Eukaryotic Gene Expression: Basics & Benefits

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Lecture 15

Signal Transduction Pathways - Introduction

Eukaryotic Gene Expression: Basics & Benefits

So far……..

1. Eukaryotic RNA polymerases and basal transcription factors
2. Diversity in core promoter elements
3. Diversity in general transcription factors
4. Proximal & Distal Promoter Elements, Enhancers and Silencers, Gene-specific Regulators
5. Transcription factors – DNA binding domains
6. Transcription factors – transcription activation domains
7. Role of chromatin in eukaryotic gene regulation
8. Role of histones in eukaryotic gene regulation
9. Role of DNA methylation in eukaryotic gene regulation
10. Chromatin remodelling & gene regulation
11. mRNA processing – Role of RNA Pol II in mRNA capping and mRNA splicing
12. mRNA processing – Role of RNA Pol II in polyadenylation and mRNA editing

Regulation of mRNA synthesis

13. Regulation of RNA Pol I transcription

Regulation of rRNA synthesis

14. Regulation of RNA Pol III transcription

Regulation of tRNA & 5S rRNA synthesis

Regulation of gene expression by signals emanating outside the nucleus

CONSTITUTIVE *VS* INDUCIBLE GENE EXPRESSION

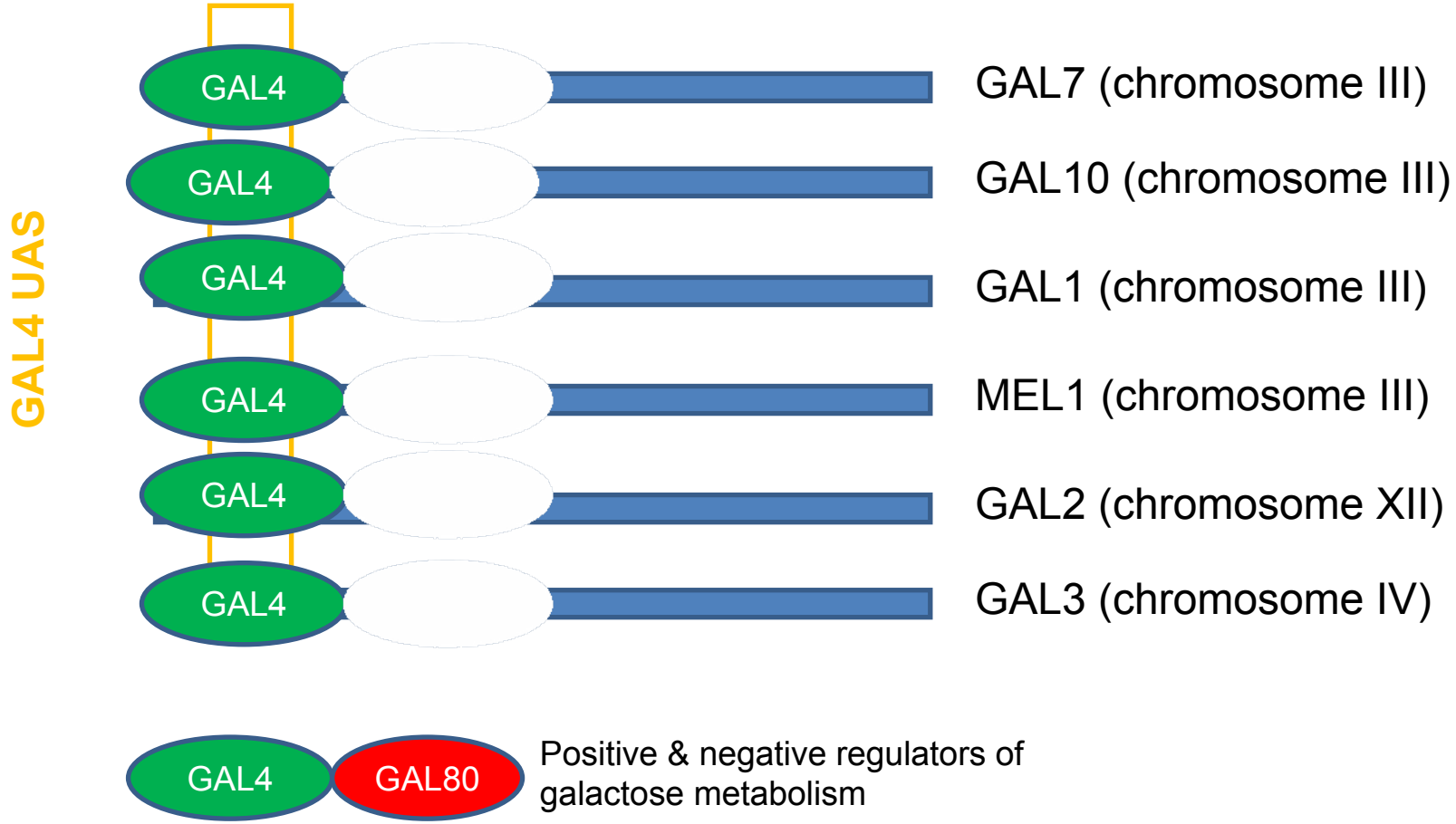
Transcription of genes inside the nucleus can lead to the synthesis of molecules that perform housekeeping functions—basic cellular processes that take place in all the different kinds of cells.

