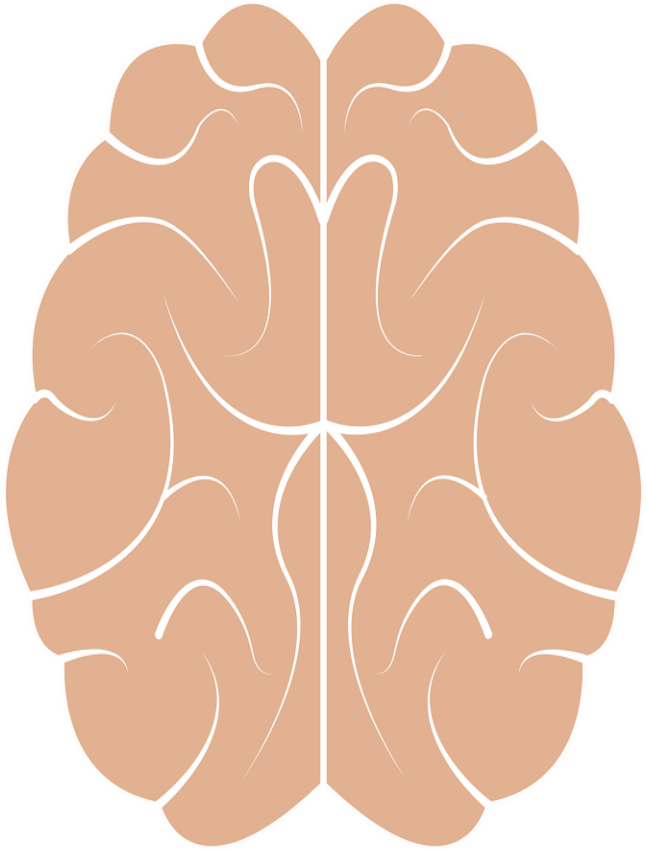


Physiology

Done By:

Rania Tayseer, Tala Quzah, Asmaa Al Shoushi

Corrected By:

Mona Eqrea

Signal Transduction

Ebaa M Alzayadneh, PhD

Integrative Physiology and Pharmacology

4. Second Messengers: for Hormones that can't cross PM

A. cAMP (most common):

i. Production:

ATP converted to cAMP by adenylate cyclase (a large multipass TM protein)

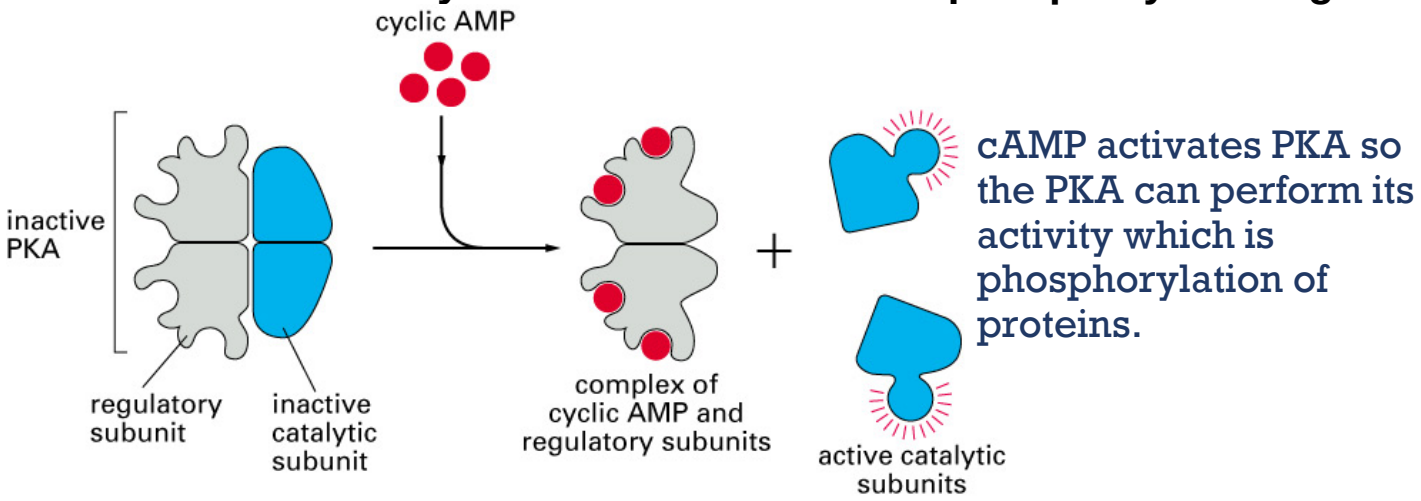
Degraded by cAMP phosphodiesterase (turned off)

ii. Action:

a. cAMP-dependent protein kinase (protein kinase A (PKA)).

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



cAMP activates PKA so the PKA can perform its activity which is phosphorylation of proteins.

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

cAMP production:

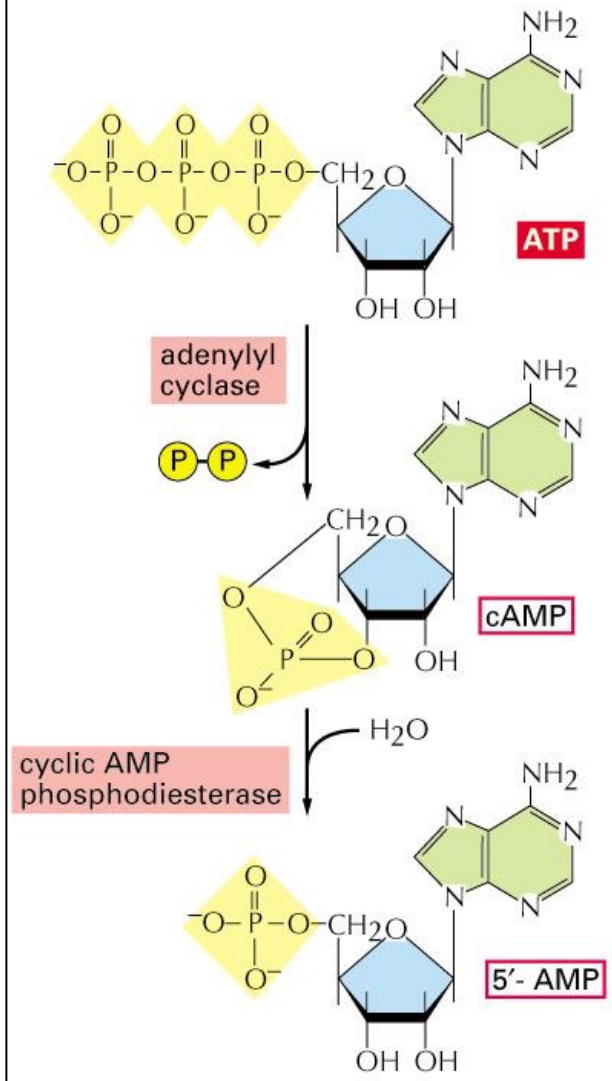


Figure 15–31. Molecular Biology of the Cell, 4th Edition.

From Janeway, Immunobiology, 5th edition

4. Second Messengers, cont.:

A. cAMP, cont.

iii. **Action:**

b. **PKA enters the nucleus and phosphorylates CREB (CRE binding protein), which binds to the cAMP response element (CRE), a regulatory DNA sequence associated with specific genes. This results in activation of transcription of those genes** (gene expression).

- Once the CREB is phosphorylated by PKA, it gets active
- The active CREB binds to a gene element in the DNA called CREB-binding element which activates (turns on) a gene expression so it will stimulate transcription, translation and producing a new protein.
- A summed up of PKA functions:

1. Activation of a gene expression
2. Phosphorylation of proteins

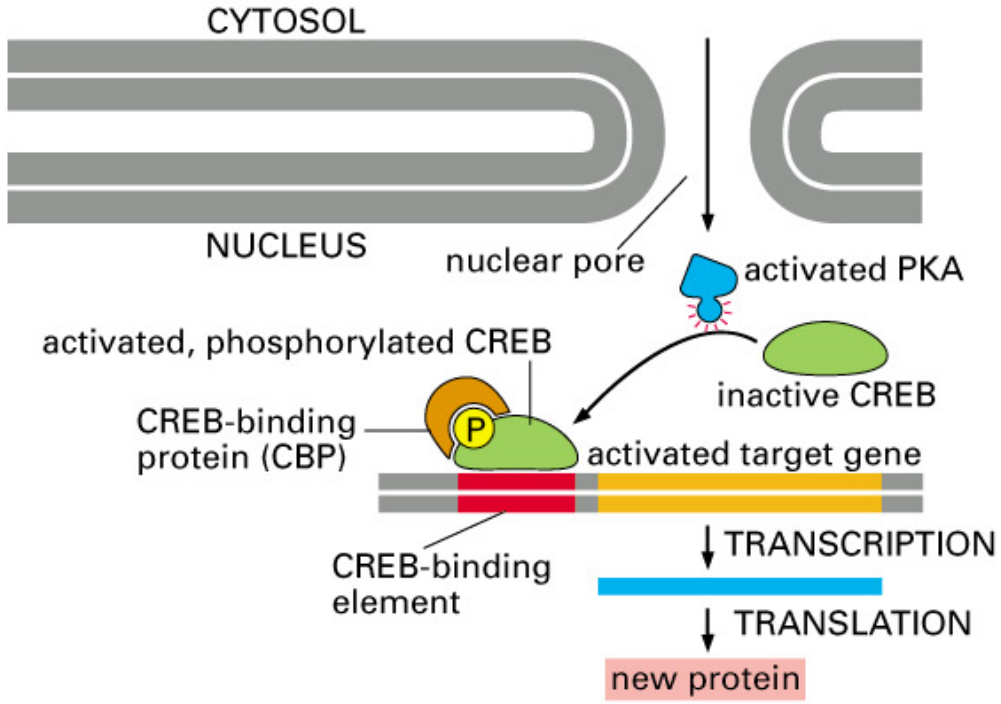


Figure 15–33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

iv. **Rapid turn on and rapid turn off of cAMP and activation by cAMP :**

Question: what turns off proteins activated by protein kinases?

v. **Amplification of signal at each step of signaling pathway - characteristic feature of signal transduction.**

- Amplification of the initial signal that was initiated by the the hormone binding to the receptor is one of the characteristics of producing cAMP.
- The cAMP do the amplification because:

1. It's produced in larger concentrations than the concentration of the hormone itself inside the cell
2. It can activate a lot of targets

4. Second Messengers, cont.:

A. cAMP, cont.:

vi. **Regulation of adenylate cyclase:**

Receptors that cause increase in cAMP do so by activating G_s, a stimulatory protein that activates adenylyl cylase.

Gs stimulates adenylyl cyclase to produce more cAMP

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

vii. **Pathogens alter cAMP production:**

Cholera toxin active subunit catalyzes transfer of ADP ribose from intracellular NAD to the α subunit of G_s, causing it to be continuously active, stimulating adenylyl cyclase indefinitely. In the digestive system**, this causes ion channels that export chloride to produce a net effux of Cl- and water, leading to severe diarrhea characteristic of cholera** which can lead to dehydration and even death if untreated.

