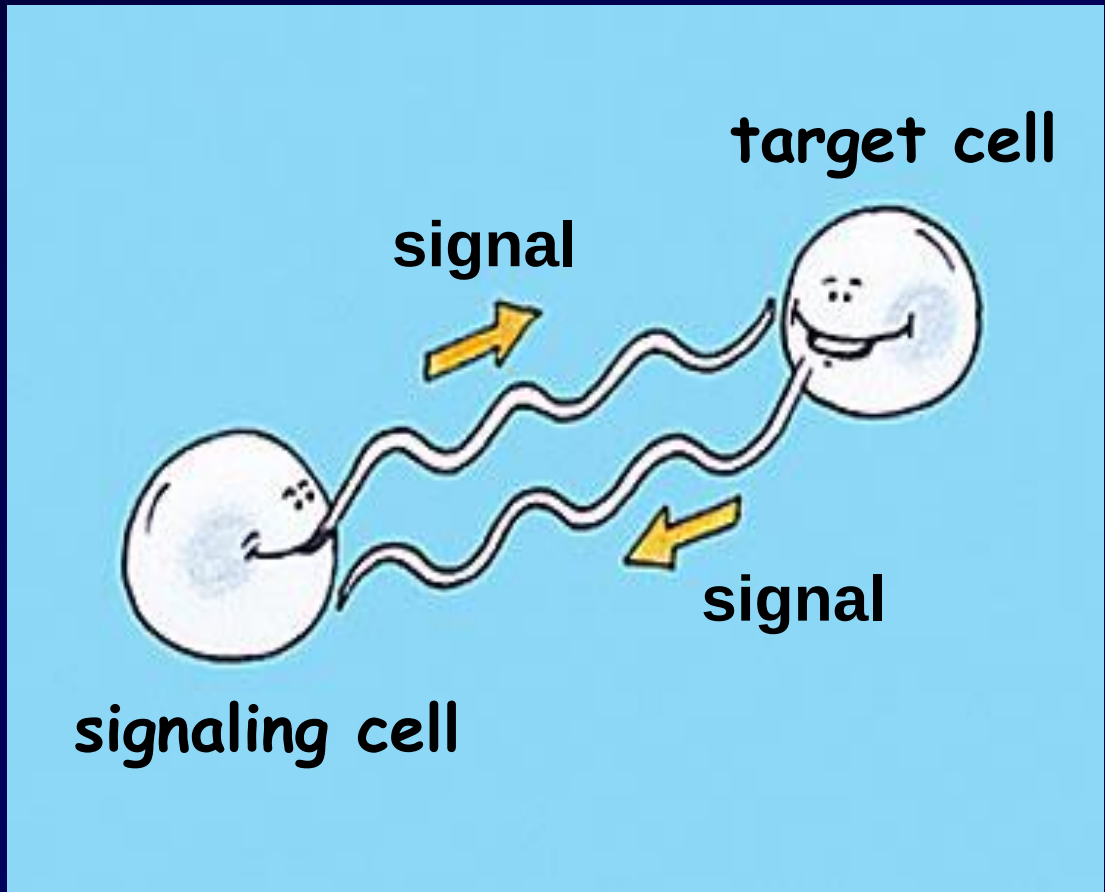
CELLULAR COMMUNICATION

A man without contact with other people.

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

Communication:

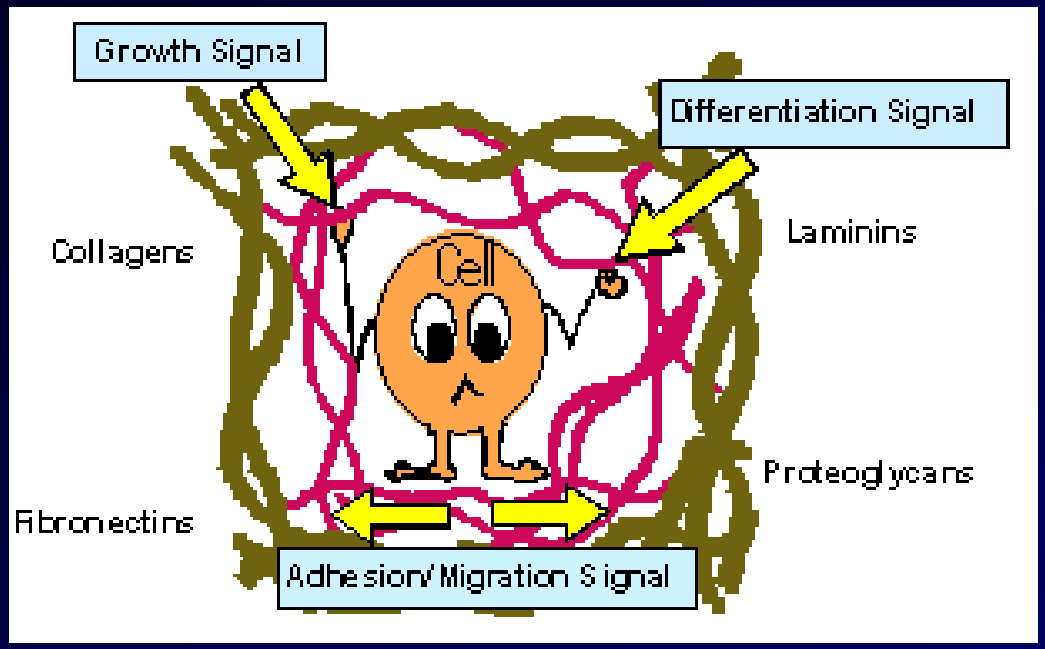
sending and receiving of signals



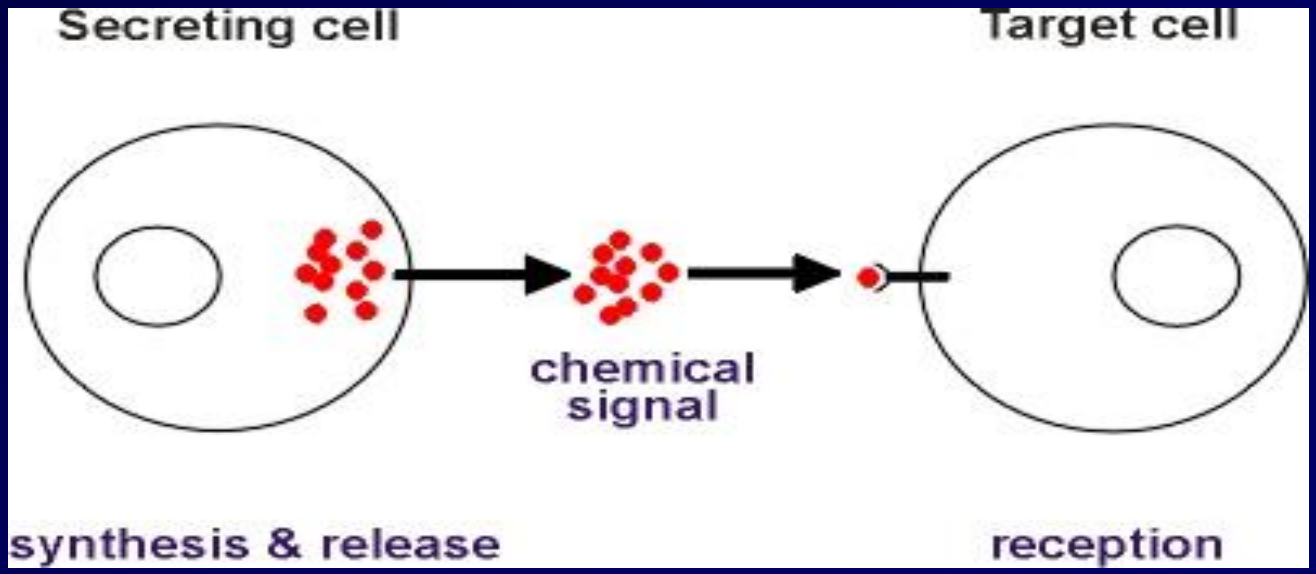
Signals:

- from the external environment (extracellular matrix)

- informations exchanged between cells

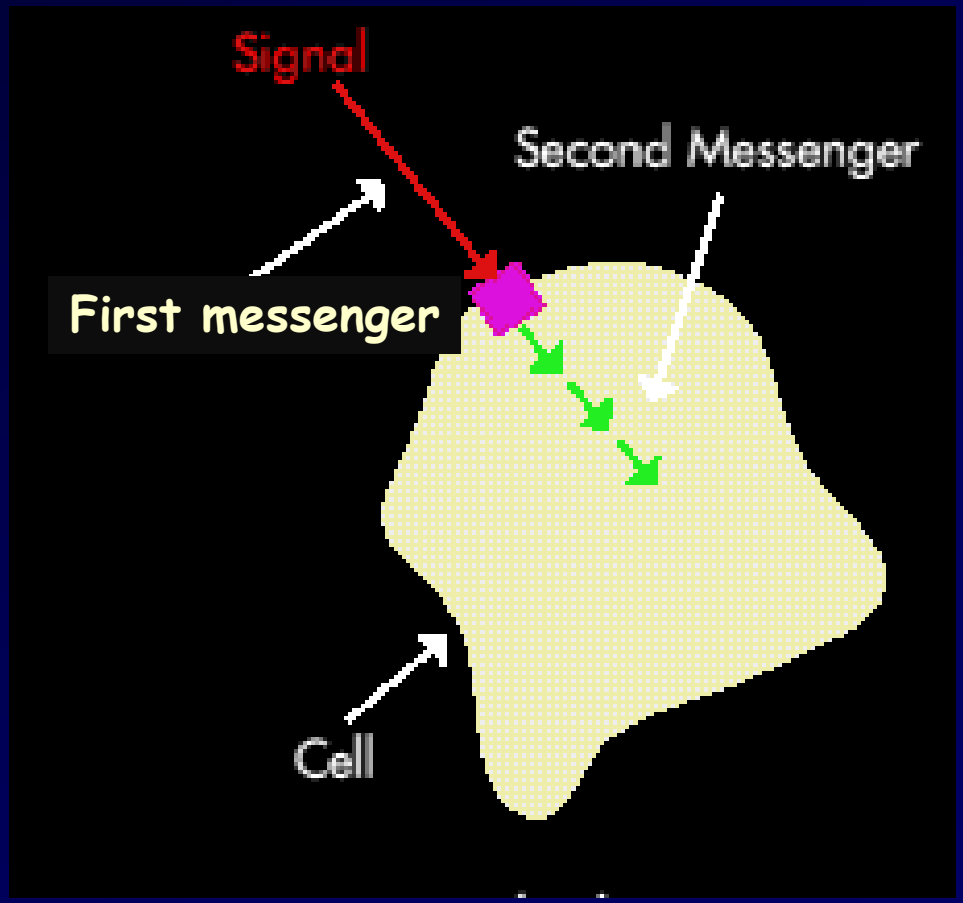


- multiple signals at the same time – contradictory?



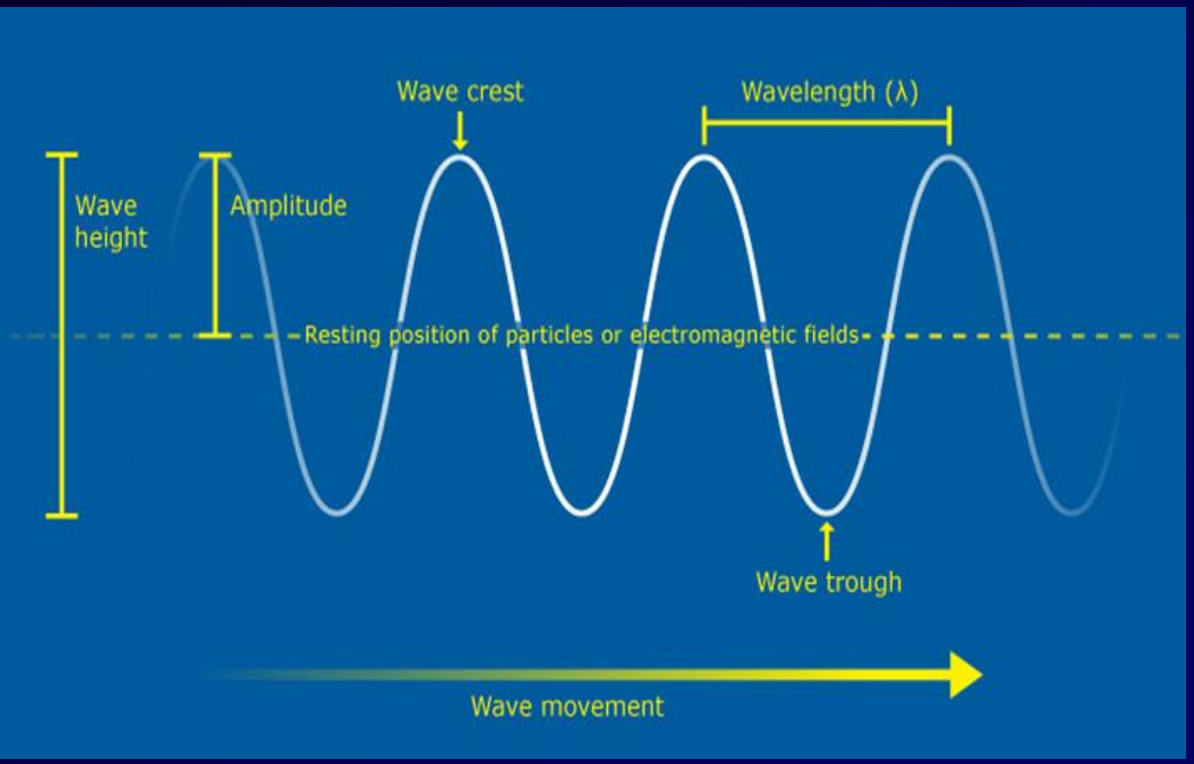
- signals - first messengers

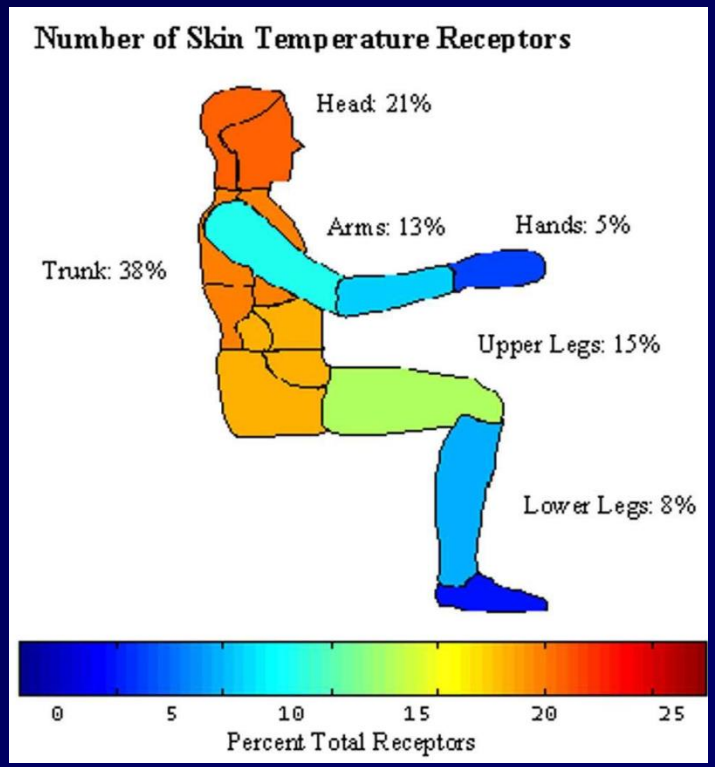
- PHYSICAL SIGNALS
- CHEMICAL SIGNALS



PHYSICAL SIGNALS – the amount and form of energy

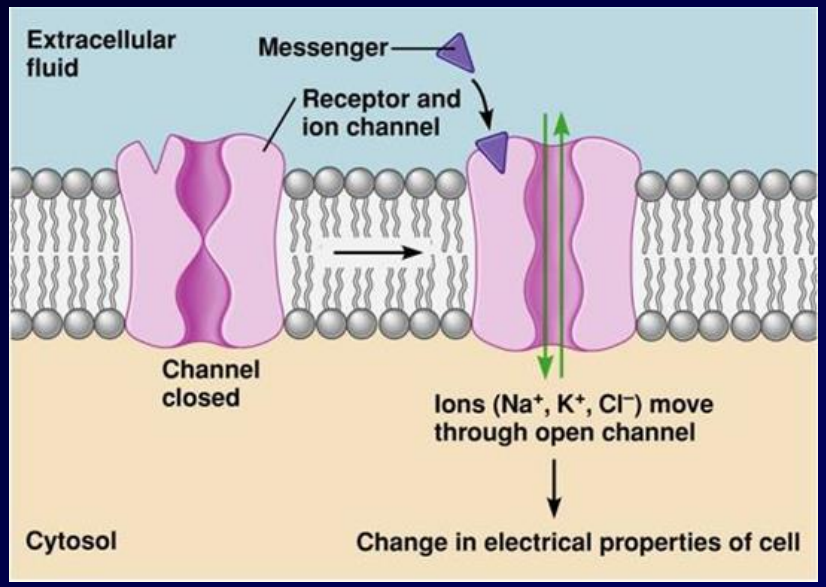
- Acoustic waves (frequency and amplitude of the wave)
- Thermal energy (temperature differences)



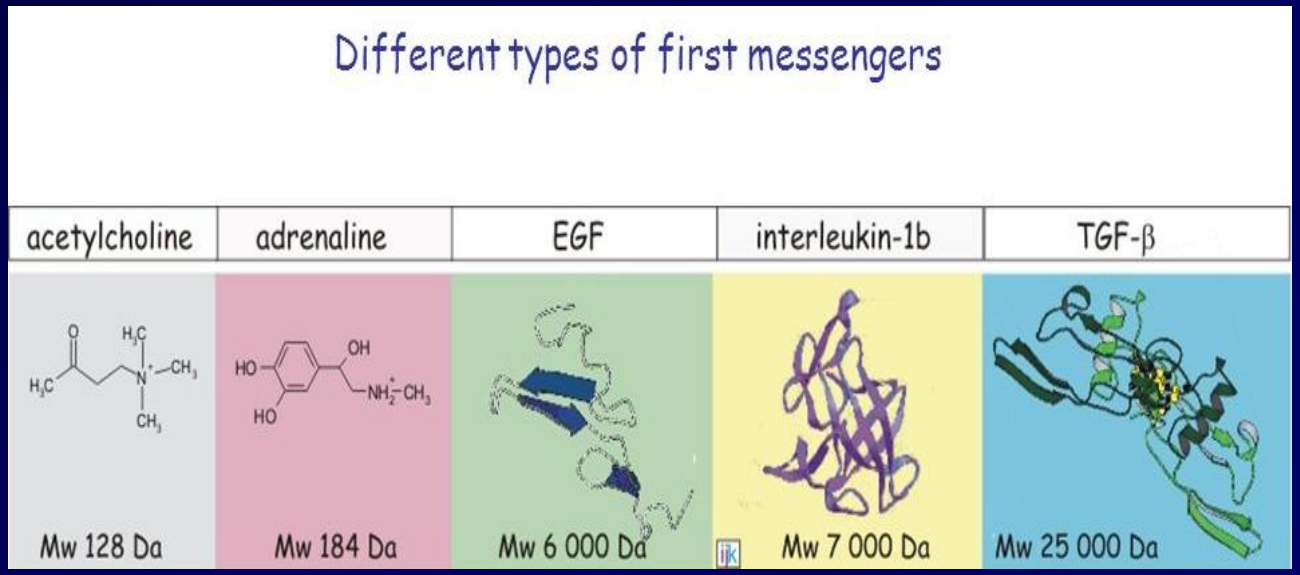


CHEMICAL SIGNALS - first messengers

- Ions
- Chemical substances (molecules)

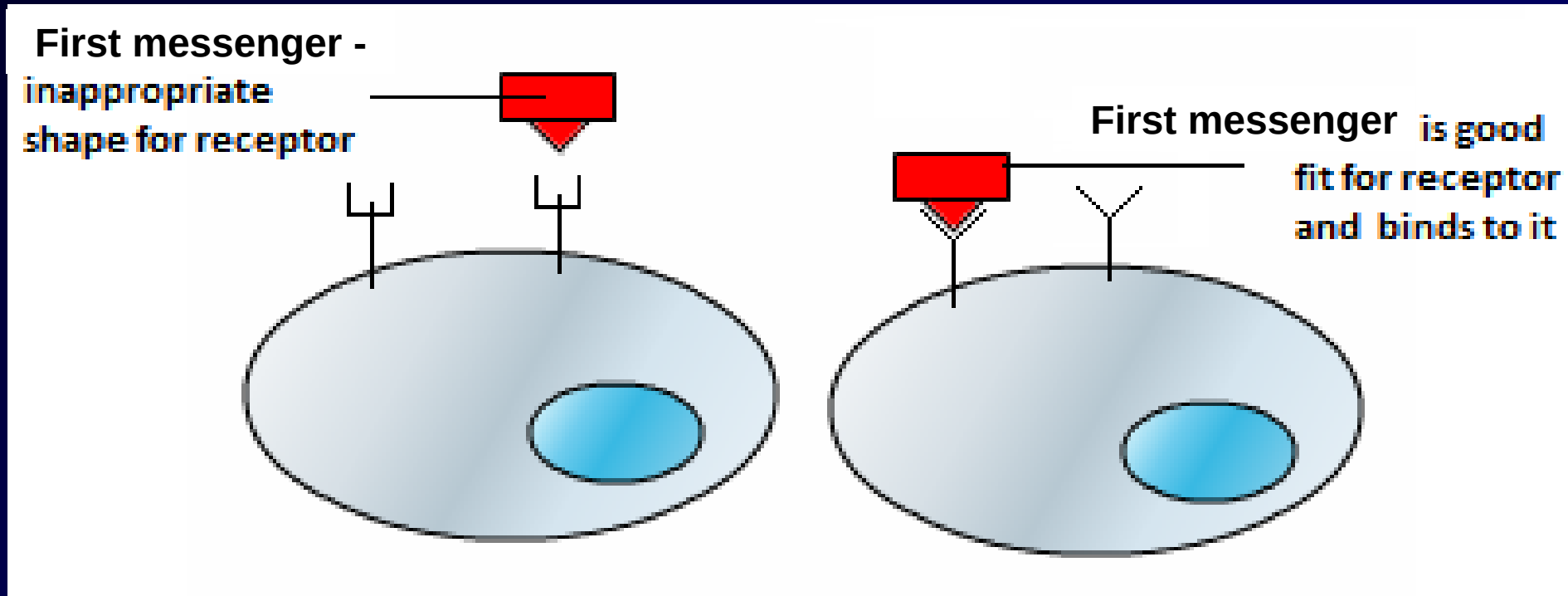


- hormones (peptides, steroids),
- cytokines,
- growth and differentiation hormones,
- chemokines

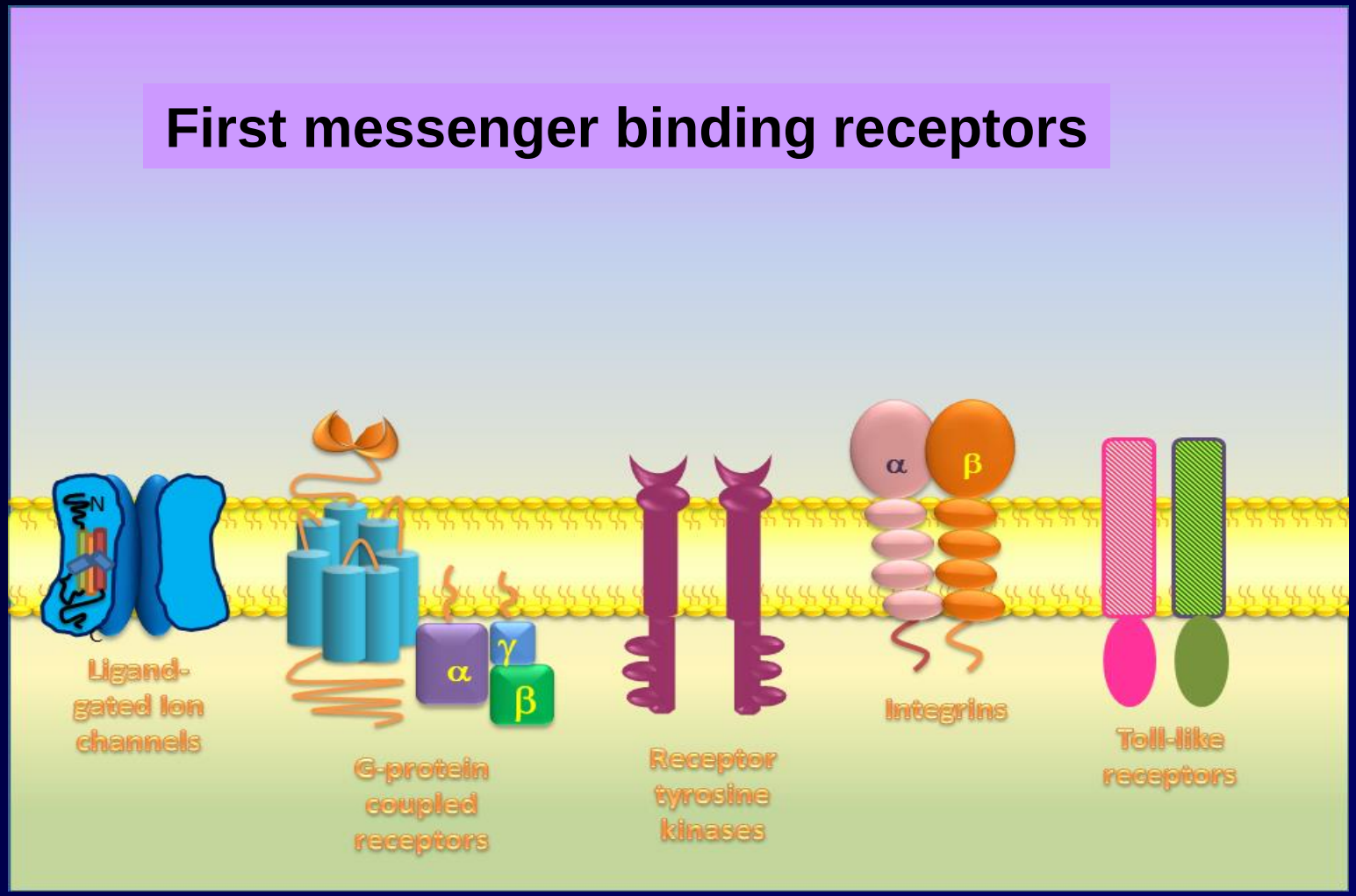


chemical signal - information depends on physico-chemical parameters of first messenger:

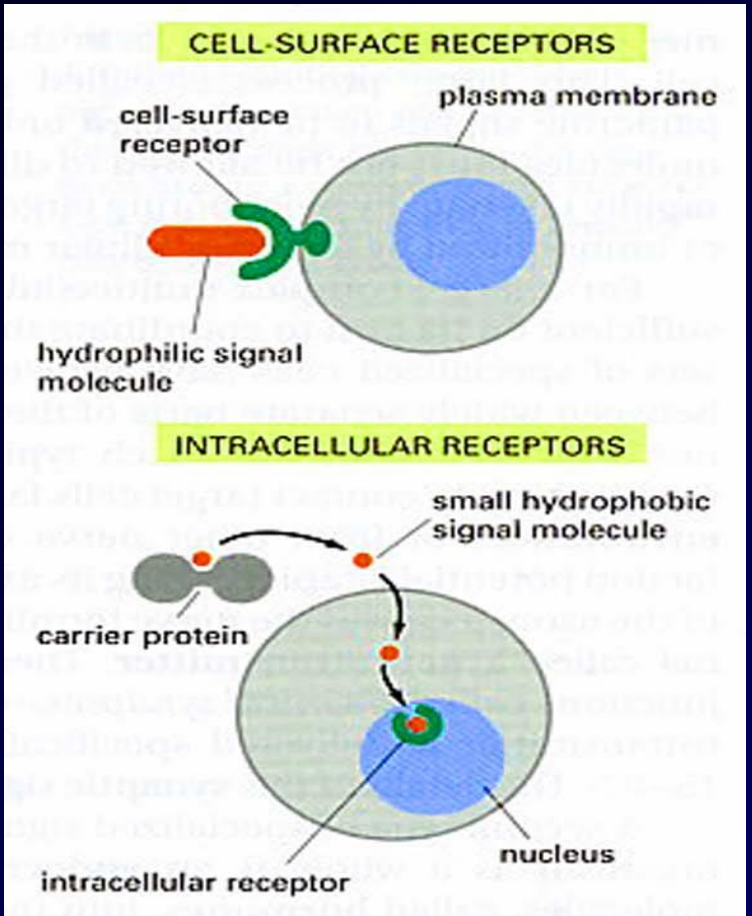
- **three-dimensional structure - ability to interact with other chemical agents**

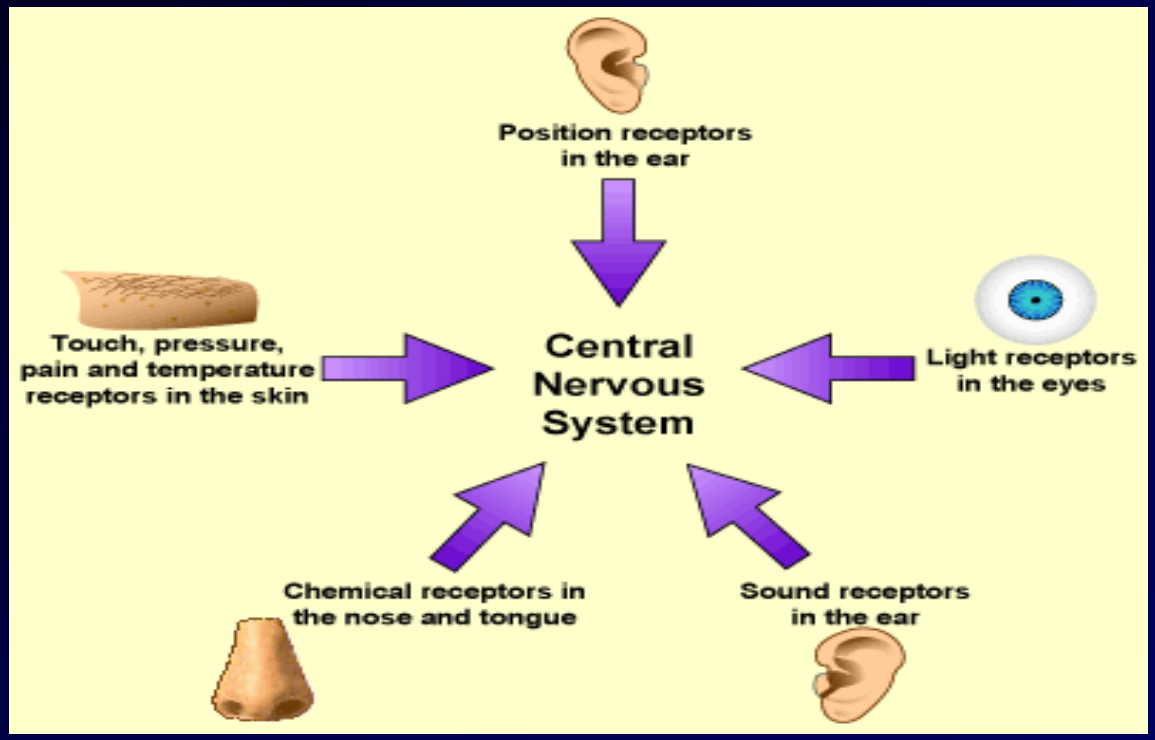


Information carried by a first messenger is received and recognized by specific structures, <u>receptors</u>.



