GROWTH FACTORS AND THEIR RECEPTORS

DR ANJUM

OBJECTIVES

- Enumerate various **growth factors** and their **receptors**
- Describe the **most common pathways** by which growth factors affect tissue repair and regeneration.

INTRODUCTION

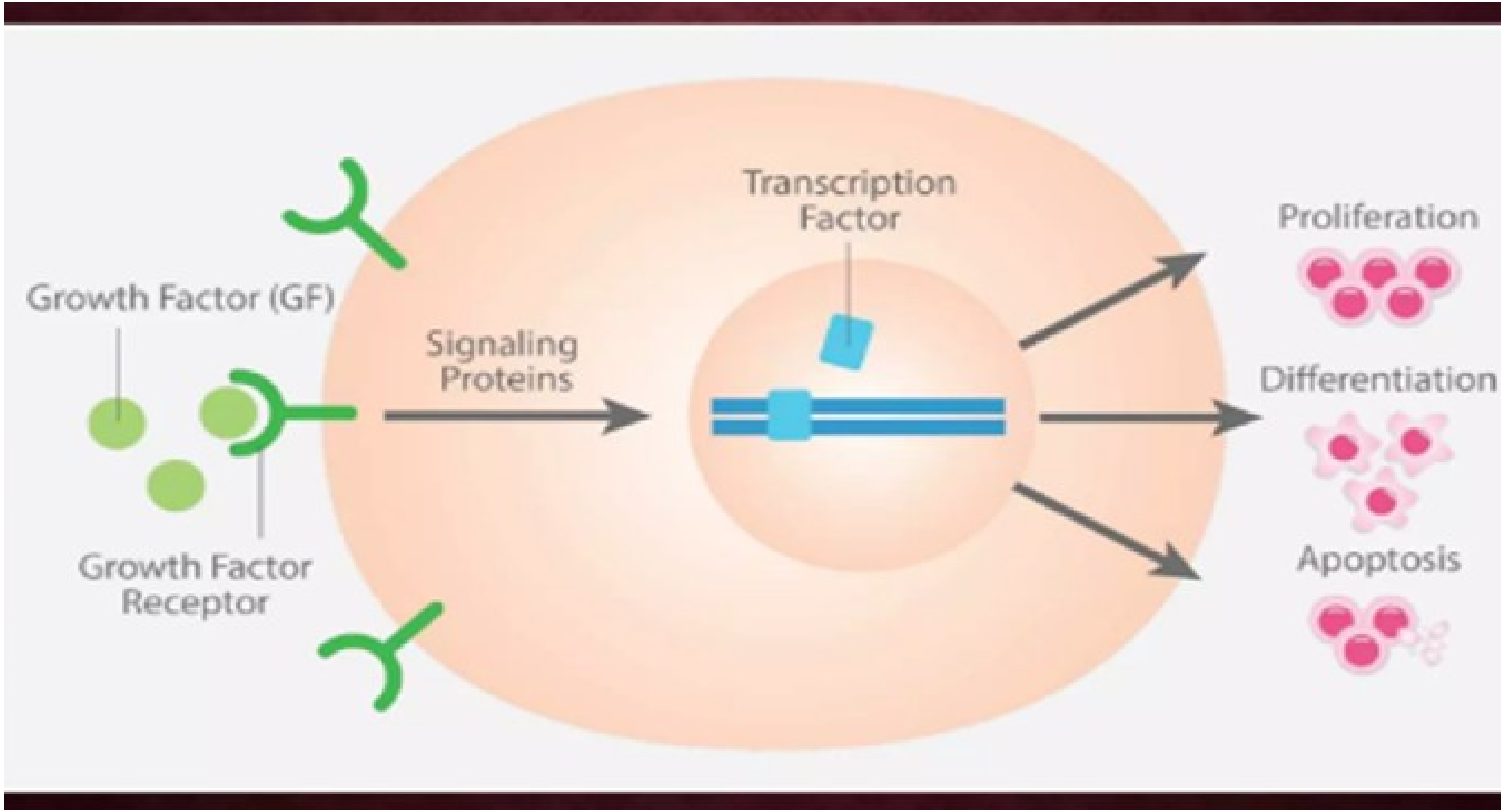
- **GROWTH FACTORS (TROPHIC FACTORS)** …. Are substances that promote cell growth.
- Growth factors are proteins that binds to their respective receptors on the cell surface…… Activation cellular proliferation and / or differentiation.
- Growth factors are molecules that function as both growth stimulator (MITOGENS) and inhibitor (NEGATIVE GROWTH FACTORS) as well.

INTRODUCTION

- **Growth factors** ….. Stimulate cell migration, act as chemotactic agents , inhibits cell migration, inhibit invasion of tumor cells .
- Modulate differentiated function of cells .
- Involved in **Apoptosis**.
- Involved in **Angiogenesis** and promote survival of cells without influencing growth and differentiation.
- **EXAMPLES……**EGF, FGF, PDGF, VEGF, IGF, TGF, ERYTHROPIEITIN

INTRODUCTION

- **Growth factors** are **polypeptides** that function as HORMONES – like regulatory signals controlling growth and differentiation.
- Growth factors are essential for cell cycle.
- These are subsets of **CYTOKINES** (diffusible signaling proteins) , stimulating growth, differentiation, survival, inflammation, and tissue repair.
- **GFs** are captured by their respective receptors.
- Interacts with membrane and cytoplasmic bound components cause alterations in gene expression of a cell …. **INDUCTIVE AGENT.**


Growth Factor (GF)
Growth Factor Receptor
Signaling Proteins
Transcription Factor
Proliferation
Differentiation
Apoptosis

INTRODUCTION

- **GROWTH FACTOR** produced by one cell and acts on the other cells…… PARACRINE REGULATION.
- Process that recaptures its own product is known as….AUTOCRINE REGULATION
- Few growth factors act during embryogenesis.

GROWTH FACTORS

- Epidermal growth factor (**EGF**)
- Platelet derived growth factor **(PDGF**)
- Fibroblast growth factors **(FGTs**)
- Transforming growth factor alpha and beta (**TGF- α, TGF-β**)
- Erythropoietin (**EPO**)
- Insulin- like factor 1 and II (**IGF- I and II**)
- Interleukin **(IL**) 1, 2 , 6 and 8
- Tumor Necrosis factor alpha and Beta **(TNF- α and TNF- β**)
- Interferon-g (**INF –g**)
- Colony stimulating factors (**CSFs**)

EPIDERMAL GROWTH FACTOR (EGF)

- **EGFs** ….. Are proteins that are found both in humans and other animals such as mice.
- **In humans**…..located in various tissues of the body.
- **Most common**…. Parotid glands and sub-mandibular glands.
- Also found in **various fluids** through out body …… urine, milk, blood plasma, and saliva.
- **More commonly present in male bodies than female**.

EGF and TGF -α

- **Both belong to EGF family and share common receptor.**
- **EGF** is mitogenic for variety of epithelial cells, hepatocytes and fibroblasts.
- **In healing wound** …..EGF produced by keratinocytes, macrophages and other inflammatory cells migrated to injured area.
- **EGF** binds to a receptor ….. EGFR with tyrosine Kinase activity.

EGF and TGF -α

- **TGF- α....**was originally extracted from sarcoma-virus transformed cells , and involved in epithelial cell proliferation in embryos and adults
- **TGF- α....**also involved in malignant transformation of normal cells to cancer.
- **TGF- α.....bind to EGFR like EGF**
- **EGRF** is referred as EGFR 1 or ERBB1
- **ERBB-2** ....also known as HER-2/ Neu is over expressed in breast cancers and is therapeutic target.

HEPATOCYTE GROWTH FACTOR ….HGF

- **HGF**….originally isolated from platelets and serum.
- Has **mitogenic effects in most epithelial cells i**ncluding Hepatocytes , cells of biliary epithelium in liver, epithelial cells of lungs , mammary glands , skin and other tissues.
- **HGF**….also produced by Fibroblasts, endothelial cells and liver parenchymal cells.
- **RECEPTOR**….. Product of proto-oncogene c-MET ….frequently over expressed **in tumors**

VASCULAR ENDOTHELIAL GROWTH FACTOR…….VEGF

1. VEGF (Vascular Endothelial GF) especially VEGF-A …….most important growth factor undergoing Physiological Angiogenesis …. proliferating endometrium.

- As well as Angiogenesis occurring in Chronic Inflammation, wound healing, tumors and Diabetic Retinopathy.
- **VEGF ……SECRETED BY MESENCHYMAL and STROMAL CELLS**… Stimulates both migration and proliferation of endothelial cells
- **VEGFR-2** ……**A Tyrosine Kinase receptor** ……most important in angiogenesis , expressed by endothelial cells and their precursors and also by tumor and other cells type

VASCULAR ENDOTHELIAL GROWTH FACTOR…….VEGF

- VEGF - (Angiogenesis and)
  increased vascular permeability

  ↓

  Exudation and deposition of plasma proteins
  Provides a stroma for the proliferating endothelial cells and fibroblasts

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

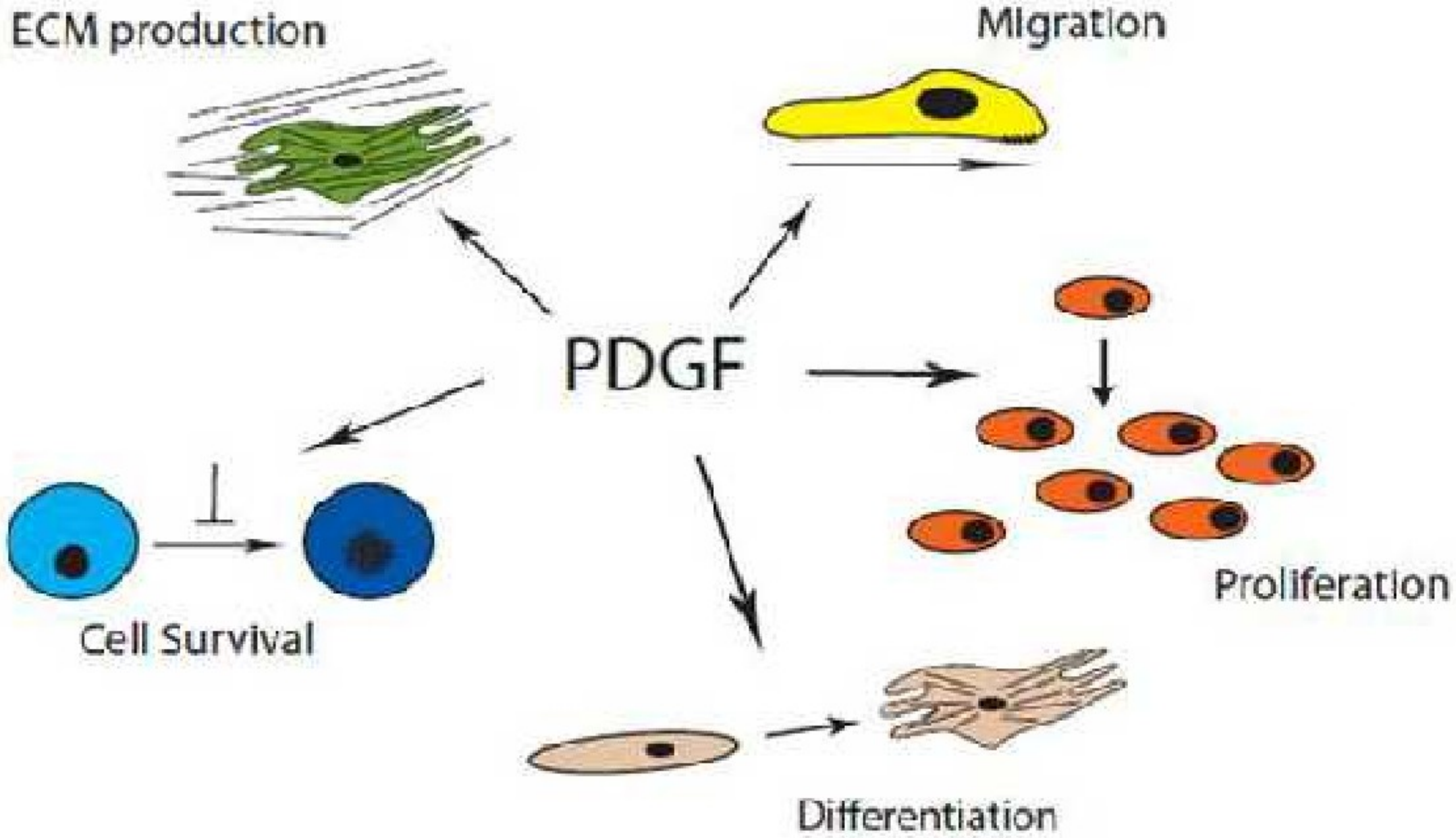
Proteins	Family members: VEGF (VEGF-A), VEGF-B, VEGF-C, VEGF-D Dimeric glycoprotein with multiple isoforms Targeted mutations in VEGF result in defective vasculogenesis and angiogenesis.
Production	Expressed at low levels in a variety of adult tissues and at higher levels in a few sites, such as podocytes in the glomerulus and cardiac myocytes
Inducing agents	Hypoxia TGF-β PDGF TGF-α
Receptors	VEGFR-1 VEGFR-2 VEGFR-3 (lymphatic endothelial cells) Targeted mutations in the receptors result in lack of vasculogenesis
Functions	Promotes angiogenesis Increases vascular permeability Stimulates endothelial cell migration Stimulates endothelial cell proliferation VEGF-C selectively induces hyperplasia of lymphatic vasculature Up-regulates endothelial expression of plasminogen activator, plasminogen activator inhibitor 1, and collagenase

