MEDICAL UNIVERSITY OF WARSAW

DEPARTMENT OF HISTOLOGY AND EMBRYOLOGY
FACULTY OF MEDICINE

Cell receptors and signal transduction

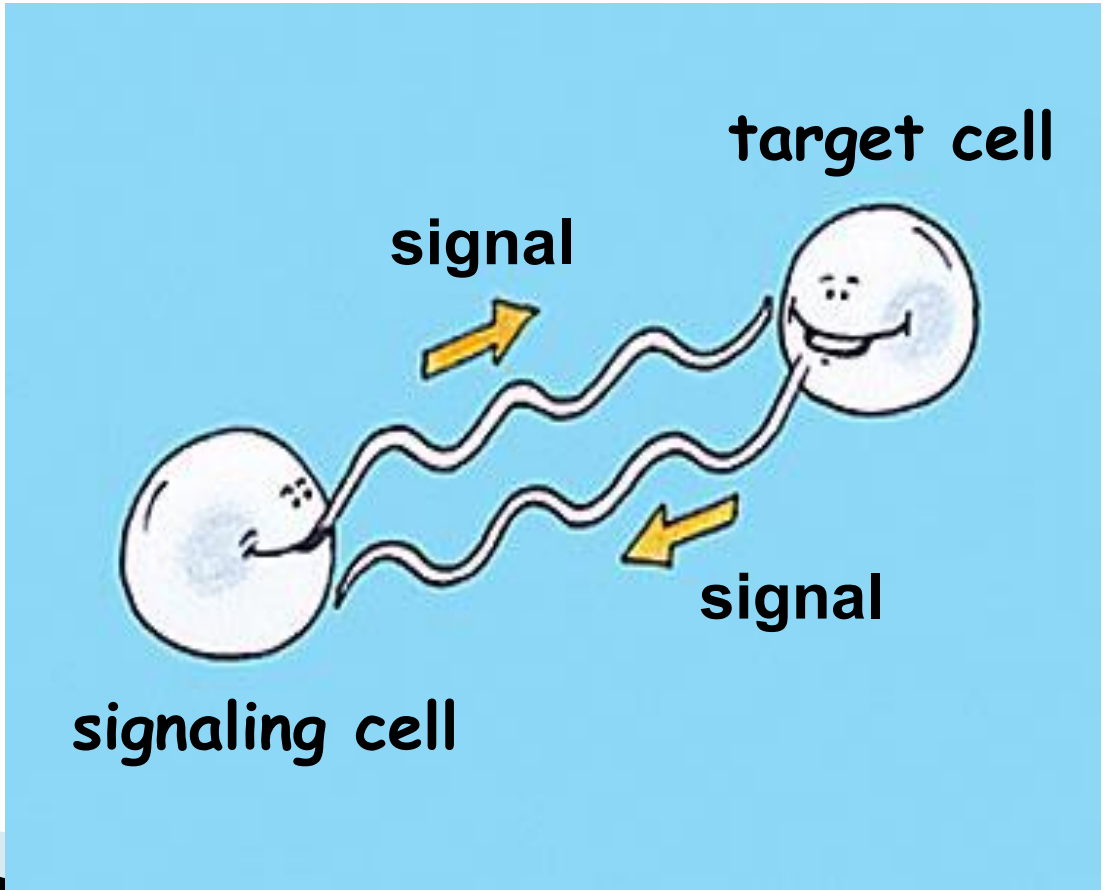
Anna Iwan

A man without contact with other people.

Communication of cells is necessary for normal function of every multicellular organism!

Communication:

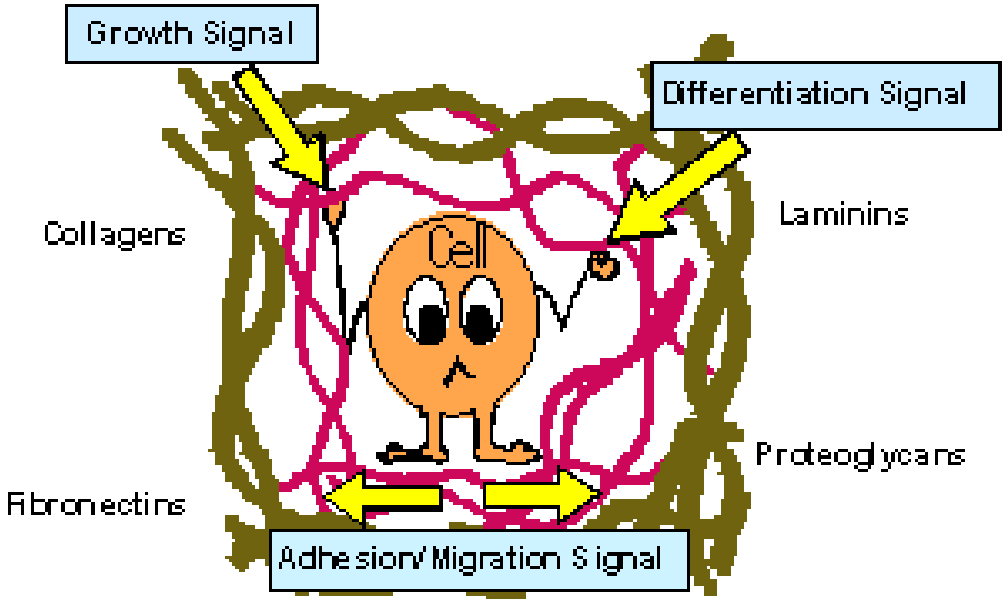
sending and receiving of signals



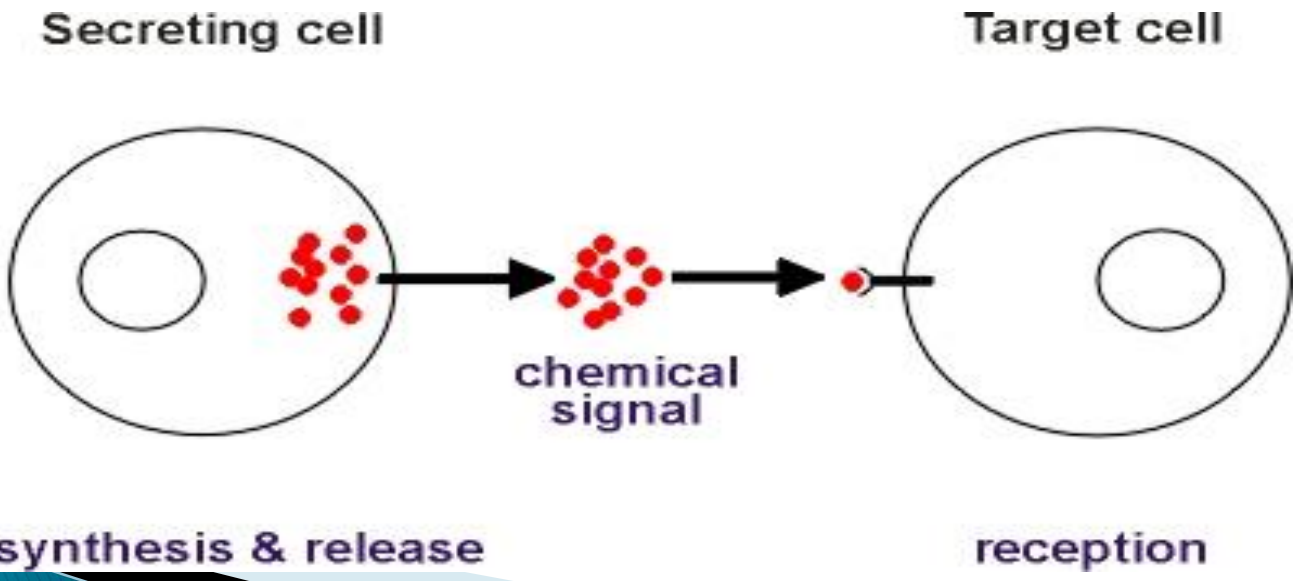
Signals:

- from the external environment (extracellular matrix)

- informations exchanged between cells

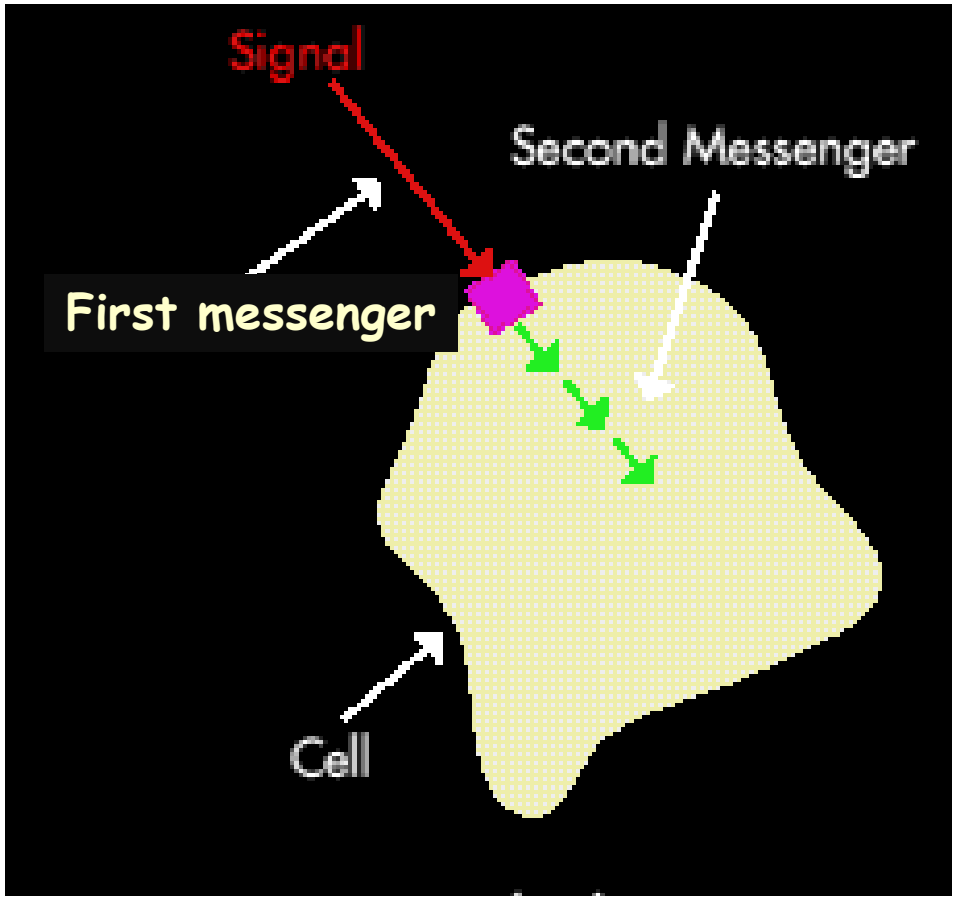


- multiple signals at the same time – contradictory?



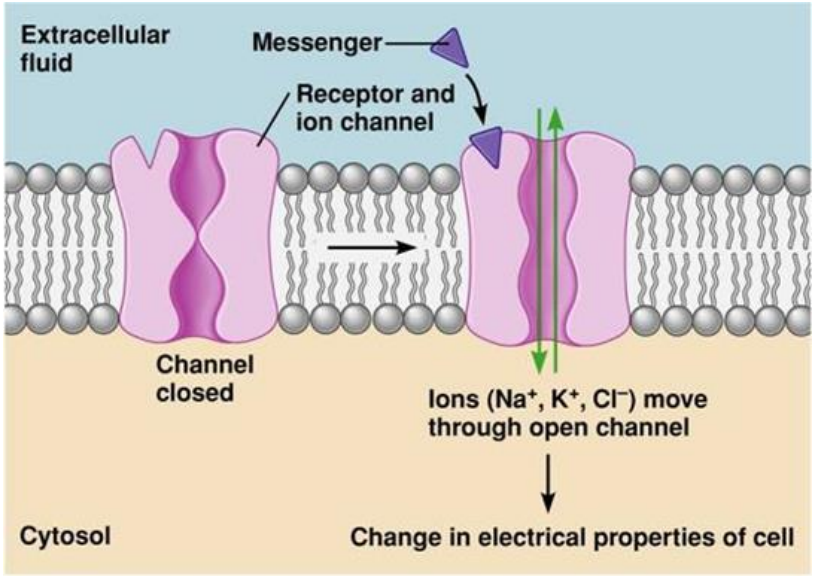
- signals - first messengers

- PHYSICAL SIGNALS
- **CHEMICAL SIGNALS**



CHEMICAL SIGNALS - first messengers

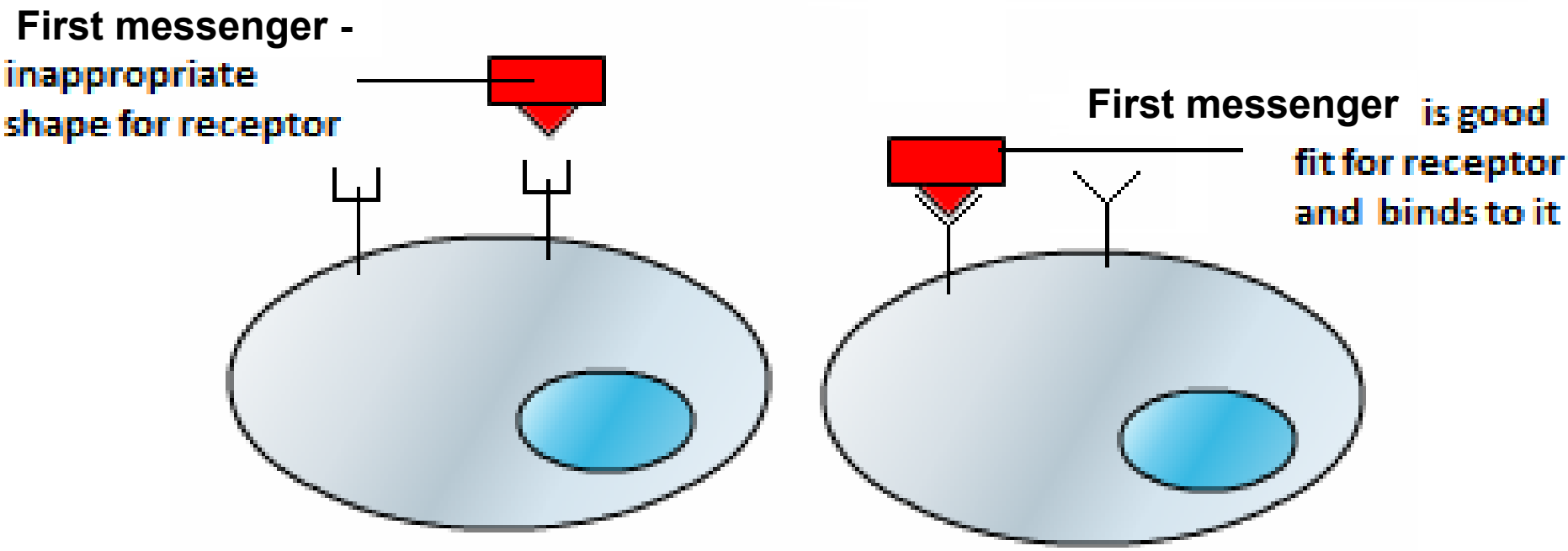
- **Ions**
- Chemical substances (molecules)



- hormones (peptides, steroids),
- cytokines,
- growth and differentiation hormones,
- chemokines

chemical signal - information depends on physico-chemical parameters of **first messenger:**

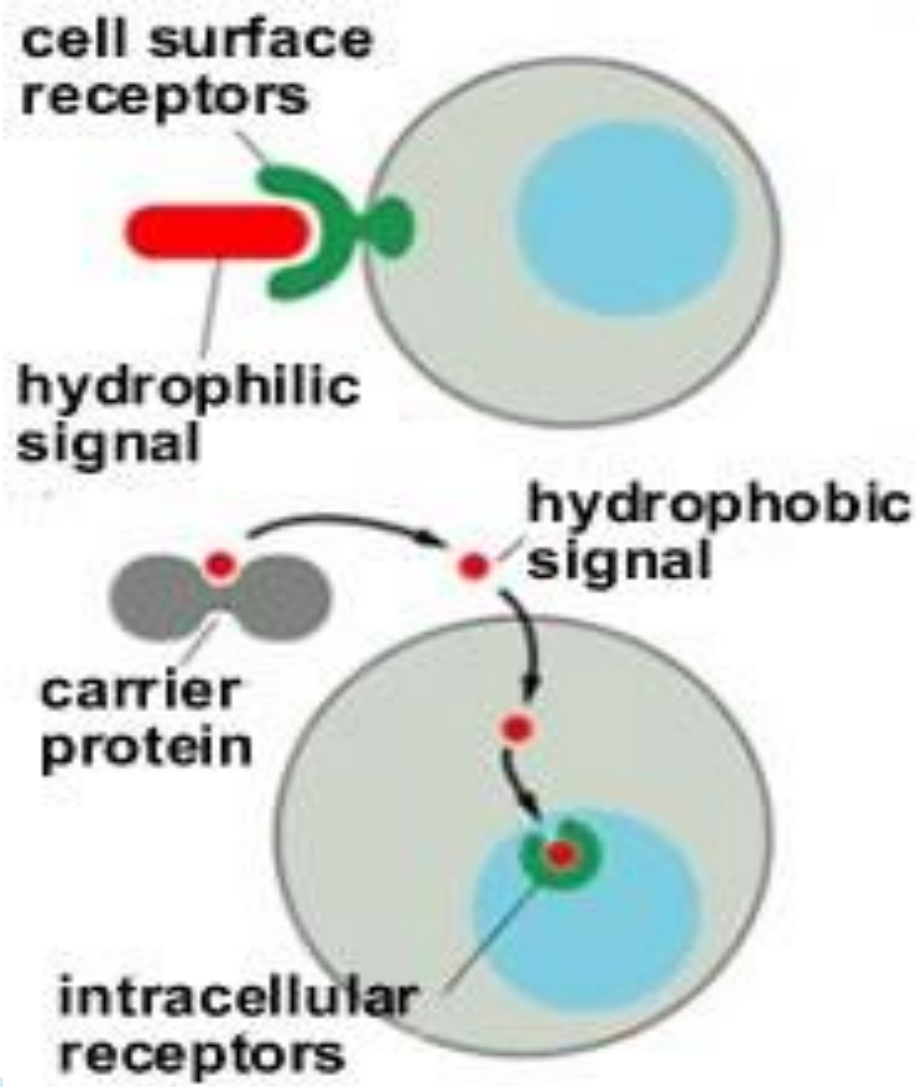
- **three-dimensional structure - ability to interact with other chemical agents**



chemical signal = first messenger = ligand
- interacts with and binds to a receptor

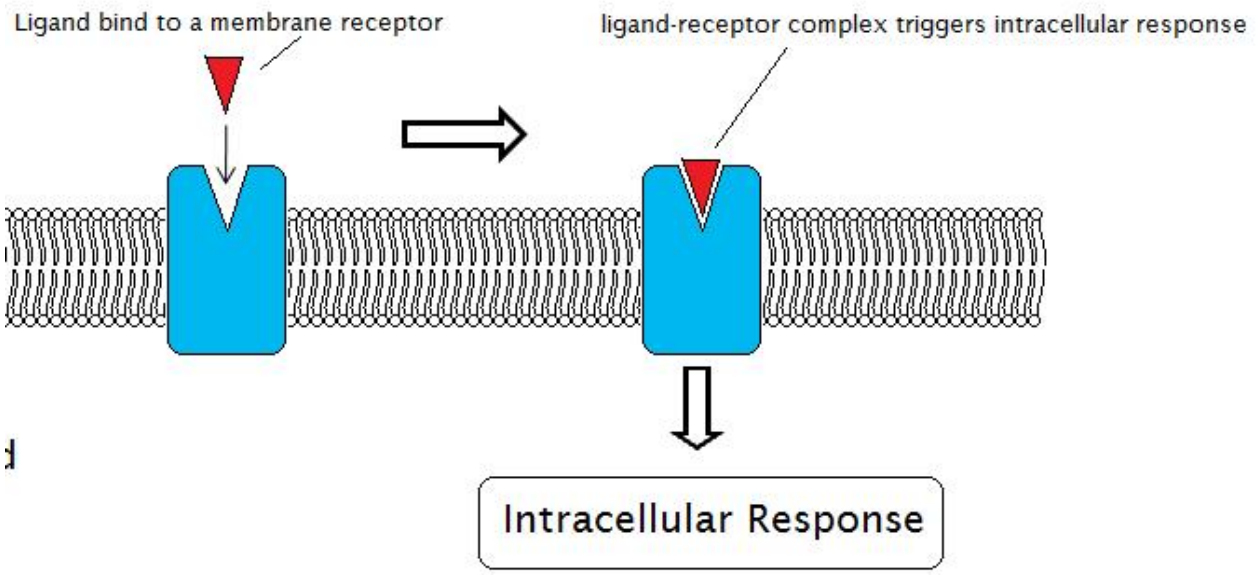
The nature and structure of the ligand determines the location of the receptor

- **cell surface receptors (peptide)**
- **intracellular receptors (steroid hormone)**
- **nuclear receptors**



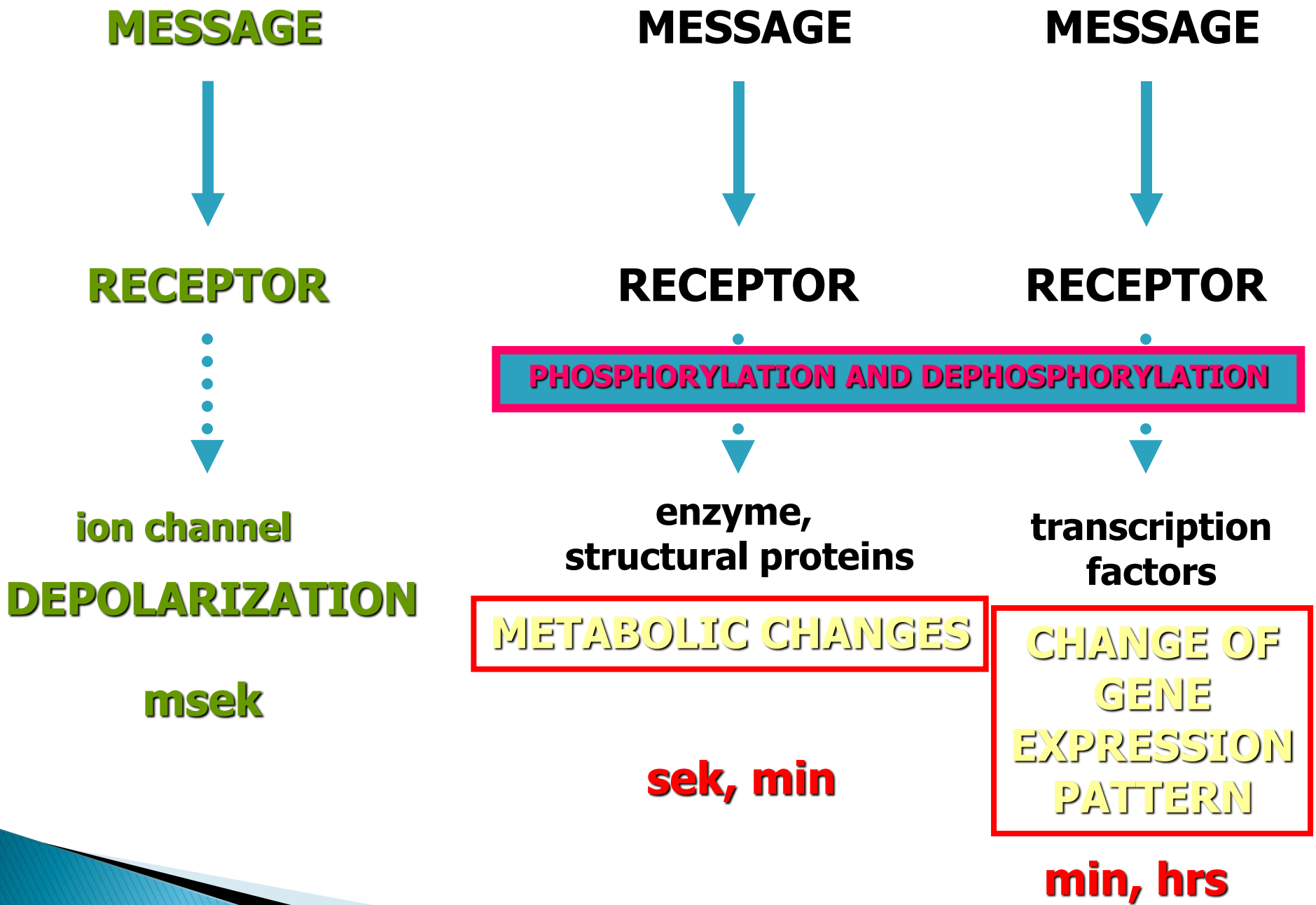
MESSAGE (first messenger, ligand)

RECEPTOR



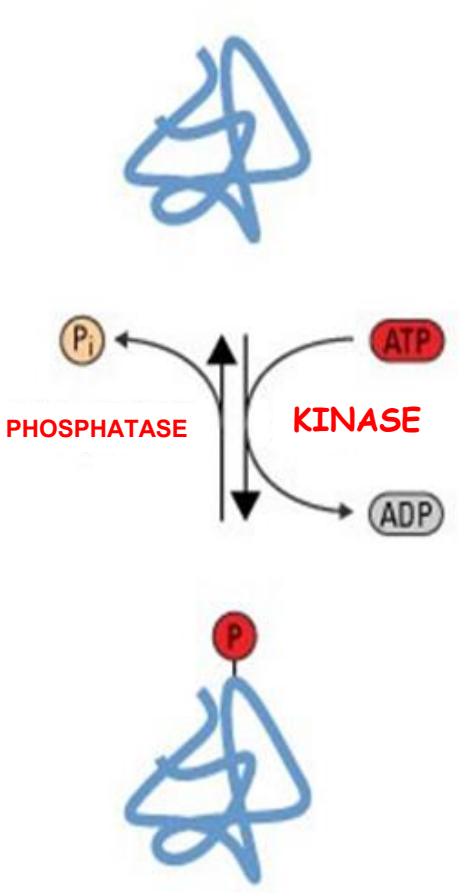
CELL REACTION

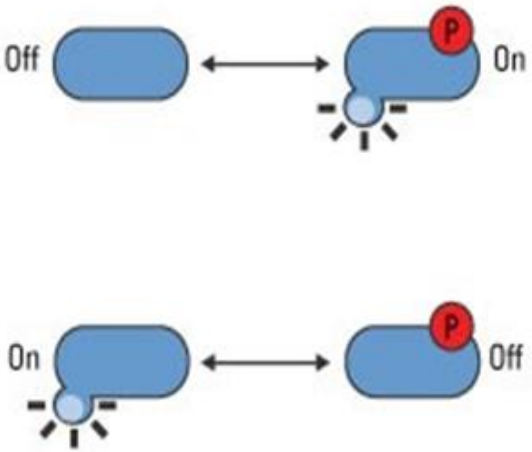
Binding of ligand – receptor - activation (conformational change, oligomerization) – **transduction of signal** (into the cell)


MESSAGE
RECEPTOR
ion channel
DEPOLARIZATION
msek
MESSAGE
RECEPTOR
PHOSPHORYLATION AND DEPHOSPHORYLATION
enzyme,
structural proteins
METABOLIC CHANGES
sek, min
MESSAGE
RECEPTOR
transcription
factors
CHANGE OF
GENE
EXPRESSION
PATTERN
min, hrs

PHOSPHORYLATION AND DEPHOSPHORYLATION OF PROTEINS

KINASES AND PHOSPHATASES





- enzymes,
- structural proteins
- transcription factors

SERINE-THREONINE KINASES

- Ca^{2+}/CaM-dependent kinase
- Kinase A
- Kinase G
- Kinase C
- Ceramide-dependent kinase
- TGFβ receptor family

