REPUBLIC ALGERIAN DEMOCRATIC AND POPULAR

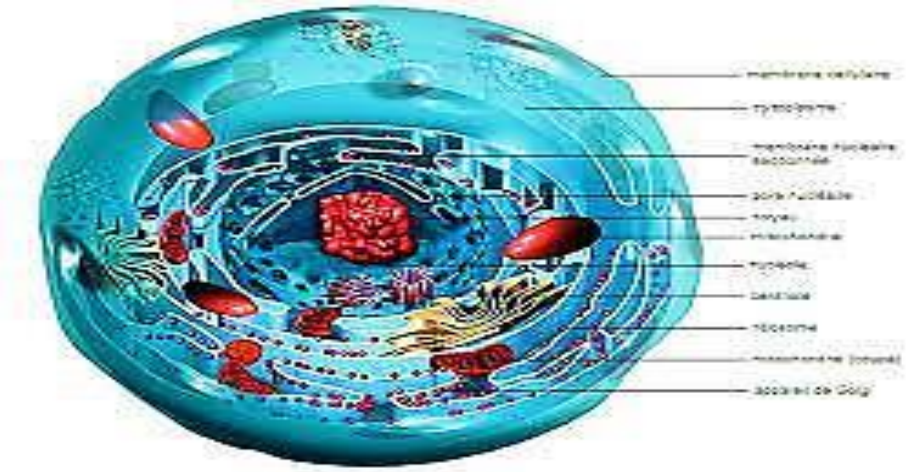
Ministry of Higher Education and Scientific Research

University of Abbes Laghrour - Khenchela –

Faculty of Natural and Life Sciences
Biology Molecular and Cellular Department

TEACHING HANDOUT

Cellular and functional biochemistry



Level: 3rd Year Licence Biochemistry

Prepared by: Dr. DOUAOUYA Lilia ***(Associate Professor A)***

2023/2024

Table of contents

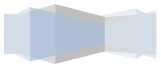
List of figures

List of figures

List of tables

Preface

This teaching handout is intended for third-year Licence Biochemistry students wishing to deepen their knowledge of cell biology in a biochemical context. Cellular and Functional Biochemistry (CFB) is broadly defined as the study of the processes and mechanisms of living organisms. The aim of this handout is to give the basics of membrane dynamics, intracellular compartmentalization, and its integration into cell function, as well as the transmission of intracellular signals from hydrophilic ligands. It also covers the interconnections between signaling networks and provides an introduction to biochemical genomics.



Chap.1. FUNCTIONAL COMPARTMENTALIZATION OF THE CELL

I. Cell theory

1. The cell is the constituent and functional unit of living organisms.
2. The body depends on t h e activity of cells, either isolated or grouped intotissues, to carry out its various functions.
3. The biochemical activities of cells are coordinated and determined by certain structures present within them.
4. The multiplication of cells enables organisms to be maintained and tomultiply.
5. This theory was formulated in 1838 by Schleiden and Schwann: the cell is theunit of life.

(All living things are cellular). This theory also suggests the presence of organelles within cells. Above all, we always wonder what characteristics cells have in common, despite their diversity. We need to know that**:**

a) The cell represents a highly organised state of matter and requires structures to be put in place to make use of external energy and matter.

b) The cell, an enclosed space that carries out exchanges with the outside world, is delimited by a membrane constituting an exchange surface that allows flows to take place.

c) Plasma membranes, despite their diversity, have, with some exceptions (certain archaeothermophiles which are unicellular prokaryotic microorganisms with a membrane made up of a single lipid layer and which live in almost all environments, particularly in extreme anaerobic, high- salinity, very hot or very deep environments), have an identical structure:

---A lipid bilayer made up of amphiphilic lipids, which acts as a basic filter, allowing hydrophobic substances to pass through and preventing hydrophilic substances fromp a s s i n g t h r o u g h .

---Transmembrane and peripheral proteins with various roles (transfer, transport, signal transduction, etc.)

d)-The membrane not only acts as a filter for molecules according to the difference in concentration (concentration gradient) but also uses energy (osmotic, chemical...) to promote endergonic flows, allows the passage of light, heat... and also ensures the transmission of information necessary for the cell's reactivity to changes in the environment and coordination with other cells. The plasma membrane therefore creates an enclosed space in constant exchange with the surrounding environment.

II. Cellular varieties

1. Eukaryotic cells

Eukaryotic cells (or true nucleus in Latin) are characterised by a nuclear envelope that divides the cell into two main compartments: the nucleus and the cytoplasm. The cytoplasm, in turn, is bounded by the plasma membrane. In the plant cell (Fig.1), the plasma membrane is covered and protected externally by a thicker cell wall pierced by canaliculi, the plasmodesmata, which communicate with neighbouring cells via fine cytoplasmic extensions. In animal cells (Fig.2), certain regions of the plasma membrane are covered by a thin layer of material usually referred to as the outer envelope.

2. Prokaryotic cells

Prokaryotic cells, such as bacteria and blue-green algae, consist of a single compartment surrounded by a plasma membrane, do not have a well-defined nucleus and have a simple internal organisation. These cells contain a single chromosome made up of a free circular or linear DNA molecule located in the cytoplasm.

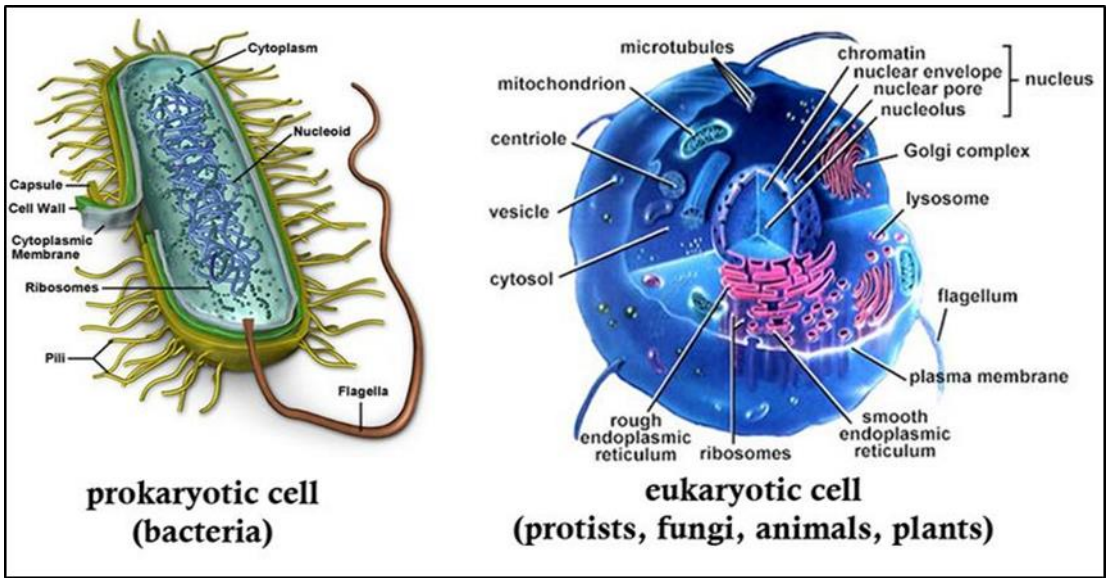


Fig 1. Prokaryotic Cell vs Eukaryotic Cell

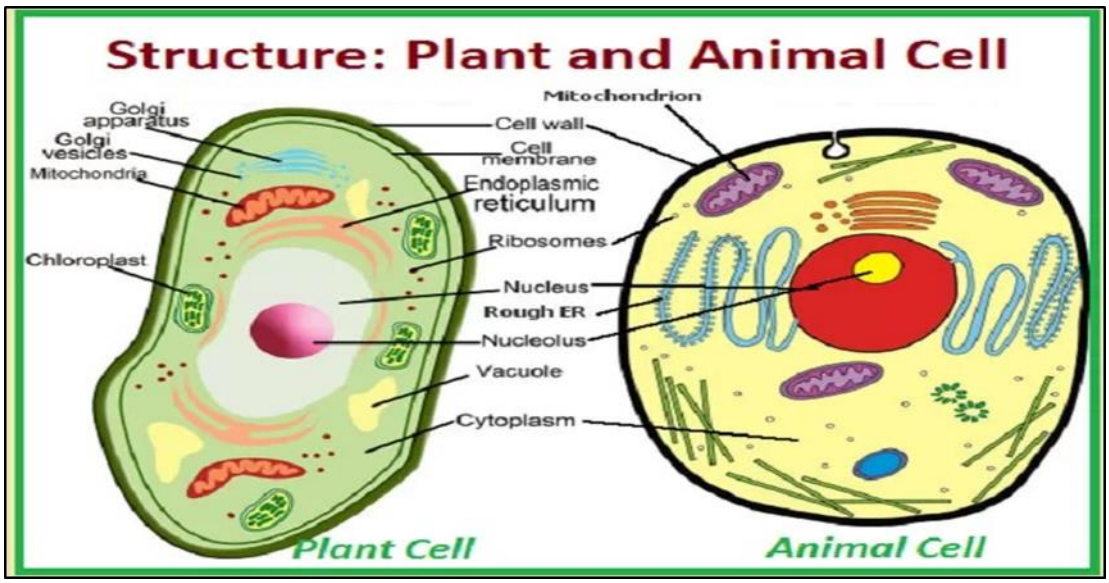


Fig 2. Plant-Animal-Cell-Structure (2)

The main differences between prokaryotic and eukaryotic cells are illustrated in the table below.

Table 1. Comparison between eukaryotic and prokaryotic cells

	Prokaryotes	**Eukaryotes**
Representatives	bacteria, archaea	protists, fungi, plants, animals
Typical size	~ 1-10 µm	~ 10-100 µm
Coretype	nucleoid; no true nucleus	real core with an envelope
DNA	circular (chromosome), with HU proteins for eubacteria	linear molecules (chromosomes) with *histone* proteins
RNA/protein synthesis	coupled to the cytoplasm	RNA synthesis in the nucleus Protein synthesis in the cytoplasm
Ribosomes	23S+16S+5S	28S+18S+5.8S+5S
Cytoplasmic structure	very few structures	highly structured by intracellular membranes and a cytoskeleton
Cell movement	flagellum made of flagellin	flagellum and cilia made of tubulin
Metabolism	anaerobic or aerobic	usually aerobic
Mitochondria	no	from one to several thousand

Chloroplasts	no	in algae and chlorophyll plants
Organisation	usually isolated cells	isolated cells, colonies, complex organisms with specialised cells
Cell division	single division	Mitosis (conformal multiplication of the cell) Meiosis (formation of gametes)

3. Eukaryotic cell: formation of communities of intracellular cells

The endosymbiotic theory (a- theory that has been demonstrated for mitochondria and chloroplasts) states that eukaryotic cells formed from a prokaryotic cell that phagocytosed and then domesticated bacteria, which are thought to be the origin ofmitochondria. The invagination of cyanobacteria gave rise to chloroplasts.

The eukaryotic cell is therefore derived from the symbiotic association of bacteria that have become totally interdependent to the point of forming a single structural and functional unit.

4. Description and function of cell components

The cell is bounded by a membrane called the plasma membrane. This provides an exchange surface for the flow of blood. Microscopic observation of cells has revealed numerous internal substances known as intracellular organelles (Figs. 1 and 2) which perform very specific functions to keep the cell alive. These include the nucleus, the endoplasmic reticulum, the Golgi apparatus, vesicles and mitochondria. These organelles are also bounded by membranes.
The cell is therefore made up of a complex system of membranes delimiting compartments within which well-defined biochemical reactions take place.

- **Cytoplasm :**

Cytoplasm is defined as the biological material contained between the plasma membrane and the nuclear envelope. It is a liquid phase, more precisely a colloidal emulsion, containing numerous organelles and structures suspended in a liquid phase called **cytosol**. It contains ribosomes, mitochondria, a cytoskeleton and a series of more or less branched vacuoles;

These vacuoles make up the endoplasmic reticulum, the Golgi apparatus, secretory vesicles, endosomes, lysosomes and phagosomes. The cytoplasm of plants also contains an organell called a plastid, which is involved in photosynthesis.

- **Cytosol**

Represents the translucent liquid (or more accurately semi-liquid) phase in which the organelles

are immersed. In biophysical terms, the exact definition of cytosol is that of a colloidal gel 4 times more viscous than water, with a neutral pH of around 7 (physiological pH of the extracellular medium is around 7.4).

- **Inclusions**

These are not functional elements but chemical substances that may or may not be present, depending on the type of cell under consideration. Examples include stored nutrients, such as glycogen granules found in abundance in liver and muscle cells, lipid droplets common in adipose cells, pigment granules (melanin) present in certain skin cells and hairs, and various types of crystals.

- **The core**

The nucleus is the organelle that gave its name to eukaryotes (eu= true, caryos= nucleus), although some may lack one at certain stages of their existence (such as mammalian red blood cells). A structure, usually spherical or ovoid, containing the bulk of the cell's genetic information.

- **Genome**

Genetic information that contains the information needed to code the other components.

- **Nucleolus**

Small spherical body in the cell nucleus of eukaryotic cells containing nucleic acids (RNA) and proteins and which is the site of ribosomal RNA synthesis. The nucleolus surrounds a region of the nucleus containing one or more chromosomes containing repeated copies of the DNA encoding ribosomal RNA.

- **Plasma membrane**

This membrane separates the living cell from its environment; it acts as a filter and a communication system with the outside world.

- **Cellulose wall**

It surrounds every plant cell and is essentially made up of polysaccharides; celluloseand pectin, hence the name "pectocellulose wall". The wall is made up of three parts: Primary wall: pecto-cellulosic.

Secondary wall: made up of cellulose and hemicellulose and enriched with phenolic compounds (lignin, suberin).

Middle lamella: this is the outermost part of the wall and is made up of pectic matteronly.

- **Cytoskeleton**

It helps maintain cell morphology, the position of organelles in the cell and thetransport of various cytoplasmic components. It also important during cell division. These include actin microfilaments, microtubules and intermediate filaments.

- **Endoplasmic reticulum (ER)**

Is an extension of the nucleus membrane. We distinguish between the smooth ER (REL) and the granular (rough) ER (RER), according to its appearance under the microscope. The smooth ER is made up of sheets or tubules. It contains receptors which bind ribosomes involved in the translation of messenger RNA for the secretion of proteins, in particular the majority of transmembrane proteins. It is also the site of lipid synthesis. From the ER, proteins are transported to the Golgi apparatus via vesicles.

- **Ribosomes**

Ribosomes are a key part of the cell's biosynthetic machinery. These organellesenable RNA to be translated into proteins.

- **Golgi apparatus**

Is a stack of membrane vesicles where glycosylation (addition of complexcarbohydrate chains) and encapsulation of secreted proteins takes place.

- **Mitochondria**

They play an important role in cell metabolism. They contain their own small piece of DNA (mitochondrial DNA). This is where cellular respiration and the production of energy, ATP, take place. This energy is essential for metabolic reactions.

- **Chloroplasts**

These organelles are present in plants and algae (photosynthetic organisms). They convert light energy from the sun into chemical energy used to make sugars from carbon dioxide. They also contain DNA.

- **Lysosomes and peroxisomes**

Intracellular organisms containing hydrolytic enzymes, they are responsible for cell lysis, i.e. the dissolution of organic elements (tissues, cells, micro-organisms) under the action of physical, chemical or enzymatic agents.

- **Vacuoles**

Inert enclaves, sometimes limited by a membrane, present in a physiological orpathological state in the cytoplasm of a cell or unicellular organism (bacteria) and which may contain various substances.

- **Centrioles**

Many animal cells have a pair of centrioles at one of their poles. These are cylindrical corpuscles made up of tubules grouped in threes. Generally located near the nucleus, they play an essential role in cell division. They form the poles that enable cell division, which is generally absent in plants.

Chap 2. BIOMEMBRANES

I. Membrane composition

1. Isolation of "plasma membrane" fractions

The development of methods of isolation of plasma membranes has made it possible to study the chemical composition of this organelle, and to conceive what its molecular architecture can be.
It is relatively difficult to obtain a fraction of plasma membranes devoid of other membrane contaminants. The problem was solved by choosing a favorable cell type. Thus, the isolation of pure plasma membranes is very easily done using red blood cells (red blood cells or erythrocytes).

1.1.The technique of isolation of plasma membranes of red blood cells

From a large amount of red blood cells it is therefore possible to recover a fraction of practically pure plasma membrane. These red blood cells are practically made up of hemoglobin surrounded by a plasma membrane (without nuclei and cytoplasmic organelles), it is sufficient to cause hemolysis by osmotic shock to obtain after centrifugation a base only consisting of plasma membranes or "phantoms of red blood cells".

1.2. Isolation method

- The red blood cells are initially separated from the serum by centrifugation to avoid contamination by the proteins of this serum.
- They are collected in a physiological medium as isotonic as possible compared to serum (NaCl is generally used at 0.9%).
- Haemolysis of red blood cells (rupture of their plasma membrane) is caused by osmotic shock. For this, the red blood cells are placed in a hypotonic solution that causes these cells to swell and burst (hemoglobin diffusion out of the cells).
- Centrifugation of lysed red blood cells to obtain a base of pure fractions of plasma membranes that are still called phantoms of red blood cells. Once the plasma membranes are isolated, we study their chemical composition.

2. Chemical composition of the plasma membrane

2.1.Definition

Cell membranes are phospholipid bilayers into which are inserted, asymmetrical manner of other structures characterizing them. In electron microscopy we observe a tri-lamination of the

membrane: a clear sheet of 3 nm surrounded by 2 dark layers of 2.5 nm each; the total thickness is therefore approximately 8 nm. This made it possible to highlight the phospholipid bilayer structure of the plasma membrane.

2.2.Membrane composition

The membranes consist (in dry membrane weight) of 40% lipids, 52% proteins and 8% carbohydrates. Taking into account the difference in weight between these classes of molecules, namely 50 lipid molecules per protein molecule.

2.3.Diversity of membrane lipids

Within the membrane lipids are present in different forms (phospholipids, glycolipids and cholesterol).

- Phospholipids: are the predominant lipids in biological membranes, they have a hydrophilic phosphorylated head and a tail formed by two aliphatic chains of saturated or unsaturated fatty acids (amphipathic molecules) (Fig.3) .

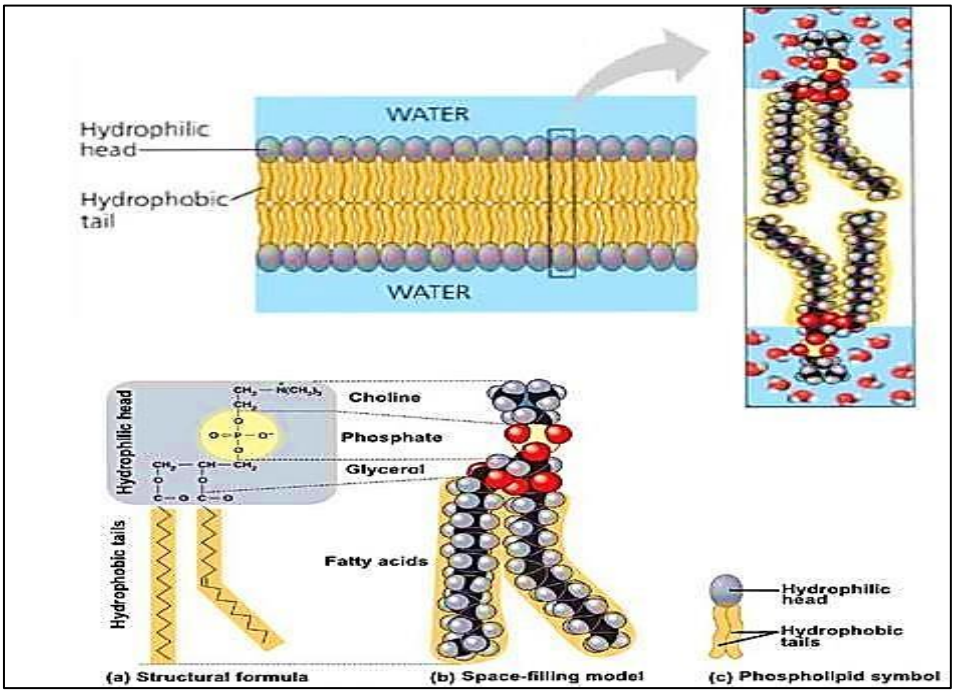


Fig 3. Phospolipid bilayer (cross section) and the structure of a phospholipid

- **Glycolipids:** Lipids whose oligosaccharide antenna is directed towards the extracellular medium. Glycolipids are of two types, glycerol glycolipids and sphingoglycolipids.
- **Cholesterol:** A steroid-like lipid. Cholesterol is only present in the membranes of animal cells. Indeed, it is absent in plant cells and bacteria. It is composed of a hydrophobic

polycyclic steroid core, a hydrophobic tail and a hydrophilic alcohol function, therefore amphiphilic. It represents about a quarter of membrane lipids and influences membrane fluidity.

3. Diversity of membrane proteins

Plasma membrane proteins are numerous and very diverse. Depending on the type of cell and cell organelle, a membrane can contain hundreds of different proteins. It is noted that the membranes of organelles are much richer in proteins, enzymes mainly, in relation to their functions. One characteristic of plasma membrane-related proteins is to have one or more hydrophobic omains that will be in contact with lipids, and one or more hydrophilic omains that will be in contact with water-rich regions of the cellular environment (cytosol, extracellular medium). Membrane proteins are amphiphilic proteins.

- **Class of membrane proteins**

Membrane proteins can be grouped into three different classes:

1) Intrinsic proteins: They enter the lipid bilayer. These are transmembrane proteins, that is, they cross the entire lipid bilayer and therefore have domains exiting the extracellular and cytoplasmic faces of the membrane.

2) Peripheral proteins: They are entirely located outside the lipid bilayer, either on the cytoplasmic or extracellular face, but are associated with the membrane by non-covalent bonds.

3) Lipid-anchored proteins: Located outside the lipid bilayer, either at the extracellular or cytoplasmic face, but united by covalence to a lipid molecule located within the bilayer.

- **Mode of association of membrane proteins with the double lipid layer** (Fig.4)

Transmembrane proteins (whether or not anchored by a fatty acid to the membrane)

1- crosses the double layer in the form of an alpha propeller.

2- crosses the double layer in the form of multiple alpha helix.

3- crosses the double layer in the barrel form of beta sheets.

Proteins associated with only one side of the membrane

4- anchored by an amphiphilic propeller.

5- covalent binding with a fatty acid (cytoplasmic).

6- covalent binding with glycosyl-phosphatidyl-inositol, GPI

7, 8- non-covalent interaction with other membrane proteins.

