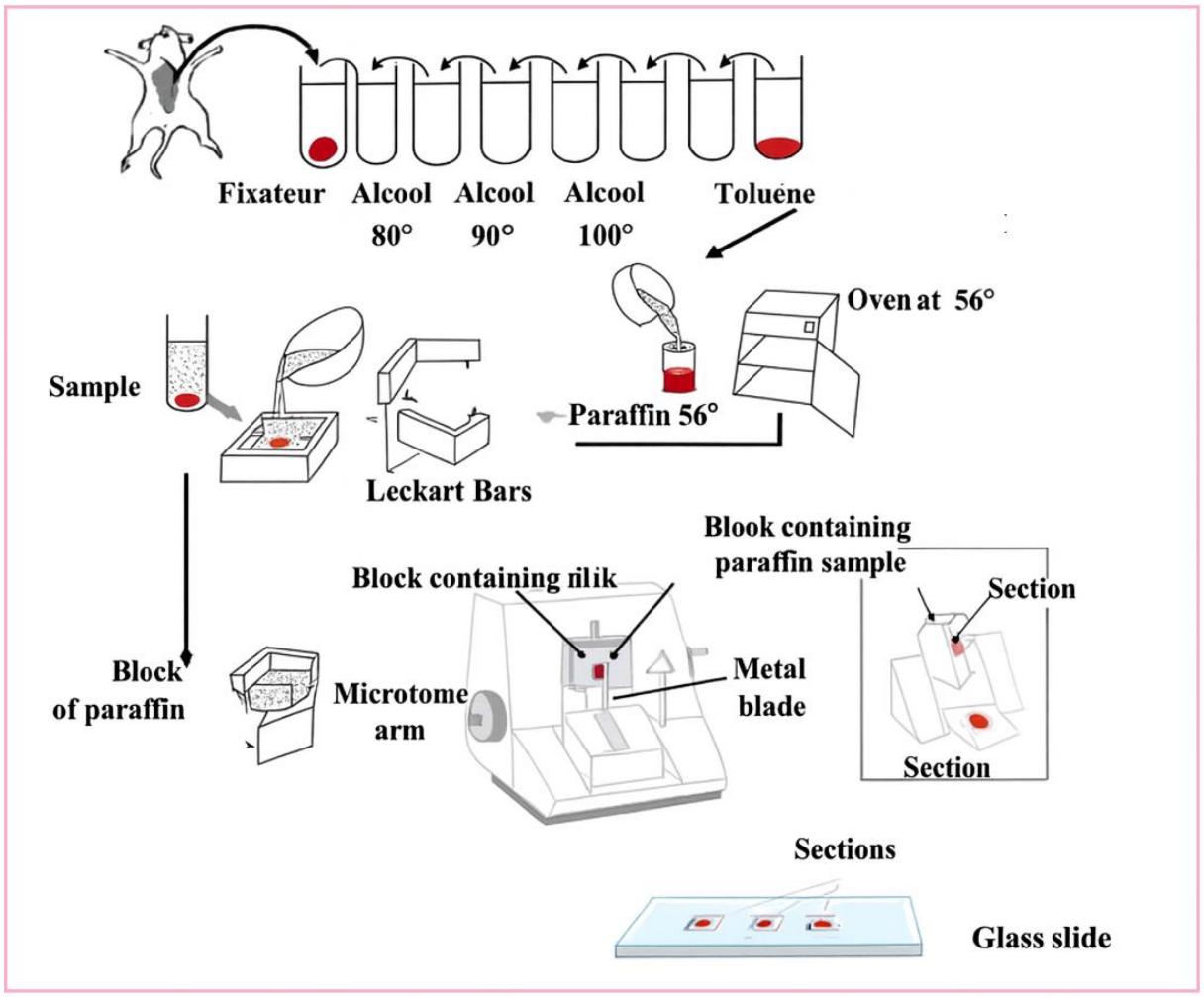


Figure 15. Procedure for sample preparation for light microscopy

2.2 Histological techniques for electron microscopy

Sample preparation for TEM is more demanding because ultrastructure must be preserved at nanometre scale and specimens must be stable in vacuum under the electron beam. The standard approach for conventional TEM is chemical fixation by double aldehyde-osmium fixation: small tissue blocks, ideally no larger than 1 mm^3, are immersed rapidly in 2-3% glutaraldehyde in 0.1 M cacodylate or phosphate buffer at pH 7.2-7.4, for 1-4 h at room temperature or at 4 °C,

to cross-link proteins and stabilise cell architecture. After thorough buffer rinses, a secondary fixation in 1-2% osmium tetroxide for 1-2 h preserves and stains membrane lipids, producing excellent contrast of bilayers and organelle membranes.

Following fixation, specimens are dehydrated in graded ethanols or acetone and infiltrated with an epoxy resin such as Epon or Araldite, which is polymerised at elevated temperature (commonly 60 °C for 24-48 h) to yield hard blocks suitable for ultrathin sectioning. Sections 60-90 nm thick are cut with a glass or diamond knife on an ultramicrotome, collected on copper or nickel grids coated with a thin support film and then subjected to contrast staining with heavy metals, typically 1-2% aqueous uranyl acetate for 10-20 min followed by lead citrate for 3-10 min, which bind preferentially to nucleic acids, membranes and protein complexes and enhance electron scattering.

An alternative to chemical fixation is cryofixation, in which specimens are vitrified by rapid freezing under high pressure or by impact against a liquid-nitrogen-cooled metal block, thereby immobilising molecules in near-native configuration without the artefacts of chemical cross-linking and dehydration. Cryofixed samples may be processed by freeze-substitution, in which water is replaced by organic solvent at low temperature in the presence of fixatives, or used directly for cryo-electron microscopy of vitreous sections, but these advanced methods exceed the requirements of routine diagnostic cytology and are mentioned here primarily to emphasise the importance of fixation speed and specimen size in preserving ultrastructure.

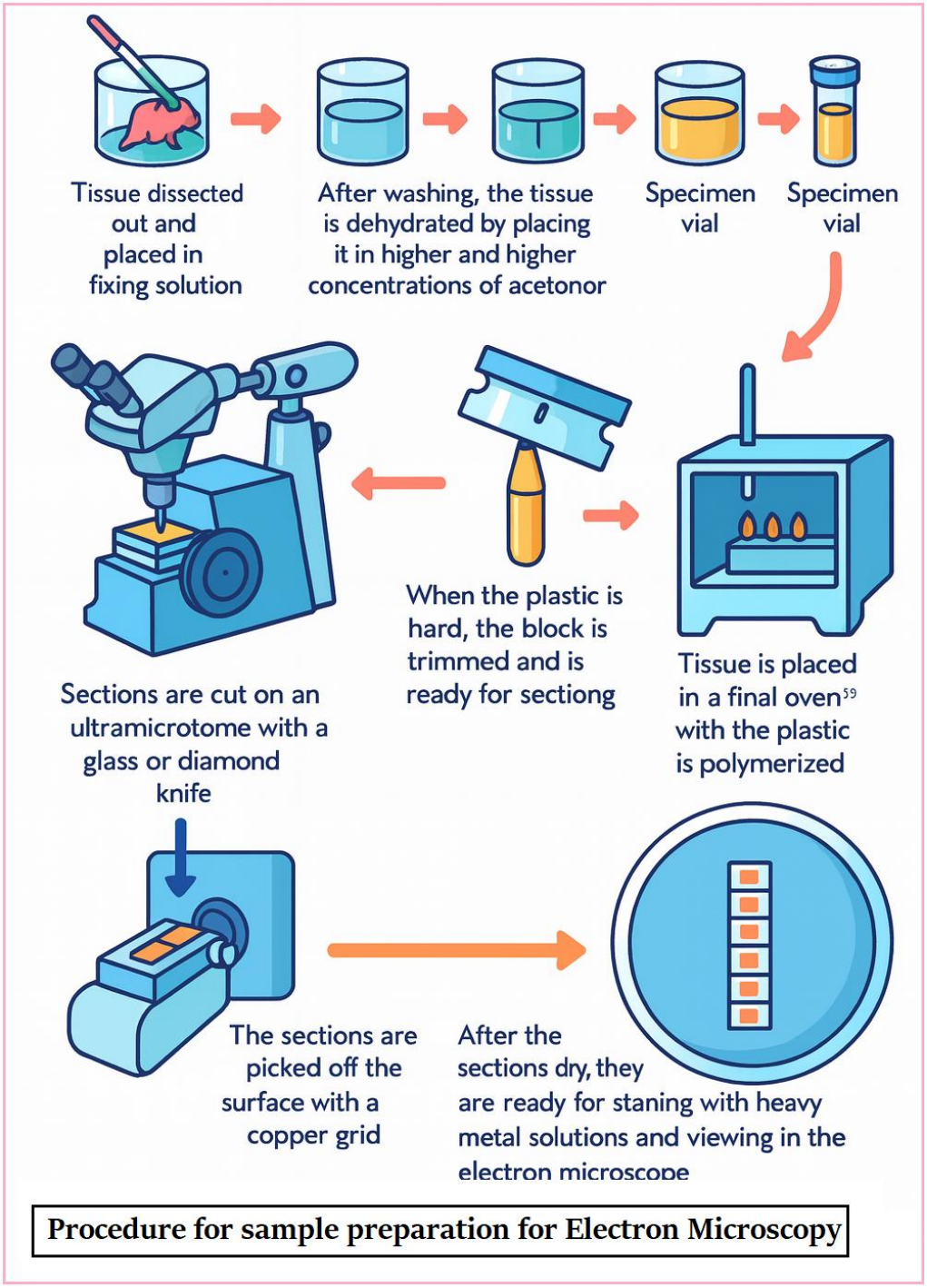


Figure 16. Procedure for sample preparation for Electron Microscopy

3 Techniques for Observing Shape and Surface

3.1 Negative contrast (negative staining)

Negative staining is a TEM technique that enhances the visibility of small particles such as viruses, ribonucleoprotein complexes or cytoskeletal fragments by surrounding them with an electron-dense, amorphous film of heavy-metal salt, so that the specimen appears as a light silhouette against a dark background. Because the stain does not usually penetrate the particle,

surface contours and overall shape are preserved with minimal preparation, making the method extremely rapid and sensitive for morphological screening of particles in biological fluids or purified preparations .

In a typical negative-staining protocol, a small volume of sample (for example 5-10 µL) containing the particles is applied to a carbon-coated copper grid that has been rendered hydrophilic by glow discharge, allowed to adsorb for about 30-60 s, then blotted gently with filter paper to leave a thin film; the grid is then washed briefly with distilled water if necessary and stained with one or more drops of heavy-metal solution such as 0.5-1% uranyl acetate at acidic pH or 1-2% phosphotungstic acid adjusted to pH 6.5-7.0. After 30-60 s of contact, excess stain is removed by blotting from the edge of the grid, which is then air-dried for several minutes to hours before examination in the TEM at 80-120 kV.

From a diagnostic perspective, negative staining is invaluable for fast visual confirmation of viruses in clinical samples, for example in suspected poxvirus or rotavirus infections, and for quality control of purified viral preparations or nanoparticles; however, the technique pres limited internal detail and is susceptible to flattening artefacts and aggregation, so it is usually complemented by resin-embedding methods when precise ultrastructural analysis is required. .

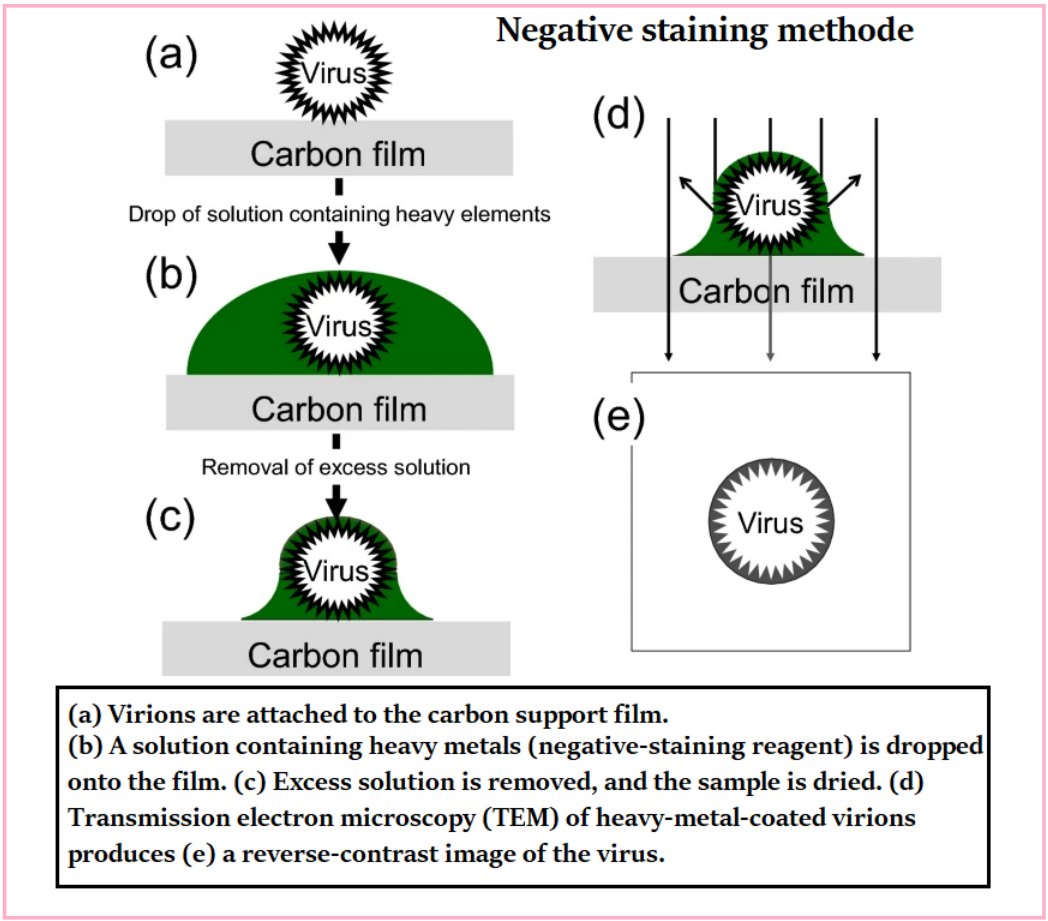


Figure 17. Negativ staining methode

3.2 Metal shadowing

Metal shadowing (including rotary shadowing and replica techniques) is an electron microscopic method in which a very thin layer of metal, often platinum - carbon, is evaporated onto a specimen at a low angle, creating a gradient of deposited metal that transforms subtle height differences into contrast in the TEM image. When combined with carbon backing and removal of underlying biological material, metal shadowing produces durable replicas that faithfully reproduce surface topography at nanometre scale, making it possible to visualise fine structures such as DNA filaments, cytoskeletal networks or membrane surfaces that pre insufficient intrinsic contrast.

In a classical rotary-shadowing protocol, macromolecular assemblies or cytoskeletal preparations are adsorbed onto freshly cleaved mica or a thin carbon film, gently fixed and dehydrated if required, then introduced into a high-vacuum coating unit where the specimen is rotated while a metal such as platinum-carbon is evaporated from an electron-beam or resistance source at a shallow angle (for example 5-7° relative to the specimen plane), followed by deposition of a stabilising carbon film. The resulting metal-carbon replica is floated off the support onto water, collected on TEM grids and, if necessary, cleaned by digestion of residual biological material with bleach or acid before imaging; careful control of metal thickness (a few nanometres) and evaporation angle is essential to avoid loss of fine detail or obscuration of the underlying structure.

Because metal shadowing emphasises surface relief, it is particularly well suited to measure filament lengths and branching patterns, to map the organisation of cytoskeletal networks, and to explore surface organisation of membranes or extracellular matrices; in many applications, it has been superseded by cryo-EM methods, but it remains an instructive technique in cell biology teaching for illustrating how specimen preparation and contrast generation influence the appearance of biological structures.

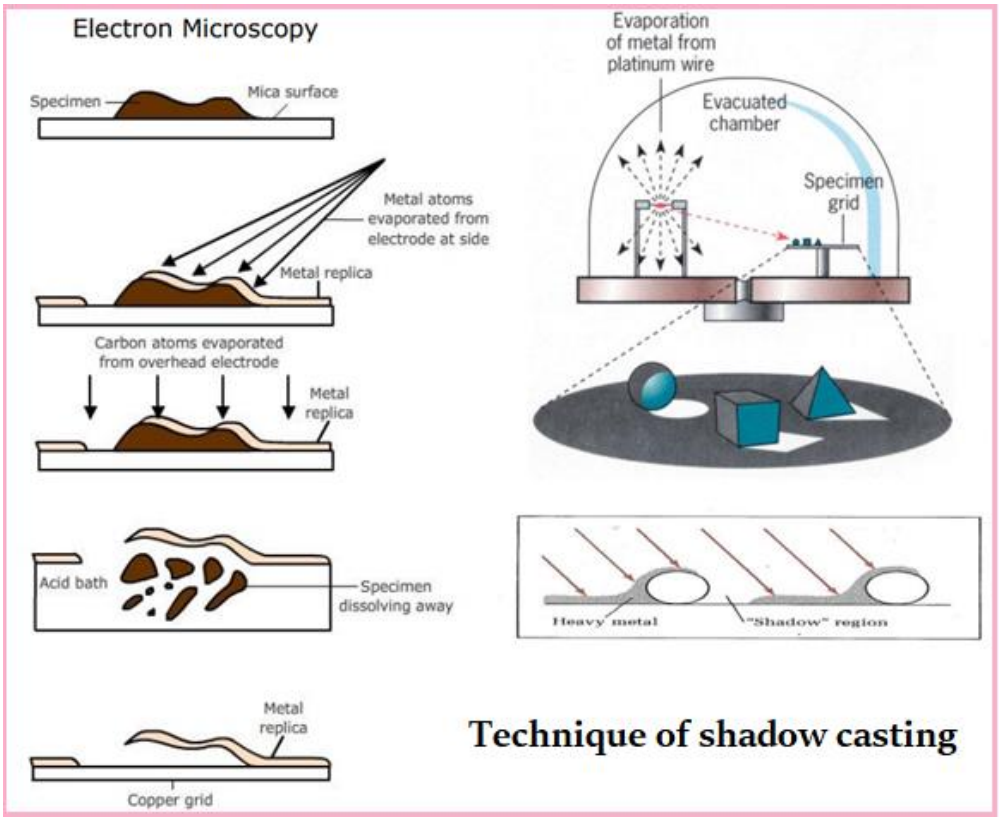


Figure 18. Technique of shadowing casting

3.3 Cryofracture (freeze fracture)

Cryofracture, usually referred to as freeze-fracture electron microscopy, is a replica technique in which specimens are rapidly frozen and then fractured under high vacuum at very low temperatures, causing membranes to split preferentially along their hydrophobic interior and exposing large planar views of membrane leaflets and intramembranous particles. The freshly exposed fracture faces are then shadowed with platinum and backed with carbon to form replicas that, after cleaning of biological material, can be examined by TEM, uniquely revealing the distribution and density of integral membrane proteins within the lipid bilayer .

In a typical protocol, tissues or cells are lightly chemically fixed in buffered glutaraldehyde, cryoprotected by infiltration with cryoprotectants such as 20-30% glycerol, then rapidly frozen, for example by immersion in liquid nitrogen-cooled Freon or by high-pressure freezing, to prevent ice crystal formation. The frozen specimens are fractured in a freeze-fracture device at temperatures around -100 to -150 °C and high vacuum, often followed by a brief “etching” step in which the temperature is raised slightly (for example to -90 °C) to sublimate a thin layer of surface ice and expose additional relief; the fracture faces are then shadowed with platinum at low angle and coated with a stabilising carbon film, after which the replicas are separated from

the biological material by digestion in strong acid or detergent and mounted on TEM grids for observation .

Freeze-fracture and related freeze-etching methods occupy a special position among EM techniques because they pre complementary “en face” views of membrane organisation compared with conventional thin sections, and were crucial in establishing the mosaic distribution of proteins in lipid bilayers and the heterogeneity of junctional domains such as tight and gap junctions. For the student of cytology, they illustrate both the power of physical methods for preserving near-native structures and the need to interpret ultrastructural images in light of the specimen preparation path that produced them .

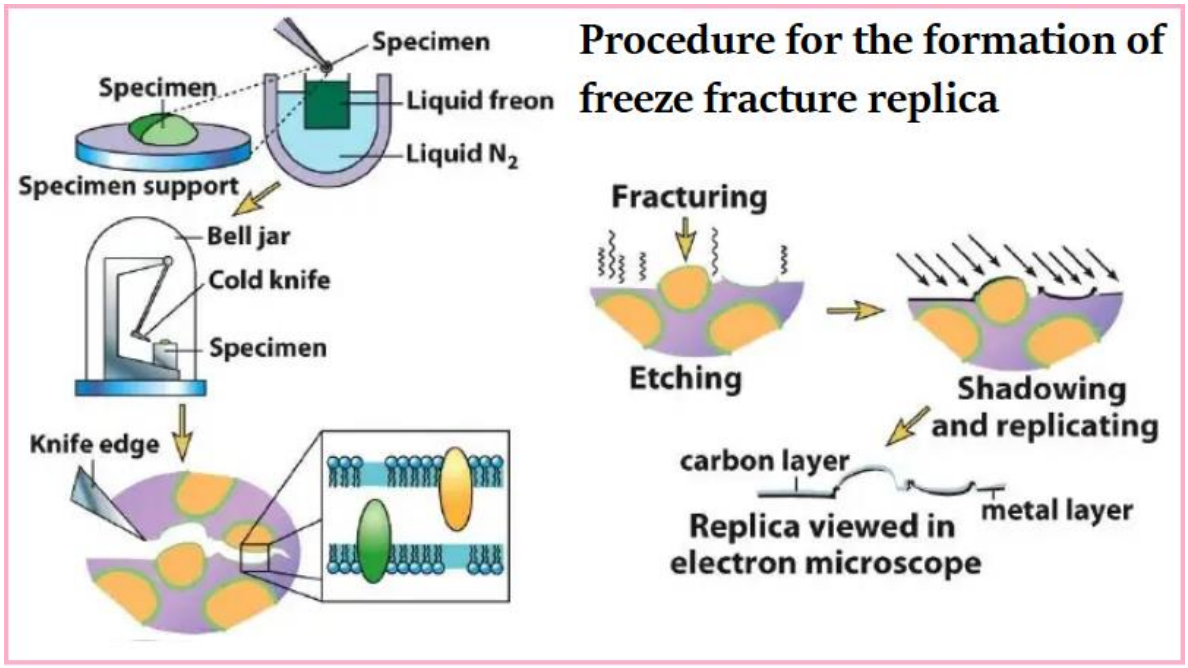


Figure 19. Procedure for the formation of freeze fracture replica

4 Centrifugation Techniques

Ultracentrifugation exploits high centrifugal forces, often 10^5-10^6 × g, generated by rapidly rotating rotors, to sediment particles such as organelles, macromolecular complexes or viruses according to their size, shape, and density, enabling cell fractionation, which separates cellular components while preserving many of their biochemical functions. In contrast to microscopy, which visualises intact cells and structures in situ, fractionation by centrifugation produces enriched preparations of nuclei, mitochondria, lysosomes, microsomes or cytosolic proteins that can be analysed biochemically, thus linking cellular morphology to function.

4.1 Differential centrifugation

Differential centrifugation separates subcellular components by subjecting a cell homogenate to a series of centrifugation steps at progressively higher centrifugal forces and durations, so that larger and denser particles sediment at lower g forces and shorter times, while smaller, lighter components require higher speeds and longer runs to form pellets. In a classic protocol derived from studies on liver or other soft tissues, cells are homogenised in an isotonic sucrose or mannitol buffer containing protective agents at 0-4 °C, then centrifuged at around 600-1000 × g for 5-10 min to remove unbroken cells, nuclei and large debris as a first pellet (P1).

The post-nuclear supernatant is then centrifuged at intermediate forces such as 10,000-20,000 × g for 15-30 min to sediment a second pellet (P2) enriched in mitochondria, lysosomes and peroxisomes, while the resulting supernatant can be further centrifuged at very high speed, for example 100,000 × g for 60-90 min in an ultracentrifuge, to yield a microsomal pellet (P3) containing fragments of endoplasmic reticulum and plasma membrane; the remaining supernatant constitutes the soluble cytosolic fraction. Each fraction can be analysed by enzyme assays or immunoblotting using marker proteins, allowing the localisation of specific activities to particular organelles, although overlap between fractions is inevitable and often requires subsequent purification.

From a practical standpoint, differential centrifugation is relatively simple and rapid, making it a standard technique in teaching laboratories and in many research protocols; however, the pellets are often heterogeneous, and shear forces during homogenisation or centrifugation can damage fragile organelles, so care must be taken in buffer design, temperature control and acceleration/deceleration profiles to preserve functional integrity. In cytology, understanding the typical sedimentation behaviour of organelles helps to interpret biochemical fractionation data in relation to the structures seen in light and electron micrographs.

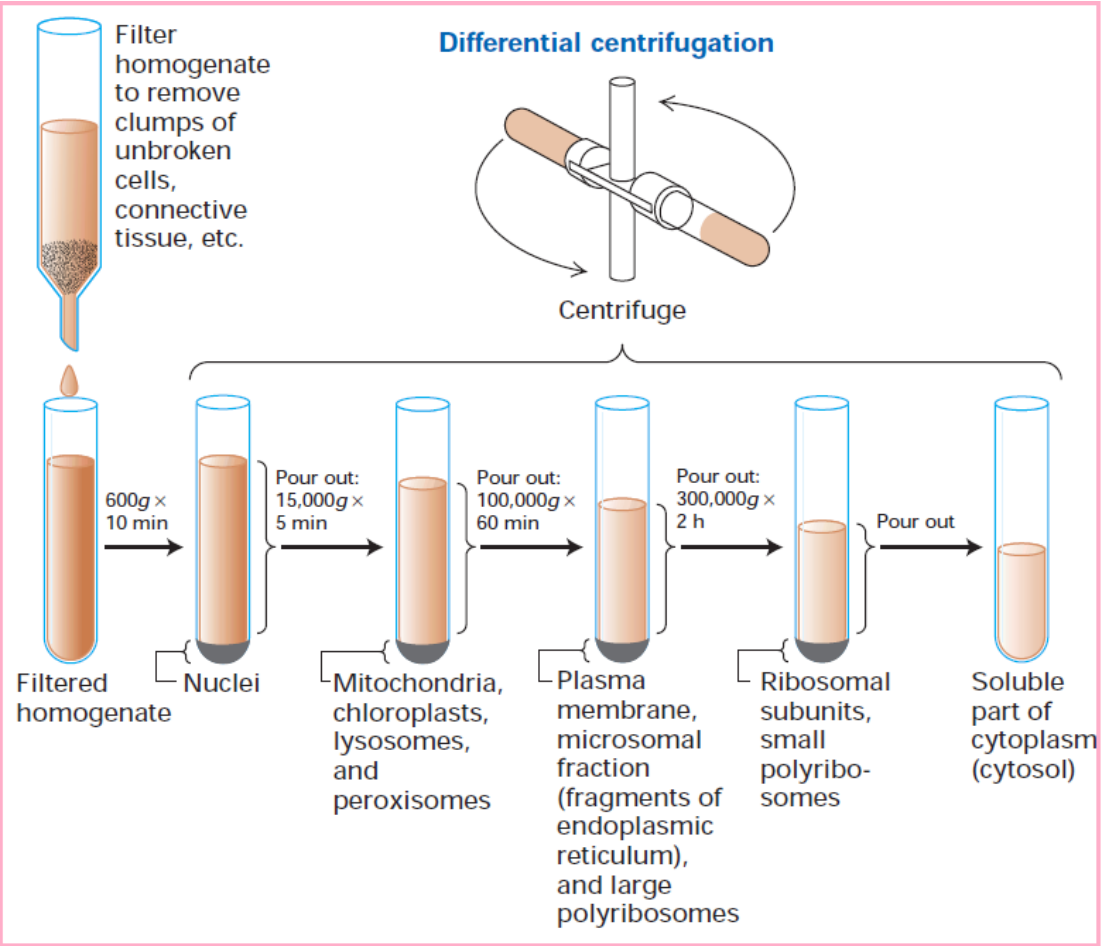


Figure 20. Separation of subcellular components by differential centrifugation.

4.2 Density-gradient ultracentrifugation

Density-gradient ultracentrifugation introduces a continuous or stepwise gradient of solute, such as sucrose, Ficoll or cesium chloride, in which particles migrate during centrifugation until they reach a region where the density of the gradient equals their own, thereby achieving separation based on buoyant density rather than solely on size and mass. Two main formats are distinguished: rate-zonal centrifugation, in which particles layered on top of a shallow gradient separate according to sedimentation rate before reaching their equilibrium position, and isopycnic (equilibrium) centrifugation, in which particles band at their buoyant density in a sufficiently long run.

In a typical organelle purification protocol, one first obtains a crude mitochondrial or membrane fraction by differential centrifugation, resuspends the pellet in 0.25 M sucrose or similar buffer and carefully layers it on top of a pre-formed sucrose gradient, for example 20-60% (w/v) sucrose in buffer, with the highest concentration at the bottom; the tubes are then centrifuged at 50,000-150,000 × g for several hours (often 2-16 h) in a swinging-bucket rotor until distinct

opaque bands appear at characteristic positions corresponding to organelles of different densities. These bands are collected from the top or bottom of the tube using pipettes or gradient fraction collectors, and the identity and purity of each fraction are assessed by marker enzymes, immunoblotting or electron microscopy.

Density-gradient methods are widely used not only for organelles but also for the purification of viruses, lipoproteins and ribosomal subunits, exploiting subtle differences in buoyant density that cannot be resolved by differential centrifugation alone; for example, many enveloped viruses band at densities around 1.18-1.22 g/mL in sucrose or iodixanol gradients. For the student of cytology, these techniques exemplify how physical properties such as density and sedimentation coefficient can be harnessed to dissect the complex organisation of the cell into operational fractions, thereby connecting structural observations in microscopy with biochemical assays of defined cellular compartments.

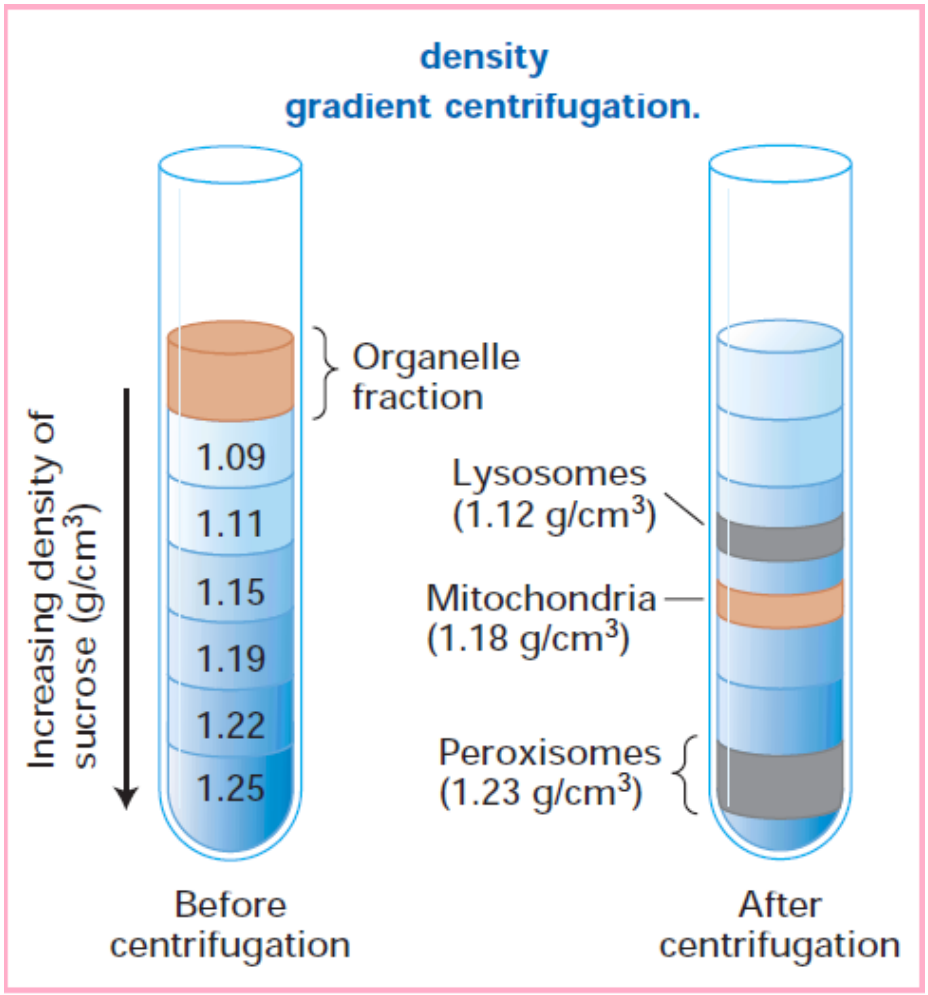


Figure 21. Density gradient centrifugation for the separation of organelles

The lesson in a nutshell

1) Why Techniques Matter

*Cell biology uses **microscopy and biochemical methods** to:*

- *Visualize cell structure and ultrastructure*
- *Study cell morphology and surface features*
- *Isolate organelles and macromolecules*

*These tools link **structure to function** in research and diagnostics.*

2) Light Microscope (LM)

Characteristics

- *Uses visible light and glass lenses*
- *Magnification: up to **1000–1500**×*
- *Can observe **living or fixed cells***

Applications

- *Cell and tissue morphology*
- *Routine histology (H&E staining)*

Variants

- *Bright-field (routine)*
- *Phase-contrast (living cells)*
- *Fluorescence (specific molecules)*

3) Electron Microscopy (EM)

Transmission EM (TEM)

- *Electrons pass through **ultrathin sections***
- *Shows **internal ultrastructure***
- *Resolution at nanometer scale*

Scanning EM (SEM)

- *Scans specimen surface*
- *Produces **3D surface images***
- *Used for microvilli, cilia, membranes*

Limits

- *Specimens must be **fixed, dehydrated, dead***
- *Expensive and complex preparation*

4) Sample Preparation for Light Microscopy

Main steps:

1. ***Fixation** (formalin)*
2. ***Dehydration** (alcohol)*
3. ***Clearing** (xylene)*
4. ***Embedding** (paraffin)*
5. ***Sectioning** (3–5 µm)*
6. ***Staining** (Hematoxylin–Eosin)*
7. ***Mounting***

5) Sample Preparation for TEM

1. *Fixation (glutaraldehyde + osmium)*
2. *Dehydration*
3. *Resin embedding*
4. *Ultrathin sectioning (60–90 nm)*
5. *Heavy metal staining (uranyl, lead)*

6) Surface & Special EM Techniques

- ***Negative staining****: viruses, small particles*
- ***Metal shadowing****: surface topography*
- ***Freeze-fracture****: membrane structure and protein distribution*

7) Cell Fractionation (Centrifugation)

Differential Centrifugation

*Separates by **size/density** through increasing speeds:*

- *Low speed → nuclei*
- *Medium → mitochondria, lysosomes*
- *High → microsomes*
- *Supernatant → cytosol*

Density Gradient Centrifugation

*Separates by **buoyant density***

- *Forms distinct bands*
- *Used for organelles, ribosomes, viruses*

CHAPTER V : PLASMA MEMBRANE AND CELLULAR EXCHANGES

Lesson Objectives

In this chapter, we'll take a look at the

- Membrane Functions
- Chemical Composition of Membranes
- Movement of Substances Across Cell Membranes

Introduction

The plasma membrane, or cell membrane, is a thin (≈7-10 nm) lipid-protein bilayer that delimits the cell, separates the intracellular from the extracellular medium, and pres the structural basis for selective exchanges, signal reception, and mechanical integrity in all human cells. In a typical human erythrocyte, the membrane consists of roughly 49% protein, 43% lipid and about 8% carbohydrate by mass, illustrating that the membrane is not merely a lipid film but a complex, dynamic organelle in its own.

The “fluid mosaic model” describes the plasma membrane as a two-dimensional fluid of lipids in which proteins are laterally mobile, while carbohydrates attached to lipids and proteins form an external glycocalyx involved in recognition and adhesion. This structural organisation underlies essential physiological functions such as maintenance of ionic gradients, transduction of hormonal and neurotransmitter signals and control of cell volume; conversely, many diseases, from hereditary spherocytosis to cystic fibrosis, are now understood as disorders of membrane components or transport processes.

1 Structure of the Plasma Membrane

At the ultrastructural level, the plasma membrane appears in transmission electron microscopy as a trilaminar “railroad track” corresponding to a phospholipid bilayer with electron-dense protein-lipid interfaces on both sides and a less dense hydrophobic layer, with an overall thickness between 5 and 10 nm in mammalian cells. Amphipathic phospholipids spontaneously assemble in aqueous media into bilayers, with hydrophobic fatty acyl chains facing inward and polar head groups oriented toward the cytosol or extracellular fluid, thereby pring a thermodynamically stable yet fluid matrix for membrane proteins .

The plasma membrane is laterally fluid: individual phospholipids undergo lateral diffusion on the order of 10^7 translocations per second, whereas spontaneous "flip-flop" between leaflets is rare in the absence of specific flippases, maintaining a stable transverse asymmetry. This asymmetry is functionally crucial: choline phospholipids such as phosphatidylcholine and sphingomyelin are enriched in the outer leaflet, whereas aminophospholipids such as phosphatidylethanolamine and phosphatidylserine are largely confined to the cytoplasmic leaflet, and exposure of phosphatidylserine on the outer surface serves as an "eat-me" signal during apoptosis and eryptosis.

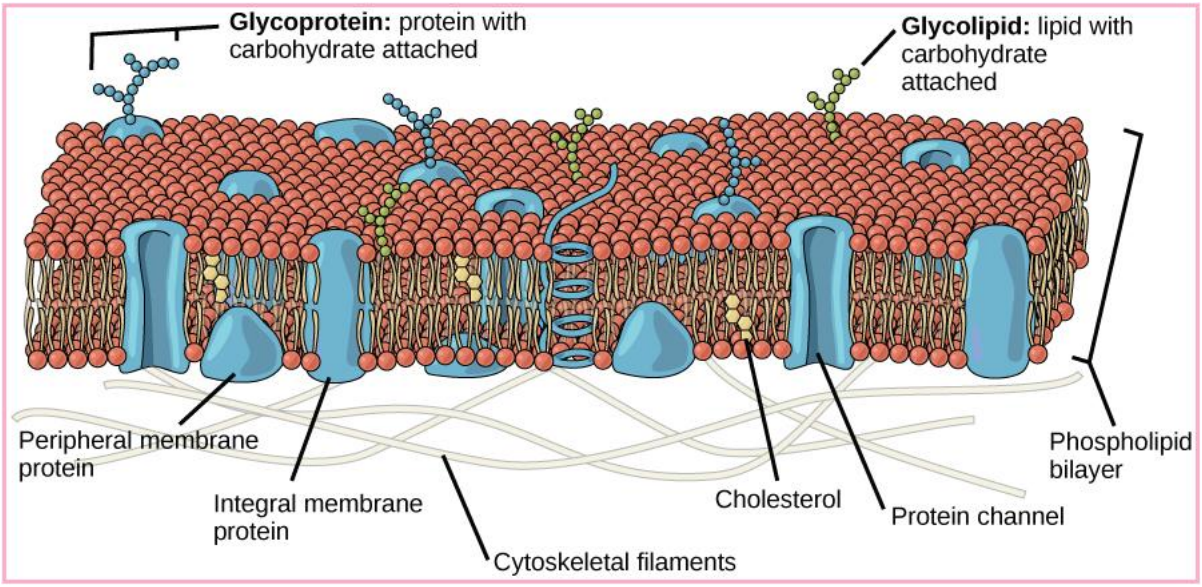


Figure 22. Molecular organization of the plasma membrane (fluid mosaic model)

2 Constituents of the Plasma Membrane

2.1 Membrane lipids

Membrane lipids are the basic structural framework and largely determine membrane fluidity and passive permeability. In human erythrocyte membranes, phospholipids and cholesterol together account for ≈45-50% of the dry mass, with glycolipids contributing a smaller fraction, consistent with detailed compositional analyses of red cell. This general proportion of lipids versus proteins (≈40-50% lipids, 50-60% proteins by mass) is typical of many mammalian plasma membranes, though absolute lipid species and ratios vary with cell type and organelle.

Phospholipids are amphipathic lipids composed of a glycerol or sphingosine backbone esterified to two hydrophobic fatty acyl chains (14-24 carbon atoms, often with one or more cis double bonds) and a polar phosphate-containing head group (e.g., choline, ethanolamine, serine, inositol). In human erythrocytes, major phospholipids include phosphatidylcholine (≈28-30% of total phospholipid), sphingomyelin (≈25-26%), phosphatidylethanolamine (≈26-27%), and

phosphatidylserine (≈12-13%). The degree of fatty acid unsaturation modulates bilayer thickness and fluidity: unsaturated chains introduce kinks, reducing packing density and increasing permeability to small solutes, which is particularly relevant in excitable membranes and thermoregulation.

Cholesterol is a rigid, amphipathic sterol whose hydroxyl group aligns near phospholipid head groups while its planar ring system intercalates among fatty acyl chains, representing about 25-30% of total red blood cell membrane lipid and a molar cholesterol/phospholipid ratio close to 0.8. Cholesterol buffers membrane fluidity by restricting acyl chain motion at high temperature and preventing tight packing at low temperature, thereby stabilising bilayer organisation and decreasing permeability to small hydrophilic molecules, which is essential for erythrocyte deformability and nerve conduction.

Glycolipids are lipids bearing one or more carbohydrate residues, typically glycosphingolipids derived from sphingosine in animal cells, located exclusively in the outer leaflet and accounting for roughly 2-5% of membrane lipid. They contribute to the glycocalyx and carry important antigenic determinants; for example, ABO blood group antigens correspond to specific terminal carbohydrate structures attached to both glycolipids and glycoproteins on the erythrocyte surface, with biochemical and genetic analyses confirming that these histo-blood group glycans modulate susceptibility to infection and thrombosis.

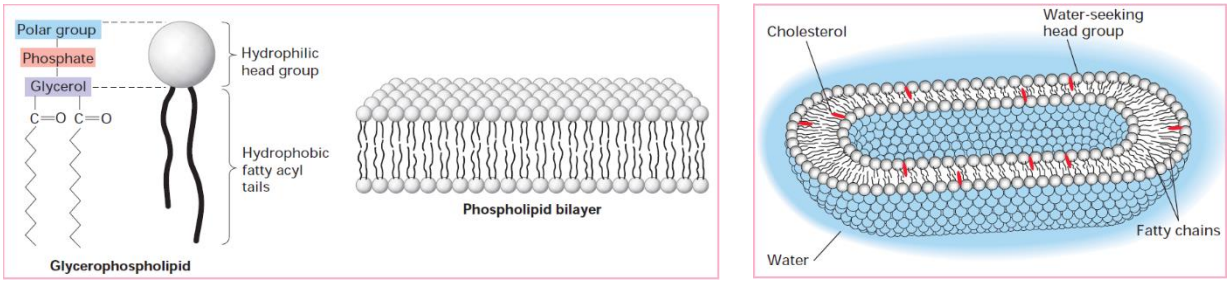


Figure 23. Structure and organization of the phospholipid bilayer of the plasma membrane.

2.2 Membrane proteins

Membrane proteins account for about half the mass of the plasma membrane and mediate most of its specific functions, including solute transport, receptor signalling, cell-cell adhesion and enzymatic catalysis. Integral membrane proteins are embedded within the lipid bilayer through one or more stretches of hydrophobic amino acids forming α-helices or β-barrels; peripheral membrane proteins are attached non-covalently to membrane surfaces or to integral proteins,

while some proteins are covalently anchored by lipid moieties such as prenyl groups or glycosylphosphatidylinositol (GPI).

Integral membrane proteins typically possess one or several transmembrane α-helices of about 20-25 hydrophobic residues and may form channels, carriers, receptors, or cell-adhesion molecules, their topology being established during biosynthesis in the endoplasmic reticulum and maintained in the mature membrane. Classical examples include voltage-gated Na^+ and K^+ channels in neurons, the Na^+/K^+-ATPase (a P-type ATPase) that maintains ionic gradients, and G protein-coupled receptors for hormones and neurotransmitters, whose overexpression or mutation contributes to diseases such as channelopathies, cardiac arrhythmias, and endocrine disorders.

Peripheral membrane proteins include cytoskeletal elements that underlie the inner leaflet and interact with integral proteins to stabilise cell shape; in erythrocytes, spectrin, ankyrin, band 3 and protein 4.2 form a membrane-skeleton network that confers the characteristic biconcave shape and high deformability required for passage through capillaries of 3-5 μm diameter (Hajjawi, 2013; King et al., 2013). Mutations in genes encoding these proteins (e.g. ANK1, SPTA1, SPTB, SLC4A1) lead to hereditary spherocytosis and elliptocytosis, in which loss of membrane surface area and altered cytoskeletal cohesion produce spherical or elliptical erythrocytes with increased osmotic fragility and haemolytic anaemia.

