Signal Transduction: Lect 4

Enzyme linked receptors, second messengers, steroids and thyroid hormone receptors.

Signaling Overview

3. Three major classes of surface receptors for signaling, cont.:

C. Enzyme-linked receptors:

1. Tyrosine kinase-linked receptors (TKRs).

***A.* Overview of TKRs:**

***1.* Cell surface receptors that are directly linked to intracellular enzymes (kinases).**

***2.* Includes receptors for most growth factors (NGF, EGF. PDGF), insulin.**

***3.* Common structure: N terminal extracellular ligand-binding domain, single TM domain, cytosolic C-terminal domain with tyrosine kinase activity.**

***4.* Can be single polypeptide or dimer.**

Examples of tyrosine kinase-linked receptors (TKRs):

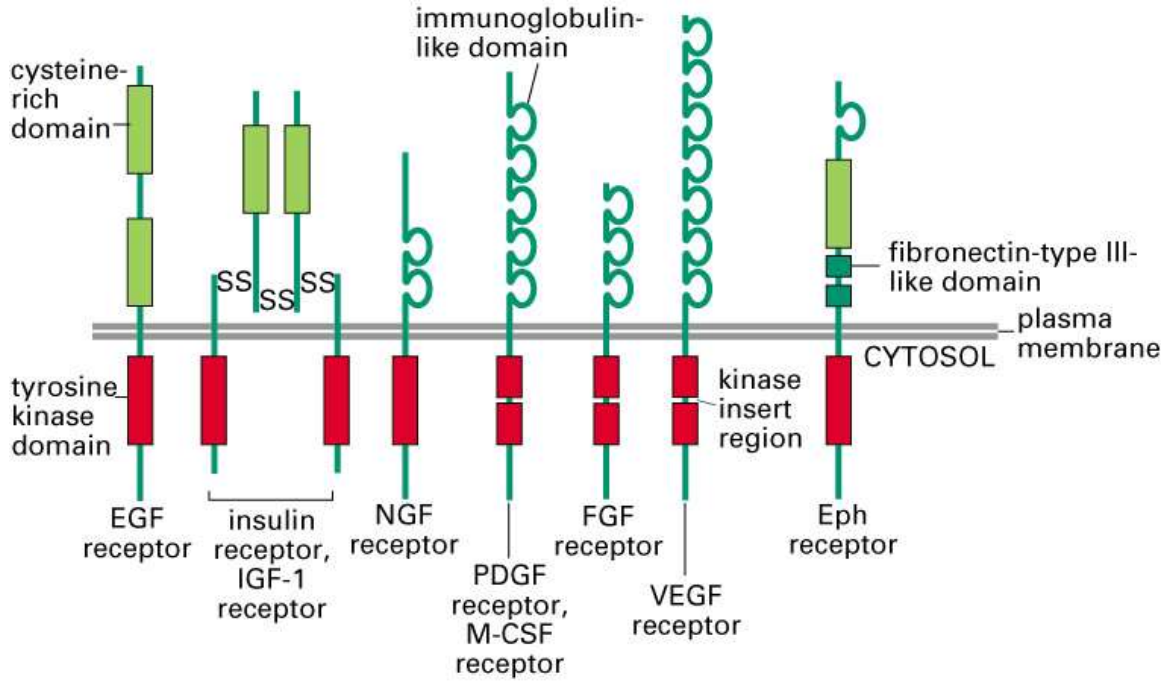


Figure 15–49. Molecular Biology of the Cell, 4th Edition.

3. Three major classes of surface receptors for signaling, cont.:

C. Enzyme-linked receptors, cont.:

1. Tyrosine kinase-linked receptors (TKRs)

***B.* Mechanism of activation of TKRs:**

***i.* ligand binding induces receptor dimerization (receptor crosslinking).**

***ii.* dimerization leads to autophosphorylation of the receptor (cross-phosphorylation).**

***iii.* phosphorylation increases kinase activity & also creates specific new binding sites.**

***iv.* proteins that bind to these new binding sites transmit intracellular signals.**

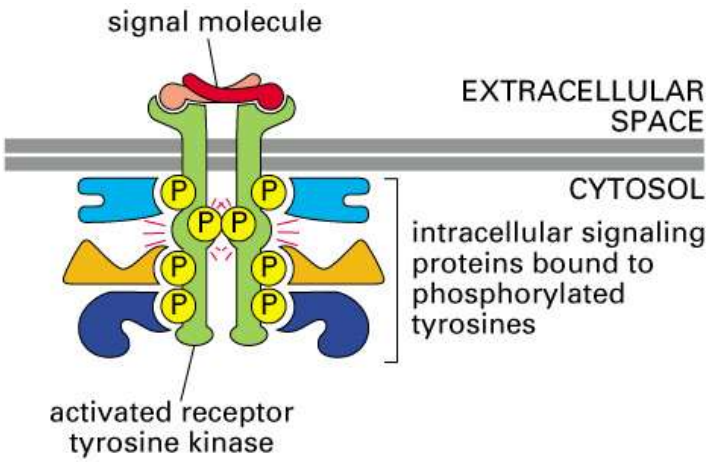


Figure 15–52. Molecular Biology of the Cell, 4th Edition.

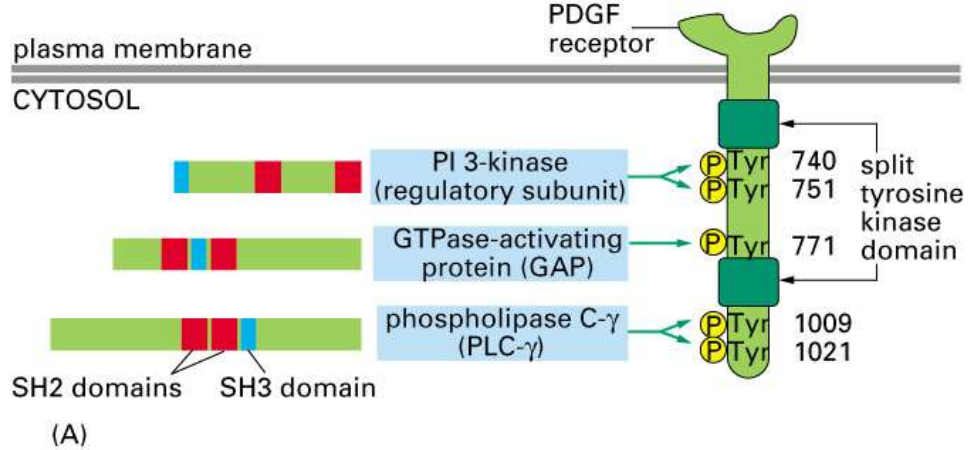


Figure 15–53 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

How receptor tyrosine kinases work together with monomeric GTPases:

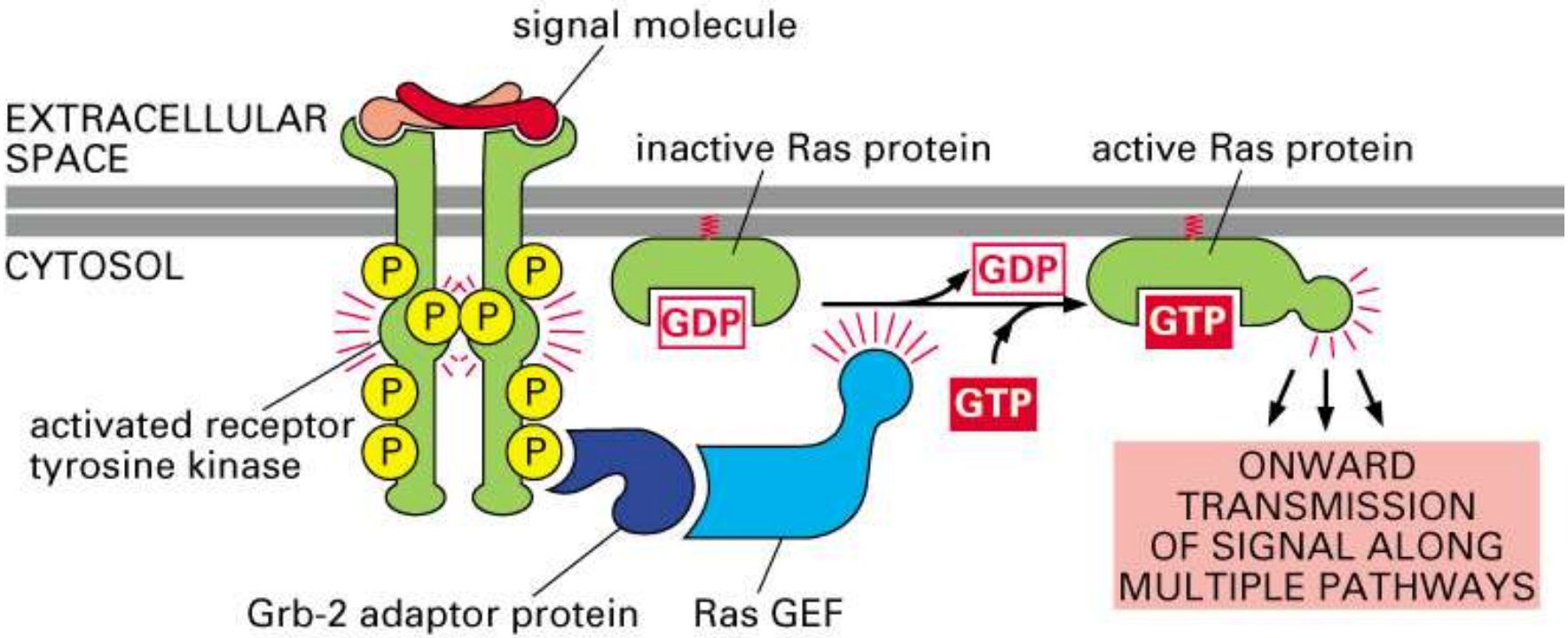
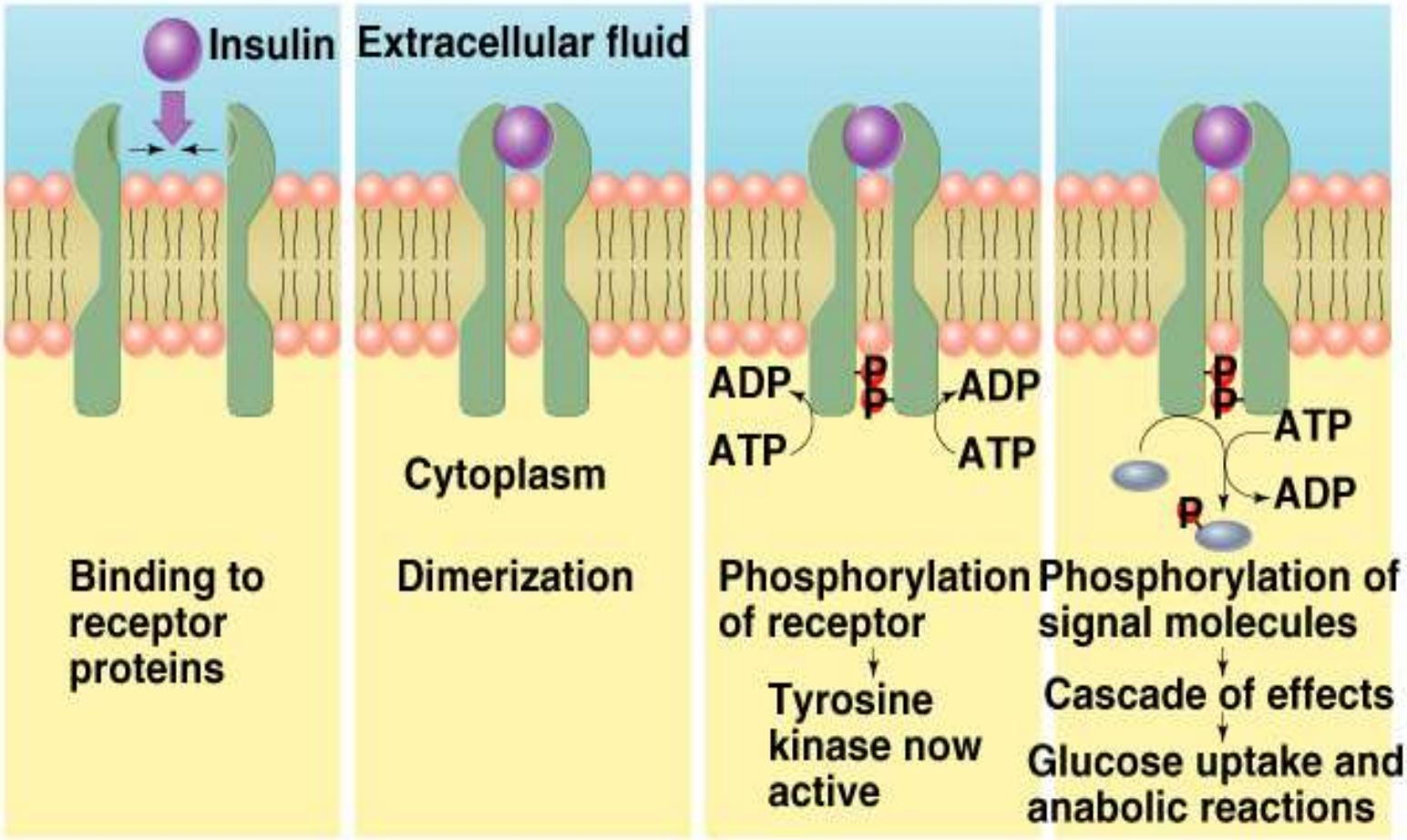


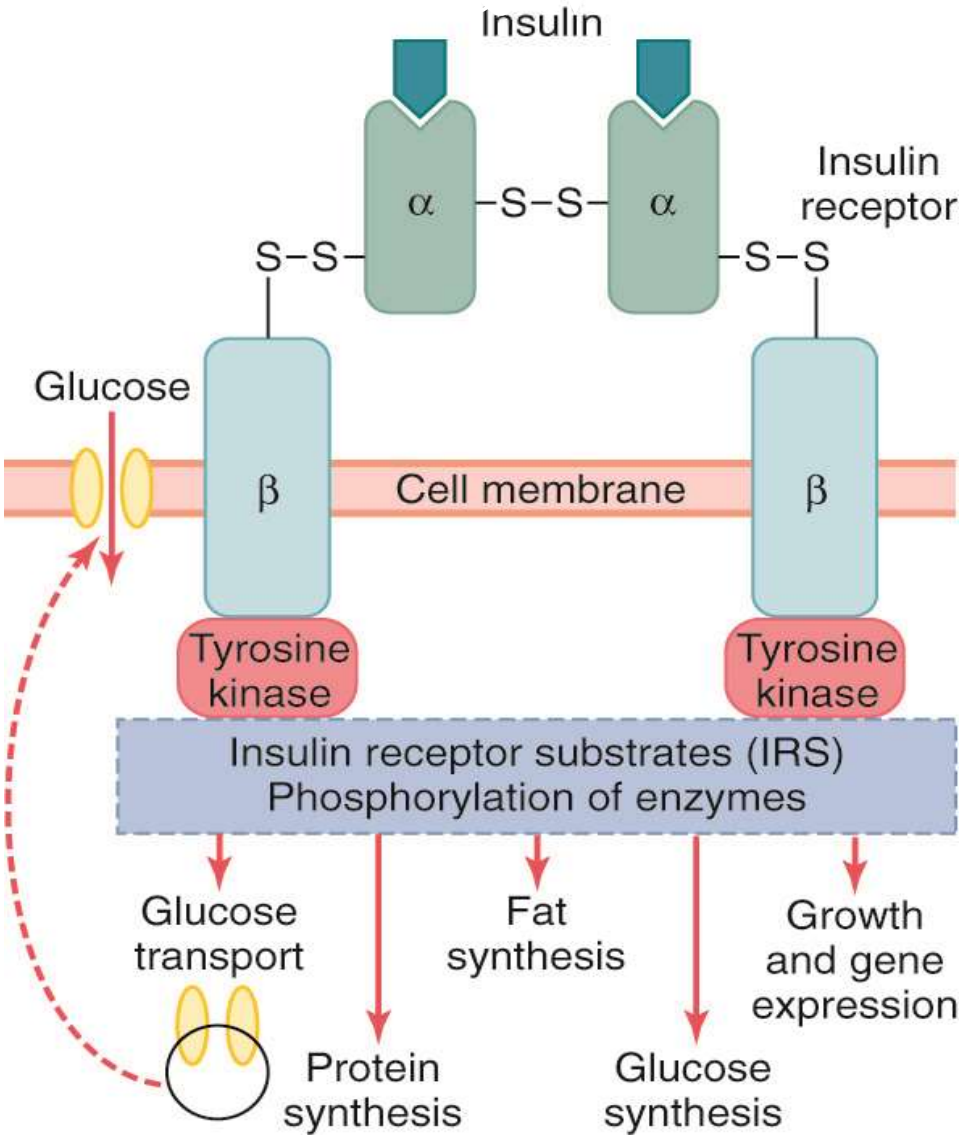
Figure 15–55. Molecular Biology of the Cell, 4th Edition.