B. cGMP:

1. **produced from GTP by guanylyl cyclase;**
2. **activates cGMP-dependent kinases** (protein kinase G (PKG)) **or other targets**
3. **example: 1. G-prot. Coupled rhodopsin photoreceptor in rod cells of retina**

2. Nitric Oxide (NO) receptor

3. Atrial natriuretic peptide (ANP) receptor

Summary of how cAMP activates transcription:

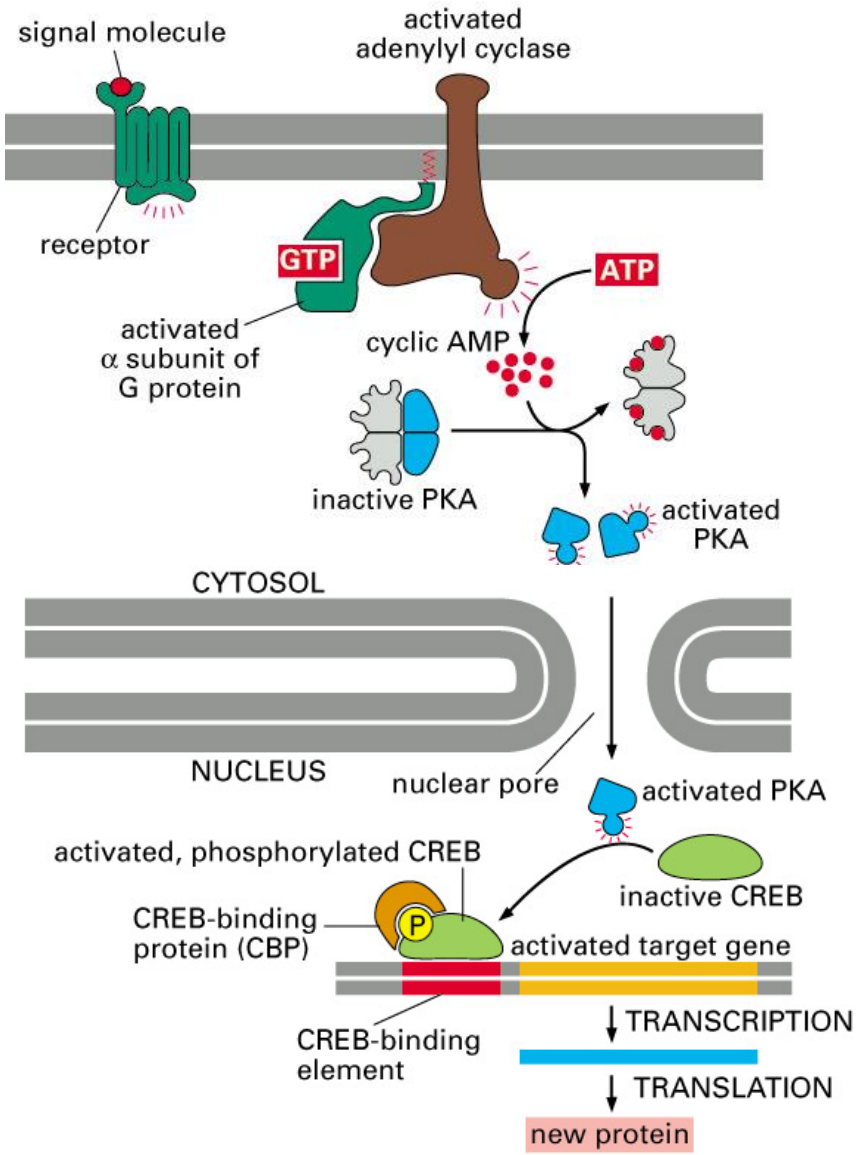


Figure 15–33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Adenylate Cyclase-cAMP

- 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.
  - Hydrolyzes cAMP to inactive fragments.

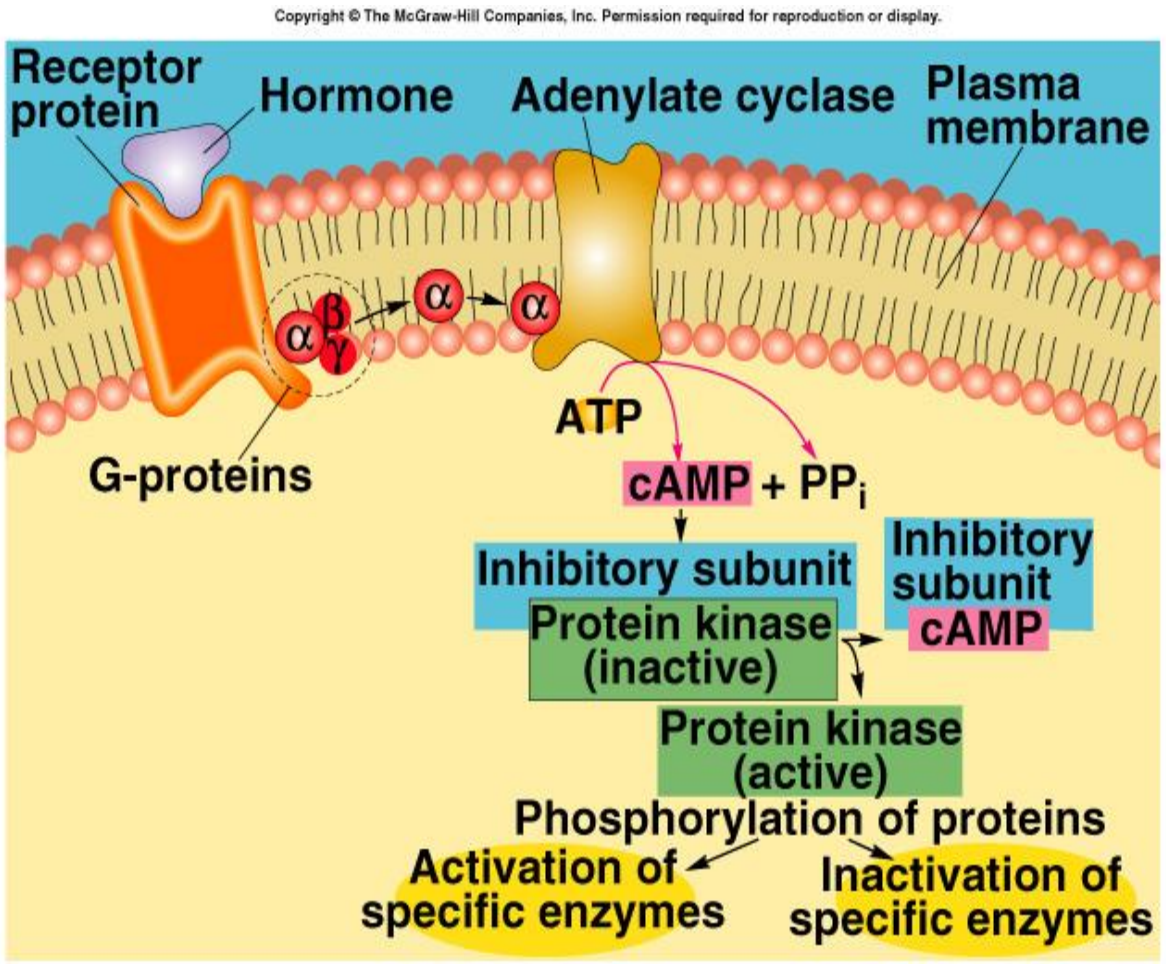
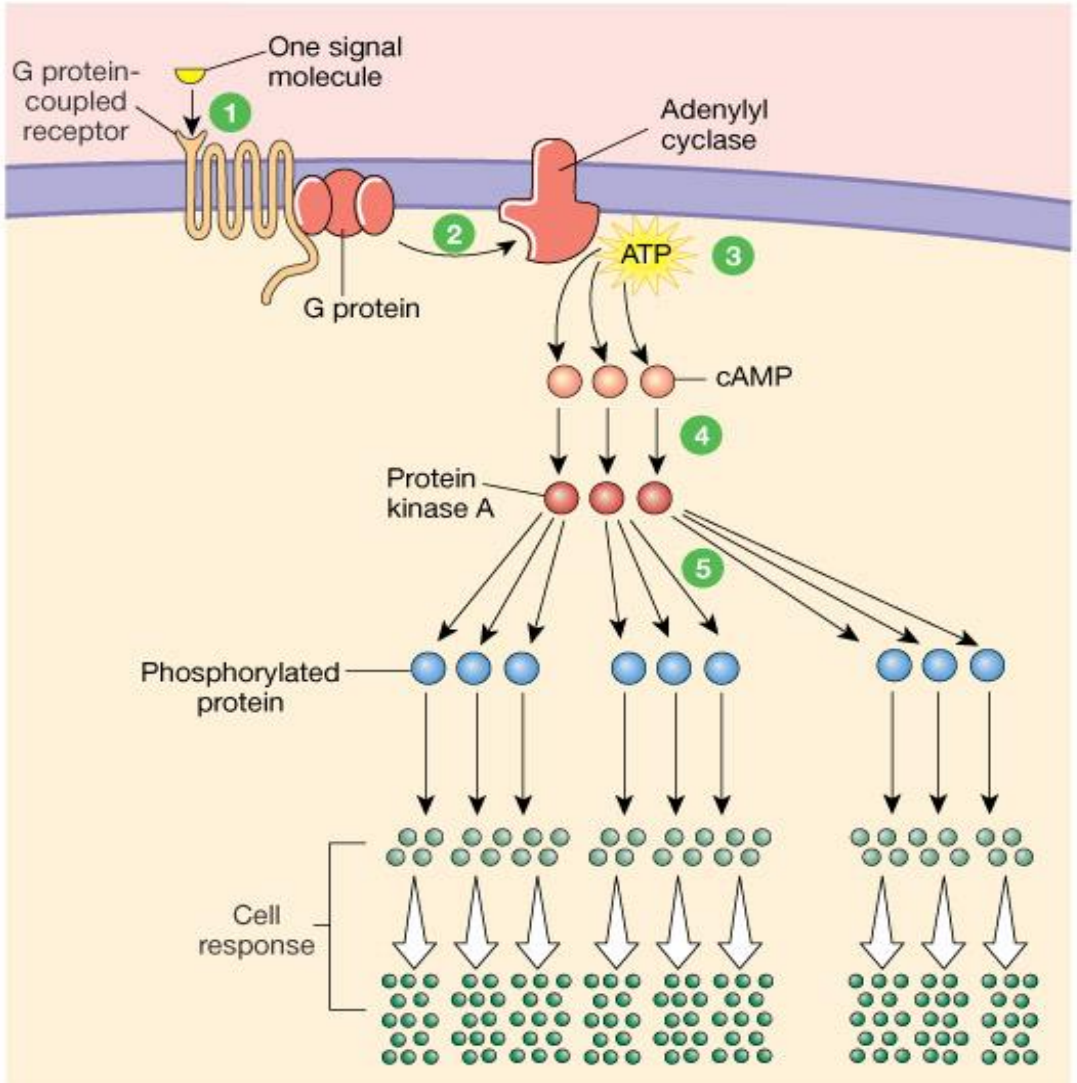


TABLE 20-3 Metabolic Responses to Hormone-Induced Rise in cAMP in Various Tissues

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

- Various metabolic responses depends on the type of the tissue
- Same hormone in different tissues leads to different metabolic responses
- This table is required and very important

G-Protein-coupled Receptors



1. Signal molecule binds to G protein-linked receptor, which activates the G protein.
2. G protein turns on adenylyl cyclase, an amplifier enzyme.
3. Adenylyl cyclase converts ATP to cyclic AMP.
4. cAMP activates protein kinase A.
5. Protein kinase A phosphorylates other proteins, leading ultimately to a cellular response.

