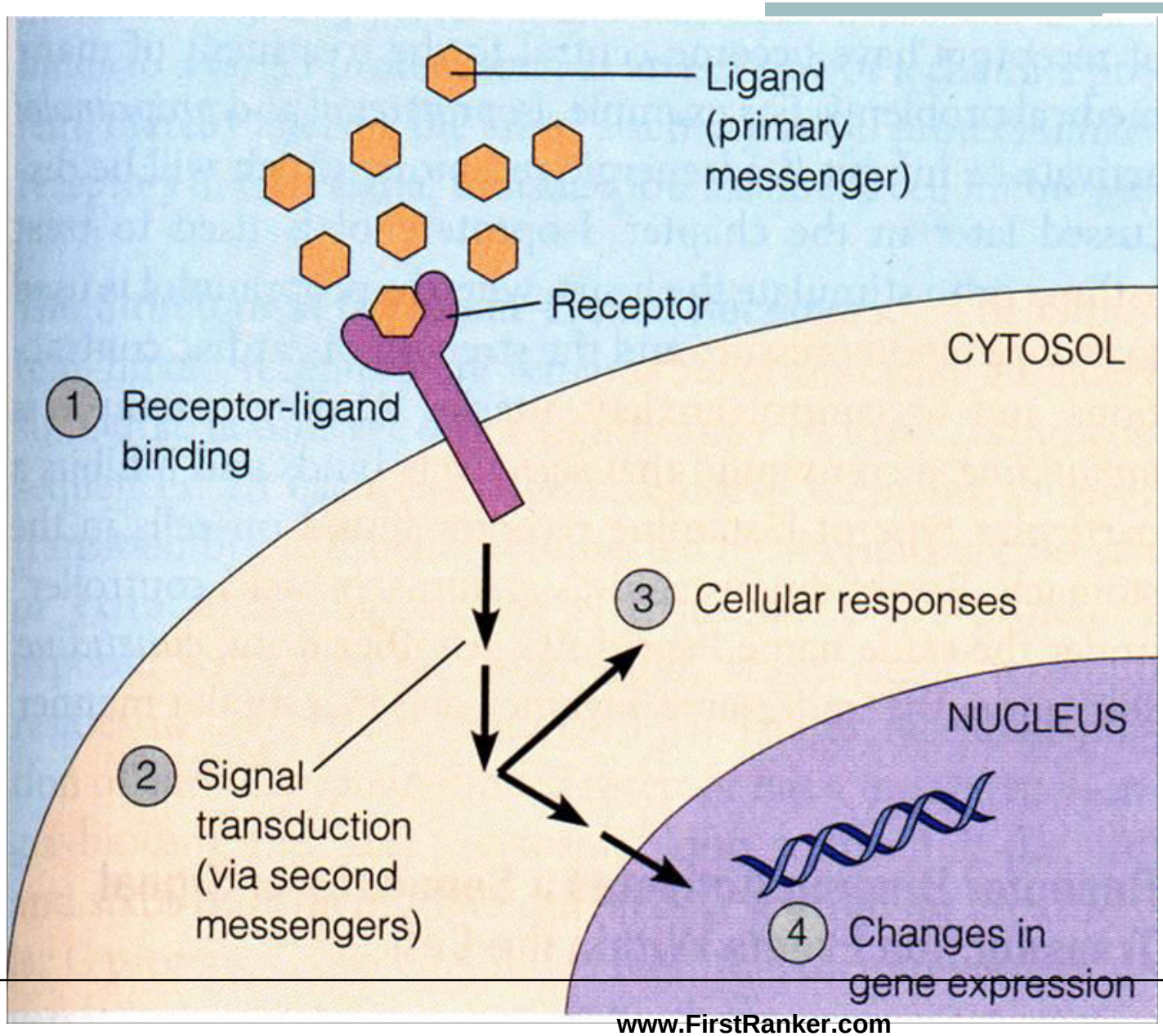
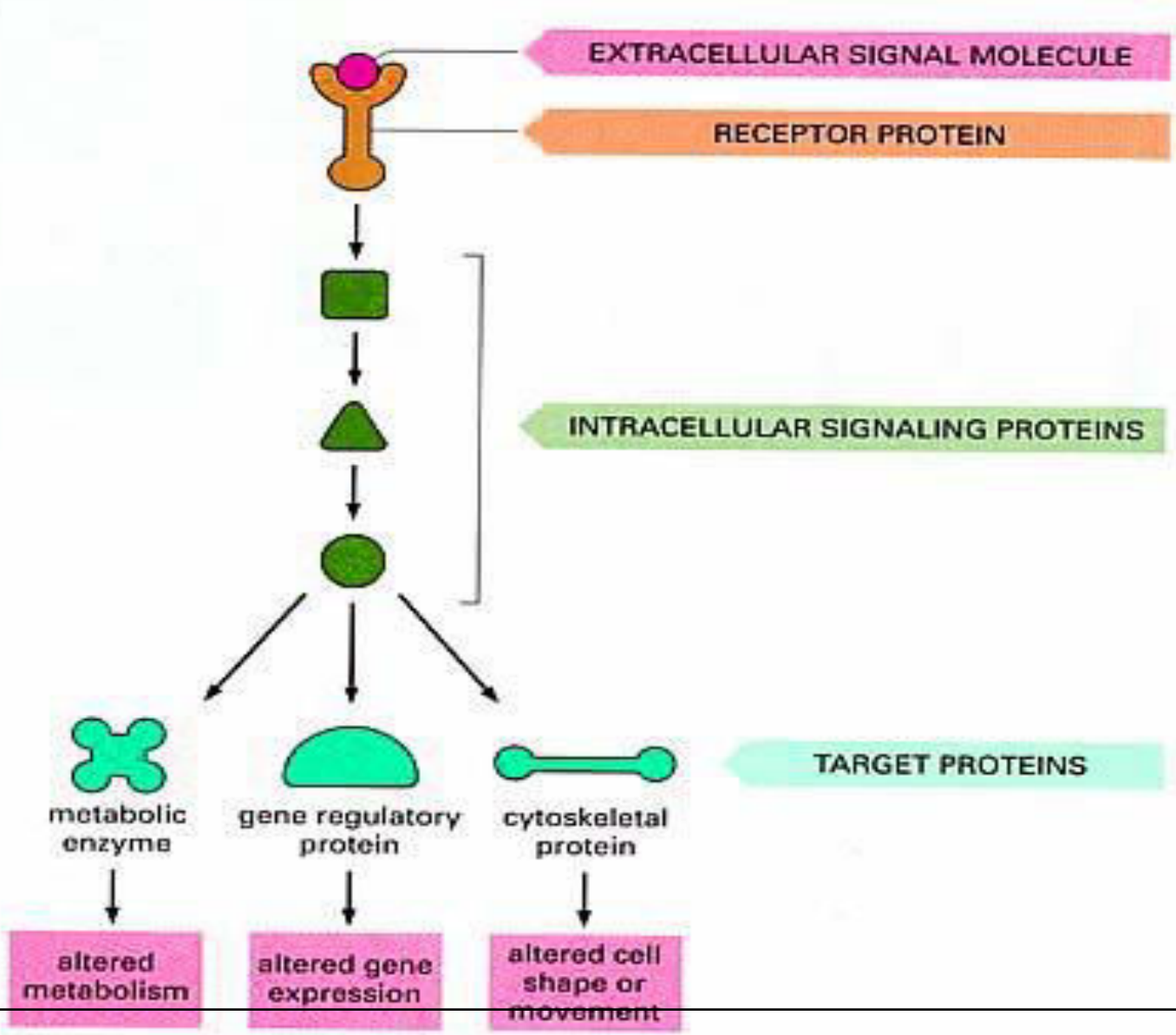
SECOND MESSANGERS

BIOCHEMISTRY

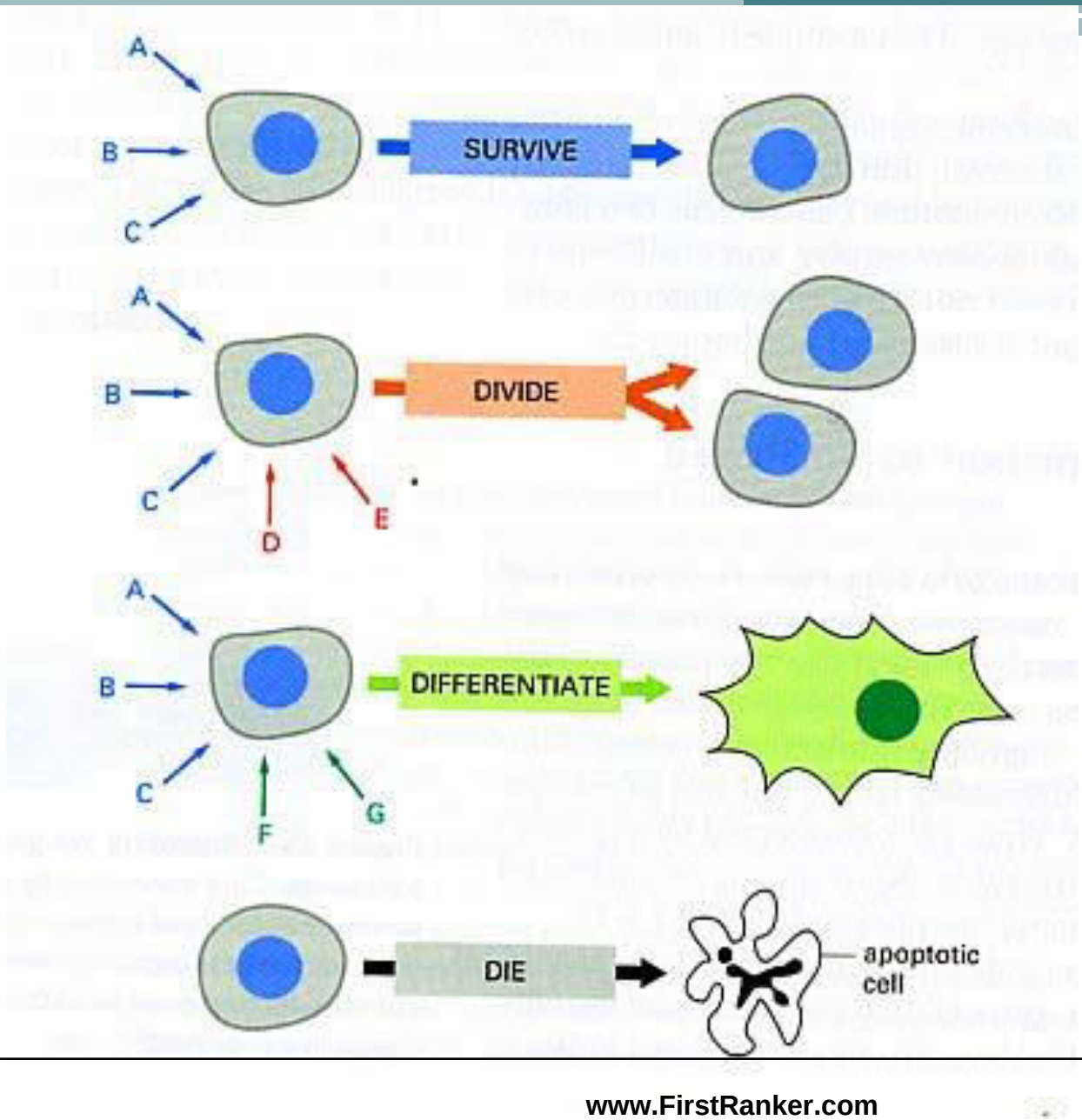
Basic Characteristics Of Cell Signaling

- **Cell must respond appropriately to external stimuli to survive.**
- **Cells respond to stimuli via cell signaling**

