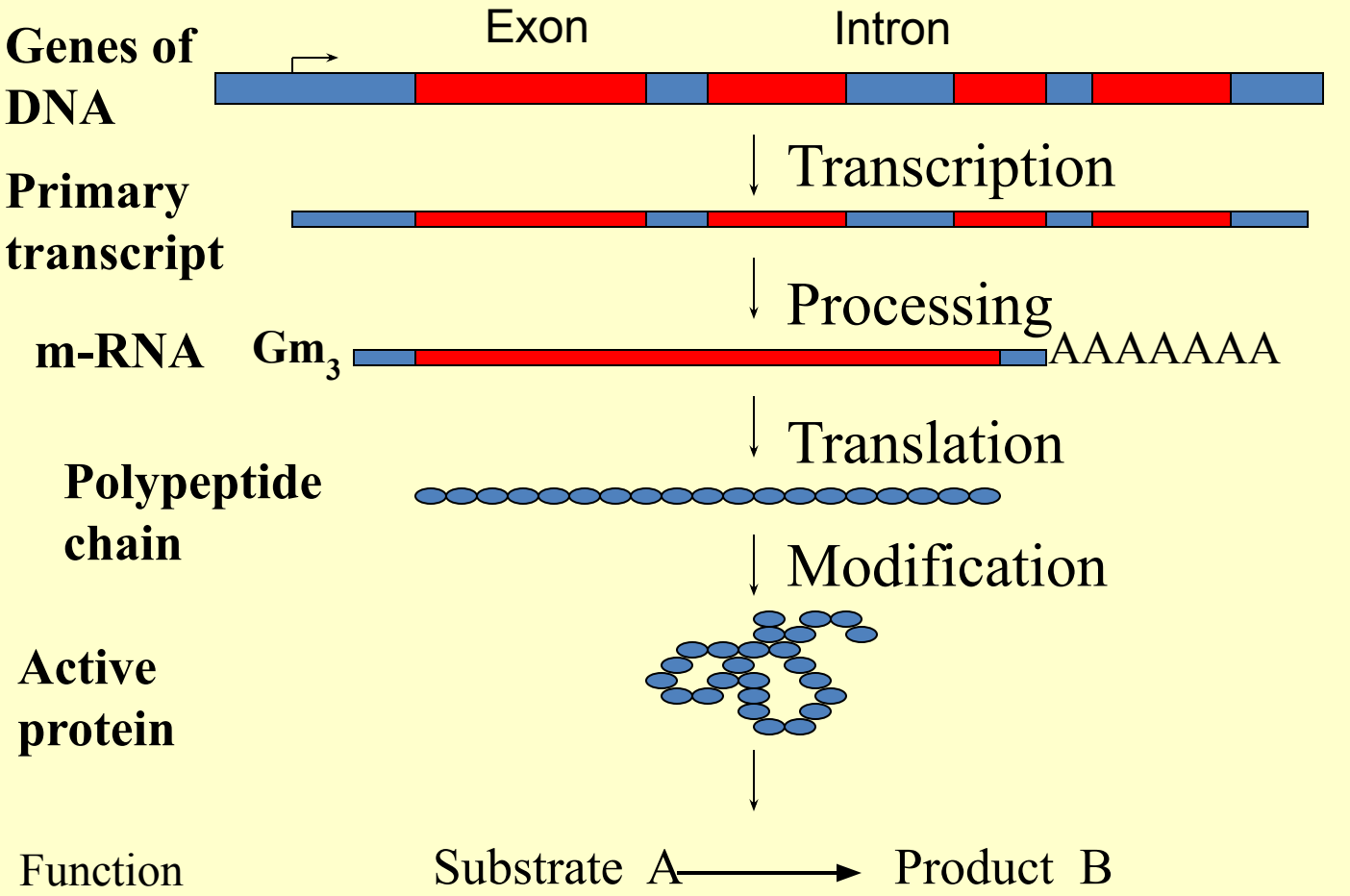
THE MINISTRY OF PUBLIC HEALTH OF UKRAINE
ZAPOROZHYE STATE MEDICAL UNIVERSITY

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

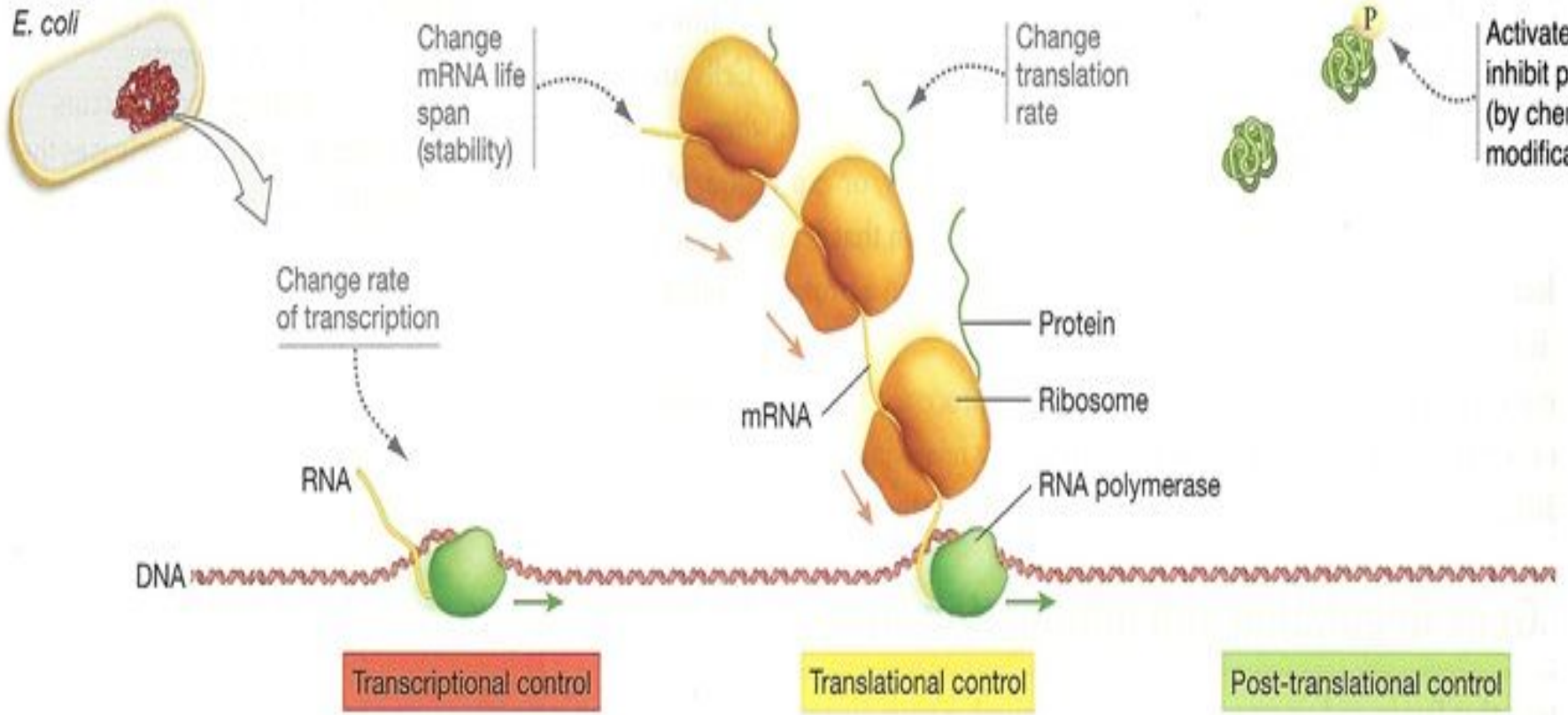
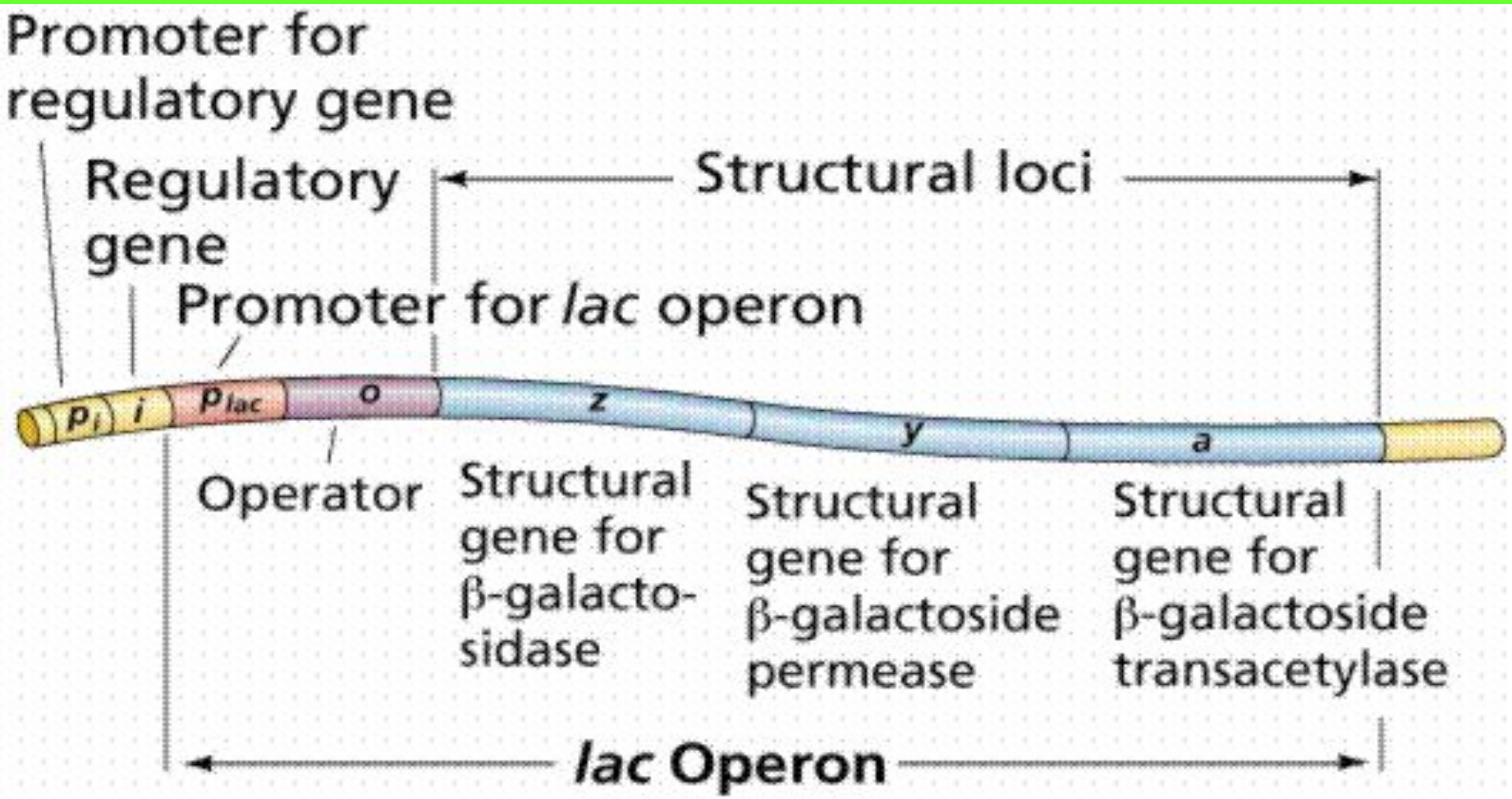


FIGURE 17.1 Gene Expression in Bacteria Can Be Regulated at Three Levels.

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

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

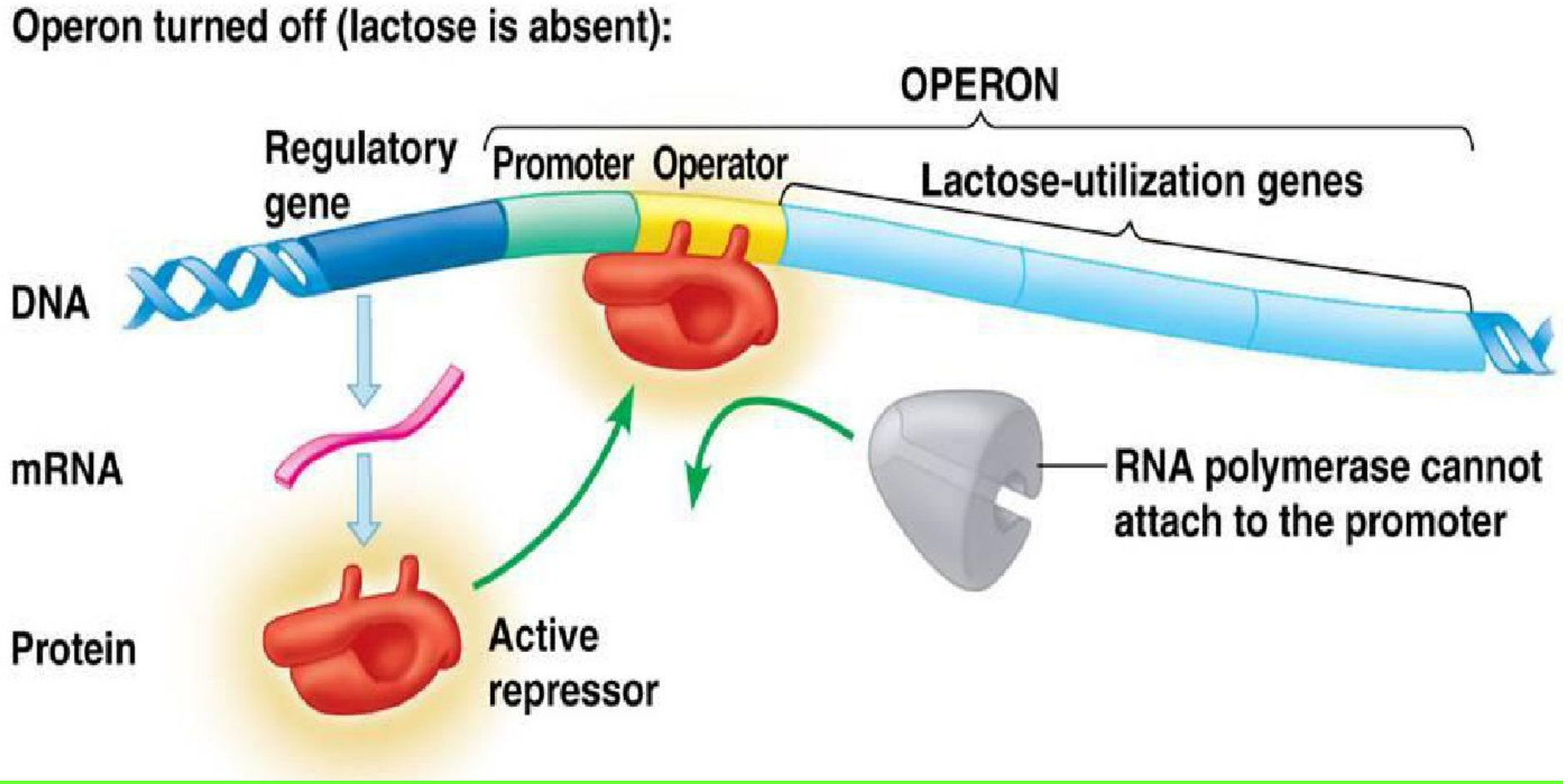
Operon is composed from promoter sequence, gene-operator, structural genes



The Lac-operon model investigated in E.coli

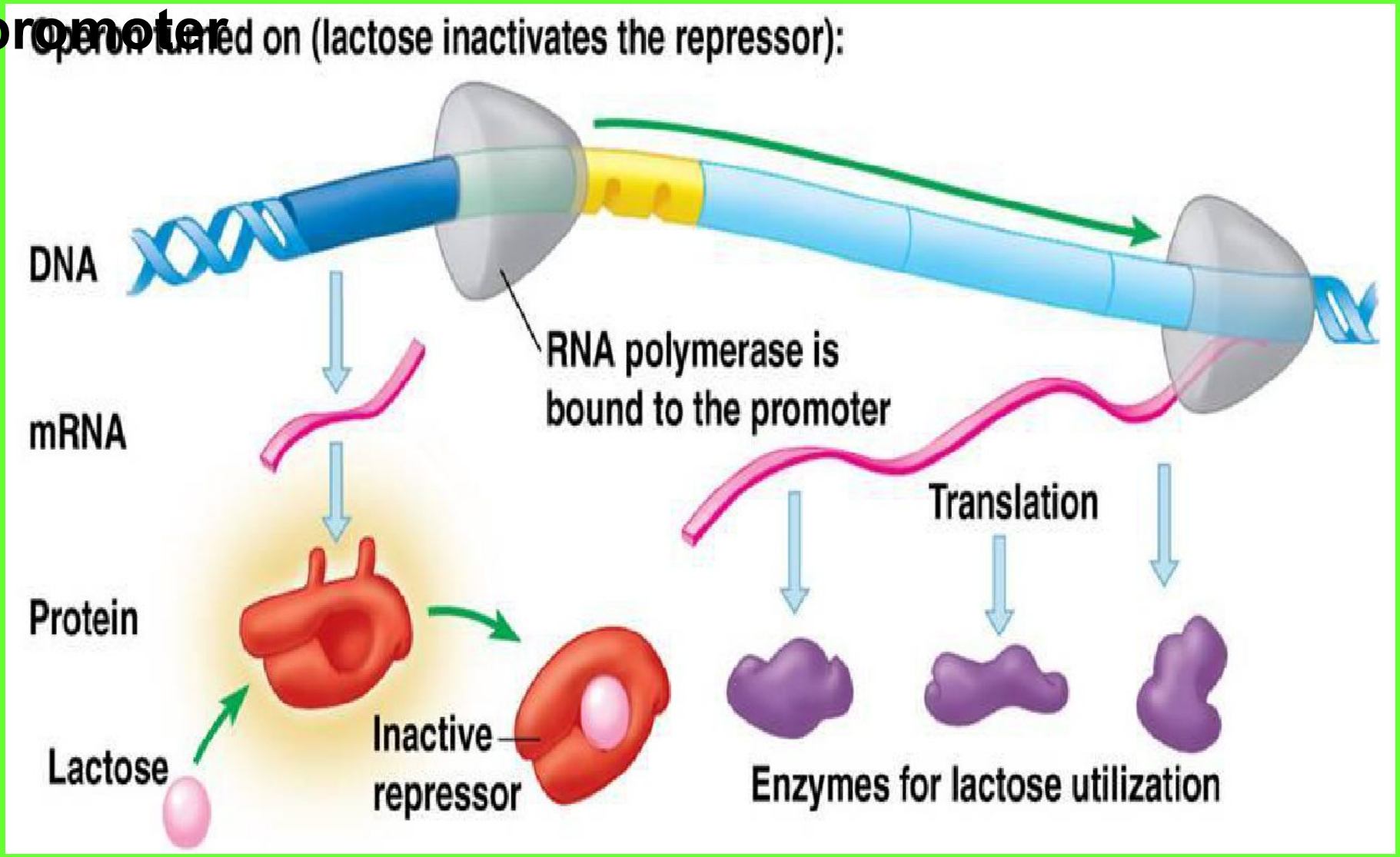
(proposed by F.Jacob and J. Monod, 1961)

