الجمهورية الجزائرية الديمقراطية الشعبية

People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

University of 20 August 1955

Skikda

Faculty of Science

Department of Natural and Life Sciences

وزارة التعليم العالي والبحث العلمي

جامعة 20 أوت 1955

سكيكدة

كلية العلوم

قسم علوم الطبيعة والحياة

Cell Biology
(Master 1 – Applied Biochemistry)

Prepared by: Dr. Bendjazia Radia

Head of Department:

President of the Faculty Scientific Council:

Academic Year 2024–2025

Preface

This course manual has been developed to facilitate the assimilation and understanding of key concepts in **cell biology**, tailored specifically for **first-year Master's students in Applied Biochemistry**. It offers a comprehensive overview of the fundamental structures and dynamic processes that govern the life of eukaryotic cells, providing students with a solid foundation for both theoretical and practical applications in modern biomedical and biotechnological research.

The content has been carefully organized to reflect the logical progression of cellular complexity. It begins with a detailed examination of the **structural and functional organization of the cell**, establishing the basis for understanding subcellular compartments and molecular interactions. This is followed by an exploration of the **extracellular matrix**, which plays a critical role in tissue architecture and intercellular communication. The course then addresses **cell differentiation**, highlighting how specialized cell types arise and are maintained.

Further sections delve into **intracellular trafficking**, which ensures the precise distribution of molecules within the cell, and the **regulation and deregulation of the cell cycle**, a key area in understanding cancer biology. We also examine the **mechanisms of communication and signal transduction**, which coordinate cellular responses to external stimuli. The process of **apoptosis**, or programmed cell death, is then discussed as a vital mechanism for maintaining cellular homeostasis. Finally, students are introduced to **cell imaging techniques**, which are essential tools for visualizing cellular structures and functions with high resolution.

This course is not only foundational for advanced modules in the Master's curriculum, but it also serves as a practical resource for students engaging in **research projects**, including **theses and doctoral work**. A deep understanding of cellular mechanisms and experimental tools will enable students to confidently design and conduct laboratory studies relevant to their field.

The pedagogical approach adopted in this manual emphasizes **clarity and accessibility**, using straightforward language supported by **practical examples**. To make the content more engaging and easier to understand, **numerous illustrations**—including simplified diagrams, photographs, and summary tables—are provided throughout the text.

As with any academic endeavor, this document may contain **errors or omissions**. Feedback, corrections, and suggestions from fellow educators and researchers are therefore **welcomed and encouraged**, in the spirit of continuous improvement and academic collaboration.

Table of Contents

Chapter 01: Structural and functional organization of cells

I. Introduction to Cells

1. Cellular theory

1.1 The axioms of cell theory

- It was in 1838 and from their observations of the living material that Matthias Jakob Schleiden and Theodor Schwann will for the first time state the term of living cells. These observations will lead them to issue this first axiom of the cellular theory: «all organisms are made up of small units: cells».
- In 1855, Virchow, a German physician, suggests that any cell comes from another cell. This is the second axiom of cell theory which states the principle of cell division. The other axioms of cell theory are:
- The cell is a living unit and the basic unit of the living, that is to say that a cell is an autonomous entity capable of carrying out a certain number of functions necessary and sufficient for its life.
- There is cellular individuality thanks to the plasma membrane that regulates the exchanges between the cell and its environment.
- The cell contains information in the form of DNA that is necessary for its functioning and reproduction. DNA may be in free form (prokaryotes) or stored in a particular structure: chromosomes, gathered in the nucleus (eukaryotes).
- These five points can be summarized as follows: the cell represents the structural and functional unit common to the organization of all living beings. As François Jacob says, “with the cell, biology has found its atom.”

1.2. Definition of cell biology

Cell biology was born from cell theory: a branch of biology that studies cells and their organelles, the vital processes that take place within them, and the mechanisms that enable their survival (reproduction, metabolism, homeostasis, negentropy, communication), not forgetting the main characteristic of the living cell: death, which can be genetically programmed (apoptosis) or the result of an attack (necrosis).

The cell is also the smallest portion of living matter that can live in isolation and reproduce. It synthesizes all of its constituents’ using elements from the extracellular environment.

1.3. The Eukaryotic Cell

1.3.1. General Information

Eukaryotic cells make up fungi, animals, and plants. Their metabolism is aerobic. They are either unicellular (e.g., yeasts) or multicellular (e.g., mammals). All eukaryotic cells have two compartments: the nucleus and the cytoplasm.

They are separated from the extracellular environment by the plasma membrane. The nucleus characterizes the eukaryotic kingdom. It is absent in prokaryotic cells.

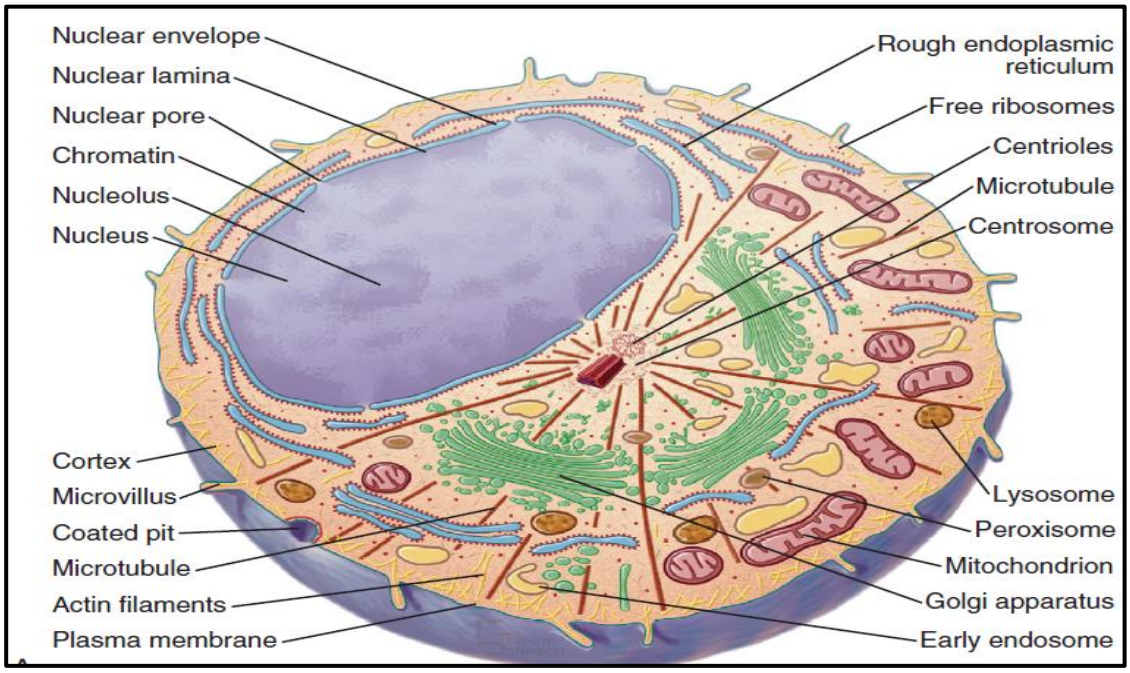


Figure 01: Basic cellular architecture of an animal eukaryotic cell with its main components.

1.3.2. Compartmentalization of the Eukaryotic Cell

a) The Eukaryotic Nucleus

The eukaryotic nucleus is a compartment delimited by the nuclear envelope, pierced by nuclear pores that allow exchanges between the nucleus and the cytoplasm (nucleocytoplasmic exchanges).

The nucleus contains DNA (Deoxyribonucleic Acid), fragmented into chromosomes, in which genetic information is stored.

b) The Cytoplasm

The cytoplasm contains several types of morphologically individualized but metabolically interactive constituents:

- The Cytosol (= hyaloplasm)

The cytosol is a kind of gel in which cellular organelles, the cytoskeleton, free ribosomes, and several types of inclusions not bounded by a membrane (lipids, glycogen) are bathed. Many biochemical reactions take place in the cytosol (e.g., glycolysis).

Note: Cytoplasm = cytosol + organelles, cytoskeleton, ribosomes, and inclusions.

- The Endomembrane System

The endomembrane system is a set of intercommunicating membrane compartments because the constituents of their membrane and the soluble molecules in their lumen (= cavity) can pass from one compartment to another through membrane flow phenomena.

These membrane compartments communicate with each other via vesicles. We thus distinguish the endoplasmic reticulum, the Golgi apparatus, endosomes, lysosomes, and vesicles.

- Mitochondria and Peroxisomes

Mitochondria ensure cellular respiration (O_2 consumption, CO_2 and H_2O production) and the synthesis of energy-rich molecules (adenosine triphosphate or ATP). They also play a major role in programmed cell death or apoptosis.

Peroxisomes are small organelles involved in the destruction of free radicals (detoxification).

- The cytoskeleton

The cytoskeleton is a network of protein filaments that maintains the cell's shape and the arrangement of its organelles in the cytoplasm. It is also responsible for cellular movement and intracellular traffic.

- The plasma membrane

This is a boundary between the cytoplasm and the extracellular environment. It enables and regulates exchanges between the intracellular and extracellular environments. It thus allows the cell to interact with its environment.

1.4. The Prokaryotic Cell

A prokaryotic cell is defined by the absence of a nucleus. Bacteria and cyanobacteria are distinguished. An example of a bacterium that has been extensively studied and commonly used in the laboratory is Escherichia coli (E. coli).

Bacteria are single-celled organisms, aerobic or anaerobic (or both).

All bacteria are surrounded by a plasma membrane. The plasma membrane is most often covered by a cell wall, of varying thickness, which gives the bacteria its shape and stiffens it.

In medical bacteriology, Gram-positive bacteria and Gram-negative bacteria are primarily distinguished using the Gram stain:

- Gram-negative bacteria stain pink: They have an outer lipid membrane and a thin cell wall.
- Gram-positive bacteria stain purple: They lack an outer membrane and have a thick cell wall.

Specific characteristics of bacteria:

- Bacteria do not have an endomembrane system (therefore, no nuclear envelope), no mitochondria, or peroxisomes.
- They do not have a cytoskeleton per se.
- Ribosomes are visible under a transmission electron microscope in the bacterial cytosol.
- Their genome is presented in the form of a single chromosome: the nucleoid. It is a double-stranded, circular DNA molecule.
- The bacterial chromosome is attached to invaginations of the plasma membrane: the mesosomes.

Bacteria can sometimes contain one or more double-stranded, circular, extrachromosomal DNA molecules: plasmids. Plasmids are facultative structures. They generally encode traits that provide a selective advantage and are not encoded by the bacterial chromosome (e.g., resistance to antibiotics, toxins). Bacteria divide by binary fission. These divisions can be very rapid (20 min in the case of E. coli under favorable conditions) if the bacteria are in a sufficient nutrient environment.

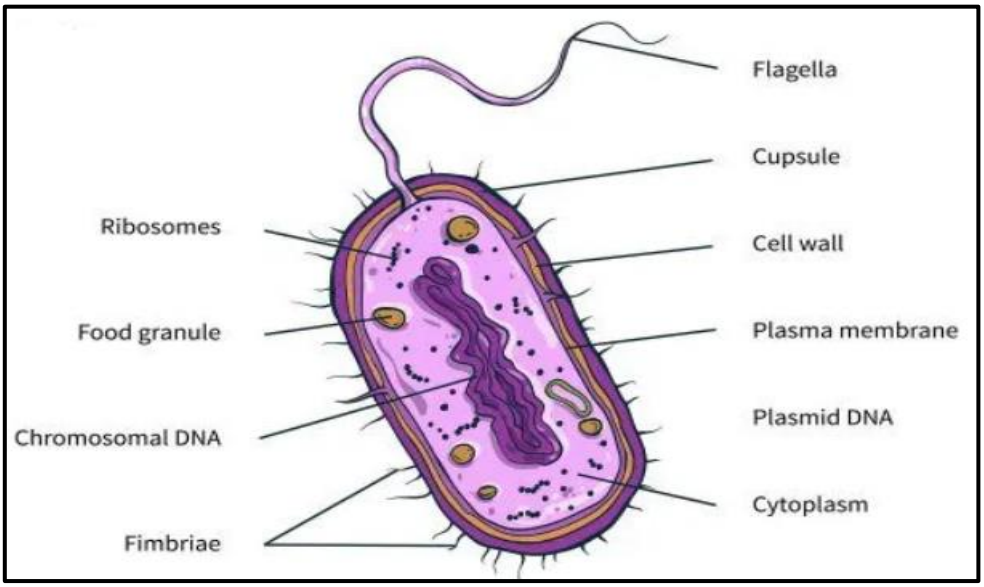


Figure 02: Bacteria Cell Diagram. Image Source: Biologyonline.com

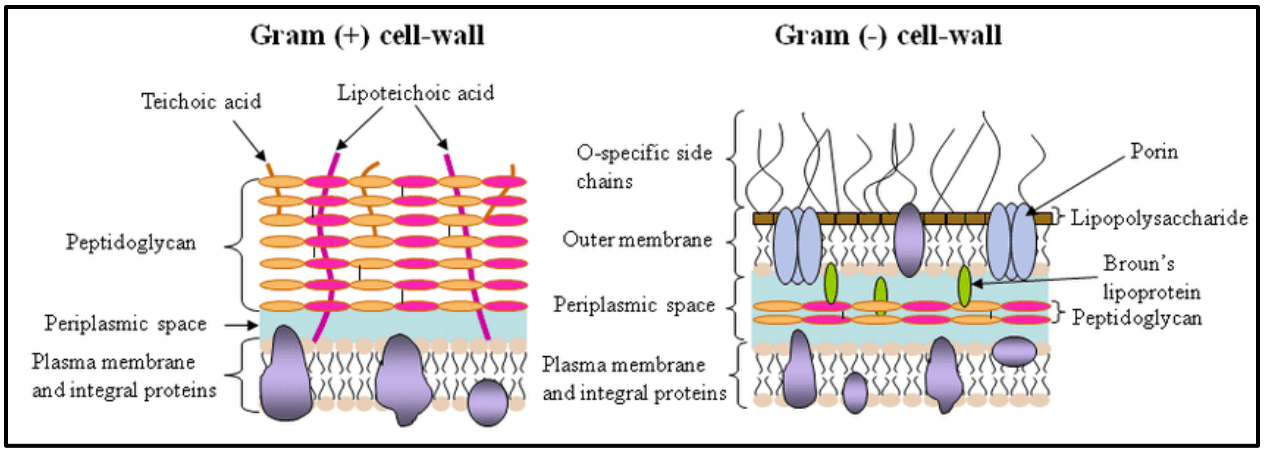


Figure 03: Schematic diagrams of Gram-positive and Gram-Negative Cell walls.

1.5. Viruses (Akaryotes)

Viruses are acellular, infectious structures consisting of at least one nucleic acid (DNA or RNA) and proteins. They depend on living cells to replicate. To do this, they are capable of profoundly and/or permanently disrupting the genetic information of the cells they infect. They are obligate intracellular parasites. We distinguish:

- ✓ Vertebrate viruses, which are very numerous, and which contain many pathogenic agents (approximately 200 species are pathogenic to humans).

- ✓ Bacterial viruses or bacteriophages.
- ✓ Viruses of algae, invertebrates, plants, etc.

1.5.1. Structure of viruses

Their size is generally between 10 and 100 nm, making them invisible under a light microscope. Therefore, electron microscopy is used. The smallest are slightly larger than ribosomes, the largest are slightly smaller than small bacteria. Viruses are essentially composed of three elements:

- a genome, or genetic material, or nucleic acid;
- a protein capsid (not always present depending on the virus);
- A lipid envelope (not always present depending on the virus...).

a) Nucleic acid

Its nature and structure are extremely variable. We distinguish:

- Viruses with double-stranded (double-stranded) or single-stranded (single-stranded) RNA.
- Viruses with double-stranded or single-stranded, linear or circular DNA.

b) Capsid

The capsid is a rigid protein shell. The capsid encloses and protects the nucleic acid. The nucleic acid + capsid combination is called a nucleocapsid. There are "naked" viruses, for which the nucleocapsid constitutes the entire virus, and enveloped viruses, for which the capsid is surrounded by a lipid envelope.

Notes: The structure of the capsid defines the shape of the virus: this is called capsid symmetry, which distinguishes two main groups: viruses with cubic symmetry (e.g., poliovirus) and viruses with helical symmetry (e.g., tobacco mosaic virus or TMV).

When the symmetry is not completely helical or icosahedral, we speak of complex viruses. They may have tails or other structures (like many bacteriophages), or have complex, multi-layered walls, like the vaccinia virus (a close relative of smallpox).

c) The lipid envelope

Viruses with an envelope are called enveloped viruses. This envelope is a lipid bilayer. It surrounds the nucleocapsid. Proteins or glycoproteins are embedded within this envelope.

Examples of viruses and their structure:

- Influenza virus: It consists of eight RNA fragments enclosed in flexural helix capsids (unlike that of TMV), all surrounded by an envelope.
- HIV is also an enveloped virus.

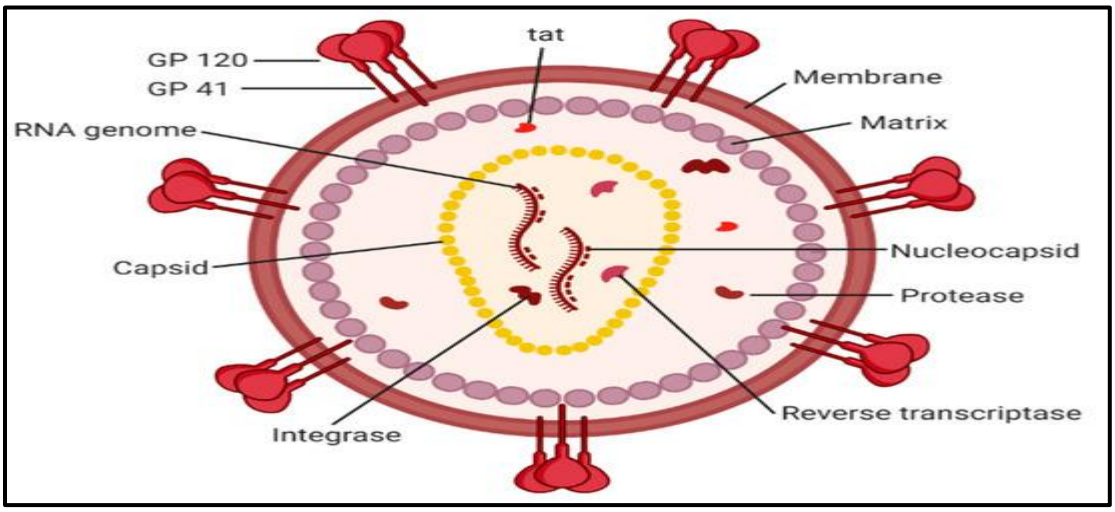


Figure 04. A schematic of the HIV-1 virion. Envelope proteins, GP41 and GP120, surround the host-derived membrane surface, which is lined internally with a layer of matrix protein. Inside the virion are viral proteins and the CA core containing the HIV-1 genome and proteins essential for infection.

1.5.2. Virus Classification

Aside from classifying viruses based on the nature of the host (animal viruses, plant viruses, bacteriophages, etc.), viruses are primarily classified according to the following criteria:

- Nature of the nucleic acid: DNA and RNA viruses;
- Type of symmetry: cubic, helical, or combined;
- Existence of an envelope: naked or enveloped viruses.

These three criteria define the family. There is also an unofficial classification used by clinicians that takes into account the host, the mode of transmission, the virus's route of entry, and its pathological effects (e.g., enteric, respiratory, and oncogenic viruses).

II. Structure and Composition of the Plasma Membrane of Eukaryotic Cells

The plasma membrane is a cell membrane. It separates the extracellular environment from the intracellular environment (= cytosol). It is thus distinguished from the cell membranes of organelles, which have a compartmentalizing role (separation of the internal compartment, or "lumen," from the cytosol).

1. Definition of the Plasma Membrane

A fluid and dynamic structure separating the intracellular environment from the extracellular environment. Its composition is linked to numerous cellular processes and contributes to the cell's identity. Average thickness of 7.5 nm, but can vary at the level of specific internal structures such as lipid rafts.

2. Characteristics of the Plasma Membrane

- Composed of a bilayer of phospholipids: ensures the stability of the membrane relative to the two fluid environments that border it (intracellular and extracellular environments).
- Contains a sterol: cholesterol, which plays a structural role. o Proteins and/or glycoproteins are inserted into the bilayer and are involved in numerous processes (transport, receptors, enzymes, adhesion, etc.).
- Asymmetric organization between the two layers is linked to the phospholipid composition, the nature of the inserted proteins, the presence or absence of carbohydrates, links to the cytoskeleton, to the extracellular matrix, etc.
- Heterogeneous chemical composition that varies from one cell type to another or between two different regions within the cell.

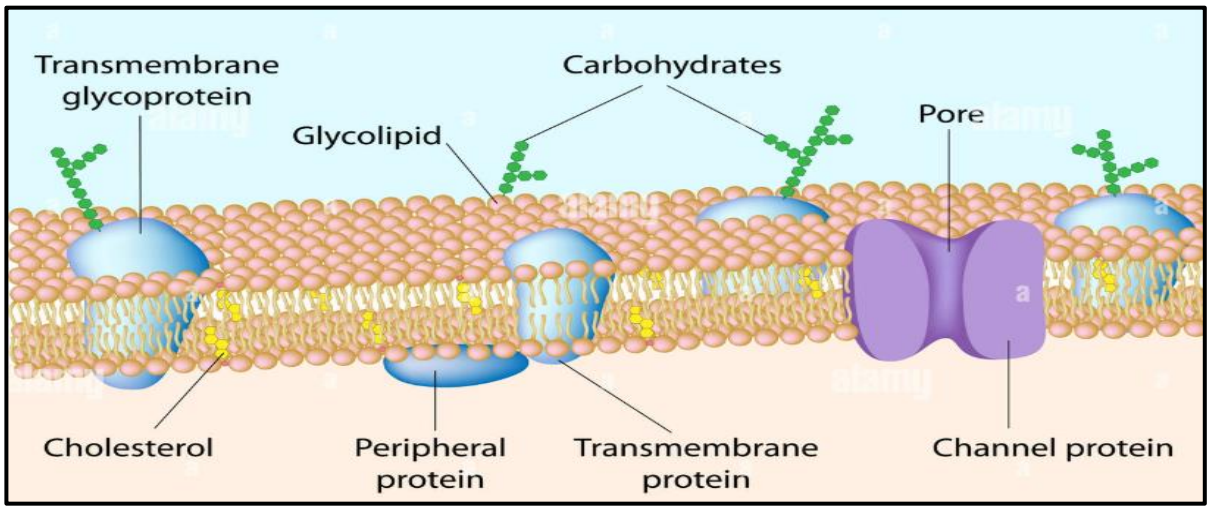


Figure 05: Structure of the plasma membrane.

3. Distribution of plasma membrane components

Thus, the plasma membrane has three types of components:

- **Membrane lipids:**
  - Glycerophospholipids (the most abundant)
  - Sphingolipids
  - Cholesterol (= steroid)
- **Membrane proteins:**
  - Intrinsic proteins
  - Anchored proteins
  - Peripheral proteins = extrinsic
- **The glycocalix = "cell coat":**

A set of glycans (carbohydrate polymers) covalently linked to the proteins and lipids of the plasma membrane (glycoproteins and glycolipids, respectively). These glycans are present only on the outer leaflet of the membrane.

Note: Since lipids are smaller molecules (MW < 1,000) than proteins (MW of a protein with four transmembrane domains: 20 to 50,000), the plasma membrane contains approximately 50 lipids for every protein.

3.1. Membrane Lipids

They represent approximately 50% of the membrane mass (= dry weight of the membrane). There are 3 types:

- Glycerophospholipids (the most abundant).
- Sphingolipids (including sphingomyelin).
- A steroid: cholesterol.

a) Glycerophospholipids: The simplest is phosphatidic acid. The others are derivatives of phosphatidic acid. We distinguish:

Phosphatidic acid: 2 fatty acids + 1 glycerol + 1 phosphate;

Other glycerophospholipids: 2 fatty acids + 1 glycerol + 1 phosphate + 1 alcohol (serine, choline, ethanolamine). Depending on the alcohol linked by an ester bond to the phosphate, we obtain: Phosphatidylserine (PS); Phosphatidylethanolamine (PE) and Phosphatidylcholine.

Glycerophospholipids ensure the membrane fluidity necessary for many cellular functions related to the plasma membrane: communication, transport, movement, and adhesion.

b) Sphingolipids: Difference from glycerophospholipids: Glycerol is replaced by sphingosine. A distinction is made between:

- Ceramide: sphingosine + fatty acid
- Sphingophospholipids: sphingosine + fatty acid + phosphate + alcohol. The best known: sphingomyelin (where alcohol = choline)
- Sphingoglycolipids including:
  - Ganglioside : ceramide + monosaccharides + sialic acid.
  - Cerebroside: ceramide + monosaccharides.
  - Sulfatide: ceramide + sulfated monosaccharides.
  - Sphingolipids primarily play a role in signal transmission and intercellular recognition.

c) Cholesterol (approximately 25% of membrane lipids):

Cholesterol is not present in the plasma membrane of prokaryotes, but is present in that of eukaryotes. In eukaryotes, cholesterol is not present in organelle membranes. It can therefore be used as a specific marker for the plasma membrane. It is distributed equally between the two layers

of the bilayer. Cholesterol is amphiphilic: hydrophilic due to its –OH group, hydrophobic due to its four carbon rings and its aliphatic side chain.

Cholesterol regulates membrane fluidity: it stiffens the membrane at high temperatures and makes it more fluid at low temperatures.

Notes:

- Most phospholipids are synthesized on the cytosolic surface of the endoplasmic reticulum membrane.
- Sphingolipids are synthesized on the luminal surface of the Golgi cisternae.

3.1.1. Properties of Membrane Lipids

1) Organization in an Aqueous Environment

When membrane lipids are in the aqueous phase, they can be organized in several different ways:

a) Lipid Bilayer

The polar heads are directed outward, in contact with the aqueous environment. The nonpolar tails are directed toward the center; they form hydrophobic interactions with each other and are protected from the aqueous environment by the polar heads. This organization corresponds to that of cell membranes.

b) Micelle

These are spherical structures in which the polar heads are oriented outward and the hydrophobic tails are in the center, protected from the aqueous environment by the polar heads. They are obtained following treatment of the plasma membrane with detergents.

c) Liposome

These are artificial structures, manufactured in vitro. Liposomes are small spherical vesicles enclosed by a lipid bilayer and filled with an aqueous medium. They can be used as vectors to deliver drugs or medications to cells because they have the ability to fuse with the plasma membrane to deliver their contents.

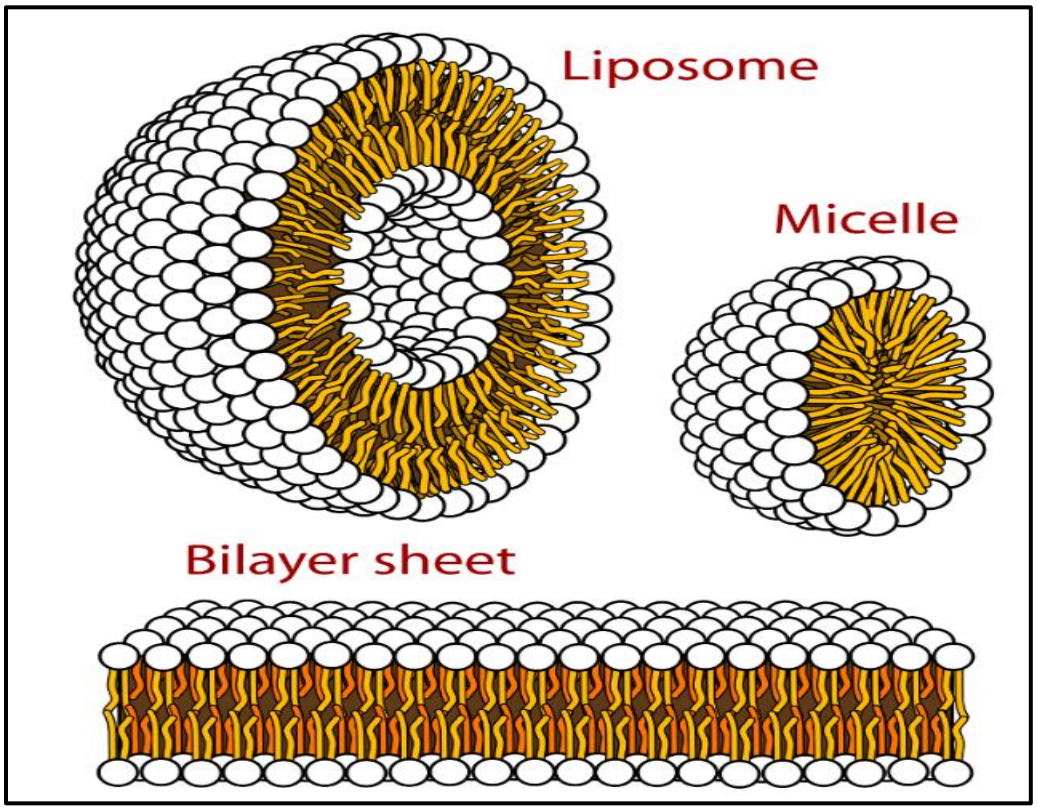


Figure 06: In an aqueous solution, phospholipids usually arrange themselves with their polar heads facing outward and their hydrophobic tails facing inward. This allows them to form lipid-bilayer spheres, single-layer spheres or lipid-bilayer sheets.

2) Lipids in the functional architecture of the plasma membrane

a) All these lipids are amphiphilic.

They have a hydrophobic and a hydrophilic part. This property allows them to organize into a bilayer encompassing intrinsic proteins. The latter interact through hydrophobic bonds thanks to their hydrophobic amino acid chains.

b) Asymmetric distribution between the two layers.

At least three reasons explain this asymmetry:

- The carbohydrate chains carried by proteins and lipids are always extracellular.
- Membrane lipids are distributed asymmetrically on the two layers.
- Membrane proteins are asymmetric.

c) Fluidity of the plasma membrane.

The plasma membrane is not a fixed structure but a very fluid one in which lipids and proteins can move. Fluidity depends on several parameters:

- The nature of the fatty acids that make up the phospholipids.
- Cholesterol quantity: Fluidity decreases as cholesterol quantity increases.
- Temperature: Fluidity increases as temperature increases.

d) Lipid Movements

Three types of movement are possible for lipids:

- Rotation
- Movement within the same layer: lateral diffusion.
- Change of layer: flip-flop or transverse diffusion. Lipid flip-flop requires the intervention of enzymes: flippases (require energy in the form of ATP).

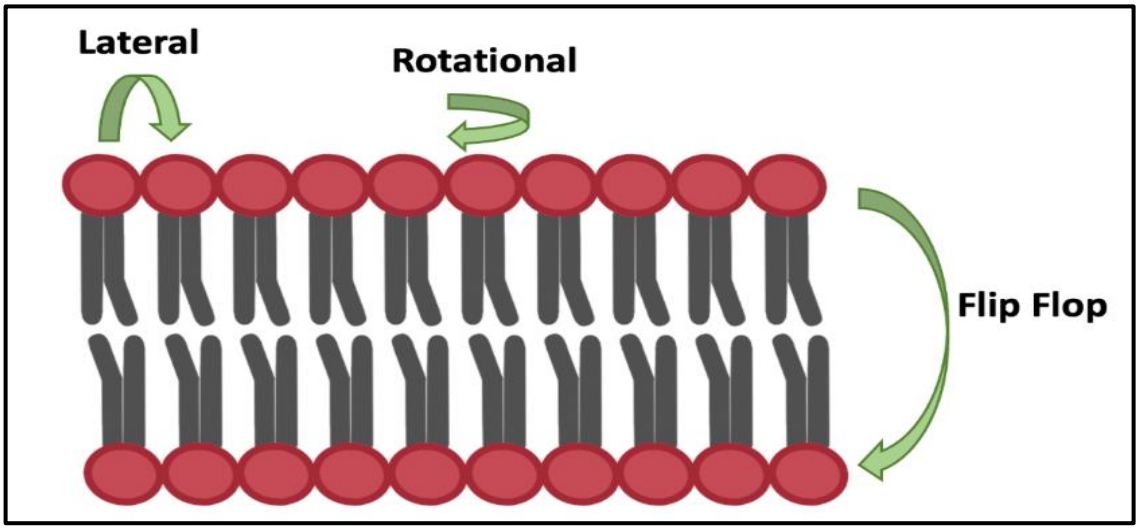


Figure 07: Examples of phospholipid movement in the cell membrane. While lateral movement is quite common, flip-flopping across the membrane is rare.

e) Lipid rafts

These are lipid microdomains also called "rafts" or DIGs (Detergent Insoluble Glycolipid-enriched microdomains). They occupy small regions of the plasma membrane and are more rigid than the

rest of the plasma membrane. They can move within the plasma membrane. These domains are enriched in cholesterol, glycolipids, sphingolipids, and glycoproteins.

- They carry caveolin aggregates on their cytosolic face.
- DIGs carry receptors on their extracellular face and serve as binding sites for extracellular proteins.
- They can cluster together to form larger domains.

3.2. Membrane Proteins

Membrane proteins perform most of the cell's specialized functions in relation to its environment. They constitute approximately 50% of the membrane's dry weight. Their classification is based on their arrangement within the membrane.

3.2.1. Intrinsic Proteins

a) Transmembrane Proteins: These proteins:

- Are very tightly bound to the membrane and therefore very difficult to extract. Cross the membrane once or several times. When they cross once, they are bitopic; when they cross several times, they are polytopic. Interactions between membrane lipids and transmembrane proteins are non-covalent and occur through the protein's hydrophobic amino acids.
- Transmembrane domains generally extend over about twenty amino acids and are often organized in an α-helix. The extracellular and cytosolic domains of these proteins are generally hydrophilic.
- The extracellular portions of the protein may be glycosylated.

Some transmembrane proteins do not cross the membrane completely and have a hairpin transmembrane domain (e.g., caveolin enters and exits on the cytosolic side). These are called monotopic proteins.

b) Anchored proteins

These proteins are associated with membrane lipids by covalent bonds. Some proteins are bound to the inner leaflet of the plasma membrane via:

– **a fatty acid**: these are acylated proteins;

– **a fatty alcohol**: these are prenylated proteins.

In both cases, the protein is covalently bound to the fatty acid or alcohol, and the latter forms hydrophobic interactions with the membrane lipids of the inner leaflet (e.g., G proteins).

There are also proteins anchored to the outer leaflet via phosphatidylinositol. These proteins are called glypiated or anchored by GPI (Glycosyl Phosphatidyl Inositol). The bond between phosphatidylinositol and protein is covalent and the fatty acids of phosphatidylinositol make hydrophobic interactions with membrane lipids of the outer leaflet of the plasma membrane.

3.2.2. Extrinsic (or peripheral) proteins

These proteins can be found on the extracellular or cytosolic side. They are never covalently bound to the lipid bilayer; they form weak interactions (hydrogen or ionic bonds) with the polar head groups of membrane lipids and the polar regions of intrinsic proteins. These proteins detach from the membrane (and thus purify) simply by changing the pH or ionic strength.

- Protein movement within the membrane:

Only two types of movement are possible for proteins: rotation; lateral diffusion (a phenomenon demonstrated by cell fusion experiments).

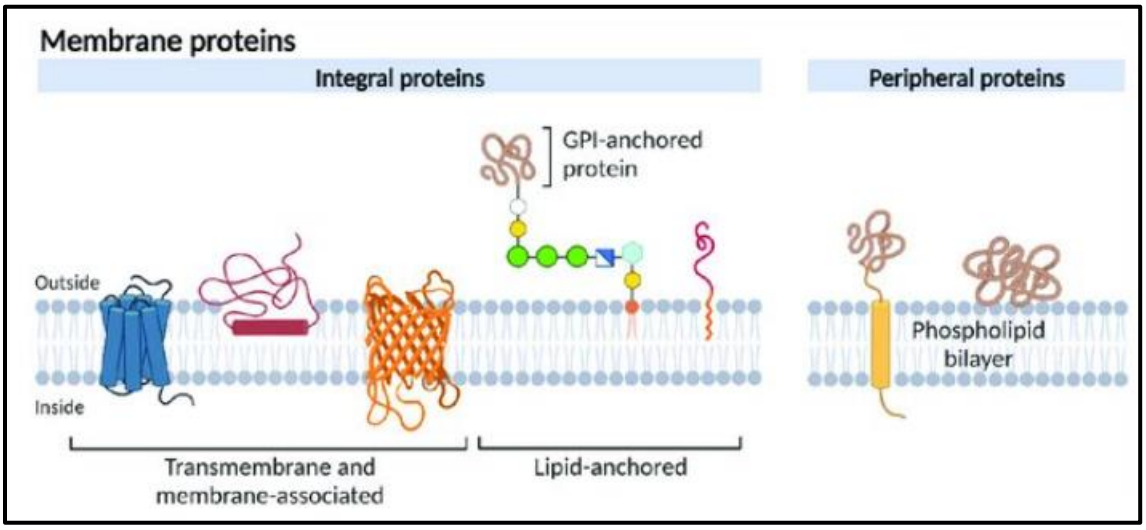


Figure 08: Schematic representation of different membrane protein types.

3.3. The Glycocalix (Cell Coat)

A set of glycans (carbohydrate polymers) covalently bound to the proteins and lipids of the plasma membrane (glycoproteins and glycolipids, respectively). These glycans are present only on the outer layer of the membrane.

The membrane proteins and lipids facing the extracellular environment are glycosylated (10% of phospholipids are glycosylated). All the carbohydrates form a coat called the glycocalix. Carbohydrates are most often heterogeneous oligosides, i.e., combinations of several known monosaccharides that are more or less modified: mannose, glucose, galactosamine, N-acetylneuraminic acid, etc. The carbohydrates that make up this coat (= glycans) represent a specific set of biological markers involved in:

- Cell recognition and identity (e.g., carbohydrate markers of blood groups on the surface of red blood cells).
- Their adhesion to their environment.
- The carbohydrate coat protects the cell surface from mechanical and chemical damage.

3.3.1. Carbohydrate Markers for Cell Identification

a) Blood Groups

The specificity of red blood cell membrane antigens, or red blood cells, depends on the nature of the constituent oligosaccharides. These oligosaccharides are carried by membrane glycolipids. Example: fucose and galactosamine are specific for group A antigens, while fucose and galactose are specific for group B antigens.

• The H antigen is the basic structure, present in type O individuals.

• The A antigen differs from H by the presence of a terminal N-acetyl-D-galactosamine.

• The B antigen differs from H by the presence of a terminal D-galactose.

b) The Major Histocompatibility Complex (MHC)

Glycoproteins of nucleated cells encoded by about twenty polyallelic genes. They are:

- **MHC I:** present on almost all cells in the body (except neurons and RBCs);

- **MHC II:** present on certain immune cells: antigen-presenting cells (macrophages, B lymphocytes, dendritic cells).

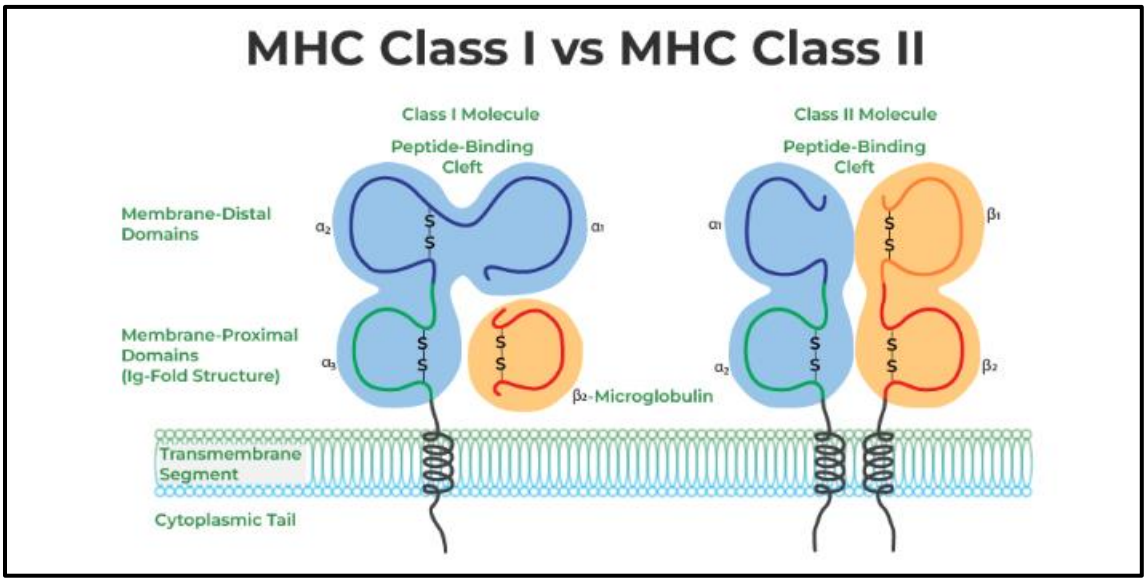


Figure 09: The major histocompatibility complex or MHC.

c) Carbohydrates and Cell Adhesion

Adhesion is linked to CAMs (cell adhesion molecules), membrane glycoproteins. Examples: selectins, integrins, cadherins. These carbohydrates play a role in:

- Cell migration. E.g., selectin and lymphocyte migration through blood vessels by diapedesis; N-cadherin and cell interaction during the development of the embryonic neural network (neural tube, for example).
- Cell/cell adhesion. E.g., E-cadherin.
- Cell/extracellular matrix adhesion. E.g., integrin.

4. Membrane Transport

4.1. General Information

To survive and/or ensure its biological function, the cell engages in exchanges with its environment: recovering the nutrients it needs, exchanging ions for activation, or eliminating molecular waste resulting from its activity. Exchanges are thus distinguished by active or passive transport.

The plasma membrane is selective: some components will be able to cross it, while others will not. The biochemical nature of the compound that must cross it is therefore essential:

4.2. Passive Transport

- Movement of solutes along their concentration gradient: from the medium with the highest solute concentration to the medium with the lowest solute concentration, until the two environments are equal in concentration.
- Does not require energy.

Simple diffusion is distinguished from facilitated diffusion.

a) Simple Diffusion

A solute directly crosses the lipid bilayer. This process therefore does not require a membrane transporter. The diffusion rate is proportional to:

- the concentration difference between the two media;
- the temperature.

And inversely proportional to the size of the element to be transported. Ex: O_2, CO_2, alcohol, fat-soluble molecules (fatty acids, steroid hormones).

b) Facilitated Diffusion

The solute passes through its interaction with a specific membrane transporter. This transport is called uniport. The transport speed is related to the number of membrane transporters. When all the transporters are saturated (occupied), the transport speed reaches a plateau and is therefore maximal. Two types of facilitated transport are distinguished:

- Channel-facilitated diffusion. E.g., Na^+ ion channel, K^+ channel, H_2O channel (aquaporin), etc.
- Carrier protein-facilitated diffusion → Requires a conformational change in the transporter. E.g., transport of glucose (GLUT) present on the basement membrane of enterocytes.

4.3. Active Transport: Primary and Secondary Active Transport

- Solute transport occurs against its concentration gradient.
- Therefore, it requires energy. Depending on the type of energy provided, primary and secondary active transport are distinguished.

Primary active transport: Energy is provided by the hydrolysis of an ATP molecule. Synonym: ATPase pump. Ex: Na^+/K^+ ATPase pump (carries 3 Na^+ out and 2 K^+ in from the cell); H^+ ATPase pump (carries H^+ into lysosomes, causing their acidification).

Secondary active transport: Energy is provided by the co-transport of a solute along its concentration gradient. A distinction is made, depending on the case:

- If solute and co-transport move in the same direction: symport.
- In the opposite direction: antiport.

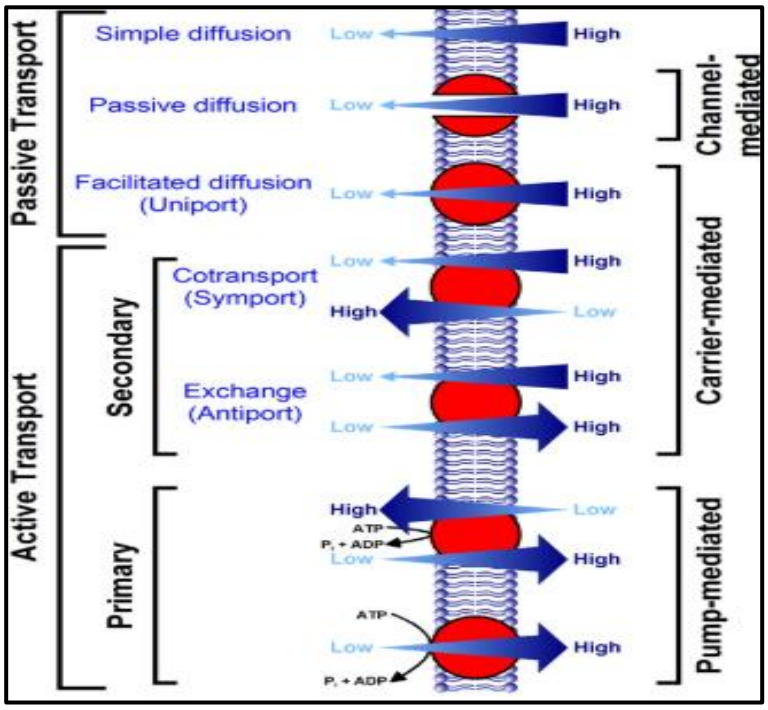


Figure 10: Summary of membrane transport.

5. Vesicular Transport

Some molecules (e.g., proteins) and particles are too large to cross membranes using membrane transporters. Their transport therefore requires movements of the plasma membrane to evacuate/ingest these molecules.

