

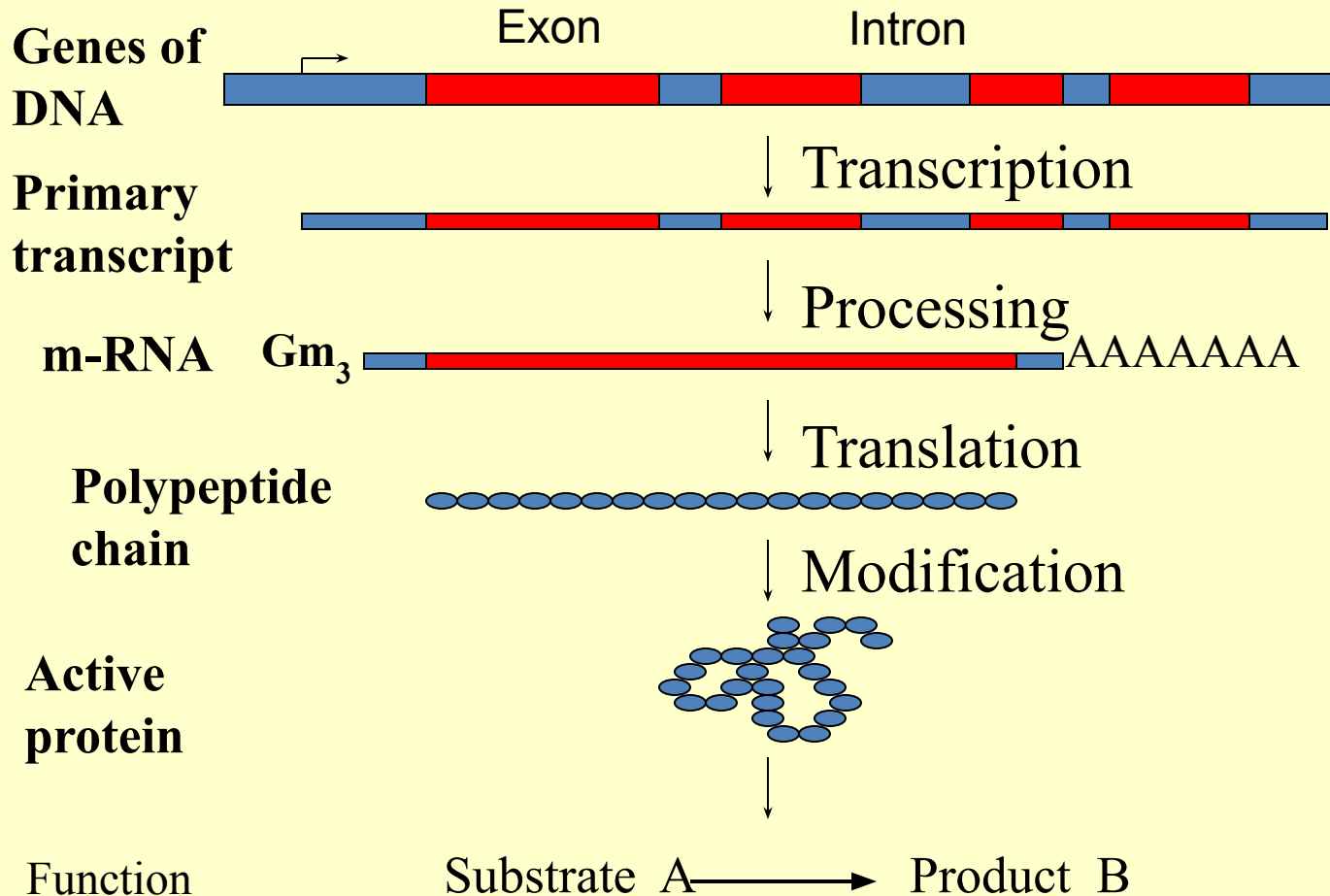
**THE MINISTRY OF PUBLIC HEALTH OF UKRAINE
ZAPOROZHYE STATE MEDICAL UNIVERSITY**

Gene Expression Regulation

Fundamentals of Biochemistry of Hormones

Produced by Ass.professor Krisanova N.V., 2015

All the levels may be regulated:



Levels of regulation in bacterial gene expression

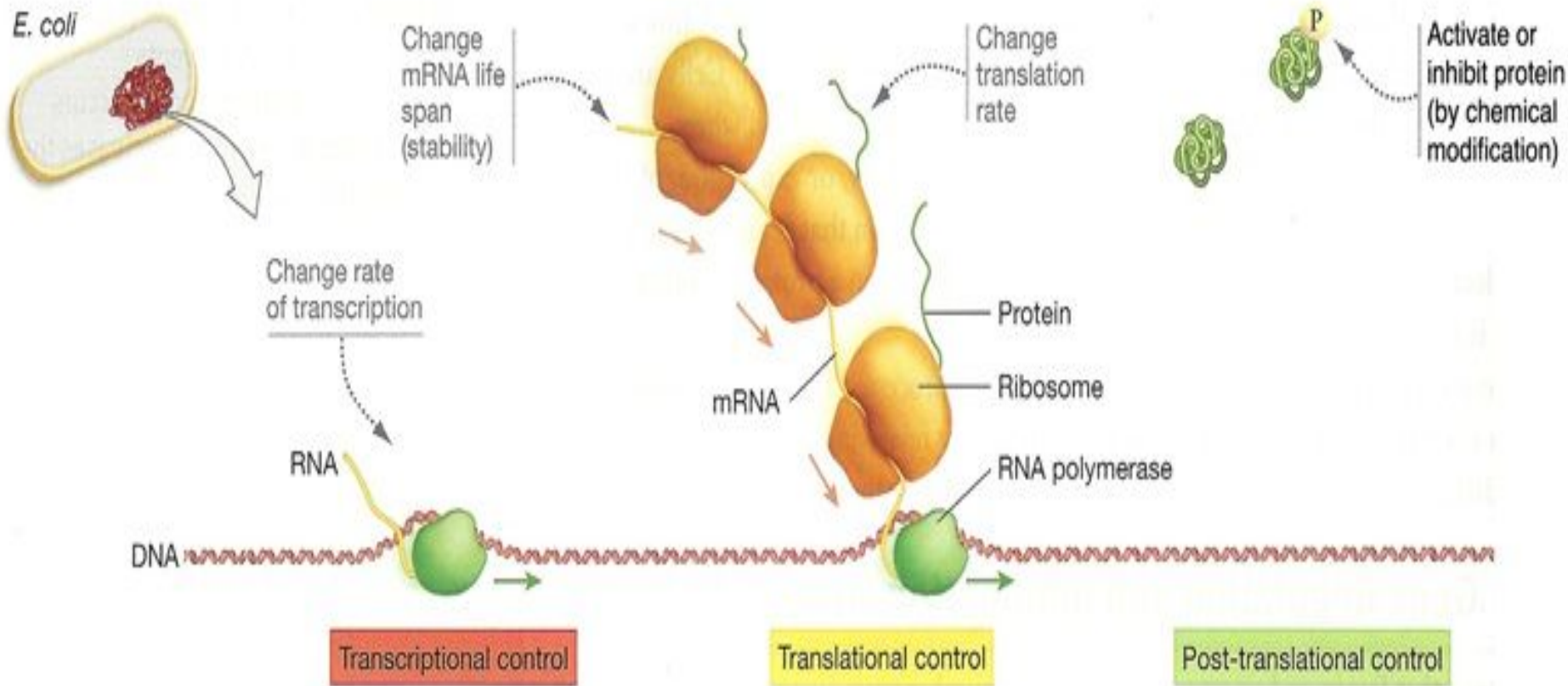
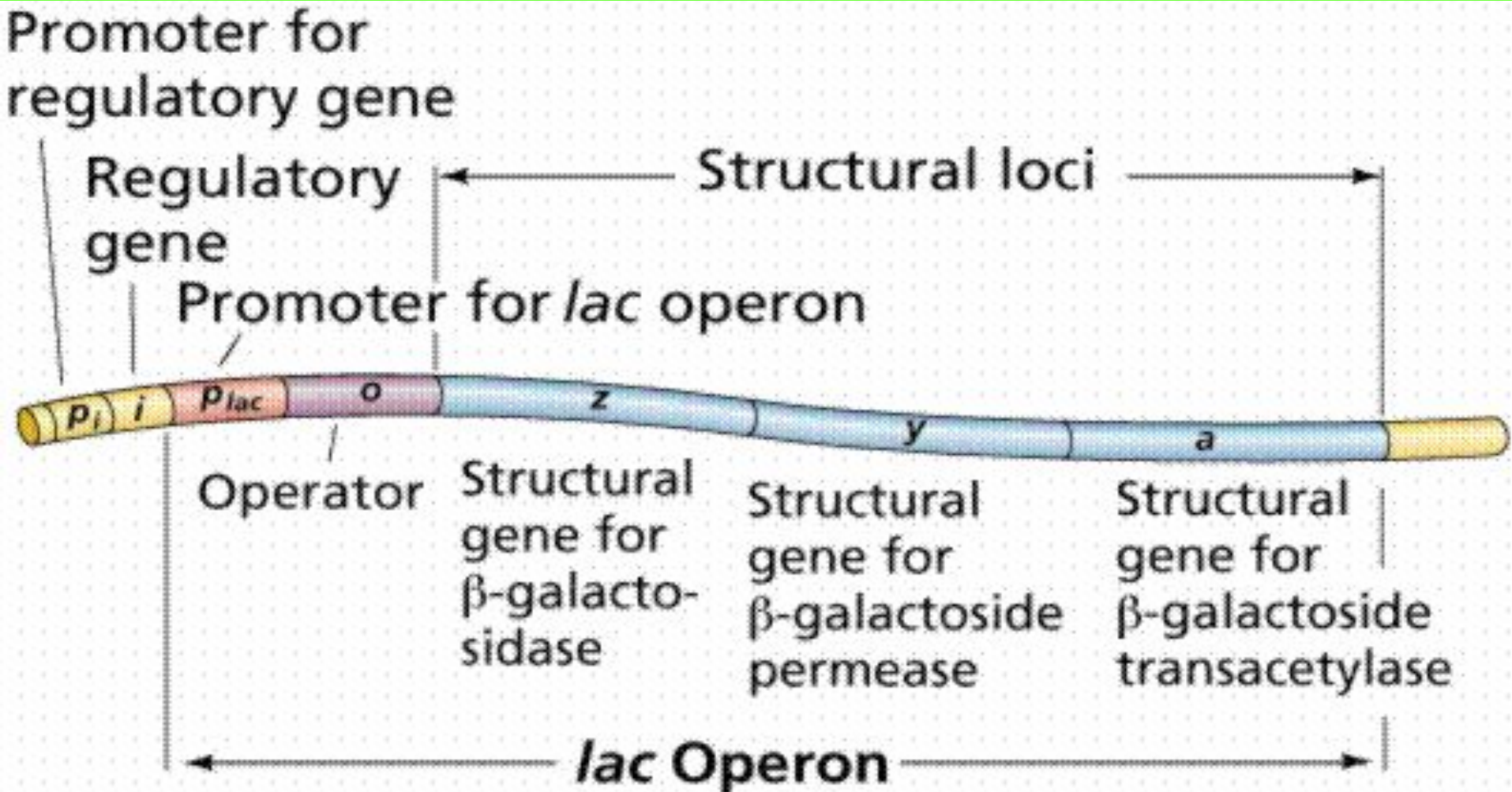


FIGURE 17.1 Gene Expression in Bacteria Can Be Regulated at Three Levels.

**All the genes of DNA in prokaryotic cell
are divided in types:**

- House keeping genes
(constitutive)**
- Inducible (structural)**
- Gene-regulators**
- Gene-operators**

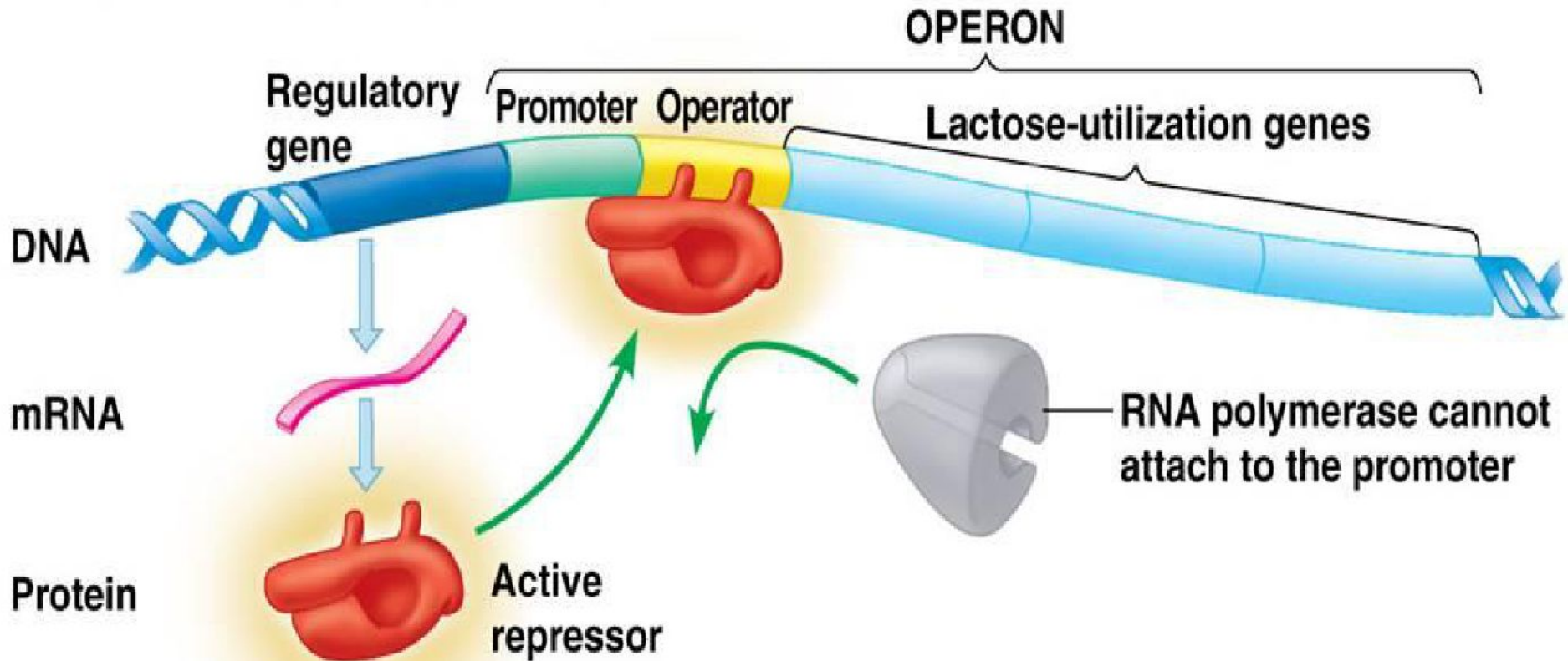
Operon is composed from promoter sequence, gene-operator, structural genes



The Lac-operon model investigated in E.coli
(proposed by F.Jacob and J. Monod, 1961)

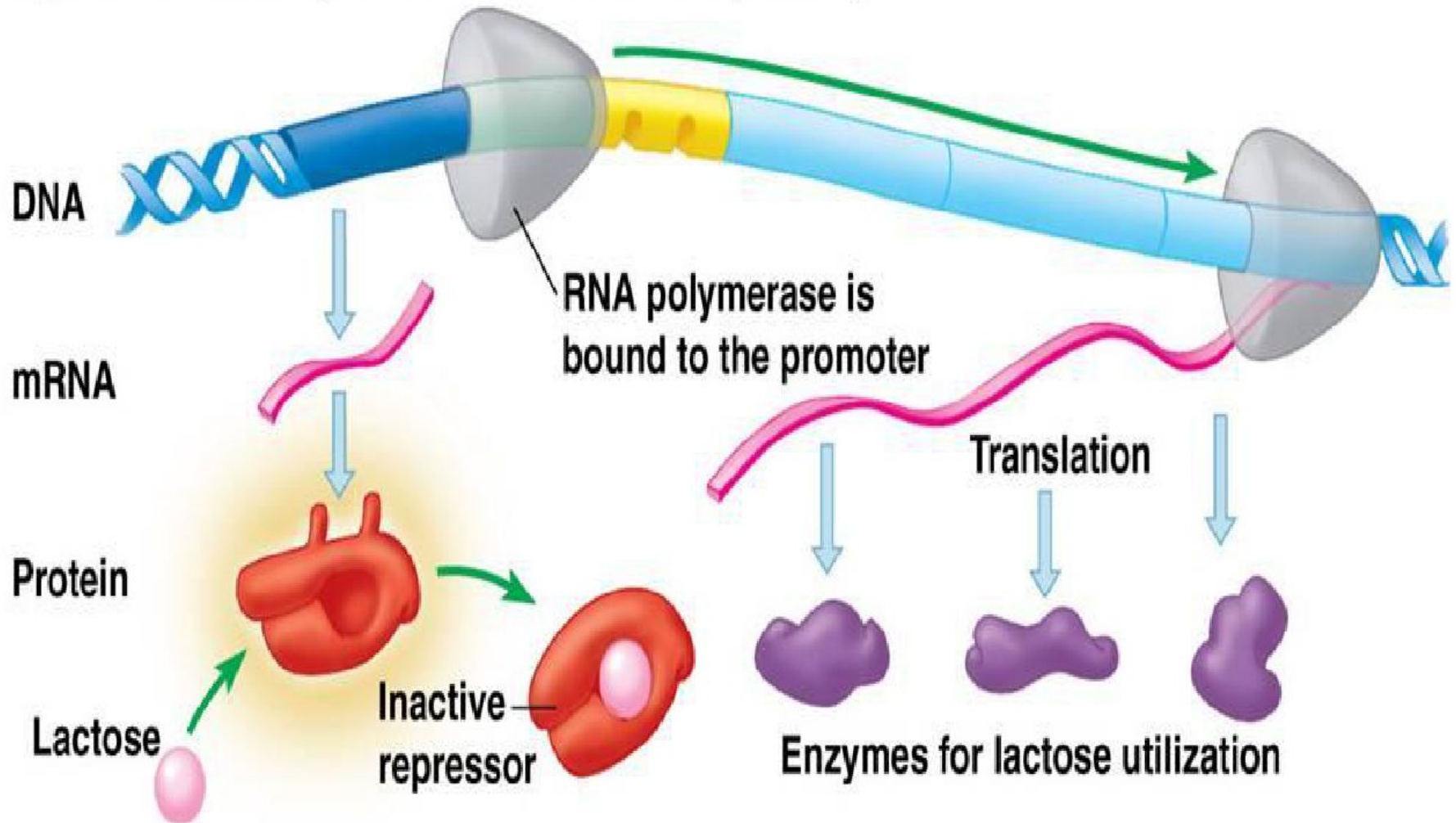
Gene-regulator is far from operon sequences, it is keeper of information about sequence of amino acid residues in protein-repressor (P-R) molecule