Glycoproteins, defined as proteins covalently linked to oligosaccharide chains, are major components of the external membrane surface and, together with glycolipids and secreted proteoglycans, build the glycocalyx that can reach tens of nanometres in thickness in endothelial cells. In erythrocytes, glycoproteins such as band 3 and glycophorins carry many blood group antigens, while in leukocytes, sialylated glycoproteins bearing sialyl-Lewis^x and related determinants mediate selectin-dependent rolling on the vascular endothelium during inflammation, linking membrane carbohydrate structures to immunological and vascular pathophysiology.

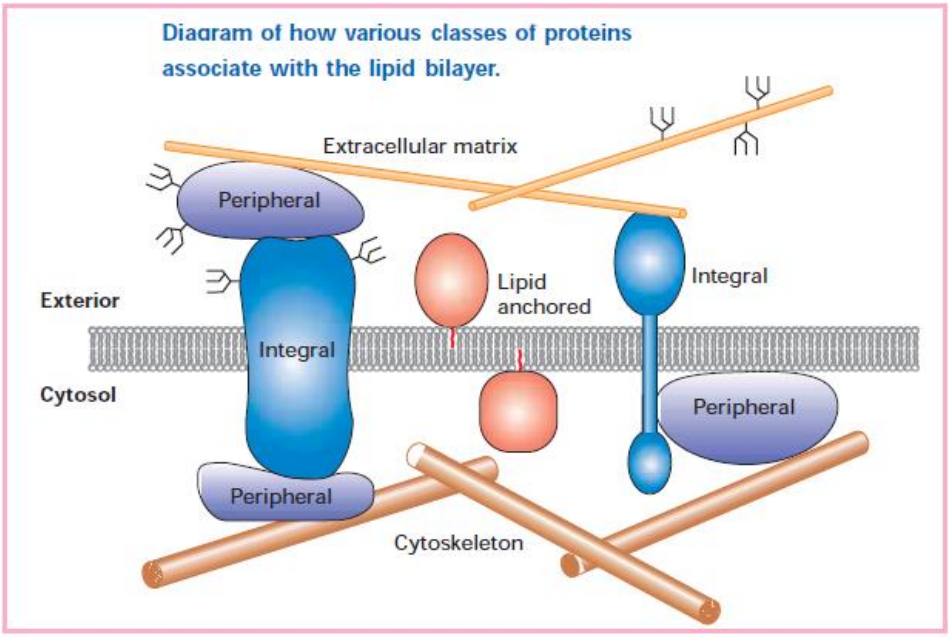


Figure 24. Proteins associate with the lipid bilayer

2.3 Membrane carbohydrates

Membrane carbohydrates, almost entirely exposed on the extracellular face, are attached to lipids (glycolipids), to proteins (glycoproteins) or form part of transmembrane proteoglycans, together constituting the glycocalyx that coats many animal cells. The glycocalyx participates in cell-cell recognition, receptor-ligand specificity and mechanical protection; for instance, ABO and related histo-blood group glycans influence host-pathogen interactions and susceptibility to infections, while endothelial glycocalyx degradation is associated with sepsis, acute kidney injury, and microvascular dysfunction.

3 Membrane Permeability and Transmembrane Transport

The lipid bilayer is intrinsically permeable to small non-polar molecules such as O_2, CO_2 and many lipophilic drugs, moderately permeable to small uncharged polar molecules like water and urea, and effectively impermeable to ions and larger polar solutes in the absence of transport proteins, as demonstrated in artificial bilayer systems and biological membranes. Selective permeability therefore results from the combined properties of the lipid matrix and an array of channels, carriers and pumps, which allow the cell to maintain intracellular ionic compositions that differ markedly from the extracellular fluid (e.g. high K^+, low Na^+ and Ca^{2+} in the cytosol).

3.1 Passive permeability

Passive transport denotes movement of solutes or water across the membrane down their electrochemical or osmotic gradients without the direct expenditure of metabolic energy by the cell. In simple diffusion, non-polar or slightly polar molecules dissolve in the lipid phase and move according to Fick's law, with flux proportional to the concentration gradient, membrane surface area, and permeability coefficient, a mechanism essential for gas exchange across the alveolo-capillary barrier .

3.1.1 Osmosis

Osmosis is the net movement of water across a selectively permeable membrane from a compartment of lower effective solute concentration (lower osmolality) toward one of higher osmolality, driven by the difference in water chemical potential. In biological membranes, water crosses both by diffusion through the lipid bilayer and, much more rapidly, through specialised water channels known as aquaporins, whose narrow pores facilitate single-file water movement while excluding ions, as shown by structural and functional studies.

In humans, normal plasma osmolality is tightly maintained between approximately 275 and 295 mOsm/kg, and isotonic solutions such as 0.9% NaCl have an effective osmolality close to this range (≈287 mOsm/kg when osmotic coefficients are considered), preventing net water shifts and volume changes in erythrocytes and other cells. Exposure of cells to hypotonic solutions leads to water influx, cell swelling and potential lysis, while hypertonic solutions cause cell shrinkage; these phenomena are routinely exploited in laboratory tests such as erythrocyte osmotic fragility, and mismanagement of intravenous fluids can precipitate cerebral oedema or dehydration .

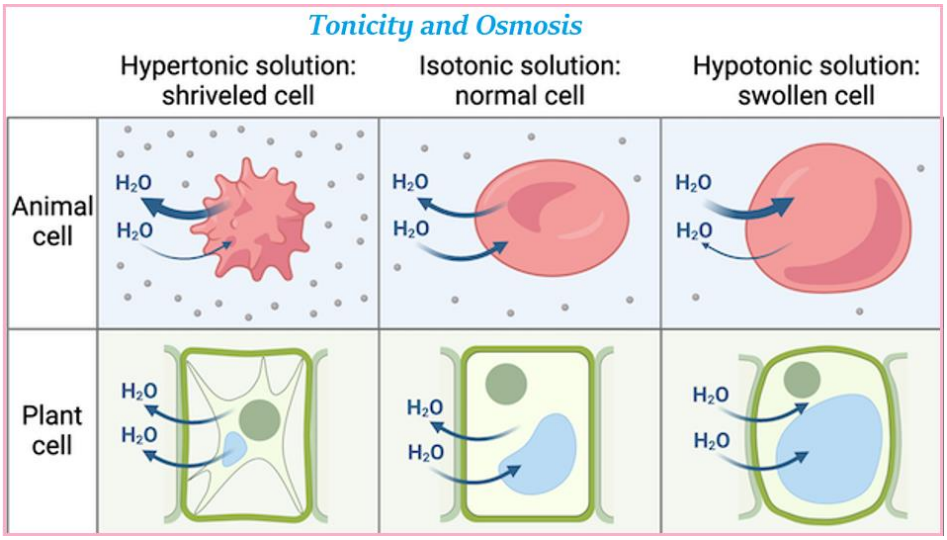


Figure 25. Tonicity and Osmosis

3.1.2 Diffusion: simple and facilitated

Simple diffusion through the lipid bilayer is limited to small non-polar molecules and some small uncharged species; many physiological solutes instead require facilitated diffusion via specific transport proteins, a process that remains passive because the solute moves down its electrochemical gradient. Channel proteins form aqueous pores that allow ions or water to cross rapidly, with selectivity filters and gating mechanisms (e.g. voltage, ligand or mechanical stimuli) controlling opening; carrier (or transporter) proteins alternately expose a substrate-binding site to each side of the membrane, showing specificity and saturable kinetics.

Facilitated diffusion is exemplified by the GLUT1 glucose transporter in erythrocytes, which mediates rapid, bidirectional, stereospecific glucose transport with a Km in the millimolar range, ensuring adequate glucose supply to tissues such as the brain while displaying saturation at high substrate concentrations. Ion channels such as inward-rectifier K^+ channels and voltage-gated Na^+ channels underlie the resting membrane potential and action potentials in excitable cells; genetic mutations in these proteins cause channelopathies presenting as epilepsy, cardiac arrhythmias or periodic paralysis, highlighting the clinical importance of facilitated diffusion mechanisms.

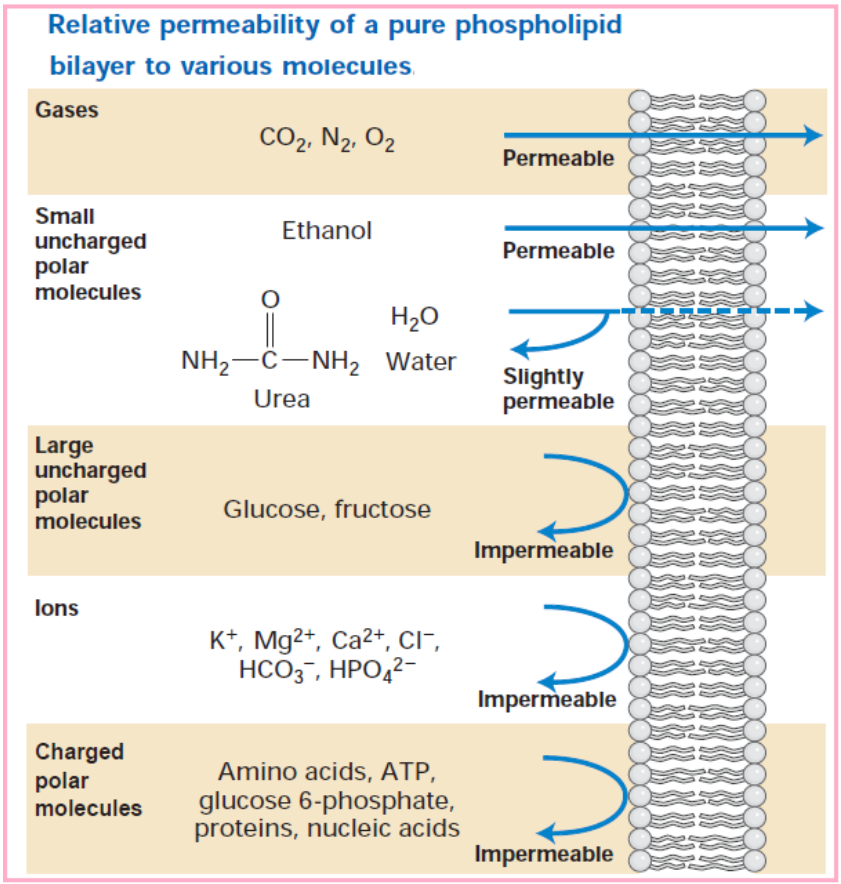


Figure 26. Relative permeability of a pure phospholipid bilayer to varios molecules

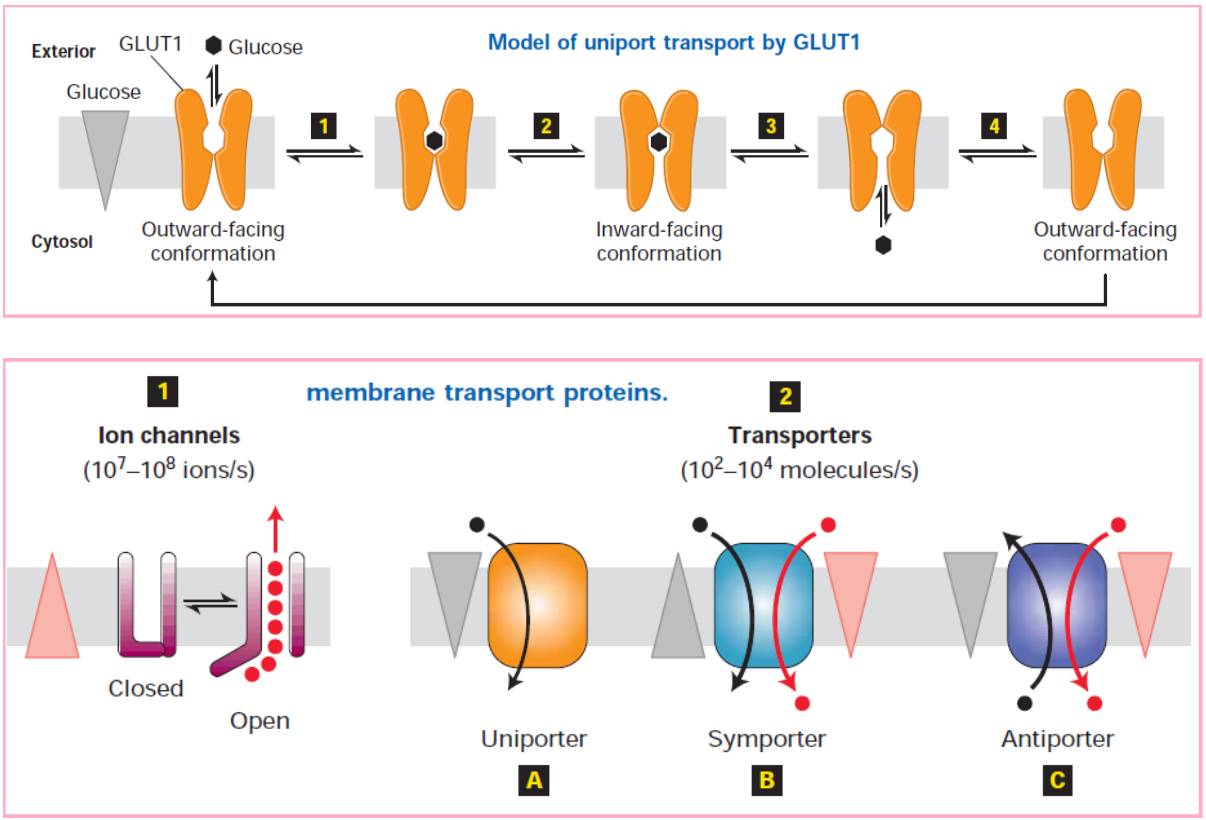


Figure 27. Membrane transport proteins: mechanisms and types of transport

3.2 Active permeability (active transport)

Active transport refers to membrane transport processes that move solutes against their electrochemical gradients, requiring direct or indirect consumption of metabolic energy. Primary active transporters, such as P-type ATPases, use the energy of ATP hydrolysis to pump ions across the membrane, whereas secondary active transporters (coupled carriers) exploit ion gradients established by primary pumps to drive the uphill movement of other solutes.

3.2.1 Primary active transport

The Na^+/K^+-ATPase is a ubiquitous P-type ATPase that extrudes three Na^+ ions from the cell and imports two K^+ ions per molecule of ATP hydrolysed, generating and maintaining steep Na^+ and K^+ gradients across the plasma membrane. These gradients are essential for the resting membrane potential, secondary active transport (e.g. Na^+-dependent nutrient uptake) and osmotic balance, and in many cell types Na^+/K^+-ATPase activity consumes 20-30% of cellular ATP, rising to ≈50-70% in neurons during intense electrical activity.

Other primary active transporters include Ca^{2+}-ATPases in the plasma membrane and sarcoplasmic reticulum, which maintain cytosolic Ca^{2+} at ≈100 nmol/L against millimolar extracellular concentrations, and the gastric H^+/K^+-ATPase in parietal cells, which secretes

protons into the gastric lumen against a gradient of more than six pH units. Clinically, cardiac glycosides such as digoxin inhibit Na^+/K^+-ATPase in cardiomyocytes, leading to increased intracellular Na^+, reduced Na^+/Ca^{2+} exchange, elevated cytosolic Ca^{2+} and augmented contractility—an illustration of how targeted modulation of primary active transport can be therapeutically useful but potentially toxic.

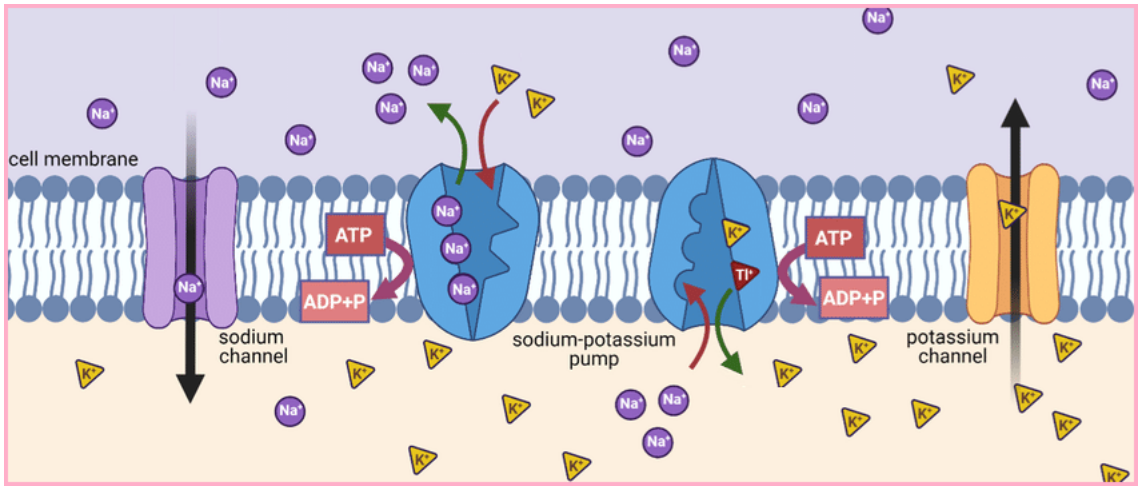


Figure 28. Primary active transport : mechanism of the Na^+/K^+ pump

3.2.2 Secondary active transport

Secondary active transporters use the energy stored in transmembrane ion gradients, typically the inwardly directed Na^+ gradient, to drive the uphill transport of other solutes; they are classified as symporters when ions and substrate move in the same direction, and antiporters when they move in opposite directions. A paradigmatic symporter is the Na^+/glucose cotransporter SGLT1 in the apical membrane of intestinal and renal epithelial cells, which couples the inward movement of two Na^+ ions to the active uptake of one glucose molecule, enabling glucose absorption even when luminal concentrations are lower than intracellular levels.

An important antiporter is the Na^+/Ca^{2+} exchanger in cardiomyocytes, which extrudes one Ca^{2+} ion in exchange for the influx of three Na^+ ions, playing a role in cardiac relaxation by removing cytosolic Ca^{2+} after each contraction. Secondary active transport is also at the heart of many pharmacological strategies: for example, SGLT2 inhibitors used in diabetes therapy modulate renal glucose reabsorption, and altered function of neurotransmitter transporters contributes to psychiatric and neurological diseases, underlining the tight connection between transporter biology and clinical practice.

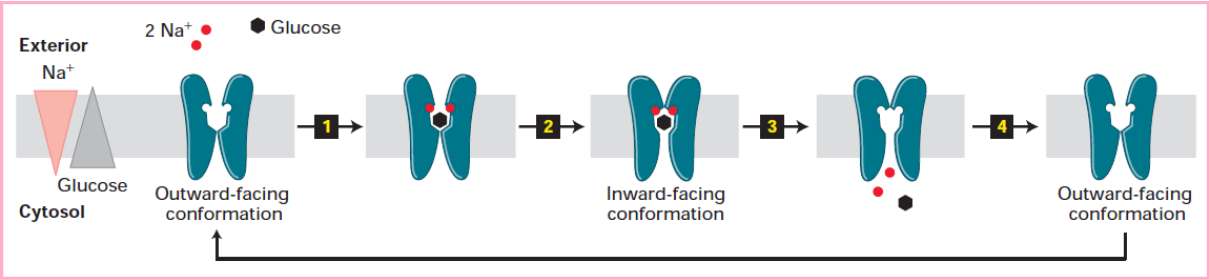


Figure 29. Sodium-dependent secondary active transport of glucose

3.2.3 Vesicular transport

Vesicular or bulk transport involves the movement of macromolecules, particles, and large volumes of fluid across the membrane via small membrane-bound vesicles, an energy-dependent process that relies on coat proteins (e.g., clathrin, COPI, COPII) and an actin/microtubule cytoskeleton. Vesicles of ≈50-100 nm diameter bud from donor membranes, are transported and fuse with target membranes, mediating endocytosis (uptake), exocytosis (secretion), and intracellular trafficking between the endoplasmic reticulum, Golgi apparatus, and other compartments.

3.2.3.1 Endocytosis

Endocytosis is the general term for processes in which the plasma membrane invaginates to internalise extracellular fluid, solutes, membrane proteins, and particles into cytoplasmic vesicles, providing a route for nutrient uptake, receptor down-regulation, and clearance of pathogens or debris. In pinocytosis ("cell drinking"), all cells continuously internalise small volumes of extracellular fluid in small vesicles, often via clathrin-coated pits, thereby sampling the environment and contributing to membrane turnover; in receptor-mediated endocytosis, specific ligands such as low-density lipoprotein (LDL) particles bind to their receptors in coated pits and are concentrated into clathrin-coated vesicles, allowing highly efficient uptake of scarce molecules.

Phagocytosis ("cell eating") is restricted in mammals to specialised cells such as macrophages, neutrophils, and dendritic cells, which engulf large particles (>0.5 μm), including bacteria, apoptotic cells, and cellular debris by extending actin-rich pseudopods around them to form phagosomes that later fuse with lysosomes. Defects in phagocytic function or signalling can lead to recurrent infections and impaired inflammation, whereas hyperactivation of phagocytosis contributes to tissue damage in autoimmune and chronic inflammatory diseases, illustrating the dual protective and destructive potential of endocytic pathways.

3.2.3.2 Exocytosis

Exocytosis is the process by which intracellular vesicles fuse with the plasma membrane, releasing their soluble contents to the extracellular space and incorporating vesicle membrane into the cell surface, thereby balancing membrane loss by endocytosis and mediating secretion. In constitutive exocytosis, present in virtually all cells, vesicles continuously deliver membrane lipids, proteins and soluble cargo such as extracellular matrix components, ensuring renewal of the plasma membrane and steady secretion; in regulated exocytosis, characteristic of specialised secretory cells, vesicles accumulate in the cytoplasm and fuse with the membrane only upon specific stimuli, often involving Ca^{2+} influx, as in synaptic vesicle fusion or hormone release from endocrine cells.

Disorders of regulated exocytosis include impaired insulin granule fusion in pancreatic β-cells in type 2 diabetes and defective mucin secretion in airway epithelia in cystic fibrosis, where mutations in the CFTR Cl^- channel alter ionic and water transport across the apical membrane, leading to dehydrated mucus and chronic infection. Conversely, uncontrolled exocytic release of neurotransmitters and inflammatory mediators contributes to epilepsy, chronic pain and asthma, highlighting the role of vesicular transport in both normal physiology and disease.

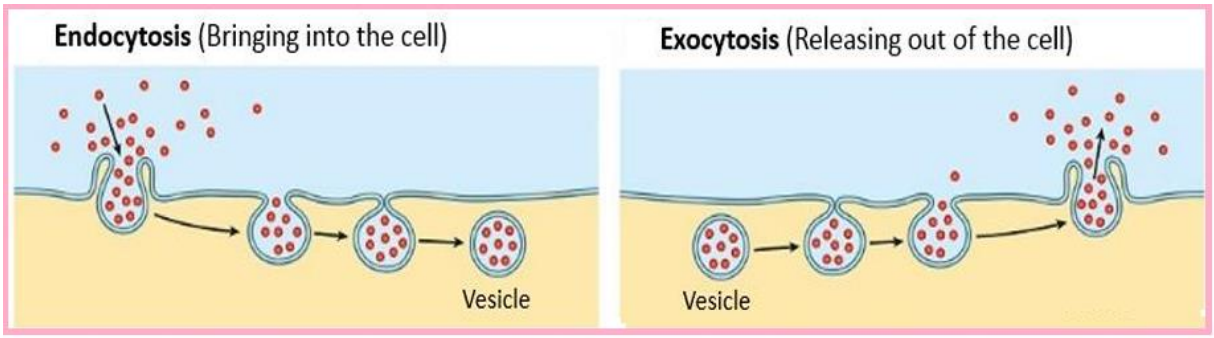


Figure 30. Mechanisms of substance uptake and release: endocytosis and exocytosis.

The lesson in a nutshell

1) Plasma Membrane: Overview

- *Thickness: **~7–10 nm***
- *Structure: **phospholipid bilayer + proteins + carbohydrates***
- *Model: **Fluid Mosaic Model***
- *Composition (approx.):*
    - *Proteins ~50%*
    - *Lipids ~40–45%*
    - *Carbohydrates ~5–10%*

Main functions

- *Selective barrier (controls entry/exit)*
- *Cell communication (receptors)*
- *Cell recognition (glycocalyx)*
- *Structural support*

2) Membrane Structure

Lipid Bilayer

- *Amphipathic phospholipids:*
    - *Hydrophilic heads (outside)*
    - *Hydrophobic tails (inside)*
- ***Lateral fluidity***
- ***Asymmetry** between inner and outer leaflets*

Cholesterol

- *Regulates **fluidity and stability***

Glycolipids

- *Located on outer surface*
- *Involved in **cell recognition (e.g., ABO groups)***

3) Membrane Proteins

Type	***Function***
Integral	*Channels, carriers, receptors*
Peripheral	*Cytoskeleton support*
Glycoproteins	*Cell recognition, adhesion*

Examples:

- *Ion channels*
- *Na^+/K^+ pump*
- *Hormone receptors*

4) Membrane Carbohydrates (Glycocalyx)

- *Only on extracellular side*
- *Functions:*
    - *Cell–cell recognition*
    - *Protection*
    - *Adhesion*
    - *Immune interactions*

5) Membrane Permeability

Lipid bilayer permeability:

- *High: **O_2, CO_2, lipid-soluble molecules***
- *Moderate: **water, small uncharged molecules***
- *Low: **ions, large polar molecules***
  → Require transport proteins

6) Passive Transport (No Energy)

Simple Diffusion

- *Movement down concentration gradient*

Facilitated Diffusion

- *Via **channels or carriers***
- *Specific and saturable*

Osmosis

- *Water moves toward **higher solute concentration***
- *Controlled by **aquaporins***
- *Effects:*
  - *Hypotonic → cell swells/lysis*
  - *Hypertonic → cell shrinks*
  - *Isotonic → no change*

7) Active Transport (Energy Required)

Primary Active Transport

- *Direct use of ATP*
- *Example: **Na^+/K^+-ATPase***
  - *3 Na^+ out / 2 K^+ in*
  - *Maintains membrane potential*

Secondary Active Transport

- *Uses ion gradient (usually Na^+)*
- *Types:*
  - ***Symport** (same direction) – Na^+/glucose*
  - ***Antiport** (opposite) – Na^+/Ca^{2+} exchanger*

8) Vesicular (Bulk) Transport

Endocytosis (into cell)

- ***Pinocytosis** – fluids*
- ***Receptor-mediated** – specific molecules*
- ***Phagocytosis** – large particles (macrophages)*

Exocytosis (out of cell)

- *Secretion of hormones, neurotransmitters*
- *Membrane renewal*

CHAPTER VI : CELL JUNCTIONS

Lesson Objectives

By the end of this lesson, students will be able to:

- Identify and describe the major types of cell junctions
- Explain the molecular composition of each type of junction
- Differentiate the structural and functional characteristics

Introduction

Cell junctions are specialized regions of the plasma membrane where two cells, or a cell and the extracellular matrix, establish close contact through organized complexes of proteins, which mechanically link the cytoskeletons of neighboring cells or create diffusion pathways and barriers. These junctions are particularly abundant in epithelia, where they cluster in the apical lateral membrane domain as the apical junctional complex, but they are also essential in cardiac muscle, smooth muscle, endothelium and many other tissues where coordinated mechanical or electrical activity is required. From a functional perspective, cell junctions are usually grouped into occluding junctions that control the paracellular pathway, anchoring junctions that confer mechanical cohesion by linking cytoskeletal filaments to the plasma membrane, and communicating junctions that mediate direct intercellular exchange of ions and small molecules.

The visualization of cell junctions illustrates the complementarity of light and electron microscopy in cytology and histology. Routine paraffin sections fixed in 10% neutral buffered formalin, corresponding to approximately 4% formaldehyde, and stained with hematoxylin–eosin (H&E) at a thickness of 3-5 µm allow recognition of the apical “terminal bar” in epithelia, which corresponds to the combined profile of tight and adherens junctions at magnifications of ×40 to ×100 with an oil-immersion objective. However, individual tight junction strands, desmosomal plaques and gap junction channels are resolved only by TEM on ultrathin sections of about 40-80 nm obtained after fixation in aldehydes (typically 2-2.5% glutaraldehyde often combined with paraformaldehyde), postfixation in 1% osmium tetroxide, resin embedding and staining with heavy metals, which allows observation at magnifications of ×10,000 and above. Accurate interpretation of junctional morphology in diagnostic cytology and histopathology therefore requires knowledge of both the underlying molecular architecture and the technical conditions that may induce artefacts such as artificial clefts or over-condensed plaques .

1. Types of Cell Junctions

From a structural and functional standpoint, three major categories of cell junctions are distinguished in human tissues : occluding junctions, anchoring junctions, and communicating junctions . Occluding junctions, exemplified by tight junctions or zonulae occludentes, seal the intercellular space near the apical pole of epithelial cells, forming a selective barrier that regulates paracellular flux and preserves the segregation of apical and basolateral membrane domains. Anchoring junctions include adherens junctions (zonulae adherentes), desmosomes (maculae adherentes) and cell-matrix junctions such as hemidesmosomes, all of which link transmembrane adhesion molecules to cytoskeletal filaments actin microfilaments in adherens junctions and fasciae adherentes, and intermediate filaments in desmosomes and hemidesmosomes thus conferring resistance to mechanical stress. Communicating junctions, represented by gap junctions, consist of arrays of intercellular channels that connect the cytoplasm of adjacent cells, allowing the diffusion of ions and small hydrophilic molecules up to about 1-1.2 kDa and thereby supporting electrical and metabolic coupling .

In epithelia, these different junction types are organized in a stereotyped vertical sequence along the lateral membrane. The most apical element of the apical junctional complex is the tight junction, immediately subjacent to which lies the zonula adherens, followed somewhat more basally by spot desmosomes distributed along the lateral surface. This arrangement is crucial for epithelial barrier and mechanical functions: the tight junction pres the primary paracellular barrier (“gate” and “fence” function), the adherens junction forms a contractile actomyosin belt that can modulate cell shape and tissue morphogenesis, and desmosomes distribute tensile forces through keratin intermediate filament networks. Disruption of any of these components can compromise epithelial integrity, as seen in inflammatory bowel disease with altered tight junctions, or in inherited desmosomal defects that predispose to cardiocutaneous syndromes.

2. Morphological Forms of Cell Junctions: Zonula, Macula and Fascia

Beyond the functional classification, cell junctions can be described morphologically as belt-like (zonula), spot-like (macula) or broad plate-like (fascia) specializations of the plasma membrane, as observed in thin sections and freeze-fracture replicas by TEM. A zonula (from Latin “little belt”) denotes a junctional complex that encircles the entire cell circumference as a continuous ring, typically located near the apical pole of polarized epithelia; both the zonula occludens (tight junction) and the zonula adherens (belt adherens junction) have this organization, forming a circumferential “adhesion belt” associated with cortical actin filaments.

In contrast, a macula (“spot”) indicates a roughly circular or oval patch, the classic example being the macula adherens or desmosome, seen by TEM as a disk-shaped junction with dense cytoplasmic plaques in each opposed cell and an intercellular space of approximately 20-35 nm containing the desmoglea.

The term fascia (“band” or “sheet”) is used for junctions that are broader than maculae but not circumferential, forming ribbon-like plaques along linear regions of cell-cell contact, as in fasciae adherentes at the transverse components of cardiac intercalated discs. Fasciae adherentes function as adherens junctions adapted to transmit contractile forces between cardiomyocytes by anchoring thin actin filaments at the ends of sarcomeres, whereas spot desmosomes interspersed within the intercalated disc pre additional tensile strength via desmin intermediate filaments. Understanding these morphological patterns helps students interpret electron micrographs and correlate the distribution of junctions with tissue-specific mechanical demands, for example the predominance of zonulae occludentes in intestinal absorptive epithelia versus the abundance of fasciae adherentes and desmosomes in contracting myocardium.

3. Tight Junctions (Zonulae Occludentes)

Tight junctions, or zonulae occludentes, are occluding junctions located at the most apical part of the lateral membrane of epithelial and endothelial cells, where they form a belt-like network of membrane “strands” that seal the intercellular cleft and regulate paracellular permeability. In conventional thin sections, tight junctions appear as close apposition or even fusion of the outer leaflets of adjacent plasma membranes over a variable length, with almost obliteration of the 10-20 nm extracellular space normally seen between cells and an intermembrane distance that may be reduced to only a few nanometres . Freeze-fracture electron microscopy reveals tight junctions as anastomosing linear arrays of intramembranous particles that encircle the cell; the number and complexity of these strands correlate with the electrical resistance and ion selectivity of the epithelium, as in the comparison between “tight” blood brain barrier endothelium and “leaky” hepatic sinusoids.

At the molecular level, tight junction strands are built primarily from claudins, a multigene family of at least 24-27 tetraspan transmembrane proteins in mammals, which form polymeric rows and confer charge and size selectivity to the paracellular pathway. Claudins associate with other transmembrane proteins including occludin and junctional adhesion molecules (JAMs), and all of these interact via their cytoplasmic tails with scaffolding proteins of the zonula occludens (ZO) family, ZO-1, ZO-2 and ZO-3, which in turn bind to F-actin, thereby linking

the tight junction to the perijunctional actomyosin ring (Schneeberger & Lynch, 2004 ; Guo et al., 2018). This molecular architecture explains two fundamental functions of tight junctions: the “gate” function, which controls ion and solute flow through paracellular pores with radii ranging from approximately 0.4 to 6 nm depending on the epithelial type, and the “fence” function, which prevents lateral diffusion of membrane proteins and lipids between apical and basolateral domains, thereby preserving epithelial polarity.

Tight junction integrity is highly sensitive to experimental and pathological conditions, a fact that has direct implications for diagnostic cytology and histology. In routine histopathology, small intestinal biopsies fixed in 10% neutral buffered formalin for 6-24 h at room temperature and processed for paraffin embedding retain sufficient ultrastructural integrity for immunohistochemical detection of tight junction proteins such as claudin-1, occludin and ZO-1, allowing semi-quantitative assessment of barrier alterations in conditions like celiac disease or inflammatory enteropathies . In contrast, TEM studies require rapid fixation of small tissue fragments (≤1 mm thickness) in 2-2.5% glutaraldehyde in buffered solution at 4-25 °C for 1-2 h, followed by osmium postfixation and embedding, because delayed or incomplete fixation leads to artifactual widening of the junctional cleft and fragmentation of tight junction strands, changes that might mimic genuine pathological barrier breakdown. Clinically, a wide range of inflammatory mediators, bacterial toxins, and ischemic insults modulate tight junction composition, often by altering claudin expression or ZO-1 phosphorylation, resulting in increased paracellular permeability that contributes to edema, diarrhea, and tissue injury in organs such as the intestine, lung, and brain.

4. Anchoring Junctions

Anchoring junctions are characterized by transmembrane adhesion molecules that connect either adjacent cells (cell-cell junctions) or cells to the extracellular matrix (cell-matrix junctions), and by cytoplasmic plaques that link these adhesion receptors to actin filaments or intermediate filaments, thereby transmitting mechanical forces across tissues. In epithelia, adherens junctions and desmosomes mediate cell-cell adhesion, whereas hemidesmosomes anchor basal epithelial cells to the basement membrane ; all three types are best appreciated at the ultrastructural level by TEM, where they appear as areas of thickened plasma membrane with underlying electron-dense plaques and characteristic intercellular or cell–matrix spaces on the order of tens of nanometres. Functionally, adherens junctions are closely linked to actin-dependent changes in cell shape and collective cell migration, while desmosomes and

hemidesmosomes pre strong, often “hyperadhesive” anchorage that maintains tissue integrity under high mechanical load, as in epidermis and myocardium.

4.1. Belt Desmosomes (Zonulae Adherentes)

The zonula adherens is an adherens junction of belt-like form that encircles the cell immediately basal to the tight junction in polarized epithelia, creating a continuous adhesion belt associated with a ring of cortical actin filaments. Ultrastructurally, the zonula adherens displays parallel plasma membranes separated by an intercellular space of approximately 10-20 nm filled with electron-dense rod-like material, corresponding to cadherin ectodomains, while its cytoplasmic face is associated with a fuzzy plaque to which actin filaments attach. The principal adhesion receptors in epithelial adherens junctions are classical cadherins, particularly E-cadherin, which engage in calcium-dependent homophilic binding between neighboring cells; the cytoplasmic tail of E-cadherin binds to catenins (β-catenin, p120-catenin, and α-catenin), which connect the cadherin complex to actin filaments via vinculin and α-actinin, thereby integrating adhesion with the actomyosin cytoskeleton.

From a methodological perspective, the zonula adherens is often recognized in light microscopy as part of the terminal bar in epithelia such as intestinal mucosa, but its molecular composition is typically studied by immunofluorescence or immunohistochemistry using antibodies against E-cadherin and catenins on formalin-fixed, paraffin-embedded sections of 3-4 μm thickness. For ultrastructural analysis, the same fixation conditions used for tight junctions, rapid aldehyde fixation followed by osmium postfixation and resin embedding with ultrathin sections of 60-80 nm, allow visualization of the adhesion belt and its associated microfilaments at TEM magnifications above ×20,000. Clinically, loss or reduction of E-cadherin expression, often detectable by immunostaining in tumor biopsies, is associated with increased invasiveness and poor prognosis in many carcinomas, reflecting the critical role of the zonula adherens in maintaining epithelial cohesion and suppressing epithelial–mesenchymal transition.

4.2. Spot Desmosomes (Maculae Adherentes)

Desmosomes, or maculae adherentes, are spot-like cell-cell anchoring junctions that link intermediate filaments keratins in epithelia and desmin in cardiac muscle to adhesion receptors of the cadherin superfamily, thereby pring strong mechanical coupling between cells. In thin sections, a typical desmosome appears as a disk-shaped structure 0.2-0.5 μm in diameter with two electron-dense plaques separated by an intercellular gap of approximately 20-35 nm (and up to about 40 nm in some tissues), whose region contains a dense midline called the

desmoglea. Bundles of intermediate filaments, with individual filament diameters of about 8-10 nm, insert into the cytoplasmic plaques and loop away into the cytoplasm, forming a three-dimensional network that distributes tensile forces over many cell-cell contacts, a feature particularly evident in stratified epithelia and in the myocardium.

The adhesive of desmosomes is formed by desmosomal cadherins, namely desmogleins (Dsg1-4) and desmocollins (Dsc1-3), which engage in calcium-dependent heterophilic interactions within the 20-30 nm intercellular space, while the cytoplasmic plaques are built from armadillo-family proteins (plakoglobin and plakophilins) and plakin-family proteins (desmoplakin) that tether the cadherins to intermediate filaments. Immunoelectron microscopy and biochemical studies have shown that desmogleins and desmocollins cluster into ordered assemblies, with recent structural work emphasizing that Ca^{2+}-dependent heterophilic binding between their extracellular domains is essential for desmosomal adhesion and can switch between a more dynamic and a "hyperadhesive" state. In routine diagnostic histology, desmosomes are not individually resolved by light microscopy but their presence is inferred from tissue context; however, immunohistochemistry for desmoglein, desmoplakin or plakoglobin on formalin-fixed, paraffin-embedded sections (3-4 μm) is widely used in dermatopathology and cardiomyopathy work-ups.

Desmosomal pathology has major clinical implications. In pemphigus vulgaris, autoantibodies target desmoglein 3 (and sometimes desmoglein 1), leading to loss of keratinocyte adhesion (acantholysis) in suprabasal epidermal layers and formation of intraepidermal blisters, which can be correlated histologically with widened intercellular spaces and reduced desmoglein staining. In arrhythmogenic right ventricular cardiomyopathy, mutations in desmosomal genes such as plakoglobin or desmoplakin weaken mechanical coupling between cardiomyocytes and disturb intercalated disc structure, thereby predisposing to ventricular arrhythmias; electron microscopy in such cases often reveals fragmented desmosomes and altered intermediate filament anchorage, though careful attention must be paid to fixation and sectioning quality to avoid artefactual disruption.

5. Communicating Junctions (Gap Junctions)

Gap junctions are specialized communicating junctions that consist of clusters of intercellular channels connecting the cytoplasm of adjacent cells, enabling direct passage of ions and small hydrophilic molecules up to approximately 1.5 nm in diameter or 1-1.2 kDa in molecular mass, thereby supporting rapid electrical and metabolic coupling in many tissues. By TEM, gap junctions appear as planar or slightly curved plaques where the two plasma membranes of

neighboring cells run closely parallel, separated by a uniform gap of about 2-4 nm much narrower than the usual 10-20 nm intercellular space in nonjunctional regions—with a characteristic pentalaminar appearance. Freeze-fracture replicas show gap junctions as arrays of closely packed intramembranous particles on one fracture face, corresponding to connexon hemichannels that span the lipid bilayer, with complementary pits on the opposite face.

Each gap junction channel is formed by the docking of two connexons, one contributed by each cell; a connexon is a hexamer of connexin proteins, which are four-pass transmembrane proteins that assemble into a hydrophilic pore of approximately 1.5-2 nm diameter. At least 21 connexin isoforms are expressed in humans, with tissue-specific patterns : Cx43 is widely expressed in the heart and many other organs, Cx26 in cochlea and epidermis, and Cx32 in peripheral nerve, among others. Gap junction channels are gated by voltage, pH and phosphorylation, and their permeability is relatively nonselective for small molecules such as Ca^{2+}, K^{+}, cyclic AMP and inositol trisphosphate, which allows propagation of action potentials in cardiac and some smooth muscles, spread of calcium waves in glia, and metabolic coordination in epithelia and osteocytes.

In routine histology, gap junctions cannot be directly resolved by light microscopy; however, immunohistochemical staining for Cx43 and other connexins on 3-5 μm paraffin sections typically after fixation in 10% neutral buffered formalin for 12-24 h and using antigen retrieval allows visualization of punctate or linear labelling along intercalated discs in myocardium or at basolateral epithelial surfaces. For ultrastructural analysis, small samples fixed rapidly in 2-2.5% glutaraldehyde and postfixed in osmium tetroxide, followed by ultrathin sectioning to 60-90 nm, pre high-contrast images of gap junction plaques at magnifications of ×30,000 and higher; improper fixation or section folds can create apparent discontinuities in plaques that must not be mistaken for genuine pathological fragmentation. Clinically, mutations in connexin genes give rise to diverse human diseases, such as Cx26-related nonsyndromic deafness, Cx32-associated Charcot-Marie-Tooth neuropathy and Cx43-linked oculodentodigital dysplasia, illustrating the crucial role of gap junctions in the physiology of excitable and non-excitable tissues.