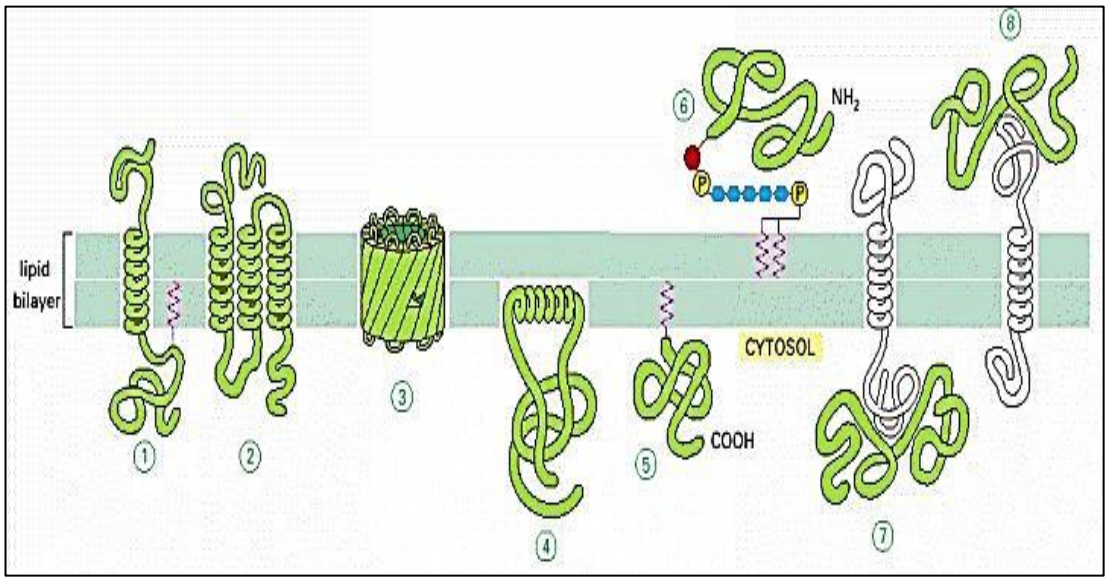


Fig 4. Various ways of membrane proteins association with the lipid bilayer

4) Membrane Carbohydrate Diversity

Most plasma membrane proteins are coupled to carbohydrates on the extracellular side of the membrane. These carbohydrates are present in the form of oligo-chains saccharides covalently bound to proteins that are then called glycoproteins like glycolipids, glycoproteins are always found in the outer half of the double lipid layer and also belong to glycocalyx. This carbohydrate-rich cell perizone plays a role in cell recognition processes and protects the cell against mechanical (blood flow), chemical (gastric acidity) and enzymatic (proteases) attacks. They are therefore present in small quantities (5 to 10% of the dry weight of PM). They have very important and varied physiological roles:

- Some families of glycoproteins are involved in the adhesion of cells with each other and with the extracellular matrix.
- Antigens of blood groups A, B present on the surface of red blood cells, are glycolipids derived from sphingomyelin.
- Galactocerebroside is the main glycolipid of myelin, present around some axons.

II. Biomolecular architecture of membranes

1. The lipid bilayer

All biomembranes form closed structures, separating the inside (light) from the outside; they have a similar **bilayer** structure. They control the movement of molecules between the inside and outside of a cell, and between the inside and outside organelles of eukaryotic cells.

Indeed, the study by electron microscopy of fine membrane sections marked with osmium

tetroxide, which is strongly attached to the polar head groups of phospholipids, reveals the en **bicouche** structure (Fig. 5).

A cross-section of all single membranes marked with osmium tetroxide resembles a railway: two fine dark lines (complexes whose head groupings are marked) separated by a uniform clear space about 2 nm wide (hydrophobic tails).

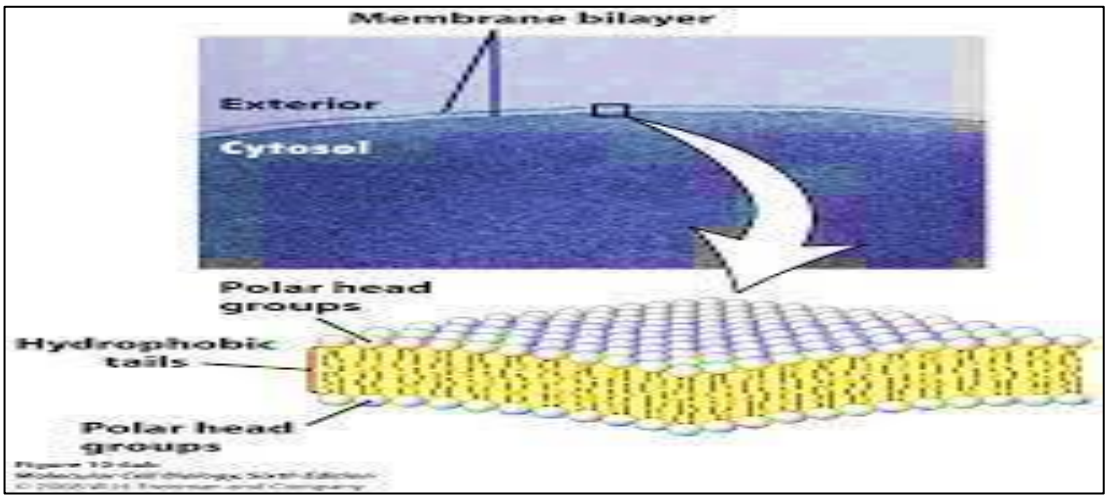


Fig 5. Bilayer structure of biomembranes.

2. The membrane model «in fluid mosaic»

The fluid mosaic model was proposed in 1972 by Singer and Nicolson. It is based on different types of experiments whose purpose was to locate proteins in the membrane edifice.

Fig.6 summarizes the main aspects of this membrane model. Lipids are organized into a bilayer made of phospholipids and cholesterol, in which there are several proteins (transmembrane, peripheral, etc.).

Features of the mosaic pattern

1. The membrane is mosaic: It is a mosaic because it consists of the juxtaposition of different elements: two layers of lipids in which globular proteins are inserted.

2. The membrane is fluid: These are quasi-fluid structures in which lipids and integrated proteins are capable of translation movements inside the entire bilamellar layer.

- Factors conditioning the fluidity of the PM:

- Temperature: whose increase leads to an acceleration of movements.
- The amount of cholesterol: Cholesterol strengthens the strength and rigidity of the membrane and corresponds to up to 50% of the total lipids of the membrane.
- Fatty acid composition: The shorter and unsaturated the carbon chains of fatty acids, the

- more fluid the membrane.
- The number of proteins: Proteins decrease membrane fluidity.

3. The membrane is asymmetrical: All biological membranes are made of sheets whose lipid compositions are different, except cholesterol which is in equivalent quantity in one or the other sheets, being able to switch easily from one to the other.

The inner sheet is characterized by phosphatidyl-serine (amphoteric) and phosphatidyl-ethanolamine (negative charge).

The outer leaf is characterized by sphingomyelin (negative charge) and phosphatidyl-choline (negative charge).

The asymmetry of lipids leads to an asymmetry of the overall load of each sheet. We also visualize an asymmetry of proteins present in the phospholipid double layer; these proteins help to characterize the membrane properties, whether on the intracellular or extracellular side.

The greatest asymmetry is that present at the level of carbohydrates, indeed all carbohydrate patterns are localized on the outer sheet of the plasma membrane (Fig.6).

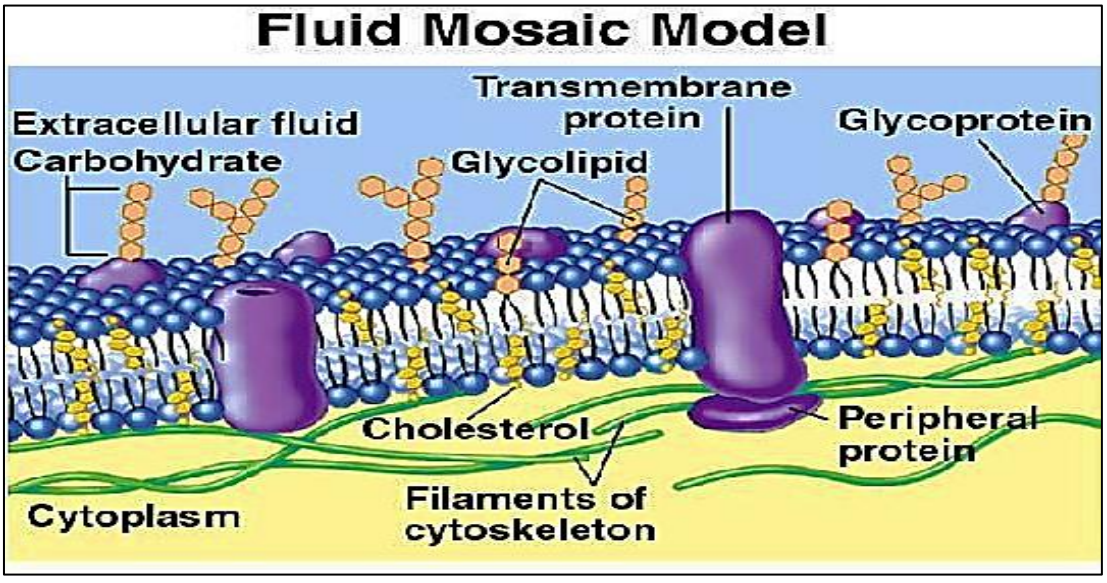


Fig 6. Fluid mosiac model of cell membrane

a. Lipid self-assembly:

Phospholipids, due to their physico-chemical properties, automatically assemble into different kinds of structures according to the environment:

Monolayers are mono-molecular layers whose hydrophilic heads are directed towards the aqueous medium and hydrophobic tails towards the lipidic medium.

Micelles are formations in the form of round droplets, or in an aqueous medium hydrophilic heads; are directed outwards from the sphere and the hydrophobic tails are directed inwards (in a lipid medium the conformation is inverse). Phospholipid lesbilayers allow the formation of spherical vesicles called dosages.

Phospholipid bilayers enter the formation of membrane bilayers. Liposomes are currently used in therapeutics to encapsulate drug substances.

b. Lipid movements

The POs have 3 types of movements (Fig.7):

- Lateral diffusion in the plane of the lipid sheet with a high speed (a lipid can change place with its neighbor 7,000,000/second).
- On-site rotation of lipids is also common.
- The transition from one sheet to another is more rare, less than once a week (flip-flop phenomenon of lipids thanks to a specialized protein or flippase and consumes energy) cholesterol easily passes from one sheet to another.

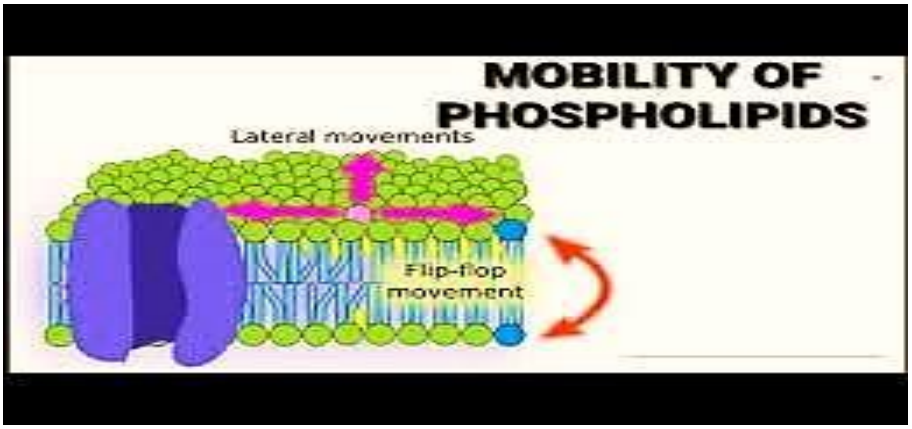


Fig 7. Mobility of phospholipids

c. Intrinsic protein movements:

- The on-site rotation of proteins is comparable to that of lipids.
- The flip-flop phenomenon of membrane proteins does not exist.
- The most important phenomenon for cellular physiology is the lateral diffusion of certain proteins.
- The movements of certain transmembrane proteins can be limited or prohibited by mechanisms that can also be associated such as their anchoring to the cytoskeleton by the extrinsic proteins of the cytosolic face, their interaction with extracellular matrix

constituents, or interaction with other proteins of the same type in the membrane or with molecules carried by two cells in contact or joint.

Chap.3. CELL STRUCTURE-FUNCTION RELATIONSHIP

I. Biosynthesis of membrane proteins and secretion proteins

Sometimes, the cell will release proteins into the external environment in order to communicate with the rest of the body: hormones, neurotransmitters, enzymes, etc. All these proteins essential to our survival are produced by a process called protein secretion (Fig.8).

The protein being secreted will pass to different positions which will each participate in its maturation, where the finished protein will be able to leave the cell. A fusion phenomenon with the membrane will then open the transport pocket, releasing the protein outside the cell. The mRNA corresponding to this protein will leave the nucleus to reach the cytosol. The mRNA will then be recognized by the ribosomes which translate it into proteins, the mRNA contains a signal which indicates to the ribosome that it will be a protein intended to be secreted; it is then placed near the endoplasmic reticulum. In this compartment, the protein, which is still in the form of a simple chain of amino acids, will then acquire a very precise and stable three-dimensional conformation. This is a step called protein folding, during which chaperone proteins intervene. These little "workers" will help the protein achieve its correct conformation.

However, if our protein fails to be folded correctly and does not reach a stable structure, it will quickly be detected by a quality control system of the endoplasmic reticulum (called ERAD for *Endoplasmic Reticulum Associated Degradation),* which then ensures degradation. misfolded protein.

Once this structure has been acquired, the protein is sent to the next compartment: the Golgi apparatus, in which it continues its maturation. In this compartment, small molecules, called residues, will be added to it. For example, a phosphate group by phosphorylation.

Once these modifications have been made to the protein, it will be taken into secretory vesicles. These kinds of transport bags will bring the protein to the membrane. Some final modifications can still be made to the protein in these vesicles. If the protein is intended to be secreted into the external environment, this protein must be soluble. And therefore the vesicle containing this protein will fuse with the membrane and allow it to be released into the external environment. This process is called exocytosis. However, if this protein is insoluble it will be attached to the cell membranes.

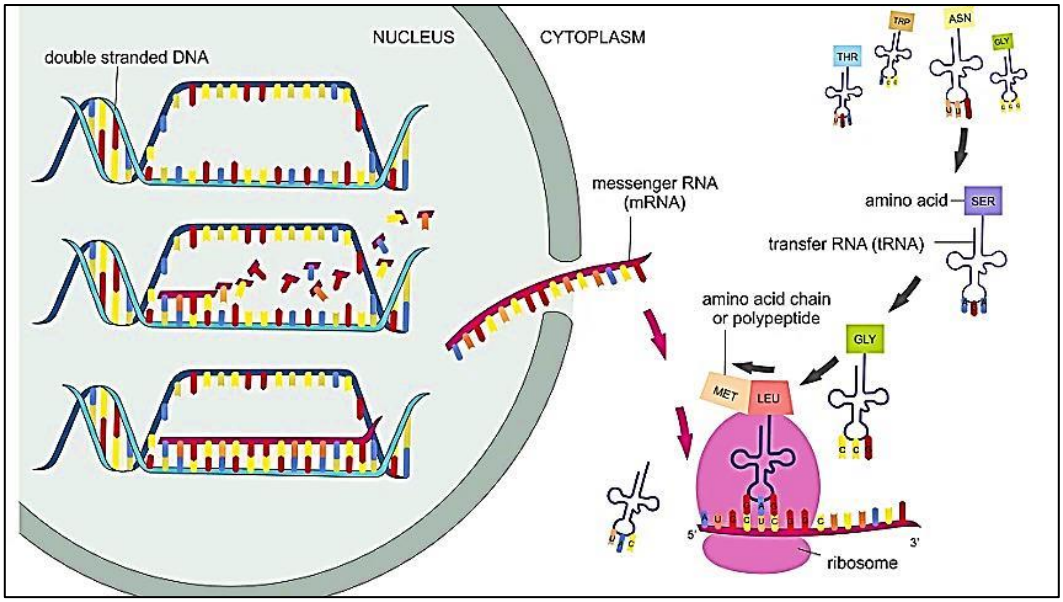


Fig.8. Protein synthesis

1. The cytoskeleton: Response of the cytoskeleton to biochemical and mechanical stimuli and its role in focal adhesion (stress fibers).

The cytoskeleton is a set of proteins assembled into a complex network of microtubules, microfilaments and intermediate filaments. Due to its name, this internal network constitutes both a "skeleton" and a "musculature" at the cellular level.

The first model, proposed by Ingber in 1993 under the name *tensegrity,* was followed by other models, each having its own constitutive equations, making it possible to predict, depending on the case, the response of the network to a torsion, shear, expansion or compression

The resistance of the cell to mechanical stress is essentially ensured by the intermediate filaments thanks to their high deformability. Microtubules and microfilaments are most often associated with the dynamic functions of the cytoskeleton, but they are also involved in the constitution of many stable structures.

These structures could not perform any function in cells if they were not associated with numerous "accessory" proteins that connect them to organelles or the plasma membrane. On the other hand, the intermediate filaments always form stable fibrous structures which have an essentially architectural role; they have only been identified, until now, in animal cells. The other two networks are common to all Eukaryotes.

In the case of large deformations occurring during major events in the life of the cell such as growth or cell division, the mechanical properties of the cells are not only due to the external lipid bilayer but also to the cytoskeleton which supports this bilayer. All eukaryotic cells1

have a cytoskeleton which gives the cell its shape, its ability to move, and which manages the internal organization of the cell. The cytoskeleton has many functions:

Cellular movements

Alongside its architectural role, the cytoskeleton has a large number of dynamic functions concerning either internal movements of the cell and its deformations, or its movement. Microtubules and actin microfilaments, present in all Eukaryotes, are responsible for these functions.

The positioning and movements of organelles:

Most of them (the endoplasmic reticulum, the Golgi saccules, and the mitochondria) are anchored on microtubules thanks to molecular motors**,** which also ensure their controlled movements depending on the stages of the cycle cellular or local physiological needs. The movements of the multiple vesicles which make the membrane compartments communicate with each other, or which allow endocytosis and exocytosis are also ensured by the cytoskeleton. Cyclosis in plants also involves these mechanisms.

During cell division, the establishment of the mitotic apparatus mobilizes all the interphase tubulin previously organized into microtubules radiating around the centrosome. This very complex structure participates in the correct separation of sister chromatids (or homologous chromosomes during meiosis I) during mitosis. Cytodieresis of animal cells uses an annular contractile device, made up of actin and myosin, whose role is to separate the cytoplasm of the cell at the end of division into two.

Cellular deformations: they involve the actin/myosin couple and are implemented during the contraction of muscle cells or during the folding of epithelia, a frequent phenomenon during embryogenesis. During the movement of isolated cells (mobile embryonic cells, tumor cells, animal cells in culture, amoebae, etc.), various actin-based structures are used, such as lamellipodia or pseudopodia; the focal contact points allow them to temporarily cling to the support and serve as a fulcrum.

Specialized locomotor structures: cilia and flagella, which are found in Protists or in many animal cells (bronchial and oviduct epithelia, or spermatozoa, for example), are made up of microtubules organized into axonemes.

Focal contacts (or focal adhesions or adhesion plaques) are point adherens junctions between the cell's plasma membrane and the underlying ECM. They form the intermediate link between

ECM molecules and actin microfilaments of the cytoskeleton. The membrane receptors ensuring cell-ECM interactions at focal contacts belong to the integrin family; the main integrin involved in focal contacts is the alpha5-beta1 integrin. Numerous intracytoplasmic proteins provide the link between the cytoplasmic domain of integrins and actin microfilaments.

The advent of microfabrication or micropatterning techniques has allowed and will allow much finer and more exhaustive measurements of the external force fields applied by cells to their environment. Mesenchymal stem cells can, for example, differentiate into neurons, myoblasts or osteoblasts depending on the rigidity of their culture substrate.

The measurement of local deformations of elastic substrates made it possible to evaluate the forces exerted on the extracellular matrix by cells during their migration at several tens of nN (nanonewtons). The existence of focal points of adhesion strengthening over time as well as that of stress fibers made up of actin filaments dictating the shape of the cell could be demonstrated.

The exercise of external stresses must be counterbalanced by a field of internal stresses supported, as in the case of focal adhesion, by the adhesion complex (the integrins) and the associated stress fibers. Thanks to the presence of transmembrane proteins, from the integrin family which will bind to the extra-cellular matrix, they will aggregate into focal complexes then focal adhesions. This adhesion complex exerts an increasing constraint on the external matrix, which has the cause and/or effect of promoting the recruitment of several dozen cyoplasmic proteins (talin, vinculin, actin, etc.). This mechanism could thus serve to transduce the stress into a chemical response. Adhesion to the extracellular matrix, depending on the transmembrane protein, is specific from one protein to another. For example, the integrin binds to vitronectin and will lead to focal adhesions rich in paxillin and vinculin, while is specific for bronectin and allows the formation of brillar adhesions rich in tensin.

2. **Fiber and muscle contraction: structure and function of microfilamentsactin and myosin**

The microfilaments of the cytoskeleton are highly developed in muscle cells and form myofibrils. Myofibrils occupy 80% of the sarcoplasm and form cylindrical elements 1 to 2 micrometers in diameter. They are arranged parallel to the long axis of the muscle fiber and they present a striation made up of alternating dark and light bands (Fig.9).