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

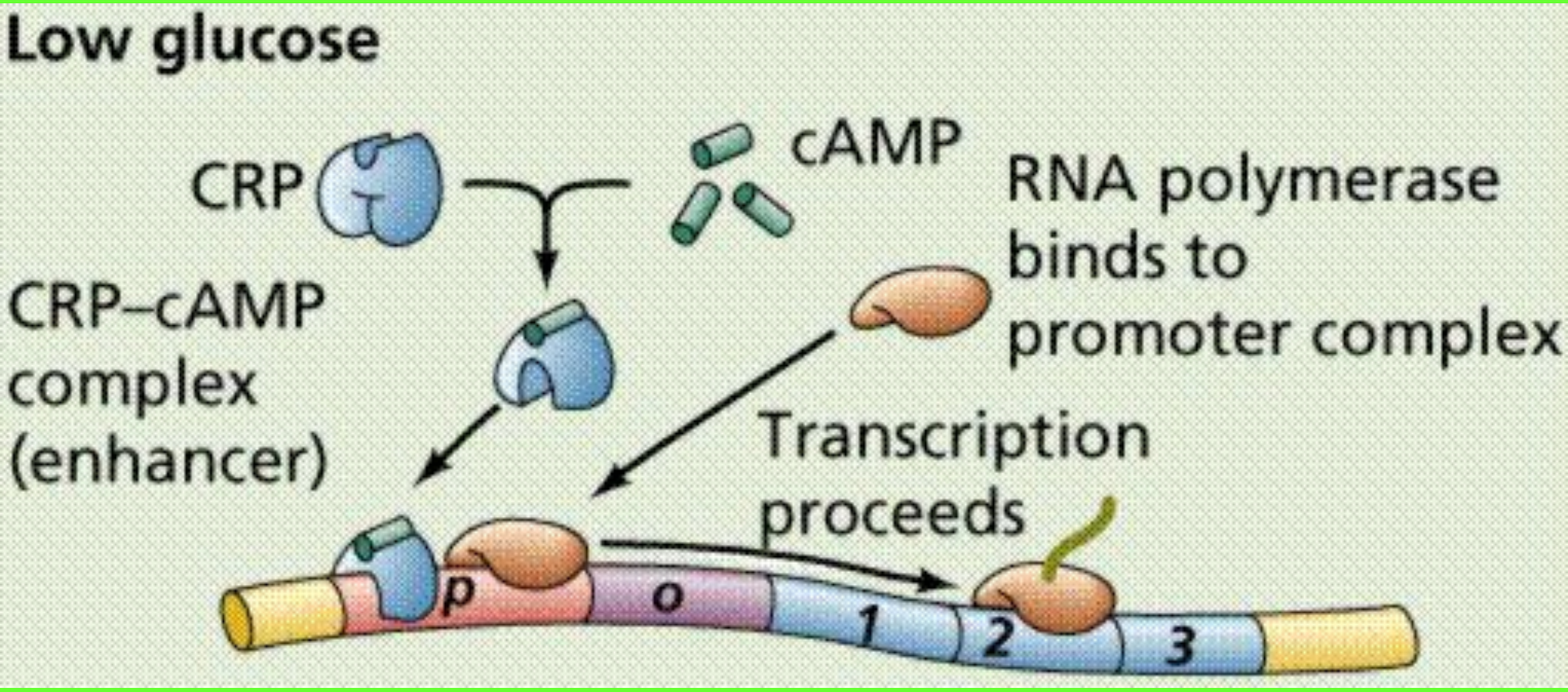


Gene-operator is placed in operon between promoter and structural genes, it has affinity to protein- repressor

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



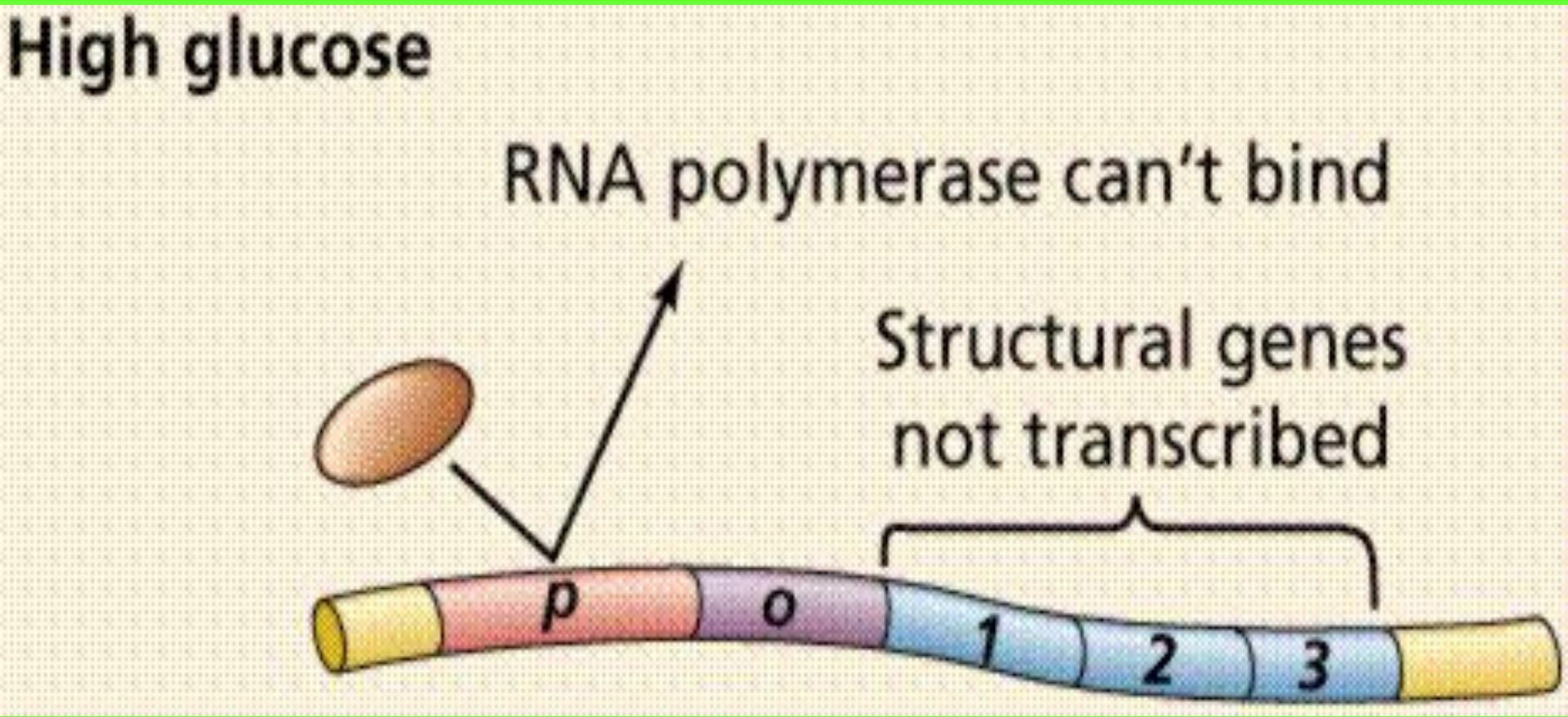
CRP-cAMP enhancer influence



CRP – Catabolite gene Reactive Protein

cAMP – cyclic AMP

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



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:

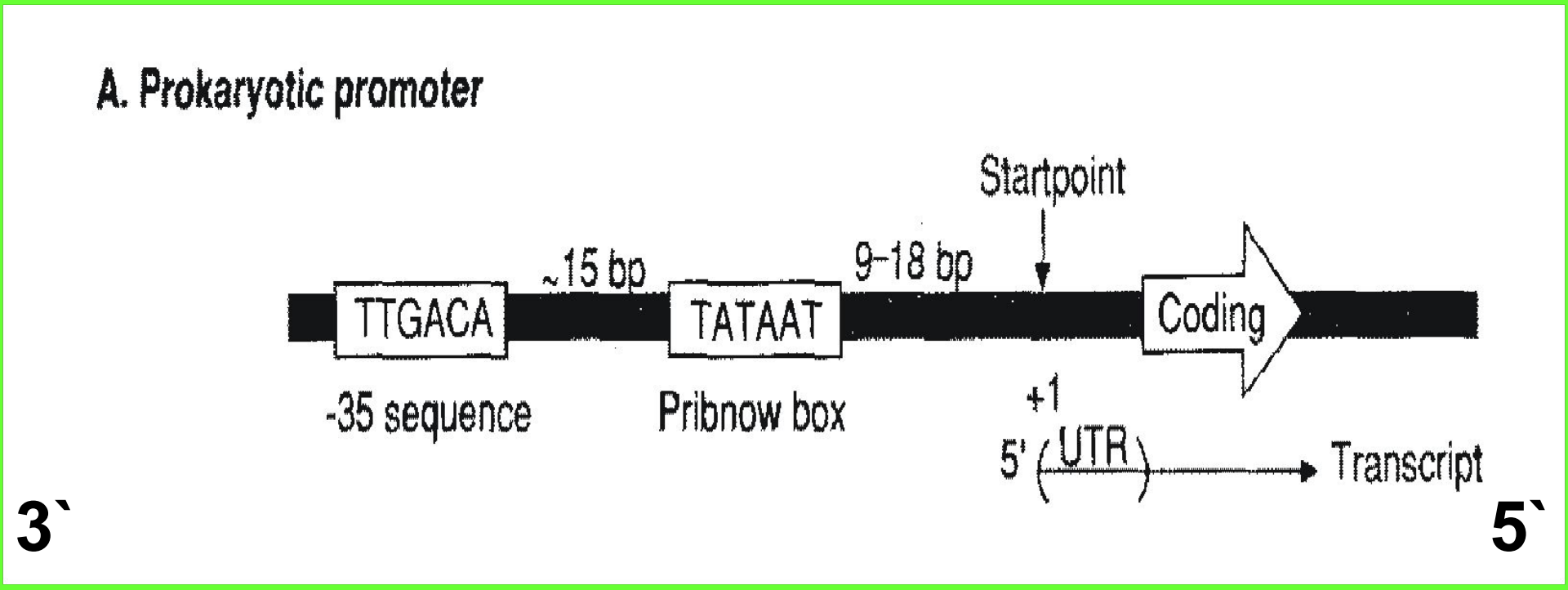
- **Methylation of histone H3 on K4 and K79 (lysine residues at the 4th and 79th position) ->-> opens the chromatin structure ->-> leading to transcriptional activation**
- **Methylation of histone H3 on K9 and K27 (lysine residues at the 9th and 27th position) ->-> condenses the chromatin structure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

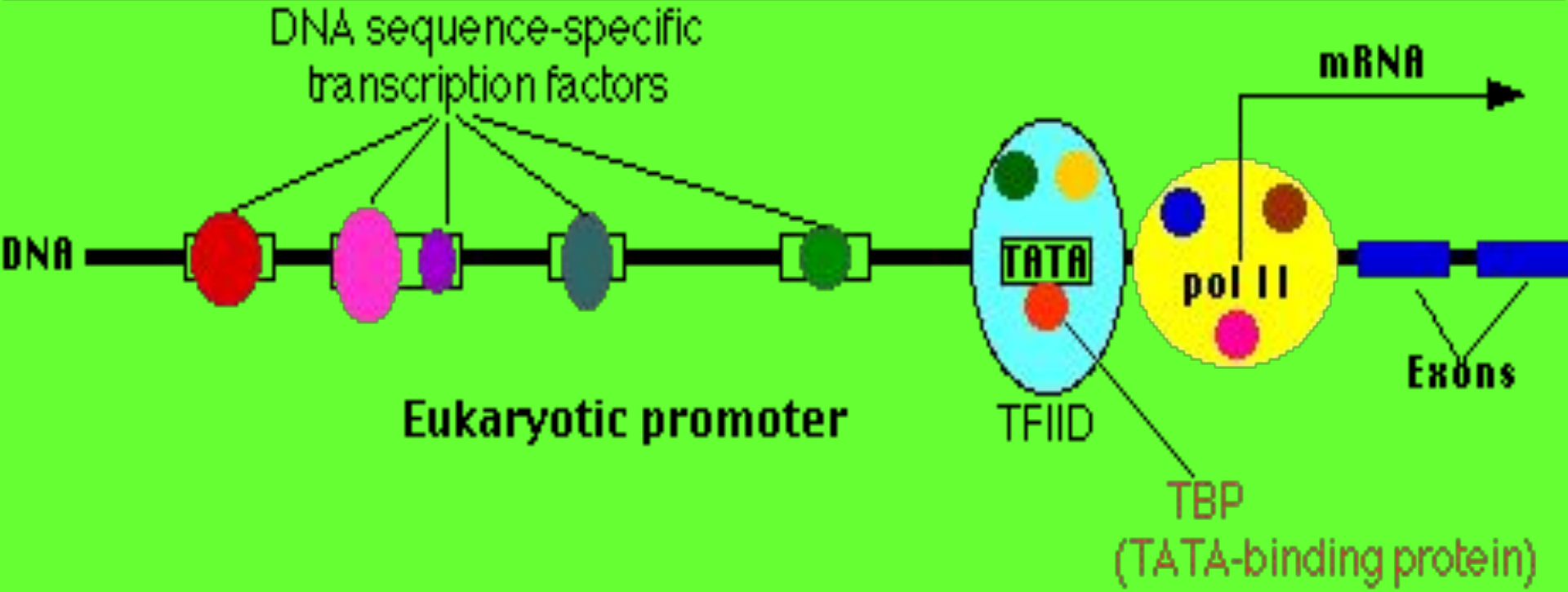
Ubiquitination

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

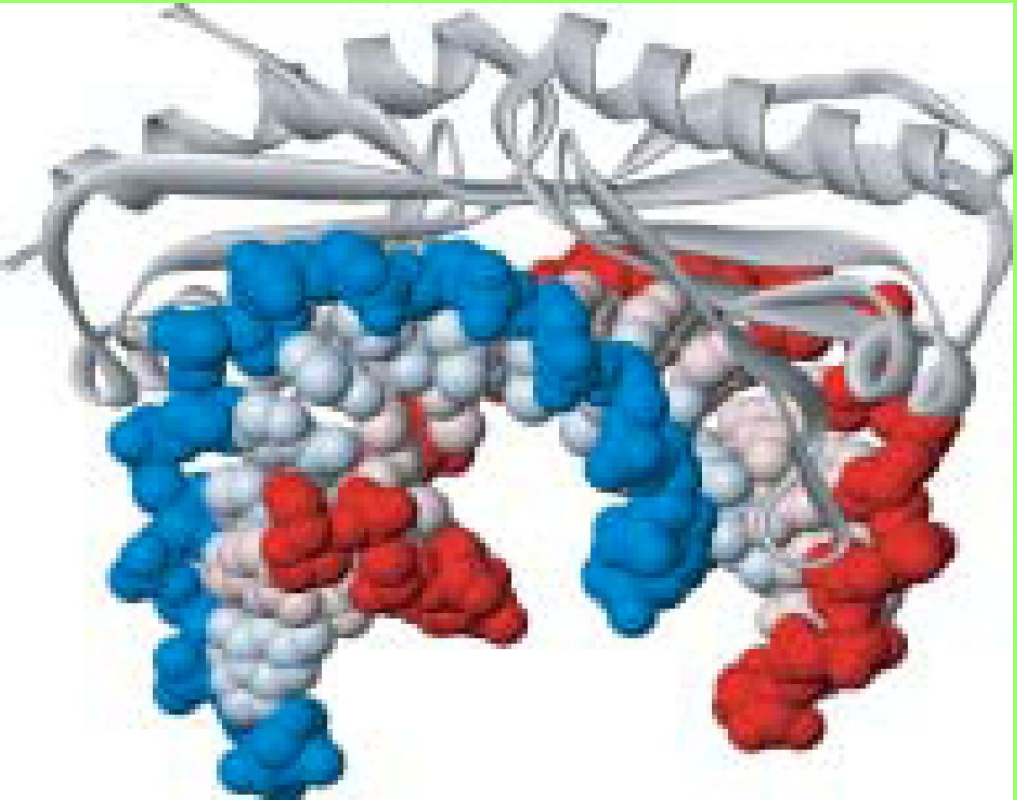
Methylation of DNA

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


A. Prokaryotic promoter
Startpoint
~15 bp
9–18 bp
TTGACA
TATAAT
Coding
-35 sequence
Pribnow box
+1
5'
UTR
Transcript
3`
5`