TYROSINE KINASES

- *Src* kinase family
- *Jak/Tyk* kinase family
- EGF/insulin receptor family

SERINE-THREONINE PHOSPHATASES

- Ca^{2+}/CaM-dependent phosphatase
- Ceramide-dependent phosphatase

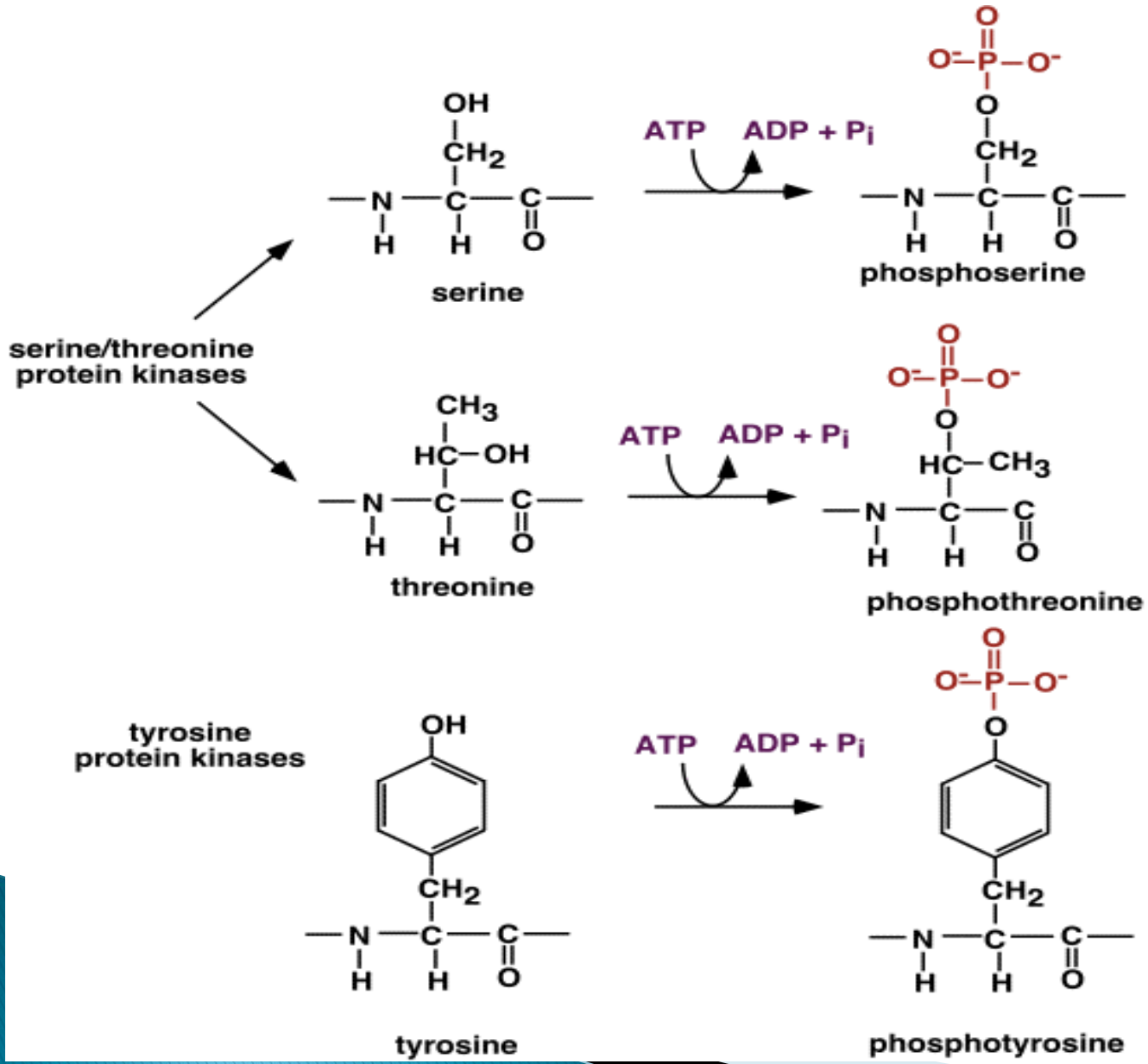
TYROSINE PHOSPHATASES

- CD45 receptor family
- Leukocyte common antigen-related family
- Human tyrosine phosphatase α family
- Human tyrosine phosphatase β family

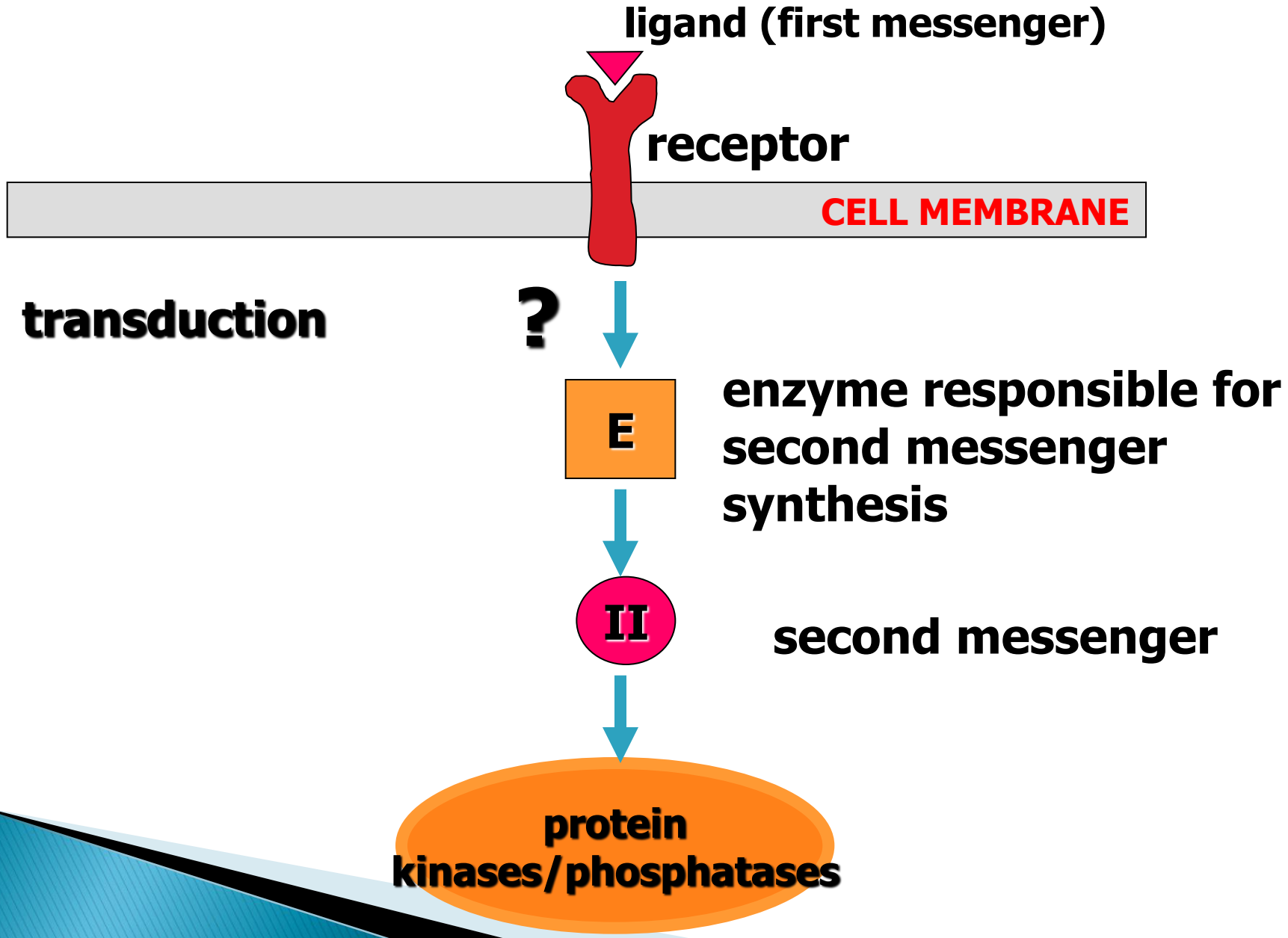
BISPECIFIC KINASES

- Mitogen-activated protein kinase kinase (MAPKK)

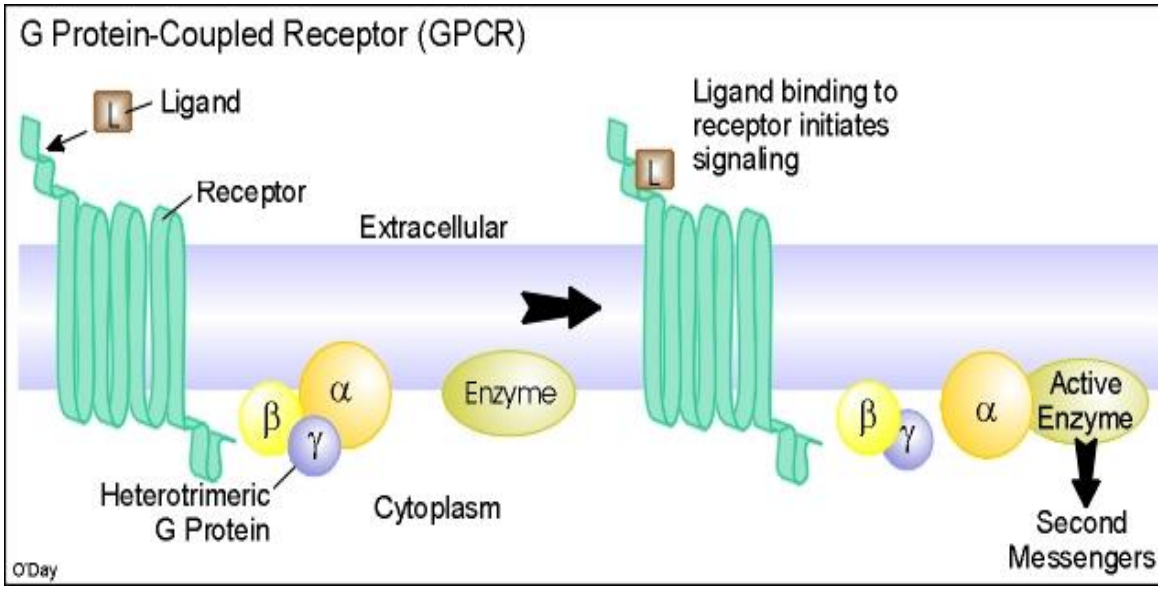
TYROSINE and SERINE/THREONINE KINASES



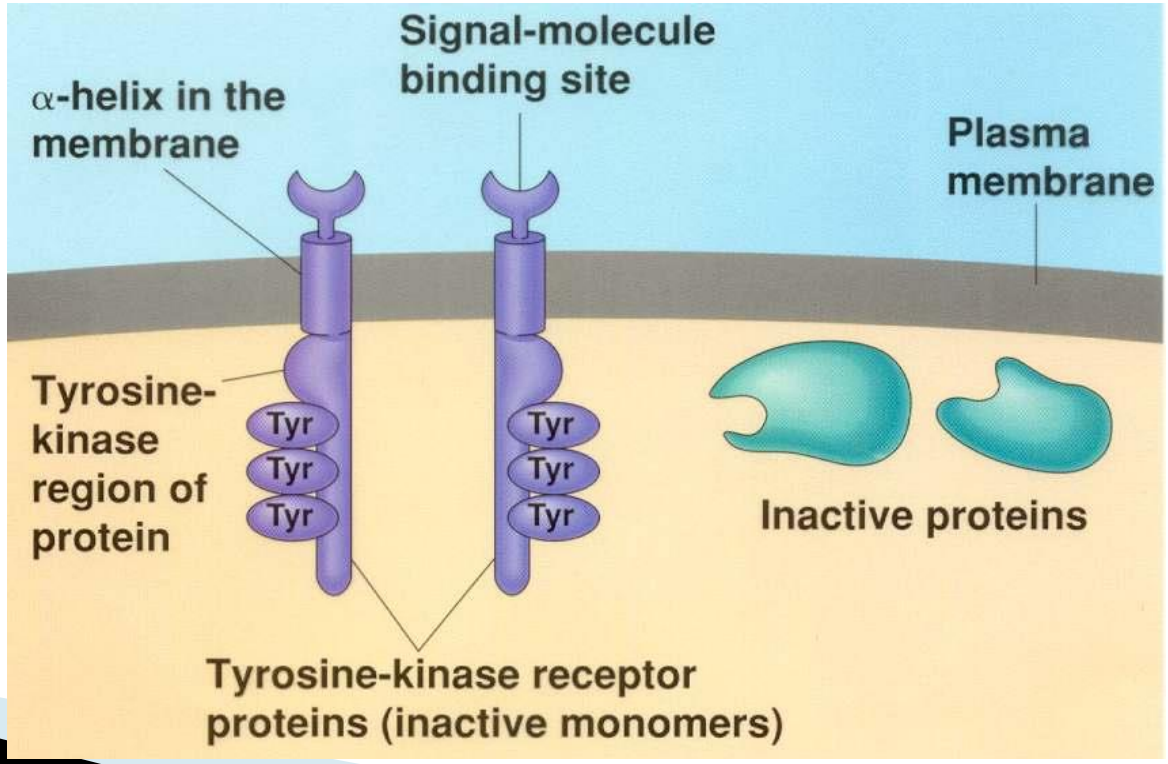
What activates protein kinases and phosphatases?



- **G-protein-linked receptors**



- **Catalytic receptors – receptors with tyrosine kinase activity**

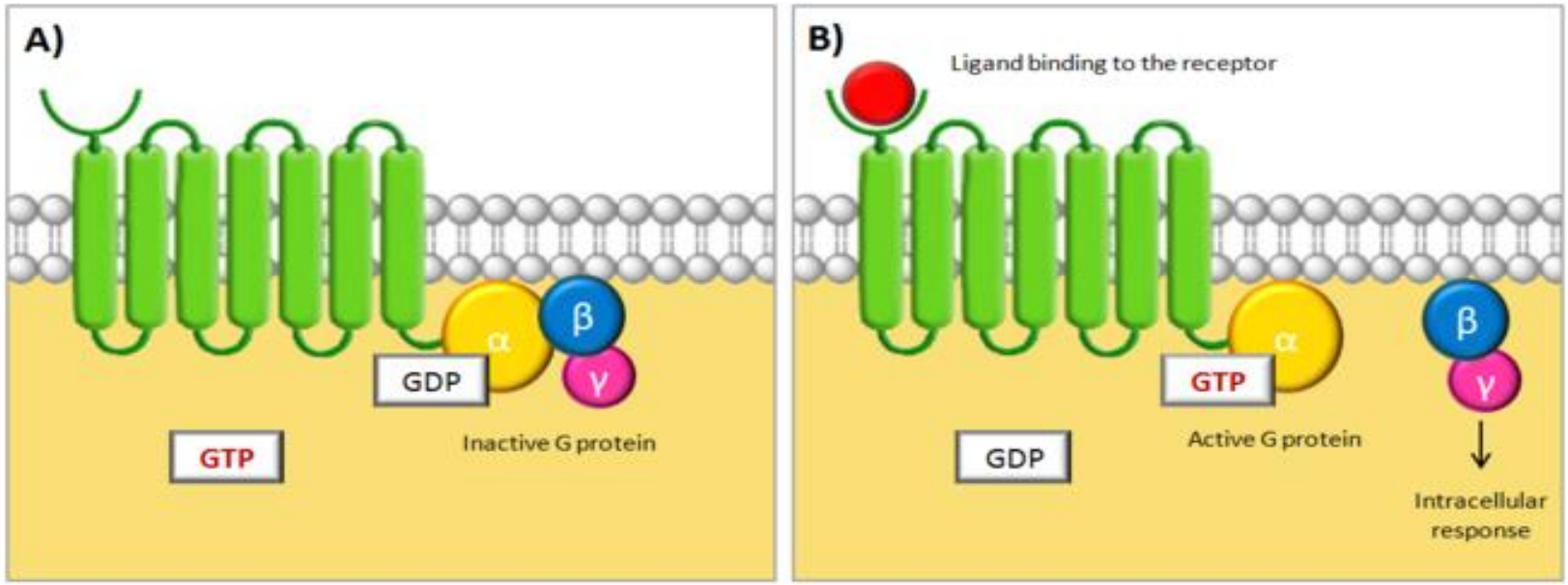


G-protein-linked receptors

- seven-pass transmembrane protein
- trimeric GTP-binding protein – G protein

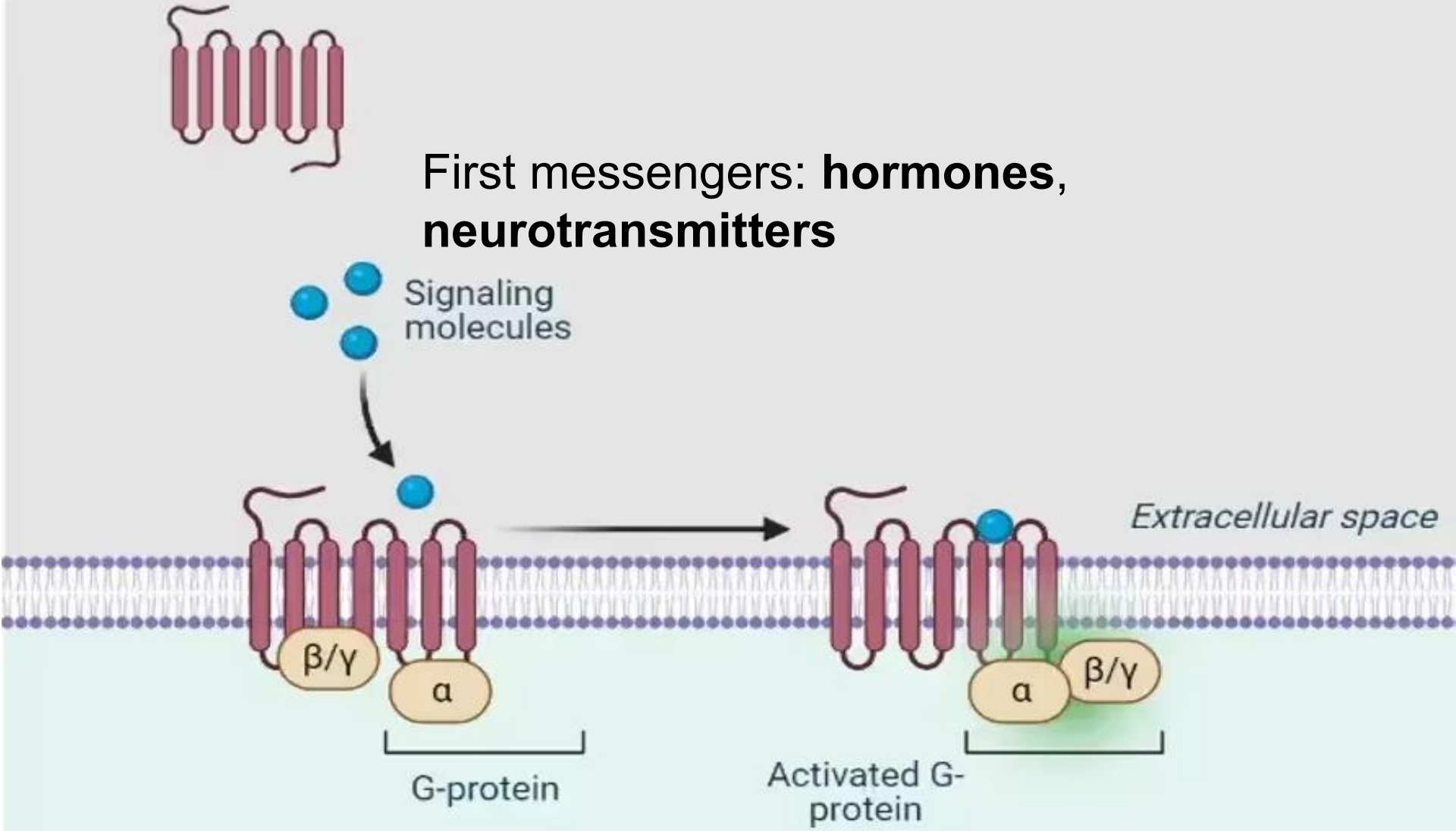
G protein

- three protein subunits: α,β and γ



In unstimulated receptor α subunit binds GDP. After ligand binding – GDP exchanged for the GTP - activation

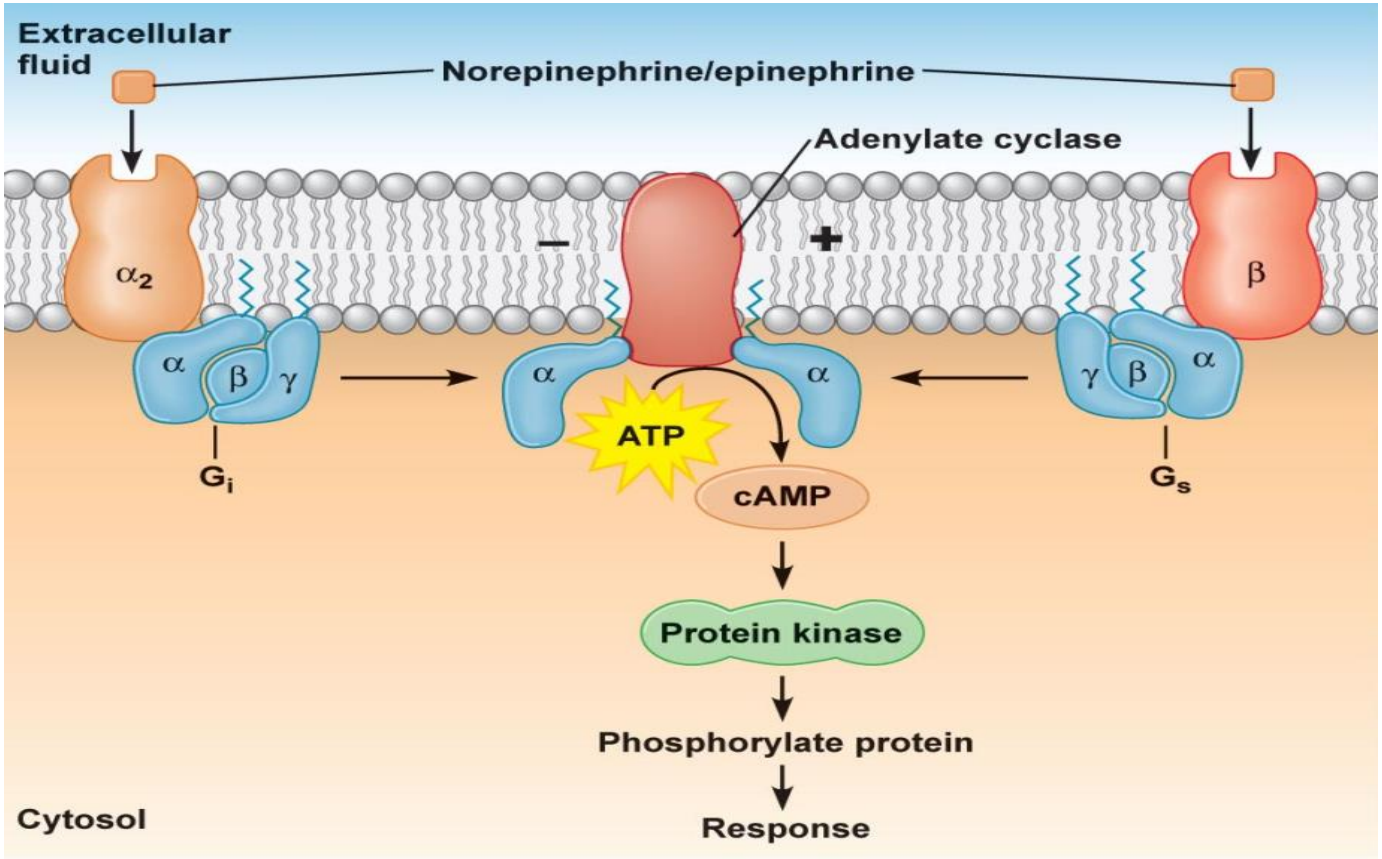
First messengers: **hormones**, **neurotransmitters**



- many tissues (cardiomyocytes), but over 90% in the brain (neurons of CNS)

G protein can activate membrane-bound enzymes

- **Adenylyl cyclase** – converts ATP to cyclic AMP – second messenger - **cAMP**



Types of G proteins

- different G proteins - various enzymes - different second messengers - different kinases

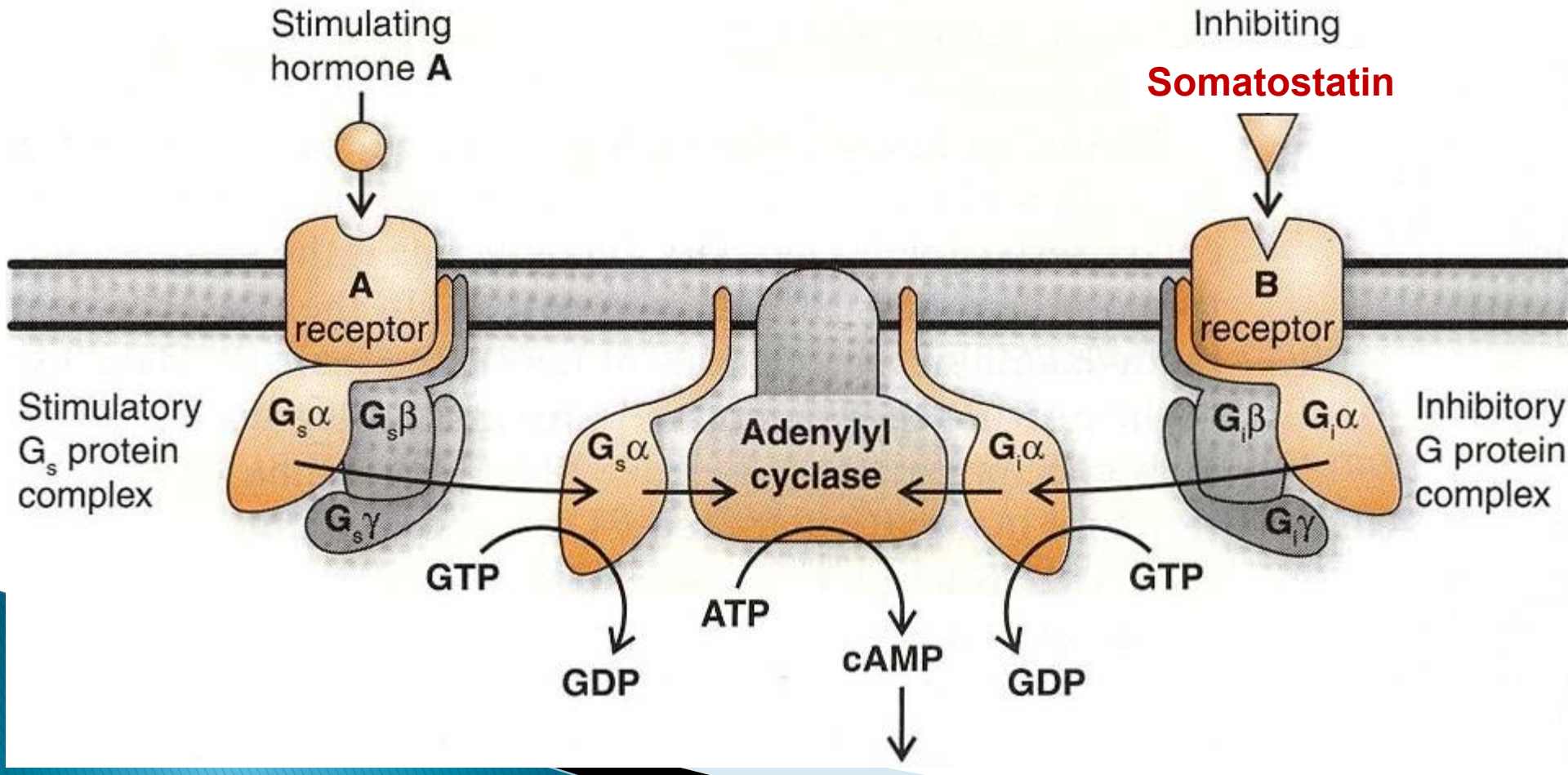
TRANSDUCING FACTOR	ENZYME	SECOND MESSENGER	KINASE
-	IP_3, ΔV	Ca^{2+}	Ca^{2+}/CaM-dependent kinase
$G_{s/i}$ protein	Adenylate cyclase	cAMP	PKA
G_q protein	PLC β	DG	PKCα, β i γ
G protein?	PLD	DG	PKCα, β i γ
G_t protein	PDE	cGMP	-
?	Guanylate cyclase	cGMP	PKG

Second messengers - **Cyclic AMP, Cyclic GMP, Inositol Triphosphate(IP3), Diacylglycerol(DG), and Calcium**