PLATELET DERIVED GROWTH FACTOR ….PDGF

- **Platelet-derived growth factor** (**PDGF**) …. is one among numerous growth factors, that regulate cell growth and division.
- A dimeric glycoprotein ……composed of two A subunits **(PDGF-AA**), two B subunits (**PDGF-BB),** or one of each (**PDGF-AB).**
- PDGF…… is a potent mitogen for cells of Mesenchymal origin, including fibroblasts , smooth muscle cells and glial cells.
- **RECEPTOR…….** **PDGFR** is classified as a receptor tyrosine kinase (RTK), a type of cell surface receptor. Two types of PDGFRs have been identified: **alpha-type and beta-type PDGFRs**

PLATELET DERIVED GROWTH FACTOR ….PDGF

- A family of molecules **released from platelets**.
- Forms of platelet-derived growth factor help to heal wounds and to repair damage to blood vessel walls.
- They also help blood vessels grow
- The **α-granules of platelets** are a major storage site for PDGF.
- **PDGF** has important functions in certain organs during embryonic development


ECM production
Migration
PDGF
Cell Survival
Proliferation
Differentiation

FIBROBLAST GROWTH FACTORS…..FGF

- **FGFs**…… large family of secretory molecules, that act through tyrosine kinase receptors known as FGF receptors.
- **Roles**…….. a wide variety of cellular functions, including cell proliferation, survival, metabolism, morphogenesis, and differentiation, as well as in tissue repair and regeneration
- **Signaling pathways….** regulated by FGFs include **RAS/mitogen-activated protein kinase (MAPK**), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)–protein kinase B (AKT), phospholipase C gamma (PLCγ), and signal transducer and activator of transcription (STAT)

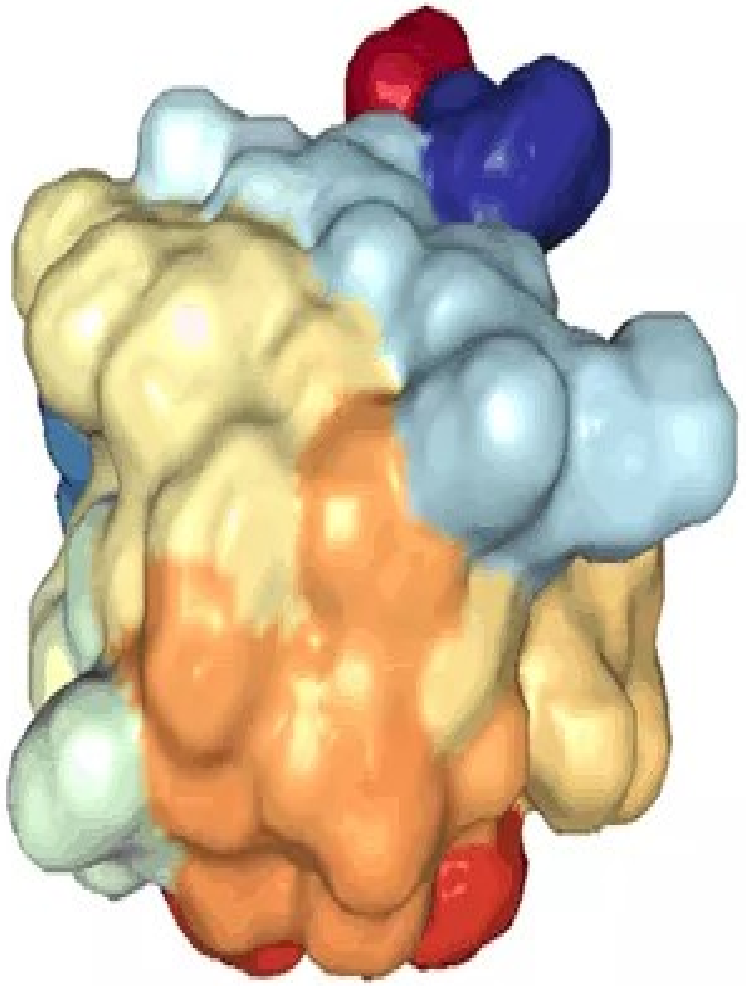
FIBROBLAST GROWTH FACTORS…..FGF

Fibroblast growth factor:

- Promotes synthesis of ECM proteins including fibronectin.
- Chemotactic for fibroblasts and endothelial cells
- Promotes angiogenesis

(A)

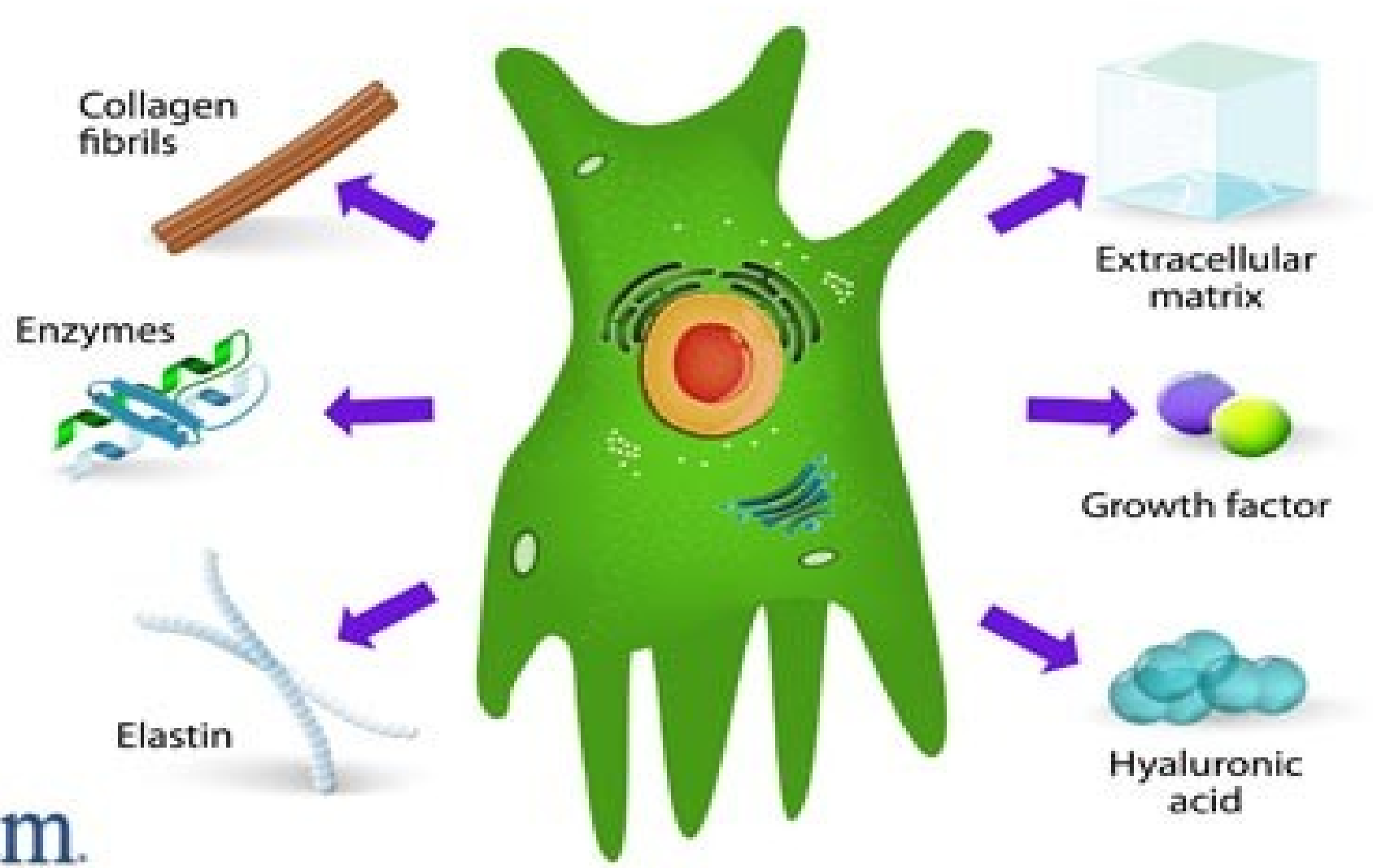
bFGF (a.k.a. FGF2)



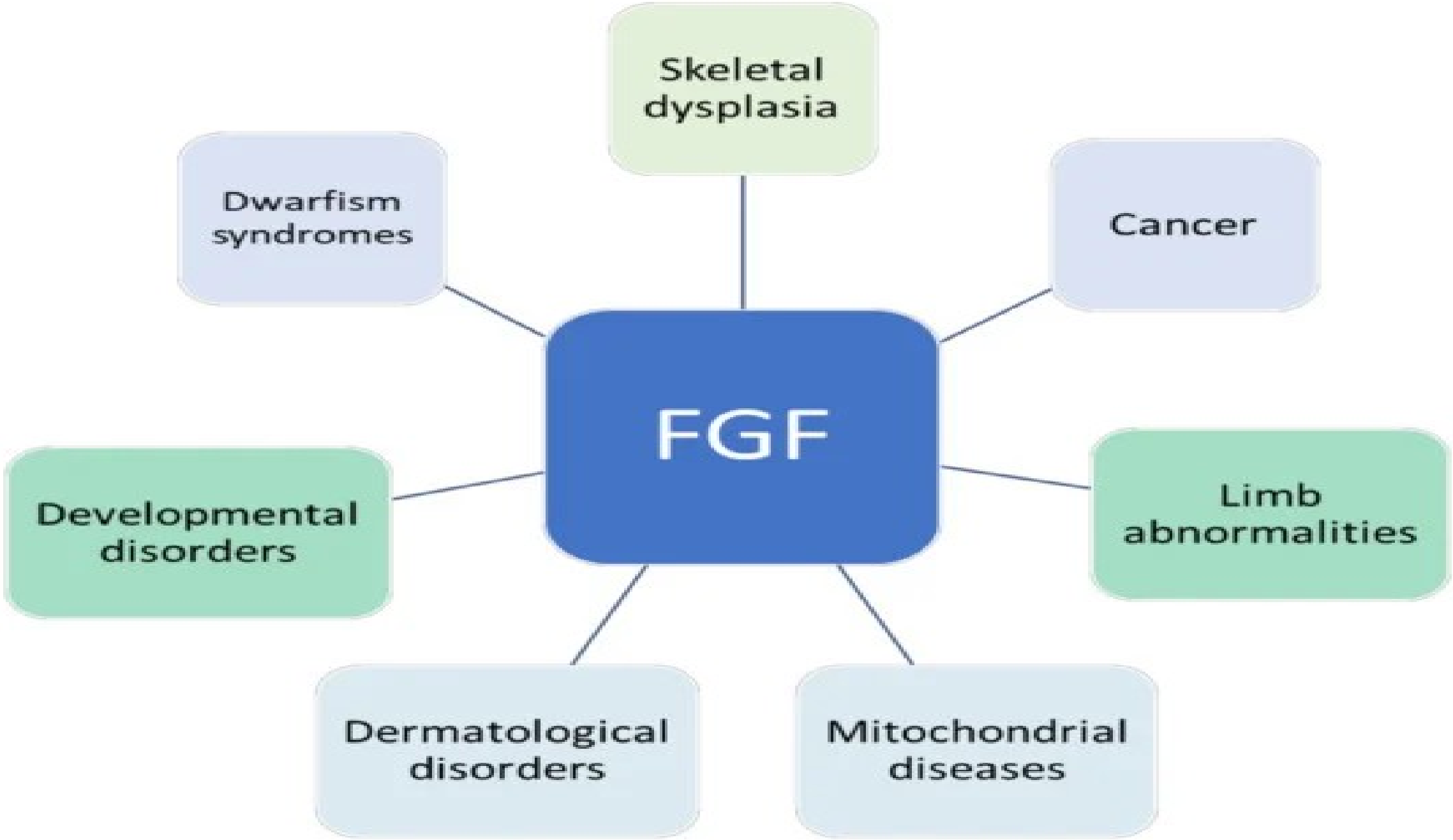
(B)

Function	Subfamily related to the function	Target cell
Cell proliferation	FGF1, FGF2	Preadipocyte
		Endothelial cell, epithelial cell,
		fibroblast cell, neural stem cell
	FGF4	Trophoblast stem cell
	FGF7, FGF10	Epithelial cell
	FGF18	Osteoblast, chondrocytes, osteoclast
Cell migration	FGF2	Astrocyte, myogenic cell
	FGF4	Myogenic cell
	FGF7	Epithelial cell, keratinocyte
	FGF8	Neural crest cell
Cell differentiation	FGF1, FGF2	Neuroepithelial
	FGF7	Keratinocyte
	FGF20	Monkey stem cell
Angiogenesis	FGF1, FGF2	Endothelial cell

FIBROBLAST



DrLam.
Body. Mind. Nutrition.