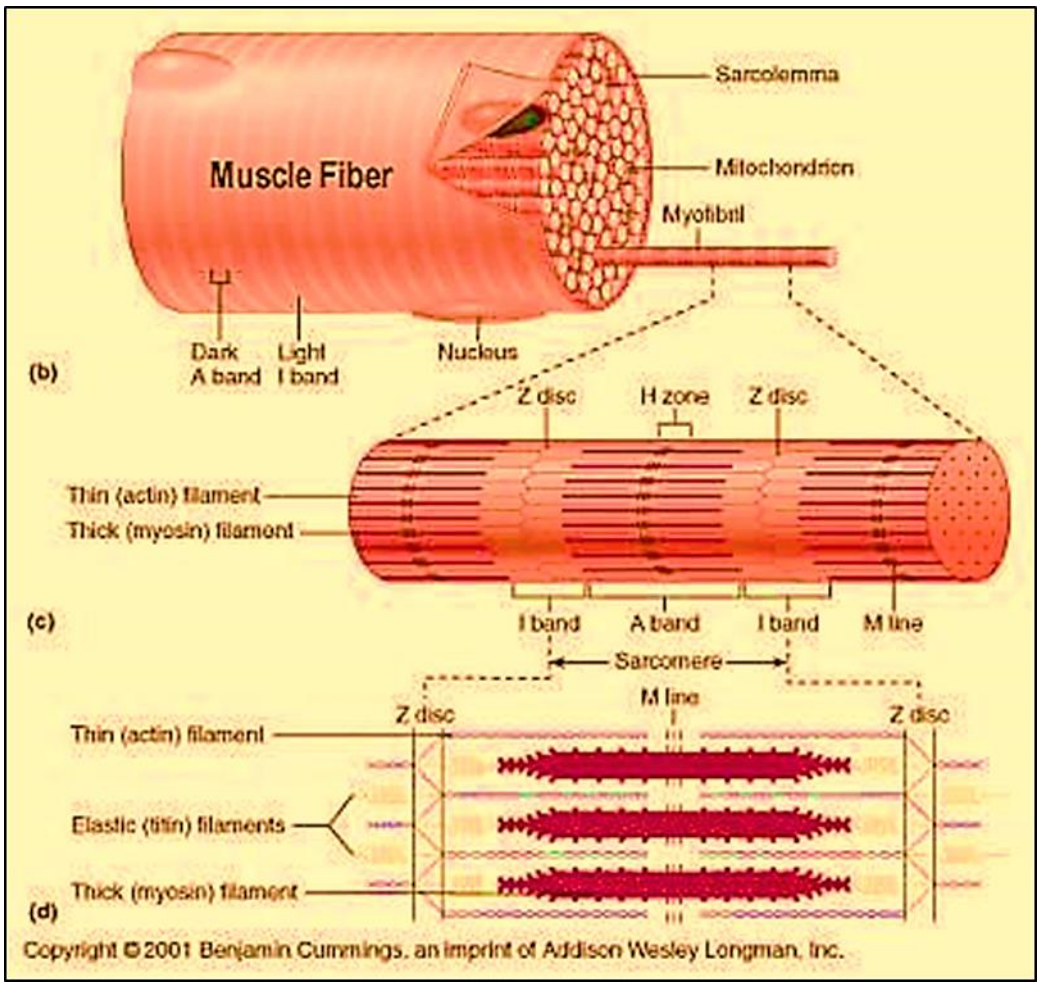


Fig.9. Muscle fibers and contractile elements

The general striation of myofibrils is the alternation of light bands or I (isotropic) and dark bands or A (anisotropic). In addition, in the light bands there is the Z streak and between two Z streaks extends the sarcomere, a muscular contractile unit.

The striation of muscle cells is explained by a particular arrangement filamentous myosin and actin molecules.

*Myosin myofilaments, also called thick filaments, constitute the dark band of the sarcomere. There are up to 1000 per sarcomere and have a diameter of 10 to 15 nm and a length of 1.6 µm. Myosin proteins look like a double articulated racket, they have one joint in the sleeves and another between the sleeve and the head. Myosin proteins have several properties: they can associate with each other to form myosin myofilaments. They can bind to actin. They can fix and hydrolyze ATP (Fig.10).

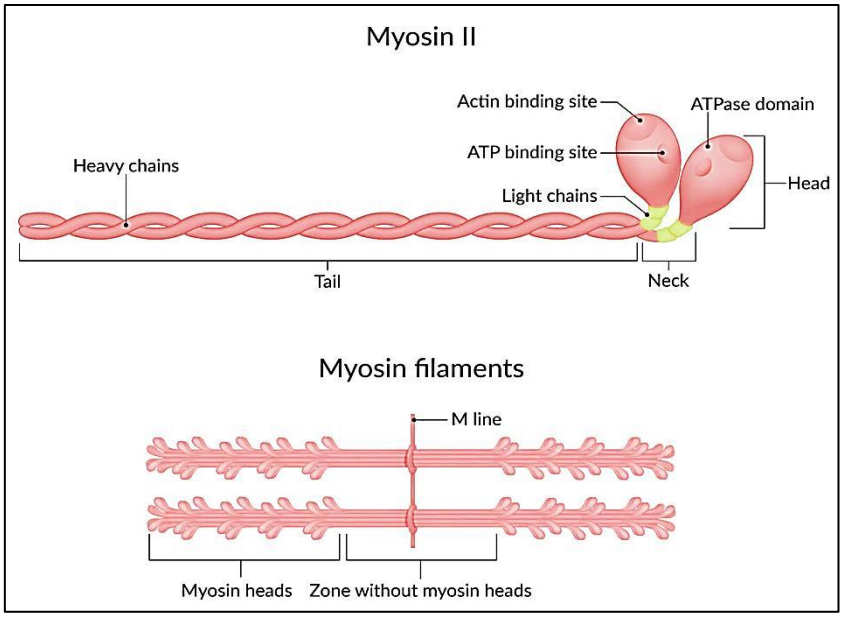


Fig.10. Myosin myofilaments.

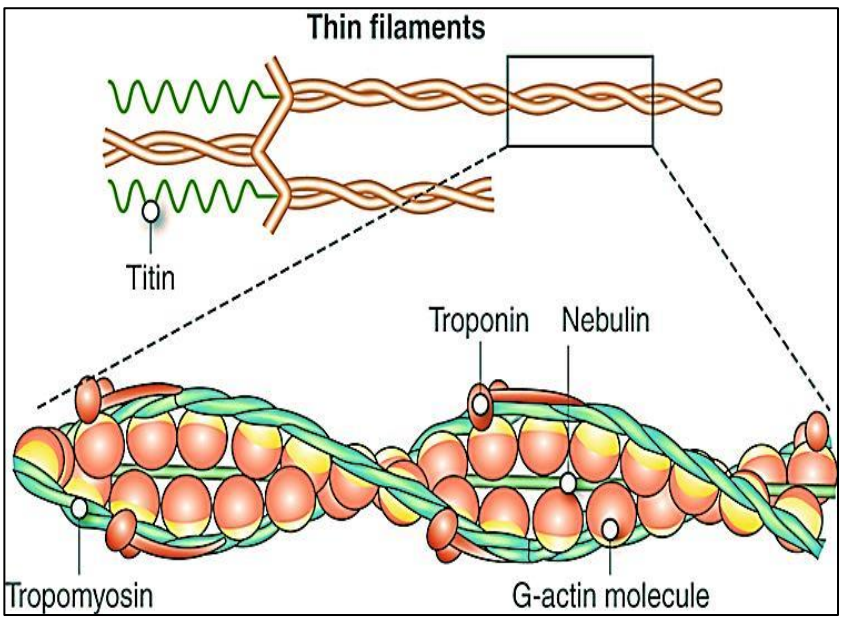


Fig.11. Actin myofilaments

On the molecular level, during muscle contraction the length of the sarcomeres decreases and the length of the myofilaments remains constant (Fig.11). Muscle contraction is a muscle shortening due to the shortening of the sarcomeres which is explained by a sliding of the myofilaments by the creation of actin-myosin bridges.

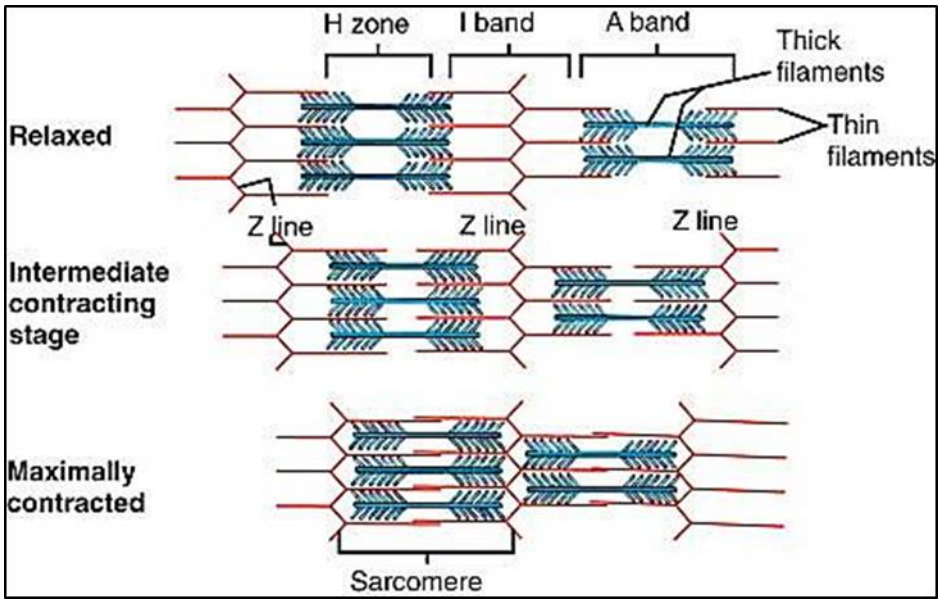


Fig.12. Mechanism of Muscle Contraction

The essential features of sliding filament theory are as follows (Figs.12, 13):

- During muscle contraction, the thin myofilaments slide inwards towards the H-zone.
- The sarcomere shortens, but the lengths of thin and thick myofilaments do not change.
- The crossbridge of the thick myofilaments connect with portions of actin of the thin myofilaments. The myosin cross bridges move on the surface of the thin myofilaments and the thin and thick myofilaments slide past each other.
- During this process, the H zone narrows and even disapperas when the thin myofilaments meet at the centre of the sarcomere.
- The length of the thick and thin filaments do not change during muscle contraction.

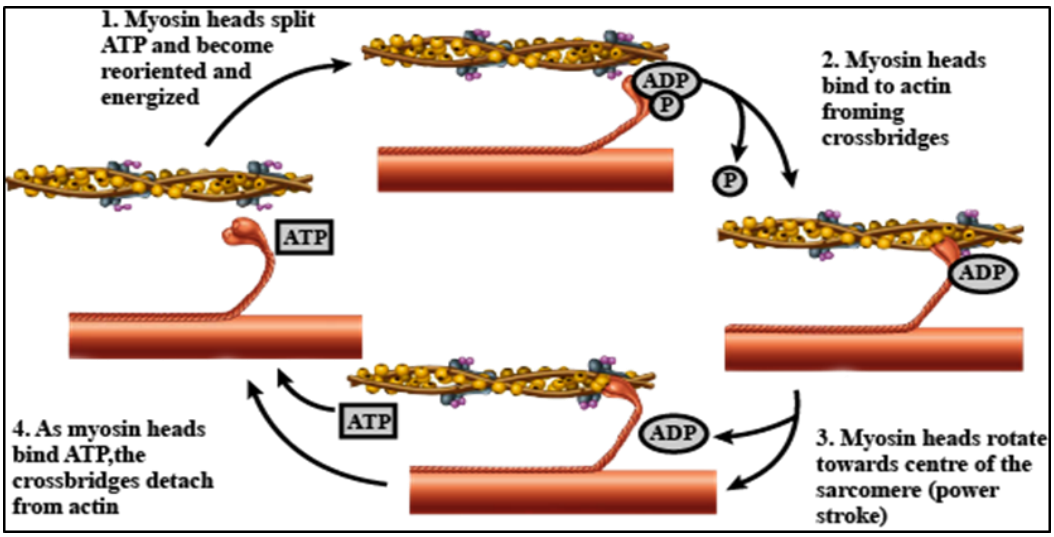


Fig.13. Molecular aspect of muscle contraction

3. The mitochondria and the oxidative phosphorylation chain

Mitochondria play an essential role in metabolic energy productionin eukaryotic cells. They are responsible for most of the useful energy derived from the breakdown of carbohydrates and fatty acids, which is converted to ATP by theprocessus de phosphorylation oxydative.

Mitochondria appear either in the form of rods with rounded ends, or in spherical form. The diameter of these organelles varies between 0.3 and 0.7µ and their lengthbetween 1 and 4 µ on average. Mitochondria are bathed in the hyaloplasm in variable numbers depending on the cell type. In a liver cell, there can be around 800, in the much larger cells like amoebas, it is estimated that there are around 50,000of mitochondria.

The mitochondrion is limited by two membranes with very different properties: The outer membrane is poor in proteins and contains a transmembrane protein, porin, which allows the passage of ions and water-soluble metabolites with a molar mass < 10,000 Da.

The internal membrane is made up of folds, often called ridges, directed inwards of the mitochondrion and arranged, most often, perpendicular to its longest axis.

The number of peaks varies depending on mitochondrial activity (cellular respiration, fatty acid oxidation, etc.). The cristae come in a wide variety of shapes (laminar, saccular, tubular, and triangular), which can co-exist in the same mitochondrion and they appear to fuse with each other and divide. The functional significance of these structures remains unknown to date.

The base of a crest is often a narrow tubular structure called a crest junction tube that establishes communication between the interior space of the crest and the intermembrane space. The surface area of the inner membrane represents between 5 and 10 times that of the outer membrane.

The inner and outer membranes can occasionally be in contact to form a transient permeability pore (PTP= Permeability Transition Pore). This is the case of the junction between the translocase (ANT =Adenine Nucleotide Translocase) located in the internal membrane and the porin or VDAC (Voltage-Dependent Anion Channel) located in the external membrane. Under normal conditions this translocase, which is an antiport system, allows the exchange of an ATP (coming from the matrix) for an ADP (from the intermembrane space).

When there is an excess of Ca++ or reactive oxygen species in the matrix, the opening of this pore is stimulated.

The inner membrane of the mitochondrion is significantly richer in proteins than the outer membrane (80%), it is poorer in lipids. It contains 5 protein complexes integrated into this membrane (Fig.14): NADH dehydrogenase (Complex I or CoI), succinate dehydrogenase

(Complex II or CoII), cytochrome c reductase (Complex III or CoIII), also known as "cytochrome b-c1 complex"), cytochrome c oxidase (Complex IV or CoIV) and ATP synthase (ComplexV or CoV). Some of its lipids are specific to this membrane such as cardiolipids and ubiquinone (coenzyme Q).

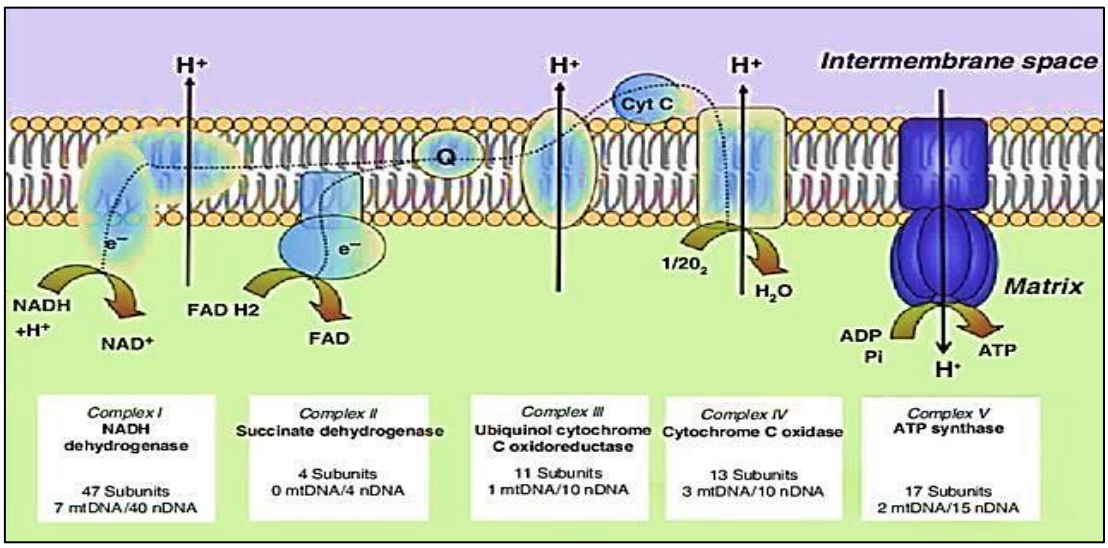


Fig.14. Mitochondrial respiratory chain

Unlike the outer membrane, the inner membrane is almost impermeable to all polar molecules (ATP ADP, Pi), anions (pyruvate) and cations (Ca++, H+, K+). The passage of all molecules requires transporters, although some uncharged molecules (of low molecular weight) can pass through. Transport proteins, antiport type and symport (shuttle system), ensure the passage of molecules through the internal membrane

Examples:

- pyruvate is transported inside the mitochondrion by the symport type transporter: H^+ /pyruvate translocase.

- incoming ADP and outgoing ATP are transported by the antiport type ANT (adenine nucleotide translocase).

- the inorganic phosphate (H_2PO_4) necessary for the phosphorylation of ADP is provided by the symport H+ /H2PO4 type transporter

- fatty acids are transported by carnitine-acylcarnitine translocase (CACT).

All Krebs cycle metabolites use specific transport proteins. Calcium uses a uniport type transporter because there are no other ions involved in transport.

The internal membrane is made up of 20 % lipids. They mainly come from endoplasmic reticulum. However, two phospholipids are synthesized in situ:

- phosphatidylethanolamine which comes from the decarboxylation of phosphatidylserine (presence of phosphatidylserine decarboxylase)
- cardiolipin which is characteristic of this membrane and which represents approximately 20% of total lipids. It is essential for the functioning of cytochrome *c* oxidase.
- The inner membrane contains very little cholesterol unlike the membrane externe.

4. Ribosome: protein synthesis, maturation and addressing of proteins

4.1.Ribosome

The recognition of a codon by the anticodon on a tRNA molecule depends on the same type of complementary base pairing used in replication and DNA transcription. However, accurate and rapid translation of mRNA into protein requires a molecular machine that can move along the mRNA, capturing Complementary tRNA molecules, hold the tRNAs in position, then covalently link the amino acids they carry to form a polypeptide chain. In prokaryotes and eukaryotes, the machine that does the work is the ribosome - a large complex made up ofseveral small proteins (ribosomal proteins) and several RNA molecules called ribosomal RNA (rRNA). A typical eukaryotic cell containsmillions of ribosomes in its cytoplasm.
Eukaryotic and prokaryotic ribosomes are very similar in structure and function. Both consist of a large subunit and a small subunit, which assemble to form a complete ribosome with a mass of several million daltons.

The large subunit with proteins designated by the prefix "L" and the small subunit with proteins designated by "S" The ribosomes of *E. coli* have a molecular weight of approximately 2.5MDa. The small ribosomal subunit consists of a single RNA (16S rRNA) and 21 proteins (S1-S21); the large subunit contains two RNAs (5S and 23S rRNA) and 34 proteins (L1-L34) (Table below). Eukaryotic ribosomes are somewhat larger; ribosomes of mammals have a molecular weight of more than 3 MDa. In mammals, the small subunit consists of a single RNA (18S rRNA) and more than 30 proteins, while the large subunit has three RNAs (5S, 5.8S and 28S sRNAs) and more than 45 proteins.

The small ribosomal subunit associates tRNAs with mRNA codons, while the large subunit catalyzes the formation of peptide bonds that covalently bind amino acids together in a

polypeptide chain. These two subunits gather on an mRNA molecule near its 5' end to start synthesis of a protein. The mRNA is then pulled through the ribosome as a long piece of ribbon. As the mRNA advances in a 5' to 3' direction, the ribosome translates its sequence nucleotide into an amino acid sequence, using tRNAs as adapters.

Each amino acid is thus added in the correct sequence at the end of the chain growing polypeptide. When protein synthesis is complete, the two sub-units of the ribosome separate.

Ribosomes work with remarkable efficiency: a eukaryotic ribosome adds about 2 amino acids to a polypeptide chain each second; a bacterial ribosome works even faster, adding about 20 amino acidsper second.

In addition to a binding site for an mRNA molecule, each ribosome contains three binding sites for tRNA molecules, called the A site, the P site and the E site (Figure belowAfter).

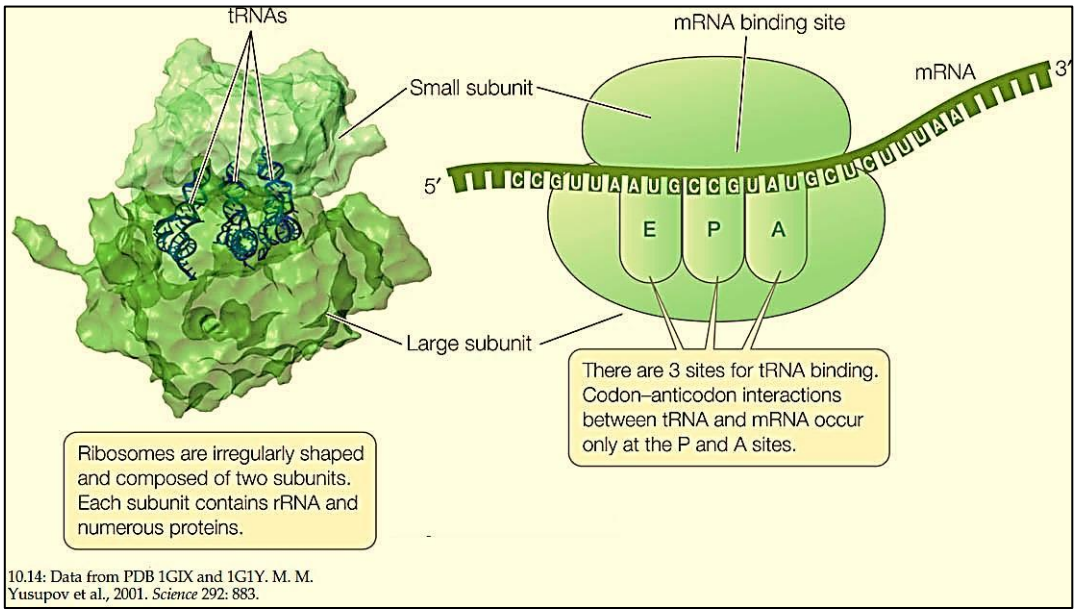


Fig.15. Role and structure of ribosom

To add an amino acid to a growing peptide chain, the charged tRNA appropriate enters site A by base pairing with the complementary codon on the mRNA molecule. Its amino acid is then linked to the peptide chain retained by the tRNAin the neighboring site P. Then the large ribosomal subunit moves forward, displacing the exhausted tRNA towards the E site before ejecting it. This cycle of reactions is repeated. Each time an amino acid is added to the polypeptide chain, the new protein becomes expanding from its amino end to its carboxyl end

until a stop codonin the mRNA is encountered.

Translation of an mRNA begins with the AUG codon, and a special charged tRNA isnecessary to initiate the translation. This initiator tRNA always carries the amino acid methionine (or a modified form of methionine, formyl-methionine, in bacteria).

Thus, newly synthesized proteins all have methionine as their first acid amino at their N-terminus, the end of a protein that is synthesized first. ThisMethionine is usually removed later by a specific protease (Fig.16).