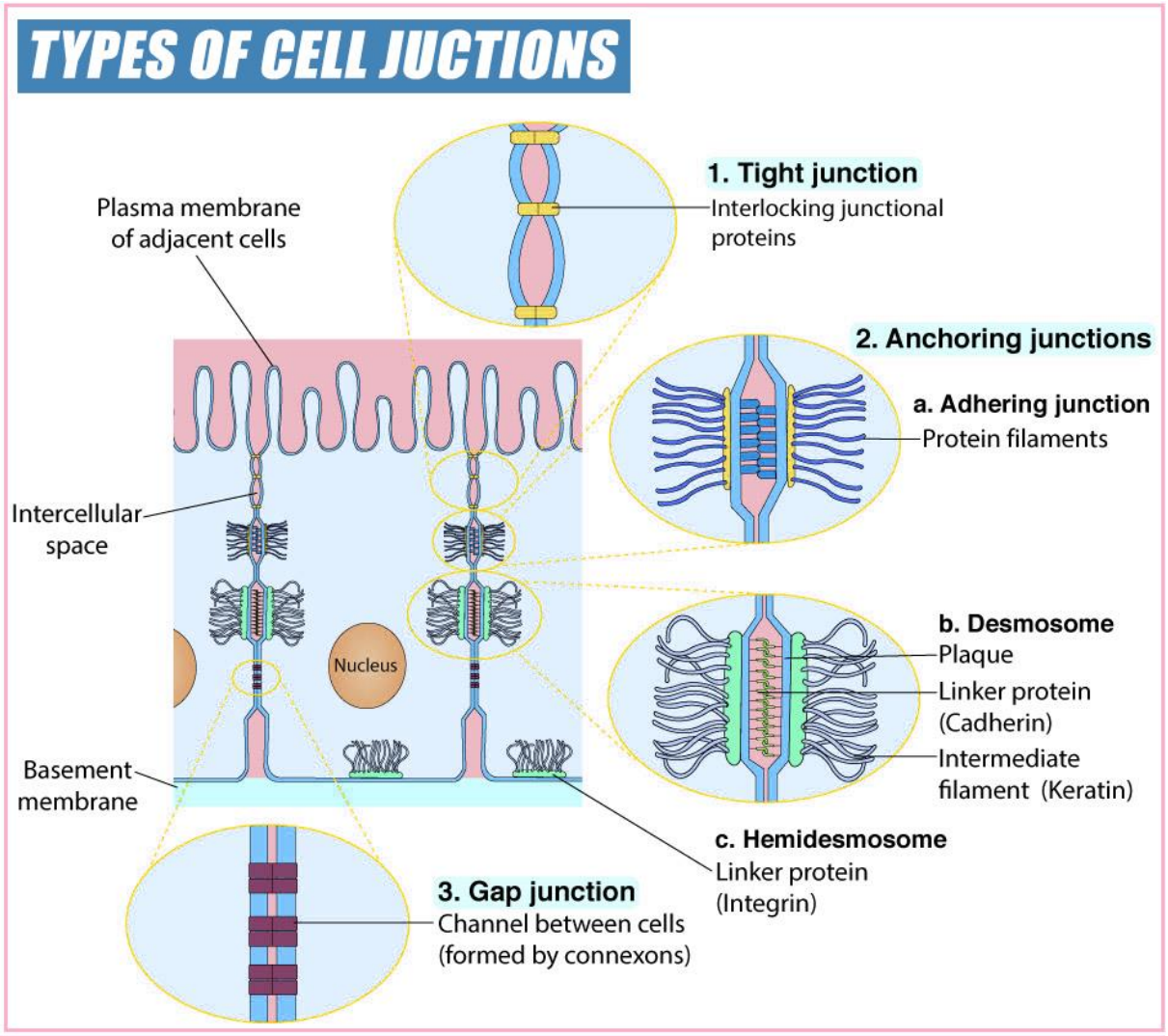
6. Hemidesmosomes

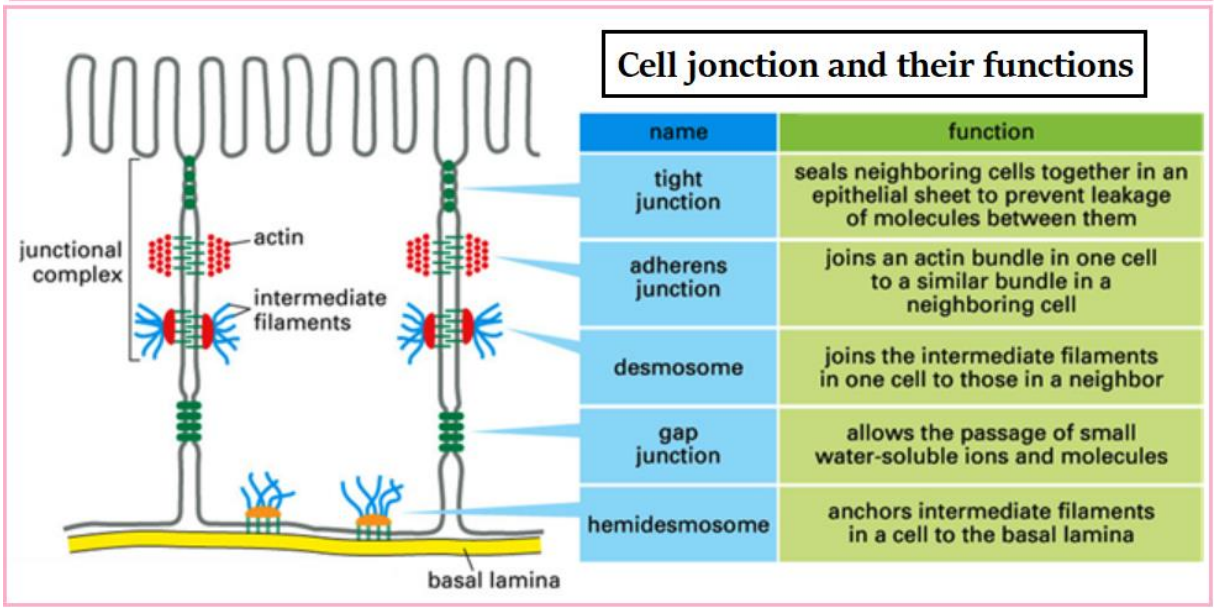
Hemidesmosomes are asymmetric cell-matrix anchoring junctions that connect the basal plasma membrane of epithelial cells to the underlying basement membrane, thereby stabilizing epithelia against mechanical forces such as friction and shear. Despite their name, hemidesmosomes are not simply “half desmosomes,” but distinct structures in which integrins,

rather than cadherins, serve as the principal adhesion receptors; in thin sections, a hemidesmosome appears as a small (typically 0.1-0.2 μm wide) electron-dense plaque on the basal cell membrane facing the lamina lucida of the basement membrane, with associated keratin intermediate filaments converging into the plaque and anchoring filaments extending into the extracellular matrix. The interspace between basal cell membrane and basal lamina in hemidesmosomal regions is on the order of tens of nanometres and contains components such as laminin-332 and type XVII collagen, which are critical for stable attachment .

At the molecular level, type I hemidesmosomes of stratified epithelia (e.g. epidermis) contain the α6β4 integrin as the main transmembrane receptor, which binds laminin-332 in the basal lamina, and a cytoplasmic plaque composed of plectin (BPAG1e), BPAG2 (type XVII collagen) and other adaptor proteins that link the integrin cytoplasmic tail to keratin intermediate filaments. Ultrastructural and biochemical studies have shown that α6β4 integrin and bullous pemphigoid antigens redistribute during epithelial wound healing, highlighting the dynamic regulation of hemidesmosomes in response to mechanical and inflammatory cues. In routine diagnostic histology, hemidesmosomes themselves are below the resolution of light microscopy, but detachment of the epidermis from the dermis at the level of the basement membrane, as seen in bullous pemphigoid, reflects disruption of hemidesmosomal components; immunofluorescence on frozen sections using antibodies against BPAG1, BPAG2 or α6β4 integrin, after fixation in 4% paraformaldehyde or cold acetone, is used to visualize these molecules and localize autoantibody deposition.

Clinically, inherited or acquired defects of hemidesmosomes produce blistering diseases characterized by epithelial fragility. In junctional epidermolysis bullosa, mutations in laminin-332 or type XVII collagen lead to cleavage within the lamina lucida and separation at the dermal–epidermal junction, consistent with the distribution of hemidesmosomal anchoring filaments observed by TEM in skin biopsies fixed in glutaraldehyde and processed for resin embedding . In bullous pemphigoid, autoantibodies directed against BPAG1e and BPAG2 cause complement activation and inflammatory cell recruitment at the basement membrane zone, weakening hemidesmosomal adhesion and producing tense subepidermal blisters ; correlating clinical, immunopathological and ultrastructural findings is essential for accurate classification of blistering disorders and demonstrates how detailed knowledge of cell junctions underpins diagnostic dermatopathology.





Cell jonction and their functions

name	function
tight junction	seals neighboring cells together in an epithelial sheet to prevent leakage of molecules between them
adherens junction	joins an actin bundle in one cell to a similar bundle in a neighboring cell
desmosome	joins the intermediate filaments in one cell to those in a neighbor
gap junction	allows the passage of small water-soluble ions and molecules
hemidesmosome	anchors intermediate filaments in a cell to the basal lamina

Figure 31. Cell junctions: structure, organization, and functions

The lesson in a nutshell

1) What are Cell Junctions?

Specialized membrane regions that:

- *Link **cell to cell** or **cell to extracellular matrix***
- *Provide **mechanical strength***
- *Control **paracellular permeability***
- *Allow **cell communication***

Three functional groups

1. ***Occluding***
2. ***Anchoring***
3. ***Communicating***

2) Organization in Epithelia (Apical → Basal)

1. *Tight junction (zonula occludens)*
2. *Adherens junction (zonula adherens)*
3. *Desmosomes (macula adherens)*
4. *Gap junctions*
5. *Hemidesmosomes (to basement membrane)*

3) Occluding Junctions

Tight Junctions (Zonula Occludens)

Function

- *Seal intercellular space*
- *Control **paracellular transport***
- *Maintain **cell polarity** (fence function)*

Main proteins

- *Claudins*
- *Occludin*
- *ZO proteins (link to actin)*

4) Anchoring Junctions

A. Adherens Junction (Zonula Adherens)

- *Belt-like around cell*
- *Connects to **actin filaments***
- *Main proteins: **E-cadherin + catenins***
- *Important for tissue cohesion and shape*

B. Desmosomes (Macula Adherens)

- *Spot-like junctions*
- *Connect **intermediate filaments (keratin/desmin)***
- *Strong resistance to mechanical stress*

Proteins

- *Desmoglein*
- *Desmocollin*
- *Desmoplakin*

C. Hemidesmosomes

- *Cell → **basement membrane***
- *Connect intermediate filaments to ECM*
- *Main receptors: **integrins (α6β4)***

5) Communicating Junctions

Gap Junctions

Function

- *Direct cytoplasmic communication*
- *Passage of ions and small molecules (<1–1.2 kDa)*
- *Electrical and metabolic coupling*

Structure

- *Channels formed by* ***connexins***
- *Two connexons (one from each cell)*

6) Morphological Types

Form	***Description***	***Example***
Zonula	*Belt-like*	*Tight, Adherens*
Macula	*Spot-like*	*Desmosome*
Fascia	*Broad band*	*Cardiac adherens*

7) Functional Summary

Junction	***Main Role***	***Cytoskeleton***
Tight	*Barrier, polarity*	*Actin*
Adherens	*Adhesion, shape*	*Actin*
Desmosome	*Mechanical strength*	*Intermediate filaments*
Hemidesmosome	*Cell–matrix anchoring*	*Intermediate filaments*
Gap	*Communication*	*None*

CHAPTER VII : INTERPHASE NUCLEUS AND THE CELL CYCLE

Lesson Objectives

By the end of this lesson, students will be able to:

- Describe the structure and organization of the interphase nucleus,
- Explain the major phases of the cell cycle (G_1, S, G_2, and M),
- Identify the key events of interphase, with a focus on DNA replication, transcriptional activity, and nuclear dynamics.

Introduction

The nucleus is the defining organelle of eukaryotic cells: it is a membrane-bound compartment, typically 5-10 µm in diameter in human somatic cells, which segregates the genome and the bulk of transcriptional machinery from the cytoplasm. This compartmentalization allows transcription, RNA processing and at least part of DNA repair to occur in a controlled nuclear microenvironment, while translation and many signalling and metabolic pathways remain cytoplasmic, thereby introducing an additional level of spatial regulation to gene expression and cell behaviour.

In routine paraffin sections fixed in 10% neutral buffered formalin (≈4% formaldehyde, typically 12-24 h at room temperature) and stained with hematoxylin-eosin (H&E), the interphase nucleus appears as a basophilic structure due to its high content of DNA and RNA, with a variable amount of condensed chromatin and one or more darker nucleoli depending on the transcriptional activity of the cell. Under the electron microscope, fixation in 2-2.5% glutaraldehyde followed by osmium tetroxide reveals the double-membrane nuclear envelope, nuclear pores and the heterogeneous density of chromatin, pring a structural basis for interpreting the light microscopic appearance of nuclei in diagnostic cytology and histopathology.

1. Morphology and structure of the interphase nucleus

In most mammalian tissues, the nucleus is approximately ovoid or spherical and usually constitutes about 10% of the cell volume ; its diameter commonly ranges from 5 to 20 µm, though extremes occur in very small lymphocytes (≈7–10 µm cell diameter) and in large, highly specialized cells such as megakaryocytes. The nuclear-to-cytoplasmic ratio is a key morphological parameter : it is relatively low in well-differentiated cells, higher in actively proliferating or less differentiated cells, and markedly increased in many malignant cells, where

nuclear enlargement (karyomegaly) and variation in nuclear size (anisokaryosis) reflect genetic instability and deregulated growth.

The internal architecture of the interphase nucleus is non-random: chromatin, nucleoli and other nuclear bodies are arranged in an ordered three-dimensional pattern, sometimes described as a "nuclear landscape," which is intimately linked to the control of gene expression, replication timing and genome stability. Cell At the light microscopic level, two main chromatin patterns can be distinguished: euchromatin, which is relatively pale and finely granular, corresponding to decondensed, transcriptionally active DNA, and heterochromatin, which is coarse, basophilic and often peripherally located, representing condensed, transcriptionally repressed regions. Electron microscopy confirms that heterochromatin forms dense aggregates under the inner nuclear membrane and around the nucleolus, whereas euchromatin occupies a more, diffuse compartment, and this spatial segregation is conserved across many cell types.

From a diagnostic perspective, alterations of nuclear morphology are criteria in cytology and histopathology. Hyperchromasia (increased chromatin staining), irregular nuclear contours, coarse clumped chromatin, prominent or multiple nucleoli, and atypical mitotic figures are hallmarks of dysplasia and malignancy and are systematically assessed in preparations such as Papanicolaou-stained cervico-vaginal smears, fine-needle aspiration cytology, and histological tumour sections. Accurate evaluation of these features requires adequate fixation, appropriate section thickness (3-5 μm for paraffin sections ; a monolayer of cells in cytological smears) and optimal nuclear staining with hematoxylin or cytological nuclear dyes, as under-fixation or over-staining can create artefactual chromatin condensation or smudged nuclei that mimic pathology.

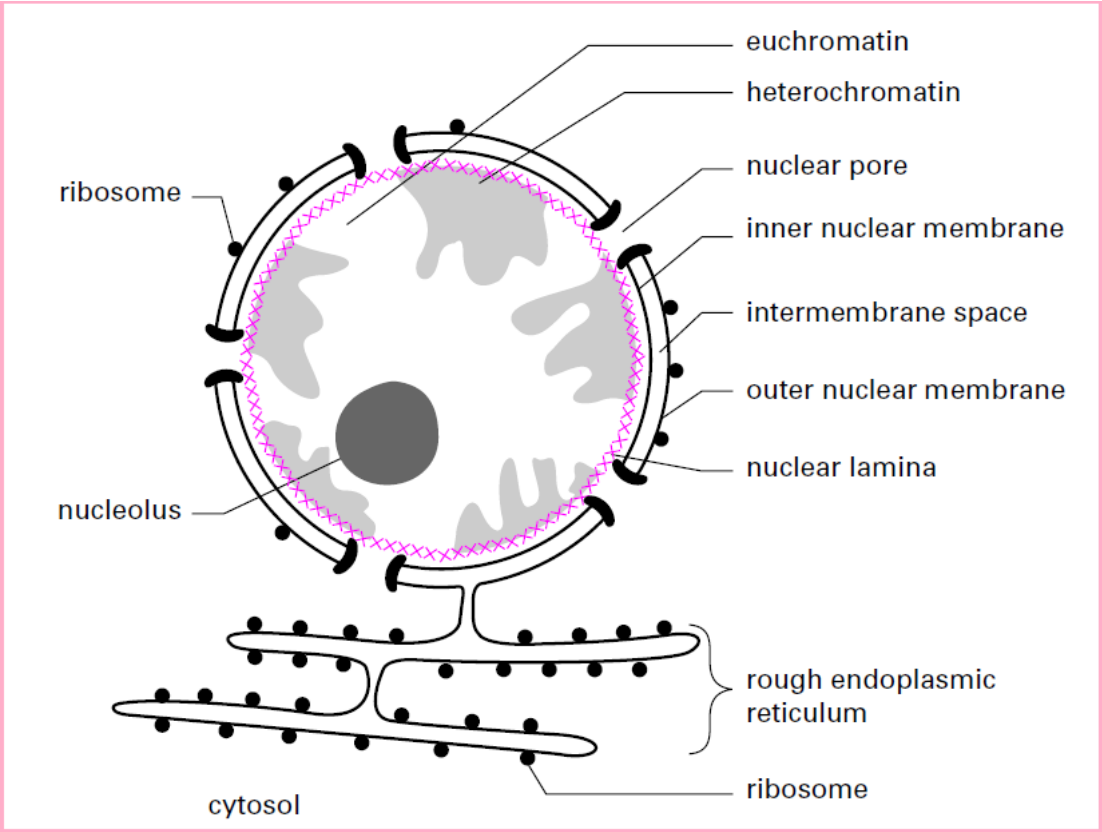


Figure 32. Structural organization of the cell nucleus

2. Constituents of the nucleus

2.1. The nuclear envelope

The nuclear envelope is a specialized double-membrane system that delimits the nucleus and separates nucleoplasm from cytoplasm; it consists of an outer nuclear membrane, an inner nuclear membrane, and an intervening perinuclear cisterna approximately 20-40 nm wide . The outer nuclear membrane is continuous with the rough endoplasmic reticulum and often bears ribosomes, whereas the inner nuclear membrane is lined by the nuclear lamina, a meshwork of A- and B-type lamins and lamin-associated proteins that confer mechanical stability and pre-anchoring sites for chromatin domains and nuclear pore complexes.

During “open” mitosis in mammalian cells, the nuclear envelope disassembles in prophase and prometaphase and later reassembles around the segregated chromatids in telophase; this dynamic behaviour depends on phosphorylation-driven disassembly of lamins and nuclear pore components and their subsequent targeted recruitment to decondensing chromatin. Mutations in lamins or lamin-associated proteins disrupt nuclear envelope integrity and nuclear mechanics, leading to laminopathies such as muscular dystrophies, lipodystrophies and the premature ageing syndrome Hutchinson–Gilford progeria; these disorders illustrate how nuclear envelope architecture is essential for normal cell function and tissue homeostasis .

Histologically, the nuclear envelope is not resolved as two separate membranes with standard light microscopy, but appears as a sharp nuclear contour whose regularity is evaluated in tumour grading systems; irregular, indented or angulated nuclear outlines often correlate with altered lamina-chromatin interactions and are common in high-grade dysplasia and carcinoma. Immunocytochemistry using antibodies against lamins or inner nuclear membrane proteins can be applied on formalin-fixed, paraffin-embedded tissue sections to demonstrate lamina defects in certain myopathies, while high-pressure freezing and cryo-electron tomography pre ultrastructural details of the envelope and its continuity with the endoplasmic reticulum in research settings.

2.2. Nuclear pores and the nuclear pore complex

Nuclear pores are large protein assemblies embedded in the nuclear envelope that mediate bidirectional transport of macromolecules between nucleoplasm and cytoplasm; each pore corresponds to a nuclear pore complex (NPC), an ~110 MDa structure in humans composed of about 1 000 protein subunits from roughly 30 different nucleoporins . Electron microscopy and biophysical modelling show that the vertebrate NPC has an outer diameter of approximately 100-120 nm, with an inner transport channel of about 40–50 nm in diameter traversing the 40-50 nm-thick nuclear envelope, and that several thousand such pores (on the order of 2 000-5 000) are distributed over the surface of a typical mammalian nucleus.

Functionally, NPCs allow passive diffusion of ions and small molecules below ≈40 kDa, whereas larger proteins and ribonucleoprotein particles, such as transcription factors, ribosomal subunits and mRNA-protein complexes, require facilitated transport mediated by karyopherins and regulated by the Ran GTPase cycle. Many nucleoporins contain intrinsically disordered phenylalanine-glycine (FG) repeat domains that form a selective permeability barrier, whose physical polymer brush versus phase-separated hydrogel remains a topic of active investigation using in vitro nanopore mimics and high-resolution structural methods. Defects in NPC components can perturb nucleo-cytoplasmic transport of transcriptional regulators and DNA repair factors, contributing to developmental abnormalities and neurodegenerative diseases, and several cancer-associated fusion proteins involve nucleoporins, underscoring the clinical importance of NPC integrity.

In standard histology the individual NPCs cannot be resolved, but their density and distribution can be visualized by immunogold labelling against nucleoporins at the electron microscope or indirectly inferred from biochemical assays measuring nuclear transport kinetics; for teaching purposes, schematic diagrams at ×50 000-×100 000 magnification help students link the

ultrastructural architecture of the NPC to its function as a gate for mRNA export and protein import.

2.3. Nucleoplasm

The nucleoplasm is the aqueous, protein-rich matrix that fills the nuclear interior and surrounds chromatin and nucleoli; it contains a high concentration of enzymes involved in DNA replication, transcription and RNA processing, as well as numerous dynamic nuclear bodies such as speckles (enriched in pre-mRNA splicing factors), Cajal bodies and promyelocytic leukaemia (PML) bodies. Although difficult to delineate as a separate entity at the light microscopic level, changes in nucleoplasmic composition can be inferred from the appearance of viral inclusion bodies, stress-induced nuclear granules, or abnormal PML body patterns in certain leukaemias, as demonstrated by immunofluorescence and immunoelectron microscopy. The nucleoplasm also serves as the milieu in which chromatin fibres and chromosome territories are suspended and rearranged during the cell cycle, so its viscoelastic properties contribute to nuclear mechanics and the mobility of genomic loci.

2.4. The nucleolus

The nucleolus is a prominent, non-membrane-bound nuclear domain where ribosomal RNA (rRNA) genes are transcribed, rRNA is processed, and the earliest steps of ribosomal subunit assembly occur; it is typically 1-2 μm in diameter in metabolically active mammalian cells and often appears as a dense, basophilic structure within the nucleus in H&E-stained sections. The nucleolus forms around nucleolar organizer regions (NORs), which are chromosomal loci bearing tandem repeats of rDNA; in human cells, these NORs reside on the short arms of the five acrocentric chromosomes, and their activity correlates with nucleolar size and number . Ultrastructurally, the nucleolus exhibits a tripartite organization into fibrillar centres, a dense fibrillar component, and a granular component, which correspond respectively to inactive rDNA, transcriptionally active rDNA with nascent transcripts, and regions where pre-ribosomal particles undergo maturation before export to the cytoplasm.

From a methodological standpoint, nucleoli are strongly basophilic and argyrophilic. Silver staining of argyrophilic nucleolar organizer region-associated proteins (AgNOR staining) highlights small black dots or clusters within nucleoli; quantitative AgNOR analyses correlate with ribosomal biogenesis rates and are widely used as proliferation markers in tumour pathology and haematology. In addition, immunohistochemical detection of nucleolar proteins such as nucleolin or nucleophosmin complements AgNOR staining in research and sometimes

in diagnostic practice by revealing nucleolar reorganization in malignancies and ribosomopathies, thereby linking alterations of nucleolar morphology to cell cycle deregulation and genomic instability.

2.5. Chromatin

Chromatin is the nucleoprotein complex that packages nuclear DNA into a compact yet accessible form; the fundamental structural unit is the nucleosome, in which ≈147 base pairs of DNA wrap around an octamer of histones, forming a "beads-on-a-string" fibre of about 10 nm in diameter that can further fold into higher-order structure. A diploid human cell contains approximately 6×10^9 base pairs of DNA (≈2 m linear length) confined within a nucleus of 5-10 μm diameter, so chromatin must be hierarchically organized to allow both dense packing and regulated access for transcription, replication and repair.

At the functional level, chromatin exists along a continuum of compaction, but histologically it is convenient to distinguish euchromatin and heterochromatin. Euchromatin is relatively decondensed, gene-rich, and transcriptionally active ; it appears pale and finely granular by light microscopy and predominates in nuclei of metabolically active or proliferating cells such as hepatocytes and tumour blasts. Heterochromatin is more condensed, gene-poor, and generally transcriptionally repressed; it tends to accumulate at the nuclear periphery, at pericentromeric regions, and around the nucleolus, and appears as coarse, deeply basophilic clumps of chromatin. Quantitative imaging has shown that heterochromatin density exceeds that of euchromatin by roughly 1.5-fold in mammalian nuclei, illustrating that the histological distinction reflects genuine biophysical differences in chromatin packing.

Chemical modifications of histones (acetylation, methylation, phosphorylation, ubiquitination) and DNA methylation constitute key epigenetic marks that remodel chromatin structure and regulate gene expression ; active promoters and enhancers are typically enriched in histone H3 lysine 4 trimethylation and acetylation, whereas repressed domains and lamina-associated domains (LADs) accumulate marks such as H3K9 and H3K27 methylation. Abnormal patterns of chromatin modification and nuclear lamina attachment contribute to oncogenesis and developmental disorders, and their morphological correlates include loss of peripheral heterochromatin, globally open or coarse chromatin, and nuclear blebs observed in a variety of cancers and laminopathies.

In routine histology, DNA-specific histochemical reactions such as the Feulgen stain, based on acid hydrolysis followed by Schiff reagent, pre-stoichiometric labelling of nuclear DNA and remain the gold standard for quantitative image cytometry of DNA ploidy, for example, in cervical smears and tumour imprints. Fluorescent DNA dyes such as DAPI or Hoechst 33342, combined with flow cytometry, allow rapid measurement of DNA content in thousands of nuclei and are routinely used to assess cell-cycle distributions (G0/G1, S, G2/M) in experimental cell populations, illustrating how chromatin-bound probes bridge the gap between nuclear structure and cell-cycle analysis.

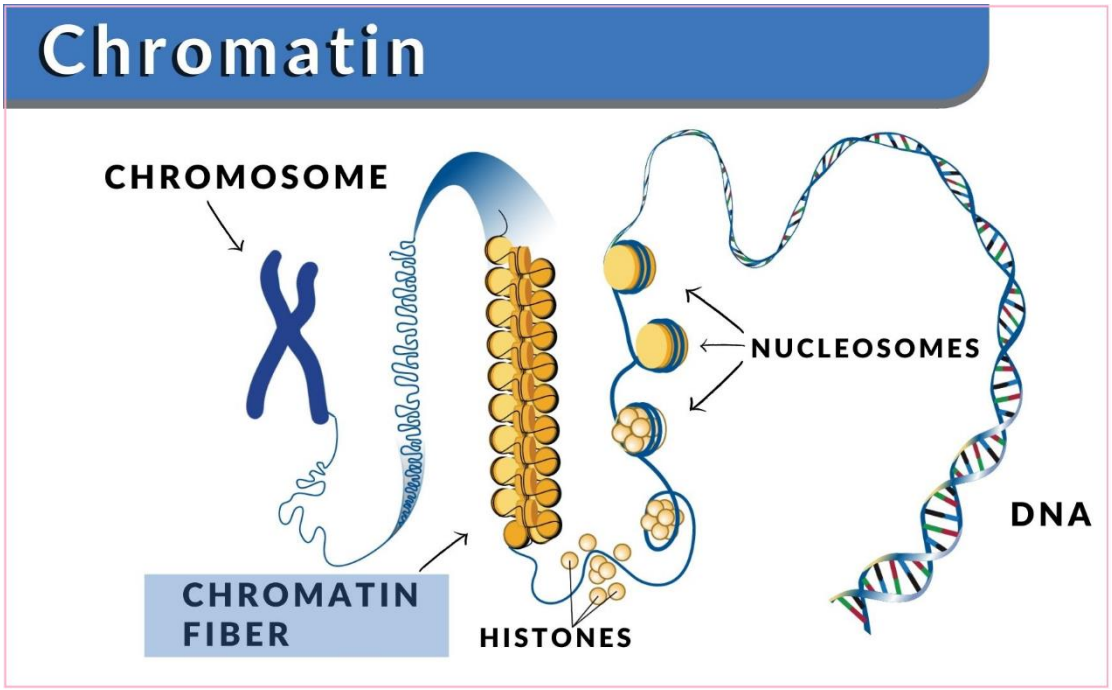


Figure 33. Chromatin structure and levels of DNA compaction

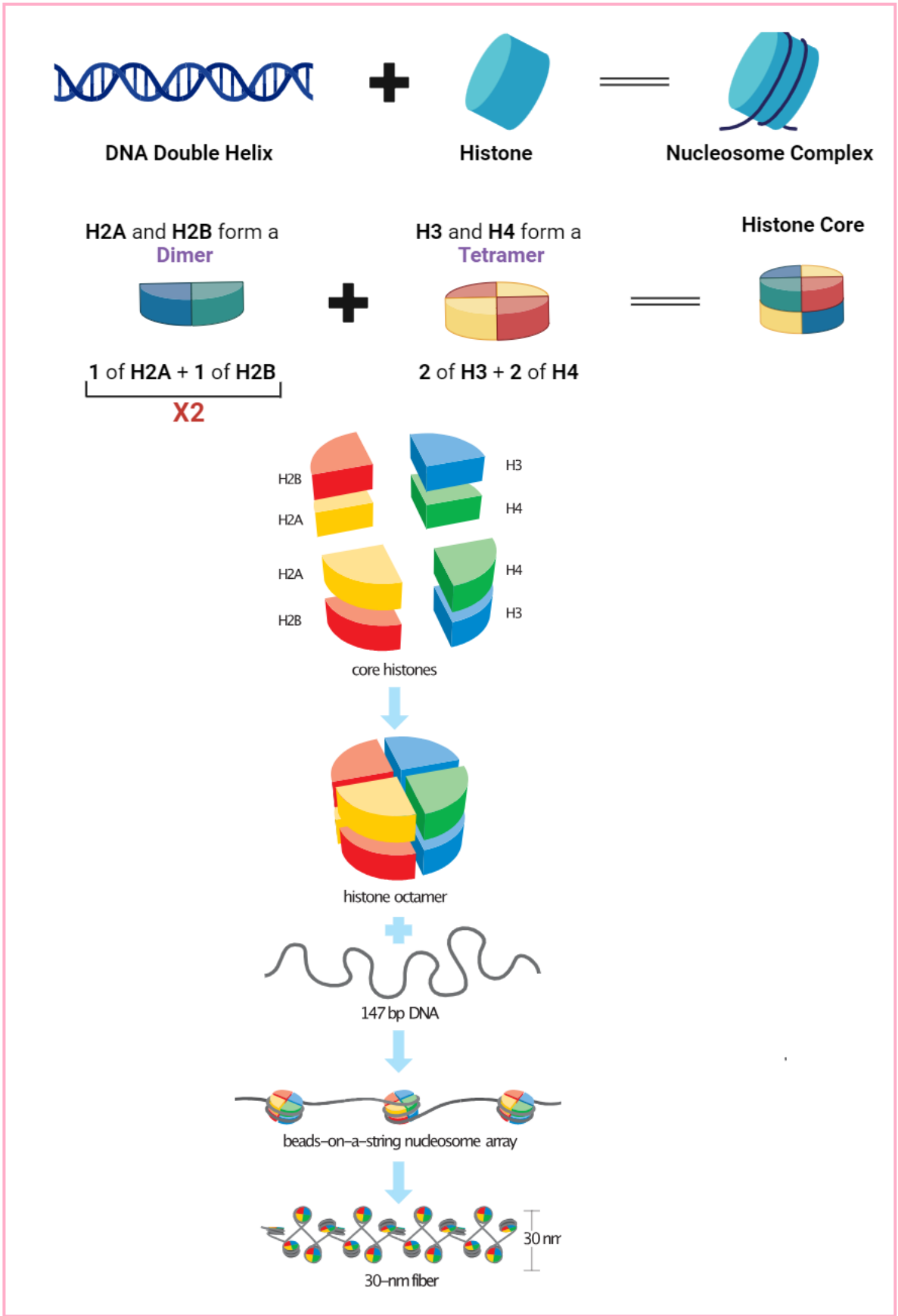


Figure 34. Nucleosome formation and chromatin organization

3. The cell cycle

The cell cycle is the ordered series of events by which a cell duplicates its genome and divides into two daughter cells; in most proliferating mammalian cells, it is conventionally divided into interphase (G_1, S and G_2 phases) and mitosis (M phase, including cytokinesis). In a typical human cell with an overall cycle time of approximately 24 h under optimal culture conditions, G_1 lasts about 7-11 h, S about 8-9 h, G_2 about 3-4 h and M about 1 h, although these values vary widely among cell types and in vivo contexts. Many differentiated cells permanently exit the cycle into a quiescent state termed G_0, while others re-enter the cycle in response to growth factors or pathological stimuli, underscoring the importance of extracellular signalling in cell-cycle control.

3.1. Interphase

Interphase comprises the bulk of the cell cycle and corresponds to the period during which the cell grows, duplicates its DNA and prepares for mitosis, while the nucleus remains intact and chromatin largely maintains an interphase configuration. Morphologically, interphase nuclei exhibit dispersed euchromatin and recognizable nucleoli, with mitotic figures absent; cytologically, nuclear size may gradually increase as the cell progresses from G_1 to G_2, reflecting accumulation of RNA and proteins and doubling of DNA content.

Phase G_1 (first gap phase) begins immediately after mitosis and is characterized by cell growth, restoration of organelles, and intense biosynthetic activity; most proteins and RNAs needed for DNA synthesis and subsequent mitosis are produced in this phase. In rapidly cycling human cells with a 24 h cycle, G_1 frequently occupies around 7-11 h and contains a critical “restriction point,” beyond which progression through S phase becomes growth factor-independent, a concept that underpins the pathological autonomy of many cancer cells. Histologically, G_1 nuclei resemble those in late telophase or early interphase, with one or a few nucleoli and relatively stable chromatin patterns ; proliferative status during G_1 can be assessed by immunohistochemical labelling of nuclear antigens expressed in cycling cells but absent in quiescent G_0 cells, notably Ki-67.

S phase (synthesis phase) is the period during which DNA replication occurs; the entire nuclear genome is duplicated, and centrosomes also replicate, resulting in cells with 4C DNA content by the end of S. In typical proliferating human cells, S phase lasts approximately 8-9 h, although its duration can be shortened in embryonic cells and lengthened in some differentiated lineages . Replication occurs in spatially and temporally regulated “replication factories,” and specific genomic regions replicate early or late in S depending on their chromatin state and nuclear

position, a principle exploited in experimental BrdU or EdU incorporation assays combined with immunofluorescence or flow cytometry to identify S-phase cells .

Phase G_2 (second gap phase) spans the interval between completion of DNA replication and entry into mitosis; during this phase, the cell verifies the integrity of replicated DNA, repairs remaining lesions, and accumulates proteins required for mitotic spindle formation and chromosome condensation. In a 24 h cycle, G_2 typically lasts about 3-4 h in mammalian cells, and progression into mitosis is governed by activation of cyclin B-CDK1 complexes, which are themselves regulated by checkpoint pathways responsive to DNA damage or incomplete replicatio. Morphologically, G_2 nuclei are similar to late S-phase nuclei but may appear slightly larger; in tumour pathology, an increased proportion of G_2/M nuclei can be inferred from DNA cytometry histograms showing a subpopulation with 4C DNA content, often associated with aggressive behaviour.

3.2. Mitosis

Mitosis (M phase) comprises nuclear division and is usually followed by cytokinesis, the division of the cytoplasm; in many human cells, mitosis occupies roughly 1 h of a 24 h cycle and is subdivided into prophase, metaphase, anaphase, and telophase, with prometaphase often distinguished for descriptive purposes. Mitoses are easily recognized in H&E-stained sections at high magnification (×40 or ×100 oil immersion objectives), and the mitotic index (number of mitoses per unit area or per high-power fields) is an important component of histological grading schemes in many malignancies, such as breast carcinoma and leiomyosarcoma .

In prophase, chromatin condenses into visible chromosomes, each consisting of two sister chromatids joined at the centromere. The nucleolus gradually disappears and the centrosomes begin to separate as the mitotic spindle forms ; the nuclear envelope starts to break down, a process that is completed during prometaphase. In metaphase, chromosomes are maximally condensed and aligned at the equatorial metaphase plate, with spindle microtubules attached to kinetochores; metaphase figures are particularly easy to recognize in histological sections and are often used as the reference phase when counting mitoses. Anaphase is defined by the synchronous separation of sister chromatids and their movement toward opposite spindle poles, while in telophase the chromosomes reach the poles, begin to decondense, the nuclear envelope reassembles around each chromatid set and nucleoli reappear; cytokinesis, mediated by an actomyosin contractile ring, usually overlaps late anaphase and telophase, producing two daughter cells.

In diagnostic practice, mitotic figures are identified on H&E sections by the presence of condensed, hyperchromatic chromatin without an intact nuclear membrane ; atypical mitoses tripolar or multipolar spindles, lagging chromosomes, or grossly asymmetrical chromatin segregation are strong indicators of malignancy and genomic instability. Immunohistochemical stains for mitosis-specific markers such as phosphorylated histone H3 (PHH3) facilitate detection of mitotic figures and improve reproducibility of mitotic counts, particularly in tumours with high mitotic rates or in small biopsies where mitoses are scarce. Combined assessment of mitotic index, Ki-67 proliferation index, and, in some settings, AgNOR counts presents a robust measure of tumour proliferative activity with prognostic significance in several cancers.

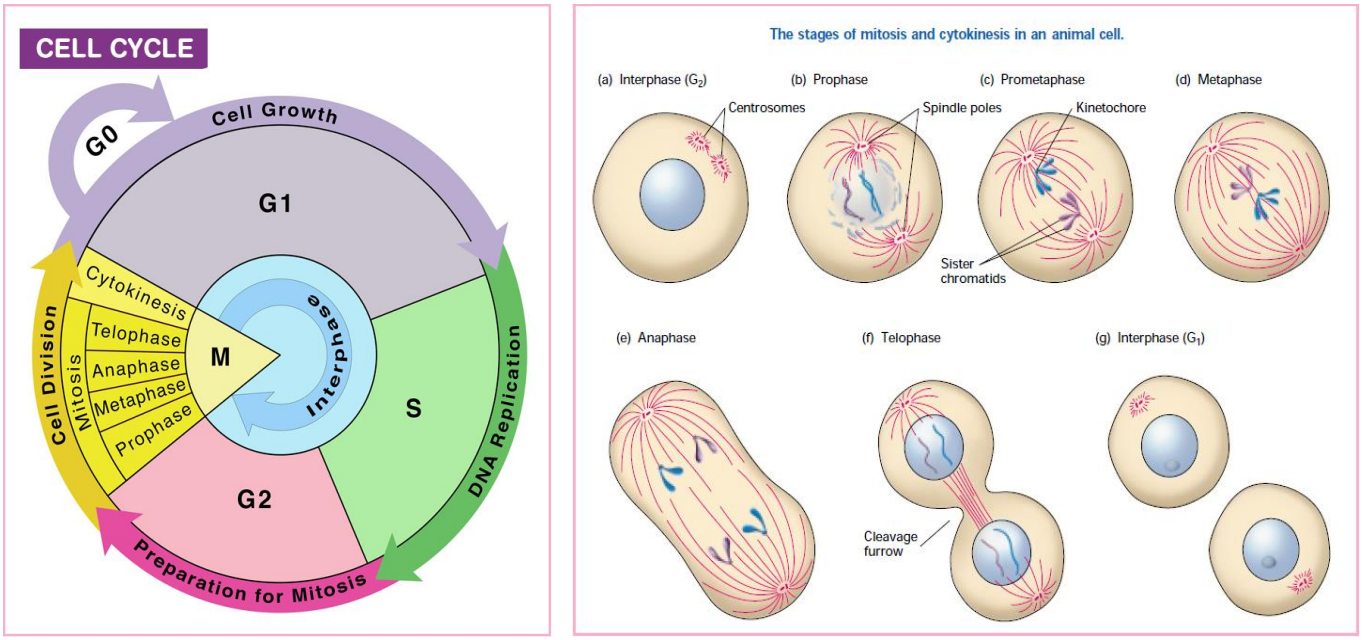


Figure 35. Cell cycle phases and cell division.

4. Genome organization in the nucleus during the cell cycle

During interphase, each chromosome occupies a distinct, although partially intermingling, spatial domain known as a chromosome territory; these territories are non-randomly arranged, with gene-rich chromosomes tending to reside toward the nuclear interior and gene-poor chromosomes more peripherally, a pattern conserved across various mammalian cell types. High-resolution microscopy and chromosome conformation capture techniques have shown that the three-dimensional arrangement of chromosome territories and sub-domains is tightly linked to gene expression programs, replication timing and DNA repair pathways, so that nuclear architecture must be understood as an additional regulatory layer over the linear genome.

A major determinant of genome organization is the interaction between chromatin and the nuclear lamina ; large genomic regions termed lamina-associated domains (LADs), collectively covering up to 40% of the genome, are typically enriched in heterochromatin and transcriptionally repressed genes and are positioned near the nuclear periphery. Dynamic changes in lamina–chromatin contacts accompany differentiation, environmental signalling and cell-cycle progression, while disruption of lamins or lamin-binding proteins in laminopathies leads to widespread mislocalization of chromatin, altered gene expression and premature cellular senescence. Nucleoli also contribute to nuclear organization by acting as hubs that attract specific chromatin regions; nucleolus-associated domains (NADs) are enriched in repetitive sequences and silent genes, and their repositioning in cancer correlates with altered rDNA transcription and genome instability.

As cells enter mitosis, the interphase nuclear architecture is dismantled : chromosome territories condense into individualized mitotic chromosomes, the nuclear envelope and lamina disassemble, and nucleoli disappear, leading to a transient state in which the genome is packaged into highly condensed, transportable units. During telophase and early G_1, the nucleus is rebuilt and chromosomes decondense; studies tracking genomic loci through the cycle indicate that chromosome territories re-establish similar, though not identical, spatial patterns from one cell generation to the next, suggesting that nuclear organization is semi-heritable yet sufficiently plastic to accommodate changes in gene expression and cell fate. Perturbations of this dynamic organization, for example, global loss of peripheral heterochromatin, abnormal nuclear shape and multiple enlarged nucleoli are characteristic of many cancers and can be appreciated on routine histological slides, where they serve as morphological surrogates for underlying genomic and epigenomic deregulation.

Understanding how nuclear structure, chromatin organization and cell-cycle progression are coordinated pres a conceptual framework for interpreting nuclear morphology in cytological and histological preparations, for using proliferation and DNA content assays in clinical diagnostics, and for appreciating how mutations in nuclear envelope and chromatin regulators drive human disease.

The lesson in a nutshell

1) The Nucleus: General Features

- *Defining organelle of **eukaryotic cells***
- *Size: **~5–10 µm***
- *Contains DNA and transcription machinery*
- *Appears **basophilic** in H&E staining*
- *Nuclear–cytoplasmic (N/C) ratio:*
    - *Low in differentiated cells*
    - *High in proliferating or cancer cells*

2) Nuclear Components

Nuclear Envelope

- ***Double membrane***
- *Outer membrane continuous with rough ER*
- *Inner membrane supported by **nuclear lamina (lamins)***
- *Breaks down during mitosis and reforms afterward*

Nuclear Pores

- *Large complexes (NPC)*
- *Allow:*
    - *Passive diffusion (< ~40 kDa)*
    - *Active transport of proteins, RNA*
- *~2,000–5,000 pores per nucleus*

Nucleoplasm

- *Protein-rich matrix*
- *Site of DNA replication, transcription, RNA processing*
- *Contains nuclear bodies (speckles, PML bodies)*

Nucleolus

- ***Ribosome production (rRNA synthesis + assembly)***
- *Size reflects metabolic/proliferative activity*
- *Often prominent in cancer cells*

Chromatin

Two forms:

Type	***Features***	***Function***
Euchromatin	*Pale, dispersed*	*Active transcription*
Heterochromatin	*Dense, peripheral*	*Inactive DNA*

Human cell DNA: ~2 m packed inside nucleus

3) The Cell Cycle Overview

Phases

- *Interphase: **G_1 + S + G_2***
- ***M phase**: mitosis + cytokinesis*

Typical duration (~24 h cycle):

- *G_1: 7–11 h*
- *S: 8–9 h*
- *G_2: 3–4 h*
- *M: ~1 h*

4) Interphase Events

G_1 Phase

- *Cell growth*
- *Protein and RNA synthesis*
- *Restriction point (commitment to division)*

S Phase

- ***DNA replication***
- *DNA content doubles (2C → 4C)*
- *Centrosome duplication*

G_2 Phase

- *DNA repair and quality control*
- *Preparation for mitosis*

5) Mitosis (M Phase)

Stage	***Key Event***
Prophase	*Chromosome condensation, nucleolus disappears*
Metaphase	*Chromosomes align at equator*
Anaphase	*Sister chromatids separate*
Telophase	*Nuclear envelope reforms*
Cytokinesis	*Cell divides*

6) Nuclear Organization in Interphase

- *Chromosomes occupy **territories***
- *Gene-rich regions → nuclear interior*
- *Gene-poor regions → periphery*
- *Interaction with nuclear lamina regulates gene expression*

7) Quick Memory Box

- ***Euchromatin = active***
- ***Heterochromatin = inactive***
- ***G_1 grow – S copy – G_2 check – M divide***

CHAPTER VIII : MITOCHONDRIA AND CELLULAR RESPIRATION

Lesson Objectives

By the end of this lesson, students will be able to:

- Describe the structure and function of mitochondria,
- Outline the major stages of cellular respiration,
- Explain how mitochondria contribute to ATP production

Introduction

Mitochondria are double-membrane-bound organelles, typically 0.5-1.0 μm in diameter and up to several micrometres in length, forming a dynamic reticular network that continuously undergoes fusion and fission in most human cells. Their primary role is the production of adenosine triphosphate (ATP) by aerobic respiration, but they also participate in calcium homeostasis, intermediary metabolism, innate immunity and regulated cell death, so that mitochondria are best understood as multifunctional signalling and metabolic hubs rather than simple "powerhouses."

The number and morphology of mitochondria vary strikingly between cell types : a hepatocyte may contain more than 1000 mitochondria distributed throughout the cytoplasm, while cardiomyocytes and slow-twitch skeletal muscle fibres show dense longitudinal and subsarcolemmal mitochondrial populations that parallel their high oxidative demand. In contrast, mature human erythrocytes have eliminated all mitochondria and rely entirely on glycolysis for ATP generation, a specialisation that optimises haemoglobin packing and gas transport.

1. Origin of mitochondria: the endosymbiotic theory

The endosymbiotic theory proposes that mitochondria derive from an ancestral alphaproteobacterium that established a stable symbiosis inside an archaeal host, giving rise to the last eukaryotic common ancestor (LECA). Historical development of this concept, from early ideas of symbiogenesis to the detailed serial endosymbiotic theory of Lynn Margulis, has been progressively substantiated by molecular, ultrastructural and phylogenomic evidence linking mitochondrial genes and proteins to extant alphaproteobacteria.

