Signal Transduction

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Integrative Physiology and Pharmacology

Introduction to Physiology (0501110)
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Subject	Lecture No.	Lecturer	Pages in the 11th edition. textbook	Pages in the 12th edition textbook
Receptors: types and adaptation - Membrane or intracellular - Ion channels - G-protein - Enzyme linked - Intracellular - Second messengers - cAMP and cGMP, Phospholipid - Calcium calmodulin and IRS	1-2		910-915	886-891
Signal Transduction (Regulation of cellular machinery) Extracellular regulators: nervous, endocrine, paracrine and autocrine	3-4		934-936 962-963	910-912 940-941
Steroids: Their Signal Transduction And Mechanism Of Action, Thyroid hormones, Nitric Oxide	5		949 954	926-927 931

Textbook: Guyton Medical Textbook of Physiology By: Guyton and Hall 12th edition
Book Chapter **Cell Signalling Biology Michael J. Berridge**

Objectives:

- Define first messenger (Hormones)
- List hormone types
- Describe receptor types
- Outline the hormone receptors interactions
- Describe second messenger mechanism of action
- List second messengers

Signaling Overview

1. Introduction
    - A. Definitions
    - B. Components involved in signaling
    - C. Types of signaling
2. Types of Signaling Ligands - cell-surface vs. intracellular
3. Three Major Classes of Signaling Receptors
    - Ion Channel-linked
    - G protein-coupled receptors (GPRs)
    - Enzyme-linked receptors
        - Tyrosine-Kinase Receptors
            - Overview
            - Mechanism of activation
            - Different ways that TKRs can be activated
            - TKs that are non-covalently linked with receptors
4. Second Messengers: cAMP, cGMP, IP3 and DAG, Ca+2, PIP3
5. Signaling Cascades
    - A. Ras GTPase
    - B. Adaptor proteins with SH2 and SH3 domains
    - C. MAP kinase pathway
    - D. 5 different kinases activated by different cascades
    - E. JAK-STAT pathway

1. Introduction

A. Definitions

Signaling: **Cell-cell communication via signals.**

Signal transduction: **Process of converting extracellular signals into intra-cellular responses.**

Ligand: **The signaling molecule.**

Receptors: **Bind specific ligands. Transmit signals to intracellular targets. Different receptors can respond differently to the same ligand.**

B. Components involved in signaling:

Ligands

Receptors

Intracellular Signaling Proteins

- ***Intermediary Proteins***
- ***Enzymes***
- ***Second Messengers***
- ***Target Proteins***
- ***Inactivating Proteins***

Overview of Signal Transduction

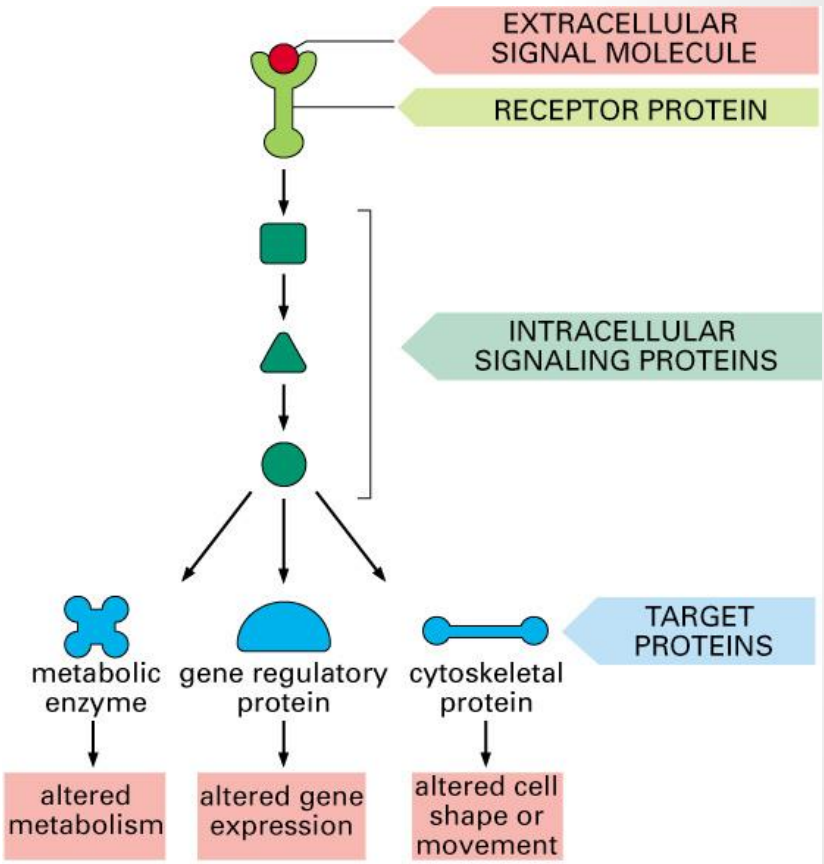
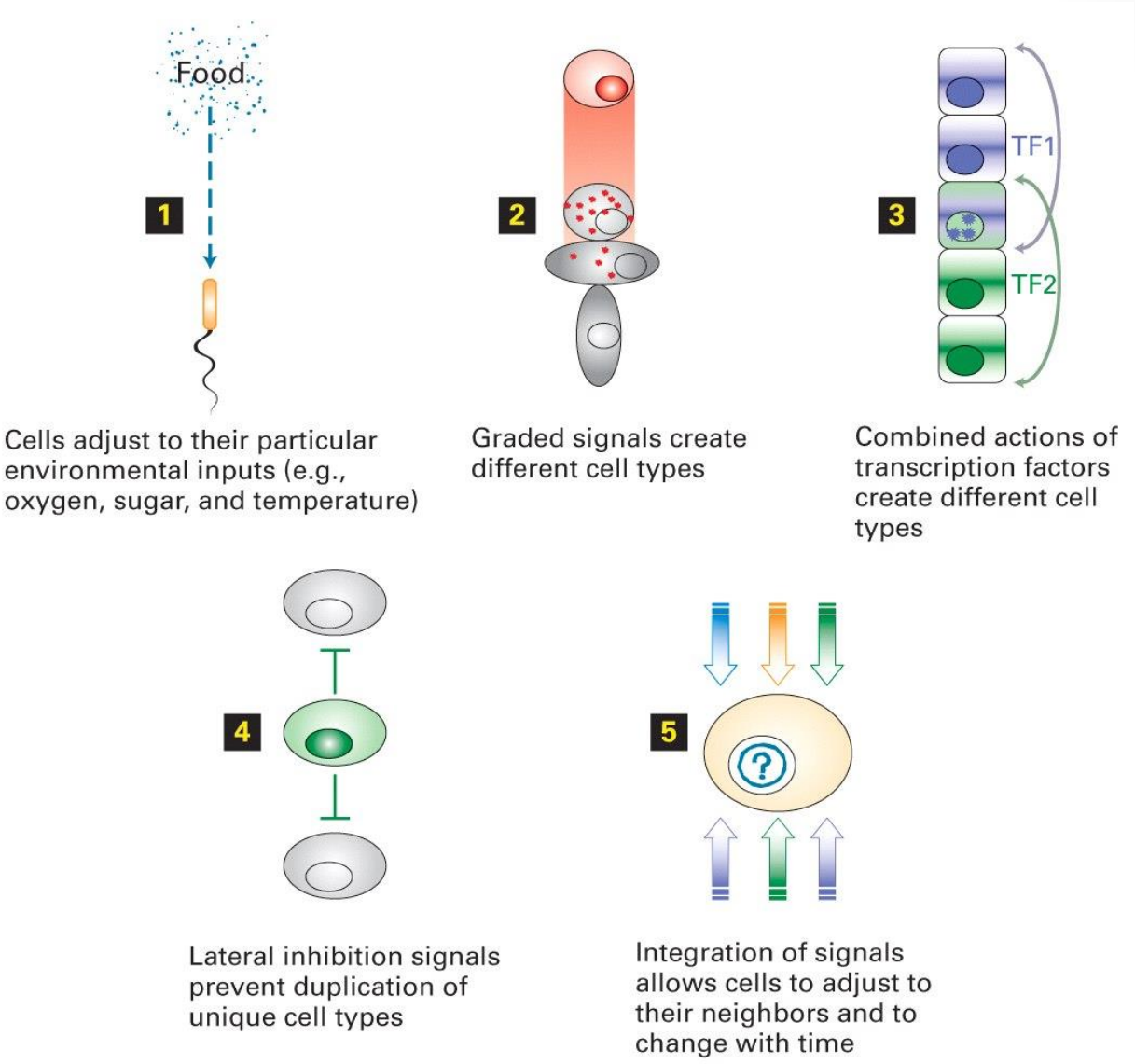
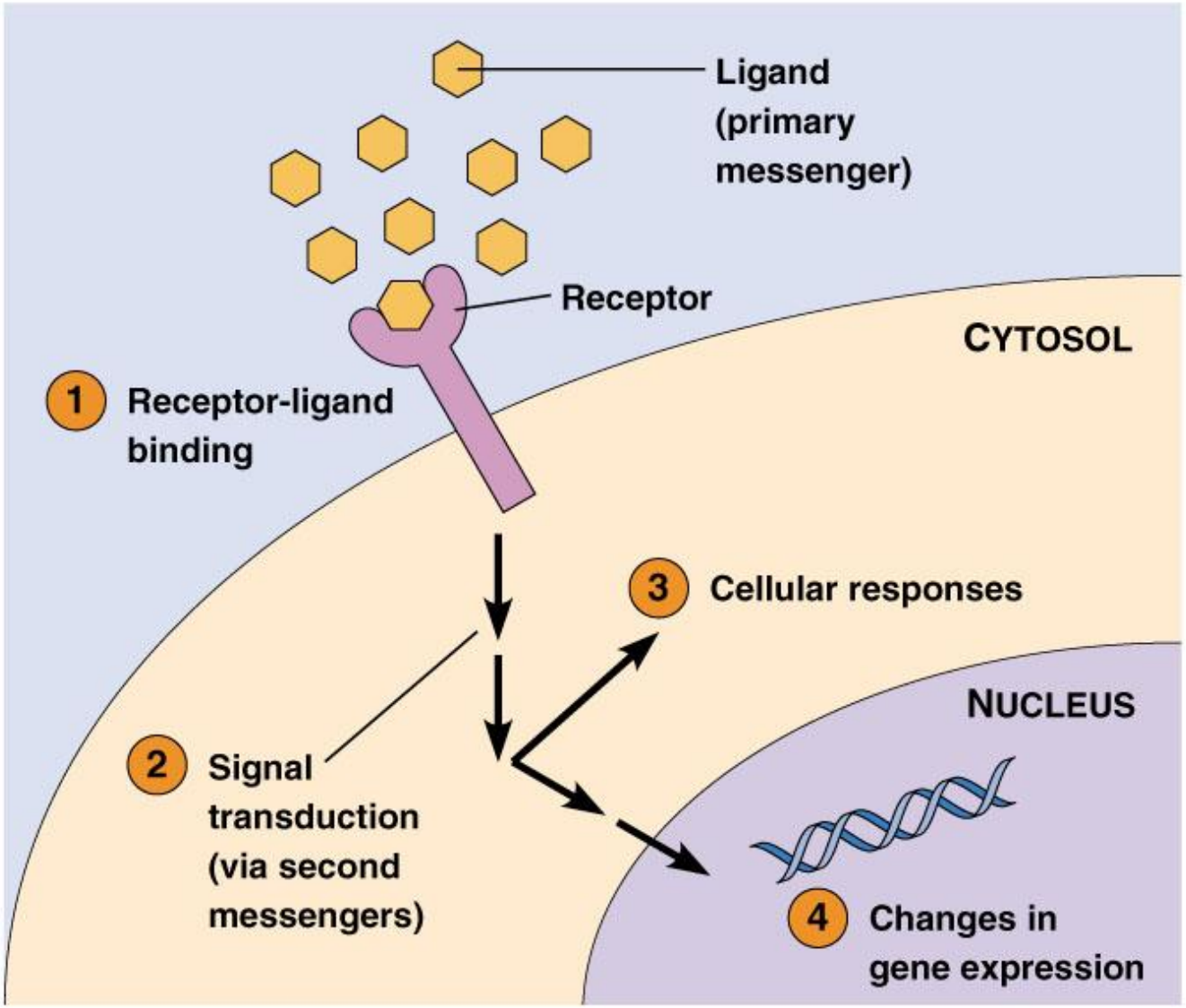


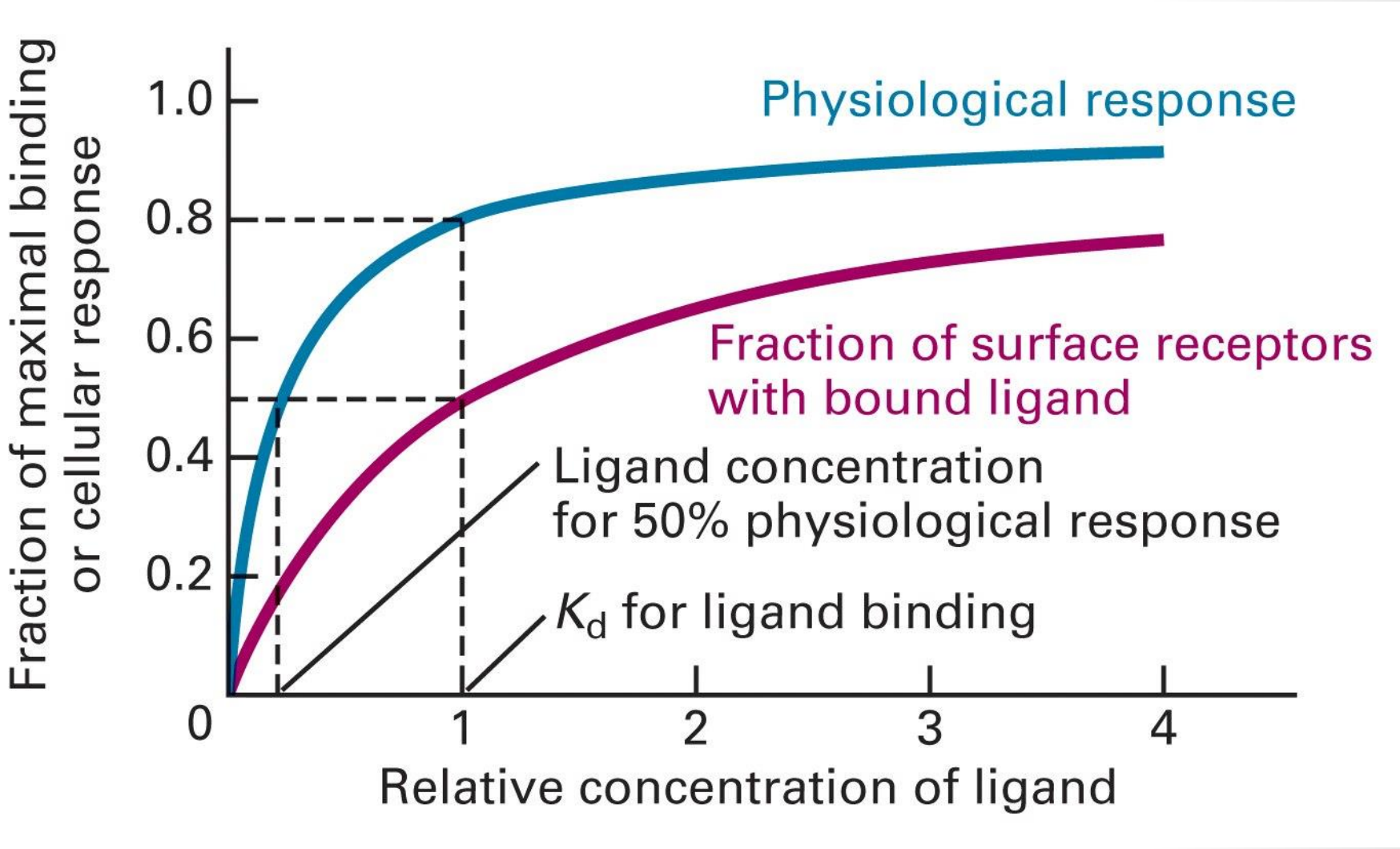
Figure 15–1. Molecular Biology of the Cell, 4th Edition.



Signaling is responsible for how cells can respond to their environment and how they can differentiate or change over time

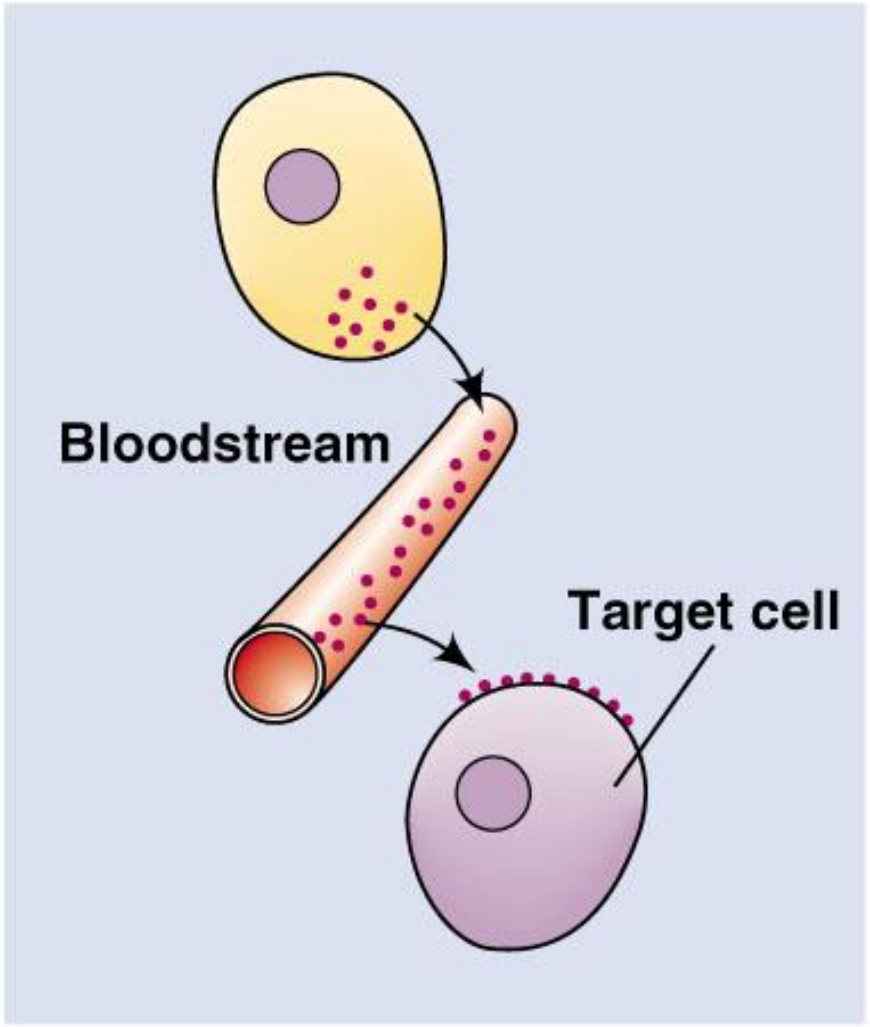
Signals get translated into cellular responses or changes in gene expression



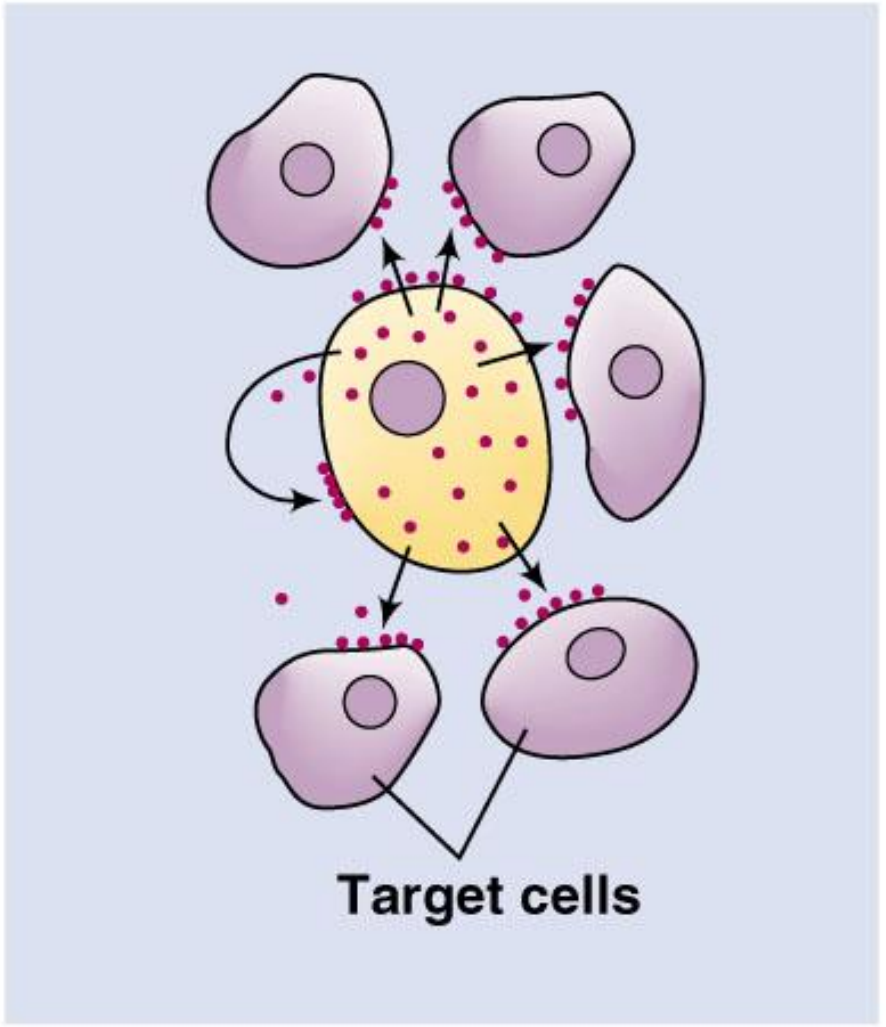


Not all of the receptor needs to be bound to induce a response

Signals can act locally or at a distance

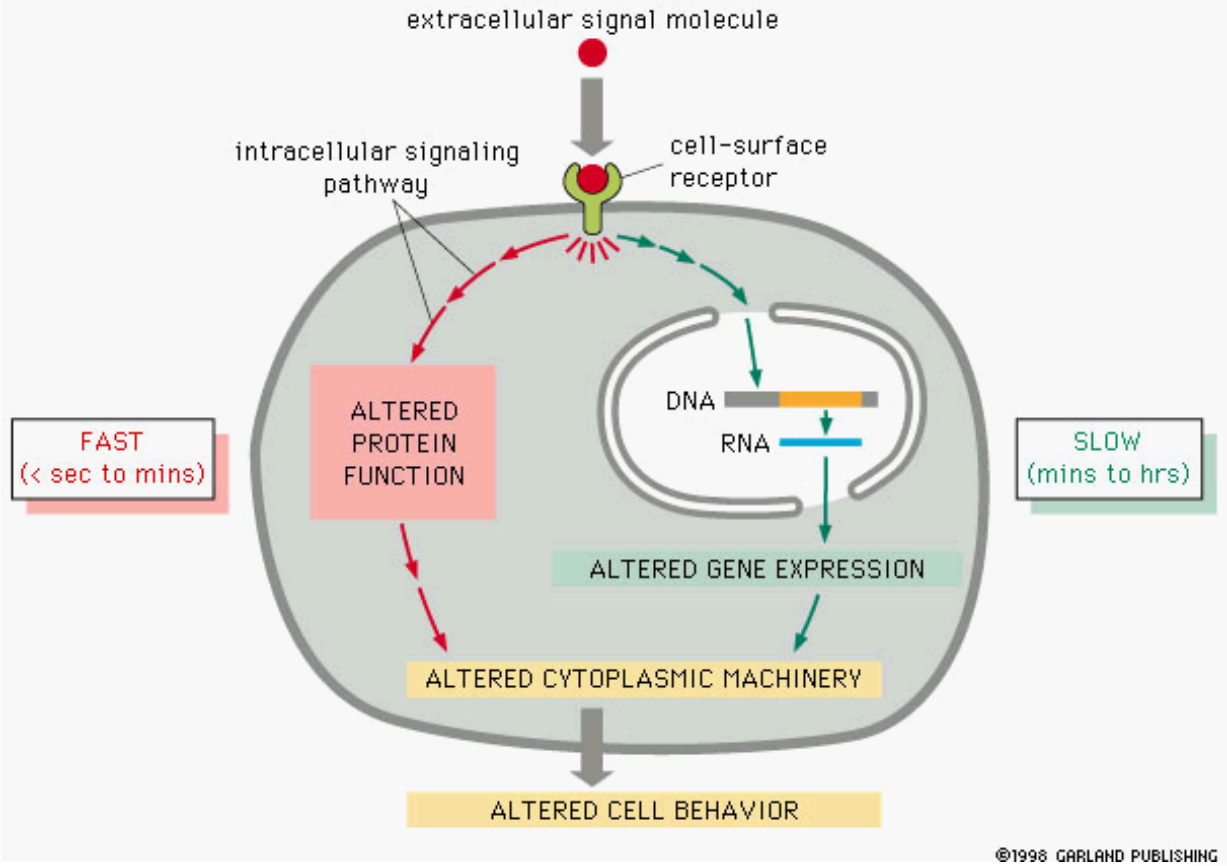


Hormones

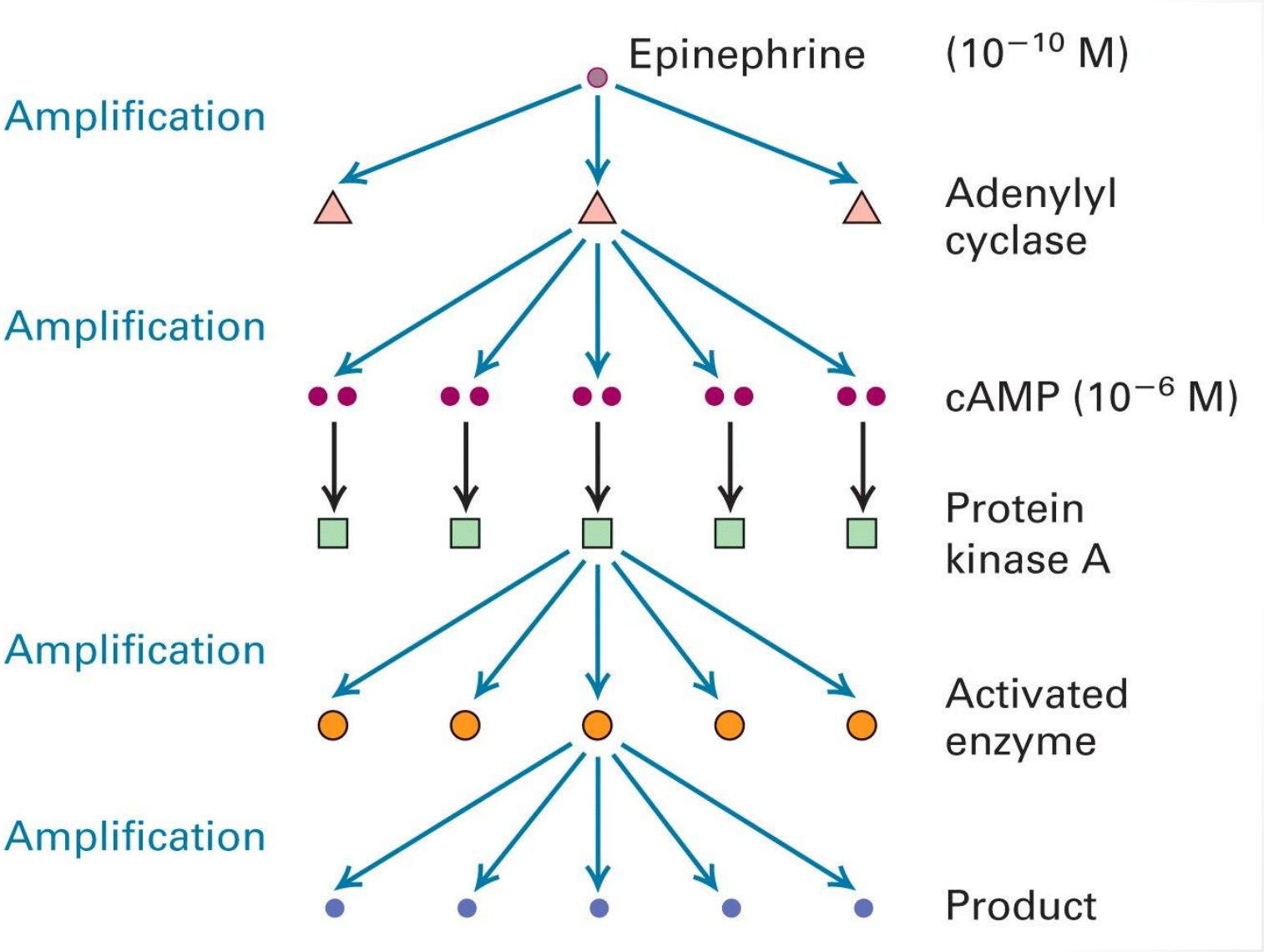


Local mediators

Responses can be fast or slow



Signals are amplified



1. Introduction

C. Types of signaling

i. Contact-dependent - via proteins in the PM:

ii. Via Secreted Signals:

a. Autocrine - via growth factors, cell that releases the signal is also the target.

b. Paracrine - via neurotransmitters and cytokines, action on adjacent target cells.

c. Endocrine - via hormones, action on distant target cells.

d. Synaptic - via neurotransmitters, action on post-synaptic cell in response to electrical stimuli

2. Types of Signaling Ligands:

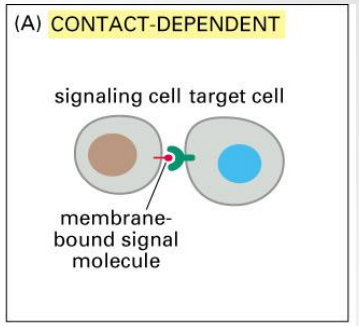
A. Ligands that bind to cell-surface receptors:

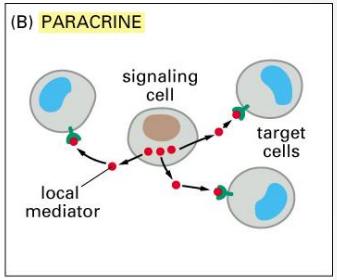
1. Neurotransmitters (NT), i.e. norepinephrine, histamine - hydrophilic (charged, polar)
2. Peptide hormones (P), i.e. insulin - can't cross membrane
3. Growth factors (GF), i.e. NGF, EGF, PDGF
4. Lipophilic signaling molecules, i.e. prostaglandins

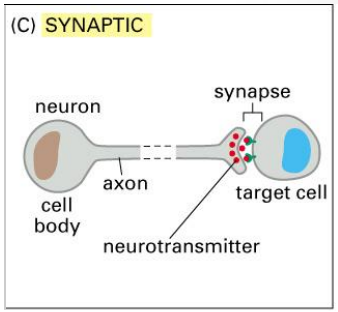
B. Ligands that bind to intracellular receptors:

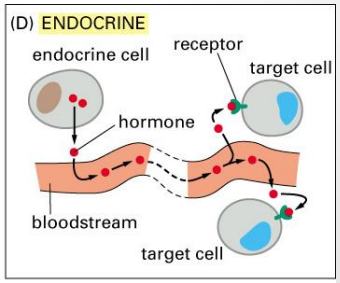
lipid soluble hormones that diffuse across the plasma membrane and interact with receptors in the cytosol or nucleus. i.e. steroids, thyroxine, retinoic acid, nitric oxide.