Skeletal dysplasia
Dwarfism syndromes
Cancer
FGF
Developmental disorders
Limb abnormalities
Dermatological disorders
Mitochondrial diseases

TGF- β and Related GROWTH FACTORS

- Is a multifunctional cytokine belonging to the transforming growth factor superfamily.
- Includes three different isoforms …… TGFB1, TGFB2, TGFB3.
- TGFB proteins are produced by all white blood cell lineages.
- Activated TGF-β complexes with other factors to form a serine/threonine kinase complex that binds to TGF-β receptors

TGF- β and Related GROWTH FACTORS

- **RECEPTORS** : TGF-β receptors are composed of both type 1 and type 2 receptor subunits.
- After the binding of TGF-β…. the type 2 receptor kinase phosphorylates and activates the type 1 receptor kinase that activates a signaling cascade.
- Leads to the activation of different regulatory proteins, inducing transcription of different target genes that function in differentiation, Chemotaxis, proliferation, and activation of many immune cells

TGF- β and Related GROWTH FACTORS

FUCTIONS:

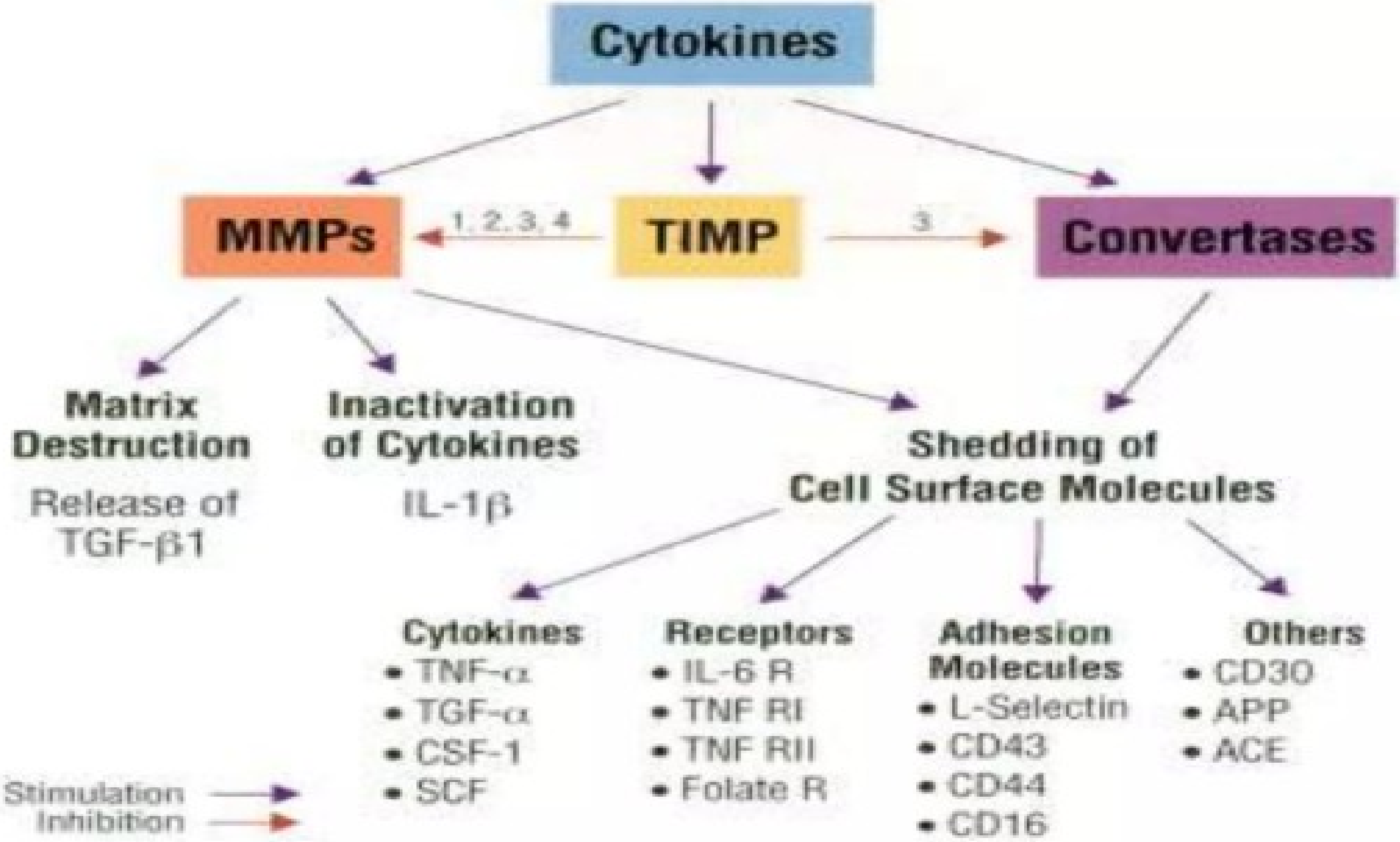
- **TGF- β**….growth inhibitor for most epithelial cells types and for leukocytes.
- **Potent fibrogenic agent**……. Stimulates fibroblasts Chemotaxis
- Enhances production of collagen , fibronectin, proteoglycans.
- **Inhibits collagen degradation** by decreasing matrix proteases.
- Involved in development of fibrosis in chronic inflammation especially in lungs, kidneys, and liver.
- Strong **anti-inflammatory effect**

MATRIX METALLOPROTEINASES …..MMPs

- **MMPs**….. Degrade ECM depends on Zn and Ca+ for their activity.
- **TIMPS** (Tissue Inhibitor of Metalloproteinase)
- **MMPs**….also have important role as EFFICIENT PROCESSING ENZYMES of many mediators like Cytokines, Chemokines , growth factors and their receptors

CYTOKINES

- **Includes a family of molecules** …….are small proteins with either paracrine or endocrine functions , which are involved in local inflammation or immunoregulation.
- So , they are unique family of growth factors.
- **Secreted primarily from leukocytes**
- Cytokines **stimulates both humoral and cellular immune** responses as well as the activation of Phagocytic cells ……..
- **LYMPHOKINES**
- **MONOKINES**.


Cytokines
MMPs
1, 2, 3, 4
TIMP
3
Convertases
Matrix Destruction
Release of TGF-β1
Inactivation of Cytokines
IL-1β
Shedding of Cell Surface Molecules
Cytokines
• TNF-α
• TGF-α
• CSF-1
• SCF
Receptors
• IL-6 R
• TNF RI
• TNF RII
• Folate R
Adhesion Molecules
• L-Selectin
• CD43
• CD44
• CD16
Others
• CD30
• APP
• ACE
Stimulation
Inhibition

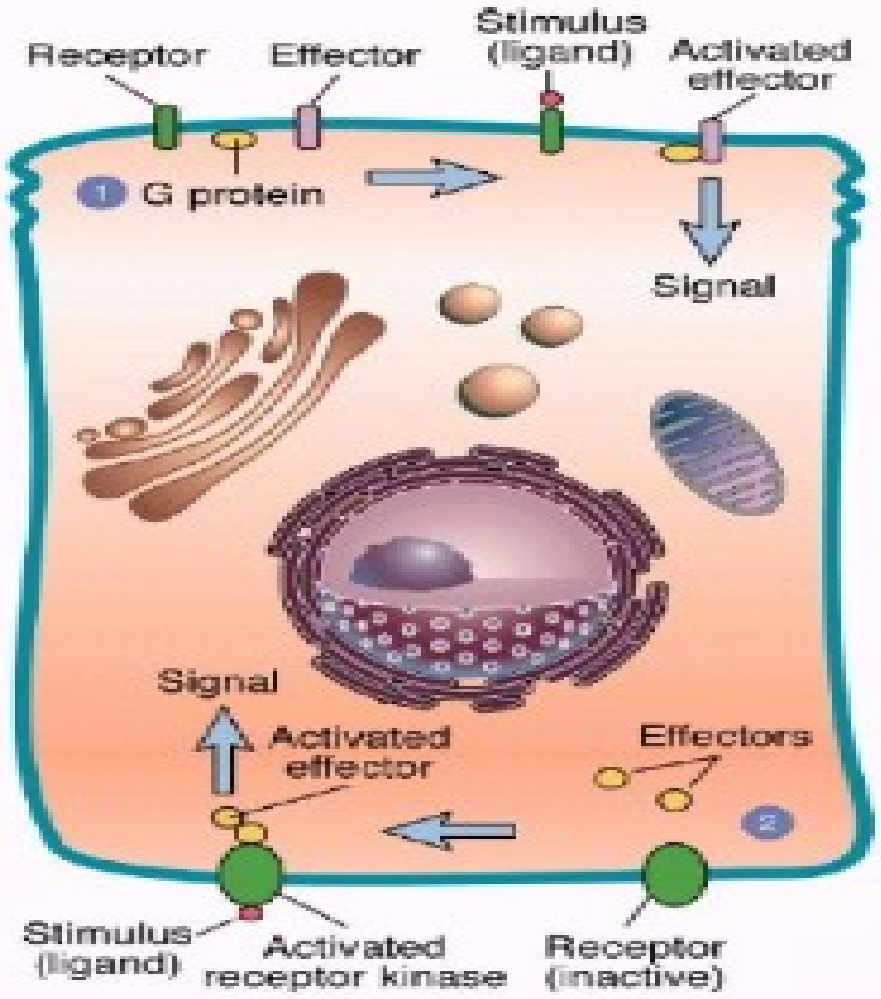
SIGNALING MECHANISM IN CELL GROWTH……INTRODUCTION

- Cells must be ready to respond to essential signals in their environment. These are often chemicals in the extracellular fluid (ECF) from:

distant locations -→ signaling by hormones;
nearby cells → cytokines;
or
even secreted by themselves .

- Long-range allostery is often a significant component of cell signaling events.

CELL SIGNALING and SIGNAL TRANSDUCTION



CELL SIGNALING is about communication between different groups of cells and tissues...how one group of cells informs another group of cells what to do.

SIGNAL TRANSDUCTION refers to how the presence of an extracellular signal can produce a change in the intracellular state of the cell without the initial signal crossing the membrane.

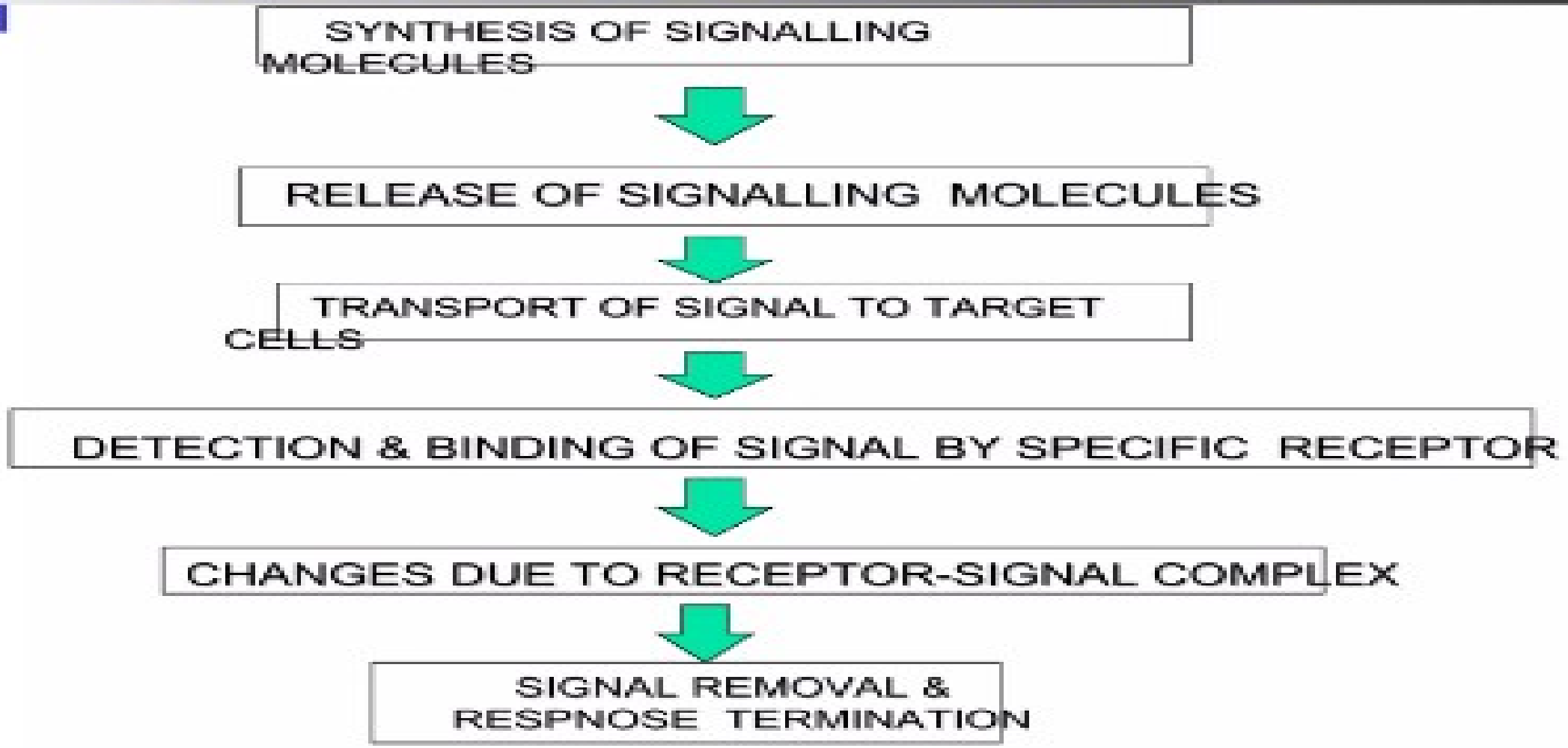
WHY CELLS COMMUNICATE ?