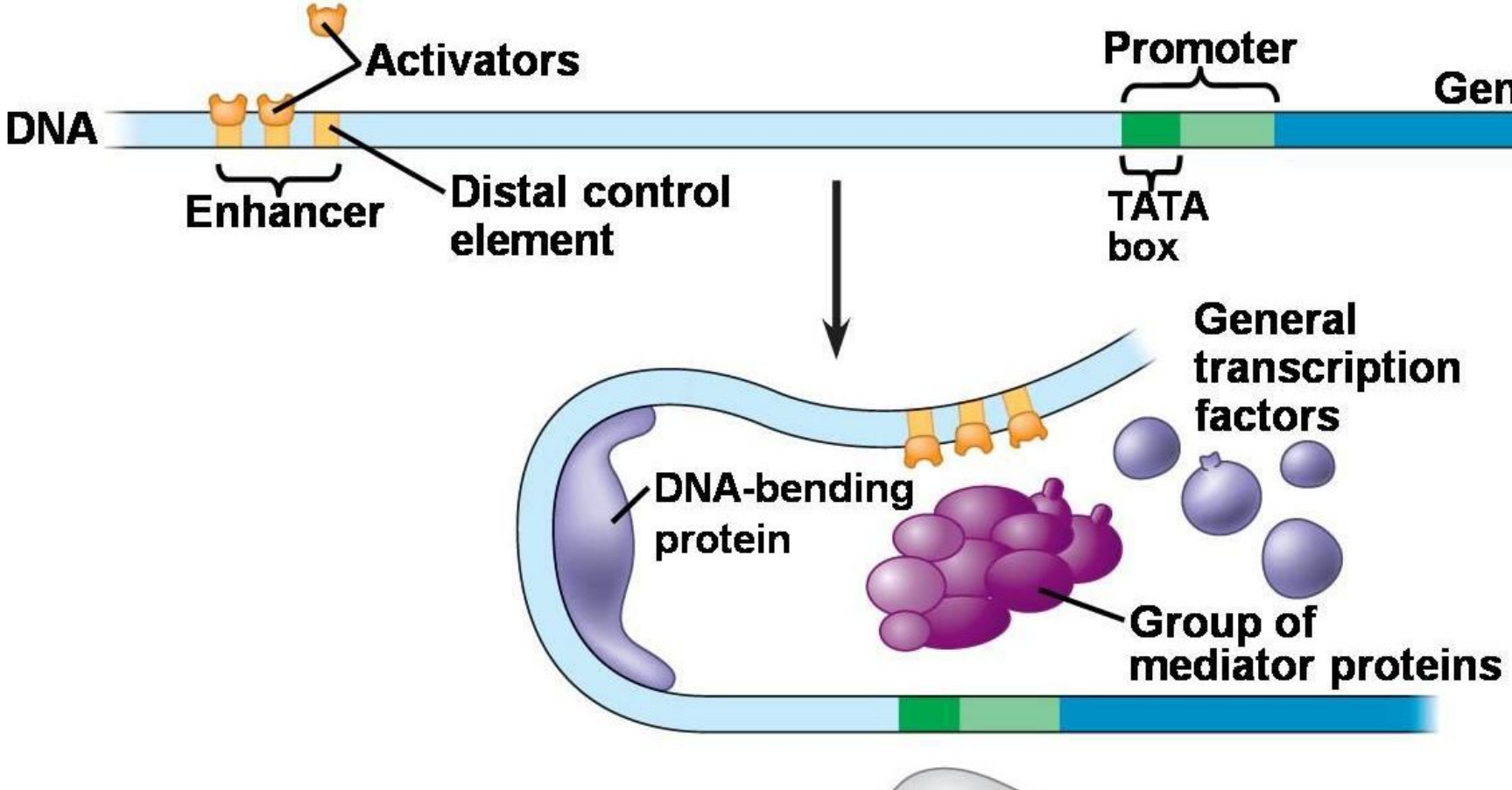

DNA sequence-specific
transcription factors
mRNA
DNA
TATA
pol II
Exons
Eukaryotic promoter
TFIID
TBP
(TATA-binding protein)

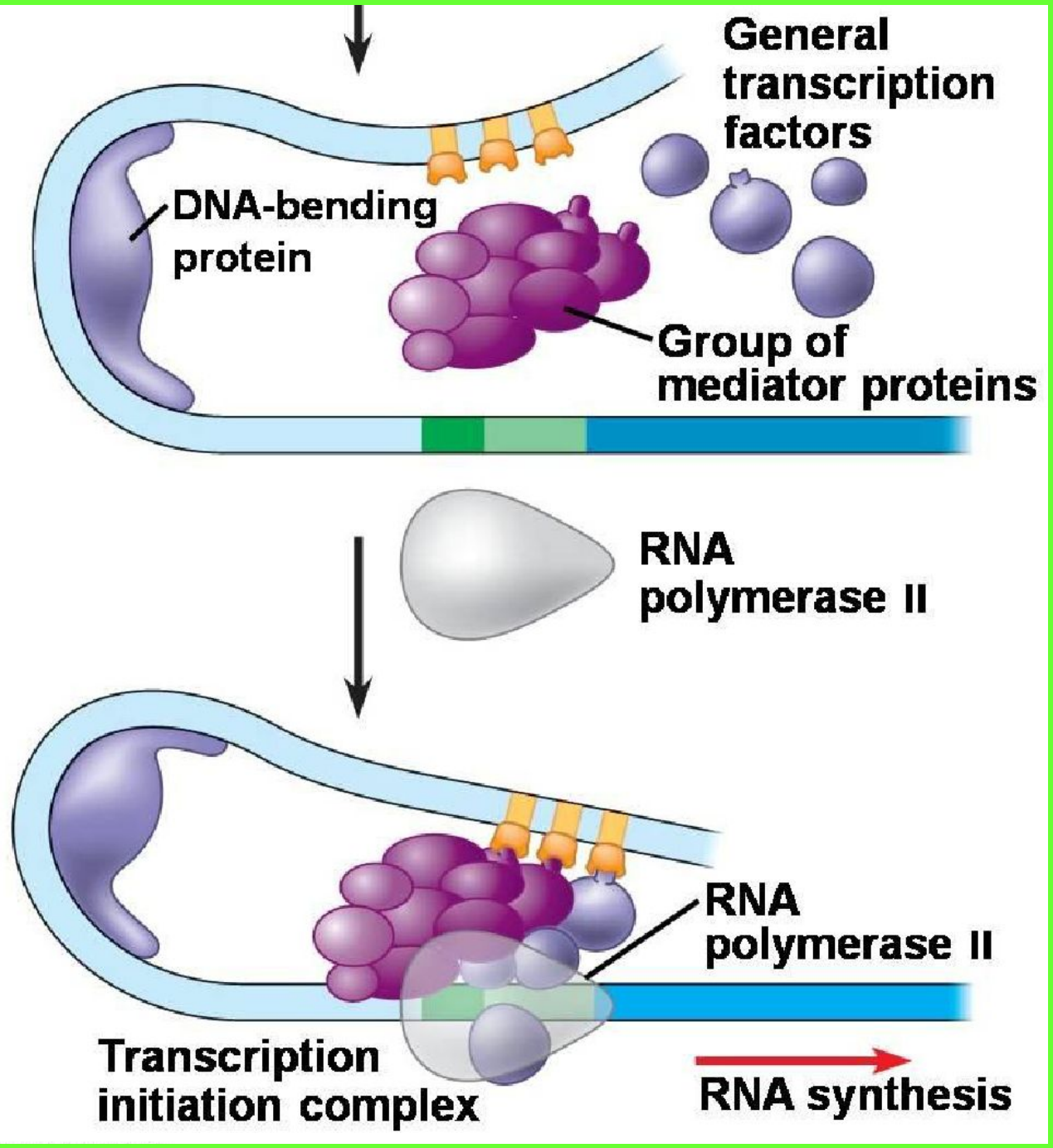
TATA-box binding protein (TBP) is found in eukaryotic cells, and it is the component of the complex TFIID containing other several proteins (TBP-associated factors) and bound to the TATA box



TATA-box binding protein (TBP)

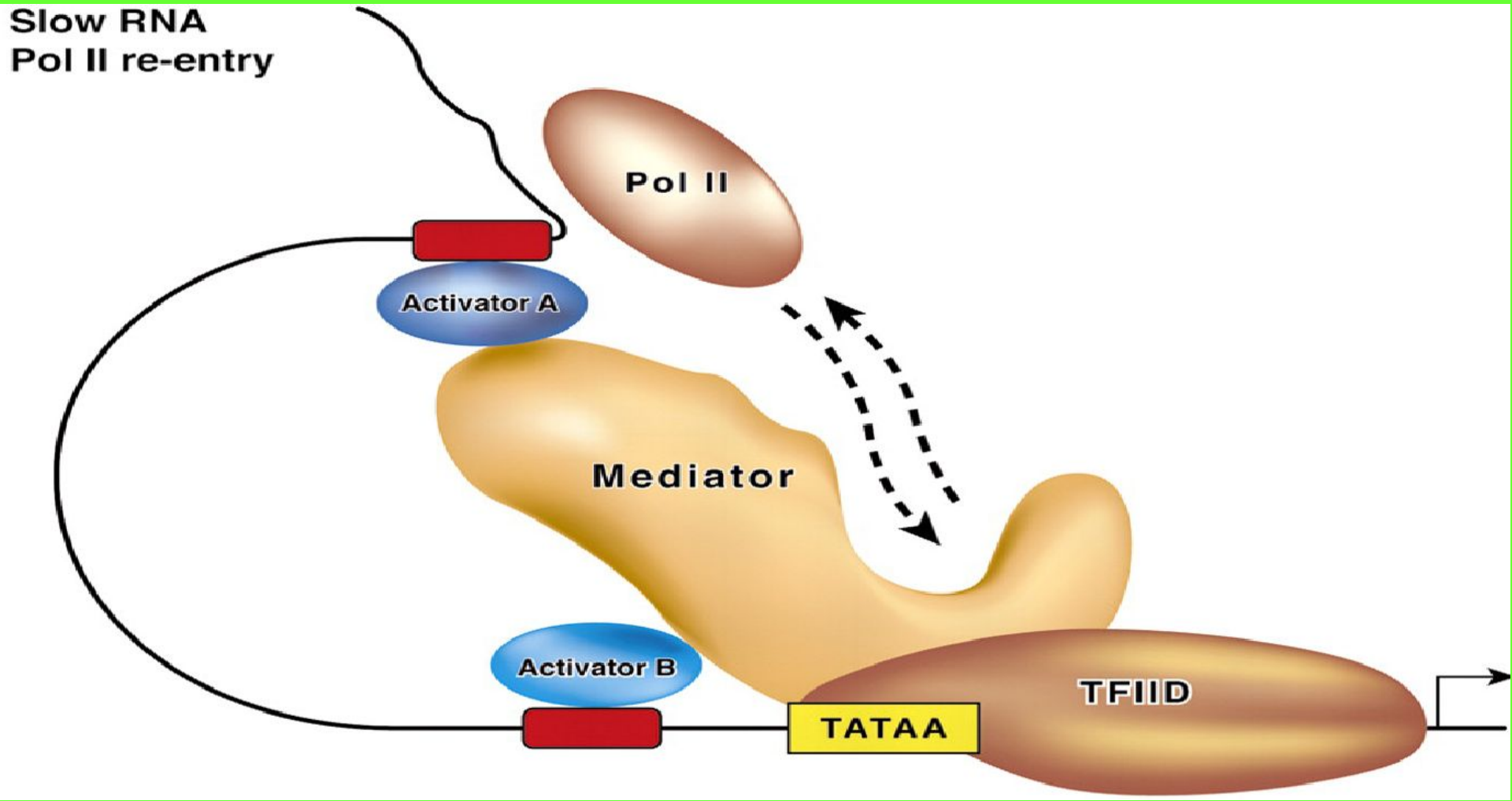
Enhancer-bending protein (EBP) changes the DNA single strand conformation to form special loop which promotes the stimulation and the increase of the rate of initiation phase of transcription.



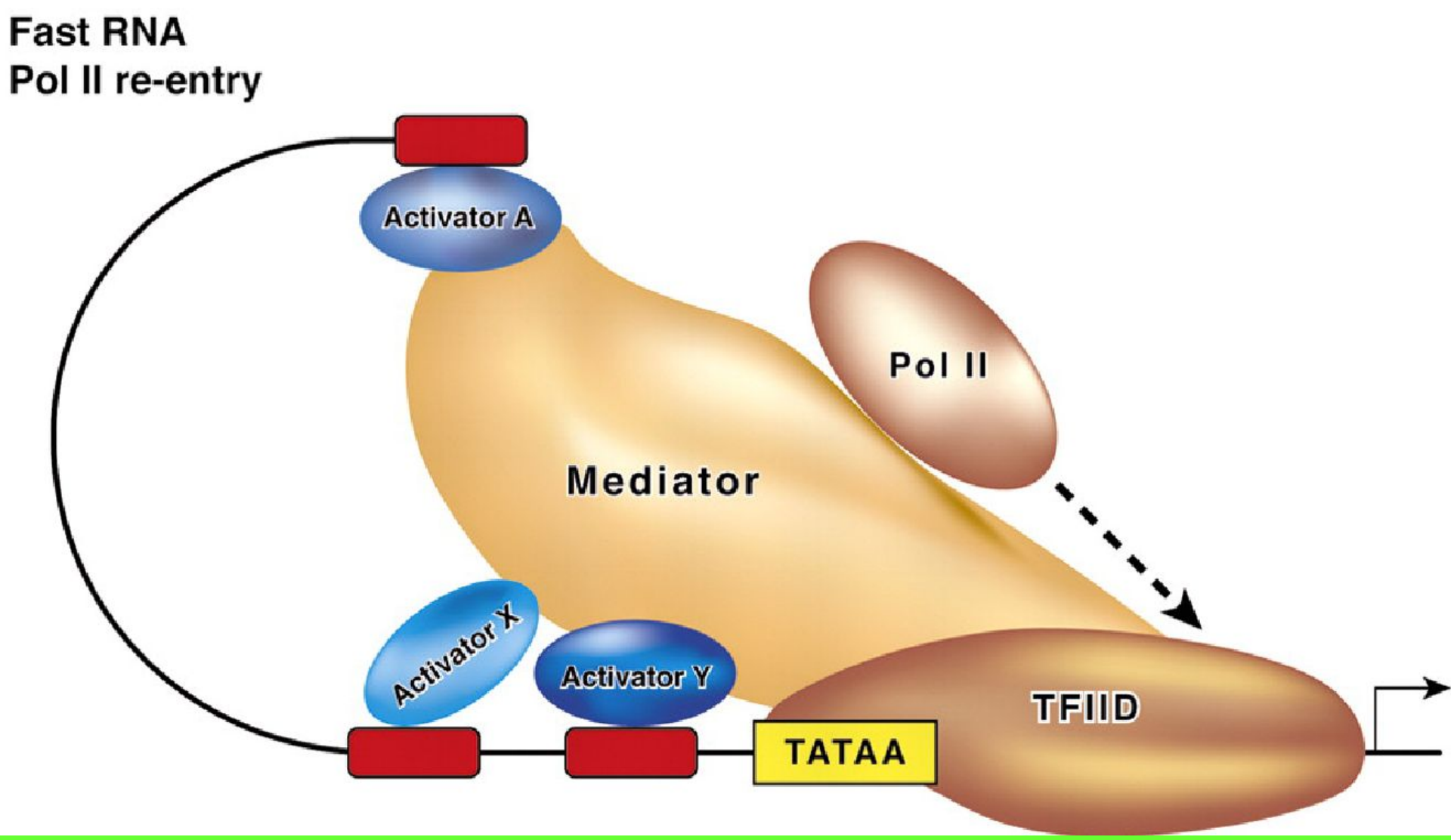


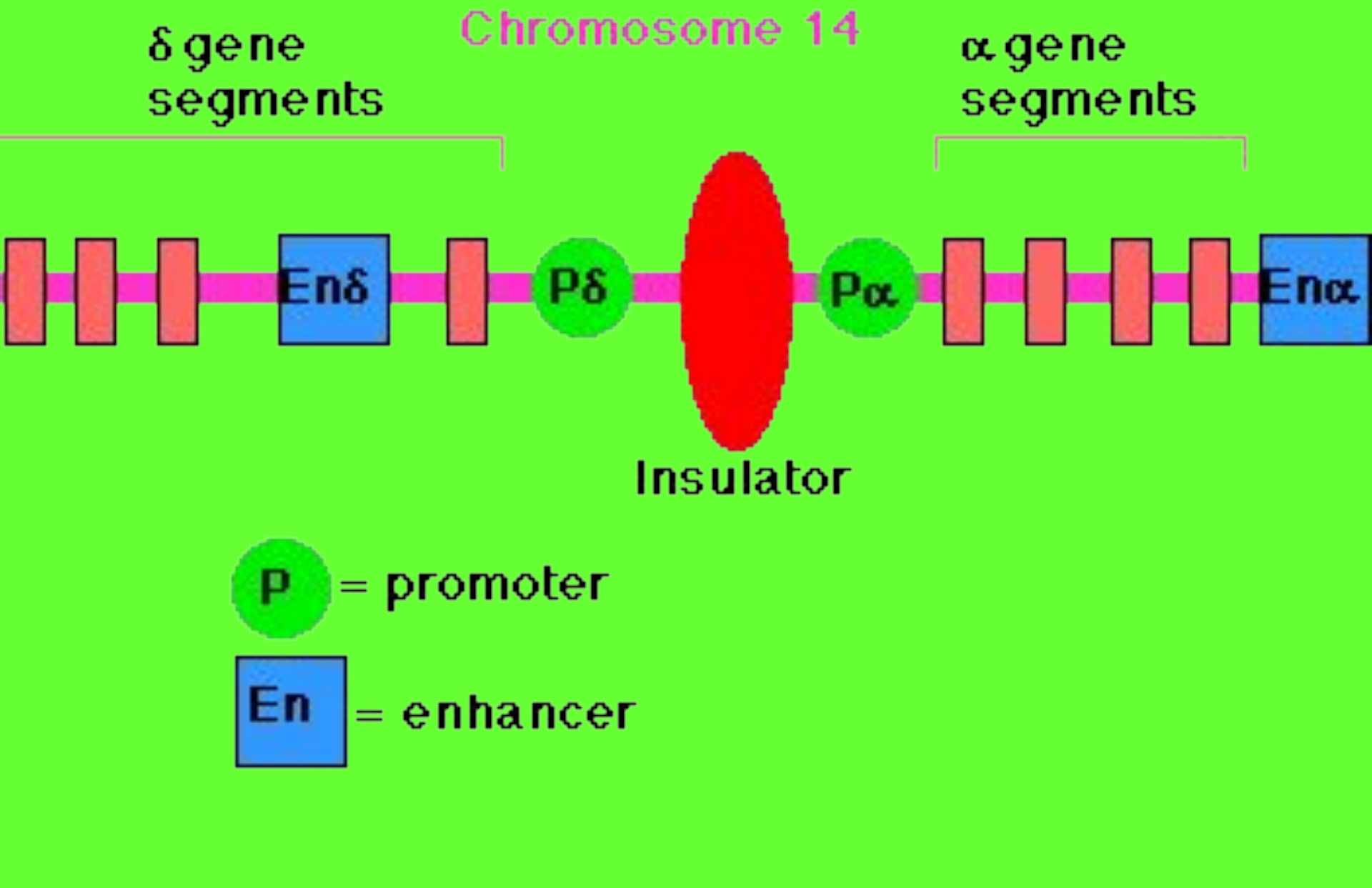
Except EBP and TF, there is the group of mediator proteins to stimulate transcription process, too