Several structural and genetic features of mitochondria support their bacterial ancestry. Mitochondria possess a double membrane, with an inner membrane enriched in the bacterial phospholipid cardiolipin, and they multiply only by binary fission, like bacteria. Human

mitochondrial DNA (mtDNA) is a circular molecule of approximately 16.6 kilobases that encodes 13 essential polypeptides of the oxidative phosphorylation (OXPHOS) system, 22 transfer RNAs and 2 ribosomal RNAs, all of which are required for intramitochondrial translation.

From a clinical perspective, the endosymbiotic origin of mitochondria explains the strictly maternal inheritance of mtDNA, the phenomenon of heteroplasmy (coexistence of mutant and wild-type mtDNA in the same cell) and the particular vulnerability of high-energy tissues, such as brain, skeletal and cardiac muscle to mtDNA mutations and respiratory chain defects .

2. Structure and composition of mitochondria

The typical mitochondrion is organised into four main compartments: an outer mitochondrial membrane (OMM), an inner mitochondrial membrane (IMM) folded into cristae, an intermembrane space between the two membranes, and a protein-rich matrix enclosed by the inner membrane. This complex architecture maximises the surface area of the inner membrane available for the respiratory chain while maintaining a chemically distinct matrix that houses enzymes of the tricarboxylic acid (TCA) cycle and mitochondrial DNA.

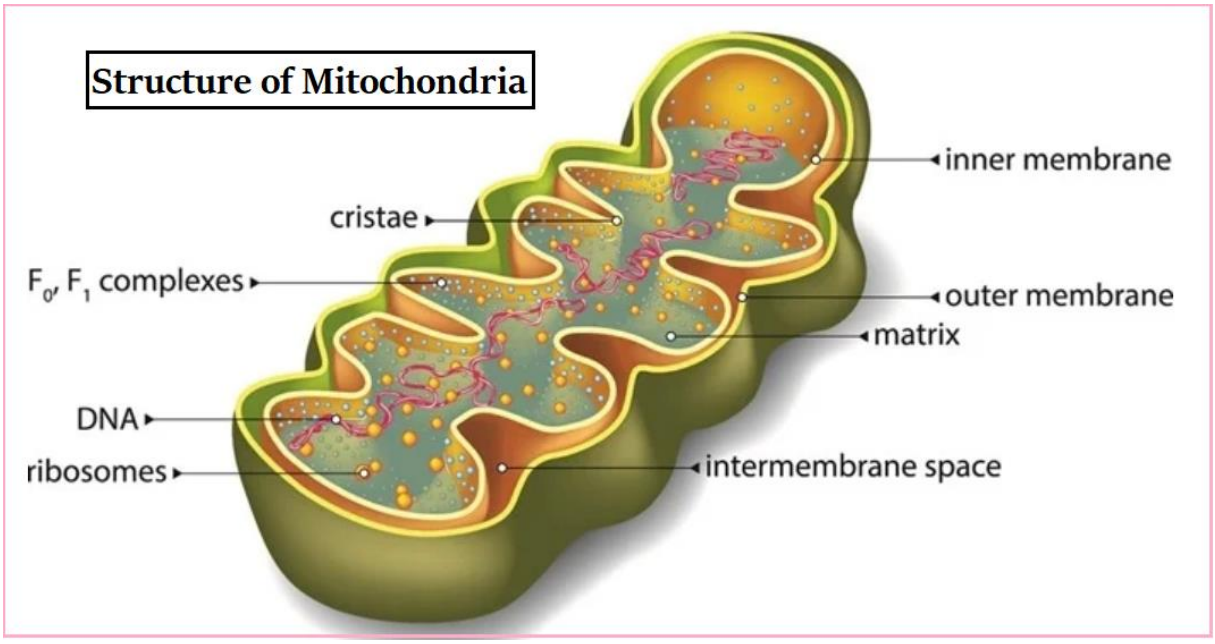


Figure 36. Structure and organization of mitochondria

2.1. The mitochondrial membranes

The outer mitochondrial membrane is a relatively smooth bilayer, about 7-10 nm thick, containing abundant β-barrel channel proteins called porins (voltage-dependent anion channels), which render it permeable to molecules up to roughly 5 kDa and thereby equilibrate many ions and metabolites between cytosol and intermembrane space (Alberts et al., 2002). It also carries components of the translocase of the outer membrane (TOM complex) responsible for importing the vast majority of nuclear-encoded mitochondrial proteins, a process that is critical for maintaining mitochondrial function and whose failure leads to profound bioenergetic defects .

The inner mitochondrial membrane is highly specialised: it is protein-rich, cholesterol-poor, and largely impermeable to ions and small solutes, so that transport requires specific carriers such as the ADP/ATP translocase or phosphate carrier. The IMM is thrown into numerous invaginations called cristae, whose density and shape correlate with metabolic activity oxidative muscle fibres and steroid-producing endocrine cells exhibit tightly packed lamellar or tubular cristae that greatly increase membrane surface area available for electron transport chain (ETC) complexes and ATP synthase.

Recent cryo-electron microscopy and super-resolution imaging studies show that complexes IV (cytochrome-c oxidase) and V (FoF_1-ATP synthase) are non-uniformly distributed within crista membranes, with ATP synthase often concentrated at crista rims and respiratory supercomplexes in planar regions, suggesting a fine spatial organisation that optimises proton-motive force utilisation.

2.2. The “cytosol” and the mitochondrial matrix

In the context of cellular respiration, it is essential to distinguish between the cytosol, the aqueous compartment of the cell outside organelles, and the mitochondrial matrix, which is the viscous, protein-rich phase within the inner membrane. The matrix contains mitochondrial DNA, mitochondrial ribosomes, tRNAs and the majority of enzymes of the TCA cycle and fatty acid β-oxidation, with a protein concentration that can exceed 500 mg/mL; this crowding favours efficient substrate channeling between successive metabolic enzymes.

Cytosolic and mitochondrial compartments are metabolically interdependent: glycolysis in the cytosol produces pyruvate and NADH, which must be channelled into mitochondrial metabolism through specific transporters and shuttle systems such as the malate–aspartate shuttle, while ATP generated in the matrix is exported to the cytosol for use by ion pumps, cytoskeletal motors and biosynthetic pathways. This tight coupling between cytosolic demand

and mitochondrial supply is reflected morphologically by the close apposition of mitochondria to sarcoplasmic reticulum in muscle or to synapses in neurons, where local ATP and calcium handling are critical.

2.3. Mitochondrial ribosomes

The mitochondrial translation machinery consists of tRNAs encoded by mtDNA and mitochondrial ribosomes (mitoribosomes), which differ markedly from cytosolic 80S ribosomes. Mammalian mitoribosomes have a sedimentation coefficient of about 55S and are composed of a 28S small subunit and a 39S large subunit, containing 12S and 16S rRNAs, respectively, and more than 70 distinct ribosomal proteins. These ribosomes translate the 13 mtDNA-encoded polypeptides, all of which are core subunits of complexes I, III, IV and V of the respiratory chain; the remaining thousands of mitochondrial proteins are encoded in the nuclear genome and imported post-translationally.

2.4. The mesosome

Historically, the bacterial “mesosome” was described as a system of invaginations of the plasma membrane in Gram-positive bacteria and was once proposed as a prokaryotic analogue of mitochondrial cristae, thus playing a role in early discussions of endosymbiotic organelles (Martin, 2017). However, careful ultrastructural work showed that the large, complex mesosomes observed in thin sections are artefacts produced by prefixation with dilute osmium tetroxide (0.1 % OsO_4) in the Ryter-Kellenberger procedure: the amount and size of mesosome-like structures increase linearly during this damaging prefixation step and disappear when specimens are fixed instead with higher concentrations of osmium tetroxide or with glutaraldehyde.

This example illustrates a fundamental principle in cytology: membrane-bound structures, including mitochondria and their cristae, must be interpreted in the light of fixation conditions. For electron microscopy of mitochondria, aldehyde fixation with 2-3 % glutaraldehyde followed by postfixation in 1 % osmium tetroxide at 4 °C is widely used to preserve crista architecture, whereas prefixation with dilute OsO_4 alone can fragment or distort membranes and generate artefacts reminiscent of mesosomes. Combined fixatives containing 3 % paraformaldehyde and 1.5 % glutaraldehyde have been shown to preserve mitochondrial morphology better than either agent alone, again underlining how technical parameters directly condition the ultrastructural appearance of organelles.

3. The cellular respiration

Cellular respiration is a multi-step process that converts the chemical energy of nutrients into ATP. In aerobic human cells, complete oxidation of one molecule of glucose can yield approximately 30-32 ATP by the coordinated action of three major stages: glycolysis in the cytosol, the tricarboxylic acid (Krebs) cycle in the mitochondrial matrix, and oxidative phosphorylation along the inner mitochondrial membrane.

3.1. Glycolysis

Glycolysis is a sequence of ten enzyme-catalysed reactions that occur entirely in the cytosol, converting one molecule of glucose ($C_6H_{12}O_6$) into two molecules of pyruvate ($C_3H_4O_3$) while generating a net gain of two ATP and two NADH per glucose by substrate-level phosphorylation. The pathway can be divided into an energy-investment phase, in which two ATP are consumed to phosphorylate glucose and fructose-6-phosphate, and an energy-payoff phase, in which four ATP are produced, one at the phosphoglycerate kinase step and one at the pyruvate kinase step for each of the two triose-phosphate molecules.

In the presence of oxygen and functional mitochondria, cytosolic NADH is re-oxidised through shuttle systems that transfer its reducing equivalents into the mitochondrial matrix, and pyruvate is imported and oxidised to acetyl-CoA by the pyruvate dehydrogenase complex . In hypoxic conditions or in cells that lack mitochondria, such as mature erythrocytes, pyruvate is reduced to lactate to regenerate NAD^+, allowing glycolysis to continue at the cost of a much lower ATP yield and predisposing tissues to lactic acidosis when oxidative phosphorylation is impaired.

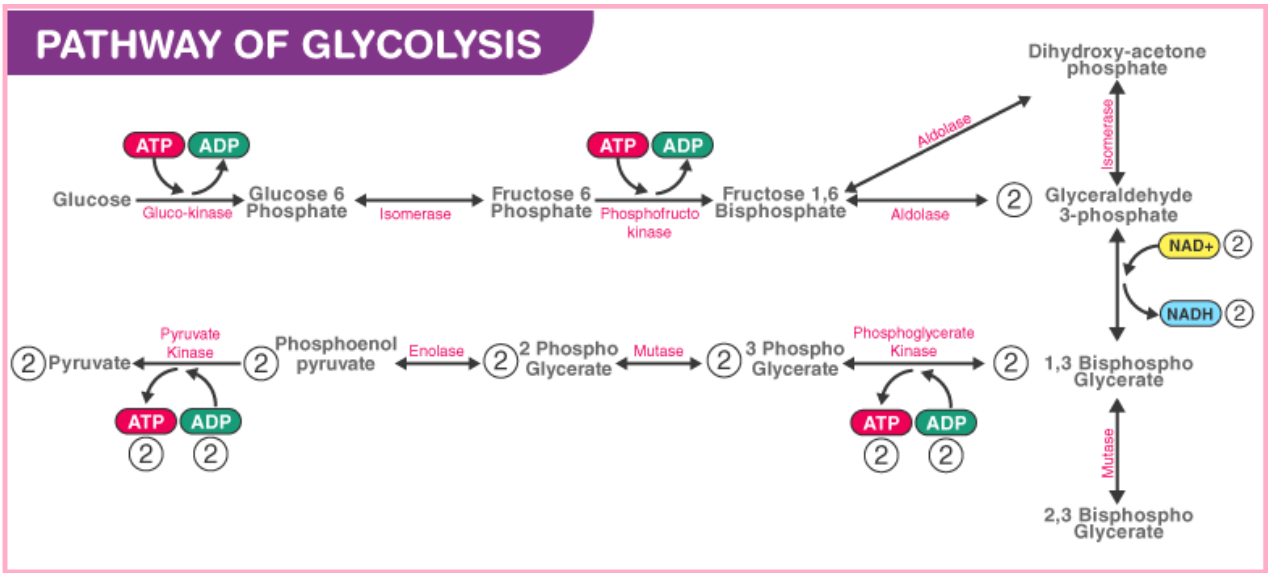


Figure 37. Pathway of Glycolysis

3.2. The Krebs (tricarboxylic acid) cycle

Within the mitochondrial matrix, the pyruvate dehydrogenase complex oxidatively decarboxylates pyruvate to acetyl-CoA, generating one NADH per pyruvate and irreversibly linking glycolysis to the TCA cycle. Each turn of the TCA cycle oxidises one acetyl-CoA to two CO_2 and generates three NADH, one $FADH_2$ and one GTP (readily converted to ATP), so that complete oxidation of one glucose (two acetyl-CoA) yields six NADH, two $FADH_2$ and two GTP from the cycle, in addition to the two NADH from pyruvate dehydrogenase.

Morphologically, tissues with high TCA activity—such as type I (slow-twitch) skeletal muscle fibres and cardiac muscle, show a very high mitochondrial density, with mitochondria packed between myofibrils and beneath the sarcolemma to supply ATP directly where cross-bridge cycling and ion pumping occur (Dubuisson et al., 2022). Histochemically, the strong expression of succinate dehydrogenase (complex II, which participates both in the TCA cycle and the ETC) in these fibres explains their intense blue staining in SDH reactions, while glycolytic type II fibres show weaker SDH activity and fewer mitochondria.

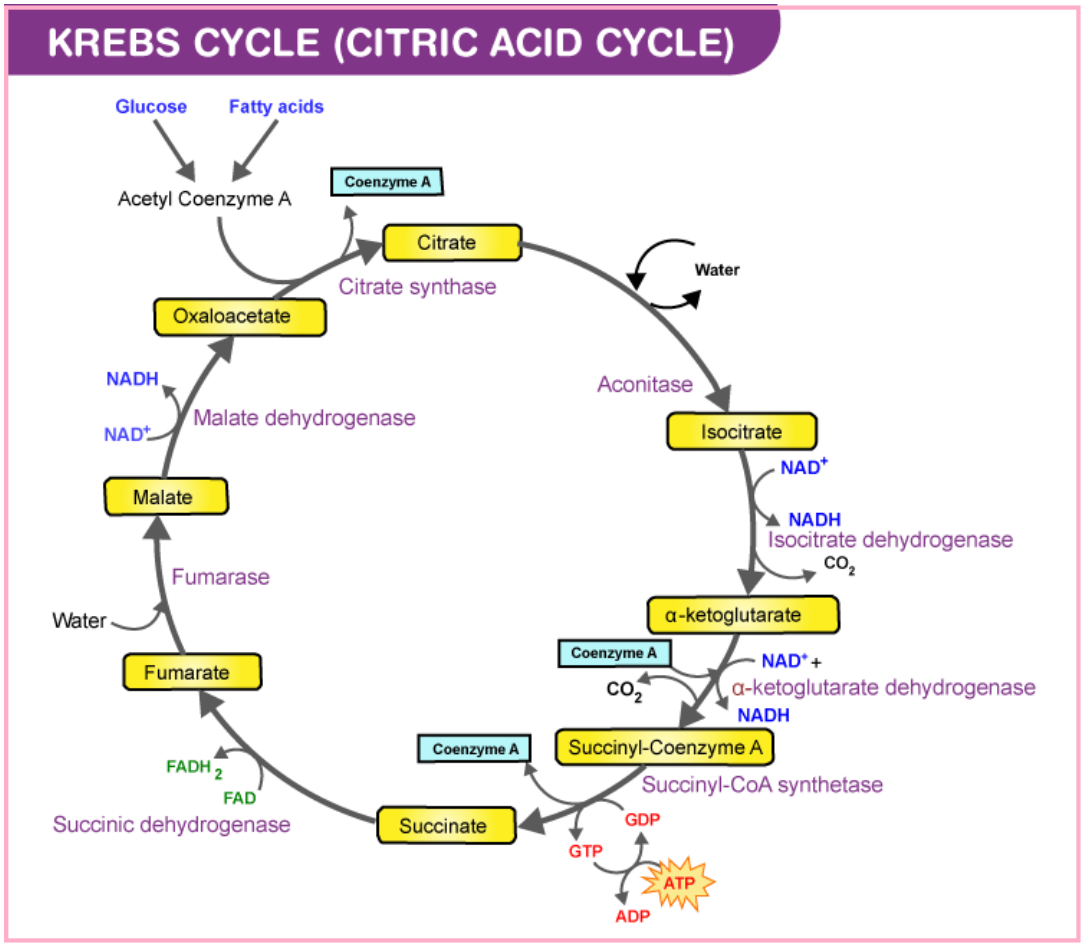


Figure 38. Krebs cycle (Citric Acid Cycle)

3.3. Oxidative phosphorylation

Oxidative phosphorylation couples electron transport along a chain of protein complexes in the inner mitochondrial membrane to ATP synthesis driven by the resulting proton-motive force. Electrons from NADH enter the chain at complex I (NADH:ubiquinone oxidoreductase), while electrons from $FADH_2$ generated by succinate dehydrogenase enter at complex II; both converge on ubiquinone, which carries electrons to complex III (cytochrome-bc_1 complex), then via cytochrome-c to complex IV (cytochrome-c oxidase), where molecular oxygen is reduced to water. As electrons flow through complexes I, III and IV, approximately 10 protons per NADH and 6 protons per $FADH_2$ are pumped from matrix to intermembrane space, generating an electrochemical gradient (proton-motive force) of around 150-200 mV across the inner membrane.

Complex V (FoF_1-ATP synthase) allows protons to flow back into the matrix, converting the energy of this gradient into mechanical rotation of its Fo c-ring and F_1 catalytic head, which synthesises ATP from ADP and inorganic phosphate. Based on experimentally determined P/O ratios, oxidation of one mitochondrial NADH yields about 2.5 ATP and oxidation of one $FADH_2$ yields about 1.5 ATP, values that are lower than classical textbook estimates but consistent with modern measurements of proton stoichiometries and membrane leak.

Disruption of oxidative phosphorylation has immediate clinical consequences. Inhibition of cytochrome-c oxidase by cyanide or carbon monoxide arrests electron flow and ATP synthesis, rapidly leading to cell death, whereas genetic defects in mtDNA-encoded respiratory chain subunits underlie disorders such as MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes) and MERRF (myoclonic epilepsy with ragged-red fibres). In muscle biopsies from patients with mitochondrial myopathies, COX/SDH double staining reveals “ragged-red” fibres with subsarcolemmal accumulations of mitochondria that are SDH-positive but COX-negative, reflecting clonal expansions of mtDNA mutations and mosaic respiratory chain deficiency.

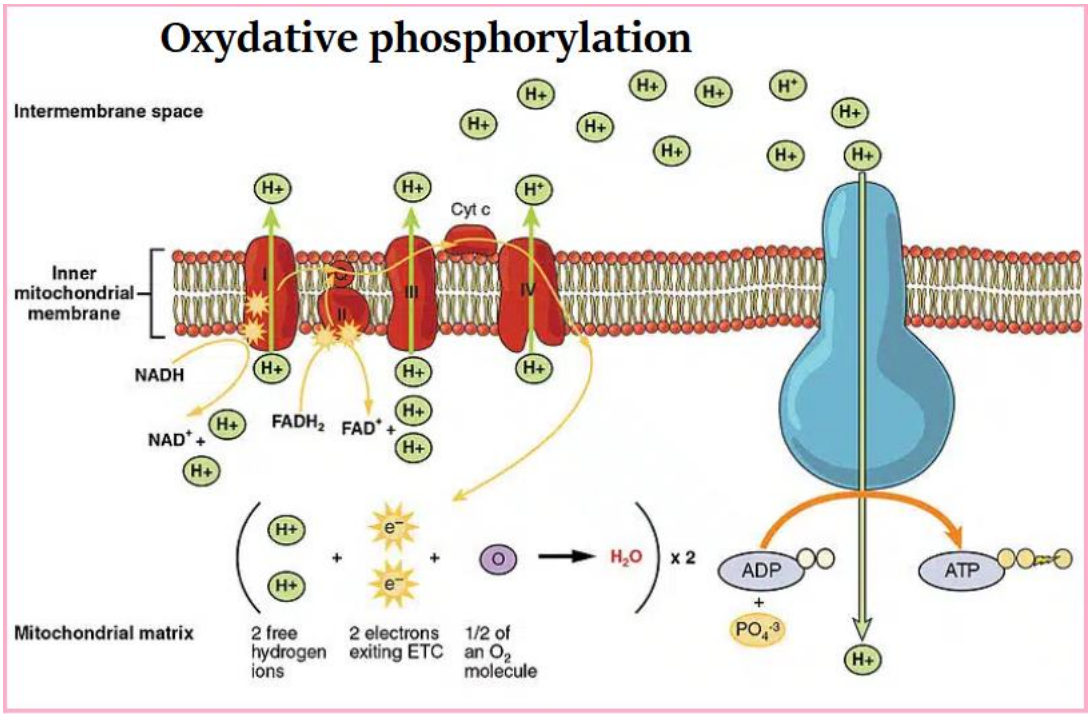


Figure 39. Oxydative phosphorylation

4. The energetic balance

Combining the yields from the different stages, complete aerobic oxidation of one molecule of glucose in a typical human cell produces approximately 30-32 ATP. Glycolysis yields 2 ATP and 2 cytosolic NADH; depending on whether the malate–aspartate or glycerol-3-phosphate shuttle is used, these NADH generate the equivalent of about 3-5 ATP. The conversion of two pyruvate molecules to two acetyl-CoA yields 2 NADH (≈ 5 ATP), and the two turns of the TCA cycle produce 6 NADH (≈ 15 ATP), 2 $FADH_2$ (≈ 3 ATP) and 2 GTP (≈ 2 ATP), giving a realistic total in the low thirties rather than the older theoretical value of 36-38 ATP.

The precise ATP yield per glucose varies between tissues and physiological states because proton leak, uncoupling proteins, variation in shuttle usage and substrate preference (glucose vs fatty acids) modulate both the efficiency of oxidative phosphorylation and the balance between carbohydrate and lipid oxidation. High-energy organs such as the brain, heart and oxidative skeletal muscle exhibit dense mitochondrial populations and finely organised cristae to sustain continuous ATP production; conversely, ischaemia, toxins or genetic defects that compromise mitochondrial function rapidly lead to ultrastructural changes such as mitochondrial swelling, loss of cristae and ultimately necrosis or apoptosis, changes that can be recognised in histological and ultrastructural examination.

ATP Production

Aerobic respiration typically produces a net total of 36 ATP per molecule of glucose consumed

A net total of 2 ATP are produced in glycolysis via substrate level phosphorylation (four are produced, but two are consumed)

A further 2 ATP are similarly produced in the Krebs cycle (one ATP per cycle – two cycles occur per glucose molecule)

Lastly, 32 ATP are produced in the electron transport chain using energy from hydrogen carriers (oxidative phosphorylation)

Hydrogen carriers produce different amounts of ATP depending on where they donate electrons to the transport chain

NADH molecules located in the matrix donate electrons to the start of the chain and produce 3 ATP per hydrogen carrier

Cytosolic NADH (from glycolysis) donate electrons later in the chain and only produce 2 ATP per hydrogen carrier

FADH2 also donates electrons later in the chain and so only produce 2 ATP per hydrogen carrier

Oxidative phosphorylation : (8 × matrix NADH) + (2 × cytosolic NADH) + (2 × FADH2) = (8 × 3) + (2 × 2) + (2 × 2) = 32 ATP

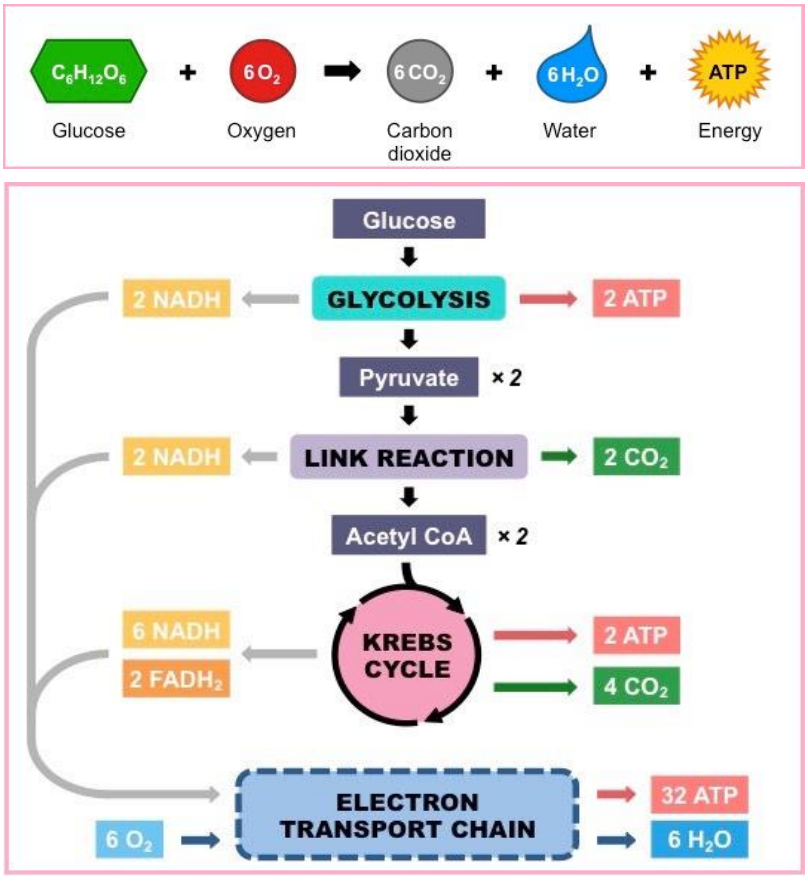


Figure 40. Aerobic cellular respiration: stages and energy yield

The lesson in a nutshell

1) Mitochondria: Overview

- *Double-membrane organelles, size **~0.5–1 µm***
- *Main function: **ATP production (aerobic respiration)***
- *Also involved in:*
  - *Calcium regulation*
  - *Metabolism*
  - *Apoptosis*
- *Number reflects energy demand (many in muscle, none in RBCs)*

2) Origin of Mitochondria

Endosymbiotic theory

- *Derived from ancestral bacteria*
- *Evidence:*
  - *Double membrane*
  - *Circular DNA (~16.6 kb)*
  - *Binary fission*
  - *Own ribosomes*
- ***Maternal inheritance** of mtDNA*

3) Mitochondrial Structure

Four Compartments

1. ***Outer membrane***
   - *Contains porins (permeable to small molecules)*
2. ***Intermembrane space***
3. ***Inner membrane***
   - *Impermeable*
   - *Forms **cristae** (increase surface area)*
   - *Contains electron transport chain (ETC)*
4. ***Matrix***
   - *Enzymes of Krebs cycle*
   - *mtDNA, tRNA, ribosomes*

4) Cellular Respiration: Overview

*Complete oxidation of glucose produces **~30–32 ATP**.*

Three main stages:

1. *Glycolysis (cytosol)*
2. *Krebs cycle (matrix)*
3. *Oxidative phosphorylation (inner membrane)*

5) Glycolysis (Cytosol)

- *Glucose → 2 pyruvate*
- *Net yield:*
  - ***2 ATP***
  - ***2 NADH***
- *Without oxygen → lactate fermentation*

6) Pyruvate Oxidation + Krebs Cycle (Matrix)
Pyruvate → Acetyl-CoA

- *Produces NADH*

Krebs Cycle (per glucose)

- ***6 NADH***
- ***2 $FADH_2$***
- ***2 GTP (≈ ATP)***
- *Releases CO_2*

7) Oxidative Phosphorylation (Inner Membrane)

- *Electrons from NADH/$FADH_2$ pass through **ETC (Complex I–IV)***
- *Protons pumped → electrochemical gradient*
- ***ATP synthase (Complex V)** produces ATP*

ATP yield:

- *NADH ≈ **3 ATP***
- *$FADH_2$ ≈ **2 ATP***
-

8) Energy Balance (per Glucose)

Stage	***ATP***
Glycolysis	*2*
Krebs (GTP)	*2*
Oxidative phosphorylation	*34*
Total	***38 ATP***

CHAPTER IX : RIBOSOMES AND PROTEIN BIOSYNTHESIS

Lesson Objectives

By the end of this lesson, students will be able to:

- Describe the structure and composition of ribosomes
- Differentiate between prokaryotic (70S) and eukaryotic (80S) ribosomes
- Explain the role of ribosomes in translating mRNA into a polypeptide
- Outline the main stages of protein synthesis

Introduction

Ribosomes are large ribonucleoprotein particles that catalyse the polymerisation of amino acids into polypeptide chains, thereby constituting the universal machinery of protein synthesis in all living cells. In human cells, cytosolic ribosomes sediment at 80S (Svedberg units, a measure of sedimentation behaviour), each being composed of a 40S small subunit and a 60S large subunit containing four ribosomal RNAs (rRNAs) and about 80 ribosomal proteins (RPs), with an overall diameter of approximately 25-30 nm. In rapidly proliferating mammalian cells, a single cell typically contains on the order of 10^6-10^7 ribosomes, and the synthesis and maintenance of this massive ribosome population consume a substantial fraction of cellular transcription and energy, underscoring why ribosomes are central to both cell growth and histological features of high proliferative activity.

From a cytological and histological perspective, ribosomes are responsible for many classical staining characteristics of the cytoplasm: because they are rich in RNA, they confer basophilia to the cytoplasm in routine haematoxylin-eosin (H&E) stains, and their high concentration on the rough endoplasmic reticulum and in the perikaryon of neurons explains the dense “Nissl substance” seen with basic dyes. In transmission electron microscopy (TEM), ribosomes appear as electron-dense granules grouped into polyribosomes or aligned along the membranes of the rough endoplasmic reticulum, structures that are optimally preserved by aldehyde fixation (for example, 2.5 % glutaraldehyde in 0.1 mol/L cacodylate buffer at 4 °C for 30-60 minutes, followed by 1 % osmium tetroxide for about 1-2 hours) before resin embedding and ultrathin sectioning. Consequently, the study of ribosomes and protein biosynthesis provides a conceptual bridge between molecular biology and the microscopic appearance of cells in both normal tissues and diagnostic cytology or histopathology.

1. Generalities on nucleic acids and amino acids

Nucleic acids are linear polymers of nucleotides, each nucleotide consisting of a pentose sugar, a phosphate group, and a nitrogenous base ; in DNA (deoxyribonucleic acid), the sugar is deoxyribose and the bases are adenine, guanine, cytosine and thymine, whereas in RNA (ribonucleic acid) the sugar is ribose and thymine is replaced by uracil. DNA forms a double helix approximately 2 nm in diameter, in which complementary base pairing allows long-term storage of genetic information, while most cellular RNAs are single-stranded molecules that adopt diverse three-dimensional conformations enabling structural and catalytic roles, as exemplified by transfer RNA (tRNA) and rRNA. In human cells, protein-coding genes are transcribed into precursor messenger RNAs (pre-mRNAs), which, after processing, yield mature mRNAs that serve as templates for translation on ribosomes, thereby embodying the central dogma DNA → RNA → protein.

The genetic code is a triplet code in which each codon, a sequence of three nucleotides in the mRNA, specifies either one of the 20 standard amino acids or a translational stop signal. Because there are $4^3 = 64$ possible codons but only 20 amino acids, the code is degenerate : most amino acids are encoded by several synonymous codons, while AUG normally functions both as the codon for methionine and as the principal start codon in eukaryotic translation, and UAA, UAG and UGA function as stop codons. The reading frame is established by the position of the start codon on the mRNA, and any shift of this frame due to insertion or deletion mutations profoundly alters the encoded polypeptide, a principle that underlies many genetic diseases associated with truncated or misfolded proteins.

Amino acids are organic molecules composed of a central α-carbon atom bearing an amino group ($–NH_2$), a carboxyl group (–COOH), a hydrogen atom, and a distinctive side chain (R group) that defines their chemical properties; at physiological pH, they are predominantly in the zwitterionic form. Peptide bonds form by condensation between the α-carboxyl group of one amino acid and the α-amino group of the next, producing linear polypeptide chains in which the sequence of residues (primary structure) determines the folding into secondary, tertiary and, where relevant, quaternary structure ; the average mass of an amino acid residue in proteins is about 110 Da, so a 500-residue protein has a mass of roughly 55 kDa. From a functional perspective, the precise sequence of amino acids encoded by an mRNA dictates the protein’s enzymatic activity, binding specificity and localisation, making the fidelity of transcription and translation cruciale for cellular homeostasis and for the interpretation of cytological abnormalities related to protein misfolding or deficiency.

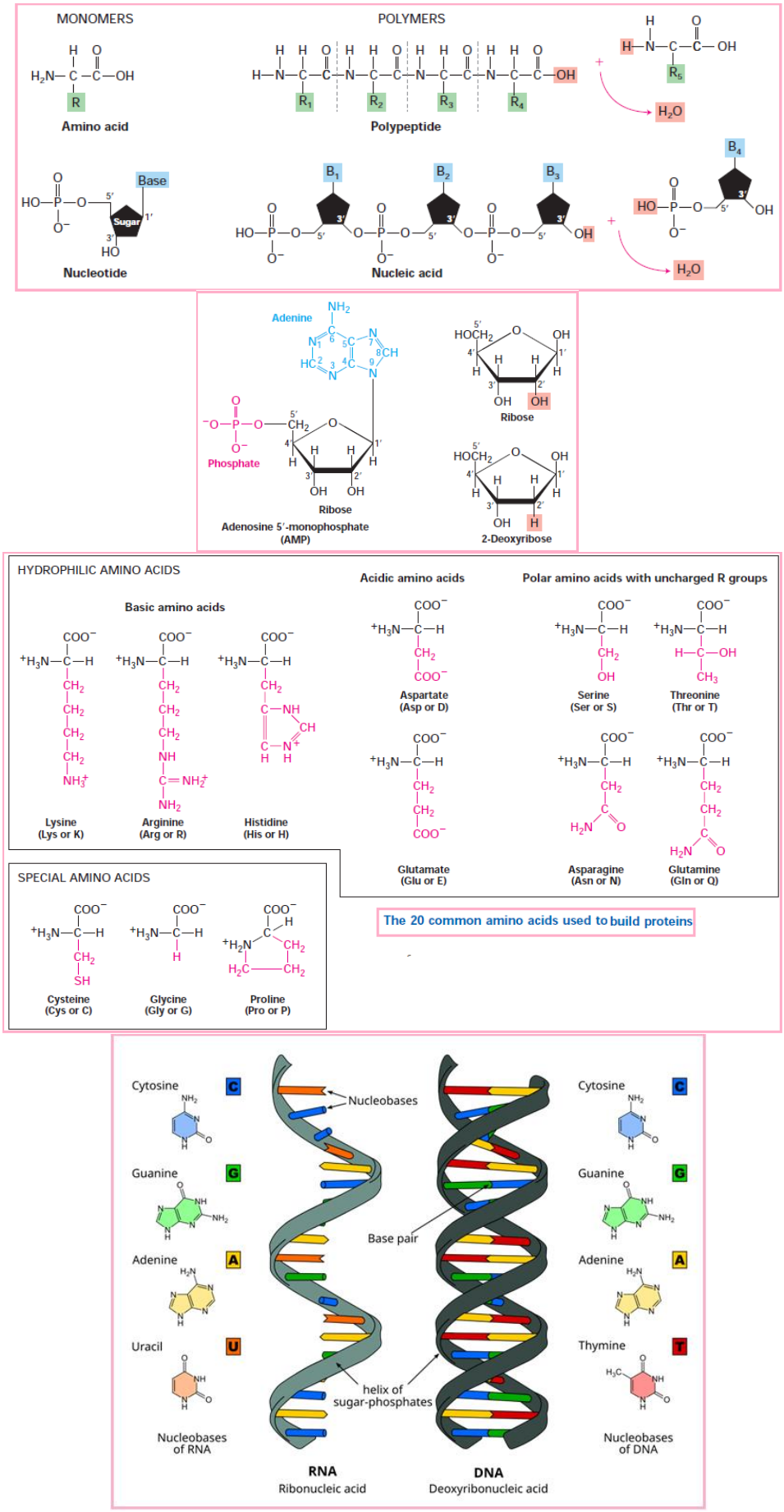


Figure 41. Fundamental Biomolecules: Amino Acids, Nucleotides, RNA and DNA

2. Structure of ribosomes

The cytosolic ribosome of human cells is an 80S particle consisting of a 40S small subunit and a 60S large subunit, each built around specific rRNAs that provide a scaffold for numerous ribosomal proteins. The 40S subunit contains a single 18S rRNA (≈1900 nucleotides) and about 33 proteins, whereas the 60S subunit contains the 28S, 5.8S and 5S rRNAs (≈5000, ≈160 and ≈120 nucleotides, respectively) and about 47 proteins; taken together, these subunits yield a ribosome with a mass of roughly 4.3 MDa and a diameter of 25-30 nm. Cryo-electron microscopy and X-ray crystallography have revealed that eukaryotic ribosomes are more complex than their bacterial counterparts, with elaborated rRNA expansion segments and additional proteins that contribute to regulatory interactions and the specificity of translation in eukaryotic cells.

Functionally, the small (40S) subunit forms the decoding centre, where codon-anticodon interactions between mRNA and tRNA are monitored, while the large (60S) subunit contains the peptidyl transferase centre that catalyses peptide bond formation and the peptide exit tunnel through which the nascent polypeptide emerges. Together, the two subunits create three tRNA-binding sites : the aminoacyl site (A site), which accepts incoming aminoacyl-tRNAs ; the peptidyl site (P site), which holds the tRNA carrying the growing polypeptide chain ; and the exit site (E site), which transiently binds deacylated tRNA prior to its dissociation. Structural and biochemical studies demonstrate that peptide bond formation is catalysed by 28S rRNA within the large subunit, establishing the ribosome as a ribozyme, while ribosomal proteins stabilise the rRNA architecture and mediate dynamic interactions with translation factors.

Comparative analyses show that eukaryotic 80S ribosomes are larger and more complex than bacterial 70S ribosomes (composed of 30S and 50S subunits), and this structural divergence underlies the selective action of many antibiotics that inhibit bacterial but not cytosolic eukaryotic translation. In contrast, mitochondrial ribosomes (mitoribosomes) in human cells are more similar to bacterial ribosomes in their sedimentation behaviour (≈55S) and sensitivity to certain antibiotics, reflecting their endosymbiotic origin and explaining why some antibacterial drugs can inadvertently affect mitochondrial protein synthesis. These structural distinctions are essential for understanding both pharmacological selectivity and ultrastructural observations in pathology, where mitochondrial ribosomal defects can manifest as myopathies with characteristic histological changes.

In routine light microscopy, individual ribosomes are below the resolution limit (~0.2 µm), but their collective presence is evident : free polyribosomes and ribosome-studded RER confer

intense basophilia to the cytoplasm of protein-secreting cells such as plasma cells and pancreatic acinar cells, reflected in their deep blue cytoplasm in H&E sections and prominent Nissl bodies in neurons stained with basic dyes. TEM resolves ribosomes as 20-30 nm dense particles arranged in rosettes or spirals (polyribosomes) or aligned along RER cisternae, features that correlate directly with high synthetic activity in secretory epithelia, haematopoietic precursors and neoplastic cells showing increased protein synthesis.

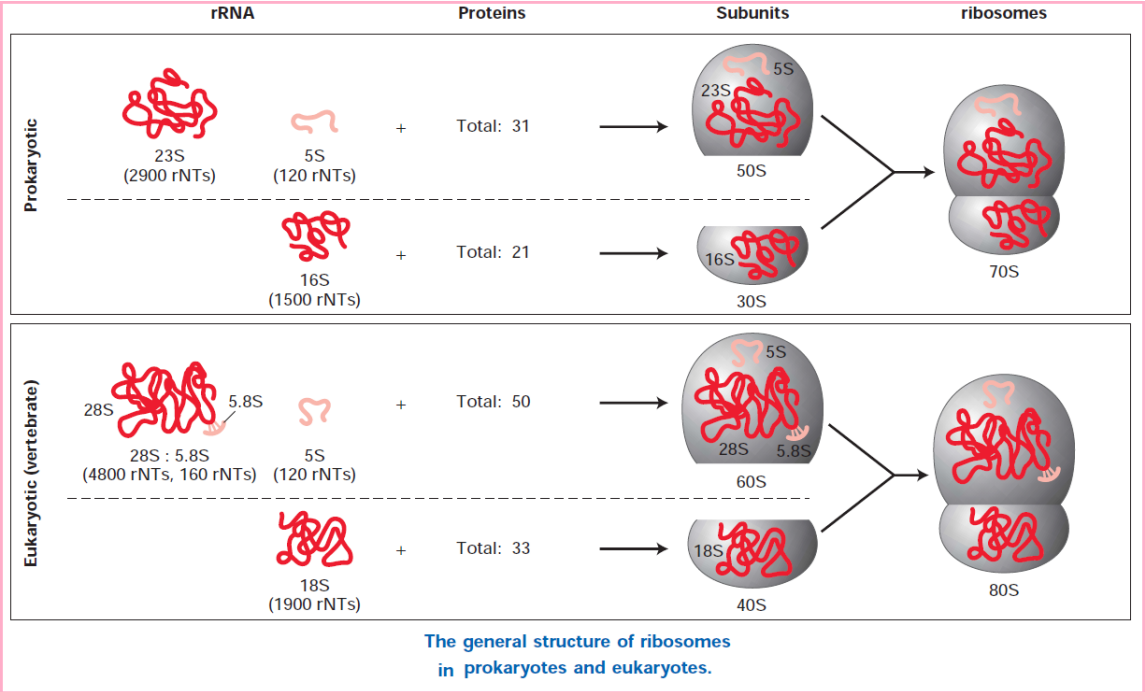


Figure 42. Comparative Structure of Prokaryotic and Eukaryotic Ribosomes

3. Biogenesis of ribosomes

Ribosome biogenesis is a highly orchestrated and energy-intensive process that integrates the activity of all three nuclear RNA polymerases, hundreds of assembly factors and small nucleolar ribonucleoproteins (snoRNPs). In a typical mammalian cell, a large fraction of total transcriptional output is devoted to ribosomal components : RNA polymerase I (Pol I) transcribes the rDNA repeats to generate a 47S pre-rRNA precursor, RNA polymerase III (Pol III) synthesises 5S rRNA and certain tRNAs, and RNA polymerase II (Pol II) transcribes the numerous ribosomal protein genes whose products are imported into the nucleus. Estimates suggest that proliferating human cells can assemble on the order of 10^5 new ribosomes per hour, requiring tight coordination between rRNA synthesis, processing and assembly to avoid imbalances that would impair growth or trigger stress responses.

The nucleolus, a prominent non-membrane-bound domain of the interphase nucleus, is the central site of early ribosome biogenesis and forms around nucleolar organiser regions

containing tandem rDNA repeats. Within the nucleolus, Pol I transcribes rDNA into the 47S pre-rRNA, which is rapidly processed through a series of endonucleolytic and exonucleolytic cleavages, accompanied by extensive nucleotide modifications (2′-O-methylation, pseudouridylation) guided by snoRNPs, to yield the mature 18S, 5.8S and 28S rRNAs incorporated into pre-40S and pre-60S subunits. These pre-ribosomal particles then undergo further maturation steps as they transit from the nucleolus through the nucleoplasm to the cytoplasm, where final rRNA processing and assembly events complete the formation of translationally competent ribosomes.

In parallel, Pol III-derived 5S rRNA and numerous ribosomal proteins synthesised in the cytosol are imported into the nucleus through nuclear pores and targeted to the nucleolus, where they assemble with pre-rRNA to form pre-subunits; this process involves an extensive network of assembly factors and quality-control checkpoints that ensure only properly formed ribosomes are exported. Export of pre-40S and pre-60S subunits from the nucleus to the cytoplasm is mediated by specific export receptors (karyopherins) that recognise export signals on the pre-ribosomal particles and rely on the Ran-GTPase system to provide directionality through the nuclear pore complex. Final cytoplasmic maturation includes release of assembly factors, incorporation of late-binding ribosomal proteins and functional proof-reading, such that only correctly assembled ribosomes participate in translation, a step whose failure can activate p53-dependent cellular stress pathways.

Genetic defects affecting ribosome biogenesis or ribosomal proteins cause a spectrum of human disorders collectively termed ribosomopathies, characterised by tissue-specific hypoplasia (notably of the bone marrow) and a paradoxically increased risk of cancer later in life. Classical examples include Diamond–Blackfan anaemia, often caused by haploinsufficiency of small- or large-subunit ribosomal proteins, Schwachman–Diamond syndrome, dyskeratosis congenita, and Treacher Collins syndrome, all of which involve impaired rRNA processing or ribosome assembly. In histology and cytology, many malignant cells display enlarged and irregular nucleoli, reflecting hyperactive ribosome biogenesis driven by oncogenic signalling pathways, a feature exploited both in grading certain tumours and in the development of experimental therapies targeting nucleolar function or Pol I activity.