5.1. Exocytosis

This involves the secretion/elimination of molecules present in the cell. Substances are enclosed in vesicles that fuse with the membrane and release their contents (e.g., waste, mucus,

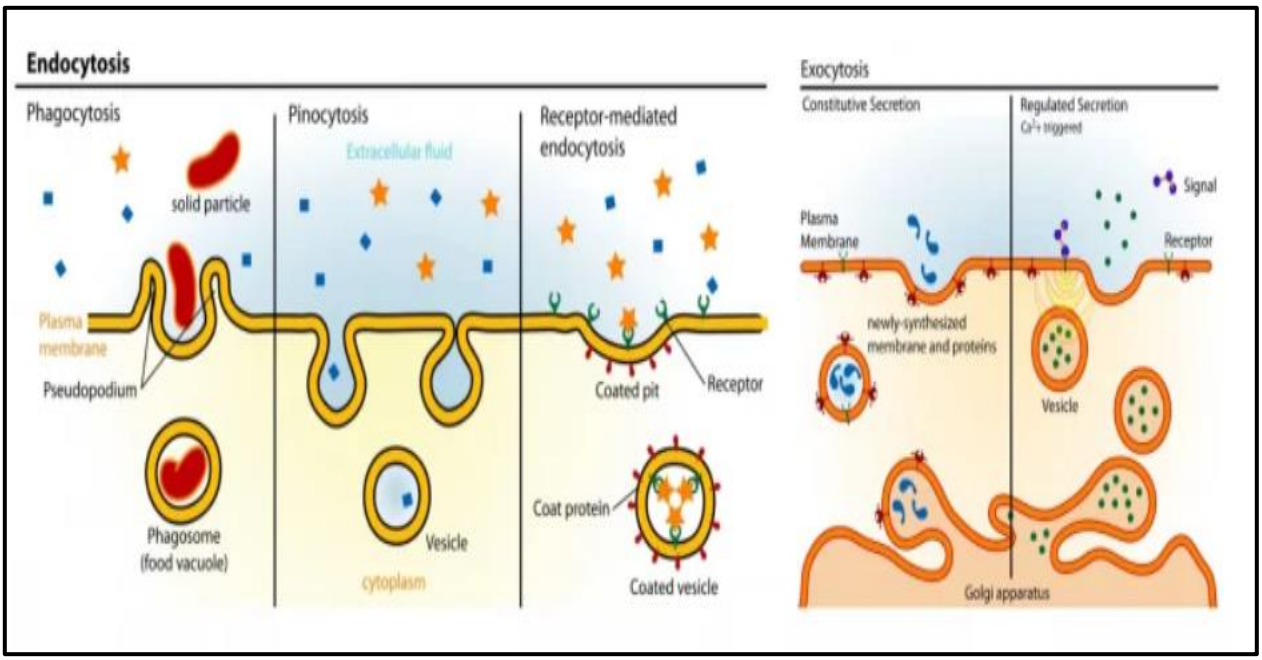
neurotransmitters, hormones) into the extracellular environment. Vesicle formation and transport are energy-consuming processes. Fusion requires vesicle/plasma membrane recognition via protein complexes (v/t SNAREs).

- **Constitutive exocytosis** is performed by all cells and releases components of the extracellular matrix (or enables the delivery of newly synthesized membrane proteins that are incorporated into the cell membrane when the vesicle fuses for transport).
- **Regulated exocytosis** occurs in cells whose primary function is secretion. Regulated secretory vesicles do not naturally bind to the plasma membrane but require an increase in calcium concentration. Ca^{2+} triggered exocytosis occurs at synapses and enables intraneuronal signaling.

5.2. Endocytosis

The process by which a cell absorbs particles or solutes by enclosing them in vesicles through invagination of the plasma membrane. Several types of endocytosis are distinguished depending on the substances ingested and their size.

a. **Receptor-mediated endocytosis:** Selective endocytosis that requires membrane receptors specific to the molecule to be ingested. The molecule/receptor complex is then endocytosed and localized in a vesicle: the early endosome. Example: Blood cholesterol is transported in the plasma associated with various molecules, including LDL (Low Density Lipoproteins). These LDLs can only release their cholesterol to the cell after binding to specific receptors on the plasma membrane. Endocytosis of the receptor/LDL pair then follows.
b. **Phagocytosis:** Endocytosis of large particles: bacteria, cellular debris. Example: phagocytosis of bacteria by macrophages (known as phagocytes).
c. **Pinocytosis:** Ingestion of suspended molecules, taken from the extracellular environment (example: lipid droplets). This is a common phenomenon in most cells (especially renal and intestinal).



Figures 11: the different types of exocytosis and endocytosis.

III. The Endomembrane System

The endomembrane system is present only in eukaryotic cells. It is a complex system made up of several cavities, vesicles, and canaliculi. The cavities are bounded by an envelope membrane and communicate with each other and with the plasma membrane, transiently, via vesicles and canaliculi.

The endomembrane system includes:

- The endoplasmic reticulum (ER);
- The nuclear envelope;
- The Golgi apparatus (GA);
- Endosomes;
- Lysosomes;
- A highly heterogeneous population of vesicles, canaliculi, and vacuoles that transit between the preceding compartments and the plasma membrane.

Membrane fluxes ensure simultaneous transport of the envelope membranes and the contents (or lumen) of the different compartments. The endomembrane system is quantitatively important in the cell. For example, in hepatocytes, it occupies 17% of the volume, and its membranes represent 58% of the total membrane surface area.

1. The Endoplasmic Reticulum (ER)

1.1. Definition and Description of the Endoplasmic Reticulum

The ER is a collection of canaliculi and vesicles that constitute a highly developed network in adult eukaryotic cells. E.g., in hepatocytes, it occupies 13% of the volume, and its membranes represent 50% of the total membrane surface area.

The composition of the ER envelope membrane is very similar to that of the plasma membrane, except that it contains little cholesterol compared to the latter. The endoplasmic reticulum is continuous with the nuclear envelope, which is part of the ER. It may or may not bear ribosomes on its cytoplasmic surface, which distinguishes the granular or rough ER (ER) from the smooth ER (SER). These two aspects correspond to different functions:

a) Functions of the Rough ER

- Synthesis and translocation of secreted, membrane-bound, and vesicle-resident proteins.
- N-glycosylation of proteins and pruning of their sugar arborization.
- Spatial conformation of proteins and quality control before their export to the Golgi apparatus.

b) Functions of the smooth ER

- Synthesis of membrane and cytosolic phospholipids.
- Synthesis of cholesterol and steroid hormones.
- Storage and release of calcium.
- Detoxification (detoxification of xenobiotics by cytochrome P450).

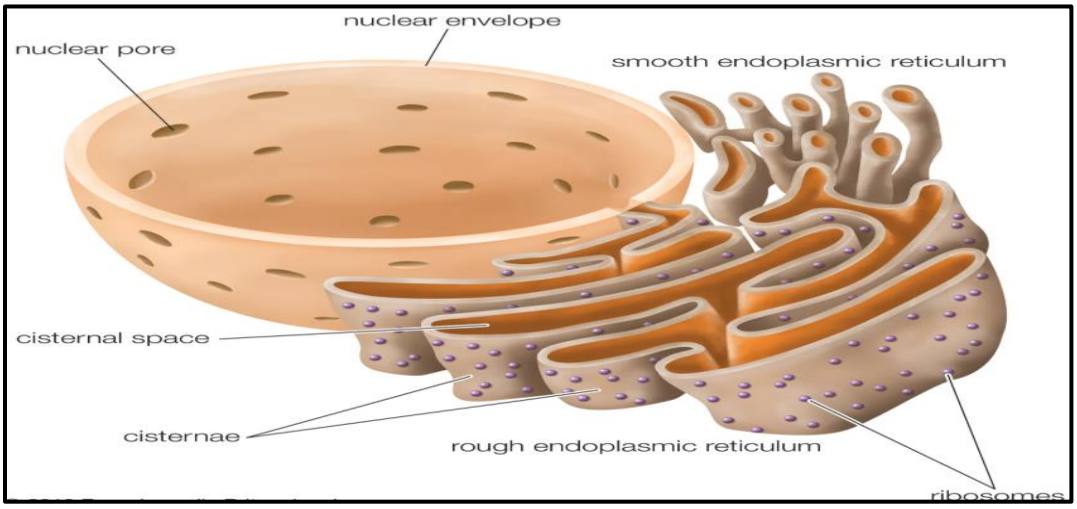


Figure 12: Rough and smooth endoplasmic reticulum.

2. The Golgi Apparatus (GA)

2.1. Definition and Description of the Golgi Apparatus

The Golgi apparatus is a polymorphic cellular organelle consisting of one or more dictyosomes (usually a single dictyosome in animal cells, and several dozen in plant cells). A dictyosome is a collection of flattened vesicles and saccules organized like a "stack of plates."

Each dictyosome is surrounded by vesicles that ensure communication between its various saccules and also between the Golgi apparatus and the rest of the endomembrane system or the plasma membrane:

The Golgi apparatus is located between the ER and the plasma membrane. It is a polarized organelle, and each dictyosome has two faces:

- the cis face, or entry face, facing the ER and the nucleus. It establishes a relationship with the ER through a set of vesicles that form the ERGIC (Endoplasmic Reticulum – Golgi Intermediate Compartment) or cis Golgi network (= CGN: Cis Golgi Network);
- the trans face or exit face, facing the plasma membrane. It is continuous with a network of canaliculi constituting the trans Golgi network (or TGN, Trans Golgi Network).

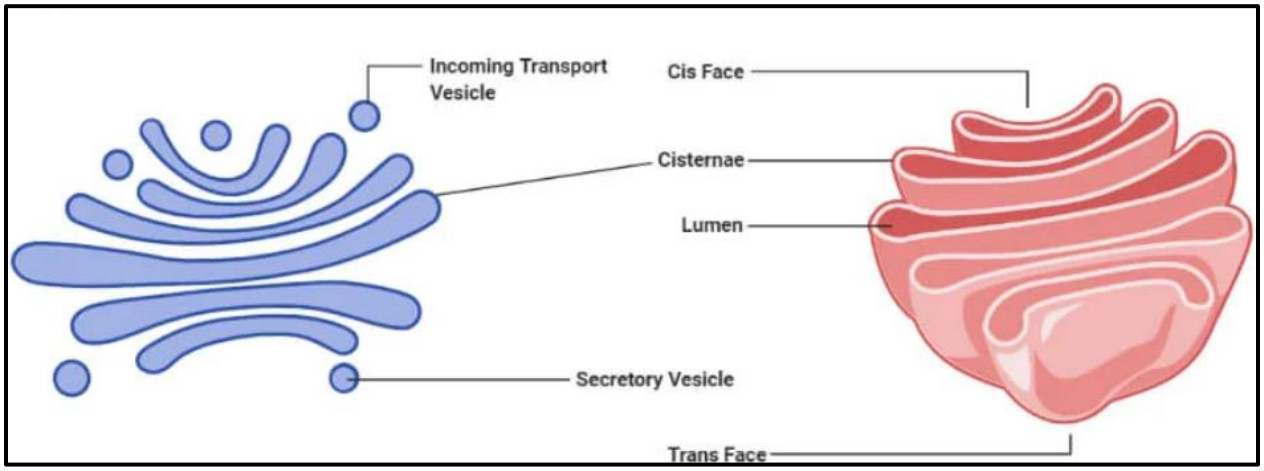


Figure 13: Organization of the Golgi apparatus and a dictyosome.

2.2. Functions of the Golgi apparatus

Very schematically, the GA receives proteins from the ER, modifies them (glycosylation, sulfation, precursor cleavage, etc.), sorts them, and then exports them to other compartments (plasma membrane, endosomes, lysosomes, etc.) or to the extracellular environment (secretion, by constitutive and regulated exocytosis):

The Golgi apparatus is the obligatory gateway for vesicle trafficking. It regulates the number of vesicles reaching the membrane and thus participates in membrane renewal. Thus, post-translational modifications carried out in the Golgi apparatus are essential for the correct targeting of proteins within the cell.

1. Protein Maturation

During their journey through the Golgi apparatus, proteins undergo numerous transformations, including several types:

- proteolytic cleavages resulting in the excision of certain polypeptide sequences,
- the addition of oligosaccharides and the possible modification of these oligosaccharides (glycosylation and pruning),
- sulfation,
- the attachment of phosphate groups.

All these transformations allow the protein to acquire its final state: this is protein maturation.

2. Sorting and Addressing

All newly synthesized proteins that pass through the Golgi saccules are sent to the correct address thanks to their markers, glycoproteins, which act as a postal code. These specific markers are recognized by membrane receptors, present on the luminal surface of Golgi membranes, which allows Golgi vesicles to act as sorters, in addition to their role in protein transport.

Thus, the Golgi apparatus is considered a sorting center from which proteins can be tracked in three main pathways in animal cells:

a) Export outside the cell

This occurs via vesicles that fuse with the plasma membrane to enable exocytosis (e.g., insulin and digestive enzymes). Oligosaccharide sequences serve as a tag for this targeting.

b) Incorporation into the plasma membrane

To enable recycling through constitutive secretion; this very important pathway allows for the renewal of the plasma membrane (transmembrane proteins).

c) The lysosomal or lytic pathway

For which the tag is a mannose 6 phosphate;

- There is therefore a transfer: REG → Transition vesicles → Golgi apparatus → Transport vesicles.

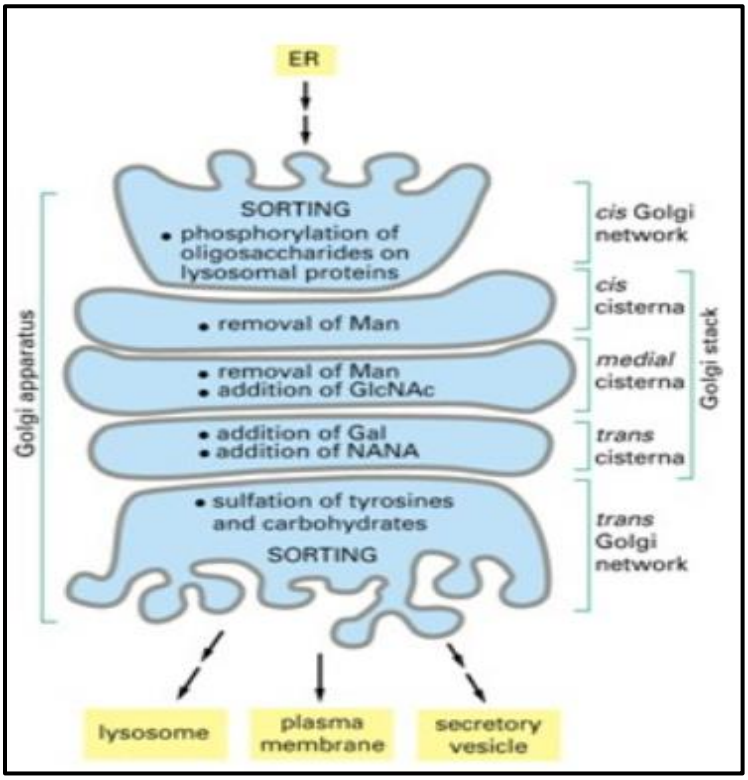


Figure 14: Examples of post-translational modifications of proteins performed by the Golgi apparatus and according to the compartment.

3. Endosomes

3.1. Definition and Origin of Endosomes

Endosomes are a morphologically very heterogeneous membrane compartment. Endosomes have several origins. They originate from:

- endocytic vesicles originating from the plasma membrane. These vesicles are smooth or coated (clathrin, caveolin) and transport molecules taken from the extracellular environment;
- transport vesicles that have budded from the trans Golgi and the TGN. They provide them with acid hydrolases and proton pumps (ATPase H^+). With this supply, the endosomes gradually transform into lysosomes.

The membrane-bound and soluble material from the endosomes is transported to the lysosomes, with which it can fuse.

3.2. Classification of Endosomes

Two classes of endosomes are distinguished based on their pH:

- Early endosomes are directly fueled by endocytosis. They have a pH close to that of the extracellular environment (7.4).
- Late endosomes have a more acidic pH (6.5), intermediate between the pH of early endosomes and that of lysosomes (5).

The maturation that transforms early endosomes into late endosomes occurs through the formation of multivesicular bodies (MVBs), which contain large amounts of invaginated membranes and internal vesicles.

MVBs gradually transform into late endosomes, either by fusing with each other or with pre-existing late endosomes. Late endosomes communicate with the trans-Golgi network via transport vesicles that deliver the proteins that will transform late endosomes into lysosomes. Thus, the endocytosed material is first found in early endosomes and then in late endosomes.

The acidification phenomenon is very important for two reasons:

1) It allows the endocytosed material (ligand) to detach from its receptor, in cases where endocytosis is specific. In this case, the receptor is often recycled to the plasma membrane via vesicles budding from early endosomes.

2) It allows the optimal functioning of the hydrolases, which begin to digest the endocytosed material.

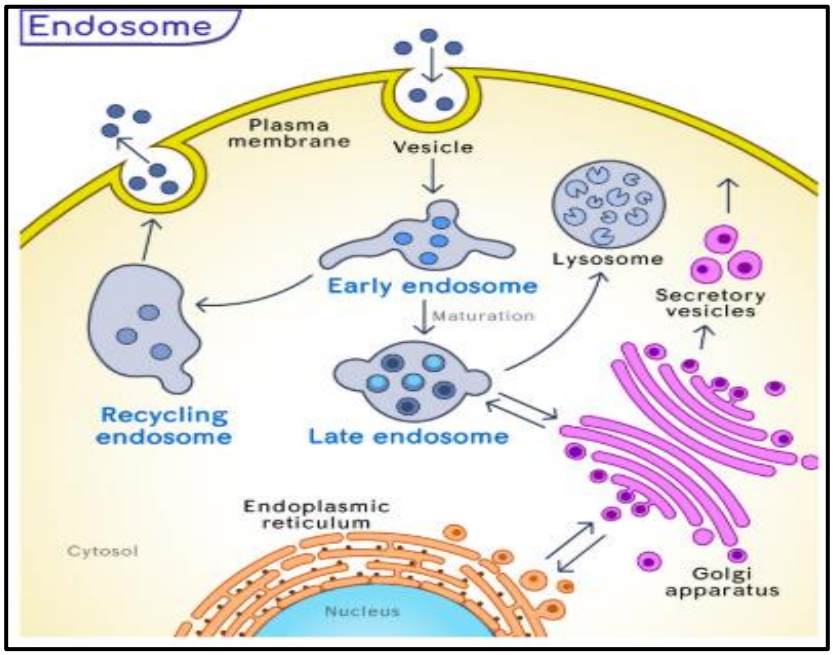


Figure 15: The endocytosis pathway from the plasma membrane to lysosomes.

4. Lysosomes

4.1. Definition of Lysosomes

A compartment with highly heterogeneous morphology, an acidic pH (pH 5), and containing numerous acid hydrolases (because they are only active at acidic pH). Acid hydrolases are enzymes capable of hydrolyzing all families of biological molecules. They are thus distinguished: nucleases (degrade DNA, RNA); proteases (degrade proteins); glycosidases (degrade carbohydrates); phosphatases (cleave phosphates); lipases (degrade lipids); sulfatases (cleave sulfate groups), etc. The diameter of lysosomes varies between 0.2 and 0.5 µm.

Lysosomes are present in all eukaryotic cells except red blood cells.

4.2. Origin of Lysosomes

Lysosomes result from the fusion of one or more transport vesicles and a vesicle (late endosome, phagosome) containing materials to be degraded.

Transport vesicles bud from the Golgi apparatus and contain, among other things, acid hydrolases and proton ATPases. They are coated with clathrin when they bud.

4.3. Characteristics of Lysosomes

a) The Lysosomal Membrane

The membrane surrounding the lysosomes has the following characteristics:

1. It keeps the system closed and prevents the escape of lysosomal enzymes into the cytosol.
2. It carries a proton ATPase that transports H+ ions from the cytosol to the lysosomal lumen, leading to its acidification.
3. It carries permeases that allow:
   - the direct entry of materials to be hydrolyzed from the cytosol into the lysosomal lumen;
   - the exit of lysosomal hydrolysis products from the lumen into the cytosol.
4. It is protected from self-digestion by an internal glycoprotein coating composed of LAMP (Lysosome Associated Membrane Protein).

LAMPs are specific markers of the lysosomal membrane.

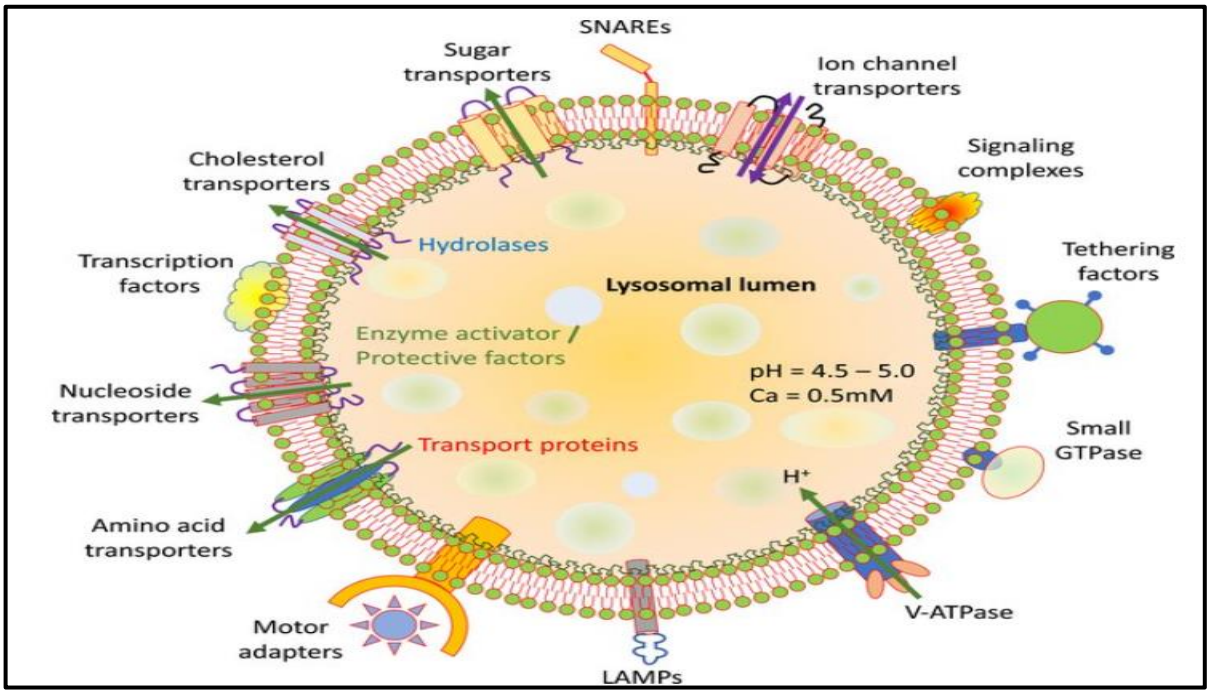


Figure 16: Schematic representation of a lysosome and its enzymatic content.

4.2. Functions of Lysosomes

The function of lysosomes is to perform intracellular digestion. Molecules to be digested in lysosomes arrive there via four pathways:

1) **Endocytosis:** Endocytic vesicles deliver molecules taken from the extracellular medium to the endosomal and then the lysosomal compartment.
2) **Phagocytosis:** The phagosome (= phagocytic vesicle) fuses with vesicles from the Golgi apparatus (= vesicles transporting acid hydrolases and proton pumps) and gradually transforms into a lysosome: formation of the phagolysosome. This mechanism is found in so-called phagocytic cells such as macrophages and neutrophilic granulocytes.
3) **Direct entry from the cytosol**: This phenomenon involves peptides and uses permeases. 4) Autophagy: This is a mechanism that allows cells to degrade their own organelles and molecules in order to ensure their renewal. The autophagic vacuole is formed from a specialized cisternae that is continuous with the trans-Golgi network.

IV: Mitochondria and Peroxisomes

1. Mitochondria

Mitochondria are organelles present in all eukaryotes (fungi, animals, and plants), but not in prokaryotes. They are closed organelles that are not part of the endomembrane system. All of a cell's mitochondria form the chondriome. The chondriome is dynamic because mitochondria can:

- move (associated with cytoskeletal elements, particularly microtubules),
- deform,
- divide by fission,
- fuse with each other.

The cell can therefore regulate the number of its mitochondria according to its metabolic activity. A hepatocyte contains between 1,000 and 2,000 mitochondria (which occupy 20% of the cell volume). In the cytoplasm, they are associated with energy reserves (glycogens and triglycerides) and located near the places of ATP consumption (e.g.: in spermatozoa, they are firmly wrapped around the flagellum).

1.1. Functions of Mitochondria

1) They produce most of the energy needed by the cell by synthesizing ATP (Adenosine Triphosphate). This aerobic process is called oxidative phosphorylation. They are the only organelles involved in cellular respiration.
2) They participate in the initiation and regulation of programmed cell death: apoptosis.
3) They participate in certain metabolic pathways by cooperating with other cellular compartments (cytosol, REL, AG, nuclear envelope, peroxisomes):
   - Synthesis of cholesterol, steroid hormones, certain membrane phospholipids, and the phospholipid portion of lipoproteins;
   - Control of cytosolic Ca^{2+} concentration;
   - Production and catabolism of superoxide ions (O_2), which are toxic to cells.

1.2. Structure of Mitochondria

1.2.1. Morphology

Mitochondria are generally ellipsoidal (diameter between 0.5 and 1 µm), but can also be spherical or filamentous. They are bounded by two concentric membranes: the inner membrane and the outer membrane, separated by the intermembrane space. These two membranes delimit an internal cavity: the matrix.

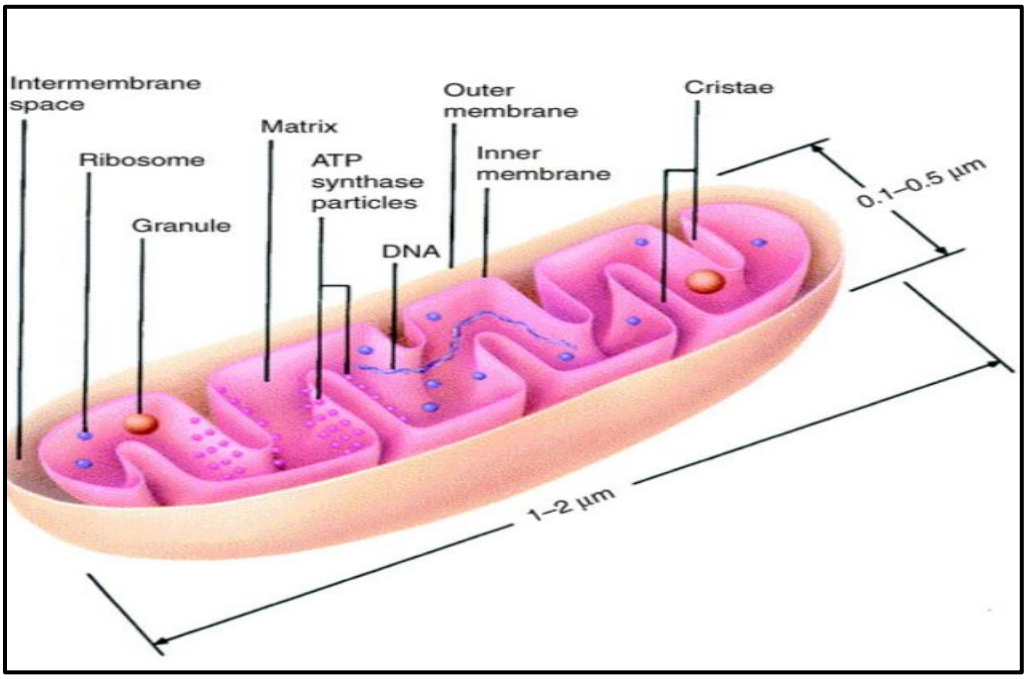


Figure 17: Ultrastructure of a mitochondria.

1.2.2. The outer membrane

It is permeable and its composition is similar to that of the plasma membrane (50% lipids, 50% proteins). It is rich in porins, permeases that form channels responsible for passive permeability to ions and small proteins (< 5 kDa).

1.2.3. The intermembrane space

Its composition is very similar to that of the cytosol. This space contains:

- molecules originating from the cytosol and crossing the outer membrane via porins;
- protons (H^+) originating from the matrix and crossing the inner membrane via certain respiratory chain complexes;
- proteins involved in apoptosis (procaspases and cytochrome c).

1.2.4. The inner membrane

It is impermeable and very rich in proteins (80% proteins, 20% lipids). It forms folds: the mitochondrial cristae. These mitochondrial cristae:

- increase the surface area of the inner membrane (factor 3 between the surface area of the inner membrane and that of the outer membrane);
- have variable morphologies depending on activity and cell type;
- are variable in number depending on ATP demand.

The inner membrane contains a class of special "double" phospholipids: cardiolipins. Cardiolipins represent 20% of the lipids in the inner membrane and are responsible for its impermeability to ions (particularly protons). Among the proteins of the inner membrane are:

- permeases, which are used for exchanges between the intermembrane space and the matrix,
- symporters (e.g., pyruvate/H^+),
- antiporters (e.g., ATP/ADP);
- respiratory chain complexes, which are electron and proton transporters;
- ATP synthases, responsible for ATP synthesis.

1.2.5. The Matrix

The matrix is very rich in enzymes because many metabolic processes take place there:

- Fatty acid beta-oxidation (= Lynen helix). This is a chain of reactions that degrade fatty acids and results in the formation of acetyl CoA and reduced coenzymes: $FADH_2$ and $NADH_2$ (= NADH, H^+).
- Pyruvate decarboxylation. This results in the formation of CO_2, acetyl CoA, and $NADH_2$. Pyruvate is produced by glycolysis, which takes place in the cytosol.
- The Krebs cycle (= citric acid cycle). This is a chain of reactions that degrades acety lCoA and results in the formation of CO_2, GTP, $FADH_2$, and $NADH_2$.

The matrix also contains nucleic acids (DNA and RNA). Mitochondrial DNA is double-stranded, circular, and small (in humans, it is approximately 16,500 base pairs). It is not associated with histones. Generally, there are several copies per mitochondria (5 to 10).

Mitochondrial DNA encodes a limited number of genes, and its transcription allows the synthesis of 13 mRNAs (messenger RNAs), 22 tRNAs (transfer RNAs), and two rRNAs (ribosomal RNAs) of 12S and 16S.

Mitochondria are therefore the site of DNA replication and transcription, as well as translation, because the matrix contains ribosomes. However, the genetic code is different from that governing the translation of RNA encoded by the nuclear genome.

Note: The protein synthesis machinery of mitochondria resembles that of bacteria. As proof, their ribosomes are sensitive to antibacterial antibiotics, and protein synthesis begins with N-formyl methionine.

1.3. Cellular Respiration

The main function of mitochondria is the production of ATP, obtained by oxidative phosphorylation through the functioning of the inner membrane's respiratory chain.

Reminders:

- Oxidation = loss of electrons (or $H = H^+ + e^-$).
- Reduction = gain of electrons (or $H = H^+ + e^-$).
- A reducing agent is a compound that provides electrons (and H^+ ions).
- An oxidizing agent is a compound that captures electrons (and H^+ ions).

1.3.1. The Respiratory Chain

Mitochondria can use pyruvate and fatty acids as fuels: pyruvate generally results from the cytoplasmic breakdown of glucose (glycolysis), while fatty acids generally come from the breakdown of glycerides.

These two fuel molecules are converted by matrix enzymes into a primary intermediate metabolite: acetyl CoA (by decarboxylation of pyruvate on the one hand and oxidation of fatty acids on the other).

The acetyl group of acetyl CoA is then oxidized by the Krebs cycle, which produces CO_2 (waste) and energy-rich electrons, which are transported by transport molecules: $FADH_2$ and $NADH_2$, which are coenzymes.

These reduced coenzymes ($FADH_2$ and $NADH_2$) will transfer their electrons to the respiratory chain complexes located in the inner membrane.

The respiratory chain consists of four sequentially ordered membrane electron transporter complexes (complexes I, II, III, and IV) linked by two mobile electron transporters (ubiquinone and cytochrome c):

- Ubiquinone (also called coenzyme Q) is a small lipophilic molecule located in the inner membrane.
- Cytochrome c is a small hemoprotein located in the intermembrane space (peripheral protein of the inner membrane).

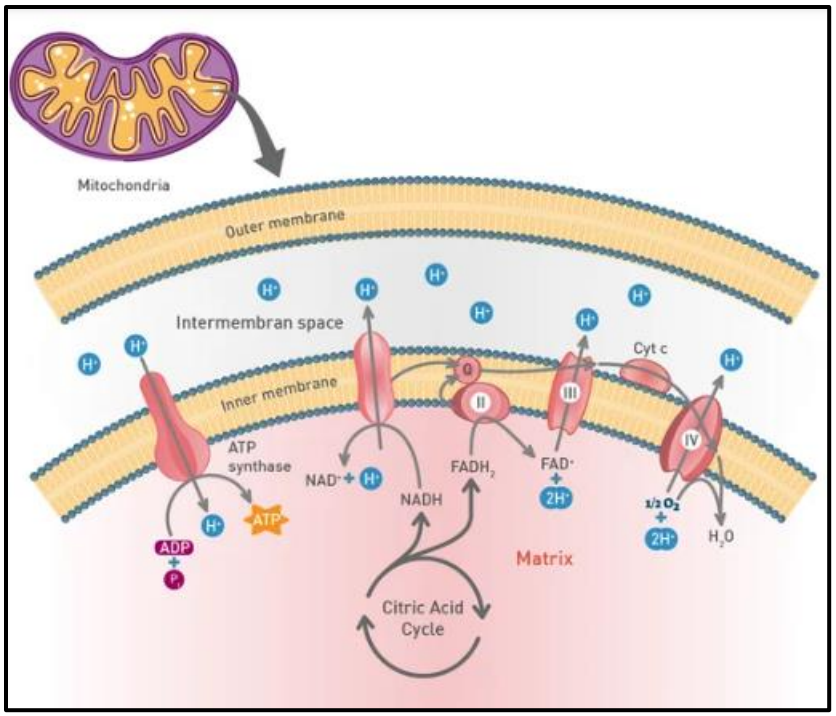


Figure 18: Summary of events and actors leading to energy production in a mitochondria.

Regarding the complexes:

Complex I (NADH dehydrogenase):

- oxidizes $NADH_2$,
- reduces ubiquinone to ubiquinol,

- is a proton pump that allows the passage of protons from the matrix to the intermembrane space.

Complex II (succinate dehydrogenase) :

- oxidizes $FADH_2$,
- reduces ubiquinone to ubiquinol,
- is not a proton pump.

Complex III (cytochrome c reductase or cytochrome b-c1)

- oxidizes ubiquinol to ubiquinone,
- reduces cytochrome c,
- is also a proton pump that allows the passage of protons from the matrix to the intermembrane space. Complex IV (cytochrome c oxidase).
- oxidizes cytochrome c,
- reduces the final acceptor of the respiratory chain (O_2) to H_2O,
- is also a proton pump that allows the passage of protons from the matrix to the intermembrane space.

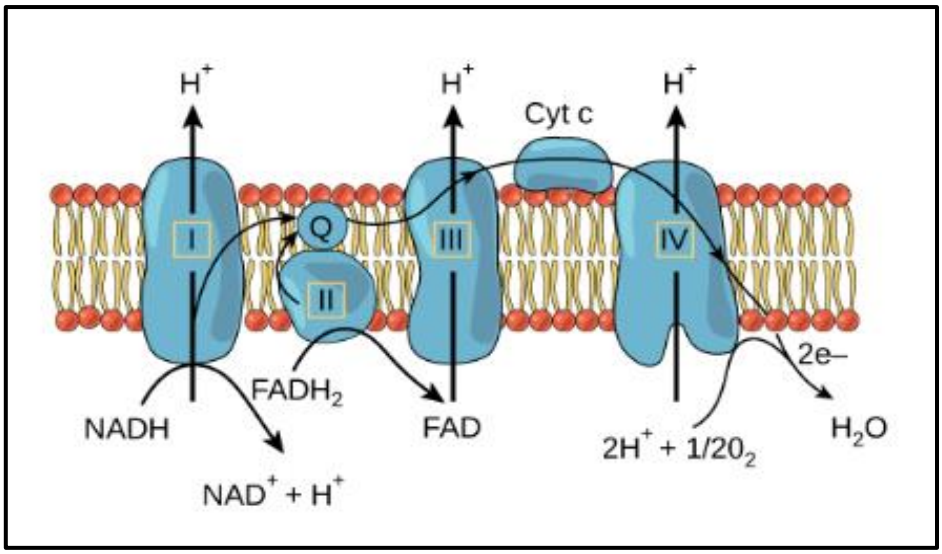


Figure 19: The electron transport chain is a set of molecules that supports a series of oxidation-reduction reactions.

1.3.2. Generation of an Electrochemical Gradient

Complexes I, III, and IV have two important properties:

- They are transmembrane;
- They transport protons (h^+) from one side of the inner membrane to the other.
- By transporting electrons, these complexes remove protons from the matrix and expel them into the intermembrane space. This proton flow generates:
- A ph gradient (δph) across the inner mitochondrial membrane: the ph of the intermembrane space is lower than that of the matrix because protons are less concentrated in the latter;
- A voltage gradient (δv) across the inner membrane: its matrix side is negative (–) and its intermembrane side is positive (+).

The ΔpH and ΔV together form an electrochemical proton gradient. This gradient is a form of energy storage that can be harvested to perform useful work as ions flow freely back across the inner membrane according to their electrochemical gradient.

1.3.3. Exploiting the Electrochemical Gradient

a) ATP Production by ATP Synthase

The electrochemical proton gradient enables the synthesis of ATP-by-ATP synthase during a process called oxidative phosphorylation.

ATP synthase is an enzyme of the inner membrane that creates a hydrophilic pathway across the membrane and allows protons to return to the matrix according to their electrochemical gradient.

This proton flow is used to drive the energetically unfavorable reaction that synthesizes ATP from ADP and inorganic phosphate (ADP + Pi → ATP).

ATP synthase, also called F0F1 ATPase, is a multi-subunit protein composed of a matrix head (F1 ATPase) and a transmembrane transporter (F0). During oxidative phosphorylation, the oxidation of:

- $FADH_2$ by the respiratory chain allows the synthesis of 1.5 (or 2) ATP thanks to ATP synthase,
- NADH2 by the respiratory chain allows the synthesis of 2.5 (or 3) ATP thanks to ATP synthase.

b) Active transport of metabolites across the inner membrane

Some metabolites, such as pyruvate or inorganic phosphate, are co-transported across the membrane with protons when the latter return to the matrix according to their electrochemical gradient. This is secondary active transport through permeases.

The voltage difference across the inner membrane activates the ATP/ADP antiport system (ATP translocase) which allows ATP (carrier of 4 negative charges) to be expelled from the matrix and replenished with ADP (carrier of 3 negative charges).

Figure 5: Examples of active transport requiring the electrochemical H^+ gradient across the inner mitochondrial membrane.

1.4. Mitochondrial Poisons

The following compounds decrease O2 consumption by mitochondria and inhibit the Krebs cycle:

- Rotenone and amytal inhibit complex I;
- Antimycin A inhibits complex III;
- Cyanide, azide, and nitric oxide (NO) inhibit complex IV;
- Oligomycin inhibits ATP synthase;
- Atractyloside inhibits ATP translocase.

Uncoupling agents such as 2,4-dinitrophenol (DNP) and arsenate are so named because they uncouple electron transport from ATP synthesis.

They cause an acceleration of the respiratory chain and the Krebs cycle and an increase in O2 consumption without ATP synthesis. Uncoupling agents act as proton transporters and provide a pathway for proton flow across the inner membrane that bypasses ATP synthase.

2. Peroxisomes

The peroxisome is a cellular organelle surrounded by a simple membrane and containing no genetic material. Peroxisomes are responsible for cell detoxification.

2.1. Characteristics of Peroxisomes

- Spherical organelles measuring 0.1 to 0.5 µm (in this case, they are called microperoxisomes or microbodies) and up to 1 µm in diameter in animals. They can reach 1.7 µm in plants.

- Present in all eukaryotic cells (except reticulocytes and red blood cells);
- All their constituent proteins are encoded by nuclear genes and originate from the cytosol.
- Most often possess a protein crystalline core (urate oxidase). • Contain oxidative enzymes: D-amino acid oxidase, urate oxidase, and catalase.

Like mitochondria, peroxisomes are essential sites for the utilization of oxygen. They use O_2 and H_2O_2 during oxidation reactions.

2.2. Enzymatic Functions of Peroxisomes

a) Oxidase Enzymes

These enzymes (D-amino acid oxidase, urate oxidase) detoxify organic molecules R, which are potentially toxic to the cell, by removing free hydrogen atoms (oxidation reaction):

$$RH_2 + O_2 \rightarrow R + H_2O_2$$

b) Catalase

Catalase uses hydrogen peroxide (H2O2) generated by other enzymes to oxidize a variety of other toxic substrates (R_) (phenols, methanoic acid, alcohol): this is called a peroxidation reaction:

$$H_2O_2 + R'H_2 \rightarrow R' + 2\ H_2O.$$

Notes:

- This type of reaction is very important in the liver and kidney cells, where peroxisomes detoxify certain toxins entering the bloodstream.
- Too much H2O2 is harmful to the cell. Thus, in the case of excess H2O2, catalase transforms it directly into water:

$$2H_2O_2 \rightarrow 2H_2O + O_2.$$

c) Beta-oxidation of long-chain fatty acids

The mechanism is similar to that of mitochondria. Peroxisomes even have exclusive use of this pathway in yeasts and plants. However, the energy balance is reduced to the production of acetyl CoA because the electrons from the reduced coenzymes ($NADH_2$ and $FADH_2$) result in the formation of hydrogen peroxide, which is detoxified on site by catalase.

d) Other functions

- Synthesis of bile acids and cholesterol.
- Catabolism of purines (xanthine oxidase).
- In chlorophyll-rich plants, peroxisomes are involved in photorespiration.
- In germinating seed cells, peroxisomes are associated with lipid bodies, from which they enable the formation of carbohydrates necessary for seedling growth. In this case, the peroxisome is called a glyoxysome.

2.3. Origin of peroxisomes: Peroxisomes have two origins:

- They either result from a fission of parental peroxisomes;
- They are formed from the ER.

All the proteins required by peroxisomes are synthesized in the cytosol. Membrane and lumen proteins are removed after translation in the cytosol. Lipids needed to make new peroxisomal membranes are also imported from the cytosol.

V. The Nucleus

1. General Information on the Nucleus

The nucleus forms a large compartment (3 to 10% of the diameter, or 20 to 25% of the total cell volume depending on the cell), present in all eukaryotic cells except red blood cells. It can be present in a single or multiple copies per cell (as in striated skeletal muscle cells, which are polynucleated).

The nucleus is the compartment delimited by the nuclear envelope that separates the nucleoplasm from the cytoplasm.

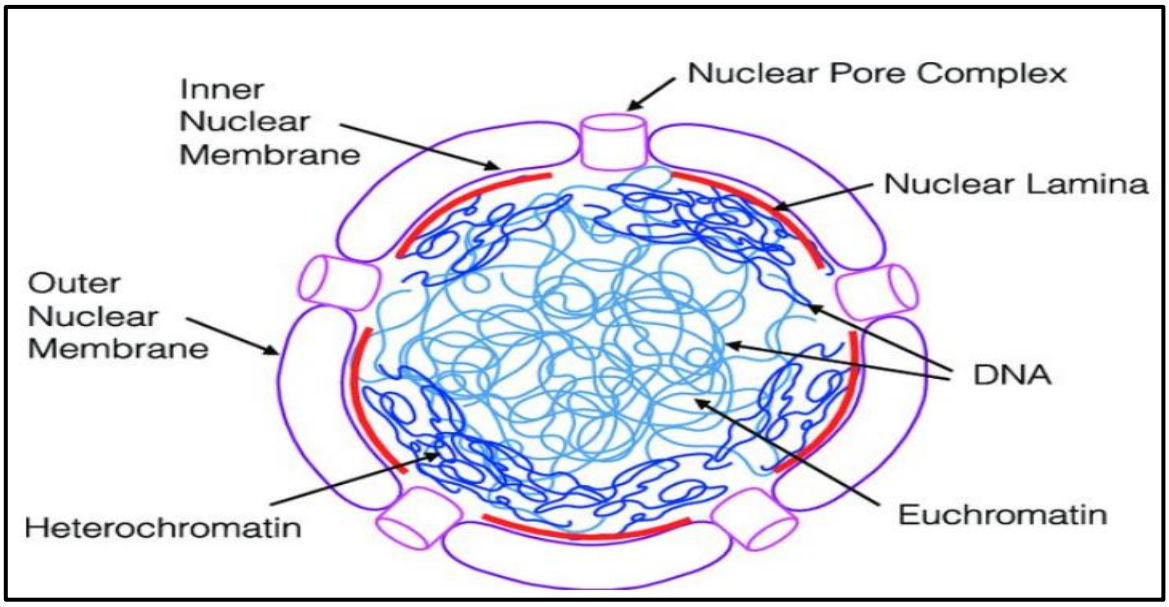


Figure 19: Diagram of a eukaryotic cell nucleus.

1.1. The Nucleoplasm

The nucleoplasm contains:

- Genetic information compacted in the form of chromatin (each chromosome occupies a defined position).
- The nuclear matrix or nucleoskeleton, which includes the nuclear lamina: a 0.2 µm thick protein layer located in contact with the inner nuclear membrane. Lamins are part of the intermediate filament family.

- One or more nucleoli.

1.2. The Nuclear Envelope

The nuclear envelope delimits the nucleoplasm, which contains genetic information compacted in the form of chromatin, and the nuclear matrix or nucleoskeleton. The nuclear envelope is composed of a double membrane (an outer membrane and an inner membrane), both sides of which are in contact with intermediate filaments:

- The outer membrane is associated with ribosomes and is continuous with the membranes of the endoplasmic reticulum. It is in contact with the perinuclear basket.
- The inner membrane is associated with a superficial intranuclear formation with a fibrous appearance: the nuclear lamina (composed of lamins, which are intermediate filaments). It also contains receptors for histones and other proteins associated with DNA.
- The nuclear envelope is also a Ca^{2+} storage site. The nuclear envelope is interrupted at several nuclear pores, which are prime sites for nucleocytoplasmic exchanges.

1.3. The nucleus, the site of gene expression

The nucleus is the place where the genetic material carried by DNA is stored, so it is the starting point for gene expression. It is estimated that there are between 20,000 and 25,000 genes in humans.

1.3.1. Gene Expression Leads to RNA (Ribonucleic Acid) Synthesis

Gene expression is a series of chemical syntheses and reactions that result in the synthesis of RNA from a DNA sequence called a gene. It should be remembered that DNA and RNA are nucleic acids.

This synthesis process, called transcription, takes place in the nucleus and leads to the formation of different types of RNA, the main ones being:

- Messenger RNAs (mRNAs), about 2% of cellular RNAs. Messenger RNA carries the genetic message leading to the synthesis of a protein during a process taking place in the cytoplasm: translation.
- Transfer RNAs (tRNAs), about 18% of cellular RNAs. They transfer amino acids to the protein being synthesized during translation.

- Ribosomal RNAs (rRNAs), about 82% of RNAs. They are part of the composition of ribosomes.
- There are other RNAs (< 1% of RNAs) involved in other processes:
  - Intranuclear: small nuclear RNAs (snRNA = *small nuclear RNA) are involved in the excision-splicing of pre-mRNAs. Small* nucleolar RNAs (snoRNAs = *small nucleolar RNAs) are involved in the* maturation of rRNAs.
  - Cytosolics: 7S RNA is part of the composition of the ribonucleic particle SRP which is involved in the translocation of proteins in the ER. *Interfering RNAs (RNAi) and microRNAs (miRNAs) are involved* in the regulation of gene expression.

1.3.2. Protein gene expression

In the case of a protein gene (DNA sequence leading to the synthesis of an mRNA), gene expression takes place in 2 steps: transcription and translation.