- information from the external environment - receptors in cells of sensory organs:



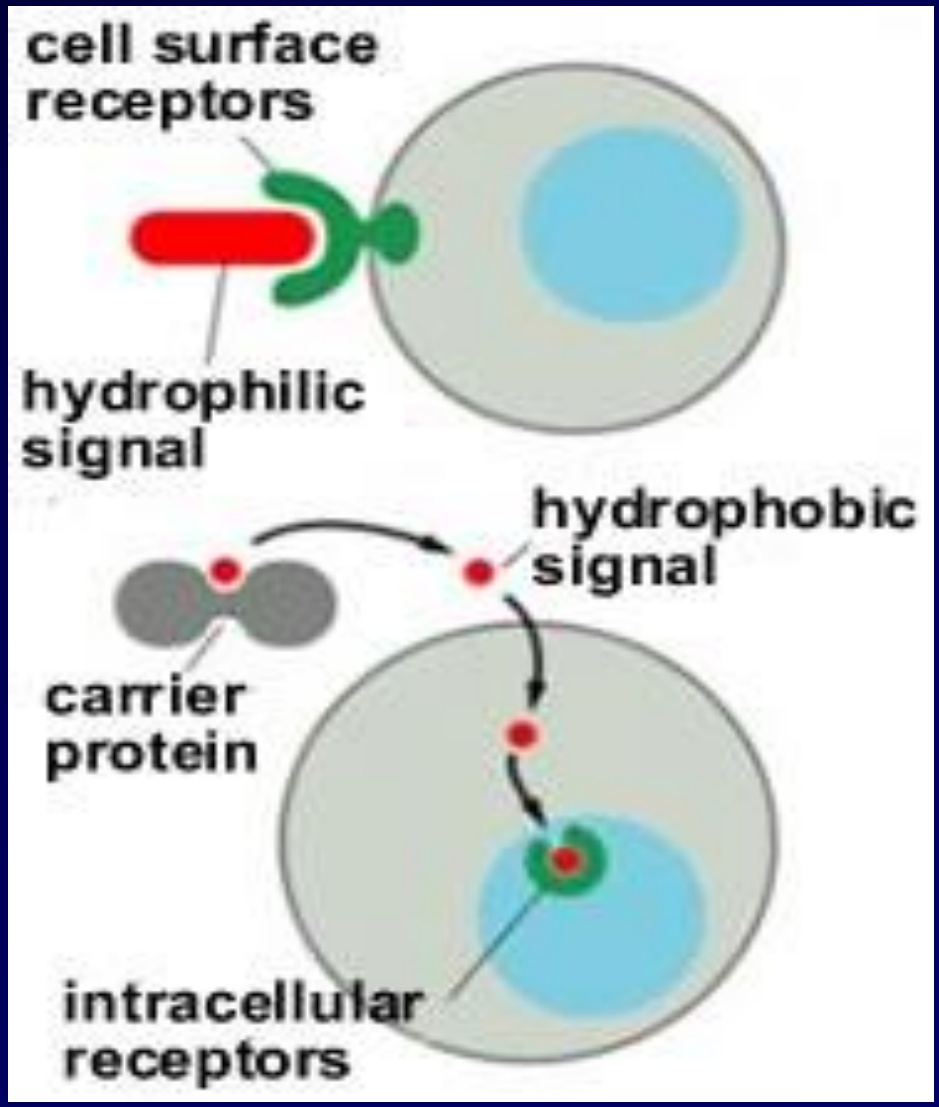


- information exchanged between cells - cellular receptors

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

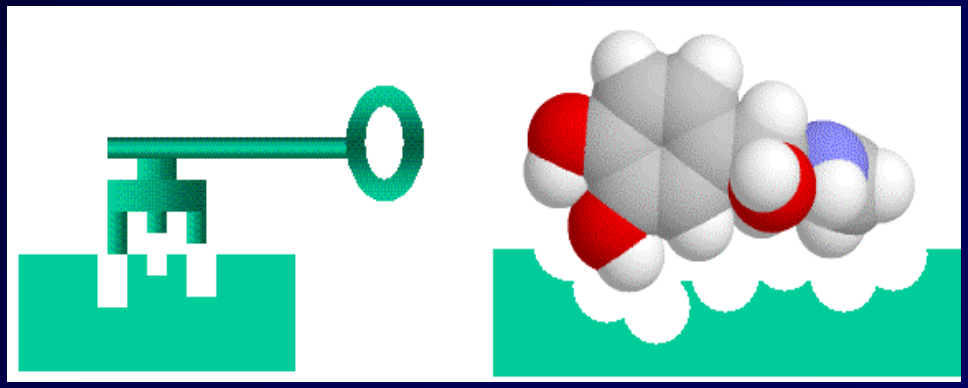
The nature and structure of the ligand determines the location of the receptor

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

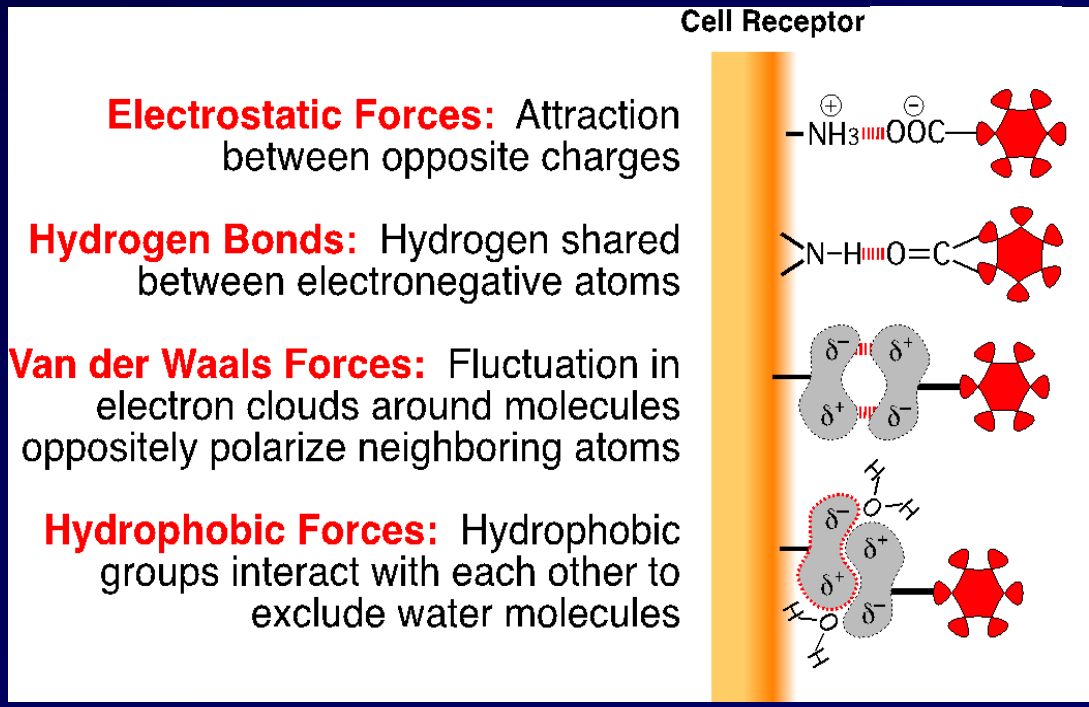


chemical messenger - receptor interactions

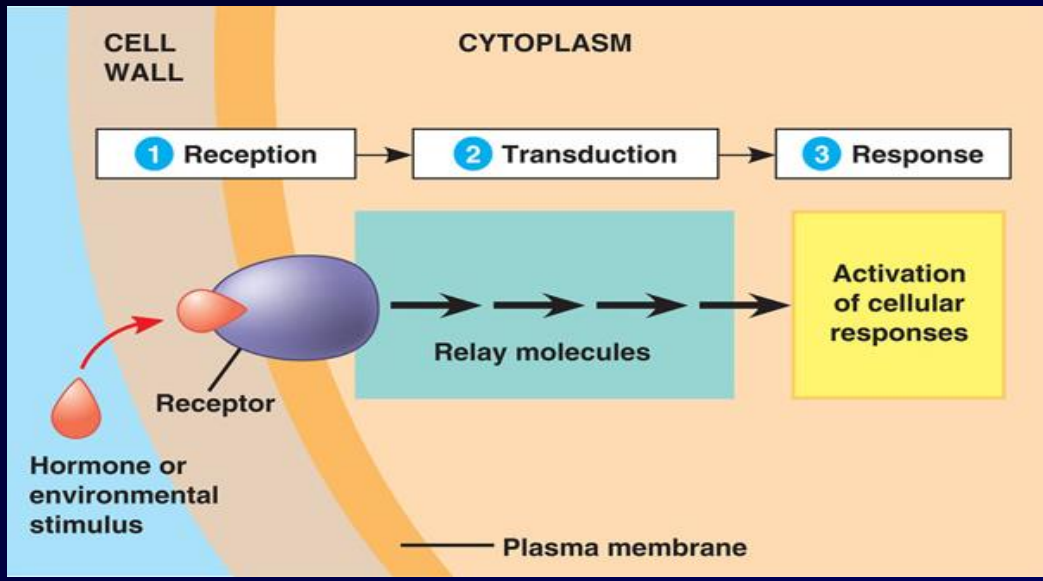
- Spatial (three dimensional) interaction between molecules (like a key and a lock)



- hydrogen bonds
- electrostatic forces
- ion bonds
- van der Waals forces

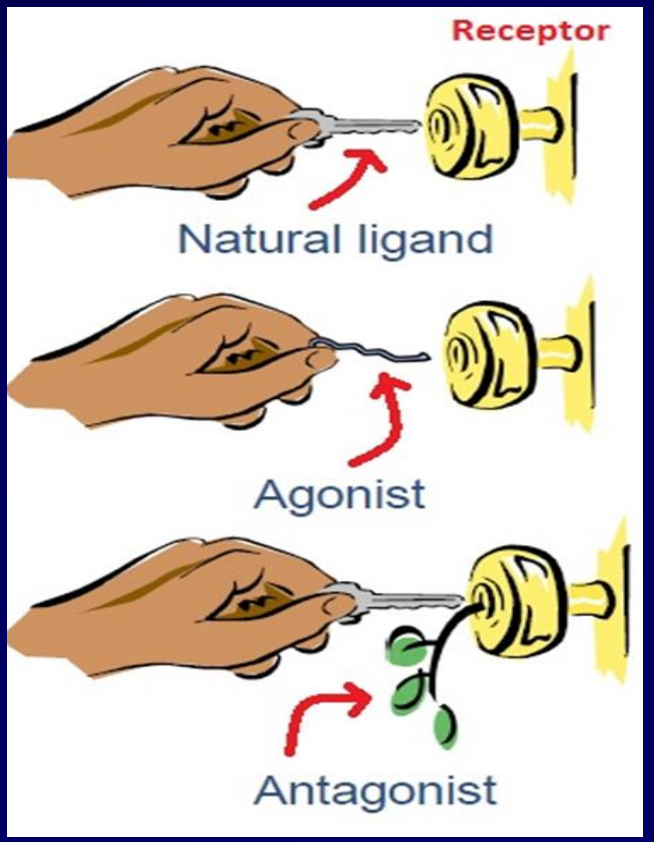


Binding of ligand – receptor -activation (conformational change, oligomerization) – **transduction of signal** (into the cell)



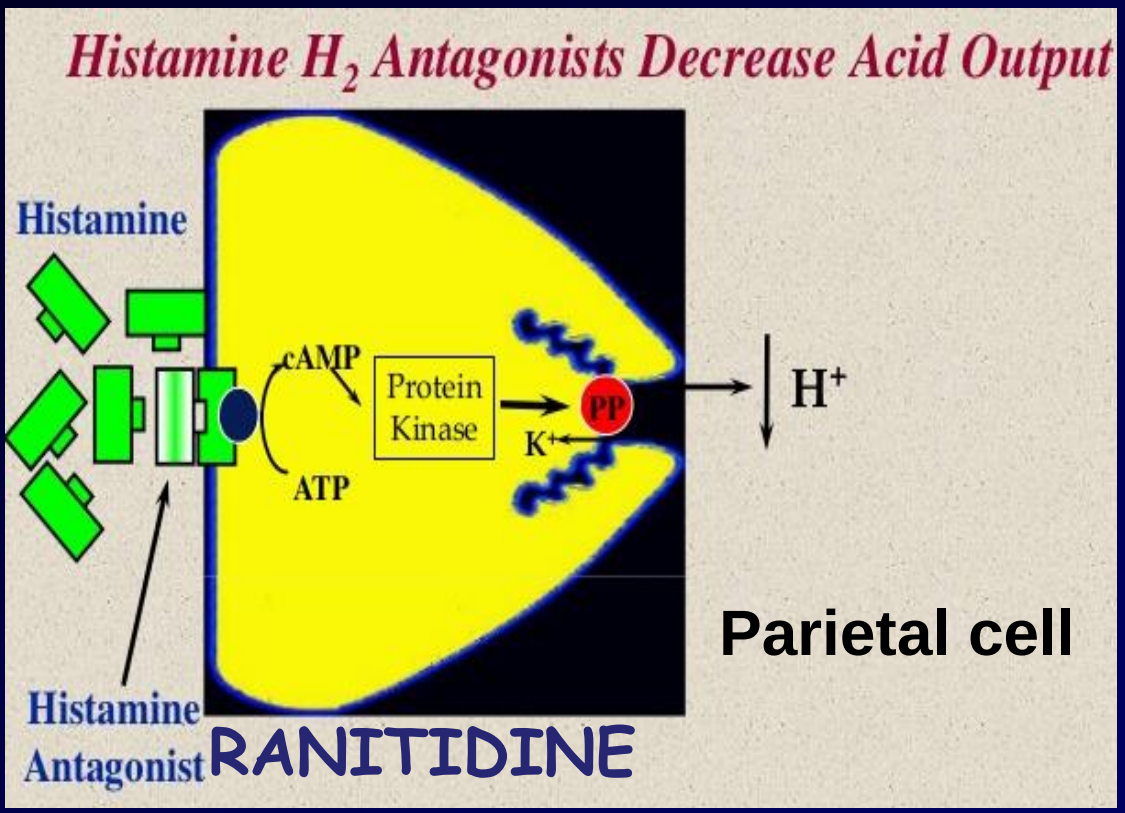
Agonist – a ligand capable of activating a specific receptor

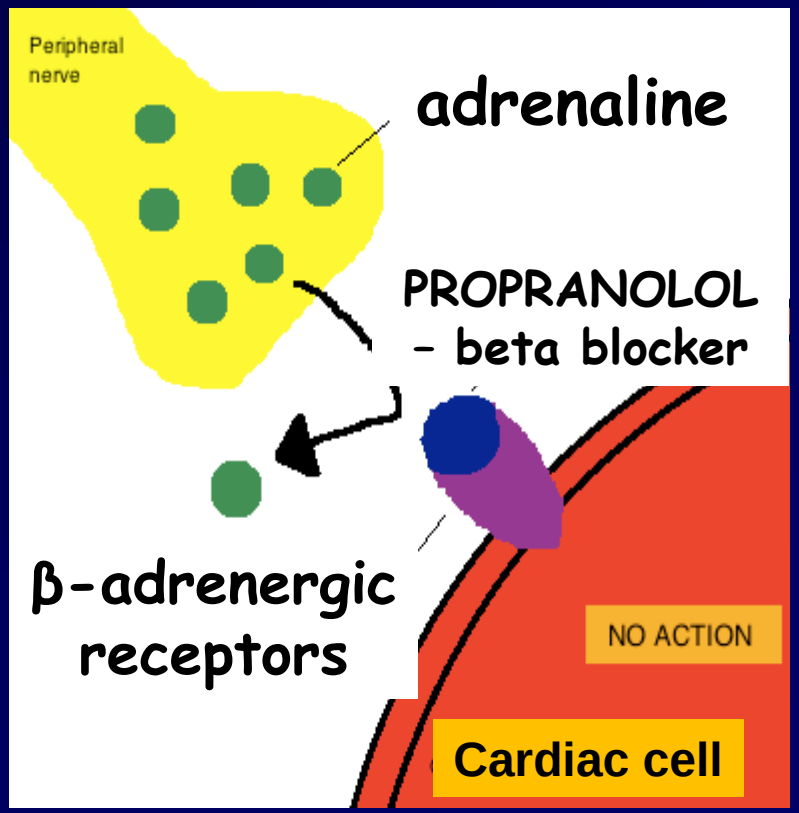
Antagonist - a ligand capable of binding to a specific receptor but does not activate it



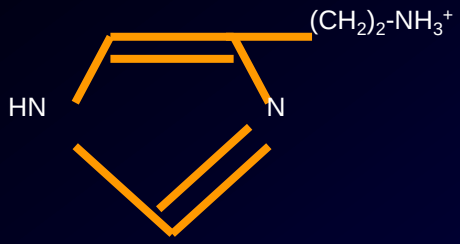
The antagonist - blocks the binding of ligand and the receptor (treatment of diseases)

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



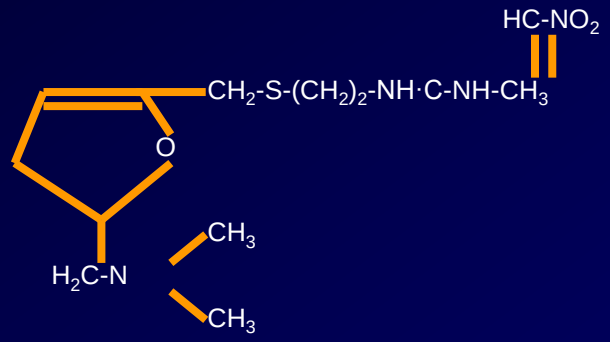


RANITIDINE - peptic ulcer disease and gastroesophageal reflux disease

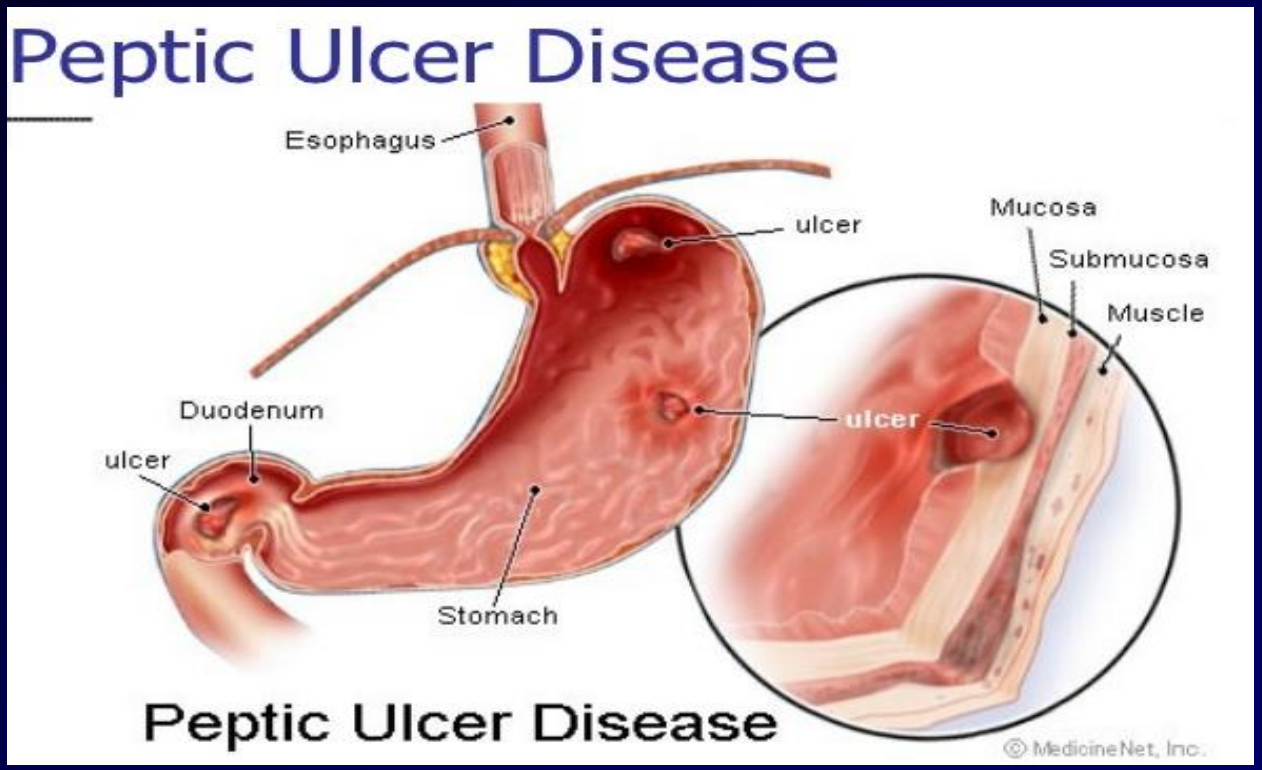


HISTAMINE

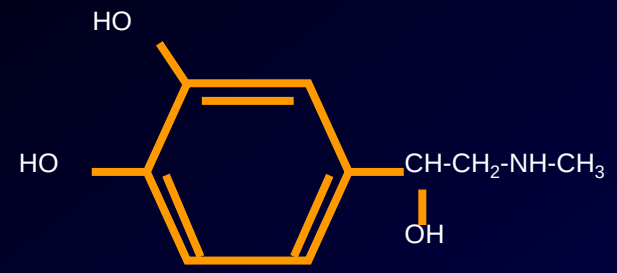
Differences in side chains only



RANITYDINE
(H2 receptor antagonist)

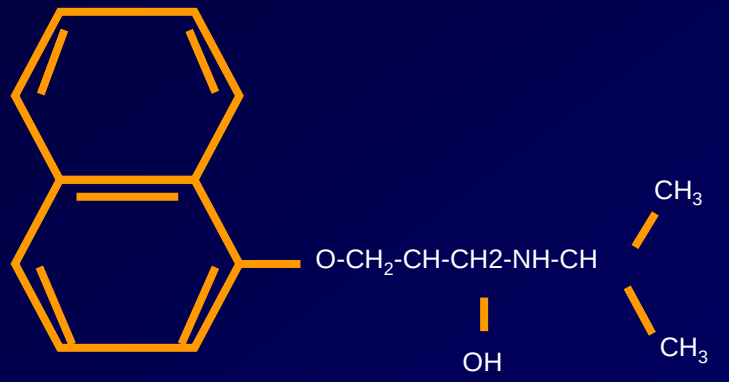


PROPRANOLOL - high blood pressure, heart dysrhythmias, heart problems in patients with angina or previous heart attacks.

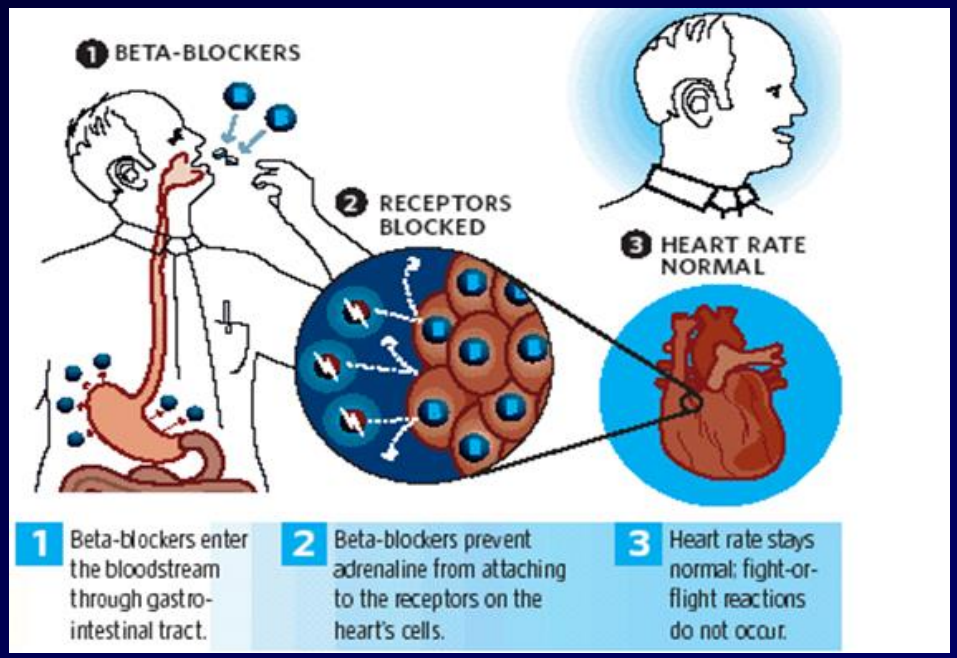


ADRENALINE

side chains differences



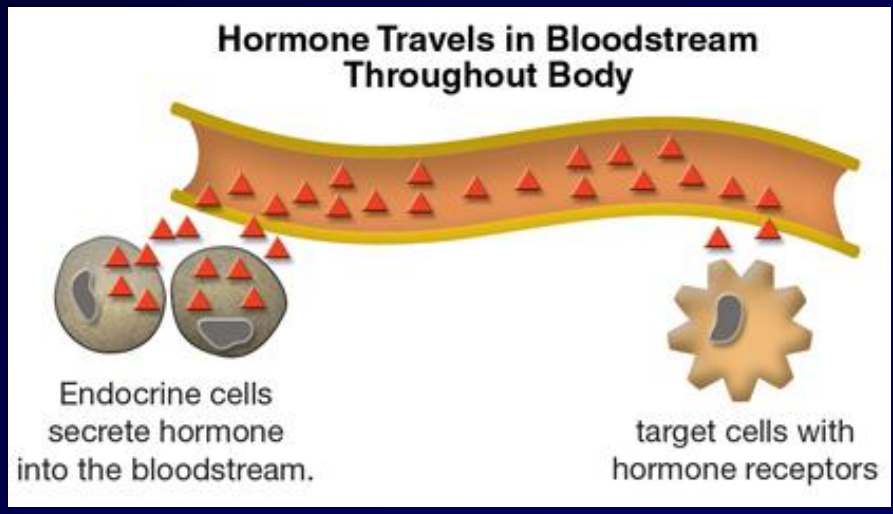
PROPRANOLOL
(β2 receptor antagonist)

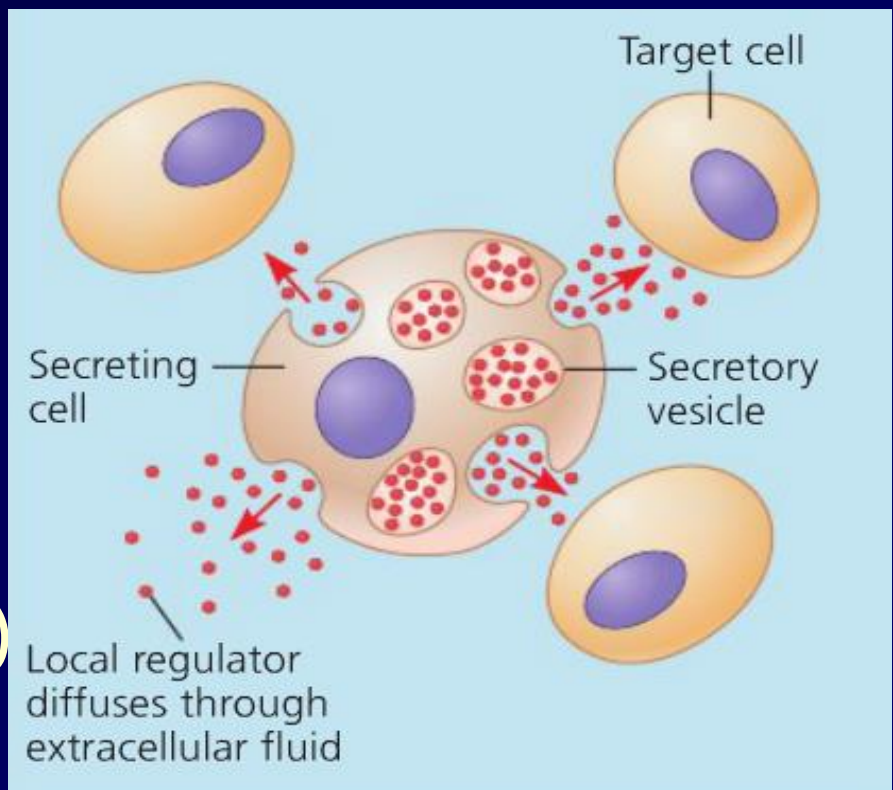


Intercellular communication - mode of signal spreading and range

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

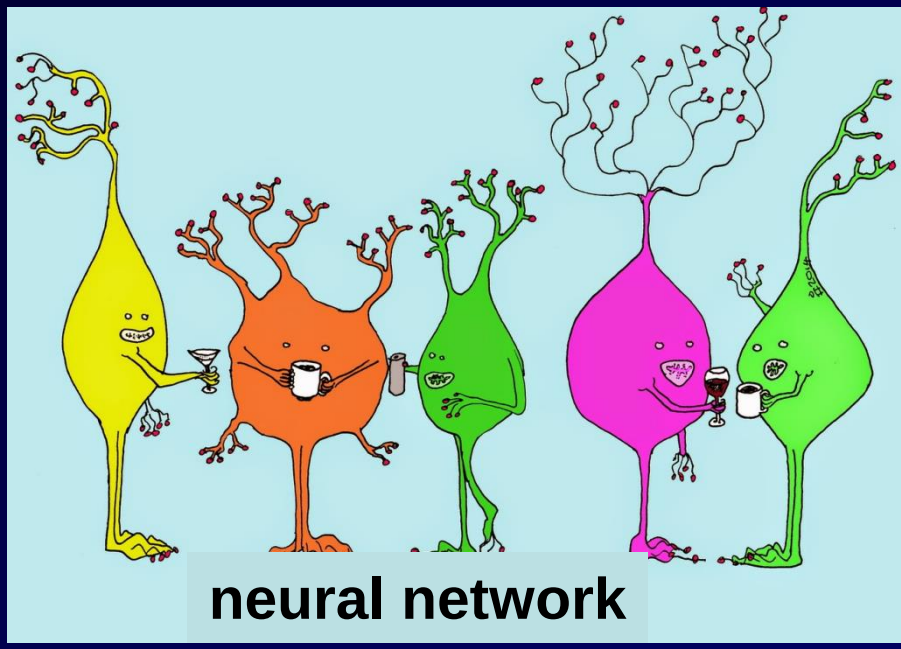
2. information acts locally
   - **paracrine communication** (cytokines, eikozanoids)



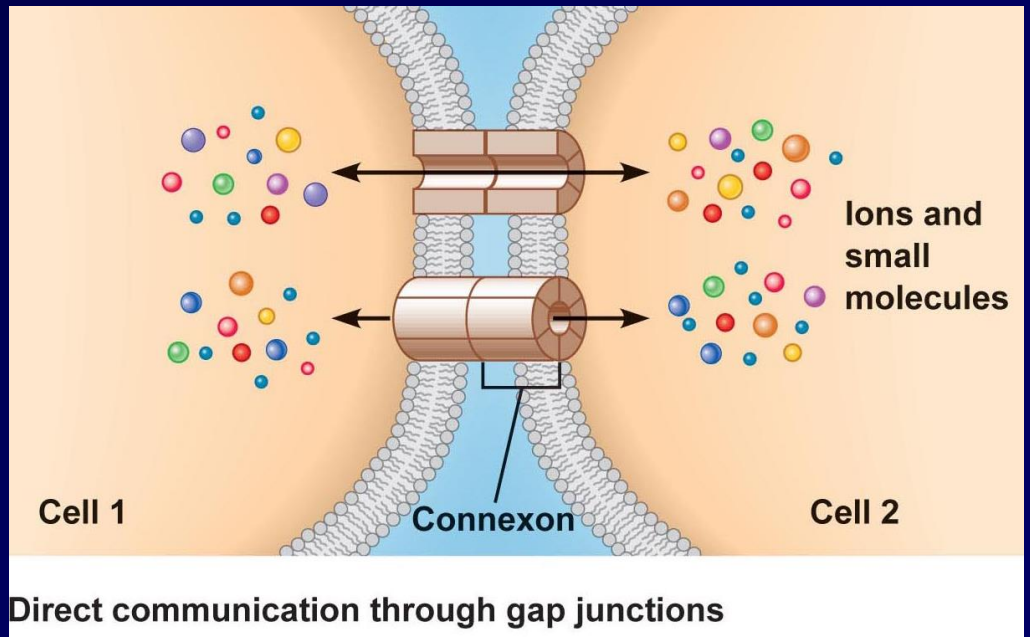


Intercellular communication - mode of signal spreading and range

3. **synaptic communication** (neurotransmitters)

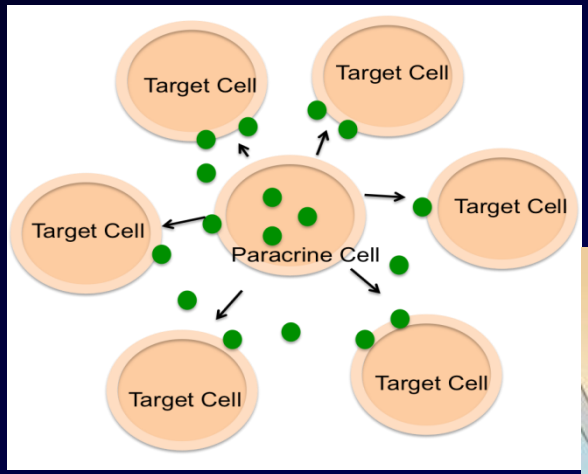


4. **metabolic connections** (gap junction) (ions, metabolites)