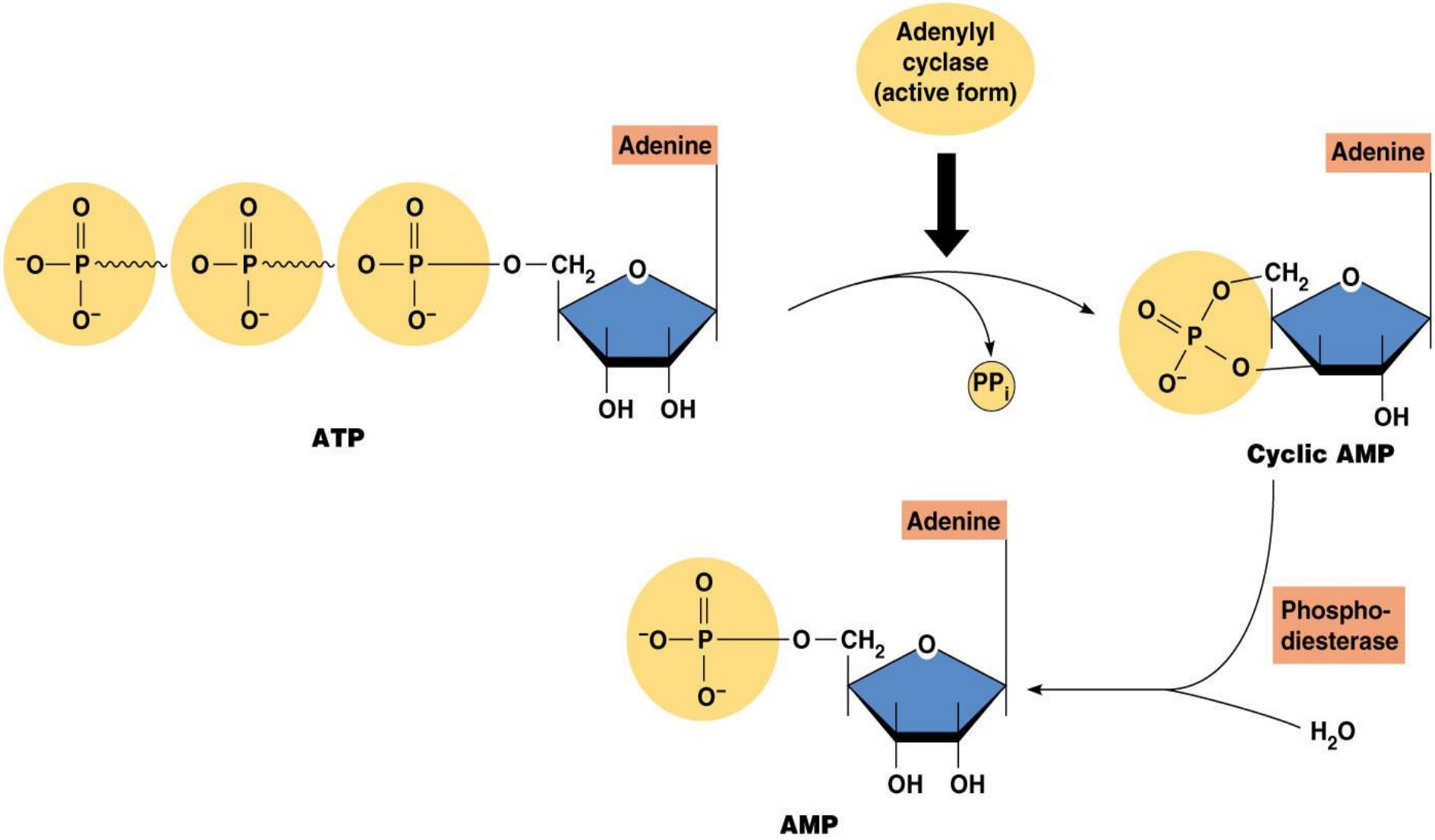
G protein

- stimulatory **Gs** protein
- inhibitory **Gi** protein

} **Adenylyl cyclase**



Cyclic AMP phosphodiesterase (PDE) converts cAMP to the AMP – elimination of signal

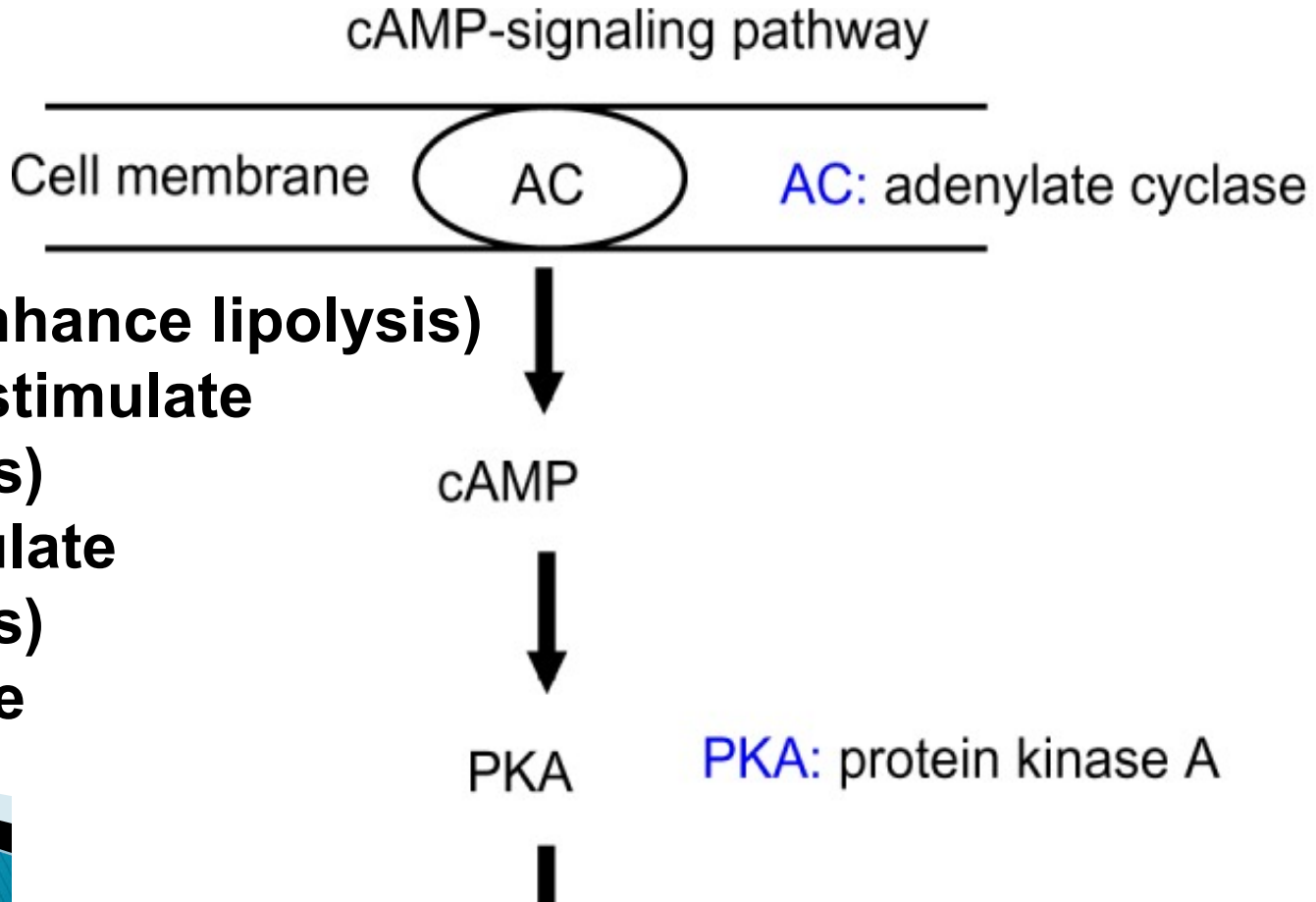


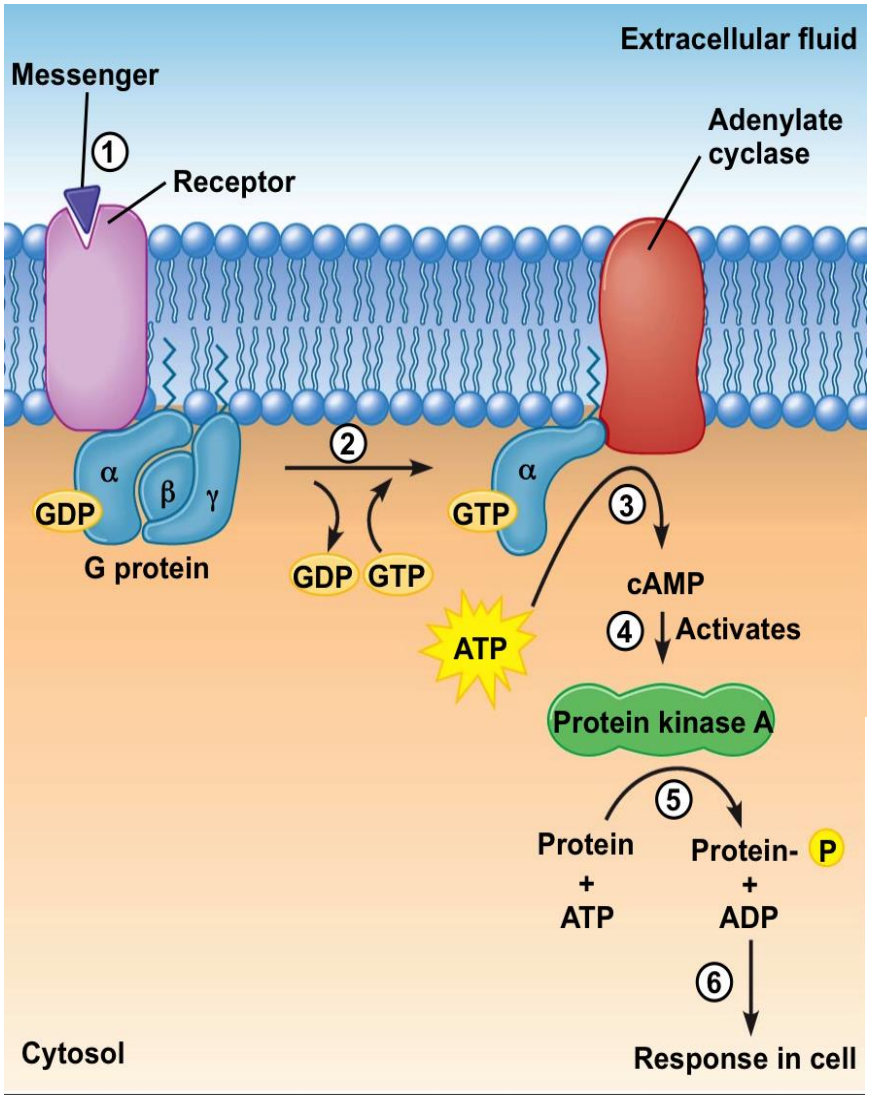
cAMP - second messenger - **function**

- hormones (**glucagon**), **adrenaline**
- **protein kinase A (PKA)** - **cAMP-dependent protein kinase** - regulation of **glycogen, sugar, and lipid metabolism**

In:

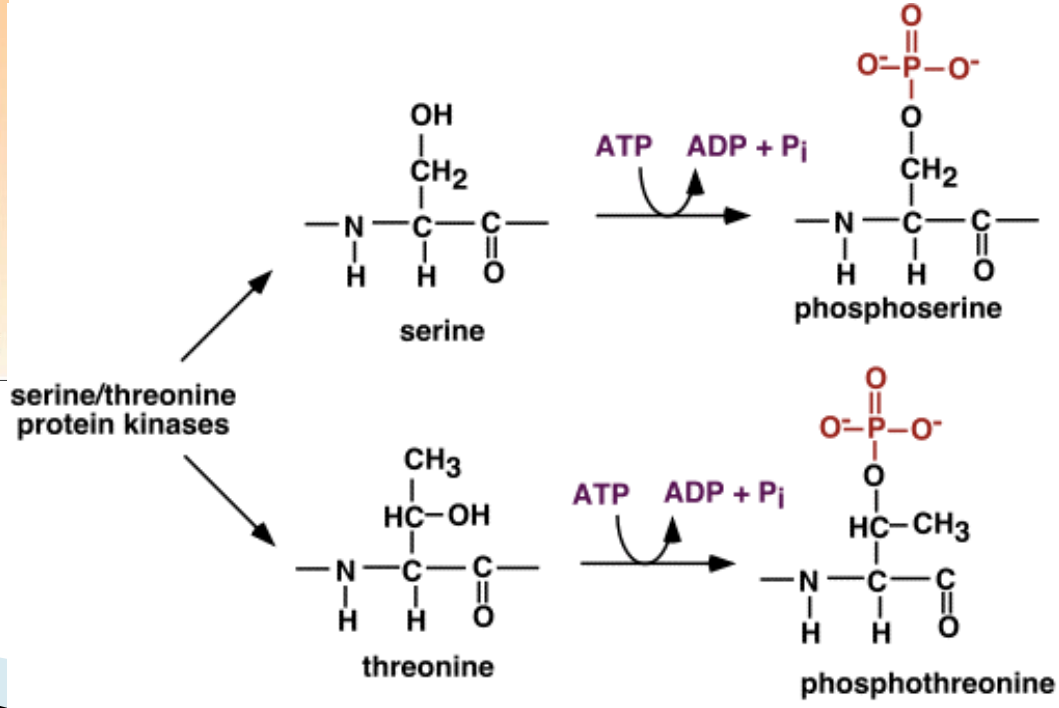
- **adipocytes (enhance lipolysis)**
- **hepatocytes (stimulate glycogenolysis)**
- **skeletal (stimulate glycogenolysis)**
- **cardiac muscle**





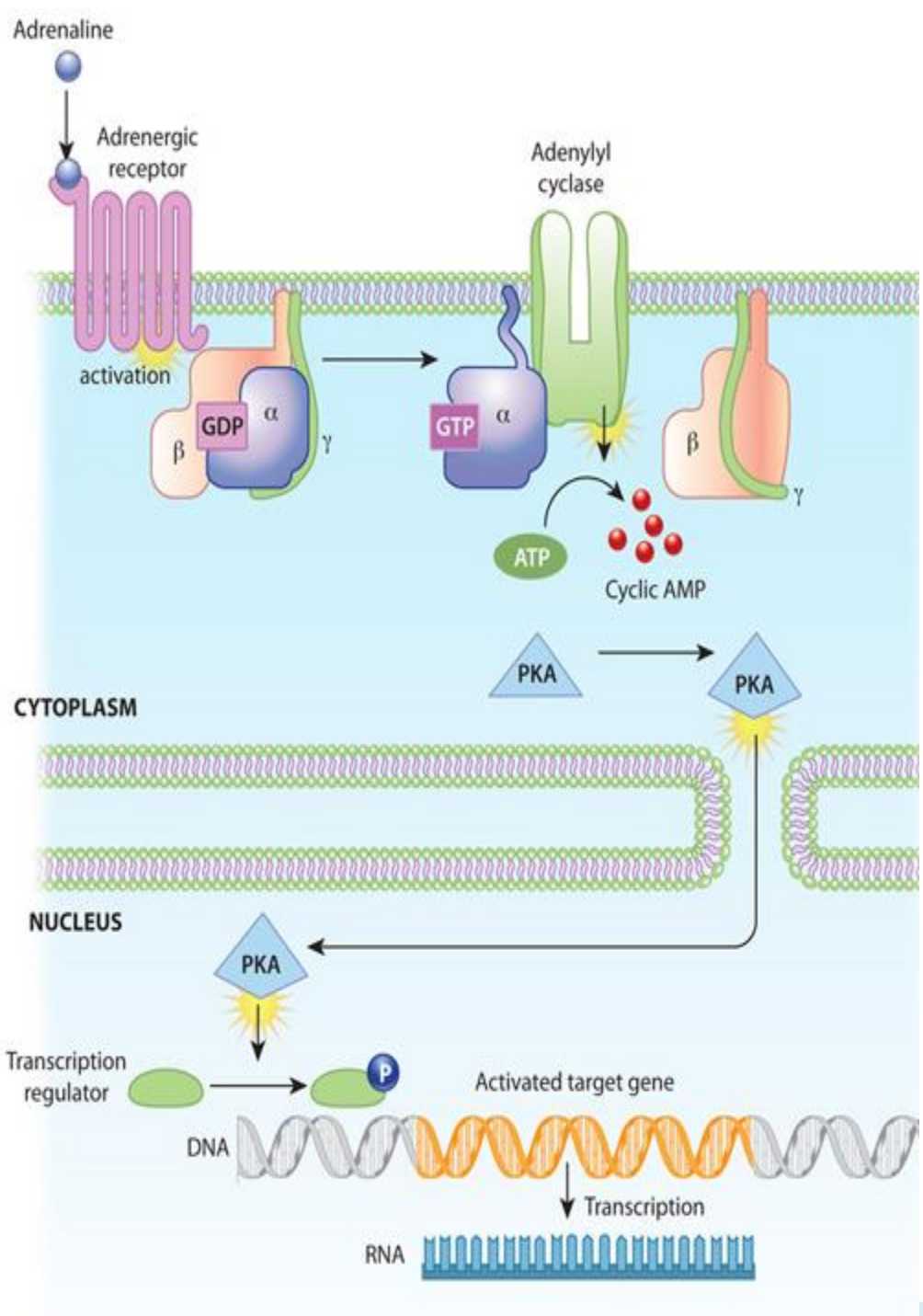
PKA – cyclic-AMP-dependent protein kinase

- phosphorylation of proteins (a serine/threonine protein kinase - phosphorylates the OH group of serine or threonine)



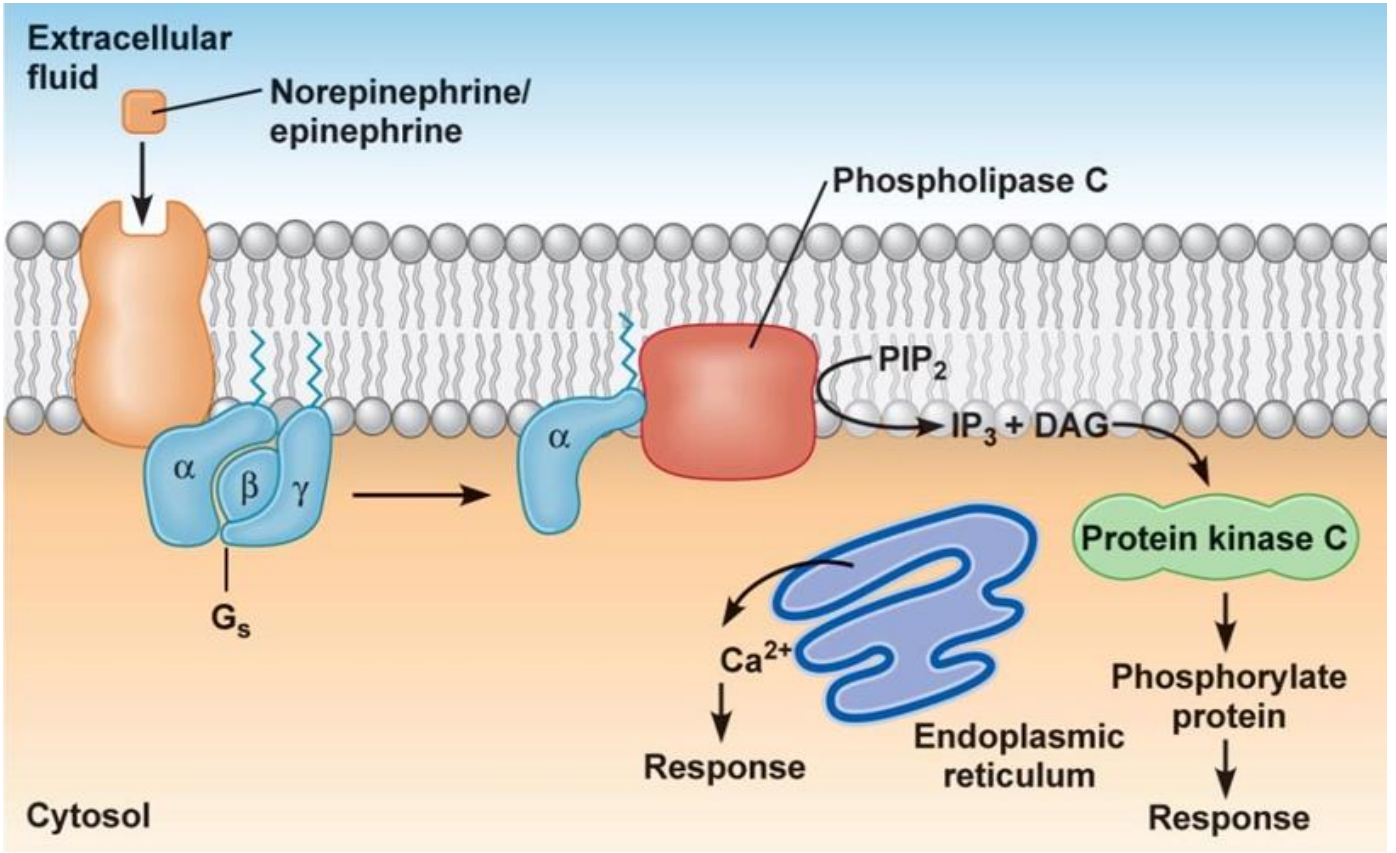
- the effects of PKA activation vary with cell type

G protein and gene expression



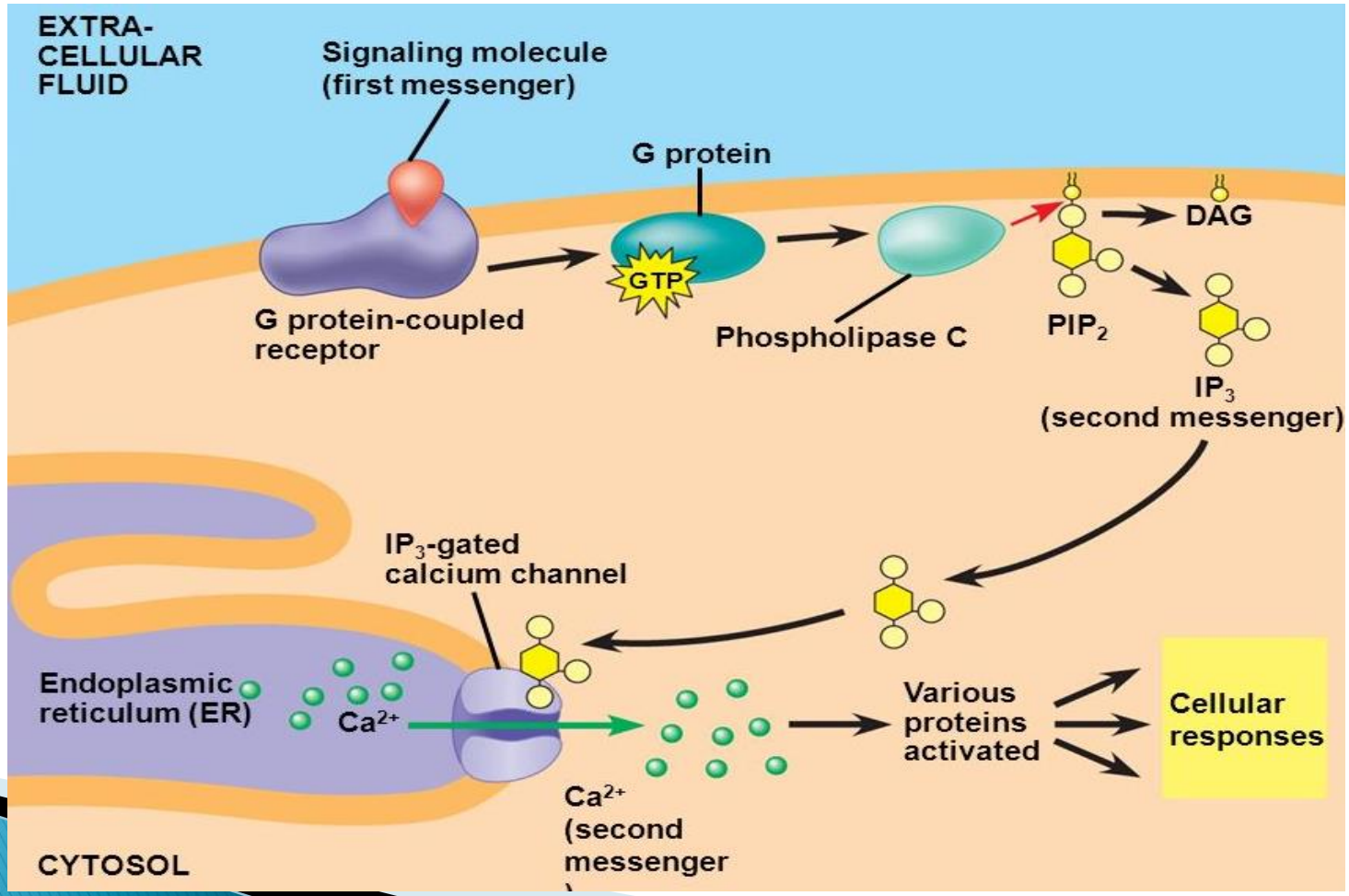
PKA – transported to the nucleus – phosphorylation of transcription factors

- **neurotransmiters, hormones, growth factors**
- **Phospholipase C** – second messengers -**inositol trisphosphate (IP3) and diacylglycerol (DAG)**