In eukaryotic cells that are compartmentalized, the two steps take place in different compartments:

1) Transcription takes place in the nucleus and gives rise to a pre-messenger RNA (pmRNA) that undergoes modifications (grouped under the term maturation) to become a mature mRNA. This mRNA then travels through the nuclear pores to pass from the nucleus to the cytosol;

2) The translation of mRNA into protein takes place in the cytosol and is partly carried out by tRNA-assisted ribosomes.

In prokaryotic cells where there is no nucleus, the mRNA is immediately translated into a protein, without maturation.

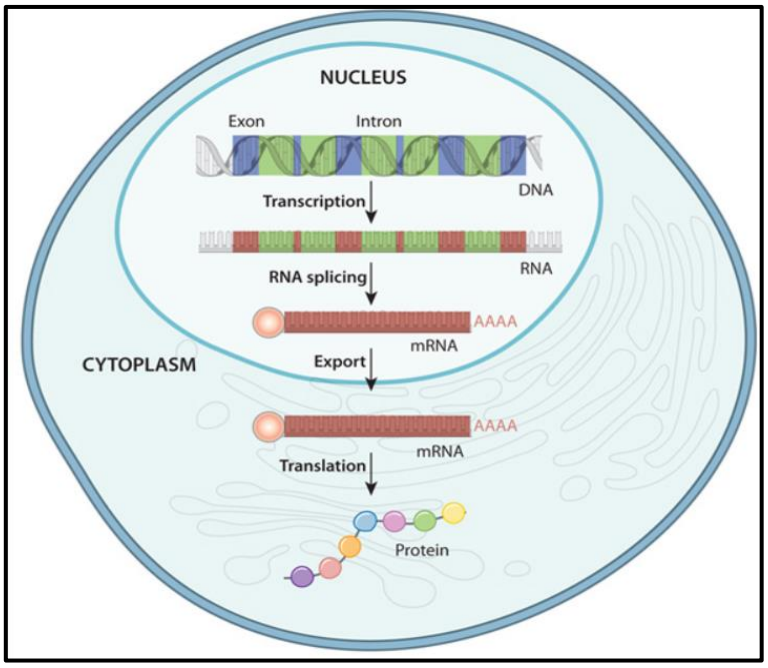


Figure 20: Summary of the steps leading from genes to proteins in eukaryotes.

1.4. Chromatin

Chromatin, present in the nucleus in a more or less compacted form, is made up of DNA (the genome) and proteins. The genome is fragmented into several molecules called chromosomes.

Somatic cells are diploid: they contain 46 chromosomes (2n) in humans. Gametes (oocytes or spermatozoa) are haploid: they contain 23 chromosomes (n). The total length of the DNA fragments corresponding to these 46 chromosomes put end to end is of the order of two meters! The condensation of DNA is therefore essential and essential for all the DNA to enter the nucleus. Chromatin is more or less spiralized and compacted depending on the functional state of the DNA. It can be:

- **Dispersed (euchromatin):** This state is observed during interphase and allows transcription;
- **Condensed (heterochromatin):** This state is observed during interphase and does not allow transcription;
- **Highly condensed (metaphasic chromosome):** This state is observed during cell divisions and does not allow transcription.

a. **Euchromatin** = Type A fibers = 11 nm in diameter

   Euchromatin corresponds to decondensed chromatin. It is made up of nucleosomal fibers (11 nm). It is accessible to RNA polymerases and is therefore active from a transcriptional point of view.

b. **Heterochromatin** (B-type fibers = 30 nm in diameter): is more electron dense than euchromatin. It is more condensed and made up of chromatin fibers (30 nm). Some of it is more concentrated at the periphery of the nucleus and around the nucleoli. 80 to 90% of nuclear DNA is in the form of heterochromatin. It is inactive from a transcriptional point of view. There are two forms:

1) Constitutive heterochromatin: It corresponds to DNA fragments that are never transcribed. It is found in the centromeres and telomeres of chromosomes and often contains repetitive sequences.

2) Facultative heterochromatin: It corresponds to DNA fragments that are not transcribed in the cell where they are observed but that can be transcribed in other cell types (or in the same cell in another state of differentiation).

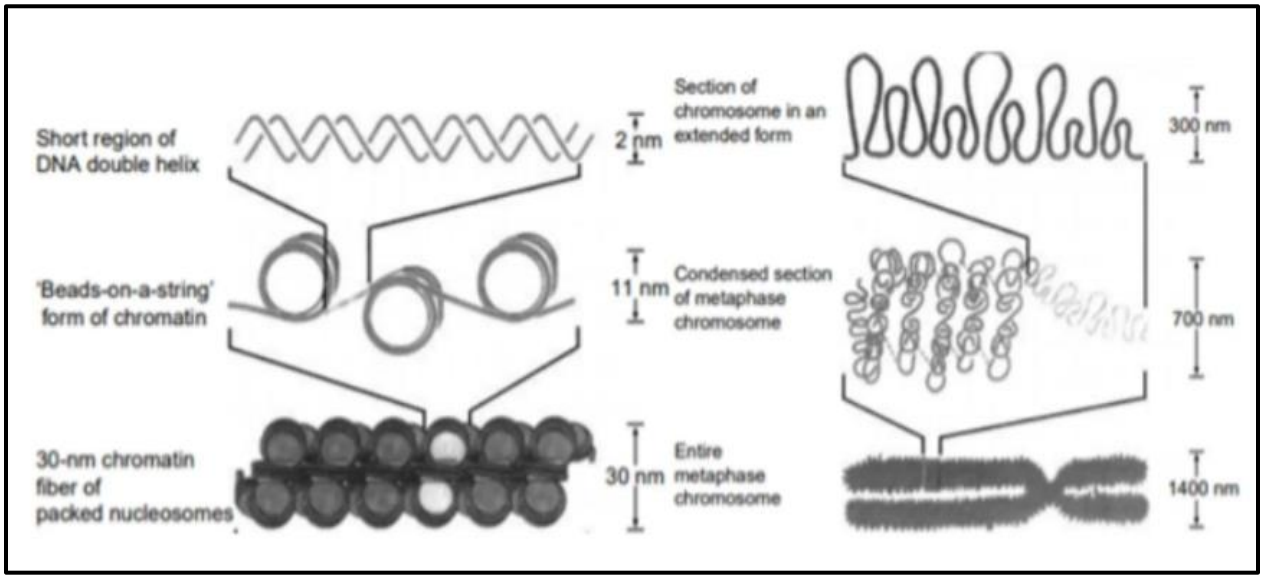


Figure 21: Chromatin packaging. The net result: each DNA molecule was packaged into a mitotic chromosome that is 10,000 times shorter than its unwound length.

1.4.1. Nucleosome and DNA Compaction

1. **First level of DNA compaction: nucleosomes**

Nucleosomes are structures in the shape of a cylinder 11 nm in diameter, formed of small basic proteins called nucleosomal histones that are positively charged (because they are rich in arginine and lysine), which facilitates their attachment to DNA, which is negatively charged because it carries phosphate groups. It is an octamer composed of 2 H2A histones, 2 H2B histones, 2 H3 histones and 2 H4 histones.

The DNA makes 2 turns (=146 base pairs) around each cylinder. Nucleosomes are separated by a short segment of DNA of varying size (60-80 bp) called a binding segment.

The set made up of the histone octamer and the 146 base pairs of DNA (1.8 times coil) associated with it represents the nucleosomal "heart" (or "nucleosome" proper). The DNA chain then resembles a string of pearls.

2. Second level of compaction: the solenoid

Nucleosomes are associated by 6 by another histone, histone H1 or binding histone, to form "solenoids". Histone H1 binds to DNA as it exits the nucleosome. The association of the nucleosome with histone H1 constitutes the chromatosome. The histone H1 molecules are linked together by peptide bonds. They are responsible for the constitution of chromatin fibers with a diameter of 30 nm or type B.

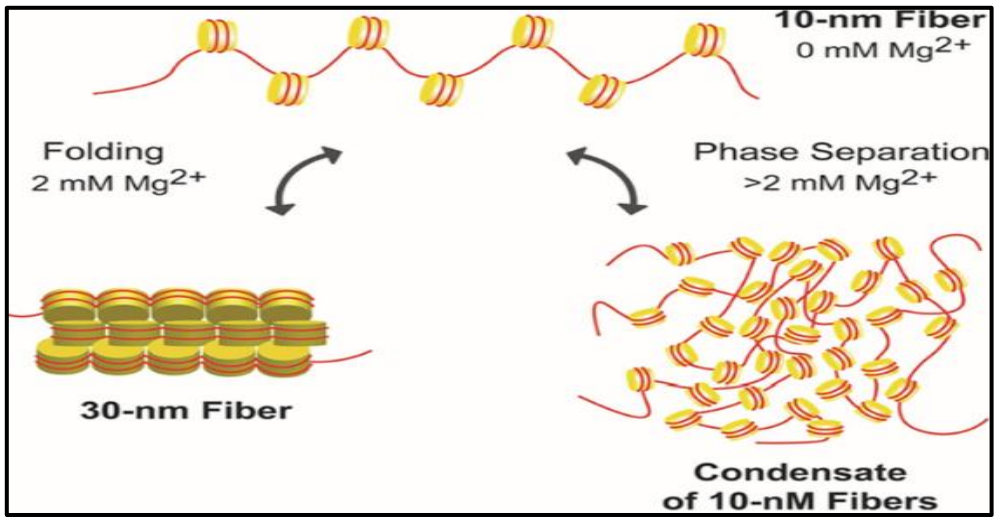


Figure 22: Upper level of condensation leading to type B chromatin fibers.

How can compaction be explained?

- Each nucleosomal histone tail is subjected to different types of covalent modifications: **lysine acetylation, lysine methylation,** and **serine phosphorylation.**
- These modifications are made after the nucleosomes have been assembled and are added and removed by enzymes in the nucleus. Example: acetyl groups are added to the N-terminal tails of histones by HAT (Histone Acetyl Transferase) or removed by HDAC (Histone Deacetylase).
- These modifications have consequences for the stability of the nucleosomes.
- For example, histone acetylation tends to destabilize the chromatin structure (decompaction) because it removes the positive charge of lysine.
- The most important effect of modifying histone tails is also that they become able to attract specific proteins to the modified chromatin segment.
- These additional proteins can increase compaction or, on the contrary, facilitate access to DNA for other proteins (e.g. proteins involved in replication, repair, transcription, etc.).

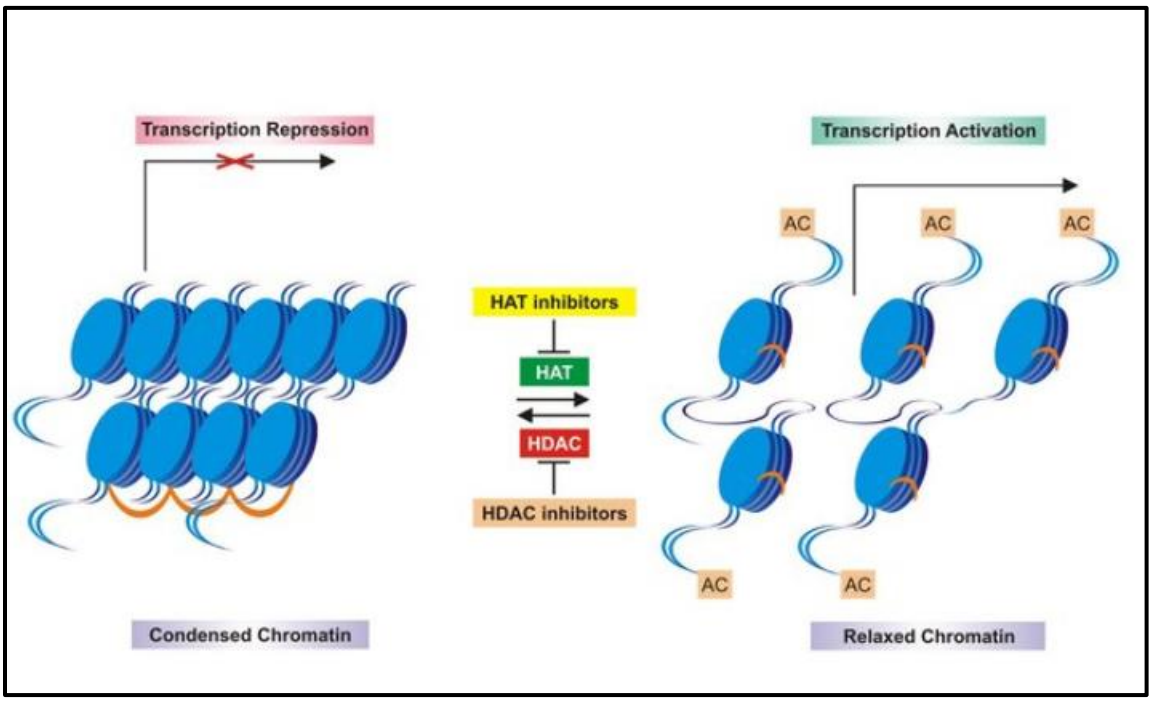


Figure 23: Acetylation and deacetylation of nucleosomal histones.

1.4.2. The metaphase chromosome

The human genome is composed of: 22 pairs of autosomes; 1 pair of gonosomes (sex chromosomes): XX or XY. It is during the mitosis metaphase that chromosomes can best be observed under an optical microscope.

Each metaphase chromosome is composed of three regions: the centromere (primary constriction); telomeres; the 2 chromatids.

The centromere: This is a constriction zone on the chromosome that is also called the primary constriction. It separates the chromatids into 2 arms. These are areas of constitutive heterochromatin containing non-coding repetitive sequences. These are the structures responsible for attaching chromosomes to the mitotic spindle.

Telomeres: These are located at the ends of chromosomes. They protect them by preventing them from fraying and welding with other chromosomes. Telomerases, which are reverse transcriptases, ensure the replication of telomeres. There is a strong correlation between telomere length and the ability of cells to proliferate. Thus, cells with short telomeres will be able to perform fewer cell divisions than cells with long telomeres.

Telomere length is related to cellular aging: telomere erosion observed during cell division is an important cause of cellular senescence.

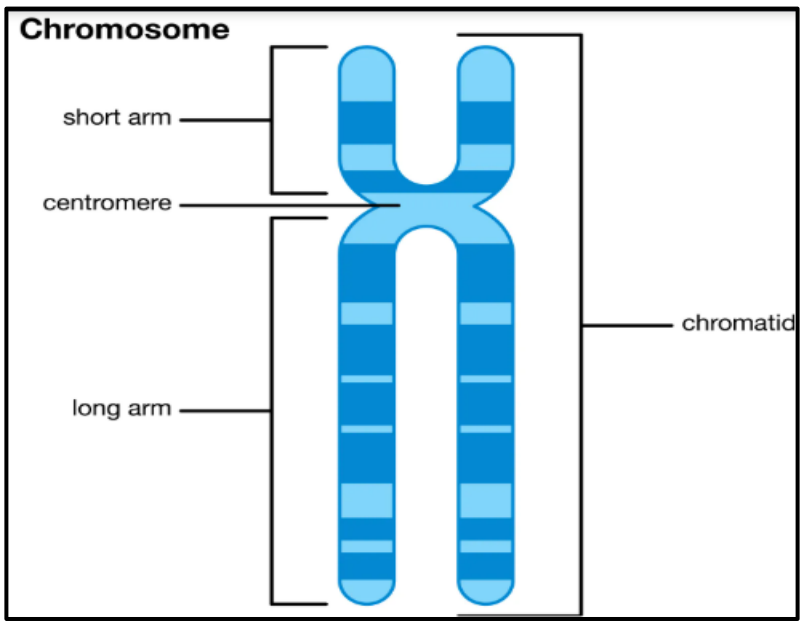


Figure 24: Diagram of the interpretation of a metaphase chromosome.

1.5. Structure of nuclear pores

A nuclear pore is a complex "basketball hoop" structure with 8th order symmetry because the periphery of the pore is bordered by a cylindrical structure, the annulus, organized according to octagonal symmetry (8 ring subunits). Their number varies according to nuclear activity and increases with it.

a) Profile view

• On the hyaloplasmic side of the complex, the pore is in relation to microfilaments (of the cytoskeleton), perpendicular to the surface of the envelope;

• Within the pore, there are two large rings of identical size anchored on both sides (diameter: 120 nm). Each of the two rings is connected to the central carrier by 8 radial arms.

• On the nucleoplasmic side, a third ring of smaller diameter is located in the nucleoplasm. It is connected to the ring of the nucleoplasmic face by microfilaments organized in a cage. The nuclear lamina is interrupted at the pore.

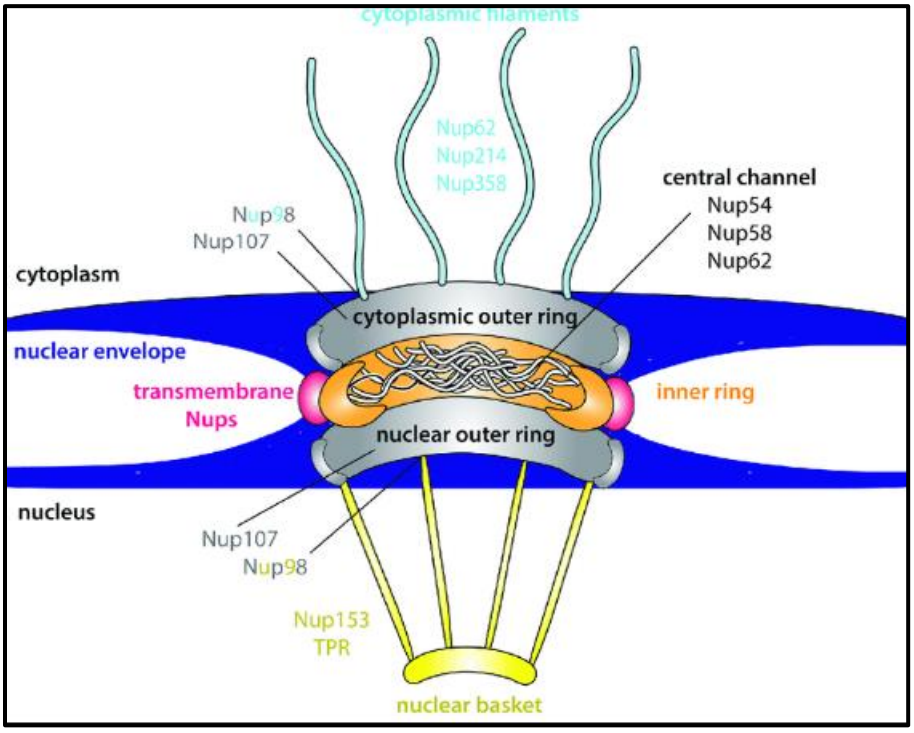


Figure 25: A nuclear pore seen from the side.

b) Top view: The pore comprises:

- a central orifice (diameter: 10-30 nm): the central transporter;
- eight lateral canals delimited by the two rings and the 2 × 8 radial arms. However, it is known that 50% of proteins are nucleoporins (= glycoproteins). They are involved in transport at the pore level by interacting with import and export protein complexes through their FG sequences (F = Phe and G = Gly).

Nucleocytoplasmic exchanges: Exchanges take place in both directions.

• From the cytosol to the nucleoplasm = Import.

• From nucleoplasm to cytosol = Export.

Examples of karyophilic proteins (those with an affinity for the nucleus include lamins and proteins associated with DNA to form chromatin, histones, proteins involved in replication and transcription (polymerases).

1. ***Transport of PM molecules < 40 kDa***: This concerns nucleotides, ions and small proteins. These molecules pass through the pores by diffusion, without energy consumption. They use the lateral channels.
2. **Transport of PM molecules > 40 kDa**: These molecules use the central transporter and their transport consumes energy. The transport is carried out in four successive stages:

Step 1: In the starting compartment, the molecule to be transported (= cargo) combines with two types of specialized proteins to form an import or export complex.

• Import complexes are made up of importin β (alone or in combination with importin α) and the Ran protein. The imported proteins carry an NLS (Nuclear Localization Signal) signal sequence;

Export complexes are formed of exportin and the Ran protein. The exported proteins carry a NES (Nuclear Exportation Signal) signal sequence.

Step 2: The importins and exportins of the complexes interact with the nucleoporins to facilitate transport.

Step 3: In the inlet compartment, the complex dissociates and releases the transported molecule.

Step 4: Imports and exportines return to the departure compartment via the central carrier.

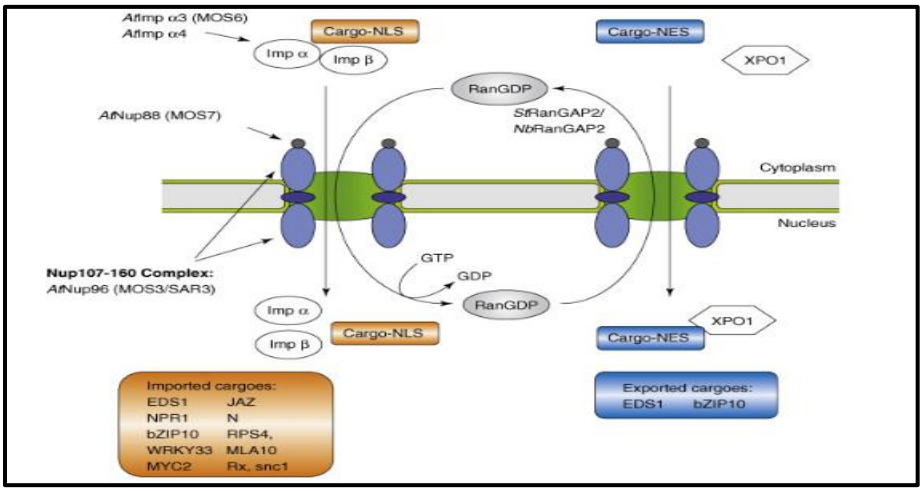


Figure 26: Import of proteins carrying the NLS sequence.

1.6. The nucleolus

The nucleolus is the site of biosynthesis of ribosomes (the cell can synthesize 2,000 to 3,000/min). The transcription of rRNAs (except 5S) and their maturation lead to the formation of ribosome subunits. There can be several nucleoli per nucleus.

The nucleolus:

- is devoid of a membrane;
- disappears at the beginning of mitosis and reconstitutes itself at the end of the latter;
- is associated with a constitutive dense heterochromatin, abundant at the periphery of the nucleolus, and which sinks into the heart of the structure by mixing closely with the other nucleolar constituents.

- **Nucleolus compartmentalization**

After observation of the nucleolus at the TEM, several compartments are observed:

• The fibrillar center: it corresponds to the intergenic spacers that are not transcribed. It can be present in one or more copies per nucleolus.

• The dense fibrillar component: it is denser to electrons and surrounds the fibrillar center. This is the site of rRNA maturation.

• The granular component: this is the storage site for preribosomal particles before export, and for various ribosomal proteins.

Nucleolar rRNA transcription and maturation

In a cell, there are several million rRNAs to synthesize, knowing that a cell contains several million ribosomes... Transcription (synthesis of RNA from DNA) is done using RNA polymerase I. This leads to the formation of a large RNA precursor: 45S RNA.

It is then cleaved into 3 fragments by the snoRNPs (Small Nucleolar RiboNucleoProtein): 18S, 5,8S and 28S with removal of the transcribed intragenic spaces.

• 18S rRNA associates with imported ribosomal proteins to form the small subunit of the eukaryotic ribosome.

• 5,8S and 28S RNAs associate with each other and with imported ribosomal proteins, and then with 5S RNA to form the large subunit of the eukaryotic ribosome.

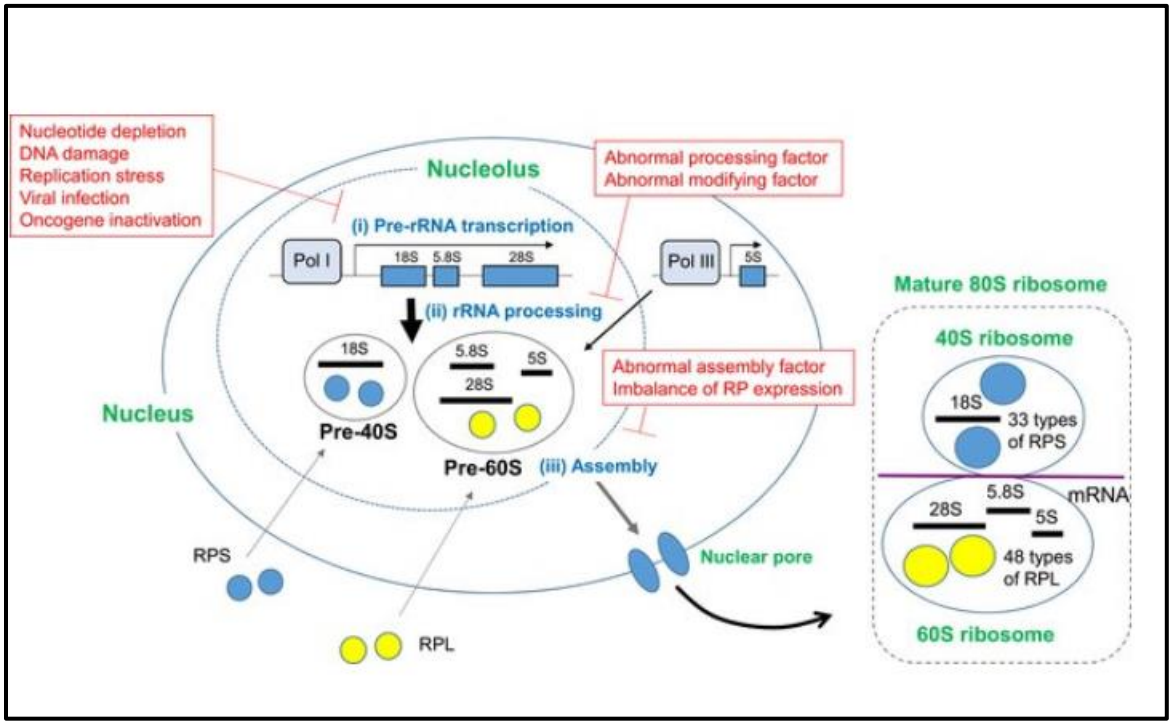


Figure 27: Synthesis of ribosomal subunits in the nucleolus.

Chapter 02: The extracellular matrix

1. Definition of the extracellular matrix

The extracellular matrix is a structured set of macromolecules (proteins, polysaccharides) synthesized by cells in their immediate environment.

In the tissues that make up an adult multicellular organism, all or part of the extracellular space is occupied by the extracellular matrix (ECM).

2. Composition of the MEC

The ECM is composed of the association of three main classes of components:

1) Very large fibrous proteins: collagen fibers and elastic fibers.

2) Less voluminous glycoproteins, allowing the adhesion of the different ECM molecules to each other and the adhesion between the ECM molecules and the cells: fibronectin and laminin are the best known.

3) Polysaccharide chains of the glycosaminoglycan (GAG) family, usually covalently bound to proteins to form proteoglycans (PGs).

These molecules trap water and form a hydrated gel: the fundamental substance.

The variation in the proportions of these three types of molecules leads to different tissue morphologies:

- In connective tissues (**e.g.,** loose, bony, cartilaginous connective tissues, etc.), the framework formed by the ECM molecules is loose and the cells, scattered throughout the ECM, can move there.
- In epithelial tissues, the cells are organized in sheets and rest on a thin ECM with a tight weft: the basal lamina.

3. Synthesis of ECM molecules

Most of the macromolecules in the ECM of connective tissues are synthesized by specialized cells: fibroblasts. In some specific connective tissues like bone and cartilage, these fibroblasts are called osteoblasts and chondroblasts, respectively.

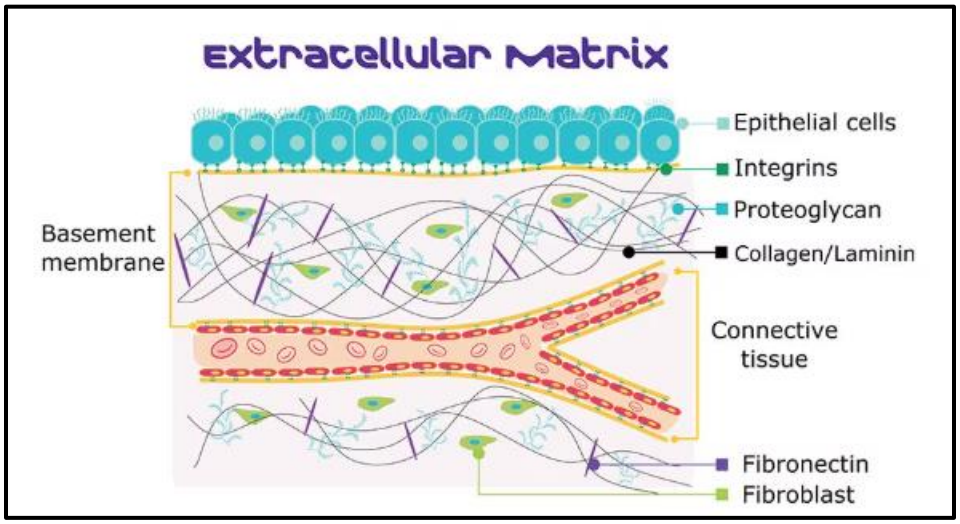


Figure 28: Illustration and arrangement of the extracellular matrix under epithelial tissue.

4. Adhesion of cells to the matrix

Cells carry receptors specific to the macromolecules of the ECM on their plasma membrane: SAMs (Substrate Adhesion Molecules), which include integrins. These receptors are responsible for the adhesion of cells to the ECM.

5. Matrix Functions

- **Support** (mostly), which keeps cells and tissues together.
- **Mechanical resistance** of tissues to compressive forces (thanks to glycosaminoglycans) and tensile forces (thanks to fibrillar collagens and elastic fibers).
- Frame for mineral deposits (e.g. construction of bones by accumulation of calcium phosphate).

ECM also acts on the behaviour of the cells that come into contact with it and influences their **shape**, **migration** but also their **survival**, **proliferation** and **development**.

The fact that a cell needs to adhere to the matrix in order to grow and proliferate, or even just survive, is called anchor dependence. This dialogue between the matrix and the cells takes place through SAMs and is called **mechanochemical transduction.**

5.1. The Fundamental Substance

A. Glycosaminoglycans (GAGs)

❖ **Structure**

GAGs are polymers composed of repeating disaccharide units. The disaccharides of GAGs have the following composition:

- The 1st monosaccharide is a uronic acid (D-glucuronic acid or L-iduronic acid),
- The 2nd monosaccharide is amino (N-acetyl-D-glucosamine or N-acetyl-D-galactosamine), and often sulfated.

GAGs are linear, unbranched, rigid structures carrying many negative charges (this is related to the presence of sulfate and carboxyl groups).

GAGs form a highly hydrated gel that fills most of the extracellular space and allows the ECM to resist compressive forces.

1. Proteoglycans (PG)

a. Structure

Proteoglycans are very heterogeneous molecules formed by the assembly of a central protein, covalently bound to GAGs (all except hyaluronic acid). This covalent binding involves the side chain of a serine of the central protein and a binding tetrasaccharide.

PGs can combine to form large aggregates. This is the case with aggrecan molecules.

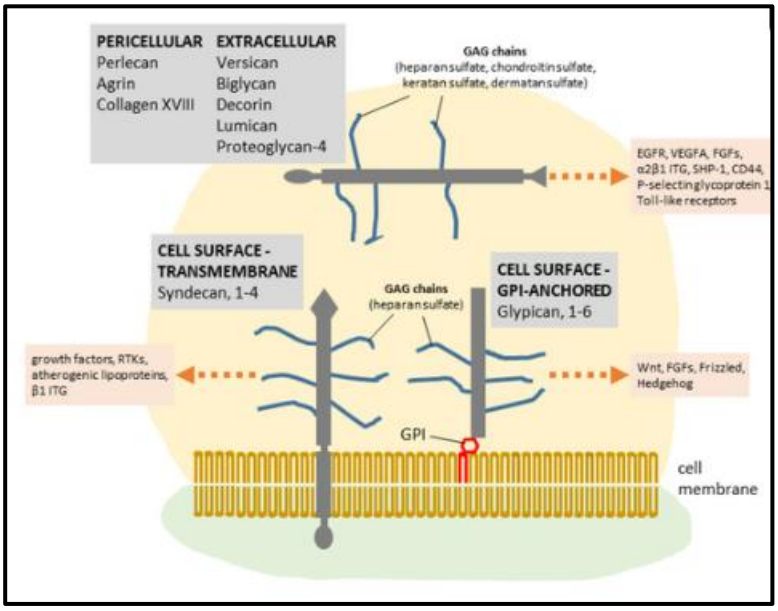


Figure 29: Binding modality between GAG and the central protein of a PG.

PGs can combine to form large aggregates. This is the case with **aggrecan molecules**.

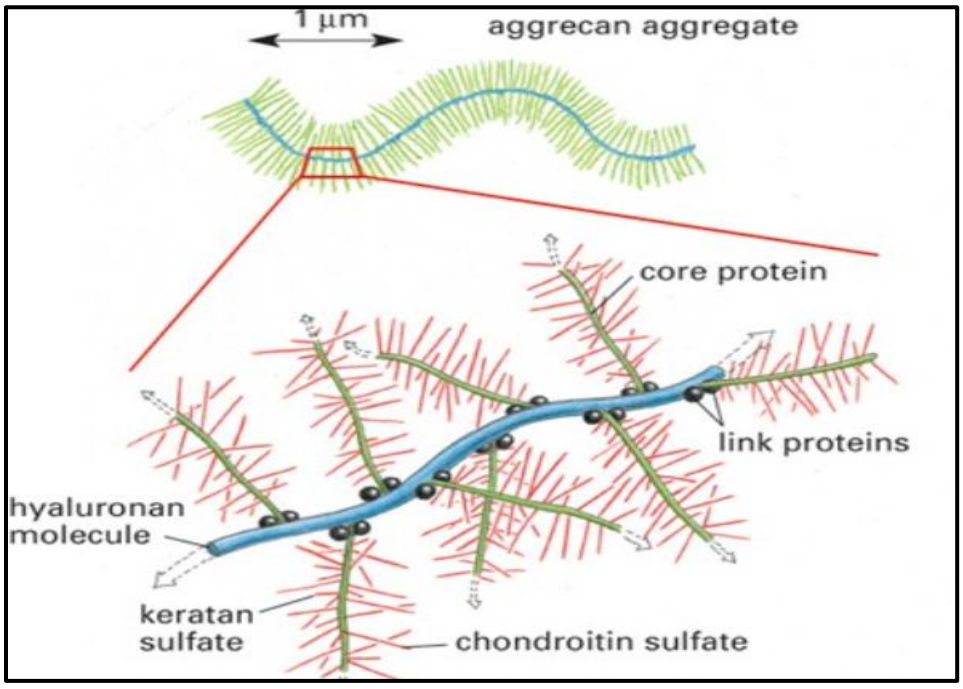


Figure 30: Aggrecan aggregate.

Aggrecan molecules, the main PG of cartilage tissues, attach laterally to a hyaluronic acid molecule. Two binding proteins ensure the non-covalent binding of each aggrecan molecule to central hyaluronic acid.

b. Synthesis

The synthesis of proteoglycans follows the following pattern:

- synthesis of the central protein in REG;
- vesicular transfer of the central protein into the FA;
- covalent binding of binding tetrasaccharides to serines;
- prolongation of disaccharide repeat sequences by specific glycosyltransferases;
- modification of certain carbohydrates (e.g. epimerization, sulfation);
- exocytosis.

c. Function of PGs

PG GAGs form gels that vary in pore size and negative charge density depending on their composition. This gives them the following roles:

- Role of selective sieve to regulate the transport of molecules or cells according to their size and charge.
- Role in cellular communication: PGs can bind signaling molecules secreted in the matrix and increase or inhibit their effects on target cells. This has been observed for growth factors such as FGF (fibroblast growth factor) and TGF-β (transforming growth factor-β) or chemokines (molecules involved in inflammatory processes).
- PGs also bind to other matrix proteins, such as proteases or protease inhibitors, and stimulate or inhibit their activity.

d. Membrane Proteoglycans

In addition to the PGs secreted in the ECM, there are also PGs expressed on the surface of the cells. These PGs insert into the plasma membrane in two ways:

- either through a hydrophobic region of their central protein;
- or through a GPI (glycosylphosphatidylinositol) anchor.

This type of PG allows the adhesion of cells to the matrix and mechanochemical transduction. This is the case of syndecanes, expressed in particular on the surface of fibroblasts and epithelial cells.

B. The Fibrous Proteins of ECM

1. Collagens

a. Structure of collagens

Collagens are a family of long, rigid fibrous proteins, present in all multicellular animals. In mammals, these are the most abundant proteins (about a quarter by weight).

They are super-helices formed by 3 polypeptide chains, called α chains = left helices with 3 amino acids per turn. Their length can reach 1,000 amino acids and they are formed by the repeat of the Glycine-X-Y triplet (where X is very often the proline and Y is hydroxyproline). Proline and glycine are two amino acids that are very important for the formation of triple helices.

b. Types of collagens

About twenty different types of collagens have been identified. Each type results from the combination of 3 of the 25 different α chains listed so far, each of which is encoded by a different gene.

Some types of collagens are composed of 2 or 3 types of α chains and others composed of a single type of α chain. There are 3 main families:

- fibrillar collagens;
- collagens associated with the fibrilles;
- collagens forming networks.

a. ***Fibrillar collagens***: are responsible for resistance to tension forces. They assemble in the matrix to form collagen fibrils with a diameter between 10 and 300 nm. The fibrils can in turn assemble to form collagen fibers, visible under an optical microscope, whose diameter can be several micrometers.

A distinction is made between:

- Type I: the main collagen in the skin and bones and representing 90% of the collagen present in the body.
- Types II, III, V and XI, also found in connective tissues.

b. *Collagens associated with fibrils*

Case of type IX and XII collagens. Their function is probably to connect the fibrils to each other and to connect the fibrils to the other components of the matrix. This function suggests their role in determining the organization of fibrils in the matrix, which varies from tissue to tissue.

c. *Network-forming collagen*

In the basal lamina, type IV collagen assemble into a flexible multi-layered sheet-like network. These molecules are more flexible than fibrillar collagens because their triple helix structure is interrupted at many points. They interact with each other through their terminal domains and assemble to form a regular loose mesh structure that serves as a framework to secure the other molecules of the basal lamina.

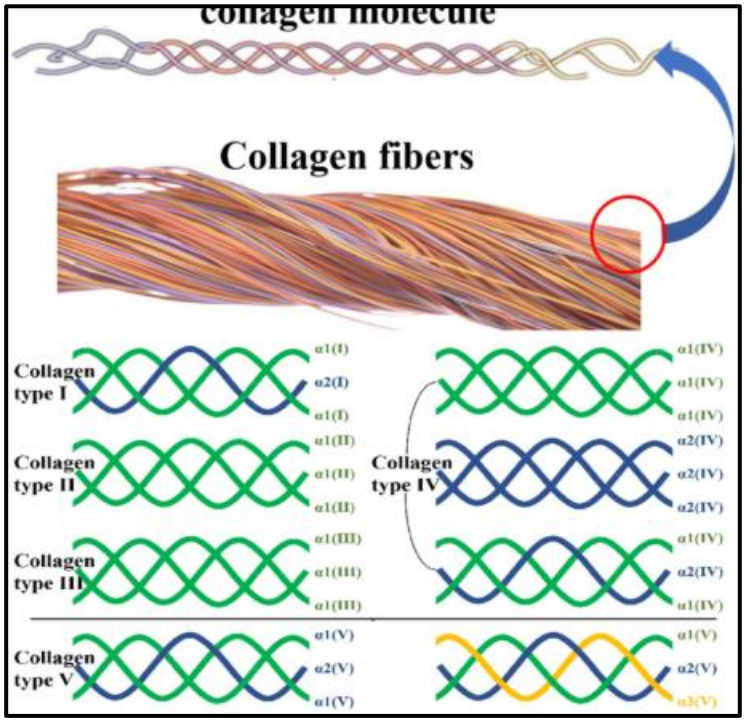


Figure 31: Collagen structure.

c. Biosynthesis of type I collagen

The synthesis steps inside the cells are those of all exported glycoproteins:

1) Summary of pro-α channels in the REG. They are protein precursors containing the ER address signal peptide at the N-terminal end, and propeptides at the N- and C-terminal ends.
2) Hydroxylation of lysine and proline residues by hydroxylases in the REG (the OH functions of hydroxyprolines form interchain H-bonds that stabilize the triple helix).
3) N-glycosylation in ERW and O-glycosylation in GA.
4) Association of 3 chains α in GA to form procollagen molecules.
5) Procollagen exocytosis. The maturation of collagen fibrils and the assembly of fibers continues outside the cell.
6) Cleavage of procollagen N- and C-terminal propeptides by specific matrix proteases. This cleavage is necessary for fibril assembly and does not occur for non-fibrillar collagens. Procollagen becomes collagen.
7) Assembly of collagen molecules into fibrils (1/4 of their length →striated appearance).
8) Assembly of collagen fibrils into fiber.

The cohesion of collagen fibrils is strengthened by the formation of covalent cross-links (bonds between Lily and OH-Lys). These bonds are made within the collagen molecule (between the alpha chains) and between the collagen molecules.

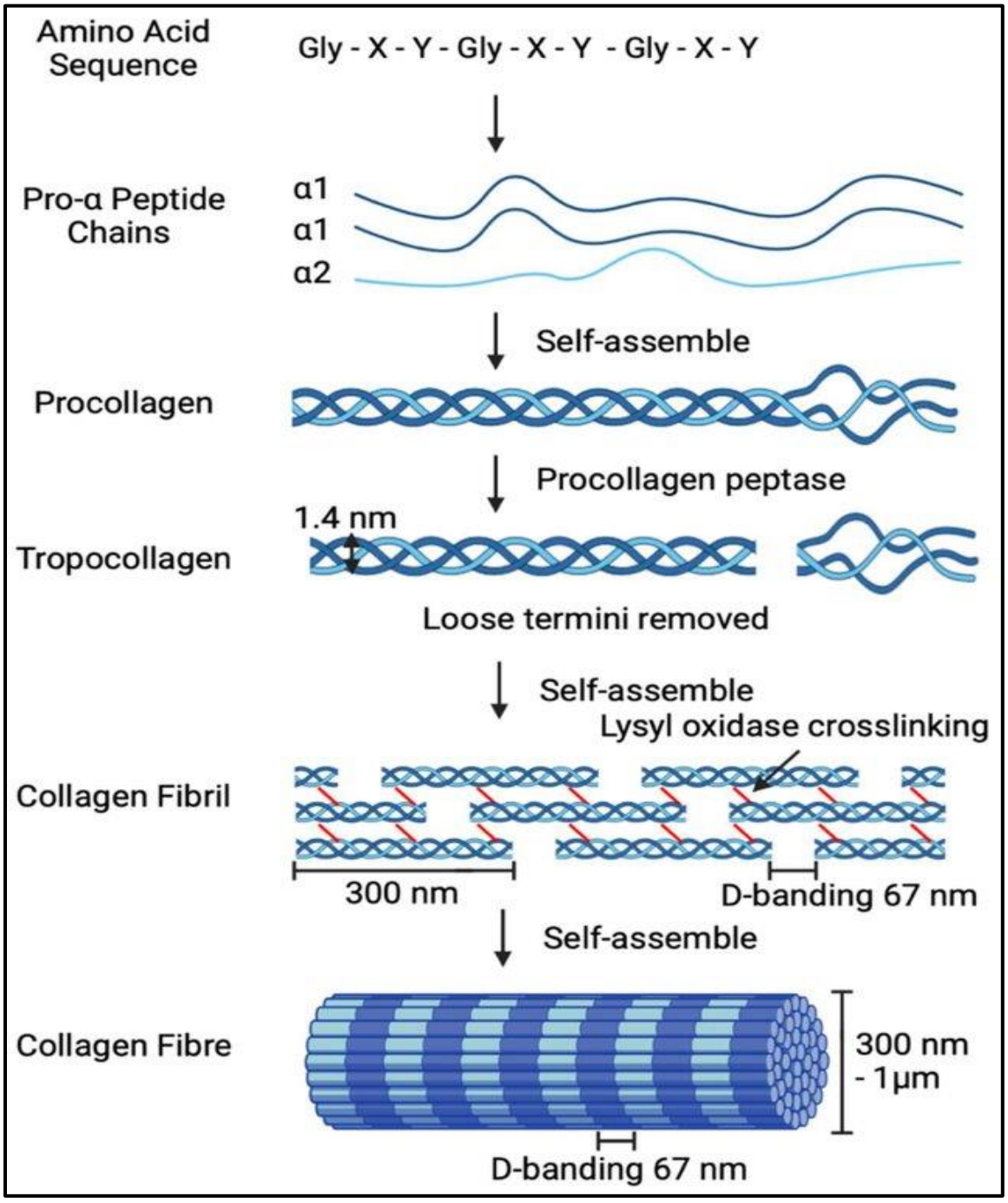


Figure 32: Biosynthesis of type I collagen.

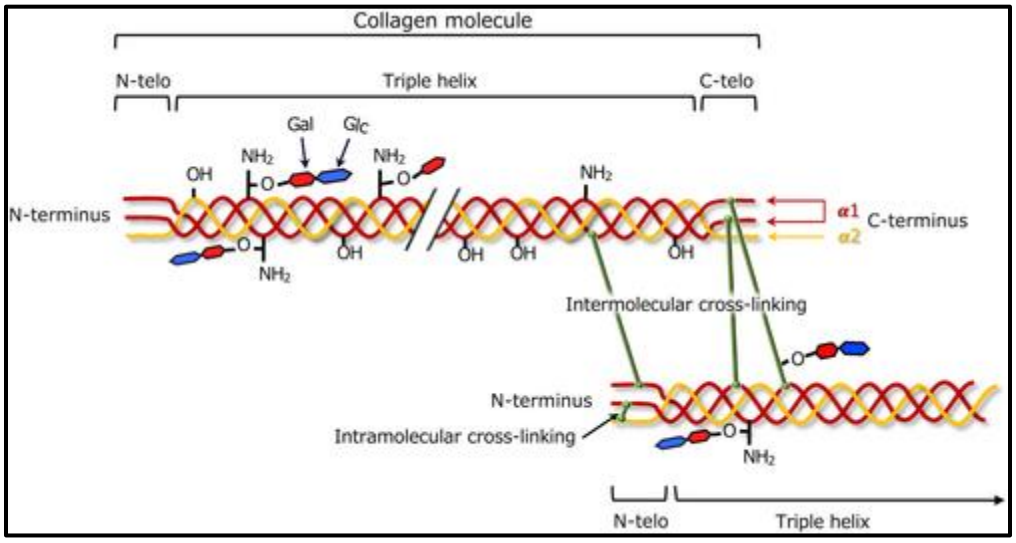


Figure 33: Cross-links of collagen fibrils.