4. Localisation of ribosomes in the cell

In the cytoplasm, ribosomes exist either as free ribosomes dispersed in the cytosol or as membrane-bound ribosomes attached to the cytosolic face of the rough endoplasmic reticulum and outer nuclear envelope; the same ribosomal pool can dynamically transition between these

states depending on the presence of signal sequences in the nascent polypeptides. Free ribosomes, often organised into polyribosomes (several ribosomes simultaneously translating a single mRNA), synthesise proteins destined to remain in the cytosol or to be imported post-translationally into the nucleus, mitochondria, peroxisomes, or other organelles, which explains the abundance of free polyribosomes in haematopoietic precursors and many metabolically active cells.

Membrane-bound ribosomes are recruited to the rough endoplasmic reticulum when the nascent polypeptide emerging from the ribosome carries an N-terminal signal peptide recognised by the signal-recognition particle (SRP), which pauses translation and targets the ribosome–nascent chain complex to SRP receptors on the ER membrane; translation then resumes with co-translational translocation of the polypeptide into the ER lumen or membrane. This pathway accounts for the synthesis of secreted proteins, lysosomal enzymes and most integral membrane proteins, and it explains why cells specialised in protein secretion, such as plasma cells and pancreatic acinar cells, show cytoplasm packed with RER cisternae, giving rise to intense basophilia in H&E and distinct patterns in cytological smears stained by May–Grünwald–Giemsa or similar Romanowsky stains.

Mitochondria possess their own ribosomes, which translate a limited set of mitochondrial genes encoding core subunits of the respiratory chain; these mitoribosomes, although functionally analogous to cytosolic ribosomes, differ considerably in rRNA/protein composition and sensitivity to inhibitors, features that must be considered when interpreting the effects of certain antibiotics or genetic defects on mitochondrial function. Pathogenic variants in mitochondrial rRNAs or proteins of the mitochondrial translation system produce characteristic clinical syndromes, including mitochondrial encephalomyopathies, which often exhibit ragged-red fibres and other distinctive histological features in muscle biopsies owing to impaired oxidative phosphorylation.

5. Steps of protein biosynthesis

5.1. Transcription

Transcription is the process by which an RNA polymerase synthesises an RNA molecule complementary to a DNA template strand, thereby generating pre-mRNA for protein-coding genes and various non-coding RNAs. In eukaryotic nuclei, RNA polymerase II (Pol II) transcribes most protein-coding genes, recognising promoter regions and producing pre-mRNAs that will later be processed into mature mRNAs, whereas Pol I and Pol III synthesise

rRNA and tRNA and other small RNAs, respectively. The transcription cycle is conventionally divided into three main phases—initiation, elongation and termination—followed by extensive mRNA processing steps that are tightly coupled to Pol II activity and essential for the production of export-competent transcripts.

5.1.1. Initiation

During transcription initiation at a Pol II promoter, a pre-initiation complex forms by the stepwise assembly of general transcription factors (TFIID, TFIIA, TFIIB, TFIIE, TFIIF and TFIIH) and Pol II at core promoter elements such as the TATA box. TFIID, which contains the TATA-binding protein (TBP) and TBP-associated factors, recognises the TATA box and bends the DNA, facilitating recruitment of other factors and Pol II to form a closed complex that subsequently transitions to an open complex as TFIIH helicase activity unwinds about 10–15 base pairs around the transcription start site. Phosphorylation of the C-terminal domain (CTD) of Pol II by TFIIH triggers promoter clearance and the release of several initiation factors, allowing Pol II to enter productive elongation while the CTD serves as a platform for factors involved in mRNA processing.

5.1.2. Elongation

In the elongation phase, Pol II moves along the template DNA at a rate of roughly 20–60 nucleotides per second, synthesising RNA in the 5′→3′ direction by catalysing the sequential addition of ribonucleoside triphosphates and using its intrinsic 3′→5′ exonuclease activity and transcription-coupled repair pathways to maintain high fidelity. Elongation is modulated by pausing and restart events, often near the promoter, which provide regulatory checkpoints for transcription factors and chromatin modifiers and integrate transcription with co-transcriptional processes such as splicing and chromatin remodelling. The density of elongating Pol II complexes and their processivity influence the level of mRNA produced, thereby controlling the amount of substrate available for translation and ultimately affecting protein abundance and cellular phenotype.

5.1.3. Termination

Termination of Pol II transcription is closely coupled to pre-mRNA 3′-end formation: after transcribing a polyadenylation signal (typically AAUAAA) and associated downstream elements, the pre-mRNA is cleaved by a multi-protein complex, and a poly(A) tail of 100–250 adenosine residues is added by poly(A) polymerase. Following cleavage, Pol II continues to

transcribe for a short distance before being released from the DNA template, a process that likely involves a “torpedo” mechanism in which a 5′→3′ exonuclease degrades the downstream RNA and destabilises the elongation complex. Proper transcription termination and polyadenylation are critical not only for mRNA stability and nuclear export, but also for preventing transcriptional interference with neighbouring genes, with defects in these processes being implicated in various genetic and neoplastic disorders.

9.6.1.4 Maturation of pre-mRNA

Eukaryotic pre-mRNAs undergo extensive maturation before they can serve as templates for translation. Immediately after initiation, the 5′ end of the nascent RNA is modified by addition of a 7-methylguanosine cap linked via a 5′–5′ triphosphate bridge, a structure that protects the mRNA from exonucleases and recruits the cap-binding complex important for splicing, nuclear export and translation initiation. Introns are removed and exons joined by the spliceosome, a large ribonucleoprotein complex composed of small nuclear RNAs and proteins, which recognises consensus sequences at exon–intron boundaries; alternative splicing, by combining exons in different patterns, markedly increases proteomic diversity and can be dysregulated in disease. Finally, cleavage and polyadenylation at the 3′ end generate the mature poly(A) tail, which enhances mRNA stability and translation efficiency; the resulting messenger RNPs are then exported through nuclear pores to the cytoplasm, where they will be engaged by ribosomes.

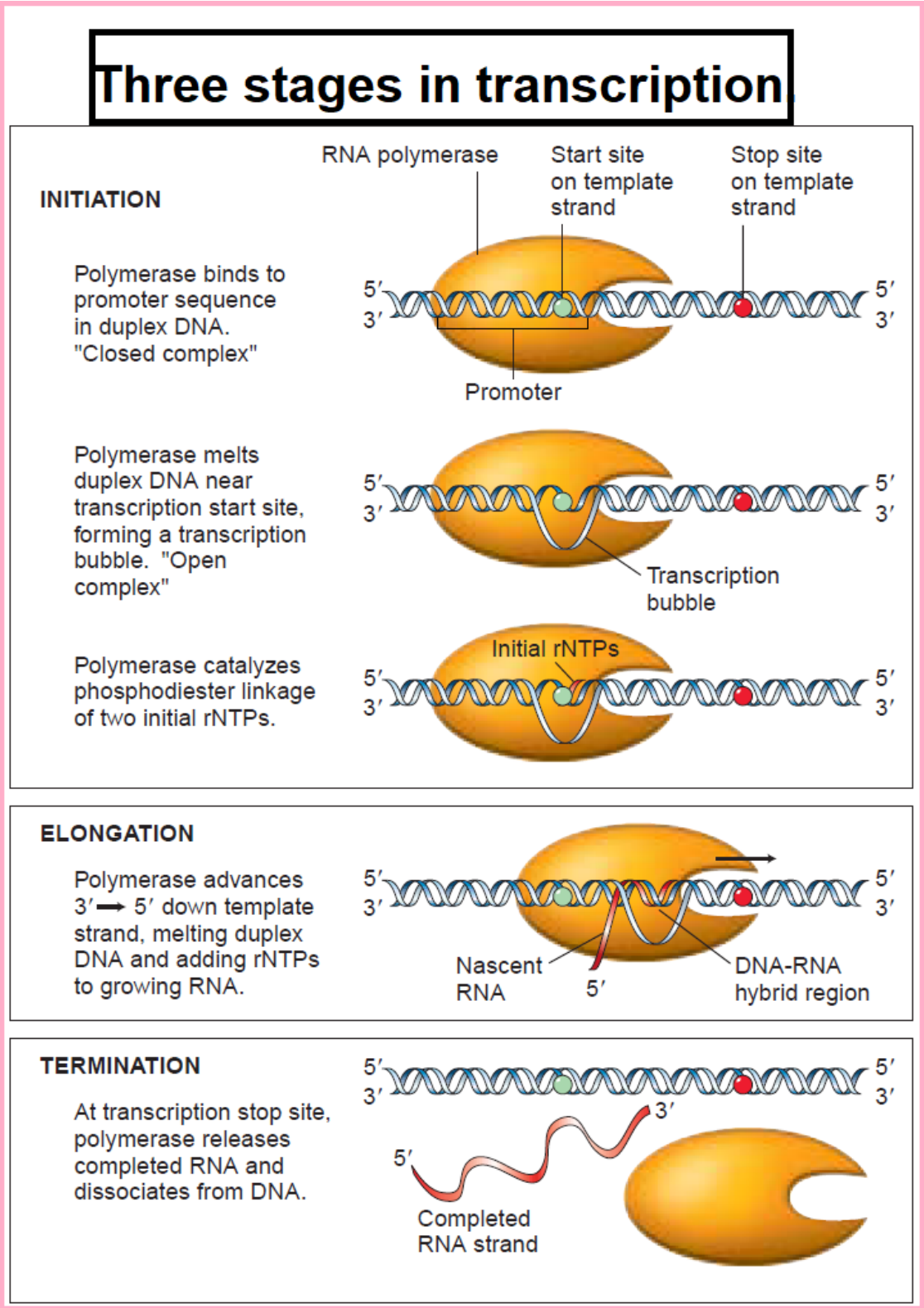


Figure 43. The Three Major Stages of Transcription

5.2. Translation

Translation is the process by which ribosomes decode the nucleotide sequence of an mRNA into the amino-acid sequence of a polypeptide, a process classically divided into activation of

amino acids, initiation, elongation, termination, and post-translational maturation. In mammalian cells, cytosolic translation typically proceeds at an average rate of a few amino acids per second per ribosome, with multiple ribosomes forming polyribosomes on a single mRNA, so that thousands of protein molecules can be produced from a single transcript before its degradation. Translation is tightly regulated at the initiation step and is modulated by signalling pathways such as mTOR, which link nutrient and growth factor availability to protein synthesis, a relationship that is profoundly altered in many cancers.

5.2.1. Activation of amino acids

Before an amino acid can be incorporated into a growing polypeptide, it must be "activated" by covalent attachment to its cognate tRNA, a reaction catalysed by aminoacyl-tRNA synthetases. Each of the 20 canonical amino acids is typically recognised by a specific aminoacyl-tRNA synthetase, which binds the amino acid and the appropriate tRNA(s) and catalyses formation of an aminoacyl-adenylate intermediate, followed by transfer of the amino acid to the 3′ end of the tRNA, creating an aminoacyl-tRNA with high free-energy content. These enzymes possess proofreading mechanisms that hydrolyse mis-activated amino acids or mis-charged tRNA species, thereby ensuring that the genetic code is translated with very low error rates (often around 10^{-4} per codon), which is essential for maintaining protein integrity and avoiding cytotoxic accumulation of misfolded proteins.

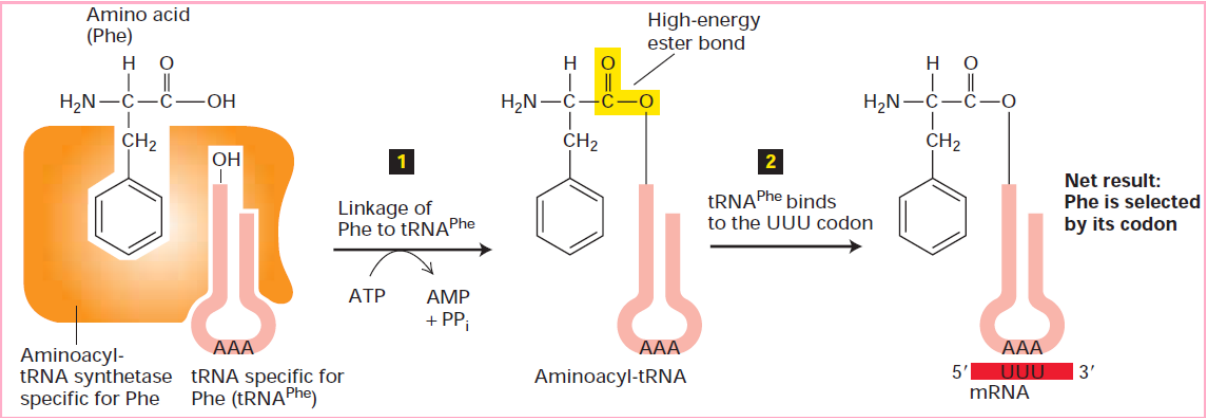


Figure 44. Mechanism of Aminoacyl-tRNA Formation and Codon Recognition

5.2.2. Initiation of translation

In cap-dependent eukaryotic translation, initiation begins with the formation of a ternary complex composed of eukaryotic initiation factor 2 (eIF2), GTP and the initiator methionyl-tRNA (Met-$tRNA_i^{Met}$), which associates with the 40S ribosomal subunit and other initiation factors (eIF1, eIF1A, eIF3, eIF5) to form a 43S pre-initiation complex. In parallel, the 5′ cap of

the mRNA is recognised by the eIF4F complex (containing eIF4E, eIF4G and eIF4A), which unwinds secondary structures in the 5′ untranslated region, allowing the 43S complex to be recruited to the mRNA and to scan in the 5′→3′ direction until it encounters an initiation codon (usually the first AUG in a favourable Kozak context). Recognition of the start codon triggers GTP hydrolysis on eIF2, release of several initiation factors, and joining of the 60S subunit, yielding an 80S initiation complex poised to enter the elongation phase with the initiator tRNA in the P site.

5.2.3. Elongation

During elongation, each cycle adds one amino acid to the growing polypeptide chain and involves three principal steps: aminoacyl-tRNA entry, peptide bond formation and translocation. In eukaryotes, aminoacyl-tRNAs are delivered to the A site of the ribosome in complex with eukaryotic elongation factor 1A (eEF1A) and GTP; only cognate tRNAs whose anticodons correctly base-pair with the mRNA codon are stabilised in the A site, while non-cognate tRNAs dissociate, providing a kinetic proofreading mechanism. Once accommodated, the peptidyl transferase centre of the large subunit catalyses peptide bond formation, transferring the nascent chain from the tRNA in the P site to the aminoacyl-tRNA in the A site; subsequently, eukaryotic elongation factor 2 (eEF2) promotes GTP-dependent translocation of the ribosome along the mRNA by one codon, moving the deacylated tRNA to the E site and the peptidyl-tRNA to the P site. This cycle repeats until a stop codon is reached, and its efficiency and accuracy are influenced by codon usage, mRNA secondary structure and the availability of tRNAs, factors that can modulate translation speed and co-translational folding of the nascent protein.

5.2.4. Termination

Termination occurs when a stop codon (UAA, UAG or UGA) enters the A site, where it is recognised not by a tRNA but by class-I release factors (eRF1 in eukaryotes), which, together with the GTPase eRF3, promote hydrolysis of the ester bond linking the polypeptide to the tRNA in the P site, thereby releasing the completed polypeptide. Subsequently, ribosome recycling factors and initiation factors such as eIF3 and ABCE1 dissociate the post-termination complex into free 40S and 60S subunits, mRNA, and deacylated tRNA, preparing these components for further rounds of translation. Errors in termination, such as stop-codon read-through or premature termination caused by nonsense mutations or aberrant mRNA surveillance, can generate truncated or elongated proteins and are associated with diverse

diseases, some of which are being targeted therapeutically by agents that modulate translational fidelity.

5.2.5. Post-translational maturation and targeting

Newly synthesised polypeptides begin to fold while still attached to the ribosome, a process assisted by molecular chaperones that prevent inappropriate aggregation and promote acquisition of the native conformation. Many proteins undergo co- and post-translational modifications, including signal peptide cleavage, glycosylation, phosphorylation, acetylation, disulphide bond formation and proteolytic processing, which are often essential for activity, stability, and correct subcellular localisation; for example, secretory and lysosomal proteins entering the ER lumen during translation are glycosylated and later sorted in the Golgi apparatus.Proteins that fail to fold correctly or that contain errors introduced during translation are recognised by quality-control systems, such as the ER-associated degradation pathway and the ubiquitin–proteasome system, preventing accumulation of misfolded proteins that could lead to cellular stress, inclusion bodies or degenerative disease.

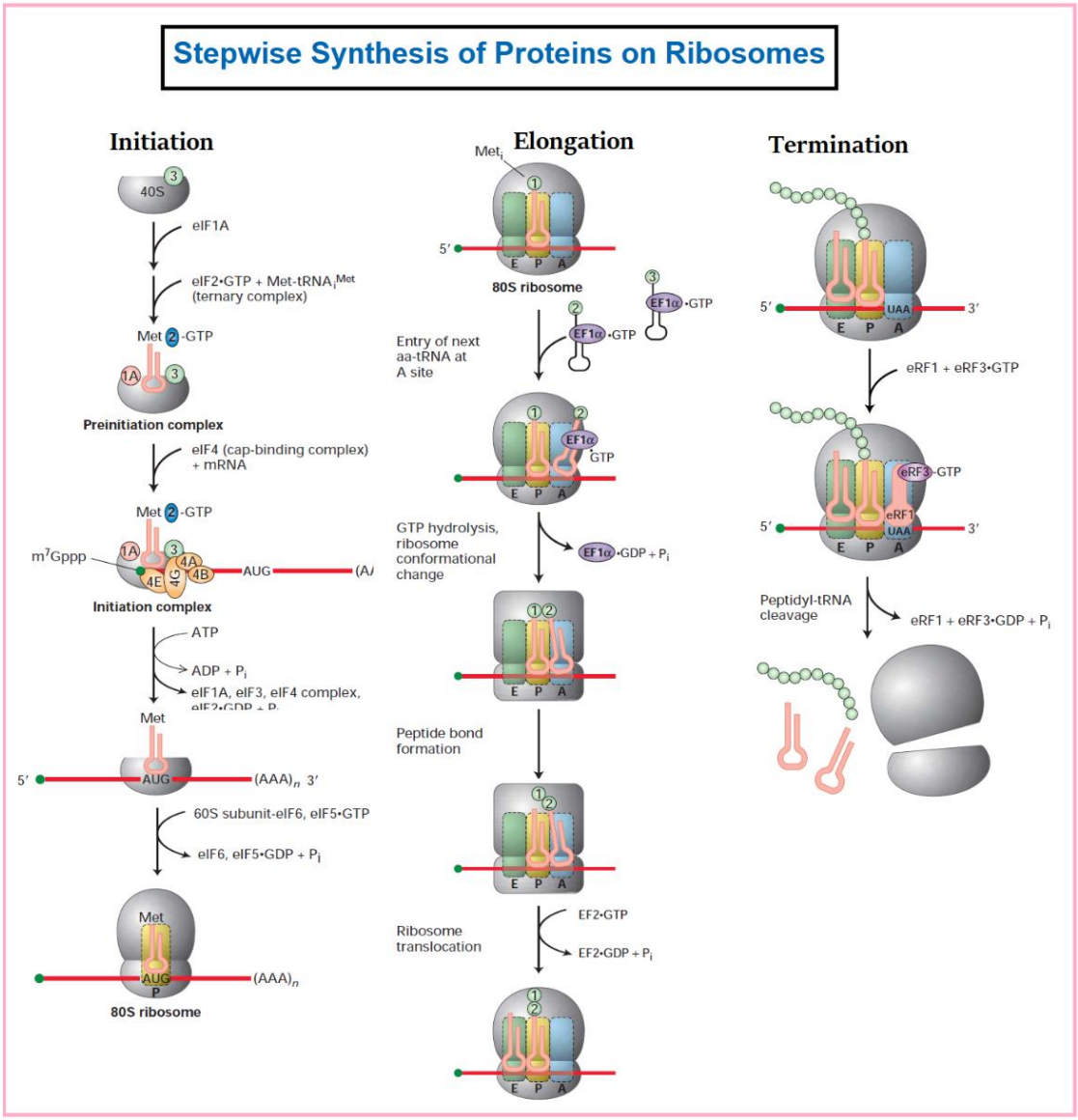


Figure 45. Stages of Protein Synthesis on Ribosomes

6. Physiological and clinical relevance of ribosomes and protein biosynthesis

The rate and regulation of ribosome biogenesis and protein synthesis are tightly coupled to cell growth, differentiation and stress responses, and they are profoundly altered in many human diseases. In normal physiology, highly secretory cells (for example, plasma cells producing immunoglobulins or exocrine gland cells secreting digestive enzymes) and rapidly proliferating cells (such as haematopoietic precursors) exhibit abundant nucleoli, dense rough endoplasmic reticulum and cytoplasmic basophilia on histological sections, reflecting massive ribosome production and active translation. In contrast, ribosomopathies arising from germline defects in ribosome biogenesis present with characteristic cytopenias, developmental abnormalities and an increased incidence of malignancy, illustrating how subtle changes in ribosomal function can produce highly tissue-specific clinical phenotypes.

Cancer cells frequently show enlarged and irregular nucleoli, increased numbers of polyribosomes, and upregulated translation initiation, features that correlate with aggressive behaviour and poor prognosis and have prompted the development of therapeutic strategies targeting ribosome biogenesis or translation factors (for example, inhibitors of Pol I transcription or mTOR-dependent initiation). In diagnostic cytology and histopathology, careful evaluation of nucleolar prominence, cytoplasmic basophilia, and the distribution of rough endoplasmic reticulum and polyribosomes contributes to the recognition of highly proliferative or protein-secreting cell populations, such as plasmacytosis in bone marrow aspirates, reactive lymphoid hyperplasia, or high-grade epithelial dysplasia. Mastery of ribosome structure, biogenesis and function, together with the mechanisms of transcription and translation outlined in this chapter, therefore provides an essential framework for interpreting both normal histological patterns and a wide range of pathological alterations seen in routine cytological and histological practice.

The lesson in a nutshell

1) General Definition

Ribosomes are ribonucleoprotein complexes responsible for translating mRNA into proteins.

- *Present in all living cells*
- *Universal machinery of protein synthesis*

2) Ribosome Structure

Eukaryotic Cytosolic Ribosome

- ***80S ribosome***
  - ***40S small subunit***
    - *Contains 18S rRNA*
  - ***60S large subunit***
    - *Contains 28S, 5.8S and 5S rRNAs*
- *Diameter: 25–30 nm*
- *Three functional sites:*
  - ***A site** (Aminoacyl)*
  - ***P site** (Peptidyl)*
  - ***E site** (Exit)*

Prokaryotic vs Eukaryotic Ribosomes

Feature	*Prokaryotes*	*Eukaryotes*
Size	*70S*	*80S*
Subunits	*30S + 50S*	*40S + 60S*

📌 *Mitochondrial ribosomes (~55S) resemble bacterial ribosomes.*

3) Cellular Localization

Free Ribosomes
Synthesize proteins destined for:

- *Cytosol*
- *Nucleus*
- *Mitochondria*
- *Peroxisomes*

Rough Endoplasmic Reticulum–Bound Ribosomes
Synthesize:

- *Secreted proteins*
- *Lysosomal proteins*
- *Membrane proteins*

📌 *Free and bound ribosomes are structurally identical.*

4) Transcription (Nucleus)

Main Stages

a. Initiation

- *RNA polymerase II binds promoter*
- *Formation of pre-initiation complex*

---

b. Elongation

- *RNA synthesized 5' → 3'*

---

c. Termination

- *Polyadenylation signal (AAUAAA)*
- *Addition of poly(A) tail*

---

mRNA Processing

- *5' cap (7-methylguanosine)*
- *Splicing (removal of introns)*
- *Polyadenylation*
- *Nuclear export*

3) Translation (Cytoplasm)

Main Stages

a. Amino Acid Activation

- *Catalyzed by aminoacyl-tRNA synthetases*
- *High fidelity (~10^{-4} error rate)*

---

b. Initiation

- *Formation of 43S pre-initiation complex*
- *Recognition of 5' cap*
- *Scanning for AUG*
- *Assembly of 80S ribosome*

---

c. Elongation

- *tRNA entry (A site)*
- *Peptide bond formation*
- *Translocation*

---

d. Termination

- *Stop codon recognition*
- *Release factors (eRF1)*
- *Ribosome dissociation*

---

e.Post-Translational Maturation

- *Protein folding (chaperones)*
- *Glycosylation*
- *Phosphorylation*
- *Signal peptide cleavage*
- *Ubiquitin–proteasome degradation*

Genetic Code

- *Triplet code (codons)*
- *64 codons*
- *AUG = start codon (Methionine)*
- *UAA, UAG, UGA = stop codons*
- *Degenerate code*

CHAPTER X : LYSOSOMES AND PEROXISOMES

Lesson Objectives

By the end of this lesson, students will be able to:

- Define lysosomes and peroxisomes,
- Explain the biogenesis of lysosomes and peroxisomes,
- Describe the major functions of lysosomes and peroxisomes,
- Discuss lysosomal andperoxisomal storage diseases

Introduction

Lysosomes are single-membrane organelles, typically 0.2-0.5 μm in diameter (occasionally up to 1.0 μm), that constitute the major catabolic compartment of animal cells, where a broad spectrum of macromolecules is degraded by acid hydrolases in an intraluminal environment with pH around 4.5-5 . Peroxisomes are similarly small, spherical organelles, usually 0.1-1.0 μm in diameter, containing more than fifty oxidative enzymes; they are defined by catalase and oxidases that generate and detoxify hydrogen peroxide and participate in specialised lipid and reactive oxygen species (ROS) metabolism. Together, lysosomes and peroxisomes integrate degradative, metabolic, and signalling pathways, and their abundance and morphology vary widely between cell types, with lysosomes especially prominent in professional phagocytes and peroxisomes abundant in hepatocytes, renal proximal tubule cells, and myelinating glia.

From a histological standpoint, lysosomes and peroxisomes are not individually resolvable by routine light microscopy, but their presence is inferred from cytoplasmic basophilia, the distribution of vacuoles and granules, and, more specifically, by histochemical reactions and immunocytochemistry. Acid phosphatase and other acid hydrolases can be demonstrated in lysosomes on cryostat sections or cell smears using modified Gomori techniques after brief fixation in low-concentration glutaraldehyde (for example, 3-6 % for 5-60 minutes), whereas catalase can be localised in peroxisomes using alkaline 3,3′-diaminobenzidine (DAB) cytochemistry in aldehyde-fixed tissues. Immunohistochemical detection of lysosome-associated membrane proteins such as LAMP-1 and LAMP-2, and of peroxisomal membrane proteins and catalase, further refines the identification and quantification of these organelles in diagnostic and research material.

1. Lysosomes

1.1. Structure and chemical composition

Lysosomes are bounded by a single phospholipid bilayer enriched in integral membrane proteins such as LAMP-1 and LAMP-2, which contribute to a dense luminal glycocalyx that protects the membrane from enzymatic digestion and mediates interactions with cytosolic factors. The lysosomal membrane contains a vacuolar H^+-ATPase that actively pumps protons into the lumen, maintaining an acidic pH of roughly 4.5–5.0, optimal for the activity of more than sixty acid hydrolases including proteases (cathepsins), nucleases, glycosidases, sulfatases, phosphatases and lipases; disruption of this proton gradient rapidly inactivates lysosomal enzymes and impairs degradation.

By transmission electron microscopy, lysosomes appear as round or oval, membrane-bound vesicles with an electron-dense, often heterogeneous matrix, sometimes containing myelin figures or residual bodies representing partially digested material; their size and appearance vary with cell type and functional state. Biochemically, the luminal hydrolases are synthesised as inactive precursors on rough endoplasmic reticulum, glycosylated in the Golgi apparatus and targeted to endosomes via mannose-6-phosphate receptors; failure of this targeting, as in I-cell disease, results in release of hydrolases into extracellular fluid and a profound intracellular deficiency.

1.2. Types of lysosomes

In classical histology, primary lysosomes are defined as small vesicles containing inactive hydrolases that have not yet participated in digestion, whereas secondary lysosomes or “digestive vacuoles” arise from fusion of primary lysosomes with endocytic vesicles, phagosomes or autophagosomes and contain both enzymes and partially degraded materia. When lysosomes fuse with autophagosomes, they form autolysosomes, whereas fusion with phagosomes in phagocytes yields phagolysosomes; residual bodies containing indigestible material may persist for long periods and, in some cells, accumulate as lipofuscin pigment, particularly in long-lived neurons and cardiomyocytes.

Modern cell biology further emphasises lysosomal heterogeneity, recognising distinct subpopulations differing in pH, enzyme content, motility and position within the cell. Late endosomes, multivesicular bodies and lysosome-related organelles (for example, melanosomes, lytic granules of cytotoxic T cells and dense granules of platelets) share components with lysosomes but have specialised cargo and trafficking pathways. Immunostaining for LAMP-1

and LAMP-2 demonstrates that not all LAMP-positive compartments are fully degradative: a significant fraction corresponds to less acidic, pre-lysosomal or signalling-competent vesicles, underscoring the functional diversity within the endo-lysosomal system.

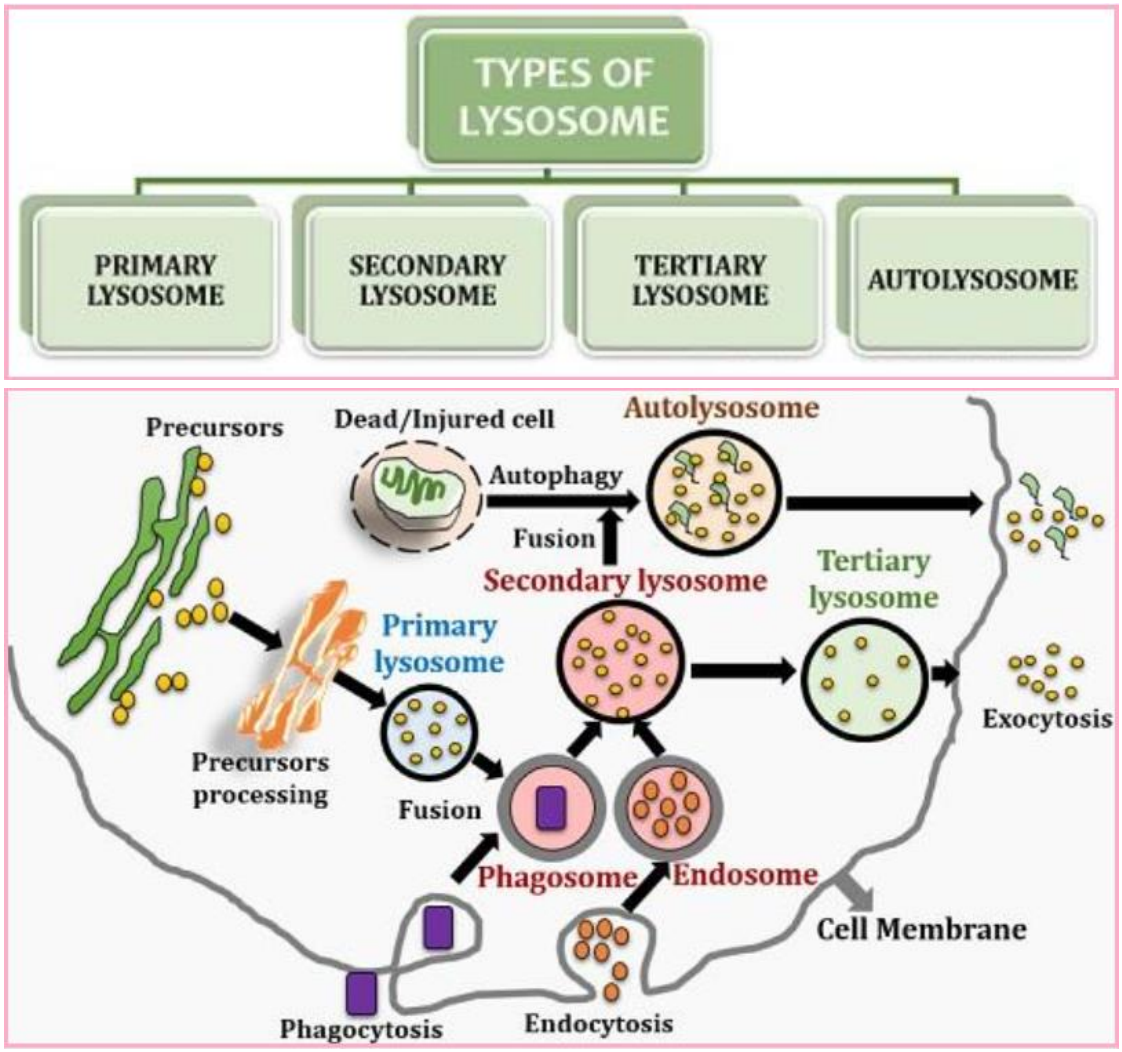


Figure 46. Types of lysosomes and their functions in intracellular digestion

1.3. Roles of lysosomes in the cell

Lysosomes receive substrates through at least three major routes: endocytosis (including receptor-mediated endocytosis of plasma membrane proteins and extracellular ligands), phagocytosis of large particles or microorganisms, and autophagy of cytoplasmic components such as organelles and protein aggregates. Macroautophagy, a conserved pathway in which double-membrane autophagosomes sequester cytoplasmic cargo and fuse with lysosomes, is essential for quality control of mitochondria and other organelles, adaptation to nutrient

starvation, and removal of aggregated proteins; defective autophagy is implicated in neurodegenerative diseases, cancer, and myopathies.

Beyond degradation, lysosomes function as metabolic and signalling hubs. The cytosolic surface of lysosomes hosts the mechanistic target of rapamycin complex 1 (mTORC1), which senses amino acids, growth factors and energy status and coordinates anabolic and catabolic pathways, thereby linking lysosomal function to cell growth and proliferation . Lysosomes also contribute to plasma membrane repair by exocytosis of lysosomal contents, to antigen processing and presentation in professional antigen-presenting cells, and to regulated secretion in specialised cells such as cytotoxic lymphocytes and osteoclasts, where “secretory lysosomes” release lytic enzymes or acids extracellularly .

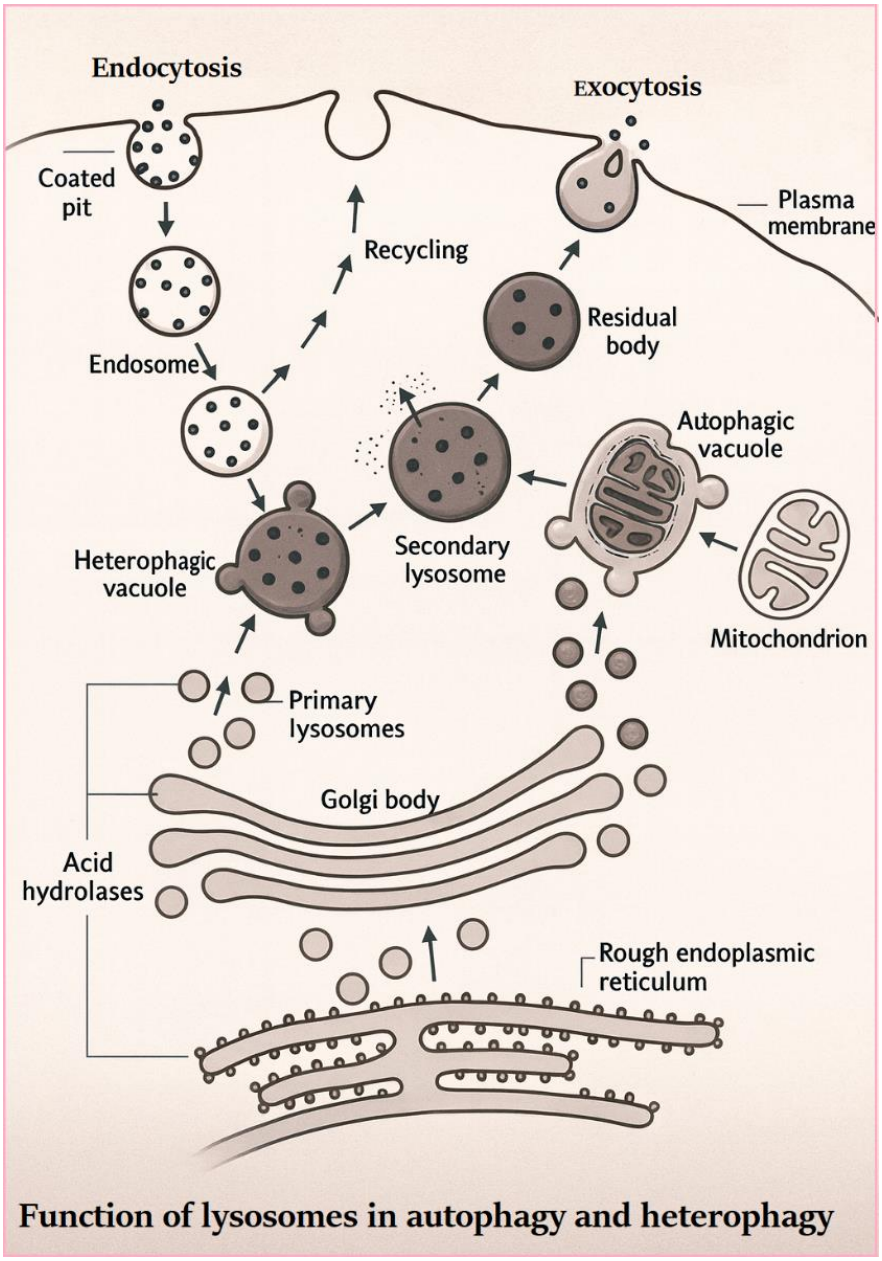


Figure 47. Role of lysosomes in autophagy and heterophagy

1.4. Diseases related to lysosomes

Lysosomal storage diseases (LSDs) are a heterogeneous group of more than 50-70 inherited metabolic disorders, each usually caused by a deficiency of a specific lysosomal enzyme, activator protein, or transporter, leading to intralysosomal accumulation of undegraded substrates such as sphingolipids, glycosaminoglycans, or glycogen. Classical examples include Gaucher disease (deficiency of glucocerebrosidase with accumulation of glucocerebroside in macrophages), Tay–Sachs disease (deficiency of β-hexosaminidase A leading to GM2 ganglioside storage in neurons), Pompe disease (acid α-glucosidase deficiency with lysosomal glycogen accumulation in muscle) and Niemann–Pick disease types A and B (sphingomyelinase deficiency with lipid storage in macrophages).

Histologically, many LSDs show enlarged, vacuolated cells with cytoplasm packed by lysosomes distended with storage material; in bone marrow or liver biopsies from Gaucher disease, macrophages exhibit a characteristic “crumpled tissue paper” appearance of the cytoplasm, while in Tay–Sachs disease neurons are ballooned by accumulated gangliosides. Diagnosis relies on measuring specific lysosomal enzyme activities in leukocytes or cultured fibroblasts, identifying pathogenic variants in the corresponding genes, and sometimes demonstrating storage material by electron microscopy; treatment options include enzyme replacement therapy, substrate reduction therapy, pharmacological chaperones and, increasingly, gene therapy.

Lysosomal dysfunction is also implicated in common acquired diseases. Impaired lysosomal acidification and autophagic flux contribute to accumulation of protein aggregates in Alzheimer and Parkinson disease, while lysosomal exocytosis and signalling pathways are increasingly recognised as therapeutic targets in cancer and inflammatory conditions. In cytology and histology, careful assessment of cytoplasmic vacuolisation, residual bodies and lipofuscin accumulation, together with enzyme histochemistry and immunostaining for lysosomal markers, provides important clues to underlying lysosomal pathology and to the proliferative and metabolic status of cells.

2. Peroxisomes

2.1. Structure and composition of peroxisomes

Peroxisomes are small, spherical or ovoid organelles bounded by a single membrane and containing a finely granular matrix enriched in oxidative enzymes, most notably catalase, which decomposes hydrogen peroxide generated by peroxisomal oxidases. They typically measure 0.1-1.0 μm in diameter and can be numerous in cells with high lipid turnover, such as

hepatocytes, renal proximal tubule cells and steroid-producing cells, where they appear in electron micrographs as electron-dense bodies sometimes with a crystalline or nucleoid-like core.

Unlike mitochondria, peroxisomes lack their own genome; all peroxisomal proteins are encoded by nuclear genes and imported post-translationally from the cytosol via specific peroxisomal targeting signals (PTS1 and PTS2) recognised by cytosolic receptors such as PEX5 and PEX7 and delivered through a dynamic translocon in the peroxisomal membrane. Peroxisomes can arise by growth and division of pre-existing organelles and by de novo biogenesis from the endoplasmic reticulum and mitochondria, processes governed by a family of peroxins encoded by PEX genes; mutations in these genes underlie the peroxisome biogenesis disorders of the Zellweger spectrum.

Cytochemically, peroxisomes are identified by their strong catalase activity, which oxidises DAB in the presence of hydrogen peroxide to yield an osmiophilic, electron-dense reaction product at the peroxisomal matrix, a method that requires prior aldehyde fixation (often with glutaraldehyde at 2-3 % in buffered solutions) and remains a standard approach for peroxisome localisation in light and electron microscopy. Immunolabelling of catalase and peroxisomal membrane proteins complements DAB staining and allows quantitative assessment of peroxisome number and distribution in experimental and pathological tissues.

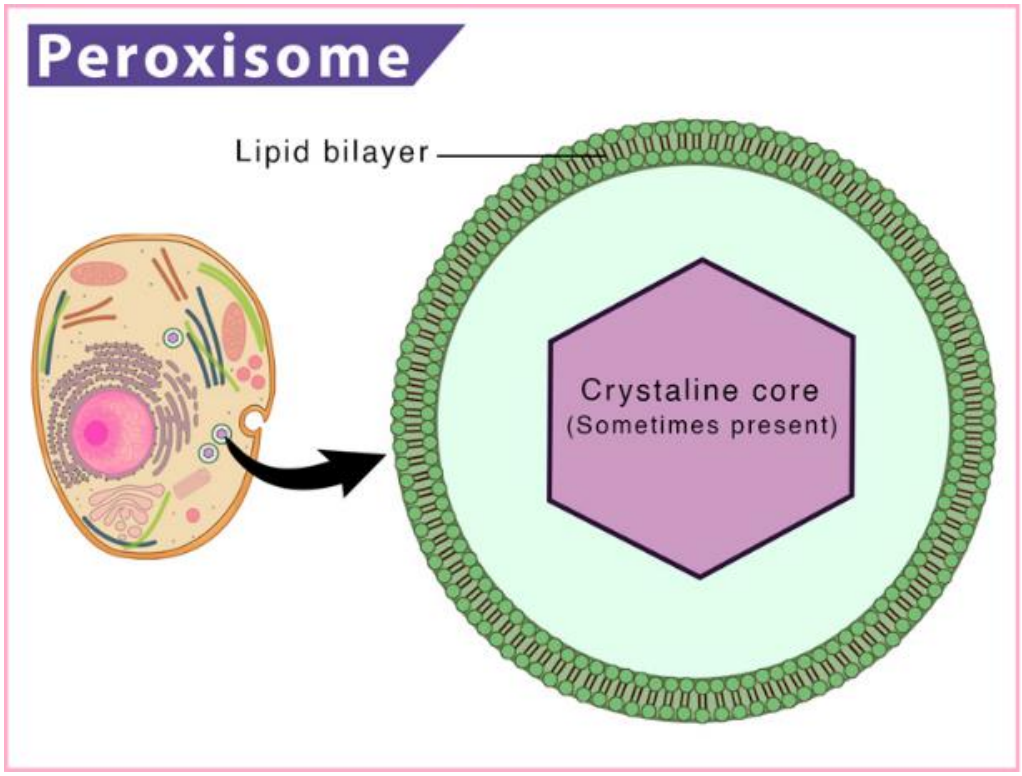


Figure 48. Peroxisome structure and organization

2.2. Roles of peroxisomes in the cell

Peroxisomes play central roles in lipid metabolism, particularly in pathways not handled efficiently by mitochondria. In mammalian cells, they catalyse β-oxidation of very-long-chain fatty acids (VLCFA; typically ≥ C22), saturation of branched-chain fatty acids such as phytanic acid via α-oxidation, early steps of bile acid synthesis from cholesterol, and synthesis of ether phospholipids (plasmalogens) that are abundant in myelin and cardiac muscle. These reactions generate hydrogen peroxide as a by-product, which is rapidly decomposed by catalase; failure to detoxify peroxides leads to oxidative damage of lipids, proteins, and DNA, highlighting the importance of peroxisomes in redox homeostasis.

Peroxisomes also participate in cellular ROS and RNS signalling, innate immunity, and antiviral defence, in part because peroxisomal membranes host pattern-recognition and adaptor proteins involved in interferon responses, and because peroxisome-mitochondria contact sites influence apoptotic and inflammatory signalling. In hepatocytes and renal epithelial cells, peroxisomes cooperate with mitochondria, smooth endoplasmic reticulum and lipid droplets to regulate triglyceride turnover and bile acid homeostasis, so that changes in peroxisome number or function are closely linked to metabolic syndromes, fatty liver disease and reproductive dysfunction.

In histology and cytology, peroxisome-rich cells may show fine granular cytoplasmic positivity in catalase-DAB reactions, whereas proliferated peroxisomes in experimental models or peroxisomal disorders sometimes appear as clusters of small, weakly DAB-positive organelles, reflecting altered enzyme content and biogenesis. Recognition of these patterns on liver biopsies or cultured fibroblasts is important for correlating biochemical abnormalities with morphological changes in peroxisomal diseases.

