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كلـــية عـــلوم الطبـــيعة والحـــياة
قسم الوراثة الجزيئية التطبيقية
People's Democratic Republic Of Algeria
Ministry of Higher Education and Scientific Research
University of Science and Technology of Oran Mohamed BOUDIAF
Faculty of Natural and Life Sciences
Department of Applied Molecular Genetics

Handout of the material

Cellular Communication and Signaling

Intended for first-year Master's students (M1)

Academic in Fundamental and Applied Genetics (GFA)
&
Professional in Cytogenetics

Presented by : Dr. BENSABER Hayette Sénia

Academic Year : 2024-2025

FOREWORD

Cellular communication is essential to the life of multicellular organisms; cells must imperatively exchange the information necessary to coordinate their actions. The principles of cellular communication are universal: signaling molecules are emitted by one cell, recognized by another, which then implements a transduction pathway for the received signal, which results in an effector system that takes the signal into account. The variety of information transmission systems is immense, both at the level of signal reception and that of the activation of effectors. However, it is certainly possible to find general patterns, common structures of the information flow, as long as we take the trouble to look for them.

The various transduction pathways are just a few examples of the many pathways that can be used by cells. This area of research is extremely active, and its therapeutic implications are potentially significant.

There were already books that discussed cellular communication and its mechanisms, renowned for their precision, detail and exhaustiveness, but there was a need for another kind of document, one that students could approach and master easily, without hiding the passion that drives researchers when faced with the challenges of this revolutionary field of biological science.

This course is a synthesis of years of teaching on cellular communication and signaling. It is intended specifically for students in the first academic year of a Master's degree in Fundamental and Applied Genetics (GFA) and also for students in the first professional year of a Master's degree in Cytogenetics to help them strengthen their knowledge and navigate through each chapter with updated knowledge of the signaling cascade.

This course will also be useful to all science, medicine and pharmacy students, as well as to many teachers in various disciplines who have a strong interest in biology, in part or in whole.

All data is presented in a clear, concise and easy-to-understand manner thanks to the presentation of numerous color diagrams and photographs illustrating the data to be acquired. Some diagrams summarize the data from the text, while others provide additional or more specific information. Handout of cellular communication and signaling

Students should find this pictorial approach well suited to revising complex signaling concepts, making them easier to remember when preparing for their exams.

ABSTRACT

Cellular communication is a fundamental process by which cells perceive and respond to their environment, ensuring the coordination of biological functions essential to maintaining homeostasis. This intercellular dialogue is based on the exchange of chemical, electrical, or mechanical signals, which can act locally (juxtacrine or paracrine communication) or remotely (endocrine or synaptic communication).

Cell signaling mechanisms typically begin with the reception of an extracellular signal via a specific receptor located on the surface or within the target cell. This interaction activates a signal transduction cascade, often composed of protein kinases, second messengers (cAMP, Ca^{2+}, IP3), and transcription factors. These cascades amplify the initial signal and lead to specific biological responses : activation or repression of genes, proliferation, differentiation, migration, or apoptosis.

Key signaling pathways include G protein-coupled receptor (GPCR), receptor tyrosine kinase (RTK), JAK/STAT, Wnt/β-catenin, and nuclear receptor pathways. Each of these pathways is finely regulated and can interact with other pathways (crosstalk), giving the cell a high degree of response plasticity. Dysfunction in cell signaling is implicated in many human pathologies, including cancers, autoimmune, neurodegenerative, and metabolic diseases.

Current research is exploring new therapeutic targets within these signaling pathways, with the goal of modulating cellular messages specifically and efficiently. Signaling biomarkers are also used in diagnosis and prognosis to assess the pathophysiological state of tissues.

Keywords : cellular communication, signaling, receptors, signal transduction, second messengers, kinases, signaling pathways, GPCR, RTK, JAK/STAT, Wnt, apoptosis, cell proliferation, pathologies, targeted therapies.

ABBREVIATIONS

ADD: Associated proteins with Death Domain

Akt: Acutely transforming retrovirus AKT8 in T-cell lymphoma (also called PKB or PKB/Akt)

cAMP: Cyclic Adenosine Monophosphate

ANF: Atrial Natriuretic Factor

Apaf-1: Apoptotic protease activating factor

APC: Adenomatous Polyposis Coli

AR: Androgen Receptor

Arf: ADP ribosylation factor

ARK (β-ARK): β-adrenergic receptor kinase

Bak: Bcl-2 homologous antagonist/killer

Bax: Bcl associated partner containing six exons

Bcl-2: B-cell lymphoma protein 2

BH: Bcl-2 homology

Bid: Bcl-2 interacting death agonist

BMP: Bone Morphogenetic Proteins

CaBPs: Calcium Binding Proteins

CAD: Caspase Activating DNase

Cadherins: Calcium dependent adhesion proteins

CaM: Calmodulin

CARD: Caspase Recruitment Domain

Caspase: Cysteine-aspartate-proteases

CBP: CREB-Binding Protein

CD: Cluster of Differentiation

Ci: Cubitus interruptus

CK1: Casein kinase 1

MHC: Major Histocompability Complex

APC: Antigen Presenting Cell

CRE: Cyclic AMP Response Element

CREB: CRE-Binding protein

DAG: Diacylglycerol

DBD: DNA Binding Domain

DD: Death Domain

DED: Death Effector Domain

DISC: Death-Inducing Signaling Complex

DSR: Death Signaling Receptors

EGF: Epidermal Growth Factor

Epac: Exchange proteins activated by cAMP

ER: Estrogen Receptor

ERK: Extracellular signal regulated protein kinase

FAK: Focal Adhesion Kinase

FGF: Fibroblast Growth Factor

FGT: General Factors of Transcription

GAP: GTPase-Activating Protein

GC: Guanylyl Cyclase

GEF: Guanine nucleotide exchange factor

cGMP: Cyclic Guanosine MonoPhosphate

GR: Glucocorticoid Receptor

Grb2: Growth factor Receptor Binding Protein-2

GRK: G-protein coupled receptor kinases

GSK-3 β: Glycogen synthase kinase-3 β

HATs: Histone acetyltransferases

HDACs: histone deacetylases

HSP: Heat Shock Protein

IAP: Inhibiting Apoptosis Protein

ICAD: CAD Inhibitor

ICAM: InterCellular Adhesion Molecules

IKK: IκB Kinase

IKKK: IκB Kinase Kinase

IL: interleukin

IL-1R: Interleukin-1 Receptor

INFα: Interferon α

IP3: Inositol tri-Phosphate

IRAQ: IL-1 Receptor-Associated Kinase

IRS: Insulin Receptor Substrates

ITAM: Immunoreceptor Tyrosine-based Activation Motif

IκB: inhibitor of nuclear factor κB

JAK: Janus Kinase

JNK: c-Jun N-terminal kinase

LAT: Linker for activation of T cells

LBD: Ligand Binding Domain

LFA: Leukocyte Function-associated Antigen

LRP: low density lipoprotein receptor related protein

MAPK: Mitogen Activated Protein Kinase

MAPKK: MAPK Kinase

MAPKKK: MAPK Kinase Kinase

MEK: MAP kinase-ERK Kinase

MLCK: Myosin light chain kinase

MR: Mineralocorticoid Receptor

MyD88: Myeloid Differentiation factor 88

NFAT: Nuclear factor of activated T cells

NF-κB: Nuclear Factor kappa B

NGF: Nerve Growth Factor

NICD: Notch intracellular domain

NLS: Nuclear Localization Signal

NO: Nitric Oxide

NOS: Nitric Oxide Synthase

PAMPs: Pathogen Associated Molecular Patterns

PDE: Phosphodiesterase

PDGF: Platelet Derived Growth Factor

PDK: Phosphoinositide-Dependent Kinase

PH: Pleckstrin Homology

Pi: inorganic phosphate

PI3-K: PhosphoInositides 3 Kinase

PIP2: Phosphatidyl Inositol bi-Phosphate

PIP3: Phosphatidyl Inositol tri-Phosphate

PKA: Protein Kinase A

PKB: Protein Kinase B (also called Akt or PKB/Akt)

PKC: Protein Kinase C

PKG: Protein Kinase G

PLC: Phospholipase C

iv

Pol II: RNA polymerase II

PPP: Proline-rich domain

PR: Progesterone Receptor

PRR: Pattern Recognition Receptors

PSGL-1: P-Selectin Glycosylated Ligand-1

PTB: Protein Tyrosine-phosphate Binding domains

PTEN: Phosphatase and tensin homolog

Rab: rat brain cDNA library

Raf: Rat fibrosarcoma

RalA: Ras-like protein

Ran: Ras-related nuclear protein

RANK: receptor activator of NFkB

Rap: Ras proximal proteins

RAR: Retinoic Acid Receptor

Ras: Rat Sarcoma

RCPG: G Protein-Coupled Receptors

RCTK: Receptor Tyrosine Kinases

RE: Responsive Element

RGCs: Receptors with guanylyl cyclase activity

RGS: Regulators of G protein Signaling

Rheb: Ras homolog enriched in brain

Rho: Ras homologous

RIP: Receptor-Interacting Protein

R-Smad: Regulated Smad

RST: Receptors with serine/threonine kinase activity

RTK: Receptor tyrosine kinase

RXR: Retinoid X acid Receptor

SARA: Smad Anchor for Receptor Activation

Ser (or S): serine

SH2: Src Homology domain 2

SH3: Src Homology domain 3

Shc: Src homology 2 (SH2)-domain-containing protein

SLP-76: Src homology 2 (SH2)-domain containing leukocyte protein of 76 kDa

SMAC: Supramolecular activation cluster

Smads: amalgamation of Sma (Small) and Mad (Mothers against Dpp). Dpp: decapentaplegic

SOS: Son of Sevenless

STAT: Signal Transducer and Activator of Transcription

TAK: TGF-β activated kinase

tBid: truncated Bid

TBP: TATA box Binding Protein

TCF: T-cell transcription factor

TCR: T-Cell Receptor

TGFs: Transforming Growth Factors

Thr (or T): Threonine

SHOOTING: Toll/IL-1R

TK: Tyrosine Kinase

TLR: Toll-like receptor

TNF: Tumor Necrosis Factor

TNFR: Tumor Necrosis Factor Receptor

TR: Thyroid hormone receptor

TRADD: TNF Receptor-Associated Death Domain

Traf6: Tumor necrosis factor receptor (TNFR)-associated factor 6

TRAP: Thyroid hormone Receptor-Associated Proteins

Tyr (or Y): Tyrosine

VDR: Vitamin D Receptor

VEGF: Vascular Endothelial Growth Factor

ZAP-70: ζ (Zeta)-chain-Associated protein-tyrosine kinase of 70 kDa

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Subject title: Cellular Communication and Signaling (CCS)

Credits: 2

Coefficients : 2

Teaching Objectives : This course will provide students with a comprehensive understanding of cell signaling and will provide an essential foundation for students in the fields of Cell Biology and Development, Biochemistry, Immunology, or Genetics. It will allow students to study the activity of immune cells dependent on expressed surface receptors. As well as the common transduction signals from different membrane receptors, this module will provide a solid understanding of the main cellular signal transduction pathways and molecular mechanisms related to immunity.

Recommended prior knowledge : Students should have a knowledge of multicellular organisms. Molecular biology, physiology, genetics.

Skills after successful completion of this subject : this module allows students to acquire a global vision of cell signaling and will constitute a common basis for all specialties in the subject.

The topics covered will be :

Content of the subject :

• Signal transduction

• Enzyme-coupled membrane receptors

• G protein-coupled receptors (GPCRs): signaling; concepts of agonist, antagonist, inverse agonist, biased ligands; dimers;

• Cell communication and signaling. Cell junctions: organization and structure.

• Cell adhesion molecules : dynamic aspects, signaling and function during BCR and TCR development :

• Signaling by antigen-specific receptors (BCR and TCR, B and T cell receptor) and the main associated co-receptors.

• Apoptosis: Morphological and biochemical characteristics

• Activation pathways and their regulation, signals emanating from apoptotic cells.

• Molecular mechanisms, physiological and pathological roles

Assessment method:

Continuous assessment Exam

GENERAL INTRODUCTION

Cellular communication is essential for the coordination and regulation of the activities of living beings, both at the organismic and cellular level. All living organisms, whether prokaryotic, eukaryotic, uni- or multicellular, constantly receive signals from their environment. These signals (which can be in the form of light, heat, odors, contact, sounds, etc.) provide information to the living being, which will process them, interpret them and respond accordingly. Similarly, for the proper functioning of a multicellular organism, the billions of cells that compose it must communicate with each other to control their growth, division, differentiation, functioning and even ... to die. This communication between cells is done by informative molecules either through gap junctions or through receptors **(1)**.

Communication through gap junctions occurs between two adjacent cells. It is generally very fast and depends on the presence of connexons that form intercellular channels allowing information to pass directly from one cell to its neighbor (see Cell Biology course - S1). This type of communication is mainly found in the heart, brain and epithelial cells. It allows the transmission of electrical charges (in the form of ionic flows) between adjacent cells, as well as low molecular weight molecules such as metabolites and second messengers.

Communication via receptors is, by far, the most common form of information transfer between cells. This is the type of communication we will study.

In several modules of this LEF Licence (M25 in S4, M27 and M30 in S5 and M35 in S6), you were able to distinguish the three intercellular communication systems that are involved in the control of a very wide variety of biological processes in animals. They can be summarized as follows :

• The hormonal system which allows to control, to monitor the growth of certain tissues, to regulate the production of substances necessary for the organism.

• The nervous system which allows nerve messages to be transmitted by synaptic transmission.

• The immune system which allows us to recognize, warn of the presence of and destroy pathogenic “intruders”.

For all three communication systems, information is transmitted through a variety of substances that exert modulating effects on different cellular activities. These substances can be hormones, growth factors, neurotransmitters, cytokines, or even components of the extracellular matrix, etc **(2)**.

Despite this subdivision into different names and designations, all these molecules have one thing in common: they all carry a message or signal. Some authors group them under the general term "signal molecules" or "semimolecules" (from the Greek: semios = signal). They are also commonly referred to by other terms: first messengers, informative molecules, modulators, mediators or ligands. These semimolecules are secreted by a cell (emitting cell or signal cell) or located on its surface and will travel to target cells. The target cells receive the signal thanks to the presence of specific receptors at their level.

In most cases, the signal molecule appears to have no function other than binding to the receptor. This binding results in changes in the receptor's properties, which triggers a series of more or less complex intracellular reactions that translate the signal into a predetermined effect.

This transfer of information from the extracellular environment to the intracellular environment where the signal is translated (or converted) into a different form, is referred to as "transduction". It is a crucial process, especially in metazoans including humans, in which it is estimated that 20% of genes are dedicated to its realization **(3).**

It is important to note that, in addition to its role in the three communication systems mentioned above, intercellular signaling also occurs during development. An animal, for example, begins with a single cell (zygote) that divides repeatedly to produce several different types of cells. This is due to the exchange of signals between neighboring cells for coordination before resulting in a complex multicellular organism.

The study of cellular communication becomes even more important when we notice that the alteration of signaling pathways is linked to several diseases.

CHAPTER I. GENERAL PRINCIPLES OF CELLULAR COMMUNICATION

The general principles of cellular communication are essential for understanding how cells interact with their environment and with other cells. Here is an overview of the main concepts of cellular communication (**Figure 1**).

- Cell surface receptors : Cell surface receptors are proteins located on the surface of cells that recognize and bind to specific molecules, such as hormones, neurotransmitters, or other cells. They then transmit signals inside the cell to trigger a response.**(1).**
- Signal transduction : Signal transduction refers to the cascade of biochemical reactions within the cell that occurs in response to the activation of surface receptors. These reactions transform the extracellular signal into an intracellular signal that regulates various cellular processes.**(2)**
- Phosphorylation cascades : Phosphorylation cascades are series of enzymatic reactions in which phosphate groups are transferred from molecule to molecule, often by protein kinases, to amplify and transmit intracellular signals. **(3).**
- Cellular responses : Cellular responses can include changes in metabolism, growth, differentiation, cell division, or programmed cell death (apoptosis), depending on the type of signal and the cellular context.**(4).**
- Intercellular communication : In addition to intracellular communication, cells can also communicate with each other via direct connections, exchanges of signaling molecules, or signals released into the extracellular environment (paracrine, endocrine, autocrine).**(5).**
- Diseases related to cell communication : Dysfunctions in cell communication can contribute to the development of many diseases, including cancer, autoimmune diseases and neurological disorders **(6).**

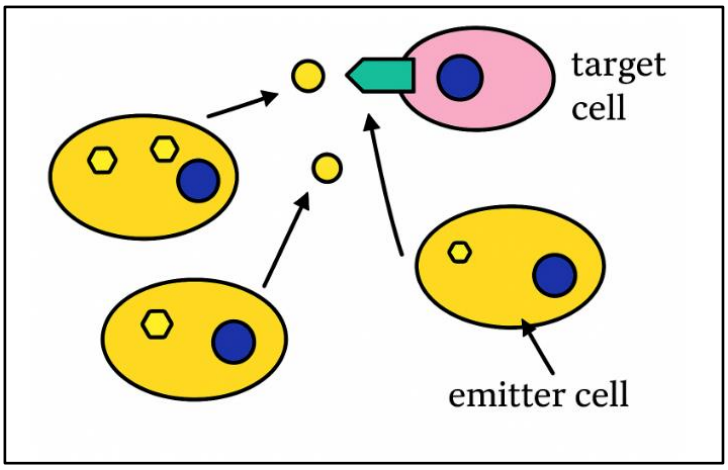


Figure 1: Cellular communication**(1)**

I.1 Different types of transmission

We can consider two types of interactions between signal-emitting cells and target cells (**figure 2**): interactions that occur via molecules released by the signal cells and those that occur through direct contact between two cells.

The different types of transmission in the context of biology and cellular communication include synaptic transmission, hormonal transmission, paracrine transmission, autocrine transmission, endocrine transmission, and contact transmission.

I.1.1. Synaptic transmission

Synaptic transmission occurs at synapses between neurons, where neurotransmitters are released into the synaptic space and act on receptors on postsynaptic cells (**figure 2d**) **(7).**

I.1.2. Paracrine transmission

In paracrine transmission, cells secrete signaling factors that act locally on neighboring cells in the same tissue or organ (**figure 2b**) **(8).**

I.1.3. Autocrine transmission

Autocrine transmission occurs when cells secrete signaling factors that act on the same cells that secreted them (**figure 2c**). The target cell is therefore itself the signal-emitting cell **(9).**

I.1.4. Endocrine transmission

Endocrine transmission involves the secretion of hormones into the bloodstream by endocrine glands distant from the target cell (**figure 2a**)**(10)**.

I.1.5. Contact transmission

Signaling involves direct contact between cells without the release of signal molecules. These remain bound to the surface of signal cells and act only on the cells with which they come into contact. Contact signaling is also called juxtacrine signaling (**figure 2e**).

I.1.6. Hormonal transmission

In hormonal transmission, hormones are secreted from endocrine glands into the bloodstream and act on target cells that express the appropriate hormone receptors**(11)**.