2. Elastic fibers

They are present in significant quantities in the ECM of tissues subject to large variations in size and shape such as the skin, blood vessels and lungs.

The main component of elastic fibers is elastin, a hydrophobic, non-glycosylated, 70 kDa protein.

Like collagen, elastin is rich in proline and glycine. However, it contains little hydroxyproline and no hydroxylysine.

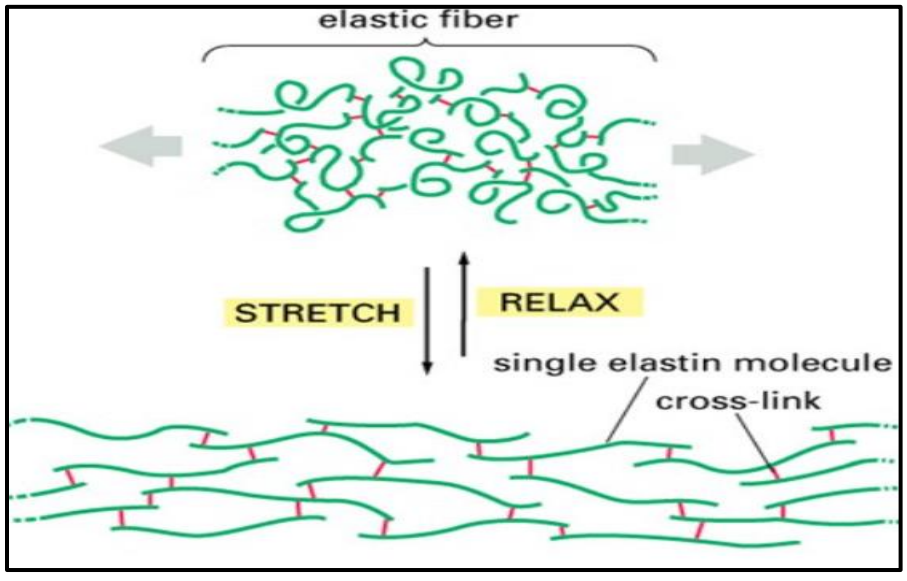


Figure 34: Network of elastin molecules.

Elastin molecules are connected to each other by covalent bonds between lysines and form a 3D network capable of large variations in size and shape.

The elastin core is covered by a sheath of microfibrils about 10 nm in diameter. Microfibrils are composed of several glycoproteins including fibrillin and fibulin.

In developing tissues, microfibrils are synthesized before elastin and appear to guide the arrangement of elastin in elastic fibers.

Elastic fibers are also associated with collagens, which limits the extent of their stretching and prevents tissue tearing.

C. Adhesion glycoproteins

Adhesion glycoproteins have the characteristic of having many binding domains: some domains are specific to other molecules in the matrix and others have an affinity for SAMs expressed on the surface of cells.

Their role is to organize the molecules of the matrix and to facilitate the anchoring of the cells to the matrix.

1. Fibronectin

a) Structure of fibronectin

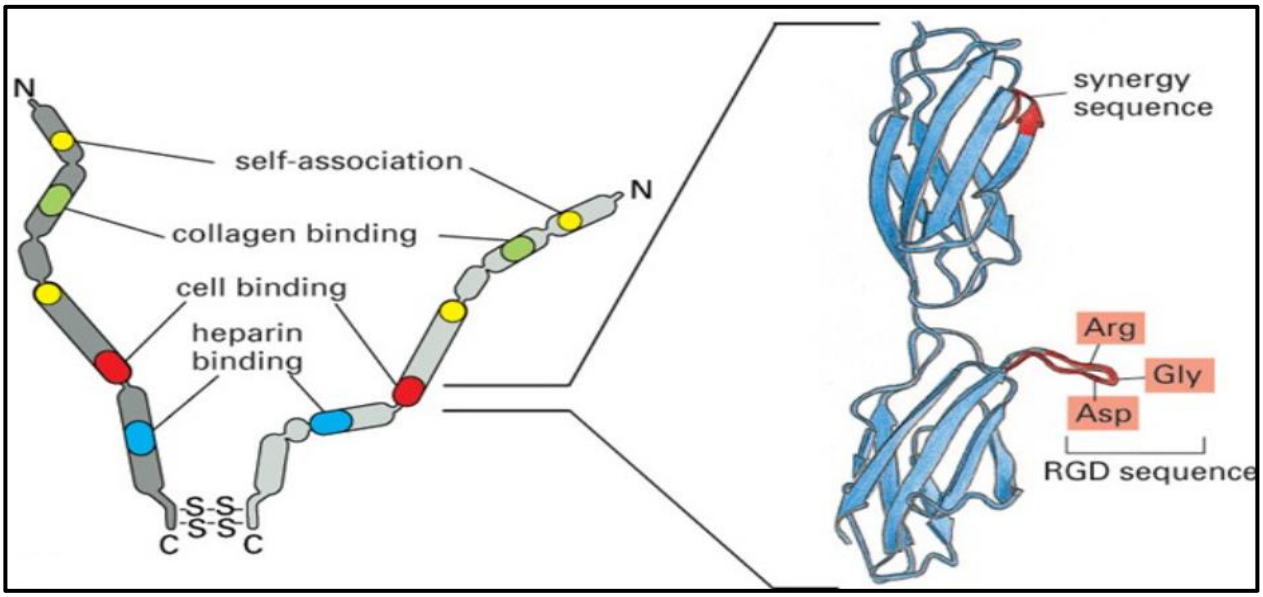


Figure 35: Structure of a fibronectin dimer.

Fibronectin is a large dimeric protein (about 220 kDa) whose polypeptide chains, connected by disulfide bridges located near their C-terminal, form a V. Each chain contains 5 to 6 domains made up of small modules repeated in series. Each domain contains binding sites for other ECM molecules (e.g., collagen, heparin) or for molecules expressed on the surface of cells (e.g., integrin).

Integrin binding sites contain a particular type of module called fibronectin type III repeat. These modules contain a specific RGD (Arg-Gly-Asp) tripeptide sequence, which is decisive in the process of binding cells to fibronectin.

The RGD sequence has since been found in many matrix proteins and plays the same role.

b) Fibronectin isoforms

The different isoforms of fibronectin are encoded by the same gene and result from the alternative splicing of mRNA.

- **Soluble fibronectin**

This is plasma fibronectin. It is the only fibronectin among the other isoforms that does not assemble into fibrils. It circulates in the blood where it promotes clotting, healing and phagocytosis.

- **Fibrillar fibronectin**

Most fibronectin isoforms come together to form highly insoluble fibrils found on the surface of cells and in the matrix.

The orientation of the fibronectin filaments of the matrix is closely related to that of the actin microfilaments inside the cells that synthesize them (e.g., fibroblasts).

c) Functions of fibrillar fibronectin

- promotes the adhesion of cells to the matrix;
- plays a role in guiding cell movements during embryogenesis.

2. Laminin

Laminin is a large glycoprotein (about 850 kDa), specific to the basal lamina. It consists of 3 chains (α, β and γ) connected by disulfide bridges. These 3 chains are wound up to form a cross-shaped

structure. There are several laminin isoforms, each formed by the combination of the different α, β and γ chains (5 types of α chains, 3 types of β chains and 3 types of γ chains have been identified).

Like fibronectin, laminin has binding sites for other molecules in the matrix (e.g., perlecan, nidogenic, collagen IV) or for molecules expressed on the surface of cells (e.g., integrin, dystroglycan).

Like type IV collagen molecules, laminin molecules assemble to form planar networks.

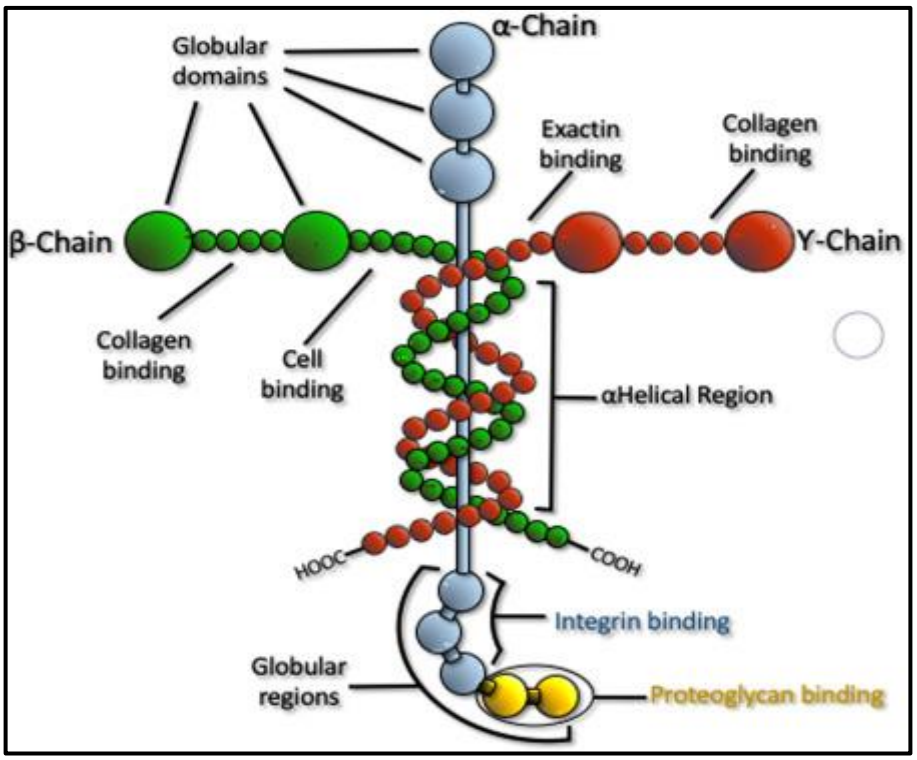


Figure 36: Structure of laminin.

D. The basal lamina

1. Distribution of the basal lamina

The basal lamina is a differentiated region of the ECM in the form of a thin layer (40 to 120 nm thick). It is observed in contact with epithelial cells arranged:

- in flat sheets resting on the basal lamina. Ex: intestinal epithelium;
- in sheets forming tubes that the basal lamina surrounds. E.g. epithelium of the renal tubules.

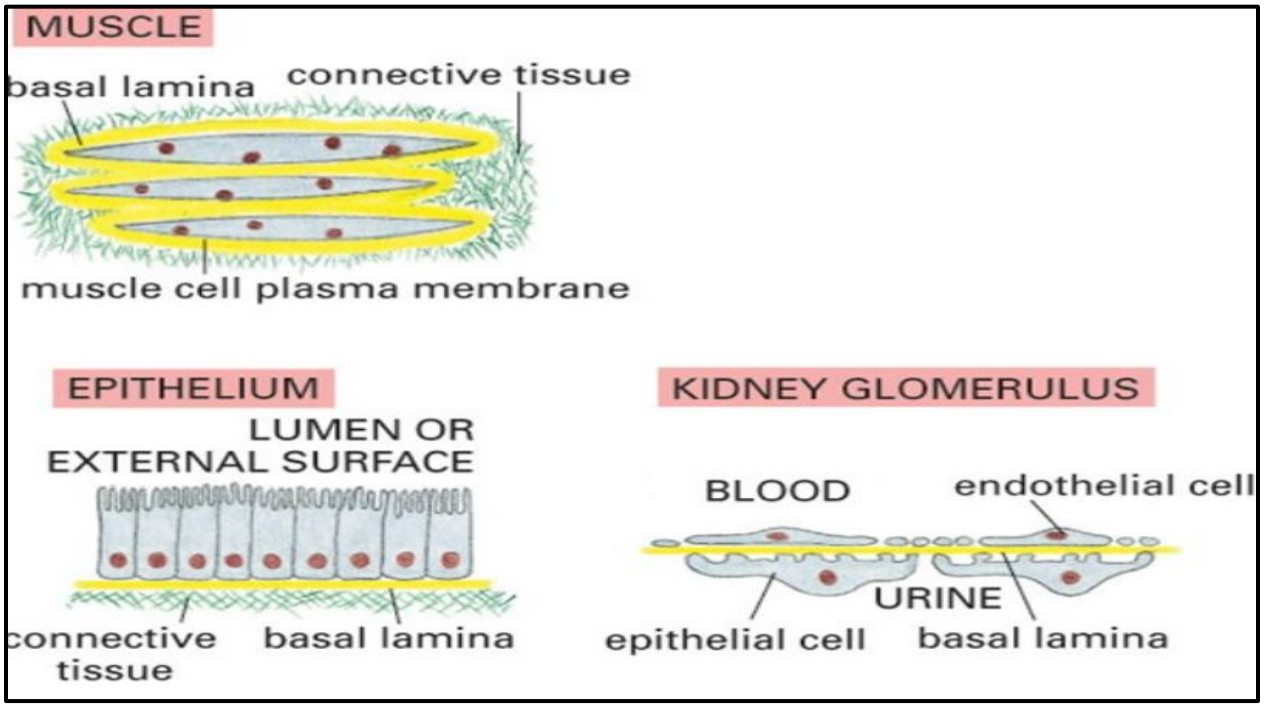


Figure 37: Examples of cells in contact with the basal lamina.

2. Basal lamina synthesis

The basal lamina is synthesized both by the adjacent cells that surround it or that are in contact with it, and by the connective cells, in a reciprocal interaction.

An experiment performed on a biopsy of human skin shows that epithelial cells of the epidermis (keratinocytes) and fibroblasts of the dermis cooperate in the synthesis of the basal lamina that separates them.

3. Composition of the basal lamina

The composition of the basal lamina varies from tissue to tissue, but always consists of an organized tangle of the following molecules: type IV collagens; laminine; perlecane (proteoglycan containing heparan sulfate); nidogenic (= entactin).

The collagen IV molecules and the laminin molecules form planar networks that the perlecane and nidogen molecules connect to each other because the latter can bind them simultaneously.

4. Functions of the basal lamina

- **Cellular boundary**

One of the roles of the basal lamina is to physically separate the epithelial cells, with which it is in contact, from connective tissue.

- **Molecular filter**

Ex: In the renal glomerulus. The basal lamina acts as a molecular filter and prevents the passage of macromolecules from the blood to the urine.

- **Selective Barrier to Cell Movement**

The basal lamina is often located at the interface between the epithelia and the connective tissue. It thus makes it possible to confine certain cells in the connective tissue by preventing their passage beyond the basal lamina.

In some pathologies, alterations in the basal lamina no longer allow this role.

Examples:

- Pulmonary fibrosis, where fibroblasts are found in the alveolar spaces.
- Metastatic cancer cells pass through the basal laminae into the bloodstream and colonize other areas of the body.

- **Tissue regeneration**

The basal lamina plays this role after tissue damage because it then serves as a framework on which regenerating cells can migrate. Thus, following the damage to tissues such as epithelia, muscles and nerves, the basal lamina serves as a guide for the migration of new cells.

6. Degradation of extracellular matrix molecules

6.1. Role of matrix molecule degradation

Matrix molecules are degraded and permanently renewed in adult tissues. The degradation of the components of the matrix is also associated with cell migration because it facilitates cell migration.

Example: Migration of leukocytes from the bloodstream to the tissues in the event of infections or lesions.

6.2. Matrix proteases

This degradation is carried out thanks to locally secreted matrix proteases. These are Ca^{2+} or Zn^{2+} dependent metalloproteinases, or serine proteases. Some have a very broad spectrum of action, others, such as collagenases, are very specific. Their targets are mainly collagen, laminin and fibronectin.

6.3. Regulation of matrix proteases

The activity of these proteases, and consequently cell migration, is tightly regulated in different ways.

a) Local activation

Case of plasmin, a serine protease that activates the dissolution of the blood clot. It is secreted as an inactive precursor abundant in the blood, plasminogen, and then activated locally by plasminogen activators such as tissue plasminogen activators (tPA).

b) Confinement by surface cell receptors

This is also the case for plasmin, where a second type of activator, the urokinase plasminogen activator (uPA), can bind to membrane receptors located at the front of migrating cells. By activating plasmin locally, the migrating cell allows a degradation of the matrix proteins that potentially blocked its path and thus makes its way through the matrix.

c) Secretion of inhibitors

Tissue Inhibitor of Metalloprotease inhibitors (= TIMPs) and serpins (serine protease inhibitors) block the activity of matrix proteases. These inhibitors are used to confine the activity of matrix proteases to specific areas and to modulate cell migration.

It has been shown that the overexpression of TIMP blocks the migration of certain cell types.

Chapter 03: Cell Differentiation

1. Definition of Cell Differentiation

Cell differentiation refers to the process by which cells from the same source gradually produce cell groups with different morphological structures and functional characteristics. Consequently, cells are spatially different, and the same cells differ over time from their previous state. The essence of cell differentiation is the selective expression of the genome in time and space.

The expression of various genes is turned on or off, and ultimately, the signature protein is produced. Generally, the process of cell differentiation is irreversible. However, under certain conditions, differentiated cells are also unstable, and their gene expression patterns can also undergo reversible changes and return to their undifferentiated state. This process is called dedifferentiation. What is a differentiated cell?

Size, shape, polarity, metabolism, and responsiveness to signals change dramatically, so that a less specialized cell becomes more specialized and acquires a more specific role. A cell that has undergone differentiation is described as differentiated.

- A differentiated cell is one that has changed shape and transitioned from a generalized cell to a more specific function.
- The term undifferentiated is used to describe a cell (or tissue) that has not yet acquired a particular structure and function. An undifferentiated cell would therefore be a primitive cell or a progenitor cell that has not yet undergone cellular differentiation.

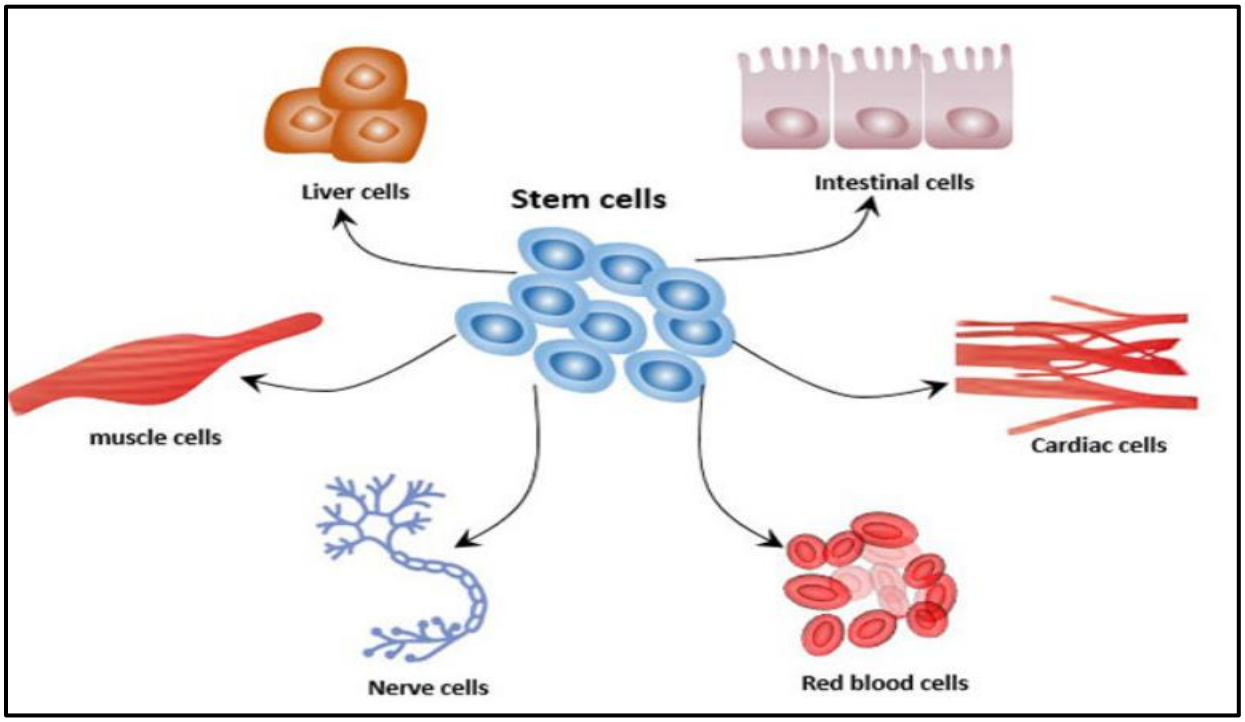


Figure 38: Cell differentiation of stem cells.

2. Characteristics of Cell Differentiation

- Cell differentiation is a fundamental process in the development of multicellular organisms. It is characterized by the progressive emergence of differentiation potential during embryonic development. Cells transition from a pluripotent state (able to become multiple cell types), to a multipotent state, and finally to a unipotent state, where they can differentiate into only one specific cell type. This transition reflects the general pattern of differentiation.
- Throughout individual development, cells undergo both temporal and spatial differentiation. This process is closely linked to cell division: while differentiation generally requires cell division, not all dividing cells undergo differentiation. Moreover, as cells become more differentiated, their capacity to divide decreases. Highly differentiated cells often lose the ability to proliferate.
- Cell differentiation is typically stable. Under normal physiological conditions, differentiated cells do not revert to an undifferentiated state or switch to another cell type. However, under specific conditions—such as experimental manipulation or in certain pathological states—cells may exhibit plasticity. In such cases, they can be reprogrammed into a less differentiated state or transdifferentiate into a different cell type.

Several factors influence cell differentiation:

- **Genetic structure**: This is the primary factor. Functional genes contain the necessary instructions that determine a cell's type and function. Mutations or structural errors in these genes can disrupt the differentiation process and overall development.
- **Environmental factors**: Elements such as temperature fluctuations, oxygen availability, and other external conditions can influence hormone activity and cellular signaling, ultimately affecting the differentiation process.

Examples of processes involving cell differentiation include:

- Regular renewal of cells (e.g., blood cells in adults).
- Maturation from embryonic to adult stages.
- Tissue repair following injury.
- Cytoplasmic influences.
- Cell-to-cell interactions.
- Hormonal signals.

Despite being specialized, differentiated cells in both animals and plants generally retain the full set of genes necessary for the development and programming of a complex organism. What distinguishes them is not the presence or absence of genes, but the pattern of gene expression. Each differentiated cell type expresses a specific subset of genes in a unique manner, defining its structure and function.

3. Importance of Cell Differentiation

- **Foundation for multicellular development**

Cell differentiation is the process by which an unspecialized cell becomes a specialized cell type. It allows a single-celled zygote to develop into a complex, multicellular organism with a wide variety of tissues and organs.

- **Essential for tissue and organ formation**

Through differentiation, cells acquire specific structures and functions, leading to the development of functional tissues (e.g., muscle, nerve, blood) and fully operational organ systems.

- **Changes in cellular properties**

During differentiation, cells undergo significant changes in size, shape, metabolism, and responsiveness to external signals. These changes are vital for ensuring that each cell type performs its intended biological role.

- **Role of gene regulation**

Differentiation is controlled by precise genetic mechanisms, especially gene regulatory networks. These networks ensure that only specific genes are activated in each cell type, maintaining the identity and function of each cell.

- **Guarantee of functional organisms**

The regulation of gene expression during differentiation helps maintain the integrity of tissues and ensures that the organism develops normally and remains functional throughout its life.

- **Impact on stem cell research**

Understanding cell differentiation has greatly influenced stem cell science. Researchers aim to harness the power of stem cells to regenerate and repair damaged tissues in medicine and therapy.

- **Unique potential of stem cells**

Stem cells are pluripotent or multipotent, meaning they can differentiate into various cell types. This makes them especially valuable in treating age-related diseases, injuries, and degenerative conditions.

- **Application in aging and tissue repair**

In elderly individuals, many cells lose their regenerative capacity. Stem cells offer a promising solution, as they can replace aged or damaged cells that can no longer divide or heal themselves.

4. Mammalian Cell Types

The mammalian body is composed of three fundamental categories of cells: somatic cells, germ cells, and stem cells.

- The adult human body contains approximately 37.2 trillion cells, nearly all of which carry one or more copies of the complete genome. An exception is found in certain cell types such as mature red blood cells, which lose their nuclei upon full differentiation.

- The majority of cells are diploid, meaning they possess two copies of each chromosome. These are known as somatic cells and include the various differentiated cells that form tissues and organs such as the skin, muscles, and internal organs. Somatic cells specialize through the process of cell differentiation, acquiring distinct structures and functions.

  In contrast:

- **Germ cells** are those that give rise to gametes—sperm and egg cells—and are thus responsible for passing genetic material from one generation to the next. They are the only cell line that is continuous across generations.
- **Stem cells** are unique in that they possess two key properties: they can self-renew indefinitely, and they can differentiate into specialized cell types. These cells play a central role in development, regeneration, and tissue repair.

  Human development begins when a sperm cell fertilizes an egg, forming a single totipotent cell capable of generating an entire organism. In the first hour's post-fertilization, the zygote divides into identical cells. Around four days after fertilization, these cells organize into a structure called the blastocyst, a hollow sphere composed of:

- An **outer layer** of cells that will contribute to the placenta.
- An **inner cell mass**, which contains pluripotent stem cells that give rise to nearly every cell type in the human body, though not a complete organism on their own.

  These pluripotent stem cells continue to differentiate into multipotent progenitor cells, which are more specialized and give rise to specific lineages of functional cells. Examples include:

- **Radial glial cells** (embryonic neural stem cells): Give rise to excitatory neurons in the fetal brain during neurogenesis.
- **Hematopoietic stem cells**: Found in bone marrow, they differentiate into red and white blood cells and platelets.
- **Mesenchymal stem cells**: Also from bone marrow, they give rise to stromal cells, adipocytes (fat cells), and osteoblasts (bone cells).
- **Epithelial stem cells**: Give rise to the various layers and types of skin cells.
- **Muscle satellite cells**: Contribute to the formation and repair of skeletal muscle tissue.

  During early embryogenesis, as cells continue to divide and specialize, the blastula (a single-layered structure) transforms into a multilayered structure through a process guided

by cell adhesion molecules—particularly those involving the amino acids arginine, glycine, asparagine, and serine. This transition gives rise to the three primary germ layers:

1. **Ectoderm** (outer layer): Develops into the skin and nervous system.
2. **Mesoderm** (middle layer): Forms muscles, bones, and connective tissues.
3. **Endoderm** (inner layer): Becomes the lining of the digestive and respiratory tracts, as well as other internal organs.

5. Cell Differentiation – Mechanism

- Cell differentiation is primarily regulated by transcription factors, which play a central role in determining which genes are expressed in a given cell. These transcription factors are influenced by chemical signals and hormones, which guide how DNA is accessed and transcribed.
- The availability and type of transcription factors in a cell are determined by both internal factors (such as the cell's own state) and external cues from surrounding cells and the organism's environment starting from fetal development and continuing throughout life.
- **DNA structure and the location of gene expression** within the genome are also key to the differentiation process. Transcription factors bind to specific DNA regions, initiating the transcription of genes into RNA, which is then translated into functional proteins. These proteins shape the identity and function of the cell.
- In addition, cells communicate with each other through cell signaling mechanisms. When cells come into close contact or crowd together, they can send signals that regulate or inhibit further differentiation, helping to maintain tissue organization and balance.

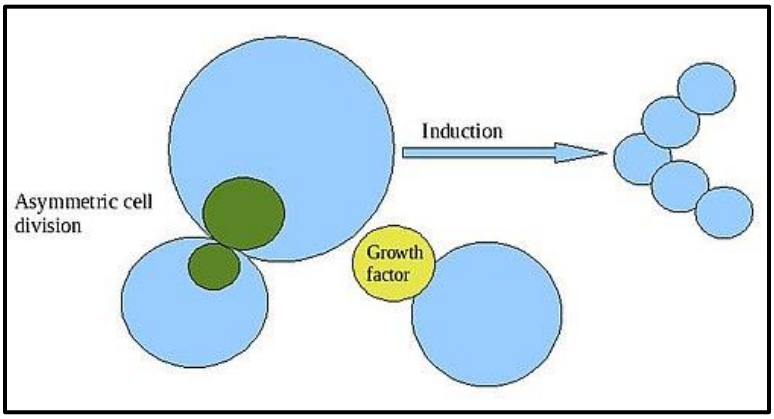


Figure 39: Mechanisms of cellular differentiation.

5.1.Gene regulatory networks

In multicellular organisms, each specialized cell type expresses only a specific subset of the total genes present in the genome. What distinguishes one cell type from another is its unique pattern of gene expression, which is tightly controlled.

Cell differentiation involves a transition from one gene expression profile to another, leading a cell to acquire a new identity and function. This transition is regulated by complex systems known as gene regulatory networks (GRNs).

A gene regulatory network is composed of interconnected genes, particularly regulatory genes and their cis-regulatory elements. These act as nodes within the network: they receive inputs (such as signals from transcription factors or environmental cues) and generate outputs that affect the expression of other genes. Through this interaction, GRNs control the timing, location, and level of gene expression during development.

The systems biology approach in developmental biology focuses on understanding how these genetic and molecular mechanisms interact to produce organized and predictable patterns in developing organisms—a process known as morphogenesis.

However, an alternative perspective has emerged in recent research. Based on the concept of stochastic (random) gene expression, it proposes that cell differentiation may not always follow a fully predetermined program. Instead, differentiation could be influenced by Darwinian selection among cells, where random gene expression variations offer certain cells a functional advantage under specific conditions. In this view, gene and protein networks may be seen more as the outcome of cellular processes, rather than the initial drivers.

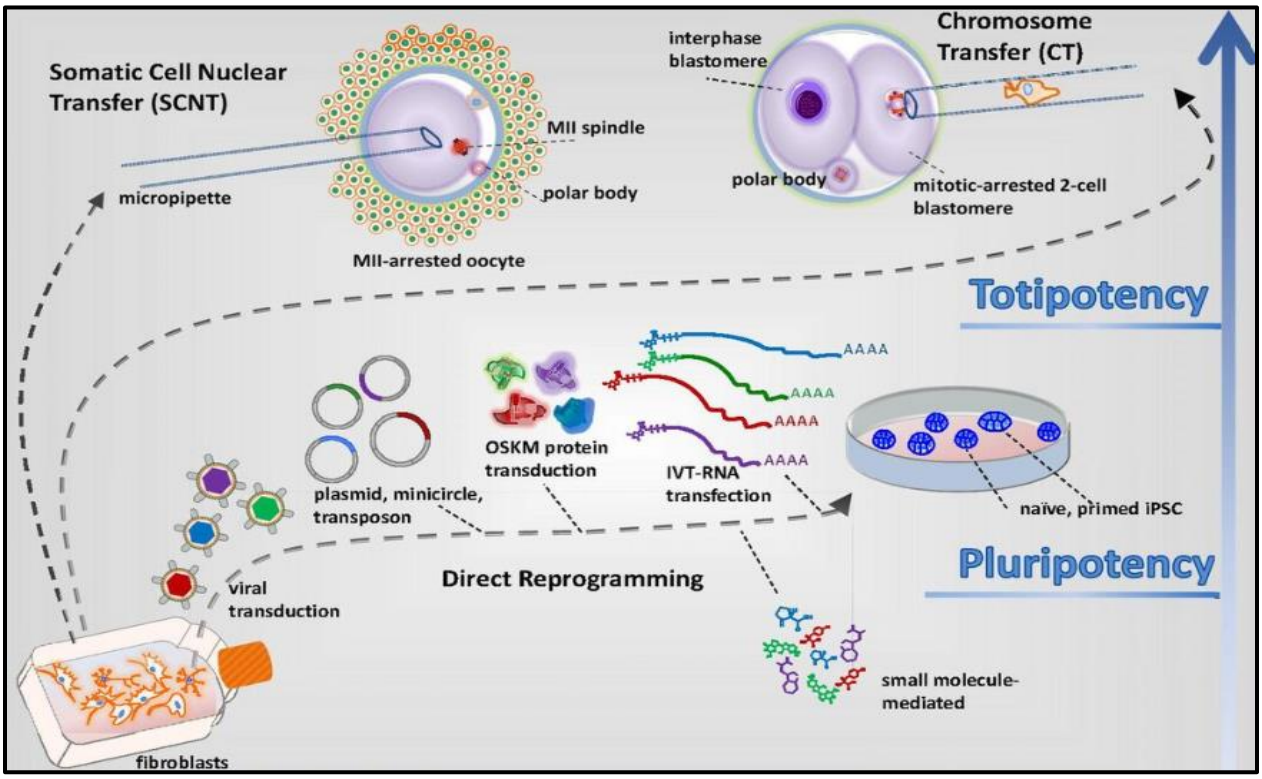


Figure 40: Diagram exposing several methods used to revert adult somatic cells to totipotency or pluripotency.

5.2.Signaling pathways

Cellular differentiation is often controlled by cell signaling. Many of the signal molecules that convey information from cell to cell during the control of cellular differentiation are called growth factors. Although the details of specific signal transduction pathways vary, these pathways often share the following general steps. A ligand produced by one cell binds to a receptor in the extracellular region of another cell, inducing a conformational change in the receptor. The shape of the cytoplasmic domain of the receptor changes, and the receptor acquires enzymatic activity. The receptor then catalyzes reactions that phosphorylate other proteins, activating them. A cascade of phosphorylation reactions eventually activates a dormant transcription factor or cytoskeletal protein, thus contributing to the differentiation process in the target cell. Cells and tissues can vary in competence, their ability to respond to external signals.

Cell differentiation involves a variety of signal transduction processes:

1. MAPK Signaling Pathway
2. Phosphatidylinositol Signaling Pathway (PLC)
3. cAMP /PKA Signaling Pathway
4. JAK-STAT Pathway
5. PI3K-AKT-mTOR Signaling Pathway
6. Notch Signaling Pathway
7. Wnt Signaling Pathway
8. TGF-β Superfamily Signaling Pathway

5.2.1. MAPK Signaling Pathway

MAPK is a mitogen-activated protein kinase, a class of protein kinases with dual phosphorylation of serine and tyrosine in the cytosol. The MAPK signaling pathway activates transcription factors and regulates gene expression through a cascade reaction (MAPKKK-MAPKK-MAPK). MAPK can cause the activation of transcription factors in the nucleus, participate in the process of signal transmission from the cell surface to the nucleus, and regulate cell proliferation and differentiation.

Currently, there are 4 known MAPK signaling pathways, including the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK, also known as SAPK), p38 and ERK5 pathways.

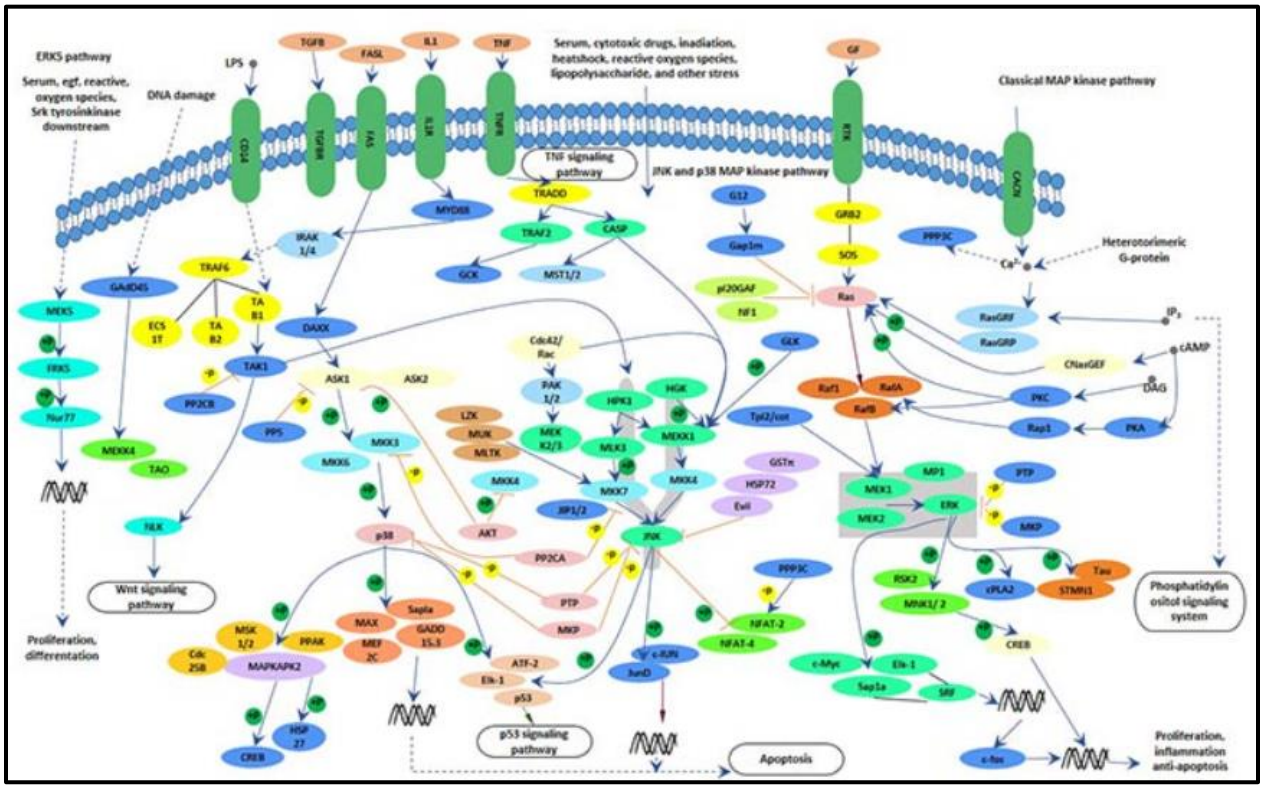


Figure 41: MAPK signaling pathway.

5.2.2. Phosphatidylinositol Signaling Pathway (PLC)

In the phosphatidylinositol signaling pathway, extracellular signaling molecules bind to G-protein-coupled receptors, activating phospholipase C (PLC-β) on the plasma membrane, which makes the phosphatidylinositol (4, 5) bisphosphate (PIP2) hydrolyzed to inositol 1, 4, 5-trisphosphate (IP3) and diacyl glycerol (DG). Therefore, the phosphatidylinositol signal pathway (PLC) is also called "double messenger system".

IP3 turns on the calcium channel and initiates downstream signals. Ca^{2+} binds to calmodulin (CaM) to form a Ca^{2+}-CaM complex, which activates adenylate cyclase (AC) and phosphodiesterase (PDEs); activates Ca^{2+}-CaM-dependent protein kinase.

DG activates PKC, phosphorylates the serine/threonine residues of proteins, and produces different cellular responses, such as cell secretion, muscle contraction, cell proliferation and differentiation. PLC-γ pathway is also involved in the differentiation of red blood cells.

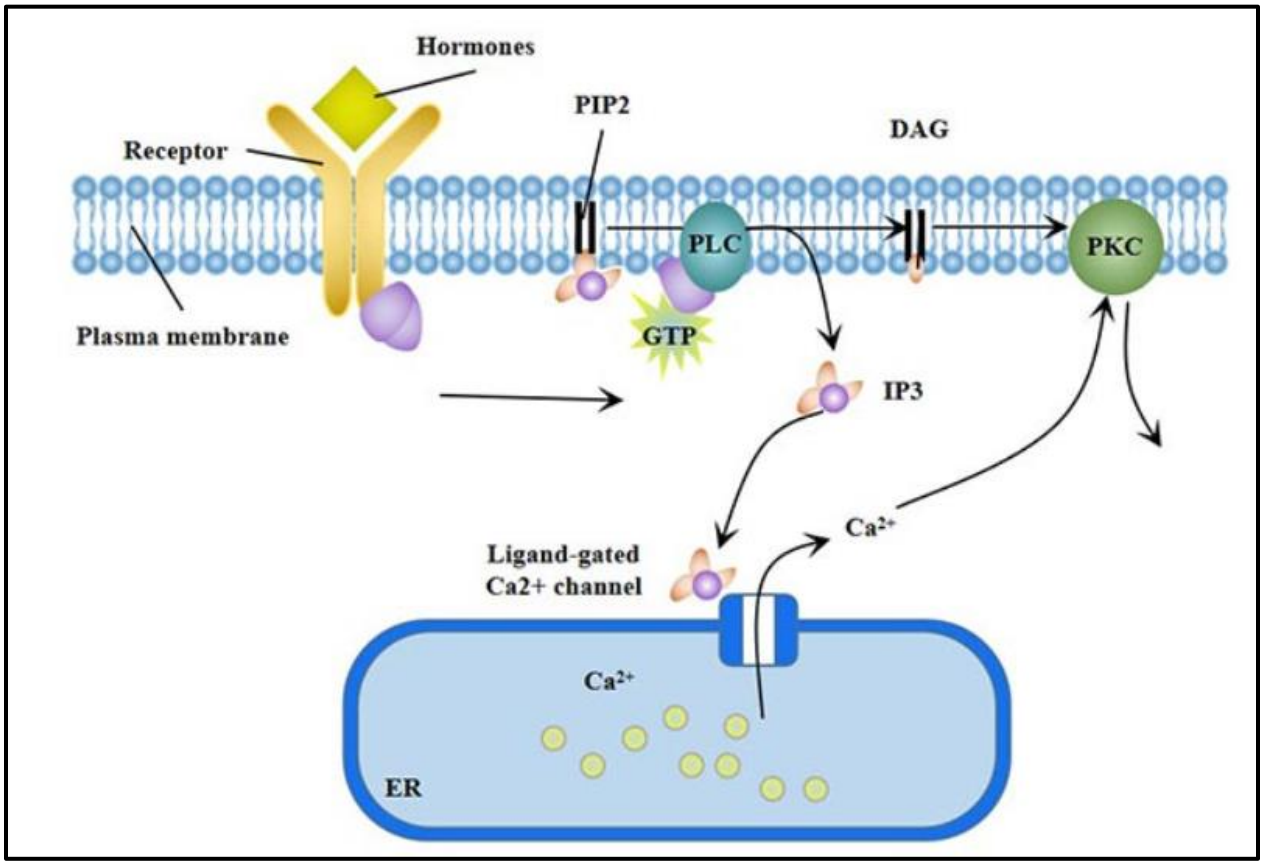


Figure 42: Phosphatidylinositol signaling pathway (PLC).

5.2.3. JAK-STAT Pathway

JAK is a tyrosine kinase whose major substrate is the transcription factor STAT. The activated STAT translocates to the nucleus and binds to the DNA sequence, thereby regulating gene expression. The JAK-STAT pathway plays an important role in cell proliferation, apoptosis and differentiation.

The main process of this signal pathway is as follows:

Ligand binding to the receptor leads to the dimerization of the receptor. Dimerized receptors activate JAK, JAK phosphorylation STAT. Phosphorylated STAT forms dimers that enter the nucleus and bind to DNA sequences to regulate gene expression.

The JAK/STAT pathway plays an important role in the proliferation and differentiation of red blood cells.

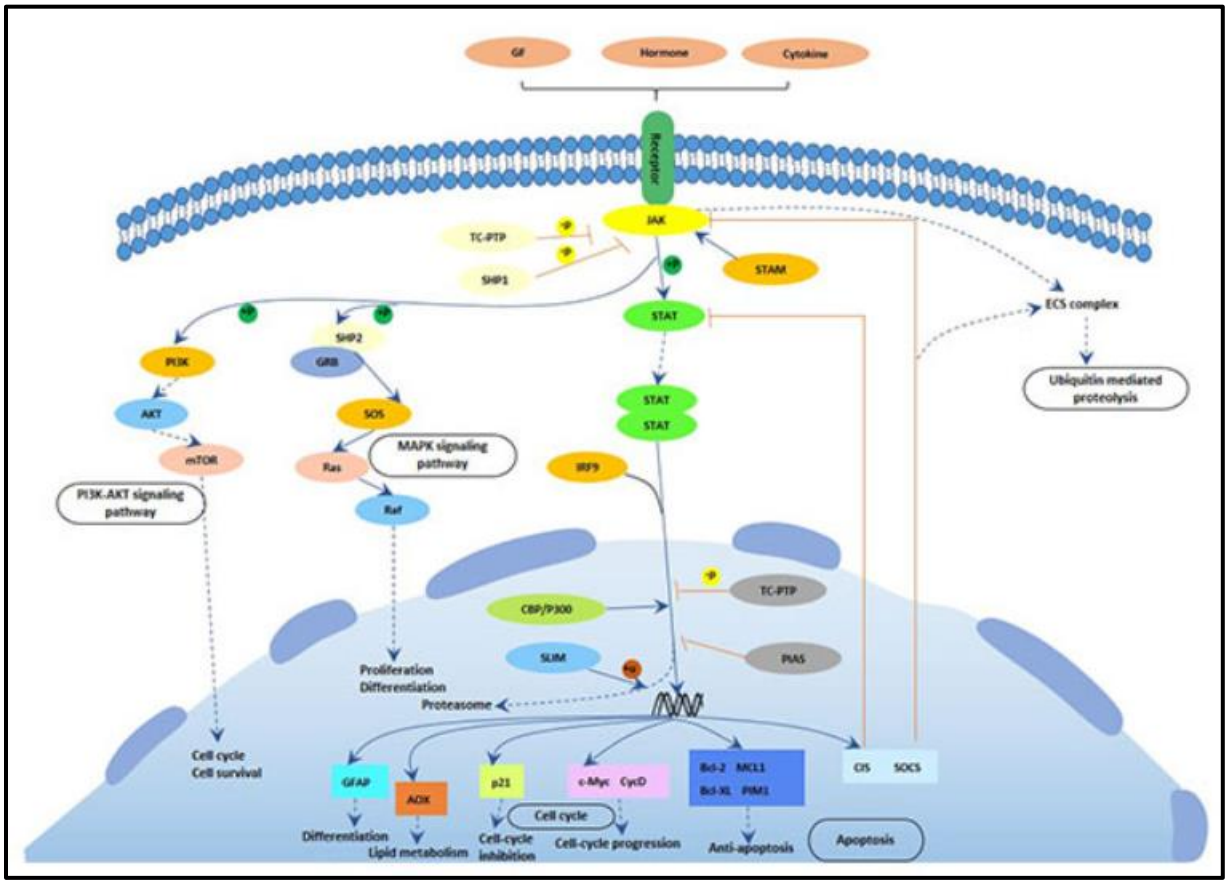


Figure 43: JAK-STAT pathway.

5.2.4. PI3K-AKT-mTOR Signaling Pathway

Mammalian target of rapamycin (mTOR) is a conserved serine/threonine protein kinase with two main forms: mTORC1 and mTORC2. Activated mTOR plays a key regulatory role in cell proliferation, differentiation and metabolism.

mTOR is mainly regulated by the PI3K/Akt/mTOR signaling pathway and LKB1/AMPK/mTOR signaling pathway. These two signaling pathways are the main pathways regulating the proliferation and differentiation of testicular supporting cells. In the mTOR signaling pathway, the deletion of the mTOR gene leads to a decrease in the number of testicular supporting cells.

Studies have shown that PI3K-activated Akt kinase plays an important role in hematopoiesis. The PI3K pathway is also important in the regeneration of small intestinal stem cells and in promoting cell differentiation.