Such genes are permanently turned on in almost all cells or tissues of an eukaryotic organism and thus are said to be **constitutively expressed.**

The expression of other genes is highly **regulated**, being turned on or off at specific stages of development or in response to specific extracellular stimuli.

Regulation of gene expression during galactose metabolism in yeast cells.

Transcriptional regulation of genes of galactose metabolism in yeast cells



The enzymes involved in galactose metabolism and transport in yeast cells are inducible and coregulated, even though the genes are located on different chromosomes.

Thus, although eukaryotic nuclear genes are not arranged into operons, they are often coordinately regulated in the cell.

Around a billion years ago, the ability of cells to communicate with extracellular signals took a great leap in complexity when eukaryotic cells began to associate together as multicellular organisms.

Along with the evolution of multicellularity came cell specialization as well as the development of tissues and organs to perform specific functions.

Coordination of the development and environmental responses in these complex multicellular organisms required an array of signaling mechanisms.

Eukaryotes developed two major communication systems

NERVOUS SYTEM

ENDOCRINE SYSTEM

It became necessary to regulate the expression of genes inside the nucleus of multicellular organisms in response to a variety of signals that are generated either within the cell or those that came from outside the cell.

Such internal as well as external signaling agents exerted their effects on gene expression by means of a series of biochemical reactions, called **signal transduction pathways,** that greatly amplify the original signal and ultimately result in the activation or repression of genes in the nucleus.

Signal transduction pathways often make use of proteins known as **receptors** that are present either on the plasma membrane or located intracellularly as well as protein phosphorylation/dephosphorylation cascades involving a series of **protein kinases and protein phosphatases**.

Enzymes catalyzing the transfer of gamma-phosphate group from ATP to the appropriate amino acids of a protein molecule are known as PROTEIN KINASES.

Protein kinases represent one of the largest protein families, which may be consist of up to 2000 members in eukaryotic genome.

More than 80% cellular events are regulated by protein phosphorylation.

Ser, Thr and Tyr are the most commonly phosphorylated amino acid residues by protein kinases.

Phosphotyrosine plays an important role in molecule recognition and formation of protein-protein complex.

Phosphorylation at serine or threonine may induce conformational change on target enzymes due to charge repulsion.

Signal transduction leading to regulation of gene expression is characterized by a maze of complex intermolecular and intramolecular interactions.

Molecules known as hormones, produced by the the endocrine system of multicellular eukaryotes became major regulators of signal transduction pathways.

Hormones are of two categories based on their ability to move across the plasma membrane:

Lipophilic hormones

They diffuse readily across the hydrophobic bilayer of the plasma membrane

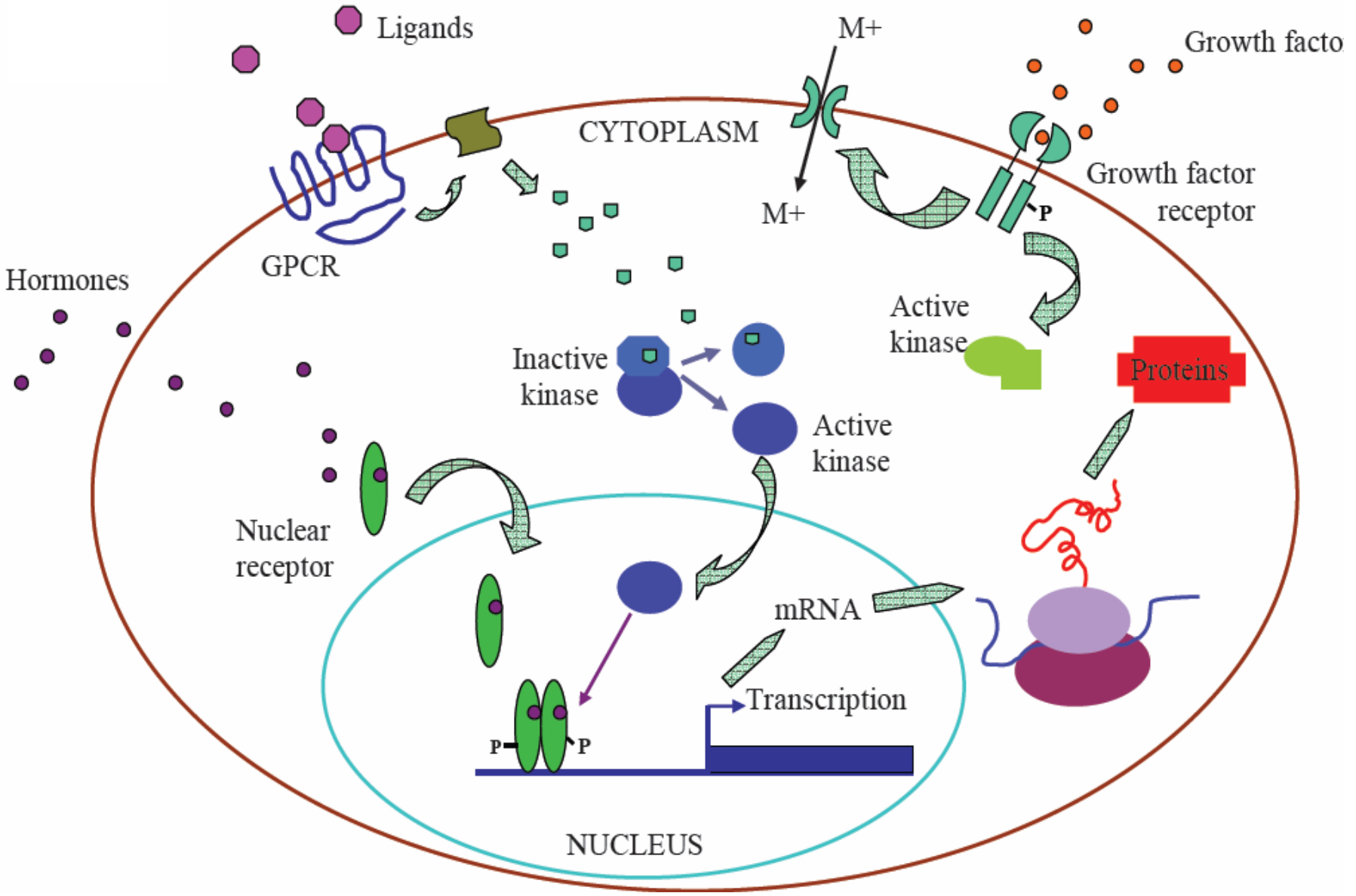
Water-soluble hormones

***These are* unable to enter the cell.**

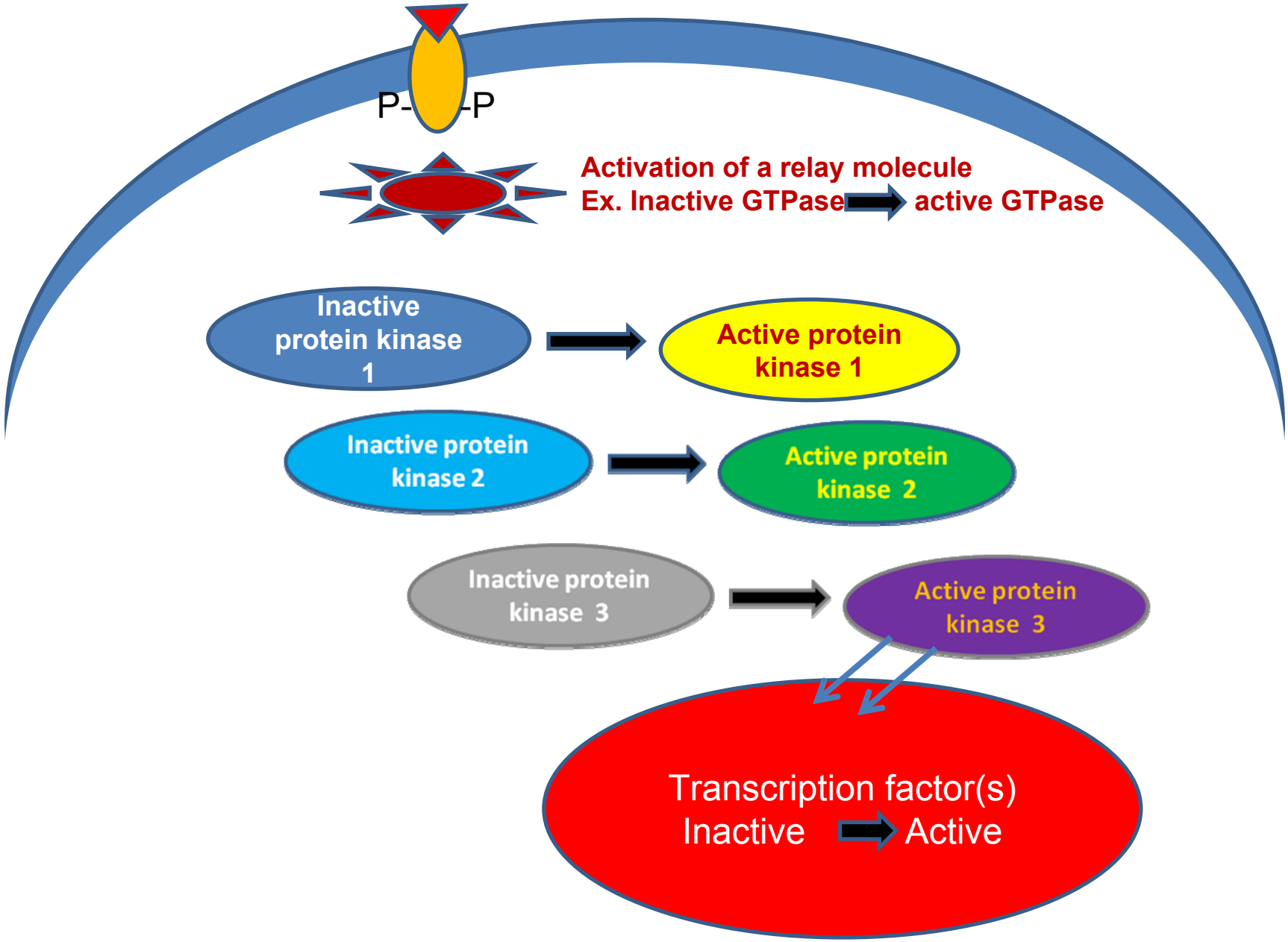
Thus, in order to regulate the expression of genes in the nucleus through these two classes of hormones, two major signal transduction pathways were evolved:

Signal transduction by water-soluble hormones through receptors located on the cell surface.