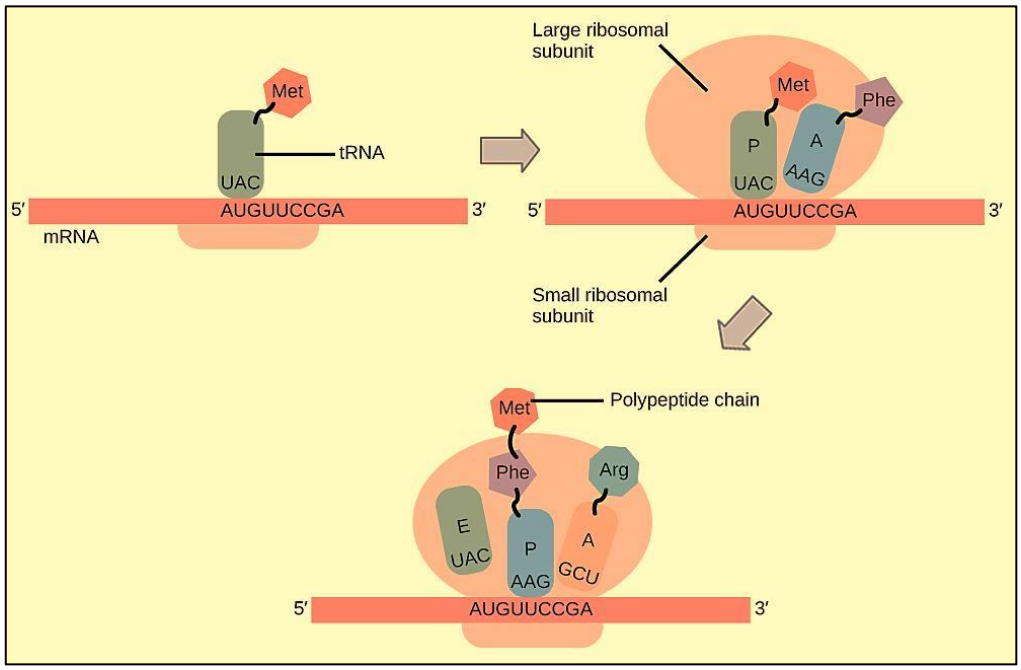


Fig.16. Steps of translation

4.2. Protein addressing and sorting

It is accepted that protein glycosylation probably serves as a means of protectionof these against possible enzymatic attacks during their transit. But the role most important of glycosylated side chains is undoubtedly to allow the addressing of proteins to the right destination. The Golgi apparatus is considered a sorting center from which 3 pathways, the main ones can be followed by proteins in animal cells (Fig.17):

- **Export outside the cell.**

  It is caused by vesicles often covered with clathrin which bud on the trans face of dictyosomes (TGN), then lose their coated with clathrin and can then fuse with the plasmalemma to allow exocytosis. This is the case for many digestive enzymes.

Oligosaccharide sequencesserve as a label for this addressing;

- **Incorporation into the plasma membrane** to allow recycling: this is what we calls the constitutive secretion. For this very important route (it allows the renewal of the plasma membrane) the addressing code is not perfectly known. It is possible that this channel corresponds to a default label addressing. Transport vesicles generally have a coatomeric coat;
- **The lysosomal pathway,** or lytic pathway for which the label, as we will see, is a mannose-6- phosphate. The transport vesicles of this pathway are covered in clathrin.

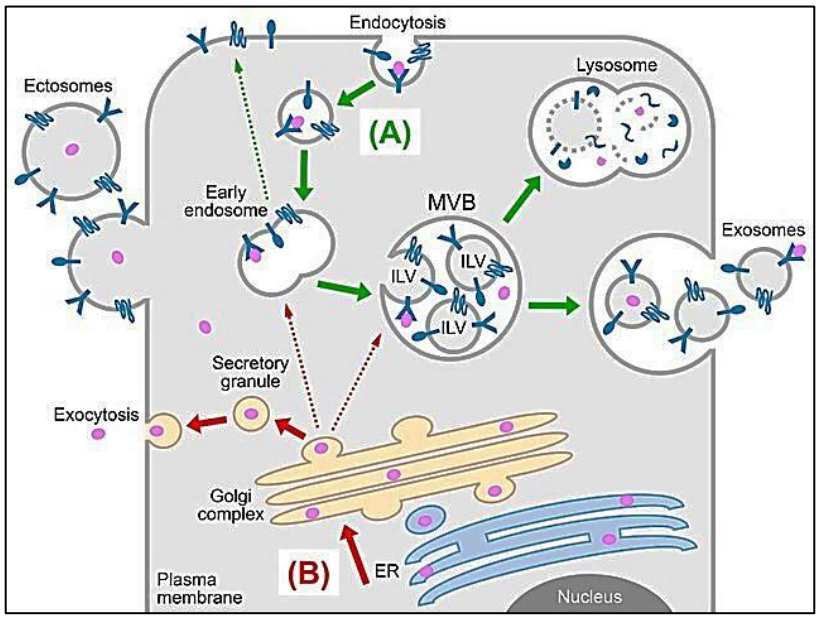


Fig.17. Schematic representation of protein sorting and secretory pathways.

(A) Cell surface receptors and other integral membrane proteins (shown in dark blue) are internalized by endocytosis and routed to early endosomes (green arrow). From there, the proteins are recycled back to the plasma membrane, or are sorted into intraluminal vesicles (ILV) that form through inward budding of endosomes and give rise to multivesicular bodies (MVB). Fusion of MVBs with lysosomes results in degradation of ILVs and their cargo, whereas fusion of MVBs with the plasma membrane results in release of ILVs as exosomes. Unlike exosomes, ectosomes (also known as microvesicles or microparticles) form through outward budding of the plasma membrane. (B) Most receptor ligands (shown in pink) are trafficked to the endoplasmic reticulum (ER) and then to the Golgi complex (red arrow). From there, the proteins are routed to endosomes, or are packaged into secretory granules that then fuse with the plasma membrane, resulting in release of proteins into the extracellular milieu.

5. The lysosomal system: structure and function

Lysosomes are cytoplasmic organelles containing acid hydrolases (cellular stomach) with maximum activity at pH5 maintained thanks to the presence in their membrane of an H+ pump. They exist in all eukaryotic cells. They have a diameter of 0.2 to 0.4, they are abundant in

hepatocytes, macrophages and granulocytes. There are two types of lysosomes:

* Primary lysosomes: these are vesicles or secretion grains which come to be formed with a homogeneous appearance and containing hydrolases.

* Secondary lysosomes: these are vacuoles with a heterogeneous appearance containing plus hydrolases the substrate being digested.

The essential role of lysosomes is the digestion of substrates of exogenous origin: heterophagy (endocytosis or phagocytosis) or endogenous: autophagy (the degradation of cellular structures (REL, mitochondria) or the destruction of the entire cell (histolysis).

After digestion, the secondary lysosomes are transformed into residual bodies rejected by exocytosis.).

Lysosomes are formed in two ways:

* From the AG: Hydrolases are synthesized in the ER then pass through the AG which give vesicles which subsequently constitute the primary lysosomes.

* From the ER: Hydrolases are synthesized in the ER then pass into the REL from which vesicles bud to give primary lysosomes without passing through the AG. Secondary lysosomes are formed by fusion of primary lysosomes with substrate-containing vesicles.

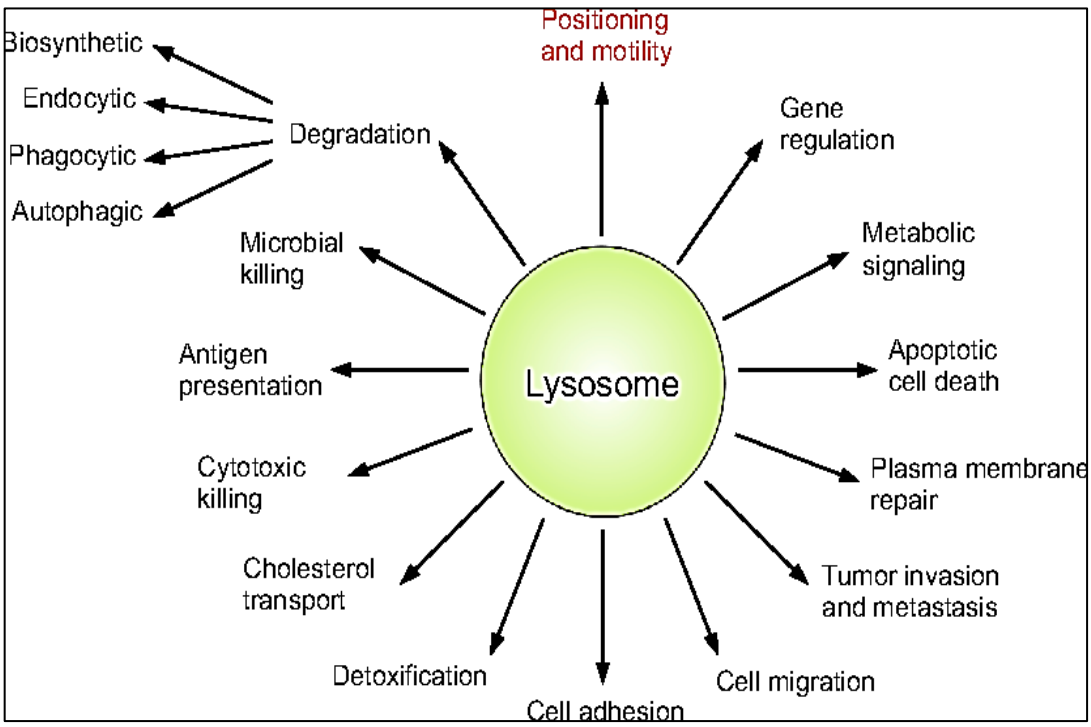


Fig.18. Multiple functions of lysosomes are influenced by their positioning and motility.

In addition to degradation, lysosomes participate in other cellular functions. Many of these functions, including endocytic, phagocytic and autophagic degradation, antigen presentation, killing of target cells by cytotoxic T-cells and NK cells, metabolic signaling, cell adhesion and migration, tumor invasion and metastasis, as well as plasma membrane repair, depend on the ability of lysosomes to move throughout the cytoplasm (Fig.18).

Chap.4. GLYCOSYLATION OF MACROMOLECULES AND BIOLOGICAL ROLE

Introduction

Glycosylation is the phenomenon of grafting carbohydrate groups, or glycans, onto proteins and lipids. It is one of the most important modifications in the synthesis of membrane and secreted proteins. The diversity of monosaccharides constituting glycans allows us to consider a multitude of possible assemblies and reflects the complexity of glycan structures and their study.

All cells have sugars on the surface which are found on lipids and proteins, called glycolipids or glycoproteins. A glycolipid has only one oligosaccharide chain; on the other hand a glycoprotein has several variable oligosaccharide chains. In nature, we find more than 100 different carbohydrate molecules, but their presence at the membrane level is approximately limited to 12 sugars, the most important of which are: D-glucose, D-galactose, D-mannose, L-fucose, L-arabinose, D-xylose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine and N-acetyl-neuraminque acid (sialic acid).

I. Glycoproteins: type of bond and interest in glycosylation

Glycoproteins are sugar chains linked to proteins; they form the majority of the mass of membrane carbohydrates. Protein glycosylation corresponds to an addition of oligosaccharides (polymers made up of a small number of simple carbohydrates or oses) during the biosynthesis of certain membrane or secreted proteins, which, in fact, become glycoproteins. This addition occurs enzymatically and in several steps in the endoplasmic reticulum and the Golgi apparatus of Eukaryotic cells. It participates in the maturation of these proteins and can have a decisive role in their function. Glycosylation concerns more than 50% of animal proteins. Glycoproteins are distributed in different cellular compartments (cytoplasm, nucleus, lysosomes, ER, Golgi or even mitochondria), most of which are secreted or associated with the plasma membrane. Among the glycoproteins, wedistinguish several large groups depending on the type of anchoring or the proportion of carbohydrates. On the basis of the bond between the protein and the glycan, two main types of glycosylation can be distinguished:

- N-glycosylation: it is the addition of an oligoside “N-acetyl-glucosamine” to an available asparagine (Asn) amino acid.

- O-glycosylation: it is the addition of carbohydrates at the level of the -OH residues of the amino acids serine and threonine of the peptide chains depending on the amino acid used.

1. N-glycosylation

N-glycosylation occurs in two steps. Firstly, a branched chain of a few oses is added cotranslationally to the protein to be modified, via the establishment of a covalent bond between a hydroxyl (-OH) of an N-acetylglucosamine located at one end of the carbohydrate chain, and the amide (-CO-NH2) of the side chain of an aspargine residue (asn) to the protein. The bond thus formed is a so-called N-glycosidic bond (Fig.19), hence the name N-glycosylation.

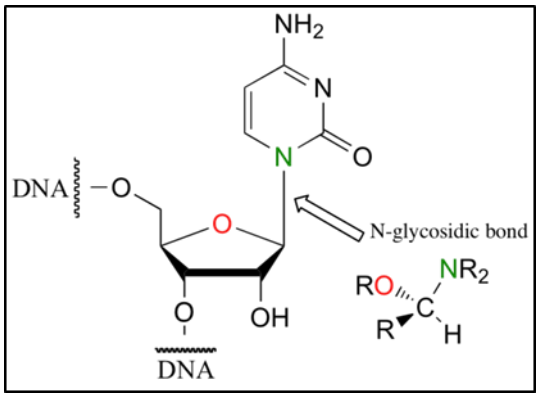


Fig.19. N-glycosidic bond

The reaction establishing this covalent bond is controlled by an enzyme, a glycosyltransferase, which is localized in the lumen of the ER. Indeed, the carbohydrate tree is initially supported by a dolichol, a lipid specifically present on the internal face of the ER membrane. It is then transferred by the glycosyltransferase to the polypeptide chain being synthesized. The initially grafted carbohydrate chain is made up of 14 bones in total. This initial carbohydrate tree is then remodeled as the protein passes through the Golgi apparatus. The modifications take place sequentially in the successive compartments of theGolgi apparatus (cis, medial, trans, and finally trans-Golgi network).

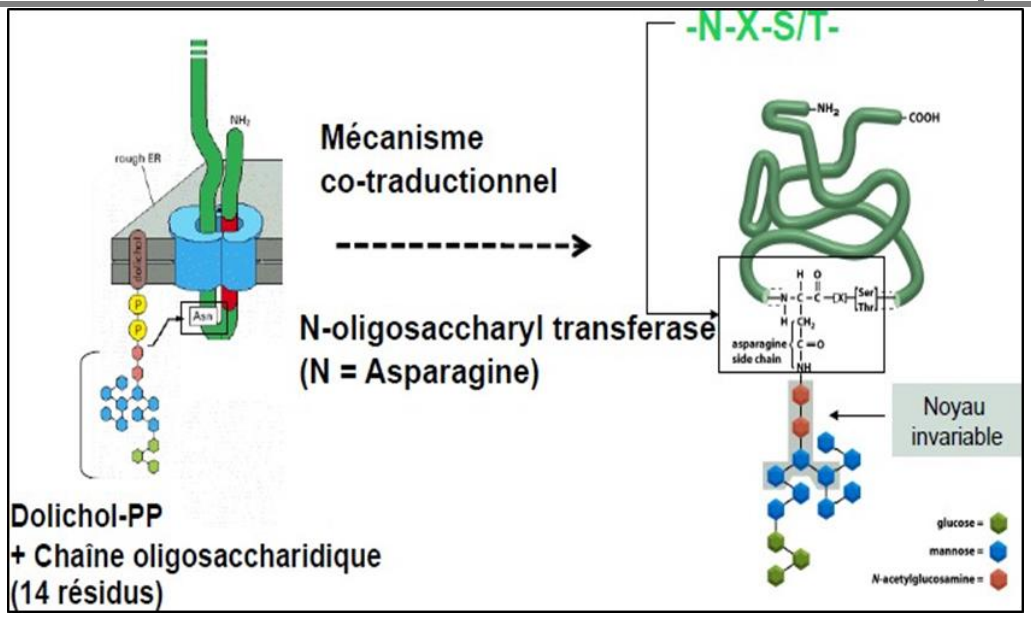


Fig.20. Protein N-glycosylation**.**

The addition of carbohydrate chains does not occur on all asparagine residues of N-glycosylated proteins. Indeed, only asparagines belonging to the two sequences Asn-X-Ser or Asn-X-Thr (where X is any amino acid except proline) can be glycosylated. These signals, although necessary, are however not sufficient and only determine potential N-glycosylation sites. Other factors come into play for the effective glycosylation of these sites, such as the structure of the protein itself or the cell type in which it is synthesized (Fig.20).

2. O-glycosylation

O-glycosylation also corresponds to the addition of an oligosaccharide to a protein, but via the establishment of a bond between an N-acetylgalactosamine and the hydroxyl group (- OH) of the side chain of a serine amino acid or threonine (Fig.21). There is, however, a special case, that of collagen, where the sugar involved in the bond is a galactose and the amino acid a hydroxylysine.

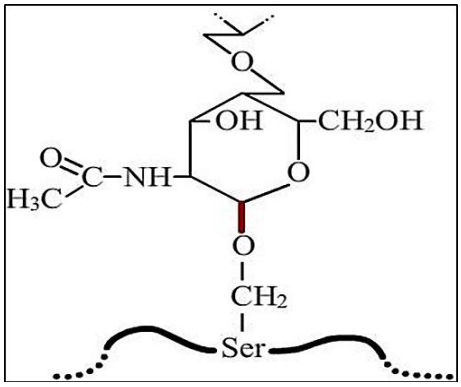


Fig.21. O-glycosidic bond

This addition takes place in the Golgi apparatus, therefore post-translationally, during the maturation phase of these glycoproteins. Once again, the mechanism is controlled enzymatically. On the other hand, the addition of residues is done sequentially one by one (if there are several of course), from residues activated by their connection with a nucleotide, and not from an oligosaccharide precursor. Ultimately, the carbohydrate chains of O-glycosylation are much shorter than in the case of N-glycosylation, with most often only 1, 2 or even 3 sugar residues. However, the chains obtained are much more varied than in the case of N-glycosylation.

3. Benefits of glycosylation

- N-glycosylation, as in the case of O-glycosylation, can affect the physical and chemical properties of proteins, changing not only molecular mass but also solubility and electrical charge.
- Membrane glycoproteins and glycolipids constitute recognition signals allowing intercellular interactions.
- Biological activity "ligand-receptor interactions" modulation of protein functions such as hormones, enzymes, immunoglobulins and growth factors,etc.
- The glycosylated portion of the glycoprotein plays a role in quality control of protein folding.
- The glycosidic substituents of glycoproteins are important sites for targeting proteins to the appropriate cellular compartment.
- Glycoconjugates are also part of the high molecular density layer, the glycocalix, which covers the surface of epithelial cells in eukaryotes. This zone participates in cellular cohesion and in the protection of the cell against physical shocks and against attack by microorganisms.
- The main glycolipids in higher animals are cerebrosides and gangliosides which seem to play a role in neurotransmission processes, in tissue immunity and in molecular recognition mechanisms between cells.

4. Molecular study of some glycoproteins

4.1. Blood group glycoproteins

Molecules capable of being recognized by the immune system and triggering immune responses are found on the surface of red blood cells (or erythrocytes). These are the erythrocyte membrane antigens. Their chemical nature is variable protein and glycoprotein or glycolipid.

The discovery of the ABO system in 1901 by Landsteiner helped explain why certain blood transfusions were successful while others ended in tragic accidents (hemolysis of the transfused erythrocytes).

ABO blood groups result from glycosylation carried out with the same enzymes as for O-glycosylation, although they are not always associated with proteins. They come from the fact that three possible conformations of an oligosaccharide grafted onto a red blood cell membrane protein give rise to three antigens: the H (or O) antigen, the A antigen and the Bantigen. Indeed, the specificity membrane antigens of red blood cells, or red blood cells, depend on the nature of the constituent oligosaccharides.

- Fucose and galactosamine specific for group A antigens.
- Focose and galactose specific for group B antigens.

These antigens are oligosaccharide chains covalently linked to glycolipids or glycoproteins in the plasma membrane. The terminal sugars of the oligosaccharides distinguish the three antigens. The presence or absence of glycosyltransferases which add galactose (Gal) or N-acetylgalactosamine (GalNAc) to the O antigen determine a person's blood type (Fig.22).

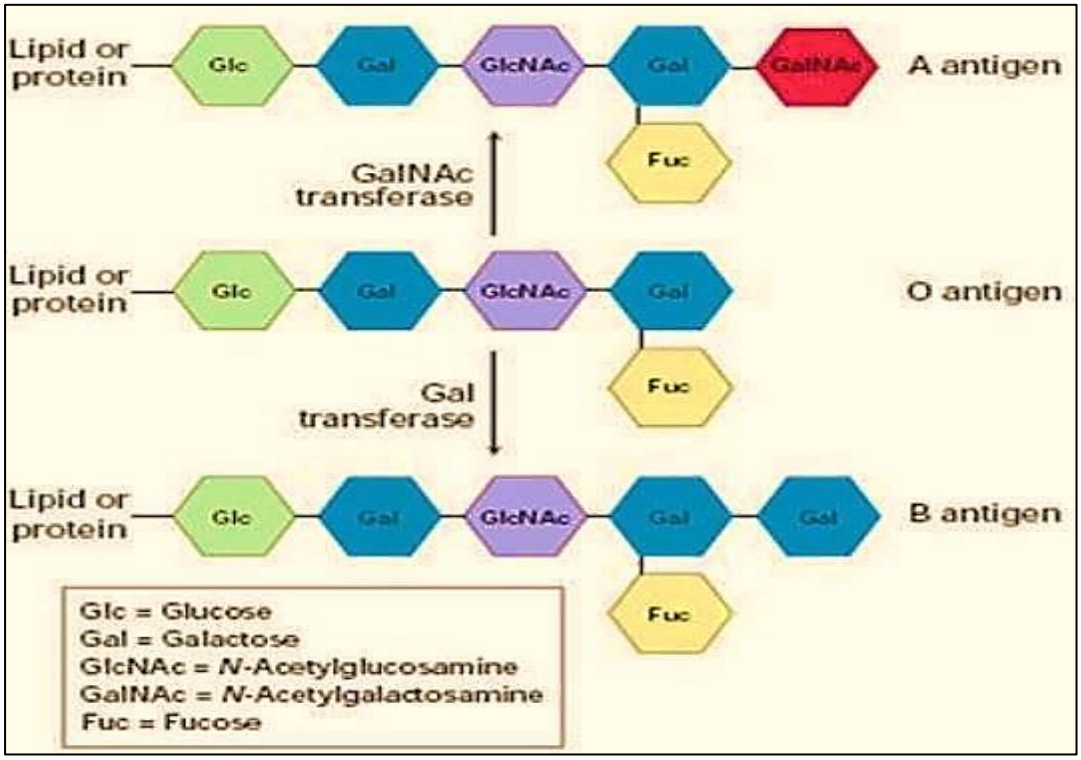


Fig.22. Blood group A and B antigens

4.2. Various human glycoproteins (lectins)

Lectins are proteins capable of recognizing and reversibly binding oligosaccharide molecules specifically. Lectins are generally classified according to their animal or plant origin and according to the saccharide structures that they recognize.