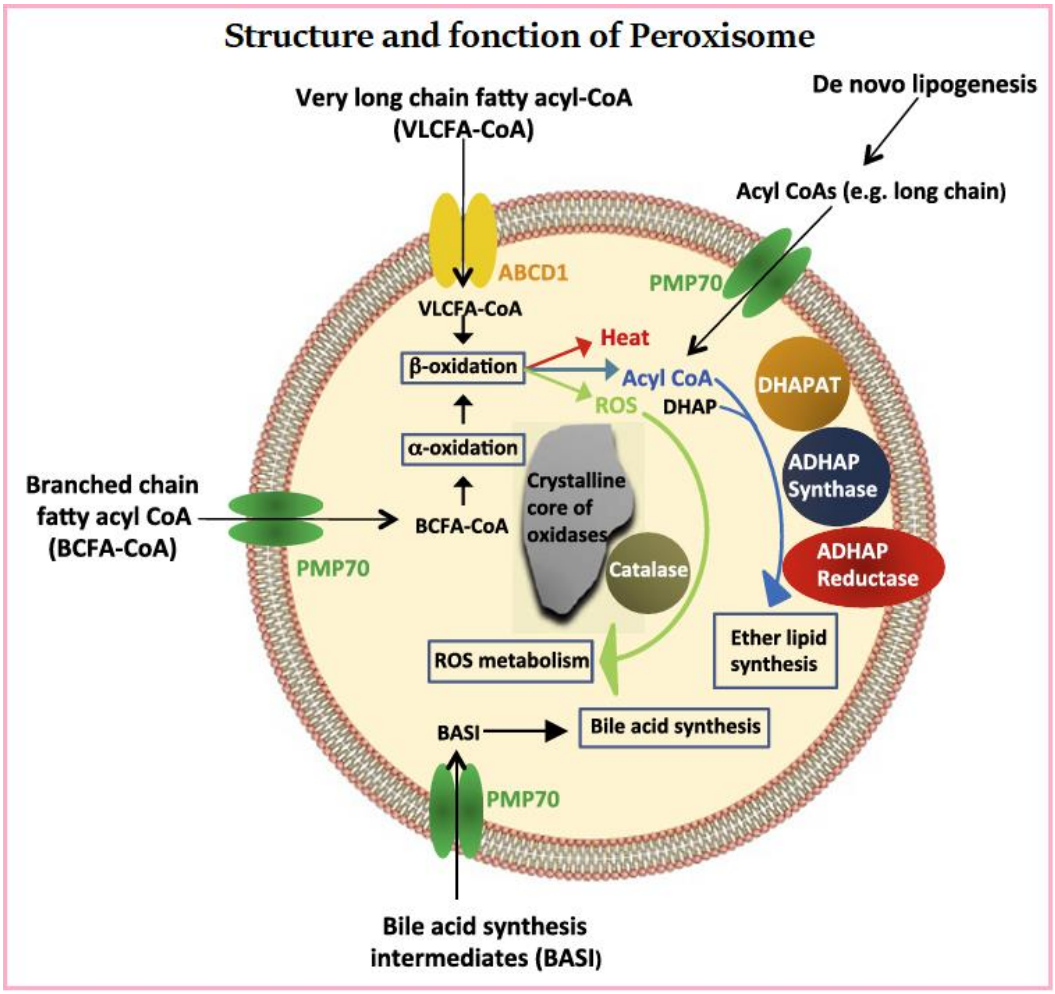


Figure 49. Peroxisome structure and metabolic functions

Table 7. Comparison Lysosome Vs Peroxisome

Comparison Table : Lysosomes vs Peroxisome		
Criterion	**Lysosome**	**Peroxisome**
Main Function	Break down biological polymers like proteins and polysaccharides	Oxidize organic compounds, breaking down metabolic hydrogen peroxides.
Composition	Consists of degradative enzymes	consist of oxidative enzymes.
Function	Responsible for the digestion in the cell	Responsible for the protection of the cell against metabolic hydrogen
Presence	Only found in animals	Found in all eukaryotes.
Origin	Derived from either Golgi apparatus or endoplasmic reticulum	Derived from the endoplasmic reticulum and are capable of replicating by themselves.
Size	Lysosomes are comparatively large in size.	Peroxisomes are small.
Signal Sequence of the Target Proteins	The proteins destined in the Lysosomes are tagged with mannose 6phosphate	The proteins destined in the peroxisomes are tagged with peroxisomal targeting signal (PTS).

2.3. Diseases related to peroxisomes

Peroxisomal disorders are broadly divided into peroxisome biogenesis disorders (PBDs), in which peroxisomes fail to assemble or import matrix proteins, and single-enzyme or single-pathway deficiencies affecting specific peroxisomal functions. The Zellweger spectrum disorders (ZSDs) represent the major subgroup of PBDs, caused by biallelic pathogenic variants in one of at least 13 PEX genes; they form a continuum from classic Zellweger syndrome presenting in the neonatal period with hypotonia, seizures, craniofacial dysmorphism, hepatomegaly and profound developmental delay, to milder infantile Refsum disease and Heimler syndrome with later onset.

Biochemically, PBDs are characterised by absence or severe reduction of functional peroxisomes and by accumulation of VLCFA, phytanic and pristanic acids, and deficiency of plasmalogens; morphologically, liver and other tissues show reduced or absent catalase-positive peroxisomes in DAB reactions, microvesicular steatosis and variable neuronal migration defects. Single-enzyme peroxisomal disorders include X-linked adrenoleukodystrophy (defective ABCD1 transporter with VLCFA accumulation and inflammatory demyelination), adult Refsum disease (phytanoyl-CoA hydroxylase deficiency causing phytanic acid accumulation and retinitis pigmentosa, neuropathy and ataxia) and isolated catalase deficiency (acatalasemia), which predisposes to oral lesions and oxidative stress.

Clinically, peroxisomal disorders often present with combinations of neurological impairment, hepatic dysfunction, adrenal insufficiency, hearing and vision loss, and skeletal anomalies, reflecting the central role of peroxisomes in myelin maintenance, hepatocellular lipid metabolism and endocrine homeostasis. Diagnosis relies on plasma and fibroblast assays of VLCFA and other peroxisomal metabolites, catalase localisation, and molecular analysis of PEX and other peroxisome-related genes; while curative therapies are limited, early detection enables supportive care and, in some conditions such as X-linked adrenoleukodystrophy, dietary interventions and haematopoietic stem cell transplantation.

The lesson in a nutshell

Lysosomes and Peroxisomes

General Overview

🔷 ***Lysosomes***

- *Single-membrane organelles*
- *Diameter: **0.2–0.5 µm***
- *Acidic lumen (pH 4.5–5)*
- *Contain **acid hydrolases***
- *Main intracellular digestive compartment*

🔷 ***Peroxisomes***

- *Single-membrane organelles*
- *Diameter: **0.1–1.0 µm***
- *Contain **oxidative enzymes (catalase, oxidases)***
- *Involved in lipid metabolism and ROS detoxification*

---

1) Lysosomes
Structure and Composition

- *Single phospholipid bilayer*
- *Membrane proteins: **LAMP-1, LAMP-2***
- *Vacuolar **H^+-ATPase** maintains acidic pH*
- *60 acid hydrolases:*
  - *Proteases (cathepsins)*
  - *Lipases*
  - *Nucleases*
  - *Glycosidases*
  - *Phosphatases*

🔎 *Enzymes are synthesized in RER → processed in Golgi → tagged with **mannose-6-phosphate (M6P)**.*

Types of Lysosomes

- ***Primary lysosomes** → contain inactive enzymes*
- ***Secondary lysosomes** → after fusion with:*
  - *Endosomes*
  - *Phagosomes → phagolysosomes*
  - *Autophagosomes → autolysosomes*
- ***Residual bodies** → indigestible material*

Functions of Lysosomes

1.Endocytosis
Receptor-mediated uptake of extracellular material
2. Phagocytosis
Degradation of microorganisms and particles
3. Autophagy
Removal of damaged organelles and protein aggregates

Additional Roles

- *Nutrient sensing (mTORC1 platform)*
- *Plasma membrane repair*
- *Antigen processing*
- *Regulated secretion*

Lysosomal Storage Diseases (LSDs)

Inherited enzyme deficiencies → substrate accumulation.
Examples

- ***Tay–Sachs disease***
  - *β-hexosaminidase A deficiency*
  - *Neuronal ganglioside accumulation*
- ***Pompe disease***
  - *Acid α-glucosidase deficiency*
  - *Glycogen accumulation*

---

2) Peroxisomes

Structure and Composition

- *Single membrane*
- *Matrix rich in:*
  - *Catalase*
  - *Oxidases*

Functions of Peroxisomes

Lipid Metabolism

- *β-oxidation of* ***very-long-chain fatty acids (VLCFA ≥ C22)***
- *α-oxidation (phytanic acid)*
- *Bile acid synthesis*
- *Plasmalogen synthesis (myelin)*

Redox Regulation

- *Produce H_2O_2*
- *Detoxified by catalase*
- *Maintain ROS balance*

Additional Roles

- *Innate immunity*
- *Cooperation with mitochondria*
- *Lipid homeostasis*

Comparison: Lysosomes vs Peroxisomes

Feature	***Lysosome***	***Peroxisome***
Main function	*Digestion*	*Oxidation*
Enzymes	*Acid hydrolases*	*Oxidases, catalase*
pH	*Acidic*	*Neutral*
Presence	*Mainly animals*	*All eukaryotes*

Peroxisomal Diseases

🔷 ***Peroxisome Biogenesis Disorders (PBD)***

- ***Zellweger syndrome***
  - *Severe neonatal disorder*
  - *VLCFA accumulation*

🔷 ***Single Enzyme Defects***

- ***X-linked adrenoleukodystrophy***
  - *ABCD1 mutation*
  - *Demyelination*

CHAPTER XI : ENDOPLASMIC RETICULUM AND GOLGI APPARATUS

Lesson Objectives

By the end of this lesson, students will be able to:

- Describe the Structure of the ER and Golgi Apparatus
- Explain the Functions of the Rough and Smooth ER
- Summarize the Functional Role of the Golgi Apparatus
- Discuss the Interactions Between the ER and Golgi Apparatus

Introduction

The endoplasmic reticulum is a continuous membrane system of branching tubules and flattened cisternae that extends throughout the cytoplasm and encloses a single lumen; in a typical animal cell its membrane accounts for more than half of the total membrane surface area, highlighting its central role in biosynthesis and intracellular transport. The ER is classically divided into rough ER (RER), whose cytosolic surface is studded with ribosomes and which is specialised for synthesis and folding of secretory and membrane proteins, and smooth ER (SER), which is ribosome-free and devoted mainly to lipid metabolism, detoxification reactions and calcium storage, with the relative abundance of each domain varying according to the functional specialisation of the cell.

The Golgi apparatus is a polarised stack of flattened membrane-bound cisternae grouped into several closely apposed stacks that are interconnected by tubules to form a perinuclear "Golgi ribbon," typically 1-3 µm across in mammalian cells, and positioned near the centrosome in most human cell types. The Golgi complex receives newly synthesised proteins and lipids from the ER, modifies them by sequential glycosylation, sulfation and proteolytic processing, and sorts them into distinct classes of transport vesicles destined for lysosomes, secretory granules, the plasma membrane or back to the ER, so that ER and Golgi together define the core of the secretory and endocytic pathways that are essential for tissue organisation, intercellular communication and extracellular matrix homeostasis.

From a technical perspective, ER and Golgi ultrastructure is best preserved in specimens fixed for transmission electron microscopy using buffered glutaraldehyde (typically 2.5-4 % in 0.1 mol/L phosphate or cacodylate buffer at 4 °C for 2-16 h), followed by postfixation with osmium tetroxide to stabilise membrane lipids before dehydration, resin embedding and ultrathin sectioning at 60-90 nm. In routine paraffin sections stained with hematoxylin–eosin, abundant RER contributes to basophilic "ergastoplasm," particularly prominent in plasma cells and

pancreatic acinar cells, whereas the Golgi apparatus appears as a pale perinuclear "Golgi zone" devoid of ribosomes; specific demonstration of the Golgi complex in light microscopy may require metal impregnation methods or immunocytochemistry.

1. Endoplasmic reticulum

The ER network consists of sheets and tubules with luminal diameters on the order of 30–50 nm, whose geometry is shaped by membrane curvature generating proteins and by interactions with the cytoskeleton, and whose luminal volume and membrane area are dynamically remodelled in response to metabolic demand and stress. Three morphological components are distinguished: flattened sheets bearing dense arrays of ribosomes that constitute the rough ER; more tubular regions with sparse or absent ribosomes that form smooth ER; and transitional ER subdomains where COPII-coated transport vesicles bud to deliver cargo to the ER-Golgi intermediate compartment (ERGIC) and then to the cis-Golgi.

In biochemical fractionation, disruption of cells followed by differential and density-gradient centrifugation generates "microsomes," small sealed vesicles derived from ER membranes; separation in sucrose gradients allows partial resolution of rough and smooth microsomal fractions, which can be used to study ER enzymes, ribosomes and luminal chaperones. Accurate interpretation of histological or ultrastructural images therefore presupposes an understanding of how these in vitro preparations relate to the continuous ER network in vivo, especially in highly secretory epithelia, hepatocytes and muscle cells.

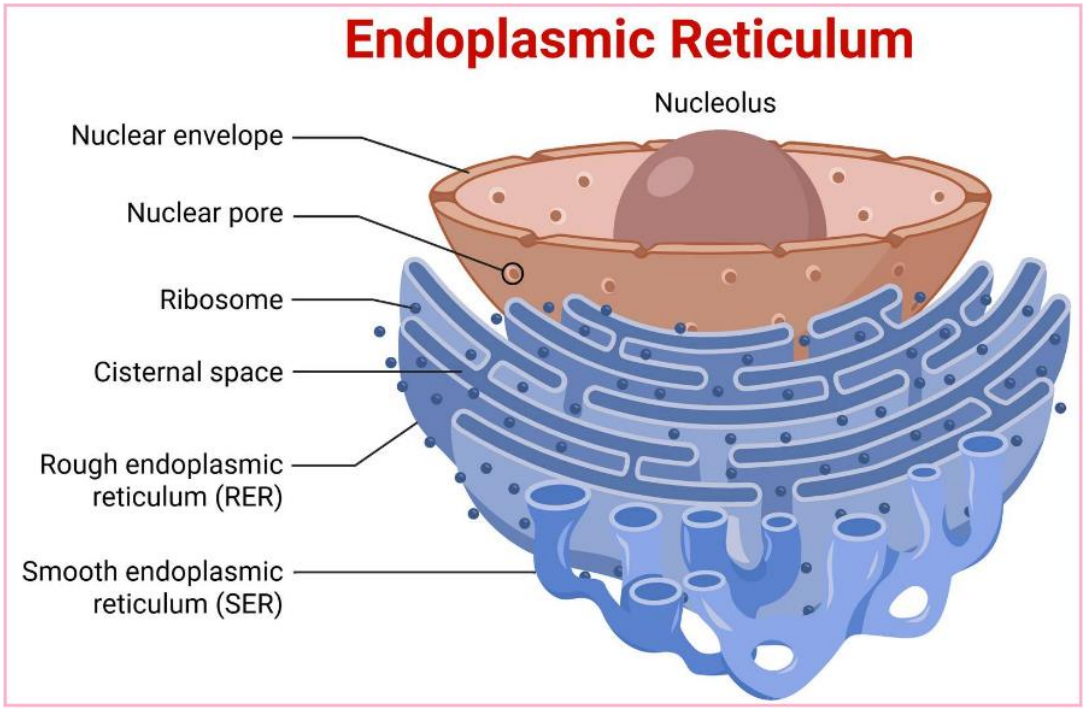


Figure 50. Rough and smooth endoplasmic reticulum: structure and organization

1.1 Smooth endoplasmic reticulum (SER)

1.1.1 Structure

Smooth ER is composed mainly of interconnected tubules with a smooth cytosolic surface because ribosomes are absent or sparse; these tubules form extensive networks that are particularly abundant in steroid-secreting endocrine cells, hepatocytes and certain muscle cells. In muscle fibres, the SER is specialised into the sarcoplasmic reticulum, a finely branched tubular system closely apposed to myofibrils and transverse tubules, whose membrane displays high densities of Ca^{2+}-ATPase pumps and ryanodine receptors that underlie excitation–contraction coupling.

The SER membrane is continuous with the RER membrane and nuclear envelope, and transitional ER domains at the interface often show a mixed morphology, with tubules bearing scattered ribosomes and specialised ER exit sites enriched in COPII-coat components that sculpt budding vesicles of approximately 50-70 nm in diameter. At the optical level, SER is not individually resolved but contributes to the eosinophilic, finely granular cytoplasm of hepatocytes and steroidogenic cells; its presence is better inferred from the paucity of basophilic ergastoplasm and from the biochemical profile of the cell, including abundant cytochrome P450 enzymes and lipid droplets.

1.1.2 Function

The SER is a major site of lipid biosynthesis, including phospholipids, cholesterol and steroid hormones, mediated by membrane-associated enzymes whose activities are up-regulated in endocrine organs such as adrenal cortex and gonads and in hepatocytes during hyperlipidaemic or toxic states. In hepatocytes, SER-associated cytochrome P450 mixed-function oxidases catalyse phase I reactions that hydroxylate hydrophobic xenobiotics, often followed by phase II conjugation in the cytosol, thereby increasing water solubility and excretability; chronic exposure to barbiturates or other inducers can lead to SER hypertrophy, visible ultrastructurally as proliferation of smooth tubules.

In muscle, the sarcoplasmic reticulum rapidly sequesters Ca^{2+} into its lumen via Ca^{2+}-ATPase at resting conditions and releases it through ligand-gated channels during depolarisation, so that the density and organisation of SER profiles parallel the contractile properties of the fibre and are altered in certain myopathies and channelopathies. More generally, SER participates in carbohydrate metabolism (for example, glucose-6-phosphatase resides in ER membranes in hepatocytes), in synthesis of lipoproteins and in the formation of transport vesicles that convey lipids and membrane components to the Golgi apparatus; disturbances in SER function

contribute to fatty liver disease, drug toxicity and dyslipidaemia, findings that correlate with altered SER abundance and morphology in histological biopsies.

1.2. Rough endoplasmic reticulum (RER)

1.2.1 Structure

Rough ER is characterised by flattened, parallel cisternae whose cytosolic surface is densely coated with ribosomes, each about 25-30 nm in diameter, giving the characteristic granular appearance in electron micrographs and intense basophilia in light microscopy due to associated ribosomal RNA. These cisternae, with luminal widths around 40–50 nm, are often arranged in stacks close to the nucleus and continuous with the outer nuclear membrane, particularly in highly secretory cells such as pancreatic acinar cells, plasma cells and fibroblasts synthesising abundant collagen.

Ribosomes associate with the RER membrane via translocon complexes when translating mRNAs encoding secretory, lysosomal or membrane proteins that carry N-terminal signal peptides recognised by the signal recognition particle; this co-translational targeting generates a dynamic mosaic in which ribosomes continuously bind and dissociate according to translational activity. By electron microscopy of glutaraldehyde–osmium fixed tissue, RER cisternae appear as electron-lucent lumina bounded by parallel membranes studded with dense particles; cup- or ring-shaped profiles may represent curved ER sheets or sectioned autophagic structures, emphasising the need to interpret these images in three dimensions and in relation to other organelles such as the Golgi apparatus and lysosomes.

1.2.2 Function

The principal function of RER is synthesis, folding and early post-translational modification of proteins destined for secretion, for insertion into membranes or for delivery to the endomembrane system. As a nascent polypeptide enters the ER lumen through the translocon, it encounters an environment rich in chaperones such as BiP/GRP78, protein disulfide isomerases and N-linked glycosylation enzymes that assist in correct folding, disulfide bond formation and quality control; misfolded proteins are retained and ultimately retro-translocated for proteasomal degradation via the ER-associated degradation (ERAD) pathway.

When folding demand exceeds capacity, unfolded or misfolded proteins accumulate and trigger the unfolded protein response (UPR), a signalling network that attempts to restore proteostasis by attenuating translation, up-regulating chaperones and enhancing degradation pathways, but which can also initiate apoptosis if stress is prolonged or severe; chronic ER stress and

maladaptive UPR activation contribute to atherosclerosis, diabetes, neurodegeneration and reproductive disorders, and correspond to morphological changes such as ER dilation, fragmentation and accumulation of autophagic vacuoles. From a diagnostic perspective, prominent RER in plasma cells, fibroblasts or tumour cells correlates with intense secretory activity, while ultrastructural evidence of dilated cisternae and electron-dense aggregates may indicate ER storage diseases or paraproteinaemias.

2. Golgi apparatus

The Golgi apparatus, or Golgi complex, consists of one or several stacks per cell, each made of 3-8 flattened cisternae, typically slightly curved with dilated rims, interconnected by tubules into a ribbon-like structure; its cis face is oriented toward the ER, whereas the trans face is oriented toward the plasma membrane and endosomal system. The Golgi stack is functionally subdivided into cis, medial and trans compartments based on the distribution of resident enzymes and trafficking machinery, and is flanked by networks of vesicles and tubules such as the ER-Golgi intermediate compartment (ERGIC) at the cis side and the trans-Golgi network (TGN) at the trans side, which function as major sorting hubs.

In electron micrographs, optimal fixation by glutaraldehyde followed by osmium tetroxide reveals the Golgi as a series of smooth, flattened cisternae separated by narrow cisternal spaces and surrounded by numerous coated and uncoated vesicles of 50-80 nm, located in the juxtanuclear region; in many endocrine and exocrine cells, the Golgi stack is closely associated with immature secretory granules, reflecting its role in condensing and packaging peptide hormones and enzymes. In routine light microscopy, the Golgi zone appears as a pale, negative image in the supranuclear cytoplasm of active secretory cells, but specific silver impregnation methods such as Golgi–Kopsch techniques, using 2-5 % potassium dichromate for several days followed by 0.5-2 % silver nitrate for 1-3 days, produce black silver chromate deposits that delineate the three-dimensional architecture of some cells and can help localise the Golgi region in neurons and certain epithelial cells.

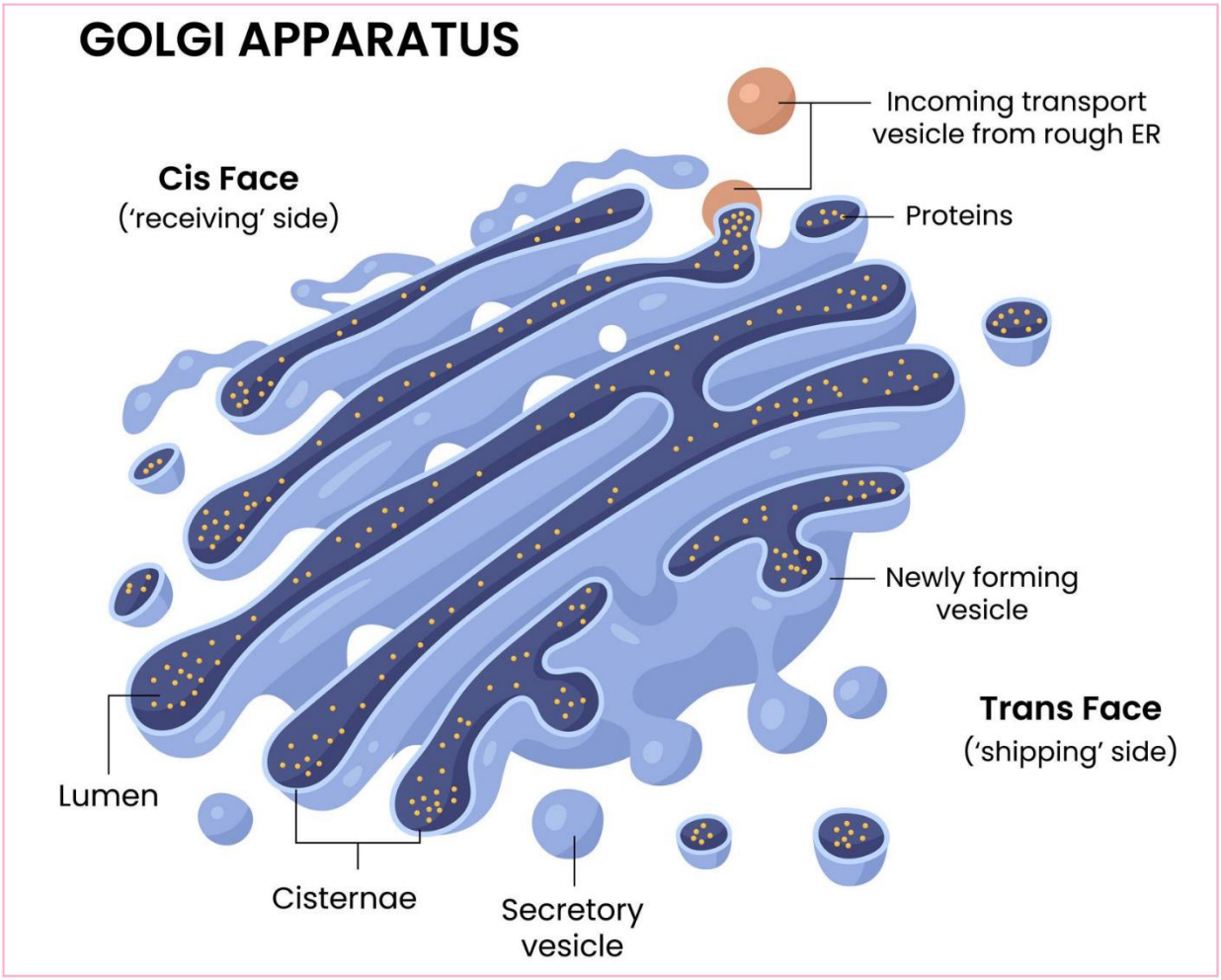


Figure 51. Architecture of the Golgi apparatus: cis and trans faces

2.1 Functional regions of the Golgi apparatus

2.1.1 Cis region

The cis-Golgi network (CGN) and the adjoining cis cisterna constitute the entry face of the Golgi apparatus, where transport carriers arriving from the ER and ERGIC fuse and deliver newly synthesised proteins and lipids; this region is enriched in enzymes involved in the early steps of N-glycan processing, such as removal of mannose residues, as well as in tethering factors and COPI-coat machinery that recycle ER-resident receptors and mis-sorted proteins back to the ER. The ER-Golgi intermediate compartment itself is a dynamic collection of vesicular-tubular clusters between ER and cis-Golgi that concentrates soluble secretory proteins by exclusion from retrograde COPI vesicles and acts as a key sorting station at the interface of these two organelles.

2.1.2 Medial region

Medial cisternae contain a different set of glycosyltransferases and glycosidases that further remodel N-linked oligosaccharides and initiate or extend O-linked glycosylation, creating progressively more complex branched glycans as cargo proteins progress through the stack; this regional specialisation underlies the stepwise nature of Golgi-dependent glycosylation, which is critical for protein stability, trafficking and function. According to cisternal maturation models, newly formed cis cisternae gradually acquire medial and then trans identities while resident enzymes recycle backward via COPI-coated vesicles, an arrangement that reconciles spatial compartmentalisation with the need to accommodate large cargo such as procollagen that cannot fit into small vesicles.

2.1.3 Trans region

The trans-Golgi cisternae and the trans-Golgi network form the exit face of the Golgi apparatus, where cargo is sorted into clathrin-coated vesicles destined for late endosomes and lysosomes, secretory granules for regulated secretion, or non-coated or differently coated carriers for constitutive secretion and plasma membrane delivery. Key events at this level include addition of terminal galactose and sialic acid residues to N-glycans, sulfation of tyrosine residues and carbohydrate chains, and recognition of lysosomal hydrolases by N-acetylglucosamine-1-phosphotransferase, which generates the mannose-6-phosphate marker required for sorting into clathrin-coated vesicles that deliver enzymes to late endosomes and lysosomes; defects in these pathways lead to abnormal serum glycoprotein patterns and lysosomal storage diseases.

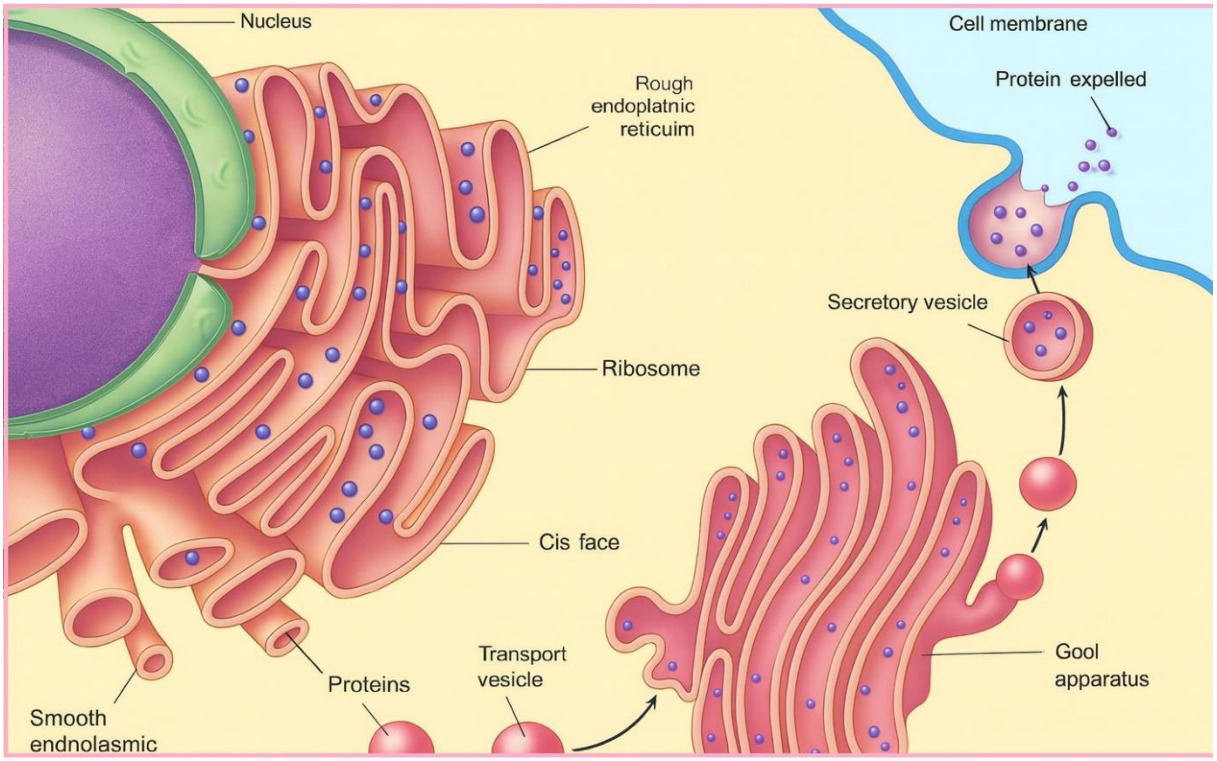


Figure 52. Protein secretory pathway: rough endoplasmic reticulum, Golgi apparatus, and exocytosis.

2.2 Functions and clinical relevance

The Golgi apparatus completes and diversifies the glycosylation of secretory and membrane proteins initiated in the ER, and thereby controls a wide range of biological processes, including cell-cell recognition, receptor function, adhesion, and the stability and trafficking of plasma proteins such as immunoglobulins, coagulation factors, and hormones . Congenital disorders of glycosylation (CDG) comprise an expanding group of inherited metabolic diseases in which defects in ER or Golgi glycosylation pathways produce multisystem phenotypes with neurodevelopmental delay, hepatopathy, coagulopathy, and endocrine dysfunction; Golgi-centred CDGs often involve mutations in glycosyltransferases, nucleotide-sugar transporters or vacuolar-type ATPases that regulate intraluminal pH, leading to characteristic abnormalities in transferrin glycoforms detectable by isoelectric focusing or mass spectrometry.

Golgi structure and function are also frequently perturbed in acquired diseases. Golgi fragmentation, defined as dispersal of the compact perinuclear ribbon into smaller ministacks or vesicles, is an early and often reversible event in many neurodegenerative diseases and in some cancers, where it may impair axonal trafficking, synaptic function and secretory pathways, and is increasingly viewed as both a marker and a potential driver of pathology. In experimental and diagnostic cytology, immunofluorescence for Golgi markers such as GM130,

giantin, TGN46 or GCC88 reveals changes in Golgi size, shape and continuity, while ultrastructural analysis in glutaraldehyde–osmium fixed specimens confirms cisternal swelling, vesiculation or loss of polarity, correlating these morphological changes with clinical phenotypes and with alterations in vesicular trafficking and stress signalling pathways .

At the practical level, understanding the organisation of the ER and the Golgi is essential for interpreting histological and cytological preparations. For example, the presence of abundant basophilic RER and a prominent pale Golgi zone in plasma cells on a blood smear or bone marrow cytology correlates with high immunoglobulin synthesis and is a key feature in diagnosing reactive plasmacytosis or plasma cell neoplasms; similarly, recognition of expanded SER in hepatocytes in biopsies from patients treated with enzyme-inducing drugs, or of fragmented Golgi in neurons in neurodegenerative disease, allows the pathologist to connect subcellular changes to functional disturbances.

The lesson in a nutshell

ENDOPLASMIC RETICULUM (ER) & GOLGI APPARATUS

1 *Endoplasmic Reticulum (ER)*

Definition

*A continuous membrane system of **flattened cisternae and branching tubules** extending throughout the cytoplasm.*

- *Encloses a single lumen*
- *Represents **more than 50% of total cellular membrane surface***
- *Continuous with the nuclear envelope*

Two types:

- ***Rough ER (RER)** → ribosome-bound*
- ***Smooth ER (SER)** → ribosome-free*

A. Smooth Endoplasmic Reticulum (SER)

Structure

- *Network of interconnected tubules*
- *Smooth cytosolic surface (no ribosomes)*
- *Continuous with RER*
- *Specialized in muscle → **Sarcoplasmic Reticulum***

Functions

- ✅ *Lipid synthesis (phospholipids, cholesterol)*
- ✅ *Steroid hormone synthesis*
- ✅ *Detoxification (Cytochrome P450 in hepatocytes)*
- ✅ *Carbohydrate metabolism (glucose-6-phosphatase)*
- ✅ *Calcium storage (especially in muscle cells)*

B. Rough Endoplasmic Reticulum (RER)

Structure

- *Flattened parallel cisternae*
- *Ribosomes (25–30 nm) attached to cytosolic surface*
- *Basophilic in light microscopy*
- *Highly developed in:*
  - *Plasma cells*
  - *Pancreatic acinar cells*
  - *Fibroblasts*

Functions

- ✅ *Synthesis of:*
  - *Secretory proteins*
  - *Membrane proteins*
  - *Lysosomal enzymes*
- ✅ *Protein folding & quality control*
- ✅ *Initial N-linked glycosylation*
- ✅ *ER-associated degradation (ERAD)*

2 *Golgi apparatus*

Definition

*A polarized stack of **3–8 flattened cisternae** forming a perinuclear ribbon.*

- ***Cis face*** → *oriented toward ER*
- ***Trans face*** → *oriented toward plasma membrane*

Central organelle of the secretory pathway.

2.1 Functional Regions

🟢 *Cis Region*

- *Entry face*
- *Receives vesicles from ER*
- *Early N-glycan processing*
- *Retrograde transport (COPI)*

🟡 *Medial Region*

- *Further glycosylation*
- *Remodeling of oligosaccharides*
- *Cisternal maturation process*

🔴 *Trans Region (Trans-Golgi Network, TGN)*

- *Final modification steps:*
  - *Addition of sialic acid*
  - *Sulfation*
- *Sorting to:*
  - *Lysosomes (mannose-6-phosphate pathway)*
  - *Secretory granules*
  - *Plasma membrane*

Overall Functions of the Golgi

- ✔ *Protein maturation*
- ✔ *Complex glycosylation*
- ✔ *Sulfation*
- ✔ *Vesicular sorting*
- ✔ *Formation of secretory granules*

CHAPTER XII : CYTOSKELETON

Lesson Objectives

By the end of this lesson, students will be able to:

- Define the cytoskeleton and explain its overall role,
- Identify the three main components of the cytoskeleton,
- Discuss the major cellular functions of the cytoskeleton

Introduction

The cytoskeleton is an extensive three-dimensional network of protein filaments that pervades the cytoplasm of eukaryotic cells, providing mechanical support, determining cell shape, organising organelle positioning and enabling cell motility and division. It is classically divided into three main systems: actin filaments or microfilaments, which are approximately 7 nm in diameter; microtubules, hollow cylinders about 24-25 nm in outer diameter; and intermediate filaments, rope-like structures of about 8-10 nm, whose diameters reflect their intermediate size between actin filaments and microtubules.

These three filament systems are functionally interdependent: actin-rich cortical networks underlie cell shape changes and migration; microtubules form the tracks for long-range vesicular transport and the mitotic spindle; and intermediate filaments provide mechanical resilience, particularly in tissues exposed to shear or tensile stress, such as epidermis, muscle and nerve. Pathological alterations of the cytoskeleton, including disruption of keratin intermediate filaments in epidermolysis bullosa simplex, microtubule dysfunction in neurodegenerative disease, and abnormal actin dynamics in cancer cell invasion, are now recognised as central contributors to human disease and can often be inferred from cytological and histological preparations.

From a methodological perspective, accurate visualisation of the cytoskeleton in diagnostic or research cytology relies on appropriate fixation and specific labeling: cells are typically fixed in 3.7-4 % formaldehyde or paraformaldehyde in buffered solution for 10-20 minutes at room temperature, permeabilised with mild detergents, and stained with fluorescent phallotoxins for F-actin or antibodies directed against α-tubulin and intermediate filament proteins, before observation by fluorescence microscopy. For example, rhodamine–phalloidin binds filamentous actin with nanomolar affinity, while anti-α-tubulin immunofluorescence delineates the microtubule array and specific antibodies against keratins, vimentin or desmin reveal

intermediate filament networks, providing a direct correlation between molecular composition and cellular morphology.

1. Constituents of the cytoskeleton

The three filament systems share the fundamental property of being polymers of smaller protein subunits, but they differ in their subunit identity, assembly dynamics and mechanical behaviour: actin filaments and microtubules are polar, nucleotide-dependent polymers exhibiting rapid turnover and regulated by a large repertoire of accessory proteins, whereas intermediate filaments are apolar, more stable assemblies whose subunits vary widely between cell types. This diversity allows each system to specialise for distinct functions while contributing to an integrated cytoskeletal network, in which cross-linking proteins and junctional complexes physically couple filaments to one another and to the plasma membrane and extracellular matrix.

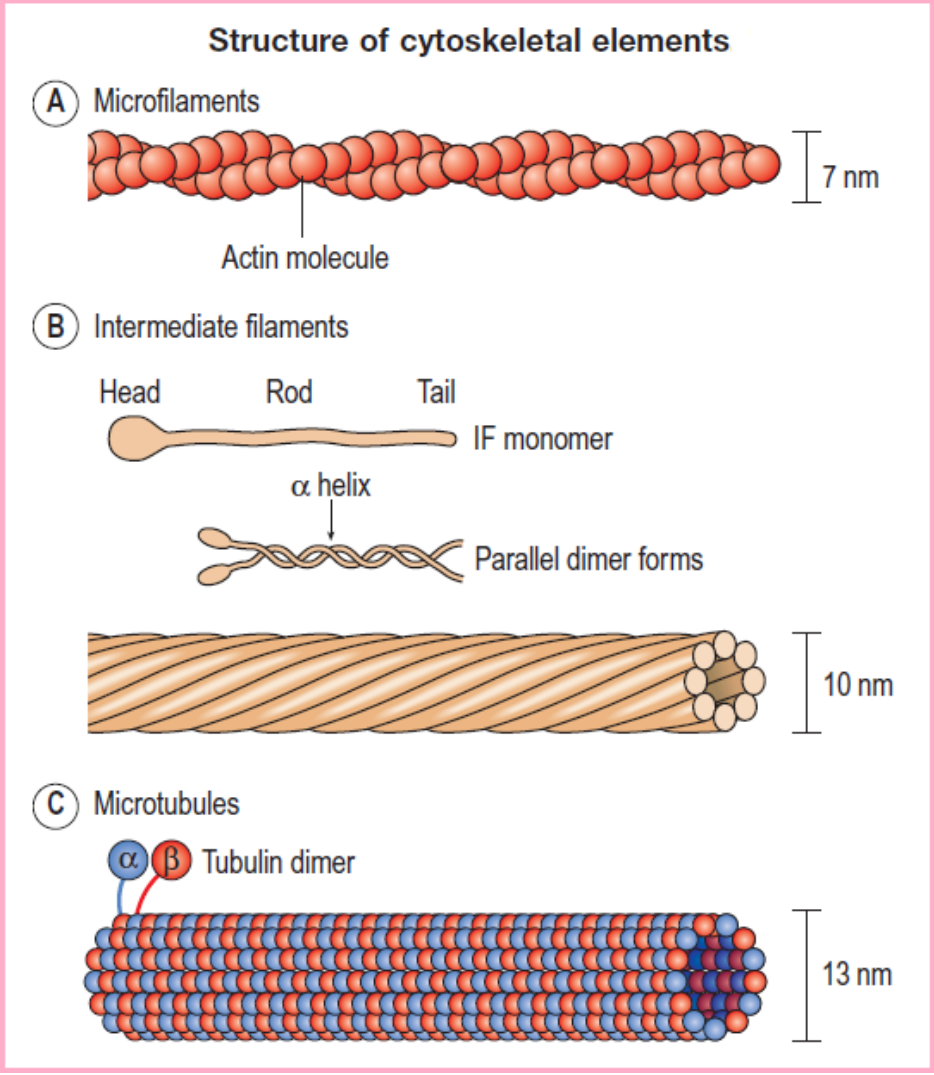


Figure 53. Structure of cytoskeletal elements

1.1. Actin microfilaments

1.1.1. Molecular architecture

Actin filaments, or microfilaments, are thin, flexible polymers formed by the head-to-tail assembly of globular actin (G-actin) monomers into a right-handed, two-stranded helical filament (F-actin) approximately 7 nm in diameter and up to several micrometres in length. Each actin monomer binds ATP or ADP, and the filament is intrinsically polar, with a barbed (plus) end that elongates more rapidly and a pointed (minus) end at which subunit dissociation is favoured; this polarity underlies directional growth, shrinkage and the ability of myosin motors to generate force along the filament. In living cells, actin filaments undergo continuous turnover through cycles of polymerisation at the plus end and depolymerisation at the minus end, a process termed "treadmilling," regulated by actin-binding proteins such as profilin, cofilin, capping proteins and nucleation factors including formins and the Arp2/3 complex.

Higher-order actin structures arise from lateral association and cross-linking of filaments into bundles and networks. Parallel bundles, stabilised by short cross-linkers such as fimbrin or fascin, form the cores of microvilli and filopodia, whereas orthogonal networks, cross-linked by more flexible proteins such as filamin, generate gel-like cortical layers beneath the plasma membrane or the branched arrays of lamellipodia. This ability to form both stiff bundles and deformable networks explains how actin can simultaneously support stable structures, like intestinal microvilli, and rapid, reversible deformations, such as those seen at the leading edge of migrating fibroblasts or in phagocytic cups.

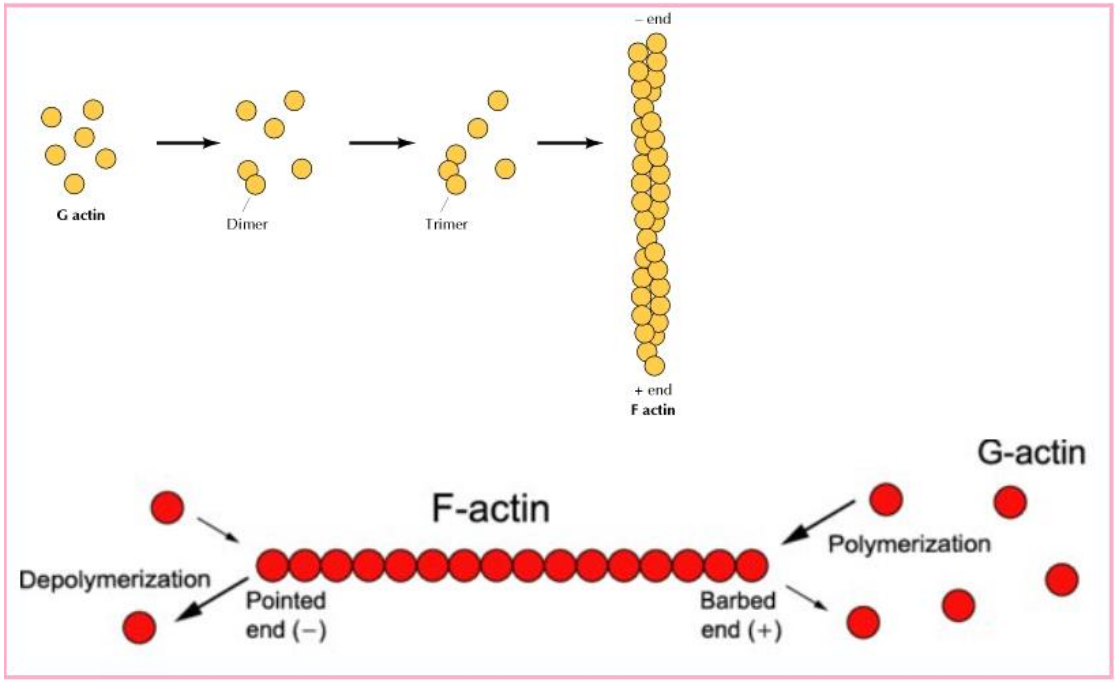


Figure 54. Actin filament dynamics: polymerization and depolymerization

1.1.2. Localisation of microfilaments

In most animal cells, actin filaments are enriched in the cell cortex, a dense meshwork immediately beneath the plasma membrane that maintains cell shape, supports surface specialisations and participates in endocytosis and exocytosis . In epithelial cells, actin bundles form the structural core of microvilli and stereocilia, where they are capped and cross-linked to confer rigidity, while in non-muscle cells, actin-rich stress fibres span the cytoplasm and insert into focal adhesions, linking the cytoskeleton to the extracellular matrix through integrins; in erythrocytes, a spectrin–actin network underlying the plasma membrane maintains the biconcave shape of red blood cells, which are typically 6-8 µm in diameter and about 2 µm in thickness. In striated muscle, actin filaments constitute the thin filaments of sarcomeres, anchored at Z-discs and interacting with myosin thick filaments to generate contraction, whereas in dividing cells actin accumulates at the equatorial cortex to form the contractile ring of cytokinesis .