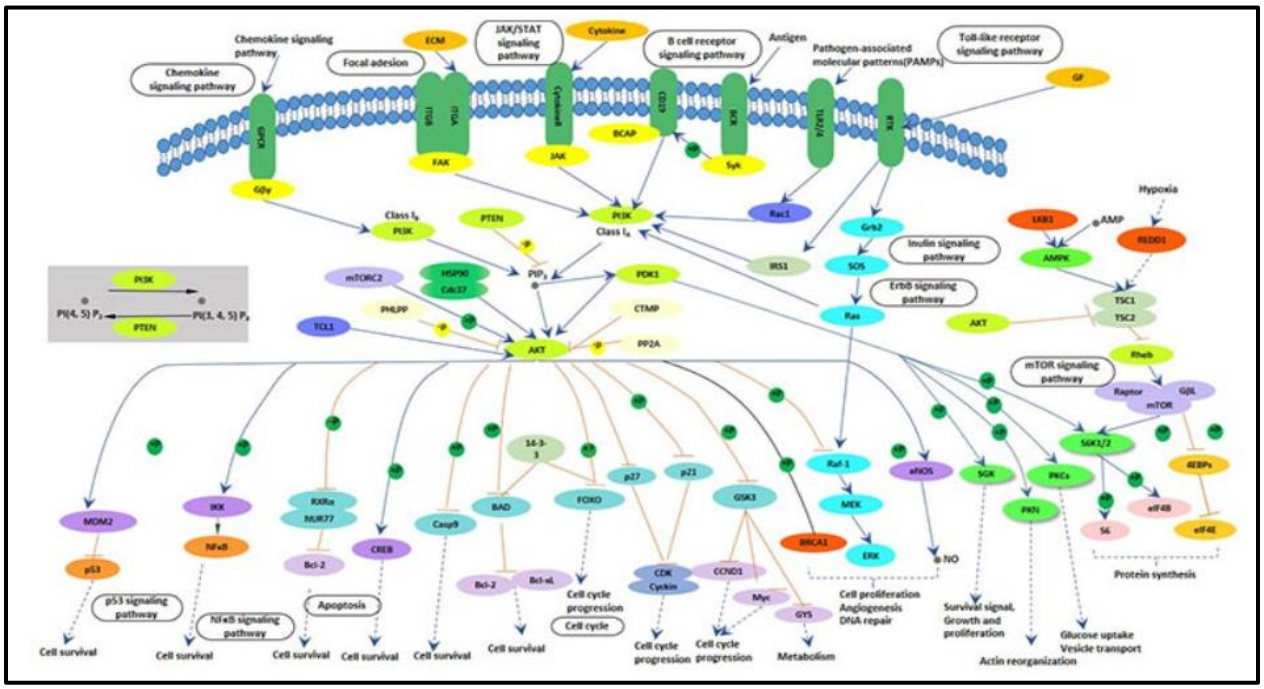


Figure 44: PI3K-AKT-mTOR signaling pathway.

6. Cell Differentiation – Errors

Abnormalities in cell differentiation can be categorized into three classes: anaplasia, dysplasia, and metaplasia.

Anaplasia: it refers to the loss of apparent differentiation which can take place in advanced stages of cancer. Early stages of cancer appear to mimic the tissue from which they originated and are organized by their differentiation pattern. With development further, variants of more irregular features are generated along with expanding malignancy. Ultimately, a highly anaplastic growth takes place where the cancerous cells have no association visibly to the parent tissue.

Dysplasia: refers to the cell arrangement typically emerging from distress in their regular growth pattern. While few are precursor lesions to cancer, some others are innocuous and relapse immediately. For instance, CIN (cervical intraepithelial neoplasia).

Metaplasia: one cell type is converted into another. Typically, it occurs when chronic damage to the tissue is succeeded by extensive regeneration. For instance, squamous metaplasia of the bronchi.

Chapter 04: Intracellular Trafficking

1. Vesicular transport

Vesicular transport is one of the most remarkable examples of cellular dynamics. It shows the extent to which the many organelles, in this case of the endomembrane system, interact and communicate with each other and with the outside of the cell through the transport vesicles. There are three pathways in vesicular transport:

- ❖ biosynthesis-secretion pathway;
- ❖ endocytosis pathways;
- ❖ return routes.

1.1.The stages of vesicular transport

Vesicular transport takes place in six stages:

1) Molecular sorting.
2) Budding of the vesicles from the donor compartment.
3) Fission: detachment of vesicles with a cytosolic protein coating (coatomers or clathrin). The vesicles "undress" thus allowing the interaction between the motor proteins of the cytoskeleton and the vesicles.
4) Vectorization: transport of vesicles between the donor compartment and the recipient compartment.
5) Anchoring of the vesicles.
6) Fusion of the vesicles with the acceptor (or receiver) compartment.

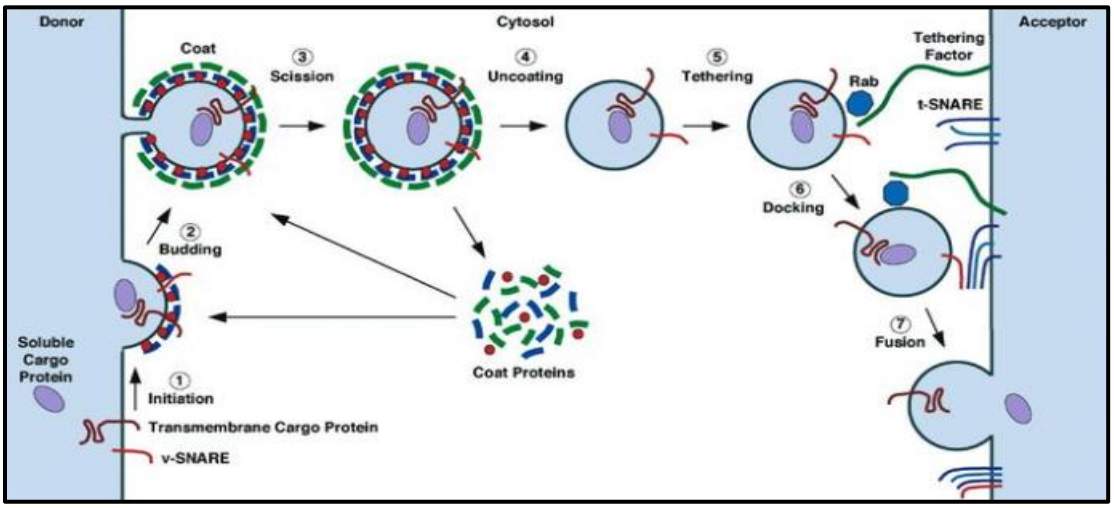


Figure 45: The stages of vesicular transport.

2. Budding, detachment of covered vesicles and loss of coating

The formation of vesicles requires the establishment of a protein coating, or mantle, on the cytosolic side. There are three types of well-characterized mantles covering the vesicles.

Each type of coating serves a different step of intracellular transport. Schematically:

- Clathrin-mantled vesicles envelop buds from the GA and plasma membrane;
- Vesicles with a COP (coating Protein) II mantle envelop buds from the ER;
- The vesicles with a COP I mantle envelop buds from the GA.
- COPI (7 subunits) and COPII (4 subunits) are also called coatomers.

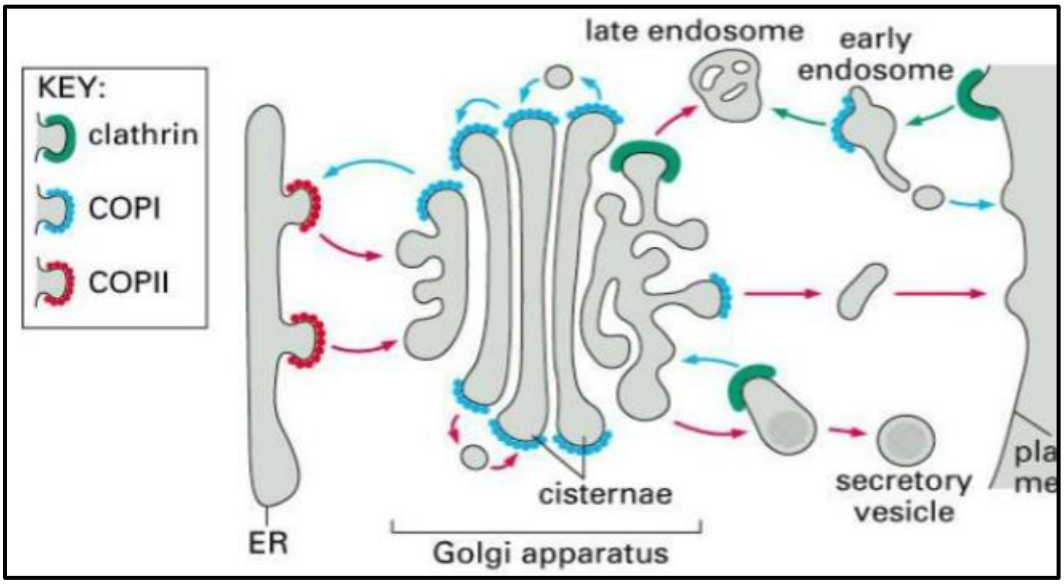


Figure 46: Use of different mantles in vesicular transport.

Red arrows: biosynthesis-secretion pathways

Green arrows: pathways of endocytosis

Blue arrows: return pathways to the plasma membrane (case of membrane receptors) and to the ER.

Several proteins participate in the formation of the coating:

- **Clathrin** = 6 protein chains (3 heavy and 3 light) organized in a 3-branched skeleton called a triskelion. The triskelions assemble in a convex basket network of hexagons and pentagons and form covered wells on the cytosolic side of the membrane.

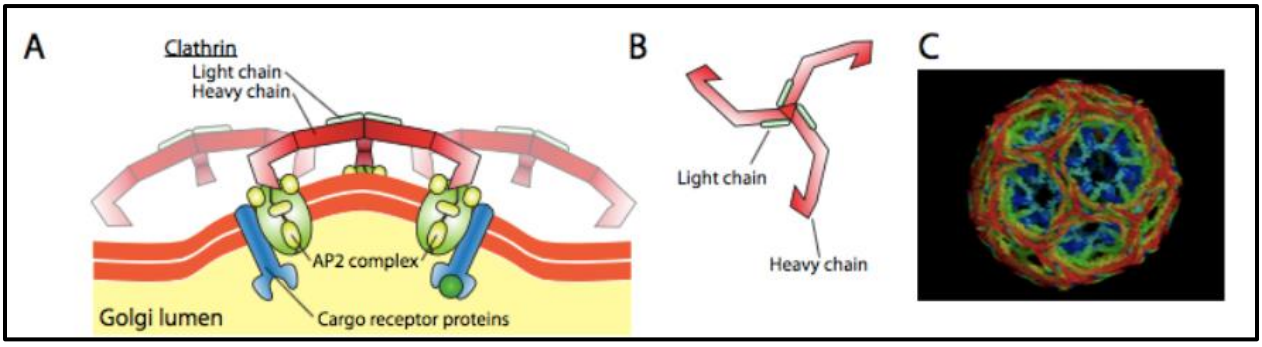


Figure 47: (A) clathrin binds to adapter proteins which are bound to transmembrane cargo receptors, linking the membrane with the clathrin. (B) A single clathrin triskelion is composed of three heavy chains and three light chains. (c) The triskelions self-assemble into a roughly spherical construct without the need for any additional energy or enzymes.

- **Adaptin** = complex with multiple subunits. It is required to unite the clathrin mantle to the membrane and to capture various transmembrane proteins, including transmembrane receptors that capture soluble loading molecules inside vesicles (= loading receptors). There are at least 4 types of adaptins, each specific to a different group of charging receptors.
- **Dynamin** is a monomeric G protein that facilitates the fusion of membranes at the "neck" of the budding vesicle. Like any G protein, it has GTPase activity.
- ARF (ADP Ribosylation factor) is a monomeric G protein that participates in the formation of vesicular coatings.

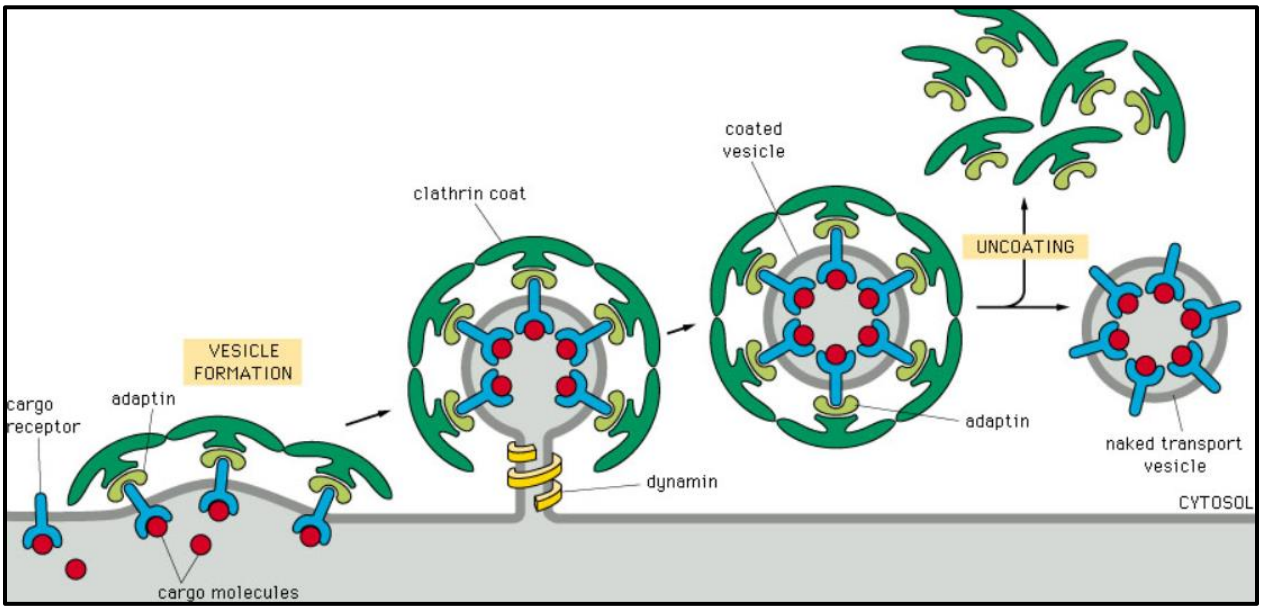


Figure 48: Steps in Clathrin Mantle Assembly and Disassembly.

Clathrin, COP I and II are distinguished by the intervention of different assembly G proteins:

- ARF is combined with COP I and clathrin coatings;
- Sar1 is associated with COP II coatings.

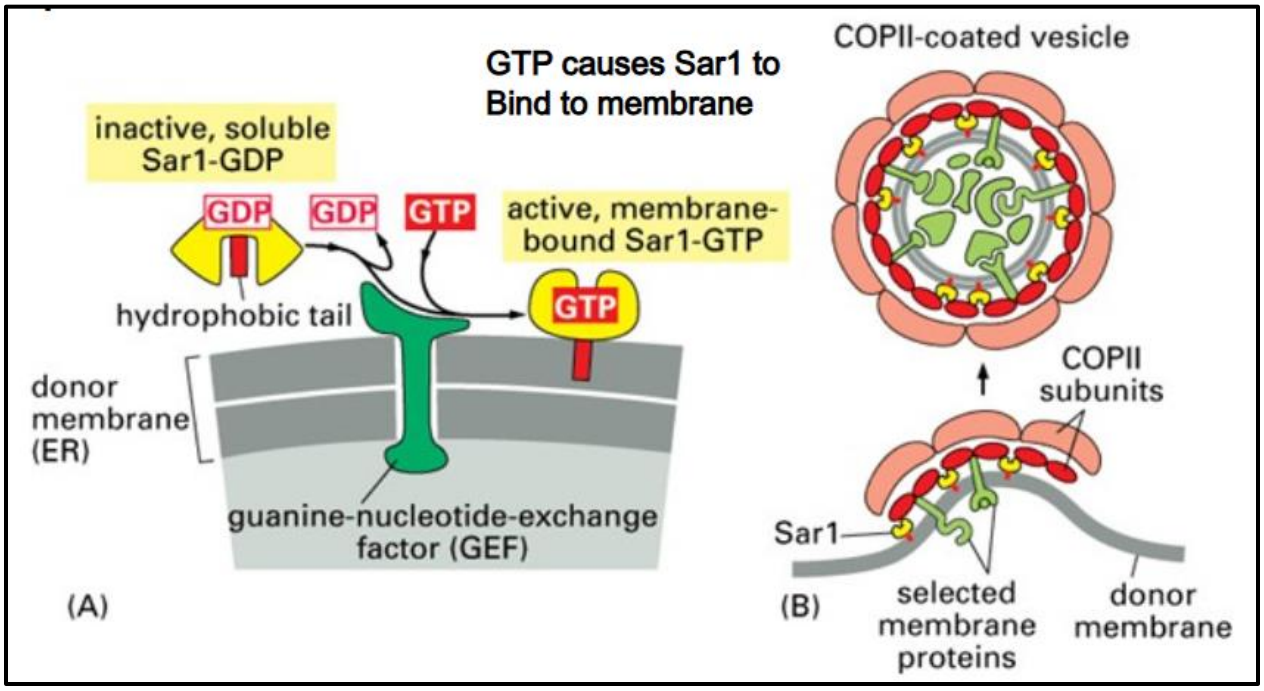


Figure 49: Formation of COP II-covered vesicles.

(A) Inactive Sar1-GDP binds to a GEF causing the GDP to be released by Sar1 and a GTP to be fixed. Sar1 then changes conformation, which allows the insertion of the hydrophobic tail into the membrane of the ER.

(B) Sar1-GTP, then active, recruits the COPII subunits on the membrane which causes the formation of a budding.

ARF works on the same principle except that it is a chain of fatty acids, covalently bound to ARF, which allows insertion into the membrane of the ER.

Summary of the different stages (for COPI and Clathrine):

1) Recruitment of adaptins and clathrin by RFA.
2) Polymerization and deformation of the coating inducing the budding of the vesicle.
3) Detachment of the covered vesicle by hydrolysis of GTP by dynamin. Undressing of the gallbladder under the action of an Hsp 70 which consumes ATP.

3. Vesicle transport

Once undressed, the vesicle is transported through the cytosol to the recipient compartment. Long-distance transport involves microtubules and motor MAP (Microtubules Associated Proteins): kinesin and dynein.

The actin microfilaments of the cortex and their associated proteins (gelsolin, short-tailed myosins) take over when approaching the plasma membrane (see Chapter 3: The cytoskeleton).

3.1. Addressing/Vectoring of Vesicular Proteins: Examples

The proteins carried in the vesicles have addressing signals that are necessary for them to reach their final destination.

Example 1: Addressing and retention signals in the ER Resident chaperone proteins of the ER such as BiP or PDI carry an addressing and retention signal in the ER located at the C-terminal end of their protein part: The KDEL (Lys-Asp-Glu-Leu) motif.

These proteins leave the ER and are transported to the GA to perfect their maturation. In the cis and median GA, the KDEL sequence is recognized by a family of membrane receptors that bind to mature ER proteins and return them to this compartment.

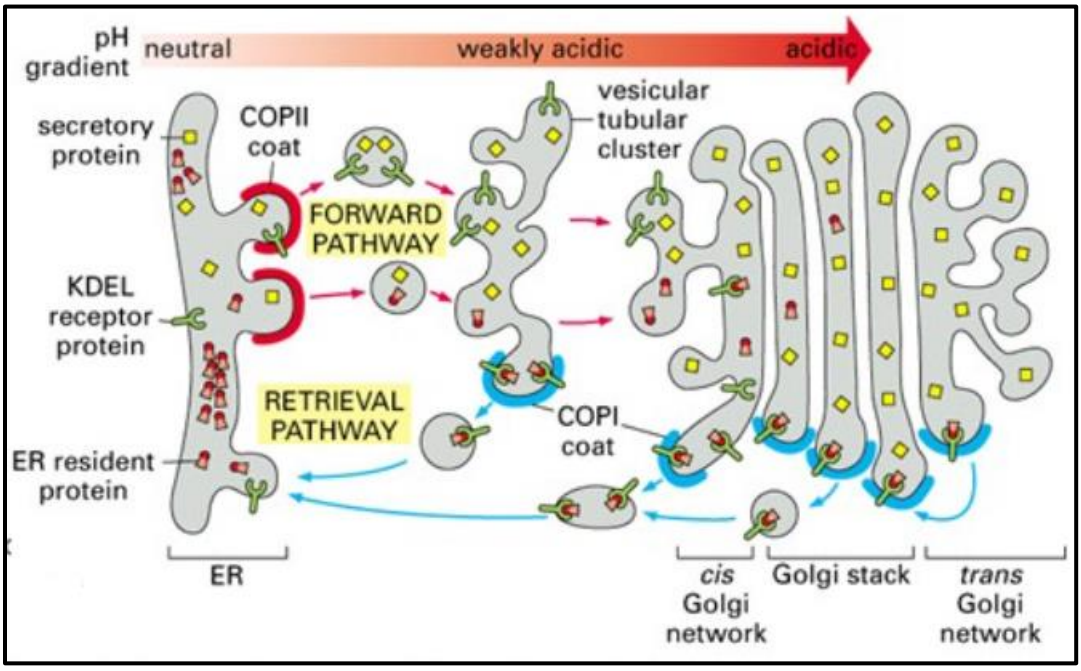


Figure 50: Role of the KDEL sequence in the reuptake of ER resident proteins.

Recognition of the KDEL motif of ER resident proteins by KDEL receptors. The return to the ER is ensured by vesicular transport. The reuptake of proteins to the ER takes place at several levels of the Golgi apparatus. In the ER (or neutral pH), proteins dissociate from the KDEL receptor which is then sent into the Golgi apparatus for reuse.

Example 2: **Lysosome Addressing Signals**

(1) Addition of the mannose-6-phosphate motif.

The soluble enzymatic glycoproteins of lysosomal lumen possess a mannose-6-phosphate (M6P) which allows them to be addressed to lysosomes.

The biosynthesis of these enzymatic glycoproteins (= enzymes... and therefore hydrolases in the case of lysosomes...) follows the following diagram:

1) biosynthesis of the protein chain in the ER: obtaining a lysosomal precursor of hydrolases;

2) N-glycosylation and modification of the sweet arborization in the rough endoplasmic reticulum;

3) vesicular transport to the Golgi apparatus;

4) modification of the sweet arborization by a GlcNAc phosphotransferase in the Golgi apparatus with the addition of an M6P.

M6P-carrying enzymes are attached in the trans Golgi apparatus by the M6P receptor. The M6P receptor addresses enzymes to the endosomal compartment (receptor-dependent transport).

In endosomes, enzymes detach from their receptor under the effect of acidic pH. The enzyme matures after phosphate removal from the mannose. The M6P receptor is recycled to the GA via clathrin-coated vesicles.

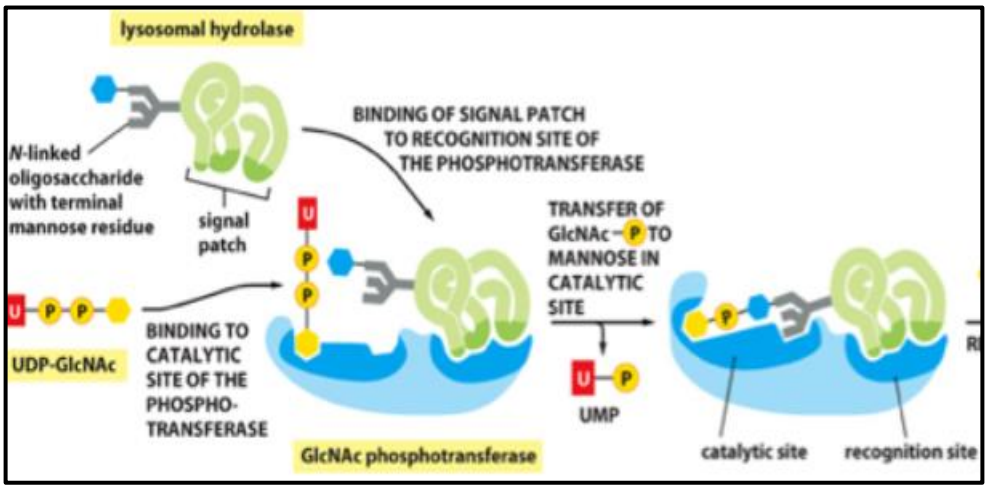


Figure 51: Management of lysosomal hydrolase by GlcNAc phosphotransferase

GlcNAc phosphotransferase, an enzyme in the Golgi apparatus, has:

- a catalytic site that binds the mannose-rich oligosaccharide and UDP-GlcNAc;
- a recognition site that fixes a signal motif present only on the surface of lysosomal hydrolases.

The GlcNAc is then cut by a second enzyme, leaving the M6P exposed on the hydrolase (not shown here).

(2) Addressing of the Mannose-6-Phosphate Labeled Enzyme.

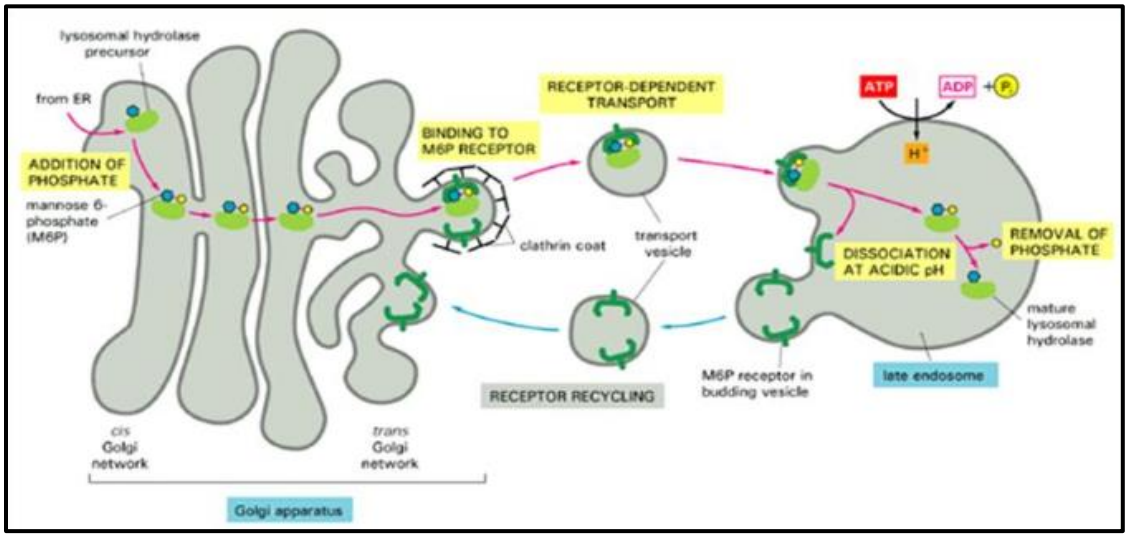


Figure 52: Transport pathway of lysosomal hydrolases to lysosomes.

4. Vesicle/recipient compartment fusion

To ensure that membrane transport takes place in an orderly manner, vesicles must be highly selective to recognize the correct target membrane with which they should fuse.

The specificity of the addressing is ensured by the display, on all transport vesicles, of surface markers that identify them according to their origin and type of load.

At the same time, the target membranes display complementary receptors that specifically recognize these markers.

This recognition step is controlled by two main classes of molecules: SNARE and Rab monomeric G proteins.

• SNARE (Soluble NSF Acceptor REceptor)

SNARE are transmembrane proteins that belong to 20 different groups in animal cells. There are two complementary groups:

- The v-SNARE, located on the vesicles (v for vesicle);
- The t-SNAREs, located on the target compartments (t for target).

Each v-SNARE specifically interacts with a complementary t-SNARE to form a trans-SNARE complex that together blocks the membranes of the vesicle and the target compartment.

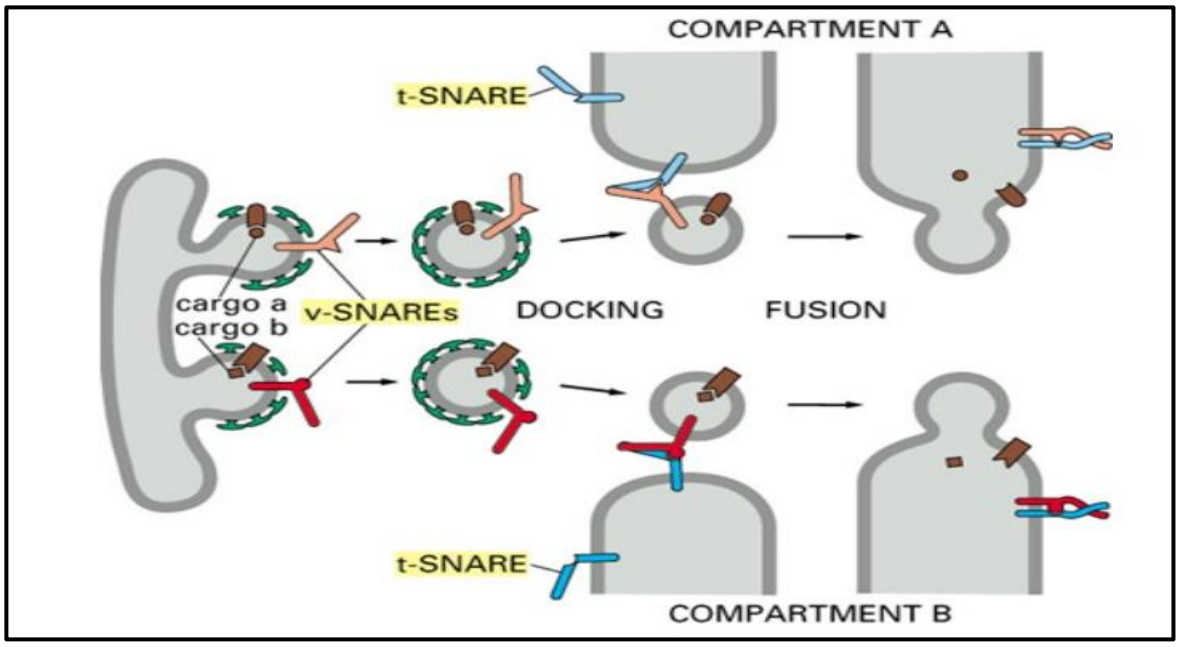


Figure 53: Role of SNAREs in vesicle addressing.

It is therefore the specificity of the SNARE interaction that determines the specificity of vesicular docking and fusion. A key protein, NSF, cycles between membranes and the cytosol and catalyzes the disassembly process between v-SNARE and t-SNARE. It is a cytosolic chaperone protein with ATPasic activity.

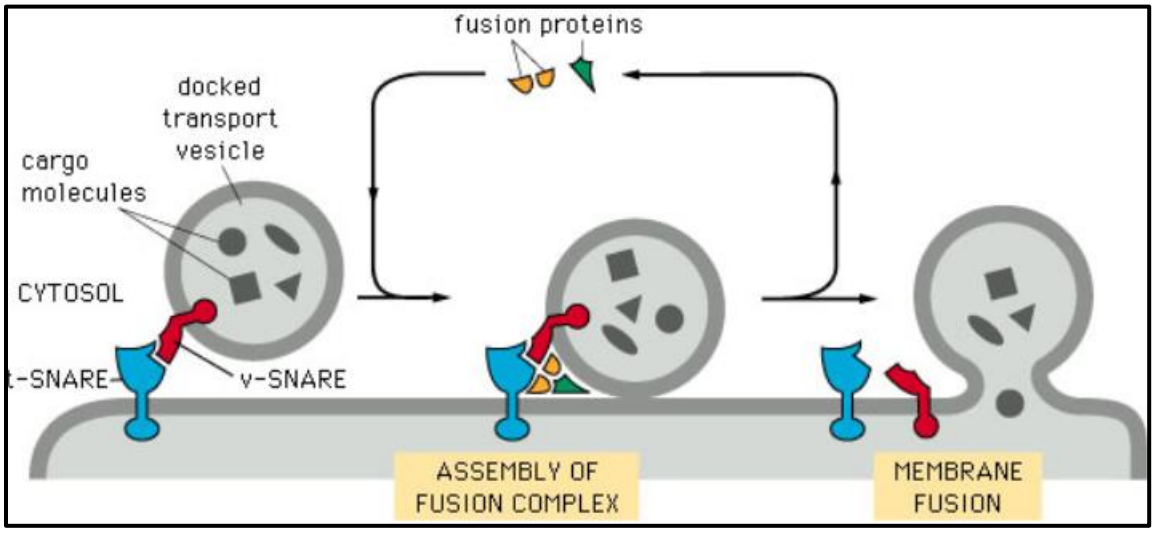


Figure 54: Dissociation of SNAREs by NSF after vesicle/membrane fusion.

After v-SNARE/t-SNARE interaction and membrane fusion, NSF binds to the SNARE complex, via adaptor proteins, and hydrolyzes ATP to detach SNAREs.

- **The monomeric G protein Rab**

Rab proteins strongly contribute to vesicular transport specificity. These are monomeric G proteins that have more than 30 known members. Like SNAREs, they are distributed specifically on the cytosolic surface of cell membranes.

Rab facilitates and regulates the speed of vesicular stowage and the correspondence between v-SNARE and t-SNARE. Like the G proteins that control mantle recruitment (ARF and Sar1), Rab performs a cycle between the membrane and the cytosol.

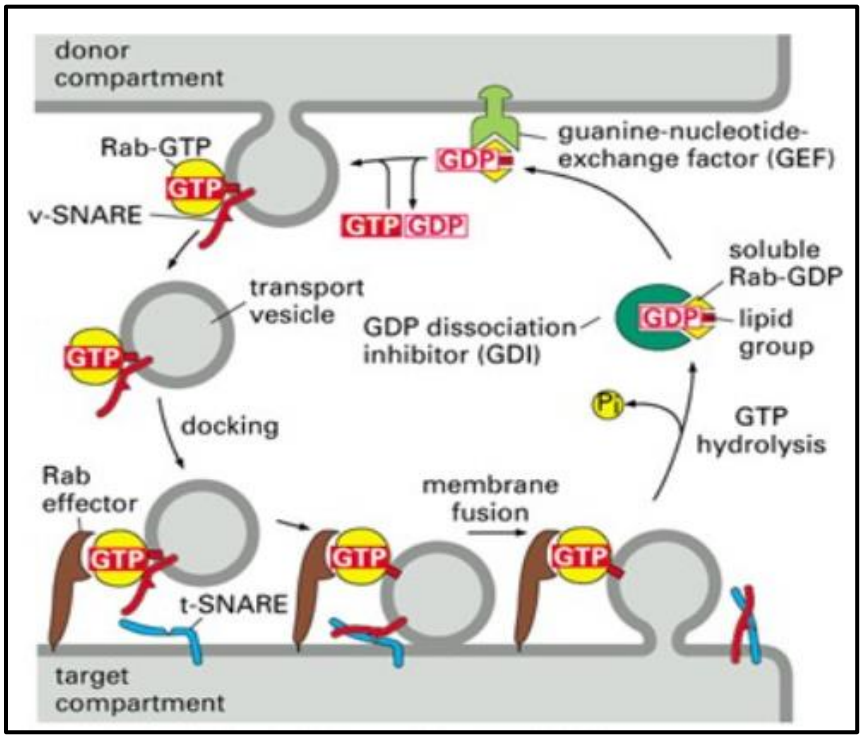


Figure 55: Involvement of Rab proteins in facilitating the docking of transport vesicles.

Chapter 05: Regulation and deregulation of the cell cycle

I. The Cell Cycle and Mitosis

1. General

The cell cycle is a series of organized and controlled events in which two daughter cells identical to the parent cell are generated. During the cell cycle, the cell performs 4 essential tasks:

- Duplication of organelles and macromolecules,
- DNA replication,
- Segregation of chromosomes into 2 identical batches,
- Separation in 2 by cytoplasmic pinching.

The cell cycle has 2 stages:

- Interphase (in 3 phases: G1, S and G2);
- Mitosis (in 6 phases: prophase, prometaphase, metaphase, anaphase, telophase and cytokinesis).

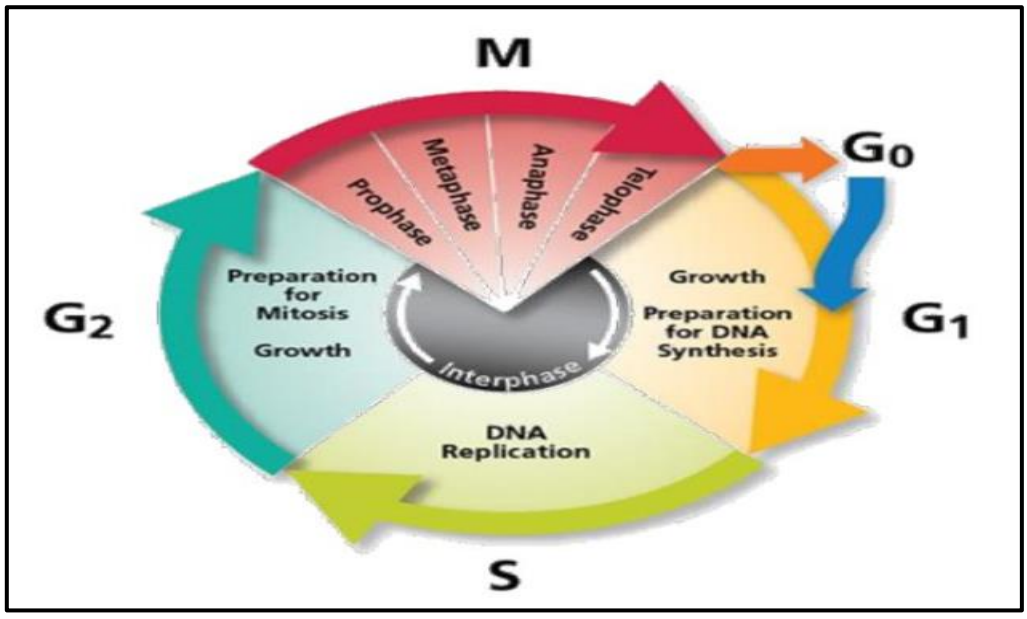


Figure 56: The different phases of the cell cycle.

Purpose of the cell cycle: to ensure cell proliferation, tissue growth and/or to replace dead cells (natural or accidental death).

The cell cycle concerns somatic cells but also germ cells before gametogenesis.

2. Cell cycle length

The length of the cell cycle is highly variable. Examples: 30 min in the xenopus embryo; 12 hours for intestinal cells; 1 year for liver cells...

In a human cell in culture, the interphase lasts an average of 23 hours. This is the period between two cell divisions. It consists of a G1 phase, an S phase and a G2 phase. The durations of the S (10-12 h) and M (1 h) phases are relatively constant. The durations of the G2 and especially G1 phases vary greatly.

3. Variation in the amount of DNA during the cell cycle

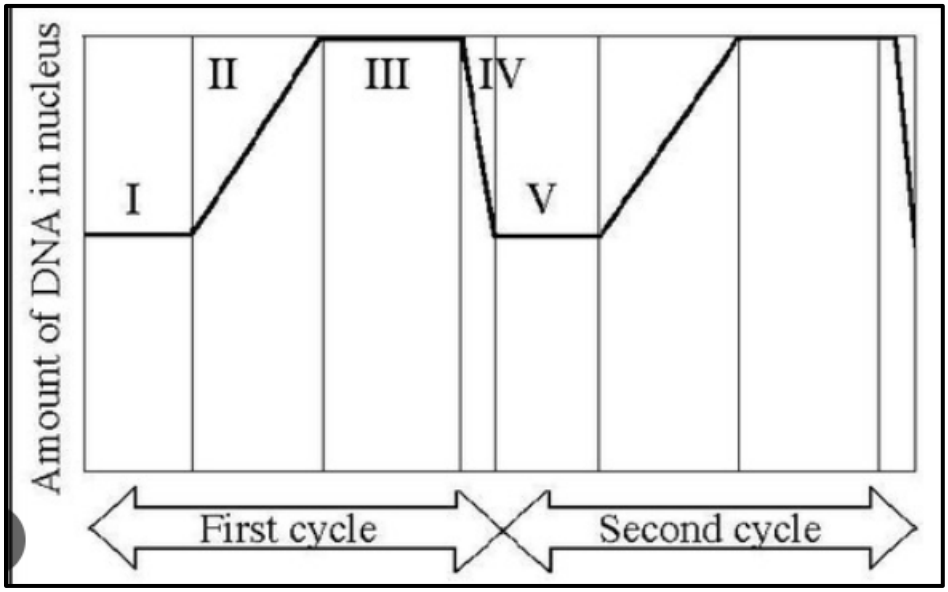


Figure 57: Variation in the amount of DNA during the cell cycle.

This variation concerns cells with 2n chromosomes called diploid. We will notice the multiplication by 2 of the amounts of DNA during the S phase (the chromosomes go from 1 to 2 chromatids), the stage of the cell cycle where DNA replication takes place.

4. The interphase

It is broken down into 3 phases: G1, S and G2.

a. Phase G1

The growth and replenishment phase during which the cell synthesizes RNA (transcription) and proteins (translation). It is also a "decision" phase.

b. Phase S

A phase essentially characterized by DNA replication. However, transcription still takes place (especially for histone genes).

DNA replication is semi-conservative: it results in the formation of two DNA molecules, each containing an old strand (= parent strand) and a new strand (= child strand = neoformed strand).

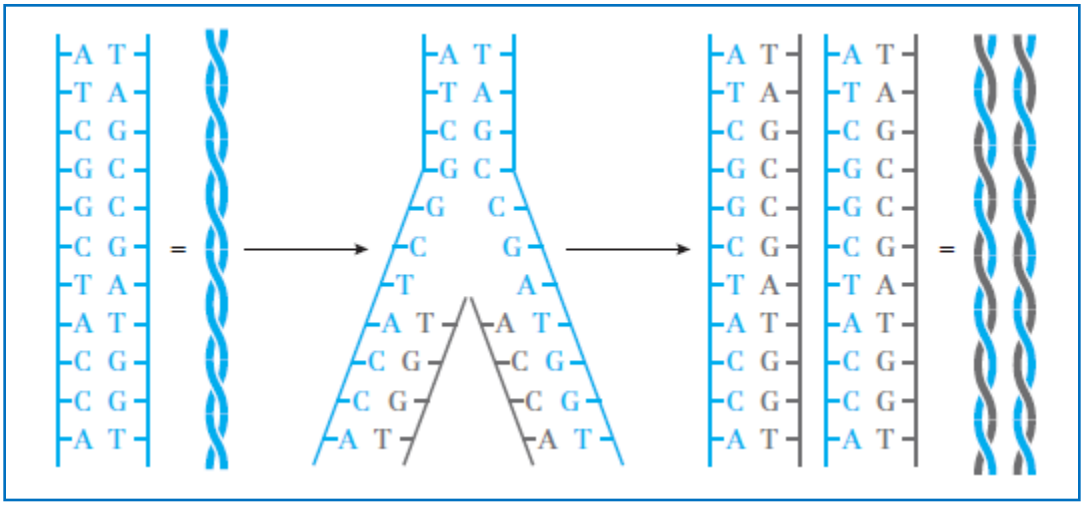


Figure 58: Simplified mechanism of DNA replication.

We also observe the establishment of cohesin = ring-shaped protein complex that encloses the two sister chromatids.

Thus, as the DNA is replicated, the two chromatids remain together.

c. Phase G2

It is a phase of waiting and control before initiating mitosis.

5. The centriolar cycle

5.1. Definition and role of the centrosome

Centrosome = Microtubule Organizing Center (MT) or MTOC. The centrosome consists of 2 centrioles perpendicular to each other and pericentriolar material. It is not bounded by a membrane and is located outside the nucleus, in the center of the cell of the majority of nucleated cells. Centrioles are composed of particularly stable TMs.

❖ ***Role of the centrosome***

1) Interphase: In G1 and G0, the network of microtubules is organized around a single centrosome often located in a central position near the nucleus. It allows the nucleation of microtubules and organizes the shape and polarity of the cell.

2) During mitosis: the centrosome, after duplication, forms the 2 poles of the mitotic spindle and thus ensures the placement of the spindle.

5.2. Centrosome Duplication

Just like DNA replication, centrosome duplication:

- takes place during phase S;
- is a semi-conservative phenomenon;
- is controlled, in part, by similar complexes (Cyclin S-Cdk complex –).

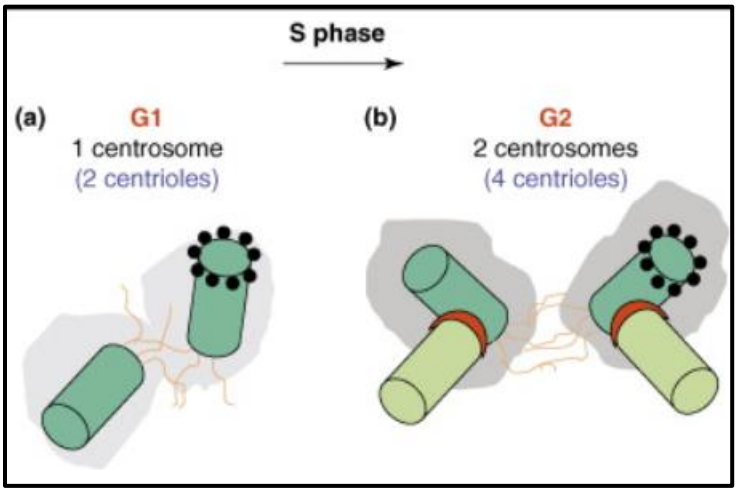


Figure 59: Centrosome Duplication.

6. The phases of mitosis

In the G2 phase, chromosomes are duplicated but decondensed and appear as chromatin fibers. The transition from an interphase state to a mitotic state is triggered and controlled by the sudden activation of the kinase activity of the MPF (Mitosis Promoting Factor): This is the G2/M transition = transition from the G2 phase of interphase to the entry into mitosis.

6.1. Prophase

- Condensation of duplicated chromatin fibers and disappearance of the nucleolus.
- Placement of kinetochores.
- Formation of mitotic microtubules.
- Centrosome separation.

Chromatin condensation

The chromosomes compact and become visible under a light microscope. The transition from an interphase chromatin fiber to a metaphase chromosome (50 times shorter) is related to:

- post-translational modifications of histones and structural proteins;
- the association with DNA of different proteins in interphase and mitosis.

These changes are controlled directly or indirectly by the MPF (Mitosis Promoting Factor = Mitosis Promoting Factor).