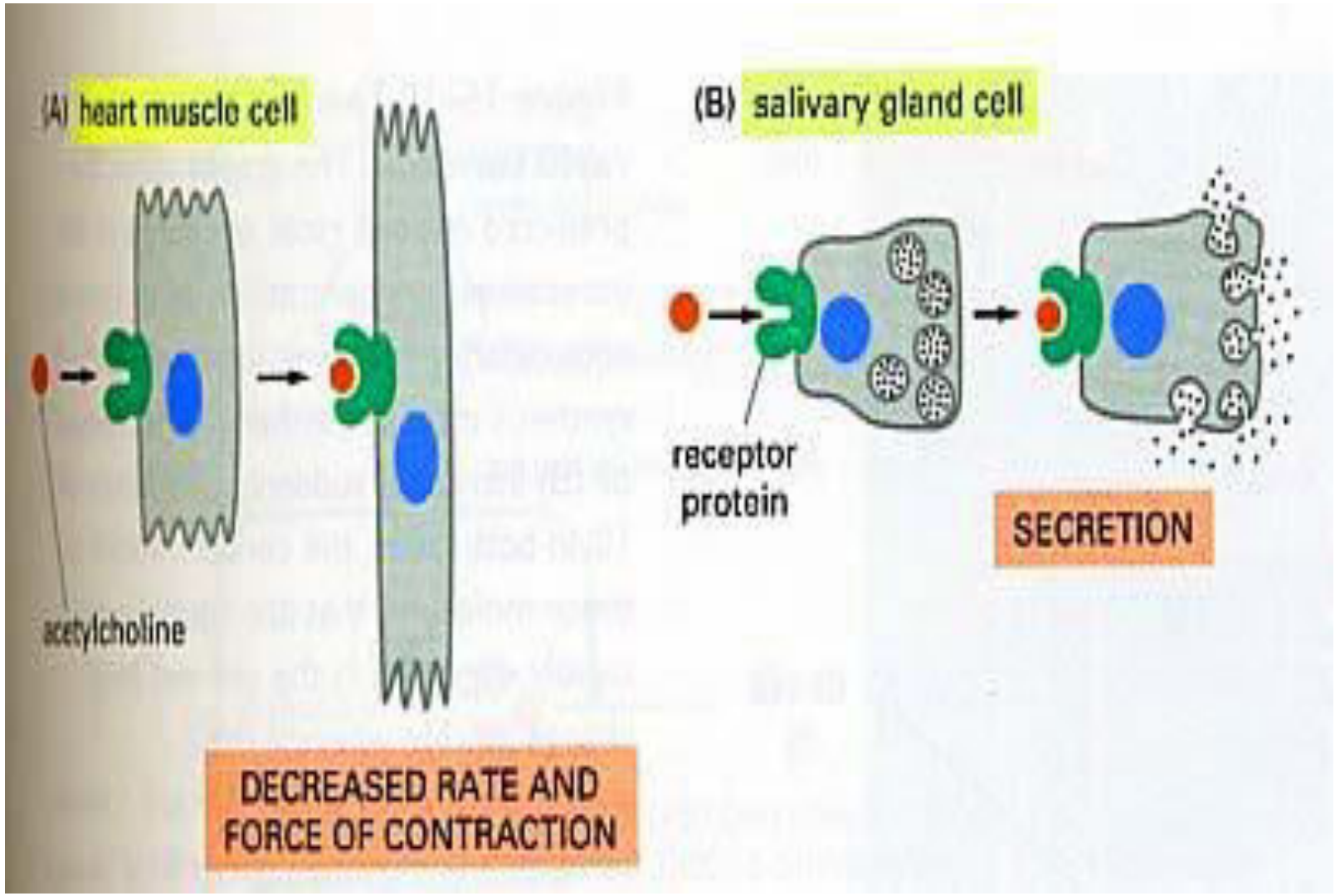




Each cell is programmed to respond to specific combinations of extracellular signal molecules

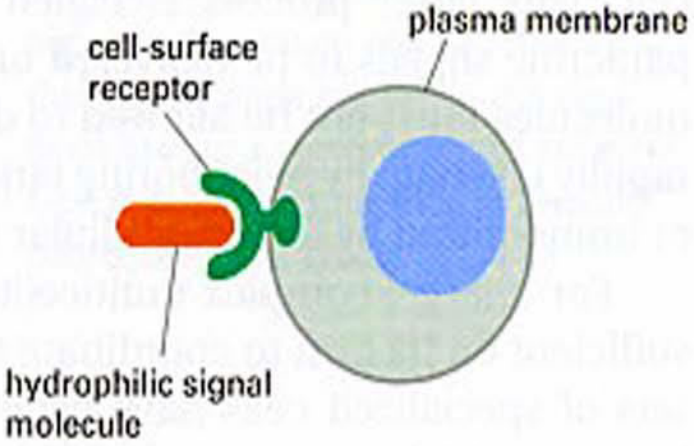

A
B
C
SURVIVE
A
B
C
D
E
DIVIDE
A
B
C
F
G
DIFFERENTIATE
DIE
apoptotic cell

Different cells can respond differently to the same extracellular signal molecule

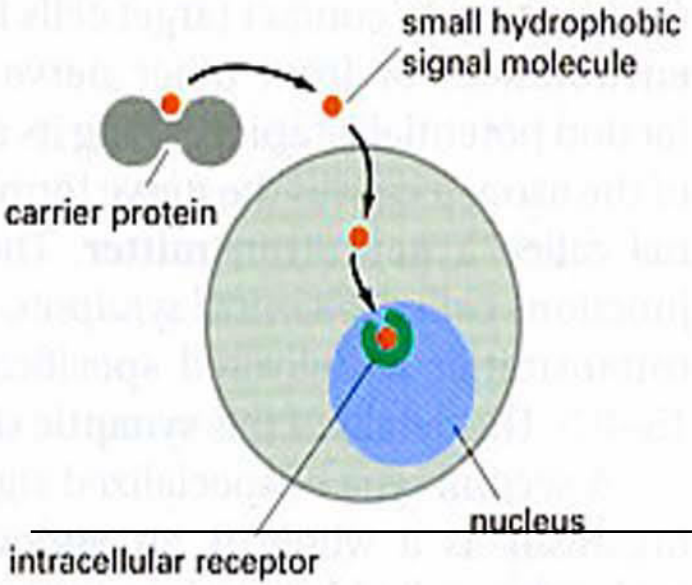

(A) heart muscle cell
acetylcholine
DECREASED RATE AND FORCE OF CONTRACTION
(B) salivary gland cell
receptor protein
SECRETION

Signal Molecules and Receptors

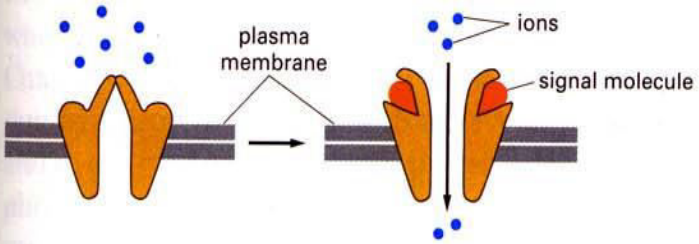
CELL-SURFACE RECEPTORS



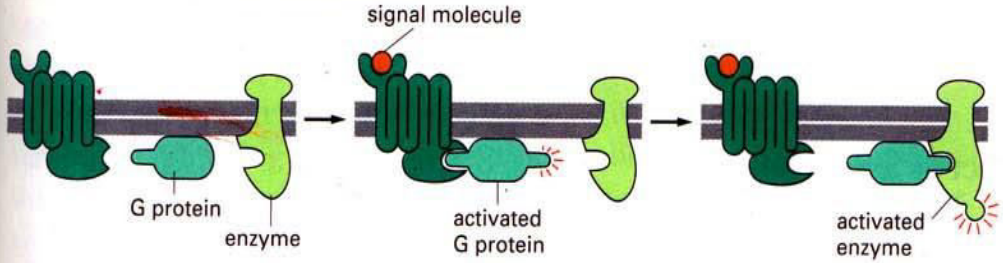
INTRACELLULAR RECEPTORS



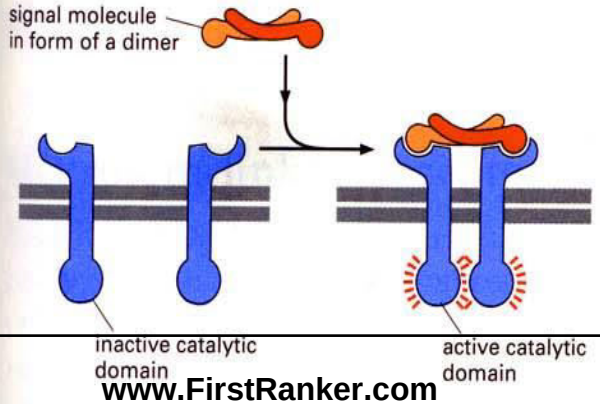
(A) ION-CHANNEL-LINKED RECEPTOR



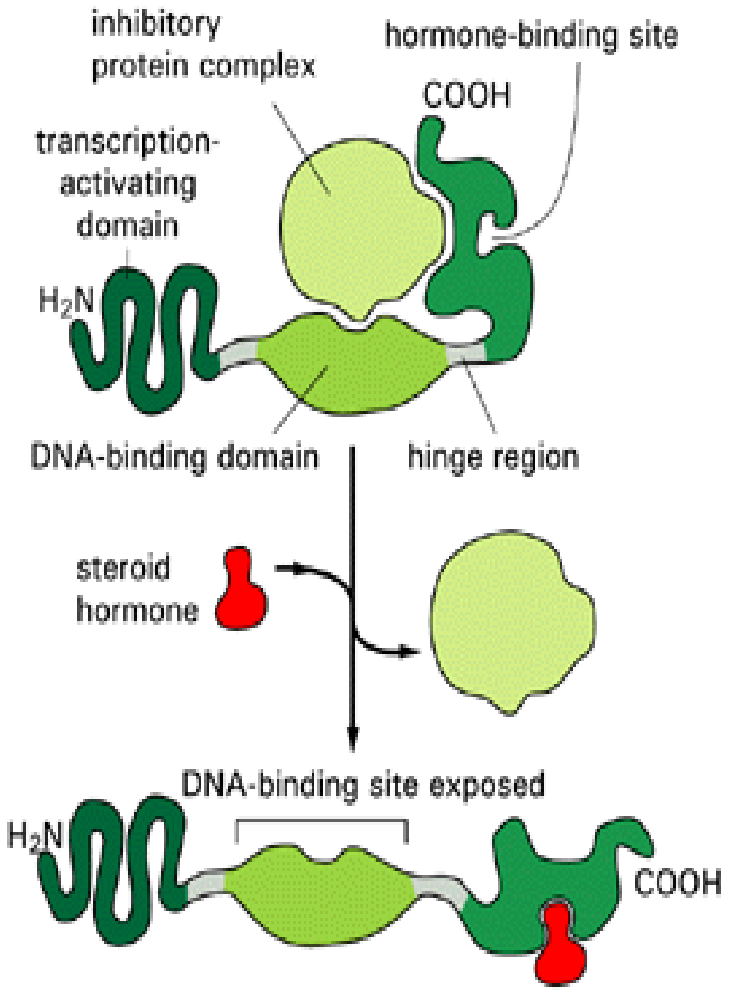
(B) G-PROTEIN-LINKED RECEPTOR



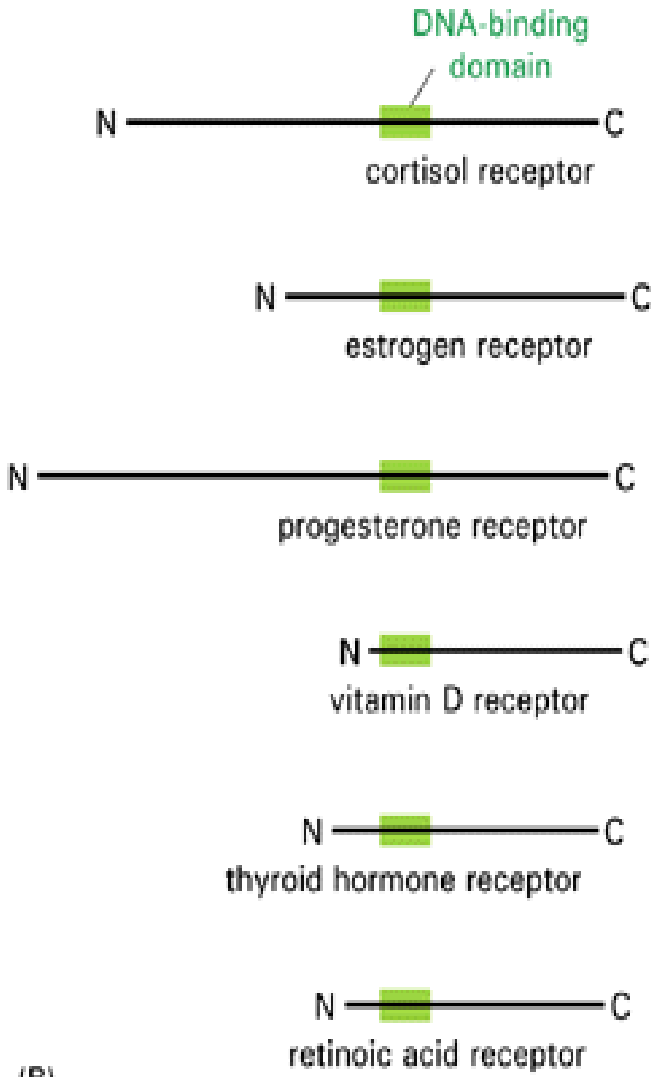
(C) ENZYME-LINKED RECEPTORS



Some small hydrophobic hormones (steroid hormones) whose receptors are intracellular gene regulatory proteins



