Physiology
Sheet No. 17

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Second Messengers: for Hormones that can't cross the plasma membrane.

- second messengers are required for intracellular signaling, especially for hormones that cannot pass the plasma membrane.

-So, they are the molecules that transmit the transduced signal by the binding of the hormone to the cell surface receptor.

Types of Second messengers to be discussed: A- cAMP. B-cGMP. C-IP3 & DAG. D- Ca^{+2}.

A. cAMP: most common.

i. Production of cAMP: ATP converted to cAMP by adenylate cyclase (a large multi pass TM protein).

Degradation (turned off) by cAMP phosphodiesterase

ii. Action of cAMP:
- their target is cAMP-dependent protein kinase (protein kinase A (PKA)).

-binding of cAMP to PKA activates it, and this activation will lead to metabolic activity of PKA in which it phosphorylates other target proteins and regulates their action and activities.

-PKA is a tetramer of catalytic and regulatory subunits, cAMP binding leads to dissociation of regulatory subunits and release of catalytic subunits which then **phosphorylate target proteins in cytoplasm**.

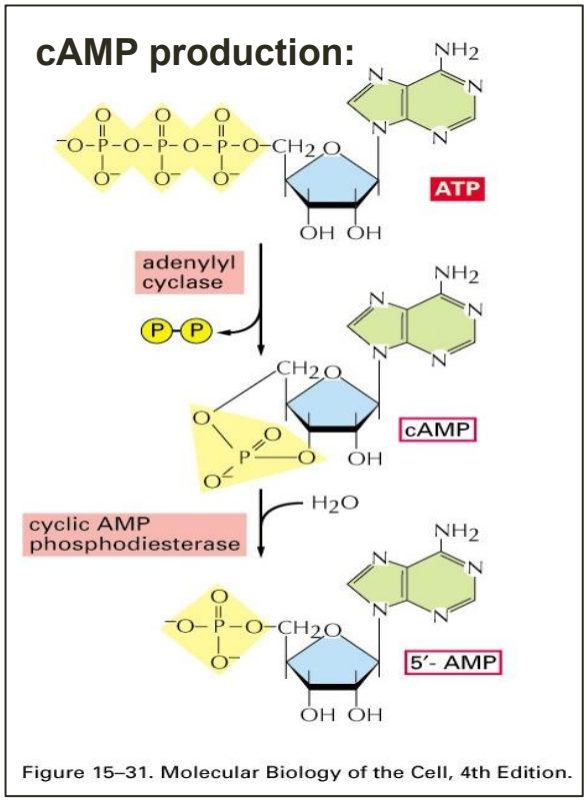


Figure 15–31. Molecular Biology of the Cell, 4th Edition.

-this photo below shows the mechanism of cAMP activation of protein kinase A:

it's a little bit detailed, you don't need to memorize all the mentioned information, just know that cAMP binding to PKA activates it, which enables PKA to phosphorylate other target proteins.

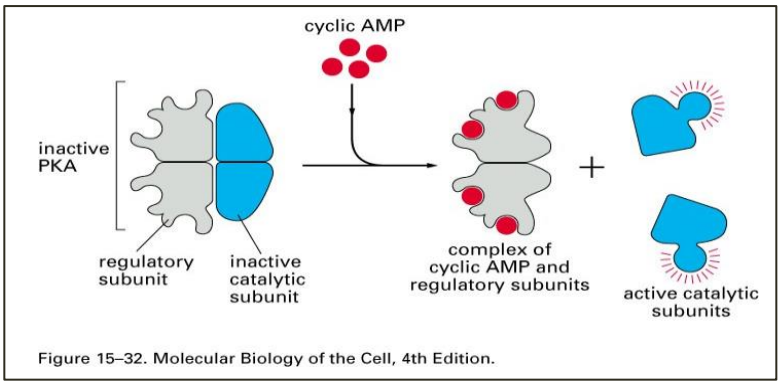


Figure 15–32. Molecular Biology of the Cell, 4th Edition.

PKA Functions: 1-phosphorylation of proteins. 2-change gene expression.

How can PKA change gene expression?

- PKA can enter the nucleus directly, any signal that enters the nucleus is called a third messenger.

-after it enters the nucleus, PKA phosphorylates **CREB;** is a CRE binding **protein**.

-**CRE** (**c**yclic-AMP **r**esponse **e**lement) is a regulatory DNA sequence associated with specific genes.

-once CREB protein is phosphorylated by PKA, it binds to the (CRE) and activates it, which results in activation of transcription of those genes adjacent to the CREB-binding element. (see the picture above)

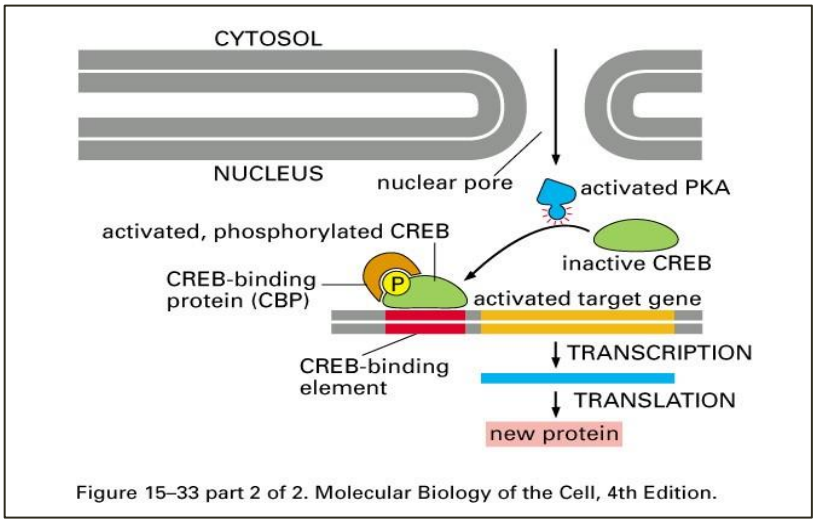


Figure 15–33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

-CREB <u>phosphorylation</u> by PKA → CREB binds to its <u>element</u> → Activation of gene <u>transcription</u>.

In conclusion: PKA throughout cAMP can change gene expression.

Amplification of signal at each step of signaling pathway:

- A characteristic feature of signal transduction in all second messengers including cAMP.
- cAMP allows hormones to have amplified effects, cAMP can be produced in higher concentrations than the concentration of the hormone itself

Rapid turn on and rapid turn off of cAMP and activation by cAMP.

Receptors that cause increase in cAMP do so by activating Gs, a stimulatory protein that activates adenylyl cyclase.
adenylyl cyclase is the enzyme that produces cAMP once its activated by the alpha α subunit of G_s.
cAMP in its role binds to PKA, and PKA performs the two functions mentioned above.

Regulation of adenylate cyclase:

Adenylyl cyclase is turned off by Gi, an inhibitory protein.

Question: what turns off proteins activated by protein kinases?

Pathogens alter cAMP production:

(abnormalities in cAMP production caused by micro-organisms).

Cholera toxin active subunit catalyzes transfer of ADP ribose from intracellular NAD to the α subunit of Gs, causing it to be **continuously active**, stimulating adenylyl cyclase indefinitely.

adenylyl cyclase would be active all the time by the continuously active alpha Gs subunit.