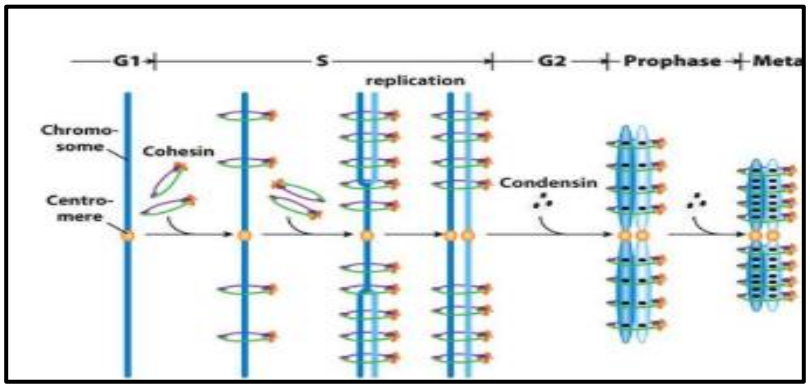


Figure 60: Roles of cohesins and condensins during the cell cycle.

a) Increased nucleosome compaction

Following histone phosphorylation:

- H1 histones are phosphorylated by MPF;
- H3 histones are phosphorylated by an MPF-activated kinase.

b) Individualization of the two chromatids

By topo-isomerase II which cuts, re-welds and unravels the DNA. Topoisomerase II is located at the base of DNA loops.

c) Formation of DNA loops (= overcoiling)

By condensins: condensin forms a ring enclosing the compaction loops of the same chromatid. Condensin is phosphorylated by MPF, which allows it to bind to DNA in prophase (it is cytoplasmic in interphase).

d) Shortening of the chromosomal axis

By the cohesines. These form a ring enclosing two chromatids.

2. Training of kinetochores

Kinetochores are a set of proteins that organize themselves on the centromere. The kinetochorian microtubules cling to the kinetochores.

3. Placement of the mitotic spindle

The spindle is set up in three steps:

a. Destruction of the interphase microtubule network and establishment of new, more dynamic microtubules.
b. Separation of duplicated centrosomes.
c. Structuring the spindle.

These steps take place thanks to the coordinated action of motor proteins of two kinds: kinesins (motor +) and dyneins (motor –).

6.2. Prometaphase

- Rupture of the nuclear envelope.
- Penetration of the MTs of the spindle into nuclear space.
- Capture of chromosomes by spindle MTs (= kinetochorian MTs).

Prometaphase is mainly characterized by the rupture of the nuclear envelope. This rupture is due to the activation of MPF. Thus, the steps leading to the rupture of the nuclear envelope are as follows:

1. Enabling MPF.
2. Phosphorylation of laminates by MPF.
3. Depolymerization of laminates and nuclear lamina.
4. Rupture of the nuclear envelope.

The rupture of the envelope has two consequences:

- allow microtubules to come into contact with chromosomes;
- allow the addition of nuclear factors stabilizing the spindle.

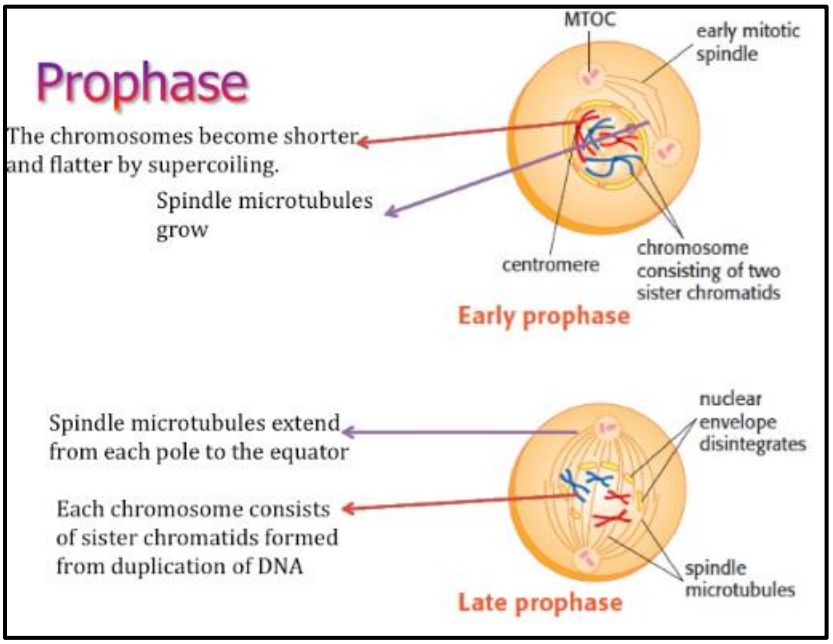


Figure 61: Prophase.

6.3. Metaphase

- Bipolar attachment of chromosomes.
- Formation of the metaphase plate.

In metaphase, all chromosomes are attached bipolarly and arranged on the equatorial plane to form the metaphase plate. Chromosomes oscillate around an equatorial position. The chromosomes are arranged in this way and maintained at the level of the mitotic spindle, which is composed of three kinds of microtubules:

- polar microtubules;
- asterian (or astral) microtubules;
- kinetochorian microtubules.

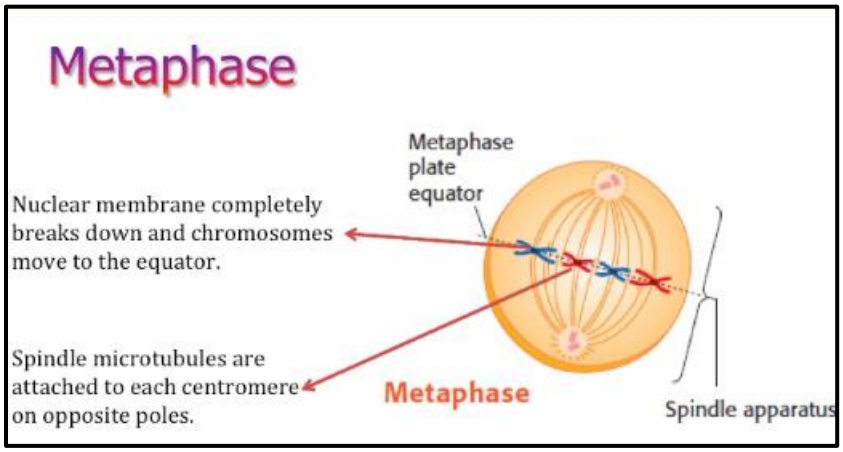


Figure 62: Arrangement of chromosomes and mitotic spindle during metaphase.

Although subjected to opposite tensions from the kinetochorian microtubules, the sister chromatids remain associated thanks to cohesin.

6.4. Anaphase

- Separation of sister chromatids.
- Movement of the child chromosomes towards the poles.

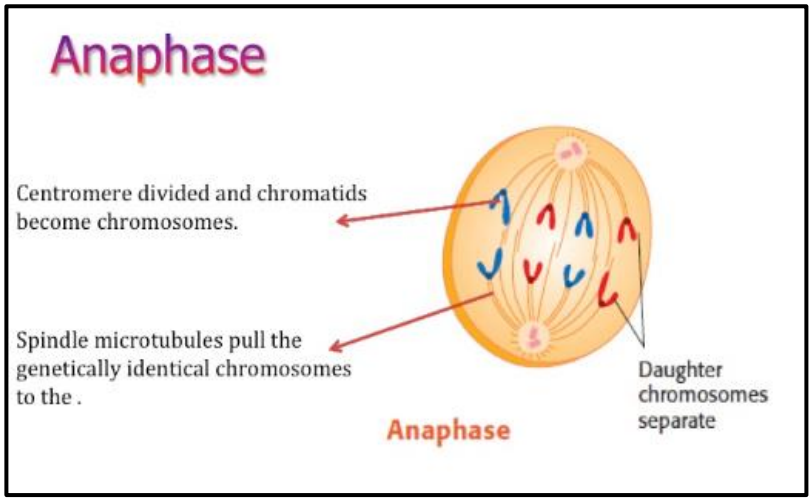


Figure 63: Anaphase.

The purpose of anaphase is to separate the sister chromatids of each chromosome and move the two identical batches of chromosomes to the poles of the spindle. Two mechanisms are needed to accomplish this:

1) Degradation of the cohesins that maintain the two sister chromatids associated. Cohesin is degraded by separation.

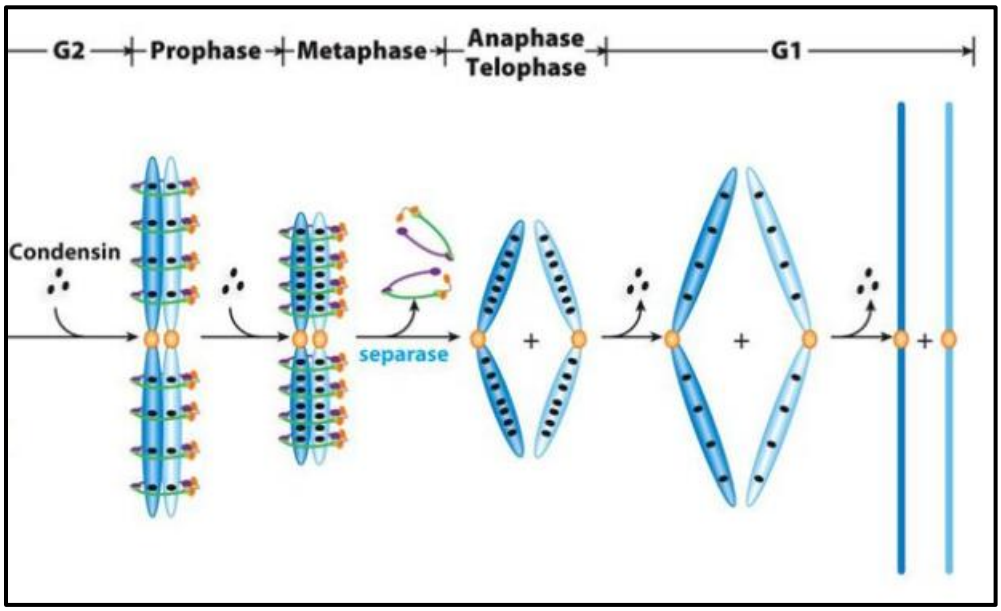


Figure 64: Consequence of the degradation of cohesins by separation.

2) Implementation of tensile forces to pull the sister chromatids towards the opposite poles.

- During anaphase A, the kinetochorian microtubules depolymerize and the length of the spindle remains constant.
- During anaphase B, the centrosomes move away and the spindle lengthens.

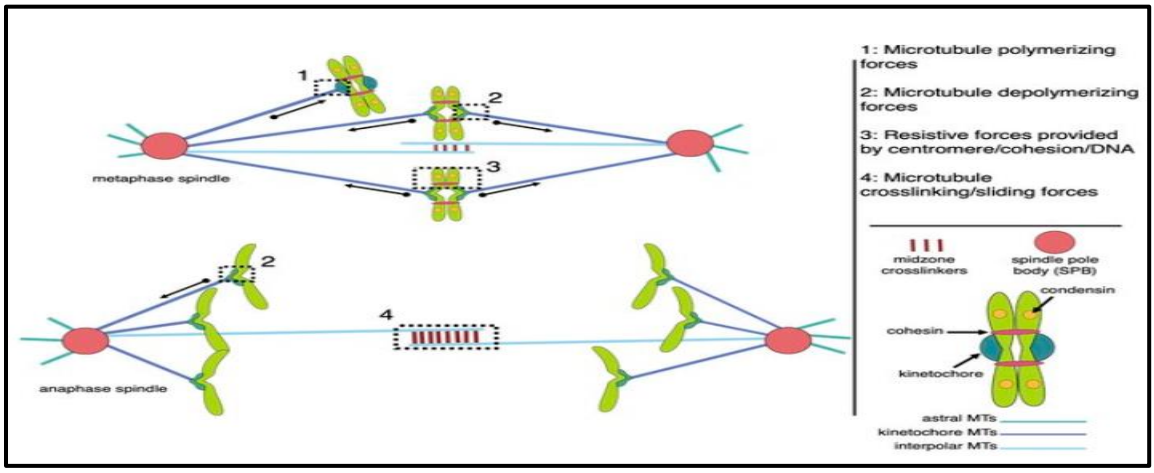


Figure 65: Chromosome separation forces during anaphase

Anaphase A is triggered first, then the two phases are concomitant.

6.5. Telophase

- Arrival of the child chromosomes at the poles.
- Reconstitution of the nuclear envelope.
- Decondensation of chromosomes.
- Placement of the contractile ring.

In telophase, MPF is inactivated and proteins (lamins, condensins, etc.), previously phosphorylated by MPF, are dephosphorylated by phosphatases.

Consequences: nuclear envelope reformation (laminates); decondensation of chromosomes (condensins).

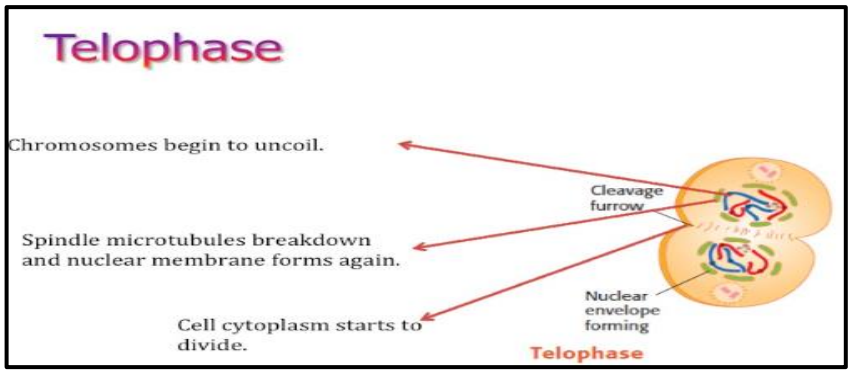


Figure 66: Telophase.

6.6. Cytokinesis

- Separation of the 2 cytoplasms.
- Reconstitution of an interphase TM network.
- Reconstitution of the nucleus.

It is the separation of the two daughter cells by division of the cytoplasm.

In animal cells, a contractile ring forms at the cell's equator. It is composed of actin and myosin. It is a transient structure that disappears once the cell is split in two.

The dephosphorylation of myosin allows it to come into contact with actin.

At the end of cytodieresis:

- the polar microtubules are constricted in the center of the cell;
- only a cytoplasmic bridge connects the two daughter cells: this is the intermediate body;
- The rupture of the intermediate body marks the end of cell division.

Note: During mitosis, the division of the nucleus (karyokinesis) precedes the division of the cytoplasm (cytodieresis or cytokinesis).

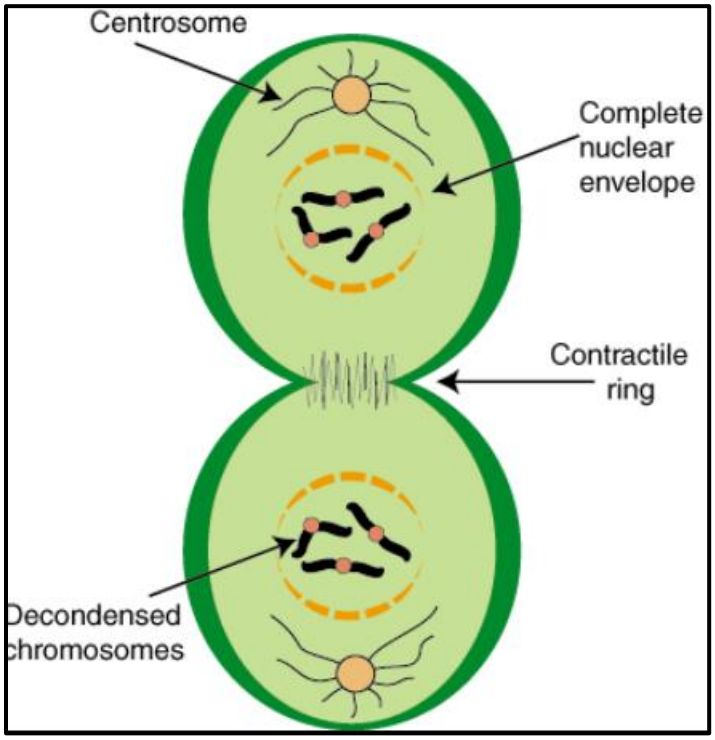


Figure 67: Cytodieresis (cytokinesis).

II: Cell Cycle Regulation

Cell divisions are essential for the development of multicellular eukaryotic organisms, but also for their survival when they reach adulthood (notion of general homeostasis).

In eukaryotes, the cell cycle is controlled by a system involving many regulatory proteins that are highly conserved during evolution.

This control system receives and integrates various signals from:

- from inside the cell (e.g., has the DNA been fully replicated?);
- from outside the cell (e.g., are growth signals present in the extracellular environment?).

Disruptions in this control system can lead to excessive cell proliferation and cancer.

1. Entry into the cell cycle

Why does a cell at the beginning of G1 or G0 begin cell division?

The decision depends on extracellular conditions (availability of nutrients) and extracellular signals from other cells (cf. mitogens).

If extracellular conditions are favorable and growth and division signals are present in the medium, the cell crosses the restriction point (R-point) and replicates its DNA.

The R-point is a critical transition point with regard to dependence on growth factors:

- ❖ before the R point, in the early G1 phase, the progression in the cycle is dependent on extracellular signals of growth and division;
- ❖ once the R-point has been passed, in the late G1 phase, the progression in the cycle is independent of extracellular signals of growth and division and the course of the cycle depends on intracellular factors.

2. Control points

Several checkpoints mark out the cell cycle. During these checkpoints, sensors analyze a series of criteria that the cell must meet in order to move on to the next phase.

- If these criteria are met, progression in the cell cycle continues normally.

- If these criteria are not met, negative intracellular signals are sent to the control system. Progression in the cell cycle is then delayed to give the cell time to rectify errors in order to meet the required criteria.

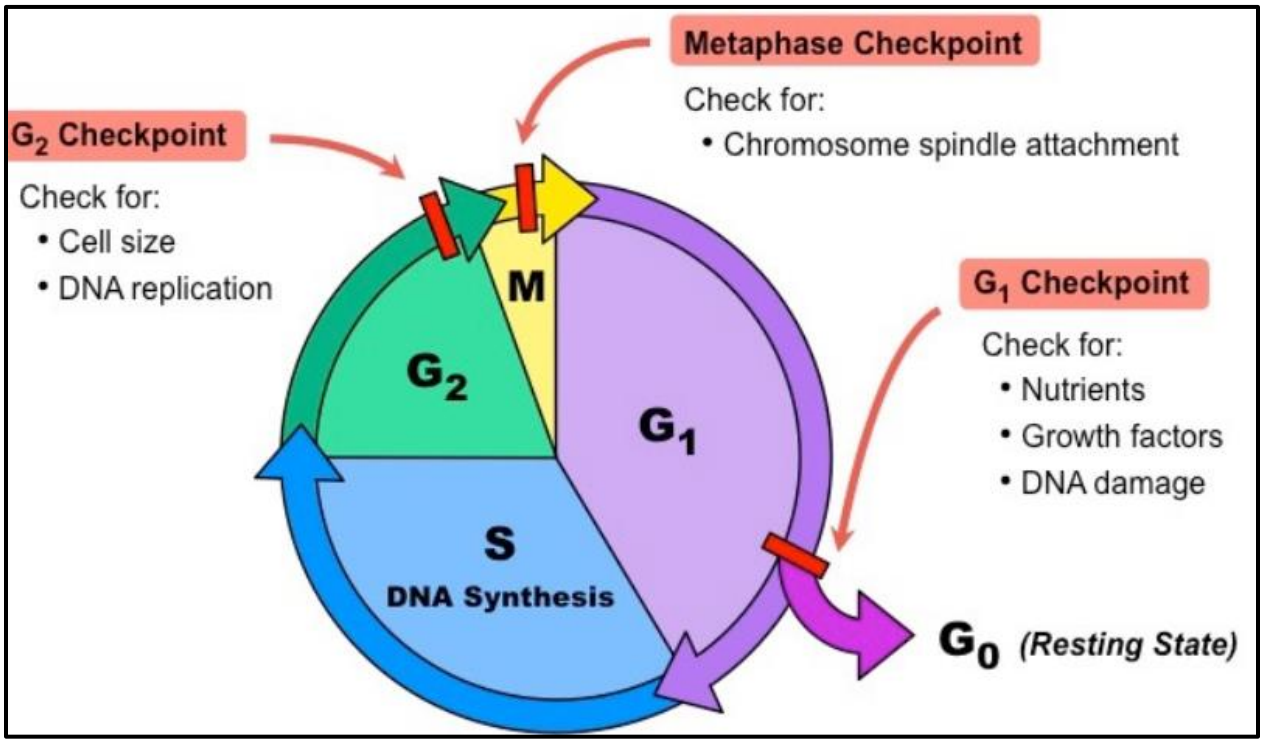


Figure 68: Cell Cycle Checkpoints.

3. Control of the cell cycle by Cdks

3.1. Cyclin-dependent kinases (Cdk)

Cyclin-dependent kinases (Cdk) are a family of protein kinases that regulate progression in the cell cycle. Each Cdk specifically recognizes a substrate (or group of substrates) that it phosphorylates using phosphate γ ATP.

The activity of Cdks varies according to the cell cycle. This results in variations in the phosphorylation of a wide variety of proteins that start or stop the successive phases of the cell cycle.

In vertebrates, four Cdks have been identified (Cdk1, Cdk2, Cdk3 and Cdk4). Their respective activity is maximal during different phases of the cell cycle and is regulated by different mechanisms.

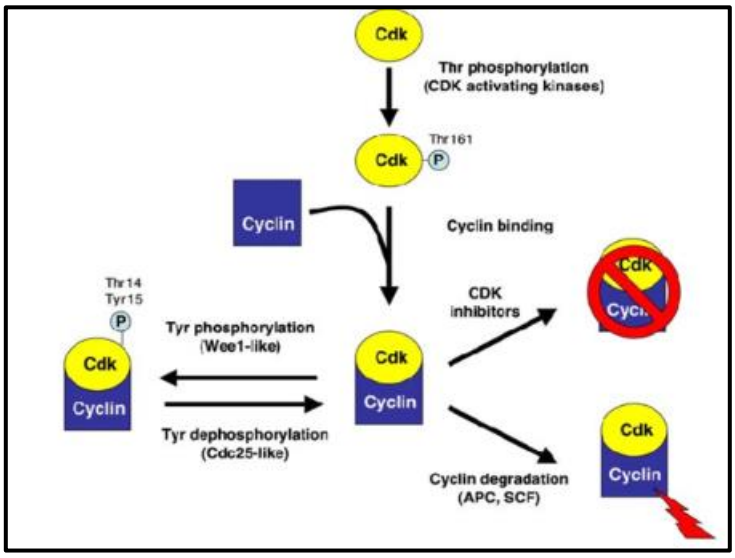


Figure 69: Mode of action of Cdk.

3.2. Regulation of the activity of the Cdk

a) Cdk/cyclin combination

Cdks have no protein kinase activity until they are attached to a cyclin. This association modifies the conformation of the kinase's active site and makes it partially active. Cyclins are a family of proteins so named because they undergo a cycle of synthesis and degradation with each cell cycle.

In eukaryotes, there are four classes of cyclins. Each cyclin-Cdk complex:

- is a heterodimer composed of 2 different subunits: a regulatory subunit (cyclin) and a catalytic subunit (Cdk);
- is activated at a different stage of the cell cycle;
- phosphorylates a different group of protein substrates and leads to distinct events.

b) Phosphorylations activating or inhibiting Cdk

Cyclin-Cdk complexes can be activated or inhibited following phosphorylation/dephosphorylation cycles. This is for example the case for the cyclin B-Cdk1 complex (MPF):

- The phosphorylation of an amino acid located near the entrance to the active site of the Cdk by a protein kinase called CAK (Cdk-Activating Kinase) fully activates the cyclin-Cdk complex.
- On the contrary, the phosphorylation of two amino acids placed below the active site of the Cdk by a protein kinase called Wee1 inactivates the cyclin-Cdk complex. The dephosphorylation of these two amino acids by a phosphatase called Cdc25 reactivates the complex.

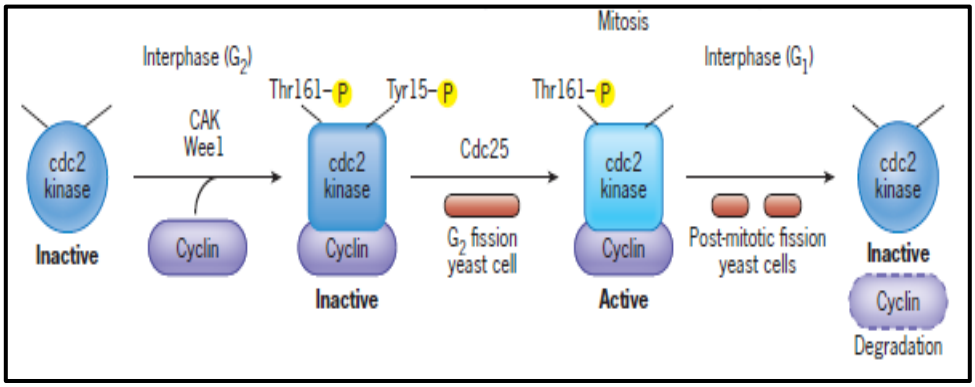


Figure 70: Regulation of Cdk by phosphorylation/dephosphorylation.

c) Cdk inhibitors: CKIs

Cyclin-Cdk complexes can be inhibited following the binding of Cdk-inhibiting proteins: the CKIs (Cyclin dependent Kinase Inhibitors).

Several CKIs have been identified and make it possible to regulate the G1 and S phases:

- p16 inhibits G1-Cdk complexes;
- p21 and p27 inhibit the G1-Cdk and G1/S-Cdk complexes.

d) Degradation of cyclins by the proteasome

Cyclin-Cdk complexes can be inactivated as a result of cyclin proteolysis by a multienzyme complex called a proteasome.

The proteasome is an enzyme complex containing proteases operating at neutral pH with ATP consumption and degrading ubiquitinylated proteins (previously coupled to ubiquitin).

The addition of ubiquitin is done through the intervention of enzymes called ubiquitin ligases. During the cell cycle, two complexes with ubiquitin ligase activity are involved: the SCF and the APC.

- **Ubiquitinylation by SCF**

During the G1 and S phases, SCF allows the ubiquitinylation and destruction of: G1/S cyclins; of certain CKIs that control the initiation of the S phase.

The activity of CFS is constant during the cell cycle, but it does not recognize its substrates until they are phosphorylated.

- **Ubiquitinylation by APC**

During phase M, APC (Anaphase Promoting Complex) allows the ubiquitinylation and destruction of: cyclins M; securine.

The activity of CFS varies during the cell cycle and the complex is activated following the addition of activating subunits such as Cdc 20.

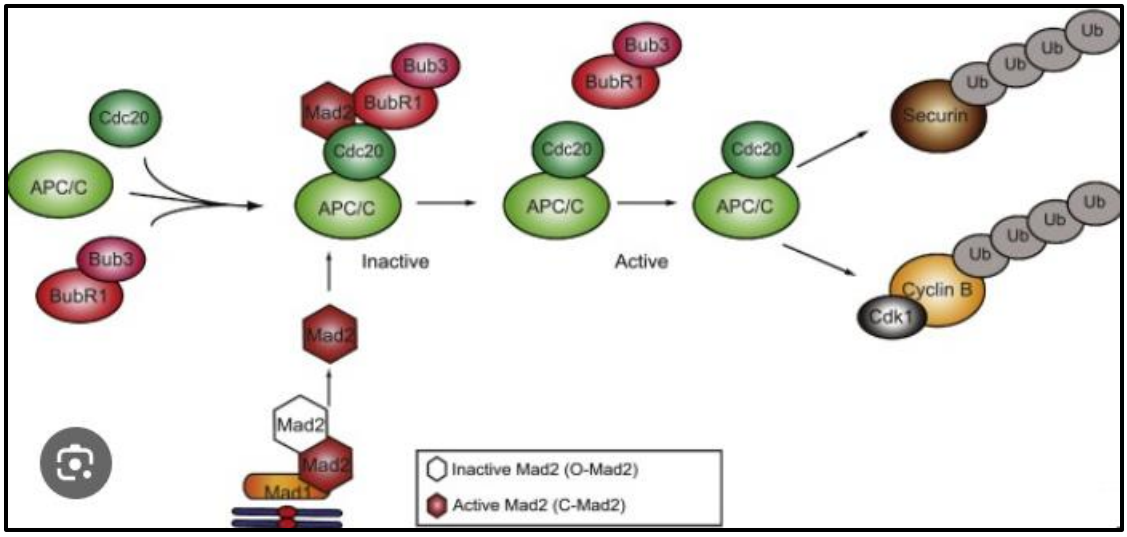


Figure 70: Mode of action of APC.

4. Extracellular control of the cell cycle (entry into the cell cycle)

4.1.Extracellular molecules controlling the cell cycle

The extracellular signaling molecules that regulate cell size and number are typically secreted soluble proteins divided into three major classes:

- mitogens, which stimulate cell division;
- growth factors, which stimulate the increase in cell mass;
- survival factors, which suppress apoptosis.

Some signaling molecules may belong to two or even three of these classes.

Careful! The term growth factor is misused to describe these three classes of factors.

a. Mitogens and their mode of action

Mitogens are a family of about fifty proteins produced by nearby cells and whose role is to stimulate cell division.

Examples: platelet-derived growth factor (PGDF = plateletderived growth factor); epidermal growth factor (EGF = epidermal growth factor); erythropoietin.

Mitogens work during the G1 phase by eliminating negative controls that block progression in the cell cycle.

- In the absence of mitogen, the inhibition of the G1-Cdk complex is maintained by CKIs and the cycle is blocked.
- In the presence of mitogens, the G1-Cdk and G1/S-Cdk complexes are activated and the cell enters the S phase.

The binding of mitogens to their enzyme receptors at the plasma membrane causes the MAP-kinase cascade to be activated, which has the effect of increasing the concentration of a transcription factor called Myc. Myc increases the transcription of several genes, including:

- the gene encoding cyclin G1 → activation of the G1-Cdk complex;
- the gene encoding a subunit of the ubiquitin ligase complex SCF
- increased degradation of p27 → increased activity of the p27 target: the G1/S-Cdk complex.

The activated G1-Cdk and G1/S-Cdk complexes phosphorylate one of their targets, the retinoblastoma protein (Rb), which causes the activation of a second transcription factor: E2F.

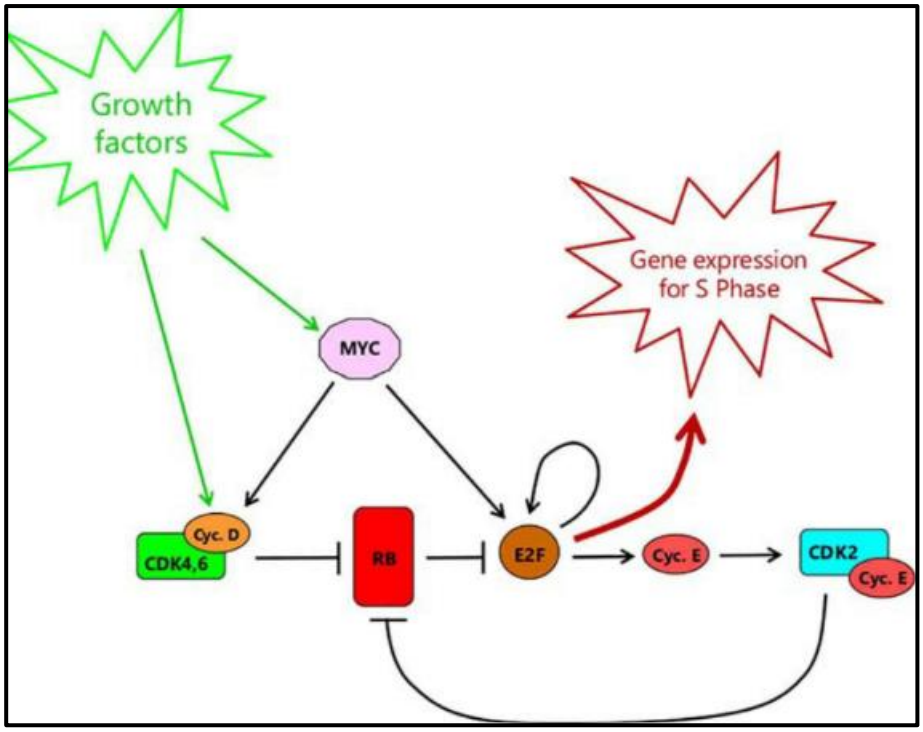


Figure 71: Intracellular consequences of mitogen activation.

5. Progression in the cell cycle

5.1. In the G1 phase: Role of the G1-Cdk complex

In phase G1, the protein retinoblastoma (Rb), an inhibitor of cell cycle progression, sequesters the transcription factor E2F.

When the cell is stimulated by mitogens, the G1-Cdk complex phosphorylates Rb, which decreases the affinity of the latter for E2F.

The release of E2F allows it to activate the transcription of several genes:

- gene encoding E2F;
- genes encoding the cyclins G1/S and S.

Impact: activation of G1/S-Cdk and S-Cdk complexes

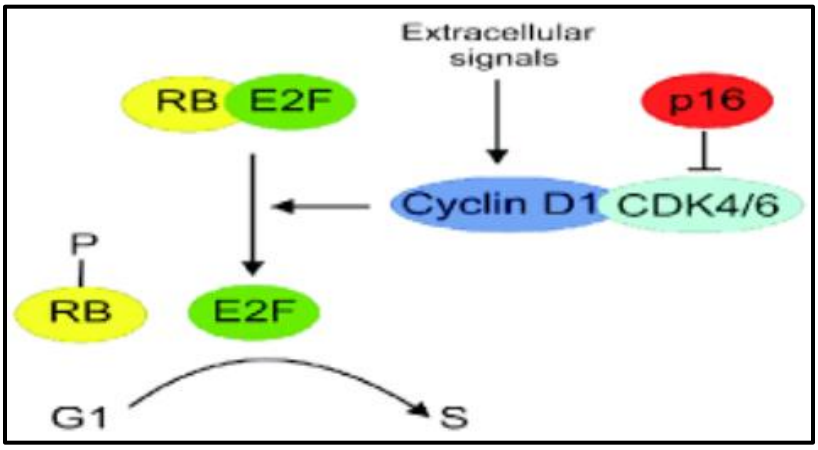


Figure 72: Sequestration and release of E2F by Rb.

5.2. In S-phase: Role of the S-Cdk complex

The **S-Cdk** complex phosphorylates proteins located at the origins of DNA replication and initiates the duplication of genetic material.

5.3. G2/M Transition and Mitosis: Roles of the M-Cdk Complex

Cyclin M synthesis increases during the G2 and M phases (transcriptional regulation). Thus, at the end of G2, the M-Cdk complex accumulates.

However, the M-Cdk complex is inactive because it carries an activating phosphate (added by CAK), but also 2 inhibitory phosphates (added by Wee1).

a) G2/M transition

The activation of the **M-Cdk complex** is conditioned by the passage of the DNA replication checkpoint at the end of G2:

- if DNA replication is not complete, sensors send a negative signal activating a protein kinase that inhibits Cdc25 phosphatase. The 2 inhibitory phosphates remain in place and the complex remains inactive;
- if DNA replication is complete, the 2 inhibitory phosphates are unhooked by the Cdc25 phosphatase and the complex is activated.

b) During mitosis

- **In prophase:**

The activation of the M-Cdk complex triggers, among others, the following phenomena:

- chromosome condensation (phosphorylation of condensins and H1 hiotones);
- rupture of the nuclear envelope (phosphorylation of lamins);
- assembly of the mitotic spindle (phosphorylation of proteins associated with microtubules);
- actin cytoskeleton rearrangement;
- reorganization of the Golgi apparatus and microtubules.

- **Metaphase/anaphase transition:**

The anaphase metaphase transition requires the activation of the APC.

The activation of the APC is conditioned by the activation of the M-Cdk complex and by the passage of the mitosis checkpoint.

- If a kinetochore is not properly attached to the spindle, sensors send a negative signal blocking the attachment of Cdc20 to the APC: APC remains inactive.
- If all the kinetochores are attached to the spindle, Cdc20 attaches to the APC and activates it.

The role of the ubiquitinyl APC is to sequester and inhibit a protease**: separation**. The degradation of the securine results in the release of the separation, which then cleaves the **cohesins**, making it possible to separate the sister chromatids during anaphase.

c) Mitosis output: inactivation of the M-Cdk complex

The exit from mitosis is due to the dephosphorylation of proteins whose phosphorylation had allowed the entry into mitosis (*via* the activation of phosphatases), but also to the inactivation of the M-Cdk complex. It is the ubiquitinylation of M cyclins by APC that leads to their degradation by the proteasome and thus the inactivation of the M-Cdk complex.

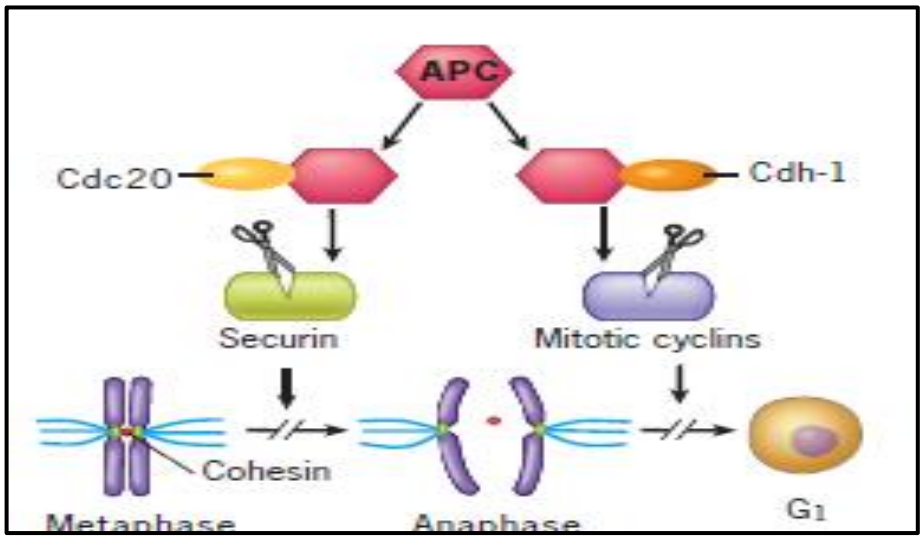


Figure 71: Targets of active APC.

6. Cycle blocking in the event of DNA damage

In the event of DNA damage, following radiation for example, the cell can block the cell cycle at two DNA damage checkpoints, one at the end of G1 and one at the end of G2.

a. G1 DNA Damage Checkpoint

A transcription factor, called p53, plays a major role in this mechanism. p53 activates the transcription of many genes, including the one that encodes p21, a CKI that inhibits the G1/S-Cdk and S-Cdk complexes.

- In the absence of DNA damage: the concentration of p53 is low because it interacts with the Mdm2 protein. Mdm2 is a ubiquitin ligase that causes the ubiquitinylation of p53 and its destruction by the proteasome.
- When DNA is damaged at the end of G1, protein kinases are activated and phosphorylate p53.

Phosphorylation of p53 decreases its affinity for Mdm2. This results in a decrease in the degradation of p53 by the proteasome and an increase in the concentration of p53 in the cell. P53 can thus activate the transcription of P21.

Once synthesized, p21 causes the inhibition of the G1/S-Cdk and S-Cdk complexes and thus the blocking of the entry into the S-phase.

b. G2 DNA Damage Checkpoint

In the G2 phase, damaged DNA sends a negative signal activating protein kinase that phosphorylate and inhibit Cdc25 phosphatase.

This signal has the same effect as the one sent when DNA replication is not complete: it keeps the M-Cdk complex inactive by preventing the removal of the 2 Cdk-inhibiting phosphates by Cdc25.

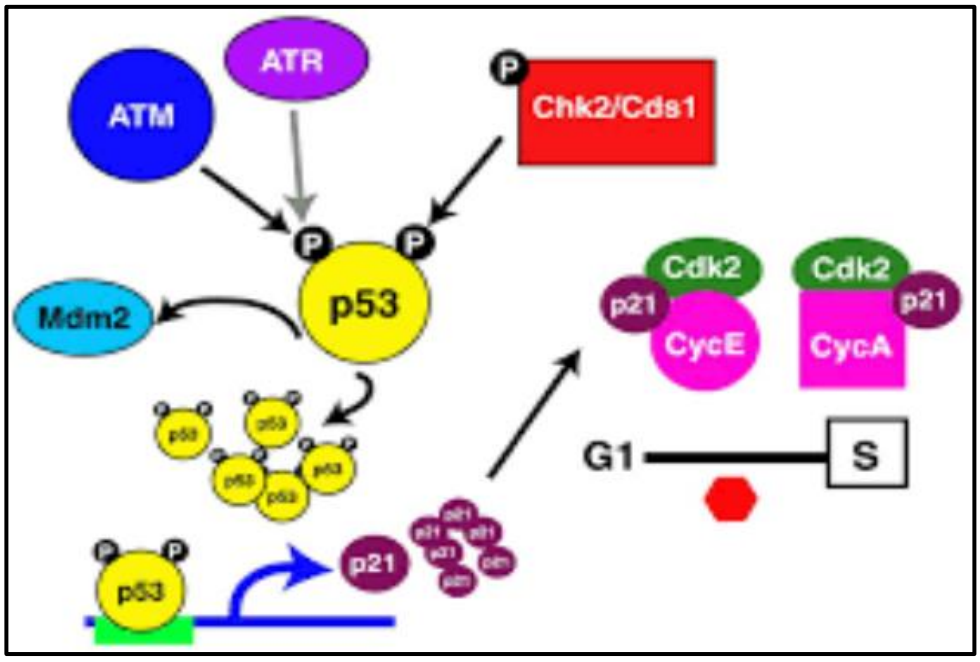


Figure 72: Role of p53 in cell cycle arrest in G1.

Chapter 06: Communication and signaling mechanism

1. The different types of cellular communications

1.1. Principles of Cellular Transmission

Intercellular communication is one of the characteristics of multicellular organisms. It is based in part on the secretion of chemical signals, or ligands, which act at more or less great distances on target cells that receive and process them. Depending on the type of signal, the consequences at the cellular level will be: cell survival, proliferation and/or differentiation. The absence of signals leading to cell death.

1.2. The four types of signage

These modes of communication can be classified according to the distance between the transmitting cell and the target cell. From the longest to the shortest distance, we find:

a) Endocrine communication

It concerns hormones: these are released into the general bloodstream. They act remotely on a cell that has a specific receptor. The delay for the signal to reach its target is long (from a few seconds to several minutes). Endocrine communication leads to a dispersion of the signal in the body (< 10-8 mol/L).

b) Paracrine communication

The signal is released into the extracellular matrix and acts only on neighboring cells.

It concerns local mediators.

Example: growth factors, mediators of inflammation.

c) Autocrine communication

The cell responds to the signal it has secreted itself.

Example: growth factors and cytokines.

d) Chemical synaptic communication

The signal is released by the presynaptic cell and acts only on the postsynaptic cell of a nearby specialized junction (chemical synapse).

There is no dispersion of the signal and the action is very fast (in the order of ms). It concerns neurotransmitters (**e.g.** acetylcholine, glutamate, norepinephrine, etc.).

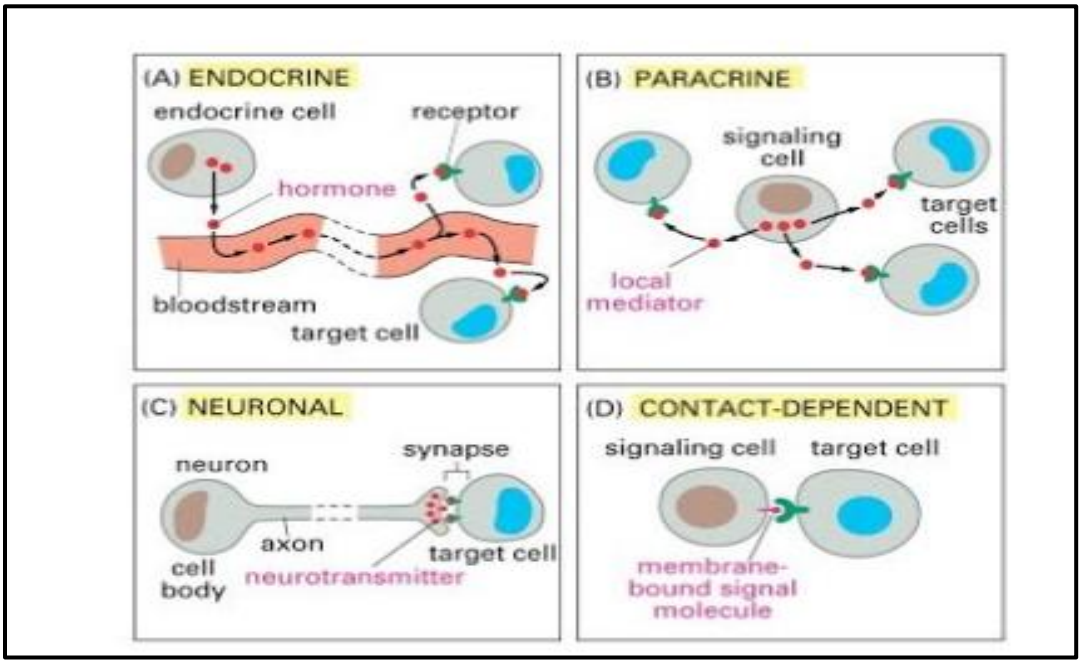


Figure 73: Differences between four modes of communication: paracrine, synaptic, endocrine and synaptic.

2. The three main types of chemical signals

2.1. Water-soluble informative molecules

a. Characteristics:

- They cannot cross the lipid bilayer of the plasma membrane.
- They act through specific receptors located on the plasma membrane of the target cell.
- Their very short lifespan (ms, s for neurotransmitters or a few minutes for hormones).

They induce rapid and short-lived responses. These responses correspond to a regulation and activation of pre-existing proteins in the target cell (enzymes, ion channels, transcriptional regulatory factors).

These molecules are:

Growth factors: these are proteins or polypeptides that play a role in cell proliferation and survival. Most often referred to as **GF**: *Growth Factor*.

Neurotransmitters: these are most often amino acid derivatives (norepinephrine, serotonin, GABA, etc.) or polypeptides that play a role in the excitation or inhibition of neurons at the synapses.

Hormones: these are molecules:

- peptides (2-100 amino acids). E.g. vasopressin, oxytocin, insulin, etc.
- protein (> 100 AA). E.g. growth hormone (GH);
- glycoproteins. Ex: LH, FSH.
- **Cytokines**: These are proteins or polypeptides that play a role in the immune response and inflammation. E.g. interleukins (IL).