4. Second Messengers, cont.:

C. IP3 and DAG:

1. Overview: Phosphotidylinositol 4,5 bisphosphate (PIP2) triggers a 2-armed signaling pathway

- ***a.*** **PIP2 is a minor PL in inner leaflet of PM 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) and inositol 1,4,5-phosphate (IP3), both of which are 2nd messengers**
- ***d.*** **PIP2 hydrolysis is activated by both GPRs and TKRs via different forms of PLC**
- ***e.*** **PLC-β is stimulated by G_q proteins while PLC-γ has SH2 domains that allow binding to activated tyrosine kinases** (different isoforms of PLC are activated by different stimuli, but both PLC-β and PLC- produce the same second messenger)

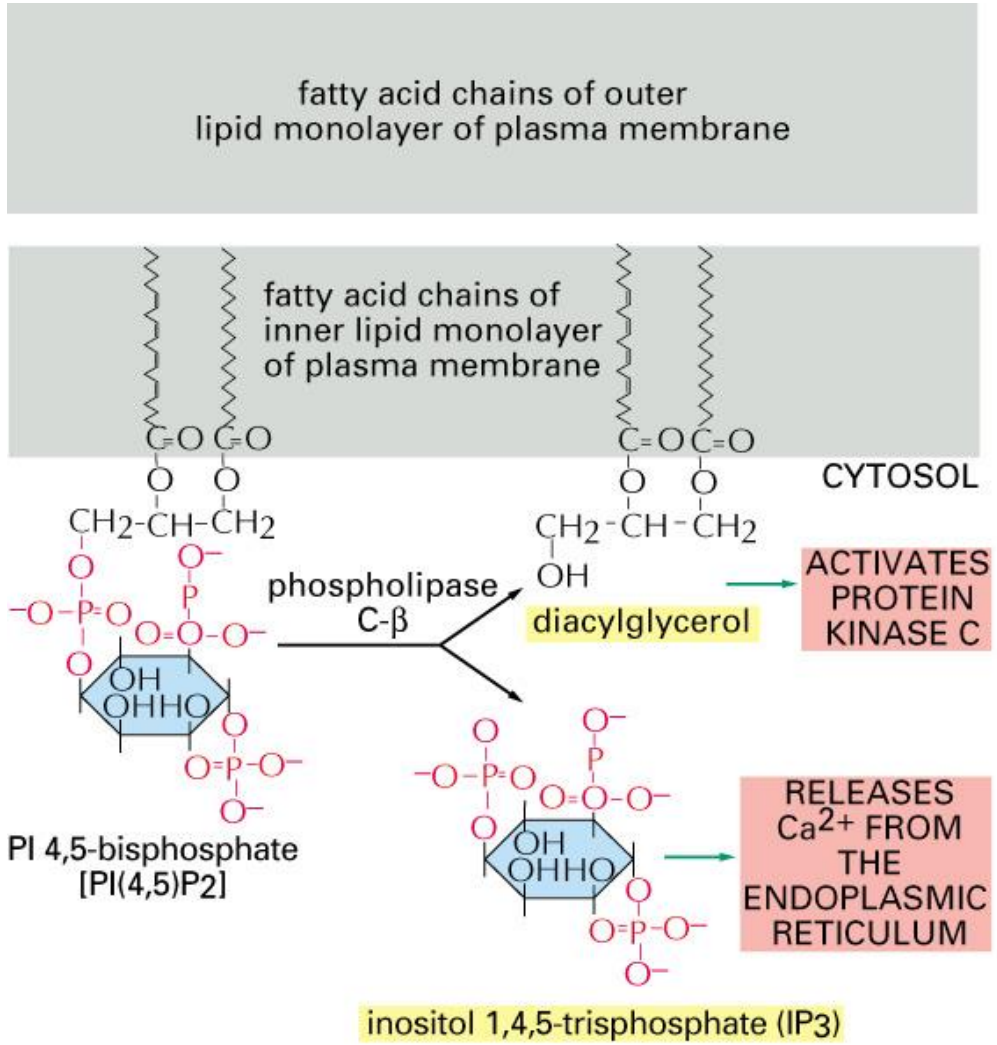
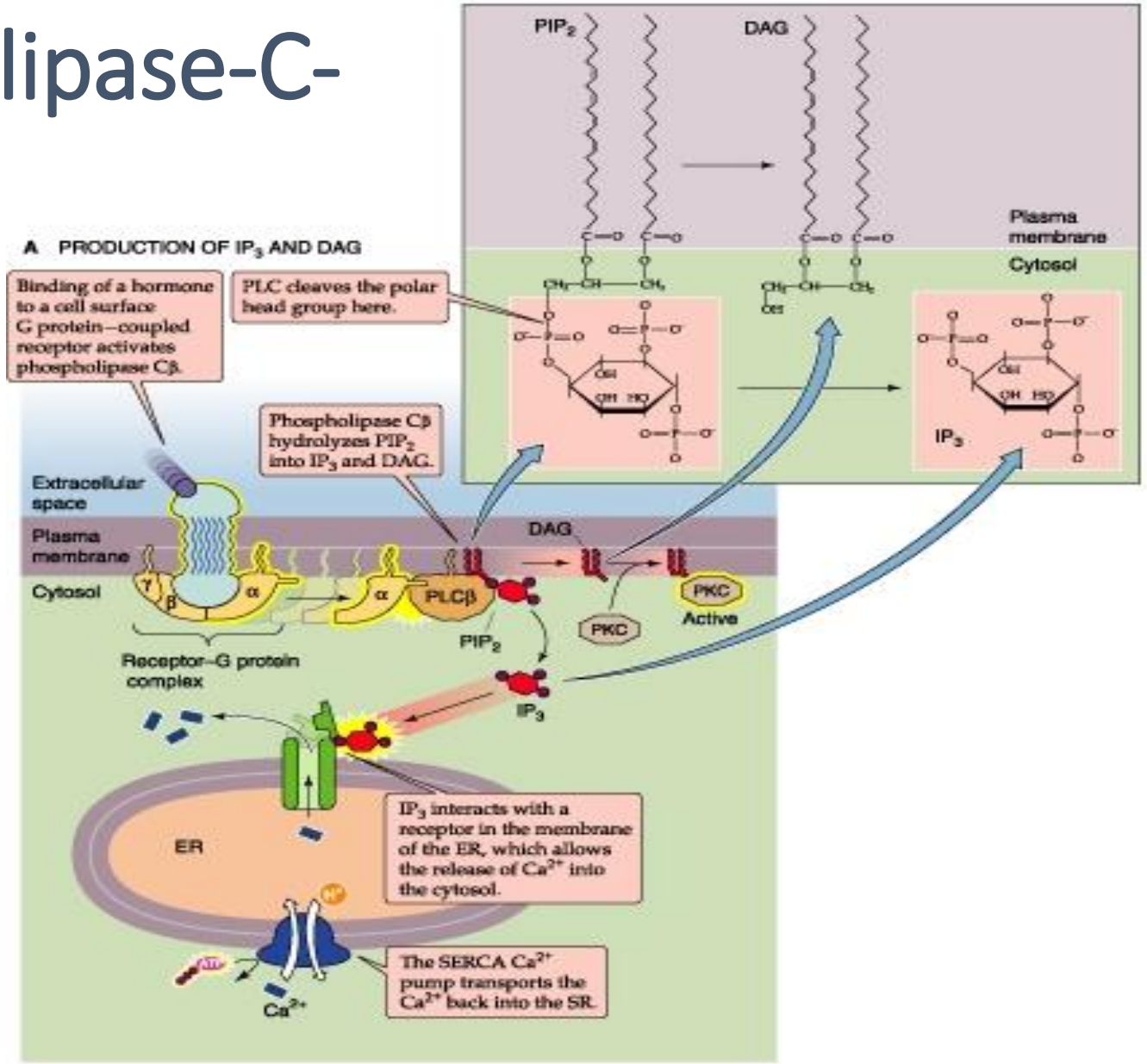


Figure 15–35. Molecular Biology of the Cell, 4th Edition.

- Phospholipase C-β is activated by alpha q subunit of G-protein

Phospholipase-C-Ca^{2+}



The function of IP3 and DAG

In this scheme you can see IP3 is generated and then it will go and activate or bind to a receptor located on the ER (Endoplasmic Reticulum) and the ER represents stores of Calcium so once IP3 binds to this channel, it will open up and allow calcium to be released from their stores into the cytosol increasing the intracellular concentration of calcium, normally the calcium intracellular is kept very low however when a signal like IP3 opens up the channels of the stores, the concentration increases transiently, this transient increase has many functions (changes) in the cell. So, what happened here is the release of calcium because of IP3. Whenever the calcium concentration increases, this increase must be transient which means that calcium must enter again and the concentration will decrease again since the concentration shouldn't be high all the time, so there are other channels which pumps the calcium back into their stores using energy.

Now what is the function of the calcium (small blue pieces) ? The calcium that had its concentration increased in the cytosol now will act as a second messenger, so now IP3 and DAG in addition to the calcium we just mentioned are all second messengers, what can this calcium do? One of the effects of calcium is that it will go and bind to a certain binding site on protein Kinase C (PKC) with the help of DAG, and activate PKC, and this activated protein will have a target, it will phosphorylate proteins and enzymes and change their functions.

- IP3 and DAG both activate PKC
- The DAG remians attached to the membrane

4. Second Messengers, cont.:

C. DAG and IP3, cont.:

2. DAG: Remains associated with the PM

a. **Stimulates the Ca^{+2}-dependent protein kinase C signaling pathway, which activates other targets including the MAP kinase cascade (see below)**

(The DAG will remian attached to the membrane and stimulate the PKC, the PKC has other targets which could be MAP kinase mitogenic pathway, which helps the cell to proliferation or to grow or survival.)

3. IP3: Small polar molecule released into cytosol

a. **Stimulates Ca^{+2} release from intracellular stores.** ***Question: where are these?***

b. **Elevated Ca^{+2} alters activities of target proteins including kinases & phosphatases**

The Gq activated the phospholipase C-Beta and it produced IP3 and DAG, the DAG remained in the membrane, and it will now activate the PKC together with calcium which is released from the calcium stores in the ER with the help of IP3 which opened the calcium channels, the calcium then binds to PKC and activates it together with DAG, so DAG and calcium together activate PKC.