Mammalian lectins are grouped into four groups, galectins, type C, type P and type I lectins. The groups are defined by sequence homology and by structural homology of the recognition sites (Fig.23).

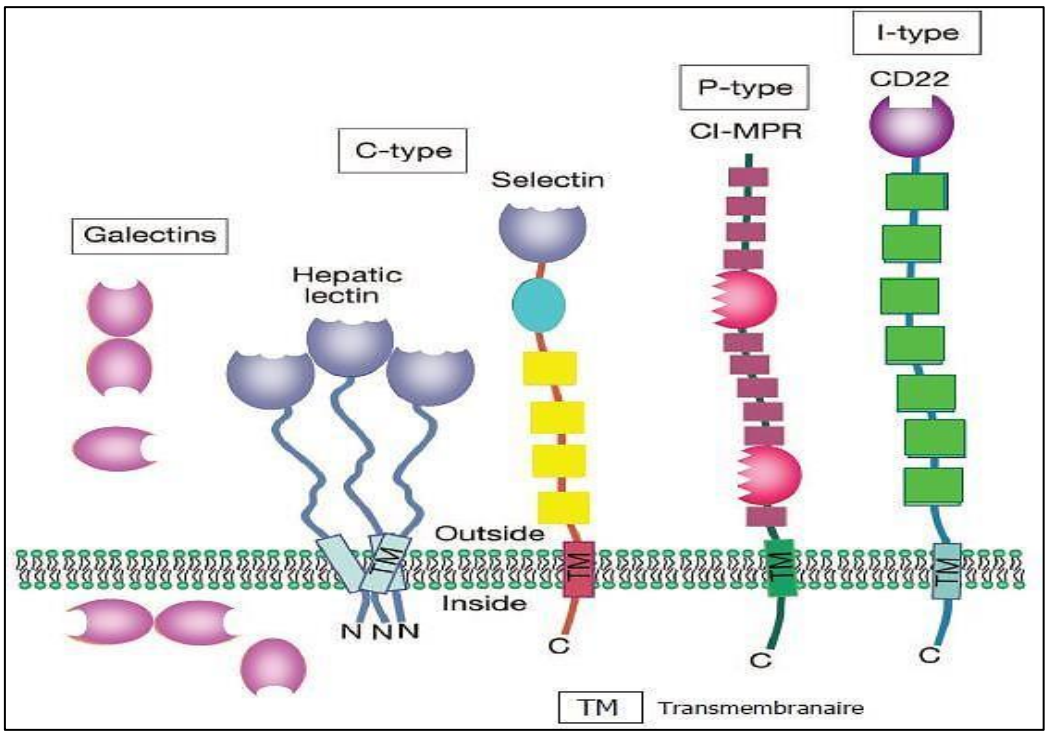


Figure.23. The main classes of lectins, based on protein structure

Galectins are defined as lectins having the capacity to fix β-Galactose residues and lactosamin structures. They are generally soluble and have a globular recognition site (Carbohydrate Recognition Domain or CRD).

C-type lectins include soluble and transmembrane molecules that recognize various sugar motifs. The interaction of these lectins with their sugar ligand most of the time requires calcium, hence their name.

P-type lectins are membrane receptors recognizing Mannose-6-phosphate (Man-6-P) motifs. They are involved in the trafficking of lysosomal enzymes.

Type I lectins belong to the immunoglobulin superfamily. Their ligand almost always has a sialic acid residue at the ends, whether on secreted or membrane glycoconjugates.

II. Glycolipids: glycerolipids, glycosphingolipids

1. Definition

Oligosaccharide units can also be found on lipids to form glycolipids. Glycolipids result from the bonding of a simple hexose or an oligosaccharide to a hydroxyl function belonging either to the glycerol of a diglyceride (glyceroglycolipid) or to lasphingosine of a ceramide (sphingolipid). Glyceroglycolipids belong to the plant kingdom. While glycosphingolipids are found in the plasma membranes of animal cells

2. Classification

Glycolipids are divided into two distinct families depending on the nature of the lipid base:

2.1. Neutral glycosphingolipids

They are sphingolipids comprising one or more saccharide residues at their polar end, so they are not charged. The simplest are the cerebrosides; their polar group is an ose linked by a β-saccharide bond to the free hydroxyl of the ceramide.

- **Cerebrogalactosides or Galactosylceramides**: They are made up of Sphingosine + AG + β DGalactose.
- **Cerebroglucides or Glucosylceramides**: They are made up of: Sphingosine + AG + β DGlucose

2.2. Acidic glycosphingolipids (gangliosides)

The most complex Glycosphingolipids; these are the gangliosides. Their saccharide end includes a sialic acid which gives them a net negative charge at pH 7.0.

Nacetylneuraminic acid is the usual sialic acid in human gangliosides. Gangliosides are especially abundant in the gray matter of the brain where they represent 6% of total lipids. Particularly abundant in nerve endings, gangliosides have been implicated in the transmission of nerve impulses at synapses. They also appear to be present in receptor sites for acetylcholine and other neurotransmitters.

Chap.5. SIGNAL TRANSDUCTION AND REGULATION OF CELL FUNCTION

Introduction

Cellular communication can be defined as follows: they are molecular signals (or messengers) emitted by a cell (called transmitter) and recognized by another cell (called the receiving cell, knowing that the transmitting cell can also be the cell receiver).

The reception of the external signal is followed by a relay inside the cell which goeslead to the amplification of the signal inducing various molecular effects as well as a change of state of the recipient cell.

- **Signaling modes**

Cell signaling modes can be classified based on the distance that separates the signal-emitting cell from the target cell (Fig.24). From the furthest distance long at the shortest distance we find:

1.Endocrine signaling: It concerns hormones (polypeptide or steroid) these are released into the general blood circulation. They act remotelyon 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 signaling results in signal dispersion throughout the body (<10-8mol/L). Examples: Thyrotrophic hormone secreted by the pituitary gland and acting on the gland Thyroid is an example of a polypeptide hormone. Estradiol, produced by the ovary and acting on the endometrium is an example of a steroid hormone. .

2.**Paracrine signaling:** The signal is released into the extracellular matrix and acts on neighboring cells. It concerns local mediators such as factors of growth and mediators of inflammation. Examples: somatostatin and glucagon acting on islet cells Neighboring Langerhans which secrete insulin.

3.Autocrine signaling: The cell responds to the signal that it itself has secreted; such as growth factors and cytokines. Examples: Prostaglandins and interleukins can act on their cellsof origin and exert autocrine control.

4.Synaptic signaling: The signal is released by the presynaptic cell and acts only on the postsynaptic cell of a neighboring specialized junction (synapse chemical). There is no dispersion of the signal and the action is very rapid (of the order of millisecond = 0.001 seconds). It concerns neurotransmitters. Examples: Acetylcholine, glutamate, norepinephrine,…….etc.

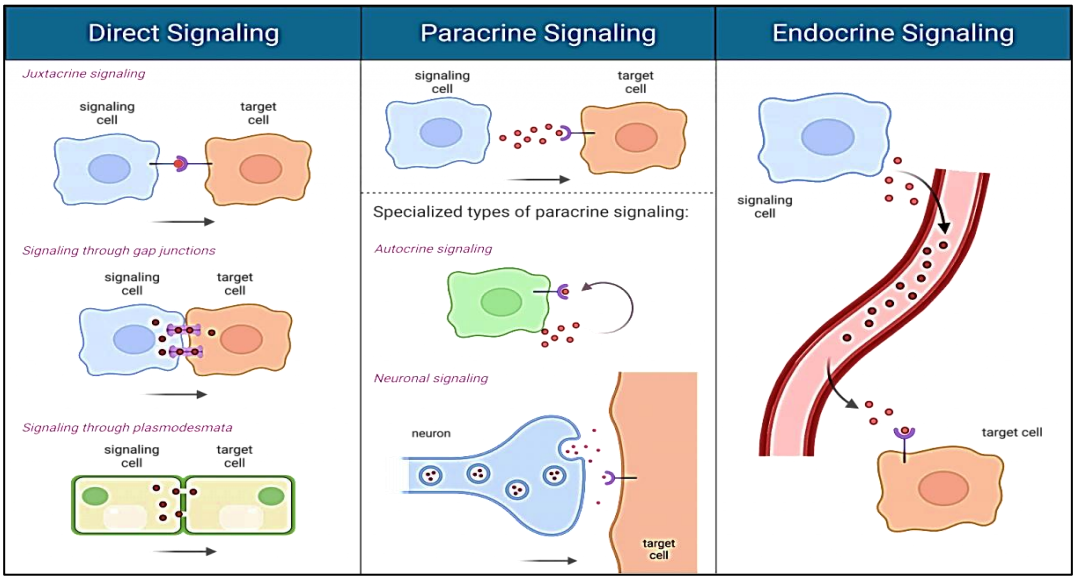


Fig.24. Types of Cell Signaling.

I. Receptors and ligands

1. Ligand

In biology, a ligand (from the Latin ligandum, binder) is a molecule that binds reversibly to a targeted macromolecule, protein or nucleic acid, playing in general a functional role: catalysis, modulation of enzymatic activity, transmissionof a signal.

This term, widely used for the study of proteins, designates molecules which interact with the protein in a non-covalent and specific manner and which play a role in its functions.

- Bonding occurs thanks to forces between molecules, such as bondsionic, hydrogen bonds and van der Waals forces.
- A ligand that can bind to a receptor, modify its function and trigger a response is called an agonist at this receptor.
- The binding of an agonist to a receptor can be characterized by the importance of the physiological response provoked and by the necessary concentration of the agonist to produce this response.
- High affinity binding requires a relatively low concentration of one ligand is enough to activate a binding site and trigger the responsephysiological.
- An agonist that does not fully activate the body's response is called a partial agonist.
- Ligands that bind to a receptor but fail to activate that response are considered receptor antagonists.
- The same ligand can have different effects depending on the target cells:pleiotropic.

- A ligand can bind several receptors.
- A receptor can bind several ligands.

1.1. Main types of ligands

1) Water-soluble informative molecules

➢ **Features**

- They cannot cross the lipid bilayer of the plasma membrane.
- They act thanks to specific receptors located on the membraneplasma of the target cell.
- Their very short lifespan (for neurotransmitters or a few minfor hormones).

They induce rapid and short-lived responses. These answers correspond regulation and activation of pre-existing proteins in the target cell (enzymes, channels ionic, and transcription 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 (noradrenaline, serotonin, GABA…etc) or polypeptides which play a role inthe excitation or inhibition of neurons at the synapses.
- **Hormones:** these are molecules:
  - ❖ Peptides (2-100 amino acids). Ex: vasopressin, oxytocin, insulin…etc.
  - ❖ Proteins (> 100 amino acids). Ex: growth hormone (GH);
  - ❖ Glycoproteins. Ex: LH, FSH.
- **Cytokines:** These are proteins or polypeptides that play a role in the immune response and inflammation. Ex: interleukins (IL).

2) Fat-soluble informative molecules

➢ **Features**

- They cross the plasma membrane by simple diffusion.
- They then activate an intracellular receptor which binds to target regions DNA and regulate gene transcription.
- They induce responses that are later and last longer. They do not act not on pre-existing proteins. These molecules are transported in the blood (case of lipid-soluble hormones) thanks to specific protein transporters before being released on contact with the plasma membrane of the target cells.

These are:

- **Thyroid hormones (Triiodothyronine "T3" and thyroxine "T4"),** derived from an amino acid: tyrosine;

- **Steroid hormones, derived from cholesterol. Ex: cortisol, estradiol, testosterone, progesterone…etc;**
- **Prostaglandins, derived from arachidonic acid (fatty acid at 20 C).**

3) Gaseous free radicals

➢ **Features**

- They diffuse freely across the plasma membrane.
- They act directly on cytosolic enzymes without intervention of a membrane or intracellular receptor. **Ex:** NO acts on a guanylate cyclasecytosolic.
- They are toxic in high concentrations.
- The best known are CO (carbon monoxide) and NO (nitrogen monoxide) (Fig.25).

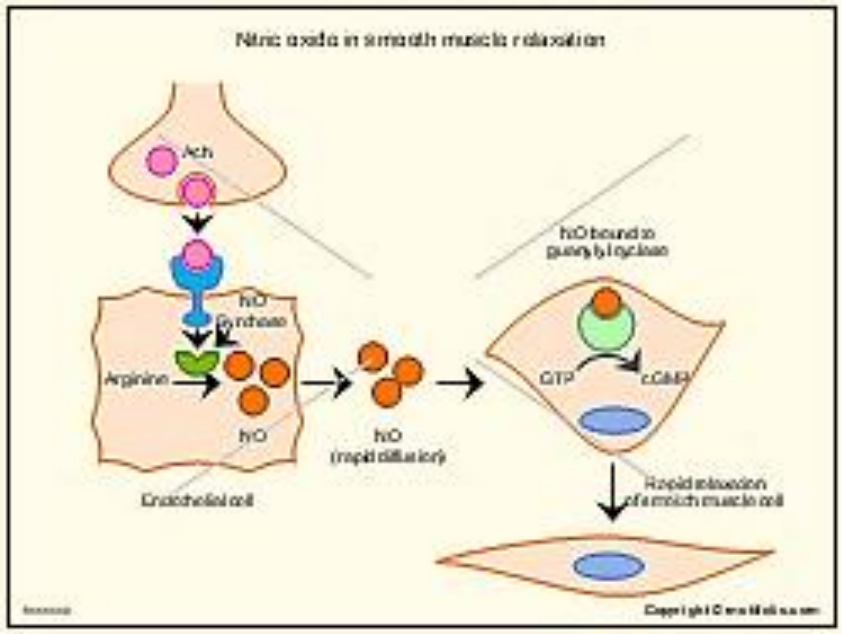


Fig.25. Nitric oxide in smooth muscle relaxation

- Agonists and antagonists

An agonist binds to the receptor and induces a response similar to that of the ligandnatural. Ex: Nicotine is the agonist of acetylcholine for its nicotinic receptor.

An antagonist attaches to the receptor but does not trigger a response. Ex: The curare is an acetylcholine antagonist at its nicotinic receptor. ***Note:*** Antagonists can be used as medications. Ex: The Antihistamines are histamine antagonists and cure the symptomsallergic.

2. **Receptor**

A receptor can be defined as a molecular structure of nature polypeptide which interacts specifically with a messenger, hormone, mediator, cytokine, or to a specific intercellular contact. This interaction creates a modification of the receptor which leads, for example, to the

opening of the channel linked to the receiver, or is transmitted via enzymatic reactions to the effector distant from the receptor. The receptors are located either at the level of the cytoplasmic membrane or at inside the cell, particularly in the nucleus. The same cell generally contains several different types of receptors.

2.1. Receiver characteristics

- **Specificity:** it fixes a given type of ligand.
- **Saturability:** the number of receptor molecules in a cell being finite number of ligand molecules that can bind is limited.
- **Reversibility:** the receptor and ligand bond being non-covalent in nature, the ligand–receptor complex dissociates when the ligand concentration decreases.
- **Coupling:** the binding of the ligand to the receptor transmits a signal to the cell; this is the most important characteristic.

2.2. Classification of receptors according to their location

Receptors are classified into two groups according to their location:

- Nuclear receptors
- Membrane receptors.
  - G protein-coupled receptors (GPCRs);
  - Ion channel receptors;
  - Enzyme receptors.

1) Nuclear receptors

These receptors constitute a superfamily of proteins that exhibit strong sequence similarities. They include 5 areas:

- The A/B domain (N-terminal end): variable domain which acts as a factor of transcription regulation = transactivation domain.
- The C domain: DNA binding domain which presents two-part architecture zinc fingers. A Zn finger = 4 Cys linked to a zinc atom. It is responsible for binding the receptor to the Hormone Response Element (HRE) of targegenes.
- Domain D: hinge domain.
- The E domain (C-terminal end): includes the ligand binding site and a nuclear localization signal (NLS) which can be masked by PARs (Proteins Associated with Receptors) and unmasked by ligand binding (Fig.26).

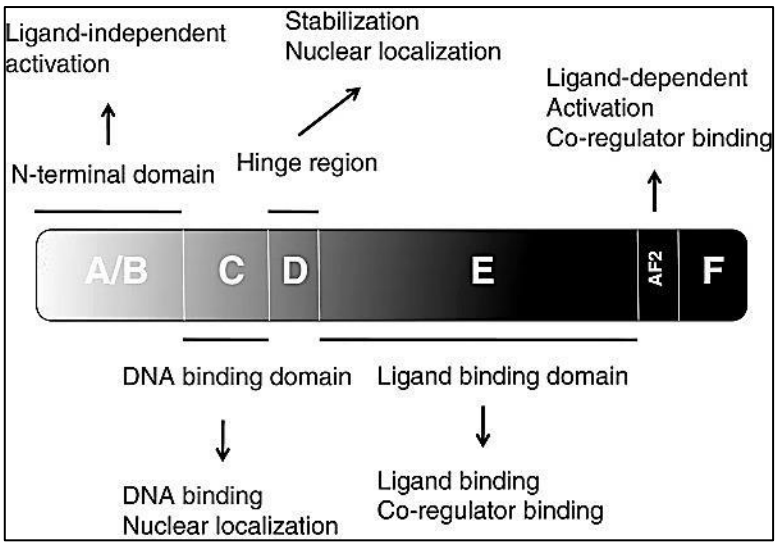


Fig.26. Schematic representation of a nuclear receptor.

1. Mechanisms of action: example of steroid hormone receptors

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

1) Binding of the hormone releases the PAR complex receptor and induces transformation of the receptor which authorizes its dimerization.

2) 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 (Fig.27).

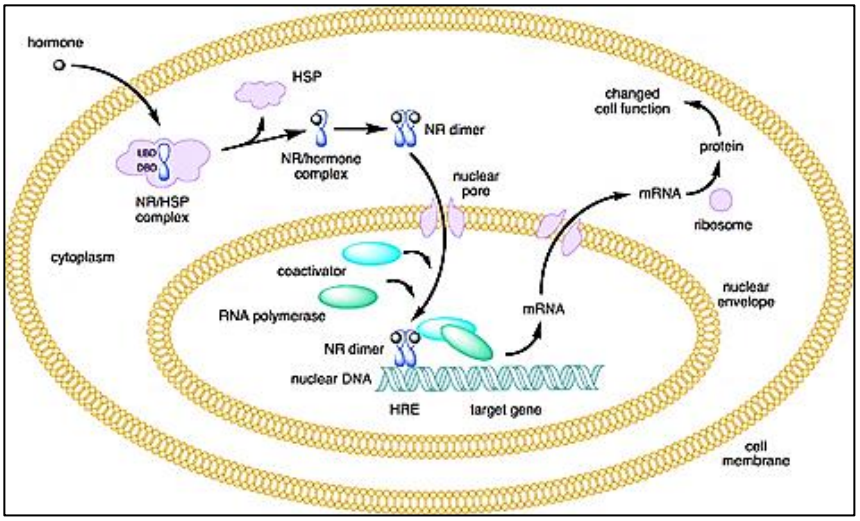


Fig.27. Operating principle of a nuclear receptor.

2. Membrane receptors

2. 1. 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 ligandspecific.

Example: The muscle nicotinic acetylcholine receptor is a pentamer of 300 kDa made up of 5 subunits:

- 2 α-subunits carrying the ligand binding sites;
- 1 β-subunit;
- 1 subunit γ or ε 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 which triggers the opening of the ion channel. Consequences: entryof Na+ causing depolarization of the muscle cell. This is how the nicotinic receptor plays an important role in transmission neuromuscular and excitation-contraction coupling (Fig.28).

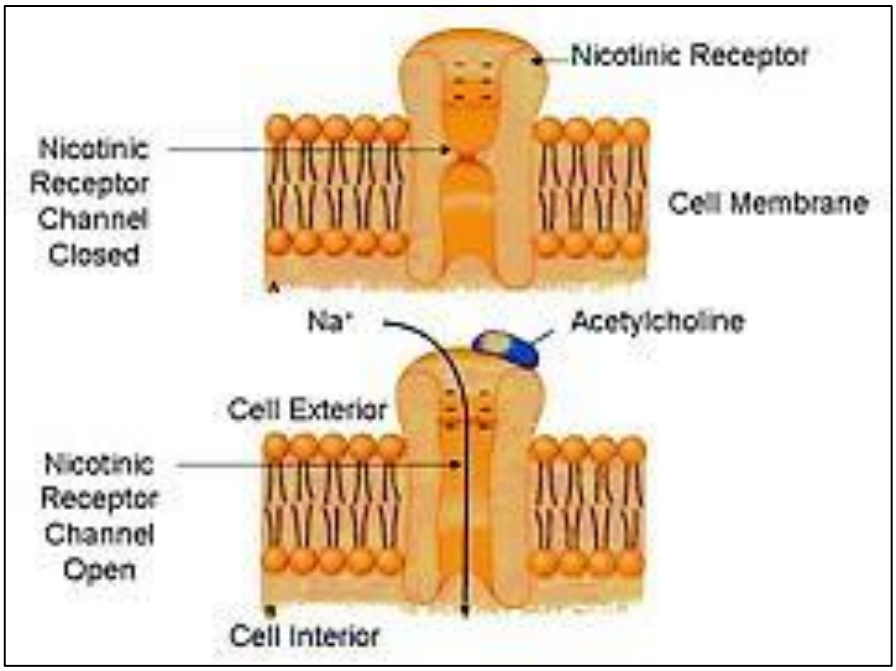


Fig.28. Operating principle of an ion channel receiver(Example: nicotinic acetylcholine receptor).

2.2. G protein-coupled membrane receptors (GPCRs)

They belong to a superfamily of proteins which have 7 domains transmembrane (7TM) (Fig.29). Their N-terminus is extracellular and they often function under homo- or heterodimer form.