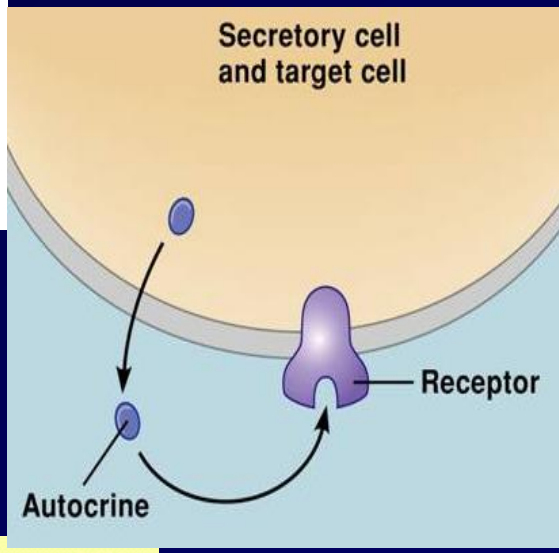


INTERCELLULAR COMMUNICATION - signal origin

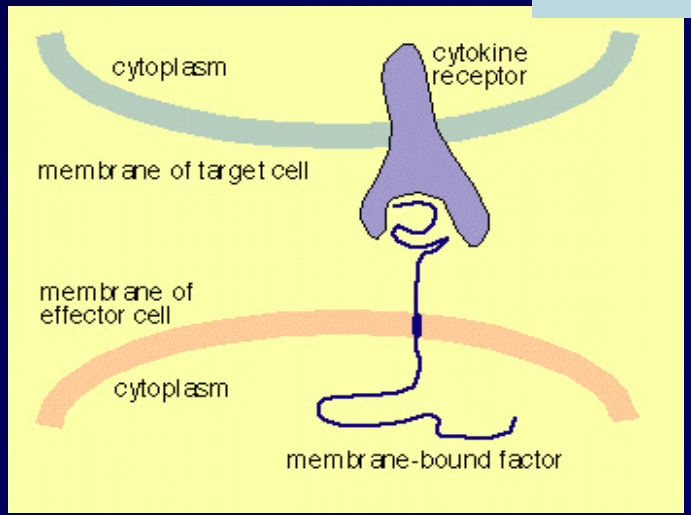
1. **paracrine** communication - signaling molecules produced by one cell act on the target cells



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

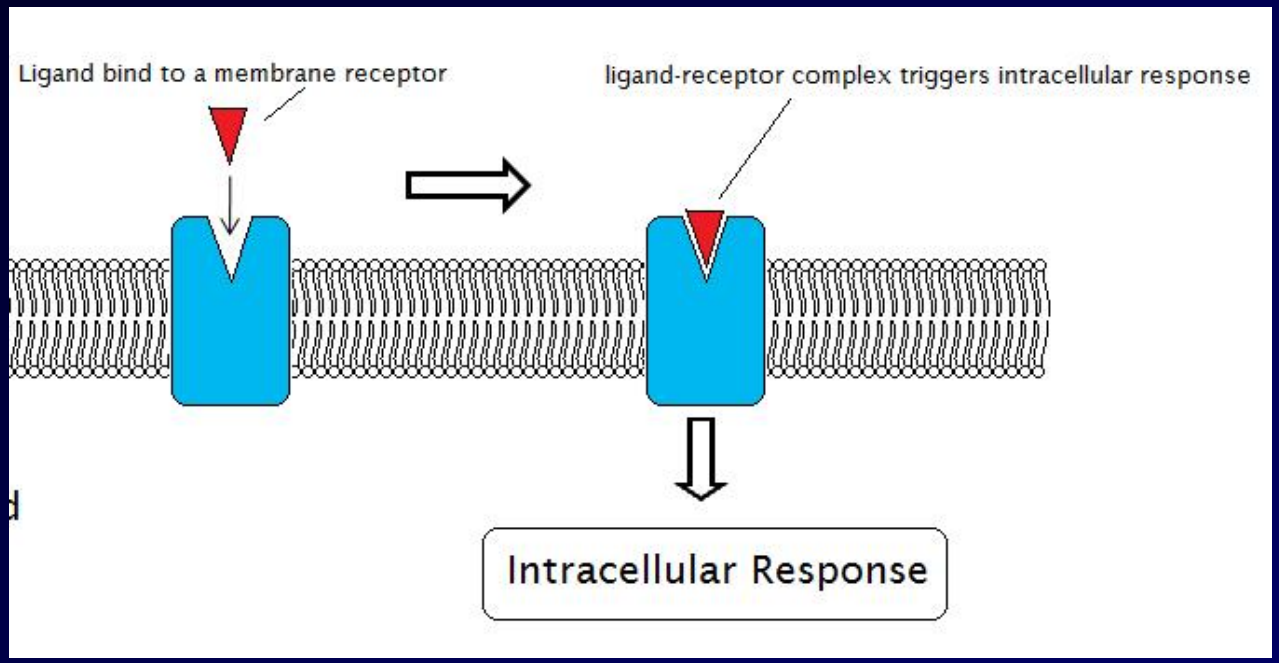


3. **juxtacrine** communication - **contact-dependent signaling** – ligand is bound to the cell



MESSAGE (ligand)

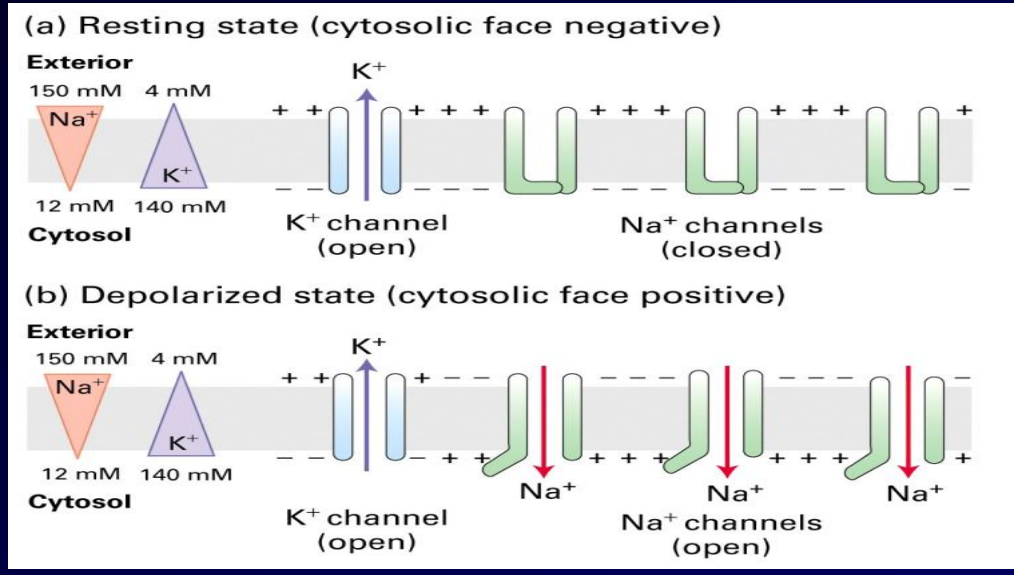
RECEPTOR

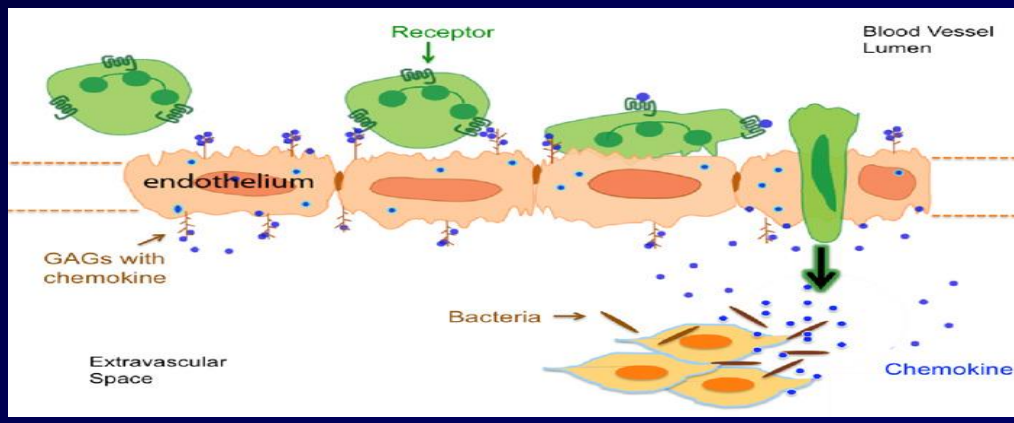


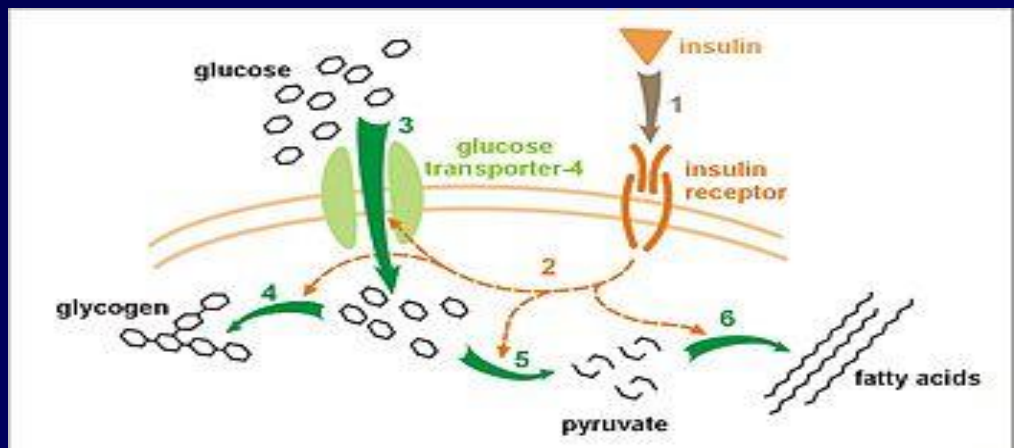
CELL REACTION

CELL REACTION

- 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





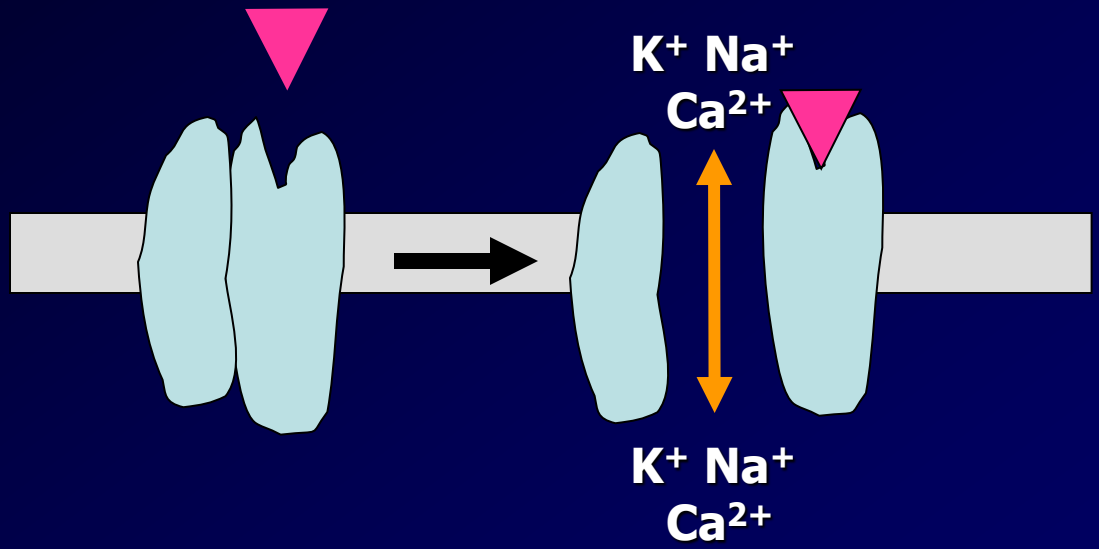


Classification of receptors

- **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

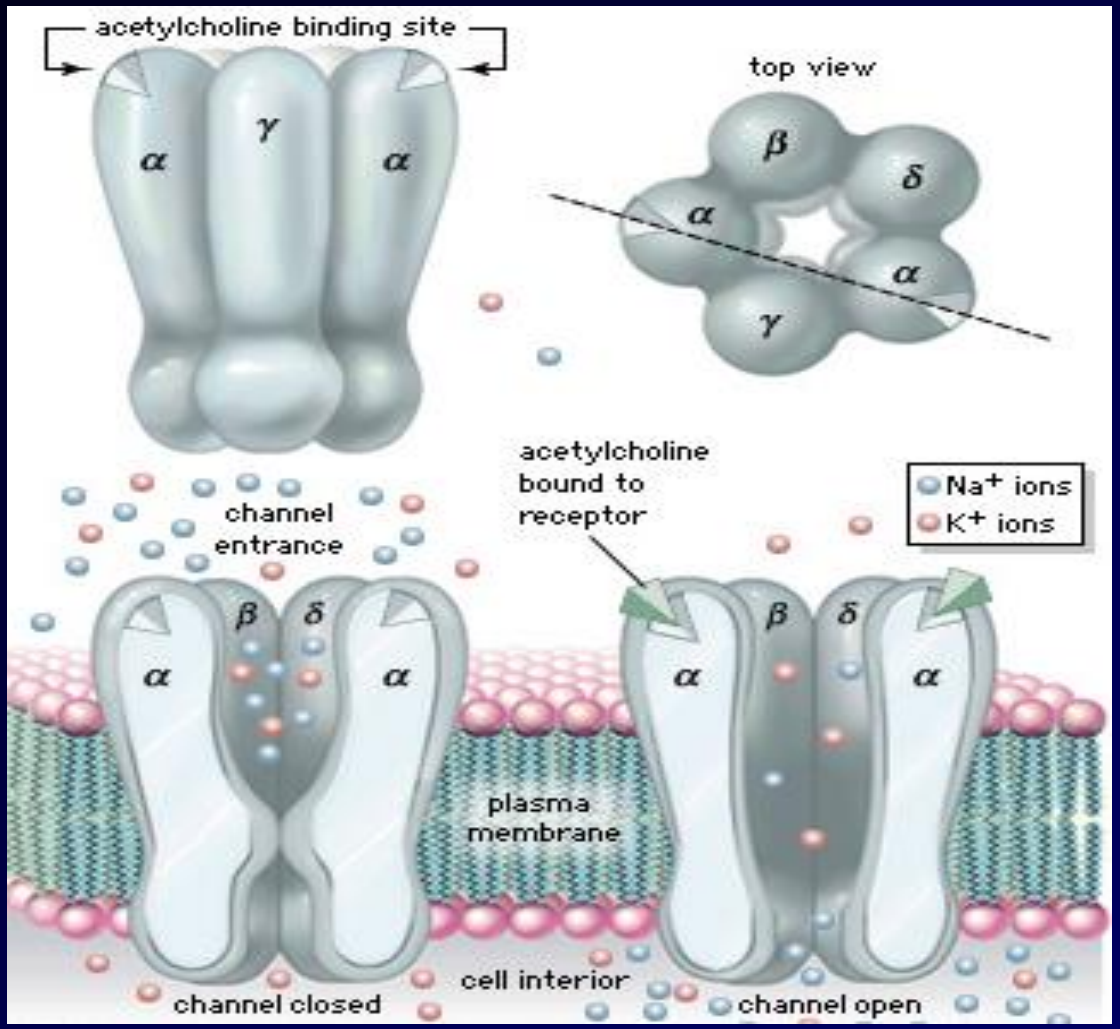
- Ligand – neurotransmitter
- Change of membrane potential
- typically composed of several different subunits



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

Nicotinic acetylcholine receptor – ligand-gated

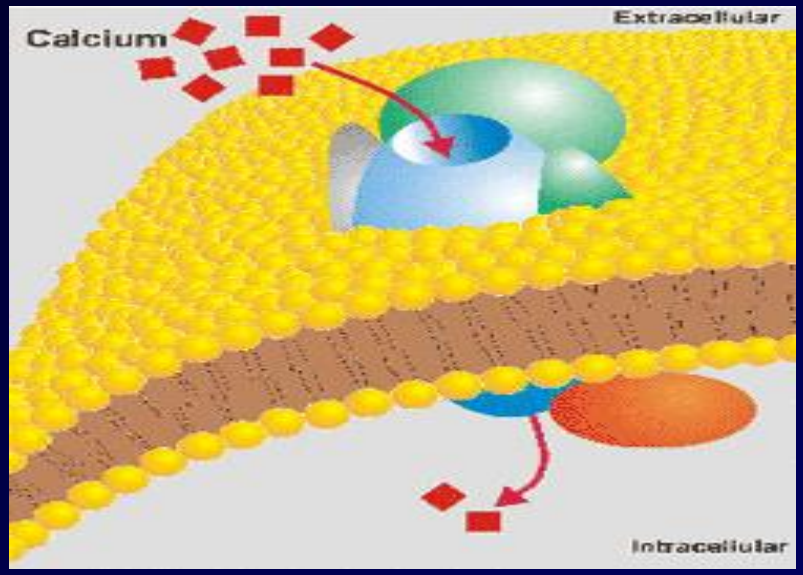
- Na+ and K+ ion channel
- five subunits - around a central pore (two of the five subunits - ability to bind **acetylcholine**)



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

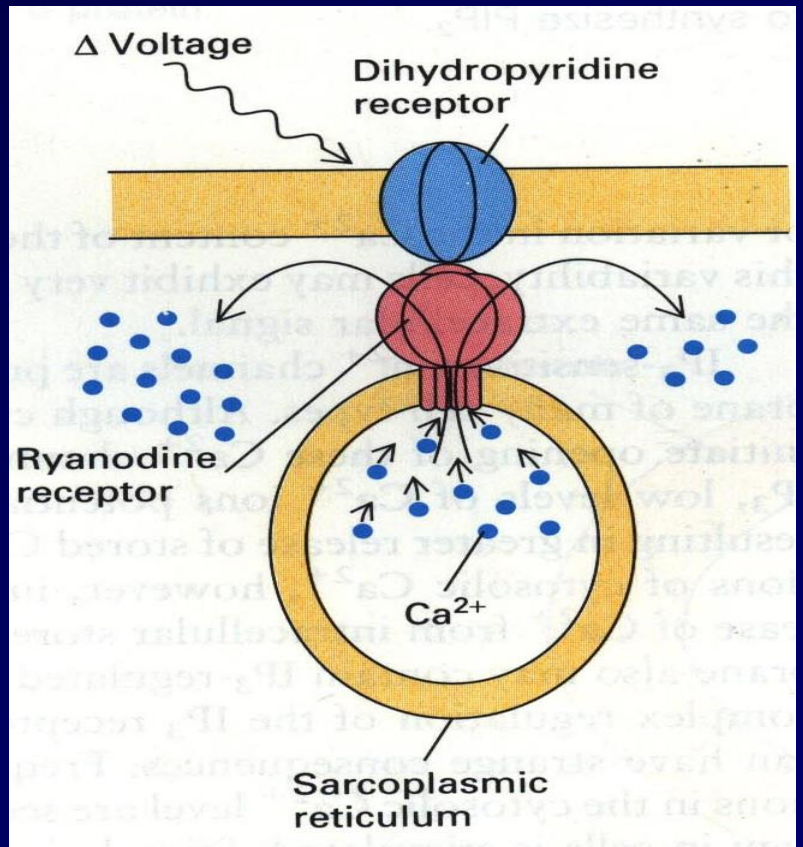
Voltage-gated calcium channels

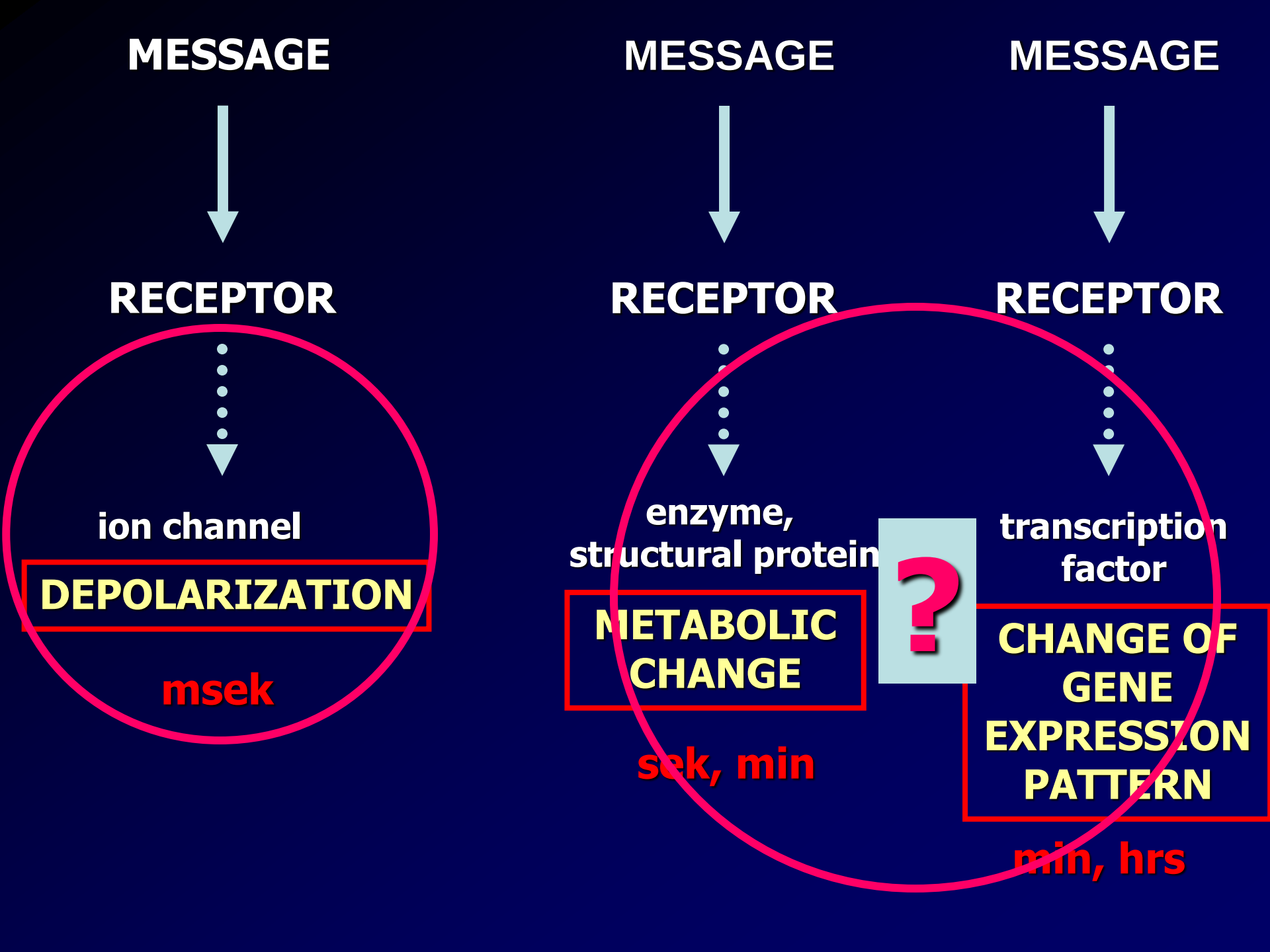
- external cell membranes
- **dihydropyridine receptors** (T tubule of skeletal muscle, associated with the **ryanodine receptor** of the sarcoplasmic reticulum)

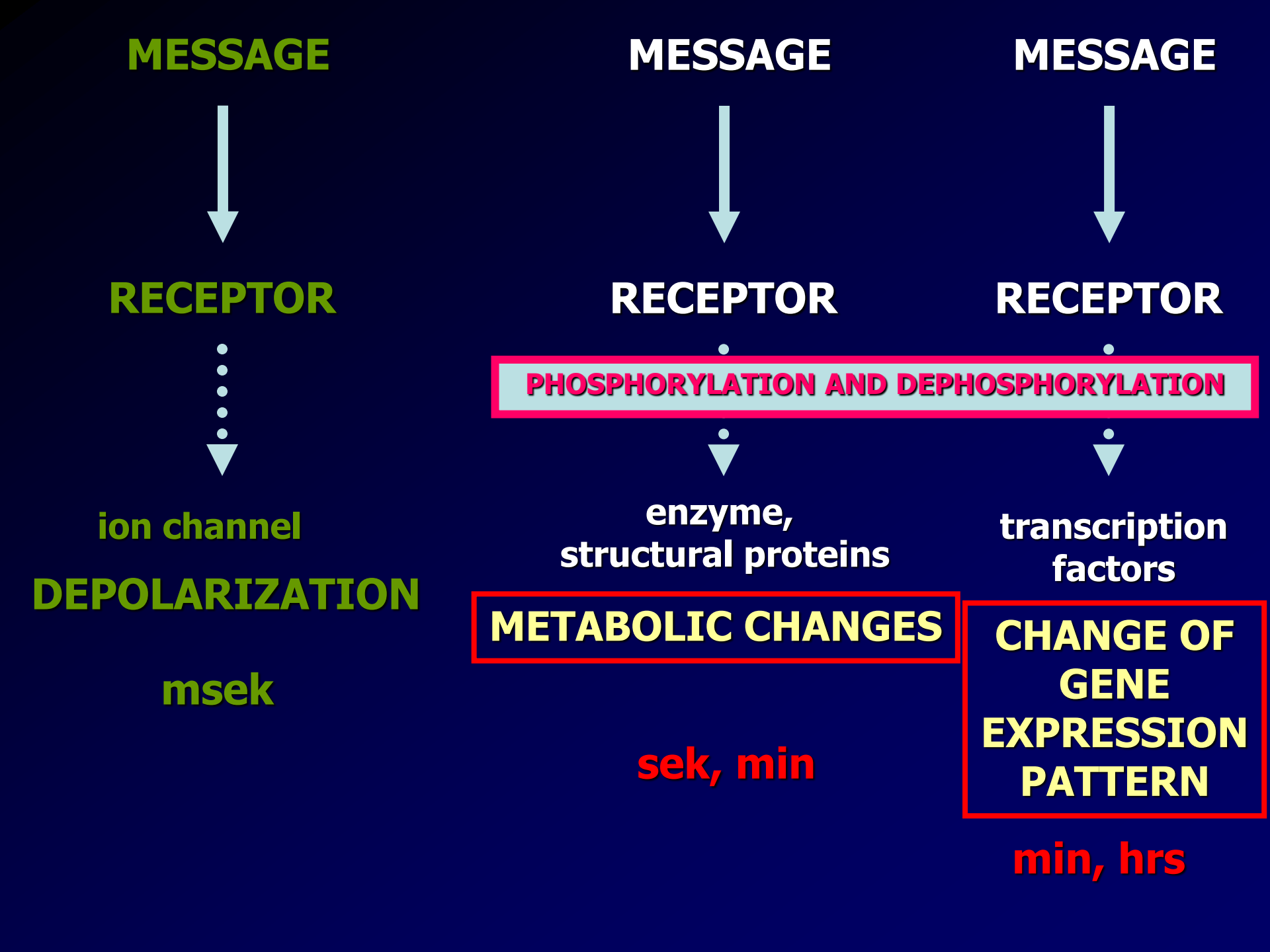


Ryanodine receptors

- mediate the release of calcium ions from the sarcoplasmic reticulum - muscle contraction




MESSAGE
RECEPTOR
ion channel
DEPOLARIZATION
msek
MESSAGE
RECEPTOR
enzyme,
structural protein
METABOLIC
CHANGE
sek, min
?
MESSAGE
RECEPTOR
transcription
factor
CHANGE OF
GENE
EXPRESSION
PATTERN
min, hrs


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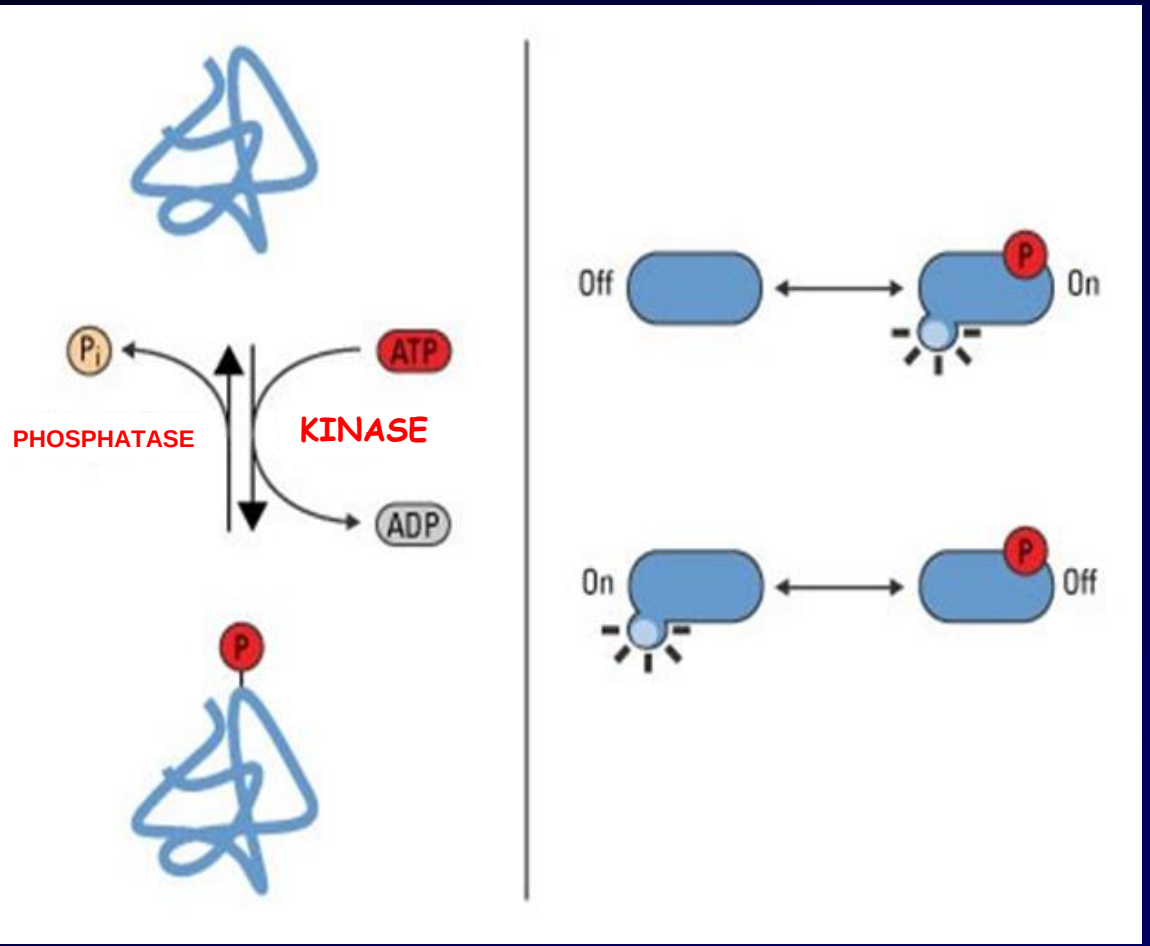
MESSAGE
RECEPTOR
transcription
factors
CHANGE OF
GENE
EXPRESSION
PATTERN
min, hrs