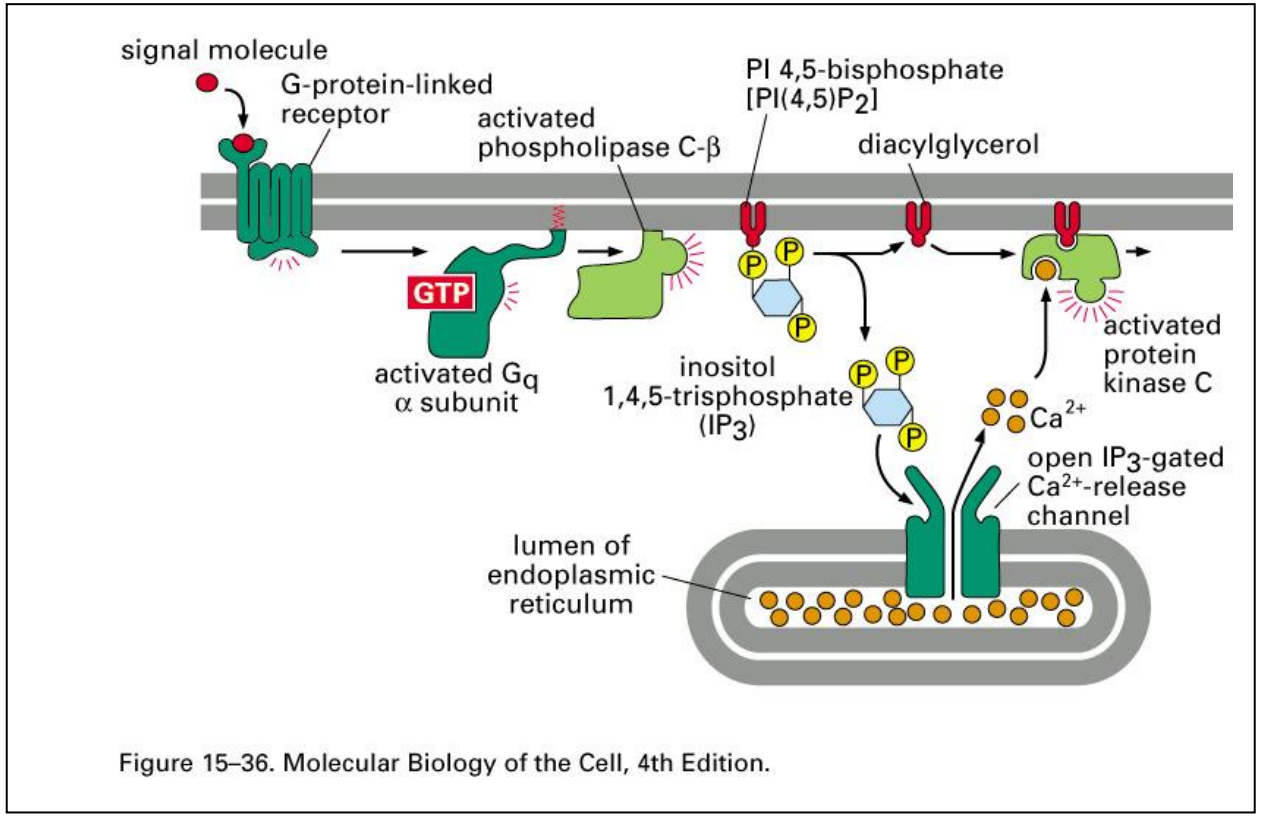
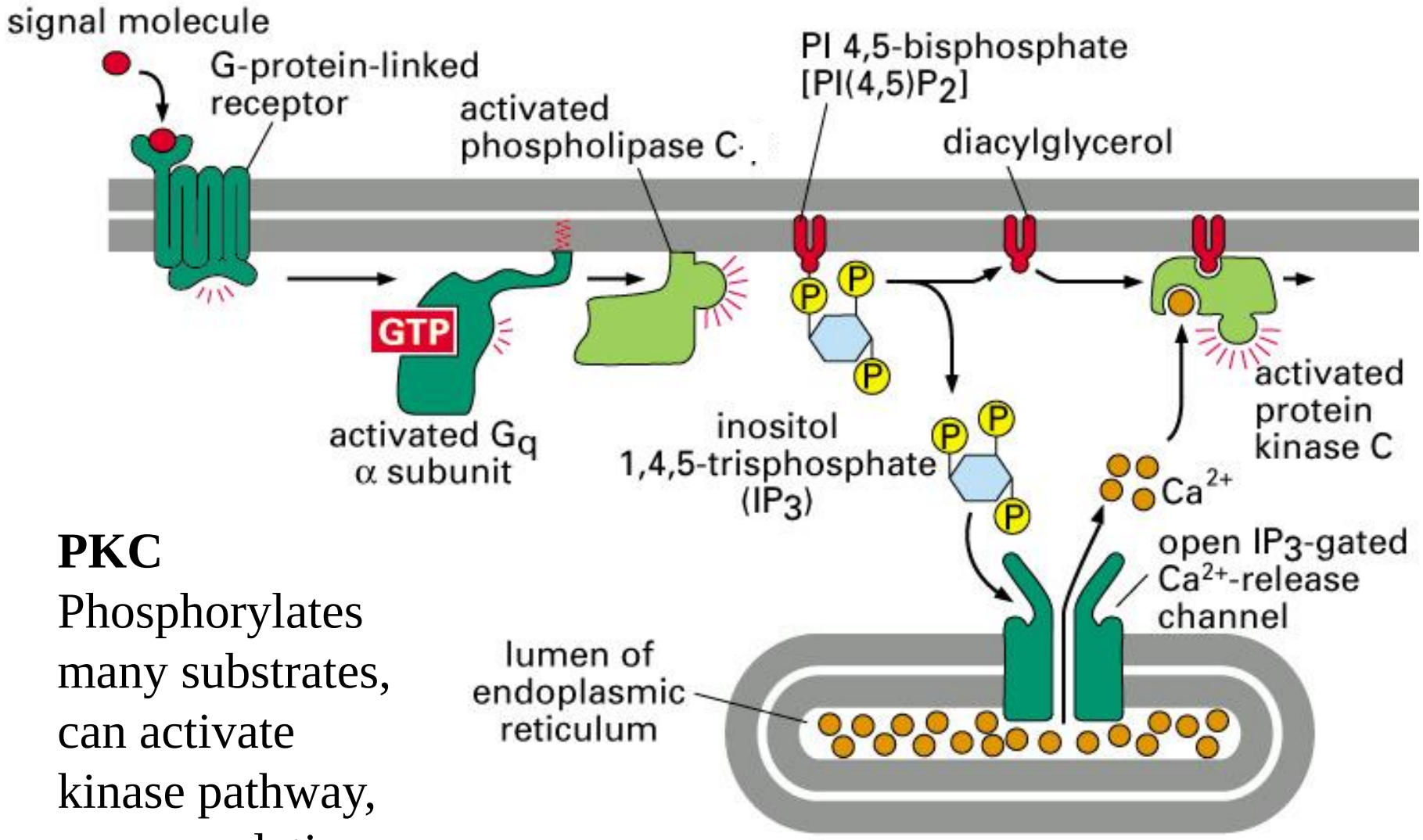


Figure 15–36. Molecular Biology of the Cell, 4th Edition.

PLC- signaling pathway



PKC

Phosphorylates many substrates, can activate kinase pathway, gene regulation

4. Second Messengers, cont.:

D. Ca^{+2} also acts as a second messenger

Ca^{+2} concentration kept low (10^{-7} M), rising locally due to transient signaling

Effects of intracellular Ca^{+2} are mediated by the Ca^{+2} binding protein calmodulin. (and activation of PKC.)

Ca+2 /calmodulin binds to target proteins, including protein kinases (Ca^{+2}calmodulin-dependent kinases; CaM-kinases), adenylyl cyclases, and phosphodiesterases, causing change in conformation and activation of these proteins. (It will phosphorylate it.)

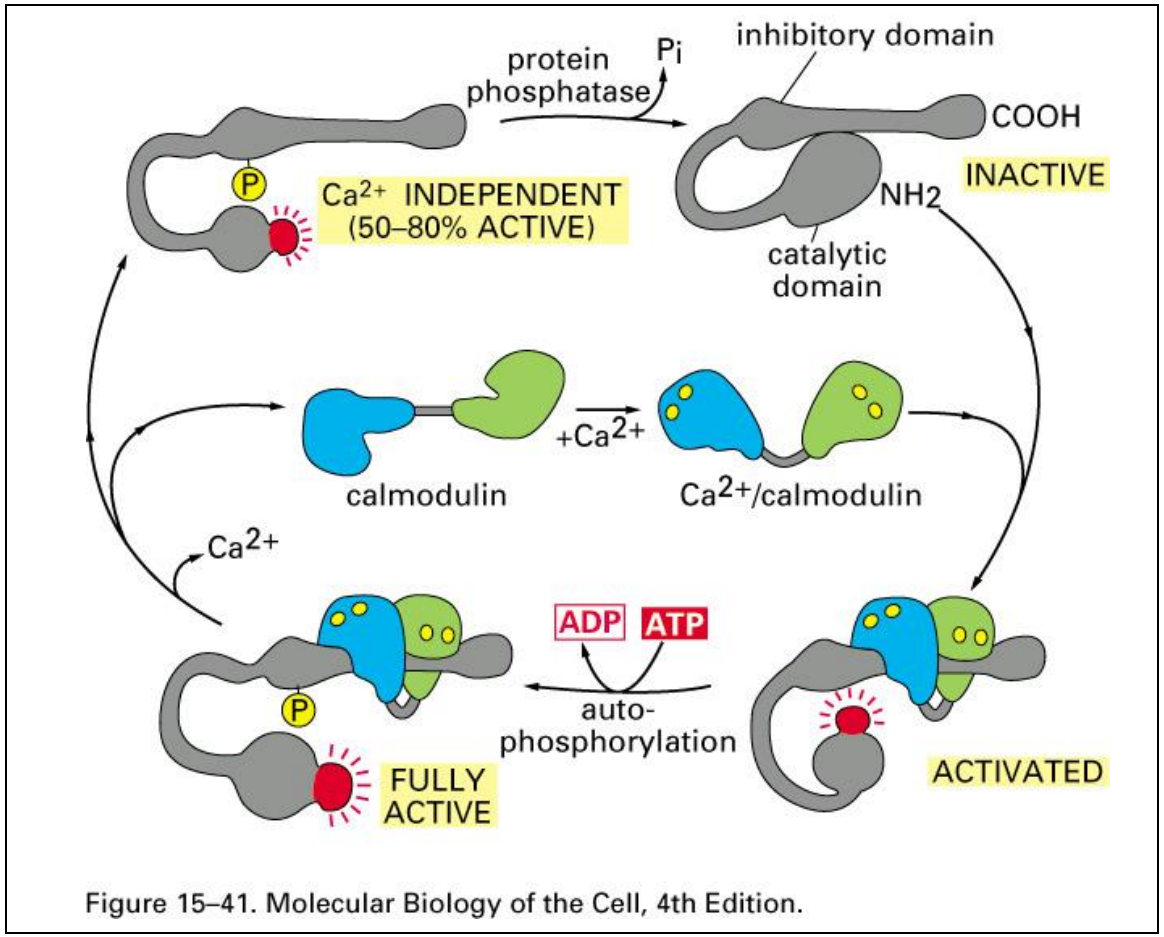
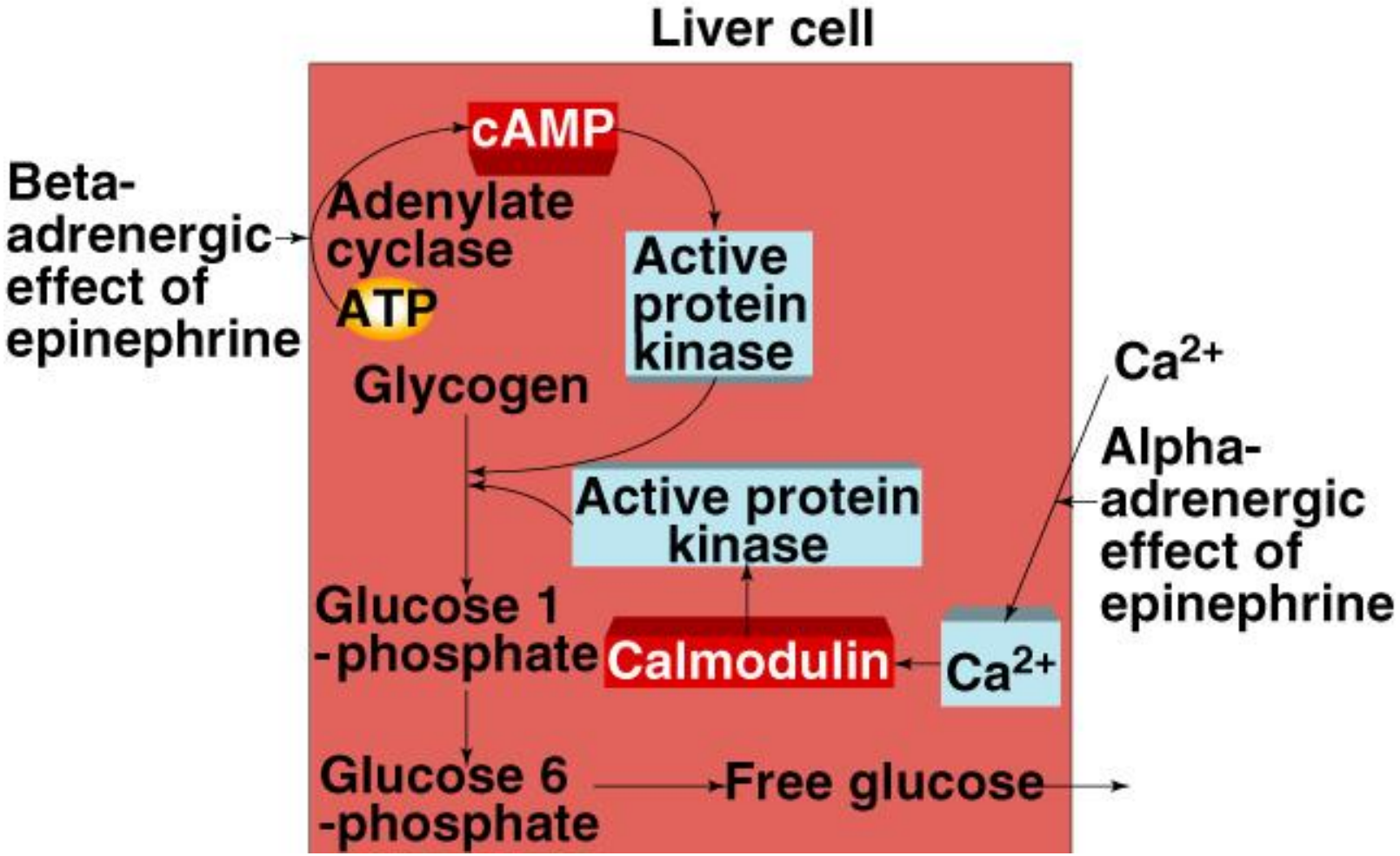


Figure 15–41. Molecular Biology of the Cell, 4th Edition.