(A)



(B)

Amino Acid Hormones

- Amino acids cannot cross the cell membrane.
- Amino acid hormones bind with a protein on the outside of the cell membrane

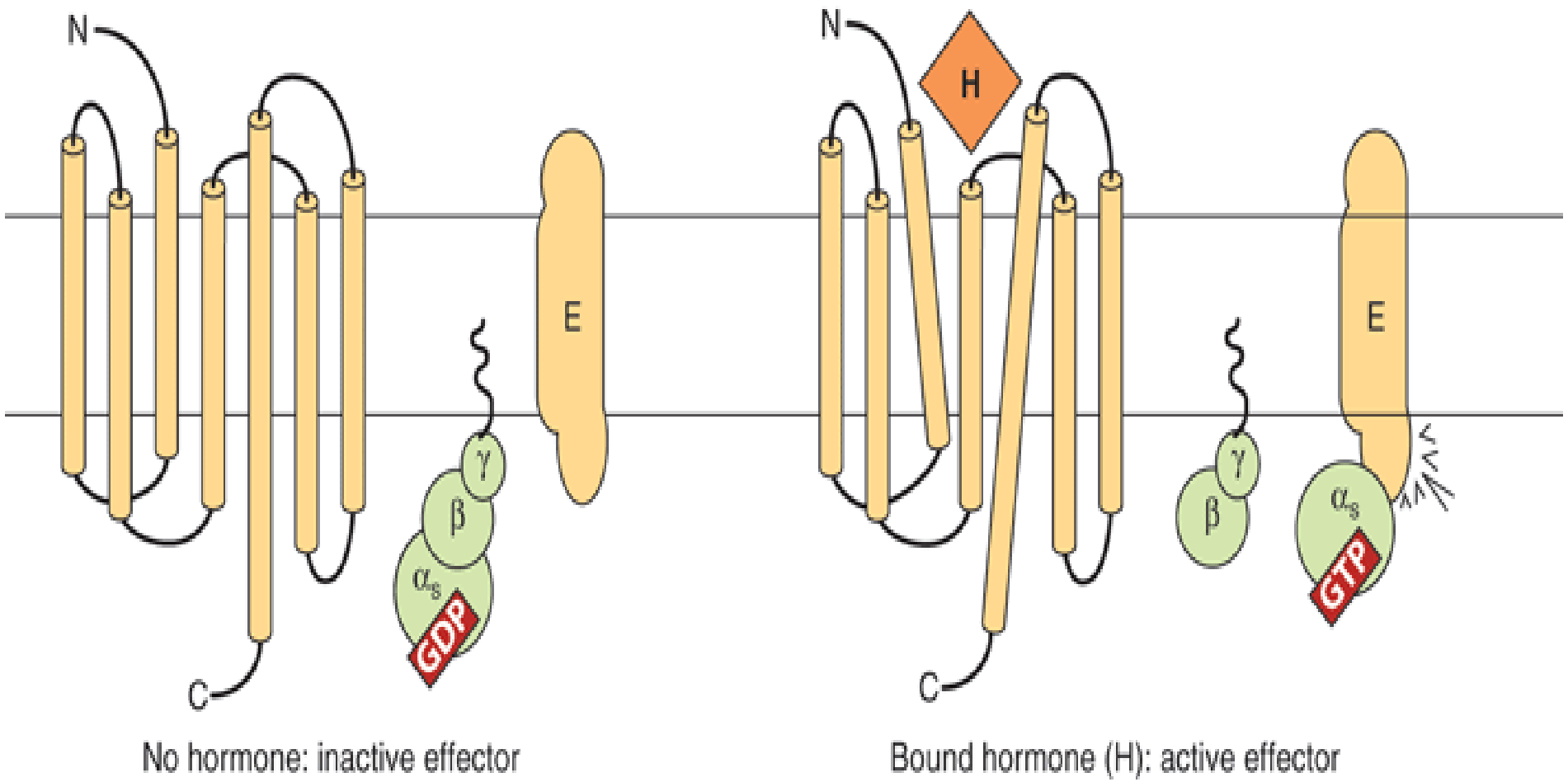
A) General Steps:

1. Hormone Receptor
2. Effectors Enzyme
3. Second Messenger
4. Metabolic Responses Triggered

G Protein-Coupled Receptors (GPCR)

- These receptors typically have seven hydrophobic plasma membrane-spanning domains.

- Many of the group II hormones bind to receptors that couple to effectors through a GTP-binding protein intermediary.



Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's*

Adenylyl Cyclase

- Different peptide hormones can either stimulate (s) or inhibit (i) the production of cAMP from adenylyl cyclase

- Two parallel systems, a stimulatory (s) one and an inhibitory (i) one, converge upon a catalytic molecule (C).

- Each consists of a receptor, R_s or R_i, and a regulatory complex, G_s and G_i

- G_s and G_i are each trimers composed of **α, β and γ subunits**.

- Because the **α subunit** in G_s differs from that in G_i the proteins, which are distinct gene products, are designated $_s$ and $_i$

Hormones That Stimulate Adenylyl Cyclase (H_s

- ACTH
- ADH
- β_2-Adrenergics
- Calcitonin
- CRH
- FSH
- Glucagon
- hCG
- LH
- MSH
- PTH
- TSH

Hormones That Inhibit Adenylyl Cyclase (H_I)

- Acetylcholine
- $_{\alpha 2}$-Adrenergics
- Angiotensin II
- Somatostatin

- The α_s protein has intrinsic GTPase activity.
- The active form, α_s·GTP, is inactivated upon hydrolysis of the GTP to GDP
- the trimeric G_s complex ($\alpha\beta\gamma$) is then re-formed and is ready for another cycle of activation.

- Cholera and pertussis toxins catalyze the ADPribosylation of α_s and $\alpha_{i\text{-}2}$ respectively.

- In the case of α_s, this modification disrupts the intrinsic GTPase activity; thus, α_s cannot reassociate with and is therefore irreversibly activated.

- ADP ribosylation of α_{i-2} prevents the dissociation of α_{i-2} from $\beta\gamma$, and free α_{i-2} thus cannot be formed.

- α_s activity in such cells is therefore unopposed.

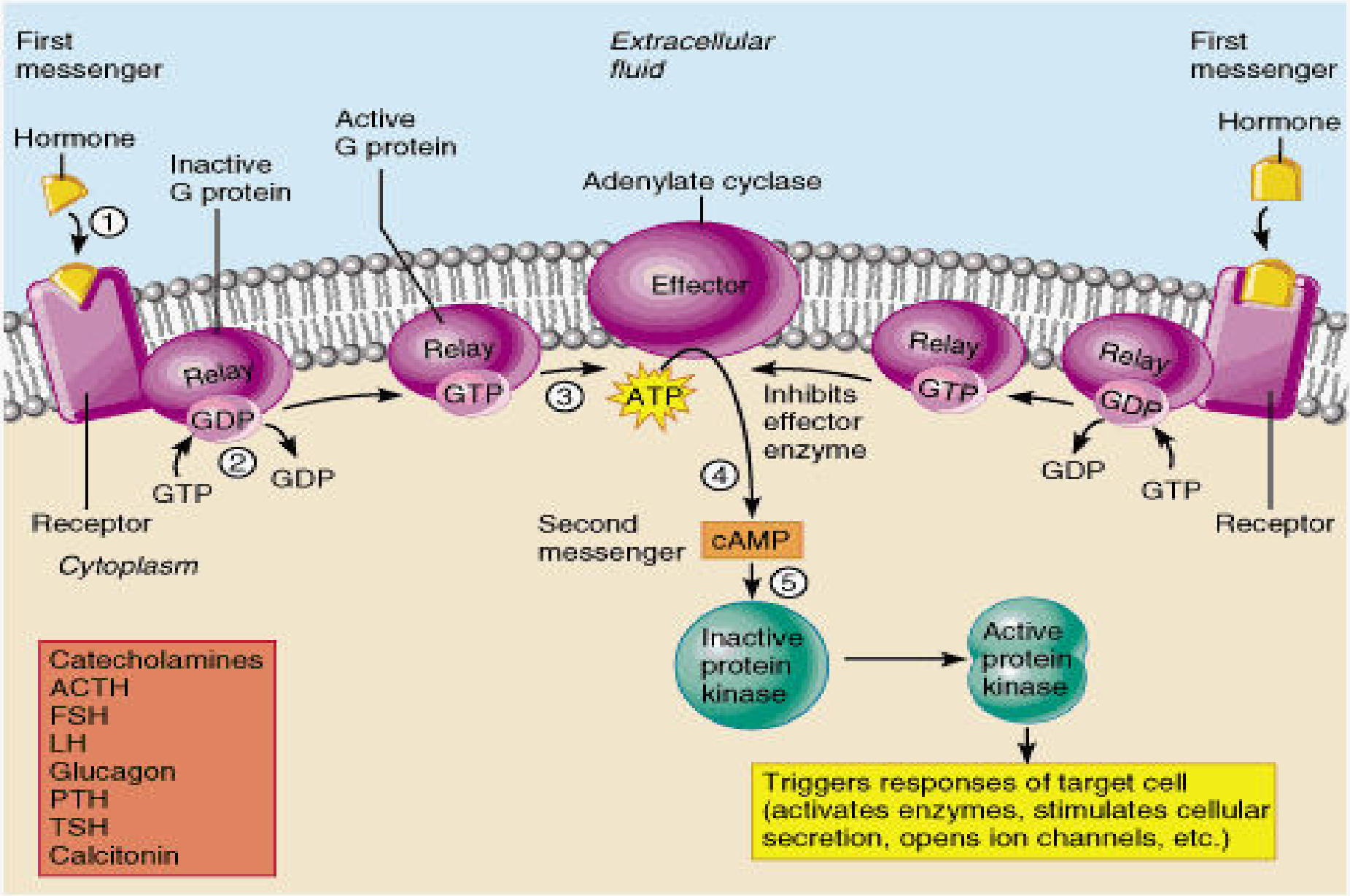
Hormones Using Adenyl Cyclase Mechanism

- ACTH
- LH
- FSH
- TSH
- ADH (v_2 receptors)
- hCG
- MSH

- CRH
- Calcitonin
- PTH
- Catecholamines (β_1, β_2 receptors)
- Angiotensin- II
- Glucagon

Some Hormones That Use the Adenylyl Cyclase–cAMP Second Messenger System

Adrenocorticotropic hormone (ACTH)
Angiotensin II (epithelial cells)
Calcitonin
Catecholamines (β receptors)
Corticotropin-releasing hormone (CRH)
Follicle-stimulating hormone (FSH)
Glucagon
Human chorionic gonadotropin (HCG)
Luteinizing hormone (LH)
Parathyroid hormone (PTH)
Secretin
Somatostatin
Thyroid-stimulating hormone (TSH)
Vasopressin (V_2 receptor, epithelial cells)



(a)

Second Messenger: Cyclic-AMP (cAMP)

1. **Hormone Receptor (first messenger)**

- Hormone binds to the plasma membrane at a specific site.
- Receptor protein changes shape and activates the **G protein.**

2. **Effector Enzyme**

- G protein complex activates **adenylate cyclase.**
- **Adenylate cyclase** breaks **GTP** to **GDP**.
- Generates **second messenger cyclic-AMP** from **ATP.**

Second Messenger

- **cyclic-AMP** moves around the cell triggering chemical reactions with protein kinases.