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

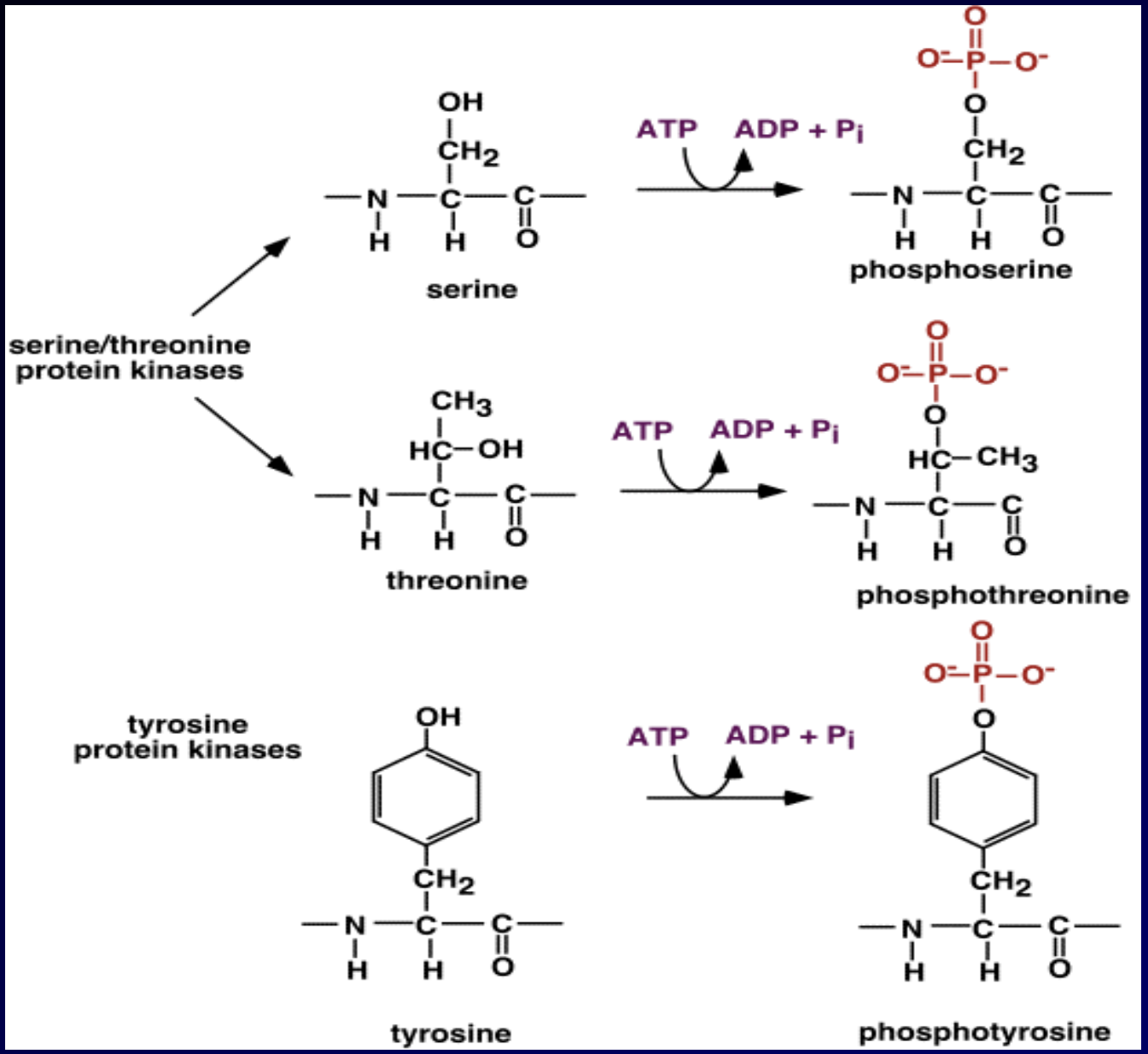
TYROSINE PHOSPHATASES

- 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



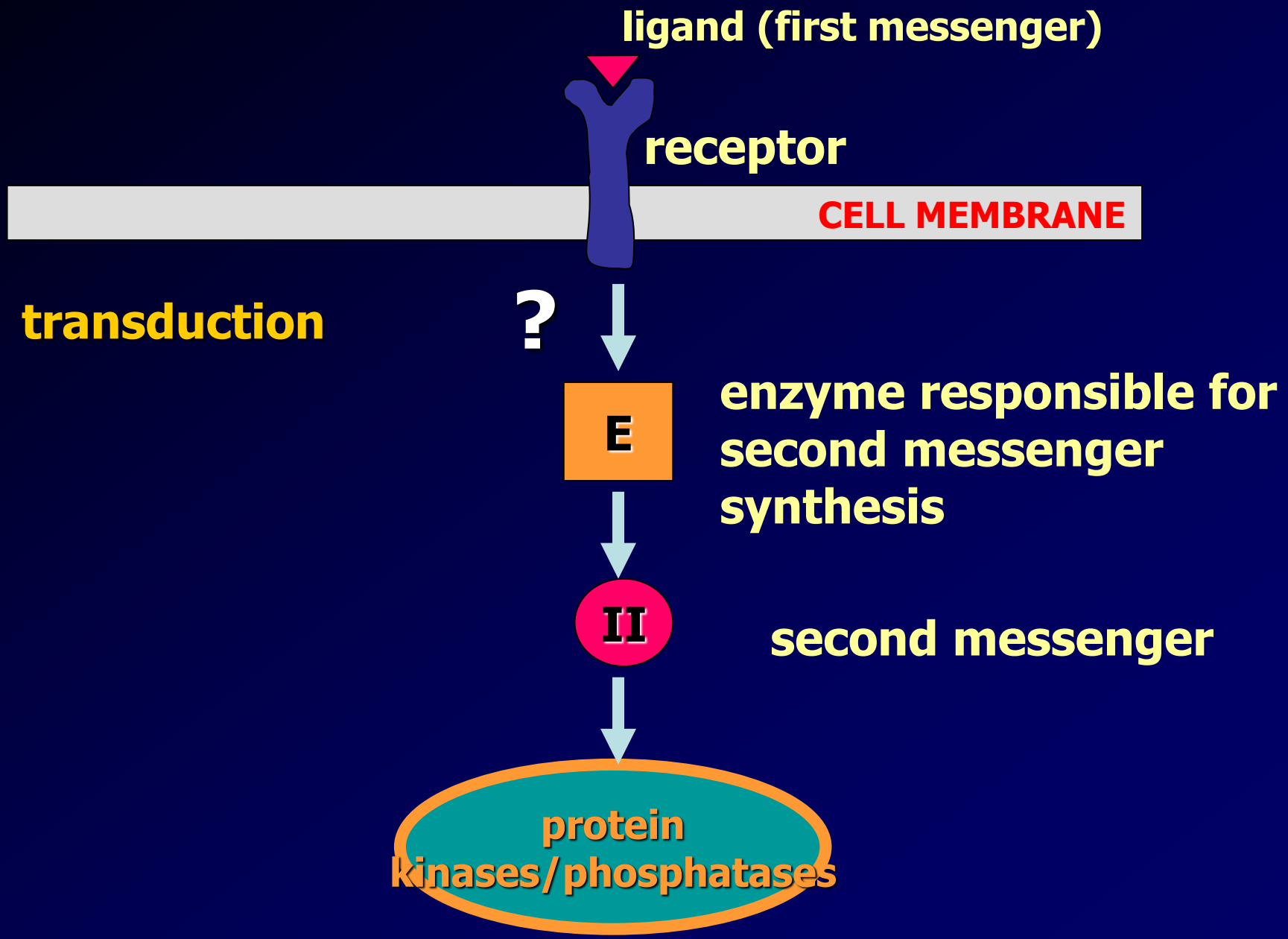
- phosphorylation of hydroxyl group

1992 Nobel Prize in Physiology or Medicine

Edwin Krebs and Edmond Fischer

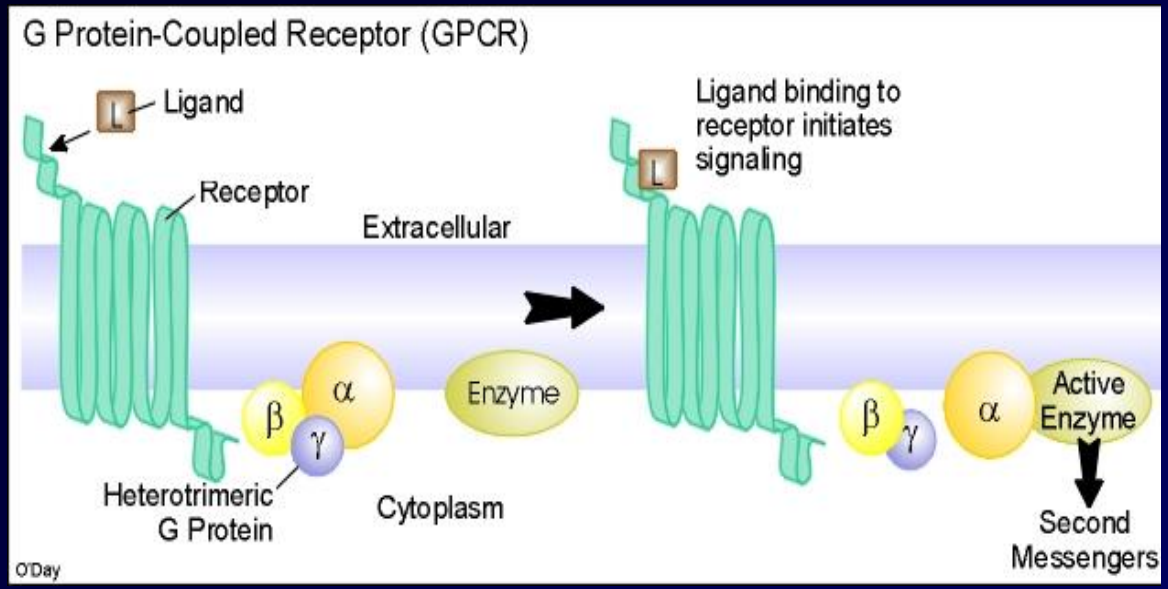
The Nobel Prize in Physiology or Medicine 1992 was awarded jointly to Edmond H. Fischer and Edwin G. Krebs **"for their discoveries concerning reversible protein phosphorylation as a biological regulatory mechanism"**

What activates protein kinases and phosphatases?

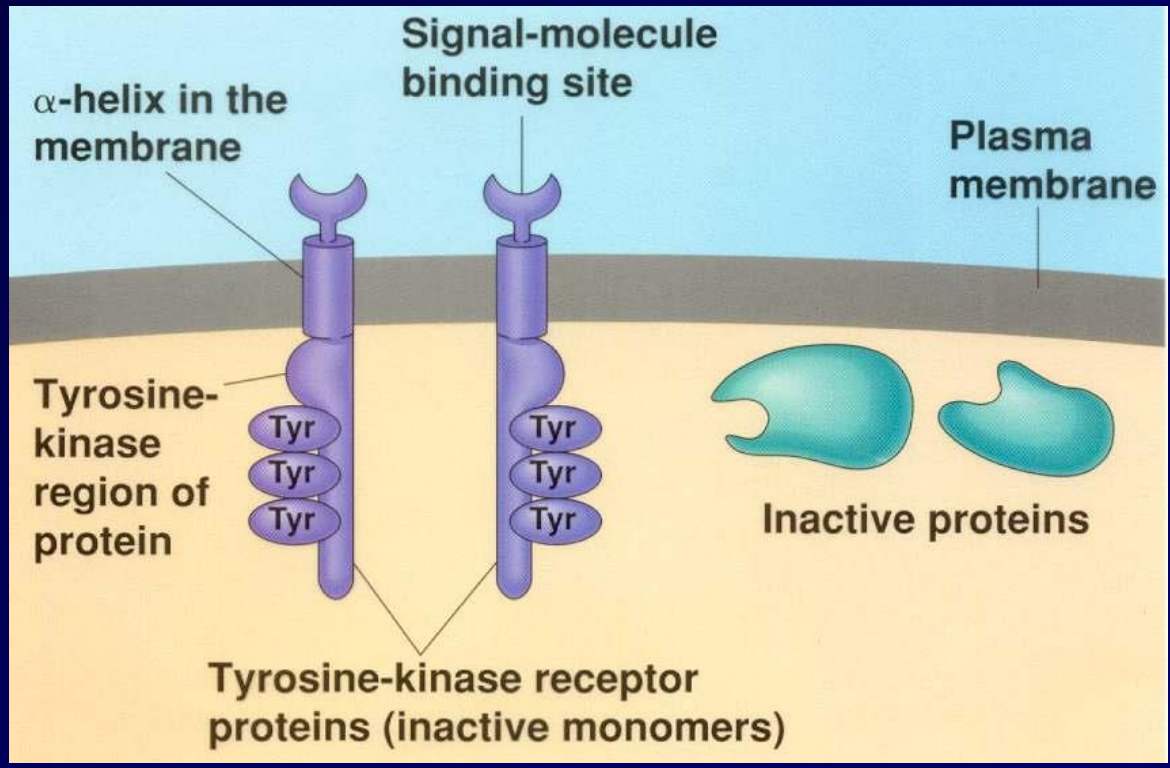


Metabotropic receptors

- G-protein-linked receptors



- Enzyme-linked receptors (tyrosine-kinase receptors)

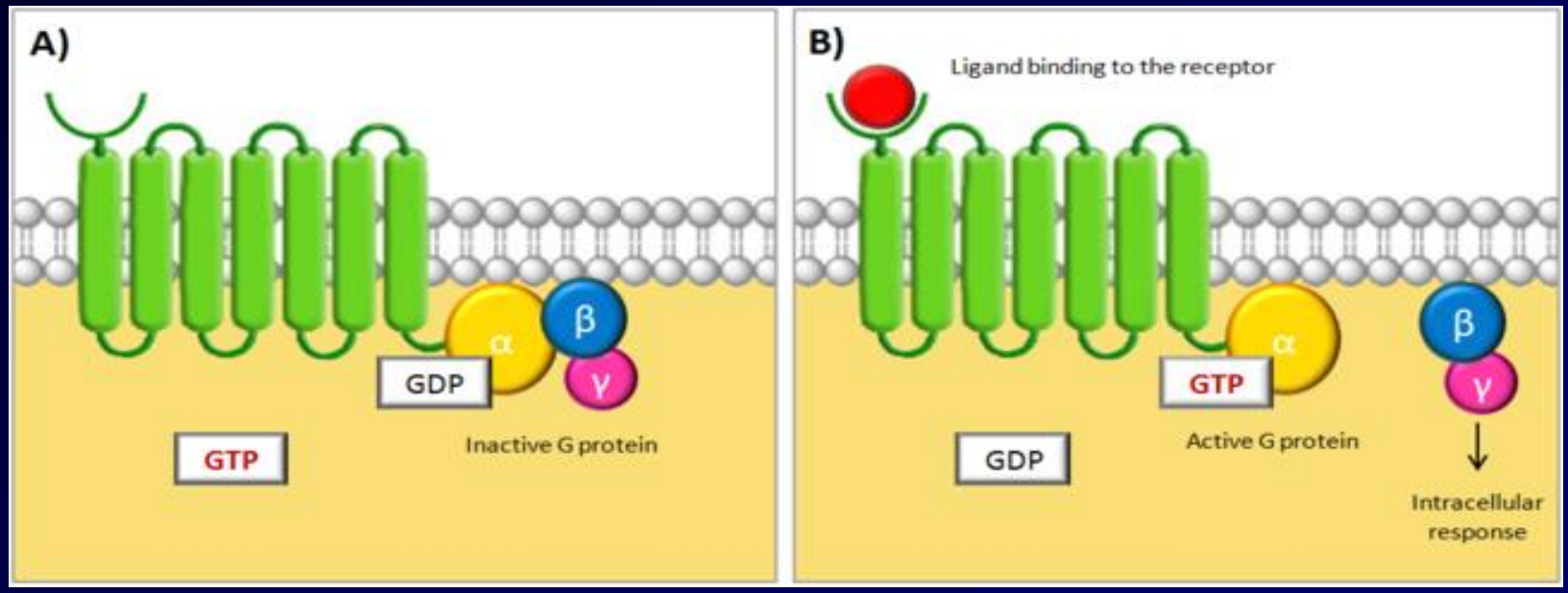


G-protein-linked receptors

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

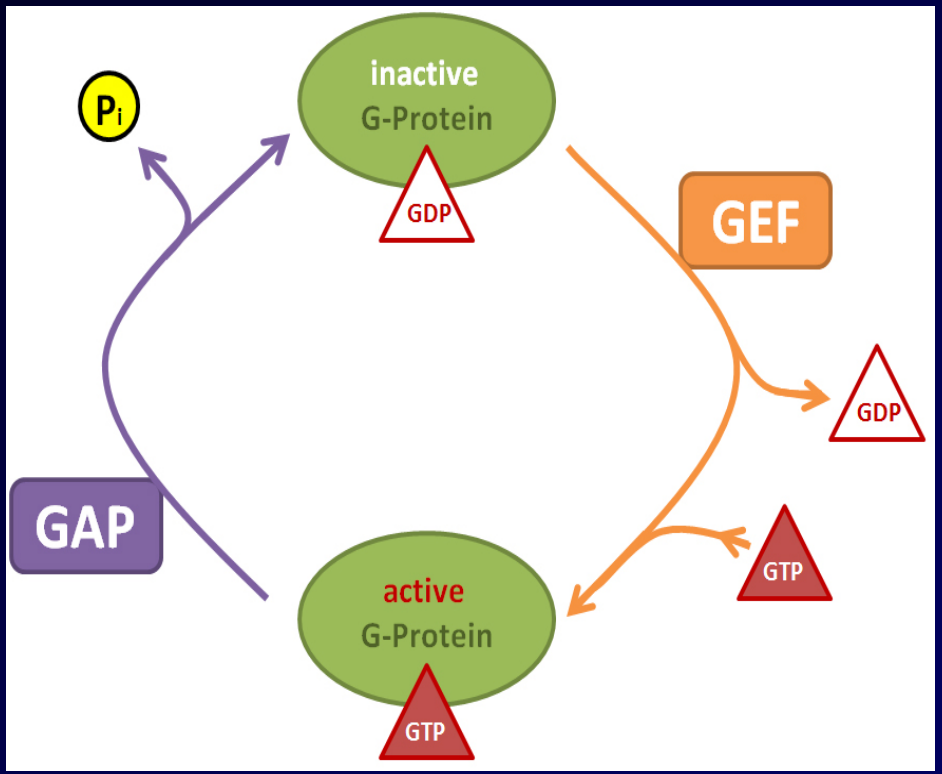
G protein

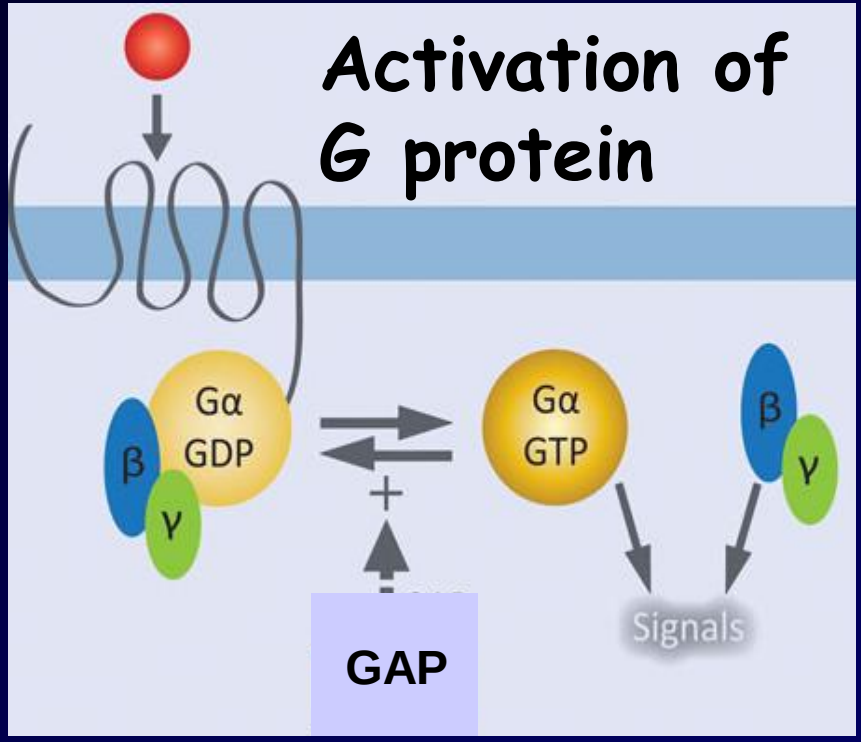
- three protein subunits: α,β and γ



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

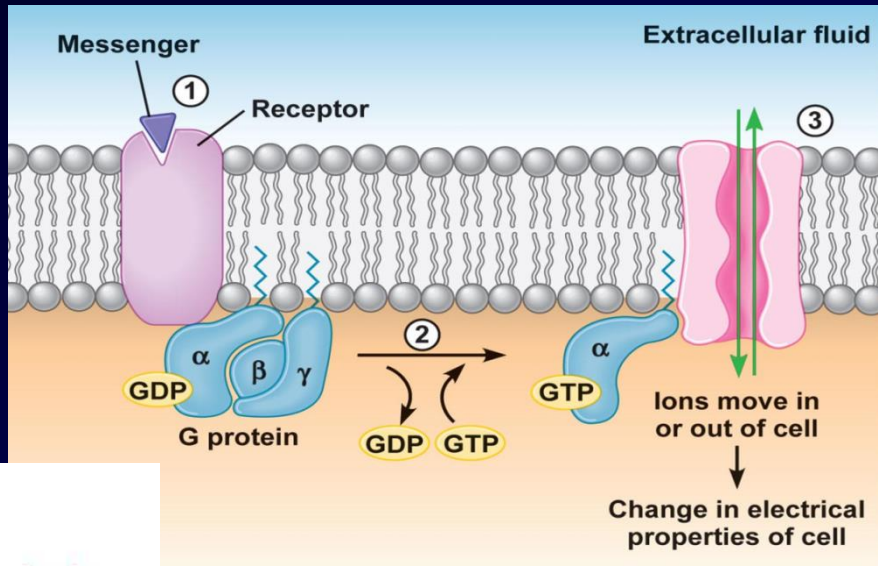
- inactive α - GDP
- binding of the ligand - conformational change o receotor - activation G protein
- GDP is exchanged for the GTP
- dissociation of the Gα subunit (which GTP) from the Gβγ dimer

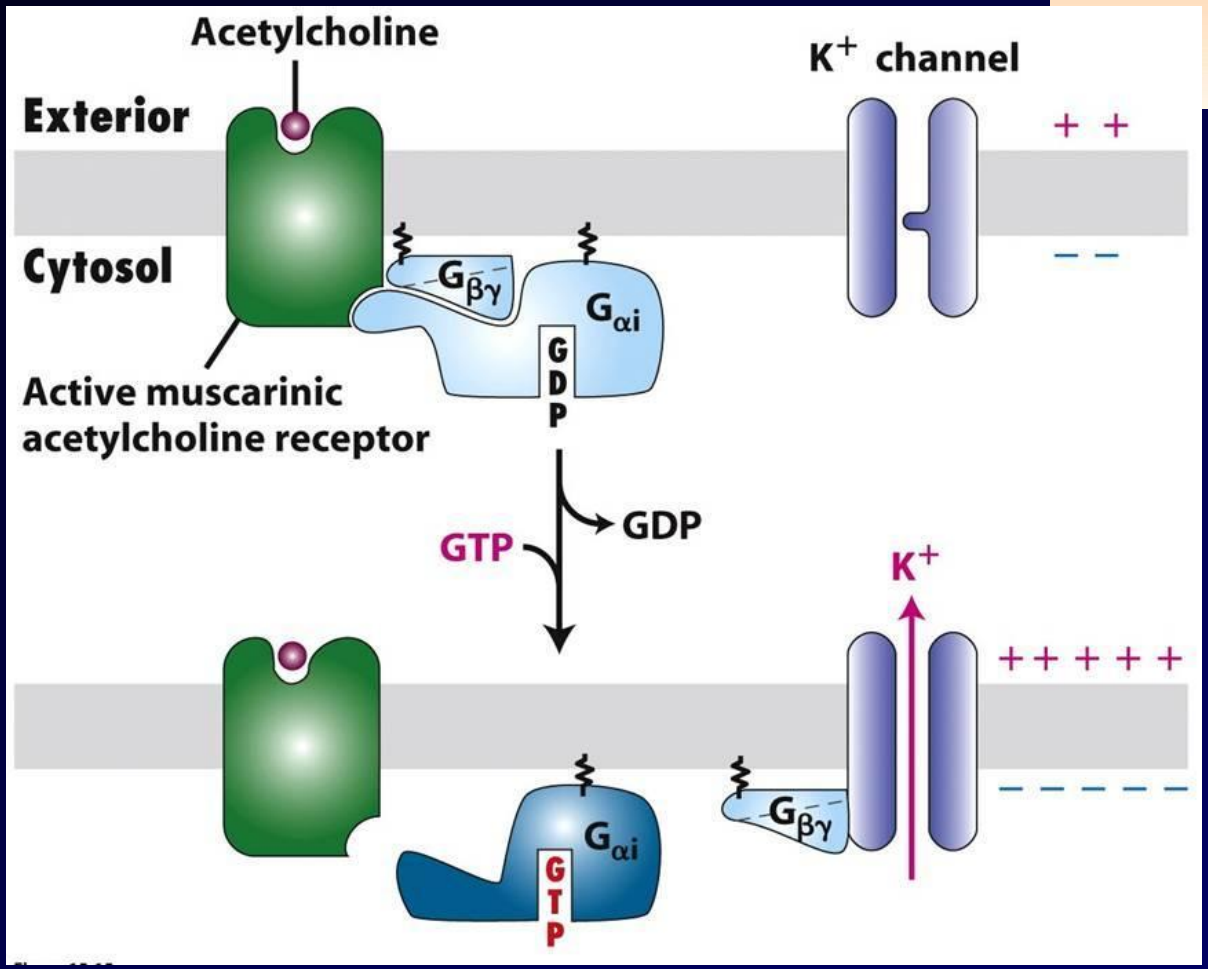




- guanine nucleotide exchange factor (**GEF**) - exchange GDP for GTP
- α subunit - GTP-hydrolyzing activity (GTPase) - hydrolyzes GTP to GDP - signal is shut off
- **GAP** - GTPase-Activating Protein

G proteins can regulate ion channels



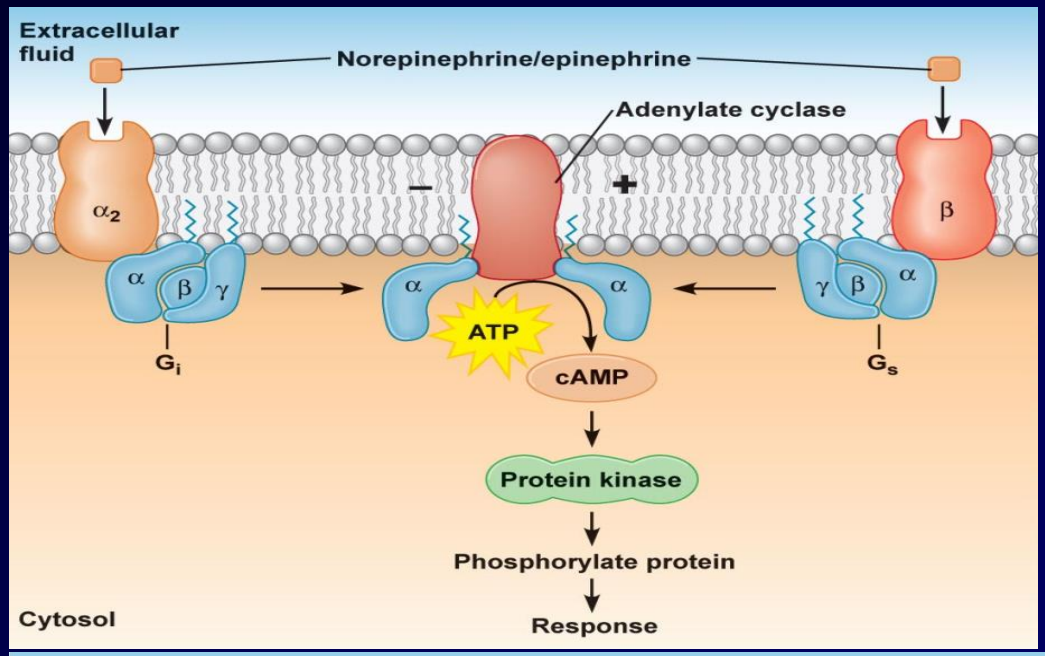


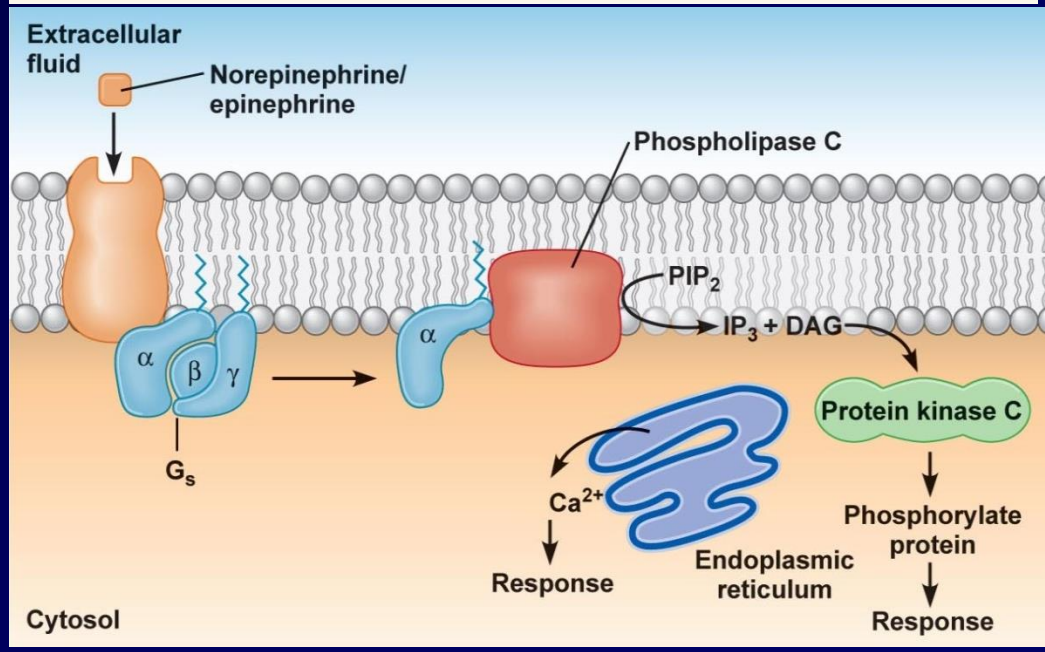
- β,γ **complex** binds to K^+ channel of heart muscle cells - inhibition of the heart activity.