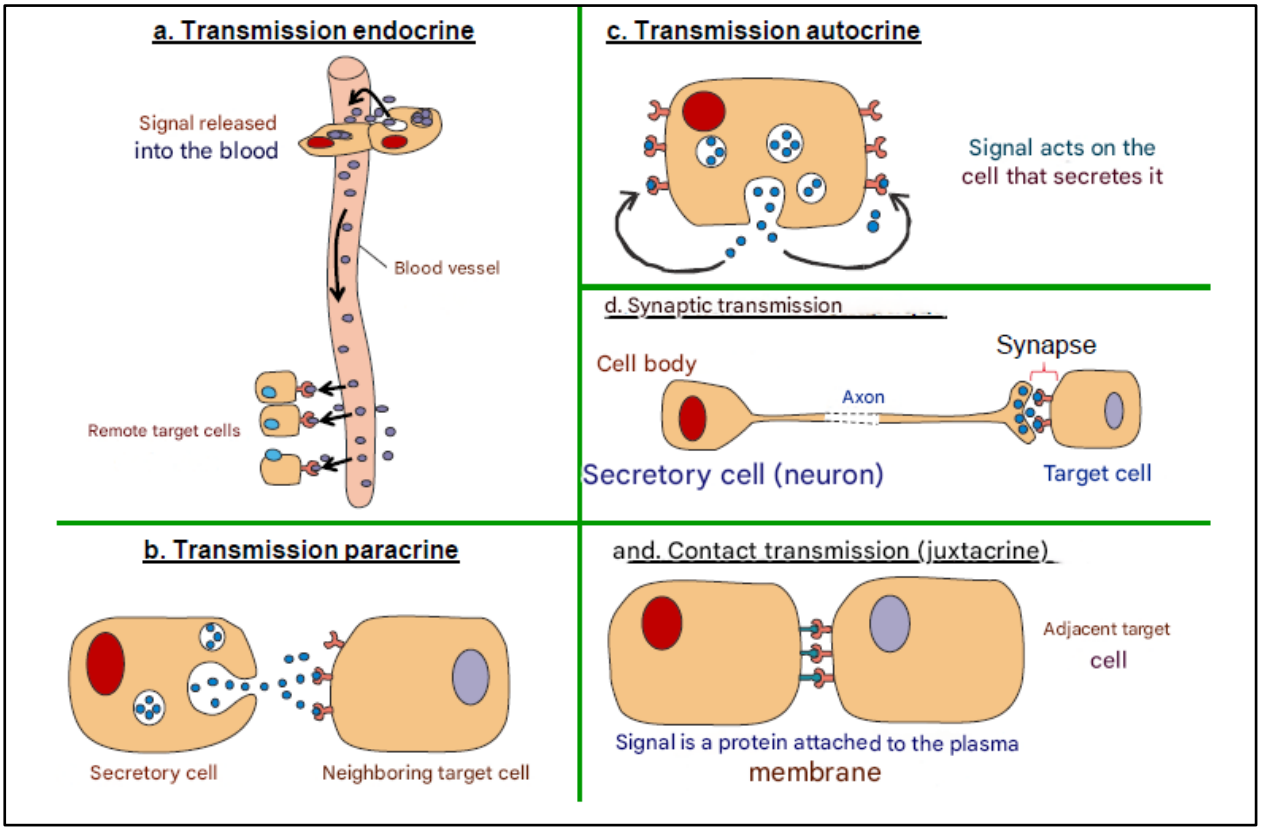


Figure 2: Different types of signal transmission between cells**(11)**

I.2.Multiplicity of responses

Response multiplicity in cellular communication refers to the diversity of responses a cell can exhibit in response to a specific signal, depending on various factors such as signal type, cell state, and environment. Here is an overview of this concept, along with references:

I.2.1. Cellular plasticity

Cells are able to respond in different ways to the same signal by adjusting their behavior and physiology, which reflects their plasticity and their ability to adapt to changing conditions.**(12)**.

1.2.2. Diversity of signaling pathways

Cells can activate different signaling pathways in response to the same stimulus, resulting in a variety of cellular responses. These pathways can interact in complex ways to regulate cellular responses.**(13)**.

I.2.3. Cellular and tissue context

Cellular responses can vary depending on the cell type, its physiological state, and its tissue environment. For example, a cancer cell may react differently to a signal than a normal cell. **(14).**

I.2.4. Temporal and spatial stimulation

The temporality and localization of stimulation can influence cellular responses. For example, prolonged exposure to a signal may result in different responses compared to short exposure**(15)**.

I.2.5. Epigenetic plasticity

Epigenetic modifications can contribute to the diversity of cellular responses by regulating gene expression without altering the DNA sequence, allowing cells to memorize and transmit information about their state (**16**).

I.3.Signal stop

Signaling shutdown mechanisms are essential for regulating cellular response to stimuli and maintaining homeostasis.

These mechanisms include receptor desensitization, regulation of the concentration of signaling molecules in the extracellular environment, and regulation of the expression and activity of proteins involved in signal transmission within the cell. **(1).**

I.4. Adaptation of the target cell

Target cell adaptation in cellular communication depends on the type of signaling involved and the receptors present on the target cell surface. Here is a general explanation of some adaptation mechanisms :

I.4.1. Desensitization of receptors

When cellular receptors are exposed to a ligand (signaling molecule) for prolonged periods, they can become less sensitive or insensitive to that ligand. This can occur through internalization of the receptors, where they are removed from the cell surface, or through post-translational modifications that impair their ability to transmit the signal.

I.4.2. Regulation of the signaling pathway

Target cells can regulate the activity of intracellular signaling pathways activated by receptors. For example, they can modulate the expression or activity of proteins involved in these pathways, in order to regulate the amplitude or duration of the cellular response.

I.4.3. Negative adaptation

Some adaptation mechanisms are designed to attenuate the cellular response to a given signal. For example, in the G protein-coupled receptor (GPCR) signaling pathway, G regulatory proteins can act as negative regulators by decreasing G protein activity, thereby attenuating the cellular response.

I.4.4. Positive adaptation

In some cases, adaptation can amplify the cellular response to a signal. For example, positive feedback mechanisms can enhance the activation of signaling pathways, thereby increasing the amplitude or duration of the cellular response.

I.4.5. Reserve receivers

Cells may have reserve receptors that can be translocated to the cell surface when needed, increasing the cell's sensitivity to a specific signal.

These adaptation mechanisms allow cells to finely regulate their responses to environmental signals, which is essential for maintaining homeostasis and responding appropriately to changes in their environment.**(1)**.

CHAPTER II. INTERCELLULAR RELATIONS

II.1 Extracellular matrix (ECM)

The extracellular matrix (ECM) is a complex network of non-cellular components found in biological tissues. It plays a crucial role in tissue structure, function, and regulation. The extracellular matrix is a three-dimensional network of proteins and polysaccharides that surrounds cells and provides structural support. It also regulates various aspects of cellular behavior, including proliferation, migration, and differentiation.**(17)**.

Here are some of its main components :

II.1.1. Polysaccharides

Polysaccharides are long, complex sugar molecules that are essential to the structure and function of the ECM. They include components such as hyaluronic acid, chondroitin sulfate, and dermatan sulfate.

II.1.2. Collagen superfamily

Collagen is the most abundant protein in the ECM. It forms strong, flexible fibers that provide strength and elasticity to tissues. There are several types of collagen, each with specific functions in different tissues.

II.1.3. Elastin

Elastin is a protein that gives tissues elasticity and resilience. It is often associated with collagen in the formation of structures such as skin, blood vessels, and lungs.

II.1.4. Fibronectin

Fibronectin is a glycoprotein that plays a crucial role in cell adhesion, cell migration, and cell growth regulation. It acts as a kind of glue that helps connect cells to the ECM and other cells.

II.1.5. Basement membranes

Basement membranes are thin, dense ECM structures that separate epithelial and connective tissues. They play an important role in regulating permeability, filtration, and support of epithelial cells.

II.1.6. Pericellular matrix

The pericellular matrix is the part of the ECM that directly surrounds cells. It provides a specialized microenvironment that regulates interactions between cells and their external environment, thus influencing their behavior, proliferation, and differentiation.

These components form a dynamic three-dimensional network that maintains the structural integrity of tissues, regulates cellular processes, and provides mechanical support to cells.**(17).**

II.2 Cell-cell or cell-ECM adhesion molecules

II.2.1 Cell interactions

A multicellular organism can only exist because each tissue has a very precise cellular organization. Therefore, during the formation of the individual and during the development of the organ, the cells or vessels must assemble : recognition systems are needed between cells of the same type, and also between cells of different types.

In the body, organs communicate with each other : specific communications.

For a cell there are 2 types of interactions : depending on the cell type one will be predominant :

- Cell-cell contact of msame type or 2 different types : 2 transmembrane proteins interact with each other

- Cell-ECM contact (everything in the body that is not cellular). A protein in the cell interacts with a protein in the ECM.

To create this organism, a multicellular assembly is required that requires these interactions.

Interaction with the ECM is predominantly observed in connective tissues. This interaction aims to ensure mechanical tension in these tissues.

Cell-cell interactions are predominant in epithelial tissues. Very little ECM. For example, in the skin: we have the BL underneath, and these interactions will allow for a solid tissue, and the mechanical tension is provided by these cell-cell interactions. Sometimes very strong cell junctions are required (**17**).

Despite everything, a hundred cell-cell adhesions are enough to ensure the organization of a tissue. And at certain points in the cell, and at certain locations in the organ: we have specialized junctions, which accept that a tissue is stretched or contracted, for example. They are essential to ensure tissue function.

Several types of interactions are observed at the level of these cells:

- Cell-cell or cell-ECM interactions that constitute the organ and that allow the transmission of signals: hence the biological role of these interactions. These interactions are fixed

- Low-affinity, labile interactions are capable of forming and unforming themselves, but there must be a lot of them: this is called the Velcro principle. These interactions allow for a modification of the cytoskeleton. The morphology of a cell is only acquired if it interacts with something else because these extracellular interactions induce intracellular interactions at the cytoskeleton level.

At the embryogenesis level, we will first have labile non-junctional contacts, allowing interactions, with an influx of signals arriving, and when they have acquired new properties, they will be directed towards other places in the organism. Then they develop fixed interactions at this level.

For example, when there is an infection, lymphocytes are directed to the site of the infection to destroy the infectious agent.

Interactions can be classified:

- Cell junctions:

- Non-cellular junctions

To find out if these interactions are essential for the survival of the organism: we take chicken retinal cells, we purify them quite efficiently, we isolate these chicken cells and we disperse them. We leave them together in the petri dish and we see that the cells recognize each other : and there is reassociation **(17)**.

But if we put antibodies against certain proteins involved in the interactions : we do not see any interactions.

We see the proteins essential for the interaction.

These interactions can take place between 2 cells:

- If they occur between two identical cells, they are homeotypic.
- But if the protein involved is carried by two different cells, we will say that it is a heterotypic interaction.

For molecules:

- If the interaction between the two cells is made by different molecules: these are heterophilic interactions
- But if identical: homophilic

If we have 2 identical molecules carried by 2 identical cells: homophilic homotypic interaction**(17)**.

II.2.2 Cell-cell adhesions: cell junctions

A cell junction involves proteins that are carried by one cell and another cell or presented by the ECM. This junction consists of two proteins, either the same or different types. Each protein is composed of a cytoskeletal component, linker molecules, a transmembrane protein, then a binding protein that helps attach the transmembrane protein to the cytoskeleton, and then the ligand on the other side.

If there is no interaction, the cytoskeleton is not organized so the cell has no particular shape.

2 types of junctions: functional classification

- Tight junctions: found in cells that are close together, the most typical being epithelial cells. These tight junctions prevent molecules from passing through ; the passage is controlled by transporters.
- Anchoring (or adhesive) junctions: role of anchoring the cell to another cell or to the matrix to fix them.

These anchor junction interactions will lead to an interaction with the cytoskeleton either:

- Via actin filaments:
  - Cell-cell: adherens junctions
  - Cell-MEC: focal contacts or adhesion plaques
- Either via the intermediate filaments:
  - Cell-cell: desmosomes
  - ECM cell: hemidesmosomes
  - Gap junction / communicating: role of passage of chemical substances or electrical information between cells.

1) **Tight junction = tight junction = zonula occludens**

The proteins involved are: claudin, occludin, JAM.

A belt is formed around the cell or around the edges of an organ. Everything that is connected to the outside will be protected. We want to weld/cement the cells together, we reattach them. Prevents the diffusion of solutes between the membranes. They are found on everything that is connected to the outside: epithelial cells, glands, etc., also in endothelial cells of the brain capillaries: blood-brain barrier, and at the level of the placenta: placental barrier.

The purpose of these tight junctions: epithelial and endothelial sealing (brain and placenta only). To prevent diffusion of small molecules such as ions or glucose: transport is done via transporters. This functional polarity (apical/basolateral domain) is maintained.

How is a tight junction made? We have a membrane protein of homophilic and homotypic type. Then we interact with the cytoskeleton.

The essential molecules of these junctions are occludin, claudins, and JAMs (Junctional Adhesion Molecules). They are either single or multiple transmembrane domains. They interact with the intermediary proteins: Z01 and Z02. These intermediary proteins interact with actin filaments via spectrin **(17).**

We have such tight junctions because these occludins etc. are placed next to each other, at a specific point, locally, we have a set of proteins which interact.

So no passage is possible: it acts as a barrier. Especially for glucose: it absolutely must pass through the transporter using signals from the transporters. In SEM, in freeze-fracture, we see the accumulation of these claudin and occludin interactions.

2) Anchor junctions

These anchor junctions allow the cell to be attached to something. A cell is connected to the cytoskeleton of another cell or to the ECM. The goal is to enable communication between the two cells: information is generated through this interaction: the two cytoskeletons will be organized in the same way. Role in mechanical tension: muscles, the epidermis, etc.

Anchoring proteins: link these connections to the cytoskeleton

Adhesion proteins: Transmembrane proteins that interact with the anchor protein and a protein from another cell or ECM(**Table I**).

Table I: Functional types **(17)**

Interaction	Cell - cell Helps maintain a compact organ		Cell - ECM	
Involves	Cadherins		Integrins	
Type of interaction	Adherens junctions	Desmosomes	Adhesion plaques (focal contacts)	Hemidesmosome
Interaction with the cytoskeleton	Actin filaments	Intermediate filaments	Actin filaments	Intermediate filaments

a. Cell-cell adhesion: adhesion junction and desmosomes

Cadherins are transmembrane proteins: there are 2 types

- Classics: depending on their location: N, E, P.
- Non-classical

They are organized into modules: 5 extracellular modules and 1 intracellular module that can interact with different partners. These junctions require calcium, so calcium ions are required. They structure these domains so that there is a possible interaction: homotypic and homophylic type.

When interacting with cadherins, the intracellular part is different:

- We interact either with actin filaments: in adherens junctions

-Either with intermediate filaments: in desmosomes

The adaptor proteins are distinct.

The zonula adherens: Involves E-cadherin, which interacts with actin because we have a whole intermediate binding complex: α-catenin, β-catenin, and p120-catenin. Interactions at the ends require the presence of this calcium. So the calcium concentration determines the strength of the interaction: the more there is, the stronger the interaction. During development, we have different calcium concentrations that allow for possible cell detachment so that the cell can migrate.

The purpose of these interactions is to connect one cell to another and orient the cytoskeleton in the same way in both cells. The actin filaments are oriented in the same way. So we have a solid structure (actin provides a certain rigidity).

Since interactions occur on molecules next to each other, the interaction is only at one end: the zipper effect. It is strong because the entire cytoskeleton is oriented and there are several attachment points, but if the calcium concentration is reduced, the cells can be detached.

Only cells that have the same protein will be able to associate.

In embryological development, we will have a compaction of the morula, thanks to these interactions. At the level of the formation of the neural tube, we induce specific information between certain types of cells. For the formation of the neural tube, certain cells must therefore migrate: at the beginning, the neural plate expresses E-cadherin, and then, the plate expresses another N-cadherin: interaction between cells that express the same type of cadherin: they group together. Allows the formation of the neural tube. This involves variations in calcium concentration to break the first interactions and replace them with interactions with the new cadherin. Some cells that will migrate at a given moment **(17).**

Cadherins are involved in cancers : absence of cadherin allows the (differentiated) cell to migrate: non-retention of the cell in its original organ type.

Cadherins play a role in maintaining the cell in its organ and in differentiations because it is labile.

Desmosomes (fibrous plaque)

Involve non-classical cadherins. They interact with other types of intermediary proteins in the cell cytoplasm.

Here they are desmogleins 1 and 3, and desmocollins. And they interact with the intermediate filaments.

Proteins involved in cadherin-intermediate filament protein interaction: desmoplakin and plakoglobin.

Intermediate filaments vary depending on the desmosome

Often it is keratin, and in certain tissues, we have specific filaments: e.g., myocardial tissue, we have desmin.

Strong interaction (the desmosome is compared to a rivet): it is a specific point of the cell that interacts with the other, in a very strong way, these interactions are not easily eliminated. Found in tissues subjected to fairly strong mechanical stress. Forms a tensile strength

Resistance to mechanical stress is only valid if there are very strong anchor points, and if they extend over a large number of cells, it is even more resistant. The pressure force is distributed across the entire tissue. This is called an adhesion belt.

Intercellular relationships play a crucial role in the functioning of multicellular tissues and organs. Some common types of intercellular relationships:

Pemphigus disease : cells no longer adhere to the basal lamina. Loss of cohesion between keratinocytes.

Blisters that appear on the skin. This can be serious if antibodies are produced against these adhesion proteins.

We have many cancers that are due to disturbances in the expression of these cadherins (we find mutations at the level of the cadherin gene sequences). This is not surprising since they play a role in development, in cell migration: the cancer cell loses its signals, there, it loses the signal that it is part of an organ, we find it in many tumors with mutations at the level of the cadherin gene sequences **(17)**.

b. Cell-ECM adhesion: focal contacts and hemidesmosomes

Table II: Focal contacts and Hemidesmosomes **(17)**

Focal Contacts	Hemidesmosomes
We can see focal contacts on our Petri dishes. The cells emit pseudopodia that interact with the surface. These adhesion plaques are anchoring junctions that involve integrins that attach cells to the ECM via actin and adaptors: vinculin, talin, and actinin. Each transmembrane protein corresponds to intermediate proteins.	Connect a cell to the ECM via integrins and another adaptor, plectin, which interacts with the intermediate filaments, keratins. A hemidesmosome resembles a desmosome, but it interacts with the ECM. However, the adaptor proteins are not the same. They are integrins instead of cadherins These are dynamic structures that can break and reassemble, allowing the cell to move or divide. A cell that must divide is forced to detach itself from its support (it still remains attached in certain places).

Hemidesmosomes are more extensive but with lower protein density (**Table II**).

NB: the same cell has several types of junctions.

3) Connecting junctions: Gap junction

They have a specific function. They don't play an adhesion role, but rather a cellular communication role. They are channels found between two identical cells, which group together in a specific location.

We have an astronomical quantity of them, we see all the channels next to each other

Thanks to these two channels, the cytoplasms of the two cells communicate and small molecules pass through these channels: ions and small chemical agents (<1.5kDa).

They are made up of small molecules called connexins: which allow the formation of connexons (association of 6 connexins): 2 connexons from 2 cells form a channel.

There is a coupling between the cell's metabolism and information of the electrical signal type.

They are found in all cells, except for a few cells: skeletal muscle, red blood cells, and certain neurons.

In EM, we see them very well. We have several gap junctions next to each other to allow molecules to pass through in large quantities.

Connexins are proteins with a complex structure: several intracellular and extracellular domains and transmembrane domains. If there is a deficiency, genetic mutation in these sequences that encode connexins, we have various diseases.