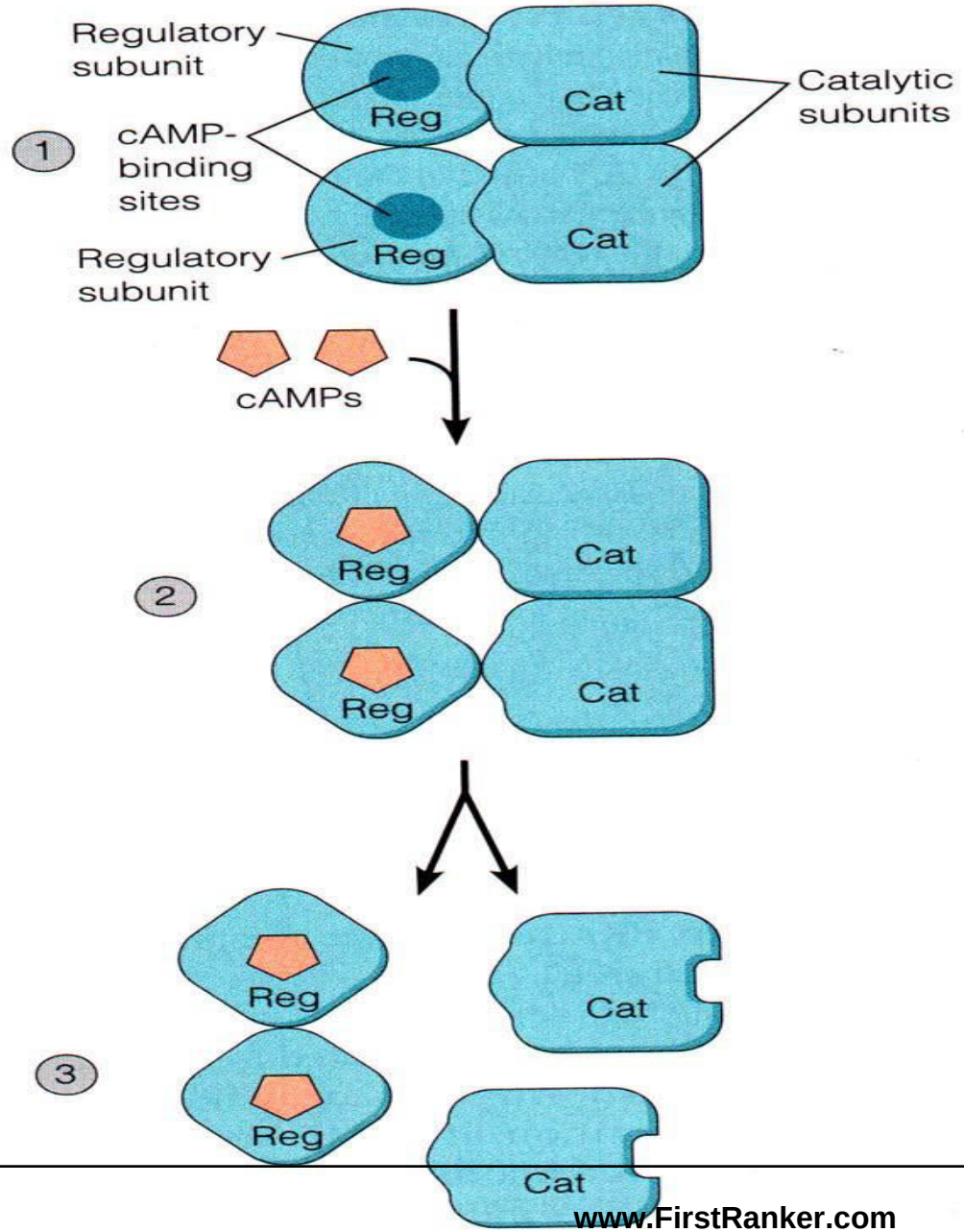
The second messenger is a kinase or phosphatase cascade:

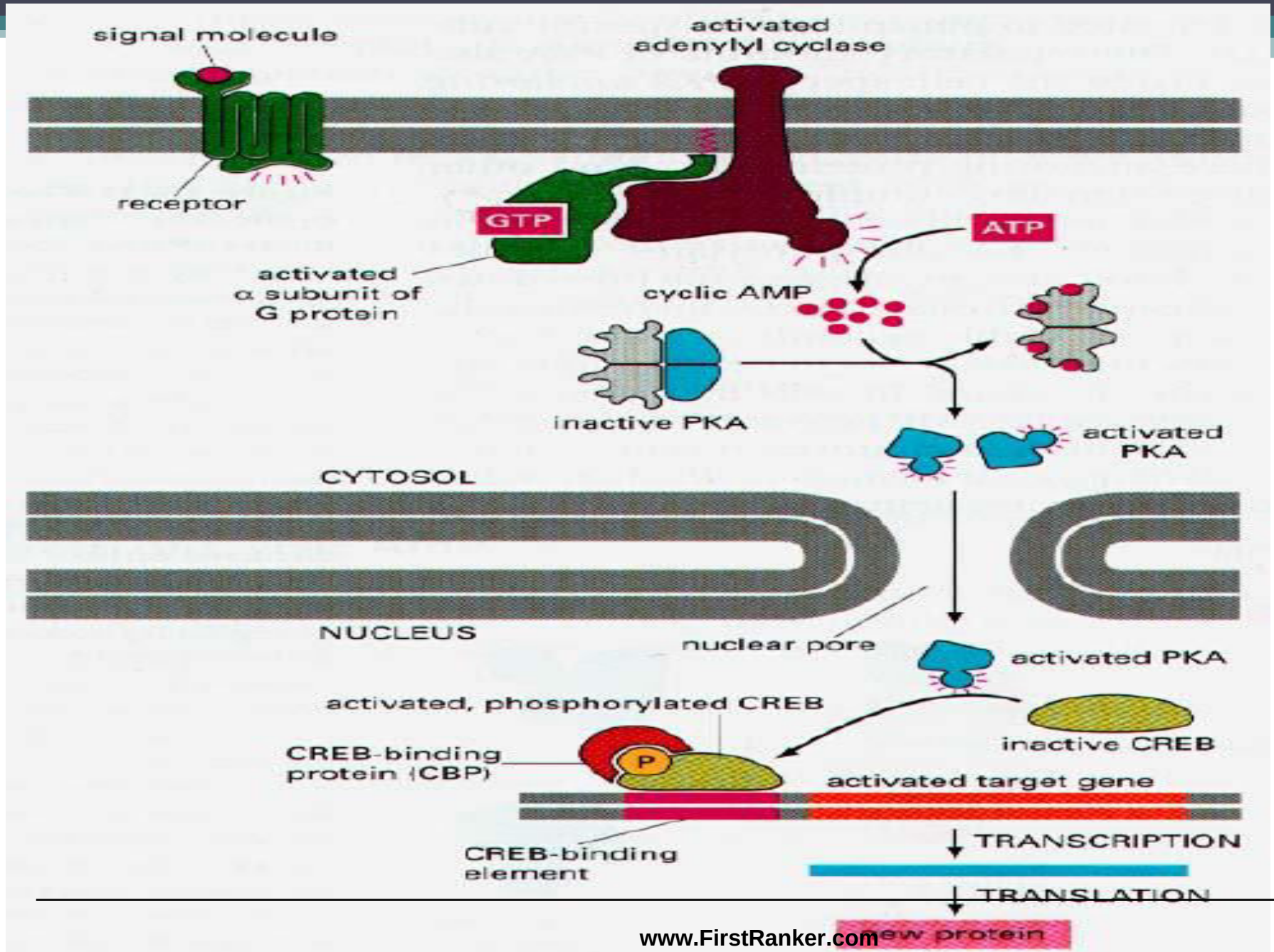
- Chorionic somatomammotropin
- Epidermal growth factor
- Erythropoietin
- Fibroblast growth factor
- Growth hormone
- Insulin
- Insulin-like growth factors I and II
- Nerve growth factor
- Platelet-derived growth factor
- Prolactin

Protein Kinase

- cAMP binds to a protein kinase called **protein kinase A (PKA)**
- It is a heterotrimeric molecule- **2 regulatory** and **2 catalytic subunits**.
- cAMP binds to the regulatory subunit and catalytic is free and activated.

- This catalytic subunit catalyzes the transfer of phosphate of ATP to other proteins, i.e. causes phosphorylation.





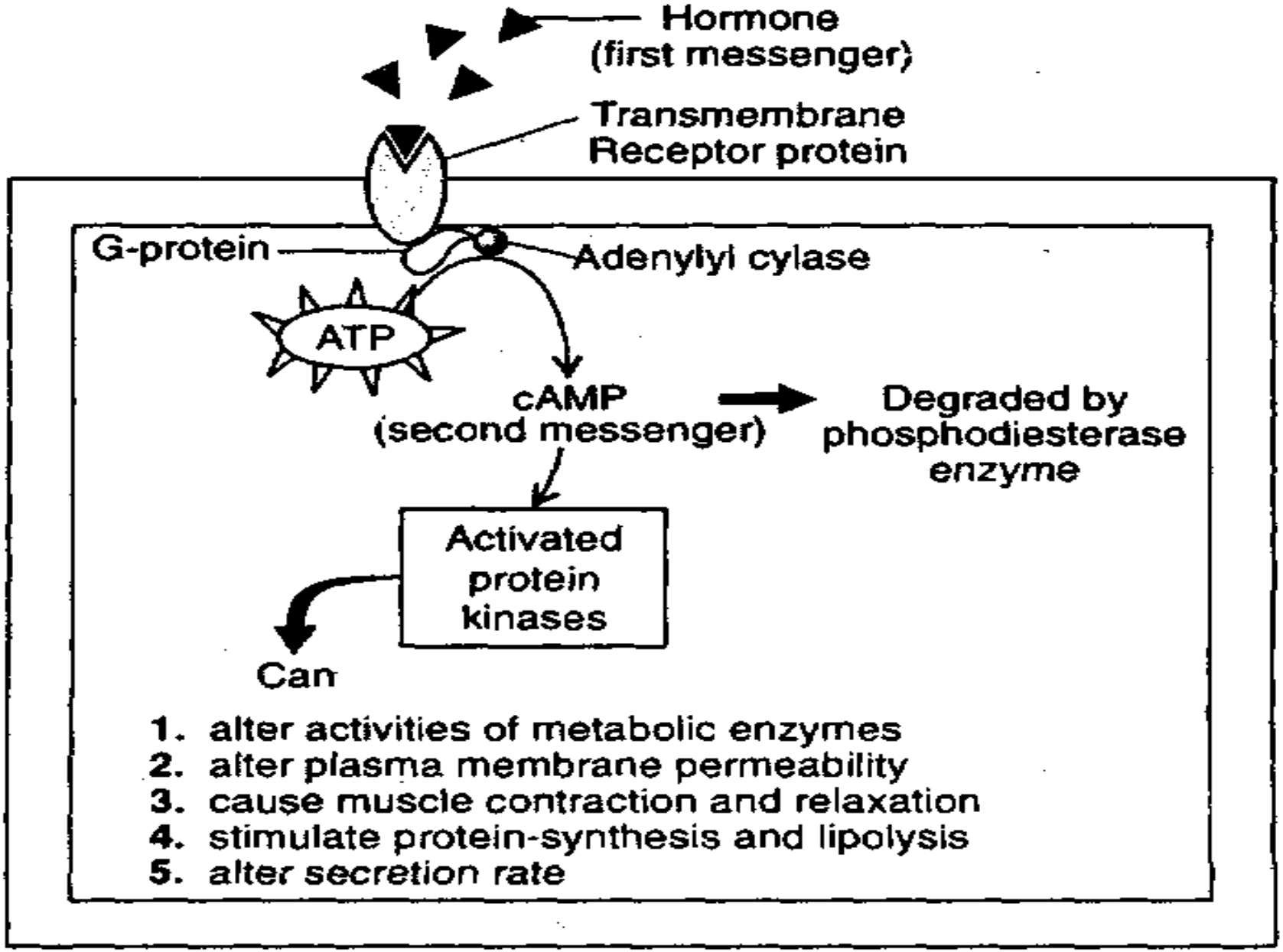


Fig. 33.3 Illustration of modifying cellular metabolism by a hormone through second messenger (cAMP)

Phosphoproteins

- There are certain substrates for phosphorylation which defines a target tissue and the extent of a particular response within a given cell.

- Control the effects of cAMP

- The control of any of the effects of cAMP, including such diverse processes as steroidogenesis, secretion, ion transport, carbohydrate and fat metabolism, enzyme induction, gene regulation, synaptic transmission, and cell growth and replication, could be conferred by a specific protein kinase, by a specific phosphatase, or by specific substrates for phosphorylation.

- **CREB** (cAMP response element binding protein)binds to CRE.

- CREB binds to a cAMP responsive element (CRE) in its nonphosphorylated state and is a weak activator of transcription.

- When phosphorylated by PKA, CREB binds the coactivator **CREB-binding protein CBP/p300** and as a result is a much more potent transcription activator.