Types of Signaling

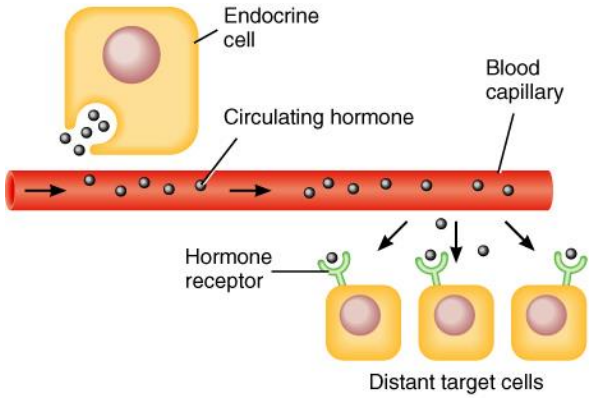




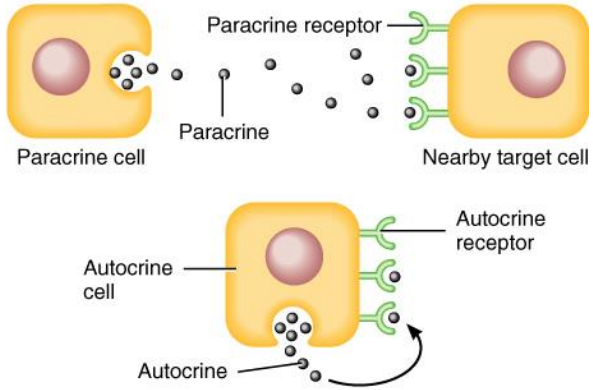




Local vs. Circulating hormones



(a) Circulating hormones



(b) Local hormones (paracrines and autocrines)

18.02

Chemical classes of hormones

- Lipid-soluble hormones- use transport proteins in the **plasma**
  - Steroid: Lipids derived from cholesterol.
    - Are lipophilic hormones.
      - Testosterone.
      - Estradiol.
      - Cortisol.
      - Progesterone.
  - Thyroid (amine but lipid soluble)
  - Nitric oxide (NO)

Chemical classes of hormones

- Water-soluble – circulate in "free" form in the plasma
  - Amines:
    - Hormones derived from tyrosine and tryptophan.
  - Polypeptides and proteins:
    - Polypeptides:
      - Chains of < 100 amino acids in length.
        - ADH.
    - Protein hormones:
      - Polypeptide chains with > 100 amino acids.
      - Growth hormone.
  - Eicosanoid (prostaglandins) derived from arachidonic acid (20 carbon 4 double bonds)

Chemical Classification of Hormones

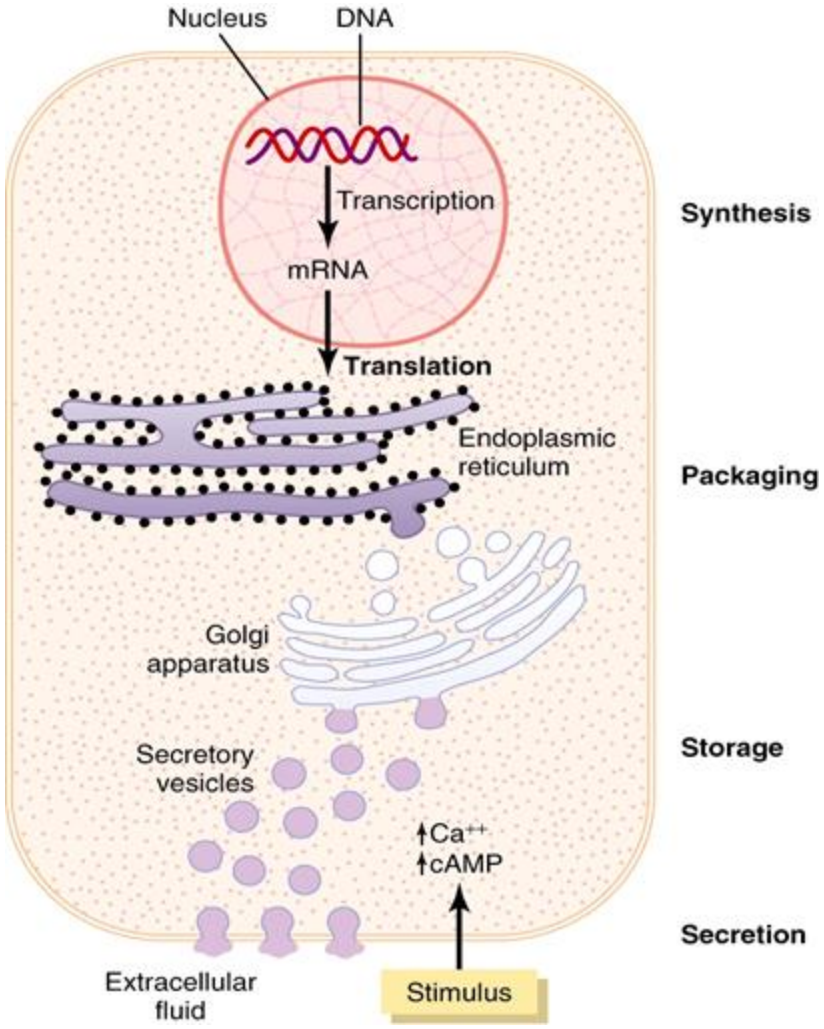
- Glycoproteins:
  - Long polypeptides (>100) bound to 1 or more carbohydrate (CHO) groups.
    - FSH and LH, TSH and hCG (human chorionic gonadotropin)

      They have α and β subunits (α is common and β is specific)
- Hormones can also be divided into:
  - Polar:
    - H_20 soluble.
  - Nonpolar (lipophilic):
    - H_20 insoluble.
      - Can gain entry into target cells.
      - Steroid hormones and T_4 (thyrxine –tetraiodothyronine))

Prohormones and Prehormones

- Prohormone:
  - Precursor is a longer chained polypeptide that is cut and spliced together to make the hormone.
    - Proinsulin – gives insulin
- Preprohormone:
  - Prohormone derived from larger precursor molecule.
    - Preproinsulin.
- Prehormone:
  - Molecules secreted by endocrine glands that are inactive until changed into hormones by target cells.
    - T_4 converted to T_3 (tri-iodothyronin).

Synthesis and secretion of peptide hormones



Chemical classification of hormones

Table 10-4 Chemical Classification and Function of Hormones

Chemical Classification	Examples	Regulated Function
Endocrine Hormones		
Amino acid derivatives	Epinephrine (adrenaline) and norepinephrine (both derived from tyrosine)	Stress responses: regulation of heart rate and blood pressure; release of glucose and fatty acids from storage sites
	Thyroxine (derived from tyrosine)	Regulation of metabolic rate
Peptides	Antidiuretic hormone (vasopressin)	Regulation of body water and blood pressure
	Hypothalamic hormones (releasing factors)	Regulation of tropic hormone release from pituitary gland
Proteins	Anterior pituitary hormones	Regulation of other endocrine systems
Steroids	Sex hormones (androgens and estrogens)	Development and control of reproductive capacity
	Corticosteroids	Stress responses; control of blood electrolytes
Paracrine Hormones		
Amino acid derivative	Histamine	Local responses to stress and injury
Arachidonic acid derivatives	Prostaglandins	Local responses to stress and injury

Peptide & Protein Hormones

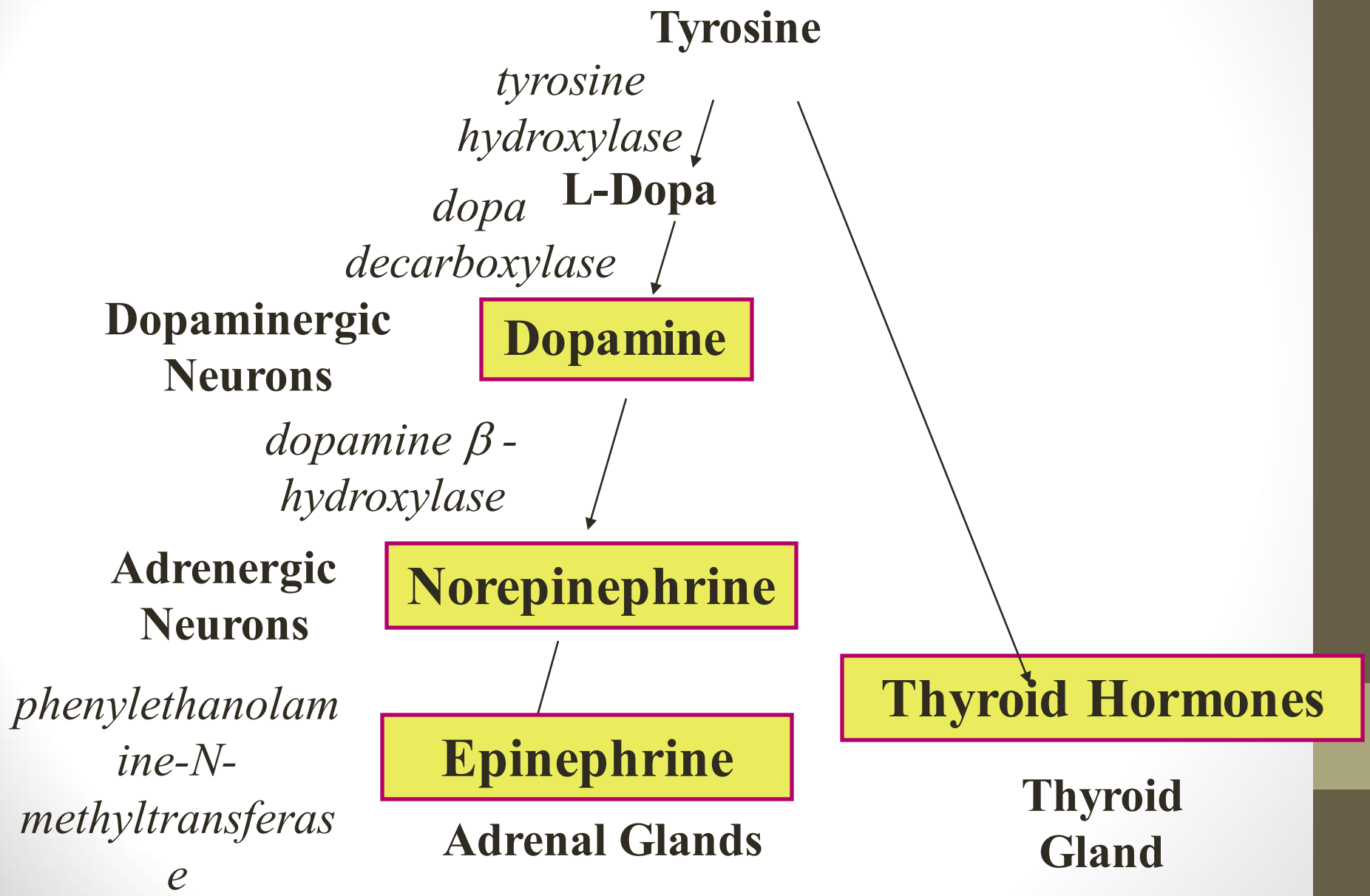
Gland/Tissue	Hormones
Hypothalamus	TRH, GnRH, CRH GHRH, Somatostatin,
Anterior pituitary	ACTH, TSH, FSH, LH, PRL, GH
Posterior pituitary	Oxytocin, ADH
Thyroid	Calcitonin
Pancreas	Insulin,Glucagon, Somatostatin
Liver	Somatomedin C (IGF-1)
Parathyroid	PTH

Gland/Tissue	Hormones
Placenta	HCG, HCS or HPL
Kidney	Renin
Heart	ANP
G.I. tract	Gastrin, CCK, Secretin, GIP, Somatostatin
Adipocyte	Leptin
Adrenal medulla	

Amine Hormones

Gland/Tissue	Hormones
Hypothalamus	▪ Dopamine
Thyroid	▪ T_3, T_4
Adrenal medulla	▪ Epinephrine and Norepinephrine (NE, EPI)

Synthesis of Amine Hormones



Steroid Hormones

Gland/Tissue	Hormones
Adrenal Cortex	▪ Cortisol, Aldosterone, Androgens
Testes	▪ Testosterone
Ovaries	▪ Estrogens, Progesterone
Corpus Luteum	▪ Estrogens, Progesterone
Placenta	▪ Estrogens, Progesterone
Kidney	▪ 1,25-Dihydroxycholecalciferol (calcitriol)

Hormone Activity

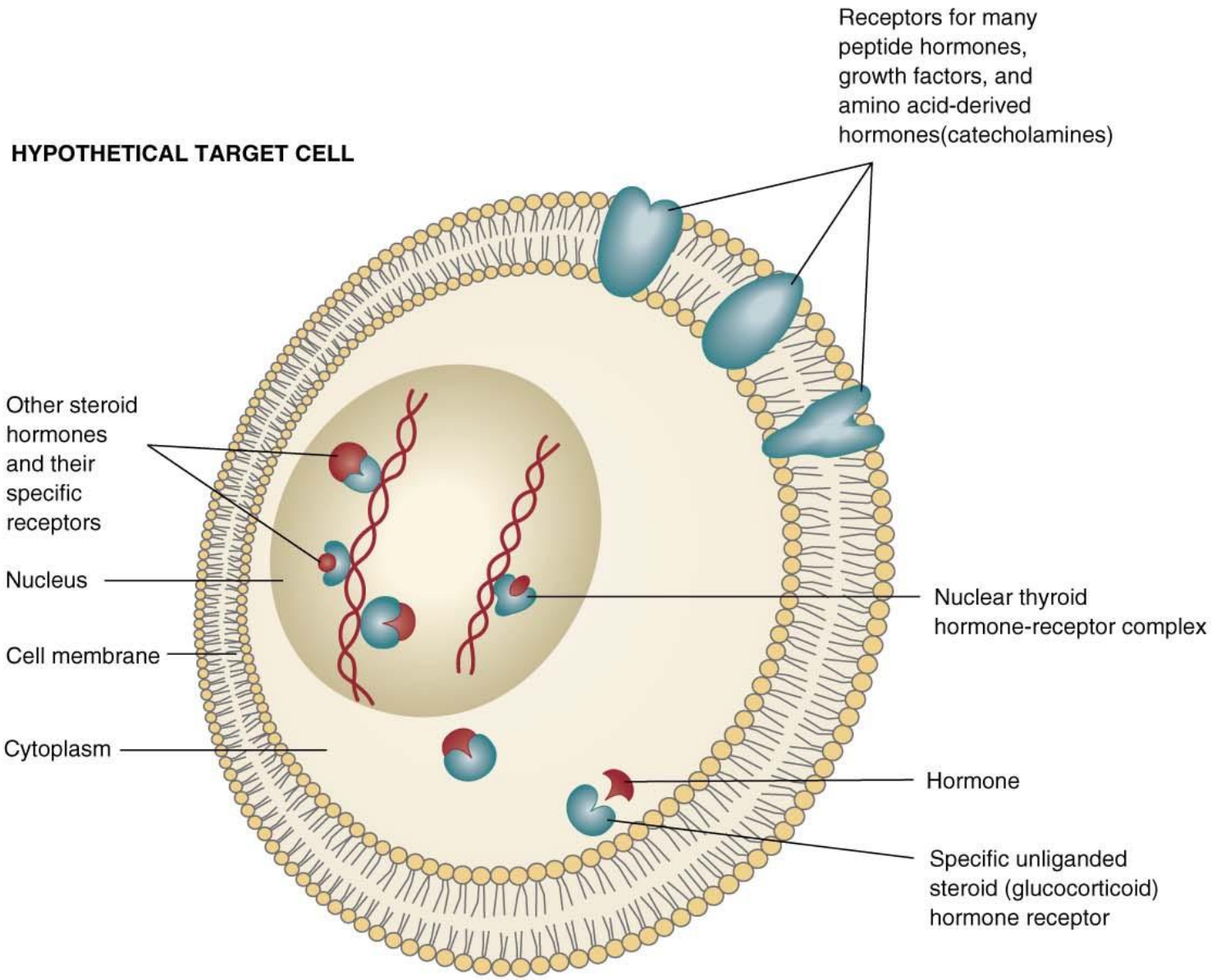
- Hormones affect only specific target tissues with specific receptors
- Receptors are dynamic and constantly synthesized and broken down
  - Down-regulation- decrease in receptor number or esponse
  - Up-regulation- increase in receptor number or activity

Effects of [Hormone] on Tissue Response

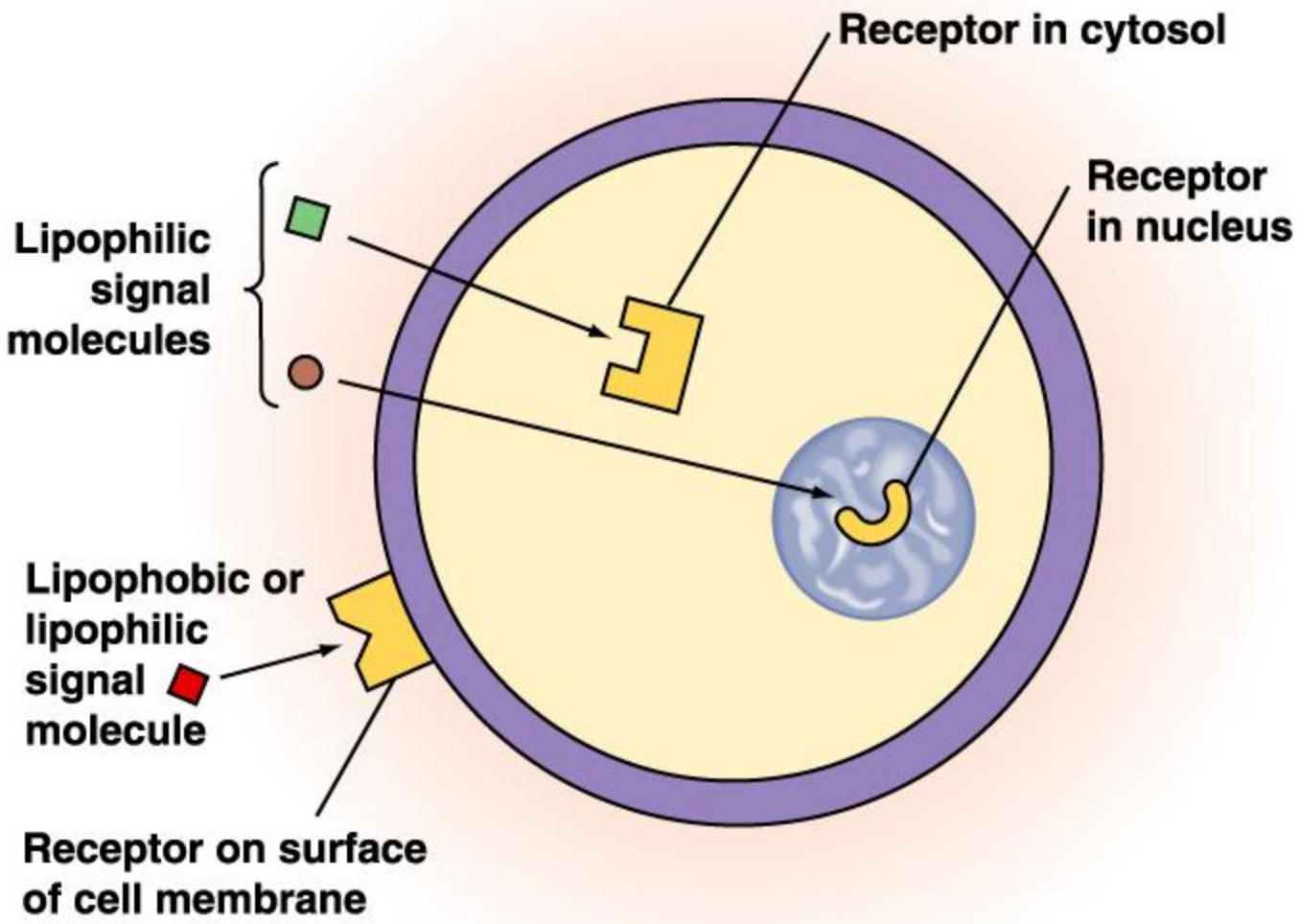
- Priming effect (upregulation):
  - Increase number of receptors formed on target cells in response to particular hormone.
  - Greater response by the target cell.
- Desensitization (downregulation):
  - Prolonged exposure to high [polypeptide hormone].
    - Subsequent exposure to the same [hormone] produces less response.
      - Decrease in number of receptors on target cells.
        - Insulin in adipose cells.
  - Pulsatile secretion may prevent downregulation.

Effects of hormone concentration on Tissue Response

- [Hormone] in blood reflects the rate of secretion.
- Half-life:
  - Time required for the blood [hormone] to be reduced to ½ reference level.
    - Minutes to days.
- Affinity of receptors to ligands, Kd
- Normal tissue responses are produced only when [hormone] are present within physiological range.
- Varying [hormone] within normal, physiological range can affect the responsiveness of target cells.



. Diagram showing the different locations of classes of hormone receptors expressed by a target cell.


Receptor in cytosol
Receptor in nucleus
Lipophilic signal molecules
Lipophobic or lipophilic signal molecule
Receptor on surface of cell membrane

Mechanisms of Hormone Action

- Hormones of same chemical class have similar mechanisms of action.
  - Similarities include:
    - Location of cellular receptor proteins depends on the chemical nature of the hormone.
    - Events that occur in the target cells.
- To respond to a hormone:
  - Target cell must have specific receptors for that hormone (specificity).
    - Hormones exhibit:
      - Affinity (bind to receptors with high bond strength).
      - Saturation (low capacity of receptors).

Mechanisms of Hormone Action

- Response depends on both hormone and target cell
- Lipid-soluble hormones bind to receptors inside target cells
- Water-soluble hormones bind to receptors on the plasma membrane
  - Activates second messenger system
  - Amplification of original small signal
- Responsiveness of target cell depends on
  - Hormone's concentration
  - Abundance of target cell receptors

Receptor

Receptors are specific membrane proteins, which are able to recognize and bind to corresponding ligand molecules, become activated, and transduce signal to next signaling molecules.

Glycoprotein or Lipoprotein

Receptors

- Specificity: The "specificity" of a ligand for a receptor is a description of how favorable the binding of the ligand for the receptor is compared with its possible binding to other types of receptors that may also be present.

- Affinity: "Affinity" simply refers to how strong the binding is (as judged by K association or K dissociation and ΔGo). "High affinity" refers to very strong binding (large negative ΔGo and a very small Kd). The association or dissociation constant is often referred to as the "affinity" or "binding" constant.

ligand

A small molecule that binds specifically to a larger one; for example, a hormone is the ligand for its specific protein receptor.