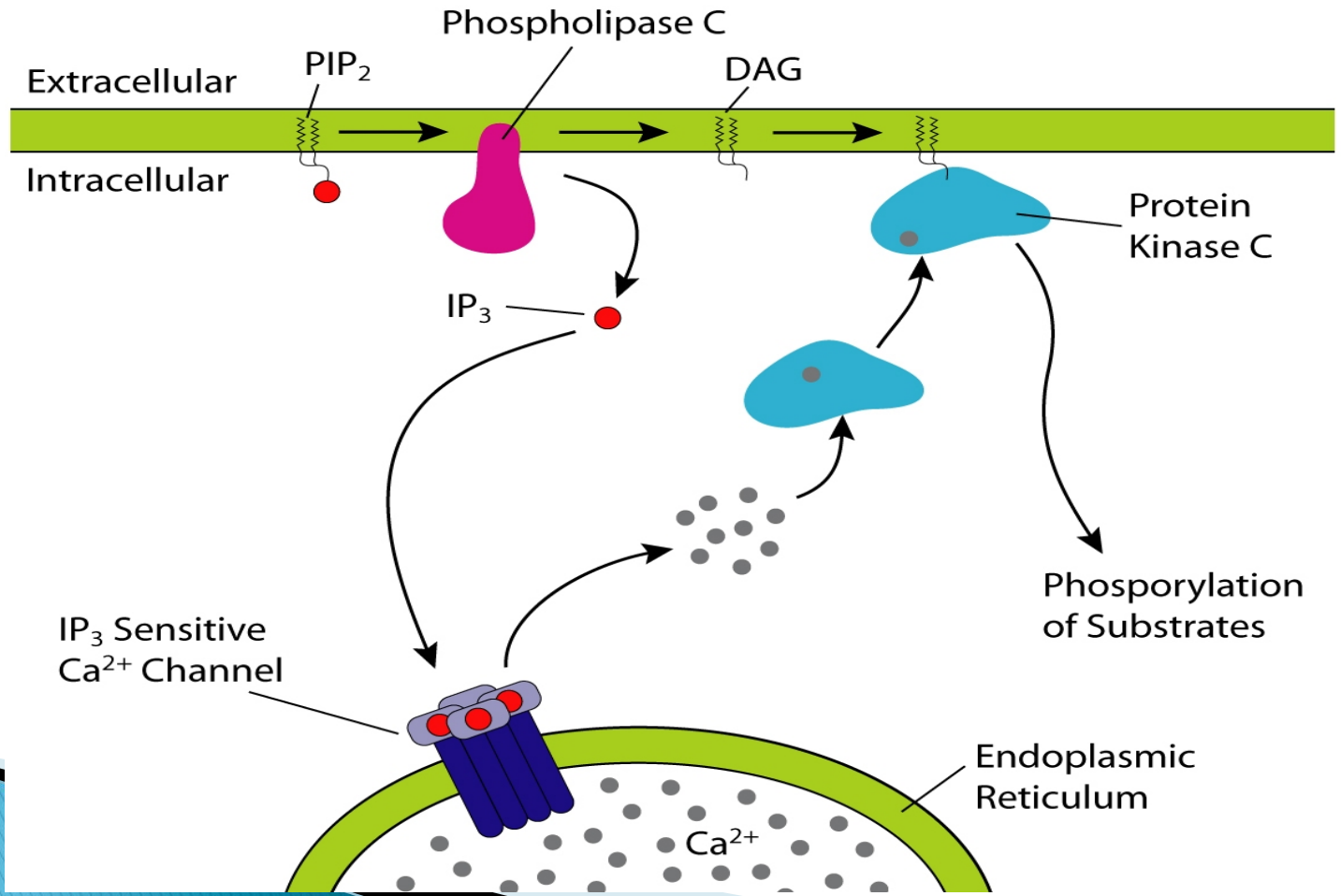


G protein activates phospholipase C

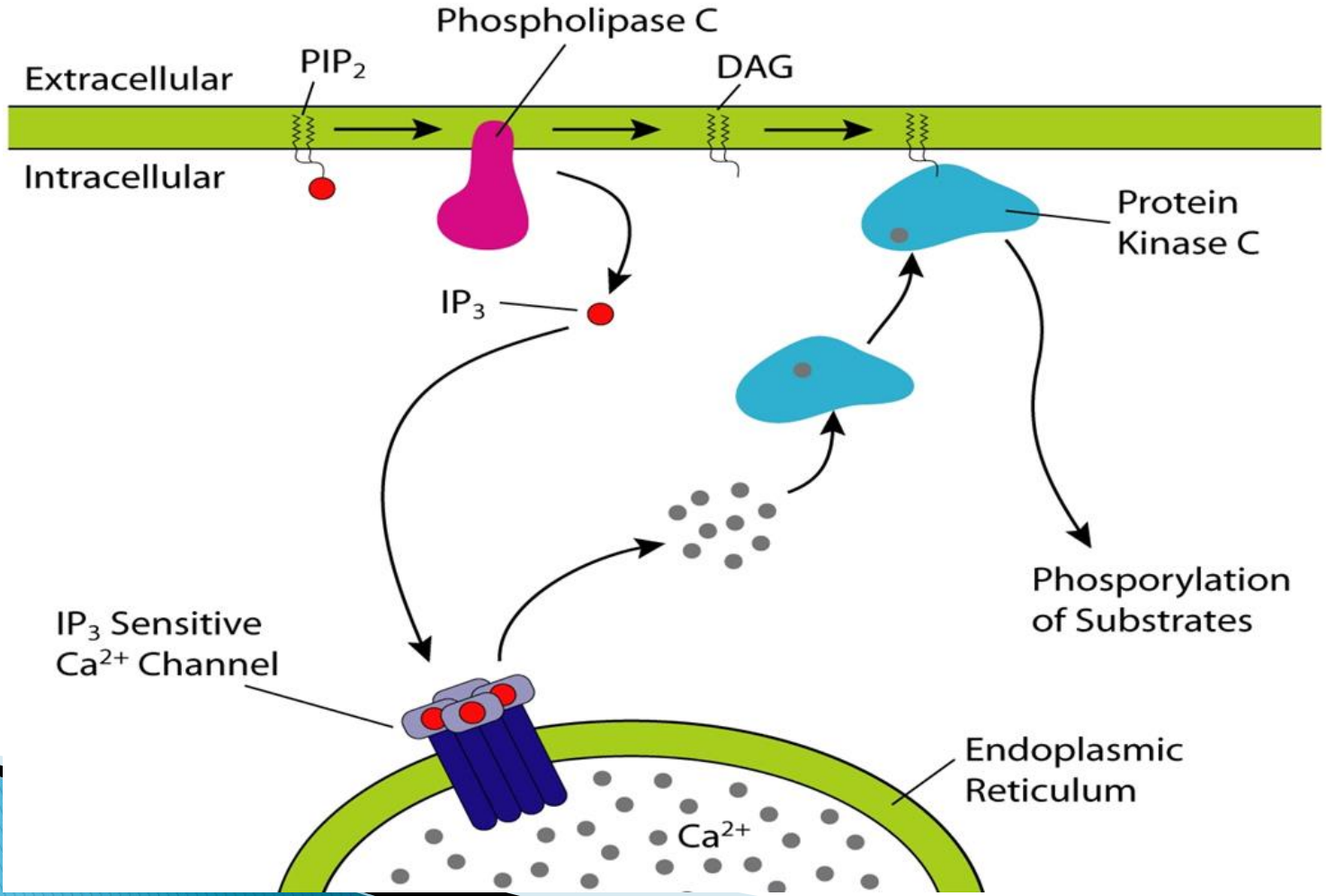
- cleaves the phospholipid **phosphatidylinositol bisphosphate (PIP_2)** into **diacyl glycerol (DAG)** and **inositol trisphosphate (IP_3).**



- DAG - bound to the membrane
- IP3 - released into the cytosol
- IP3 diffuses through the cytosol to bind to IP3 receptors (calcium channels in the smooth endoplasmic reticulum).

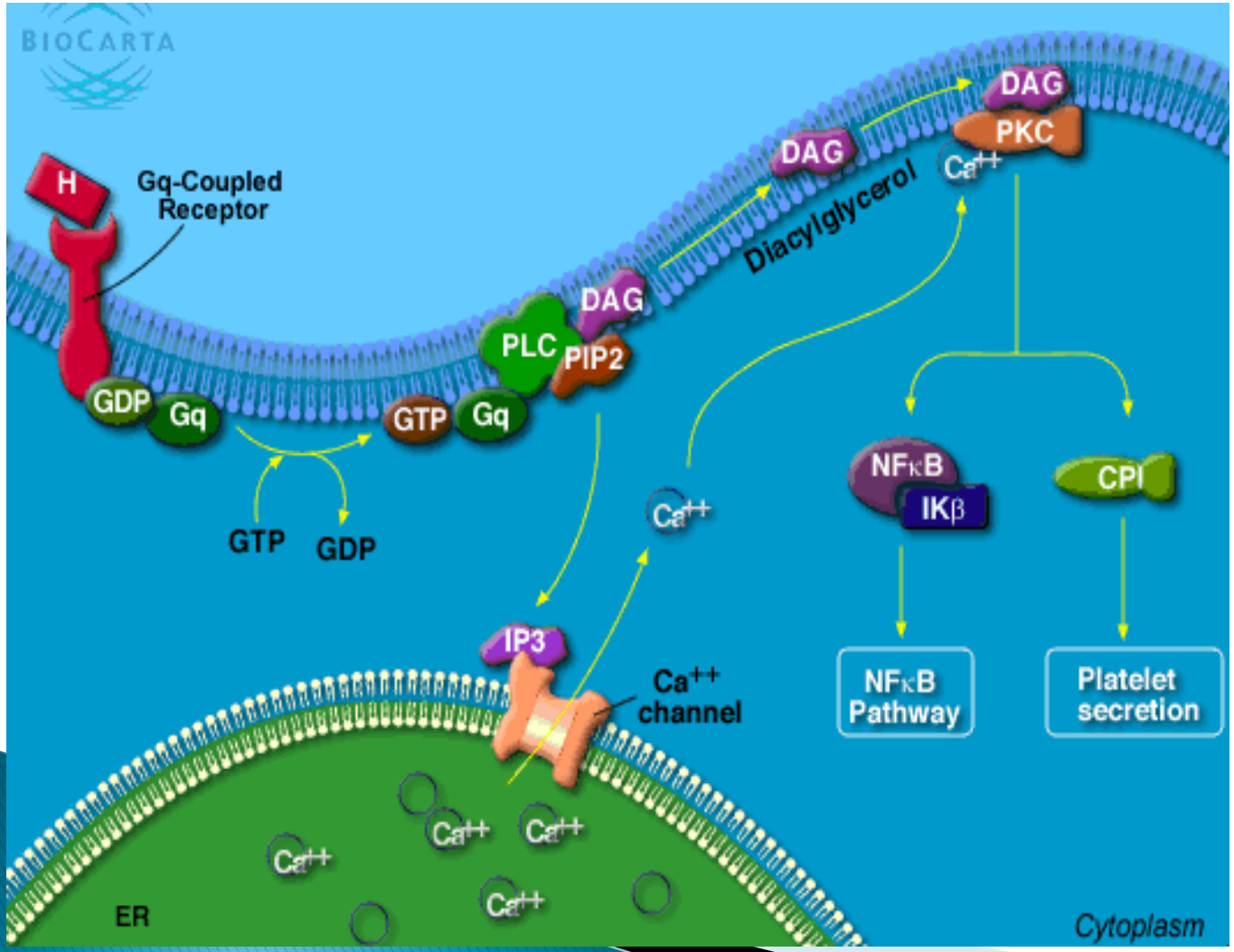


- calcium ions - a cascade of intracellular changes and activity
- calcium and DAG together activate **protein kinase C** - phosphorylation other molecules - cellular activity



Protein kinase C – serine-threonine kinase

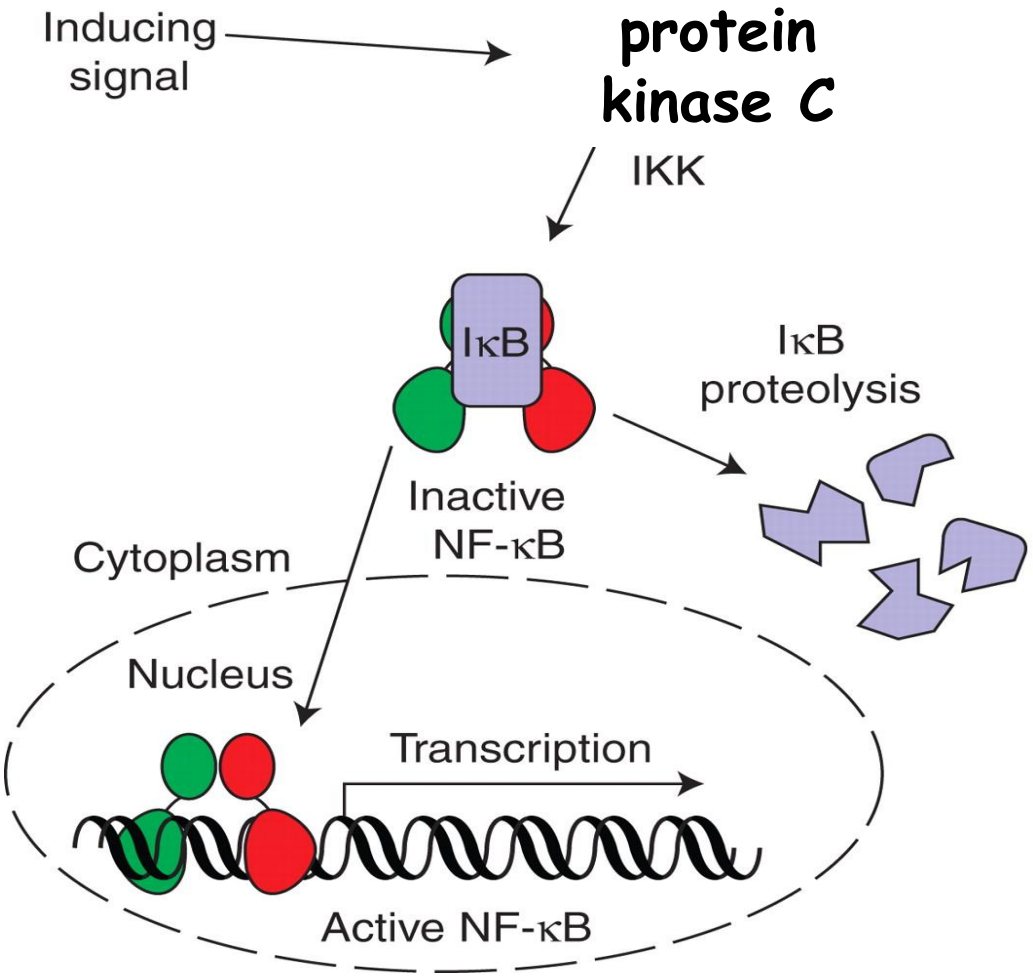
- phosphorylation of hydroxyl groups of serine and threonine amino acid residues on proteins



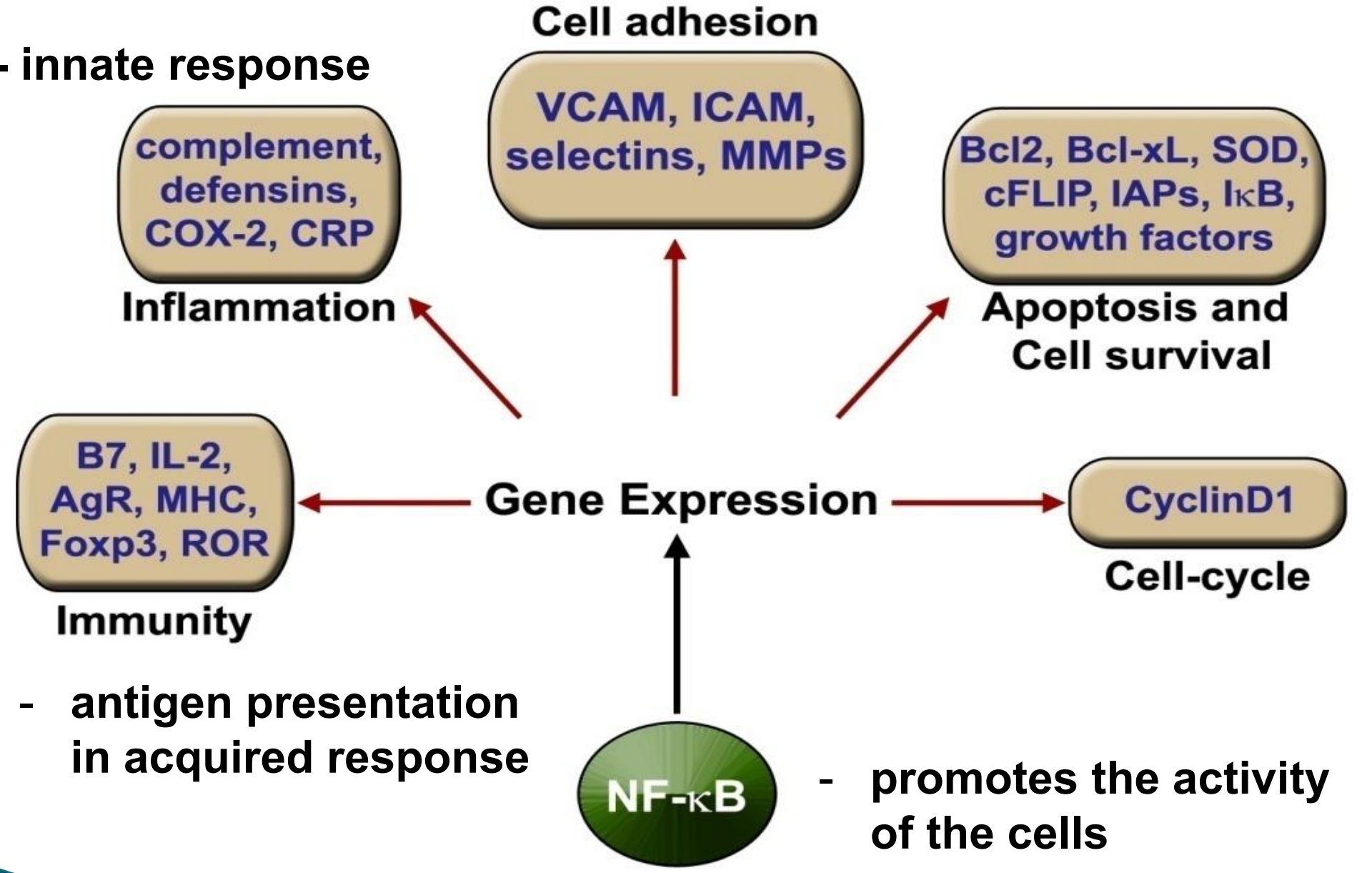
- activated by calcium ions (Ca2+) and diacylglycerol (DAG)
- a multiplicity of functions - induces NF-κB

NF-κB

- a protein complex - controls transcription of DNA(acts as transcription factor)



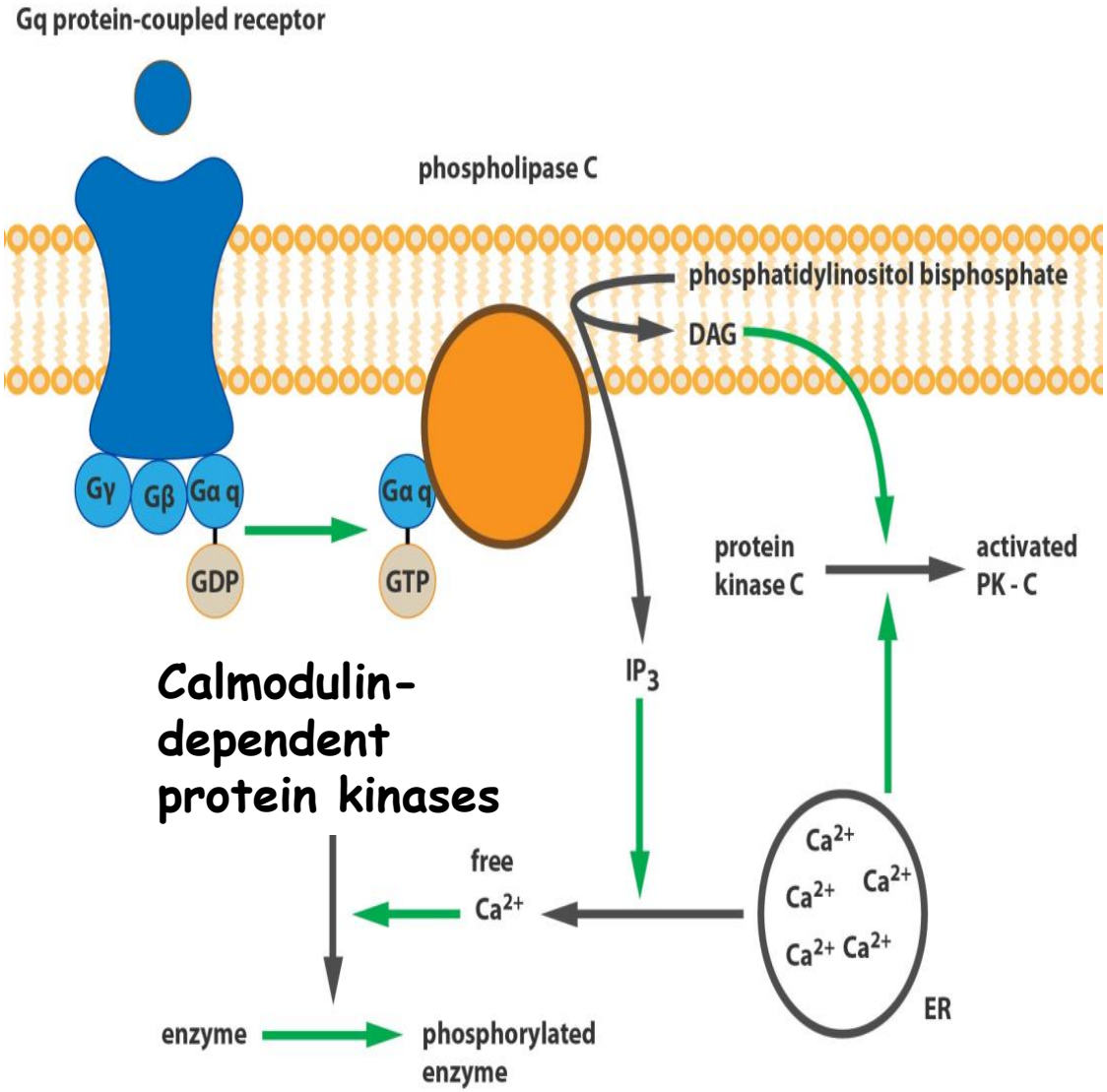
- NF-κB - in cytosol with the inhibitory protein IκB.
- After phosphorylation - IκB dissociates and is degradated by the proteasome.
- The activated NF-κB - translocated into the nucleus - binds to DNA – transcription of genes.



- involved in the immune response to infection
  - cytokine production and cell survival

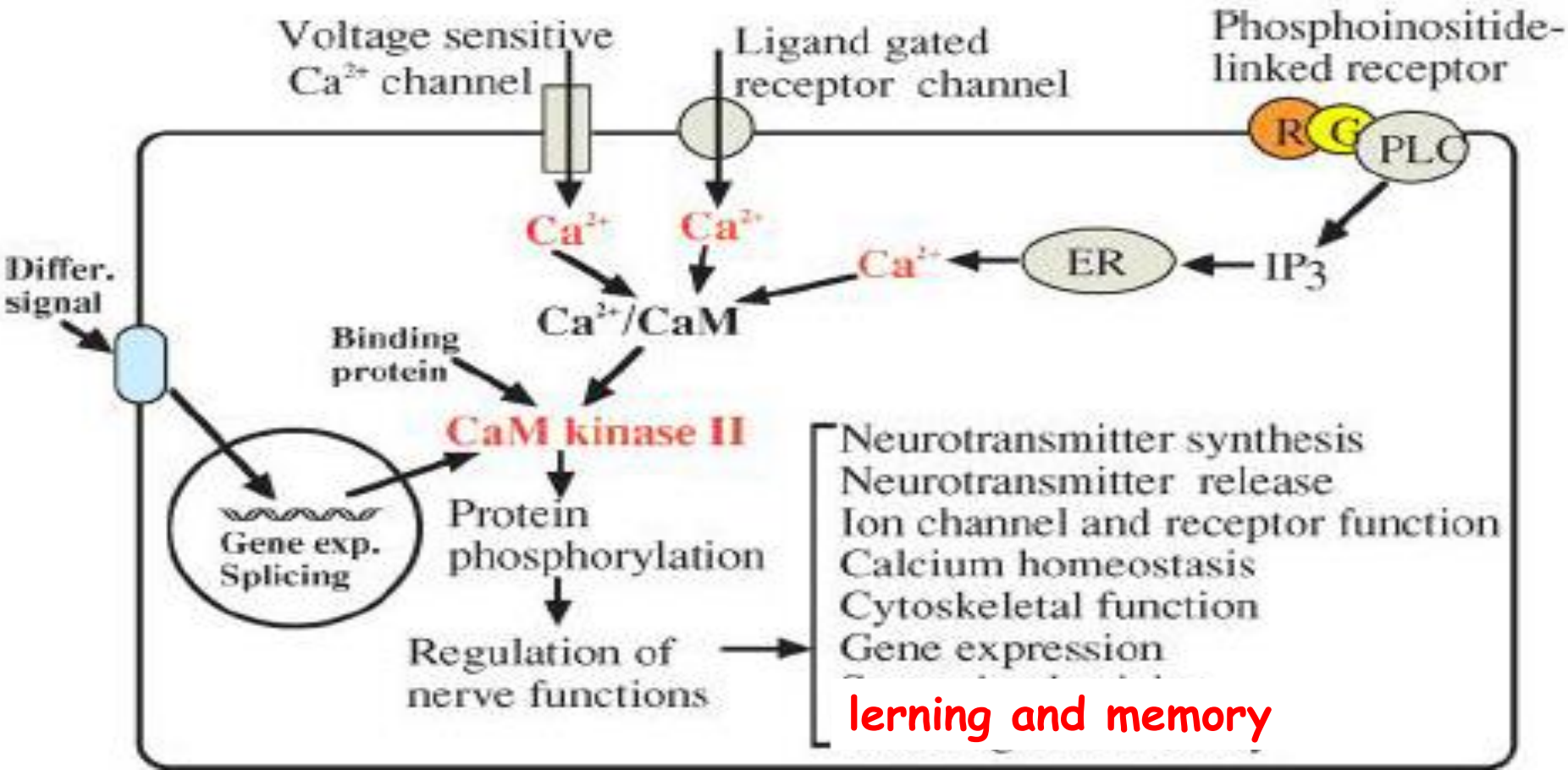
Calcium ions

- second messenger (bound by calmodulin - **Calmodulin-dependent protein kinases (CaM-kinases)**

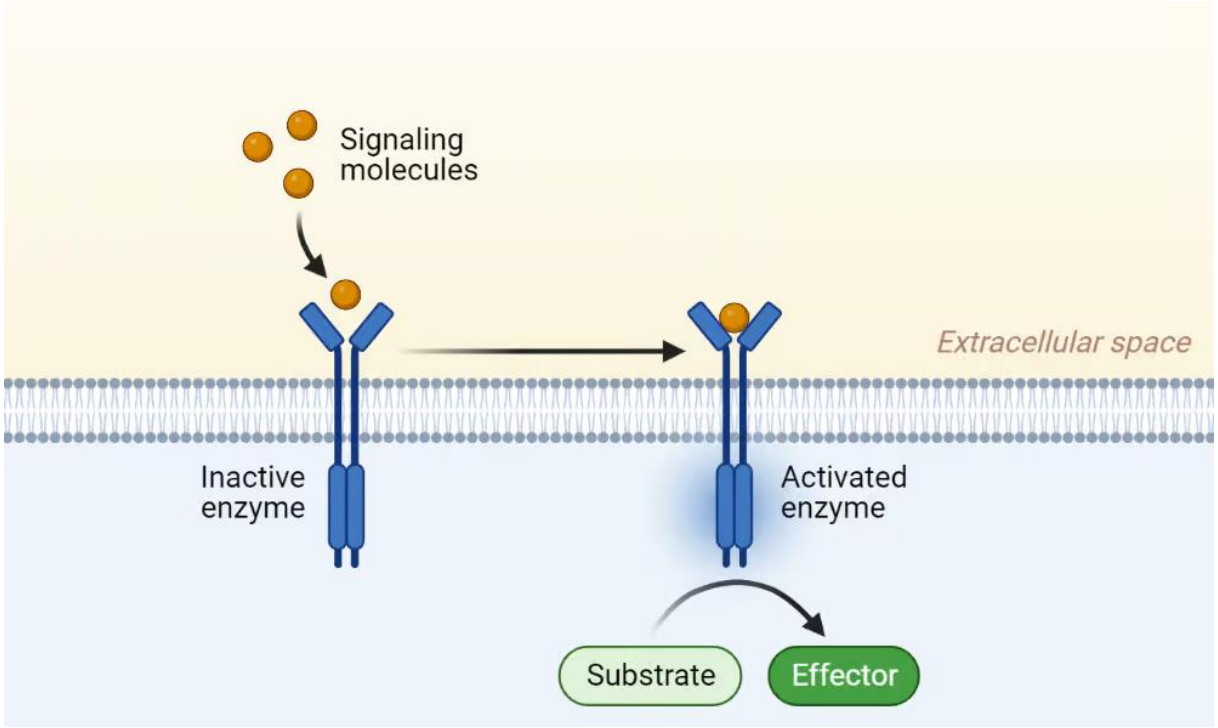


Ca 2+/Calmodulin-dependent protein kinase (CaM-kinases) - serine-threonine kinase

- in nerve cells – neurotransmitter synthesis and release
- learning and memory (dysregulation of CaM-kinases - Alzheimer's disease?)