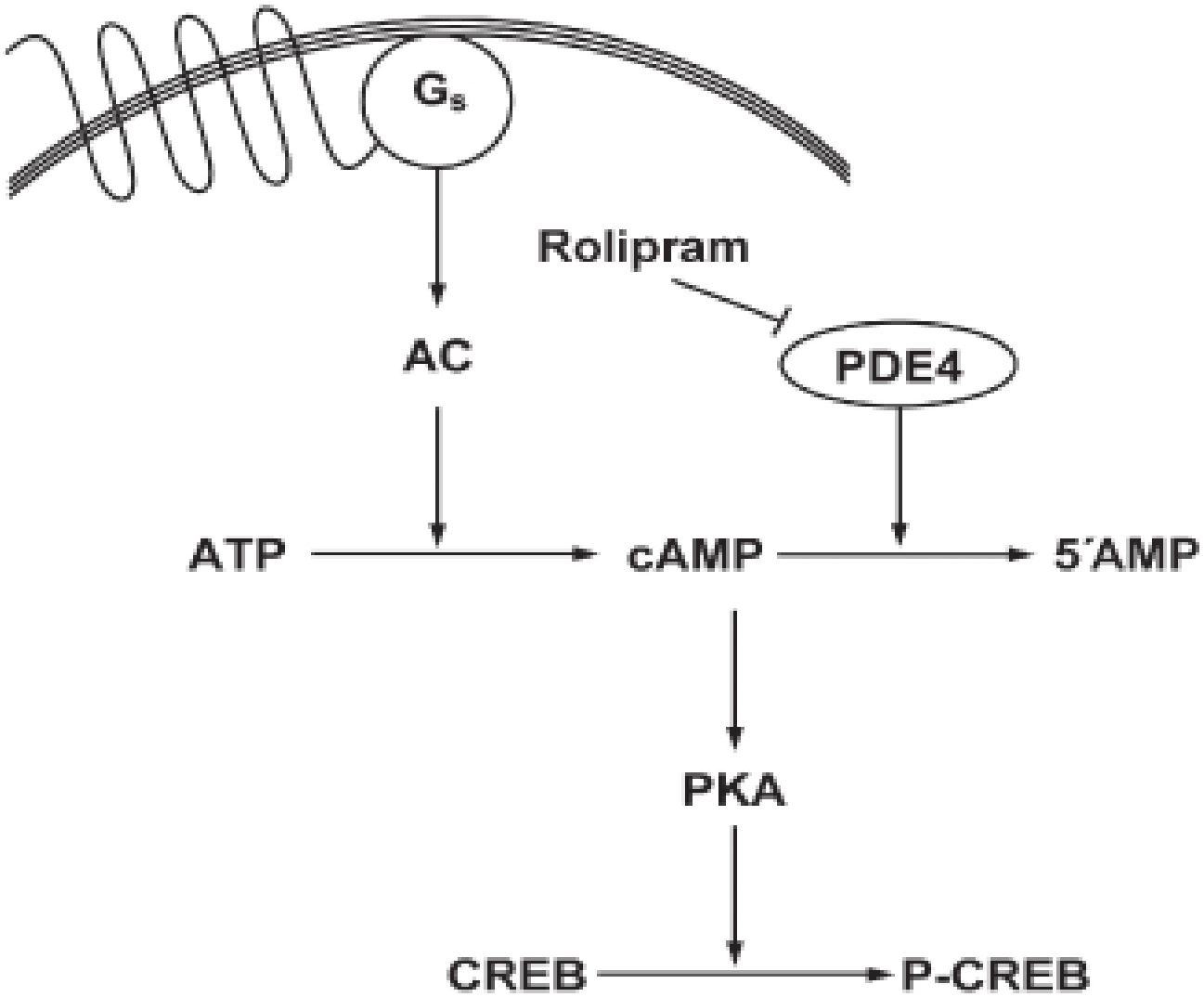
Phosphodiestrases

- They terminate the action of **cAMP** by its hydrolysis to **5´-AMP**.

- Thus they increase the intracellular signals and prolong the action of hormones through this signal.

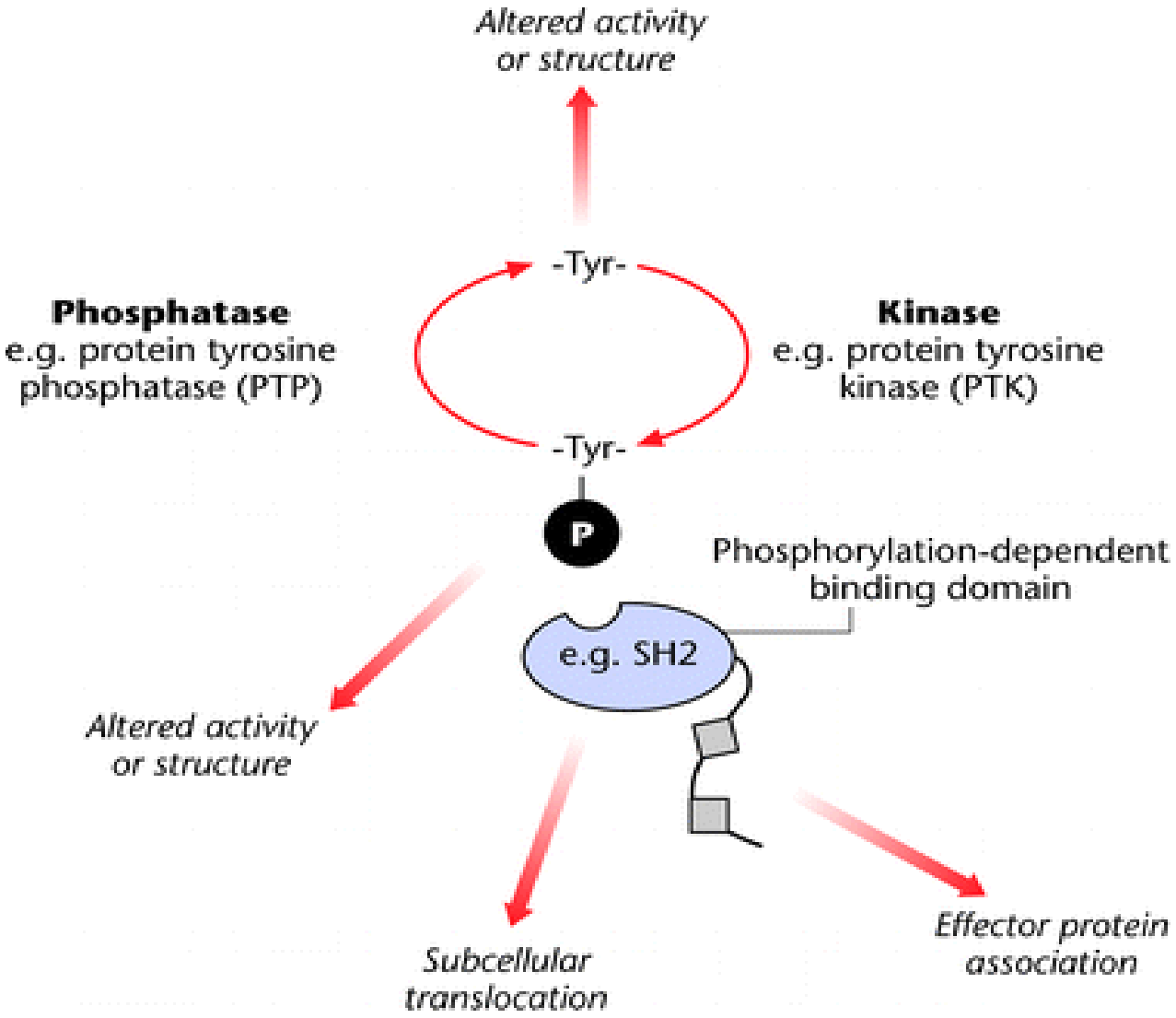
- These are subject to regulation by their substrates, cAMP and cGMP; by hormones; and by intracellular messengers such as calcium, probably acting through calmodulin.

- Most significant inhibitors of phosphodiestrase are methylated xanthine derivatives such as caffeine



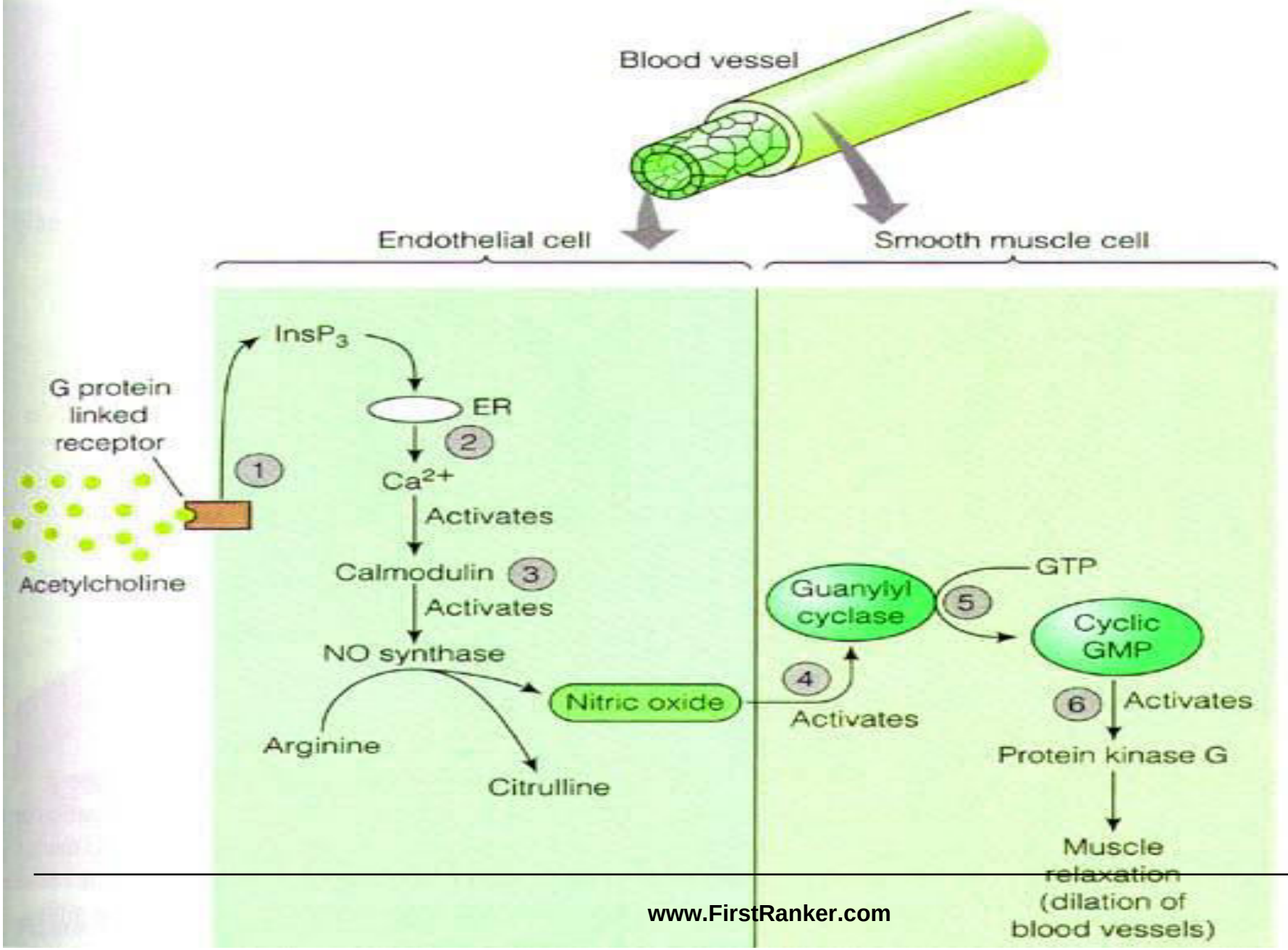
Phosphatases

- They are responsible for protein dephosphorylation.