1.1.3. Disposition of microfilaments

The spatial disposition of actin filaments determines the mechanical properties of the cytoplasm and the modes of cell movement. In lamellipodia, Arp2/3-dependent branching produces a dense, two-dimensional dendritic network of short filaments oriented at characteristic angles, enabling broad, sheet-like protrusions and efficient exploration of the substrate; genetic disruption of Arp2/3 in fibroblasts abolishes lamellipodia and impairs persistent directional migration. By contrast, filopodia contain long, parallel bundles of actin filaments generated by formins and bundled by fascin; these slender protrusions function as sensory organelles that probe substrate rigidity and guidance cues, and their density correlates with fibroblast spreading and migration on rigid matrices. In the equatorial region of cytokinetic cells, actin and non-muscle myosin II form a circular contractile ring whose architecture evolves from a band of actomyosin clusters into a continuous ring, highlighting how local reorganisation of actin from networks to bundles accompanies force generation and cell division.

In histological and cytological preparations, these actin structures are best demonstrated by fluorescent phallotoxins such as rhodamine-phalloidin applied at nanomolar concentrations to fixed, permeabilised cells; this yields high-contrast labeling of cortical actin, stress fibres, microvilli and contractile rings, while careful choice of fixation conditions (e.g. 3.7-4 % formaldehyde in phosphate-buffered saline) is essential to preserve actin cables and avoid artefactual clustering. These staining patterns allow direct visualisation of actin rearrangements

during phagocytosis, cell division or migration in cultured cells and cytological smears, providing a powerful link between molecular mechanism and observed morphology.

1.2. Microtubules

1.2.1. Molecular composition

Microtubules are long, hollow cylinders composed of 13 parallel protofilaments, each formed by head-to-tail stacking of α/β-tubulin heterodimers; in most animal cells, they have an outer diameter of about 24-25 nm and can extend many micrometres across the cytoplasm. Like actin filaments, microtubules are polar polymers with structurally distinct plus and minus ends, but in this case, the plus end typically grows and shrinks rapidly, while the minus end is anchored at a microtubule-organising centre (MTOC) such as the centrosome. Tubulin dimers bind GTP, with the GTP on β-tubulin being hydrolysed after incorporation into the polymer; loss of a protective GTP cap at the plus end triggers a stochastic transition from growth to rapid shrinkage, a behaviour known as dynamic instability that allows microtubules to probe intracellular space and rapidly reorganise during mitosis and migration.

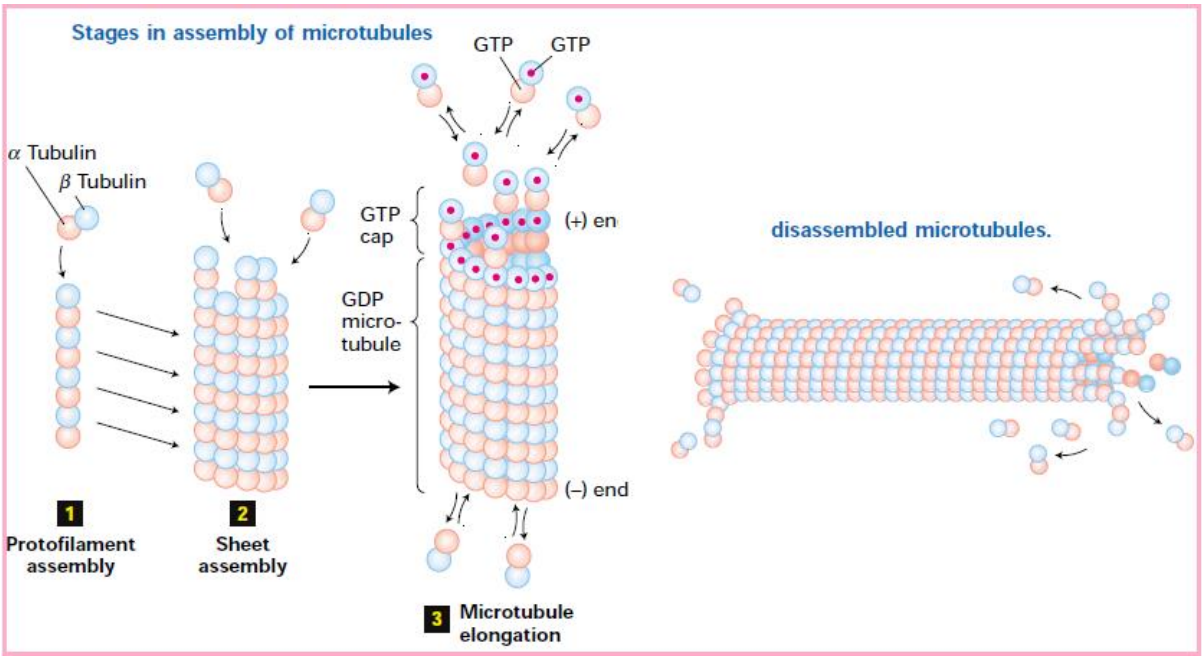


Figure 55. Stages in assembly of microtubules

1.2.2 Biogenesis and centrosomes

In most animal cells, the principal MTOC is the centrosome, composed of a pair of orthogonally oriented centrioles surrounded by an electron-dense pericentriolar material rich in γ-tubulin ring complexes that nucleate new microtubules. The canonical centriole is a cylindrical structure

approximately 0.5 μm in length and 0.2 μm in diameter built from nine microtubule triplets, and each centrosome contains a “mother” and “daughter” centriole that duplicate once per cell cycle, ensuring formation of the bipolar spindle in mitosis. Aberrant centriole number or structure leads to supernumerary centrosomes, abnormal spindle geometry and chromosome mis-segregation, features frequently observed in solid tumours and linked to chromosomal instability and aneuploidy in carcinogenesis.

1.2.3 Labile microtubules

Labile microtubules are highly dynamic and undergo rapid cycles of growth and shrinkage on timescales of seconds to minutes; they are predominant in the mitotic spindle, at the leading edge of migrating cells and in neuronal growth cones. Dynamic instability enables “search-and-capture” of kinetochores during prometaphase and continual remodeling of cell polarity, but also makes microtubules sensitive to drugs such as colchicine, nocodazole and the vinca alkaloids, which destabilise microtubules, and taxanes such as paclitaxel, which stabilise them; these agents are widely used in chemotherapy to block mitosis but can cause dose-limiting neuropathy by perturbing axonal microtubules. In immunofluorescence microscopy, labile microtubules are readily visualised by anti-α- or anti-β-tubulin antibodies after fixation in 3.7 % formaldehyde, revealing dense astral arrays in mitotic cells and fine radial networks in interphase, with rapid loss of polymer following nocodazole treatment .

1.2.4 Stable microtubules and centrioles

Stable microtubules exhibit slower turnover, resist depolymerisation and are often enriched in post-translational modifications such as acetylation or detyrosination; they are prominent in axons, where they maintain long-range order, and in axonemal structures of cilia and flagella, where a highly regular 9+2 microtubule arrangement provides the scaffold for dynein-driven beating (Gundersen et al., 1984; Caplow & Shanks, 1990). Centrioles themselves represent an extreme of microtubule stability, and when docked to the plasma membrane, they function as basal bodies that nucleate the ciliary axoneme; defects in centriole-to-basal-body conversion or axonemal assembly underlie a spectrum of ciliopathies characterised by respiratory infections, infertility and situs anomalies (Kobayashi & Dynlacht, 2011; Bettencourt-Dias & Glover, 2013).

1.2.5 Microtubule-associated proteins

Microtubule-associated proteins (MAPs) regulate microtubule stability, organisation and interaction with other cell components. Structural MAPs such as MAP2 and tau bind along the length of microtubules and promote their bundling and spacing, particularly in neurons, where they are critical for axonal integrity; hyperphosphorylated tau forms neurofibrillary tangles in Alzheimer disease and other tauopathies, illustrating how perturbation of MAPs can lead to characteristic cytopathology. Plus-end tracking proteins ("+TIPs"), including end-binding proteins (EBs), localise to growing microtubule ends and mediate interactions with actin and the cortex, whereas motor MAPs kinesins and dyneins convert the chemical energy of ATP hydrolysis into directed movement of cargos such as vesicles, mitochondria and chromosomes along microtubule tracks.

Kinesin-1, one of the best-characterised motors, typically moves towards microtubule plus ends at velocities on the order of 0.5-1 μm/s, whereas cytoplasmic dynein moves towards minus ends with comparable or slightly higher velocities, allowing long-range anterograde and retrograde transport over distances of tens of micrometres in axons and other elongated cells. Disruption of these motors or of microtubule integrity leads to accumulation of vesicles and organelles, impaired synaptic function and neurodegeneration, findings that can be correlated with altered microtubule staining patterns in immunofluorescence and ultrastructural accumulations of vesicles and swollen axons in neuropathology.

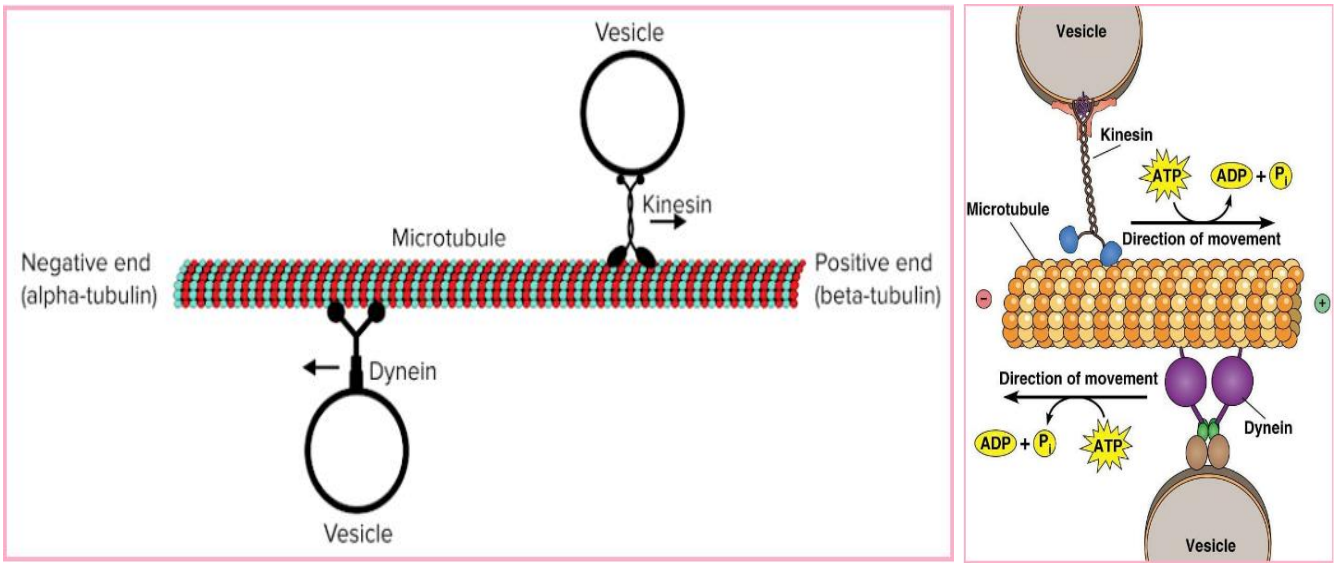


Figure 56. Intracellular transport along microtubules: molecular motors kinesin and dynein

1.3. Intermediate filaments

1.3.1. Molecular composition and classes

Intermediate filaments (IFs) are a diverse family of cytoskeletal proteins that assemble into apolar, rope-like filaments approximately 8-10 nm in diameter, providing tensile strength and resistance to mechanical deformation in cells and tissues. IF proteins share a common domain organisation, with a central α-helical rod flanked by non-helical head and tail domains; staggered coiled-coil dimers associate laterally into tetramers, which then assemble end-to-end into unit-length filaments that anneal into mature filaments, explaining their characteristic rope-like appearance in electron micrographs.

Multiple IF classes are distinguished by sequence and tissue distribution: type I and II keratins are expressed in epithelia; type III proteins include vimentin in mesenchymal cells, desmin in muscle and GFAP in astrocytes; type IV includes neurofilament proteins in neurons; and type V lamins form the nuclear lamina underlying the inner nuclear membrane in almost all somatic cells. Keratin intermediate filaments in basal keratinocytes, for example, form a cytoplasmic network that connects desmosomes and hemidesmosomes, allowing the epidermis to withstand mechanical friction, while desmin filaments link Z-discs of adjacent myofibrils and connect the contractile apparatus to the sarcolemma in muscle.

Clinically, mutations in IF genes cause tissue-specific fragility disorders. Epidermolysis bullosa simplex results from dominant-negative mutations in keratin 5 or keratin 14 that weaken the basal keratinocyte IF network, leading to cell rupture and intraepidermal blistering after minimal trauma, while LMNA mutations affecting lamin A/C underlie Hutchinson-Gilford progeria and a spectrum of cardiomyopathies and muscular dystrophies due to defective nuclear lamina integrity. In diagnostic histopathology and cytology, immunostaining for keratins, vimentin, desmin, GFAP or neurofilaments is indispensable for determining the lineage of poorly differentiated tumours and for assessing muscle regeneration, underscoring the central role of IFs as both structural elements and diagnostic markers.

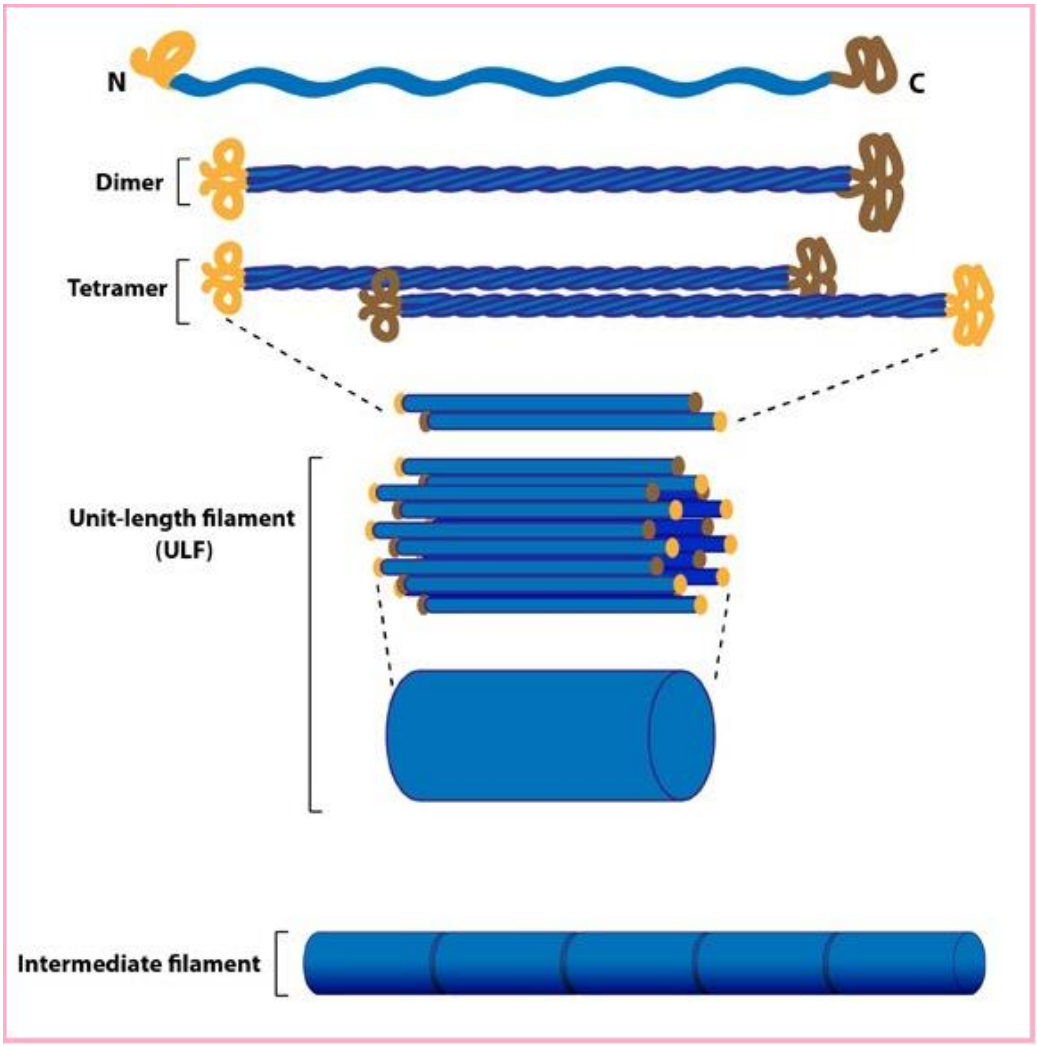


Figure 57. Assembly and organization of intermediate filaments

2. Functions of the cytoskeleton

The cytoskeleton integrates mechanical support with dynamic processes, coordinating vesicular trafficking, contractility, division and migration through the concerted action of its three filament systems and associated motors and regulatory proteins. Actin filaments and myosins are primarily responsible for generating contractile forces and shape changes at the cortex; microtubules and their motors mediate long-range intracellular transport and chromosome movements; and intermediate filaments distribute and absorb mechanical stress, stabilising organelles such as the nucleus and maintaining tissue integrity, especially where cells experience repeated deformation.

2.1. Vesicular transport

Intracellular vesicular transport depends critically on microtubules, which serve as tracks along which kinesin and dynein motors move cargo in anterograde (towards the plus end) and

retrograde (towards the minus end) directions, respectively; such transport ensures delivery of secretory vesicles from the Golgi to the plasma membrane, recycling of endosomes, and positioning of mitochondria and other organelles. Kinesin-1 typically moves at approximately 1 μm/s along microtubules, and similar velocities have been reported for dynein-dynactin complexes in living cells, allowing rapid translocation over tens of micrometres, which is particularly important in neurons where vesicles and mitochondria must travel along axons that can be centimetres long.

Close to the plasma membrane, short-range transport and docking of vesicles often rely on actin and myosin motors, such as myosin V and VI, which move cargo along cortical actin networks towards sites of exocytosis or endocytosis. Disruption of microtubules or motor proteins leads to accumulation of vesicles near the nucleus, Golgi fragmentation and impaired secretion, features observed ultrastructurally and by immunofluorescence in certain neurodegenerative diseases and in cells treated with microtubule-targeting chemotherapeutic agents.

2.2. Muscle contraction

In striated muscle, the cytoskeleton forms the molecular basis of the sliding filament mechanism. Sarcomeres, the fundamental contractile units with an optimal resting length around 2.2 μm in human cardiac and skeletal muscle, consist of thin filaments of actin anchored at Z-discs and thick filaments of myosin II in the A-band; during contraction, myosin heads cyclically bind to actin and, fuelled by ATP hydrolysis, generate force that slides thin filaments towards the centre of the sarcomere. Desmin intermediate filaments surround the Z-discs and connect adjacent myofibrils to each other and to the sarcolemma at costameres, ensuring lateral force transmission and alignment, while microtubules and actin cytoskeleton elements participate in positioning mitochondria and nuclei, coordinating energy supply and gene expression with mechanical activity.

Pathological alterations of cytoskeletal proteins produce characteristic histological changes in muscle. Mutations in desmin or associated proteins lead to desmin-related myopathies, with disorganised myofibrils and accumulation of desmin-positive aggregates; defects in dystrophin and associated costameric components result in muscular dystrophies with fibre necrosis and regeneration; and abnormal microtubule organisation contributes to cardiomyopathies, all of which can be appreciated on light and electron microscopy and confirmed by immunohistochemistry.

2.3. Cell division (mitosis and cytokinesis)

During mitosis, the microtubule cytoskeleton is dramatically reorganised into the mitotic spindle, a bipolar structure composed of astral, kinetochore and interpolar microtubules that orchestrates chromosome alignment at metaphase and segregation at anaphase. Microtubules emanating from centrosomes undergo dynamic instability, repeatedly growing and shrinking until their plus ends are captured by kinetochores; proper attachment and tension are monitored by the spindle assembly checkpoint, whose failure leads to aneuploidy, a hallmark of many cancers, often accompanied by centrosome amplification and abnormal spindle morphology observable in tumour cytology.

Cytokinesis, the physical separation of daughter cells, is driven in animal cells by an actomyosin contractile ring that forms at the equatorial cortex during late anaphase and telophase. This ring comprises filamentous actin, non-muscle myosin II, septins and scaffolding proteins that assemble into an equatorial band of clusters, which then coalesce into a continuous ring whose constriction, coordinated with membrane insertion, produces the cleavage furrow and midbody. Concomitantly, the nuclear lamina composed of lamins A/C and B is dismantled by phosphorylation at prophase and reassembled at telophase; laminopathies affecting lamin A/C can delay or perturb this process, resulting in abnormal nuclear morphology and defects in cell division seen in progeroid syndromes and some cardiomyopathies.

2.4. Amoeboid movements and fibroblast migration

Cell migration in many contexts, including wound healing, immune surveillance and cancer invasion, relies on highly coordinated changes in the actin cytoskeleton and its coupling to adhesion sites and the extracellular matrix. In mesenchymal-type migration, as exemplified by fibroblasts, polymerisation of actin at the leading edge generates lamellipodia and filopodia that protrude the membrane, while integrin-based focal adhesions form under these protrusions to provide traction; Arp2/3-dependent branched actin networks and formin-generated bundles cooperate to shape these structures and determine persistence and directionality of movement . Traction forces are generated as myosin II contracts actin stress fibres linked to focal adhesions, pulling the cell body forward, while adhesive contacts at the rear are disassembled, a cyclical process whose efficiency depends on the mechanical interplay between actin polymerisation, myosin contractility and adhesion dynamics.

Amoeboid migration, observed in many immune cells and metastatic cancer cells, is characterised by a more rounded morphology, high cortical actomyosin contractility and less reliance on strong focal adhesions; cells often move by extending and retracting blebs-

membrane protrusions driven by local cortical rupture and actomyosin contraction—allowing rapid movement through confined 3-D environments. High Rho-ROCK-myosin II activity in the cortex promotes this amoeboid state, and cells can switch between mesenchymal and amoeboid modes of migration depending on matrix density and stiffness, a plasticity that complicates anti-metastatic therapies and is increasingly appreciated in epithelial–mesenchymal transition studies. In cytological preparations and live-cell imaging, these distinct migration modes correspond to recognisable actin patterns broad lamellipodia and prominent stress fibres in fibroblast-like cells versus cortical actin and dynamic blebs in amoeboid cells, highlighting the diagnostic and interpretive value of cytoskeletal staining in understanding cell behaviour.

The lesson in a nutshell

Definition and General Role

*The **cytoskeleton** is a three-dimensional network of protein filaments that:*

- *Maintains cell shape*
- *Provides mechanical support*
- *Organizes organelles*
- *Enables intracellular transport*
- *Drives cell division and migration*

*It consists of **three major filament systems**:*

Component	***Diameter***	***Main Role***
Actin filaments (microfilaments)	*~7 nm*	*Cell shape & movement*
Microtubules	*~24–25 nm*	*Transport & mitosis*
Intermediate filaments	*~8–10 nm*	*Mechanical strength*

1) *Actin Filaments (Microfilaments)*

Structure

- *Polymer of **G-actin → F-actin***
- *Polar filament:*
  - *Plus (barbed) end → fast growth*
  - *Minus (pointed) end → slower growth*
- *ATP-dependent polymerization*
- *Dynamic turnover → **treadmilling***

Localization

- *Cell cortex*
- *Microvilli*
- *Stress fibers*
- *Contractile ring*
- *Sarcomeres (muscle)*

Functions

- *Cell motility (lamellipodia, filopodia)*
- *Phagocytosis*
- *Cytokinesis*
- *Muscle contraction (with myosin)*

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2)Microtubules

Structure

- *Hollow cylinders*
- *13 protofilaments*
- *Built from α/β-tubulin dimers*
- *GTP-dependent assembly*
- *Exhibit **dynamic instability***

Microtubule Organizing Center (MTOC)

- *Centrosome = 2 centrioles*
- *Centriole: 9 triplets arrangement*
- *Nucleation via γ-tubulin*

Functions

1 Intracellular Transport

- *Kinesin → plus end (anterograde)*
- *Dynein → minus end (retrograde)*

2 Mitosis

- *Form mitotic spindle*
- *Chromosome segregation*

3 Cilia & Flagella

- *9+2 arrangement*
- *Basal bodies derived from centrioles*

3)Intermediate Filaments (IFs)

Structure

- *Rope-like*
- *Non-polar*
- *More stable than actin/microtubules*
- *Provide tensile strength*

Major Classes

Type	***Protein***	***Location***
I & II	*Keratins*	*Epithelia*
III	*Vimentin*	*Mesenchymal cells*
III	*Desmin*	*Muscle*
III	*GFAP*	*Astrocytes*
IV	*Neurofilaments*	*Neurons*
V	*Lamins*	*Nuclear lamina*

Major Cellular Functions of the Cytoskeleton

Vesicular Transport

- *Microtubules = long-range tracks*
- *Actin = short-range transport*
- *Essential for secretion and endocytosis*

Muscle Contraction

- *Actin + Myosin II*
- *Sarcomere length ≈ 2.2 μm*
- *Desmin stabilizes Z-discs*

Cell Division

Mitosis

- *Mitotic spindle (microtubules)*
- *Centrosome duplication*
- *Chromosome segregation*

Cytokinesis

- *Actomyosin contractile ring*

Cell Migration

Mesenchymal migration

- *Lamellipodia*
- *Focal adhesions*
- *Stress fibers*

Amoeboid migration

- *Rounded cells*
- *Blebbing*
- *High cortical contractility*

Important in cancer metastasis.

References

Aardema, H., Lolicato, F., van Tol, H. T. A., et al. (2022). The key role of peroxisomes in follicular growth, oocyte maturation, fertilization and preimplantation embryo development. Oxidative Medicine and Cellular Longevity, 2022, 7982344.

Abergel, C., Legendre, M., & Claverie, J.-M. (2015). The rapidly expanding universe of giant viruses: Mimivirus, Pandoravirus, Pithovirus and Mollivirus. FEMS Microbiology Reviews, 39(6), 779–796.

Abraham, Z., Peled, E., & Gur, D. (2018). Kinesin and dynein mechanics: Measurement methods and transport regulation in cells. Biophysical Reviews, 10(5), 1313–1331.

Adewoyin, A. S. (2014). Peripheral blood film – A review. Annals of Ibadan Postgraduate Medicine, 12(2), 71–79.

Agellon, L. B. (2021). Cellular biology of the endoplasmic reticulum. Springer.

Ahn, J., Jo, I., & Shin, H. (2022). Crystal structure of progeria mutant S143F lamin A/C reveals altered filament assembly and nuclear lamina stability. Communications Biology, 5, 126.

Akasaka, M. (1965). Influence of glutaraldehyde fixation on acid phosphatase activity. Acta Histochemica et Cytochemica, 6(1), 21–32.

Al-Khateeb, A. A. O., et al. (2025). Factors influencing histology slide quality in surgical pathology. Saudi Journal of Medicine and Public Health, 5(1), 1–10.

Alamoudi, W. A., et al. (2018). A simplified colorimetric method for rapid detection of cell viability based on Janus Green B. Journal of BUON, 23(5), 1505–1514.

Alberts, B. (2002). An overview of the cell cycle. In B. Alberts et al., (4th ed.). Bookshelf.

Alberts, B. (2002). Looking at the structure of cells in the microscope. In B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, & P. Walter, Molecular Biology of the Cell (4th ed.). New York, NY: Garland Science. (Available from NCBI Bookshelf)

Alberts, B., et al. (2015). Molecular biology of the cell (6th ed.). Garland Science.

Alberts, B., Heald, R., Johnson, A., Morgan, D., Raff, M., Roberts, K., & Walter, P. (2022). Molecular biology of the cell (7th ed.). W. W. Norton & Company.

Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., & Walter, P. (2014). (6th ed.). Garland.

Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., & Walter, P. (2015). (6th ed.). Garland.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). Looking at the structure of cells in the microscope. In Molecular biology of the cell (4th ed.). Garland Science.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). Molecular biology of the cell (4th ed.). Garland Science. Bookshelf chapter “The Mitochondrion.”

Alonso-Matilla, R., Goychuk, A., & Thievessen, I. (2025). Physical principles and mechanisms of cell migration. Nature Reviews Physics, 7, 1–20.

Anders, K. R., et al. (2001). Dominant-lethal α-tubulin mutants defective in microtubule depolymerization in yeast. Molecular Biology of the Cell, 12(12), 3973–3986.

Anderson, J. M., & Van Itallie, C. M. (2009). Molecular structure and function of the tight junction. In L. González-Mariscal (Ed.), Tight junctions (pp. 1–19). Landes Bio.

Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H. L., Coulson, A. R., Drouin, J., ... Young, I. G. (1981). Sequence and organization of the human mitochondrial genome. , 290(5806), 457–465.
Aoki, T. (2017). A comprehensive review of our current understanding of red blood cell (RBC) glycoproteins. Membranes, 7(4), 56.

Appenzeller-Herzog, C., & Hauri, H.-P. (2006). The ER–Golgi intermediate compartment (ERGIC): In search of its identity and function. Journal of Cell Science, 119(11), 2173–2183.

Avila, J., Lucas, J. J., Pérez, M., & Hernández, F. (2004). Role of tau protein in both physiological and pathological conditions. Physiological Reviews, 84(2), 361–384.

Balen, B. (2013). Biomembranes: Structure and function [Teaching material]. University of Zagreb.

Ballabio, A. (2009). Lysosomal storage disorders: From storage to cellular damage. Biochimica et Biophysica Acta, 1793(4), 684–696.

Baltimore, D. (1971). Expression of animal virus genomes. Bacteriological Reviews, 35(3), 235–241.

Baron, S. (Ed.). (1996). Medical Microbiology (4th ed.). Galveston, TX: University of Texas Medical Branch at Galveston. (Available from NCBI Bookshelf)

Baron, S. (Ed.). (1996). Medical microbiology (4th ed.). University of Texas Medical Branch at Galveston. Chapter 41: Structure and classification of viruses (H. R. Gelderblom).

Barral, D. C., & Kirchhausen, T. (2022). Current methods to analyse lysosome morphology, positioning and function. Traffic, 23(7), 536–555.

Baumgart, E. (2003). A review of morphological techniques for detection of peroxisomes. Journal of Microscopy, 212(Pt 2), 89–100.

Beggs, R. R., Byrne, E. F. X., & Garrod, D. (2022). Desmosomes undergo dynamic architectural changes in response to signalling and disease. Cell Adhesion & Migration, 16(1), 141–155.

Berg, J. M., Tymoczko, J. L., Gatto, G. J., & Stryer, L. (2019). Biochemistry (9th ed.). W. H. Freeman.

Bertucci, F., Finetti, P., Cervera, N., Esterni, B., Hermitte, F., Viens, P., & Birnbaum, D. (2013). Comparison of the prognostic value of genomic grade index, histological grade and Ki-67 in early breast cancer. , 24(3), 625–632.

Bettencourt-Dias, M., & Glover, D. M. (2013). Q&A: Who needs a centrosome? BMC Biology, 11, 28.

Beznoussenko, G. V., & Mironov, A. A. (2022). Comparison of the cisterna maturation–progression model with the kiss-and-run model of intra-Golgi transport: Role of COPI vesicles. International Journal of Molecular Sciences, 23(7), 3590.

Bharathi, P., et al. (2019). UNIT-III ultrastructure and functions of cell organelles. Department of Zoology, Government College for Women, Kumbakonam.

Bianconi, E., Piovesan, A., Facchin, F., Beraudi, A., Casadei, R., Frabetti, F., … Canaider, S. (2013). An estimation of the number of cells in the human body. Annals of Human Biology, 40(6), 463–471.

Bickmore, W. A. (2013). The spatial organization of the human genome. Cell, 153(1), 13–26.

Biesterfeld, S., Gerres, K., Böcking, A., & Schramm, M. (2011). Feulgen staining remains the gold standard for precise DNA image cytometry. Anticancer Research, 31(1), 53–58.

Biggiogera, M. (2024). A brief history of the Feulgen reaction. Histochemistry and Cell Biology.
biology of the cell (4th ed.). Garland Science.

Blaustein, M. P., & Lederer, W. J. (1999). Sodium/calcium exchange: Its physiological implications. Physiological Reviews, 79(3), 763–854.

Bohnsack, K. E., & Bohnsack, M. T. (2019). Uncovering the assembly pathway of human ribosomes and its emerging links to disease. The EMBO Journal, 38(2), e100278.

Bonam, S. R., Wang, F., & Muller, S. (2019). Lysosomes as a therapeutic target. Nature Reviews Drug Discovery, 18(12), 923–948.

Bose, M., Sklirou, E., Horsley, A., et al. (2022). Characterization of severity in Zellweger spectrum disorder. Cells, 11(12), 1891.

Bowne-Anderson, H., Zanic, M., Kauer, M., & Howard, J. (2013). Microtubule dynamic instability: A new model with coupled GTP hydrolysis and multistep catastrophe. FEBS Letters, 587(8), 1038–1046.

Boyman, L., Karbowski, M., & Lederer, W. J. (2020). Regulation of mitochondrial ATP production: Ca^{2+} signalling and quality control. Trends in Molecular Medicine, 26(1), 21–39.

Branco, M. R., & Pombo, A. (2006). Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. Biology, 4(5), e138.

Brandon, L. (2004). Subcellular fractionation. University of Alabama at Birmingham, Cell Biology teaching notes.

Braverman, N. E., Moser, A. B., & Steinberg, S. J. (2016). Peroxisome biogenesis disorders in the Zellweger spectrum: An overview of current diagnosis, clinical manifestations, and treatment guidelines. Molecular Genetics and Metabolism, 117(3), 313–321.

Britannica. (2025). Smooth endoplasmic reticulum (SER). Encyclopaedia Britannica.
Brown, A., et al. (2014). Structure of the large ribosomal subunit from human mitochondria. Proceedings of the National Academy of Sciences, 111(2), 728–733.

Brunk, U. T. (1972). The demonstration of acid phosphatase in cultured cells after fixation with glutaraldehyde. Histochemie, 30(1), 1–9.

Busch, H. (Ed.). (1974). The cell nucleus. Volume I. Academic Press.

Cambridge University Press. (2016). Cell structure and function [Excerpt]. Cambridge University Press.

Canene-Adams, K. (2013). Preparation of formalin-fixed paraffin-embedded tissue for immunohistochemistry. In Methods in enzymology (Vol. 533, pp. 225–233). Elsevier.

Cann, A. J. (2012). Principles of molecular virology (5th ed.). Academic Press.
Capetanaki, Y., Bloch, R. J., Kouloumenta, A., Mavroidis, M., & Psarras, S. (2007). Desmin and its role in muscle structure and function. FEBS Journal, 274(6), 1472–1489.

Caplow, M., & Shanks, J. (1990). Evidence that microtubule depolymerization proceeds by loss of a single layer of tubulin. Journal of Cell Biology, 111(1), 223–229.

Carattoli, A. (2013). Plasmids and the spread of resistance. International Journal of Medical Microbiology, 303(6–7), 298–304. https://doi.org/10.1016/j.ijmm.2013.01.001

Carpita, N. C., & Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. The Plant Journal, 3(1), 1–30.

Carson, J. L., Collier, A. M., & Goldsmith, J. D. (2014). Fundamental technical elements of freeze-fracture/freeze-etch. Microscopy and Microanalysis, 20(S3), 966–967.

Castejón, O. J., & Caraballo, A. J. (1980). Light microscopy, scanning and transmission electron microscopy of nervous tissue. Scanning Microscopy International, 2, 12–34.

Castellano, M. J., Woldringh, C. L., & al. (2020). The nuclear pore complex as an entropic gate. Journal of Physics A: Mathematical and Theoretical, 53(20), 205601.

Centers for Disease Control and Prevention (CDC). (2024). Negative staining electron microscope protocol for rash illness and poxvirus diagnosis.

Chandler, D. E. (1986). Rotary shadowing with platinum–carbon in biological electron microscopy: A review of methods and applications. Journal of Electron Microscopy Technique, 3(3), 305–335.

Chang, T.-Y., et al. (2004). Fractionation of mouse liver homogenate by differential and sucrose density gradient centrifugation. Journal of Biological Chemistry, 279(19), 19374–19381.

Chaudhry, R., & Varacallo, M. (2025). Biochemistry, glycolysis. In StatPearls. StatPearls Publishing.

Chavan, I. S., Raval, N., & Rajagopalan, V. (2025). Lysosome heterogeneity and diversity mapped through its membrane protein landscape. Cellular and Molecular Life Sciences.

Chen, J., et al. (2024). An aldehyde-crosslinking mitochondrial probe for STED super-resolution microscopy. Proceedings of the National Academy of Sciences, 121(e2317703121).

Chen, Y.-L., et al. (2025). Reversible and effective cell cycle synchronization method using CDK1 inhibitors. , 8(5), e202403000.

Cheng, X.-T., Xie, Y.-X., Zhou, B., Huang, N., Farfel-Becker, T., & Sheng, Z.-H. (2018). Characterization of LAMP1-labelled nondegradative lysosomal and endocytic compartments in neurons. Journal of Cell Biology, 217(9), 3127–3139.

Chi, Q., Yin, T., & Gregersen, H. (2014). Rear actomyosin contractility-driven directional cell migration in three-dimensional matrices. Journal of the Royal Society Interface, 11(94), 20131072.

Cinci, L., et al. (2014). Peroxisome determination in optical microscopy: A useful tool for cell biology. Methods in Cell Biology, 124, 1–16.

Clark, M. A., Choi, J., & Douglas, M. (2018). Biology 2e. Houston, TX: OpenStax, Rice University.

Cook, N. R., Row, P. E., & Davidson, H. W. (2004). Lysosome associated membrane protein 1 (Lamp1) regulates late endosome fusion with lysosomes. Traffic, 5(9), 593–604.

Cooper, G. M. (2000). The Cell: A Molecular Approach (2nd ed.). Sunderland, MA: Sinauer Associates. (Available from NCBI Bookshelf)

Cooper, G. M., & Hausman, R. E. (2000). The cell: A molecular approach (2nd ed.). Sinauer Associates
.
Coulombe, P. A., & Kerns, M. L. (2009). Epidermolysis bullosa simplex: A paradigm for disorders of tissue fragility. Journal of Clinical Investigation, 119(7), 1784–1793.

Cylwik, B., Gruszczyńska, J., Chrostek, L., & Gruszewska, E. (2013). Congenital disorders of glycosylation. Part I. Defects of protein N-glycosylation. Acta Biochimica Polonica, 60(2), 151–161.

Da Costa, L., Galimand, J., Fenneteau, O., & Mohandas, N. (2013). Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders. Best Practice & Research Clinical Haematology, 26(4), 285–302.

Dalton, L. (2024). DNA, chromosomes, and the interphase nucleus. In Cell biology .

Dalton, L. (2024). The cytoskeleton. In L. Dalton & R. Young, Fundamentals of Cell Biology. Corvallis, OR: Oregon State University / Open Textbook Library.

Daniels, G. (2007). Functions of red cell surface proteins. Vox Sanguinis, 93(4), 331–340.

Dauban, L., et al. (2025). Interactions between the genome and the nuclear lamina in shaping three-dimensional genome organization. Structural & Molecular Biology.

De Magistris, P., & Antonin, W. (2018). The dynamic of the nuclear envelope. Current Biology, 28(8), R487–R497.

Dean, L. (2005). The ABO blood group. In Blood groups and red cell antigens. National Center for Biotechnology Information (US).

Debec, A., Sullivan, W., & Bettencourt-Dias, M. (2010). Centrioles: Active players or passengers during mitosis? Cellular and Molecular Life Sciences, 67(13), 2173–2194.

Derenzini, M. (2001). Silver-stained nucleolar organizer regions (AgNOR) in tumour pathology. Pathology, Research and Practice, 197(6), 349–354.

Deshpande, O., & Mohiuddin, S. S. (2025). Biochemistry, oxidative phosphorylation. In StatPearls. StatPearls Publishing.

Dever, T. E., & Green, R. (2012). The elongation, termination, and recycling phases of translation in eukaryotes. Cold Spring Harbor Perspectives in Biology, 4(7), a013706.

DiMauro, S., & Schon, E. A. (2003). Mitochondrial respiratory-chain diseases. The New England Journal of Medicine, 348(26), 2656–2668. https://doi.org/10.1056/NEJMra022003

Dimchev, G., et al. (2021). Induced Arp2/3 complex depletion increases FMNL formin expression and filopodia formation. Frontiers in Cell and Developmental Biology, 9, 634708.

Dotz, V., & Wuhrer, M. (2019). Histo-blood group glycans in the context of personalized medicine. Biochimica et Biophysica Acta – General Subjects, 1863(9), 1463–1473.
Dráber, P., Sulimenko, V., & Dráberová, E. (2012). Cytoskeleton in mast cell signaling. Frontiers in Immunology, 3, 130.

Dubois, M.-L., Boisvert, F.-M., & al. (2016). The nucleolus: Structure and function. In The nucleolus (Springer).

Dubuisson, N., et al. (2022). Histological methods to assess skeletal muscle mitochondrial function and morphology. International Journal of Molecular Sciences, 23(24), 16080.

Dumančić, E. (2023). Beginners' guide to sample preparation techniques for electron microscopy. Periodicum Biologorum, 125(2), 189–199.

Dörner, K., et al. (2023). Ribosome biogenesis factors-from names to functions. The EMBO Journal, 42(4), e112699.

D'Souza, A. I., et al. (2023). Vesicles driven by dynein and kinesin exhibit directional reversals by mechanical motor interplay. Nature Communications, 14, 6850.

Ebersold, H. R., Cordier, J. L., & Lüthy, P. (1981). Bacterial mesosomes: Method dependent artifacts. Archives of Microbiology, 130(1), 19–22. https://doi.org/10.1007/BF00527066

Ebner, M., & Huber, L. A. (2025). The biology of lysosomes: From order to disorder. Histochemistry and Cell Biology. (ed.). Garland.

Elblbesy, M. A. (2018). Analytical study of normal erythrocytes and iron deficiency anemia using light scattering. International Journal of Hematology-Oncology and Stem Cell Research, 12(4), 260–268.

Ellenberg, J. (2013). Dynamics of nuclear envelope proteins during the cell cycle in mammalian cells. In The nuclear envelope (Bookshelf).

Endoplasmic Reticulum Lesson. (2014). Institute of Lifelong Learning, University of Delhi.

Epel, B. L. (1994). Plasmodesmata: Composition, structure and trafficking. Plant Molecular Biology, 26(5), 1349–1356.

Eriksson, J. E., et al. (2009). Introducing intermediate filaments: From discovery to disease. Journal of Clinical Investigation, 119(7), 1763–1771.

Fahimi, H. D. (2009). Peroxisomes: 40 years of histochemical staining, personal reminiscences and current problems. Histochemistry and Cell Biology, 131(4), 437–446.

Fahimi, H. D. (2017). Cytochemical detection of peroxisomes in light and electron microscopy. In Methods in Molecular Biology (Vol. 1595, pp. 141–154).

Fauquet, C. M. (2008). Taxonomy, classification and nomenclature of viruses. In B. W. J. Mahy & M. H. V. Van Regenmortel (Eds.), Encyclopedia of virology (3rd ed., pp. 15–23). Elsevier.

Flint, S. J., Enquist, L. W., Racaniello, V. R., & Skalka, A.-M. (2015). Principles of virology (4th ed., Vol. 1–2). ASM Press.

Foertsch, F., et al. (2017). Imaging cytochrome-c oxidase and FoF_1-ATP synthase in mitochondrial cristae of living human cells by FLIM and super-resolution microscopy. Preprint (:1702.00512).