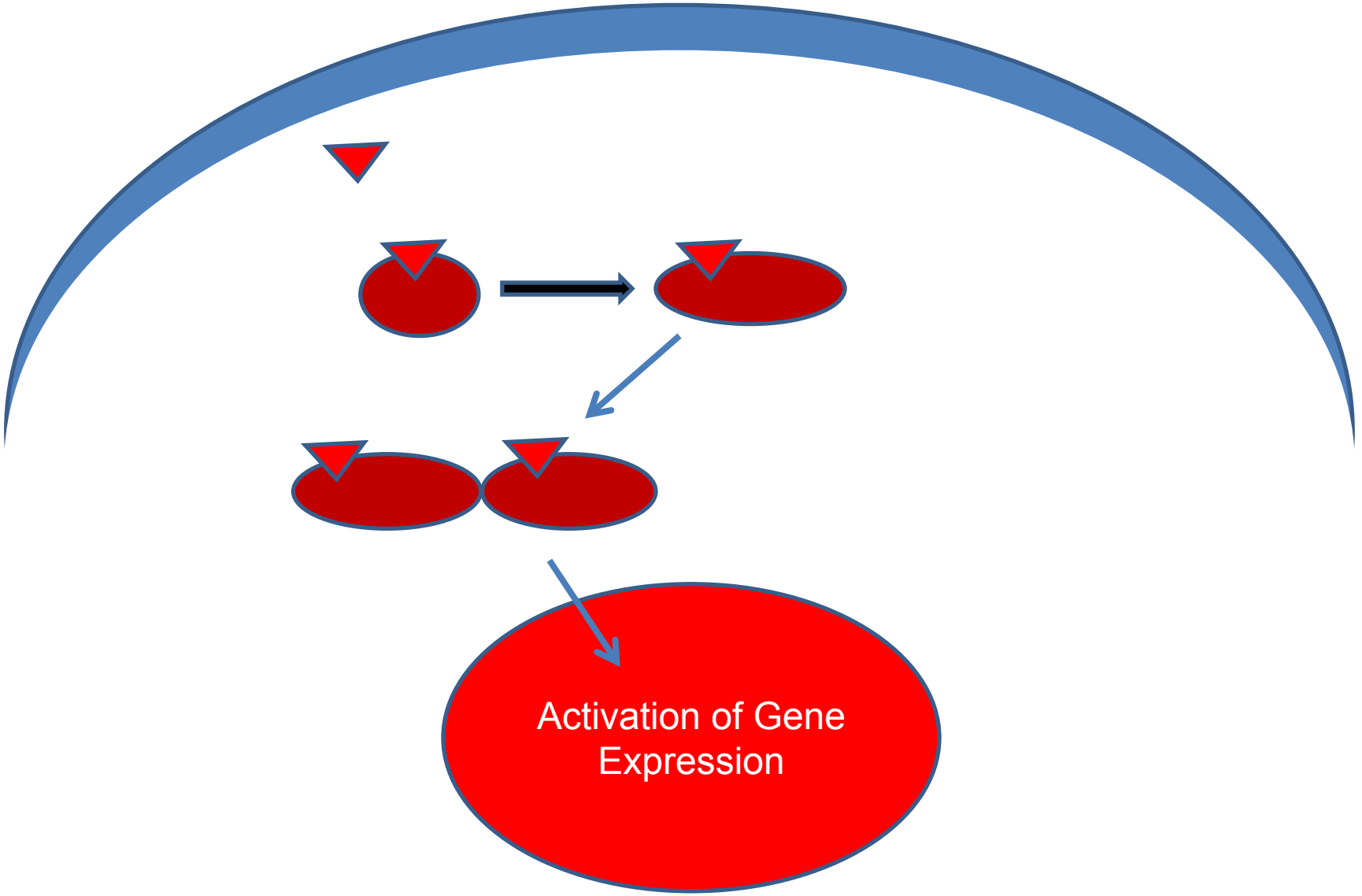
Signal transduction by lipophilic hormones through receptors present in the cytoplasm or nucleus


Ligands
M+
Growth facto
CYTOPLASM
Growth factor
receptor
M+
P
GPCR
Hormones
Active
kinase
Inactive
kinase
Proteins
Active
kinase
Nuclear
receptor
mRNA
Transcription
P
P
NUCLEUS

Signal transduction by water-soluble hormones through receptors located on the cell surface.



Signal transduction by lipophilic hormones through intracelluar receptors

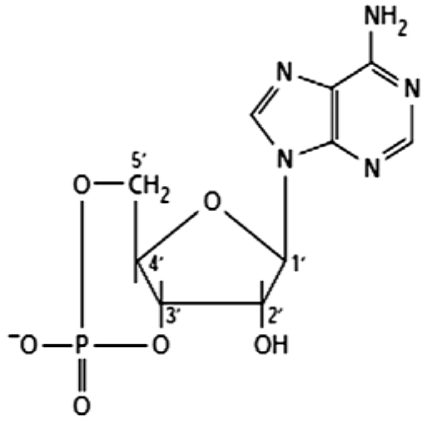


Often, numerous signals are required to turn on or turn off the expression a specific gene and each of these signals is transmitted to the gene by a separate regulator.