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



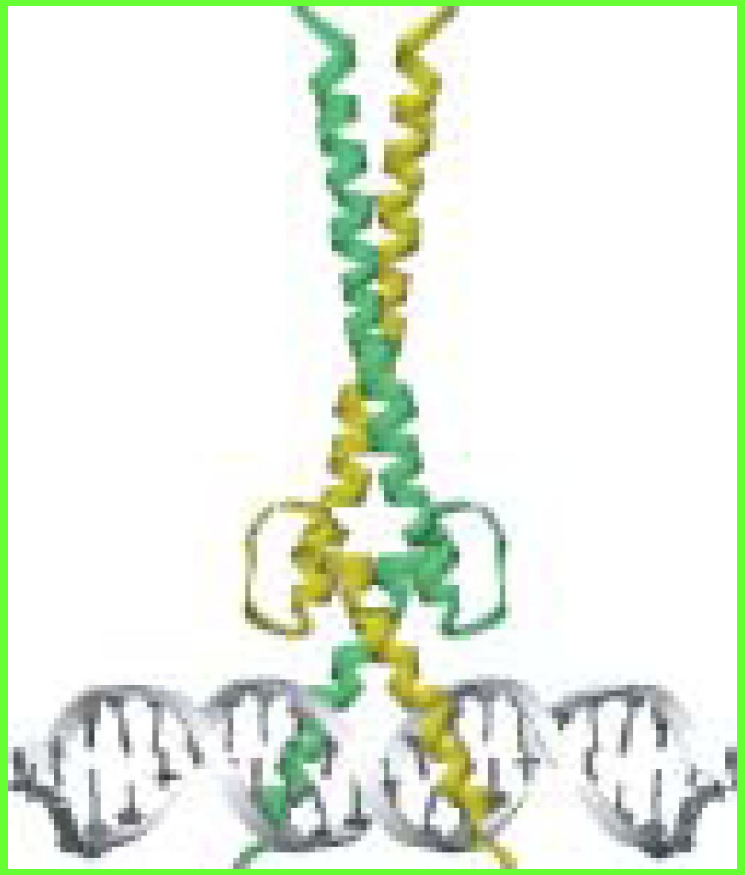
Proteins-mediators are in close relations with general transcription factors placed in the complex TFIID




Chromosome 14
δ gene segments
α gene segments
Enδ
Pδ
Pα
Enα
Insulator
P = promoter
En = enhancer

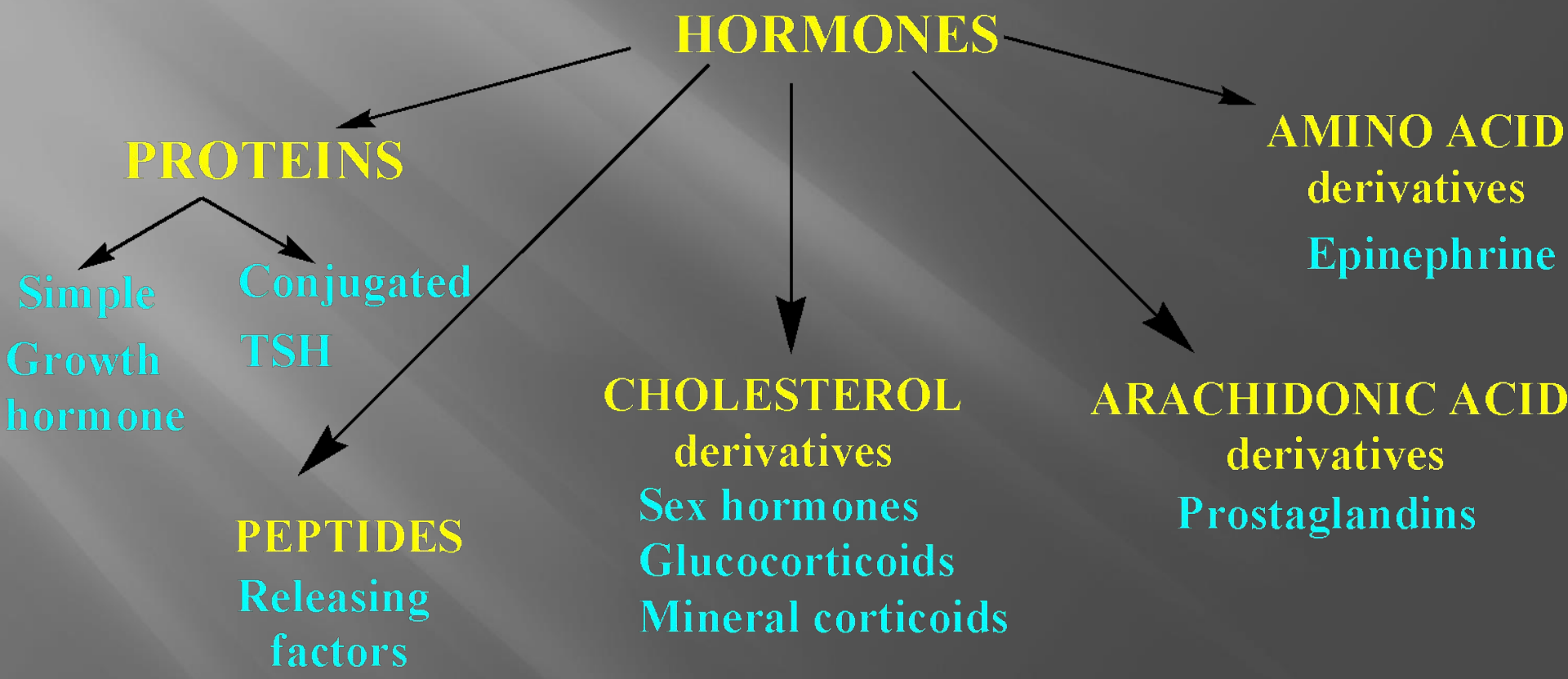
Interaction of homodimeric leucine-zipper (A) and basic helix-loop-helix (B) proteins with DNA

(A)

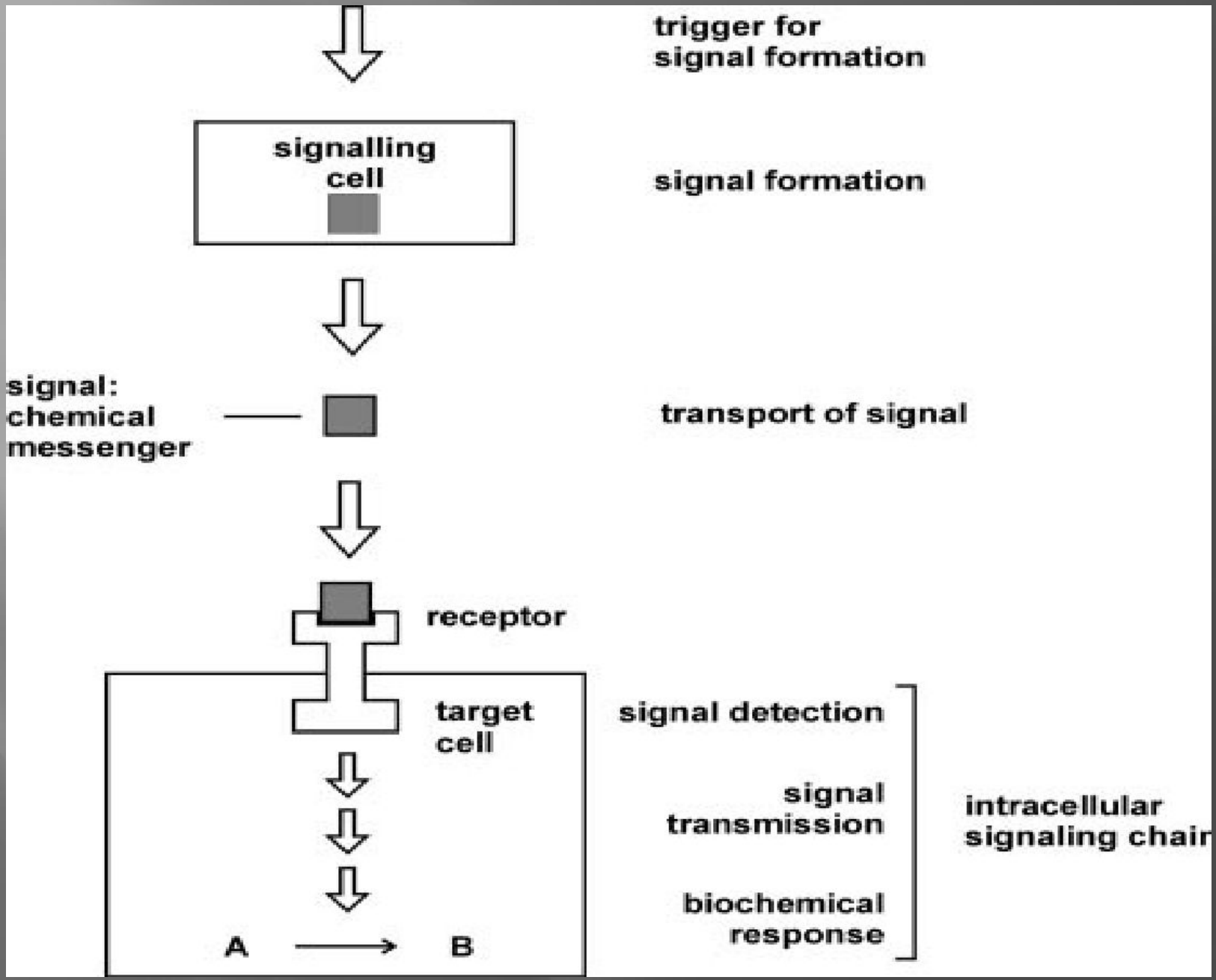


(B)

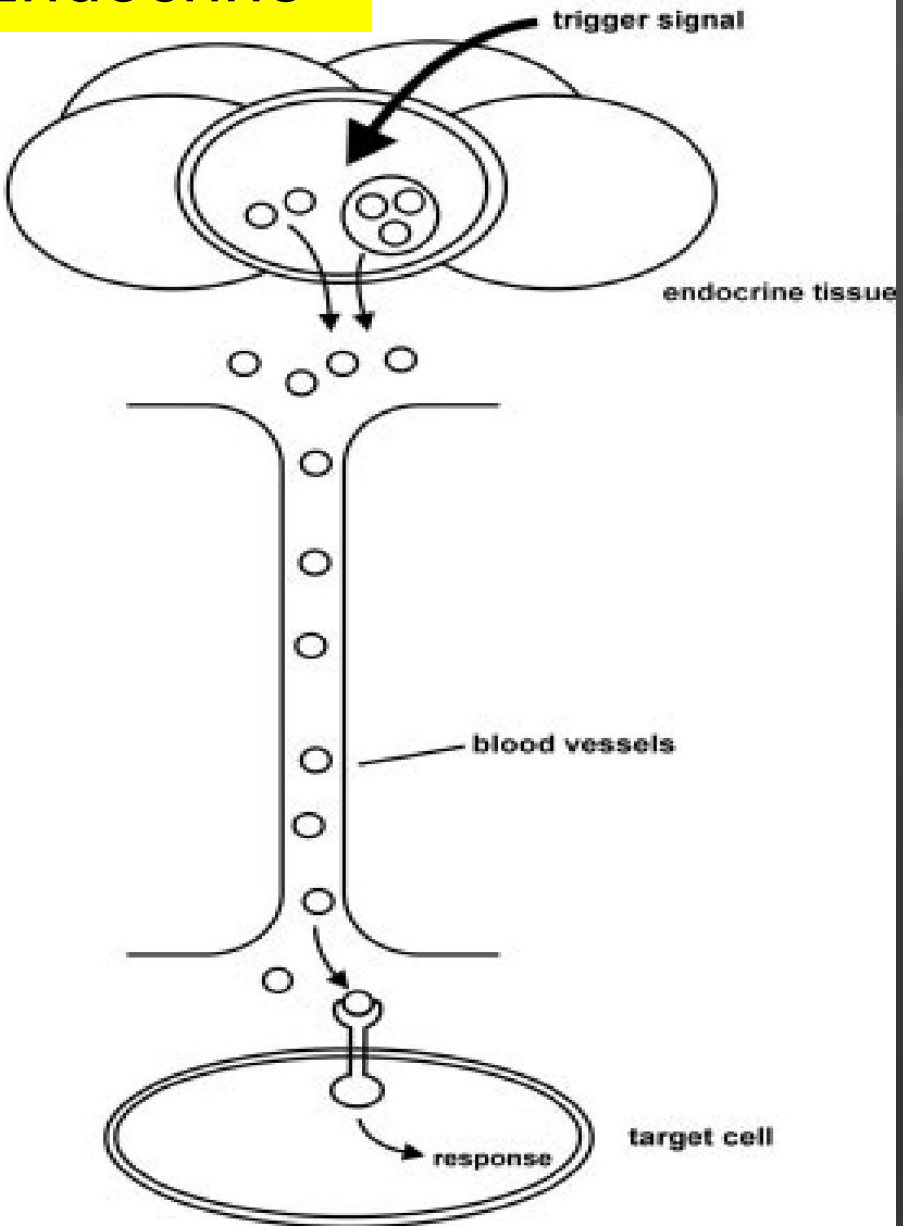
Classification of hormones according chemical nature



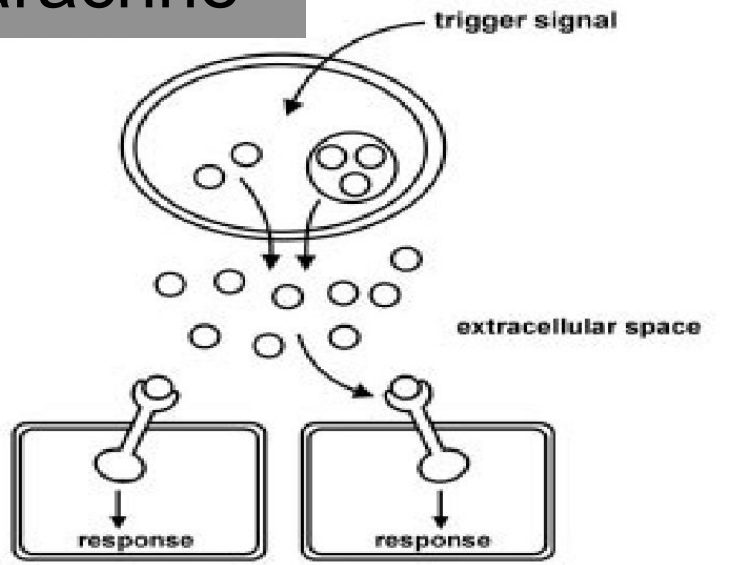
INTERCELLULAR MECHANISM of COMMUNICATION