- **Membrane receptors**

  Membrane Glycoprotein

- **Intracellular receptors**

  Cytosol or nuclei

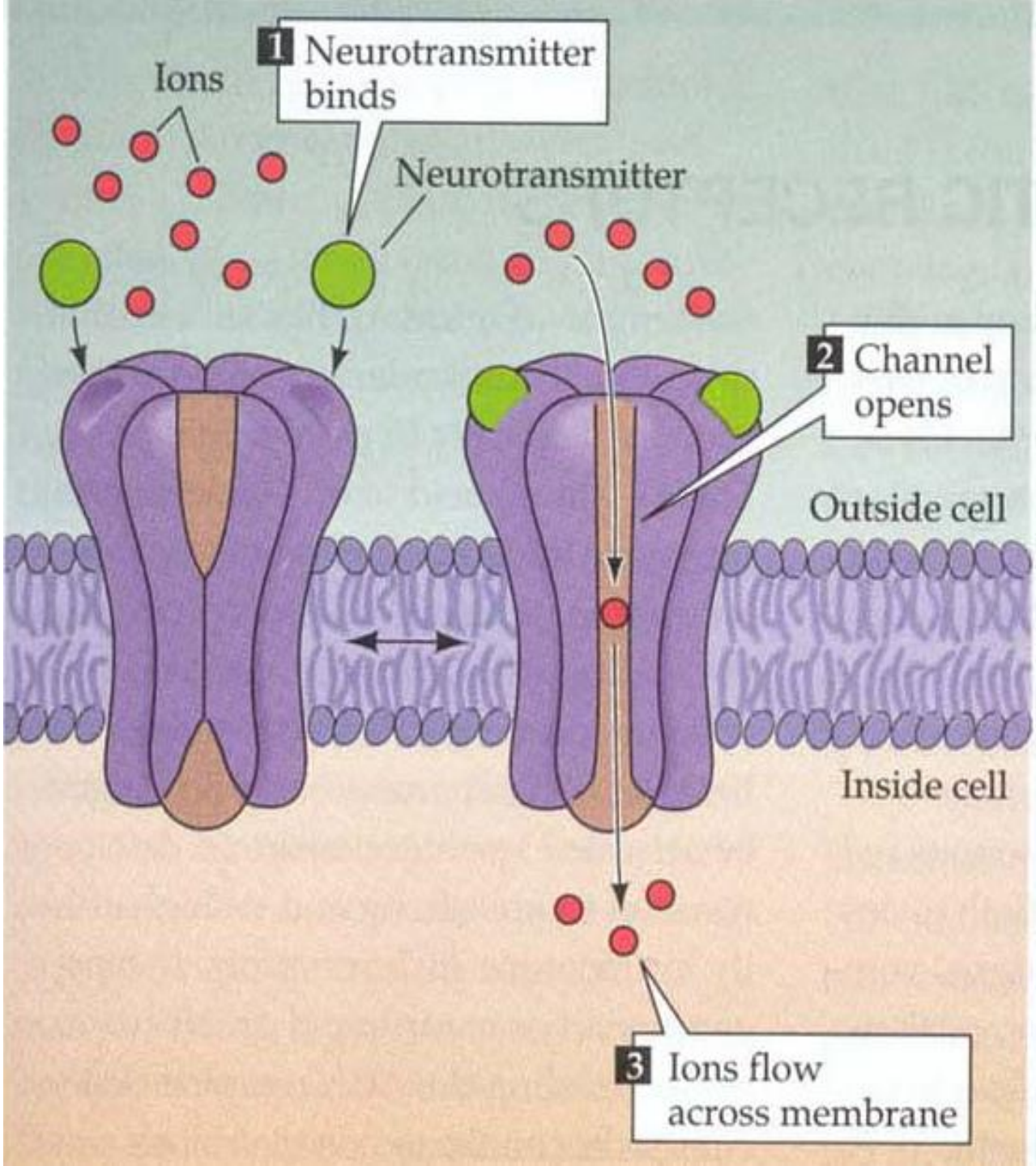
  DNA binding protein

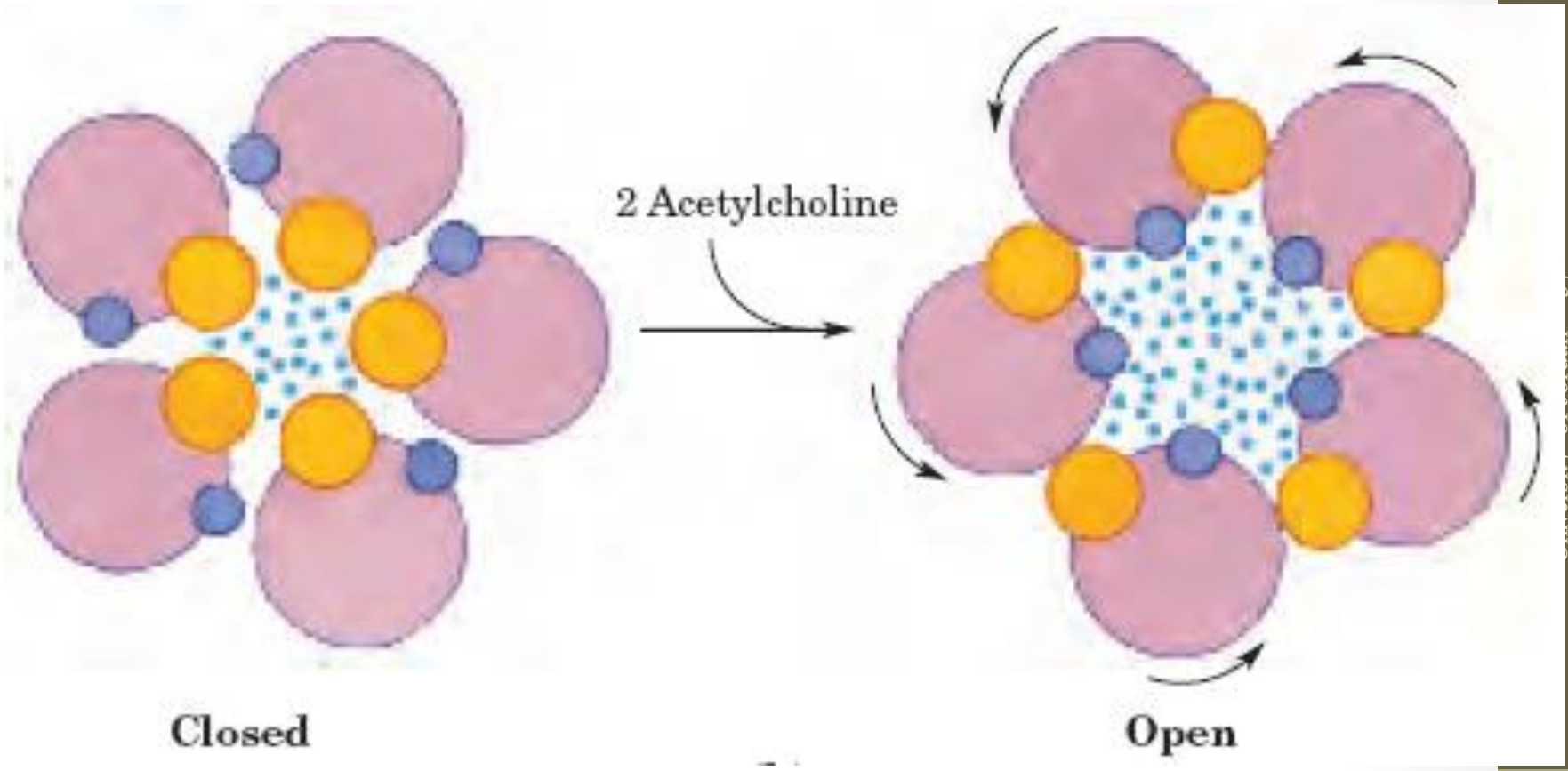
1. membrane receptors

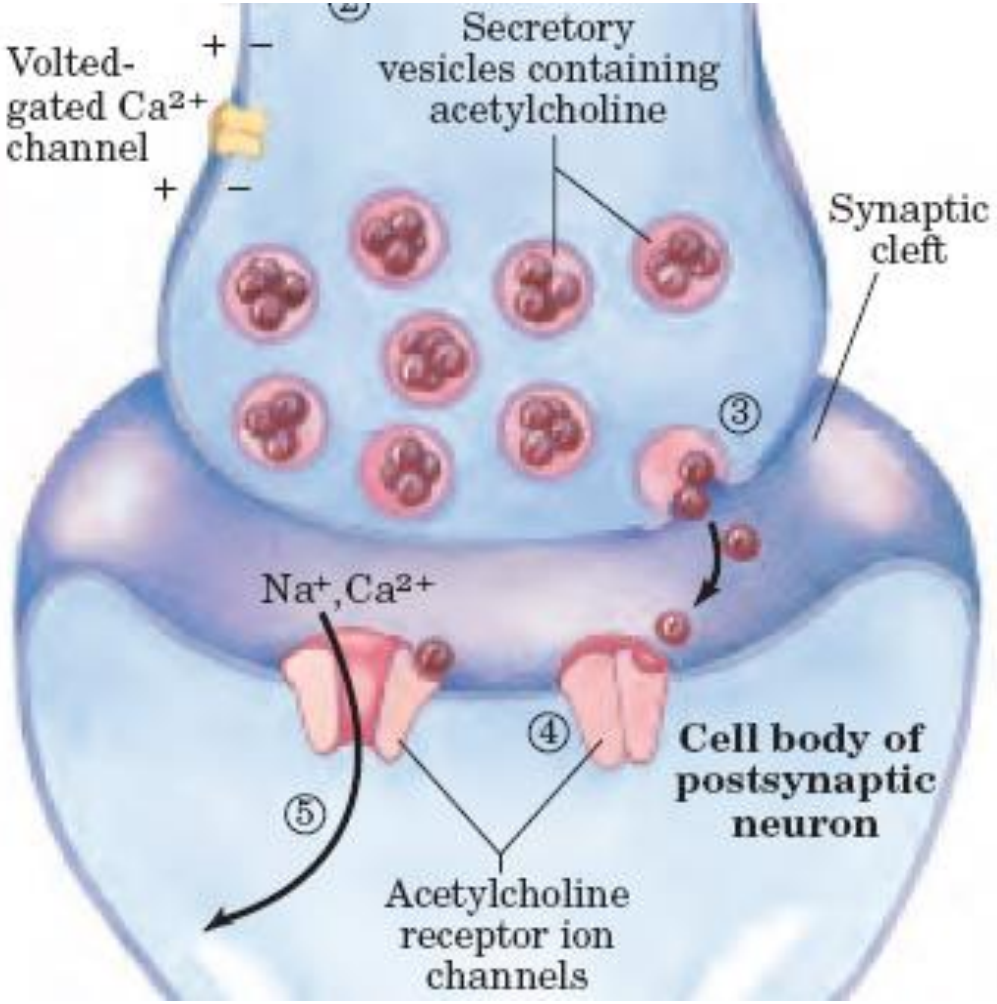
1- Ligand-gate ion channels type

(cyclic receptor)

ligand→receptor→ion channel open or close


Ions
1 Neurotransmitter binds
Neurotransmitter
2 Channel opens
Outside cell
Inside cell
3 Ions flow across membrane


2 Acetylcholine
Closed
Open


Volted-
gated Ca2+
channel
+ −
+ −
Secretory
vesicles containing
acetylcholine
Synaptic
cleft
③
Na+, Ca2+
④
Cell body of
postsynaptic
neuron
⑤
Acetylcholine
receptor ion
channels

Signaling Overview

3. Three major classes of surface receptors for signaling :

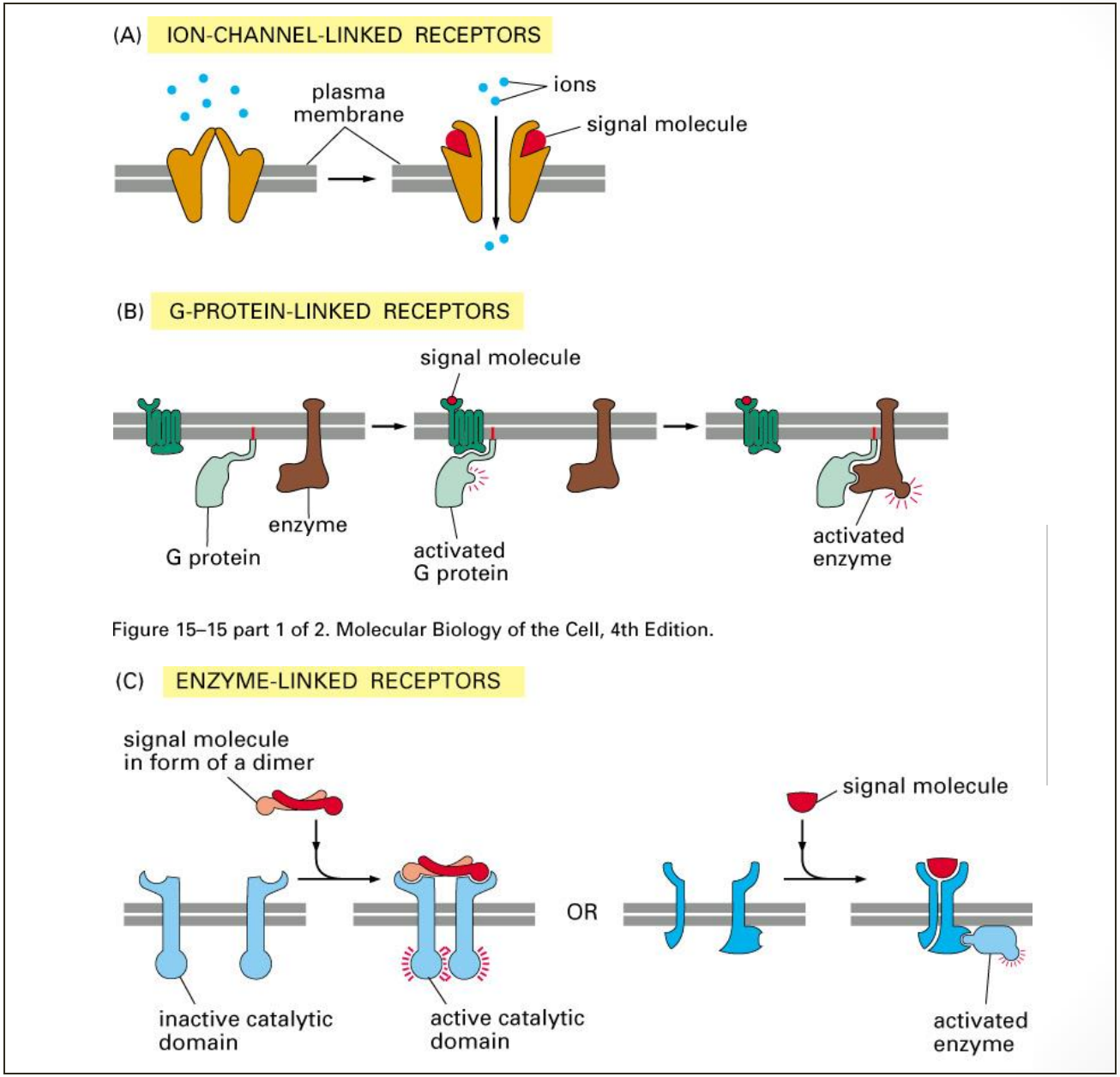
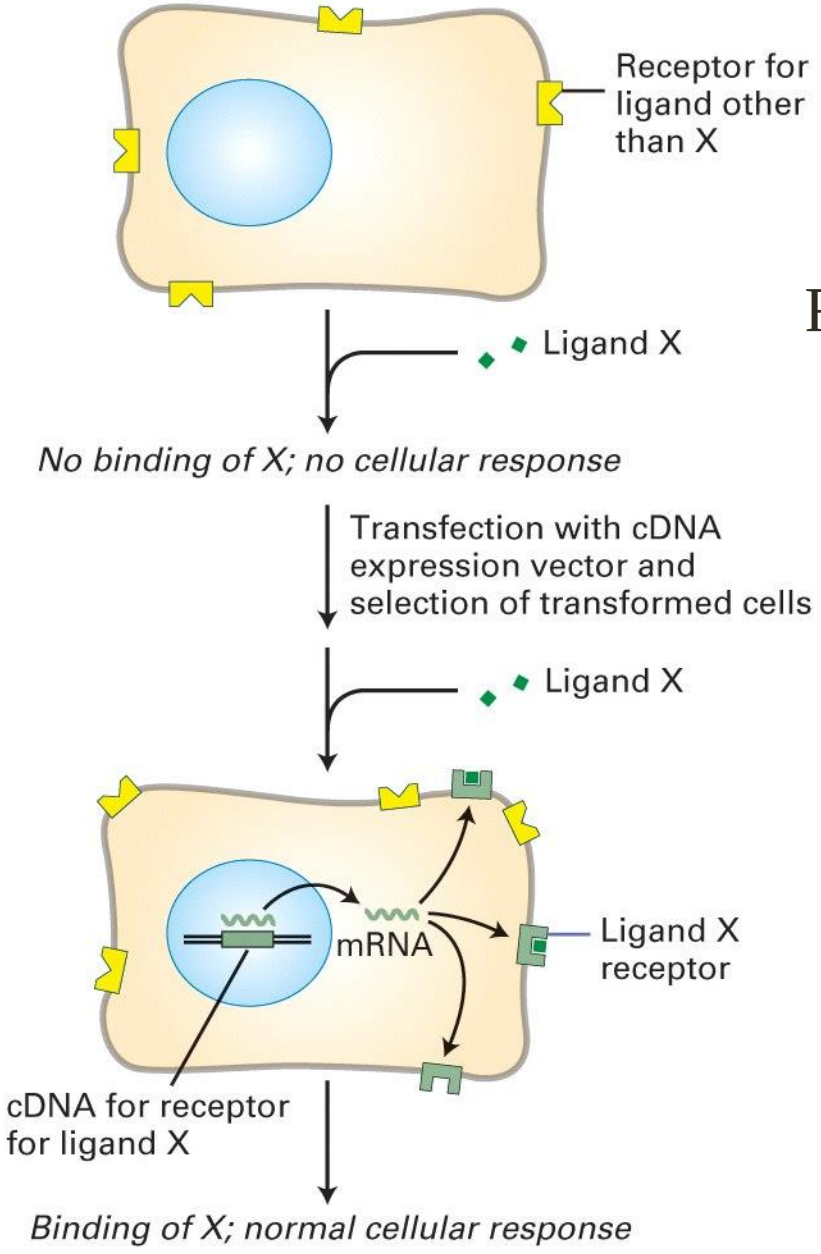


Figure 15–15 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



Receptors determine response

No receptor - no response

Signaling Overview

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

A. Ion Channels:

B. G protein-coupled receptors (GPRs): largest family of cell surface receptors; present in all eukaryotes; ex: adrenergic receptors, opioid receptors.

1. Overview:

- ***a.*** **7 trans-membrane spanning domains**
- ***b.*** **Act as receptors for many different ligands including NT, H**
- **c. Large amount of receptor diversity, but common mechanism of action**
- ***d.*** **Transmit signals to intracellular targets via G proteins**
- ***e.*** **Targets are plasma membrane bound enzymes or ion channels**

2. Mechanism of Activation of GPRs:

- ***a.*** **Binding of ligand to extracellular domain of GPRs induces conformational change that allows cytosolic domain of the receptor to bind to inactive G protein at inner face of PM.**
- ***b.*** **This interaction activates the G protein, which dissociates from the receptor**
- ***c.*** **Activated G protein α subunit can now bind GTP instead of GDP, causing dissociation into activated α vs. βγ subunits. Each of these can go on to activate target proteins.**

C. Enzyme-linked receptors:

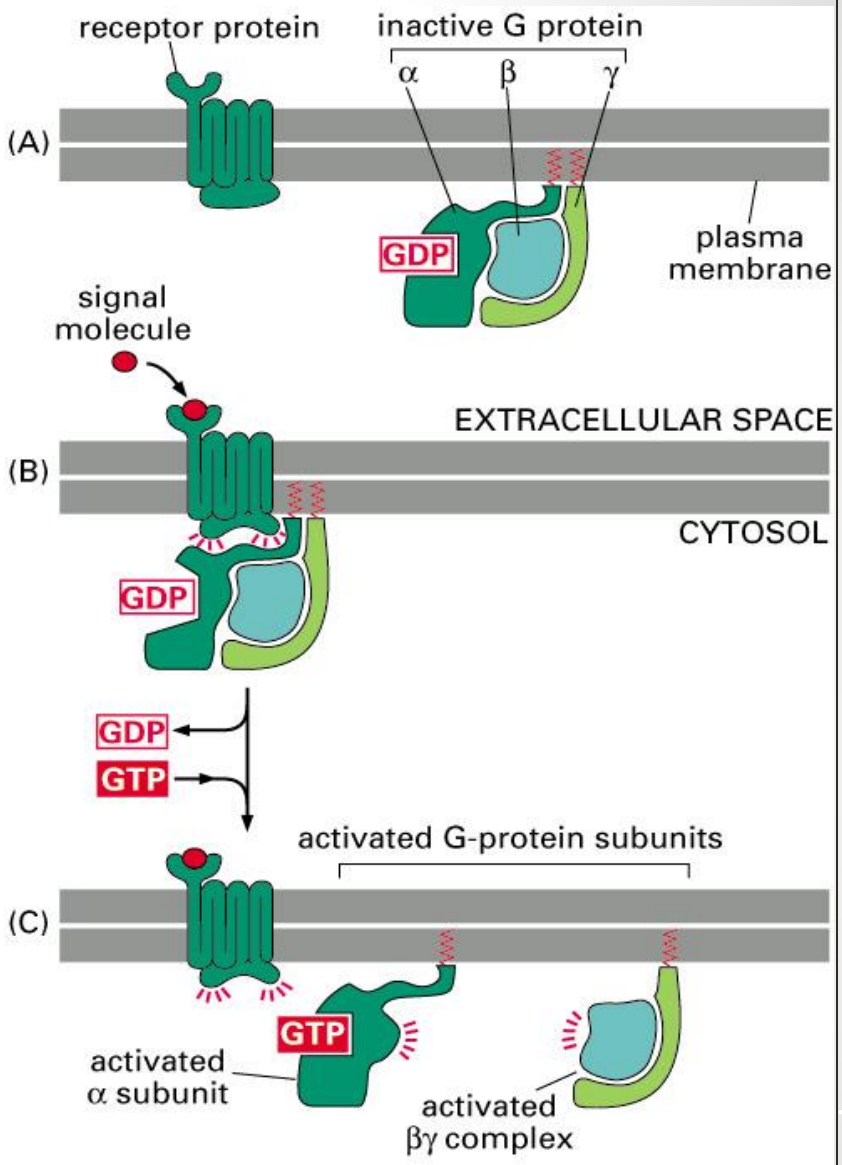


Figure 15–28. Molecular Biology of the Cell, 4th Edition.

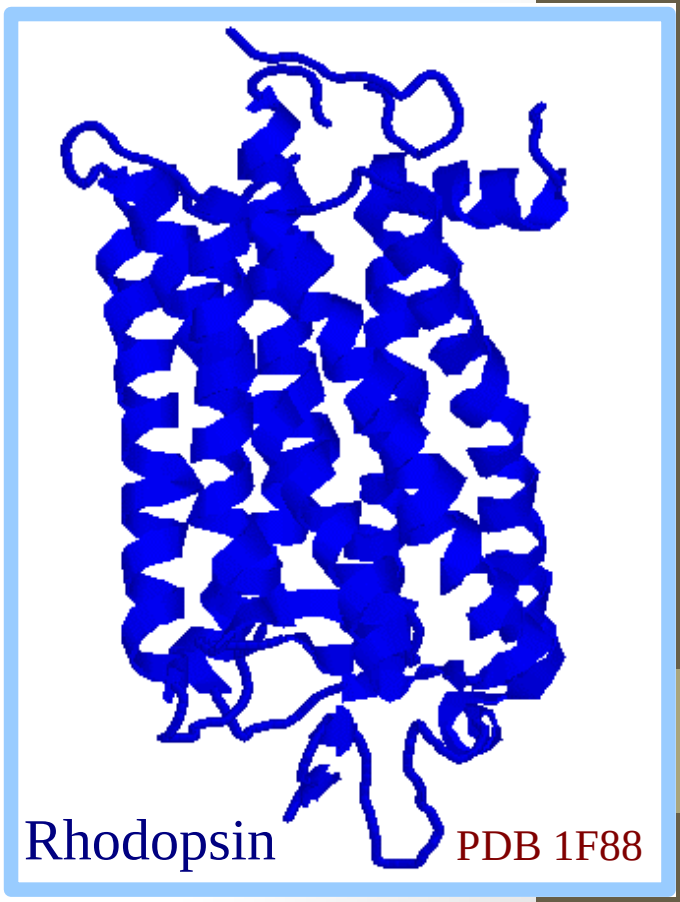
G Protein Signal Cascade

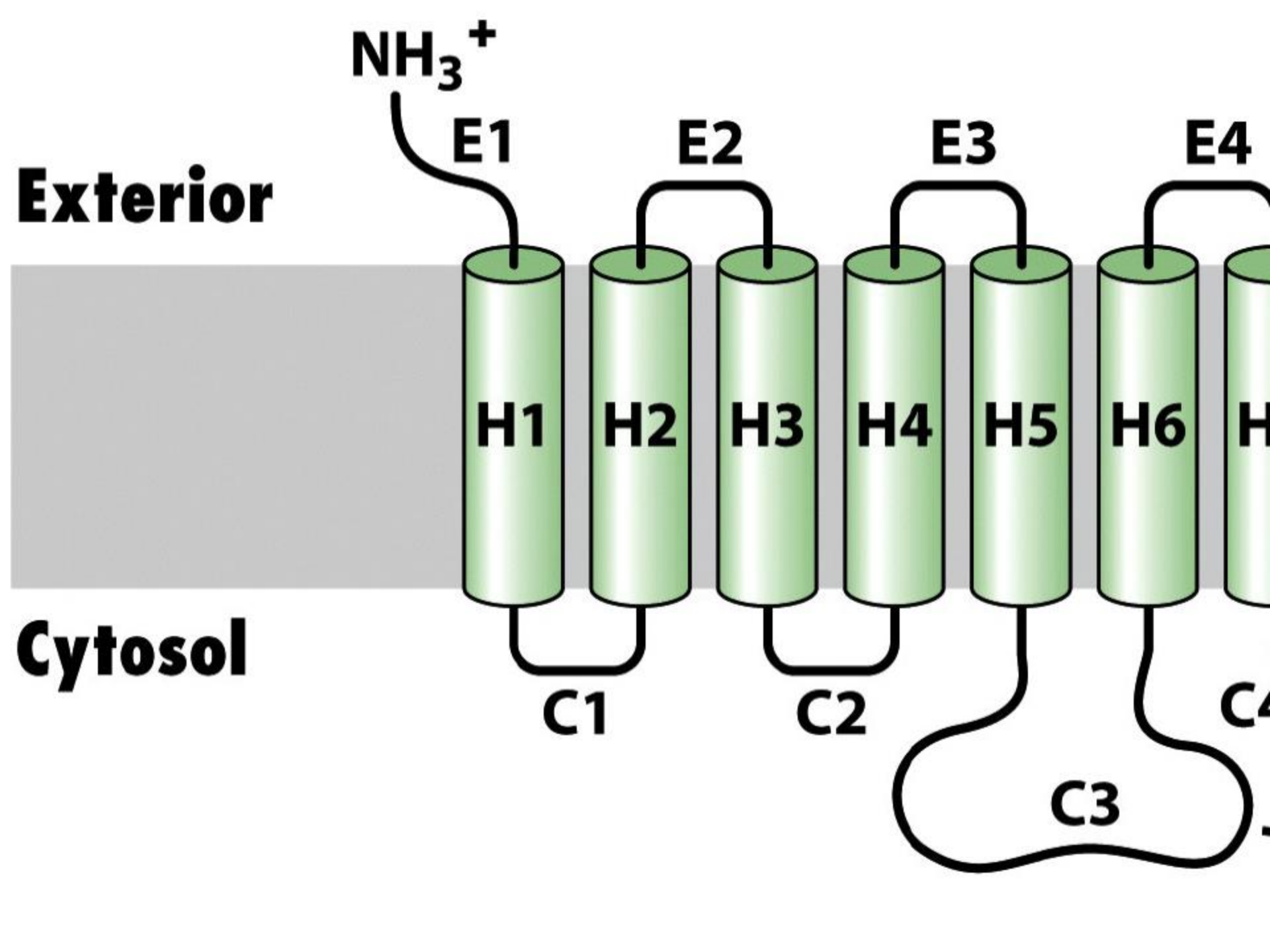
Most signal molecules targeted to a cell bind at the cell surface to **receptors** embedded in the **plasma membrane**.

Only signal molecules able to cross the plasma membrane (e.g., steroid hormones) interact with intracellular receptors.

A large family of **cell surface receptors** have a common structural motif, **7 transmembrane α-helices**.

Rhodopsin was the first of these to have its 7-helix **structure** confirmed by X-ray crystallography (in retina, low light vision).




NH3+
E1
E2
E3
E4
Exterior
H1
H2
H3
H4
H5
H6
Cytosol
C1
C2
C3

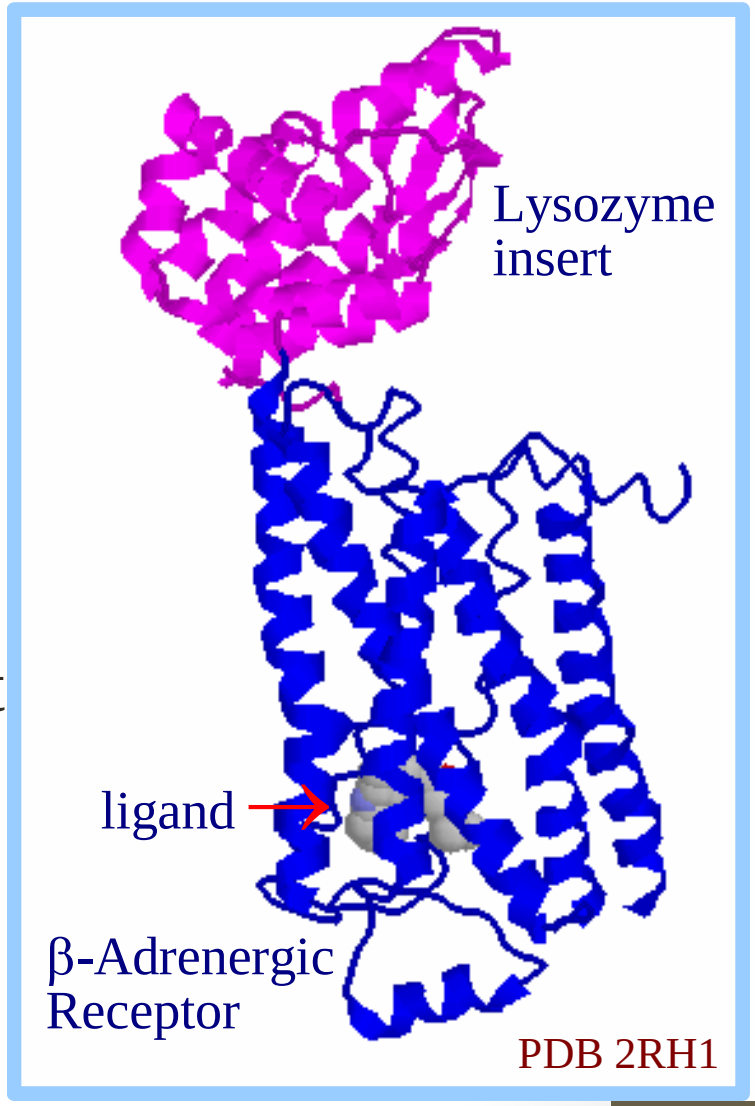
G Protein Signal Cascade

- **Rhodopsin** is unique.

  It senses **light**, via a bound chromophore, retinal.

- **Most 7-helix receptors** have domains facing the **extracellular** side of the plasma membrane that recognize & bind **signal molecules** (**ligands**).

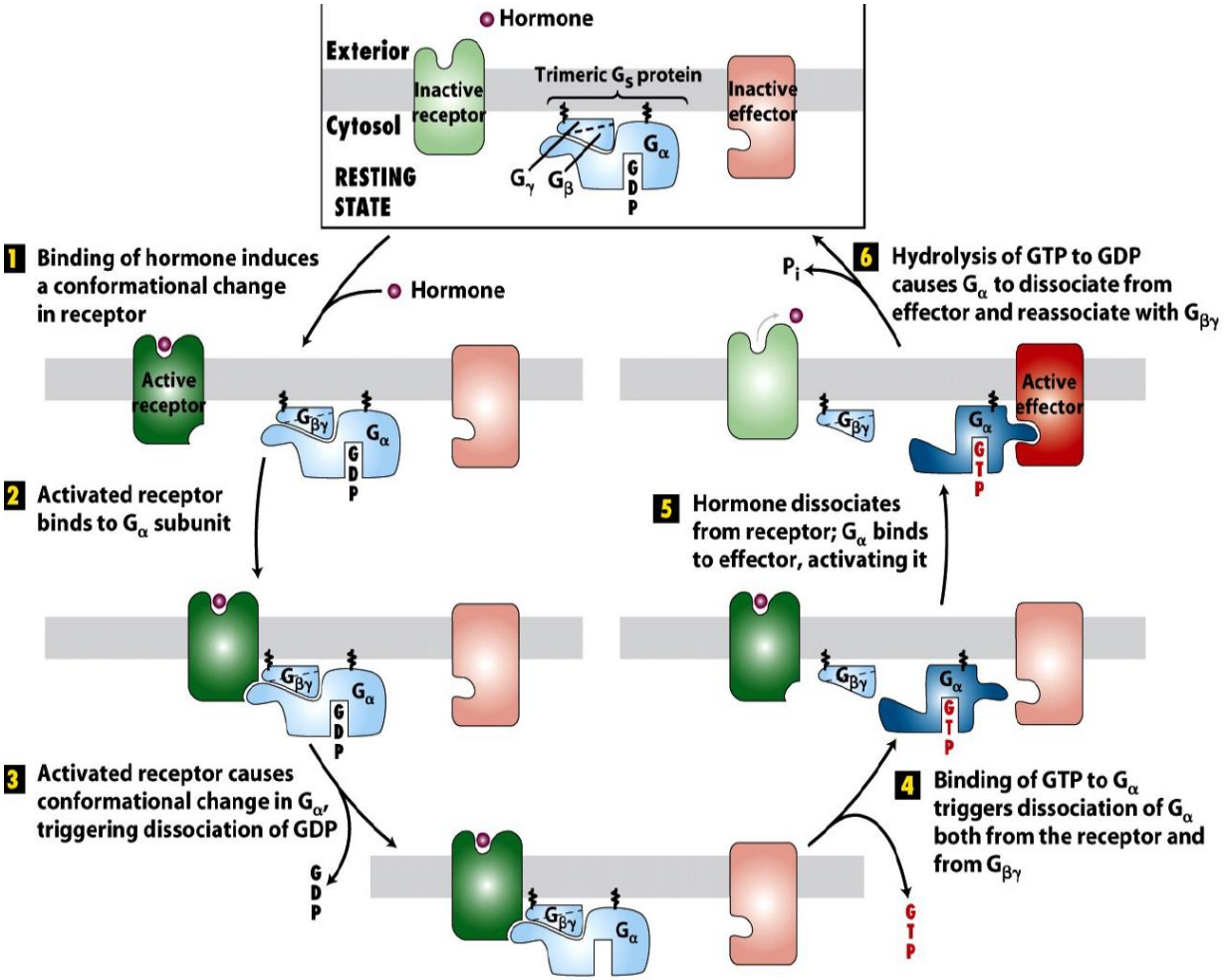
  E.g., the **β-adrenergic receptor** is activated by epinephrine & norepinephrine.