Fontana, P., et al. (2022). Structure of the cytoplasmic ring of the nuclear pore complex by cryo-EM. , 376(6595), eabm9326.
Franke, W. W., et al. (1988). The nuclear envelope and the architecture of the nuclear periphery. Cell Biology International Reports, 12(9), 669–695.

Freeze, H. H., & Ng, B. G. (2011). Golgi glycosylation and human inherited diseases. Cold Spring Harbor Perspectives in Biology, 3(9), a005371.

Fritz, A. J., Sehgal, N., Rapić-Otrin, V., & Bustin, M. (2016). Organization of chromosome territories in the interphase nucleus. Genes, Chromosomes & Cancer, 55(5), 289–298.

Furuse, M. (2010). Molecular basis of the structure of tight junctions. Cold Spring Harbor Perspectives in Biology, 2(1), a002907.

Förster, C. (2008). Tight junctions and the modulation of barrier function in disease. Histochemistry and Cell Biology, 130(1), 55–70.

Gablishvili, M., et al. (2023). Prognostic value of mitotic index and Bcl-2 expression in uterine leiomyosarcoma. Brazilian Journal of Pathology and Laboratory Medicine, 59(1), e0052022.

Gajewska, M., Rutkowska, E., Kwiecień, I., Rzepecki, P., & Sułek, K. (2022). Analysis of argyrophilic nucleolar organizer regions (AgNORs) in acute leukemia in adults. Diagnostics, 12(4), 832.

Gallanti, A., et al. (1992). Desmin and vimentin as markers of regeneration in muscle diseases. Acta Neuropathologica, 85(1), 88–92.

Garrod, D., & Chidgey, M. (2008). Desmosome structure, composition and function. Biochimica et Biophysica Acta – Biomembranes, 1778(3), 572–587.

Gartner, L. P., & Hiatt, J. L. (2014). Color Textbook of Histology (4th ed.). Philadelphia, PA: Elsevier.
Gelderblom, H. R. (1996). Structure and classification of viruses. In S. Baron (Ed.), Medical microbiology (4th ed.). University of Texas Medical Branch at Galveston.

Georgatos, S. D., & al. (2001). The inner nuclear membrane: Simple, or very complex? The EMBO Journal, 20(12), 2989–2994.

Ghochani, M., Nulton, J. D., Salamon, P., Frey, T. G., Rabinovitch, A., & Baljon, A. R. C. (2010). Tensile forces and shape entropy explain observed crista structure in mitochondria. Biophysical Journal, 99(2), 3244–3254.

Gipson, I. K., Spurr-Michaud, S., Tisdale, A., & Elwell, J. (1993). Redistribution of the hemidesmosome components α6β4 integrin and bullous pemphigoid antigens during epithelial wound healing. Journal of Histochemistry & Cytochemistry, 41(9), 1291–1301.

Gonzalo, S. (2016). The nuclear lamina in health and disease. Current Opinion in Cell Biology, 40, 24–31.

Gonzalo, S. (2017). A premature aging disease caused by LMNA gene mutations. Mechanisms of Ageing and Development, 165, 16–21.

Gould, E. A., Meyers, G., Monath, T., Pletnev, A., Rico-Hesse, R., Smith, D. B., Stapleton, J. T., & ICTV Report Consortium. (2017). ICTV virus taxonomy profile: Flaviviridae. Journal of General Virology, 98(1), 2–3.

Graham, L., & Orenstein, J. M. (2007). Processing tissue and cells for electron microscopy in diagnostic pathology and research. Nature Protocols, 2(10), 2439–2450. https://doi.org/10.1038/nprot.2007.304
Graham, L., & Orenstein, J. M. (2007). Processing tissue and cells for transmission electron microscopy in diagnostic pathology and research. Protocols, 2(10), 2439–2450.

Gray, M. W. (2017). Lynn Margulis and the endosymbiont hypothesis: 50 years later. Molecular Biology and Evolution, 34(5), 1253–1255.

Green, K. J., & Gaudry, C. A. (2000). Are desmosomes more than tethers for intermediate filaments? Reviews Molecular Cell Biology, 1(3), 208–216.

Green, K. J., Broussard, J. A., & Epperson, E. A. (2019). Essential contributors to an integrated intercellular junction system. , 8, 2150.

Guo, W., Wang, P., Liu, Z., & Ye, P. (2018). Analysis of differential expression of tight junction proteins in gingival epithelial cells of chronic periodontitis. International Journal of Oral , 10(4), 31.

Guzel, E., Arlier, S., Guzeloglu-Kayisli, O., et al. (2017). Endoplasmic reticulum stress and homeostasis in reproductive physiology and pathology. International Journal of Molecular Sciences, 18(4), 792.

Habbane, M., et al. (2021). Human mitochondrial DNA: Particularities and pathologies. Pan African Medical Journal, 40, 15.

Hajjawi, O. S. (2013). Human red blood cells-1. Manuscript.

Hampoelz, B., et al. (2023). Nuclear envelope assembly and dynamics during the cell cycle. Current Opinion in Cell Biology, 79, 102135.

Handy, J. M., et al. (2024). Ups and downs of lysosomal pH: Conflicting roles of LAMP family proteins. Autophagy, 20(1), 1–14.

Harrison, C. M. H. (1971). The arrangement of chromatin in the interphase nucleus. Chromosoma, 33(1), 79–90.

Hatton, I. A., Galbraith, E. D., Merleau, N. S. C., Miettinen, T. P., Smith, B. M., & Shander, J. A. (2023). The human cell count and size distribution. Proceedings of the National Academy of Sciences, 120(39), e2303077120.

Hegazy, M., Xu, X., Guo, L., & Guo, Z. (2021). Desmosomal cadherins in health and disease. Cell Communication and Adhesion, 27(1), 1–12.

Held, P. (2015). Cell fixation methods for fluorescence microscopy. Technologies White Paper 5994-2778EN.

Held, P. (2015). Sample preparation for fluorescence microscopy: Fixation and permeabilization. Agilent Technologies White Paper 5994-2778EN.

Hendricks, G. M. (2013). Metal shadowing for electron microscopy. In J. Kuo (Ed.), Electron microscopy: Methods and protocols (pp. 65–75). Humana Press.

Henson, J. H., et al. (2017). The ultrastructural organization of actin and myosin II in the contractile ring. Molecular Biology of the Cell, 28(20), 2742–2756.

Henson, J. H., et al. (2024). Cytokinetic contractile ring structural progression in an echinoderm embryo. Frontiers in Cell and Developmental Biology, 12, 1483345.

Hetzer, M. W. (2010). The nuclear envelope. Cold Spring Harbor Perspectives in Biology, 2(3), a000539.
Hine, R. (1999). Membrane. In The Facts on File dictionary of biology (3rd ed., p. 198). Facts On File.

Hinkle, P. C. (2005). P/O ratios of mitochondrial oxidative phosphorylation. Biochimica et Biophysica Acta (Bioenergetics), 1706(1–2), 1–11.

Hino, H., & Nagata, K. (1981). Size of desmosomes and hemidesmosomes in normal human skin and in bullous diseases. Acta Dermato-Venereologica, 61(2), 133–138.

Histology Guide. (2024). Golgi apparatus (Golgi's method). HistologyGuide.com.
Huang, X., et al. (2023). Mitochondrial cristae in health and disease. Journal of Molecular Structure, 1279, 134843.

Hyams, C., Camberlein, E., Cohen, J. M., Bax, K., & Brown, J. S. (2010). The Streptococcus pneumoniae capsule inhibits complement activity and neutrophil phagocytosis by multiple mechanisms. Infection and Immunity, 78(2), 704–715. https://doi.org/10.1128/IAI.00881-09

ICTV (International Committee on Taxonomy of Viruses). (2024). About virus taxonomic classification. Retrieved from the ICTV website.

Ifuji, A., et al. (2017). A novel immunofluorescence method to visualize antiparallel microtubule overlaps at the midzone. Experimental Cell Research, 360(2), 245–252.

IHC World. (2024). Electron microscopy negative staining protocol (Method 2).

Ikegami, K., et al. (2020). Phosphorylated lamin A/C in the nuclear interior binds active enhancers associated with abnormal transcription in progeria. Developmental Cell, 52(5), 699–713.

Jannink, I., Van Diest, P. J., & Baak, J. P. A. (1995). Classical and random mitotic activity assessments with reference to prognostic value in breast cancer. Histopathology, 27(2), 135–141.

Jia, X., Yuan, S., Wang, Y., & Liu, Y. (2024). Protein translation: Biological processes and therapeutic opportunities. Signal Transduction and Targeted Therapy, 9, 149.

Jiao, L., Liu, X., Liu, Y., & Xu, J. (2023). Ribosome biogenesis in disease: New players and therapeutic avenues. Signal Transduction and Targeted Therapy, 8, 32.

Jimson, S., et al. (2013). Lysosomal storage diseases: A review with dental and orofacial manifestations. Journal of Dr. NTR University of Health Sciences, 2(1), 22–29.

Johnston, R., et al. (2024). Roles of ribosomal RNA in health and disease. Frontiers in RNA Research, 3, 1331185.

Jongebloed, W. L., et al. (1999). Modern methods for preparing biological specimens for field emission scanning electron microscopy. Scanning, 21(6), 372–383.

Junqueira, L. C., & Carneiro, J. (2013). Junqueira’s basic histology: Text and atlas (13th ed.). McGraw-Hill.
Junqueira, L. C., & Carneiro, J. (2013). Junqueira’s Basic Histology: Text and Atlas (13th ed.). New York, NY: McGraw-Hill.

Junqueira, L. C., & Carneiro, J. (2018). Junqueira’s basic histology: Text and atlas (15th ed.). McGraw-Hill.

Junqueira, L. C., & Mescher, A. L. (2021). Junqueira’s basic histology: Text and atlas (16th ed.). McGraw-Hill.
Junqueira’s Basic Histology (Mescher, A. L.). (2018). Junqueira’s basic histology: Text and atlas (15th ed.). McGraw-Hill.

Kahyaoğlu, F., Alpay, M., & Sahin, B. (2016). Light microscopic determination of tissue fixation quality: A practical approach. European Journal of Medicine, 22(3), 120–124.

Kalafati, E. (2022). Mimiviruses: Giant viruses with novel and intriguing features. Microorganisms, 10(5), 1011.

Kasman, L. M., & Porter, L. D. (2022). Bacteriophages. In StatPearls. StatPearls Publishing.

Kierszenbaum, A. L., & Tres, L. (2020). Histology and cell biology: An introduction to pathology (5th ed.). Elsevier.

Kim, S. Y., et al. (2022). Neuronal mitochondrial morphology is significantly affected by changes in fixative composition. Frontiers in Cellular Neuroscience, 16, 966708.

Kim, Y., Sharov, A. A., McDole, K., & Fan, C.-M. (2019). Role of lamins in 3D genome organization and global gene regulation. Nucleus, 10(1), 33–41.

Kind, J., & van Steensel, B. (2010). Genome–nuclear lamina interactions and gene regulation. Current Opinion in Cell Biology, 22(3), 320–325.

King, M. J., Garçon, L., Hoyer, J. D., Iolascon, A., Picard, V., Stewart, G., … Zanella, A. (2013). ICSH guidelines for the laboratory diagnosis of nonimmune hereditary red cell membrane disorders. International Journal of Laboratory Hematology, 35(3), 222–230.

Kirichenko, E. Y., Ryabinina, M. N., & Moss, S. E. (2021). Structure and functions of gap junctions and their role in development and disease. Cells, 10(8), 1953.

Kliuchnikov, E., et al. (2022). Microtubule assembly and disassembly dynamics model. Journal of Biosafety and Biosecurity, 4(1), 18–26.

Klouwer, F. C. C., Berendse, K., Ferdinandusse, S., et al. (2015). Zellweger spectrum disorders: Clinical overview and management approach. Orphanet Journal of Rare Diseases, 10, 151.

Klumperman, J. (2011). Architecture of the mammalian Golgi. Cold Spring Harbor Perspectives in Biology, 3(7), a005181.

Kobayashi, T., & Dynlacht, B. D. (2011). Regulating the transition from centriole to basal body. Journal of Cell Biology, 193(3), 435–444.

Kohanski, M. A., Dwyer, D. J., & Collins, J. J. (2010). How antibiotics kill bacteria: From targets to networks. Nature Reviews Microbiology, 8(6), 423–435. https://doi.org/10.1038/nrmicro2333

Koonin, E. V. (2021a). Viruses defined by the position of the virosphere within the replicator space. Current Opinion in Virology, 47, 81–87.

Koonin, E. V. (2021b). The Baltimore classification of viruses 50 years later: How does it stand in the light of virus evolution? Virology, 556, 1–7.

Koonin, E. V., & Starokadomskyy, P. (2016). Are viruses alive? The replicator paradigm sheds decisive light on an old but misguided question. Studies in History and Philosophy of Biological and Biomedical Sciences, 59, 125–134.
Krasnow, S. H. (1980). Structure of erythrocyte membrane and its transport function. Annals of Clinical and Laboratory , 10(3), 209–221.

Krause, C., et al. (2009). Rational diagnostic strategy for Zellweger syndrome and other peroxisome biogenesis disorders. European Journal of Human Genetics, 17(4), 431–444.

Krystofiak, E. S., et al. (2019). Carbon replicas reveal double-stranded structure of tight junction strands. Communications Biology, 2, 227.

Kubitscheck, U., et al. (2024). Nuclear transport and the dynamics of ribosome biogenesis. Current Opinion in Cell Biology, 82, 102217.

Kumar, N. M., & Gilula, N. B. (1996). The gap junction communication channel. Cell, 84(3), 381–388.
Kumar, R., et al. (2024). The peroxisome: An update on mysteries 3.0. Histochemistry and Cell Biology, 161(1), 1–26.

Kumar, V., Abbas, A. K., & Aster, J. C. (2021). Robbins and Cotran pathologic basis of disease (10th ed.). Elsevier.

Kumar, V., Abbas, A. K., & Aster, J. C. (2021). Robbins and Cotran Pathologic Basis of Disease (10th ed.). Philadelphia, PA: Elsevier.

La Rosa, S., et al. (2023). Diagnostic, prognostic, and predictive role of Ki-67 in neuroendocrine neoplasms. Cells, 12(3), 495.

Lackmann, F. (2017). Nucleolar ribosome assembly. Doctoral dissertation, Uppsala University.
Lammerding, J. (2011). Mechanics of the nucleus. The Journal of Cell Biology, 194(4), 475–485. https://doi.org/10.1083/jcb.201102113

Lefeber, D. J., Morava, E., & Jaeken, J. (2022). Congenital disorders of glycosylation. In GeneReviews. University of Washington, Seattle.

Li, J., et al. (2005). Spectrin-level modeling of the erythrocyte cytoskeleton and optical properties. Biophysical Journal, 88(5), 3707–3719.

Lin, D. H., & Hoelz, A. (2019). The structure of the nuclear pore complex (an update). Annual Review of Biochemistry, 88, 725–783.

Linders, P. T. A., Mertens, B. J. G., van Zadelhoff, G., & Lefeber, D. J. (2020). Sugary logistics gone wrong: Membrane trafficking and congenital disorders of glycosylation. International Journal of Molecular Sciences, 21(13), 4654.

Lindström, M. S., Jurada, D., Bursac, S., Orsolic, I., Bartek, J., & Volarević, S. (2018). Nucleolus as an emerging hub in maintenance of genome stability and cancer pathogenesis. Oncogene, 37(18), 2351–2363.

Liu, F., & Zhou, Z. H. (2011). Comparative virion structures of human herpesviruses. In A. Arvin et al. (Eds.), Human herpesviruses: Biology, therapy, and immunoprophylaxis. Cambridge University Press.

Liu, J., Wang, H., & Zhang, Y. (2021). The role of the Golgi apparatus in disease. International Journal of Molecular Medicine, 47(2), 75.

Lodhi, I. J., & Semenkovich, C. F. (2014). Peroxisomes: A nexus for lipid metabolism and cellular signaling. Cell Metabolism, 19(3), 380–392.
Lodhi, I. J., & Semenkovich, C. F. (2014). Peroxisomes: A nexus for lipid metabolism and cellular signalling. Cell Metabolism, 19(3), 380–392.

Longo, G. M. C., & Pombo, A. (2021). Territories or spaghetti? Chromosome organization exposed. Reviews Molecular Cell Biology, 22(5), 299–300.

Loomis, C., & Alu, M. (2016). Detailed discussion of tissue prep and formalin/formaldehyde fixation recommendations [Technical protocol]. Experimental Pathology Research Laboratory, NYU Langone Medical Center.

Louten, J. (2016). Virus structure and classification. In Essential human virology (pp. 19–43). Elsevier.

Lovy, J. (2014). Electron microscopy for diagnostic virology. In AFS–FHS blue book: Suggested procedures for the detection and identification of certain finfish and shellfish pathogens (Section 2.1.2).

Machamer, C. E. (2015). The Golgi complex in stress and death. Frontiers in Neuroscience, 9, 421.

Macnab, R. M. (2003). How bacteria assemble flagella. Annual Review of Microbiology, 57, 77–100. https://doi.org/10.1146/annurev.micro.57.030502.090832

Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., & Stahl, D. A. (2018). Brock Biology of Microorganisms (15th ed.). New York, NY: Pearson.

Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., & Stahl, D. A. (2018). Brock biology of microorganisms (15th ed.). Pearson.

Madsen-Østerbye, J., et al. (2022). Biology and model predictions of the dynamics of lamina–chromatin interactions. in Cell and Developmental Biology, 10, 913458.

Madyastha, K. M., & Subramanian, S. (1977). Subcellular localisation of a cytochrome P-450-dependent monoterpene hydroxylase. Biochemical Journal, 164(1), 51–59.

Mall, M. A., & Hartl, D. (2014). CFTR: Cystic fibrosis and beyond. European Respiratory Journal, 44(4), 1042–1054.

Manso, J. A., Mateos-Cáceres, P. J., & Talavera-González, A. (2019). Integrin α6β4 recognition of a linear motif of bullous pemphigoid antigen BP230 controls its recruitment to hemidesmosomes. Matrix Biology, 80, 23–35.

Maraldi, N. M., Lattanzi, G., Cenni, V., & al. (2006). Nuclear envelope proteins and chromatin arrangement. European Journal of Histochemistry, 50(1), 5–14.

Martin, W. F. (2017). Physiology, anaerobes, and the origin of mitosing cells 50 years on. Journal of Theoretical Biology, 434, 2–10.

Martínez-Alonso, E., & Malhotra, V. (2013). Morpho-functional architecture of the Golgi complex in mammalian cells. Frontiers in Endocrinology, 4, 41.

Martínez-Menárguez, J. A., et al. (1999). Vesicular tubular clusters between the ER and Golgi mediate concentration of soluble secretory proteins by exclusion from COPI-coated vesicles. Cell, 98(1), 81–90.

Marzuca-Nassr, G. N., Vitzel, K. F., Mancilla-Solorza, E., & Márquez, J. L. (2018). Sarcomere structure: The importance of desmin protein in muscle atrophy. International Journal of Morphology, 36(2), 576–584.

Mattout, A., Meshorer, E., & al. (2015). Chromatin states and nuclear organization in development. Genome Biology, 16, 206.

Mazzarello, P. (1999). A unifying concept: The history of cell theory. Nature Cell Biology, 1(1), E13–E15.

Medical Cytology Lecture Notes. (2019). The plasma membrane [Lecture notes]. Department of Anatomy, Mustansiriyah University.

Megías, M. (2019). Histological techniques: Light microscopes. Universidade de Vigo, Histology web notes.

Mehta, N. G. (1980). ABO(H) blood group antigens of the human erythrocyte membrane. Immunogenetics, 11(5), 429–439.

Meng, W., & Takeichi, M. (2009). Adherens junction: Molecular architecture and regulation. Cold Spring Harbor Perspectives in Biology, 1(6), a002899.

Mescher, A. L. (2021). Junqueira's basic histology: Text and atlas (16th ed.). McGraw Hill.

Meyer, H. W. (2001). Freeze-fracture studies on lipids and membranes. Current Opinion in Colloid & Interface , 6(5–6), 439–448.

Meyer, H. W., & Verkman, A. S. (2001). Freeze-fracture and freeze-etch electron microscopy of biological membranes. Micron, 32(4), 379–389.

Miller, A. L. (2011). The contractile ring. Current Biology, 21(24), R976–R978.

Mills, E. W., & Green, R. (2017). Ribosomopathies: There's strength in numbers. Science, 358(6363), eaan2755.

Milo, R., & Phillips, R. (2015). Cell biology by the numbers. Garland Science.

Moraleva, A. A., et al. (2022). Eukaryotic ribosome biogenesis: The 60S subunit. , 14(3), 4–19.

Moritz, M. L. (2019). Why 0.9% saline is isotonic: Understanding the aqueous phase of plasma and the difference between osmolarity and osmolality. Pediatric Nephrology, 34(7), 1299–1300.

Morrison, O., & Thakur, J. (2021). Molecular complexes at euchromatin, heterochromatin and centromeric chromatin. International Journal of Molecular s, 22(13), 6922.

Mostafa, H. K. K., et al. (2022). Different cells of the human body: Categories and morpho-functional features. Journal of the Medical Colleges of Uganda, 1(2), e10–e27.

Moura, J. P., et al. (2025). Mitochondria: An overview of their origin, genome, architecture, and dynamics. Progress in Biophysics and Molecular Biology, 186, 1–25.

Moxon, E. R., & Kroll, J. S. (1990). The role of bacterial polysaccharide capsules as virulence factors. Current Topics in Microbiology and Immunology, 150, 65–85.

Mustikaningrum, F. (2022). Histology slide preparation and analysis (Doctoral thesis). University of Nottingham.

Müller, D. J., Hand, G. M., Engel, A., & Sosinsky, G. E. (2002). Conformational changes in surface structures of isolated connexin 26 gap junctions. The EMBO Journal, 21(14), 3598–3607.
Narla, A., & Ebert, B. L. (2010). Ribosomopathies: Human disorders of ribosome dysfunction. Blood, 115(16), 3196–3205.

Nath, S. (2019). Integration of demand and supply sides in the ATP energy economics of cells. Biophysical Chemistry, 252, 106208.

National Institute of General Medical Sciences (NIGMS). (2005). Inside the cell (NIH Publication No. 05-1051). U.S. Department of Health and Human Services, National Institutes of Health.

National Institute of General Medical Sciences (NIGMS). (2005). Inside the Cell (NIH publication). Bethesda, MD: National Institutes of Health.

National Library of Medicine. (2003). Cytology. In Medical dictionary [NCBI Bookshelf]. National Library of Medicine.

Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J., & Setlow, P. (2000). Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments. Microbiology and Molecular Biology Reviews, 64(3), 548–572. https://doi.org/10.1128/MMBR.64.3.548-572.2000

Niessen, C. M. (2007). Tight junctions/adherens junctions: Basic structure and function. Journal of Investigative Dermatology, 127(11), 2525–2532
.
Nigg, E. A., & Holland, A. J. (2018). Once and only once: Mechanisms of centriole duplication and their deregulation in disease. Nature Reviews Molecular Cell Biology, 19(5), 297–312.

Nigg, E. A., & Raff, J. W. (2009). Centrioles, centrosomes, and cilia in health and disease. Cell, 139(4), 663–678.

Norman, A., Hansen, L. H., & Sørensen, S. J. (2009). Conjugative plasmids: Vessels of the communal gene pool. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1527), 2275–2289. https://doi.org/10.1098/rstb.2009.0037

Oakes, S. A., & Papa, F. R. (2015). The role of endoplasmic reticulum stress in human pathology. Annual Review of Pathology, 10, 173–194.

Ofner, D., et al. (1996). Demonstration of silver-stained nucleolar organizer regions and their prognostic value in breast carcinoma. Virchows Archiv, 429(1), 63–69.

OpenStax. (2013). The cell cycle. In Concepts of biology. OpenStax CNX.

OpenStax. (2016). Themes and concepts of biology. In Biology 2e. OpenStax, Rice University.

Oshima, R. G. (2007). Intermediate filaments: A historical perspective. Experimental Cell Research, 313(10), 1981–1994. https://doi.org/10.1016/j.yexcr.2007.04.007

Ostashevsky, J. Y. (2002). A polymer model for large-scale chromatin organization. , 13(7), 2157–2169.

Padh, H. (1992). Organelle isolation and marker enzyme assay. In Proceedings of the Association for Biology Laboratory Education (Vol. 13, pp. 93–131).

Parada, L. A., Misteli, T., & al. (2002). Chromosome positioning in the interphase nucleus. Trends in Cell Biology, 12(9), 425–432.

Parkinson-Lawrence, E. J., Shandala, T., Prodoehl, M., Plew, R., Borlace, G. N., & Brooks, D. A. (2005). Immunochemical analysis of CD107a (LAMP1). Cell Biology International, 29(9), 803–812.
Parry, N. (2024). Histology slide preparation: Five important steps.

Paul, R., et al. (2020). Quantitative absorption imaging of red blood cells to determine their morphology and hemoglobin content. RSC Advances, 10(60), 36421–36430.

Pawlina, W. (2023). Histology: A text and atlas: With correlated cell and molecular biology (9th ed.). Wolters Kluwer.

Pawlina, W., & Ross, M. H. (2019). Histology: A text and atlas with correlated cell and molecular biology (8th ed.). Wolters Kluwer.

Pellegrini, L., Wetzel, A., Grannó, S., Heaton, G., & Harvey, K. (2017). Back to the tubule: Microtubule dynamics in Parkinson's disease. Cellular and Molecular Life Sciences, 74(3), 409–434.

Pellett, P. E. (2014). Basics of virology. In D. M. Knipe & P. M. Howley (Eds.), Fields virology (6th ed.). Lippincott Williams & Wilkins.

Pfister, S., King, J., & Böttcher, B. (2023). Structural conservation of HBV-like capsid proteins over 400 million years of evolution. Communications, 14, 1432.

Pollard, T. D., & Cooper, J. A. (2009). Actin, a central player in cell shape and movement. Science, 326(5957), 1208–1212.

Poswar, F. O., Vairo, F., Maluf, S. W., et al. (2019). Lysosomal diseases: Overview on current diagnosis and treatment. Genetics and Molecular Biology, 42(1), 165–177.

Proft, T., & Baker, E. N. (2009). Pili in Gram-positive bacteria: Structure, assembly and their role in disease. Cellular and Molecular Life Sciences, 66(4), 613–635.

Qin, Y., et al. (2021). The combination of paraformaldehyde and glutaraldehyde preserves mitochondrial morphology better than either alone. The EMBO Journal, 40(e95709).

Rahner, C., Mitic, L. L., & Anderson, J. M. (1996). Structure–function correlation of tight junctional permeability. Gastroenterology, 111(2), 404–413.

Raices, M., & D'Angelo, M. A. (2022). Structure, maintenance, and regulation of nuclear pore complexes. Current Opinion in Cell Biology, 74, 41–49.

Rajkumar, V., & Riemer, C. (2023). Lysosomal storage disease. In StatPearls. StatPearls Publishing.
Raju, K. (2016). Evolution of Pap stain. Biomedical Research and Therapy, 3(6), 629–635
.
Rampazzo, A., Calore, M., van Hengel, J., & van Roy, F. (2014). Intercalated discs and arrhythmogenic cardiomyopathy. Circulation: Cardiovascular Genetics, 7(6), 930–940.

Raska, I., Shaw, P. J., & Cmarko, D. (2006). Structure and function of the nucleolus in the spotlight. Current Opinion in Cell Biology, 18(3), 325–334.

Rehfeld, A., Nylander, M., & Karnov, K. (2017). Histological methods. In Compendium of histology. Springer.

Review. (2022). Principle, applications and types of centrifugation. Journal of Analytical & Bioanalytical Techniques, 13(1), 1–5.

Rivas-Manzano, P., et al. (2021). Silver staining methods for nucleolar organizer regions and Golgi–Kopsch techniques. Histology and Histopathology, 36(2), 195–205.
Rodier, C., et al. (2025). Muscle growth by sarcomere divisions. Science Advances, 11, eadw9445.

Roger, A. J., Muñoz-Gómez, S. A., & Kamikawa, R. (2017). The origin and diversification of mitochondria. Current Biology, 27(21), R1177–R1192.

Roizman, B. (1996). Multiplication. In S. Baron (Ed.), Medical microbiology (4th ed.). University of Texas Medical Branch at Galveston.

Rosoklija, G., et al. (2003). Optimization of Golgi methods for impregnation of brain tissue from humans and monkeys. Journal of Neuroscience Methods, 131(1–2), 1–10.

Ross, J. M. (2011). Visualization of mitochondrial respiratory function using cytochrome-c oxidase/succinate dehydrogenase double-labelling histochemistry. Journal of Visualized Experiments, 23, e3266.

Ross, M. H., & Pawlina, W. (2019). Histology: A text and atlas with correlated cell and molecular biology (8th ed.). Wolters Kluwer.

Ross, M. H., & Pawlina, W. (2019). Histology: A text and atlas: With correlated cell and molecular biology (8th ed.). Wolters Kluwer.

Roy, B., et al. (2024). Serial section histopathology image dataset of colon and liver tissue. Scientific Data, 11, 123.

Ruprecht, V., et al. (2015). Cortical contractility triggers a stochastic switch to fast amoeboid cell motility. Cell, 160(4), 673–685.

Ryan, K. J., & Ray, C. G. (Eds.). (2004). Sherris Medical Microbiology (4th ed.). New York, NY: McGraw-Hill.

Salton, M. R. J., & Kim, K.-S. (1996). Structure. In S. Baron (Ed.), Medical Microbiology (4th ed.). Galveston, TX: University of Texas Medical Branch at Galveston. (Available from NCBI Bookshelf)
Sanchez Carrillo, I. B., et al. (2025). In situ architecture of the nuclear pore complex of higher plants. Plants.

Sanjib, S. (2020). Cell cycle & its regulation (lecture notes).

Sanvictores, T., & Ismail, F. (2023). Histology, rough endoplasmic reticulum. In StatPearls. StatPearls Publishing.

Schick, J., & Martiel, J.-L. (2022). Blebs—Formation, regulation, positioning, and role in amoeboid cell migration. Frontiers in Cell and Developmental Biology, 10, 926394.

Schneeberger, E. E., & Lynch, R. D. (2004). The tight junction: A multifunctional complex. American Journal of Physiology – Cell Physiology, 286(6), C1213–C1228.

Schon, E. A. (2012). Human mitochondrial DNA: Roles of inherited and somatic mutations. Reviews Genetics, 13(12), 878–890.

Schrader, M., & Fahimi, H. D. (2006). Peroxisomes and oxidative stress. Biochimica et Biophysica Acta – Molecular Cell Research, 1763(12), 1755–1766. https://doi.org/10.1016/j.bbamcr.2006.09.006

Schrader, M., & Fahimi, H. D. (2008). The peroxisome: Still a mysterious organelle. Histochemistry and Cell Biology, 129(4), 421–440.
Schrand, A. M., Rahman, M. F., Hussain, S. M., Schlager, J. J., Smith, D. A., & Ali, S. F. (2010). Preparation of cells for assessing ultrastructural localization of nanoparticles with transmission electron microscopy. Nature Protocols, 5(4), 744–757.

Schreiber, K. H., & Kennedy, B. K. (2013). When lamins go bad: Nuclear structure and disease. Cell, 152(6), 1365–1375.

Schwarz, D. S., & Blower, M. D. (2016). The endoplasmic reticulum: Structure, function and response to cellular signaling. Cellular and Molecular Life Sciences, 73(1), 79–94.

Schöfer, C., & Weipoltshammer, K. (2018). Nucleolus and chromatin. Histochemistry and Cell Biology, 150(5), 361–379.

Scott, I., & Logan, D. C. (2004). The birth of cell biology. New Phytologist, 163(1), 7–9.

Seigneuret, F., et al. (2025). The HBV large envelope protein initiates virion assembly via nucleocapsid–envelope interactions. Cellular and Molecular Life s.

Seligman, A. M., Karnovsky, M. J., Wasserkrug, H. L., & Hanker, J. S. (1958). Nondroplet ultrastructural demonstration of succinic dehydrogenase activity with new electron-opaque complex. Science, 125(3251), 104–105.

Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. PLOS Biology, 14(8), e1002533.

Severs, N. J. (1985). Intercellular junctions and the cardiac intercalated disk. In The cardiac muscle cell (pp. 371–399). Springer.

Severs, N. J. (2007). Freeze-fracture electron microscopy. Protocols, 2(3), 547–576.

Shah, M. M. (2022). Physiology, plasma osmolality and oncotic pressure. In StatPearls. StatPearls Publishing.

Shah, S. B., et al. (2004). Structural and functional roles of desmin in mouse skeletal muscle. Biophysical Journal, 86(5), 3432–3444.

Sheikh, F., Ross, R. S., & Chen, J. (2009). Cell–cell connection to cardiac disease. Trends in Cardiovascular Medicine, 19(6), 182–190.

Sheth, J., et al. (2023). Lysosomal storage disorders: From biology to the clinic with emerging technologies. The Lancet Regional Health – Southeast Asia, 13, 100124.

Shevelyov, Y. Y., & Nurminsky, D. I. (2023). Interactions of chromatin with the nuclear lamina and nuclear pore complexes. International Journal of Molecular s, 24(21), 15771.

Shore, D., & Tollervey, D. (2022). Ribosome biogenesis and the control of cell proliferation. Current Opinion in Cell Biology, 74, 1–8.

Sicari, D., et al. (2020). A guide to assessing endoplasmic reticulum homeostasis and stress in mammalian systems. FEBS Journal, 287(1), 27–45.

Silhavy, T. J., Kahne, D., & Walker, S. (2010). The bacterial cell envelope. Cold Spring Harbor Perspectives in Biology, 2(5), a000414.

Silva, M. T., Sousa, J. C., Polónia, J. J., Macedo, M. A., & Parente, A. M. (1976). Bacterial mesosomes: Real structures or artifacts? Biochimica et Biophysica Acta, 443(1), 92–105.

Simón, D., et al. (2024). Two decades ago, giant viruses were discovered. in Microbiology, 15, 1356711.

Singer, S. J., & Nicolson, G. L. (1972). The fluid mosaic model of the structure of cell membranes. , 175(4023), 720–731.

Sirri, V., Urcuqui-Inchima, S., Roussel, P., & Hernandez-Verdun, D. (2007). Nucleolus: The fascinating nuclear body. Histochemistry and Cell Biology, 129(1), 13–31.

Smith, A. C. (2021). Gram stain protocol. Washington, DC: American Society for Microbiology. Retrieved from

Smith, D. G. (2023). Vesicle-mediated cellular transport. Research Starters: Anatomy and Physiology. Information Services.

Sonenberg, N., & Hinnebusch, A. G. (2009). Regulation of translation initiation in eukaryotes: Mechanisms and biological targets. Cell, 136(4), 731–745.

Squire, J. M. (2016). Muscle contraction: Sliding filament history, sarcomere dynamics and the two-state model. Biophysical Reviews, 8(Suppl 1), 291–323.

Steinberg, S. J., et al. (2020). Zellweger spectrum disorder. In GeneReviews. University of Washington, Seattle.

Steinert, P. M., & Roop, D. R. (1996). Molecular and cellular biology of intermediate filaments. Annual Review of Biochemistry, 65, 615–663.

Stokes, D. L. (2007). Desmosomes from a structural perspective. Current Opinion in Cell Biology, 19(5), 565–571.

Sun, A. (2018). Lysosomal storage disease overview. Acta Medica Academica, 47(1), 1–12.

Sun, Y., Liu, Y., & Yan, X. (2020). Fine structure of the intercalated disc and cardiac junctions in vertebrates. Applied Microscopy, 50, 11.

Suraneni, P., et al. (2012). The Arp2/3 complex is required for lamellipodia extension and directional fibroblast migration. Journal of Cell Biology, 197(2), 239–251.

Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's theory and practice of histological techniques (8th ed.). Elsevier.

Szul, T., & Sztul, E. (2011). COPII and COPI traffic at the ER–Golgi interface. Physiology, 26(5), 348–364.

Tafforeau, L., et al. (2013). The complexity of human ribosome biogenesis revealed by systematic nucleolar screening of pre-rRNA processing factors. Molecular Cell, 52(4), 539–551.

Taiz, L., & Zeiger, E. (2010). Plant Physiology (5th ed.). Sunderland, MA: Sinauer Associates.

Tetzlaff, M. T., et al. (2013). Immunodetection of phosphohistone H3 as a surrogate marker for mitotic figures in melanoma. Modern Pathology, 26(9), 1150–1157.

Thermo Fisher Scientific. (2024). Actin staining protocol using fluorescent phallotoxins. Technical Protocol.

Tonog, P. (2022). Normal saline. In StatPearls. StatPearls Publishing.

Torres-Fernández, O. (2006). The Golgi silver impregnation method. Biomédica, 26(1), 133–142.

Tripathi, N., & Sapra, A. (2023). Gram staining. In StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing. Retrieved from

University of Geneva Faculty of Medicine EM Facility. (2023). Negative staining TEM: Protocol and recommendations.

University of Maryland. (2023). Cell fractionation based on density gradient. Department of Chemical and Biomolecular Engineering teaching notes.

University of Minnesota. (2019). Definition of life. In Introductory biology: Evolutionary and ecological perspectives. University of Minnesota Libraries Publishing.

University of Nebraska–Lincoln Microscopy Facility. (2021). TEM fixation protocols. Nebraska Center for Biotechnology, University of Nebraska–Lincoln.

van der Gulik, P. T. S., Massar, S., Gilis, D., Buhrman, H., & Rooman, M. (2017). In defence of the three-domains of life paradigm. BMC Evolutionary Biology, 17, 218.

Van Itallie, C. M., & Anderson, J. M. (2014). Claudins and other tight junction proteins. Cold Spring Harbor Perspectives in Biology, 6(1), a016857.

van Prooijen-Knegt, A. C., et al. (1980). Quantitative aspects of the cytochemical Feulgen-DNA reaction. Histochemical Journal, 12(1), 45–62.

van Steenhoven, J. E. C., et al. (2020). Assessment of tumour proliferation by use of the mitotic activity index and phosphohistone H3. Histopathology, 77(2), 191–202.

Vanslembrouck, B., Van den Broeck, W., & De Vylder, D. (2022). Microscopic visualization of cell–cell adhesion complexes in epithelia and endothelium. in Cell and Developmental Biology, 10, 819534.

Vasicova, P., et al. (2016). Formaldehyde fixation is detrimental to actin cables in glucose-depleted yeast cells. Microbial Cell, 3(5), 175–184.

Verma, V., et al. (2024). Human dynein–dynactin is a fast processive motor in living cells. eLife, 13, e94963.
Vermij, S. H., Abriel, H., & van Veen, T. A. B. (2017). Refining the molecular organization of the cardiac intercalated disc. Cardiovascular Research, 113(3), 259–275.

Vidak, S., & Foisner, R. (2016). Molecular insights into the premature aging disease progeria. Histochemistry and Cell Biology, 145(4), 401–417.

Vincent, A. E., et al. (2018). Multilevel heterogeneity of mitochondrial respiratory chain deficiency in ageing human muscle. The Journal of Pathology, 246(3), 396–407.

Virchow, R. (1989). Cellular pathology as based upon physiological and pathological histology (F. Chance, Trans.). Classics of Medicine Library. (Original work published 1858).

Voigt, T., et al. (2005). Osmium postfixation in transmission electron microscopy. Microscopy and Microanalysis, 11(Suppl. 2), 530–531.

Volpi, A., et al. (2004). Prognostic relevance of histological grade and its components in breast carcinoma. British Journal of Cancer, 90(1), 36–41.

Vorsanova, S. G., et al. (2010). Human interphase chromosomes: A review of available molecular cytogenetic data. Molecular Cytogenetics, 3, 1.

Vrdoljak-Mozetić, D., et al. (2024). Pitfalls in gynecological cytology: Review of common sources of error. Diagnostic Cytopathology.

Walko, G., Castañón, M. J., & Wiche, G. (2015). Molecular architecture and function of the hemidesmosome. Cell and Tissue Research, 360(3), 529–544.

Wanders, R. J. A., et al. (2023). The physiological functions of human peroxisomes. Physiological Reviews, 103(3), 1895–1979.

Wang, H., & Joffré, E. (2025). Plasmid copy number as a modulator in bacterial pathogenesis and antibiotic resistance. npj Antimicrobials and Resistance, 3, 72.

Wang, H., et al. (2020). To study the effect of oxygen carrying capacity on expressed changes of erythrocyte membrane protein in different storage times. Pakistan Journal of Medical s, 36(4), 123–131.

Wang, L., Zeng, L., Zhang, Y., et al. (2022). Endoplasmic reticulum stress and the unfolded protein response in neurodegenerative diseases. Frontiers in Cellular Neuroscience, 16, 864426.

Wang, X., Han, Q., & Chen, Y. (2016). Exploring tight junction alteration using double fluorescent labeling in chronic periodontitis. Scientific Reports, 6, 32218.

Wang, Z., Li, J., & Chu, K. (2021). Regulation of cell cycle progression by growth factor signalling. Cells, 10(12), 3327.

Waschke, J. (2008). The desmosome and pemphigus. Histochemistry and Cell Biology, 130(1), 21–54.

Washington University Laboratory. (2024). Succinate dehydrogenase (SDH) histochemistry protocol. .wustl.edu.

Whittaker, R. H. (1969). New concepts of kingdoms of organisms. Science, 163(3863), 150–160. https://doi.org/10.1126/science.163.3863.150

Wijaya, C. S., et al. (2024). Reevaluating Golgi fragmentation and its implications in disease. Cell Regeneration, 13, 22.

Wilke, S., Krausze, J., & Büssow, K. (2012). Crystal structure of the conserved domain of the DC lysosomal associated membrane protein. BMC Biology, 10, 62.

Wilson, D. N. (2012). The structural basis for ribosome activity in peptide bond formation, translocation, termination and rescue. Cold Spring Harbor Perspectives in Biology, 4(5), a012278.

Winey, M., Meehl, J. B., O'Toole, E. T., & Giddings, T. H. (2014). Conventional transmission electron microscopy. , 25(3), 319–323.

Woese, C. R., Kandler, O., & Wheelis, M. L. (1990). Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. Proceedings of the National Academy of Sciences of the United States of America, 87(12), 4576–4579. https://doi.org/10.1073/pnas.87.12.4576

Wong, S., Guo, W.-H., & Wang, Y.-L. (2014). Fibroblasts probe substrate rigidity with filopodia extensions before occupying an area. Proceedings of the National Academy of Sciences, 111(48), 17176–17181.

Woodford, P. (2012). Cytology. Australian Prescriber, 35(1), 12–15.

Worman, H. J., & Bonne, G. (2007). Nuclear lamins and laminopathies. Journal of Clinical Investigation, 117(6), 1825–1832.

Worman, H. J., & Bonne, G. (2007). "Laminopathies": A wide spectrum of human diseases. Experimental Cell Research, 313(10), 2121–2133.

Wright, E. M., Loo, D. D. F., & Hirayama, B. A. (2011). Biology of human sodium glucose transporters. Physiological Reviews, 91(2), 733–794

Wu, Y., Kanchanawong, P., & Zaidel-Bar, R. (2015). Actin-delimited adhesion-independent clustering of E-cadherin forms the nanoscale building blocks of adherens junctions. Developmental Cell, 32(2), 139–154.

Wulf, E., Deboben, A., Bautz, F. A., Faulstich, H., & Wieland, T. (1979). Fluorescent phallotoxin, a tool for the visualization of cellular actin. Proceedings of the National Academy of Sciences, 76(9), 4498–4502.

Xiang, Q., et al. (2025). ITCH regulates Golgi integrity and proteotoxicity in neurodegeneration. Science Advances, 11(4), eado4330.

Yamaguchi, M., et al. (2020). Good ultrastructural preservation of human tissues and cells by glutaraldehyde fixation followed by rapid freezing. Cytologia, 85(1), 11–18.

Yip, R., et al. (2010). Yip1A structures the mammalian endoplasmic reticulum. Molecular Biology of the Cell, 21(13), 2262–2275.

Yu, S., Zhang, Y., & Li, Y. (2023). Zonula occludens proteins signaling in inflammation and cancer. International Journal of Biological s, 19(12), 3804–3818.

Yuan, S., et al. (2024). Translation machinery: The basis of translational control. Science China Life Sciences, 67(4), 647–667.

Yusupova, G., & Yusupov, M. (2017). High-resolution structure of the eukaryotic ribosome and its complexes with inhibitors. Philosophical Transactions of the Royal Society B, 372(1716), 20160184.

Zhang, R., Alushin, G. M., Brown, A., & Nogales, E. (2015). Mechanistic origin of microtubule dynamic instability and its modulation by EB proteins. Cell, 162(4), 849–859.

Zhou, X., et al. (2017). Density imaging of heterochromatin in live cells using soft X-ray microscopy. , 28(15), 2146–2154.

Zhou, Z., et al. (2021). Chromatin hierarchical branching visualized at the nanoscale. Nanoscale Advances, 3(5), 1178–1188.

Zidovska, A. (2020). The rich inner life of the cell nucleus: Dynamic organization, active flows, and emergent rheology. Biophysical Reviews, 12(2), 109–118.