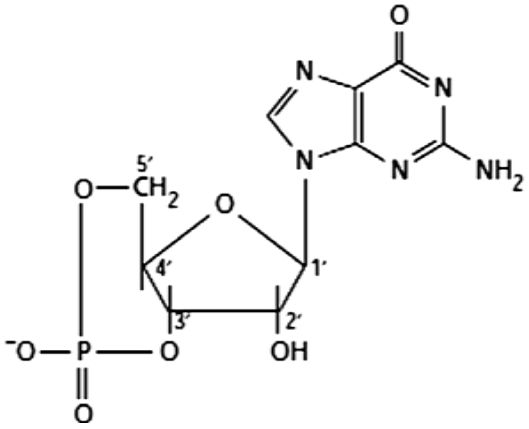
Thus, multiple signalling molecules and multiple transcription factors act together leading to synergistic activation or repression of transcription of specific genes.

Signal transduction pathways often involve the generation of second messengers inside the cell that greatly amplify the original signal.

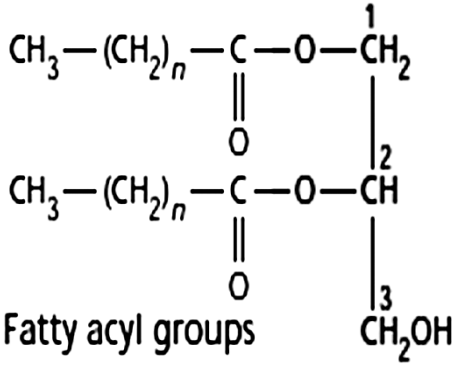
For example, interaction of a single hormone molecule with a membrane receptor can lead to the activation of an enzyme that produces hundreds of molecules of a second messenger.



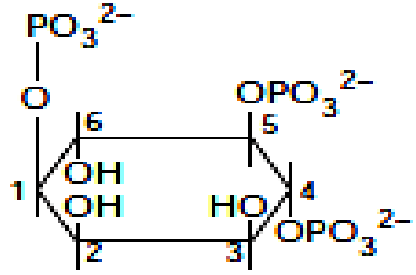
3′,5′-cyclic AMP (cAMP)



3′,5′-cyclic GMP(cGMP)



1,2- diacylglycerol (DAG)



inositol 1,4,5-trisphosphate (IP3)

Nitric oxide (NO)

Calcium (Ca^{2+})

The second messengers in turn bind to specific regulatory proteins inducing a conformational change that leads to their activation.

Once activated, these proteins go on to regulate the activity of numerous other proteins including transcription factors in the cell.

Cell surface receptors and G proteins

Water-soluble mammalian hormones bind to cell surface receptors that interact with signal-transducing, heterotrimeric GTP-binding regulatory proteins or **G Proteins** which are GTPases that undergo conformation change on GTP binding and activate an **effector enzyme** which **g**enerates an intracellular second messenger (Ex. cAMP).

Receptors interacting with trimeric G-proteins contain **seven-transmembrane alpha helices**

GPCR SIGNALLING

Amines, nucleotides, eicosanoids, lipid moieties

Peptide hormones

Proteases (thrombin)

Glycoprotein hormones (LH, FSH, hCG, TSH)