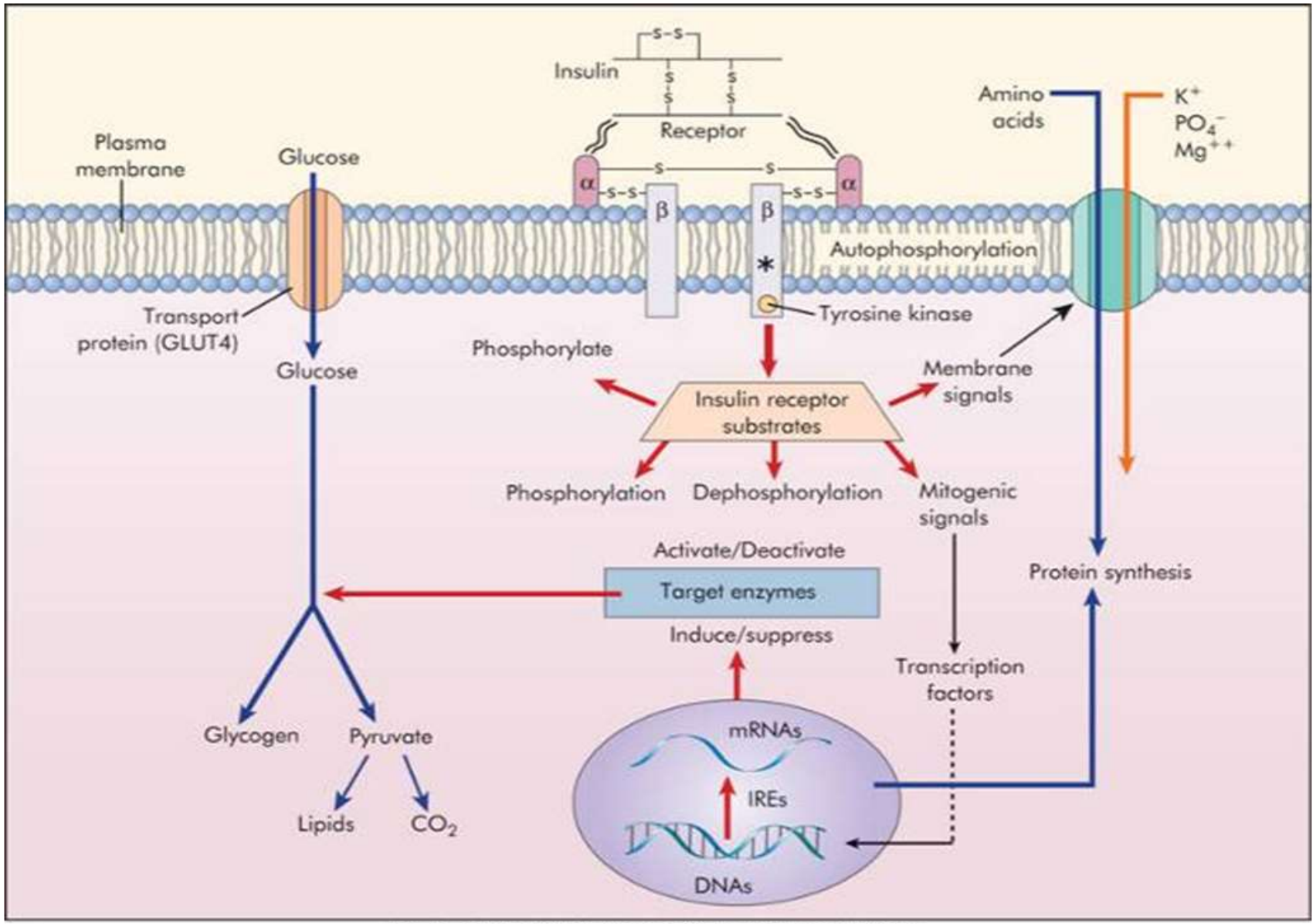
Tyrosine Kinase

- Insulin receptor consists of 2 units that dimerize when they bind with insulin.
  - Insulin binds to ligand–binding site on plasma membrane, activating enzymatic site in the cytoplasm.
- Autophosphorylation occurs, increasing tyrosine kinase activity.
- Activates signaling molecules.
  - Stimulate glycogen, fat and protein synthesis.
  - Stimulate insertion of GLUT-4 carrier proteins.

Tyrosine Kinase

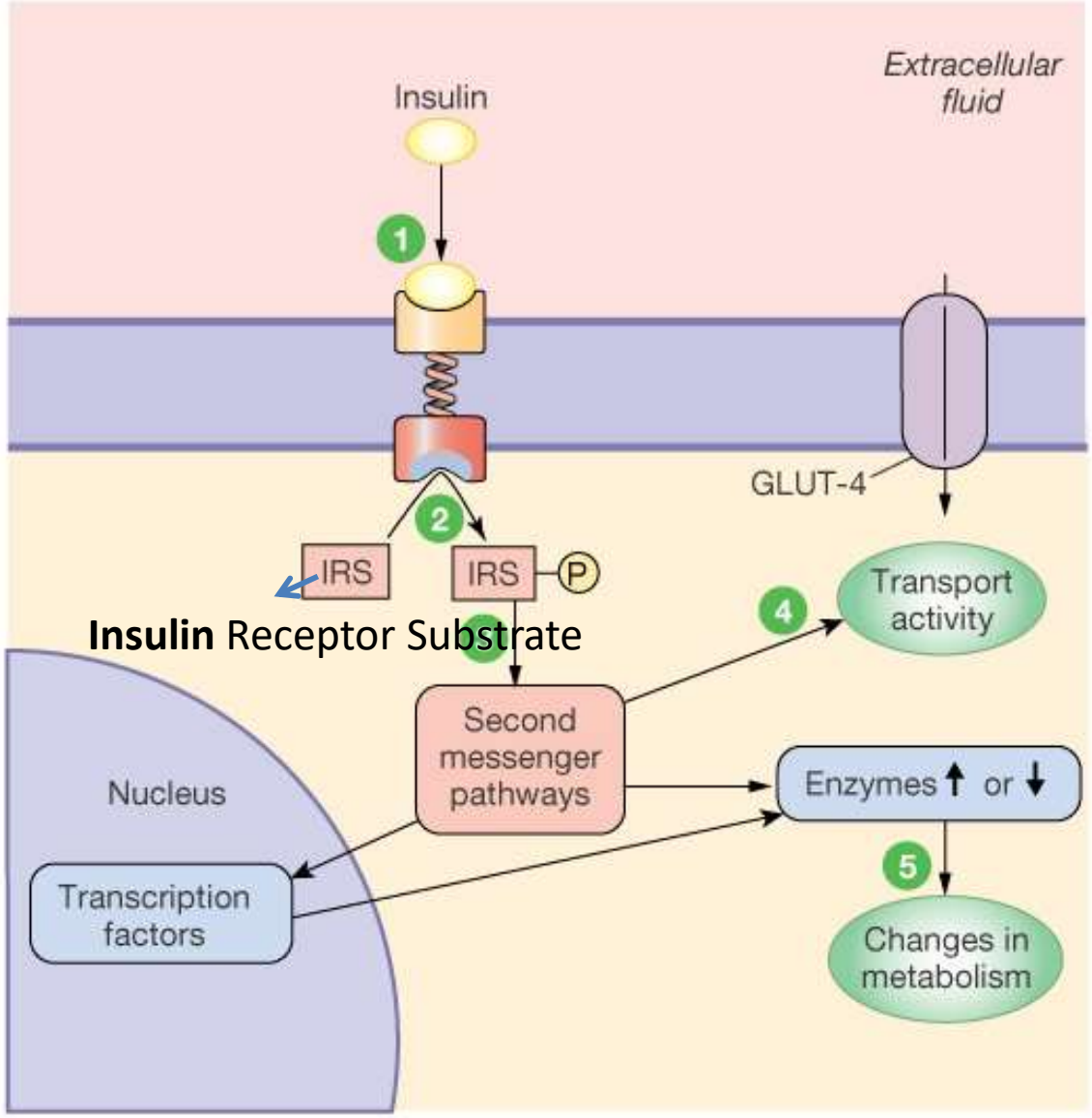






From Berne RM, Levy MN: *Principles of physiology*, ed 3, St Louis, 2000, Mosby.

Insulin Action on Cells:



1. Insulin binds to tyrosine kinase receptor.
2. Receptor phosphorylates insulin-receptor substrates (IRS).
3. Second messenger pathways alter protein synthesis and existing proteins.
4. Membrane transport is modified.
5. Cell metabolism is changed.

Signaling Overview

3. Three major classes of surface receptors for signaling, cont.:

C. Enzyme-linked receptors, cont.:

2. TKs non-covalently associated with receptor (includes cytokine receptors, T & B cell receptors) = NRTKs

Cytokine receptors, as well as T and B cell receptors, stimulate tyrosine kinases that are non-covalently associated with receptor.

A. Overview

1. **N-term. extracell. ligand-binding domain, transmemb α helix,C-term. cytosolic domain**
2. **Cytosolic domain has no catalytic (kinase) activity**
3. **Acts in conjuction with a non-receptor tyrosine kinase that is activated as a result of ligand binding.**
4. **Activation is similar to that of RTKs: ligand binding causes cross phosphorylation of associated tyrosine kinases that phosphorylate the receptor, providing phosphotyrosine binding sites for recruitment of proteins with SH2 domains.**

This example is not required

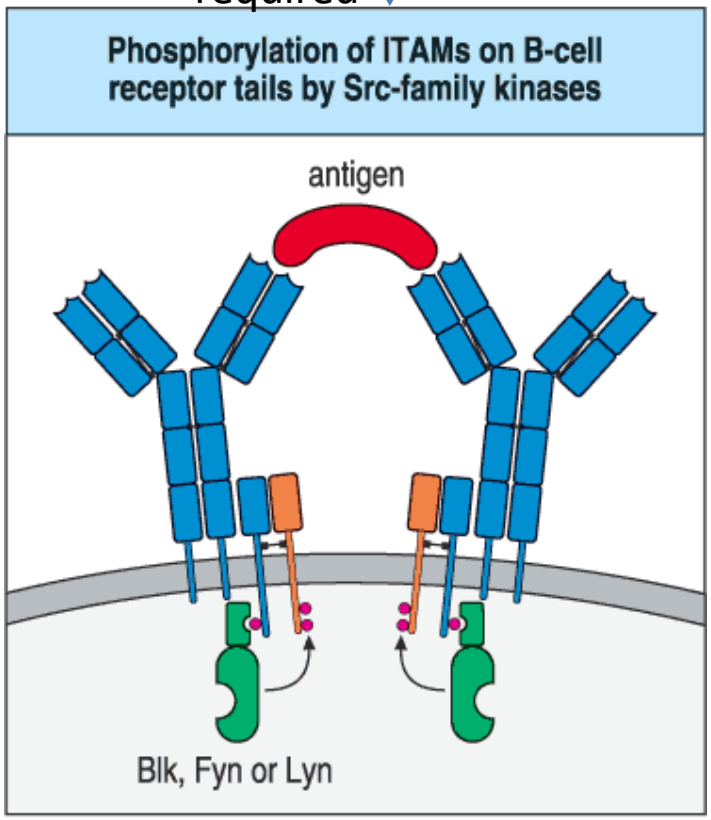


Fig 6.9 © 2001 Garland Science

From Janeway, Immunobiology, 5th edition

3. Three major classes of surface receptors for signaling, cont.:

C. Enzyme-linked receptors, cont.:

B. **Two kinds of kinases associate with NRTKs:**

1. Src family protein kinases - important for B and T cell signaling (not requiredd)

2. Janus kinases (JAK) - universally required for signaling from cytokine receptors. (Leptin) (required example)

C. Receptors can be linked to or associated with other enzymes, besides TKs, i.e.

Protein-tyrosine phosphatases (remove phosphates, thereby terminate signals initiated by protein-tyrosine kinases).