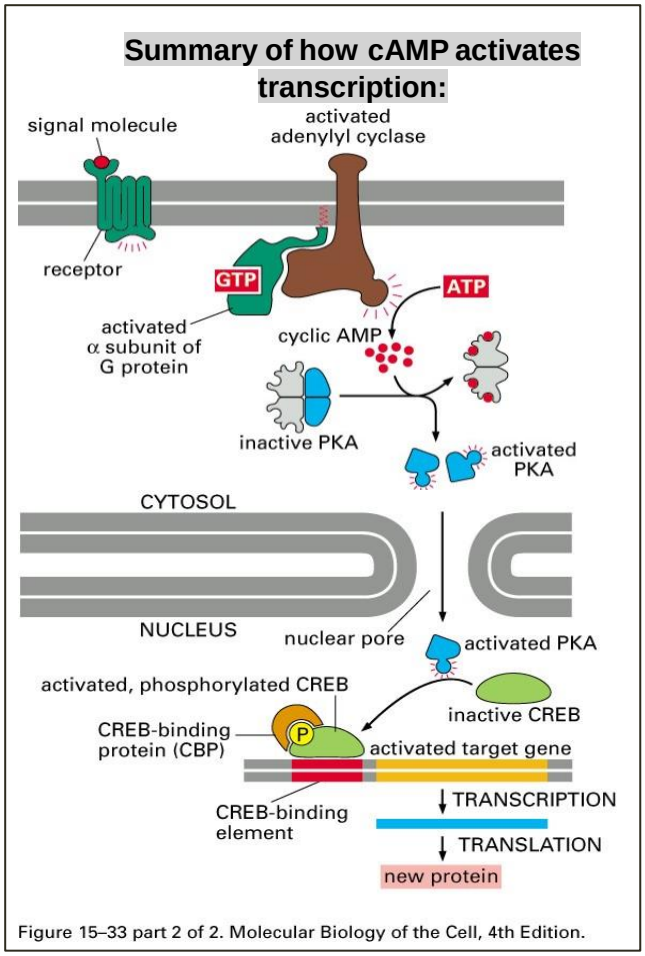


Figure 15–33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

This causes ion channels in GI tract that export chloride to produce a net efflux (**secretion**) of chloride ions Cl^- and water, leading to severe diarrhea, a characteristic of cholera.

-if cAMP weren't turned off, this would cause severe and deleterious effects on our body that can be fatal like diarrhea.

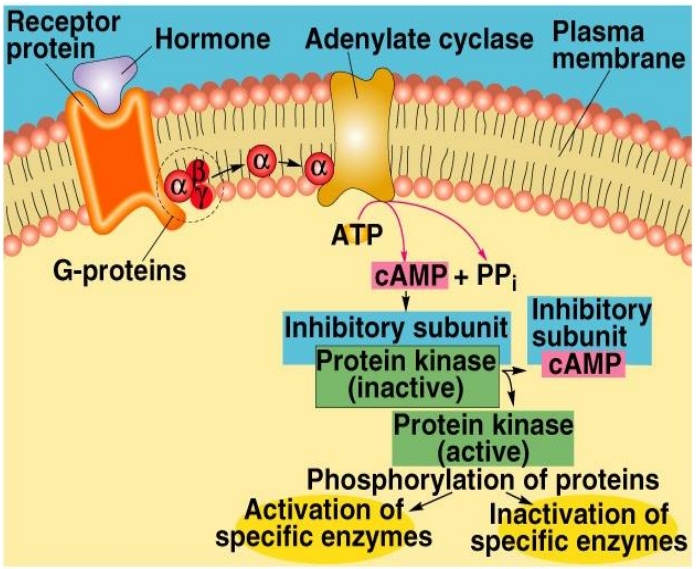
Adenylate Cyclase-cAMP (summary)

• Phosphorylates enzymes within the cell to produce hormone's effects.

• Modulates activity of enzymes present in the cell .

• Alters metabolism of the cell .

• cAMP inactivated by phosphodiesterase that hydrolyzes cAMP to inactive fragments.



B. cGMP: cyclic guanine monophosphate.

1. produced from GTP by **guanylyl** cyclase.
2. target: activates cGMP-dependent kinases (protein kinase G) or other targets.
3. example on signaling pathway that produces cGMP as a 2nd messenger:

A- G-protein Coupled rhodopsin photoreceptor in rod cells of retina.
B- Nitric oxide signaling.
C-AMP signaling.

Several metabolic responses caused by a rise in intracellular cAMP in different tissues:

TABLE 20-3 Metabolic Responses to Hormone-Induced Rise in cAMP in Various Tissues

Tissue	Hormone Inducing Rise in cAMP	Metabolic Response
Adipose	Epinephrine; ACTH; glucagon	Increase in hydrolysis of triglyceride; decrease in amino acid uptake
Liver	Epinephrine; norepinephrine; glucagon	Increase in conversion of glycogen to glucose; inhibition of synthesis of glycogen; increase in amino acid uptake; increase in gluconeogenesis (synthesis of glucose from amino acids)
Ovarian follicle	FSH; LH	Increase in synthesis of estrogen, progesterone
Adrenal cortex	ACTH	Increase in synthesis of aldosterone, cortisol
Cardiac muscle cells	Epinephrine	Increase in contraction rate
Thyroid	TSH	Secretion of thyroxine
Bone cells	Parathyroid hormone	Increase in resorption of calcium from bone
Skeletal muscle	Epinephrine	Conversion of glycogen to glucose
Intestine	Epinephrine	Fluid secretion
Kidney	Vasopressin	Resorption of water
Blood platelets	Prostaglandin I	Inhibition of aggregation and secretion

-the hormones in the table cause rise in intracellular cAMP through cell surface G-protein coupled receptors.

-Various metabolic responses depend on the tissue itself, different responses might include the same receptor and 2nd messenger, this is mainly due to the different cell types in different tissues.

Example: rise in cAMP caused by the same hormones (like epinephrine or glucagon) in the adipose tissue will cause hydrolysis of triglycerides, however in the liver the response will be conversion of glycogen to glucose (inhibition of glycogen), increase in amino acid uptake; increase in glucose synthesis from amino acids in a process known as gluconeogenesis.

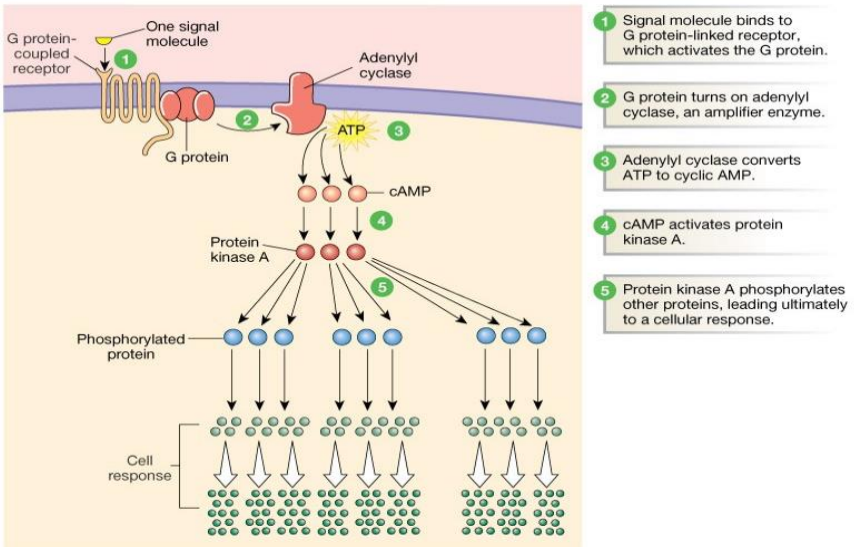
In skeletal muscles, epinephrine raises cAMP concentration which stimulates glycogen conversion into glucose -similar to the response in the liver-.

In cardiac muscles increase in cAMP by epinephrine as well causes increase in the contraction rate (heart rate).