Operon turned off (lactose is absent):



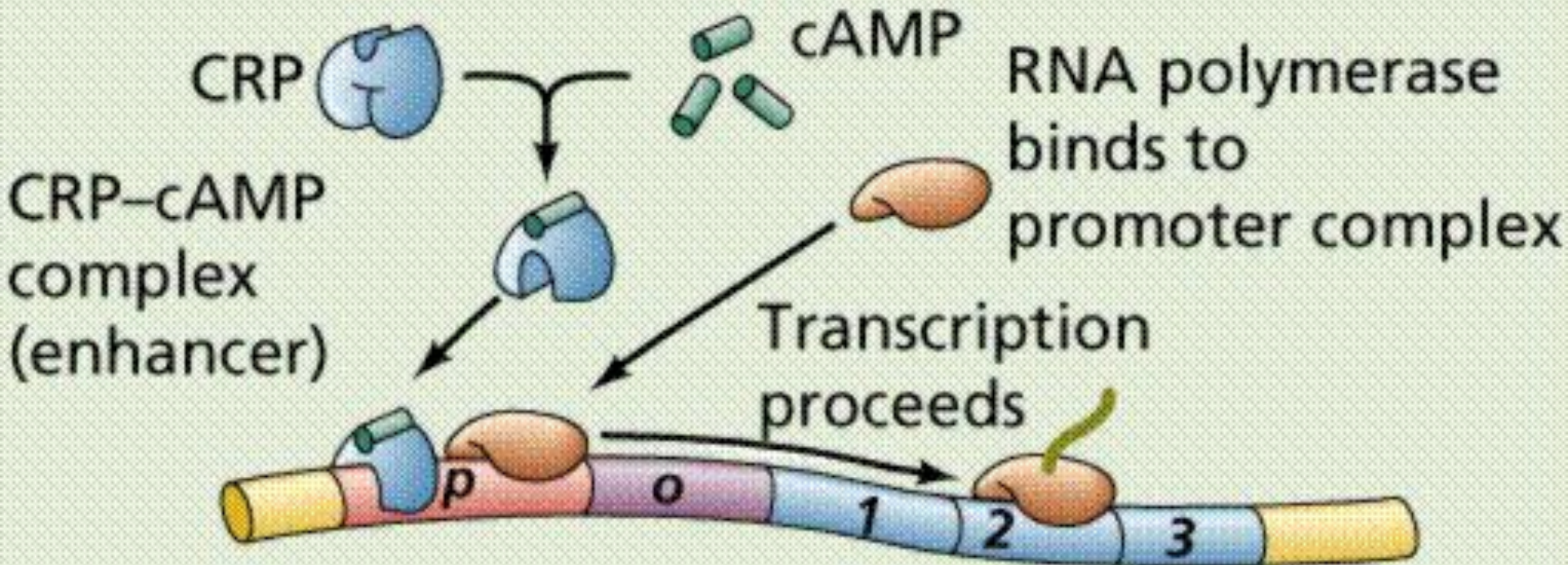
Gene-operator is placed in operon between promoter and structural genes, it has affinity to protein-repressor

Lactose is inducer of transcription made on Lac-operon because of its ability to block activity of P-R and thus to induce mRNA linkage to the promoter
operon turned on (lactose inactivates the repressor):



CRP-cAMP enhancer influence

Low glucose

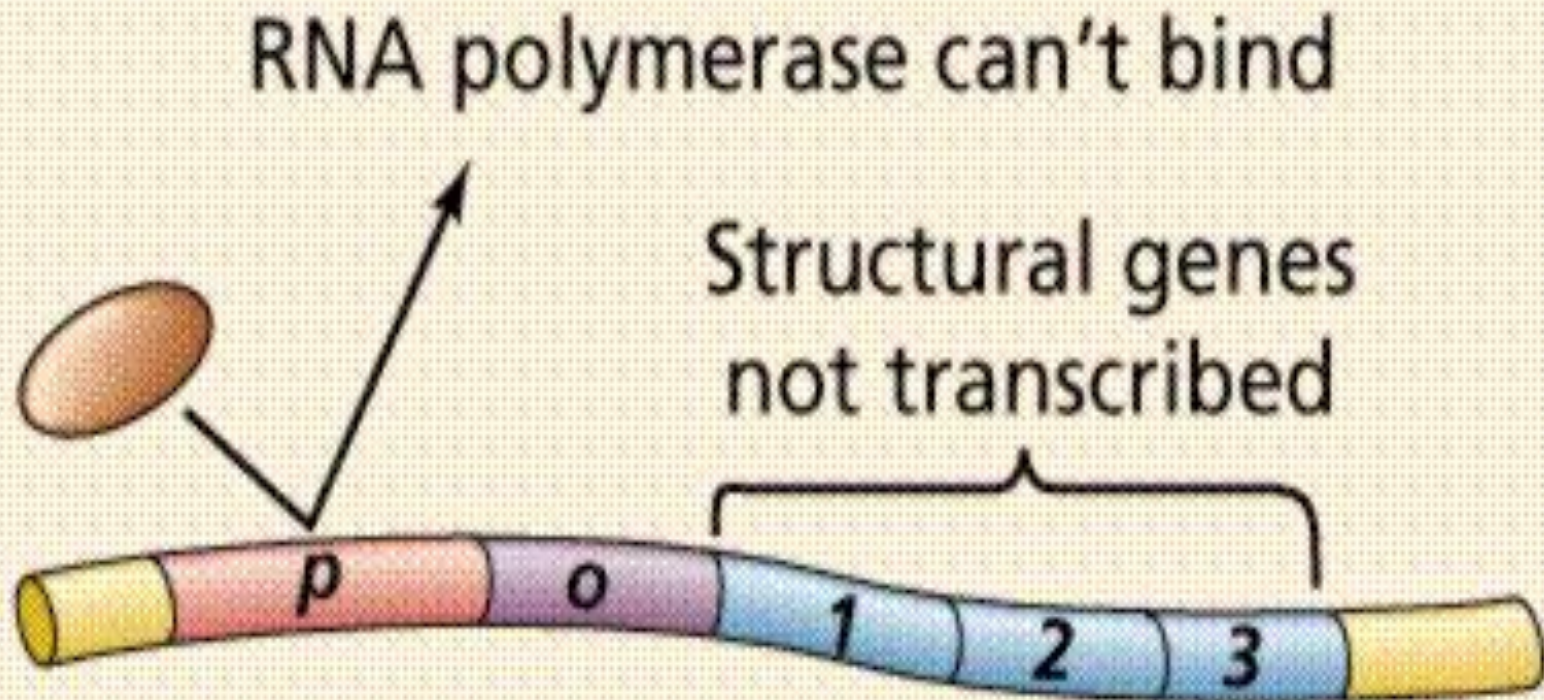


CRP – Catabolite gene Reactive Protein

cAMP – cyclic AMP

**The higher Glucose or Glycerol levels
in the intracellular space
the lower levels of cAMP**

High glucose



Different Genes are found in eukaryotic DNA

- **House keeping genes**
- **Genes required during cellular differentiation**
- **Genes which get triggered as a response to some external factors**
- **Genes which get triggered during apoptosis**

Points for Gene Expression in Eukaryotes

- Synthesis of proteins is controlled right from the chromatin stage.**
- Expression of gene is controlled at many steps during the process of transcription and translation.**

Two forms of chromatin :

- **Euchromatin** – A lesser coiled transcriptionally active region which can be easily accessed by the RNA polymerases.
- **Heterochromatin** – A highly condensed transcriptionally inactive region. The genes in this region cannot be accessed by the RNA polymerases for active transcription .

Mechanisms which affect the chromatin structure and hence the expression of gene are:

- **Acetylation of Histones** : ↑ Acetylation
----↓ Condensation of DNA -----

↑ Transcription of genes in that region

- **Methylation of histone H4 on R4**
(arginine residue at the 4th position) ->->
opens the chromatin structure ->->
leading to transcriptional activation

Mechanisms which affect the chromatin structure and hence the expression of gene are:

- **Methylation of histone H3 on K4 and K79 (lysine residues at the 4th and 79th position) ->-> opens the chromatin structure ->-> leading to transcriptional activation**
- **Methylation of histone H3 on K9 and K27 (lysine residues at the 9th and 27th position) ->-> condenses the chromatin structure ->-> leading to transcriptional inactivation**

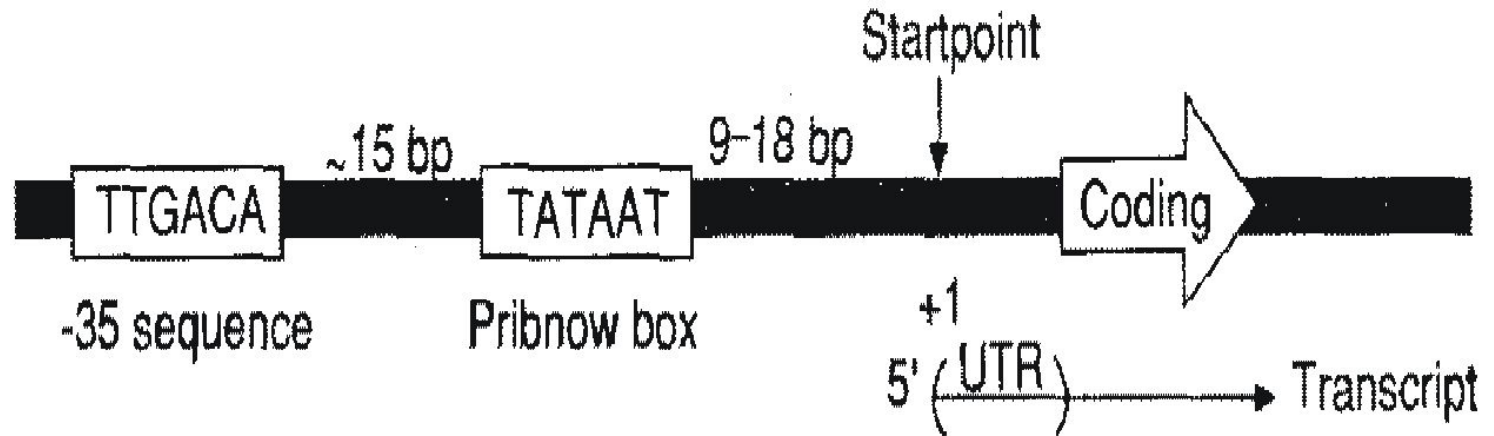
Ubiquitination

- Ubiquitination of H2A – Transcriptional inactivation
- Ubiquitination of H2B - Transcriptional activation

Methylation of DNA

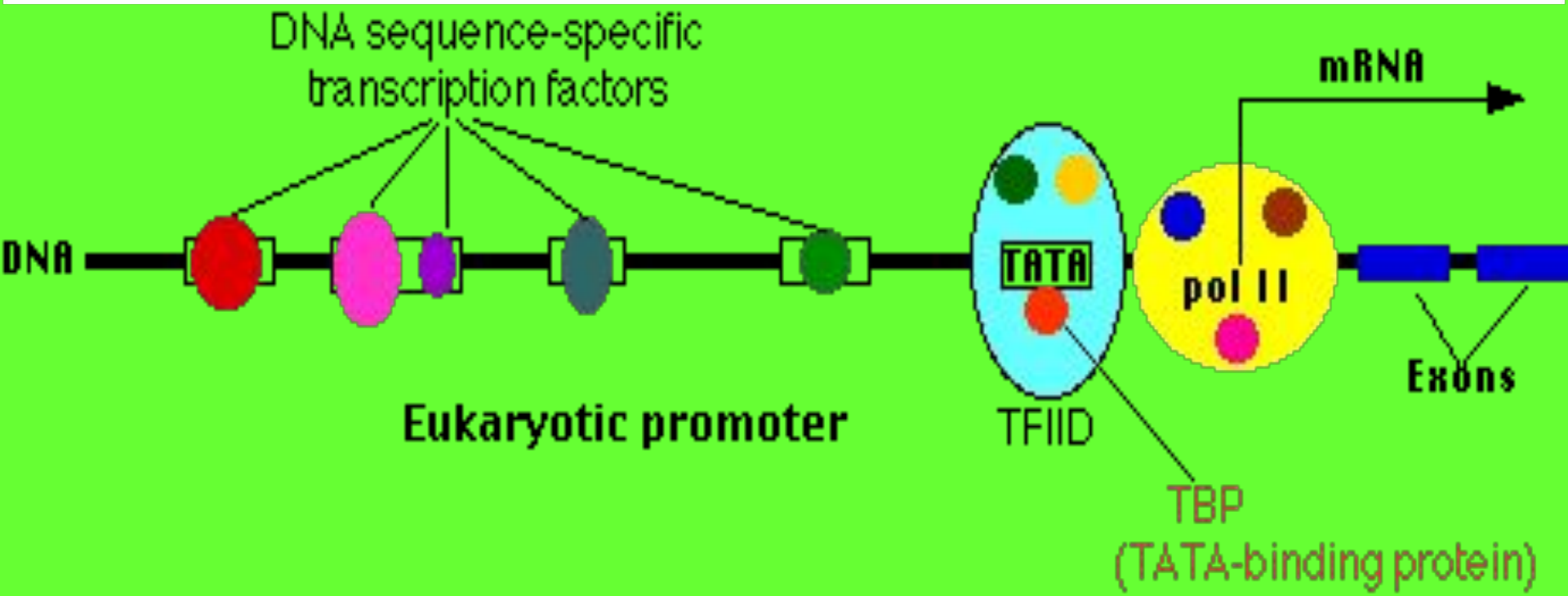
- Target sites of methylation are - The cytidine residues which exist as a dinucleotide, CG (written as CpG)
- ↑ methylated cytidine -- ↓ Transcriptional activity

A. Prokaryotic promoter

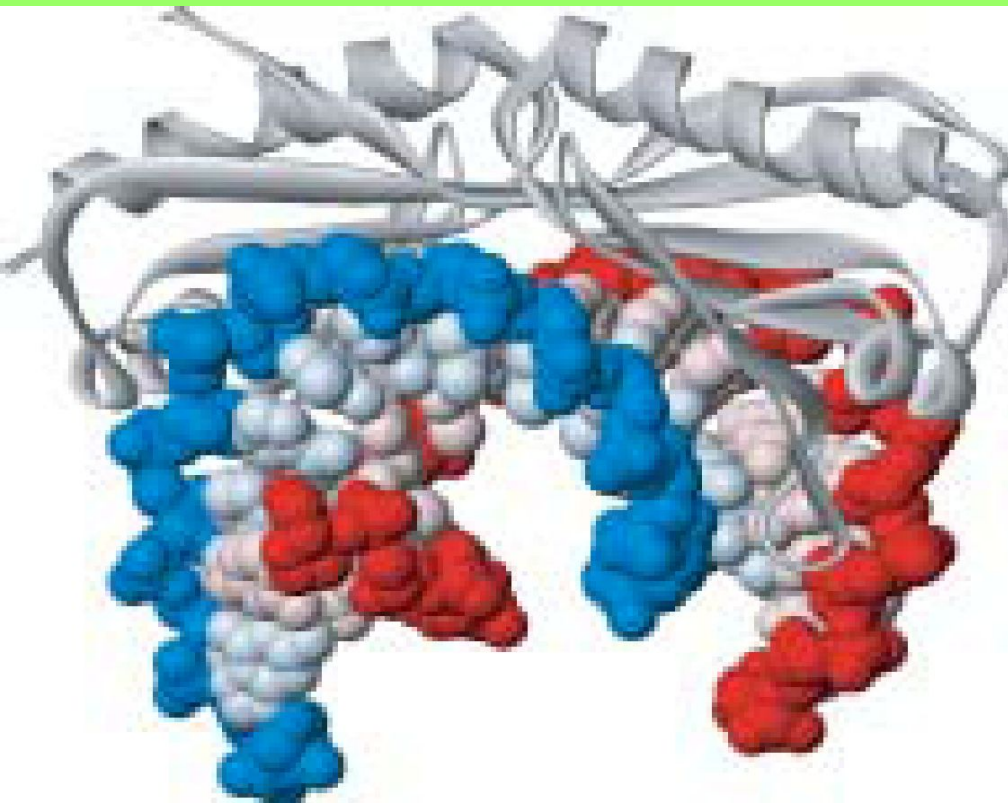


3'