Serine/ threonine kinases, i.e. TGF-β (required example)

Guanylyl cyclases:(required example)

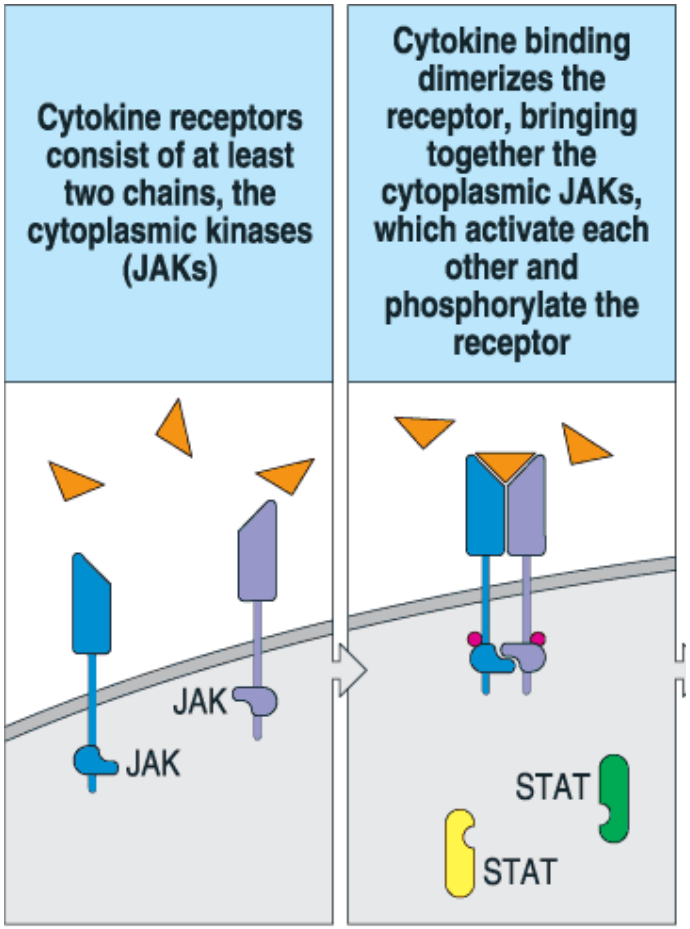
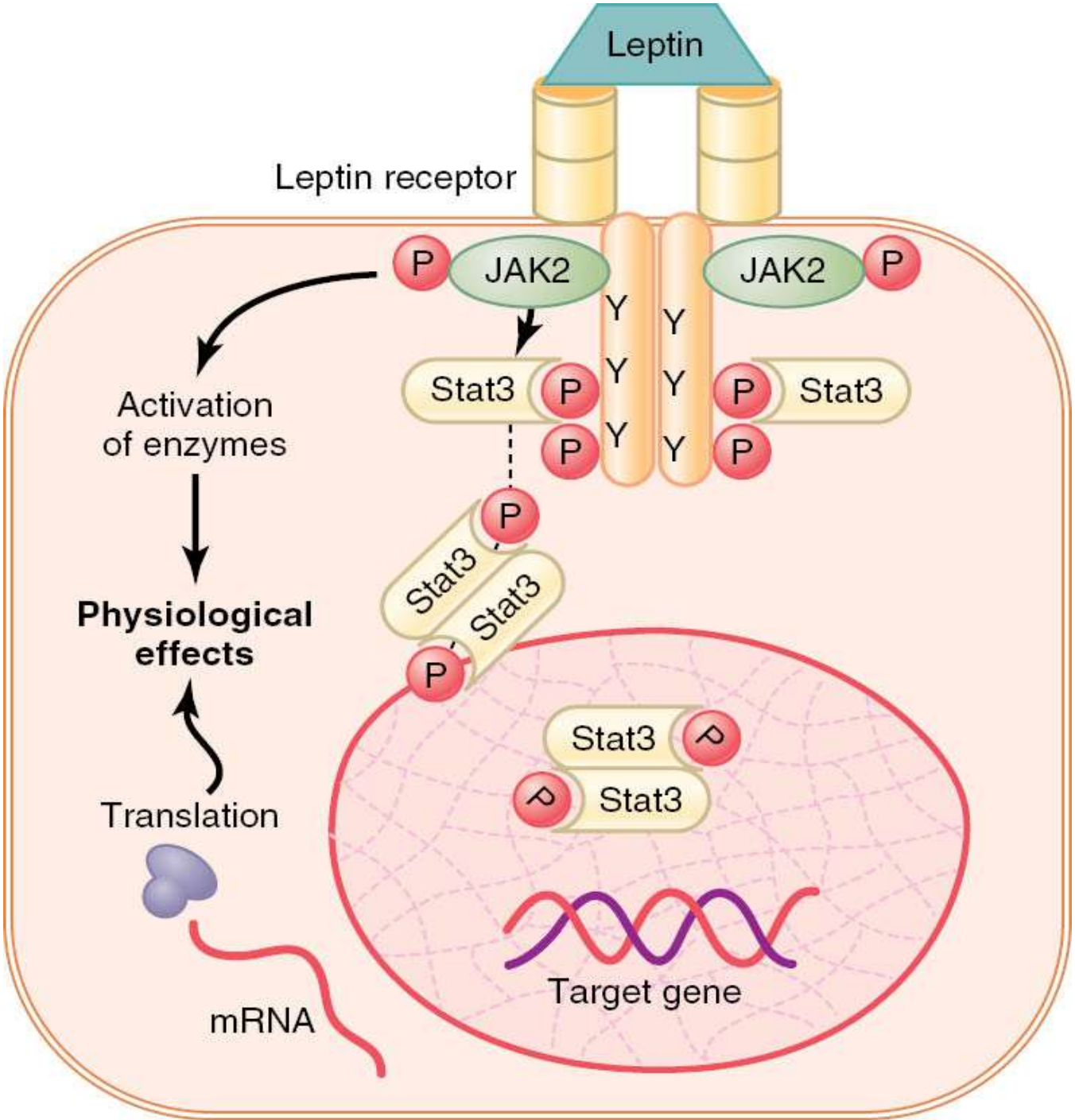


Fig 6.22 © 2001 Garland Science

From Janeway, Immunobiology, 5th edition

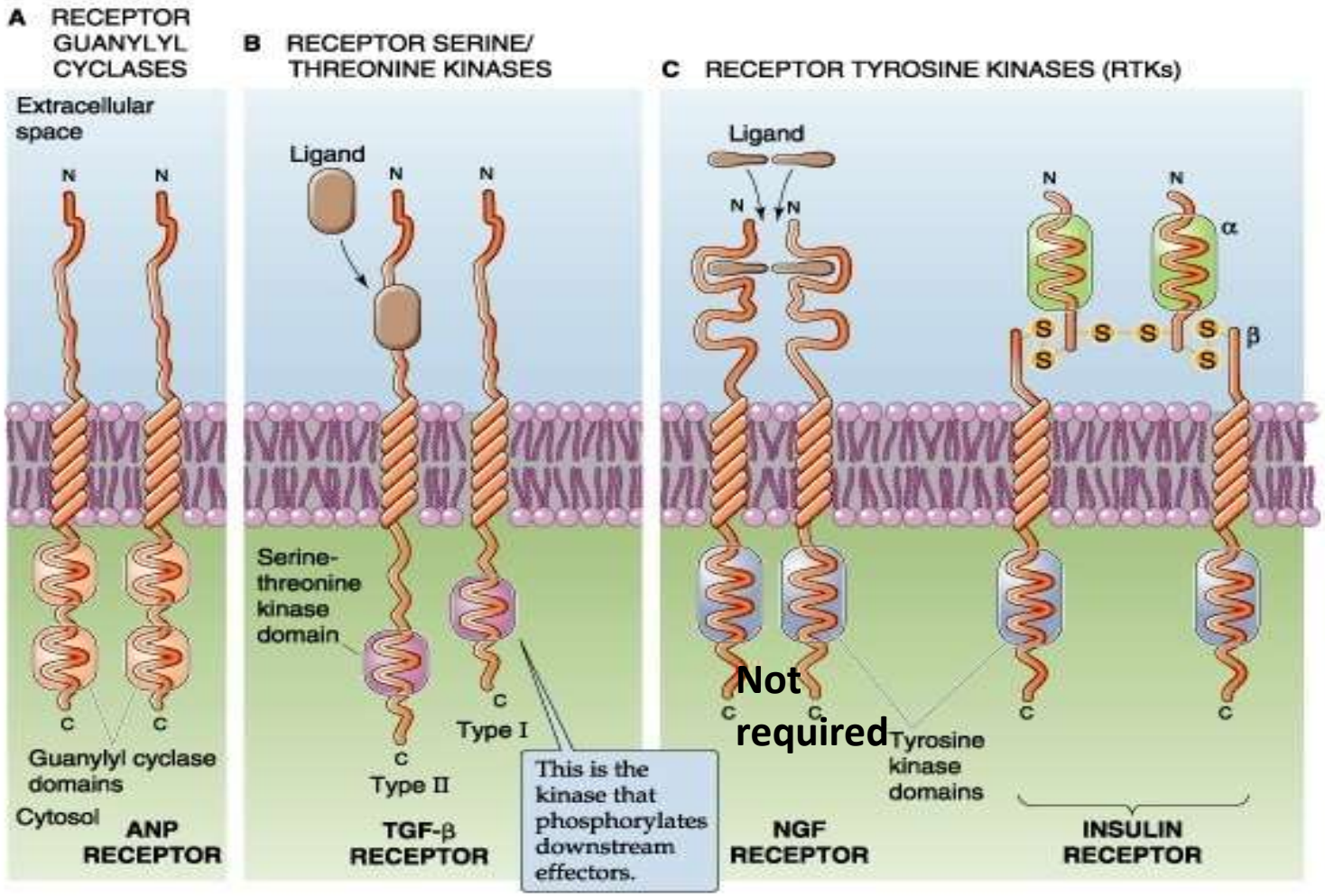
Enzyme-linked Receptor (the Leptin receptor)
JAK= Janus Kinase
STAT= Signal Transducer and Activator of Transcription



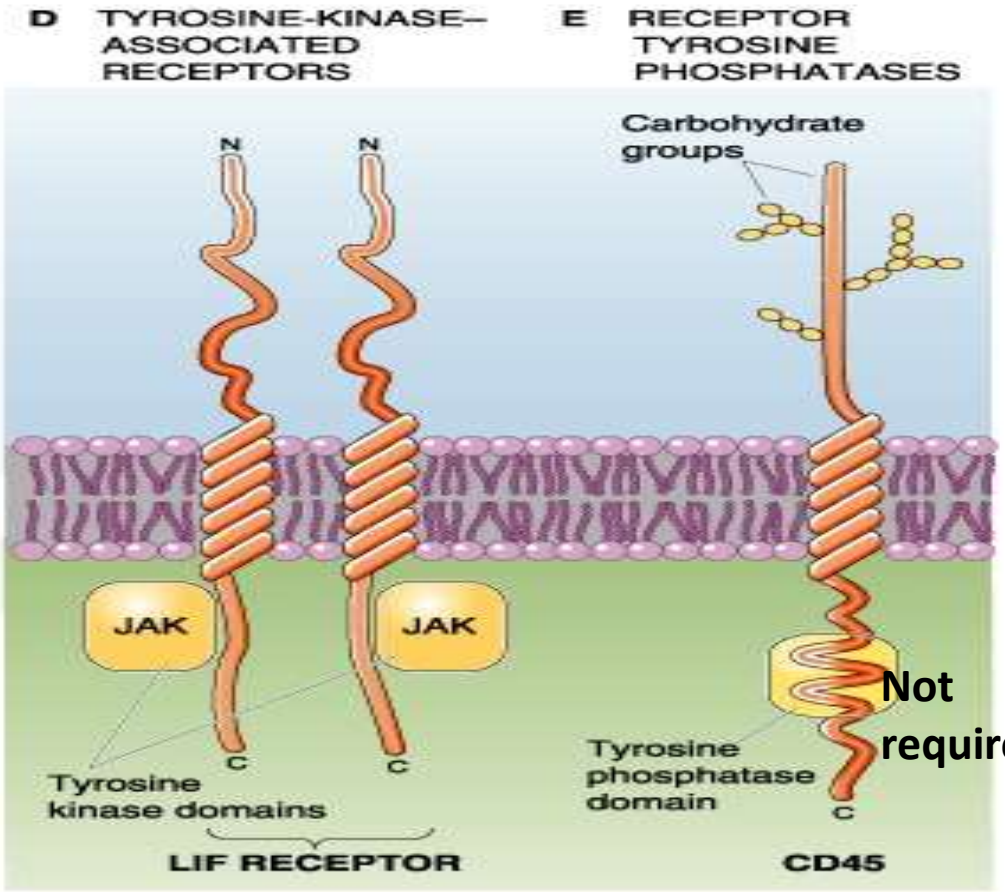
Enzyme-linked receptor (the leptin receptor)

- The receptor exists as a homodimer (two identical parts)
- Leptin binds to the extracellular part of the receptor
- This causes activation of the intracellular associated janus kinas 2
- This causes phosphorylation of signal transducer and activator of transcription (STAT) proteins
- This then activates the transcription of target genes and synthesis of proteins
- JAK 2 phosphorylation also activates several other enzyme systems that mediate some of the more rapid effects of leptin

Tyrosine Kinase



Tyrosine Kinase



Not required

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

A. cAMP:

i. Production:

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

Degraded by cAMP phosphodiesterase

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 proteins in cytoplasm:

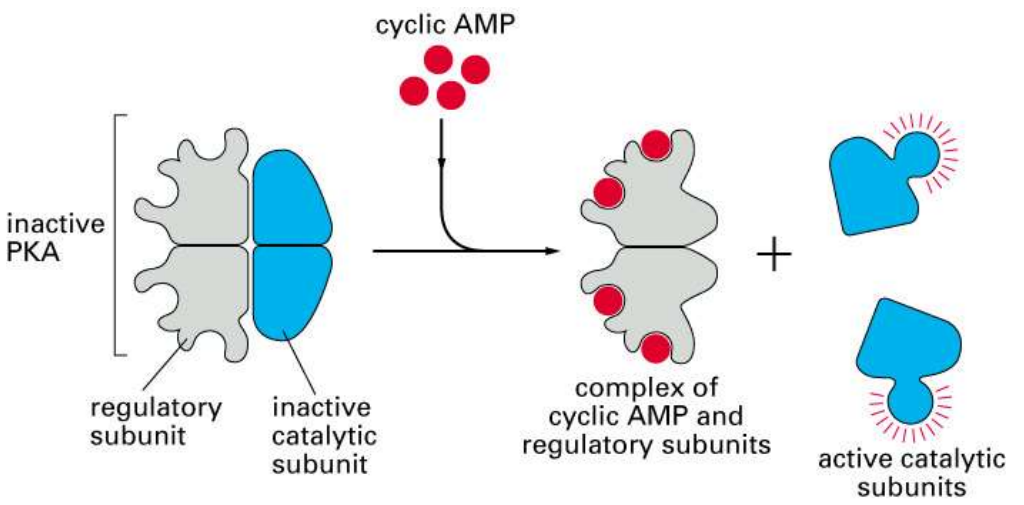


Figure 15–32. Molecular Biology of the Cell, 4th Edition.

cAMP production:

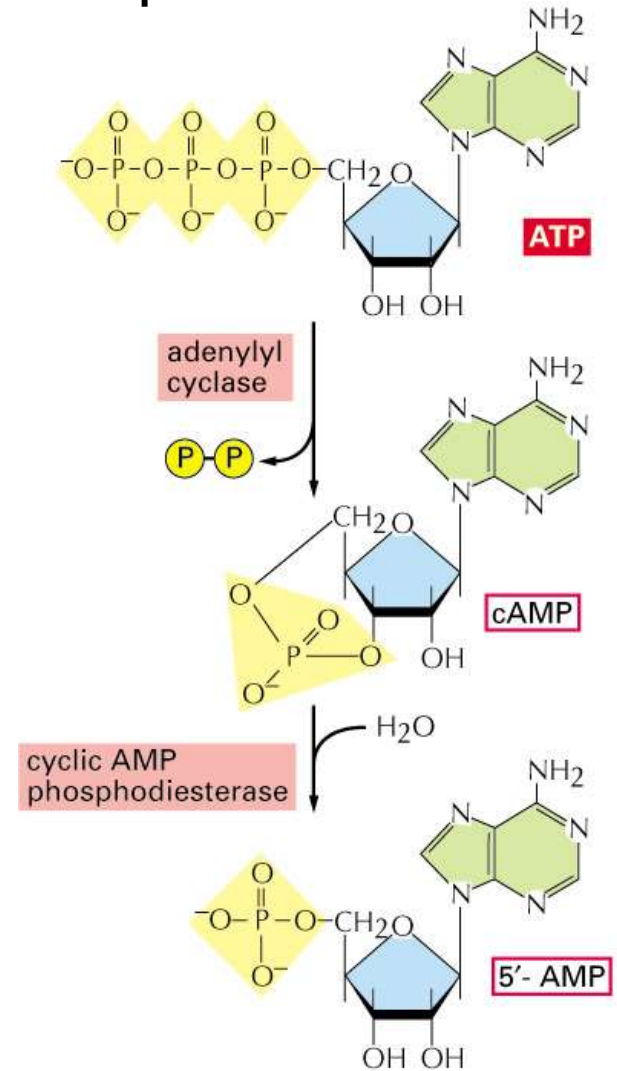


Figure 15–31. Molecular Biology of the Cell, 4th Edition.