Calcium, Glutamine, GABA

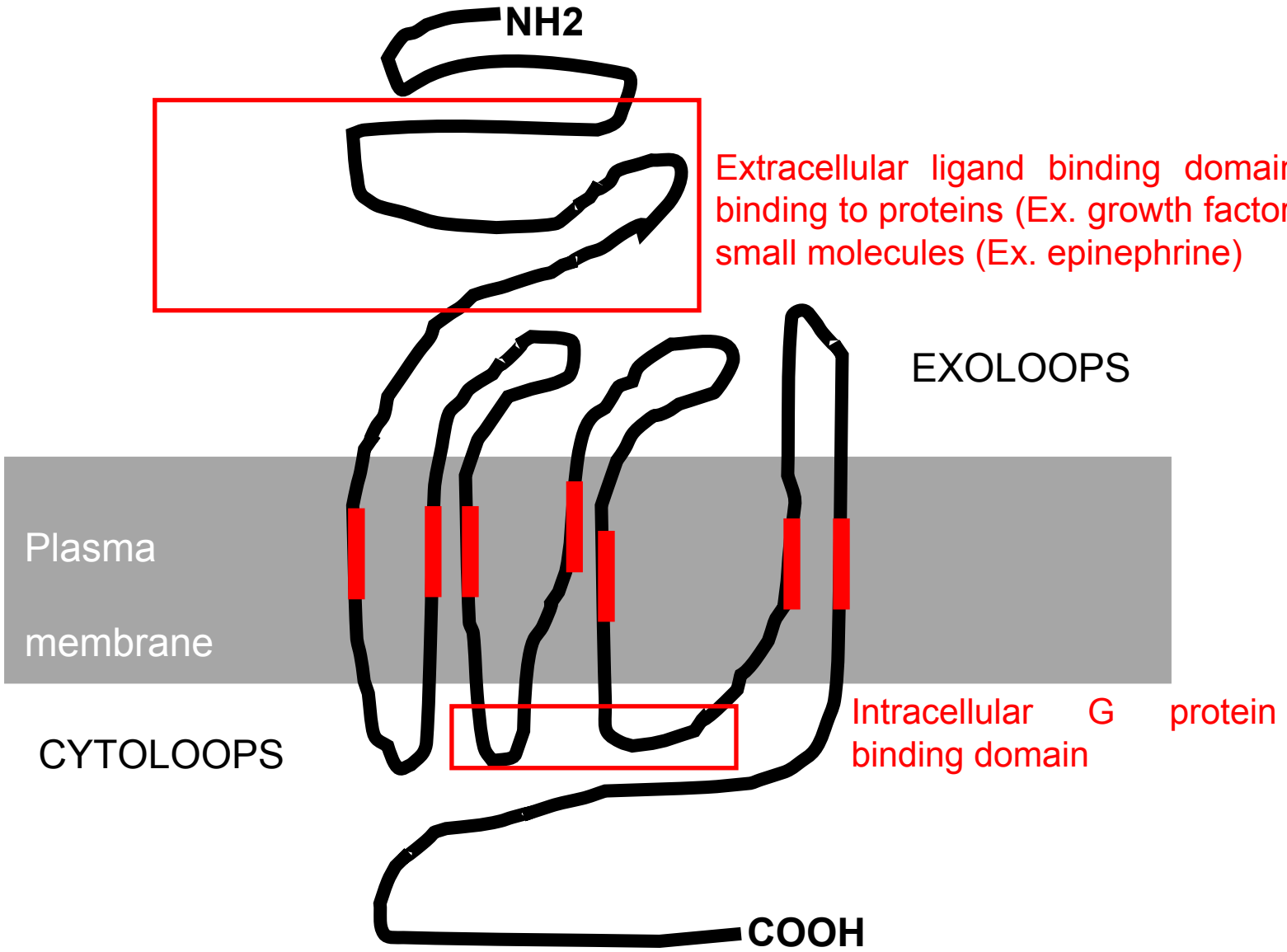
J. Biol. Chem. 273:17299-302, 1998

Heterotrimeric G-protein is composed of three subunits (alpha, beta and gamma).

The alpha subunit (~40 kDa) contains GTPase activity

the beta (~37 kDa) and gamma (8.4 kDa) subunits form a dimer and can only be dissociated by denaturation.

Seven-spanning receptors that interact with trimeric G-proteins



The heterotrimeric G proteins are distinct from the monomeric G proteins, (Ex. Ras)

Heterotrimeric G proteins cycle between active and inactive forms, thus acting as molecular switches.

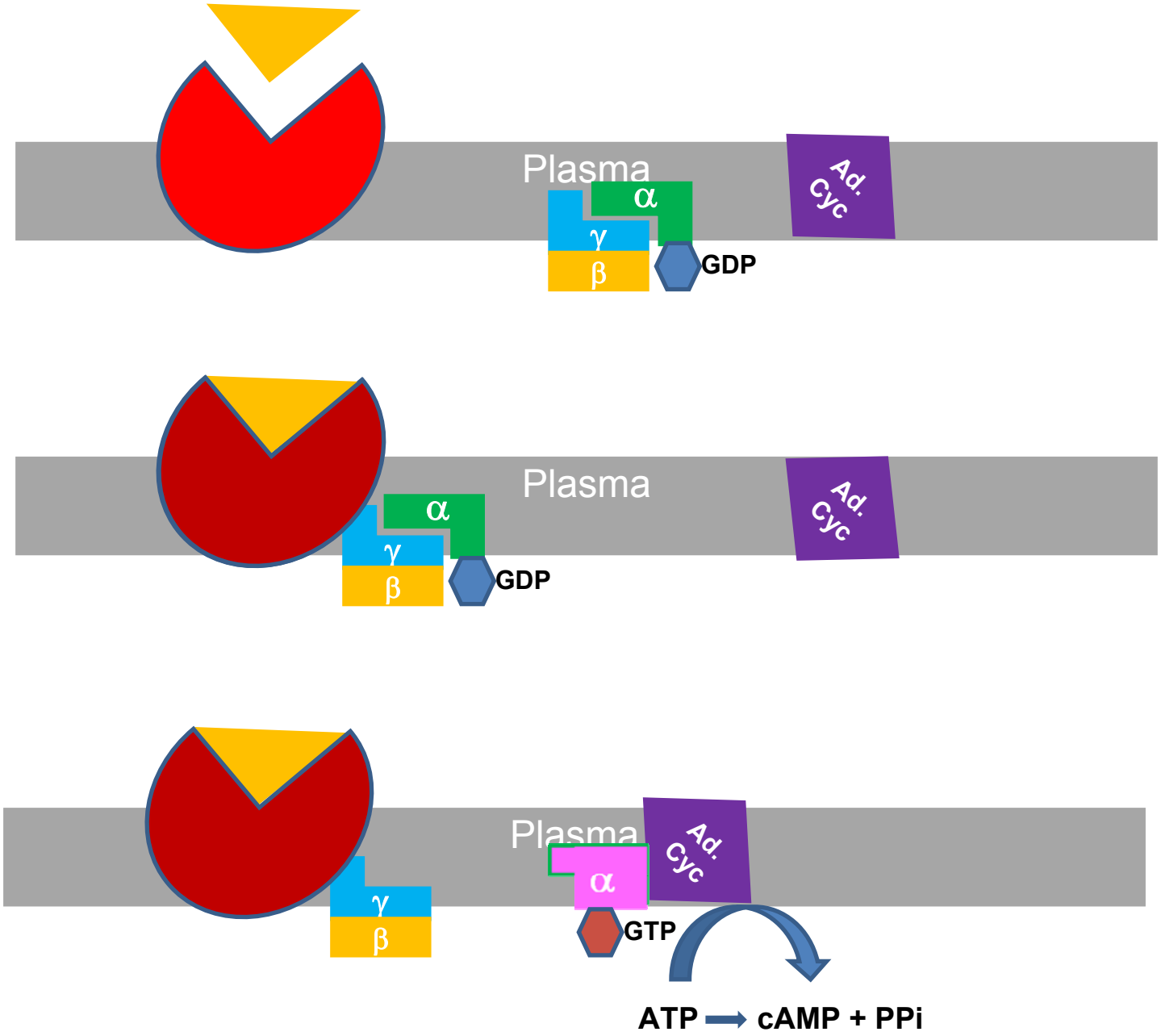
The β and γ subunits form a tight complex that anchors the trimeric G protein to the membrane on the cytoplasmic side.