Altered activity
or structure
-Tyr-
Phosphatase
e.g. protein tyrosine
phosphatase (PTP)
Kinase
e.g. protein tyrosine
kinase (PTK)
-Tyr-
P
Phosphorylation-dependent
binding domain
e.g. SH2
Altered activity
or structure
Subcellular
translocation
Effector protein
association

The second messenger is cGMP

- Atrial natriuretic factor
- Nitric oxide



- **NO – guanyl cyclase (soluble form)** – GTP –cGMP – PKG- muscle relaxation

- **Atrial natriuretic peptide -guanyl cyclase (membrane bound form)** – GTP –cGMP – PKG- natriuresis, diuresis, vasodilatation, inhibition of aldosterone secretion

- Increased **cGMP** activates cGMP dependent protein kinase- **protein kinase G** which in turn phosphorylate the proteins

- Muscle relaxation and dilatation of blood vessels

- **Nitroprusside, Nitroglycerin, Nitric oxide, sodium azide all are vasodilators.**

Second Messenger is Calcium or PI

- Acetylcholine
- Angiotensin II
- Antidiuretic hormone
- Cholecystokinin
- Gastrin
- Gonadotrophin releasing hormone
- Oxytocin
- PDGF
- TRH

- The extracellular calcium (Ca^{2+}) concentration is about 5 mmol/L and is very rigidly controlled. Although substantial amounts of calcium are associated with intracellular organelles such as mitochondria and the endoplasmic reticulum, the intracellular concentration of free or ionized calcium (Ca^{2+}) is very low: 0.05–10 mol/L

- In spite of this large concentration gradient and a favorable trans-membrane electrical gradient, Ca^{2+} is restrained from entering the cell

- prolonged elevation of Ca^{2+} in the cell is very toxic

- Na^{+}/Ca^{2+} exchange
- Ca^{2+}/proton ATPase-dependent pump that extrudes Ca^{2+} in exchange for H^{+}
- Ca^{2+}-ATPases pump Ca^{2+} from the cytosol to the lumen of the endoplasmic reticulum

- There are three ways of changing cytosolic Ca^{2+}:

(1) Certain hormones by binding to receptors that are themselves Ca^{2+} channels, enhance membrane permeability to Ca^{2+} and thereby increase Ca^{2+} influx.

(2) Hormones also indirectly promote Ca^{2+} influx by modulating the membrane potential at the plasma membrane. Membrane depolarization opens voltage-gated Ca^{2+} channels and allows for Ca^{2+} influx.

(3) Ca^{2+} can be mobilized from the endoplasmic reticulum, and possibly from mitochondrial pools

Enzymes and Proteins Regulated by Calcium or Calmodulin

- Adenylyl cyclase
- Ca^{2+}-dependent protein kinases
- Ca^{2+}-Mg^{2+}-ATPase
- Ca^{2+}-phospholipid-dependent protein kinase
- Cyclic nucleotide phosphodiesterase

- Some cytoskeletal proteins
- Some ion channels (eg, L-type calcium channels)
- Nitric oxide synthase
- Phosphorylase kinase
- Phosphoprotein phosphatase 2B
- Some receptors (eg, NMDA-type glutamate receptor)

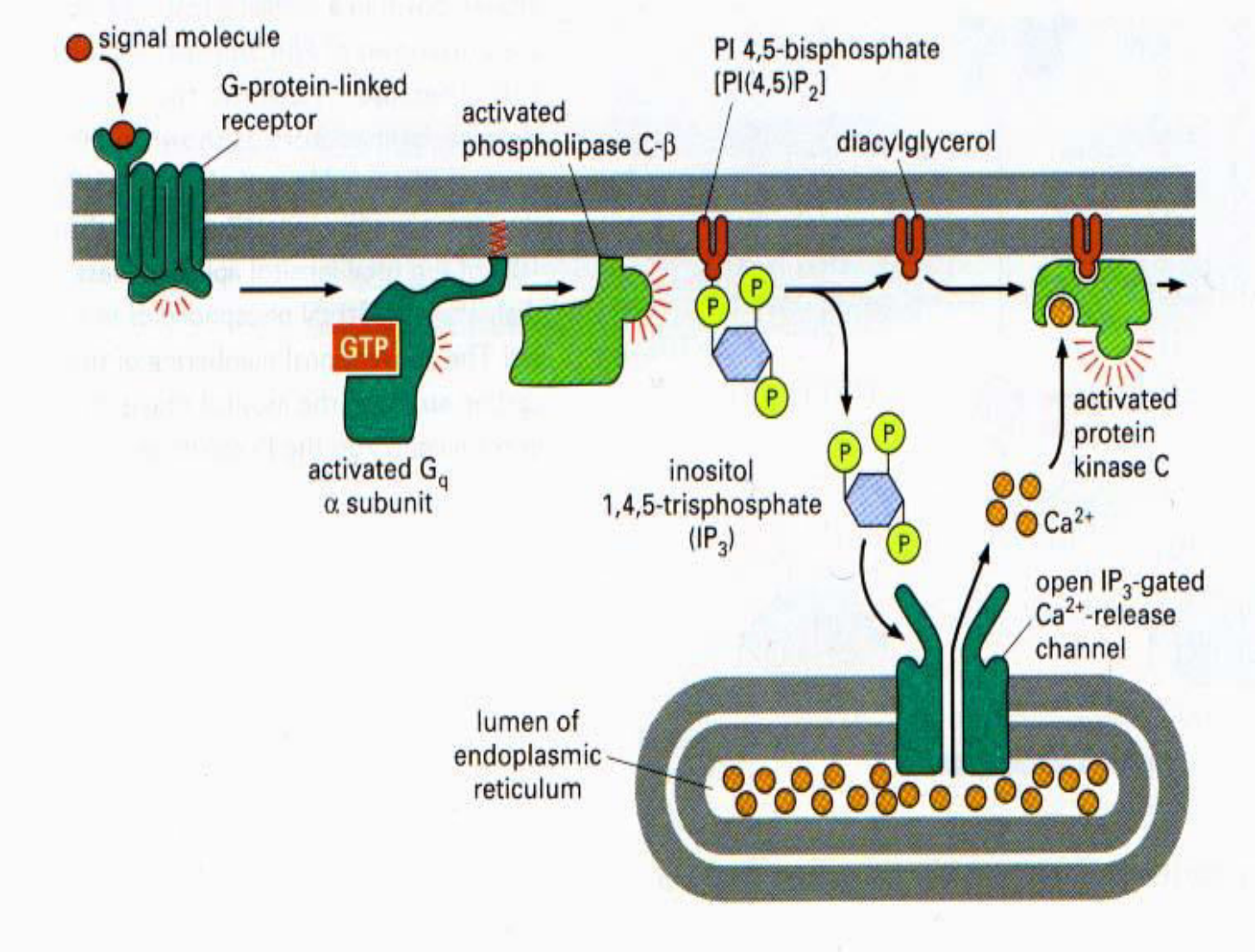
- Calmodulin is a calcium dependent protein and it is similar to troponin C.
- It has four binding sites for calcium.
- Cell surface receptors such as those for acetylcholine, ADH and α1-catecholamines function through this pathway.

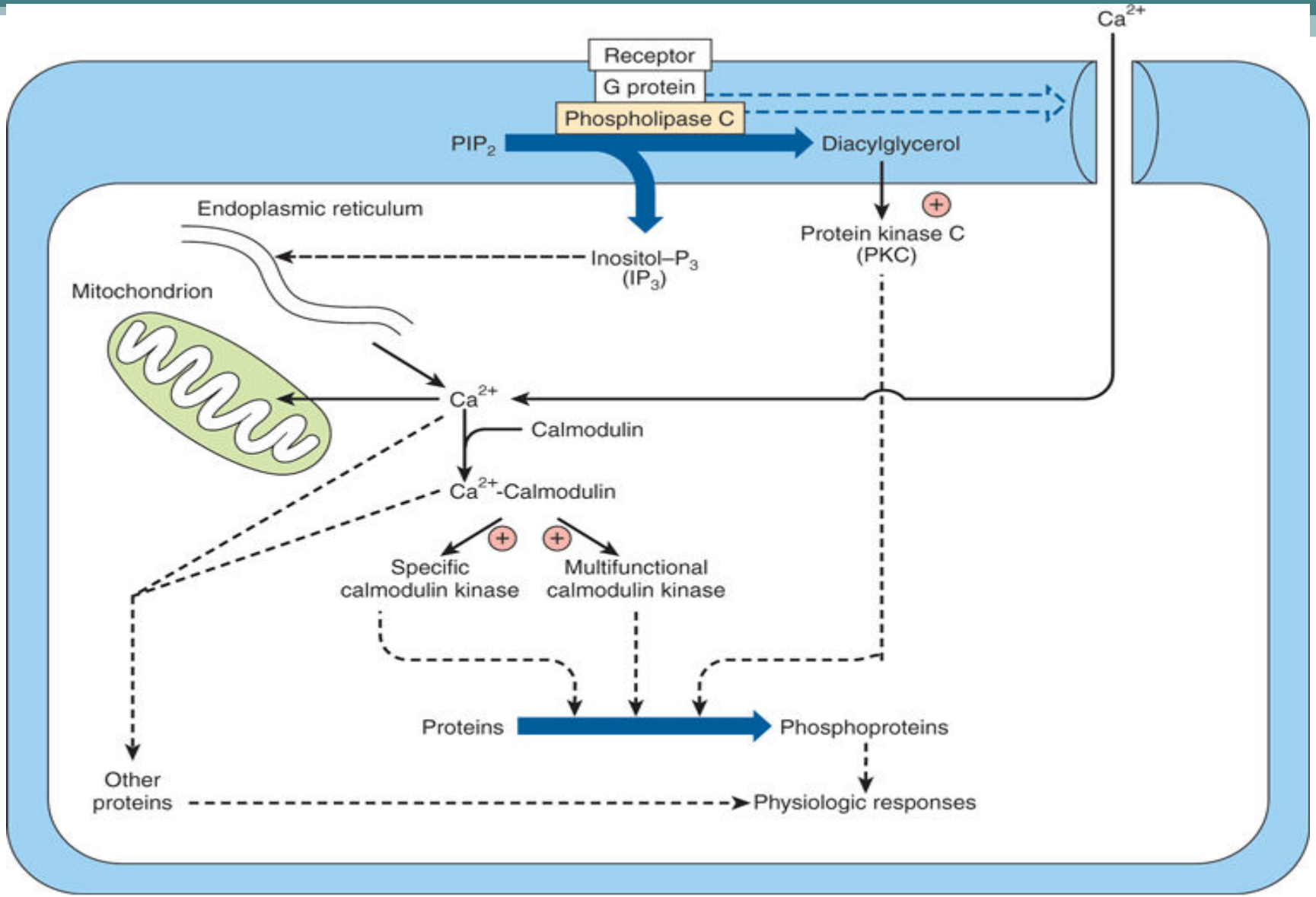
- Their binding activates phospholipase C.
- Phospholipase C catalyzes the hydrolysis of phosphatidylinositol 4,5 bisphosphate to **inositol triphosphate(IP3) and 1,2 diacylglycerol.**

- 1,2 diacylglycerol activates protein kinase C (PKC).
- PKC phosphorylate other substrates
- IP3 causes the release of calcium from endoplasmic reticulum and mitochondria