G Protein Signal Cascade

The **signal** is usually passed from a **7-helix receptor** to an intracellular **G-protein**.

- Seven-helix receptors are thus called **GPCR**, or **G-P**rotein-**C**oupled **R**eceptors.
- Approx. 800 different GPCRs are encoded in the human genome.


Hormone
Exterior
Cytosol
RESTING STATE
Inactive receptor
Trimeric G_s protein
Inactive effector
G_γ
G_β
G_α
GDP
1 Binding of hormone induces a conformational change in receptor
Hormone
Active receptor
$G_{\beta\gamma}$
2 Activated receptor binds to G_α subunit
3 Activated receptor causes conformational change in G_α, triggering dissociation of GDP
4 Binding of GTP to G_α triggers dissociation of G_α both from the receptor and from $G_{\beta\gamma}$
GTP
5 Hormone dissociates from receptor; G_α binds to effector, activating it
Active effector
6 Hydrolysis of GTP to GDP causes G_α to dissociate from effector and reassociate with $G_{\beta\gamma}$
P_i

igure 15-13

G Protein Signal Cascade

- **G-proteins** are **heterotrimeric**, with 3 subunits **α**, **β**, **γ**.
- A G-protein that activates cyclic-AMP formation within a cell is called a **stimulatory G-protein**, designated $\mathbf{G_s}$ with alpha subunit $\mathbf{G_{s\alpha}}$.
- $\mathbf{G_s}$ is activated, e.g., by receptors for the hormones **epinephrine** and **glucagon**.

  The **β-adrenergic receptor** is the **GPCR** for epinephrine.

TABLE 15-1 Major Classes of Mammalian Trimeric G Proteins and Their Effectors*

G_α CLASS	ASSOCIATED EFFECTOR	2ND MESSENGER	RECEPTOR EXAMPLES
$G_{\alpha s}$	Adenylyl cyclase	cAMP (increased)	β-Adrenergic (epinephrine) receptor; receptors for glucagon, serotonin, vasopressin
$G_{\alpha i}$	Adenylyl cyclase K^+ channel ($G_{\beta\gamma}$ activates effector)	cAMP (decreased) Change in membrane potential	α_2-Adrenergic receptor Muscarinic acetylcholine receptor
$G_{\alpha olf}$	Adenylyl cyclase	cAMP (increased)	Odorant receptors in nose
$G_{\alpha q}$	Phospholipase C	IP_3, DAG (increased)	α_1-Adrenergic receptor
$G_{\alpha o}$	Phospholipase C	IP_3, DAG (increased)	Acetylcholine receptor in endothelial cells
$G_{\alpha t}$	cGMP phosphodiesterase	cGMP (decreased)	Rhodopsin (light receptor) in rod cells

*A given G_α subclass may be associated with more than one effector protein. To date, only one major $G_{\alpha s}$ has been identified, but multiple $G_{\alpha q}$ and $G_{\alpha i}$ proteins have been described. Effector proteins commonly are regulated by G_α but in some cases by $G_{\beta\gamma}$ or the combined action of G_α and $G_{\beta\gamma}$.
IP_3 = inositol 1,4,5-trisphosphate; DAG = 1,2-diacylglycerol.
SOURCES: See L. Birnbaumer, 1992, *Cell* **71**:1069; Z. Farfel et al., 1999, *New Eng. J. Med.* **340**:1012; and K. Pierce et al., 2002, *Nature Rev. Mol. Cell Biol.* **3**:639.

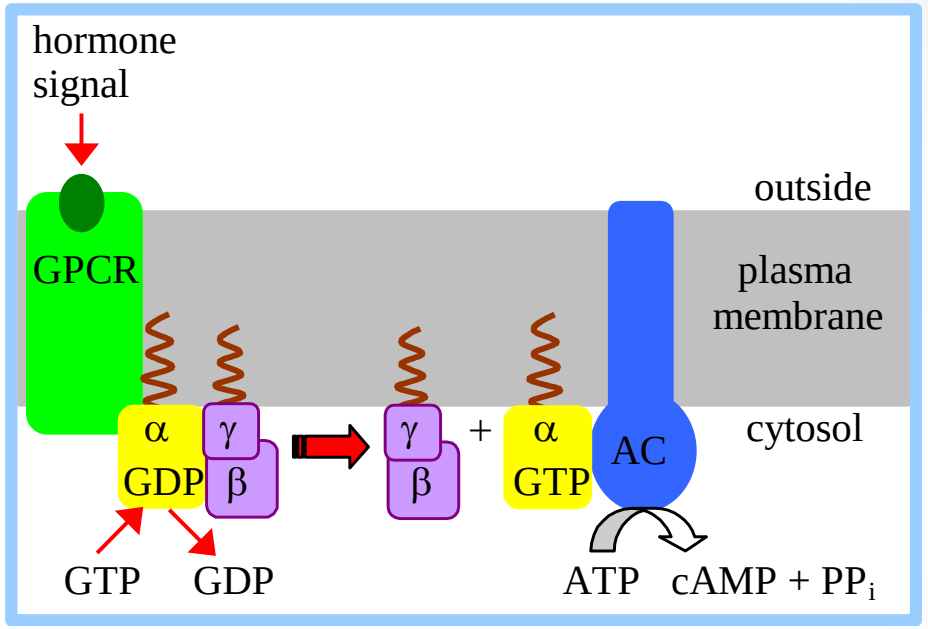
Table 15-1
Molecular Cell Biology, Sixth Edition

Summary of Hormones signaling pathways

IP_3	**cAMP**	**cGMP**	**Tyrosine kinase - intrinsic**	**Tyrosine kinase - receptor associated**	**Steroid**
GnRH	FSH	ANP	Insulin	Prolactin	Glucocorticoid
Gastrin	LH	NO (EDRF)	IGF-1	Cytokines (IL-2,6,8)	Estrogen
Oxytocin	ACTH		FGF	GH	Progesterone
TRH	TSH		PDGF		Testosterone
ADH (V_1)	CRH				Aldosterone
Histamine (H_1)	hCG				Vitamin D
Angiotensin II	PTH				T_3/T_4
	Calcitonin				Cortisol
	Glucagon				
	GHRH (can act via IP_3 as well)				

G Protein Signal Cascade

- The **α** subunit of a G-protein (G_α) binds **GTP**, & can hydrolyze it to GDP + P_i.



- **α** & **γ** subunits have covalently attached **lipid anchors** that bind a G-protein to the plasma membrane cytosolic surface.
- **Adenylate Cyclase** (AC) is a transmembrane protein, with cytosolic domains forming the catalytic site.

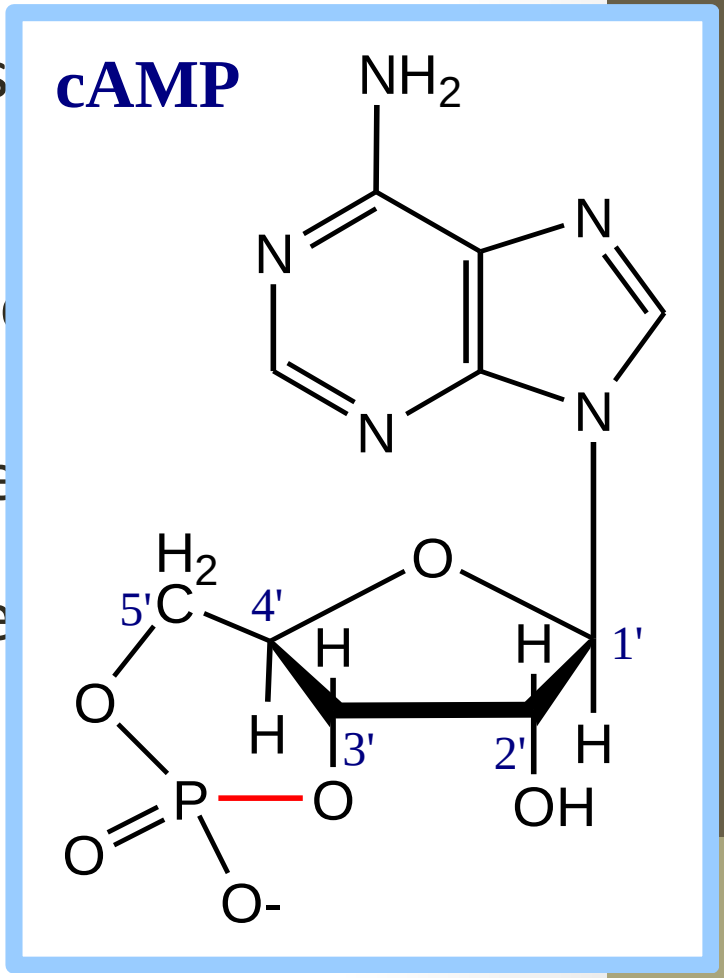
Adenylate Cyclase

Adenylate Cyclase (Adenylyl Cyclas

ATP → cAMP + PP_i

Binding of certain **hormones** (e.g.,
the outer surface of a cell activates
Cyclase to form cAMP within the ce

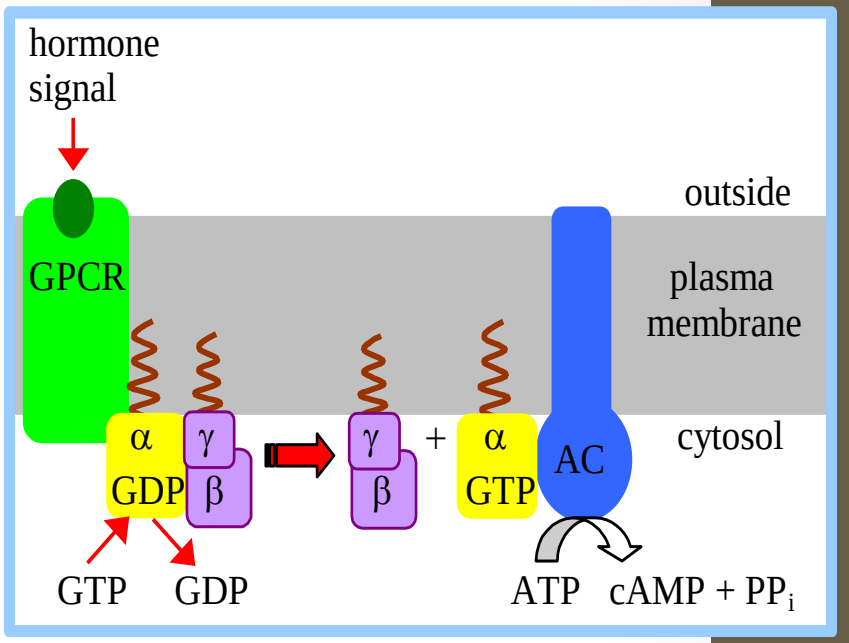
Cyclic AMP is thus considered to be
messenger.

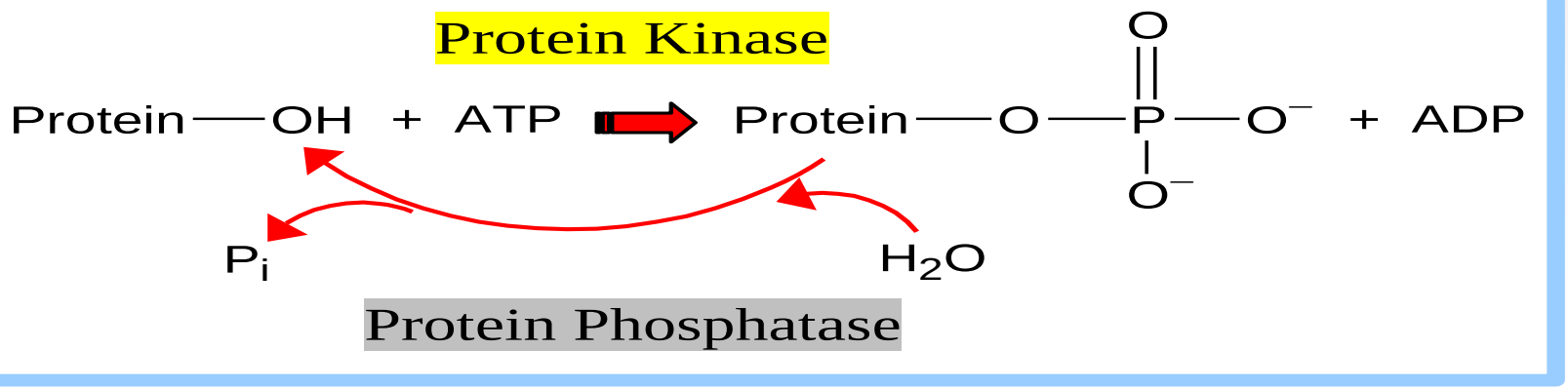


G Protein Signal Cascade

4. **Adenylate Cyclase**, activated by the stimulatory G_{α}-GTP, catalyzes synthesis of **cAMP**.

5. **Protein Kinase A** (cAMP Dependent Protein Kinase) catalyzes transfer of phosphate from ATP to serine or threonine residues of various cellular proteins, altering their activity.





Protein kinases and phosphatases are themselves regulated by complex signal cascades. For example:

- Some protein kinases are activated by **Ca^{++}-calmodulin**.
- **Protein Kinase A** is activated by **cyclic-AMP** (cAMP).

Protein Kinase A (cAMP-Dependent Protein Kinase) transfers P_i from ATP to OH of a Ser or Thr in a particular 5-amino acid sequence.

Protein Kinase A in the resting state is a complex of:

- 2 catalytic subunits (**C**)
- 2 regulatory subunits (**R**).

R_2C_2 : When each (**R**) binds 2 cAMP, a conformational change causes (**R**) to release (**C**).

The catalytic subunits can then catalyze phosphorylation of Ser or Thr on target proteins.

PKIs, Protein Kinase Inhibitors, modulate activity of the catalytic subunits (**C**).

Turn off of the signal:

1. **G_α** hydrolyzes GTP to GDP + P_i. (**GTPase**).

   The presence of **GDP** on G_α causes it to rebind to the inhibitory **βγ** complex.

   Adenylate Cyclase is no longer activated.

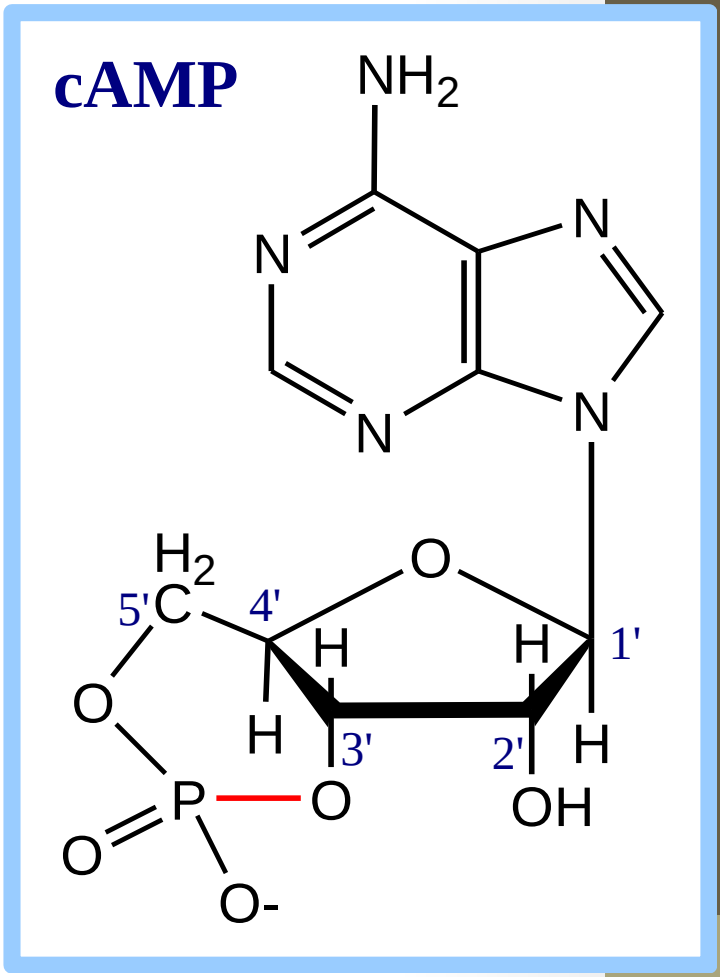
2. **Phosphodiesterases** catalyze hydrolysis of **cAMP → AMP**.

Phosphodiesterase enzymes catalyze:

cAMP + H_2O → AMP

The phosphodiesterase that cleaves cAMP is activated by phosphorylation catalyzed by Protein Kinase A.

Thus **cAMP stimulates its own degradation**, leading to rapid turnoff of a cAMP signal.



3. **Receptor desensitization** varies with the hormone.

- In some cases the **activated receptor** is **phosphorylated** via a G-protein Receptor Kinase.
- The phosphorylated receptor then may bind to a protein **β-arrestin.**
- **β-Arrestin** promotes **removal of the receptor** from the membrane by clathrin-mediated endocytosis.
- **β-Arrestin** may also bind a cytosolic **Phosphodiesterase**, bringing this enzyme close to where cAMP is being produced, contributing to signal turnoff.

4. **Protein Phosphatase** catalyzes removal by hydrolysis of phosphates that were attached to proteins via Protein Kinase A.

- **Different** isoforms of G_α have different signal roles. E.g.:
  - The **stimulatory $G_{s\alpha}$**, when it binds GTP, **activates** Adenylate cyclase.
  - An **inhibitory $G_{i\alpha}$**, when it binds GTP, **inhibits** Adenylate cyclase.

    Different effectors & their receptors induce **$G_{i\alpha}$** to exchange GDP for GTP than those that activate **$G_{s\alpha}$**.
- The complex of **$G_{\beta,\gamma}$** that is released when G_α binds GTP is itself an effector that binds to and **activates or inhibits** several other proteins.

  E.g., **$G_{\beta,\gamma}$ inhibits** one of several isoforms of **Adenylate Cyclase**, contributing to rapid signal turnoff in cells that express that enzyme.

Small GTP-binding proteins include (roles indicated):

- **initiation & elongation factors** (protein synthesis).
- **Ras** (growth factor signal cascades).
- **Rab** (vesicle targeting and fusion).
- **ARF** (forming vesicle coatomer coats).
- **Ran** (transport of proteins into & out of the nucleus).
- **Rho** (regulation of actin cytoskeleton)

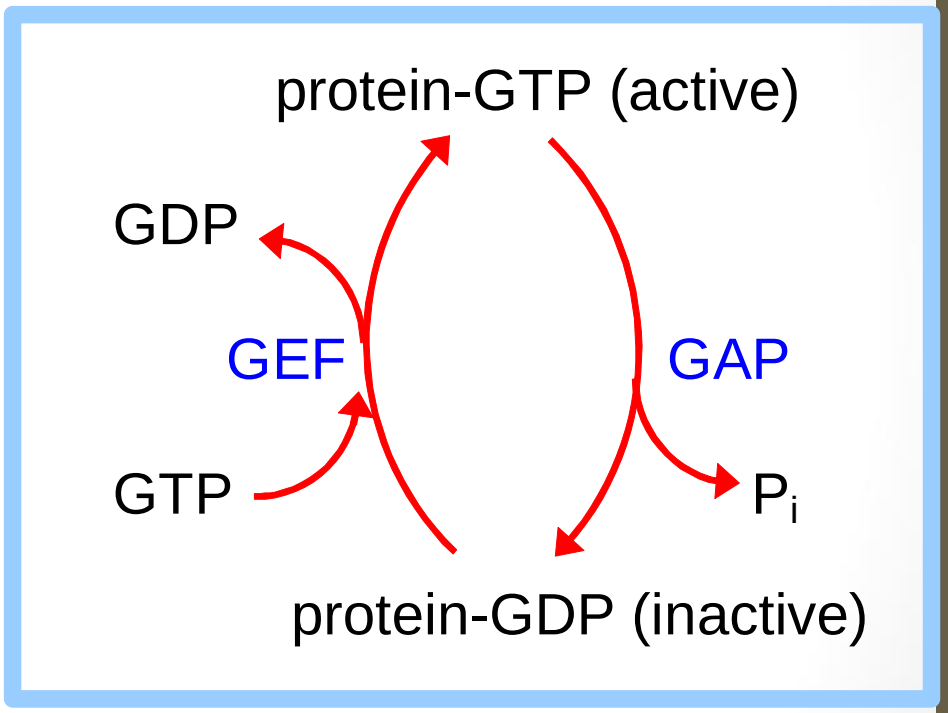
All GTP-binding proteins differ in conformation depending on whether GDP or GTP is present at their nucleotide binding site.

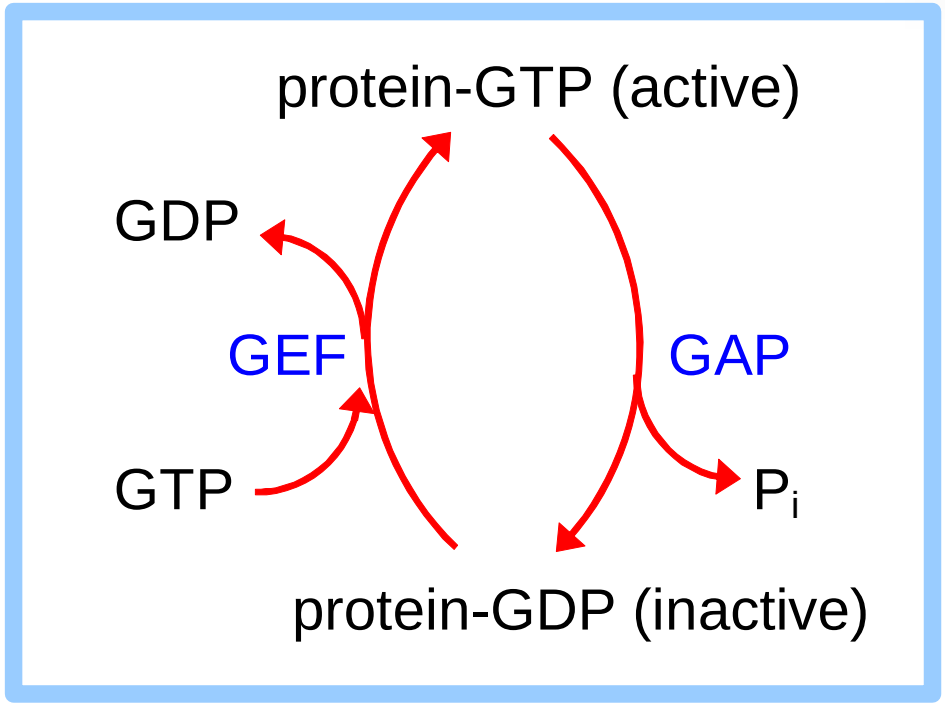
Generally, **GTP** binding induces the **active** state.

Most GTP-binding proteins depend on **helper proteins**:

GAPs,

GTPase **A**ctivating **P**roteins, promote GTP hydrolysis.

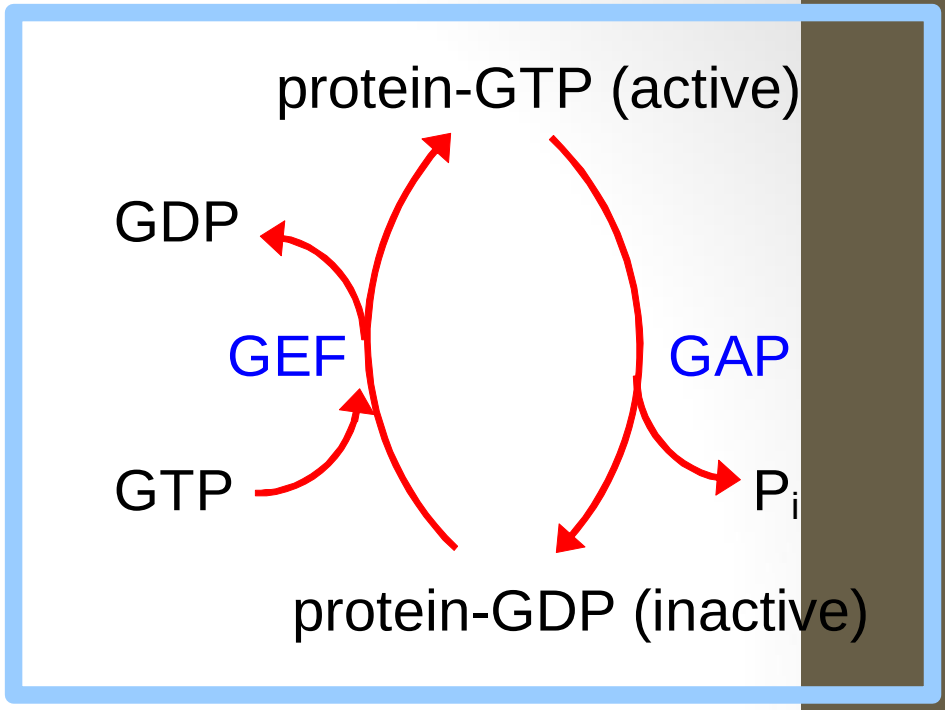




- **G_α** of a heterotrimeric G protein has innate capability for GTP hydrolysis.

  It has the essential arginine residue normally provided by a GAP for small GTP-binding proteins.

GEFs, **G**uanine **N**ucleotide **E**xchange Factors, promote GDP/GTP exchange.



- An activated **receptor** (GPCR) normally serves as **GEF** for a heterotrimeric G-protein.

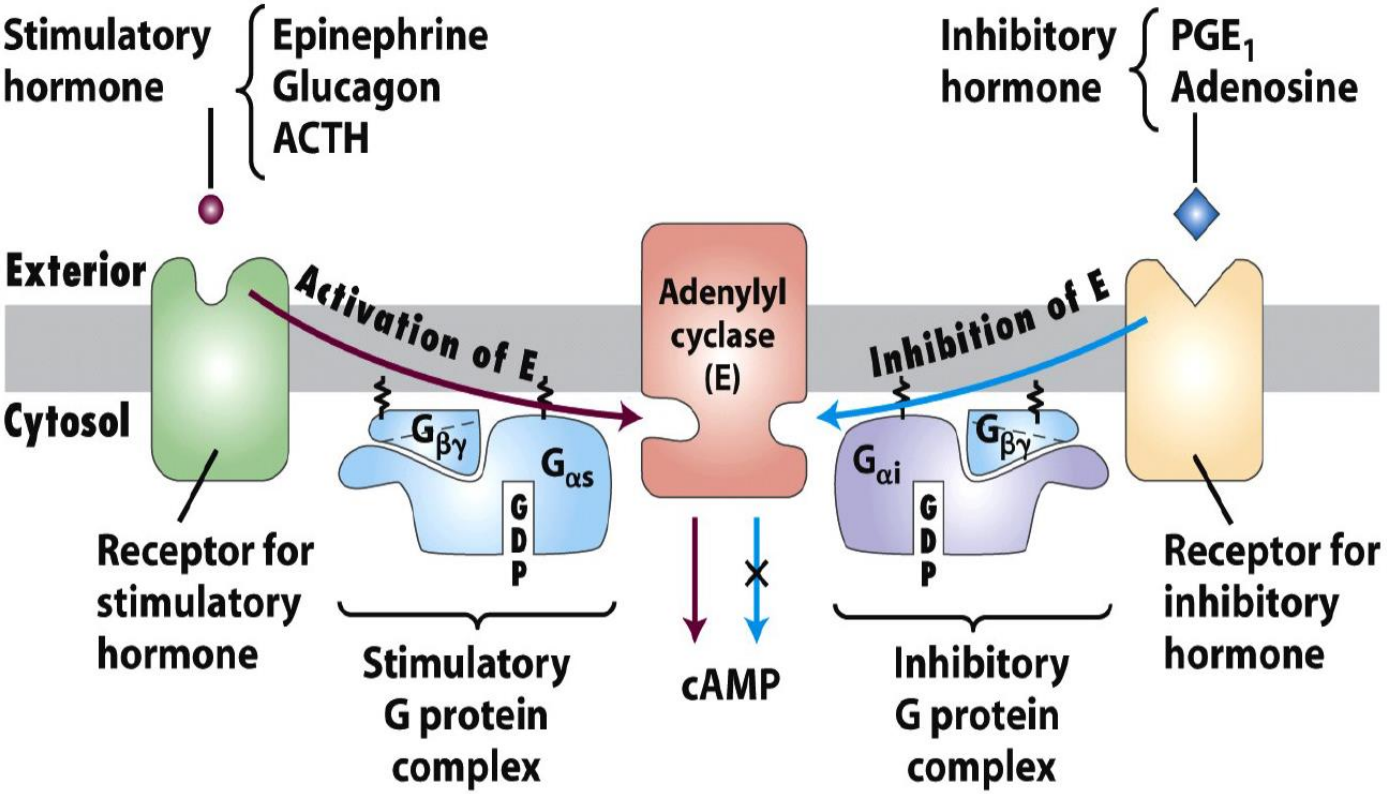

Stimulatory hormone
Epinephrine
Glucagon
ACTH
Inhibitory hormone
PGE1
Adenosine
Exterior
Cytosol
Activation of E
Inhibition of E
Adenylyl cyclase (E)
Gβγ
Gαs
Gαi
GDP
Receptor for stimulatory hormone
Receptor for inhibitory hormone
Stimulatory G protein complex
Inhibitory G protein complex
cAMP

Figure 15-21

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, and Src.**

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):

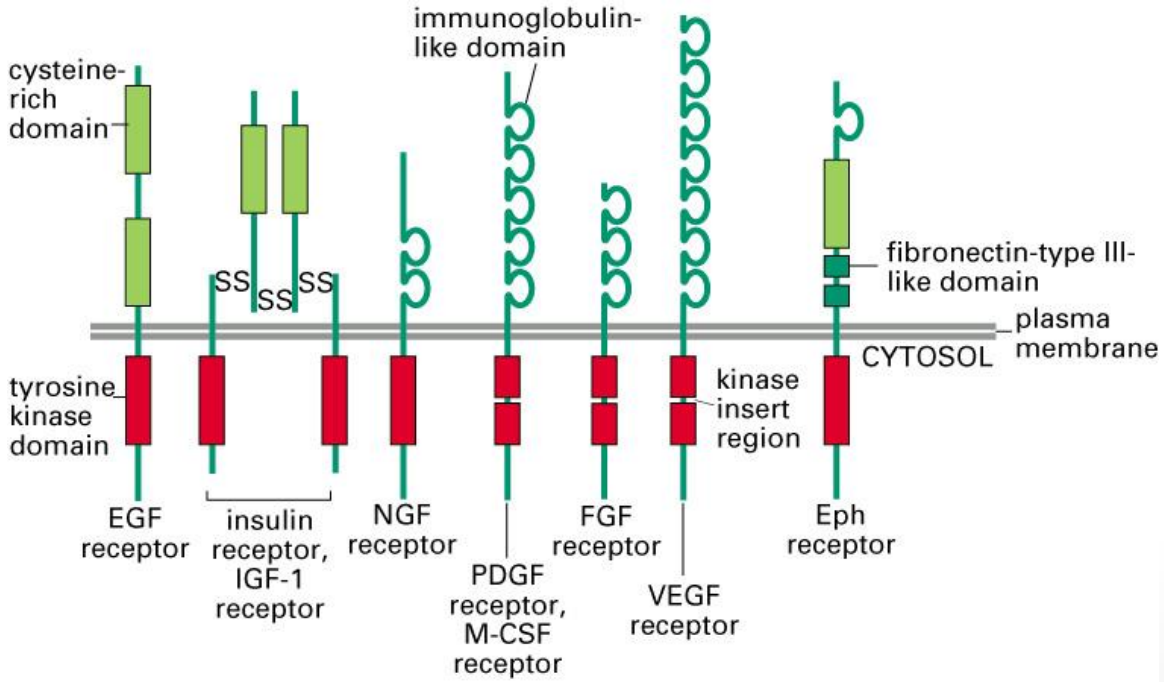


Figure 15–49. Molecular Biology of the Cell, 4th Edition.