5'

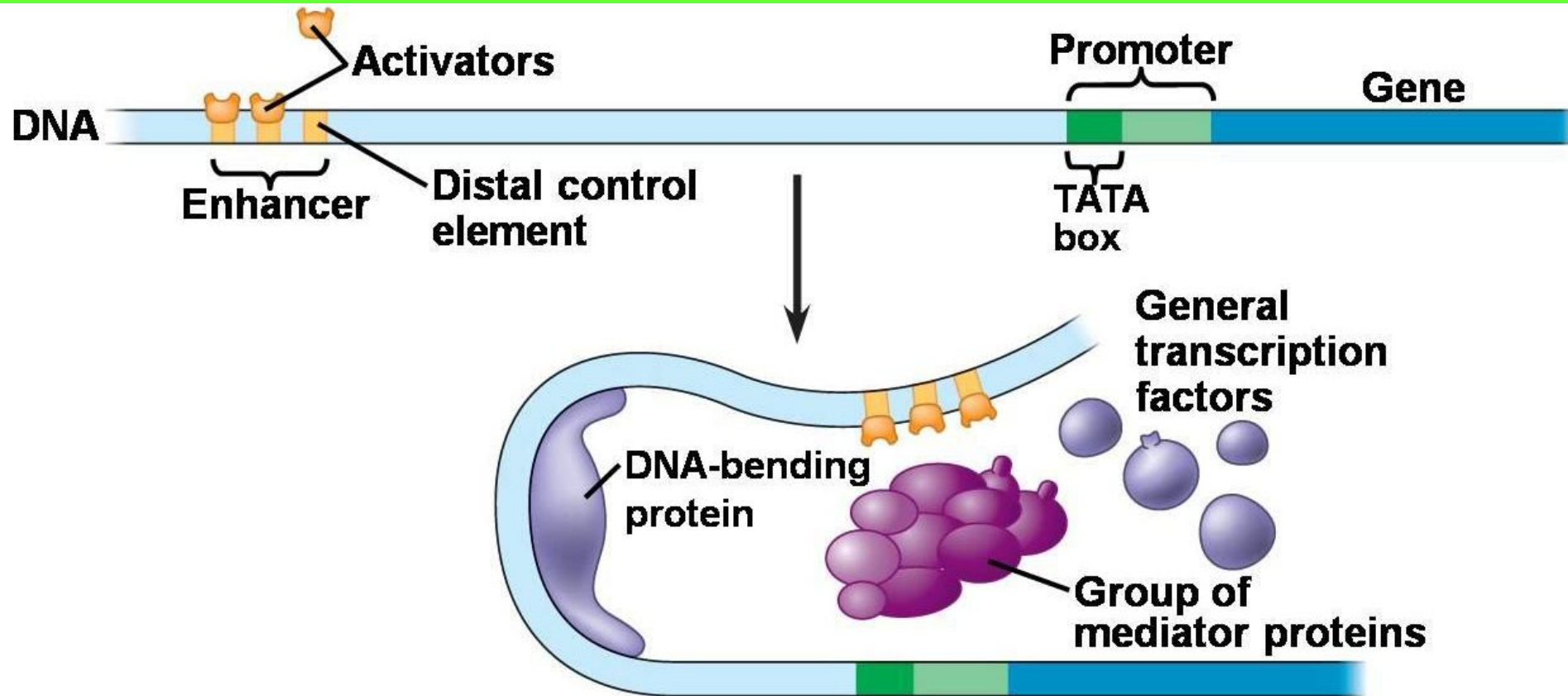


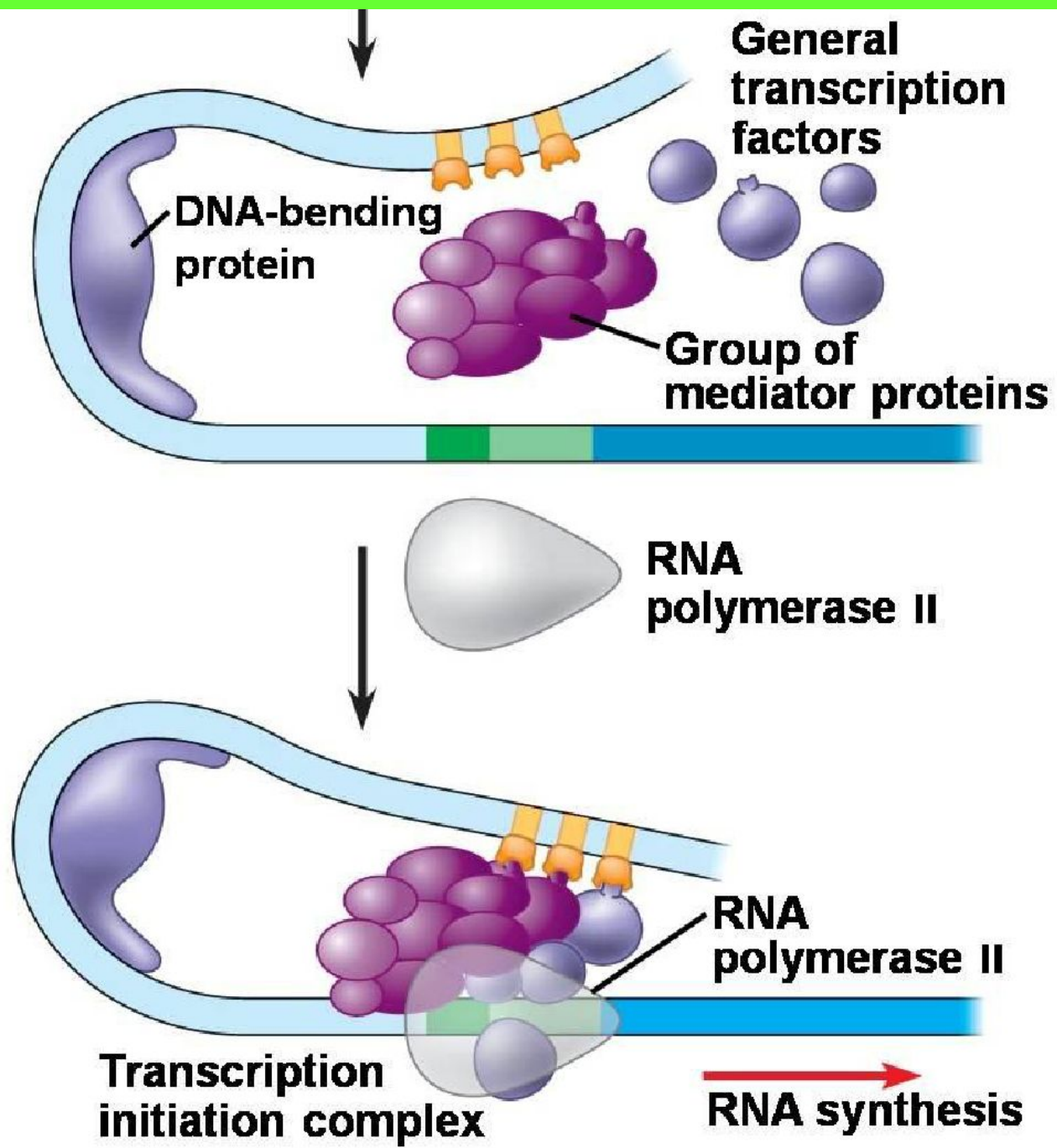
TATA-box binding protein (TBP) is found in eukaryotic cells, and it is the component of the complex TFIID containing other several proteins (TBP-associated factors) and bound to the TATA box



TATA-box binding protein (TBP)

Enhancer-bending protein (EBP) changes the DNA single strand conformation to form special loop which promotes the stimulation and the increase of the rate of initiation phase of transcription.

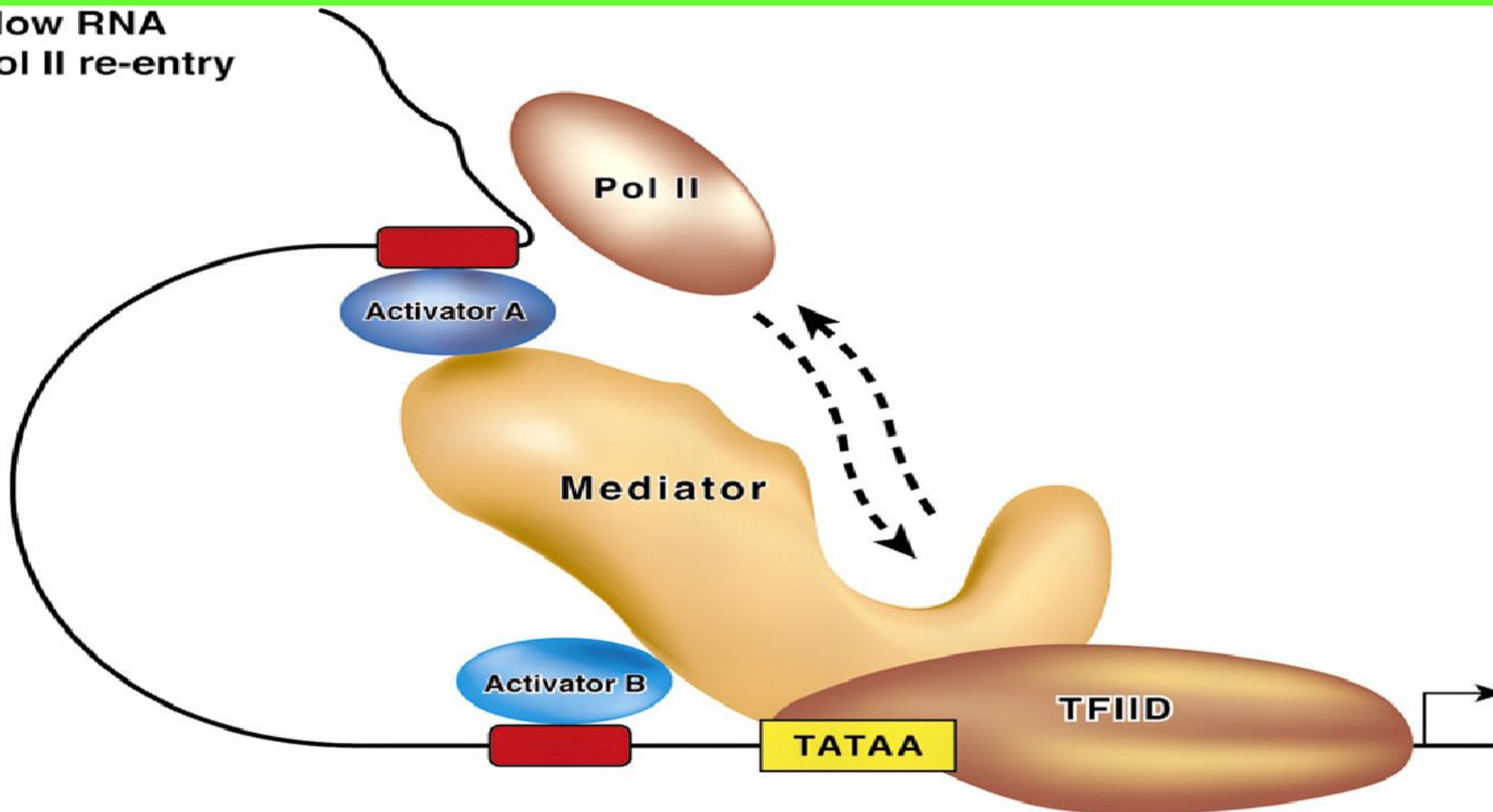




Except EBP and TF, there is the group of mediator proteins to stimulate transcription process, too

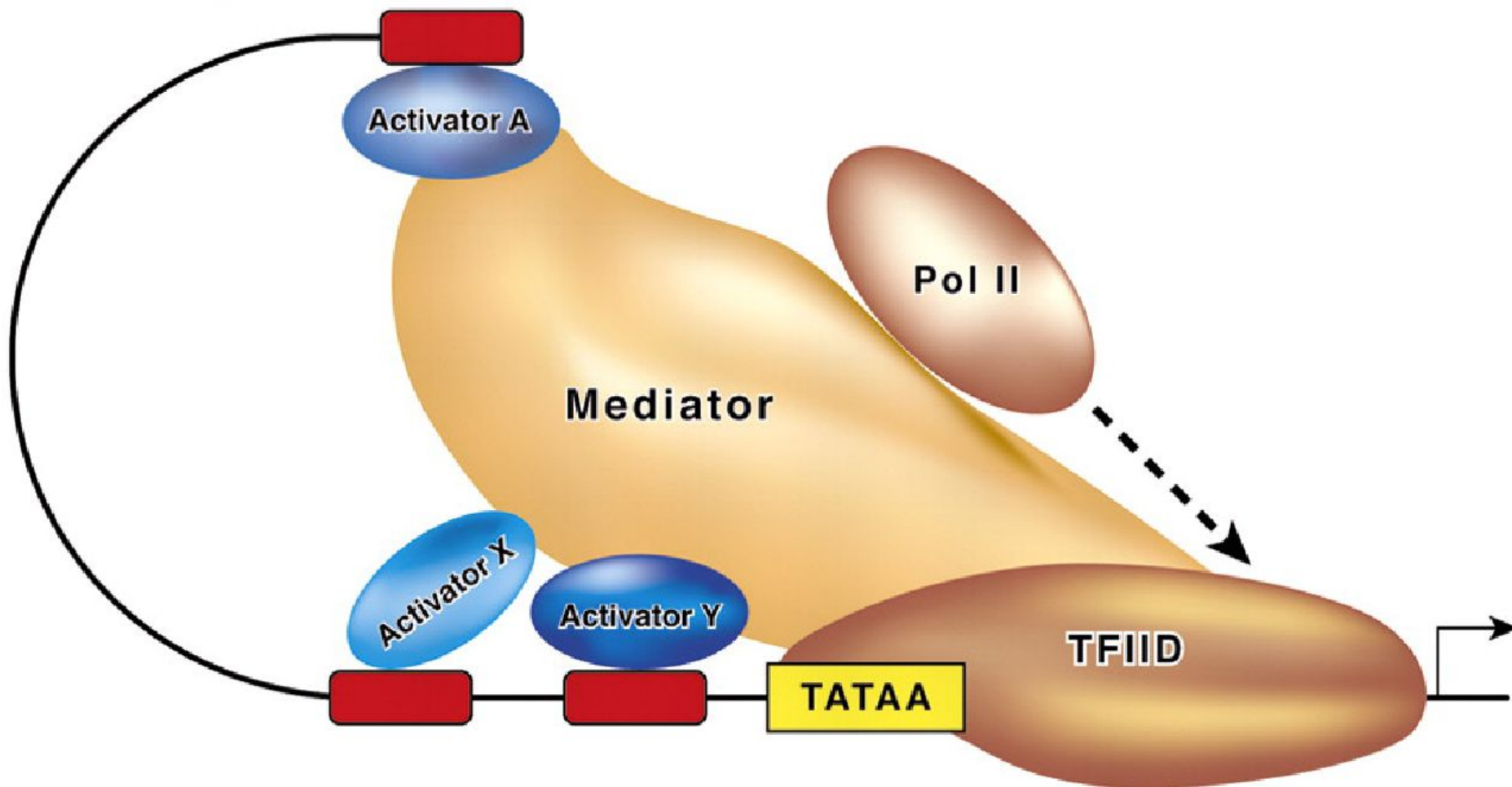
Proteins-mediators can control the rate of transcription due to their ability to change conformation of their molecules

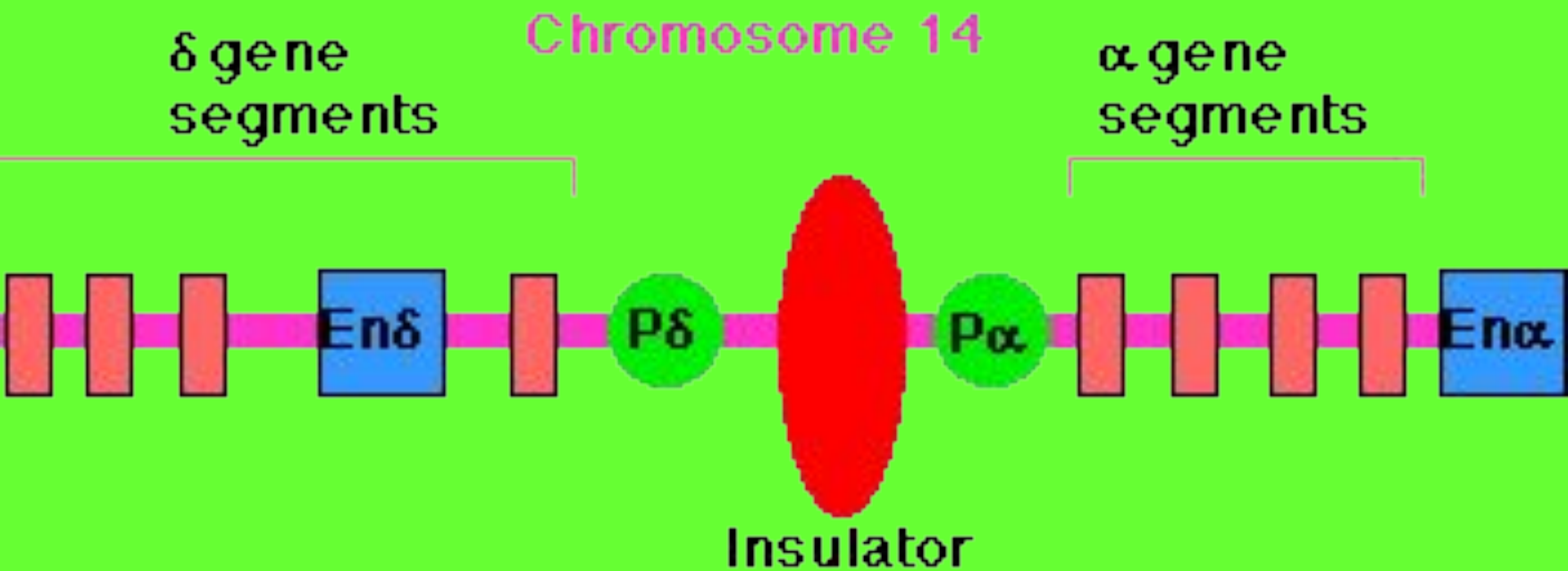
Slow RNA
Pol II re-entry



Proteins-mediators are in close relations with general transcription factors placed in the complex TFIID