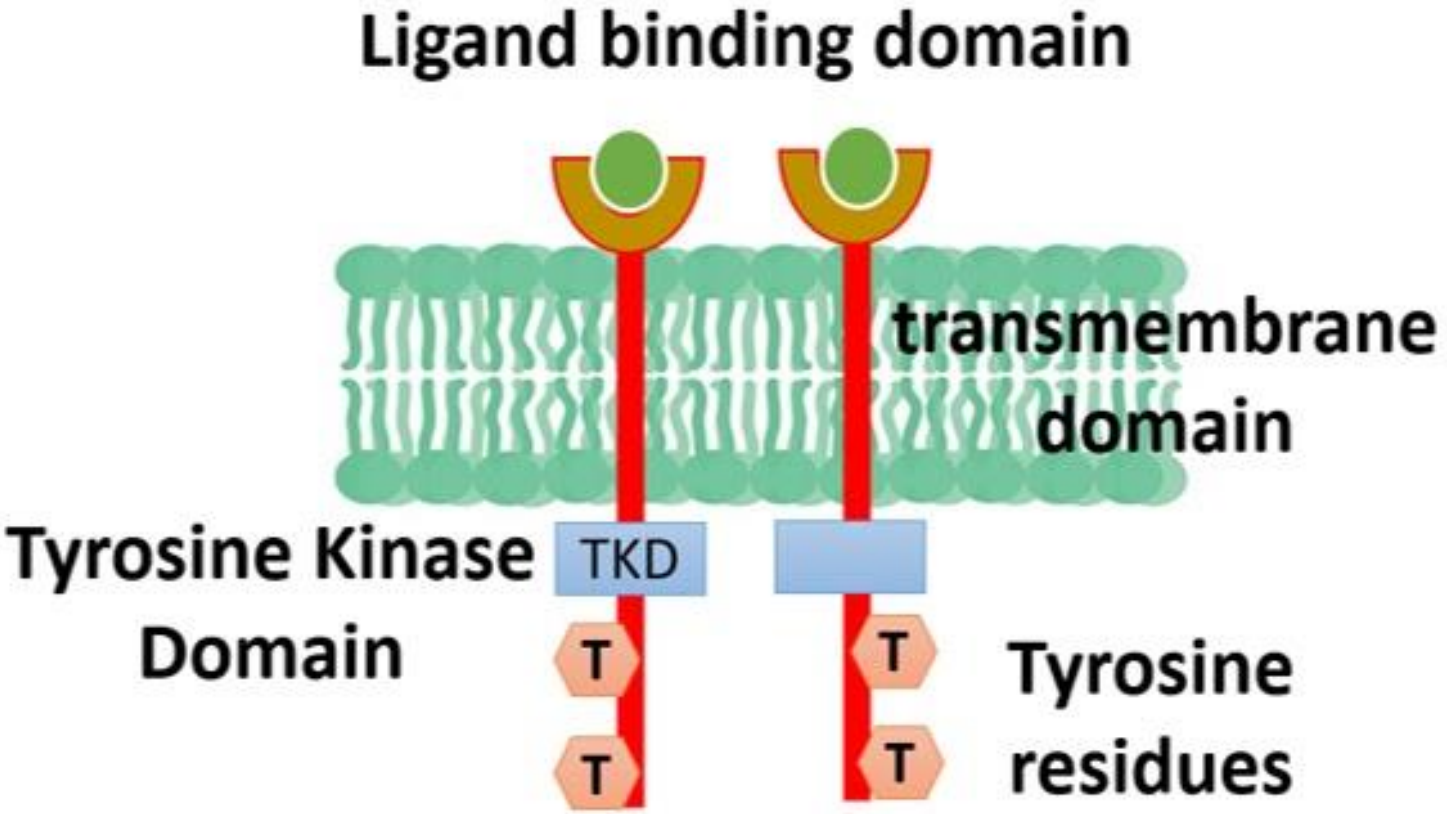
Enzyme-linked receptors (catalytic receptors)



- **Receptors tyrosine kinase** (growth factors, cytokines, and hormones)
- cell growth, proliferation or differentiation, cancers

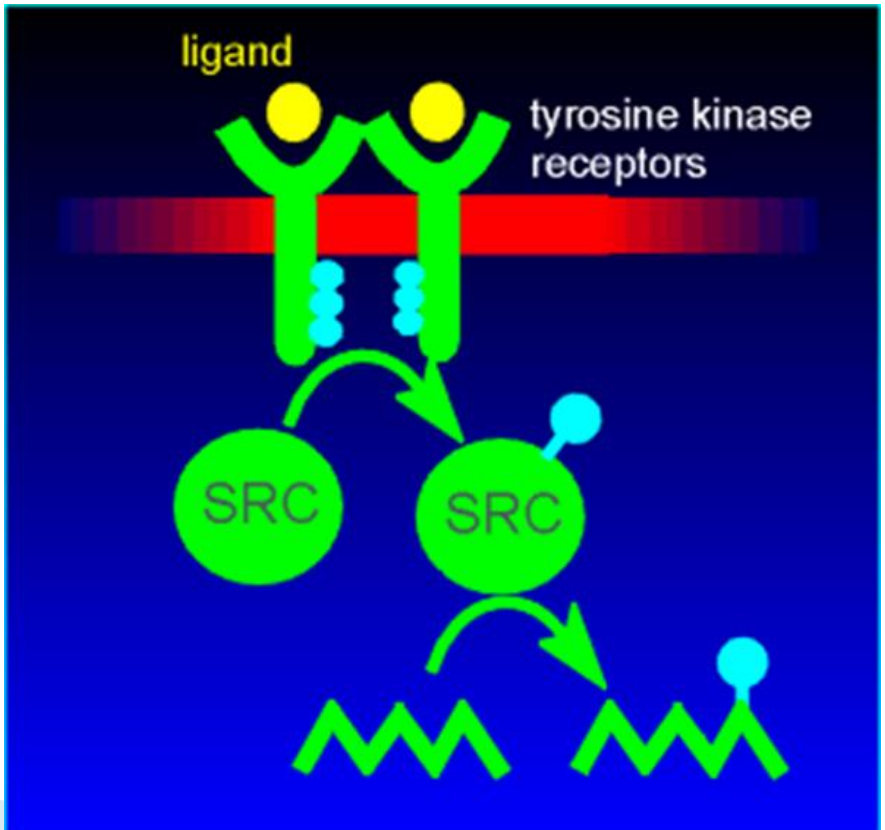
Receptors with intrinsic tyrosine kinase activity

- transmembrane proteins
- extracellular - ligand-binding domain,
- cytoplasmic domain (with tyrosine kinase activity)

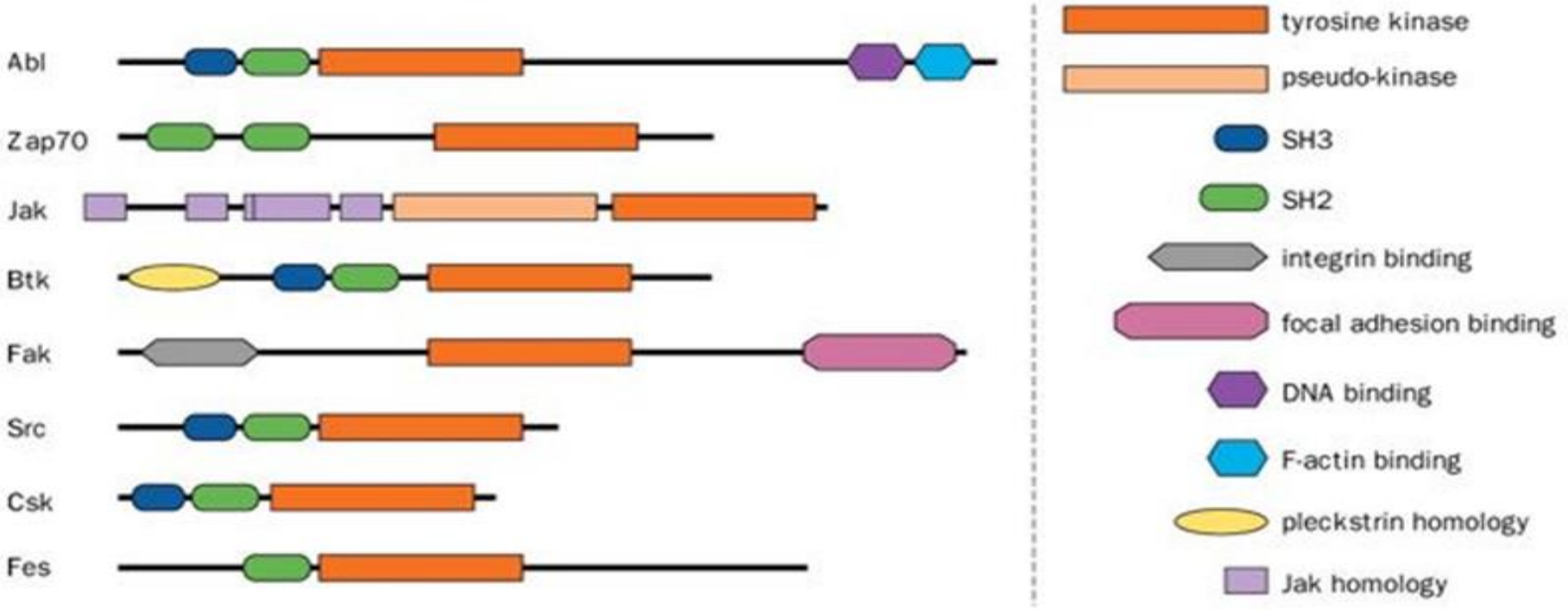


Receptors for some cytokines (interleukins), hormones (prolactin, growth hormone)

- do not possess their own **tyrosine kinase activity**
- Form complexes (activate) nonreceptor **tyrosine kinases**

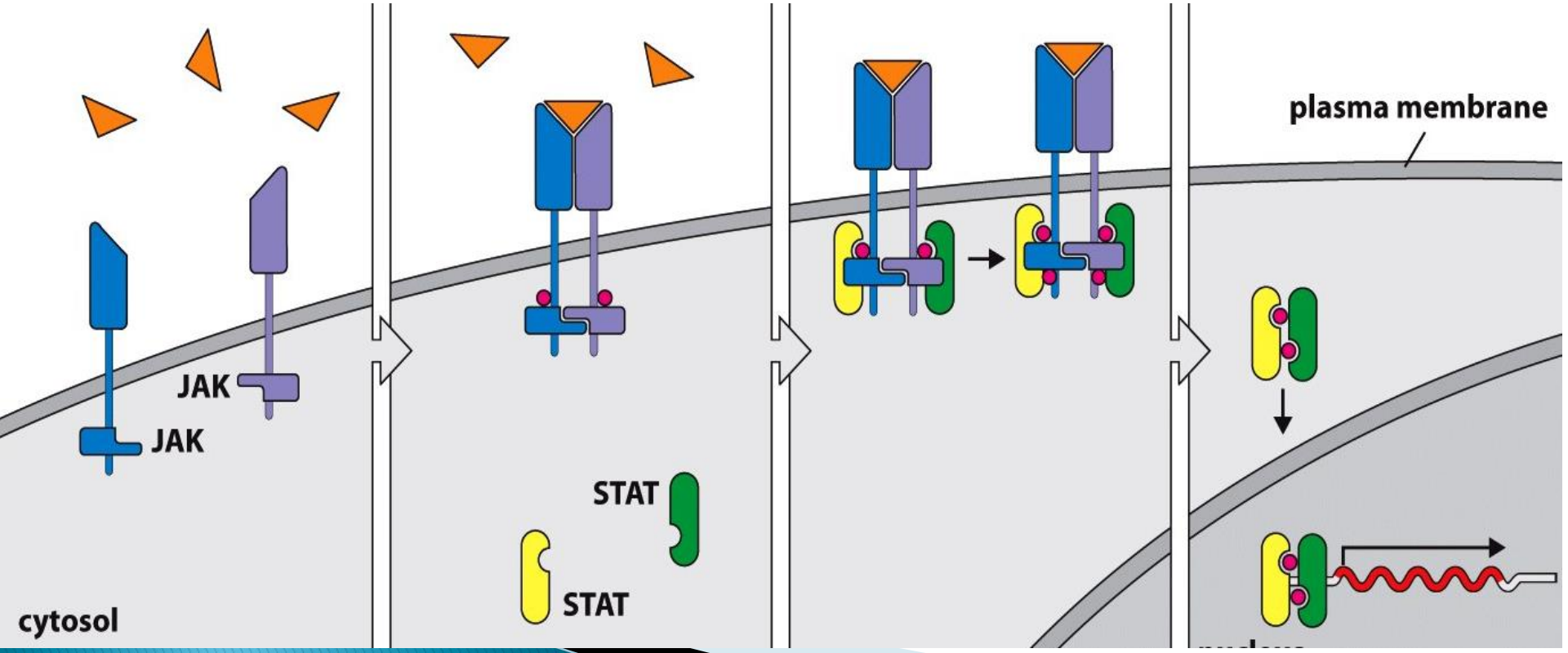


- **RECEPTOR TYROSINE KINASES**
- **NONRECEPTOR (membrane) TYROSINE KINASES**
  SRC (sarcoma) family (Src, Fyn, Lck…)
- **CYTOPLASMIC TYROSINE KINASES**
  JAK (Janus kinase) – STATs phosphorylation

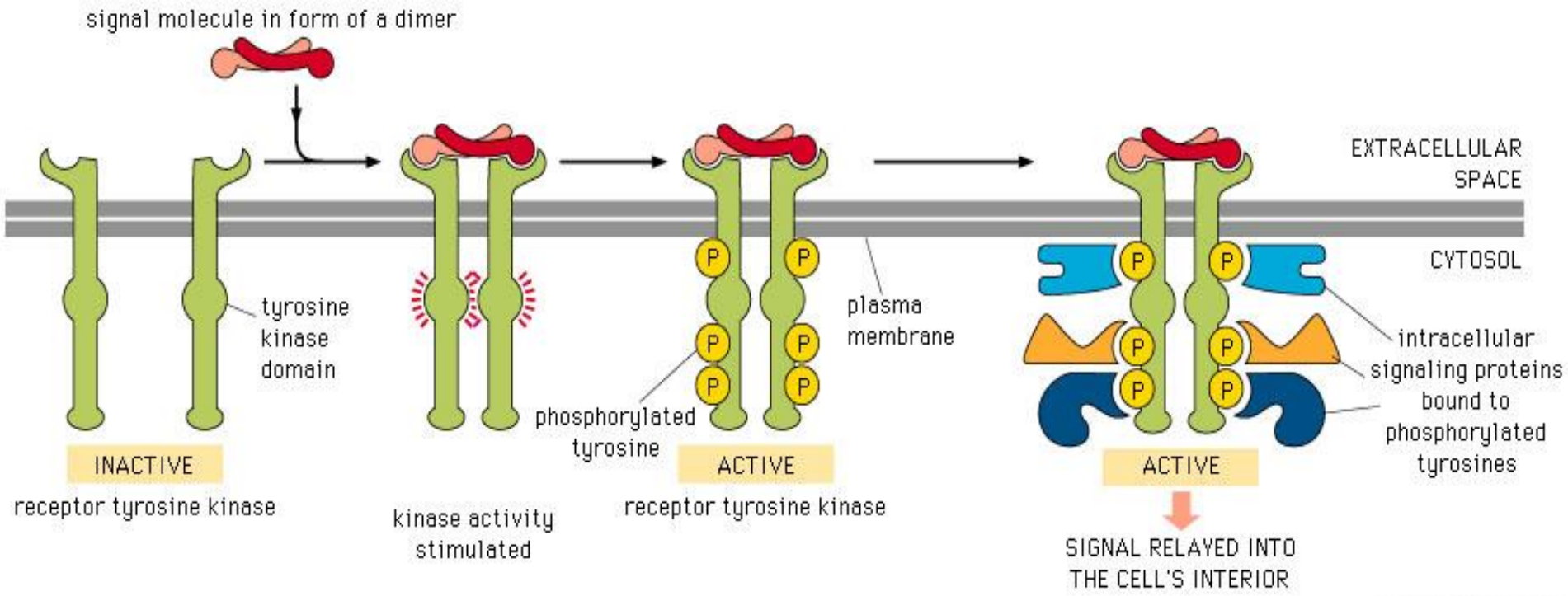


Cytokine receptors - have no intrinsic enzyme activity

- complexes with cytoplasmic tyrosine kinase –**JAK (Janus kinase)**
- JAK phosphorylates the transcription factor **STAT (Signal Transducer and Activator of Transcription)**
- STAT to the nucleus – stimulation of gene transcription (cytokine-inducible genes)



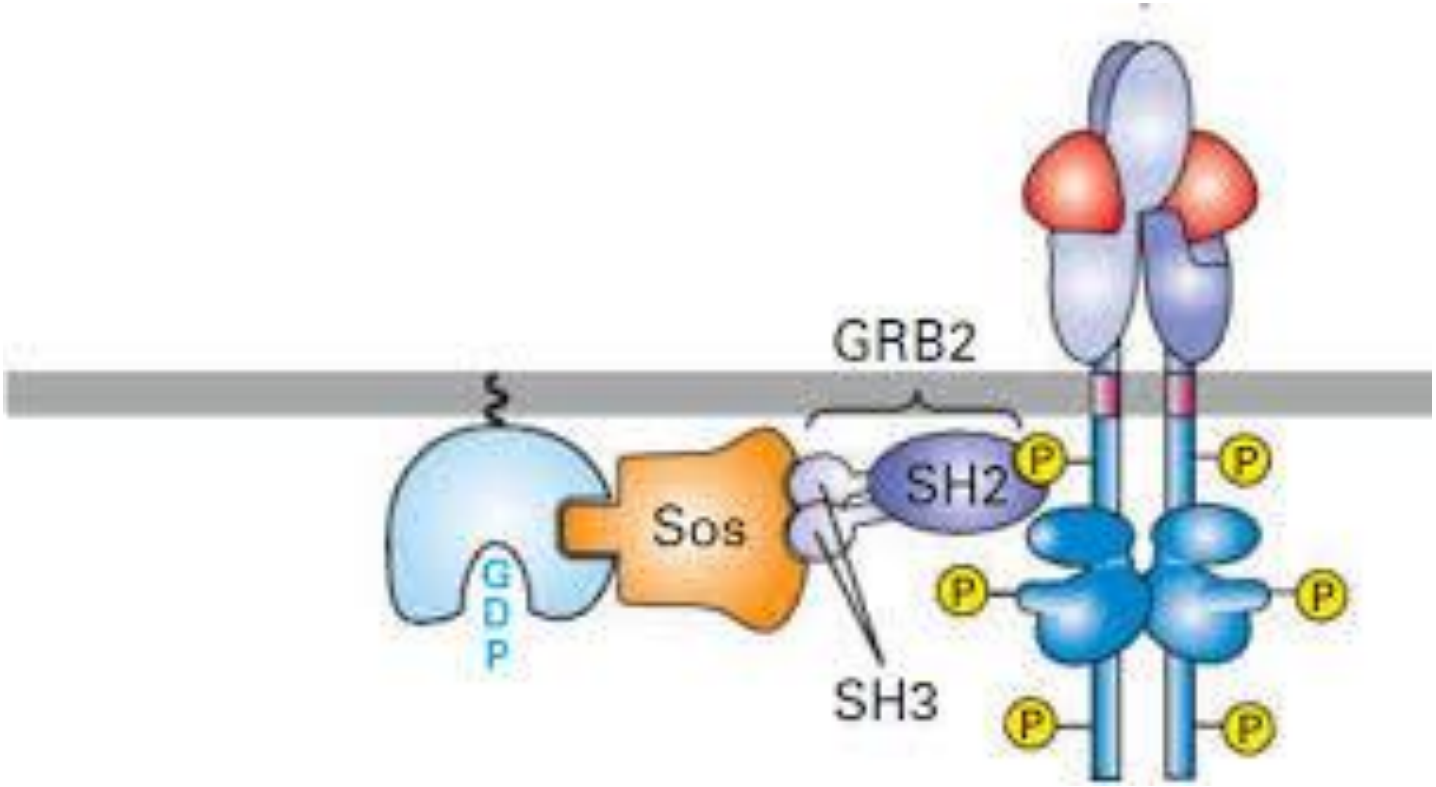
Receptors tyrosine kinase



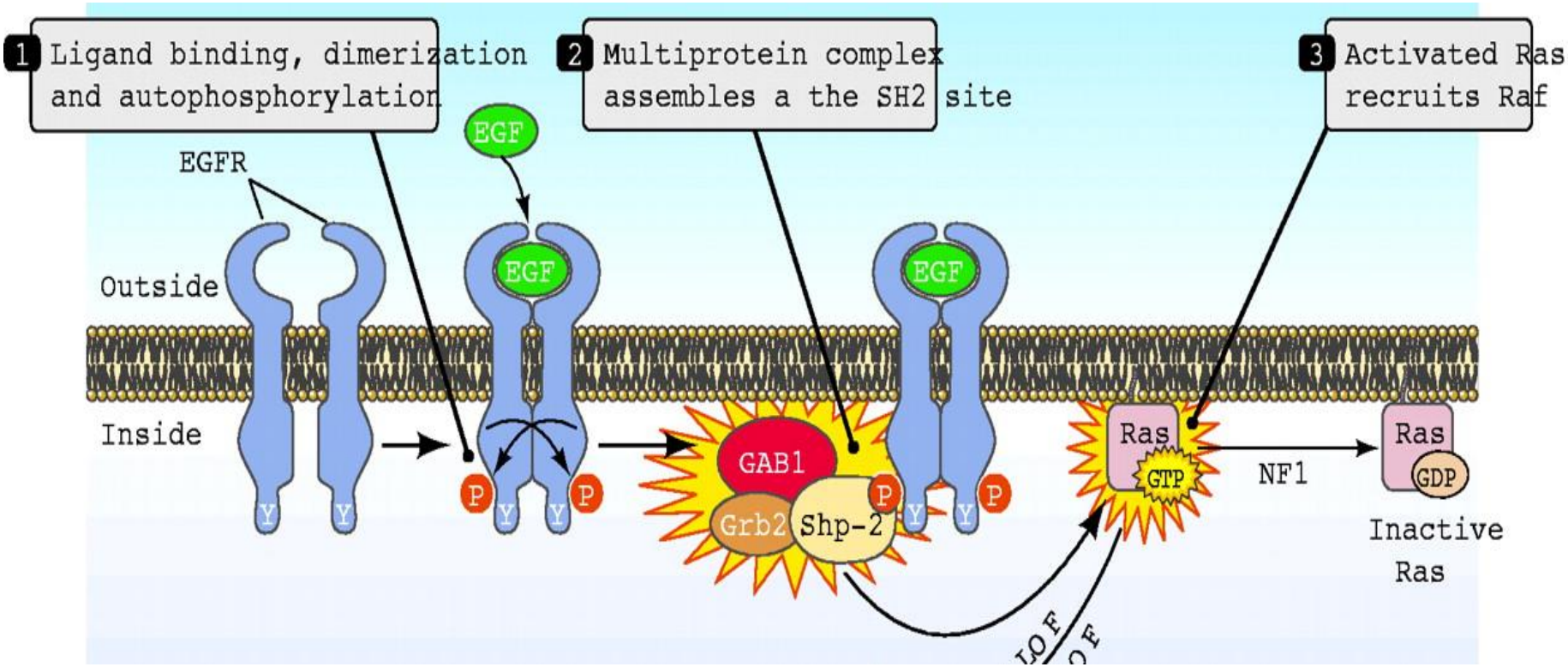
1. Dimerization of receptors (kinase activity stimulation)
2. Phosphorylation of the tyrosine in the cytoplasmic portion of receptor monomer
3. Binding intracellular signaling (**adaptor**) proteins

Adaptor proteins

- SH2, SH3 highly conserved domeins (**S**rc **h**omology)
- Initiation of signal transduction pathways



Receptor tyrosine kinase and Ras protein



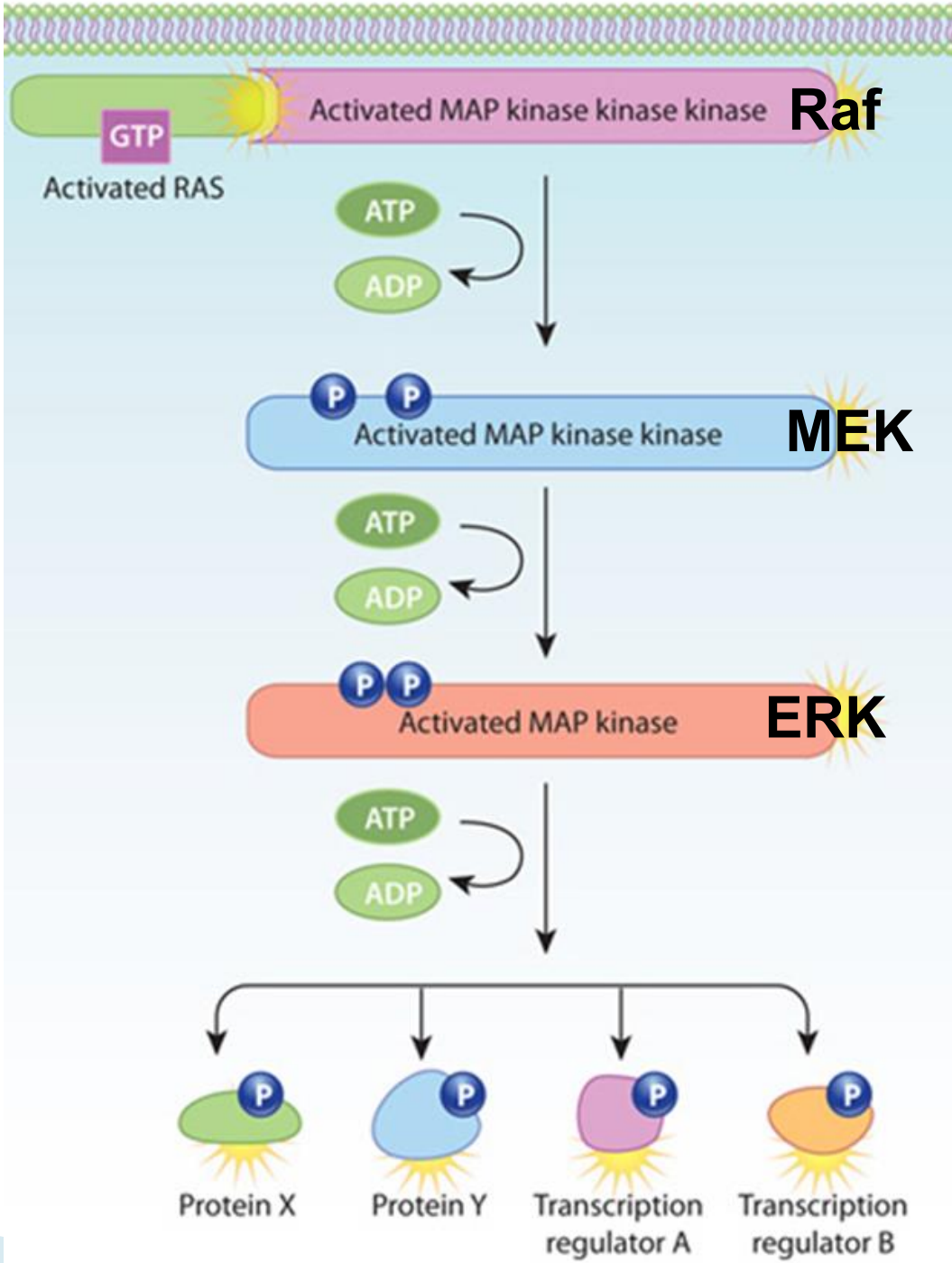
Ras protein – monomeric GTP-binding protein

- resembles α subunit of G protein

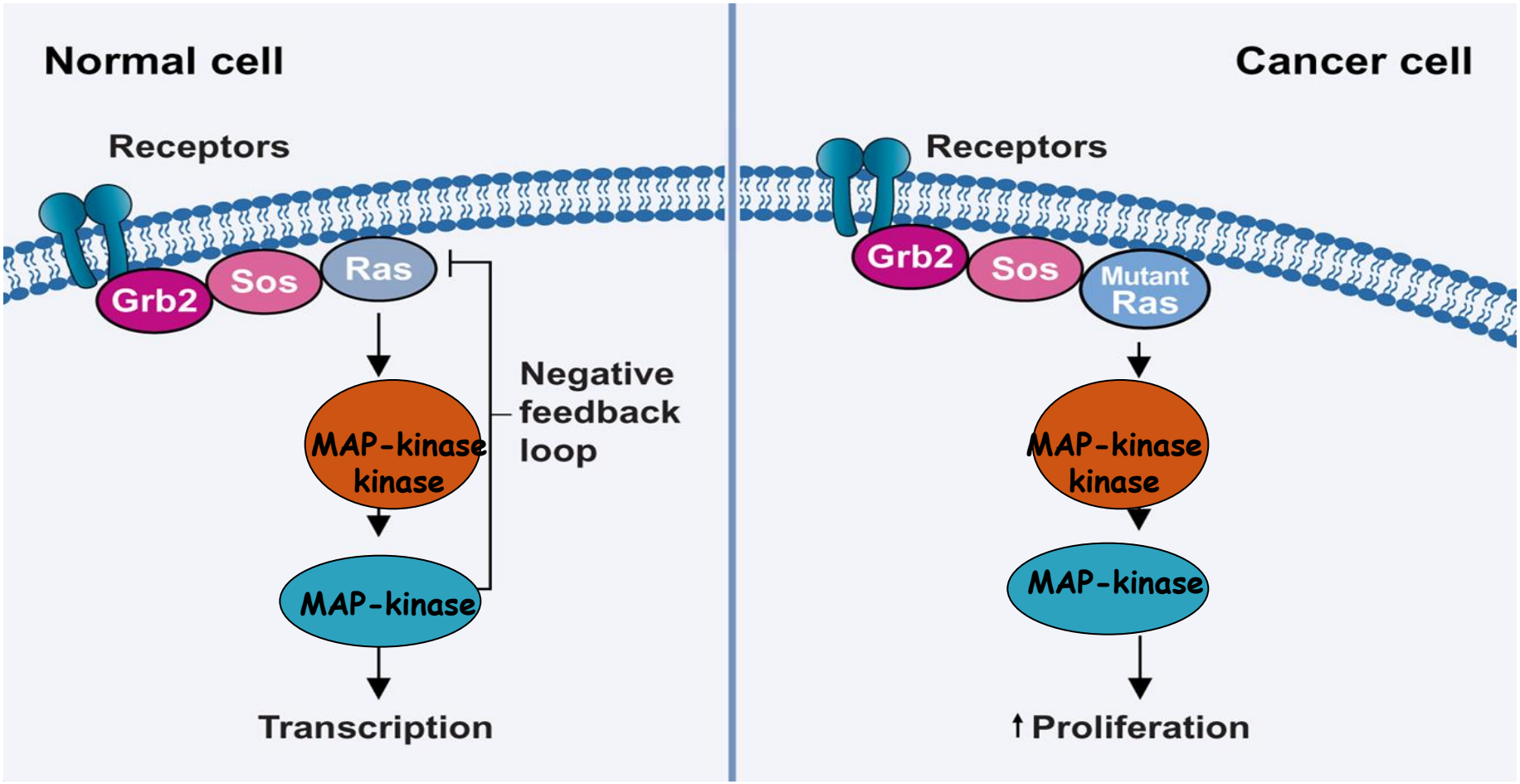
active Ras activates MAP-kinases cascade