*Read the table and the effect of each hormone on its target tissue; vasopressin increases cAMP in the kidney which stimulates water reabsorption.

G-Protein Coupled Receptors

this picture demonstrates the concept of signal amplification by cAMP, one signal molecule causes production of multiple cAMP, that activates multiple PKA, which causes phosphorylation of many target proteins



*Second Messengers, continuation:

C. IP3 and DAG:

General overview: Phosphatidylinositol 4,5-bisphosphate (PIP2) triggers a 2-armed signaling pathway.

alpha q subunit of G-protein activates phospholipase-C that converts a phospholipid that's present in the inner lipid monolayer of PM and known as PIP2.

a. PIP2 is a minor phospholipid in inner leaflet of the plasma membrane's bilayer that is produced by phosphorylation of phosphatidyl- inositol and is involved in signaling.
b. Ligand binding to certain receptors stimulates PIP2 hydrolysis by phospholipase-C (PLC).

c. This produces diacylglycerol (DAG) that's still connected to the plasma membrane and inositol 1,4,5-triphosphate (IP3) that's free in the cytosol, both of which are 2nd messengers with different actions.

PIP2 $\xrightarrow{\text{phospholipase-C}}$ IP3 + DAG

d. PIP2 hydrolysis is activated by both GPRs and TKRs via different forms of PLC.

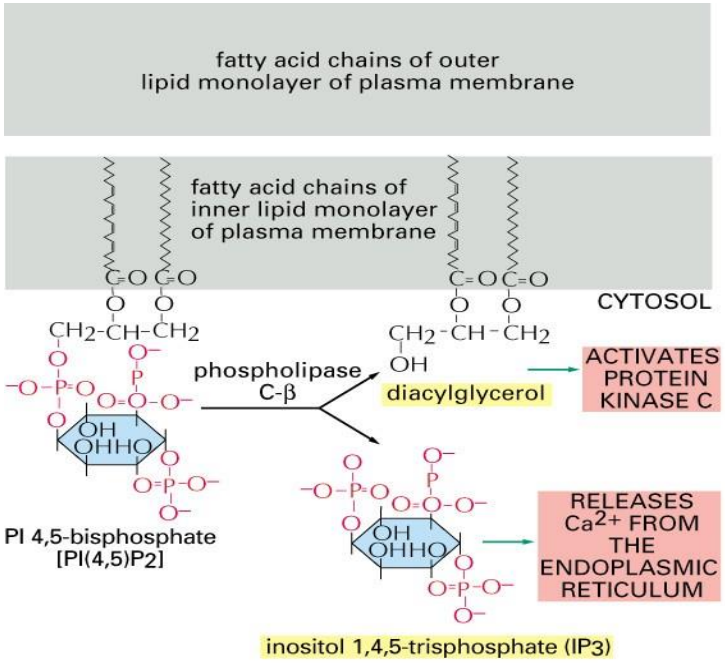


Figure 15–35. Molecular Biology of the Cell, 4th Edition.

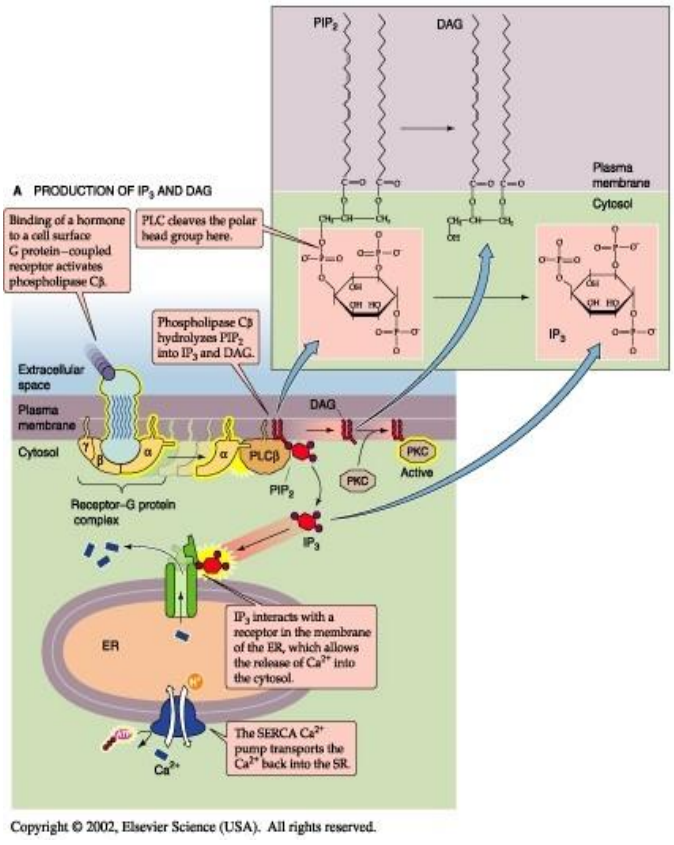
pay attention to the fact that different isoforms of phospholipase-C are activated by different stimuli: phospholipase-C beta (PLC-β) is stimulated by G_q proteins while phospholipase-C gamma (PLC-γ) has SH2 domains that allow binding to activated tyrosine kinase linked receptors.

so, both (PLC-β) and (PLC-γ) are activated through different receptors but produce the same 2nd messenger by the end.

this schematic picture shows that PLC produces two types of second messengers: IP3 and DAG from PIP2.

Action of IP3: because its free in the cytosol, it will bind to a receptor on the endoplasmic reticulum (ER) membrane which results in opening calcium ion channels on ER membrane and release of calcium ions from their stores into the cytosol increasing intracellular calcium levels.

USUALLY, intracellular Ca^{+2} levels are kept very low.



but when it comes to IP3 signaling calcium levels are increased transiently, in which calcium has effects such as activating a protein kinase C PKC along with DAG.

DAG + calcium ions activate PKC

There's another type of channel that's called SERCA: turns off the calcium signal, pumps the calcium ions back to their stores in the ER to bring calcium levels back to their normal state. (blue colored channel in the picture above)

Summary of DAG and IP3 actions:

- **DAG**: Remains associated with the PM
- Stimulates the Ca^{+2}-dependent protein kinase C signaling pathway, which activates other targets including the MAP kinase cascade (see picture below)

- **IP3**: Small polar molecule released into cytosol.
- Stimulates Ca^{+2} release from intracellular stores. (ER)
- Elevated Ca^{+2} alters activities of target proteins including kinases & phosphatases.