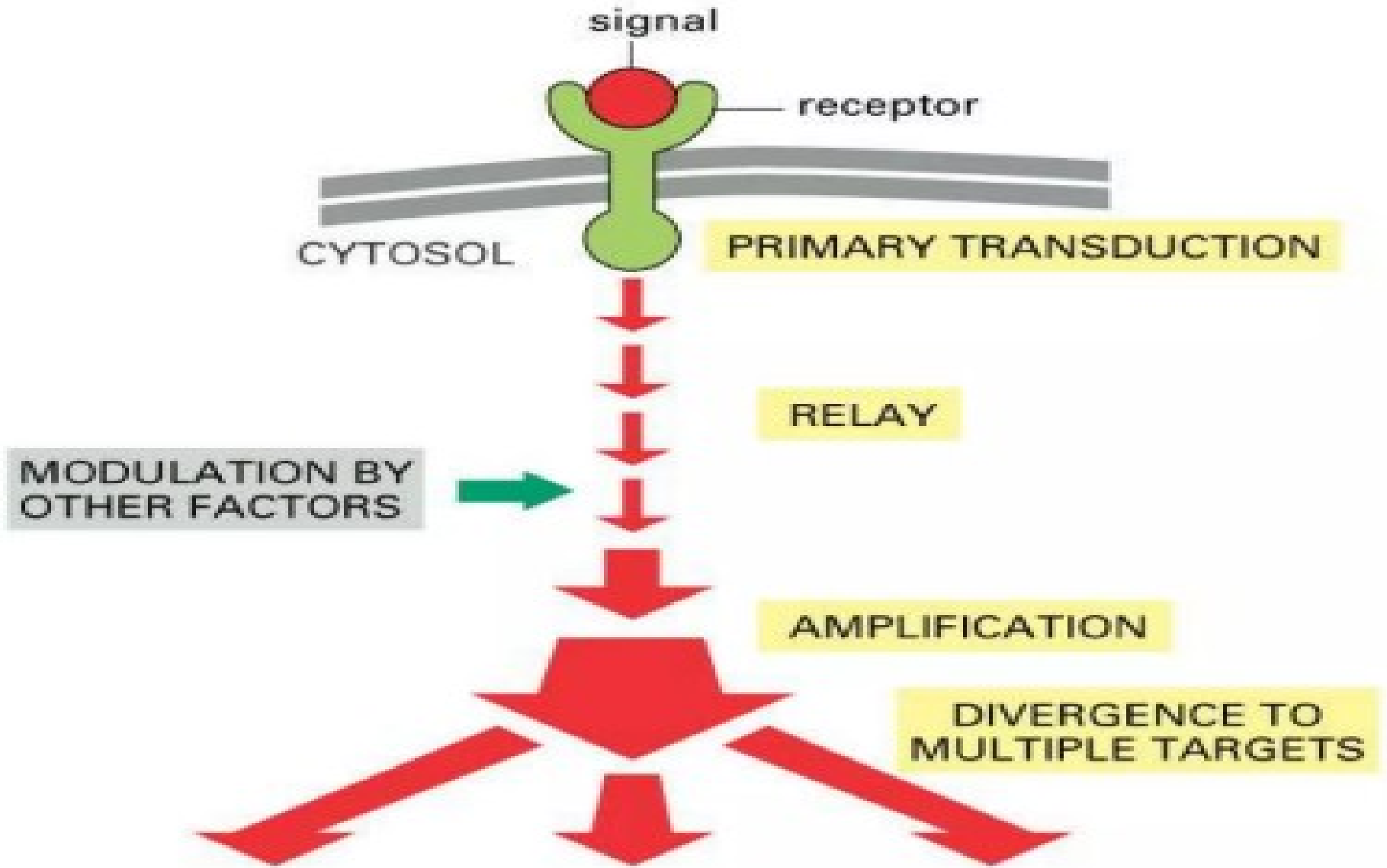
- During development, cells differentiate to adopt specialized roles
- Cells need to know whether to live, die or divide.
- Neurotransmission
- Regulation of metabolism
- Contraction – Expansion
- Secondary sexual characteristics

STEPS

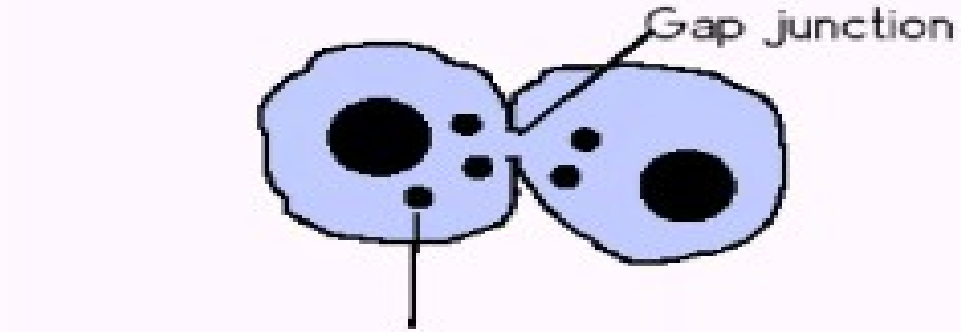
- Biosynthesis and release of the signal.
- Transport of signals to target cell
- Transduction in target cell.
- Alteration of cell growth and metabolism.
- Termination of signals

STEPS IN CELL SIGNALING



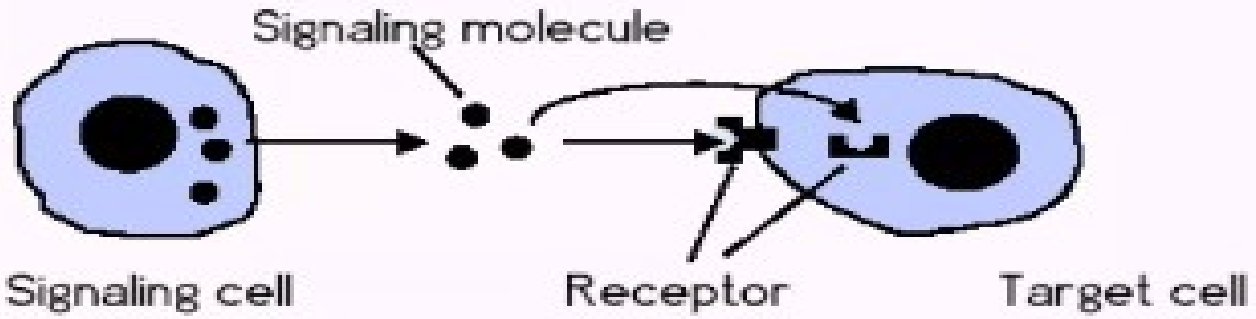

signal
receptor
CYTOSOL
PRIMARY TRANSDUCTION
RELAY
MODULATION BY
OTHER FACTORS
AMPLIFICATION
DIVERGENCE TO
MULTIPLE TARGETS

MODES OF SIGNALING



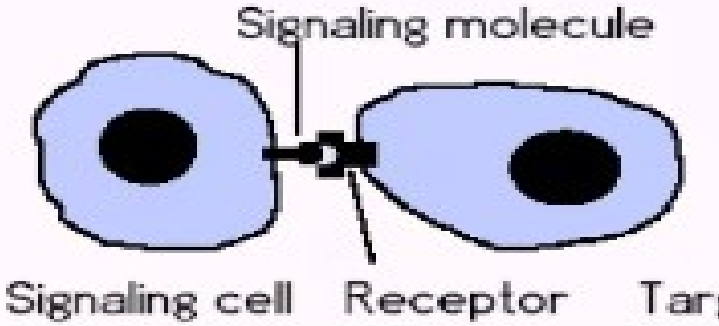
1

Signaling by cytoplasmic bridge



2

Signaling by secreted molecules



3

Signaling by plasma membrane-bound molecules

SIGNALING MECHANISM IN CELL GROWTH

- All growth factors bind to their specific receptors which deliver signals to the target cells.
- These signals have **two general effects** :

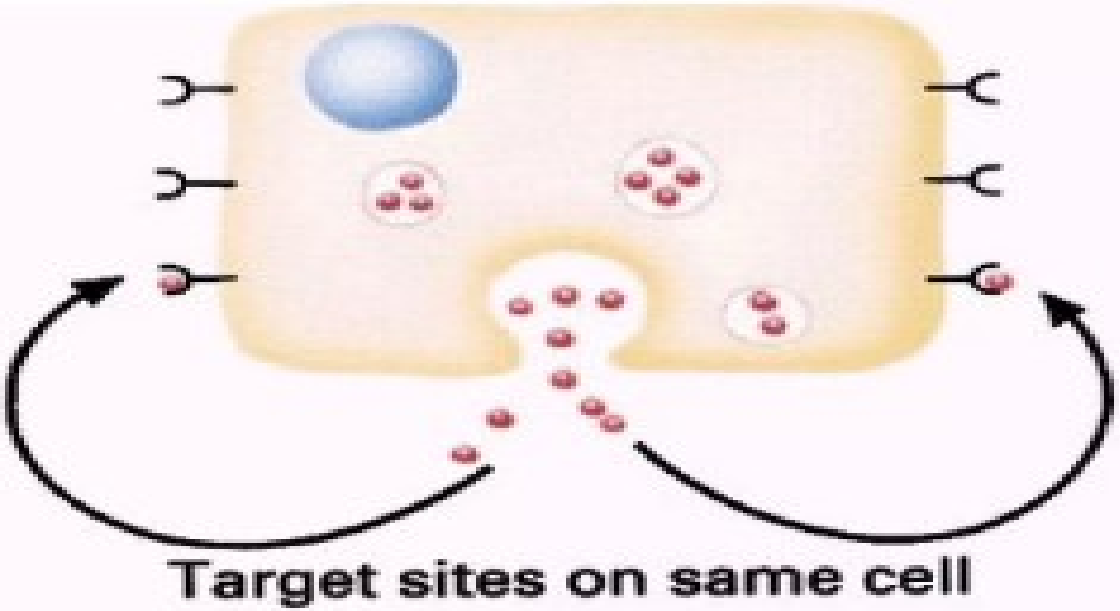
1. Stimulate the transcription of many genes , silent in the resting cells.
2. Several of these genes regulate the entry cells into the cell-cycle and their passage through various stages of cell cycle

SIGNALING MECHANISM IN CELL GROWTH

- **CLASSIFICATION OF INTRACELLULAR SIGNALING:**

- AUTOCRINE .....signals target the cell itself.....immune cells
- PARACRINE....signals target the cells in the vicinity of the emitting cell ..... neurotransmitters an example
- ENDOCRINE....signals target distant cells ..... Hormones
- JUXTACRINE....signals target adjacent (touching) cells....signals transmitted along cell membranes via protein or lipid component integral to the membrane , capable of affecting the emitter cell or cells immediately adjacent