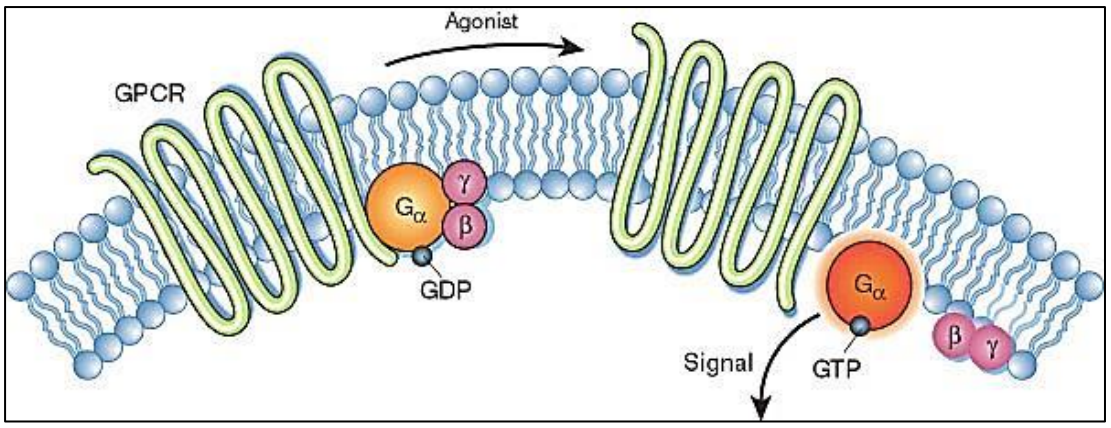


Fig.29. G protein-coupled membrane receptor (GPCR).

The GPCR signaling pathway involves 6 partners:

- The first messenger which is an extracellular ligand. Ex: norepinephrine, glucagon.
- GPCRs.
- Heterotrimeric G proteins "G = Guanine nucleotide binding proteins" (=transducers).
- Primary effectors which are ion channels or enzymes. Ex: adenylate cyclase, phospholipid C…etc.
- Second messengers whose intracellular concentration is controlled by primary effectors. Ex: cAMP, Ca2+…etc.
- Secondary effectors activated by second messengers. Ex: protein kinase A activated by cAMP.

The fixing of the first messenger on the RCPG was successful after a very important amplification phenomenon, the modification of numerous cellular activities (Fig.30).

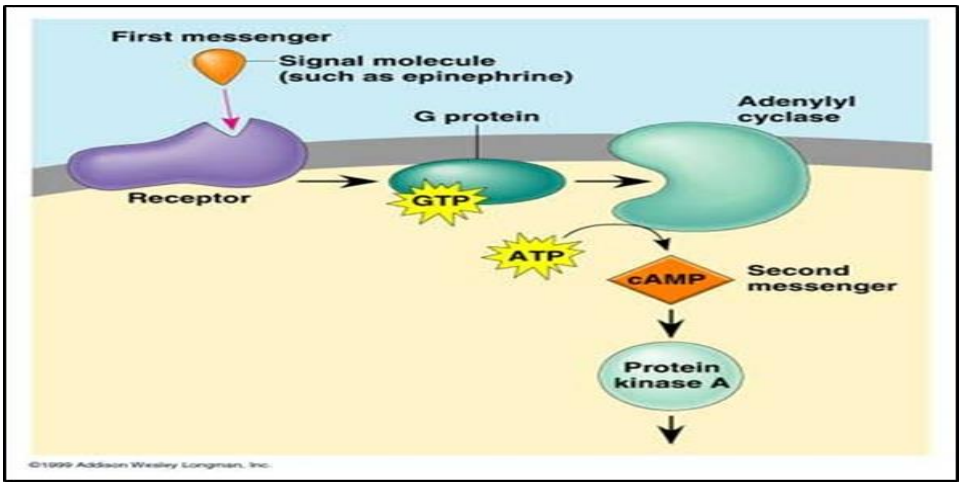


Fig.30. Events resulting from the binding of a water-soluble information molecule to a GPCR

2.3. Enzyme receptors

They own:

- a single transmembrane domain;
- a glycosylated N-terminal extracellular domain which binds the ligand;
- a C-terminal cytoplasmic end which carries intrinsic enzymatic activity or is directly associated with an enzyme (Fig.31).

Their characteristics:

- They are inactive in the monomer state and act mostly in dimer form.
- There are several classes of receptor enzymes.
- The most widespread are receptors with tyrosine kinase activity.
- They play a determining role in the action of growth factors (PDGF: Platelet-Derived Growth Factor, EGF: Epidermal Growth Factor…etc) andinsulin.

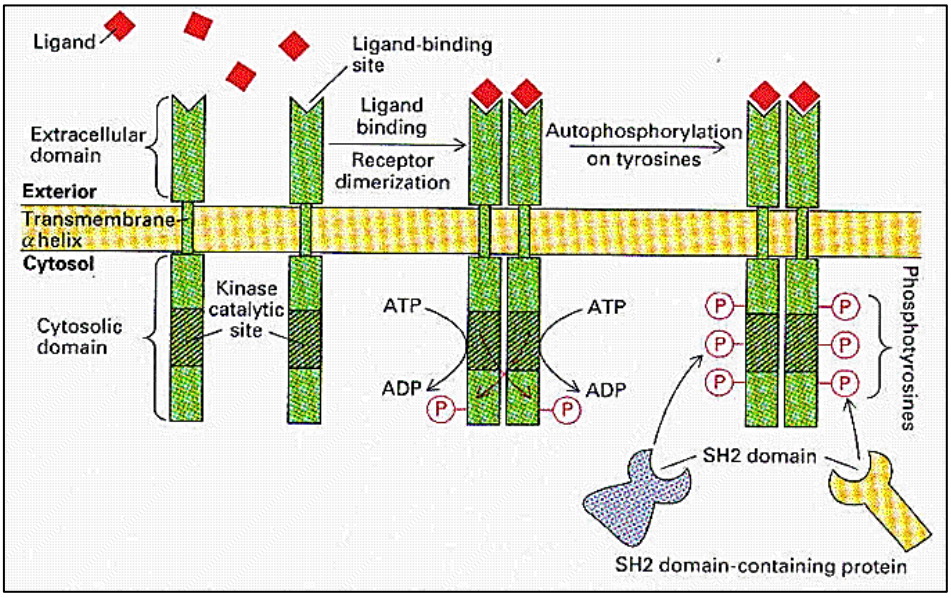


Fig .31. Enzyme-Linked Receptors.

II. Transducers and coupling factors

The transduction of signals perceived by receptors involves multiple processes, but the general mechanisms involved are few in number, the main ones being:

- ✓ The "recruitment" of proteins capable of interacting with others: there are many

"adaptor" proteins;

- ✓ Phosphorylation and dephosphorylation reactions by kinases and phosphatases, which modify the three-dimensional conformation of proteins and therefore their reactivity;
- ✓ Small G proteins are involved in an almost constant mechanism of exchange and hydrolysis of guanylic nucleotides;
- ✓ The production of intracellular "second messengers" relaying the information provided at membrane level.

Finally, the end-effectors are also very diverse, but here again it is possible to group them into a few entities:

- ✓ Transcriptional regulators, commonly known as "transcription factors",
- ✓ which control the transcription of target genes; these are the most general and most frequently encountered effectors downstream of signal transduction pathways;
- ✓ Translational regulators, which are directly involved in a number of signaling pathways and affect the level of protein synthesis;
- ✓ Cytoskeletal or extracellular matrix proteins, which control cell adhesion,motility and dispersion;
- ✓ Finally, ion channels, which we find here as effectors, are involved in synaptic transmission in particular, but not only (Fig.32).

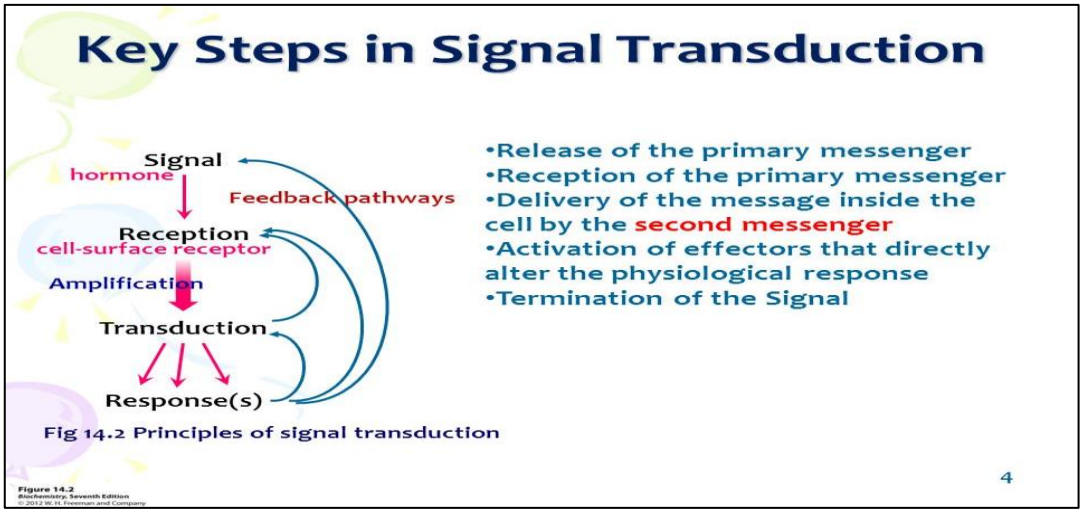


Fig.32. Key streps in signal transduction.

1. Scaffold adapters and proteins

There are adaptor proteins and scaffolding proteins. All can link different signalling proteins and are essential for many signalling pathways despite their lack of intrinsic enzymatic activity.

However, scaffolding proteins are generally larger and act as a platform for the assembly of proteins to allow the induction of more diverse signalling.

A. Adaptor proteins

Adaptor proteins are small proteins in the "second messenger" family, usually containing 2 to 3 domains.

These consensus structural domains (SH2, SH3, PTB, PH, etc) are necessary for protein-protein and protein-lipid interactions and signal transduction.

These domains enable them to connect several proteins together over a precise period of time, and to optimally localise different multi-protein complexes in the cell and positively or negatively modulate signal transduction. This assembly of signalling complexes by adaptor proteins is found for all types of membrane receptors, whatever the cellular response involved: proliferation, cell death, differentiation, motility and polarisation, and whatever the cell type (e.g. growth factors activate a family of "second messengers" called adaptor proteins).

B. Protein-protein interaction domain

A protein domain is a part of a protein capable of adopting a structure that is autonomous or partially autonomous from the rest of the molecule. This structure is generally compact and stable. It is a modular element in the structure of proteins, which can be composed of the assembly of several of these domains.

This is known as a multidomain protein. They can carry out certain functions specific to the complete protein: binding ligands, interacting with other macromolecules, etc.

A given protein domain is generally characterised by its three-dimensional structure and by a certain number of amino acids conserved in its primary structure. The same protein domain may be present in different proteins with different functions.

The typical size of a protein domain varies between 30 and 500 amino acids, with an average of around one hundred amino acids.

The most frequent protein-protein interaction domains are:

1. SH domains (Src homology domain)

Protein-protein interaction domains play a major role in signal transmission from receptors with tyrosine kinase activity.

Activation of these receptors initially leads to autophosphorylation of certain tyrosine residues located in the cytoplasmic domain of the protein. Secondly, they create recognition sites for other proteins, which are then phosphorylated and activated in turn, triggering the signal transduction cascade, e.g. β receptors for PDGF (Platlet-Derived Growth Factor).

Receptors with phosphorylated tyrosines specifically bind certain proteins with SH2 domains

(for Src Homology region 2; Src: pronounced "sarc", from the word sarcoma).

a. SH2 domains

The SH2 (Src homology 2) domains are protein domains of around 100 amino acids, present in many cytoplasmic proteins involved in signal transmission relayed by proteins with tyrosine kinase activity.

The binding of an SH2 domain to a peptide sequence is conditioned by the presence of a phosphorylated tyrosine, and the specificity of the interaction is dictated by the nature of the carboxy-terminal amino acids in relation to this tyrosine.

For example, the SH2 domain of the p85 subunit of PI3 kinase (Phosphatidyl Inositol3 kinase) binds to a Y(p) XXM motif (where X is any amino acid and M is a methionine) (Fig.33).

The SH2 domains are a fundamental component of the protein interaction network set up during the activation of membrane receptors with tyrosine kinase activity. They enable cytosolic enzyme activities to be localised on the membrane, enzymes to come into contact with substrates and enzyme activities to be modulated.

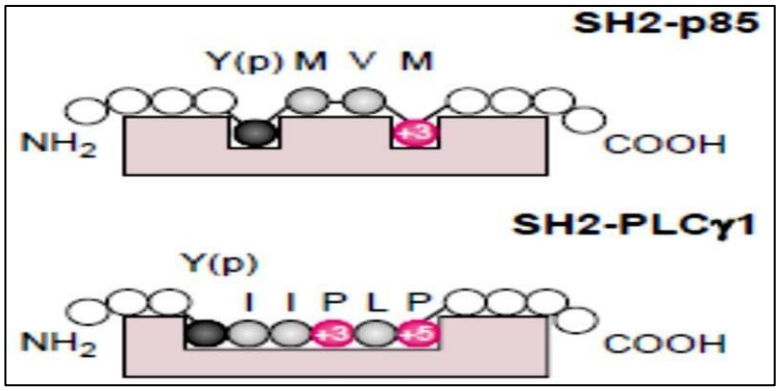


Fig.33. Recognition specificity of SH2 domains.

The SH2 domains complex phosphorylated tyrosine and amino acids in the carboxy-terminal position relative to it, in particular in position +3 for the p85 subunit of PI3 kinase and +3 and +5 for PLCγ1 *(PhosphoLipase* Cγ*). Single letter code for amino acids: I: Ile; L: Leu; M: Met; P: Pro;V: Val.*

b. SH3 domains

Other proteins have domains known as SH3 (Src Homology region 3). These domains have a strong affinity for proline-rich sequences, which tend to adopt a poly-proline structure. Some proteins have both SH2 and SH3 domains. This is the case, for example, with PI3 kinase.

These enzymes, activated by their binding, can bind other proteins with proline-rich sequences via their SH3 domains, activating them in turn.

c. PTB (phosphotyrosine binding) domains

Larger than SH2 domains (around 200 amino acids), PTB domains can also form a complex with a peptide phosphorylated on a tyrosine residue. The PTB domain of SHC was the first to be identified.

The SHC protein is represented by three isoforms, p66, p52 and p46, encoded by a single gene. The amino-terminal part of SHC could bind a protein phosphorylated on atyrosine residue despite the absence of an SH2 domain.

Since then, this interaction has been demonstrated with other membrane receptors with tyrosine kinase activity, such as TRKA (nerve growth factor receptor), FLT4/VEGFR3 (vascular endothelial growth factor-C receptor) and IGFI-R (insulin growth factor-I receptor), as well as cytokine receptors (GM-CSF and IL-2 receptors).

The amino-terminal region of IRS-1 (insulin receptor substrate-1), another signal transduction adaptor protein, bound to the insulin receptor in a similar way to the PTB domain of SHC. The term PTB is generally preferred to designate this domain (Figs 34,35).

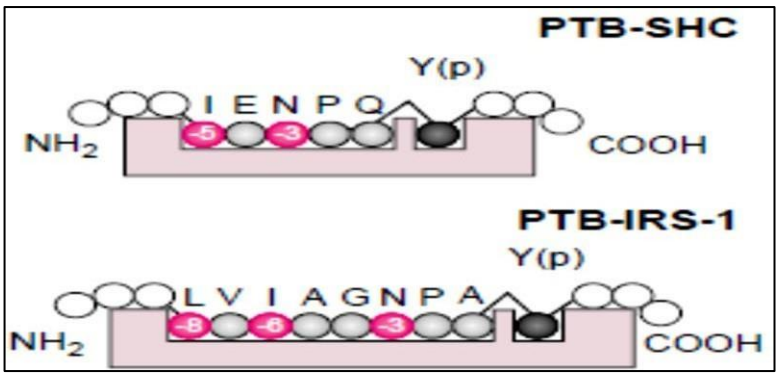


Fig.34. Recognition specificity of PTB domains.

The PTB domains recognise amino acids in the amino-terminal position relative to tyrosine: positions -3 and -5 for SHC and -8, -6 and -3 for IRS-1. One-letter code for amino acids : A: Ala; E: Glu; G: Gly; I: Ile; L: Leu; N: Asn; P: Pro; Q: Gln; V: Val .

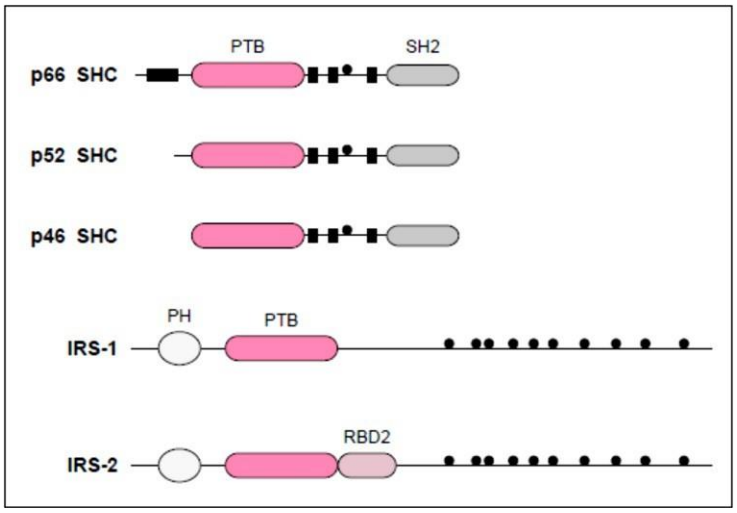


Fig.35. Structure of SHC and IRS-1.

d. PH domains

The PH domain (around 120 amino acids) was first defined in pleckstrin, the main substrate of protein kinase C in blood platelets. This domain is present not only in proteins involved in signal transmission (protein kinases, nucleotide exchange factors, GTPases, phospholipases, adaptor proteins), but also in cytoskeletal proteins such as spectrin andsyntrophin.

Certain phospholipids, in particular the bis- and trisphosphate phosphatidylinositols PtdIns (4,5) P2 and PtdIns(1,4,5) P3, as well as Gβγ proteins, can interact with these domains. These protein-protein interaction domains are not unique to adaptor proteins, but are also present in other proteins, enzymes and transcription factors (Fig.36).

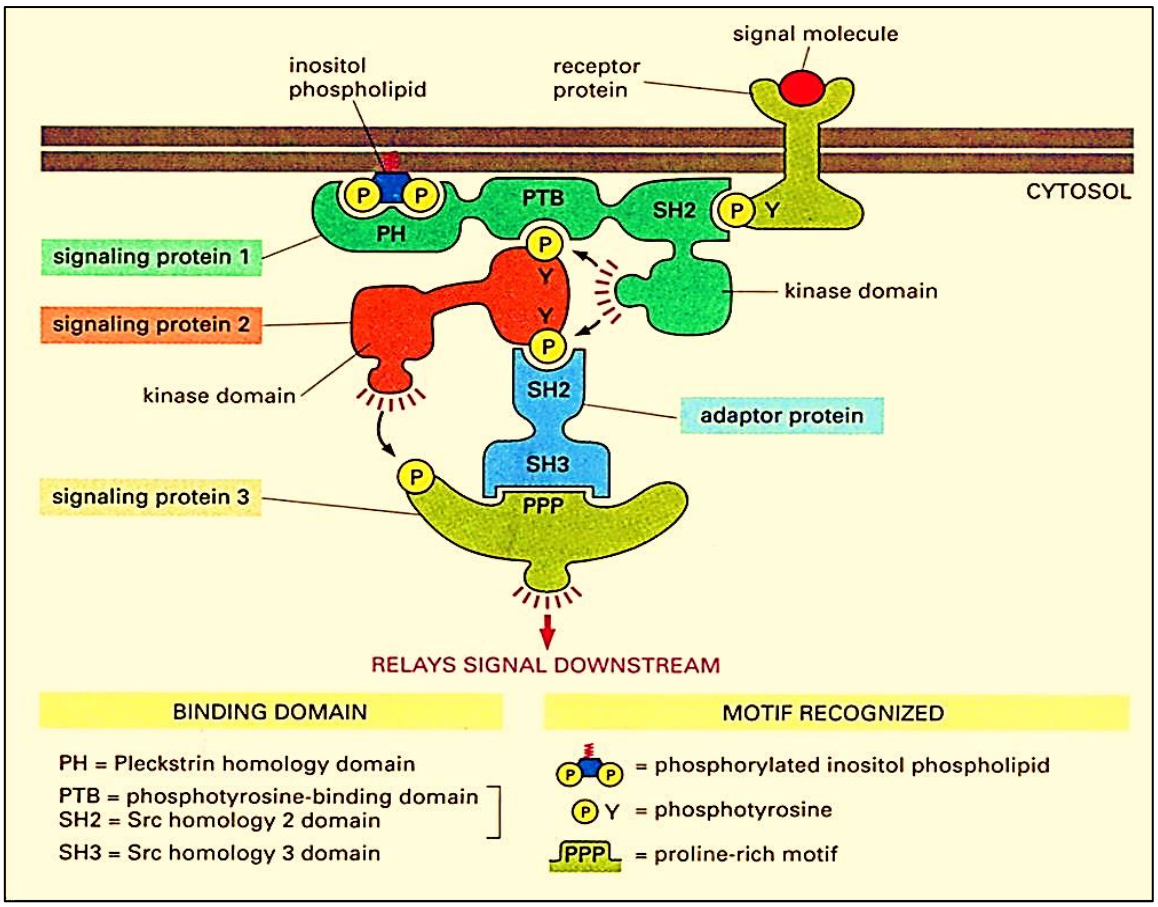


Fig.36. Protein interaction domains.

2. Adaptor proteins with SH2 domains

2.1. Grb2 protein (growth factor receptor bound protein 2)

The mammalian Grb2 protein consists of an SH2 domain and 2 SH3 domains. The SH2 domain of Grb2 binds to a peptide sequence of a receptor with activated tyrosine kinase activity. The two SH3 domains bind to proline-rich Sos (Son Of Sevenless) sequences.

2.2. SHC protein (SH2 and collagen homology domain containing protein)

The 62 KDa SHC protein plays a role similar to that of IRS (Insulin receptor substrate) as an adaptor protein between the insulin receptor and certain cellular effectors, at least those in the signalling pathway passing through Ras (These are small GTPases which belong to the family of monomeric G proteins. They are small single-chain proteins of 150 amino acids with a mass of 21 kDa).

The SHC protein contains an SH2 domain that can interact with phosphorylated tyrosines of activated receptors and a domain homologous to the α1 chain of collagen. Phosphorylation of SHC allows association with Grb2.

3. Small G proteins

The small G proteins, known as "G" proteins because they bind a guanylic nucleotide (GTP or GDP), have intrinsic GTPase activity. They form a Ras superfamily with more than 150 members of 20 to 40 kDa divided into 5 subgroups according to their sequence and functional similarities, including the Ras, Rho, Rab, Arf and Ran proteins (Table 2).

Table 2. Distribution of small G proteins in the subfamilies of the Ras superfamily

Subgroup	Number of members	Cellular functions
Ras	36	Gene expression
Rho	20	Gene expression; organisation of the cytoskeleton
Arf	27	Vesicular transport
Rab	61	Vesicular transport
Ran	1	Nuclear transport and cell cycle regulation
Other	9	/

They are anchored to the inner surface of the plasma membrane and coexist in two inter-convertible forms: an inactive form bound to GDP and an active form bound to GTP.