G protein can activate membrane-bound enzymes

- **Adenylyl cyclase** – most frequent target enzyme – second messenger - **cAMP**

- **Phospholipase C** – second messengers - **inositol trisphosphate (IP3) and diacylglycerol (DAG)**





Types of G proteins

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

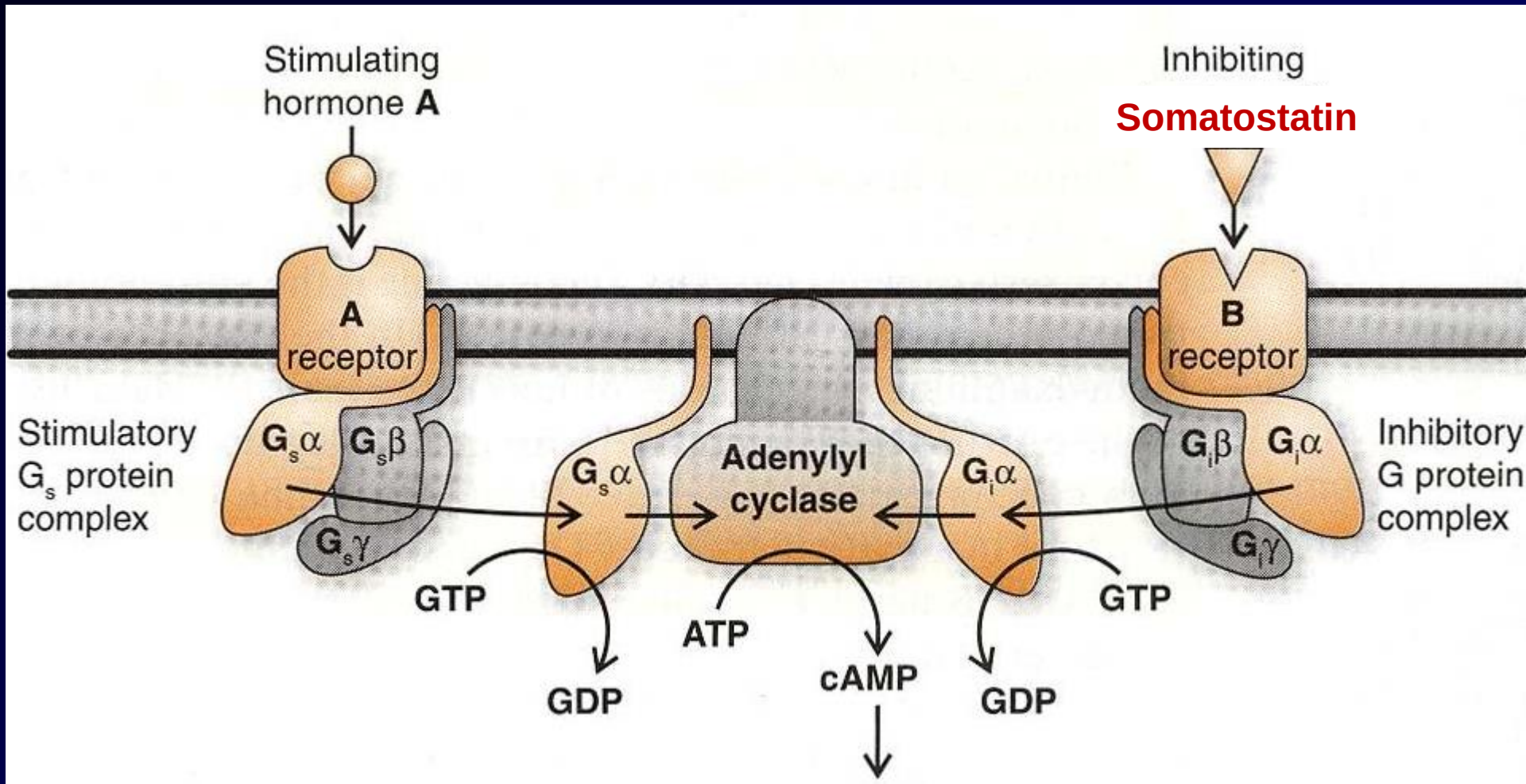
TRANSDUCING FACTOR	ENZYME	SECOND MESSENGER	KINASE
-	IP_3, ΔV	Ca^{2+}	Ca^{2+}/CaM-dependent kinase
$G_{s/i}$ protein	Adenylate cyclase	cAMP	PKA
G_q protein	PLC β	DG	PKCα, β i γ
G protein?	PLD	DG	PKCα, β i γ
G_t protein	PDE	cGMP	-
?	Guanylate cyclase	cGMP	PKG

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

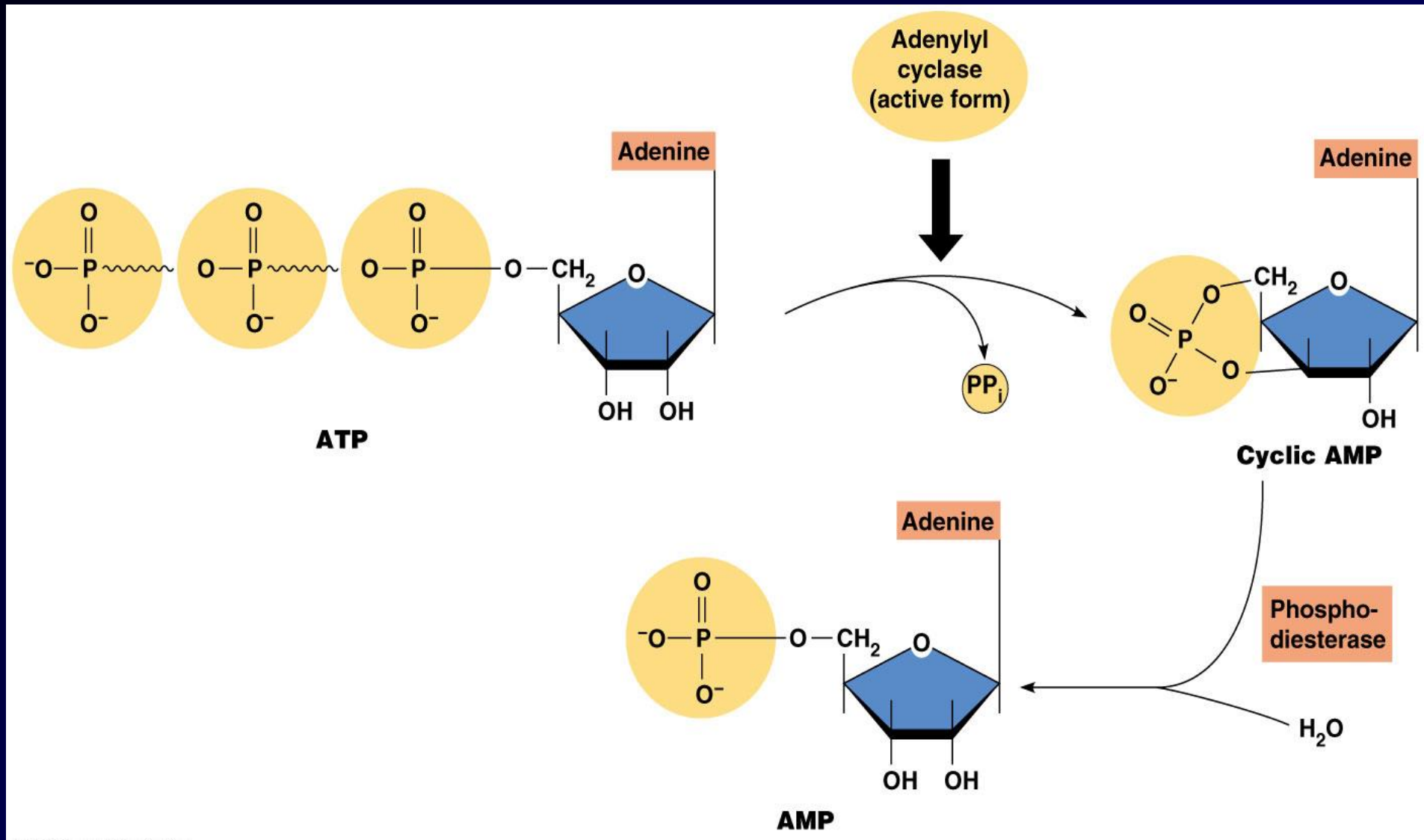
G protein

- stimulatory Gs protein
- inhibitory Gi protein

Adenylyl cyclase

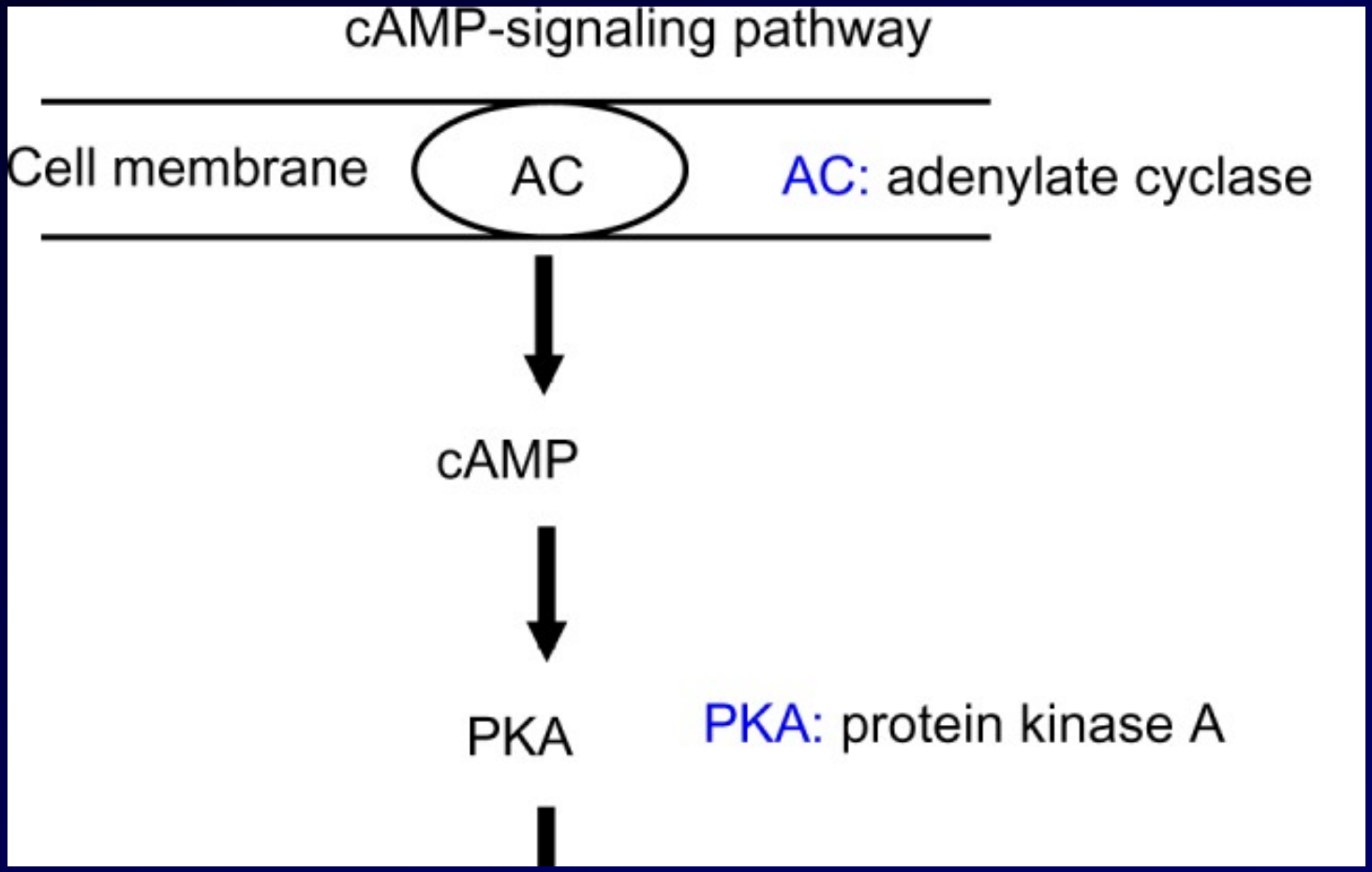


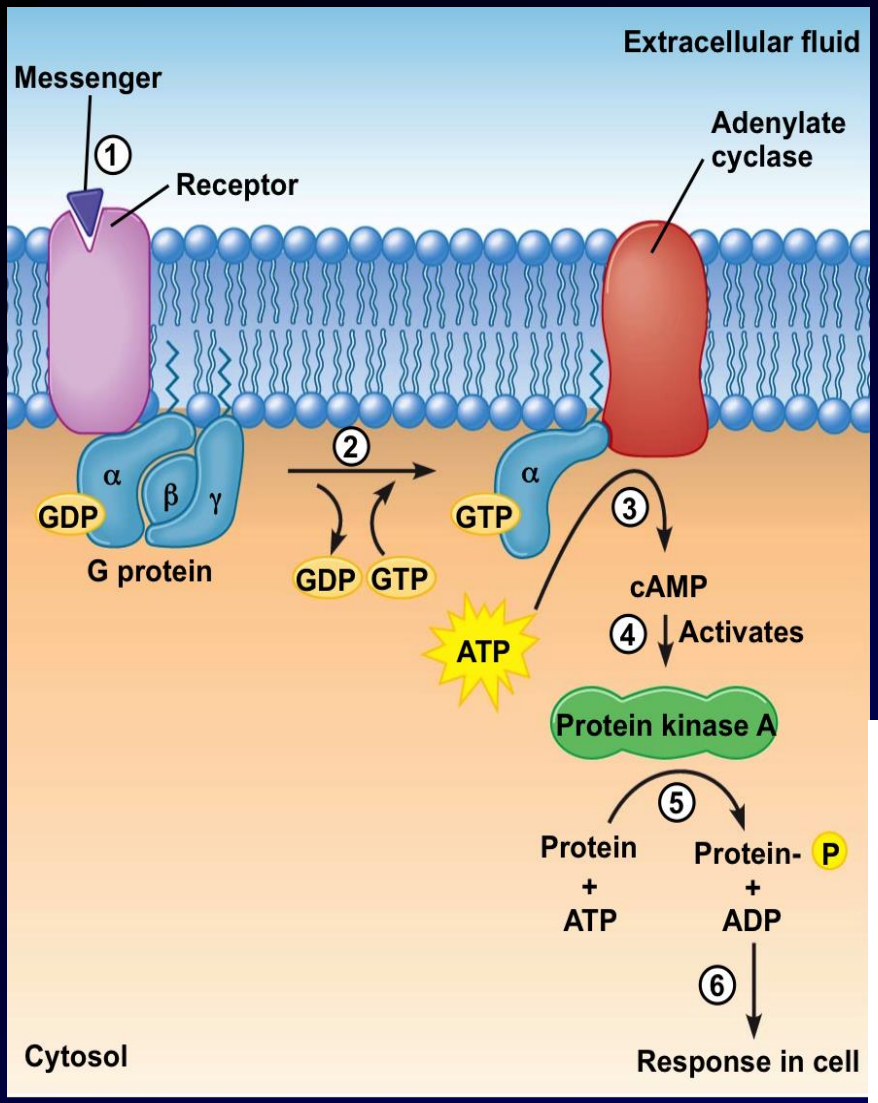
Cyclic AMP phosphodiesterase (PDE) converts cAMP to the AMP – elimination of signal



cAMP - second messenger - **function**

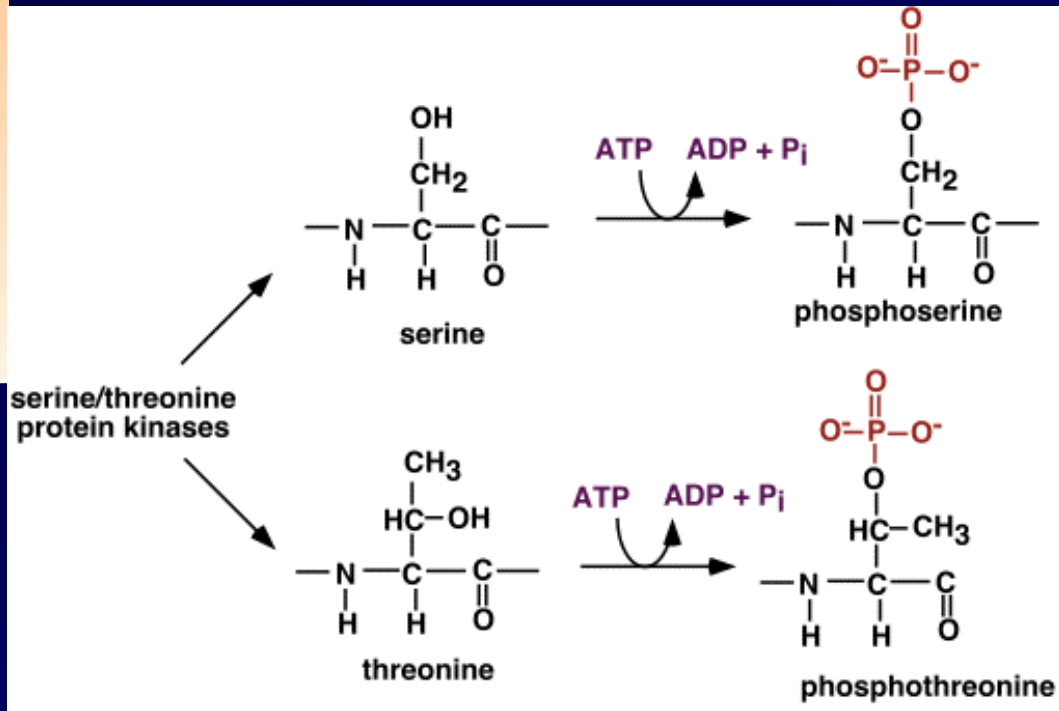
- hormones (glucagon), adrenaline
- **protein kinase A (PKA)** - **cAMP-dependent protein kinase** - regulation of glycogen, sugar, and lipid metabolism





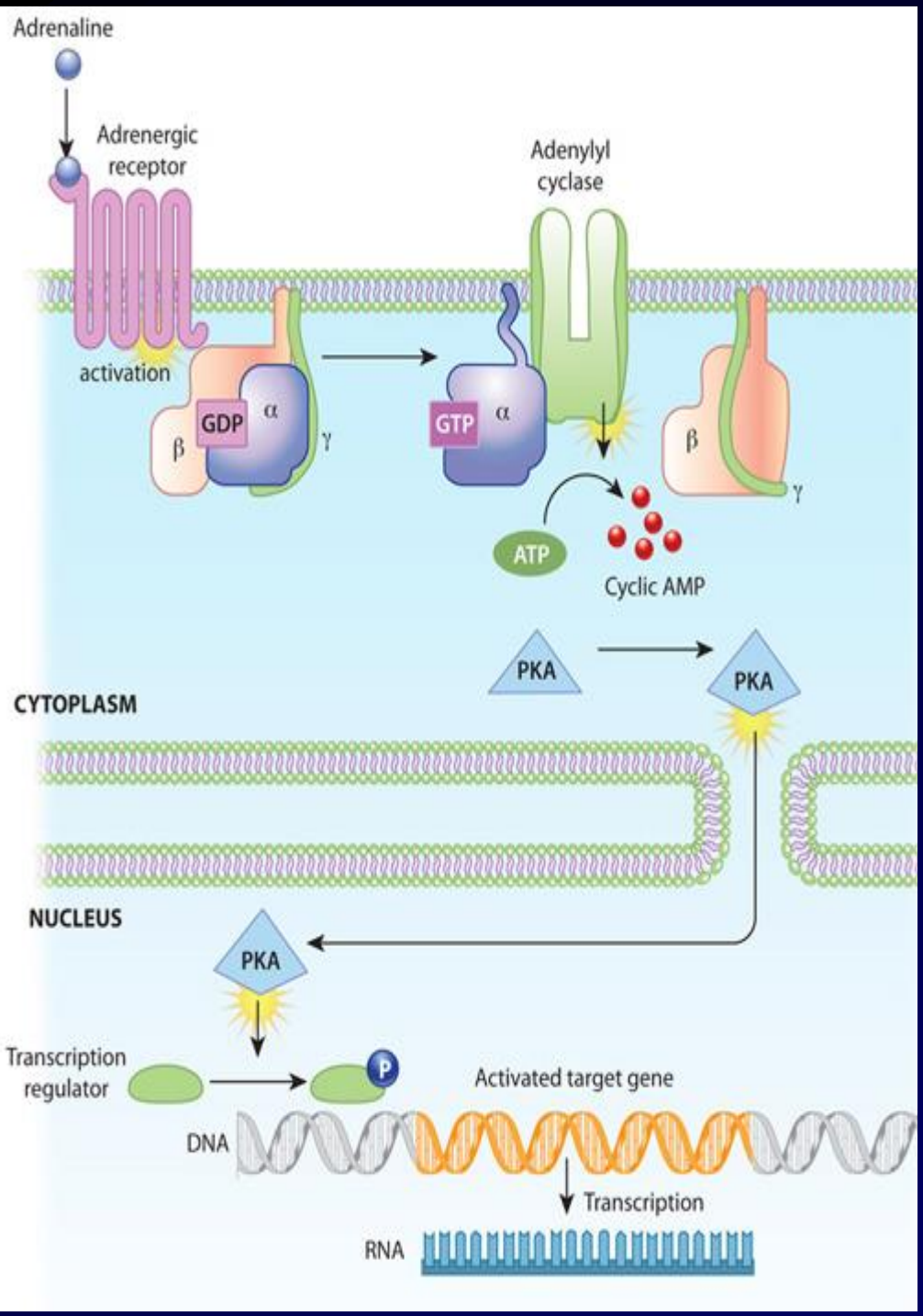
PKA – cyclic-AMP-dependent protein kinase

- 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

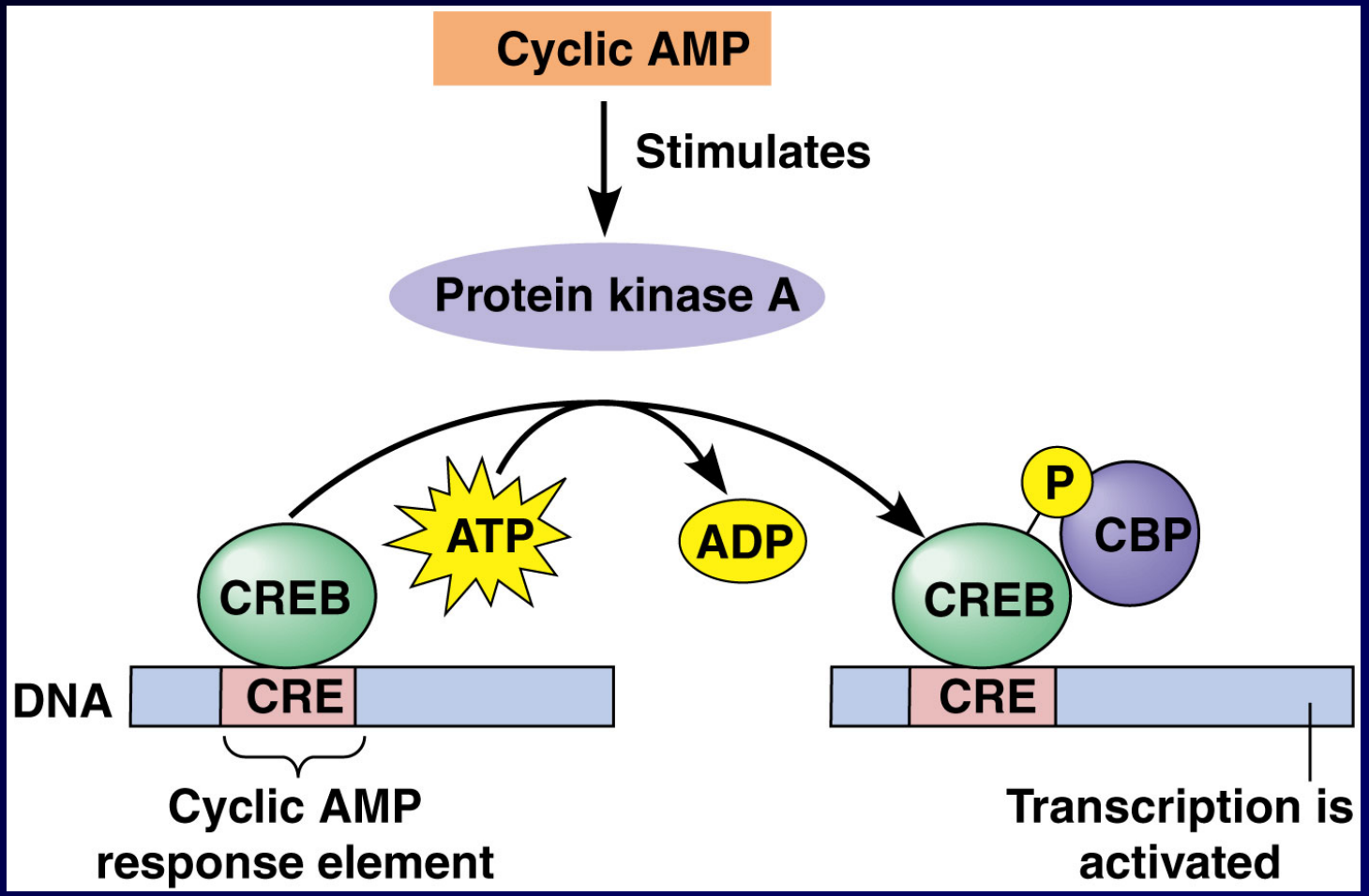
G protein and gene expression



PKA – transported to the nucleus – phosphorylation of transcription factors

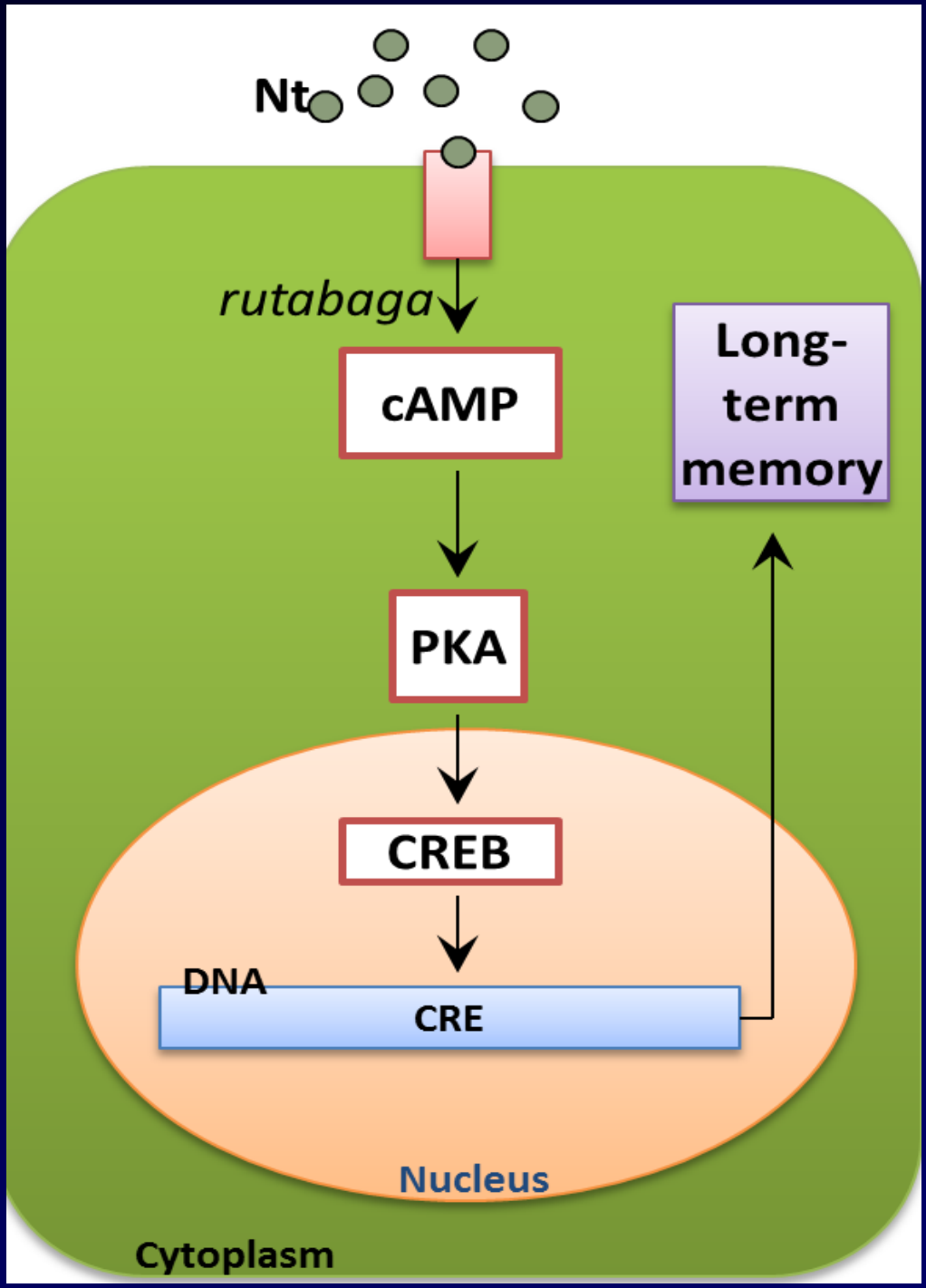
CREB - (cAMP response element-binding protein)

- transcription factor – activated by cAMP or Ca^{2+} and protein kinase A – transported to the nucleus – transcription of genes



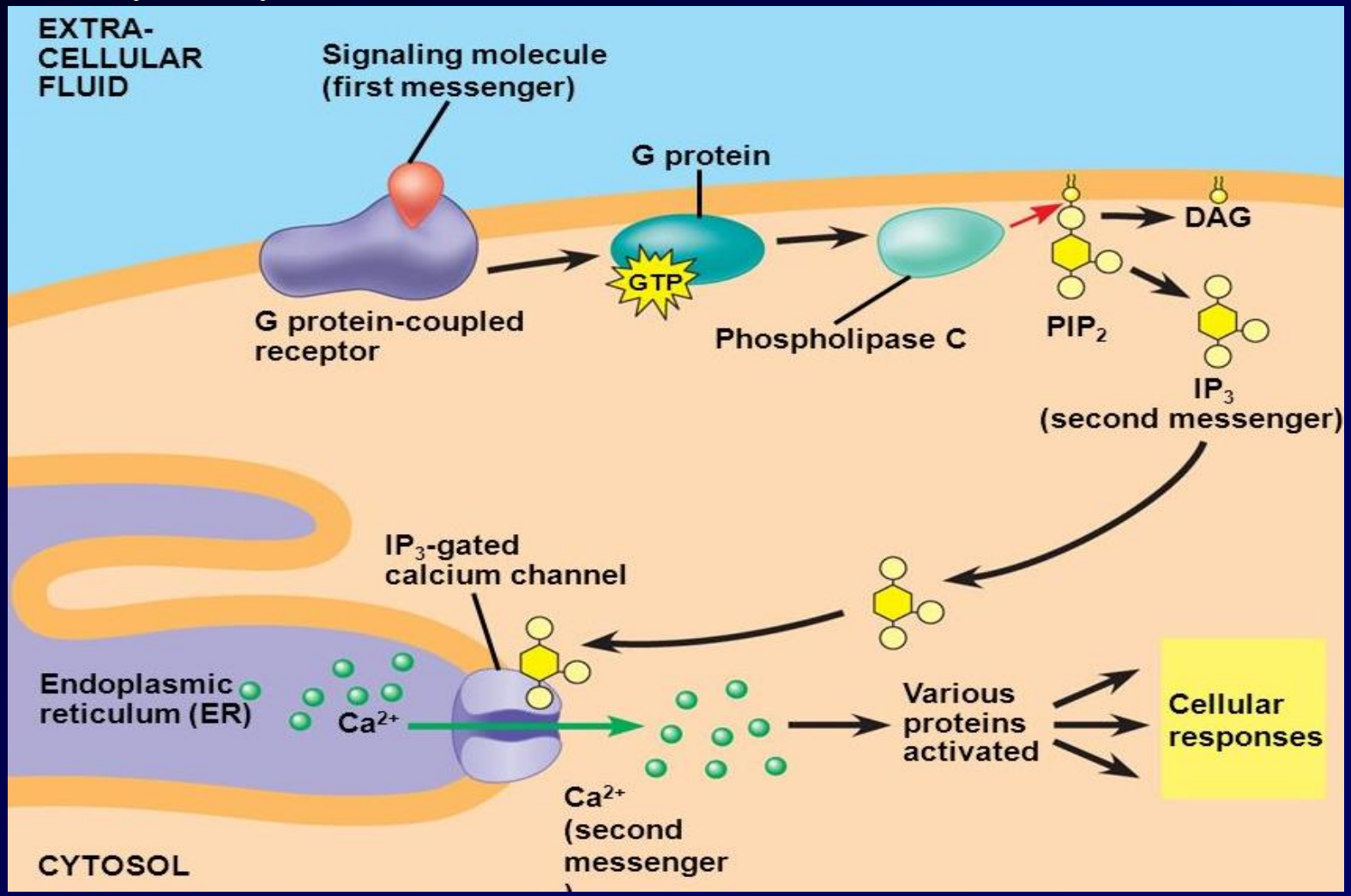
CREB – function

- survival of neurons
- in neurons - involved in the formation of long-term memories