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.**

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.**

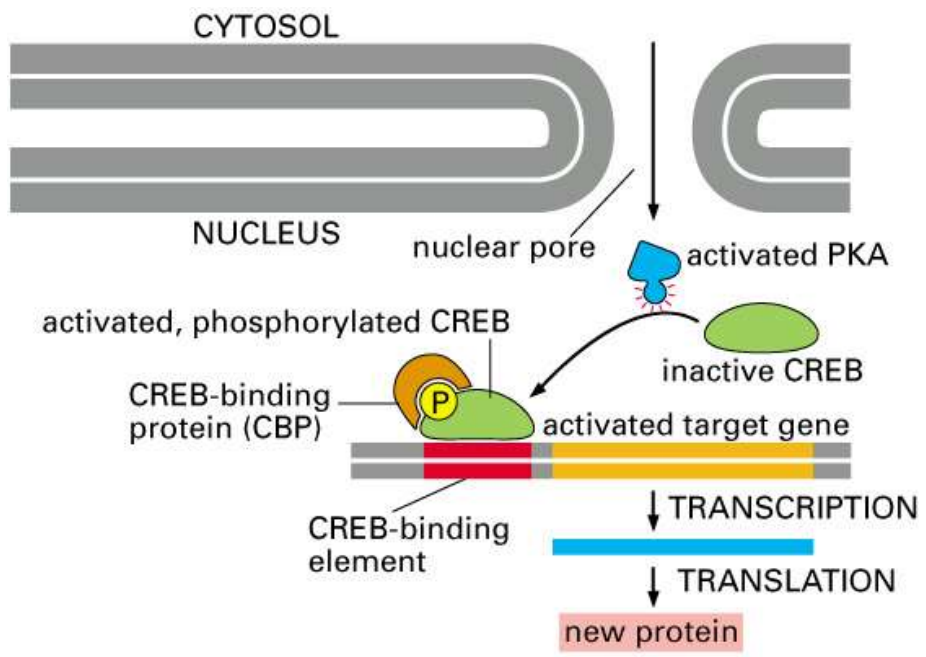


Figure 15–33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

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.

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. This causes ion channels that export chloride to produce a net effux of Cl- and water, leading to severe diarrhea characteristic of cholera.

B. cGMP:

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

Summary of how cAMP activates transcription:

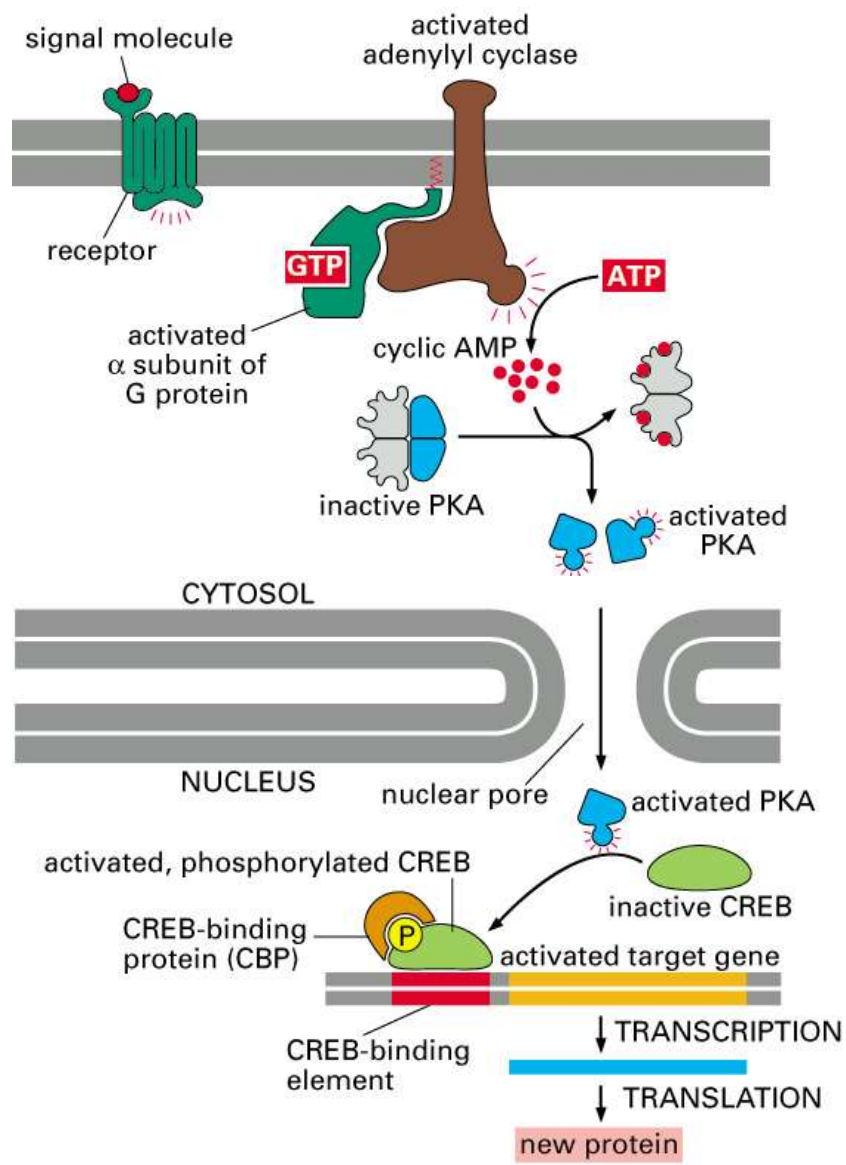
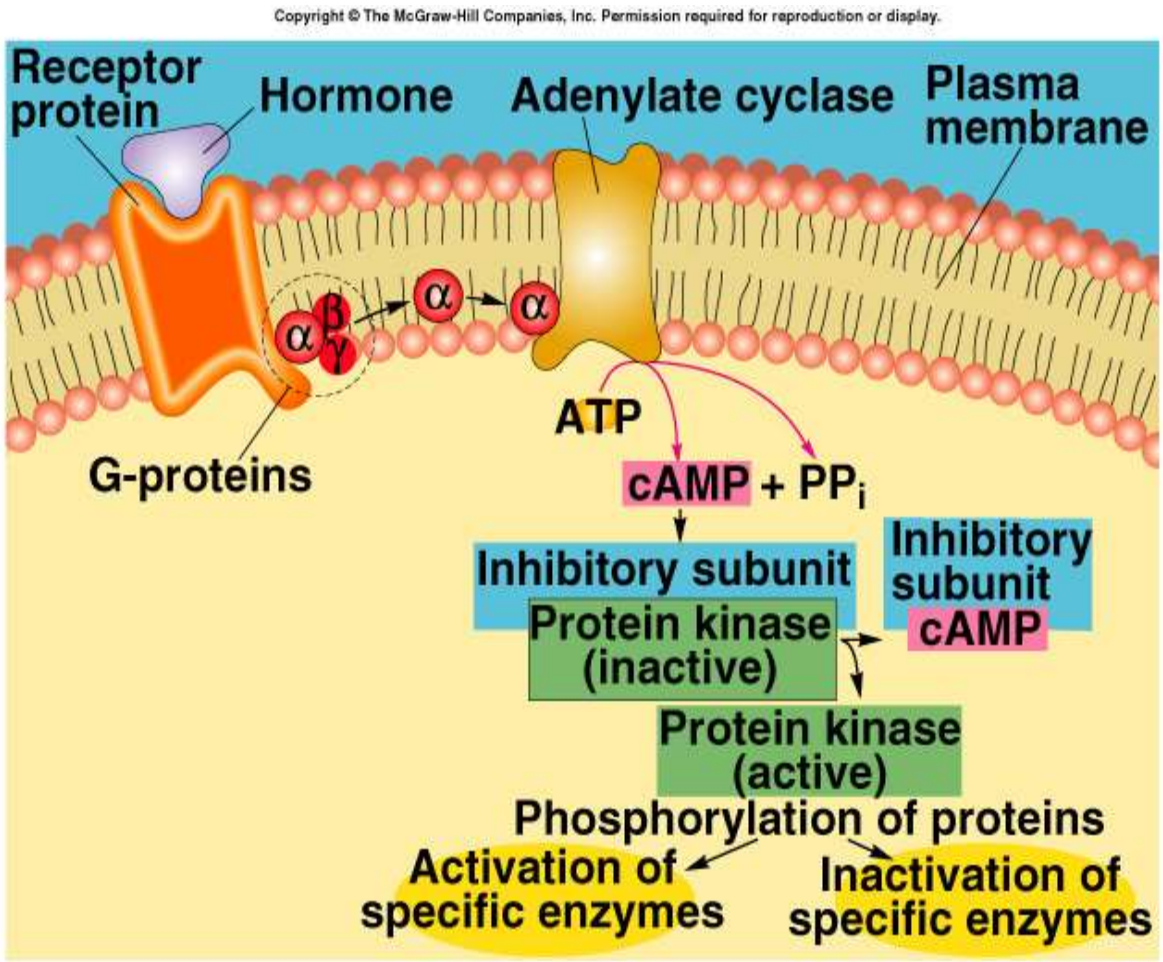


Figure 15–33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

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.

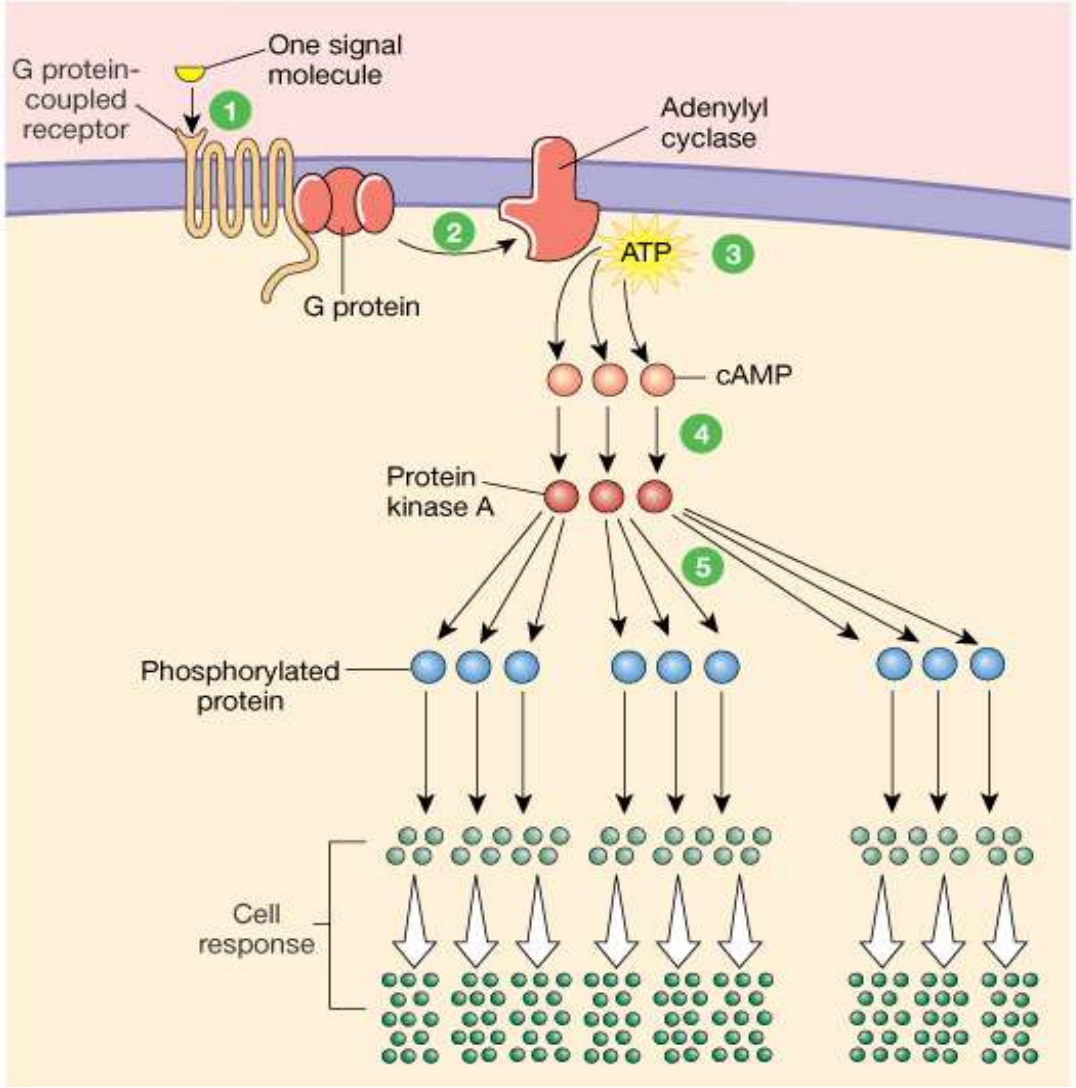


Not for memorization, just see the different cAMP mediated processes by different hormones