Since we have very large families of connexins, we have very different connexons: connexons are hetero-oligomers: different, specific permeability. The probability of passage depends on the affinity of the molecule with the connexon: different passage speed. Very important functional consequences.

Functions of these gap junctions: consequences of the passage of molecules are different: the second messenger is synthesized in the cell. These variations, these increased productions of metabolites induce passages through neighboring cells: cAMP produced passes into neighboring cells. This is what we find in the contraction of the intestine or heart cells, or movement of cilia, or astrocytes: the cells must contract at the same time: functional connection between the cells. The metabolite information spreads only if the cells are linked by gap junctions: coordinated function **(17)**.

These gap junctions are more or less closed depending on the concentrations of H+ ions or calcium ions.

Essential function for the life of the organs in which they are found.

II.2.3 Intercellular adhesion

This is the prerequisite step for adhesion. These cells must arrive there, and so for them to follow a good path, they must adhere to the anchoring site. Intercellular adhesion phenomena between cells of different types are required for there to be attachment.

It requires cell adhesions, specifically: selection.

These routes, guidance paths to follow, are the ECM. These mechanisms are carried out by chemotaxis or chemorepulsion. These intercellular adhesions are essential for determining the… of organisms. It involves a certain number of proteins.

II.2.4 Ca2+-dependent adhesion

II.2.4.1 Cadherins

Involved in fixed cell adhesion and cell adhesion.

Calcium-dependent glycoproteins.

Associate in the membrane

Non-junctional and junctional cell adhesion.

Often dimers but we can have a higher number of proteins.

Intermediates for interaction with actin: catenins. Always have a transmembrane domain and an extracellular domain.

This structuring depends on calcium: the more calcium you add, the more rigid the structure is: this rigid structure is needed so that it is not too loose, and therefore so that it is recognizable. This dynamic due to calcium concentrations allows for non-functional junctions.

These interactions are obligatory and specific to allow the junction between the exterior and the cytoskeleton of the cell (actin filaments).

These cadherins are involved in many cellular mechanisms important for cell life

Some interactions will be specific to certain types of cadherin. In particular, N-cadherin interacts with FGFR-type receptors. This interaction is very important because it increases cell mobility and protects against apoptosis. These signals pass through other intermediaries.

Cadherin can also associate with the PDGF receptor: increased mobility at the level of the lamellipodia: allowing them to move forward. We only have a part of the cell that will attach to the support of the lamellipodia.

E-cadherin intervenes by competing with p120 catenin: no extracellular intermediary.

II.2.4.2 Selectins

It depends on calcium, so it's dynamic. The structures are different, so are the interactions.

Heterophile transmembrane proteins: they never interact with another selectin but with another partner.

Roles: The most important is the movement of leukocytes in the blood and tissues. This process involves transient molecular adhesions.

Selectin and its partner on another cell make transient interactions. These interactions also occur between two different cells: interaction between leukocyte and endothelial cell: heterophilic and heterotypic binding **(17)**.

Selectins recognize sugars on certain proteins (glycoproteins)

There are several types of selectin: 3 major types

- On leukocytes

- On the activated endothelial cell (it knows that it must allow an interaction with leukocyte

- Platelet, non-activated endothelial cell, or inflammatory reaction.

They are transmembrane proteins, and have a highly conserved domain that binds to a specific sugar (oligosaccharide) present either on an ECM protein or on a protein from another cell.

All these proteins are related to the cytoskeleton: here it also interacts with actin.

The extracellular domain is characterized by the lectin domain, specific to a given sugar, carried by another protein. It sorts and recognizes a particular sugar. In addition, there is an EGF domain, and domains that allow the binding of complement proteins: a link with immune reactions.

It is the selectin-sugar interaction that is calcium dependent.

The particularity of selectins is that they have a redundancy of information. They are carried by both the endothelial cell and the leukocyte:

- On the endothelial cell: E and P selectin

- Leuco: L-selectin

They recognize glycoproteins on leukocytes and endothelial cells: they are all different. There is cross-recognition between the two cell types.

These recognitions allow transient and therefore non-junctional interactions: we do not have long-term interactions.

The goal is that when a tissue is damaged, the vx of the tissue must receive this information to stop the rolling of leukocytes in the blood and tell them to enter the tissue at that level.

Leukocytes interact with the vessel wall and tissue.

When there is an infection in a given area, a macrophage detects the infection and therefore gives an initial signal : it releases two histamines and thrombin. Then the endothelial cells receive this information, become activated, and respond to this information by expressing a specific lectin on their surface.

Then this lectin is recognized by the selectin present on the leukocytes: L-selectin: it is always present on the leukocytes (constitutive expression). This interaction leads to a slowing down of the leukocytes before they stop. The leukocyte stops at this point: there is an adhesion between this leukocyte and the endothelial cell.

In the endothelial cell, we also have expression of platelet activation factors (PAF). They are placed on the surface, complementary to the expression of the lectin. Endothelial cell Expression of lectin + a P-selectin. So we increase the control.

Before the activation of the endothelial cell, this P-selectin is present but intracellular, so after activation there is presentation of this P-selectin on the surface of the endothelial cell: exocytosis.

The leukocyte itself contains lectins constitutively (always present on the surface glycoprotein). These leukocyte lectins recognize P-selectins on the endothelial cell. They also recognize the PAFPAF receptor, which is present on the leukocyte.

The 2 recognitions endothelial lectin / leuco selectin + leuco lectin / endothelial selectin: slowing down of leuco on the vessel walls.

These selectins are search sites for excessive inflammatory reactions. Molecules are being sought to inhibit this recognition **(17)**.

II.2.5 Ca2+-independent adhesion: IgCAM

They are immunoglobulin type (structure). They are calcium-independent transmembrane glycoproteins. They play a number of roles in the cell, including:

- Intercellular adhesion between cells of the same type: only for neurons: N CAM

- Intercellular adhesion between cells of different types: I CAM of endothelial cells (blood cells)

They can interact between mole and cell of the same type or heterophilic heterotypic.

They have specific functional domains in addition to the Ig like domains.

There is the last protein 120, which is fixed in the membrane but does not enter, there is no intracellular domain. These CAMs can interact between molecules of the same type and cells of the same type or heterotypic/heterophilic.

They are involved in different types of interactions.

In terms of quantity, those in neurons are the most abundant, followed by those on blood cells.

Interactions occur with integrins; Role in the inflammatory reaction.

Interactions with integrins

On the leukocyte we have alpha beta 2 type integrins. And also Ig type ICAMs present before there is interaction

These leukocytes interact with endothelial molecules, the integrin interacts with a partner of the endothelial cell: but for this interaction to take place, the integrin must not be located on the membrane, the structure of the integrin must be modified so that it acquires an activated conformation. This change in conformation is due to the signal given by the PAF. The integrin can thus recognize the ICAM present before activation.

These are strong interactions between ICAM integrins. (Whereas for selectin/lectin: weak interaction)

This interaction with ICAM is long-lasting and strong, allowing the passage of leukocytes through the blood vessel.

At the level of passage in the tissue, we have very strong interactions which call upon JAMs (proteins involved in tight junctions).

Conformational changes: Intracellular partners are required. Depending on the conformation, the integrin is not read in the same way. On the other hand, isomerization phenomena are involved in recognition.

Role of CAMs in the development of the central nervous system:

These CAMs interact in a homotypic manner: two molecules of the same type interact with each other. We have what is called the adhesive form. We have a network that is established: therefore the neurons are linked to each other: passage of a signal through these interactions.

If we are in the presence of polysialic acid (present in the ECM): interacts with one end of the NCAM: therefore not available to bind to another NCAM therefore we can have attachment – detachment.

Important role in embryogenesis: to enable aggregation, disaggregation, and reaggregation (e.g. cell migration)

(Membership always takes place before joining (with the JAMs))

II.2.5.1 Integrins: transmembrane ECM adhesion receptors

Integrins are transmembrane proteins that are called receptors because they bind to protein ligands found in the ECM. They play a role in cell attachment to dispersed ECM or the basal lamina.

Very important role because it is a receiver of signals from the environment: sensors of what is happening with the outside because they interact with molecules from the outside (MEC): signals of death, differentiation, etc.

As they are transmembrane and adhesion molecules: they interact with the cytoskeleton.

Present in almost all cell types. Very abundant and not strong interactions: therefore strong response due to multiplicity.

Bond dependent on either calcium or magnesium

Alpha-beta heterodimer. 2 different chains: alpha and beta. (18 possible alpha and 9 beta) so many possible combinations. Does not exist in monomeric form, only dimeric.

Different types of association between alpha beta, and each su is in a given tissue type.

This heterodimer can interact with its partner via the upper part: extracellular terminal part.

This recognition comes through various reasons including GRD.

Binding with cytoskeletal molecules (acin): via talin and alpha actinin.

The preferred ligand is fibronectin. It contains a number of domains. It is found in the ECM, like collagen. It can bind to integrin and collagen.

At the internal level, it is only the beta chain that interacts with the cytoskeleton (unlike the extracellular part, it is alpha and beta) **(17)**.

Different integrins of an A cell capable of interacting with the same ECM molecule. Ex: collagen 1 can interact with alpha1/beta1, alpha2/beta 1, and alpha3/beta1. Resulting in different physiological responses.

The same integrin can bind different ligands: several biological effects: e.g. alpja2/beta1 interacts with laminin, collagen 4 and collagen 1.

And there are integrins that bind to a single particular molecule: e.g. alpha5/beta1 with fibronectin.

Each particular alpha-beta association is present on a particular cell type and has a particular role. The molecules involved are of different types

Domaine EILDV can also be involved in these recognitions.

Ligands are ECM molecules: major interactions that involve very broad cellular responses. And a possible ligand is fibrinogen: reaction at the level of the coagulation phenomenon. And possible interaction with ICAMs: role in adhesion of leukocytes during their migration to the inflammatory site, and also at the IV of development in gastrulation (mesodermal migration)**(18)**.

These integrins are found at the level of hemi-desmosomes and focal contacts

Regulation of integrin binding.

Interaction between integrin and ECM can occur, but this is only observed if a signal is received at the cell level. Ligand binds to a receptor that induces a structural modification of the integrin, which can then interact with a specific ligand of the ECM.

Integrins connect cells to the ECM or to Ig family receptors.

We can have an extracellular signal. Either the signal is:

- Extracellular: the integrin is modified so that it acts in a specific way

- Intracellular: same

We have an integrin-ligand interaction, then oligomerization phenomenon (they will localize to a particular place in the cell) + recruitment of other adaptor proteins and cytosolic kinases. So a new function of the cell: we activate different signaling pathways: migration, adhesion, proliferation, differentiation, shape and polarity **(18)**.

These processes are found in the adult cell (immune response, healing, coagulation, etc.) as well as in embryogenesis.

This out-in signal:

- Reorganization of the cytoskeleton: modification of cell mobility and shape

- Cell proliferation or differentiation

- Formation of focal contacts

Phosphorylation mechanism as an activator: (very important in biological systems)

ECM/integrin interaction phosphorylation of integrin intracellular domains interaction with cytoskeleton

Out-in signal: Change in expression or mobility: signal comes from inside the cell.

If we no longer interact with the ECM, we no longer interact with the cytoskeleton: this can happen in the case of cell division: when the cell has to divide, the cytoskeleton must not interact with integrins. **(1).**

Signal redundancy: Between selectins and lectins of leukocytes and endothelial cells: several signals are essential for the signal. Redundancy to avoid errors and also to ensure that the organism survives even if there is a small defect in a given location.

Roles of alpha2beta3 integrins in coagulation

Integrin activation conformational change of the cytoplasmic domain due to inside-out signal: increase in their affinity for fibrinogen. Aggregation can be blocked by RGD peptides

Diseases related to integrin regulation problems:

- Overexpressed alpha5/beta3 integrins: unregulated proliferation

- Metastatic process: loss of expression of certain integrins in tumor cells

- Cell death: if integrins are absent or not bound to molecules: the anti-apoptotic pathway is not activated, therefore the cell dies.

Genetic mutations at the level of α2b/β3: pathology: bleeding disorders.

The ten integrins:

There are proteins that degrade integrins or alter integrin-ligand interaction.

Ex: in snake venom: blocks coagulation. Contains the RGD sequence and interferes with adhesion

Integrin-MEC: allows cell de-adhesion and migration

In our country, ADAM proteins contain disintegrin and metalloprotease motifs: they modify interactions.

Intervene during spz and ovule fusion and myoblast fusion during myogenesis. **(19).**

II.3 Cell junctions

Tight junctions, anchoring junctions (desmosomes, hemidesmosomes, and adherens junctions), and gap junctions are specialized structures that connect adjacent cells and facilitate communication between them. **(1)**.

II.3.1 Tight junctions

Tight junctions, also known as airtight junctions, are specialized cellular structures found in the epithelial tissues of multicellular organisms. They are an essential part of epithelial barriers, selectively regulating the passage of molecules and ions across intercellular spaces.

These junctions are formed by transmembrane proteins present on adjacent cell membranes, which combine to form tight bonds. The main proteins involved in the formation of tight junctions include claudins, occludins, and junctins.

Tight junctions play crucial roles in several biological processes, including maintaining the integrity of the epithelial barrier by preventing the diffusion of substances between cells, polarizing epithelial cells by defining distinct apical and basolateral domains, and regulating ion transport and selective permeability.

Due to their functional importance, tight junctions are implicated in various pathologies, including inflammatory bowel diseases, neurological disorders, and cardiovascular diseases. Research on tight junctions is therefore crucial for understanding the mechanisms underlying these diseases and for developing new therapeutic strategies **(1)**..

II.3.2 Anchor junctions

Anchor Junctions in Cell Biology: In the field of cell biology, anchor junctions refer to specialized structures present between adjacent cells or between cells and the extracellular matrix, which help maintain tissue cohesion and transmit mechanical forces. Anchor junctions include structures such as cell junctions, desmosomes, hemidesmosomes, etc. **(18).**

II.3.3 Adherent junctions

Adherens junctions are specialized cellular structures found in epithelial tissues and some connective tissues that play a critical role in maintaining tissue integrity and transmitting mechanical forces between cells.

Here are some key points about adherent junctions:

Main function : Adherens junctions are responsible for cell adhesion, i.e., the connection between adjacent cells and between cells and the extracellular matrix. They also contribute to the formation of tissue barriers and participate in the transmission of mechanical and biochemical signals between cells.

Structure : Adherens junctions consist primarily of transmembrane proteins called cadherins, which associate with each other to form binding complexes between cells. Inside the cell, cadherins are bound to cytoplasmic proteins such as β-catenin, α-catenin, and vinculin, which anchor adherens junctions to the actin cytoskeleton.

Types of adherent junctions: There are several types of adherens junctions, including classical adherens junctions (zonula adhaerens), desmosomes, and focal junctions. Each of these types of adherens junctions has specific functions and structures adapted to the needs of different tissues and physiological situations.

Regulation : Adherent junctions are dynamically regulated in response to various cellular signals, such as mechanical, chemical, and electrical signals. The regulation of adherens junctions is essential for morphogenesis, cell migration, wound healing, and other physiological processes.

Involvement in diseases : Dysfunctions of adherens junctions can contribute to the development of various diseases, including autoimmune diseases, embryonic developmental disorders, cardiovascular diseases, and cancers. For example, mutations in genes encoding cadherins or associated proteins can lead to developmental disorders or cancer metastasis. **(13).**

In summary, adherens junctions are crucial cellular structures for maintaining tissue integrity, regulating cellular interactions, and responding to mechanical forces in various physiological and pathological settings.

CHAPTER III SIGNALING MOLECULES AND THEIR RECEPTORS

Signaling molecules are chemicals produced by cells to communicate with other cells in the body. They play a crucial role in regulating various biological processes, such as growth, development, cell differentiation, metabolism, immune response, and neuronal communication. Signaling molecules can act at short distances (paracrine or neurotransmitters) or long distances (hormones), and they interact with specific receptors on the cell surface or inside the cell. **(4)**.

Receptors are proteins located on the cell surface or inside the cell that recognize and bind to signaling molecules. When a signaling molecule binds to its receptor, it triggers a series of biochemical reactions inside the cell, leading to a specific cellular response. Receptors can be of different types, such as G protein-coupled receptors, enzymatic receptors, nuclear receptors, and others. **(1).**

In summary, signaling molecules and their receptors form a complex communication system that allows cells to regulate their activity and coordinate biological functions throughout the body.

III.1. Different types of signals

III.1.1. Nitric oxide and carbon monoxide

Nitric oxide (NO) and carbon monoxide (CO) are two endogenous signaling gases, meaning they are naturally produced by the body and play a crucial role in regulating various biological processes.

III.1.2. Nitric oxide (NO)

Nitric oxide is an important signaling molecule in many biological processes, including the regulation of vascular tone, neurotransmission, and the immune system.

Nitric oxide is a small gaseous molecule produced by various cell types, including vascular endothelial cells, neurons, and immune cells.

It acts as a cellular messenger in many physiological processes, including blood pressure regulation, vasodilation, neurotransmission, and modulation of the immune system **(20)**.

Nitric oxide is synthesized from arginine by the enzyme nitric oxide synthase (NOS), and it exerts its effects by activating soluble guanylate cyclase (sGC), leading to increased intracellular levels of cyclic GMP (cGMP).

Dysfunctions in the nitric oxide signaling pathway are associated with various pathologies, including cardiovascular, neurological, and inflammatory diseases.**(20)**.

III.1.3. Carbon monoxide (CO)

Although often associated with toxicity when inhaled in large amounts, carbon monoxide is also an important mediator in the body, playing a role in cell signaling and the regulation of physiological function.**(21)**

Carbon monoxide is an odorless, colorless gas produced by the breakdown of hemoglobin and by certain enzymes, such as heme oxygenase, in various tissues of the body**(22)**.

Although it is often associated with toxicity when inhaled in large amounts, at physiological concentrations it plays an important role in cell signaling.

Carbon monoxide can act as a messenger in various signaling pathways, including regulation of vasodilation, modulation of the immune system, and protection against oxidative stress. **(23).**

Its effects are mediated in part by activation of soluble guanylate cyclase (sGC), leading to increased levels of cyclic GMP (cGMP), similar to nitric oxide **(24).**

III.1.4. Amines

Amines are a class of organic compounds that can act as signaling molecules in the body, playing a role in neurotransmission, hormonal regulation, and other biological processes. **(25).**

These contain an amine functional group (NH2). They can be classified into several categories, including biogenic amines such as dopamine, serotonin, and adrenaline, which act as neurotransmitters, as well as aromatic amines such as histamine and adrenaline.**(26)**.

III.1.5. Thyroid hormones

Thyroid hormones, such as thyroxine (T4) and triiodothyronine (T3), are chemicals produced by the thyroid gland that play a crucial role in controlling metabolism, growth, and development. **(27).**

Thyroid hormones are secreted by the thyroid gland and act on many tissues throughout the body, regulating metabolism, growth, and development. **(28).**

III.1.6. Lipid derivatives

Lipid derivatives are lipid-derived molecules that often act as signaling mediators in various biological processes **(29).**

Lipid derivatives include a variety of molecules, including eicosanoids (such as prostaglandins, leukotrienes, and thromboxanes), sphingolipids (such as sphingosine-1-phosphate), lysophospholipids (such as lysophosphatidate), and other lipid-derived compounds**(30)**.