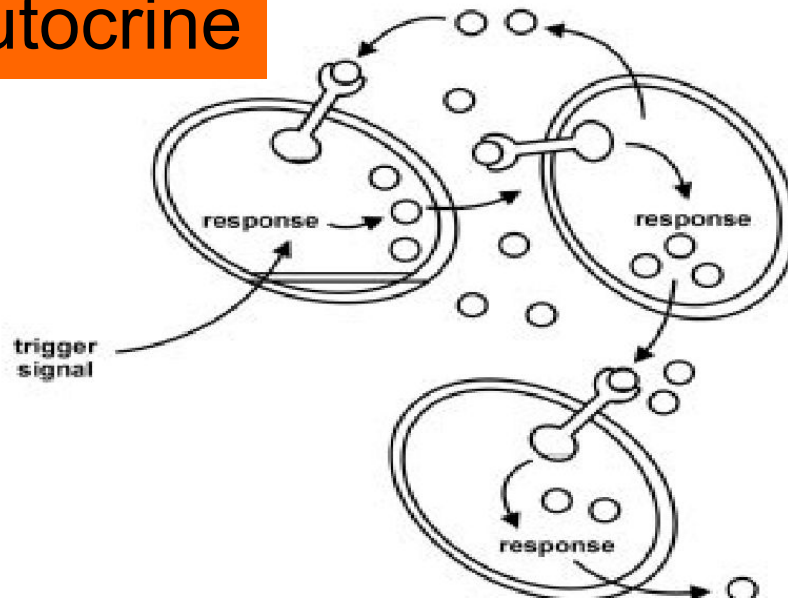
Endocrine

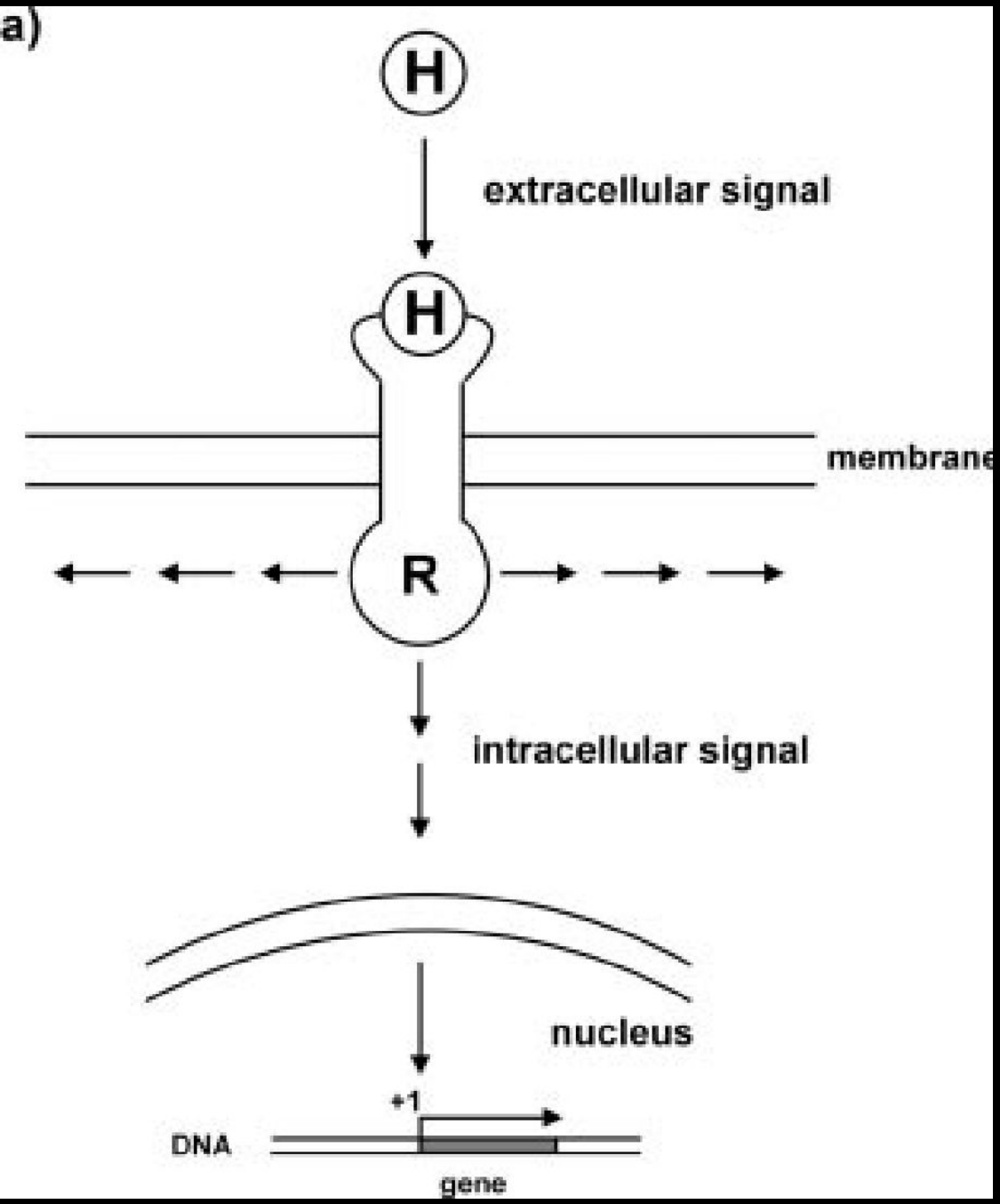


Paracrine

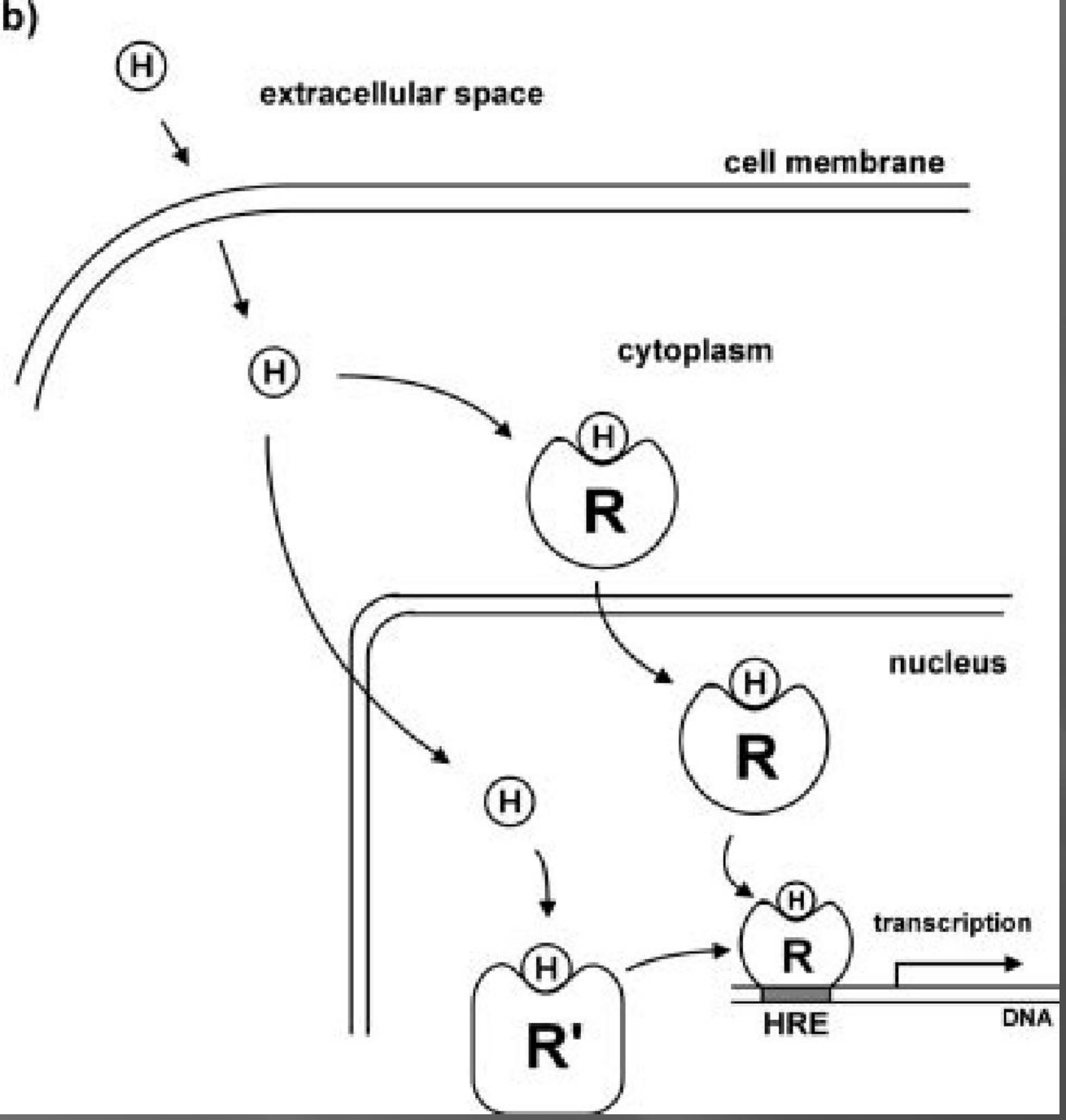


Autocrine

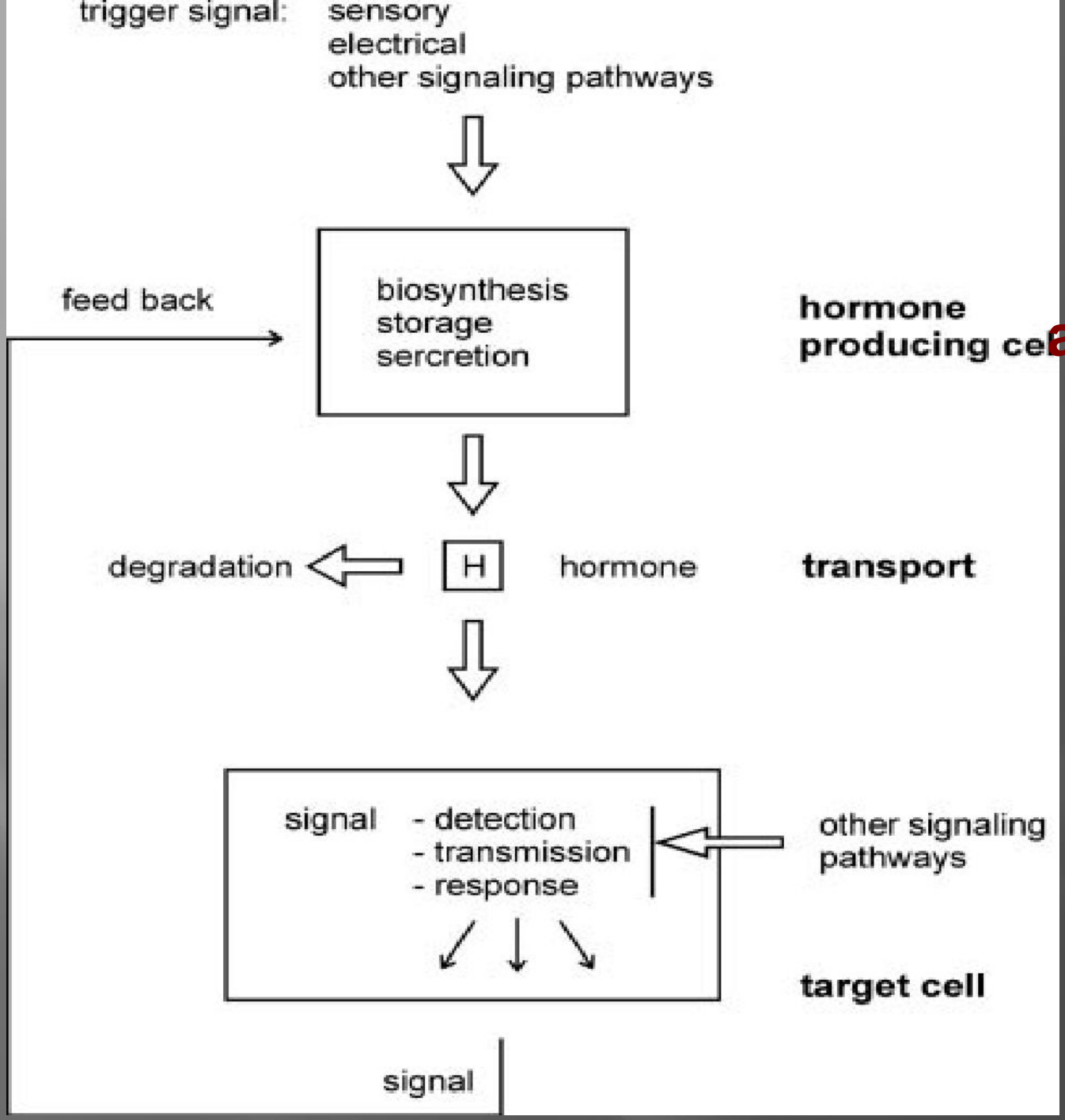




The receptor (**R**) for **hydrophilic hormones** (**H**) is located in the cellular membrane of target cell



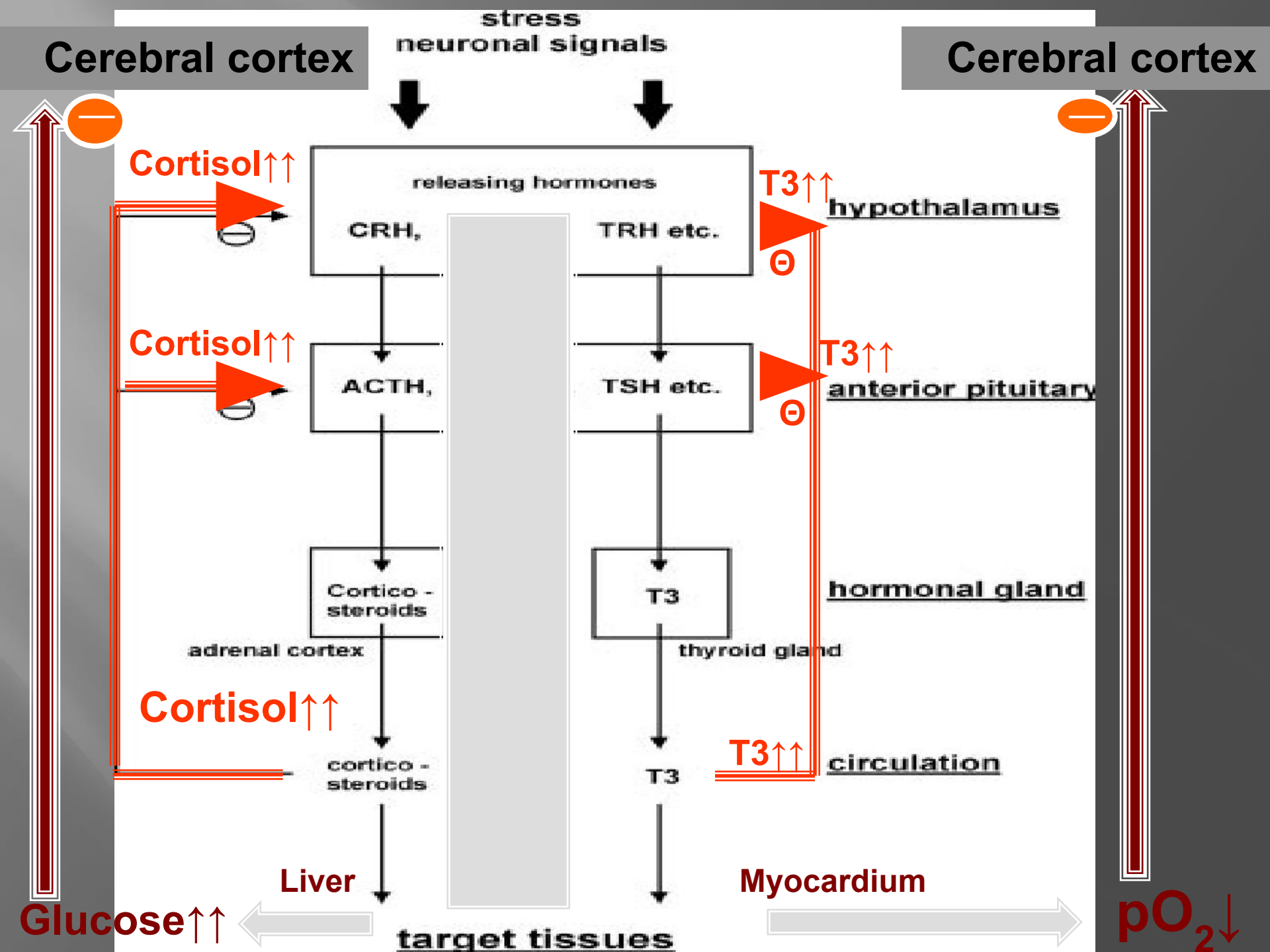
Lipophilic hormones (H) may be linked to cytoplasmic (R) and nuclear (R`) receptors



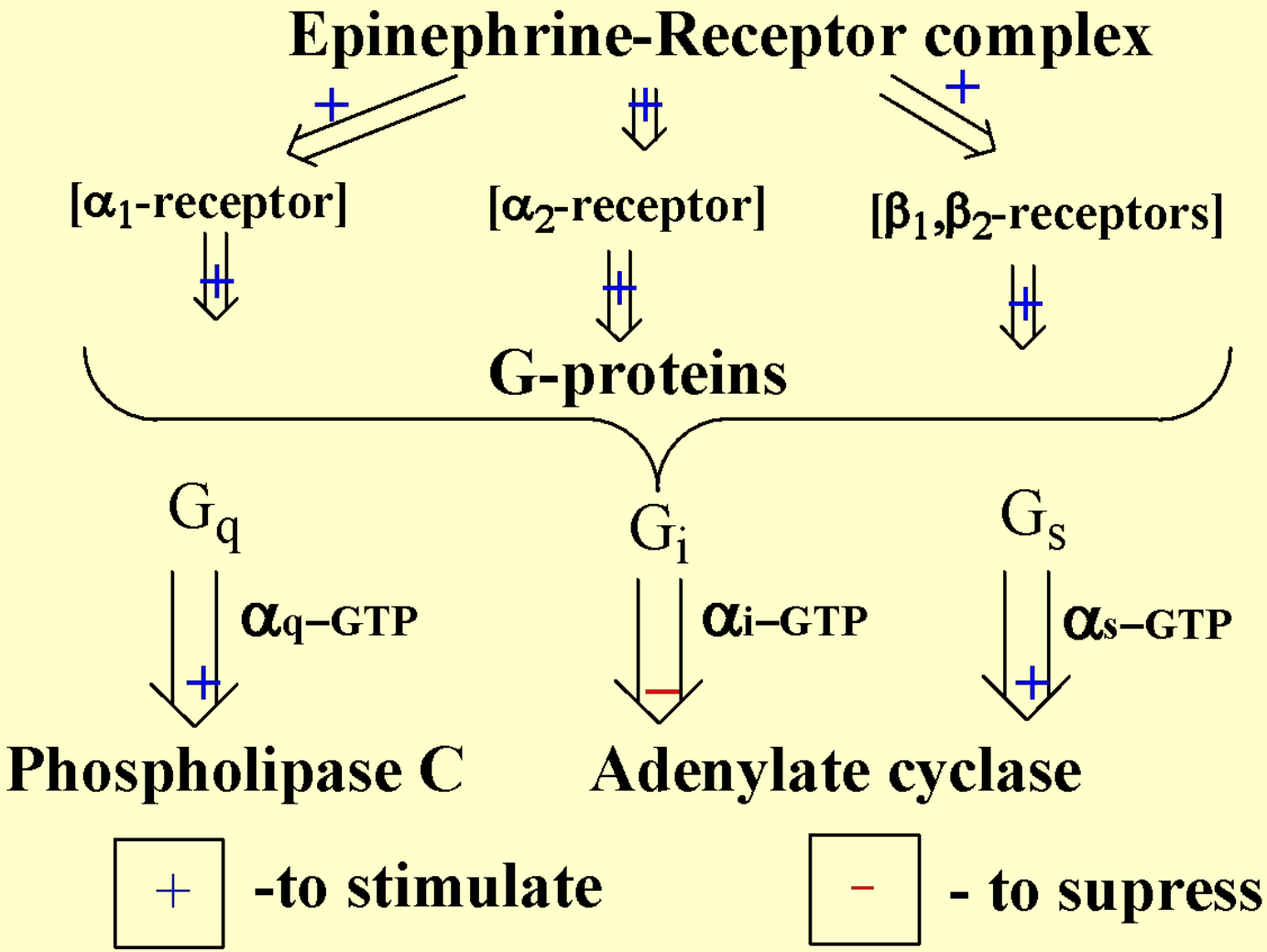
The feed-forward and feed-back control of a hormone level in the blood

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
Cortisol↑↑
releasing hormones
CRH,
TRH etc.
T3↑↑
hypothalamus
Θ
Cortisol↑↑
ACTH,
TSH etc.
T3↑↑
anterior pituitary
Θ
Cortico -
steroids
T3
hormonal gland
adrenal cortex
thyroid gland
Cortisol↑↑
cortico -
steroids
T3
T3↑↑
circulation
Liver
Myocardium
Glucose↑↑
target tissues
pO2↓