Structure of GPCR
Annu. Rev. Pharmacol. Toxicol. 37:167,1997)

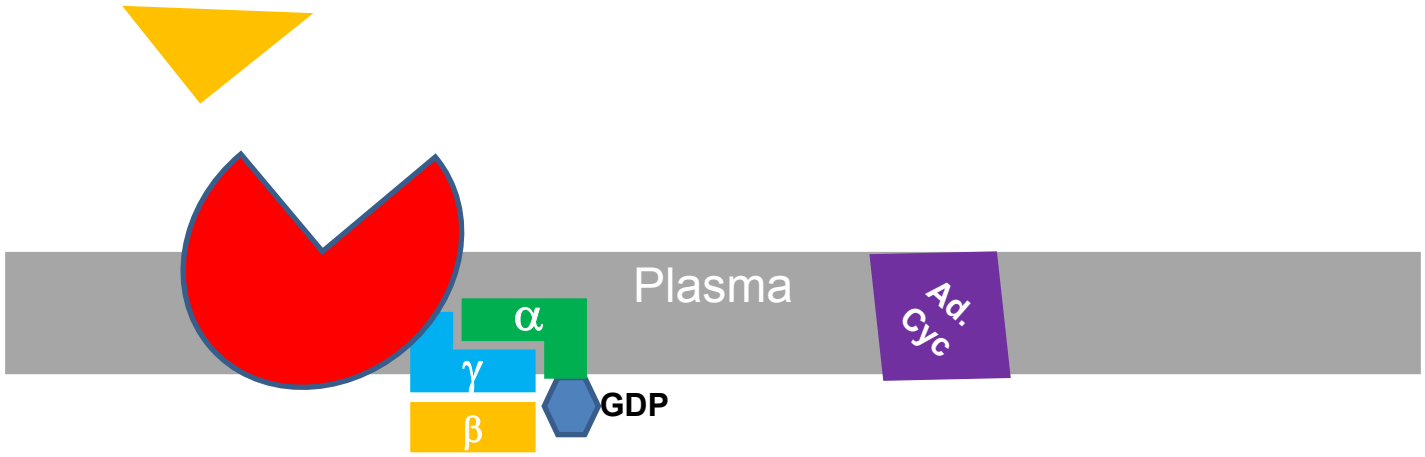
The G protein becomes activated upon binding to the ligand activated seven-spanning receptor. In its inactive form, G protein exists as a trimer with GDP bound to the α subunit. Binding to the receptor–ligand complex induces the α subunit to exchange GDP for GTP.

This exchange causes the α subunit to dissociate from β and γ, allowing α to associate instead with an effector enzyme such as *adenylate cyclase and the* GTPase activity of the **α subunit is activated.**

***GTP is* hydrolyzed to GDP, thereby inactivating the α subunit, which in turn inactivates adenylyl cyclase. The α subunit bound to GDP reassociates with the β and γ subunits and can then be reactivated by associating with the hormone– receptor complex.**



GTPase activity of α subunit is activated when it binds to adenylate cyclase. Adenylate cyclase catalyzes synthesis of cAMP from ATP. ***GTP is*** hydrolyzed to GDP, thereby inactivating the α subunit, which in turn inactivates adenylyl cyclase



Following GTP hydrolysis, the GDP-bound α subunit of G protein reassociates with the heterotrimeric G protein and is ready to be reactivated by a second hormonal stimulus.

Mol Pharmacol 72:219-230, 2007

Science 296:1636-1639,2002

Activation of adenylate cyclase by heterotrimeric G proteins increases the concentration of cAMP in the cell, which otherwise is maintained at a low level by cyclic AMP phosphodiesterase, which hydrolyzes cAMP to 5′-AMP.