Fast RNA
Pol II re-entry

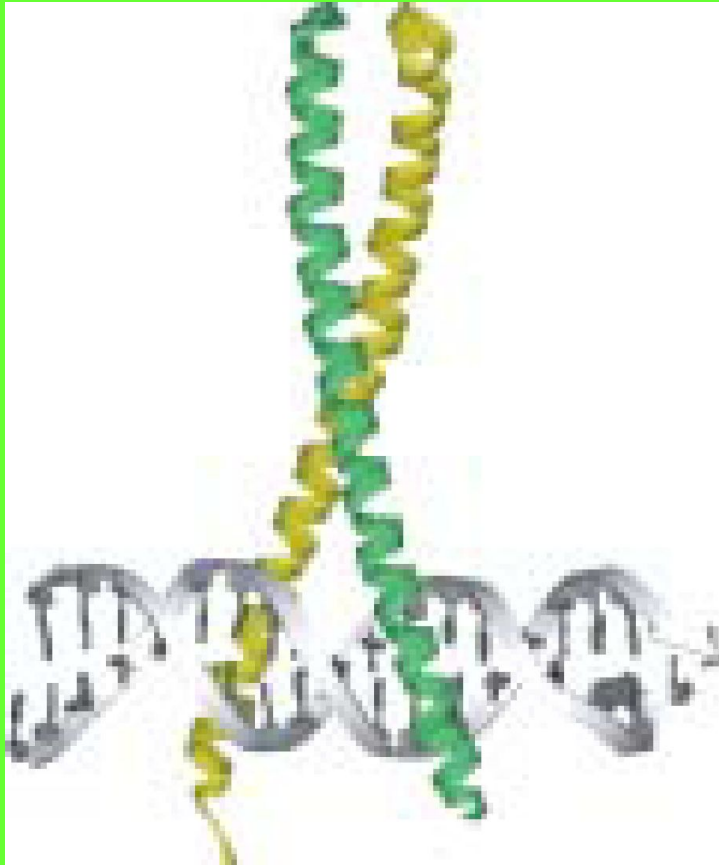




P = promoter

En = enhancer

Interaction of homodimeric leucine-zipper (A) and basic helix-loop-helix (B) proteins with DNA

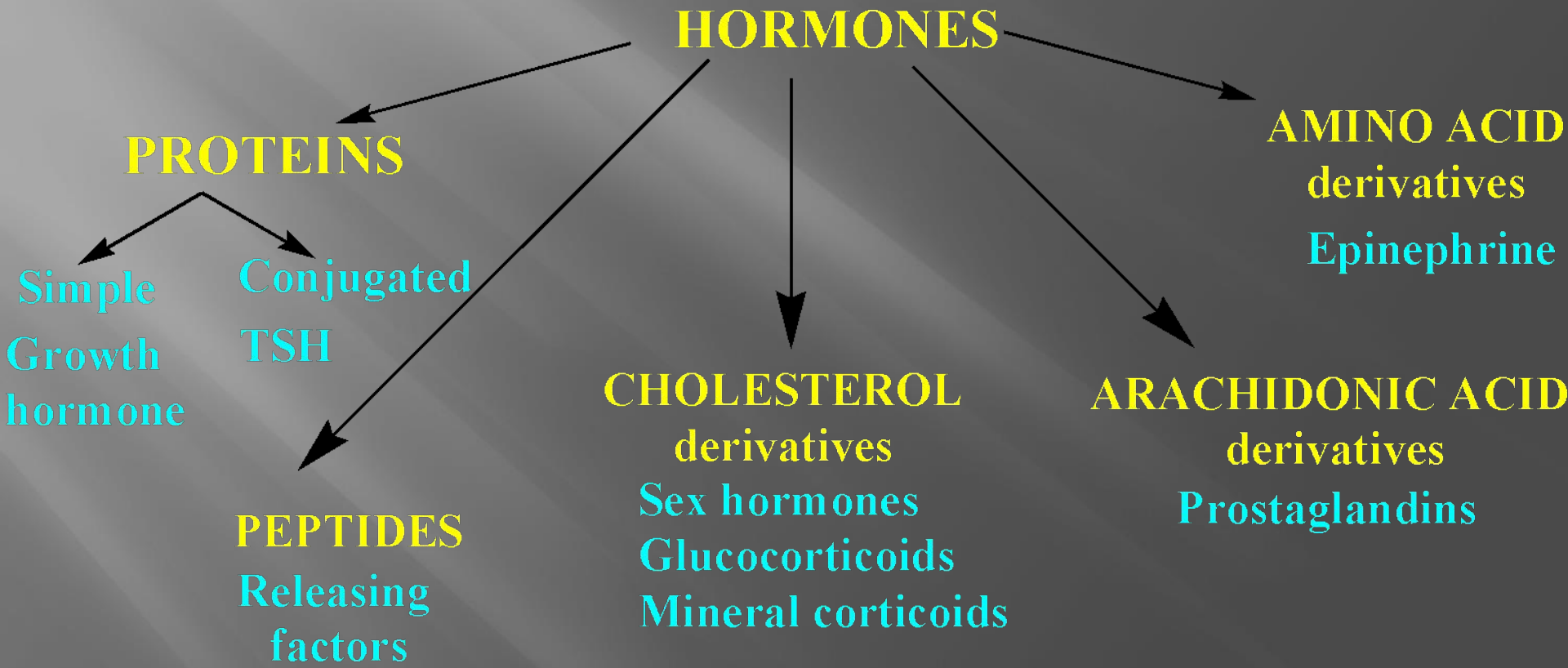


(A)

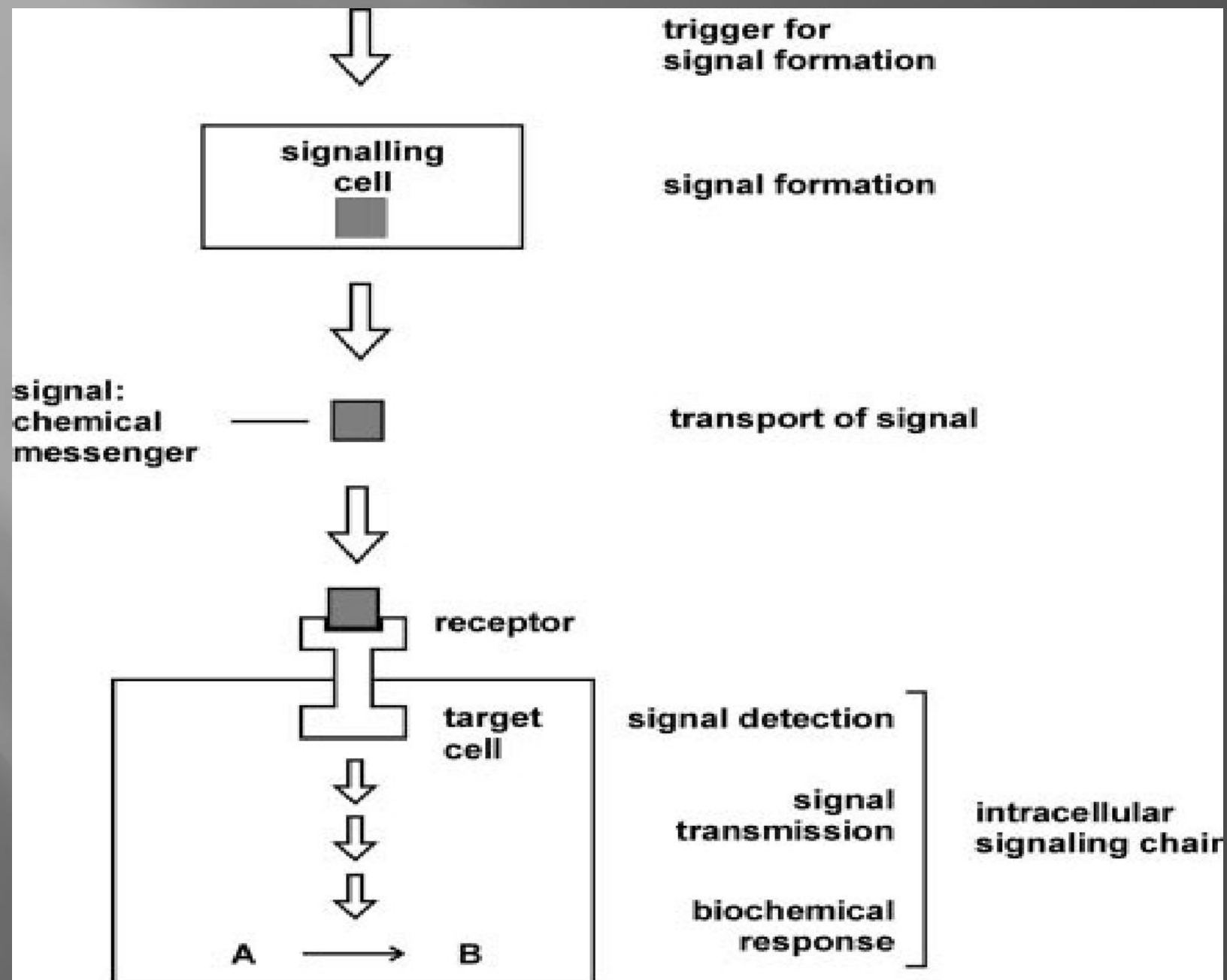


(B)

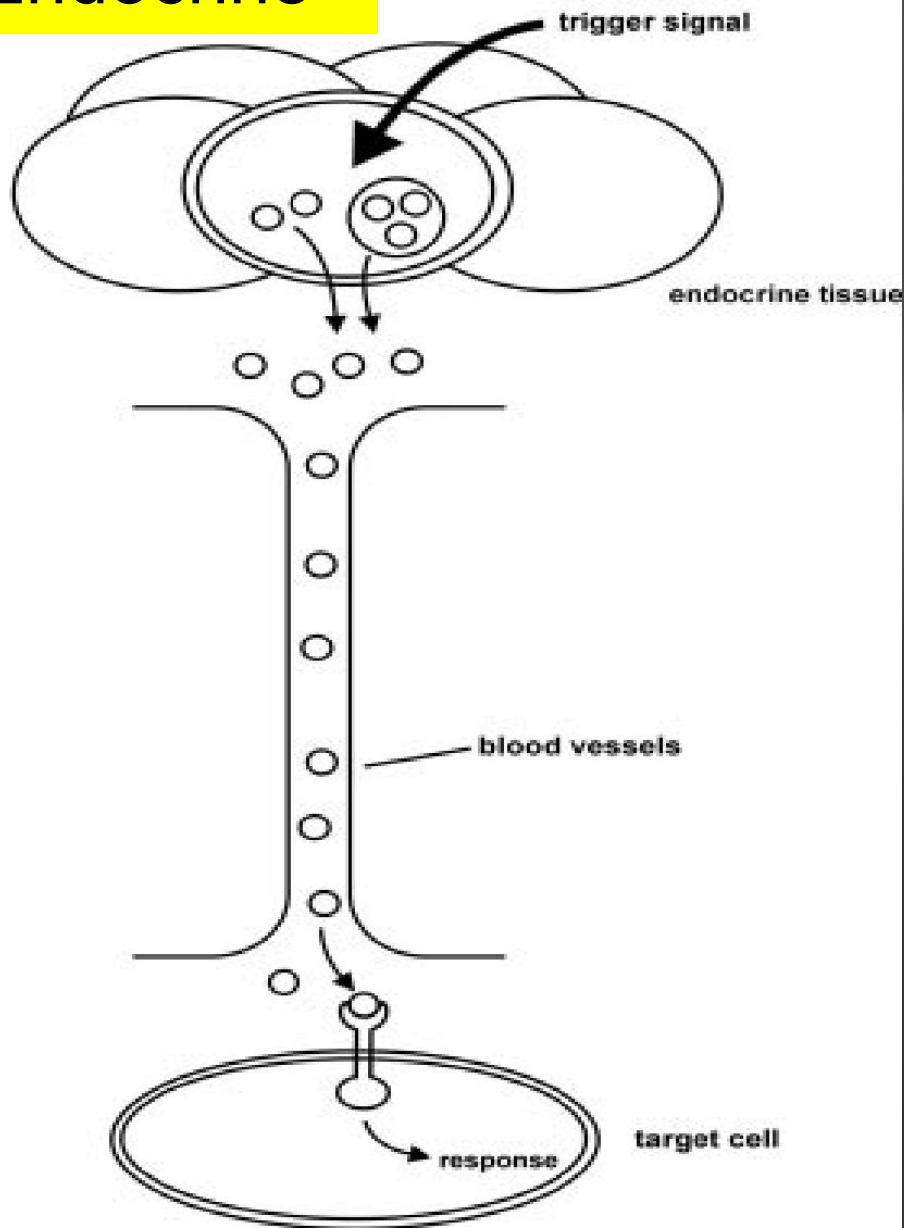
Classification of hormones according chemical nature



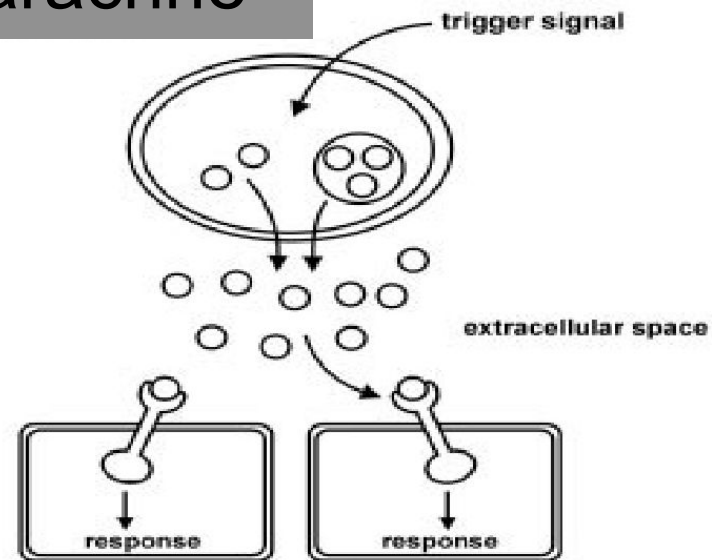
INTERCELLULAR MECHANISM of COMMUNICATION