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.**

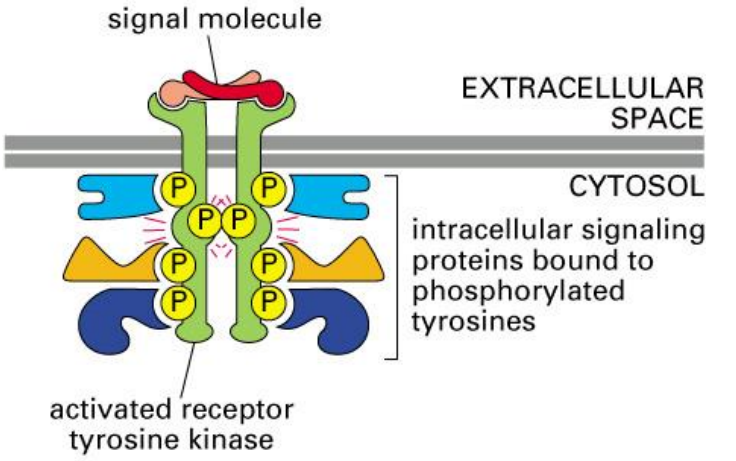


Figure 15–52. Molecular Biology of the Cell, 4th Edition.

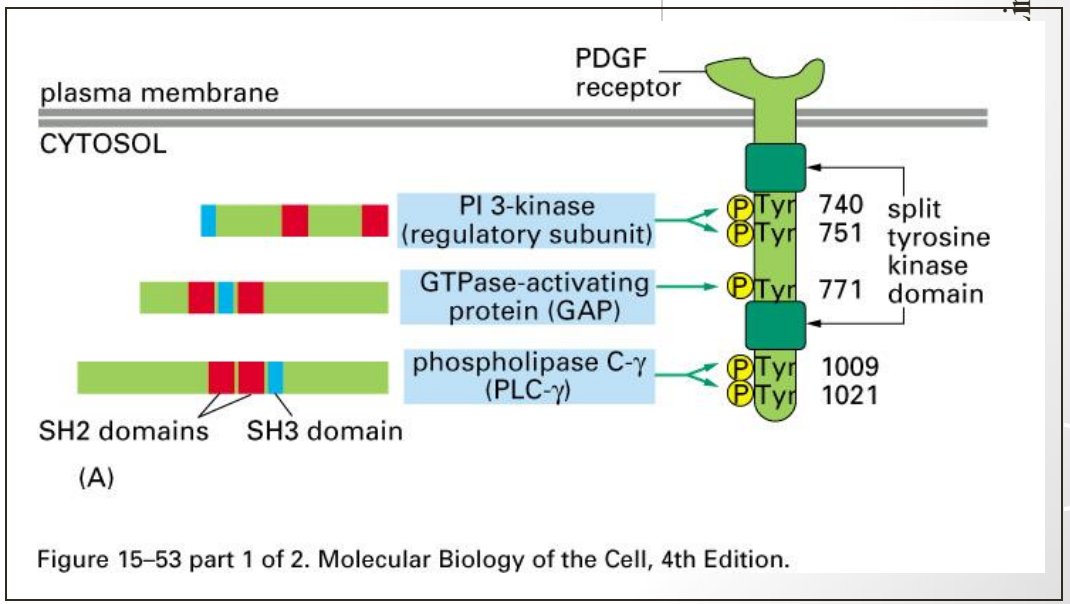
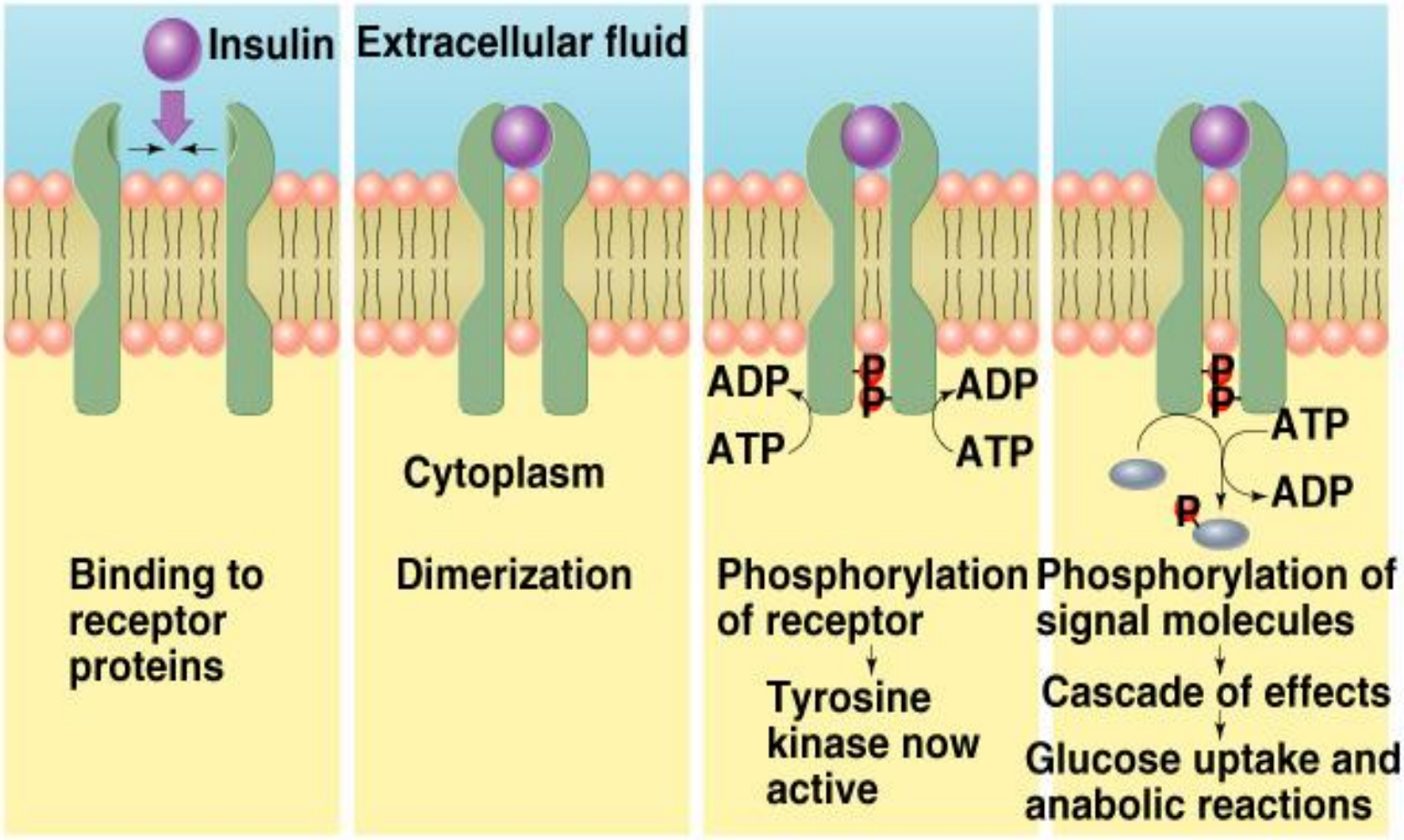


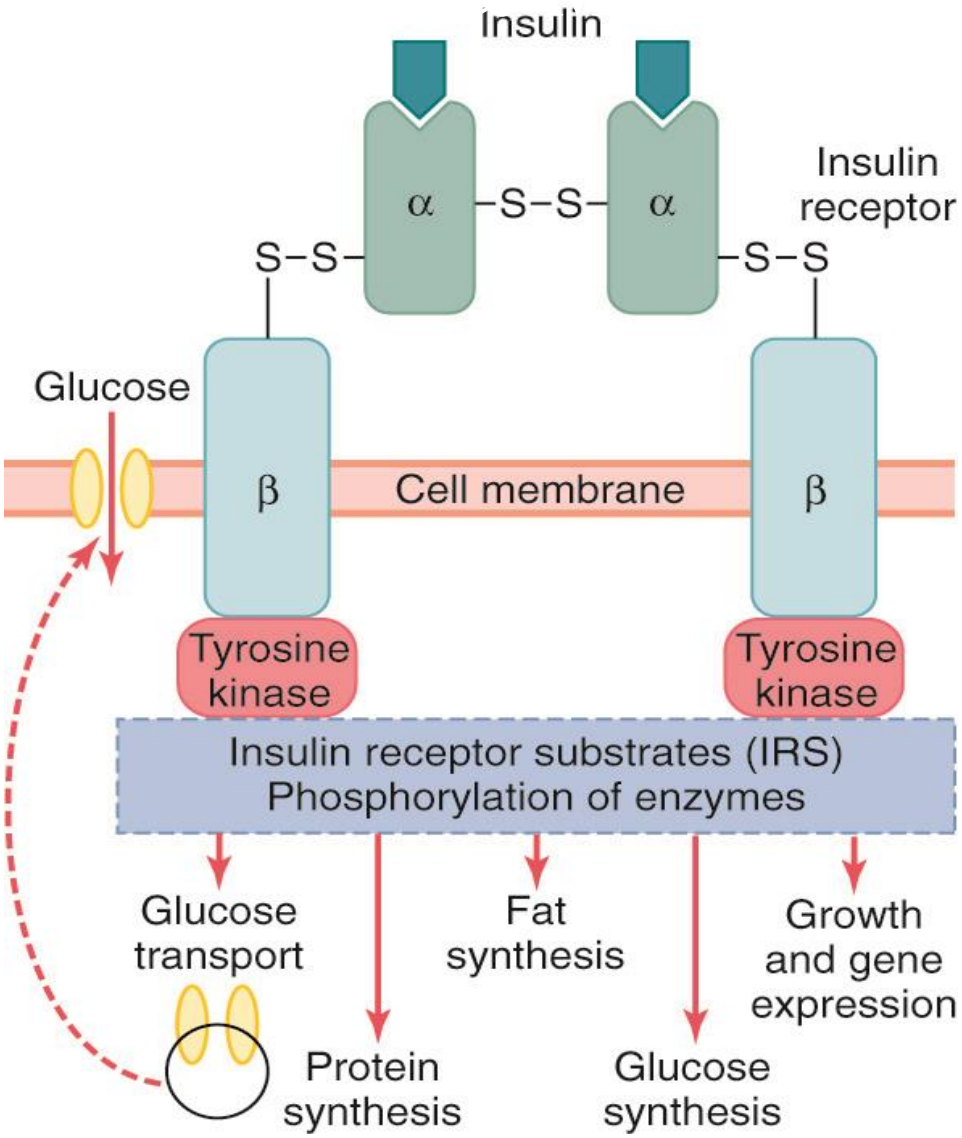
Figure 15–53 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

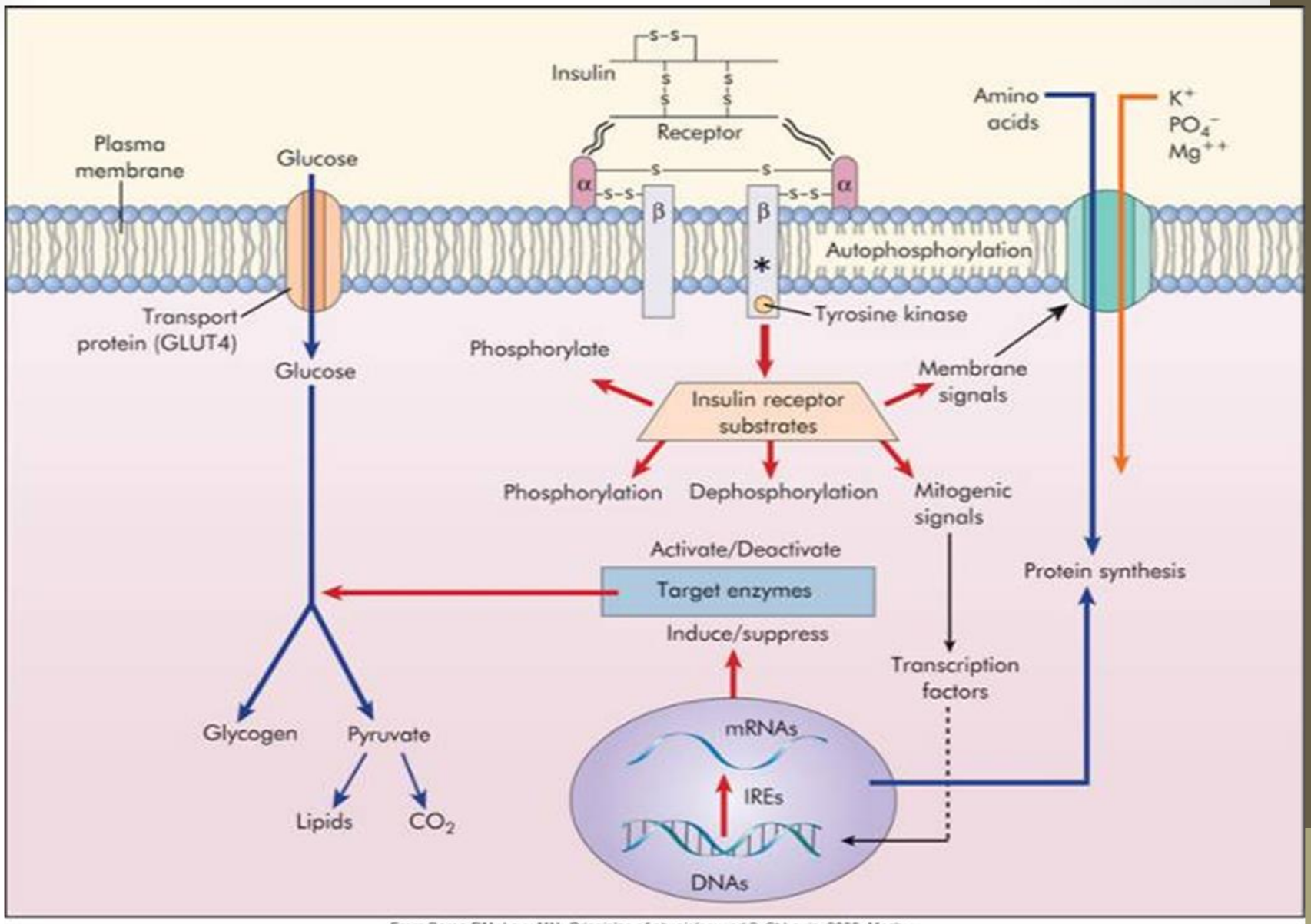
Tyrosine Kinase

- 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




Insulin
Insulin receptor
α
S–S
α
S–S
S–S
Glucose
β
Cell membrane
β
Tyrosine kinase
Tyrosine kinase
Insulin receptor substrates (IRS)
Phosphorylation of enzymes
Glucose transport
Protein synthesis
Fat synthesis
Glucose synthesis
Growth and gene expression



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

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.**

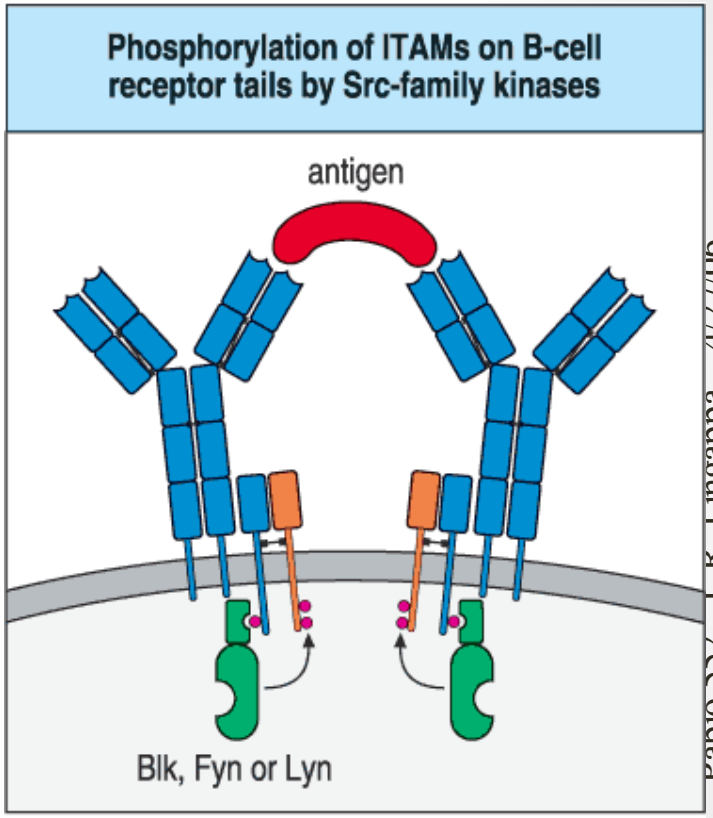


Fig 6.9 © 2001 Garland Science

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

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

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

Guanylyl cyclases

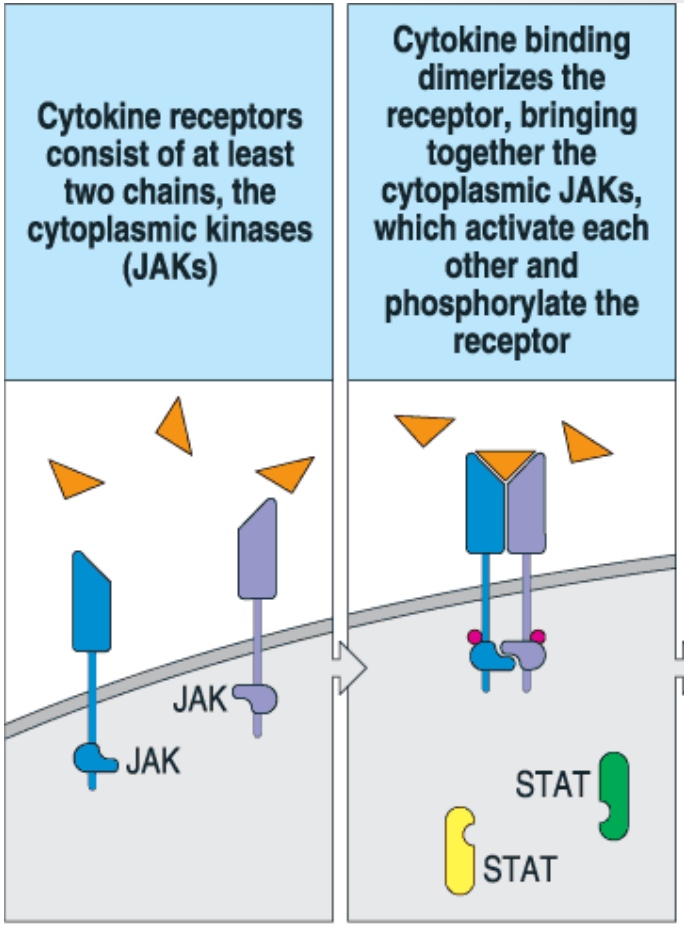
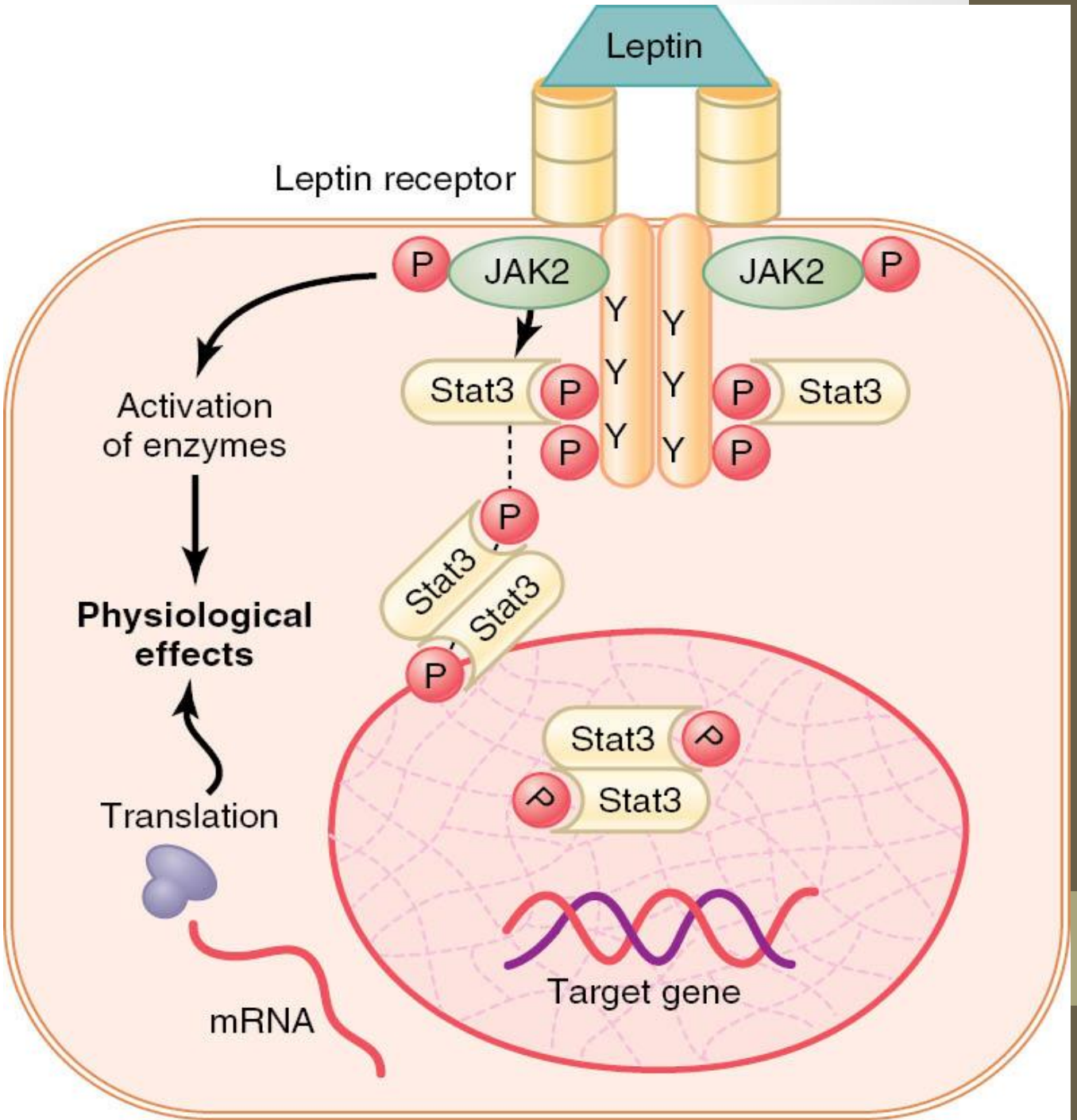


Fig 6.22 © 2001 Garland Science

From Janeway, Immunobiology, 5th edition

Pabio 552, J. R. Lingappa 4/27/06

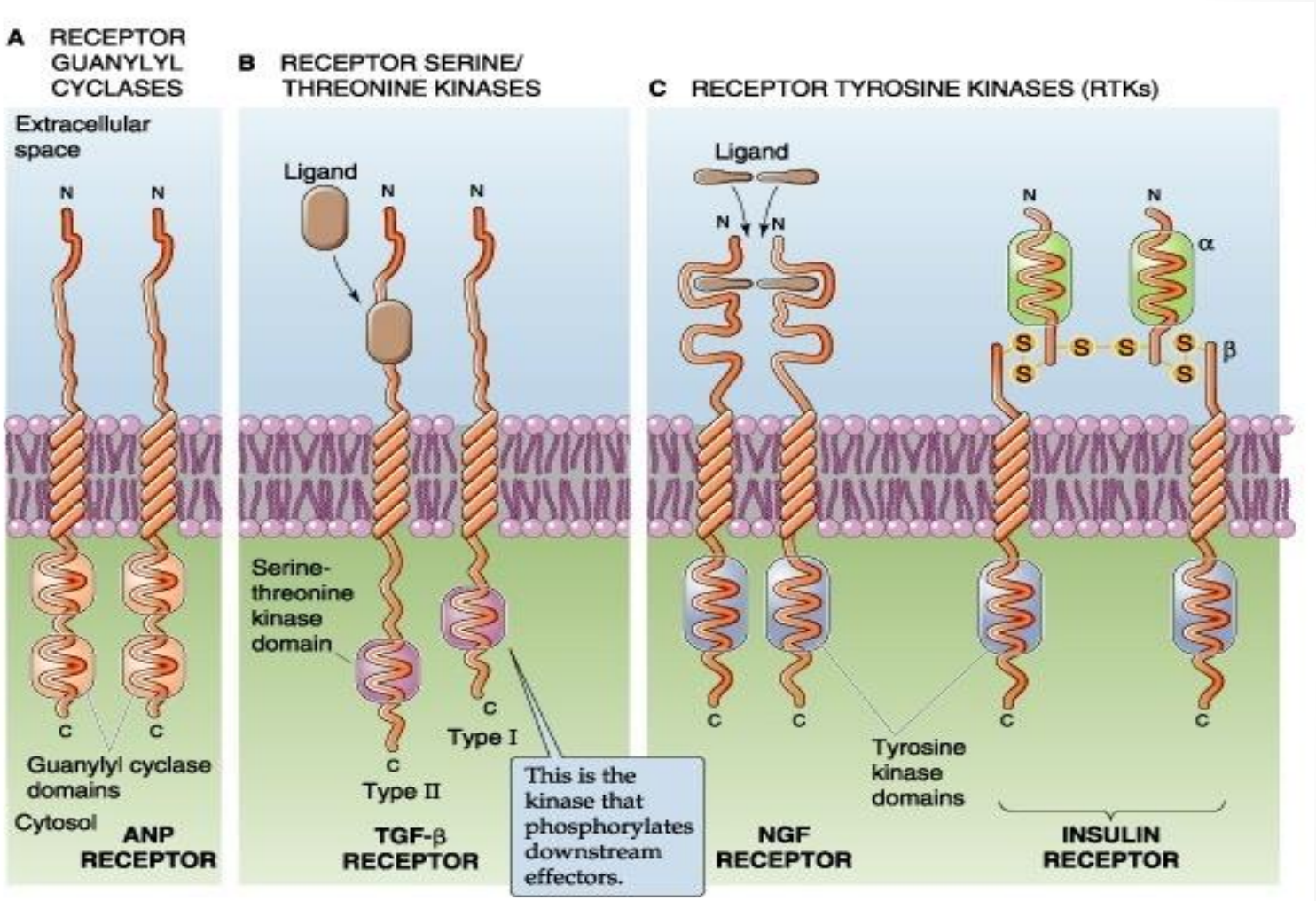
Enzyme-linked Receptor (the Leptin receptor)
JAK= Janus Kinase
STAT= Signal Transducer and Activator of Transcription