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

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**

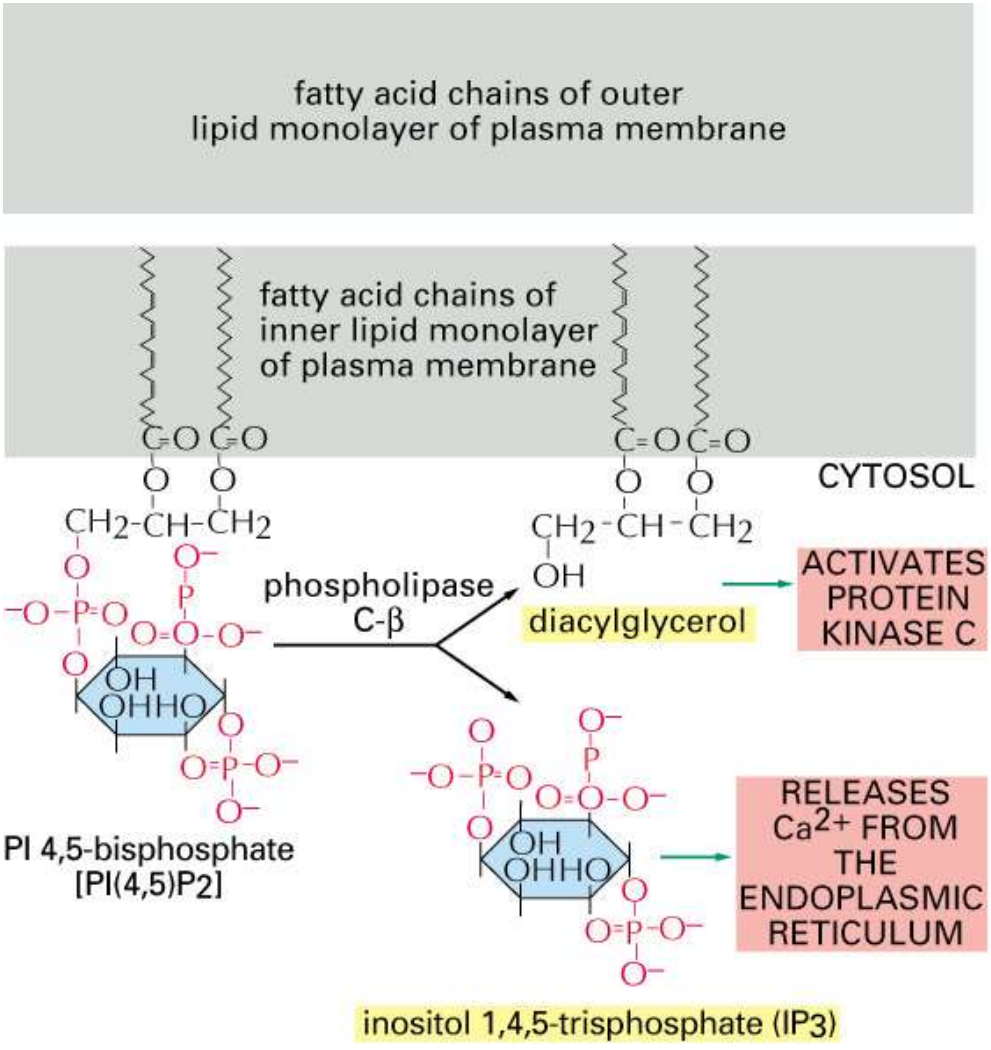
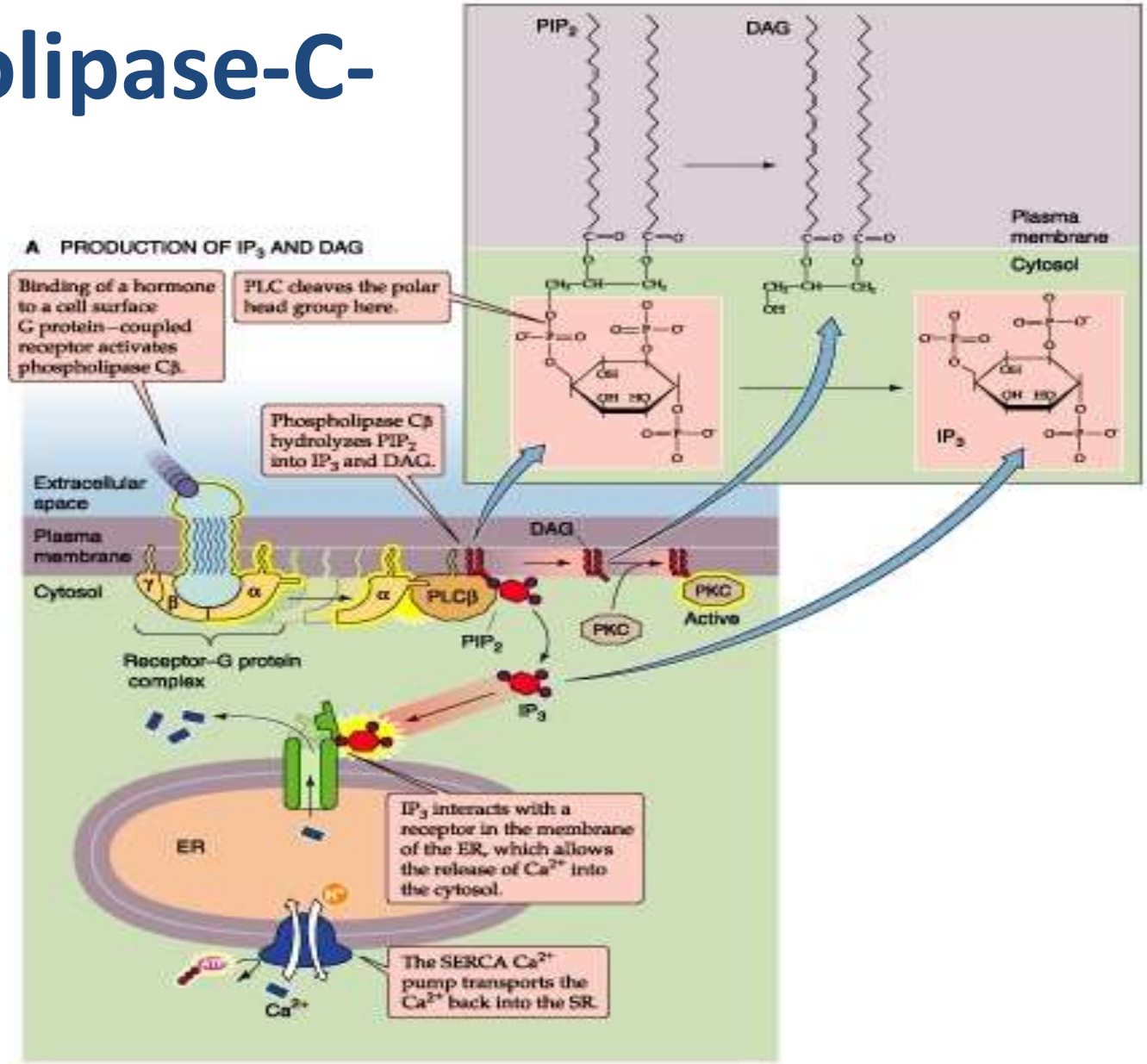


Figure 15–35. Molecular Biology of the Cell, 4th Edition.

Phospholipase-C-Ca^{2+}



Signaling Overview

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)**

b. **Can also be cleaved to form another messenger, eicosanoids, which include prostaglandins**

c. **Tumor producing phorbol esters mimic DAG and thereby stimulate protein kinase C**

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**

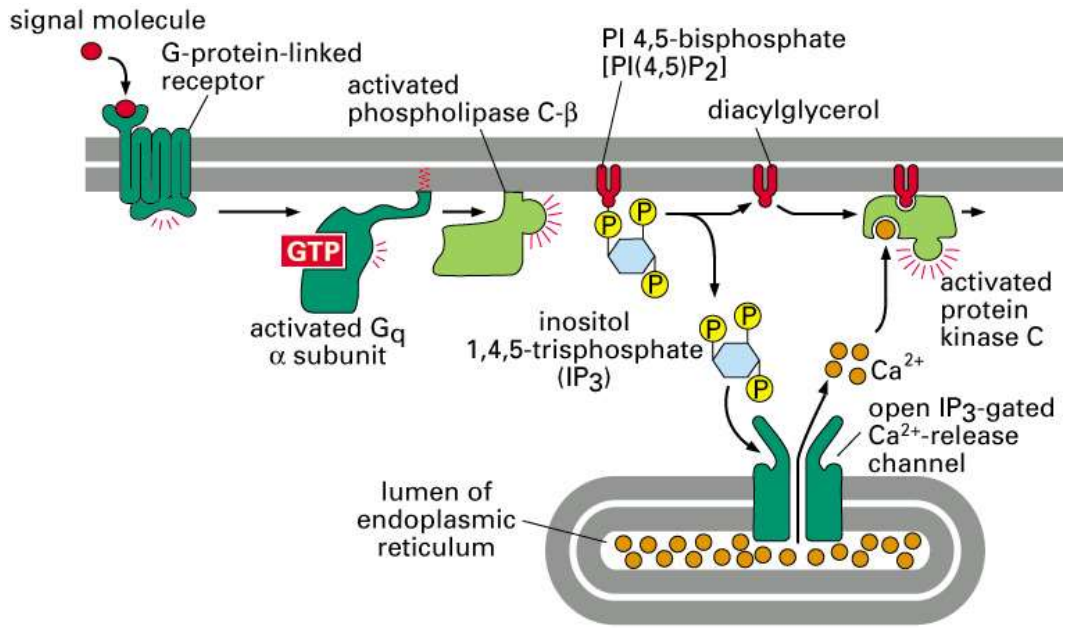
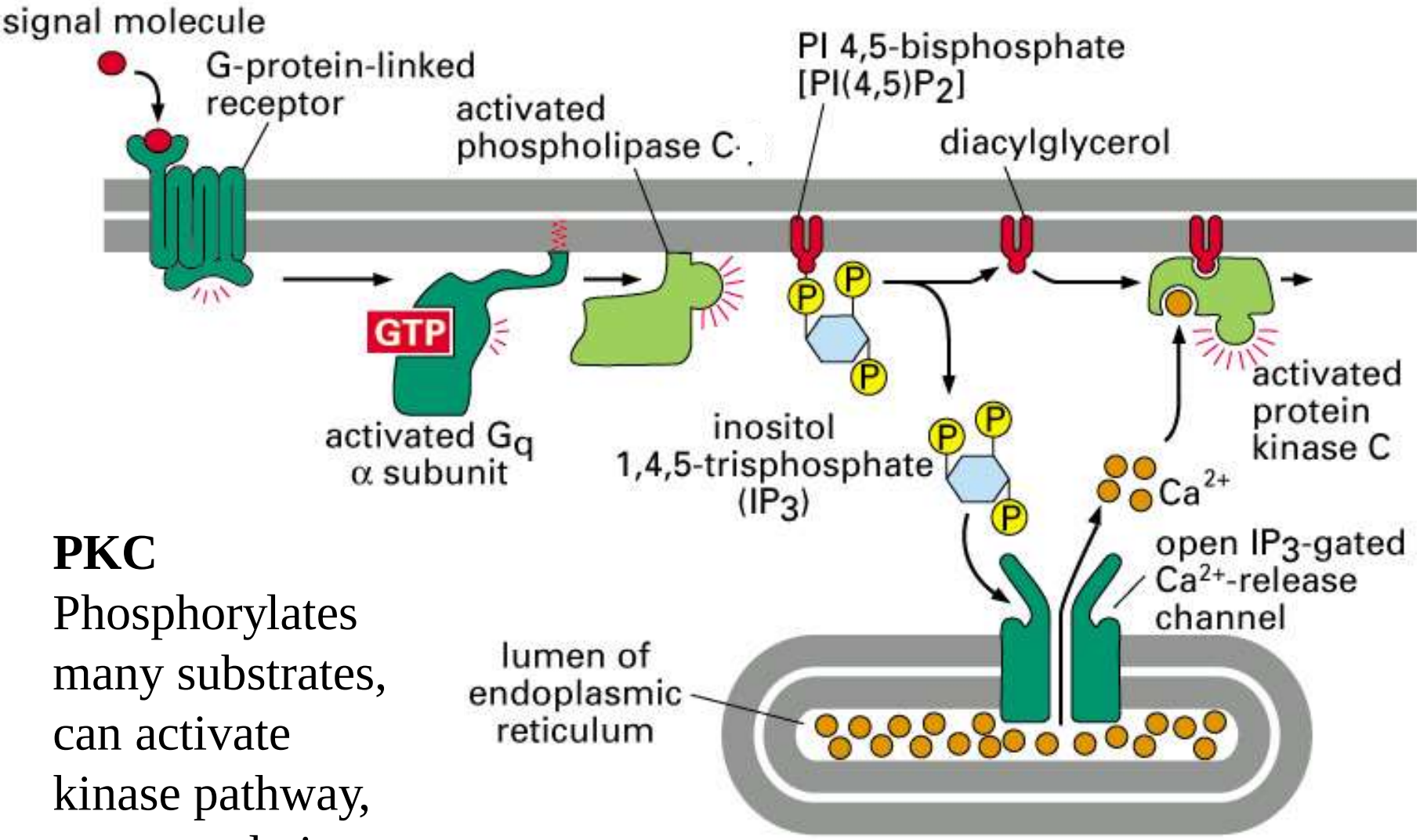


Figure 15–36. Molecular Biology of the Cell, 4th Edition.

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.

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.