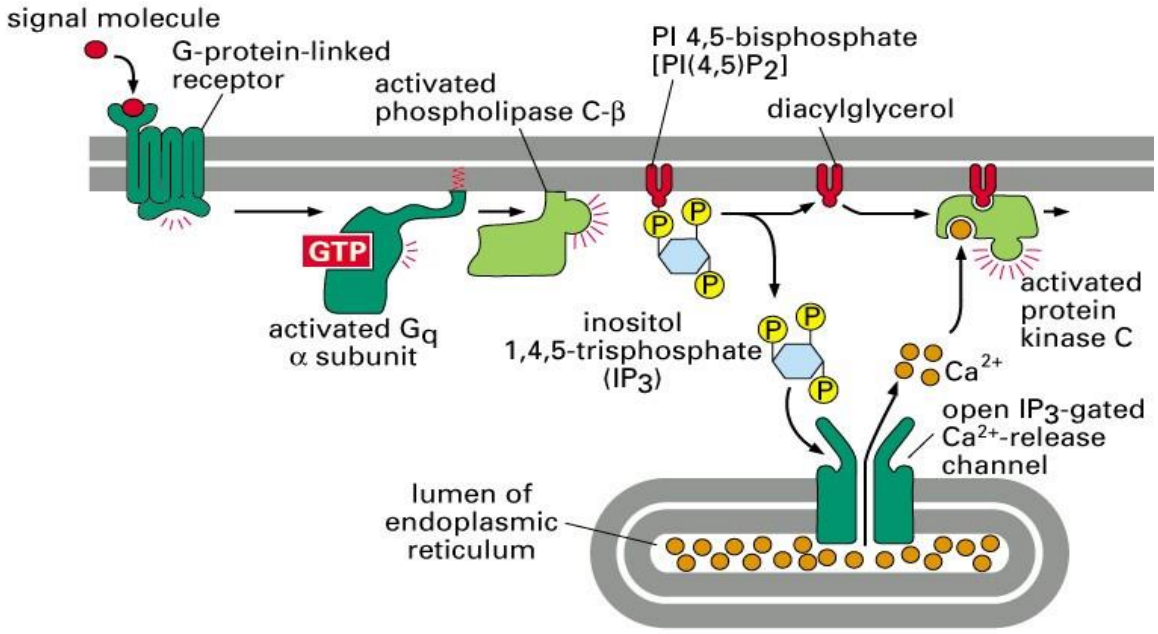
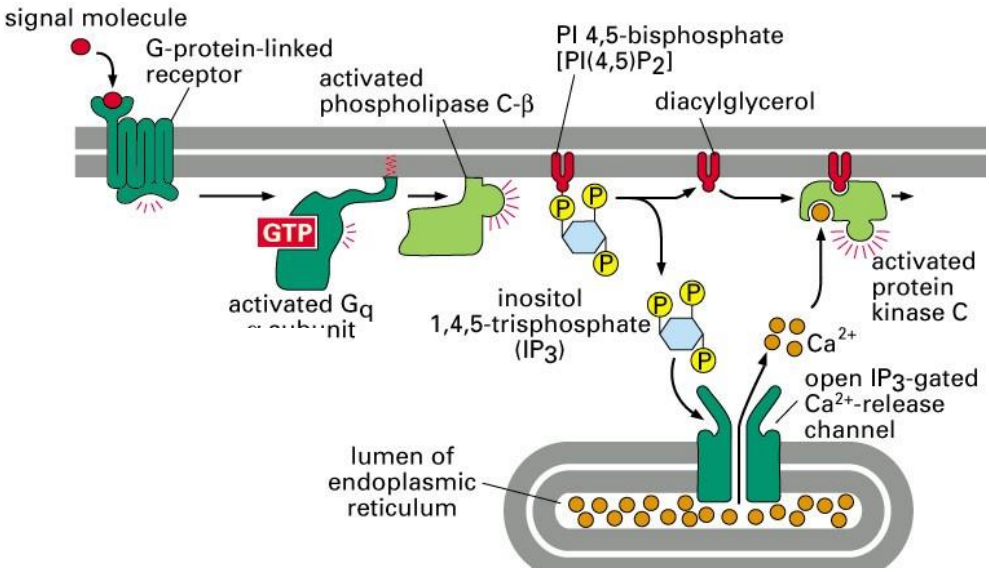


Figure 15–36. Molecular Biology of the Cell, 4th Edition.

PLC- signaling pathway summary

this schematic shows PLC- β

when it's activated by G-protein coupled to Gq alpha subunit which eventually results in IP3 and DAG production.



IP3 releases Ca^{+2} ions.

Ca^{+2} and DAG activates PKC.

PKC (protein kinase C) phosphorylates many substrates that can activate kinase pathway and gene regulation.

*Second Messengers continuation:

D- Ca^{+2} also acts a second messenger:

Ca^{+2} concentration is kept low (10^{-7} M) and rises locally due to transient signaling via IP3.

Ca^{+2} acts as a second messenger on its one of its target proteins: calmodulin.

so, the effects of intracellular Ca^{+2} are mediated by the Ca^{+2} binding protein calmodulin.

the conformation of calmodulin changes when calcium binds to it, forming Ca^{+2}/ calmodulin complex.

Ca+2 /calmodulin complex binds to other target proteins, regulate their activity, and fully activate them.

Examples on Ca+2 /calmodulin target protein: protein kinases (Ca^{+2} calmodulin-dependent kinases; CaM-kinases), adenylyl cyclases, and phosphodiesterases, causing change in conformation and activation of these proteins.

in this picture: Ca+2 /calmodulin complex binds to an enzyme (protein phosphatase) and fully activates it by facilitating its auto-phosphorylation process.

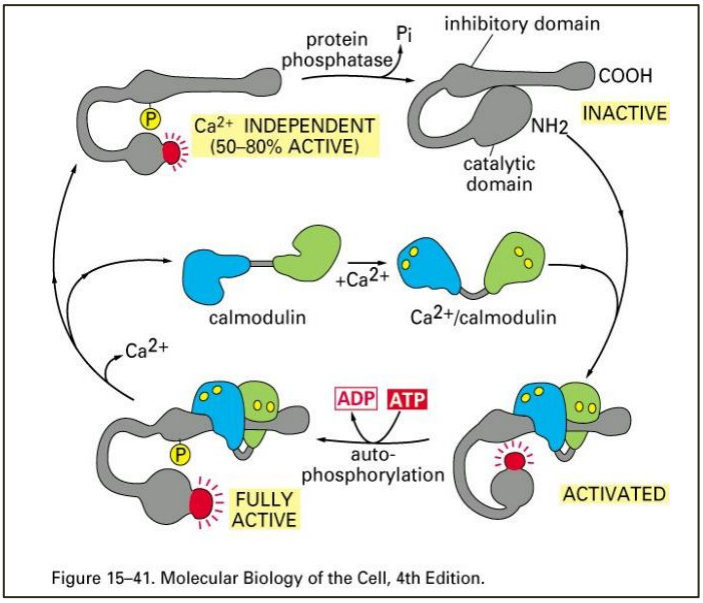
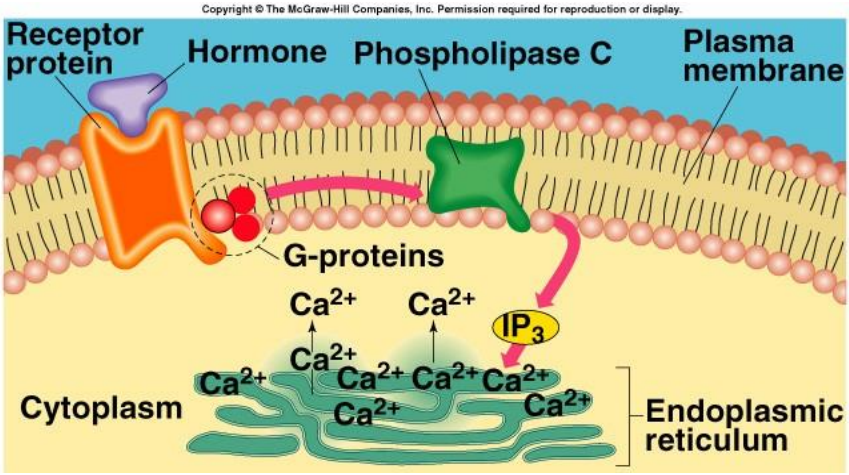


Figure 15–41. Molecular Biology of the Cell, 4th Edition.