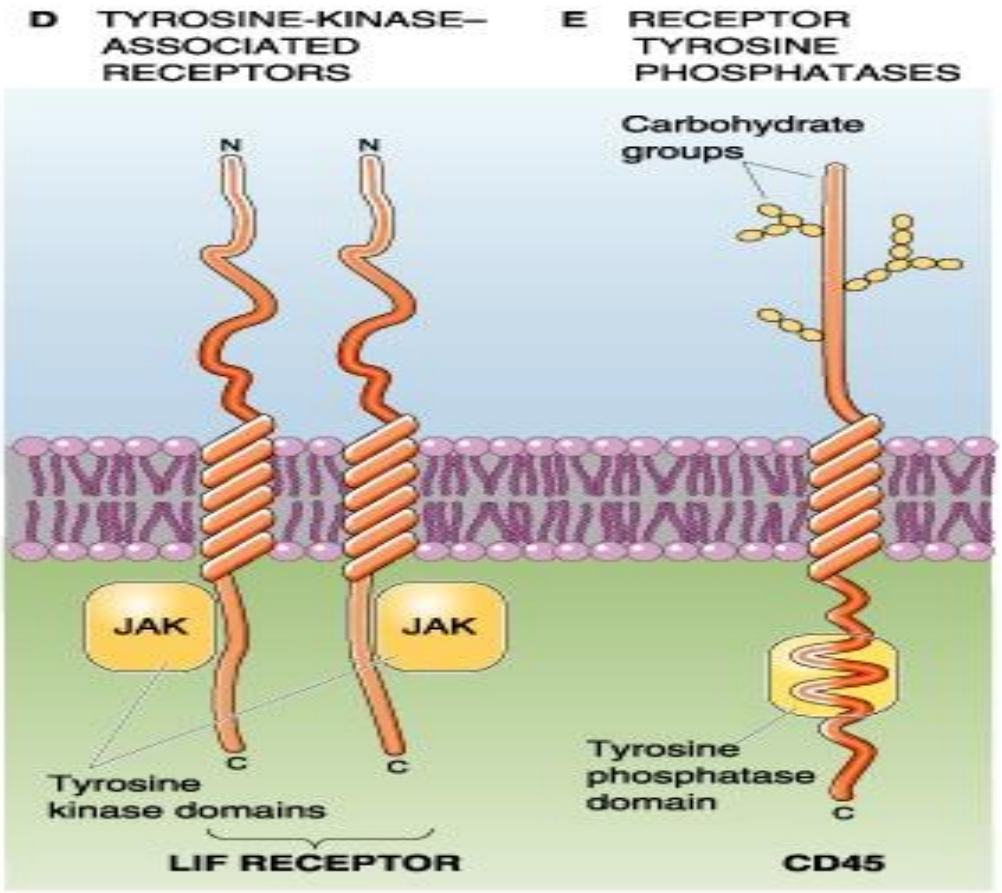


Enzyme-linked receptor (the leptin receptor)

- 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

Enzyme linked receptors





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:

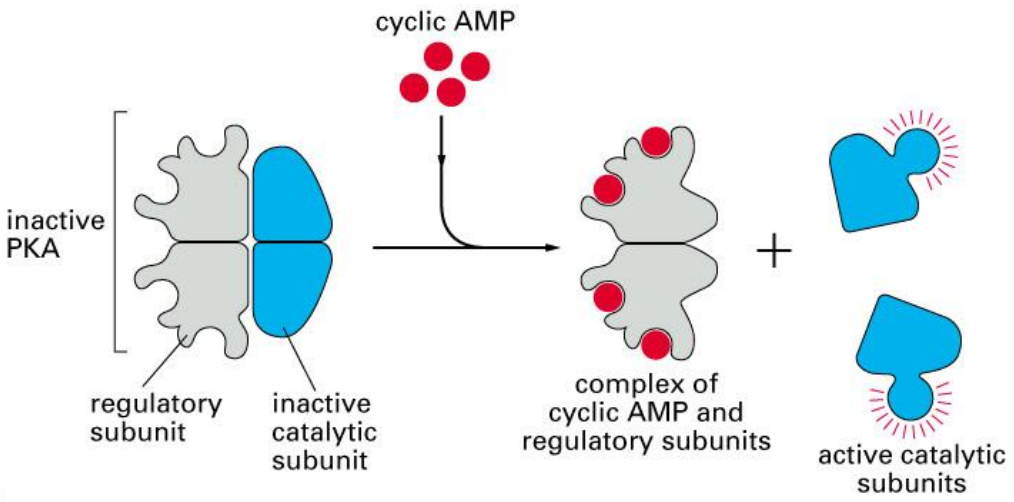


Figure 15–32. Molecular Biology of the Cell, 4th Edition.

cAMP production:

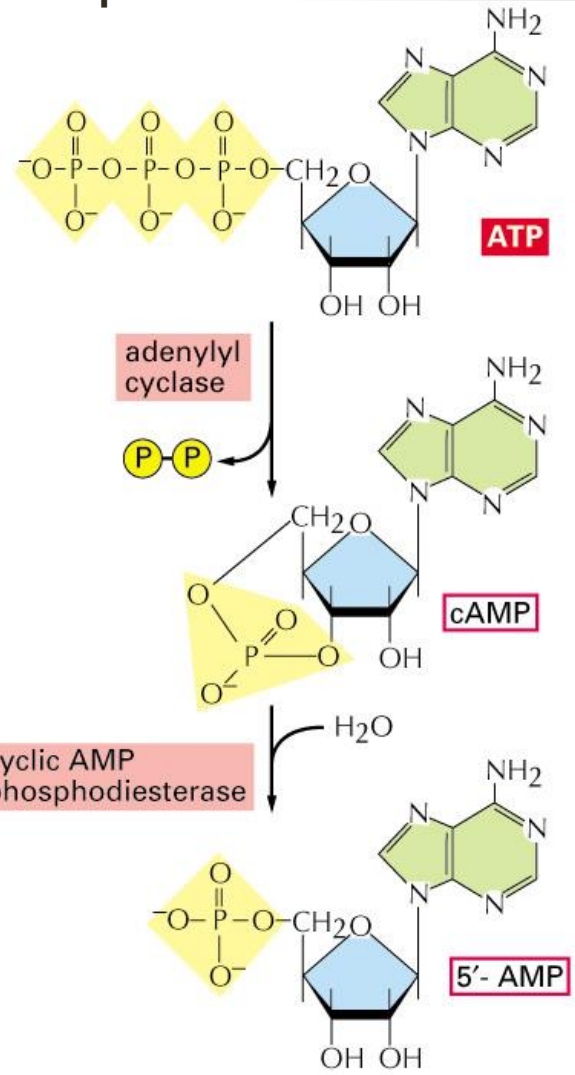


Figure 15–31. Molecular Biology of the Cell, 4th Edition.

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.**

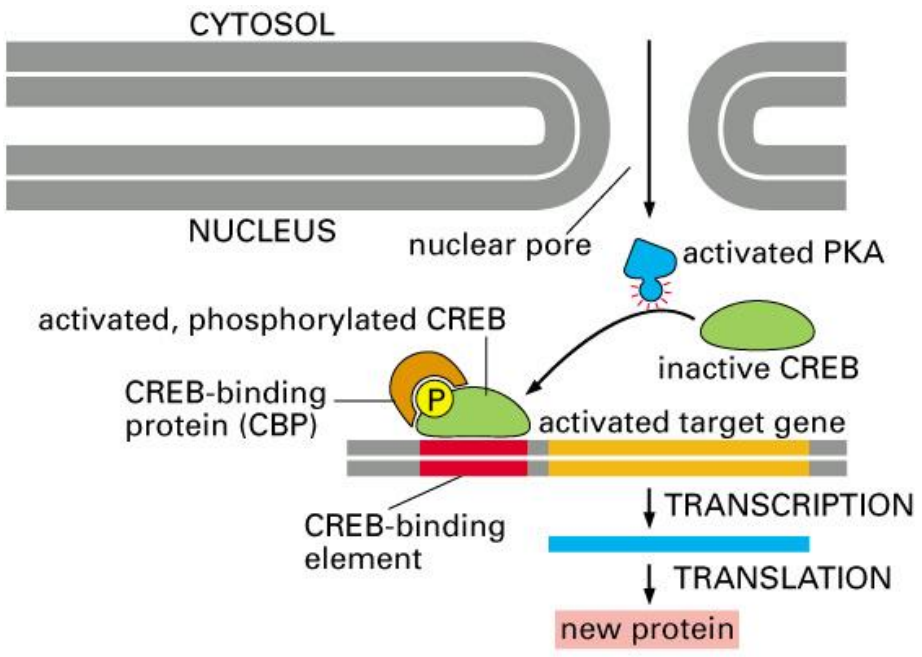


Figure 15–33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

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:

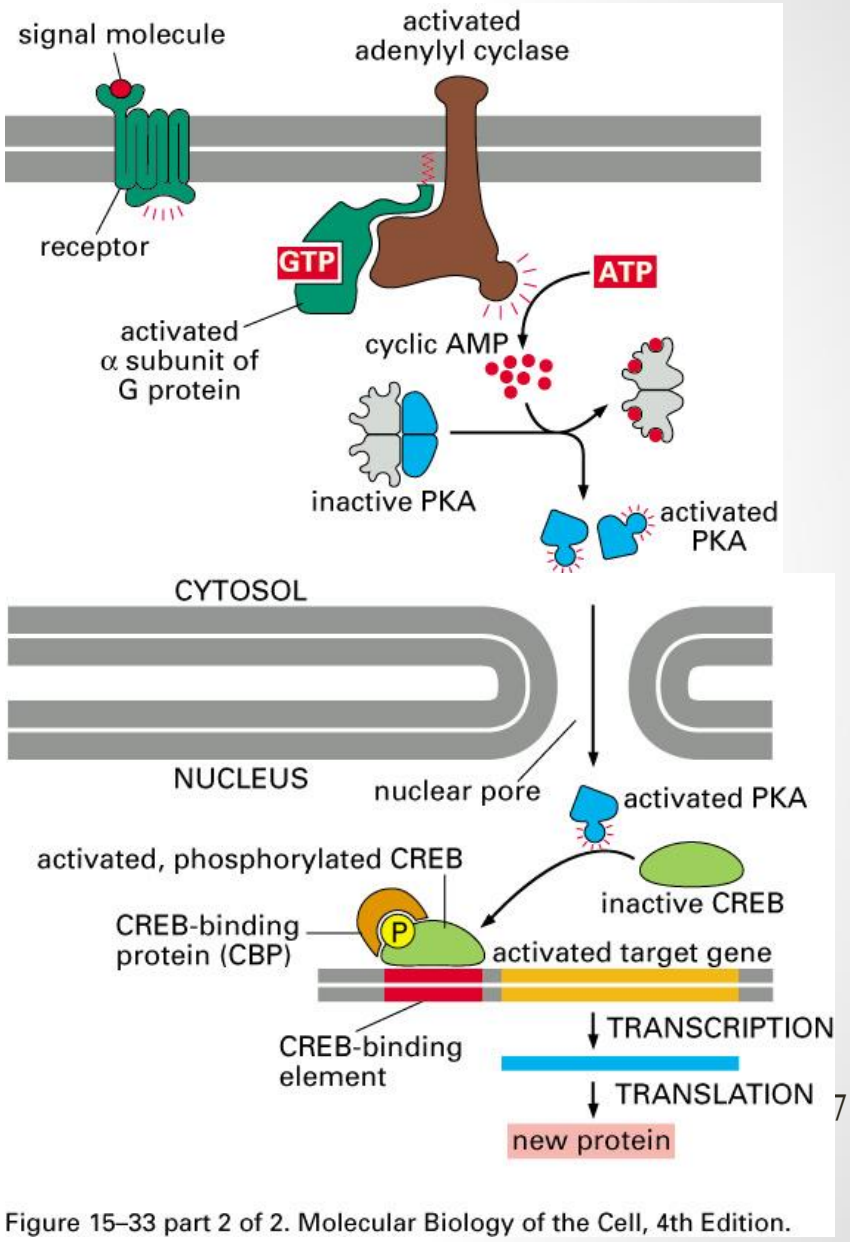


Figure 15–33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

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.

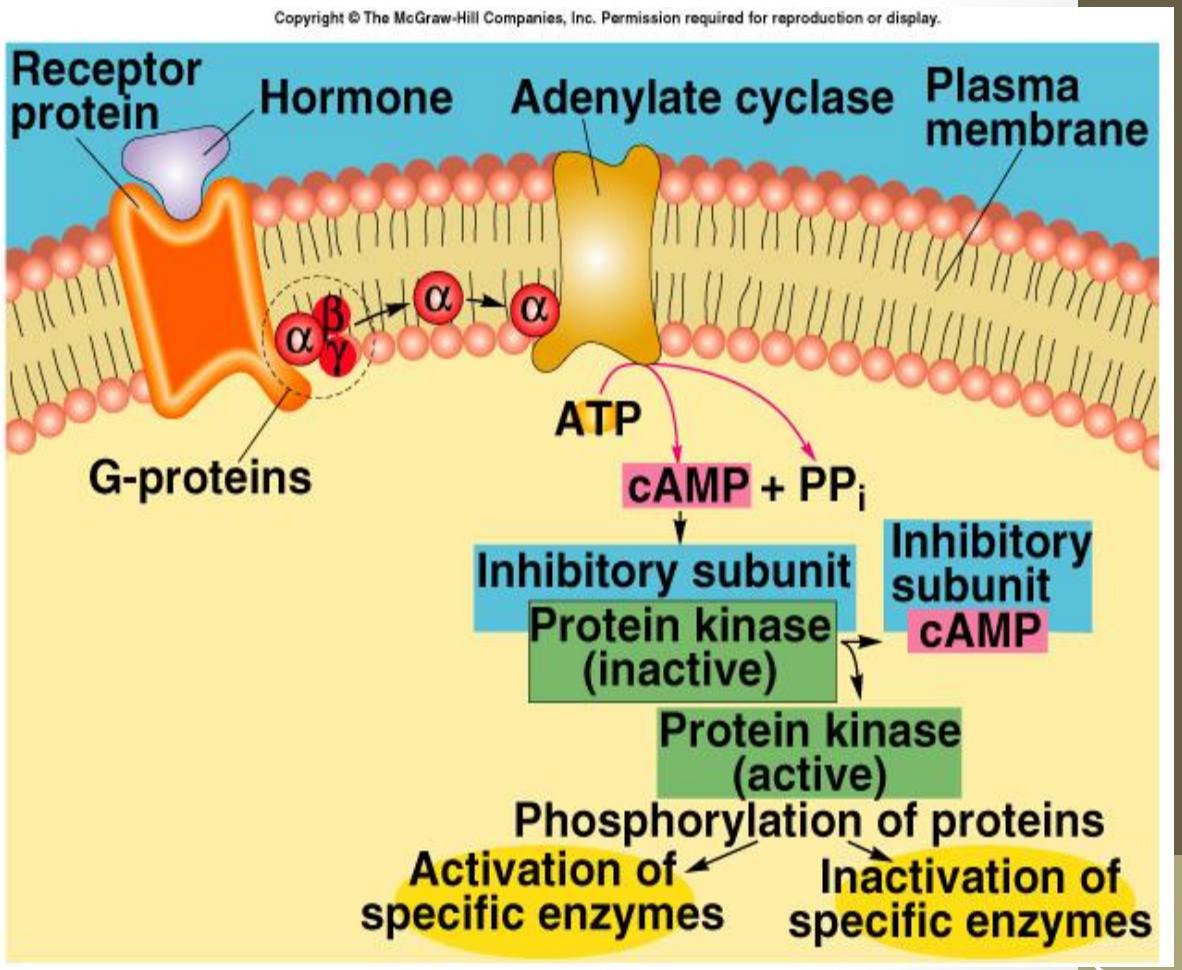
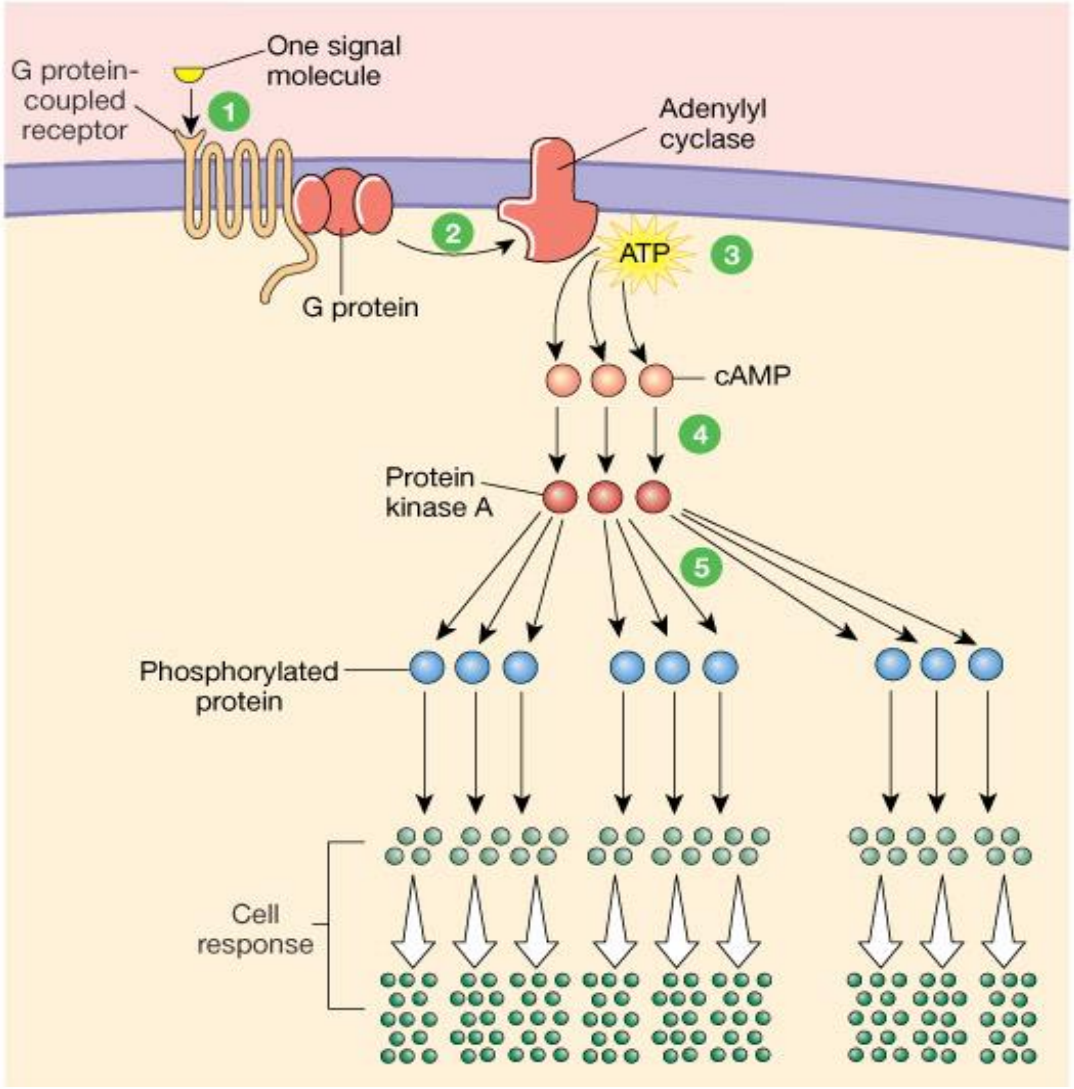


TABLE 20-3 Metabolic Responses to Hormone-Induced Rise in cAMP in Various Tissues

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**

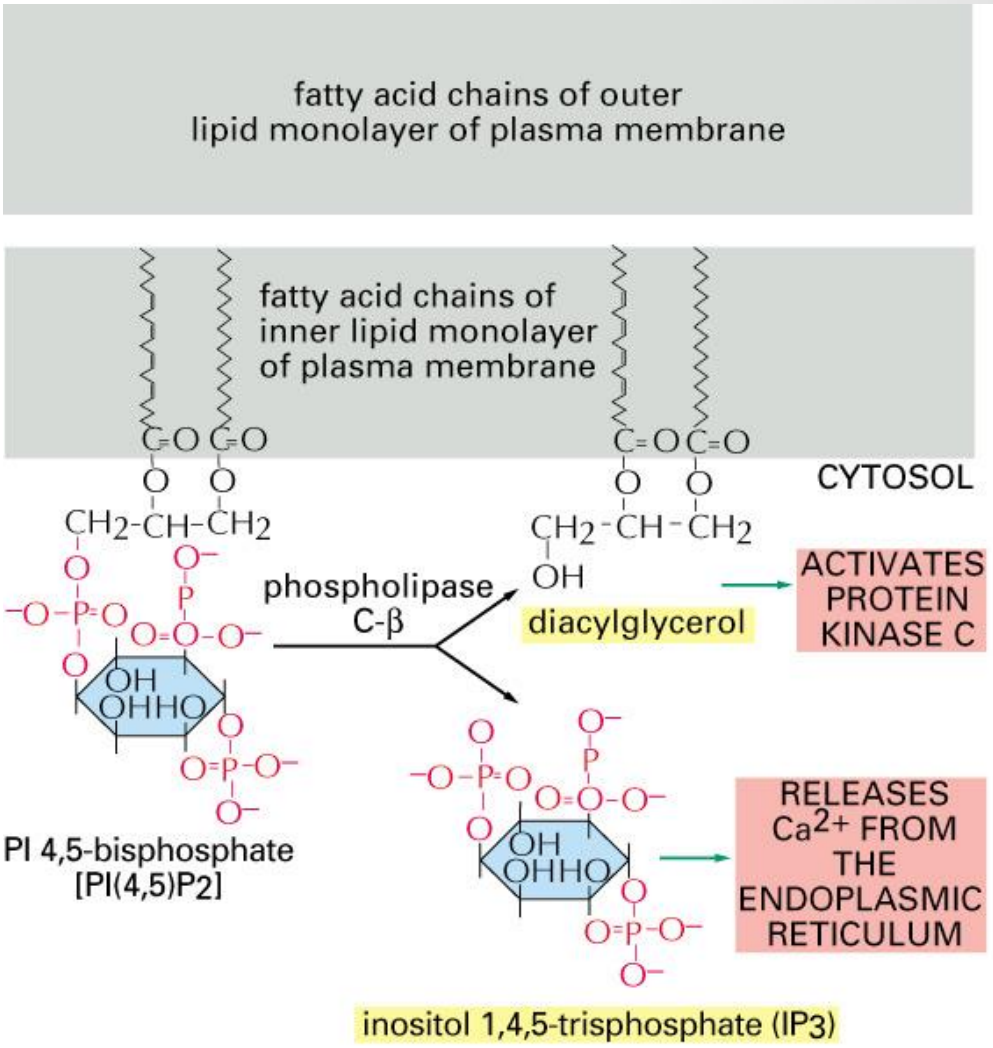
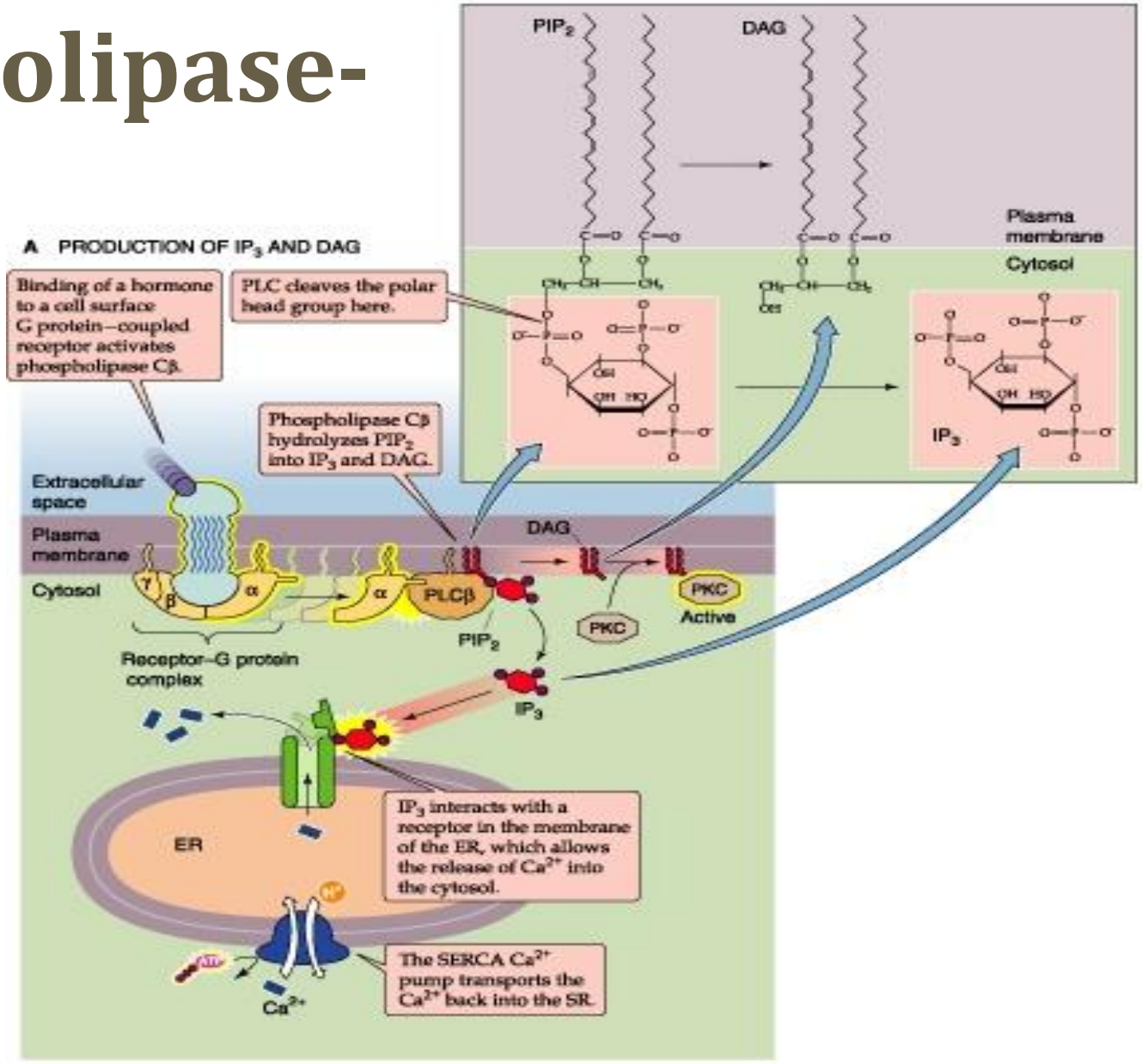


Figure 15–35. Molecular Biology of the Cell, 4th Edition.

Phospholipase-C-Ca^{2+}



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)**

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**

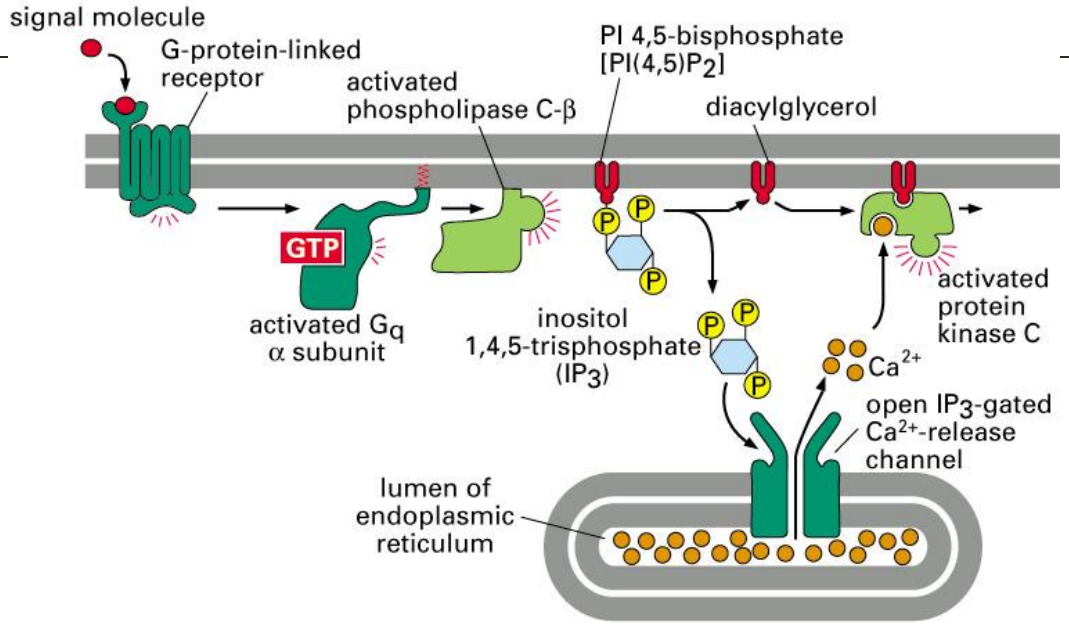
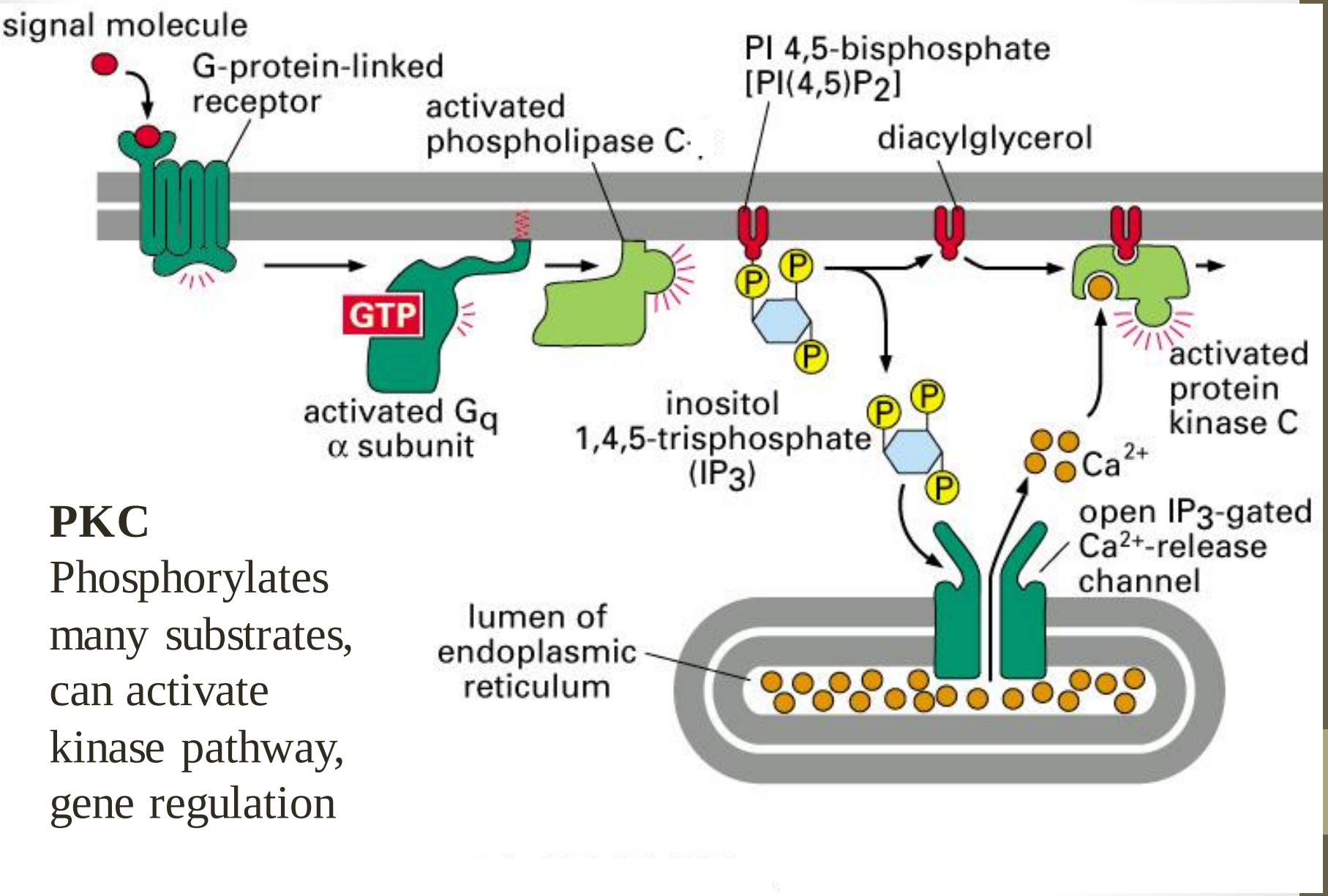


Figure 15–36. Molecular Biology of the Cell, 4th Edition.