MAP-kinase - Mitogen-activated protein kinase

- phosphorylates structural proteins and transcription factors
- **proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis.**



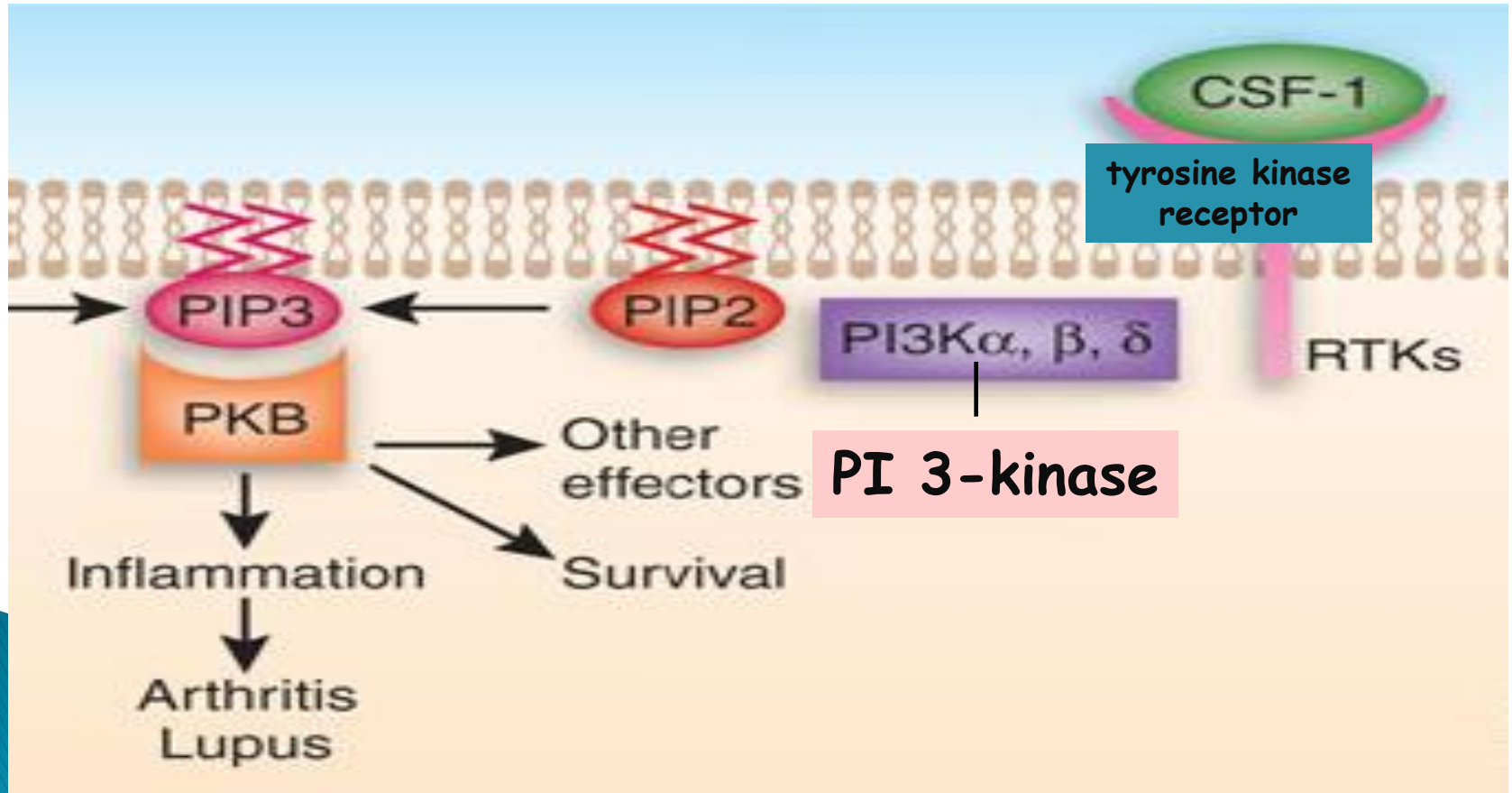
Ras activation - cell growth and proliferation – mutations, in which the protein is still active - cancer



The mutant protein Ras is not inhibited by the negative feedback mechanism - cancer

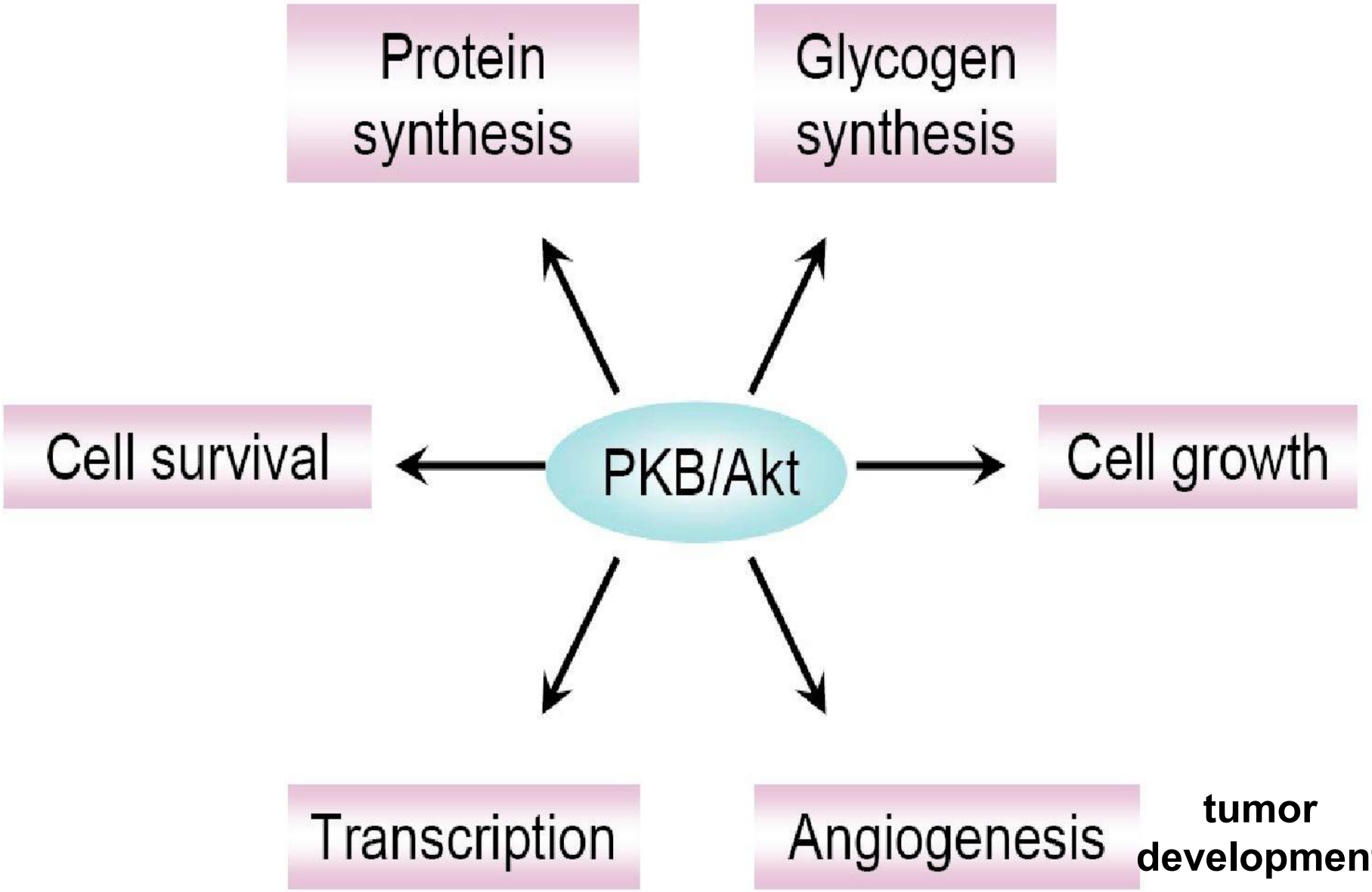
Catalytic receptors also stimulate Phosphatidyl-inositol 3-kinase (PI 3-kinase)

- phosphorylates inositol phospholipids of plasma membrane (PIP2 to PIP3)
- PIP3 - activation of protein **kinase B (PKB)**



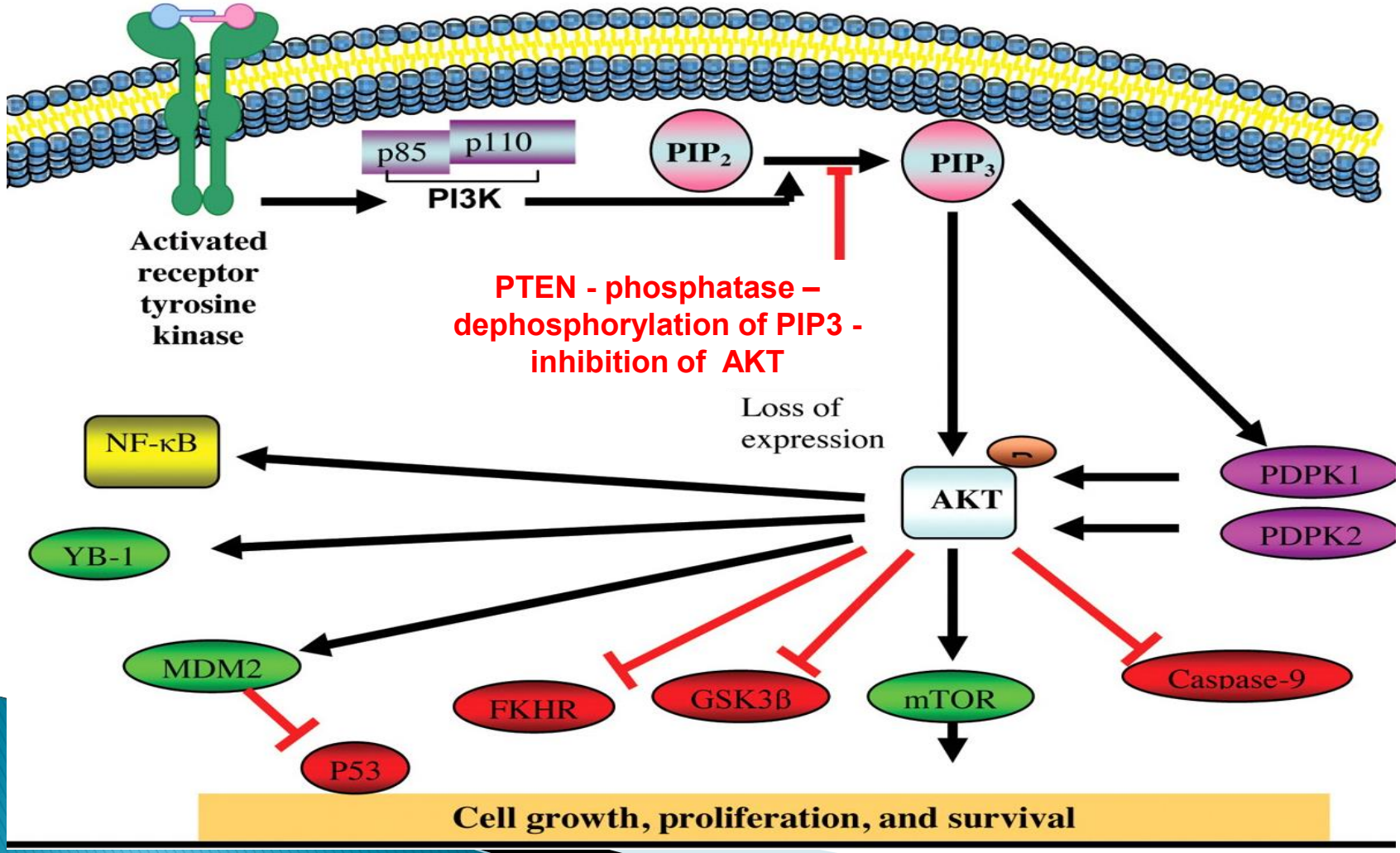
Protein kinase B – kinase Akt

- a serine/threonine-specific protein kinase

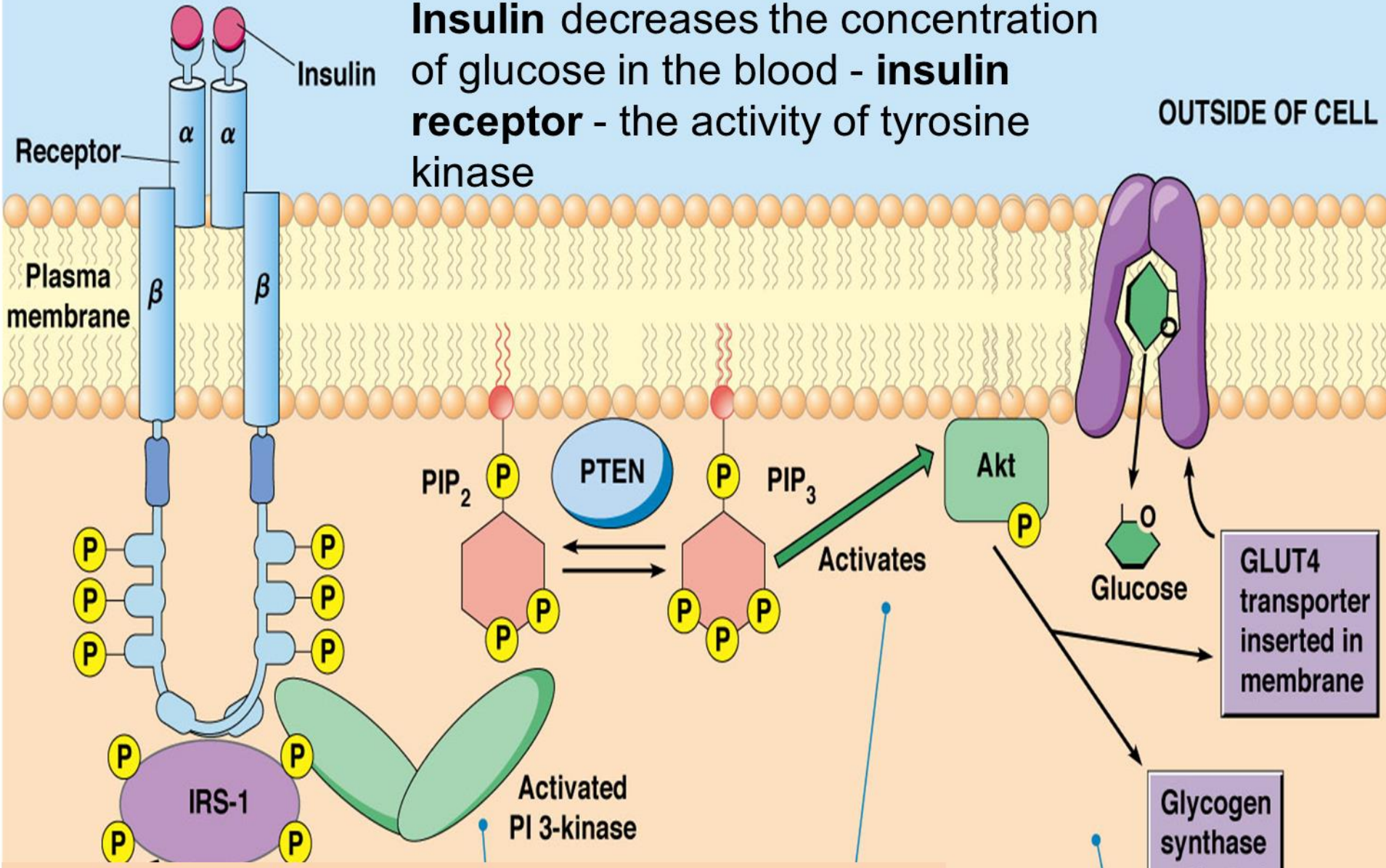


Akt kinase in cancer

- aberrant activation of Akt (mutations of Akt, inactivation of PTEN) - glioblastoma, ovarian, pancreatic and breast cancers



Insulin decreases the concentration of glucose in the blood - **insulin receptor** - the activity of tyrosine kinase

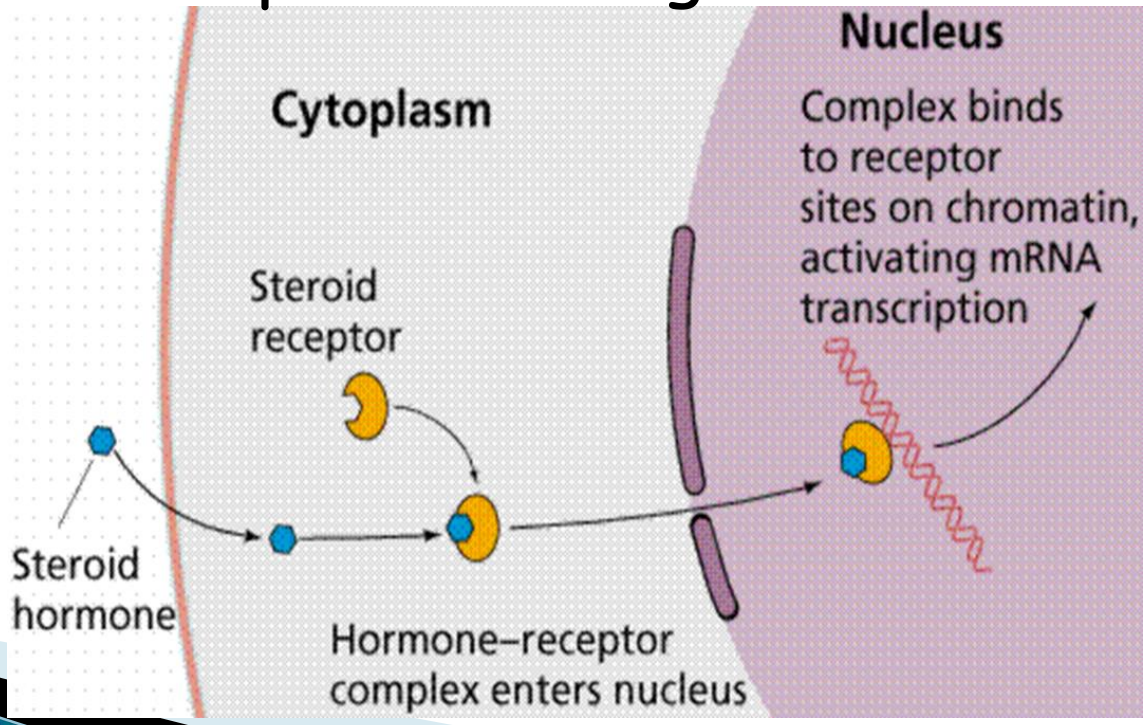


Insulin receptor substrate 1 (IRS-1)

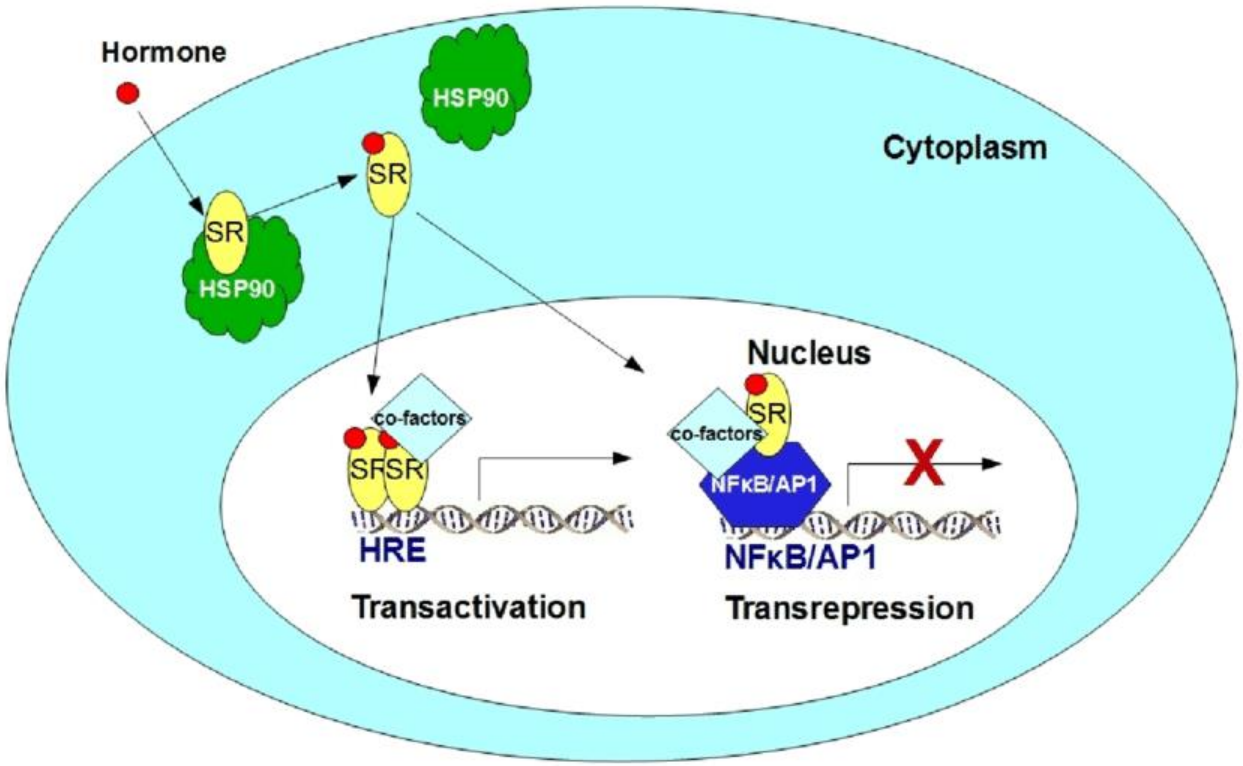
- recruitment of GRB2- activation of Ras pathway
- or activation of PI3 kinase - Akt

Steroid receptor signaling

Nuclear-initiated steroid signaling (NISS)
- Intracellular receptors - lipophilic substances – **steroid hormones** (androgens, estrogens, glucocorticoids, progesterone, vitamins A and D) – act as l**igand-activated transcription factors** - bind to DNA - regulate the expression of genes

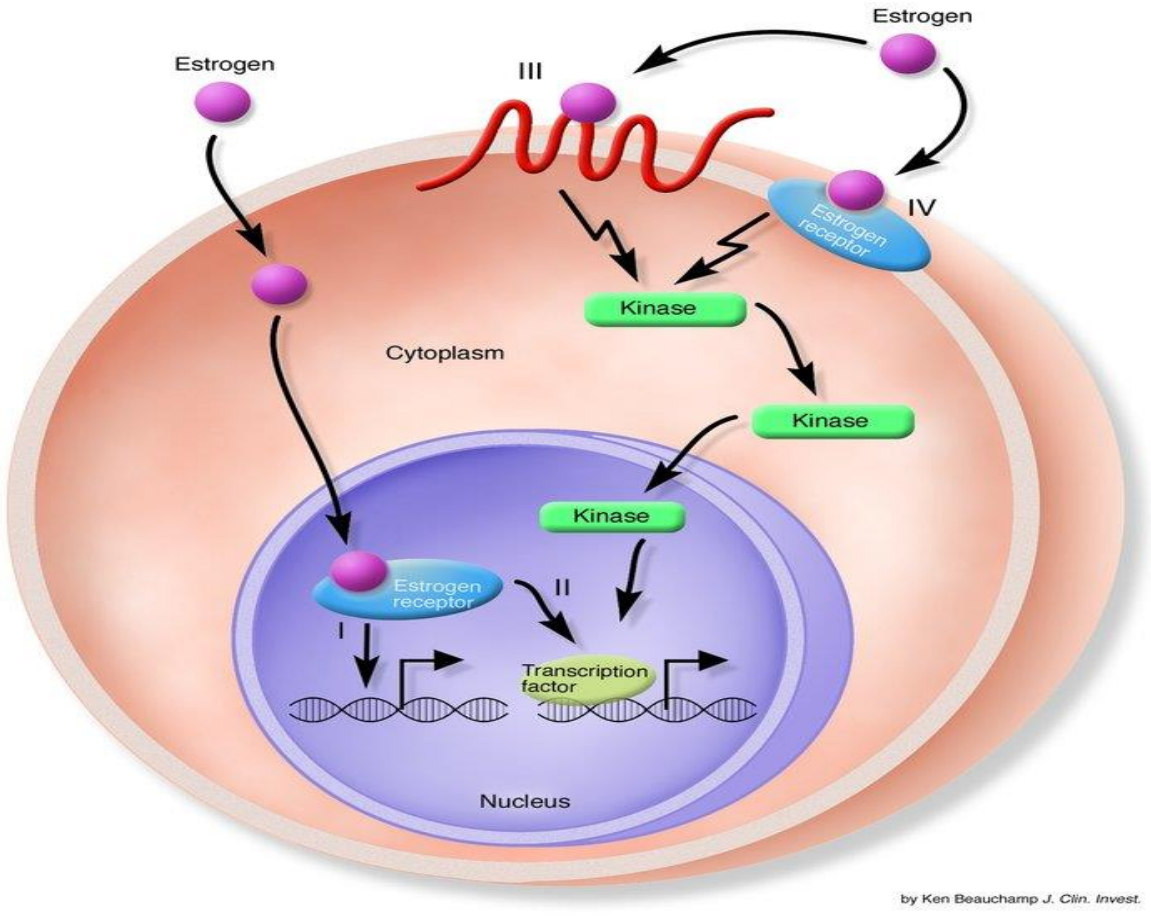


Steroid hormone-receptor complex binds to the **hormone-response element (HRE)** in the promoter (or enhancer) region of the gene - transcription

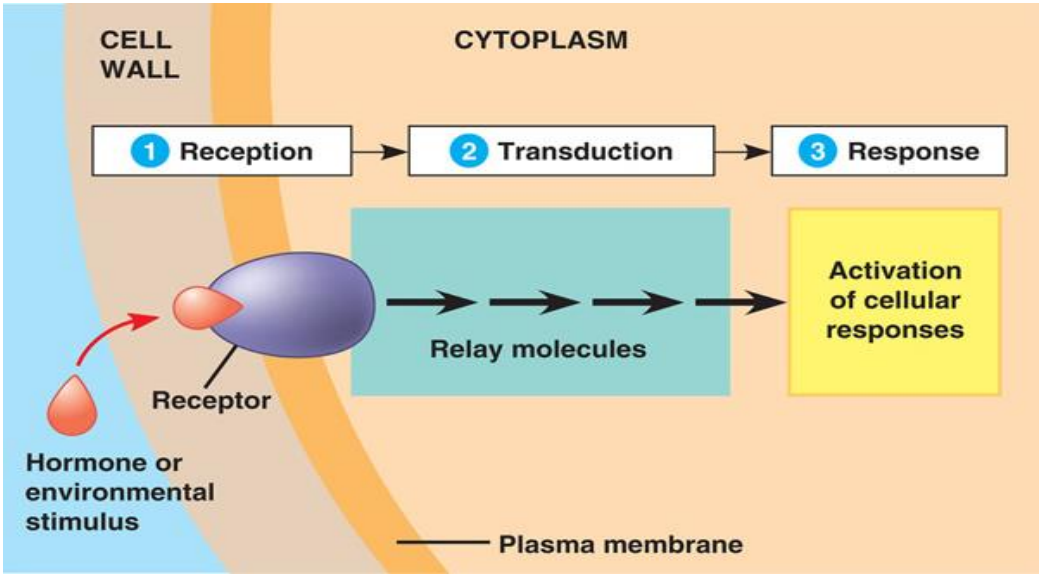


Membrane-initiated steroid signaling

- Repid effect (within seconds) of steroid hormones
- G-protein-linked receptors, catalytic receptors….
- Protein kinase activation (phosphorylation of proteins), Ras protein activation….