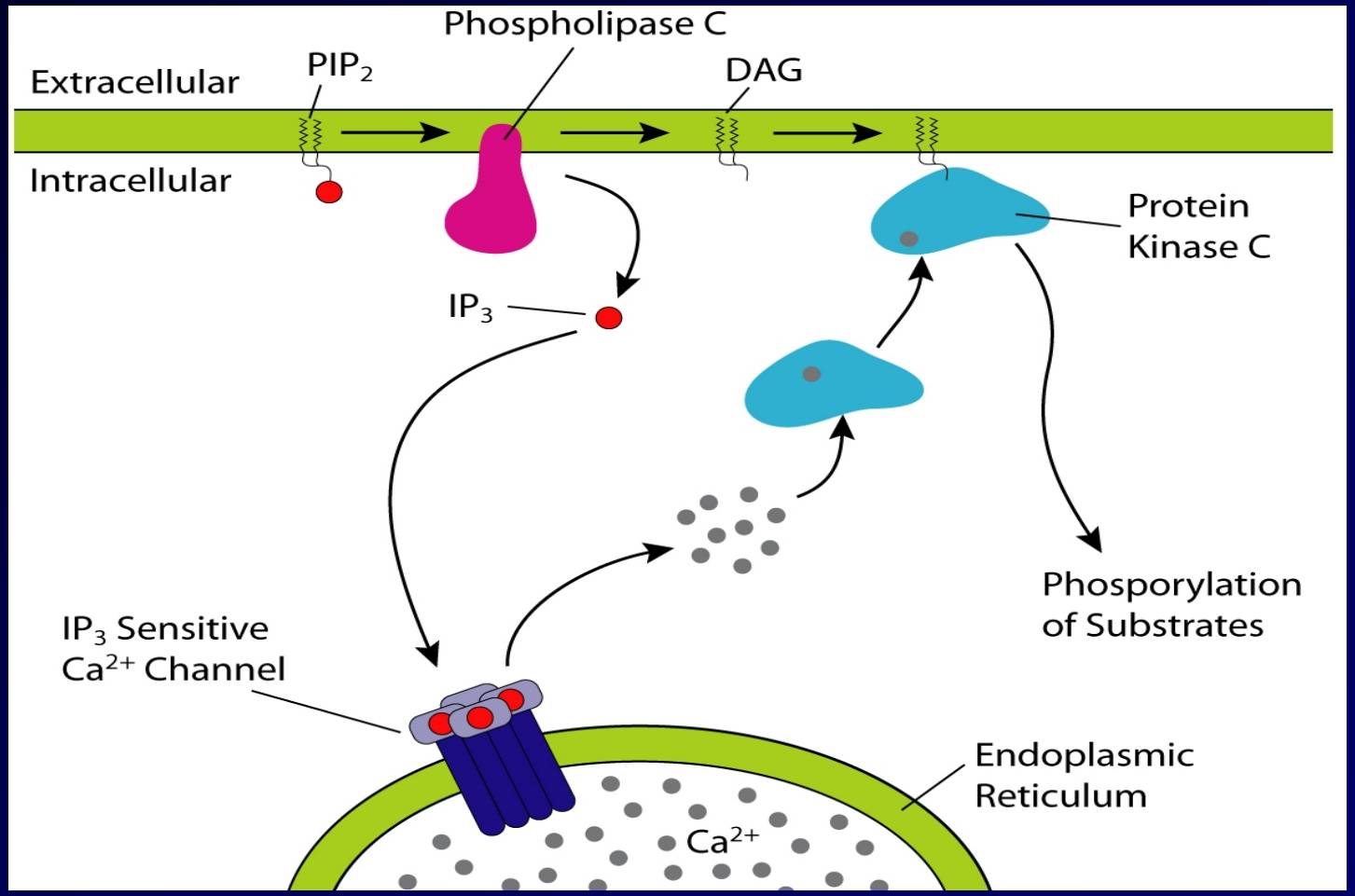


G protein activates phospholipase C

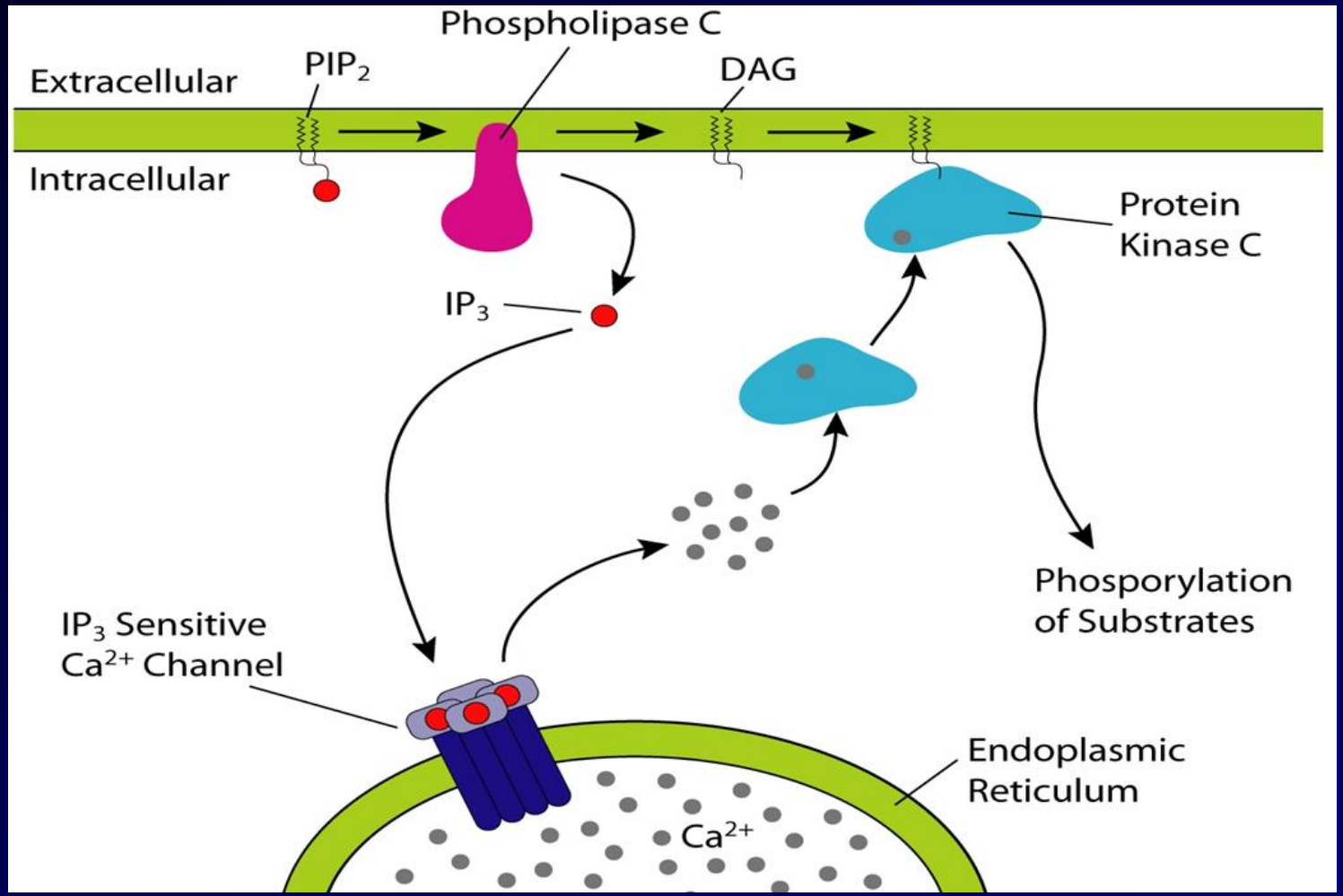
- cleaves the phospholipid **phosphatidylinositol bisphosphate** (PIP_2) into **diacyl glycerol** (DAG) and **inositol trisphosphate** (IP_3).



- 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).

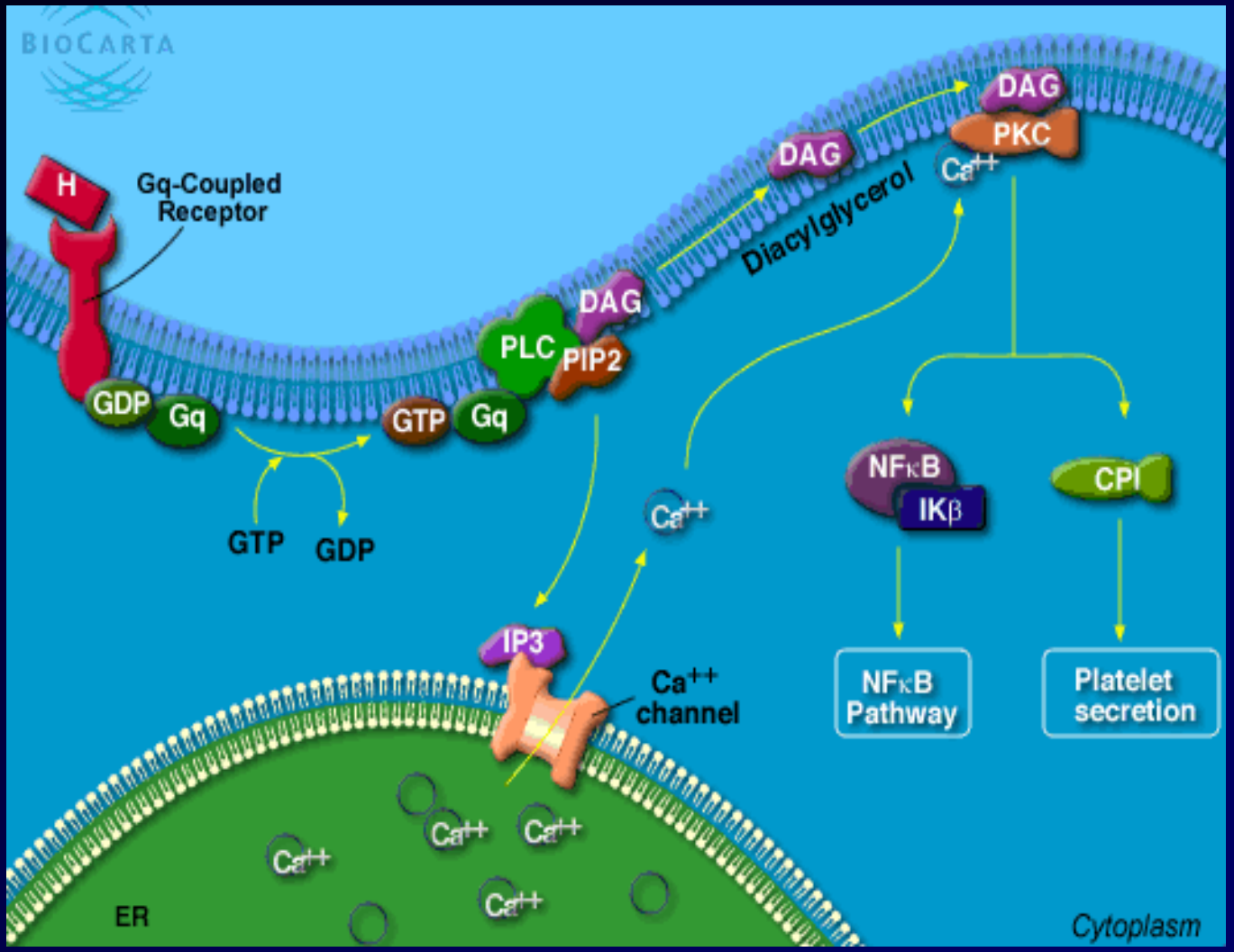


- calcium ions - a cascade of intracellular changes and activity
- calcium and DAG together activate **protein kinase C** - phosphorylation other molecules - cellular activity



Protein kinase C – serine-threonine kinase

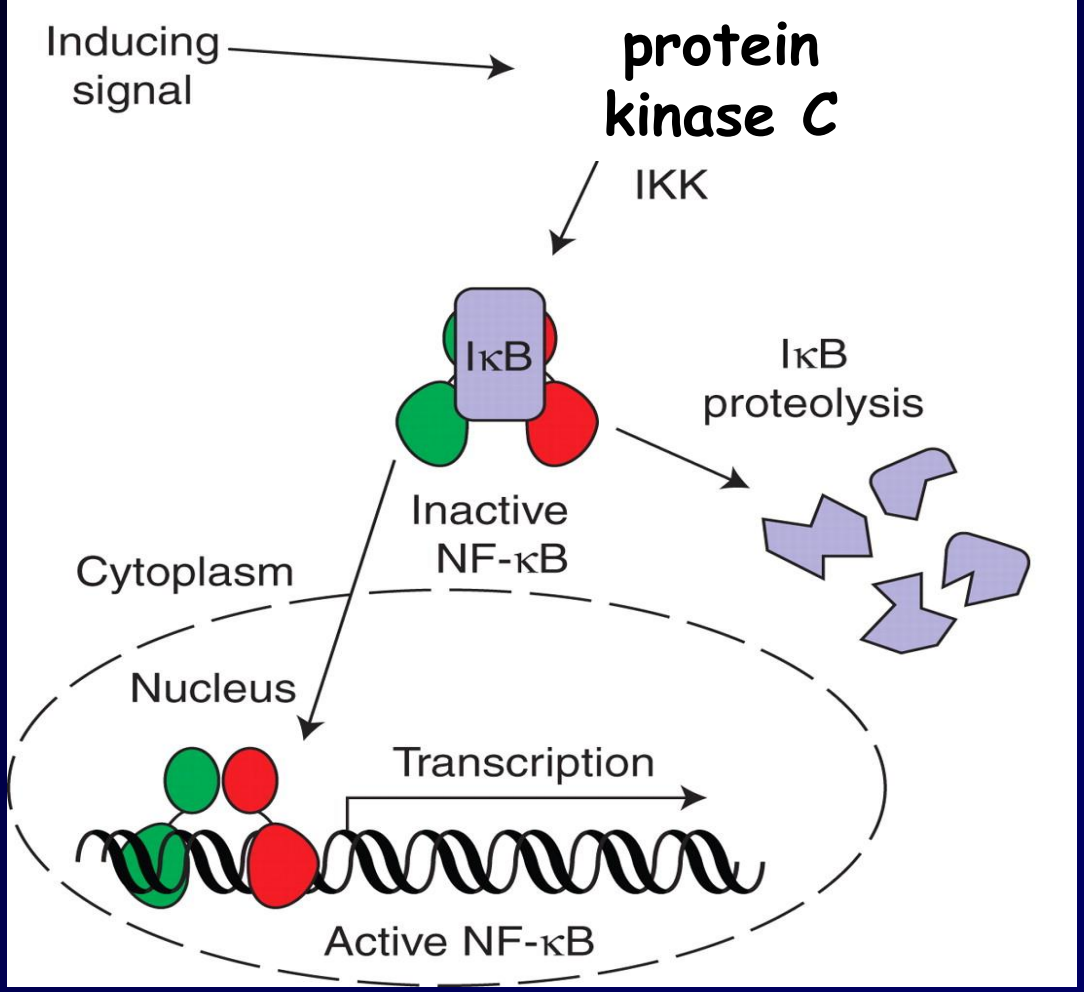
- phosphorylation of hydroxyl groups of serine and threonine amino acid residues on proteins



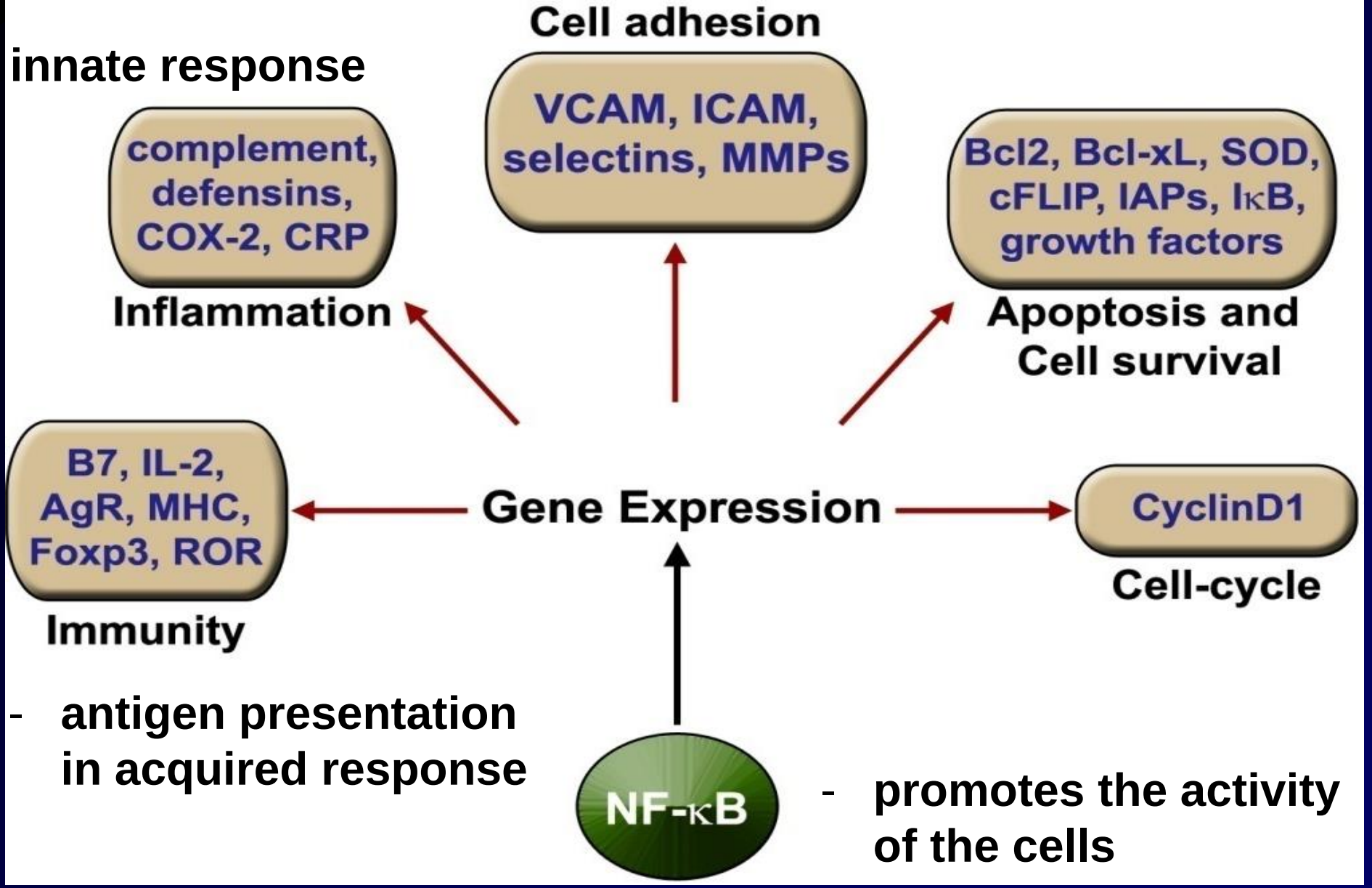
- activated by calcium ions (Ca2+) and diacylglycerol (DAG)
- a multiplicity of functions - induces NF-κB

NF-κB

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



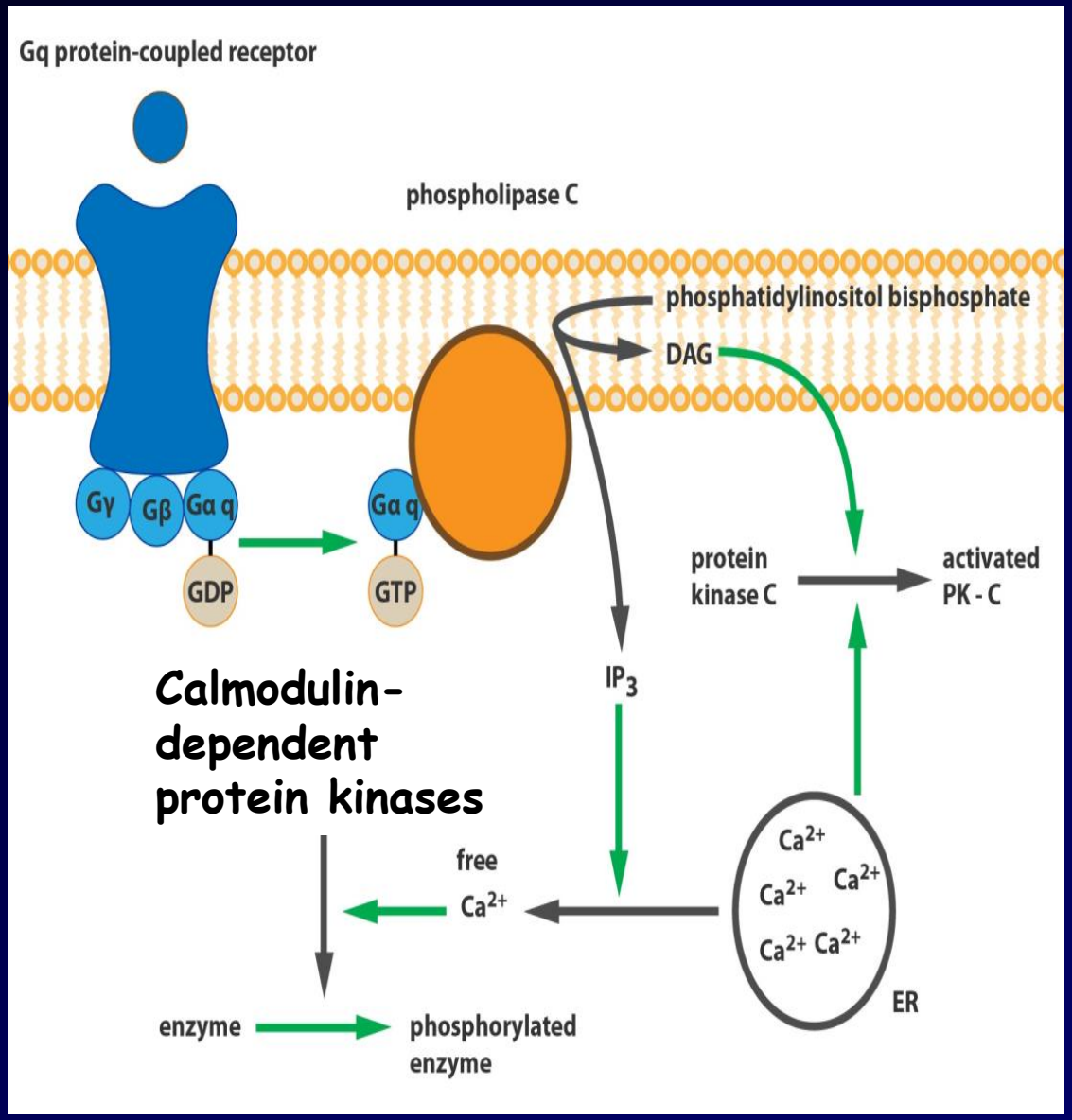
- NF-κB - in cytosol with the inhibitory protein IκB.
- After phosphorylation - IκB dissociates and is degradated by the proteasome.
- The activated NF-κB - translocated into the nucleus - binds to DNA – transcription of genes.



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

Calcium ions

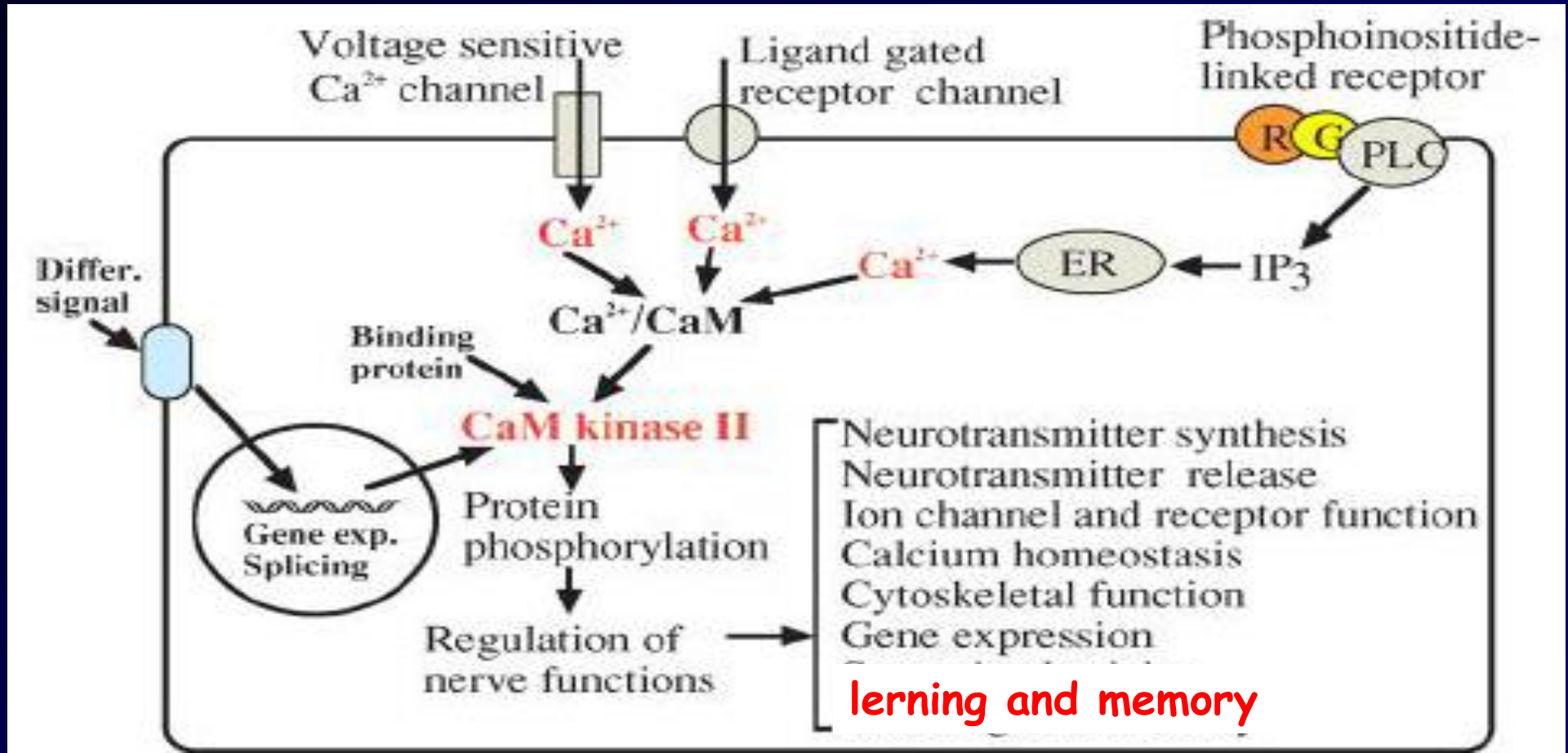
- second messenger (bound by calmodulin - **Calmodulin-dependent protein kinases**



- **phospholipase C** - phosphatidylinositol bisphosphate (**PIP2**) into diacyl glycerol (**DAG**) and inositol trisphosphate (**IP3**).
- **IP3** - calcium ions

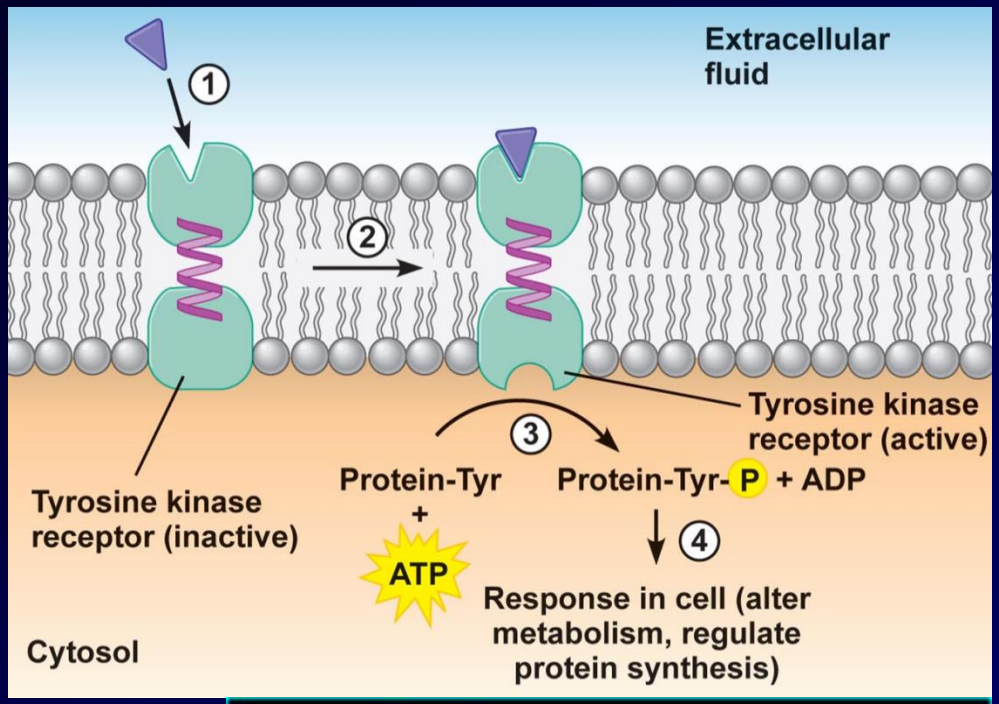
Ca 2+/Calmodulin-dependent protein kinase (CaM-kinases) - serine-threonine kinase

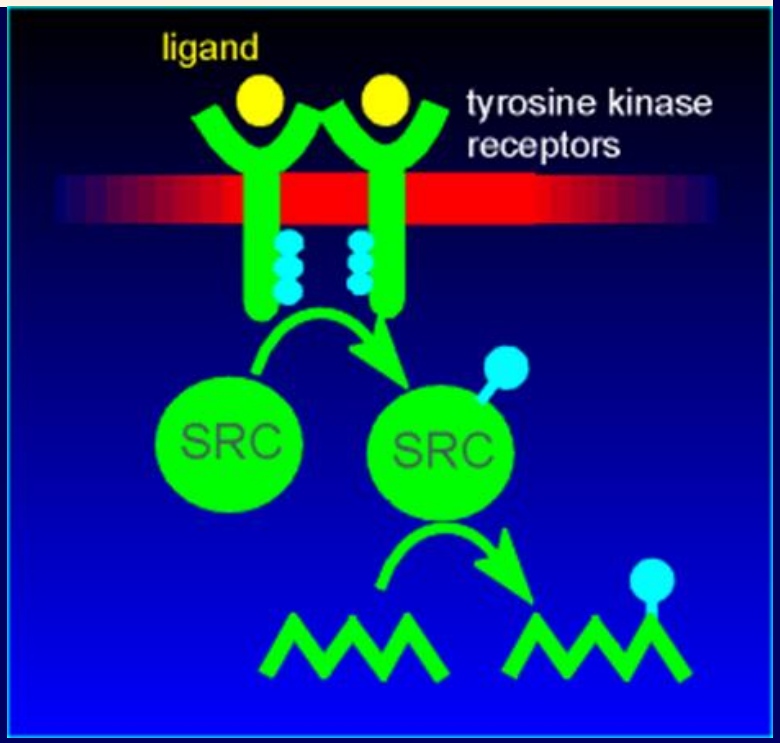
- in nerve cells – neurotransmitter synthesis and release
- learning and memory (dysregulation of CaM-kinases - Alzheimer's disease?)