AUTOCRINE SIGNALING



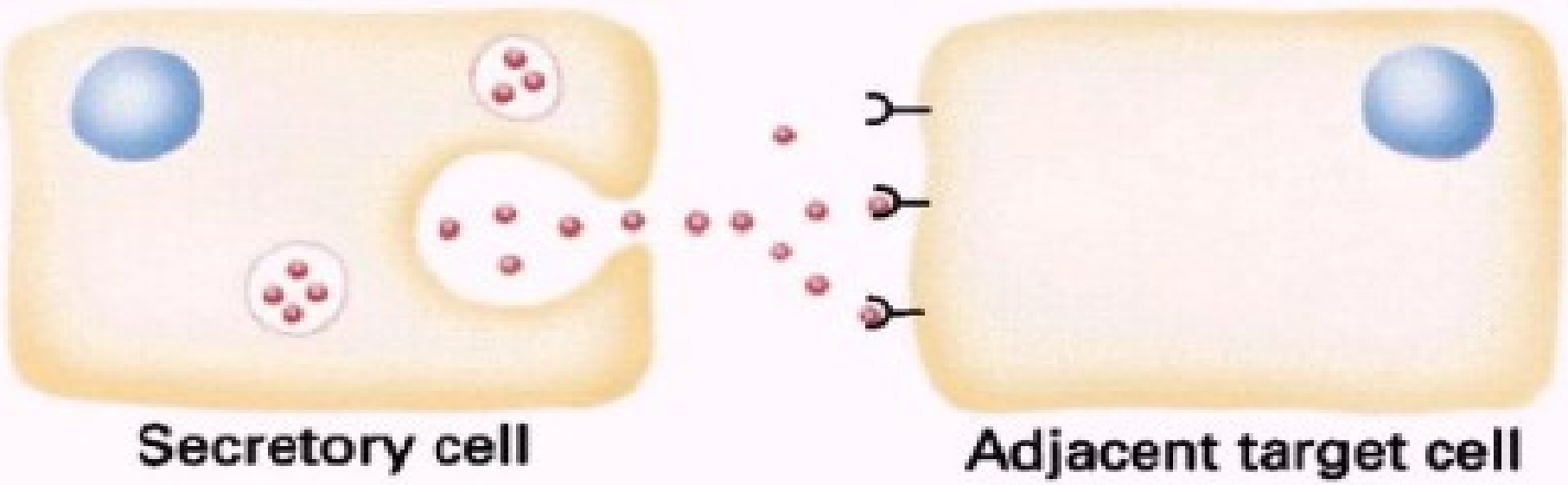
Extracellular signal

Receptor

Membrane-attached signal

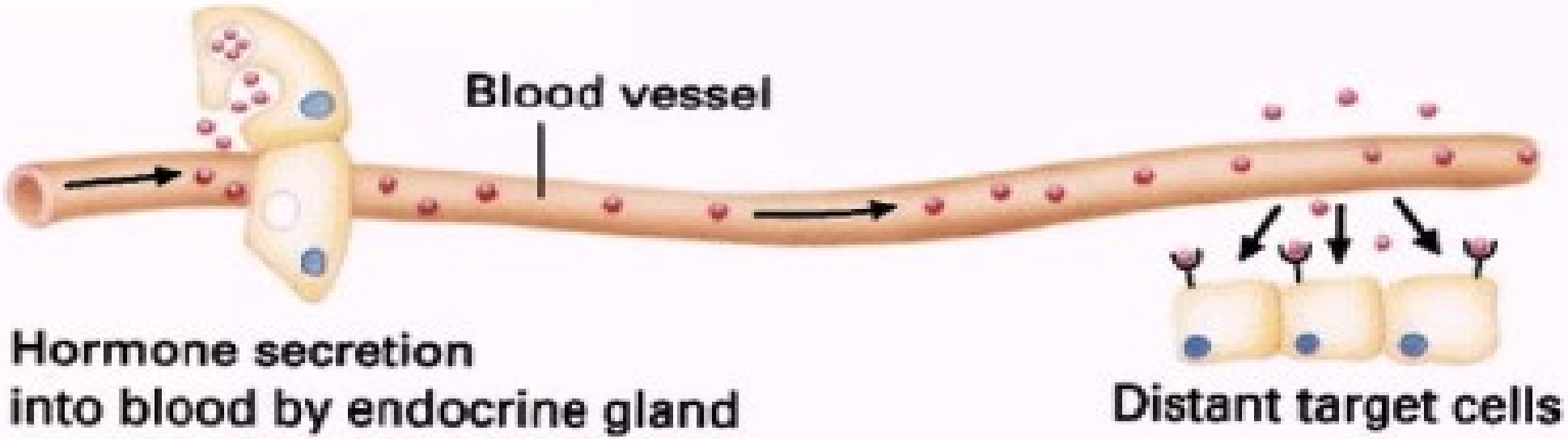
Examples: cytokines/immune cells, growth factors

PARACRINE SIGNALING



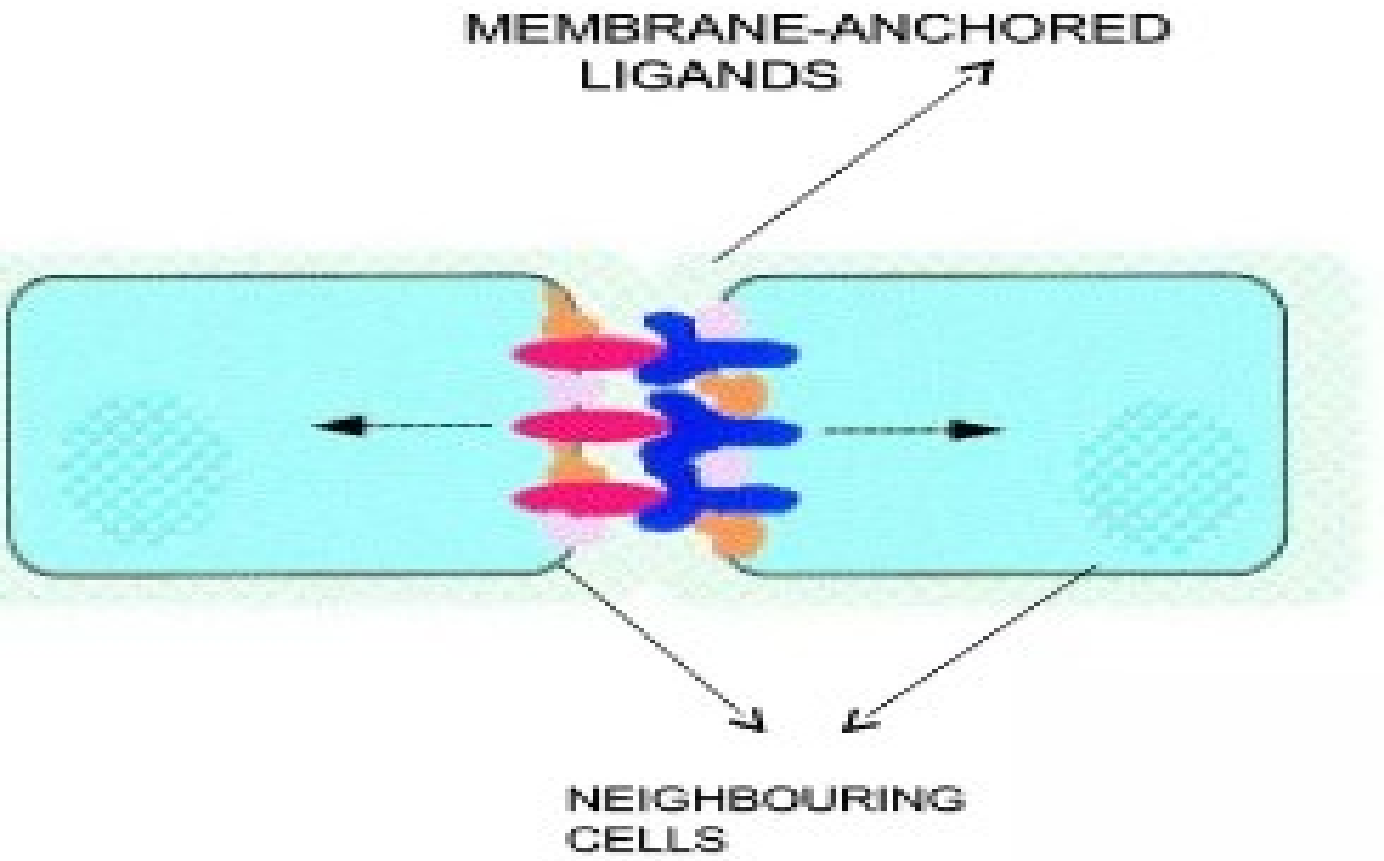
Examples: nerve–nerve, nerve–muscle cells, cytokines

ENDOCRINE SIGNALING



Examples: peptide and steroid hormones

JUXTACRINE SIGNALING

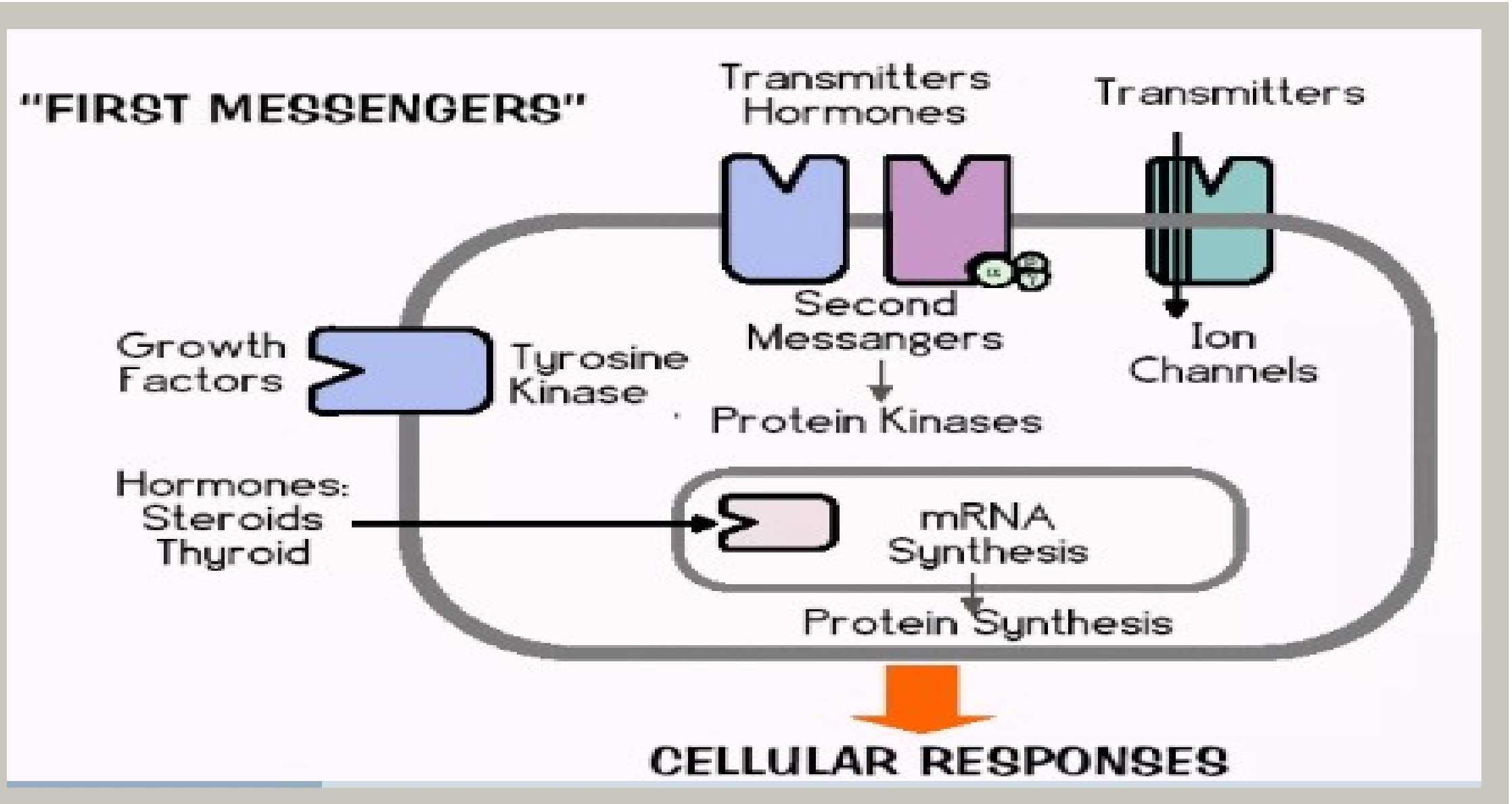


e.g.*Notch signalling.

SIGNALING MOLECULES

Type of molecule	Local mediator	Neurotransmitter	Hormone
Peptides	Kinin	Neuropeptides	Vasopressin
Polypeptides	---	---	Insulin
Amino acids and Derivatives	Histamine	Glutamate	Epinephrine
Fatty acid Derivatives	Prostaglandins Leukotrienes PAF	---	Testosterone
Other small Molecules	Cytokines Chemokines	Acetylcholine	---

CELL SIGNALING And SIGNAL TRANSDUCTION



SIGNAL TRANSDUCTION PATHWAYS

1. **SIGNAL RECOGNITION …….** • Ligand binding , cell contact
2. **SIGNAL TRANSDUCTION……** • Transfer of signals to cell interior
   • Modulate the activity of protein kinases and phosphatases
3. **RESPONSE……** • Phosphorylation state of targets
   • Modulation of effector activity
   • Reversibility of response

RECEPTORS ?????