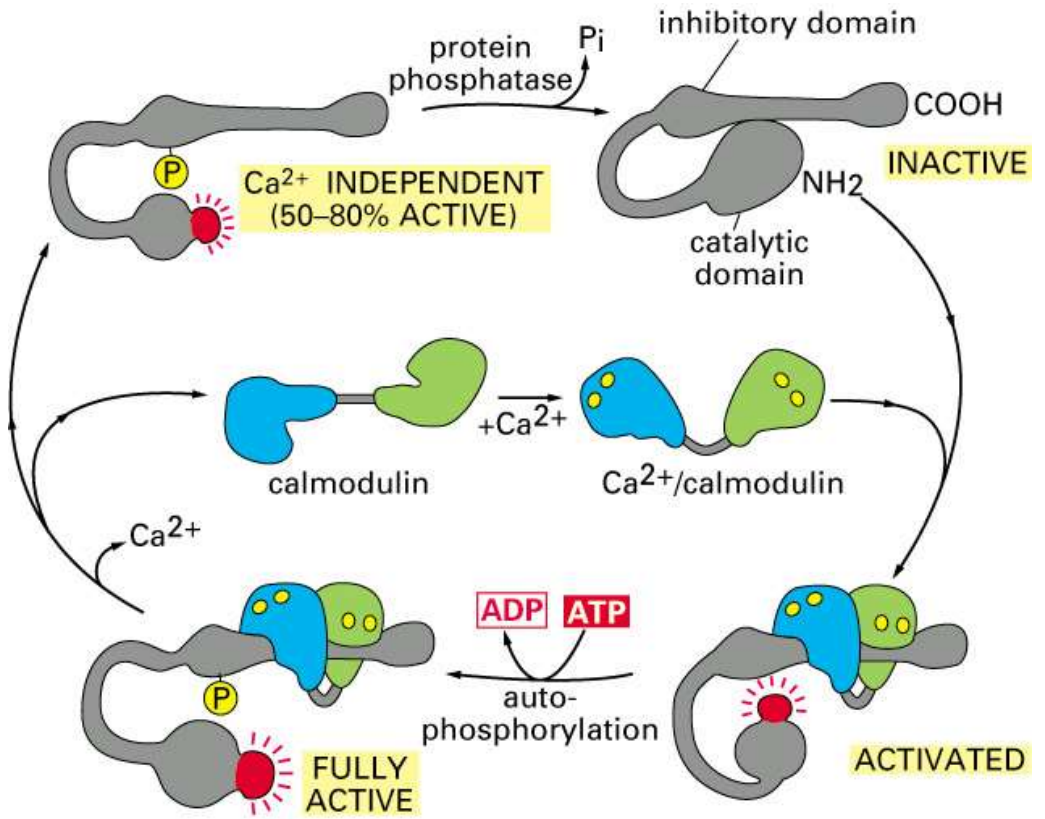
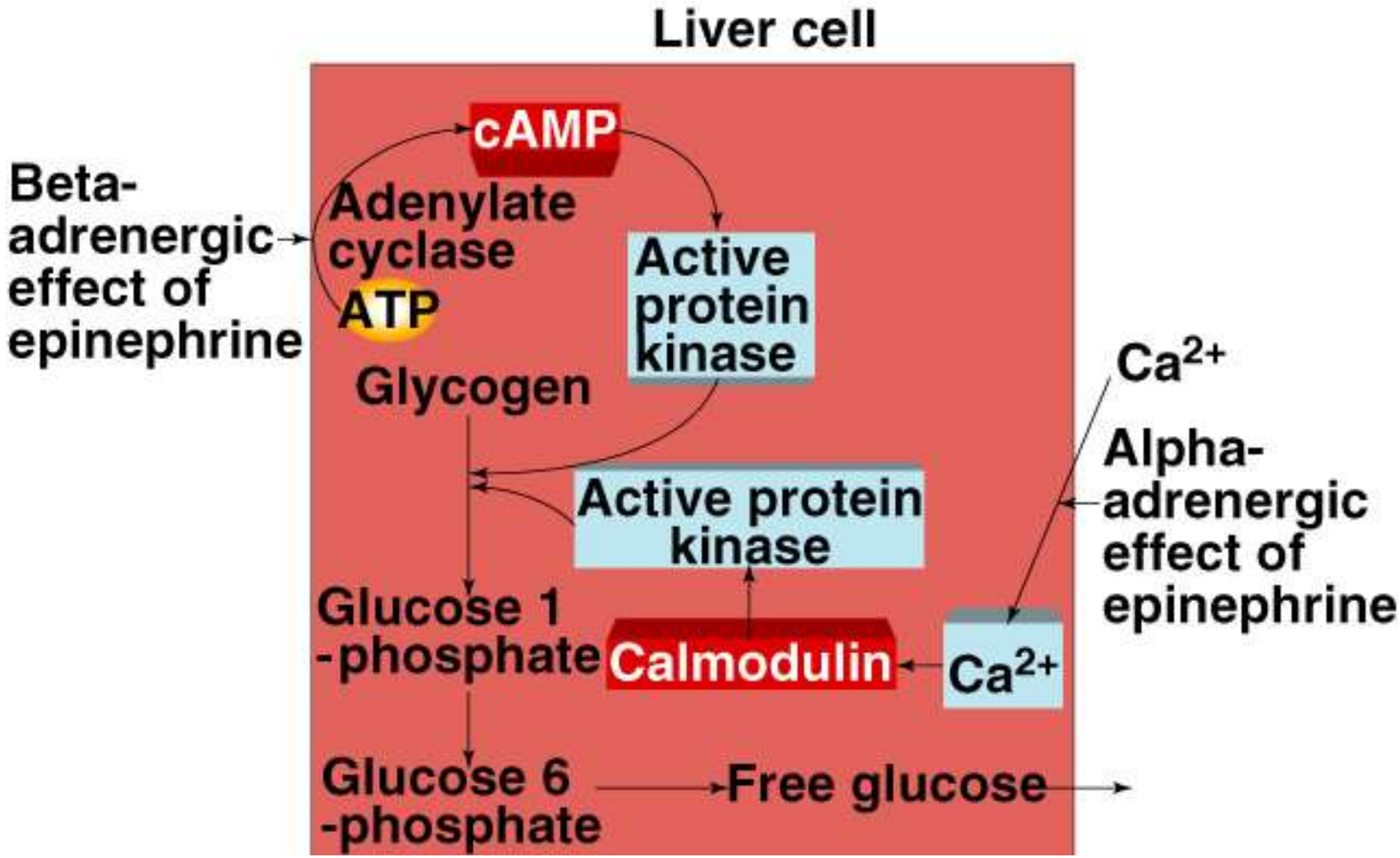


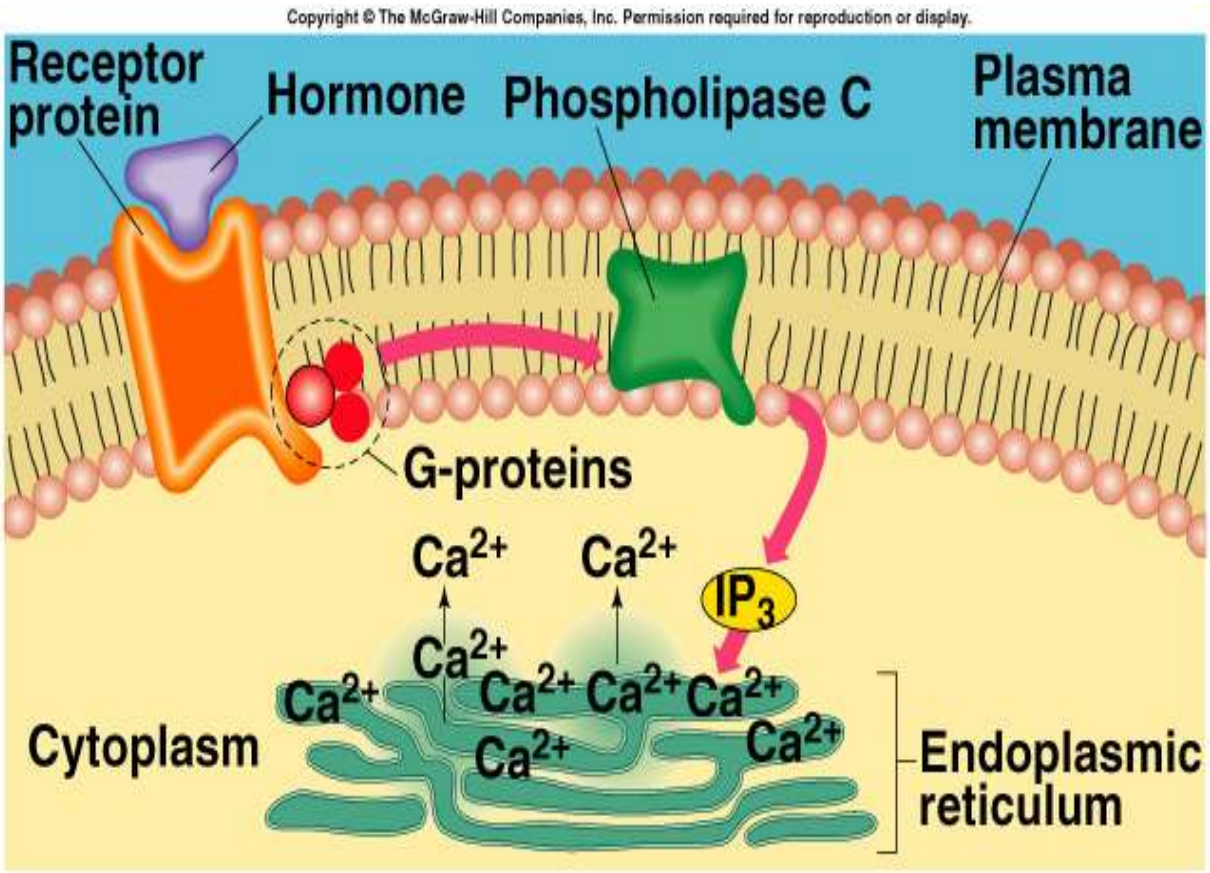
Figure 15–41. Molecular Biology of the Cell, 4th Edition.

Epinephrine Can Act Through Two 2nd Messenger Systems

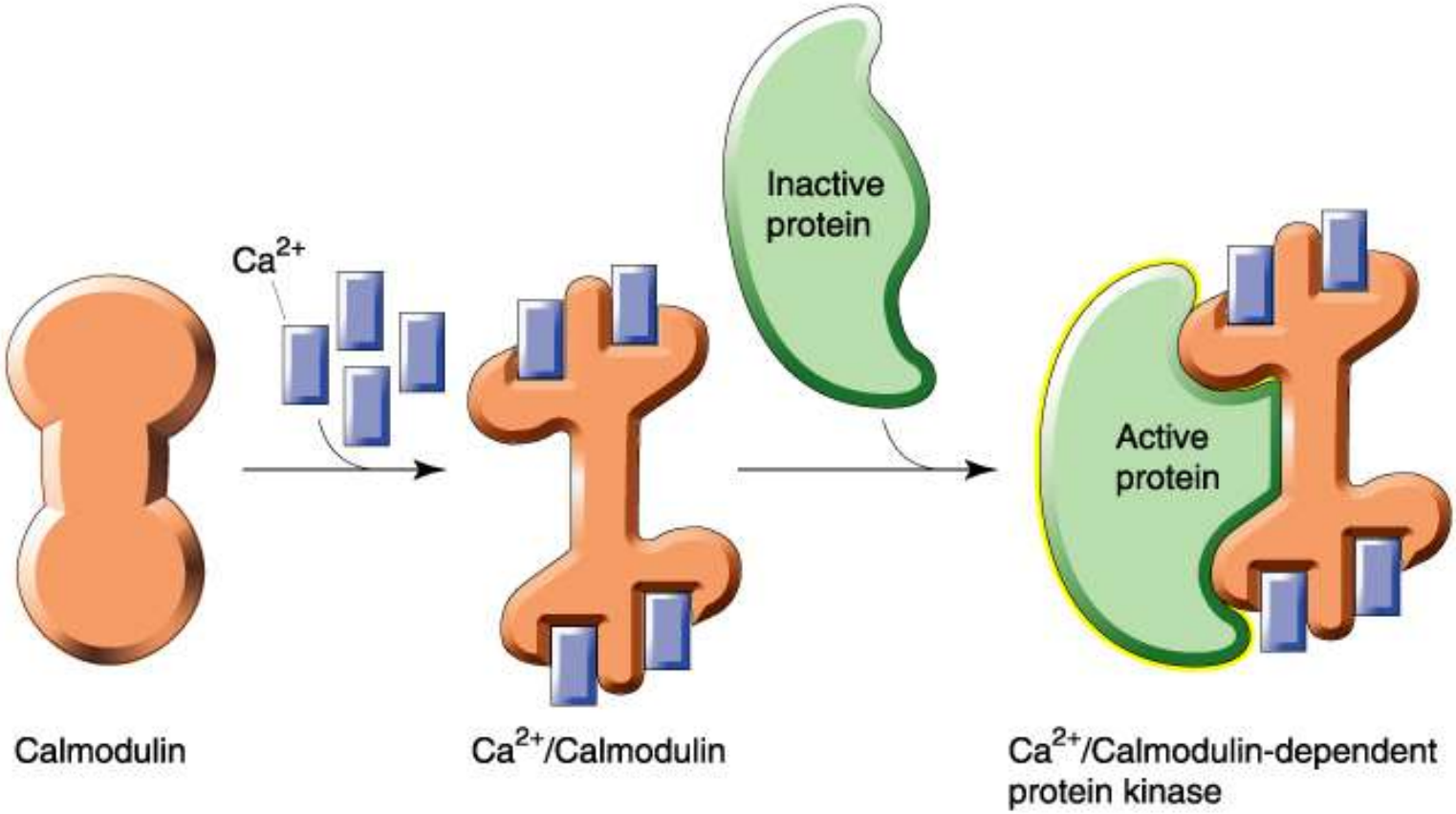


Ca^{2+}- Calmodulin (continued)

- 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)

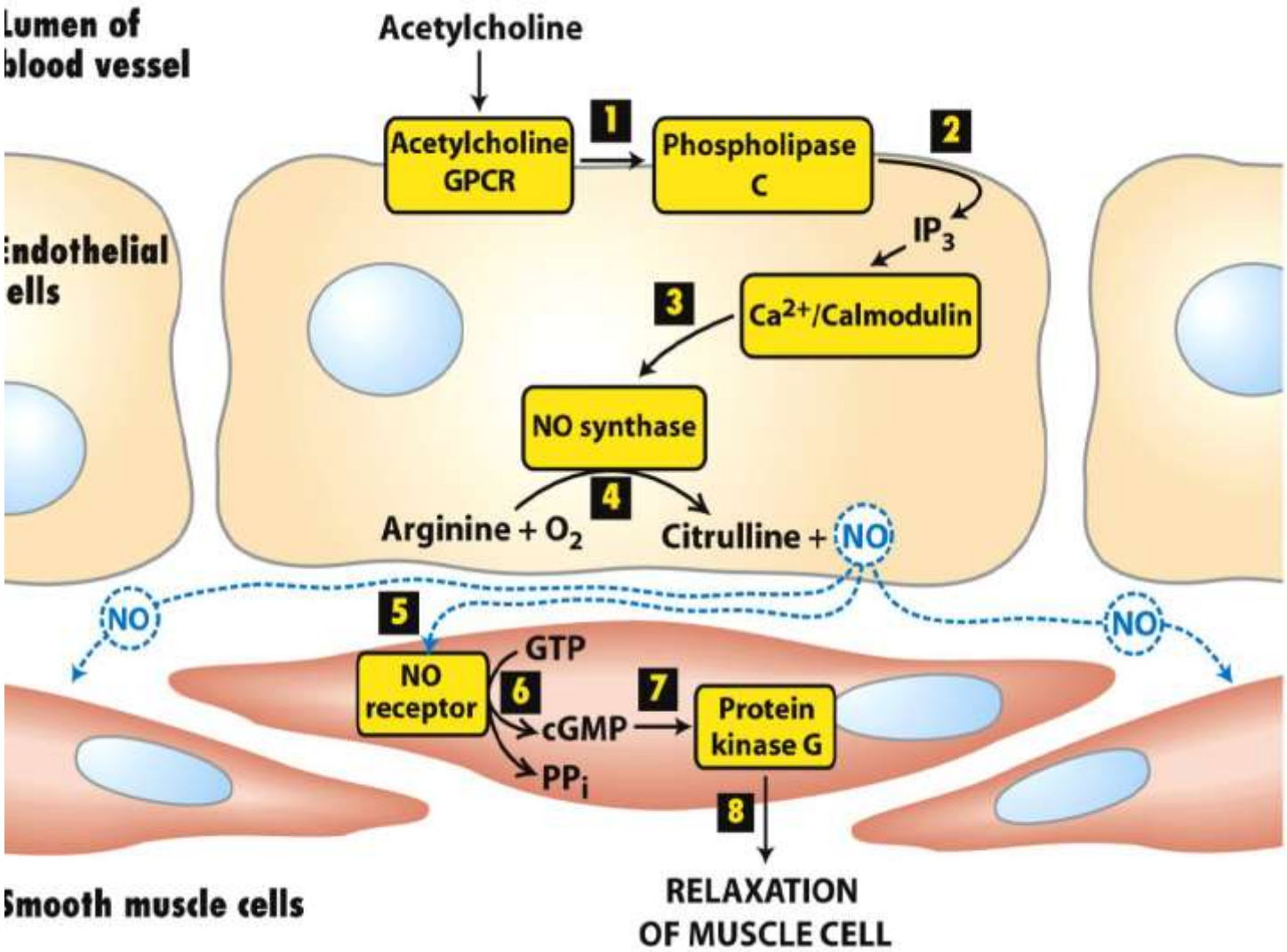


Guanylate cyclase (GC) receptor

Membrane receptor –ANP

Soluble receptor – NO, CO

NO signaling(required example)



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. Inhibition of apoptosis

just underlined info required in this slide

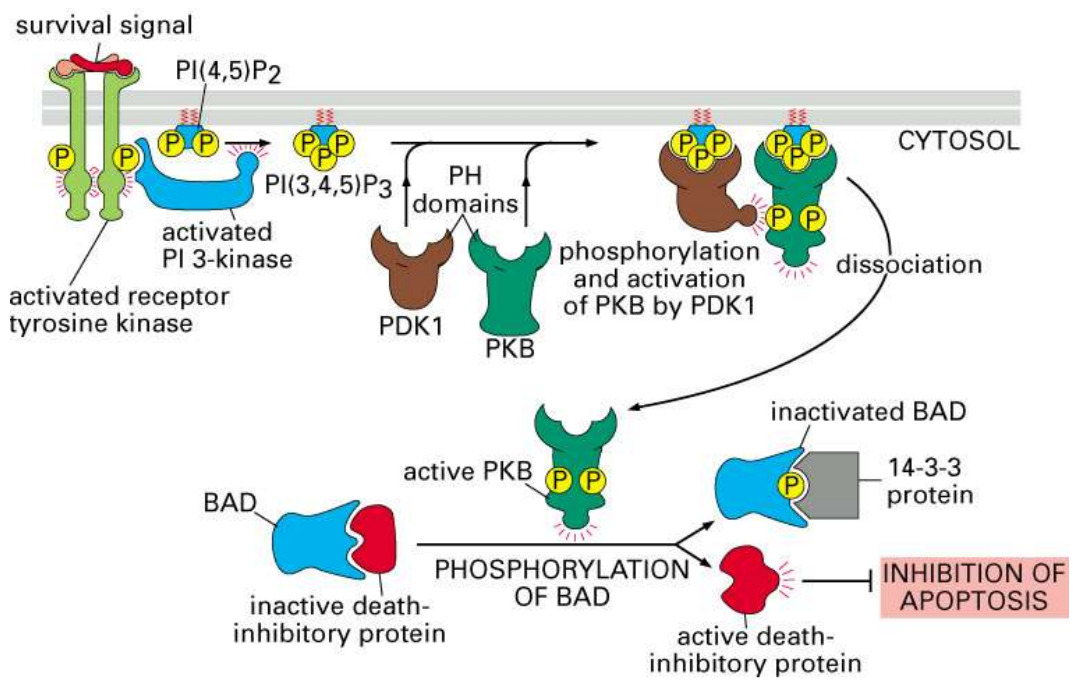


Figure 15–60. Molecular Biology of the Cell, 4th Edition.