Ca^{2+}- Calmodulin :

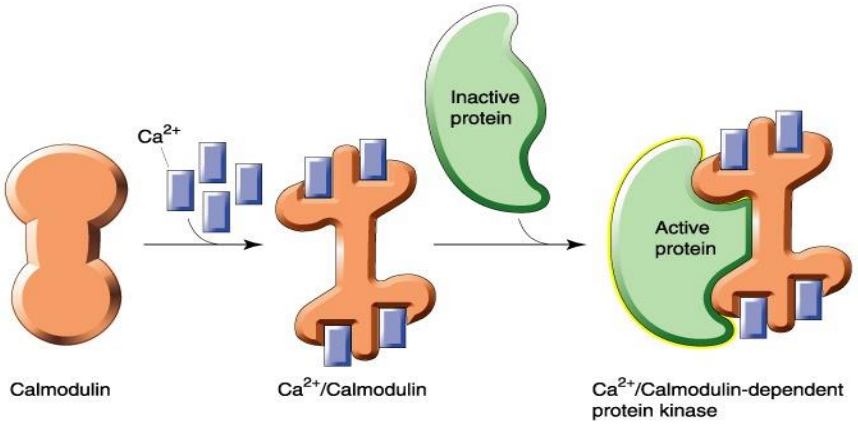
(summary of calcium-calmodulin complex)

- Ca^{2+} diffuses into the cytoplasm.
- Ca^{2+} binds to calmodulin.
- Calmodulin activates specific protein kinase enzymes.
- Alters the metabolism of the cell, producing the hormone's effects.



The mechanism of enzyme activation by calcium calmodulin

Ca+2 /calmodulin complex activates target proteins by changing their conformational structure which results in activity changes.



Epinephrine Can Act Through Two 2nd Messenger Systems

the same hormone can active two different types of receptors which results in production of two different types of second messengers in the same cell.

Example: (Epinephrine effect on a liver cell)

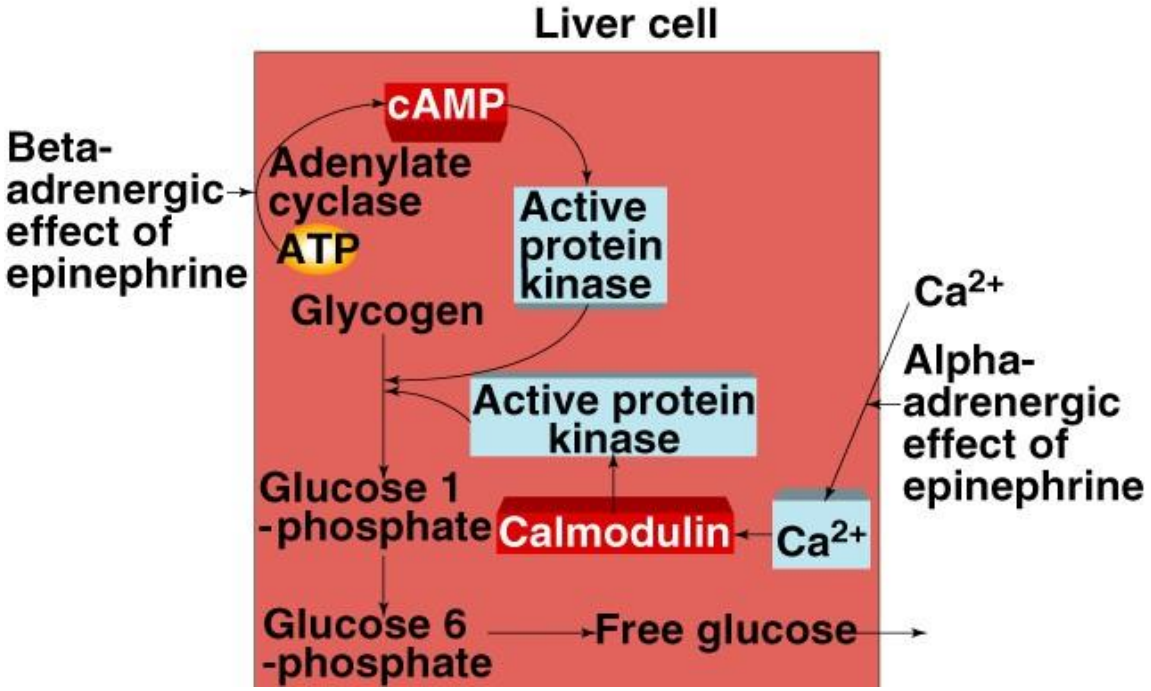
epinephrine binding to beta-adrenergic receptors, which are G-protein coupled receptors, results in increase of cAMP 2nd messenger, and activation of protein kinase A consequently.

protein kinase A increase glycogen metabolism and production of glucose in the liver.

The other pathway epinephrine can cause by binding to α_1 (Alpha)-**adrenergic** receptor that is associated with $G_{\alpha q}$ subunit that activates phospholipase C and produces IP3 and DAG. (check table 15-1 in slide 48)

This results in calcium production as a 2nd messenger, Ca^{+2} binds to calmodulin, as we mentioned before Ca+2 /calmodulin activates protein kinases which also increases the glycogen metabolism.

So two second messengers (cAMP and Ca^{+2}) mediated the function of the liver cell.



ANP

Atrial natriuretic peptide is a hormone secrete by the atrium, and an anti-hypertensive agent.

its main **function** is to lower blood pressure

Guanylate cyclase (GC) receptors

very important in: ANP and Nitric oxide signaling.

Examples: 1- Membrane receptor-ANP*. 2- Soluble receptor-NO*,CO

NO signaling

NO synthesis:

in blood vessels there are endothelial cells and smooth muscle cells.

in endothelial cells there are acetylcholine G-protein coupled receptors that binds with acetyl choline. (associated with $\mathbf{G_{\alpha o}}$ subunit)

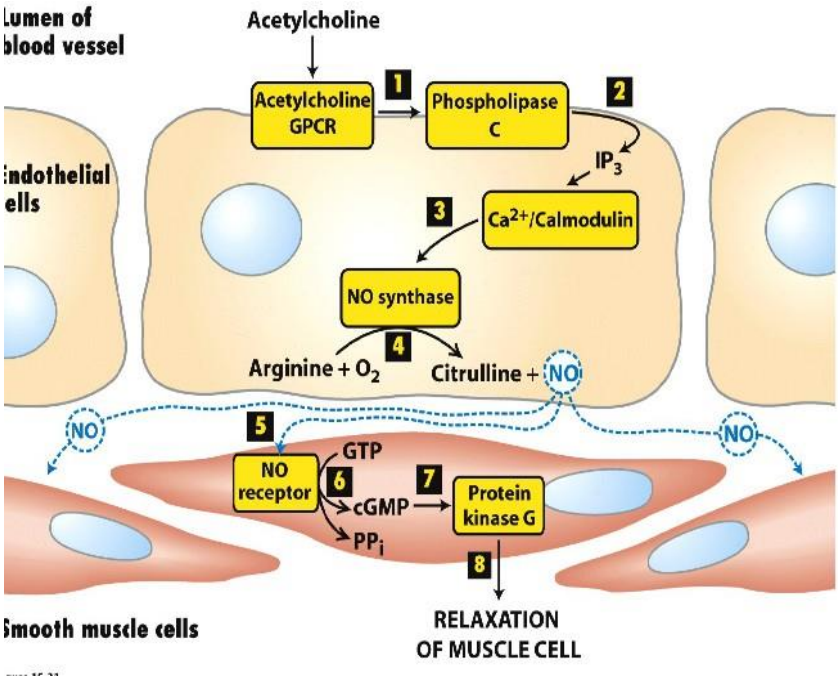
the binding induces activation of PLC, PLC → IP3 → Ca^{+2} /calmodulin complex activates an enzyme called nitric oxide synthase that synthesizes NO from arginine amino acid. Arginine $\xrightarrow{\text{NO synthase}}$ **NO** + citrulline

nitric oxide is a gas that can freely diffuse across the PM and goes to adjacent smooth muscle cells as well. once NO enter the smooth muscle cells it will bind to a receptor called NO receptor, which is a soluble receptor meaning its inside the cell's cytosol.

this receptor contains guanylyl cyclase activity, which converts GTP into cGMP.