- Are proteins associated with cell membranes or located within the cells .
- Receptors “ recognizes ” signaling molecules by binding to them.
- Binding of receptors by signaling molecules …… cell behavior changes

RECEPTORS CATEGORIES

Can be separated into **4** classes:

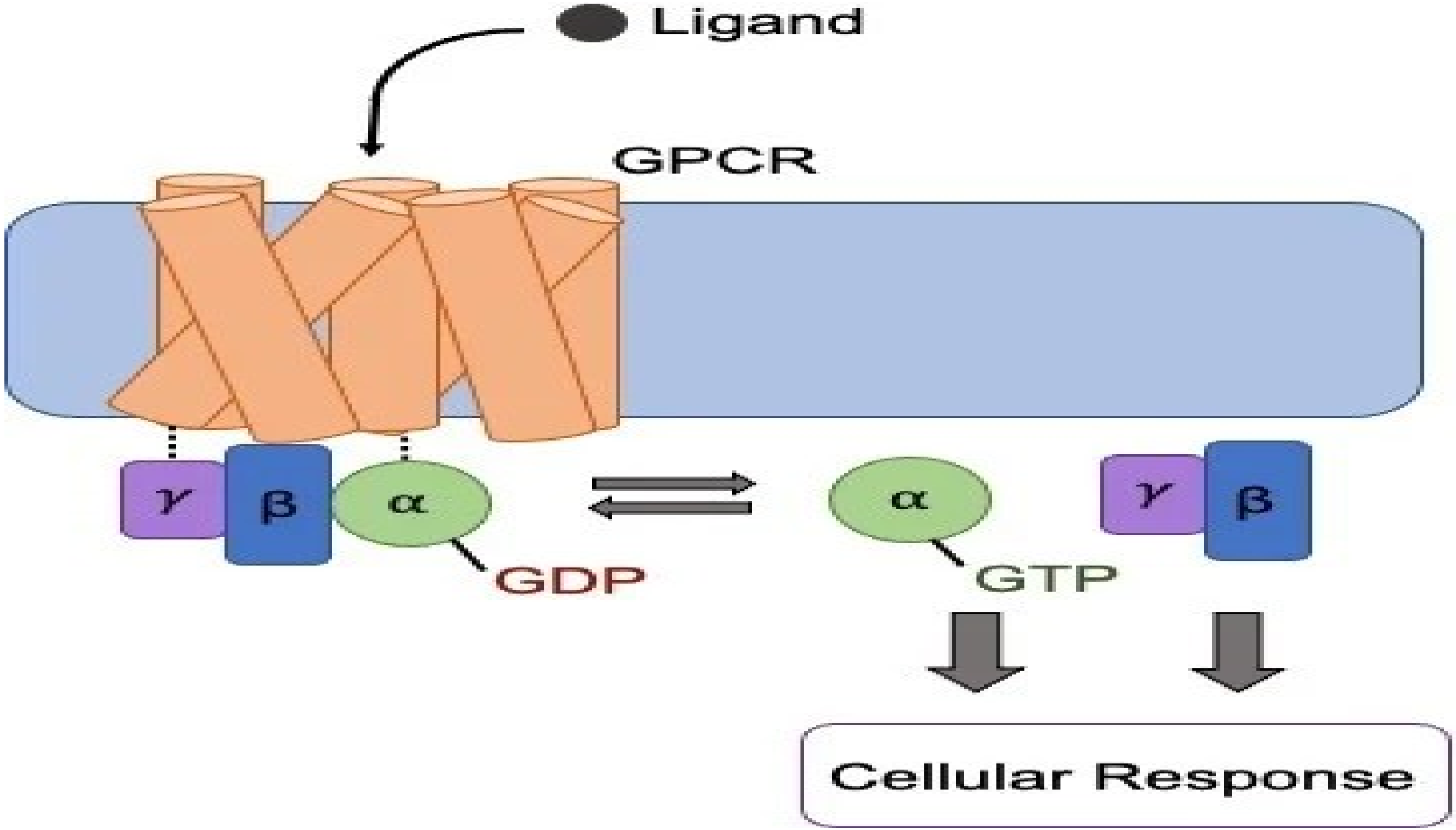
1. G protein-linked receptors (GPCRs)
2. Ion-channel receptors
3. Receptors lacking intrinsic catalytic activity but directly associated with cytosolic protein tyrosine kinases
4. Receptors with intrinsic enzymatic activity (RTKs)

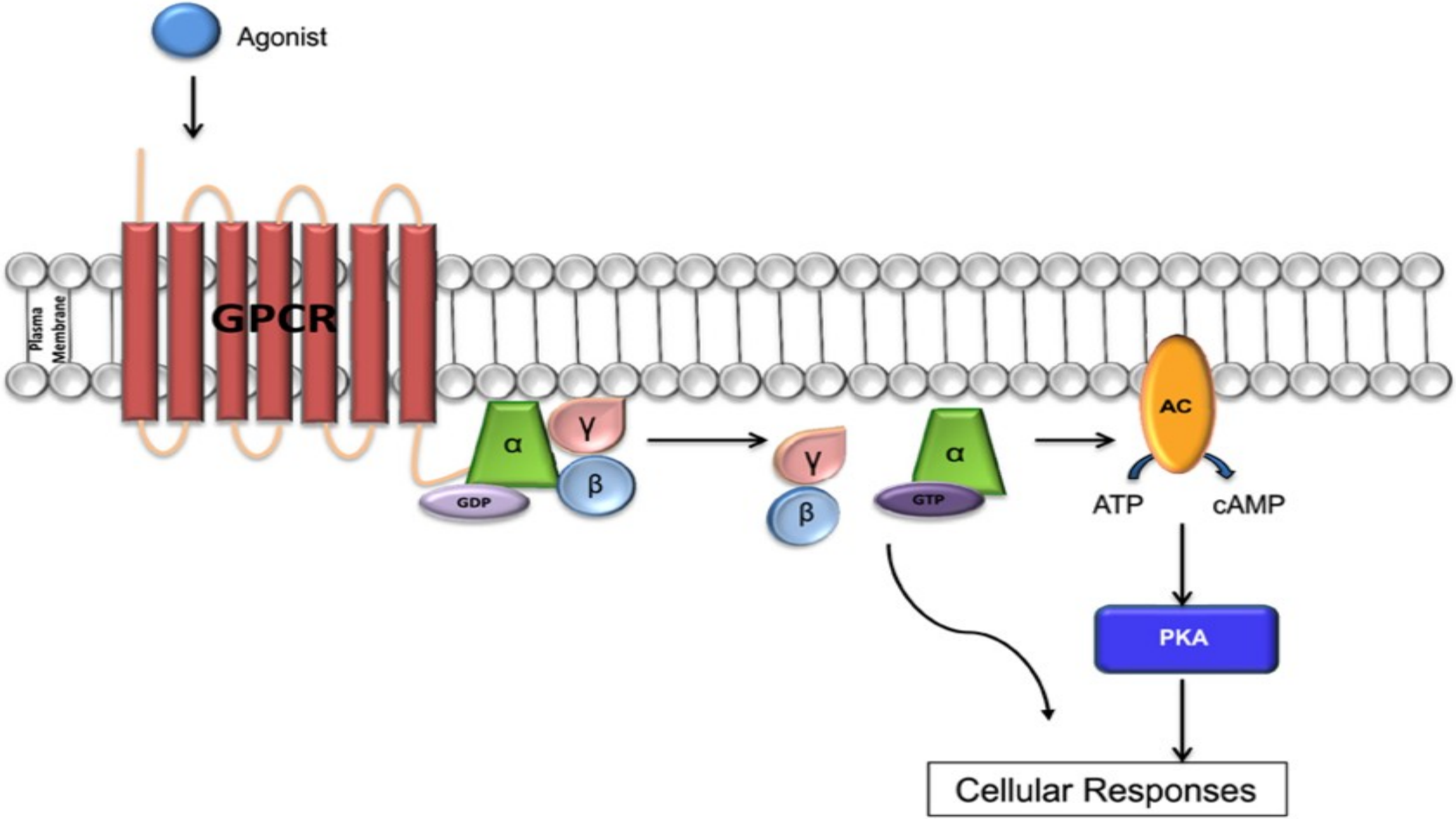
SEVEN TRANSMEMBRANE G - PROTEIN COUPLED RECEPTORS..... (GPCRs)

- So named as they contain **7 α – helices** .
- Constitute largest family of plasma membrane receptors .
- Transmit signals into the cell through trimeric GTP- binding proteins (G-proteins).
- Ligand signal through this..... Vasopressin, Serotonin, Histamine , Epinephrine, Norepinephrine, calcitonin , glucagon, PTH, and number of drugs as well .

SEVEN TRANSMEMBRANE G - PROTEIN COUPLED RECEPTORS ..... (GPCRs)

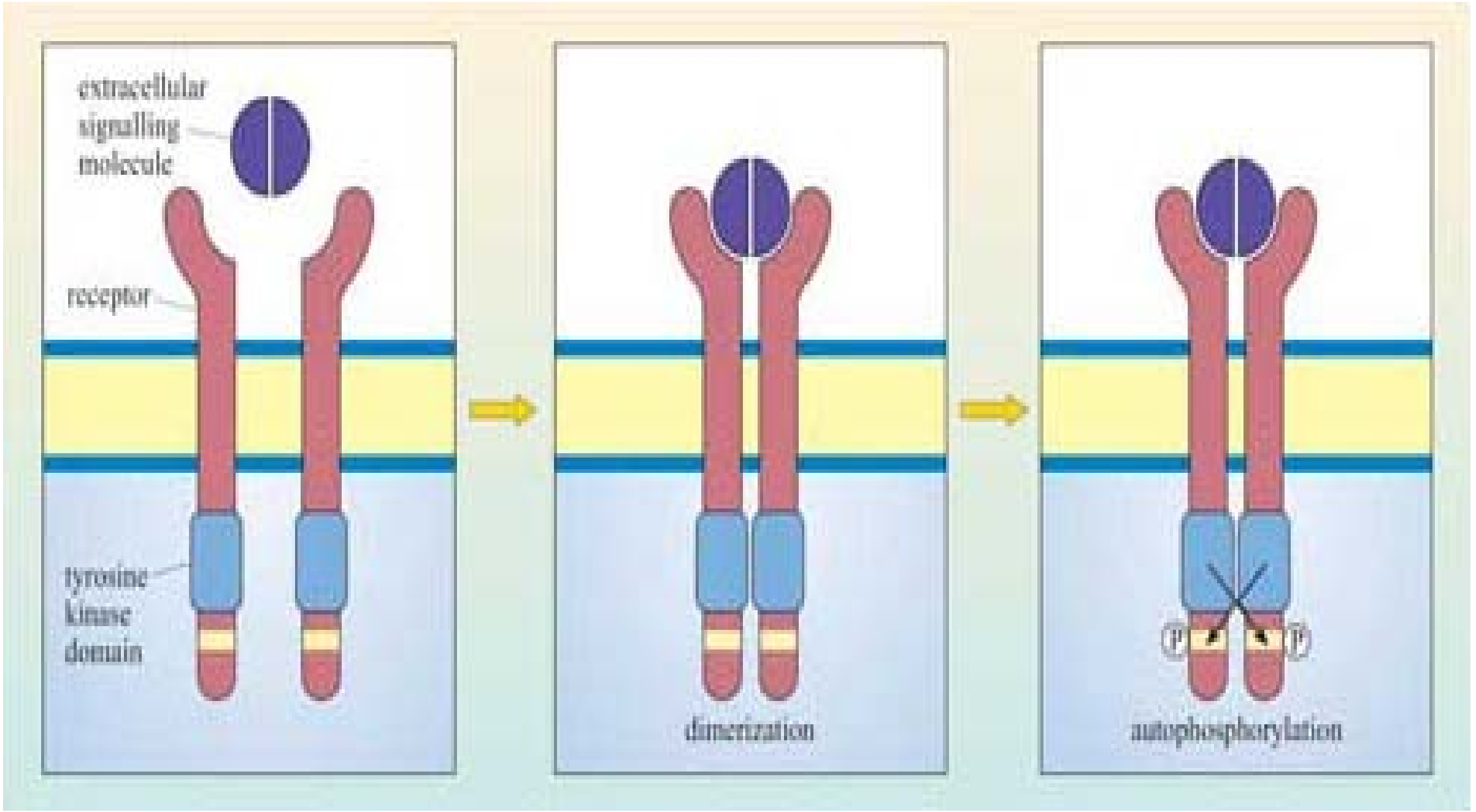
- Binding of ligand to receptor induces **activation of G- proteins**.
- By exchange **of GDP (inactive protein) to GTP (active form).**
- This signal transduction involves " calcium and adenosine 3', 5' cyclic monophosphate (c AMP) as second messenger".
- Leads to the production of Inositol 1, 4, 5 triphosphate (IP3)..... releasing Ca from Endoplasmic reticulum.
- Ca+ signal targets ..... cytoskeletal proteins , potassium and chloride ion pumps etc.
- Ca+ targets protein kinase A and cAMP gated ion channels.....important in **vision and olfactory sensing**


Ligand
GPCR
γ
β
α
GDP
α
GTP
γ
β
Cellular Response


Agonist
GPCR
Plasma Membrane
α
γ
β
GDP
γ
β
α
GTP
AC
ATP
cAMP
PKA
Cellular Responses