The interaction of a small G protein with a particular protein upstream of the signalling pathway causes the pathway to switch from an inactive to an active state.

All G proteins function according to the same principle, similar to that of α subunits of heterotrimeric G proteins:

1. They can form a complex with guanine-based nucleotides.
2. They bind GTP and can hydrolyse it to GDP, but their hydrolytic activity is low.
3. They can therefore form stable complexes with one or other of the nucleotides that determine their active or inactive state, as they lead to a change in the conformation of the protein at its catalytic domain.

3.1. Activation and inactivation of small G proteins

Three groups of proteins are involved in the activation cycle of small G proteins:

1. GTPase dissociation inhibitors (GDIs) keep the protein bound to the GDP in an inactive conformation. 2 GDIs are known: Rab GDI and Rho GDI;

2. GTPase activating proteins (GAPs) which catalyse the intrinsic GTPase activity of the G protein and therefore participate in the inactivation of the G protein.

3. Guanine nucleotide exchange factor proteins (GEF), which catalyse the exchange of GDP for GTP, thereby activating the G protein.

3.2. Regulatory proteins associated with small G

1. **GDI proteins (**GTPase dissociation inhibitors**)**

GDIs can bind to proteins with a geranylgeranyl residue, freeing these proteins from their membrane anchorage. This removal from the membrane helps to inhibit small G proteins, which are less likely to be activated in the cytosol. In addition, GDIs cover the effector binding site, prevent nucleotide exchange and inhibit GTP hydrolysis by small G proteins;

2. **GAP proteins** (GTPase Activating Protein)

GAPs interact with the GTPase domain of the protein and promote the GTP hydrolysis reaction. They promote the alignment of water molecules with the catalytic site of the proteins, thereby accelerating GTP catalysis. They also promote protein stability and facilitate the protein's interaction with GTP.

3. **GEP** (GTP/GDP Exchange Pro**tein**)/ **GEF** (guanine nucleotide exchange factor)

4. **Exchange proteins.**

The dissociation of GDP and small G proteins requires the intervention of a GEF which participates in the exchange of GDP for GTP by stabilising G proteins not bound to GTP or by catalysing the dissociation of GDP from its binding site and promoting interaction with GTP.

However, stabilisation of the unbound form of the G protein increases exposure of the GTP binding site but also promotes dissociation of the GEF. GEFs have several protein- protein and protein-lipid interaction sites. These sites enable them to be recruited to the membrane and act in response to an extracellular signal (Fig.37).

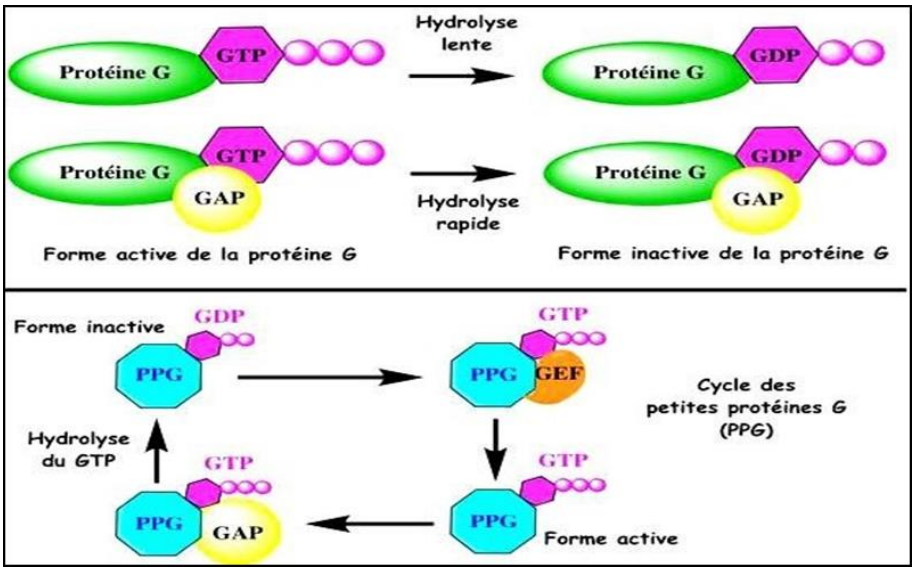


Fig.37. GDP/GTP cycle of G proteins.

C. Scaffolding proteins and anchoring proteins

Some proteins are involved in the formation of these complexes and may act solely as the

structure of the complex, such as scaffolding proteins, or may participate in signalling reactions, such as anchoring proteins.

1. Scaffolds proteins

Receptors with tyrosine kinase function can modulate signalling in several ways, either following direct binding of the proteins required for their signalling or via the binding of scaffolding proteins. Scaffolding proteins are proteins without enzymatic activity that modulate the interaction of other signalling molecules.

They establish a signalling platform following the recruitment of various enzymes and proteins, thereby amplifying this signalling. They also enable spatial and temporal regulation of intracellular signalling following the formation of specific protein complexes (Figs 38, 39).

Scaffolding proteins participate in the formation of complexes but may also be involved in the specificity of cell signalling by promoting signal transduction at the level ofthe proteins with which they may associate. In addition, phosphorylation of these proteins can modify their affinity for the associated proteins and thus modulate the specificity, duration or amplitude of the signal. There are several scaffolding proteins, such as the IRS1 family, MyD88 and Gab1.

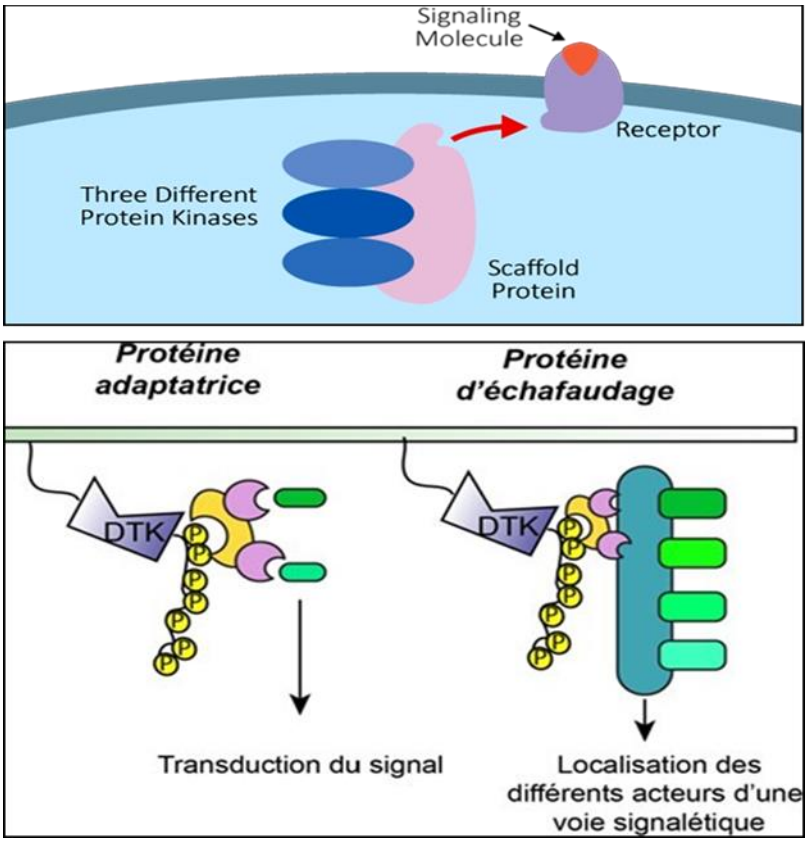


Fig.38. Difference between adaptor proteins and scaffolding proteins.

2. Anchoring proteins

Anchoring proteins enable the formation of protein complexes and facilitate interactions between proteins with complementary roles, usually in accordance with their subcellular location. They can also participate in the cell signalling of the proteins with which they are associated.

Several proteins, such as A-kinase anchoring protein (AKAP) (Fig49) and RACKs, play a major role in cell signalling because they can associate with kinases and phosphatases and bring the different interaction sites into contact.

Some proteins, such as certain small G proteins, require membrane anchoring to be activated and need to be translocated to different compartments such as the Golgi apparatus or the plasma membrane.

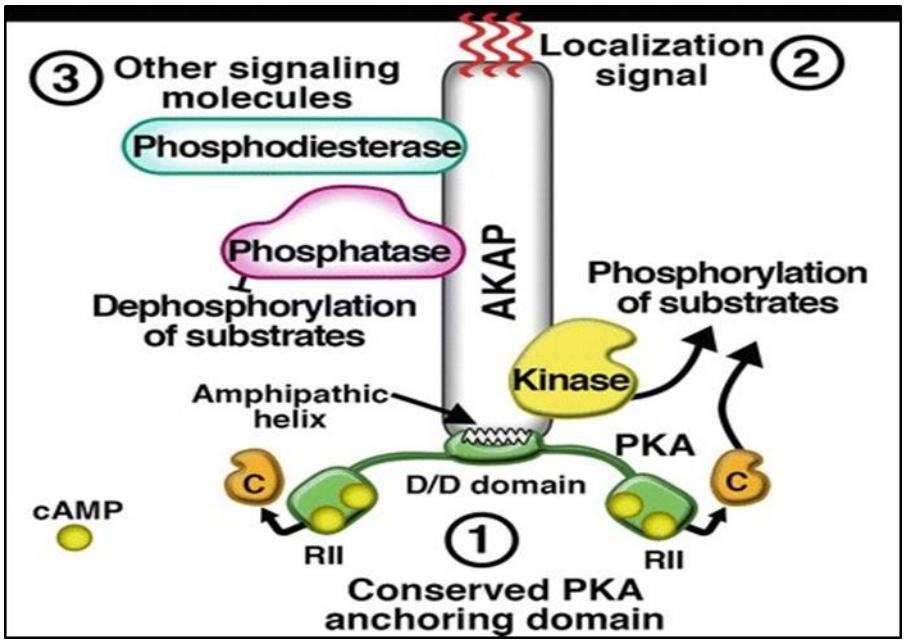


Fig.39. The A kinase anchoring protein (AKAP).

III. Signal amplification via second messengers

Secondary messengers, or second messengers, are molecules that enable the transduction of a signal from outside a cell to its interior or surface. Generally, a ligand (a hormone) binds to a membrane receptor. This binding results in the release of a secondary messenger into the cytoplasm or the plasma membrane, depending on its chemical affinity (hydrophilic/hydrophobic). This secondary messenger can trigger a cascade of reactions (amplifying the signal) leading to a cellular response (transcription of the target gene, release of the contents of exocytosis vesicles, etc.).

These secondary messengers can be of various types: lipids, inorganic ions (Ca, Mg), cyclic nucleotides, gases (nitric oxide).

A second messenger has the following characteristics:

- It is a small molecule that is diffusible in the plasma membrane if it is hydrophobic and in the cytosol if it is hydrophilic.
- It acts as an intracellular ligand, usually activating protein kinases, but also ion channels or interacting with other proteins.
- Its levels, resulting from its synthesis and catabolism, are finely regulated.

1. Phospholipases C and D/DAG/IP3/Ca^{+2} cascade

1.1. Heterotrimeric G proteins

The G protein is a heterotrimeric protein anchored in the inner leaflet of the plasma membrane. It exists in 3 main classes:

1. Gs: stimulates adenylate cyclase;
2. Gi: inhibits adenylate cyclase;
3. Gq: stimulates phospholipase C.

They belong to a large superfamily of proteins that bind GTP and hydrolyse it to GDP. They are made up of 3 subunits (SU):

1. A **SU** α (39 - 45kDa) which binds GDP and GTP and has GTPase activity;
2. A **SU** β (32 kDa) and;
3. A **SU** γ (8 kDa) which form an indissociable dimer.

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

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

1. Binding of the first messenger to the GPCR activates the G protein and triggers the exchange of one molecule of GDP for one molecule of GTP at the SU α;
2. This exchange leads to the dissociation of the trimeric complex and the SU α separates from the other two;
3. α and βγ modulate the activity of many primary effectors:

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

Following detachment of the first messenger, SU α exerts a GTPase activity that leads to hydrolysis of GTP and reconstitution of the inactive trimeric form (bound to GDP)(Fig.40).

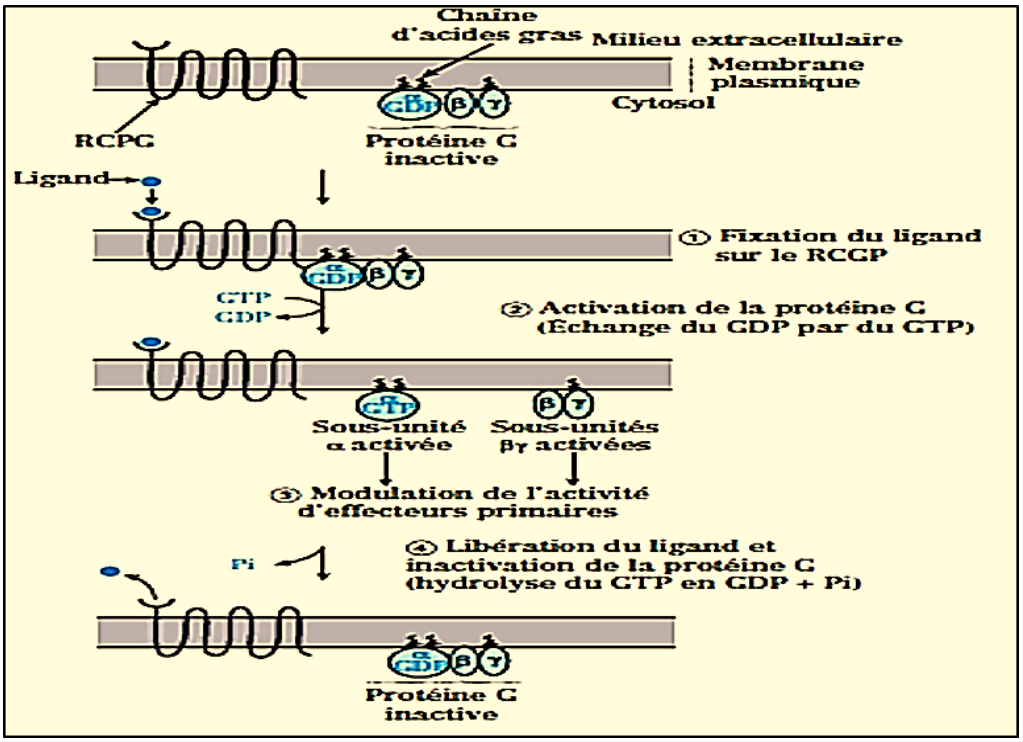


Fig.40. How a G protein-coupled receptor (GPCR) works.

1.2. Heterotrimeric G protein targets

a) The adenylate cyclase/AMPc pathway

Adenylate cyclase is a transmembrane enzyme whose active site faces the cytosol. When activated by $SU_{\alpha s}$, it catalyses the conversion of ATP into cAMP, a small soluble molecule that spreads into 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 numerous substrates, which considerably amplifies the effects of extracellular signals (Fig.41).

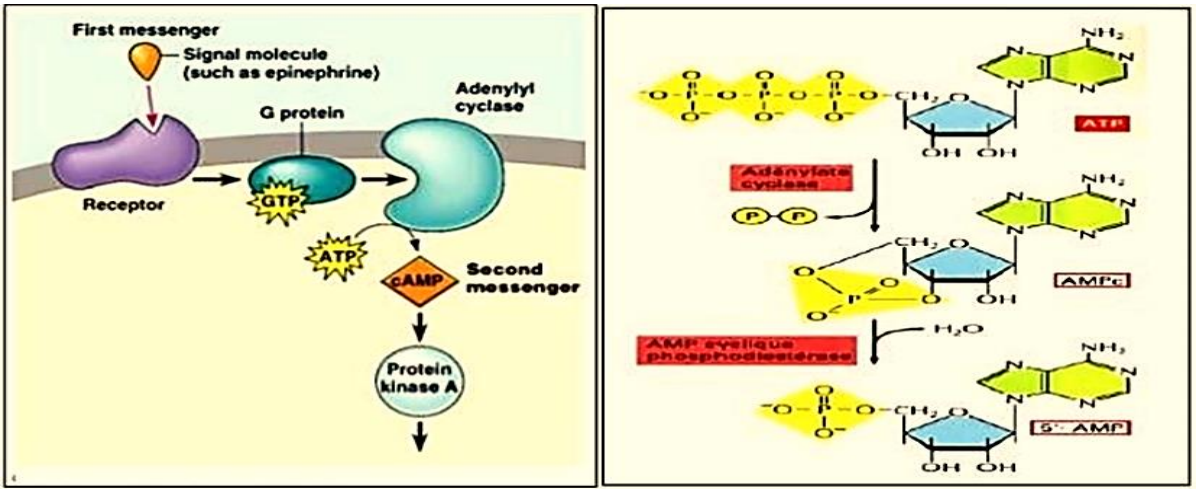


Fig.41. Mechanism of activation of cyclic AMP and adenylate cyclase

b) The phospholipase C (PLC) pathway - IP3, DAG and Ca+2

This pathway is activated by a cytosolic enzyme located close to the plasma membrane: PLC. When PLC is activated by SUαq, it hydrolyses PIP2 (Phosphatidyl-Inositol 4, 5- bisphosphate), a component of the inner leaflet of the plasma membrane.

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

IP3 leaves the membrane to bind to its receptor, located on the membrane of the LER (Smooth Endoplasmic Reticulum). This receptor is a Ca+2 channel which opens and allows the release of Ca+2 into the cytoplasm.

Ca+2 ions bind to and activate calmodulin. Calmodulin is then able to activate a number of enzymes, including Ca2+ /calmodulin-dependent protein kinases (CaM Kinase).

DAG activates a PKC (calcium-dependent protein kinase). It phosphorylates numerous substrates which relay the message, in particular transcription factors.

c) The ion channel pathway

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

Example: M2 muscarinic 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 opening of K+ channels: this opening causes the outflow of K+ and increases the difficulty of depolarising the cell and contributes to the inhibitory effect of acetylcholine on the heart (Fig.42).

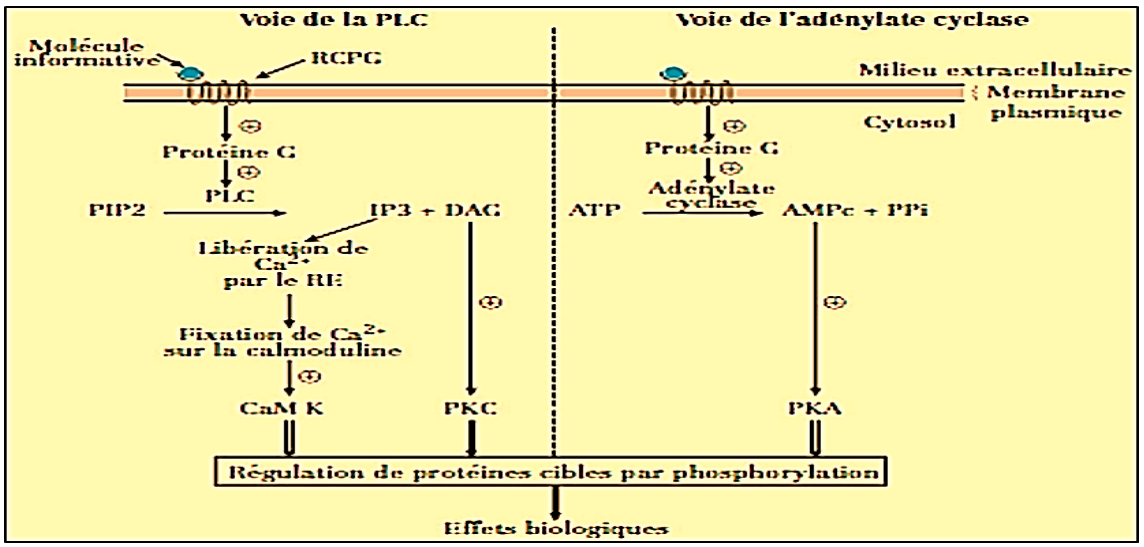


Fig.42. Comparison of the adenylate cyclase and PLC pathways following binding of a water-soluble molecule to a GPCR.

2. Phospholipase A2/ eicosanoid cascades

2.1. Eicosanoids

Eicosanoids are oxygenated derivatives of polyunsaturated fatty acids (arachidonic acid), present in almost every cell in the body with the exception of erythrocytes. They are local hormones that react to chemical stimuli. Their action is mainly paracrine and autocrine, and they appear in the blood only in minute quantities. In addition, they have a short lifespan of 1 to 2 minutes, as they are rapidly inactivated by reduction of the double bonds and dehydration of the hydroxyl groups. They act as second messengers for a number of hydrophilic hormones, TSH and ACTH.

2.2. Synthesis

They are synthesised from C20 arachidonic acid, formed from essential fatty acids (PUFAs: Polyunsaturated Fatty Acids) and then incorporated into membrane phospholipids using phospholipase A2 (PLA2).

From arachidonic acid, various enzymatic reactions produce the two major families: Prostanoids (Prostaglandin, Prostacyclin, Thromboxane) and Leukotrienes (Fig.43).

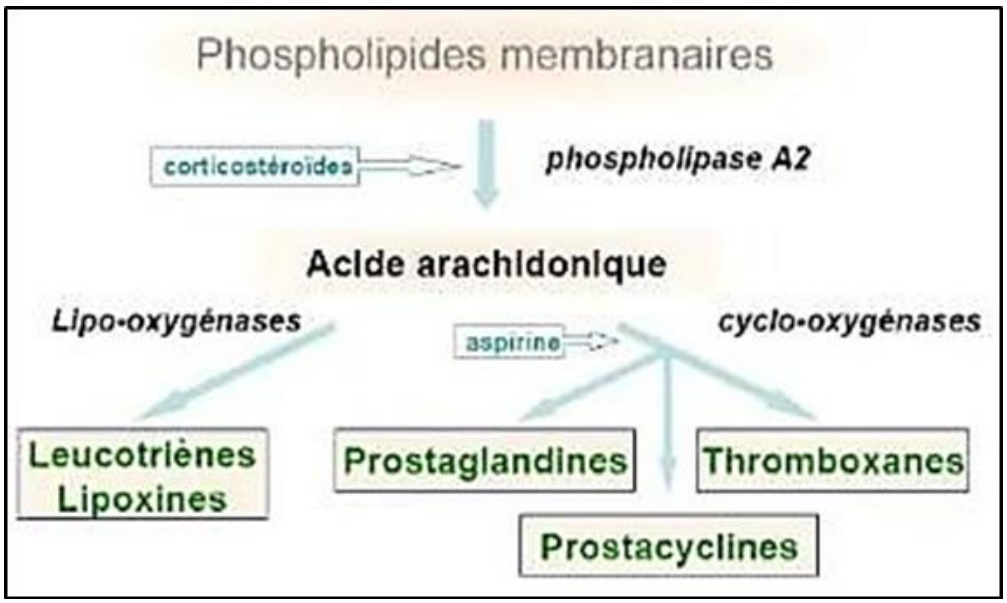


Fig.43. Synthesis of eicosanoids.