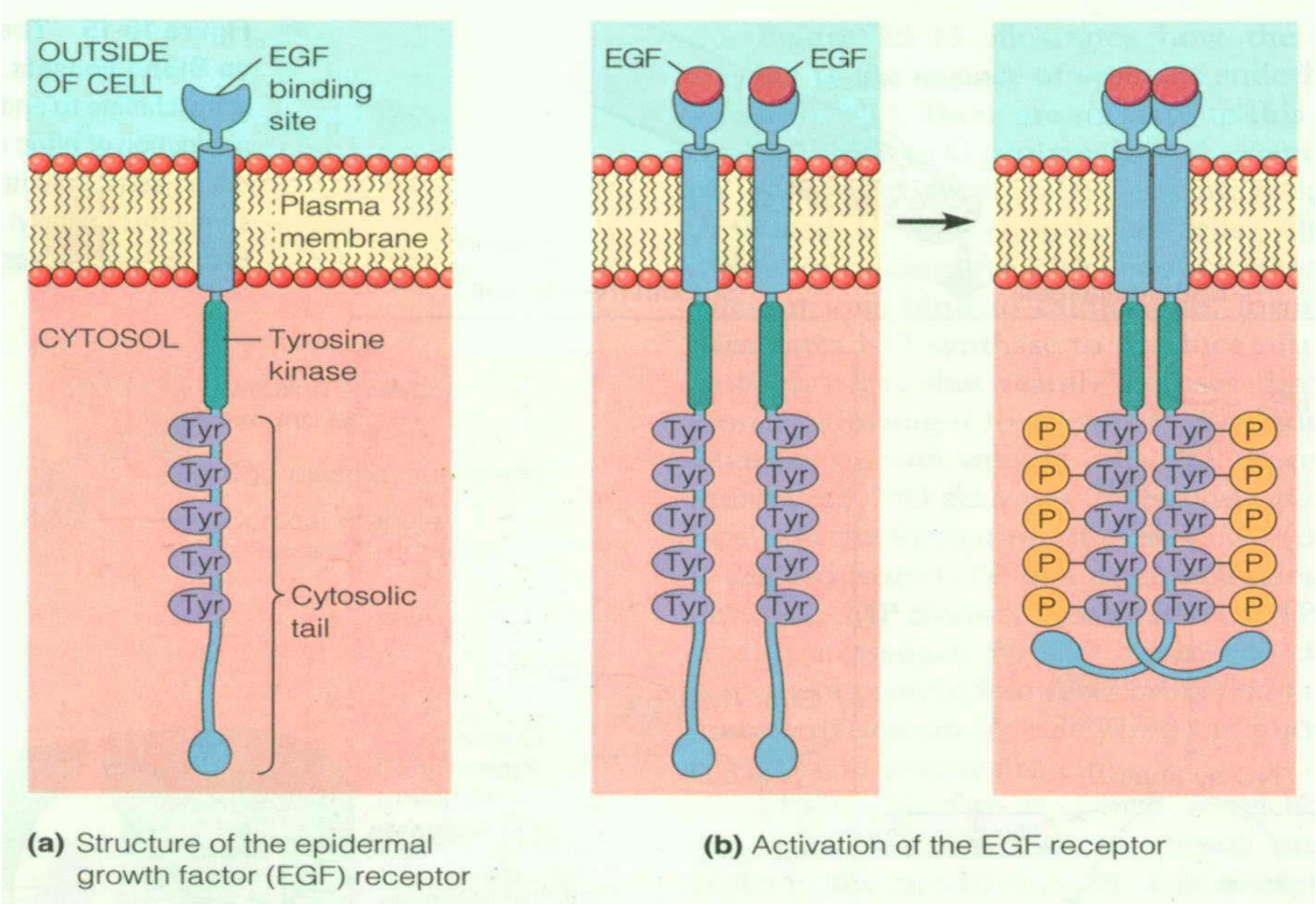
- The normal calcium ion concentration in most cells of the body is 10^{-8} to 10^{-7} mol/L, which is not enough to activate the calmodulin system.

- But when the calcium ion concentration rises to 10^{-6} to 10^{-5} mol/L, enough binding occurs to cause all the intracellular actions of calmodulin.


signal molecule
G-protein-linked receptor
activated phospholipase C-β
PI 4,5-bisphosphate [PI(4,5)P2]
diacylglycerol
GTP
activated Gq α subunit
inositol 1,4,5-trisphosphate (IP3)
activated protein kinase C
Ca2+
open IP3-gated Ca2+-release channel
lumen of endoplasmic reticulum
P



Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 28th Edition*: http://www.accessmedicine.com



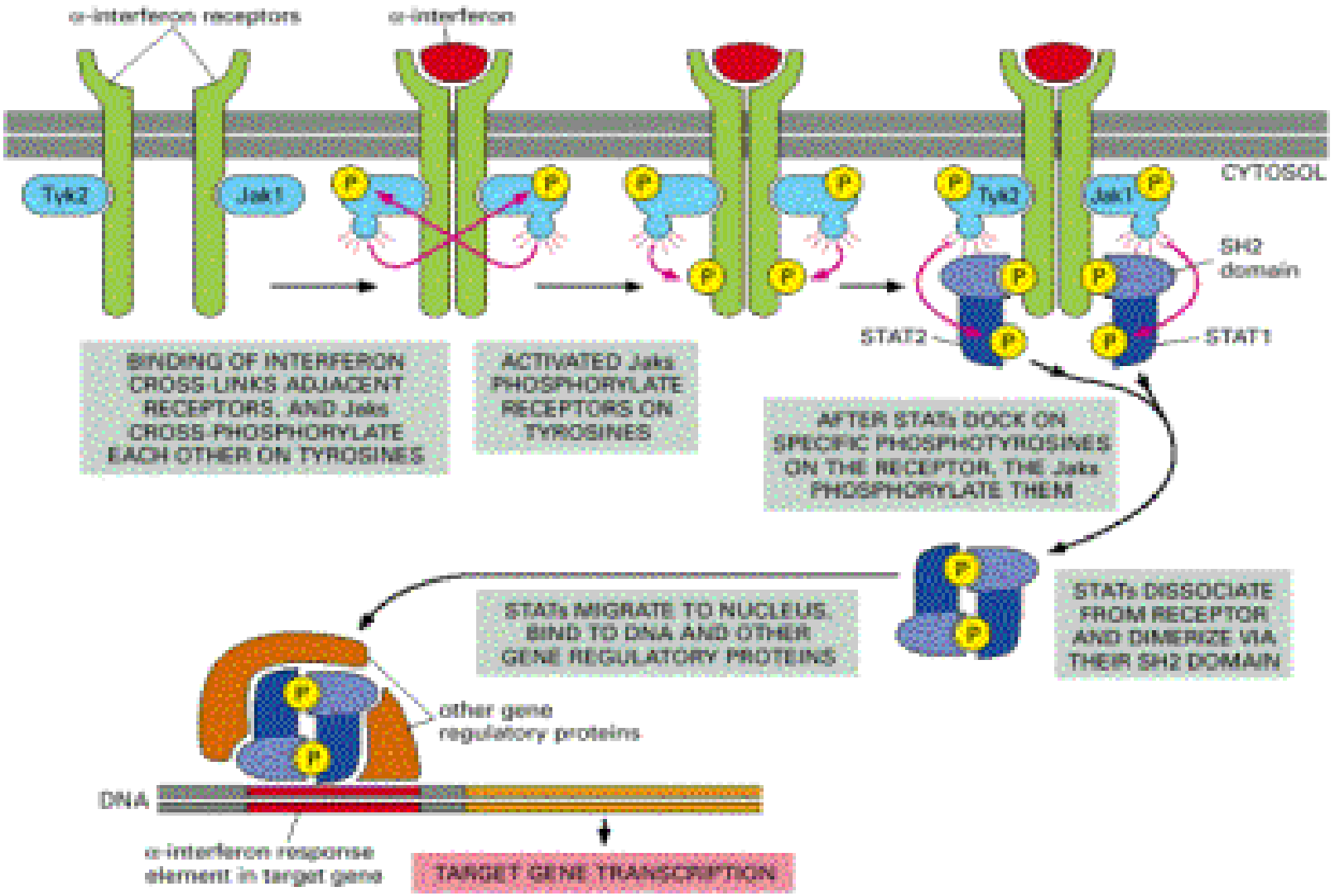
(a) Structure of the epidermal growth factor (EGF) receptor

(b) Activation of the EGF receptor

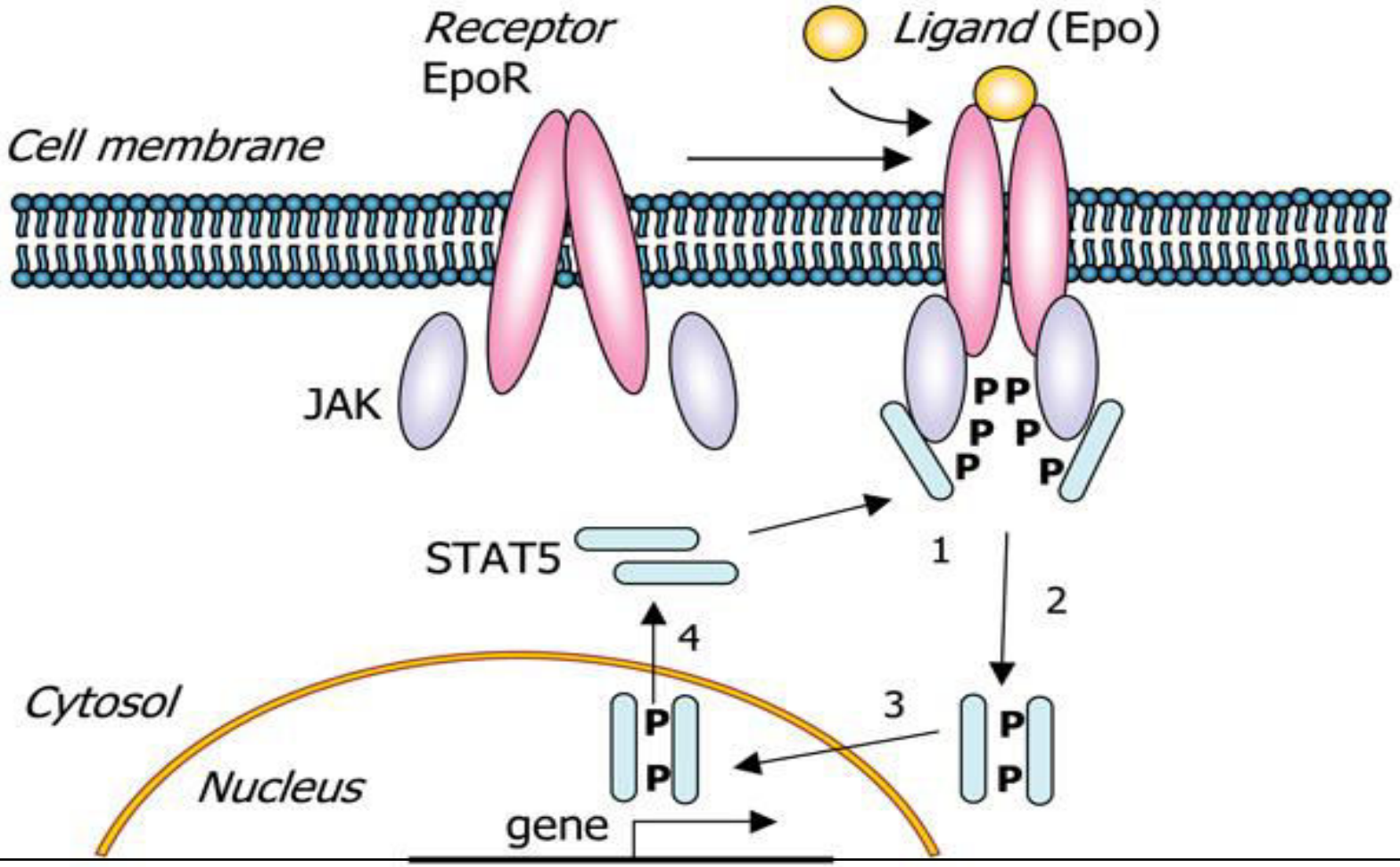
JAK-STAT pathway

- Growth hormone
- Prolactin
- Erythropoietin
- Cytokines

All these activate cytoplasmic protein tyrosine kinases such as: Tyk-2, Jak-1or 2.


α-interferon receptors
α-interferon
Tyk2
Jak1
CYTOSOL
SH2 domain
STAT2
STAT1
BINDING OF INTERFERON CROSS-LINKS ADJACENT RECEPTORS, AND Jaks CROSS-PHOSPHORYLATE EACH OTHER ON TYROSINES
ACTIVATED Jaks PHOSPHORYLATE RECEPTORS ON TYROSINES
AFTER STATs DOCK ON SPECIFIC PHOSPHOTYROSINES ON THE RECEPTOR, THE Jaks PHOSPHORYLATE THEM
STATs DISSOCIATE FROM RECEPTOR AND DIMERIZE VIA THEIR SH2 DOMAIN
STATs MIGRATE TO NUCLEUS, BIND TO DNA AND OTHER GENE REGULATORY PROTEINS
other gene regulatory proteins
DNA
α-interferon response element in target gene
TARGET GENE TRANSCRIPTION