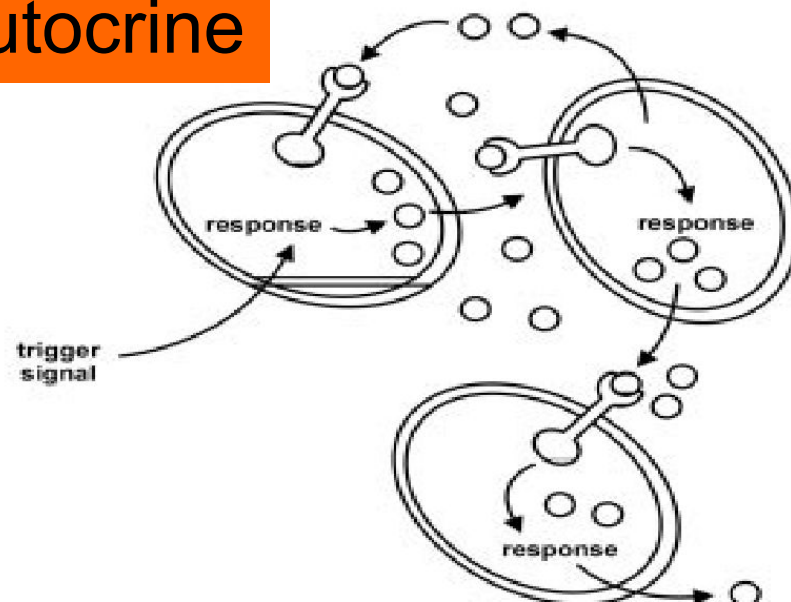
Endocrine



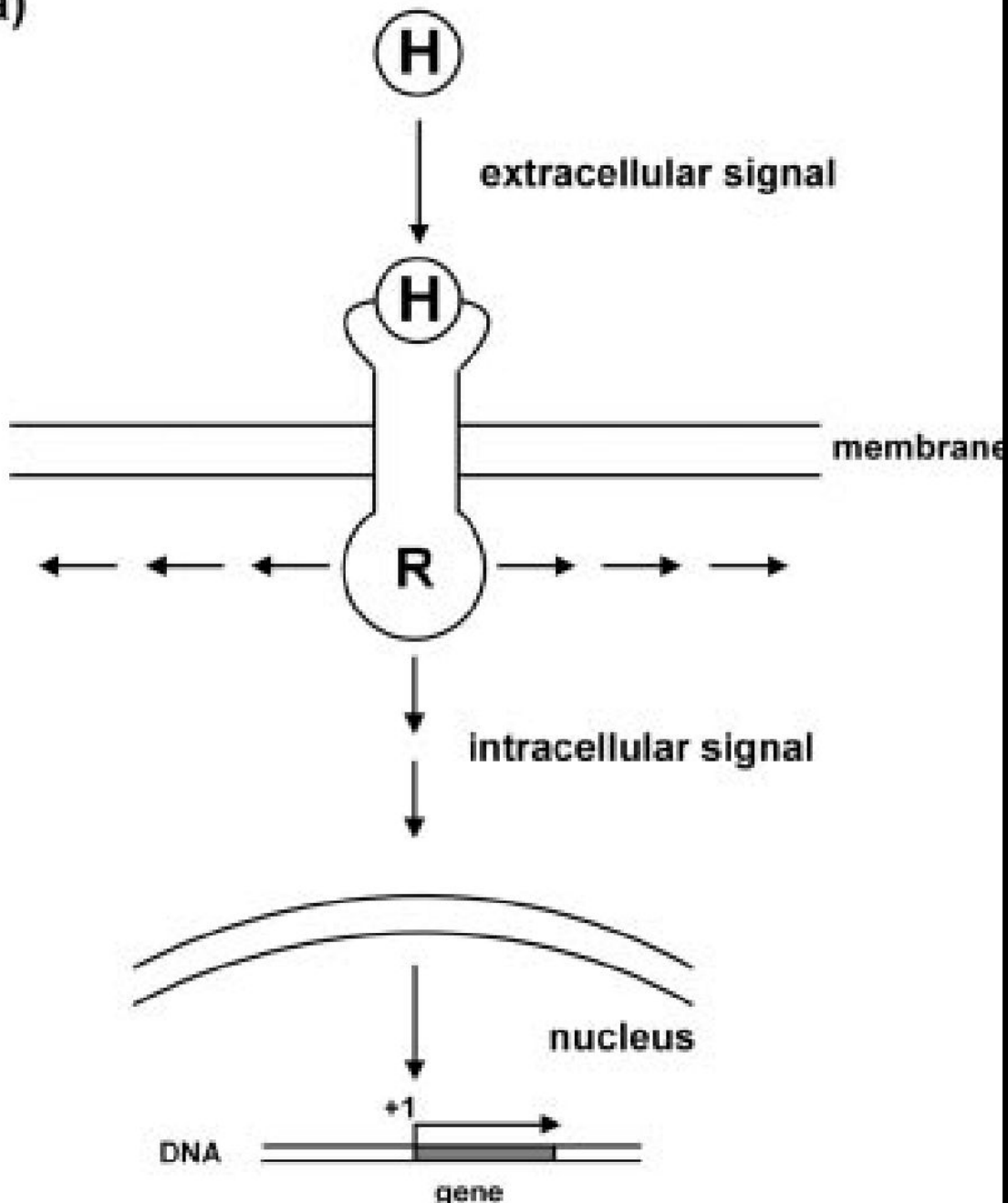
Paracrine



Autocrine

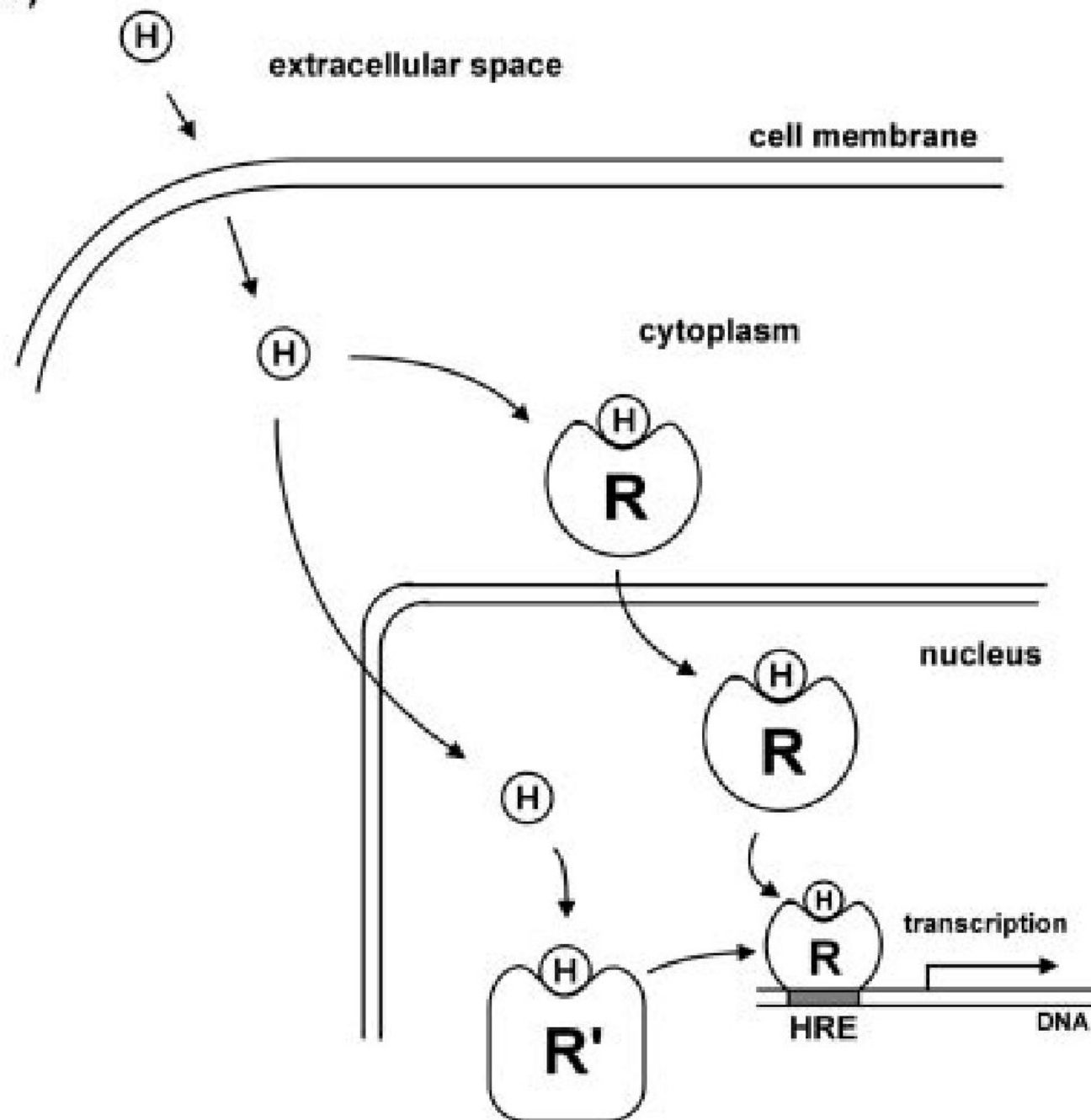


a)

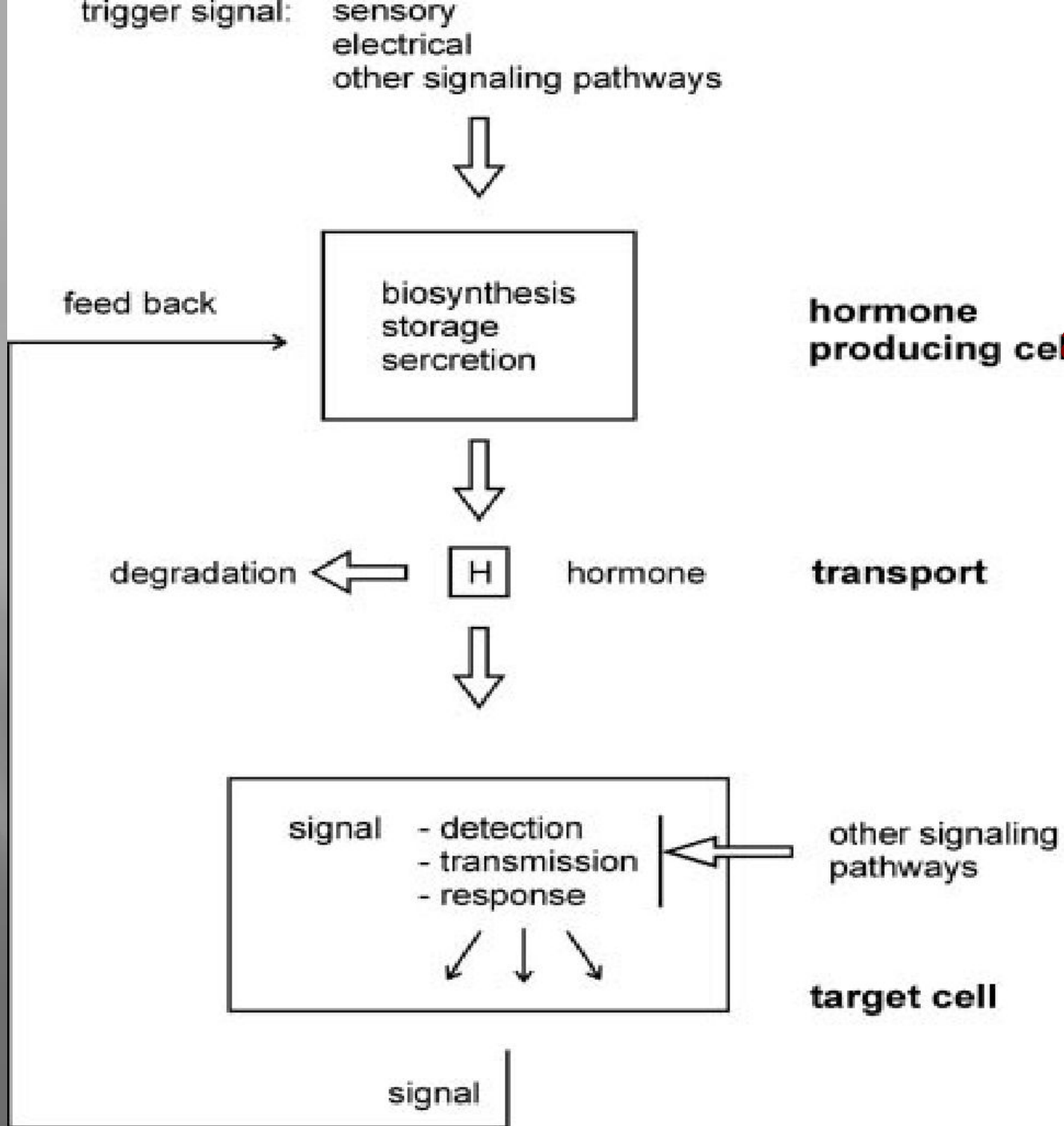


The receptor (**R**)
for
hydrophilic
hormones (H)
is located
in the cellular
membrane
of target cell

b)



Lipophilic hormones (H) may be linked to cytoplasmic (R) and nuclear (R') receptors



The feed-forward and feed-back control of a hormone level in the blood

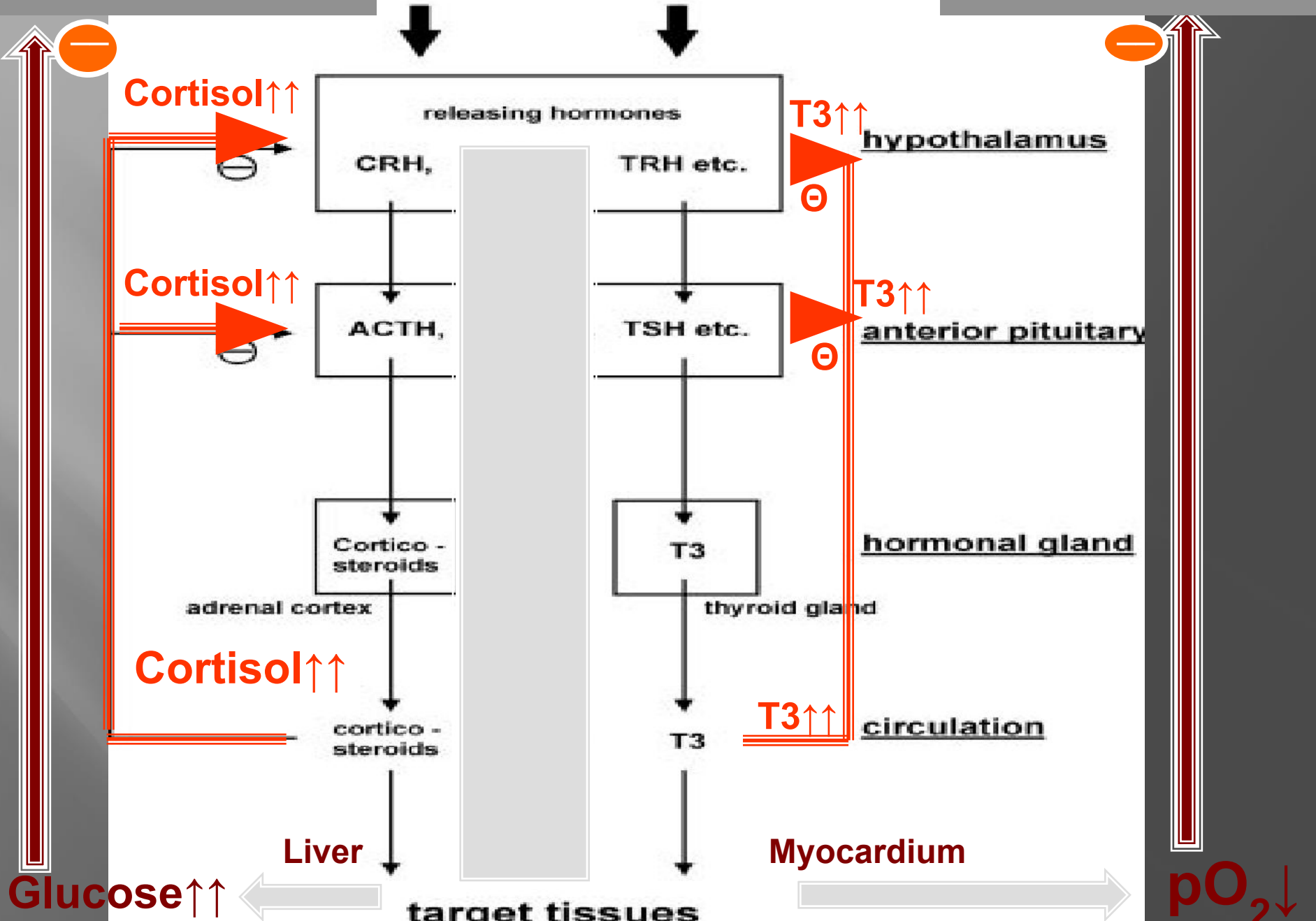
**All of the steps below
are subject to regulation:**

- **biosynthesis of the hormone**
- **storage, secretion of the hormone**
- **transport of the hormone to the target cell**
- **reception of the signal by the hormone receptor**
- **transmission and amplification of the signal, biochemical reaction in the target cell**
- **degradation and excretion of the hormone**