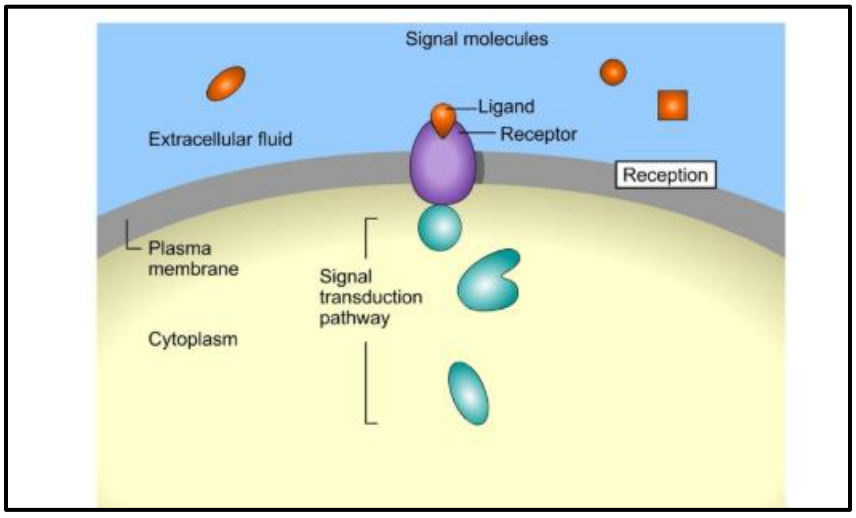


Figure 74: Signaling by water-soluble molecules.

2.2. Fat-soluble informative molecules

Characteristics:

- They cross the plasma membrane by simple diffusion.
- They then activate an intracellular receptor that binds to target regions of DNA and regulate gene transcription.
- They induce later and longer-lasting responses. They do not act on pre-existing proteins.

These molecules are transported in the blood (in the case of fat-soluble hormones) thanks to specific protein transporters before being released on contact with the plasma membrane of the target cells. They are:

- **thyroid hormones (T3 and T4),** derived from an amino acid: tyrosine;
- Steroid **hormones**, derived from cholesterol. **E.g.** cortisol, estradiol, testosterone, progesterone...
- **Prostaglandins**, derived from arachidonic acid (fatty acid at 20 C).

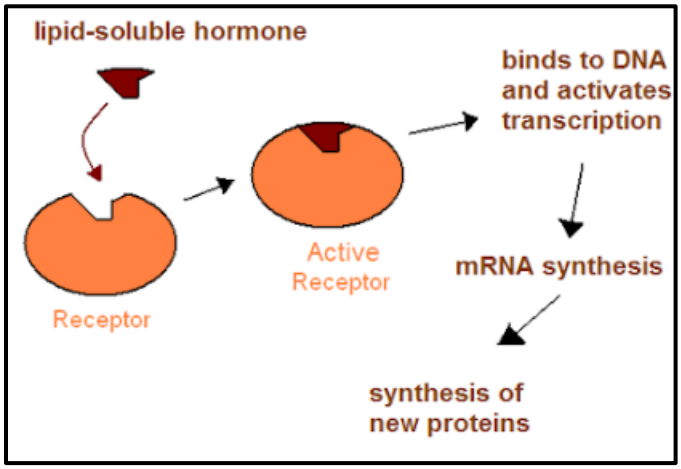


Figure 75: Signaling by fat-soluble molecules.

2.3. Gas Free Radicals

Characteristics:

- They diffuse freely across the plasma membrane.
- They act directly on cytosolic enzymes without the intervention of a membrane or intracellular receptor.

  Example: NO acts on a cytosolic guanylate cyclase.

- They are toxic in high concentrations.
- The best known are **CO** (carbon monoxide) and **NO** (nitric oxide).

2.4. Agonists and antagonists

- An **agonist** binds to the receptor and induces a response similar to that of the natural ligand.

Ex: Nicotine is the acetylcholine agonist for its nicotinic receptor.

- An **antagonist** attaches to the receiver but does not trigger a response.

  Ex: **Curare** is an antagonist of acetylcholine for its nicotinic receptor.

Note: Antagonists can be used as medications.

Ex: Antihistamines are histamine antagonists and cure allergic symptoms.

3. Fat-soluble signals and their receptors

3.1. Structure of Nuclear Receptors

These receptors constitute a superfamily of proteins that have strong sequence similarities.

They include 5 areas:

- The **A/B domain** (N-terminal end): variable domain that acts as a **transcription regulatory factor = transactivation domain.**
- Domain **C**: a DNA binding domain that has a **zinc-fingered** architecture. A finger of Zn = 4 Cys bound to a zinc atom. It is responsible for binding the receptor to the **ERH** (Hormone Response Element or HRE) region of the target genes.
- Domain **D**: pivotal domain.
- The **E domain (**C-terminal end**):** includes the ligand binding site and a nuclear localization signal (**NLS**) that can be masked by **RECEPTOR-Associated Proteins** (**RAPs**) and unmasked by ligand binding.

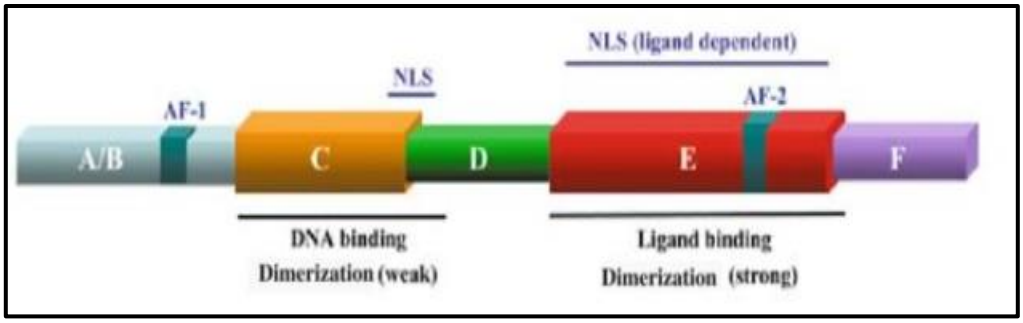


Figure 76: Schematic representation of a nuclear receptor.

3.2. Mechanisms of action: example of steroid hormone receptors

Free receptors are attached to several proteins (PAR: Hsp70, Hsp90) to form an inactive complex, i.e. unable to bind to DNA. PARs mask the zinc finger and NLS. The free receptor associated with PAR is activated by the binding of the hormone.

i. The binding of the hormone releases the receptor from the PAR complex and induces a transconformation of the receptor that allows its dimerization.
ii. The NLS is unmasked and the hormone-receptor complex is translocated into the nucleus where it can attach to the ERH region of a gene.

The overall response to a steroid hormone is twofold:

1) The induction of a few specific genes is said to be primary: the genes are transcribed into mRNA which are exported to the cytosol and translated into proteins.

2) Some of these proteins (primary response) may in turn act as regulatory factors for gene transcription. This is the secondary answer. They can have an effect on:

- **inhibitor** on the genes of the primary response (negative feedback);
- **stimulator** on other genes, characteristic of the secondary response.

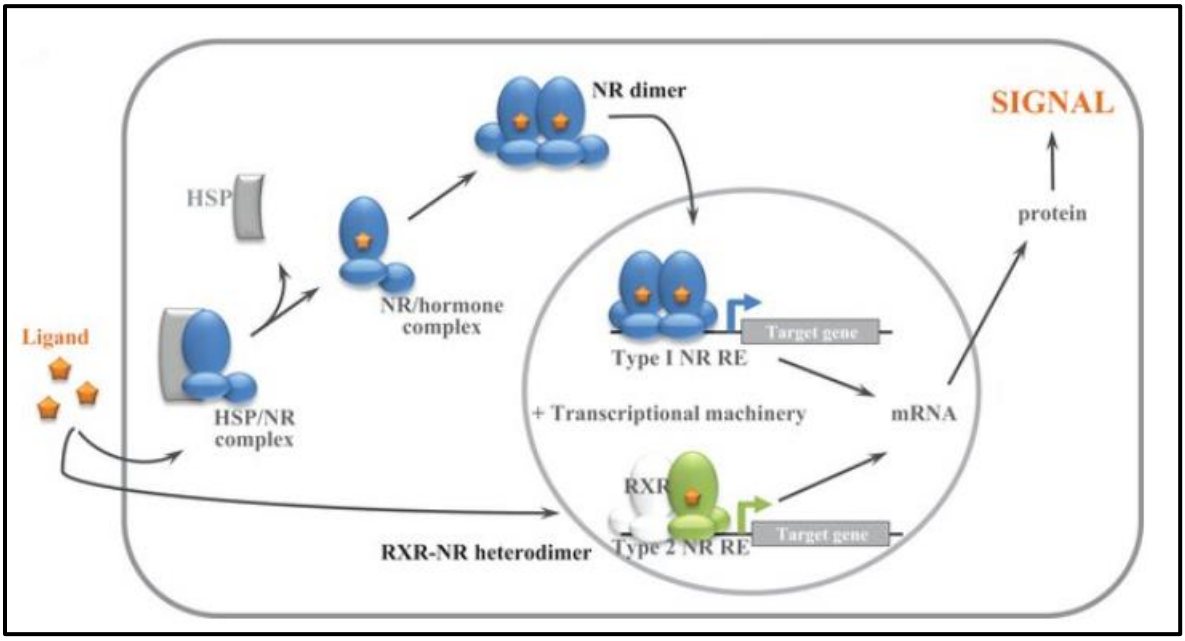


Figure 77: Schematic model of Nuclear Receptor function.

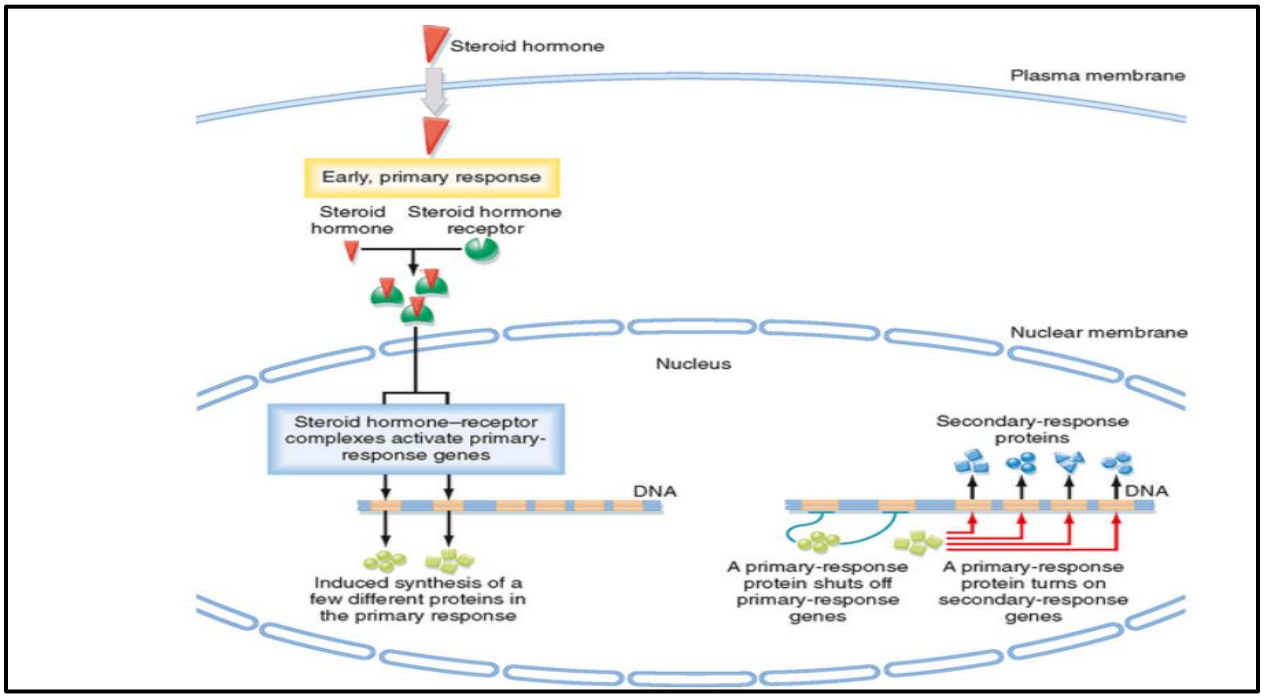


Figure 78: Primary and secondary responses following the attachment of a *steroid hormone/receptor* complex to DNA.

4. Water-soluble signals and ion channel receptors

It is a superfamily of multimeric receptors in which each monomer has 4 transmembrane domains. Their opening is triggered by the binding of their specific ligand.

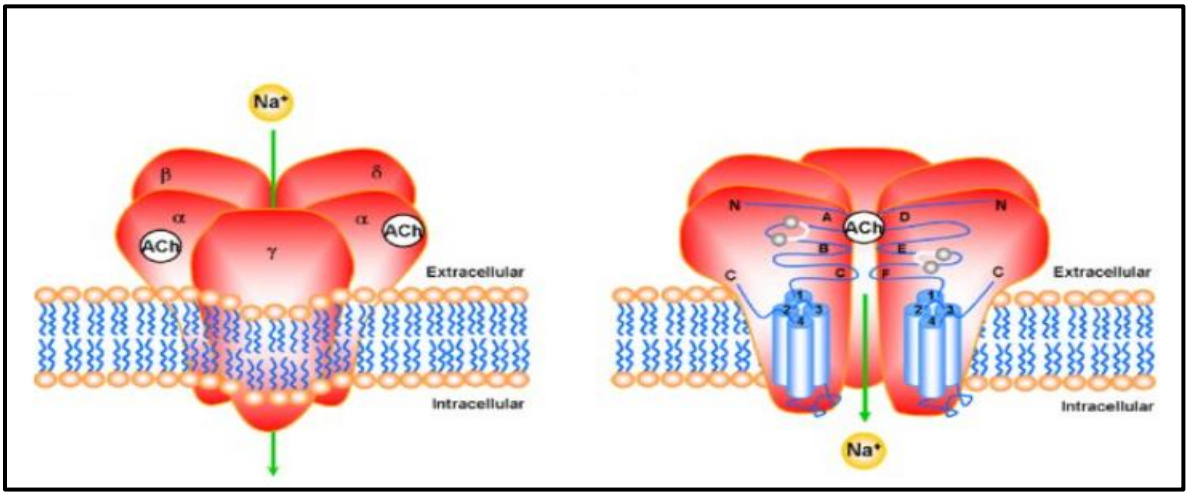


Figure 79: Principle of operation of a channel receptor (here nicotinic acetylcholine receptor).

Example: The nicotinic muscle acetylcholine receptor is a 300 kDa pentamer formed by 5 subunits: 2 α subunits carrying the ligand binding sites, 1 β subunit, 1 γ subunit and 1 δ subunit.

These **5 subunits** delimit the ion channel.

The binding of acetylcholine to each α subunit causes a reorganization of the structure of the **5** subunits that triggers the opening of the ion channel. Consequences: entry of Na+ causing a depolarization of the muscle cell.

This is how the nicotinic receptor plays an important role in neuromuscular transmission and excitation-contraction coupling.

5. Water-soluble signals and G-protein-coupled membrane receptors (GPCRs)

5.1. GPCR Structure

They belong to a superfamily of proteins that have **7 transmembrane domains**. Their **N-terminal is extracellular** and they **often function as homo- or heterodimer**.

5.2. GPCR Activation Cascade

The GPCR signaling pathway involves 6 partners:

- The first messenger which is an extracellular ligand. **Ex:** norepinephrine, glucagon.
- GPCRs.
- Heterotrimeric G proteins (= transducers).
- Primary effectors which are ion channels or enzymes.
  E.g. adenylate cyclase, phospholipase C...
- Second messengers whose intracellular concentration is controlled by primary effectors. Ex: cAMP, Ca2+...
- Secondary effectors activated by second messengers. E.g., protein kinase A activated by cAMP.
  - The binding of the first messenger to the GPCR leads to the modification of many cellular activities after a very important amplification phenomenon.

5.3. Heterotrimeric G proteins

They belong to a large superfamily of proteins that bind GTP and hydrolyze it to GDP. They are composed of 3 subunits (SUs):

- an SU α which sets the GDP and GTP and has a GTPase activity;
- an SU β and an SU γ which form an inseparable dimer.

ED α and ED γ are covalently bound to fatty acids, allowing them to temporarily anchor to the cytosolic leaflet of the plasma membrane.

The transfer of information between the GPCR and the primary effector relies on the functional cycle of G proteins:

1) The binding of the first messenger to the GPCR activates the G protein and triggers the exchange of a GDP molecule by a GTP molecule at the level of the SU α.

2) This exchange induces the dissociation of the trimeric complex and the α SU separates from the other two.

3) α and βγ modulate the activity of many primary effectors:

- the ED α of Gs proteins stimulates adenylate cyclase;
- ED α of Gi proteins inhibits adenylate cyclase;
- the ED α of Gq proteins stimulates phospholipase C (PLC);
- the βγ dimer of G proteins activates channels at K^+.

Following the detachment of the first messenger, the α ED exerts a GTPase activity that leads to the hydrolysis of the GTP and the reconstitution of the inactive trimeric form (bound to GDP).

5.4. Heterotrimeric G protein targets

a) The adenylate cyclase – cAMP pathway

Adenylate cyclase is a transmembrane enzyme whose active site is turned towards the cytosol.

When activated by the αs SU, it catalyzes the transformation of ATP into cAMP, a small soluble molecule that spreads through the cytosol and acts as a second messenger.

The main effect of cAMP is the activation of PKA, a cAMP-dependent protein kinase.

This PKA can then phosphorylate many substrates, which greatly amplifies the effects of extracellular signals.

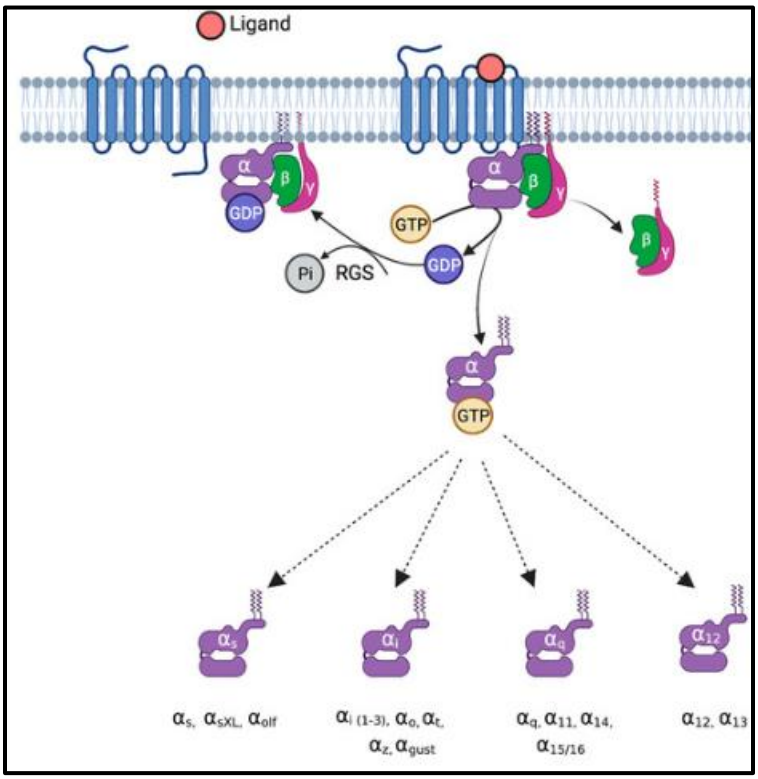


Figure 80: G-proteins (GPCR) and their mechanism of activation.

b) The phospholipase C (PLC) pathway – IP3, DAG and Ca^{2+}

This pathway is activated by a cytosolic enzyme located near the plasma membrane: PLC.

When PLC is activated by SU αq, it hydrolyzes PIP2 (Phosphatidyl-Inositol 4,5 bisphosphate), a component of the inner layer of the plasma membrane.

Hydrolysis produces DAG (DiAcylGlycerol), which remains in the membrane, and IP3 (Inositol Tri Phosphate), a small soluble molecule.

The IP3 leaves the membrane to attach to its receiver, located on the membrane of the REL. This receptor is a Ca2+ channel that opens and allows the release of Ca^{2+} into the cytoplasm.

Ca2+ ions bind to and activate calmodulin. This then becomes capable of activating many enzymes including Ca2+/calmodulin-dependent protein kinases (CaM Kinase).

The DAG activates a **PKC** (Calcium-Dependent Protein Kinase). It phosphorylates many substrates that relay the message, in particular transcription factors.

c) The ion channel pathway

The G protein can also directly activate or inactivate the plasma membrane channels of the target cell, thus altering its permeability and excitability.

Example: **muscarinic M2 acetylcholine receptors** located on cardiac muscle cells (nicotinic receptors are located on skeletal muscles and nerve cells).

These receptors activate the G protein whose βγ subunits cause the channels to open to K^+: this opening causes the exit of K^+ and increases the difficulty in depolarizing the cell and contributes to the inhibitory effect of acetylcholine on the heart.

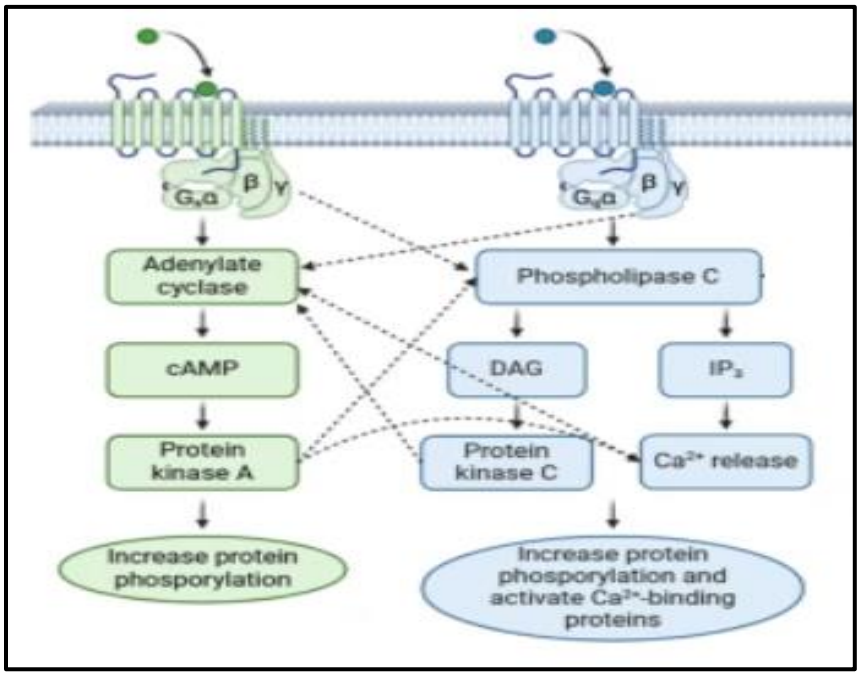


Figure 81: Comparison of adenylate cyclase and PLC pathways following the binding of a water-soluble molecule to a GPCR.

Note: Simplified diagram for clarity. G protein, PIP2, PLC and adenylate cyclase are bound to the plasma membrane.

6. Water-soluble signals and enzyme receptors

6.1. Structure: They have:

- a single transmembrane domain;
- a glycosylated N-terminal extracellular domain that binds the ligand;
- a C-terminal cytoplasmic end that carries intrinsic enzyme activity or is directly associated with an enzyme.

6.2. Features

They are inactive in the monomer state and act mostly as a dimer. There are several classes of enzyme receptors. The most widespread are receptors with tyrosine kinase activity. They play a decisive role in the action of growth factors (PDGF, EGF, etc.) and insulin.

6.3. Receptor tyrosine kinases: Example of GF growth factor receptors

The successive binding of 2 ligand molecules induces the dimerization of the receptor and its autophosphorylation which then allows it to recruit associated proteins. This binding allows the receptor to activate the monomeric G protein Ras. This activation is indirect and involves:

- an intermediate protein, which binds to the receptor (Grb2);
- a protein that binds to Grb2 and stimulates the exchange of GDP by GTP at the level of Ras (Sos = GEF).

Activated Ras induces a phosphorylation cascade in which a series of protein kinases interact sequentially: MAP-kinasekinase-kinases (= Raf) and MAP-kinase-kinases (= MEK). The last kinase is a MAP kinase (*Mitogene Activated Protein Kinase*).

This cascade leads to the modification of cytosolic protein activity and the activation of transcription factors. This pathway helps regulate cell proliferation, differentiation, and survival.

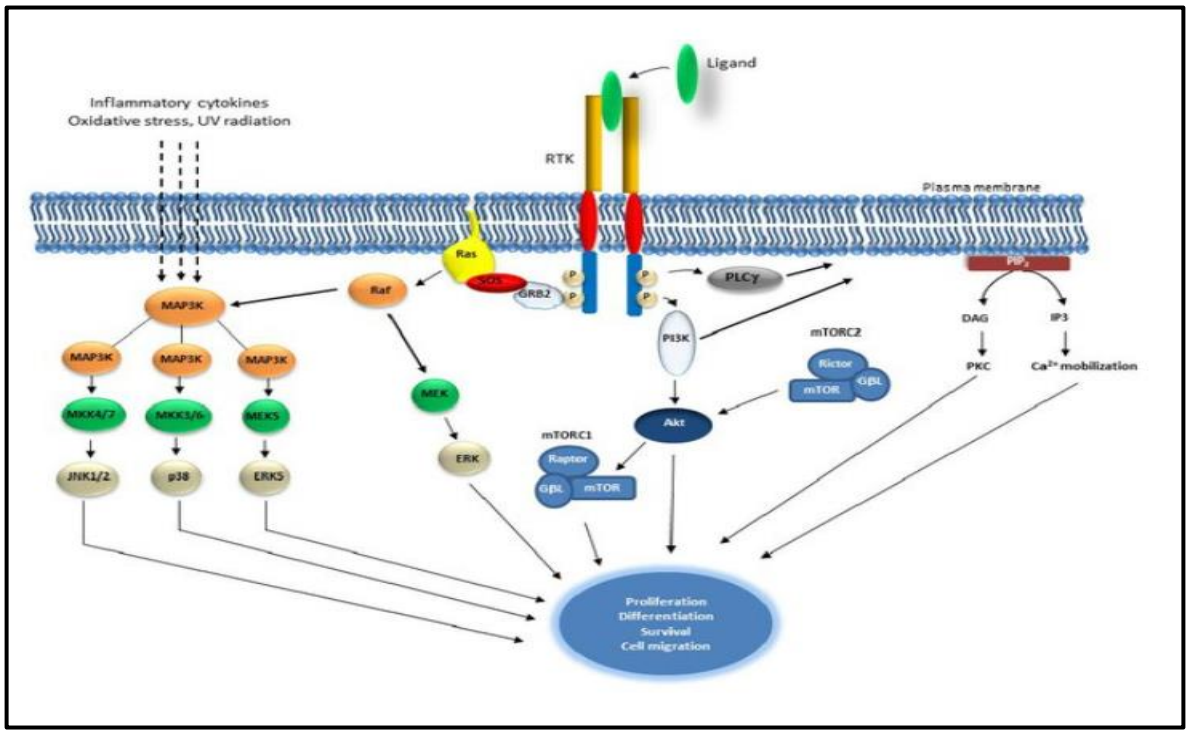


Figure 82: Activation of a receptor with tyrosine kinase activity and intracellular consequences.

Note: GTPase Activating Protein **(**GAP*) proteins* stimulate the hydrolysis of GTP by Ras and render it inactive.

Chapter 07: Apoptosis

1. Definition of Apoptosis

Apoptosis is a form of cell death also known as "programmed cell death," in which a suicide program is activated inside the cell. Apoptosis is a normal, highly regulated phenomenon that allows multicellular organisms:

- regulate their number of cells;
- eliminate normal cells that are no longer needed;
- eliminate abnormal cells that represent a danger to the body (infected cells, cells with mutated DNA, etc.).

Necrosis, on the other hand, is accidental cell death.

2. Roles of Apoptosis

a) Apoptosis and development

- Ex. 1: *Caenorhabditis elegans*. A transparent worm 1 mm long, living in sand and with a very short lifespan (2 weeks). The egg cell produces 1,090 cells, 131 of which die by apoptosis during its development.
- Ex. 2: Sculpture of the fingers of the mouse paw. The legs appear as a "shovel-like" structure, and the fingers only separate when the cells between them die by apoptosis.
- Ex. 3: Immune system. In the thymus, self-recognizing T cells are eliminated by apoptosis to avoid autoimmune reactions.

b) Apoptosis in adult tissues

In adult tissues, apoptosis balances cell division exactly. If it didn't, the tissue would grow or shrink. Example: Rat liver:

- If part of the liver is shed in an adult rat, the proliferation of liver tissue increases to make up for the loss.
- If a rat is treated with phenobarbital (= drug that stimulates cell division and therefore enlargement of the liver), after stopping treatment, apoptosis increases until the liver returns to its original size (about 1 week).

3. Caspases, proteases of apoptosis

3.1. Definition of caspases

Apoptosis depends on a family of proteases: caspases. These contain a cysteine at their active site and cut their protein substrate at the level of specific aspartic acids. All cells synthesize caspases in the form of inactive precursors: procaspases. The activation of procaspases is tightly regulated.

There are two families of caspases: initiating caspases (e.g. caspases 8 and 9) and effector caspases (e.g. caspase 3).

a) Activation of caspases

Following the activation of apoptosis, adaptor proteins, such as FADD and Apaf-1, allow the formation of aggregates containing many initiating procaspase molecules. This results in their mutual severance and activation.

Once activated, the initiating caspases cut and activate other procaspases: the effector procaspases.

Activated effector procaspases are substrates of other caspases, but also key proteins such as:

- nuclear laminates. Their degradation leads to fragmentation of the nucleus;
- the ICADs. ICADs (Inhibitors of CADs) are DNAse inhibitors called CADs (Caspase Activated DNAse). The ICADs associate with the CADs, hide their NLS (= addressing sequence to the kernel – see sheet 50) and sequester them in the cytosol. The degradation of the ICADs by caspases releases the CADs that enter the nucleus and cut the DNA into regular segments.
- apoptosis-regulating proteins such as Bid.

These chain reactions are called the amplifying proteolytic cascade.

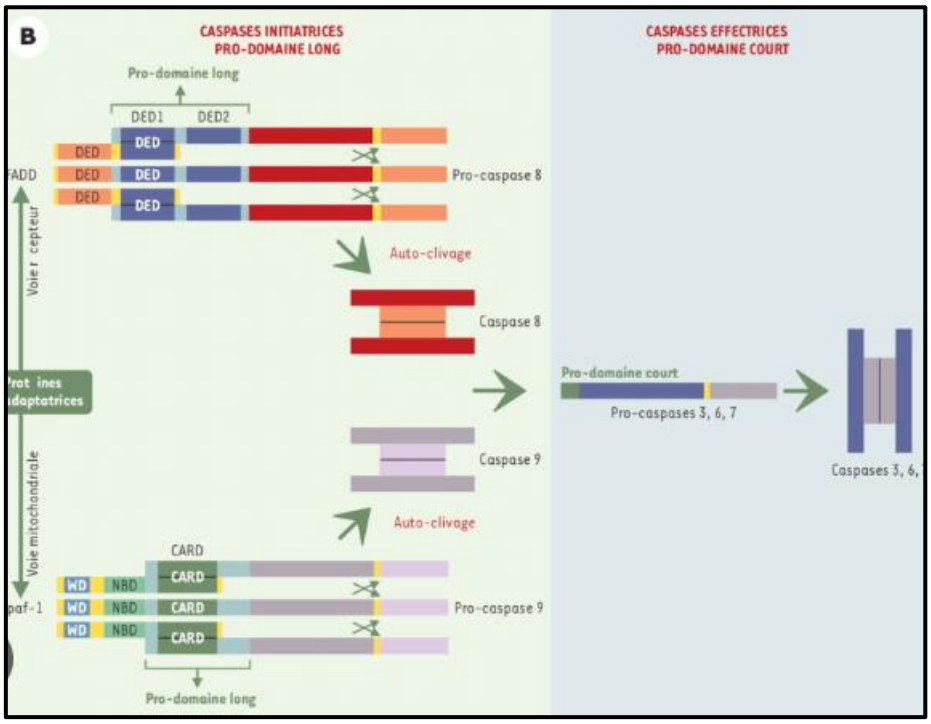


Figure 83: Diagram of the activation of procaspases in caspase.

Activation of apoptosis can be done:

- from outside the cell (extrinsic pathway): this is the death receptor pathway;
- from inside the cell (intrinsic pathway): this is the mitochondrial pathway.

3.2. The Death Receptor Pathway

3.2.1. An example of a death receiver: The Fas receiver

T lymphocytes induce the death of their target cells by apoptosis. These lymphocytes express a protein called the Fas ligand (Fas-L). Fas-L binds specifically to a receptor expressed on the surface of target cells: the Fas death receptor (Fas-R). Fas-L/Fas-R binding leads to the death of target cells.

The extracellular part of Fas-R carries cysteine-rich CRD (Cystein Rich Domain) domains that recognize the Fas ligand (Fas-L). The cytosolic part carries a DD domain (Death Domain), which is essential for signal transduction.

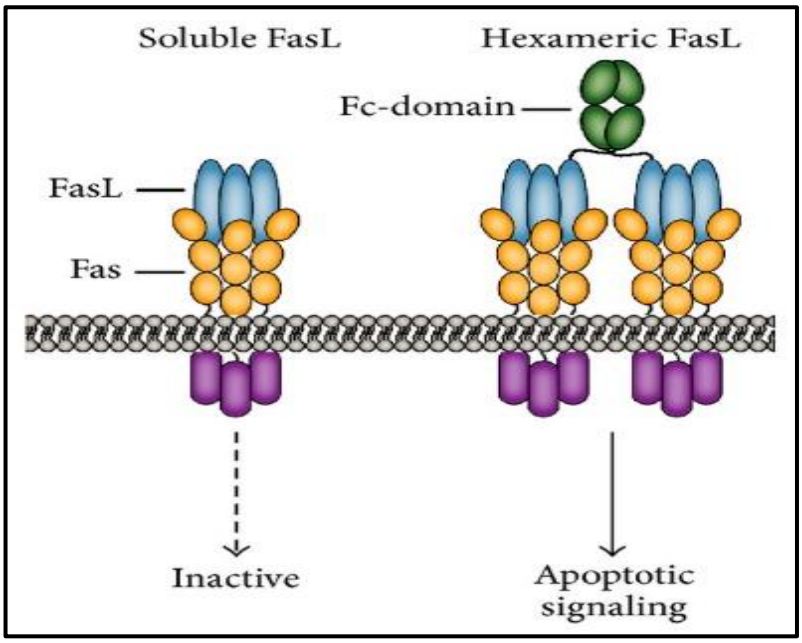


Figure 84: Structure of the fas receptor

3.2.2. Signal transduction (example of Fas receivers)

1) Fas-L binds to the extracellular part of Fas-R.
2) Fas-R is trimerized → Change in conformation of the DD domains.

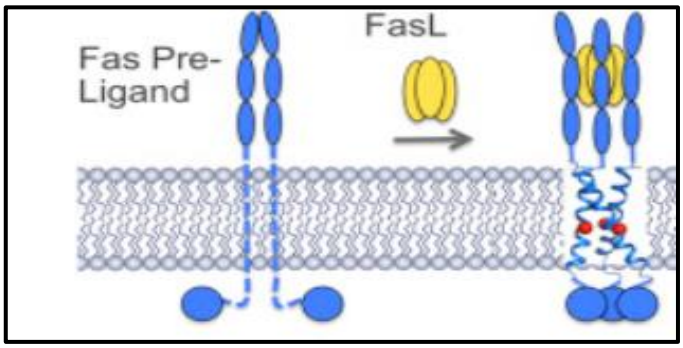


Figure 85: Binding of Fas-L to the Fas receptor.

3) Establishment of the DISC (Death Inducing Signaling Complex) submembrane complex:

The DISC complex results from the association of Fas-R with FADD and procaspases below the plasma membrane 8.

FADD is an adaptor protein linking Fas-R to procaspases 8. FADD has one DD domain and two DED (Death Effector Domain) domains. FADD performs homophile interactions with the DD domain of Fas R and with the DED prodomains of procaspases 8.

4) The consequence of the formation of the DISC complex is the aggregation of procaspases 8 which leads to their self-activation by auto-proteolysis. Caspases 8 in turn activate procaspases 3.

Remark:

- Procaspase 8 is initiating. It contains a DED prodomain that allows its interaction with FADD.
- Procaspase 3 is effector. It does not contain a prodomain.

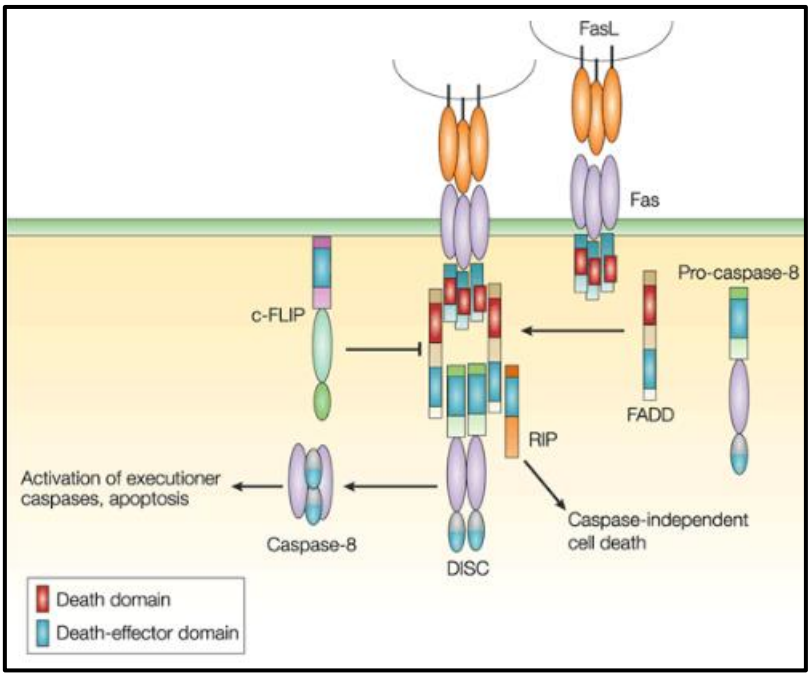


Figure 86: Formation of the DISC submembrane complex and activation of the proteolytic cascade.

4. The Mitochondrial Pathway

4.1. Apoptosome Formation

The mitochondrial pathway involves the formation of a cytosolic complex called an apoptosome. This includes:

- Cytochrome c: mobile transporter of electrons in the mitochondrial respiratory chain. It is normally located in the intermembrane space of the mitochondria.
- Procaspase 9: initiating procaspase that has a CARD prodomain.

- Apaf-1 (Apoptotic protease activating factor-1): an adaptor protein that links cytochrome c to procaspases 9.

Apaf-1 has a CARD domain that interacts homophilically with the CARD prodomain of procaspases 9 and a WD40 domain that allows it to interact with cytochrome c.

4.2. Regulation of the mitochondrial pathway

a) Proteins of the Bcl-2 family

Proteins of the Bcl-2 family have a role in the regulation of the mitochondrial pathway:

- Bcl-2 and Bcl-XL are anti-apototic because they block the release of cytochrome c from the mitochondria.
- Bad, Bax, Bak and Bid are pro-aptotic and act through different mechanisms:
  - Bad binds to certain anti-apoptotic molecules and inactivates them;
  - Bax and Bak stimulate the release of cytochrome c from the mitochondria;
  - Bid activates Bax and Bak.

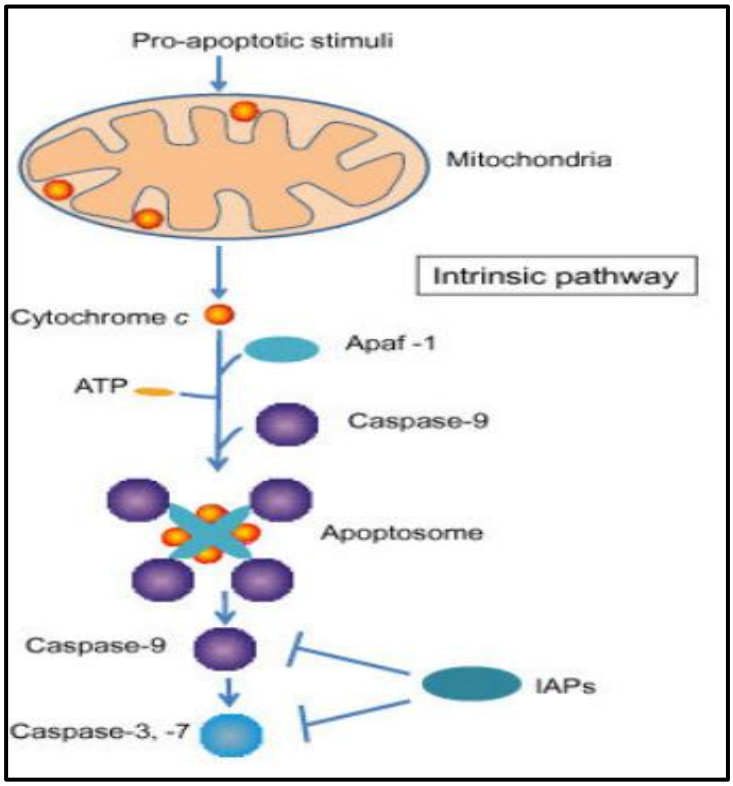


Figure 87: Summary of the course of the mitochondrial pathway of apoptosis.

1. Mitochondrial cytochrome C release into the cytosol.
2. Recruitment of Apaf-1 at the cytochrome c level.
3. Recruitment of procaspases 9 and establishment of the apoptosome (for reasons of simplification, only 2 molecules of Apaf-1 are represented in the apoptosome instead of 7).
4. Mutual activation of procaspases 9 by mutual proteolysis.
5. Activation of procaspases 3 (effectors) by caspases 9.

b) Triggering the mitochondrial pathway

The mitochondrial pathway can be triggered in two ways:

1) When the death receptor pathway is activated, the 8 caspases cleave Bid resulting in its activation. Bid activates Bax and Bak.

2) When DNA is damaged, the gene regulatory protein p53 accumulates and stimulates the transcription of genes that encode pro-apoptotic proteins such as Bid, Bax and Bak.

In both cases, the consequence is the release of cytochrome c by the mitochondria.

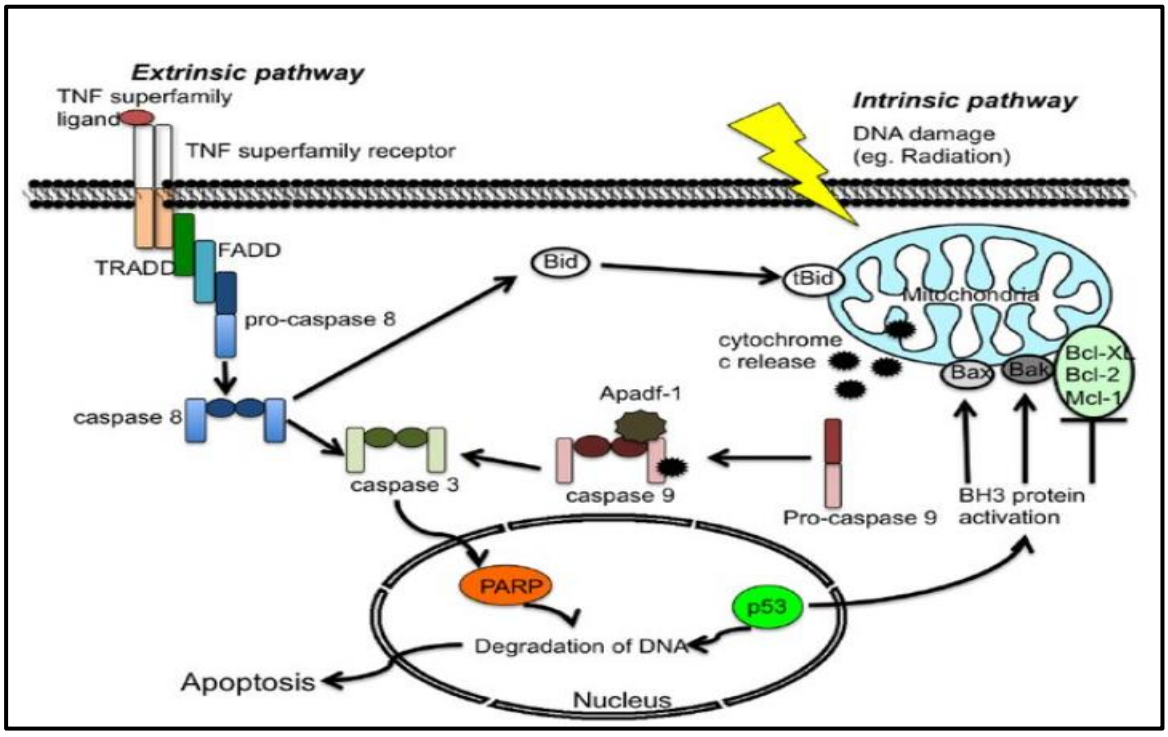


Figure 89: Generalized overview of the extrinsic and intrinsic apoptosis pathways.

Chapter 08: Cellular imaging techniques

Because of the very small size of the subject matter, cell and molecular biology is more dependent on the development of new instruments and technologies than any other branch of biology. Consequently, it is difficult to learn about cell and molecular biology without also learning about the technology that is required to collect data.

The simplest microscope is a single lens, and as we saw in the Introduction this was for many years the best microscope. *Simple microscopes*, as they are called, can give high-resolution images, and once we understand how one lens works, it is equally easy to see how they are combined to form both ancient and modern *compound microscopes*.

1. The Light Microscope

Microscopes are instruments that produce an enlarged image of an object. A light source, which may be external to the microscope or built into its base, illuminates the specimen. The substage *condenser lens* gathers the diffuse rays from the light source and illuminates the specimen with a small cone of bright light that allows very small parts of the specimen to be seen after magnification.

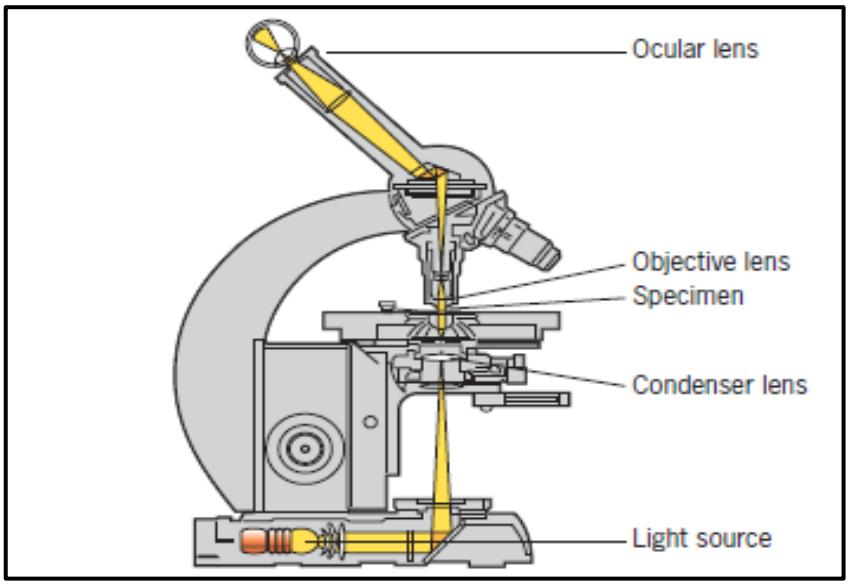


Figure 90: Sectional diagram through a compound light microscope.