III.1.7. Steroids and retinoids

Steroids and retinoids are organic compounds involved in the regulation of many biological processes, including cell growth, development, and differentiation. **(31).**

Steroids, such as cortisol and sex hormones (estrogen, progesterone, testosterone), as well as retinoids, such as retinoic acid (vitamin A), are fat-soluble compounds that exert biological effects by binding to specific nuclear receptors in the cell nucleus**(32)**.

III.1.8. Amino acid neurotransmitters

Amino acid-based neurotransmitters play an essential role in the transmission of nerve signals in the central and peripheral nervous system **(33).**

Amino acid-based neurotransmitters include glutamate, GABA (gamma-aminobutyric acid), glycine, and other amino acids that act as mediators of synaptic transmission in the brain and nervous system **(34).**

III.1.9. Peptide mediators

Peptide mediators are signaling molecules composed of chains of amino acids called peptides. They play an important role in signal transmission in the nervous system and many other biological processes. **(35)**.

Peptide mediators are signaling molecules composed of short chains of amino acids, called peptides. They act as neurotransmitters, hormones, or growth factors, and regulate a variety of biological processes, including nervous system regulation, pain modulation, appetite and behavior regulation, and immune response.**(36)**.

III.1.10. Protein mediators

Protein mediators are proteins that act as chemical messengers in biological processes, regulating various cellular and physiological functions **(37)**.

Protein mediators are proteins that act as signals in cells and tissues, regulating a variety of biological processes, including cell growth, differentiation, programmed cell death (apoptosis), immune response, and cell signaling. **(38).**

III.2. Receivers

Receptors are specialized proteins or molecular structures found on or within cells. Their function is to specifically recognize and bind to signaling molecules, such as hormones, neurotransmitters, or other chemical messengers, to trigger a specific cellular response.

When a signaling molecule binds to its receptor, it triggers a series of biochemical events inside the cell, which can include changes in cellular metabolism, activation of intracellular signaling pathways, regulation of gene expression, and other cellular responses. **(39).**

Receptors can be of different types, including G protein-coupled receptors (GPCRs), enzymatic receptors (such as receptor tyrosine kinases), ion channel receptors, and nuclear receptors. Each type of receptor has specific structural characteristics and signaling mechanisms that determine its function and role in regulating biological processes.

Receptors are membrane or intracellular proteins that detect and transmit molecular signals from the extracellular or intracellular environment to the interior of the cell, thereby triggering specific responses**(4)**.

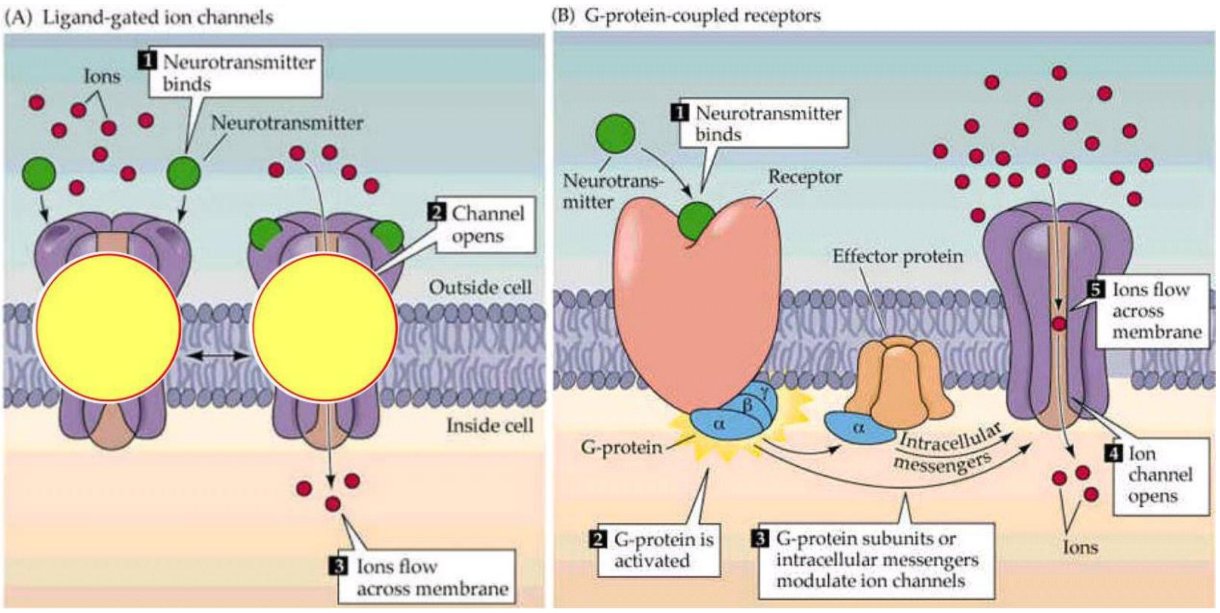
They play an essential role in the regulation of various biological processes, such as growth, differentiation, immune response, sensory perception and neuronal information transmission.

In summary, receptors are key components of the cell signaling system, allowing cells to detect and respond to signals from their environment in a precise and coordinated manner.

III.2.1. Ion channel receptors

Ion channel receptors are membrane proteins that function both as receptors for signaling molecules and as ion channels. They are involved in the transmission of electrical and chemical signals across cell membranes. When activated by the binding of a specific signaling molecule, these receptors undergo a conformational change that causes the ion channel to open, allowing the selective passage of ions across the cell membrane. **(40).**

These ion channels can be selective for certain ions, such as sodium (Na+), potassium (K+), calcium (Ca2+), or chloride (Cl-), and their opening leads to changes in the cell's membrane potential, which can trigger specific cellular responses, such as muscle contraction, neurotransmission, or the generation of action potentials in excitable cells such as neurons. **(41)**(**figure 3**).



Ionotropic **Metabotropic**

Figure 3: Ion channel receptors**(41)**

III.2.2. Enzyme receptors

Enzyme receptors, also called enzyme-active receptors, are membrane proteins that have a dual function: they act both as receptors for specific signaling molecules and as enzymes that catalyze specific chemical reactions. When a signaling molecule binds to these receptors, it triggers a cascade of biochemical reactions within the cell, catalyzed by the receptor's enzymatic activity. These reactions may include the phosphorylation or dephosphorylation of proteins, regulation of the enzymatic activity of other proteins, or other molecular modifications that result in a specific cellular response. **(42)**.

There are several classes of enzyme receptors, including tyrosine kinase receptors, serine/threonine kinase receptors, and guanylate cyclase receptors, among others. Each class of enzyme receptor catalyzes specific biochemical reactions in response to the binding of its corresponding signaling molecule.**(43)(figure 4).**

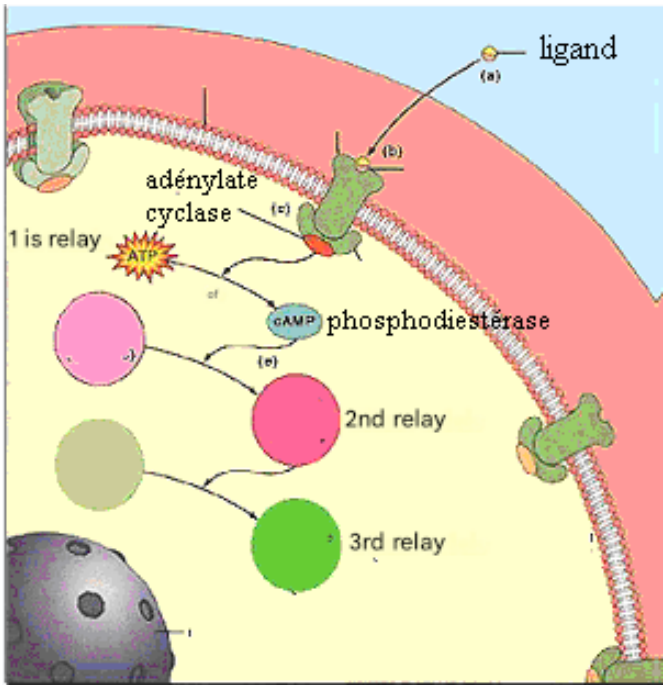


Figure 4:Enzyme receptors**(43)**

III.2.2.1. Tyrosine Kinase

Tyrosine kinase is an enzyme that catalyzes the phosphorylation of tyrosine residues in target proteins. It plays a critical role in the regulation of many cellular processes, including cell growth, differentiation, proliferation, migration, and cell survival.

Phosphorylation of tyrosine residues by tyrosine kinases can serve as an intracellular transduction signal, modulating the activity of various effector proteins, such as enzymes, transcription factors, or structural proteins **(38)**.

There are two main classes of tyrosine kinases: receptor tyrosine kinases (RTKs) and cytoplasmic (or non-receptor) tyrosine kinases.

RTKs are transmembrane receptors that are activated by the binding of specific ligands to their extracellular domain, resulting in the phosphorylation of tyrosine residues in their intracellular domain and the activation of cellular signaling pathways.**(38)**.

Cytoplasmic tyrosine kinases, on the other hand, are located in the cell cytoplasm and are activated by various mechanisms, including association with adaptor proteins or post-translational modifications.

Tyrosine kinases are involved in many cellular signaling pathways, some of which are associated with human diseases, including cancer. Indeed, mutations or overexpression of tyrosine kinases can lead to abnormal activation of cellular signaling and tumor progression. **(44).**

III.2.2.2. Serine/Threonine Kinase

Serine/threonine kinases are a class of protein enzymes that catalyze the phosphorylation of serine and threonine residues in target proteins. The phosphorylation of serine and threonine residues is an important regulatory mechanism in cell signaling, controlling many biological processes such as cell growth, differentiation, cell division, migration, and programmed cell death (apoptosis).

Serine/threonine kinases are involved in a wide range of cellular signaling pathways, often acting in cascade with other proteins to transmit signals within the cell. These signaling pathways can be activated by various extracellular stimuli such as hormones, growth factors, cytokines, and neurotransmitters. **(45).**

Serine/threonine kinases are also implicated in numerous human diseases, including cancer, neurodegenerative diseases, cardiovascular diseases, and metabolic disorders. Mutations or abnormal regulation of these enzymes can disrupt cell signaling and contribute to the development and progression of these diseases.

There are many different serine/threonine kinases in human cells, each with specific substrates and functions.

Some of the most well-known serine/threonine kinases include protein kinase A (PKA), protein kinase C (PKC), protein kinase B (PKB or Akt), and mTOR (mammalian target of kanamycin) protein kinase. **(46).**

III.2.2.3. Tyrosine Phosphatase

Tyrosine phosphatases are a class of enzymes that catalyze the dephosphorylation of tyrosine residues in target proteins. Unlike tyrosine kinases, which add a phosphate group to tyrosine residues, tyrosine phosphatases work by removing these phosphate groups, thereby regulating the activity of phosphorylated target proteins. This regulation is crucial in many biological processes, including cell signaling, cell growth, differentiation, cell division, and the immune response.

Tyrosine phosphatases are involved in a variety of cellular signaling pathways and often interact with tyrosine kinases to maintain the phosphorylation/dephosphorylation balance necessary for proper cellular signaling. Imbalances in this regulation can lead to diseases, including cancer, autoimmune diseases, metabolic disorders, and neurodegenerative diseases. **(47)**.

There are several families of tyrosine phosphatases, including catalytic domain phosphatases (e.g., protein tyrosine phosphatases, PTPs) and protein tyrosine phosphatase motif phosphatases (PTPs), which share similar structures and catalytic mechanisms. Each tyrosine phosphatase family has its own specific substrates and functions in regulating cell signaling.

Tyrosine phosphatases are potential targets for drug development, particularly in the treatment of cancer and other diseases where cell signaling is dysregulated. By selectively modulating the activity of tyrosine phosphatases, it is possible to precisely regulate cell signaling and specifically target pathological processes. **(48).**

III.2.2.4. Guanylate cyclase

Guanylate cyclase is an enzyme that catalyzes the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), a secondary signaling molecule important in many biological processes. This reaction is regulated by various extracellular stimuli, including hormones, neurotransmitters, and growth factors.

Guanylate cyclase can be classified into two main types:

III.2.2.4.1. Soluble guanylate cyclase (GCs)

GCs are present in the cell cytosol and are activated by various molecules, such as nitric oxide (NO) and carbon monoxide (CO).

Once activated, GCs catalyzes the conversion of GTP to cGMP, which acts as an intracellular secondary messenger, modulating various cellular processes, including smooth muscle relaxation, blood pressure regulation, neurotransmission, and cell growth regulation.**(49)**.

III.2.2.4.2. Membrane guanylate cyclase (GCm)

GCm is associated with the cell membrane and acts as a G protein-coupled receptor (GPCR).

It is activated by the binding of specific ligands to its extracellular domain, which triggers an intracellular signaling cascade resulting in the activation of membrane guanylate cyclase.

Once activated, GCm also catalyzes the conversion of GTP to cGMP, thereby regulating various cellular processes, including muscle relaxation, blood pressure regulation, and sensory perception.**(49)**.

cGMP signaling is involved in many important cellular functions, including vasodilation, regulation of calcium homeostasis, immune response, and regulation of cell growth. Dysfunctions in the guanylate cyclase signaling pathway are associated with various diseases, including cardiovascular disease, neurological disorders, and gastrointestinal disorders. **(50).**

III.2.3. G protein-coupled receptors

G protein-coupled receptors (GPCRs), also known as seven-transmembrane receptors, are a large family of membrane receptors involved in extracellular signal transduction into the cell interior. They play a critical role in regulating a wide variety of physiological processes, including sensory perception, neurotransmission, mood regulation, metabolic regulation, immune response, and cell growth.

The structure of GPCRs comprises seven transmembrane domains connected by extracellular and intracellular loops **(51)**..

When a specific ligand binds to the extracellular domain of the GPCR, it induces a conformational change in the receptor that activates a heterotrimeric protein called receptor-associated G protein. This activation leads to the dissociation of the G protein complex into α and βγ subunits, which can then regulate the activity of various cellular effectors, such as enzymes and ion channels **(51)**.

GPCRs are classified into several families based on their sequence, structure, and signaling mechanisms. Some of the most studied GPCR families include Gs protein-coupled receptors (stimulatory), Gi/o protein-coupled receptors (inhibitory), Gq/11 protein-coupled receptors (phospholipase C activators), and G12/13 protein-coupled receptors.

GPCRs are important targets for drug development, and many drugs currently on the market specifically target these receptors to treat a variety of diseases, including cardiovascular disease, neurological disorders, metabolic disorders, and cancer **(52).**

III.2.4. Receptors associated with protein kinases

Protein kinase-associated receptors are a class of membrane receptors that, when activated by the binding of a specific ligand, activate an intracellular signaling cascade involving protein kinases. These receptors play a critical role in the regulation of various cellular processes, including growth, differentiation, cell survival, and response to extracellular stimuli **(38).**

There are several types of protein kinase receptors, each activating specific signaling pathways. Examples of protein kinase receptors include:

III.2.4.1. Receptor tyrosine kinase (RTK) activity

These receptors possess intrinsic tyrosine kinase enzymatic activity in their intracellular domain. When a ligand binds to the extracellular domain of RTK, it induces dimerization of the receptor and activation of its tyrosine kinase activity.

RTKs then activate an intracellular tyrosine phosphorylation cascade, recruiting adaptor proteins and activating cellular signaling pathways such as the Ras/MAP kinase pathway and the PI3-kinase/Akt pathway. **(38).**

III.2.4.2. Receptors with serine/threonine kinase activity

These receptors activate serine/threonine protein kinases once activated by their ligand.

An important example is the activin receptor, which regulates cell growth and differentiation by activating mitogen-activated kinase (MAPK) and extracellular protein-activated kinase (ERK) signaling.**(38).**

III.2.4.3. Receptors associated with the protein kinase Jak (Janus Kinase)

These receptors activate Janus kinases that phosphorylate cytoplasmic substrate proteins, including STAT (Signal Transducer and Activator of Transcription) transcription factors, leading to their translocation into the nucleus and regulation of gene expression.

Activation of protein kinase-associated receptors leads to a variety of cellular responses depending on the receptor type, ligand, and activated signaling pathways. Dysfunctions in these signaling pathways can contribute to the development of diseases, including cancer, cardiovascular disease, and metabolic disorders.**(53)**.

III.2.5. Nuclear receptors

Nuclear receptors are a special class of receptor proteins that act as intracellular transcription factors, regulating gene expression in response to the binding of specific ligands. Unlike many other receptors that transmit signals within the cell through cytoplasmic signaling cascades, nuclear receptors directly affect gene transcription by modulating the activity of gene promoters.

Here are some important characteristics of nuclear receptors :

- **Structure** : Nuclear receptors have a modular structure that includes a ligand-binding domain (LBD) and a DNA-binding domain (DBD). The LBD is responsible for ligand binding, while the DBD recognizes specific DNA sequences on the promoters of target genes.
- **Activation** : When a specific ligand binds to the ligand-binding domain of the nuclear receptor, it induces a conformational change in the receptor protein that exposes transcriptional activation domains. These domains then allow the receptor to recruit transcriptional coactivators and interact with the transcription machinery to upregulate or downregulate the expression of target genes(**54**)**(figure 5)**.

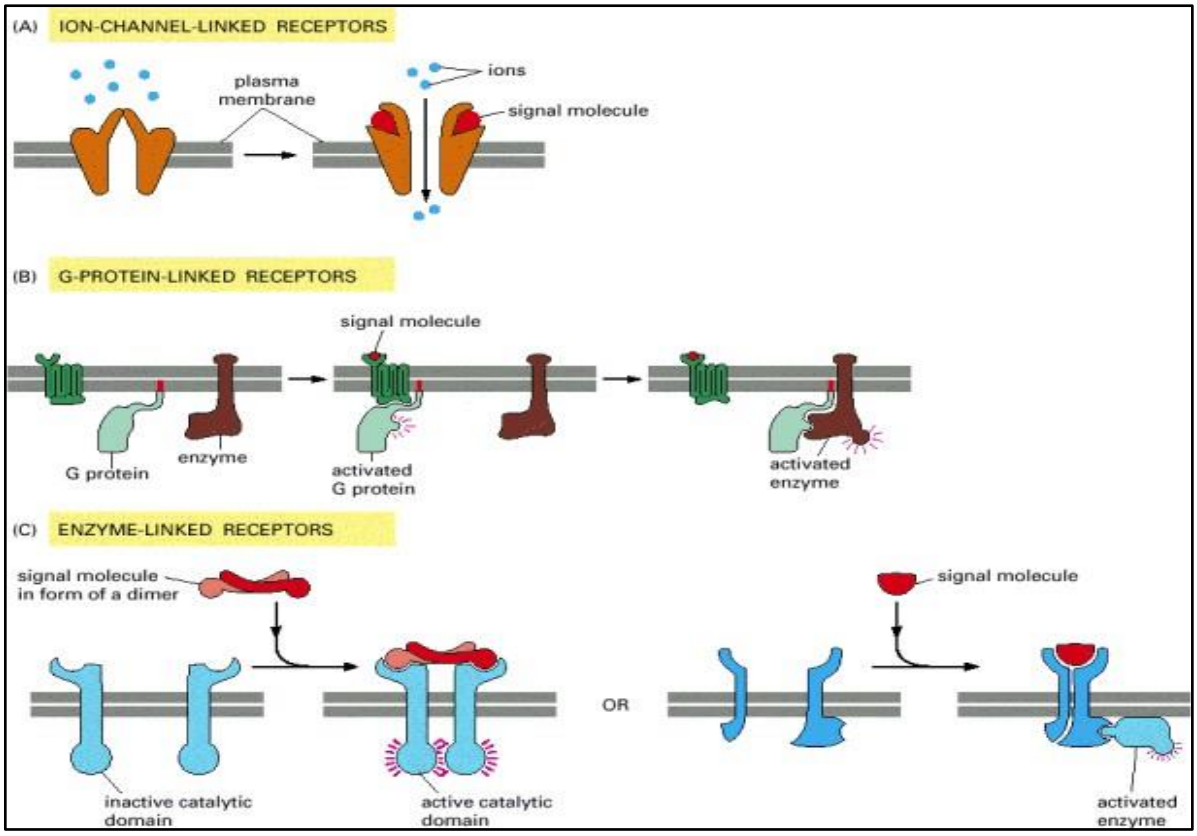


Figure 5: Differentreceptor types **(54)**

- **Variety of ligands**: Nuclear receptors can be activated by a wide variety of ligands, including steroid hormones, thyroid hormones, fatty acids, fat-soluble vitamins, and other molecules. Each nuclear receptor has its own specific ligands and regulates a distinct set of target genes **(55)**.
- **Regulation of biological processes**: Nuclear receptors play essential roles in the regulation of many biological processes, including embryonic development, cell differentiation, metabolism, stress response, and immune response **(56)**.
- Malfunctions in nuclear receptors can lead to a wide range of diseases, including endocrine diseases, metabolic disorders, cardiovascular diseases, and cancers. Due to their central role in regulating gene expression, nuclear receptors are important targets for drug development **(57)**.