Signaling Overview

5. Signaling Cascades, cont.:

5 downstream kinases activated by different signaling cascades

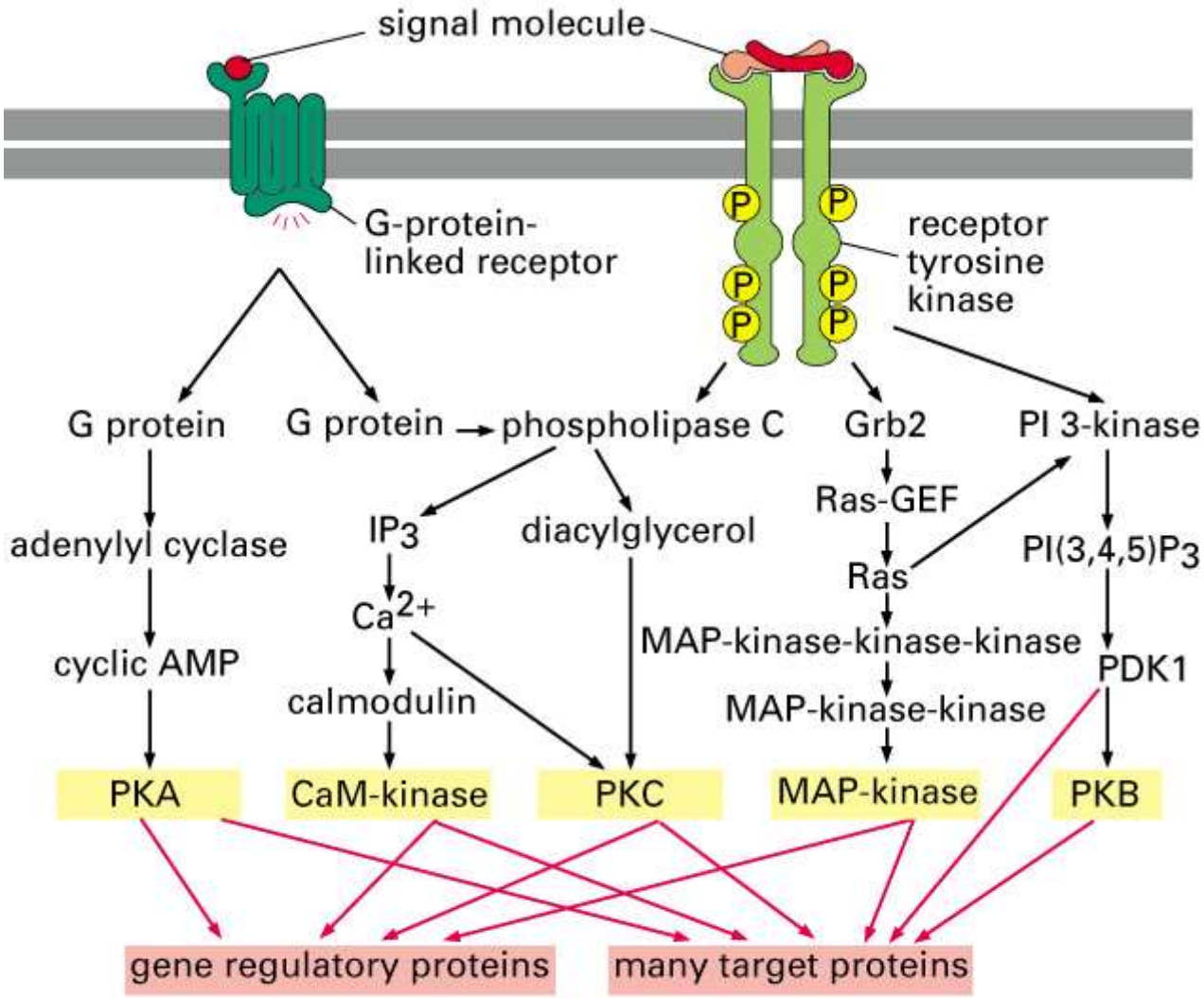
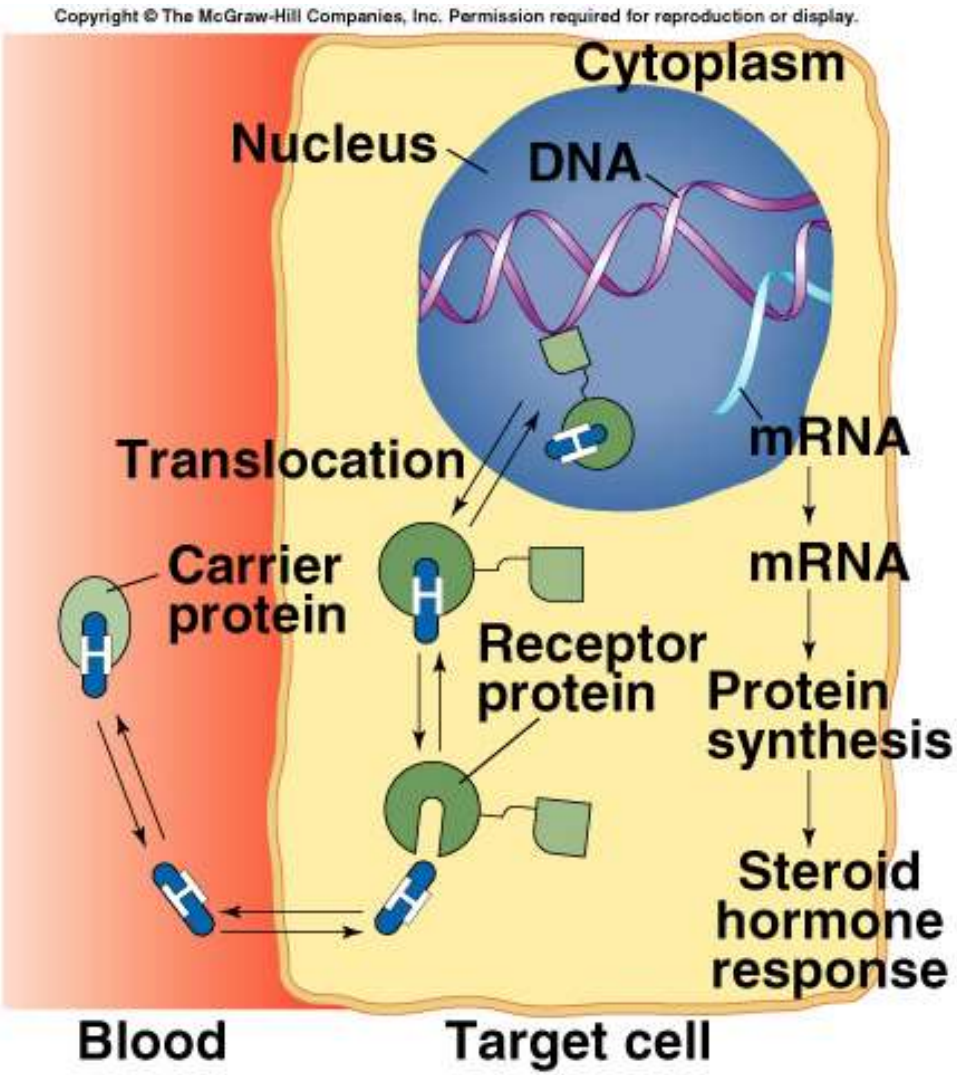


Figure 15–61. Molecular Biology of the Cell, 4th Edition.

Hormones That Bind to Nuclear Receptor Proteins

- 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.

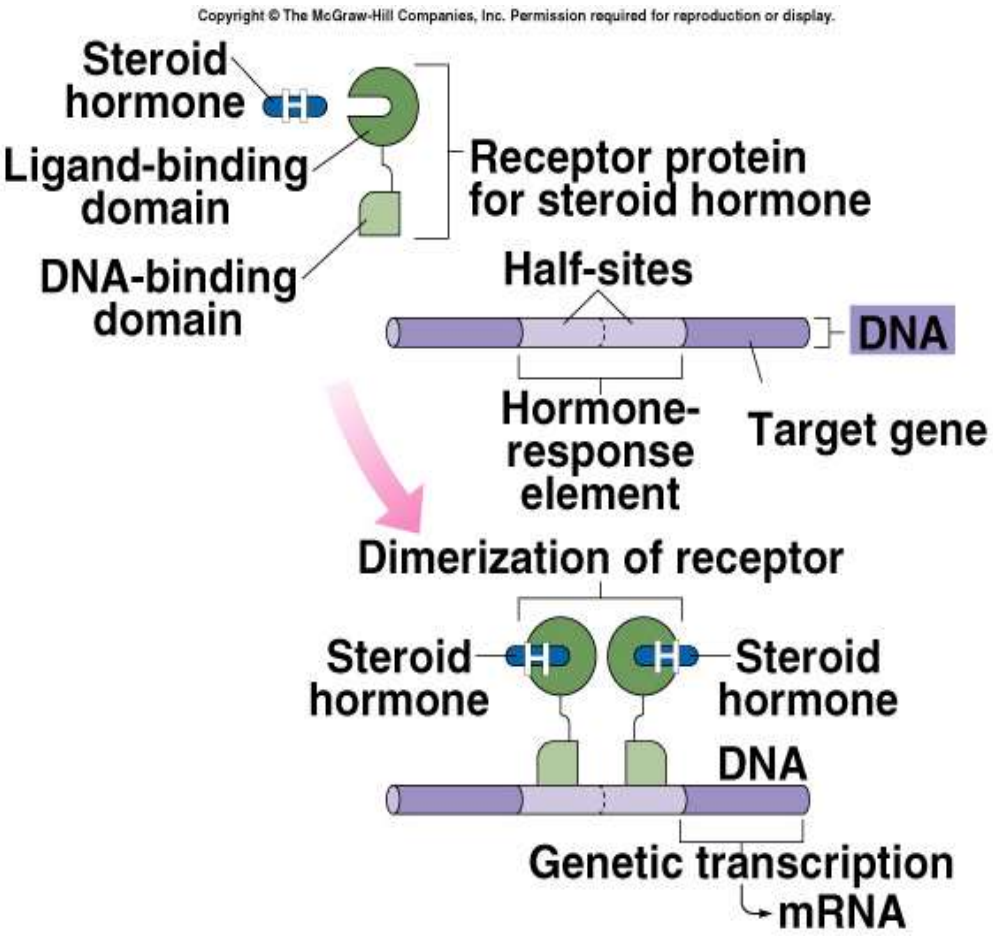


Nuclear Hormone Receptors

- 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.

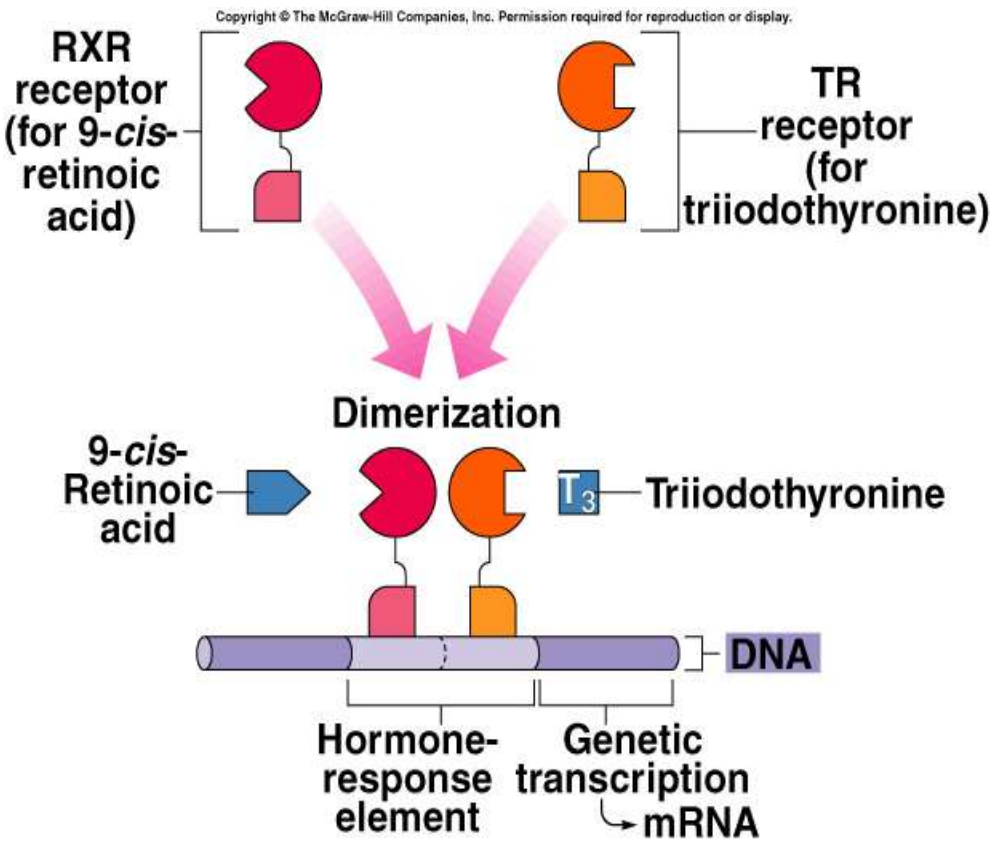
Mechanisms of Steroid Hormone Action

- 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.