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.

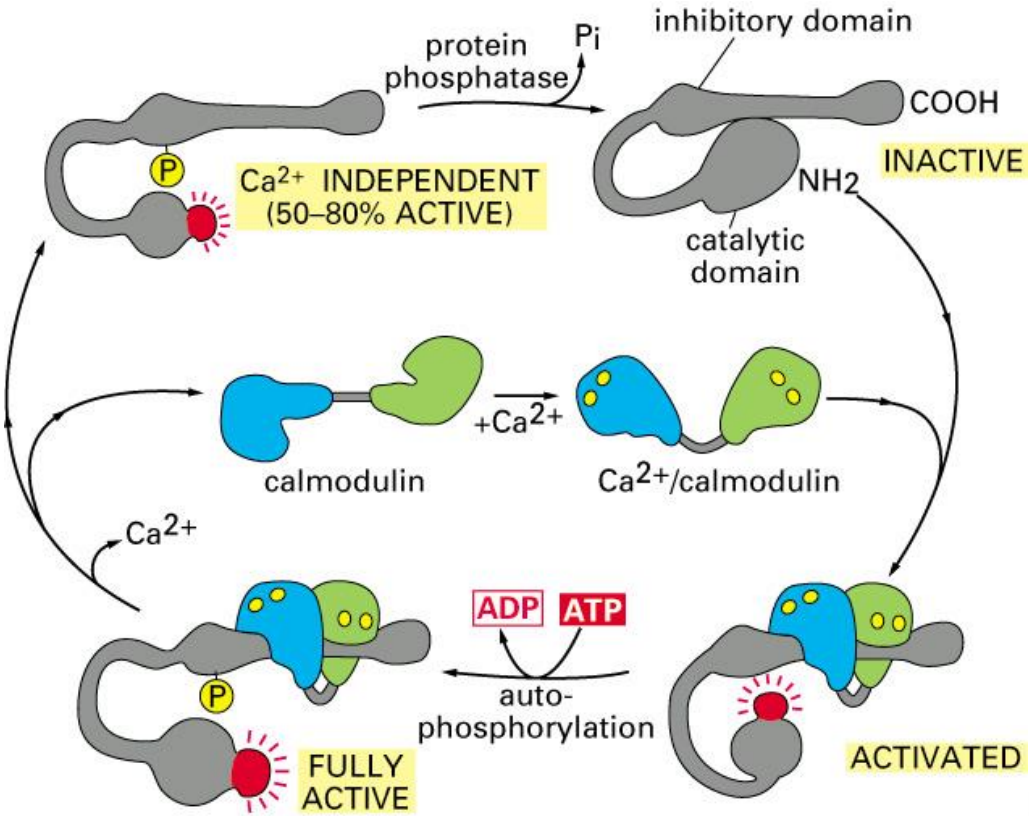
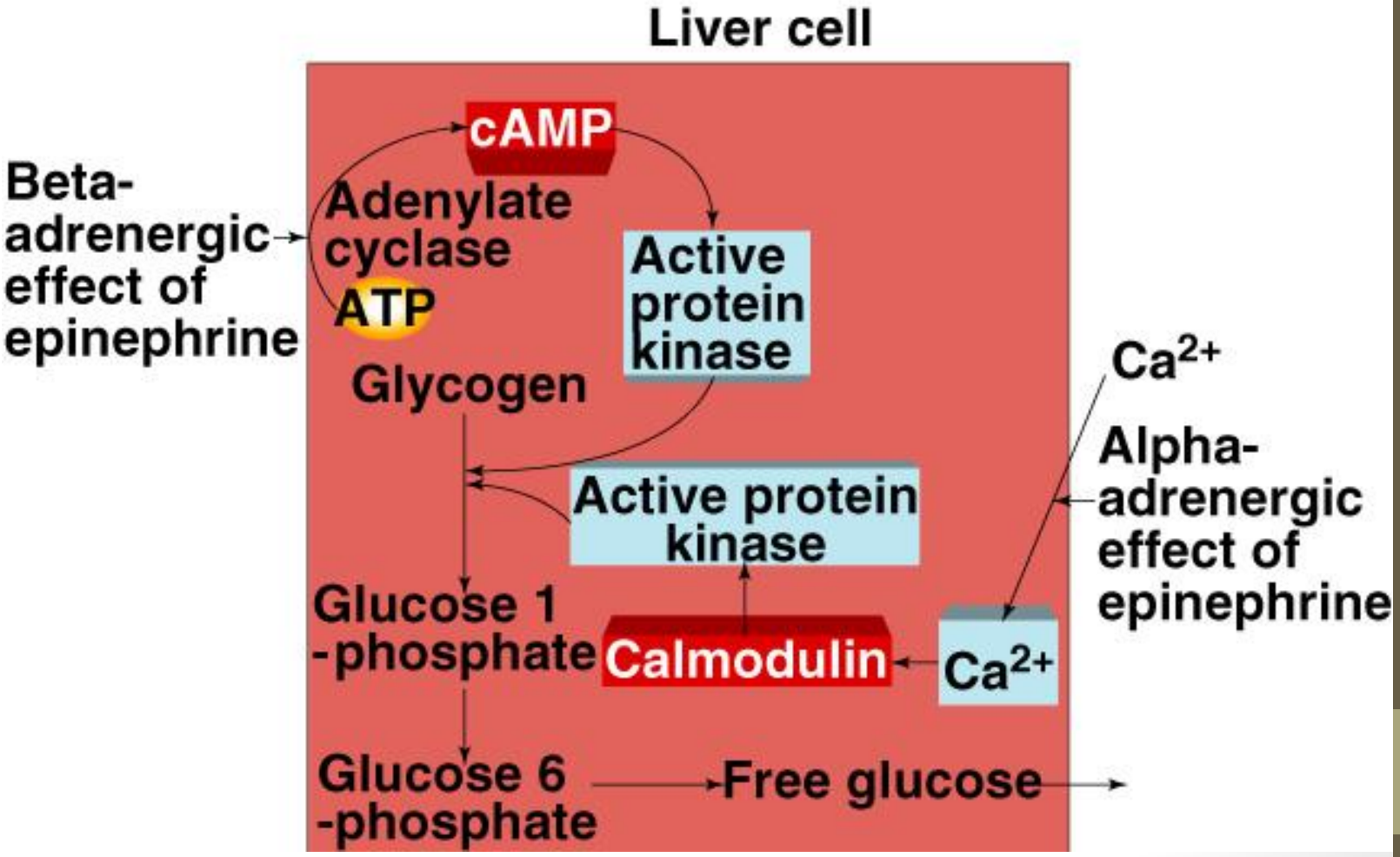


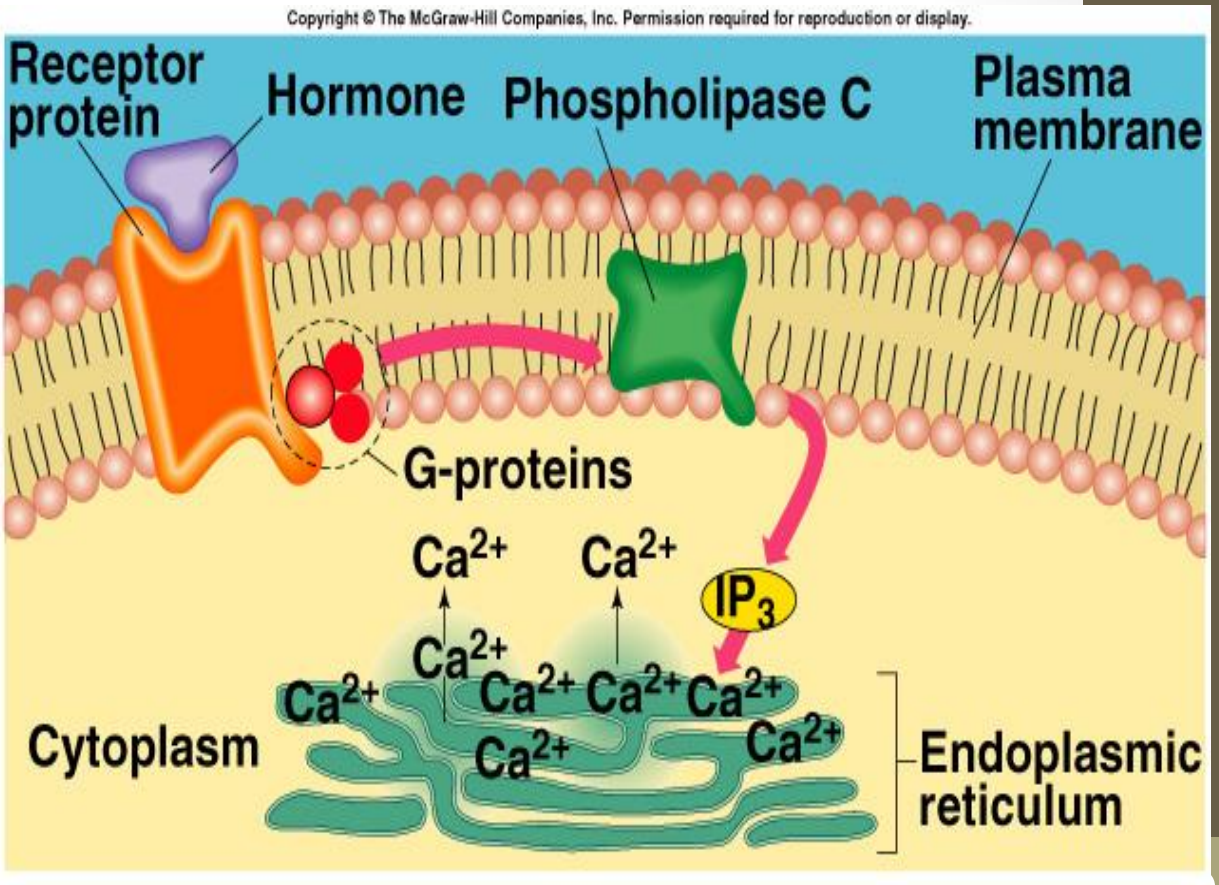
Figure 15–41. Molecular Biology of the Cell, 4th Edition.

Epinephrine Can Act Through Two 2nd Messenger Systems

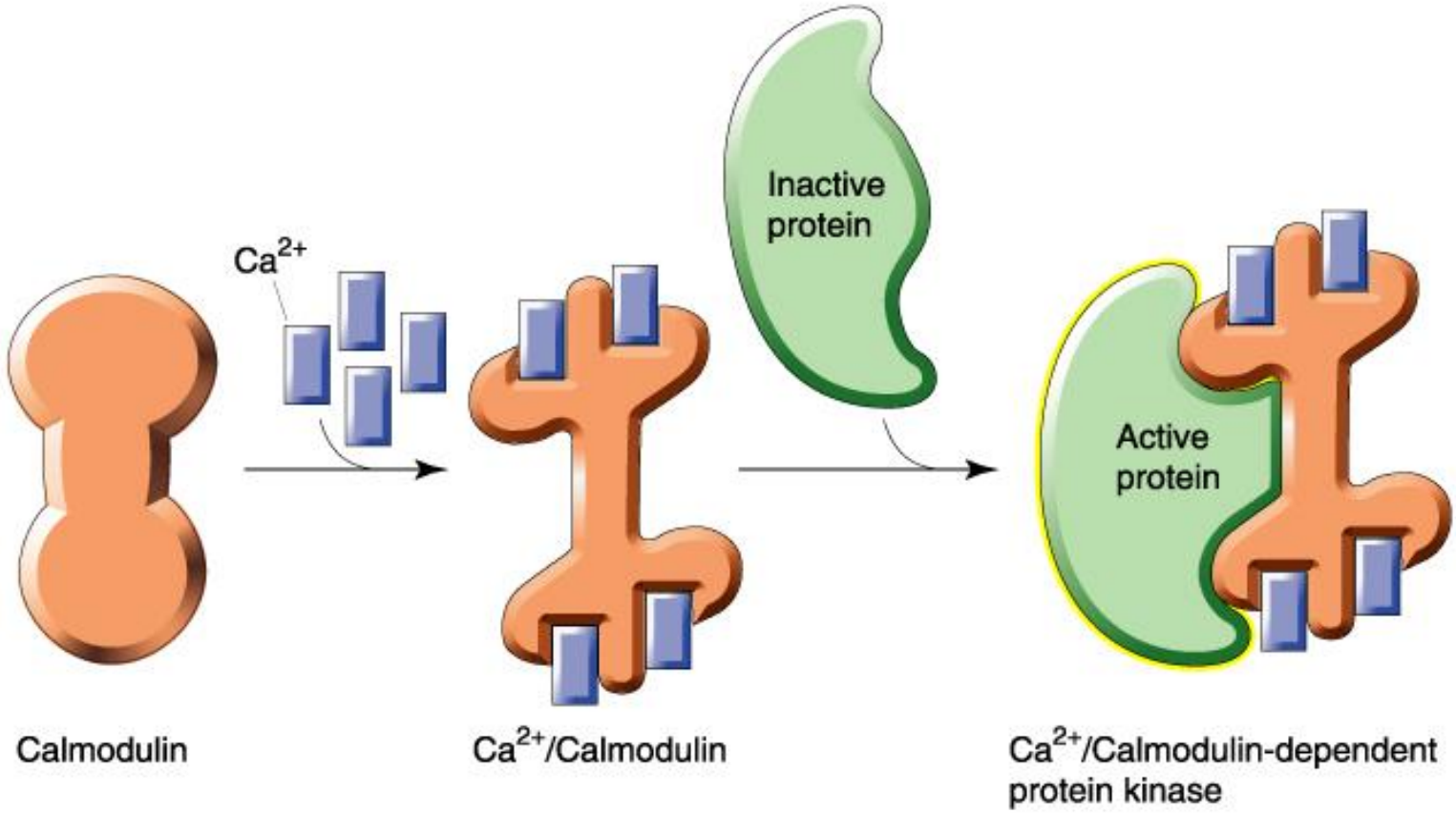


Ca^{2+}- Calmodulin (continued)

- 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)

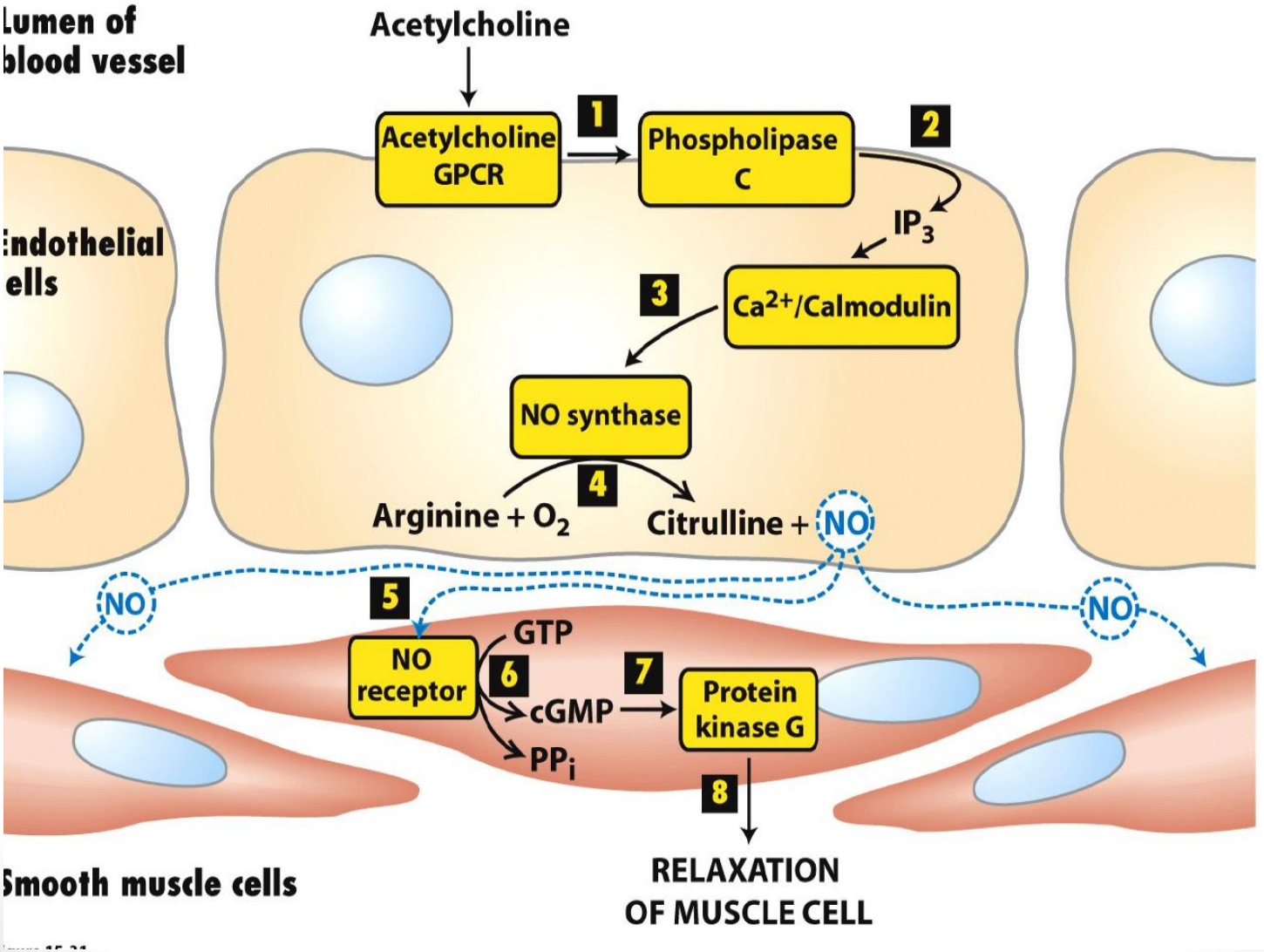


Guanylate cyclase (GC) receptor

Membrane receptor –ANP

Soluble receptor – NO, CO

NO signaling



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.

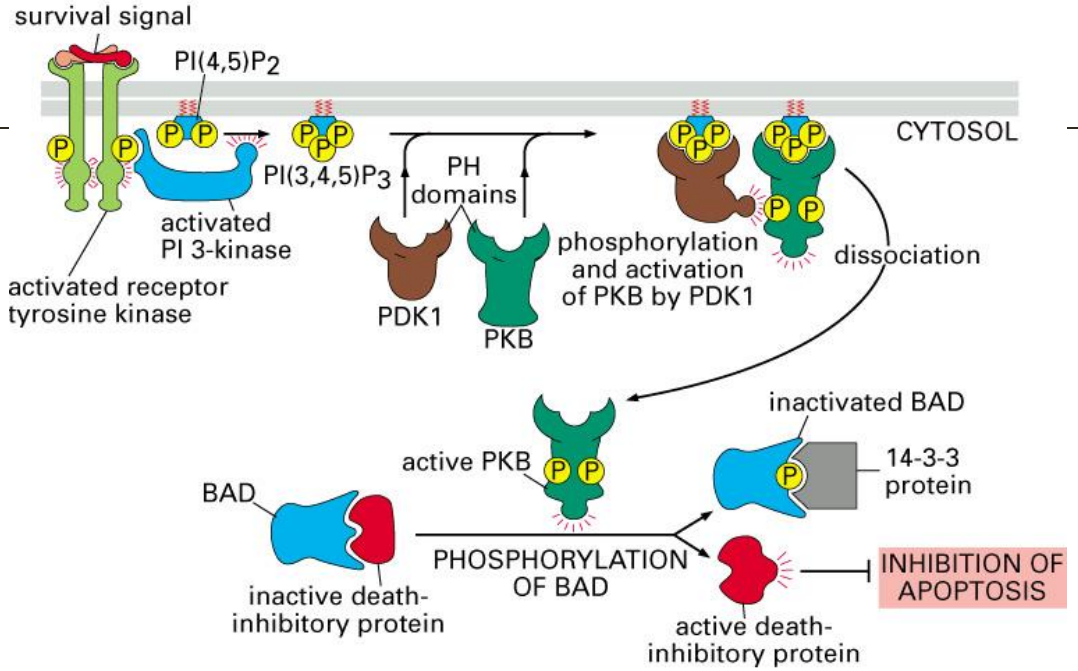


Figure 15–60. Molecular Biology of the Cell, 4th Edition.

5. Signaling Cascades, cont.:

5 downstream kinases activated by different signaling cascades

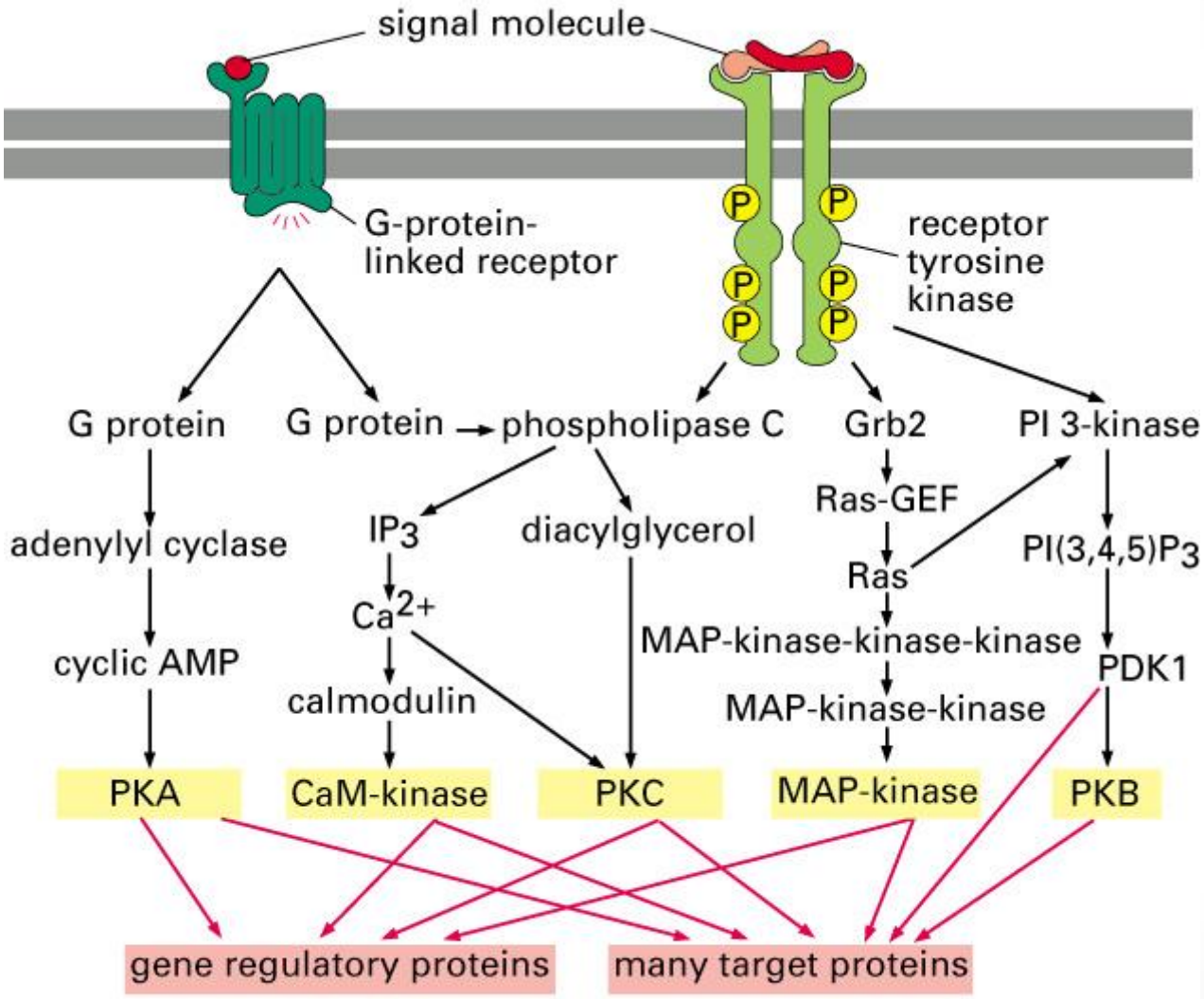
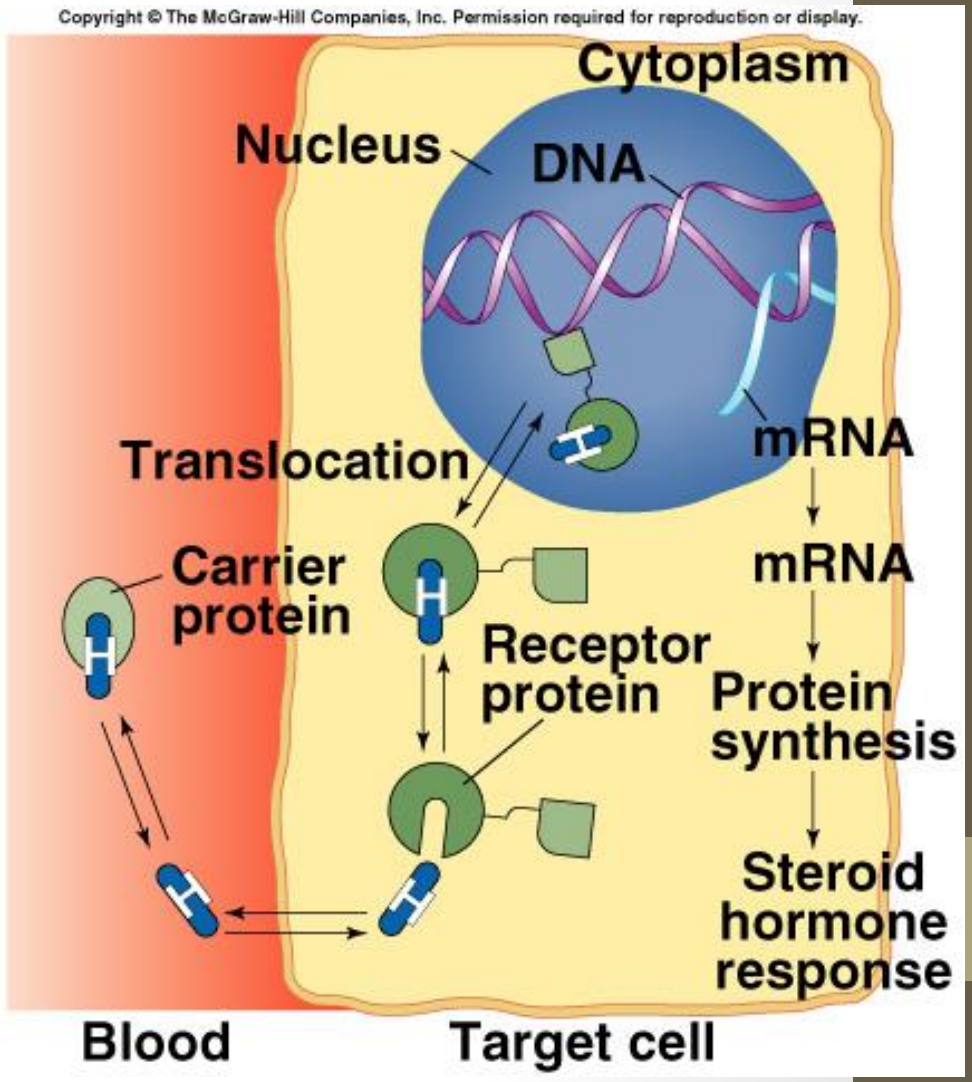


Figure 15–61. Molecular Biology of the Cell, 4th Edition.