Enzyme-linked receptors

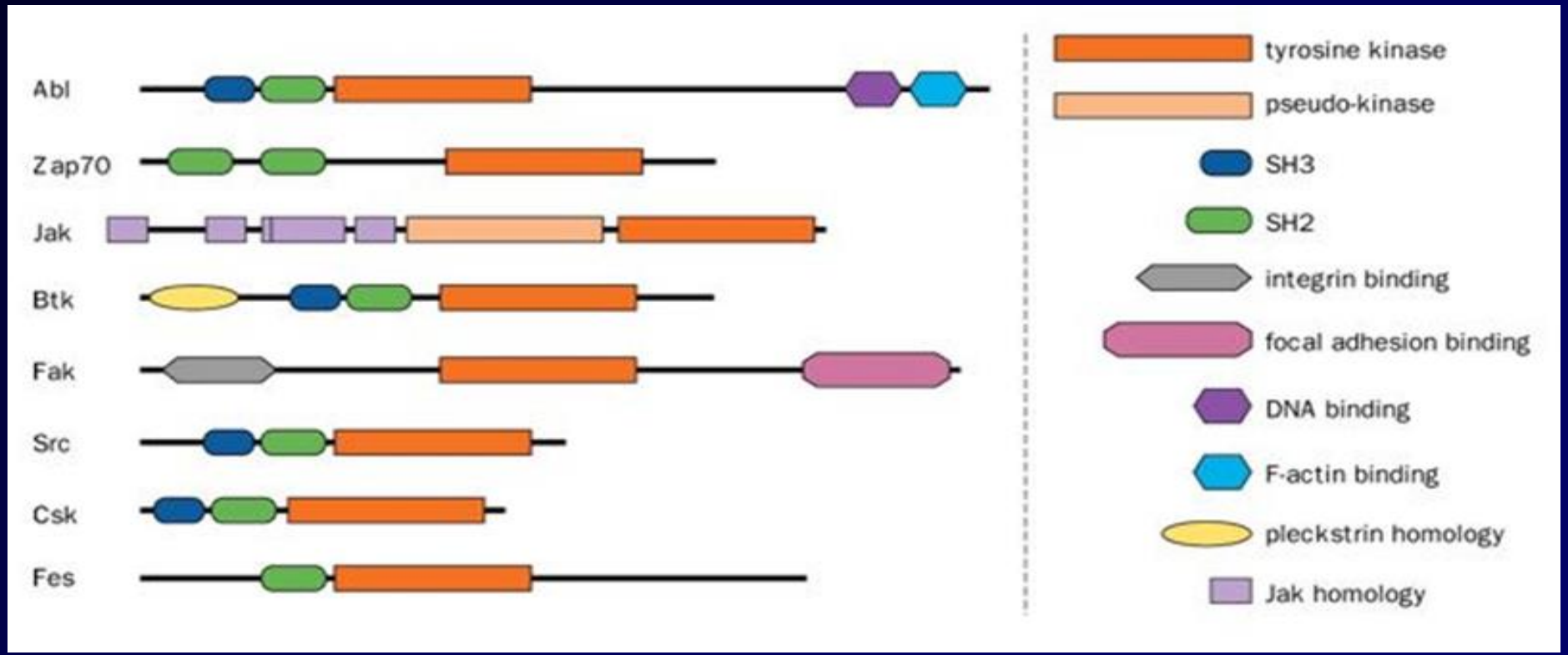
- transmembrane proteins - two domains
- extracellular - ligand-binding domain,
- cytoplasmic domain
    1. has an activity of an enzyme
    2. forms a complex with an enzyme (**tyrosine kinase**)
- **Receptors tyrosine kinase** (growth factors, cytokines, and hormones)
- cell growth, proliferation or differentiation, cancers



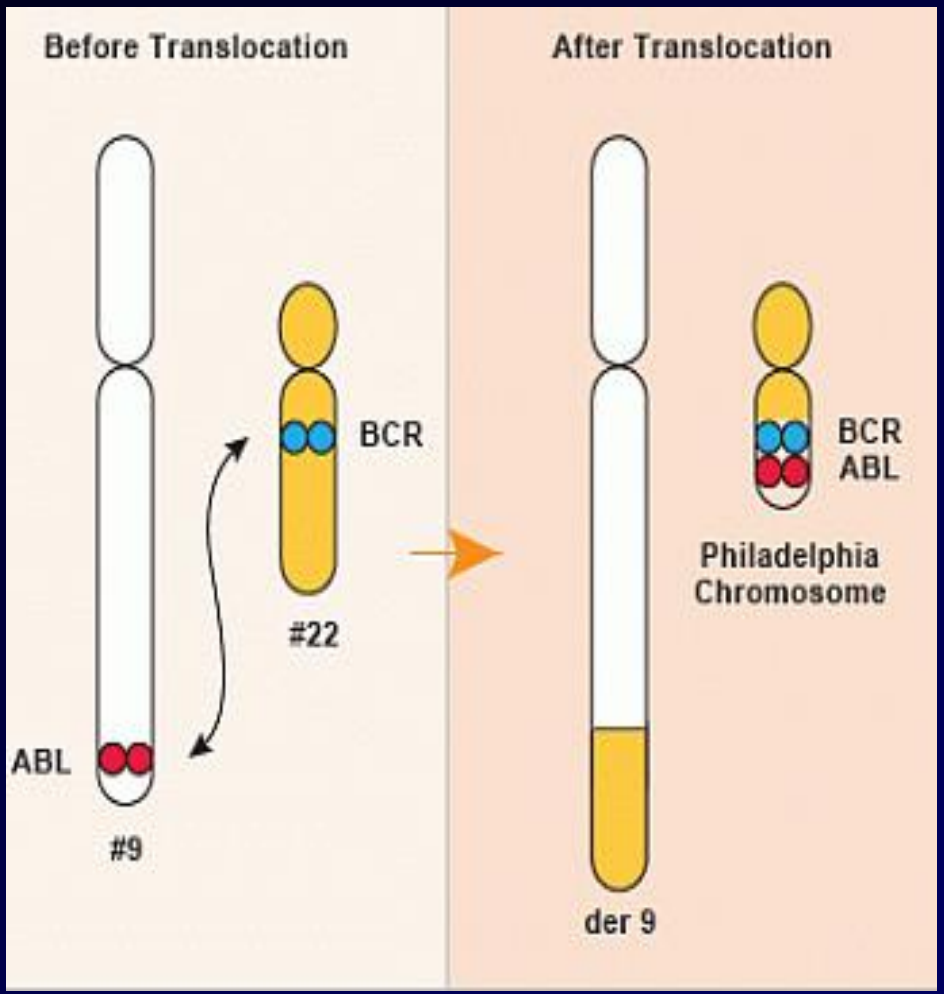


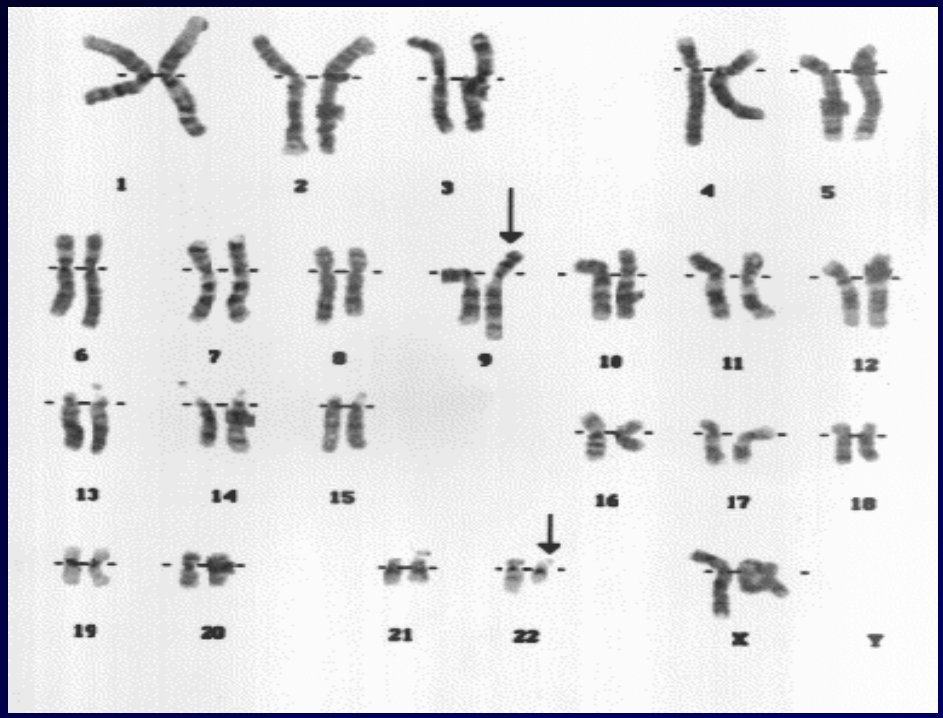
TYROSINE KINASES

- RECEPTOR TYROSINE KINASES
- NON-RECEPTOR (membrane) TYROSINE KINASES
  - SRC (sarcoma)
- CYTOPLASMIC TYROSINE KINASES
  - JAK/TYK (Janus kinase)



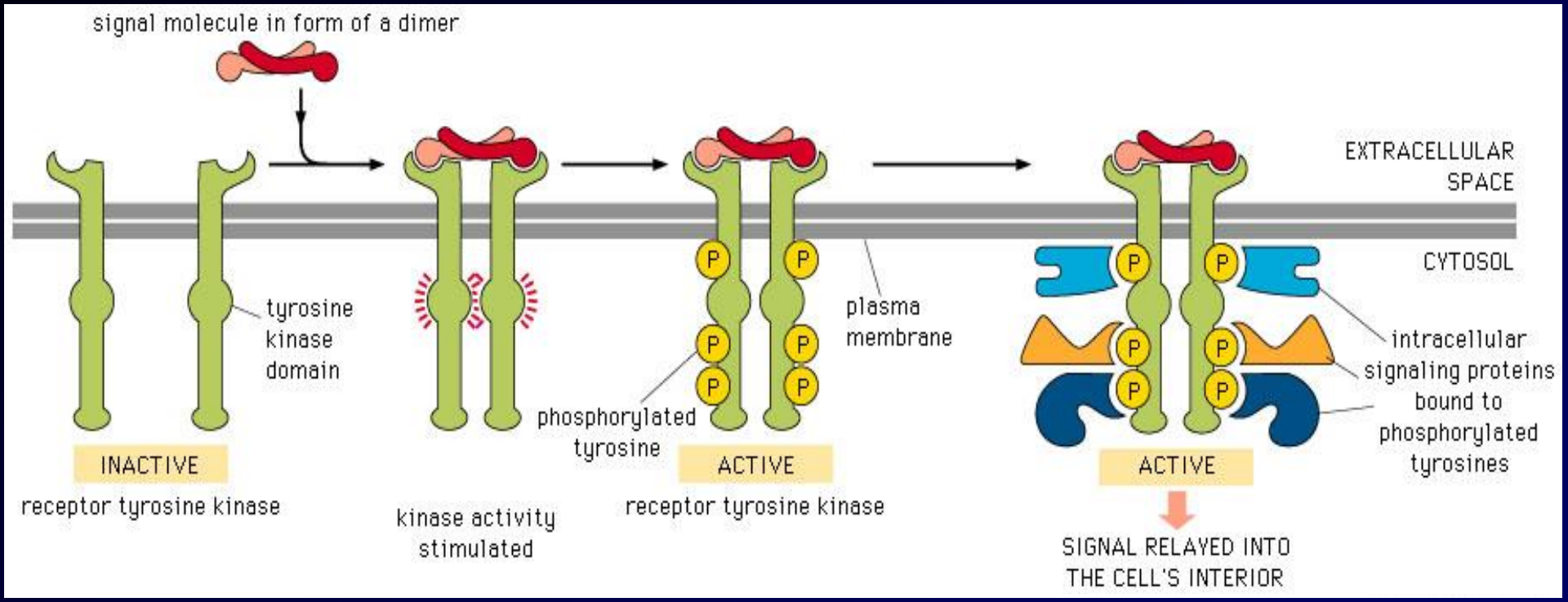
Philadelphia chromosome - a chromosomal abnormality - **chronic leukemia** (acute leukemia).





- Translocation of Abl gene from chromosome 9 to a part of the BCR ("breakpoint cluster region") gene on chromosome 22.
- fusion gene Bcr-abl - fusion protein – **oncogene** unregulated cell division.

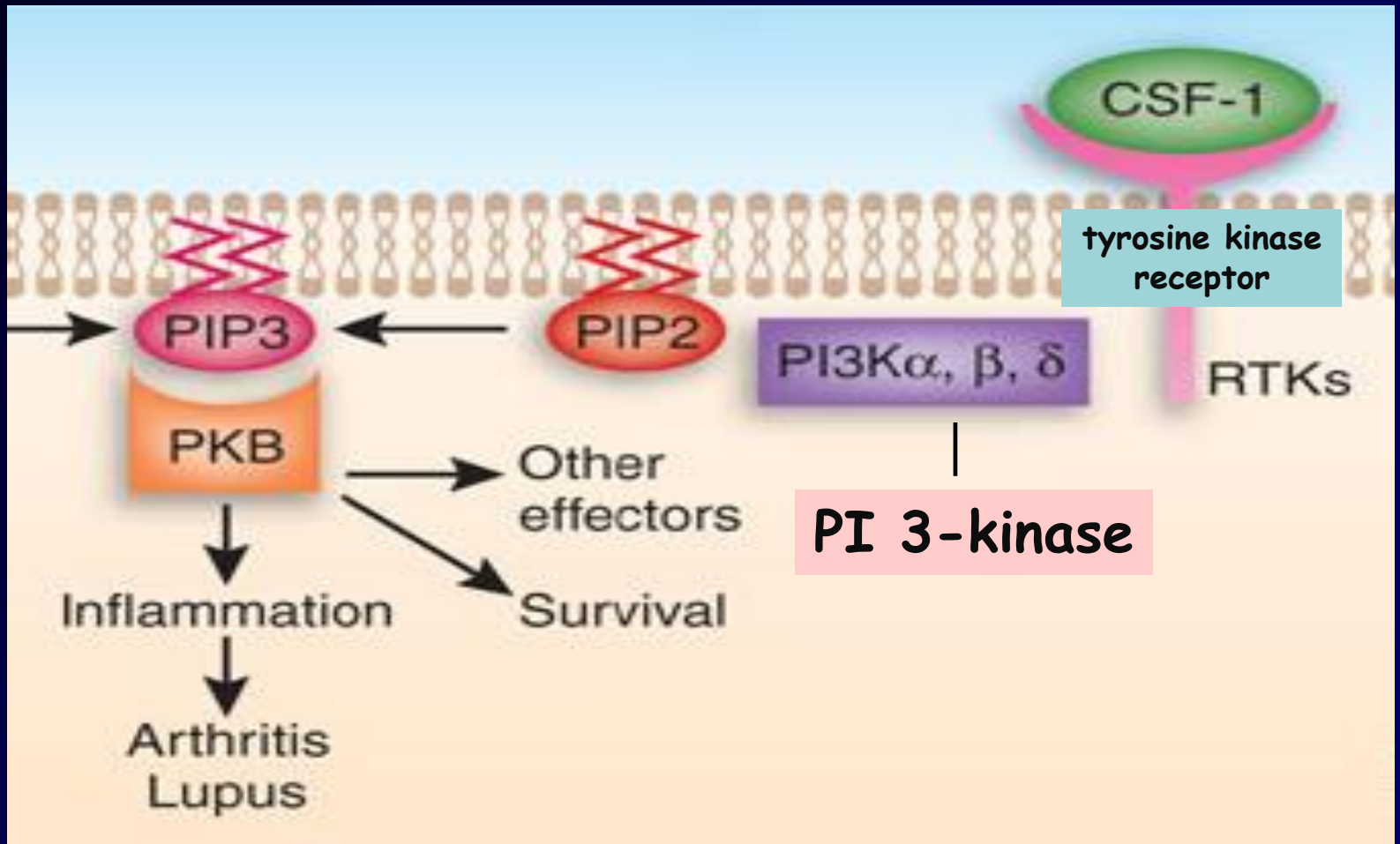
Receptors tyrosine kinase



1. Dimerization of receptors (kinase activity stimulation)
2. Phosphorylation of the tyrosine in the cytoplasmic portion of receptor monomer
3. Binding intracellular signaling proteins
4. Initiation of signal transduction pathways

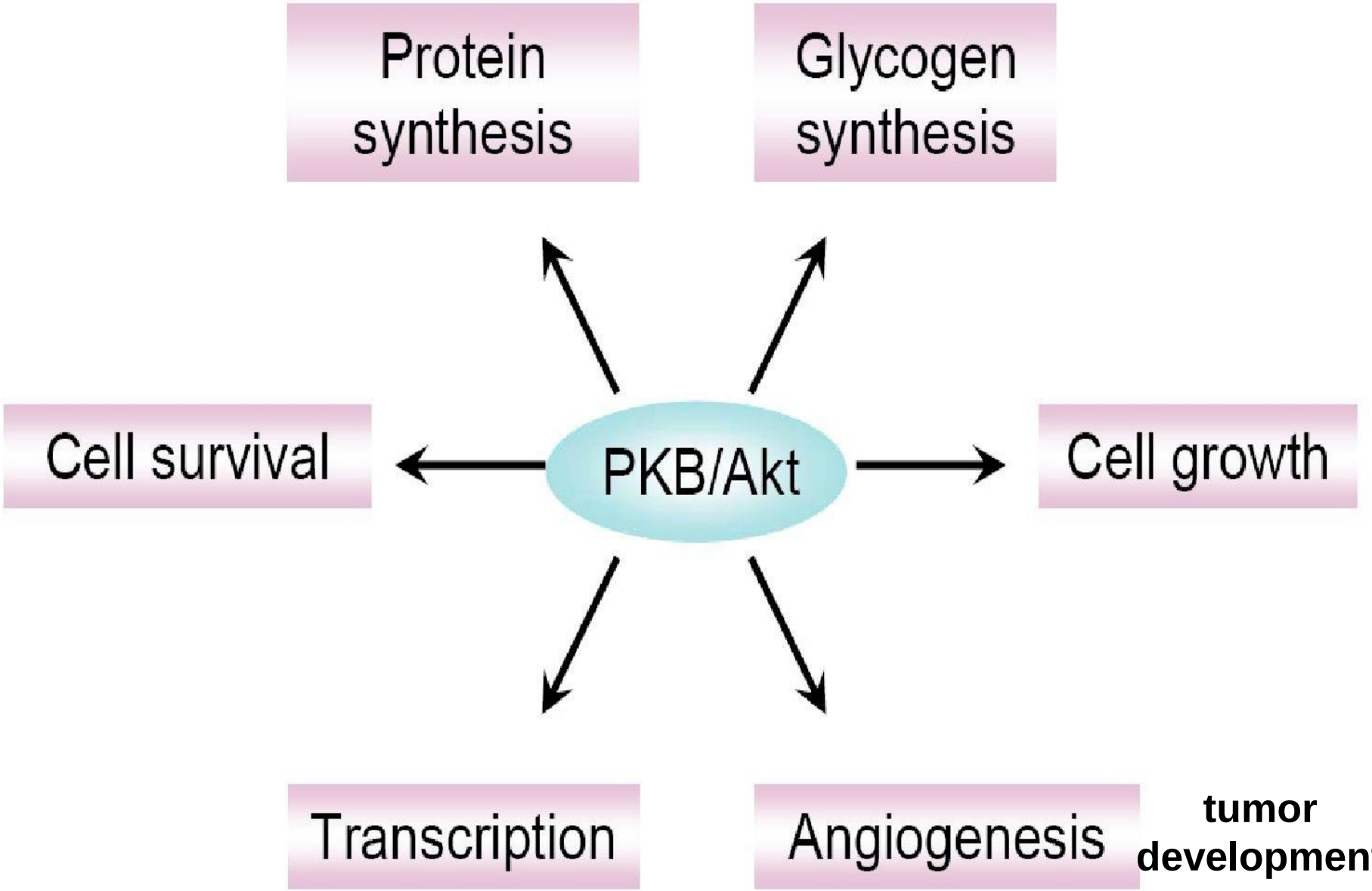
Phosphatidyl-inositol 3-kinase (PI 3-kinase)

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



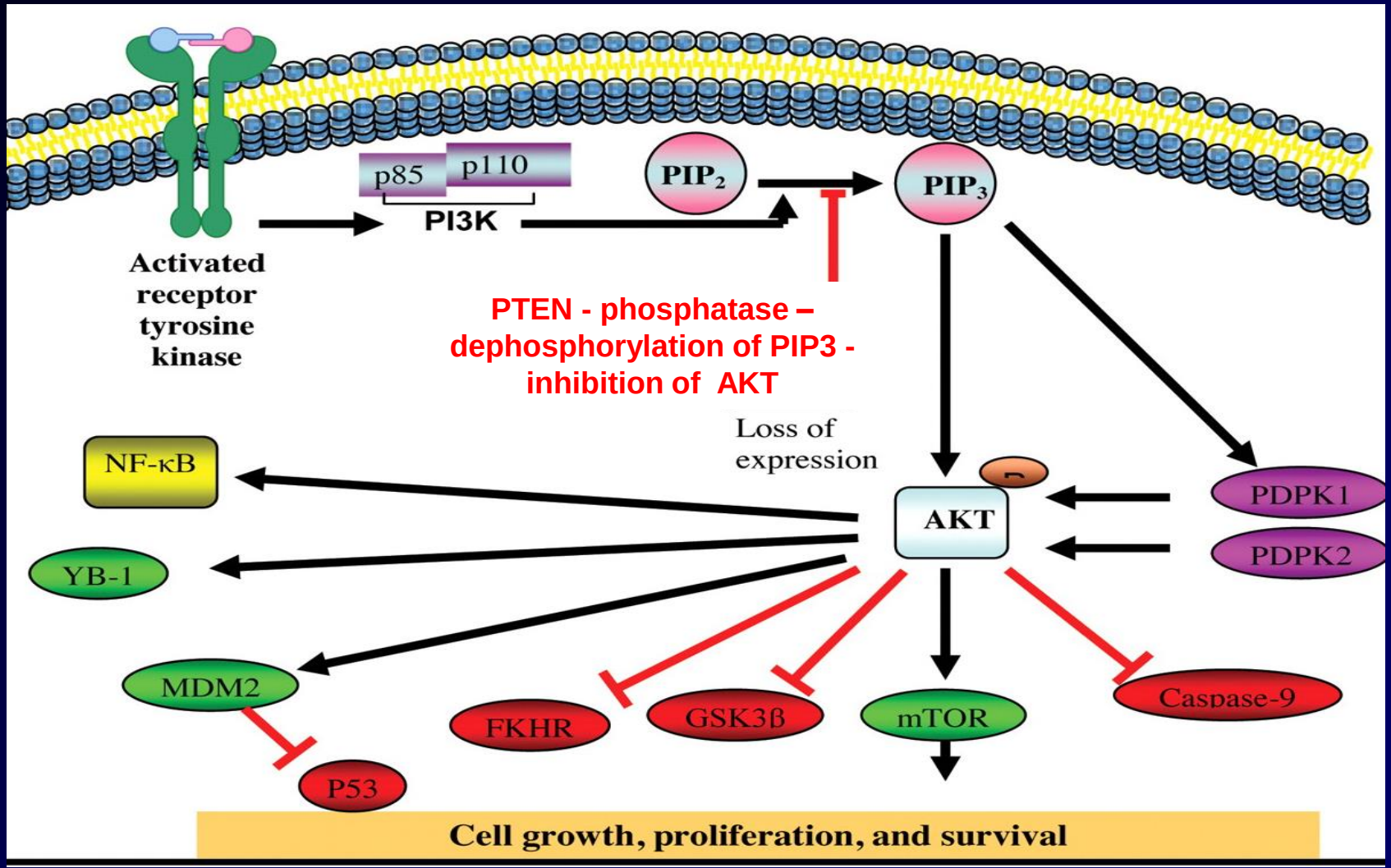
Protein kinase B – kinase Akt

- a serine/threonine-specific protein kinase

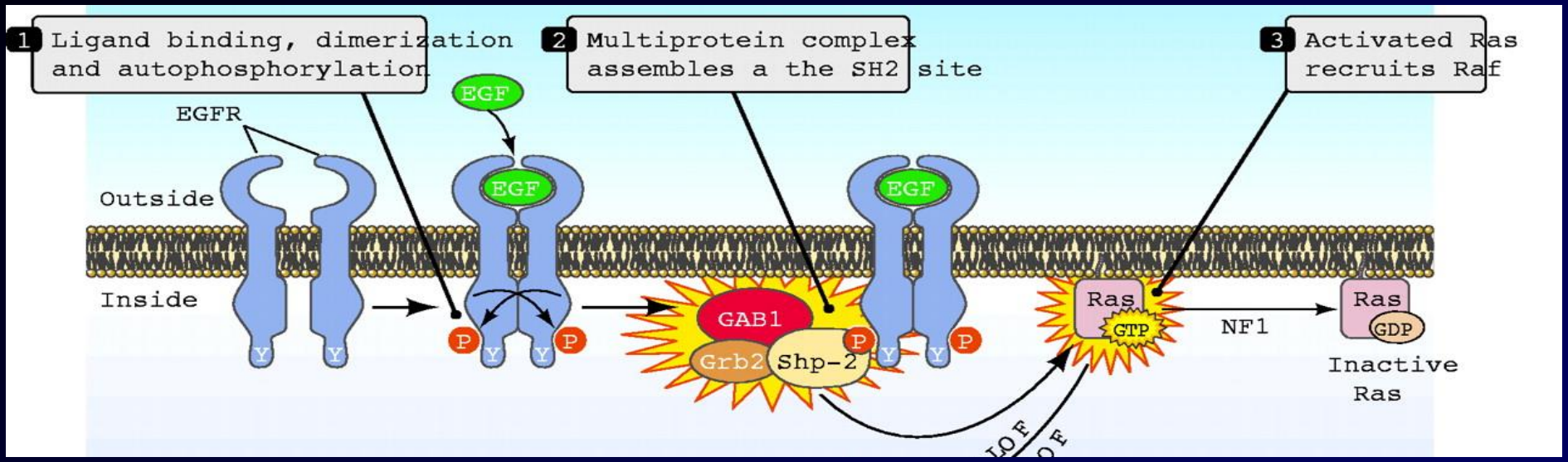


Akt kinase in cancer

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

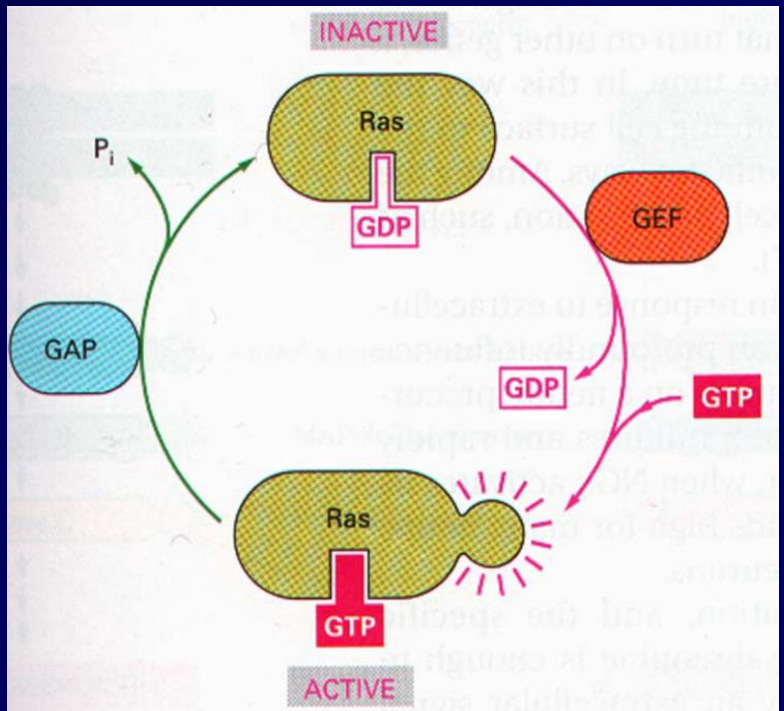


Receptor tyrosine kinase and Ras protein



Ras protein – monomeric GTP-binding protein

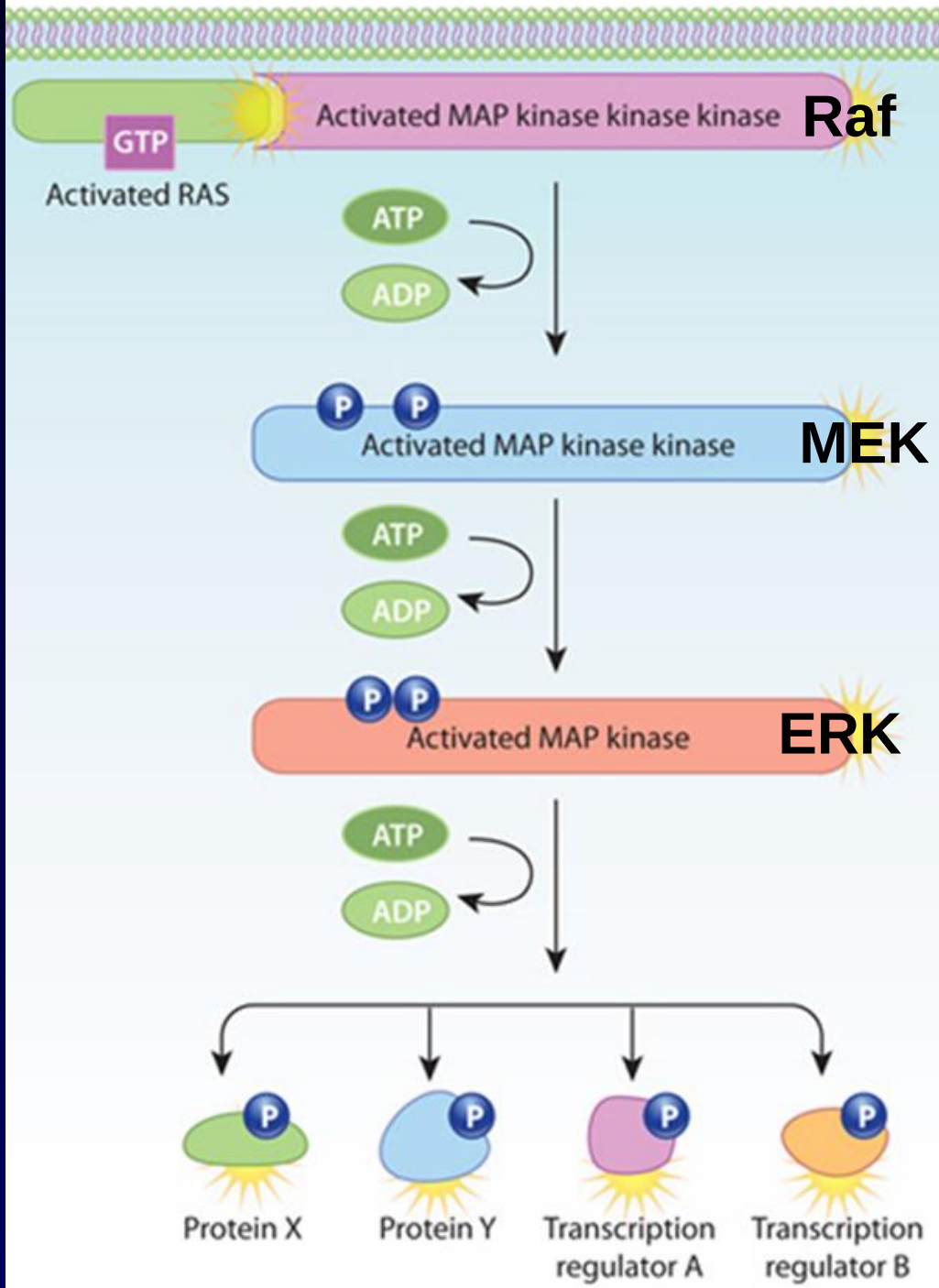
- resembles α subunit of G protein
- inactive form - GDP, active – GTP
- Guanine nucleotide exchange factors (GEF) - exchange of GDP to GTP
- GTPase-Activating Protein (GAP)



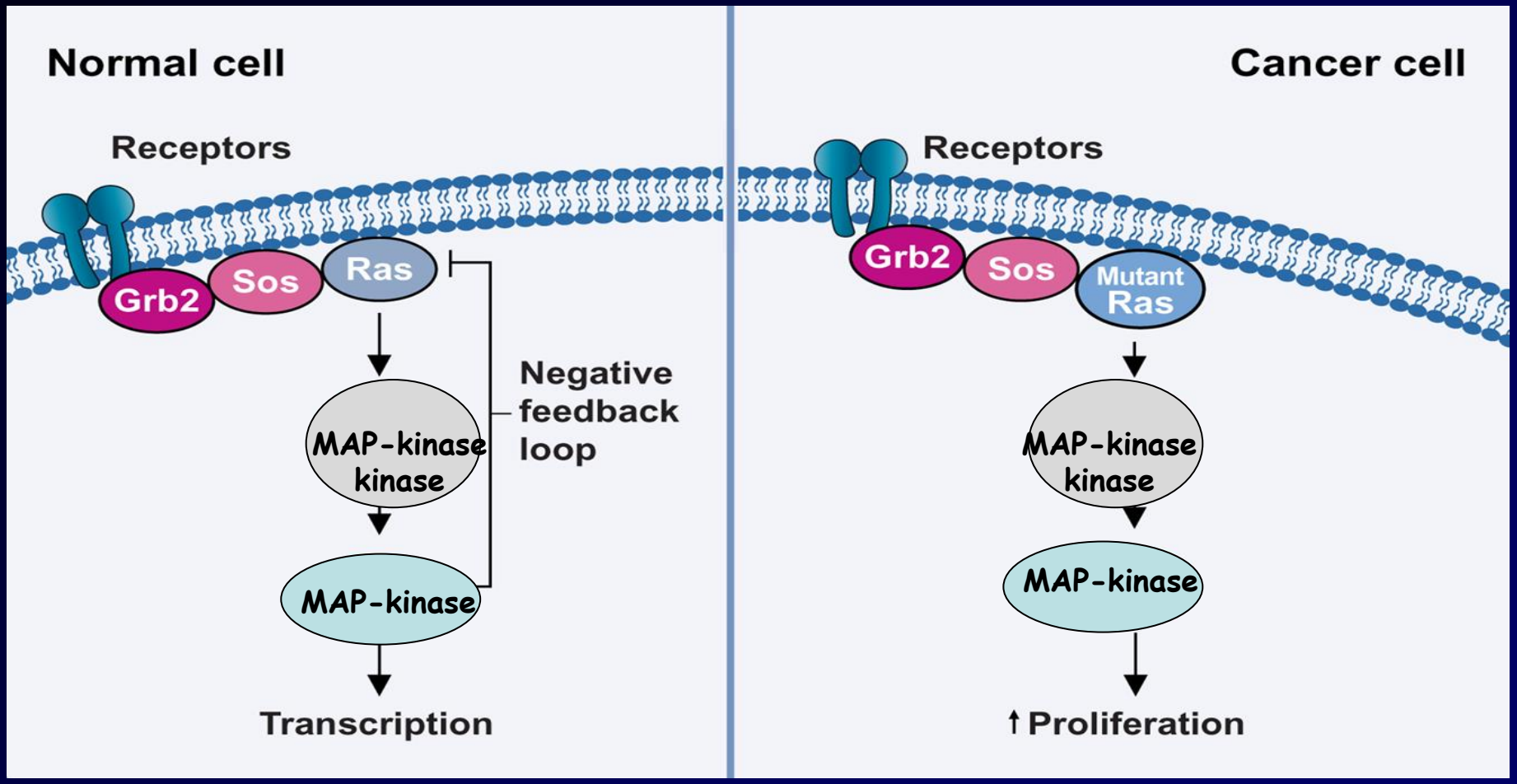
active Ras activates MAP-kinases cascade