III.3. Modifications involved in signal transduction

Post-translational modifications play a crucial role in cellular signal transduction by regulating protein activity, localization, and interaction. Some of the main modifications involved in signal transduction include:

III.3.1. Phosphorylation and dephosphorylation

Phosphorylation of serine, threonine, and tyrosine residues by protein kinases is one of the most important modifications in signal transduction. It can activate or deactivate target proteins, thereby altering their functional activity **(58)**.

Phosphorylation and dephosphorylation are fundamental processes regulating cell signaling that involve the addition or removal of phosphate groups (PO43-) on amino acid residues of proteins **(59)**.

Dephosphorylation, catalyzed by phosphatases, removes phosphate groups from proteins, reversing the effects of phosphorylation.

- **Acetylation and deacetylation**

Acetylation of lysine residues by histone acetyltransferases (HATs) can open chromatin and activate gene transcription. Deacetylation by histone deacetylases (HDACs) has the opposite effect, repressing transcription **(60)**.

- **Methylation and demethylation**

Methylation of arginine and lysine residues by methyltransferases can regulate protein activity. Demethylation, catalyzed by demethylases, removes methyl groups and thus alters protein-protein interactions **(61)**.

- **Ubiquitination and deubiquitination**

Ubiquitination involves attaching ubiquitin chains to target proteins, regulating their stability, subcellular localization, and activity. Deubiquitination removes ubiquitin chains and can reverse these effects **(62)**.

- **Sumoylation**

Sumoylation involves attaching the SUMO (small ubiquitin-like modifier) peptide to target proteins, modulating their activity, localization, and interactions with other proteins **(62)**.

- **Glycosylation**

Glycosylation involves adding carbohydrate chains to proteins, influencing their stability, function, and location.

These post-translational modifications are involved in a variety of signaling pathways and biological processes, and their dysregulation is associated with many diseases, including cancer, neurological diseases, and metabolic disorders **(63)**.

III.3.1.1. Phosphorylation

Phosphorylation involves adding a phosphate group to an amino acid residue, usually serine, threonine, or tyrosine, of a target protein. This process is catalyzed by enzymes called kinases, which transfer the phosphate group from a phosphate donor molecule, often adenosine triphosphate (ATP).

Phosphorylation can activate or deactivate a protein, altering its three-dimensional structure and biological function. For example, in many signaling pathways, the phosphorylation of proteins by kinases leads to their activation, which triggers a cascade of biochemical reactions in the cell **(64)**.

III.3.1.2. Dephosphorylation

Dephosphorylation is the reverse process of phosphorylation, where a phosphate group is removed from an amino acid residue by enzymes called phosphatases. These enzymes hydrolyze the bond between the phosphate and the amino acid residue, thereby regulating the activity of phosphorylated proteins.

Dephosphorylation can restore a protein to its baseline state, either deactivating it or preparing it for a new round of phosphorylation and activation. It also allows for the temporal and spatial regulation of signaling pathways by controlling the duration and intensity of cellular responses.

Phosphorylation and dephosphorylation are ubiquitous regulatory mechanisms in cell signaling, involved in a variety of biological processes, including cell growth, differentiation, programmed cell death (apoptosis), metabolism, stress response, and cell division. Their dysfunction is associated with many diseases, including cancer, cardiovascular disease, and neurological disorders **(65).**

III.3.2. Phosphorylation by protein kinases

Phosphorylation by protein kinases is a central process in the regulation of cell signaling. Protein kinases are enzymes responsible for transferring a phosphate group from a donor molecule, usually adenosine triphosphate (ATP), to a specific amino acid residue (serine, threonine, or tyrosine) on a target protein. This process changes the electrical charge of the target protein and can alter its three-dimensional structure, stability, and/or functional activity **(59)**.

Here are some key points regarding phosphorylation by protein kinases:

Activation of protein kinases: Protein kinases can be activated by different mechanisms, including extracellular signals (e.g., hormones or growth factors) or intracellular regulatory proteins.

Substrate specificity: Each protein kinase specifically targets particular amino acid residues on specific target proteins. This specificity is determined by the surrounding amino acid sequence, the three-dimensional structure of the protein kinase, and other factors **(59).**

Consequences of phosphorylation: Phosphorylation by protein kinases can have various functional consequences on target proteins, such as activation, deactivation, regulation of protein-protein interaction, subcellular localization, and degradation.

Signaling networks: Protein kinases often act within complex signaling networks, where the coordinated phosphorylation and dephosphorylation of multiple proteins regulate diverse cellular processes, such as growth, differentiation, programmed cell death, and stress response.

Regulation of protein kinase activity: Protein kinases themselves are often regulated by phosphorylation, either by autophosphorylation mechanisms or by phosphorylation by other protein kinases or by phosphatases.

Phosphorylation by protein kinases is an essential mechanism in cell signaling, allowing cells to respond in a precise and coordinated manner to extracellular and intracellular signals. Its dysfunction is implicated in numerous diseases, making it an important target for the development of pharmacological therapies **(66)**.

III.3.3. Dephosphorylation by protein phosphatases

Dephosphorylation by protein phosphatases is a critical process in regulating cell signaling, countering the action of protein kinases by removing phosphate groups from phosphorylated amino acid residues of target proteins. Protein phosphatases are the enzymes responsible for this dephosphorylation reaction.

Here are some key points about this process :

- **Types of protein phosphatases** : There are several classes of protein phosphatases, but the two main ones are serine/threonine phosphatases (PP1, PP2A, PP2B, PP4, PP5, PP6, PP7) and tyrosine phosphatases (PTPs).
- **Substrate specificity** : Like protein kinases, protein phosphatases exhibit some substrate specificity. However, they tend to be less specific than kinases. Some phosphatases specifically target phosphorylated serine/threonine residues, while others target phosphorylated tyrosine residues.
- **Regulation of protein phosphatase activity** : Protein phosphatases are regulated by various mechanisms, including covalent modifications such as phosphorylation, interactions with regulatory proteins, conformational changes, and subcellular localization. **(67).**
- **Consequences of dephosphorylation**: Dephosphorylation by protein phosphatases can have various functional consequences on target proteins, such as deactivation, regulation of protein-protein interaction, regulation of subcellular localization, and protein stabilization.
- **Signaling networks** : Protein phosphatases often act in coordination with protein kinases to regulate cellular signaling pathways. This balance between phosphorylation and dephosphorylation is crucial for the proper functioning of biological processes.

Dephosphorylation by protein phosphatases is therefore an essential mechanism for regulating cell signaling by allowing the extinction of signals activated by phosphorylation. Any malfunction in this process can lead to serious consequences for the cell and can be associated with various diseases. **(68).**

CHAPTER IV. SIGNALLING ROUTES INTRACELLULAR

IV.1. Adenylyl cyclase – cAMP pathway

IV.1.1. Adenylyl cyclase

Adenylyl cyclase (or adenylate cyclase) is a membrane enzyme consisting of 12 transmembrane regions distributed in 2 identical motifs with 6 α helices each.

Currently, there are 9 known isoforms of membrane adenylyl cyclase. Adenylyl cyclase (AC) is activated by Gαs-type subunits. It catalyzes the reaction of formation of cyclic AMP (cAMP), which is the second messenger produced, from ATP

IV.1.2. Activation-inactivation cycle of adenylyl cyclase

- Binding with the ligand causes a change in the conformation of the receptor. It will associate with the Gαs protein.
- This association results in a change in the conformation of the Gαs subunit. It replaces GDP with GTP and dissociates from Gβγ.
- The Gαs subunit binds to adenylyl cyclase, activating it. cAMP is synthesized.
- Hydrolysis of GTP to GDP results in the dissociation of Gαs with adenylyl cyclase and its reassociation with Gβγ. AC then becomes inactive again.

In some cases, ligands cause not stimulation but inhibition of adenylyl cyclase activity. In these cases, the G protein involved is then called Gi protein (i for inhibitory) whose GTP-bound αi subunit (αi.GTP) inhibits AC activity. The intracellular concentration of cAMP is controlled on the one hand, at the level of its synthesis by adenylyl cyclase, but also at the level of its degradation into non-cyclic (and inactive) 5'AMP by a phosphodiesterase (PDE) which attenuates the response to the signal.

The main site of action of cAMP is an enzyme, protein kinase A (A for cAMP-dependent). However, cAMP can act directly on certain types of ion channels, particularly in the case of GPCRs in the olfactory system. **(69).**

IV.1.3. Protein kinase A

Protein kinase A (PKA) is a cytoplasmic enzyme that catalyzes the phosphorylation of specific proteins on certain of their serine and/or threonine residues.

- **PKA activation**

Protein kinase A is composed of 4 subunits: two catalytic (C) subunits and two regulatory (R) subunits.

PKA target genes contain, in their regulatory region, a DNA sequence called CRE (Cyclic AMP Response Element). In this case, the catalytic C subunits of activated PKA enter the nucleus where they phosphorylate a transcription factor called CREB (CRE-Binding protein). Phosphorylated CREB recognizes and binds to the CRE sequence, then recruits a transcription coactivator CBP (CREB-Binding Protein); which allows the modulation of the transcription of these genes.

- **Effects of toxins**

Some toxins, such as those responsible for cholera and whooping cough, affect G protein function through ADP-ribosylation. ADP-ribose is transferred from intracellular NAD+ to the α subunit.

- **There cholera toxin**released by the bacterium Vibrio cholerae, is an enzyme that modifies the αs subunit of the Gs protein, so that it can no longer hydrolyze its GTP when it is in the α.GTP form. Consequently, it remains irreversibly activated and constantly stimulates adenylyl cyclase. The resulting high concentration of cAMP causes large losses of salts and water in the epithelial cells of the intestine, causing diarrhea and dehydration characteristic of cholera.
- **Whooping cough toxin**released by the bacterium Bordetella pertussis, causes irreversible inactivation of the Gi protein following ADP-ribosylation at the C-terminal level of the αi subunit. The Gi protein cannot couple with the receptor and therefore remains stuck in the inactive state. It can no longer inhibit adenylyl cyclase and consequently, the concentration of cAMP increases. When the infection reaches the respiratory tract, the pulmonary epithelial cells secrete abundant fluid and mucus causing the characteristic cough of whooping cough**(69)**(**figure 6**).

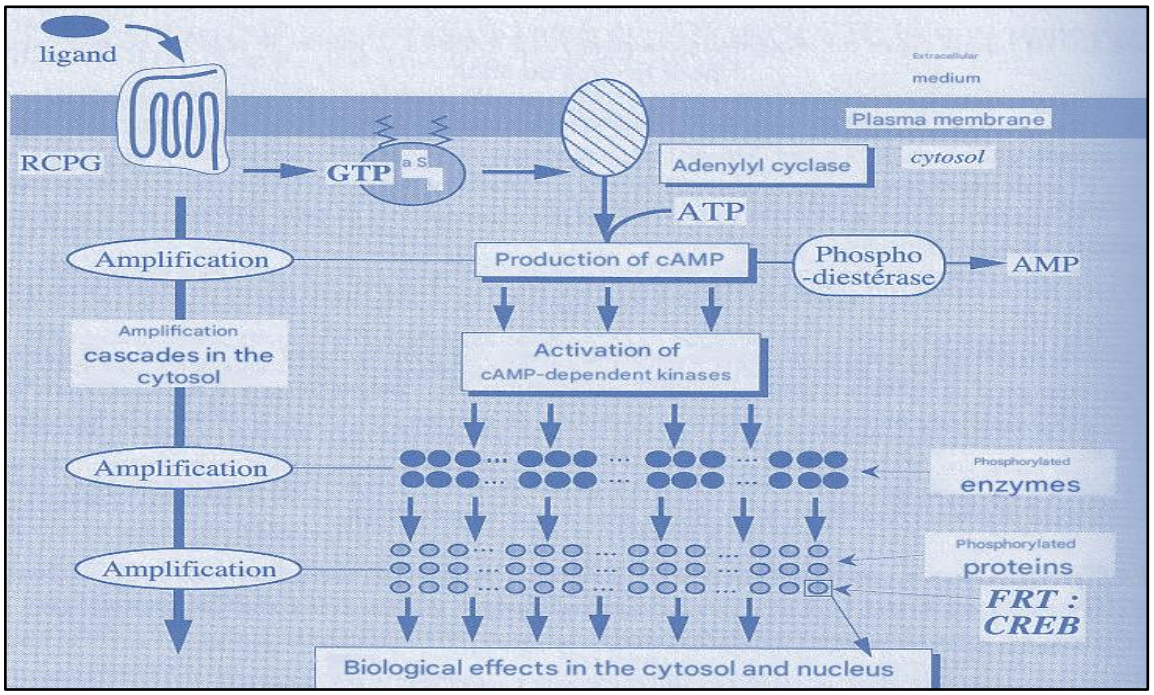


Figure 6: Cascade of amplifications triggered by the activation of adenyl cyclase and the production of cyclic AMP**(69)**

IV.2. Phospholipase C –Ca++ pathway

Several GPCRs exert their effect through G proteins (Gq family) that activate phospholipase C (PLC-β) in a similar manner to how Gs activates adenylyl cyclase.

Phospholipases are enzymes that hydrolyze the ester bonds of phospholipids. Phospholipase C (PLC), activated by the Gαq subunit, catalyzes the hydrolysis reaction of PIP2 (phosphatidyl inositol biphosphate) present in the inner leaflet of the plasma membrane into two second messengers: IP3 (inositol 1,4,5-triphosphate) and DAG (diacylglycerol).

These two messengers stimulate two downstream signaling cascade pathways: that of protein kinase C and that of Ca++ mobilization.

There are two forms of PLC: PLC-β and PLCγ. PLC-β is activated by a G protein. PLC γ contains SH2 domains that allow it to associate with a tyrosine kinase receptor.

Tyrosine phosphorylation increases PLC-γ activity which in turn stimulates PIP 2 degradation.

- DAG, which remains embedded in the membrane, will activate protein kinase C (PKC), a serine threonine kinase that activates other intracellular targets.

- The IP3, a water-soluble molecule, will diffuse through the cytoplasm to act on receptors specific to it, located in the membrane of the endoplasmic reticulum.
- It thus activates the opening of Ca2+ channels at the level of this structure, promoting the release of Ca2+ and its accumulation in the cytoplasm. The released calcium ions, in the case of PKC, play a role in the movement of this enzyme from the cytosol to the plasma membrane where it is activated by DAG**(69)**(**figure 7**).

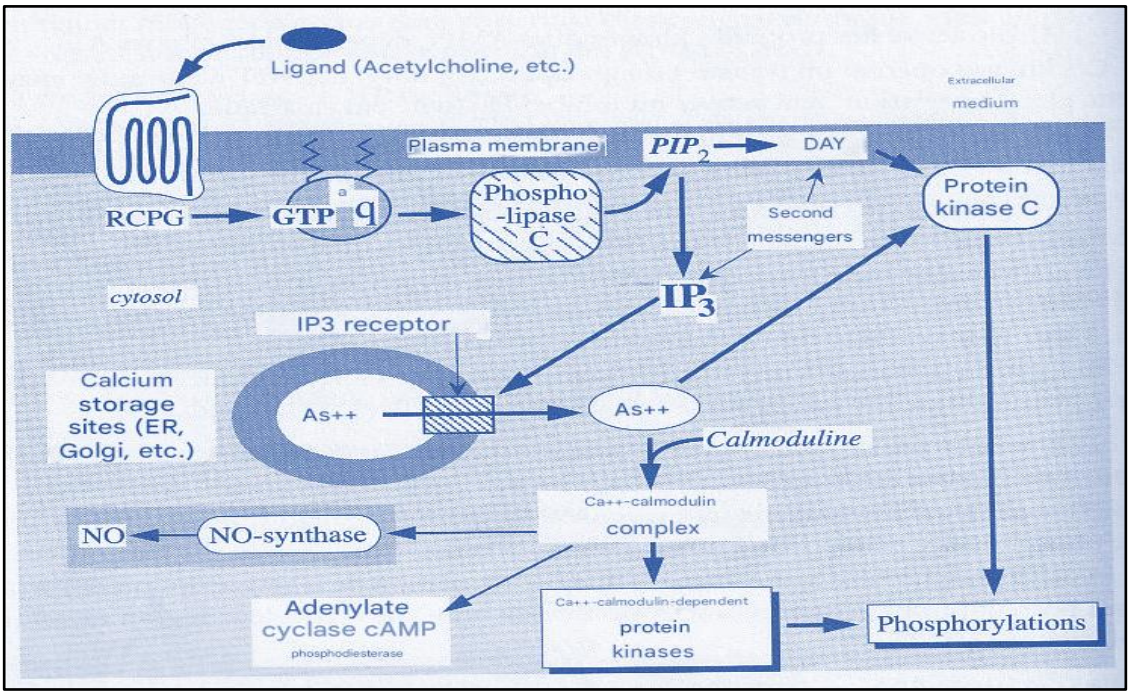


Figure 7: Activation pathway G protein, phospholipase C, phosphoinositide**(69)**

IV.3. cGMP pathway

In the transmission of visual signals in vertebrates, the functioning of cation channels (Na+, Ca++), responsible for the generation and propagation of the action potential in these cells, is under the control of cyclic GMP: these channels are called GMPC-dependent cation channels.

The concentration of this nucleotide depends on its rapid synthesis (by a guanylate cyclase) and its rapid degradation (by a phosphodiesterase).

a) This pathway has been particularly studied in the case of the rod-type photoreceptor cell of the retina. This cell is responsible for monochromatic vision in low light, while the second type of photoreceptor cell, the cone, is involved in color vision in strong light:

- The outer segment of the rods is formed of stacked discs (about 1,000 per cell) which contain a photosensitive molecule, rhodopsin.
- The plasma membrane surrounding the outer segment contains cyclic GMP-gated cation channels, which are opened in the dark by GMPC molecules that are attached to their cytosolic face.

b) Light activates rhodopsin

- It is a 7-transmembrane domain chromoprotein, comprising a protein moiety, opsin, and a photon-absorbing chromophore, 11-cis-retinal.