Types of signal transmission due to G-proteins

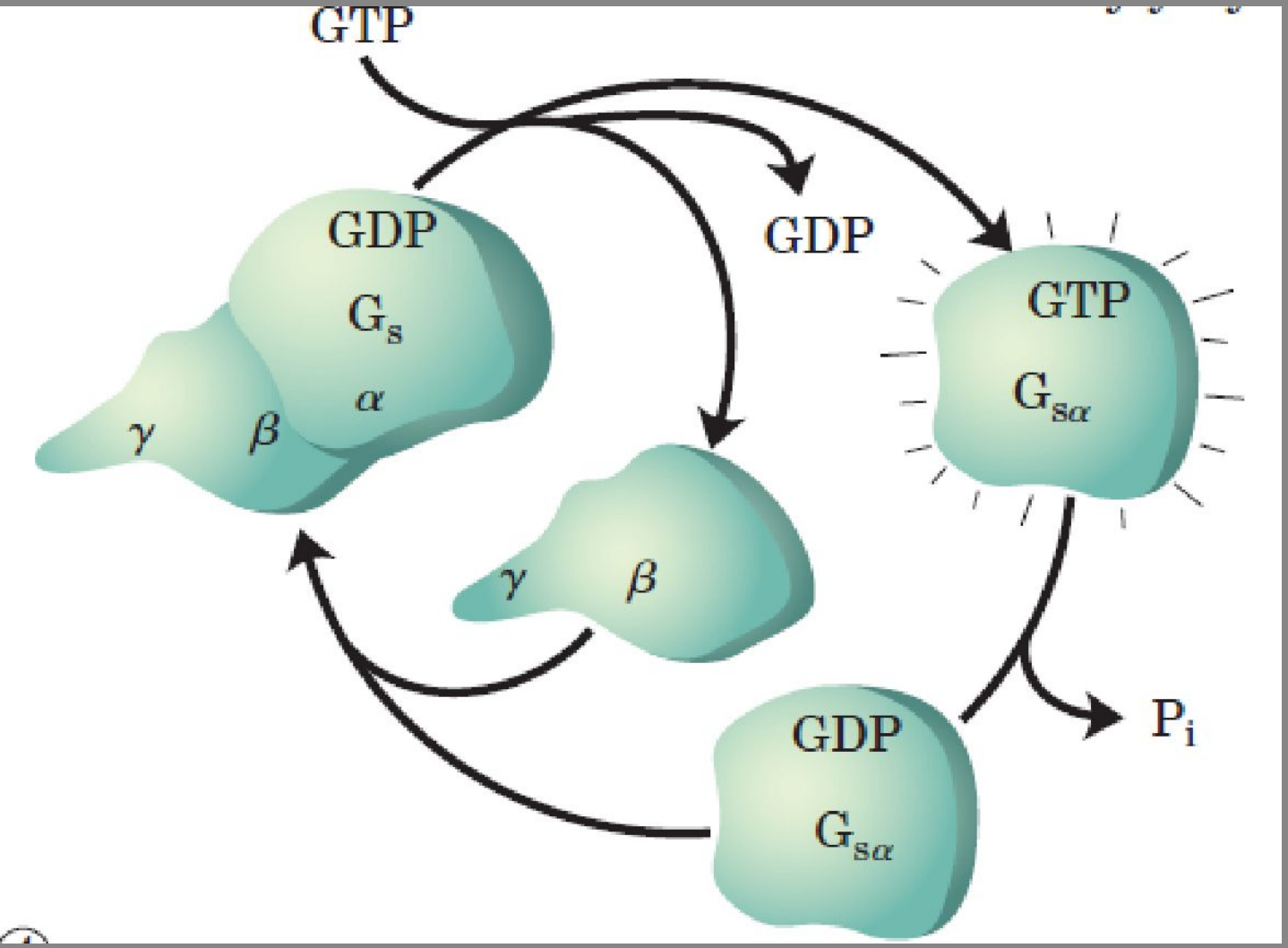


Inactive Gs protein is composed from three subunits: α, β, γ .

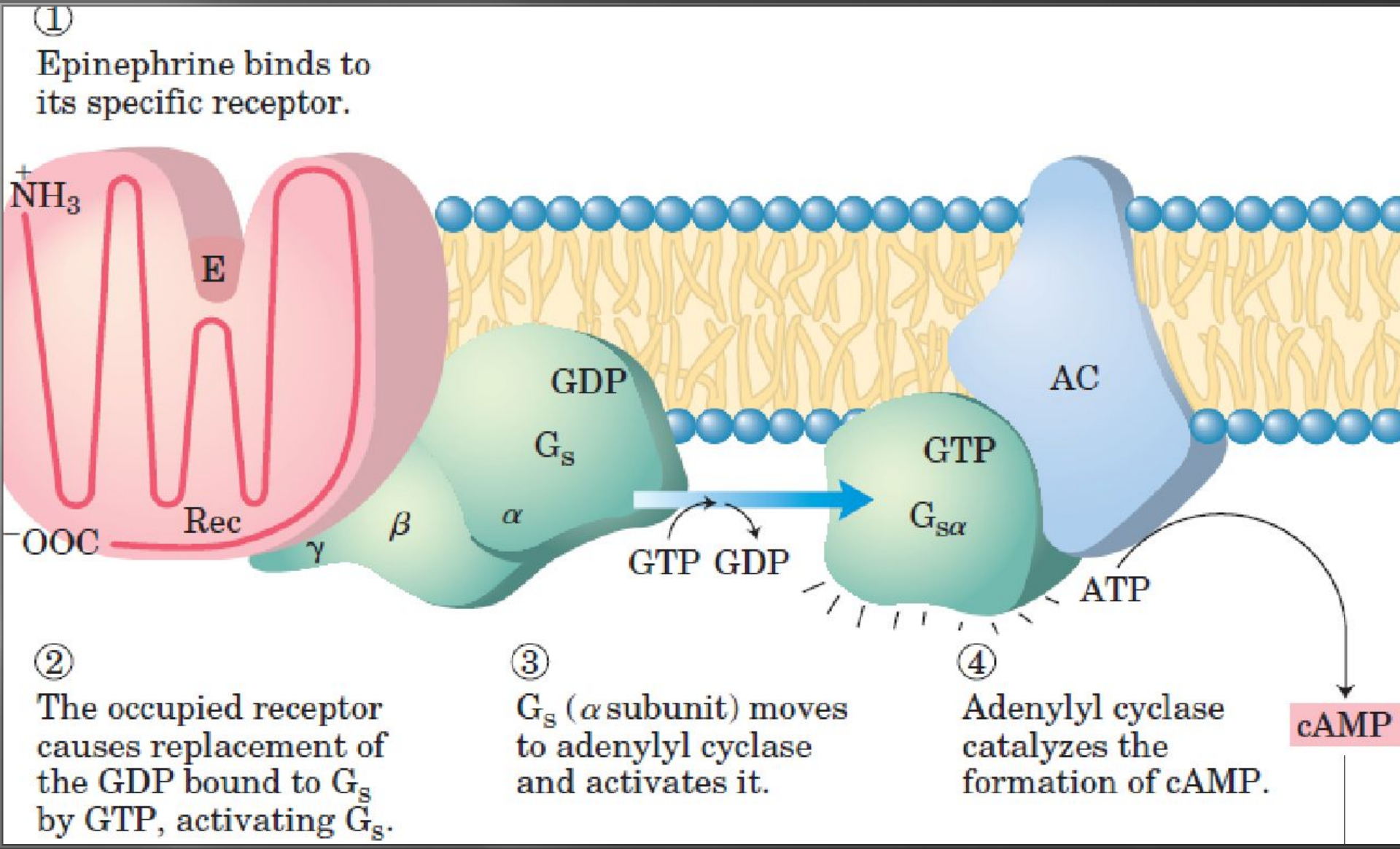
Hormone-receptor complex can stimulate Gs - it means :

dissociation of Gs to dimmer and single α−subunit linked

to GDP that is formed from GTP



Gsα−GDP is named active Gs protein


①
Epinephrine binds to its specific receptor.
NH_3^+
E
Rec
^-OOC
GDP
G_s
β
α
γ
GTP GDP
GTP
$G_{s\alpha}$
AC
ATP
cAMP
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.

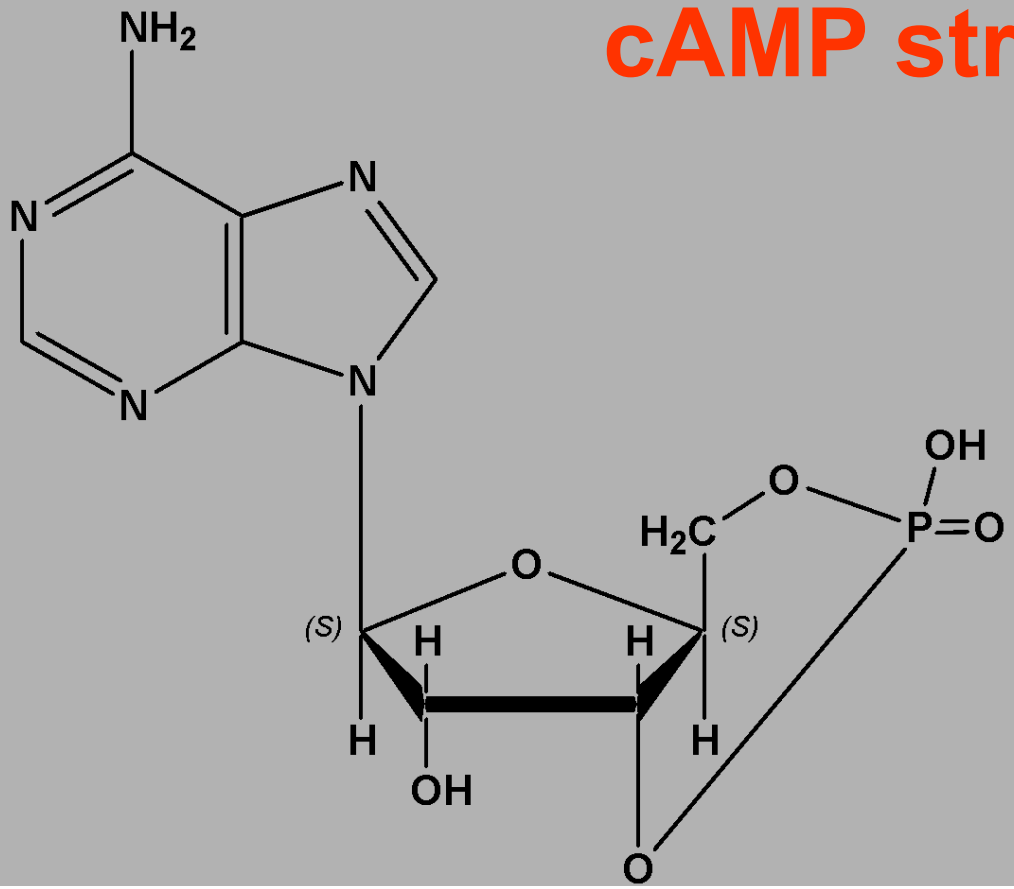
Some factors influenced G-proteins

- **Cholera toxin** modifies α-subunit of Gs 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 Gi to allow Adenylate cyclase to produce cAMP in excess levels

GAP function:

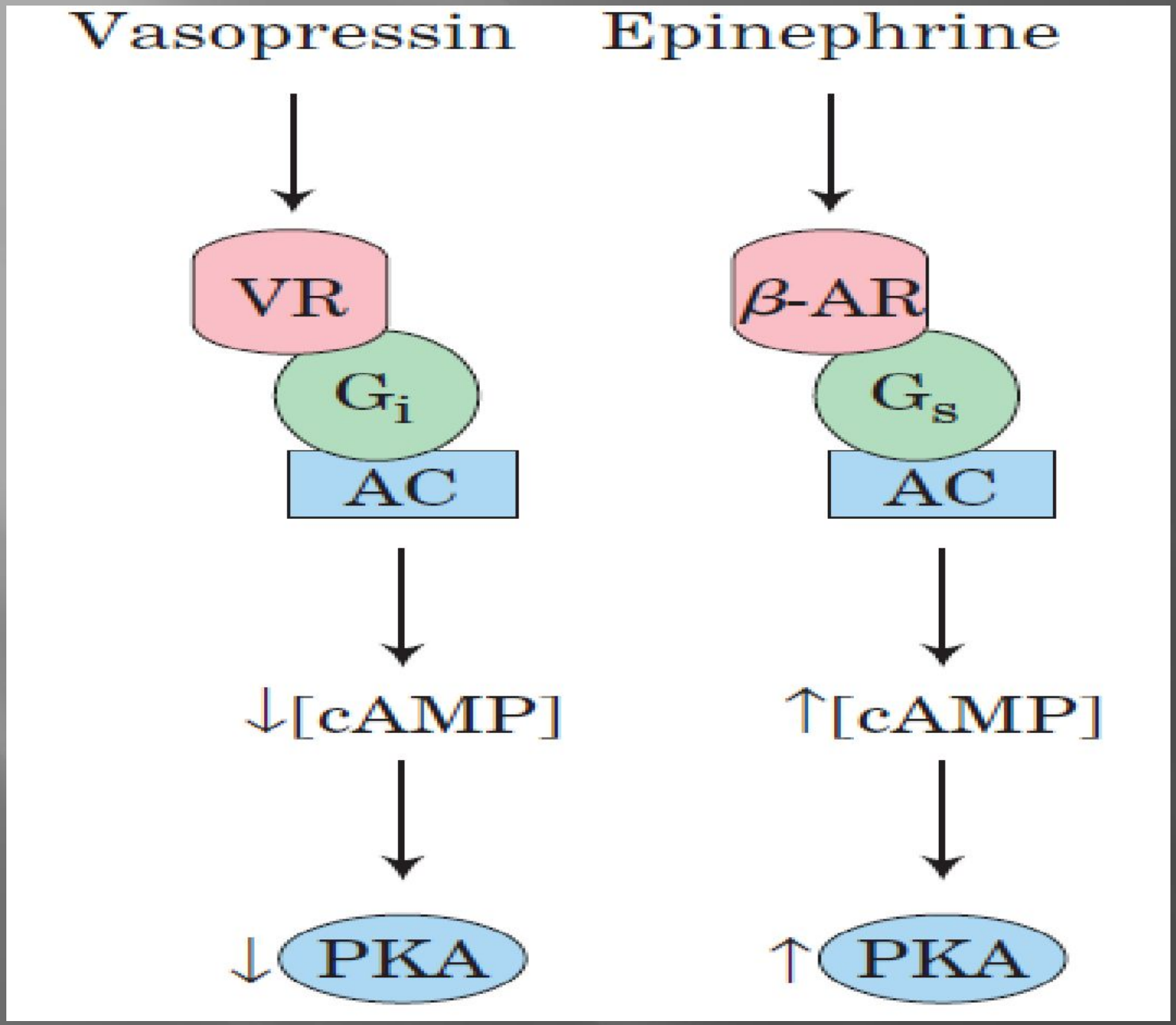
GTPase-Activating Proteins, or **GAPs** can bind to activated G-proteins and stimulate their GTPase activity, with the result of terminating the signaling event. GAPs are also known as regulator of G protein signaling proteins, or RGS proteins, and these proteins are crucial in controlling the activity of G proteins. **GAP role is to turn the G protein activity off .**