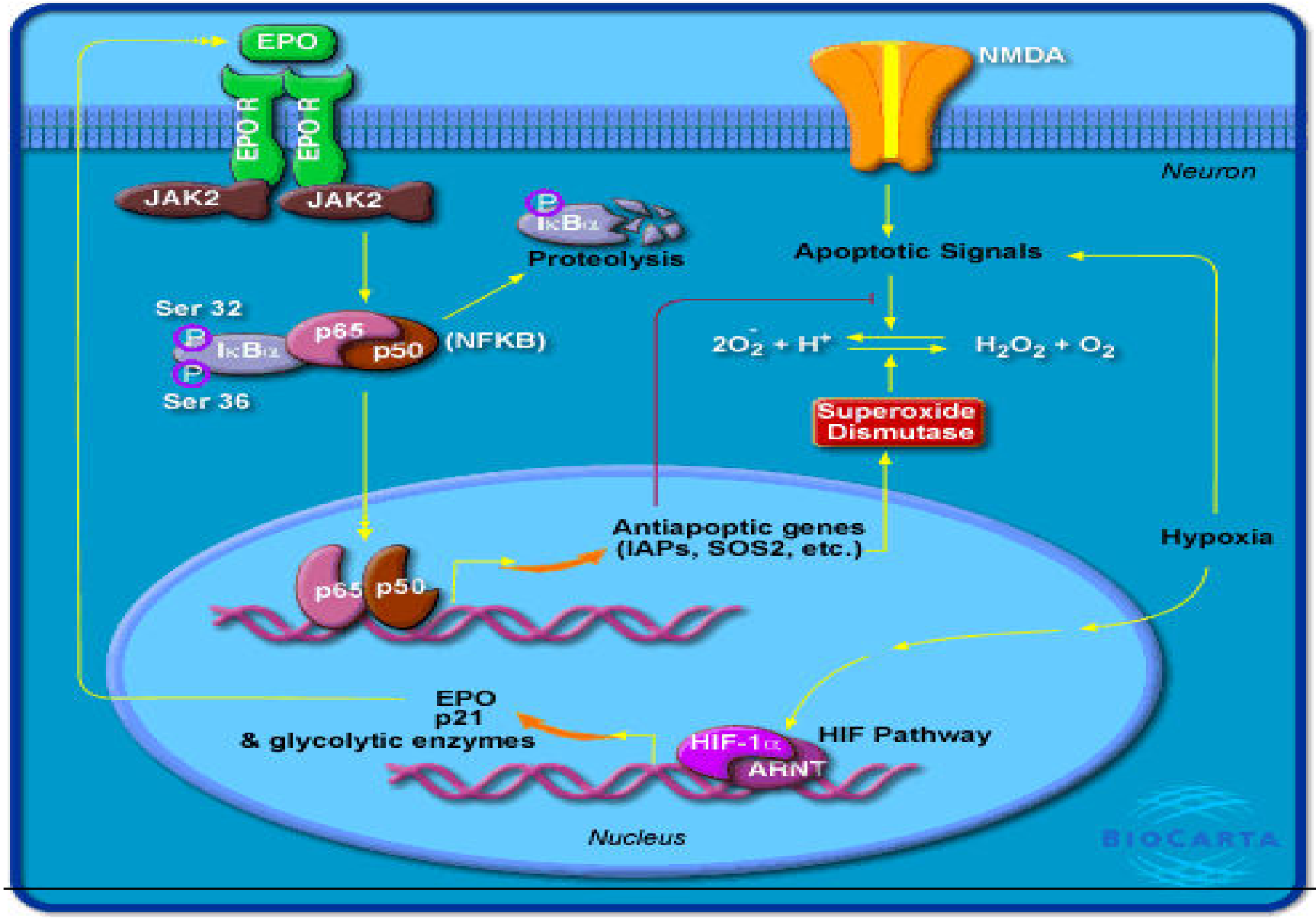
Jak-Stat pathway

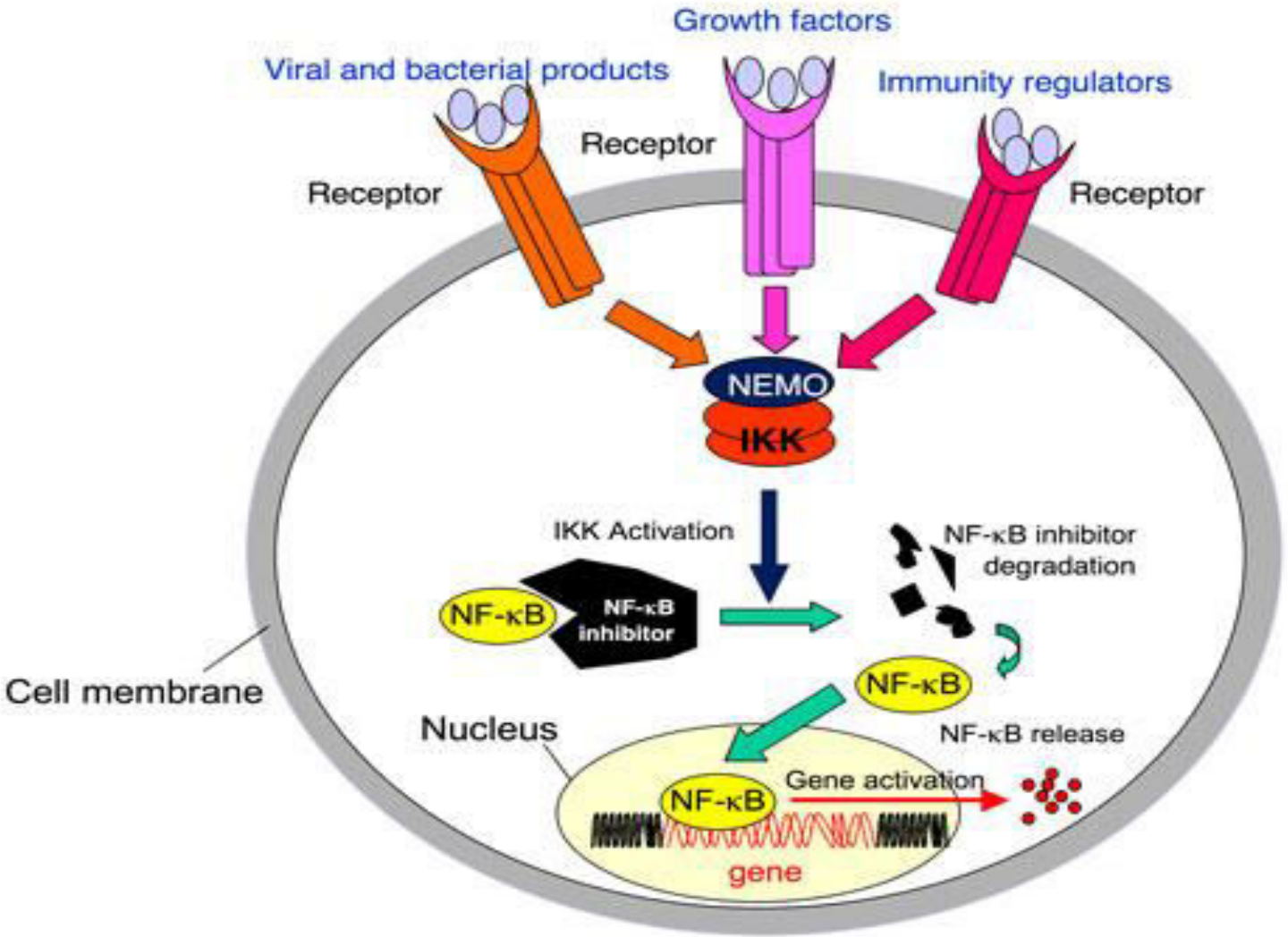


NF-kB pathway

- Extracellular stimuli such as pro inflammatory cytokines, reactive oxygen species and mitogens.

- All these activate this pathway.

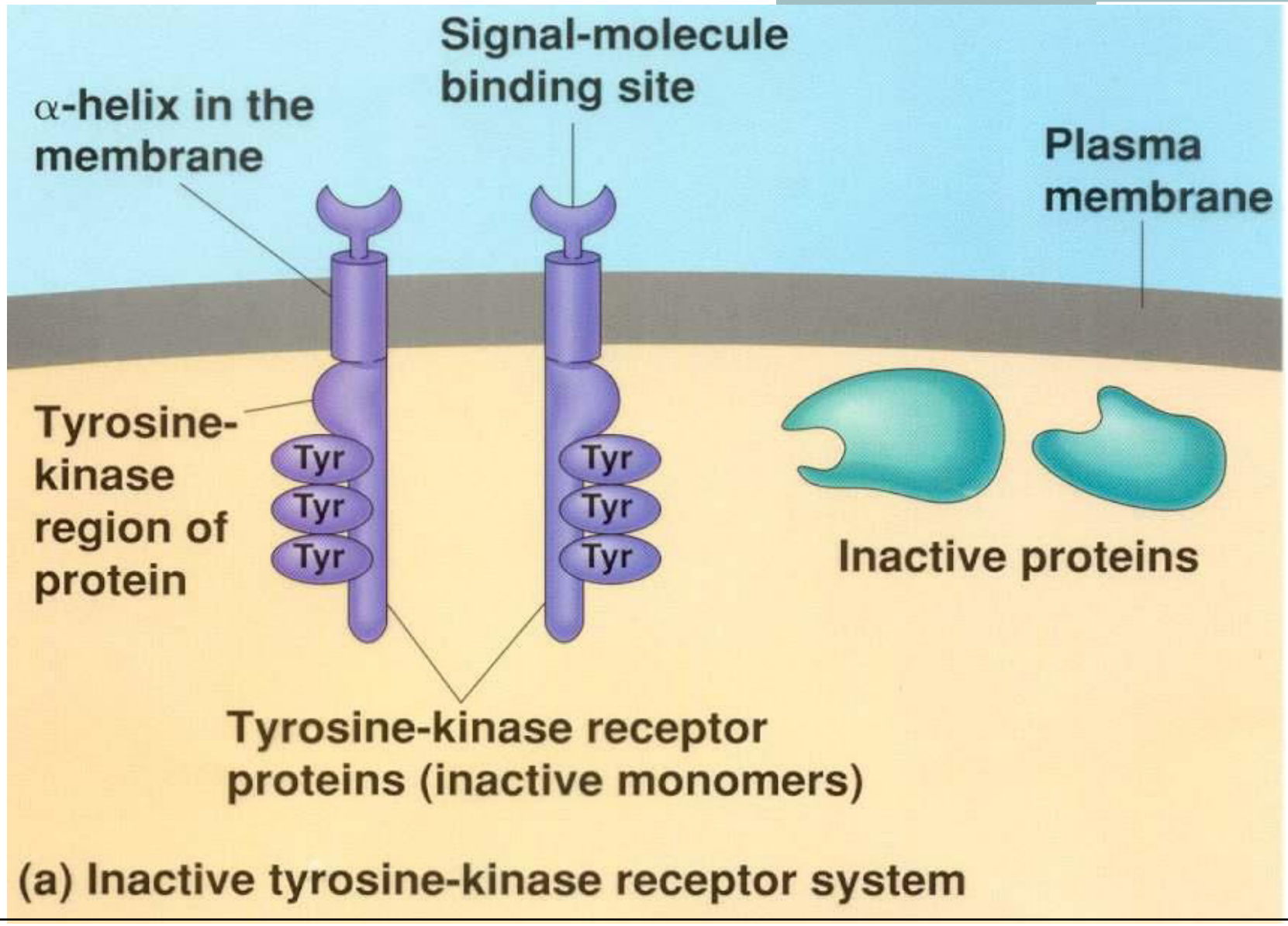

EPO
EPO R
EPO R
JAK2
JAK2
NMDA
Neuron
IκBα
Proteolysis
Apoptotic Signals
Ser 32
IκBα
p65
p50
(NFKB)
Ser 36
$2O_2^- + H^+$
$H_2O_2 + O_2$
Superoxide Dismutase
Antiapoptic genes
(IAPs, SOS2, etc.)
Hypoxia
p65 p50
EPO
p21
& glycolytic enzymes
HIF-1α
ARNT
HIF Pathway
Nucleus
BIOCARTA


Growth factors
Viral and bacterial products
Immunity regulators
Receptor
Receptor
Receptor
NEMO
IKK
IKK Activation
NF-κB inhibitor degradation
NF-κB
NF-κB inhibitor
NF-κB
Cell membrane
Nucleus
NF-κB release
Gene activation
NF-κB
gene

- Glucocorticoid hormones are therapeutically used for the treatment of inflammatory and immune diseases.
- These actions are modulated by the inhibition of NF-kB pathway.

Tyrosine Kinase pathway

- Insulin, epidermal growth factor and insulin like growth factor-I receptors have intrinsic protein tyrosine kinase activity.


Signal-molecule binding site
α-helix in the membrane
Plasma membrane
Tyrosine-kinase region of protein
Tyr
Tyr
Tyr
Tyr
Tyr
Tyr
Inactive proteins
Tyrosine-kinase receptor proteins (inactive monomers)
(a) Inactive tyrosine-kinase receptor system

- The activation of a Tyrosine-Kinase Receptor occurs as follows:
  - Two signal molecule binds to two nearby Tyrosine-Kinase Receptors, causing them to aggregate, forming a dimer
  - The formation of a dimer activated the Tyrosine-Kinase portion of each polypeptide
  - The activated Tyrosine-Kinases phosphorylate the Tyrosine residues on the protein