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.

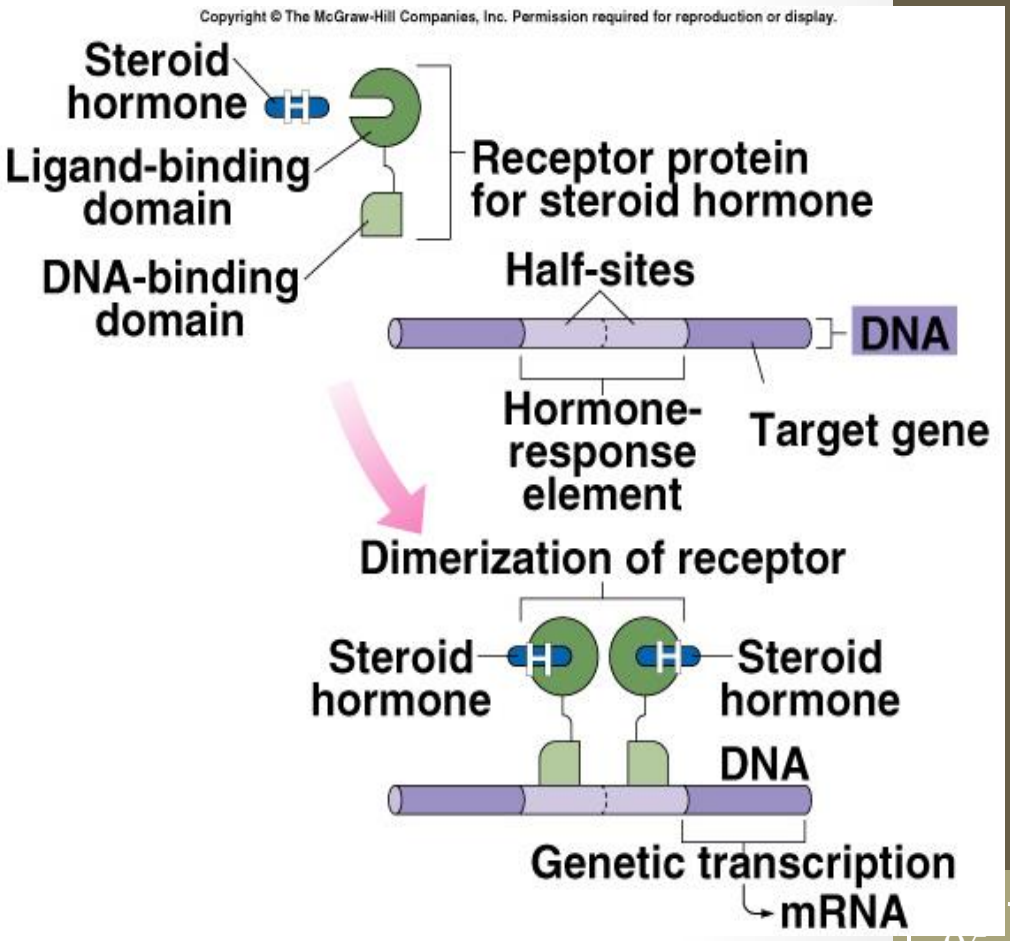


Nuclear Hormone Receptors

- 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.

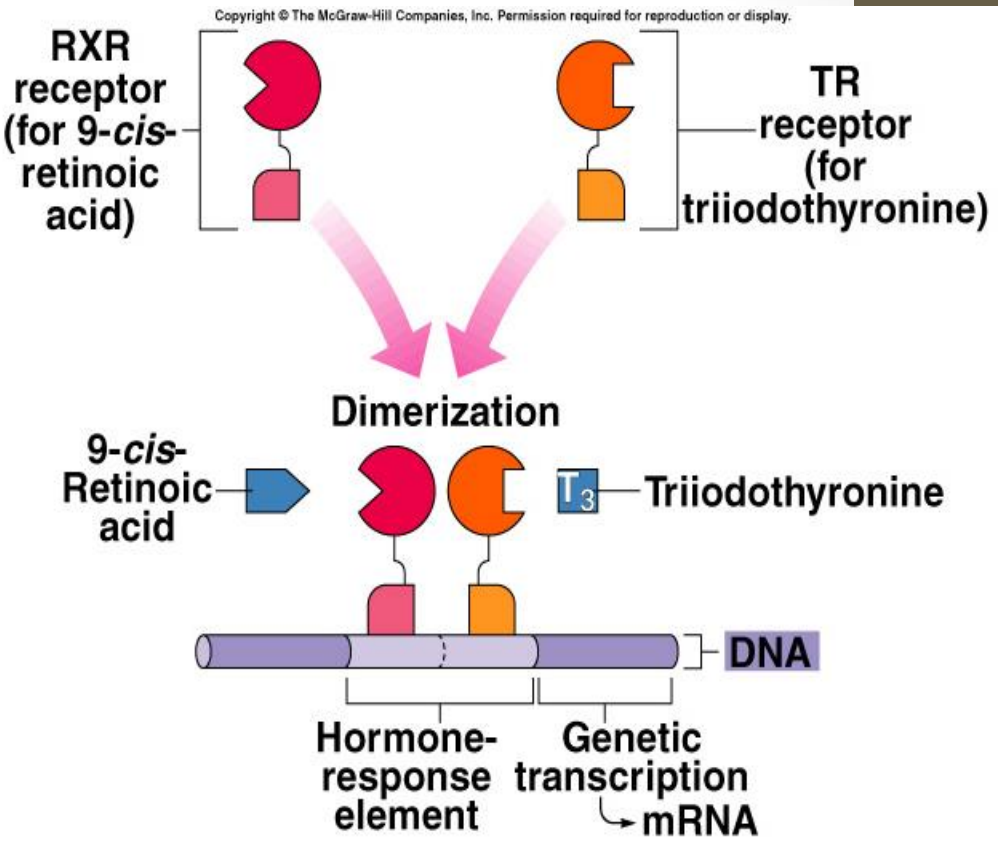
Mechanisms of Steroid Hormone Action

- 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.

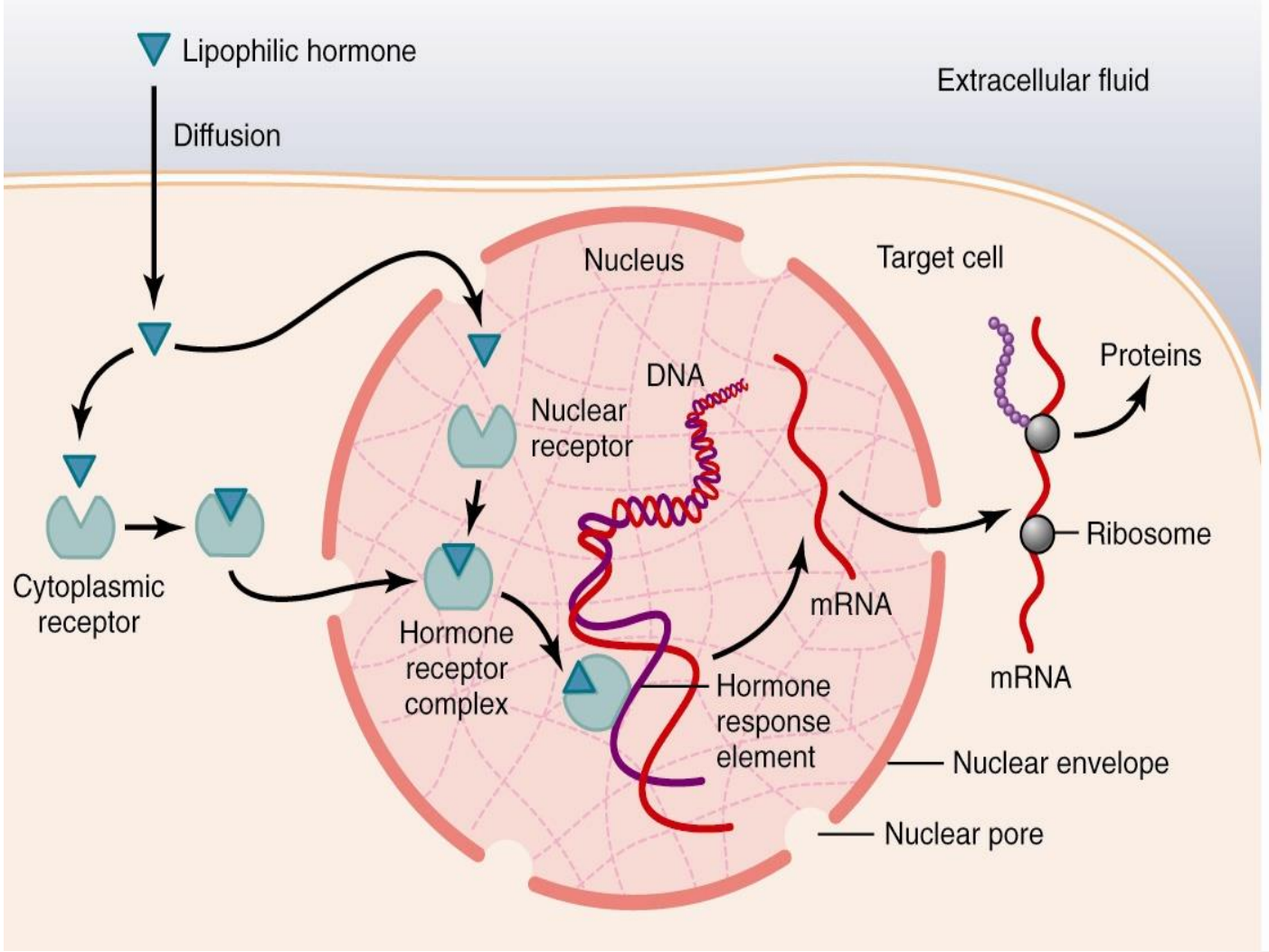


Mechanism of Thyroid Hormone Action

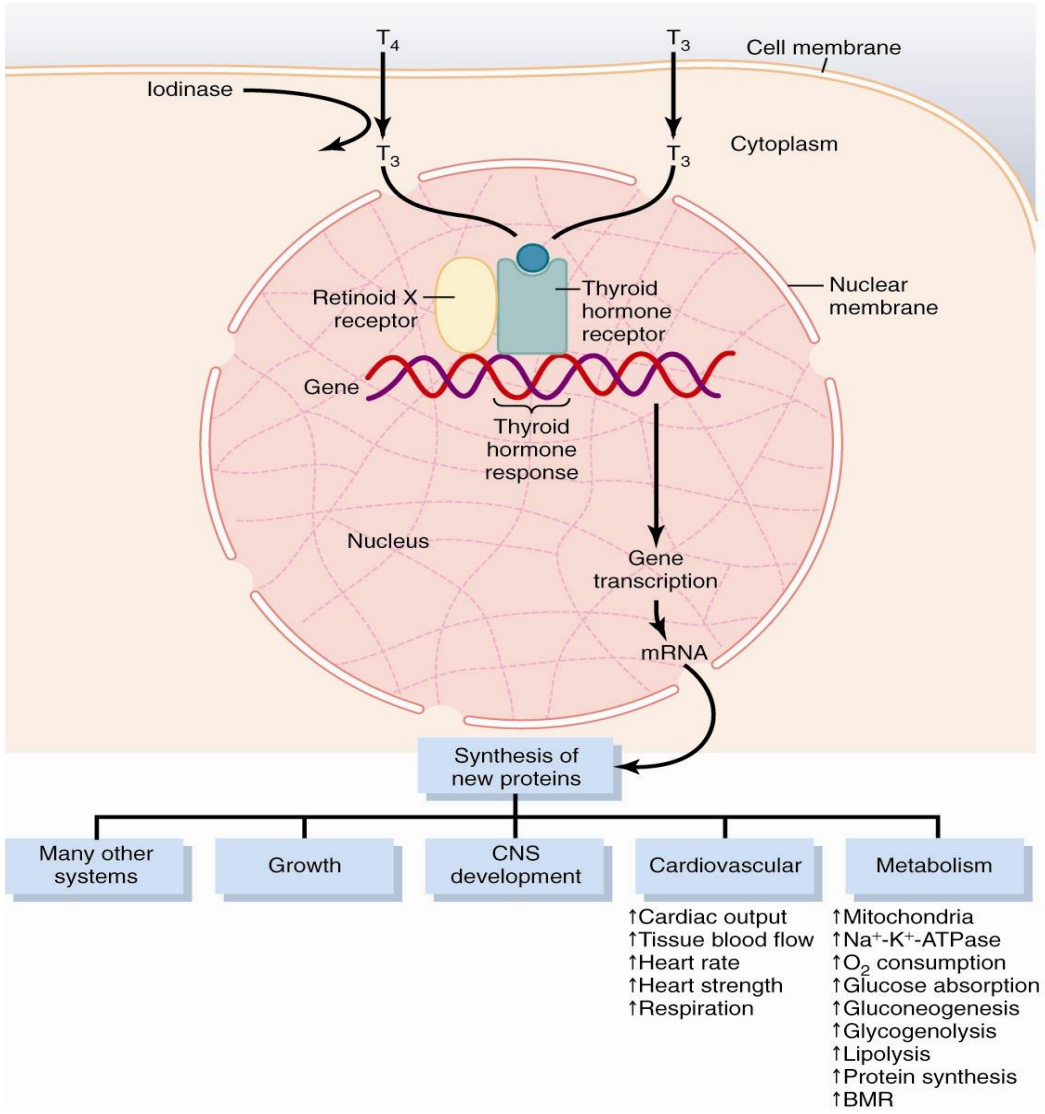
- T_4 passes into cytoplasm and is converted to T_3.
- Receptor proteins located in nucleus.
  - T_3 binds to ligand-binding domain.
  - Other half-site is vitamin A derivative (9-cis-retinoic) acid.
    - DNA-binding domain can then bind to the half-site of the HRE.
  - Two partners can bind to the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



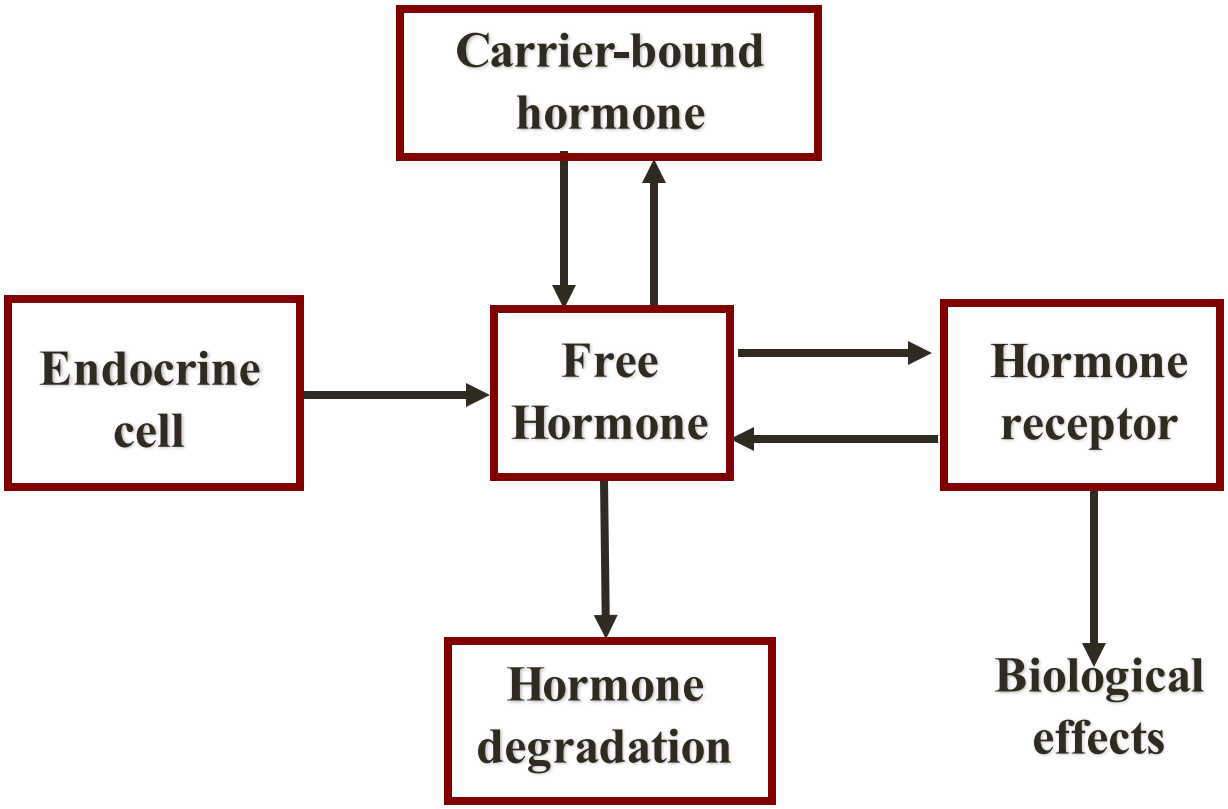
Steroid & Thyroid Hormones - Mechanism of Action



Actions of Thyroid Hormones



Determinants of Free Hormone Receptor Binding

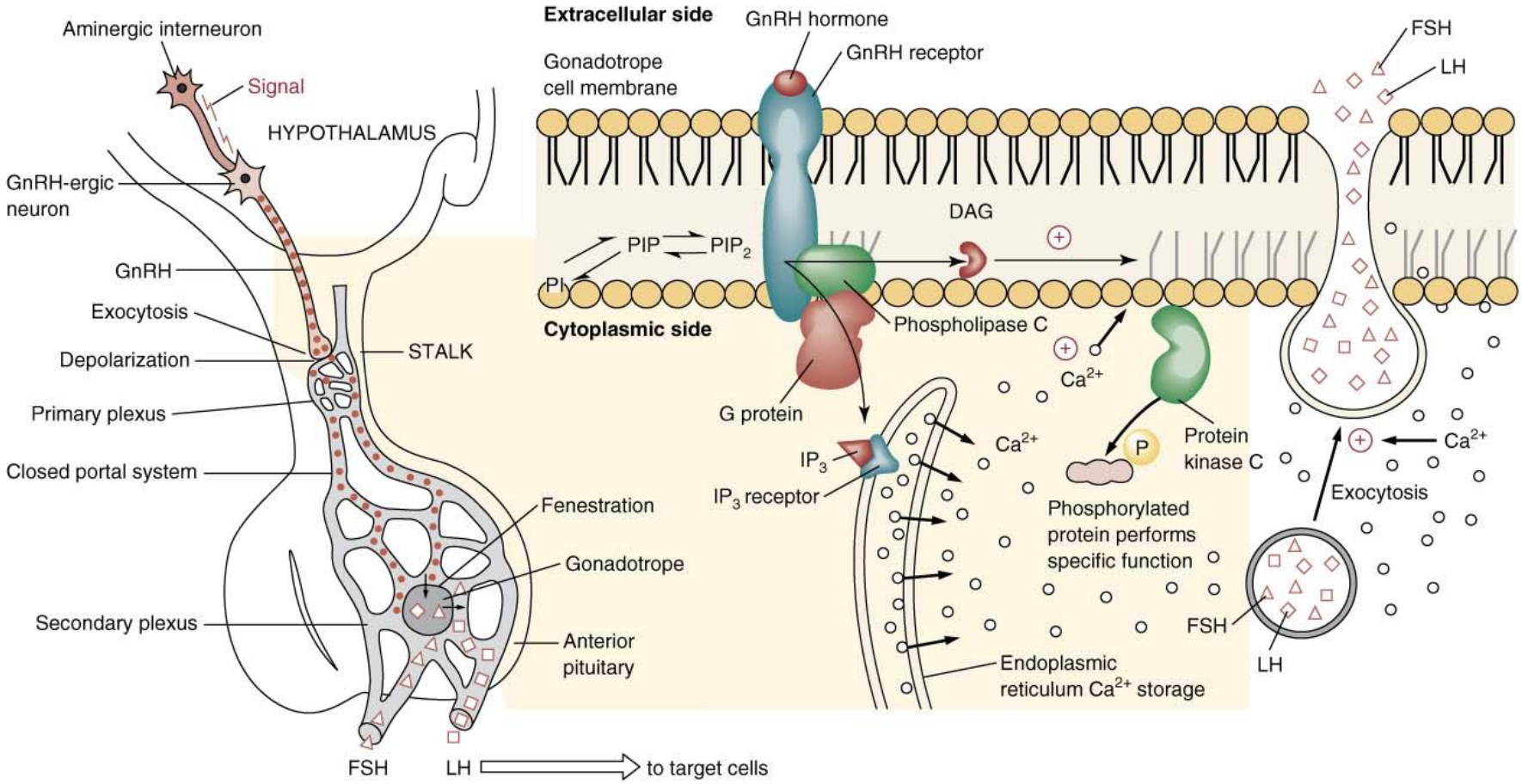


Correlation of Plasma Half-Life & Metabolic Clearance of Hormones with Degree of Protein Binding

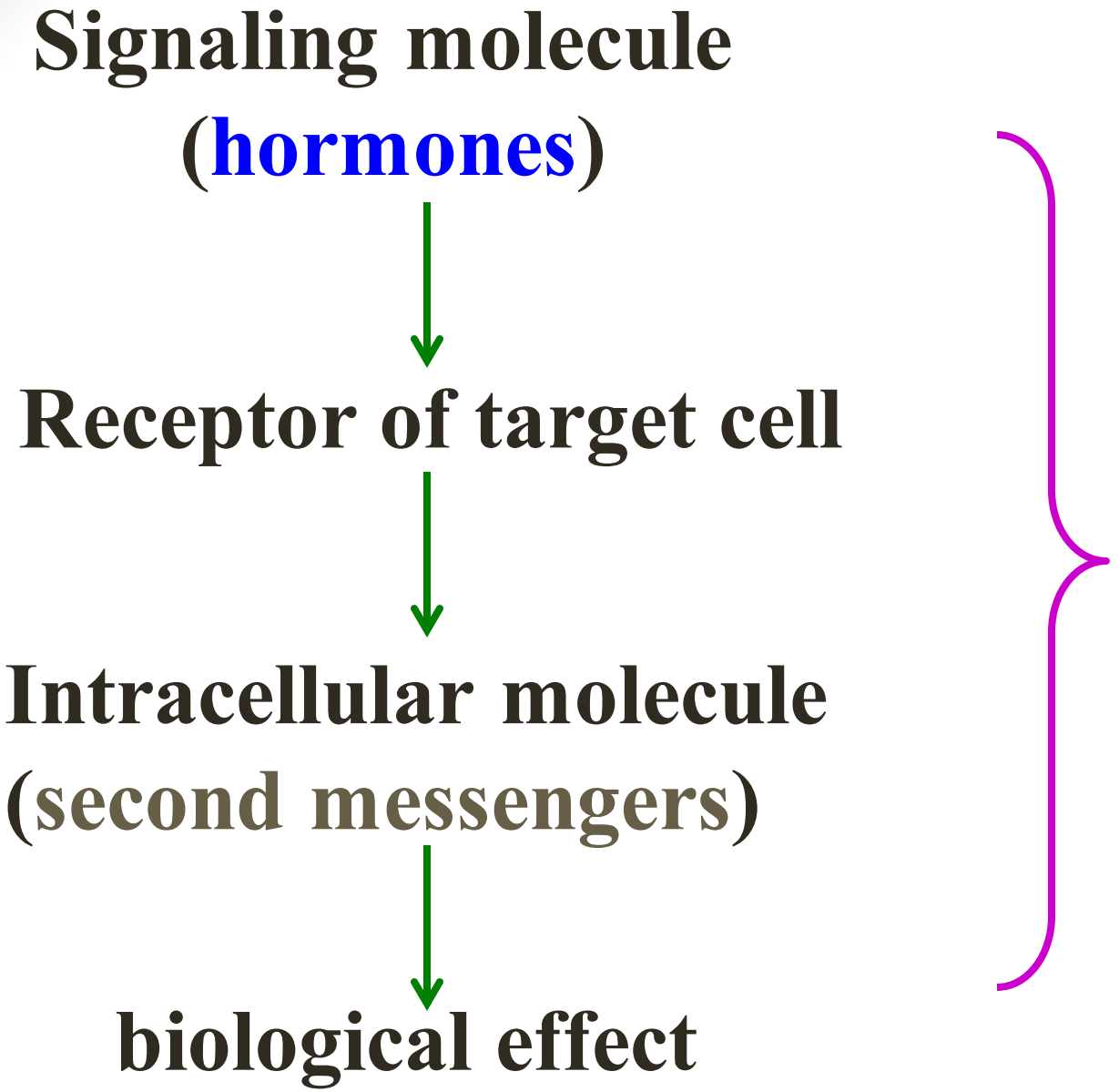
Hormone	Protein binding (%)	Plasma half-life	Metabolic clearance (ml/minute)
Thyroid			
Thyroxine	99.97	6 days	0.7
Triiodothyronine	99.7	1 day	18
Steroids			
Cortisol	94	100 min	140
Testosterone	89	85 min	860
Aldosterone	15	25 min	1100
Proteins			
Thyrotropin	little	50 min	50
Insulin	little	8 min	800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

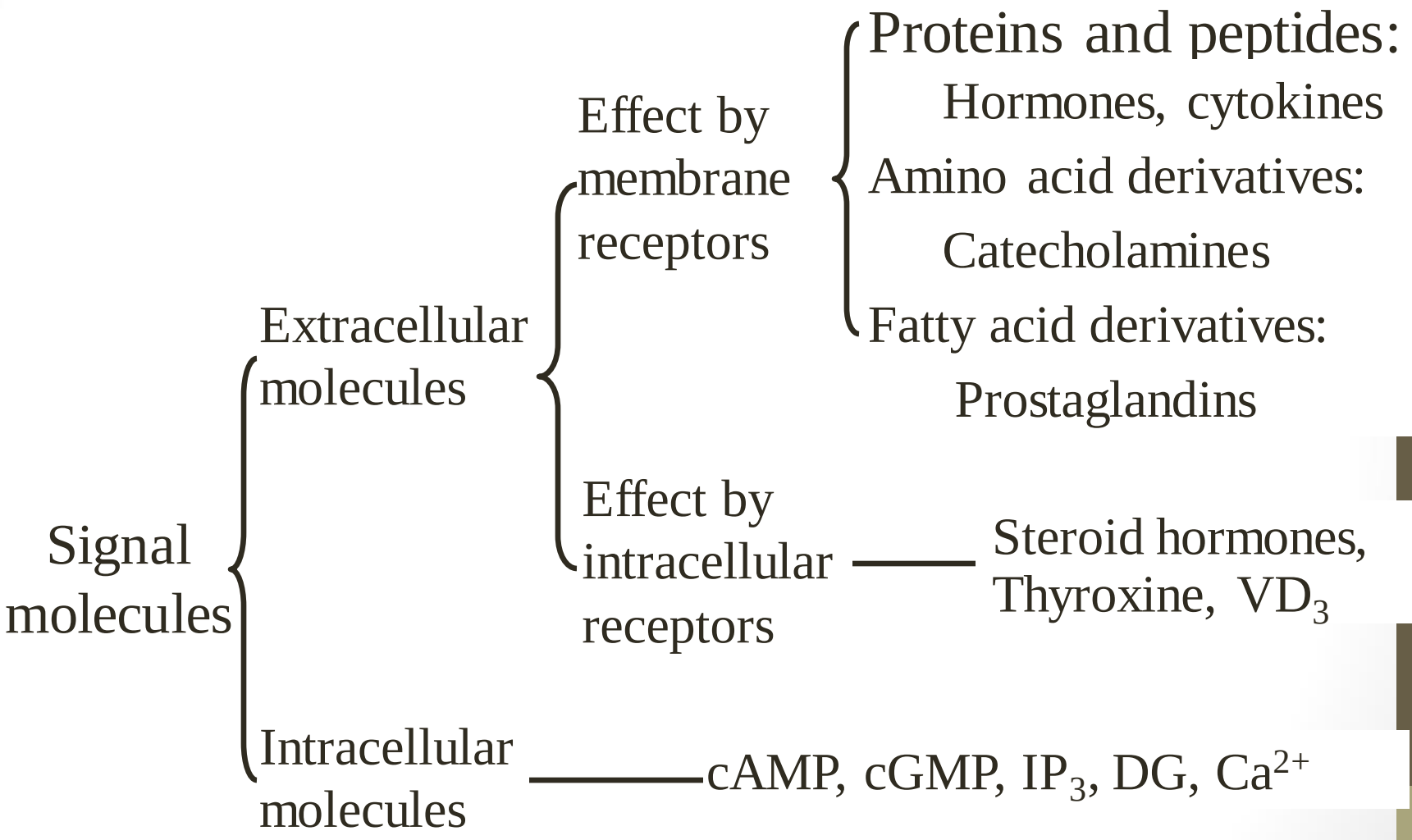


Regulation of secretion of LH and FSH by protein kinase C.


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.


Signal molecules
Extracellular molecules
Effect by membrane receptors
Proteins and peptides:
Hormones, cytokines
Amino acid derivatives:
Catecholamines
Fatty acid derivatives:
Prostaglandins
Effect by intracellular receptors
Steroid hormones, Thyroxine, VD_3
Intracellular molecules
cAMP, cGMP, IP_3, DG, Ca^{2+}