cAMP structure

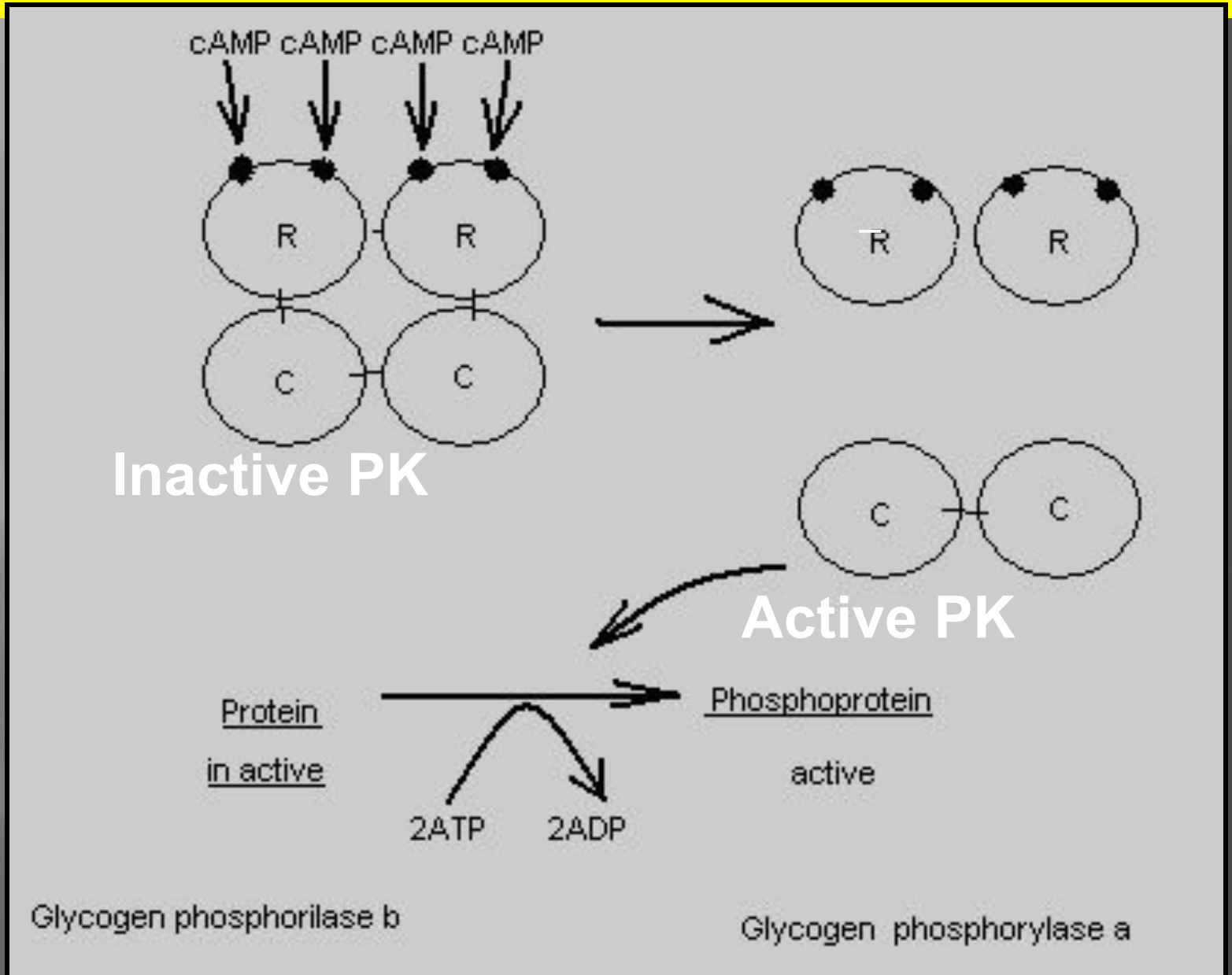


PDE – Phosphodiesterase

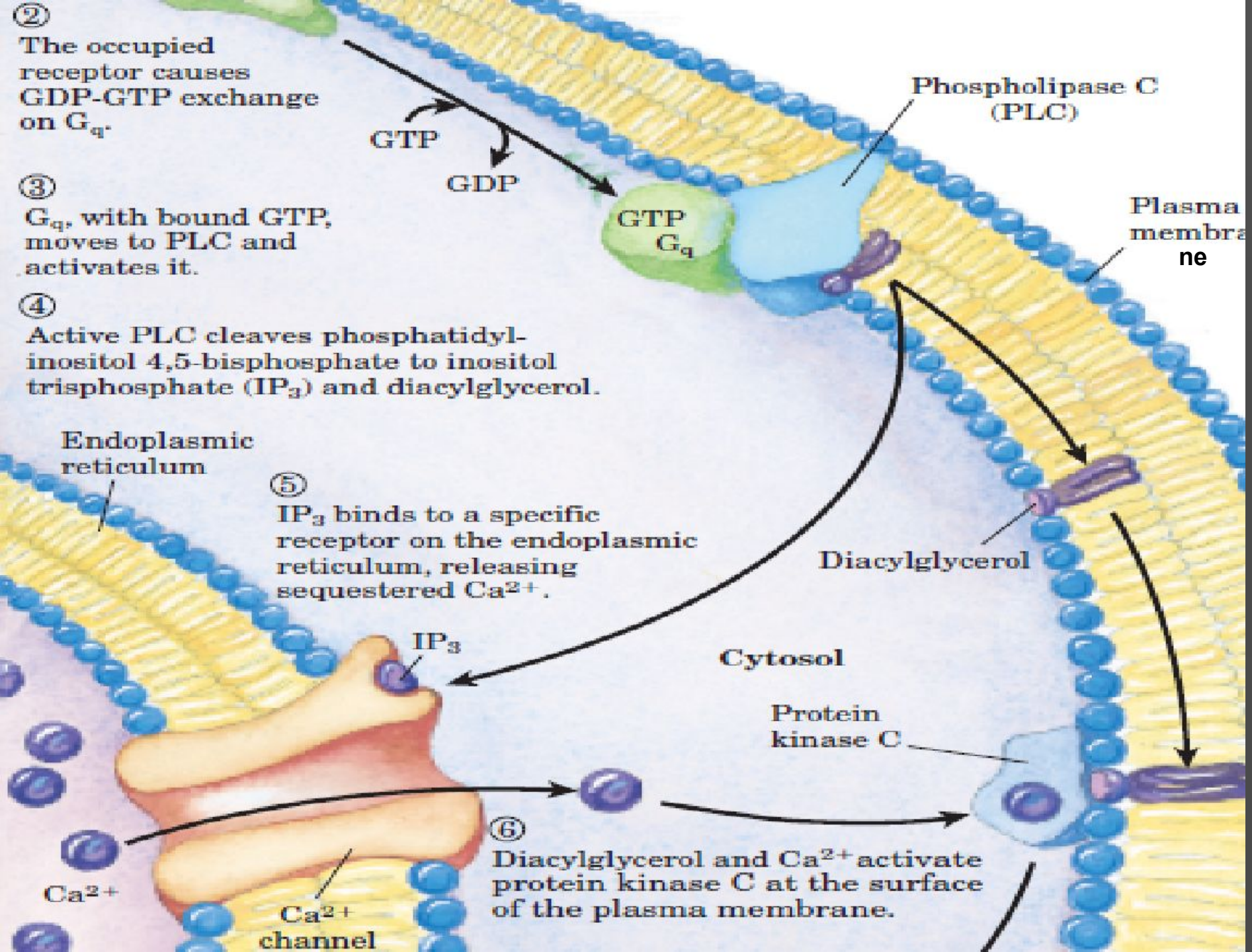
Inhibitors: metyl xanthines


Vasopressin
Epinephrine
VR
G_i
AC
β-AR
G_s
AC
↓[cAMP]
↑[cAMP]
↓ PKA
↑ PKA

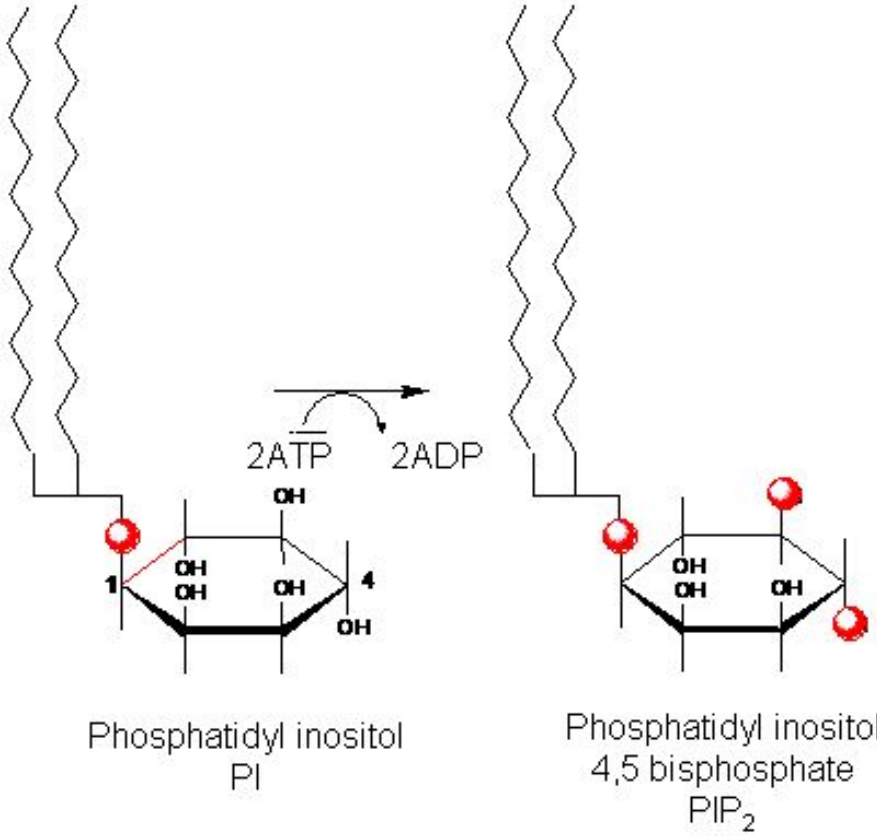
cAMP-dependent protein kinase (PK) activation



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

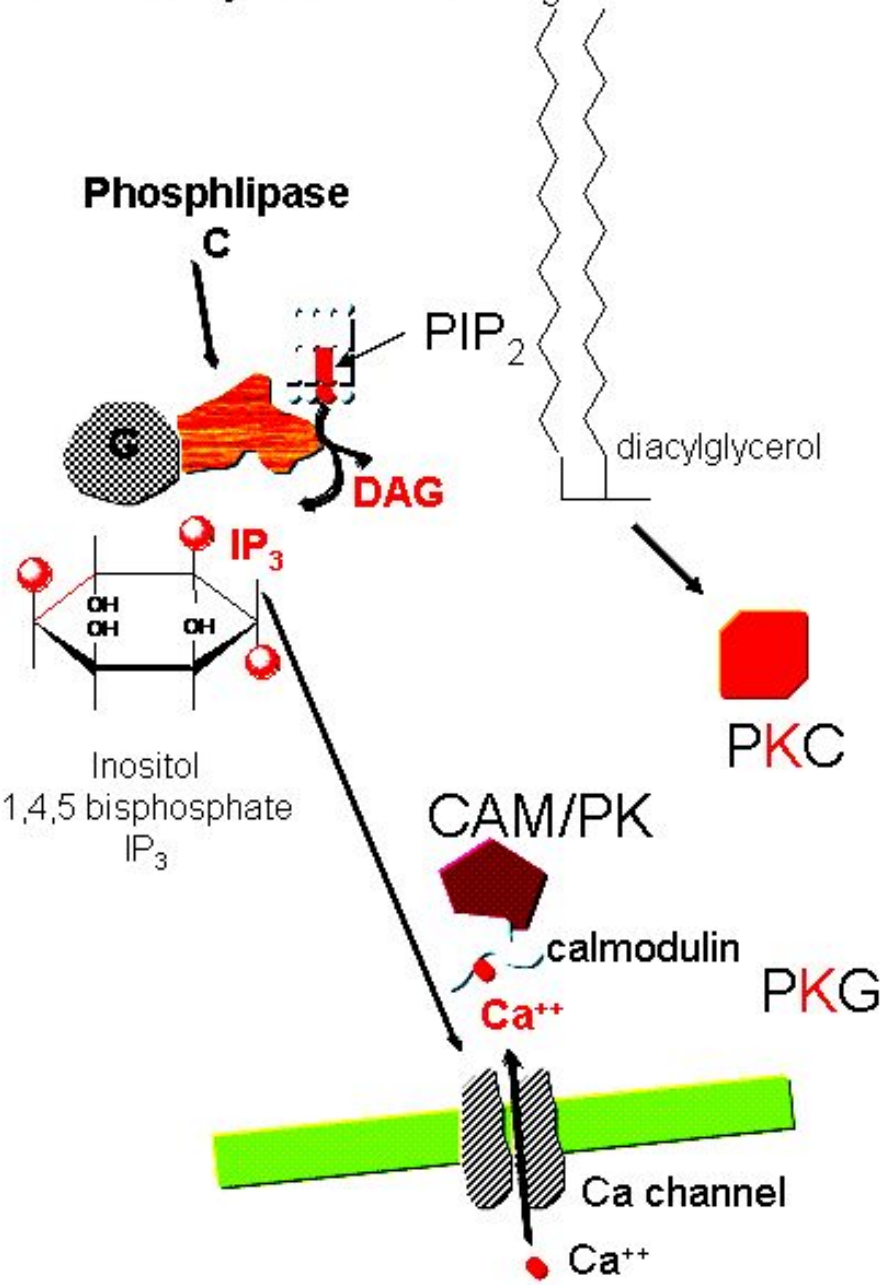

② The occupied receptor causes GDP-GTP exchange on G_q.
GTP
GDP
Phospholipase C (PLC)
③ G_q, with bound GTP, moves to PLC and activates it.
GTP G_q
Plasma membra ne
④ Active PLC cleaves phosphatidyl-inositol 4,5-bisphosphate to inositol trisphosphate (IP_3) and diacylglycerol.
Endoplasmic reticulum
⑤ IP_3 binds to a specific receptor on the endoplasmic reticulum, releasing sequestered Ca^{2+}.
Diacylglycerol
IP_3
Cytosol
Protein kinase C
Ca^{2+}
Ca^{2+} channel
⑥ Diacylglycerol and Ca^{2+} activate protein kinase C at the surface of the plasma membrane.

Activation of PKC and Ca^{2+} Channels by DAG and IP_3

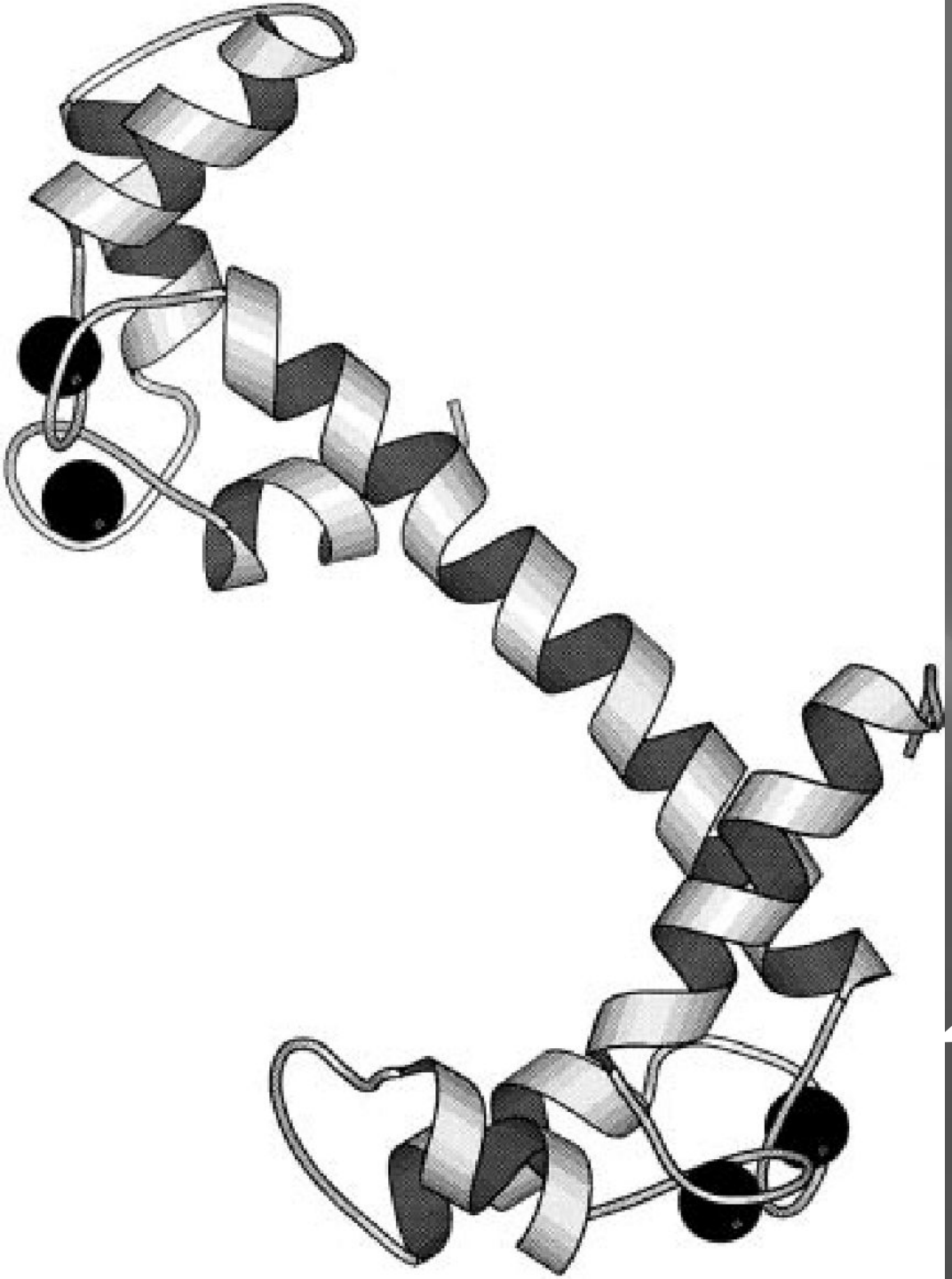


Phosphatidyl inositol
PI

Phosphatidyl inositol
4,5 bisphosphate
PIP_2



Inositol
1,4,5 bisphosphate
IP_3

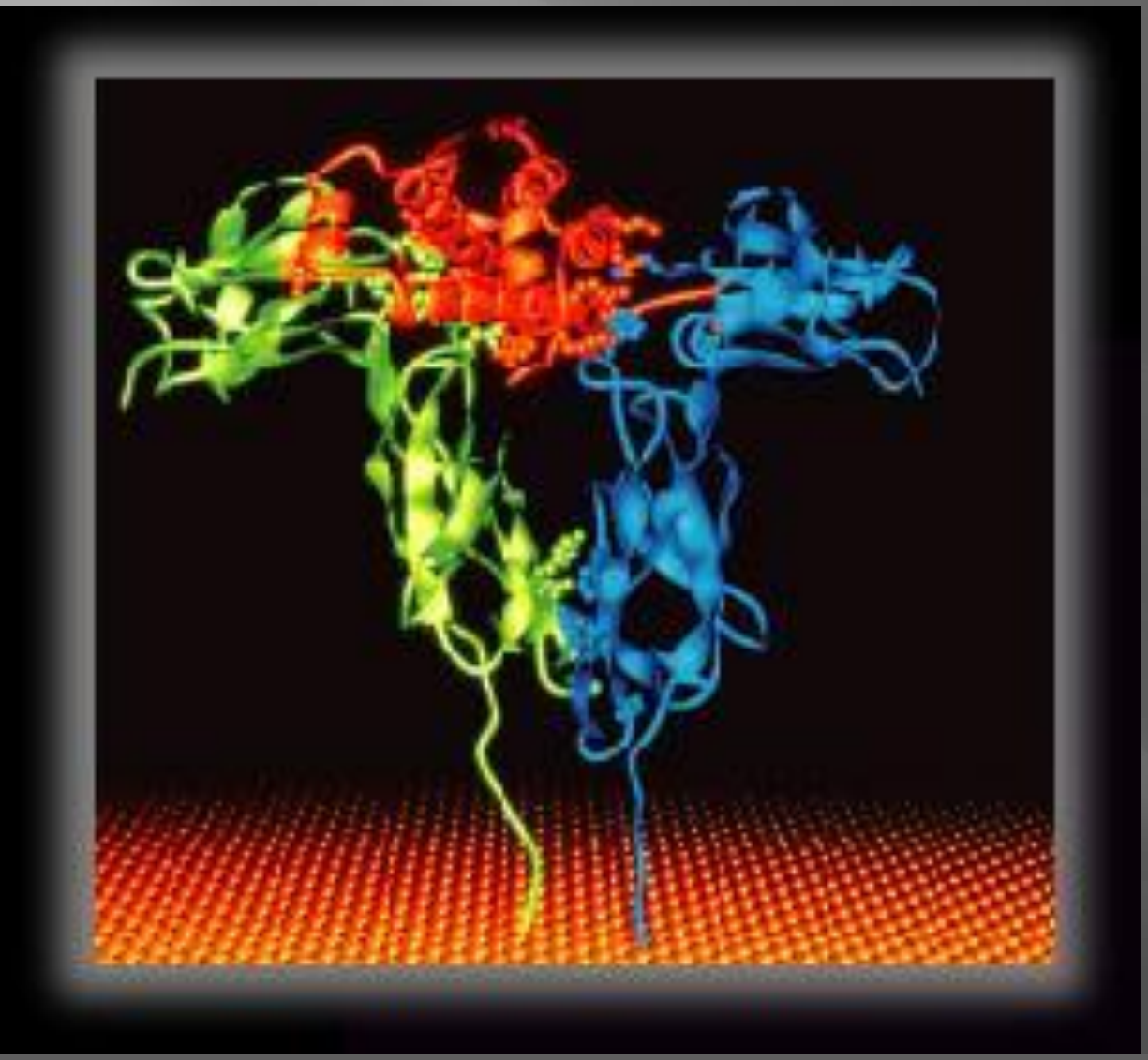


Calmodulin-4Ca $^{2+}$ complex

Ca^{2+}

Examples of different signals, receptors, G_α like-subunits, second messenger changes, and affected intracellular enzymes