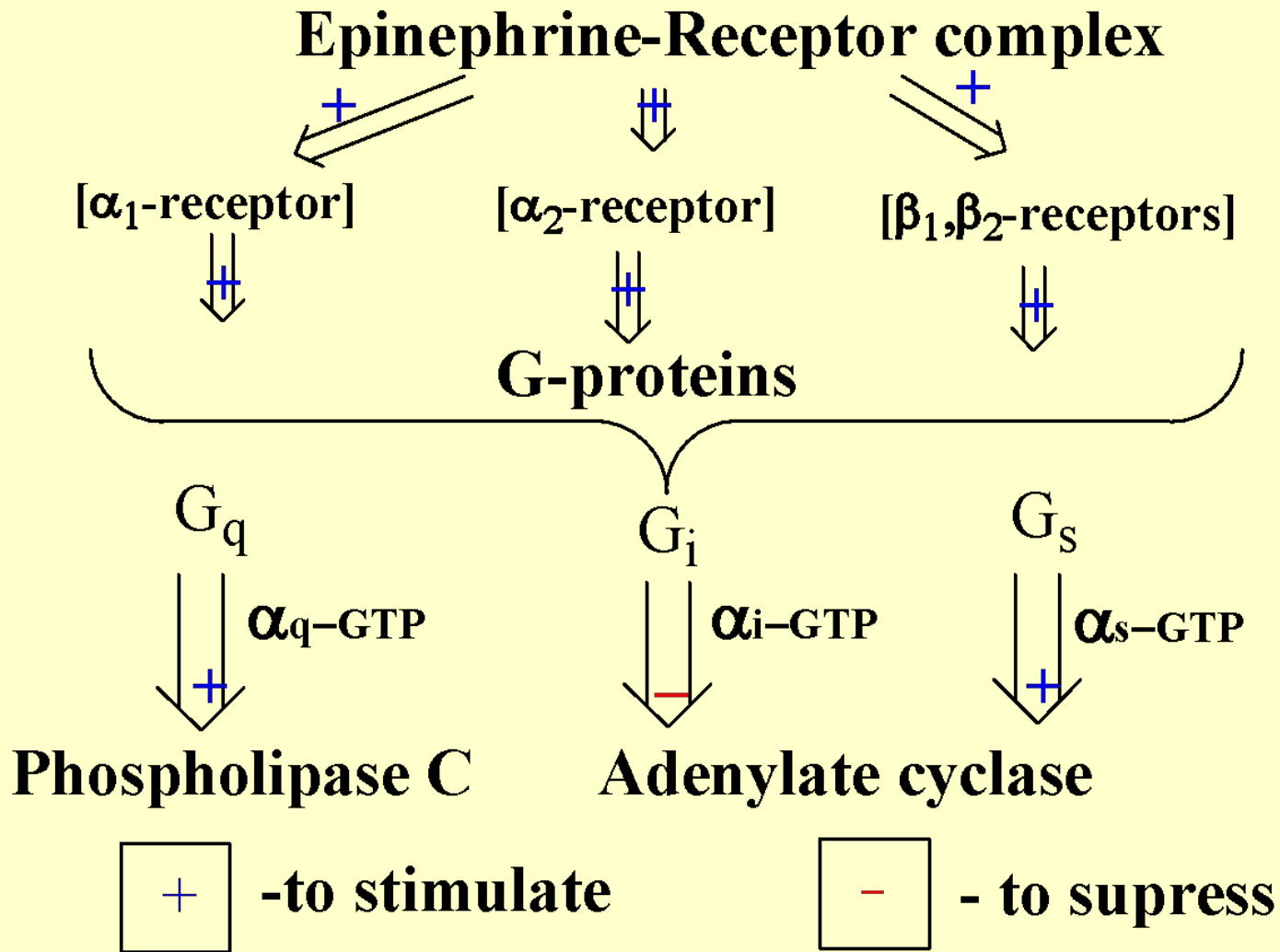
Cerebral cortex

stress neuronal signals

Cerebral cortex

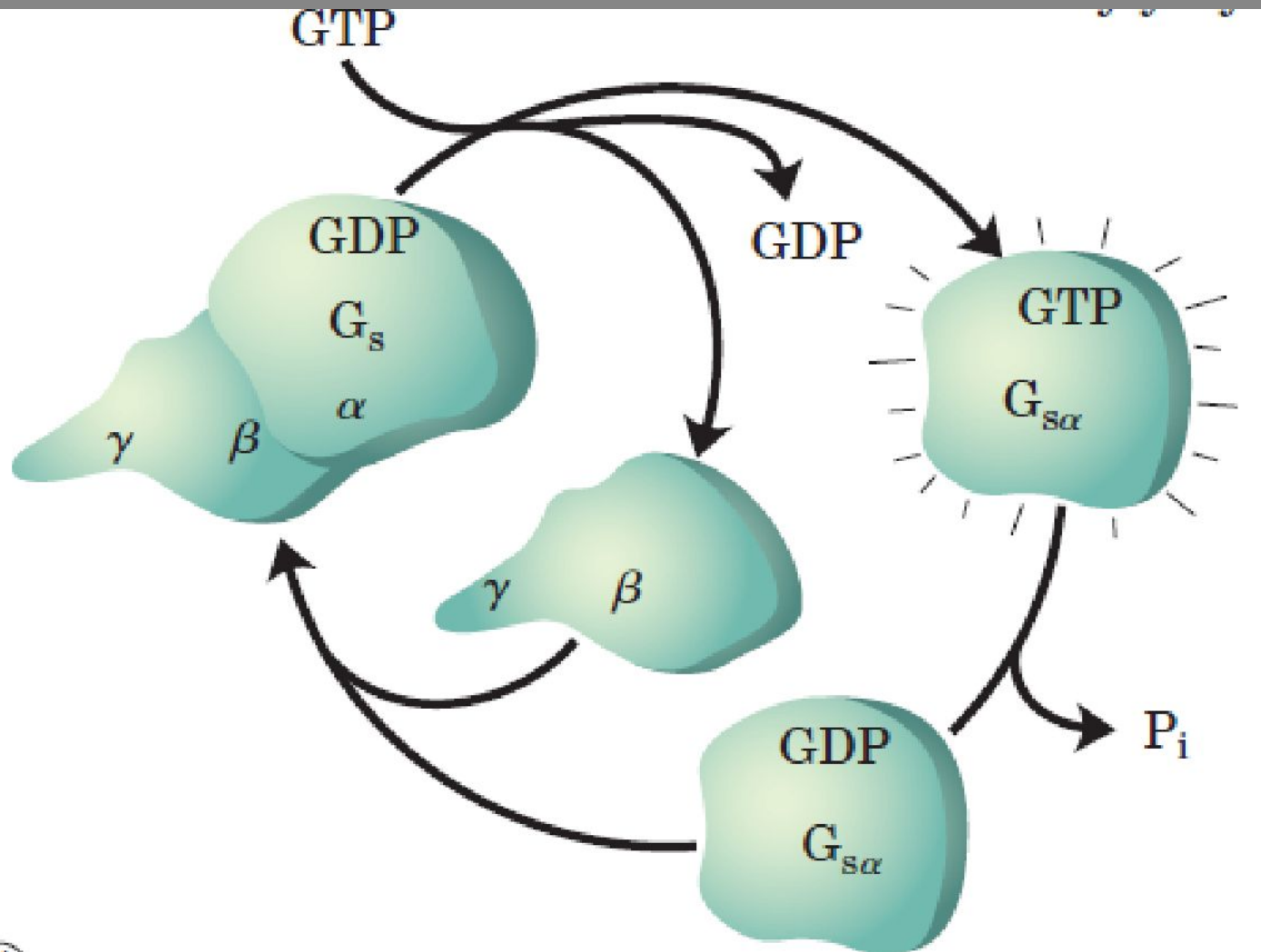


Types of signal transmission due to G-proteins



Inactive Gs protein is composed from three subunits: α , β , γ .

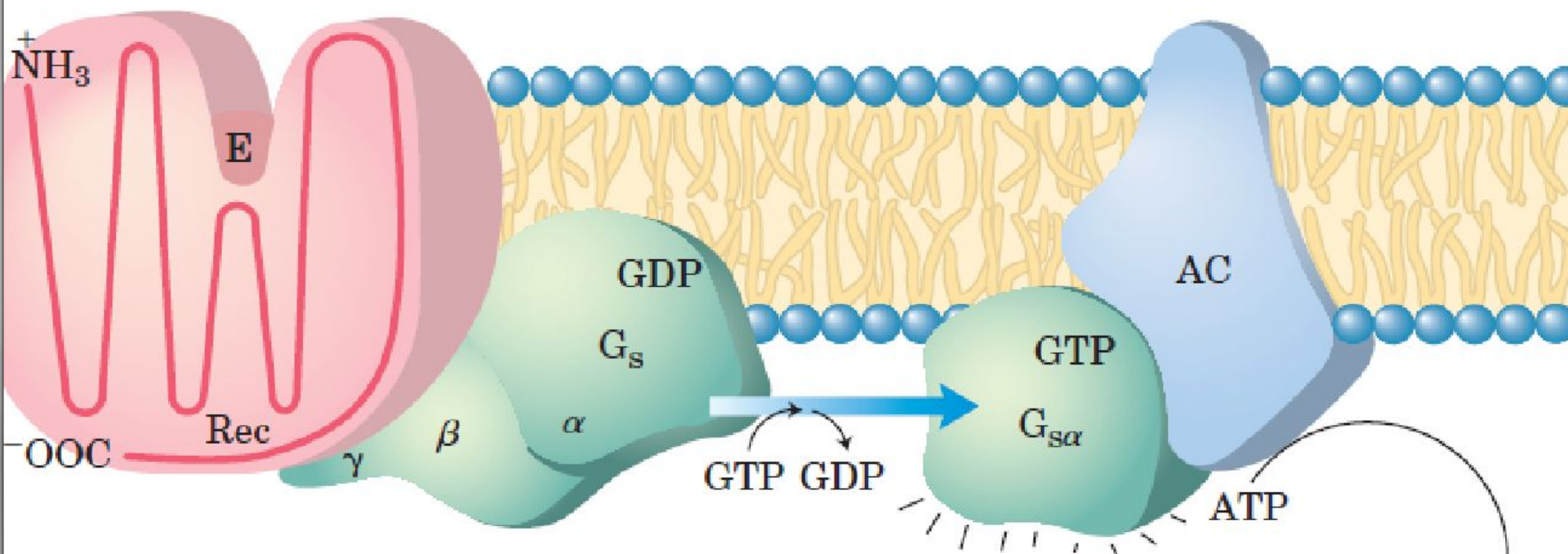
Hormone-receptor complex can stimulate Gs - it means :
dissociation of Gs to dimer and single α -subunit linked
to GDP that is formed from GTP



Gs α -GTP
is named
active Gs
protein

①

Epinephrine binds to its specific receptor.



②

The occupied receptor causes replacement of the GDP bound to G_s by GTP, activating G_s .

③

G_s (α subunit) moves to adenylyl cyclase and activates it.

④

Adenylyl cyclase catalyzes the formation of cAMP.

cAMP

Some factors influenced G-proteins

- **Cholera toxin** modifies α -subunit of G_s as the result - the block of hydrolysis of GTP to GDP and superstimulation of Adenylate cyclase
- **Pertussis toxin** (produced at whooping cough) modifies α -subunit of G_i to allow Adenylate cyclase to produce cAMP in excess levels

GAP function:

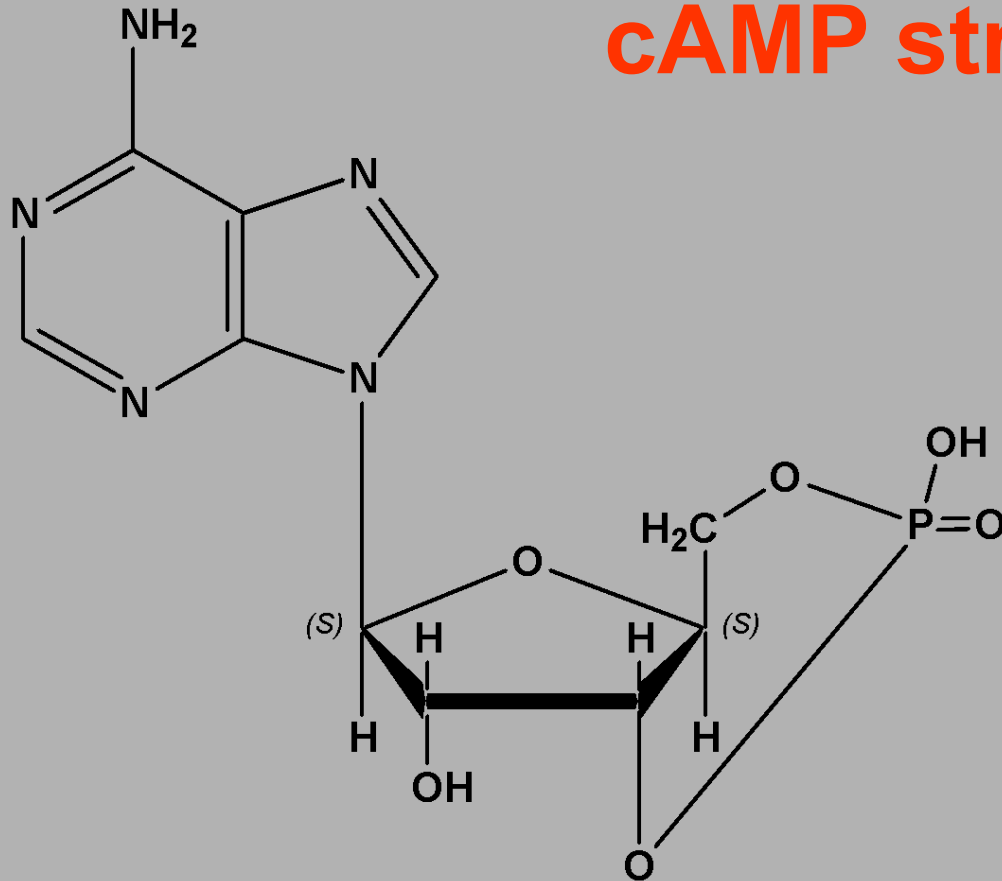
GTPase-Activating Proteins, or GAPs

can bind to activated G-proteins and stimulate their GTPase activity, with the result of terminating the signaling event.

GAPs are also known as regulator of G protein signaling proteins, or RGS proteins, and these proteins are crucial in controlling the activity of G proteins.

GAP role is to turn the G protein activity off .

cAMP structure



PDE

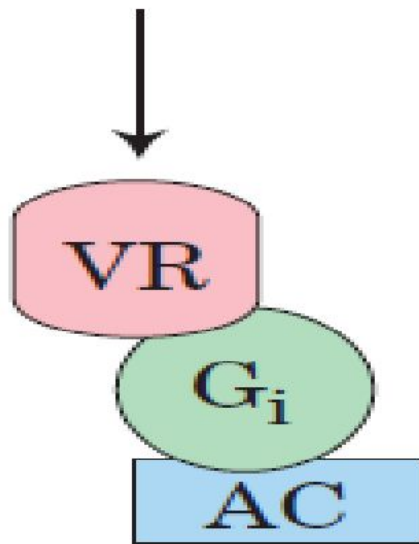
cAMP

AMP

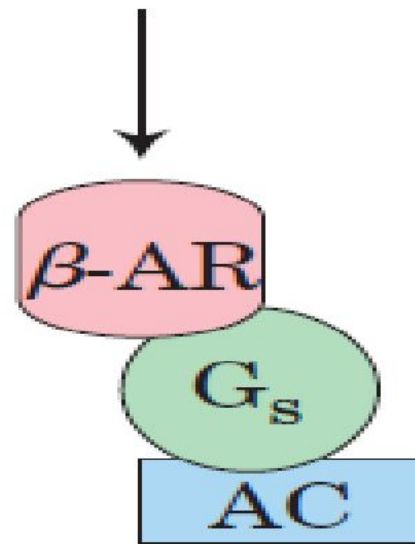
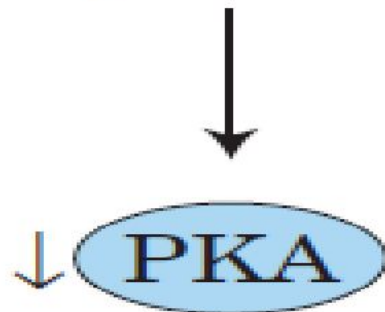
PDE – Phosphodiesterase
Inhibitors: methyl xanthines

Vasopressin

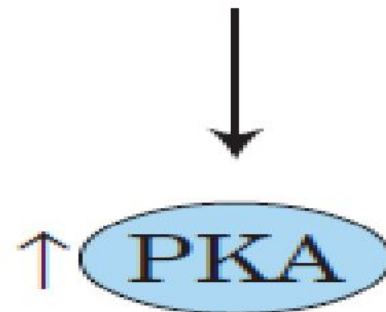
Epinephrine



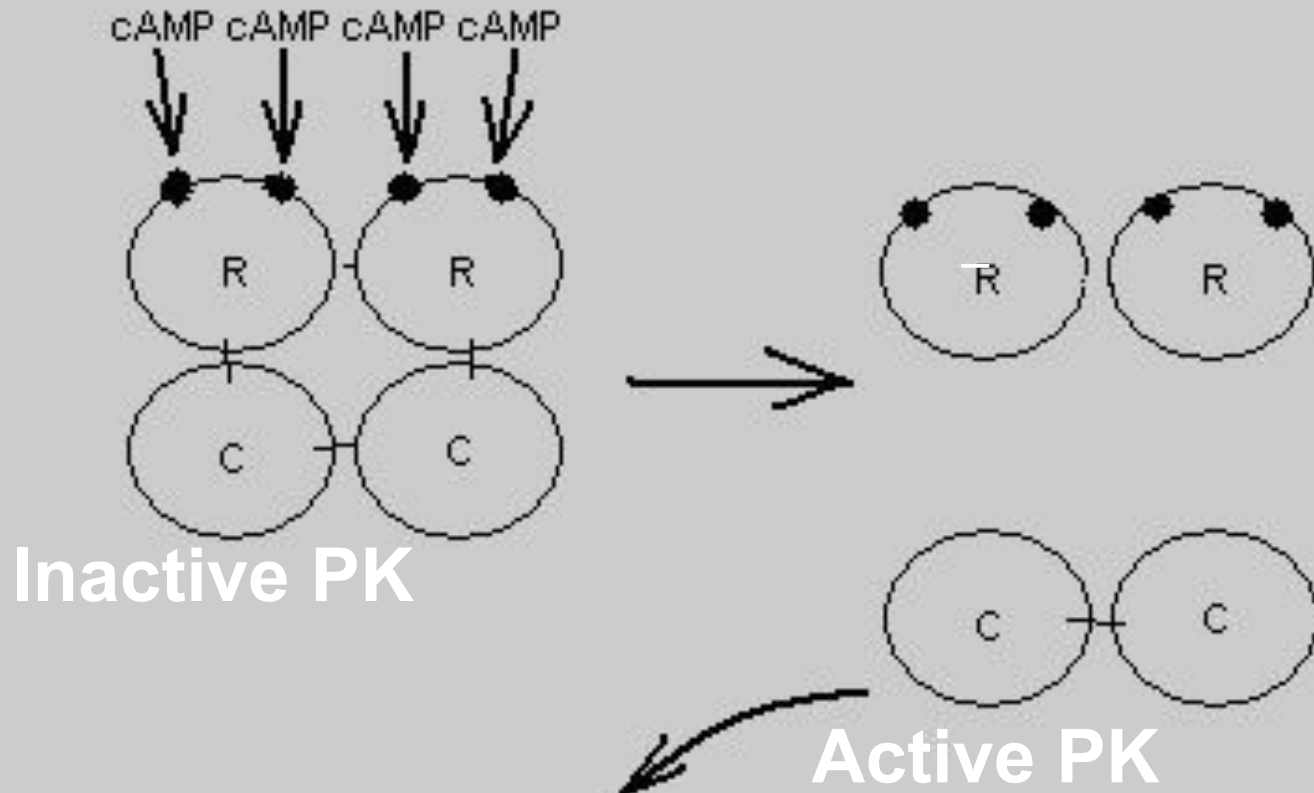
↓[cAMP]