The light rays focused on the specimen by the condenser lens are then collected by the microscope's objective lens. From this point, we need to consider two sets of light rays that enter the objective lens: those that the specimen has altered and those that it hasn't. The latter group consists of light from the condenser that passes directly into the objective lens, forming

the background light of the visual field. The former group of light rays emanates from the many parts of the specimen and forms the image of the specimen.

These light rays are brought to focus by the objective lens to form a real, enlarged image of the object within the column of the microscope. The image formed by the objective lens is used as an object by a second lens system, the *ocular lens*, to form an enlarged and virtual image.

A third lens system located in the front part of the eye uses the virtual image produced by the ocular lens as an object to produce a real image on the retina. When the focusing knob of the light microscope is turned, the relative distance between the specimen and the objective lens changes, allowing the final image to become focused precisely on the plane of the retina. The total magnification attained by the microscope is the product of the magnifications produced by the objective lens and the ocular lens.

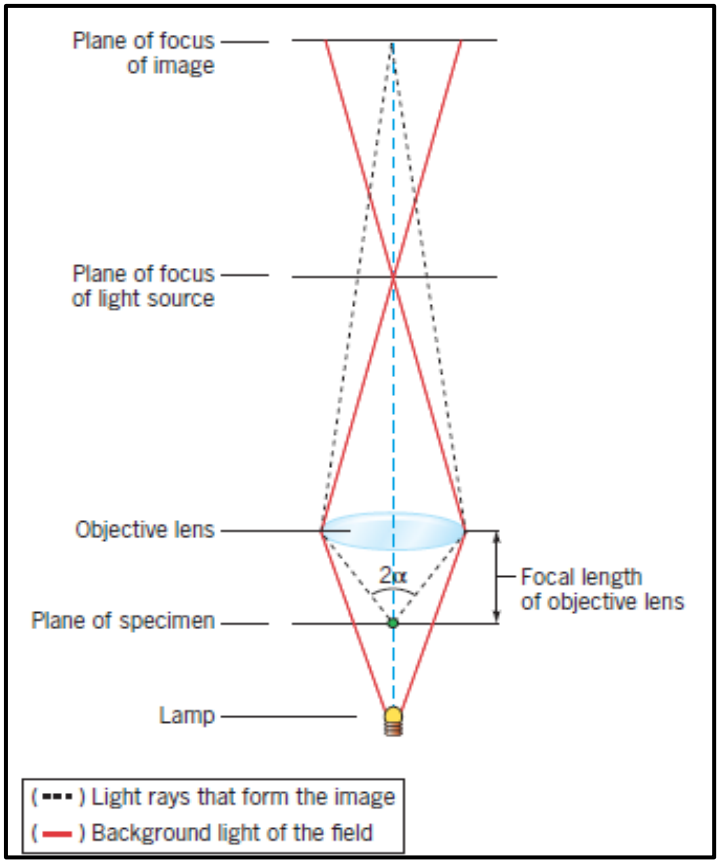


Figure 91: The paths taken by light rays that form the image of the specimen and those that form the background light of the field.

1.2. Resolution

This passage explains that while magnification enlarges an image, it doesn't guarantee better image quality or detail. Using higher-power ocular lenses (like switching from 5x to 10x) can help distinguish closely spaced structures like chromosomes by spreading the image over more retinal area. However, further increasing magnification (like to 20x) without added image detail leads to *empty magnification*. The true quality of an image depends on resolution the ability to distinguish two nearby points. Resolution is limited by light diffraction, which causes points to appear as disks; if the disks overlap, the points can't be seen separately.

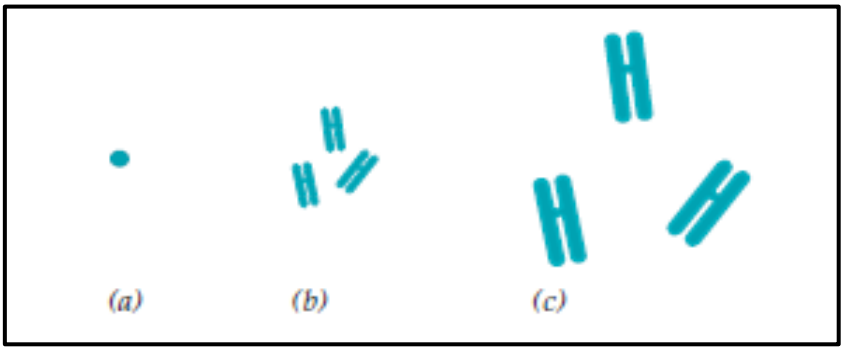


Figure 92: Magnification versus resolution**.** The transition from (a) to (b) provides the observer with increased magnification and resolution, whereas the transition from (*b*) to (*c*) provides only increased magnification (empty magnification). In fact, the

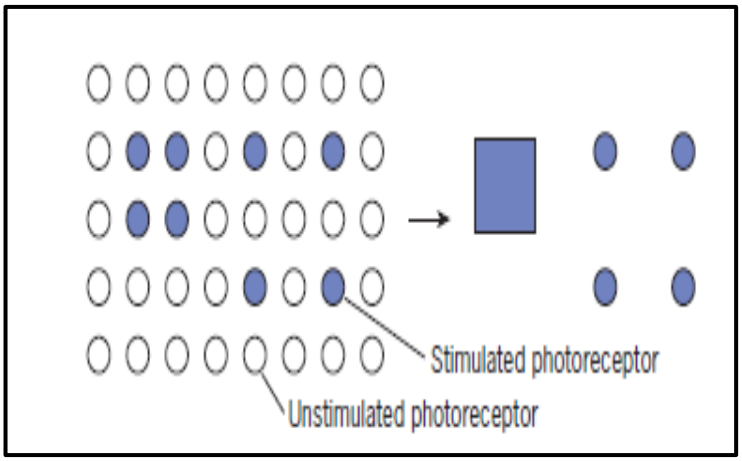


Figure 93: The resolving power of the eye. A highly schematic illustration of the relationship between the stimulation of individual photoreceptors (left) and the resulting scene one would perceive (right). The diagram illustrates the value of having the image fall over a sufficiently large area of the retina.

The resolving power of a microscope is limited by the wavelength of light and the numerical aperture (N.A.) of the objective lens, described by the formula:

$$\mathbf{d = 0.61\lambda / (n \sin \alpha)}$$

d: is the minimum resolvable distance,

λ is the wavelength of light,

n is the refractive index, and α is the half-angle of the light cone entering the lens.

The maximum N.A. is about 1.0 for air and 1.5 for oil-immersion lenses. A light microscope can magnify usefully up to 500–1000× its N.A.; beyond this lies "empty magnification." The best resolution possible is around 0.2 µm, enough to see large organelles. Resolution is also limited by lens imperfections (aberrations), which are corrected using multiple lens elements.

1.3.visibility in microscopy

This passage focuses on visibility in microscopy, which refers to how easily an object can be seen not just whether it's present. Visibility depends on contrast, or how differently an object affects light compared to its background. For example, a glass bead in oil with the same refractive index becomes invisible because it blends into the background. Similarly, transparent cells can be hard to see under a light microscope. To improve contrast, staining is often used. Stains absorb certain wavelengths of light, coloring specific structures in the specimen and increasing their visibility. However, most stains are toxic or require conditions that make them unsuitable for living cells, like the Feulgen stain, which is specific for DNA but requires acid treatment.

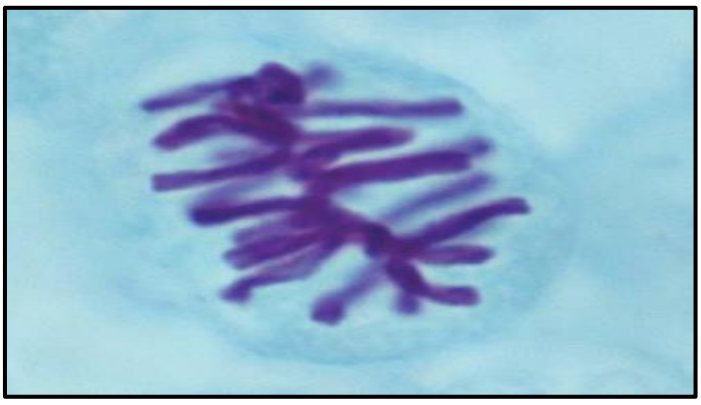


Figure 94: The Feulgen stain. This staining procedure is specific for DNA as indicated by the localization of the dye to the chromosomes of this onion root tip cell that was in metaphase of mitosis at the time it was fixed.

2. Bright-Field and Phase-Contrast Microscopy

Different types of light microscopes use different types of illumination. In a bright-field microscope, the cone of light that illuminates the specimen is seen as a bright background against which the image of the specimen must be contrasted. Bright-field microscopy is ideally suited for specimens of high contrast, such as stained sections of tissues, but it may not provide optimal visibility for other specimens.

2.1. Bright-field light microscopy

Bright-field light microscopy involves examining either whole mounts (intact specimens) or sections (thin slices of tissue). Since most plant and animal tissues are opaque, they must be thinly sliced for observation. Before sectioning, the tissue is fixed using chemicals like formaldehyde to preserve its structure. It is then dehydrated, embedded in paraffin wax for support, and sliced. The wax is later removed, and the tissue is stained to enhance contrast. Finally, a coverslip is placed over the sample using a medium with the same refractive index as the glass for clear viewing.

2.2.Phase-contrast microscopy

Phase-contrast microscopy enhances the visibility of transparent, unstained specimens like living cells by converting differences in refractive index into differences in brightness. Since different cell components (e.g., DNA, proteins, vacuoles) have different refractive indices, they affect light differently. This technique separates and manipulates direct and diffracted light to create contrast. While it doesn't necessarily reveal new structures, it allows real-time observation of living cells, making it valuable in research and teaching. However, it has some drawbacks, like reduced resolution and halo artifacts.

A related method, Differential Interference Contrast (DIC) or Nomarski microscopy, minimizes these artifacts and produces high-contrast images with a 3D appearance, especially at structural edges.

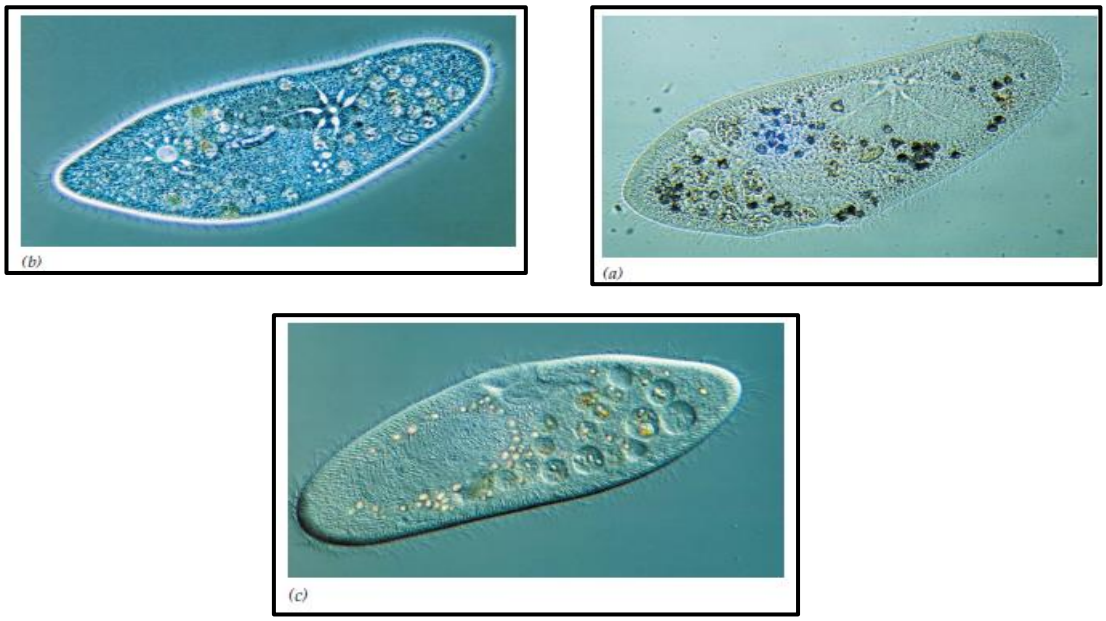


Figure 95: A comparison of cells seen with different types of light microscopes. Light micrographs of a ciliated protist as observed under bright-field (*a*), phase-contrast (*b*), and differential interference contrast (DIC) (or Nomarski) optics (*c*). The organism is barely visible under brightfield illumination but clearly seen under phase-contrast and DIC microscopy.

2.3.Fluorescence microscopy

Fluorescence microscopy has revolutionized cell biology by allowing the visualization of molecular events in living cells. Fluorophores—molecules that absorb light at one wavelength and emit it at another—can be chemically attached to proteins or introduced via genetic engineering (e.g., GFP from jellyfish). GFP and its color variants (BFP, YFP, CFP, DsRed, etc.) enable real-time tracking of proteins and cellular dynamics.

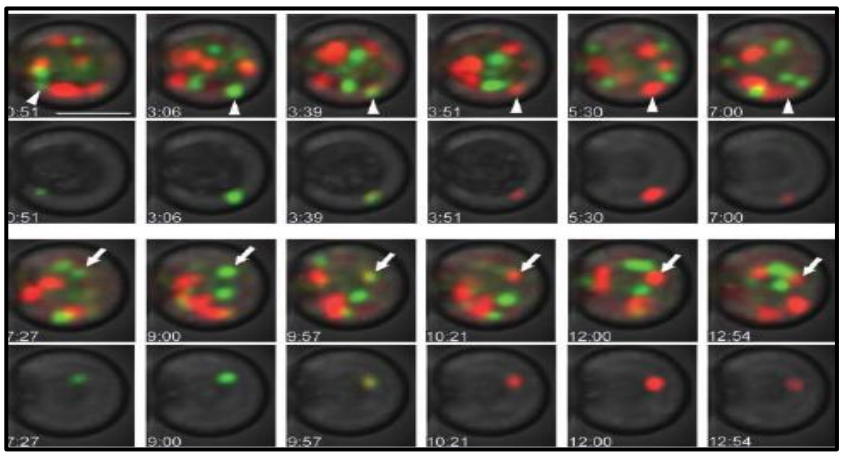


Figure 96: Using dual-label fluorescence to follow dynamic events within cellular organelles of living cells.

Key Techniques:

- **Immunofluorescence**: Antibodies tagged with fluorophores locate specific proteins in cells.
- **Live-cell imaging**: Fluorescent proteins track movement and interactions of molecules in real time.
- **FRET (Förster Resonance Energy Transfer)**: Measures nanoscale distances between two fluorophores to study protein conformational changes or interactions.
- **FRAP (Fluorescence Recovery After Photobleaching)**: Measures protein movement and turnover by bleaching a fluorescent area and observing recovery.
- **Multiphoton microscopy**: Uses long-wavelength, low-energy photons for deep tissue imaging (up to 200 µm), with minimal damage.

Automated imaging and analysis software enhance the power of fluorescence microscopy for large-scale experiments, such as gene function screens using siRNA libraries.

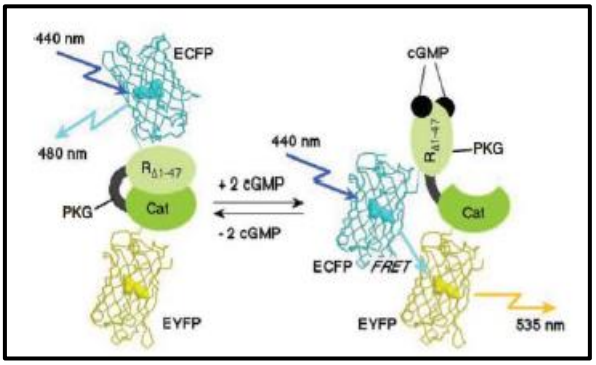


Figure 97: Fluorescence resonance energy transfer (FRET).

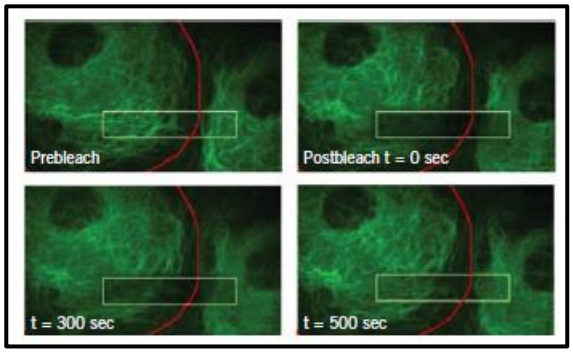


Figure 98: Micrographs from a FRAP experiment performed on interphase (non-dividing) cells expressing GFPtubulin. Two side-by-side cells were bleached in the boxed region with a laser,

and the cells were then imaged over time. The fluorescence signal in the bleached region recovers over the time course of the experiment.

2.4.Laser Scanning Confocal Microscopy

Laser Scanning Confocal Microscopy is an advanced technique that allows researchers to obtain sharp, high-resolution images of thin slices (optical sections) within thick specimens. Unlike conventional light microscopes, which suffer from blurred images due to out-of-focus light from other planes, confocal microscopes eliminate this problem.

Key Features:

- Uses a focused laser beam to scan across a specimen at a single depth.
- The specimen is fluorescently labeled, and only light from the focal plane passes through a pinhole aperture, filtering out out-of-focus light.
- Produces clear images of individual optical sections.
- Multiple optical sections can be digitally stacked to reconstruct a 3D model of the specimen.
- Invented by Marvin Minsky in the 1950s, it has become essential in modern biological imaging.

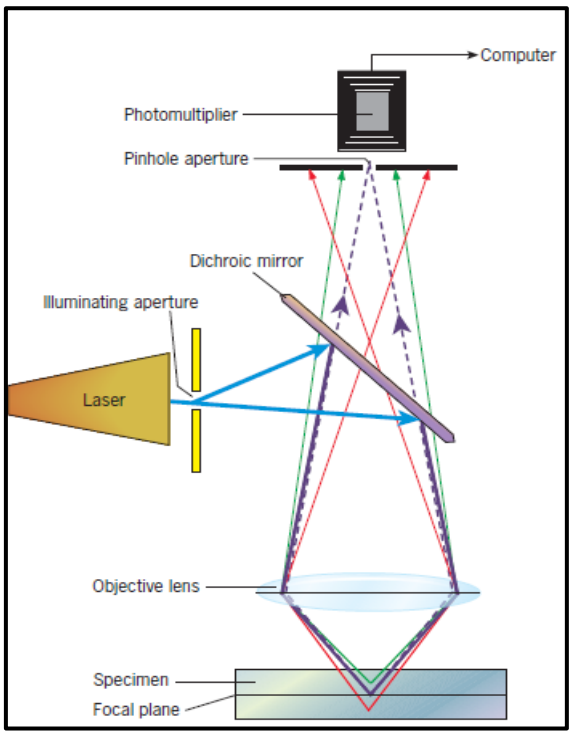


Figure 99: Laser scanning confocal fluorescence microscopy.

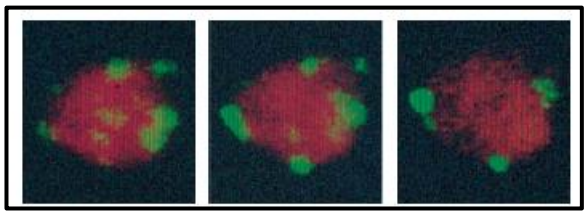


Figure 100: Confocal fluorescence micrographs of three separate optical sections, each 0.3 µm thick, of a yeast nucleus stained with two different fluorescently labeled antibodies. The red fluorescent antibody has stained the DNA within the nucleus, and the green fluorescent antibody has stained a telomere-binding protein that is localized at the periphery of the nucleus.

2.5.Light Sheet Fluorescence Microscopy (LSFM)

Light Sheet Fluorescence Microscopy (LSFM) is a powerful imaging technique used for non-invasive, long-term observation of large, three-dimensional biological samples, such as whole embryos.

Key Points:

- A thin sheet of laser light illuminates a single plane of the sample, perpendicular to the observation direction.
- Allows 3D imaging over time (4D imaging: 3D + time) with minimal photodamage and photobleaching.
- Suitable for live imaging of entire organisms (e.g., zebrafish, fly, worm embryos) at subsecond intervals.
- In 2014, Eric Betzig introduced an advanced version called lattice light sheet microscopy, which increases speed and resolution.
- This method is ideal for tracking dynamic cellular and molecular processes in living systems.

Figure 101: Live neutrophil-like cell imaged by lattice light sheet microscopy and digitally rendered.

3. Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is a powerful imaging technique that uses electrons transmitted through a specimen to generate highly detailed images.

Key Points:

- TEM is different from SEM, which uses electrons reflected off the surface; TEM deals with transmitted electrons.
- It offers much greater resolution than light microscopy due to the shorter wavelength of electrons.
- Electron wavelength is determined by the accelerating voltage applied in the microscope (typically 10,000–100,000 volts).
- At 60,000 V, the electron wavelength is about 0.05 Å, enabling visualization of fine cellular structures far beyond the light microscope's capabilities.
- The resolution depends on the electron's wavelength, which is inversely proportional to its energy (determined by voltage).

While the theoretical resolution of TEM can reach ~0.03 Å, practical limitations—mainly spherical aberration—reduce it to about 3–5 Å, and even 10–15 Å for biological specimens.

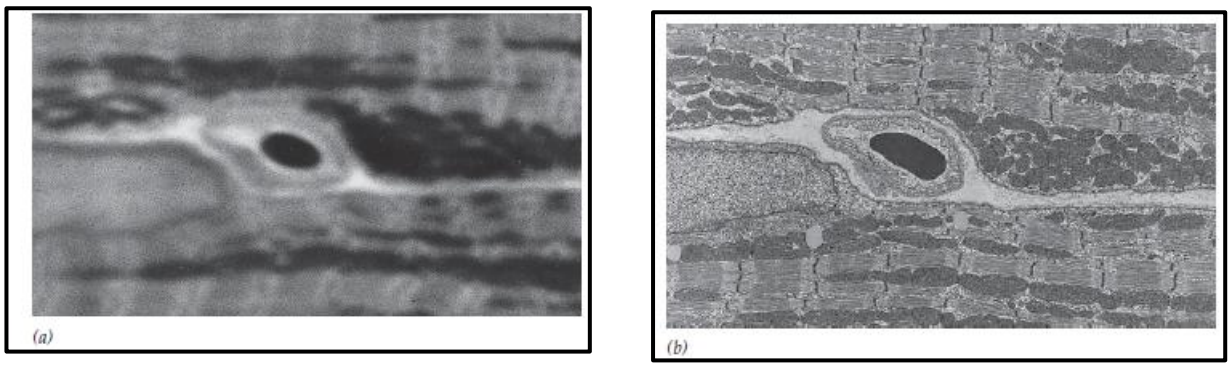


Figure 102: A comparison between the information contained in images taken by a (a) light and (b) electron microscope.

How a TEM works:

- The microscope consists of a vacuum-sealed column to prevent electrons from scattering.
- At the top, a tungsten filament (cathode) emits electrons when heated. These are accelerated by high voltage toward the anode.
- Electromagnetic lenses (controlled by electric current) focus the electron beam.
- Condenser lenses focus the beam on the specimen, which is placed on a small metal grid.
- Magnification is adjusted by changing lens currents, ranging from 1,000x to 250,000x.
- Electrons that pass through the specimen strike a phosphorescent screen, converting them into visible light to form the final image.

Image formation in TEM relies on differential electron scattering:

- Without a specimen, the electron beam evenly lights the screen.
- With a specimen, some electrons are scattered by atoms in the sample and can't pass through the aperture, so they don’t contribute to the image.
- Scattering is stronger where there are larger atomic nuclei, but biological materials (mainly composed of light atoms like C, O, N, H) scatter electrons poorly.

To enhance contrast:

- Samples are stained with heavy metals (e.g. uranium, lead) which bind to specific cell parts.
- Areas with more heavy metal scatter more electrons, allowing fewer to reach the screen, making those areas appear darker.

Image capture:

- Originally done on photographic film, which is sensitive to electrons.
- Today, digital detectors (CCD sensors) are more common: electrons are converted into photons and then recorded digitally.

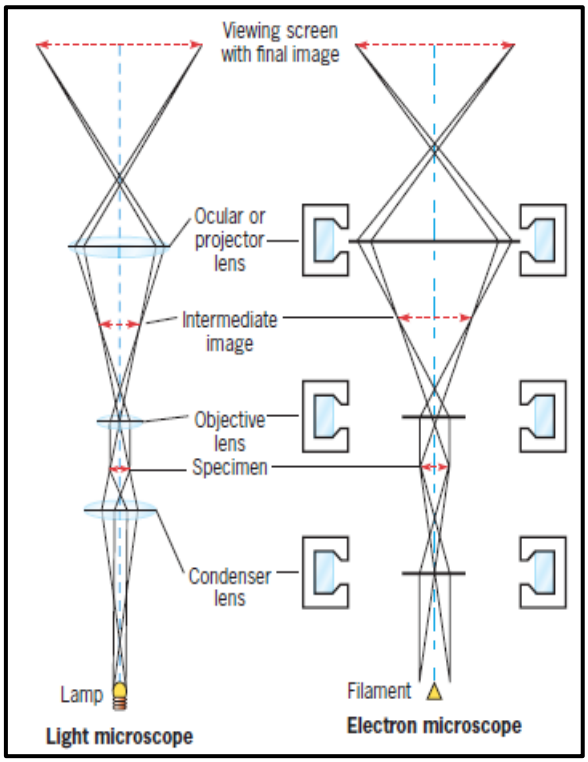


Figure 103: A comparison of the lens systems of a light and electron microscope.

3.1.1. Specimen Preparation for Electron Microscopy

Preparing samples for electron microscopy requires meticulous steps due to the high resolution and sensitivity of the technique:

- **Fixation** is crucial to preserve fine cellular details. Common fixatives are:
  - **Glutaraldehyde**: Cross-links proteins by reacting with amino groups.
  - **Osmium tetroxide**: Binds to fatty acids, preserving membranes.

To avoid artifacts, multiple fixation methods are used or cryofixation techniques may be applied.

- **After fixation:**
  - Tissue is dehydrated (usually with alcohol),
  - Then embedded in epoxy resin for support.
- **Sectioning:**
  - For TEM, ultrathin sections (<0.1 µm) are needed—much thinner than light microscopy (5 µm).

- A diamond or glass knife cuts these thin slices which float on water and are collected on a metal grid.

- **Staining:**
  - Sections are stained with heavy metals (e.g., uranyl acetate, lead citrate) to increase contrast by scattering electrons.
  - For detecting specific molecules, metal-tagged antibodies can be used—best applied on tissues embedded in acrylic resins for permeability.

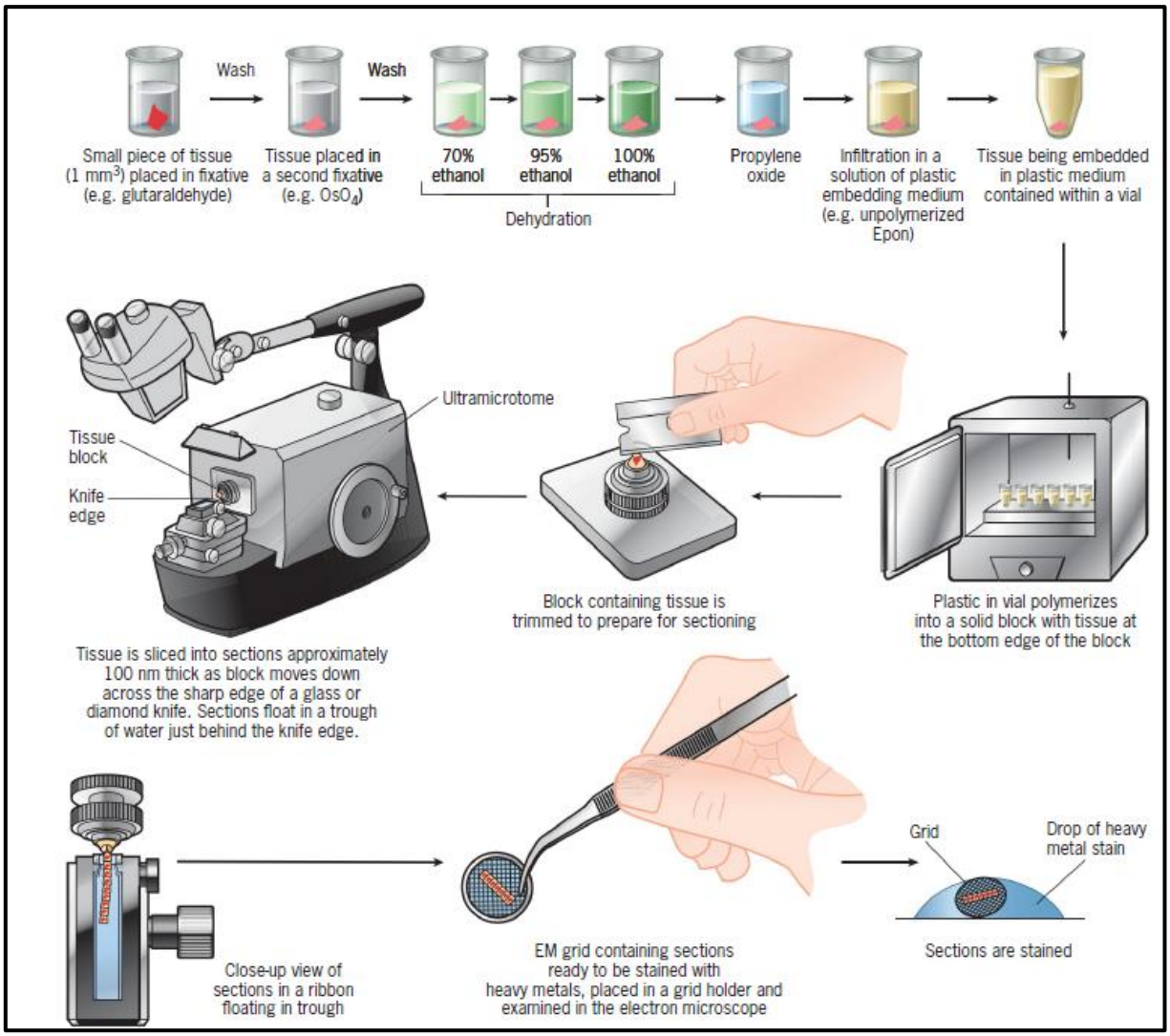


Figure 104: Preparation of a specimen for observation in the electron microscope.

3.1.2. Cryofixation and the Use of Frozen Specimens

Cryofixation is a method where biological specimens are rapidly frozen rather than chemically fixed and embedded. It preserves structures in a near-native state and avoids the artifacts caused by chemical fixatives. This is especially useful for:

- **Enzyme studies**, where activity can be lost with chemical fixation.
- **Fast diagnoses** during surgery (e.g. frozen section analysis by pathologists).

Key techniques include:

- **Cryo-electron tomography (cryo-ET)**: Creates 3D images of flash-frozen samples by collecting 2D images at different angles and reconstructing them digitally—similar to CT scans in medicine. It enables nanoscale imaging of intact, hydrated cells or molecular complexes (like ribosomes).
- **Advantages**: Preserves structure without altering macromolecules; minimal artifacts.
- **Main challenge**: **Ice crystal formation**, which damages cells. This is avoided by ultrarapid freezing:
    - **Small samples**: Plunged into liquid propane.
    - **Larger samples**: Use high-pressure freezing with jets of liquid nitrogen, which slows ice crystal growth by lowering the freezing point.

Even after freezing, specimens can be sectioned using a cryomicrotome and examined under a microscope, just like paraffin or resin-embedded tissues.

Electron microscopy is useful for studying very small structures like viruses, ribosomes, protein complexes, and individual macromolecules. Two common techniques that enhance visibility by improving contrast are:

a. Negative Staining

- Involves depositing heavy metals (e.g., uranyl acetate) everywhere on the grid except where the specimen lies.
- The particles block metal deposition and thus appear lighter against a dark background.
- Useful for imaging small particles such as viruses and proteins.

b. Shadow Casting

- Specimens are placed in a vacuum chamber with a metal filament (usually platinum with carbon).
- The metal is evaporated at an angle, coating only one side of each particle and casting a shadow on the opposite side.
- In the image:
    - **Metal-coated areas = dark**.
    - **Shadowed (uncoated) areas = bright**.
- The final printed image is **reversed** (a negative), making the particle appear illuminated with a **3D effect**.

Both techniques greatly enhance contrast and can reveal fine surface details.

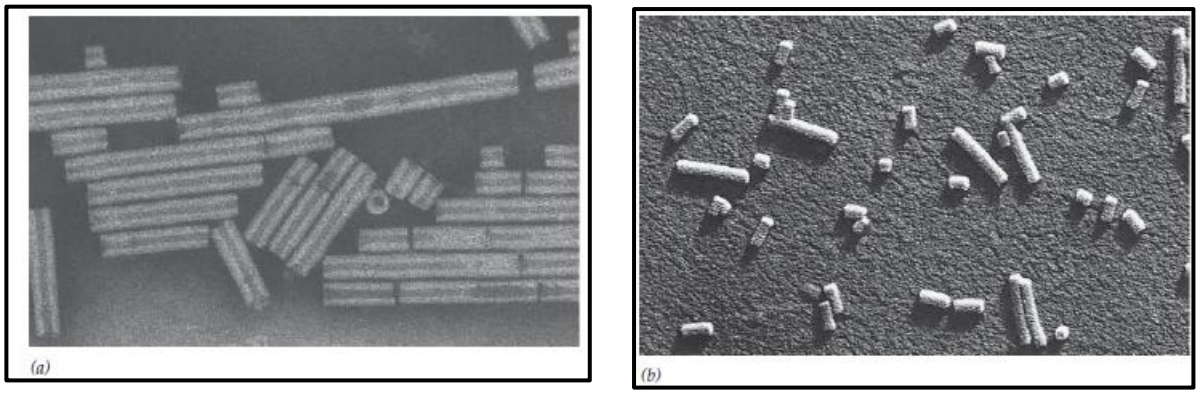


Figure 105: Examples of negatively stained and metal-shadowed specimens. Electron micrographs of a tobacco rattle virus after negative staining with potassium phosphotungstate (*a*) or shadow casting with chromium (*b*).

3.1.3. Freeze-Fracture Replication

Freeze-fracture replication is a powerful electron microscopy technique for studying frozen cells and their internal membrane structures:

◇ Procedure Overview:

1. **Rapid Freezing:** Tissue is quickly frozen to preserve its native state.

2. **Fracturing:** A cold knife splits the frozen specimen along natural planes—often through the middle of membranes.
3. **Shadowing and Replication:**
    - A thin layer of heavy metal is deposited at an angle to enhance surface detail (like shadow casting).
    - Then a carbon layer is added to stabilize the replica.
4. **Removal:** The biological tissue is thawed and discarded; only the metal-carbon replica is examined under the electron microscope.

◇ Freeze Etching (Optional Enhancement):

- Before shadowing, the fractured surface is exposed to mild heat in a vacuum, causing some ice to sublime.
- This "etches" the surface and reveals more structural detail—especially useful for visualizing cytoskeletal and organelle architecture.

◇ Applications:

- Ideal for analyzing the arrangement of membrane proteins.
- Provided key evidence for the fluid mosaic model of membranes.
- Deep-etching, developed by John Heuser, allows even more detailed imaging of organelle and cytoskeletal structures in 3D relief.

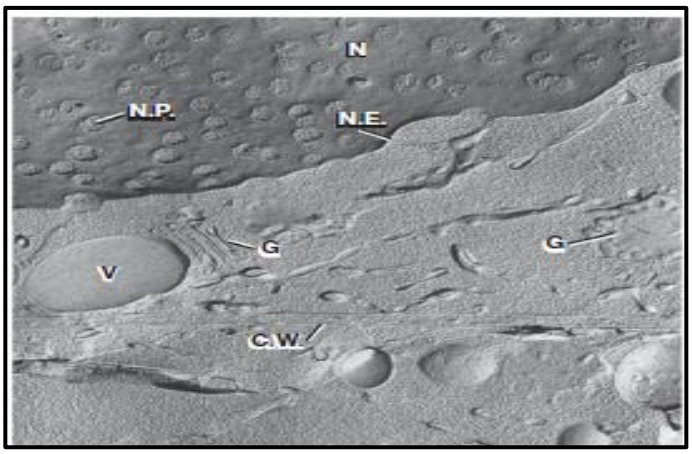


Figure 106: Replica of a freeze-fractured onion root cell showing the nuclear envelope (N.E.) with its pores (N.P.), the Golgi complex (G), a cytoplasmic vacuole (V), and the cell wall (C.W.).

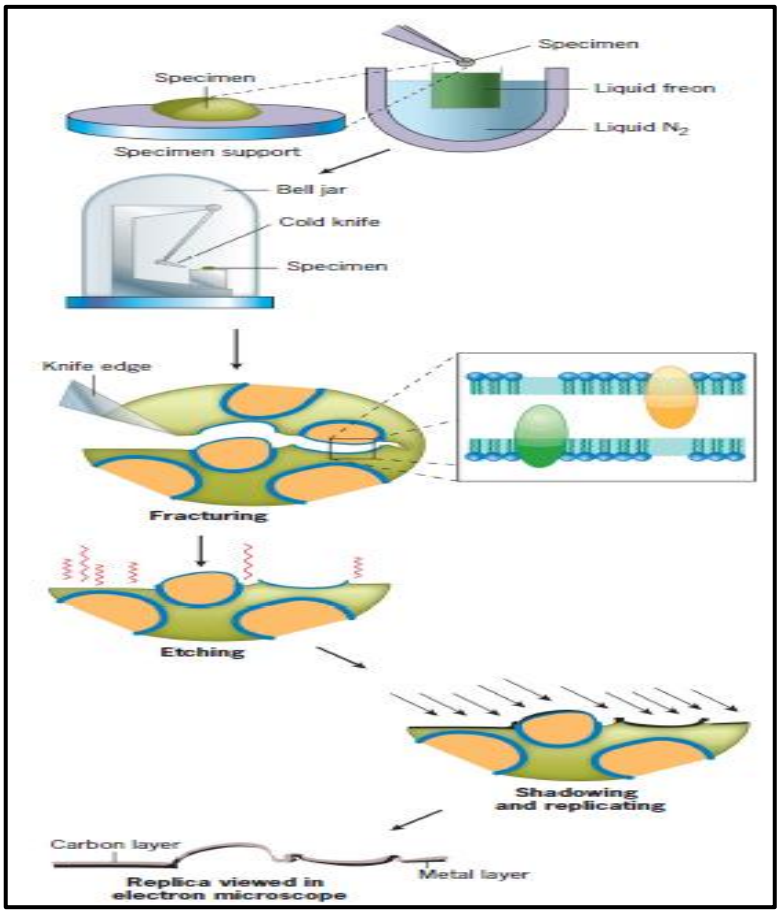


Figure 107: Procedure for the formation of freeze-fracture replicas. Freeze etching is an optional step in which a thin layer of covering ice is evaporated to reveal additional information about the structure of the fractured specimen.

4. Scanning Electron Microscopy (SEM)

SEM is mainly used to examine surface structures of specimens, from tiny viruses to large organisms, unlike TEM which reveals internal structures.

- Preparation Steps:

1. **Fixation:** Specimens are chemically fixed to preserve their structure.
2. **Dehydration:** Water is removed using alcohols.
3. **Critical-Point Drying:**
    - Replaces water with a transitional fluid (like CO_2).

- Uses high temperature and pressure to vaporize the fluid without surface tension, preventing distortion of 3D shapes.

4. **Metal Coating:** A thin layer of metal (e.g., gold or platinum) is applied to make the specimen conductive and electron-reflective.

◆ How SEM Works:

- A focused electron beam scans the surface of the specimen.
- Secondary and backscattered electrons emitted by the specimen are detected.
- These electron signals are used to control the intensity of a synchronized beam on a cathode-ray screen, forming a detailed 3D image of the surface.

◆ Key Features of SEM:

- Magnification: ~15x to 150,000x.
- Resolution: < 5 nanometers with advanced models.
- Depth of focus: ~500x better than a light microscope.
- Surface Visualization: Ideal for examining surface morphology and cellular extensions.
- Can also be used with gold-labeled antibodies to locate specific proteins on the cell surface.

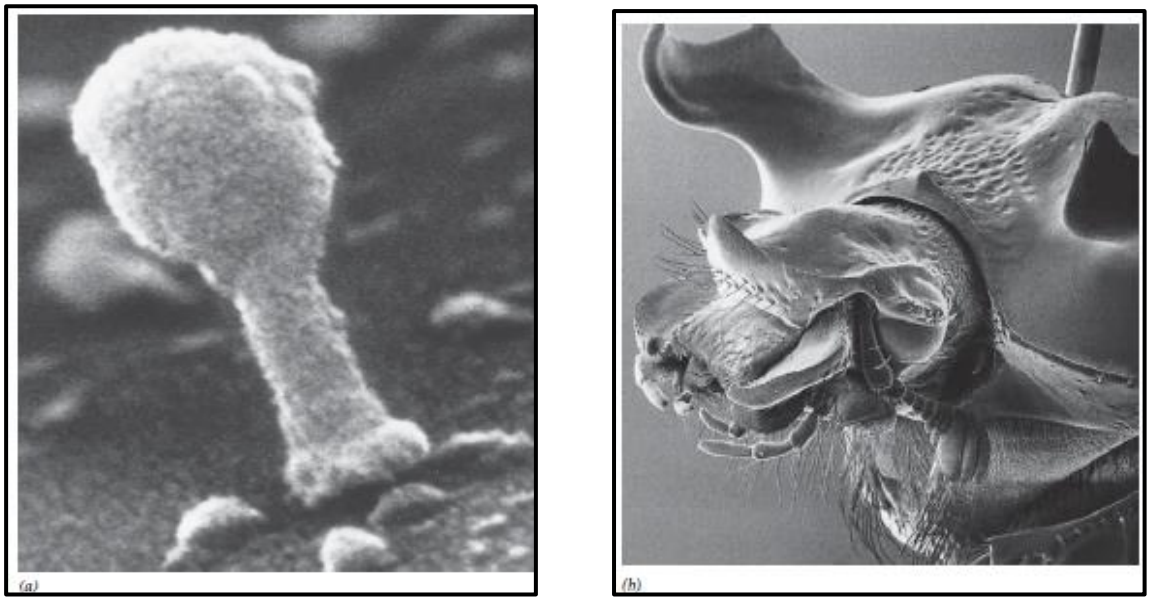


Figure 108: Scanning electron microscopy. Scanning electron micrographs of (*a*) a T4 bacteriophage (275,000) and (*b*) the head of an insect (40).

5. Atomic Force Microscopy

AFM is a high-resolution scanning instrument (not an electron microscope) used in nanotechnology and molecular biology to visualize individual molecules and measure mechanical properties.

- How AFM Works:
  - A sharp tip (probe) scans the surface of a specimen.
  - The tip is attached to a tiny oscillating cantilever.
  - As the tip encounters surface features, the frequency of oscillation changes, which are converted into a 3D topographic image.
- Advantages:
  - Unlike X-ray crystallography or cryo-EM, AFM provides images of individual molecules (not averaged structures).
  - High-speed AFM (HS-AFM) allows researchers to capture real-time sequences, like a molecular movie — e.g., watching a myosin V molecule walk along actin.
- Other Uses:
  - The AFM tip can manipulate molecules — it can push, pull, or stretch them to measure mechanical strength.
  - The tip can also be functionalized with ligands to measure binding affinity to receptors.

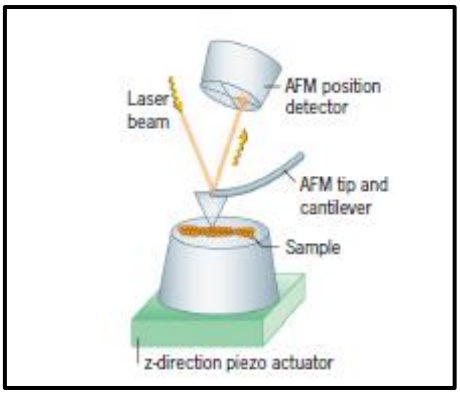


Figure 109: High-speed atomic force microscopy.

📚 Bibliographic References

1. Structural and Functional Organization of the Cell

- Alberts et al., *Molecular Biology of the Cell*, 6th ed., Garland Science, 2014 — Chapters 1–4
- Karp, G., *Cell and Molecular Biology: Concepts and Experiments*, 8th ed., Wiley, 2018 — Chapters 1–3
- Lodish et al., *Molecular Cell Biology*, 9th ed., W.H. Freeman, 2021 — Chapters 1–3

2. The Extracellular Matrix

- Alberts et al., *Molecular Biology of the Cell*, Chapter 19
- Lodish et al., *Molecular Cell Biology*, Chapter 20
- Cooper & Hausman, *The Cell: A Molecular Approach*, Chapter 15

3. Cell Differentiation

- Lodish et al., *Molecular Cell Biology*, Chapter 22
- Karp, G., *Cell and Molecular Biology*, Chapter 16
- Alberts et al., *Molecular Biology of the Cell*, Chapter 21

4. Intracellular Trafficking

- Alberts et al., *Molecular Biology of the Cell*, Chapters 12–13
- Lodish et al., *Molecular Cell Biology*, Chapters 13–14
- Cooper & Hausman, *The Cell*, Chapter 9

5. Regulation and Deregulation of the Cell Cycle

- Alberts et al., *Molecular Biology of the Cell*, Chapter 17
- Karp, G., *Cell and Molecular Biology*, Chapter 10
- Lodish et al., *Molecular Cell Biology*, Chapter 19

6. Cell Communication and Signal Transduction

- Alberts et al., *Molecular Biology of the Cell*, Chapters 15–16
- Lodish et al., *Molecular Cell Biology*, Chapters 15–16
- Karp, G., *Cell and Molecular Biology*, Chapters 7–9

7. Apoptosis

- Alberts et al., *Molecular Biology of the Cell*, Chapter 18
- Lodish et al., *Molecular Cell Biology*, Chapter 18
- Cooper & Hausman, *The Cell*, Chapter 18

8. Cell Imaging Techniques

- Alberts et al., *Molecular Biology of the Cell*, Appendix & Chapter 9
- Karp, G., *Cell and Molecular Biology*, Experimental Highlights & Appendix
- JoVE – *Journal of Visualized Experiments*, Cell Biology Section: https://www.jove.com/

📚 **Additional Useful Resources:**

- **PubMed Central**: https://www.ncbi.nlm.nih.gov/pmc/
  ➤ Free access to a wide range of peer-reviewed scientific articles.
- **Nature Reviews Molecular Cell Biology**
  ➤ A leading journal to follow the latest developments in the field.
- **JoVE (Journal of Visualized Experiments)** – *Cell Biology section*
  ➤ Provides experimental protocols demonstrated through high-quality video.