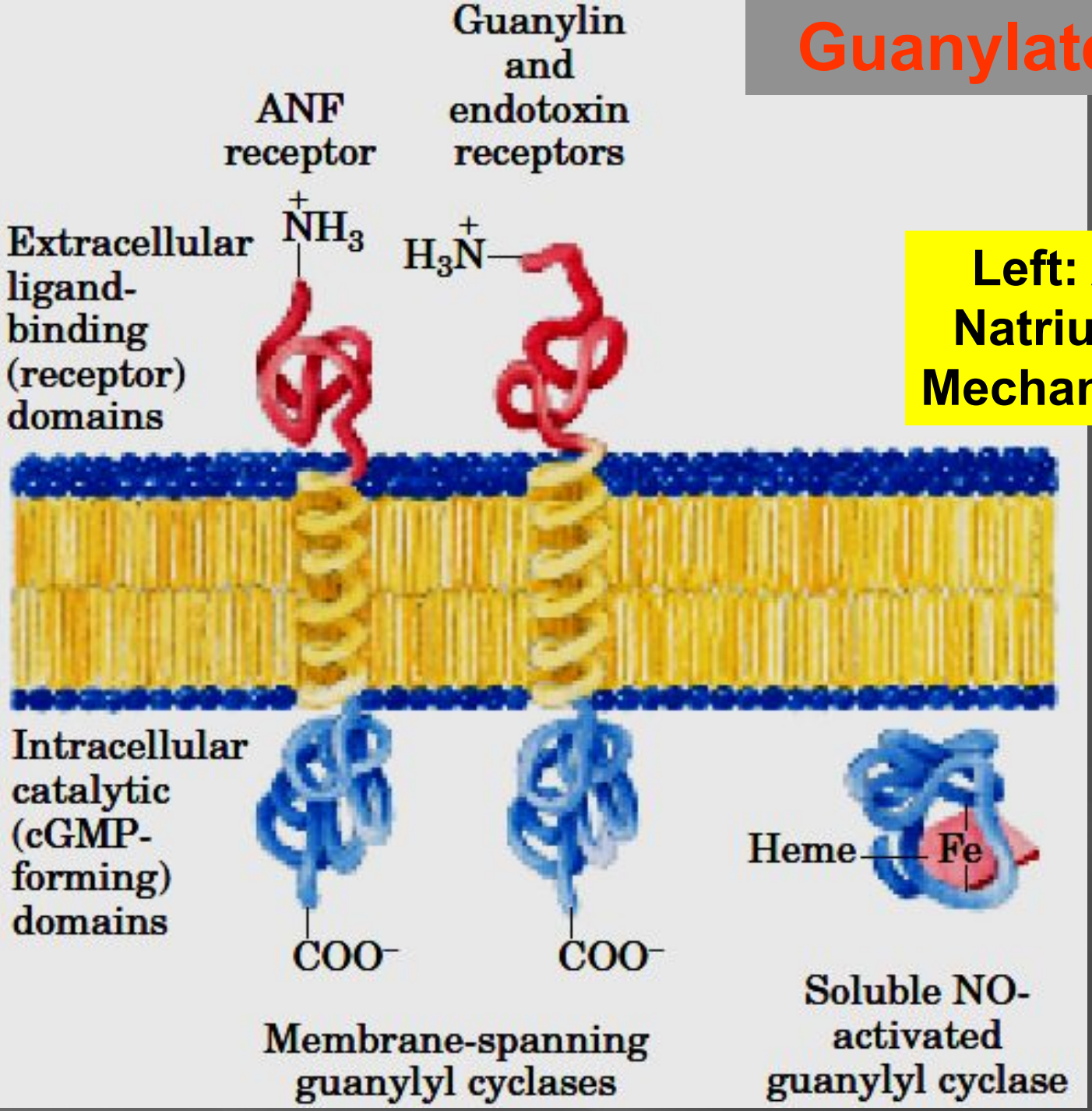
Signal	Vasopressin	Epinephrine	Light
receptor	VR	β-adrenergic	Rhodopsin
G_α like-subunit	G_i	G_s	Transducin
coupled enzyme	Adenylate cyclase	Adenylate cyclase	Phosphodi-esterase
Secondary me-ssenger	↓cAMP	↑cAMP	↓ cGMP
protein affected	↓ PK-A	↑ PK-A	↓ Ca^{2+}, Na^+ perm.



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*).

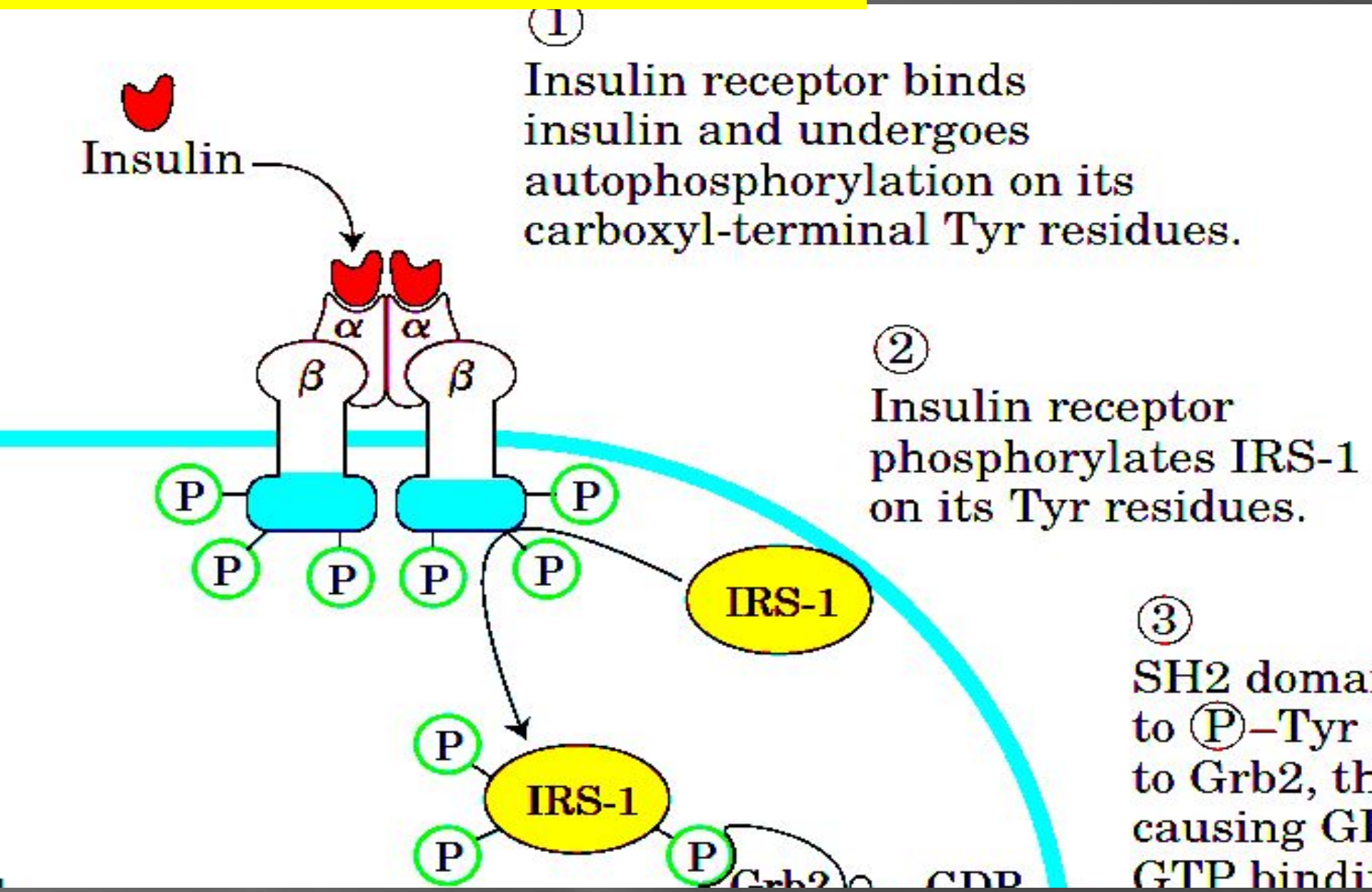
X-ray structure by and drawing courtesy of Abraham de Vos and Anthony Kossiakoff, Genentech Inc., South San Francisco, California.

Guanylate cyclases



Left: ANF –Atrial Natriuretic Factor Mechanism of action

Structure of Insulin Receptor



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

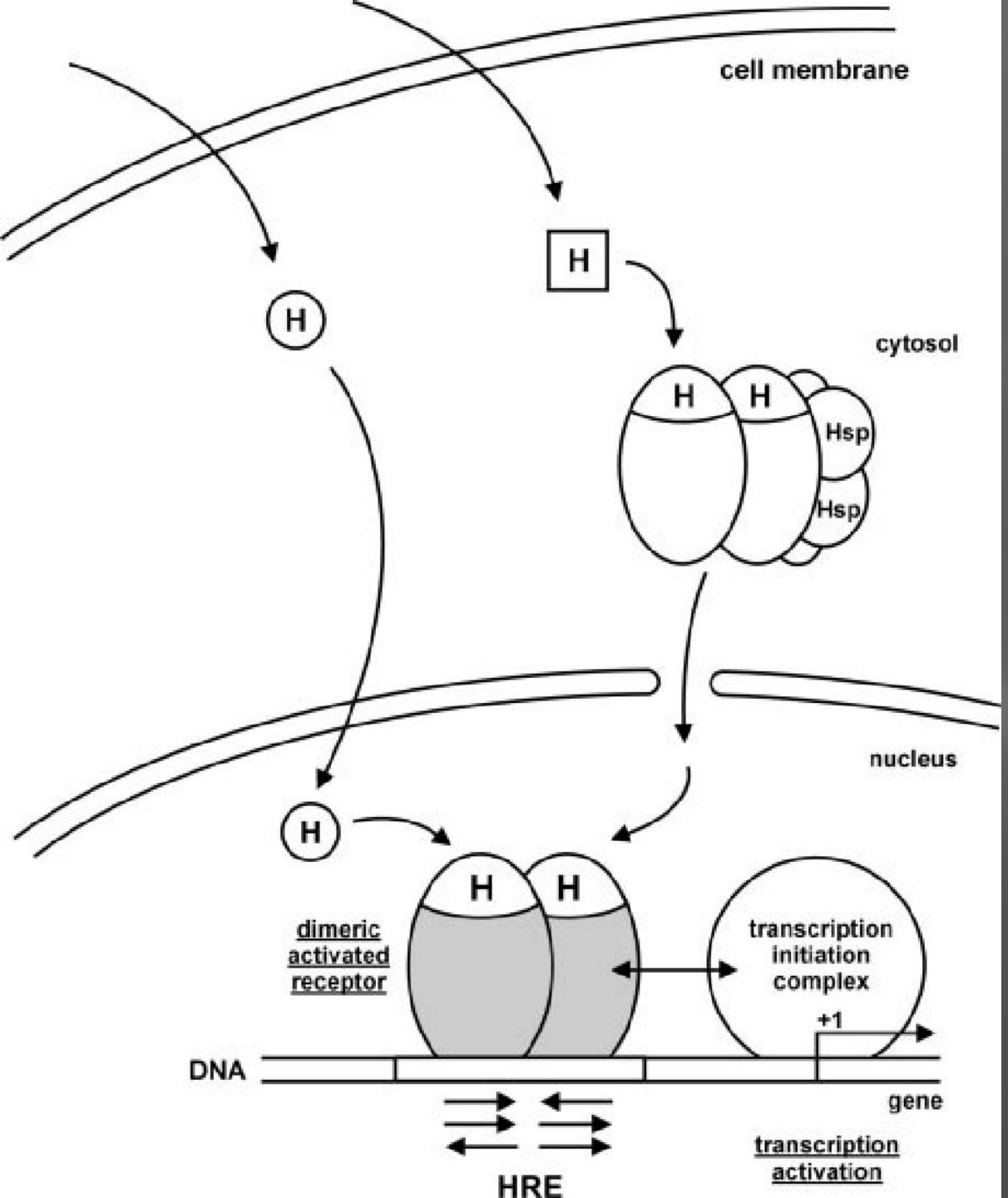
Dephosphorylation

Stimulation of Acetyl-CoAcarboxylase, Glycogen synthetase, Pyruvate dehydrogenase, Pyruvate kinase, Phosphofructokinase

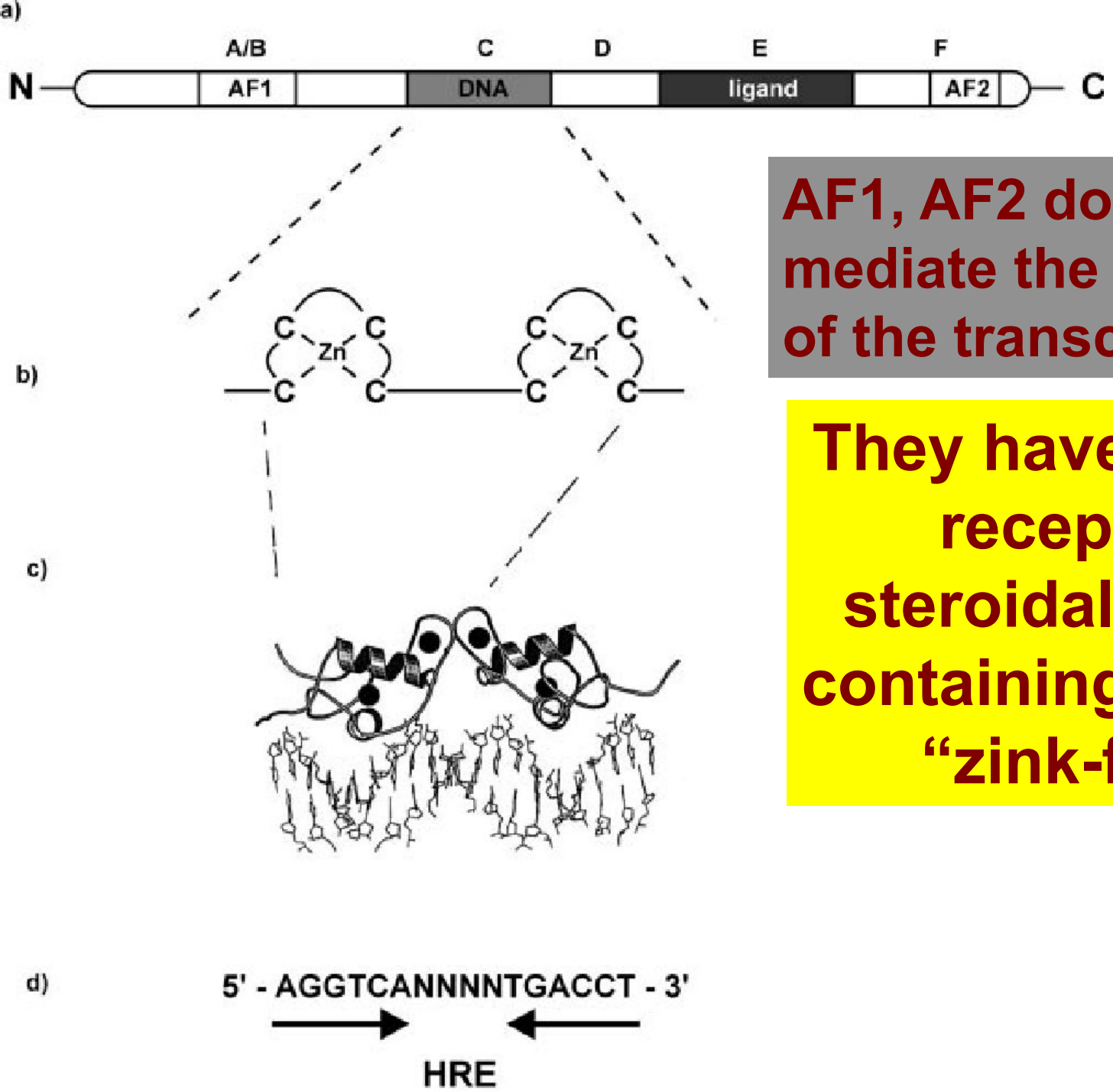
Suppression of Glycogen phosphorylase α, Triacylglycerol lipase

Stimulation of gene expression for Tyrosine aminotransferase, Palmitate synthetase, Pyruvate kinase, Glucokinase, STH, Albumins, Ovalbumin

Suppression of gene expression for Phosphoenol-pyruvate carboxykinase

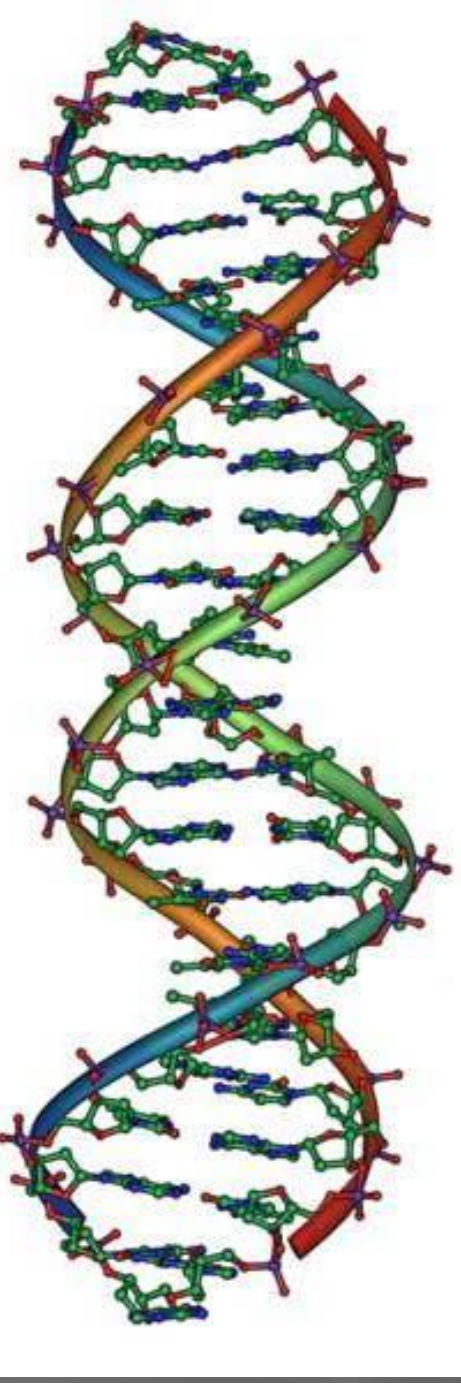


The Mechanism of action for Lipophilic Hormones (H); HRE –Hormone Response Elements



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