↑[cAMP]



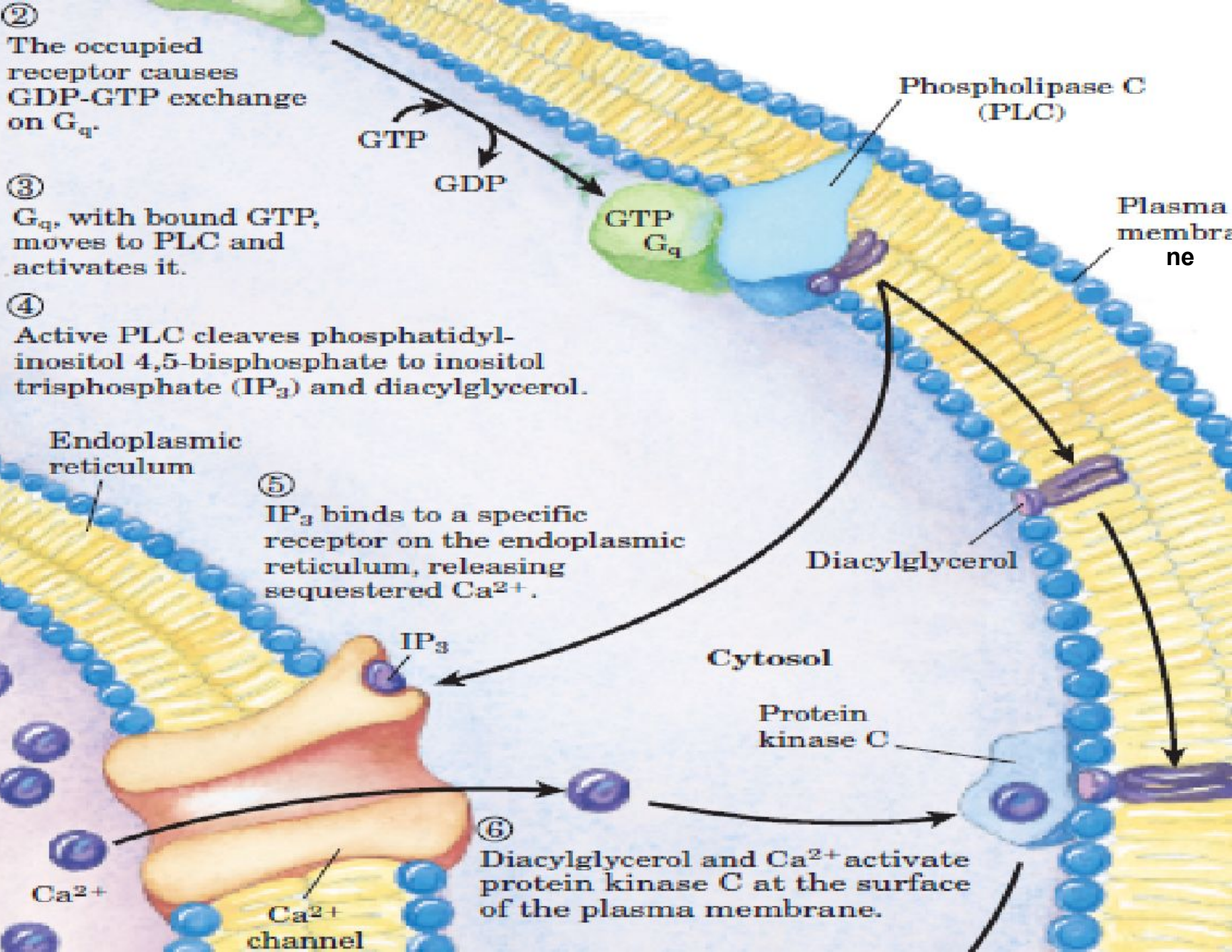
cAMP-dependent protein kinase (PK) activation



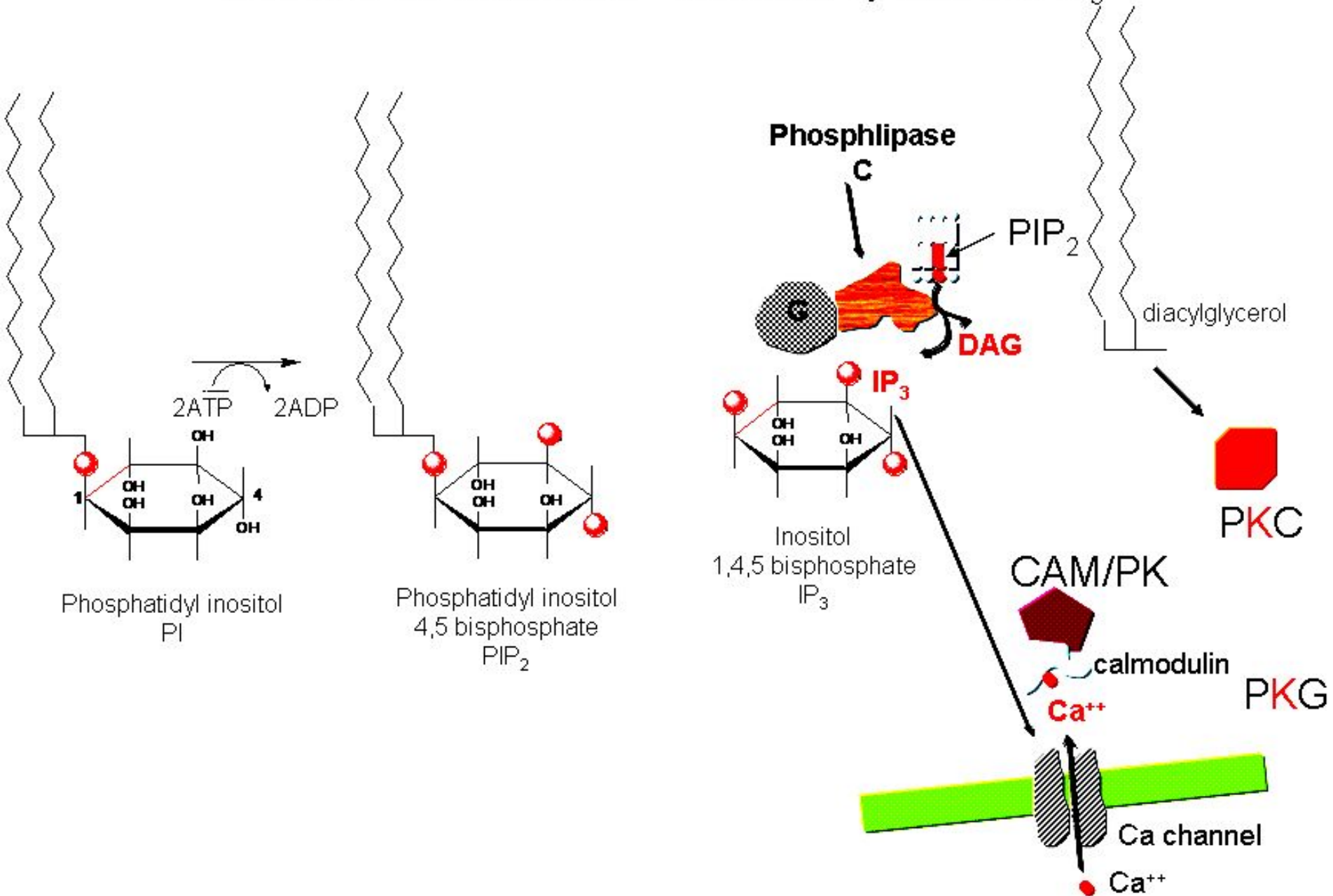
Glycogen phosphorilase b

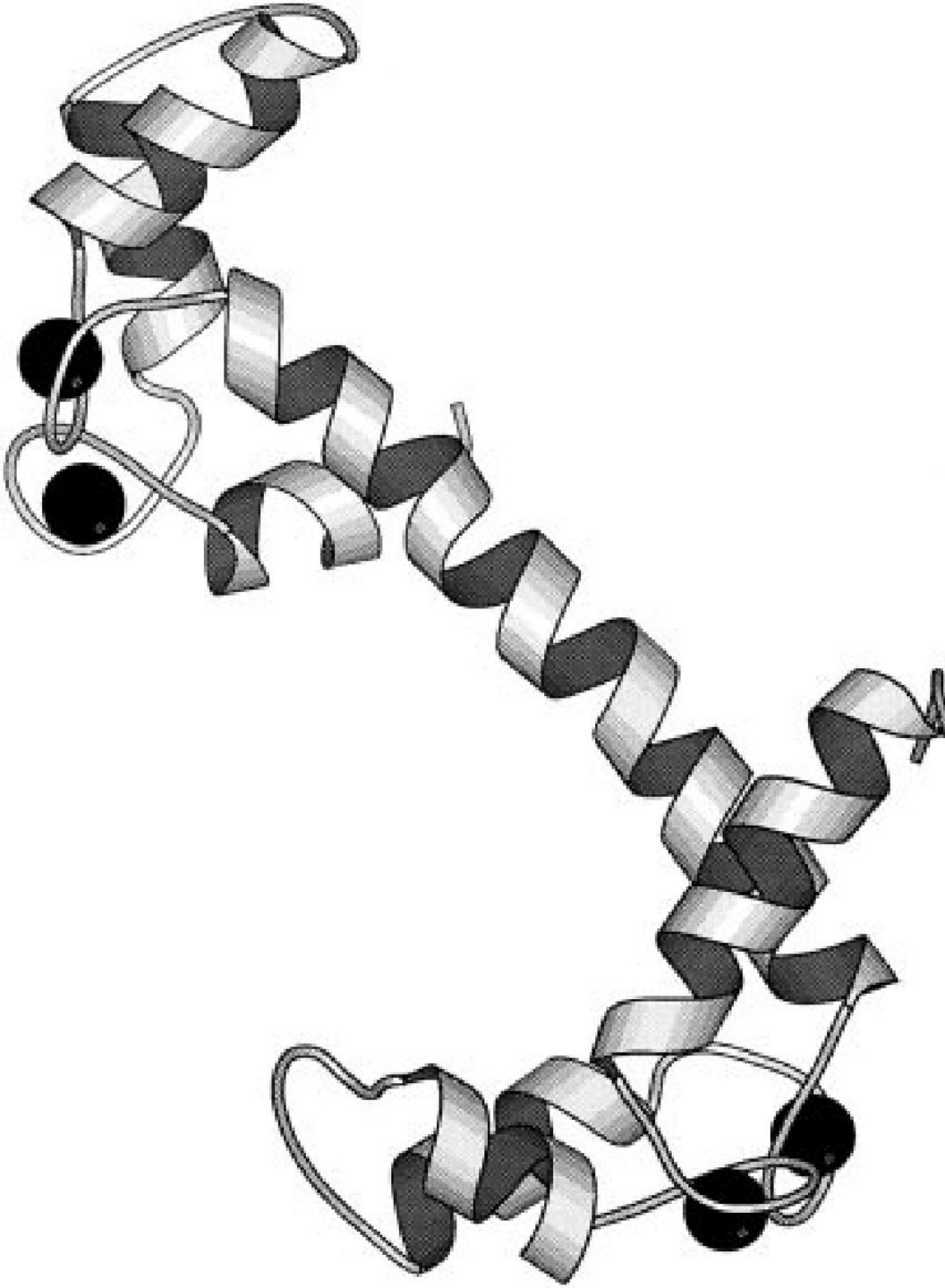
Glycogen phosphorylase a

Enzyme or protein phosphorylated by PK	Pathway catalyzed by the enzyme
Glycogen Synthase	Glycogen synthesis
Phosphorylase Kinase	Glycogen breakdown
Pyruvate Kinase	Glycolysis
Pyruvate Dehydrogenase	Pyruvate to acetyl-CoA
Hormone-sensitive Lipase	Triacylglycerol breakdown
Tyrosine Hydroxylase	Formation of DOPA, dopamine, norepinephrine



Activation of PKC and Ca²⁺ Channels by DAG and IP₃





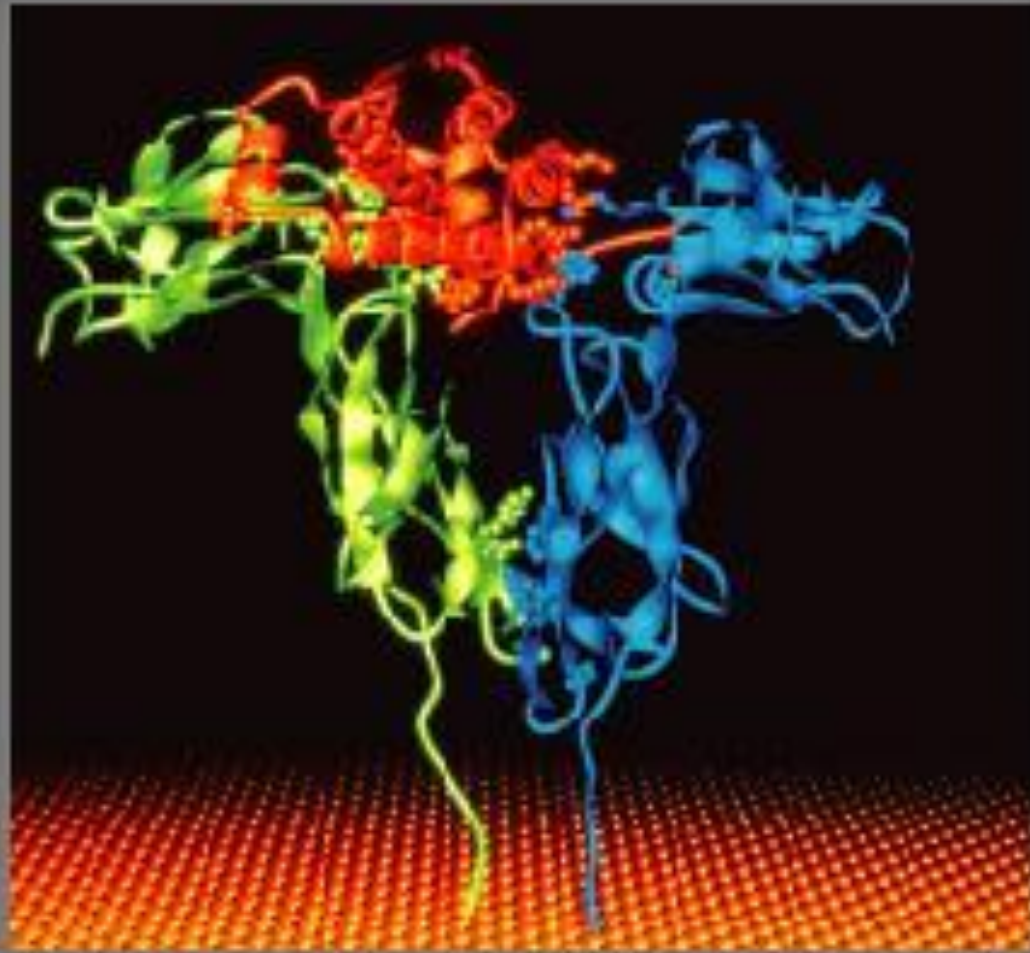
**Calmodulin-4Ca²⁺
complex**

Ca²⁺



Examples of different signals, receptors, G_{α} like-subunits, second messenger changes, and affected intracellular enzymes