-The energy of a photon isomerizes retinal and activates opsin. This photochemical process causes a change in the three-dimensional conformation of opsin in a very short time (10 ms).

c) A trimeric G protein, transducin is activated by light photon-stimulated rhodopsin:

-The α subunit of transducin separates from the βγ dimer.

-The α subunit activates a phosphodiester of cyclic GMP

d) Hydrolysis of GMPC causes its cytosolic concentration to drop

-Cation channels close, inducing membrane hyperpolarization and inhibiting synaptic transmission from the photoreceptor to retinal neurons

-The light signal was transformed into an electrical signal.

-The signal amplification phenomenon is considerable in the case of vision. A single absorbed photon results in the hydrolysis of 500 GMPC molecules and the closure of 250 cation channels. In total, in one second, this cascade results in the hydrolysis of 105 GMPC molecules per quantum of light **(69)** (**figure 8**).

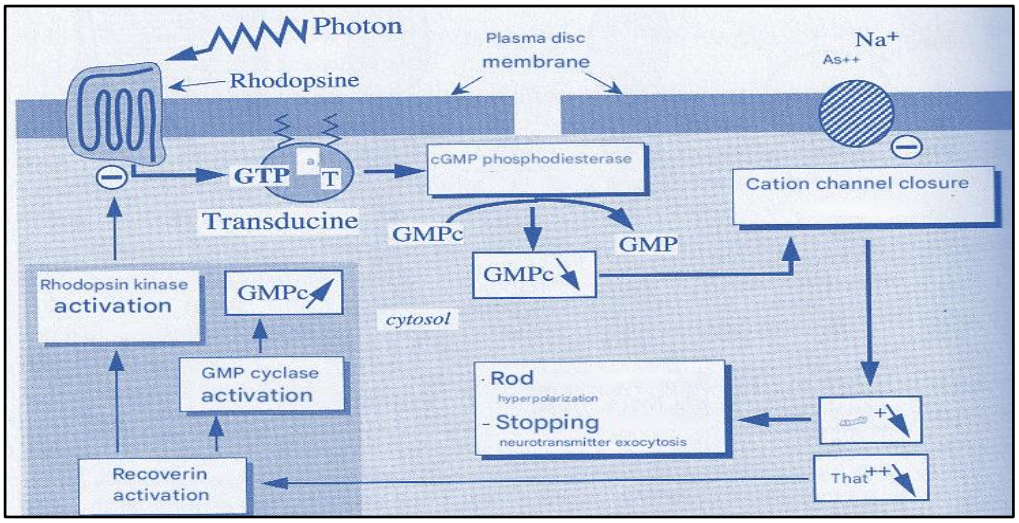


Figure 8 :GMP pathwayC (phenomenon of signal transduction and its amplification after the reception of a single photon of light)**(69)**

IV.4. Ca++-calmodulin pathway

While the diacylglycerol second messenger remains associated with the plasma membrane, the other PIP2-derived IP3 second messenger is released into the cytosol to activate ion pumps and mobilize Ca++ from these intracellular storage sites. High concentrations of Ca++ in the cytosol (from a basal level of 0.1 µM to a concentration of 1.0 µM after release into the cytosol) activate several Ca++-dependent protein kinases and phosphatases.

Calmodulin is a Ca++-dependent protein activated when the Ca++ concentration reaches 0.5 µM. Ca++-Calmodulin complexes bind to many target proteins in the cytosol to regulate cellular responses. It should be noted that Ca++ is an important second messenger and its intracellular concentration can be increased not only by release from intracellular storage sites but also by calcium entry into the cell from the extracellular medium **(70)**.

IV.5. NF-κB transcription factor signaling pathway

Nuclear factor NF-κB is a family of 5 DNA-binding proteins that regulate the expression of a large number of genes involved in diverse biological functions such as immunity, inflammation, development, and apoptosis. They consist of homodimers and heterodimers that are sequestered in the absence of activation in the cytoplasm by associating with NF-κB inhibitory (IκB) proteins **(70).**

IV.5.1. Components of the NF-κB signaling pathway

Thus, the NF-κB signaling pathway is made up of NF-κB dimers, IκB proteins, IKK complexes and intracellular adaptor proteins, notably the TRAF (TNF receptor associated factors) family**(70)**.

The NF-κB family consists of five members in mammals: p65 (RelA), RelB, c-Rel, p50 (NF-κB1), and p52 (NF-κB2). NF-κB family members share a Rel homologous domain (RHD) at their N-terminus that allows for DNA binding and the formation of homo- or heterodimers. NF-κB family members differ at their C-terminus and in their mode of synthesis. RelA/p65, RelB, and c-Rel possess a transactivation domain (TAD) that is required for NF-κB transcriptional activity and are synthesized in their mature form. In contrast, NF-κB1/p50 and NF-κB/p52 lack a transactivation domain and are synthesized by proteolysis from their polypeptide precursor p105 and p100, respectively **(70)**.

Proteolysis of p105 to p50 is constitutive, while that of p100 to p52 is tightly regulated and inducible.

Members of the IκB family are structurally related proteins that possess repeated motifs.

IV.5.2. Classical or canonical pathway of NF-κB activation

The classical or canonical pathway is activated by many stimuli such as inflammatory cytokines, bacterial or viral products, stress, oxygen-derived free radicals, ultraviolet and ionizing radiation, etc. These signals induce the degradation of IκBα and the nuclear accumulation, essentially, of the RelA-p50 dimer which regulates the expression of genes involved in the immune response and cell death **(70)**.

IV.5.3. Non-classical or alternative (non-canonical) pathway of NF-κB activation

It is activated by receptors involved in the organogenesis of lymphoid tissues and the development of lymphocytes, such as the lymphotoxin-β receptor or the B cell-activating factor (BAFF) receptor **(70)**(**figure 9**).

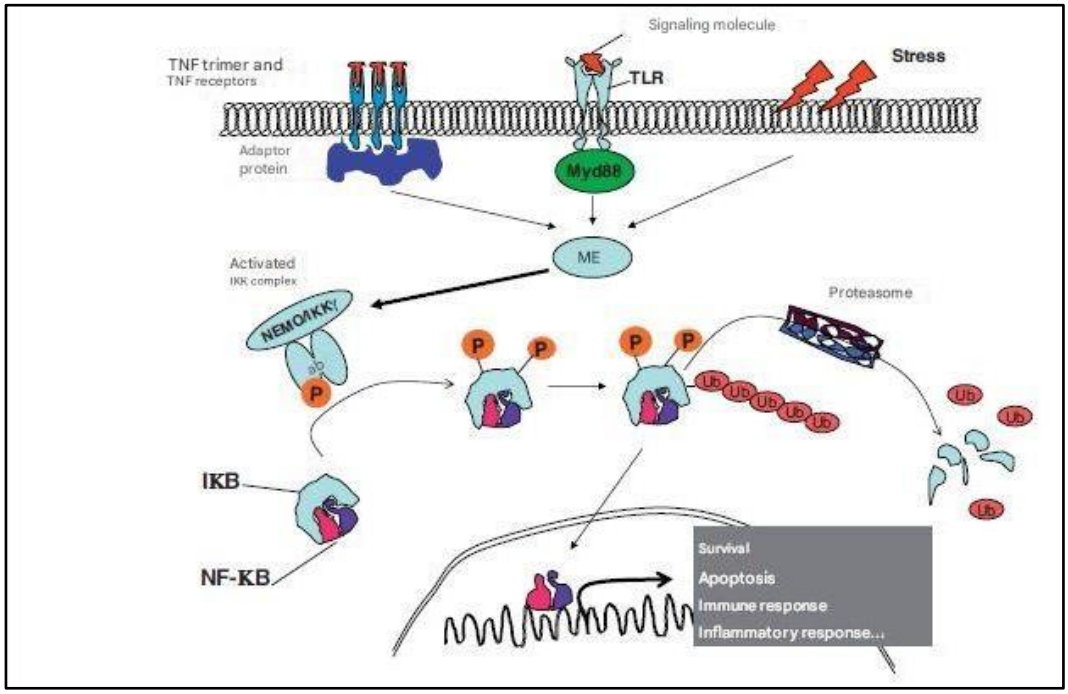


Figure 9: Wayof NF-κB **(70)**

NF-κB is sequestered in the cytosol by inhibitory proteins (IκB). Activation of the NF-κB pathway by TNFα receptors, TLR receptors (receptors belonging to the superfamily of IL-1 receptors and Toll receptors) or stress (ultraviolet radiation, ionizing radiation, oxygen derivatives) stimulates the phosphorylation of the IκB kinase-kinases (IKK) complex which is a trimer associating the regulatory subunit NEMO (NF-κB essential regulator, also known as IKKγ) and the two catalytic subunits IKKα and IKKβ. Activated IKK induces the phosphorylation of IκB then its ubiquitinylation (Ub) and its degradation by the proteasome. The degradation of IκB then allows NF-κB to move into the nucleus and activate the synthesis of specific proteins involved in the regulation of cell survival and apoptosis, the immune and inflammatory response, etc. NIK: NF-κB-inducing kinases. MyD88: myeloid differentiating factor

IV.6. MAP kinase (MAPK) pathway

The MAP kinase (mitogen activated protein kinase) pathway is one of the main pathways for transmitting proliferation signals provided by growth factors such as NGF (nerve growth factor).

Mitogen-activated protein (MAP) kinases are members of the protein kinase superfamily. They are ubiquitous proteins and important mediators involved in the transduction of extracellular signals from the plasma membrane to the nucleus.

MAP kinases are proteins that possess kinase activity that phosphorylate serine/threonines in response to extracellular stimuli such as mitogens and thus regulate various cellular activities such as gene expression, mitosis, differentiation, cell proliferation and survival and its corollary, apoptosis or programmed cell death **(70)**.

MAP kinases are activated by various types of signals, including mitogens, cytokines, T cell antigens, pheromones, phorbol esters, UV (A, B, C), ionizing radiation, osmotic stress, heat shock, oxidative stress; they initiate a variety of cellular responses.

The MAPK pathway can be activated by a wide variety of external or internal stimuli, but the classic activation pattern of this pathway involves the binding of a ligand to its membrane receptor. This binding induces a conformational change in the receptor, which leads to phosphorylation of the receptor itself or of proteins associated with it. This generally leads to the direct or indirect activation of proteins of the G protein family, which are coupled to the receptor or in free form, such as the Ras protein**(70)**.

A characteristic feature of all MAP kinases is their activation by double phosphorylation at Tyrosine and Threonine residues present in a consensus motif Thr-X-Tyr (X = Glu, Gly or Pro). MAP kinases are finely regulated by phosphatases which, by dephosphorylation of a single residue, inactivate them. In response to external stimuli, they regulate the transcriptional activity of several transcription factors via phosphorylations at activating or inhibitory regulatory sites, and thus allow the expression of a wide variety of genes **(70)**.

The activation of the receptor and associated proteins is located upstream of the MAPK cascade which is made up of three kinases: a MAPK kinase kinase (MAP3K) activated by extracellular stimuli, which phosphorylates and activates a MAPK kinase (MAP2K or MKK) on its serine and threonine residues, which in turn activates the MAPK kinase through the phosphorylation of its serine and threonine residues.

The latter, translocated into the cell nucleus, then phosphorylates the transcription factors which activate the transcription of all the genes responsible for DNA replication and the initiation of the cell cycle (DNA polymerases, cyclins, etc.).

MAPKs are divided into three subfamiliesdefined by the last elements of the cascade, which all comprise several isoforms, are the pathways of the kinases ERK1 and ERK2 (extracellular signal-regulated kinases), p38 MAP kinases (with 4 isoforms called α, β, γ and δ) and C-Jun N-terminal kinases (JNK1, JNK2 and JNK3).

MAP kinases are ubiquitously expressed and involved in many biological processes. ERK1 and ERK2 usually regulate cell proliferation, survival, and differentiation. MAP kinases p38 and c-JUN are involved in inflammatory response, cell death, extracellular matrix remodeling, etc.**(70)**(**figure 10**).

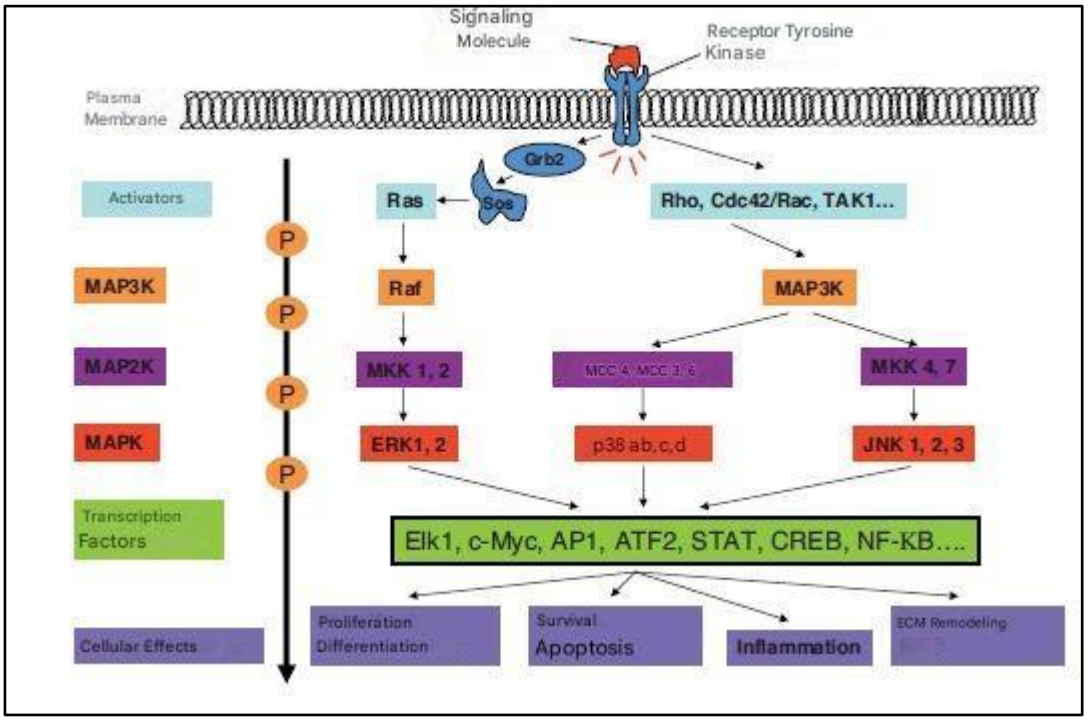


Figure 10:MAP kinase pathway **(70)**

The MAP kinase (mitogen-activated protein kinase) pathways are divided into three pathways: ERK (extracellular signal-regulated kinases), p38, and JNK (C-Jun N-terminal kinases). These three proteins have multiple isoforms and are the final protein kinases in the three respective pathways. Each protein kinase is activated by specific MAPK kinases (MKK or MAP2K), which are themselves activated by other kinases, MAPK kinase kinases (MAP3Ks). MAP3Ks have specific activators depending on the initial extracellular signal and the cell type.

IV.6.1. Ras/MAPK pathway or ERK1/2 pathway

Before developing the Ras/MAPK signaling pathway, we will first focus on the Ras protein, which is the main player in activating this pathway, and then we will focus on other signaling pathways.

IV.6.1.1. Presentation of the Ras family

The Ras protein superfamily is a family of small GTPases (guanosine triphosphate hydrolases) comprising about twenty members divided into several subfamilies. These are small monomeric proteins capable of binding GDP or GTP, which have been highly conserved during evolution. In humans, approximately 150 of these small signaling proteins are expressed and serve to regulate growth, cell mobility, morphogenesis and membrane trafficking. This superfamily includes 5 subgroups: Ras, Rho, Ran, Rab and Arf which are associated with specific functions.

- **Ras Family**

The Ras family (Ras sarcoma) consists of 36 members. Activated Ras interacts with numerous effectors that regulate signaling pathways, which control gene expression and the regulation of cell proliferation, differentiation, and survival.

The MAPK pathway remains the best-identified Ras-mediated signaling pathway.

- **Rho family**

Proteins in this family regulate actin organization, cell cycle progression, and gene expression. Twenty members have been identified, with RhoA, Rac1, and Cdc42 being the most studied. Some of these proteins are membrane-bound, while others are primarily cytoplasmic. For example, RhoB is localized to endosomes and Cdc42 to the endoplasmic reticulum. Their localization may depend on their interaction with other proteins such as guanine nucleotide dissociation inhibitors (GDIs), and this localization may be regulated during the GTPases cycle. Although Miro proteins were first described as Rho proteins, it has emerged that these proteins constitute a separate group. Miro proteins are localized to mitochondria.

- **Rab Family**

Rab proteins (Ras-like proteins in brain) comprise 61 members. They regulate vesicular and secretory transport.

Rab proteins will be localized in specific compartments according to their functions. Rab5 is localized in early endosomes and regulates clathrin-coated vesicle-mediated transport.

- **Ran Family**

Ran (Ras-like nuclear) proteins are the most abundant GTPases in cells and the best known for their essential function in the nucleocytoplasmic transport of RNA and proteins.

The nuclear Ran-GTP protein interacts with importins, exportins, or cargo proteins. These proteins are involved in DNA replication and nuclear envelope assembly.

- **Arf Family**

Proteins of the Arf (ADP ribosylation factor) family are involved in the regulation of vesicular transport. The Arf1 protein is the best characterized.

IV.6.1.2. Regulation of Ras activation

Small G proteins exist in two conformational states dependent on the nature of the bound nucleotide and corresponding to different functional states of the protein, the GTP-bound form being active while the GDP-bound form is inactive. Only the active form is capable of binding effectors. The transition between these two states is highly controlled and regulated by different pathways (the GTPases cycle).

This cycle is regulated by two classes of proteins: GAP proteins (GTPases Activating proteins), which promote the GTPasic activity of small G proteins, allowing the formation of the inactive form bound to GDP, and GEF proteins (Guanine Exchange Factor), which allow the formation of the active form bound to GTP. Comparison of the three-dimensional structures of the GDP-bound or GTP-bound states essentially shows that two regions of the protein change depending on the nucleotide; these are called switch I and II regions. These two structures are not fixed in the GDP-bound conformation, but are stabilized in a specific position in the GTP form. The two switch regions form the effector domain of Ras and GTPases. The effectors interact at these two regions.

There is a class of inhibitory proteins called GDIs (guanine nucleotide dissociation inhibitors), which are part of a subclass of small GTPases. The precise role of these proteins in the GTPase cycle is not very clear **(69)**.

IV.6.1.3. Ras protein

a) Structure of Ras isoforms

The prototype of the small G protein family is the Ras oncogene, which is a small 21 kDa protein. In mammals, there are four isoforms of the Ras protein encoded by three different genes: H-Ras, N-Ras, and K-Ras4A and 4B.

The first 85 amino acids are identical in all four proteins and allow the binding of guanosine diphosphate (GDP) and guanosine triphosphate (GTP).

The G domain is composed of the P-loop, which binds the γ-phosphate of GTP, and switch I and II, regulating binding to Ras regulators and effectors.

b) Ras activation

Following stimulation of receptor tyrosine kinase activity, phosphorylated tyrosines of the receptor serve as anchoring sites for SH2 domain adaptor proteins such as Grb2 (growth factor receptor binding Homology 2) or Shc. Through its two SH3 domains, Grb2 is constitutively associated with the proline-rich carboxy terminal domain of Sos (Son of Sevenless), the exchange factor of the small G protein Ras. Thus, binding of Sos to the receptor via Grb2 allows its membrane relocalization close to its substrate, which it activates. Ras GEFs are divided into three families: Sos, GRF, and GRP. While Sos1 and Sos2 are ubiquitously expressed, Ras-GRF and Ras-GRP are abundant in the brain. Sos exists in an autoinhibited state; therefore, interactions with Ras modulate Sos activity.