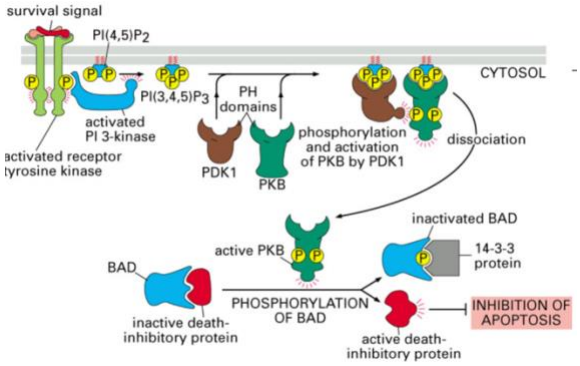
cGMP is a 2nd messenger than bind to protein kinase G, this will induce relaxation of the muscle cell which results in vasodilation of vessels, which is very important in blood flow efficiency.

NO is an important anti-platelet and vasodilator.



LAST SECOND MESSENGER : PIP3 (like IP3 but produced from PIP2 to PIP3 by PI3 kinase)

*details of its signalling pathway are not required , just know that it is a 2nd messenger and that it contributes in the survival of the cell by inhibition of apoptosis by PDK and PKB.



DIVERSION & CONVERSION

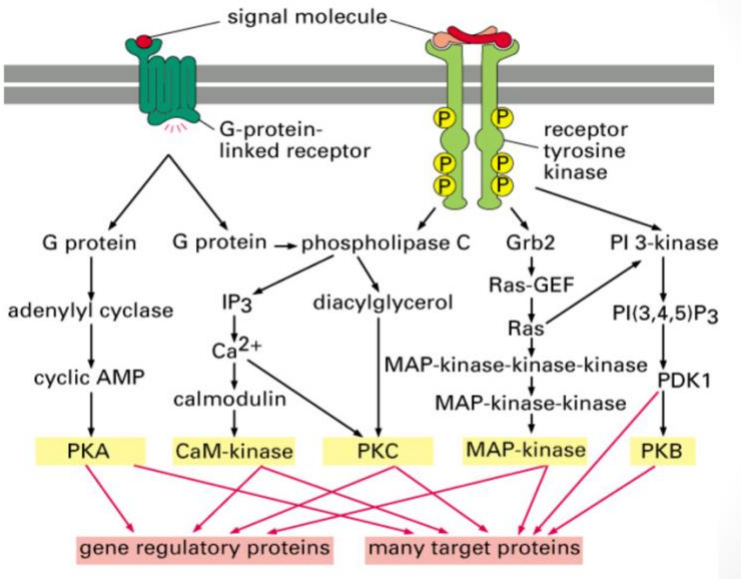
*the same enzyme could be activated by different types of receptors

Ex: phospholipase C can be activated by G- protein coupled receptor and tyrosine kinase receptors.

*the same receptor can affect different pathways that are activated by another receptor

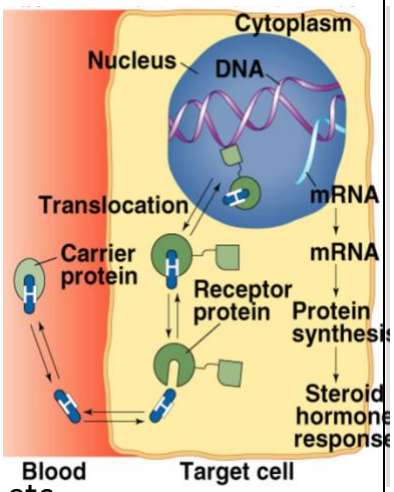
Ex: tyrosine kinase can affect G-protein coupled receptor's pathways.

Also, integration can occur, like calcium , when activates PKC that is activated from another signalling pathway.



INTRACELLULAR RECEPTORS: 1- STEROID RECEPTORS. 2- THYROID RECEPTORS.

#Their receptors can be:1- nuclear. 2-cytosolic.

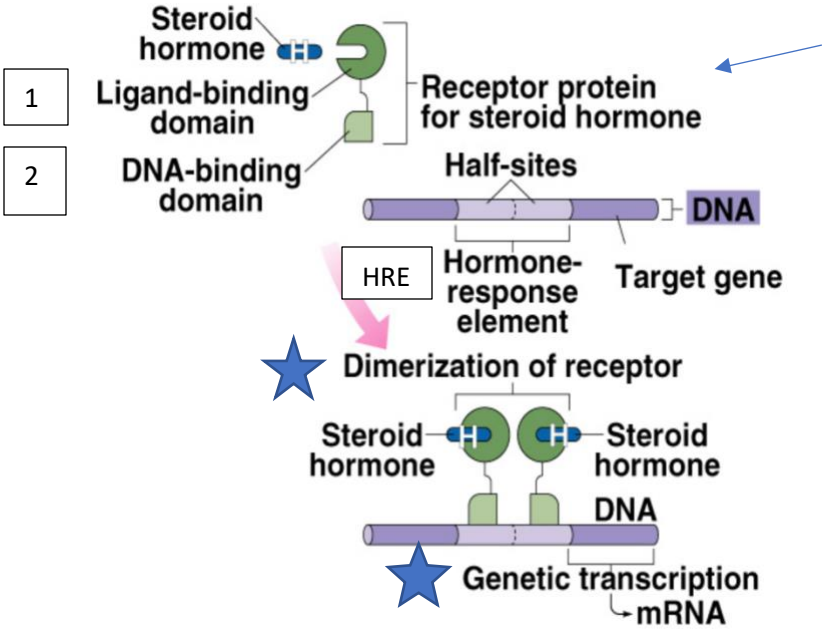


#(For lipophilic hormones) they can go directly to the nucleus or

This is a general scheme to describe the steroid hormone that is translocated by a carrier protein in plasma of blood (by dissociation) , and binds to a receptor in the cell, that enters the nucleus and bind to DNA and change the gene expression.

1. Mechanism of steroid hormones:
   - For steroid hormones like :sex hormones, testosterone, cortisol, etc.
   - The nuclear receptor has 2 main regions, ligand (hormone)-binding protein AND DNA-binding protein.

   Steroid receptors are located in cytoplasm and in the nucleus.
   - Function within cell to activate genetic transcription.
   - Messenger RNA directs synthesis of specific enzyme proteins that change metabolism.
   - Receptor must be activated by binding to hormone **before** binding to specific region of DNA called HRE (hormone responsive element) .
   - HRE is located adjacent to gene that will be transcribed.



Summary of the mechanism of steroid hormones:

• Cytoplasmic receptor binds to steroid hormone.

• Translocates to nucleus.