RECEPTORS WITH INTRINSIC TYROSINE KINASE ACTIVITY….RTKs

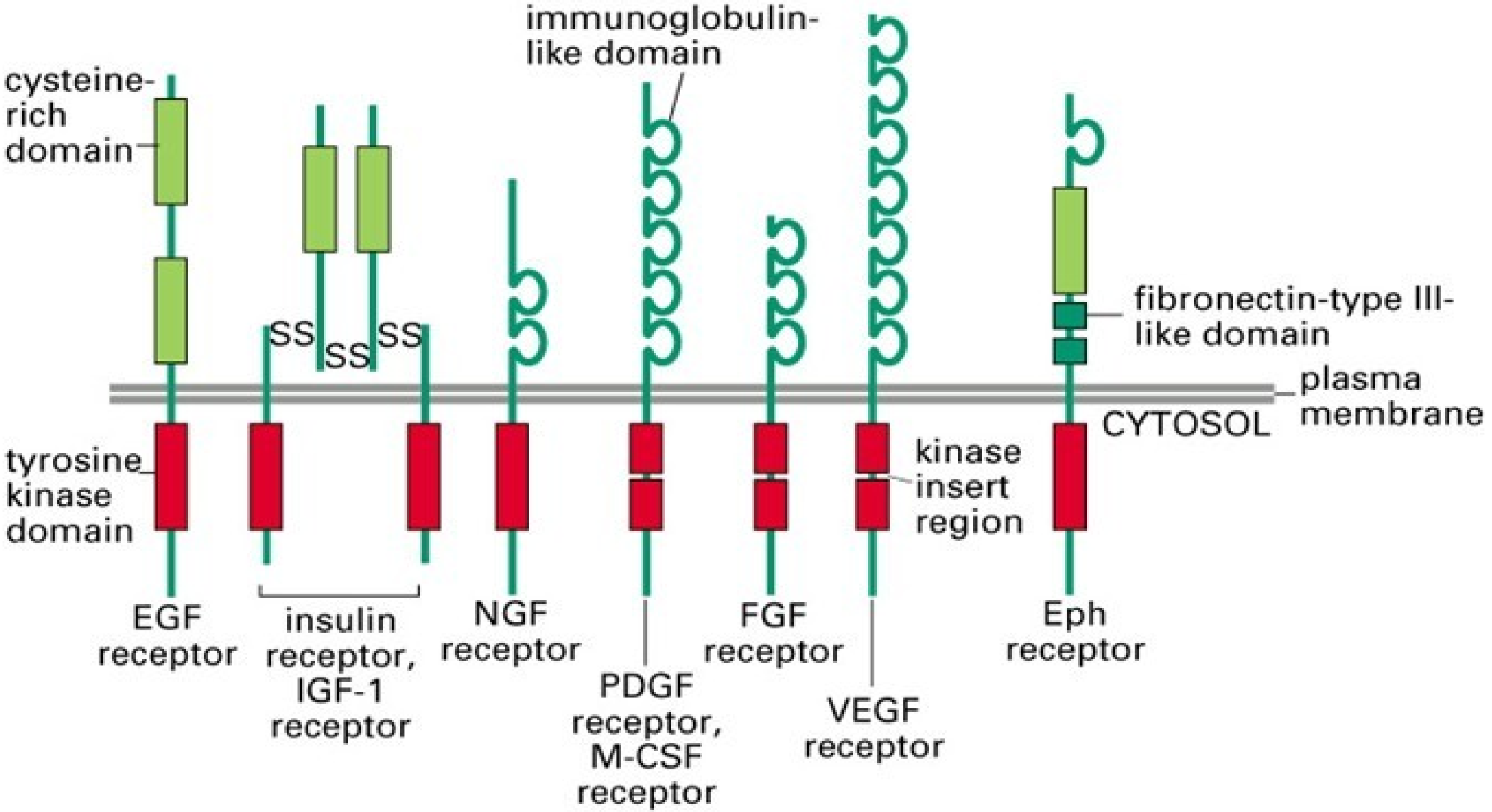
- This group includes…..EGF, TGF-α , PDGF, VEGF , FGF , HGF., Insulin receptors
- **RTKs**….. differ from other cell surface receptors in that they contain **intrinsic enzyme activity.**
- It has extracellular ligand-binding domain (trans-membrane region) and a cytoplasmic tail having intrinsic tyrosine kinase activity.


extracellular
signalling
molecule
receptor
tyrosine
kinase
domain
dimerization
P
P
autophosphorylation

RTKs

- **Binding of ligand induces**…. dimerization of the receptor, Tyrosine phosphorylation , activation of receptor Tyrosine Kinase

- Active kinase then phosphorylated and activate nearby EFFECTOR molecules.
- EFFECTOR molecules include…. Phospholipases…..which catalyzes membrane phospholipids into IP3 (increase Ca+ concentration) and Diacylglycerol (activates serine –threonine kinase protein C ..PKC) .
- PKC in turns activates various transcription factors involved in cell proliferation and in inhibition of Apoptosis
- Transcription factors also stimulate the production of various GFs , GFRs and proteins that causes the entry of cell into cell cycle .


cysteine-rich domain
immunoglobulin-like domain
fibronectin-type III-like domain
plasma membrane
CYTOSOL
tyrosine kinase domain
kinase insert region
SS
SS
SS
EGF receptor
insulin receptor, IGF-1 receptor
NGF receptor
PDGF receptor, M-CSF receptor
FGF receptor
VEGF receptor
Eph receptor

RECEPTORS LACKING INTRINSIC KINASE ACTIVITY

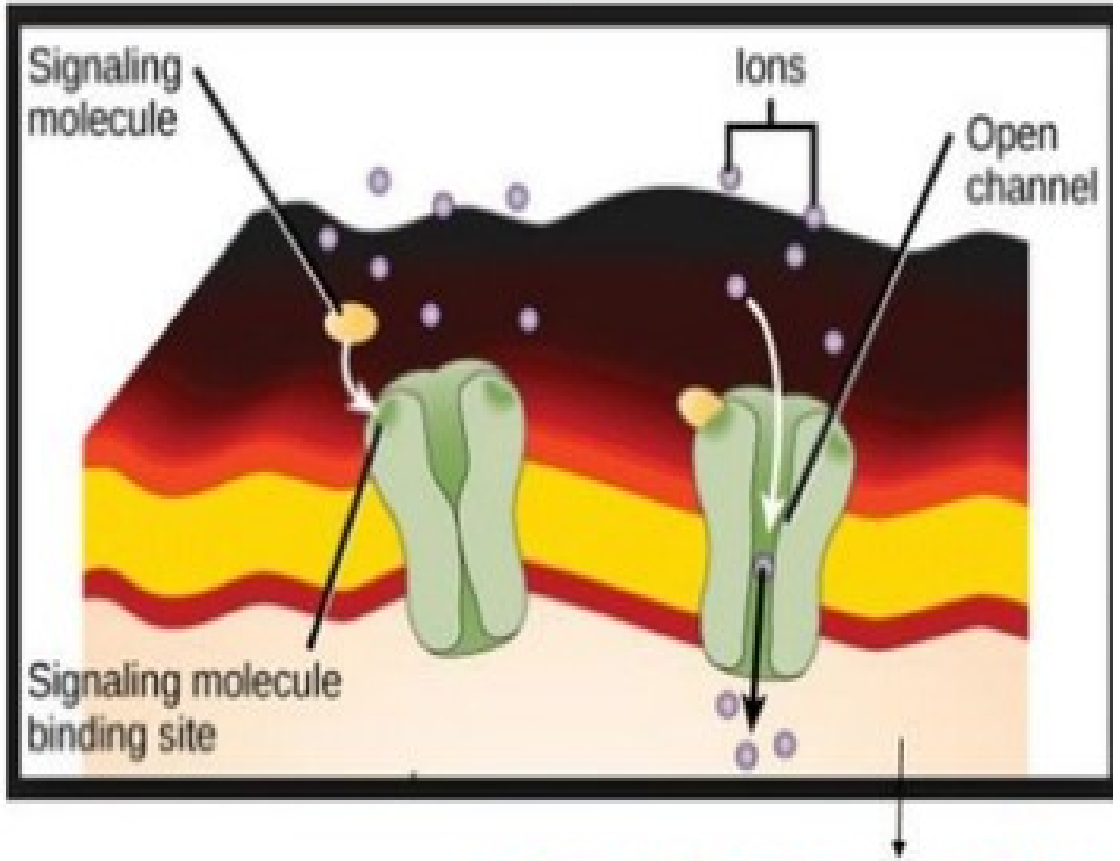
- They recruit **KINASES**.
- Ligands for these receptors include many Cytokines….IL 1 and 3 . Interferons α , β , and ϒ, erythropoietin growth hormone , and prolactin.
- Receptors transmit extracellular signals to the nucleus by activating ,members of the JAK (Janus Kinase) family of proteins.
- JAKs link the receptors with and activate cytoplasmic transcription factors called STATs (signal transducers and Activation of Transcription) ……induces gene transcription in nucleus.

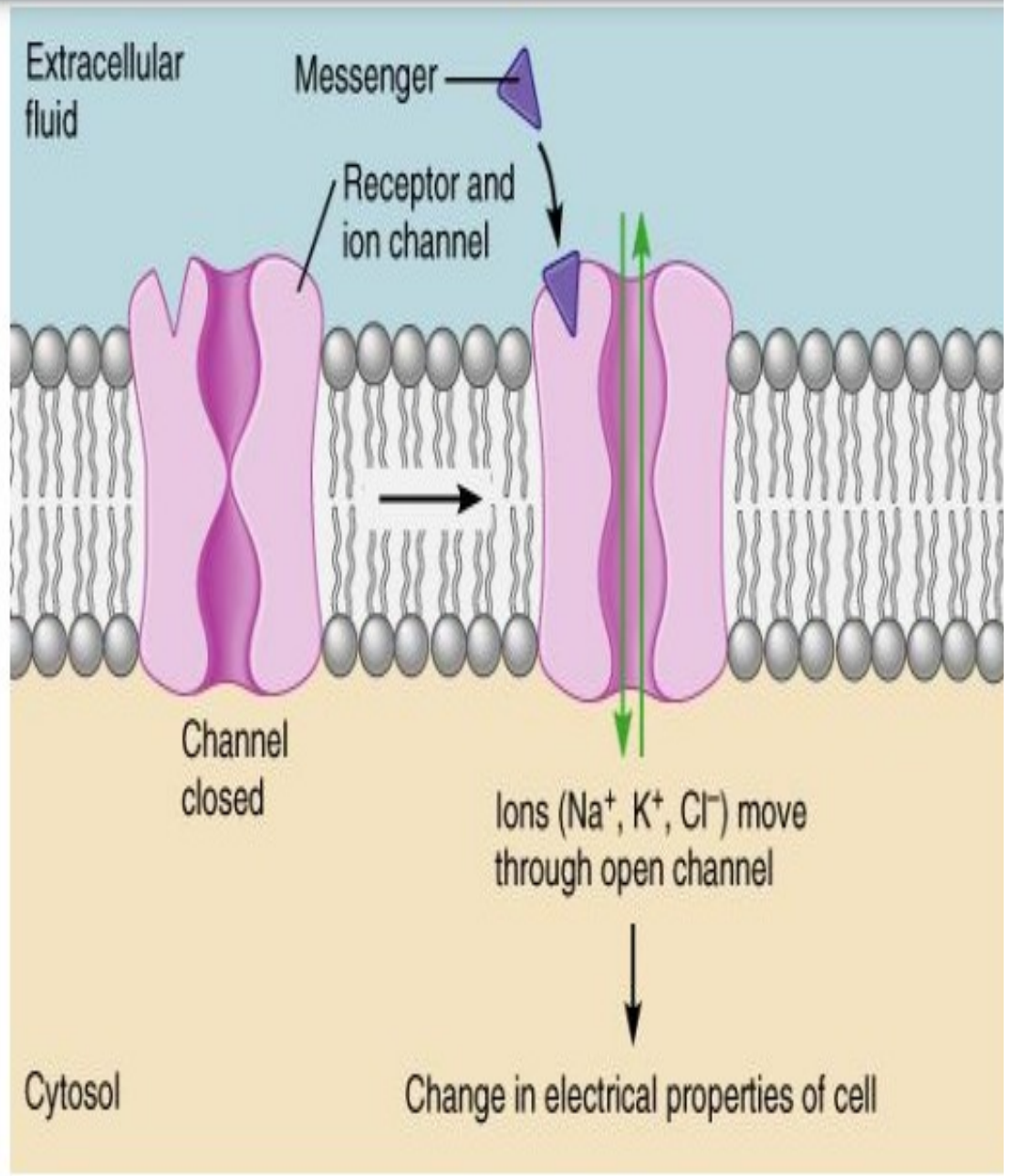
STEROIDS HORMONE RECEPTORS

- Ligands for these receptors diffuse through cell membrane
- Then binds to the receptors located in the nucleus and less so in cytoplasm.
- Receptors …… transcription factors , bind the ligand and activate transcription.
- Estrogen receptor important in breast cancers is located in cytoplasm
- Other examples….. Thyroid hormone , vitamin D and retinoid

ION CHANNELS RECEPTORS

- Cell membrane bound receptor.
- Also known as ionotropic receptor & ligand gated channels.
- Act through synaptic signaling.
- Convert chemical signals to electric signals.
- Essential in neuronal activities.
- Changing the ion permeability of plasma membrane is the major mechanism.





- **FUNCTIONS :**
- Establishing a resting membrane potential, -
- Shaping action potentials and other electrical signals by gating the flow of ions across the cell membrane, -
- Controlling the flow of ions across membranes, - regulating cell volume.
- Their activation translates into a rapid physiological effect.

ION CHANNELS RECEPTORS

- **Ion channels** are membrane proteins, which play a principal role in regulating cellular excitability.
- They are found in virtually all cells, and are of crucial physiological importance.
- Based on the stimulus to which they respond, ion channels are divided into **three super families**:

1. Voltage-gated,
2. Ligand-gated and
3. Mechano-sensitive ion channels.

VOLTAGE-GATED ION CHANNELS

- Voltage-gated channels are highly selective for a specific ion, i.e., Na+, K+, Ca2+, and Cl-.
- **Voltage-gated Na+ channel** - These channels are responsible for the generation of action potentials of long duration, and thus are targets of local anesthetics, such as lidocaine and benzocaine.
- **Voltage-gated Ca2+ channels –** They regulate intracellular Ca2+ concentrations.
- One of the most important processes regulated by these channels is the release of neurotransmitters at synapses.
- Calcium channel blockers are valuable in treating a variety of conditions ranging from heart disease to anxiety disorders.