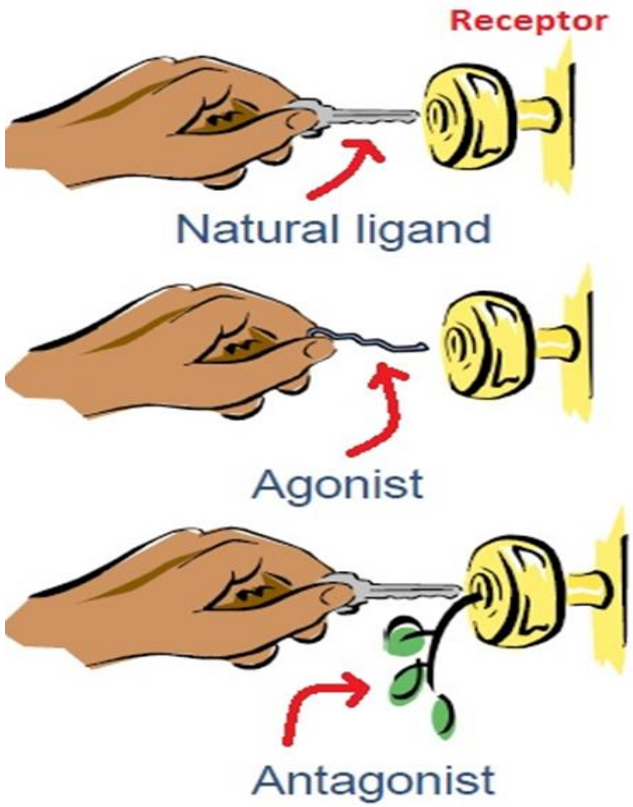


Binding of ligand – receptor -activation (conformational change, oligomerization) – **transduction of signal** (into the cell)



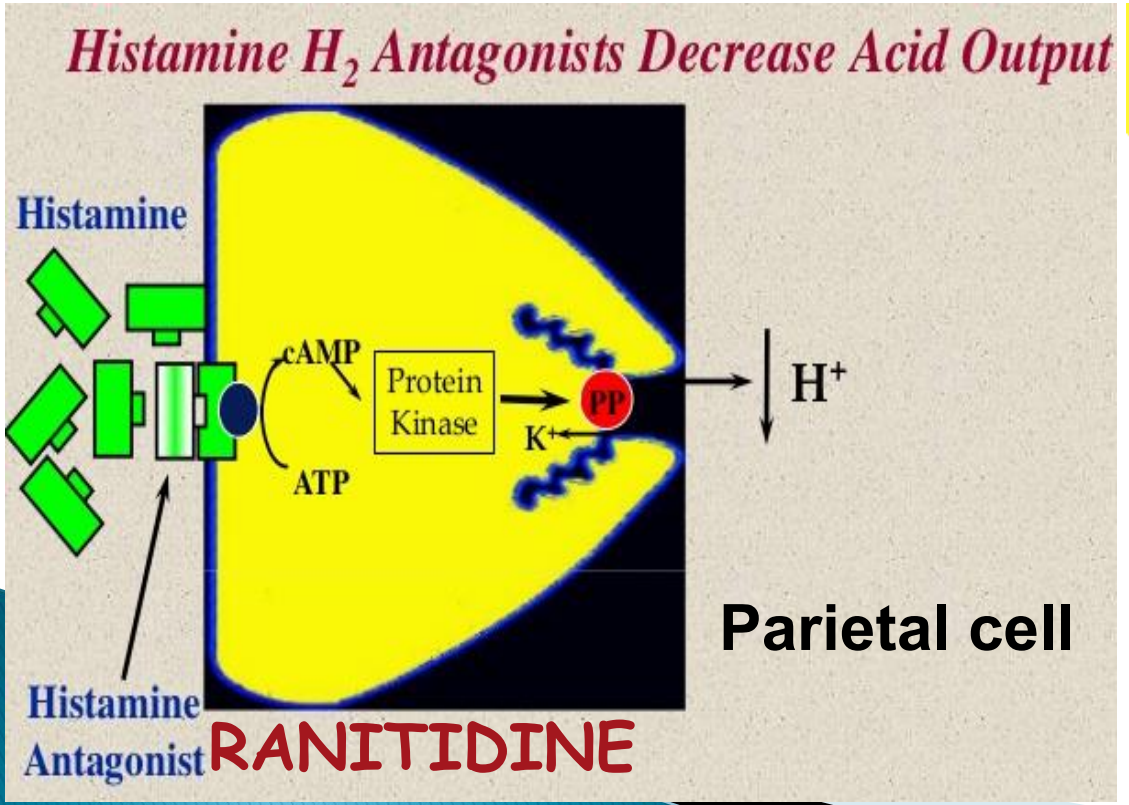
Agonist – a ligand capable of activating a specific receptor

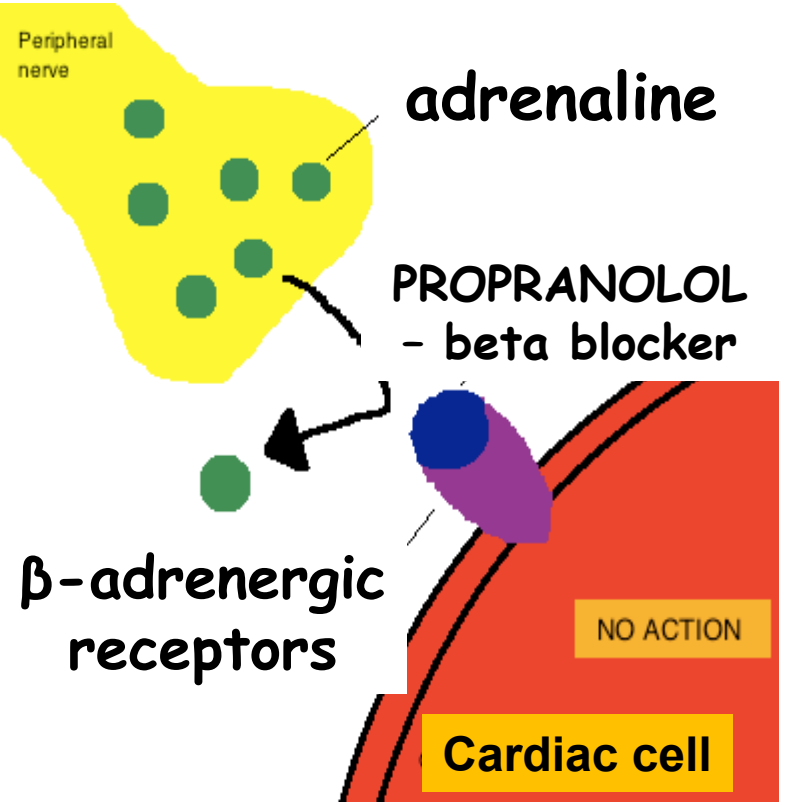
Antagonist - a ligand capable of binding to a specific receptor but does not activate it



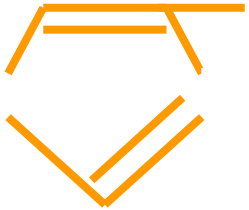
The antagonist - blocks the binding of ligand and the receptor (treatment of diseases)

- RANITIDINE - a histamine H2-receptor antagonist - inhibits stomach acid production
- PROPRANOLOL - beta blocker - blocks the action of β-adrenergic receptors



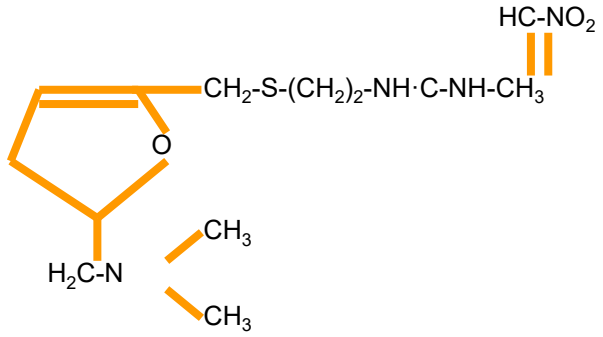


RANITIDINE - peptic ulcer disease and gastroesophageal reflux disease

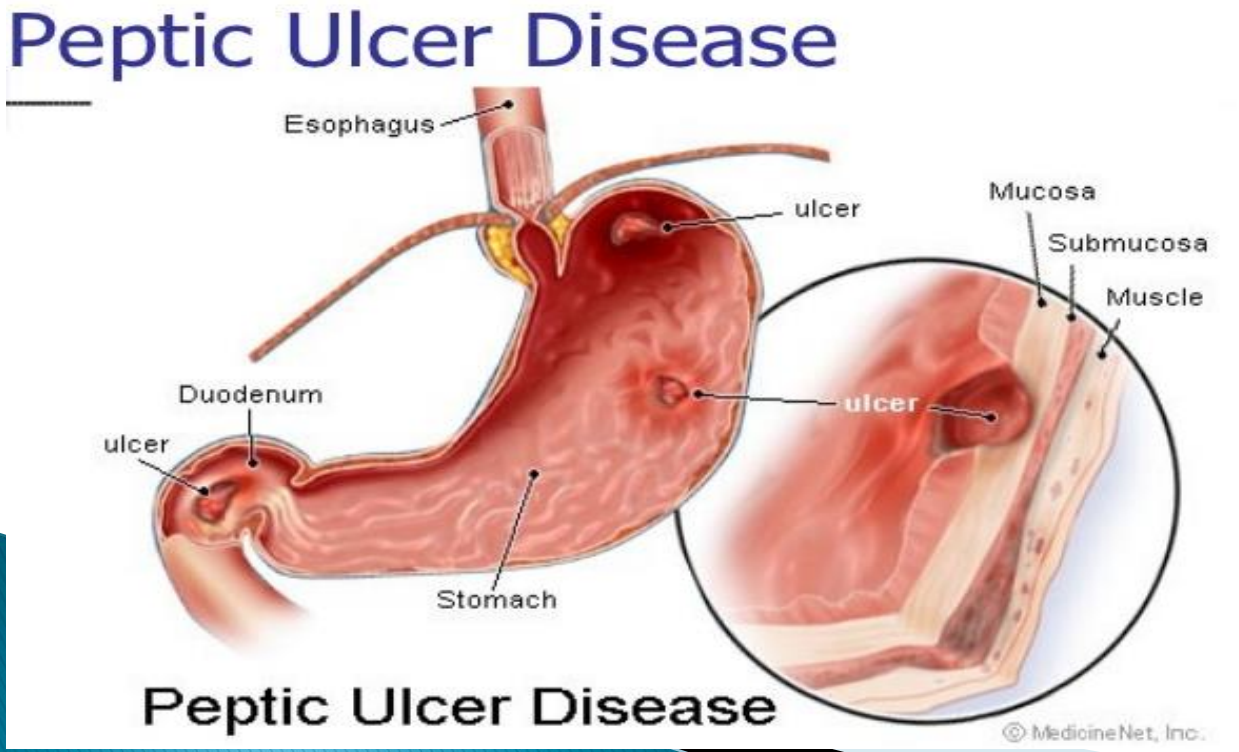


HISTAMINE

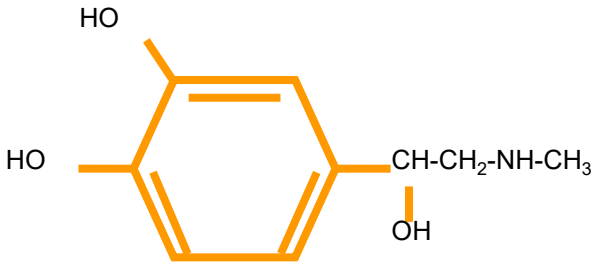
Differences in side chains only



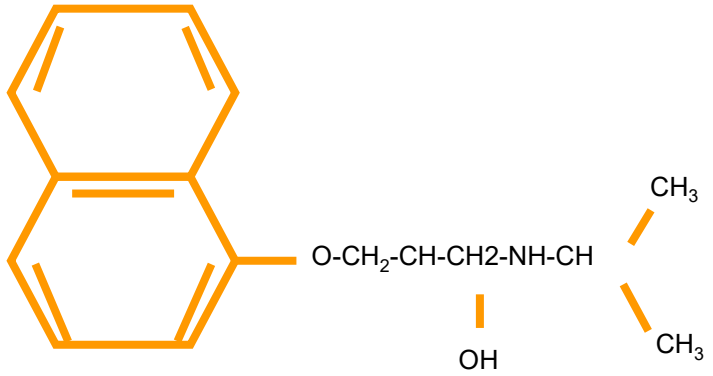
RANITYDINE
(H2 receptor antagonist)



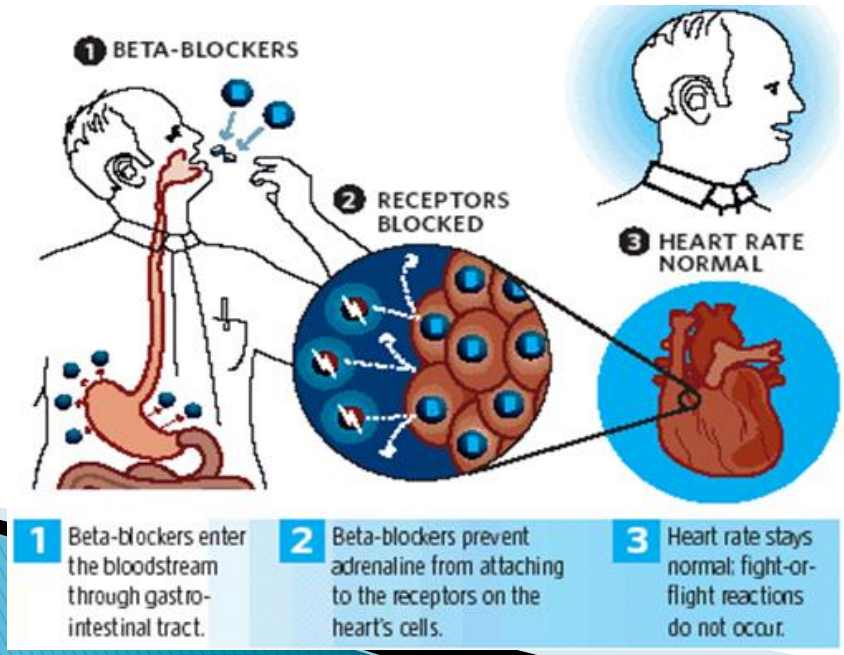
PROPRANOLOL - high blood pressure, heart dysrhythmias, heart problems in patients with angina or previous heart attacks.



ADRENALINE



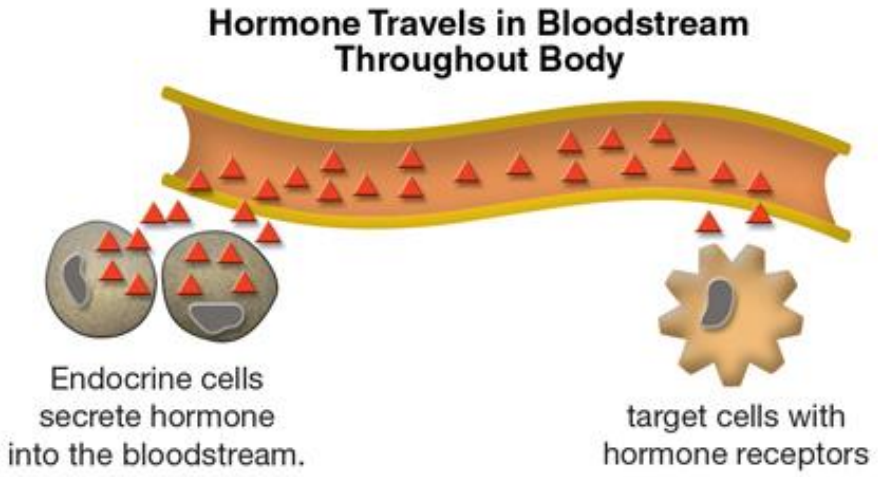
PROPRANOLOL
(β2 receptor antagonist)

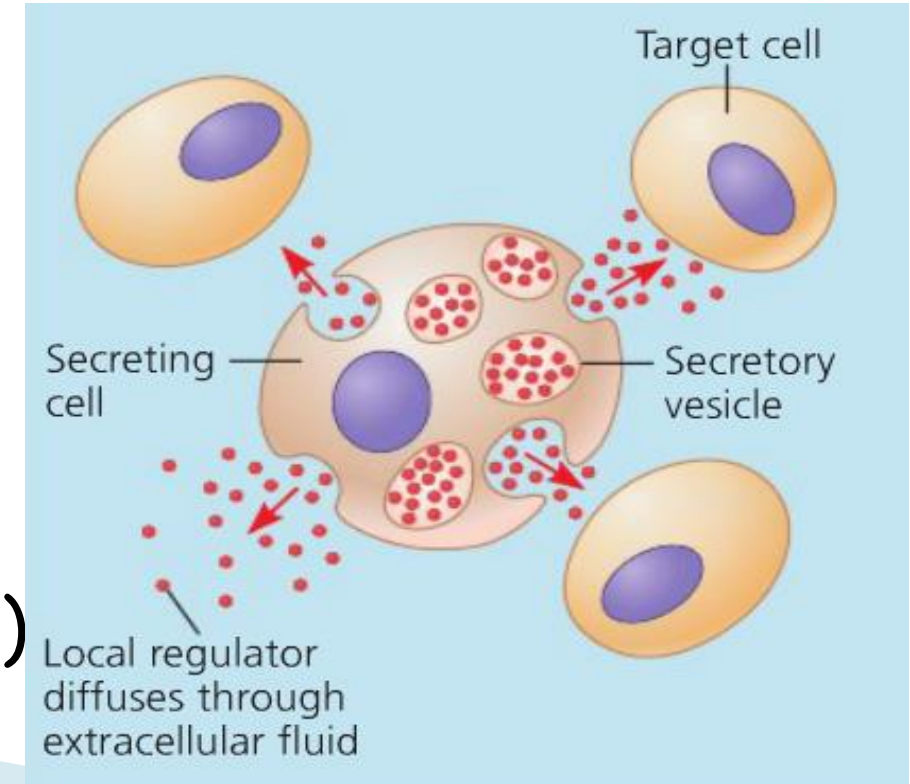


Intercellular communication - mode of signal spreading and range

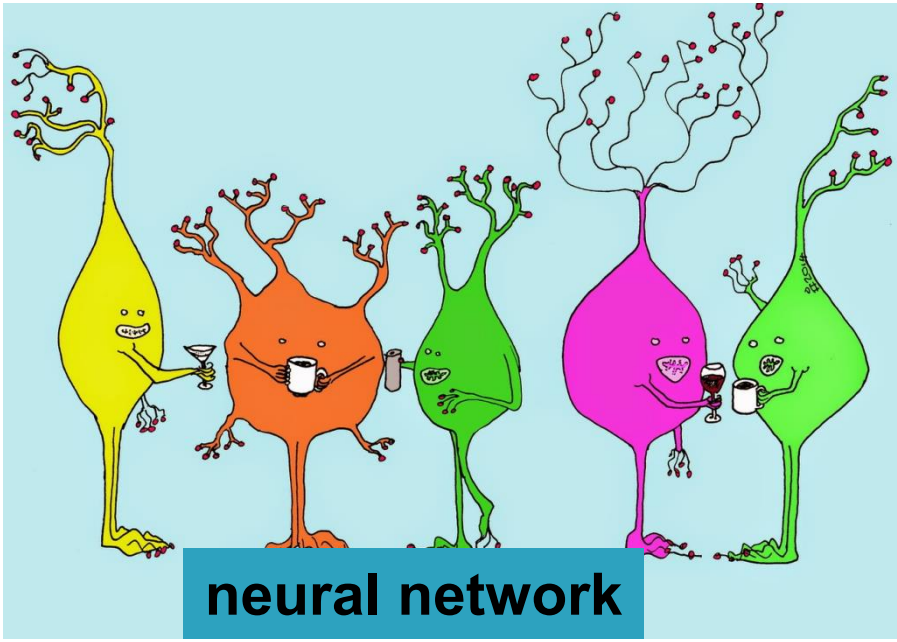
1. information should be available for many different cells in the body
   - **endocrine communication** (peptide and steroid hormones, vitamins)

2. information acts locally
   - **paracrine communication** (cytokines, eikozanoids)



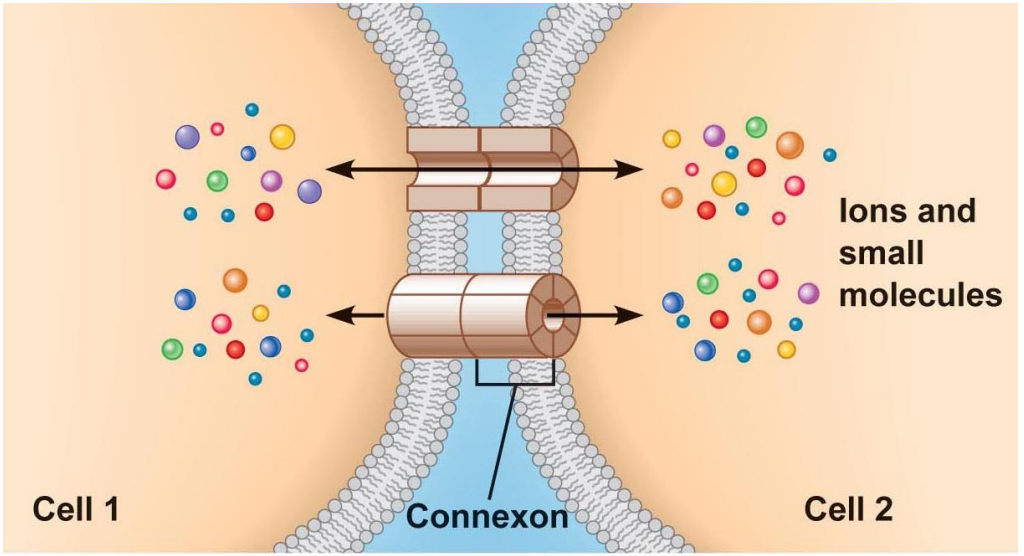


Intercellular communication - mode of signal spreading and range



3. **synaptic communication** (neurotransmitters)

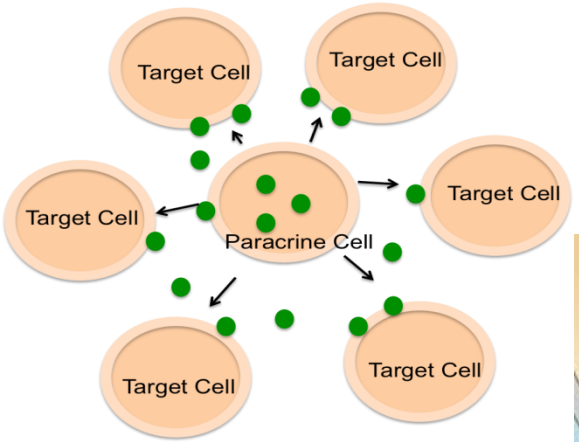
4. **metabolic connections** (gap junction) (ions, metabolites)



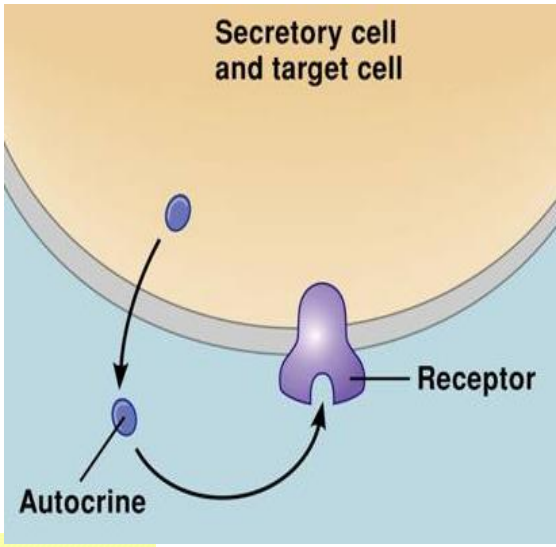
Direct communication through gap junctions

INTERCELLULAR COMMUNICATION - signal origin

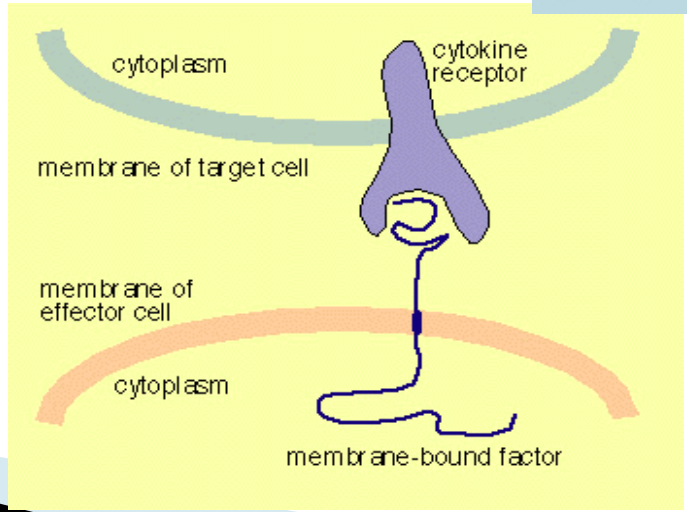
1. **paracrine** communication - signaling molecules produced by one cell act on the target cells



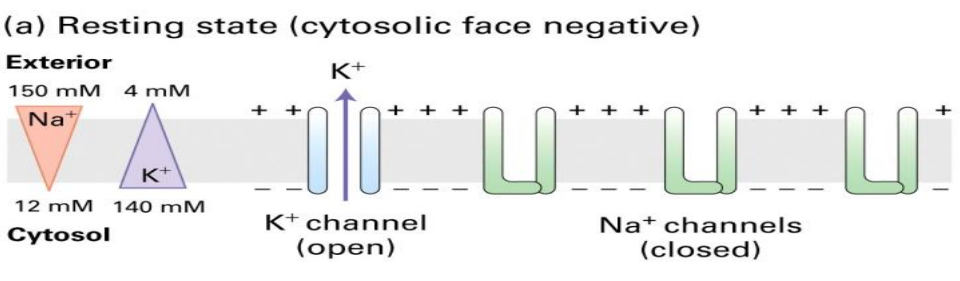
2. **autocrine** communication - cell secretes the signal that binds to the receptor on the same cell