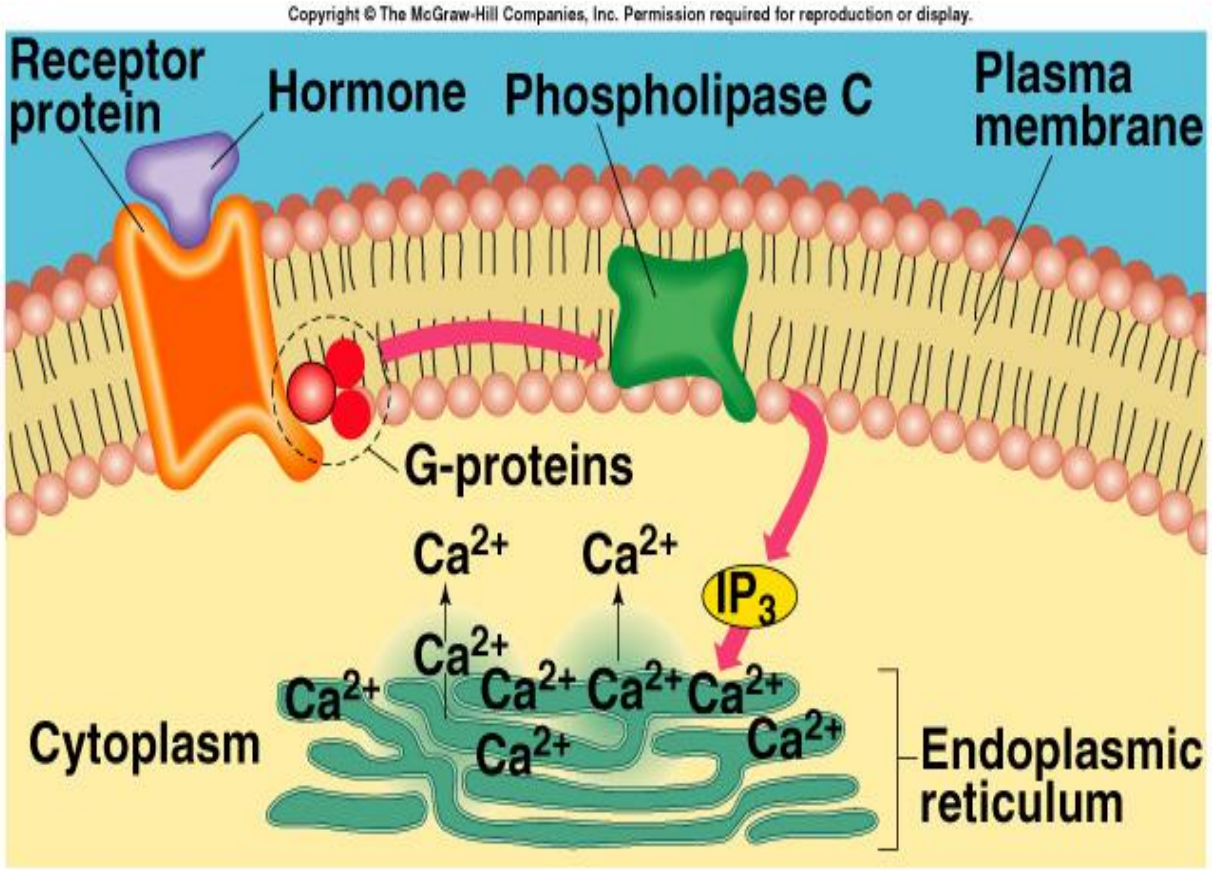
Epinephrine Can Act Through Two 2nd Messenger Systems

Now in this graph you can see that epinephrine can act through two types of receptors and generate two diiferent second messengers (when a hormone binds to different receptors the outcome, or second messengers could be different).
For example, the liver cell, once the epinephrine is bound to Beta-adrenergic receptor which is Gprotein coubled to s or Gs, it will increase cAMP, which will activate protein Kinase a which will then do its function, the epinephrine could also bind to alpha one-adrenergic receptor which generates DAG and IP3 (which is linked to alpha o that has the same effect as alpha q) will release calcium from its stores and bind to calmodulin and activate protein Kianses like cAMP kinase and this could also be involved in Glycogen breakdown to give glucose. So, by two types of messengers, we have mediation of the action of epinephrine.



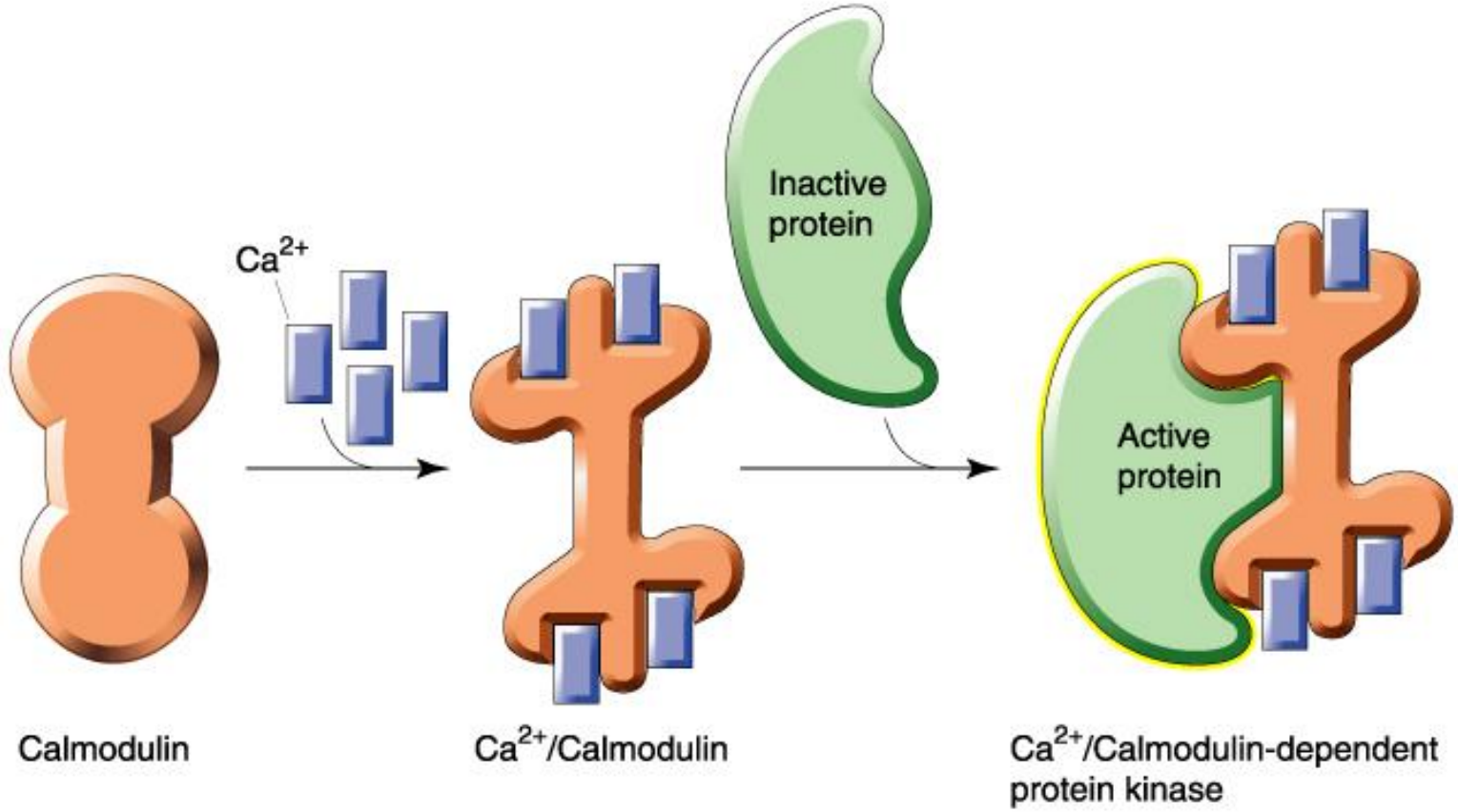
Ca^{2+}- Calmodulin (continued)

- 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.



Ca^{2+}- Calmodulin (continued)

Notice here how the calcium binding to the calmodulin changes its conformation, resulting in activation of protein.



Guanylate cyclase (GC) receptor

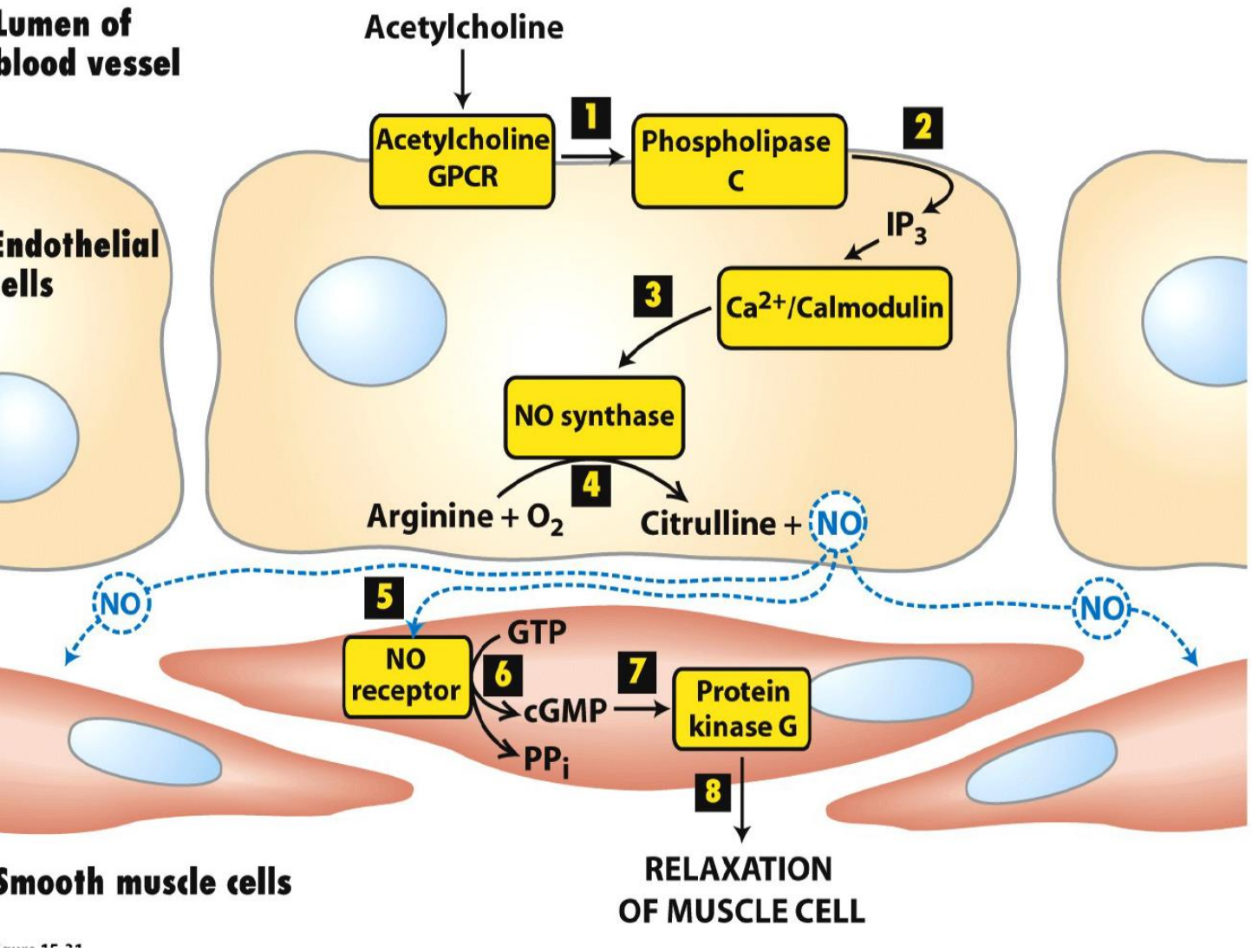
(Is related to enzyme-linked receptors)

Membrane receptor –ANP

Soluble receptor – NO, CO

(Is found in the cytosol)

NO signaling



NO (nitric oxide) Signaling is very important in vascular functions (blood vessels), what happens here is that the blood vessel wall consists of the endothelial cell lining, and we also have smooth muscle cell layer.