Binding of Ras-GDP to the allosteric site of Sos, which is distal to the catalytic site, induces low GEF activity, which then allows Ras-GTP production. Ras-GTP then binds with higher affinity to the same site. Mutations in Sos that disrupt their autoinhibition have recently been identified in patients with Noonan syndrome.

c) Mechanisms of negative regulation of Ras activation

GAP proteins play an important role in Ras regulation. These proteins help interrupt the signal produced by Ras activation. There are at least 160 human genes that encode GAP proteins.

The first member of the GAP family to be identified was p120GAP, which was the first protein discovered to interact with the effector domain of Ras.

The p120GAP protein can associate with the activated PDGF receptor through its SH2 domains or through an adaptor protein p62Dok. In addition to a catalytic domain, this protein has SH2, SH3, and PH domains, and phospholipid-binding motifs. GAP proteins are regulated by intramolecular interactions. Thus, the PH domain of p120 RasGAP regulates the activity of its catalytic domain. The p120 RasGAP protein appears to be regulated by proteolysis and is degraded by cleavage by certain caspases.

Other GAP proteins have been identified, such as neurofibromin. The latter is encoded by the tumor suppressor gene NF1. Mutations in this gene are responsible for the inherited genetic disease neurofibromatosis type I (NFI).

Neurofibromin is phosphorylated at multiple sites in its C-terminal region by protein kinase A. This phosphorylation allows the interaction of neurofibromin with 14-3-3 proteins and is correlated with a reduction in Ras-GAP activity. Neurofibromin undergoes rapid proteolytic degradation by ubiquitination. This regulation of neurofibromin may be explained by an increase in the duration and intensity of Ras activation. Studies have shown that phospholipids and fatty acids have inhibitory effects on the catalytic activity of neurofibromin and p120 RasGAP **(69).**

d) Ras effectors

Activation of receptors such as GPCRs, RTKs, or integrins by different ligands triggers the activation of Ras proteins, which then recruit a number of effectors. The binding of these effectors to Ras proteins triggers distinct signaling cascades. Ras effectors are characterized by the presence of a Ras-Binding Domain (RBD) or a Ras-Associating Domain (RA).

In 1993, the Raf protein kinase was identified as a major effector of Ras. Raf proteins are the first kinases in the MAPK cascade. The other major effector of Ras is the PI3K protein. Indeed, there is a direct interaction between the p110 catalytic subunit of PI3K and the activated Ras protein. This interaction is mediated by an RBD domain that PI3K possesses. Among Ras isoforms, H-Ras is the most potent activator of PI3K compared to K-Ras **(69)**(**figure 11**).

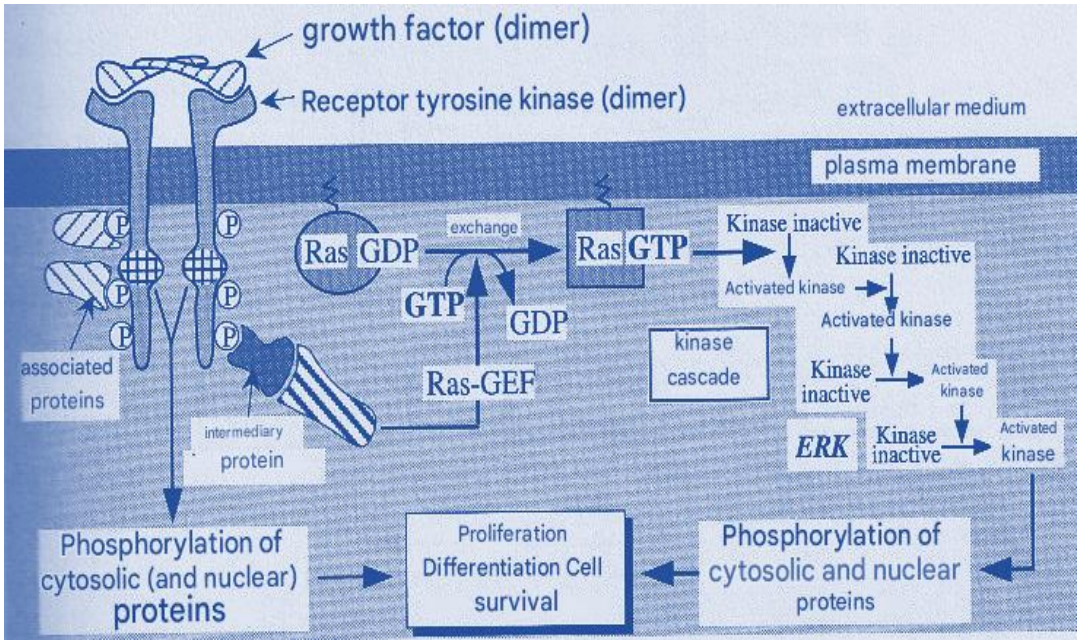


Figure 11: Ras activation by a receptor tyrosine kinase**(69)**

IV.6.1.4. Description of the Ras/MAPK pathway or ERK1/2 pathway

ERK1 and 2 have 83% identical amino acids and are expressed in all tissues. They are highly activated by growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF), and by phorbol esters, which, through membrane receptors such as receptor tyrosine kinases (RTKs) or G-protein-coupled receptors, transmit activating signals to the Raf/MEK/ERK cascade via different isoforms of the Ras protein **(71)**.

MEK1/2 selectively activate ERK 1/2 MAP kinases by phosphorylation at a conserved TEY (Thr-Glu-Tyr) motif present in the activation loop, according to a distributive mechanism.

ERK1 and ERK2 are 43 and 41 kDa proteins that are 85% homologous. ERK in turn phosphorylates many substrates that are involved in the regulation of cell proliferation and differentiation.

The MEK/ERK pathway is an intracellular signaling pathway characterized by a protein phosphorylation cascade leading to a cellular response. It is activated by growth factors, hormones, or cytokines that act through membrane receptor tyrosine kinases.

Ligand binding to the extracellular domain of the receptor causes its dimerization, which triggers autophosphorylation of its cytoplasmic domain on tyrosine residues, giving rise to new protein motifs. These allow the anchoring to the receptor of so-called adaptor proteins, such as GRB2, containing SH2 or PTB (phosphotyrosine-binding domains).

These proteins will then recruit the Sos protein, which is a GDP exchange factor for RAS proteins. These are monomeric G proteins (or GTPases) that cycle between an inactive state bound to GDP and an active state bound to GTP. The proximity of SOS and RAS will promote the exchange of GDP by GTP, inducing its activation. Thus activated, RAS will bind to various effectors including the C-RAF kinase, which will phosphorylate and activate the MEK1 and MEK2 kinases, which will themselves phosphorylate and activate the ERK1 and ERK2 kinases, then phosphorylate cytosolic proteins such as S6 kinase or nuclear transcription factors such as ELK, ETS, or fos, which are at the origin of cellular effects **(71).**

IV.6.1.5. Regulation of the MEK/ERK pathway

The MEK/ERK pathway is tightly regulated, notably by negative feedback exerted by ERK. Phosphorylation of SOS by ERK limits its association with GRB2, thus preventing its recruitment to the plasma membrane. Similarly, ERK is capable of directly phosphorylating RAF and the intracellular domain of the EGF receptor, inducing their inactivation. Furthermore, transcription factors of the ETS family stimulate the synthesis of inhibitors of the MEK/ERK pathway, such as Sprouty proteins or phosphatases of the DUSP (Dual Specific Phosphatases) family. The MEK/ERK pathway is activated and interacts with many other signaling pathways, establishing cross-regulations between these pathways. These complex regulations are often at the origin of the limitation of anti-tumor effects observed with pathway-specific inhibitors.

Indeed, blocking one pathway induces compensatory activations of other signaling cascades, such as the activation of membrane tyrosine kinases such as Met, IGFR1, PDGFRβ, EGFR or C-KIT, thus overcoming the pharmacological effects of inhibitors **(71)**(**figure 12**).

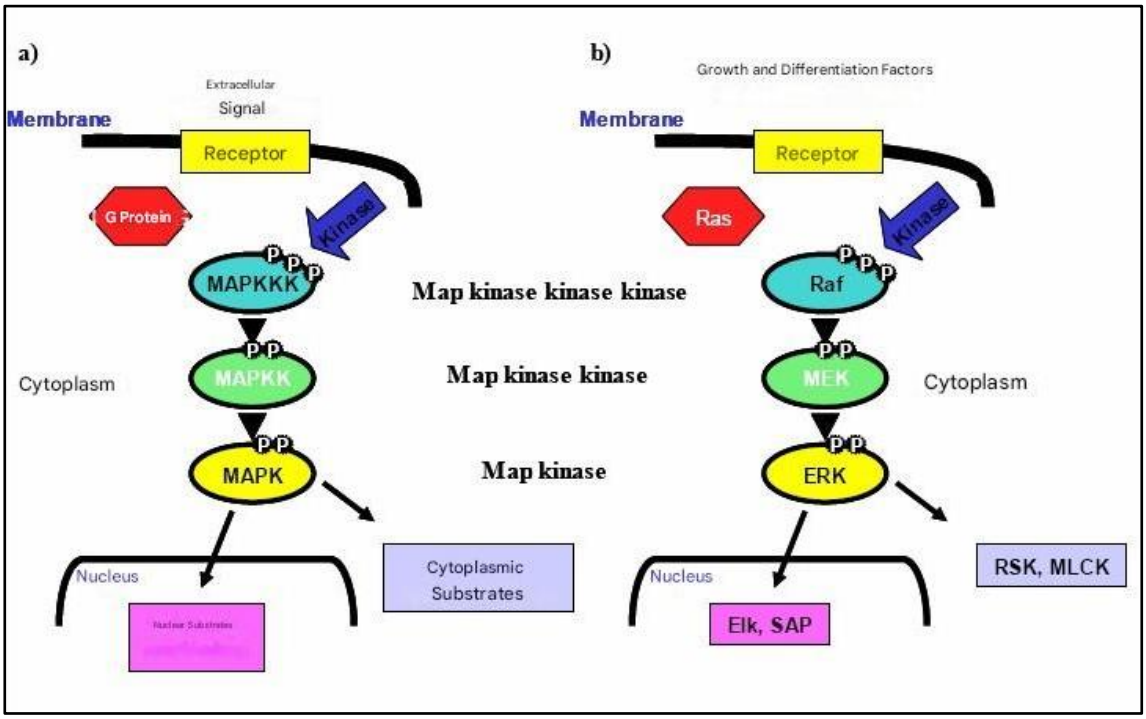


Figure 12: Schematic of MAPK pathway activation **(71)**

IV.6.2. JNK Way

The MAP kinase JNK pathwayis mainly activated by cytokines and cellular stresses such as osmotic shock, oxidative stress or radiation, so JNK MAP kinases were initially named stress-activated protein kinases (SAPKs). AP-1 family transcription factors are among the most important targets of JNK. These are heterodimers formed from Fos, Maf, ATF and/or members of the Jun family (c-Jun, JunB, JunD). AP-1 factors link activation of the JNK pathway to the induction of several target genes involved in proliferation, apoptosis, inflammation and DNA repair.

Thus, the JNK pathway is involved in growth, nervous system development, as well as in the response to cytokines and insulin. It therefore represents a prime target for the treatment of Parkinson's disease, inflammation, diabetes, cancer, and many other pathologies.

JUN N-terminal kinases, JNK or SAPK (Stress-activated protein kinase), numbering three, encoded by three genes leading to the production of at least ten isoforms. The identified isoforms are JNK1/SAPKγ, JNK2/SAPKα, and JNK3/SAPKβ, their catalytic domains present 85% homology are activated in response to pro-inflammatory cytokines, stresses such as heat, ionizing radiation, DNA damage or oxidative stress, and incidentally by growth factors. TNF (Tumor necrosis factor) and WNT ligands also activate this signaling pathway **(71).**

JNK/SAPKs are activated by phosphorylation on two sites, tyrosine and threonine, like all other MAP kinases. Activation of the JNK pathway involves a large number of MAP3Ks whose action converges towards the activation of a limited number of MAP2Ks. Indeed, only two members of the MAP2K family, MKK4 (SEK1, MEK4, JNKK1, SKK1) and MKK7 (MEK7, JNKK2, SKK4), have been implicated in the JNK/SAPK pathways. Activation of the JNK pathway is generally associated with the regulation of cell death processes, insulin signal transduction and cell cycle regulation **(71)**.

- ✓ **MAPKKK of the JNK route**

Several MAPKKXs activate the JNK pathway to varying degrees of intensity. Although this specificity appears to be related to the nature of the activating stimulus, it remains highly cryptic. Nevertheless, MEKKÏ is the first identified activator of the JNK pathway and the most potent.

Subsequently, several other MAPKKKs of this pathway were discovered:

MEKK2 and MEKK3, MEKK4, Mixed-lineage kinase 2 (MLK2) and MLK3, Apoptosis signal-regulating kinase 1 (ASK1) and ASK2 and TGF-β-activated kinase 1 (TAK1).

In addition, other proteins such as Dual leucine zipper-bearing kinase (DLK) as well as Thousand and one amino acid kinase 1 (TAO1) and TAO2 can also activate this pathway, but to a lesser extent.

Because there are so many of them, gene inactivation of these different MAPKKKs did not allow the identification of any of them as being essential to the JNK pathway. Indeed, the absence of MEKK1 or other MAPKKKs does not prevent activation of the JNK pathway by stresses such as ultraviolet (UV) rays. These data suggest that several MAPKKKs are activated by the same stress, or that the MAPKKK critical to the JNK pathway has not yet been inactivated **(71)**.

IV.6.3. p38 pathway

The p38 MAP kinase pathway is a preferred relay for stress and inflammation signals. It is activated by UV rays, thermal or osmotic shock, as well as by growth factors and several inflammatory cytokines.

Furthermore, activation of the p38 pathway is itself essential for the production of pro-inflammatory cytokines, antioxidant enzymes, tissue remodeling proteins, and many other inflammatory molecules. Finally, the p38 pathway is involved in the proliferation and differentiation of immune system cells **(72)**.

The p38 MAPK family consists of four isoforms derived from the expression of different genes: p38α (MAPK14), p38β (MAPK11), p38γ (MAPK12) and p38δ (MAPK13). The amino acid sequences of p38α and p38β are 75% similar, those of p38γ and p38δ are 70% similar. In contrast, p38γ and p38δ share only about 60% identity with p38α. The expression of these isoforms differs depending on the tissue considered.

These factors activate MAP3Ks named MEKK4, TAK1 or ASK1 which in turn activate MAP2Ks involved in the p38 pathway but can also activate the JNK pathway. Two MAP2K proteins, MEK3 (MKK3) and MEK6 (MKK6), strongly activate p38 MAP kinases. MEK3 appears to promote the phosphorylation of p38α and the p38β isoforms, whereas MEK6 efficiently phosphorylates all members of the p38 family.

Activation of the p38 pathway is generally associated with the activation of transcription factors and protein kinases involved in the regulation of cell death, differentiation and inflammatory response processes **(72)**.

Additionally, activation of the p38 pathway is itself essential for the production of pro-inflammatory cytokines, antioxidant enzymes, tissue remodeling proteins, and many other inflammatory molecules. Finally, the p38 pathway is involved in the proliferation and differentiation of immune system cells **(72)**.

IV.6.3.1. Components of the p38 pathway

Often activated by the same stimuli, the p38 pathway shares several important MAPKKKs with the JNK pathway. These include TAK1, ASK1, DLK, MEKK1, MEKK3, MEKK4, MLK1, MLK2, and MLK3, overexpression of which activates both the p38 and JNK pathways with varying efficiencies.

The MAPKKs MKK3 and MKK6 are 80% identical. Gene inactivation of either leads to immunological defects that do not affect mouse viability or fertility. In contrast, double inactivation of MKK3 and MKK6 results in lethality resulting from severe vascularization defects. While MKK6 efficiently activates all four p38 isoforms, MKK3 is unable to activate p38β. Furthermore, the MAPKK MKK4, activated in the JNK pathway, can lead to the activation of p38α and p38δ under certain cellular conditions.

MKK4 contributes to UV-induced activation of p38 MAP kinases, but only MKK3 and MKK6 mediate p38 activation by Tumor necrosis factor α (TNFα). In summary, MKK3 and MKK6 each exert specificity despite their largely redundant action, and they are considered the major MAPKKs of the p38 pathway **(72)**(**figure 13**).

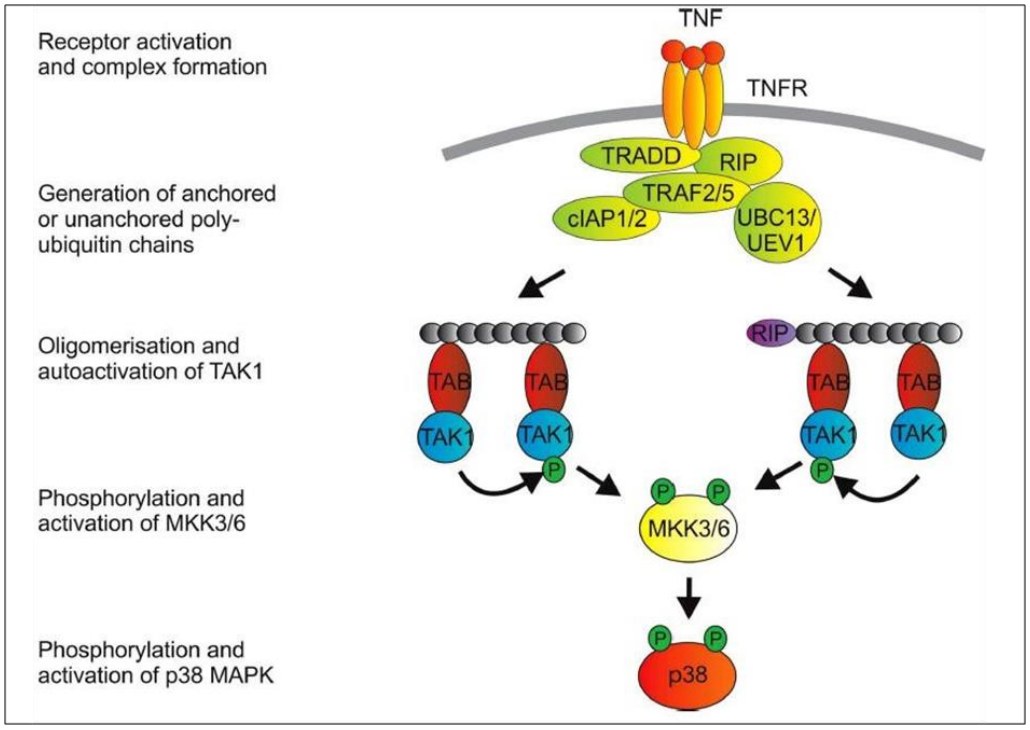


Figure 13: Activation of p38 Map kinase **(72)**

IV.7. JAK/STAT signaling pathway

The JAK/STAT signaling pathway is involved in regulating cellular responses to cytokines and growth factors. Following activation by a cytokine or growth factor, the signaling pathway uses JAK (Janus kinases) and STAT (Signal transducers and activators of transcription) proteins to transmit the extracellular signal to the nucleus, where the activated STAT proteins modulate gene expression. This signaling pathway plays a critical role in cell proliferation, differentiation, and apoptosis. It is particularly important in hematopoiesis.