2.3. Mode of action of eicosanoids:

Once secreted by the cells, eicosanoids bind to GPCR receptors, which generally activate adenylate cyclase, leading to the synthesis of cAMP as a second messenger. There are 8 types of surface receptor for E:

- ✓ **RDP:** with prostaglandins **D**;
- ✓ **REP1:** to prostaglandins **E1**;
- ✓ **REP2:** to prostaglandins **E2**;
- ✓ **REP3:** to prostaglandins **E3**;
- ✓ **REP4:** to prostaglandins **E4**;
- ✓ **RFP:** to prostaglandins **F**;
- ✓ **RIP:** Prostacyclins;
- ✓ **RTP:** Thromboxanes.

Eicosanoids are lipid molecules, so they can also be ligands for intracellular receptors:PPARs (α, β, γ).

RIP example:

In blood vessels, PG12 binds to a GPCR and causes muscles to relax. The initial mechanism is the binding of the molecule to its GPCR. Several second messengers may be activated:

- ➢ cAMP, which subsequently cleaves a PKA;
- ➢ PLA2 activates the production of arachidonic acid and LOX (Lipo-oxygenase)
- ➢ PLC activates the release of IP3 and Ca2+, the latter playing a role in vasodilatation and

smooth muscle contraction.

For example the PLC:

When PGI2 binds to its RIP, it activates the G protein, which in turn activates PLC. PLC transforms PGI2 into IP3 and DAG. DAG activates PKC, which is responsible for enzyme phosphorylation, cell differentiation and exocytosis.... It can also be metabolised to form PLs. IP3 activates a channel in the LER, which opens and releases the Ca2+ store.

The cytoplasmic concentration of Ca2+ increases and activates calmodulin (which contains 4 Ca2+ binding sites).

The Ca2+/Calmodulin complex stimulates a large number of Ca2+-dependent processes, such as the activation of a cytoplasmic kinase, MLCK (Myosin Light Chain Kinase), which increases smooth muscle contraction and at the same time activates:

- ✓ AMP_C phosphodiesterase: inhibits AMP_C;
- ✓ GMP_C phosphodiesterase: inhibits GMP_C (GMP_C is used for muscle relaxation and therefore vasodilation);
- ✓ PLA2 for greater release of arachidonic acid and therefore synthesis of G proteins;
- ✓ ATPase to supply energy to an organism (generally under stress);
- ✓ Tyrosine and phenylalanine hydrolases for the synthesis of catecholamines.

2.4. Effects on various physiological processes

Prostanoids (PGE2, PGI2):

- ✓ Inhibits secretion of HCl and Pepsin;
- ✓ Stimulates the secretion of bicarbonates;
- ✓ Stimulates the production of protective mucus;
- ✓ Stimulates mucosal irrigation;
- ✓ Protective effect on the gastric mucosa.

3. Cyclic NO/GMP cascade and guanylate cyclase

Cyclic guanosine monophosphate (GMPc) was identified in different cells with fluctuating concentrations in response to different signals. GMPc emerged as an excellent second messenger (Fig.44).

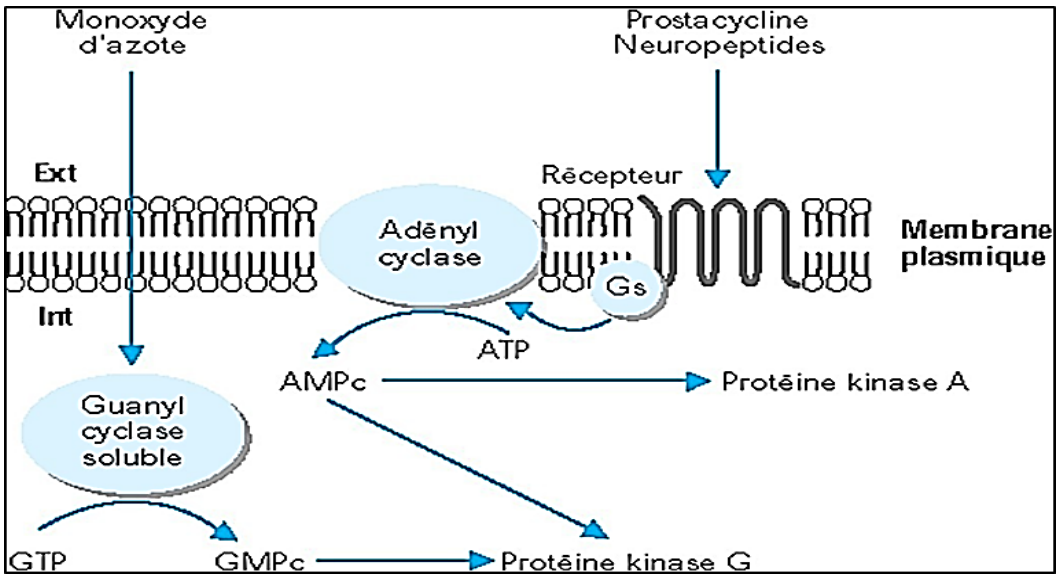


Fig.44. Mechanism of activation of cyclic GMP and guanylate cyclase

Like the adenylate cyclase and phospholipase C systems, the guanylate cyclase system amplifies a hormonal message, leading to a cellular response.

Guanylate cyclase degrades GTP into cyclic GMP or GMPc (second messenger). There are two forms of Guanylate cyclase:

✓ A membrane form;

✓ A cytosolic form, known as soluble.

Soluble Guanylate Cyclase consists of a NO-sensitive α β heterodimer (nitric oxide: NO is produced by the catabolism of arginine). Binding of NO to α and β activates Guanylate Cyclase.

Inactivation is achieved by hydrolysis of GMPc to 5'GMP using a phosphodiesterase (PDE) (isoforms of that involved in the Adenylate Cyclase/AMPc/PKA system).

VII. Signal amplification via MAP Kinases cascades

1. Protein Kinase (A, B, C, CAM, MAP)

Protein kinases are enzymes that catalyze the transfer of a group phosphate (P) of adenosine triphosphate (ATP) on the hydroxyl of the side chains of amino acids with an alcohol function: serine, threonine and tyrosine.

The amino acids tyrosine (Y), serine (S) and threonine (T) all contain an alcohol function that can fix a P. We distinguish within proteins Tyrosine Kinases (PTK), receptor tyrosine kinase (RTK) activity and cytoplasmic PTKs. THE

Protein kinases are involved in regulating the activity of target proteins. Phosphorylation is a major post-translational modification of proteins, which is involved in a very large number of cellular processes (differentiation, division, proliferation, apoptosis, etc.) and in particular in

signaling mechanisms.

Phosphorylation induces structural and therefore very functional modifications. Important of the target protein which has the consequences:

- An increase or inhibition of its enzymatic activity;
- A change in its cellular location in certain cases (transcription factors for example) and ; Association with other proteins.

Phosphorylation is a modification that is reversed by dephosphorylation catalyzed by protein phosphatases.

1.1. Protein Kinase A

Protein kinase A (PKA) refers to the family of enzymes whose activity isdependent on the level of cyclic AMP (cAMP) in the cell. Protein kinase A has many functions in the cell, in particular itregulates glycogen, sugar and lipid metabolisms.

1.2. Protein Kinase B

Akt or protein kinase B (PKB); It is an essential protein in signaling mammalian cells. Akt1 is involved in the survival signaling pathway cellular, by inhibiting apoptosis. Akt1 is also capable of inducing protein biosynthesis, and is therefore a key element in the cellular phenomena leading to skeletal muscle hypertrophy and tissue growth in general. From the moment Akt can block apoptosis and thereby promote cell survival, Akt2 is a factor involved in cellular insulin signaling. It is necessary for the transport of glucose.

1.3. Protein kinase C

Protein kinases C (PKC) are cytoplasmic enzymes with serine activity threonine kinase whose involvement in oncogenesis proves to be complex. Of the deregulations of expression of certain PKCs have been reported in different tumors, but there is great variability in the roles of these enzymes depending on the isoform considered or the type cell studied (pro- or antiproliferative action).

Even if the understanding of the role of PKC in tumor development is stillincomplete, different agents are now being evaluated. This review offers a summary of the available data, with particular emphasis on the resultsclinics.

1.4. CAM protein kinase

Ca2+/calmodulin-dependent protein kinases or CAM kinases are Serine-Threonine-dependent kinases regulated by the Ca2+/calmodulin complex.

CaMKII is involved in numerous signaling cascades, and is an important putative mediator of

learning and memory.

CaMKII is also necessary for calcium homeostasis and calcium reuptake in cardiomyocytes, the transport of chlorine ions into the epithelia, positive selectionT cells, and activation of CD8 T cells.

1.5. MAP protein kinase

Mitogen-activated protein kinases (MAPKs) are a set of protein kinases necessary for the induction of mitosis in eukaryotic cells.

MAPKs are involved in a certain number of events in the life of the cell, such as mitosis, but also very linked to apoptotic phenomena, the differentiation or cell survival. This is done in response to various external signals: mitogenic factors (PDGF, for example), cellular osmotic stress, shockthermal or even a certain number of cytokines.

MAPKs are found in animal, plant and human cells and inmushrooms.

2. Tyrosine kinase receptors: Example of growth factor receptors GF

The successive binding of 2 ligand molecules induces 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 which binds to Grb2 and stimulates the exchange of GDP by GTP at the level of Ras (Sos = GEF).

Activated Ras induces a cascade of phosphorylations 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 results in the modification of activity of cytosolic proteins and in activation of transcription factors. This pathway regulates cell proliferation, differentiation and survival (Fig.45).

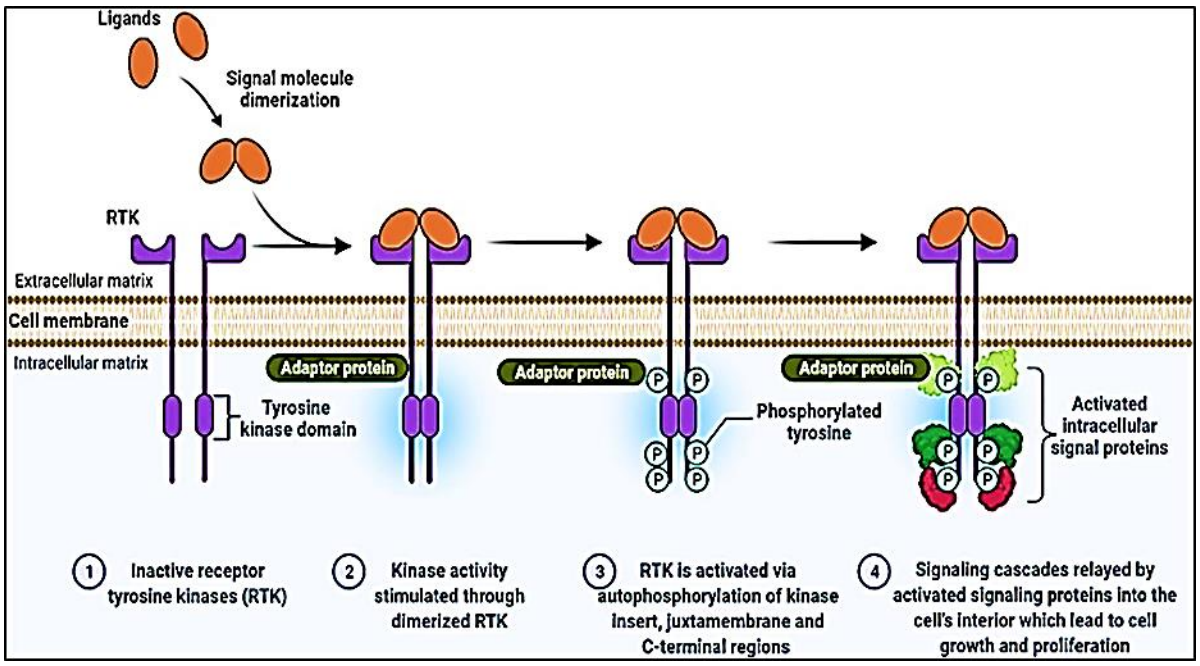


Fig.45. Classical model of RTK activation mechanism.

Chap 6. SIGNALING ABNORMALITY AND PATHOLOGY

1. RAS proteins

Ras proteins are a family of proteins, with a role as proto-oncogene. A One tumor in four in humans has a mutation in this gene. The proteins from this gene have a molecular weight of 21000 dalton hence their names *p21*.

They act on several metabolic pathways by activation of kinases;

They are involved in the regulation of proliferation, differentiation and cell survival as well as in the organization of the cytoskeleton. They promote autophagy, which results in the cancerous transformation of cells (Fig.46).

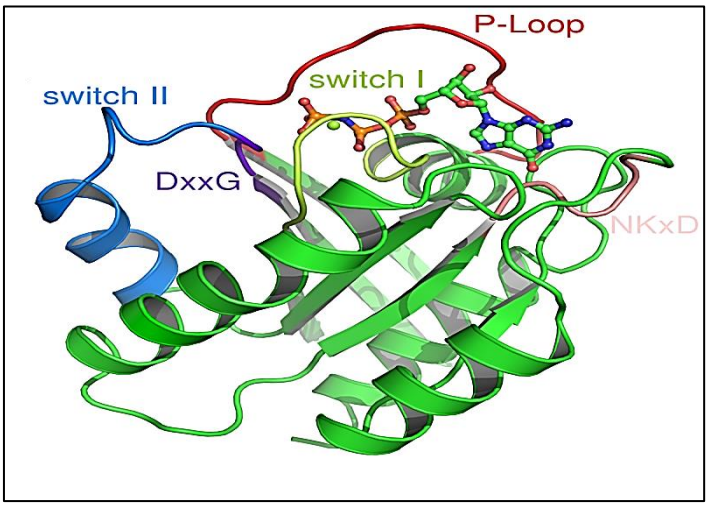


Fig.46. The RAS protein.

2. Oncoproteins

It is a protein whose synthesis in the body is controlled by an oncogene, that is to say an altered gene, likely to be involved in the appearance of a tumor.

3. EGFR

Epidermal growth factor receptor (EGFR) is a protein transmembrane monomeric with tyrosine kinase activity. It becomes an oncogene by acquisition of an activating mutation, located essentially in exons 19 or 21 of the gene (Fig.47).

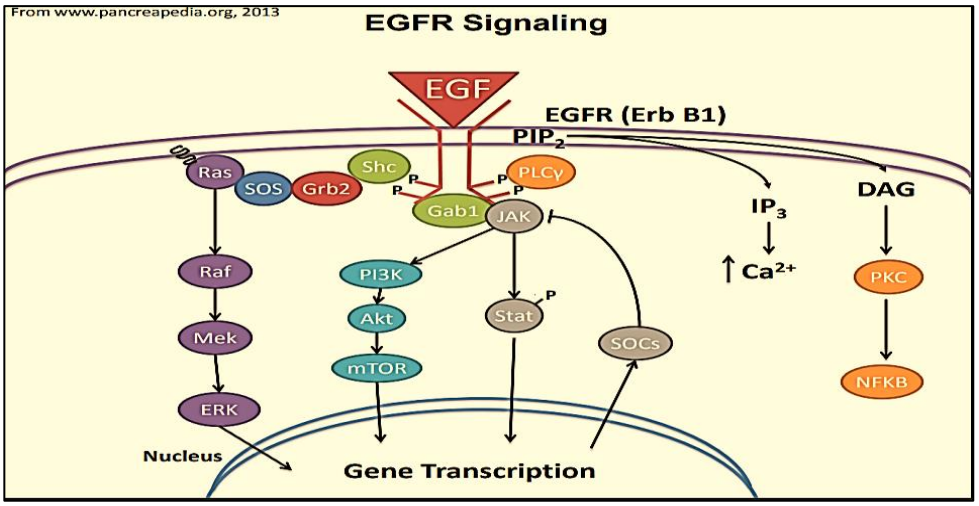


Fig.47. EGFR signaling

4. Oncogenes

Any cellular gene, called proto-oncogene, likely to become, following a qualitative or quantitative modification, a transforming gene, that is to say a gene capable to experimentally confer the cancerous phenotype (transformation) to a normal cell eukaryotic.

Alteration of one allele is sufficient to cause abnormal activation.

Oncogenes are divided into 6 major classes based on oncoproteins for which they code, we will cite the most important in our studies which is EGFR.

Their activation mechanism is multiple, we will mention that of the deletion which results most often to a loss of function, which can sometimes lead to abnormal activation if it affects a regulatory region

5. Abnormal EGFR activation in cancers

EGFR has been shown to play an important role in the genesis of many epithelial cancers including colorectal cancers where this receptor is overexpressed in 30 to 85 % of cases.

a. Oncogenic activation of EGFR in cancers can occur through several mechanisms: Increase in its expression linked to an increase in the number of receptors forsurface of the cell. This mechanism may result from an increase in transcription or increased stability of the protein;

b. Mutations of the EGFR gene at the extracellular domain, responsible for the synthesis of an abnormal receptor having lost this domain and activated in a mannerconstitutive;

c. Increase in the quantity of EGFR ligands, in particular TGFÿ, responsible for an autocrine loop of continuous activation of EGFR;

d. Amplification of the EGFR gene, frequently found in bronchocancerspulmonary, ENT

and colorectal.

Receptor mutations in non-somatic cells are associated with severalforms of cancer, particularly breast and lung (Fig.48).

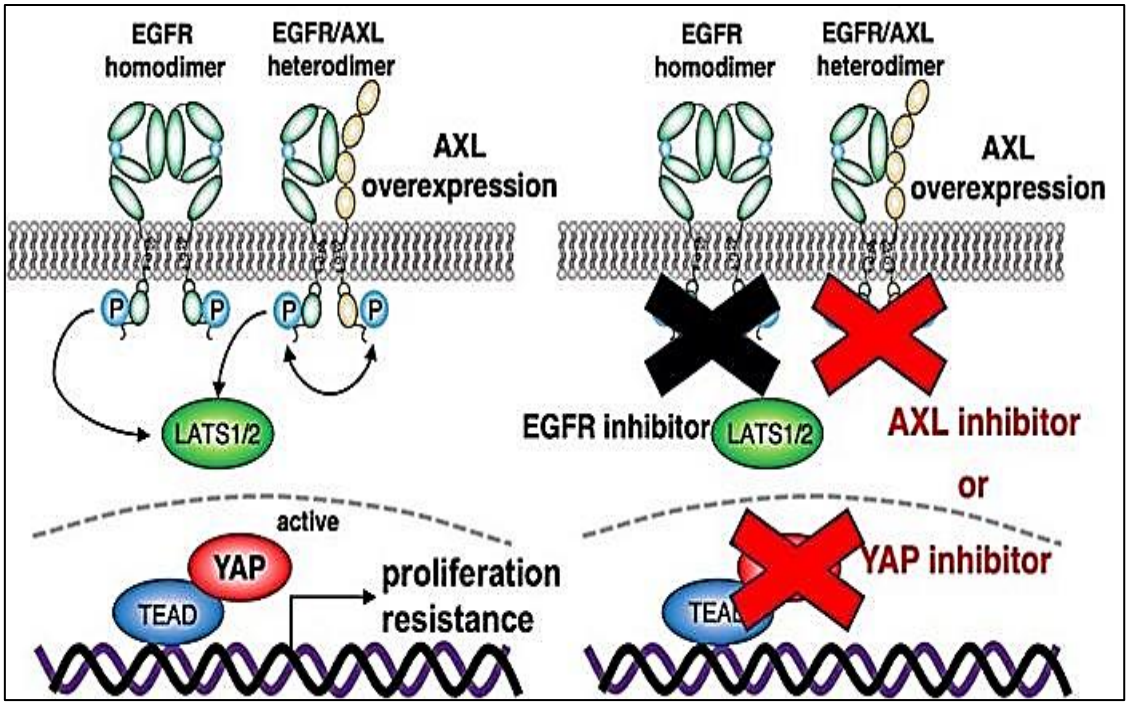


Fig.48. A combination of cancer inhibitors shows success in slowing tumor growth

6. Pathology linked to abnormal activation of the RAS/MAPK pathway

Concerning RAS proteins, KRAS is one of the most frequent oncogenes activated in cancers since approximately 20% of human tumors have a mutationactivator of this gene.

Cancers with a high prevalence of KRAS mutations are cancers of the pancreas, colorectal, bile ducts, bronchopulmonary cancers.

Activation of the RAS protein occurs through the presence of a missense mutation of KRAS which give them oncogenic power. The presence of such mutations at the level tumor is responsible for acquired activation of the RAS/MAPK pathway downstream of EGFR, and totally independent of the binding of the ligand to the latter, which gives the cells resistance to anti-EGFR antibodies in colorectal cancers.

Bibliographic references

Alok Raghav, Jamal Ahmad (2022). Fundamentals of Glycosylation. Royaume-Uni: IntechOpen.

Chanoine, C., Charbonnier, F. (2018). Biologie cellulaire et moléculaire de la cellule eucaryote: UE2. France: Ellipses.

Charest-Morin, X. (2018). Ligands biotechnologiques des récepteurs couplés aux protéines G: vers des applications diagnostiques ou thérapeutiques. Canada: Université Laval.

Ciesielski, F. (2006). Etude de la relation entre structure et fonction du récepteur de la vitamine D. France.

Colas, C. (2011). Etude Des Interactions Des Récepteurs Nicotiniques Avec Leurs Ligands. Allemagne: Editions Universitaires européennes.

Guidelli, R. (2016). Bioelectrochemistry of Biomembranes and Biomimetic Membranes. Royaume-Uni: Wiley.

Iwasa, J., Karp, G., Marshall, W. F. (2018). Biologie cellulaire et moléculaire de Karp. Belgique: De Boeck supérieur.

John Libbey Eurotext (2014). Anomalies moléculaires des cancers: ciblage thérapeutique. France.

Jos A.F.op den Kamp (2013). Molecular Dynamics of Biomembranes. Allemagne: Springer Berlin Heidelberg.

Moussard, C. (2020). Biochimie et biologie moléculaire. Belgique: De Boeck supérieur.

Robert, J. (2011). Signalisation cellulaire et cancer. Ukraine: Springer Paris.

Universalis, E. (2016). Structure en biologie: Les Grands Articles d'Universalis. Belgique: Primento Digital Publishing.