Now, acetylcholine will bind to its receptor which is acetylcholine GPCR (coubled to q) which will activate phsopholipase C, it will produce IP3 and DAG, IP3 will induce calcium release, calcium will bind to calmodulin and then calcium/calmodulin complex will activate an enzyme called Nitric Oxide synthase in the endothelial cell, this NO synthase will convery Arginine amino acid into citrulline and produce NO in this reaction, since NO is a gas, it will diffuse freely from the endothelial cell into the smooth muscle cell, it will find a receptor for it called NO receptor which produces cGMP (a second messenger), which will bind to protein kinase G and cause vasorelaxation because it will cause relaxation of the muscle, therefore the blood vessel will also have relaxation and its diameter will increase and improve the blood flow in this blood vessel.

Signaling Overview

4. Second Messengers, cont.:

E. PIP3:

PIP2 phosphorylated by PI 3-kinase, resulting in PIP3, which is also a 2nd messenger.

PI 3-kinase can be activated by GPRs or TKRs.

One target of PIP3 is a protein-serine/threonine kinase called Akt, or protein kinase B,

which becomes activated by a kinase called PDK1.

PIP3 binds to Akt at the pleckstrin homology domain.

Activation of Akt leads to regulation of target molecules, including BAD, which is pro-apoptotic and becomes inactivated by phosphorylation.

This mechanism is important for the survival of the cell or inhibition of Apoptosis using a pathway called PKB and PDK 1.

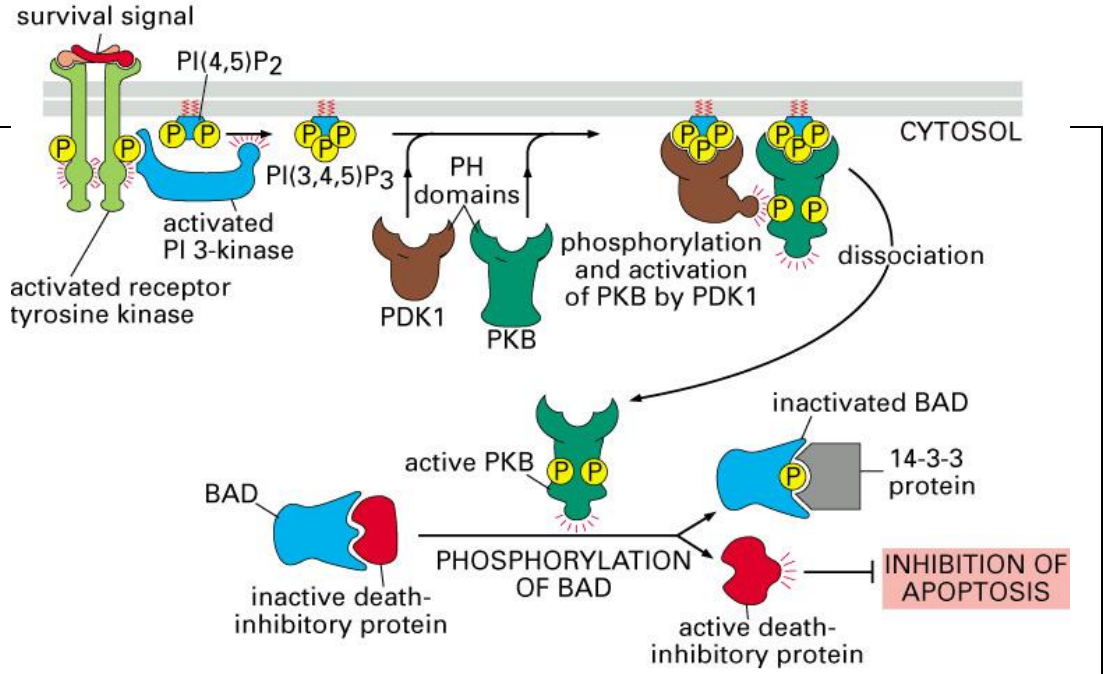


Figure 15–60. Molecular Biology of the Cell, 4th Edition.

Signaling Overview

5. Signaling Cascades, cont.:

5 downstream kinases activated by different signaling cascades

This is an overview of everything we've talked about.

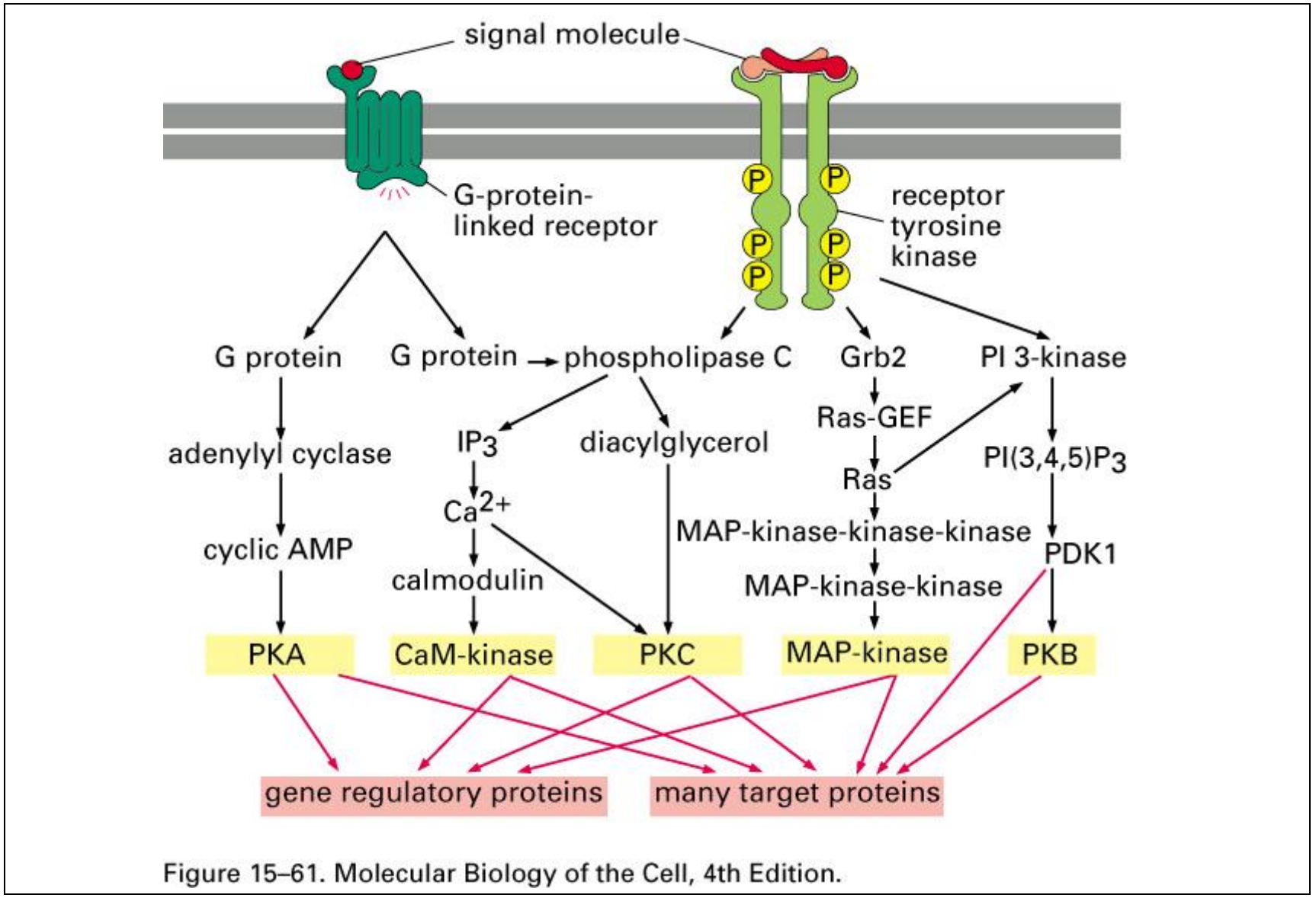


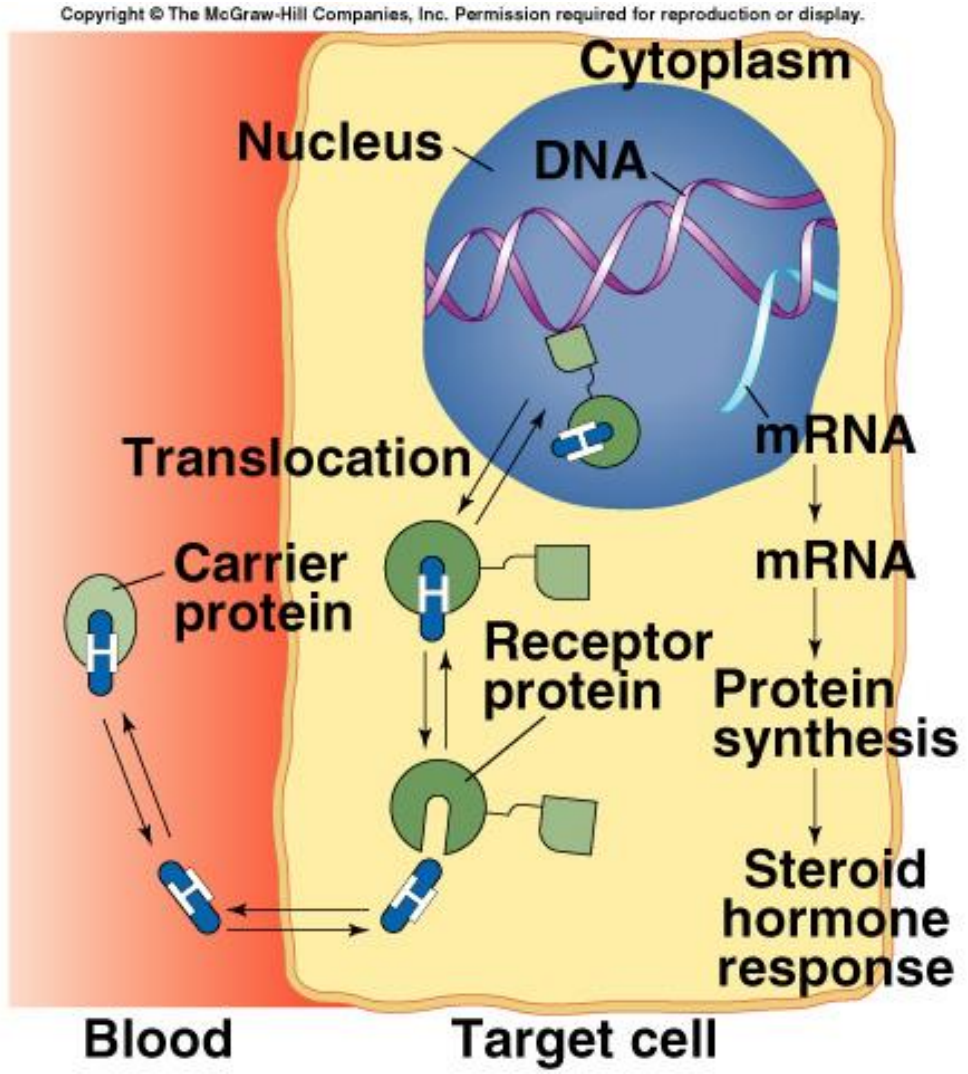
Figure 15–61. Molecular Biology of the Cell, 4th Edition.

Hormones That Bind to Nuclear Receptor Proteins

(Intracellular receptors : Thyroid and Steroid receptors.)

- Lipophilic steroid and thyroid hormones are attached to plasma carrier proteins.
  - Hormones dissociate from carrier proteins to pass through lipid component of the target plasma membrane.
- Receptors for the lipophilic hormones are known as nuclear hormone receptors.