JAK proteins, which have tyrosine kinase activity, bind to certain cytokine receptors. Binding of the ligand to its receptor will activate JAK **(70)**.

The increased kinase activity of JAK will result in increased phosphorylation of tyrosine residues on the receptor and thus create sites of interaction with proteins that contain SH2 domains that bind phosphotyrosines. However, STAT proteins have SH2 domains capable of binding these phosphotyrosine residues, which are thus recruited to the receptors and are themselves phosphorylated at their tyrosine residues by JAKs. These phosphotyrosines will then serve as binding sites for the SH2 domains of other STAT molecules, thus promoting their dimerization. Thus, different STAT proteins can form homodimers or heterodimers. These STAT dimers, thus activated, will translocate to the cell nucleus and activate the transcription of target genes **(70)**(**figure 14**).

Furthermore, STATs can also be directly phosphorylated at their tyrosine residues by tyrosine kinases present at the receptor level (e.g., EGF receptor or "epidermal growth factor") or by c-src type tyrosine kinases.

The STAT pathway is often accompanied by negative feedback. STAT also stimulates the production of inhibitory proteins such as SOCS3 (suppressor of cytokine signaling 3), which inactivates JAK, and STAT5, which inactivates phosphorylated STAT through feedback. The other deactivation mechanism involves tyrosine phosphatases. Thus, JAKs are inhibited by the tyrosine phosphatase SHP-1**(73)**(**Figure 15**).

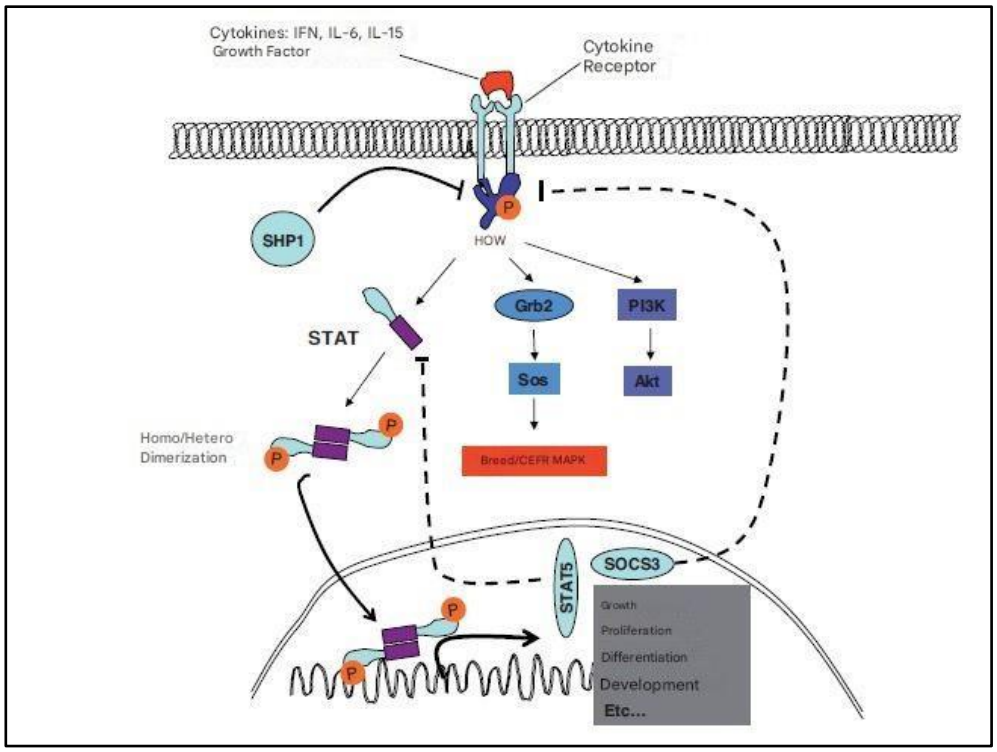


Figure 14:JAK/STAT activation pathway **(70)**

Janus Kinases (JAK) are intracytoplasmic tyrosine kinases that can be activated by cytokine receptors. Activated JAK stimulates the phosphorylation of STAT (signal transducers and activator of transcription) which can then form homo/heterodimers. STAT dimers migrate into the nuclear nucleus and stimulate target genes that cause the modifications of cellular behavior. STAT also induces the production of proteins that exert negative feedback on the JAK pathway such as proteins SOCS3 (suppressor of cytokine signaling 3) and STAT5. JAK can be inhibited by a tyrosine phosphatase, SHP1. Furthermore, activation JAK can also stimulate the Ras/MAPK ERK1/2 pathways and the PI3K and Akt pathway.

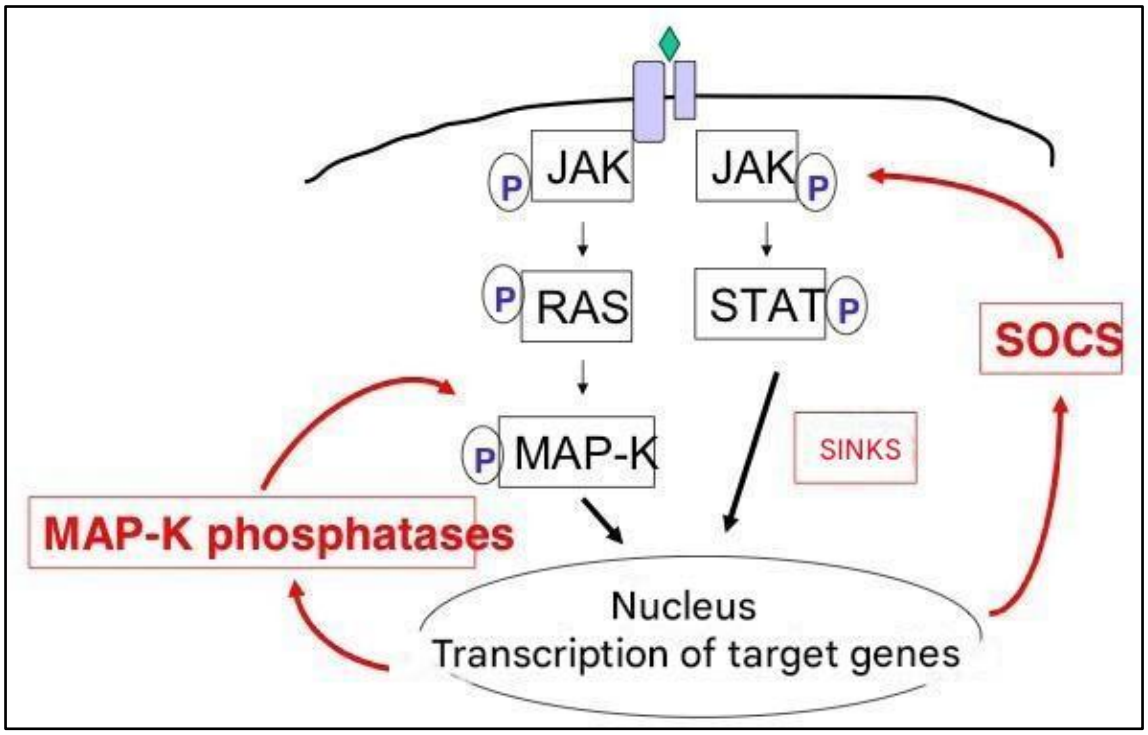


Figure 15: Inhibition of the intracellular signaling pathway JAK/STAT and MAPK**(73)**

- MAPK phosphatases for the MAPK pathway
- SOCS for the STAT track
- PIAS for the STAT route

IV.8. Phosphatidylinositol-3-kinase/Akt (PI3K/Akt) pathway

The phosphatidylinositol-3-kinase (PI3K) pathway is one of the signaling pathways that opens downstream of the interaction of a growth factor with a receptor tyrosine kinase (RTK). It therefore follows a path parallel to the MAP kinase pathway. Like the latter, it is implemented following the recognition of a phosphotyrosine of the receptor activated by an adaptor protein and involves sequential activations of kinases leading to multiple effects on the transcription of genes involved in cell proliferation, differentiation and survival **(73)**.

This pathway is interconnected in particular with the MAP kinase pathway at the RAS level; it is also capable of integrating metabolic and nutritional signals that serve to link cell growth and proliferation to nutrient availability. It is one of the major pathways of insulin action.

- **From phosphatidylinositol-3-kinase to AKT proteins**

The main phosphatidylinositol-3-kinase (PI3 kinase) is a heterodimer composed of two subunits, a catalytic subunit (p110, PIK3CA) carrying lipid kinase activity, and a regulatory subunit (p85, PIK3R1) with an SH2 domain that allows it to recognize phosphotyrosines of activated RTKs, and to transmit this activation to the catalytic subunit. Indirectly, the activation of p85 can occur via an adaptor protein, IRS1 or 2 (Insulin receptor substrate 1 or 2), itself phosphorylated by certain activated receptors such as IGF1R (Insulin-like growth factor 1 receptor), and recognized by an SH2 domain of p85 **(73).**

PI3 kinase can also be activated by the RAS adaptor protein that we saw in the MAP kinase pathway. Indeed, the catalytic subunit has, on the C-terminal side, a recognition domain for activated RAS. This is a major interconnection between the two signaling pathways, and the therapeutic consequences of this interconnection are important in the context of targeted therapies. Finally, PI3 kinase can also be activated by G protein-coupled receptors (GPCRs).

PI3 kinase ensures the 3-phosphorylation of a particular membrane lipid, phosphatidylinositol-4,5-diphosphate, in order to transform it into phosphatidylinositol-3,4,5-triphosphate. Unlike the signaling pathway involving a phospholipase C, which releases a triphosphoinositol (IP3) as a second messenger, it is the presence of the phosphate in 3 of inositol which constitutes the message itself, because this phosphate is likely to be recognized by proteins possessing a particular domain called PH (Pleckstrin-homology domain) **(73)**.

There are actually four class I PI3 kinases, denoted PIK3CA to PIK3CD, with precise tissue and functional specificities, which have a p110α, β, γ or δ catalytic subunit and which are activated by various types of regulatory subunits. These PI3 kinases differ in particular in their ability to be activated by an RTK, a GPCR, a cytokine, an integrin and/or a RAS protein. The functions of class II and III PI3 kinases are less well understood than those of class I PI3 kinases and concern membrane trafficking and receptor internalization **(73)**.

They also differ in their precise lipid substrate from the inositide class.

The presence of a 3-phosphate group on inositide allows the recruitment to the membrane of PH-domain protein kinases, in particular PDK1 (Phosphoinositide-dependent kinase 1) and the protein AKT, formerly called protein kinase B (PKB).

Probably due to a specific arrangement at the membrane level, linked to the recognition of phosphoinositide, PDK1 is likely to phosphorylate and activate an AKT protein. This phosphorylation occurs on threonine 308, at the catalytic site. The AKT protein is responsible for the activation of multiple effectors **(74)**(**figure 16**).

There are three isoforms of Akt named Akt1, Akt2 and Akt3. All Akt isoforms share a plekstrin homology (PH) domain that preferentially binds to PIP3. This interaction between the PH domain of Akt and PIP3 leads to conformational changes in Akt that lead to the unmasking of two phosphorylation sites (Thr308 and Ser473). Akt phosphorylation is then regulated by the protein kinase PDK1 (3'-phosphoinositide-dependent kinase 1) which is also recruited by its PH domain to the PIP3 production site.

Once activated, Akt phosphorylates various substrates that are involved in the regulation of cell proliferation, survival and metabolism **(75)**(**figure 17**).

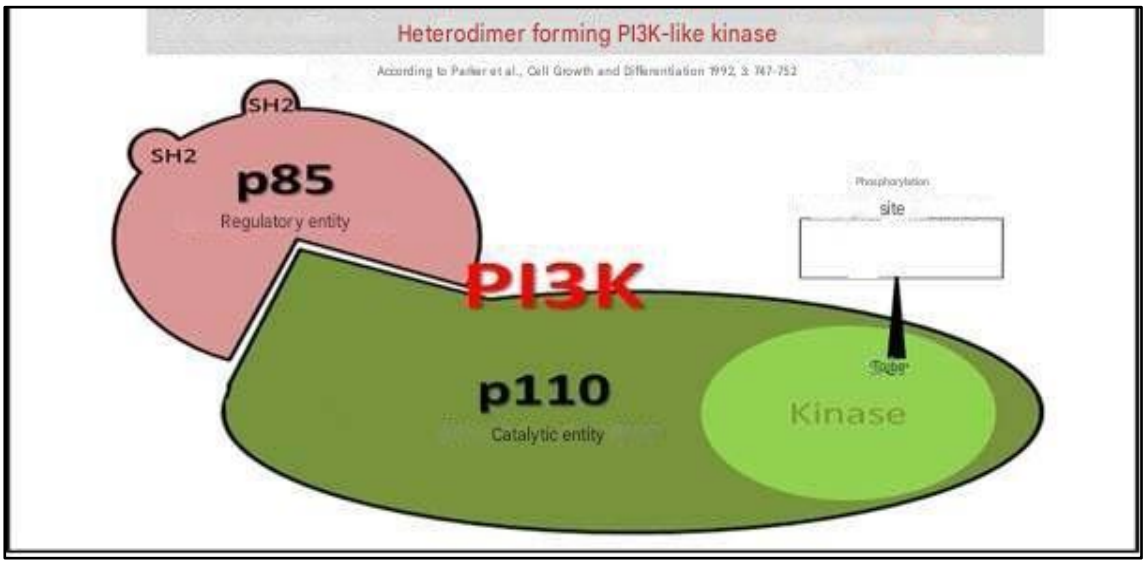


Figure 16: Schematic representation of the structure of PI3K-like kinase *(74)*

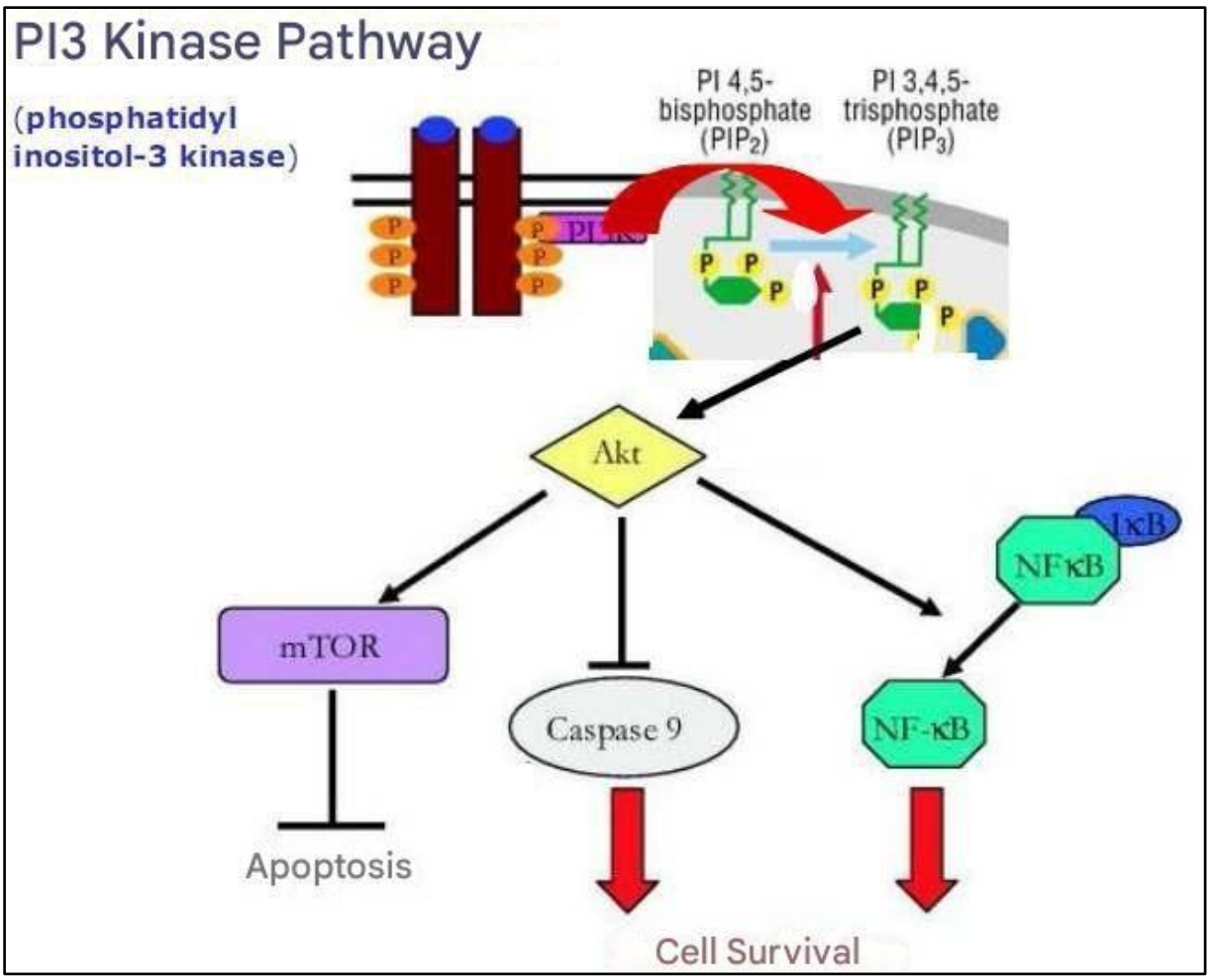


Figure 17:PI3 kinase pathway**(75)**

V. GENERAL CONCLUSION

Cell communication and signaling are fundamental processes that regulate the functioning of living organisms, ensuring coordination between cells and their environment. These mechanisms are essential for maintaining homeostasis, responding to external and internal stimuli, and for the development and survival of multicellular organisms.

Cell signaling systems rely on complex interactions between ligands, receptors, intracellular messengers, and molecular effectors. The diversity of signaling pathways, including those dependent on G proteins, receptor tyrosine kinases, and ion channels, allows for a wide variety of cellular responses tailored to physiological needs.

One of the most remarkable aspects of cell signaling is its specificity and fine regulation. Cells are able to integrate multiple signals simultaneously, thanks to modulation mechanisms such as phosphorylation, methylation, and ubiquitination. Furthermore, feedback loops and interactions between different signaling pathways ensure the precision of biological responses and avoid pathological disruptions.

However, alterations in these signaling mechanisms can lead to various pathologies, including cancer, autoimmune diseases, and neurodegenerative disorders. For example, deregulation of the MAPK, PI3K/AKT, or Notch pathways is frequently implicated in tumor transformation and cancer progression. The in-depth study of these pathways now makes it possible to develop targeted therapies aimed at specifically inhibiting the molecules involved in these deregulations.

Furthermore, recent advances in molecular biology and biotechnology have led to a better understanding of signaling mechanisms and their therapeutic modulation. The rise of approaches such as transcriptomics, proteomics, and genome editing (CRISPR-Cas9) opens new perspectives in the manipulation of signaling pathways to treat various pathologies.

Cellular communication and signaling are dynamic and complex processes that play a crucial role in the biology of living organisms. A thorough understanding of these mechanisms remains a major challenge in cell biology and medicine, offering promising prospects for the development of new targeted and personalized therapeutic strategies.

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