The cAMP thus generated then activates protein kinase A

In unstimulated cells, PKA is in the inactive state because of the presence of a pair of inhibitory subunits. On cAMP binding, these inhibitory subunits, dissociate from the two catalytic subunits, thereby activating the catalytic subunits.

The activated catalytic subunits then phosphorylate specific serine or threonine residues of either enzymes such as glycogen phosphorylase kinase or transcription factors such as CREB.

Activation of metabolic enzymes by PKA

When phosphorylated by PKA, glycogen phosphorylase kinase phosphorylates (activates) *glycogen phosphorylase*, the enzyme that breaks down glycogen in muscle cells to glucose-1-phosphate.

Activation of transcription factors by PKA

CREB (cyclicAMP response element binding protein) binds to the cAMP response elements (CRE), in the promoter regions of genes that are regulated by cAMP.

G protein signalling

smell and taste
(~1000 types of receptors)
Perception of light
neurotransmission
function of endocrine
and exocrine glands
chemotaxis
endocytosis
control of blood pressure
embryogenesis
development
cell growth and differentiation
HIV infection
oncogenesis

Gutkind, J. S., *J. Biol. Chem.* 273:1839-42,1998;
Marinissen, M. J. and Gutkind, J. S., *Trend Pharmacol. Sci.* 22:368-76,2001)

Mutations in GPCRs result in constitutive signalling leading to a number of diseases:

Familial hypoparathyroidism, familial male precocious puberty, Jansen metaphyseal chondroplasis, congenital night blindness, hyperfunctional thyroid nodules, and familial non-autoimmune hyperthyroidism

Trends Endocrinol. Metabol. 9:27,1998;
Trends Endocrinol. Metabol. 9:133,1998;
Pharmacol Rev 59:225-250, 2007;
Biochim Biophys Acta.1768: 994-1005,2007

Receptor/Gene name	Mutation	Disease
Calcium-Sensing (CaS)/CaSR	Multiple (e.g. Arg185Gln)	Autosomal Dominant Hypocalcemia (ADH) Sporadic Hypoparathyroidism Familial Hypoparathyroidism
CXCR4	Multiple (e.g. Ser338X)	WHIM syndrome
Endothelin receptor B (ET_B)/EDNRB	Multiple (e.g. Trp276Cys)	Hirschsprung's disease
Follicle-stimulating hormone (FSH)/FSHR	Multiple (e.g. Ala189Val)	Female infertility
N-formyl-peptide (FPR)/FPR1	Phe110Ser, Cys126Trp	Juvenile periodontitis
Frizzled (FZD_4)/FZD4	Multiple (e.g. Arg417Gln)	Familial exudative vitreoretinopathy (FEVR)
Goandotropin-releasing hormone (GnRH)/ GNRHR	Multiple (e.g. Arg262Gln)	Hypogonadotropic hypogonadism (HH)
GPR54/GPR54	Multiple (e.g. Cys223Arg)	Hypogonadotropic hypogonadism (HH)
GPR56/GPR56	Multiple (e.g. Cys223Arg)	Bilateral frontoparietal polymicrogyria (BFPP)
vGPCR/KSHV-GPCR	(constitutively active)	Kaposi's sarcoma (KS)
Relaxin family peptide receptor 2 (RXFP2)/LGR8	Multiple (e.g. Thr222Pro)	Cryptorchidism
MASS1 (also called VLGR1, USH2C)/ MASS1	Multiple (e.g. Ser2652X))	Usher syndrome Febrile seizures (FS)
Melanocortin (MC_4)/MC4R	Multiple (e.g. Pro78Leu)	Dominant and recessive obesity
Rhodopsin/RHO	Multiple (e.g. Pro23His)	Retinitis pigmentosa (RP)
Vasopressin receptor (V_2)/AVPR2	Multiple (e.g. Arg113Trp)	Nephrogenic diabetes insipidus (NDI)

Receptor	Polymorphisms	Examples of disease associations
β_1 Adrenergic receptor	Arg389Gly	Heart failure
β_2 Adrenergic receptor	Multiple	Hypertension, Asthma
β_3 Adrenergic receptor	Trp64Arg	Obesity
CC chemokine receptor 2 (CCR2)	Val64Ile	Delayed progression of AIDS
CC chemokine receptor 5 (CCR5)	Multiple	Associated with progression of AIDS
Dopamine receptor 2 (D_2)	3'UTR52A/G	Associated with depression and anxiety
Dopamine receptor 3 (D_3)	Ser9Gly, Promoter SNPs	Haplotype associated with schizophrenia
Muscarinic receptor subtype 3 (M_3)	Promoter haplotype	Possible association with asthma and atopy
Neuropeptide S receptor (NPSR; also called GPR154, GPRA)	Haplotypes H1, H5 Asn107Ile, rs324981	Asthma susceptibility
$P2Y_{12}$	CA deletion at Codon 240	Associated with bleeding diathesis

Signal Transduction Pathways

G-protein coupled receptors
Small G-proteins
Intracellular receptors
Ser/Thr protein kinases
Receptor Tyrosine protein kinases
Phosphatases
Calcium Signaling
NO Signaling