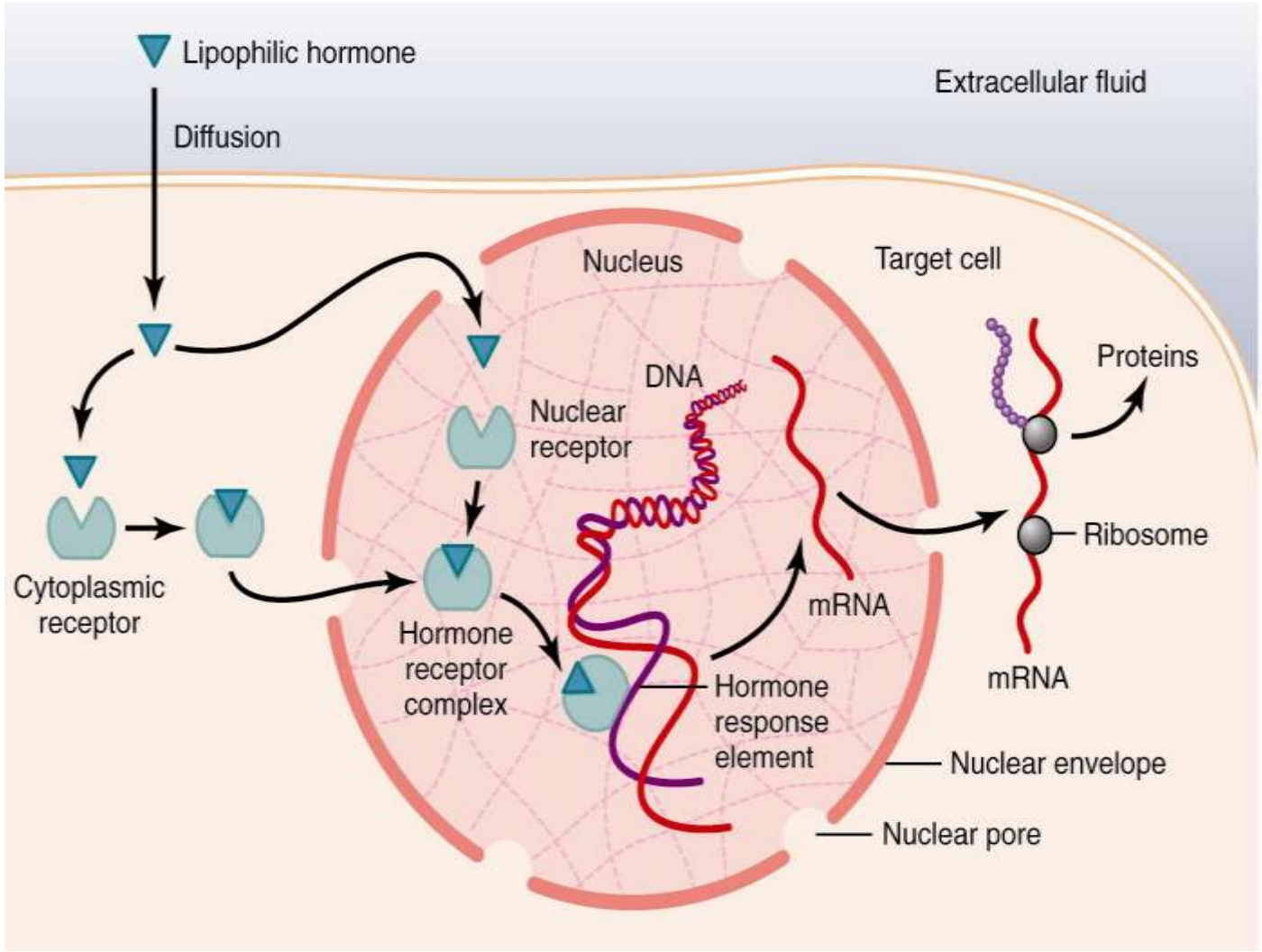


Mechanism of Thyroid Hormone Action

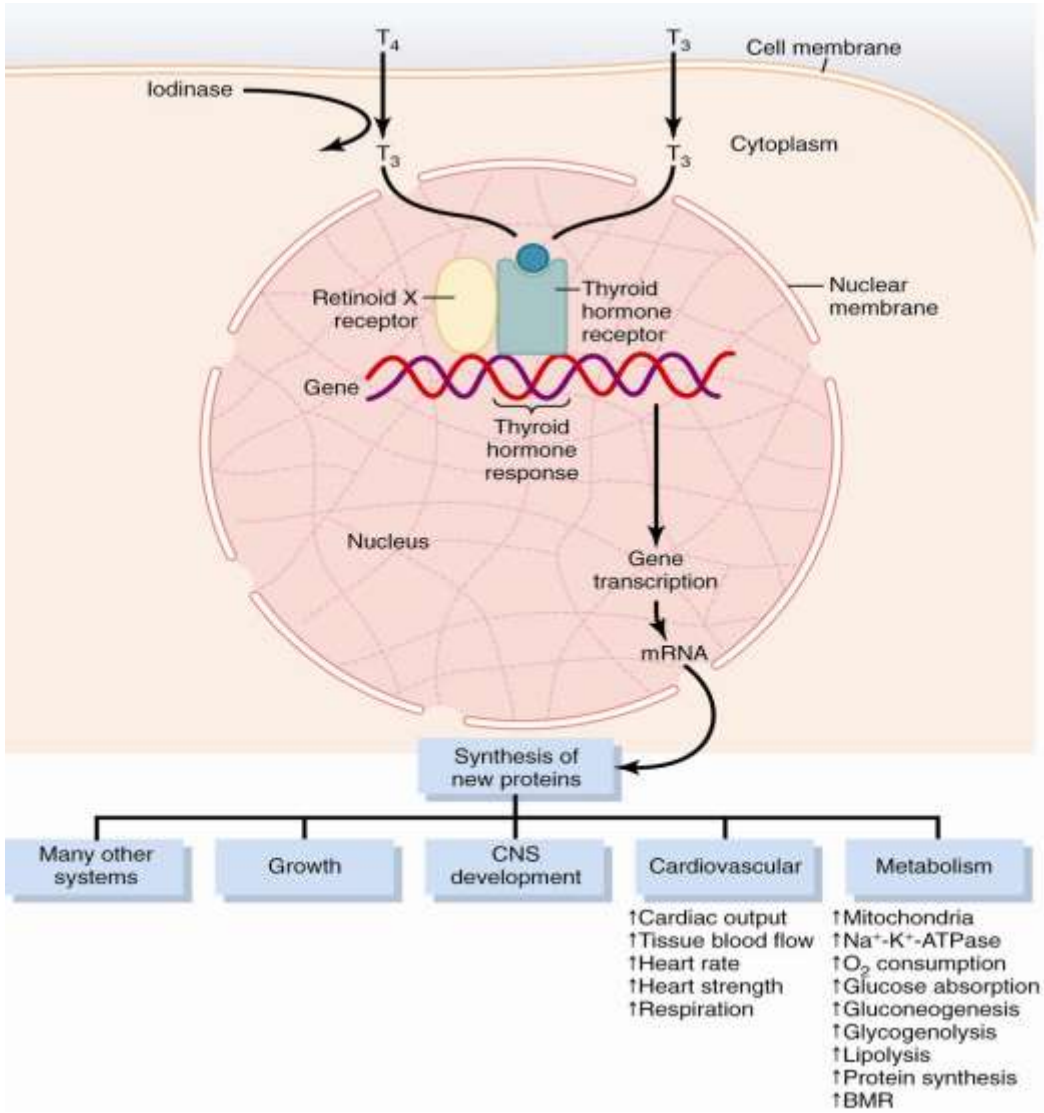
- T_4 passes into cytoplasm and is converted to T_3.
- Receptor proteins located in nucleus.
  - T_3 binds to ligand-binding domain.
  - Other half-site is vitamin A derivative (9-cis-retinoic) acid.
    - DNA-binding domain can then bind to the half-site of the HRE.
  - Two partners can bind to the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



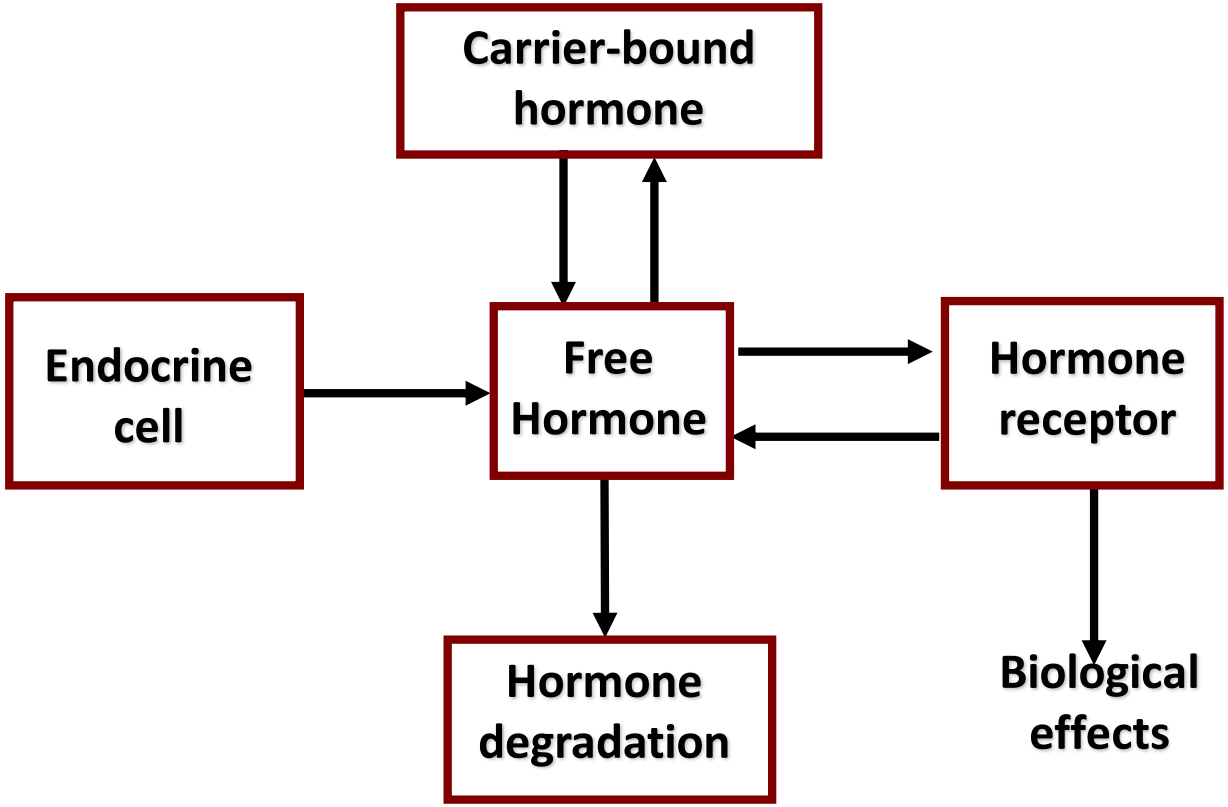
Steroid & Thyroid Hormones - Mechanism of Action



Actions of Thyroid Hormones



Determinants of Free Hormone Receptor Binding

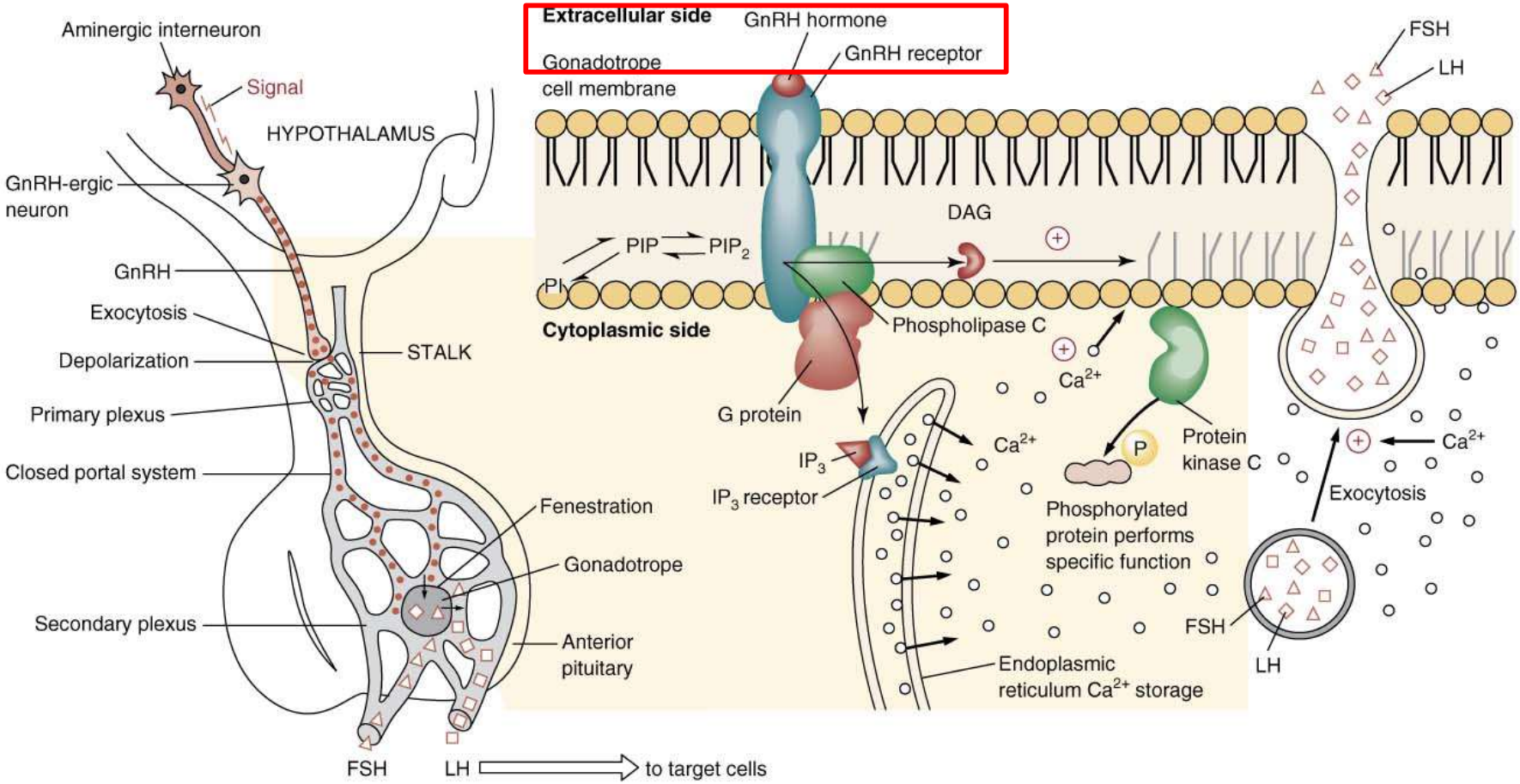


Correlation of Plasma Half-Life & Metabolic Clearance of Hormones with Degree of Protein Binding

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

Circulating Transport Proteins

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



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

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