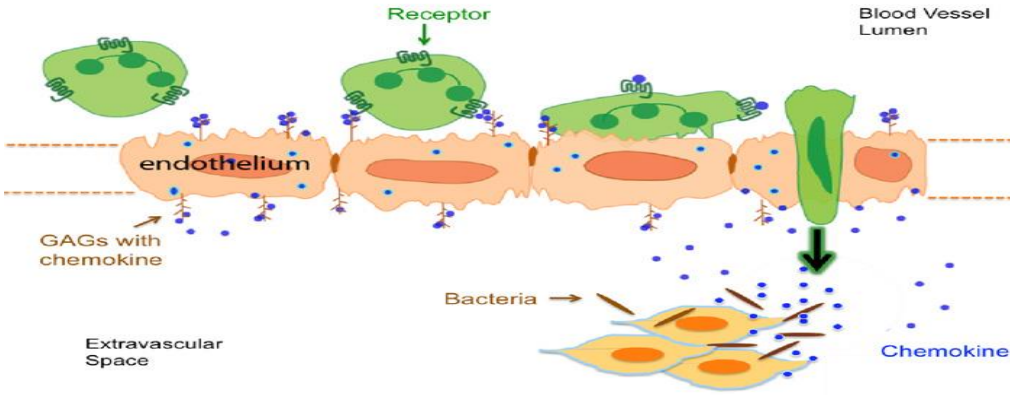


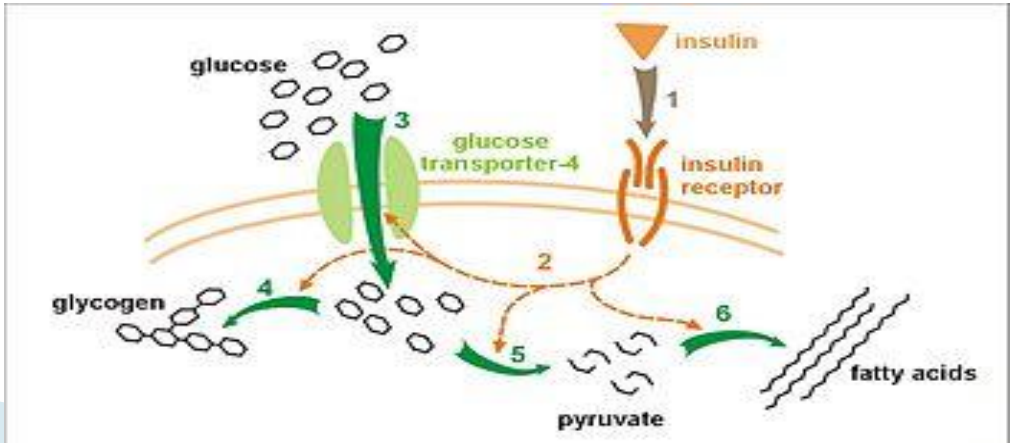
3. **juxtacrine** communication - **contact-dependent signaling** - ligand is bound to the cell



- Cell membrane depolarization - ion channels
- Change of cell shape - cytoskeleton rearrangement and change of cell adhesion (adhesion molecules)
- Change of cell metabolism –
  1. enzymatic activity
  2. gene expression





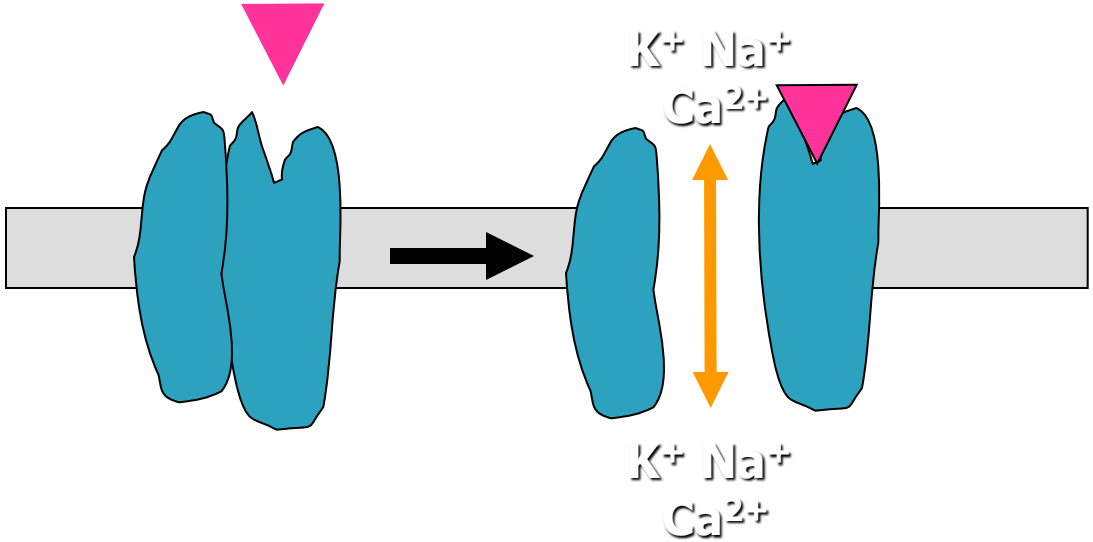


Classification of receptors

- **ionotropic receptors** - cell membrane depolarization
- **metabotropic receptors** - change of cell metabolism
    - modification of structural proteins - a change of cell shape
    - change of cell metabolism - enzymatic activity
    - change of gene expression - transcription factors

IONOTROPIC RECEPTORS
LIGAND- OR VOLTAGE-GATED ION CHANNELS

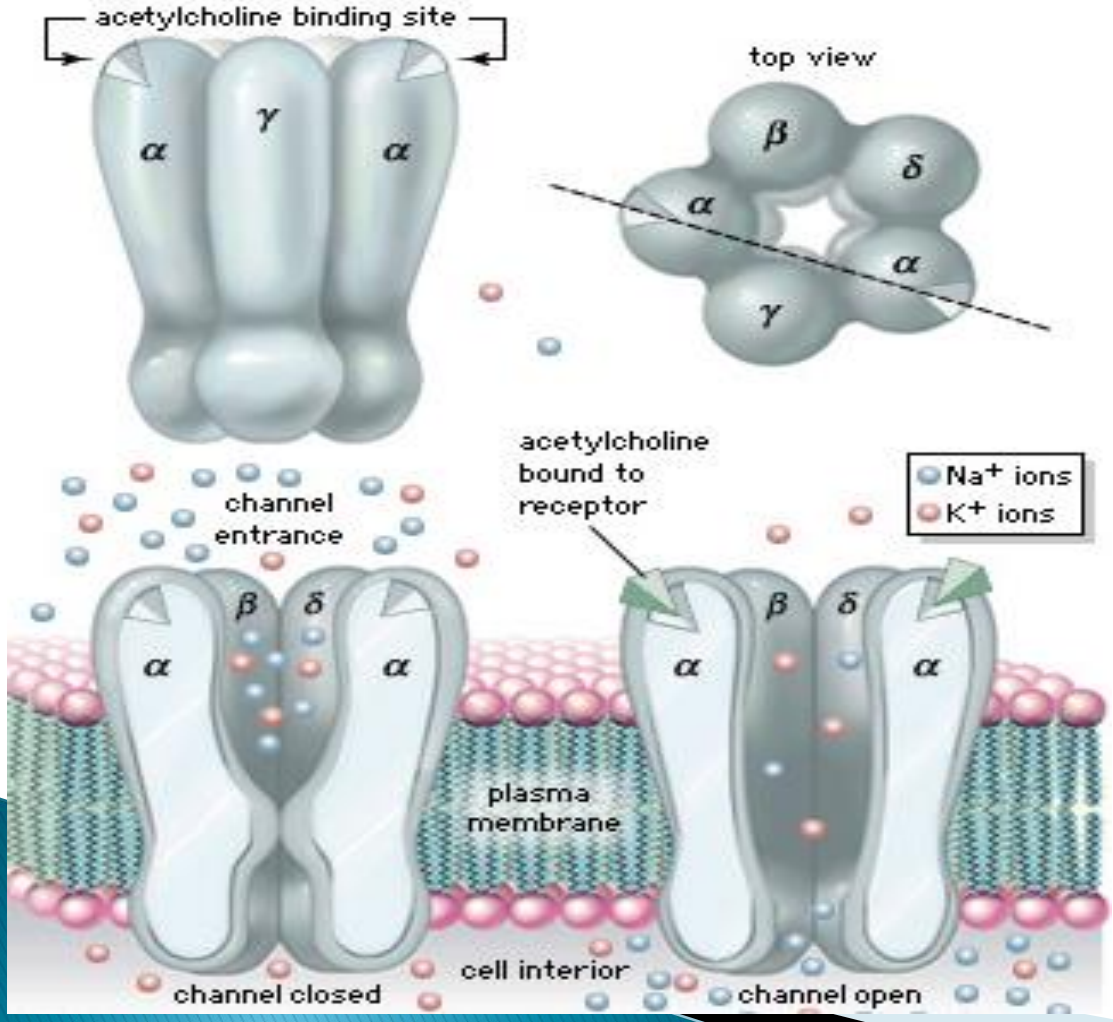
- Ligand – neurotransmitter
- Change of membrane potential
- typically composed of several different subunits



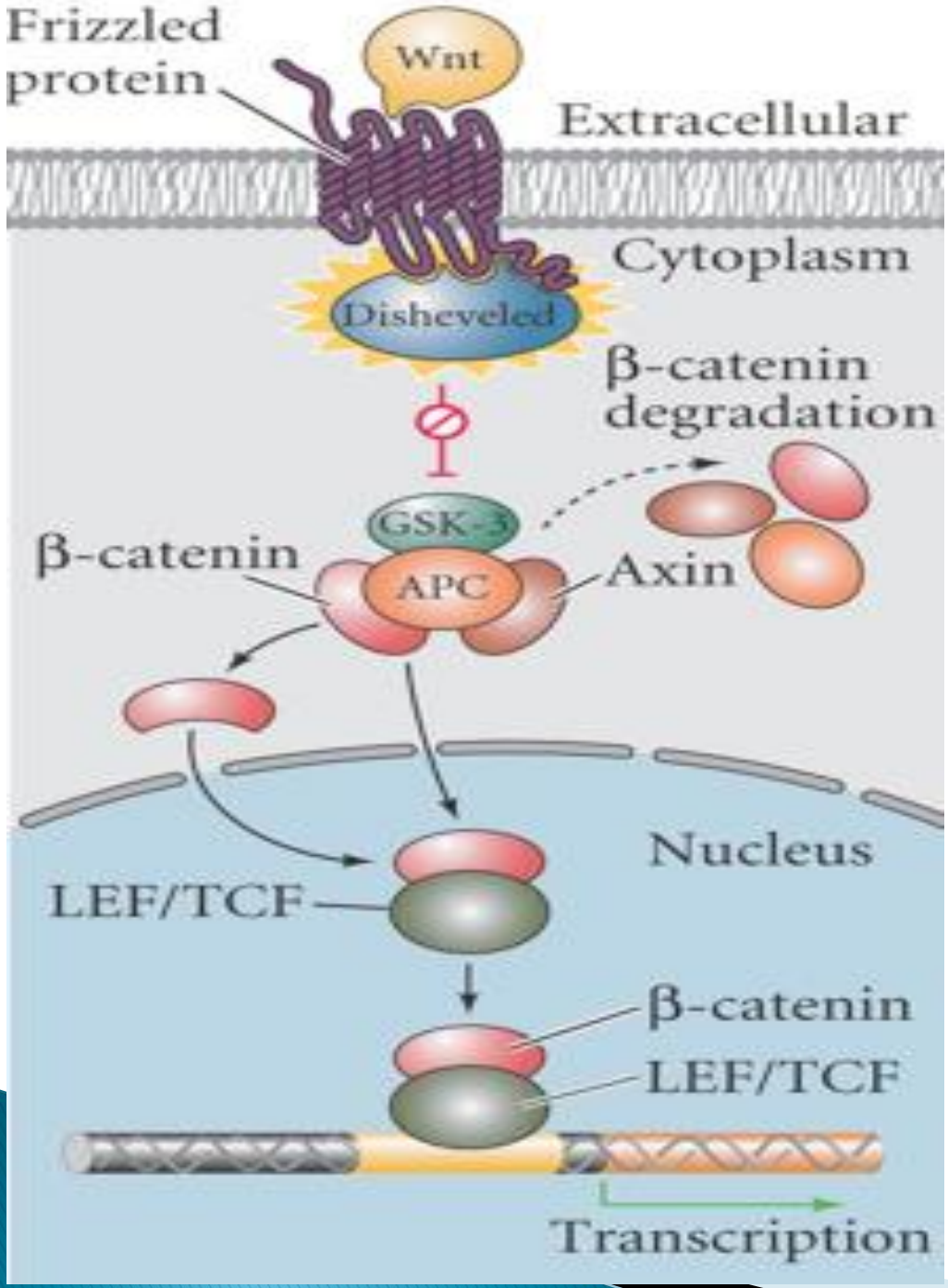
BIOLOGICAL EFFECT: rapid change of ion concentration membrane depolarization (miliseconds)

Nicotinic acetylcholine receptor – ligand-gated

- Na+ and K+ ion channel
- five subunits - around a central pore (two of the five subunits - ability to bind **acetylcholine**)

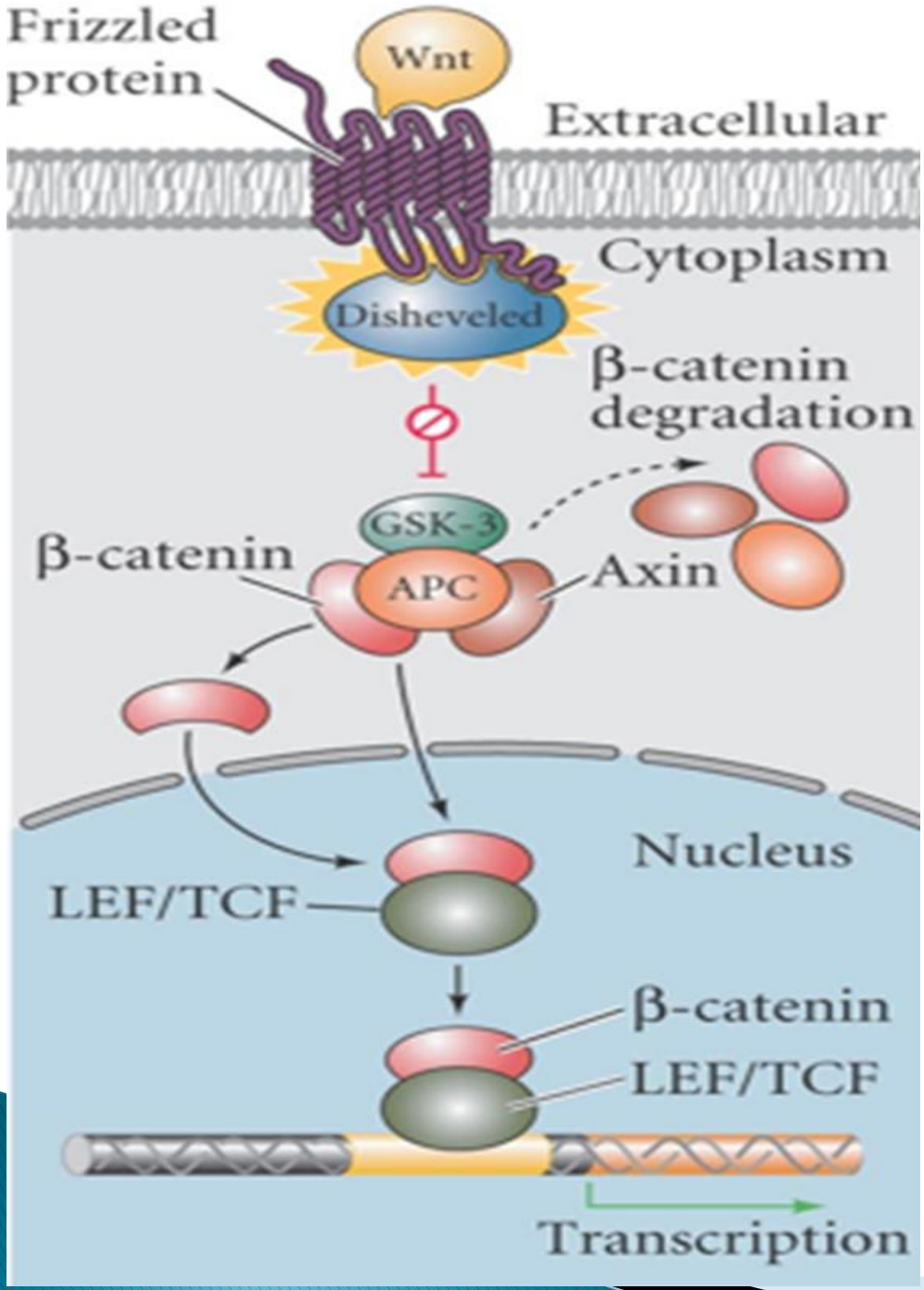


binding of acetylcholine - receptor undergoes conformational changes - the opening of the channel and the free flow of Na+ and K+ ions



Wnt signaling pathway

- activated by binding a **Wnt-protein** ligand to a **Frizzled receptor** associated with **Dishevelled** protein
- accumulation of **β-catenins** in the cytoplasm - translocation into the nucleus - activation of transcription factors **TCF/LEF**
- cell proliferation

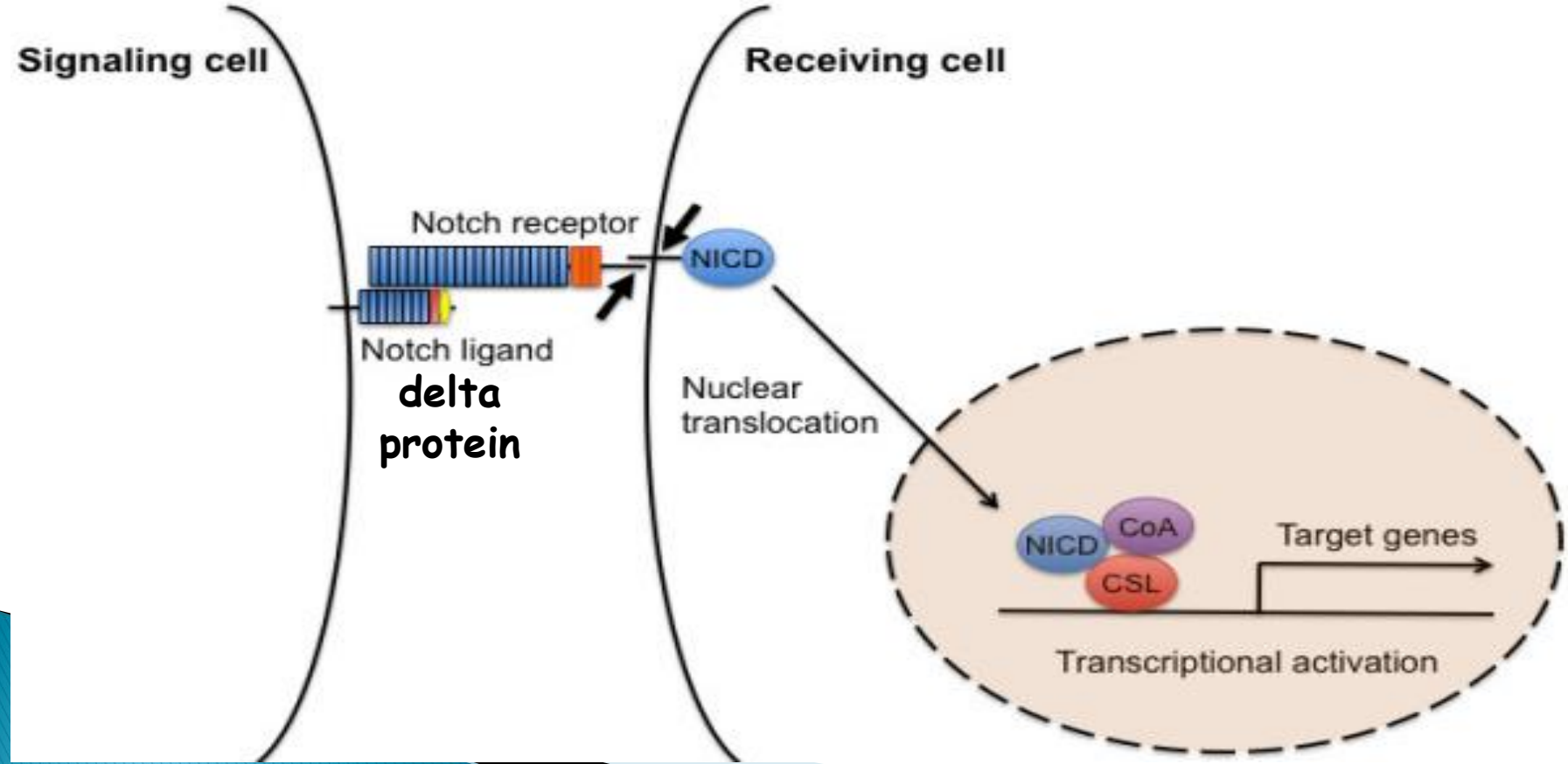


Wnt signaling pathway

- without Wnt signaling - β-catenins degraded (destruction complex APC/Axin/β-catenins).
- mutations in APC, β-catenins, - **colorectal, breast and prostate cancer**)
- APC - tumor suppressor gene (prevents the uncontrolled growth of cells)

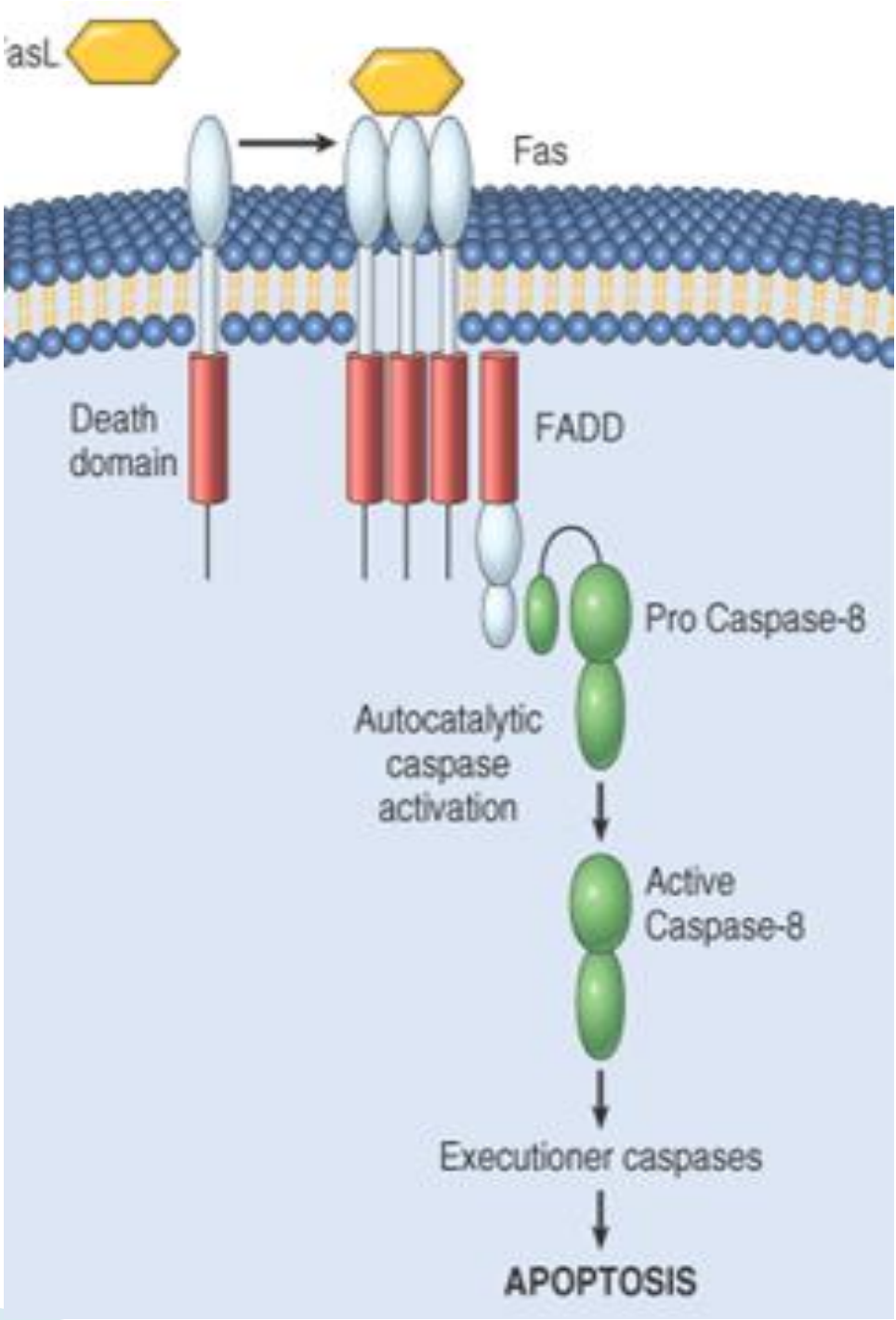
Notch signaling pathway – juxtacrine signaling (contact-dependent) - ligand – **delta protein**

- cleaved Notch intracellular domain migrates to the nucleus – transcription factor
- **neurogenesis**, embryo polarity (anterior-posterior polarity and left-right asymmetry)



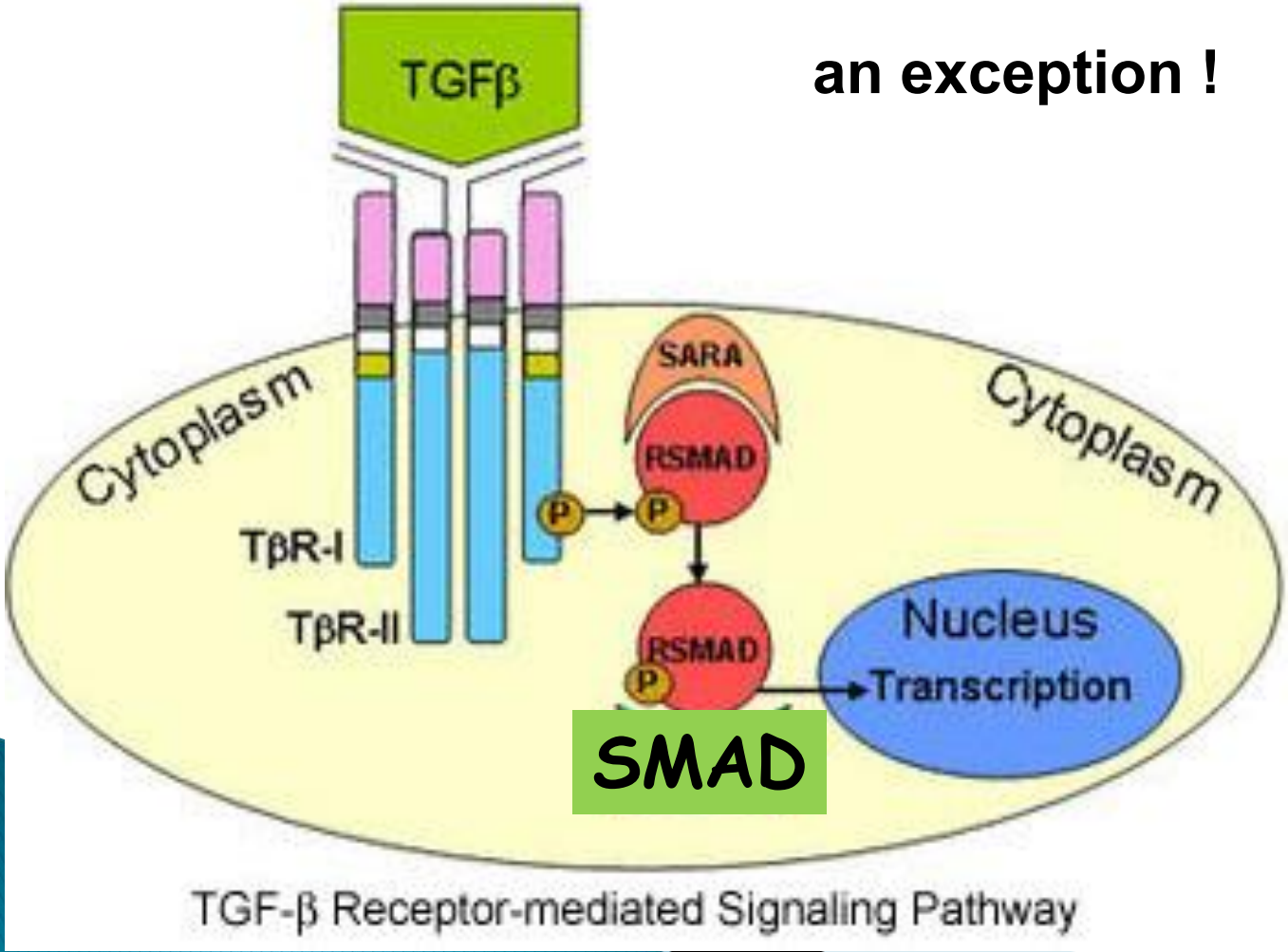
Proapoptotic receptors - Death Receptors

- ligands: **TNF, Fas ligand, TRAIL**
- activation - apoptosis
- **death domain (DD)**
- activation of **caspases** (cysteine proteases)
- **apoptosis** (programmed cell death)



TGF-β receptors - serine/threonine kinase receptors!!

- form dimers and phosphorylate transcription factors SMADs
- SMADs regulate transcription of genes



TGF-β Receptor-mediated Signaling Pathway

an exception !

- in embryonic development (cell growth, cell differentiation, apoptosis)