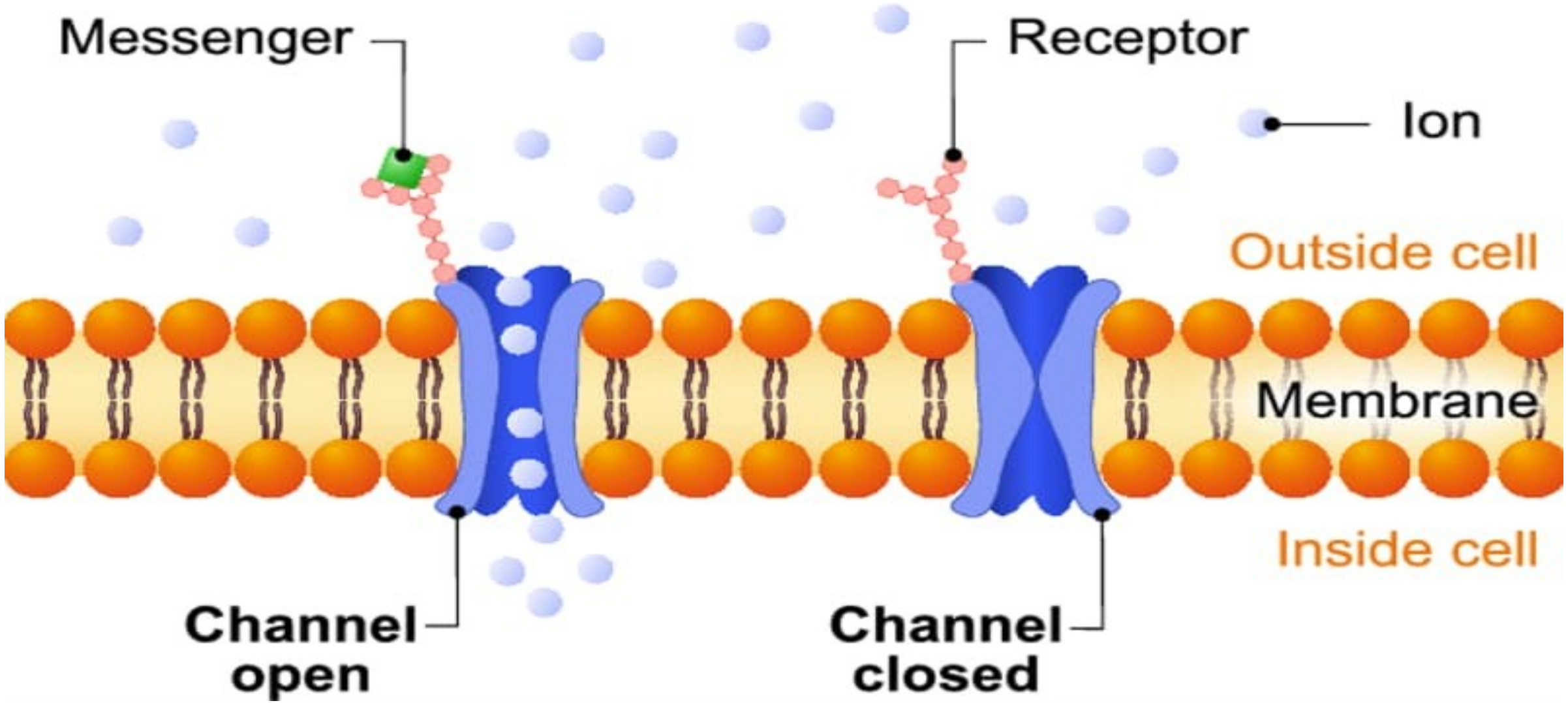
VOLTAGE-GATED ION CHANNELS

- **Voltage-gated K+ channels** – They constitute **the largest** and the most diverse class of voltage-gated ion channels.
- They are imperative in generating the **resting membrane potential**.
- **Voltage-gated Cl- channel**s - These channels are present in every type of neuron and are involved in regulating excitability and cell volume.
- They are also known to contribute to the **resting membrane potential**

LIGAND-GATED ION CHANNELS

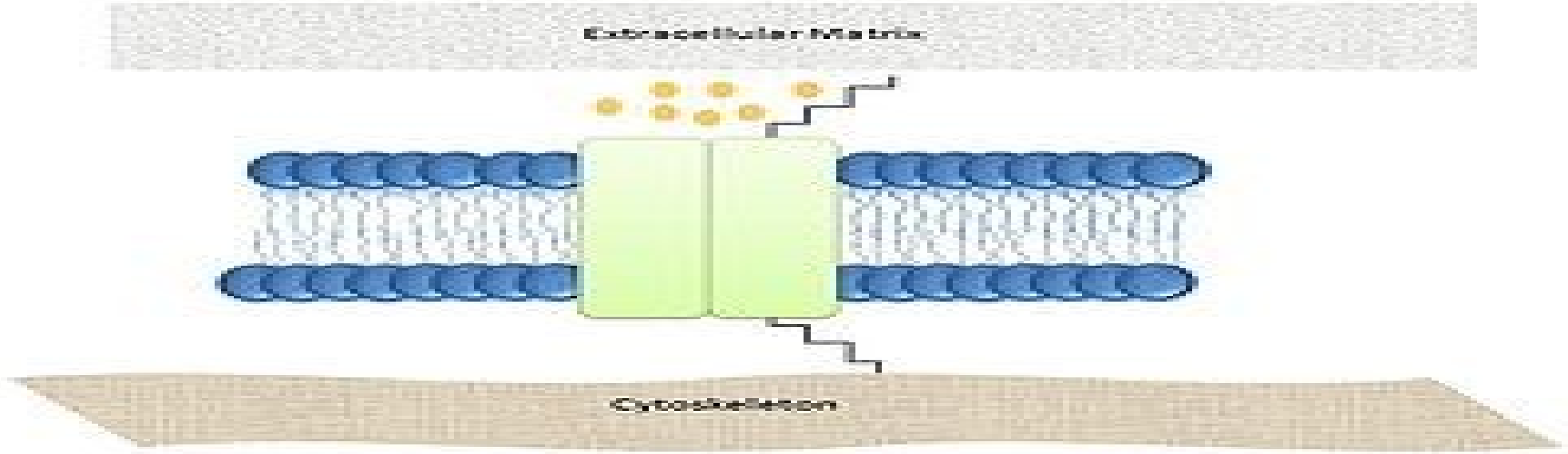
- **LGIC** are integral membrane proteins that contain a pore which allows the regulated flow of selected ions across the plasma membrane.
- Ion flux is passive and driven by the electrochemical gradient for the permeant ions.
- **LGIC** , are targets for many drugs, such as anesthetics, antipsychotics, and antidepressants.

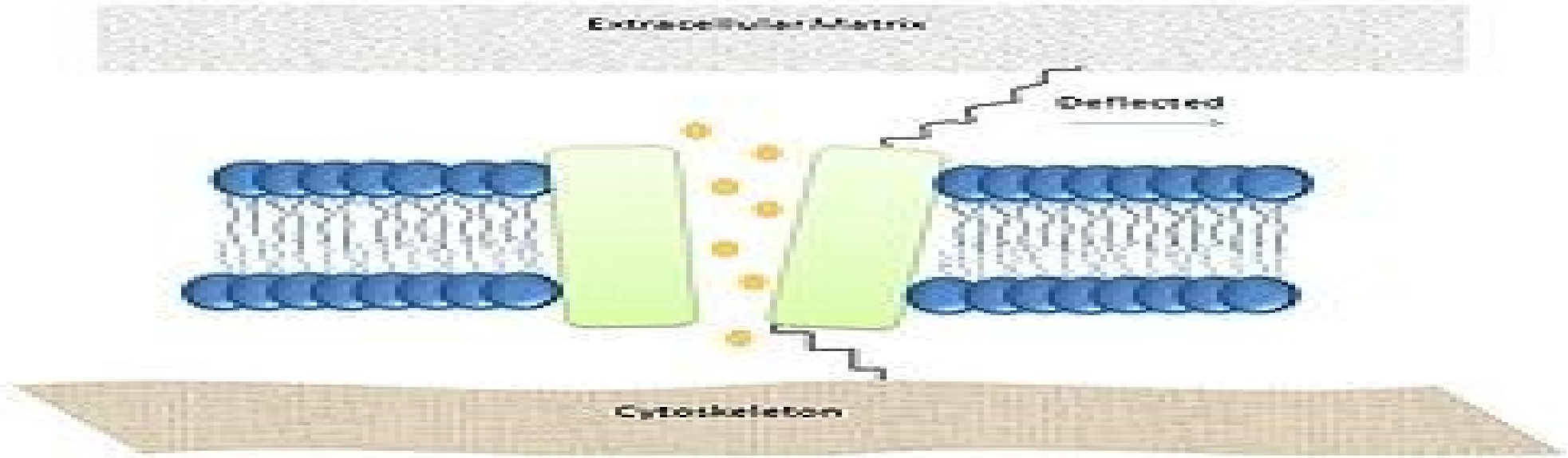
Ligand-gated ion channel



MECHANO-SENSITIVE ION CHANNELS

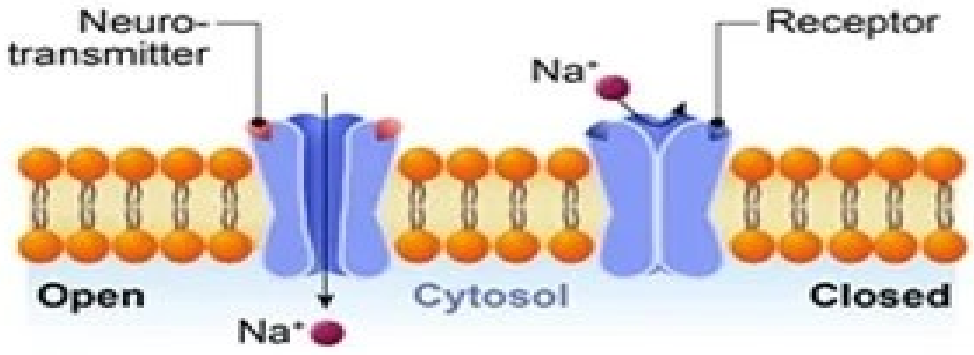
- Subset of proteins that translate mechanical signals into a biochemical response.
- Mechano-sensitive ion channels play important roles in cellular functions, such as **gene expression, cell division, migration, cell adhesion, and fluid homeostasis**


Extracellular Matrix
Cytoskeleton

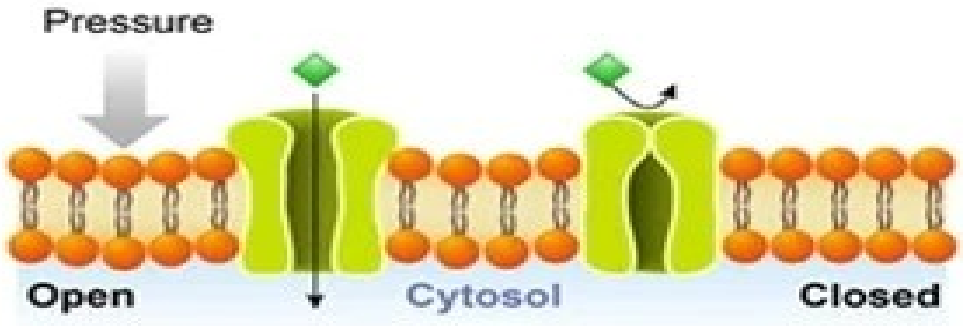

Extracellular Matrix
Deflected
Cytoskeleton

ION CHANNEL

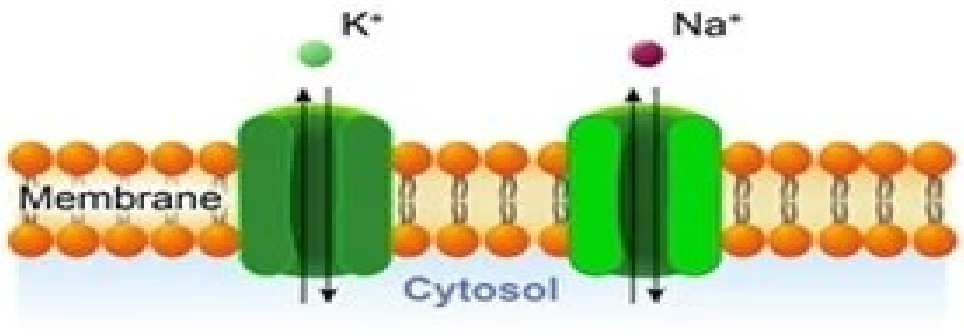
Ligand-gated



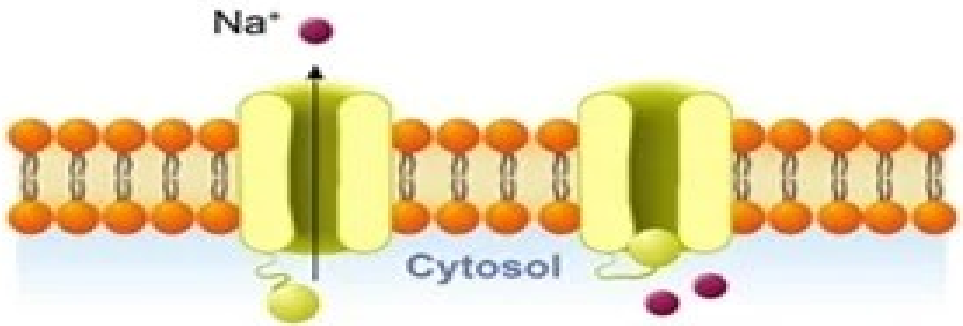
Mechanically-gated



Always open



Voltage-gated



Thank You
For Your Attention