  They can be nuclear or cytocellular that can go to the nucleus



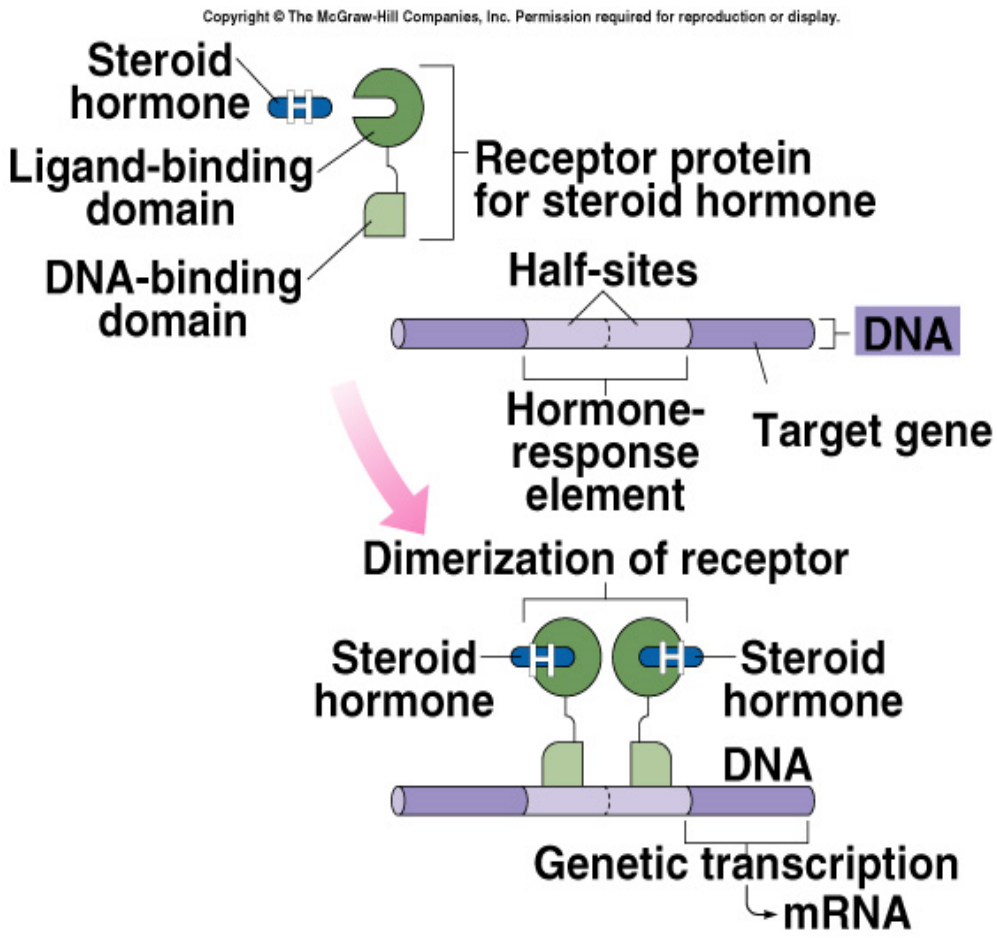
Nuclear Hormone Receptors

- 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.
- Each nuclear hormone receptor has 2 regions:
  - A ligand (hormone)-binding domain.
  - DNA-binding domain.
- Receptor must be activated by binding to hormone before binding to specific region of DNA called HRE (hormone responsive element).
  - Located adjacent to gene that will be transcribed.

Another characteristic of nuclear hormone receptors that they should bind to DNA as dimers, means to receptor bind to hormone should be dimerizes to enable activate genetic transcription.

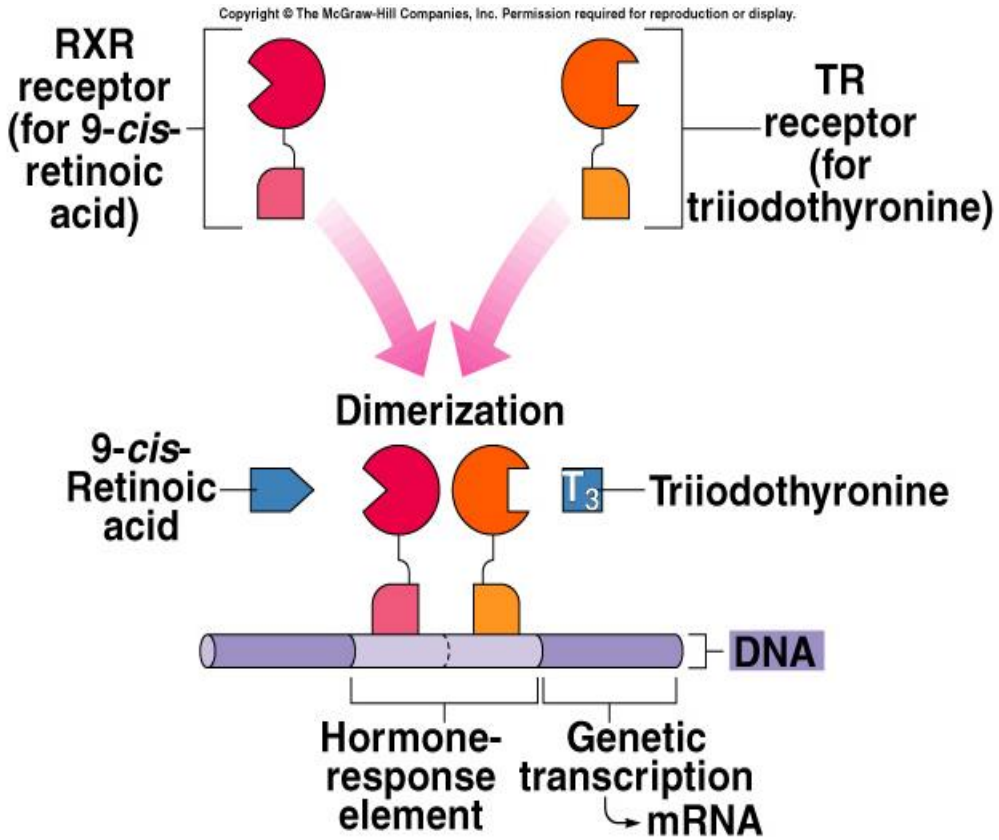
Mechanisms of Steroid Hormone Action

- 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.



Mechanism of Thyroid Hormone Action

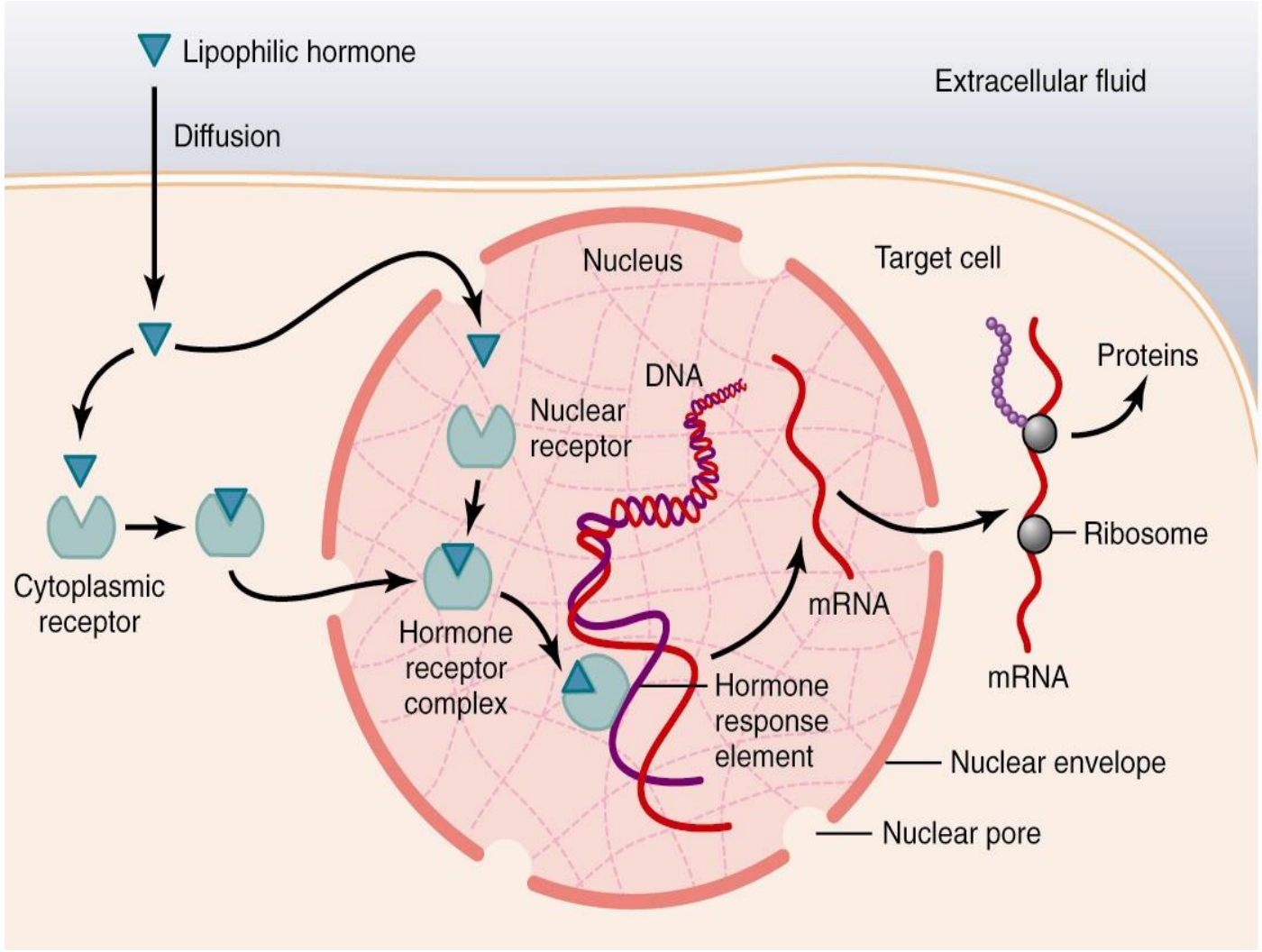
- T_4 passes into cytoplasm and is converted to T_3.
- Receptor proteins located in nucleus.
  - T_3 binds to ligand-binding domain.
  - Other half-site is vitamin A derivative (9-cis-retinoic) acid.
    - DNA-binding domain can then bind to the half-site of the HRE.
  - Two partners can bind to the DNA to activate HRE.
    - Stimulate transcription of genes.



Kinds of thyroid hormone:
T3 (triiodothyronine)
T4 (tetraiodothyronine)
TR receptor (for triiodothyronine) dimerized with another receptor that is RXR receptor.(because they are different receptors called heterodimer).