MAP-kinase - Mitogen-activated protein kinase

- serine/threonine-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



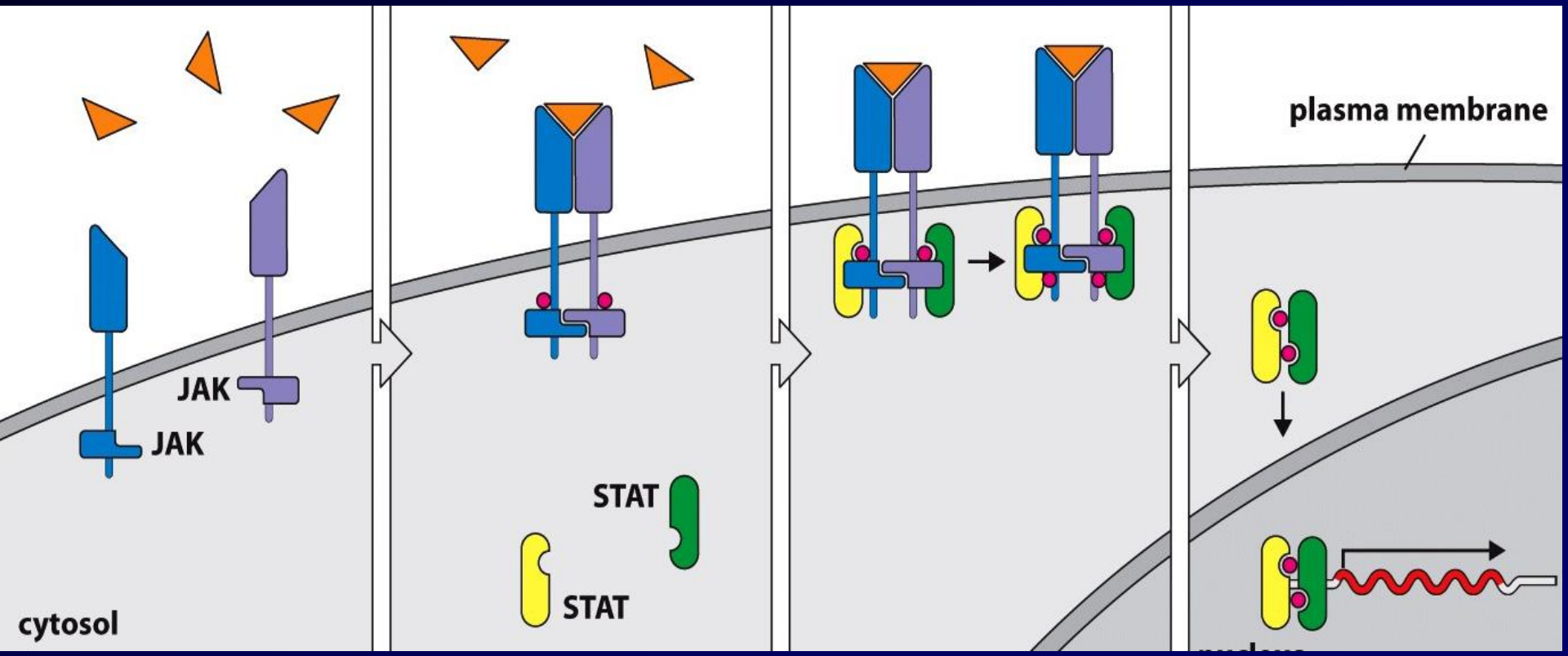
The mutant protein Ras is not inhibited by the negative feedback mechanism - cancer

Mutations that permanently active Ras are found in 20% to 90% of all human tumors

Tumor type	Incidence of ras mutations
Pancreatic Adenocarcinoma	90%
Colon Adenoma	50%
Colon Adenocarcinoma	50%
Seminoma	40%
Lung Adenocarcinoma	30%
Myelodisplatic Syndrome	30%
Acute Myelogenous leukemia	30%
Keratinoacanthoma	30%
Thyroid carcinoma	25%
Melanomas	20%
Bladder carcinoma	6%

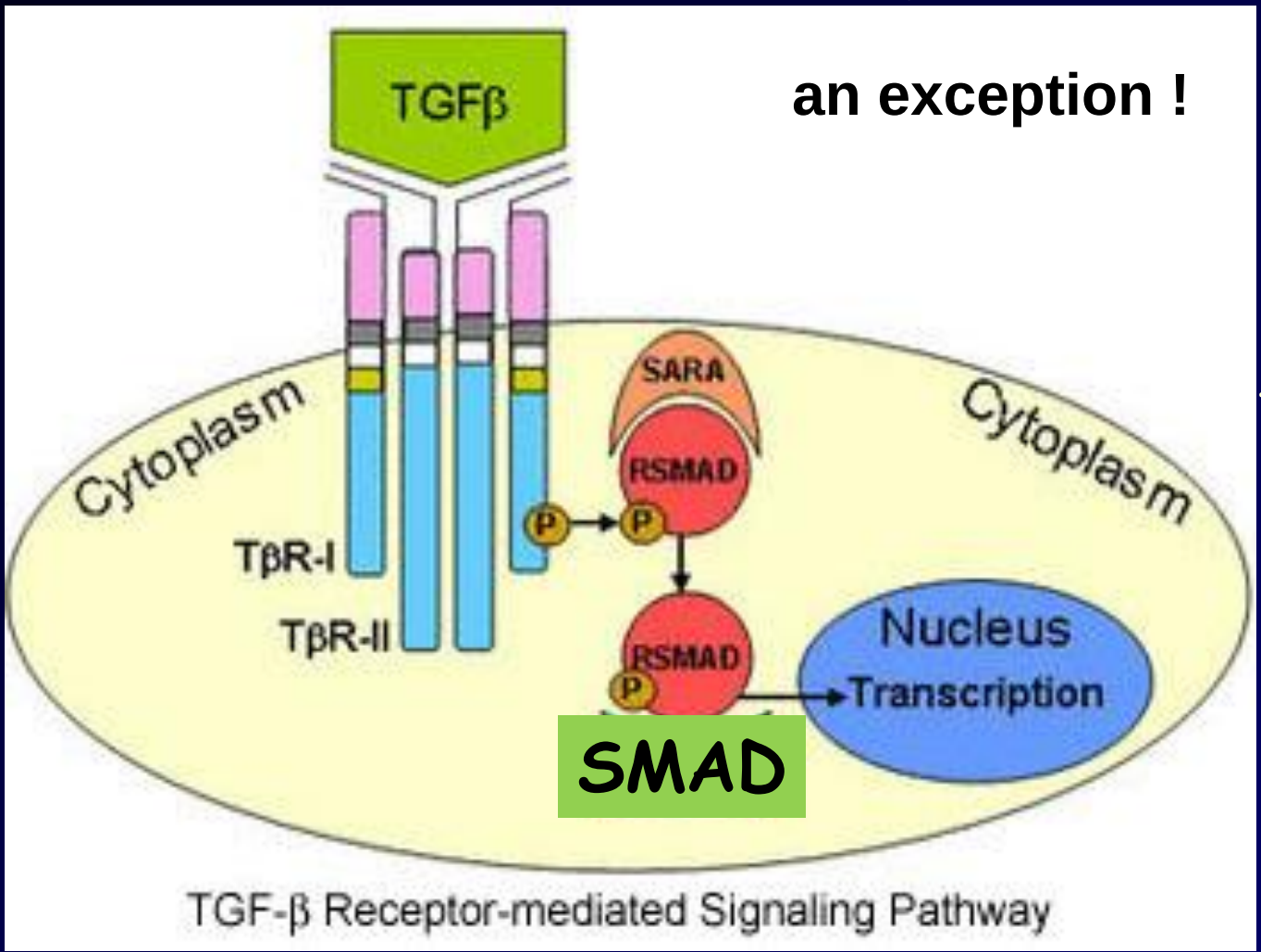
Cytokine receptors - have no intrinsic enzyme activity

- 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)



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

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

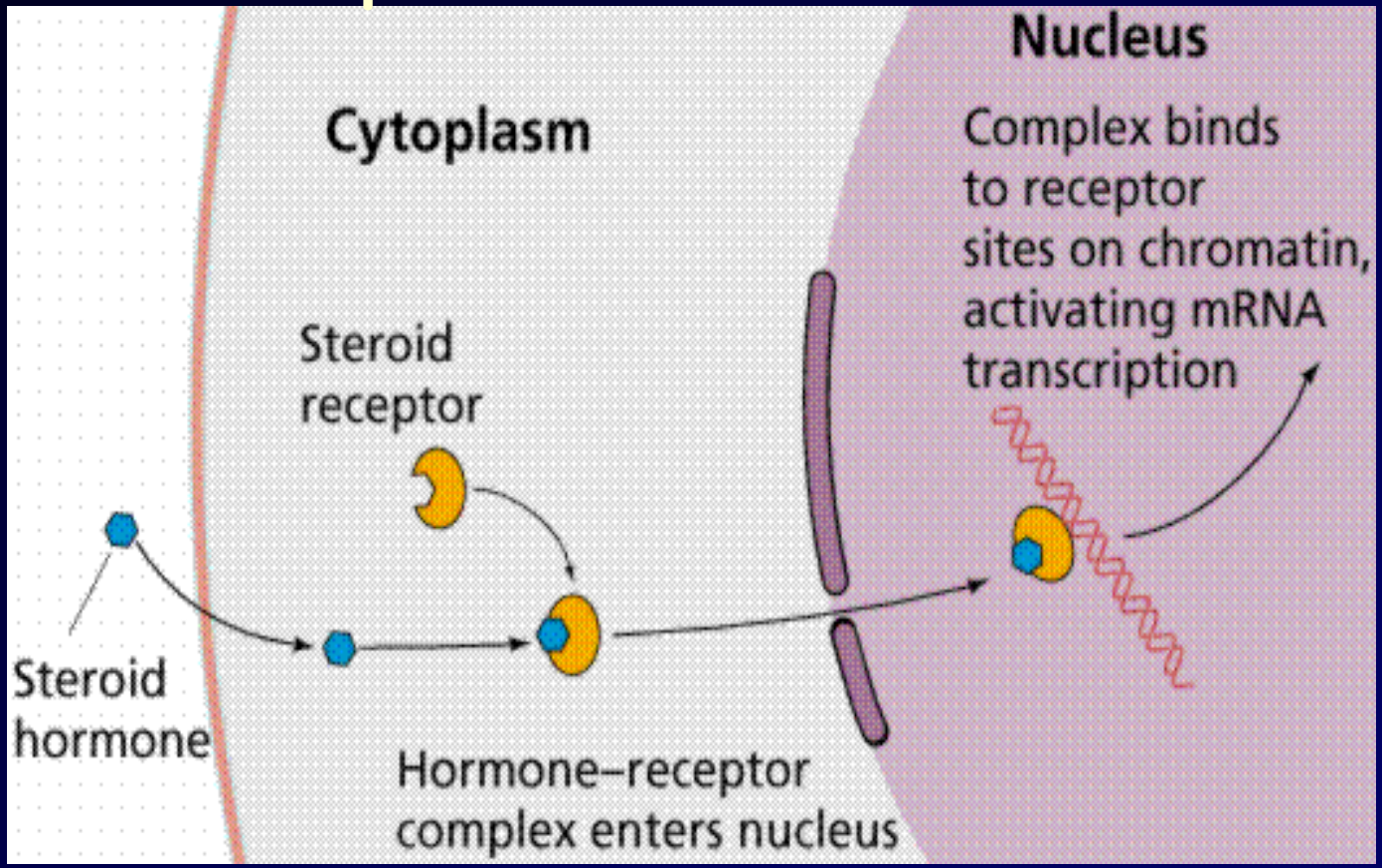


TGF-β Receptor-mediated Signaling Pathway

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

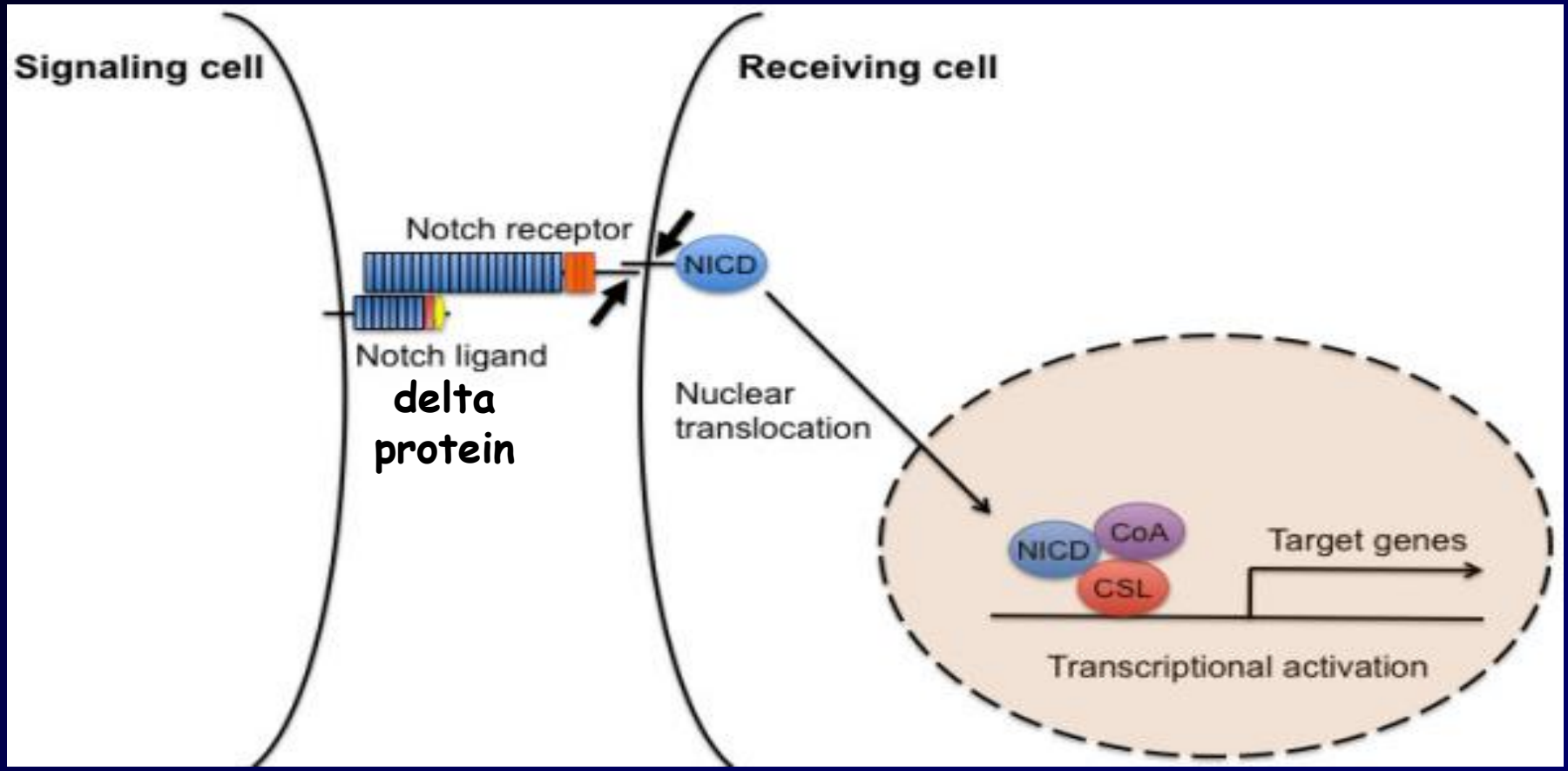
NUCLEAR RECEPTORS

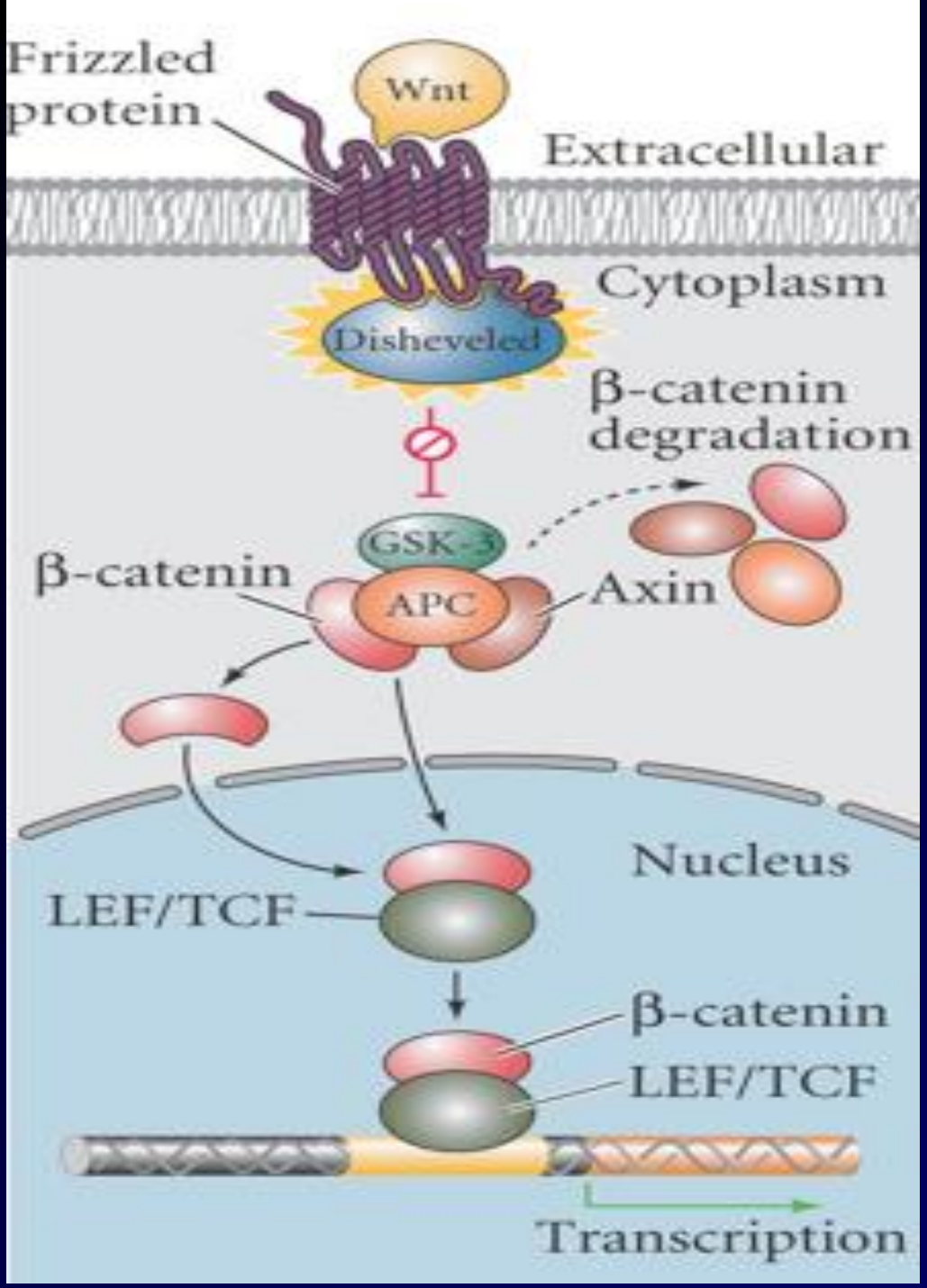
- Ligands - lipophilic substances – steroid hormones (androgens, estrogens, glucocorticoids, progesterone), thyroid hormones, vitamins A and D, and eicosanoids
- bind to DNA - regulate the expression of genes - **transcription factors**



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

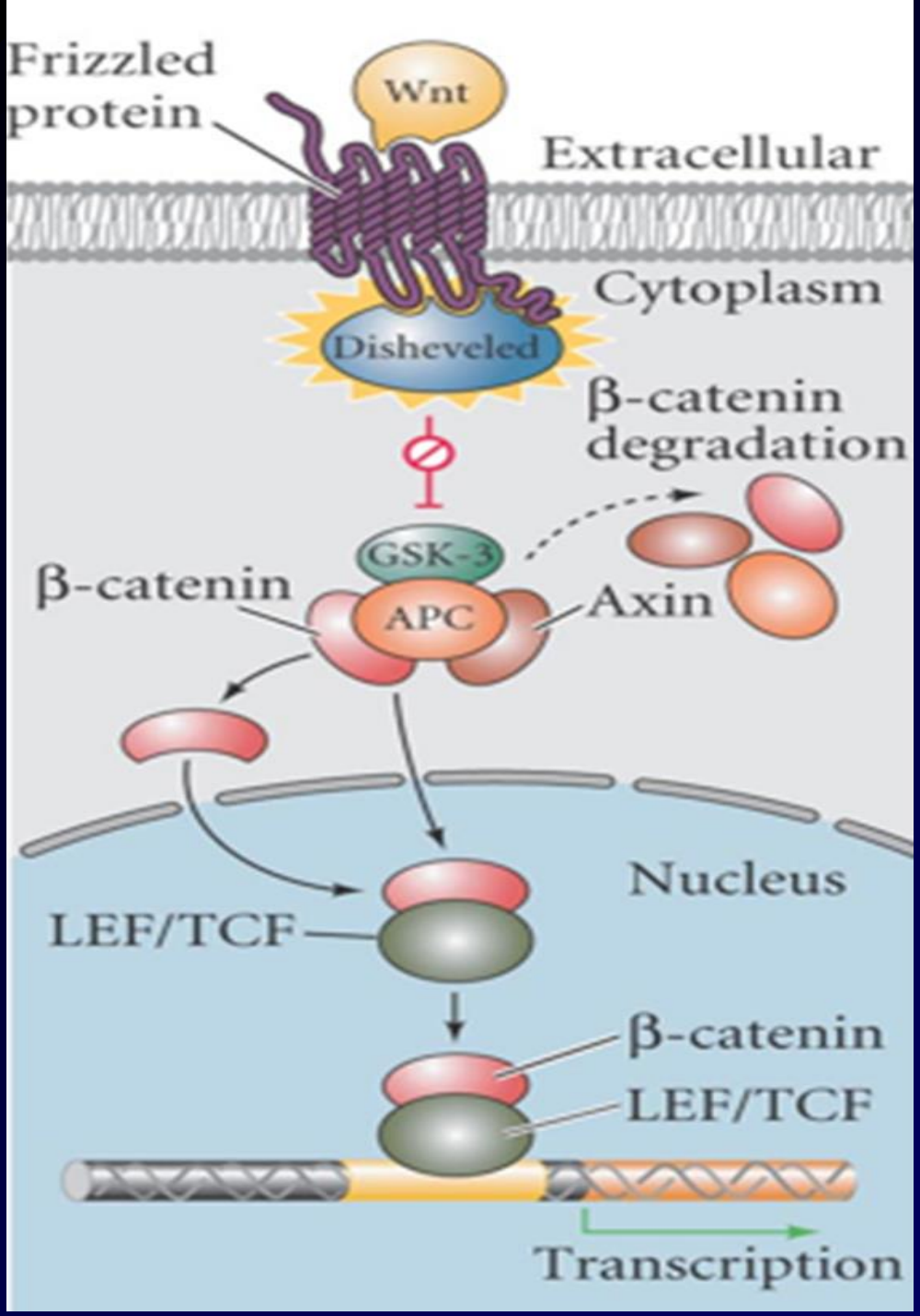
- cleaved Notch intracellular domain migrates to the nucleus – transcription factor
- **neurogenesis**, embryo polarity (anterior-posterior polarity and left-right asymmetry)





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

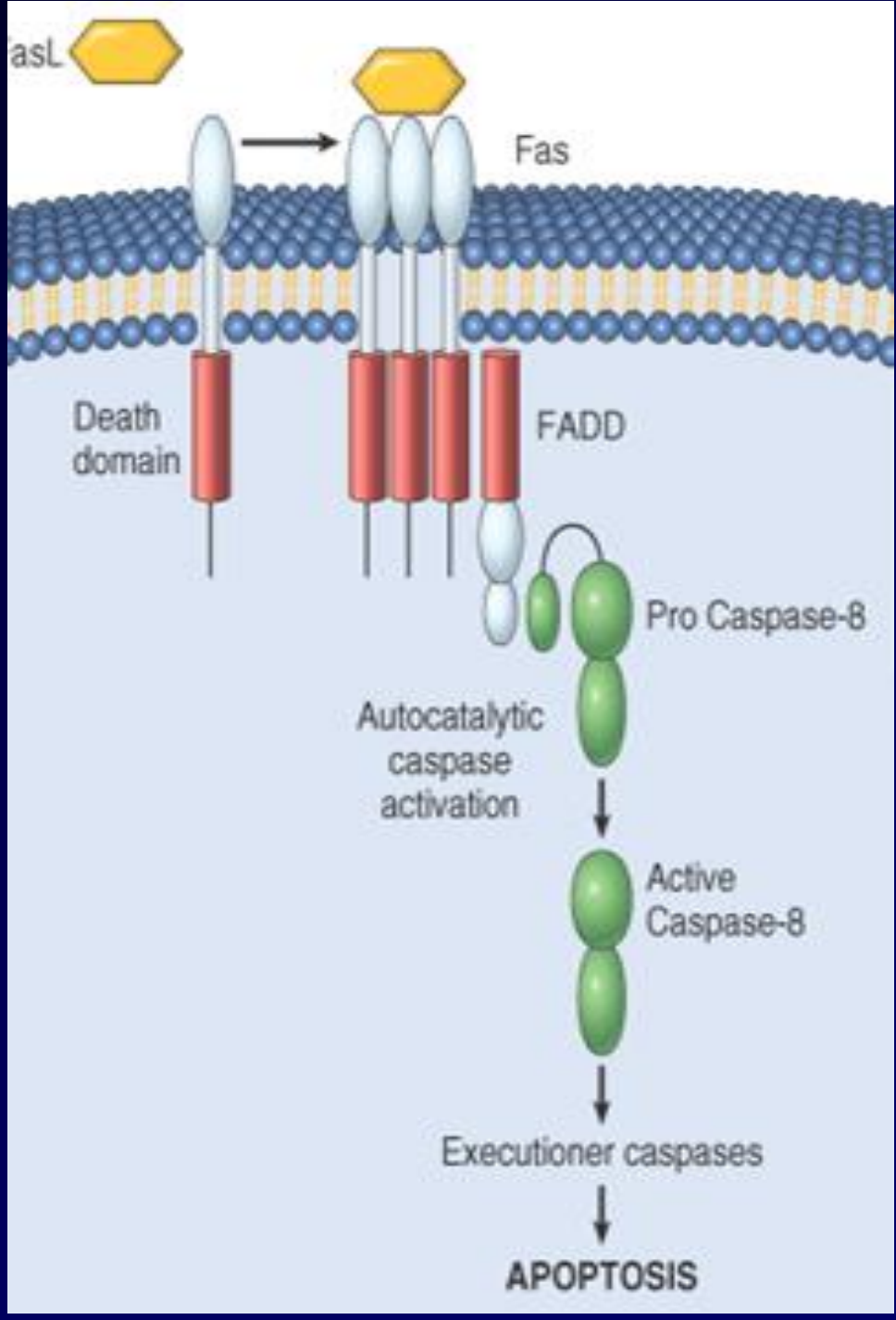


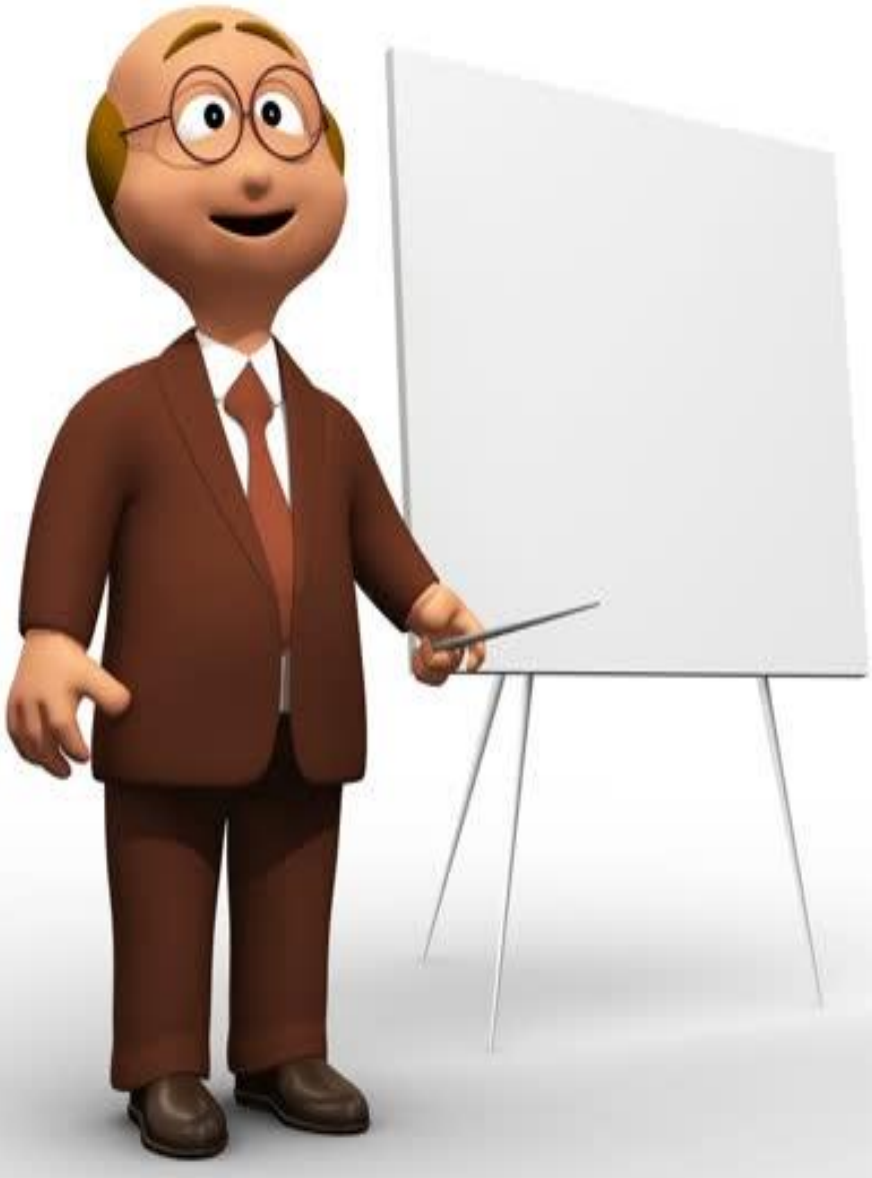
Wnt signaling pathway

- 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)

Proapoptotic receptors - Death Receptors

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





Thank you for your attention

1. Antagonist vs agonist - mechanism of action - therapeutic application
2. Types of intercellular communication
3. Kinds of receptors - location, exerted effect
4. Types of ionotropic receptors
5. Types of kinases and phosphatases and their activity
6. Kinds of metabotropic receptors
7. G-protein-linked receptors - structure and their activation
8. Enzymes activated by G proteins
9. Kinds of second messengers - function of phosphodiesterase
10. Adenylyl cyclase - cAMP - PKA – cyclic-AMP-dependent protein kinase pathway
11. Activation and function transcription factor CREB
12. Function of phospholipase C
13. Activation and function of protein kinase C (PKC)
14. Activation and function of transcription factor NF-κB
15. Activation and function of Ca 2+/Calmodulin-dependent protein kinase (CaM-kinases)
16. Enzyme-linked receptors - receptors tyrosine kinase
17. Types of tyrosine kinases
19. Mechanism of activation of receptors with the activity of tyrosine kinase.
20. Protein kinase B – kinase Akt - activation and function
21. Ras protein - activation and function - MAP-kinase - Mitogen-activated protein kinase
22. Nuclear receptors
23. Notch signaling pathway - juxtacrine signaling (contact-dependent)