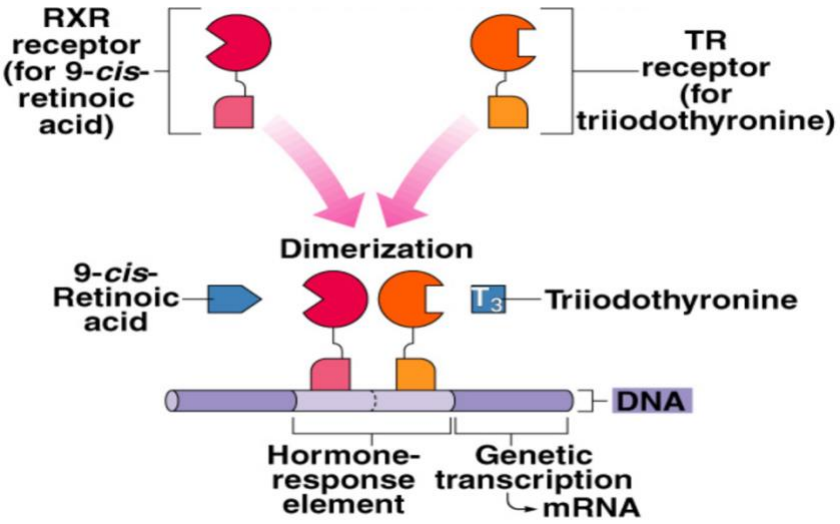
• DNA-binding domain binds to specific HRE of the DNA.

• Dimerization occurs.

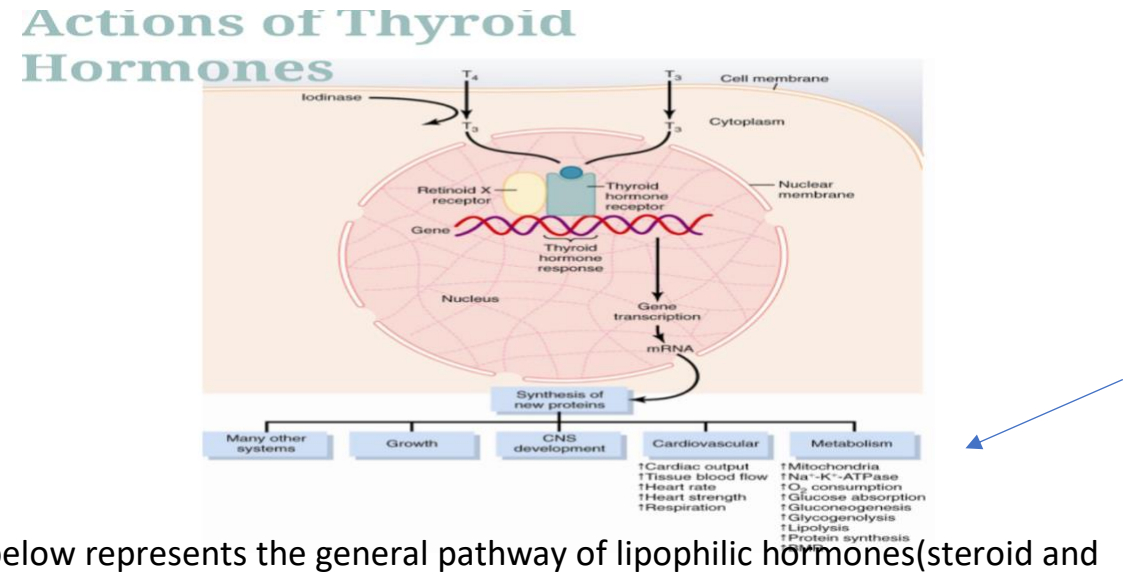
• Process of 2 receptor units coming together at the 2 half-sites.

• Stimulates transcription of particular genes.

2. The mechanism of thyroid hormones:
   •thyroid hormones like T3 and T4 (most of thyroid hormones that can be found in the blood is T4)
   •T4 passes into cytoplasm and is converted to T3 by iodinase.
   Receptor proteins located in nucleus.
   • T3 (we can call it “the active form”) binds to ligand- binding domain on its specific receptor ;TR receptor (for triiodothyronine)
   • Other (ligand) half-site is vitamin A derivative (9-cis- retinoic) acid on RXR receptor.
   TR receptor dimerises with RXR receptor to form a **heterodimer** .
   • DNA-binding domain can then bind to the half-site of the HRE (hormone response element) adjacent to the to be transcripted gene.
   • Two partners can bind to the DNA to activate HRE.
   • Stimulate gene transcription.

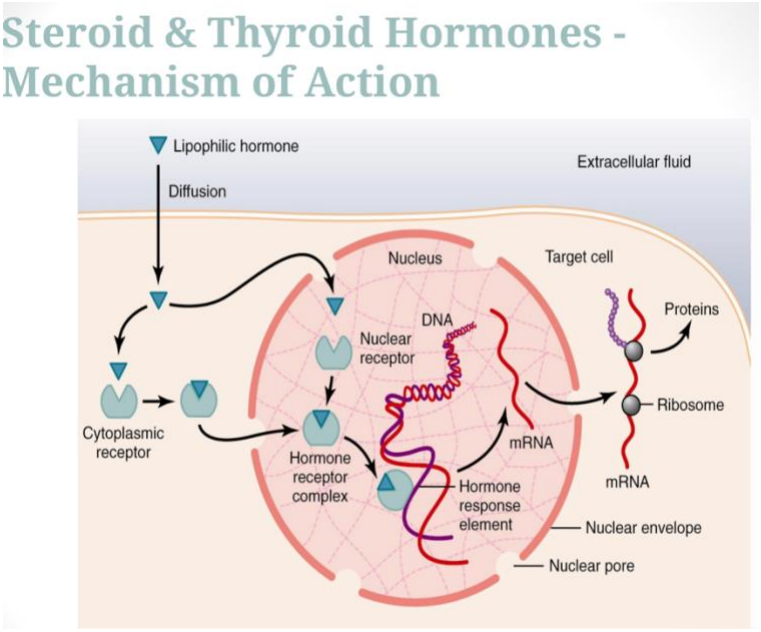


Summary of thyroid hormone mechanism and some effects:

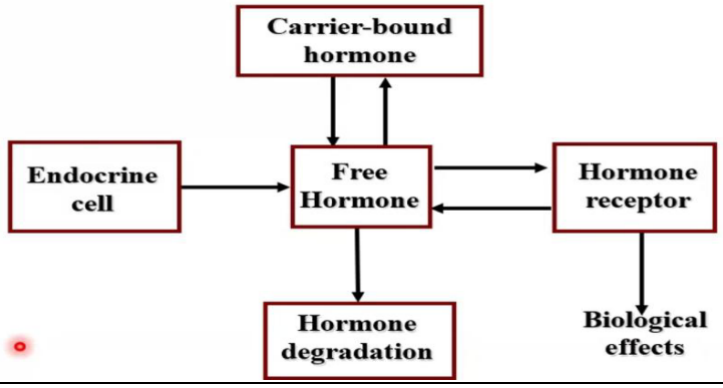


This pic below represents the general pathway of lipophilic hormones(steroid and thyroid in general):

Any lipophilic hormone, either bind directly to the nuclear receptor or go to a cytosolic receptor then translocated to the nucleus.



What determines the activity of a hormone? (It's binding)



1.the concentration of the hormone that is available for binding.(conc. Of free hormone level) and that's determined by secretion of the endocrine system.

2.the conc. Of carrier-bound hormone.

3.the conc. Of hormones bound to the receptor.

4.the level of hormone degradation (clearance) in the body; to go out from it.

Clearance is the rate of disappearance from plasma/ conc. In plasma.

…….