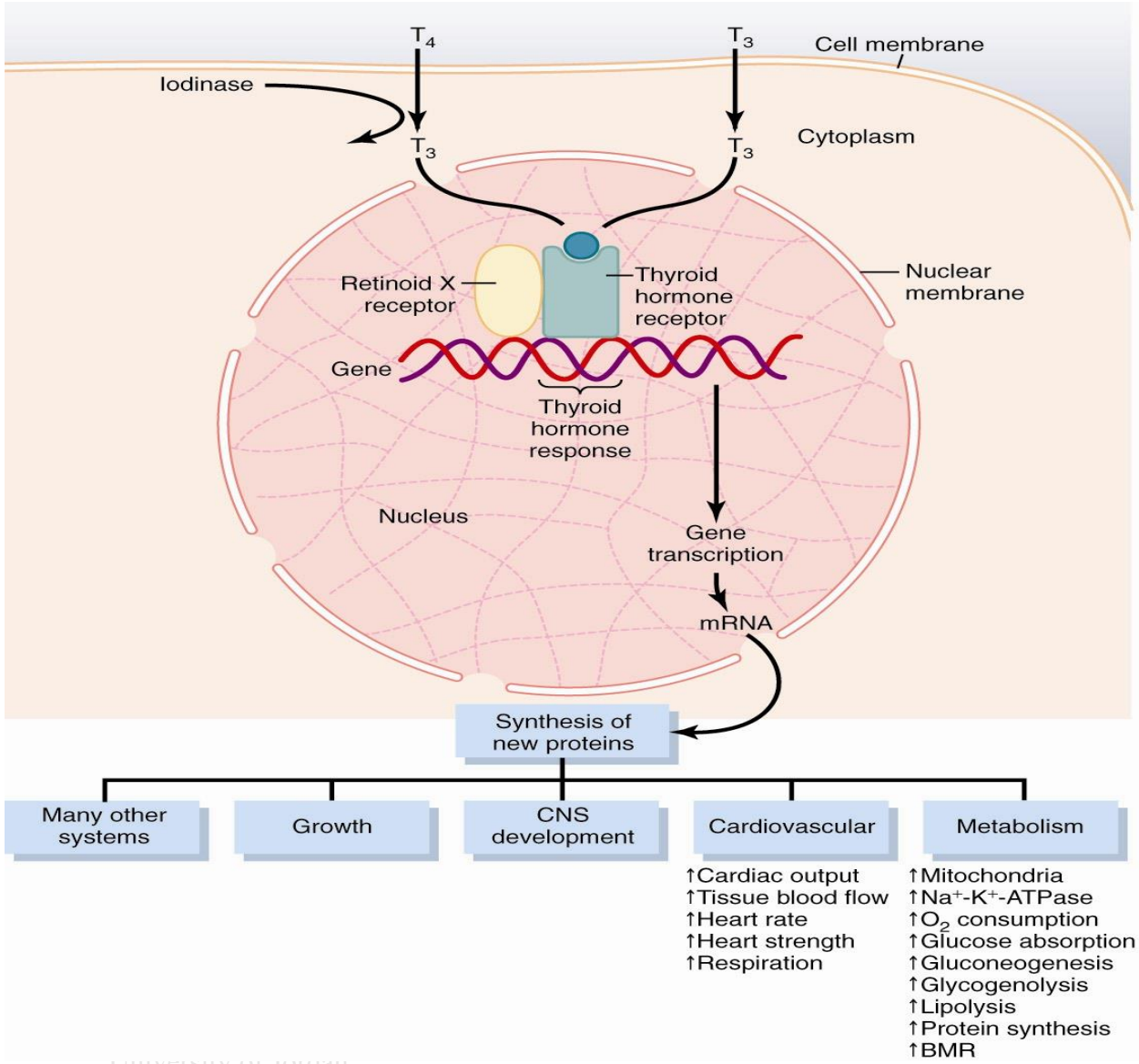
Steroid & Thyroid Hormones - Mechanism of Action

Either the hormones go direct to the nuclear receptors or bind to cytoplasmic receptors that translocate to the nucleus.



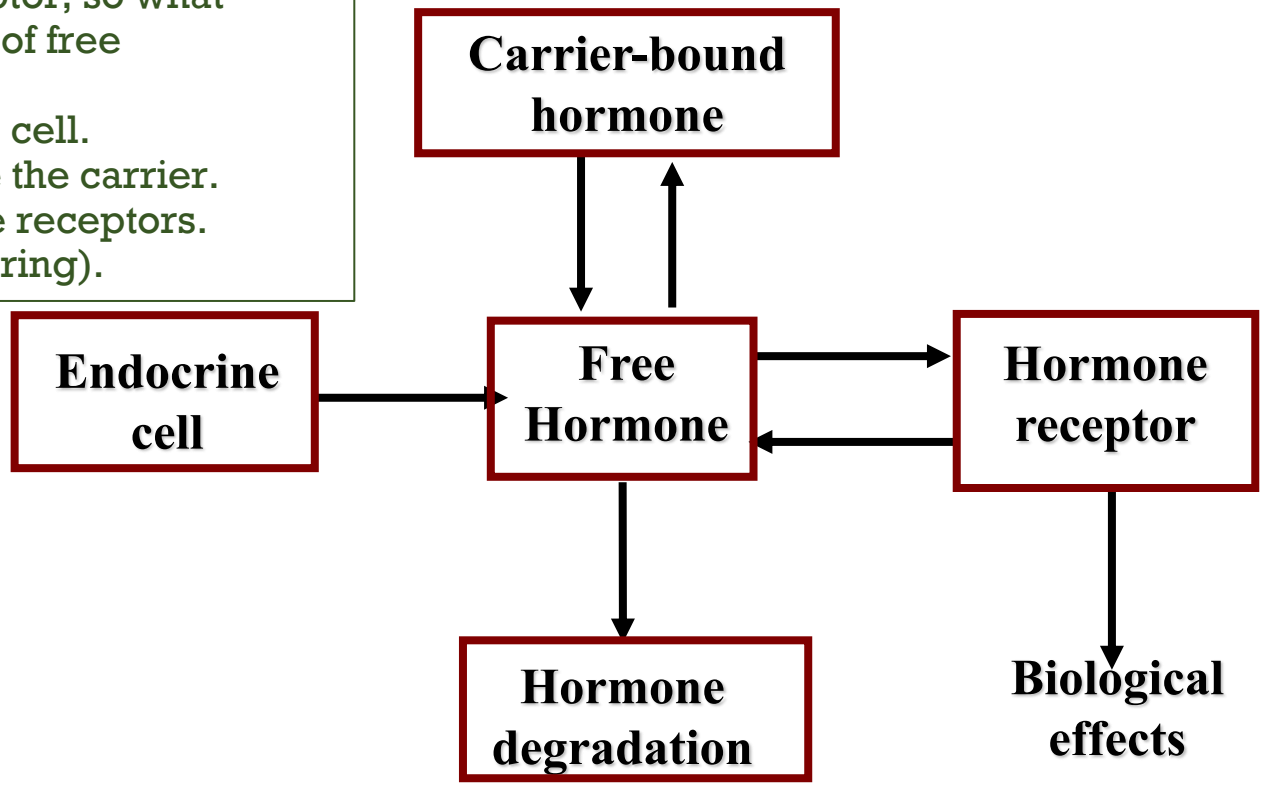
Actions of Thyroid Hormones

T3 go inside the nucleus and bind with thyroid hormone receptor and dimerizes then stimulates transcription of particular genes.
Synthesis of new proteins involved a lot of mechanism such as:
1)metabolism that include increasing metabolic rate, lipolysis and gluconeogenesis(glucose production).
2)cardiovascular that include increasing the heart rate and cardiac output and so on.
3)also affect on CNS development of infants and embryos and affect the growth of children.



Determinants of Free Hormone Receptor Binding

What determine the activity of the hormones?
The concentration of free hormones that are available to bind to the receptor, so what determine the concentration of free hormones?
1)the secretion by endocrine cell.
2)how many hormones leave the carrier.
3)binding the hormone to the receptors.
4)hormone degradation(clearing).



Correlation of Plasma Half-Life & Metabolic Clearance of Hormones with Degree of Protein Binding

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

One of the most important relationship is protein binding percentage9how much hormones bind to the receptors in plasma.
Thyroxine is T4.
The percentage protein binding affect the half-life. when the binding percentage of hormone with protein is high, plasma half-life will be high too.
When hormones bind to proteins will be protected from clearance because of that the half-life become longer.
Clearance represent how much fast the body get rid of hormones (ml/minute).
When the binding percentage high, the clearance is low.

Circulating Transport Proteins

Hormones transport in plasma maybe bind to specific protein or nonspecific protein.

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

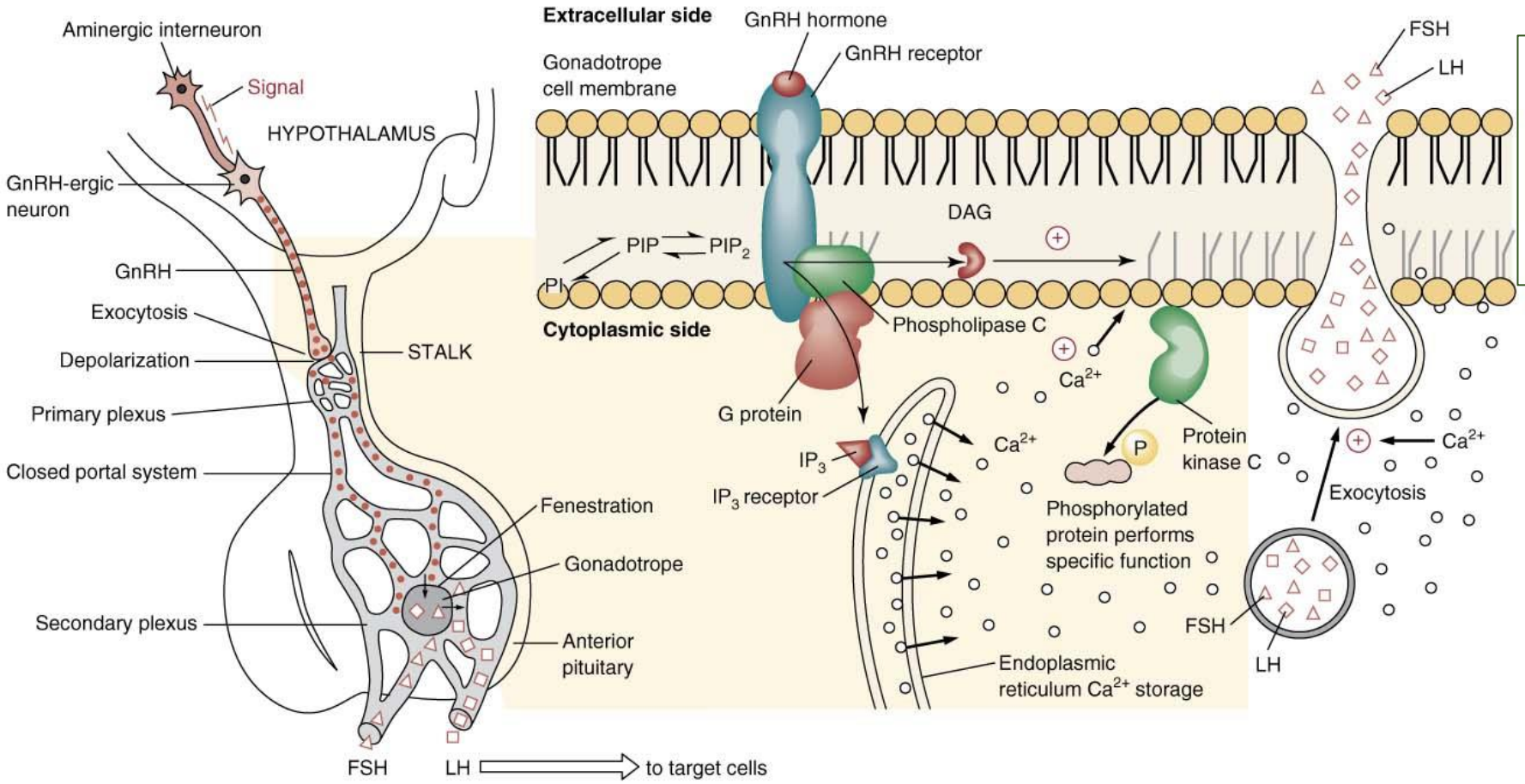


Figure 23.31. Regulation of secretion of LH and FSH by protein kinase C.

The anterior pituitary gland secrete LH(luteinizing hormone) and FSH (follicle stimulating hormone) that is female sex hormone which regulate ovarian function.

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

Hypothalamus released GnRH (gonadotrophin releasing hormone) signal that go through blood stream to anterior pituitary and bind to receptor called GnRH receptor(which is G protein coupled to Q), that will activate phospholipase C and producing DAG and IP3.
IP3 release calcium from its store.
Calcium and DAG will activate protein kinase C(PKc) that activate vesical contain LH+FSH so it released by exocytosis, then LH+FSH go to the target cell.

Signaling molecule
(hormones)

↓

Receptor of target cell

↓

Intracellular molecule
(second messengers)

↓

biological effect

Signal transduction

Third messengers:

Third messengers are the molecules which transmit message from outside to inside of nucleous or from inside to outside of nucleous, also called DNA binding protein.