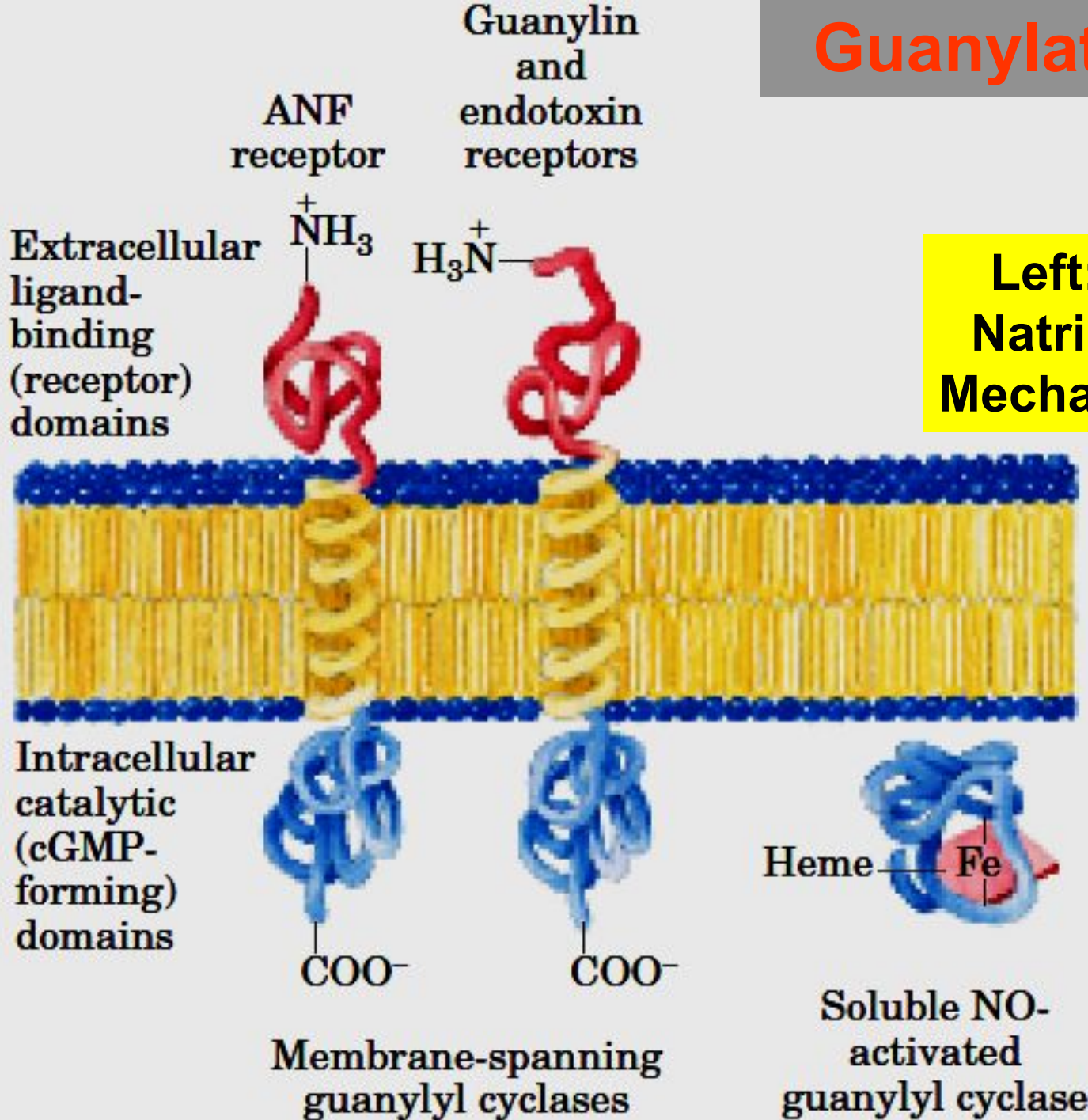
Signal	Vasopressin	Epinephrine	Light
receptor	VR	β-adrenergic	Rhodopsin
G_{α} like-subunit	G_i	G_s	Transducin
coupled enzyme	Adenylate cyclase	Adenylate cyclase	Phosphodi-esterase
Secondary messenger	\downarrow cAMP	\uparrow cAMP	\downarrow cGMP
protein affected	\downarrow PK-A	\uparrow PK-A	\downarrow Ca^{2+}, Na^+ perm.



Complex of human growth hormone and its receptor. Two identical molecules of the receptor extracellular domain (*blue and green ribbon models*) bind a single molecule of growth hormone (*red*).

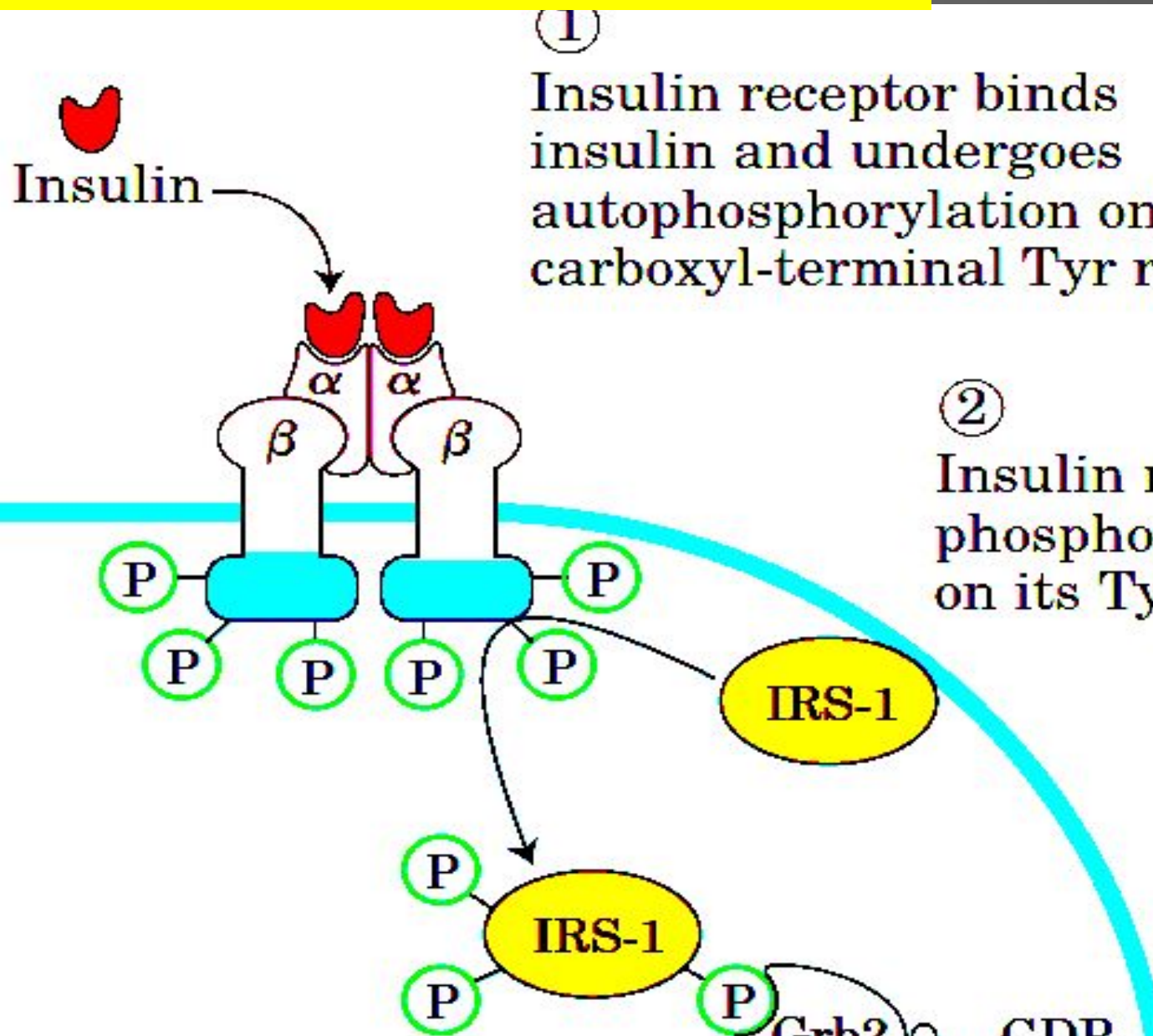
X-ray structure by and drawing courtesy of Abraham de Vos and Anthony Kossiakoff, Genentech Inc., South San Francisco, California.

Guanylate cyclases



Left: ANF –Atrial Natriuretic Factor Mechanism of action

Structure of Insulin Receptor



① Insulin receptor binds insulin and undergoes autophosphorylation on its carboxyl-terminal Tyr residues.

② Insulin receptor phosphorylates IRS-1 on its Tyr residues.

③ SH2 domain of Grb2 binds to P-Tyr on IRS-1, causing Grb2 to bind to CDP, leading to GTP binding to CDP.

INSULIN-RECEPTOR COMPLEX

Effects in the intracellular space

after autophosphorylation:

Glucose transport
stimulation across the
cellular membrane

**Phosphorylation of
Phosphodiesterase:**
as the result the cAMP ↓

**Stimulation of
phosphoprotein
phosphatases**

Dephosphorylation

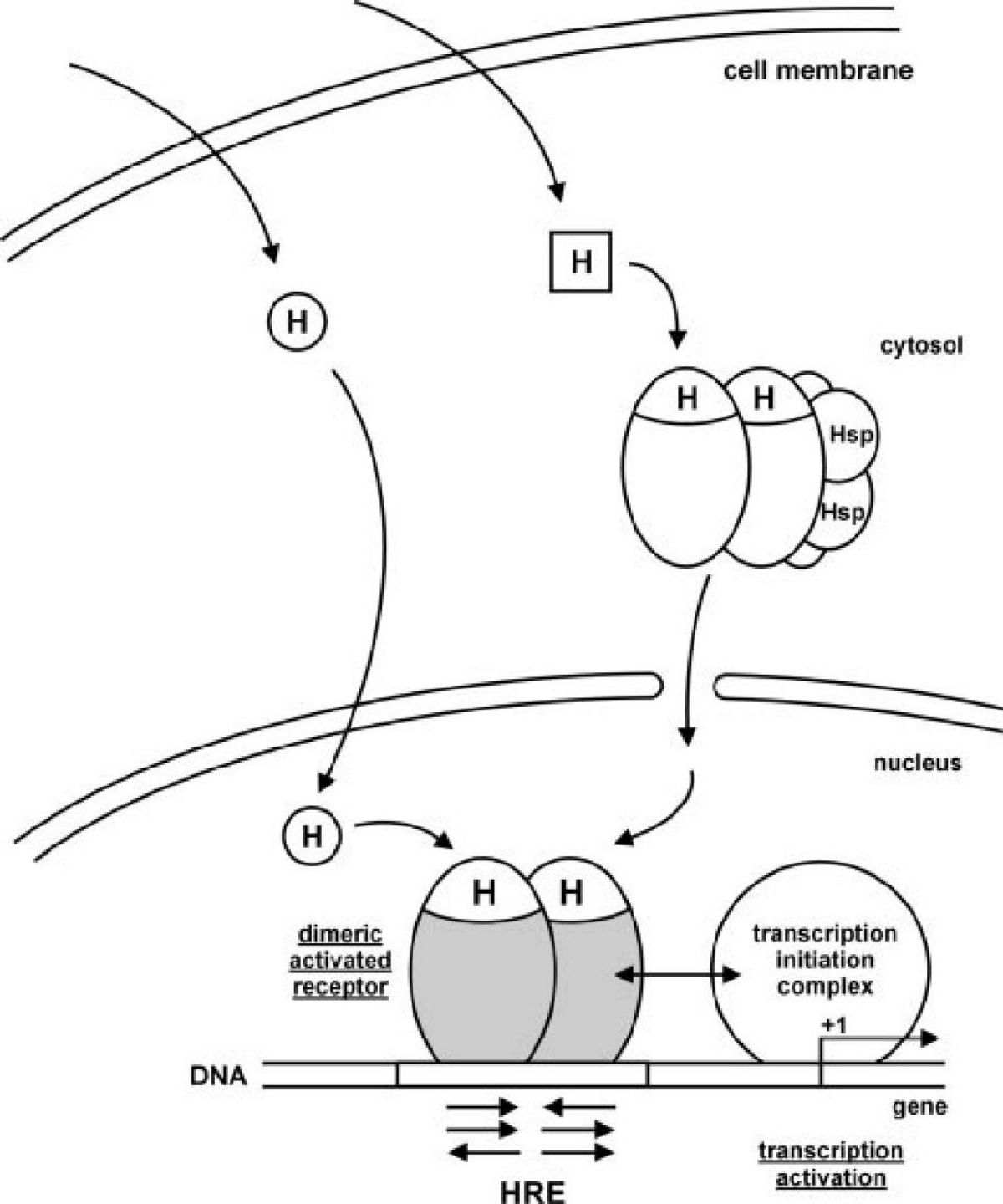
Stimulation of
Acetyl-CoA carboxylase,
Glycogen synthetase,
Pyruvate dehydrogenase,
Pyruvate kinase,
Phosphofructokinase

Suppression of
Glycogen
phosphorylase α ,
Triacylglycerol
lipase

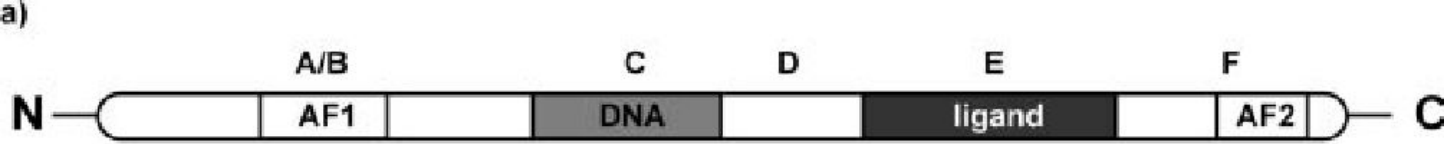
**Stimulation of
gene expression**
for

Tyrosine
aminotransferase,
Palmitate
synthetase,
Pyruvate kinase,
Glucokinase, STH,
Albumins,
Ovalbumin

**Suppression of
gene expression**
for Phosphoenol-
pyruvate
carboxykinase

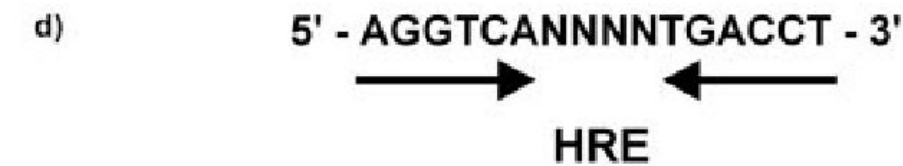
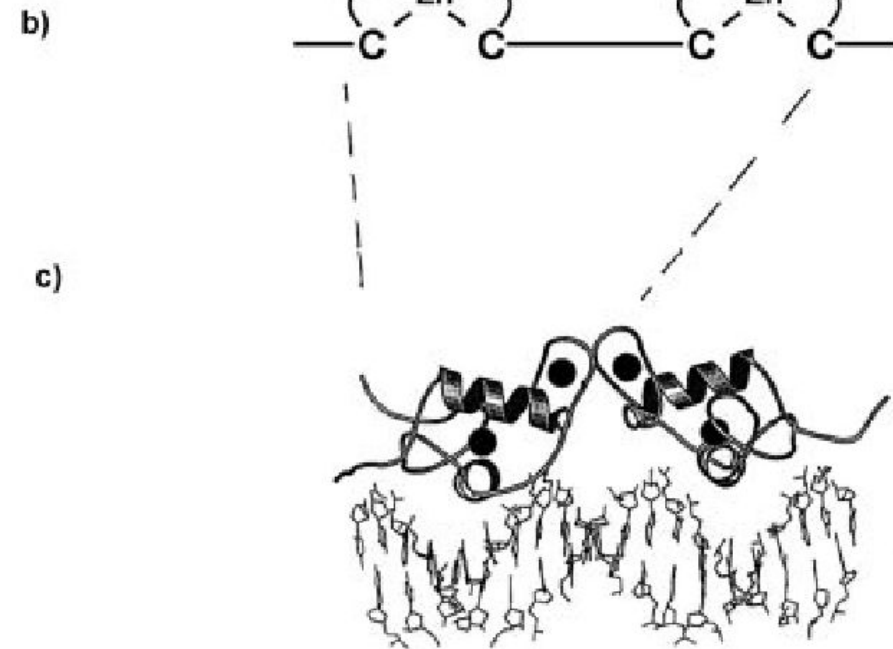


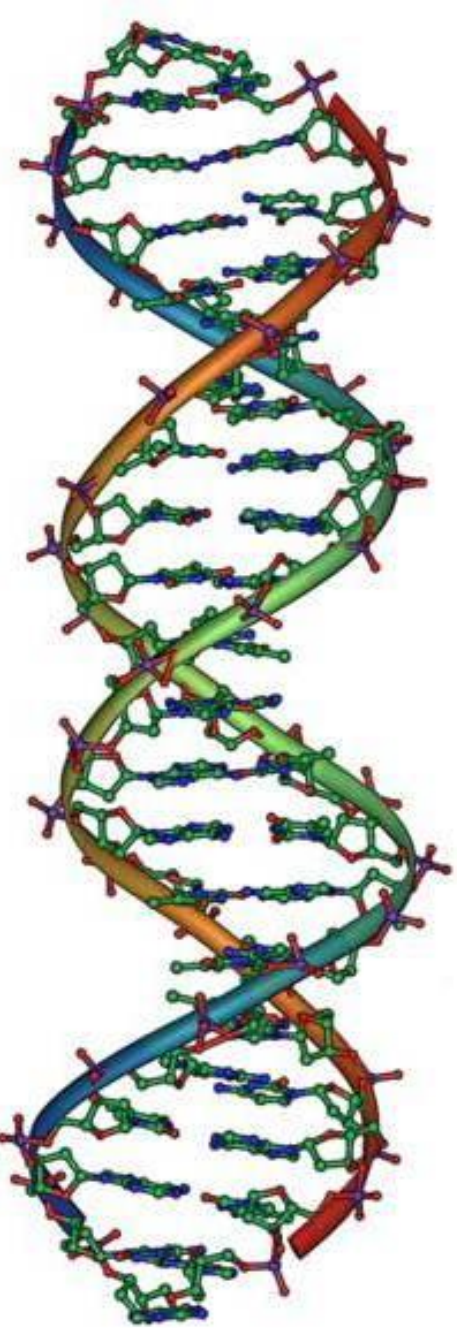
**The
Mechanism of
action
for Lipophilic
Hormones (H);
HRE
–Hormone
Response
Elements**



AF1, AF2 domains that mediate the stimulation of the transcription

They have affinity to receptors of steroidal hormone containing so named “zink-fingers”





THANK YOU
For
ATTENTION