How much the hormone is bound to the transport protein in the plasma

The half-life of clearance of hormone

How much ml/min the plasma is cleared from the hormone (rate)

Hormone	Protein binding (%)	Plasma half-life	Metabolic clearance (ml/minute)
Thyroid			
Thyroxine	99.97	6 days	0.7
Triiodothyronine	99.7	1 day	18
Steroids			
Cortisol	94	100 min	140
Testosterone	89	85 min	860
Aldosterone	15	25 min	1100
Proteins			
Thyrotropin	little	50 min	50
Insulin	little	8 min	800
Antidiuretic hormone	little	8 min	600

Dr.Ebaa said Know the hormones with high and low % of binding

The higher protein binding -> more hormones are "protected" from clearance-> much time is needed	Increasing in protein binding means increasing protection so increased plasma half-life and so on little amount of that hormone is cleared per ml of plasma

These transport protein in the plasma can be specific/non specific to the hormone

Circulating Transport Proteins

Required

Transport Protein	Principle Hormone Transported
Specific	
Corticosteroid binding globulin (CBG, transcortin)	Cortisol, aldosterone
Thyroxine binding globulin (TBG)	Thyroxine, triiodothyronine
Sex hormone-binding globulin (SHBG)	Testosterone, estrogen
Nonspecific	
Albumin	Most steroids, thyroxine triiodothyronine
Transthyretin (prealbumin)	Thyroxine, some steroids

The last signalling pathway to be talked about :

FSH and LH secretion regulation by PKC (just follow the steps to understand it)

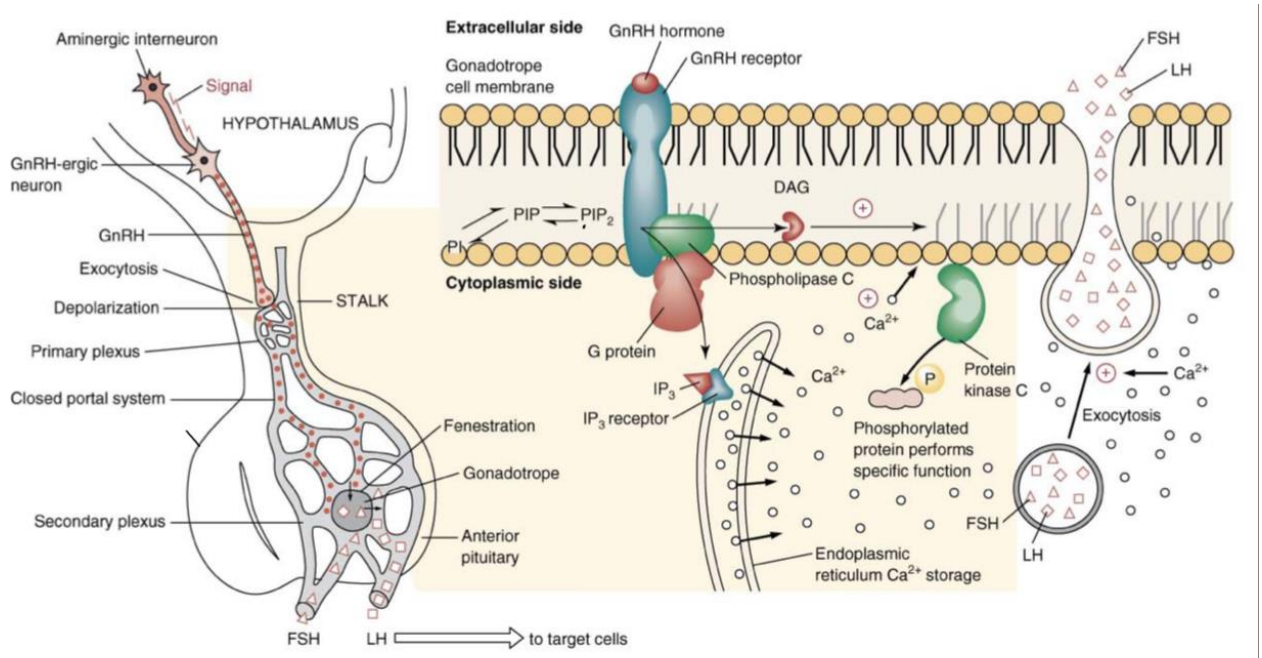
FSH and LH are female sex hormones that are secreted by anterior pituitary gland.

1.hypothalamus secretes GnRH (gonadotropin releasing hormone) reaching the anterior pituitary gland where its receptors are located (GnRH- receptor)

2.GnRH-receptors are G-protein coupled receptor (with G aq subunit) that activate the phospholipase C to produce DAG and IP3 so increases the Ca++ in the cytosol (as we've learned)

3. So now DAG and Ca++ (2nd messenger) activate protein kinase C to perform specific functions.

4.also the Ca++ activates the exocytosis of the vesicles containing FSH &LH to go to the future ovaries.



Regulation of secretion of LH and FSH by protein kinase C.

Summary: Hypothalamus

↓

GnRH

↓

Anterior Pituitary gland (GnRH – receptor)

↓

GnRH → GPCR → Gαq

↓

activate PLC

↙ ↘

DAG IP3

↓

↑ Ca^{++}

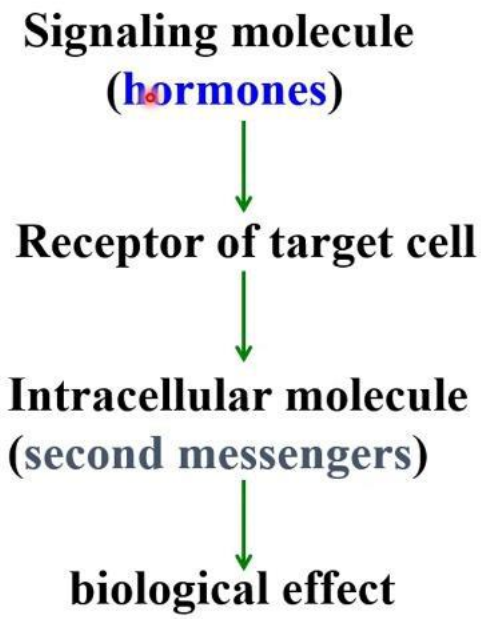
↓

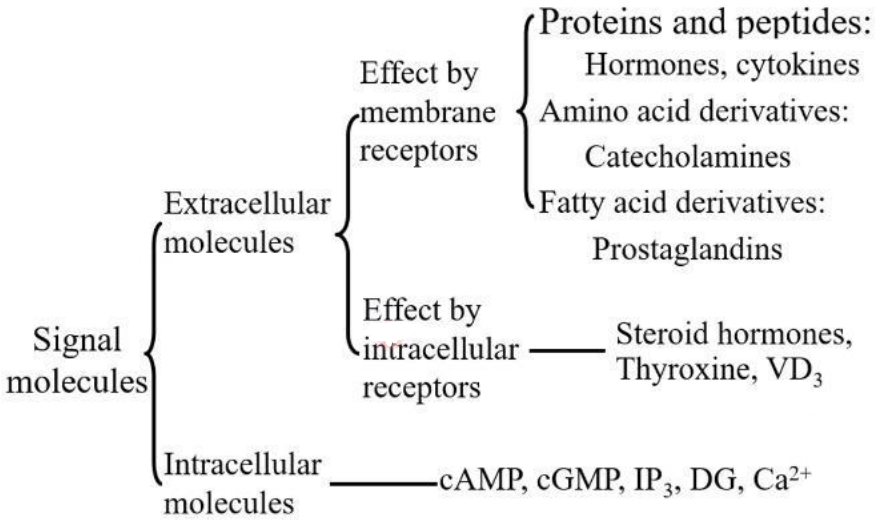
activate PKC

↓

Exocytosis LH FSH →

Generally:





***Third messengers:**

Third messengers are the molecules which transmit message from outside to inside of nucleus or from inside to outside of nucleus, also called DNA binding protein.(carrying a signal enter the nucleus)

THANK YOU 💜🤲 ادعولنا